Geographic distribution, evolution, and disease importance of species within the Neotropical *Anopheles albitarsis* Group (Diptera, Culicidae)

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ABSTRACT: The *Anopheles albitarsis* group of mosquitoes comprises eight recognized species and one mitochondrial lineage. Our knowledge of malaria vectorial importance and the distribution and evolution of these taxa is incomplete. We constructed ecological niche models (ENMs) for these taxa and used hypothesized phylogenetic relationships and ENMs to investigate environmental and ecological divergence associated with speciation events. Two major clades were identified, one north (Clade 1) and one south (Clade 2) of the Amazon River that likely is or was a barrier to mosquito movement. Clade 1 species occur more often in higher average temperature locations than Clade 2 species, and taxon splits within Clade 1 corresponded with a greater divergence of variables related to precipitation than was the case within Clade 2. Comparison of the ecological profiles of sympatric species and sister species support the idea that phylogenetic proximity is related to ecological similarity. *Anopheles albitarsis* I, *An. janconnae,* and *An. marajoara* ENMs had the highest percentage of their predicted suitable habitat overlapping distribution models of *Plasmodium falciparum* and *P. vivax*, and warrant additional studies of the transmission potential of these species. Phylogenetic proximity may be related to malaria vectorial importance within the Albitarsis Group. ***Journal of Vector Ecology* 39 (1): 168-181. 2014.**

Keyword Index: Mosquito, ecological niche models, malaria, phylogenetics, SEEVA, Albitarsis Group.

INTRODUCTION

The *Anopheles* (*Nyssorhynchus*) *albitarsis* group comprises eight recognized species: *An. albitarsis* Lynch Arribalzaga, *An. deaneorum* Rosa-Freitas, *An. janconnae* Wilkerson and Sallum (previously *An. albitarsis* E), *An. marajoara* Galvão and Damasceno, *An. oryzalimnetes* Wilkerson and Motoki (previously *An. albitarsis* B), *An. albitarsis* F, *An. albitarsis* G, and *An. albitarsis* I; and the mitochondrial lineage, *An. albitarsis* H (Motoki et al. 2009, Ruiz-Lopez et al. 2012). The range of the group includes northern Colombia, Venezuela, Trinidad and Tobago, to Argentina (Ruiz-Lopez et al. 2012). These authors note that members of this group have also been reported from Bolivia, Costa Rica, French Guiana, Guatemala, Guyana, Panama, Peru, Suriname, and Uruguay, but specimens were not available to them for verification. McKeon et al. (2013) analyzed larval habitat characteristics (temperature, water chemistry, turbidity, water movement, shade, vegetation) of five species of *Anopheles* (*Nyssorhynchus*) in the Amazon, Brazil. They concluded that while *An. janconnae* and *An. oryzalimnetes* are habitat specialists, *An. marajoara* could not be so easily characterized. The Albitarsis Group is involved in malaria transmission in South America (Klein et al. 1991a, b, Conn et al. 2002, Póvoa et al. 2006), but our knowledge of the vectorial importance, distribution, evolution, and ecological requirements of each individual species is incomplete.

According to Hanley et al. (2007), the concept of ecological niche assumes that a species can survive in a certain hypervolume of biotic and abiotic factors. Knowledge of evolutionary relationships may allow the identification of abiotic or ecological characters that exhibit phylogenetic constraints or changing importance within a lineage. Such characters could be considered for inclusion in ecological niche models (ENMs) that map the potential distribution of particular species. Some authors have concluded that niches can be conserved between sister species (e.g., Peterson et al. 1999, Kozak and Wiens 2006), and that even subtle changes in climatic gradients can promote geographic fragmentation into allopatric lineages. For example, past climate changes may have isolated montane species by making intervening lowland areas inhospitable. Others have found that closely related species can show considerable niche plasticity (Losos and Glor 2003, Graham et al. 2004), suggesting that divergence estimated by morphology or molecular genetics may underestimate the extent of niche divergence. Kozak and Wiens (2006) argue that conclusions about the role of natural selection in speciation based on comparisons of sister species' climatic niche models should be informed by knowledge of the specific role that climatic factors play in divergence.

We were interested in exploring the following evolutionary

questions:

- What changes occur in the environmental requirements of species and species clades as they have evolved?
- Which environmental variable most strongly differentiates sister groups?
- Is there greater ecological divergence between allopatric sister species than sympatric sister species?
- Can phylogeny inform the vector potential of individual species?

We constructed ENMs to species of the Albitarsis Group based on the geographical origin of molecularly verified specimens, and environmental and bioclimatic data at these locations, using the program Maxent (Phillips et al. 2006). A similar approach was undertaken for the SE Asian *An. dirus* complex (Obsomer et al. 2012). We used the program FingerPrint 1.0 (Hanley et al. 2007) to visualize the species' ecological niche as defined by the Maxent distribution models for each species. The program SEEVA (Struwe et al. 2011) was used to explore environmental and ecological divergence associated with evolutionary splits, using a pre-defined hypothesis of phylogenetic relationships within the Albitarsis Group (Ruiz-Lopez et al. 2012) and the respective environmental and climatic data at collection localities. This approach has the potential to identify ecological adaptations that accompany or are instrumental in speciation. Actual and potential mosquito distribution was overlaid on models of malaria distribution (Guerra et al. 2008) to explore the possible vectorial importance of the member species.

MATERIALS AND METHODS

Specimens used

Specimens were collected over a period of 20 years by RCW, JFR, MMP, EB, and MAS and were individually identified by retrospective correlation of mtDNA *cytochrome c oxidase* I (*COI*) gene sequences (corresponding to the DNA barcoding region) with those 565 sequences published in Ruiz-Lopez et al. (2012). These include *An. albitarsis* s.s. (GenBank: JQ615201–JQ615309], *An. deaneorum* (JQ615310–JQ6153450, *An. janconnae* (JQ615346–JQ615441), *An. marajoara* (JQ615442–JQ615511), *An. oryzalimnetes* (JQ615512–JQ615562), *An. albitarsis* F (JQ614998–JQ615041), *An. albitarsis* G (JQ615042–JQ615146), *An. albitarsis* H (JQ615147–JQ615188), and *An. albitarsis* I (JQ615189–JQ615200). The full data set comprised 1,141 specimens, including those in Ruiz-Lopez et al. (2012). Data revealed 232 unique species-locality combinations of which 199 were unique at the resolution of the niche modeling (*An. albitarsis,* n = 38; *An. deaneorum,* n = 17; *An. janconnae,* n = 22; *An. marajoara,* n = 16; *An. oryzalimnetes,* n = 67; *An. albitarsis* F, n = 8; *An. albitarsis* G, n = 10; *An. albitarsis* I, n = 3; *An. albitarsis* H, n = 18). Novel sequences generated here are available under GenBank Accession numbers KJ011904-KJ012004 and KJ492398-KJ492558. Detailed individual specimen level collection data are available in the Mosquito Barcoding Initiative projects on the Barcode of Life (BOLD) website (www.boldsystems.org) under the Mosquitoes of the World container project, "MBIAA: *Anopheles albitarsis* complex", and in VectorMap (www.vectormap.org). Voucher material, including DNA extracts, are housed at the Smithsonian Institution (National Museum of Natural History), Washington DC, the Natural History Museum, London, and University of São Paulo, Brazil.

Ecological niche modeling

ENMs were constructed using Maxent 3.3.3k (Phillips et al. 2006). Input layers were: WorldClim bioclimatic variables, hydrological variables (elevation, aspect, slope, flow accumulation, flow direction, compound topographic index (= Topo Index)), and soil layers (world soil suborders) (see Table 1). All geographical information systems (GIS) analyses were undertaken using ESRI's ArcMap 10. All variables were resampled to 0.04 degrees resolution and clipped by region via mask (Spatial Analyst Tools/Extraction/Extract by Mask). The following Maxent parameters were used: Bootstrapping, ten replicates, 0 random test percentage, 10,000 iterations, no clamping / extrapolation.

