Spintharus flavidus in the Caribbean – a 30 million year biogeographical history and radiation of a 'widespread species'

Austin Dziki, Greta Binford, Jonathan A Coddington, Ingi Agnarsson

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.

1 ***Spintharus flavidus* in the Caribbean – a 30 million year biogeographical**
2 **history and radiation of a 'widespread species'**

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11 **ABSTRACT**

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

34

35 Subjects Biogeography, Taxonomy, Biodiversity

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

38

INTRODUCTION

40 Archipelagos represent unique conditions to study gene flow and diversification (Agnarsson &
41 Kuntner 2012; Gillespie & Roderick 2002; Losos & Ricklefs 2010; Ricklefs & Bermingham
42 2008; Warren et al. 2015). Islands are situated within a matrix of oceanic barriers that restrict
43 gene flow in proportion to the geographic isolation of an island and the dispersal ability of a
44 given taxon (Agnarsson et al. 2014; Claramunt et al. 2012; Diamond et al. 1976; Warren et al.
45 2015). They also provide opportunity for adaptive radiations within islands, particularly for
46 dispersal-limited taxa.
47   Among archipelagos, the Caribbean is particularly rich as an arena for biogeographic
48 analyses. The region is composed of a range of old continental fragments (Greater Antilles, ~40
49 my) and relatively recent volcanic islands (Lesser Antilles, <10 my) (Iturralde-Vinent &
50 MacPhee 1999; Iturralde-Vinent 2006), and features rich biodiversity and high levels of
51 endemism (Hedges 1996a; Hedges & Heinicke 2007; Losos & DeQueiroz 1997; Myers et al.
52 2000; Ricklefs & Bermingham 2008). The proximity of these islands to ancient continents (N.
53 and S. America), creates potential for a dynamic interchange of taxa between continents and
54 islands (Bellemain & Ricklefs 2008; Heaney 2007). The geological history of the islands has
55 created unique conditions for colonization and speciation. Over the last 40 million years, the
56 Greater Antilles landmasses have emerged, moved, sometimes amalgamated, and perhaps,
57 connected to South America via a former land bridge; the Greater Antilles and Aves Ridge Land
58 Bridge (GAARlandia) hypothesis (Ali 2012; Iturralde-Vinent & MacPhee 1999). Ricklefs &
59 Bermingham (2008) portrayed the Caribbean as "a laboratory of biogeography and evolution"
60 ideally suited to study replicate patterns of allopatric speciation and evolutionary radiations.
61   Arthropods can strongly test biogeographical patterns (Gillespie & Roderick 2002). They
62 can be abundant enough to be easily sampled without deleterious population effects, and they
63 have short generation times, compared to vertebrates and many plants. Short generation times
64 and large brood sizes allow them to evolve and diversify quickly. Like vertebrates and plants,
65 arthropods span the spectrum of dispersal abilities from extremely poor to excellent. Widespread
66 species tend to be good dispersers and for a 'naturally' widespread species to persist in the
67 Caribbean, gene flow among islands must be sufficient. Thus, for lineages with relatively poor
68 dispersal ability, widespread species are improbable taxonomic hypotheses.
69   The taxonomic hypothesis *Spintharus "flavidus"* circumscribes a widespread spider
70 species found throughout the Caribbean and from northern N. America to Brazil (Levi 1954;
71 Levi 1963). This species has been documented ballooning (aerial dispersal on silk threads (Bell
72 et al. 2005)) and might be expected to maintain gene flow across oceanic barriers. However, the
73 single ballooning record was a short distance dispersal, and other information on the distribution
74 and biology of *Spintharus* suggest long distance dispersal is rare. *Spintharus* have a somewhat
75 cryptic habitat, often found in leaf litter or the undersides of low-level leaves. The genus is old;
76 its sister lineage *Episinus* occurs in 45 myo Baltic amber, but, unlike *Episinus*, *Spintharus* is
77 restricted to the Americas. *Spintharus* includes just two species, the putatively widespread *S.
78 flavidus* and *S. gracilis* restricted to Brazil (Levi 1954; Levi 1963). A third species *S. argenteus*
79 (Dyal 1935) from Pakistan is clearly misplaced in the genus based on its original description
80 (Levi 1954), it's proper placement is unclear but it may be a tetragnathid (Agnarsson pers. obs.).
81 *Spintharus flavidus* varies greatly in color and genitalic morphology—usually species-specific in
82 spiders—thus hinting at greater species diversity. Levi (1954, 1963), however, did not see clear

