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1 **Phylogeography of the Rufous Vanga and the role of bioclimatic transition**
2 **zones in promoting speciation within Madagascar**

3
4 **Running title: Phylogeography of the Rufous Vanga**

5
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21
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23 diversification

24

25

26 **Abstract**

27 Madagascar is known as a biodiversity hotspot, providing an ideal natural laboratory for
28 investigating the processes of avian diversification. Yet, the phylogeography of
29 Madagascar's avifauna is still largely unexamined. In this study, we evaluated
30 phylogeographic patterns and species limits within the Rufous Vanga, *Schetba rufa*, a
31 monotypic genus of forest-dwelling birds endemic to the island. Using an integrative
32 taxonomic approach, we synthesized data from over 4,000 ultra-conserved element (UCE)
33 loci, mitochondrial DNA, multivariate morphometrics, and ecological niche modeling to
34 uncover two reciprocally monophyletic, geographically circumscribed, and morphologically
35 distinct clades of *Schetba*. The two lineages are restricted to eastern and western
36 Madagascar, respectively, with distributions broadly consistent with previously described
37 subspecies. Based on their genetic and morphological distinctiveness, the two subspecies
38 merit recognition as separate species. The bioclimatic transition between the humid east
39 and dry west of Madagascar likely promoted population subdivision and drove speciation in
40 *Schetba* during the Pleistocene. Our study is the first evidence that an East-West bioclimatic
41 transition zone played a role in the speciation of birds within Madagascar.

42

43

44 **Introduction**

45

46 Madagascar is a biodiversity hotspot that has been described as a model region for plant
47 and animal diversification studies (Wilmé *et al.*, 2006; Yoder & Heckman, 2006; Vences *et*
48 *al.*, 2009; Brown *et al.*, 2014). The island's long history of geographic isolation (approx. 88
49 million years; Storey *et al.*, 1995), coupled with its complex landscape heterogeneity,
50 provided ample opportunities for in-situ lineage diversification resulting in a unique and
51 largely endemic biota (de Wit, 2003; Wilmé *et al.*, 2006). However, phylogeographic
52 structure within the Malagasy avifauna is still largely unexamined, with no published genetic
53 data for almost half of the island's endemic species (Reddy, 2014). Recent discoveries of
54 cryptic species-level diversity within Malagasy birds (Younger *et al.*, 2018), small mammals
55 (Everson *et al.*, 2016; Hotaling *et al.*, 2016; Everson *et al.*, 2018), reptiles (Florio *et al.*, 2012),
56 and amphibians (Brown *et al.*, 2014), coupled with alarming rates of deforestation
57 (Vieilledent *et al.*, 2018), highlight the need for further efforts to comprehend the full

58 breadth of the biodiversity endemic to Madagascar.

59

60 Bioclimatically, the forests of Madagascar can be coarsely subdivided into the humid east and
61 the dry west (Gautier & Goodman, 2003). The eastern edge of the island is characterized by
62 evergreen forest with high precipitation levels, and elevation increases sharply from the coast
63 to the montane forests of the Central Highlands. The western portion of the island receives
64 considerably less precipitation and experiences a pronounced dry season. The biome consists
65 of dry deciduous forest throughout most of the west, spiny bush formations in the subarid
66 southwest, and some smaller areas of subhumid forest. The Central Highlands is situated
67 between the east and west biomes, and is thought to have historically consisted of a matrix
68 of forest and wooded grasslands (Yoder *et al.*, 2016), but now has little remaining native
69 forest habit.

70

71 The abrupt bioclimatic transition between eastern and western Madagascar has been
72 hypothesized to act as a facilitator for speciation via ecogeographic isolation (Yoder &
73 Heckman, 2006; Vences *et al.*, 2009). According to this hypothesis, populations of a
74 widespread, generalist ancestral species differentially adapted to conditions in the humid
75 east versus the dry west, producing sister taxa with minimal overlap in their environmental
76 niches. Following this initial divergence, secondary subdivision of populations may occur
77 within the eastern and western bioclimatic zones. Vences *et al.*, (2009) also proposed an
78 alternative mechanism for east-west divergences without adaptation termed the 'Western
79 rainforest refugia mechanism'. In this scenario, an ancestral species adapted to humid forest
80 habitat may have been widespread during warmer (wet) periods of the Pleistocene, but then
81 became isolated in forest refugia during glacial (dry) periods, eventually speciating in allopatry
82 without ecological divergence (Vences *et al.*, 2009). Under this model, sister lineages in
83 eastern and western Madagascar would be expected to occupy similar environmental niches.
84 The east-west bioclimatic transition appears to have facilitated speciation in a range of taxa,
85 including reptiles (Nussbaum & Raxworthy, 1994; Nussbaum & Raxworthy, 1998; Raxworthy
86 *et al.*, 2007; Orozco-Terwengel *et al.*, 2008; Florio *et al.*, 2012), amphibians (Vences *et al.*,
87 2000; Andreone *et al.*, 2002; Vences & Glaw, 2002; Köhler *et al.*, 2007), insects (Lees *et al.*,
88 2003), and mammals (Everson *et al.*, 2016; Yoder *et al.*, 2016). So far, little is known about an
89 east-west speciation pattern in birds. A study of Malagasy Scops-owl (*Otus rutilus*) found a

90 pattern of subtle genetic differentiation between east and west Madagascar, suggesting the
91 bioclimatic transition could contribute to population divergence in birds (Fuchs *et al.*, 2007).

92

93 *Schetba*, or the Rufous Vanga, is a monotypic genus within an endemic Malagasy radiation
94 of songbirds, the Vangidae (Yamagishi *et al.*, 2001; Reddy *et al.*, 2012; Jønsson *et al.*, 2012).
95 The current taxonomy of the genus comprises a single species, *S. rufa*, with two subspecies:
96 *S. r. rufa* (Linnaeus, 1766) and *S. r. occidentalis* (Delacour, 1931). *S. r. rufa* occupies the
97 humid and littoral forests of eastern Madagascar, whereas *S. r. occidentalis* is found in the
98 deciduous forests and subhumid forests of western Madagascar (Goodman & Raherilalao,
99 2013; Schulenberg, 2013). Given the geographic division of these subspecies in the east and
100 west, it is possible that the bioclimatic transition between these regions played a role in the
101 divergence of *Schetba*. However, the degree of divergence between these subspecies, which
102 are based on slight differences in bill dimensions and plumage, has not been corroborated
103 with genetic or ecological data. Previous genetic studies each included only a single
104 representative of *S. rufa* (Yamagishi *et al.*, 2001; Jønsson *et al.*, 2012; Reddy *et al.*, 2012).
105 Furthermore, the distributional limits of *S. r. occidentalis* and *S. r. rufa* are somewhat ill-
106 defined and might be attributed to clinal variation (Delacour, 1932; Schulenberg, 2013).
107 Given the dearth of conclusive information, some have suggested that *S. rufa* may be better
108 regarded as monotypic (Schulenberg, 2013).

109

110 Here, we aim to (1) clarify the taxonomy of *Schetba* in light of recent findings of cryptic
111 species-level diversity within Madagascar (e.g. Younger *et al.*, 2018), and (2) investigate
112 whether the bioclimatic transition between the humid east and dry west of Madagascar
113 may have facilitated speciation within birds. We synthesized data from over 4,000 ultra-
114 conserved element (UCE) loci, mitochondrial DNA, morphometrics, and ecological niche
115 modeling in an integrative systematics approach to assess species limits and explore
116 phylogeographic patterns within the genus.

