ORIGINAL ARTICLE

Older than the islands: origin and diversification of Galápagos leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) by multiple colonizations

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ABSTRACT

Aim We re-examine the biogeography of the leaf-toed geckos (*Phyllodactylus*) endemic to the Galápagos Islands. Our aim was to (1) test the multiple-colonization hypothesis against the single-colonization scenario proposed for most terrestrial organisms in the archipelago, (2) estimate the age of colonization of *Phyllodactylus*, and (3) evaluate the roles of dispersal and vicariance in the evolution of these lizards.

Location Galápagos archipelago, Ecuador.

Methods Phylogenetic relationships were evaluated with maximum-likelihood and Bayesian methods, including the estimation of divergence times and species trees. Previous biogeographical hypotheses were evaluated with tree-topology tests.

Results Our results support a multiple-colonization scenario: all but one species of *Phyllodactylus* in the Galápagos Islands belonged to a single radiation. The founders of this radiation colonized the archipelago 13.2 Ma when the islands currently above water had not yet emerged. Ten million years later, the ancestors of *Phyllodactylus darwini* colonized San Cristóbal.

Main conclusions As with other Galápagos organisms, the Pacific coast of South America seems to have been the source for the founders that led to the oldest radiation of leaf-toed geckos. Unlike most Galápagos endemics, however, *P. darwini* might have originated in the Andes. Our phylogenetic hypotheses and recent palaeogeographical data support both dispersal and vicariance as mechanisms leading to the radiation of leaf-toed geckos in the Galápagos Islands.

Keywords
Dispersal, Galápagos, island biogeography, island evolution, lizards, multiple colonizations, *Phyllodactylus*, phylogeny, vicariance.

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INTRODUCTION

The relatively well-known geological history of the Galápagos Islands has provided an excellent framework for evolutionary and biogeographical studies. The oceanic islands in this archipelago formed as the Nazca Plate moved over a volcanic hotspot, resulting in clusters of islands whose ages increase eastwards along the plate (White *et al.*, 1993). The oldest islands – Española, San Cristóbal and Santa Fe (Cox, 1983; Geist, 1996; Geist *et al.*, 2014) – are thus located in the south-eastern part of the archipelago (Fig. 1). The age of the

modern Galápagos islands has been estimated at 3–4 Ma based on geological and fossil evidence (Cox, 1983; Hickman & Lipps, 1985; White *et al.*, 1993; Geist, 1996; Geist *et al.*, 2014). Further research on drowned seamounts east of the oldest islands led to a revised estimate of 14 Ma (Werner *et al.*, 1999). Recent palaeogeographical data suggest that proto-Galápagos islands were available for colonization at least 9 Ma, suggesting that terrestrial organisms had the potential to colonize and evolve since at least that time (Poulakakis *et al.*, 2012; Geist *et al.*, 2014). Furthermore, the estimated age of the Galápagos volcanic hotspot is 80–90 Ma

(Christie et al., 1992; Hoernle et al., 2002). This has important implications for evolutionary studies of Galápagos organisms, because precursors of these islands may have been available for colonization for a longer period of time than was previously thought.

Island colonization by dispersal has been advocated as the main mechanism leading to allopatric speciation in Galápagos land organisms. Recent palaeogeographical studies suggest, however, that several large islands of the central and western archipelago (together with their satellites) were connected during the Pleistocene, and that more minor islands and islets were exposed above water at that time (Poulakakis et al., 2012; Geist et al., 2014; Ali & Aitchison, 2014). These past connections among islands suggest that vicariance may be a more important mechanism for island biogeography in the Galápagos Islands than was previously thought.

The biota of the Galápagos Islands has attracted the attention of evolutionary biologists worldwide ever since Darwin visited the islands in 1835. Nonetheless, the evolution of leaf-toed Phyllodactylus geckos remains poorly understood compared to other Galápagos vertebrates. Strikingly, no phylogenetic tree of Galápagos Phyllodactylus based on DNA sequence data has ever been published. The genus includes approximately 50 species, and occurs from southern North America to southern South America and in the Caribbean (Dixon & Huey, 1970; Torres-Carvajal et al., 2013). Among Pacific oceanic islands, leaf-toed geckos have only colonized Malpelo (P. transversalis) and the Galápagos archipelago. Six species of Phyllodactylus are endemic to the Galápagos Islands (P. barringtonensis Van Denburgh, 1912; P. baurii Garman, 1892; P. darwini Taylor, 1942; P. galapagensis Peters, 1869; P. gilberti Heller, 1903; P. leei Cope, 1889), and

one (P. reissii Peters, 1862) arrived from the mainland approximately 40 years ago (Hoogmoed, 1989). Wright (1983) presented the first evolutionary study of Phyllodactylus in the Galápagos Islands and proposed three colonization events to explain their diversification.

In this study, we investigate the origin, colonization and diversification of Galápagos-endemic leaf-toed geckos based on multilocus analyses of DNA sequence data of a large sample of specimens from the Galápagos Islands and the South American mainland. The objectives of our study were: (1) to infer the phylogeny of Galápagos Phyllodactylus and its closest relatives on the continent; (2) to test the multiple-colonization hypothesis against a single-colonization scenario; (3) to estimate the age of colonization; and (4) to evaluate dispersal and vicariance events based on phylogenetic and palaeogeographical data.

MATERIALS AND METHODS

Character and taxon sampling

We analysed 5136 nucleotides (nt) encompassing six nuclear genes and four mitochondrial genes. The nuclear genes were: brain-derived neurotrophic factor (BDNF); oocyte maturation factor MOS (CMOS); recombination-activating gene 1 (RAG1); recombination-activating gene 2 (RAG2); acetylcholinergic receptor M4 (ACM4); and phosducin (PDC). The mitochondrial genes were NADH dehydrogenase subunit 4 (ND4) and a continuous fragment comprising 12S rRNA, tRNA^{Val} and 16S rRNA.

We obtained sequence data from 38 specimens from eight major islands and one islet, representing five of the six
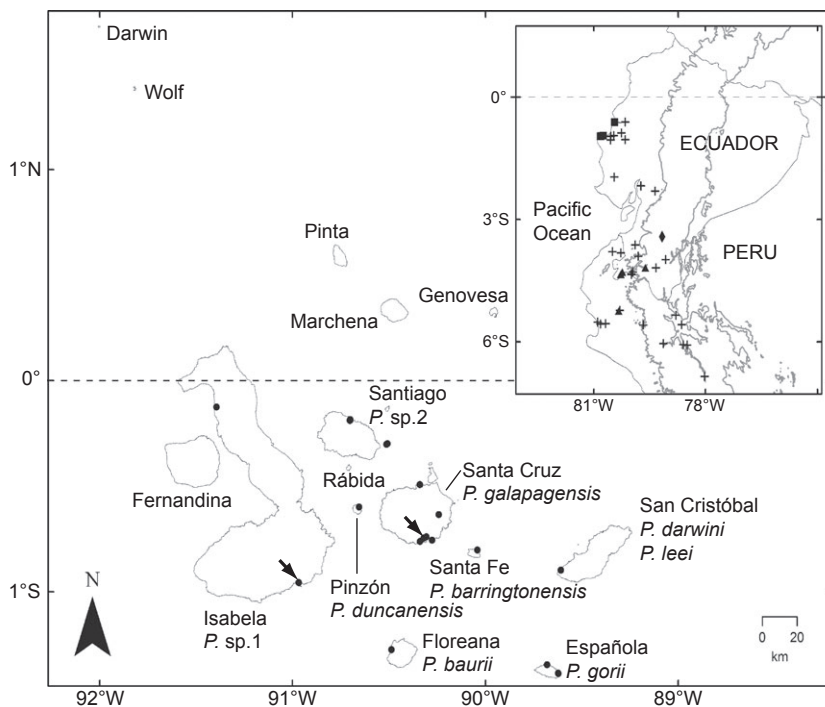
Figure 1 Map of north-western South America (inset) and the Galápagos Islands showing sampling localities. Species names of Phyllodactylus occurring on each sampled island are provided below each island name following the taxonomic arrangement explained in Appendix S3. Mainland species include P. kofordi (triangles), P. leoni (diamond), P. pumilus (squares) and P. reissii (crosses). Data points for the last species were displaced 2.5 km east for clarity. The arrows on Isabela and Santa Cruz islands indicate collection localities for P. reissii. The 1000-m contour in the Andes is shown in grey on the inset map.

