Journal of Biogeography (*J. Biogeogr.*) (2016)

Journal of Biogeography

ORIGINAL ARTICLE

Gecko phylogeography in the Western Indian Ocean region: the oldest clade of *Ebenavia inunguis* lives on the youngest island

Oliver Hawlitschek[1,2]*, Emmanuel F. A. Toussaint[3], Philip-Sebastian Gehring[4], Fanomezana M. Ratsoavina[5], Nik Cole[6,7], Angelica Crottini[8], Joachim Nopper[9], Athena W. Lam[1], Miguel Vences[10] and Frank Glaw[1]

[1]*Department of Herpetology, Zoologische Staatssammlung München (ZSM-SNSB), Department Herpetology, 81247 Munich, Germany,* [2]*Institut de Biologia Evolutiva (CSIC-UPF), Animal Biodiversity and Evolution Program, 08003 Barcelona, Spain,* [3]*Division of Entomology, Biodiversity Institute and Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence 66045, KS, USA,* [4]*Fakultät für Biologie Universität Bielefeld, Abt. Biologiedidaktik, 33615 Bielefeld, Germany,* [5]*Département de Biologie Animale, Université d'Antananarivo, Antananarivo 101, Madagascar,* [6]*Durrell Wildlife Conservation Trust, Trinity, Jersey Channel Islands,* [7]*Mauritian Wildlife Foundation, Vacoas, Mauritius,* [8]*CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, 4485-661 Vairão, Portugal,* [9]*Animal Ecology and Conservation, Zoological Institute, Universität Hamburg, 20146 Hamburg, Germany,* [10]*Zoological Institute, Technische Universität Braunschweig, 38106 Braunschweig, Germany*

*Correspondence: Oliver Hawlitschek, Institut de Biologia Evolutiva (CSIC-UPF), Passeig Marítim de la Barceloneta 37, 08003 Barcelona, Spain.
E-mail: oliver.hawlitschek@gmx.de.

ABSTRACT

Aim We studied the gecko genus *Ebenavia* to reconstruct its colonization history, test for anthropogenic versus natural dispersal out of Madagascar, and correlate divergence date estimates of our phylogeny with geological age estimates of islands in the region.

Location Madagascar and surrounding islands of the Western Indian Ocean (Comoros, Mayotte, Mauritius, Pemba).

Methods We reconstructed the phylogeny of *Ebenavia* covering its entire geographical range using a molecular data set of three mitochondrial and two nuclear markers. We estimated divergence times based on calibrations using (1) previously calculated mutation rates of mitochondrial markers, (2) a combination of these rates with old or (3) young geological age estimates for some of the islands inhabited by the genus, and (4) an independent data set with fossil outgroup calibration points.

Results *Ebenavia inunguis*, one of two recognized species of the genus, comprises multiple ancient evolutionary lineages. The earliest divergence within this complex (Miocene, 13–20 Ma; 95% credibility interval [CI]: 4–29 Ma) separates the population of the Comoros Islands, excluding Mayotte, from all other lineages. The age estimates for island lineages coincide with the geological age estimates of the islands except for Grand Comoro, where the age of the local clade (3–5 Ma; 95% CI: 2–7 Ma) significantly predates the estimated island age (0·5 Ma). A clade from north Madagascar + Mayotte + Pemba is estimated to have diverged from an eastern Malagasy clade in the Miocene.

Main Conclusions Our results suggest that Grand Comoro Island is geologically older than previously estimated. The islands of the Comoros and Pemba were probably colonized via natural dispersal out of Madagascar (> 1000 km in the case of Pemba). Mauritius was most likely colonized only recently from eastern Madagascar via human translocation.

Keywords

Comoros, Madagascar, Mauritius, Mayotte, molecular clock, Pemba Island, phylogeny, reptiles

INTRODUCTION

Charles Darwin (1859) already noted that reptiles were prime examples for studying the colonization history of oceanic islands by non-flying terrestrial animals. Their slow metabolic rate compared to mammals, and their resilience to desiccation and osmotic stress as compared to amphibians are seen as pre-adaptations for the successful colonization of new terrestrial habitats via overseas dispersal. Consequently, many oceanic islands are rich in reptile species, but poor in or devoid of amphibians and non-flying mammals (de Queiroz, 2005). The ability to disperse across very large

http://wileyonlinelibrary.com/journal/jbi
doi:10.1111/jbi.12912

distances was demonstrated in particular for geckos (Carranza *et al.*, 2000; Gamble *et al.*, 2008, 2011).

Overseas dispersal played a major role in biota assembly across the Western Indian Ocean region (Vences *et al.*, 2003; Yoder & Nowak, 2006; Warren *et al.*, 2010; Townsend *et al.*, 2011; Crottini *et al.*, 2012; Samonds *et al.*, 2012). Despite its relatively low number of islands, the area comprises ancient continental fragments of Gondwanan origin (Madagascar, the granitic Seychelles), continental islands that were connected with the mainland at low sea levels (the Zanzibar Archipelago and islands off Madagascar), and oceanic islands. The latter comprise most islands of the Seychelles and the volcanic hotspot archipelagos of the Comoros and Mascarenes. Age estimates exist for all major oceanic islands (Montaggioni & Nougier, 1981; Emerick & Duncan, 1982; Nougier *et al.*, 1986; Saddul, 1995; see also Sheth *et al.*, 2003; Debeuf, 2009) and for the splits of Madagascar from the African continent and of the Seychelles from Gondwana and India (Plummer & Belle, 1995; Ali & Aitchison, 2008; Collier *et al.*, 2008; Gibbons *et al.*, 2013). However, the oceanic islands of the Western Indian Ocean lack true fossils (Hume *et al.*, 2011) and the Cenozoic animal fossil record of Madagascar is particularly poor (Goodman & Benstead, 2003). Consequently, geological age estimates have been used for the calibration of molecular clocks in many studies on regional biota (Vences *et al.*, 2003; Warren *et al.*, 2003, 2005, 2006; Rocha *et al.*, 2007; Fuchs *et al.*, 2008; Crottini *et al.*, 2012).

The careful use of oceanic island age estimates for time-tree calibration has the theoretical advantage of providing maximum age constraints of relatively shallow divergences, compared to fossils, which tend to provide minimum age boundaries and are therefore mostly useful for deeper clades. This approach has been challenged by several studies that estimated clades of a variety of organisms being older than the purported ages of the islands to which they are endemic. The most prominent example is that of the Galápagos islands, in which the divergence between island clades of endemic iguanas (Rassmann, 1997) and weevils (Sequeira *et al.*, 2000) were estimated considerably older than the archipelago on which they lived (see review in Parent *et al.*, 2008). Another well-known example among reptiles are the native anoles of Barbados Island, whose age was estimated to 5–6 million years ago (Ma), while the geological age of Barbados was estimated to be *c.* 1 Ma (Thorpe *et al.*, 2005). Recently, a clade of stick insects endemic to the Mascarenes was estimated to pre-date the age of the oldest extant island of the group by 6–31 million years (Bradler *et al.*, 2015). However, it is still open to discussion how common such 'rock *versus* clock' discrepancies in volcanic islands are. Some of the earlier studies were based only on mitochondrial DNA and assumptions on mutation rates that might provide age overestimates (Grechko, 2013). More recent studies have provided younger estimates for some of the taxa involved (e.g. MacLeod *et al.*, 2015, for Galápagos iguanas).

The discrepancies between geological age estimates of islands and age estimates for their endemic clades have been reviewed by a number of authors (Heads, 2011; Hipsley & Müller, 2014; Ho *et al.*, 2015). Two plausible explanations exist: first, the clade of the species in question is actually older than the island it is endemic to, either because its closest mainland relative has gone extinct after the colonization event, or because the colonization took place via stepping stones that today no longer exist (Renner *et al.*, 2010). Second, the geological age estimates for the island do not correctly reflect the time span available for its colonization. Most geological age estimates of oceanic islands are based on surface rocks that may be younger than the age of first emergence of the island, for example, if older volcanoes become active after a period of inactivity, whereas older and deeper layers of rock that would allow correct age estimates are inaccessible for sampling (Heads, 2011).

