

# A comparative analysis of island floras challenges taxonomy-based biogeographical models of speciation

Javier Igea,<sup>1</sup> Diego Bogarín,<sup>2</sup> Alexander S. T. Papadopoulos,<sup>1,3</sup> and Vincent Savolainen<sup>1,4</sup>

<sup>1</sup>Department of Life Sciences, Imperial College London, Silwood Park Campus, Ascot, SL5 7PY, United Kingdom

<sup>2</sup>Jardín Botánico Lankester, Universidad de Costa Rica, P.O. Box 302–7050, Cartago, Costa Rica

<sup>3</sup>Current Address: Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, United Kingdom

<sup>4</sup>E-mail: v.savolainen@imperial.ac.uk

Received September 2, 2014

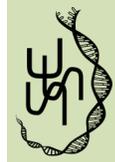
Accepted November 28, 2014

Speciation on islands, and particularly the divergence of species *in situ*, has long been debated. Here, we present one of the first, complete assessments of the geographic modes of speciation for the flora of a small oceanic island. Cocos Island (Costa Rica) is pristine; it is located 550 km off the Pacific coast of Central America. It harbors 189 native plant species, 33 of which are endemic. Using phylogenetic data from insular and mainland congeneric species, we show that all of the endemic species are derived from independent colonization events rather than *in situ* speciation. This is in sharp contrast to the results of a study carried out in a comparable system, Lord Howe Island (Australia), where as much as 8.2% of the plant species were the product of sympatric speciation. Differences in physiography and age between the islands may be responsible for the contrasting patterns of speciation observed. Importantly, comparing phylogenetic assessments of the modes of speciation with taxonomy-based measures shows that widely used island biogeography approaches overestimate rates of *in situ* speciation.

**KEY WORDS:** Cladogenesis, Cocos Island, phylogeny, sympatric speciation.

Islands have provided endless research opportunities for systematists and ecologists. In 1967, MacArthur and Wilson set the foundations for island biogeography, providing a theoretical framework to predict species formation, immigration, and extinction (MacArthur and Wilson 1967; Losos and Ricklefs 2009a). Oceanic islands are thought to have simplified histories compared to continents and serve as “natural laboratories” for ecology and evolution (Whittaker and Fernández-Palacios 2007). For example, remote oceanic islands can act as “blank slates” from which one can infer the colonization and diversification processes that shaped the species assemblies found today (Losos and Ricklefs 2009b). Hence, islands often become the focus of scientific debates. One such point of contention is whether species can evolve (cladogenesis) on small islands where geographic isolation is unlikely, that is, speciation in the face of gene flow. Lord Howe Island (LHI) in the Tasman Sea has provided several such cases of

sympatric speciation in plants (Savolainen et al. 2006; Papadopoulos et al. 2011). Although the possibility of an allopatric phase has been proposed (Stuessy 2006; Stuessy et al. 2014), assessments suggest this is not the case (Savolainen et al. 2006; Papadopoulos et al. 2014). Island biogeographers have used area to determine the probability for cladogenesis to occur on islands for a wide range of taxa (e.g., Coyne and Price 2000; Kisel and Barraclough 2010). Other researchers have made similar predictions based on physiographical parameters, such as maximum island elevation, habitat heterogeneity, and age (e.g., Stuessy et al. 2006). These models of island biogeography are often developed using taxonomic classifications (Bunnefeld and Phillimore 2012; Patiño et al. 2013), with congeneric endemics considered to be the products of *in situ* cladogenesis. This can be problematic when there are biases in taxonomic effort or when multiple colonizations of congeners have occurred (Gray and Cavers 2014).



Here, we examined speciation on a remote island in the Pacific Ocean, Cocos Island (also known as *Isla del Coco*), with two objectives. First, we evaluated the various modes of speciation that lead to the present-day plant diversity on Cocos Island, which we compare with LHI. Second, we used the Cocos Island and LHI floras to evaluate how taxonomic versus phylogenetic assessments may affect inferences from models of island biogeography.

Cocos Island is a remote island that lies 550 km off of the Pacific coast of Costa Rica, and 680 km to the north of the Galapagos archipelago. It was discovered in the 16th century and is now a Costa Rican National Park (Fig. 1A) as well as one of the world's largest, tropical, uninhabited islands. It is the only emergent part of the Cocos Ridge and was formed as a result of volcanic activity by the Galapagos hotspot between 1.9 and 2.4 million years ago (Bellon et al. 1983; Castillo et al. 1988). Its high annual rainfall (5000–7000 mm, Alfaro 2008) makes it one of the few islands in the Eastern tropical Pacific that harbors a tropical rainforest. It was designated a World Heritage Site by UNESCO in 1997. Unlike surrounding islands, including the Galapagos, Malpelo, and Clipperton, the vegetation of Cocos Island is thought to be rather similar to the original mainland source regions (Hogue and Miller 1981). The flora of Cocos Island now comprises 189 native species, 33 of which are endemic (Trusty et al. 2006). Due to its wet climate, it has a particularly high number of fern species, which account for 42% of the native flora and half of the endemic species.

Like Cocos Island, LHI is a small oceanic island. It is less than 16 km<sup>2</sup> and is situated 580 km off the eastern coast of Australia. LHI is pristine, with less than 20% of its vegetation disturbed by human settlements. It is also a UNESCO World Heritage Site and a permanent park reserve now protects 70% of the island. Formed by volcanic activity 6.4–6.9 million years ago, LHI is older than Cocos Island. LHI is subtropical, with a more heterogeneous landscape and a greater maximum elevation compared to Cocos Island (Pickard 1983). Despite some differences, these islands provide a great opportunity to assess the influence of colonization and different modes of speciation. Following Papadopoulos et al. (2011), we present one of the first, complete phylogenetic analyses of the modes of speciation of the flora of an oceanic island. Using data from mainland relatives and fossil calibrations, we compare Cocos Island and LHI's histories to contribute to current debates on sympatric speciation. Finally, we demonstrate the importance of incorporating phylogenetic trees into analyses of island biogeography.

