



Symposium Article

Archipelago-Wide Patterns of Colonization and Speciation Among an Endemic Radiation of Galápagos Land Snails

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Abstract

Newly arrived species on young or remote islands are likely to encounter less predation and competition than source populations on continental landmasses. The associated ecological release might facilitate divergence and speciation as colonizing lineages fill previously unoccupied niche space. Characterizing the sequence and timing of colonization on islands represents the first step in determining the relative contributions of geographical isolation and ecological factors in lineage diversification. Herein, we use genome-scale data to estimate timing of colonization in *Naesiotus* snails to the Galápagos islands from mainland South America. We test inter-island patterns of colonization and within-island radiations to understand their contribution to community assembly. Partly contradicting previously published topologies, phylogenetic reconstructions suggest that most *Naesiotus* species form island-specific clades, with within-island speciation dominating cladogenesis. Galápagos *Naesiotus* also adhere to the island progression rule, with colonization proceeding from old to young islands and within-island diversification occurring earlier on older islands. Our work provides a framework for evaluating the contribution of colonization and in situ speciation to the diversity of other Galápagos lineages.

Subject areas: Population structure and phylogeography, Molecular systematics and phylogenetics

Keywords: Bulimulidae, in situ speciation, island biogeography, island progression rule, *Naesiotus*, overseas dispersal

Oceanic islands are dynamic ecosystems with discrete community assemblages resulting from the interplay of colonization, extinction, and in situ (i.e., within-island) speciation (MacArthur and Wilson

1963, 1967). The contribution of within-island speciation to species diversity on islands increases with isolation from continental landmasses and is most noticeable on remote archipelagos where

successful colonization is less frequent (Ricklefs and Bermingham 2004; Whittaker et al. 2008; Gillespie and Baldwin 2010; Heaney 2000). Immigrants on remote islands are likely to encounter fewer predators and competitors and are therefore excellent models to examine the manifestation of adaptive radiation following colonization of previously unoccupied niche space (Emerson and Oromí 2005; Emerson and Gillespie 2008). Characterizing the timing of colonization of archipelagos and between islands represents a crucial step in understanding factors that regulate the formation of species diversity on islands.

Despite isolation from continental ecosystems, many archipelagos around the world are replete with endemic biota widely ranging in mobility, including birds (Lerner et al. 2011; Illera et al. 2012), marine fishes (Piñeros et al. 2019), angiosperms (Weigelt et al. 2016), lizards (Losos et al. 2006; Losos 2009; Phillips et al. 2019), frogs (Maddock et al. 2014; Bell et al. 2015a, 2015b; Labisko et al. 2019), terrestrial arthropods (Gillespie 2004; Garb and Gillespie 2006; Borges and Hortal 2009; Hendrickx et al. 2015; Kitson et al., 2018), and terrestrial snails (Cowie 1992; Chiba 1996, 1999; Goodacre 2002; Triantis et al. 2016). When colonization of remote archipelagos does occur, in situ speciation and subsequent adaptive radiation facilitated by the availability of unoccupied niche space can become dominant processes (Losos 1996; Losos et al. 1998; Schluter 2000; Gillespie 2002; Gillespie and Baldwin 2010). This is particularly common in organisms that are likely to successfully colonize remote land masses (e.g., generalists, self-fertilizing, or clonal species), while subsequently becoming separated from the vector of transportation to the archipelago or losing dispersal abilities at a local scale (thereby limiting gene flow across geographical boundaries). With broad geographical distributions and high levels of local adaptation, such lineages are ideal models for studying the role of colonization in seeding adaptive radiation within archipelagos. Limited gene flow among isolated populations can maintain local genetic differences that serve as a source for phylogenetically informative loci for delineating lineages and estimating biogeographic patterns. Thus, lineage affinities as revealed through molecular phylogenetics can be indicative of historic corridors among present-day localities and provide the key elements to compare present phylogeographic structure against past island geological formation.

Volcanic islands are particularly informative to retrace macroevolutionary processes, as the absence of prior continental connection eliminates the possibility for long-standing ancestral populations. Archipelagos of volcanic origin often display a chronological sequence of island formation and are frequently colonized as each landmass emerges (Shaw and Gillespie 2016). This results in biotic communities concurrently found at different stages of assembly, allowing for the study of evolutionary processes and community assembly through time. Finally, the emergence time of volcanic islands provides a framework in which to test the progression of colonization and speciation of the lineages as islands are sequentially formed.

One such archipelago that displays these properties is the Galápagos Islands, which has served as a model for island biogeography and diversification involving many taxonomic groups (Caccone et al. 2002; Parent and Crespi 2006; Benavides et al. 2009; de Busschere et al. 2010; Torres-Carvajal et al. 2014; Zaher et al. 2018). Geological ages for all major islands and volcanoes have been estimated, with all currently subaerial islands originating within the past 4 million years and several forming within the last million years (White et al. 1993; Geist et al. 2014; Geist, personal communication). Buoyed by an established geologic history, evolutionary biologists have leveraged this information to test patterns and timing of

inter-island dispersal in endemic Galápagos taxa (Caccone et al. 2002; Parent et al. 2008; Benavides et al. 2009; Torres-Carvajal et al. 2014; Zaher et al. 2018). With 61 validly described species (Shoobs and Rosenberg, in preparation) and at least another 10 species awaiting formal description (Shoobs, Kraemer, Parent, unpublished data), Galápagos endemic land snails of the genus *Naesiotus* (formerly placed in the genus *Bulimulus*) form the most species-rich lineage on these islands (Parent et al. 2008). The high number of *Naesiotus* species on each island (in some cases more than 20) is in sharp contrast to most other Galápagos lineages that have produced complexes of single-island endemics with no or very limited within-island speciation, including *Microlophus* lizards (Benavides et al. 2009), *Phyllodactylus* geckos (Torres-Carvajal et al. 2014), and *Pseudalsophis* snakes (Zaher et al. 2018), but see de Busschere et al. (2010). *Naesiotus* snails have radiated into all but coastal terrestrial habitats in Galápagos (Parent and Crespi 2006). On the mid-age and older islands (e.g., Floreana, San Cristóbal, Santa Cruz), it is common to find several *Naesiotus* species in sympatry partitioning space among different local microhabitats, according to substrate, plant species, vegetation type, height and angle (e.g., under rocks vs. particular heights on particular plant species, etc.; Parent and Crespi 2009).