Stepping stones very likely existed in the Seychellean Arc and along the Réunion (Mascarenes) and Karthala (Comoros) Hotspots in times of lower sea levels (Hijmans *et al.*, 2005; Miller *et al.*, 2005; Warren *et al.*, 2010; Strickland *et al.*, 2013; see discussion for the Mascarenes in Bradler *et al.*, 2015), and many volcanoes are active today or were repeatedly active until very recent geological times, which may have biased geological dating attempts towards younger age estimates (Emerick & Duncan, 1982).

The biogeography of the largest island, Madagascar, was largely influenced by other factors. While as many as 18 biogeographical regions have been recognized on Madagascar (Wilmé *et al.*, 2006), the most pronounced distinctions are those of the arid west *versus* the humid coastal east, and of the north < 15° southern latitude *versus* the remainder of the island (Brown *et al.*, 2014). Previous studies have pointed out the existence of deeply divergent clades within genera, species complexes, or species of vertebrates in the Malagasy north (Boumans *et al.*, 2007). Unlike the rest of the islands, this region was under monsoon influence since the end of the Mesozoic and, with changes in intensity, mostly remained so during the Palaeogene (Wells, 2003; Boos & Kuang, 2010; Buerki *et al.*, 2013; Ohba *et al.*, 2016).

Our study focuses on *Ebenavia inunguis* Boettger, 1878; a small nocturnal gecko that is relatively rarely seen but widespread in Madagascar and the surrounding islands. It inhabits the north including Nosy Be Island, the east including some offshore islets, and parts of the central highlands of Madagascar. It is also found in all major islands of the Comoros, in Mauritius, and Pemba Island. The origin of these insular populations by human translocation *versus* natural dispersal has not been clarified so far. The second species of the genus, *E. maintimainty* Nussbaum & Raxworthy, 1998, is known only from a small range around its type locality in south-western Madagascar.

We here apply a newly sequenced, geographically comprehensive set of DNA sequences of *E. inunguis* to (1) reconstruct the phylogenetic relationships of regional clades and estimate their divergence times, (2) test the hypothesis that all populations of *E. inunguis* outside Madagascar originate from natural overseas dispersal, and (3) assess the fit of the divergence time estimates with geological age estimates of the islands in the Western Indian Ocean region.

MATERIALS AND METHODS

We included 52 samples of *Ebenavia inunguis* and two of *E. maintimainty* in our dataset, representing altogether 31 localities across the entire known distribution range of the genus. From a further 20 samples and nine localities, only COI data were available (see Appendix S2, Table S1).

A more detailed description of all methods used, including additional analyses, is given in Appendix S1. We amplified three mitochondrial gene fragments, 12S rRNA (12S), cytochrome C oxidase subunit 1 (COI), and cytochrome b (CYTB), and two nuclear fragments, the recombination activating gene 2 (RAG2) and the prolactin receptor (PRLR), resulting in a data set of 2,251 bp. All PCR protocols follow Hawlitschek *et al.* (2012) and Hawlitschek & Glaw (2013). For a subset of specimens we also amplified the recombination activating gene 1 (RAG1) and brain-derived neurotrophic factor (BDNF) following the protocols of Crottini *et al.* (2012). Data were submitted to BOLD (process IDs EBINU001–15 to 064–15) and GenBank (accession numbers LT591928 to LT592132).

We conducted Bayesian inference analyses on the aligned and partitioned data set in MrBayes 3·2·0 (Ronquist *et al.*, 2012) with two runs and four chains with 100,000,000 generations. Furthermore, we included the 20 samples for which only COI was available in a separate Mrbayes COI tree. In addition, we conducted maximum likelihood analyses of the concatenated data set with 1000 bootstrap replicates in raxmlGUI 1·0 (Stamatakis, 2006; Silvestro & Michalak, 2012).

In order to independently assess molecular dating results, five specimens were chosen to represent the major clades retrieved in our main phylogenetic analyses. RAG1 and BDNF sequences of these specimens were added to the original alignment of Crottini *et al.* (2012).

We employed four dating strategies, one of them based on a completely independent set of molecular data and calibration framework, for estimating the divergence times within *Ebenavia*. The goal of this approach was the cross-validation of our calibration methods to assess the accuracy of our dating results (see Near *et al.*, 2005). We used beast 1·8·1 (Drummond *et al.*, 2012) with our main data set. The MrBayes topology was fixed as the starting tree. We assumed an uncorrelated relaxed clock model for all partitions and conducted runs both under a Yule and a birth-death model. We used substitution rates inferred from the phylogenies of geckos from the Canary Islands (Carranza & Arnold, 2012). BEAST analyses were run for 75,000,000 generations, sampling every 100 generations. Log files were checked for convergence of model parameters as indicated by effective sample sizes (ESS) > 200 in tracer v1·5 (http://beast.bio.ed.ac.uk/Tracer).

We used a number of geological age estimates for the Comoros Islands with considerable variation between older and younger estimates for the calibration of our divergence time estimates with three strategies: (1) No calibration points, dating based exclusively on the substitution rates of 12S and CYTB. (2) Calibration of *Ebenavia* lineages from Mayotte (15 Ma), Mohéli + Grand Comoro (age of Mohéli: 5·0 Ma), and Anjouan + Mohéli + Grand Comoro (age of Anjouan: 11·5 Ma), based on the estimates of the oldest possible geological ages (Montaggioni & Nougier, 1981; Nougier *et al.*, 1986), and the age of Mauritius (8·3 Ma; Sheth *et al.*, 2003). (3) Same as (2), but using the geological age estimates of Emerick & Duncan (1982) based on the oldest surface lavas of the Comoros (Mayotte: 5·4 Ma, Mohéli: 2·81 Ma, Anjouan: 3·5 Ma).

A divergence time dating analysis was also conducted with the RAG1 and BDNF data set using Multidivtime (Thorne & Kishino, 2002). This data set contains representatives of all major vertebrate groups from Madagascar and numerous outgroups, allowing a molecular dating analysis with as many as 48 cross-validated age constraints across the vertebrate tree of life. We implemented these constraints and all other analytical settings as Crottini *et al.* (2012); the only difference to the published analytical protocol was the inclusion of five additional *Ebenavia* terminals.

Finally, we conducted reconstructions of the ancestral areas of the major clades and subclades of *Ebenavia* using the R package 'BioGeoBEARS' (Matzke, 2013; Appendix S1).