Model output is a continuous variable ranging from 0 to 100, indicating relative suitability. The median model output was given a threshold of the lowest value associated with an observed presence record, i.e., the minimum training presence (MTP) value (Pearson et al. 2007), averaged across the ten replicates. To facilitate model analysis, we created binary predictions of presence and absence by classifying as "present" any cell with suitability greater than or equal to the MTP.

SEEVA analysis

For the SEEVA analysis, we used the phylogeny suggested by Ruiz-Lopez et al. (2012), based on DNA barcodes (658 bp of the mtDNA cytochrome C oxidase gene - *COI*), and converted to NEXUS format, and extracted environmental data from collection localities, using ArcMap 10. Environmental variables used in the SEEVA analyses are listed in Table 1. SEEVA was limited to four states or categories per environmental layer, so each layer was re-categorized to fit this schema for the analyses. Quantitative variables were scored as ordered, continuous data divided into four quartile classes. Qualitative variables (soil, geology, and vegetation type) were treated as non-ordered, categorical data. When state frequencies were very low, we followed the approach of Struwe et al. (2011) by combining related types or (rarely) omitted the very few records with rare types present in fewer than four individuals.

Eight phylogenetic nodes or junctions occur in the phylogeny of Ruiz-Lopez et al. (2012) (Figure 1), leading to eight (independent) tests for each environmental feature. Node 1 combines the lineage pairs: *An. marajoara*, *An. albitarsis* G, *An. albitarsis* H, *An. deaneorum*, *An. oryzalimnetes*, *An. albitarsis* & *An. albitarsis* F, *An. janconnae*, *An. albitarsis* I. Node 2: *An. marajoara*, *An. albitarsis* G, *An. albitarsis* H, *An. deaneorum* & *An. oryzalimnetes*, *An. albitarsis*. Node 3: *An. marajoara*, *An. albitarsis* G, *An. albitarsis* H & *An. deaneorum*. Node 4: *An. marajoara*, *An. albitarsis* G & *An. albitarsis* H. Node 5: *An. marajoara* & *An. albitarsis* G. Node 6: *An. oryzalimnetes* & *An. albitarsis*. Node 7: *An. albitarsis* F & *An. janconnae*, *An. albitarsis* I. Node 8: *An. janconnae* & *An. albitarsis* I.

SEEVA calculates two indices to measure the strength of the phylogenetic–ecological association. The Impact Index is independent of sample size and degrees of freedom thereby providing a measure of the skewness for each node and variable; the Diversity Index is a proportional measure, bounded on the interval (0, 1). The Diversity Index conveys the magnitude of

Table 1. Environmental and climate layer information used for the Maxent, SEEVA and Fingerprint analyses (x = included in analysis).

Analysis type			Layer name	Layer source
Maxent	SEEVA	Fingerprint		
	X	1	BIO1 = Annual Mean Temperature	http://www.worldclim.org/
X	X	2	BIO2 = Mean Diurnal Range (Mean monthly (max temp - min temp))	http://www.worldclim.org/
	X	3	BIO3 = Isothermality (BIO2/BIO7) (* 100)	http://www.worldclim.org/
X	X	4	BIO4 = Temperature Seasonality (SD*100)	http://www.worldclim.org/
	X	5	BIO5 = Max Temperature of Warmest Month	http://www.worldclim.org/
X	X	6	BIO6 = Min Temperature of Coldest Month	http://www.worldclim.org/
X	X	7	BIO7 = Temperature Annual Range (BIO5-BIO6)	http://www.worldclim.org/
X	X	8	BIO8 = Mean Temperature of Wettest Quarter	http://www.worldclim.org/
X	X	9	BIO9 = Mean Temperature of Driest Quarter	http://www.worldclim.org/
X	X	10	BIO10 = Mean Temperature of Warmest Quarter	http://www.worldclim.org/
X	X	11	BIO11 = Mean Temperature of Coldest Quarter	http://www.worldclim.org/
X	X	12	BIO12 = Annual Precipitation	http://www.worldclim.org/
	X	13	BIO13 = Precipitation of Wettest Month	http://www.worldclim.org/
	X	14	BIO14 = Precipitation of Driest Month	http://www.worldclim.org/
	X	15	BIO15 = Precipitation Seasonality (Coefficient of Variation)	http://www.worldclim.org/
X	X	16	BIO16 = Precipitation of Wettest Quarter	http://www.worldclim.org/
X	X	17	BIO17 = Precipitation of Driest Quarter	http://www.worldclim.org/
X	X	18	BIO18 = Precipitation of Warmest Quarter	http://www.worldclim.org/
X	X	19	BIO19 = Precipitation of Coldest Quarter	http://www.worldclim.org/
X	X	20	HYDRO-1K, Aspect	http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro/samerica
X	X	21	HYDRO-1K, Elevation	http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro/samerica
X	X	22	HYDRO-1K, Flow acceleration	http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro/samerica
X	X	23	HYDRO-1K, Flow direction	http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro/samerica
X	X	24	HYDRO-1K, Slope	http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro/samerica
X	X	25	HYDRO-1K, Topo Index	http://eros.usgs.gov/#/Find_Data/Products_and_Data_Available/gtopo30/hydro/samerica
X		26	Soils suborders	http://soils.usda.gov/use/worldsoils/mapindex/
		27	Landcover	http://glcf.umiacs.umd.edu/data/landcover/
	X	28	LandScan Land Cover Classes	http://www.usgs.gov/
	X	29	Treecover	http://glcf.umiacs.umd.edu/data/landcover/
	X		Water holding capacity	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Net primary production	http://daac.ornl.gov/NPP/npp_home.shtml
	X		Major Habitat Code (Ecoregions 1998 – 1999)	P. Hearn, Jr., T. Hare, P. Schruben, D. Sherrill, C. Lamar, P. Tsushima, 2003. Global GIS Database: Digital Atlas of the Earth, USGS Digital Data Series DDS-62-H, Flagstaff, AZ.
	X		Biological Distinctiveness Index (Ecoregions 1998–99)	P. Hearn, Jr. et al. 2003.
	X		Geological age	P. Hearn, Jr. et al. 2003.
	X		Soil Temperature Regimes	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Soil organic carbon	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Soil moisture regimes	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Soil inorganic carbon	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Phosphorus retention potential	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Inherent land quality	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Biomes	http://soils.usda.gov/use/worldsoils/mapindex/
	X		Anthropic landscapes	http://soils.usda.gov/use/worldsoils/mapindex/
	X		*Plasmodium falciparum* (stable)	http://www.map.ox.ac.uk/
	X		*Plasmodium falciparum* (unstable)	http://www.map.ox.ac.uk/
	X		*Plasmodium vivax*	http://www.map.ox.ac.uk/
	X		LandScan 2011	http://www.ornl.gov/sci/landscan/
	X		Cattle density	http://www.fao.org/AG/AGAInfo/resources/en/glw/GLW_dens.html

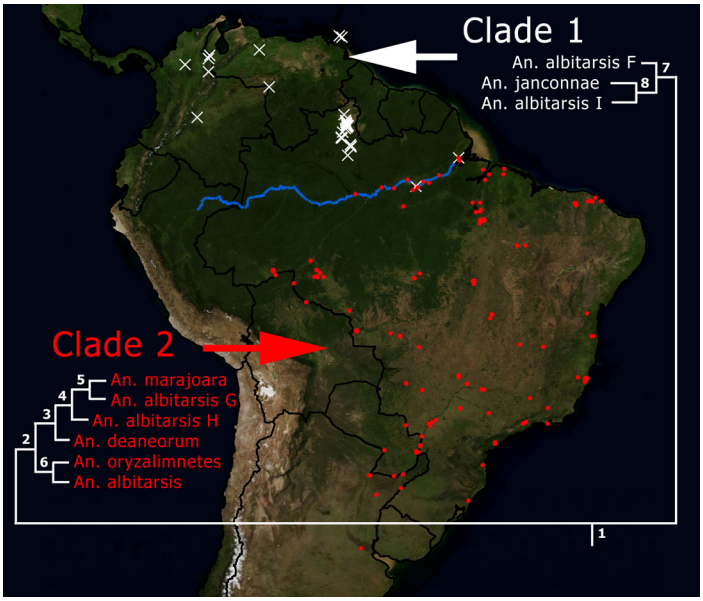

Figure 1. Location of collection points for species according to membership of the two major basal clades within the Albitarsis Group. Nodes in the phylogeny are numbered 1 to 8. Note the disjunction in the distribution north and south of the Amazon River (blue line).

divergence better than the impact factor (Struwe et al. 2011), and we only report the former here. Nodes that have evolved a diversity of adaptations or ecological requirements are more likely to show a high index of diversity. For measuring statistical significance, we used a Bonferroni correction for an alpha of 0.05, which amounts to declaring a significant result for a particular node only if $P = 0.0063$, which is equivalent to an experiment-wise error rate of $a = 1 - (1 - 0.0063)^8 \approx 8*(0.0063) = 0.05$ for the set of eight independent nodal tests (Struwe et al. 2011).