83 geographical patterns to this variation. Specimens from a single locality differed so continuously
84 in color and genitalia that he believed it to be one widespread and variable species.
85   Preliminary analyses of the COI gene indicate high levels of molecular diversity within *S.*
86 *flavidus* and contest the widespread species hypothesis. Here, we use molecular phylogenetics to
87 study patterns of diversification in a 'widespread' spider species, specifically to test the
88 hypothesis that it represents single species capable of frequent dispersal across moderate
89 stretches of ocean. While our taxon sampling is not designed for detailed biogeographical
90 analyses, samples from the continental landmasses (N., C. and S. America) permit preliminary
91 evaluation of the route, number, and timing of colonization events. We also use our data to
92 propose an initial biogeographical hypothesis for *Spintharus* that can readily be tested as more
93 data accumulate, especially from South America.

94

95 **MATERIALS AND METHODS**

96 **Specimen sampling and DNA extraction and assembly**

97 The CarBio team (www.islandbiogeography.org) collected specimens from Cuba, the Dominican
98 Republic, Puerto Rico, Jamaica, the Lesser Antilles, Florida, South Carolina, Costa Rica,
99 Mexico, and Columbia between 2011 and 2015. All specimens were collected under appropriate
100 permits: **Puerto Rico,** DRNA: 2011-IC-035 (O-VS-PVS15-SJ-00474-08042011); **Jamaica**,
101 NEPA, reference number #18/27;
102 **USA**, USDI National Park Service, EVER-2013-SCI-0028; **Costa Rica**, SINAC, pasaporte
103 científico no. 05933, resolución no. 019-2013-SINAC; **Cuba**, Departamento de Recursos
104 Naturales; **Dominican Republic**, Secretaría de Estado de Medio Ambiente y Recursos
105 Naturales, **Mexico**, SEMARNAT scientific collector permit FAUT-0175 issued to Dr. Oscar
106 Federico Francke Ballve, Oficio no. SGPA/DGVS/10102/13; **Colombia**, [list to be completed].
107 We used standard protocols for aerial search, beating, sifting, and cryptic methods (Coddington
108 et al. 2009; Coddington et al. 1991). Spiders were immediately fixed in 95% ethanol and stored
109 at -20˚C (UVM Natural History Museum). Phenotype vouchers will also be deposited at the
110 USNM (Smithsonian Institution).
111   We chose 195 individuals from the field samples for molecular analyses, representing
112 each collecting locality with several specimens (targeting 4-5), when available (Figure 1). DNA
113 was extracted from 1-4 legs from each individual and isolated using a Qiagen DNeasy Tissue kit
114 using the kit protocol (Qiagen, Valencia, CA, USA). DNA from some specimens was isolated
115 from leg samples at the Smithsonian Institute (SI) in Washington, DC using an
116 Autogenprep965for an automated phenol:chloroform extraction. We initially sequenced a
117 fragment of the mitochondrial COI (cytochrome c oxidase subunit 1) to establish basic patterns
118 of phylogenetic relationships and obtain an initial estimate of diversification patterns through
119 'DNA barcoding' (Hebert et al. 2003). Upon discovering deep divergences among isolated
120 'populations' with COI, we additionally sequenced the mitochondrial ribosomal 16S rRNA, and
121 the nuclear Internal Transcribed Spacer unit 2(ITS2) for selected exemplars from each clade
122 indicated by COI. These molecular markers have proven successful in similar phylogenetic
123 studies of spiders ranging from low taxonomic levels to divergences as deep as the age of the
124 Caribbean (Agnarsson 2010; Agnarsson & Rayor 2013; Kuntner & Agnarsson 2011a; Kuntner &
125 Agnarsson 2011b). To amplify the COI, 16S, and ITS2, we used LCOI 1490 & HCOI 2198, 16S