RESULTS

A more detailed description of all results is given in Appendix S1. *Ebenavia maintimainty* was recovered as the sister group to the *E. inunguis* complex (Fig. 1). This complex contained three major and largely allopatric clades: (A) The Comoros clade, comprising samples from the Comoros islands of Anjouan, Mohéli and Grand Comoro, recovered as the sister group to all other clades; (B) The north clade, including samples from localities in the very north of Madagascar and also from the islands of Mayotte (Comoros Archipelago) and Pemba (Zanzibar Archipelago off Tanzania); and (C) the east clade with samples from most of Madagascar's east coast and Sambirano region including the offshore island of Nosy Be, but excluding north Madagascar. The east clade is further subdivided into three subclades: (Ca) comprising samples from higher elevations of east Madagascar; (Cb) with a sample from Nosy Be; and (Cc) comprising all other east coast lowland samples. Additional samples are placed in these clades in the MrBayes COI tree (Fig. 2)

Divergence time estimates derived from our five-gene-fragment data set in beast and the Multidivtime analyses are provided in (Table 1). They indicate ancient divergences

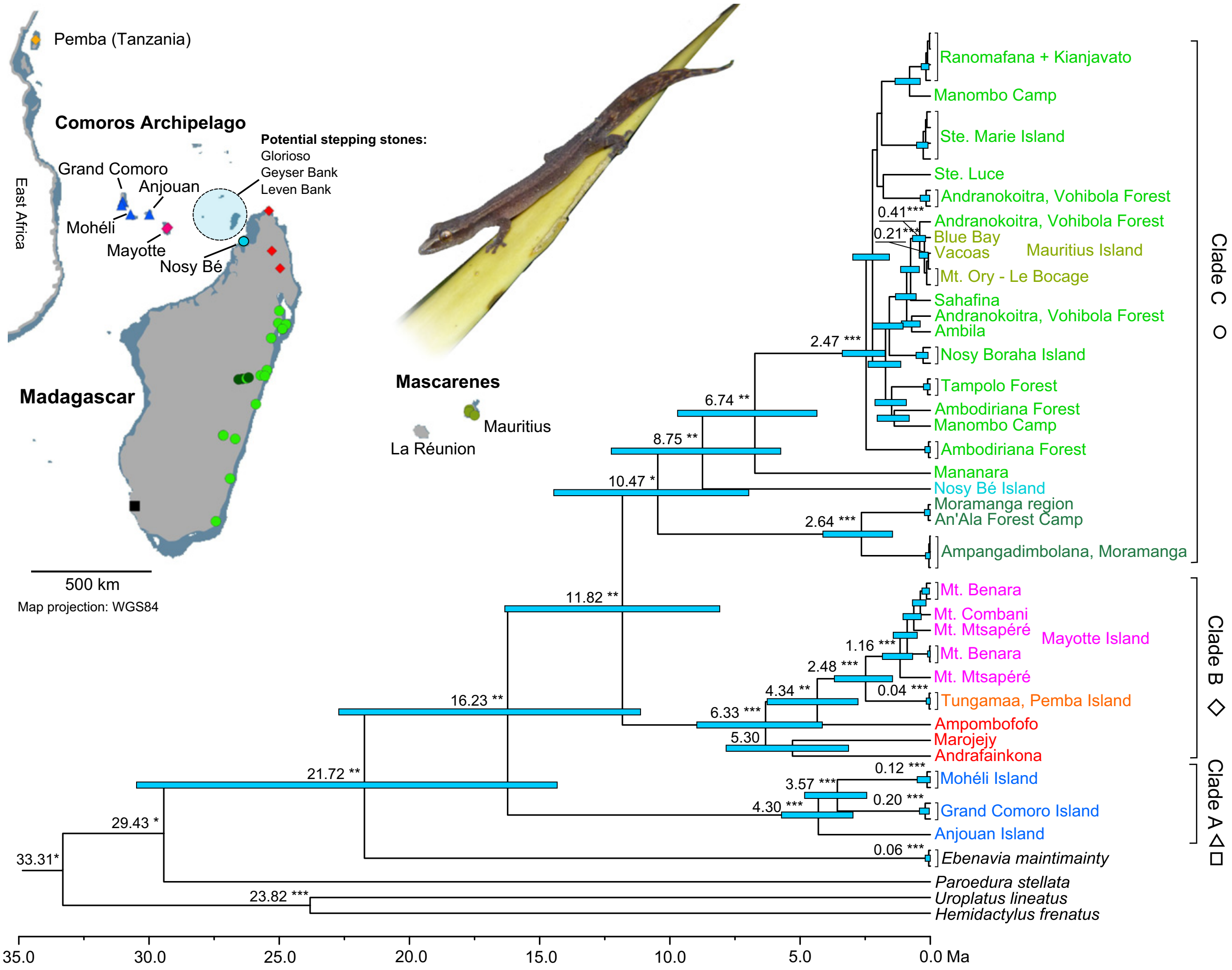

Figure 1 A chronogram of the *Ebenavia inunguis* complex, with outgroups, created in BEAST with island age estimates from Emerick & Duncan (1982) and a Yule process (see Table 1). Node ages are given above nodes in million years. Node bars indicate 95% credibility intervals of ages. Asterisks following node ages indicate support values of 0·9 or better; each one asterisk is displayed for BEAST posterior/MrBayes posterior probability/RAxML bootstrap. The inlay map shows sampling localities with colours corresponding to the distribution of deep lineages. The dark blue area is terrestrial surface at lower sea levels during the Last Glacial Maximum (from Hijmans *et al.*, 2005), showing now sunken islands as potential stepping stones. The depicted specimen is from Singani, Grand Comoro, Comoros.

among the main clades within *E. inunguis*. The mean divergence age between the two species of *Ebenavia*, across the different analyses, was estimated between 21·7 and 26·3 Ma (95% credibility interval: 12·33–36·80 Ma), whereas the oldest divergence within *E. inunguis* was recovered at 13–20 Ma (95% CI: 5–29 Ma). BEAST estimates of this split are about 16–20 Ma (95% CI: 11–27), whereas Multidivtime estimated a slightly younger age and a wide credibility interval of 13 Ma (95% CI: 5–29). The Multidivtime analysis based on the RAG1 and BDNF data set yielded results largely compatible with those from the BEAST analysis.

The results of the ancestral area reconstructions are given in Appendix S3, Table S2. Madagascar is recovered as the most probable ancestral area for the entire *Ebenavia inunguis* complex under the DEC+j and BAYAREAlike+j models, whereas DIVAlike+j recovers the Comoros. The latter model scores best, albeit only with a marginal difference, in all model tests.

DISCUSSION

The age of Grand Comoro: molecular clock versus geological dating

Notably, the most basal split within the *Ebenavia inunguis* complex separates the clade inhabiting the Comoros except Mayotte from all other clades. Our age estimates coincide with or even pre-date the oldest proposed geological age of Mayotte (15 Ma), the oldest extant island of the Comoros Archipelago (Montaggioni & Nougier, 1981). This suggests that the Comoros clade may have colonized the oldest extant island immediately after its emergence, or that it had already existed

