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**Spintharus flavidus** in the Caribbean - a 30 million year biogeographical history and radiation of a ‘widespread species’

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The Caribbean island biota is characterized by high levels of endemism, the result of an interplay between colonization opportunities on islands and effective oceanic barriers among them. A relatively small percentage of the biota is represented by ‘widespread species’, presumably taxa for which oceanic barriers are ineffective. Few studies have explored in detail the genetic structure of widespread Caribbean taxa. The cobweb spider *Spintharus flavidus* Hentz, 1850 (Theridiidae) is one of two described *Spintharus* species and is unique in being widely distributed from northern N. America to Brazil and throughout the Caribbean. As a taxonomic hypothesis, *Spintharus “flavidus”* predicts maintenance of gene flow among Caribbean islands, a prediction that seems contradicted by known *S. flavidus* biology, which suggests limited dispersal ability. As part of an extensive survey of Caribbean arachnids (project CarBio), we conducted the first molecular phylogenetic analysis of *S. flavidus* with the primary goal of testing the ‘widespread species’ hypothesis. Our results, while limited to three molecular loci, reject the hypothesis of a single widespread species. Instead this lineage seems to represent a radiation with at least 16 species in the Caribbean region. Nearly all are short range endemics with several distinct mainland groups and others being single island endemics. While limited taxon sampling, with a single specimen from S. America, constrains what we can infer about the biogeographical history of the lineage, clear patterns still emerge. Consistent with limited overwater dispersal, we find evidence for a single colonization of the Caribbean about 30 million years ago, coinciding with the timing of the GAARLandia landbridge hypothesis. In sum, *S. “flavidus”* is not a single species capable of frequent overwater dispersal, but rather a 30 my old radiation of single island endemics that provides preliminary support for a complex and contested geological hypothesis.
**Spintharus flavidus** in the Caribbean – a 30 million year biogeographical history and radiation of a ‘widespread species’

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**ABSTRACT**

The Caribbean island biota is characterized by high levels of endemism, the result of an interplay between colonization opportunities on islands and effective oceanic barriers among them. A relatively small percentage of the biota is represented by ‘widespread species’, presumably taxa for which oceanic barriers are ineffective. Few studies have explored in detail the genetic structure of widespread Caribbean taxa. The cobweb spider *Spintharus flavidus* Hentz, 1850 (Theridiidae) is one of two described *Spintharus* species and is unique in being widely distributed from northern N. America to Brazil and throughout the Caribbean. As a taxonomic hypothesis, *Spintharus “flavidus”* predicts maintenance of gene flow among Caribbean islands, a prediction that seems contradicted by known *S. flavidus* biology, which suggests limited dispersal ability. As part of an extensive survey of Caribbean arachnids (project CarBio), we conducted the first molecular phylogenetic analysis of *S. flavidus* with the primary goal of testing the ‘widespread species’ hypothesis. Our results, while limited to three molecular loci, reject the hypothesis of a single widespread species. Instead this lineage seems to represent a radiation with at least 16 species in the Caribbean region. Nearly all are short range endemics with several distinct mainland groups and others being single island endemics. While limited taxon sampling, with a single specimen from S. America, constrains what we can infer about the biogeographical history of the lineage, clear patterns still emerge. Consistent with limited overwater dispersal, we find evidence for a single colonization of the Caribbean about 30 million years ago, coinciding with the timing of the GAARLandia landbridge hypothesis. In sum, *S. “flavidus”* is not a single species capable of frequent overwater dispersal, but rather a 30 my old radiation of single island endemics that provides preliminary support for a complex and contested geological hypothesis.

**Subjects** Biogeography, Taxonomy, Biodiversity

**Keywords** *Spintharus*, Theridiidae, GAARLandia, Evolution, Caribbean, Adaptive Radiation, Dispersal, zoology, Arachnology
INTRODUCTION

Archipelagos represent unique conditions to study gene flow and diversification (Agnarsson & Kuntner 2012; Gillespie & Roderick 2002; Losos & Ricklefs 2010; Ricklefs & Bermingham 2008; Warren et al. 2015). Islands are situated within a matrix of oceanic barriers that restrict gene flow in proportion to the geographic isolation of an island and the dispersal ability of a given taxon (Agnarsson et al. 2014; Claramunt et al. 2012; Diamond et al. 1976; Warren et al. 2015). They also provide opportunity for adaptive radiations within islands, particularly for dispersal-limited taxa.