126   A & 16S B, and ITS 4 & ITS 5.8 primers respectively (Folmer et al. 1994; White et al. 1990).
127   Standard PCR protocols were used as described in (Agnarsson 2010; Agnarsson et al. 2007). The
128   PCR products were purified using Exosap kits and purified PCR products sequenced at the
129   University of Arizona, Beckman Genomics, or the SI. All sequences will be deposited in
130   GenBank [accession numbers not yet available]. COI, 16s, and ITS2 sequences from *Anelosimus*
131   and *Episinus* species were downloaded from GenBank and used as outgroups along with a
132   *Chrysso* specimen we sequenced here.
133   The chromatographs were interpreted with Phred 45 and Phrap (Green 2009; Green &
134   Ewing 2002) using the Chromaseq module (Maddison & Maddison 2011a) in the program
135   Mesquite (Maddison & Maddison 2011b) and edited by hand. The alignments for the COI
136   sequences were trivial with no implied indels, and were done in Mesquite through ClustalW. The
137   16S and ITS2 sequences were aligned using the program MAFFT (Katoh 2013) through the
138   online server portal http://mafft.cbrc.jp/alignment/server. Other than setting the tree building
139   number and maxiterate to the maximum, we used default MAFFT settings.

140

141   *Phylogenetic and biogeographical analyses*

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

145   We used MrBayes V3.2.3 on XSDE (Ronquist & Huelsenbeck 2003) through the online portal
146   CIPRES (Miller et al. 2010), to run a Bayesian analysis for mtDNA (COI plus 16S) and nuDNA
147   (ITS2) separately and for the concatenated three loci. We used Mesquite to concatenate loci and
148   to partition the analysis by locus. The Bayesian analyses ran MC^3 (Metropolis coupled Markov
149   chain Monte Carlo) for 50,000,000 generations, sampling every 1,000 generations, and we used
150   Tracer (Drummond & Rambaut 2007), to assure proper mixing of chains and sufficient
151   sampling. ML searches were done in Garli 2.0, (Zwickl 2006) and repeated 100 times and the
152   tree maximizing likelihood of the data was preferred.
153
154   Analyses of node ages were done in BEAST 1.8 (Heled & Drummond 2010). We pruned the
155   matrix to include 2-3 exemplars with minimum missing data from each major clade, and
156   constrained the monophyly of *Spintharus* and that of Dominican *Spintharus*, because all prior
157   analyses justified their monophyly. We employed GTR + G + I model for the concatenated
158   matrix with a Yule process tree prior, and a UPGMA starting tree. Two chains of 100 million
159   generations were run and, convergence and correct mixing of the chains were monitored using
160   TRACER version 1.5. We estimated node ages using a relaxed exponential clock calibrated with
161   a Dominican amber fossil of *Spintharus* that is dated to about 15-20 mya (Wunderlich 1988).
162   This sets the minimum age of the genus, but more importantly, the colonization of Hispaniola at
163   15 mya and was implemented using exponential priors on both nodes with a mean of 5 and offset
164   of 15 my – spanning about 15-35 my. Many fossils of the closely related Spintharinae genus
165   *Episinus* s. l. occur in Dominican and Baltic amber (Wunderlich 1988; Wunderlich 2008), the
166   latter dating to approximately 44 my. Hence we constrained the root of Spintharinae (*Spintharus*
167   plus *Episinus*) with an exponential prior with an offset of 44 my and mean of 15, spanning
168   approximately 40-100 my, to the approximate origin of Theridiidae (Bond et al. 2014, Liu et al.

169 resubmitted). A recent study on Theridiidae estimates the origin of Spintharinae between 55-45
170 mya (Liu et al. resubmitted)
171
172 We inferred ancestral ranges using RASP 3.2 (Yu et al. 2015) inputting the preferred Bayesian
173 tree and a set of 100 post-burnin trees. We defined areas as each of the Caribbean islands, and S.
174 America, and N. America (including USA, Costa Rica, and Mexico). S-DIVA, S-DEC and
175 Bayesian Binary ancestral area analyses were run limiting areas to two, as all putative species
176 level clades are restricted to one area, and without dispersal constraints. We exported results as
177 tables and graphics and the latter we touched up in Adobe Illustrator.

178

179 ***Species delimitation, distribution, and photo-documentation***

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

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

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

204

205 **RESULTS**

206 ***Specimen sampling and DNA extraction and assembly***

207 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.

211

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

248 represent 11 of the 22 initial species hypotheses, and five species whose delimitation is broader
249 than the original hypotheses (Table 1, Figure 1).

