Evidence for Miocene overwater colonization in Caribbean Cyrtognatha spiders

*Klemen Čandek1, Ingi Agnarsson2,4, Greta Binford3, Matjaž Kuntner1,4,5,6
1 Evolutionary Zoology Laboratory, Institute of Biology, Scientific Research Centre, Slovenian Academy of Sciences and Arts, Ljubljana, Slovenia
2 Department of Biology, University of Vermont, Burlington, VT, USA
3 Department of Biology, Lewis and Clark College, Portland, OR, USA
4 Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington D.C., USA
5 College of Life Sciences, Hubei University, Wuhan, Hubei, China
6 Evolutionary Zoology Laboratory, Department of Organisms and Ecosystems Research, National Institute of Biology, Ljubljana, Slovenia

Abstract

Island systems provide excellent arenas to test evolutionary hypotheses pertaining to gene flow and diversification of dispersal-limited organisms. Here we focus on an orbweaver spider genus Cyrtognatha (Tetragnathidae) from the Caribbean, with the aims to reconstruct its evolutionary history, describe its biogeographic history in the archipelago, and to estimate the timing and route of Caribbean colonization. Specifically, we test if Cyrtognatha biogeographic history is consistent with an ancient vicariant scenario (the GAARlandia landbridge hypothesis) or overwater dispersal. We reconstructed a species level phylogeny based on one mitochondrial (CO1) and one nuclear (28S) marker. We then used this topology to constrain a time-calibrated mtDNA phylogeny, for subsequent biogeographical analyses of over 100 originally sampled Cyrtognatha individuals. Our results suggest a monophyletic radiation of Caribbean Cyrtognatha, containing 11 to 14 species that are exclusively single island endemics. Our analyses refute vicariance and instead support an overwater colonization to the Caribbean in mid-Miocene. Having colonized Hispaniola first, Cyrtognatha subsequently dispersed to, and diversified on, the other islands of the Greater, and Lesser Antilles.

Introduction

Island biogeography is concerned with colonization and diversification of organisms on islands, including empirical tests of evolutionary hypotheses pertaining to gene flow in dispersal-limited organisms1,2. Islands are geographically widespread and diverse, and vary in shapes and sizes, age and geologic origins, and show different degrees of isolation. Darwin already recognized that this combination of attributes makes islands appealing objects of scientific study4. Modern biogeography recognizes the interplay among island histories, the specifics of their geography, and various attributes of organisms that inhabit them5.

Amongst island systems some of the best studied in terms of biogeographic research are Hawaii5–8, Galapagos9–11, Azores12–14, Canary15–17 and Solomon18–20 islands, as well as large continental fragments such as Madagascar21–23 and New Zealand24–26. However, the Caribbean island system27–30 is the single most ‘published’ island system in biogeography literature (Google Scholar title hits 237 compared with 195 for the second, New Zealand). The Caribbean Basin, also known as West Indies, lies in the tropical zone between South and North American continents, and to the east of the Gulf of Mexico. Combining over 700 islands, the Caribbean is considered among the world’s biodiversity
hotspots. In its most broad categorization, the Caribbean comprises three regions: (1) Greater Antilles with the largest islands of Cuba, Hispaniola (Dominican Republic and Haiti), Puerto Rico and Jamaica representing 90% of all land in Caribbean Sea; (2) Lesser Antilles with numerous smaller, mostly volcanic, islands and (3) the Lucayan platform archipelago (the Bahamas).

The Greater Antillean islands of Cuba, Hispaniola, and Puerto Rico, but not Jamaica, are parts of the old proto-Antillean arc that began its formation in the distant past, over 130 million years ago (MYA). Through Caribbean plate tectonics, the proto-Antillean arc drifted eastward until settling at its current location around 58 MYA. Researchers disagree on the timing of the proto-Antillean arc connection with South or North America in the Cretaceous or even on the existence of such a connection. However, that distant past may have had little biological relevance for current biotas due to a catastrophic effect of the bolide that crashed into Yucatan around 65 MYA which arthropods would likely not have survived. The emergence of the Greater Antilles as relevant biogeographic units is therefore more recent. Various studies estimate that earliest contiguous permanent dry land on the Greater Antilles has existed since the middle Eocene, approximately 40 MYA.

Although it may be possible that the Greater Antilles have remained isolated from continental landmasses since the early Cenozoic, a hypothesized land bridge potentially existed around 35-33 MYA. This land bridge, known as GAARlandia (Greater Antilles – Aves Ridge), is hypothesized to have connected the Greater Antilles with the South American continent for about 2 million years, due to a sea level drop and subsequent exposure of land at Aves Ridge. As a means of biotic evolution on the Greater Antilles, the GAARlandia hypothesis allows for a combination of overland dispersal and subsequent vicariance and can be tested with the help of time calibrated phylogenies and fossils. While patterns of relationships that are consistent with predictions based on GAARlandia have been found in some lineages it is not a good model for explaining the biogeographical history of others.

Among the islands forming the Greater Antilles, Jamaica is a geological special case since it was originally a part of the Central American tectonic plate. Jamaica emerged as an island around 40 MYA but remained partially or fully submerged until its reemergence in mid Miocene around 15 MYA and was never part of the hypothetical GAARlandia landbridge. Consequently, Jamaica’s biota is distinct from other regions of Greater Antilles.

The Lesser Antilles formed more recently. Northward of Guadalupe they split into two arches of distinct origins. The older, outer arch formed volcanically in Eocene-Oligocene, but its islands are largely composed of limestone and have undergone orogenic uplift since the Miocene. The Lesser Antilles’ inner arc is of more recent volcanic origin (<10 MYA) and its islands continue to be formed. With no history of continental connection, most of the Lesser Antilles have been completely isolated for at least a few million years, and thus their biotas must have originated via overwater dispersal.

Spiders and other arachnids are emerging as model organisms for researching biogeography of the Caribbean. Spiders are globally distributed and hyperdiverse (~47,000 described of roughly 100,000 estimated species) organisms that vary greatly in size, morphology and behavior, habitat specificity, and importantly, in their dispersal biology. While some spiders show good active dispersal, others are limited in their cursorial activities but exhibit varying passive dispersal potential. Many species are able to passively drift on air currents with behavior called ballooning to colonize new areas. Some genera of spiders, like Tetragnatha or Nephila are known to easily cross geographic barriers, disperse large distances, and are one of the first colonizers of newly formed islands. These are considered to be excellent aerial dispersers, while other lineages are not as
successful. For example, the primitively segmented spiders, family Liphistiidae and the mygalomorph trapdoor spiders, likely do not balloon and have highly sedentary lifestyle imposing strict limits on their dispersal potential. As a consequence, bodies of sea water or even rivers represent barriers that limit their gene flow, which leads to micro-allopatric speciation\(^{75-80}\). Unlike the above clear-cut examples, the dispersal biology of most spider lineages is unknown, and their biogeographic patterns poorly understood.

This research focuses on the tetragnathid spider genus *Cyrtognatha* and its biogeography in the Caribbean. *Cyrtognatha* is distributed from Argentina to southern Mexico and the Caribbean\(^{65,81}\). A recent revision recognized 21 species of *Cyrtognatha* but cautioned that only a fraction of its diversity is known\(^{82}\). Its biology is poorly understood as these spiders are rarely collected and studied (a single Google scholar title hit vs 187 title hits for *Tetragnatha*). Considering their phylogenetic proximity to *Tetragnatha*, as well as its described web architecture, it seems likely that *Cyrtognatha* species disperse by ballooning\(^{83-85}\). Through an intensive inventory of Caribbean arachnids, we obtained a rich original sample of *Cyrtognatha* that allows for the first reconstruction of their biogeographic history in the Caribbean. We use molecular phylogenies to reconstruct *Cyrtognatha* evolutionary history with particular reference to the Caribbean, and compare estimates of clade ages with geological history of the islands. We use this combined evidence to test the vicariant versus dispersal explanations of Caribbean colonization, and to look for a broad agreement of *Cyrtognatha* biogeographic patterns with the GAARlandia landbridge hypothesis. We also greatly expand understanding of *Cyrtognatha* diversity in the Caribbean region.

**Materials and Methods**

**Sampling and identification**

Material for our research was collected as a part of a large-scale Caribbean Biogeography (CarBio) project. Extensive sampling was conducted across Caribbean islands, using visual aerial search (day and night), and beating\(^{66,86}\). Collected material was fixed in 96% ethanol and stored at -20/-80 °C until DNA extraction. Species identification was often impossible due to juvenile individuals or lack of match with the described species (Table 1).