The Galápagos Islands are an exceedingly well-placed archipelago to test mechanisms of within-island speciation as they contain multiple islands and volcanoes that formed more or less at the same time (e.g., Española/San Cristóbal, Floreana/Santa Cruz, Figure 1), which can be used as replicates at similar stages of community assembly. In particular, the island of Isabela is composed of 6 independently formed volcanoes that emerged within tens of thousands of years of one another (White et al. 1993). *Naesiotus* snails have colonized most major islands, all volcanoes on Isabela, and many of the smaller islets in the Galápagos. Most species are thought to be single-island endemics and past phylogenetic work suggests that some island assemblages are (at least in part) the result of within-island speciation (Parent and Crespi 2006). Previous work has estimated phylogenetic relationships and inter-island colonization patterns based on a 2-gene phylogeny for 35 species of Galápagos *Naesiotus* (Parent and Crespi 2006). The phylogenetic placement of roughly half of Galápagos *Naesiotus* species remains to be determined and the relationships within and between species and island lineages have yet to be resolved. Herein, we seek to address these deficiencies and use genome-wide data for 95 populations of 45 species of Galápagos *Naesiotus* and one mainland *Naesiotus* species to reconstruct the timing of the initial overseas colonization followed by the sequential inter-island colonization and within-island speciation across the archipelago. Using our phylogenetic reconstruction, we then test the following hypotheses related to diversification of Galápagos *Naesiotus*: 1) inter-island colonization tracks the sequence of emergence of islands (i.e., inter-island colonization follows the progression rule), 2) inter-island colonization is rare and island clades form monophyletic groups, and 3) island community assembly in *Naesiotus* snails is driven by habitat diversity so that inter-island colonization events are followed by extensive within-island diversification.

Methods

Sample Collection and DNA Sequencing

Naesiotus specimens ($N = 151$; Figure 1 and Supplementary Table S1) from field collections (2000–2016) were selected for RAD sequencing representing both taxonomic and geographic breadth

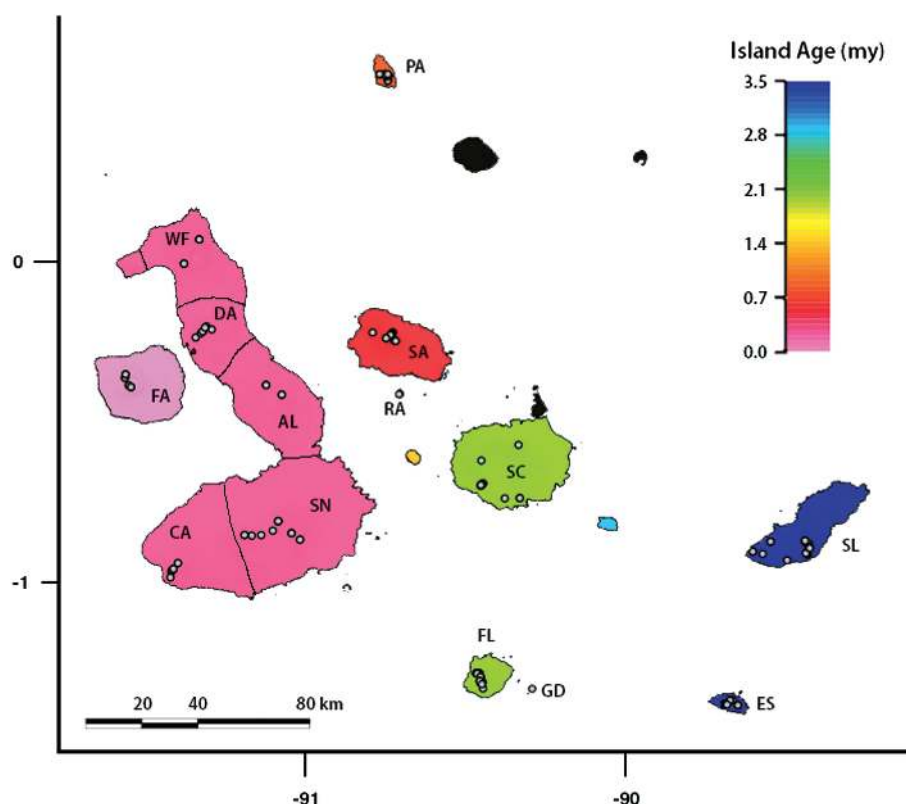


Figure 1. Map of sampling localities in Galápagos used in this study. Islands are colored according to median estimates of emergence time from Geist et al. (2014) and Geist (unpublished data). *Naesiotes* snails are thought to be absent on islands colored in black. ES = Española, FA = Fernandina, FL = Floreana, PA = Pinta, RA = Rábida, SC = Santa Cruz, SA = Santiago, SL = San Cristóbal. Isabela samples are partitioned by volcano: AL = Alcedo, DA = Darwin, CA = Cerro Azul, SN = Sierra Negra, WF = Wolf.

of the genus in the Galápagos Islands with one additional species from mainland Ecuador. In addition to including specimens from most major islands, we included specimens from 5 of 6 volcanoes on the island of Isabela (Alcedo, Cerro Azul, Darwin, Sierra Negra, Wolf). Given the independent formation and geologic history of each Isabela volcano (Geist et al. 2014) and the barren lava flows greatly limiting terrestrial dispersal between them, we treat them herein as separate islands. After collection in the field, snails were preserved in 100% ethanol and once back in the lab, genomic DNA was extracted using Qiagen DNeasy Blood and Tissue Kits following the manufacturer's protocol (Qiagen Inc., Hilden, Germany). Quality of DNA was assessed by identifying a high molecular weight band (>10 Kb) on a 1% agarose gel, and DNA concentration was quantified on a Qubit 4 Fluorometer. Library preparation followed the bestRAD protocol (Krohn et al. 2018). Samples were digested with the restriction enzyme SbfI followed by ligation of unique 8-bp barcoded biotinylated adapters. After this step, all samples were pooled and randomly sheared to ~400 bp fragments using a Covaris M220 shearing focused ultrasonicator. The multiplexed libraries were enriched for adapter-ligated fragments and size-selected for 300–400 bp fragments using AMPure magnetic beads, and then standardized to a concentration of 10 nM. Finally, the multiplexed libraries were sequenced on two 150 bp paired-end lanes on an Illumina HiSeq 4000 at the University of Oregon's Genomic Core Facility. Raw Illumina reads were pre-processed with custom Perl scripts to filter for reads containing the SbfI cut site. The processed reads were then used for de novo RAD loci assembly using ipyrad v.0.7.29 (Eaton 2014; Eaton and Overcast 2018). To optimize for

