PATTERNS OF ENDEMISM AND SPECIES RICHNESS IN MALAGASY COPHYLINE FROGS SUPPORT A KEY ROLE OF MOUNTAINOUS AREAS FOR SPECIATION

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Cophyline narrow-mouthed frogs (Anura: Microhylidae) are a diverse endemic radiation of Madagascar. Cophylines contain a high proportion of range restricted species and constitute a good model system to understand patterns of evolutionary diversification in tropical ecosystems. We combine spatial and phylogenetic analyses for a near-complete taxon sample to test competing explanations for patterns of species richness (SR) and endemism. Our reconstruction of the phylogeny of cophylines indicates the presence of 22 new species and several instances of nonmonophyly. We found a strong historical signal in current cophyline ranges indicating a high degree of spatial niche conservatism in clade diversification, with clades occurring in the North of Madagascar constituting the most derived in the phylogeny. We identified six positively correlated centers of SR and endemism that can neither be explained by stochastic models such as elevational or latitudinal mid-domain effect, nor by low-elevation river catchments. Instead, the locations of these centers in areas spanning a high altitudinal range in combination with specific climatic parameters support a key role of mountainous areas for speciation of these anurans, although we cannot exclude an influence of habitat loss due to human impact. High conservation priority is ascribed to these areas.

KEY WORDS: Amphibia, Cophylinae, hotspot, Madagascar, phylogeography, Microhylidae.

On a global scale, Madagascar belongs to the world's top 12 "mega diversity" hotspots with high levels of taxonomic endemism and species diversity (Myers et al. 2000; Goodman and Benstead 2005). Understanding mechanisms that shaped the present patterns of species richness (SR) and endemism within such species-rich tropical environments is a fundamental question in evolutionary biology (Moritz et al. 2000; Wiens and Donoghue 2004). Yet, these processes are not well understood for Madagascar, mainly due to missing taxonomic, phylogenetic, and distributional information.

Biogeographic patterns of endemism have previously been studied for Malagasy mammals (Martin 1972; Wilmé et al. 2006; Wilmé and Callmander 2006), reptiles (Blanc 1971; Lang 1990; Raxworthy and Nussbaum 1995; Raxworthy and Nussbaum 1997), and amphibians (Blommers-Schlösser and Blanc 1993; Lees 1996; Lees et al. 1999). Raxworthy and Nussbaum (1996) found areas of endemism to be congruent among almost 360 vertebrate species belonging to four groups (amphibians, reptiles, birds, nonvolant mammals) and identified four regions of endemism for amphibians and reptiles, whereas no clear predictions could be made for birds and mammals. Recent studies suggest that diversification processes in Madagascar occur within distinct ecoregions (e.g., Wilmé et al. 2006; Wilmé and Callmander 2006; Boumans et al. 2007). Wilmé et al. (2006) proposed that these ecoregions may correspond to watersheds; past climatic changes would have led to a contraction of forests and restriction of forest-depending taxa to the surroundings of rivers in these watersheds. River catchments with sources at relatively low elevations would have been separated by arid intervening areas that constituted barriers to gene flow; these catchments thus served as zones of isolation, leading to the speciation of locally endemic taxa, whereas river catchments with sources at higher elevations served as zones of retreat and dispersion and hence today contain proportionately lower levels of endemism. As an alternative hypothesis it has been suggested that the barriers to gene flow were and are the large rivers themselves, especially at low elevations where they are widest, and thus could promote lineage diversification (Pastorini et al. 2003; Louis et al. 2005; Vieites et al. 2006; Boumans et al. 2007).

In a different line of argument, several studies have emphasized the high species diversity of the mountain massifs in northern Madagascar and their putative function as refugia, centers of clade origin and endemism (Raxworthy and Nussbaum 1997; Andreone et al. 2000 and ref. therein; Yoder and Heckman 2006; Boumans et al. 2007). In a variety of reptile species, basal intraspecific lineages and highest haplotype diversity occur in this region (Boumans et al. 2007). Other authors (Lees 1996; Lees et al. 1999) identified centers of SR for several animal taxa, including frogs, in the Northern Central East of Madagascar, specifically in the area of Andasibe (= Périnet), indicating

the possibility of a stochastic community assemblage and contradicting the existence of a strict biogeographical zonation of Madagascar.

In this article, we integrate GIS-based records with phylogenetic reconstruction and multivariate analyses of climatic and habitat information to infer patterns of SR and endemism in an endemic Malagasy vertebrate radiation with near-complete taxon sample. We determine whether centers of SR and endemism are congruent with the ecoregions proposed by Wilmé et al. (2006), and test four hypotheses for their formation. These are (1) the mid-domain effect (MDE, i.e., stochastic community assemblage), (2) recent historical processes of habitat fragmentation and loss, (3) key ecological parameters, and (4) spatial niche conservatism and phylogeographic history.

(1) The mid-domain effect provides a null model for spatial variation in SR and has been assumed to explain the species distribution patterns of a variety of vertebrate and invertebrate species in Madagascar (Lees et al. 1999; Colwell and Hurtt 1994; Colwell and Lees 2000). Under the MDE, if species ranges are distributed randomly within a given domain lacking any biological response to environmental gradients, a peak of SR will occur in the center of the domain (Laurie and Silander 2002). Regarding the apparent biogeographic subdivision of Madagascar (Koechlin 1972; Martin 1972; Goodman and Ganzhorn 2004; Wilmé et al. 2006), the MDE should be considered a null model rather than a hypothesis explaining biological reality (Colwell et al. 2004); in fact, a recent study showed that SR patterns in Malagasy birds and small mammals do not follow the patterns predicted under the MDE (Kerr et al. 2006; but see Lees and Colwell 2007). Under the MDE, we would expect a peak in SR in the center of Madagascar, whereas no predictions for patterns of endemism can be made.

(2) Spatial variability of SR and endemism could alternatively be caused by a recent historical process, namely habitat fragmentation and loss due to human impact. Currently Madagascar retains only $\leq 10\%$ of its natural habitat (Green and Sussman 1990; Dufils 2003; Goodman and Benstead 2005). Under this scenario, centers of endemism and SR would be simply determined by the amount of remaining primary habitat (Kerr et al. 2006). Madagascar's animal taxa are characterized by generally small distribution areas and high levels of endemism, and thus entire species are likely to be threatened by medium to small-scale habitat loss (Goodman and Benstead 2005). We would expect areas with a larger coverage with primary vegetation to harbor more intact animal communities and thus have a higher probability of being species-rich than areas retaining only small patches of primary habitat. In contrast, such fragmented areas will tend to be occupied by fewer species, and these areas may display a higher degree of endemism due to the loss of large parts of the ranges of the taxa inhabiting these areas.

(3) A third hypothesis explaining patterns of SR and endemism is that certain environmental key factors favor the presence of a higher number of species or of a higher number of range-restricted species. In this case, species dispersing stochastically would randomly assemble in areas characterized by these environmental factors, e.g., climatic parameters, topographic structure, or vegetation heterogeneity. As a result we would expect strong correlations between SR or endemism and the determining factors, but not necessarily a correlation between SR and endemism. Stochastic community assembly is mainly expected in groups of wide-ranging and highly vagile species that is usually not assumed for amphibians (Duellman and Trueb 1986). However, recent evidence indicates that wide-ranging amphibian species exist (Fouquet et al. 2007) and that also in some Malagasy frogs, recent gene flow or fast dispersal characterizes at least some species (Rabemananjara et al. 2007). Usually, contemporary climate conditions are seen as important in maintaining SR (Currie et al. 1999; Jetz et al. 2004), but also as an influential predictor for centers of endemism (Jetz et al. 2004). Montane areas, in particular montane forests, have repeatedly been demonstrated to be key areas for the occurrence of narrowly distributed species in Africa (Diamond and Hamilton 1980; Collar and Stuart 1988; Johnson et al. 1998; Linder 2001).

(4) Lastly, current patterns of SR and endemism can be a result of spatial niche conservatism (Wiens and Graham 2005) and thus be correlated with the phylogeographic history of the radiation in question. The spatial variation of endemism and SR within an evolutionary lineage is ultimately caused by its species ranges, which are the outcome of the evolutionary history of that lineage (Ricklefs and Schluter 1993; Jetz et al. 2004; Wiens and Donoghue 2004). Under a scenario of constant speciation rates not influenced by environmental factors, and applied to species with low dispersal abilities, areas of clade origin will contain a higher number of species as well as many endemics because in these areas there was more time available for diversification, as compared to areas that were only recently colonized by representatives of the lineage under study. Under this hypothesis we expect a strong phylogenetic signal in present-day distribution patterns revealing historical centers of clade origin (cf. Smith et al. 2007). We also expect centers of SR to harbor more endemics (Jetz et al. 2004).

