DOI: 10.1111/jzs.12387

ORIGINAL ARTICLE

WILEY

Colonization history of Galapagos giant tortoises: Insights from mitogenomes support the progression rule

Nikos Poulakakis^{1,2} | Joshua M. Miller³ | Evelyn L. Jensen³ Adalgisa Caccone³

Luciano B. Beheregaray⁴ | Michael A. Russello⁵ | Scott Glaberman⁶ | Jeffrey Boore⁷ |

¹Department of Biology, School of Sciences and Engineering, University of Crete, Heraklio, Greece

²Natural History Museum of Crete, School of Sciences and Engineering, University of Crete, Heraklio, Greece

³Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT, USA

⁴College of Science and Engineering, Flinders University, Adelaide, SA, Australia

⁵Department of Biology, The University of British Columbia, Kelowna, BC, Canada

⁶Department of Environmental Science and Policy, George Mason University, Fairfax, VA, USA

⁷Providence St. Joseph Health and Institute for Systems Biology, Seattle, WA, USA

Correspondence

Adalgisa Caccone, Department of Ecology and Evolutionary Biology, Yale University, 21 Sachem St. New Haven, CT 06520, USA. Email: adalgisa.caccone@yale.edu

Funding information

National Geographic Society; The Eppley Foundation; Galapagos Conservancy; Mohamed Bin Zayed Species Conservation Fund; Turtle Conservancy; Swiss Friends of Galapagos

Abstract

Galapagos giant tortoises (Chelonoidis spp.) are a group of large, long-lived reptiles that includes 14 species, 11 of which are extant and threatened by human activities and introductions of non-native species. Here, we evaluated the phylogenetic relationships of all extant and two extinct species (Chelonoidis abingdonii from the island of Pinta and Chelonoidis niger from the island of Floreana) using Bayesian and maximum likelihood analysis of complete or nearly complete mitochondrial genomes. We also provide an updated phylogeographic scenario of their colonization of the Galapagos Islands using chrono-phylogenetic and biogeographic approaches. The resulting phylogenetic trees show three major groups of species: one from the southern, central, and western Galapagos Islands; the second from the northwestern islands; and the third group from the northern, central, and eastern Galapagos Islands. The time-calibrated phylogenetic and ancestral area reconstructions generally align with the geologic ages of the islands. The divergence of the Galapagos giant tortoises from their South American ancestor likely occurred in the upper Miocene. Their diversification on the Galapagos adheres to the island progression rule, starting in the Pleistocene with the dispersal of the ancestral form from the two oldest islands (San Cristóbal and Española) to Santa Cruz, Santiago, and Pinta, followed by multiple colonizations from different sources within the archipelago. Our work provides an example of how to reconstruct the history of endangered taxa in spite of extinctions and human-mediated dispersal events and provides a framework for evaluating the contribution of colonization and in situ speciation to the diversity of other Galapagos lineages.

KEYWORDS

ancestral area reconstruction, Bayesian inference, Chelonoidis, maximum likelihood, timecalibrated phylogeny

Poulakakis and Miller contributed equally to this work

Contributing authors: Nikos Poulakakis (poulakakis@nhmc.uoc.gr), Joshua M. Miller (millerjm86@gmail.com), Evelyn L. Jensen (evelyn.jensen@yale.edu), Luciano B. Beheregaray (luciano.beheregaray@flinders.edu.au), Michael A. Russello (michael.russello@ubc.ca), Scott Glaberman (sglaberm@gmu.edu), Jeffrey Boore (Jeffrey.Boore@providence.org)

INTRODUCTION 1

Oceanic islands are excellent systems to observe and interpret patterns of evolution due to their well-defined and often young geological histories, relative isolation, distinct boundaries, heterogeneous environments, and simplified biotas (Losos & Ricklefs, 2009). Dispersal, rather than geological vicariance, appears to be the principal driver of range evolution and subsequent speciation, regardless of the geological histories and ages of island systems (Iwanycki Ahlstrand et al., 2019). Advances in molecular phylogenetic/divergence analyses in tandem with increasing availability of geologic data have allowed for the ages of oceanic islands to be used for calibrating molecular clocks in many studies of insular biota. The use of island age-based calibrations has the theoretical advantage of providing maximum age constraints for dating relatively shallow divergences, in comparison with the use of fossils that tend to provide minimum ages more appropriate for inferring the timeframe of deeper splits (Hawlitschek et al., 2017). However, this approach has been challenged by several studies that estimated clade ages for a variety of organisms that were older than the purported ages of the islands to which they are endemic. Prominent examples include the divergences between island clades of endemic iguanas (MacLeod et al., 2015; Rassmann, 1997) and weevils (Segueira, Lanteri, Scataglini, Confalonieri, & Farrell, 2000) from the Galapagos Islands that were all estimated to be considerably older than the current archipelago (Parent, Caccone, & Petren, 2008). These discrepancies could be due to (a) species clades being older than the islands to which they are endemic (e.g., the colonization took place via stepping stones between islands that no longer exist (Renner, Strijk, Strasberg, & Thebaud, 2010)) or (b) geological age estimates for the islands not accurately reflecting the time span available for colonization.

The Galapagos Islands, a volcanic archipelago located ~900 km off the coast of Ecuador, are a celebrated model of island systems, with notable examples of adaptive radiations (Parent et al., 2008). Endemic reptiles, in particular, are prime examples for studying the colonization

extinct with asterisk (*)

history of oceanic islands given their slow metabolic rates, as well as their resilience to desiccation and osmotic stress. Together, these characteristics have been considered pre-adaptations for the successful colonization of new terrestrial habitats via overseas dispersal (Hawlitschek et al., 2017). Galapagos giant tortoises (Chelonoidis, Fitzinger 1835) are flagship species for ongoing restoration efforts in the archipelago (Benitez-Capistros, Huge, Dahdouh-Guebas, & Koedam, 2016), not only as iconic representatives of the endemic biodiversity at risk, but also due to their function as native mega-herbivores, crucial for maintaining ecosystem health (Blake, Guezou, Deem, Yackulic, & Cabrera, 2015; Gibbs, Sterling, & Zabala, 2010). These long-lived reptiles are considered the largest living ectothermic terrestrial vertebrates, and include 14 species, with a single species per island except for Santa Cruz, that hosts two species, and Isabela, which has a different endemic species associated with each of its five major volcanoes (Rhodin et al., 2017) (Figure 1). In addition to the 14 named species, the extinct population from Santa Fé probably belonged to a distinct, still undescribed, species (Poulakakis, Russello, Geist, &

Since first being discovered, human activities have dramatically altered ecosystems in the Galapagos Islands (Guezou et al., 2010; Phillips, Wiedenfeld, & Snell, 2012). Over the past three centuries, harvesting for food and oil by whalers, sealers and buccaneers, and predation and/or competition with non-native species has contributed to the extinction of three species of Galapagos giant tortoise (one more is possibly extinct) and dramatic declines of the others (Harper & Carrion, 2011; Jiménez-Uzcátegui et al., 2008; Townsend, 1925). All Galapagos giant tortoises are listed in the IUCN Red List of endangered species (IUCN, 2020). The extinct species include Chelonoidis abingdonii (Günther, 1877) and Chelonoidis niger (Quoy and Gaimard, 1824b) from the islands of Pinta and Floreana, respectively, and the undescribed, but genetically distinct, lineage from Santa Fé (Poulakakis et al., 2012). Among the extant species, three are listed as vulnerable (Chelonoidis becki (Rothschild, 1901), Chelonoidis vandenburghi



Caccone, 2012).

VILEY-

(DeSola, 1930), and Chelonoidis duncanensis (Pritchard, 1996)), three as endangered (Chelonoidis vicina (Günther, 1875a), Chelonoidis chathamensis (Van Denburgh, 1907), and Chelonoidis microphyes (Günther, 1875)), and six as critically endangered (Chelonoidis guntheri (Baur, 1889), Chelonoidis hoodensis (Van Denburgh, 1907), Chelonoidis donfaustoi (Poulakakis, Edwards, Caccone, 2015), Chelonoidis porteri (Rothschild, 1903), Chelonoidis darwini (Van Denburgh, 1907), and Chelonoidis phantasticus (Van Denburgh, 1907) [possibly extinct]). To protect these species and others, concerted efforts are being made including restoring habitats in the Galapagos (Campbell, Donlan, Cruz, & Carrion, 2004; Hamann, 1993; Harper & Carrion, 2011), captive breeding and repatriation of native species (e.g., Fabiani et al., 2011; Jensen, Edwards, et al., 2018; Milinkovitch et al., 2004; Quinzin et al., 2019), and introducing closely related species to islands where the native one has been extirpated (e.g., releasing Española tortoises on Santa Fé: Tapia et al., 2016).