## Materials and Methods

### DETERMINATION OF THE BIOGEOGRAPHICAL SOURCE REGIONS

We followed Papadopoulos et al. (2011) to determine the likely source regions for the flora of Cocos Island. These areas were

determined for all nonendemic native plant species, that is, excluding endemics (which by definition evolved in situ), as well as 74 recent invasive species (which are not relevant to our analyses). Worldwide presence/absence data for the target species were gathered for 38 regions as defined in van Balgooy (1971), with modifications to further partition South America (Table S1). The probability of a given region to be the source for any given species on Cocos Island was calculated as:

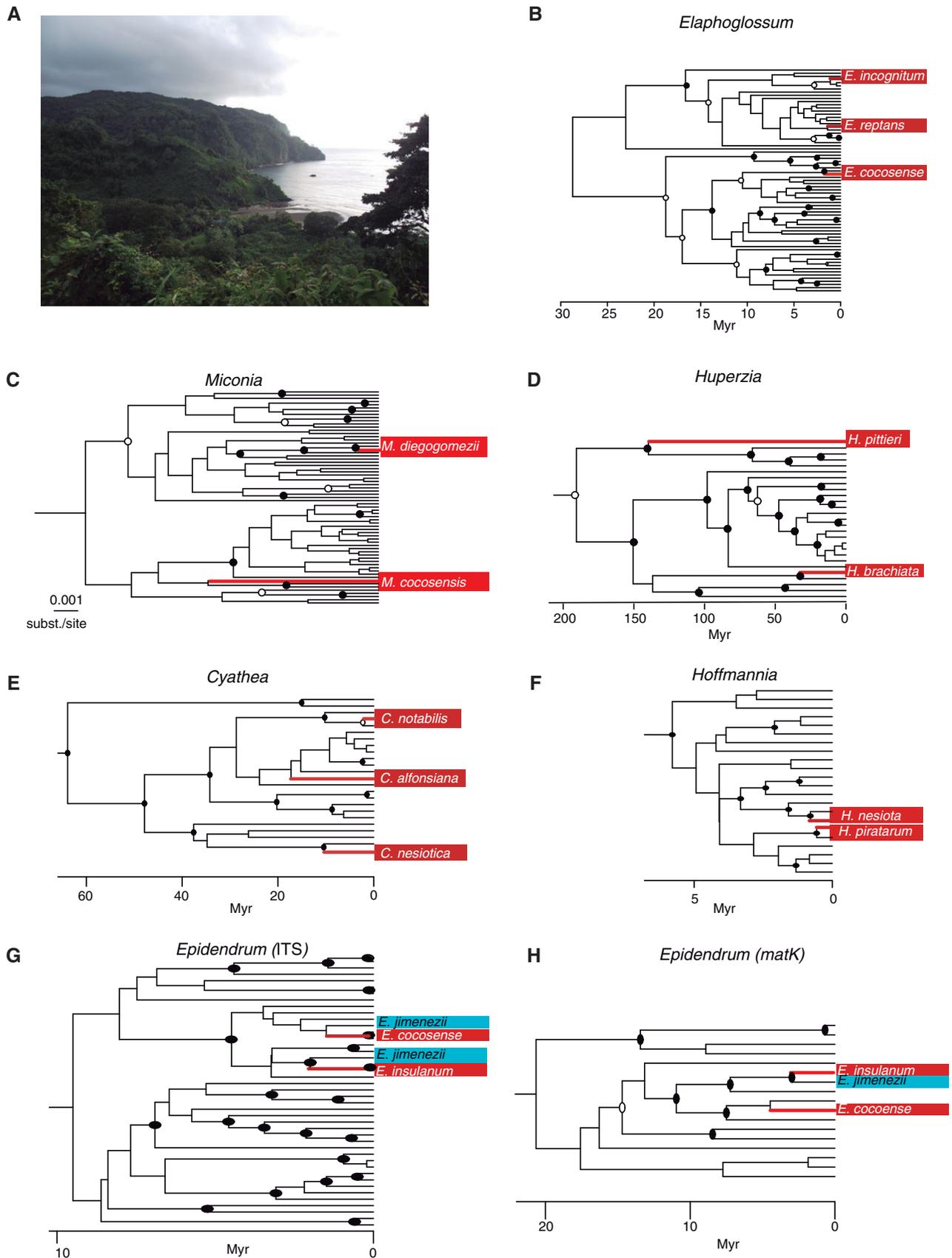
$$p_i = \frac{1}{N_s} \sum_{j=1}^{N_s} \left( \frac{O_{i,j}}{\sum_{i=1}^{N_R} O_{i,j}} \right)$$

where  $N_s$  is the number of species included,  $N_R$  is the number of source regions, and  $o_{i,j}$  is the presence (1) or absence (0) of species  $j$  in region  $i$  (Papadopoulos et al. 2011); data are presented in Table S1.

Checklists were compiled for all source regions for genera with more than one species on Cocos Island using regional floras (Foster 1958; Howard 1989; Brako and Zarucchi 1993; Davidse et al. 1994; Boggan et al. 1997; Jørgensen and León-Yáñez 1999; Acevedo-Rodríguez and Strong 2007; Orlando Rangel-Ch 2007; Hokche et al. 2008; Sarukhan 2008; Forzza 2010; Idarraga Piedrahita et al. 2011; Basualdo 2013). In addition to sequencing plant species from Cocos Island (see below), we mined Genbank for DNA sequences of related congeneric species. To reconstruct the phylogeny of the flora of Cocos Island, we selected the DNA markers with the highest taxonomic coverage for any given genus and relevant source regions. For three genera (*Epidendrum*, *Eugenia*, and *Paspalum*), additional markers were also selected because they were available for a large number of species (see below).

### DNA SEQUENCING

Plant tissues for DNA sequencing were collected in the field and dried using silica gel. In some cases, samples were also obtained from herbarium collections (Table S2). A modified version of Doyle and Doyle's CTAB protocol (Doyle and Doyle 1987; Csiba and Powell 2006) was used to extract genomic DNA from plant leaves, which was then purified using a DNEasy Plant Mini Kit (QIAGEN). For the herbarium extractions, DNA was precipitated in isopropanol for two weeks before amplification. Polymerase chain reaction (PCR) was carried out in a 25  $\mu$ L volume reactions containing 12.5  $\mu$ L of REDTaq<sup>®</sup> ReadyMix<sup>™</sup> PCR Reaction Mix (Sigma), primers (0.2  $\mu$ M final concentration; Table S3), bovine serum albumin (1  $\mu$ L of 0.4% solution), and 50–100 ng of DNA. For herbarium specimens, genus-specific primers for shorter fragments were designed to ensure amplification of degraded DNA (Table S3). PCRs were carried out with the following parameters: initial four min at 94°C; then 34 cycles of 60 sec at 94°C, 30–60 sec at 50–60°C (depending on the primer combination used; see Table S3) and 60 sec at 72°C; and a final extension for 10 min at 72°C. PCR products were purified using ExoSAP-IT (Affymetrix)



**Figure 1.** (A) View of Wafer Bay on Cocos Island. (B–H) Phylogenetic relationships of congeneric endemics on Cocos Island. Endemic lineages are marked with a red box. *Epidendrum jimenezii*, more likely a hybrid between *E. cocoense* and *E. insulanum*, is shown in blue.

and sequenced using forward and reverse primers and Big Dye terminator version 3.1 chemistry (Life Technologies). Sequence reactions were run on an Applied Biosystems 3730 capillary DNA automated sequencer. Electropherograms were edited and assembled using Geneious Pro version 6.1.5 (Biomatters).