sufficient divergence between samples for phylogenetic analysis, we generated 6 primary datasets for analyses. We manipulated the clustering threshold using either 85%, 88%, or 91%, and for each clustering threshold we tested 2 datasets: one contained all loci removed that were not present in at least 4 samples (quartet dataset) and the second removed all loci that were not present in at least 10 samples (Bayesian dataset). Failing to find significant differences in topology among the 6 datasets, we opted to use the 85% threshold with a minimum coverage of 4 samples per locus to include a broader set of data in our analyses.

Phylogenetic Analyses

We employed 3 approaches to evaluate the phylogenetic relationships among our taxa to account for possible biases between methods. First, a species tree method based on the multi-species coalescent (MSC) was used to estimate a phylogeny with singular value decomposition (SVD) quartets (Chifman and Kubatko 2014), a method that analyzes sequence data on a site-by-site basis. This approach utilizes site pattern probability distributions and applies algebraic statistics to calculate the SVD score for all taxa-quartets at unlinked SNPs under the assumption that each site has its own genealogy drawn from the MSC. These quartets of taxa were assembled into a species tree (Supplementary Figure S2) using the Quartet FM algorithm (Reaz et al. 2014) in PAUP* (Swofford 2003) for 50 000 quartets. All quartets were evaluated and support for nodes was inferred using 1000 nonparametric bootstrap replicates using the previously assembled quartet dataset. We also tested the

topology of the SVD quartets phylogeny by inferring a species tree using a Bayesian coalescent method implemented in the SNAPP plugin (Bryant et al. 2012) using BEAST2. To reduce the computational intensity required by our full dataset, we only included one individual per species (selected by choosing the highest coverage individual for each species), unless preliminary analyses suggested the possibility of paraphyly, in which case one individual per clade within a species was included.

We also constructed a data matrix of concatenated RADseq loci. RADseq loci can be concatenated to infer phylogenies, and the resulting concatenation matrices commonly produce accurate inferences (Ree and Hipp 2015). We initially implemented a Bayesian approach to estimate phylogenetic relationships using BEAST 1.8.3 (Rambaut and Drummond 2007) to verify the topology of the unrooted SVD quartet tree. For this analysis, we tested different datasets to determine if the amount of data included would impact our results. First, an alignment concatenating all SNPs from the SVD quartet dataset was run for 100 million generations under an HKY model of evolution due to the onerous size of the dataset (223,392 SNPs for 153 taxa). We also compared this topology (Supplementary Figure S3) to a reduced alignment (15,535 SNPs) containing all SNPs from each locus that was present for a minimum of 10 individuals and contained a maximum of 20 SNPs per locus. The Bayesian analyses (using the 223,392 and 15,535 SNPs datasets) produced identical topologies with respect to all inter-island relationships among clades, so we proceeded with downstream analyses using the reduced dataset. Previous work suggests that dating phylogenies using only SNPs can bias estimates of divergence (Leaché et al. 2015). Therefore, we performed analyses with and without invariant sites to evaluate their impact on our date estimates. The BEAST output was analyzed using Tracer v1.6 (Rambaut et al. 2014) to verify an effective sample size exceeding 200 for all parameters being estimated, and TreeAnnotator was used to produce a maximum clade credibility tree from the post-burn-in trees.

Divergence Dating

We estimated the timing of *Naesiotus* divergence events by inferring an absolute evolutionary timescale via Bayesian time calibration of the phylogenetic tree. Given the lack of information about a general mutation rate for gastropod RAD loci, we used the oldest estimate for time of emergence of the first-known subaerial land mass in the Galápagos to calibrate our phylogeny. Age estimates from geological studies of the archipelago based on known subaerial land masses from the Carnegie Ridge formerly placed the emergence of now submerged islands between 5 and 9 million years ago (mya) (Christie et al. 1992), with a maximum time of emergence at 14 mya (Werner et al. 1999). However, recent work suggests that currently submerged landmasses in Galápagos may have been emergent 20 mya (Orellana-Roviroso and Richards 2018). In all divergence dating analyses, a lognormal prior distribution was placed at 4.0 mya (due to the oldest proposed emergence of any currently subaerial islands in the Galápagos archipelago), but the maximum age was constrained to 20 mya with the minimum age specified as 0. Initially, *Oreohelix* (family Oreohelidae) was used as an outgroup based on its close phylogenetic relationship to Bulimulidae inferred from whole mitochondrial genome data (Linscott and Parent 2019). Preliminary analyses rooted with *Oreohelix* recovered all Galápagos *Naesiotus* as monophyletic with respect to the mainland samples used in the present study. Therefore, the mainland Ecuador *Naesiotus* from the present study was used to root trees in subsequent analyses.

For the Bayesian approach to estimating the timing of divergence events, analyses were conducted in BEAST under the GTR+ Γ model of evolution, a relaxed lognormal molecular clock, and a birth-death model tree prior. The analysis consisted of 50 million generations with a sampling interval of 5000 and a burn-in of 25%. The BEAST output was analyzed using Tracer v1.4 (Rambaut and Drummond 2007) to verify an effective sample size exceeding 200 for all parameters being estimated, and TreeAnnotator was used to produce a maximum clade credibility tree from the post-burn-in trees. To evaluate the adherence of Galápagos *Naesiotus* to the island progression rule (i.e., a sequence of colonization from old to young islands, Funk and Wagner 1995; Juan et al. 2000), we used a general linear model as implemented in R (R Core Team 2016) to test for a relationship between clade age (calculated as the mean estimate of divergence within each island or volcano from the Bayesian phylogeny) and island age (Geist et al. 2014; Geist, unpublished data).