Mitochondrial DNA (mtDNA) has a long history of use for phylogenetic and phylogeographic studies (Avise, Giblin-Davidson, Laerm, Patton, & Lansman, 1979; Avise, Lansman, & Shade, 1979; Moritz, Dowling, & Brown, 1987), especially for studies of recently diverged lineages or rapid radiations (Moore, 1995). Traditionally, such analyses were done with one or a few mtDNA genes, for example, cytochrome oxidase 1 (cox1) (Hebert, Ratnasingham, & deWaard, 2003). However, advances in high-throughput sequencing technologies coupled with novel bioinformatic methods for mtDNA genome reconstruction (Hahn, Bachmann, & Chevreux, 2013; Machado, Lyra, & Grant, 2016; Miller, Malenfant, Moore, & Coltman, 2012) have enabled full mitogenome analyses that increase phylogenetic resolution compared to those based on single genes (Gibb et al., 2016; Morin et al., 2010).

Previous phylogenetic studies of Galapagos giant tortoises have largely relied on single or multiple mtDNA regions (Beheregaray, Ciofi, Caccone, Gibbs, & Powell, 2003; Beheregaray et al., 2004; Caccone et al., 2002; Caccone, Gibbs, Ketmaier, Suatoni, & Powell, 1999; Poulakakis et al., 2008, 2012; Russello, Beheregaray, et al., 2007; Russello et al., 2005) and nuclear introns (Caccone et al., 2004). However, the phylogenetic relationships of these species are still partly unclear, likely due to the use of molecular character data largely restricted to a small portion of the mitogenome having insufficient power to resolve the rapid and recent radiation of the group (the oldest extant island dates to ~3 million years ago (Geist, 1996; Geist, Mcbirney, & Duncan, 1986; Geist, Snell, Snell, Goddard, & Kurz, 2014)). These limitations also apply to our ability to accurately estimate the timing and pattern of inter-island colonization, including the relative roles of vicariance and dispersal (Beheregaray, Ciofi, Caccone, et al., 2003; Beheregaray et al., 2004; Caccone et al., 1999; Ciofi et al., 2006; Poulakakis et al., 2012).

To overcome these limitations, here we used complete or nearly complete mtDNA genomes of all extant species and two extinct ones (*C. abingdonii* from the island of Pinta and *C. niger* from the island of Floreana) to reconstruct the phylogeny of Galapagos giant tortoises, infer processes underlying diversification, and estimate the timing of island colonization.

2 | MATERIALS AND METHODS

2.1 | Data collection

Unique circumstances have allowed us to recover DNA for two extinct species from fresh tissue, as opposed to preserved material. For C. abingdonii, we used blood samples collected from the last living tortoise from this species, a male named "Lonesome George" that died in 2012. For C. niger, we recovered the "extinct" mtDNA genome from a living tortoise. This was enabled by previous studies which identified mixed ancestry in tortoises living on northern Isabela Island, the likely outcome of relatively recent hybridization events between the extant endemic species, C. becki, and C. niger individuals endemic to Floreana Island, brought to those shores by humans (Garrick et al., 2012; Poulakakis et al., 2008). Thus, the individual used in this study to recover the C. niger mtDNA genome can be considered a "genomic archive," negating the need for ancient DNA methods to recover the mtDNA genome of an extinct species. For the remaining species, DNA was extracted from blood stored in 100 mM Tris/100 mM EDTA/2% SDS buffer by using the DNeasy Blood & Tissue Kit (QIAGEN). All blood samples were collected previously by A. Caccone, but remain the property of the Galapagos National Park, and are not deposited as vouchered specimens in a museum.

We constructed complete or nearly complete mtDNA genomes for 13 Galapagos giant tortoise species (Table 1), including 11 extant and two extinct species (*C. niger* and *C. abingdonii*), as well as almost complete mtDNA genomes for two of the three South American *Chelonoidis* species, the red-footed (*Chelonoidis carbonarius* (Spix, 1824)) and yellow-footed (*Chelonoidis denticulatus* (Linnaeus, 1776)) tortoises, using both Ion Torrent and Sanger sequencing methods (for more details, Alignment S1, Tables S1 and S2, and Figure S1). These mtDNA genome sequences were combined with those available for *Chelonoidis chilensis* (Gray, 1870a), the closest living relative of the Galapagos giant tortoises (Caccone et al., 1999; Poulakakis et al., 2008), *C. alburyonon* (Franz and Franz, 2009), an extinct species from the Bahamas, and two more distantly related African species, *Centrochelys sulcata* (Miller, 1779) and *Stigmochelys pardalis* (Bell 1828a) (Kehlmaier et al., 2017) (see Table 1).

Ion Torrent and Sanger sequences were imported to Geneious version 6.0.6 (https://www.geneious.com), aligned with MUSCLE (Edgar, 2004) and checked by eye for alignment quality. Sequences for the two red-footed tortoises were identical, so analyses for that taxon are based on a single sample.

2.2 | Sequence diversity analyses

For the phylogenetic and phylogeographic analyses, we created a dataset of 24 mitochondrial genome sequences, 15 sequences from the 11 extant and the two extinct species of Galapagos giant tortoises, and nine sequences from the six outgroup taxa (see Table 1). Previous mtDNA sequence data were obtained from Jensen, Miller, et al. (2018),

TABLE 1 List of Galapagos giant tortoise and outgroup taxa used in this study, along with geographic location, construction method, GenBank accession numbers (in parathneses, the corresponding code on the figures of phylogenetic trees is given), and references [p.s. present study, 1: Jensen, Miller, et al. (2018), 2: Kehlmaier et al. (2017), 3: Parham et al. (2006)]

Species	Population	Island	Methodology	Accession no	Reference
C. abingdonii	Pinta	Pinta	MITObim	MT017690	p.s.
C. becki	Puerto Bravo	Isabela	Sanger	MT017693	p.s.
C. chathamensis	San Cristóbal	San Cristóbal	Sanger	MT017692	p.s.
C. darwini	Santiago	Santiago	Sanger	MT017694	p.s.
C. donfaustoi	Cerro Fatal	Santa Cruz	Sanger	MT017695	p.s.
C. niger	Floreana	Floreana	Ion Torrent	MT017691	p.s.
C. duncanensis	Pinzón	Pinzón	Sanger	MT017697 (1), MG912828 (2)	p.s. & 1
C. guntheri	Roca Union	Isabela	Sanger	MT017700	p.s.
C. hoodensis	Española	Española	Sanger	MT017696	p.s.
C. microphyes	Volcano Darwin	Isabela	Sanger	MT017702	p.s.
C. porteri	La Caseta	Santa Cruz	Sanger	MT017698	p.s.
C. vandenburghi	Volcano Alcedo	Isabela	Sanger	MT017701	p.s.
C. vicina	West Cerro Azul	Isabela	Sanger	LT599486 (1), MT017699 (2)	p.s. & 2
C. alburyorum	Bahamas		MITObim	LT599482	2
C. chilensis	South America		MITObim	LT599484	2
C. carbonarius	South America		Ion Torrent	MT017704 (1), MT708501 (2), LT599483 (3)	p.s. & 2
C. denticulatus	South America		Ion Torrent	LT599485 (1), MT017703 (2).	p.s. & 2
Centrochelys sulcata	Africa			LT599487	2
Stigmochelys pardalis	Africa			DQ080041	3

Kehlmaier et al. (2017), and Parham, Feldman, and Boore (2006). Due to the availability of previously published sequences, two species of Galapagos giant tortoises (*C. vicina* and *C. duncanensis*) are represented by two sequences in our dataset. All sequences were initially aligned in Geneious using MUSCLE (Edgar, 2004), with a final alignment length of 19,152 base pairs. Sequence divergences were estimated in MEGA7 (Kumar, Stecher, & Tamura, 2016), using the *p*-distance model among the Galapagos giant tortoise species and the outgroup species.

2.3 | Phylogenetic analyses

We extracted individual gene regions based on annotations of the Pinta Island tortoise (*C. abingdonii*) mtDNA genome. These were then realigned with MUSCLE and concatenated into a super matrix for determination of appropriate substitution models using Partitionfinder2 (PF2) (Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2017). The final super matrix of 16,437 bases included all 13 protein coding genes, two ribosomal RNAs, and 21 of 22 tRNAs (Table S3). We excluded tRNA-Phe as partial sequences were present in only two of 17 taxa. PF2 was run with linked branch lengths using a greedy algorithm and specifying codon positions for each of the protein coding genes. Optimal models were chosen using the Bayesian information criterion.

Bayesian inference (BI) analyses were performed in MrBayes v.3.2.7 (Ronquist et al., 2012) conducting four runs and using eight sampling chains for each run based on the partition results and models

revealed in PF2. Each chain ran for 2,000,000 generations, sampling every 1,000 generations. Several MCMC diagnostics were used to check for convergence and stationarity (the plot of the generation vs. the log probability of the data [the log likelihood values], the average standard deviation of split frequencies, the average potential scale reduction factor [PSRF], and the minimum value of estimated sample sizes [ESS]). The first 25% of trees were discarded as burn-in, as a measure to sample from the stationary distribution and avoid the possibility of including random, sub-optimal trees. A 50% majority rule consensus tree was then produced from the posterior distribution of trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade. Posterior probabilities ≥0.95 indicate statistically significant support (Huelsenbeck & Ronquist, 2001).