recognized endemic species of *Phyllodactylus* from the Galápagos Islands (*P. barringtonensis*, *P. baurii*, *P. darwini*, *P. galapagensis* and *P. leei*). We also obtained data from four specimens of the introduced species *P. reissii* from Isabela and Santa Cruz islands, and 62 specimens representing four species (*P. kofordi*, *P. leoni*, *P. pumilus* and *P. reissii*) from mainland Ecuador and Peru (Fig. 1). In addition, we used GenBank sequences of mainland *P. reissii* and 29 outgroup taxa. The number of outgroup taxa varied between analyses as explained below. Voucher and locality data for the sequences generated in this study are available in GenBank (*BDNF*: KJ914270–KJ914362; *CMOS*: KJ914099–KJ914187; *RAG1*: KJ913916–KJ914007; *RAG2*: KJ913823–KJ913915; *ACM4*: KJ914456–KJ914542; *PDC*: KJ914008–KJ914098; *ND4*: KJ914188–KJ914269; 12S rRNA–tRNA^{Val}–16S rRNA: KJ914363–KJ914455) and Torres-Carvajal *et al.* (2013), and those for the outgroup taxa are given by Gamble *et al.* (2011) and Blair *et al.* (2009). The alignments and phylogenetic trees are available in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S15954).

Laboratory protocols

Total genomic DNA was digested and extracted from liver or muscle tissue using a guanidinium isothiocyanate extraction protocol. Tissue samples were first mixed with proteinase K and lysis buffer and digested overnight prior to extraction. DNA samples were quantified using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA), resuspended, and diluted to 25 ng μL^{-1} in ddH$_2$O prior to amplification. Primers and amplification protocols follow Gamble *et al.* (2008) and Blair *et al.* (2009).

Alignment and model selection

Data were assembled and aligned in Geneious 5.5 (Drummond *et al.*, 2010) under the default settings for mafft (Katoh & Toh, 2010). Ribosomal (12S, tRNA^{Val} and 16S) gene regions with multiple gaps were realigned to minimize indels and optimize nucleotide identities among different individuals. *ND4* and nuclear gene sequences were translated into amino acids in mesquite 2.75 (Maddison & Maddison, 2011) to confirm the alignment. Evolutionary models for each gene were selected in jModelTest 2 (Darriba *et al.*, 2012) according to the Bayesian information criterion (see Table S1 in Appendix S1 of the Supporting Information).

Concatenated tree estimation

Phylogenetic relationships based on gene trees were assessed under Bayesian and maximum-likelihood (ML) approaches. We used a data matrix of 5136 nt and 108 terminals, including all samples of *Phyllodactylus* from the Galápagos Islands and mainland South America, as well as four outgroup taxa (*P. nocticolus*, *P. unctus*, *P. xanti* and *Tarentola mauritanica*). For both Bayesian and ML analyses, data were partitioned by

gene (nine partitions), except for the tRNA^{Val} gene, which was included in the same partition as 12S rRNA.

Bayesian phylogenetic analyses were performed in MrBayes 3.2.1 (Ronquist *et al.*, 2012) and beast 1.7.5 (Drummond *et al.*, 2012). To reduce the chance of converging on a local optimum, four runs of 10^8 generations each were performed in both analyses. Trees were sampled every 10,000 generations, resulting in 10,000 saved trees per analysis. Stationarity was confirmed by plotting the log-likelihood (–ln *L*) per generation in the program Tracer 1.5 (Rambaut & Drummond, 2007). The standard deviation of the partition frequencies and the potential scale-reduction factor (Gelman & Rubin, 1992) were also used as convergence diagnostics in MrBayes for the posterior probabilities of bipartitions and branch lengths, respectively. Adequacy of mixing was assessed by examining the acceptance rates for the parameters in MrBayes. Independence of samples for both MrBayes and beast analyses was assessed by examining the effective sample sizes (ESS) in Tracer. Given that some parameters in the beast analysis showed poor convergence and had low ESS values (< 100) even after performing several runs, we used the simplest model (HKY) for all partitions in this analysis.

After analysing convergence, mixing and sampling, the first 1000 trees in the sample were discarded as burn-in from each run. We then confirmed that the four runs of each Bayesian analysis reached stationarity at a similar likelihood score and that the topologies were similar. We used the resulting 36,000 trees from each analysis to calculate posterior probabilities (PP) for each bipartition in a 50% majority-rule consensus tree for the MrBayes analysis, and a maximum clade credibility tree using TreeAnnotator 1.6.1 (Rambaut & Drummond, 2010) for the beast analysis.

The ML tree was obtained using the same partition scheme and inferred models as in the Bayesian analysis (see above). We selected the best tree from 10 runs performed in Garli 2.0 (Zwickl, 2006) using the default settings. These runs resulted in similar trees with similar log-likelihoods, indicating that additional runs were not necessary. Support for individual nodes was assessed with nonparametric bootstrapping (BP) with 100 pseudoreplicates and parameters estimated from each resampled dataset under the model selected for the original dataset.

Divergence time and species tree estimation

Island endemics are not necessarily younger than the islands they inhabit, because they may have survived on former land nearby that has since been submerged. We do not therefore consider island ages to be appropriate for calibrating divergence-time estimation, especially if other kinds of data (e.g. fossils) are available. We conducted a chronophylogenetic analysis (i.e. co-estimation of rates, divergence times and phylogeny) under the Bayesian framework implemented in beast 1.7.5 (Drummond *et al.*, 2012), using node-age information from the time-calibrated gecko phylogeny presented

by Gamble *et al.* (2011), which was based on fossil and biogeographical data. Using the same genes (*ACM4*, *CMOS*, *PDC*, *RAG1* and *RAG2*) employed by Gamble *et al.* (2011), we constructed a data matrix of 42 taxa and 1818 nt including GenBank sequences of all species of Phyllodactylidae from that study, and 13 new sequences of representatives of *Phyllodactylus* from the Galápagos Islands and mainland South America. The main purpose of this analysis was to estimate the age of colonization of the Galápagos archipelago by leaf-toed geckos. Based on the posterior stem ages calculated by Gamble *et al.* (2011: Table 2), we applied the following calibrations: (1) a normally distributed prior (95% CI, 58–87 Ma; mean 72 Ma) representing the maximum age of *Thecadactylus*; (2) a normally distributed prior (95% CI, 53–81 Ma; mean of 67 Ma) representing the maximum age of the clade including *Garthia*, *Gymnodactylus*, *Homonota*, *Phyllodactylus* and *Phyllopezus*; and (3) a normally distributed prior (95% CI, 7–17 Ma; mean of 12 Ma) representing the maximum age of *Tarentola americana*. Model selection and other analytical details are the same as those explained above for the phylogenetic analyses, except that the number of generations was reduced to 10^7.

