Molecular Phylogenetics and Evolution 103 (2016) 1–5

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

Present diversity of Galápagos leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) stems from three independent colonization events

Omar Torres-Carvajal [a,*], Andrea Rodríguez-Guerra [a], Jaime A. Chaves [b]

[a] *Museo de Zoología, Escuela de Biología, Pontificia Universidad Católica del Ecuador, Avenida 12 de Octubre y Roca, Apartado 17-01-2184, Quito, Ecuador*
[b] *Universidad San Francisco de Quito, Colegio de Ciencias Biológicas y Ambientales - Extensión Galápagos, Campus Cumbayá, Casilla Postal 17-1200-841, Quito, Ecuador*

ARTICLE INFO

Article history:
Received 11 February 2016
Revised 23 May 2016
Accepted 7 July 2016
Available online 9 July 2016

Keywords:
Galápagos
Island biogeography
Lizards
Multiple colonizations
Phylogeny
Species tree

ABSTRACT

We re-examined the biogeography of the leaf-toed geckos (*Phyllodactylus*) endemic to the Galápagos Islands by analyzing for the first time samples of *P. gilberti*, a species endemic to Wolf island, in a phylogenetic framework. Our aim was to test the three-colonizations scenario previously proposed for these lizards and estimate the age of each colonization event. To achieve this we estimated simultaneously a species tree and divergence times with Bayesian methods. Our results supported the three-colonizations scenario. Similar to a previous hypothesis, the species tree obtained here showed that most species of *Phyllodactylus* are nested in a single clade with an age between 5.49 and 13.8 Ma, whereas a second independent colonization corresponding to *P. darwini* from San Cristóbal island occurred 3.03 Ma ago. The species from Wolf island, *P. gilberti*, stems from a more recent colonization event (0.69 Ma). Thus, present diversity of Galápagos leaf-toed geckos stems from three independent, asynchronous colonization events. As with other Galápagos organisms, the Pacific coast of South America seems to be the source for the founders of *P. gilberti*.

1. Introduction

Located about 960 km west from the coast of Ecuador, the Galápagos archipelago is widely recognized as one of the best model systems for evolutionary and biogeographic studies (e.g., Emerson, 2002; Grant and Grant, 2014; Losos and Ricklefs, 2009). Such studies have benefited from the relatively well-known geological history of these oceanic islands, which have been forming for millions of years as the Nazca Plate moves eastwards over a volcanic hotspot. As a result, island age increases eastwards along the plate, with the oldest islands—Española, San Cristóbal, Santa Fé—located in the south-eastern part of the archipelago (Geist et al., 2014; White et al., 1993). The northern islands (Genovesa, Marchena, Pinta, Wolf and Darwin), however, are not typical hotspot islands in that they originated from hotspot-derived plume material flowing within the mantle towards the Galápagos Spreading Center, the boundary between the Cocos and Nazca plates (Geist et al., 2014; Harpp and Geist, 2002; Harpp et al., 2002).

Among evolutionary studies of Galápagos land vertebrates, Darwin and Wolf islands have received little attention as they have not been colonized by non-volant land vertebrates, except for Wolfs leaf-toed geckos, *Phyllodactylus gilberti* Heller 1903. Darwin and Wolf, two of the smallest islands of the archipelago, are located in its remote far north. Darwin has a surface area of 106.3 ha and Wolf is approximately 134.4 ha (Black, 1973). These islands are small caps on mostly submarine volcanoes (Geist et al., 2014). The age of Darwin is about 400 ka, whereas Wolf is between 0.88 ± 0.13 and 1.60 ± 0.07 Ma old (White et al., 1993).

Wolf's leaf-toed geckos represent one of eight named species of endemic leaf-toed geckos known to occur in the Galápagos (*P. barringtonensis* Van Denburgh, 1912; *P. baurii* Garman, 1892; *P. darwini* Taylor, 1942; *P. duncanensis* Van Denburgh 1912; *P. galapagensis* Peters, 1869; *P. gorii* Lanza, 1973; *P. leei* Cope, 1889); in addition, two undescribed species might occur in Isabela and Santiago, respectively (Torres-Carvajal et al., 2014). Based on genetic similarity of allozyme data, Wright (1983) hypothesized that leaf-toed gecko diversity in the Galápagos stems from three colonization events: (i) one that led to the establishment of *P. gilberti* in Wolf; (ii) a second one that led to the establishment of *P. darwini* in San Cristóbal, and (iii) a third one that explains the remaining diversity of leaf-toed geckos in the archipelago, although not necessarily in that order. Wright's hypothesis is very significant for the evolution and biogeography of Galápagos fauna, as other animal radiations in the archipelago stem from a single colonization event, except for lava lizards, which have colonized the islands twice (Benavides et al., 2009; Wright, 1983). In a recent

---

* Corresponding author.
E-mail address: omartorcar@gmail.com (O. Torres-Carvajal).

http://dx.doi.org/10.1016/j.ympev.2016.07.006

study, Torres-Carvajal et al. (2014) confirmed Wright's hypotheses (ii) and (iii) using phylogenetic analyses of DNA sequence data. Here we use genomic samples of *P. gilberti* from Wolf Island to test Wright's hypothesis that this species originated from an independent colonization event. By inferring a dated species tree including all recognized species of Galápagos leaf-toed geckos, we confirm that these reptiles are unique among terrestrial animals in the archipelago in that their origin stems from three independent and asynchronous colonization events.