Among archipelagos, the Caribbean is particularly rich as an arena for biogeographic analyses. The region is composed of a range of old continental fragments (Greater Antilles, ~40 my) and relatively recent volcanic islands (Lesser Antilles, <10 my) (Iturralde-Vinent & MacPhee 1999; Iturralde-Vinent 2006), and features rich biodiversity and high levels of endemism (Hedges 1996a; Hedges & Heinicke 2007; Losos & DeQueiroz 1997; Myers et al. 2000; Ricklefs & Bermingham 2008). The proximity of these islands to ancient continents (N. and S. America), creates potential for a dynamic interchange of taxa between continents and islands (Bellemain & Ricklefs 2008; Heaney 2007). The geological history of the islands has created unique conditions for colonization and speciation. Over the last 40 million years, the Greater Antilles landmasses have emerged, moved, sometimes amalgamated, and perhaps, connected to South America via a former land bridge; the Greater Antilles and Aves Ridge Land Bridge (GAARLandia) hypothesis (Ali 2012; Iturralde-Vinent & MacPhee 1999). Ricklefs & Bermingham (2008) portrayed the Caribbean as “a laboratory of biogeography and evolution“ ideally suited to study replicate patterns of allopatric speciation and evolutionary radiations.

Arthropods can strongly test biogeographical patterns (Gillespie & Roderick 2002). They can be abundant enough to be easily sampled without deleterious population effects, and they have short generation times, compared to vertebrates and many plants. Short generation times and large brood sizes allow them to evolve and diversify quickly. Like vertebrates and plants, arthropods span the spectrum of dispersal abilities from extremely poor to excellent. Widespread species tend to be good dispersers and for a ‘naturally’ widespread species to persist in the Caribbean, gene flow among islands must be sufficient. Thus, for lineages with relatively poor dispersal ability, widespread species are improbable taxonomic hypotheses.

The taxonomic hypothesis *Spintharus “flavidus”* circumscribes a widespread spider species found throughout the Caribbean and from northern N. America to Brazil (Levi 1954; Levi 1963). This species has been documented ballooning (aerial dispersal on silk threads (Bell et al. 2005)) and might be expected to maintain gene flow across oceanic barriers. However, the single ballooning record was a short distance dispersal, and other information on the distribution and biology of *Spintharus* suggest long distance dispersal is rare. *Spintharus* have a somewhat cryptic habitat, often found in leaf litter or the undersides of low-level leaves. The genus is old; its sister lineage *Episinus* occurs in 45 myo Baltic amber, but, unlike *Episinus, Spintharus* is restricted to the Americas. *Spintharus* includes just two species, the putatively widespread *S. flavidus* and *S. gracilis* restricted to Brazil (Levi 1954; Levi 1963). A third species *S. argenteus* (Dyal 1935) from Pakistan is clearly misplaced in the genus based on its original description (Levi 1954), it’s proper placement is unclear but it may be a tetragnathid (Agnarsson pers. obs.). *Spintharus flavidus* varies greatly in color and genitalic morphology—usually species-specific in spiders—thus hinting at greater species diversity. Levi (1954, 1963), however, did not see clear
geographical patterns to this variation. Specimens from a single locality differed so continuously in color and genitalia that he believed it to be one widespread and variable species.

Preliminary analyses of the COI gene indicate high levels of molecular diversity within S. *flavidus* and contest the widespread species hypothesis. Here, we use molecular phylogenetics to study patterns of diversification in a ‘widespread’ spider species, specifically to test the hypothesis that it represents single species capable of frequent dispersal across moderate stretches of ocean. While our taxon sampling is not designed for detailed biogeographical analyses, samples from the continental landmasses (N., C. and S. America) permit preliminary evaluation of the route, number, and timing of colonization events. We also use our data to propose an initial biogeographical hypothesis for *Spintharus* that can readily be tested as more data accumulate, especially from South America.