Maximum-likelihood (ML) analyses were performed using RAxML v.8.1.21 as implemented in raxmIGUI v.1.6 (Silvestro & Michalak, 2011) based on the partition results and models revealed in PF2. The best ML tree for each dataset was selected from 50 MI searches, and the statistical confidence of the branches was further assessed based on 1,000 thorough bootstrap replicates (Felsenstein, 1985).

2.4 | Molecular divergence dating

For the estimation of divergence times, we used three methods implemented in the programs StarBEAST2 (v.0.13.5; Ogilvie,

NILEY-

POULAKAKIS ET AL.

Bouckaert, & Drummond, 2017), MCMCTree incorporated in PAML (v.4.9; Yang, 2007), and MrBayes (Ronquist et al., 2012). Details of priors and specifications are given in Supplementary Material.

To estimate the divergence times, we used two calibration points: The first was the split between C. carbonarius and C. denticulatus, and the second was between C. chilensis and the Galapagos giant tortoises. Two recent studies suggest different times for these splits. The first one (Kehlmaier et al., 2017) places the split of C. carbonarius and C. denticulatus at 13.5-11.8 Mya and that of C. chilensis and the Galapagos giant tortoises at 11.95 Mya, while the second (Pereira, Sterli, Moreira, & Schrago, 2017) presents a much older split at 27 and 25 Mya, respectively. The large difference between these two studies could be due to the different approaches applied. The first study, Kehlmaier et al. (2017), was conducted with BEAST based on two calibration points using normal distribution priors, while the second. Pereira et al. (2017). was conducted under a Bavesian framework in MCMCTree based on 22 calibration points. However, the use of a normal distribution is not always appropriate for calibrating a node using fossil information (see manual of BEAST). The aforementioned data (2 vs. 22 calibration points and appropriateness of normal distribution for fossil information) suggest that the estimations of the second study might be more accurate than the first one. Nevertheless, we applied both sets of calibration points and used AICM in Tracer to test which model (the model with the first set of calibration points from Kehlmaier et al. (2017) or the model with the second set of calibration points from Pereira et al. (2017)) better fit our data.

To evaluate the adherence of Galapagos giant tortoises to the island progression rule (i.e., a sequence of colonization from old to young islands) (Funk & Wagner, 1995), we used a general linear model in MS Excel (2016) to test for a relationship between clade age (calculated as the mean estimate of divergence within each island from the Bayesian phylogeny in starBEAST) and island age (Geist et al., 2014).

2.5 | Model-based biogeographical inference

To reconstruct the ancestral distribution of Galapagos giant tortoises species, we analyzed the Bayesian time calibrated tree by employing the R package "BioGeoBEARS" (Matzke, 2013a, 2013b), using each island as a separate region. This analysis enables probabilistic inference of ancestral geographical ranges and statistical comparisons of different models of range expansion: dispersal extinction cladogenesis (DEC; Ree & Smith, 2008), dispersal vicariance analysis (DIVA; Ronquist, 1997), and Bayesian analysis of biogeography (BAYAREA; Landis, Matzke, Moore, & Huelsenbeck, 2013) using a time-calibrated species tree. These models were tested with and without a "jump dispersal" parameter J that allows for founder-event speciation (Templeton, 2008). This is an important parameter when modeling speciation on remote oceanic islands (Matzke, 2014), as it allows the modeling of a long-distance colonization event of a small number of individuals

founding a population that then becomes genetically isolated from the ancestral population, a phenomenon guite common in island systems. A total of six models were used (DEC-LIKE, DEC-LIKE+J, DIVA-LIKE, DIVA-LIKE+J, BAYAREA-LIKE, BAYAREA-LIKE+J; for more details, see manual of BioGeoBEARS (http://phylo.wikid ot.com/biogeobears). The maximum clade credibility tree from BEAST2 was used as the input file to estimate the probabilities of ancestral ranges at internal nodes of the phylogeny, including only Galapagos giant tortoise species. Each individual was designated as present or absent on each of the eight islands. We performed model selection by comparing Akaike information criterion (AIC) values and AIC weights (AICw) on time-stratified models to determine the most appropriate model for the colonization history of Galapagos giant tortoises. In this analysis, the probability of dispersal and island occupancy was restricted by estimates of island emergence time (Geist, 1996; Geist et al., 2014). The oldest extant islands are estimated to have emerged ~4.0 Mya for San Cristóbal and 3.5 Mya for Española (Christie et al., 1992; Geist et al., 2014). Based on these dates and assuming that the initial founding of the Galapagos was a single event on one island (either Española or San Cristóbal, or on a proto-island when they were still a single island), we tested all possible scenarios for the patterns of colonization in Galapagos.

3 | RESULTS

The newly assembled Pinta Island tortoise (*C. abingdonii*) mitochondrial genome was circular and 16,449 bp long, constructed from 88,837 mapped reads (average coverage of 537), and included a complete set of 13 protein coding genes, two rRNA genes, and 22 tRNA genes. This annotated sequence has been made available on GenBank (Accession Number MT017690).

For all 11 extant Galapagos giant tortoise species, shotgun Sanger sequences were assembled into contiguous fragments of 15,647 or 15,648 bp (GenBank accession numbers MT017692–MT017702) representing ~95% of the complete *C. abingdonii* genome. Relative to the genome from *C. abingdonii*, data obtained using Sanger sequencing did not contain 507 bp on the 5' end of the genome and 281 bp on the 3' end (Figure S1).

Ion Torrent sequencing for four samples (two *C. carbonarius*, one *C. denticulatus*, and one *C. niger*) produced 205,448 total reads with a mean read length of 144 bp. The number of reads per individual ranged from 12,406 (*C. niger*) to 63,969 (one of the *C. carbonarius* individuals) with an average (\pm SD) of 46,836 \pm 22,807 reads. Mean read depth was 576 \pm 287 across all samples, with per sample averages ranging from 177 to 742. Through Sanger sequencing, we were able to assemble an additional ~4,800 bp to extend the mtDNA sequence for each of these four samples. Final aligned sequences (GenBank accession numbers MT017691, MT017703, and MT017704) ranged from 14,156 bp (*C. niger*) to 15,751 bp (*C. carbonarius*), representing ~86% to 95% of the complete mtDNA as compared with *C. abingdonii*.

1267

WILEN

Average *p*-distances between the Galapagos giant tortoise species and the outgroup species were 7.6%, 8.5%, 9.4%, and 10% for *Chelonoidis alburyorum*, *C. chilensis*, *C. denticulatus*, and *C. carbonarius*, respectively. The average difference among Galapagos giant tortoise sequences was 0.7%, whereas the pairwise *p*-distances ranged from 0.08% to 1.11% among Galapagos giant tortoise species and from 0.08% to 12.3% among all taxa, including the outgroups (Table S4).

3.1 | Phylogenetic trees

The best-fit partitioning scheme for each downstream analysis and the selected nucleotide substitution models are presented in Table S5. The ML and BI analyses resulted in a phylogenetic tree with lnL = -51,660.94 and lnL = -48,351.69 (harmonic mean), respectively. All MCMC diagnostic metrics indicated that the iterations of BI analysis reached convergence and stationarity. The average standard deviation of split frequencies was smaller than 0.001 (when this value approaches zero, the tree samples are more similar), the plot of generation versus log-likelihood of the data had characteristic "whitenoise" morphology after burn-in. In addition, for all parameters, the PSRF values were near 1.00 (range 0.999–1.000) and ESS values were well over 200 for all parameters (minimum ESS was 347.2).

Tree topologies from the Bayesian and maximum-likelihood analyses were identical (Figure 2) and had high support values for all nodes (Bayesian posterior probabilities 1.00, bootstrap supports >80), with the exception of the sister group relationship of *C. becki* and *C. darwini* with *C. abingdonii*, *C. hoodensis*, *C. chathamensis*, and *C. donfaustoi* (bootstrap value 62).

Galapagos giant tortoises formed a single monophyletic group, with a sister group relationship with one of the three extant South American *Chelonoidis* species, the Chaco tortoises (*C. chilensis*), with the extinct Bahamas species (*C. alburyorum*), being the next closest relative, followed by a clade including the other two extant South American congeneric species (*C. denticulatus* and *C. carbonarius*). Within the Galapagos giant tortoise clade, two major clusters (clades) were recognized. The first clade includes the species from the central, south, and west islands of the archipelago (Isabela, Santa Cruz, Floreana, and Pinzón). The second clade is divided into two subclades. One subclade includes the four species from the eastern, central, and northern Galapagos islands (Española, San Cristóbal, eastern Santa Cruz, and Pinta). The other subclade include the species from the northwestern part of Galapagos (Santiago and northern Isabela).

Finally, we note that the two *C. vicina* specimens are not clustered together in the phylogenetic tree. This could be due to the fact that the second specimen (with Accession Number LT599486, Kehlmaier et al. (2017)) was taken from the Reptile Zoo Happ (Klagenfurt) in Austria and might have been incorrectly assigned to that species, whereas the *C. vicina* mtDNA genome generated



FIGURE 2 Bayesian inference (BI) tree based on the complete mitochondrial genomes of Galapagos giant tortoise and outgroup taxa. The posterior probabilities for BI and bootstrap support for Maximum-likelihood (ML) are given on top of the branches. Asterisks indicate full support by both methods (ML/BI).The red section corresponds to Galapagos giant tortoises. Cross indicates extinct species. The drawings of the Galapagos giant tortoises were obtained with permission from Tropical Herping (Arteaga, Bustamante, Vieira, Tapia, & Guayasamin, 2019) VILEY-

here was from a wild collected sample from the distribution area of *C. vicina* on Isabela, suggesting that this genealogical lineage corresponds to that species.