We also estimated a Galápagos *Phyllodactylus* species tree from the multilocus trees under a coalescent model – and simultaneously estimated divergence times – using the *BEAST method (Heled & Drummond, 2010) implemented in beast 1.7.5. For this analysis, we excluded the three North American species of *Phyllodactylus* from the matrix used in the concatenated tree estimation, and assigned the remaining 105 sequences to 15 species. This a priori species assignment was based on current taxonomy as well as the concatenated trees obtained in this study and included 10 recognized species and five putative species. To calibrate the divergence dating, we used two estimated node ages from the previous analysis to set normal priors for the Galápagos major clade (see Results) and the South American *Phyllodactylus* clade (means of 5.17 and 23.97 Ma, respectively). The analyses were conducted under a model with uncorrelated substitution rates among branches and the rate for each branch drawn independently from an underlying lognormal distribution (Drummond *et al.*, 2006); a Yule prior was used for the species tree. Other details for this analysis are the same as those described above for the concatenated tree estimation in beast.

Alternative hypotheses test

Because previous work (Wright, 1983) and our data support more than one colonization for *Phyllodactylus* (see Results), we tested the alternative single-colonization scenario using a Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999) as implemented in paup* (Swofford, 2003). For this test, we used the ML tree obtained in this study (two colonizations) and the alternative ML single-colonization tree (i.e. monophyly of Galápagos *Phyllodactylus*). The latter was constructed in Mesquite 2.75 (Maddison & Maddison,

2011) by collapsing all terminals corresponding to *Phyllodactylus* from the Galápagos into a single clade and leaving relationships among this clade and other species of *Phyllodactylus* as unresolved. This alternative tree was loaded into Garli 2.0 (Zwickl, 2006) as a constraint tree and enforced in an ML analysis. After obtaining the single-colonization ML tree, the SH test was performed in paup* with 10,000 RELL (resampling estimated log-likelihoods) bootstrap replicates.

Wright (1983) published a phenogram of *Phyllodactylus* from the Galápagos Islands (Fig. 2 in Wright, 1983) without including mainland taxa. Following the procedure described above, we also tested Wright's hypothesis under the SH test. In order to build the constraint tree, we modified the relationships between Galápagos taxa in our ML tree to match Wright's phenogram, except for *P. darwini*. According to Wright's tree, this species is sister to other *Phyllodactylus* in the Galápagos, but Wright (1983) did not assume that all species of *Phyllodactylus* from the Galápagos formed a monophyletic group. In fact, he recognized *P. darwini* as being distantly related to other *Phyllodactylus* in the Galápagos Islands. Because our ML tree (see below) is concordant with Wright's results regarding *P. darwini*, placing it as sister to other Galápagos *Phyllodactylus* in our constraint tree would have biased the results of the SH test against Wright's hypothesis.

RESULTS

Tree estimation from concatenated data

The genetic variation among sampled gene regions is summarized in Table S1; *ND4* was the most informative region (see Fig. S1 in Appendix S1), with sequence divergence ranging from 0.00 to 0.33 (mean = 0.22 ± 0.04 SD; see Table S2 & Fig. S2 in Appendix S1). Bayesian analyses and the ML analysis yielded largely congruent phylogenetic trees, in which South American species of *Phyllodactylus* were split into two main clades with maximum support values in all analyses, herein named clades A and B. The tree obtained in beast (Fig. S3 in Appendix S2) differed from those from MrBayes (Fig. 2) and Garli (Fig. S4 in Appendix S2) in placing the North American *Phyllodactylus* clade (*P. xanti* (*P. nocticolus*, *P. unctus*)) sister to Clade A, with strong support (PP = 1). The trees obtained in MrBayes and Garli recovered clades A and B as sister taxa, albeit with weak support (PP = 0.82; BP = 0.61). Nonetheless, we chose the MrBayes tree to represent the latter topology (Fig. 2) as it is congruent with a tree containing a larger taxonomic sampling (see 'Divergence time estimation' below).

Clade A contained the mainland coastal species *P. reissii* as sister (PP = 1, BP = 1) to a strongly supported clade (PP = 1; BP = 1) that comprised all the species of *Phyllodactylus* from the Galápagos Islands included in this study, except for *P. darwini* from San Cristóbal. This last species was deeply nested within Clade B as the sister to *P. leoni* (PP = 1; BP = 0.76), an Andean species from southern
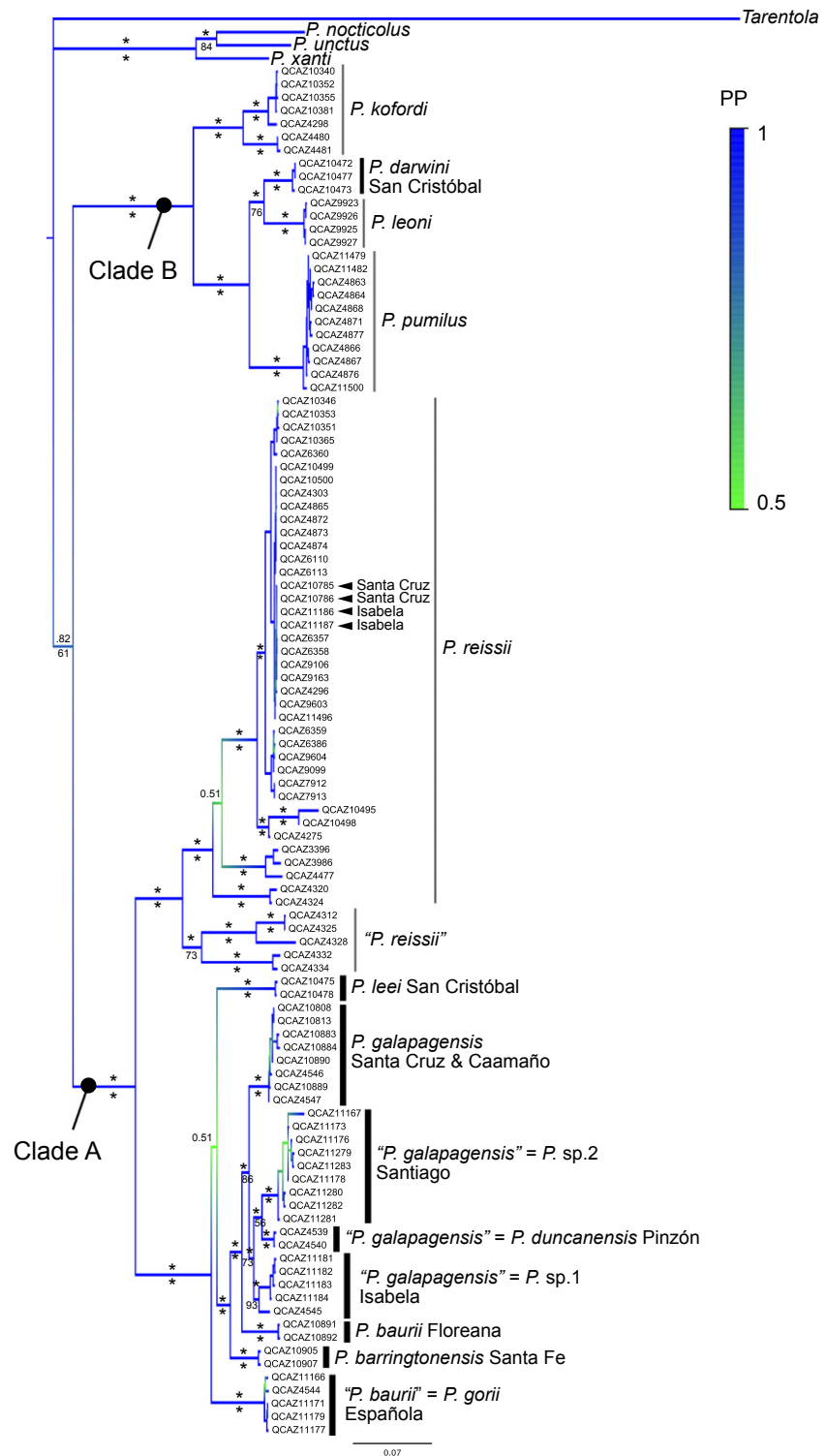
Figure 2 Phylogeny of *Phyllodactylus* from the Galápagos and the continent. This majority-rule (50%) consensus tree of 36,000 trees was obtained from a Bayesian analysis of 108 specimens and 5136 nucleotides. The data set was divided into nine partitions corresponding to three mitochondrial and six nuclear gene regions. Numbers on branches correspond to posterior probability (PP, above) and maximum-likelihood bootstrap (below) values. Asterisks correspond to posterior probability values > 0.99 and bootstrap values > 95%. On short branches, support values are not shown as numbers for clarity. Voucher numbers of sequences obtained in this study are indicated for each terminal. Island names are shown for specimens from the Galápagos Islands (black vertical bars). Arrows indicate specimens of *P. reissii* collected in the Galápagos Islands. For the taxonomic changes proposed in this paper (see Appendix S3), currently recognized names are placed in inverted commas.