117 **Materials and Methods**

118 **Taxon sampling**

119 We sampled *Schetba* from across its geographic range in order to assess phylogeographic
120 patterns, subspecies definitions, and subspecies distributional limits (Figure 1). Tissue
121 samples used for genotyping are associated with vouchered specimens held at the Field
122 Museum of Natural History (FMNH; Chicago) and the Mention Zoologie et Biologie Animale,
123 Université d'Antananarivo (UADBA; Antananarivo, formerly Département de Biologie
124 Animale). We genotyped 27 individuals of *S. rufa*, plus two outgroup species of Vangidae
125 (*Euryceros prevostii* and *Newtonia amphichroa*). Morphometric data was collected from 20
126 adult *S. rufa* study skins (five individuals of each sex for each subspecies) in the FMNH and
127 American Museum of Natural History (AMNH; New York) collections. For detailed location
128 information (locality, latitude, and longitude), accession numbers, and data collected from
129 each specimen, please refer to Supplementary Table 1.

130 **Sequencing**

131 DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit following the
132 manufacturer's protocol. UCE libraries for 28 taxa (27 *Schetba* plus *Euryceros* outgroup)
133 were prepared following described methods (Faircloth *et al.*, 2012; McCormack *et al.*, 2013)
134 with minor modifications. Briefly, purified DNA was normalized to 10 ng/μL and fragmented
135 via sonication (Covaris, Model #M220) to approximately 550 base pairs (bp). Samples were
136 end-repaired, A-tailed and Illumina TruSeqHT adapters were ligated using either a TruSeq
137 DNA HT Sample Prep Kit (Illumina) or a KAPA Hyper Prep Kit (Kapa Biosystems), following
138 the manufacturer's instructions. Libraries were then amplified by limited-cycle (16–18) PCR
139 using Kapa HiFi DNA polymerase (Kapa Biosystems), normalized, and pooled into sets
140 consisting of eight libraries each (along with taxa for other studies) with a total of 500 ng of
141 sample. We enriched these pooled libraries for 5,060 UCE loci using MYbaits capture kits
142 (Terapods 5K v1, MYcroarray) following the manufacturer's instructions. Enriched libraries
143 were quantified using qPCR (Kapa Library Quantification Kit) and a Qubit Fluorometer
144 (Invitrogen), normalized, and pair-end sequenced (2 x 250 bp) on the Illumina HiSeq2500
145 platform. DNA sequence reads are archived on NCBI SRA (XXXXX).

146

147 We amplified and sequenced the mitochondrial gene NADH dehydrogenase 3 (ND3) for 26
148 taxa (including outgroups *Euryceros* and *Newtonia*) using standard PCR and Sanger
149 sequencing methods with primers ND3-L10751 (5'-GACTTCCAATCTTTAAAATCTGG-3') and
150 ND3-H11151 (5'-GATTTGTTGAGCCGAAATCAAC-3'). We used Geneious 9.0.5 for alignment
151 and sequences were deposited in GenBank (TBA — TBA). We also extracted mitochondrial
152 cytochrome b (CYTB) sequences from off-target contigs of the UCE protocol using the
153 Megablast function within Geneious 9.0.5, and successfully recovered CYTB for 24 of the *S.*
154 *rufa* individuals.

155

156 **Bioinformatics**

157 We used the PHYLUCE 1.5 package (Faircloth, 2015) to prepare alignments of UCE loci for
158 phylogenetic analysis. The demultiplexed reads were trimmed to remove adapters and low-
159 quality bases using Illumiprocessor (Faircloth, 2013), then assembled into contigs using
160 Trinity 2.0.4 (Grabherr *et al.*, 2011). UCE loci were extracted from among the contigs using
161 PHYLUCE and then aligned with MAFFT 7 (Katoh *et al.*, 2002; Katoh & Standley, 2013). The
162 alignments were trimmed using the edge-trimming algorithm available in PHYLUCE, and
163 then a data matrix of 75% completeness was generated, where 'completeness' refers to the
164 minimum number of taxa sequenced for a locus to be included in the matrix.

165

166 We prepared a dataset of single nucleotide polymorphisms (SNPs) for the 27 *Schetba*
167 individuals, following the methods of the seqcap_pop pipeline (Harvey *et al.*, 2016), with
168 some modifications. In brief, following cleaning of the reads with Illumiprocessor, we used
169 Trinity 2.0.4 to assemble reads across all specimens into contigs *de novo*. Contigs matching
170 UCE probes were then extracted using PHYLUCE and were used as a reference for SNP
171 calling. The reads for each individual were mapped to the reference contigs using BWA (Li &
172 Durbin, 2009), with a maximum of four mismatches allowed per read. We used SAMtools (Li
173 *et al.*, 2009) and Picard (<http://broadinstitute.github.io/picard/>) to convert sam files to bam
174 format, soft-clip reads beyond the reference, add read groups for each sample, and then
175 merge bam files across all samples in the dataset. We used the Genome Analysis Toolkit
176 (GATK; McKenna *et al.*, 2010) to realign reads and indels, call SNPs, annotate SNPs and
177 indels, mask indels, remove SNPs with a quality score < Q30, and to conduct read-backed

178 phasing. At this point we output a dataset of phased SNPs in vcf format for further filtering.
179 We filtered the SNP dataset using VCFtools 0.1.15 (Danecek *et al.*, 2011): we specified a
180 minimum read depth of three for a genotype call; removed any SNPs with a minor allele
181 count < 2 (these are potential sequencing errors and generally uninformative loci);
182 restricted to biallelic SNPs; and removed any variants not genotyped in 100% of individuals.
183 We then used a custom python script to select one SNP at random per contig to reduce
184 linkage in the final dataset. VCFtools 0.1.15 was used to calculate mean sequencing
185 coverage of each SNP. Because our analysis found two highly distinct groups within *Schetba*
186 (East and West groups), we also prepared separate SNP datasets for each of these group to
187 allow for separate clustering analyses within the East and West to detect fine-scale genetic
188 structure. After the final filtering with VCFtools on the entire dataset as described, we
189 divided the dataset into East and West datasets, then applied a minor allele count filter to
190 remove positions that are invariant within these groups, and finally selected one SNP at
191 random per contig. PGDSpider 2.1.0.0 (Lischer & Excoffier, 2012) was used to convert vcf
192 files into other formats required for analysis.

193

194 **Phylogenetic analysis**

195 We inferred maximum likelihood (ML) phylogenies for the UCE dataset using RAxML 8.2.7
196 (Stamatakis, 2014). We performed both unpartitioned and partitioned concatenated
197 analyses. To find the most appropriate partitioning scheme for the UCE dataset we used the
198 Sliding-Window Site Characteristics (SWSC) entropy based method (Tagliacollo & Lanfear,
199 2018) to generate partitions that account for within-locus heterogeneity (e.g., the flanking
200 regions of UCE loci are typically more variable than the ultraconserved core). These
201 partitions were then input to PartitionFinder 2 (Lanfear *et al.*, 2014; Lanfear *et al.*, 2016), to
202 estimate the optimal partitioning scheme for phylogenetic analysis by grouping together
203 similar subsets from the SWSC output. For each RAxML analysis, we conducted rapid
204 bootstrapping analysis and a search for the best-scoring ML tree in a single program run,
205 using the MRE-based bootstopping criterion (Pattengale *et al.*, 2010) to ascertain when
206 sufficient bootstrap replicates had been generated. All searches were conducted under the
207 GTR GAMMA site-rate substitution model.