**Molecular procedures**

DNA isolation took place at University of Vermont (Vermont, USA; UVM) using QIAGEN DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA), at the Smithsonian Institute in Washington, DC using an Autogenprep965 for an automated phenol chloroform extraction, and at EZ Lab (Ljubljana, Slovenia).

The latter protocol involved robotic DNA extraction using Mag MAX™ Express magnetic particle processor Type 700 with DNA Multisample kit (Applied Biosystems, Foster City, CA, USA) and following modified protocols (Vidergar, Toplak & Kuntner, 2014).

We targeted two genetic markers: (1) the standard Cytochrome C oxidase subunit 1 (CO1) barcoding region, which has repeatedly been shown to be taxonomically informative in species delimitation\(^{87,88}\), and (2) the nuclear 28S gene for a subset of terminals representing all sampled species. We used the forward LCO1490 (GGTCAACAAATCTAAGATATTGG)\(^{89}\) and the reverse C1-N-2776 (GGATAATCAGAATATCGTCGAGG)\(^{90}\) for CO1 amplification. The standard reaction volume was 25 µL containing 5 µL of Promega’s GoTaq Flexi Buffer and 0.15 µL of GoTaq Flexi Polymerase, 0.5 µL dNTP’s (2 mM each, Biotools), 2.3 µL MgCl\(_2\) (25 mM, Promega), 0.5 µL of each primer (20 µM), 0.15 µL BSA (10 mg/mL; Promega), 2 µL DNA template and the rest was sterile distilled water. We used
the following PCR cycling protocol: an initial denaturation step of 5 min at 94 °C followed by 20
touch-up method cycles of 60 s at 94°C, 90 s at 44 °C, 1 min at 72 °C, followed by 15 cycles of
90 s at 94 °C, 90 s at 53.5 °C, 60 s at 72 °C and the final extension period of 7 min at 72 °C.

The primer pair for 28S were the forward 28Sa (also known as 28S-D3A;
GACCGTCTTGAAACACCGGA)\(^9\) and the reverse 28S-rD5b (CCACAGCGCCAGTTCTGCTTAC)\(^10\). The
standard reaction volume was 35 µL containing 7.1 µL of Promega’s GoTaq Flexi Buffer and 0.2 µL of
GoTaq Flexi Polymerase, 2.9 µL dNTP’s (2 mM each, Biotools), 3.2 µL MgCl\(_2\) (25 mM, Promega), 0.7 µL
of each primer (20 µM), 0.2 µL BSA (10 mg/mL; Promega), 1 µL DNA template and the rest was sterile
distilled water. We used the following PCR cycling protocol: an initial denaturation step of 7 min at 96
°C followed by 20 touch-down method cycles of 45 s at 96 °C, 45 s at 62 °C → 52 °C, 60 s at 72 °C,
followed by 15 cycles of 45 s at 96 °C, 45 s at 52 °C, 60 s at 72 °C and the final extension period of 10
min at 72 °C. The PCR products were purified and sequenced at Macrogen (Amsterdam, NL).

We used Geneious v. 5.6.7\(^9\) for sequence assembly, editing and proofreading. For alignment, we
used the default settings and the automatic optimization option in the online version of MAFFT\(^94\). We
concatenated the CO1 and 28S matrices in Mesquite\(^85\).

We obtained 103 original Cyrtognatha CO1 sequences and mined four additional Cyrtognatha CO1
sequences from GenBank (Table 1). Moreover, we added three CO1 sequences from GenBank (Arkys
cornutus, Metellina mengei, Pachygnatha degeeri) and three original CO1 sequences (Leucauge
argyra, Chrysometa linguiformis, Tetragnatha elongata) to be used as outgroups. We obtained 22
original sequences of 28S gene fragment representing all putative species of Cyrtognatha and
included one from GenBank. Additionally, we incorporated three original 28S sequences (Leucauge
argyra, Chrysometa linguiformis, Tetragnatha elongata) and a single one from GenBank (Arkys
cornutus) to be used as outgroups (Table 1). The concatenated matrix contained 1244 nucleotides
(663 for CO1 and 581 for 28S).

Species delimitation

Because the current taxonomy of Cyrtognatha based on morphology is highly incomplete\(^82\), we
undertook species delimitation using CO1 data. To determine the molecular taxonomic operational
units (MOTUs), we used four different species delimitation methods, each with its online application:
PPT (Poisson tree process)\(^96\), mPTP (multi-rate Poisson tree process)\(^97\), GMYC (generalized mixed yule
coalendent)\(^98\) and ABGD (automatic barcode gap discovery)\(^99\). We ran these species delimitation
analyses using the default settings, with the input tree for GYMC from BEAST2\(^100\) and the input trees
for PPT and mPTP from MEGA 6.0\(^101\).

Phylogenetic analyses

We used MrBayes\(^102\) to reconstruct an all-terminal phylogeny for a complete set of our original
Cyrtognatha material and outgroups using CO1 (Table 1). For Bayesian analysis we used the
Generalised time-reversible model with gamma distribution and invariant sites (GTR+G+I) as
suggested by AIC and BIC criterion in jModelTest\(^2\). We ran two independent runs, each with four
MCMC chains, for 100 million generations, with a sampling frequency of 1000 and relative burn-in
set to 25%. The starting tree was random.

For a species level phylogeny, we then selected two individuals per MOTU and added 28S sequence
data for two partitions and analyzed this concatenated dataset under a Bayesian framework. As
above, jModelTest\(^2\) suggested GTR+G+I as the appropriate model, this time for both partitions.
These analyses had 28 terminals including outgroups (Table 1). The settings in MrBayes were as
above, but the number of MCMC generations was set to 30 million. Due to high mutation rates in
noncoding parts of nuclear genes like 28S, insertions and deletions accumulate through
evolution\textsuperscript{104,105}, resulting in numerous gaps in a sequence alignment. We treated gaps as missing data
but also ran additional analyses applying simple gap coding with FastGap\textsuperscript{106}.

**Molecular dating analyses**

We used BEAST\textsuperscript{200} for time calibrated phylogeny reconstruction (chronogram). We used a single
CO1 sequence per MOTU and trimmed the sequences to approximately equal lengths. We then
modified the xml file in BEAUti\textsuperscript{100} to run three different analyses. The first analysis was run using
GTR+G+I as suggested by jModelTest2. The second analysis employed the package and model
bModelTest\textsuperscript{107}. The third analysis used the package and RBS model\textsuperscript{108}. All parameters were set to be
estimated by BEAST. We calibrated a relaxed log normal clock following Bidegaray-Batista and
Areno\textsuperscript{109} with the ucl.d.mean set as normal distribution with mean value of 0.0112 and standard
deviation of 0.001, and the ucl.d.stdev set to exponential distribution with the mean of 0.666. We ran
an additional analysis using a fossil calibration point on the basal node of Caribbean *Cyrtognatha*
clade. *Cyrtognatha weitschatii*, known from Dominican amber of Hispaniola, is hypothesized to be
13.65 – 20.41 million years old. We used an exponential prior with 95 % confidence interval spanning
from a hard lower bound at 13.65 MYA to the soft upper bound at 41 MYA. This upper bound
corresponds with the time of Hispaniola appearance. We selected Yule process as a tree prior and
constrained the entire topology for BEAST analyses based on the results from the above described
species level phylogeny. The trees were summarized with TreeAnnotator\textsuperscript{109}, with 20% burn-in based
on a Tracer\textsuperscript{110} analysis, target tree set as Maximum clade credibility tree and node heights as median
heights.

All metafiles from BEAST and MrBayes were evaluated in Tracer to determine burn-in, to examine
ESS values and to check for chain convergence. For visualization of trees we used FigTree\textsuperscript{111}. All
MrBayes and BEAST analyses were run on CIPRES portal\textsuperscript{112}.