Ancestral Area Estimation

To reconstruct the ancestral distribution of Galápagos *Naesiotus* species in our dataset, we analyzed the Bayesian time-calibrated tree by employing the R package “BioGeoBEARS” (Matzke 2013), using each island (or volcano within Isabela) as a separate region. One advantage of BioGeoBEARS is that it allows for the comparison of the 3 most frequently used methods for ancestral area estimation: dispersal vicariance analysis (DIVA; Ronquist 1997), dispersal extinction cladogenesis analysis (DEC; Ree and Smith 2008), and Bayesian analysis of biogeography (BAYAREALIKE; Landis et al. 2013). The DIVALIKE model is similar to DEC in that it allows narrow vicariance as well as widespread vicariance, but does not include sympatric speciation (Ronquist 1997). Here, widespread vicariance may be demonstrated as an ancestor with a distribution ABCD splitting into 2 distributions AB and CD. The DEC model focuses on narrow vicariance, or allopatric speciation due to separation of the geographical range (when an ancestor with distribution ABC splits into 2 distributions A and BC) and also allows for sympatric speciation (when an ancestor with a distribution ABC splits into 2 distributions A and ABC; Ronquist and Sanmartín 2011). Finally, the BAYAREALIKE model is a modification of DEC without cladogenesis. The DEC, DIVALIKE, and BAYAREALIKE models have 2 free parameters that specify the rate of range expansion (d) and of range contraction per million years (e), while each models’ alternative adds 1 or 2 free parameters, j and x (see Matzke 2014 for further explanation). The j parameter, corresponding to the probability of founder-event dispersal (Matzke 2014), was initially implemented for island systems, in which new lineages may be established by colonization of a new island without a widespread ancestor (Clark et al. 2008). The x parameter allows dispersal probability between ranges to be altered as a function of distance (Van Dam et al. 2016). The distance matrix between geographical ranges (i.e., islands or volcanoes) was calculated by generating a pairwise distance matrix in kilometers between the highest elevation point of each island or volcano (for Isabela).

To infer ancestral ranges at internal nodes of the phylogeny, each individual was designated as present or absent on each of the 12 islands or volcanoes within Isabela in our study. We performed model selection by comparing Akaike information criterion (AIC) values and AIC weights on time-stratified and non-stratified models to determine the most appropriate model(s) for the colonization history of Galápagos *Naesiotus*. In the time-stratified models, probability of dispersal and island occupancy were restricted by estimates of island

emergence time (Geist et al. 2014; Geist, unpublished data), while in the non-stratified models these constraints were relaxed (equal probability of dispersal between all islands and occupancy of any island possible at any time). However, in all cases non-stratified models yielded a higher likelihood, so we do not present the results of the time-stratified models here.

After determining the most appropriate biogeographic model given our data, 500 biogeographical stochastic maps were simulated under the chosen model parameters. However, recent criticisms of both DEC models and the j parameter do not assume probabilistic equivalency of all modes of speciation (Ree and Sanmartín 2018). Therefore, here we consider the best non-DEC model without the j parameter. Counts of cladogenesis events associated with vicariance, within-island speciation, founder-event dispersal, as well as dispersal events between ranges were recorded in each model. Mean counts were then determined by averaging over the entire map to determine the most likely modes of speciation and paths of dispersal across the archipelago.

Results

Phylogenetic Reconstruction and Divergence Dating

All analyses, regardless of the phylogenetic method or assembly dataset, recovered 2 major clades of island populations that split along a southeast-northwest division with the southeastern clade comprised of all populations from Española, Floreana (plus nearby Gardner), and San Cristóbal, and the northwestern group containing all populations from Fernandina, Isabela (all volcanoes), Pinta, Rábida, Santiago, and Santa Cruz (Figures 2–4). Our analysis estimated a basal divergence of 3.2 mya (95% HPD 2.1–6.7 mya), with the southeast clade 2.0 mya (1.1–4.3 mya) coalescing at a younger date than the northwest lineage 2.6 mya (1.4–5.5 mya; Figure 2, Table 1). In all analyses, island-monophyletic clades were found for Española, Floreana, Gardner, Pinta, Rábida, and San Cristóbal. Santiago contained 3 independent lineages, with no mean estimate of within-island cladogenesis older than 0.2 mya (Table 1). One Santiago population was sister to the Rábida clade in all analyses, another Santiago clade was sister to all of Santa Cruz + Isabela, while the third Santiago group was nested within an otherwise monophyletic Santa Cruz clade.

In all analyses, Fernandina *Naesiotus* are found to be nested within Isabela. While the Isabela snails generally group by volcano, there were some violations of monophyly (Figures 2 and 3). Alcedo forms 2 lineages, one of which (Alcedo 1) is sister to all of Isabela + Fernandina, and the other (Alcedo 2) sister to a Darwin + Fernandina clade, with one Fernandina population nested within Darwin, forming a clade sister to the rest of Fernandina. Wolf is monophyletic and sister to the Alcedo 2 + Darwin + Fernandina clade. The populations on Volcán Wolf were recovered as sister to all Isabela + Fernandina except Alcedo 1. For all analyses, Cerro Azul forms a clade nested within Sierra Negra, which has 2 major lineages.

Ancestral Area Estimation and Colonization History

The DEC + j + x model from the non-stratified analysis was found to have the lowest AIC score and greatest AIC weight (AIC: 119.3, AIC weight: 0.62; Table 2). However, as stated in the methods, serious concerns have been raised regarding both the DEC model and the j parameter of founder-event-speciation (Ree and Sanmartín 2018). We present the results from all models tested in Table 2, but given

