



## Long-distance dispersal and inter-island colonization across the western Malagasy Region explain diversification in brush-warblers (Passeriformes: *Nesillas*)

JEROME FUCHS<sup>1\*</sup>, DELPHINE LEMOINE<sup>1</sup>, JUAN LUIS PARRA<sup>2</sup>, JEAN-MARC PONS<sup>1</sup>, MARIE JEANNE RAHERILALAO<sup>3</sup>, ROBERT PRYS-JONES<sup>4</sup>, CHRISTOPHE THEBAUD<sup>5</sup>, BEN H. WARREN<sup>6</sup> and STEVEN M. GOODMAN<sup>3,7</sup>

<sup>1</sup>Département Systématique et Evolution, UMR7205, Institut de Systématique, Evolution, Biodiversité CNRS MNHN UPMC EPHE, Sorbonne Universités, Muséum National d'Histoire Naturelle, CP 51, 57 rue Cuvier, F-75231, Paris Cedex 05, France

<sup>2</sup>Grupo de Ecología y Evolución de Vertebrados, Instituto de Biología, Universidad de Antioquia, Calle 67 No. 53-108, Medellín, Colombia

<sup>3</sup>Association Vahatra, BP 3972, Antananarivo, 101, Madagascar

<sup>4</sup>Bird Group, Department of Life Sciences, Natural History Museum, Tring, Herts HP23 6AP, UK

<sup>5</sup>Laboratoire Evolution et Diversité Biologique (EDB), UMR 5174, Centre National de la Recherche Scientifique (CNRS) – Université Paul Sabatier – ENFA, F-31062, Toulouse, Cédex 9, France

<sup>6</sup>Department of Systematic and Evolutionary Botany, University of Zurich, Zollikerstrasse 107, Zurich, 8008, Switzerland

<sup>7</sup>Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL, 60605, USA

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The present study examines the colonization history and phylogeography of the brush-warblers (*Nesillas*), a genus of passerines endemic to islands of the western Indian Ocean (Madagascar, Comoros, and Aldabra Atoll). The phylogeny of all recognized *Nesillas* taxa was reconstructed employing Bayesian phylogenetic methods and divergence times were estimated using a range of substitution rates and clock assumptions. Spatiotemporal patterns of population expansion were inferred and niches of different lineages were compared using ecological niche modelling. Our results indicate that taxa endemic to the Comoros are paraphyletic and that the two endemic species on Madagascar (*Nesillas typica* and *Nesillas lantzii*) are not sister taxa. The brush-warblers started to diversify approximately 1.6 Mya, commencing with the separation of the clade formed by two species endemic to the Comoros (*Nesillas brevicaudata* and *Nesillas mariae*) from the rest of the genus. The lineages leading to the two Malagasy species diverged approximately 0.9 Mya; each with significantly different modern ecological niches and the subject of separate demographic processes. Patterns of diversification and endemism in *Nesillas* were shaped by multiple long distance dispersal events and inter-island colonization, a recurring pattern for different lineages on western Indian Ocean islands. The diversification dynamics observed for *Nesillas* are also consistent with the taxon cycle hypothesis. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 873– 889.

**KEYWORDS:** demographic history – ecological niche modelling – island biogeography – MAXENT – mitochondrial sequence data – phylogenetic constraints – taxon cycle.

\*Corresponding author. E-mail: fuchs@mnhn.fr

## INTRODUCTION

Madagascar is one of the most important regions on Earth for biodiversity, with levels of endemism reaching 100% in native amphibians and terrestrial mammals, 92% in reptiles, and 44% in birds (Vences *et al.*, 2009). The current consensus about the origin of the island's biota is that most endemic lineages arrived by over-water dispersal during the last 65.5 Myr and that relatively few lineages are the result of vicariance associated with the fragmentation of Gondwana, which took place well over 100 Myr (Yoder & Nowak, 2006; Warren *et al.*, 2010; Samonds *et al.*, 2012). Most avian lineages colonized Madagascar, Aldabra Atoll, and the volcanic Comoro Archipelago from Africa (Warren *et al.*, 2003; Marks & Willard, 2005; Pasquet *et al.*, 2007; Fuchs, Johnson & Mindell, 2015b), Indo-Malaya (Warren *et al.*, 2005; Fuchs *et al.*, 2008; Sheldon *et al.*, 2009), and, to a lesser extent, from Australasia (Warren *et al.*, 2013; Cibois *et al.*, 2014). The majority of the colonization events occurred during the last 4 Myr (Warren *et al.*, 2003, 2005; Fuchs *et al.*, 2008, 2015b; Kundu *et al.*, 2012), with few lineages composed of endemic families or subfamilies being older than 20 Myr (Fuchs, Fjeldså & Pasquet, 2006; Jønsson *et al.*, 2012; Reddy *et al.*, 2012).

Although Madagascar exhibits a distinct signature of extensive diversification in five lineages (Mesitornithidae, Bernieridae, Vangidae, Couinae, Brachypteraciidae), it is striking that only four of the other independent avian colonizations of the Indian Ocean islands (*Tachybaptus*, *Nesillas*, *Monticola*, *Foudia*) have given rise to more than one species on Madagascar. Two of these lineages (*Nesillas*, *Foudia*) are endemic to the Malagasy Region (Madagascar and neighbouring archipelagos), where they appear to have radiated with a greater number of taxa on surrounding islands (Comoros, Seychelles, and, in the case of *Foudia*, the Mascarenes) than on Madagascar itself (Warren *et al.*, 2012). Although details remain to be clarified with regard to Malagasy Region birds, such complex patterns of distribution and diversity across the region must relate primarily to the combined effects of biogeographical history and a series of taxon-specific features, such as dispersal ability, niche traits, and competitiveness (Gillespie & Baldwin, 2009).

The origin and patterns of colonization of species found in the Comoros and Aldabra may be explained by immigration events from Madagascar and, to a lesser extent, by direct colonizations from Africa, Indo-Malaya or Australasia. However, colonization patterns across multiple islands such as those found in the Malagasy Region are difficult to unravel because, once the initial successful colonization for a

given organism on an island took place, dispersion between nearby islands may have occurred quickly and potentially randomly across a lineage (Fuchs *et al.*, 2008), possibly as a consequence of the close distance of certain islands (40–80 km in the Comoros Archipelago). The islands making up the Comoros have few known Quaternary fossils (Hume & Middleton, 2011) despite having origins 10–15 Mya (Emerick & Duncan, 1982; Nougier, Cantagrel & Karche, 1986; Michon, 2016). Given the small size of islands in this archipelago (largest being 1150 km<sup>2</sup>), as well as recent human-induced environmental change, extinction may have played an important role in moulding extant avian communities.

