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Single-Island Endemism despite Repeated Dispersal in Caribbean *Micrathena* (Araneae: Araneidae): An Updated Phylogeographic Analysis

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Abstract: Island biogeographers have long sought to elucidate the mechanisms behind biodiversity genesis. The Caribbean presents a unique stage on which to analyze the diversification process, due to the geologic diversity among the islands and the rich biotic diversity with high levels of island endemism. The colonization of such islands may reflect geologic heterogeneity through vicariant processes and/ or involve long-distance overwater dispersal. Here, we explore the phylogeography of the Caribbean and proximal mainland spiny orbweavers (*Micrathena*, Araneae), an American spider lineage that is the most diverse in the tropics and is found throughout the Caribbean. We specifically test whether the vicariant colonization via the contested GAARlandia landbridge (putatively emergent 33–35 mya), long-distance dispersal (LDD), or both processes best explain the modern *Micrathena* distribution. We reconstruct the phylogeny and test biogeographic hypotheses using a 'target gene approach' with three molecular markers (CO1, ITS-2, and 16S rRNA). Phylogenetic analyses support the monophyly of the genus but reject the monophyly of Caribbean *Micrathena*. Biogeographical analyses support five independent colonizations of the region via multiple overwater dispersal events, primarily from North/Central America, although the genus is South American in origin. There is no evidence for dispersal to the Greater Antilles during the timespan of GAARlandia. Our phylogeny implies greater species richness in the Caribbean than previously known, with two putative species of *M. forcipata* that are each single-island endemics, as well as deep divergences between the Mexican and Floridian *M. sagittata. Micrathena* is an unusual lineage among arachnids, having colonized the Caribbean multiple times via overwater dispersal after the submergence of GAARlandia. On the other hand, single-island endemism and undiscovered diversity are nearly universal among all but the most dispersal-prone arachnid groups in the Caribbean.

Keywords: phylogeny; Caribbean biogeography; GAARlandia; arachnid; araneae; *Micrathena*; vicariance; long distance dispersal

1. Introduction

Understanding the evolutionary machinery of biodiversity genesis in island systems has long been a focus of fundamental biological research [1–4]. Islands serve as discrete, isolated systems in which to study the generation of biodiversity, resulting from complex patterns of (sometimes) repeated colonization, radiation, and extinction. The isolated nature of islands also allows for the evolution of increased magnitudes of endemic forms; archipelagos facilitate these processes, which are replicated continuously across the entire system [5–7]. Such biodiversity is exemplified within Caribbean archipelagoes and can be observed across taxonomic groups, including arthropods, amphibians, fish, mammals, birds, and plants [7,8]. The proximity of the Caribbean islands to continental blocks has

resulted in the production of a unique assemblage of endemic biota, while still being remote enough for the formation of effective oceanic barriers for dispersal [7].

The geologic history of the Caribbean is intrinsically coupled with this biological diversity, and the region itself is composed of islands with varying geologic origins and different regional tectonic influences [9–12]. This complex geology includes old islands such as the Greater Antilles, which have been emergent for at least 40 million years (mid-Eocene) [13] and younger, primarily volcanic islands (e.g., Lesser Antilles) that emerged less than 10 mya (upper Miocene). The distinct geologic history of each island in the Caribbean should be reflected in the modern patterns of organismal diversity, resulting from its colonization via long-distance dispersal and/or vicariant processes, potentially leading to diversification. Newer volcanic islands and isolated limestone/sedimentary oceanic islands, separated from other landmasses by large swaths of ocean, will likely have species assemblages exclusively resulting from long-distance dispersal from the mainland or other island sources. Continental islands, such as the Greater Antilles, are much older island systems with a complex history of islands becoming emergent or submerged, and splintering and rejoining [12,14,15]. Unraveling the role of LDD and vicariance for a specific group depends on the geology of an individual island, in conjunction with the biology of that lineage [14–18]. As these islands are deferentially isolated from continents, the dispersal ability of a selected lineage is especially significant in understanding its historical colonization of the Caribbean [19].

The GAARlandia (Greater Antilles Aves Ridge) landbridge is a hypothetical sub-aerial connection between South America and the Greater Antilles, in which parts of the previously submerged Aves Ridge became exposed as a consequence of dropping sea levels and the Greater Antillean uplift during the Eocene-Oligocene transition (35–33 mya) [20,21]. This ephemeral connection would have permitted direct overland colonization of South American taxa to the Greater Antilles, followed by the subsequent diversification and speciation as organisms filled previously empty niches before the landbridge was re-submerged around 30 mya [20]. The GAARlandia hypothesis, therefore, predicts the simultaneous colonization across diverse taxa to the Greater Antilles within this timespan, a readily testable biological prediction that has recently been evaluated in a variety of Caribbean biogeographic studies across multiple arthropod taxa [14,16,22–36]. While recent chronostratigraphic data suggests the emergence of a landmass between Puerto Rico and the Lesser Antilles in the mid-Eocene, corresponding with crustal shortening and thickening that is consistent with GAARlandia [37], the hypothesis remains contested due to limited [38,39] or conflicting geological and paleo-oceanographic data [40,41]. Ali and Hedges [40], and others cited therein, also emphasize that biogeographic evidence, consistent with the hypothesis, may offer only weak support due to ambiguity in lineage dating. Recent meta-analyses, uniting multiple studies, generally rejected the role of GAARlandia in the biogeography of Caribbean land vertebrates [40], continuing this active debate.

This complex geologic and evolutionary history can be clarified with phylogeographic evidence from densely sampled, regionally-focused clades. Spiders have increasingly been used, in recent years, as biogeographical models not only in the Caribbean but on global and finer scales [23,42–46], as they form a hyperdiverse group with corresponding diversity in dispersal ability and lineage age. While much of the historical research concerning Caribbean biogeography has been vertebrate-based [14,34,47–49], invertebrates, such as arachnids, can provide fine-scale signals of historical dispersal and colonization [16,50]. Recent evidence from these animals have found mixed support for vicariance and LDD, with a large diversity of focal lineages [16,23,26,29,31,32,36,51,52].

Micrathena, the spiny orbweavers (Araneae, Araneidae), are a colorful, highly ornate, and sexually dimorphic group of 119 New World species, distributed from northern Argentina, throughout the Caribbean and Central America, to the New York state, and into southern Ontario [53,54]. Members of the genus reside in forests or woodlands, constructing webs in the understory up to approximately 4 m off the ground [55]. The large, colorful adult females

are sedentary and solitary, while the much tinier males wander in search of a mate, preferably a penultimate-instar female (as noted in the case of *Microthena gracilis*) [55]. Ballooning behavior has only been formally observed in the juveniles of *Microthena sagittata* [56] but the biogeographic patterns [36,51,53] suggest that it may have played a role in overwater dispersal in the Caribbean.

About 67 *Microthena* species are South American endemics (most found in Colombia and Brazil), with an additional 25 potentially widespread species that have part of their range in South America [57]. Fourteen species are Central American endemics, and eight are Caribbean endemics. Of the eight Caribbean species, four are known single-island endemics: two from Cuba (*M. banksi* and *M. cubana*), one from Jamaica (*M. rufopuncata*), and one from Hispaniola (*M. similis*). In addition, *Microthena forcipata* from Cuba and Hispaniola, and *Microthena militaris* from Puerto Rico and Hispaniola, have recently been suggested to represent clearly divergent lineages, potentially yielding four additional single-island endemics in the Caribbean [51]. Four species are found in North America (*M. funebris*, *M. gracilis*, *M. mitrata*, and *M. sagittata*), and each of these species is in the Caribbean. A previous phylogeographic analysis of Caribbean *Microthena* by McHugh et al. [51] proposed three Caribbean species-groups (the *militaris* group, the *furcula* group, and the *gracilis* group), in agreement with studies by Magalhães et al. [51,53]. Each of these species groups included members of the North, Central, and South American *Microthena*, indicating that Caribbean *Microthena* are not monophyletic, and that colonization of the Caribbean must have been repetitive [51]. Similar patterns are found in some other members of Araneidae (I. Agnarsson unpublished data).

This paper expands on the work of McHugh et al. [51] with increased taxon sampling of Caribbean *Microthena* and additional North and South American mainland species (Colombia and Florida). These additional taxa allow more refined tests of patterns of single-island endemism and more a rigorous evaluation of factors influencing divergence patterns. McHugh et al. [51] rejected the hypothesis that *Microthena* colonized the Greater Antilles via the GAARlandia landbridge. Here, we explicitly test the dispersal route using our additional data on previously omitted and undersampled species that help clarify patterns and timelines for the Caribbean colonization in the genus. These tests strengthen our understanding of the continental-island interchange and other biogeographic patterns of *Microthena* within the region.

2. Materials and Methods

2.1. Specimen and Taxon Sampling

Microthena specimens were collected in the field from 1997–2015 (Table 1, Figure 1). Specimens were stored at −20 °C in 95% ethanol at the University of Vermont. In this work, we added 50 individuals, representing 14 additional *Microthena* species, to the previous McHugh et al. [51] *Microthena* phylogeography study (*M. duodecimspinosa*, *M. lucasi*, *M. sp* (putative species) *M. mitrata*, *M. beta*, *M. cornuta*, *M. embira*, *M. exlinae*, *M. miles*, *M. perfida*, *M. reimoseri*, *M. spinulata*, *M. triangularispinosa*, and *M. yanomami* (Table 1)). We also added previously represented species from new localities: *M. gracilis* from Florida; *M. horrida* from Jamaica; *M. militaris* from Dominica; *M. sagittata* from Florida and Mexico; *M. schreibersi* from Colombia, Trinidad, and Costa Rica; *M. sexspinosa* from Colombia; and expanded sites of *M. forcipata* from Cuba, which were sampled on CarBio trips from 2012–2015 (Table 1). We used a specimen of *Achaearanea* sp. (Theridiidae) as the primary outgroup, along with five araneid members: two *Argiope* specimens and three *Gasteracantha cancriformis* individuals. The outgroups included some relatively near relatives of *Microthena* [58], along with more distantly related araneid members in *Argiope* [49], with members of Theridiidae being used to root the tree.

Table 1. Taxon sampling table with barcodes, locality data, and GenBank accession numbers. "x" denotes GenBank submission in progress.