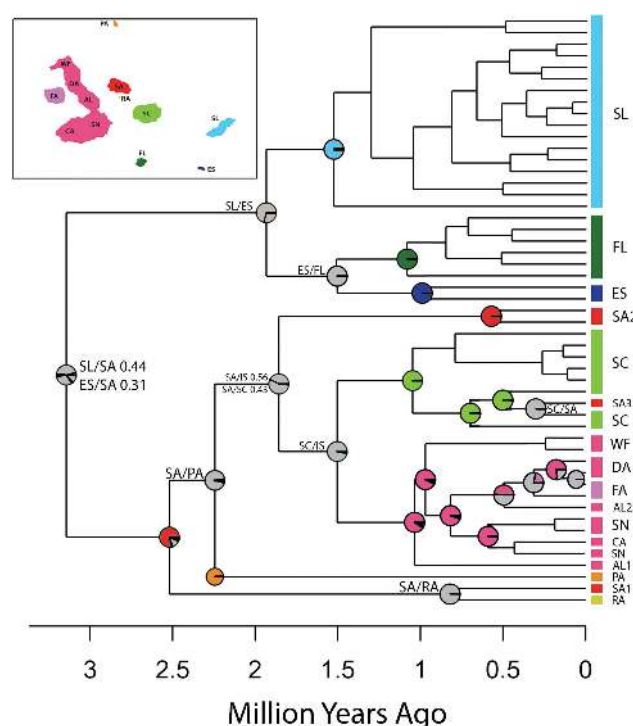


Figure 2. Ancestral area estimation using the time-calibrated Bayesian inference phylogeny. Probabilities of ancestors at each island are reconstructed on all major nodes. Probabilities of multi-island distributions are in gray and the most likely combinations listed on the node if no single-island state exceeds 60%. Colors match islands from inset map. Colors for San Cristóbal and Floreana differ slightly from Figure 1 to distinguish their clades and node probabilities from Española and Santa Cruz, respectively. Bayesian Posterior Probabilities for this tree can be viewed in Supplementary Figure S5. Volcano and island abbreviations as in Figure 1, with the addition of IS = Isabela.

these reservations, we restrict our discussion to the most favored model not affected by these concerns: DIVALIKE + x , from the non-stratified analysis. There are 3 major biogeographical findings resulting from this model. First, the basal node was recovered with several polymodal states, none of which exceeds 45% probability. The mean age estimate for this node is 3.2 mya, which predates the estimated geological age of most current islands. Second, the ancestor of the southeastern lineage has the highest probability (71%) of occupying both Española and San Cristóbal. Third, all within-island radiations stemmed from nodes with high probabilities (posterior = 1) of an ancestor within their home island.

When the x parameter (distance-dispersal scaling exponent) was included in the ancestral area estimation, all non- j parameter models recovered a higher d (rate of range expansion; Table 2), because a higher x lowers probability of dispersal. This is unsurprising as d inversely scales with dispersal probability, which is directly impacted by the scaling factor of the distance-dispersal matrix scaling exponent x . With lower probability of dispersal between sites (x), there is a corresponding increase in the rate of dispersal (d and j), so estimates for d and j significantly increase with more negative values of x (Van Dam and Matzke 2016). There were stark differences in the parameter estimates between the stratified and non-stratified models for d , j and x (the dispersal, jump-dispersal, and distance-dispersal scaling exponent parameters, respectively) (Table 2). Non-stratified models estimated a value of x that corresponded roughly to an

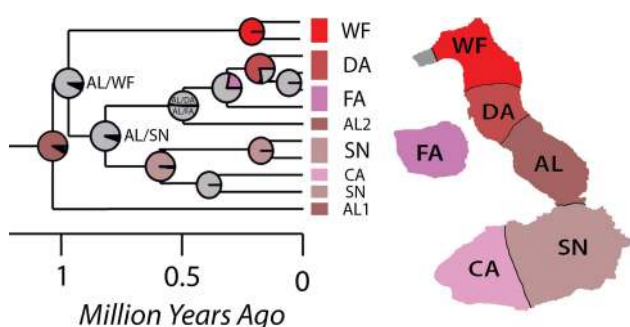


Figure 3. The time-calibrated phylogenetic reconstruction (Bayesian) and ancestral area estimation for Isabela and Fernandina. Volcano and island abbreviations as in Figure 1.

Table 1. Estimated dates and 95% confidence intervals (CIs) for all major nodes in the phylogenies

	Oldest within-island divergence	95% CI
Galapagos <i>Naesiotus</i>	3.2	2.1–6.7
Southeast Lineage	2.0	1.1–4.3
San Cristobal	1.6	0.9–3.4
Espanola + Floreana	1.6	0.9–3.4
Espanola	1.1	0.5–2.3
Floreana + Gardner	1.1	0.6–2.5
Floreana	0.9	0.5–2.0
Gardner	0.2	0.04–0.5
Northwest lineage	2.6	1.4–5.5
Rabida + Santiago 1	0.9	0.4–2.1
Rabida	0.2	0.08–0.5
Santiago 1	0.2	0.04–0.5
Pinta + Santa Cruz + Santiago 2&3 + Isabela	2.3	1.3–4.9
Pinta	0.2	0.07–0.5
Santa Cruz + Santiago 2&3 + Isabela	1.9	1.1–4.1
Santiago 2	0.6	0.3–1.5
Santa Cruz + Santiago 3 + Isabela	1.6	0.9–3.4
Santiago 3	0.1	0.03–0.2
Isabela + Fernandina	1.1	0.6–2.4
Alcedo 1	0.1	0.02–0.3
Rest of Isabela + Fernandina	1.0	0.5–2.2
Wolf	0.3	0.1–0.7
Rest of Isabela + Fernandina	0.9	0.4–1.9
Sierra Negra + Cerro Azul	0.7	0.3–1.4
Sierra Negra 1	0.4	0.2–0.9
Sierra Negra 2 + Cerro Azul	0.5	0.3–1.1
Sierra Negra 2	0.3	0.1–0.8
Cerro Azul	0.4	0.2–0.9
Alcedo 2 + Darwin + Fernandina	0.6	0.2–1.2
Alcedo 2	0.1	0.03–0.3
Darwin + Fernandina	0.4	0.2–0.9
Fernandina 1	0.2	0.06–0.4
Darwin + Fernandina 2	0.2	0.09–0.6

In the case of radiations composed of multiple lineages within an island or volcano, the oldest mean estimate of within-island divergence is presented.

inverse square law of dispersal probability ($x = -1.3$ for DIVALIKE + x), where the probability of dispersal decreased exponentially at a rate of $1/\text{distance}^{1.3}$.

Mean counts of cladogenesis events over the biogeographic stochastic maps indicate that the greatest proportion of cladogenic

events are associated with within-island sympatry (56%), whereas vicariance and dispersal had lower probabilities (23% and 21% respectively; Table 3). We found a significant linear positive relationship between clad age and island geological age ($R^2 = 0.65$, $p = 0.008$; Figure 6), and lineages on younger islands started speciating within-island sooner after colonization than on older islands (i.e., deviation from the 1:1 relationship increases with island age in Figure 6).

