Huntsmen of the Caribbean: Multiple tests of the GAARlandia hypothesis

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ABSTRACT

The origin of the Caribbean biota remains debated, but amassing evidence suggests important roles of both dispersal and vicariance events in the colonization the archipelago. The most prominent vicariance hypothesis is colonization over the GAARlandia land bridge that putatively connected the Greater Antilles to South America around 33 mya. This hypothesis has received support from studies of individual lineages, but its main prediction—the simultaneous colonization of multiple lineages during that time window—requires further unambiguous corroboration. Here, we examine the phylogenetic structure of huntsman spiders (Sparassidae) of the Caribbean. Huntsman spiders are appropriate models for this question, as they are expected to be dispersal limited as substrate and foliage dwelling spiders that rarely balloon, yet are found on some volcanic islands, and thus at least some overwater dispersal must have occurred. We focus on the Caribbean endemic *Neostasina*, but also include Caribbean *Olios*, for a deeper biogeographical understanding. We use two mitochondrial and four nuclear markers to reconstruct dated phylogenetic trees and to test taxonomic and biogeographic hypotheses. Our analyses strongly support the monophyly of *Neostasina* and the polyphyly of *Olios*, with a new clade endemic to the Caribbean. Both *Neostasina* and Caribbean *Olios* occur on the Greater and Lesser Antilles and independently colonized the Caribbean around 36–28 mya. Hypothesis testing in BioGeoBEARS suggests a role of the GAARlandia landbridge in the colonization of both clades. The 'Olios-like' clade, in contrast, is restricted to the southern Lesser Antilles and shows a biogeographic history consistent with colonization from S. America, probably within the last 10 my. Thus, many spider lineages on the Greater Antilles seem to have colonized the Caribbean during a relatively short time span approximately coinciding with the proposed timing of GAARlandia. The synchronous colonization of multiple lineages suggests a temporary land connection. However, the main problem in concluding synchronous events across lineages in this study, as in most others, is the ambiguity in chronogram analyses meaning that many different patterns can be 'consistent' with GAARlandia, thus potentially providing a false positive result. Broad comparative biogeographical studies such as the CarBio project will offer the best opportunity to multiply test shared biogeographic patterns among independent lineages. The current paper contributes evidence from multiple lineages that will contribute to this synthesis.

1. Introduction

The Caribbean region is a Conservation International hotspot of biological diversity, with large numbers of endemic birds, reptiles, plants, fish and arthropods (Dziki et al., 2015; Myers et al., 2000; Ricklefs and Bermingham, 2008; Weaver et al., 2016). This region includes a large number of islands and islets, forming an archipelago over 4000 km long. Geological evidence suggests that, post the bolide impact around 65 mya that for example drove dinosaurs to extinction and may have wiped out all terrestrial Caribbean life, the Greater Antilles landmasses have emerged, submerged, moved, sometimes amalgamated, and only relatively recently taken their current shape and
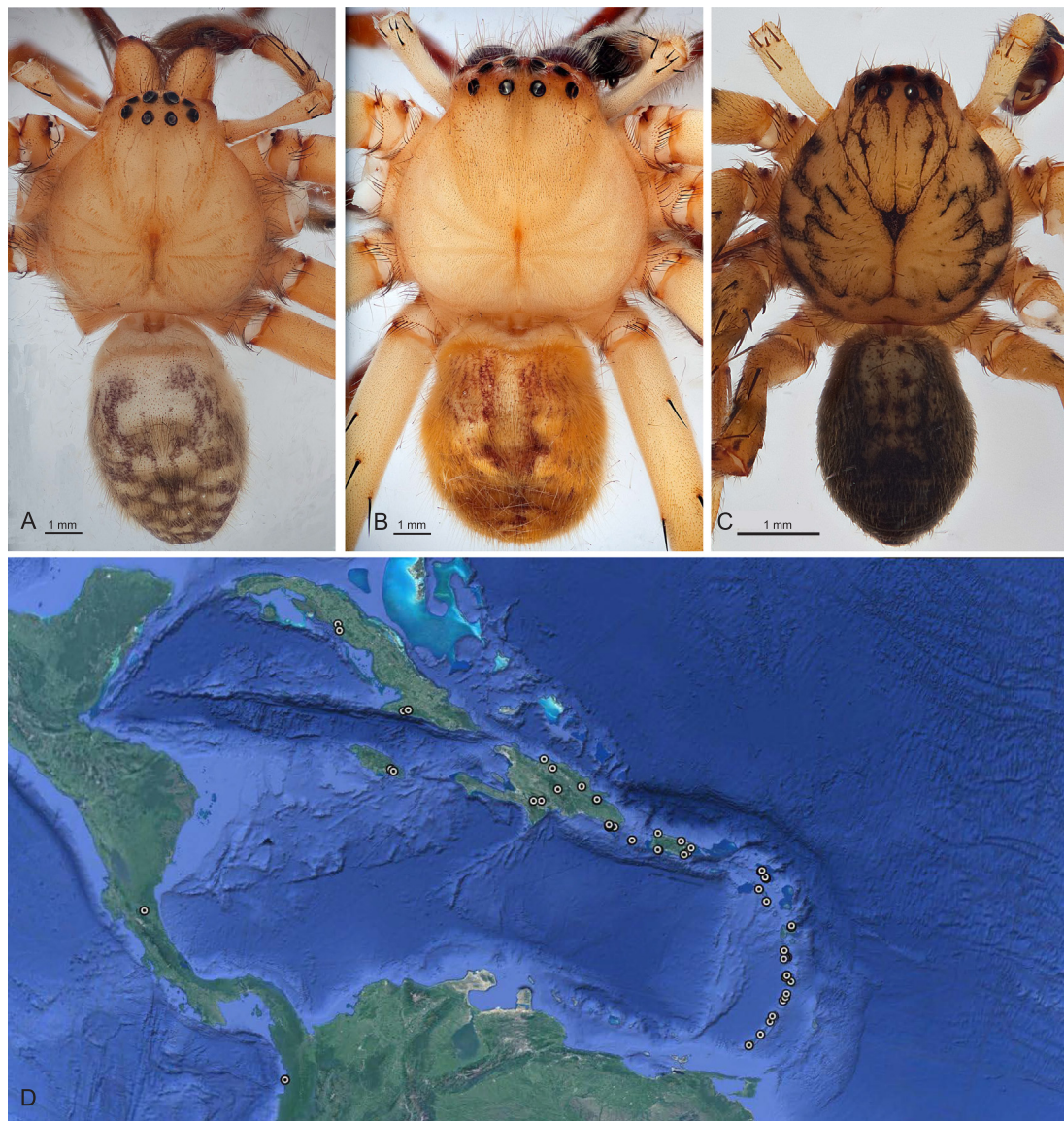
Fig. 1. The three main lineages of Sparassidae from the Caribbean (A–C) and a map of localities where Sparassidae were freshly sampled in this project (D). Further data from South America and Old World representatives came from Genbank. A, *Olios* sp 4; B, "*Olios*" *antiguensis*; C, *Neostasina* sp.