### PHYLOGENETIC ANALYSES

To determine which species are sisters and could have diverged on Cocos Island, we conducted phylogenetic analyses for each genus that had more than one native species (Table S4). Multiple sequence alignments were then built for each genus using MAFFT version 6.8 (Kato and Toh 2008). Phylogenetic relationships were determined using a Bayesian approach, as implemented in BEAST version 1.7.5 (Drummond and Rambaut 2007). For each marker, the best fitting model of evolution was selected using Akaike's information criterion using jModeltest version 2.1.2 (Darriba et al. 2012). A birth-and-death model was assumed and molecular clock tests were carried out using MEGA version 6.0.5 (Tamura et al. 2013) to assess the null hypothesis of a single evolutionary rate for the entire phylogenetic tree. If this was rejected, an uncorrelated lognormal relaxed clock was used. To date divergence times of congeneric species on the island, whenever possible the trees were calibrated either using fossils or secondary calibration points from other studies (Table S5). Markov chains were run until the stationary distribution was reached; convergence and effective sample size were assessed using Tracer version 1.5 in the Beast package.

### ISLAND TOPOGRAPHY

To compare the topography of Cocos Island with LHI, we created a 10 × 10 m digital elevation model (DEM) for Cocos Island in ArcGIS version 9 using 50 m contour shapefiles provided by Peter Minton (EVS Islands). This model and a 10 × 10 m DEM of LHI (Papadopoulos et al. 2013) were used to measure the standard deviation of elevation for both islands. Digitized maps of the geology of each island were converted into raster layers.

### SPECIATION MODES

For comparative purposes, the contribution of different speciation modes to the current flora of Cocos Island was assessed using the same criteria as in Papadopoulos et al. (2011) (Fig. S1). Each species from Cocos Island was assigned to one of the following categories: (1) colonization, that is, a nonendemic native species with no congeneric on the island; (2) allopatric speciation, that is, an endemic species with no congeneric on the island; (3) sympatric speciation, that is, two endemic species that are each other's closest relatives and whose divergence time is confidently dated within the age of the island; (4) hybrid species, as indicated by intermixed nuclear DNA from other species; (5) equivocal, that is, species for which allopatric or sympatric origin are equally

probable; (6) unknown, that is, species for which phylogenetic data were not available to assess relationships with other congeneric species. Of particular interest were eight genera that have at least two endemic species on Cocos Island (*Cyathea*, *Elaphoglossum*, *Epidendrum*, *Eugenia*, *Hoffmannia*, *Huperzia*, *Miconia*, and *Thelypteris*); that is, they represent potential candidates for sympatric speciation.

### REANALYSIS OF KISEL AND BARRACLOUGH AND STUESSY ET AL.'S DATASETS

We also compared our estimates of speciation modes with two other biogeographical analyses of islands. First, we examined Kisel and Barraclough (2010), in which the relationship between the probability of speciation and island area across 64 oceanic islands and archipelagos was assessed, including Cocos Island and LHI. In their analysis, the probability of speciation on a given island was calculated as the proportion of genera with more than one endemic species on the island over the total number of endemic lineages. Their figures were estimated using published taxonomies and corrected with phylogenetic trees whenever available (30% of the cases). Here, we calculated the maximum proportion of sympatric speciation events detected among endemic lineages from the phylogenetic analyses of Cocos and LHI and also used individual regression models following Kisel and Barraclough (2010). Second, we assessed data from Stuessy et al. (2006), who analyzed levels of anagenetic speciation in the angiosperm floras of oceanic and continental islands. We calculated the percentage of endemic species that were a product of anagenetic and cladogenetic speciation on LHI and Cocos Island using their taxonomy-based approach and the phylogenetic analysis presented here, as well as correlations as described in Stuessy et al. (2006).

## Results

### ORIGINS OF THE FLORA OF COCOS ISLAND

We inferred the most likely source regions for the 154 native plant species on Cocos Island: these were Central America, Colombia, Ecuador, Venezuela, Mexico, Peru, Greater Antilles, Guyanas, Brazil, Bolivia-Paraguay, and Lesser Antilles, in decreasing order of colonization frequency. The corresponding  $p_i$  values (i.e., probability of a given region to be the source of a species taken at random from the Cocos pool of species) ranged from 0.14 to 0.04 (Table S1). Altogether, these 11 focal regions account for the majority of the dispersal events to Cocos Island (total  $p_i = 0.86$ ).

We also sequenced 121 samples from 25 of the 27 genera that have more than one congeneric species on Cocos Island (Table S4). These, when added to DNA sequences downloaded from Genbank totaled up to over 1400 DNA sequences that were used to build genus-level phylogenetic trees (Figs. S2–S25). These

trees encompass 71 species from Cocos Island in addition to relatives from source regions or other parts of the world (the mean sampling of the focal regions was 15%; Table S6). We used one genetic locus per genus, except for *Epidendrum*, *Paspalum*, and *Eugenia*, where both nuclear and plastid markers were obtained and analyzed separately to check for incongruences between the two genomes. In each case, the results from each partition were topologically congruent with regard to the placement of the Cocos Island species (Figs. S7 and S18).

Our analyses included phylogenetic trees for seven genera with more than one endemic species on Cocos Island (*Cyathea*, *Elaphoglossum*, *Epidendrum*, *Eugenia*, *Hoffmannia*, *Huperzia*, *Miconia*). We were not able to obtain sequences for the genus *Thelypteris*, of which two endemic species occur on Cocos; potentially, they could represent an undetected cladogenetic event. Apart from these, we did not recover any sister relationships for species from Cocos Island, indicating that sympatric speciation has not taken place on the island (Fig. 1B–H). Instead, 15 independent colonizations followed by anagenetic speciation are responsible for the presence of these species on Cocos Island.