Species fingerprinting

The program FingerPrint 1.0 (Hanley et al. 2007) was used to visualize the species' ecological niche, using the Maxent distribution models for each species, and the results projected into a visualization matrix. Species fingerprinting maps the probability of a species' existence through the entire range of each of the environmental factors, allowing ecological comparison across many species. Similarity indices comparing the level of niche overlap between species were calculated and the index over all environmental factors was estimated as a mean of all similarity indices. The program's default settings were chosen for the cut-off probability (70% - the lowest probability, below which the species' survival is unlikely) and the method (mean). The sequence and list of environmental and climate layers used in the Fingerprint analyses are given in Table 1.

Species and malaria distribution overlap

The overlap of malaria and individual species predicted suitable habitat was quantified in ArcMap 10 (Raster Calculator and Zonal histogram tools), using the mean of ten mosquito models rendered to presence or absence *via* the MTP, and *Plasmodium falciparum* (stable: PfAPI ≥ 0.1 per thousand per annum, and unstable: PfAPI < 0.1 per thousand pa) for 2007 (Guerra et al. 2008) and *P. vivax* models for 2008 from the Malaria Atlas Project (http://www.map.ox.ac.uk/).

RESULTS

The earliest phylogenetic split (node 1) separates Clade 1 (*An. albitarsis* F, *An. albitarsis* I, *An. janconnae* - north of the Amazon River from Clade 2 (all other species - south of the Amazon River) (Figure 1). The location of collection points shows that exceptions to this north-south distribution pattern are few, and comprise locations near the Amazon River and the species *An. albitarsis* G, *An. janconnae,* and *An. marajoara*. Interestingly, these species are all terminal taxa according to the phylogeny for this group. According to Ruiz-Lopez et al. (2012), *An. janconnae* (Clade 1) and *An. marajoara* (Clade 2) are sympatric in Pará state, Brazil.

Ecological niche modelling

The ENMs (median values) are shown in Figure 2. Except for a few areas, habitat suitability derived from the ENMs support the hypothesis that Clade 1 and Clade 2 species are north and south of the Amazon River, respectively.

Relative contributions of the environmental variables to the Maxent models are shown in Table 2. For percent contribution, soils had a high value across all species, but particularly for *An. albitarsis* F (58.8%). Precipitation contributions were high for many species, but hydrological variables were higher for *An. deaneorum* (25.8%), and temperature variables were higher for *An. albitarsis*, *An. albitarsis* F, *An. albitarsis* G, and *An. albitarsis* I (19.1–35.7%). Phylogenetically terminal (sister) taxa differed in the contribution of temperature vs precipitation. For example, for *An. albitarsis* I, a temperature variable (Bio11) contributed the most (35.5%) to the model compared to precipitation (Bio19, 31.3%) for the sister taxon *An. janconnae*. When a variables' contribution to the model was seen in isolation (see Table 2), soils were no longer as important, except for *An. albitarsis* F. Altitude was uniformly unimportant for Clade 1 species, yet was important for species in Clade 2. Temperature variables more commonly gave the highest values for basal taxa, such as *An. albitarsis* F, *An. albitarsis,* and *An. oryzalimnetes*.

SEEVA

Environmental features that showed phylogenetic 'signal' from the SEEVA analysis are shown in Figures 3 and 4. For the 47 environmental features considered, 92 of the 376 nodal comparisons were significant ($P < 0.0063$, indicated by *), and 57 had Index of Diversity values >0.5. Node 8 shows greatest ecological divergence, with the highest number of significant values, and nodes 2, 3, and 6 show the least ecological divergence with no significant values. The absence of any strong environmental effect on the *An. oryzalimnetes* and *An. albitarsis* clade is noteworthy, suggesting that ecological divergence (as measured here) did not play a role or accompany speciation in this clade. No nodes for any of the environmental layers that gave an Index of Diversity >0.5 were statistically significant, suggesting caution is needed in the interpretation of results. Node 1 had high (>0.5) Index of Diversity figures only for the temperature-related variables (BIO1, 2, 3, 6, 9, 10), with Clade 1 mainly restricted to warmer locations compared to a wider spectrum of temperature conditions recorded

Table 2. Relative contributions of environmental variables to Maxent model for species within the Albitarsis Group. Percent contribution (above) of each variable when all variables are considered together and permutation importance (below) when the contribution of each variable in calculated independent of other variables. The two highest values for each model are shaded, taxa are grouped according to clade and reciprocal monophyly (sister taxa in parentheses), and variables are grouped according to type (temperature, precipitation, hydrology, and soil).

Percent contribution										
Type	Variable	Clade 1	Clade 1	Clade 1	Clade 2	Clade 2	Clade 2	Clade 2	Clade 2	Clade 2
		An. albitarsis F	*(An. albitarsis* I	*An. janconnae)*	*(An. albitarsis*	*An. oryzalimnetes)*	*An. deaneorum*	*An. albitarsis* H	*(An. marajoara*	*An. albitarsis* G*)*
Temp	bio_2	0.9	0.5	0.3	0.5	1.7	0	0.1	1	2
Temp	bio_4	19.1	2.1	1.8	35.7	5.5	4.6	1.4	2.1	0.1
Temp	bio_6	10.4	0.3	0.9	0	1.3	3.4	0.4	1.9	33.8
Temp	bio_7	2.7	0	0.2	0.5	2.3	1.3	0.1	0.1	2.8
Temp	bio_8	0	16.8	0	3.8	0.8	0.1	2.8	0	0
Temp	bio_9	2.5	0.1	0.5	0.3	4.1	0	0.1	0.5	1.4
Temp	bio_10	0.2	0	0.2	0.5	7.7	0	0.8	1.9	0
Temp	bio_11	1.2	35.5	2.5	0.5	1.2	0	1.5	0.5	0.3
Precip	bio_12	0	3.4	8.8	10.4	3.5	1.3	5.3	0.4	0.6
Precip	bio_16	0.1	0	0.1	1.5	9.4	1.2	3.3	26.5	0.6
Precip	bio_17	0.4	1.1	4.8	10.6	8.2	4.8	15.9	7.1	0.2
Precip	bio_18	0.6	11.4	6.7	2.2	8.3	0.6	0.3	11.5	6.6
Precip	bio_19	0	3.7	31.3	0.3	5.4	4.9	10.3	1.9	1.2
Hydro	h_aspect	0	1.8	4.5	1.7	3	6.1	2	4.8	4
Hydro	h_dem	2.5	0	2.9	3.7	7.4	6.5	2.9	19.3	6.7
Hydro	h_flowacc	0	0	1.2	2.1	2.9	25.8	3.2	4.7	0
Hydro	h_flowdir	0.1	3.1	1.5	1.1	1.3	3	11.6	3.6	0.6
Hydro	h_slope	0.4	0	2	1.6	4.9	8.6	5.8	2	0.3
Hydro	h_ topoind	0.1	0.8	0.1	0.4	0.2	0.1	0.4	1.7	1.1
Soil	soilso	58.8	19.5	29.8	22.7	20.7	27.5	31.9	8.4	37.7
Permutation importance										
Temp	bio_2	2.5	1.3	2.1	0.1	1.6	0.1	0.5	2.3	1.9
Temp	bio_4	20.5	6.5	0.1	43.7	7.6	3.1	1.7	12.6	0.1
Temp	bio_6	1.3	0.9	0.6	0	0.3	0.3	0.2	0.1	0
Temp	bio_7	10.7	0	8.3	1.4	1.6	0	0	0	36.9
Temp	bio_8	0	43.2	2.8	3.8	3.9	0.3	10	0.6	0.1
Temp	bio_9	10.5	8.3	9.8	0.4	19.6	0	0.1	0	13.6
Temp	bio_10	0.1	0	1.2	1.7	4.5	0.1	1.2	0.2	0
Temp	bio_11	0.6	33.5	14.6	1	0.8	0	0	0.8	0
Precip	bio_12	0	5	28.4	6.5	2.8	0.5	1.2	2.5	3.9
Precip	bio_16	0.8	0	1.1	1.9	6	1	0.2	18.8	0.4
Precip	bio_17	1.8	0	7.9	3.9	11.3	1.2	34.9	17.4	0.4
Precip	bio_18	0.4	0	0.5	1.6	5.4	1	0.4	3.3	7
Precip	bio_19	0.1	1.1	7.3	0.9	1.2	14.8	12.3	0.7	0.5
Hydro	h_aspect	0	0	0.4	0.6	1.4	0.3	1	3.3	0.8
Hydro	h_dem	7.7	0	6	16	20.2	63.5	20.9	21.5	31.5
Hydro	h_flowacc	0	0	0.1	1	0.3	5.3	1.1	2.9	0
Hydro	h_flowdir	0.2	0.2	1	0.2	0.3	1	5.9	1.8	0.4
Hydro	h_slope	0.7	0	5.9	7.7	3.8	1.7	4.4	6.6	0.4
Hydro	h_ topoind	0	0	0	1.6	0.7	0.2	0.6	2.3	0.2
Soil	soilso	42.1	0	1.7	6.1	6.6	5.6	3.5	2.1	1.8