position (Iturralde-Vinent, 1998; Iturralde-Vinent and MacPhee, 1999; Iturralde-Vinent, 2006). Importantly, no island has been continuously above water since the dramatic impact. Yet, some islands, notably the Greater Antilles, are relatively old *as islands*, and their current biota is thought to have around 40 million years (my) biogeographical history, during which the islands have been continuously emerged (Iturralde-Vinent, 1998; Iturralde-Vinent and MacPhee, 1999; Iturralde-Vinent, 2006). Others, including the Lesser Antilles, are much more recent (< 10 my) volcanic islands and contain biota that has reached them via overwater dispersal in the last few million years. Geological data furthermore have been used to propose that the Greater Antilles and Aves Ridge formed a *more or less* continuous land bridge with South America around 30–33 mya; the GAARlandia hypothesis (Iturralde-Vinent, 1998; Iturralde-Vinent and MacPhee, 1999; Iturralde-Vinent, 2006).

The GAARlandia hypothesis remains contested (Ali, 2012), but its major strength lies in providing detailed and testable geological and biological predictions that have inspired much research. Its clear geological predictions largely remain to be tested, a task that would take extensive geological drilling across the region. Biologically, it predicts simultaneous overland colonization of the Greater Antilles by multiple

lineages, notably among lineages that may not have the ability to cross oceanic barriers. This key prediction of the GAARlandia hypothesis underlies many recent studies of Caribbean biogeography (Agnarsson et al., 2016, 2018; Antonelli et al., 2015; Cosgrove et al., 2016; Crews and Gillespie, 2010; Davalos, 2004; de Weerd et al., 2016; Dziki et al., 2015; Esposito et al., 2015; Fabre et al., 2014; Kay, 2015; Masonick et al., 2017; Matos-Maravi et al., 2014; McHugh et al., 2014; Nieto-Blazquez et al., 2017; Rican et al., 2013; Rodriguez et al., 2015; Sato et al., 2016; Shpeley et al., 2017; Tagliacollo et al., 2017; Weaver et al., 2016). Direct support has been garnered from some studies, ambiguous support from others, and for many lineages this proposed land bridge has clearly not played any role (e.g. Agnarsson et al., 2016; Yusseff-Vanegas and Agnarsson, 2017).

The challenges in testing the GAARlandia hypotheses are many, most notably in accurate dating of trees using molecular data. Virtually all studies to date rely on chronograms obtained with a handful of genetic markers and a limited fossil record that at best yield ambiguous dating with wide error margins. Furthermore, comparison among different lineages that have different histories, fossil record, rates of molecular evolution, is challenging. In addition, different studies face

different challenges in terms of sampling (in part organism dependent), data and methodology of choice, and even practical aspects such as available funding. Hence, it can be difficult to compare the results of independent single-lineage studies. A potentially strong test of the hypothesis could come from a single lineage that has colonized the islands multiple times, and is sampled across the archipelago with a comparable effort. In such a case, the fossil record is shared, molecular rates of evolution may be relatively homogenous within the lineage, and data and methods are comparable as they derive from a single analysis. Huntsman spiders of the Caribbean offer such an opportunity, which is the impetus for the current study.

The huntsman spiders (Sparassidae) are a diverse family composed of 88 genera and 1224 species, distributed worldwide, with over 28 genera and 230 species from the Americas (WSC, 2018). The taxonomy of huntsman spiders has advanced relatively rapidly in recent years (Agnarsson and Rayor, 2013; Jager and Krehenwinkel, 2015; Jager et al., 2015; Moradmand et al., 2014; Pinto and Rheims, 2016; Rheims, 2015; Rheims and Alayon, 2016; Zhang et al., 2017), yet only a few molecular phylogenies have been published for the family, or any group within (Agnarsson and Rayor, 2013; Cao et al., 2016; Moradmand et al., 2014). Our knowledge of huntsman spiders of the Caribbean is limited (for detail see 'taxonomic summary' under Methods) but currently known are eight genera and 38 species, a small subset of the continental fauna. That the Caribbean contains a small subset of American genera is not surprising, but highlights the importance of oceanic barriers (or filters) to dispersal.

Being medium to large sized, substrate and foliage dwelling and web less spiders, sparassids appear not well equipped for overwater dispersal and have not been directly recorded ballooning (Bell et al., 2005). Hence, the subset of sparassids that are found in the Caribbean may be, at least in part, expected to represent lineages that were able to colonize during a relatively brief period of (more or less) continuous land connection between the American continent and the major Caribbean island—GAARlandia. Of Caribbean sparassids, three are currently considered widespread, that is, found on more than one island, while the remaining 24 sparassids are thought to be single island endemics. These include eight species from Cuba, seven from Jamaica, three from Virgin Island, three from Hispaniola and one each form Antigua and Puerto Rico (WSC, 2018). For example, *Olios sanctivincenti* (Simon, 1898) and *Pseudosparianthis ravida* Simon, 1898, are only known from St. Vincent; *Decaphora cubana* (Banks, 1909) and *Stasina rangelensis* Franganillo, 1935 only from Cuba; *P. jayuyae* Petrunkevitch only from Puerto Rico; while *Olios antiguensis* (Keyserling, 1880) is more widespread in the Caribbean. The genus *Neostasina* (Rheims and Alayon, 2016) (Fig. 1), with 27 described species, is the only currently known genus endemic to the Caribbean region. It is found across the major islands and neighboring continental landmasses (Rheims and Alayon, 2016).