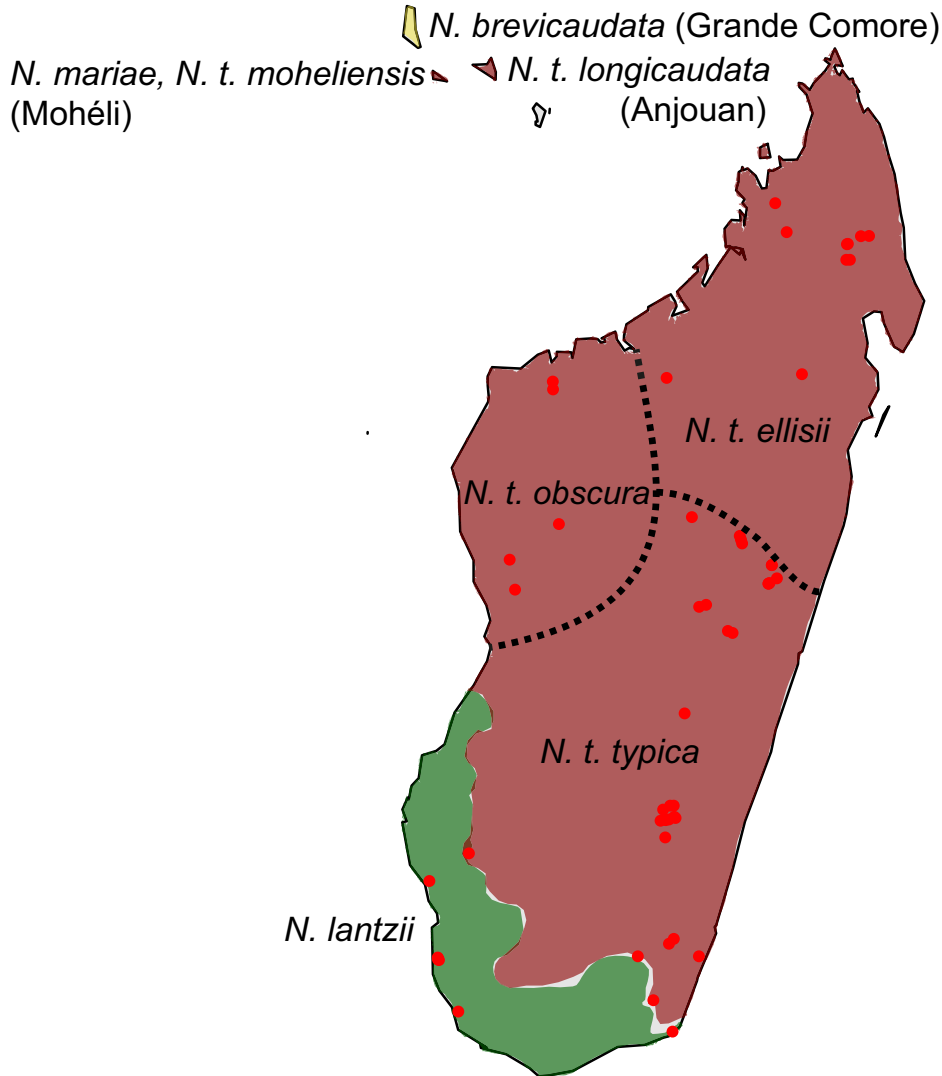
In a striking pattern among the bird lineages that occur on both Madagascar and the Comoros, some taxa are inexplicably absent from some islands in the Comoros (e.g. *Turdus bewsheri* and *Nesillas* absent on Mayotte; *Dicrurus* absent on Mohéli; *Hypsipetes parvirostris*, *Coracina cinerea*, *Cinnyris notatus*, and *Cyanolanius madagascarinus* absent on Mayotte and Anjouan), as well as from the Seychelles and Mascarenes. From a biogeographical perspective, several models have been formulated to explain the spatiotemporal dynamics of taxa on archipelagos that rely upon absence of a given species from certain islands within an island group and differences in occupied habitats among conspecific or congeneric taxa occupying different islands (Ricklefs, 1970, 2010). In this context, the Malagasy Region, with land masses of different geological origins, represents an interesting system for examining the dynamics of inter-island colonization and diversification patterns with respect to shaping the present diversity and the distribution of lineages that occur on Madagascar (ancient), the Comoros (*in situ* volcanic), and Aldabra (*in situ* coral atoll).

The Old World passerine family Acrocephalidae (Fregin *et al.*, 2009, 2012; Arbabi, Gonzalez & Wink, 2014) consists of six genera with remarkable dispersal abilities, having colonized many remote oceanic islands (Cibois, Thibault & Pasquet, 2007). One of these genera, *Nesillas* (brush-warblers), consists of five species endemic to the Malagasy Region, including Madagascar (*Nesillas typica typica* and *Nesillas lantzii*), the Comoros Archipelago (*Nesillas mariae* and *Nesillas typica moheliensis* on Mohéli, *Nesillas brevicaudata* on Grande Comore, *Nesillas typica longicaudata* on Anjouan), and the recently extinct *Nesillas aldabrana* on Aldabra Atoll (Fig. 1). All five species have or had allopatric or parapatric distributions, making *Nesillas* an ideal study system by which to understand the mechanisms underlying diversification at the scale of Madagascar and the surrounding islands. *Nesillas* spp. can usually be

found in areas of intact native forest vegetation, scrub, and thickets. On Madagascar and Anjouan, *N. typica* also occurs in heavily degraded non-native

or mixed vegetation, such as in villages and city gardens, attesting to the behavioural flexibility in this genus (Safford & Hawkins, 2013).

➤ *N. aldabrana* (Aldabra)



**Figure 1.** Distribution of *Nesillas* species (dots indicate sampling localities) based on BirdLife International and NatureServe (2013) and subspecies from Kennerley *et al.* (2010). According to recent taxonomic treatments (Kennerley *et al.*, 2010), *Nesillas typica* is polytypic and includes five subspecies: *Nesillas typica typica*, *Nesillas typica ellisii*, and *Nesillas typica obscura* on Madagascar; *Nesillas typica moheliensis* on Mohéli; and *Nesillas typica longicaudata* on Anjouan. The separation of *N. t. ellisii* from *N. t. typica* is not recognized in all studies (Alström *et al.*, 2006; Dickinson & Christidis, 2014). The two species on Madagascar, which differ in plumage coloration and call (Kennerley *et al.*, 2010; Safford & Hawkins, 2013), have parapatric distributions (Goodman & Raherilalao, 2013) and no evidence of interbreeding is known in the zone of overlap. *Nesillas lantzii* only occurs in the southern part of Madagascar, whereas *N. typica* is widely distributed over much of the rest of the island. Two taxa, *N. t. moheliensis* and *N. mariae*, co-occur on Mohéli. Maps were made using the R (R CoreTeam, 2013) libraries *maps* and *mapdata* (Becker & Wilks, 2013), *maptools* (Bivand & Lewin-Koh, 2014), and *scales* (Wickham, 2014).

According to the recent taxonomic treatment of Kennerley, Pearson & Small (2010), *N. typica* is polytypic and includes five subspecies: *Nesillas typica typica*, *Nesillas typica ellisii*, and *Nesillas typica obscura* on Madagascar; *N. t. moheliensis* on Mohéli; and *N. t. longicaudata* on Anjouan. Certain studies (e.g. Alström *et al.*, 2006; Dickinson & Christidis, 2014) do not recognize the separation of *N. t. ellisii* from *N. t. typica*. The two Malagasy taxa, *N. typica* and *N. lantzii*, differ in plumage coloration and call (Kennerley *et al.*, 2010; Safford & Hawkins, 2013). *Nesillas lantzii* is restricted to the south, whereas *N. typica* is widely distributed in the central and northern portions of the island, with no known interbreeding in the zone of overlap (Goodman & Raherilalao, 2013). Two taxa, *N. t. moheliensis* and *N. mariae*, occur on Mohéli. Their phenotypic affinities to other taxa and corresponding taxonomic placements suggest that this island was colonized twice (Louette *et al.*, 1988). *Nesillas* is absent from Mayotte, the eastern-most island of the Comoros and geographically the closest to Madagascar. On Aldabra, *N. aldabrana* was always extremely rare and now appears to be extinct (Prÿs-Jones, 1979; Safford & Hawkins, 2013).

In the present study, we use mitochondrial sequence data from all known taxa, together with a combination of population genetic and phylogenetic approaches, to clarify the diversification history of *Nesillas* in the Malagasy Region. We aim to: (1) infer the number and possible direction of colonization events between Madagascar and surrounding islands; (2) investigate the phylogeographical structure of the two Madagascar *Nesillas* spp., which have large distributions relative to the taxa on neighbouring islands, and determine whether environmental suitability could explain the parapatric distribution of the Madagascar species; and (3) examine the relative importance of ecological niche and phylogenetic constraints on colonization history by testing if more closely-related lineages tend to have similar ecological envelopes compared to distant taxa. We discuss our results in light of the taxon cycle theory aiming to explain the current distributions of island species for which there are few convincing examples (but see Ricklefs & Bermingham, 2002; Jönsson *et al.*, 2014).

## MATERIAL AND METHODS

### TAXONOMIC SAMPLING

We obtained genetic samples for 122 individuals of *Nesillas*, representing all extant taxa and the recently extinct *N. aldabrana* (Fig. 1; see Supporting information, Table S1). As outgroups, we used

sequences from *Sylvia atricapilla*, *Acrocephalus scirpaceus*, and *Phragmaticola aedon*. All the material used in the present study originates from pre-existing museum collections and no animal was collected specifically for this study.