Hypotheses (3) and (4) are not mutually exclusive. Speciation could be favored by environmental characteristics that are found only in particular areas, in which case environmental key factors and spatial niche conservatism would be reinforcing each other. For example, species living in mountainous forests have been demonstrated to have a higher frequency of speciation (Graves 1988; Rahbek 1997; Smith et al. 1997; Schneider and Moritz 1999; Moritz et al. 2000; Jetz et al. 2004) which may explain the higher

SR in these areas. Hence, a phylogenetic signal in spatial patterns and a correlation of SR and endemism, in the absence of environmental correlates of richness and endemism, favors hypothesis (4). Correlation of SR and endemism with habitat features, but not necessarily with each other, and without a clear phylogenetic signal, favors hypothesis (3). A combination of these results favors a combination of hypotheses (3) and (4).

In this study, we test these alternative scenarios in a subfamily of narrow-mouthed frogs (Microhylidae: Cophylinae) which represents one of three endemic amphibian radiations in Madagascar. These frogs are mostly restricted to primary habitat and, like amphibians in general, can be assumed to exhibit limited dispersal capabilities. All but three cophyline species are distributed exclusively in humid eastern Madagascar including the Sambirano region (Glaw et al. 2007).

Cophylines currently contain 44 described species exhibiting a remarkable diversity of ecological life styles (Andreone et al. 2005). This species diversity may be linked to the specialized reproductive mode of this lineage, with endotrophic (nonfeeding) tadpoles (Andreone et al. 2005). So far, supraspecific classification of cophylines has been difficult due to their reduced bioacoustic repertoires, miniaturized body size of many species in concert with the reduction of morphological characteristics (e.g., digits), as well as possible convergent evolution of morphological traits such as terminal disks on fingers in arboreal species (cf. Blommers-Schlösser 1976; Wu 1994; Andreone et al. 2005). The use of molecular and karyological characters on limited sets of cophylines has already led to generic rearrangements and revealed cryptic diversity within this lineage (Andreone et al. 2005; Frost et al. 2006; Aprea et al. 2007).

Here we establish a molecular phylogeny for 36 of the 44 currently known cophyline species, and add sequences for 23 potentially new species to clarify evolutionary relationships among them. We use the phylogeny of these frogs in concert with a GIS-referenced locality database to (1) identify centers of SR and endemism for this endemic radiation, and (2) explain the observed patterns. Because Madagascar is among the most critical geographic priorities for conservation (Myers et al 2000; Yoder et al. 2005), we will (3) discuss the usefulness of these results to predict areas of high conservation priority in Madagascar.

Materials and Methods
MOLECULAR DATA ANALYSIS

We determined DNA sequences from 36 of the 44 currently described cophyline species (www.amphibiaweb.org; *Plethodontohyla angulifera* is a dubious taxon not considered here) and complemented the dataset with sequences for *Rhombophryne coudreaui, R. serratopalpebrosa* and *Cophyla occultans* obtained

from Andreone et al. (2005). Species missing in our dataset are: *Anodonthyla hutchisoni, Platypelis alticola, P. cowani, P. tsaratananaensis, P. tetra, P. mavomavo, Plethodontohyla guentherpetersi,* and unfortunately the monotypic *Madecassophryne truebae*. We furthermore added sequences of 23 potentially new cophyline species. For voucher specimen and locality information see online Supplementary Table S1. We analyzed fragments of the mitochondrial 12S rRNA, tRNA$_{Val}$, 16S rRNA, and Cytochrome *b* (*cob*) genes with a total length of 3273 bp (3216 bp after exclusion of ambiguous sites). The alignment was submitted to TreeBase (http://www.treebase.org; final accession number S2013) and sequences were submitted to GenBank (accession numbers EU340992-EU341121; see online Supplementary Table S1, Benson et al., 2004). PCR was performed using primers from Goebel et al. (1999) and thermocycling protocols described in Darst and Cannatella (2004) for 12S rRNA and 16S rRNA and in Chiari et al. (2004) for *cob*. Sequencing reactions were performed with the forward primers and resolved on automated sequencers. The obtained sequences were edited conservatively and verified as cophyline DNA via BLAST searches. *Phrynomantis* sp. (D. Cannatella, unpubl. data) was used as outgroup taxon. Sequences of scaphiophrynine and dyscophine Malagasy microhylids, *Scaphiophryne boribory, S. madagascariensis, S. marmorata, S. brevis, S. calcarata, Dyscophus insularis, D. antongilii,* and *Paradoxophyla palmata* (see online Supplementary Table S1) were included as hierarchical outgroups. In addition, a second, reduced dataset was compiled using a limited set of 15 of the above-mentioned taxa (representing all major clades of Malagasy cophylines except *Madecassophryne*) with the addition of 1380 bp of the nuclear Rag-1 gene (from the dataset of Van der Meijden et al. 2007), together with seven newly determined sequences for this gene; GenBank accession numbers EU341122-EU341128, see online Supplementary Table S1) with *Paradoxophyla* defined as outgroup taxon. For methods of phylogeny reconstruction see online Supplementary material S2.

IDENTIFYING CENTERS OF ENDEMISM AND SPECIES RICHNESS

Centers of endemism and SR were identified both one- and two-dimensionally. For two-dimensional analysis, we used locality information obtained from an extensive database (Glaw and Vences 2007) to construct GIS-referenced distribution maps of all cophyline species with ArcView GIS (ver. 3.2a, Esri © 1992–2000; ESRI, Redlands, CA). Minimum Convex Polygons (MCP) were assumed to represent conservative measures for actual species distributions as implied by the presence locality data. A digital map of Madagascar was partitioned into vector grid cells using the "Grid Make" extension in ArcView (© D. M. Tranel). Because an area of endemism can be defined as the smallest area to which two or more taxa are limited in distribution (Crisp et al. 2001), grid

cell size is an important variable in identifying centers of SR and endemism: proportionally small grids produce a higher number of endemism and SR centers, whereas very large grids can confound ecological factors that vary on a smaller scale that determine these centers and may blur the attempt to find adaptive explanations (Crisp et al. 2001); thus, the appropriate grid cell size varies according to the question of interest. We chose a grid cell size of 82×63 km $= 5166$ km^2, dividing Madagascar into 24 latitudinal and 8 longitudinal grid rows, which roughly matches the scale of sampling density in our locality database as well as the scale on which ecological parameters seem to vary (M. Vences and F. Glaw, pers. obs.). For each grid cell we subsequently computed values measuring degree of endemism and SR (1) separately for each of the major cophyline lineages and (2) for all cophylines combined.

We defined endemism according to Crisp et al. (2001), using the corrected weighted endemism (CWE), where the proportion of endemics in a grid cell is the weighted endemism (all species per grid counted, plus weighting each species with the inverse of its cell range) divided by the total number of species in a cell. We chose to use the CWE, because this index is least related to SR (Crisp et al. 2001; Linder 2001). Values for CWE were computed using the ArcView extension "Endemicity Tools" (provided by N. Danho). By definition, if no species occur in a certain grid cell, the CWE is not defined. Therefore "empty" grid cells were excluded from further analyses. Two-dimensional SR was defined as the number of species in a grid cell. Major biogeographic regions in Madagascar are referred to as in Boumans et al. (2007).

One-dimensional CWE and SR were computed from the two-dimensional dataset as arithmetic means for CWE / species counts over all grids per latitudinal grid category. This is a valid procedure, because all except three species of cophylines show an exclusively eastern-skewed distribution (Colwell et al. 2004), and thus no significant area effects are expected. Furthermore, one-dimensional SR was plotted with the software RangeModel (a Monte Carlo Simulation tool for assessing geometric constraints on SR, see Colwell 2006) by transforming each species MCP distribution into a set of coordinates for its latitudinal borders and midpoint. Both one-dimensional SR and endemism were again calculated separately for each of the major cophyline lineages, and for all cophylines combined.

HYPOTHESIS TESTING

To test among the four hypotheses, we established three datasets. These were distinguished by their case units. These were (1) grid cells with nonzero values for SR and endemism independent from their position within the Madagascar grid, (2) 24 latitudinal grid cell categories associated with variables averaged over latitude, (3) cophyline species. If not stated otherwise, statistical analyses were performed with STATISTICA (version 7.1 StatSoft, Tulsa, OK).

Following Lees et al. (1999), we tested for the mid-domain effect using RangeModel (R. Colwell, Storrs, CT), where a simulated species distribution expected under the MDE can be generated and compared to the empirical species distribution of the dataset. This test was performed using cophyline species and geographical coordinates for latitudinal species distributions. Congruence between the empirical cophyline distributions and the simulated distribution was tested using Mann–Whitney U-test and two-sample Kolmogorov–Smirnov test.