The goal of our study is to provide the first time-calibrated phylogenetic estimate of huntsman spiders of the Caribbean in order to (1) test the monophyly of *Neostasina*, (2) evaluate monophyly of other Caribbean sparassid lineages, (3) critically examine classifications of the huntsman spiders in Caribbean, and (4) to discuss the broad biogeographic patterns of Caribbean huntsman spiders through a comparative test of the GAARlandia hypothesis.

2. Materials and methods

2.1. Taxonomic summary of Caribbean Sparassidae

Sparassidae occurs worldwide with representatives in all zoogeographical regions except Antarctica. The Neotropical fauna is represented by 237 species distributed in 29 genera. Of these, 24 are exclusively Neotropical and five include species with Neotropical distribution but are probably misplaced in these genera [*Olios* Walckenaer (68 species), *Heteropoda* Latreille (4 species), *Eusparassus* Simon (1

species), *Nonianus* Simon (2 species) and *Stasina* Simon (4 species)]. Thirteen genera are restricted to South America and five occur in both South and Central Americas. Only *Curicaberis* Rheims, *Decaphora* Franganillo, *Macrinus* Simon and *Prusias* Simon include species distributed in Mexico and southern USA (WSC, 2018). The Caribbean sparassids amount to 38 species, distributed in eight genera. Of these, five belong to the subfamily Sparianthinae. *Neostasina* Rheims & Alayón, with 27 species, and the monotypic *Diminutella* Rheims & Alayon, are endemic to the region (WSC, 2018). *Decaphora* Franganillo, includes one Caribbean species, *D. cubana* (Banks), from Cuba (Rheims and Alayón, 2014), *Stasina* Simon includes *S. rangelensis* from Cuba and *Pseudosparianthis* Simon, includes *P. jayuyae* Petrunkevitch, from Puerto Rico and *P. ravida* Simon, from St. Vincent (WSC, 2018), the latter also widely spread through northern South America (CAR, unpublished data). Sparassinae is represented in the Caribbean by two genera. *Macrinus* Simon includes, *M. calypso* Rheims from Tobago and *Olios* Walckenaer includes *O. antiguensis* (Keyserling), from Antigua. *O. fuhrmanni* Strand from St. Thomas, *Olios sanctivicenti* (Simon) from St. Vincent and *O. trinitatis* Strand from Trinidad. *Olios santivicenti* and *O. trinitatis* belong to a group of species that will probably be transferred to the genus *Sadala* Simon (currently a junior synonym of *Olios*). This genus is diverse including several species that are currently erroneously allocated in other genera and a large number of new species. Mostly they are distributed throughout the southern Caribbean islands, northern and central South America. *Sparianthina parang* Rheims is the sole representative of the so called Herepodinae *sensu latu* (Moradmand et al., 2014) and occurs in Tobago.

2.2. Specimen and taxon sampling

The CarBio team (www.islandbiogeography.org) collected specimens from Cuba, the Dominican Republic, Puerto Rico, Jamaica, the Lesser Antilles, Costa Rica, USA, and Columbia between 2011 and 2016 (Fig. 1). 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, PE 2012/05, 2012003 and 2012001; **Dominican Republic**, Ministerio de Medio Ambiente y Recursos Naturales, no 0577, **Colombia**, Authoridad Nacional de Licencias Ambientales, 18.497.666 issued to Alexander Gómez Mejía; **Martinique**, Ministère de L'Écologie, du Développement Durable, et de L'Énergie; **Nevis**, Nevis Historical & Conservation Society, no F001; **Barbados**, Ministry of Environment and Drainage, no 8434/56/1 Vol. II.

Comparable standard spider sampling methods (Coddington et al., 2009, 1991) were used on all islands. Spiders were fixed in 95% ethanol and stored at −20 °C at the UVM Natural History Museum. DNA barcoding vouchers will be deposited at the USNM (Smithsonian Institution). This paper reports on the huntsman spiders collected during CarBio island trips, resulting in 132 individuals from 59 localities, including 64 adult specimens and 68 juveniles representing four genera and over 30 putative species (Table S2, Fig. 2). The paper is the first to produce DNA sequences of multiple species of the recently described genus *Neostasina*. To identify species we used Rheims and Alayón (2016). Non of the sampled sparassids from mainland USA were found to belong to *Neostasina* or related groups and were thus not included.