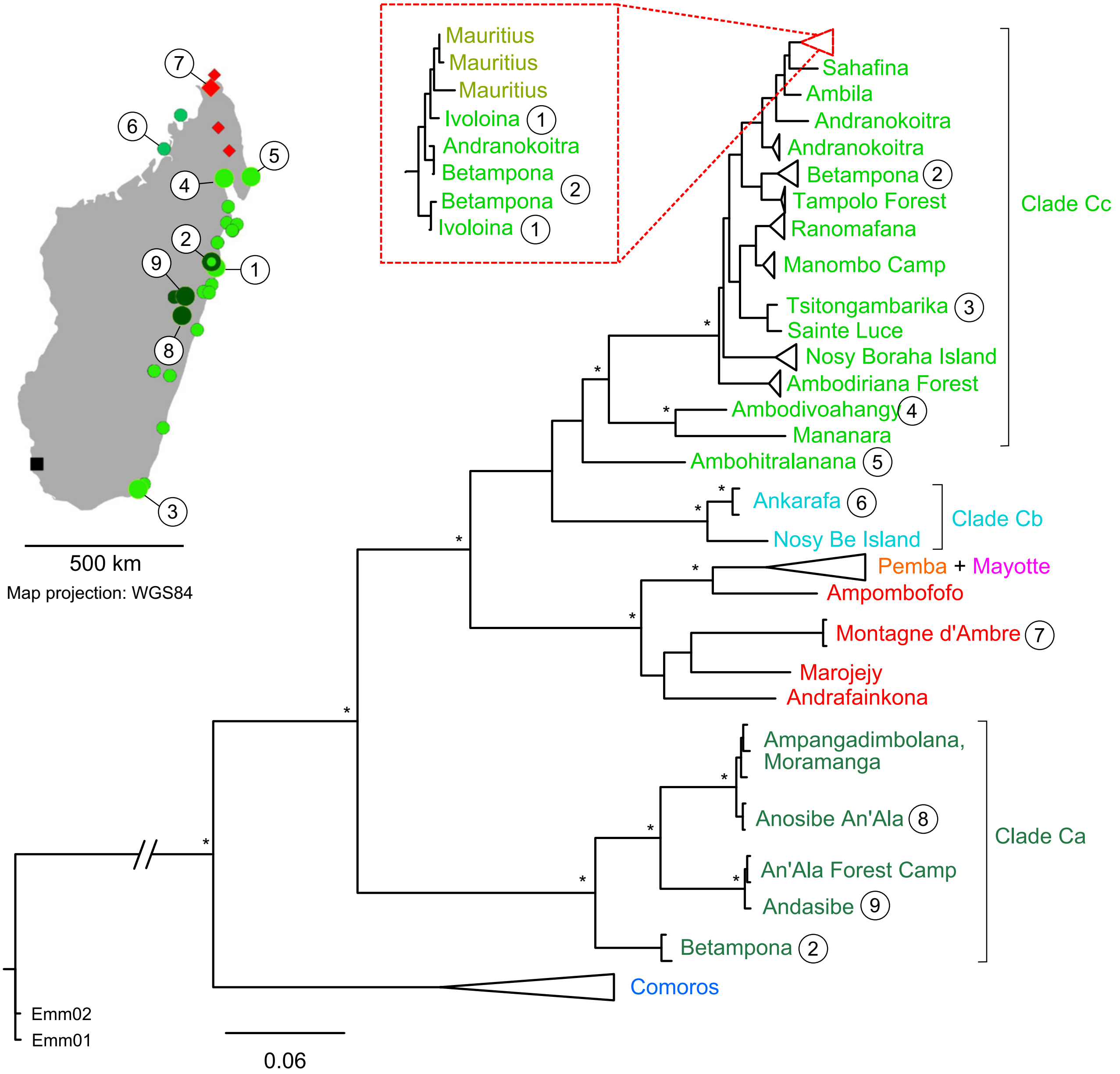

Figure 2 Bayesian phylogram of *Ebenavia* based on the COI marker alone. Asterisks at nodes indicate support values of 0·9 or better. Sampling localities not represented in the chronogram in Fig. 1 are highlighted with numbers 1 to 9 and represented by enlarged symbols in the inlay map.

on older islands that are now submerged. The seamounts of the Geyser Bank and the Leven Bank are located between the extant islands of the Comoros and Madagascar and, based on their bathymetry, must have been exposed during periods of sea level regression, such as those of the Pleistocene glaciations, but very likely also at earlier times (Rohling *et al.*, 2014). They might have provided stepping stones between Madagascar and the present-day Comoros (Fig. 1).

Dispersal from Madagascar to the Comoros is favoured by the direction of marine currents. The South Equatorial Current, coming from the East, passes the northern tip of Madagascar and continues to the Comoros (Louette *et al.*, 2004; Carton & Giese, 2008); this circulation pattern was probably active since ca. 35 Ma and therefore covers the geological time span relevant to *Ebenavia* (Ali & Huber, 2010; Townsend *et al.*, 2011; Tolley *et al.*, 2013). Consequently, much of the Comoran biota, particularly reptiles, is of Malagasy ancestry (e.g. Rocha *et al.*, 2007; Hawlitschek *et al.*, 2012; Hawlitschek & Glaw, 2013). Surprisingly, our ancestral area reconstruction (Appendix S1 and Appendix S3, Table S2) does not unambiguously support a Malagasy origin of the extant lineages of the *E. inunguis* complex; instead, the most highly supported model (DIVAlike+j) favours a Comoran origin, which would mean that the ancestors of the Malagasy clades of this complex had colonized Madagascar from the Comoros. At first sight, this scenario seems much less

Table 1 A comparison of estimated divergence times of nodes in the phylogeny of the *Ebenavia inunguis* complex. Ages are given in million years, with 95% Bayesian credibility interval ranges in brackets. The three calibration schemes used under BEAST are 'None' (no calibration points, based only on mutation rates), 'Old' (using the oldest available age estimates for the Comoros Islands by Montaggioni & Nougier, 1981 and Nougier, 1986) and 'Young' (using the most commonly applied age estimates for the Comoros Islands by Emerick & Duncan, 1982). All calibration schemes were run each under a Yule Process and with a birth-death (BD) model. Furthermore, the ages for nodes retrieved in the MULTIDIVTIME analysis are given in the column 'Multi-Div'. *In BEAST with Yule model, the node of *Paroedura/Ebenavia* only had a posterior probability of < 0·5, and therefore, no credibility intervals of the age estimates can be given. In runs with birth-death model, the node was not retrieved.

Node	None/Yule	Old/Yule	Young/Yule	None/BD	Old/BD	Young/BD	Multi-Div
*Paroedura/Ebenavia**	32·98	35·20	29·43	–	–	–	68·28 (100·03–41·50)
E. maintimainty/E. inunguis	24·65 (35·54–15·10)	26·34 (36·80–16·46)	21·72 (30·48–12·33)	24·94 (33·81–17·89)	25·63 (34·20–18·59)	24·48 (32·74–17·90)	–
Comoros/rest of *E. inunguis*	18·65 (26·85–11·79)	19·62 (27·23–12·48)	16·23 (22·71–11·13)	17·61 (23·41–12·81)	18·12 (24·26–13·27)	17·10 (22·42–12·94)	13·28 (28·85–4·84)
Anjouan/Grand Comoro + Mohéli	5·63 (8·64–3·25)	5·97 (8·50–3·73)	4·30 (5·71–2·97)	4·85 (6·75–3·12)	5·21 (7·22–3·49)	4·27 (5·51–3·13)	–
Grand Comoro/Mohéli	4·67 (7·16–2·59)	4·96 (7·09–3·05)	3·57 (4·83–2·44)	4·07 (5·79–2·52)	4·29 (5·94–2·83)	3·25 (4·67–2·45)	–
Within Grand Comoro	0·22 (0·50–0·05)	0·24 (0·53–0·05)	0·20 (0·42–0·04)	0·19 (0·39–0·04)	0·19 (0·42–0·05)	0·17 (0·37–0·04)	–
Within Mohéli	0·13 (0·56–0·00)	0·15 (0·64–0·00)	0·12 (0·50–0·00)	0·10 (0·40–0·00)	0·10 (0·40–0·00)	0·10 (0·37–0·00)	–
North/Highland + East + Nosy Be	13·45 (19·14–8·86)	14·04 (19·51–9·23)	11·82 (16·34–8·09)	12·25 (15·97–9·29)	12·49 (16·08–9·43)	11·99 (15·19–9·26)	10·14 (22·80–3·51)
North Madagascar/Pemba + Mayotte	4·91 (7·43–2·90)	5·18 (7·53–3·09)	4·34 (6·26–2·78)	4·22 (5·87–2·91)	4·36 (5·97–2·97)	4·17 (5·68–2·95)	–
Pemba/Mayotte	2·79 (4·36–1·60)	2·94 (4·40–1·71)	2·48 (3·68–1·45)	2·31 (3·31–1·46)	2·39 (3·46–1·54)	2·29 (3·26–1·50)	–
Within Pemba	0·05 (0·17–0·00)	0·05 (0·18–0·00)	0·04 (0·16–0·00)	0·04 (0·15–0·00)	0·04 (0·14–0·00)	0·04 (0·13–0·00)	–
Within Mayotte	1·30 (2·05–0·71)	1·41 (2·18–0·79)	1·16 (1·84–0·68)	1·05 (1·54–0·61)	1·09 (1·63–0·66)	1·07 (1·57–0·67)	–
Highland/Nosy Be + East	11·92 (16·83–7·46)	12·46 (17·41–8·08)	10·47 (14·46–6·97)	10·91 (14·31–7·94)	11·09 (14·23–8·27)	10·51 (13·38–7·93)	–
Nosy Be/East	9·97 (14·51–6·31)	10·34 (14·52–6·55)	8·75 (12·25–5·74)	9·06 (11·98–6·54)	9·31 (12·16–6·74)	8·88 (11·36–6·55)	–
Mananara/Rest of East	7·67 (11·26–4·64)	7·95 (11·26–4·77)	6·74 (9·70–4·35)	6·94 (9·48–4·85)	7·15 (9·60–5·11)	6·76 (8·91–4·77)	–
Basal within Rest of East	2·75 (3·89–1·79)	2·92 (3·99–1·91)	2·47 (3·34–1·73)	2·37 (3·02–1·81)	2·43 (3·12–1·34)	2·31 (2·92–1·78)	–
Mauritius/Rest of East	0·45 (0·78–0·20)	0·47 (0·82–0·22)	0·41 (0·68–0·18)	0·35 (0·59–0·18)	0·37 (0·61–0·18)	0·35 (0·57–0·17)	–
Basal within Mauritius	0·24 (0·47–0·09)	0·26 (0·49–0·08)	0·21 (0·42–0·08)	0·19 (0·36–0·07)	0·20 (0·36–0·08)	0·19 (0·34–0·07)	–