### LABORATORY PROTOCOLS

DNA was extracted from tissue or blood using the Qiagen extraction kit (Qiagen) in accordance with the manufacturer's instructions, and from toe pad samples as described by Warren *et al.* (2005). We sequenced a fragment of the mitochondrial genome corresponding to the loci tRNA<sub>Met</sub> and ND2 using primers L5140 (5'-CTRGTGATAAAGTGRRCAAGAGG-3'; present study) and H6313 (5'-CTCTTATT-TAAGGCTTTGAAGGC-3'; Johnson & Sorenson, 1998). The polymerase chain reaction (PCR) amplification protocol included an initial denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 75 s and was terminated by a final elongation at 72 °C for 15 min. Sequences from toe pads of the *N. aldabrana* type specimens were obtained by performing several overlapping PCR amplifications (size 200–350 bp) using *Nesillas*-specific primers designed in the present study. Newly generated sequences have been deposited in Genbank (accession numbers KX133722–KX133843).

### PHYLOGENETIC RECONSTRUCTION AND MITOCHONDRIAL (MT)DNA DIVERGENCE TIMES

Gene tree reconstructions of haplotypes were performed using Bayesian inference and maximum likelihood, as implemented in MrBayes, version 3.2 (Ronquist *et al.*, 2012) and RAxML (Stamatakis, 2014), respectively, on the CIPRES 3.1 gateway server (<https://www.phylo.org/>; Miller, Pfeiffer & Schwartz, 2010). We used the *nst = mixed* option such that model uncertainty was taken into account during the phylogenetic reconstruction, and incorporated rate variation using the *gamma* setting. Four independent analyses consisting of four Metropolis-coupled Markov chain Monte Carlo (MCMC) chains (one cold and three heated) were run for  $20 \times 10^6$  iterations, with trees sampled every 1000 iterations. We tried several prior distributions for the branch-length parameters (exp: 10 to exp: 500).

We estimated the time to most recent common ancestor among the *Nesillas* haplotypes using BEAST, version 1.8 (Drummond & Rambaut, 2007). We performed analyses with the strict and uncorrelated lognormal molecular clock models (Drummond *et al.*, 2006). The substitution models for the two loci (ND2 and tRNA<sub>Met</sub>) were selected using TOPALI



(Milne *et al.*, 2009) under the Bayesian information criterion. We implemented the Trn + I (ND2) and JC (tRNA<sub>Met</sub>) substitution models and used a Yule tree prior. MCMC chains were run for  $5 \times 10^7$  steps and were sampled every  $10^3$  steps. Inferring within species divergence times is challenging because internal fossil calibration is seldom possible. To circumvent this problem, we used two substitution rates and their associated uncertainties to calibrate the trees. Lerner *et al.* (2011), employing complete mtDNA genomes from the honeycreepers (Passeriformes, Drepanididae) and calibration points based on the age of volcanic islands in the Hawaiian Archipelago, proposed a new substitution rate for ND2 [0.029 s/s/l/Myr; 95% highest posterior density (HPD): 0.024–0.034 s/s/l/Myr]. Subramanian *et al.* (2009) suggested that time dependency phenomena could primarily be attributed to nonsynonymous substitutions. They estimated the rate of evolution at four-fold degenerated sites from complete mtDNA sequences of *Pygoscelis adeliae* (a species of penguin) to be 0.073 (95% HPD: 0.025–0.123 s/s/l/Myr); we also made use of this rate to estimate divergence times among *Nesillas*.

We compared the obtained divergence times based on the molecular rates with those derived from a biogeographical calibration, corresponding to the split between *N. aldabrana* and its sister-group dating back to no more than 0.125 Myr. This date corresponds to the oldest age that would have been feasible for the colonization of Aldabra Atoll by the *N. aldabrana* lineage. The Aldabra Atoll was apparently inundated until 0.125 Mya (Thomson & Walton, 1972), an event that probably eliminated all terrestrial biota. All other islands from the Aldabra group (Assumption, Cosmoledo and Astove) are estimated to have emerged within the last 15 000 years (Radtkey, 1996).

We used TRACER, version 1.5 (Rambaut & Drummond, 2007) to ensure that our effective sample size of the underlying posterior distribution was sufficiently large ( $> 100$ ) for a meaningful estimation of parameters.

#### POPULATION GENETIC ANALYSES AND DEMOGRAPHIC HISTORY

To determine whether demographic histories within *Nesillas* are comparable, we used the McDonald–Kreitman test (MK test; McDonald & Kreitman, 1991) in DNASP, version 5.0 (Librado & Rozas, 2009) to examine evidence of selection on ND2. Significance was assessed using Fischer's exact test and a threshold of 0.05.

Haplotype diversity ( $H_D$ ), nucleotide diversity ( $\pi$ ) and Watterson's theta ( $\theta$ ) were estimated with

DNASP for each subspecies or clade recovered in our Bayesian inference topology. We employed Fu's  $F_S$  test (1000 replicates) and the Ramos-Onsins and Rozas  $R_2$  statistic (Ramos-Onsins & Rozas, 2002) to detect signatures of demographic change. We used TCS, version 1.21 (Clement, Posada & Crandall, 2000) to reconstruct a 95% statistical parsimony network.

We generated extended Bayesian skyline plots (Drummond *et al.*, 2005) to reconstruct the demographic history of *N. typica* and *N. lantzii* on Madagascar; these graphs plot effective population size through time, providing a temporal reference to demographic events such as bottlenecks and expansions. We ran three replicate analyses, each  $1 \times 10^8$  iterations long, as implemented in BEAST, version 1.8 (Drummond & Rambaut, 2007). We used the rate proposed by Lerner *et al.* (2011) for ND2 (0.029 s/s/l/Myr; 95% HPD: 0.024–0.034 s/s/l/Myr). We assessed convergence primarily by comparing the resulting replicate plots, making sure each replicate produced the same demographic pattern.

#### SPATIAL DYNAMICS

To reconstruct spatial dynamics in the Madagascar populations of *N. typica*, the species with the largest distribution range within the genus and occupying most of the island, we considered phylogenetic diffusion models in continuous space (Lemey *et al.*, 2010; Pybus *et al.*, 2012), as implemented in BEAST, version 1.8 (Drummond & Rambaut, 2007). We used the rate proposed by Lerner *et al.* (2011) for ND2 (0.029 s/s/l/Myr; 95% HPD: 0.024–0.034 s/s/l/Myr) and a HKY+G substitution model. We assessed convergence primarily by comparing the resulting replicate plots, making sure each replicate produced the same demographic pattern. Summaries of the spatial spread through time were obtained using SPREAD (Bielejec *et al.*, 2011) and visualized in Google Earth (<http://earth.google.com>). We used the CIPRES 3.1 gateway server ([www.cipres.org](http://www.cipres.org)) to run all BEAST analyses.

#### NICHE MODELLING

To determine whether environmental suitability could explain the parapatric distribution of Madagascar *Nesillas* spp., we used ecological niche modelling to derive expectations of the geographical and environmental distribution of the two lineages. Two of the investigators in the present study (MJR and SMG) compiled and curated a specimen and observation database for all occurrences of *Nesillas* on Madagascar (102 records for *N. lantzii* and 388 for *N. typica*). We used a maximum entropy (MAXENT)

algorithm (Phillips, Anderson & Schapire, 2006) to relate the environments associated with occurrence data to environments across the study area, defined as the polygon delimited by the coordinates: 42.6, 52, -26.9, -8.1 ( $x_{\min}$ ,  $x_{\max}$ ,  $y_{\min}$ ,  $y_{\max}$ ) that included surrounding islands of interest (see Supporting information, Fig. S1). MAXENT is a presence only algorithm that uses occurrence coordinates as well as different bioclimatic layers to estimate the use and availability of environments (Phillips *et al.*, 2006). The MAXENT algorithm assumes that occurrences were sampled in a similar fashion to the background points (i.e. at random within the study area). Because this assumption is most likely incorrect, we decided to include a bias map indicating the areas with most access (e.g. close to roads) or where most sampling effort occurred on Madagascar. We quantified sampling effort by counting the number of years in which there was a bird observation made or a specimen collected on Madagascar. The data were downloaded for all bird species from the GBIF portal (39748 records in total) and rasterized to the same extent and resolution as the climate data (study area and 30 s resolution). The value given to each pixel was the number of unique years for which there was at least a single record. We used this effort map to indicate the relative probability of sampling a pixel to generate the background sample.