3.2 | Molecular divergence dating and model-based biogeographical inference

According to the BEAST2, the model based on Pereira et al. (2017) estimated differentiation of Galapagos giant tortoises starting at 1.54 Mya (Figure 3), while using the Kehlmaier et al. (2017) estimates, divergence started at 0.63 Mya (Figure S2). Model selection analysis in Tracer revealed a better score for the model based on calibration from Pereira et al. (2017) than those from Kehlmaier et al. (2017) (AICM = 96,832.90 [$SE \pm 0.242$] and AICM = 96,745.64 [$SE \pm 0.52$], respectively). Therefore, all the remaining analyses in BEAST2, MCMCTree and MrBayes were performed using only the calibration points from Pereira et al. (2017). In starBEAST analyses in BEAST2, the model selection analysis revealed that the birth-death model for speciation, linear with constant root populations, and relaxed lognormal clock were the best among the 12 models compared (Table S6). Based on this model, the phylogenetic tree has a InL = -48,352.26 with high effective sample sizes for all parameters (posterior ESS)

values >200). In the MCMCTree, the analysis also resulted in high posterior ESS values (>2,000) for all parameters, and convergence was reached prior to 200,000 generations (InL = -22.32). In MrBayes, the analysis resulted in a phylogenetic tree with InL = -48,399.3. In all analyses, the estimation of divergence times with starBEAST, MCMCTree, and MrBayes led to similar ages, with, based on our samples, the start of the Galapagos giant tortoise radiation dated between 1.63 and 1.52 Mya (node G; Table 2).

BioGeoBEARS model comparisons showed that the DIVALIKE, DEC, and BAYAREA models with the +J parameter that allows for founder-event speciation are a better fit to our data than the same models without the +J parameter (Table 3). Among them, the DIVALIKE+J model was the best fit to the data and most likely to infer the correct ancestral range at each node. We also found a significant linear positive relationship between clade age and island geological age, when using either minimum or maximum ages for island emergence (adjusted $R^2 > .801$, p < .001 and $R^2 > .855$, p < .001, respectively; Figure S3).

Our data suggested that the history of the Galapagos giant tortoise radiation started in the early Pleistocene, at which point the oldest extant islands of San Cristóbal and Española were united as a single landmass. Thus, the biogeographic analysis recovered both islands as the most probable ancestral area for the Galapagos giant



FIGURE 3 The calibrated starBEAST2 species tree of the chronophylogenetic analysis based on the complete mitochondrial genome dataset. The red section corresponds to Galapagos giant tortoises. Cross indicates extinct species. The posterior probabilities are given above the branches. Cp1 and cp2 are the calibration points. The calculated node ages of the major nodes (A–R, see Table 3) are given below the branches. The respective credible intervals (95% HPD) and corresponding values from the other two methods of dating are given in Table 3. Note that in species tree analysis, all specimens of the same species have been collapsed in a single branch

TABLE 2 The estimated divergence times for all splits (see Figure 3) based on the three different methods (starBEAST, MrBayes, and MCMCTree)

	Divergence times in Mya					
Split	starBEAST	MrBayes	MCMCTree			
A	41.22 (39.19-43.49)	40.49 (40.01-42.42)	33.66 (40.01-42.42)			
В	38.59 (36.74-40.27)	37.55 (34.82-39.75)	31.15 (34.82–39.75)			
С	33.65 (31.99-35.15)	32.68 (30.96-35.32)	29.65 (30.96-35.32)			
D	28.00 (27.81-28.20)	27.65 (25.76–29.57)	27.44 (25.76-29.57)			
E	28.00 (26.44-29.32)	27.52 (25.49-29.85)	27.39 (25.49-29.85)			
F	25.99 (25.80-26.18)	23.85 (22.13-26.78)	25.51 (22.13-26.78)			
G	1.54 (1.27–1.79)	1.63 (1.37-1.81)	1.52 (0.83-3.02)			
Н	1.02 (0.75-1.28)	1.19 (0.99–1.36)	1.02 (0.52–2.05)			
I	0.55 (0.36-0.73)	0.64 (0.52–0.76)	0.57 (0.28-1.14)			
J	0.41 (0.24-0.57)	0.57 (0.46-0.69)	0.47 (0.23-0.96)			
К	0.13 (0.07-0.20)	0.29 (0.22-0.36)	0.23 (0.11-0.48)			
L	0.05 (0.00-0.09)	0.15 (0.10-0.20)	0.08 (0.02-0.19)			
Μ	0.04 (0.00-0.11)	0.15 (0.10-0.21)	0.10 (0.03-0.25)			
Ν	1.09 (0.80-1.37)	1.24 (0.98–1.39)	1.08 (0.55-2.04)			
0	0.72 (0.53-0.91)	0.80 (0.66–0.98)	0.68 (0.32-1.37)			
Р	0.25 (0.06-0.43)	0.40 (0.30-0.52)	0.32 (0.10-0.76)			
Q	0.33 (0.13-0.53)	0.47 (0.36-0.80)	0.35 (0.11-0.83)			
R	0.03 (0.00-0.07)	0.14 (0.10-0.21)	0.08 (0.02-0.27)			

Note: The calibration points are in bold.

TABLE 3 Biogeographical model selection with and without founder-event speciation (+J) based on the lowest corrected Akaike information criterion (AICc) values

Initial founding		LnL	Number of parameters	d	е	j	AICc	AICc_wt
San Cristóbal	DIVALIKE+J	-24.38	3	1.00E-12	1.00E-12	0.18	57.42	0.66
	DEC+J	-25.41	3	1.00E-12	1.00E-12	0.22	59.49	0.23
	BAYAREALIKE+J	-26.21	3	1.00E-07	1.00E-07	0.21	61.08	0.11
	DIVALIKE	-38.29	2	0.16	0.40	0	81.79	3.40E-06
	DEC	-40.74	2	0.17	0.72	0	86.68	2.90E-07
	BAYAREALIKE	-43.64	2	0.19	1.54	0	92.49	1.60E-08

Note: d, rate of dispersal; e, rate of extinction; j, relative probability of founder-event speciation at cladogenesis.

tortoises radiation (Figure 4). From this landmass, colonization occurred ~1.54 Mya (1.52–1.63 Mya) to a united landmass of what is now Santa Cruz, Floreana, and Pinzón (Figure 4c). The descendents of this ancestral lineage remained only on the island of Pinzón (*C. duncanensis*; E in Figure 4a,b), when the united landmass started to divide and the giant tortoises subsequently colonized the now separate island of Santa Cruz at 1.02 Mya, giving rise to *C. porteri* (D in Figure 4a,b). Afterward, two other colonization events from the island of Santa Cruz occurred: one to the island of Floreana (*C. niger*; 0.55 Mya) and the other to southern Isabela at 0.41 Mya, which gave rise to the four southernmost species on Isabela (*C. vicina, C. vandenburghi, C. microphyes*, and *C. guntheri*). Around 1.09 Mya, colonization occurred from San Cristóbal to Santiago Island, leading to the lineage that gave rise *C. darwini.* Later, overseas dispersal from the now divided landmass of San Cristóbal occurred to give rise to *C. hoodensis* on Española (0.72–0.80 Mya) and *C. donfaustoi* on eastern Santa Cruz (0.33–0.47 Mya). This reconstruction suggests that the current species on Española is derived from a second colonization event, rather than from the ancestral lineage that colonized the united landmass of San Cristóbal and Española. Colonization occurred from Española to Pinta at 0.25 Mya, which, although peculiar due to the geographic distance between two islands, is probably a natural dispersal, given the prevailing northwesterly currents (Pak & Zaneveld, 1973; Russello, Hyseni, et al., 2007). The most recent colonization was from Santiago to northern Isabela Island at 0.03 Mya, giving rise to *C. becki*.

WILEN



FIGURE 4 (a) Results of ancestral area estimations in BioGeoBEARs with non-Galapagos taxa removed for clarity. The DIVALIKE+J reconstruction model, where ancestral lineages could disperse in any direction but distance among islands influenced colonization likelihood. Colors in the boxes highlighting each island in the map below to show current distributions and ancestral colonization patterns. (b) The biogeographic scenario generated based on the present BioGeoBEARs analysis, in which the differentiation of the lineages of Santa Cruz, Floreana, and Pinzón is the result of three dispresals and one extinction events (one dispersal from San Cristóbal/Española to the united landmass Santa Cruz, Floreana, and Pinzón, one extinction from Santa Cruz, Floreana, and two more dispersal events from Pinzón to Santa Cruz and Floreana, respectively). (c) The pageographic map of the Galapagos Islands in Middle Pleistocene (~1 Mya) (redrawn from Poulakakis et al. (2012)). Cross indicates extinct species

4 | DISCUSSION

4.1 | MtDNA phylogenetic history

Despite years of study, DNA-based research, coupled with thorough character and taxonomic sampling of both extant and extinct taxa, is still yielding insights into the phylogenetic history of the giant tortoises of the Galapagos. Using between 1,600- and 5,300-bp-long mtDNA fragments and nearly complete taxon sampling, several previous studies provided a phylogeny of this group (Caccone et al., 1999, 2002; Poulakakis et al., 2012). However, uncertainties remained as to the placement of several clades that were not strongly supported by all phylogenetic analyses. Here, we have generated the most robust mtDNA phylogeny of Galapagos giant tortoises to date by substantially increasing the amount of mitochondrial sequence data, enhancing taxon sampling (all but two species: C. phantasticus [possibly extinct] from Fernandina and an undescribed species from Santa Fé [extinct]), and applying comprehensive analytical methods to reconstruct and evaluate trees.