We performed a regression analysis to investigate the relationship between remaining primary vegetation coverage and the patterns of SR and endemism on the dataset containing nonzero grid cells. Values for amount of remaining primary vegetation (area in km^2) were obtained by intersecting the nonzero grid cells with a map of the remaining primary vegetation in ArcView (obtained from Du Puy and Moat 1996, 1998). Furthermore we tested with a Kruskal–Wallis ANOVA if the predictor "coverage of remaining primary vegetation" depends also on the altitudinal range covered by the grid cells by defining two altitudinal range categories (0–950 m, 951–1800 m; see below).

We tested if environmental factors are predictors for patterns of SR and CWE with multiple regressions. We used nonzero grid cells to test the effects of mean, minimal, and maximal altitude in the cells, altitudinal range spanned by the cells, and heterogeneity of vegetation (number of different vegetation types) on SR and CWE. The primary vegetation types used in this analysis were: mangrove, deciduous seasonally dry western forest, western coastal forest, deciduous dry southern forest, eastern coastal forest, evergreen humid forest (low altitude), evergreen humid forest (mid altitude), evergreen humid forest (lower montane), montane (*Philippia*) scrubland, evergreen sclerophyllous *Uapaca*, and marshland. Using the dataset of cophyline species we tested if specific climatic parameters favor narrowly distributed (distribution area ≤ 1 km^2, in contrast to widespread, distribution area ≥ 1 km^2) and thus potentially endemic species.

The effect of current climate on patterns of CWE was analyzed using 21 climatic variables from the WorldClim database version 1.4 (Hijmans et al. 2005) as predictors. These 21 climatic parameters were subjected to a factor analysis (PCA) in Varimax-raw rotated coordinate system prior to analysis to detect patterns of covariation and to reduce the number of operational variables. We performed nonparametric distance-based variance analyses using the software DISTLM version 5, (McArdle and Anderson 2001; Anderson 2001, 2004) to infer the effect of climate parameters on distribution area size classes while controlling for latitude. We subsequently performed a hierarchical partitioning analysis using the hier.part package in R (Chevan and Sutherland 1991; MacNally 2000, 2002; Walsh and MacNally 2003; MacNally and

Walsh 2004) to quantify the contribution of each predictor variable to the total explained variance, and to identify the climate variables that have the highest independent influence on cophyline range size classes.

Furthermore, latitudinal co-distribution of altitudinal range with SR and CWE was tested for latitudinal grid cells. Congruence between these distributions was determined using Mann–Whitney U-test and two-sample Kolmogorov–Smirnov test in STATISTICA (V7.1 StatSoft, © 1984-2005). We did not test for latitudinal co-variation of SR and CWE with climatic parameters as we could not obtain reliable climatic data for each grid cell

The evolutionary history of latitudinal species distribution midpoints was traced on a phylogeny of cophylines under the Maximum Parsimony criterion of character state reconstruction in Mesquite (Maddison and Maddison 2006) to estimate areas of clade origin. The phylogeny used was the all-taxon Bayesian tree with deep relationships (among genera) constrained following the additional analysis including Rag-1 sequences. The accuracy of trait reconstruction was confirmed by testing for the strength of phylogenetic signal in the latitudinal midpoint data, and whether they evolve under a Brownian motion model using the software CONTINUOUS (ver.1.0.d13, Pagel 1999, following Smith et al. 2007). We furthermore tested for correlation between SR and CWE both independently of latitude (only nonzero grid cells) and using latitudinal grid cells.

Results
COPHYLINE PHYLOGENY: GENUS LEVEL REARRANGEMENTS AND PARALLEL MINIATURIZATION

The combined analysis of 12S rRNA, 16S rRNA, and $tRNA_{Val}$ sequences resulted in a resolved phylogenetic hypothesis (Fig. 1). Support values were high for most relationships among species and for monophyly of genera, except those that were nonmonophyletic (see below). Basal relationships among genera were not supported except the placement of *Cophyla* sister to *Platypelis*, and the clade containing *Rhombophryne* and *Stumpffia*.

Of the 23 potentially new cophyline species, we found levels of genetic distinctness comparable to those of already described species among 22 specimens. This suggests they represent valid undescribed species (Fig. 1). Preliminary morphological and bioacoustic data are available for many of them (Glaw and Vences 2007) and support this conclusion. However, we refrain from a more detailed analysis of species status here.

Our data confirm that the genus *Plethodontohyla* as previously understood is not monophyletic. Two species (*Pl. coronata* and *Pl. minuta*) are firmly placed in *Rhombophryne*. Of the species currently classified in *Platypelis*, *P. occultans* is placed in a clade with the two species of *Cophyla*.
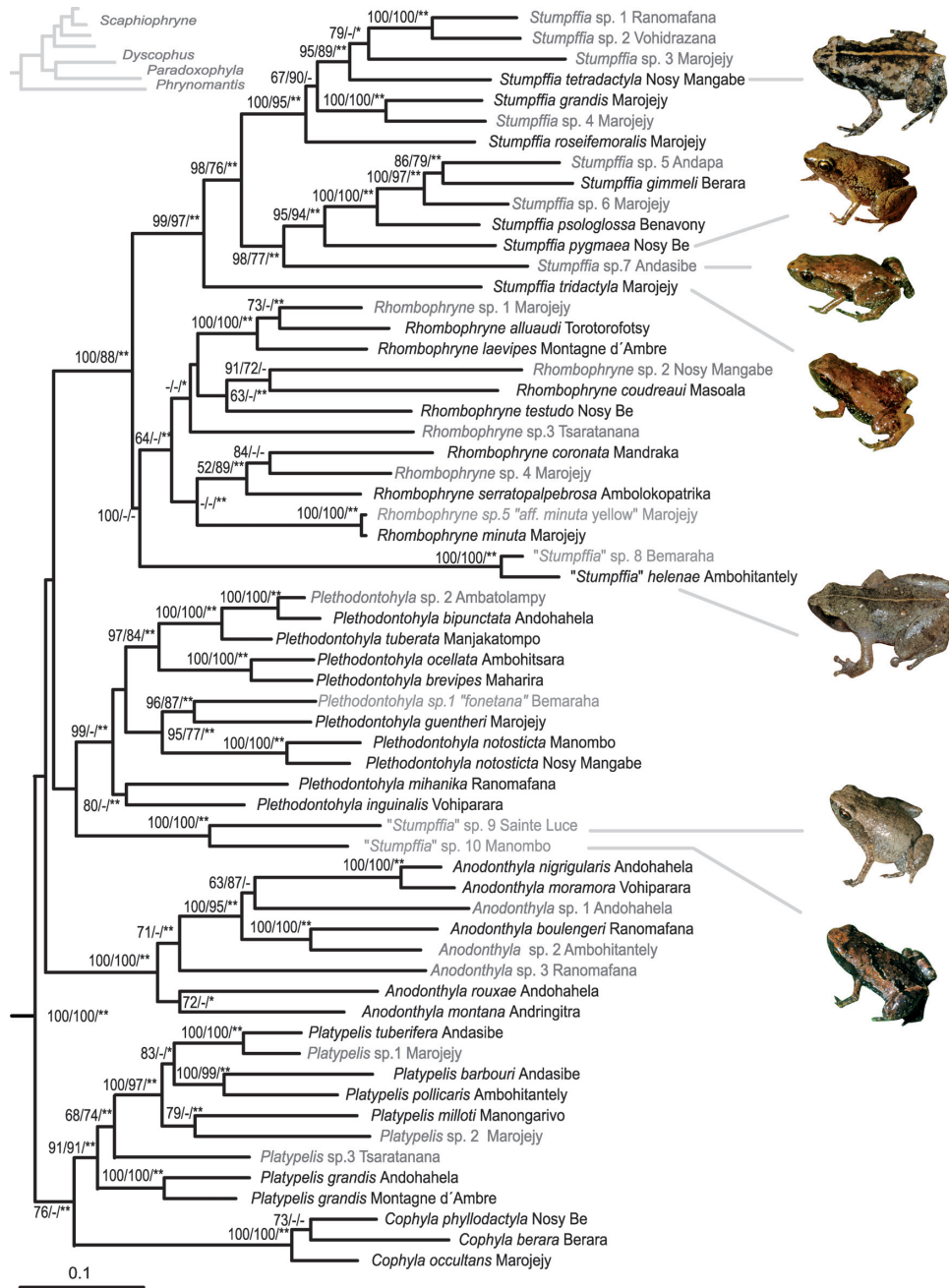
Figure 1. Bayesian phylogeny for the combined dataset of 12S rRNA, tRNA$_{Val}$, 16S rRNA, and *cob* gene fragments. Images show miniaturized cophyline species assigned to *Stumpffia*. In the western and southern *Stumpffia* clades, the genus name is written in quotation marks as these lineages represent undescribed genera. Values on nodes refer to ML bootstrap values ($\geq$ 50%), MP bootstrap values ($\geq$ 50%), and Bayesian posterior probabilities (asterisks $\geq$ 95%, double asterisks $\geq$ 99%). Gray taxon names indicate species that are yet undescribed.