Ecuador; together with *P. pumilus* from the Pacific coast in Ecuador, these species formed a clade (PP = 1; BP = 1), which was in turn sister to *P. kofordi* (PP = 1; BP = 1) from Andean foothills in southern Ecuador and northern Peru. Thus, species of *Phyllodactylus* in the Galápagos originated from at least two colonization events (Fig. 2). Specimens of *P. reissii* from Santa Cruz and Isabela were deeply nested

within the *P. reissii* clade, together with specimens from several localities along the coast of Ecuador and Peru; their branches were as short as those representing most specimens of this species (Fig. 2).

Among Galápagos leaf-toed geckos, the following species or island groups were recovered with strong support (PP = 1; BP > 0.9): *P. darwini* (San Cristóbal); *P. baurii*

Table 1 Results of the Shimodaira–Hasegawa tests of alternative phylogenetic hypotheses of relationships among species of *Phyllodactylus* from the Galápagos Islands. The observed difference ($\delta \ln L$) in log-likelihood score ($-\ln L$) between each alternative hypothesis and the maximum-likelihood concatenated tree obtained in this study are presented, along with the *P*-values for each test. Significance level: $\alpha = 0.05$.

Alternative hypothesis	$-\ln L$	$\delta \ln L$	P
This study	33490.47812	–	–
Wright (1983: Fig. 2)	33540.26251	49.78439	0.0059
Monophyly of *Phyllodactylus* endemic taxa in Galápagos	34059.67677	569.19865	0.0000

(Española); *P. leei* (San Cristóbal); *P. barringtonensis* (Santa Fe); *P. baurii* (Floreana); *P. galapagensis* (Santa Cruz and Caamaño islet); *P. galapagensis* (Isabela); *P. galapagensis* (Pinzón); and *P. galapagensis* (Santiago). *Phyllodactylus baurii* is paraphyletic in our tree (Fig. 2); we therefore recognize the species in Española as *P. gorii* (see Appendix S3). Even though '*P. galapagensis*' is monophyletic with strong support in the Bayesian analysis (PP = 1; BP = 0.86), it contains four well-supported clades corresponding to four different islands or island groups, which we argue represent different species; we recognize the species on Pinzón as *P. duncanensis* (see Appendix S3).

In the SH tests comparing our best ML topology to both the monophyly of Galápagos *Phyllodactylus* (i.e. single-colonization scenario) and the hypothesis presented by Wright (1983), the alternative topologies represented significantly worse alternatives than our best tree (see Table 1). We therefore consider our concatenated tree (Fig. 2) to be the best working phylogenetic hypothesis for *Phyllodactylus* from the Galápagos Islands.

Species-tree estimation

The species tree (Fig. 3) was largely concordant with the concatenated tree described above (Fig. 2). In terms of topology, the only differences were the position of *P. leei* from San Cristóbal and *P. baurii* from Española. The former was recovered as sister to all other Galápagos species in Clade A in the species tree, whereas the latter replaced it in the concatenated tree. Nonetheless, the corresponding bipartitions had low support in both cases (PP = 0.39 and 0.51, respectively). Another difference was that the relationships among Galápagos *Phyllodactylus* in Clade A had low support values (PP < 0.8) in the species tree, whereas the corresponding species/island groups in the concatenated tree were strongly supported. In contrast, *P. darwini* was recovered as sister to *P. leoni* with strong support (PP = 0.92) in Clade B.

Divergence-time estimation

The topology of the phylogeny of Phyllodactylidae based on nuclear markers (Fig. 4) was congruent with the concatenated-gene and species trees described above. The monophyly of *Phyllodactylus* and its basal split into a North American and a South American clade was strongly supported (PP = 1). In contrast, as expected from the low informativeness of nuclear loci (Table S1 and Fig. S1 in Appendix S1), the relationships among Galápagos taxa were poorly resolved (Fig. 4). Despite this, the Galápagos clade (excluding *P. darwini*) was strongly supported (PP = 1) and had an estimated node age (i.e. minimum age) of 5.17 Ma, with a 95% highest posterior density (HPD) interval (i.e. the shortest interval that contains 95% of the sampled values) of 2.63–8.02 Ma. Unlike the concatenated gene and species trees, *P. darwini* was sister to *P. pumilus* in this tree, albeit with moderate support (PP = 0.87); together they were sister to *P. leoni* (PP = 1). The estimated node ages were 4.32 Ma (HPD, 1.66–7.14 Ma) and 5.84 Ma (HPD, 2.79-9.31 Ma), respectively (Fig. 3).

The species tree (Fig. 3) yielded an estimated node age of 5.45 Ma (HPD, 4.54–6.36 Ma) for the Galápagos clade (PP = 1). This age represents the split between *P. leei* (San Cristóbal) and all other species in this clade. The next species to split off was *P. baurii* (Española) at 4.68 Ma (HPD, 3.30–5.95 Ma), followed by *P. barringtonensis* (Santa Fe) at 3.81 Ma (HPD, 2.46–5.30 Ma), and *P. baurii* (Floreana) at 3.02 Ma (HPD, 1.74–4.39 Ma). The remaining taxa corresponded to *P. galapagensis* as currently recognized, where the oldest split corresponded to the population from Santa Cruz (2.38 Ma; HPD, 1.21–3.56 Ma), followed by the population from Isabela (1.87 Ma; HPD, 0.87–2.95 Ma) and more recently the split between the populations on Pinzón and Santiago (1.16 Ma; HPD, 0.24–2.13 Ma). Regarding the second colonization event, the split between *P. darwini* from San Cristóbal and its sister Andean species *P. leoni* was estimated to have occurred 3.09 Ma (HPD, 0.27–6.20 Ma).

DISCUSSION

The origin of Galápagos leaf-toed geckos

Dispersal is fundamental to the evolution of biodiversity in oceanic islands (Cowie & Holland, 2006). Capable of travelling long distances by passive drift on ocean currents (de Queiroz, 2005), geckos are among the best non-volant vertebrates at overseas dispersal. Some species have reached remote islands in the Pacific Ocean (Zug, 2013); others have crossed the Atlantic Ocean (Carranza *et al.*, 2000). Leaf-toed geckos and several other Galápagos endemics possibly arrived in this archipelago via drifting events enhanced by the Humboldt Current, which attained its present conditions during the Miocene (Amiot *et al.*, 2008; Sepulchre *et al.*, 2009). This oceanic current runs north along mainland South America and then diverts westward at around the latitude of the Galápagos Islands. Based on genetic similarities in allozyme data, Wright (1983) hypothesized that the diversity of leaf-toed geckos in the Galápagos Islands stems from three colonization events (Fig. 5), similar to the situation reported for