In comparison with previous studies, there are several topological differences and almost all nodes that were weakly supported in previous studies received strong statistical support (Caccone et al., 1999; Kehlmaier et al., 2017; Poulakakis et al., 2012) (Figure 2). Considering the topological differences, within the clade of the central, south, and west islands of the archipelago (Isabela, Santa Cruz, Floreana, and Pinzón), *C. duncanensis* from the island of Pinzón is in a

basal position relative to the other species as in previous studies, but C. porteri from western Santa Cruz, rather than extinct C. niger species from Floreana, is the sister taxon to the four species from central and southern Isabela, C. vicina, C. guntheri, C. vandenburghi, and C. microphyes. In the second clade, although the same grouping was recovered by previous mtDNA analyses, our results based on nearly complete mtDNA sequences provides, for the first time, relatively strong support for these clades according to both Bayesian (posterior probability = 1.00) and maximum-likelihood (bootstrap value = 62) methods. Our results also confirmed the genetic separation of the two species co-occurring on Santa Cruz island, one (C. porteri) living in the south and southwest of the island, and the other (C. donfaustoi) found on the eastern parts (Beheregaray, Ciofi, Caccone, et al., 2003; Beheregaray et al., 2004; Poulakakis et al., 2015; Russello et al., 2005). These two species are linked through the deepest node within the Galapagos giant tortoises, suggesting independent colonization events.

4.2 | Island progression rule and colonization scenario

Not only did the model based on the calibration points retrieved from Pereira et al. (2017) have a better fit than those of Kehlmaier et al. (2017), the phylogeographic reconstruction was more plausible. Specifically, using the model of Kehlmaier et al. (2017), the colonization history was stochastic, did not follow a palaeogeographic pattern, and occurred at an exceptionally rapid pace, producing in \sim 600,000 years at least 14 genetically distinct mtDNA lineages, ranging in divergence from 0.1% to 1.1% (average 0.7%; Table 2). On the other hand, using the model of Pereira et al. (2017), colonization events occur from older to younger islands and do not pre-date the island ages.

We found that the Galapagos giant tortoise species coalesced around 1.52–1.63 Mya, a timing that seems plausible, being almost half the age of the oldest islands in the archipelago (San Cristóbal [2.4–4.0 Mya] and Española [3.0–3.5 Mya]). This dating also gives ample opportunity for enough vegetation to develop on these islands to sustain a tortoise population.

Our finding of within-island Galapagos giant tortoise assemblages resulting from a single colonization with subsequent radiation on most islands (excluding Isabela and Santa Cruz Islands for which two colonization events each occurred) confirms previous phylogeographic studies based on a smaller subset of mtDNA data (Poulakakis et al., 2012). The finding of a significant positive relationship between island clade and geological ages (Figure S3) implies that clades are colonizing and diversifying on islands after their emergence from the sea. Therefore, our phylogenetic and ancestral range reconstruction suggests that Galapagos giant tortoises generally follow the island progression rule, as with other terrestrial groups (Parent & Crespi, 2006). Galapagos giant tortoises are not good swimmers, but they float (drifting on currents) and can survive without food or freshwater for up to 6 months (Pak & Zaneveld, 1973). Within the Galapagos, prevailing currents run in a northwesterly direction, which is also the approximate order of emergence of the islands from older to younger ones (Pak & Zaneveld, 1973). This means that organisms with passive dispersal and low active dispersal abilities, like the Galapagos giant tortoises, are likely to diverge according to the island progression rule and colonize remote geographic areas, such as oceanic islands, in sequence, while adapting and diversifying locally (Beheregaray et al., 2004; Caccone et al., 2002). A similar trend has been observed in several animal groups in Galapagos (Parent et al., 2008), including spiders (De Busschere et al., 2010), beetles (Sequeira et al., 2000), and marine iguanas (Steinfartz et al., 2009), as well as in other oceanic islands, such as Tahiti and Hawaii (Haponski, Lee, & Foighil, 2019; Holland & Hadfield, 2004).

The estimated times of coalescence for each island radiation are younger than the maximum (and in most cases minimum) date of island emergence (Geist et al., 2014) and the reconstruction of ancestral ranges inferred that ancestral species were present on contemporary subaerial islands. Based on the ancestral range reconstruction, Galapagos giant tortoises started their diversification from the oldest islands of the archipelago, San Cristóbal and Española (see Figure 4), presumably living on a proto-island that eventually split to form the two islands. This is in agreement with a recent palaeogeographic visualization through simulated island configurations that showed these two islands were connected as recently as 2–1.5 Mya (Karnauskas, Mittelstaedt, & Murtugudde, 2017). This is true for other islands as well. The central Galapagos islands, which include the modern-day islands of Santa Cruz and Floreana and later on the islands of Santiago, Santa Cruz, Pinzón, Floreana, and Rabida at the end, were also united in a single landmass 2–1 Mya (Geist et al., 2014; Karnauskas et al., 2017).

The scenario deemed to be the most likely in the BioGeoBEARS analysis has four major discrepancies with the colonization pattern proposed in previous studies (Beheregaray et al., 2004; Poulakakis et al., 2012). The first one is that the ancestral reconstruction area was not San Cristóbal or Española, but a proto-island that later gave rise to San Cristóbal and Española. The second is that the species currently living on Española (C. hoodensis) did not derive directly from the ancestral lineage living on this proto-island, but resulted from a more recent colonization event from San Cristóbal, well after the two islands separated. The third discrepancy has to do with the source of colonization of southern Isabela, which according to this study was western Santa Cruz rather than Floreana. The fourth discrepancy involves the lineages on the islands of Santa Cruz. Pinzón. and Floreana, which were proposed in Poulakakis et al. (2012) to have arose through vicariance. In that scenario, the ancestral lineage from a united landmass of Santa Cruz, Floreana, and Pinzón became the present-day lineages through vicariance with the isolation of Pinzón Island (C. duncanensis; 1.02 Mya), and Floreana Island (C. niger; 0.55 Mya) from the remnant landmass of Santa Cruz (C. porteri). The scenario supported by our analyses here instead involves three dispersal events and one extinction event (one dispersal from San Cristóbal/Española to the united landmass Santa Cruz, Floreana, and Pinzón, one extinction from Santa Cruz, Floreana, and subsequent dispersal events from Pinzón to Santa Cruz, and then Santa Cruz to Floreana).

As with previous studies of this group, we found that Galapagos giant tortoises were highly divergent from extant mainland taxa, and within the radiation, all lineages were phylogenetically distinct from one another and, as such, should be treated as different conservation units. Among the Galapagos giant tortoises, divergence estimates were highly concordant between the full mtDNA genomes and previous estimates from the d-loop only (Garrick et al., 2015), but not between mtDNA genome *p*-distances and *F*_{st} estimates based on genome-wide SNPs (Miller et al., 2018). The lower correlation between divergence estimates from nuclear versus mitochondrial markers had previously been reported for this group (Miller et al., 2018).

Mitochondrial DNA markers have traditionally been considered well-suited for studies involving recently diverged lineages or rapid radiations (Moore, 1995), given the fast mutation rate relative to the nuclear genome, matrilineal inheritance, and infrequency of recombination of the mtDNA genome. However, in some cases, the mtDNA and nuclear genome have different inheritance patterns, which can lead to discordance between phylogenetic reconstructions obtained using these two genomes (Mallo & Posada, 2016). Such mito-nuclear discordance has been observed in numerous taxa and may be attributed to a variety of biogeographic or biological causes including selection on mitochondrial genes or geographic isolation followed by secondary contact. In addition to the faster rate of sorting of variation in the mtDNA compared to the nuclear genome, volcanic activity might cause

1272

VILEY-

recurrent population bottlenecks that can influence biogeographic history and patterns of evolutionary divergence in lineages inhabiting young volcanic oceanic archipelagoes. This is thought to be the case for the Galapagos giant tortoise from the Volcano Alcedo (*C. vandenburghi*), where rapid mtDNA sorting and evolutionary diversification were thought to be influenced by a volcanic eruption (Beheregaray, Ciofi, Geist, et al., 2003). Thus, the mtDNA reconstruction presented in this study, although statistically robust, may or may not reflect the evolutionary history of the entire radiation.