**MATERIALS AND METHODS**

**Specimen sampling and DNA extraction and assembly**

The CarBio team ([www.islandbiogeography.org](http://www.islandbiogeography.org)) collected specimens from Cuba, the Dominican Republic, Puerto Rico, Jamaica, the Lesser Antilles, Florida, South Carolina, Costa Rica, Mexico, and Columbia between 2011 and 2015. All specimens were collected under appropriate permits: **Puerto Rico**, DRNA: 2011-IC-035 (O-VS-PVS15-SJ-00474-08042011); **Jamaica**, NEPA, reference number #18/27; **USA**, USDI National Park Service, EVER-2013-SCI-0028; **Costa Rica**, SINAC, pasaporte científico no. 05933, resolución no. 019-2013-SINAC; **Cuba**, Departamento de Recursos Naturales; **Dominican Republic**, Secretaría de Estado de Medio Ambiente y Recursos Naturales; **Mexico**, SEMARNAT scientific collector permit FAUT-0175 issued to Dr. Oscar Federico Francke Balle; **Colombia**, [list to be completed].

We used standard protocols for aerial search, beating, sifting, and cryptic methods (Coddington et al. 2009; Coddington et al. 1991). Spiders were immediately fixed in 95% ethanol and stored at -20°C (UVM Natural History Museum). Phenotype vouchers will also be deposited at the USNM (Smithsonian Institution).

We chose 195 individuals from the field samples for molecular analyses, representing each collecting locality with several specimens (targeting 4-5), when available (Figure 1). DNA was extracted from 1-4 legs from each individual and isolated using a Qiagen DNeasy Tissue kit using the kit protocol (Qiagen, Valencia, CA, USA). DNA from some specimens was isolated from leg samples at the Smithsonian Institute (SI) in Washington, DC using an Autogenprep965 for an automated phenol:chloroform extraction. We initially sequenced a fragment of the mitochondrial COI (cytochrome c oxidase subunit 1) to establish basic patterns of phylogenetic relationships and obtain an initial estimate of diversification patterns through ‘DNA barcoding’ (Hebert et al. 2003). Upon discovering deep divergences among isolated ‘populations’ with COI, we additionally sequenced the mitochondrial ribosomal 16S rRNA, and the nuclear Internal Transcribed Spacer unit 2 (ITS2) for selected exemplars from each clade indicated by COI. These molecular markers have proven successful in similar phylogenetic studies of spiders ranging from low taxonomic levels to diversifications as deep as the age of the Caribbean (Agnarsson 2010; Agnarsson & Rayor 2013; Kuntner & Agnarsson 2011a; Kuntner & Agnarsson 2011b). To amplify the COI, 16S, and ITS2, we used LCO1490 & HCO1298, 16S
A & 16S B, and ITS 4 & ITS 5.8 primers respectively (Folmer et al. 1994; White et al. 1990). Standard PCR protocols were used as described in (Agnarsson 2010; Agnarsson et al. 2007). The PCR products were purified using Exosap kits and purified PCR products sequenced at the University of Arizona, Beckman Genomics, or the SI. All sequences will be deposited in GenBank [accession numbers not yet available]. COI, 16s, and ITS2 sequences from *Anelosimus* and *Episinus* species were downloaded from GenBank and used as outgroups along with a *Chrysso* specimen we sequenced here.

The chromatographs were interpreted with Phred 45 and Phrap (Green 2009; Green & Ewing 2002) using the Chromaseq module (Maddison & Maddison 2011a) in the program Mesquite (Maddison & Maddison 2011b) and edited by hand. The alignments for the COI sequences were trivial with no implied indels, and were done in Mesquite through ClustalW. The 16S and ITS2 sequences were aligned using the program MAFFT (Katoh 2013) through the online server portal http://mafft.cbrc.jp/alignment/server. Other than setting the tree building number and maxiterate to the maximum, we used default MAFFT settings.