Additionally, we used data from GenBank (mostly Old World and S. American species)—maximizing representation from the Caribbean and neighboring landmasses as available on the database—for six species of *Heteropoda*, eight species of *Eusparassus* and eleven species of *Olios*, and also added non sparassid outgroups to root the trees. These represent all species with available data for *Eusparassus* and *Olios* that are key ingroups in this study, and the *Heteropoda* species with maximum data on Genbank to improve robustness of phylogenetic relationship among non-Caribbean taxa. Outgroups choice for sparassids is not obvious as
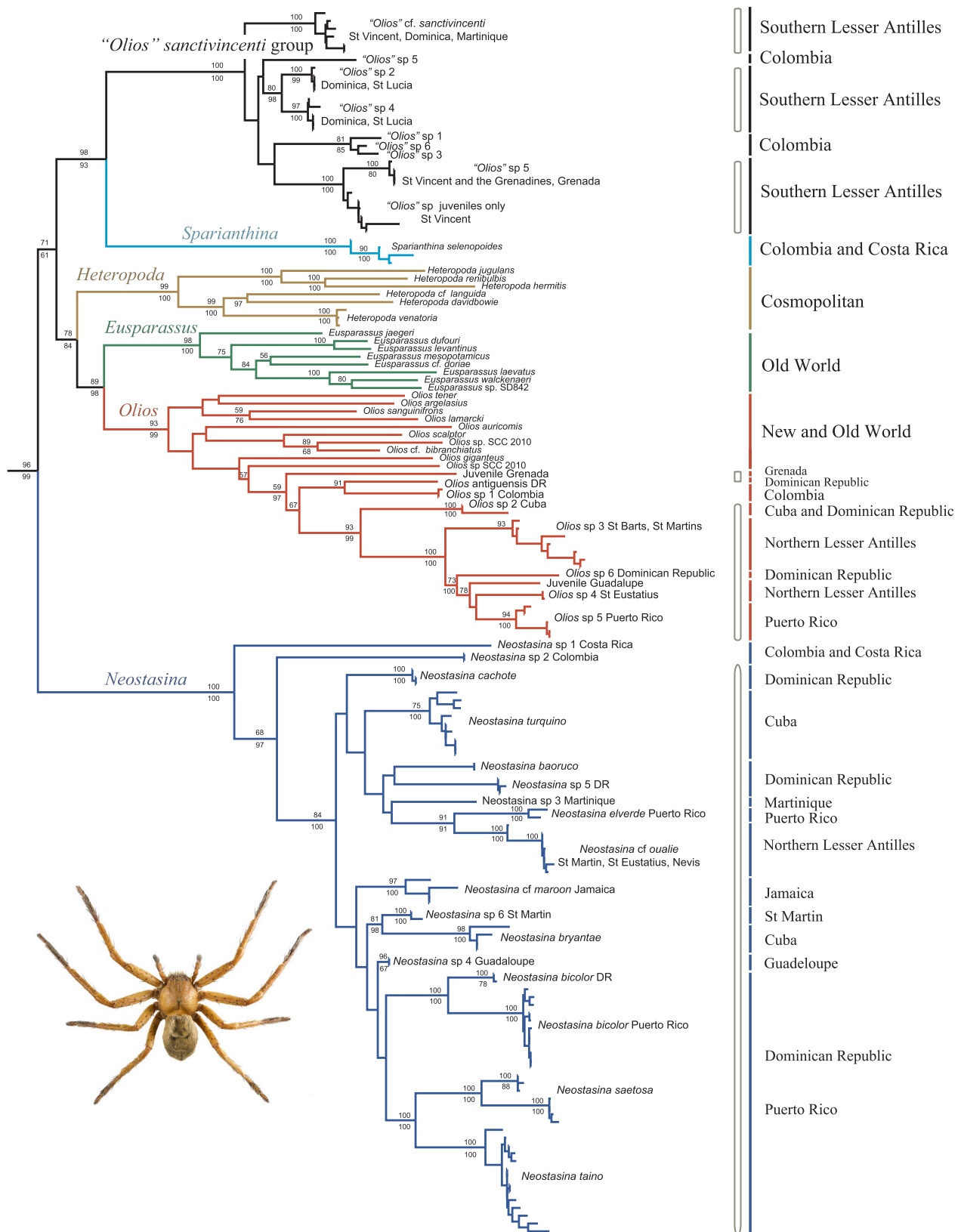
Fig. 2. Results of a Maximum likelihood analysis of all data concatenated. Outgroups are omitted for clarity. Clades are colored by genus. Numbers below nodes are Bayesian posterior probability values, numbers above are ML bootstrap support. Results from a Bayesian analyses are largely congruent (see Fig. S2). Inset image is of *Olios argelasius*, sourced from Wikipedia Commons.

they appear to be a monophyletic lineage sister to all other RTA spiders (Moradmand et al., 2014), a huge and highly morphologically diverse lineage. We represent the RTA clade with four species: Hisponinae

(Salticidae), *Paraphidippus* (Salticidae), *Philodromus* (Philodromidae), and *Thanatus* (Philodromidae). For primary outgroups we chose taxa outside, but not distantly related to, the RTA clade: *Oecobius*

(Oecobiidae), and two species of *Hersilia* (Hersiliidae). For accession numbers, see Table S2.

2.3. DNA extraction and assembly

We extracted DNA from two to four legs of each specimen using Universal Gen DNA Kit (CWBIO, Beijing, China). To amplify the COI, 16S, 18S, 28S, H3 and ITS2, we used LCOI-1490 & HCOI-2198, 16S A & 16S B, 18S a & 9r, 28S C & 28S O, H3Af & H3aR, and ITS 4 & ITS 5.8 primers respectively (Folmer et al., 1994; Simon et al., 1994; White et al., 1990). Standard PCR protocols were used as described elsewhere (Agnarsson, 2010; Agnarsson et al., 2007). Amplified fragments were sequenced in both directions by the Tsingke Biological Technology (Wuhan, China) and then assembled and proofread using the Chromaseq module in Mesquite (Maddison and Maddison, 2011a, 2011b) employing Phred and Phrap (Green, 1999; Green and Ewing, 2002).

2.4. Alignment and phylogenetic analyses

We aligned all sequences using MAFFT (Katoh, 2013) through the EMBL-EBI online portal with 100 tree rebuilding replications and 100 max iterations for a thorough search otherwise using default settings.

Protein coding gene sequences were translated to amino acids and confirmed to contain no stop codons. All original sequences were verified to belong to Sparassidae using BLASTn in GenBank. The six gene alignments were concatenated in Mesquite. We conducted Bayesian and maximum-likelihood analyses to estimate phylogenetic relationships among species. In all analyses, gaps and ambiguous bases were treated as missing data. We created eight data partitions for sensitivity analyses to explore potential data conflict: 16S, 18S, 28S, H3, ITS2, COI1st, COI2nd and COI3rd. The appropriate models for the Bayesian analysis were selected with jModelTest2 on XSEDE (2.1.6) (Darriba et al., 2012) using the Akaike information criterion (AIC) (Posada and Buckley, 2004). These were: GTR + I + G for 16S, 18S, 28S, ITS, COI1st and COI2nd, GTR + G for COI3rd and SYM + I + G for H3. Data matrices were analyzed using Bayesian inference with MrBayes 3.2.6 (Ronquist et al., 2012) on XSEDE. The Markov chain Monte Carlo search for each matrix ran with four chains for 50,000,000 generations sampling the Markov chain every 1000 generations, and the sample points of the first 12,500,000 generations were discarded as 'burnin', after which the chains had reached stationarity as determined by analysis in Tracer (Rambaut and Drummond, 2007). Maximum likelihood analyses were done with RAxML HPC2 (Stamatakis, 2006) on XSEDE on the focal matrix with same partitions as implemented in the Bayesian analysis, but using a GTR + I + G model for all partitions, keeping other parameters as default. All large analyses were run in parallel on the CIPRES cluster at the San Diego Supercomputing Center (Miller et al., 2010).