Therefore, it may be useful to produce a phylogenetic tree of the Galapagos giant tortoises based on nuclear markers for comparison. A first attempt toward this goal, using ~4,000 bp of nuclear DNA, failed to resolve phylogenetic relationships within the Galapagos giant tortoises clade, most likely because of the relatively small number of variable sites available in the analyzed fragments, despite being introns (Caccone et al., 2004). More recently, single nucleotide polymorphism (SNP)-based analyses have been used to infer patterns of genetic differentiation and diversity within and among the extant species of Galapagos giant tortoises (Gaughran et al., 2018; Miller et al., 2018), but a direct comparison to this type of broad study is not possible because there is no comparable set of markers for the extinct taxa. Future analyses based on genome-wide SNPs from all species will provide added resolution and allow us to assess the degree to which the mtDNA and nuclear genome provide a concordant reconstruction of the species history and to investigate any possible sources of discordance.

Overall, our reconstruction of Galapagos giant tortoises' diversification across the islands includes a combination of dispersal and extinction events, confirming the role of dispersal in shaping island biogeography, as in many other oceanic systems (Benavides, Baum, Mcclellan, & Jack, 2007; Gentile et al., 2009; Parent et al., 2008; Rassmann, Tautz, Trillmich, & Gliddon, 1997; Sato et al., 2001; Schmitz, Cibois, & Landry, 2007; Sequeira, Lanteri, Albelo, Bhattacharya, & Sijapati, 2008). These findings stress the importance of not assuming that oceanic island systems fit a single model of diversification and the importance of taking into account the geological history and the biology of the study system. Such detailed considerations would not have been possible without the robust phylogeny produced by whole mitochondrial genomes.

ACKNOWLEDGEMENTS

We thank Carol Mariani for assistance with the Ion Torrent sequencing and Kirstin Dion and Allana Pike for assistance with the Sanger sequencing. This work was supported by a number of agencies that funded either the field expeditions to gather the samples or the genetic analyses. They include the National Geographic Society, The Eppley Foundation, the Galapagos Conservancy, and the Mohamed Bin Zayed Species Conservation Fund, Turtle Conservancy, and Swiss Friends of Galapagos. L.B. Beheregaray was supported by the Gaylord Donnelley Environmental Fellowship (YIBS). We especially are thankful for the support and logistic help of the Galapagos National Park Directorate.

ORCID

Nikos Poulakakis https://orcid.org/0000-0002-9982-7416 Joshua M. Miller https://orcid.org/0000-0002-4019-7675

REFERENCES

- Arteaga, A., Bustamante, L., Vieira, J., Tapia, W., & Guayasamin, J. M. (2019). Reptiles of the Galápagos. Quito, Ecuador: Tropical Herping.
- Avise, J. C., Giblin-Davidson, C., Laerm, J., Patton, J. C., & Lansman, R. A. (1979). Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proceedings of California Academy of Science, 76, 6694–6698. https://doi.org/10.1073/pnas.76.12.6694
- Avise, J. C., Lansman, R. A., & Shade, R. O. (1979). Use of restriction endonucleases to measure mitochondrial-DNA sequence relatedness in natural-populations. 1. Population-structure and evolution in the genus *Peromyscus. Genetics*, 92, 279–295.
- Beheregaray, L. B., Ciofi, C., Caccone, A., Gibbs, J. P., & Powell, J. R. (2003). Genetic divergence, phylogeography and conservation units of giant tortoises from Santa Cruz and Pinzon, Galápagos Islands. *Conservation Genetics*, 4, 31–46.
- Beheregaray, L. B., Ciofi, C., Geist, D., Gibbs, J. P., Caccone, A., & Powell, J. R. (2003). Genes Record a Prehistoric Volcano Eruption in the Galápagos. *Science*, 302, 75.
- Beheregaray, L. B., Gibbs, J. P., Havill, N., Fritts, T. H., Powell, J. R., & Caccone, A. (2004). Giant tortoises are not so slow: Rapid diversification and biogeographic consensus in the Galápagos. Proceedings of the National Academy of Sciences of the United States of America, 101, 6514–6519.
- Benavides, E., Baum, R., Mcclellan, D., & Jack, W. J. (2007). Molecular phylogenetics of the lizard genus *Microlophus* (Squamata: Tropiduridae): Aligning and retrieving indel signal from nuclear Introns. *Systematic Biology*, *56*, 776–797. https://doi.org/10.1080/10635150701618527
- Benitez-Capistros, F., Huge, J., Dahdouh-Guebas, F., & Koedam, N. (2016). Exploring conservation discourses in the Galapagos Islands: A case study of the Galapagos giant tortoises. *Ambio*, 45, 706–724. https://doi.org/10.1007/s13280-016-0774-9
- Blake, S., Guezou, A., Deem, S. L., Yackulic, C. B., & Cabrera, F. (2015). The dominance of introduced plant species in the diets of Migratory Galapagos Tortoises increases with elevation on a human-occupied Island. *Biotropica*, 47, 246–258. https://doi.org/10.1111/btp.12195
- Caccone, A., Gentile, G., Burns, C. E., Sezzi, E., Bergman, W., Ruelle, M., ... Powell, J. R. (2004). Extreme difference in rate of mitochondrial and nuclear DNA evolution in a large ectotherm, Galápagos tortoises. *Molecular Phylogenetics and Evolution*, 31, 794–798. https:// doi.org/10.1016/j.ympev.2004.02.004
- 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.
- Caccone, A., Gibbs, J. P., Ketmaier, V., Suatoni, E., & Powell, J. R. (1999). Origin and evolutionary relationships of giant Galapagos tortoises. Proceedings of the National Academy of Sciences of the United States of America, 96, 13223–13228. https://doi.org/10.1073/ pnas.96.23.13223
- Campbell, K., Donlan, C. J., Cruz, F., & Carrion, V. (2004). Eradication of feral goats Capra hircus from Pinta Island, Galapagos, Ecuador. Oryx, 38, 328–333.
- 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 Galápagos hotspot imply extended speciation times. *Nature*, 355, 246–248.
- Ciofi, C., Wilson, G. A., Beheregaray, L. B., Marquez, C., Gibbs, J. P., Tapia, W., ... Powell, J. R. (2006). Phylogeographic history and gene flow among giant Galápagos tortoises on southern Isabela

Island. Genetics, 172, 1727-1744. https://doi.org/10.1534/genet ics.105.047860

- De Busschere, C., Hendrickx, F., Van Belleghem, S. M., Backeljau, T., Lens, L., & Baert, L. (2010). Parallel habitat specialization within the wolf spider genus *Hogna* from the Galapagos. *Molecular Ecology*, 19, 4029–4045.
- Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*, 1792–1797. https://doi.org/10.1093/nar/gkh340
- Fabiani, A., Trucchi, E., Rosa, S., Marquez, C., Snell, H. L., Snell, H. M., ... Gentile, G. (2011). Conservation of Galápagos land iguanas: Genetic monitoring and predictions of a long-term program on the island of Santa Cruz. Animal Conservation, 14, 419–429. https://doi. org/10.1111/j.1469-1795.2011.00442.x
- Felsenstein, J. (1985). Confidence-limits on phylogenies an approach using the bootstrap. *Evolution*, *39*, 783–791.
- Funk, V. A., & Wagner, W. L. (1995). Biogeographic patterns in the Hawaiian Islands. In W. L. Wagner & V. L. Funk (Eds.), *Hawaiian biogeography: Evolution on a hot spot archipelago* (pp. 379-419). Washington, DC: Smithsonian Institution Press.
- Garrick, R. C., Benavides, E., Russello, M. A., Gibbs, J. P., Poulakakis, N., Dion, K. B., ... Caccone, A. (2012). Genetic rediscovery of an 'extinct' Galapagos giant tortoise species. *Current Biology*, 22, R10–R11.
- Garrick, R. C., Kajdacsi, B., Russello, M. A., Benavides, E., Hyseni, C., Gibbs, J. P., ... Caccone, A. (2015). Naturally rare versus newly rare: Demographic inferences on two timescales inform conservation of Galapagos giant tortoises. *Ecology and Evolution*, 5, 676–694.
- Gaughran, S. J., Quinzin, M. C., Miller, J. M., Garrick, R. C., Edwards, D. L., Russello, M. A., ... Caccone, A. (2018). Theory, practice, and conservation in the age of genomics: The Galapagos giant tortoise as a case study. *Evolutionary Applications*, 11, 1084–1093.
- Geist, D. (1996). On the emergence and submergence of the Galápagos Islands. Noticias de Galápagos, 56, 5–8.
- Geist, D. J., Mcbirney, A. R., & Duncan, R. A. (1986). Geology and petrogenesis of Lavas from San Cristóbal Island, Galápagos Archipelago. *Geological Society of America Bulletin*, 97, 555–566.
- Geist, D. J., Snell, H., Snell, H., Goddard, C., & Kurz, M. D. (2014). A Paleogeographic model of the Galápagos Islands and biogeographical and evolutionary implications. In K. S. Harpp, E. Mittelstaedt, N. d'Ozouville & D. W. Graham (Eds.), *The Galápagos: A natural laboratory for the earth sciences* (pp. 145–166). Hoboken, NJ: John Wiley & Sons Inc.
- Gentile, G., Fabiani, A., Marquez, C., Snell, H. L., Snell, H. M., Tapia, W., & Sbordoni, V. (2009). An overlooked pink species of land iguana in the Galápagos. Proceedings of the National Academy of Sciences of the United States of America, 106, 507–511.
- Gibb, G. C., Condamine, F. L., Kuch, M., Enk, J., Moraes-Barros, N., Superina, M., ... Delsuc, F. (2016). Shotgun mitogenomics provides a reference phylogenetic framework and timescale for living Xenarthrans. *Molecular Biology and Evolution*, 33, 621–642. https:// doi.org/10.1093/molbev/msv250
- Gibbs, J. P., Sterling, E. J., & Zabala, F. J. (2010). Giant Tortoises as ecological engineers: A long-term quasi-experiment in the Galápagos Islands. *Biotropica*, 42, 208–214. https://doi. org/10.1111/j.1744-7429.2009.00552.x
- Guezou, A., Trueman, M., Buddenhagen, C. E., Chamorro, S., Guerrero, A. M., Pozo, P., & Atkinson, R. (2010). An extensive alien plant inventory from the inhabited areas of Galápagos. *PLoS ONE*, *5*, e10276.
- Hahn, C., Bachmann, L., & Chevreux, B. (2013). Reconstructing mitochondrial genomes directly from genomic next-generation sequencing reads-a baiting and iterative mapping approach. *Nucleic Acids Research*, 41(13), e129. https://doi.org/10.1093/nar/gkt371
- Hamann, O. (1993). On vegetation recovery, goats and giant tortoises on Pinta Island, Galapagos, Ecuador. *Biodiversity and Conservation*, 2, 138–151.