Members of the Acrocephalidae, which includes *Nesillas*, are recognized for their considerable dispersal capacity and are found in a wide range of habitats; thus, we had no a priori information to choose particular habitats in our analyses. We used the 19 bioclimatic variables available at Worldclim ([www.worldclim.org](http://www.worldclim.org); Hijmans *et al.*, 2005) that summarize climatic mean and extreme tendencies for temperature and precipitation available during the period 1950–2000. Because the occurrence and background data were aggregated, we removed environmental bias by filtering out occurrences that were closer than 1 °C and < 100 mm in annual precipitation (Boria *et al.*, 2014). This left us with 29 occurrence points for *N. lantzii*, 139 for *N. typica*, and 273 background points, which formed the MAXENT input data (see Supporting information Fig. S1) for the study area. Model performance was evaluated with the area under the receiving operator curve (AUC) and comparing the density of predicted values for the background vs. the presence points.

After evaluating the performance of the current niche model, it was used to predict the potential geographical distribution of the two species under past climates during the mid-Holocene (~6000 years ago), Last Glacial Maximum (~21 000 years ago) and the Last Interglacial (~130 000 years ago). The climate layers for these periods were obtained from the

Worldclim website. To evaluate the dynamics of the potential geographical distribution from the perspective of available climates and under the assumption of niche constancy, we converted the continuous output of the MAXENT model to a binary (presence–absence) map applying the minimum training presence criterion threshold. We measured the area of presence during the four periods (three past and present) after projecting each map to the Madagascar Laborde Tan1925 projection.

To quantify niche similarity between the two species, we used the method proposed by Broennimann *et al.* (2012) to compare niches in environmental space. To do this, we employed the same set of occurrence and background points and included all pixels on Madagascar and the surrounding islands (Aldabra, Grande Comore, Mohéli, and Anjouan). This method estimates an occurrence density surface in environmental space, considering the occupancy and availability of environments. These surfaces can then be compared using the same methods developed by Warren, Glor & Turelli (2008) to evaluate the hypothesis of niche equivalency (i.e. are two niches identical?) and the hypothesis of niche similarity (are two niches more or less similar than expected under a null model of random distribution of points?). We report the results of each test and show the estimated occurrence density surfaces in environmental space.

## RESULTS

### PHYLOGENETIC RELATIONSHIPS AND HAPLOTYPE NETWORKS

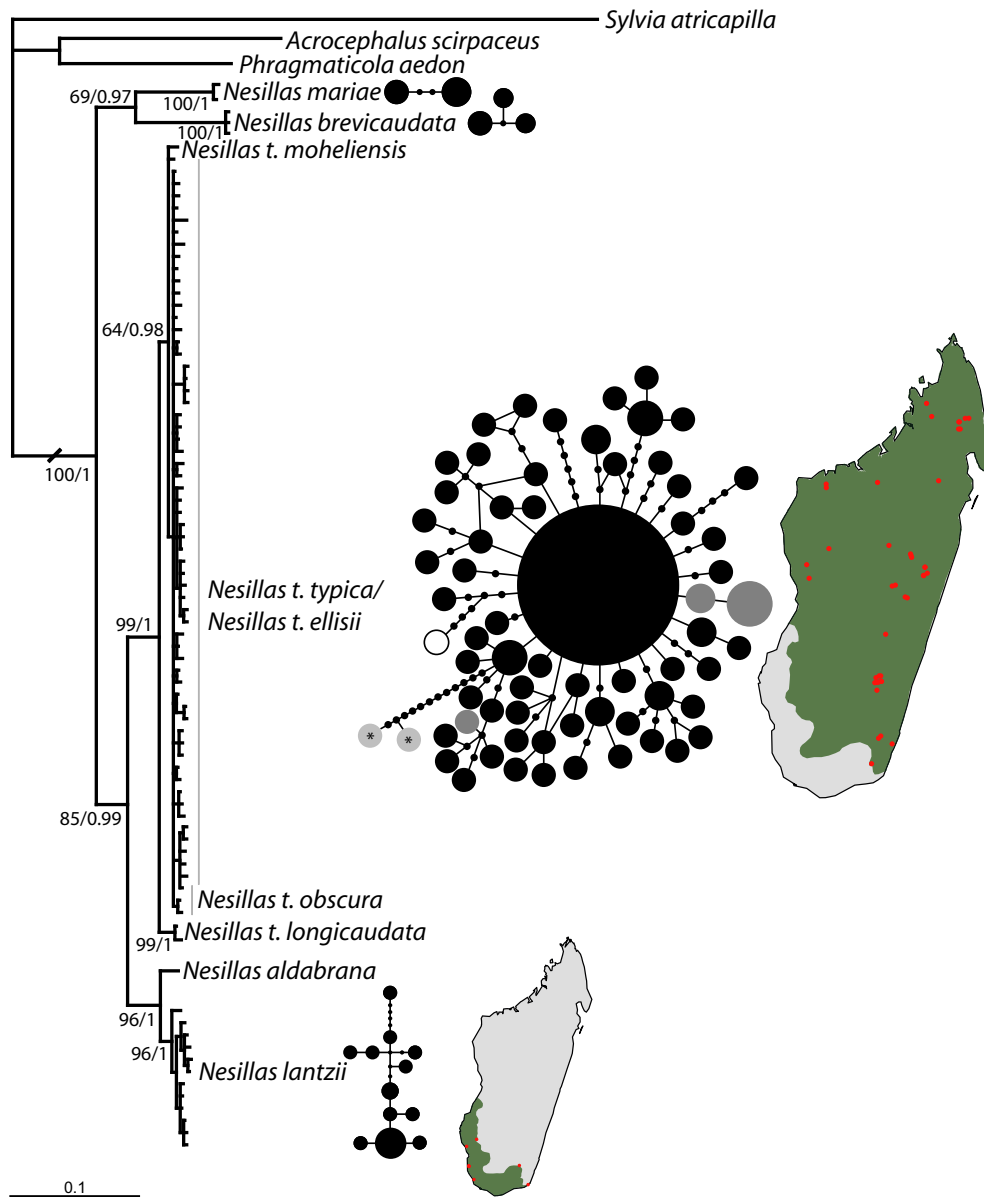
We analyzed 1088 bp of mitochondrial DNA (47 bp of tRNA<sub>Met</sub>, 1041 bp of ND2) for the 122 individual *Nesillas* collected across the range of the genus. The 50% majority-rule consensus tree from the Bayesian inference and the maximum likelihood topology of the mitochondrial haplotypes are identical with respect to relationships among taxa, and differ only with respect to haplotypes within subspecies (Fig. 2). A first lineage consists of two species that are endemic to the Comoros, *N. mariae* (Mohéli) and *N. brevicaudata* (Grande Comore) [posterior probability (PP) = 0.97]. This lineage is sister to the clade that includes all *N. typica* subpp., *N. lantzii*, and the extinct *N. aldabrana* (PP = 0.99), with the latter being sister to *N. lantzii* (PP = 1). Within the monophyletic *N. typica* (PP = 1), the subspecies *longicaudata* (Anjouan) is sister to all other taxa (PP = 0.97), whereas the other Comorian subspecies *moheliensis* from Mohéli is nested within the Madagascar *N. typica* haplotypes. Hence, the two Comorian *N. typica* subspecies are not each other's closest relatives.

Genetic structure within the two Madagascar species (*N. typica* and *N. lantzii*) is very limited, as inferred from the star-like shape of the haplotype network (Fig. 2).

rates vs. calibration of island history, strict clock evolution or not) (Table 1). Indeed, the analyses performed under a strict molecular model and the ND2 or the four-fold rate indicated that *Nesillas* began to diversify approximately 1.6 Mya (ND2 rate 95% HPD: 1.2–2.0; four-fold: 95% HPD: 0.7–2.8). The divergence between *N. lantzii* and *N. typica* occurred approximately 0.9 Mya (ND2 rate 95% HPD: 0.6–1.1; four-fold: 95% HPD: 0.3–1.3) and the colonization of

MTDNA DIVERGENCE TIMES

The divergence time analyses yielded notably different results depending on the assumptions (molecular



**Figure 2.** The majority rule (50%) consensus tree resulting from the Bayesian analyses of the unique haplotypes for all recognized forms of *Nesillas*. Numbers close to nodes refer to bootstrap values and posterior probabilities for nodes involving relationships among taxa. Maps derived as in Fig. 1. The minimum spanning networks indicate the relationships among the haplotypes obtained using TCS (Clement *et al.*, 2000), with circle size proportional to the number of individuals. Colour codes for the *Nesillas typica* sub-network are black (*Nesillas typica typica*/*Nesillas typica ellisii*), grey (*Nesillas typica obscura*), light grey with asterisk (*Nesillas typica longicaudata*), and white (*Nesillas typica moheliensis*).