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

257 ***Biogeographical patterns***

258 The dating analysis in BEAST suggests that the N. American plus Caribbean clade diverged
259 from the sister clade represented by the Colombian taxon, between 37-29 mya, a time window
260 consistent with GAARlandia (Figure 2). The Caribbean and the N. American clades diverged
261 between 27-29 mya. Divergences corresponding to Greater Antilles island clades are old (>
262 20mya), except Puerto Rico that appears to contain a recent lineage. Some Lesser Antilles
263 islands contain lineages estimated to be older than the currently hypothesized age of the islands.

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

271

272 **DISCUSSION**

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

288   of populations, and some limited evidence for long distance dispersal (colonization of the Lesser
289   Antilles and Puerto Rico across water).

290   This is not an isolated finding. The CarBio project (islandbiogeography.org) has found evidence
291   of other multiple single island endemics among hypothetical 'widespread species' of putatively
292   poor dispersers (McHugh et al. 2014, Esposito et al. 2015, Petersen et al. in prep., Nishida et al.
293   in prep., Lequier et al. in prep.). In other groups of animals and plants, dispersal limited taxa also
294   tend to form island endemics rather than widespread species (Ricklefs & Bermingham 2008). In
295   contrast, some other spider lineages with a long history in the Caribbean seem to be excellent
296   dispersers and truly widespread, such as the garden spider (*Argiope*, LeQuier et al. in review),
297   several tetragnathid species, and others (Agnarsson et al. unpublished data). Dispersal ability is a
298   key parameter in biogeography, as the Intermediate Dispersal Model predicts (Agnarsson et al.
299   2014; Claramunt et al. 2012; Diamond et al. 1976).

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

305   Mechanisms and routes of colonization in the Caribbean are diverse across different organisms
306   (Ricklefs & Bermingham 2008). Among relatively poor dispersers, however, evidence mounts
307   for an important, temporary (35-30 mya) overland dispersal route, the GAARlandia landbridge
308   (Ali 2012; Hedges 1996b; Hedges 2006; Iturralde-Vinent 1998; Iturralde-Vinent & MacPhee
309   1999; Iturralde-Vinent 2006). "GAARlandia" lineages form monophyletic Caribbean clades
310   dating to this narrow time window, such as: several spiders and scorpions (Binford et al. 2008;
311   Crews & Gillespie 2010; McHugh et al. 2014) (Esposito et al. in review); toads (Alonso et al.
312   2012), mammals (Davalos 2004) (but see (Fabre et al. 2014)); cichlid fishes (Rican et al. 2013);
313   butterflies (Matos-Maravi et al. 2014; Pena et al. 2010), spurge plants (Euphorbiaceae) (van Ee
314   et al. 2008), and many others (Ricklefs & Bermingham 2008).

315   Although we emphasize the preliminary nature of these biogeographical analyses for *Spintharus*,
316   these spiders also support colonization via GAARlandia (Figures 2-3). The Caribbean *Spintharus*
317   form a clade and the Caribbean plus N. America a more inclusive clade, and the estimated timing
318   of colonization of the Caribbean plus N. America at about 32 mya agrees with the hypothesized
319   age of the land bridge (Figure 2). Ancestral area reconstruction also supports a single origin on a
320   mix of mainland and islands, consistent with GAARlandia (Figure 3). These results are
321   necessarily preliminary due to lack of sampling of S. America other than a specimen from
322   Colombia. However, available morphological evidence suggests that S. American specimens are
323   more similar to the sampled Colombian specimen than the Caribbean + N. American clade (Levi
324   1963). Furthermore, the other known *Spintharus* species is restricted to S. America (although it,
325   too, may be a species complex). With the strength of this combined evidence, further sampling of
326   S. American taxa seems unlikely to refute the Caribbean + N. American monophyly.

327   The sister relationship between the Caribbean clade and specimens from the Yucatan peninsula
328   is intriguing (Figure 1). Historically, the Yucatan peninsula was an island that subsequently
329   joined Central America. This island, situated close to ancient Cuba, arguably was part of the

330 Caribbean archipelago. The sister relationship between N. America and the archipelago is, in
331 turn, frequently observed in other taxa, such as other lineages of spiders (Binford et al. 2008).

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

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

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

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

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

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570   at Austin.