Discussion

We find that the Galápagos *Naesiotus* species included in our study coalesce around 3.2 mya (Figure 2 and Supplementary Figure S5), around the median estimates of emergence for the 2 oldest islands in the archipelago: San Cristóbal (2.4–4.0 mya) and Española (3.0–3.5 mya). Using the geological formation of islands to calibrate the timing of phylogenetic divergence can be circular. However, the prior we used here (4 mya with a log-normal distribution ranging from 20 to 0 mya) allowed for the exploration of a wide range of possible values of coalescent time and did not strictly dictate the age estimate we recovered. Furthermore, we are using the estimated dates of emergence of the current oldest island (4 mya) and the age of the oldest known submerged island (20 mya) as the only geological dates to inform our prior. The date of geological formation for any additional islands making up the archipelago are not used to calibrate more recent nodes in the phylogeny, which means that estimates of the order of inter-island colonization and within-island speciation subsequent to the initial overseas colonization are independent from (i.e., not constrained by) geological date estimates. With no fossils known for the group and no reliable molecular clock, calibration using selected geological formation estimates is currently the best option for our system. While there is an element of circularity to this method, the date of the ancestral node was assigned a maximum date of 20 mya as a prior (per Orellana-Roviroso and Richards 2018), which it never approached. Furthermore, re-running this method with the maximum age set at 4 mya (based on only the current islands), 9 mya (Christie et al. 1992), and 14 mya as priors (Werner et al. 1999; analyses not shown), consistently resulted in the estimated age of the basal node for Galápagos *Naesiotus* falling in the 3.0–3.5 mya range. These results are in line with an initial divergence of *Naesiotus* snails within Galápagos occurring on the currently emerged land mass. The timing of the initial cross-oceanic colonization by *Naesiotus* could be further evaluated by including lineages of Galápagos *Naesiotus* currently missing in our analysis (e.g., from Pinzón and Santa Fé islands).

Currently available data do not allow for a robust analysis of mainland-island colonization dynamics. *Naesiotus* congeners occurring on nearby mainland Ecuador and Peru (Breure and Coppo 1978, Breure 1979, Richardson 1995) could have colonized the islands more than once. However, species-level relationships within *Naesiotus* and other bulimulid snails are not well established due to currently limited representation on phylogenies of the geographical and taxonomic breadth of the group. This work, requiring extensive additional sampling on the mainland, will be the focus of future research and will allow us to best address the frequency and sequence of mainland-island colonization.

Our finding that *Naesiotus* diversity results from a single colonization with subsequent within-island radiation for most single-island assemblages (with the exceptions of Fernandina and Santiago), partially contradicts the more common pattern of island paraphyly found by previous phylogenetic studies mostly based on

mitochondrial DNA (mtDNA, Figure 5; Parent and Crespi 2006; Kraemer et al. 2019). The present study did not include mtDNA, and a more detailed evaluation of the extent of mito-nuclear discordance in this system is beyond the scope of this article. Nevertheless, our use of thousands of independent evolving SNPs allows us to accurately resolve relationships among Galápagos *Naesiotus* clades

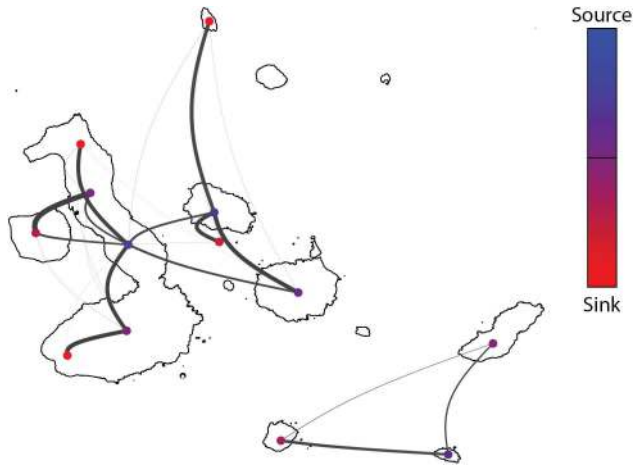


Figure 4. Dispersal network of Galápagos *Naesiotus* inferred from biogeographical stochastic mapping. Wider and darker lines indicate a greater proportion of dispersal. Weight for the interconnecting edges is the proportion of dispersal coming into the island from each island (each island's edges must sum to a weight of 1). Volcano and island abbreviations as in Figure 1.

and assess the biogeographic history of the group. This assertion is further supported by our use of multiple phylogenetic analyses that recovered similar topologies and branch lengths that are congruent with respect to the composition and origination of most major clades. It is currently unclear to what extent gene flow within islands could have contributed to the intra-island relationships of *Naesiotus* and if recent hybridization and introgression may obscure older biogeographic patterns.

We find that estimated dates of coalescence for each within-island radiation are younger than maximum (and in most cases minimum) dates of island emergence as put forth in Geist et al. (2014) with 2 exceptions: the lineages on Fernandina and Isabela originated earlier (0.2 and 1.1 mya, respectively; Figure 3, Table 1) than when the islands emerged (0.06 and 0.6 mya, respectively). However, in both cases, the mean estimate of island emergence equals the bottom limit of the 95% confidence interval around any inter-island divergence and the wide confidence intervals around these mean dates leave some uncertainty. One possible biological explanation for the inference of clades originating prior to the estimated time of island emergence is that multiple sister lineages might have colonized the same island, then went extinct on the islands where they originated from. Our phylogeny could also be missing extant species that if included, could result in a different ancestral range reconstruction. For example, several species from Santa Cruz are missing due to rarity or extinction (Parent, unpublished data). We find evidence for paraphyly of some Galápagos *Naesiotus* species (Supplementary Figure S4), suggesting that the current taxonomy may be underestimating species diversity. The recovery of paraphyly for multiple species in the current and past studies (Parent and Crespi 2006; Kraemer et al. 2019) illustrates a need for comprehensive assessment of genetic diversity and

Table 2. Likelihood results for all models tested in the ancestral area estimation (BioGeoBEARS) using both stratified and non-stratified models