2. Materials and methods

2.1. Sampling and laboratory protocols

We obtained sequence data of two specimens of *Phyllodactylus gilberti* from Wolf Island (Genbank accession numbers KX268798–KX268815), and combined them with 104 publically available sequences of species of *Phyllodactylus* from Galápagos and continental South America (Fig. 1; Torres-Carvajal et al., 2014). The gecko *Tarentola mauritanica* (Genbank accession number EU443255) was used as outgroup.

We analyzed 5136 nucleotides (nt) encompassing six nuclear genes and four mitochondrial genes. Nuclear genes included 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). Mitochondrial genes included NADH dehydrogenase subunit 4 (ND4), and a continuous fragment including 12S rRNA, tRNA^{Val}, and 16S rRNA.

Total genomic DNA was digested and extracted from muscle tissue (tail tips) 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, Inc), re-suspended and diluted to 25 ng/µl in ddH2O prior to amplification. Primers and amplification protocols are the same as those presented previously (Torres-Carvajal et al., 2014).

2.2. Phylogenetic analyses

Data were assembled and aligned in Geneious v7 (Kearse et al., 2012) under default settings for Mafft (Katoh and Toh, 2010). Ribosomal (12S, tRNA^{Val}, 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 for confirmation of alignment. We used the same evolutionary models as in Torres-Carvajal et al. (2014).

We estimated a species tree from the multilocus trees under a coalescent model and simultaneously estimated divergence times using *Beast version 1.8 (Drummond et al., 2012). Based on previous results (Torres-Carvajal et al., 2014), we assigned the 107 sequences to 16 species including *P. gilberti*, and calibrated divergence dating using two estimated node ages to set normal priors for the Galápagos main radiation (i.e., the *galapagensis* clade; mean = 5.17 m.a.) and the South American *Phyllodactylus* clade (mean = 23.97 m.a.). The analyses were conducted under a model with uncorrelated substitution rates among branches and the rate for each branch independently drawn from an underlying lognormal distribution (Drummond et al., 2006); a Yule prior was used for the species tree. To reduce the chance of converging on a local optimum, four runs of 10^8 generations each were performed. Trees were sampled every 10,000 generations resulting in 10,000 saved trees per analysis. Stationarity was confirmed by plotting the $-\ln L$ per generation in the program Tracer v1.5 (Rambaut and Drummond, 2007). Independence of samples was assessed by examining the effective sample sizes (ESS) in Tracer. After analyzing convergence, mixing, and sampling, the first 25% of 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 resultant trees of each analysis to calculate posterior probabilities (PP) for each bipartition in a maximum clade credibility tree using TreeAnnotator 1.8 (Rambaut and Drummond, 2016).

3. Results

The species tree (Fig. 1) was largely concordant with the phylogenetic tree presented by Torres-Carvajal et al. (2014; their Fig. 3); therefore here we present only the results related to *Phyllodactylus gilberti*. This species is sister to *P. reissii* from the Pacific coast in Ecuador and Peru (Fig. 1) with maximum support (PP = 1). Together they form a clade sister (PP = 0.98) to an unnamed species of *Phyllodactylus* from northern Peru, herein referred to as "*P. reissii*" because of morphological similarity with *P. reissii*. The clade ("*P. reissii*", (*P. gilberti*, *P. reissii*) is sister (PP = 1) to a clade containing all species of *Phyllodactylus* (herein named *galapagensis* clade) from Galápagos except *P. darwini*. Thus, among species of *Phyllodactylus* from the Galápagos, *P. gilberti* originated from an independent colonization event.

Similar to the hypothesis presented by Torres-Carvajal et al. (2014), the species tree (Fig. 1) yielded an estimated node age of 5.49 Ma for the *galapagensis* clade, with a 95% highest posterior density (HPD) interval (i.e., the shortest interval that contains 95% of the sampled values) of 4.54–6.39 Ma, as well as a stem age of 13.8 Ma (HPD = 7.92–20.21 Ma). The split between *P. darwini* from San Cristóbal and its sister Andean species *P. leoni* occurred 3.03 Ma (HPD = 0.22–5.87 Ma), whereas the split between *P. gilberti* and its sister species *P. reissii* was estimated to have occurred 0.69 Ma (HPD = 0.04–1.56 Ma).

4. Discussion

Leaf-toed geckos are unique among terrestrial animals endemic to the Galápagos in that their present diversity is the result of three independent colonizations. Other terrestrial animal taxa in the archipelago stem from a single colonization (see Table 2 in Torres-Carvajal et al., 2014), except for lava lizards, which have colonized the Galápagos twice (Benavides et al., 2009; Wright, 1983). A three-colonization scenario was originally proposed for Galápagos leaf-toed geckos by Wright (1983) using genetic distance data, and two of those colonizations were later corroborated by Torres-Carvajal et al. (2014) using DNA-sequence data in a phylogenetic framework. Here we include for the first time samples of Wolf's leaf-toed gecko, *P. gilberti*, and find strong support for Wright's (1983) hypothesis that this species stems from a third colonization event (Fig. 1). A multiple-colonization scenario for Galápagos geckos is not difficult to explain as these reptiles are capable of travelling long distances by passive drift on ocean currents (de Queiroz, 2005). Nonetheless, to our knowledge a three-colonization scenario for geckos in other archipelagos of the world has only been proposed for *Tarentola* in the Canary islands (Carranza et al., 2002).

The Galápagos archipelago is washed by different ocean currents that might represent the invasion routes used by some of the ancestors of its present terrestrial fauna. The Humboldt Current carries cold water northwards along the west coast of South America from the Antarctic to the equator, where it turns westwards to form the South Equatorial Current-South before reaching the Galápagos (Palacios, 2004). These ocean currents most likely were used