2.5. Estimation of divergence times, fossil calibration, and biogeographical analyses

Analyses of divergence times were done in BEAST 1.8 (Drummond and Rambaut, 2007; Drummond et al., 2012). We constrained the monophyly of *Eusparassus and Neostasina*, based on prior analyses in MrBayes. We employed GTR + G + I model for the concatenated matrix with a Yule process tree prior, and a UPGMA starting tree. We also ran separate analysis using partitioned by gene, a dating analysis of COI alone calibrated by estimated substitution rates for that gene, and a dating analysis combined with COI substitution rates and fossil calibration. Two chains of 400 million generations were run for each analysis and convergence and appropriate mixing of the chains were monitored using Tracer 1.5. Priors were set to default other than detailed below. We estimated node ages using a relaxed clock based on spider-specific substitution rate estimates for mitochondrial genes across several spider groups (Bidegaray-Batista and Arnedo, 2011; Kuntner et al., 2013). Following these studies, the mitochondrial

substitution rate parameter (ucld.mean) was assigned a normal prior with mean = 0.0112 and SD = 0.001. For the nuclear genes substitution mean starting rates were set at an order of magnitude slower than the reported mitochondrial rate (see Kuntner et al., 2013) and assigned uniform flat priors. We also calibrated three nodes of the tree with fossils following the logic and approach of (Moradmand et al., 2014)). (1) Oecobiidae–Hersiliidae node: Penney et al. (2012) state comparable divergence times between Eresidae, Oecobiidae and Hersiliidae. The common ancestor is assumed to be at least as old as the oldest fossil within this group; a fossil Oecobiidae from Lebanese amber, estimated at 125–135 MA (Wunderlich, 2008; Dunlop et al., 2017; Selden and Penney, 2010). This fossil was implemented with parameters as follows: mean 2.3, stdev 1, offset 123 = 5%: 124.9 MA, median: 133 MA, 95%: 174 MA, following Wood et al. (2013). (2) Salticidae–Philodromidae node: Dunlop et al. (2017) provide convincing salticid fossil record suggesting that salticids did not occur prior to Eocene Baltic amber. This record was implemented with the following parameters: mean 2.3, stdev 1, offset 43 = 5%: 44.9 MA, median: 53 MA, 95%: 94.7 MA). (3) *Eusparassus* node was set to a minimum age based on the amber fossil *E. crassipes* (Dunlop et al., 2017) and the maximum age based on the biogeographic evidence, assuming post Gondwanan origin of Eusparassus (as they occur only in the Old World) with the following parameters: mean 1.7, stdev 1.51, offset 43.5 = 5%: 43.96 MA, median: 48.97 MA, 95%: 109.1 MA).

We used BioGeoBEARS in R (Matzke, 2013) to estimate ancestral ranges. Maximum range was constrained to two areas, given the predominant pattern of single island endemism. We defined eight geographic regions as Old World (OW), North America (NM), South America (SA), Cuba (CU), Hispaniola (HI), Jamaica (JA), Puerto Rico (PR), northern Lesser Antilles (north of Guadalupe, NL), and southern Lesser Antilles (SL). For additional tests of the GAARlandia hypothesis we also ran analyses with the Greater Antilles defined as a single region, representing the hypothetical GAARlandia landbridge.

To test the GAARlandia model, we applied probabilities to geology-based time slices (Iturralde-Vinent and MacPhee, 1999; Iturralde-Vinent, 2006), following Chamberland et al. (2018) and Weaver et al. (2016). GAARlandia was modeled as probable Greater Antilles land connection to South America, as well as inter-island connections from 33 to 30 Ma. Other than this connection the two alternative models shared the opening of the Mona Passage (23 Ma) and the Windward Passage (15 Ma) (Iturralde-Vinent, 2006). We applied the following models: Dispersal-Extinction-Cladogenesis DEC and DEC + J, the latter accounting for founder event speciation. The degree to which DEC + J accurately models founder event speciation, and how it can be compared with DEC is debated (Ree and Sanmartin, 2018). Our emphasis here was to test the GAARlandia hypothesis under both models. The likelihood scores for the GAARlandia analysis were compared to test for significance (a natural log of 2 was considered significant (Ree & Smith, 2008). Akaike information criterion (AIC) and relative likelihood were used to asses the model probabilities given the data (Table S1).

3. Results

3.1. Sequence characteristics

A total of 528 sequences were successfully generated from two mtDNA and four nDNA fragments for 132 sparassid specimens. We obtained 83, 74, 76, 92, 106, and 94 sequences for *COI*, 16S, 28S, 18S, ITS2, and *H3* genes from all samples, respectively. The aligned sequences amounted to 1240 base pairs (bp) for *COI*, 512 bp for *16S*, 854 bp for 28S rRNA, 759 bp for 18S rRNA, 454 bp for ITS2, and 370 bp for *H3*. Transitions and transversions within the two mtDNA and four nDNA partitions accumulated in a linear way and gave no indication of saturation. All sequences are deposited in GenBank (Table S1).

3.2. Phylogeny

The Bayesian inference and maximum likelihood results were largely congruent (Fig. 2. S1), with the common pattern that bootstrap likelihood supports were distinctly lower than Bayesian posterior probabilities (Fig. S1). The monophyly of Sparassidae was robustly supported. Except for the genus *Olios*, expected a priori to be non-monophyletic, other Sparassidae genera are well supported. The Sparassidae analyzed here can be divided into two major clades: I, the genus *Neostasina*, and II, the remaining sparassids. Clade II has five subclades, *Sparianthina*, *O.* cf. *sanctivincenti* group, *Heteropoda*, the "true"*Olios* group and *Eusparassus*. *Sparianthina* clustered *O.* cf. *sanctivincenti* group; *Heteropoda* and the "true" *Olios* group clustered *Eusparassus*.