Genus	Species	Barcode	Country/Region	Latitude	Longitude	16S	CO1	ITS2
Micrathena	*annulata*	MIC007	Brazil	26.08933S	48.64006W		KJ157272	
Micrathena	*aureola*	MIC009	Brazil	4.904167S	42.79083W		KJ157249	
Micrathena	*banksi*	784750	Cuba	20.05269N	76.50296W	KJ156991	KJ157215	KJ157104
Micrathena	*banksi*	784760	Cuba	20.0107N	76.8843W	KJ156992	KJ157216	
Micrathena	*banksi*	784976	Cuba	20.00939N	76.89402W	KJ156993	KJ157217	KJ157105
Micrathena	*banksi*	785101	Cuba	20.00939N	76.89402W	KJ156994	KJ157220	KJ157106
Micrathena	*banksi*	785175	Cuba	20.33178N	74.56919W	KJ156995	KJ157219	KJ157107
Micrathena	*banksi*	787933	Cuba	20.01742N	76.89781W	KJ156996	KJ157218	KJ157108
Micrathena	*beta*	MIC238	Peru	4.5674444S	73.45925W		KX687306	
Micrathena	*bimucronata*	MIC123	Costa Rica	10.233518N	84.075411W		KJ157236	
Micrathena	*brevipes*	MIC121	Costa Rica	9.552960N	83.112910W		KJ157223	
Micrathena	*cornuta*	MIC199	Peru	12.8088056S	69.30175W		KX687309	
Micrathena	*cubana*	784355	Cuba	20.01309N	76.83400W	KJ156997	KJ157224	KJ157109
Micrathena	*cubana*	784820	Cuba	20.00874N	76.88777W	KJ156998	KJ157225	KJ157110
Micrathena	*cubana*	785048	Cuba	22.65707N	83.70161W	KJ156999	KJ157226	KJ157111
Micrathena	*cubana*	787840	Cuba	20.33178N	74.56919W	KJ157000	KJ157227	
Micrathena	*digitata*	MIC017	Brazil	11.39983S	40.52206W		KJ157238	
Micrathena	*duodecimspinosa*	00004833A	Costa Rica	San Antonio de Escazú			x	x
Micrathena	*embira*	MIC182	Brazil	9.642419S	41.446727W		KX687311	
Micrathena	*exlinae*	MIC147	Brazil	0.99185S	62.15915W		KX687313	
Micrathena	*forcipata*	00002846A	Cuba	Juan Gonzalez, Guamá			x	x
Micrathena	*forcipata*	00002848A	Cuba	20.01309N	76.83400W		x	x
Micrathena	*forcipata*	00002845A	Cuba	20.01309N	76.83400W		x	x
Micrathena	*forcipata*	784425	Cuba	20.00939N	76.89402W	KJ157002	KJ157256	KJ157113
Micrathena	*forcipata*	787842	Cuba	20.33178N	74.56919W	KJ157003	KJ157257	
Micrathena	*forcipata*	782311	Hispaniola	18.355536N	68.61825W	KJ157004	KJ157258	
Micrathena	*forcipata*	782434	Hispaniola	19.34405N	69.46635W	KJ157005	KJ157260	KJ157114
Micrathena	*forcipata*	784362	Hispaniola	18.32902N	68.80995W	KJ157006	KJ157264	KJ157115
Micrathena	*forcipata*	784366	Hispaniola	18.32902N	68.80995W		KJ157271	KJ157116
Micrathena	*forcipata*	784447	Hispaniola	18.2205360N	68.480607W	KJ157007	KJ157261	KJ157117
Micrathena	*forcipata*	785054	Hispaniola	19.746175N	71.257726W	KJ157008	KJ157263	KJ157118
Micrathena	*forcipata*	785282	Hispaniola	18.355536N	68.6185W	KJ157009	KJ157259	KJ157119
Micrathena	*forcipata*	785682	Hispaniola	18.2205360N	68.480607W	KJ157010	KJ157	
Micrathena	*forcipata*	787132	Hispaniola	18.310010 N	71.6000 W		KJ157265	
Micrathena	*forcipata*	787135	Hispaniola	18.310010 N	71.6000 W	KJ157011	KJ157266	
Micrathena	*forcipata*	787150	Hispaniola	18.310010 N	71.6000 W	KJ157012	KJ157267	KJ157121
Micrathena	*forcipata*	787153	Hispaniola	18.310010 N	71.6000 W	KJ157013	KJ157269	KJ157122
Micrathena	*forcipata*	787210	Hispaniola	18.310010 N	71.6000 W	KJ157014	KJ157268	KJ157123
Micrathena	*forcipata*	787243	Hispaniola	18.310010 N	71.6000 W	KJ157015	KJ157270	KJ157124

Table 1. *Cont.*

Genus	Species	Barcode	Country/Region	Latitude	Longitude	16S	CO1	ITS2
Micrathena	*furcata*	MIC037	Brazil	27.66667 S	49.01667W		KJ157242	
Micrathena	*gracilis*	10000619A	FL, USA	29.4776N	82.5627W		x	x
Micrathena	*gracilis*	10000629A	FL, USA	29.62986N	82.29880W		x	
Micrathena	*gracilis*	10000627A	FL, USA	29.62986N	82.29880W		x	
Micrathena	*gracilis*	10000638A	FL, USA	29.63680N	82.23961W		x	x
Micrathena	*gracilis*	10000644A	FL, USA	29.46368N	82.52898W		x	
Micrathena	*gracilis*	10000642A	FL, USA	29.62688N	82.29878W		x	
Micrathena	*gracilis*	10000643A	FL, USA	29.62688N	82.29878W		x	
Micrathena	*gracilis*	00000804A	NC, USA	35.44842N	81.58694W		KJ157250	KJ157188
Micrathena	*gracilis*	00000954A	SC, USA	33.03913N	79.56459W	KJ157084	KJ157252	KJ157192
Micrathena	*gracilis*	00000935A	SC, USA	33.03913N	79.56459W	KJ157083	KJ157254	KJ157191
Micrathena	*gracilis*	00000889A	SC, USA	33.03913N	79.56459W	KJ157082	KJ157251	KJ157190
Micrathena	*gracilis*	00000984A	SC, USA	33.03913N	79.56459W	KJ157086	KJ157253	KJ157194
Micrathena	*gracilis*	00000988A	SC, USA	33.03913N	79.56459W	KJ157087	KJ157255	KJ157195
Micrathena	*gracilis*	00002487A	NY, USA	42.01807N	73.91707W	KJ157088		KJ157196
Micrathena	*gracilis*	00002501A	NY, USA	42.01807N	73.91707W	KJ157089		KJ157197
Micrathena	*gracilis*	00000976A	SC, USA	33.03913N	79.56459W	KJ157085		KJ157193
Micrathena	*horrida*	MIC042	Brazil	16.59553S	41.57925W		KJ157248	
Micrathena	*horrida*	MIC122	Costa Rica	10.233518N	84.075411W		KJ157245	
Micrathena	*horrida*	00003552A	Jamaica	18.1635N	77.39410W		x	x
Micrathena	*horrida*	784351	Cuba	20.00939N	76.89402W	KJ157016	KJ157243	KJ157125
Micrathena	*horrida*	784751	Cuba	20.00939N	76.89402W	KJ157017	KJ157246	KJ157126
Micrathena	*horrida*	787913	Cuba	20.00939N	76.89402W	KJ157018	KJ157247	KJ157127
Micrathena	*horrida*	787919	Cuba	20.00939N	76.89402W	KJ157019	KJ157244	KJ157128
Micrathena	*lucasi*	00004785A	Costa Rica	San Antonio de Escazú				
Micrathena	*macfarlanei*	MIC054	Brazil	19.65000S	42.56667W		KJ157241	
Micrathena	*miles*	MIC142	Peru	3.82975S	73.375333W		KX687317	
Micrathena	*militaris*	10000526A	Dominica	15.32710N	61.3381W		x	x
Micrathena	*militaris*	10000528A	Dominica	15.32710N	61.3381W		x	x
Micrathena	*militaris*	782365	Hispaniola	18.355536N	068.61825W	KJ157020		KJ157129
Micrathena	*militaris*	784338	Hispaniola	18.32902N	068.80995W	KJ157021	KJ157273	
Micrathena	*militaris*	784363	Hispaniola	18.32902N	068.80995W	KJ157022	KJ157293	KJ157130
Micrathena	*militaris*	784403	Hispaniola	18.32902N	068.80995W	KJ157023	KJ157298	KJ157131
Micrathena	*militaris*	784430	Hispaniola	18.32902N	068.80995W	KJ157024		KJ157132
Micrathena	*militaris*	784448	Hispaniola	18.32902N	068.80995W	KJ157025	KJ157294	KJ157133
Micrathena	*militaris*	784458	Hispaniola	18.32902N	068.80995W	KJ157026		KJ157134
Micrathena	*militaris*	784503	Hispaniola	18.3150011N	71.580556W	KJ157027	KJ157300	KJ157135
Micrathena	*militaris*	784531	Hispaniola	18.355536N	068.61825W	KJ157028		KJ157136
Micrathena	*militaris*	784566	Hispaniola	18.32902N	068.80995W	KJ157029	KJ157296	KJ157137
Micrathena	*militaris*	784671	Hispaniola	19.06707N	069.46355W	KJ157030		KJ157138
Micrathena	*militaris*	784721	Hispaniola	18.32902N	068.80995W	KJ157031	KJ157310	KJ157139