**Phylogenetic and biogeographical analyses**

The aligned sequences for COI, 16S, and ITS2 were tested for the best fitting substitution model using the program Jmodeltest 2.1.7 (Darriba et al. 2012). The best models for each gene, among the 24 models available in MrBayes, were GTR+G for 16S and ITS2 and GTR+I+G for COI.

We used MrBayes V3.2.3 on XSDE (Ronquist & Huelsenbeck 2003) through the online portal CIPRES (Miller et al. 2010), to run a Bayesian analysis for mtDNA (COI plus 16S) and nuDNA (ITS2) separately and for the concatenated three loci. We used Mesquite to concatenate loci and to partition the analysis by locus. The Bayesian analyses ran MC3 (Metropolis coupled Markov chain Monte Carlo) for 50,000,000 generations, sampling every 1,000 generations, and we used Tracer (Drummond & Rambaut 2007), to assure proper mixing of chains and sufficient sampling. ML searches were done in Garli 2.0, (Zwickl 2006) and repeated 100 times and the tree maximizing likelihood of the data was preferred.

Analyses of node ages were done in BEAST 1.8 (Heled & Drummond 2010). We pruned the matrix to include 2-3 exemplars with minimum missing data from each major clade, and constrained the monophyly of *Spintharus* and that of Dominican *Spintharus*, because all prior analyses justified their monophyly. We employed GTR + G + I model for the concatenated matrix with a Yule process tree prior, and a UPGMA starting tree. Two chains of 100 million generations were run and, convergence and correct mixing of the chains were monitored using TRACER version 1.5. We estimated node ages using a relaxed exponential clock calibrated with a Dominican amber fossil of *Spintharus* that is dated to about 15-20 mya (Wunderlich 1988). This sets the minimum age of the genus, but more importantly, the colonization of Hispaniola at 15 mya and was implemented using exponential priors on both nodes with a mean of 5 and offset of 15 my – spanning about 15-35 my. Many fossils of the closely related Spinharinae genus *Episinus* s. l. occur in Dominican and Baltic amber (Wunderlich 1988; Wunderlich 2008), the latter dating to approximately 44 my. Hence we constrained the root of Spinharinae (*Spintharus* plus *Episinus*) with an exponential prior with an offset of 44 my and mean of 15, spanning approximately 40-100 my, to the approximate origin of Theridiidae (Bond et al. 2014, Liu et al.)
A recent study on Theridiidae estimates the origin of Spintharinae between 55-45 mya (Liu et al. resubmitted). We inferred ancestral ranges using RASP 3.2 (Yu et al. 2015) inputting the preferred Bayesian tree and a set of 100 post-burnin trees. We defined areas as each of the Caribbean islands, and S. America, and N. America (including USA, Costa Rica, and Mexico). S-DIVA, S-DEC and Bayesian Binary ancestral area analyses were run limiting areas to two, as all putative species level clades are restricted to one area, and without dispersal constraints. We exported results as tables and graphics and the latter we touched up in Adobe Illustrator.

Species delimitation, distribution, and photo-documentation

We calculated distances among clades suggested by the barcoding analysis of the COI data using MEGA6 (Table 1). The phylogenetic results and genetic distance measures provide initial species hypotheses. Various species delimitation methods were then used to help estimate number of species in this radiation using COI or the three loci dataset/tree depending on method. We used the species delimitation plugin in Geneious (Kearse et al. 2012; Masters et al. 2011) to estimate species limits under Rosenberg’s reciprocal monophyly P(AB) (Rosenberg 2007) and Rodrigo’s P(RD) method (Rodrigo et al. 2008). We also estimated the probability of population identification of a hypothetical sample based on the groups being tested (P ID(Strict) and P ID (Liberal)). The genealogical sorting index (gsi) statistic (Cummings et al. 2008) was calculated using the gsi webserver (http://genealogicalsorting.org) on the estimated tree and an assignment file that contained the same user specified groups identified in the Geneious plugin. Finally we used a single locus Bayesian implementation (bPTP) of the Poisson tree processes model (Zhang et al. 2013) to infer putative species boundaries on a given single locus phylogenetic input tree. The analysis was run as a rooted tree with outgroups removed for 100,000 generations with 10% burnin removed.