**Ancestral area estimation**

We used BioGeoBEARS\textsuperscript{113} in R version 3.5.0\textsuperscript{114} to estimate ancestral range of *Cyrtognatha* in the
Caribbean. We used a BEAST produced ultrametric tree from the above described molecular dating
analysis as an input. We removed the outgroup *Tetragnatha elongata* and conducted the analyses
with the 13 *Cyrtognatha* MOTUs from six areas (Hispaniola, Jamaica, Puerto Rico, Cuba, Lesser
Antilles and Panama). We estimated the ancestral range of species with all models implemented in
BioGeoBEARS: DEC (+J), DIVALIKE (+J) and BAYAREALIKE (+J). We used log-likelihoods (LnL) with
Akaike information criterion (AIC) and sample-size corrected AIC (AICc) scores to test each model’s
suitability for our data. All of our *Cyrtognatha*’s MOTUs are single island endemics, therefore we
were able to reduce the parameter “max_range_size” to two\textsuperscript{29,115,116}.

**Results**

We collected 103 *Cyrtognatha* individuals from Cuba, Jamaica, Dominican Republic/Hispaniola,
Puerto Rico and Lesser Antilles (Fig. 1, Table 1). We confirmed that all individuals are morphologically
*Cyrtognatha*. Most species are undescribed but we identified two known species: *C. espanola* (Bryant, 1945) and *C. elyunquensis* (Petrunkevitch, 1930) comb. nov., previously placed in
*Tetragnatha*\textsuperscript{65}.
We obtained CO1 sequences for all *Cyrtognatha* individuals. Using computational methods for species delimitation our *Cyrtognatha* dataset is estimated to contain from 11 to 14 MOTUs (Supplementary Note S1). The results from PTP, mPTP and ABGD were mostly consistent, disagreeing only on the status of three putative species. To these species that are supported by some but not all analyses, we added the label B or C after the species name: *Cyrtognatha* SP10B, *Cyrtognatha* SP2B and *Cyrtognatha* SP2C. On the other hand, we dismiss the results from GMYC analyses using either a single versus multiple threshold option, which failed to recover reliable MOTUs. The composition of our dataset is most likely not compatible with GMYC method as it cannot detect switches between inter- and intraspecific branching patterns, offering us from 1 to 39 MOTUs.

The two gene and the CO1 phylogenies yielded nearly identical networks for the Caribbean taxa, but strikingly different phylogenies due to different root placement (Fig. 2, Supplementary Fig. S1).

Therefore, we ran an additional analysis constraining the root of the mtDNA phylogeny to reflect that of the two gene phylogeny, with otherwise the same settings (Fig. 1, Supplementary Fig. S2). Our unconstrained all-terminal Bayesian phylogeny supports Caribbean *Cyrtognatha* monophyly, albeit with only three non-Caribbean samples. Most terminal clades were well supported with lower supports for deeper nodes (Supplementary Fig. S1). This phylogeny strongly recovers all putative species groups as single island endemics. Furthermore, all geographic areas harbor monophyletic lineages, with the exception of Hispaniola that supports two independent clades. The unconstrained all-terminal CO1 phylogeny recovers the Lesser Antillean clade as sister to all other Caribbean taxa. However, this relationship is not recapitulated in the concatenated, species level, phylogeny (Fig. 2, Supplementary Fig. S3). The concatenated phylogeny also supports monophyly of the Caribbean taxa but recovers the clade of *C. espanola* and *C. SP12* from Hispaniola as sister to all other Caribbean *Cyrtognatha*. The species level phylogeny is generally better supported, with the exception of a clade unifying species from Lesser Antilles, Cuba, Hispaniola and Puerto Rico. In both Bayesian analyses the chains successfully converged and ESS as well as PRSF values of summarized MCMC runs parameters were appropriate.

Chronograms produced by BEAST, using either exclusively CO1 mutation rate or incorporating the additional fossil for time calibration, exhibited very similar time estimates (Fig. 3, Supplementary Fig. S5). We decided to proceed with the mutation rate-only calibrated phylogeny for further analyses because it is less likely to contain known potential biases when calibrating with scarcely available fossils and geological information. The molecular dating analyses based on the three different models in BEAST largely agreed on node ages with less than 1 million years variation. However, the log files from the chronogram based on GTR+G+I model consistently exhibited low ESS values (<50), even with MCMC number of generations having been increased to 200 million. The analyses using the remaining models, RBS and bModelTest, were more appropriate since MCMC chains successfully converged, and the lowest ESS values were 981 and 2214 respectively, thus far exceeding the suggested 200. Additional examination of the log files produced by bModelTest phylogeny with bModelAnalyzer from AppStore.exe revealed that MCMC chains spent most time in modified TN93 model with the code 123143 which contributed for 49.56% of posterior probability (for details on bModelTest method of model selection see ). The BEAST chronogram using bModelTest (Fig. 3, Supplementary Fig. S4) yielded the best supported results, amongst the above mentioned approaches, based on ESS values, and was therefore used in subsequent biogeographical analyses.

This chronogram (Fig. 3) supports a scenario in which *Cyrtognatha* diverged from its sister genus *Tetragnatha* at 18.7 MYA (95% HDP: 12.8 – 26.7 MYA). The Caribbean clade is estimated to have split from the mainland *Cyrtognatha* (represented here by a species from Panama) 15.0 MYA (95% HDP: 10.5 – 20.7 MYA). The clade with lineages represented on Lesser Antilles diverged from those on Greater Antilles at 11.5 MYA (95% HDP: 8.3 – 15.6 MYA).
The comparison of all six models of ancestral area estimation with BioGeoBEARS recovered DIVALIKE+J as most suitable for our data due to highest LnL scores in both AIC and AICc tests (Supplementary Table S1). The estimation of ancestral states suggests that the most recent common ancestor of all Caribbean Cyrtognatha in our dataset most likely (62%) resided on Hispaniola (Fig. 4). Moreover, all the Greater Antillean island clades as well as the Lesser Antillean clade most likely originated from Hispaniola with the following probability: Jamaican clade (47%), Lesser Antillean clade (40%), Cuban clade (63%) and Puerto Rican clade (89%) (Fig. 4, Supplementary Table S1).

Discussion

We reconstruct the first Cyrtognatha phylogeny using molecular data from over 100 individuals of this rarely collected group. Our results support Cyrtognatha as a relatively young clade, having diverged from a common ancestor with its sister genus Tetragnatha, in early- to mid-Miocene, and colonized the Caribbean in mid-Miocene. As we discuss below, these estimated ages, combined with the phylogenetic patterns, refute vicariant explanations of their Caribbean origin, including the GAARlandia hypothesis. Instead, the patterns suggest colonization of Hispaniola, and subsequent dispersal to other islands.

The all-terminal phylogeny (Fig. 1) reveals clear patterns of exclusively single island endemic (SIE) species. This holds true even for the three MOTUs on the Lesser Antilles island group, as they appear on Guadelupe, St. Lucia and Grenada (Table 1). Even in the absence of the oceanic barriers, i.e. within the larger islands, we find evidence of short range endemism. While we do not claim to have thorough regional sampling, we find patterns of local endemism in regions where our sampling is particularly dense, providing the strongest test with available data. Many Caribbean spiders such as Spintharus, Micrathena, Selenops and Nops, as well as other arachnid lineages such as Amblypygi and Pseudoscorpions, demonstrate a similar pattern. The distribution and quantity of SIEs depends on island properties such as maximum elevation, size, isolation and geological age. As we discuss below, these estimated ages, combined with the phylogenetic patterns, refute vicariant explanations of their Caribbean origin, including the GAARlandia hypothesis. Instead, the patterns suggest colonization of Hispaniola, and subsequent dispersal to other islands.

Cyrtognatha most likely colonized the Caribbean through long distance overwater dispersal. An ancient vicariant hypothesis would predict that the early proto-Antilles were connected to the continental America and were colonized in the distant past, possibly over 70 MYA and the GAARlandia landbridge putatively existed around 35-33 MYA. These hypothetical scenarios are clearly not reflected in our BEAST chronogram (Fig. 3) in which we estimate that the Caribbean Cyrtognatha split from its continental population as late as 13.2 MYA. This suggests that the genus Cyrtognatha is much younger than the most reasonable possible vicariant timeframe. Likewise, the reconstructed biogeographic patterns fail to support vicariant scenarios. First, the Jamaican lineage split from Hispaniola soon after colonization (12.2 MYA) even though Jamaica was never a part of the...
proto-Antilles. Secondly, Puerto Rico was a part of the proto-Antilles but was colonized only recently (4.8 MYA). The results of Jamaican and Puerto Rican colonization from Hispaniola thus are most consistent with a scenario of colonization by overwater dispersal.