The two samples of miniaturized terrestrial cophylines from South Eastern Madagascar (localities Manombo and Nahampoana/Sainte Luce), assigned to the genus *Stumpffia*, turned out to be unrelated to other *Stumpffia*. This placement is also supported by the topology including the Rag-1 fragment (see online Supplementary Fig. S3). Adding to the heterogeneity of *Stumpffia*, the only two species with enlarged finger disks (*S. helenae* and one undescribed species from Bemaraha) are also deeply genetically divergent. They are placed close to *Rhombophryne* in the full-taxon dataset, and sister to other *Stumpffia* species in the dataset including Rag-1 (see online Supplementary Fig. S3). Considering their distribution, we will refer to these species in the following as southern and western clades of *Stumpffia*. The analysis containing fewer taxa but including Rag-1 sequences provided a more robust

hypothesis on intrageneric relationships that we used for further analyses (see online Supplementary Fig. S3). The phylogenetic positions of the southern and western *Stumpffia* clades do not receive high support. Among the cophyline genera, *Rhombophryne* and *Stumpffia* (including the western *Stumpffia* clade) comprise a monophyletic lineage. *Platypelis* and *Cophyla* comprise the second well-supported clade. The placement of this group sister to the *Stumpffia*/*Rhombophryne* clade receives strong bootstrap support only in MP analysis. The placement of *Anodonthyla* sister to the above-mentioned group is well supported in ML and Bayesian analyses but not in MP, retaining *Plethodontohyla* (including the southern *Stumpffia* clade) as the most basal cophyline lineage.

CENTERS OF SPECIES RICHNESS AND ENDEMISM AND THEIR RELATIONSHIPS

The dataset of all cophylines combined revealed that SR and endemism are concentrated in particular regions of Madagascar (Fig. 2). We identified four areas with highest scores for each SR (centers of SR, CSR), and four areas with highest values of corrected weighted endemism (centers of endemism, CE). Two of these roughly coincide among the two analyses: The northern CSR involves several grid cells that are also included in the northern CE, although the highest scores do not refer to the same grid cells. The southeastern CSR has the highest score in one grid cell that also forms the southeastern CE. Furthermore, a central eastern and a southern CSR, and a western and a central CE are apparent. Online Supplementary Fig. S4 shows the identified areas of SR and endemism for the major cophyline lineages.

The three grid cells that constitute the northern CE, two of which also are part of the northern CSR, correspond almost perfectly to the major massifs in this region: from west to east, these are Manongarivo (with Manongarivo Special Reserve), Tsaratanana (with Tsaratanana Strict Nature Reserve), and Marojejy (including the National Parks Marojejy and Anjanaharibe-Sud,
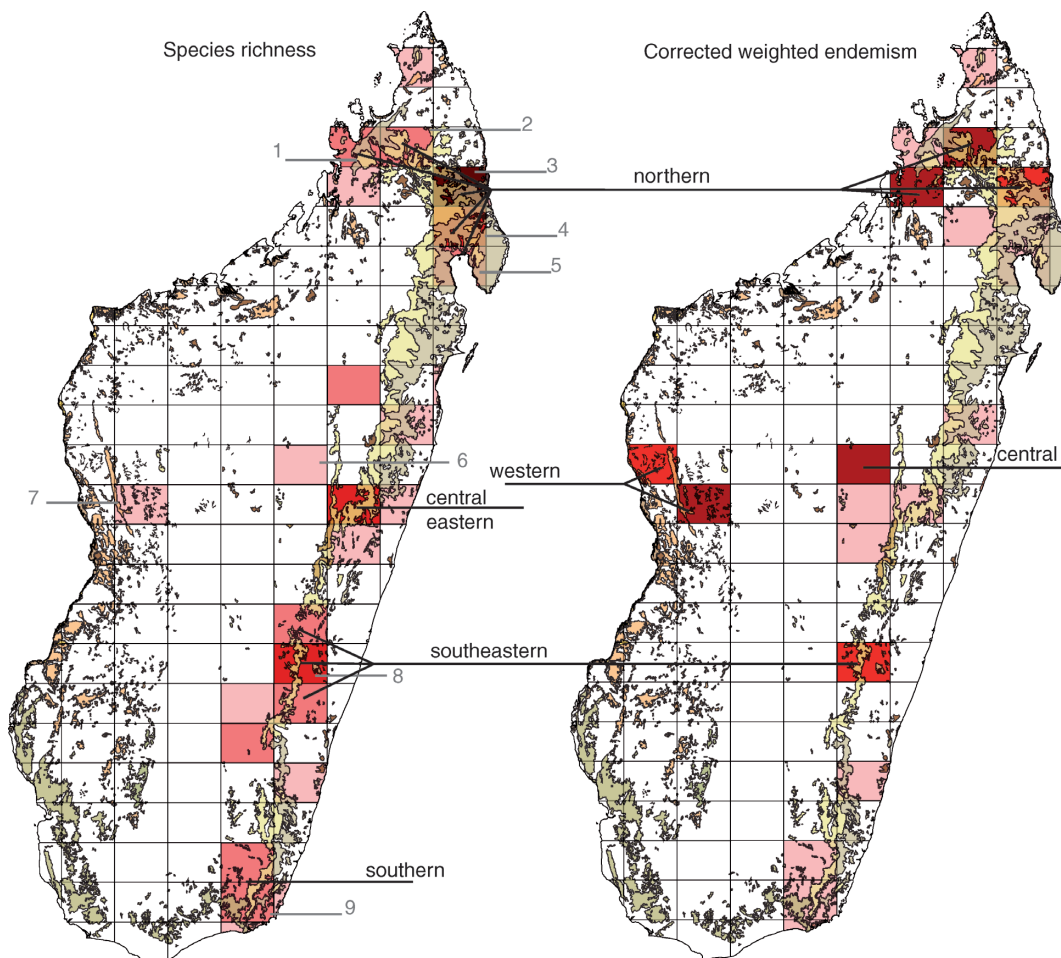
Figure 2. Two-dimensional patterns of species richness and endemism for all cophylines. Overlaid is the remaining primary vegetation. The grid shades represent (from light to dark) 0.2–0.4; 0.41–0.6 (not present); 0.61–0.8; and 0.81–1.0 for CWE, and empirical species counts per grid cell (maximally 26 in the Marojejy cell), respectively. Centers of species richness and centers of endemism are named. Grid cells including geographic localities mentioned in the text are indicated by gray numbers. 1 = Manongarivo; 2 = Tsaratanan; 3 = Marojejy; 4 = Masoala-Anjanaharibe; 5 = Masoala; 6 = Ambohitantely; 7 = Tsingy de Bemaraha; 8 = Ranomafana; 9 = Andohahela.

Fig. 2). Various additional grids in the north are identified as a CE (but bearing lower values for corrected weighted endemism than the three grid cells mentioned above): these correspond to the Sambirano region, especially the island of Nosy Be, the Montagne d' Ambre massif, and the area of Masoala National Park and Nosy Mangabe Island. Except for *Anodonthyla*, *Plethodontohyla*, and the southern and western *Stumpffia* clades, all major lineages of cophylines show high values for SR and endemism in this area. The central CE receives its importance mainly due to the presence of an endemic representative of the western *Stumpffia* clade (*S. helenae*) and an undescribed endemic species of *Anodonthyla* that are so far only known from the Ambohitantely Special Reserve that is located within that grid. In the West, due mainly to the presence of two endemic species (an undescribed species of the western *Stumpffia* clade and one of *Plethodontohyla*, which are the only cophylines endemic to western Madagascar), the two grids containing Tsingy de Bemaraha National Park are also highlighted as CE. The second (southeastern) hotspot for combined endemism and SR contains the area of Ranomafana National Park and, besides harboring high SR, receives high values for endemism. Endemic to this area are several members of *Anodonthyla*, *Stumpffia*, (and to a lower extent) *Plethodontohyla*, and *Platypelis*. The central eastern CSR contains Andasibe, a confirmed hotspot for Malagasy amphibian diversity (Lees 1996; Glaw and Vences 2007). The southern CSR is formed by Andohahela National Park and the Anosy/Vohimena chains. Several cophylines from this area are endemic (among them *M. truebae, Anodonthyla nigrigularis, A. rouxae,* and *Anodonthyla* sp. 1).

That the latter area was not identified as a CE is due to the fact that the localities in which these taxa occur are allocated to different grid cells, providing an example of the disadvantage associated with the method of a priori dividing an area into grid cells irrespective of actual ecological boundaries. The whole area between Ranomafana and Andohahela has not been sufficiently surveyed yet, and future studies may reveal additional still unknown species and range extensions.