Table 1. *Cont.*

| Genus	Species	Barcode	Country/Region	Latitude	Longitude	16S	CO1	ITS2
Micrathena	*militaris*	784759	Hispaniola	18.355536N	068.61825W	KJ157032	KJ157277	KJ157140
Micrathena	*militaris*	784762	Hispaniola	18.2205360N	68.4806070W	KJ157033		KJ157141
Micrathena	*militaris*	784772	Hispaniola	18.32902N	068.80995W	KJ157034	KJ157287	KJ157142
Micrathena	*militaris*	784806	Hispaniola			KJ157035		KJ157143
Micrathena	*militaris*	784926	Hispaniola			KJ157036		KJ157144
Micrathena	*militaris*	785066	Hispaniola	19.06707N	069.46355W	KJ157037		KJ157145
Micrathena	*militaris*	785080	Hispaniola	18.32902N	068.80995W	KJ157038	KJ157274	KJ157146
Micrathena	*militaris*	785099	Hispaniola	18.32902N	068.80995W		KJ157313	
Micrathena	*militaris*	785128	Hispaniola	18.355536N	068.61825W	KJ157039		KJ157147
Micrathena	*militaris*	785144	Hispaniola	19.746175N	71.257726W	KJ157040		KJ157148
Micrathena	*militaris*	785169	Hispaniola	18.355536N	068.61825W	KJ157041	KJ157290	KJ157149
Micrathena	*militaris*	785173	Hispaniola	19.06707N	069.46355W	KJ157042	KJ157314	KJ157150
Micrathena	*militaris*	785174	Hispaniola	19.06707N	069.46355W	KJ157043	KJ157292	KJ157151
Micrathena	*militaris*	785194	Hispaniola	18.355536N	068.61825W	KJ157044		
Micrathena	*militaris*	785208	Hispaniola	18.2205360N	68.4806070W	KJ157045	KJ157297	KJ157152
Micrathena	*militaris*	785219	Hispaniola	18.355536N	068.61825W	KJ157046	KJ157286	KJ157153
Micrathena	*militaris*	785263	Hispaniola	18.355536N	068.61825W	KJ157047		KJ157154
Micrathena	*militaris*	785273	Hispaniola	19.432213N	070.371412W	KJ157048	KJ157275	KJ157155
Micrathena	*militaris*	785280	Hispaniola	18.32902N	068.80995W	KJ157049	KJ157315	KJ157156
Micrathena	*militaris*	785312	Hispaniola	19.34405N	069.46635W	KJ157050	KJ157280	KJ157157
Micrathena	*militaris*	785401	Hispaniola	19.06707N	069.46355W	KJ157051	KJ157276	KJ157158
Micrathena	*militaris*	785402	Hispaniola	19.34405N	069.46635W	KJ157052	KJ157285	KJ157159
Micrathena	*militaris*	785423	Hispaniola	18.355536N	068.61825W	KJ157053		KJ157160
Micrathena	*militaris*	785461	Hispaniola	19.06707N	069.46355W	KJ157054	KJ157281	
Micrathena	*militaris*	785502	Hispaniola	19.06707N	069.46355W	KJ157055	KJ157301	KJ157161
Micrathena	*militaris*	785512	Hispaniola	19.06707N	069.46355W	KJ157056	KJ157316	KJ157162
Micrathena	*militaris*	785524	Hispaniola	18.355536N	068.61825W	KJ157057	KJ157311	KJ157163
Micrathena	*militaris*	785527	Hispaniola	19.34405N	069.46635W	KJ157058	KJ157279	KJ157164
Micrathena	*militaris*	785563	Hispaniola	19.06707N	069.46355W	KJ157059	KJ157295	KJ157165
Micrathena	*militaris*	785604	Hispaniola	19.06707N	069.46355W	KJ157060	KJ157288	KJ157166
Micrathena	*militaris*	785706	Hispaniola	19.06707N	069.46355W	KJ157061	KJ157278	KJ157167
Micrathena	*militaris*	785709	Hispaniola	19.06707N	069.46355W		KJ157312	KJ157168
Micrathena	*militaris*	785722	Hispaniola	19.06707N	069.46355W	KJ157062	KJ157283	KJ157169
Micrathena	*militaris*	785729	Hispaniola	19.34405N	069.46635W	KJ157063	KJ157284	KJ157170
Micrathena	*militaris*	785743	Hispaniola	19.06707N	069.46355W	KJ157064	KJ157282	KJ157171
Micrathena	*militaris*	785769	Hispaniola	19.06707N	069.46355W	KJ157065		KJ157172
Micrathena	*militaris*	787068	Hispaniola	18.980122N	70.798425W	KJ157066	KJ157299	KJ157173
Micrathena	*militaris*	787106	Hispaniola	18.980122N	70.798425W	KJ157067	KJ157289	KJ157174
Micrathena	*militaris*	787148	Hispaniola	18.3150011N	71.580556W	KJ157068	KJ157291	KJ157175
Micrathena	*militaris*	787152	Hispaniola	18.3150011N	71.580556W	KJ157069		KJ157176
Micrathena	*militaris*	787166	Hispaniola	18.3150011N	71.580556W	KJ157070		KJ157177
Micrathena	*militaris*	787190	Hispaniola	18.3150011N	71.580556W	KJ157071		KJ157178

Table 1. *Cont.*

Genus	Species	Barcode	Country/Region	Latitude	Longitude	16S	CO1	ITS2
Micrathena	*militaris*	787208	Hispaniola	18.3150011N	71.580556W	KJ157072		KJ157179
Micrathena	*militaris*	787212	Hispaniola	18.3150011N	71.580556W	KJ157073		KJ157180
Micrathena	*militaris*	787214	Hispaniola	18.3150011N	71.580556W	KJ157001		KJ157112
Micrathena	*militaris*	392672	Puerto Rico	17.971472N	66.867958W	KJ157074	KJ157302	KJ157181
Micrathena	*militaris*	392677	Puerto Rico	17.971472N	66.867958W	KJ157075	KJ157303	KJ157182
Micrathena	*militaris*	782048	Puerto Rico	18.414373N	66.728722W	KJ157076	KJ157307	KJ157183
Micrathena	*militaris*	782126	Puerto Rico	18.173264N	66.590149W	KJ157077	KJ157308	KJ157184
Micrathena	*militaris*	782153	Puerto Rico	18.414373N	66.728722W	KJ157078	KJ157306	KJ157185
Micrathena	*militaris*	782174	Puerto Rico	18.414373N	66.728722W	KJ157079	KJ157304	KJ157186
Micrathena	*militaris*	782201	Puerto Rico	18.032518N	67.094653W	KJ157080	KJ157305	KJ157187
Micrathena	*militaris*	783400	Puerto Rico	18.45226N	66.59711W		KJ157309	
Micrathena	*mitrata*	10000679A	Mexico	19.79357N	104.0554W		x	x
Micrathena	*mitrata*	00002849A	Mexico	19.79357N	104.0554W		x	x
Micrathena	*nigrichelis*	MIC056	Brazil	20.43481S	43.50906W		KJ157239	
Micrathena	*perfida*	MIC026	Brazil	24.387111S	47.017583W		KX687318	
Micrathena	*plana*	MIC062	Brazil	16.53294S	41.51042W		KJ157240	
Micrathena	*reimoseri*	MIC072	Brazil	11.399833S	40.522056W		KX687321	
Micrathena	*saccata*	MIC076	Brazil	1.424828S	48.43802W		KJ157237	
Micrathena	*sagittata*	10000618A	FL, USA	29.4776N	082.5627W		x	
Micrathena	*sagittata*	10000621A	FL, USA	29.63703N	082.23976W		x	
Micrathena	*sagittata*	10000631A	FL, USA	29.62986N	082.29880W		x	x
Micrathena	*sagittata*	10000633A	FL, USA	29.62986N	082.29880W		x	
Micrathena	*sagittata*	10000636A	FL, USA	29.63680N	082.23961W		x	x
Micrathena	*sagittata*	10000634A	FL, USA	29.46397N	082.55285W		x	x
Micrathena	*sagittata*	10000639A	FL, USA	29.63680N	082.23961W		x	
Micrathena	*sagittata*	10000640A	FL, USA	29.62688N	082.29878W		x	
Micrathena	*sagittata*	00002847A	Mexico	18.18963N	89.46333W		x	
Micrathena	*sagittata*	00000833A	SC, USA	33.03913 N	79.56459W	KJ157081	KJ157221	KJ157189
Micrathena	*schreibersi*	00002357A	Colombia	Bucaramanga			x	
Micrathena	*schreibersi*	10000650A	Colombia	8.39104N	77.21548W		x	
Micrathena	*schreibersi*	10000652A	Colombia	8.39104N	77.21548W		x	
Micrathena	*schreibersi*	10000653A	Colombia	8.39104N	77.21548W		x	x
Micrathena	*schreibersi*	10000664A	Colombia	8.424N	77.29216W		x	
Micrathena	*schreibersi*	10000673A	Colombia	8.39104N	77.21548W		x	
Micrathena	*schreibersi*	10000658A	Colombia	8.39104N	77.21548W		x	
Micrathena	*schreibersi*	10000651A	Colombia	8.39104N	77.21548W		x	x
Micrathena	*schreibersi*	10000663A	Colombia	8.424N	77.29216W		x	
Micrathena	*schreibersi*	10000665A	Colombia	8.424N	77.29216W		x	x
Micrathena	*schreibersi*	00004787A	Colombia	10.21192N	75.25403W		x	x
Micrathena	*schreibersi*	00004818A	Trinidad				x	x
Micrathena	*schreibersi*	00002900A	Costa Rica	10.430686N	84.007089W		x	x
Micrathena	*schreibersi*	00000936A	Colombia	7.062695N	73.073058W	KJ157090	KJ157318	KJ157198
Micrathena	*schreibersi*	00002357A	Colombia	7.062695N	73.073058W	KJ157092	KJ157319	KJ157199