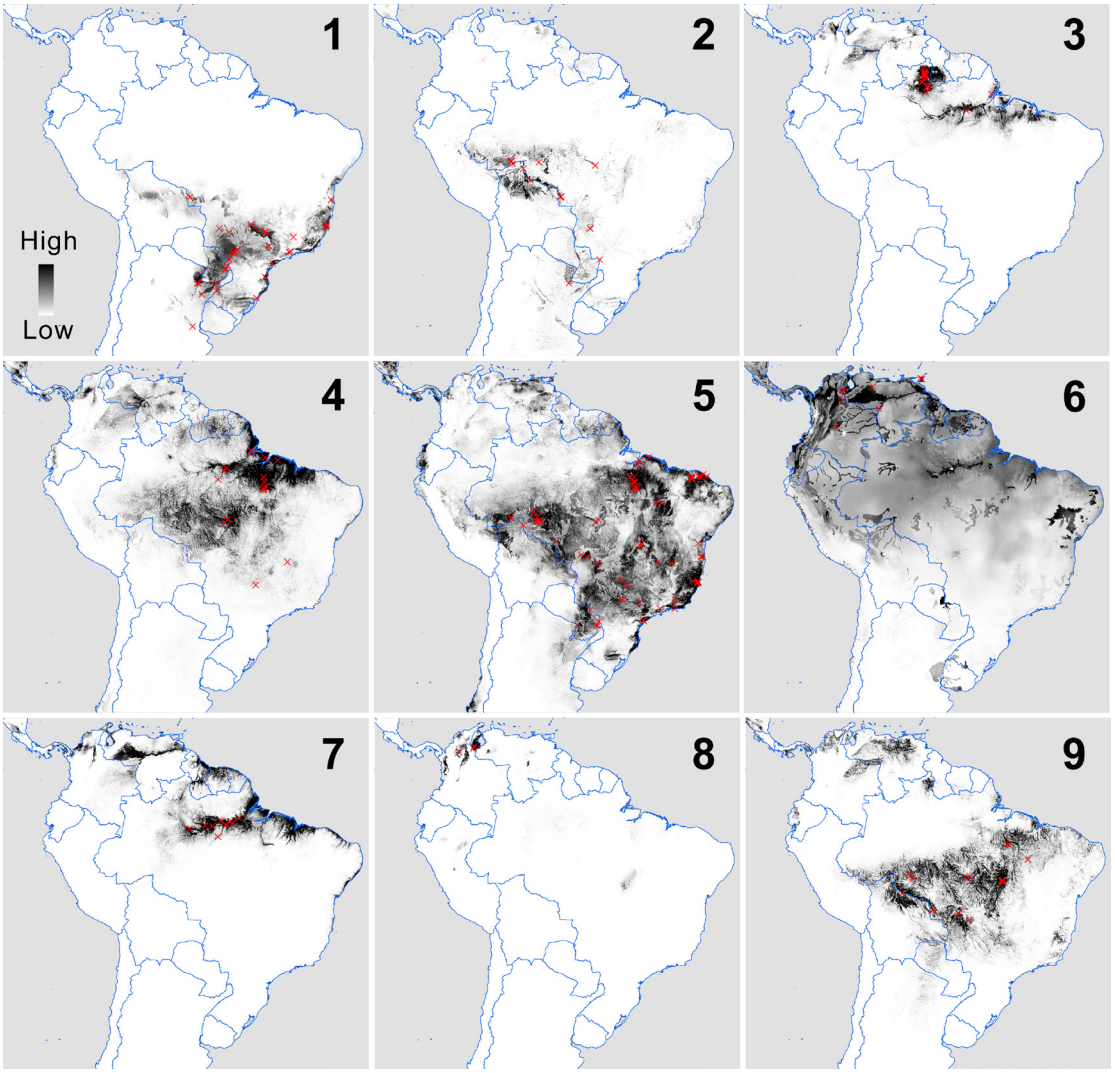

Figure 2. Maxent ecological niche models for nine species within the Albitarsis Group. 1. *Anopheles albitarsis*, 2. *An. deaneorum*, 3. *An. janconnae*, 4. *An. marajoara*, 5. *An. oryzalimnetes*, 6. *An. albitarsis* F, 7. *An. albitarsis* G, 8. *An. albitarsis* I, and 9. *An. albitarsis* H. The higher the predicted habitat suitability, the darker the shade. Collection points used in the models for each species are shown as red crosses.

for species within Clade 2. Node 4 showed high Index of Diversity figures for a variety of climate (temperature and precipitation) variables, and for elevation. Node 5 showed high figures for soil-related variables and one precipitation variable (BIO13). Node 7 showed high figures for a variety of variables except temperature. Node 8 showed the greatest number and variety of variables with high Index of Diversity figures. However, numbers of *An. albitarsis* I were low (n = 3), suggesting caution should be used in the interpretation of these results for this taxon.

Comparison of the average nodal Index of Diversity revealed that Clade 1 was ecologically more divergent than Clade 2: 15 of 47 environmental features had values >0.5 (three of which were >0.75) for Clade 1, compared to none of 47 for Clade 2. For Clade 1, climate features with high diversity were all precipitation related rather than temperature related. For example, precipitation of the warmest quarter was higher and precipitation seasonality was lower in locations where *An. albitarsis* F and *An. albitarsis* I occurred compared to those for *An. janconnae* (data not shown).