We also calculated that colonization without speciation was responsible for 75.3% of the island species, whereas allopatric speciation (i.e., colonization followed by anagenetic speciation) accounted for 15.1% of the species (Fig. 2A). Hybridization was found in one case in *Epidendrum* (see below). Equivocal speciation modes remain for 3.2% of the species, and we could not obtain data for 5.9% of the species of Cocos Island (Fig. 2A).

*Eugenia* (Myrtaceae) was examined in more details due to the high level of morphological similarity between the described endemic species. Sequence data were obtained for *E. pacifica* and *E. cocosensis*, which had been described as separate species by Barrie (2005). The main character used to distinguish between the two species was leaf width: *E. pacifica* leaves should be wider than 2.5 cm, whereas *E. cocosensis* leaves should be narrower than 2 cm (Barrie 2005; Trusty et al. 2006). We obtained plastid *rbcL* and nuclear internal transcribed spacer (ITS) and 5S DNA sequences for three individuals of each species, but each locus was identical within and across species. We also plotted the distribution of leaf widths from 21 herbarium specimens (18 *E. pacifica* and three *E. cocosensis*), which showed that there was no distinctive threshold separating the two species (Fig. S26). This, along with the lack of genetic differentiation, indicates that *E. pacifica* and *E. cocosensis* are more likely to represent a single species; therefore, we considered them as such in our analyses.

In *Epidendrum* (Orchidaceae), morphological studies suggested that the rare *E. jimenezii* might be a hybrid between *E. cocoense* and *E. insulanum* (Bogarin et al. 2011; Trusty et al. 2011). We obtained DNA sequence data for plastid *matK* and nuclear ITS loci for all three species on Cocos Island (Fig. 1G, H). Although *E. jimenezii* and *E. insulanum* had identical *matK*

sequences, two different ITS sequences were amplified from within *E. jimenezii*: each of which was identical either to ITS in *E. cocoense* or in *E. insulanum*. This confirms that *E. jimenezii* is likely to be of hybrid origin. However, additional data would be necessary to determine whether there are self-sustaining populations of *E. jimenezii* and that it truly represents a distinct species. The presence of private genetic polymorphisms in *E. jimenezii* would support the former case. Alternatively, *E. jimenezii* individuals may be the product of occasional hybridization events.

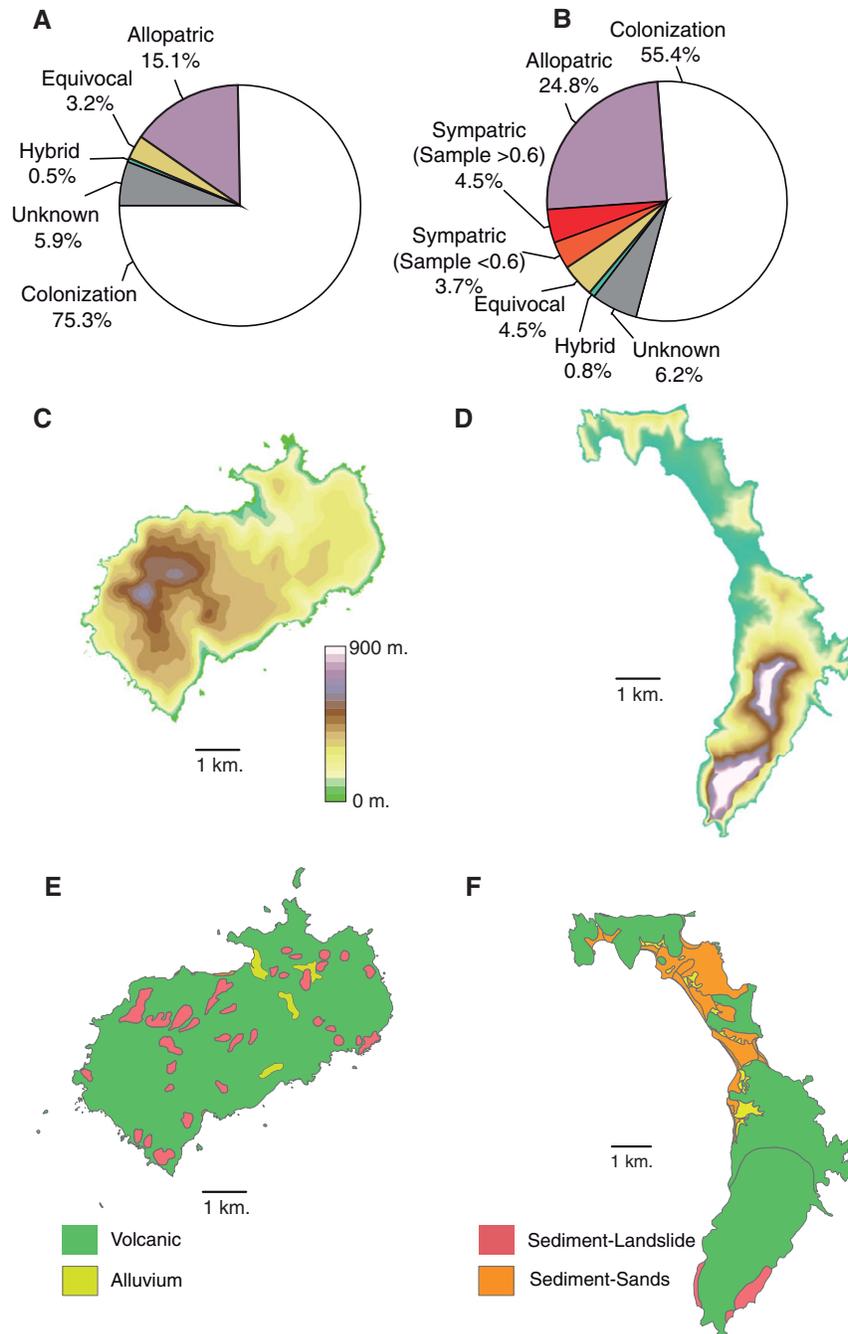
## ISLAND BIOGEOGRAPHICAL MODELS

We used our estimates of the speciation modes for Cocos Island, with those from LHI, to reevaluate results from Kisel and Barraclough (2010) and Stuessy et al. (2006). Kisel and Barraclough (2010) assessed the relationship between the probability of cladogenetic speciation and island area, reporting values of 0.20 and 0.31 for the angiosperms of LHI and Cocos Island, respectively, and 0.54 and 0.43 for their fern floras. In contrast, probabilities estimated here using phylogenetic information were much lower: 0.09 and 0.00 for angiosperms, and 0.18 and 0.00 for ferns on LHI and Cocos Island, respectively (Fig. 3A, B). The positive correlation between the probability of speciation in angiosperms and the area of the island is maintained after correcting the data with our phylogenetic information (Fig. 3A). Originally, no speciation-area relationship was evident in ferns (Kisel and Barraclough 2010), however, after the addition of phylogenetic information a positive correlation was uncovered (Fig. 3B). Of course, we would argue that this new correlation potentially is still flawed given that not all datapoint are based on phylogenetic information; yet, increasing phylogenetic information for two of the islands does help recover some expected patterns such as the speciation-area relationships.