The mid-Miocene (ca. 15 MYA) is considered as the start of the modern Earth in that the climate began to stabilize and the ocean currents started to take their current form. This combination of events enabled the colonization of the Caribbean islands from eastern-northern parts of South America for example via vegetation rafts passively drifting with water currents. That also meant that the wind directions and the hurricane paths most likely resembled those of today from East to West direction. In fact, hurricanes may create numerous dispersal/colonization opportunities, especially for the organisms with poor active dispersal abilities. Wind directions and tropical storms are relevant for tetragnathid spiders like *Cyrtognatha* that disperse by ballooning and could facilitate their colonization of the Caribbean islands in a stepping stone or leap-frog manner.

With the examination of the relationships in the time calibrated phylogeny (Fig. 3), we predict that a single colonization of the Caribbean from the continental America occurred sometime between 10.5 and 20.7 MYA. The most likely scenario indicates the original colonization of the Greater Antilles (Hispaniola; Fig. 4). Such patterns of colonization of Greater Antilles in Miocene are also evident in many other lineages including vertebrates, invertebrates and plants. A more rigorous test of number and directionality of colonization pathways would require more thorough sampling across potential source populations on the mainland.

Our inference of ancestral ranges proposes an early within island diversification of *Cyrtognatha* ancestors occupying Hispaniola and predict that Hispaniola is the ancestral area for all Caribbean clades (Fig. 4, Supplementary Fig. S5). The path of colonization does not resemble a straightforward pattern such as the stepping-stone pattern. The colonization sequence seems more random or resembles a “leap-frog” pattern. In our case the clear example of island being “leap frogged” is Puerto Rico. A leap frog pattern could indicate a role of hurricanes in movement among Caribbean islands.

**Conclusions**

Our phylogenetic analysis of the tetragnathid spider genus *Cyrtognatha* facilitates reconstruction of its biogeographic history in the Caribbean. The ancestor of this relatively young lineage appears to have colonized the Caribbean overwater in the Miocene and further diversified into an exclusively single island endemic biogeographic pattern seen today. Further sampling of these rarely collected spiders in continental America is needed to confirm the timing, number and source of colonization of the Caribbean and to contrast those from other Caribbean spider clades. For example, *Spintharus* and *Deinopis* (Chamberland et al. ‘in review’) patterns clearly support ancient vicariance, but *Argiope* readily disperses among the islands. *Tetragnatha*, the sister lineage of *Cyrtognatha*, may prove to be of particular interest in comparison, because its biogeographic history on the islands may mirror their global tendency towards repeated colonization of even most remote islands.

**References**

1. Losos, J. B., Ricklefs, R. E. & MacArthur, R. H. *The Theory of Island Biogeography Revisited* (eds. Losos, J. B. & Ricklefs, R. E.) 476p (Princeton University Press, 2009).

2. Henderson, S. J. & Whittaker, R. J. Islands in *Encyclopedia of Life Sciences* (John Wiley & Sons,
350  2003).
351  3. Weigelt, P., Jetz, W. & Kreft, H. Bioclimatic and physical characterization of the world’s
352  islands. *Proc. Natl. Acad. Sci.* **110**, 15307–15312 (2013).
353  4. Darwin, C. *On the Origin of Species by Means of Natural Selection* (Murray, London, 1859).
354  5. Warren, B. H. *et al.* Islands as model systems in ecology and evolution: prospects fifty years
355  after MacArthur-Wilson. *Ecol. Lett.* **18**, 200–217 (2015).
356  6. O’Grady, P. & DeSalle, R. Out of Hawaii: the origin and biogeography of the genus Scaptomyza
357  (Diptera: Drosophilidae). *Biol. Lett.* **4**, 195–199 (2008).
358  7. Ricklefs, R. E. Historical Biogeography and Extinction in the Hawaiian Honeycreepers. *Am. Nat.*
359  **190**, E106–E111 (2017).
360  8. Kennedy, S. R., Dawson, T. E. & Gillespie, R. G. Stable isotopes of Hawaiian spiders reflect
361  substrate properties along a chronosequence. *PeerJ* **6**, e4527 (2018).
362  9. Edgar, G. J., Banks, S., Fariña, J. M., Calvopiña, M. & Martínez, C. Regional biogeography of
363  shallow reef fish and macro-invertebrate communities in the Galapagos archipelago. *J.
364  Biogeogr.* **31**, 1107–1124 (2004).
365  10. Fournié, G. *et al.* Biogeography of parasitic nematode communities in the Galápagos giant
tortoise: Implications for conservation management. *PLoS One* **10**, e0135684 (2015).
366  11. Carvajal-Endara, S., Hendry, A. P., Emery, N. C. & Davies, T. J. Habitat filtering not dispersal
limitation shapes oceanic island floras: species assembly of the Galápagos archipelago. *Ecol.
Lett.* **20**, 495–504 (2017).
367  12. Fattorini, S., Rigal, F., Cardoso, P. & Borges, P. A. V. Using species abundance distribution
models and diversity indices for biogeographical analyses. *Acta Oecologica* **70**, 21–28 (2016).
368  13. Ávila, S. P. *et al.* How did they get here? The biogeography of the marine molluscs of the
Azores. *Bull. la Soc. Geol. Fr.* **180**, 295–307 (2009).
369  14. Schaefer, H. *et al.* The Linnean shortfall in oceanic island biogeography: A case study in the
Azores. *J. Biogeogr.* **38**, 1345–1355 (2011).
370  15. Planas, E. & Ribera, C. Description of six new species of L oxosceles (Araneae: Sicariidae)
endemic to the Canary Islands and the utility of DNA barcoding for their fast and accurate
identification. *Zool. J. Linn. Soc.* **174**, 47–73 (2015).
371  16. Steinbauer, M. J. *et al.* Biogeographic ranges do not support niche theory in radiating Canary
Island plant clades. *Glob. Ecol. Biogeogr.* **25**, 792–804 (2016).
372  17. Tomé, B. *et al.* Along for the ride or missing it altogether: Exploring the host specificity and
diversity of haemogregarines in the Canary Islands. *Parasites and Vectors* **11**, 190 (2018).
373  18. Oliver, P. M., Travers, S. L., Richmond, J. O., Pikacha, P. & Fisher, R. N. At the end of the line:
Independent overwater colonizations of the Solomon Islands by a hyperdiverse trans-
Wallacean lizard lineage (Cyrtodactylus: Gekkota: Squamata). *Zool. J. Linn. Soc.* **182**, 681–694
(2018).
374  19. Pikacha, P., Morrison, C., Filardi, C. E. & Leung, L. Factors affecting frog species richness in the
Solomon Islands. *Pacific Conserv. Biol.* **23**, 387–398 (2017).
375  20. Weeks, B. C. *et al.* New Behavioral, Ecological, and Biogeographic Data On the Montane
Avifauna of Kolombangara, Solomon Islands. *Wilson J. Ornithol.* **129**, 676–700 (2017).
21. Agnarsson, I. et al. Systematics of the Madagascar Anelosimus spiders: Remarkable local richness and endemism, and dual colonization from the Americas. *Zookeys* **2015**, 13–52 (2015).

22. Bacon, C. D., Simmons, M. P., Archer, R. H., Zhao, L. C. & Andriantiana, J. Biogeography of the Malagasy Celastraceae: Multiple independent origins followed by widespread dispersal of genera from Madagascar. *Mol. Phylogenet. Evol.* **94**, 365–382 (2016).

23. Lei, R. et al. Phylogenomic reconstruction of sportive lemurs (genus Lepilemur) recovered from mitogenomes with inferences for Madagascar biogeography. *J. Hered.* **108**, 107–119 (2017).

24. Wallis, G. P. et al. Rapid biological speciation driven by tectonic evolution in New Zealand. *Nat. Geosci.* **9**, 140 (2016).

25. Opell, B. D., Helweg, S. G. & Kiser, K. M. Phylogeography of Australian and New Zealand spray zone spiders (Anyphaenidae: Amaurobioides): Moa’s Ark loses a few more passengers. *Biol. J. Linn. Soc.* **118**, 959–969 (2016).

26. McCulloch, G. A., Wallis, G. P. & Waters, J. M. Does wing size shape insect biogeography? Evidence from a diverse regional stonefly assemblage. *Glob. Ecol. Biogeogr.* **26**, 93–101 (2017).