Despite these average tendencies, individual nodes within Clade 1 and 2 could show high values for the Index of Diversity. For example: Clade 1 was notable in that no collections were made from the Woodland landscape category (Figure 3). Only *An. albitarsis* G from Clade 2 was absent from Woodland, although numbers were low. Clade 1 also was absent from locations with the lowest category of inorganic carbon (data not shown). For node 8, *An. janconnae* was located in areas with lower host (human) density than *An. albitarsis* I (data not shown). Members of node 6 (*An. oryzalimnetes* and *An. albitarsis*) tended to be associated with higher densities of humans. Within Clade 1, significant divergence occurred in temperature and precipitation variables, as well as altitude. Higher temperature range, especially with lower temperature conditions and lower precipitation in the driest and coldest time of the year, is a feature of locations supporting *An. albitarsis* H, probably due to the higher elevation of inland collection locations. Within Clade 2, significant divergence occurs, mainly in characteristics of the land rather than climate. These land conditions include: soil suborder, soil phosphorus retention potential, soil moisture regime, soil inorganic carbon, soil water holding capacity, inherent land quality, biome type, and Biological Distinctiveness Index.

Where comparisons of sister species were possible (nodes 5, 6, and 8) there was no consistency in the degree of divergences, which ranged from no appreciable divergence to large divergences, mainly in non-climate variables.

The youngest geological age category (Tertiary and Quaternary) made up the majority of sites from where the

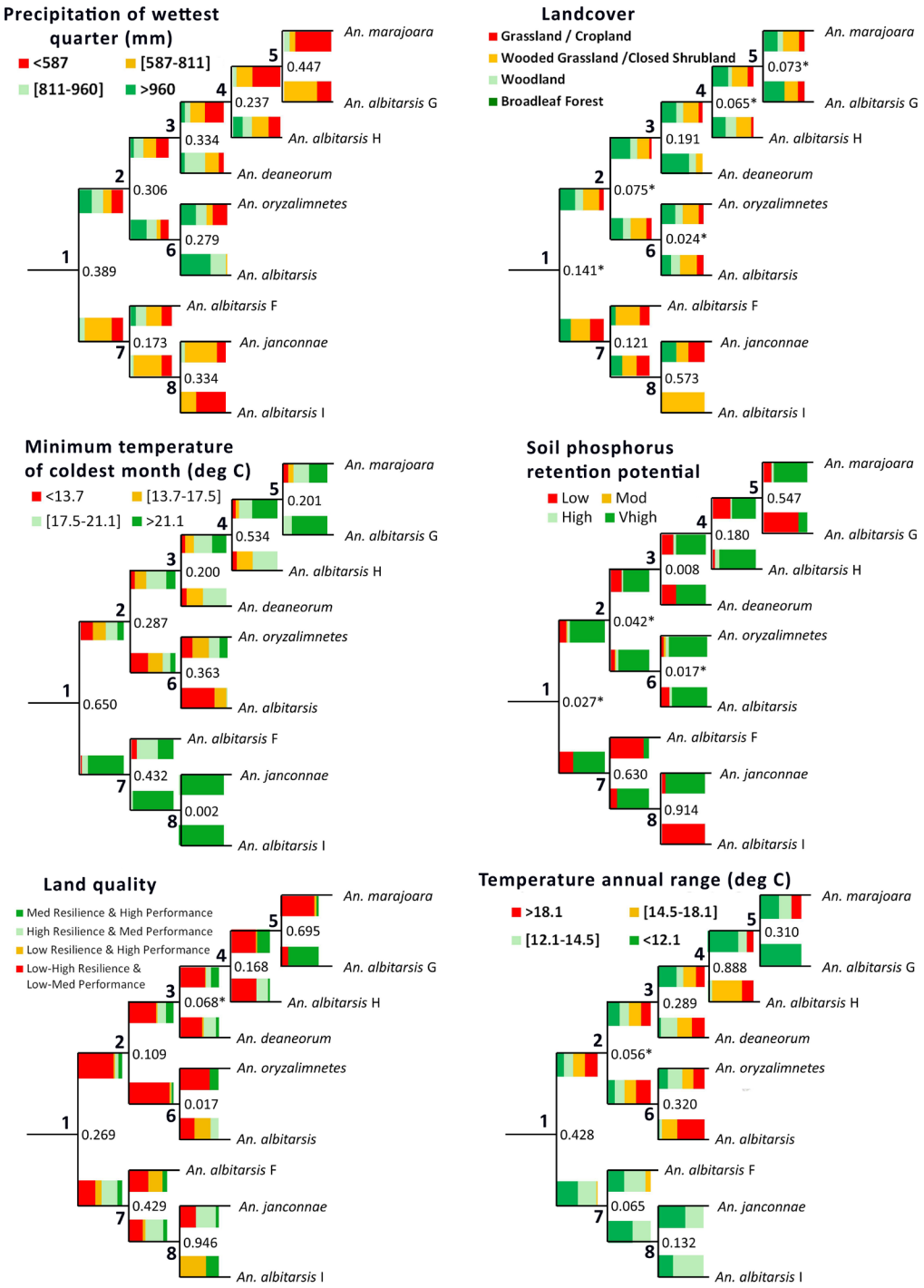

Figure 3. Example 1 output from the program SEEVA for variables mapped on a phylogram of the Albitarsis Group, based on mtDNA *COI* sequence data (Ruiz-Lopez et al. 2012). The bars indicate proportion of categories present at each sister group. Decimal numbers are the Index of Diversity and asterisks indicate statistically significant indices ($P < 0.0063$). Nodes in the phylogeny are numbered 1 to 8.