Figure 3 *Phyllodactylus* species tree, with divergence time estimates in millions of years. The species tree is a maximum clade credibility tree of 36,000 samples obtained from a *BEAST analysis of 105 specimens and nine gene regions (5136 nt). The numbers beside branches correspond to posterior probability (PP) values. Node bars represent 95% highest posterior density (HPD) intervals of sampled age-estimate values. Species from the Galápagos Islands (with island of occurrence in parentheses) are shaded in grey.
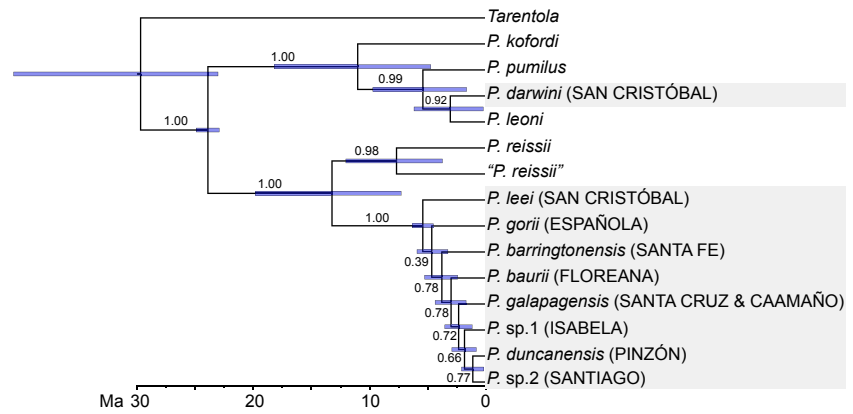
Figure 4 Phylogenetic tree of Phyllodactylidae, with divergence time estimates in millions of years. This maximum clade credibility tree of 36,000 samples was obtained from a Bayesian analysis of 42 taxa and five nuclear loci (1818 nt). Asterisks correspond to posterior probability (PP) values > 0.90; PP values < 80% are not shown for clarity. Galápagos species are shaded in grey.
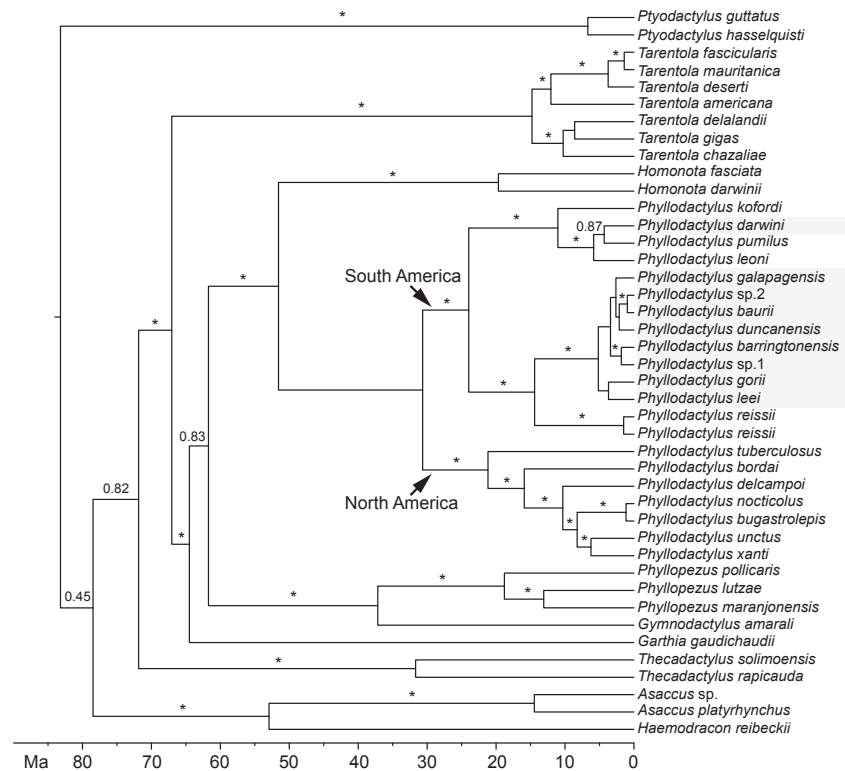
Tarentola geckos in the Canary Islands (Carranza *et al.*, 2002). One event gave rise to the population on Wolf Island currently recognized as *P. gilberti*. Because we were unable to obtain samples of this species, this colonization event could not be tested in a phylogenetic framework. The other two events proposed by Wright (1983), however, are in agreement with our results in that one led to the establishment of the single species *P. darwini* on San Cristóbal, whereas the other explains the remaining diversity of leaf-toed geckos in the archipelago (Fig. 5). This colonization pattern contrasts with the single-colonization scenario (rejected by our data) proposed for all other groups of Galápagos terrestrial animals except *Microlophus* lava lizards (Table 2). The intervals between colonization events differ strongly, however, between lava lizards and leaf-toed geckos. Lava lizards colonized the

archipelago twice within a 2.5-Myr period (Benavides *et al.*, 2009), whereas the two colonization events by leaf-toed geckos reported in this paper are 10 Myr apart (Table 2). Multiple colonizations have been detected among Galápagos plants (Andrus *et al.*, 2009) and, although rare, they form part of the biogeography of other oceanic islands worldwide, such as Hawaii, the Society Islands, the Marquesas Islands and the Canary Islands (Juan *et al.*, 2000; Cowie & Holland, 2008; Gillespie *et al.*, 2008).

Our phylogenetic hypotheses provide information for the first time on putative mainland relatives of Galápagos leaf-toed geckos. These results, however, should be interpreted with caution, as many species of *Phyllodactylus* from the Pacific coast south of Ecuador (e.g. *P. angustidigitus*, *P. clinatus*, *P. gerrhopygus* and *P. microphyllus*) were not included
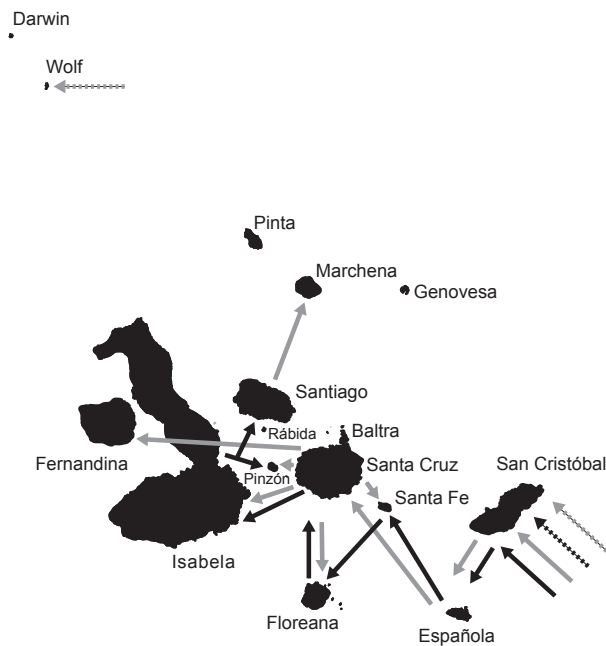
Figure 5 Colonization routes within the *Phyllodactylus* Galápagos radiation inferred from the phylogenetic hypotheses obtained in this study (black arrows) under a strict dispersal model. The colonization hypothesis proposed by Wright (1983) is also presented (grey arrows). Solid arrows correspond to the major radiation of leaf-toed geckos in the Galápagos. Dotted arrows correspond to separate colonization events in San Cristóbal (*P. darwini*) and Wolf (*P. gilberti*; not included in this study).

in our analyses. The closest relative of the western radiation (Clade A in Fig. 2) of Galápagos leaf-toed geckos is *Phyllodactylus reissii* (Figs 2–4), a species that naturally occurs along the Pacific coast of Ecuador and north-western Peru (Dixon & Huey, 1970). Nonetheless, our phylogenetic trees (Figs 2 & 3) suggest that *P. reissii* as currently recognized probably represents a species complex. Even though we sampled extensively throughout the coastal region of South America closest to the Galápagos (Fig. 1), the closest relatives of the Galápagos western radiation of leaf-toed geckos could live south of our sampling area, similar to the situation determined for Galápagos giant tortoises (Caccone *et al.*, 1999).