**Table 1.** Divergence time estimates within different clades of the genus *Nesillas*

Model	ND2 clock Lerner	ND2 uncorrelated Lerner	Four-fold clock	ND2 clock Aldabra	ND2 uncorrelated Aldabra
<i>Nesillas</i>	1.6 (1.2–2.0)	0.9 (0.6–1.3)	1.6 (0.7–2.8)	0.6 (0.4–0.8)	0.26 (0.14–0.40)
<i>Nesillas brevicaudata</i> / <i>Nesillas mariae</i>	1.3 (0.9–1.7)	0.5 (0.2–0.9)	1.3 (0.5–2.2)	0.5 (0.3–0.7)	0.16 (0.05–0.3)
<i>Nesillas lantzii</i> / <i>Nesillas typica</i>	0.9 (0.6–1.1)	0.7 (0.4–1.0)	0.8 (0.3–1.3)	0.3 (0.2–0.4)	0.21 (0.1–0.3)
<i>Nesillas lantzii</i> / <i>Nesillas aldabranus</i>	0.3 (0.2–0.5)	0.4 (0.2–0.6)	0.4 (0.15–0.75)	<b>0.122 (0.10–0.14)</b>	<b>0.122 (0.10–0.14)</b>
<i>Nesillas lantzii</i>	0.2 (0.1–0.3)	0.25 (0.1–0.4)	0.25 (0.1–0.4)	0.1 (0.03–0.15)	0.08 (0.04–0.12)
<i>Nesillas typica typica</i> / <i>Nesillas typica</i> <i>longicaudata</i>	0.4 (0.2–0.5)	0.5 (0.3–0.7)	NA	0.12 (0.07–0.2)	0.14 (0.07–0.22)
<i>Nesillas typica</i>	0.2 (0.15–0.3)	0.37 (0.2–0.5)	0.4 (0.2–0.7)	0.1 (0.05–0.12)	0.11 (0.05–0.17)

Values shown in bold represent the biogeographical calibration point. NA, not available.

Aldabra approximately 0.3 Mya (ND2 rate 95% HPD: 0.2–0.5; four-fold: 95% HPD: 0.1–0.4). The analyses utilizing the biogeographical calibration point (inundation of the Aldabra Atoll) yielded estimates that are approximately four times more recent (e.g. divergence *N. lantzii*/*N. typica* 0.3 Mya; 95% HPD: 0.2–0.4). The estimate for the coefficient of variation was very high (> 1.5), indicating substantial rate variation. The estimates from the two independent molecular rates were very similar and we consider them as the best estimates. However, we highlight that the divergence times obtained using calibrated island history would not alter the hypotheses regarding the biogeographical patterns discussed.

#### POPULATION GENETICS AND DEMOGRAPHIC HISTORY

The MK test was significant at the 0.05 threshold when we specified non-*Nesillas* outgroups (*S. atricapilla*,  $P < 0.0001$ ; *A. scirpaceus*,  $P = 0.005$ ; *P. aedon*,  $P = 0.0025$ ), whereas it was nonsignificant when either *N. mariae* ( $P = 0.45$ ) or *N. brevicaudata* ( $P = 0.36$ ) were used as outgroups of the Madagascar clade (i.e. including the node representing the last common ancestor of *N. lantzii* and *N. typica*). Because the level of significance increased with phylogenetic distance, this result suggests that homoplasy of synonymous substitutions may explain the significant  $P$ -value of the MK test with non-*Nesillas* outgroups. The nonsignificant  $P$ -values for the within *Nesillas* tests suggest that the different mitochondrial lineages are not under different selective constraints and that their demographic history can be compared.

Population genetics summary statistics ( $H_D$ , Watterson's  $\theta$  and  $\pi$ ) indicate that Malagasy *N. typica* has more mitochondrial genetic diversity than *N. lantzii* (see Supporting information, Table S2). The forms found on the Comoros had lower genetic diversity than the two Madagascar taxa, both with distinctly larger distributions, although the limited number of tissue specimens for the Comoros taxa does not allow us to definitely rule out sample size as an explanation for this difference.

Population size change was detected for *N. typica* based on all three methods because both the summary statistics (Fu's  $F_S$  and  $R_2$ ) and the extended Bayesian skyline plot (*demographic.populationSizeChanges* = 1.69 with 0 being excluded) supported an increase in population size, although the magnitude was limited (see Supporting information, Fig. 2). For *N. lantzii*, support for population expansion was less apparent; only Fu's  $F_S$  was significantly negative and the posterior distribution of the *demographic.populationSizeChanges* included 0 (mean = 0.909; highest posterior probability was for  $k = 1$ ; see Supporting information, Fig. S2).

#### SPATIAL DYNAMICS

Reconstruction of the dispersal pattern using the continuous phylogeographical model for the *N. typica* populations on Madagascar indicated continuous population expansion in the east, starting in the northern central zone and then spreading in easterly and southerly directions. The *N. typica* populations in central-north-western Madagascar (*N. t. obscura*) were derived from a first jump dispersal event consisting of two distinct haplotypes (FMNH 429325,



429326, 431268, 431269, BW-410), followed by a second independent dispersal (FMNH 395986) (see Supporting information, Fig. S3).

NICHE MODELLING

The model performances for the two Malagasy *Nesillas* spp. were different: *N. lantzii* had an excellent performance with AUC values above 0.9, whereas, for *N. typica*, the AUC was moderate (0.8). The predicted MAXENT values for the occurrences of each species in relation to the background samples were notably different (see Supporting information, Fig. S4) suggesting a preference for particular environments available within Madagascar. *Nesillas lantzii* occurrences were associated mostly with warm temperatures (> 20°C) and low annual precipitation (< 1000 mm) and were in dry environments, whereas *N. typica* occurrences were associated with a wider range of temperatures (11–27°C) and higher annual precipitation (> 1000 mm) and were in more mesic areas. These strict delimitations show some variation, best exemplified by the passage of *N. lantzii* from the dry western slopes of the Anosyenne Mountains to the more mesic eastern slopes in the vicinity of Tolagnaro (FMNH 352925–352926), indicating some habitat adaptability in a zone of rapid ecological transition (Goodman *et al.*, 1997).

Models projected back into four different periods of the Quaternary showed similar patterns of geographical distribution to the current one with an expansion of *N. lantzii* during the mid-Holocene, whereas

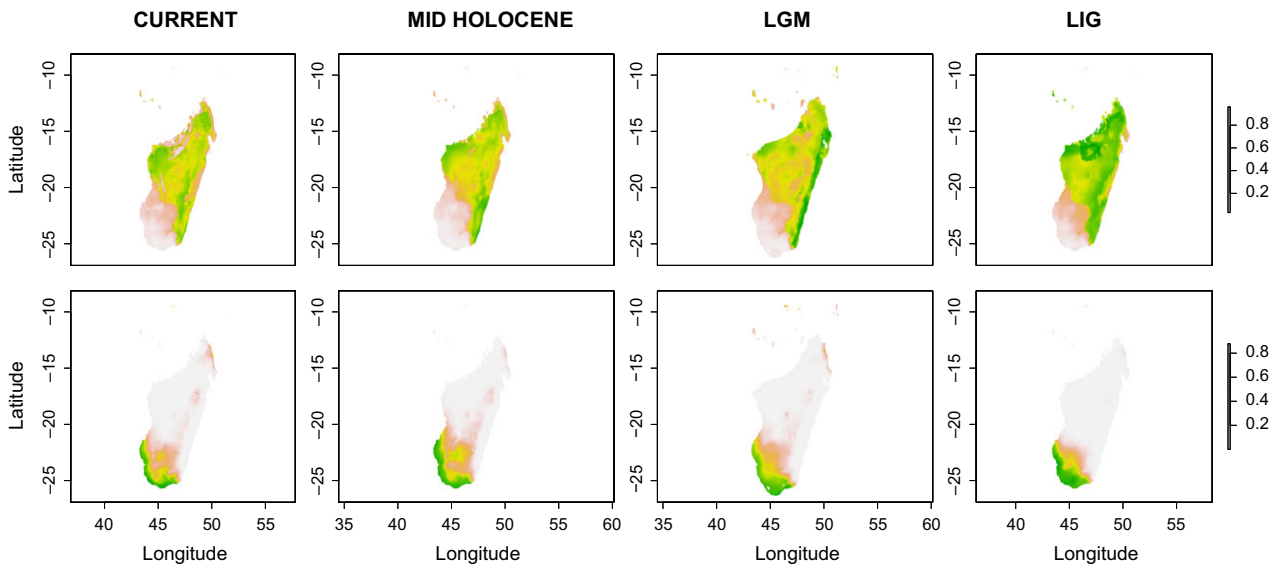
there was an expansion of *N. typica* during the Last Glacial Maximum (Fig. 3). Nevertheless, shifts in geographical distribution appear to be mostly driven by sea level changes and the associated coastline shape. For example, during the LGM, considerable lowland areas were exposed with suitable conditions, which were occupied by these species (Table 2).