One-dimensional measures of SR and endemism for the major cophyline lineages reveal genus-specific peaks and thus significant area effects. *Anodonthyla*, the western *Stumpffia* clade, and *Plethodontohyla* have their maximal SR in the latitudinal center of Madagascar, contrasted by all other cophylines that show SR peaks in the latitudinal north. An exception is the southern *Stumpffia* clade that has a strongly south-skewed SR pattern. Similarly, the corrected weighted endemism of all lineages except *Plethodontohyla* shows significant area effects (see online Supplementary Fig. S4).

HYPOTHESIS TESTING

Both a two-tailed Kolmogorov–Smirnov test (for curve shape) and a Mann–Whitney U-test (for curve medians) proved that that the cophyline dataset and the simulated dataset were not drawn from similar distributions (Table 1, Fig. 3A), rejecting a mid-domain effect. Because this test already yielded negative results, we did not test for a two-dimensional MDE or incorporate correction factors, such as band area, as suggested by Kerr et al. (2006).

Table 1. Kolmogorov–Smirnov two-sample tests (A) for differences in curve shape and less sensitive Mann–Whitney U-tests (B) for differences in curve medians for latitudinal variation of altitudinal range, species richness, corrected weighted endemism, and test for a latitudinal MDE. Both the Kolmogorov–Smirnov test and the first Mann–Whitney U-test reveal significant differences between latitudinal variation in empirical species richness and a simulated dataset, and thus the absence of a latitudinal MDE. The second and third Kolmogorov–Smirnov and Mann–Whitney U- tests reveal no significant differences in the latitudinal distributions of altitudinal range and species richness/corrected weighted endemism. Units are latitudinal grid cell categories. "Max neg diff" = maximum negative difference, "Max. pos. diff" = maximum positive difference.

A					
Dataset 1	Dataset 2	Max neg diff	Max pos diff	*P*-level	
Cophyline species richness	Simulated dataset	−0.617	0	$P \leq 0.001$	
Altitudinal range	Cophyline species richness	−0.083	0.25	$P \geq 0.1$	
Altitudinal range	Cophyline endemism	−0.125	0.208	$P \geq 0.1$	

B					
Dataset 1	Dataset 2	Rank sum Group 1	Rank sum Group 2	*U*	*Z*	*P*-level
Cophyline species richness	Simulated dataset	2383	4877	553	−6.545	$P \leq 0.0001$
Altitudinal range	Cophyline species richness	620	556	256	0.6598	$P \geq 0.51$
Altitudinal range	Cophyline endemism	591	585	285	0.0619	$P \geq 0.95$
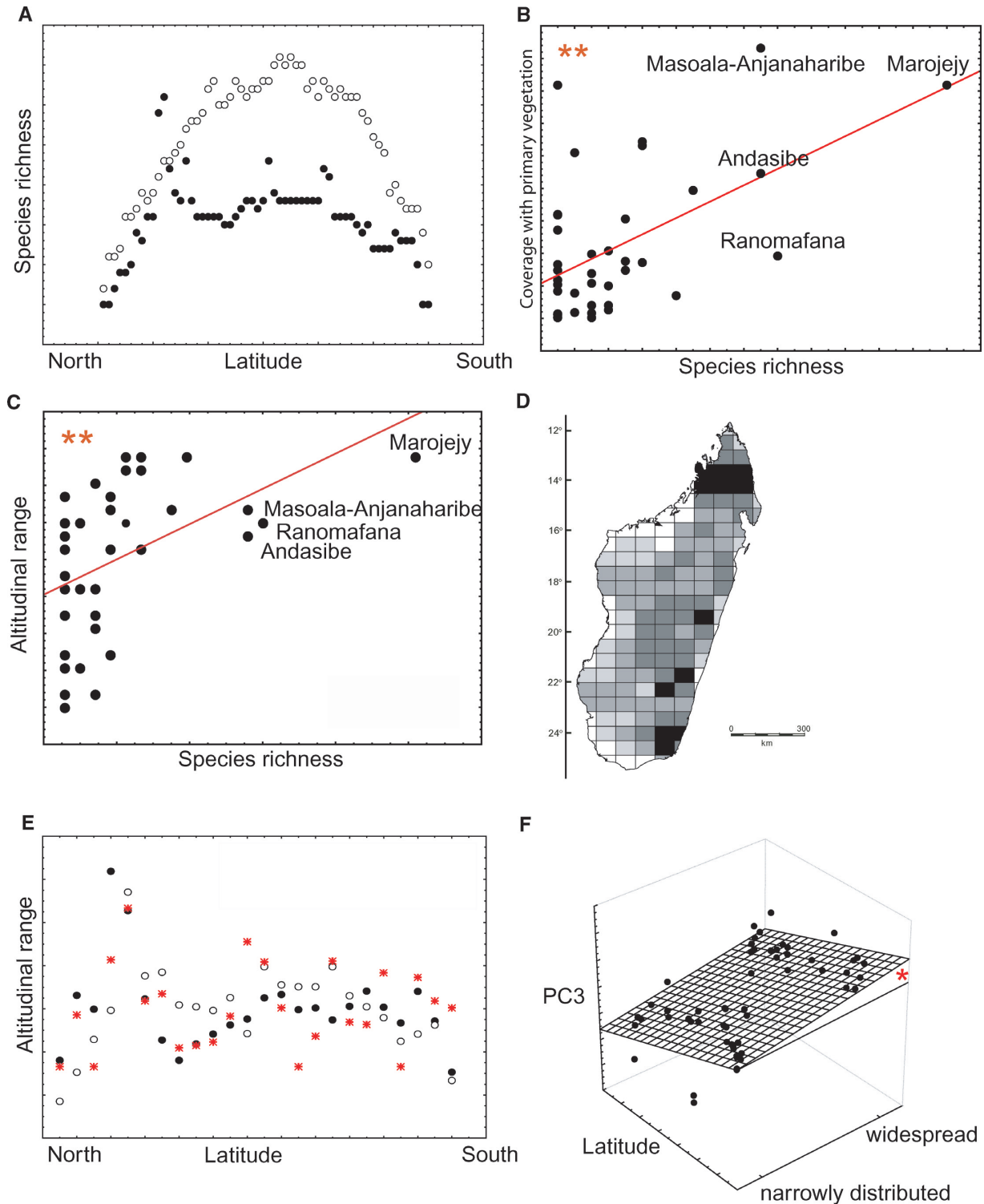
Figure 3. Correlations of climatic and habitat factors with species richness and endemism in cophylines. "Latitude" corresponds to latitude values given in 3D. (A) Graphical representation of RangeModel test for the MDE in one-dimensional species richness. Empty circles: simulated distribution; filled circles: empirical distribution of cophyline species richness. (B, C) Correlation scatterplots for species richness with the environmental factors altitudinal range and coverage with remaining primary vegetation. (D) Map of spatial distribution of "altitudinal range" in the Madagascar grid. Shades from transparent to black denote altitude spans from 0–300, 301–700, 701–1100, 1101–1600, 1601–1800 m. (E) Latitudinal covariation of cophyline species richness (empty circles) and endemism (stars) with altitudinal range (filled circles) (F) Graphical representation of the relationship between climate PC3, range size classes, and latitude. The star indicates the difference in PC3 between widespread and narrowly distributed species (= slope of the plane) independently from latitudinal variation.

Table 2. Multiple regression of remaining habitat (Veg cov) and the five environmental factors habitat heterogeneity (Hab het), mean altitude (Alt mean), minimal altitude (Alt min), maximal altitude (Alt max), and altitudinal range (Alt range) on species richness (SR) and endemism (CWE) values within grid cells, and their univariate effects. Significant effects are marked in bold.

	All effects F	All effects P	Veg cov F	Veg cov P	Hab het F	Hab het P	Alt mean F
CWE	0.881	0.521	0.178	0.676	0.999	0.324	1.402
SR	**3.152**	**0.016**	**14.263**	**0.0006**	1.534	0.223	0.074

	Alt mean P	Alt min F	Alt min P	Alt max F	Alt max P	Alt range F	Alt range P
CWE	0.244	0.401	0.531	1.384	0.247	0.091	0.765
SR	0.786	1.547	0.222	3.192	0.083	**8.72**	**0.005**

Following the methods of Kerr et al. (2006) we tested for the effect of habitat features on the number of species and on the number of range-restricted species among nonzero grid cells. Variation of CWE was not explained by any of these predictors, but SR was significantly predicted by these six habitat features (Table 2). Mean, minimal, and maximal altitude in the cells as well as heterogeneity of vegetation had no significant univariate effects on SR. However, we found a univariate significant effect of primary vegetation coverage on SR, these two values being positively correlated (Table 2, Fig. 3B).