27. Agnarsson, I. et al. Phylogeography of a good Caribbean disperser: Argiope argentata (Araneae, Araneidae) and a new ‘cryptic’ species from Cuba. *Zookeys* **2016**, 25–44 (2016).

28. Reynolds, R. G. et al. Archipelagic genetics in a widespread Caribbean anole. *J. Biogeogr.* **44**, 2631–2647 (2017).

29. Tucker, D. B., Hedges, S. B., Colli, G. R., Pyron, R. A. & Sites, J. W. Genomic timetree and historical biogeography of Caribbean island ameiva lizards (Pholidoscelis : Teiidae). *Ecol. Evol.* **7**, 7080–7090 (2017).

30. Ricklefs, R. E. & Bermingham, E. The West Indies as a laboratory of biogeography and evolution. *Philos. Trans. R. Soc. B Biol. Sci.* **363**, 2393–2413 (2008).

31. Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J. Biodiversity hotspots for conservation priorities. *Nature* **403**, 853 (2000).

32. Mittermeier, R. A., Turner, W. R., Larsen, F. W., Brooks, T. M. & Gascon, C. Global Biodiversity Conservation: The Critical Role of Hotspots in *Biodiversity Hotspots* (eds. Zachos, F. E. & Habel, J. C.) 3–22 (Springer, 2011).

33. Bowen, B. W., Rocha, L. A., Toonen, R. J. & Karl, S. A. The origins of tropical marine biodiversity. *Trends Ecol. Evol.* **28**, 359–366 (2013).

34. Pindell, J. L., Maresch, W. V., Martens, U. & Stanek, K. The Greater Antillean Arc: Early Cretaceous origin and proposed relationship to Central American subduction mélanges: Implications for models of Caribbean evolution. *Int. Geol. Rev.* **54**, 131–143 (2012).

35. Lomolino, M. V., Riddle, B. R. & Whittaker, R. J. *Biogeography: Biological Diversity across Space and Time* (Sinauer Associates Inc., 2017).

36. Hedges, S. B., Hass, C. A. & Maxson, L. R. Caribbean biogeography: molecular evidence for dispersal in West Indian terrestrial vertebrates. *Proc. Natl. Acad. Sci.* **89**, 1909–1913 (1992).

37. Penney, D. & Selden, P. A. Spinning with the dinosaurs: the fossil record of spiders. *Geol. Today* **23**, 231–237 (2007).
38. Iturralde-Vinent, M. A. & MacPhee, R. D. E. Paleogeography of the Caribbean Region: Implications for Cenozoic Biogeography. Bull. Am. Museum Nat. Hist. 238, 1–95 (1999).

39. MacPhee, R. D. E. & Grimaldi, D. A. Mammal bones in Dominican amber. Nature 380, 489–490 (1996).

40. MacPhee, R. D. E. & Iturralde-Vinent, M. A. First Tertiary Land Mammal from Greater Antille: An Early Miocene Sloth (Xenarthra, Megalonychidae) From Cuba. Am. Museum Novit. 1–95 (1994).

41. Iturralde-Vinent, M. A. Meso-Cenozoic Caribbean Paleogeography: Implications for the Historical Biogeography of the Region. Int. Geol. Rev. 48, 791–827 (2006).

42. Graham, A. Geohistory models and Cenozoic paleoenvironments of the Caribbean region. Syst. Bot. 28, 378–386 (2003).

43. Ali, J. R. Colonizing the Caribbean: Is the GAARlandia land-bridge hypothesis gaining a foothold? J. Biogeogr. 39, 431–433 (2012).

44. Dziika, A., Binford, G. J., Coddington, J. A. & Agnarsson, I. Spintharus flavidus in the Caribbean - a 30 million year biogeographical history and radiation of a ‘widespread species’. PeerJ 3, e1422 (2015).

45. Říčan, O., Piálek, L., Zardoya, R., Doadrio, I. & Zrzavý, J. Biogeography of the Mesoamerican Cichlidae (Teleostei: Cichlidae): colonization through the GAARlandia land bridge and early diversification. J. Biogeogr. 40, 579–593 (2013).

46. Alonso, R., Crawford, A. J. & Bermingham, E. Molecular phylogeny of an endemic radiation of Cuban toads (Bufo: Peltophryne) based on mitochondrial and nuclear genes. J. Biogeogr. 39, 434–451 (2012).

47. Weaver, P. F., Cruz, A., Johnson, S., Dupin, J. & Weaver, K. F. Colonizing the Caribbean: biogeography and evolution of livebearing fishes of the genus Limia (Poeciliidae). J. Biogeogr. 43, 1808–1819 (2016).

48. Agnarsson, I. et al. A radiation of the ornate Caribbean ‘smiley-faced spiders’, with descriptions of 15 new species (Araneae: Theridiidae, Spintharus). Zool. J. Linn. Soc. 182, 758–790 (2018).

49. Uit de Weerd, D. R., Robinson, D. G. & Rosenberg, G. Evolutionary and biogeographical history of the land snail family Urocoptidae (Gastropoda: Pulmonata) across the Caribbean region. J. Biogeogr. 43, 763–777 (2016).

50. Nieto-Blázquez, M. E., Antonelli, A. & Roncal, J. Historical Biogeography of endemic seed plant genera in the Caribbean: Did GAARlandia play a role? Ecol. Evol. 7, 10158–10174 (2017).

51. Pindell, J. L., Kennan, Stanek, K. P., Maresch, W. V. & Draper, G. Foundations of Gulf of Mexico and Caribbean evolution: Geol. Acta 4, 303–341 (2009).

52. Boschman, L. M., van Hinsbergen, D. J. J., Torsvik, T. H., Spakman, W. & Pindell, J. L. Kinematic reconstruction of the Caribbean region since the Early Jurassic. Earth-Science Rev. 138, 102–136 (2014).

53. Morgan, G. S. Quaternary land vertebrates of Jamaica in Biostratigraphy of Jamaica (eds. Wright, R. E. & Robinson, E.) 417–442 (Geological Society of America Memoir, 1993).

54. Buskirk, R. E. Zoogeographic patterns and tectonic history of Jamaica and the northern Caribbean. J. Biogeogr. 12, 445 (1985).
55. Nagle, F., Stipp, J. J. & Fisher, D. E. K-Ar geochronology of the Limestone Caribbees and Martinique, Lesser Antilles, West Indies. Earth Planet. Sci. Lett. 29, 401–412 (1976).

56. Peck, S. B. Diversity and distribution of beetles (Insecta: Coleoptera) of the northern Leeward Islands. Maarten Insecta Mundi 678 (2011).

57. Ricklefs, R. E. & Lovette, I. J. The roles of island area per se and habitat diversity in the species-area relationships of four Lesser Antillean faunal groups. J. Anim. Ecol. 68, 1142–1160 (1999).

58. Garmon, W. T., Allen, C. D. & Groom, K. M. Landscapes and Landforms of the Lesser Antilles. (Springer, 2017).

59. Munch, P. et al. Pliocene to Pleistocene carbonate systems of the Guadeloupe archipelago, French Lesser Antilles: A land and sea study (the KaShallow project). Bull. la Soc. Geol. Fr. 184, 99–110 (2013).

60. Hedges, S. B. Historical Biogeography of West Indian Vertebrates. Annu. Rev. Ecol. Syst. 27, 163–196 (1996).

61. Surget-Groba, Y. & Thorpe, R. S. A likelihood framework analysis of an island radiation: phylogeography of the Lesser Antillean gecko Sphaerodactylus vincenti in comparison with the anole Anolis roquet. J. Biogeogr. 40, 105–116 (2013).

62. Crews, S. C., Puente-Rolón, A. R., Rutstein, E. & Gillespie, R. G. A comparison of populations of island and adjacent mainland species of Caribbean Selenops (Araneae: Selenopidae) spiders. Mol. Phylogenet. Evol. 54, 970–83 (2010).

63. Cosgrove, J. G., Agnarsson, I., Harvey, M. S. & Binford, G. J. Pseudoscorpion diversity and distribution in the West Indies: sequence data confirm single island endemism for some clades, but not others. J. Arachnol. 44, 257–271 (2016).

64. Esposito, L. A. et al. Islands within islands: Diversification of tailless whip spiders (Amblypygi, Phrynus) in Caribbean caves. Mol. Phylogenet. Evol. 93, 107–117 (2015).