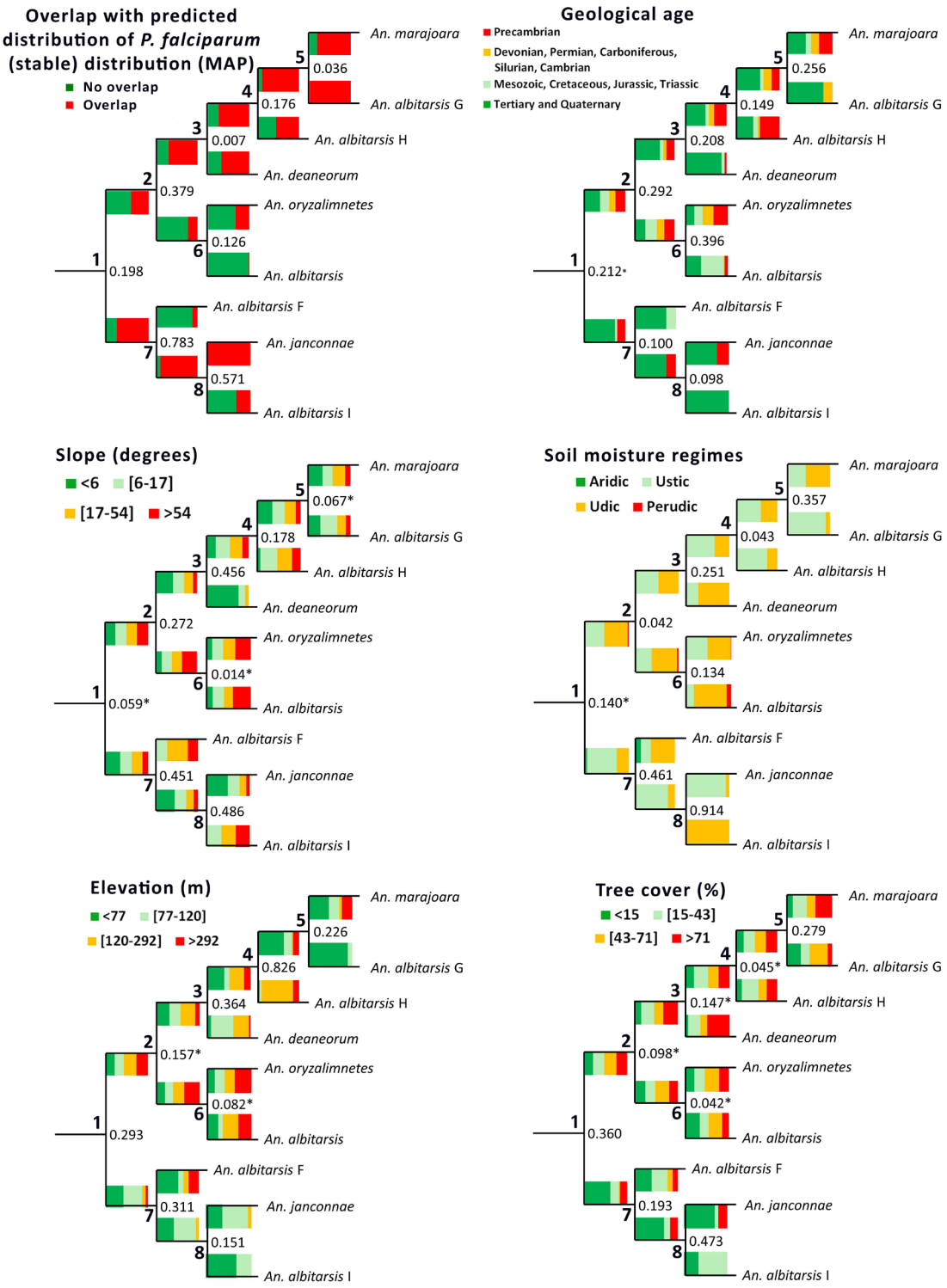

Figure 4. Example 2 output from the program SEEVA for variables mapped on a phylogram of the Albitarsis Group, based on mtDNA *COI* sequence data (Ruiz-Lopez et al. 2012). The bars indicate proportion of categories present at each sister group. Decimal numbers are the Index of Diversity and asterisks indicate statistically significant indices ($P < 0.0063$). Nodes in the phylogeny are numbered 1 to 8.

Albitarsis Group were collected (Figure 4).

Species fingerprinting

The results of the FingerPrint analysis are shown in Figure 5. Hydrological layers (e.g., low values of flow acceleration and higher values of Topo Index) appear important for the ecological niche of *An. oryzalimnetes* and soil types for *An. albitarsis* F. Lower mean annual temperature range is more important for *An. albitarsis* F than for *An. albitarsis*; *An. albitarsis* F and *An. albitarsis* I have higher precipitation requirements than the other species. Temperature and hydrological features appear to be unimportant for the ecological niche of *An. albitarsis* I, and temperature and precipitation appear to be unimportant for *An. janconnae*. A phylogram constructed in MEGA 5.02 (Tamura et al. 2011) using dissimilarity values output from FingerPrint (Figure 6) differed from the DNA-derived phylogeny, with the relatively unrelated *An. albitarsis* F (Clade 1) and *An. oryzalimnetes* (Clade 2) being ecologically distinct from each other and the other species. *Anopheles albitarsis* F differed mainly in temperature and precipitation variables and slope, and *An. oryzalimnetes* differed mainly in hydrological variables (aspect, Topo Index, and flow acceleration and direction), and both species differed simultaneously in soils, land cover, and flow direction. The output matrix of the mean Full Dissimilarity Index from FingerPrint (Figure 6) showed low values for sister species within Clade 1 (n = 1.00), high values for non-sister species within Clade 1 (n = 8.00), and intermediate values for Clade 2 sister and non-sister comparisons (n = 4.00 and 3.85, respectively). The average mean Full Dissimilarity Index for Clade 1 was 5.67 and for Clade 2 was 3.87. The greatest dissimilarity values were between *An. albitarsis* I and *An. oryzalimnetes*, which showed non-overlap in precipitation variables (Figure 5). Dissimilarity is perhaps not surprising given the non-overlapping and restricted predicted habitat suitability of *An. albitarsis* I, and extensive distribution of *An. oryzalimnetes* (see Figure 2). However, low dissimilarity was seen between other non-overlapping species (e.g., *An. albitarsis* I and *An. deaneorum*).

The comparison of sister species (nodes 5, 6, and 8) gave an average dissimilarity score of 3.00, compared to an overall average of 4.31 (Figure 6), supporting the idea that phylogenetic proximity is related to ecological similarity, i.e., that niches are conserved. As noted by Ruiz-Lopez et al. (2012) for a subset of the current data, some species and lineages were found to be sympatric: *An. marajoara* with *An. oryzalimnetes* and *An. janconnae* in Pará, Brazil; *An. albitarsis* H, *An. deaneorum*, *An. marajaora,* and *An. oryzalimnetes* in Mato Grosso state, Brazil; *An. oryzalimnetes* and *An. albitarsis* in São Paulo state, Brazil, among others. The average dissimilarity score for these sympatric species was 4.78 (Figure 6), suggesting that geographic proximity was not correlated with ecological similarity.

DISCUSSION

Zeisset and Beebee (2008) discussed five factors that have been used to explain biogeographic patterns in tropical South America. Of these, the barrier effect of major Amazonian rivers (the River hypothesis), the Refuge hypothesis, and the Climatic/River refuge hypothesis are the most relevant to mosquitoes. Major rivers, but not usually minor tributaries or headwaters, can act as partial barriers to gene flow in some animals (Zeisset and Beebee 2008). Molecular evidence suggests that the Amazon River has been a significant barrier for several taxa, including monkeys (Lavergne et al. 2010), tapirs (de Thoisy et al. 2010), carnivores (Eizirik et al. 2001), and birds (Fernandes et al. 2012). Geological data suggest that the Amazon River attained its current flow during the late Pleistocene (Rossetti et al. 2005), and Solomon et al. (2008) noted that diversification at time subsequent to the formation of the Amazon River (5–12 mya) would be consistent with the riverine barrier hypothesis. Pedro et al. (2010) analyzed a fragment of the *COI* mitochondrial gene of *An. triannulatus* (Neiva and Pinto) and *An. darlingi* Root and concluded that river and mountain barriers block gene flow for more than 1,000 km for both species and likely played a part in historical gene flow. They found that populations northeast of the Amazon and in southeastern Brazil are generally reciprocally monophyletic to the remaining groups. They speculated that human-aided transport and unassisted dispersal across the islands of the Amazon delta may have played a part in explaining cross Amazon River haplotypes. Although dating of the phylogenetic nodes of the Albitarsis Group has yet to be done, the role of the Amazon River in the speciation of this group appears important, initially in the separation of Clade 1 and 2 and more recently in the co-location near the Amazon River of the sister taxa *An. marajoara* and *An. albitarsis* G. A geographically more detailed study of vector species distribution in relation to the Amazon River appears warranted.

Sister species couplets are: *An. marajoara* - *An. albitarsis* G (node 5, overlapping distribution, ecological divergence in soil features), *An. albitarsis* - *An. oryzalimnetes* (node 6, overlapping southern distribution, no strong ecological divergence in characters considered here), and *An. janconnae* - *An. albitarsis* I (node 8, disjunct distribution, greatest ecological divergence). *Anopheles deaneorum* and *An. albitarsis* H are sympatric, with overlapping middle to western Amazonian distribution. Sister species either had overlapping or sympatric potential distribution with little ecological divergence, or allopatric potential distribution with great ecological divergence. Although numbers are low, phylogenetic proximity more often corresponds with geographic and ecological proximity.