For each putative species-level clade, representatives from all localities were chosen for taxonomic photography. The spiders were positioned in Germ-X hand sanitizer (65% ethanol) and covered in 95% ethanol. The photographs were taken with the Visionary Digital BK Laboratory System, using a Canon 5D camera, a 65 mm macro zoom lens. Photo stacks of 30-50 slices were then compiled using the program Helicon Focus 5.3. The image was then edited in Photoshop CS6 to balance light quality, adjust for brightness, remove background blemishes, and provide a scale.

We used the online program GPS Visualizer (http://www.gpsvisualizer.com) to plot localities (Figure 1).

RESULTS

Specimen sampling and DNA extraction and assembly

Of the 195 individuals chosen for DNA work, 186 yielded quality DNA and 175 were
208 successfully amplified for COI. The subset of 186 taxa that was chosen for additional sequencing
209 yielded 180 16S and 79 ITS2 sequences, representing all major clades. In all, the concatenated
210 matrix contains 1572 bp of which 668 are COI, 682 are 16S and 312 ITS2.

Phylogenetics

213 Bayesian inferences of the concatenated COI, 16s, and ITS2 sequences from *S. flavidus* provide
214 a robust and well-resolved phylogenetic hypothesis (Figure 1). Maximum likelihood analyses of
215 the concatenated matrix yield nearly identical results. Independent mDNA and nuDNA were
216 highly congruent with one another and with the concatenated analysis. No strongly supported
217 clades in either gene tree contradict the concatenated tree, rather areas of disagreement reflect
218 lack of resolution in gene trees.

219 All analyses support the monophyly of *Spintharus* and of the Caribbean taxa. Furthermore, the
220 N. American + Caribbean specimens form a clade and within that the specimens from the
221 Yucatan peninsula are sister to the islands clade. Deep genetic divergences occur within the
222 USA, especially between USA1 (from Genbank) and the remaining specimens. Within the
223 Caribbean two main clades are supported. The first contains two Cuban and one Hispaniolan
224 clade, of three or more species each, and the Hispaniolan clade nests among the Cuban clades.
225 The second contains the remaining islands, Jamaica, Puerto Rico, and the Lesser Antilles. Puerto
226 Rican specimens nest within a Lesser Antilles clade.

227 Separate analyses of mtDNA and nuDNA markers reveal general congruence among the
228 independent lines of evidence. Both support the monophyly of *Spintharus*, N. America plus the
229 Caribbean, Yucatan plus Caribbean and the Caribbean islands. Both recover the two Cuban
230 clades, one sister to a Hispaniolan clade, and both nest Puerto Rico within the Lesser Antilles.
231 Both resolve most putative monophyletic species, and all islands are monophyletic (except the
232 two Cuban clades discussed above).

Species delimitation, distribution, and photo-documentation

234 Bayesian inferences of the concatenated COI, 16s, and ITS2 yield 22 distinct and well supported
235 lineages. Most are independently recovered in the mtDNA and ITS2 datasets (Supplementary
236 Materials). These 22 lineages, of which 20 include multiple specimens and two single specimens,
237 constitute our initial species hypotheses (Table 1). Most putative species lineages show genetic
238 distances >5%, and nearly all are separated by a ‘barcoding gap’ (Table 1). Shallower
239 divergences, between 1.9-4% are found between clades Cuba 1 and 2 versus Cuba 3, and
240 between USA 1 and 2, and between St. Lucia and Grenada (Table 1). The results of various
241 methods of species delimitation reject the single widespread species hypothesis. The bPTP
242 analysis estimated between 17-31 species, including all the initial 22 species hypotheses but
243 supported one USA species. The maximum likelihood and the Bayesian tree supported 17-19
244 species, congruent with the minimum estimate from the bPTP analyses. Other species
245 delimitation methods yielded similar results. Eighteen putative species had P ID (liberal) of 89 or
246 higher, 19 had significant Rosenberg values and 18 had GSI values of >82, thereof 14 = 100. In
247 general methods were congruent and supported at least the 16 circled species in Figure 1. These
represent 11 of the 22 initial species hypotheses, and five species whose delimitation is broader than the original hypotheses (Table 1, Figure 1).