The genus *Olios* is confirmed polyphyletic (Rheims, 2010; Moradmand et al., 2014). One of the '*Olios*' clades, is the *O.* cf. *sanctivincenti* group, including *O. sanctivincenti* and several undescribed species, distributed in southern Lesser Antilles and adjacent South America (here including samples from Colombia). This group appears to represent a new undescribed genus (or possibly a resurrected *Sadala*, C. Rheims, pers. obs). The other clade here analyzed contains the "true" *Olios* group, including the old world species, and some undescribed species distributed in northern Lesser Antilles and Colombia.

The monophyly of *Neostasina* is well supported as a genus of subfamily Sparianthinae, as expected from recently proposed morphological diagnosis (Rheims and Alayon, 2016). Our phylogeny also supports Sparianthinae as sister to the remaining Sparassidae.

3.3. Divergence times

Analyses in BEAST indicate that lineages present on the Greater Antilles, and lineages only found on the Lesser Antilles, differ in their time of arrival to the islands (Fig. 3). The Greater Antilles lineages, *Neostasina* and the "true" *Olios* are estimated to be around 30 mya, and thus colonized the Caribbean within a timeframe consistent with the GAARlandia hypothesis (Figs. 3 and 4). The two other lineages (within the *O. sanctivincenti* group) are estimated to be younger, though their age is not easily delimited due to limited mainland sampling of this group. The earliest splits within each lineage in the Caribbean occur around 7–8 mya, consistent with the age of the Lesser Antilles, and thus these lineages likely arrived via overwater dispersal to the volcanic Lesser Antilles. The earliest speciation event (split) within Caribbean *Neostasina* is dated to about 28 mya, while the split between mainland and Caribbean species dates to about 36 mya. Colonization of the Caribbean likely took place sometime during that timespan surrounding the timing of GAARlandia (33–30 mya). *Olios* is more ambiguous as the Caribbean species are not monophyletic and three species, one each from Colombia, Grenada, and Dominican Republic differ in placement among analyses. The tree resulting from the Bayesian analysis of all data in MrBayes supports the monophyly of the Caribbean species, excluding one specimen from the Dominican Republic. This clade dates to about 41–30 mya, depending on these ambiguous relationships.

3.4. Biogeographical patterns

Analyses in BioGeoBEARS suggest three to four colonization events of the Caribbean by huntsman spiders (Figs. 4, S2). Sparassids clearly colonized the archipelago twice (*Neostasina* and *Olios*) during the early history of the modern Caribbean islands. Colombian or Colombian plus Central American species are sister to each group, suggesting colonization from South and/or Central America. Within the two groups occurring on the Greater Antilles, colonization events occur near or at the proposed timespan of GAARlandia. In contrast, the southern Lesser Antilles appear to have been colonized more recently from South America by the *O.* cf. *sanctivincenti* group, probably twice. Hypothesizing two colonization events within the *O.* cf. *sanctivincenti* group, rather than a single event with a 'reverse colonization' back to the mainland, appears more consistent with the age of islands and more likely colonization from continent to islands rather than vice versa.

Within our focal clade here, *Neostasina*, colonization via the Greater Antilles is consistent with the data, with basal splits occurring within Hispaniola and possibly Puerto Rico. None of the Major Antilles support monophyletic lineages, indicating movement among islands subsequent to the colonization of the Caribbean, or a widespread distribution on GAARlandia.

Hypothesis testing in BioGeobEARS indicated a significantly better fit of hypotheses (either under DEC or DEC + J, and under GAARlandia vs 9 area coding) that included the GAARLandia model (Table S1).

4. Discussion

Our results indicate that within the huntsman spider family (Sparassidae), multiple lineages have colonized the Caribbean independently, and at different times (Figs. 2–4). These lineages can be divided in two groups: those that are more widespread and occur on the Greater Antilles, and those that are limited to the southern part of the Lesser Antilles. Our focal lineage for which we have the most complete sampling, the Caribbean area endemic *Neostasina*, shows patterns of relationship, timing, and biogeographical reconstructions most consistent with a single colonization of the Caribbean islands during, or very close to, the timespan of GAARlandia (Figs. 2–4). Though this timing is consistent, this genus is found across both the Greater and Lesser Antilles. Thus, transoceanic dispersal events necessarily explain at least its occurrence on the volcanic Lesser Antilles. We cannot therefore rule out that an ancient dispersal event also explains the original Caribbean colonization.

This reinforces the dilemma of what mode of colonization is more likely, vicariance or dispersal? Alignment of multiple colonization events in time with a geological corridor could provide rationale to prefer one explanation over the other. Thus, it is noteworthy that another sparassid lineage present on the Greater Antilles, *Olios*, also colonized the Caribbean at a similar time. Two such independent events within a single lineage that coincide with a hypothetical landbridge might tip the balance towards a vicariance hypothesis as a more general explanation of the patterns (as dispersal leaves the timing unexplained). This hypothesis is further supported by the BioGeoBEARS analyses (Table S1, Fig. 4). However, uncertainties in relationships among some species in *Olios*, reflected in differences among the ML, Bayesian, and time constrained trees, and relatively broad error margins in age estimations, mean that the timing results are not unambiguous, and that a direct comparison of the two lineages is not straightforward. This may in part be due to missing species from Mexico in this study (note there are no known species of *Neostasina* from Mexico). In some other studies of Caribbean spiders (Dziki et al., 2015), Mexican specimens have formed the sister group to a Caribbean clade and thus may clearly influence the estimated timing of colonization events.