Distribution patterns

Based on present day distribution, some speculation about past distributions at the time of speciation is possible. For example, the sister taxa *An. janconnae* and *An. albitarsis* I may have arisen in allopatry at the eastern and western edges, respectively, of a more widespread ancestral taxon. The geographically intermediate distribution of the phylogenetically basal *An. albitarsis* F possibly reflects the ancestral ecological niche of Clade 1. For example, a hotter, drier past climate may have isolated this ancestral species to higher, cooler areas, enabling allopatric speciation of intervening lowland areas to occur. *Anopheles albitarsis* appears to have specialized to more southern environments, and *An. oryzalimnetes* retained the more widespread ancestral (node 1 and 2) niche, judging from the similarity of the latter species to the hypothesized ancestral ecological spectra (e.g., temperature annual range, Figure 4). These sister taxa are unique in their occurrence in dry environments – the deserts and xeric shrub lands biome – an adaptation that may have played a role in the evolution of this

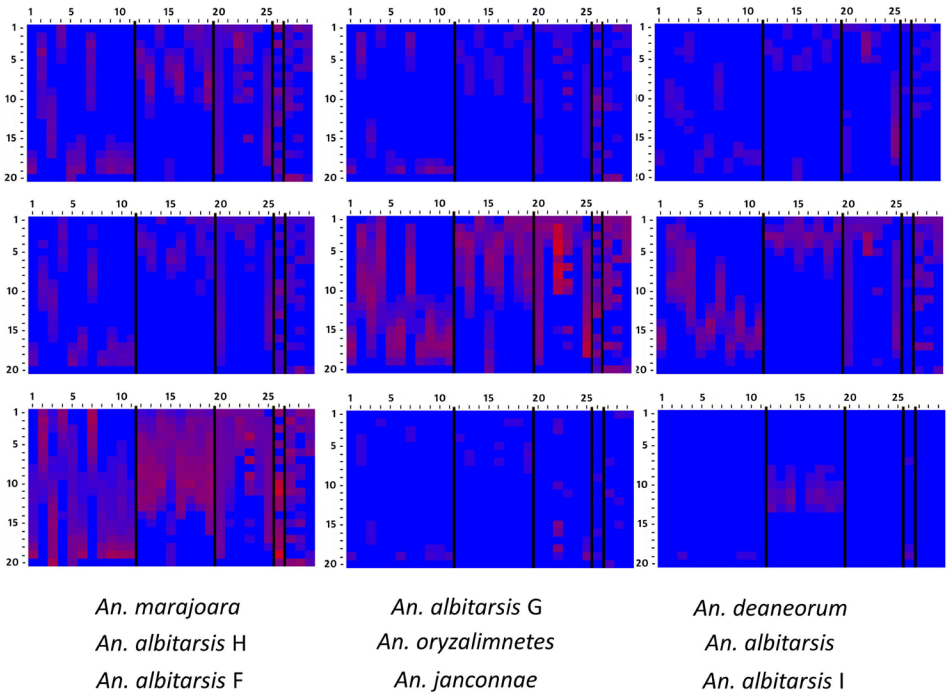

Figure 5. Visual representation of the ecological fingerprint for 29 environmental variables (columns in the matrix for each species) according to the nine species within the Albitarsis Group (the more red the color, the higher the probability of occurrence and the more important for the ecological niche of that species). The numbers across the top of each species matrix refer to the different environmental layers used in the modeling and are divided by a solid black vertical line into general types. From left to right these are: temperature (1 – 11), precipitation (12 – 19), hydrological (20 – 25), soil (26), and landscape variables (27 – 29) (see methods). The values on the left refer to 20 different value levels for each environmental layer, e.g. for temperature, low = 1 and high = 20. The bottom row of three species comprise Clade 1 taxa.

	An. deaneorum	An. albitarsis	An. albitarsis I	An. albitarsis H	An. albitarsis G	An. albitarsis F	An. oryzalimnetes	An. marajoara	An. janconnae
An. deaneorum									
An. albitarsis	3								
An. albitarsis I	1	4							
An. albitarsis H	1	3	2						
An. albitarsis G	1	4	1	1					
An. albitarsis F	8	8	8	7	7				
An. oryzalimnetes	8	6	9	7	8	7			
An. marajoara	2	4	3	2	2	6	6		
An. janconnae	1	3	1	1	1	8	8	3	

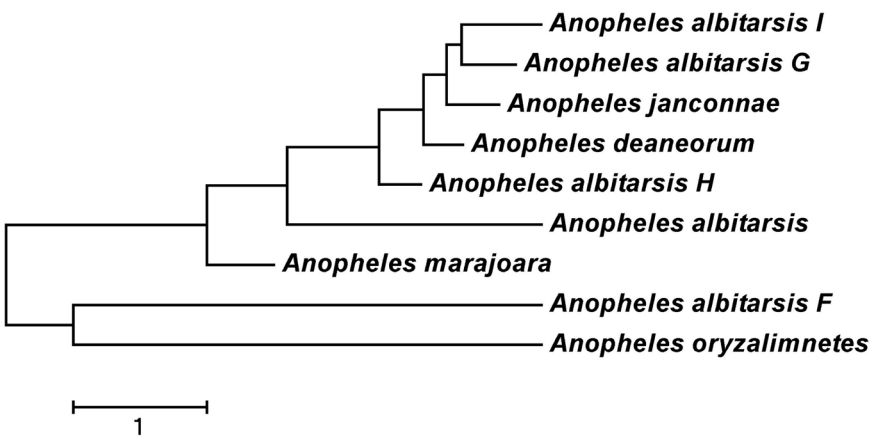

Figure 6. Matrix of mean P based Full Dissimilarity Index output for species within the Albitarsis Group from the program FingerPrint. Those values with a blue background refer to Clade 1 comparisons, white refers to Clade 2, and grey refers to cross clade comparisons. Values with a red font refer to sister taxa comparisons, black font refers to non-sister taxa comparisons. Also shown is a Neighbor Joining phylogram, constructed in MEGA 5.02, using the matrix values, to further illustrate the relative ecological similarity of species within the Albitarsis Group.

clade. Although roughly co-located in western Brazil, *An. albitarsis* H appears to have become specialized for hillier, higher elevation and less-forested locations that are hotter and drier than the more ancestral *An. deaneorum*. The clade comprising *An. marajoara* and *An. albitarsis* G occurs in the proximity of the Amazon River and shows evidence of ecological divergence in features of the soil rather than climate, e.g., *An. albitarsis* G occurs in locations with wetter soils characterized by lower soil phosphorus retention potential. Soil, organic detritus, and aquatic chemistry have powerful inter-related effects on mosquito distribution and abundance (Gardner et al. 2013). Access to a robust molecularly-dated phylogenetic tree and projecting what we know about the ecological niche and geological history onto scenarios of past climate may show spatial habitat suitability patterns that could shed light on the factors involved in speciation in the Albitarsis Group.

We found a widespread occurrence of *An. oryzalimnetes* and more ancestral-like environmental spectra of this species (Figures 3 and 4). McKeon et al. (2013) considered this species an evolutionary specialist and found that the number of larval habitats that were shared among this and other species was one of the lowest within the study sites sampled. The fingerprint analysis reported here confirmed that this species is very different in its associated climatic and environmental characteristics compared to the other species, which suggests the exploitation of a unique niche (but not a geographically limited niche). McKeon et al. (2013) based their findings on the observation of a more restricted distribution of this species than we detected and on its abundance being associated with the water chemistry variables that they measured. The present study measured occurrence of adults over a wider geographic area and may better reflect more evolutionarily constrained physiological tolerance. Also, McKeon et al. (2013) measured larval abundance, which is partially related to more plastic behavioral oviposition preferences and less easily interpreted optimal conditions for growth. A combination of a detailed understanding of the phylogeography of the species within the Albitarsis Group across their distribution range and studies such as McKeon et al. (2013) that investigate the nature and degree of tolerance in different geographical populations of the different species with varying exposures to water chemistry may assist an understanding of the population structure and history of adaptations within this group.

Limitations of this study include: the phylogenetic hypothesis (branching order) is based on a single gene and may not be correct; collection sites may be subject to sampling bias and were sometimes few in number (e.g., very low numbers for *An. albitarsis* I, $n = 3$); additional cryptic species may be present that were not accounted for; categorization of layers to four states may not represent biological reality and could distort the analysis; correlations of ecology and phylogeny do not prove causality thereby limiting our conclusions; limits on distribution may be historical rather than relating to ecological niche; and the limited resolution of the remote sensing data may make species with different ecologies but the same general location appear closer in their niche requirements than is the case. The biological reality of ecological niche predictions could be improved with additional collection locations, especially those remote from sites already sampled. According to the SEEVA manual, the generic null hypothesis for all tests is that the cross-classified factors are completely independent, and the hypothesis driving the work is that species or clade distributions are influenced by specific environmental variables, as shown by non-random associations of particular species or clades and their environmental situations. However, it should not be assumed that environmental variables are independent, and an assumption of independency is not necessary for the SEEVA analysis. In general, correlations between divergence and environmental variables can be inferred as trends and tendencies within phylogenetic lineages and not as the definite cause for the divergence until verified by further research. The role of ecology in speciation more widely is unclear, but the observation of apparent niche conservatism in this study argues against it in the Albitarsis Group. However, as stated by Svennson (2012), "the ecology of species differences is not the same as ecological speciation, just like the genetics of species differences does not equate to the genetics of speciation."