probable than the colonization of the Comoros from a Malagasy source population which also gave rise to the extant *E. inunguis* clades of Madagascar. However, some likely cases of the re-colonization of mainland areas from islands are known (Nicholson *et al.*, 2005; Bellemain & Ricklefs, 2008), and our example reminds us that apparently obvious biogeographical explanations may be complicated by our lack of knowledge on palaeocurrents, stepping stones, and extinction events.

Within the Comoros clade, our divergence time estimates of the island populations coincide with the geological age estimates for Anjouan (divergence 4·3 Ma; oldest island age estimate 11·5 Ma, based on oldest surface lavas 3·5 Ma) and Mohéli (divergence 3·6 Ma; oldest island age estimate 5 Ma, based on oldest surface lavas 2·81 Ma) (Montaggioni & Nougier, 1981; Emerick & Duncan, 1982; Nougier *et al.*, 1986). We see this agreement as support of our strategy of calibration and cross-validation. Despite local disagreements between individual gene trees *versus* haplotype networks (see also Appendix S1 and Appendix S3) and evidence for haplotype sharing between lineages the clade age estimates for these islands support the robustness of our calibration.

In contrast to Anjouan and Mohéli, we find a strong discrepancy between estimated clade and island age in the lineage of the youngest and volcanically still active island Grand Comoro. The oldest geological age estimate for the island is 0·5 Ma, whereas the split between the Grand Comoro and Mohéli populations is dated at ca. 3–5 Ma (95% CI: 2–7 Ma). Even the average estimated divergence between the two haplotypes sampled from Grand Comoro is 0·2 Ma (95% CI: 0·04–0·53 Ma), which is older than many geological age estimates for the island, for example, of 0·13 ± 0·02 Ma by Emerick & Duncan (1982). The youngest estimated island age is 0·01 ± 0·01 Ma (Hajash & Armstrong, 1972), which is even lower than the lowest border of our credibility interval.

One potential biogeographical explanation for the ancient age of the endemic Grand Comoro lineage is that this lineage may have previously existed elsewhere. It might have colonized Grand Comoro only recently by way of stepping stone islands on which it finally became extinct. Indications for the extinction and re-colonization of Comoran islands by reptiles exist (Hawlitschek & Glaw, 2013). However, *Ebenavia* is not the only case in which the divergence between Grand Comoro and other Comoran populations, as well as the genetic diversity within Grand Comoro, point to an older age than geologically estimated. This is the case in chameleons (Rocha *et al.*, 2005a; Tolley *et al.*, 2013), day geckos (Rocha *et al.*, 2007), snakes (Hawlitschek *et al.*, 2012), and ground geckos (Hawlitschek & Glaw, 2013). Pasquet *et al.* (2007) found that the inclusion of the age of Grand Comoro as a calibration point for the divergence time estimates of drongo birds (Dicruridae) led to much younger overall age estimates compared to other calibration patterns, and that the Grand Comoro clade of these birds was significantly older than 0·5 Ma. If the hypothesis that all these populations existed elsewhere before colonizing Grand Comoro was true, at least some survivors might still be expected to live on other extant islands in sympatry with divergent endemic clades. Also, the question arises why exactly Grand Comoro and none of the other islands of the archipelago functions as a refuge for old clades.

Incorrect geological age estimates for Grand Comoro provide an alternative and rather plausible explanation for the old age of the Grand Comoro lineage. All geological age estimates for the island are based on rocks exposed on the surface of the island (Emerick & Duncan, 1982). These surface rocks might cover older volcanic rocks that might provide older and more reliable age estimates, but they are often inaccessible to geologists. Grand Comoro consists of two volcanoes, the young and active Karthala and the older and now inactive La Grille. Most surface rocks are of Karthala origin, and also La Grille had probably been active and producing surface lavas long after the origin of Grand Comoro. Such events have often not been taken into account in studies on geological age estimates, although most geologists are aware that the mechanisms of the formation of volcanic islands are still poorly understood (Sherrod, 2009). Grand Comoro thus provides a clear example for the caveats connected to geological calibration points in molecular clock studies.

Extinction and re-founding of island populations

The situation of *Ebenavia* in the Comoros is further complicated by the fact that the oldest island Mayotte is not inhabited by *E. inunguis* of the Comoros clade (clade A), but by the Malagasy north clade (clade B). A similar situation was detected in *Paroedura* ground geckos (Hawlitschek & Glaw, 2013), where the lineage from the Comoros except Mayotte is the sister group to the lineage comprising species from Madagascar and Mayotte. It is unknown in both examples whether Mayotte was ever successfully colonized by the same clade that colonized the remaining islands of the archipelago. If this was the case, the earlier populations either became extinct after being out-competed by the later colonizers or a local extinction event exterminated the populations on Mayotte and made way for a new colonization from Madagascar. Potential evidence for the extinction hypothesis is provided by the active volcanism of the Karthala Hotspot, by geologically recent eruptions also on the older islands (Nougier *et al.*, 1986; Debeuf, 2009), and by indications of extinction events also on other islands of the archipelago (e.g. Anjouan: Hawlitschek & Glaw, 2013). If the earlier colonizers were out-competed instead of being wiped out by a catastrophic event, then remnants of the Comoros clade might still be found in the genome of the extant population of Mayotte. Neither mtDNA nor the nuclear markers studied provide any indication for such a scenario, as was also shown for *Paroedura* (Hawlitschek & Glaw, 2013). However, the relatively small genetic data set typically used in biogeographical studies, such as ours, may be insufficient to trace these genetic remnants. In either case, the short geographical distance between Madagascar and Mayotte, and the direction of the marine currents, may have allowed the relatively fast re-colonization of Mayotte.

The recent colonization of islands out of Madagascar

Outside of Madagascar *Ebenavia inunguis* inhabits not only the Comoros, but also the islands of Mauritius and Pemba. The least divergent island population is that of Mauritius, which is phylogenetically nested within the east coast lowland subclade (Cc) and very closely related to a COI haplotype from Ivoloina (Appendix S3, Fig. S2, S3), close to a major shipping centre to Mauritius. The earliest dated *Ebenavia* records in Mauritius are from 1948 when the species was collected in Moka (Vinson & Vinson, 1969), located *c.* 6 km from the port of Mauritius' capital Port Louis. These data strongly suggest that the Mauritian population of *Ebenavia* goes back to passive human translocation of at least several females, as is the case for many other reptile species of the Western Indian Ocean region (Rocha *et al.*, 2005b, 2007, 2010). The introduction might have been conducted via shipping traffic from Toamasina to Port Louis. This view is supported by the present-day direction of marine currents between Madagascar and Mauritius, which do not favour dispersal in this direction (Cheke & Hume, 2008). Furthermore, *Ebenavia* still has a relatively patchy distribution on Mauritius, often near urban areas (NC, unpublished data), which is in agreement with a recent introduction.