208

209 We also inferred a phylogeny under the multispecies coalescent method. Gene-tree based
210 coalescent methods may have reduced accuracy when inadequately resolved gene trees are
211 included, which can result from using loci with low phylogenetic signal (Gatesy & Springer,
212 2014; Xi *et al.*, 2015; Hosner *et al.*, 2016; Meiklejohn *et al.*, 2016). We therefore selected the
213 25% of UCE loci with the greatest number of parsimony informative sites for analysis. This
214 subset contained 1,062 loci with between five and 26 parsimony informative sites each. A
215 gene tree was estimated for each locus with 100 ML searches under GTR GAMMA using
216 RAxML, and these were then reconciled into a gene tree-species tree using ASTRAL 4.10.12
217 with default settings (Mirarab & Warnow, 2015).

218

219 **Divergence time estimation**

220 We performed time-calibrated Bayesian phylogenetic analyses on mtDNA sequences (ND3
221 and CYTB) using BEAST 2.4.4 (Bouckaert *et al.*, 2014) to estimate divergence times among
222 *Schetba* lineages. The mtDNA genes were used because estimates of divergence rates in
223 birds are available for these loci (Lerner, Meyer, James, Hofreiter, & Fleischer, 2011; Weir &
224 Schluter, 2008). Furthermore, the mtDNA gene trees resolved the same well-supported
225 clades as the UCE dataset. The data was partitioned into ND3 and CYTB, with nucleotide
226 substitution models specified as HKY for both genes to reflect the optimal models selected
227 by PartitionFinder 2 (Lanfear *et al.*, 2016). We used the Yule tree prior with a strict
228 molecular clock. The molecular clock was calibrated using two different reference rates; (1)
229 the divergence rate of CYTB for Passeriformes of 2.07% (\pm 0.20) per million years (Weir &
230 Schluter, 2008; lognormal, mean = 0.01035, SD = 0.05); and (2) the substitution rates
231 estimated for ND3 and CYTB for Hawaiian honeycreepers (Lerner *et al.*, 2011; ND3:
232 lognormal, mean = 0.024, SD = 0.09; CYTB: lognormal, mean = 0.014, SD = 0.05). Two
233 independent analyses were performed for each to ensure reproducibility of the posterior
234 distributions. The MCMCs were run until convergence of the posteriors, as confirmed using
235 Tracer v1.6 (Rambaut & Drummond, 2007). We estimated maximum clade credibility trees
236 with mean node heights from each posterior after removing the first 10% of samples as
237 burn-in.

238

239 **Genetic clustering analyses and summary statistics**

240 To estimate the number of genetic clusters in the *Schetba* SNP dataset, we performed
241 Discriminant Analysis of Principal Components method (DAPC; Jombart *et al.*, 2010), and
242 Bayesian clustering within Structure 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). The
243 DAPC method, implemented in *adegenet* (Jombart, 2008; Jombart & Ahmed, 2011), creates
244 discriminant functions to maximize variance among, whilst minimizing variance within,
245 genetic clusters. The most likely number of clusters in the dataset, and the assignment of
246 individuals to those clusters, was estimated using successive *K*-means clustering, with the
247 number of clusters selected based on minimum BIC. Then DAPC was performed, using the
248 cross-validation method (1000 replicates) to determine the optimal number of PCs to retain.
249 Finally, we plotted the posterior membership probability of all *Schetba* taxa to the genetic
250 clusters.

251

252 For a given number of clusters (*K*), Structure identifies genetic clusters within the dataset
253 and estimates the corresponding membership coefficients for each. We performed
254 Structure analyses for the entire *Schetba* dataset, as well as for *S. r. rufa* and *S. r.*
255 *occidentalis* separately in order to detect any fine-scale genetic differentiation within the
256 eastern and western sectors of the island. For all analyses, we used the admixture model
257 with correlated allele frequencies and ran the model without sampling locations as priors.
258 For each dataset, we performed an initial run of 100,000 generations, discarding the first
259 50,000 as burn-in, with *K* = 1 and lambda allowed to vary in order to estimate a value for
260 lambda (the allele frequencies prior) for the dataset. For subsequent runs, the value of
261 lambda was set to the estimated value, and the number of clusters was allowed to vary
262 from *K* = 1 to *K* = 10 (for the full dataset), and from *K* = 1 to *K* = 5 for the analyses on the East
263 and West groups. Each analysis was run for 500,000 generations, discarding the first
264 100,000 as burn-in, and repeated ten times. We used Structure Harvester Web 0.6.94 (Earl,
265 2012) to assess convergence across replicates, to determine the most optimal value of *K* for
266 the three datasets (based on the log likelihood of each value of *K*, and the Evanno method
267 (Evanno *et al.*, 2005)), and to prepare input files for CLUMPP 1.1.2 (Jakobsson & Rosenberg,
268 2007). CLUMPP was then used to calculate average membership coefficients from across
269 the replicates. Distruct 1.1 (Rosenberg, 2004) was used to visualize the final results for
270 several values of *K*, in order to better understand the levels of genetic structure within
271 *Schetba*. Previous work suggests that a “true” value of *K* does not usually exist (Gilbert *et al.*,

272 2012; Benestan *et al.*, 2016; Janes *et al.*, 2017), and that in order to gain insight into
273 different levels of genetic structure it is best practice to view multiple K -values.

274

275 We used Genodive 2.0b27 (Meirmans & Van Tienderen, 2004) to calculate the Weir and
276 Cockerham unbiased weighted F_{ST} estimator (Weir & Cockerham, 1984) between the East
277 and West clades, with significance calculated using 10,000 permutations of the data. We
278 also used Genodive to calculate observed (H_o) and expected (H_s) heterozygosity.

279

280 **Ecological niche modeling**

281 Our occurrence dataset comprised a total of 16 spatially unique latitude/longitude
282 combinations for *S. r. occidentalis* and 18 for *S. r. rufa*. Bioclimatic variables for Madagascar
283 were used to summarize aspects of temperature and precipitation from the latter half of
284 the 20th century (Hijmans *et al.*, 2005), as well as for the Last Glacial Maximum (LGM;
285 ~21,000 years BP; under both Community Climate System Model (CCSM) and Model for
286 Interdisciplinary Research on Climate (MIROC) scenarios). We used bioclimatic GIS layers
287 (<http://www.worldclim.org>) at a spatial resolution of 2.5 arc-minutes. To account for
288 dimensionality across environmental spaces and time scales, we used a subset of six of the
289 19 layers that showed lowest correlation ($p < 0.7$): annual mean temperature (bio1), mean
290 diurnal range (bio2), maximum temperature of warmest month (bio5), annual precipitation
291 (bio12), precipitation of wettest month (bio13), and precipitation of driest month (bio14).
292 We used MaxEnt v.3.4.1 (Phillips *et al.*, 2006) to construct ecological niche models of each
293 subspecies. Owing to the low number of unique occurrences for each subspecies, we set the
294 algorithm to perform cross validation with five replicates and a 10% training presence
295 threshold. The spatial extent of our model training was kept at the level of the entire island
296 of Madagascar, while our models were run using climatic variables for the present time
297 frame and then projected onto LGM past conditions (CCSM and MIROC scenarios). We
298 performed an additional MaxEnt run with all occurrence points of the two taxa combined (*S.*
299 *rufa* sensu lato; total of 34 unique points), in order to examine potential ecological and
300 biogeographical divergences and breaks within this taxon. For this run we once again used
301 cross validation with five replicates and a 10% training presence threshold. Niche similarity
302 between the two taxa was assessed by calculating Schoener's D metric using the Maxent

303 estimates of present-day potential distributions. To evaluate statistical significance of the
304 niche similarity measure, we generated a null distribution of D values for each of the two
305 taxa through 100 simulated models based on the same environmental layers and
306 background extent, and random samples of background in place of occurrence records
307 (Warren *et al.*, 2008).