The geographical distribution of the ancestor of *P. darwini* might differ from that of the western radiation. Its sister species, *P. leoni* (Fig. 3), occurs at mid-elevations in the Andes in southern Ecuador, at nearly 2000 m (Torres-Carvajal *et al.*, 2013). This area corresponds to the upper basin of the Jubones River, one of the major rivers in southern Ecuador that drains into the Pacific Ocean. One possibility is that the ancestor of *P. darwini* and *P. leoni* occurred in the Pacific lowlands nearby, from where it dispersed both to the Galápagos and the Andes. An alternative scenario is also possible. According to our data, the colonization leading to *P. darwini* occurred nearly 3 Ma. At this time, the Andes in southern Ecuador had formed (Simpson, 1979), suggesting that the Jubones River was available as a dispersal route from the

Andes to the Pacific. It is therefore possible that the ancestor of *P. darwini* and *P. leoni* occurred in the Andes, and the founders that evolved into *P. darwini* first dispersed downstream along the Jubones River before drifting on the Humboldt Current towards the Galápagos Islands. Thus, unlike most organisms in this archipelago, *P. darwini* might have its origins in the Andes.

The age of Galápagos leaf-toed geckos

The oldest islands in the Galápagos archipelago, Española and San Cristóbal, are thought to have emerged approximately 2–4 Ma (Geist *et al.*, 2014). Except for marine iguanas, age estimates imply that most colonization events of Galápagos-endemic vertebrates occurred when the present islands were above sea level (Wright, 1983; Caccone *et al.*, 2002; Petren *et al.*, 2005; Arbogast *et al.*, 2006; Benavides *et al.*, 2009). Our species tree yielded an estimated minimum age of 5.45 Ma (HPD, 4.54–6.36 Ma) for the clade representing the western radiation of Galápagos leaf-toed geckos (Fig. 3), which fully overlaps with the wider range estimate of 2.10–8.40 Ma obtained by Wright (1983). The maximum age estimate for the same clade (i.e. the split between the '*P. reissii*' clade and the Galápagos lineage) was, however, 13.24 Ma (HPD, 7.29–19.80 Ma), which means that the absolute age of this radiation is somewhere between 4.54 and 19.80 Ma. This age estimate does not quite overlap with that of the oldest islands currently above sea level, suggesting that the first leaf-toed gecko founders may have landed on what is now submerged island territory. This idea is supported by recent palaeogeographical evidence that suggests there were proto-Galápagos islands (now submerged) available for colonization at least 9 Ma (Geist *et al.*, 2014). The later independent arrival of the ancestor of *P. darwini* to San Cristóbal 3.09 Ma (HPD, 0.27–6.20 Ma) probably happened when this island had already emerged (i.e. 2.4–4.0 Ma; Geist *et al.*, 2014). Although rare, taxa older than the oceanic islands they inhabit have also been documented elsewhere, such as the endemic lobeliads of Hawaii (Givnish *et al.*, 2009).

Radiations of Galápagos-endemic animals have been estimated to be 1–6 Myr old (Caccone *et al.*, 2002; Petren *et al.*, 2005; Arbogast *et al.*, 2006; Schmitz *et al.*, 2007; Benavides *et al.*, 2009), with the notable exceptions of marine iguanas and *Galapaganus* weevils at one extreme (10–20 Ma and > 8.6 Ma, respectively; Wyles & Sarich, 1983; Rassmann, 1997; Sequeira *et al.*, 2008), and Galápagos hawks at the other (< 300 kyr; Bollmer *et al.*, 2006). These age values should be taken with caution, because they were estimated using different methods and are not necessarily comparable. Some of these age estimates were obtained under calibrated phylogenetic analyses (e.g. Benavides *et al.*, 2009), whereas others were based on simple conversion of observed genetic distances into evolutionary divergence times based on a previously estimated rate of evolution (e.g. Arbogast *et al.*, 2006). Furthermore, age estimates might represent different 'types' of age. For example, the node age estimate of clade X

Table 2 Summary of recent studies that estimate the age and number of colonizations of Galápagos-endemic terrestrial fauna. Taxon with number of recognized taxa by each study in parentheses, number of sampled taxa, number of sampled islands with total number of inhabited islands in parentheses, type of genetic markers, number of inferred colonizations, maximum estimated age of colonization in millions of years, and references are presented.

Taxon	Sampled taxa	Sampled islands*	Genetic markers	Number of colonizations	Maximum (stem) age of each colonization (Ma)	References
Non-avian reptiles						
Leaf-toed geckos (6)	6	11 (11)	Allozymes	3	4.7 (western radiation)	Wright (1983)
					4.7 (*P. gilberti*)	Wright (1983)
					6.4 (*P. darwini*)	Wright (1983)
Leaf-toed geckos (11)	10	8 (11)	mtDNA, nDNA	2	13.2 (western radiation)	This study
					3.1 (*P. darwini*)	This study
Lava lizards (7)	7	11 (11)	Allozymes	2	9.5 (eastern radiation)	Wright (1983)
					9.5 (western radiation)	Wright (1983)
Lava lizards (7)	7	7 (11)	Albumins	3	20 (*M. delanonis*)	Lopez et al. (1992)
					11–13 (western radiation)	Lopez et al. (1992)
					6 (eastern radiation)	Lopez et al. (1992)
Lava lizards (10)	10	11 (11)	mtDNA, nDNA	2	2.09–2.79 (eastern radiation)	Benavides et al. (2009)
					3.69–4.54 (western radiation)	Benavides et al. (2009)
Iguanas (3)	2	? (14)	mtDNA	1	13.3–19.1	Rassmann (1997)
Iguanas (3)	3	? (14)	Albumins	1–2	> 15–20†	Wyles & Sarich (1983)
Galápagos tortoises (11)	11	7 (7)	mtDNA	1	2–3	Caccone et al. (2002)
Birds						
Darwin's finches (13)	12	5	mtDNA	1	2.3	Sato et al. (2001)
Mockingbirds (4)	4	9 (13)	mtDNA	1	1.6–5.5	Arbogast et al. (2006)
Galápagos hawk (1)	1	10 (10)	mtDNA, minisatellites	1	0.3	Bollmer et al. (2006)
Insects						
Galagete moths (12)	12	13 (13)	mtDNA, nDNA	1	> 2.9–3.7†	Schmitz et al. (2007)
Galapaganus weevils (10)	8	8 (11)	mtDNA	1	> 8.6–11.5†	Sequeira et al. (2008)

*Only major islands were considered (Darwin, Wolf, Pinta, Marchena, Genovesa, Fernandina, Isabela, Santiago, Pinzón, Santa Cruz, Santa Fe, Floreana, Española and San Cristóbal). Some species also occur in nearby smaller islands and islets.
†Minimum (node) age estimate.
mtDNA, mitochondrial DNA; nDNA, nuclear DNA.

represents the minimum age of that clade (i.e. clade X is at least that old) but reveals nothing about the possible maximum age (i.e. stem age) or the absolute age of clade X.

The roles of dispersal and vicariance

The general dynamic theory (GDT) of oceanic island biogeography was proposed recently (Whittaker et al., 2008, 2010) to provide a framework for explaining patterns of biodiversity, endemism and diversification on oceanic islands, based on biological processes (migration, speciation and extinction) and the life cycle of oceanic islands. Consistent with the GDT, a stepwise colonization down a linear island chain from the oldest to the youngest island, known as the 'progression rule' (Funk & Wagner, 1995), has been proposed as a biogeographical hypothesis for explaining diversity in some island archipelagos, including Hawaii and the Canary Islands (Funk & Wagner, 1995; Juan et al., 2000). The Galápagos Islands are not linearly arranged by age; instead, they form clusters of islands of similar age. The oldest cluster of islands is in the east and the ages of the clusters decrease towards the west (Cox,

1983; White et al., 1993; Geist, 1996). If the progression rule applies in the Galápagos, a general east-to-west colonization pattern is therefore expected. As with most vertebrate endemics (see Table 2 in Parent et al., 2008), Galápagos leaf-toed geckos follow this pattern. Based on our phylogenetic hypotheses (Figs 2 & 3), the founder events that led to most of the diversification of *Phyllodactylus* in the Galápagos occurred in the following sequence (oldest to youngest) under a strict dispersal model: Española or San Cristóbal, Santa Fe, Floreana, Santa Cruz, Isabela, Pinzón and Santiago (Fig. 5).