571 ***Figure legends***

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

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

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

590

591

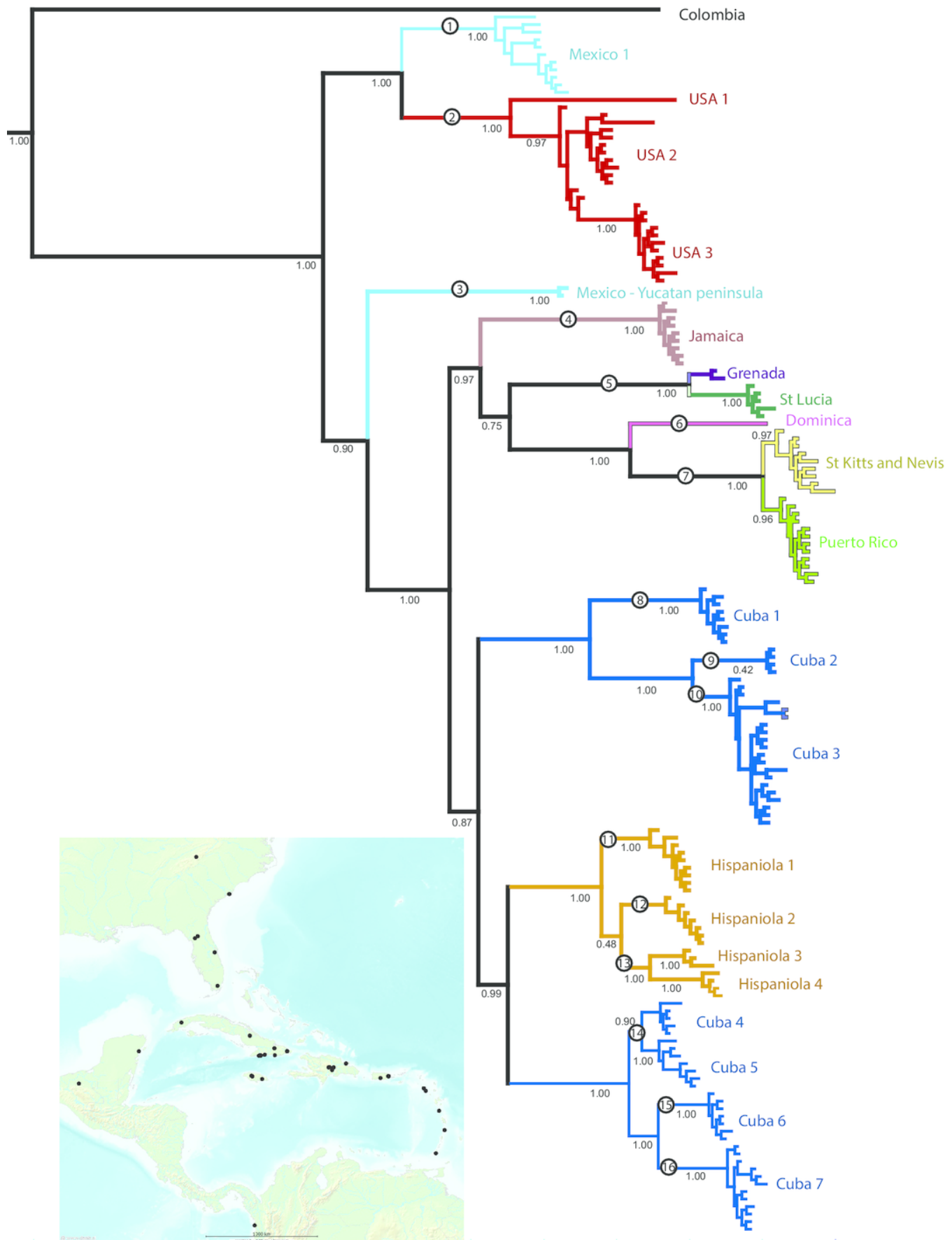
results of species delimitation analyses

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.