All 16 species are narrow range endemics, either restricted to continents or are single island endemics, except species 7, with distinct populations on St. Kitts, Nevis and Puerto Rico, and species 5, with distinct populations on Grenada and St. Lucia. The only islands that share haplotypes are St. Kitts and Nevis, small keys separated by less than 30 km of shallow ocean. The sister clade to the St. Kitts and Nevis clade is, in contrast, Puerto Rico separated by over 250 km. The largest Greater Antilles islands, Cuba and Hispaniola, harbor minor within-island radiations resulting in multiple species-level clades.

**Biogeographical patterns**

The dating analysis in BEAST suggests that the N. American plus Caribbean clade diverged from the sister clade represented by the Colombian taxon, between 37-29 mya, a time window consistent with GAARlandia (Figure 2). The Caribbean and the N. American clades diverged between 27-29 mya. Divergences corresponding to Greater Antilles island clades are old (>20mya), except Puerto Rico that appears to contain a recent lineage. Some Lesser Antilles islands contain lineages estimated to be older than the currently hypothesized age of the islands.

The RASP analyses of ancestral areas is consistent with the single colonization of N. America plus the Caribbean, and the single colonization of the islands though inferential power is limited due to only a single specimen from S. America. The common ancestor of the islands is reconstructed to have occurred on mainland and islands (Cuba), consistent with the GAARlandia hypothesis, and Hispaniola is reconstructed to have been colonized from Cuba. The colonization of Puerto Rico is supported as a relatively recent (~6 mya) event via ancestors in the Lesser Antilles.

**DISCUSSION**

Island archipelagos, terrestrial habitat surrounded by aquatic barriers, have long offered unique insight into processes of diversification (Baldwin & Sanderson 1998; Darwin 1859; Gillespie & Roderick 2002; Losos et al. 1998). Widespread species on archipelagos represent taxonomic hypotheses that predict ongoing gene flow. Such hypotheses are plausible for excellent dispersers but are rendered less and less probable as dispersal ability of organisms decreases, until finally ocean barriers become completely effective (Agnarsson et al. 2014; Agnarsson & Kuntner 2012; Claramunt et al. 2012; Diamond et al. 1976). Our molecular analyses refute the current taxonomy of *Spintharus flavidus*. Phylogenetic and species delimitation analyses of both mitochondrial and nuclear genes, independently and combined, strongly reject the single-species hypothesis and suggest that *S. flavidus* is a radiation of short range endemics (Harvey 2002) in the Caribbean. Morphological evidence supports the multiple species hypothesis (Figure 3) with some highly distinct forms and, though polymorphic, consistent color patterns within islands. This finding is consistent with the limited dispersal ability of this lineage as suggested by its biology and habitat. Furthermore, despite the relatively old age of the *flavidus* clade, it is restricted to the Americas (unlike its sister taxon *Episinus*), shows small scale genetic structuring
of populations, and some limited evidence for long distance dispersal (colonization of the Lesser Antilles and Puerto Rico across water).

This is not an isolated finding. The CarBio project (islandbiogeography.org) has found evidence of other multiple single island endemics among hypothetical ‘widespread species’ of putatively poor dispersers (McHugh et al. 2014, Esposito et al. 2015, Petersen et al. in prep., Nishida et al. in prep., LeQuier et al. in prep.). In other groups of animals and plants, dispersal limited taxa also tend to form island endemics rather than widespread species (Rickles & Bermingham 2008). In contrast, some other spider lineages with a long history in the Caribbean seem to be excellent dispersers and truly widespread, such as the garden spider (*Argiope*, LeQuier et al. in review), several tetragnathid species, and others (Agnarsson et al. unpublished data). Dispersal ability is a key parameter in biogeography, as the Intermediate Dispersal Model predicts (Agnarsson et al. 2014; Claramunt et al. 2012; Diamond et al. 1976).