308

309 **Morphological variation**

310 We measured 20 *Schetba* skin specimens (10 per subspecies) to examine morphological
311 variation. One of us (TOH) took standard linear measurements of bill length from the crown
312 to tip (BL), bill width at the anterior edge of nares (BW), bill depth at nares (BD), tarsus
313 length (TL), hallux length (HL), tail length (Tail), and wing chord length (WL). These
314 measurements followed the descriptions in (Baldwin *et al.*, 1931). Wing and tail lengths
315 were measured with a wing rule to an accuracy of 1 mm, all other measurements were
316 taken with Mitutoyo Digital Calipers to an accuracy of 0.01 mm. All measurements were
317 repeated three times, checked for outliers (by confirming that all measurements for an
318 individual were within one standard deviation), and then averaged. The summary statistics
319 of these measurements for the two clades are given in Supplementary Table 2. We first
320 tested whether males and females exhibit significant variation by conducting an ANOVA for
321 each variable between sexes within each clade. Next, we log-transformed and standardized
322 all measurements and conducted principal components analysis (PCA) on all specimens to
323 examine the morphological variation between the two genetic clades. We conducted a
324 multivariate analysis of variance (MANOVA) to determine whether the centroids of the two
325 clades were statistically different. There were five specimens for which wing measurements
326 could not be made and since missing data is problematic in multivariate analyses, we
327 removed wing length and used only the remaining six variables for these analyses. We also
328 conducted ANOVA tests for each measured trait with clade as a factor to determine which
329 traits differed significantly between clades. We used the R statistical package for all
330 statistical analyses.

331

332 **Results**

333 **Sequence capture of UCE loci**

334 After removal of adapters, low quality bases and unpaired reads, an average of 350 million
335 bp of sequence per individual remained (46 million – 589 million bp). These reads were
336 assembled into an average of 15,448 contigs per individual, with a mean contig length of
337 508 bp. An average of 4,235 UCE loci were recovered per individual (3,139–4,421), with
338 4,951 UCE loci recovered across all taxa. The 75% complete data matrix used for analysis
339 consisted of 4,243 loci with a mean locus length of 784 bp. The concatenated alignment was
340 3,328,172 bp in length, and contained 15,392 parsimony informative sites.

341

342 The recovered UCE loci contained a total of 56,701 SNPs. Our filtering protocols reduced this
343 to 12,045 SNPs, and after thinning to one SNP per contig our final dataset contained 3,609
344 SNPs for use in subsequent analyses. The mean sequencing coverage of these SNPs was 68X.
345 The SNP datasets we prepared for *S. r. rufa* and *S. r. occidentalis* contained 2,873 and 3,044
346 SNPs, respectively.

347

348 **Phylogenetic relationships**

349 Our phylogenetic analyses converged on a strongly supported topology showing a clear
350 division of *S. rufa* into two reciprocally monophyletic clades (Figure 2), corresponding to
351 eastern and western Madagascar. The ML phylogenies also indicated several well-supported
352 clades within each of the eastern and western clades, corresponding with latitudinal
353 subdivision (details in section on fine-scale genetic structure, below). The topology
354 recovered from ML analysis of the 4,243 UCE loci dataset was robust to partitioning scheme
355 (Figure 2, Supplementary Figure 1). The ASTRAL species tree constructed from the 1,062
356 most informative UCE loci had 100% support for the eastern and western clades, and had a
357 normalized quartet score of 0.42 (Supplementary Figure 2). The sub-clades within the
358 eastern and western clades were less well supported in the ASTRAL tree, indicating a degree
359 of either incomplete lineage sorting or gene flow, as expected for intraspecific comparisons.

360

361 The eastern and western clades were also reciprocally monophyletic and 100% supported in
362 the mitochondrial tree (phylogeny not shown). We estimated that the divergence of eastern
363 and western clades of *S. rufa* occurred approximately 854,000 years ago (median estimate,

364 95% HPD: 0.582 – 1.16 MYA), based on the Weir & Schluter (2008) calibration for all
365 Passeriformes. Our estimates of divergence times based on the two calibration strategies
366 had overlapping 95% HPDs, with a slightly younger estimate of lineage divergence based on
367 substitution rates in Hawaiian honeycreepers (Lerner *et al.*, 2011) of 0.536 MYA (median
368 estimate, 95% HPD: 0.380 – 0.736 MYA).

369

370 **Clustering analyses and differentiation measures (eastern vs. western *Schetba rufa*)**

371 The optimal number of genetic clusters in our Structure analysis of the 27 *S. rufa* individuals
372 was $K = 2$, based on both the maximum posterior log likelihood and the rate of change in log
373 probability (deltaK, Evanno method). Assignments of individuals to these clusters was
374 consistent with the results of our phylogenetic analyses, dividing *S. rufa* into two genetic
375 groups originating in eastern and western Madagascar (Figure 3a, Figure 2). Successive K -
376 means clustering also clearly indicated $K = 2$ as the most likely number of clusters, and DAPC
377 was able to differentiate between these with 100% support (root mean squared error = 0),
378 even when only a single PC was retained for analysis (Supplementary Figure 3a). The
379 posterior membership probabilities for all taxa were 100% to their respective clusters in
380 both Structure and DAPC, with no evidence of admixture between the east and west groups
381 (Figure 3a, Supplementary Figure 3b).

382

383 Our estimate of F_{ST} between the east and west groups was 0.256 (95%CI: 0.235 – 0.277, p -
384 value < 0.0001), suggesting strong, statistically significant genetic differentiation between
385 them. There were 103 fixed SNPs between the two clades (across the full SNP dataset). The
386 expected (H_S) heterozygosity for the western clade was greater than that of the eastern
387 clade (0.143, 95%CI: 0.138 – 0.147; compared to 0.133, 95%CI: 0.128 – 0.138).

388

389 **Fine-scale genetic structure**

390 To investigate finer-scale divergences within the eastern and western groups of *S. rufa*, we
391 conducted further Structure analyses on these two groups separately. For the *S. r. rufa* (the
392 eastern clade), the posterior log likelihood was maximized at $K = 3$, whereas deltaK was
393 maximized at $K = 2$. In the two-cluster scenario, individuals from the northeast humid forest
394 (Masoala National Park) are clearly differentiated from those in the southeastern humid

395 forest with minimal admixture (Figure 3b). In the three-cluster scenario, this division
396 between northeast and southeast is still apparent, and three individuals from the
397 northwestern sector of Masoala National Park (near Hiaraka village) are largely assigned to a
398 third cluster, distinct from the other Masoala National Park individuals (Figure 3c). The four-
399 cluster scenario is consistent with this finding, showing no further genetic structure (Figure
400 3d). In our phylogenetic analysis, the individuals from the southeastern forest were
401 monophyletic with 100% bootstrap support, but the individuals from Masoala National Park
402 were paraphyletic, with those individuals from the eastern sector (Sarahandrano Forest)
403 appearing the most divergent (Figure 2). Based on this inconsistency regarding genetic
404 subdivision in Masoala, we conclude that there are most likely two genetic populations of *S.*
405 *rufa* in the eastern humid forests; in Masoala National Park and in the southeastern region.