Table 1. *Cont.*

Genus	Species	Barcode	Country/Region	Latitude	Longitude	16S	CO1	ITS2
Micrathena	*sexspinosa*	10000690A	Colombia	8.35249N	77.22118W		x	
Micrathena	*sexspinosa*	10000659A	Colombia	8.35249N	77.22118W		x	
Micrathena	*sexspinosa*	10000674A	Colombia	8.35249N	77.22118W		x	x
Micrathena	*sexspinosa*	10000677A	Colombia	11.120083N	74.082805W		x	
Micrathena	*sexspinosa*	10000683A	Colombia	11.120083N	74.082805W		x	
Micrathena	*sexspinosa*	10000669A	Colombia	8.39104N	77.21548W		x	x
Micrathena	*sexspinosa*	10000670A	Colombia	8.39104N	77.21548W		x	x
Micrathena	*sexspinosa*	10000681A	Colombia	8.35249N	77.22118W		x	
Micrathena	*sexspinosa*	10000678A	Colombia	8.35249N	77.22118W		x	
Micrathena	*sexspinosa*	00000987A	Colombia	7.062695N	73.073058W	KJ157091	KJ157222	
Micrathena	*similis*	785024	Hispaniola	19.34405N	69.46635W	KJ157093	KJ157228	KJ157200
Micrathena	*similis*	785496	Hispaniola	19.34405N	69.46635W	KJ157094	KJ157232	KJ157201
Micrathena	*similis*	787265	Hispaniola	19.05116N	70.88866W	KJ157095	KJ157233	KJ157202
Micrathena	*similis*	787297	Hispaniola	19.05116N	70.88866W	KJ157096		KJ157203
Micrathena	*similis*	787308	Hispaniola	19.03627N	70.54337W	KJ157097	KJ157229	KJ157204
Micrathena	*similis*	787309	Hispaniola	19.05116N	70.88866W	KJ157098		KJ157205
Micrathena	*similis*	787311	Hispaniola	19.05116N	70.88866W		KJ157235	KJ157206
Micrathena	*similis*	787318	Hispaniola	19.03627N	70.54337W	KJ157099	KJ157234	KJ157207
Micrathena	*similis*	787320	Hispaniola	19.05116N	70.88866W	KJ157100	KJ157230	KJ157208
Micrathena	*similis*	787322	Hispaniola	19.05116N	70.88866W	KJ157101	KJ157231	KJ157209
Micrathena	sp.	10000656A	Colombia	11.120083N	74.082805W		x	
Micrathena	sp.	10000671A	Colombia	11.120083N	74.082805W		x	x
Micrathena	sp.	00006693A	Colombia	11.120083N	74.082805W		x	x
Micrathena	*spinulata*	MIC205	Mexico	19.1381667N	97.2045W		KX687324	
Micrathena	*triangularispinosa*	MIC156	Brazil	0.97799S	62.10292W		KX687327	
Micrathena	*yanomami*	MIC193	Peru	13.055639S	71.546194W		KX687332	
Outgroups								
Achaearanea	sp.	784841	Cuba	21.59166N	77.78822W		KJ157211	
Argiope	*lobata*	Arg0160	Spain	Missing GPS data		KJ156988		KJ157103
Gasteracantha	*cancriformis*	787198	Hispaniola	18.3150011N	71.580556W	KJ156989	KJ157212	
Gasteracantha	*cancriformis*	784515	Hispaniola	18.2205260N	68.480607W		KJ157213	
Gasteracantha	*cancriformis*	782149	Puerto Rico	18. 172979N	66.491798W	KJ156990	KJ157214	
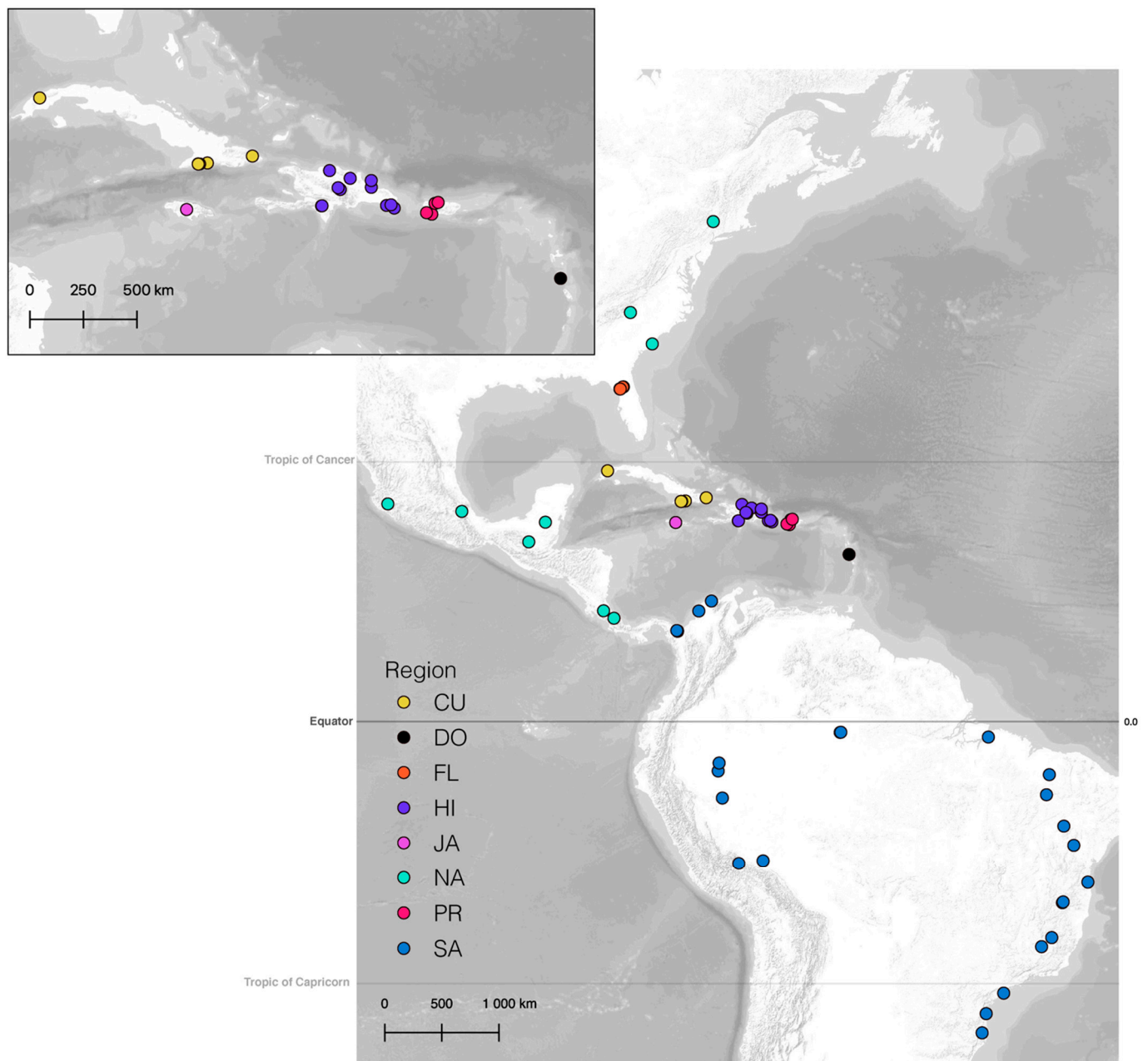
Figure 1. Map of collection localities of all specimens included in analysis. Points are colored by biogeographic area assigned for BioGeoBEARS analysis (see supporting material).

2.2. Tissue Extraction and PCR

Tissue samples were taken from the right legs, and DNA was isolated using the QIA-GEN DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA, USA). Fragments of one mitochondrial locus (CO1: cytochrome c oxidase subunit 1) and one nuclear locus (ITS-2: internal transcribed spacer 2) were sequenced. The 16S data, along with the previous ITS-2 and CO1 data, were retrieved from McHugh et al. [51]. Both ITS-2 and CO1 have demonstrated utility in illuminating relationships between species-level and low-level taxonomic clades in previous arachnid phylogenetics studies [59,60]. The CO1 locus was amplified using the primers *Jerry* [61] and C1-N-2776 [62] for the majority of specimens (*n* = 43), while a select number were amplified using LCO1490 [63] and C1-N-2776 (*n* = 7), which resulted in a higher success rate of amplification within this group. The ITS2 locus was amplified using the primers ITS5.8S and ITS4 [64]. The conditions for each PCR are listed in Table 2. Sanger sequencing was conducted by the University of Vermont Cancer Center DNA Analysis Facility within

the Vermont Integrative Genomics Resource (VIGR) facility. Additional sequences used to inform deficiencies in our South American *Micrathena* collection were retrieved from GenBank. All novel sequences have been submitted to GenBank (in progress).

Table 2. Polymerase chain reaction (PCR) conditions for ITS-2 and CO1. Conditions were split for CO1, given that two sets of primers were used.

Polymerase Chain Reaction (PCR) Conditions				
Gene	**Forward Primer**	**Reverse Primer**	**Annealing Temp. (°C)**	**Fragment Length (bp)**
Internal transcribed spacer 2 (ITS-2)	ITS4	ITS5.8	47	350–500
	Jerry	C1-N-2776	46	~1250
Cytochrome oxidase subunit 1 (CO1)	LCO11490	C1-N-2776	48	~1250

2.3. Alignment and Phylogeny Building

Phred and Phrap [65,66] were used to compile sequence chromatograms. Chromatograms were inspected and sequences were edited using the Chromaseq module [67] within the program Mesquite 3.61 [68]. Sequences were aligned using the MAFFT online service [69] with gaps treated as missing characters and all other settings set to default. The substitution models and partitioning schemes for a Bayesian analysis were selected with PartitionFinder 2.1 [70], using AIC (Akaike's information criterion) [71] amongst the 24 available models in MrBayes [72]. Sequence data were partitioned by gene, and additionally by codon, for CO1 as input for PartitionFinder. We ran a Bayesian inference using the CIPRES online portal [73] on a concatenated matrix where each locus was separately partitioned using MrBayes 3.2.7.a [72]. The Markov Chain Monte Carlo (MCMC) algorithm was run with four chains for 30,000,000 generations, sampling every 1000 generations. Tracer 1.71 [74] was used to verify the proper mixing of chains, to confirm that stationarity had been achieved, and to determine the adequate burn-in.

2.4. Divergence Time Estimation and Biogeographic Modeling

To estimate node ages among *Micrathena*, we used BEAST 2.60 [75] under a relaxed clock model. Because the South American species only had CO1 sequence data available, we used only this locus in the BEAST analysis. Terminal taxa were pruned for redundancy so that one representative of each critical species remained. BEAST analyses for CO1 were run with both an alignment partitioned by codon, using the best-fit models extracted from PartitionFinder [70] (GTR + I + Γ for position 1, TVM + I + Γ for position 2, and TRN + Γ for position 3), along with an unpartitioned analysis, which was run using the best-fit model for CO1 overall (GTR + I + Γ). Both analyses returned identical results. The analyses in *BEAST* were run for 30,000,000 generations, sampling every 1000 generations with a Yule Tree prior. *Micrathena*, along with closely related lineages, lack a fossil record, so the phylogeny was calibrated using the estimated age of Araneidae and the most recent common ancestor (MRCA), including Theridiidae and Araneidae derived from a recent fossil calibrated study by Kuntner et al. [76]. The minimum age of Araneidae was set as a normal prior with a mean of 70 million years and a standard deviation of 3. The minimum age of Theridiidae + Araneidae was also set as a normal prior with a mean of 100 million years and a standard deviation of 9; both prior distributions covered the 95% confidence intervals derived from Kuntner et al. [76]. Based on the estimated substitution rates of CO1 that have been found to be consistent across spider lineages [76,77], the mitochondrial substitution rate parameter (ucld.mean) mean value was set to 0.0112 and the s.d. was set to 0.001. We confined the monophyly of *Micrathena* based on the results of our Bayesian analyses. *Tracer 1.7* [74] was

again utilized to visualize the results of our node age estimation analysis, to determine burn-in and to check for stationarity.