The first two components of the principal component analysis performed for the niche comparison accounted for 75% of the total environmental variation within the study area. The niches of the two species were not identical ( $P = 0.01$ ) and the metric of similarity was close to 0 ( $D = 0.144$ ) indicating that the environments used by these two species are significantly different (see Supporting information, Fig. S5A, B). However, it was not possible to reject the hypothesis that the niches of the two species

**Table 2.** Changes in area (km<sup>2</sup>) occupied by *Nesillas typica* and *Nesillas lantzii* during four different periods over the last 130 000 years

	<i>Nesillas typica</i>	<i>Nesillas lantzii</i>
Current	428956.3	102168.7
Mid-Holocene	437959.5	144120.9
Last Glacial Maximum	564237.4	130448.4
Last Interglacial Maximum	520790.4	99022.6

Area is derived based on binary maps generated by applying the minimum training presence threshold.



**Figure 3.** Potential geographical distribution of *Nesillas typica* (upper row) and *Nesillas lantzii* (lower row) for each of four periods: current, mid-Holocene (~6000 years ago), Last Glacial Maximum (~21 000 years ago), and last interglacial (~130 000 years ago). Colours indicate relative probability of occurrence (grey, low; green, high).

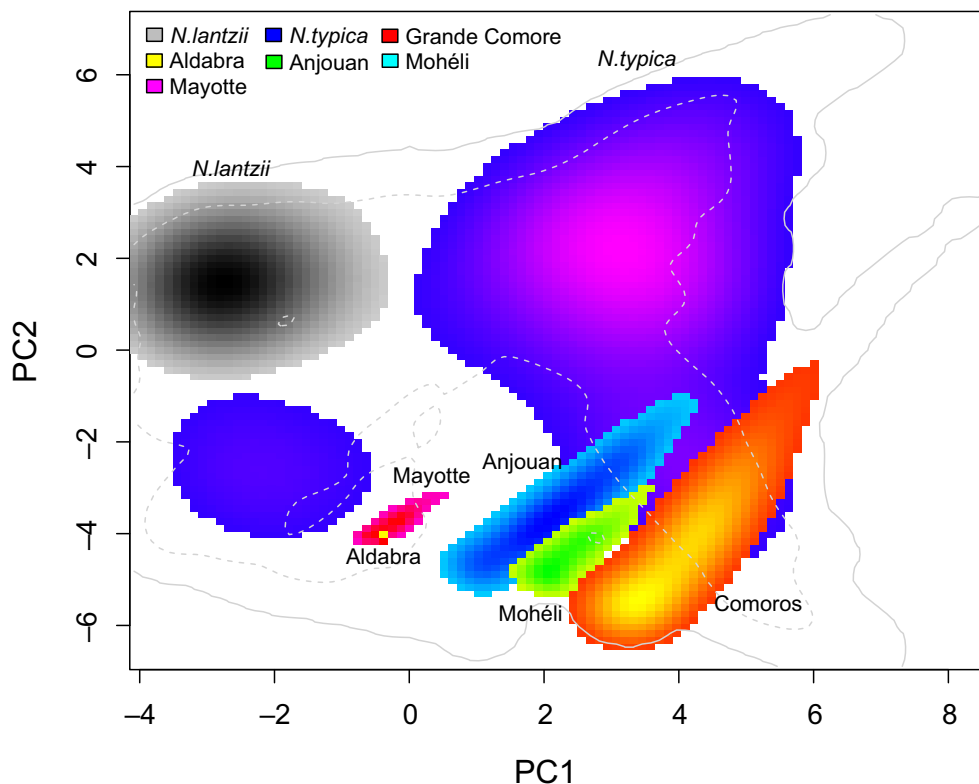
were more different than expected under a null model where the centre of occurrence density is randomly allocated within the environmental space available in the study area. Indeed, when fixing the points of *N. typica* and randomly allocating the centre of the occurrence density of *N. lantzii* in the environmental space available on Madagascar, the niches are more similar than expected under the null model ( $P = 0.03$ ; see Supporting information, Fig. S5C). Finally, it was evident that the environments available on the surrounding islands are marginal or unique with respect to those on Madagascar (Fig. 4).

## DISCUSSION

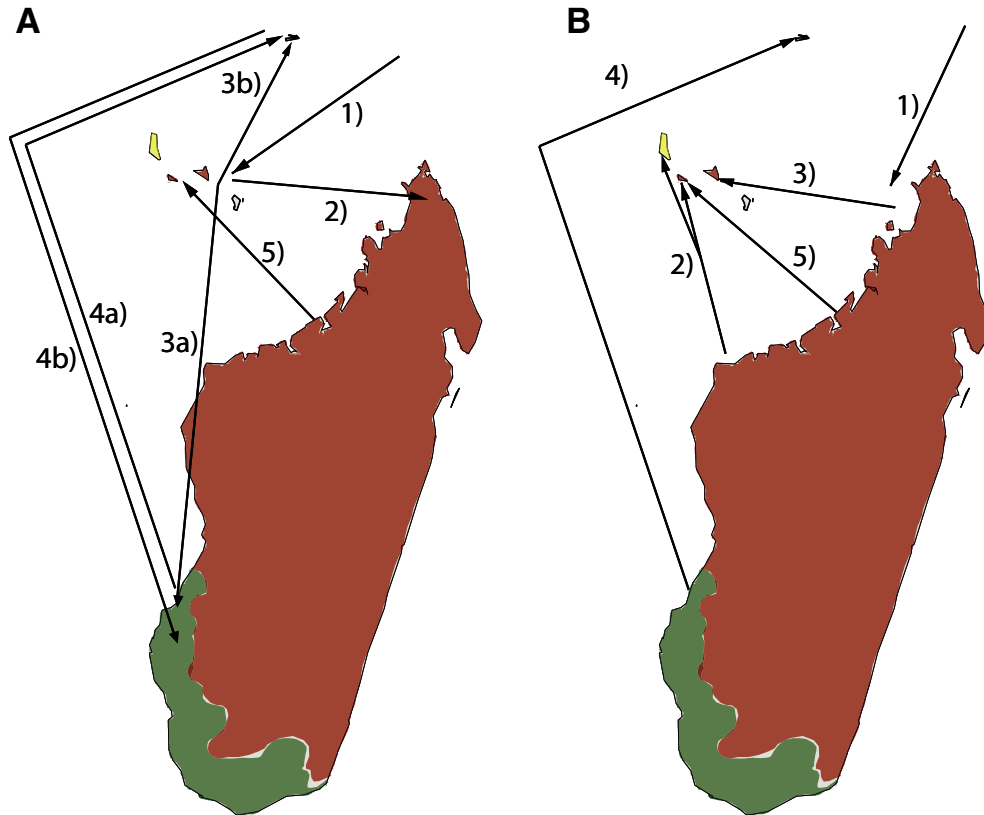
### MULTIPLE *NESILLAS* COLONIZATION EVENTS BETWEEN MADAGASCAR AND SURROUNDING ARCHIPELAGOS