MODEL	AIC	AIC Weight	LnL	Parameters	<i>d</i>	<i>e</i>	<i>j</i>	<i>x</i>
BAYAREALIKE	225.9	0.00	-110.9	2	0.0	0.5	0.0	0.0
BAYAREALIKE_J	147.9	0.00	-70.9	3	0.0	0.0	0.0	0.0
BAYAREALIKE_Jx	127.1	0.01	-59.5	4	0.0	0.0	1.0	-2.1
BAYAREALIKE_x	212.7	0.00	-103.4	3	3.7	0.5	0.0	-1.1
DEC	183.6	0.00	-89.8	2	0.0	0.1	0.0	0.0
DEC_J	143.3	0.00	-68.7	3	0.0	0.0	0.0	0.0
DEC_Jx	120.4	0.37	-56.2	4	0.0	0.0	3.0	-2.3
DEC_x	169.3	0.00	-81.6	3	7.9	0.1	0.0	-1.3
DIVALIKE	164.7	0.00	-80.3	2	0.0	0.0	0.0	0.0
DIVALIKE_J	141.1	0.00	-67.6	3	0.0	0.0	0.0	0.0
DIVALIKE_Jx	119.3	0.62	-55.7	4	0.0	0.0	1.9	-1.8
DIVALIKE_x	155.8	0.00	-74.9	3	8.0	0.0	0.0	-1.3

Model with the highest AIC score is in bold. *d* = dispersal, *e* = extinction, *j* = founder-event speciation, *x* = power that the dispersal probability matrix is scaled by distance.

Table 3. Summary of biogeographical stochastic mapping for *Naesiotus* cladogenic events using DIVALIKE+*x*

Mode	Type	Mean counts	Percent explained
Dispersal	Range expansion	13.32 (0.53)	21%
	Range contraction	0	0%
Within-area speciation	Sympatry	35.68 (0.53)	56%
	Subset-speciation	0	0%
Vicariance	Vicariance	14.32 (0.53)	23%

Means counts and standard deviation (SD) are reported for different event types.

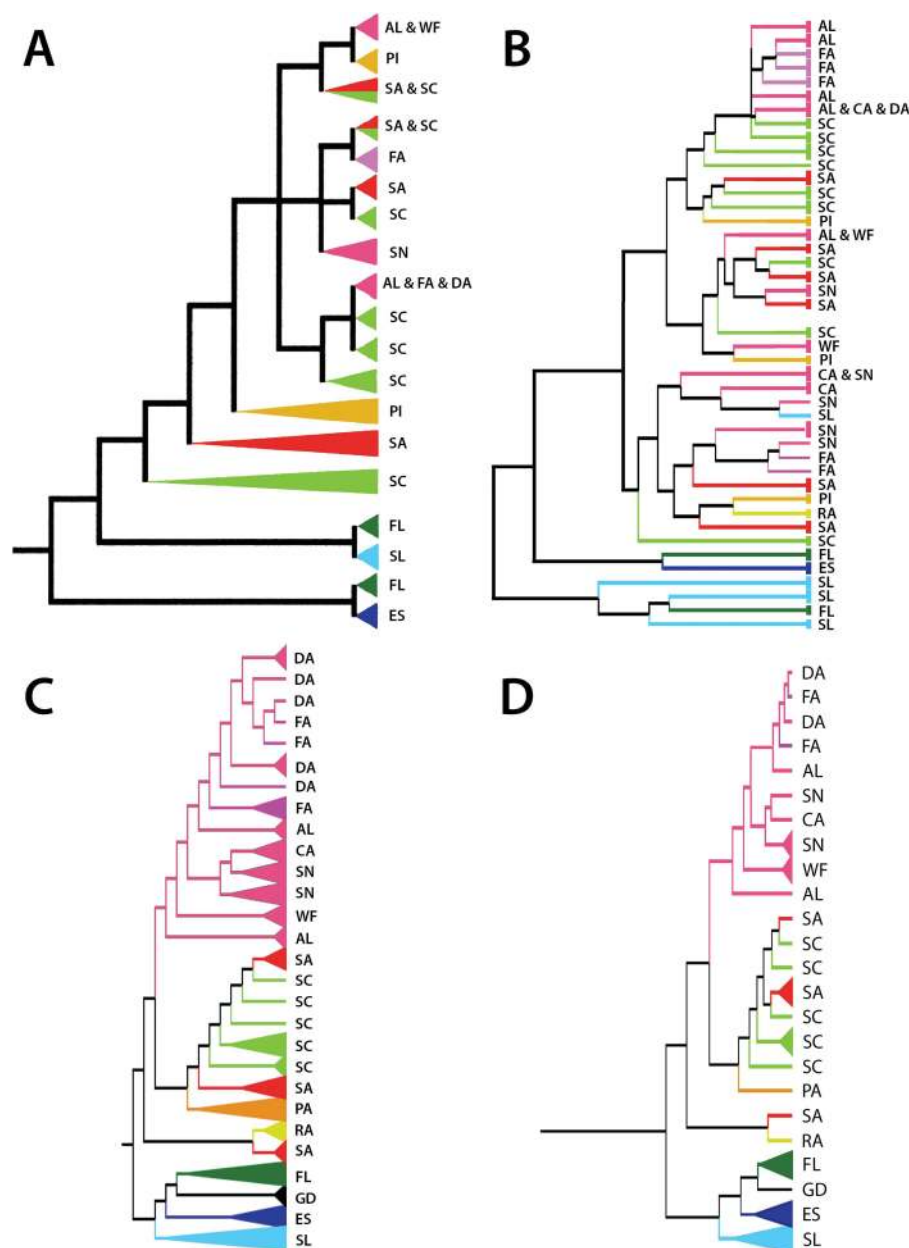


Figure 5. Different phylogenetic hypotheses for the evolutionary histories of Galápagos *Naesiotus* from (A) Parent and Crespi 2006 based on CO1 and ITS, (B) Kraemer et al. 2019 based on mtDNA, (C) SVD quartets estimation from this study, (D) Bayesian phylogeny from this study. Volcano and island abbreviations as in Figure 1.

species delimitation in *Naesiotus*, which will form the focus of future work. Further investigation of gene flow among intra-island lineages will be required to better understand population-scale dynamics in Galápagos *Naesiotus*.