An ancestral range analysis was conducted using the BioGeoBEARS v.1.1.2 package in R [78]. The maximum range was constrained to three areas, due to the widespread distribution of some focal taxa. In this analysis, we employed our CO1 dated phylogeny with terminals pruned to represent single species or genetically distinct single-island endemics based on our Bayesian tree. We defined seven geographic areas: North America (NA), South America (SA), Florida (FL), Cuba (CU), Hispaniola (HI), Jamaica (JA), and Puerto Rico (PR) (see Supplementary File S1). Mexico, and all of Central America north of Panama, were included as part of North America, given that the edge of the Maya Block in southern Mexico corresponds to the southernmost boundary of the North American Tectonic Plate and that the Chorotega and Chortís blocks of Central America were associated with North America as a geologic entity for our focal time period [79–81]. Florida was coded as a separate entity from North America, as the land was unavailable until about 5 mya [82].

We tested a GAARlandia model and a no-GAARlandia model (the distribution was explained by overwater dispersal) by applying probabilities to paleogeographical-based time slices coded on the emergence or submergence of the defined areas at a given period, following Chamberland et al. [46] and Tong et al. [31] (see Supplementary Material). GAARlandia was modeled as the connections between islands making up the Greater Antilles, along with their connection to South America from 35–30 mya [20,21]. We also modeled the geologic splits among the Greater Antillean islands in both the GAARlandia and no-GAARlandia models, specifically the opening of the Mona Passage between Hispaniola and Puerto Rico at 23 mya, and the opening of the Windward Passage, separating Cuba and Hispaniola, at 15 mya [20]. In addition, we encoded for the fluctuating emergence of Jamaica at various periods, and on the timing of the appearance and distance of Central America to other landmasses within the region [20]. In *BioGeoBEARS* and within *R*, we applied the dispersal-extinction-cladogenesis (DEC) and DEC + J models, the latter of which accounts for founder-event speciation. It should be mentioned that the DEC + J model has been criticized as a poor explanator of geographic range evolution due to its parameterization of the speciation mode, as opposed to speciation rate [83]. Here, we tested DEC and DEC + J under the no-GAARlandia and GAARlandia models. The Akaike information criterion (AIC) [71] and relative likelihoods were used to assess model probabilities, given the data. We compared the likelihood scores obtained from each run to test for significance (ΔAICc of 2 was considered significant) [84].

2.5. Specimen Photography

Specimen photographs, depicting morphological variation between the populations or species, were taken using a Canon 5D camera with a 65 mm macro $5\times$ zoom lens attached to the Visionary Digital BK laboratory system rig (Dun Inc., Palmyra, VA, USA). Specimens were placed in a dish filled with alcohol-based hand sanitizer (65% ethanol), and covered with a thin film of 95% ethanol to in order to produce a clear image. Multiple image slices were stacked using the Helicon Focus [85] and were refined in Adobe Photoshop 22.1, where dust and other residues were removed from the background and the image was fine-tuned to adjust for contrast and sharpness. Scale measurements for each specimen were also added via Photoshop. Figures were generated and edited using Adobe Illustrator and exported as PDFs.

3. Results

3.1. Sequence Alignment

A total of 76 sequences were generated from the CO1 and ITS2 fragments of the *Micrathena* sample set ($n_{CO1} = 50$, $n_{ITS2} = 26$). These were combined with sequences retrieved from data generated by McHugh et al. [51] to form a combined dataset of 405 sequences ($n_{CO1} = 164$, $n_{ITS2} = 131$, $n_{16S} = 110$), representing 189 individuals. The additional 24 CO1

sequences, representing unaccounted-for species, were retrieved from GenBank. Alignment lengths were CO1-1162 bp, 16S-458 bp, and ITS2-554 bp for a total of 2174 base pairs.

3.2. Phylogenetics

Relationships based on the Bayesian inference were robustly supported, with posterior probability values of most nodes >0.95 (Figure 2). Relationships within *Micrathena militaris* showed considerably lower support than the other nodes along the tree, as did some of the other fine-scale relationships highlighted in this analysis (mostly individual specimens representing tree tips) (Figures 2–5). However, support for major clade divisions and deep-rooted nodes remained consistently robust throughout the concatenated phylogeny (Figure 2).

Our results support the monophyly of *Micrathena*, but reject the monophyly of Caribbean *Micrathena* (Figures 2–5). All named *Micrathena* species were monophyletic. Caribbean taxa are distributed among three species groups, previously defined by Magalhães and Santos [53] (Figure 3). We identified Caribbean *Micrathena* to belong to the nominal *militaris*-group, including *M. sexspinosa*, *M. militaris*, *M. sagittata*, and *M. banksi* (Figure 3). In addition, we substantiated the *furcula*-group, containing *M. cubana* and *M. similis*.

The *gracilis*-group, including *M. gracilis* and *M. horrida*, was additionally delineated but did not include *M. forcipata* in our multilocus analysis (Figure 3). Instead, we found that *Micrathena forcipata* was located as a sister to *M. schreibersi*, together forming the sister group to the *furcula* group. However, the topology of our CO1 trees indicated that the positionality of the *furcula* group (*M. cubana and M. similis*) and *M. schreibersi* were unstable. In our CO1 analysis, *M. schreibersi* is sister to the *gracilis*-group, instead of *M. forcipata*, while both *M. schreibersi* and the *gracilis*-group were, together, sisters to *M. forcipata* (Figure 4).

Our analysis also produced evidence in support of single-island endemism and island monophyly of *Micrathena forcipata*. High levels of island genetic structuring and relatively deep divergences were observed between *M. forcipata* from Cuba and *M. forcipata* from Hispaniola (Figures 2–5). At a finer scale, *M. forcipata* groups from Hispaniola further demonstrated intra-island structuring (Figure 2).