This traditional interpretation of island colonization strictly focused on dispersal assumes that island geology is fixed in space and time. However, as acknowledged by the GDT, oceanic islands like those that make up the Galápagos archipelago are dynamic entities that experience dramatic geological changes over time (Whittaker et al., 2008, 2010). Recent palaeogeographical studies suggest that the Galápagos archipelago experienced several episodes of island connection and island isolation during the Pleistocene, providing opportunities for contact and isolation among populations of land-locked vertebrates (Poulakakis et al., 2012; Geist et al., 2014;

Ali & Aitchison, 2014). Some authors propose the existence of a large island that linked Floreana and Santa Cruz 2 Ma, as well as Rábida, Santiago, Pinzón and possibly Santa Fe 1 Ma (Poulakakis *et al.*, 2012; Geist *et al.*, 2014). More recently, Ali & Aitchison (2014) inferred several coalescence episodes among the central and western Galápagos islands over the last 700,000 years. It stands to reason that species of *Phyllodactylus* from these islands might have speciated in response to vicariance events (i.e. the division of an older large island into several smaller islands) rather than inter-island dispersal events. Vicariance and dispersal scenarios are both, however, consistent with the phylogeny presented in this paper (Figs 2 & 3), in which species from Floreana, Santa Cruz, Santiago, Pinzón, Santa Fe and Isabela are nested in the same clade. Our estimates for the maximum age of this clade (4.68 Ma, Fig. 3) and the splitting events within it are older than those for the corresponding islands according to the palaeogeographical studies mentioned above. Nonetheless, this does not invalidate the vicariance scenario, because a large island, where the volcanoes that later formed the central and western islands emerged, was probably present at that time. These palaeogeographical studies challenge traditional, dispersal-based biogeographical reconstructions for other terrestrial organisms in the Galápagos Islands, such as the lava lizards (Benavides *et al.*, 2009; Geist *et al.*, 2014) and giant tortoises (Caccone *et al.*, 2002; Poulakakis *et al.*, 2012), and highlight the importance of considering both dispersal and vicariance in the interpretation of biogeographical patterns on oceanic islands.

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REFERENCES

Ali, J.R. & Aitchison, J.C. (2014) Exploring the combined role of eustasy and oceanic island thermal subsidence in shaping biodiversity on the Galápagos. *Journal of Biogeography*, **41**, 1227–1241.

Amiot, R., Göhlich, U.B., Lécuyer, C., de Muizon, C., Cappetta, H., Fourel, F., Héran, M.-A. & Martineau, F. (2008) Oxygen isotope compositions of phosphate from Middle Miocene–Early Pliocene marine vertebrates of Peru. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **264**, 85–92.

Andrus, N., Tye, A., Nesom, G., Bogler, D., Lewis, C., Noyes, R., Jaramillo, P. & Francisco-Ortega, J. (2009) Phylogenetics of *Darwiniothamnus* (Asteraceae: Astereae) – molecular evidence for multiple origins in the endemic flora of the Galápagos Islands. *Journal of Biogeography*, **36**, 1055–1069.

Arbogast, B.S., Drovetski, S.V., Curry, R.L., Boag, P.T., Seutin, G., Grant, P.R., Grant, B.R. & Anderson, D.J. (2006) The origin and diversification of Galapagos mockingbirds. *Evolution*, **60**, 370–382.

Benavides, E., Baum, R., Snell, H.M., Snell, H.L. & Sites, J.W., Jr (2009) Island biogeography of Galápagos lava lizards (Tropiduridae: *Microlophus*): species diversity and colonization of the archipelago. *Evolution*, **63**, 1606–1626.

Blair, C., Méndez de la Cruz, F.R., Ngo, A., Lindell, J., Lathrop, A. & Murphy, R.W. (2009) Molecular phylogenetics and taxonomy of leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) inhabiting the peninsula of Baja California. *Zootaxa*, **2027**, 28–42.

Bollmer, J.L., Kimball, R.T., Whiteman, N.K., Sarasola, J.H. & Parker, P.G. (2006) Phylogeography of the Galápagos hawk (*Buteo galapagoensis*): a recent arrival to the Galápagos Islands. *Molecular Phylogenetics and Evolution*, **39**, 237–247.

Caccone, A., Gibbs, J.P., Ketmaier, V., Suatoni, E. & Powell, J.R. (1999) Origin and evolutionary relationships of giant Galápagos tortoises. *Proceedings of the National Academy of Sciences USA*, **96**, 13223–13228.

Caccone, A., Gentile, G., Gibbs, J.P., Fritts, T.H., Snell, H.L., Betts, J. & Powell, J.R. (2002) Phylogeography and history of giant Galápagos tortoises. *Evolution*, **56**, 2052–2066.

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., Mateo, J.A. & Geniez, P. (2002) Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution*, **23**, 244–256.

Christie, D.M., Duncan, R.A., McBirney, A.R., Richards, M.A., White, W.M., Harpp, K.S. & Fox, C.G. (1992) Drowned islands downstream from the Galapagos hotspot imply extended speciation times. *Nature*, **355**, 246–248.

Cowie, R.H. & Holland, B.S. (2006) Dispersal is fundamental to biogeography and the evolution of biodiversity on oceanic islands. *Journal of Biogeography*, **33**, 193–198.

Cowie, R.H. & Holland, B.S. (2008) Molecular biogeography and diversification of the endemic terrestrial fauna of the Hawaiian Islands. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 3363–3376.

Cox, A. (1983) Ages of the Galápagos Islands. *Patterns of evolution in Galápagos organisms* (ed. by R.I. Bowman, M. Berson and A.E. Levinton), pp. 11–24. American Association for the Advancement of Science, Pacific Division, San Francisco, CA.

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**, 772.

Dixon, J.R. & Huey, R.B. (1970) Systematics of the lizards of the gekkonid genus *Phyllodactylus* of mainland South America. *Los Angeles County Museum Contributions in Science*, **192**, 1–78.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. (2006) Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**, e88.

Drummond, A.J., Ashton, B., Buxton, S., Cheung, M., Cooper, A., Heled, J., Kearse, M., Moir, R., Stones-Havas, S., Sturrock, S., Thierer, T. & Wilson, A. (2010) *Geneious v5.5*. Biomatters, Auckland, New Zealand. Available at: http://www.geneious.com/.

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.

Funk, V.A. & Wagner, W.L. (1995) Biogeographic patterns in the Hawaiian Islands. *Hawaiian biogeography: evolution on a hot spot archipelago* (ed. by W.L. Wagner and V.A. Funk), pp. 379–419. Smithsonian Institution Press, Washington, DC.

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.

Geist, D. (1996) On the emergence and submergence of the Galápagos Islands. *Noticias de Galápagos*, **56**, 5–8.

Geist, D., Snell, H., Snell, H., Goddard, C. & Kurz, M. (2014) Paleogeography of the Galápagos Islands and biogeographical implications. *The Galápagos: a natural laboratory for the earth sciences* (ed. by K. Harpp, E. Mittelstaedt, N. d'Ozouville and D.W. Graham). American Geophysical Union, Washington, DC.