Our phylogenetic analyses yielded unexpected results associated with both the relationships among recognized *Nesillas* taxa and their patterns of genetic differentiation. Divergence of this genus from the

rest of the Acrocephalidae represents the deepest split within a family well known for its dispersal ability (Cibois *et al.*, 2007). Our analyses reveal that the deepest split within *Nesillas* separates the sister species *N. mariae* (Mohéli) and *N. brevicaudata* (Grande Comore) from the rest of the genus. The second primary lineage includes *N. typica* and the sister species *N. lantzii*/*N. aldabrana*. All *N. typica* subspecies cluster together and within this clade, the deepest split separates the form *longicaudata* (Anjouan) from all other subspecies. The other subspecies occurring in the Comoros (*N. t. moheliensis*) appears to be nested within or sister to the Madagascar *N. typica* haplotypes. Hence, the overall mitochondrial topology recovers the Comorian taxa as paraphyletic. This result is consistent with an initial colonization of Madagascar by *Nesillas* from the Comoro Archipelago, followed by multiple dispersal events between Madagascar, Aldabra Atoll and back to the Comoros (Fig. 5A) (but see Kirkendale & Meyer, 2004). As for the genera *Foudia* (for which nuclear data are provided in Warren *et al.*, 2012) and *Coracopsis* (Kundu *et al.*, 2012), it is possible



**Figure 4.** Occurrence of *Nesillas lantzii* and *Nesillas typica* in environmental space. Colours indicate the occurrence densities (uncorrected for environmental availability) of each lineage and the environments available on five adjacent islands: Grande Comoro, Mohéli, Anjouan, Mayotte, and Aldabra. Gray contour lines indicate all and 50% of the environmental space available in the study area. PC, principal component.



**Figure 5.** Summary of the two primary hypotheses regarding the colonization history of the western Indian Ocean islands by the members of the genus *Nesillas*. A, biogeographical history implying: (1) initial colonization of Comoros Archipelago followed by a first speciation event giving rise to the taxa *Nesillas mariae* (Mohéli) and *Nesillas brevicaudata* (Grande Comore); (2) a colonization of Madagascar giving rise to *Nesillas typica* and (3a) a colonization of Madagascar giving rise to *Nesillas lantzii* followed by a (4a) dispersal to Aldabra giving rise to *Nesillas aldabrana*. The alternative scenario with a first colonization of Aldabra giving rise to the taxon *N. aldabrana* (3b) followed by (4b) the colonization of Madagascar by the ancestor of *N. lantzii* is also shown. The last dispersal (5) from Madagascar gave rise to *Nesillas typica moheliensis*. The number of dispersal events between the Comoros Archipelago, Aldabra, and Madagascar (that is not considered within Comoros dispersal) is four. B, biogeographical history implying: (1) initial colonization of Madagascar followed by (2) a first dispersal event to the Comoros (giving rise to the taxa *N. mariae*, Mohéli and *N. brevicaudata*, Grande Comore), an *in situ* speciation event on Madagascar giving rise to *N. lantzii* and *N. typica*, followed by three independent colonizations of the Comoros and Aldabra giving rise to *Nesillas typica longicaudata* (3), *N. aldabrana* (4), and *N. t. moheliensis* (5). The number of dispersal events between the Comoros Archipelago, Aldabra and Madagascar (that is not considering within Comoros dispersal) is four. In both (A) and (B), the orientation of the arrow (1) is not intended to indicate the geographical source from which the western Indian Ocean islands were originally colonized. On Madagascar, the distribution of *N. typica* is shown in reddish-brown and that of *N. lantzii* is shown in green.

that Madagascar could have been successfully colonized independently and at approximately the same time by two closely-related lineages. However, the alternative scenario of an initial colonization of the Comoros from Madagascar, which lead to the sister species *N. mariae* (Mohéli) and *N. brevicaudata* (Grande Comore), followed by two independent colonizations of Anjouan and Mohéli from Madagascar by *N. typica* and one colonization of Aldabra from Madagascar by *N. lantzii* remains plausible (Fig. 5B) (Emerson, 2002; Kirkendale & Meyer, 2004).

The current knowledge on the biogeography of certain volant vertebrates in the Malagasy Region largely includes double colonization of Madagascar by closely-related lineages of independent continental origin (i.e. Nectariniidae, Warren *et al.*, 2003) or a single colonization of Madagascar (*Otus*, Fuchs *et al.*, 2007, 2008; *Dicrurus*, Pasquet *et al.*, 2007; Fuchs *et al.*, 2013) or multiple exchanges between Madagascar and the Comoros (*Miniopterus* bats, Weyeneth *et al.*, 2008). Taking a broader view of long-range dispersal amongst birds, the colonization of either

mainland or larger areas from islands has received much attention in recent years (Bellemain & Ricklefs, 2008), with the likely colonization of South and Central America from the West Indies (*Coereba*, Bellemain, Bermingham & Ricklefs, 2008), colonization of Australia from Pacific islands (*Monarcha*, Filardi & Moyle, 2005), and colonization of Indo-Malaya by Bornean (*Alophoixus*, Fuchs *et al.*, 2015a) or by western Indian Ocean lineages (*Otus*, Fuchs *et al.*, 2008). Arbabi *et al.* (2014), using the same ND2 rate as ourselves (0.029 s/s/Myr, Lerner *et al.*, 2011), as well as sequences from two other mitochondrial genes, estimated that the split between *Nesillas* and other Acrocephalidae occurred approximately 14 Mya (95% CI: 12–16.3). On the basis of the dataset generated in the present study, this split could have occurred more recently (4.1 Mya, 95% HPD 3.1–5.0; ND2 rate, strict clock) and these significant estimate differences are not related to sampling (i.e. phylogeny vs. phylogeography). Indeed, we performed a complementary set of analyses (using ND2) with one individual per Acrocephalidae species sampled by Arbabi *et al.* (2014), as well as the *Nesillas* taxa included here, and found that the basal divergence within the Acrocephalidae occurred approximately 4.7 Mya (95% HPD: 3.5–5.9 Myr). Hence, it appears that the differences might be related to either locus specific effects or the misspecification of the substitution rates in the analyses. Although the exact timing of colonization may be uncertain, both the 14 Mya (Arbabi *et al.*, 2014) or the 4.1–4.7 Mya (present study) estimates could be in accordance with the hypothesis that *Nesillas* first colonized the volcanic Comoro Archipelago before Madagascar because the oldest island (Mayotte) was formed *in situ* during this period (7.7 Mya, origin of volcanism 10–20 Mya) (Nougier *et al.*, 1986; Michon, 2016) and magmatic activity giving rise to the other three islands (Anjouan, Mohéli, and Grande Comore) began approximately 10 Mya (Michon, 2016). Hence, there is no discrepancy between the geological data and the molecular estimates because at least a portion of the Comoro Archipelago was exposed when *Nesillas* diverged from the other Acrocephalidae. Thus, these two estimates are not in conflict with the hypothesis of colonization of Madagascar from the Comoros, which is also suggested by our genetic data, although the alternative hypothesis of colonization of the Comoros from Madagascar is also compatible. We also highlight the discrepancy between the molecular estimates of Arbabi *et al.* (2014) and ourselves, and this difference warrants further clarification. An emerging pattern from the literature is the paraphyletic topology of taxa from the Comoros with respect to those on Madagascar; specifically, the basal position of certain endemic bird taxa of Grande

Comore and Mohéli (Warren *et al.*, 2003; Pasquet *et al.*, 2007; Fuchs *et al.*, 2008; present study). Relative to the older and more eroded Anjouan and Mayotte, Mohéli and Grande Comore currently incorporate a wider range of altitudes (Grande Comore is the largest island in the archipelago, and also contains the highest summit, Mount Karthala), with more remaining natural forest cover. Hence, these two islands may have ensured longer-term survival for *Nesillas* than Mayotte (where no members of the genus currently occur) or Anjouan.