406

407 For the individuals from western Madagascar, the optimal number of clusters in our
408 Structure analyses was four, based on both the posterior log likelihood and deltaK. In a two-
409 cluster scenario (Figure 3e), the individuals from the northwest forest (Namoroka and
410 Ankarafantsika) were differentiated from the rest of the western clade, a split which was
411 also supported in our phylogeny (Figure 2). When $K = 3$ further subdivision is apparent, with
412 Namoroka and Ankarafantsika individuals largely assigned to distinct clusters (Figure 3f).
413 These groups are located south and north of the Betsiboka River, respectively, and this split
414 has 100% support in our phylogenetic analysis (Figure 2). In the four-cluster scenario, there
415 is further divergence between individuals from the southwest and central-west regions
416 (Figure 3g); this split has 100% support in our phylogeny (Figure 2). Therefore, it appears
417 that there are four genetically differentiated populations in western Madagascar, separated
418 latitudinally. Overall, our genetic data provide evidence for an initial divergence in the *S.*
419 *rufa* complex between the east and west of Madagascar during the mid-Pleistocene,
420 followed by more recent divergences within these two regions, which perhaps reflect the
421 fragmented nature of Madagascar's forest habitat and/or low levels of dispersal of *Schetba*.

422

423

424 **Ecological niche modeling**

425 Our ecological niche models for *Schetba* provided a good fit to their contemporary
426 distribution (Goodman & Raherilalao, 2013; Schulenberg, 2013), with the caveat that the

427 actual inhabited area is smaller than predicted in the model owing to recent deforestation
428 (Vieilledent *et al.*, 2018). Of the five model replicates for the separate subspecies (*S. r.*
429 *occidentalis* and *S. r. rufa*) and the single taxon (*S. rufa s.l.*), we selected the run with the
430 best performance (highest AUC values and lowest testing data omission error) for further
431 interpretation.

432

433 Our combined single taxon ecological niche model (pooled dataset of 34 unique points)
434 recovered two distinct areas of suitability (Figure 4), corresponding to the eastern and
435 western clades evident in our phylogenetic analyses. Separate MaxEnt models of each
436 subspecies (*S. r. occidentalis* and *S. r. rufa*) produced similar geographic signatures, with the
437 individual models showing suitable habitat in western and eastern Madagascar,
438 respectively. Slight differences in the individual models compared to the pooled dataset (*S.*
439 *rufa s.l.*) were observed in an apparent connection between the two subdivided habitats in
440 western Madagascar, which correspond to the genetic break between the northwest forest
441 (Namoroka/Ankarafantsika) and the remainder of the western clade of *S. r. occidentalis*
442 (Figures 2, 3e). While this subdivision was not recovered in the present-day model of *S. r.*
443 *occidentalis*, this separation was visible in the LGM model projections for this taxon. Models
444 of the combined dataset also differed from the individual models for *S. r. occidentalis* and *S.*
445 *r. rufa*, by rendering areas of eastern Madagascar as largely habitable by *Schetba* during the
446 LGM scenarios, but omitting suitable habitats in the northwest. The individual model for *S. r.*
447 *occidentalis* produced models with suitable areas in western Madagascar during both LGM
448 scenarios (Figure 4). Notable for the *S. r. occidentalis* models is the difference between the
449 CCSM and MIROC LGM scenarios, where under the former scenario only the southwestern
450 region of the island is predicted as having large extents of suitable areas, while the
451 northwestern suitable area is reduced to a smaller, isolated patch (Figure 4). The observed
452 niche similarity between the two taxa based on Schoener's *D* was 0.182. This value was
453 outside the lower bound of the 95% confidence interval of the simulated null distributions
454 of *D* values, indicating that the niches of the two taxa are significantly dissimilar.

455

456

457 **Morphological variation**

458 There was no significant difference between sexes within each clade based on our ANOVA,
459 so we used all individuals together for subsequent analyses. Univariate ANOVA of each
460 measurement separately showed that *S. r. rufa* and *S. r. occidentalis* were significantly
461 different in terms of bill length, bill depth, tarsus, and tail length (Supplementary Table 2).
462 We used all 20 individuals and six variables (removing wing length due to missing data) for
463 the PCA, which resulted in six PCs, with the first four explaining more than 90% of the
464 variance (see Supplementary Table 3). The two *Schetba* clades formed distinct clusters in
465 morphospace (Figure 5; Supplementary Figure 4). Our MANOVA test determined that the
466 clade centroids were significantly different ($p < 0.001$).

467

468 **Discussion**

469

470 **Previously unrecognized species diversity within *Schetba***

471 We found that the two *Schetba rufa* subspecies are geographically, genetically, ecologically,
472 and morphologically distinct. The *S. r. rufa* and *S. r. occidentalis* lineages are restricted to
473 eastern and western Madagascar, respectively, occupying distinct ecological niches
474 separated by a large expanse of unfavorable habitat (the Central Highlands). The subspecies
475 formed reciprocally monophyletic clades in all of our analyses. We estimate that these
476 lineages diverged 854,000 years ago (95% HPD: 0.582 – 1.16 MYA), and have since
477 accumulated fixed SNP differences in their nuclear genomes and diverged in their genetic
478 diversity levels. They have also diverged in morphology, such that *S. r. occidentalis* has a
479 significantly longer tail, longer tarsus, and longer and heavier bill than *S. r. rufa*. This result is
480 consistent with other morphological studies (Schulenberg, 2013).

481

482 The genetic and morphological differences described here suggest that the two *S. rufa*
483 subspecies merit recognition as separate species. We therefore propose that within the
484 currently defined *S. rufa*, the western subspecies, *occidentalis*, should be elevated to species
485 level, *S. occidentalis*. We suggest the common name ‘Western Rufous Vanga’ for this new
486 species, to reflect its geographic distribution. The eastern subspecies, *rufa*, would remain *S.*
487 *rufa*. A full description for the *S. r. occidentalis* subspecies already exists (Delacour, 1931),
488 therefore we do not include a species description for *S. occidentalis* here. These two species

489 are on separate evolutionary trajectories, and their distinctiveness should be taken into
490 consideration in future conservation plans and biodiversity studies. Only by recognizing and
491 conserving the full spectrum of genetic and morphological variation can the adaptive
492 potential of *Schetba* be maximized (Funk *et al.*, 2012; D'Amen *et al.*, 2013).

493

494 Our discovery of unrecognized species-level diversity within *Schetba*, coupled with the
495 recent discovery of other cryptic species diversity within the endemic Vangidae family
496 (Younger *et al.*, 2018), suggests that the avian species richness of Madagascar may still be
497 underestimated. This is concerning given the high rates of deforestation and forest
498 fragmentation (Vieilledent *et al.*, 2018) that are currently threatening the island's avifauna.
499 Recent efforts in avian taxonomy suggest that unrecognized species may be a widespread
500 problem, leading to substantial underestimates of avian biodiversity levels and fine-scale
501 endemism (Barrowclough *et al.*, 2016; Hosner *et al.*, 2018). Given that most conservation
502 plans rely on species-level designations (Barrowclough *et al.*, 2016), it is crucial to continue
503 efforts to comprehend the full breadth of avian species diversity.