65. World Spider Catalog. World Spider Catalog Version 19.0 http://wsc.nmbe.ch, (2018).

66. Agnarsson, I., Coddington, J. A. & Kuntner, M. Systematics: Progress in the study of spider diversity and evolution in Spider Research in the 21st Century: Trends and Perspectives (ed. Penney, D.) (Siris scientific press, 2013).

67. Bell, J. R., Bohan, D. A., Shaw, E. M. & Weyman, G. S. Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. Bull. Entomol. Res. 95, 69–114 (2005).

68. Wu, L., Si, X., Didham, R. K., Ge, D. & Ding, P. Dispersal modality determines the relative partitioning of beta diversity in spider assemblages on subtropical land-bridge islands. J. Biogeogr. 44, 2121–2131 (2017).

69. Lee, V. M. J., Kuntner, M. & Li, D. Ballooning behavior in the golden orbweb spider Nephila pilipes (Araneae: Nephilidae). Front. Ecol. Evol. 3, 2 (2015).

70. Lafage, D., Sibelle, C., Secondi, J., Canard, A. & Pétillon, J. Short-term resilience of arthropod assemblages after spring flood, with focus on spiders (Arachnida: Araneae) and carabids (Coleoptera: Carabidae). Ecohydrology 8, 1584–1599 (2015).

71. Reynolds, A. M., Bohan, D. A. & Bell, J. R. Ballooning dispersal in arthropod taxa: conditions at take-off. Biol. Lett. 3, 237–40 (2007).

72. Kuntner, M. & Agnarsson, I. Phylogeography of a successful aerial disperser: The golden orb spider Nephila on Indian Ocean islands. BMC Evol. Biol. 11, (2011).
73. Szymkowiak, P., Górski, G. & Bajerlein, D. Passive dispersal in arachnids. *Biol. Lett* **44**, 75–101 (2007).

74. Edwards, J. S. & Thornton, I. W. B. Colonization of an Island Volcano, Long Island, Papua New Guinea, and an Emergent Island, Motmot, in Its Caldera Lake. VI. The Pioneer Arthropod Community of Motmot. *Source J. Biogeogr.* **28**, 1379–1388 (2016).

75. Xu, X. *et al.* A genus-level taxonomic review of primitively segmented spiders (Mesotherelae, Liphistiidae). *Zookeys* **488**, 121–151 (2015).

76. Xu, X. *et al.* Pre-Pleistocene geological events shaping diversification and distribution of primitively segmented spiders on East Asian margins. *J. Biogeogr.* **43**, 1004–1019 (2016).

77. Xu, X. *et al.* Extant primitively segmented spiders have recently diversified from an ancient lineage. *Proc. R. Soc. B Biol. Sci.* **282**, 20142486–20142486 (2015).

78. Bond, J. E., Hedin, M. C., Ramirez, M. G. & Opell, B. D. Deep molecular divergence in the absence of morphological and ecological change in the californian coastal dune endemic trapdoor spider *Aptostichus simus*. *Mol. Ecol.* **10**, 899–910 (2001).

79. Bond, J. E., Beamer, D. A., Lamb, T. & Hedin, M. C. Combining genetic and geospatial analyses to infer population extinction in mygalomorph spiders endemic to the Los Angeles region. *Anim. Conserv.* **9**, 145–157 (2006).

80. Hedin, M. C., Starrett, J. & Hayashi, C. Crossing the uncrossable: Novel trans-valley biogeographic patterns revealed in the genetic history of low-dispersal mygalomorph spiders (Antrodiaetidae, *Antrodiaetus*) from California. *Mol. Ecol.* **22**, 508–526 (2013).

81. GBIF. Global Biodiversity Information Facility (GBIF). *Nat. Hist.* (2012).

82. Dimitrov, D. & Hormiga, G. Revision and Cladistic Analysis of the Orbweaving Spider Genus *Cyrtognatha*. *Bull. Am. Museum Nat. Hist.* **1881**, 140 (2009).

83. Dimitrov, D. *et al.* Tangled in a sparse spider web: single origin of orb weavers and their spinning work unravelled by denser taxonomic sampling. *Proc. R. Soc. B Biol. Sci.* **279**, 1341–1350 (2012).

84. Wheeler, W. C. *et al.* The spider tree of life: phylogeny of Araneae based on target-gene analyses from an extensive taxon sampling. *Cladistics* **33**, 574–616 (2017).

85. Alvarez-Padilla, F., Dimitrov, D., Giribet, G. & Hormiga, G. Phylogenetic relationships of the spider family Tetragnathidae (Araneae, Araneoidea) based on morphological and DNA sequence data. *Cladistics* **25**, 109–146 (2009).

86. Coddington, J. A., Griswold, C. E., Silva, D., Penaranda, E. & Larcher, S. F. Designing and testing sampling protocols to estimate biodiversity in tropical ecosystems. (Int. Congr. Syst. Evol. Biol. 1991).

87. Hebert, P. D. N., Cywinska, A., Ball, S. L. & deWaard, J. R. Biological identifications through DNA barcodes. *Proc. Biol. Sci.* **270**, 313–321 (2003).

88. Čandek, K. & Kuntner, M. DNA barcoding gap: Reliable species identification over morphological and geographical scales. *Mol. Ecol. Resour.* **15**, 268-277 (2015).

89. Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* **3**, 294–299 (1994).

90. Hedin, M. C. & Maddison, W. P. A Combined Molecular Approach to Phylogeny of the Jumping
Spider Subfamily Dendryphantinae (Araneae: Salticidae). *Mol. Phylogenet. Evol.* **18**, 386–403 (2001).

91. Nunn, G. B., Theisen, B. F., Christensen, B. & Arctander, P. Simplicity-correlated size growth of the nuclear 28S ribosomal RNA D3 expansion segment in the crustacean order isopoda. *J. Mol. Evol.* **42**, 211–223 (1996).

92. Whiting, M. F. Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera. *Zool. Scr.* **31**, 93–104 (2002).

93. Kearse, M. *et al.* Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649 (2012).

94. Katoh, K. & Standley, D. M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).

95. Maddison, W. & Maddison, D. Mesquite: a modular system for evolutionary analysis version 3.51 [http://www.mesquiteproject.org](http://www.mesquiteproject.org) (2018).

96. Zhang, J., Kapli, P., Pavlidis, P. & Stamatakis, A. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics* **29**, 2869–76 (2013).

97. Kapli, P. *et al.* Multi-rate Poisson tree processes for single-species delimitation under maximum likelihood and Markov chain Monte Carlo. *Bioinformatics* **33**, 1630–1638 (2017).

98. Fujisawa, T. & Barraclough, T. G. Delimiting species using single-locus data and the generalized mixed yule coalescent approach: A revised method and evaluation on simulated data sets. *Syst. Biol.* **62**, 707–724 (2013).

99. Puillandre, N., Lambert, A., Brouillet, S. & Achaz, G. ABGD, automatic barcode gap discovery for primary species delimitation. *Mol. Ecol.* **21**, 1864–1877 (2012).

100. Bouckaert, R. *et al.* Beast2: A software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**, 1003537 (2013).

101. Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729 (2013).

102. Huelsenbeck, J. P. & Ronquist, F. MR BAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754–755 (2001).

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

104. Golenberg, E. M., Clegg, M. T., Durbin, M. L., Doebley, J. & Ma, D. P. Evolution of a noncoding region of the chloroplast genome. *Mol. Phylogenet. Evol.* **2**, 52–64 (1993).

105. Kelchner, S. A. The Evolution of Non-Coding Chloroplast DNA and Its Application in Plant Systematics. *Ann. Missouri Bot. Gard.* **87**, 482 (2000).

106. Borchsenius, F. FastGap 1.2. [http://www.auobot.dk/FastGap_home.htm](http://www.auobot.dk/FastGap_home.htm) (2009).

107. Bouckaert, R. & Drummond, A. J. bModelTest: Bayesian phylogenetic site model averaging and model comparison. *BMC Evol. Biol.* **17**, 42 (2017).

108. Bouckaert, R., Alvarado-Mora, M. V & Rebello Pinho, J. R. Evolutionary rates and HBV: issues of rate estimation with Bayesian molecular methods. *Antivir. Ther.* **18**, 497–503 (2013).

109. Bidegaray-Batista, L. & Arnedo, M. A. Gone with the plate: the opening of the Western
Mediterranean basin drove the diversification of ground-dweller spiders. *BMC Evol. Biol.* **11**, 317 (2011).

Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* 1–3 (2018).

Rambaut, A. FigTree v1.4.3. http://tree.bio.ed.ac.uk/software/figtree/ (2018).

Miller, M. A., Pfeiffer, W. & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees in *Gateway Computing Environments Workshop* (2010).

Matzke, N. J. Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Front. Biogeogr.* **5**, 242-248 (2013).

R Core Team. *R*: A language and environment for statistical computing. R Foundation for Statistical Computing https://cran.r-project.org/ (2018).

Lam, A. R., Stigall, A. L. & Matzke, N. J. Dispersal in the Ordovician: Speciation patterns and paleobiogeographic analyses of brachiopods and trilobites. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **489**, 147–165 (2018).

Pereira, A. G. & Schrago, C. G. Arrival and diversification of mabuyine skinks (Squamata: Scincidae) in the Neotropics based on a fossil-calibrated timetree. *PeerJ* **5**, e3194 (2017).

Heads, M. Old taxa on young Islands: A critique of the use of Island age to date Island-endemic clades and calibrate phylogenies. *Syst. Biol.* **60**, 204–218 (2011).

Hipsley, C. A. & Müller, J. Beyond fossil calibrations: Realities of molecular clock practices in evolutionary biology. *Front. Genet.* **5**, 138 (2014).

McHugh, A., Yablonsky, C., Binford, G. J. & Agnarsson, I. Molecular phylogenetics of Caribbean *Micrathena* (Araneae: Araneidae) suggests multiple colonisation events and single island endemism. *Invertebr. Syst.* **28**, 337–349 (2014).

Crews, S. C. & Gillespie, R. G. Molecular systematics of *Selenops* spiders (Araneae: Selenopidae) from North and Central America: Implications for Caribbean biogeography. *Biol. J. Linn. Soc.* **101**, 288–322 (2010).

Sánchez-Ruiz, A., Brescovit, A. D. & Alayón, G. Four new caponiids species (Araneae, Caponiidae) from the West Indies and redescription of *Nops blandus* (Bryant). *Zootaxa* **3972**, 43–64 (2015).

Kallimanis, A. S. *et al.* Biogeographical determinants for total and endemic species richness in a continental archipelago. *Biodivers. Conserv.* **19**, 1225–1235 (2010).

Gray, A. & Cavers, S. Island biogeography, the effects of taxonomic effort and the importance of island niche diversity to single-island endemic species. *Syst. Biol.* **63**, 55–65 (2014).

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

Dalsgaard, B. *et al.* Determinants of bird species richness, endemism, and island network roles in Wallacea and the West Indies: Is geography sufficient or does current and historical climate matter? *Ecol. Evol.* **4**, 4019–4031 (2014).
127. Steinbauer, M. J., Otto, R., Naranjo-Cigala, A., Beierkühlein, C. & Fernández-Palacios, J. M. Increase of island endemism with altitude - speciation processes on oceanic islands. *Ecography (Cop.). 35*, 23–32 (2012).

128. Bellemain, E. & Ricklefs, R. E. Are islands the end of the colonization road? *Trends Ecol. Evol.* **23**, 461–468 (2008).

129. Silvertown, J., Francisco-Ortega, J. & Carine, M. A. The monophyly of island radiations: An evaluation of niche pre-emption and some alternative explanations. *J. Ecol. 93*, 653–657 (2005).

130. Mwangi, P. N. et al. Niche pre-emption increases with species richness in experimental plant communities. *J. Ecol. 95*, 65–78 (2007).

131. Wiens, J. A. *The Ecology of Bird Communities: Volume 2: Processes and Variations*, (Cambridge University Press, 1989).

132. Fukami, T. Historical contingency in community assembly: Integrating niches, species pools, and priority effects. *Annu. Rev. Ecol. Evol. Syst. 46*, 1–23 (2015).

133. Esselstyn, J. A., Maher, S. P. & Brown, R. M. Species interactions during diversification and community assembly in an Island radiation of shrews. *PLoS One 6*, e21885 (2011).

134. Garb, J. E. & Gillespie, R. G. Island hopping across the central Pacific: Mitochondrial DNA detects sequential colonization of the Austral Islands by crab spiders (Aranaeae: Thomisidae). *J. Biogeogr. 33*, 201–220 (2006).

135. Hedges, S. B. Biogeography of the West Indies: An overview in *Biogeography of the West Indies: Patterns and perspectives* (eds. Woods, C. A. & Sergile, F. E.) (CRC Press, 2001).

136. Pound, M. J., Haywood, A. M., Salzmann, U. & Riding, J. B. Global vegetation dynamics and latitudinal temperature gradients during the mid to late Miocene (15.97-5.33Ma). *Earth Sci. Rev. 112*, 1–22 (2012).

137. Ketmaier, V., Giusti, F. & Caccone, A. Molecular phylogeny and historical biogeography of the land snail genus Solatopupa (Pulmonata) in the peri-Tyrrhenian area. *Mol. Phylogenet. Evol. 39*, 439–451 (2006).

138. Quan, C., Liu, Y.-S., Tang, H. & Utесh, T. Miocene shift of European atmospheric circulation from trade wind to westerlies. *Sci. Rep. 4*, 5660 (2015).

139. Andraca-Gómez, G. et al. A potential invasion route of Cactoblastis cactorum within the Caribbean region matches historical hurricane trajectories. *Biol. Invasions 17*, 1397–1406 (2015).

140. Censky, E. J., Hodge, K. & Dudley, J. Over-water dispersal of lizards due to hurricanes. *Nature 395*, 556 (1998).

141. Gilpin, M. E. The role of stepping-stone islands. *Theor. Popul. Biol. 17*, 247–253 (1980).

142. Baskin, J. N. & Williams, E. E. The Lesser Antillean Ameiva (Sauria, Teiidae): Re-evaluation, zoogrography and the effects of predation. *Stud. Fauna Curacao other Caribb. Islands 23*, 144–176 (1966).

143. Fabre, P.-H. et al. Rodents of the Caribbean: origin and diversification of hutias unravelled by next-generation museomics. *Biol. Lett. 10*, (2014).

144. Hedges, S. B. Paleogeography of the Antilles and origin of West Indian terrestrial vertebrates. *Ann. Missouri Bot. Gard. 93*, 231–244 (2006).
145. Dávalos, L. M. Phylogeny and biogeography of Caribbean mammals. *Biol. J. Linn. Soc.* **81**, 373–394 (2004).

146. Reynolds, R. G. *et al.* Molecular phylogeny and historical biogeography of West Indian boid snakes (*Chilabothrus*). *Mol. Phylogenet. Evol.* **68**, 461–470 (2013).

147. MacPhee, R. D. E. ‘First’ appearances in the Cenozoic land-mammal record of the Greater Antilles: Significance and comparison with South American and Antarctic records. *J. Biogeogr.* **32**, 551–564 (2005).

148. Cervantes, A., Fuentes, S., Gutiérrez, J., Magallón, S. & Borsch, T. Successive arrivals since the Miocene shaped the diversity of the Caribbean Acalyphoideae (*Euphorbiaceae*). *J. Biogeogr.* **43**, 1773–1785 (2016).

149. Si, X. *et al.* Functional and phylogenetic structure of island bird communities. *J. Anim. Ecol.* **86**, 532–542 (2017).

150. Pyron, R. A. & Burbrink, F. T. Ecological and evolutionary determinants of species richness and phylogenetic diversity for island snakes. *Glob. Ecol. Biogeogr.* **23**, 848–856 (2014).

151. Glor, R. E., Losos, J. B. & Larson, A. Out of Cuba: Overwater dispersal and speciation among lizards in the *Anolis carolinensis* subgroup. *Mol. Ecol.* **14**, 2419–2432 (2005).

Competing Interests

The authors declare no competing interests.

Contributions

Research design: K.Č., I.A., G.B., M.K. Material acquisition and molecular procedures: I.A., G.B., K.Č., M.K. Data analyses: K.Č. K.Č. wrote the first draft of the paper. All authors contributed to writing and revising the paper.

Data availability

All data generated in this study and protocols needed to replicate it are included in this published article and its Supplementary material files.