Regardless, there is reasonably strong support for GAARlandia from our data. *Neostasina* forms a Caribbean clade and in *Olios* the Bayesian tree for all but one of the Caribbean island species form a clade. In both cases the colonization is indicated at around 30 mya, though again, not with high precision. At the very least, it is clear that the two lineages that arrived to the island early in the biogeographical history of the Caribbean, at around the same time as did a variety of other animal and plant lineages (Ricklefs and Bermingham, 2008). Further independent evidence may come from the remaining Caribbean sparassids. In contrast to *Neostasina* and *Olios*, the Caribbean island species of the *O. sanctivincenti* group are restricted to the southern Lesser Antilles, an island chain with maximum age of some 8 my. These species do not form a monophyletic group and either represent two relatively recent (< 8 my) colonization events to the islands, or an older colonization involving 'reverse colonization' back to S. America. We prefer the former explanation as it is consistent with the age of these islands, as well as their broader biogeography. The biota of the southern Lesser
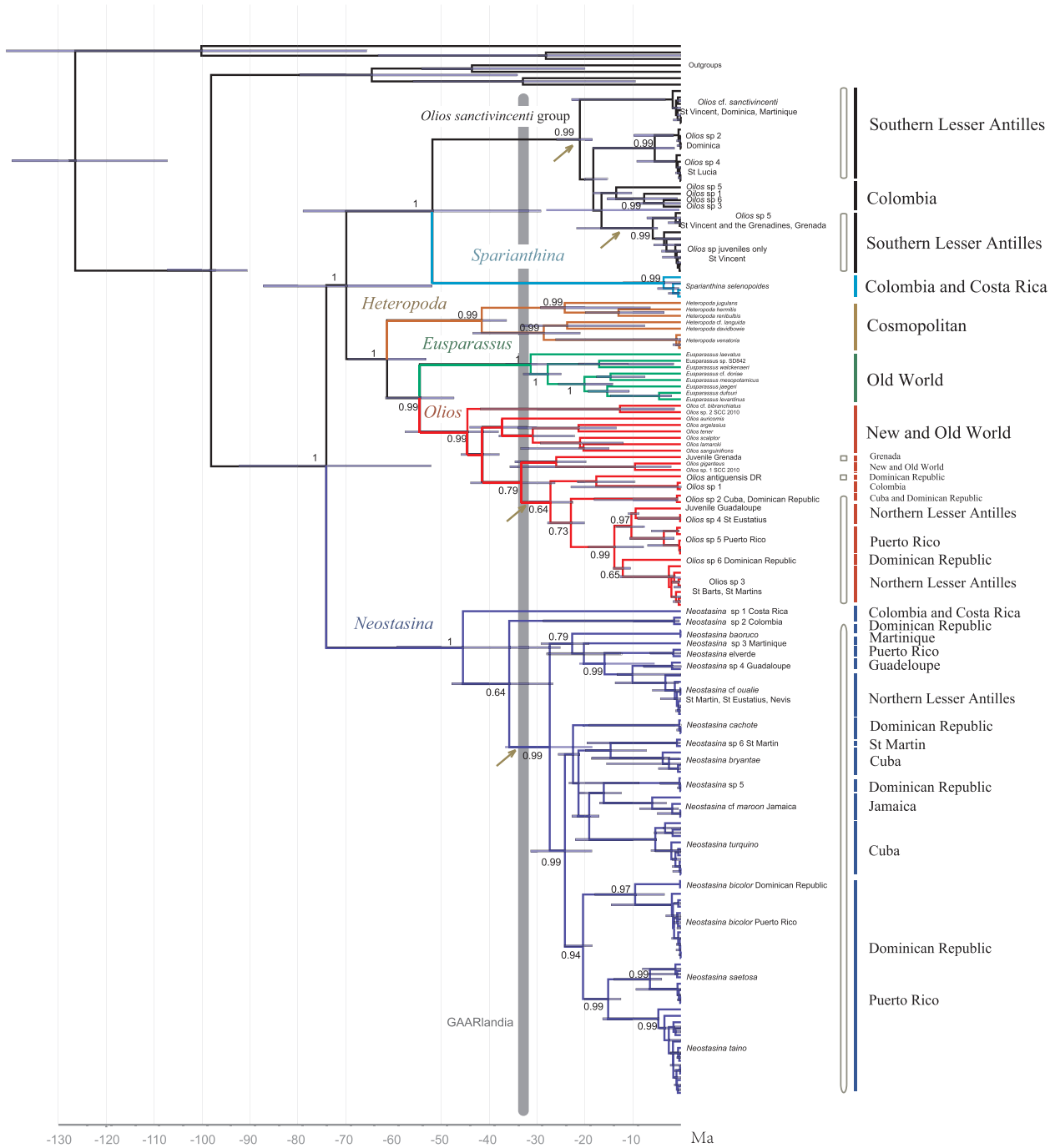
Fig. 3. Clade age estimation from BEAST. Numbers on nodes are posterior probability values from the BEAST analysis. Gray vertical bar indicates the approximate time-span of the GAARlandia landbridge. Blue horizontal bars refer to ambiguity in time estimation. Arrows indicate putative colonization events. Further sampling will be required to determine the number of colonization events in the *Olios sanctivincenti* group.

Antilles is strongly S. American, frequently containing taxa relatively recently colonized from S. America, and that are not found in the Greater Antilles. If a dispersal hypothesis best explained the origin of Caribbean *Neostasina* and *Olios*, we might expect to also observe more recent colonization events to the Lesser Antilles, such as in *O. sanctvincenti*, however, those are not implied by the data.

We note that complete taxon sampling is, of course, the most desirable evidence on which to base tests of biogeographical hypotheses. This study, like most others, is constrained by incomplete, though quite thorough, taxon sampling. For example, we sampled at least 16 *Neostasina* species (plus likely additional species representing separate island population of described species such as *N. saetosa* and *N. bicolor*,

see Fig. 2). Fourteen *Neostasina* species were not sampled, mainly representing species from localities not surveyed in Jamaica and Cuba. Furthermore, the true species richness of the lineages studied herein is unknown; no doubt future work will discover many new endemic species both on the islands and the neighboring continental landmasses. What is likely to be the impact of these missing species on our conclusions? It is difficult to speculate, but we would expect that just like the sampled species, the missing species would fall within the Caribbean clade and be unlikely to significantly alter estimated timing of colonization of the islands.