504

505 **Phylogeography of *Schetba***

506

507 Our genetic data indicate an initial divergence in *Schetba* between the east and west of
508 Madagascar during the mid-Pleistocene. Although other studies have proposed that the
509 bioclimatic transition between the humid east and dry west of Madagascar may promote
510 population subdivision and speciation (Yoder & Heckman, 2006; Vences *et al.*, 2009), this is
511 the first evidence for this speciation mechanism in birds. Sister species pairs restricted to east
512 and west Madagascar could form via predominantly adaptive processes (i.e. ecogeographic
513 isolation), or via non-adaptive processes (i.e. biogeographic isolation) (Vences *et al.*, 2009). In
514 the case of *Schetba*, the two species differ in ecological niche based on our models (Figure 4),
515 therefore adaptive processes most likely played a role in their divergence. These results fit
516 the hypothesis for ecogeographic isolation, with sister taxa in east and west Madagascar that
517 differ in ecological niche. The divergence of *Schetba* does not appear to be consistent with
518 the 'Western rainforest refugia' speciation mechanism put forward by Vences *et al.* (2009),
519 given that the two taxa have significantly dissimilar environmental niches. Interestingly, based
520 on its distribution, *S. occidentalis* does not appear to be a strictly dry-adapted species. It

521 occupies subhumid and deciduous forests, and is not found in the arid spiny bush habitat. For
522 example, there is a population of *S. occidentalis* in the high elevation areas of the subhumid
523 forest of Analavelona (Figure 1), and the species is not found in the non-forested area
524 surrounding the massif. The flora of the Analavelona region shares characteristics of the mid-
525 altitude forests of the east, and has been considered a Pleistocene relict when portions of
526 southwestern Madagascar was wetter than today (Goodman *et al.*, 2018).

527

528 Biogeographic isolation may have also played a role in the divergence of *S. occidentalis* and *S.*
529 *rufa*. Our ecological niche models for *Schetba* recovered two distinct areas of suitable habitat
530 in east and west Madagascar, separated by a large expanse of unfavorable habitat in central
531 Madagascar. The natural forest habitats of the Central Highlands have been degraded over
532 hundreds of years (Green & Sussman, 1990; Gade, 1996), but during the Pleistocene this
533 region may have consisted of mosaic habitat of wooded savannah and closed canopy forests
534 (Yoder *et al.*, 2016). Whether this region has been a biogeographic barrier to *Schetba* dispersal
535 over the past 854,000 years is unclear. *Schetba* has a broad elevational range (0 – 1829 m,
536 (Goodman & Raheirilalao, 2013)) and, hence, in principal could disperse across these
537 highlands given the necessary ecological conditions. On the other hand, both species are
538 strictly closed canopy forest dependent and found in large tracts of relatively undisturbed
539 forest habitat (Schulenberg, 2013), therefore wooded savannah habitat may have acted as a
540 biogeographic barrier to dispersal.

541

542 Overall, especially given our low number of occurrence records for ecological niche modeling,
543 we cannot say conclusively whether ecogeographic or biogeographic isolation was the
544 predominant cause of speciation, and it may be the case that both ecological and
545 biogeographic mechanisms played a significant role in generating and maintain these species.

546

547 **Concluding remarks**

548

549 Madagascar has been considered a model region for species diversification studies, yet the
550 phylogeography and diversification processes of the island's avifauna are still largely
551 unexamined. Here we provide the first evidence that the bioclimatic transition between the
552 humid east and dry west of Madagascar has facilitated speciation within birds. More

553 importantly, our findings of unrecognized diversity within *Schetba*, and cryptic diversity
554 within *Newtonia* (Younger *et al.*, 2018), suggest there may be other species awaiting
555 recognition in this biodiversity hotspot. Appreciating the full spectrum of diversity is likely to
556 alter conservation priorities for Madagascar, and we urge that further studies are needed to
557 quantify the island's biodiversity before it is lost to deforestation.

558

559

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561

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569

570

571 **Data Accessibility**

572

573 The Illumina short reads are available from the NCBI sequence read archive, link_TBA and
574 Sanger sequences are available from GenBank link_TBA.

575

576

577

578

579 **Author Contributions**

580

581 JY collected, analyzed, and interpreted the data, wrote the manuscript, and participated in
582 conceiving and designing the study. PD carried out phylogenetic analyses, AN conducted
583 ecological niche modeling, TOH collected the morphometric data. MJR collected genetic
584 samples. SMG collected genetic samples, and participated in interpreting the data and
585 conceiving the study. SR conceived and designed the study, and carried out morphometric
586 analyses.