Acknowledgements

We thank the entire CarBio team (http://www.islandbiogeography.org/participants.html) for collecting the material across the Caribbean. Moreover, we thank Lisa Chamberland and other members of the Agnarsson lab (http://www.theridiidae.com/lab-members.html) for the help with material sorting and molecular procedures. This work was supported by grants from the National Science Foundation (DEB-1314749, DEB-1050253), and the Slovenian Research Agency (J1-6729, P1-0236, BI-US/17-18-011).
Figure legends

Figure 1: A) Map of the Caribbean with indicated sampling localities. B) The all-terminal mitochondrial Bayesian phylogeny of Cyrtognatha. Branch colors match those of the islands in A. Notice that all putative species form exclusively single island endemic pattern.

Figure 2: Species level Bayesian phylogeny of Cyrtognatha based on CO1 and 28S. Relationships agree with Cyrtognatha and Caribbean Cyrtognatha monophyly.

Figure 3: Time-calibrated BEAST phylogeny of Cyrtognatha. This chronogram suggests Cyrtognatha colonized the Caribbean in mid-Miocene and refutes ancient vicariant scenarios. The lack of any land bridge connection of the Caribbean with mainland at least since early Oligocene (cca. 33 MYA; GAARandia) suggests that colonization happened by overwater dispersal. Confidence intervals of clade ages agree with geological history of Caribbean islands.

Figure 4: Ancestral area estimation of Cyrtognatha with BioGeoBEARS. The biogeographical analysis, using the most suitable model for our data (DIVALIKE + J, max_range_size = 2), revealed that Hispaniola was most likely colonized first. Following colonization, Cyrtognatha diversified within Hispaniola and subsequently dispersed from there to all other islands of the Caribbean.

Table 1: Detailed information on Cyrtognatha specimens and outgroups.

| Genus      | Species/ MOTU | Voucher code | Location   | Lat.     | Lon.     | CO1 accession number | 28S accession number |
|------------|---------------|--------------|------------|----------|----------|----------------------|----------------------|
| Cyrtognatha | elyunquensis  | 00392873     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00392894     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00392865     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00392845     | Puerto Rico| 18.17213 | -65.77074|                      |                      |
| Cyrtognatha | elyunquensis  | 00392808     | Puerto Rico| 18.28925 | -65.77877|                      |                      |
| Cyrtognatha | elyunquensis  | 00392813     | Puerto Rico| 18.28925 | -65.77877|                      |                      |
| Cyrtognatha | elyunquensis  | 00392764     | Puerto Rico| 18.28925 | -65.77877|                      |                      |
| Cyrtognatha | elyunquensis  | 00392745     | Puerto Rico| 18.28925 | -65.77877|                      |                      |
| Cyrtognatha | elyunquensis  | 00392732     | Puerto Rico| 18.28925 | -65.77877|                      |                      |
| Cyrtognatha | elyunquensis  | 00392911     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00392843     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00392763     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00392853     | Puerto Rico| 18.29574 | -65.79065|                      |                      |
| Cyrtognatha | elyunquensis  | 00782105     | Puerto Rico| 18.17326 | -66.59015|                      |                      |
| Cyrtognatha | elyunquensis  | 00782116     | Puerto Rico| 18.17326 | -66.59015|                      |                      |
| Cyrtognatha | espanola      | 00782473     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00782595     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00782517     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00782495     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00782505     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00782511     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00785757     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Cyrtognatha | espanola      | 00784817     | Hispaniola | 19.35504 | -070.111 |                      |                      |
| Species      | Code          | Location     | Latitude  | Longitude |
|--------------|---------------|--------------|-----------|-----------|
| Cyrtognatha  | espanola      | Hispaniola   | 19.35504  | -070.111  |
|              | 00784739      |              |           |           |
|              | 00785433      |              |           |           |
|              | 00785697      |              |           |           |
|              | 00782558      |              |           |           |
|              | 0078592       |              |           |           |
|              | 00785705      |              |           |           |
|              | 00785514      |              |           |           |
|              | 00782583      |              |           | -69.46635|
|              | 00782543      |              |           |           |
|              |              |              |           |           |
|              | 00785647      |              |           |           |
|              | 00785737      |              |           |           |
|              | 00785592      |              |           |           |
|              | 00785514      |              |           |           |
|              | 00784482      |              |           |           |
|              | 00787050      | Hispaniola   | N/A       | N/A       |
|              | 00787032      | Hispaniola   | N/A       | N/A       |
|              | 00787053      | Hispaniola   | N/A       | N/A       |
|              | 00787040      | Hispaniola   | N/A       | N/A       |
|              | 00787095      | Hispaniola   | 19.03750  | -70.96918 |
|              | 00786963      | Hispaniola   | 18.82208  | -70.6838  |
|              | 00784541      | Hispaniola   | 18.09786  | -71.18925 |
|              | 00002436A     | Jamaica      | 18.04833  | -76.61814 |
|              | 00002399A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00002589A     | Jamaica      | 18.04833  | -76.61814 |
|              | 00002990A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004283A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004492A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004414A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004313A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004384A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00003825A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00003822A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004474A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004456A     | Jamaica      | 18.05350  | -76.59950 |
|              | 00004029A     | Jamaica      | 18.05350  | -76.59950 |
| Infraseries | Name | Accession | Country | Latitude | Longitude |
|------------|------|-----------|---------|----------|-----------|
| Cyrtognatha | SP4  | 00004432A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004294A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004170A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004335A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004444A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004206A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004209A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004202A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004462A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004310A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004010A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP4  | 00004136A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP5  | 00003183A | Jamaica | 18.34769 | -77.64158 |
| Cyrtognatha | SP5  | 00002964A | Jamaica | 18.34769 | -77.64158 |
| Cyrtognatha | SP6  | 00003815A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP6  | 00003173A | Jamaica | 18.05350 | -76.59950 |
| Cyrtognatha | SP7  | 00782814  | Cuba    | 20.01309 | -76.83400 |
| Cyrtognatha | SP7  | 00782885  | Cuba    | 20.01309 | -76.83400 |
| Cyrtognatha | SP7  | 00782894  | Cuba    | 20.01309 | -76.83400 |
| Cyrtognatha | SP7  | 00000564A | Cuba    | 20.31504 | -76.55337 |
| Cyrtognatha | SP7  | 00002323A | Cuba    | 20.31504 | -76.55337 |
| Cyrtognatha | SP7  | 00000317A | Cuba    | 20.31504 | -76.55337 |
| Cyrtognatha | SP7  | 00784348  | Cuba    | 20.00939 | -76.89402 |
| Cyrtognatha | SP8  | 00784418  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | SP8  | 00784494  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | SP8  | 00787280  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | SP8  | 00784608  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | SP8  | 00787174  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | SP8  | 00787178  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | SP10 | 00001639A | Grenada | 12.09501 | -61.69500 |
| Cyrtognatha | SP10 | 00001673A | Grenada | 12.09501 | -61.69500 |
| Cyrtognatha | SP10 | 00001688A | Grenada | 12.09501 | -61.69500 |
| Cyrtognatha | SP10 | 00001792A | Grenada | 12.09501 | -61.69500 |
| Cyrtognatha | SP10B| 00001680A | Saint Lucia | 13.96448 | -60.94473 |
| Cyrtognatha | SP12 | 00787269  | Hispaniola | 19.05116 | -70.88866 |
| Cyrtognatha | atopica GB | N/A | N/A | N/A | GU129638 |
| Cyrtognatha | sp. GB | N/A | N/A | N/A | GU129629 |
| Cyrtognatha | sp. GB | N/A | Panama | N/A | GU129630 | GU129609 |
| Cyrtognatha | sp. GB | N/A | Panama | N/A | GU129629 |
| Arkys | comatus GB | N/A | N/A | N/A | FJ607556 | KY016938 |
| Chrysoneta | linguisformis | 00784514 | Cuba | 22.56010 | -83.83318 |
| Leucauge | argyra | 00782551 | Hispaniola | 19.35504 | -70.11100 |
| Metellina | mengeli GB | N/A | N/A | N/A | KY269213 |
| Pachygnatha | degeeri GB | N/A | N/A | N/A | KY268868 |
Figure 1

A) 

B) 

Outgroups: SP12 Hispaniola
SP2C Hispaniola
SP2CBS Hispaniola
SP8 Hispaniola

eyunate: Puerto Rico

SP1 Guadeloupe
SP12B Saint Lucia
SP10 Grenada
SP5 Jamaica
SP2 Jamaica

SP4 Jamaica

*** > 95% support
** > 90% support

0.07