- Haponski, A. E., Lee, T., & Foighil, D. O. (2019). Deconstructing an infamous extinction crisis: Survival of partula species on Moorea and Tahiti. *Evolutionary Applications*, 12, 1017–1033.
- Harper, G. A., & Carrion, V. (2011). Introduced rodents in the Galapagos: Colonisation, removal and the future. In C. R. Veitch, M. N. Clout & D. R. Towns (Eds.), *Island invasives: Eradication and management* (pp. 63–66). Gland, Switzerland: IUCN.
- Hawlitschek, O., Toussaint, E. F. A., Gehring, P. S., Ratsoavina, F. M., Cole, N., Crottini, A., ... Glaw, F. (2017). Gecko phylogeography in the Western Indian Ocean region: The oldest clade of *Ebenavia inunguis* lives on the youngest island. *Journal of Biogeography*, 44, 409–420.
- Hebert, P. D. N., Ratnasingham, S., & deWaard, J. R. (2003). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society B-Biological Sciences*, 270, S96–S99.
- Holland, B. S., & Hadfield, M. G. (2004). Origin and diversification of the endemic Hawaiian tree snails (Achatinellidae : Achatinellinae) based on molecular evidence. *Molecular Phylogenetics and Evolution*, 32, 588–600. https://doi.org/10.1016/j.ympev.2004.01.003
- Huelsenbeck, J. P., & Ronquist, F. (2001). MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755. https://doi. org/10.1093/bioinformatics/17.8.754
- IUCN. (2020). The IUCN red list of threatened species. version 2020-1. https://www.iucnredlist.org. Accessed March 19, 2020.
- Iwanycki Ahlstrand, N., Verstraete, B., Hassemer, G., Dunbar-Co, S., Hoggard, R., Meudt, H. M., & Rønsted, N. (2019). Ancestral range reconstruction of remote oceanic island species of *Plantago* (Plantaginaceae) reveals differing scales and modes of dispersal. *Journal of Biogeography*, 46, 706–722.
- Jensen, E. L., Edwards, D. L., Garrick, R. C., Miller, J. M., Gibbs, J. P., Cayot, L. J., ... Russello, M. A. (2018). Population genomics through time provides insights into the consequences of decline and rapid demographic recovery through head-starting in a Galapagos giant tortoise. Evolutionary Applications, 11, 1811–1821. https://doi. org/10.1111/eva.12682
- Jensen, E. L., Miller, J. M., Edwards, D. L., Garrick, R. C., Tapia, W., Caccone, A., & Russello, M. A. (2018). Temporal mitogenomics of the Galapagos Giant Tortoise from Pinzon reveals potential biases in population genetic inference. *Journal of Heredity*, 109, 631–640.
- Jiménez-Uzcátegui, G., Milstead, B., Marquez, C., Zabala, J., Buitrón, P., Llerena, A., ... Zabala-Albizua, J. (2008). Galápagos vertebrates: Endangered status and conservation actions. In *Galápagos Report*: 2006-2007 (pp. 104–110). CDF, GNP and INGALA.
- Karnauskas, K. B., Mittelstaedt, E., & Murtugudde, R. (2017). Paleoceanography of the eastern equatorial Pacific over the past 4 million years and the geologic origins of modern Galápagos upwelling. *Earth and Planetary Science Letters*, 460, 22–28. https://doi. org/10.1016/j.epsl.2016.12.005
- Kehlmaier, C., Barlow, A., Hastings, A. K., Vamberger, M., Paijmans, J. L. A., Steadman, D. W., ... Fritz, U. (2017). Tropical ancient DNA reveals relationships of the extinct Bahamian giant tortoise Chelonoidis alburyorum. Proceedings of the Royal Society B-Biological Sciences, 284(1846), 20162235.
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874. https://doi.org/10.1093/molbe v/msw054
- Landis, M. J., Matzke, N. J., Moore, B. R., & Huelsenbeck, J. P. (2013). Bayesian analysis of biogeography when the number of areas is large. *Systematic Biology*, 62, 789–804. https://doi.org/10.1093/sysbio/ syt040
- Lanfear, R., Frandsen, P. B., Wright, A. M., Senfeld, T., & Calcott, B. (2017). PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution*, 34, 772–773.

1274 | WILEY-

- Losos, J. B., & Ricklefs, R. E. (2009). Adaptation and diversification on islands. *Nature*, 457, 830–836. https://doi.org/10.1038/nature07893
- Machado, D. J., Lyra, M. L., & Grant, T. (2016). Mitogenome assembly from genomic multiplex libraries: Comparison of strategies and novel mitogenomes for five species of frogs. *Molecular Ecology Resources*, 16, 686–693. https://doi.org/10.1111/1755-0998.12492
- MacLeod, A., Rodriguez, A., Vences, M., Orozco-terWengel, P., Garcia, C., Trillmich, F., ... Steinfartz, S. (2015). Hybridization masks speciation in the evolutionary history of the Galapagos marine iguana. Proceedings of the Royal Society B-Biological Sciences, 282, 20150425.
- Mallo, D., & Posada, D. (2016). Multilocus inference of species trees and DNA barcoding. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 371(1702), 20150335.
- Matzke, N. J. (2013a). BioGeoBEARS: BioGeography with Bayesian (and Likelihood) evolutionary analysis in R scripts. R Package v. 0.2.1. Retrieved from http://CRAN.R-project.org/package=BioGeoBEARS
- Matzke, N. J. (2013b). 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. https://doi.org/10.21425/F55419694
- Matzke, N. J. (2014). Model selection in historical biogeography reveals that founder-event speciation is a crucial process in island clades. *Systematic Biology*, 63, 951–970. https://doi.org/10.1093/sysbio/ syu056
- Milinkovitch, M. C., Monteyne, D., Gibbs, J. P., Fritts, T. H., Tapia, W., Snell, H. L., ... Powell, J. R. (2004). Genetic analysis of a successful repatriation programme: Giant Galápagos tortoises. *Proceedings of the Royal Society B-Biological Sciences*, 271, 341–345. https://doi. org/10.1098/rspb.2003.2607
- Miller, J. M., Malenfant, R. M., Moore, S. S., & Coltman, D. W. (2012). Short reads, circular genome: Skimming SOLiD sequence to construct the bighorn sheep mitochondrial genome. *Journal of Heredity*, 103, 140–146. https://doi.org/10.1093/jhered/esr104
- Miller, J. M., Quinzin, M. C., Edwards, D. L., Eaton, D. A. R., Jensen, E. L., Russello, M. A., ... Caccone, A. (2018). Genome-wide assessment of diversity and divergence among extant galapagos giant tortoise species. *Journal of Heredity*, 109, 611–619. https://doi.org/10.1093/jhered/esy031
- Moore, W. S. (1995). Inferring phylogenies from mtDNA variation: Mitochondrial-gene trees versus nuclear-gene trees. Evolution, 49, 718-726. https://doi.org/10.2307/2410325
- Morin, P. A., Archer, F. I., Foote, A. D., Vilstrup, J., Allen, E. E., Wade, P., ... Harkins, T. (2010). Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research*, 20, 908–916. https://doi.org/10.1101/ gr.102954.109
- Moritz, C., Dowling, T. E., & Brown, W. M. (1987). Evolution of animal mitochondrial-DNA - relevance for population biology and systematics. *Annual Review of Ecology and Systematics*, 18, 269–292. https://doi. org/10.1146/annurev.es.18.110187.001413
- Ogilvie, H. A., Bouckaert, R. R., & Drummond, A. J. (2017). StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. *Molecular Biology and Evolution*, 34, 2101–2114. https:// doi.org/10.1093/molbev/msx126
- Pak, H., & Zaneveld, J. R. V. (1973). The Cromwell current on the east side of the Galapagos Islands. *Journal of Geophysical Research*, 78, 7845–7859. https://doi.org/10.1029/JC078i033p07845
- 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.
- Parent, C. E., & Crespi, B. J. (2006). Sequential colonization and diversification of Galápagos endemic land snail genus *Bulimulus* (Gastropoda, Stylommatophora). *Evolution*, 60, 2311–2328.