We find that stratified (as compared to non-stratified) models altered our estimates of dispersal probabilities between geographic ranges. The lower estimates of x in the stratified models suggest a possible role for the timing of island formation in determining dispersal-distance probabilities. Discrepancy between the models may be heavily influenced by the fact that we are treating islands as static entities through time in the non-stratified models, as these models did not account for changes in island size and associated geologic connections over time. For islands forming in sequence,

allowing occupancy and dispersal to possible geographic range states prior to their emergence may upwardly bias our estimates of x by forcing the non-stratified model to consider dispersal from the oldest islands to younger islands prior to their emergence. However, many of the current islands were likely connected to one another within the last 0.5 million years (Ali and Aitchison 2014; Karnauskas et al. 2017; Norder et al. 2019), and the island geography is thought to have changed dramatically due to fluctuating sea levels since the last glacial maximum (Geist et al. 2014). As an example, our ancestral range reconstructions suggest the ancestor for the southeastern clade as simultaneously occupying both San Cristóbal and Española (71%, Figure 2). However, these 2 islands were most likely previously connected as recently as 2 mya

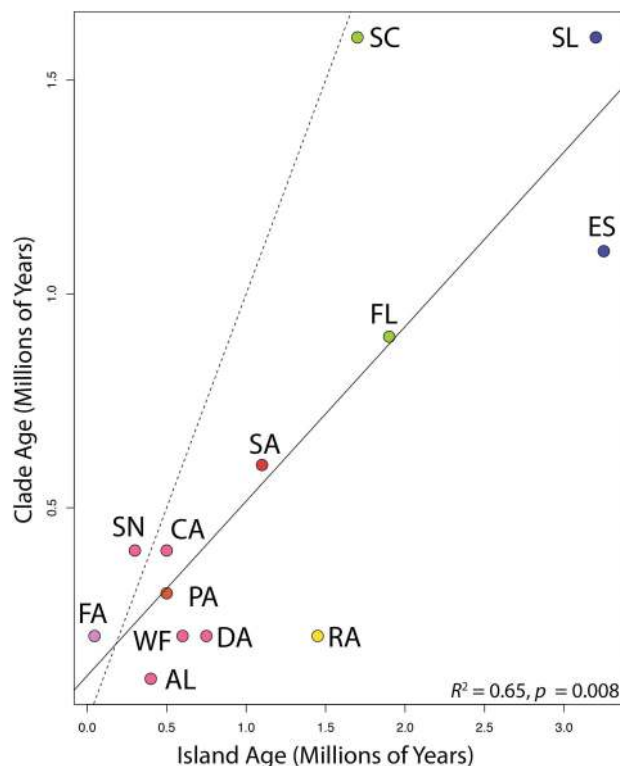


Figure 6. Linear relationship (solid line) between mean age of emergence for each island and mean estimation of divergence for *Naesiotus* within each island based on the Bayesian phylogeny. Colors correspond to island age (Figure 1). Dotted gray line denotes a 1:1 relationship between island and clade age, which would reflect within-island diversification immediately following colonization. Volcano and island abbreviations as in Figure 1.

(Karnauskas et al. 2017), so the ancestor of this clade presumably had a distribution across the combined landmass. The past connectivity between islands could also explain our finding of ancestral species inferred to occur on islands prior to their emergence and might contribute to the relatively poor performance of the stratified models (i.e., higher AIC values for every model tested in the stratified analysis compared to the same model in the non-stratified analysis).

There is a strong significant positive relationship between island clade age and island geological age (Figure 6), indicating that clades are colonizing and diversifying on islands as they appear. The basal node of the Galápagos radiation is the only phylogenetic node that is constrained based on island geological formation, and other more recent nodes are all free to take any younger age value and are estimated independently from geological date estimates. Our phylogenetic and ancestral range reconstruction, therefore, suggest that Galápagos *Naesiotus* generally follow the island progression rule (Wagner and Funk 1995), consistent with the findings of Parent and Crespi (2006). A similar trend has been found in other clades of snails diversifying on island systems, including Hawaii (Holland and Hadfield 2004; Rundell et al. 2004) and Tahiti (Haponski et al. 2019). In Galápagos, this pattern has been observed in a range of taxonomic groups (Parent et al. 2008), including endemic *Hogna* spiders (de Busschere et al. 2010), *Stomion* and *Galapaganus* beetles (Finston and Peck 1997, 2004; Johnson et al. 2000; Sequeira et al. 2000, 2008) and *Chelonoidis* tortoises (Caccone et al. 2002; Beheregaray et al. 2004; but see

Poulakakis et al. 2012). Land snails have previously been shown to adhere to the island progression rule (Holland and Hadfield 2004), as they can colonize broad geographic ranges in sequence while adapting and diversifying locally.

Previous work on Galápagos *Naesiotus* has identified factors important in promoting species richness resulting from within-island speciation and between-island colonization (Parent and Crespi 2006; Losos and Parent 2009). This past work, based on a 2-locus phylogeny and a smaller set of species (Figure 5A), highlighted the important contribution of within-island speciation to species richness on most islands. Many other taxonomic groups in the Galápagos are composed of single-island endemics (e.g., Benavides et al. 2009; Torres-Carvajal et al. 2014; Zaher et al. 2018), which also point to limited inter-island colonization. Here we also find that colonization between islands is rare for Galápagos *Naesiotus*. In contrast, a signature of inter-island gene flow was detected in Galápagos *Hogna* spiders, which presumably allowed for the introgression of key adaptive alleles in species distributed across islands and contributing to parallel evolution of morphological traits in this group (de Busschere et al. 2015). Colonization and speciation of Galápagos *Naesiotus* (the most species-rich adaptive radiation of Galápagos) reveals diversity predominantly attributed to within-island speciation. On an archipelago that is not particularly known for high species richness, these findings highlight the immense contribution of in situ speciation to diversity. Future studies should focus on the ecological conditions facilitating adaptation and the maintenance of genetic differences between *Naesiotus* populations within islands. Ultimately, understanding the dynamics and contribution of inter-island dispersal and in situ speciation to insular diversity will contribute to a better understanding of the factors affecting the rate and trajectory of community assembly. The importance of within-island radiation to species richness observed here establishes a need for similar studies in other island systems, which will then allow for cross-archipelago studies of the ecological, geological, geographical, and lineage-specific conditions leading to insular diversification.

Supplementary Material

Supplementary data are available at *Journal of Heredity* online.

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Data Accessibility

All sequences can be found on the Sequence Read Archive.

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