A Puerto Rican *M. militaris* clade was nested within Hispaniolan *M. militaris*; thus, it is not a single-island endemic (Figure 2). *Micrathena horrida* from Cuba, Jamaica, and Central America were not found to be genetically distinct from one another, but were distinct from South American *M. horrida* (Figures 2–5). Furthermore, *M. sagittata* from Mexico, North America (South Carolina), and Florida were genetically distinct from one another, and may represent isolated, morphologically similar, but distinguishable species (Figures 2 and 3, L. Shapiro unpublished data). A putative new species, sister to *M. nigrichelis*, was additionally delineated, here denoted as *M.* sp. (Figure 2). In the Bayesian analysis two South American *Micrathena*: *M. perfida* and *M. beta* were used as outgroups, as they were found to be sister to the least inclusive clade containing Caribbean *Micrathena* (Figure 2).
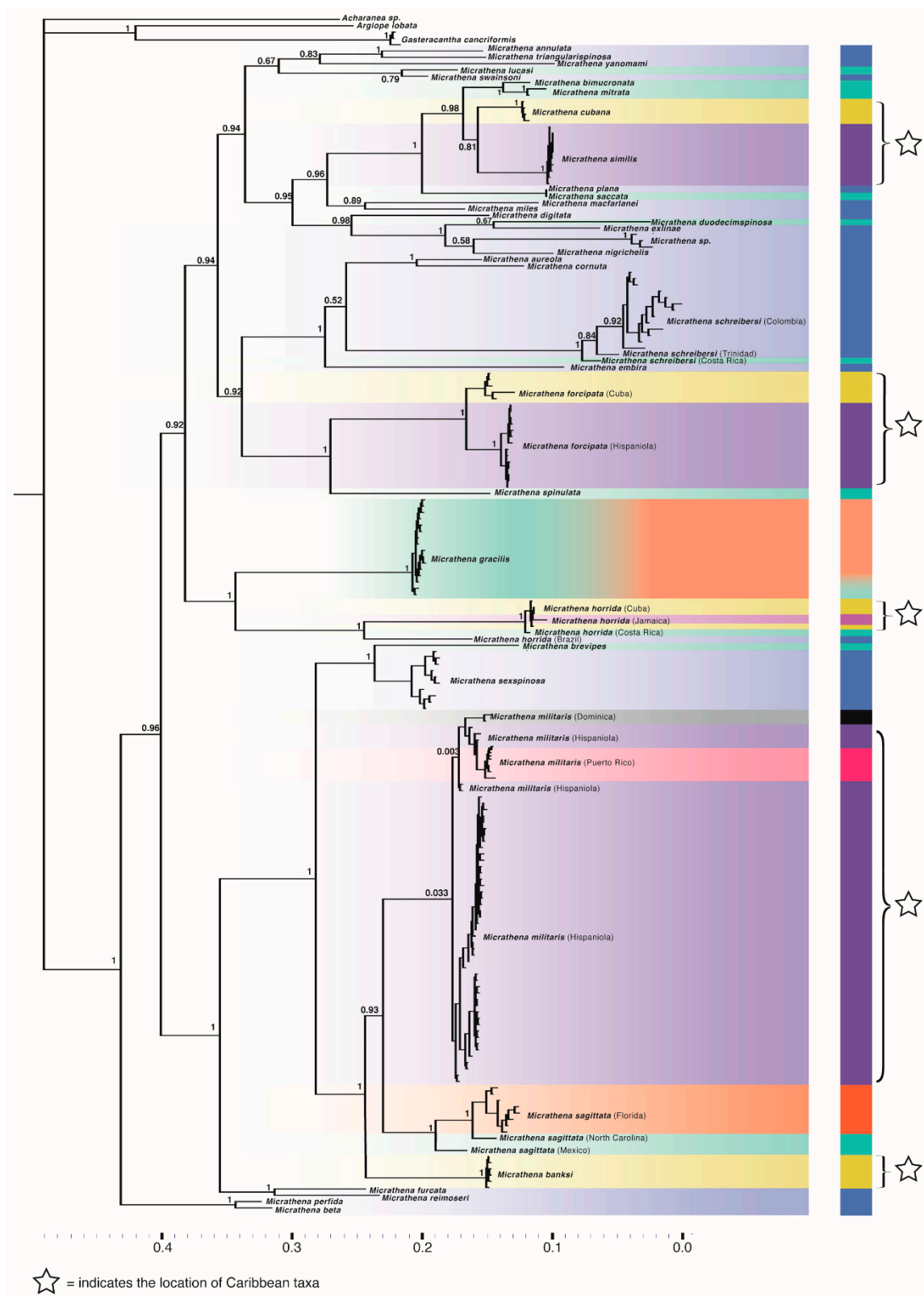
Figure 2. Complete consensus tree from MrBayes concatenated analysis depicting relationships among all sampled *Micrathena* species. Outgroups are located at the top of the phylogeny. Here, terminal individual labels have been replaced with species names along with locality. Overlaying colors are in accordance with color-coded map areas. *M. gracilis* was sampled from both North America and Florida and, therefore, is shaded with an analogous gradient. Stars represent the placement of Caribbean groups within the phylogeny. Posterior probability values are indicated.
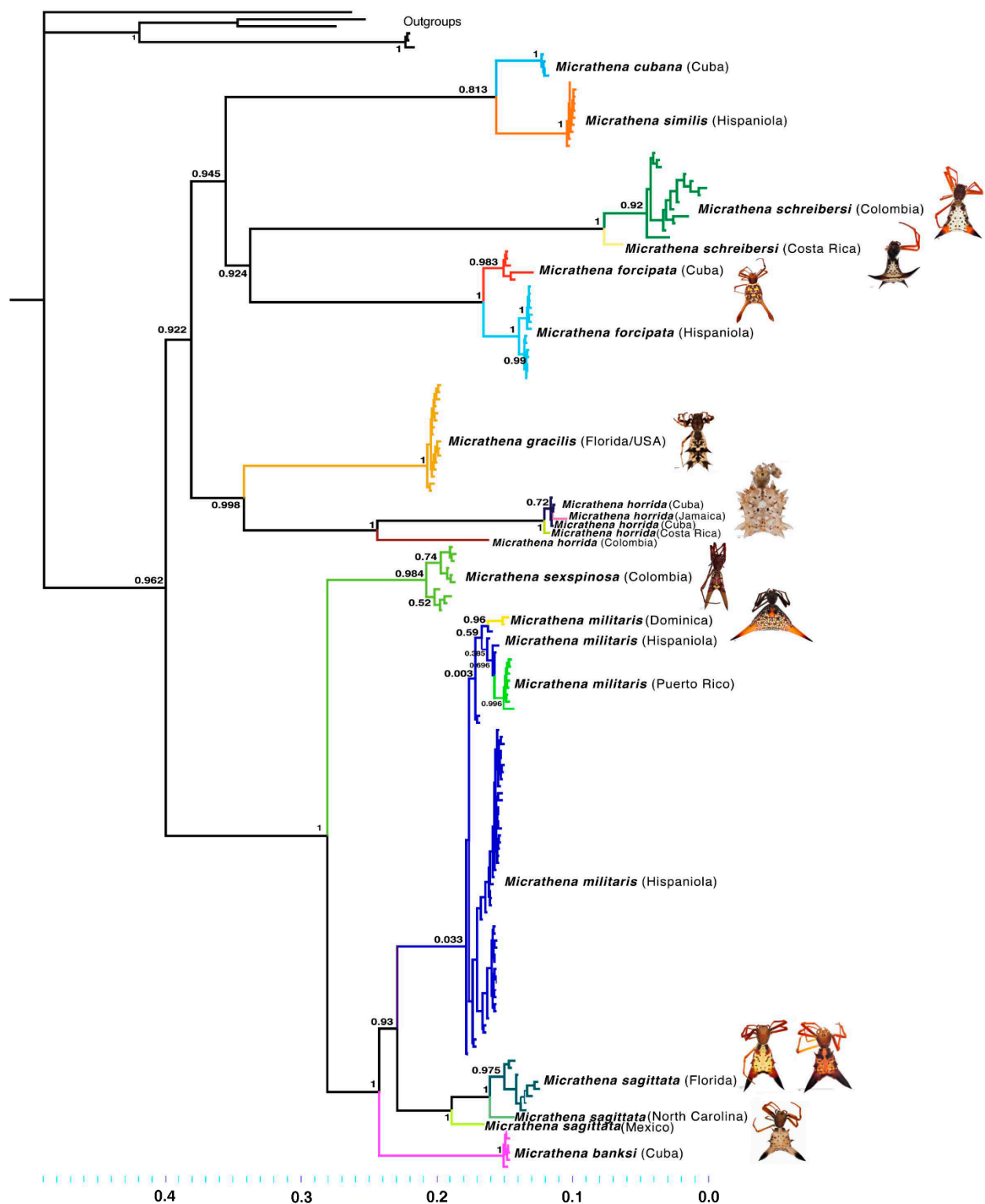
Figure 3. Pruned Bayesian inference tree depicting relationships among Caribbean species groups with associated posterior probability values. Branches are colored by species and individual taxa and have been replaced by species names at tips, but full clade structure is preserved. *Micrathena* dorsal habitus images represent adjacently located taxa. Branches are proportional to evolutionary distances.
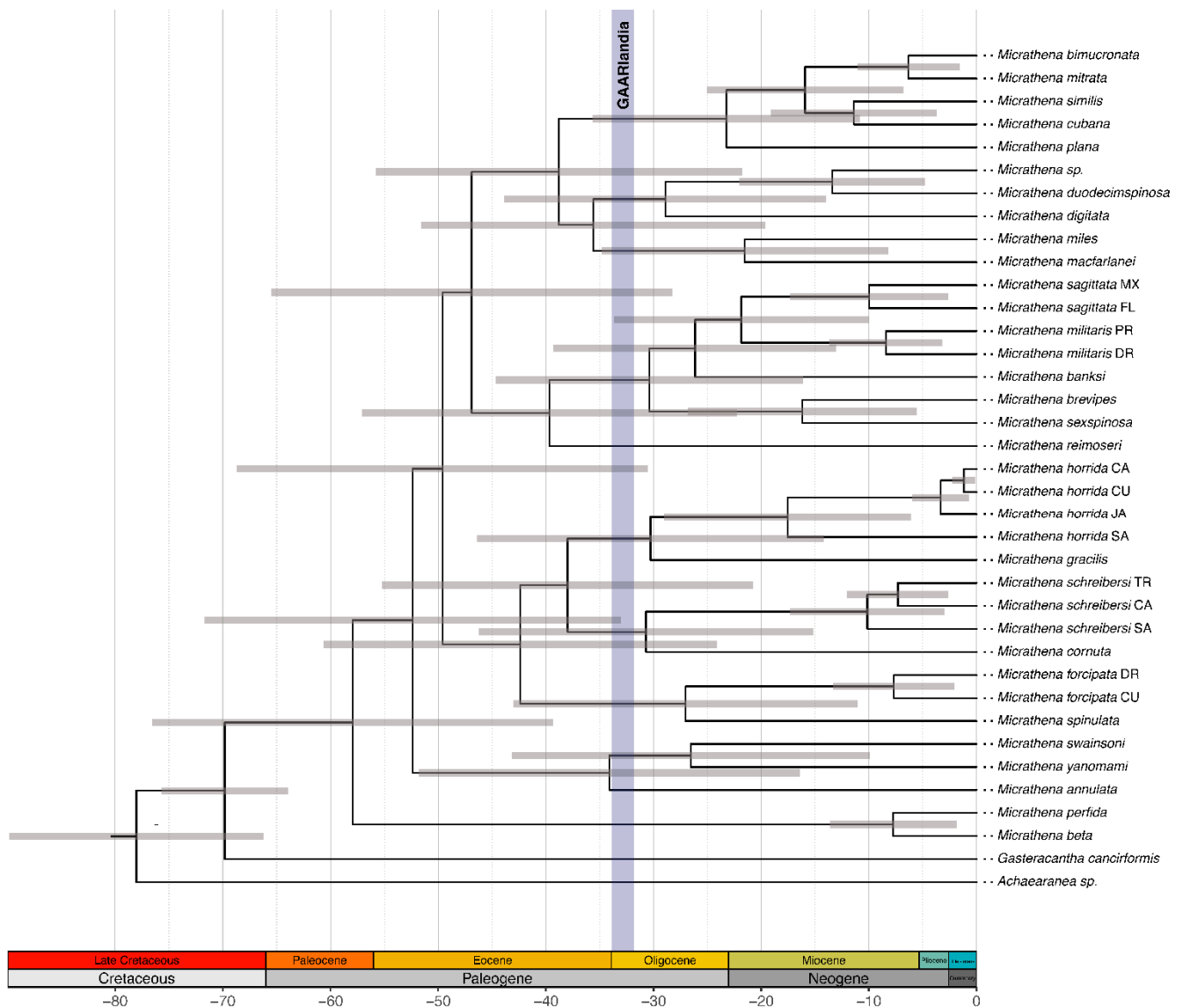
Figure 4. BEAST divergence time estimations of pruned taxa from CO1 data. Grey error bars show error margins around splits calculated in BEAST. Bottom scale is in millions of years and indicates associated geologic time units (periods on lower scale, epochs on upper scale). The timing of the GAARlandia landbridge is also shown from 33–35 Ma. Regional codes associated with taxon names are as follows: CA = Central America, CU = Cuba, DR = Dominican Republic, FL = Florida, JA = Jamaica, MX = Mexico, PR = Puerto Rico, TR = Trinidad.

Figure 5. Ancestral range estimation output from BioGeoBEARS on the DEC + J no-GAARlandia model. Colored nodes indicate the most probable range of the MRCA (most recent common ancestor); SA = South America, NA = North America + Central America, CU = Cuba, PR = Puerto Rico, HI = Hispaniola, FL = Florida, JA = Jamaica. Some boxes indicate multiple probable ranges. Boxes are colored by species area labels (See Figure 1). Relevant geologic events corresponding with BioGeoBEARS time slice inputs (see Supplementary Material) are indicated by dotted lines.