On Pemba Island, *E. inunguis* is reported from two localized populations in the north and south (Pakenham, 1983), suggesting a wider distribution around the island. The divergence between the Pemba population and its closest relative in the north Clade is dated at *c.* 2–3 Ma (95% CI: 1–4 Ma). The age of the clade strongly supports the view of a natural long-distance overseas dispersal (> 1000 km) from north Madagascar, Mayotte, or from one of the sunken stepping stone islands between Madagascar and the Comoros. We cannot rule out the possibility that the Pemba individuals were translocated from an unsampled population in north Madagascar or Mayotte. Our ancestral area reconstructions are not informative on this event. However, the possibility that the observed patterns are the result of natural dispersal from Madagascar is supported by prevailing marine currents and is demonstrated in the parallel example of *Phelsuma parkeri*, a gecko endemic to Pemba Island whose sister species inhabits north Madagascar (Rocha *et al.*, 2007, 2009).

Evolutionary steps towards east–west-vicariance in Madagascar

According to our phylogenetic reconstruction, *Ebenavia* follows a common pattern of Malagasy biogeography with the most ancient divergence between *E. maintimainty* in the arid south-west and the *E. inunguis* complex distributed mainly in the humid east (Boumans *et al.*, 2007). Most native reptiles colonized the Comoros from north-west or north Madagascar (Hawlitschek & Glaw, 2013; Hawlitschek *et al.*, 2016). In *Ebenavia*, this might indicate an initial split between the south-west and north-west/north with subsequent dispersal to the Comoros, lineage diversification in the north and finally the east, a pattern similar to that found in *Phelsuma* day geckos (Gehring *et al.*, 2013).

CONCLUSIONS

The deep divergences, old divergence time estimates, and the geographical isolation of some populations within *Ebenavia inunguis* support the view of a species complex. However, species delimitation and taxonomic revision are beyond the scope of this paper and will be addressed elsewhere.

The discrepancy between the molecular dating of *Ebenavia* versus geological age estimates of Grand Comoro provides a clear example of how uncertainties in the dating of volcanic islands, as well as a lack of data on stepping-stones and possible past extinction events, may complicate molecular dating. At the same time, the coincidence of our divergence time estimates of endemic *Ebenavia* lineages of other islands with the corresponding island age estimates shows that if properly cross-validated, geological age estimates of oceanic islands can provide reliable biogeographical calibration points for divergence time dating.

ACKNOWLEDGEMENTS

We are grateful to the following people for their participation and invaluable help in the field surveys: F. Andreone, J. Berger, H.-P. Berghof, P. Bora, B. Brückmann, B. Brenzinger, M. Coates, K. Glaw, M. Hiermeier, J. Köhler, M. Kotrba, K. Mebert, R. Mootoocurpen, M. Pabijan, M. Puente, L. Raharivololoniaina, E. Rajeriarison, T. Rajoafiarison, A. Rakotoarison, F. Randrianasolo, R.D. Randrianiaina, S. Rasamison, A. Razafimanantsoa, D. Rödder, G.M. Rosa, V. Tatayah, M. Thomas, D.R. Vieites, as well as M. 'Kamal' Badroudine, H. Sinane, Y. Ibrahim, S. Moussa, K. Green, H. Doulton, I. Said, A. Houmadi and other members of the staff of Bristol Conservation and Science Foundation's project in the Comoros (www.bscf.org.uk/comoros), and D. Laybourne, G. Viscardi, G. Decalf, M. Charpentier, C. Dionisio, M. Baudouin and the other members of the DAF, DEAL, and Naturalistes Mayotte. Authorities at Antananarivo (Madagascar), Moroni (Comoros), Mamoudzou (Mayotte, France), Zanzibar (Tanzania, for Pemba Island), and Port Louis (Mauritius) are acknowledged for issuing collection and export permits. Furthermore, we thank M. Balke, Munich, for providing lab infrastructure, M. Franzen, Munich, for general support, O. Madsen, Wageningen, for conducting the MULTIDIVTIME runs, the EMBL/EBI team for handling the GenBank entries, and the BOLD team for handling the DNA Barcode data entry. Finally, we thank the editor Krystal Tolley and three anonymous referees for their extremely helpful comments.

The research was financially supported by grants of the Volkswagen Foundation to PSG, MV and FG; the Mohamed bin Zayed Species Conservation Fund (project 0925157) to FG and OH, by DAAD grant D/09/49634 to OH, by SuLaMa/BMBF (FKZ 01LL0914) to JN, by an Investigador FCT (IF) grant from the Portuguese 'Fundaçao para a Ciencia e a Tecnologia' (IF/00209/2014) and by the Saint Louis Zoo Field Conservation program (grant: FRC #12–12) to AC, and by the 'Deutsche Forschungsgemeinschaft' DFG (GL 314/1) to FG and DFG (BA2152/7-1) to M. Balke, Munich. The 'Freunde der Zoologischen Staatssammlung München', the Society of the University of Munich and EES funding program of the University of Munich provided additional financial support.

REFERENCES

Ali, J.R. & Aitchison, J.C. (2008) Gondwana to Asia: plate tectonics, paleogeography and the biological connectivity of the Indian sub-continent from the Middle Jurassic through latest Eocene (166-35 Ma). *Earth-Science Reviews*, **88**, 145–166.

Ali, J.R. & Huber, M. (2010) Mammalian biodiversity on Madagascar controlled by ocean currents. *Nature*, **463**, 653–656.

Bellemain, E. & Ricklefs, R.E. (2008) Are islands the end of the colonization road? *Trends in Ecology and Evolution*, **23**, 461–468.

Boettger, O. (1878) Die Reptilien und Amphibien von Madagascar. *Abhandlungen der Senckenbergischen naturforschenden Gesellschaft*, **11**, 1–56.

Boos, W.R. & Kuang, Z. (2010) Dominant control of the South Asian monsoon by orographic insulation versus plateau heating. *Nature*, **463**, 218–222.

Boumans, L., Vieites, D.R., Glaw, F. & Vences, M. (2007) Geographical patterns of deep mitochondrial differentiation in widespread Malagasy reptiles. *Molecular Phylogenetics and Evolution*, **45**, 822–839.

Bradler, S., Cliquennois, N. & Buckley, T.R. (2015) Single origin of the Mascarene stick insects: ancient radiation on sunken islands? *BMC Evolutionary Biology*, **15**, 196.

Brown, J.L., Cameron, A., Yoder, A.D. & Vences, M. (2014) A necessarily complex model to explain the biogeography of the amphibians and reptiles of Madagascar. *Nature Communications*, **5**, 5046.

Buerki, S., Devey, D.S., Callmander, M.W., Phillipson, P.B. & Forest, F. (2013) Spatio-temporal history of the endemic genera of Madagascar. *Botanical Journal of the Linnean Society*, **171**, 304–329.

Carranza, S., Arnold, E.N., Mateo, J.A. & López-Jurado, L.F. (2000) Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 637–649.

Carranza, S. & Arnold, E.N. (2012) A review of the geckos of the genus Hemidactylus (Squamata: Gekkonidae) from Oman based on morphology, mitochondrial and nuclear data, with descriptions of eight new species. *Zootaxa*, **3378**, 1–85.

Carton, J.A. & Giese, B.S. (2008) A reanalysis of ocean climate using simple ocean data assimilation (SODA). *Monthly Weather Review*, **136**, 2999–3017.