#### WHAT COULD EXPLAIN GAPS IN THE DISTRIBUTION OF COMORIAN BIRDS?

The presence of poorly differentiated *N. typica* populations across two islands (*N. t. longicaudata* on Anjouan and *N. t. moheliensis* on Mohéli) and the single-island endemism of the two highly differentiated Comorian species (*N. brevicaudata* on Grande Comore and *N. mariae* on Mohéli) suggest that colonization history of *Nesillas* may represent an example of a ‘taxon cycle’ (Ricklefs, 1970; Ricklefs & Cox, 1972, 1978; Ricklefs & Bermingham, 2002), which involves four stages: (1) initial expansion where a taxon colonizes a series of islands within an archipelago, usually the lowland portions; followed by (2) a slowdown in geographical expansion and the onset of phenotypic differentiation; and subsequent (3) extinction of populations on the smallest islands or with less habitat complexity. The final stage (4) results in the survival of closely-related taxa usually confined to large islands or higher elevational zones. The information reported here suggests that *Nesillas* went through a complete taxon cycle in the case of the *N. mariae*/*N. brevicaudata* complex. The possible onset of a second cycle stage is shown by the colonization of Mohéli by *N. t. moheliensis*, where this subspecies has a different environment preference compared to those on Anjouan and Madagascar and is almost syntopic with *N. mariae* (Louette *et al.*, 1988). The complex biogeographical scenario that we describe is also consistent with the patterns highlighted in several other avian systems from the Malagasy Region, including the old taxa inhabiting the highlands of Mohéli and Grande Comore, as well as the possible recent secondary colonization of lowland areas of this archipelago (Warren *et al.*, 2005, 2006, 2013). The secondary colonization of a particular volcanic island for a second cycle appears to be more random (colonization occurring on Anjouan, Mohéli, and Mayotte, or any possible combination). Another consistent aspect is that all the bird lineages on western Indian Ocean islands that possibly went through an apparent taxon cycle



probably colonized the region within the last 5 Myr (Warren *et al.*, 2005, 2006, 2013).

Larger populations have a lower probability of extinction than smaller populations (Allendorf & Lusk, 2007) or, expressed in a different manner, populations occurring in larger areas have a higher chance of survival than populations in smaller areas (MacArthur & Wilson, 1963). These different aspects would imply that, for lineages going through a taxon cycle, larger islands or those with more heterogeneous habitat would hold taxa from the initial stage (e.g. *N. brevicaudata* on Grande Comore). In the present case, however, and in contrast to that is expected under the taxon cycle model, *N. mariae* differentiated and persisted on Mohéli, which is currently small and with little topographical complexity. These discrepancies highlight the difficulty of understanding how immigration–speciation–extinction dynamics affect current patterns of diversity and distribution, and the contribution of geographical context, even in an apparently simple situation such as that reported in the present study.

#### LONG DISTANCE DISPERSAL AND DIVERSIFICATION IN THE WESTERN INDIAN OCEAN ISLANDS

The Brush-warblers lineages endemic to Anjouan (*N. t. longicaudata*) and Aldabra (*N. aldabrana*) are most closely related to Malagasy forms (*N. typica* and *N. lantzii*, respectively). Although the strong biogeographical link between Aldabra and Madagascar is a repeated relationship across numerous bird lineages (Warren *et al.*, 2003, 2005, 2012; Pasquet *et al.*, 2007; Fuchs *et al.*, 2008), the pattern seen here in *Nesillas* is unusual in that, *N. aldabrana* is sister to *N. lantzii*, endemic to the south-western Madagascar, making these two taxa the most geographically distant within the genus. Given the distribution of *N. typica* in the region between Aldabra and the range of *N. lantzii*, colonization either occurred by a stepping stone model (and unknown extinction has obscured the pattern) or by long distance dispersal between similar dry habitats. Although unexpected, the latter is consistent with the remarkable dispersal abilities of Acrocephalidae warblers (Cibois *et al.*, 2007). In this context, the absence of *Nesillas* on the Seychelles and the Mascarenes is unexpected given the high faunal affinities between all these islands (Warren *et al.*, 2005, 2006). The Mascarenes and Seychelles both host an endemic species of *Acrocephalus*. It is thus possible that the limited size of these islands (Aldabra, Comoros Archipelago, Mascarenes, Seychelles) prevents the coexistence of different Acrocephalidae lineages, whereas the larger size of Madagascar allowed the colonization of the islands by both *Acrocephalus* and

*Nesillas*, which occur in sympatry at some sites (Goodman & Raherilalao, 2013).

#### PROCESSES DRIVING THE SPECIES AND DISTRIBUTION LIMITS ON MADAGASCAR

Based on current data, the two extant species of *Nesillas* on Madagascar are not sister species and have a notably similar age (Table 1); this is irrespective of the substitution rate employed. Because these species have non-overlapping distributions (Fig. 1) corresponding to different bioclimatic regions (Cornet, 1974), the dissimilar ecological requirements could have promoted or enhanced their differentiation. Indeed, our ecological niche modelling analyses indicated that the environments occupied by the two species are divergent, with a metric of similarity close to zero ( $D = 0.144$ ).

Projection of the ecological niche models onto the environmental layers implying climatic optima and minima (mid-Holocene ~6000 years ago, Last Glacial Maximum ~21 000 years ago and Last Interglacial ~130 000 years ago) suggests little change through time in the distribution of optimal environment for *N. typica* and *N. lantzii*. Given that the ecological niches are so distinct between these two species, it is possible that their geographical overlap was always limited. In this context, it is also worth noting that the location and extent of the optimal habitat have apparently not changed subsequent to the last interglacial. Hence, even though these two species appear to have the capacity to disperse over long distances, their establishment across the whole of Madagascar may have been precluded either by their distinct ecological requirements or biotic interactions such as competitive exclusion or host–parasite interactions. Our niche equivalency and similarity results indicate that the evolution of ecological niches in this group might be consistent with the idea of niche conservatism, where lineages are similar at a broad scale but show distinct differences at finer scales. Thus, for *Nesillas*, niche conservatism or competition with a close relative with similar morphology may have prevented adaptation to a broader ecological niche (i.e. all terrestrial environments of Madagascar). This also presents a challenge to the colonization of other islands in the archipelago because the environments available in these islands are quite different and sometimes unique (Fig. 4).

Our ecological niche modelling and genetic results are congruent and indicate no clear evidence for range extension of *N. lantzii* over time (based on extended Bayesian skyline plots and  $R_2$ ; Fu's  $F_S$ ) but do provide stronger evidence of population expansion for *N. typica*. The SPREAD analyses suggested that the geographical expansion of *N. typica* on

Madagascar started in the central eastern area, with a gradual latitudinal expansion towards the east. Hence, the morphological distinctiveness of the populations distributed in western Madagascar (darker plumage colour of *N. t. obscura*) might be best attributed to factors linked to recent evolution or phenotypic plasticity rather than isolation in deep geological time.

Although possible, our analyses could not confirm that the two *Nesillas* species occurring on Madagascar speciated *in situ*, leaving the possibility that the two lineages were already divergent before colonizing the island. The pattern recovered here is thus not directly comparable to colonization–speciation scenarios recently presented for other volant vertebrates of Madagascar. These patterns include very strong north–south genetic structure (Cruaud *et al.*, 2011; Weyeneth, Goodman & Ruedi, 2011; Block *et al.*, 2015), to weak but existing east–west genetic structure (Fuchs *et al.*, 2007), to no genetic structure (Fuchs *et al.*, 2013). Within the most widely distributed taxon, *N. typica*, the shape of the haplotype network and the results from the SPREAD analyses suggest recent expansion and lack of mitochondrial differentiation across its distribution.

The lack of genetic differentiation of *N. typica* on Madagascar needs to be considered in the period implied by its divergence (at most 0.5 Myr) from its closest relative (*N. t. longicaudata*). It can be postulated that insufficient time has passed for genetic differentiation to take place, particularly considering its generalist ecological requirements. Indeed, large areas of suitable habitat were present on Madagascar subsequent to the Last Interglacial Maximum, allowing continuous gene flow across populations. Hence, the patterns encountered for *N. typica* appear to be similar to that of another generalist species, *Dicrurus forficatus* (Fuchs *et al.*, 2013), where colonization is recent and the continuous presence of large areas of suitable conditions did not appear to create barriers to gene flow, nor result in phylogenetically structured populations.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1.** Map showing the study area (black dashed line), and the occurrences used in the model for *Nesillas lantzii* (black points), *Nesillas typica* (red points), and the background sample (grey points).

**Figure S2.** Extended Bayesian skyline plots for *Nesillas lantzii* (left) and *Nesillas typica* (right; Madagascar populations only).

**Figure S3.** Results of the SPREAD analyses.

**Figure S4.** Results of ecological niche model performance for the two *Nesillas* lineages.

**Figure S5.** Results of the niche equivalency and niche similarity tests between *Nesillas lantzii* and *Nesillas typica*.

**Table S1.** List of samples used in the present study.

**Table S2.** Genetic diversity and summary statistics obtained using DNASP, version 5 (Librado & Rozas, 2009) for the different primary lineages of *Nesillas*.