Species and malaria distribution overlap

Collection locations and ENMs can be overlaid with human and malaria models to incriminate vector species (by eliminating from contention those species that do not coincide with malaria) and to predict the likely species encountered in malaria field studies. Given the caveats that correlation does not imply causation, that this study does not consider the role of non-Albitarsis Group vectors, and that 'anophelism without malaria' (Fantini 1994) is possible, collection locations and ENMs available in this study can be used to explore the potential importance of individual species in transmission. The expectation for a species that is a 'good' vector throughout its range is that it will have a high co-occurrence with malaria. If it does not, then the mosquito or *Plasmodium* models are inaccurate or malaria distribution depends on other (non-vector) factors. The expectation for a species that is a 'poor' vector throughout its range is that it will have a low co-occurrence with malaria. If it does not, then the non-vector species is usually sympatric with vector species, the result occurs through chance, or both mosquito and malaria distribution are responding to an unknown third factor.

At least three species are vectors of human malaria parasites in Brazil: *An. deaneorum* (Klein et al. 1991a, b), *An. marajoara* (Conn et al. 2002), and *An. janconnae* (as *An. albitarsis* E, Póvoa et al. 2006). Collection locations of these species (plus *An. albitarsis* G and H) largely coincide with areas predicted suitable for *P. falciparum* (Figure 4). Interestingly, *An. janconnae* and *An. albitarsis* G were unique in that they were always in areas predicted suitable for *P. falciparum* (stable) and *P. vivax* but never in areas that were predicted to be suitable for *P. falciparum* (unstable).

The percentage overlap of ENMs with malaria distribution models is shown in Figure 7. On average, higher percentage of overlap is observed for *P. vivax* compared with *P. falciparum*, and for *P. falciparum* (stable) compared with *P. falciparum* (unstable). The widespread *An. oryzalimnetes* was predicted to most often coincide with malaria (data not given), but when considering the percentage of this species' suitable habitat that coincides with malaria the result is very different. *Anopheles albitarsis* I, *An. janconnae,* and *An. marajoara* had the highest levels of overlap with *P. falciparum* and *P. vivax*, as a percentage of the total area predicted suitable for each mosquito species. Moderate to

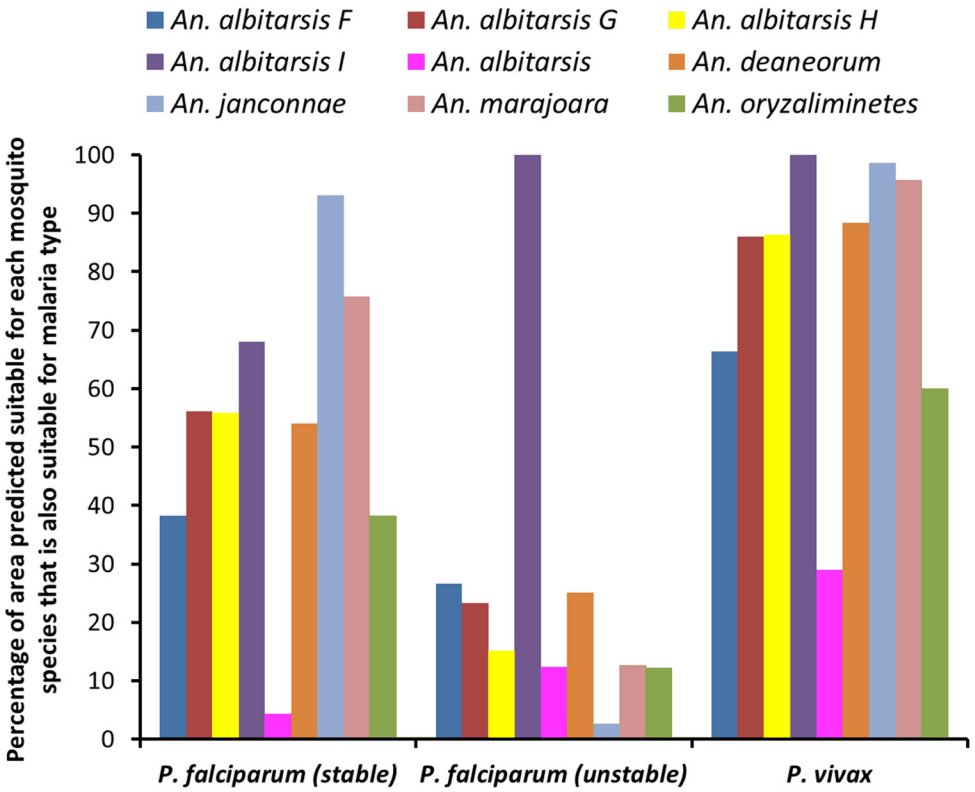

Figure 7. Percentage of the area predicted suitable, based on niche models, for each species within the Albitarsis Group that is also suitable for malaria type according to models of malaria distribution for 2007/2008 from the Malaria Atlas Project.

high percentages of overlap were noted for *An. albitarsis* G, *An. albitarsis* H, and *An. deaneorum*. The sister taxa *An. oryzalimnetes* and *An. albitarsis* showed the lowest percentage of overlap and, on this basis, do not appear to be important malaria vectors. This observation also suggests that phylogenetic proximity may be related to malaria vectorial importance within the Albitarsis Group. Gutiérrez et al. (2010) used the phylogenetic proximity to *An. janconnae* of a new lineage (= *An. albitarsis* I, Ruiz-Lopez et al. 2012) from Colombia to argue for its possible role in malaria transmission. Mapping malaria to a vector species phylogeny is not meant to imply that speciation is related to malaria, but this type of analysis could reveal phylogenetic constraints and pre-adaptations important to malaria transmission or vector control.

The current results agree with the list of known vector species above and also supports the suggestion of a role in malaria transmission for *An. albitarsis* I (Gutiérrez et al. 2010). Low collection numbers of this species limit conclusions, but the present results are suggestive and warrant additional studies to be undertaken on the transmission potential of this species.

Other information can be considered in relation to vectorial importance. For example, *An. marajoara,* followed by *An. deaneorum* and *An. albitarsis H,* tended to be associated with low to medium human densities (data not shown), as expected in rural areas where malaria is more likely and with land of higher vulnerability to anthropogenic activities (i.e., lower Inherent Land Quality, see Figure 3). By contrast, *An. albitarsis* G and *An. janconnae* collection locations tended to be in areas with higher land quality. Low resilience and performance soils in agricultural areas are likely to be related to lower crop yields and increased levels of poverty, poorer nutrition, and lower access to health services; poverty is a risk factor for malaria (Tusting et al. 2013).

Knowledge of species distributions may assist vector control workers to identify the likely members of the Albitarsis Group, regardless of their vectorial importance, in given areas. ENMs may also suggest new areas for mosquito surveys and geographically isolated populations that are genetically distinct. For example, the vicinity of São Luís, Maranhão, was not sampled but appears suitable for *An. marajoara* and *An. albitarsis* G. Analyses such as from SEEVA may shed light on the evolutionary history and likely responses of individual species to aspects of climate/environmental change. For example, long term changes in precipitation of the wettest quarter may have very different effects on the abundance and distribution of *An. marajoara* and *An. albitarsis* G compared to *An. albitarsis* (see Figure 3). Knowledge of the abiotic conditions, biotic factors, geographic connectivity, and evolutionary capacity of a species to adapt to new conditions is necessary to fully appreciate the relationship between a species' fundamental ecological niche and the geographic areas where it is found (Soberón and Peterson 2005). This knowledge could also suggest how a species might react (e.g., González et al. 2010) given future anthropogenic changes in aspects of the climate, habitat, and the types of species interactions, including with hosts and pathogens, for an area of interest.

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