Stuessy et al. (2006) found a positive correlation between elevation and the proportion of anagenetic evolution on island angiosperms. Taxonomy-based estimates of the proportion of endemics resulting from anagenesis were 55% for Cocos Island and 75% for LHI. Using phylogenetic information, we increased these figures to 100% for Cocos Island and 86% for LHI (Fig. 3C). The resulting correlations revealed a tighter fit to the data using some additional phylogenetic information ( $r^2 = 0.58$  in Stuessy et al. vs.  $r^2 = 0.74$  here), as well as stronger significance ( $P = 0.02$  in Stuessy et al. vs.  $P = 0.0002$  here) (Fig 3C); again, these correlations may still be biased by taxonomic-based datapoints.

## Discussion and Conclusion

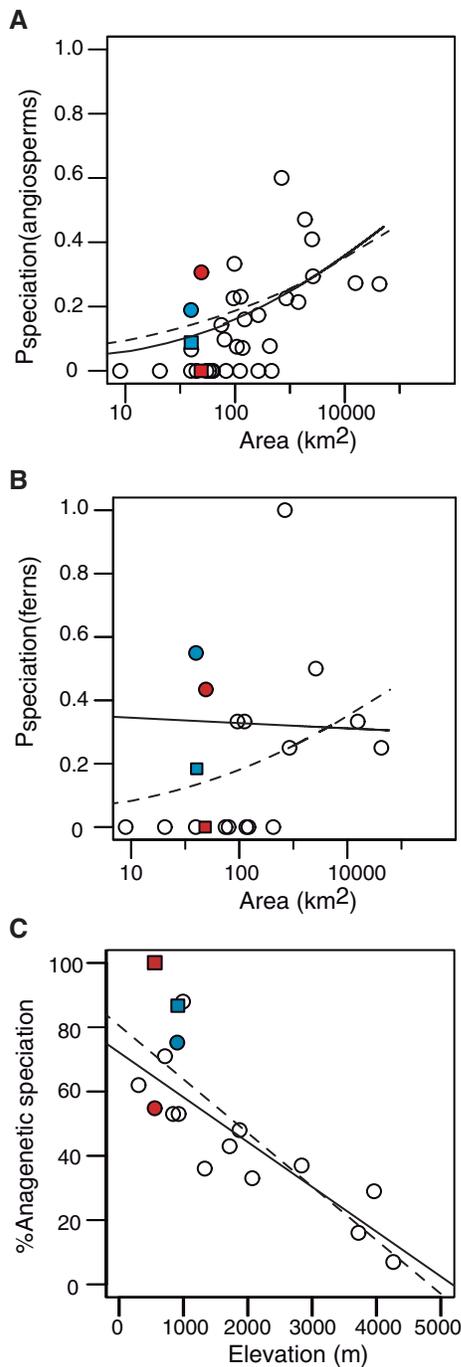
Species formation on oceanic islands can be the result of anagenesis (which increases global diversity but does not affect the net species diversity on the island) or cladogenesis (divergence into two species—a global and insular increase in diversity). On



**Figure 2.** Comparisons between Cocos Island and LHI. Modes of speciation for the flora of Cocos Island (A; with all detailed phylogenetic trees presented in Figs. S2–S25) and that of LHI (B, redrawn from Papadopulos et al. 2011); DEMs for Cocos Island (C) and LHI (D); geologic maps of Cocos Island (E) and LHI (F). The percentages of the different soil types on Cocos and LHI are, respectively: volcanic (90.26% and 77.75%); alluvium (1.67% and 3.1%); sediment landslide (7.91% and 2.73%); and sediment sands (0.15% and 16.43%).

Cocos Island 17% of the plant species are endemic, however, none of these species appear to be derived from in situ cladogenetic events. Conversely, several lineages of angiosperms and ferns were found to be the result of in situ speciation on LHI, accounting for up to 8.2% of the current flora. The absence of cladogenetic events on Cocos Island, despite having a substantial

number of congeneric endemic species, has broader implications. The remoteness of Cocos Island suggests that multiple independent colonizations by congenics should be minimal, and they are more likely to represent sister species. Island biogeographical analyses often assume that congeneric endemic species evolved in situ on remote oceanic islands and archipelagos (Coyne and



**Figure 3.** Biogeographical models of speciation. (A) Relationship between area and the probability of speciation in angiosperms from 32 oceanic islands and archipelagos (Kisel and Barraclough 2010); (B) relationship between area and the probability of speciation in ferns from 17 oceanic islands and archipelagos (Kisel and Barraclough 2010); (C) relationship between island elevation and the proportion of anagenetic speciation from 13 islands (Stuessy et al. 2006). Taxonomy-based estimates are represented by circles; phylogeny-based estimates from this study are represented by squares; data from Cocos Island are shown in red and data from Lord Howe Island are shown in blue. Linear regression from the taxonomy-based and the phylogeny-based data are shown with solid and dotted lines, respectively.

Price 2000; Emerson and Kolm 2005; Kisel and Barraclough 2010). We compared the probabilities of speciation obtained in two studies, which used taxonomy-based approaches, with our estimates using phylogenetic information. Our results show that the taxonomy-based studies overestimated the probability of cladogenetic speciation (Fig. 3; and see Table S7 for several examples). Kisel and Barraclough (2010) suggested that cladogenetic speciation on small islands is rare in most taxa. Although the estimates of cladogenetic speciation in their study are too high, and the estimated parameters may be somewhat inaccurate as a result, the overall conclusion may remain unaffected. In most cases, the error associated with taxonomy-based measures of speciation may be difficult to determine for any specific study as the variation in the accuracy of taxonomies is unpredictable. Therefore, it is not possible to assess the effect that it has had on the results of biogeographic studies using these measurements in each specific case. This is particularly true for those studies that have used single island endemics on archipelagos for their models, as the effect of extinction events on estimates is difficult to assess too. In a general sense, the model parameters that are estimated from taxonomy-based measurements should be treated with caution and the conclusions drawn from these studies should be considered in light of the potential errors that these measures clearly contain.