Altitudinal range was also positively correlated with SR ($r = 0.4769$; $P = 0.003$, Fig. 3C) and CWE ($r = 0.1936$; $P = 0.24$, not shown), although the latter correlation was not significant. Multiple regression found altitudinal range to be a second predictor of two-dimensional SR with a significant univariate effect (Table 2). In contrast to the two-dimensional grid cell-based analysis, both latitudinal SR and endemism significantly covaried with altitudinal range spanned by the latitudinal grid cells categories (as confirmed by both Kolmogorov–Smirnov and Mann–Whitney U-tests (Table 1, Fig. 3D, E), showing maximal values in the north of Madagascar as well as in the southeast.

Which of the two variables, altitudinal range or coverage with remaining primary habitat, constitutes the better predictor for centers of SR in cophylines cannot be estimated, because only one grid cell with a lower altitudinal range (900 m) is largely covered by primary vegetation (and contains just one species). Grid cells spanning a larger altitudinal range (951–1800 m) are covered by significantly more primary vegetation than grid cells spanning only a small altitudinal range (0–950 m; Kruskal–Wallis $P = 0.011$, correlation of raw data $r = 0.49$, $P = 0.002$, not shown).

We subsequently tested if specific climatic parameters favor the presence of narrowly distributed and thus potentially endemic species. The nonparametric distance-based variance analysis revealed a significant effect of latitude on variance of the 21 climatic variables ($P = 0.0001$ not shown). This corresponds to the fact that most endemic species occur in northern Madagascar, and latitudinal species distribution was therefore included as a

covariable in subsequent analyses. To prevent errors due to area effects, we excluded the cophyline species endemic to the West of Madagascar (Tsingy de Bemaraha) from these analyses (cf. Colwell et al. 2004). A factor analysis of the 21 climatic variables revealed a high level of covariation among them, extracting three principal components (PCs; explaining 48.6%, 28.9%, and 15.5% of total variance, respectively, see online Supplementary Table S5). The nonparametric distance-based variance analysis revealed PC3 as having a significant effect on the two range size categories (widespread vs. narrowly distributed; Table 3, Fig. 3F). Hierarchical partitioning analysis of a regression model (Table 3) revealed PC3 as having the strongest independent influence on range size classes (57.43%), even more than latitude (38.73%). Both PC1 and PC2 did not explain variance in range size classes, which was also confirmed by the hierarchical partitioning analysis (0.85% and 2.99% independently explained variance, respectively). The variables with significant factor loadings for PC3 were those coding for precipitation of wettest month, and of wettest and warmest quarters.

Character tracing of latitudinal species distribution midpoints together with the one-dimensional analyses of SR and CWE revealed remarkable regional endemism and a strong geographical conservatism among the major cophyline lineages (Fig. 4 and online Supplementary Fig. S4). The estimated maximum-likelihood

Table 3. Univariate regression of climate PCs on cophyline range size classes ("spread" = widespread vs. narrowly distributed) including latitude as a covariate, with 9999 permutations for each test (distance measure = Euclidean distances). "expl. var." shows percent of independently explained variance as found by hierarchical partitioning analysis of each predictor variable.

Dependent	Predictor	Covariate	P-level	expl. var.
Spread	pc1	latitude	0.6898	0.85
Spread	pc2	latitude	0.2069	2.99
Spread	**pc3**	**latitude**	**0.0265**	**57.43**
Spread	latitude	—	—	38.73
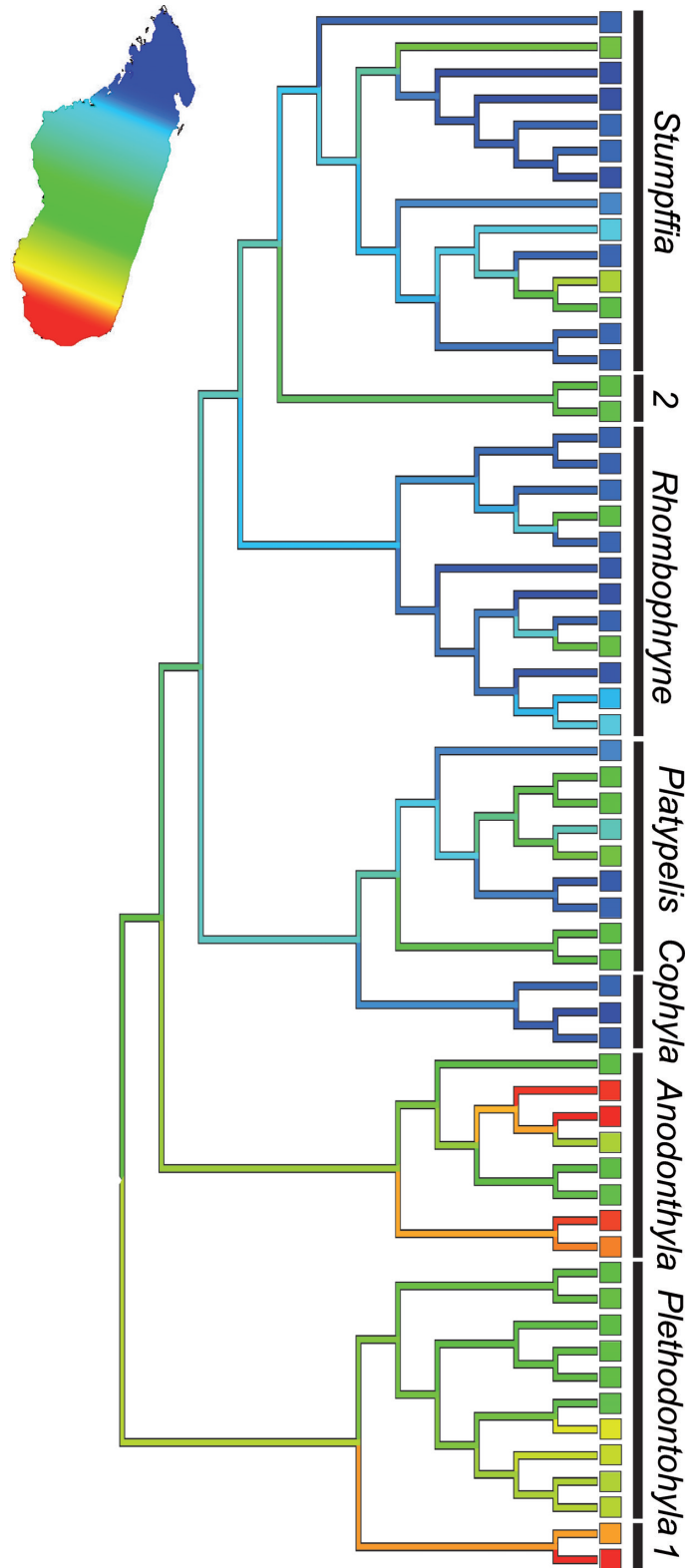
Figure 4. Maximum parsimony character tracing of latitudinal distribution midpoints (on colored branches) to identify areas of clade origin. blue = north; green = center; orange = south (see miniature map). The latitudinal midpoint of the domain lies at −18.78°. *Plethodontohyla*, *Anodonthyla* and the western (2) and southern (1) *Stumpffia* clades share latitudinal distribution areas in the south and center, whereas *Stumpffia*, *Platypelis*, *Rhombophryne* and *Cophyla* have distribution midpoints in the latitudinal center and north. The analysis is based on the phylogeny shown in Figure 1, with relationships among deep clades adjusted according to the nuclear Rag-1 gene in a reduced set of taxa (see online Supplementary Fig. S3).

value for phylogenetic correlation of latitudinal midpoints (lnL = −137.588, computed under the directional random-walk model as preferred by the data), was tested with hierarchical likelihood-ratio tests against the hypotheses of (1) no phylogenetic conservatism of the data ($\lambda = 0$, $P \leq 0.00001$, lnL = −156.331) and of (2) an evolution of the data under a Brownian motion model ($\lambda = 1$, $P = 1$, lnL = −137.588). These results confirm a strong phylogenetic signal in the data and their evolution under a purely Brownian motion model (cf. Smith et al. 2007).

The analyses of one-dimensional endemism (see online Supplementary Fig. S4) show that *Rhombophryne* and *Stumpffia* have their diversity and endemism centered in northern Madagascar where also *Platypelis* and *Cophyla* have most of their endemic species; *Plethodontohyla* is widespread with a tendency of being more diverse in southern Madagascar; *Anodonthyla*, and the southern *Stumpffia* clade have most or all endemics in the south; the two species of the western *Stumpffia* clade are distributed in central-eastern and western Madagascar. Ancestral character state reconstruction using parsimony revealed that *Plethodontohyla*, *Anodonthyla* and the southern *Stumpffia* clade share latitudinal distribution midpoints in southern and central Madagascar (Fig. 4, red to green shades), whereas *Stumpffia*, *Platypelis*, *Rhombophryne*, and *Cophyla* share distribution latitudinal midpoints in central and northern Madagascar (Fig. 4, green to blue shades).

Despite the fact that only two grid cells within the major centers of SR and endemism are identical, all cophyline lineages show a significant or highly significant positive correlation of latitudinal SR and endemism (see online Supplementary Fig. S6).