The most convincing evidence favoring GAARlandia would be precise and highly consistent dates found for multiple lineages.
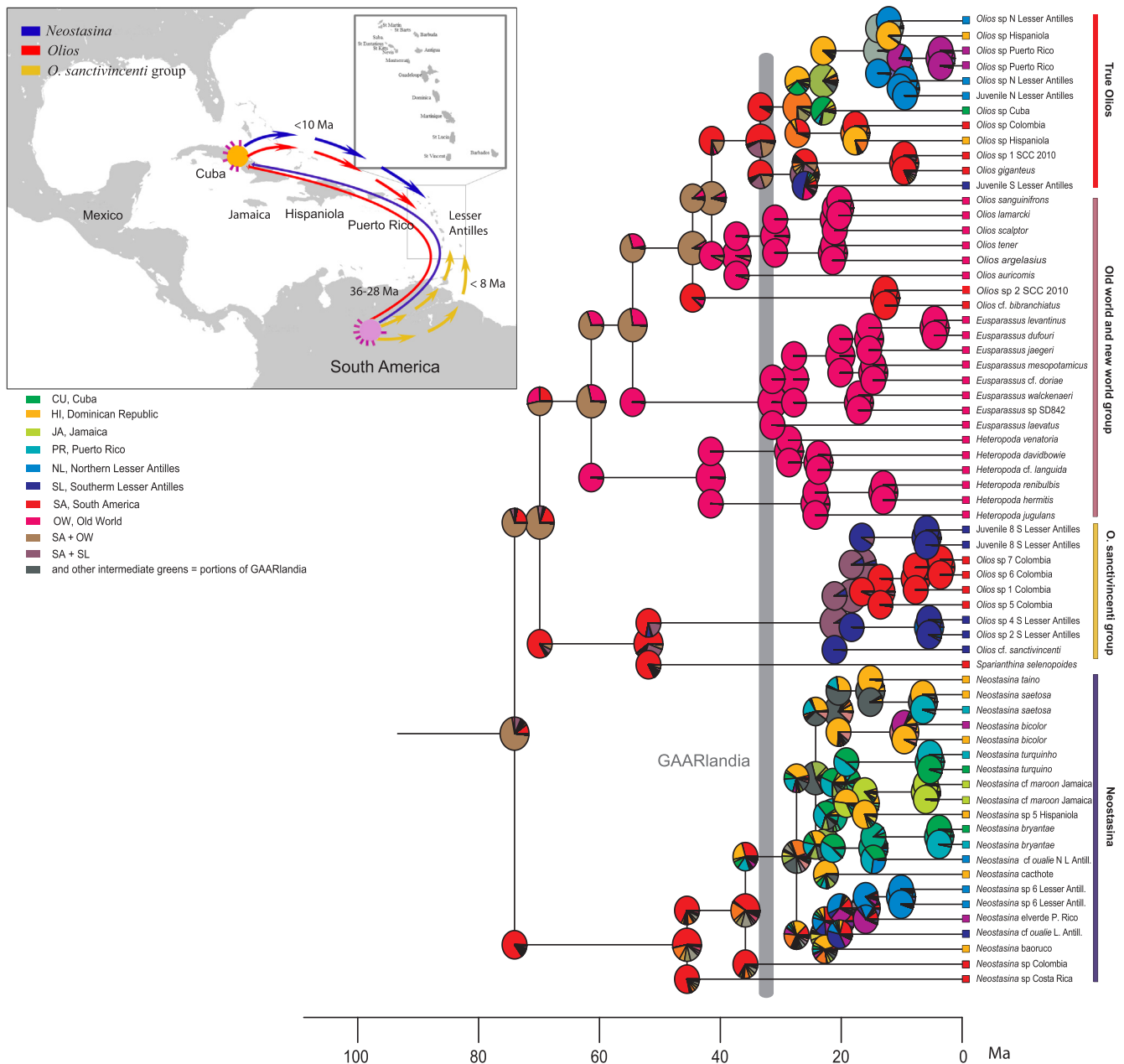
Fig. 4. Results of ancestral area reconstruction from BioGeoBears, treating each island as a separate region (see Fig. S2 for a coding scheme uniting the Greater Antilles into a 'GAARlandia' landmass). Grey vertical bar indicates the time-span of the GAARlandia landbridge. Both lineages occurring on the Greater Antilles colonized in a time frame consistent with GAARlandia, while two much more recent colonization events are postulated for the Southern Lesser Antilles *O. sanctivincenti* group.

Unfortunately, as is true for this and most similar studies (Agnarsson et al., 2016, 2018; Antonelli et al., 2015; Cosgrove et al., 2016; Crews and Gillespie, 2010; Davalos, 2004; de Weerd et al., 2016; Dziki et al., 2015; Esposito et al., 2015; Fabre et al., 2014; Kay, 2015; Masonick et al., 2017; Matos-Maravi et al., 2014; McHugh et al., 2014; Nieto-Blazquez et al., 2017; Rican et al., 2013; Rodriguez et al., 2015; Sato et al., 2016; Shpeley et al., 2017; Tagliacollo et al., 2017; Weaver et al., 2016; Yusseff-Vanegas and Agnarsson, 2016, 2017), time calibrated trees are rarely so precise. In other words, a lot of results are 'consistent' with GAARlandia, but is it because the data support the hypothesis or that the results are simply too vague to reject it? A node dated 15–40 mya and another dated 25–50 mya are both 'consistent' with GAARlandia, but also with a variety of other hypotheses. This is one of the longstanding questions in biogeography in general and that of the Caribbean in particular (Ali, 2012; Chamberland et al., submitted;

Nieto-Blazquez et al., 2017; Rican et al., 2013; Ricklefs and Bermingham, 2008). We cannot aim to solve that here, but rather to provide additional lineages that can be combined for future mega analyses.

In sum, we present the first phylogenetic analysis of Caribbean Sparassidae, including exemplars from the entire family but with a focus on the endemic genus *Neostasina*. Phylogenetic trees, dates, and biogeographical reconstructions suggest early Caribbean colonization in two lineages with diversity concentrated in the Greater Antilles and northern Lesser Antilles, which we propose to tentatively support the GAARlandia hypotheses. On the other hand, more recent transoceanic dispersal events better explain the origin of the southern Lesser Antilles sparassids. Our dataset is valuable as the first insight into the evolutionary history of huntsman spiders of the Caribbean, and in providing datapoints for a broad comparative biogeographical analysis of large

number of lineages that will constitute a strong biological test of the debated geological hypothesis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2018.09.017.

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