Cheke, A. & Hume, J. (2008) *Lost land of the Dodo: an ecological history of Mauritius. Réunion & Rodrigues*. Poyser, London.

Collier, J.S., Sansom, V., Ishizuka, O., Taylor, R.N., Minshull, T.A. & Whitmarsh, R.B. (2008) Age of Seychelles-India break-up. *Earth and Planetary Science Letters*, **272**, 264–277.

Crottini, A., Madsen, O., Poux, C., Strauss, A., Vieites, D.R. & Vences, M. (2012) Vertebrate time-tree elucidates the biogeographic pattern of a major biotic change around the K-T boundary in Madagascar. *Proceedings of the National Academy of Sciences of the USA*, **109**, 5358–5363.

Darwin, C. (1859) *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life*. John Murray, London.

Debeuf, D. (2009) *Étude de l'évolution volcano-structurale et magmatique de Mayotte, Archipel des Comores, océan Indien: approches structurale, pétrographique, géochimique et géochronologique*, Dissertation. Université de La Réunion, La Réunion.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Emerick, C.M. & Duncan, R.A. (1982) Age progressive volcanism in the Comores Archipelago, western Indian Ocean and implications for Somali plate tectonics. *Earth and Planetary Science Letters*, **60**, 415–428.

Fuchs, J., Pons, J., Goodman, S.M., Bretagnolle, V., Melo, M., Bowie, R.C.K., Currie, D., Safford, R., Virani, M.Z., Thomsett, S., Hija, A., Cruaud, C. & Pasquet, E. (2008) Tracing the colonization history of the Indian Ocean scops-owls (Strigiformes: *Otus*) with further insight into the spatio-temporal origin of the Malagasy avifauna. *BMC Evolutionary Biology*, **8**, 197.

Gamble, T., Bauer, A.M., Greenbaum, E. & Jackman, T.R. (2008) Out of the blue: a novel, trans-atlantic clade of geckos (Gekkota, Squamata). *Zoologica Scripta*, **37**, 355–366.

Gamble, T., Bauer, A.M., Colli, G.R., Greenbaum, E., Jackman, T.R., Vitt, L.J. & Simons, A.M. (2011) Coming to America: multiple origins of New World geckos. *Journal of Evolutionary Biology*, **24**, 231–244.

Gehring, P.-S., Glaw, F., Gehara, M., Ratsoavina, F.M. & Vences, M. (2013) Northern origin and diversification in the central lowlands? – Complex phylogeography and taxonomy of widespread day geckos (*Phelsuma*) from Madagascar. *Organisms Diversity & Evolution*, **13**, 605–620.

Gibbons, A.D., Whittaker, J.M. & Müller, R.D. (2013) The breakup of East Gondwana: assimilating constraints from Cretaceous ocean basins around India into a best-fit tectonic model. *Journal of Geophysical Research: Solid Earth*, **118**, 808–822.

Goodman, S.M. & Benstead, J.P. (eds., 2003) *The Natural History of Madagascar*. University of Chicago Press, Chicago.

Grechko, V.V. (2013) The problems of molecular phylogenetics with the example of squamate reptiles: mitochondrial DNA markers. *Molecular Biology*, **47**, 55–74.

Hajash, A. & Armstrong, R.L. (1972) Paleomagnetic and radiometric evidence for the age of the Comores Islands, west central Indian Ocean. *Earth and Planetary Science Letters*, **16**, 231–236.

Hawlitschek, O. & Glaw, F. (2013) The complex colonization history of nocturnal geckos (*Paroedura*) in the Comoros Archipelago. *Zoologica Scripta*, **42**, 135–150.

Hawlitschek, O., Nagy, Z.T. & Glaw, F. (2012) Island evolution and systematic revision of Comoran snakes: why and when subspecies still make sense. *PLoS ONE*, **7**, e42970.

Hawlitschek, O., Scherz, M.D., Straube, N. & Glaw, F. (2016) Resurrection of the Comoran fish scale gecko *Geckolepis humbloti* Vaillant, 1887 reveals a disjunct distribution caused by natural overseas dispersal. *Organisms Diversity & Evolution*, **16**, 289–298.

Heads, M. (2011) Old taxa on young islands: a critique of the use of island age to date island-endemic clades and calibrate phylogenies. *Systematic Biology*, **60**, 204–218.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hipsley, C.A. & Müller, J. (2014) Beyond fossil calibrations: realities of molecular clock practices in evolutionary biology. *Frontiers in Genetics*, **5**, 1–11.

Ho, S.Y.W., Tong, K.J., Foster, C.S.P., Ritchie, A.M., Lo, N. & Crisp, M.D. (2015) Biogeographic calibrations for the molecular clock. *Biology Letters*, **11**, 20150194.

Hume, J., Middleton, G., Box, P.O. & Bay, S. (2011) A preliminary vertebrate palaeontological cave survey of the Comoros Islands. *Phelsuma*, **19**, 27–40.

Louette, M., Meirte, D. & Jocqué, R. (2004) *La faune terrestre de l'archipel des Comores*. MRAC, Tervuren.

MacLeod, A., Rodríguez, A., Vences, M., Orozco-terWengel, P., García, C., Trillmich, F., Gentile, G., Caccone, A., Quezada, G. & Steinfartz, S. (2015) Hybridization masks speciation in the evolutionary history of the Galápagos marine iguana. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20150425.

Matzke, N.J. (2013) Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography*, **5**, 242–248.

Miller, K.G., Kominz, M.A., Browning, J.V., Wright, J.D., Mountain, G.S., Katz, M.E., Sugarman, P.J., Cramer, B.S., Christie-Blick, N. & Pekar, S.F. (2005) The phanerozoic record of global sea-level change. *Science*, **310**, 1293–1298.

Montaggioni, L.F. & Nougier, J. (1981) Les enclaves de roches détritiques dans les volcans d'Anjouan (Archipel des Comores). Origine et interprétation dans le cadre de l'évolution du Canal de Mozambique. *Bulletin de la Société Géologique de France*, **Series 7, 23**, 595–601.

Near, T.J., Meylan, P.A. & Shaffer, H.B. (2005) Assessing concordance of fossil calibration points in molecular clock studies: an example using turtles. *The American Naturalist*, **165**, 137–146.

Nicholson, K.E., Glor, R.E., Kolbe, J.J., Larson, A., Blair, Hedges S. & Losos, J.B. (2005) Mainland colonization by island lizards. *Journal of Biogeography*, **32**, 929–938.

Nougier, J., Cantagrel, J.M. & Karche, J.P. (1986) The Comores Archipelago in the western Indian Ocean: volcanology, geochronology and geodynamic setting. *Journal of African Earth Sciences (1983)*, **5**, 135–144.

Nussbaum, R.A. & Raxworthy, C.J. (1998) Revision of the genus *Ebenavia* Boettger (Reptilia: Squamata: Gekkonidae). *Herpetologica*, **54**, 18–34.

Ohba, M., Samonds, K.E., LaFleur, M., Ali, J.R. & Godfrey, L.R. (2016) Madagascar's climate at the K/P boundary and its impact on the island's biotic suite. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **441**, 688–695.

Pakenham, R.H.W. (1983) The reptiles and amphibians of Zanzibar and Pemba Islands (with a note on the freshwater fishes). *Journal of the East Africa Natural Society and National Museum*, **177**, 1–43.

Parent, C.E., Caccone, A. & Petren, K. (2008) Colonization and diversification of Galápagos terrestrial fauna: a phylogenetic and biogeographical synthesis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 3347–3361.

Pasquet, E., Pons, J.M., Fuchs, J., Cruaud, C. & Bretagnolle, V. (2007) Evolutionary history and biogeography of the drongos (Dicruridae), a tropical Old World clade of corvoid passerines. *Molecular Phylogenetics and Evolution*, **45**, 158–167.