The majority of our analyses were based on single-gene phylogenetic trees, which, in theory, may not reflect the true species tree due to hybridization and/or incomplete lineage sorting (Maddison 1997; Brito and Edwards 2009). This is an acute issue when the ratio between the time separating successive branching events in the tree and the population size is small (Nichols 2001), which is potentially the situation here. Therefore, real sister species relationships for an endemic pair of species, with a divergence postdating the formation of the island, could have been missed by our analyses. However, this is unlikely as in each genus, numerous lineages and large evolutionary distances separated Cocos Island endemic species (see Fig. 1B–H). Despite an average of 60 species per phylogenetic tree, completeness (i.e., the proportion of species included from each genus originating in the hyperdiverse regions of Central and South America) was low. This may affect the validity of the resulting topologies (Heath et al. 2008; Nabhan and Sarkar 2012) but again, we argue that it should not impact our conclusions of the modes of speciation on the island due to the unambiguously large evolutionary distances between Cocos endemics. More densely sampled, multilocus phylogenetic trees would be needed to accurately infer colonization times and establish the nearest relatives of Cocos Island species.

Our results may also be affected by extinction on Cocos Island. Cladogenetic events followed by extinction of all the endemic members of the clade but one would lead to an underestimation of sympatric speciation. Unfortunately no data are available

to determine whether this is the case on Cocos Island. It may have an influence on the apparent lack of cladogenesis on the island as small islands have been associated with higher extinction rates (McArthur and Wilson 1967). Furthermore, a colonizer of the island that became extinct in the mainland source regions would be erroneously classified as an endemic (paleoendemic). This would result in an overestimation of the rate of anagenesis, an acute issue for studies incorporating the number of single island endemics as a parameter.

Despite the caveats above, our results confirm that taxonomy is not a reliable determinant of sister relationships in endemic island species, but this also raises a potential source of error. It is possible that two sister endemic species from Cocos have been erroneously classified into different genera, a scenario that is undetectable using this approach. However, a close examination of all the endemic species on the island reveals no clear candidates for this type of phenomenon. The majority of “anagenetic” species on the island belong to different families. Those that do belong to the same family are either in different subfamilies or show a suite of clear morphological differences. Therefore, it seems highly unlikely that any potential cladogenetic event on the island would have remained undetected by our analyses due to taxonomic errors.

Ecological speciation has been suggested as the process driving speciation in several plants on LHI. For example, the sister palm species of the endemic genus *Howea* evolved following edaphic adaptation and assortative mating through displaced flowering phenologies (Savolainen et al. 2006). Adaptation to elevation appears to have played a major role in the split between *Metrosideros nervulosa* and *M. sclerocarpa*, as well as the radiation of several species in *Coprosma* (Papadopoulos et al. 2013). Signatures of local adaptation have also been detected in numerous other LHI species that have not yet differentiated (Papadopoulos et al. 2014). The remarkable frequency of “speciation with gene flow” found on LHI (Fig. 2B) is thought to have been driven by ecological factors that are not unique to this island, suggesting that sympatric speciation could be widespread (Papadopoulos et al. 2011). However, the results we present here, that is, no in situ species split on Cocos Island, confirm earlier expectations of infrequent sympatric speciation, at least in birds (Coyne and Price 2000). Why, then, are there such different patterns of speciation between these two islands?

Both Cocos Island and LHI are of volcanic origin and are equally isolated from other landmasses. A suite of factors has been shown to influence the speciation and extinction rate dynamics and the rate of endemism on oceanic islands, including age, habitat heterogeneity, and isolation. Cocos is roughly three times younger than LHI, which has afforded less time for colonizers to arrive on Cocos and to evolve into distinct species or for cladogenesis to occur. This may explain the smaller number of

endemic plant species—33 on Cocos and 90 on LHI. However, the observed pattern of speciation contradicts theoretical predictions about the influence of island age on in situ speciation rates. It has been proposed that the contribution of cladogenesis to species diversity is higher on young islands than old islands (Whittaker et al. 2008). The total life span of Cocos Island may be short, which might imply it has already reached a relatively more mature phase despite its younger absolute age. Alternatively there may be differences in the carrying capacity of both islands. The size of both islands is comparable, with LHI slightly smaller. The maximum elevation of an island has traditionally been used as a proxy for habitat diversity (Whittaker et al. 2007; Steinbauer et al. 2012): Cocos Island stands at 552 m above sea level (Fig. 2C), whereas LHI is 875 m at its highest (Fig. 2D). The standard deviation of elevation is also lower for Cocos Island (113.2 vs. 183.9 for LHI), consistent with a more homogeneous environment. Furthermore, Cocos Island is mainly composed of volcanic rocks (Fig. 2E), while 16% of LHI is covered by calcareous formations (Pickard 1983; Fig. 2F). Surveys of the vegetation of LHI have shown that there is a substantial variety of distinct habitats, with 25 vegetative associations (Pickard 1983), whereas Cocos Island has only seven major vegetation types (Trusty et al. 2006). Cocos Island is almost entirely covered by rainforest with low tree diversity (Trusty et al. 2006). Other vegetation is restricted to bayshores, landslides, and cliffs. Compared to Cocos Island, the greater variety of ecological niches on LHI may provide an enhanced opportunity for adaptation and speciation.

Colonization has been important in shaping the flora of Cocos Island. Although it is only responsible for 55% of the species on LHI, it accounts for 75% of those on Cocos Island (Fig. 2A, B). Both islands are equally distant from other landmasses and have similar values of proportion of surrounding landmass (SLMP): SLMP = 0.259 for LHI and SLMP = 0.3023 for Cocos Island. This measure of isolation accounts for the proportion of surrounding landmasses measured in several buffering distances and has recently been proposed as the best isolation metric to explain island plant diversity (Weigelt and Kreft 2013; Weigelt et al. 2013). Presently, Cocos Island is the only component of the Cocos Ridge that stands above sea level. However, many of the underwater seamounts located in the Cocos Plate and in the nearby Nazca Plate may have been emergent within the last few million years (Werner and Hoernle 2003). These seamounts may have constituted a temporary stepping-stone system for potential mainland colonizers to reach Cocos Island more readily. More frequent gene flow between the mainland and the island colonizer populations may have hindered speciation on Cocos Island.