Discussion
COPHYLINE SYSTEMATICS AND ECOMORPHOLOGICAL EVOLUTION

Our reconstruction of the phylogeny of Malagasy cophylines and assessment of their genetic levels of divergence adds 22 candidate species to the taxonomic inventory of this group. The data suggest the need for generic rearrangements within the subfamily Cophylinae. The paraphyly of *Plethodontohyla* was first demonstrated by Andreone et al. (2005) and later formalized by Frost et al. (2006) who transferred three species of *Plethodontohyla* to *Rhombophryne*. We here provide phylogenetic information for additional species and consequently follow Glaw and Vences (2007) to transfer the taxa *minuta* and *coronata* from *Plethodontohyla* to *Rhombophryne*, and of *occultans* from *Platypelis* to *Cophyla*. We also provide novel evidence for the existence of at least one cryptic lineage of miniaturized cophylines that could eventually merit future recognition as a new genus.

Our taxon sample lacks eight described cophyline species: five *Platypelis*, one *Anodonthyla*, the single known species of

Madecassophryne, and "*Plethodontohyla*" *guentherpetersi*. Four of these *Platypelis* species (*P. alticola, P. mavomavo, P. tetra,* and *P. tsaratananaensis*) are endemic to the Tsaratanana massif and the northeast of Madagascar, *P. alticola* being known from a single specimen from the Tsaratanana Massif. The fifth species, *P. cowani,* is known from two localities in the northeast and southern central east, and its taxonomy is in need of revision. Overall, the inclusion of these species is unlikely to change the biogeographic patterns identified herein. Instead, it would even reinforce the high species diversity and endemism of *Platypelis* in northern Madagascar. *Anodonthyla hutchisoni* is an endemic species from the northeast (and the northernmost occurring *Anodonthyla*), relatively closely related to *A. boulengeri*. The taxon "*Plethodontohyla*" *guentherpetersi*, is a high-elevation endemic from the Tsaratanana Massif. It would be the only *Plethodontohyla* species endemic to this massif, and it is more likely that this species will in fact turn out to belong to *Rhombophryne*. This would further reinforce the northern CE and CSR of this genus. The eighth species and only genus missing from our analysis is *M. truebae,* which is endemic to the Anosy and Andohahela mountains in the southeast.

Our phylogenetic analysis revealed several further instances of parallel ecomorphological adaptations within the Cophylinae. A high degree of homoplasy is identified with respect to miniaturization (adult specimens ≤ 15 mm) (Clarke 1996; Yeh 2002; for data on cophyline body size see Glaw and Vences 2007) that occurred in at least four occasions in the Cophylinae (Fig. 1) and has so far confounded the discovery of two genetically distinct cophyline lineages (the southern and western clades assigned to *Stumpffia*). Taxonomically, our data indicate recognition of at least the southern clade of *Stumpffia* as a new, distinct genus, but further studies of these specimens are necessary to clarify their taxonomic status.

EXPLAINING SPATIAL VARIATION IN COPHYLINE ENDEMISM AND SPECIES RICHNESS

We identified six centers of SR and endemism for cophylines. The grid cells with highest ratings for both measures—the northern, central, central-eastern, western, and southeastern CSRs and CEs—are only identical in two grid cells that additionally received highest values for both measures. These are in the northern CSR and CE (including Marojejy and Anjanaharibe-Sud) and in the southeastern CSR and CE (including Ranomafana National Park).

Although our analyses are based on a sampling throughout all biogeographic regions, two of the inferred centers of endemism in eastern Madagascar (Ranomafana and Andasibe) coincide with especially well-sampled localities. We acknowledge the possibility that several of the taxa endemic to these sites might have slightly larger distribution areas. However, the fact that these local

endemics are not known from other adjacent, intensively surveyed areas, and that correspondingly the local endemics from Andohahela, Marojejy, and Tsaratanana have not been found in other intensively surveyed areas at moderate distances, corroborates a high local endemism in this group and validates the data basis of the analyses performed herein. Furthermore, our conservative method of representing distribution areas as MCP should account for sampling biases because species recorded from two well-sampled sites will also be assumed to occur in intervening undersampled areas.

The mid-domain effect can be rejected as an explanation of latitudinal variation in patterns of SR in Malagasy cophylines. *Plethodontohyla* is the only cophyline genus exhibiting an SR distribution similar to the one expected under the MDE, and was the only cophyline genus included in the study of Lees et al. (1999). Kerr et al. (2006) who rejected the MDE hypothesis for Malagasy birds and small mammals did not include any amphibians. Because various authors already emphasized the biogeographic subdivision of Madagascar and its relevance to Malagasy vertebrate lineages (Koechlin 1972; Martin 1972; Goodman and Ganzhorn 2004; Wilmé et al. 2006; Wilmé and Callmander 2006), this result was not unexpected. However, it differs from previous analyses based on all Malagasy amphibian species (Lees 1996; Lees et al. 1999) where a clear peak of SR in central eastern Madagascar, around the village of Andasibe (= Périnet) was found. As geographical distribution patterns of the investigated cophyline lineages may be phylogenetically influenced we can reinforce the hypothesis that the MDE should not be considered an appropriate null model in related taxa (Davies et al. 2005). However, the stochastic effects of the MDE may become recognizable with a broad taxonomic sampling that includes (1) groups with generalists and specialists adapted to the full variety of environmental conditions present over the geographic area investigated, and (2) a sufficient number of phylogenetic lineages that differ in their origins and thus centers of speciation.

Our data failed to reject the hypothesis that recent human-induced habitat loss may determine current spatial patterns of SR. However, this effect could not be discerned from the effect of an environmental factor, namely the altitudinal range spanned by the grid cells, because only one cell largely covered with primary vegetation contained only a limited altitudinal range. Although the scenario that more primary vegetation would have persisted exclusively on steep slopes unsuitable for agriculture appears plausible, and differences in SR would thus be a product of extinction processes, two arguments prevented us from accepting this hypothesis as a primary cause. First, several grid cells with high primary vegetation coverage did not harbor any cophyline species (these were not analyzed as we could only use nonzero value grid cells). Second, we did not find an influence of remaining primary vegetation on patterns of endemism, although such an influence could be a further indicator for large-scale extinction processes.

In fact, the geographical distribution of altitudinal ranges can explain both spatial variation of SR and endemism in Malagasy cophylines, with two maxima in the northeast and southern central east of Madagascar. A higher altitudinal range in a defined area can harbor a greater variety of microhabitats, leading to an ecotone of narrow homothermous elevational bands, which can result in areas containing slopes (specifically mountain chains) being more diverse (e.g., Rahbek and Graves 2001; Jetz and Rahbek 2002; Jetz et al. 2004). Our results are congruent with previous studies that found altitudinal range (sometimes equated with "topographic heterogeneity") to be a strong predictor of centers of endemism in sub-Saharan birds (Jetz and Rahbek 2002; Jetz et al. 2004), and of SR in South American birds (e.g., Rahbek and Graves 2001). It has been suggested that a correlation of SR with altitude may resemble a hump-shaped mid-elevational diversity pattern (Smith et al. 2007; Wiens et al. 2007), making mid altitudes most species-rich. However, our results of altitudinal range do not support this hypothesis, because the spatial distribution of mid-elevations in Madagascar is clearly distinguishable from the pattern in altitudinal range, SR, and endemism. The spatial variation in patterns of cophyline endemism can be furthermore explained by the variance in certain climatic parameters after controlling for latitudinal climate variation. Specific climatic parameters may have favored the formation of disjunct centers of endemism: endemic species were characteristically present in areas in which precipitation of wettest month and of wettest and warmest quarters was lower than in localities harboring widespread species.

All major cophyline lineages show SR peaks (except *Anodonthyla*) and maximal levels of endemism at specific latitudes. Maximum parsimony character tracing revealed a strong phylogenetic signal in latitudinal species distributions. Species of the major cophyline lineages have latitudinal distribution midpoints either from southern central east to the northern central east (*Plethodontohyla,* the southern *Stumpffia* clade*, Anodonthyla*), or from the northern central east to northern Madagascar (*Stumpffia, Rhombophryne, Platypelis and Cophyla*) (cf. Andreone et al. 2005).

On a global scale, centers of SR and endemism have been found to be nonoverlapping in amphibians (Grenyer et al. 2006). On a finer spatial scale Ricketts (2001) found positive correlations between richness and endemism among 110 ecoregions in North American amphibians. We found a similar pattern, identifying latitudinal endemism and SR as highly positively correlated within and among all cophyline genera with the highest values in these identical grid cells. A correlation between SR and endemism is supposedly caused by roughly log-normal range size frequency distributions, with the majority of species inhabiting only small ranges (Gaston 1998, 2003; Crisp et al. 2001), which

also applies to Malagasy cophylines (F. Glaw and M. Vences, unpubl. data). Furthermore there is a chance that species-rich patches display higher levels of endemism by chance alone (Jetz et al. 2004).