Plummer, P.S. & Belle, E.R. (1995) Mesozoic tectono-stratigraphic evolution of the Seychelles microcontinent. *Sedimentary Geology*, **96**, 73–91.

de Queiroz, A. (2005) The resurrection of oceanic dispersal in historical biogeography. *Trends in Ecology and Evolution*, **20**, 68–73.

Rassmann, K. (1997) Evolutionary age of the Galápagos iguanas predates the age of the present Galápagos islands. *Molecular Phylogenetics and Evolution*, **7**, 158–172.

Renner, S.S., Strijk, J.S., Strasberg, D. & Thébaud, C. (2010) Biogeography of the Monimiaceae (Laurales): a role for East Gondwana and long-distance dispersal, but not West Gondwana. *Journal of Biogeography*, **37**, 1227–1238.

Rocha, S., Carretero, M.A. & Harris, D.J. (2005a) Mitochondrial DNA sequence data suggests two independent colonizations of the Comoros archipelago by chameleons of the genus *Furcifer*. *Belgian Journal of Zoology*, **135**, 39–42.

Rocha, S., Carretero, M.A. & Harris, D.J. (2005b) Diversity and phylogenetic relationships of *Hemidactylus* geckos from the Comoro islands. *Molecular Phylogenetics and Evolution*, **35**, 292–299.

Rocha, S., Posada, D., Carretero, M.A. & Harris, D.J. (2007) Phylogenetic affinities of Comoroan and East African day geckos (genus *Phelsuma*): multiple natural colonisations, introductions and island radiations. *Molecular Phylogenetics and Evolution*, **43**, 685–692.

Rocha, S., Vences, M., Glaw, F., Posada, D. & Harris, D.J. (2009) Multigene phylogeny of Malagasy day geckos of the genus *Phelsuma*. *Molecular Phylogenetics and Evolution*, **52**, 530–537.

Rocha, S., Carretero, M.A. & Harris, D.J. (2010) On the diversity, colonization patterns and status of *Hemidactylus* spp. (Reptilia: Gekkonidae) from the Western Indian Ocean Islands. *Herpetological Journal*, **20**, 83–89.

Rohling, E.J., Foster, G.L., Grant, K.M., Marino, G., Roberts, A.P., Tamisiea, M.E. & Williams, F. (2014) Sea-level and deep-sea-temperature variability over the past 5·3 million years. *Nature*, **508**, 477–482.

Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3·2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, **61**, 539–542.

Saddul, P. (1995) *Mauritius – a geomorphological analysis*. Mahatma Gandhi Institute, Mauritius.

Samonds, K.E., Godfrey, L.R., Ali, J.R., Goodman, S.M., Vences, M., Sutherland, M.R., Irwin, M.T. & Krause, D.W. (2012) Spatial and temporal arrival patterns of Madagascar's vertebrate fauna explained by distance, ocean currents, and ancestor type. *Proceedings of the National Academy of Sciences of the USA*, **109**, 5352–5357.

Sequeira, A.A., Lanteri, A.A., Scataglini, M.A., Confalonieri, V.A. & Farrell, B.D. (2000) Are flightless *Galapaganus* weevils older than the Galapagos islands they inhabit? *Heredity*, **85**, 20–29.

Sherrod, D.R. (2009) Hawaiian Islands – geology. *Encyclopedia of Islands* (ed. by R.G. Gillespie and D.A. Clague), pp. 404–410. University of California Press, Berkeley.

Sheth, H.C., Mahoney, J.J. & Baxter, A.N. (2003) Geochemistry of lavas from Mauritius, Indian Ocean: mantle sources and petrogenesis. *International Geology Review*, **45**, 780–797.

Silvestro, D. & Michalak, I. (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution*, **12**, 335–337.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Strickland, M.S., Hawlena, D., Reese, A., Bradford, M.A. & Schmitz, O.J. (2013) Trophic cascade alters ecosystem carbon exchange. *Proceedings of the National Academy of Sciences of the USA*, **110**, 11035–11038.

Thorne, J.L. & Kishino, H. (2002) Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology*, **51**, 689–702.

Thorpe, R.S., Leadbeater, D.L. & Pook, C.E. (2005) Molecular clocks and geological dates: cytochrome b of *Anolis extremus* substantially contradicts dating of Barbados emergence. *Molecular Ecology*, **14**, 2087–2096.

Tolley, K.A., Townsend, T.M. & Vences, M. (2013) Large-scale phylogeny of chameleons suggests African origins and Eocene diversification. *Proceedings of the Royal Society B: Biological Sciences*, **280**, 20130184.

Townsend, T.M., Tolley, K.A., Glaw, F., Böhme, W. & Vences, M. (2011) Eastward from Africa: palaeocurrent-mediated chameleon dispersal to the Seychelles islands. *Biology Letters*, **7**, 225–228.

Vences, M., Vieites, D.R., Glaw, F., Brinkmann, H., Kosuch, J., Veith, M. & Meyer, A. (2003) Multiple overseas dispersal in amphibians. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 2435–2442.

Vinson, J. & Vinson, J.M. (1969) The saurian fauna of the Mascarene Islands. *The Mauritius Institute Bulletin*, **6**, 203–220.

Warren, B.H., Bermingham, E., Bowie, R.C.K., Prys-Jones, R.P. & Thébaud, C. (2003) Molecular phylogeography reveals island colonization history and diversification of western Indian Ocean sunbirds (*Nectarinia*: Nectariniidae). *Molecular Phylogenetics and Evolution*, **29**, 67–85.

Warren, B.H., Bermingam, E., Prys-Jones, R.P. & Thébaud, C. (2005) Tracking island colonization history and phenotypic shifts in Indian Ocean bulbuls (*Hypsipetes*: Pycnonotidae). *Biological Journal of the Linnean Society*, **85**, 271–287.

Warren, B.H., Bermingham, E., Prys-Jones, R.P. & Thébaud, C. (2006) Immigration, species radiation and extinction in a highly diverse songbird lineage: white-eyes on Indian Ocean islands. *Molecular Ecology*, **15**, 3769–3786.

Warren, B.H., Strasberg, D., Bruggemann, J.H., Prys-Jones, R.P. & Thébaud, C. (2010) Why does the biota of the Madagascar region have such a strong Asiatic flavour? *Cladistics*, **26**, 526–538.

Wells, N.A. (2003) Some hypotheses on the Mesozoic and Cenozoic palaeoenvironmental history of Madagascar. *The Natural History of Madagascar* (ed. by S.M. Goodman and J.P. Benstead), pp. 16–34. University of Chicago Press, Chicago.

Wilmé, L., Goodman, S.M. & Ganzhorn, J.U. (2006) Biogeographic evolution of Madagascar's microendemic biota. *Science*, **312**, 1063–1065.

Yoder, A.D. & Nowak, M.D. (2006) Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 405–431.

SUPPORTING INFORMATION

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

Appendix S1 Extended materials, methods and results.
Appendix S2 Data of samples used in this study.
Appendix S3 Additional figures.

BIOSKETCHES

Oliver Hawlitschek is an evolutionary biologist interested in speciation, species delimitation, and biogeography, with a focus on the Western Indian Ocean region. He conducted his doctoral dissertation in the lab of Frank Glaw at the Bavarian State Collection of Zoology, Munich, Germany.

Frank Glaw and **Miguel Vences** have studied the herpetofauna of Madagascar and surrounding islands since 1987 in collaboration with the University of Antananarivo, Madagascar.

Author contributions: OH and FG designed the study. FG and MV provided the institutional framework. OH, PSG, FR, NC, AC, JN, MV and FG provided samples. OH conducted DNA sequencing, phylogenetic, and molecular clock analyses. AWL and AC conducted further DNA sequencing. MV conducted further molecular clock analyses. EFAT constructed the haplotype networks. OH led the writing of the manuscript. All authors contributed to the writing.

Editor: Krystal Tolley.