Overall, our findings call for caution when applying general measures to quantify speciation on islands, as accurate hypothesis testing in island biogeography clearly requires phylogenetic information. To build these phylogenetic trees, collecting plants

and animals from the world's 100,000 islands will be a huge task, but the benefit of such enterprise for understanding the origin and maintenance of biodiversity will be considerable.

### ACKNOWLEDGMENTS

We thank the rangers and volunteers at Cocos Island National Park, especially R. Cubero, for their help in the field, A. Estrada (CR National Herbarium) and A. Cascante (USJ Herbarium) for helping with taxonomic identifications, J. Warner and F. Pupulin for the logistics in Costa Rica, P. Minton and I. Lysenko for their help with the maps, T. Barraclough and J. Garcia-Porta for comments, the Vice-Rector of Research at the University of Costa Rica for supporting research permits, F. Quirós Brenes for granting collecting permits, and the Darwin Initiative, the UK Royal Society, and the European Research Council for funding.

### DATA ARCHIVING

All the sequences obtained in this study have been deposited in GenBank under accession numbers KM495080-KM495225 and KM583468-KM583472 (see Table S2). Alignments and trees have been deposited in TreeBASE under accession number S16313.

### LITERATURE CITED

- Acevedo-Rodríguez, P., and M. T. Strong. 2007. Catalogue of the seed plants of the West Indies Website.
- Alfaro, E. J. 2008. Ciclo diario y anual de variables troposféricas y oceánicas en la Isla del Coco, Costa Rica. *Rev. Biol. Trop.* 52:19–29.
- Barrie, F. R. 2005. Thirty-five new species of *Eugenia* (Myrtaceae) from Mesoamerica. *Novon* 15:4–49.
- Basualdo, I. 2013. Inventario Biológico de Paraguay. Tropicos, Missouri Botanical Garden, St. Louis, MO.
- Bellon, H., R. Saenz, and J. Tournon. 1983. K-Ar radiometric ages of lavas from Cocos Island (Eastern Pacific). *Mar. Geol.* 54:M17–M23.
- Bogarin, D., J. Warner, M. P. Powell, and V. Savolainen. 2011. The orchid flora of Cocos Island National Park, Puntarenas, Costa Rica. *Bot. J. Linn. Soc.* 166:20–39.
- Boggan, J., V. Funk, C. Kelloff, M. Hoff, G. Cremers, and C. Feuillet. 1997. Checklist of the plants of the Guianas (Guyana, Surinam, French Guiana). 2nd ed. Biological Diversity of the Guianas Program, Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, D.C.
- Brako, L., and J. L. Zarucchi. 1993. Catalogue of the flowering plants and gymnosperms of Peru. *Monogr. Syst. Bot. Missouri Bot. Gard.* 45:1286.
- Brito, P. H., and S. V. Edwards. 2009. Multilocus phylogeography and phylogenetics using sequence-based markers. *Genetica* 135:439–455.
- Bunnefeld, N., and A. B. Phillimore. 2012. Island, archipelago and taxon effects: mixed models as a means of dealing with the imperfect design of nature's experiments. *Ecography* 35:15–22.
- Castillo, P., R. Batiza, D. Vanko, E. Malavassi, J. Barquero, and E. Fernandez. 1988. Anomalously young volcanoes on old hot-spot traces: I. Geology and petrology of Cocos Island. *Geol. Soc. Am. Bull.* 100:1400–1414.
- Coyne, J. A., and T. D. Price. 2000. Little evidence for sympatric speciation in island birds. *Evolution* 54:2166–2171.
- Csiba, L., and M. P. Powell. 2006. DNA extraction protocols. Pp. 48–51 in V. Savolainen, M. P. Powell, K. Davis, G. Reeves, and A. Corthals, eds. *DNA and tissue banking for biodiversity and conservation: theory, practice and uses*. Royal Botanic Gardens, Kew, Richmond, Surrey, U.K.
- Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* 9:772.
- Davidse, G., M. Sousa Sánchez, and “Flora Mesoamericana Committee.” 1994. *Flora Mesoamericana*. Missouri Botanical Garden, Universidad Autónoma de Mexico, Natural History Museum, Mexico, D.F.
- Doyle, J. J., and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* 19:11–15.
- Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* 7:214.
- Emerson, B. C., and N. Kolm. 2005. Species diversity can drive speciation. *Nature* 434:1015–1017.
- Forzza, R. C. 2010. *Catálogo de Plantas e Fungos do Brasil*. Andrea Jakobsson Estudio, Jardim Botânico do Rio de Janeiro, Brasil.
- Foster, R. C. 1958. A catalogue of the ferns and flowering plants of Bolivia. *Contr. Gray Herb* 184:1–223.
- Gray, A., and S. Cavers. 2014. Island biogeography, the effects of taxonomic effort and the importance of island niche diversity to single-island endemic species. *Syst. Biol.* 63:55–65.
- Heath, T. A., S. M. Hedtke, and D. M. Hillis. 2008. Taxon sampling and the accuracy of phylogenetic analyses. *J. Syst. Evol.* 46:239–257.
- Hogue, C. L., and S. E. Miller. 1981. Entomofauna of Cocos Island, Costa Rica. *Atoll Research Bulletin* No. 250.
- Hokche, O., P. B. Berry, and O. Huber. 2008. Nuevo catálogo de la flora vascular de Venezuela. *Fundacion Instituto Botanico de Venezuela, Caracas, Venezuela*.
- Howard, R. A. 1989. *Flora of the Lesser Antilles: Leeward and Windward Islands*. Arnold Arboretum of Harvard University, Cambridge, MA.
- Idarraga Piedrahita, A., R. del, C. Ortiz, R. Callejas-Posada, and M. Merello. 2011. *Flora of Antioquia: Catálogo de las plantas vasculares del Departamento de Antioquia*. Missouri Botanical Garden, Universidad de Antioquia, Medellín, Colombia.
- Jørgensen, P. M., and S. León-Yáñez (eds.). 1999. Catalogue of the vascular plants of Ecuador. Pp. i–viii, 1–1182 in *Monographs in systematic botany from the Missouri Botanical Garden*. Vol. 75. Missouri Botanical Garden Press, St. Louis, MO.
- Katoh, K., and H. Toh. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief. Bioinform.* 9:286–298.
- Kisel, Y., and T. G. Barraclough. 2010. Speciation has a spatial scale that depends on levels of gene flow. *Am. Nat.* 175:316–334.
- Losos, J. B., and R. E. Ricklefs. 2009a. The theory of island biogeography revisited. Princeton Univ. Press, Princeton, NJ.
- . 2009b. Adaptation and diversification on islands. *Nature* 457:830–836.
- Maddison, W. P. 1997. Gene trees in species trees. *Syst. Biol.* 46:523–536.
- McArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. *Monogr. Popul. Biol.* 1:1–203.
- Nabhan, A. R., and I. N. Sarkar. 2012. The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. *Brief Bioinform.* 13:122–134.
- Nichols, R. 2001. Gene trees and species trees are not the same. *Trends Ecol. Evol.* 16:358–364.
- Orlando Rangel-Ch, J. 2007. *Colombia Diversidad Biótica*. Instituto de Ciencias Naturales, Facultad de Ciencias. Universidad Nacional de Colombia, Bogotá, Colombia.
- Papadopulos, A. S. T., W. J. Baker, D. Crayn, R. K. Butlin, R. G. Kynast, I. Hutton, and V. Savolainen. 2011. Speciation with gene flow on Lord Howe Island. *Proc. Natl. Acad. Sci. USA* 108:13188–13193.
- Papadopulos, A. S. T., Z. Price, C. Devaux, H. Hipperson, C. M. Smadja, I. Hutton, W. J. Baker, R. K. Butlin, and V. Savolainen. 2013. A comparative analysis of the mechanisms underlying speciation on Lord Howe Island. *J. Evol. Biol.* 26:733–745.