- Parham, J. F., Feldman, C. R., & Boore, J. L. (2006). The complete mitochondrial genome of the enigmatic bigheaded turtle (*Platysternon*): Description of unusual genomic features and the reconciliation of phylogenetic hypotheses based on mitochondrial and nuclear DNA. *BMC Evolutionary Biology*, 6, 11.
- Pereira, A. G., Sterli, J., Moreira, F. R. R., & Schrago, C. G. (2017). Multilocus phylogeny and statistical biogeography clarify the evolutionary history of major lineages of turtles. *Molecular Phylogenetics and Evolution*, 113, 59–66. https://doi.org/10.1016/j. ympev.2017.05.008
- Phillips, R. B., Wiedenfeld, D. A., & Snell, H. L. (2012). Current status of alien vertebrates in the Galápagos Islands: Invasion history, distribution, and potential impacts. *Biological Invasions*, 14, 461–480. https:// doi.org/10.1007/s10530-011-0090-z
- Poulakakis, N., Edwards, D. L., Chiari, Y., Garrick, R. C., Russello, M. A., Benavides, E., ... Caccone, A. (2015). Description of a new galapagos giant Tortoise species (*Chelonoidis*; Testudines: Testudinidae) from cerro fatal on Santa Cruz Island. *PLoS ONE*, 10, e0138779. https:// doi.org/10.1371/journal.pone.0138779
- Poulakakis, N., Glaberman, S., Russello, M., Beheregaray, L. B., Ciofi, C., Powell, J. R., & Caccone, A. (2008). Historical DNA analysis reveals living descendants of an extinct species of Galápagos tortoise. *Proceedings of the National Academy of Sciences of the United States* of America, 105, 15464–15469. https://doi.org/10.1073/pnas.08053 40105
- 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. https://doi.org/10.1111/j.1365-294X.2011.05370.x
- Quinzin, M. C., Sandoval-Castillo, J., Miller, J. M., Beheregaray, L. B., Russello, M. A., Hunter, E. A., ... Caccone, A. (2019). Genetically informed captive breeding of hybrids of an extinct species of Galapagos tortoise. *Conservation Biology*, 33, 1404–1414. https://doi. org/10.1111/cobi.13319
- Rassmann, K. (1997). Evolutionary age of the Galápagos iguanas predates the age of the present Galápagos Islands. *Molecular Phylogenetics and Evolution*, 7, 158–172. https://doi.org/10.1006/mpev.1996.0386
- Rassmann, K., Tautz, D., Trillmich, F., & Gliddon, C. (1997). The microevolution of the Galápagos marine iguana *Amblyrhynchus cristatus* assessed by nuclear and mitochondrial genetic analyses. *Molecular Ecology*, 6, 437-452.
- Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Systematic Biology, 57, 4–14. https://doi.org/10.1080/10635 150701883881
- Renner, S. S., Strijk, J. S., Strasberg, D., & Thebaud, 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. https://doi.org/10.1111/j.1365-2699.2010.02319.x
- Rhodin, A. G. J., Iverson, J. B., Bour, R., Fritz, U., Georges, A., Shaffer, H.
 B., & van Dijk, P. P. (2017). Turtles of the world: Annotated checklist and atlas of taxonomy, synonymy, distribution, and conservation status. In A. G. J. Rhodin, J. B. Iverson, P. P. van Dijk, R. A. Saumure, K. A. Buhlmann, P. C. H. Pritchard & R. A. Mittermeier (Eds.), *Conservation biology of freshwater turtles and tortoises: A compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group.* (pp. 1–292). Lunenburg, MA: Chelonian Research Monographs.
- Ronquist, F. (1997). Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. Systematic Biology, 46, 195–203. https://doi.org/10.1093/sysbio/46.1.195
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Hohna, S., ... Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542. https://doi.org/10.1093/sysbio/ sys029

- Russello, M. A., Beheregaray, L. B., Gibbs, J. P., Fritts, T. H., Havill, N., Powell, J. R., & Caccone, A. (2007). Lonesome George is not alone among Galápagos tortoises. *Current Biology*, 17, R317–R318. https:// doi.org/10.1016/j.cub.2007.03.002
- Russello, M. A., Glaberman, S., Gibbs, J. P., Marquez, C., Powell, J. R., & Caccone, A. (2005). A cryptic taxon of Galápagos tortoise in conservation peril. *Biology Letters*, 1, 287–290. https://doi.org/10.1098/ rsbl.2005.0317
- Russello, M. A., Hyseni, C., Gibbs, J. P., Cruz, S., Marquez, C., Tapia, W., ... Caccone, A. (2007). Lineage identification of Galápagos tortoises in captivity worldwide. *Animal Conservation*, 10, 304–311. https://doi. org/10.1111/j.1469-1795.2007.00113.x
- 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. https://doi.org/10.1093/oxfordjournals.molbev.a003806
- 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. https://doi.org/10.1016/j.ympev.2007.05.010
- Sequeira, A. S., Lanteri, A. A., Albelo, R., Bhattacharya, S., & Sijapati, M. (2008). Colonization history, ecological shifts and diversification in the evolution of endemic Galápagos weevils. *Molecular Ecology*, 17, 1089–1107. https://doi.org/10.1111/j.1365-294X.2007.03642.x
- Sequeira, A. S., 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.
- Silvestro, D., & Michalak, I. (2011). raxmlGUI: A graphical front-end for RAxML. Organisms Diversity & Evolution, 12, 335-337.
- Steinfartz, S., Glaberman, S., Lanterbecq, D., Russello, M. A., Rosa, S., Hanley, T. C., ... Caccone, A. (2009). Progressive colonization and restricted gene flow shape island-dependent population structure in Galapagos marine iguanas (Amblyrhynchus cristatus). BMC Evolutionary Biology, 9.
- Tapia, W., Gibbs, J. P., Rueda, D., Carrión, J., Villalba, F., Málaga, J., ... Cayot, L. J. (2016). Giant Tortoise restoration initiative: Beyond rescue to full recovery. *Galapagos Report*, 173–180.
- Templeton, A. R. (2008). The reality and importance of founder speciation in evolution. *BioEssays*, 30, 470–479. https://doi.org/10.1002/ bies.20745
- Townsend, C. H. (1925). The Galápagos tortoises in relation to the whaling industry: A study of old logbooks. *Zoologica*, 4, 55–135.
- Yang, Z. H. (2007). PAML 4: Phylogenetic analysis by maximum likelihood. *Molecular Biology and Evolution*, 24, 1586–1591. https://doi. org/10.1093/molbev/msm088

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Data collection and analyses

This file includes details on data collection and analyses performed in the present study and supplementary Figures S1, S2, S3 and the supplementary Tables S1, S2, S3, S4, S5, and S6.

Figure S1. The complete mitochondrial genome of *Chelonoidis abingdonii* and the relative position of the fragments used in this study.

Figure S2. The starBEAST tree and the time of divergences for each node based on the calibration points retrieved from Kehlmaier et al. (2017).

Figure S3. Linear relationship between minimum and maximum age of emergence for each island and mean estimation of divergence for Galapagos giant tortoises within each island based on the starBEAST chronophylogenetic analysis.

 Table S1. Primer sequences used for generation of mtDNA genomes

 for the 11 extant species of Galapagos giant tortoise included in this

 study

Table S2. Primer sequences used for generation of mtDNA genomes for the extinct Floreana Galapagos giant tortoise (*Chelonoidis niger*), the red-footed (*Chelonoidis carbonarius*), and yellow-footed (*Chelonoidis denticulatus*) tortoises.

Table S4. Pairwise genetic *p*-distances of the complete mtDNA genomes among the Galapagos giant tortoises and the outgroup taxa. Table S5. Partitioning schemes and best-fit models of sequence evolution selected in PartitionFinder2 (PF) for downstream analyses.

Table S6. The marginal likelihood of the 12 different models in star-BEAST analyses based on the Akaike's information criterion through Markov chain Monte Carlo simulation (AICM; *SE* estimated from 1,000 bootstrap replicates).

Alignment S1. The alignment of the mtDNA genomes used in this study in phylip format (Galapagos_Tortoises_mtDNA_dataset.phy).

How to cite this article: Poulakakis N, Miller JM, Jensen EL, et al. Colonization history of Galapagos giant tortoises: Insights from mitogenomes support the progression rule. *J Zool Syst Evol Res.* 2020;58:1262–1275. <u>https://doi.org/10.1111/jzs.12387</u>