587

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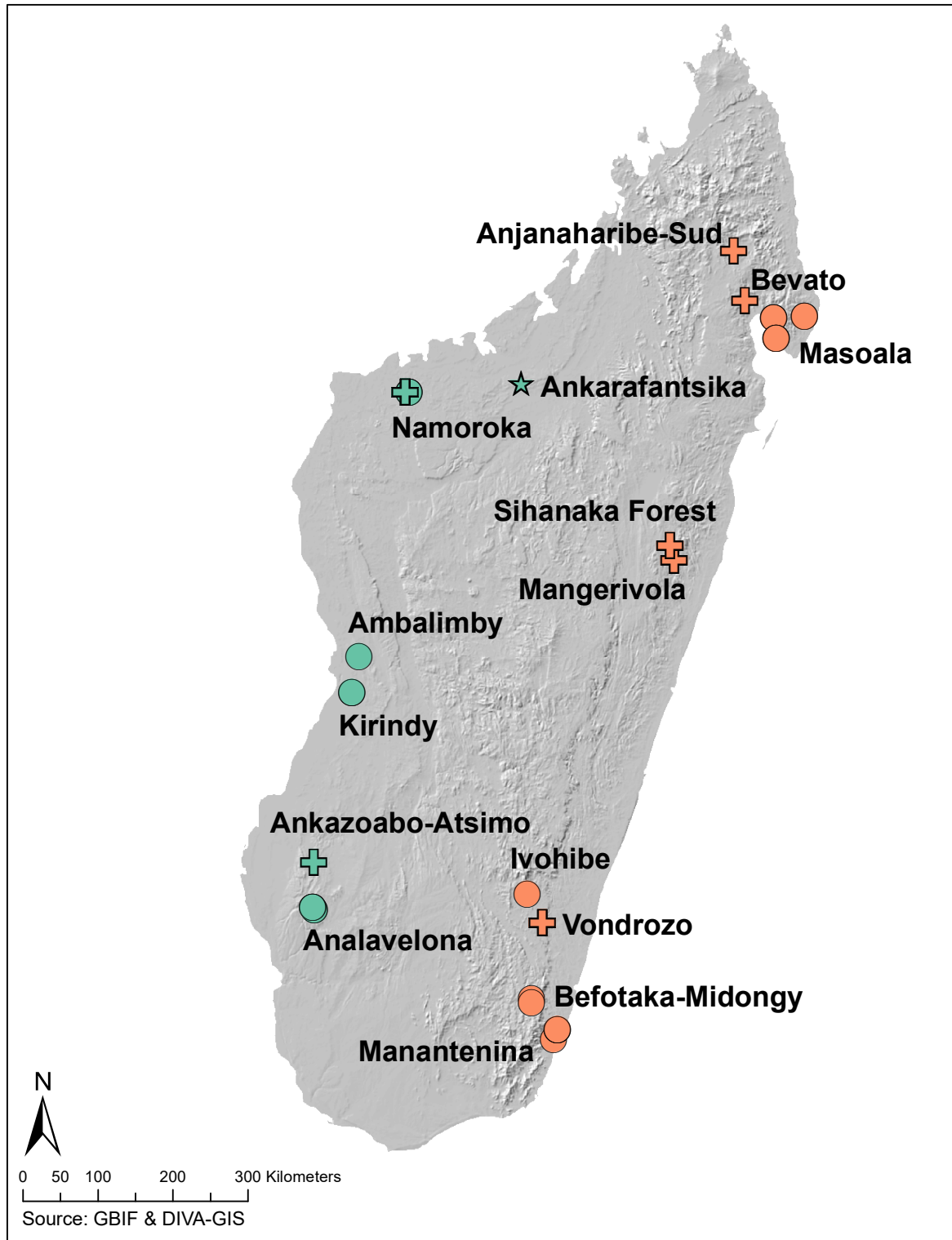
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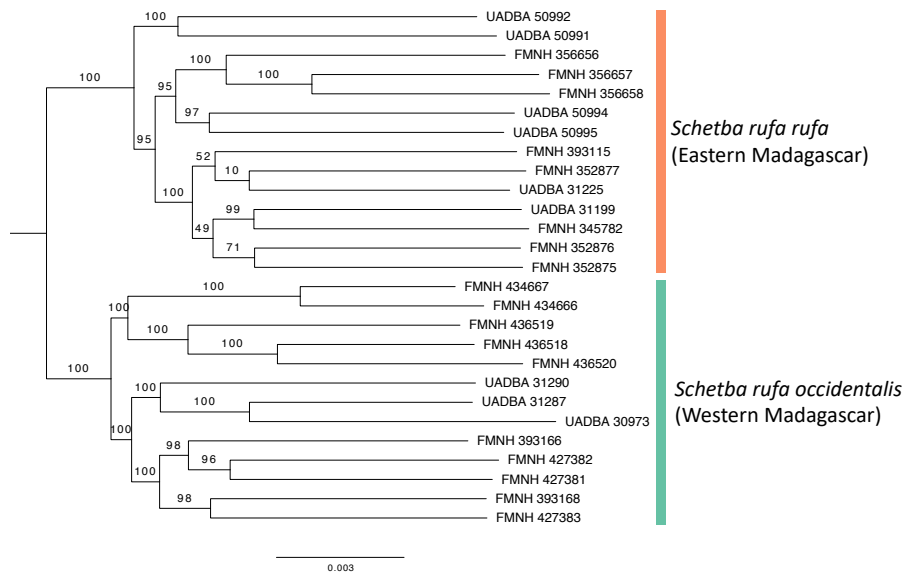
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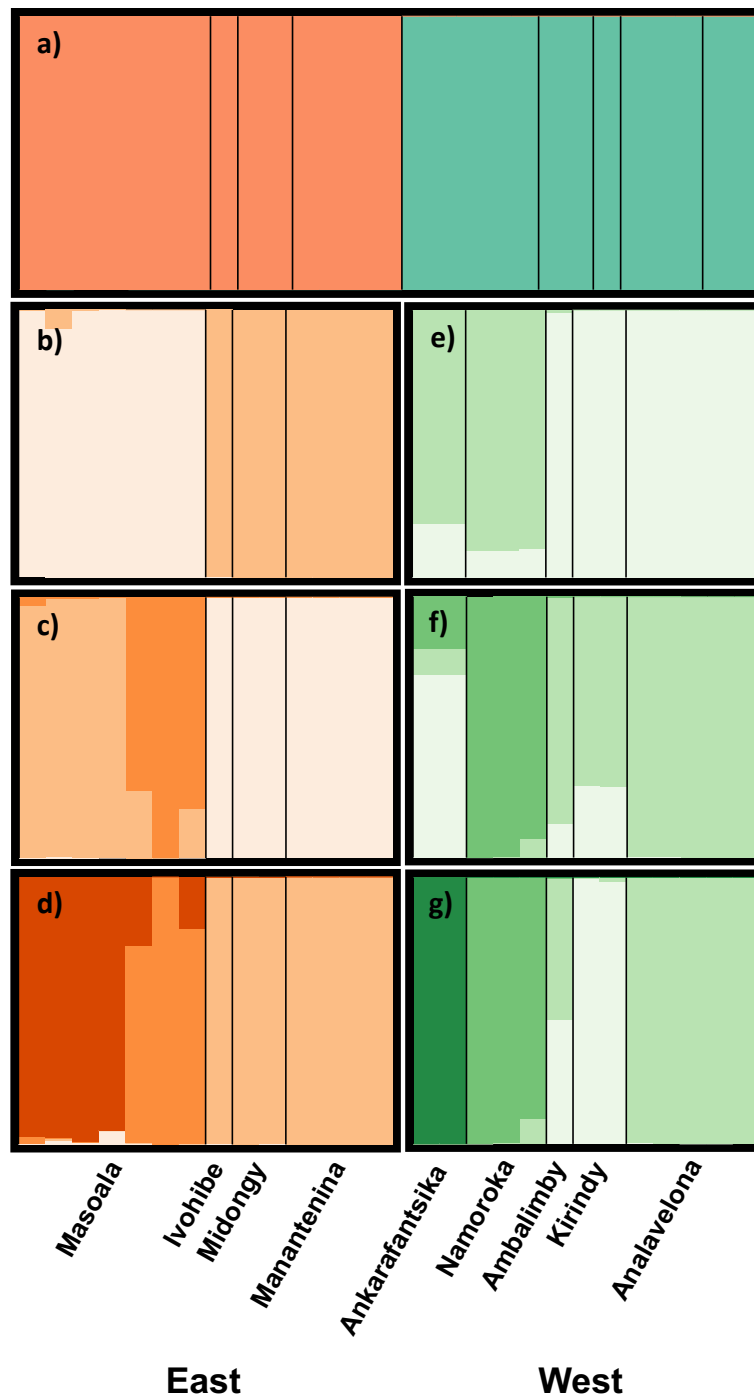