3.3. Divergence Times

Only CO1 data were used to build our dated phylogeny, as sequences were available for various South American taxa for which data on other loci were absent. BEAST analyses indicated that the age of Araneidae was estimated at 70 my (64–76), while the age of the Araneidae–Theridiidae split was placed at 78 my (67–91) (Figure 4). The age of *Microthena* was estimated to be around 58 my (33–71) (Paleocene, Thanetian, supported by Garrison et al. [86]), corroborating that they are representative of a relatively old New World araneid lineage and were present in the Caribbean region within the timing of the GAARlandia landbridge (Figure 4). Caribbean lineages diverged from mainland groups at variable geologic timepoints, with the oldest split dating back to around 30 mya between Cuba and North America and, additionally, implied five possible colonizations of the Caribbean (Figure 4). More recent Caribbean taxa, exemplified by *M. cubana* and *M. similis*, split from their Mexican and Central American relatives (*M. mitrata* and *M. bimucronata*) at approximately 16 mya (Figure 4). The Caribbean and Central American lineages of *M. horrida* split from South American *M. horrida* at around 17 mya (Figure 4). Deep divergences between Mexican and Floridian *M. sagittata* were also suggested, with a split occurring approximately 10 mya (Figures 2–4). Caribbean *Microthena* were ostensibly polyphyletic (Figures 2–5).

For further detail on topological comparisons between the Bayesian and CO1 BEAST trees, see Supplementary File S3.

3.4. Biogeographic Patterns

3.4.1. Overview

The ancestral range reconstruction in *BioGeoBEARS* suggested five independent colonizations of the Caribbean by *Microthena* (the *similis/cubana* clade, *banksi* clade, *militaris* clade, *horrida* clade, and *forcipata* clade) (Figure 5). The larger *banksi/militaris* group is considered a Caribbean clade, but *M. banksi* and *M. militaris* from Hispaniola and Puerto Rico each arrived to the Greater Antilles independently (Figure 6). *Microthena* originated in South America; an early branching South American lineage is sister to a lineage represented by another South American clade that is then, in turn, sister to the rest of the genus, including further South American members and those found in North and Central America and the Caribbean (Figure 5). There existed an early split between South and North American *Microthena* 52 million years ago and, subsequently, multiple bifurcations between North/Central and South American *Microthena* occurred thereafter (Figure 5). These results indicated that a fraction of *Microthena*, other than the *swainsoni* and *perfida* clades, were indeed North American/Central American in origin, the ancestor having split from South America at this 52 mya timepoint, and this clade originating in North America 50 million years ago (Figure 5).

Four of the five clades containing Greater Antillean taxa are North American/Central American in origin (Figure 5). *M. horrida* is the exception, with South America denoted as ancestral, originating about 17 ma (Figure 5). However the common ancestor of *M. horrida* and *M. gracilis* appears to be North American (30 Ma) (Figure 5). While Cuba is resolved as ancestral to the entirety of the sagitatta/militaris clade (including *M. banksi*), North America is the origin of *M. militaris* from both Puerto Rico and Hispaniola (its pre-dispersal to Puerto Rico was approximately 21 ma) (Figure 5). After colonization from South America, *M. horrida* appears to have diversified to form the Central American, Jamaican, and Cuban clades. Jamaican *M. horrida* split off from this group first at 3.3 Ma, with North/Central American *M. horrida* and Cuban *M. horrida* subsequently bifurcating at 1.18 Ma (Figure 5).
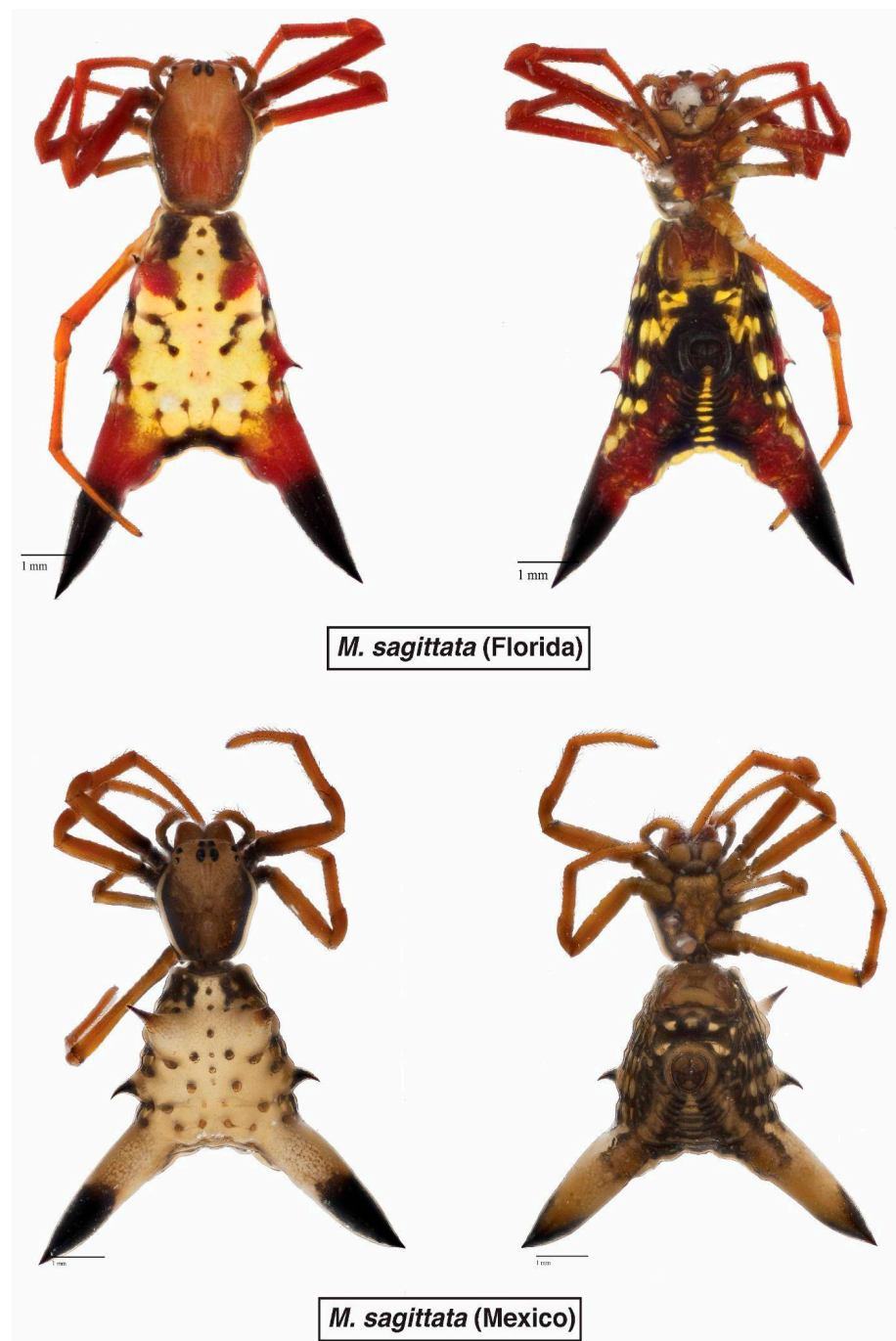
Figure 6. High-resolution composite photographs of female *M. sagittata* specimens from Florida and Mexico depicting morphological variation between populations. Images are of dorsal and ventral habitus of each specimen. Scale bars are associated with each photograph (all lines are 1 mm in length). Habitus shape, along with posterior spine proportion and form, differ between the two groups, although spine number is consistent. Posterior spines of *M. sagittata* from Mexico appear more rounded and wider-set than Floridian *M. sagittata.* Obvious differences in coloration are apparent, with Mexican *M. sagittata* lacking the bright red and yellow pigmentation of Floridian *M. sagittata* on dorsal and ventral sides. Further sampling of Mexican *M. sagittata* is necessary to ensure within-population morphology is consistently distinct from Floridian *M. sagittata*.

Cuba was the first of the Greater Antillean islands to be colonized by South and North/Central American ancestors among all Caribbean groups in our analyses, preceding dispersal to other Caribbean islands (Puerto Rico, Hispaniola, or Jamaica (or mainland sources

in select aforementioned cases)) (Figure 5). The initial splits between mainland and Cuban taxa occur at 27 Ma (in the *M. spinulata*/*M. forcipata* group), 17 Ma (amongst *M. horrida*), 30 Ma (in the *M. militaris* clade), and 16 Ma (within the *M. simils*/*M.cubana*/*M. mitrata* clade) (Figure 5).

We additionally observed multiple inter-island colonization events within the Greater Antilles; this included movement from Puerto Rico to Hispaniola at 8 mya within *M. militaris*, and two Cuba–Hispaniola splits at 7 and 11 mya within *M. forcipata* and between *M. cubana* and *M similis* (Figure 5).

3.4.2. Vicariance vs. Long Distance Dispersal

The DEC + J no-GAARlandia hypothesis demonstrated the best statistical fit, given our input phylogeny, applied time-slices, and affiliated chrono-geographical probabilities (Table 3). The model comparison using AICc also distinguished the BAYAREALIKE + J as significant (Table 3). The top three models determined by AICc were all representative of no-GAARlandia hypotheses (Table 3) with mixed support for lower-ranked models, although none are of statistical significance (Table 3). Both the model ranking and Bio-GeoBEARS results are in agreement that colonization events are not tied to dispersal via the GAARlandia landbridge.

Table 3. BioGeoBEARS model probabilities and rankings. Six models were used in our analysis (DEC, DEC + J, BAYAREALIKE, BAYAREALIKE + J, DIVALIKE, DIVALIKE + J) to test data in the presence or absence of GAARlandia (GAARlandia and no-GAARlandia models). *LnL* is log likelihood, *d* is dispersal rate, *e* is extinction rate, *j* is the relative probability of founder event speciation at cladogenesis, *AICc* is Akaike's information criterion (with correction for smaller sample sizes), *AICc weight* is the normalized relative model likelihood, and *ΔAICc* is AIC—min(AIC).