The implications of our findings for conservation are profound. Instead of a common, widespread species unlikely to rank highly in conservation priorities, multiple, narrowly endemic species exist that merit independent conservation evaluation and effort. As congruent evidence from multiple lineages for small endemic areas accumulates, the basic units of conservation strategy both multiply and shrink in size.

Mechanisms and routes of colonization in the Caribbean are diverse across different organisms (Rickles & Bermingham 2008). Among relatively poor dispersers, however, evidence mounts for an important, temporary (35-30 mya) overland dispersal route, the GAARandia landbridge (Ali 2012; Hedges 1996b; Hedges 2006; Iturralde-Vincent 1998; Iturralde-Vincent & MacPhee 1999; Iturralde-Vincent 2006). “GAARandia” lineages form monophyletic Caribbean clades dating to this narrow time window, such as: several spiders and scorpions (Binford et al. 2008; Crews & Gillespie 2010; McHugh et al. 2014) (Esposito et al. in review); toads (Alonso et al. 2012), mammals (Davalos 2004) (but see (Fabre et al. 2014)); cichlid fishes (Rican et al. 2013); butterflies (Matos-Maravi et al. 2014; Pena et al. 2010), spurge plants (Euphorbiaceae) (van Ee et al. 2008), and many others (Rickles & Bermingham 2008).

Although we emphasize the preliminary nature of these biogeographical analyses for *Spintharus*, these spiders also support colonization via GAARandia (Figures 2-3). The Caribbean *Spintharus* form a clade and the Caribbean plus N. America a more inclusive clade, and the estimated timing of colonization of the Caribbean plus N. America at about 32 mya agrees with the hypothesized age of the land bridge (Figure 2). Ancestral area reconstruction also supports a single origin on a mix of mainland and islands, consistent with GAARandia (Figure 3). These results are necessarily preliminary due to lack of sampling of S. America other than a specimen from Colombia. However, available morphological evidence suggests that S. American specimens are more similar to the sampled Colombian specimen than the Caribbean + N. American clade (Levi 1963). Furthermore, the other known *Spintharus* species is restricted to S. America (although it, too, may be a species complex). With the strength of this combined evidence, further sampling of S. American taxa seems unlikely to refute the Caribbean + N. American monophyly.

The sister relationship between the Caribbean clade and specimens from the Yucatan peninsula is intriguing (Figure 1). Historically, the Yucatan peninsula was an island that subsequently joined Central America. This island, situated close to ancient Cuba, arguably was part of the
Caribbean archipelago. The sister relationship between N. America and the archipelago is, in turn, frequently observed in other taxa, such as other lineages of spiders (Binford et al. 2008).

Two main lineages occur in the Caribbean (Figure 1). The first comprises Cuban and Hispaniolan taxa. These islands are not only adjacent, but stayed connected for a time even after GAARlandia broke up and lineages on these islands are often close relatives (Iturralde-Vinent & MacPhee 1999; Iturralde-Vinent 2006). Furthermore, both islands include geological subunits that were separate islands during the formation of the Caribbean. Hispaniola is composed of two islands that eventually fused, and Cuba has four highland regions that were all islands at some point. Our findings agree with this geology; one Cuban lineage is sister to Hispaniolan taxa rather than the other Cuban clade, and their ages predate the Cuba-Hispaniola separation (The Cuban Hispaniola node dates to 17.8 mya).

The other main Caribbean clade comprises Jamaican, Puerto Rican, and Lesser Antillean species. Several patterns are noteworthy. Jamaica was colonized early on, consistent with its old age. However, the Lesser Antilles seems to have been colonized 18-19 mya, long before the age of existing islands (Figure 2). Endemic island taxa can predate the islands they currently occur on (Heads 2011). Such lineages may have occupied older, now submerged, islands or they could have gone extinct from the mainland or other older islands. The latter absences could also be false due to sparse sampling. Other taxa, like Lesser Antillean geckos dating back some 13-14 mya, also significantly predate their islands and are thought to have colonized islands now long gone (Thorpe et al. 2008).