- Papadopoulos, A. S. T., M. Kaye, C. Devaux, H. Hipperson, J. Lighten, L. T. Dunning, I. Hutton, W. J. Baker, R. K. Butlin, and V. Savolainen. 2014. Evaluation of genetic isolation within an island flora reveals unusually widespread local adaptation and supports sympatric speciation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369.
- Patíño, J., F. Guilhaumon, R. J. Whittaker, K. A. Triantis, S. R. Gradstein, L. Hedenäs, J. M. González Mancebo, and A. Vanderpoorten. 2013. Accounting for data heterogeneity in patterns of biodiversity: an application of linear mixed effect models to the oceanic island biogeography of spore-producing plants. *Ecography* 36:904–913.
- Pickard, J. 1983. Vegetation of Lord Howe Island. *Cunninghamia* 1:133–265.
- Sarukhan, J. 2008. Catalogo taxonomico de especies de Mexico. *In* Capital Natural de Mexico, Vol. 1: conocimiento actual de la biodiversidad. Conabio, México.
- Savolainen, V., M.-C. Anstett, C. Lexer, I. Hutton, J. J. Clarkson, M. V. Norup, M. P. Powell, D. Springate, N. Salamin, and W. J. Baker. 2006. Sympatric speciation in palms on an oceanic island. *Nature* 441:210–213.
- Steinbauer, M. J., R. Otto, A. Naranjo-Cigala, C. Beierkuhnlein, and J. M. Fernández-Palacios. 2012. Increase of island endemism with altitude—speciation processes on oceanic islands. *Ecography* 35:23–32.
- Stuessy, T. F. 2006. Evolutionary biology: sympatric plant speciation in islands? *Nature* 443:E12, discussion E12–E13.
- Stuessy, T. F., G. Jakubowsky, R. S. Gomez, M. Pfosser, P. M. Schluter, T. Fer, B.-Y. Sun, and H. Kato. 2006. Anagenetic evolution in island plants. *J. Biogeogr.* 33:1259–1265.
- Stuessy, T. F., K. Takayama, P. López-Sepúlveda, and D. J. Crawford. 2014. Interpretation of patterns of genetic variation in endemic plant species of oceanic islands. *Bot. J. Linn. Soc.* 174:276–288.
- Tamura, K., G. Stecher, D. Peterson, A. Filipowski, and S. Kumar. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30:2725–2729.
- Trusty, J. L., H. C. Kesler, and G. H. Delgado. 2006. Vascular flora of Isla del Coco, Costa Rica. *Proc. Calif. Acad. Sci.* 57:247.
- Trusty, J. L., H. C. Kesler, J. Rodriguez, and J. Francisco-Ortega. 2011. Applying IUCN Red List criteria on a small island. Pp. 452–473 *in* D. Bramwell and J. Caujapé-Castells, eds. *The biology of island floras*. Cambridge Univ. Press, Cambridge, U.K.
- Van Balgooy, M. M. J. 1971. Plant geography of the Pacific as based on a census of phanerogam genera. *Blumea* 6:1–222.
- Weigelt, P., and H. Krefl. 2013. Quantifying island isolation—insights from global patterns of insular plant species richness. *Ecography* 36:417–429.
- Weigelt, P., W. Jetz, and H. Krefl. 2013. Bioclimatic and physical characterization of the world's islands. *Proc. Natl. Acad. Sci. USA* 110:15307–15312.
- Werner, R., and K. Hoernle. 2003. New volcanological and volatile data provide strong support for the continuous existence of Galápagos Islands over the past 17 million years. *Int. J. Earth Sci.* 92:904–911.
- Whittaker, R. J., and J. M. Fernández-Palacios. 2007. *Island biogeography: ecology, evolution, and conservation*. Oxford Univ. Press, Oxford, U.K.
- Whittaker, R. J., R. J. Ladle, M. B. Araújo, J. María Fernández-Palacios, J. Domingo Delgado, and J. Ramón Arévalo. 2007. The island immaturity—speciation pulse model of island evolution: an alternative to the “diversity begets diversity” model. *Ecography* 30:321–327.
- Whittaker, R. J., K. A. Triantis, and R. J. Ladle. 2008. A general dynamic theory of oceanic island biogeography. *J. Biogeogr.* 35:977–994.

Associate Editor: F. Burbrink  
Handling Editor: R. Shaw

## Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Figure S1.** Possible speciation scenarios on Cocos Island.

**Figures S2–S25.** Bayesian phylogenetic reconstruction for genera with more than one species on Cocos Island.

**Figure S26.** Leaf width of 21 specimens of *Eugenia pacifica* and *E. cocosensis* from Cocos Island.

**Table S1.** Presence/absence matrix of Cocos Island native species in 38 biogeographic regions.

**Table S2.** Samples obtained for this study.

**Table S3.** Primers used in this study.

**Table S4.** Species present on Cocos Island used in this study.

**Table S5.** Parameters and calibrations for BEAST phylogenetic analyses.

**Table S6.** Number of species within each genus on Cocos Island and each of the focal regions.

**Table S7.** Examples of island biogeography studies that employ taxonomy-based measures with the corresponding main features.