Gelman, A. & Rubin, D.B. (1992) Inference from iterative simulation using multiple sequences. *Statistical Science*, **7**, 457–472.

Gillespie, R.G., Claridge, E.M. & Goodacre, S.L. (2008) Biogeography of the fauna of French Polynesia: diversification within and between a series of hot spot archipelagos. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 3335–3346.

Givnish, T.J., Millam, K.C., Mast, A.R., Paterson, T.B., Theim, T.J., Hipp, A.L., Henss, J.M., Smith, J.F., Wood, K.R. & Sytsma, K.J. (2009) Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). *Proceedings of the Royal Society B: Biological Sciences*, **276**, 407–416.

Heled, J. & Drummond, A.J. (2010) Bayesian inference of species trees from multilocus data. *Molecular Biology and Evolution*, **27**, 570–580.

Hickman, C.S. & Lipps, J.H. (1985) Geologic youth of Galápagos islands confirmed by marine stratigraphy and paleontology. *Science*, **227**, 1578–1580.

Hoernle, K., van den Bogaard, P., Werner, R., Lissinna, B., Hauff, F., Alvarado, G. & Garbe-Schönberg, D. (2002) Missing history (16–71 Ma) of the Galápagos hotspot: implications for the tectonic and biological evolution of the Americas. *Geology*, **30**, 795–798.

Hoogmoed, M.S. (1989) Introduced geckos in Puerto Ayora, Santa Cruz, with remarks on other areas. *Noticias de Galápagos*, **47**, 12–16.

Juan, C., Emerson, B.C., Oromí, P. & Hewitt, G.M. (2000) Colonization and diversification: towards a phylogeographic synthesis for the Canary Islands. *Trends in Ecology and Evolution*, **15**, 104–109.

Katoh, K. & Toh, H. (2010) Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics*, **26**, 1899–1900.

Lopez, T.J., Hauselman, E.D., Maxson, L.R. & Wright, J.W. (1992) Preliminary analysis of phylogenetic relationships among Galapagos Island lizards of the genus *Tropidurus*. *Amphibia-Reptilia*, **13**, 327–339.

Maddison, W.P. & Maddison, D.R. (2011) *Mesquite: a modular system for evolutionary analysis*. Available at: http://mesquiteproject.org/.

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.

Petren, K., Grant, P.R., Grant, B.R. & Keller, L.F. (2005) Comparative landscape genetics and the adaptive radiation of Darwin's finches: the role of peripheral isolation. *Molecular Ecology*, **14**, 2943–2957.

Poulakakis, N., Russello, M., Geist, D. & Caccone, A. (2012) Unravelling the peculiarities of island life: vicariance, dispersal and the diversification of the extinct and extant giant Galápagos tortoises. *Molecular Ecology*, **21**, 160–173.

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

Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.4*. University of Edinburgh, Edinburgh, UK. Available at: http://beast.bio.ed.ac.uk/Tracer.

Rambaut, A. & Drummond, A.J. (2010) *TreeAnnotator version 1.6.1*. University of Edinburgh, Edinburgh, UK. Available at: http://beast.bio.ed.ac.uk/.

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

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.

Sato, A., Tichy, H., O'hUigin, C., Grant, P.R., Grant, B.R. & Klein, J. (2001) On the origin of Darwin's finches. *Molecular Biology and Evolution*, **18**, 299–311.

Schmitz, P., Cibois, A. & Landry, B. (2007) Molecular phylogeny and dating of an insular endemic moth radiation inferred from mitochondrial and nuclear genes: the genus *Galagete* (Lepidoptera: Autostichidae) of the Galapagos Islands. *Molecular Phylogenetics and Evolution*, **45**, 180–192.

Sepulchre, P., Sloan, L.C., Snyder, M. & Fiechter, J. (2009) Impacts of Andean uplift on the Humboldt Current system: a climate model sensitivity study. *Paleoceanography*, **24**, PA4215.

Sequeira, A.S., Lanteri, A.A., Albelo, L.R., Bhattacharya, S. & Sijapati, M. (2008) Colonization history, ecological shifts and diversification in the evolution of endemic Galápagos weevils. *Molecular Ecology*, **17**, 1089–1107.

Shimodaira, H. & Hasegawa, M. (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution*, **16**, 1114–1116.

Simpson, B.B. (1979) Quaternary biogeography of the high montane regions of South America. *The South American herpetofauna: its origin, evolution and dispersal* (ed. by W.E. Duellman), pp. 157–188. University of Kansas Natural History Museum, Lawrence, KS.

Swofford, D.L. (2003) *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.* University of Kansas Natural Sinauer Associates, Sunderland, MA.

Torres-Carvajal, O., Carvajal-Campos, A., Barnes, C.W., Nicholls, G. & Pozo-Andrade, M.J. (2013) A new Andean species of Leaf-toed Gecko (Phyllodactylidae: *Phyllodactylus*) from Ecuador. *Journal of Herpetology*, **47**, 384–390.

Werner, R., Hoernle, K., van den Bogaard, P., Ranero, C., von Huene, R. & Korich, D. (1999) Drowned 14-m.y.-old Galápagos archipelago off the coast of Costa Rica: implications for tectonic and evolutionary models. *Geology*, **27**, 499–502.

White, W.M., McBirney, A.R. & Duncan, R.A. (1993) Petrology and geochemistry of the Galápagos Islands: portrait of a pathological mantle plume. *Journal of Geophysical Research*, **98**, 19533–19563.

Whittaker, R.J., Triantis, K.A. & Ladle, R.J. (2008) A general dynamic theory of oceanic island biogeography. *Journal of Biogeography*, **35**, 977–994.

Whittaker, R.J., Triantis, K.A. & Ladle, R.J. (2010) A general dynamic theory of oceanic island biogeography: extending the MacArthur–Wilson theory to accommodate the rise and fall of volcanic islands. *The theory of island biogeography revisited* (ed. by J.B. Losos and R.E. Ricklefs), pp. 88–115. Princeton University Press, Princeton, NJ.

Wright, J.W. (1983) The evolution and biogeography of the lizards of the Galápagos Archipelago: evolutionary genetics of *Phyllodactylus* and *Tropidurus* populations. *Patterns of evolution in Galápagos organisms* (ed. by R.I. Bowman, M. Berson and A.E. Levinton), pp. 123–155. American Association for the Advancement of Science, Pacific Division, San Francisco, CA.

Wyles, J.S. & Sarich, V.M. (1983) Are the Galápagos iguanas older than the Galapagos? Molecular evolution and colonization models for the archipelago. *Patterns of evolution in Galápagos organisms* (ed. by R.I. Bowman, M. Berson and A.E. Levinton), pp. 177–185. American Association for the Advancement of Science, Pacific Division, San Francisco, CA.

Zug, G. (2013) *Reptiles and amphibians of the Pacific islands: a comprehensive guide.* University of California Press, Berkeley, CA.

Zwickl, D.J. (2006) *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.* PhD Thesis, University of Texas at Austin, Austin, TX.

SUPPORTING INFORMATION

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

Appendix S1 Genetic variation among island and mainland samples of *Phyllodactylus* included in this study. (Tables S1 & S2, Figs S1 & S2)

Appendix S2 Additional phylogenetic trees obtained in this study (Figs S3 & S4).

Appendix S3 Diversity and taxonomy of Galápagos leaf-toed geckos.

BIOSKETCH

Omar Torres-Carvajal is interested in the theory and practice of phylogenetic systematics, particularly as they relate to the evolutionary biology of reptiles. He has focused his research on the biogeography and systematics of South American reptiles.

Author contributions: O.T-C. designed the study; O.T-C. and W.T. conducted the fieldwork; C.W.B., M.J.P-A. and G.N. carried out laboratory work; O.T-C. analysed the data; O.T-C. wrote the first complete version of the manuscript; C.W.B. and W.T. read and commented on the manuscript.

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