Puerto Rico evidently was colonized relatively recently via the Lesser Antilles (6-7 mya, Figs. 1, 3). The results suggest ‘island hopping’ from Grenada and St. Lucia in the south, through Dominica and then St. Kitts and Nevis in the north. Puerto Rican taxa are usually related to other Greater Antillean groups. Spintharus are probably capable of overwater dispersal over short distances. Additional sampling in the Lesser Antilles may reveal more evidence for stepping stone-like colonization. Thus, we can predict the approximate phylogenetic placement of as yet unsampled areas such as Barbados, Martinique, Guadalupe, Anguilla, and the Virgin Islands, forming a grade in among the Lesser Antilles taxa sampled here.

Interestingly the southern Lesser Antilles islands in many taxa are S. American in origin, and not a part of Caribbean clades (e.g. Esposito et al. in review). Further sampling of Spintharus in Central America and the Lesser Antilles, with focus on the southernmost islands of Trinidad and Tobago as well as the neighboring regions of Venezuela and Columbia are priorities.

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Table 1. Summary of species delimitation. Species hypotheses (first column) represent colored and numbered clades on Figure 1. The various measures of distance and other of isolation and exclusivity metrics of these clades follow including: distance (D), the probability of population identification of a hypothetical sample based on the groups being tested (P ID(Strict) and P ID (Liberal)), Rosenberg’s reciprocal monophyly (P(AB)), the genealogical sorting index (gsi), and a single locus Bayesian implementation of the Poisson tree processes model (bPTP). Sp congru. refers to species hypothesis that are congruent with all methods, and Sp cons. is our conservative estimate of actual species richness based on agreement among all methods and >2% mtDNA sequence divergence.


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**Figure legends**

Figure 1. Results of a bayesian analysis of the three concatenated loci summarized in terms of region and clade uniqueness. Outgroups are omitted for clarity. Color coded and numbered clades represent our initial species hypotheses based on this tree and barcoding gaps. Encircled numbers on nodes refer to our ‘conservative’ estimtes of actual species richness (see Table 1). Numbers below nodes are posterior probability values. Results from maximum likelihood analyses of the same dataset are largely congruent. For details of specimens in each clade see supplementary Nexus file and Table S1. Inset map shows collecting sites for this study.

Figure 2. Results of a dated BEAST analysis. Numbers on scale and nodes are in mya. Stars indicate calibration points of the analysis. Blue bar represents the span of the GAARLandia landbridge and the arrow points to the timing of colonizaiton of the Caribbean plus N. America. The age of the Caribbean island clade is estimated between 33-19 mya.

Figure 3. Results of a preliminary RASP biogeographical analysis of ancestral areas under the Bayesian binary model. The results indicate colonization of the N. American + Caribbean clade from S America, and subsequently the Caribbean from N. America (Yucatan). The ancestral state for the Caribbean is a mizsture of islands and continent. Hispaniola was colonized from Cuba and the results indicate the colonization of Puerto Rico via the Lesser Antilles. Inset photographs are of adult females from the corresponding area on the cladogram, showing a part of the diversity of external morphology, especially coloration, in this clade.
Table 1 (on next page)

results of species delimitation analyses

Summary of species delimitation. Species hypotheses (first column) represent colored and numbered clades on Figure 1. The various measures of distance and other of isolation and exclusivity metrics of these clades follow including: distance (D), the probability of population identification of a hypothetical sample based on the groups being tested (P ID(Strict) and P ID (Liberal)), Rosenberg’s reciprocal monophyly (P(AB)), the genealogical sorting index (gsi), and a single locus Bayesian implementation of the Poisson tree processes model (bPTP). Sp congru. refers to species hypothesis that are congruent with all methods, and Sp cons. is our conservative estimate of actual species richness based on agreement among all methods and >2% mtDNA sequence divergence.
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dated phylogenetic tree

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biogeographical analysis

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