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 817 **Figure 1. Map of study sampling sites, with *S. r. rufa* indicated by orange icons, and *S. r.***
 818 ***occidentalis* by green icons.** Green star indicates western population outside the
 819 documented range of *S. r. occidentalis*, but confirmed as *occidentalis* in this study. Circles
 820 indicate genetic sampling, crosses indicate morphological sampling only. See Table S1 for
 821 latitude/longitude and accession numbers.
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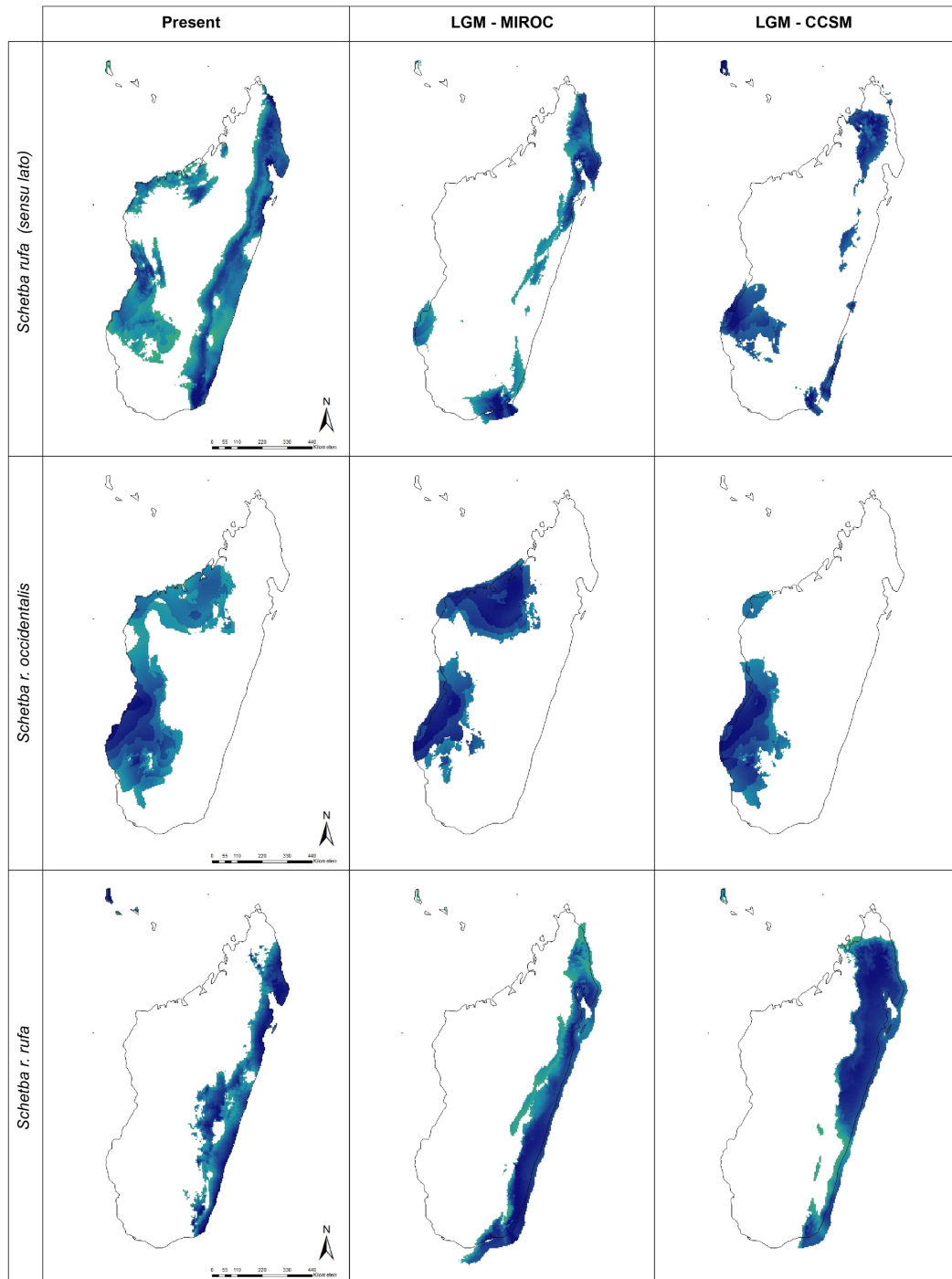
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Figure 2. Phylogenetic relationships within *Schetba*. Partitioned maximum-likelihood phylogeny of 4,243 concatenated UCE loci (3,328,172 bp). Support values are shown for nodes that received >70% bootstrap support.

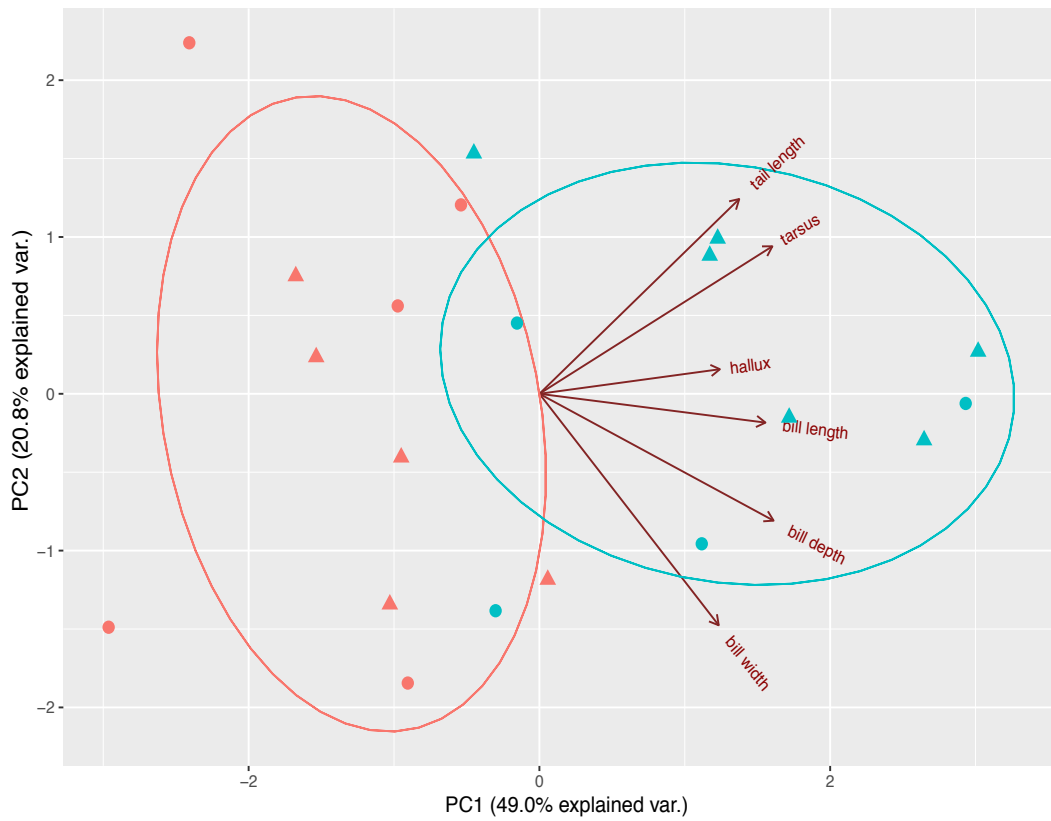


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Figure 3. Structure plot showing the membership coefficients for *Schetba* individuals to genetic clusters. (a) All *Schetba*, assigned to two genetic clusters ($K = 2$). All individuals from eastern Madagascar have 100% assignment to the orange cluster, whereas all western Madagascar individuals have 100% assignment to the green cluster. Panels (b) – (d); assignment of *S. r. rufa* and *S. r. occidentalis* individuals for $K = 2 - K = 4$. Labels refer to the area of collection of individuals.



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836 **Figure 4. Ecological niche models for *Schetba***, demonstrating suitable habitat in the present
837 and at the Last Glacial Maximum based on two alternate climate scenarios (MIROC and
838 CCSM). Top row represents the best model (AUC = 0.712; omission error = 0.286)
839 for *Schetba rufa (sensu lato)* obtained from the pooled set of occurrences (N = 34). The
840 middle row corresponds to the best model (AUC = 0.891; omission error = 0.000) for *S. r.*
841 *occidentalis* (N = 16), while the bottom row shows the best model output (AUC = 0.925;
842 omission error = 0.000) for *S. r. rufa* (N = 14). Dark blue areas represent higher occurrence
843 probability, while light blue and turquoise indicates lower presence probability.
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 846 **Figure 5. Principal components analysis of morphometric comparisons across *Schetba*.**
 847 Biplot of PC1 versus PC2, which together explain ~70% of the variation. Centroids of each
 848 clade (orange = *S. r. rufa*; green = *S. r. occidentalis*) were significantly different ($p < 0.001$)
 849 according to a MANOVA. Circles indicate 95% confidence ellipses around the centroid of
 850 each clade; symbols indicate sex (dots = females; triangles = males). Since there was no
 851 significant difference between sex, all individuals of each clade were analyzed together.
 852 Arrowed lines show direction and magnitude of the coefficients of each variable
 853 (abbreviations in text).

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