Conclusively, our results are both in agreement with the hypothesis assuming community assembly triggered by key environmental factors, and with the hypothesis of regional phylogenetic diversification. The scenario of community assembly as being solely triggered by ecological key factors implies the existence of a large pool of vagile species having large distribution areas. Among all terrestrial vertebrates, amphibians are in general known for their limited dispersal capabilities, often specialized breeding biology, and thus a subsequent adaptation to specific microhabitats (Duellman and Trueb 1986; Vences and Wake 2007). Nevertheless, in Madagascar, several widespread amphibian species show little genetic variation across their whole range. This applies for instance to the *Mantella baroni*/*M. nigricans* complex of mantelline frogs, which share identical mitochondrial haplotypes across distances of about 700 km (Rabemananjara et al. 2007). In contrast, our molecular data, together with those of Andreone et al. (2005) provide evidence for a low dispersal potential of cophylines: most species have narrow ranges, and in the widespread species studied (*Platypelis grandis, Plethodontohyla notosticta*), the populations from northern and southern Madagascar show a strong genetic differentiation. At least one other taxon (*Stumpffia tridactyla*), which was considered to be widespread in the northeast and northern central east (e.g., Glaw and Vences 1994) turned out to consist of two unrelated lineages (*S. tridactyla* and *S.* sp. 7) which makes the sole application of the community assembly hypothesis less likely and confirms that current centers of SR and endemism indeed correspond to historical centers of clade origin and speciation.

EVOLUTIONARY PROCESSES INFLUENCING COPHYLINE DISTRIBUTION PATTERNS

It has been suggested that centers of endemism, when overlapping with centers of SR, are the manifestation of historical centers of clade origin and speciation that can still be seen in present-day distribution patterns (Ricklefs and Schluter 1993; Jetz et al. 2004). In our example, this would identify the areas of northeast, Sambirano, and north, as well as southern central east as centers of historical cophyline diversification.

The strong phylogenetic signal in present-day distribution patterns furthermore indicates a relative rarity of range shifts since the split of the major evolutionary cophyline lineages. This partly explains also the deviation from the mid-domain effect, as well as the higher species-level endemism near these centers. The historical explanation for the highest levels of SR and endemism in northern Madagascar is simply that the majority of cophyline species diversified in northern Madagascar (four genera), con-

trasted by only two radiations in central/southern Madagascar. Northern Madagascar also has the highest incidence of mountainous areas spanning from very low elevations up to almost 3000 m (Marojejy, Manongarivo, and Tsaratanana massifs). The similarity of range size frequency distributions among the cophyline lineages eventually resulted in higher levels of endemism and SR in northern Madagascar. In contrast to previous studies (Smith et al. 2007; Wiens et al. 2007), we do not find evidence for a historical role of specific elevational zones in clade diversification (elevational mid-domain effect), nor a latitudinal mid-domain effect. Instead, a strong combined effect of latitudinal conservatism in clade diversification, centers of diversification in areas spanning a high altitudinal range (cf. Moritz et al. 2000) in combination with current climatic parameters explains patterns of SR and endemism. This combined effect may even provide a suitable explanation for the evolutionary history of other Malagasy vertebrates. It remains to be tested if not only the net difference of altitude within a given area, but also the number of slopes and their orientation (the geographical habitat complexity) may be an explanatory variable for patterns of endemism and SR.

The cophyline lineages occurring in the highly diverse northern area of SR and endemism constitute the most derived clades in the phylogeny, which is in agreement with a hypothesis of mountainous areas as centers of diversification and possibly acting as "species pumps" (in contrast to "montane museums," cf. Smith et al. 2007). Mountainous areas have served as historical centers of speciation, because both adaptive (1) and vicariant (2) speciation (for definitions see Vences and Wake 2007) are likely to be favored in these areas:

(1) Slopes are thought to contain more homothermous bands than level regions, which should increase community complexity and thus the probability for frequent adaptive (parapatric or sympatric) speciation due to differential selection across environmental gradients (Smith et al. 1997; Schneider and Moritz 1999).

(2) It has been suggested that altitudinal range promotes more opportunities for allopatric speciation over time, as topographic heterogeneity could reflect potential barriers increasing the probability of vicariant speciation (Graves 1988; Rahbek 1997; Moritz et al. 2000; Jetz et al. 2004).

Montane areas, in particular montane forests, have repeatedly been demonstrated to be key areas for the occurrence of narrowly distributed species in Africa (Diamond and Hamilton 1980; Collar and Stuart 1988; Johnson et al. 1998; Linder 2001). Furthermore, the higher habitat diversity might have allowed these regions to act more successfully as refuges during climatic shifts (in conformity with Wilmé et al. 2006). Raxworthy and Nussbaum (1995) have proposed this factor to explain the high degree of species diversity and endemism of dwarf chameleons, *Brookesia*, in northern Madagascar.

According to Ray and Adams (2001), rainforest during the Pleistocene glaciations was restricted to northern and northeastern Madagascar, whereas the east coast was covered by tropical woodland. If a similar pattern existed in the lower and mid-Tertiary, lineages retreating to these rainforest refuges could have consequently encountered increased opportunities for speciation (and for survival of locally endemic taxa) in mountainous areas, especially in northern Madagascar.

The alternative hypothesis of Wilmé et al. (2006) predicts a higher degree of endemism in watersheds isolated from the major summits and thus with a lower headwater origin, due to an increased frequency of biological isolation of such watersheds during glacial maxima. This hypothesis therefore identifies coastal and lowland regions of Madagascar as centers of speciation and thus of endemism. Our results are in sharp contrast to predictions of this hypothesis, because cophyline endemism was not correlated with low altitudes but instead with mountainous areas containing high altitudinal ranges on a small spatial scale—corresponding to the retreat-dispersion watersheds that should, according to Wilmé et al. (2006), contain proportionately lower levels of endemism (see online Supplementary Fig. S7). The hypothesis of Wilmé et al. (2006) was mainly based on distribution patterns of lemurs, and our data indicate it may apply only to groups of vagile animals with a preference for lowland forests.

IMPACT ON CONSERVATION PRIORITIES

Having identified centers of SR and endemism for Malagasy cophylines, we can point out the importance of two geographic regions for conservation priorities. First, Ranomafana National Park and its vicinities display high levels of SR and endemism due to a large coverage with remaining primary vegetation as well as a high altitudinal range. The equally high altitudinal range spanned by the slopes of the Andohahela massif makes it a high-priority area for future amphibian surveys, as it exhibits similar characteristics to the Ranomafana center of SR and endemism, and several areas of high SR and endemism have already been identified along its axis. Furthermore, we recommend a conservation focus on northern Madagascar. The majority (∼75%) of narrowly distributed and thus potentially endemic cophyline species reach their southernmost distribution borders at approximately 20°S latitude, with highest levels of endemism in the Marojejy, Manongarivo, and Tsaratanana massifs, which warrants highest conservation priority for these areas.

This work adds a case study to examples in which areas of SR and endemism are not necessarily identical, but positively correlated on a latitudinal scale for an endemic radiation of Malagasy vertebrates (Crisp et al. 2001; Linder 2001; Ricketts 2001, but see Orme et al. 2005). Therefore, at least for this evolutionary lineage, endemism and SR are of equal importance for conservation measures, pointing out the overall need for larger protected areas covered by significant amounts of primary vegetation and including heterogeneous topology (cf. Moritz et al. 2000).

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Supplementary Material

The following supplementary material is available for this article:

Appendix S1: Methods of phylogeny reconstruction.

Figure S1. Bayesian phylogeny for the reduced dataset of 12S rRNA, tRNAVal, 16S rRNA, *cob* and Rag-1 gene fragments.

Figure S2. One and two-dimensional patterns of endemism and species richness in the major cophyline lineages.

Figure S3. Correlation of latitudinal species richness and endemism, Asterisks indicate significant correlations ≤ 0.05, double asterisks indicate significant correlations ≤ 0.01.

Figure S4. Patterns of cophyline endemism (squares) and centers of endemism according to Wilm'e et al., 2006 (White areas: Retreat-dispersion watersheds; light grey areas: Centers of endemism).

Table S1. Overview of voucher specimen, locality information and Genbank accession numbers for the molecular dataset (12S rRNA - 16S rRNA fragment, *cob*, Rag- 1 if present).

Table S2. PCA of the 21 climate variables in Varimax-raw rotated coordinate system.

Table S3. Character set models for the two molecular datasets used in the different partitioning strategies in the Bayesian analyses; "pos" = codon position.

Table S4. Table comparing 2ln Bayes factors computed from the Harmonic Means of log Likelihood for the seven different partitions in the all-taxa dataset and the five different partitions in the reduced dataset including Rag-1.

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