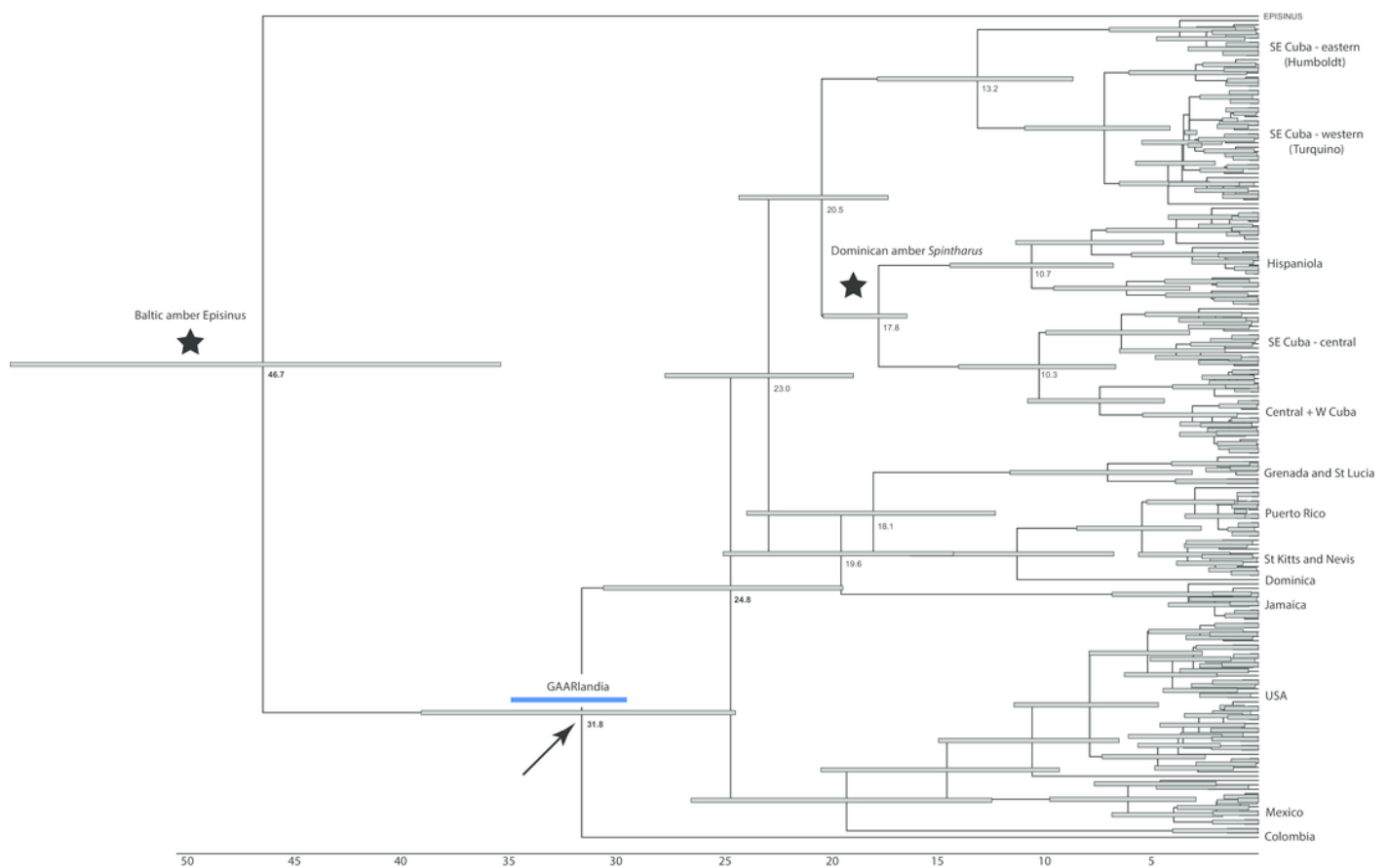
Sp Hyp.	Mono	D Intra	D Inter	Dtra/Dter	P ID(Strict)	P ID(Liberal)	P(AB)	GSI	bPTP	Sp congru.	Sp cons.
Mex 1	yes	0.008	0.041	0.19	0.66 (0.49, 0.84)	0.90 (0.75, 1.0)	3.10E-04	1	Y	1	1
USA 1	yes	n/a	0.005	n/a	n/a	0.96 (0.83, 1.0)	0.02	0.61	N	2	2
USA 3	yes	0.001	0.012	0.12	0.71 (0.54, 0.89)	0.94 (0.80, 1.0)	0.17	1	N		
USA 2	no	0.005	0.005	1.01	0.33 (0.22, 0.44)	0.68 (0.61, 0.74)	NA	0.83	N		
Mex 2	yes	0.001	0.063	0.02	0.58 (0.43, 0.73)	0.97 (0.82, 1.0)	1.60E-05	1	Y	3	3
Jam	yes	0.003	0.059	0.05	0.76 (0.58, 0.93)	0.98 (0.84, 1.0)	4.20E-04	1	Y	4	4
Grenada	yes	0.002	0.014	0.15	0.52 (0.36, 0.67)	0.89 (0.74, 1.0)	0.05	1	Y	5	5
St Lucia	yes	0.003	0.014	0.2	0.66 (0.48, 0.84)	0.90 (0.75, 1.0)	0.05	1	Y	6	
St Kitts	yes	0.003	0.01	0.32	0.58 (0.40, 0.75)	0.82 (0.68, 0.97)	0.02	0.83	Y	7	6
PR	yes	0.002	0.01	0.21	0.65 (0.47, 0.83)	0.89 (0.74, 1.0)	0.02	1	Y		
Dom	yes	n/a	0.006	n/a	n/a	0.96 (0.83, 1.0)	0.1	1	Y	8	7
CU1	yes	0.002	0.043	0.04	0.77 (0.59, 0.94)	0.99 (0.84, 1.0)	5.80E-04	1	Y	9	8
CU2	yes	0.001	0.021	0.07	0.75 (0.57, 0.92)	0.97 (0.83, 1.0)	1.85E-03	1	Y	10	9
CU3	yes	0.006	0.021	0.29	0.81 (0.70, 0.91)	0.93 (0.86, 0.99)	1.85E-03	1	Y	11	10
DR1	yes	0.004	0.022	0.18	0.67 (0.49, 0.85)	0.90 (0.76, 1.0)	1.80E-04	1	Y	12	11
DR2	yes	0.003	0.02	0.14	0.88 (0.77, 0.99)	0.96 (0.90, 1.0)	4.40E-05	1	Y	13	12
DR3	yes	0.003	0.016	0.2	0.65 (0.48, 0.83)	0.89 (0.75, 1.0)	0.01	1	Y	14	13
DR4	yes	0.003	0.016	0.18	0.75 (0.60, 0.89)	0.94 (0.83, 1.0)	0.01	0.83	Y	15	
CU4	yes	0.003	0.011	0.3	0.73 (0.61, 0.86)	0.93 (0.83, 1.0)	1.06E-03	1	Y	16	14
CU5	yes	0.004	0.011	0.38	0.61 (0.47, 0.75)	0.87 (0.76, 0.98)	1.06E-03	0.83	Y		
CU6	yes	0.002	0.019	0.09	0.73 (0.56, 0.91)	0.96 (0.81, 1.0)	0.01	0.92	Y	17	15
CU7	yes	0.004	0.021	0.2	0.74 (0.59, 0.88)	0.93 (0.83, 1.0)	0.01	1	Y	18	16