Model	LnL	Number of Parameters	d	e	j	AICc	AICc Weight	ΔAICc
DEC + J no-GAARlandia	−81.87	3	0.0041	0.0011	0.2	170.5	0.56	0
BAYAREALIKE + J no-GAARlandia	−82.46	3	0.0019	0.01	0.2	171.7	0.31	1.2
DIVALIKE + J no-GAARlandia	−83.53	3	0.0048	0.001	0.2	173.8	0.11	3.3
BAYAREALIKE + J GAARlandia	−85.26	3	0.023	0.011	0.8	177.3	0.019	6.8
DIVALIKE no-GAARlandia	−95.23	2	0.013	0.0033	0	194.8	2.9×10^{-6}	24.3
DEC + J GAARlandia	−94.48	3	0.025	1.00×10^{-12}	2.4	195.7	1.90×10^{-6}	25.2
DIVALIKE + J GAARlandia	−97.42	3	0.027	1.00×10^{-12}	1.7	201.6	9.90×10^{-8}	31.1
DEC no-GAARlandia	−99.69	2	0.013	0.0063	0	203.8	3.40×10^{-8}	33.3
BAYAREALIKE no-GAARlandia	−107.9	2	0.017	0.025	0	220.2	8.90×10^{-12}	49.7
BAYAREALIKE GAARlandia	−112	2	0.24	0.025	0	228.4	1.50×10^{-13}	57.9
DIVALIKE GAARlandia	−112.8	2	0.11	0.0058	0	230	6.90×10^{-14}	59.5
DEC GAARlandia	−112.9	2	0.16	0.01	0	230.2	6.00×10^{-14}	59.7

4. Discussion

Molecular analyses, with the expanded taxon sampling of *Microthena*, resolved the genus as monophyletic with polyphyletic Caribbean taxa (Figures 2–5), consistent with the findings of McHugh et al. [51], Crews and Esposito [36], and Magalhães and Santos [53] (Figures 2–5). We detected five independent colonization events to the Caribbean from varying mainland sources (Figure 5). While South America was the ancestral *Microthena* range, four of the five Caribbean groups were actually North American/Central American in origin (Figure 5), corroborating evidence by other authors [36]. Crews and Esposito [36] found evidence that *Microthena* had repeatedly dispersed to the Caribbean (six times) and suggested that GAARlandia likely played some role in this dispersal. We did not find evidence for the latter hypothesis [36,51]. Rather, the BioGeoBEARS results and the biogeographic model ranking indicated that *Microthena* colonized the Caribbean multiple times, but each time outside of the timespan of the proposed GAARlandia landbridge.

In addition to the dispersal from continental sources, we found evidence for movement among islands, as well as the reverse colonization of North America from Cuba (Figure 5). The phenomenon of movement from island-to-continent has been documented in other spider lineages, including *Deinopis* [46] and *Tetragnatha* [87], adding to the growing frequency of this pattern observed in arachnids, even across groups with variable dispersal strategies [87]. Movement among the Greater Antillean islands reflected both long-distance dispersal and the dispersal to nearby islands (e.g., two pairs of HI-CU sister taxa and the *M. militaris* groups from PR and HI) (Figures 2–5).

Independent dispersals at various geologic timepoints (Figure 5) suggested that stochastic events, such as extreme weather events (e.g., hurricanes) or ocean currents, could have played a role in transporting *Microthena* across the Caribbean, as proposed for other arthropod groups [88–90]. Given that the Caribbean lineages of *Microthena* have a North/Central American origin, the loop current, wrapping around the Gulf of Mexico, entering by the Yucatán peninsula, and exiting via the straights of Florida [91], may be of particular import as it brushes close to Greater Antillean islands. The long-distance dispersal, via rafting in arachnids, has been documented in *Moggridgea* mygalomorphs in Australia [92] and in *Amaurobioides* [93]. Paleocurrent directionality in the Caribbean, which most likely mirrors that of the Holocene (although a thruway between the Atlantic and Pacific existed before the closure of the Panama isthmus at 3.5 Ma) [94–96], and it can be hypothesized that the dispersal routes that allowed *Microthena* to colonize the Caribbean reflect modern and paleooceanographic dynamics. Future investigations may consider integrating paleowind and paleocurrent data to better explain fine-scale dispersal routes of Caribbean colonization that criss-cross the region. While such analyses have been undertaken for Caribbean mammals in terms of utilizing "floating islands" [97], these data have not been applied to biogeographic investigations of spiders. However, hurricanes (with modern directionality) have been shown to be a mechanism important in arthropod dispersal [90] and the dispersal effects have also been empirically noted [89]. The habitat choice in *Microthena*, often occupying the center of wide-open spaces in forests where the web and animal are readily exposed to weather conditions reaching inside the forest, could render them relatively prone to weather-related involuntary aerial dispersal.

This study adds to the growing composite of data suggesting manifold Caribbean dispersals in *Microthena* and indicates that, although they are considered relatively poor dispersers due to their apparent bulkiness and elaborate spine coverage, *Microthena* may actually be relatively proficient dispersers. We would predict this dispersal would mostly occur as juveniles, when they are less heavily ornamented. Other large araneids, including *Nephila* [98] and various *Argiope* and *Araneus* species, do balloon [56]. Not much is known about the physical capacity for dispersal in *Microthena*, and biogeographic investigations may benefit from increased physiological and behavioral analyses of the genus.

We recovered four distinct *Microthena* clades containing Caribbean taxa, which roughly correspond to the species-groups defined by Magalhães and Santos [53] and are corroborated by McHugh et al. [51]: the *militaris*-group, the *gracilis*-group, and the *furcula*-group + *M. forcipata*

(Figure 3, Table 4). Like McHugh et al. [51], our analyses do not place *M. forcipata* within the *gracilis* group. However, the placement of *M. forcipata* differs from McHugh et al. [51] and is influenced by taxon sampling and phylogenetic methods (Table 4). It is likely that gaps in taxon sampling are responsible for the instability of *M. schreibersi* and the *furcula* group, that is noted between the multilocus and the CO1 analyses.

Table 4. Comparisons between species-group delineations for three *Micrathena* phylogenetic analyses performed by Magalhaēs et al. [53], McHugh et al. [51], and this investigation (multilocus datset, Figures 1 and 2). Caribbean species groups are listed along with species belonging to that group in each study. Additional notes on the differing position of *M. schreibersi*, as it relates to these groups, the study by McHugh et al. [51], and this analysis, are listed as footnotes.

Species-Group	Magalhaēs et al., 2012	McHugh et al., 2014	Current *Micrathena* Study
furcula	*M. cubana, M. similis*	*M. cubana, M. similis*	*M. cubana, M. similis*
militaris	*M. banksi, M. militaris, M. sagittata, M. sexspinosa*	*M. banksi, M. militaris, M. sagittata, M. sexspinosa*	*M. banksi, M. militaris, M. sagittata, M. sexspinosa*
gracilis	*M. horrida, M. gracilis, M. forcipata*	*M. horrida, M. gracilis* [1]	*M. horrida, M. gracilis* [2]

[1] *M. schreibersi* is the sister to the *gracilis* group; *M. forcipata* is the sister to the *furcula* group. [2] *M. schreibersi* is the sister to *M. forcipata*, and both are sisters to the *furcula* group.

Our analyses indicated deep divergences within 'widespread taxa', suggesting that such taxa would be better characterized as multiple single-island endemics. For example, *M. forcipata* from Cuba and Hispaniola are genetically distinct from one another, as indicated by deep branching separating the two on the phylogeny. These taxa may also be distinguishable based on morphology (Figure 3 and L. Shapiro's unpublished data). The divergence among these similar taxa is likely due to the segregation of these two islands by the Windward Passage, acting as a geographic barrier post-dispersal (Figures 2–5). While McHugh et al. [51] also determined that the *M. militaris* groups represent single-island endemics from Puerto Rico and Hispaniola, we found that, although *M. militaris* from Puerto Rico are monophyletic, they are nested within the Hispaniolan members of the species, hence rejecting a model of purely single-island endemics in this genus (Figure 2).

Genetic divergences between *M. sagittata* from North America (North Carolina), Florida, and Mexico were also noted in our analyses, where the Mexican *M. sagittata* is the sister to the North American group (Figures 2 and 3). Morphological distinctions between Mexican *M. sagittata*, in comparison to our *M. sagittata* sample from Florida, can be clearly observed (Figure 6). An additional putative, currently undescribed sister species to *M. nigrichelis* was identified in the phylogeny, *Micrathena* sp. The preliminary habitus photographs of *M.* sp. are displayed in Figure 7. Integrative genetic and morphological analyses are currently underway to solidify evidence for the species delimitations of new clades and divergent species uncovered in this study.

Our work, combined with previous biogeographic analyses, substantiates *Micrathena* spiders as an excellent model for Caribbean biogeography of a dispersal-prone lineage. The additional depth in taxon sampling of *Micrathena* and the related genera, especially across Central and South America, as well as expanded data with next-generation sequencing and the greater availability of fossil evidence for calibration, will add to the resolution of factors influencing biodiversity in this region.

Figure 7. High-resolution composite photographs of putative new species *M.* sp. from Colombia. Photographs depict dorsal and ventral habitus of a female specimen. Future studies will hopefully provide more data detailing important morphological characters. Scale is depicted at the bottom of each photograph.

5. Conclusions

We present a detailed molecular phylogenetic and biogeographic analysis of *Microthena*, demonstrating that the group likely colonized the Caribbean region multiple times independently during the last 30 million years, and that diversification was likely a result of multiple overwater dispersal events and not GAARlandia vicariance. This finding suggests that *Microthena*, while potentially dispersal-limited due to its size and morphology, have nevertheless been carried across oceanic barriers to colonize Caribbean islands five times in 30 million years, perhaps as juveniles. We found interesting evidence for single-island endemics in *M. forcipata* and have unveiled the cryptic diversity in *M. sagittata* and within the genus altogether. Further studies will focus on taxonomic examinations of potential species uncovered in this phylogeny.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d14020128/s1, File S1: Dispersal probabilities and geography input for BioGeoBEARS, File S2: List of *Microthena* species in study, File S3: Comparison of concatenated Bayesian and BEAST phylogenies, File S4: Raw BEAST.xml output file.

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