1

phylogenetic tree

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.

2

dated phylogenetic tree

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.

3

biogeographical analysis

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.

Result of combined:

A - S America
B - N America
C - Cuba
D - Hispaniola (DR)
E - Puerto Rico
F - St Lucia
G - Grenada
H - Dominica
I - St Kitts and Nevis
J - Jamaica

Spintharus Columbia 00004378A
Spintharus MX 00006462A
Spintharus MX 00006359A
Spintharus flavidus ARNEDO
Spintharus SEUS 00001262A
Spintharus SEUS 00001078A
Spintharus SEUS 00003938A
Spintharus SEUS 00003462A
Spintharus SEUS 00003819A
Spintharus MX 00006349A
Spintharus MX 00006443A
Spintharus Jamaica 00003602A
Spintharus JA 00003068A
Spintharus LA 00001773A
Spintharus LA 00001606A
Spintharus LA 00001712A
Spintharus LA 00001800A
Spintharus LA 00002152A
Spintharus LA 00000648A
Spintharus Lesser Antilles 00001536A
Spintharus PR 00782101
Spintharus PR 00392921
Spintharus CU 00787852
Spintharus CU 00785195
Spintharus CU 00005529A
Spintharus CU 00005478A
Spintharus CU 00004590A
Spintharus CU 00004082A
Spintharus CU 00004623A
Spintharus CU 00004621A
Spintharus DR 00785670
Spintharus DR 00784899
Spintharus DR 00786970
Spintharus DR 00786971
Spintharus DR 00787274
Spintharus DR 00787316
Spintharus DR 00787310
Spintharus DR 00787259
Spintharus CU 00784459
Spintharus CU 00787473
Spintharus CU 00004619A
Spintharus CU 00004718A
Spintharus CU 00783213
Spintharus CU 00784389
Spintharus CU 00783202