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SPINY SPIDERS AND SPINY TREES: MOLECULAR PHYLOGENETICS AND  
BIOGEOGRAPHIC RECONSTRUCTION REVEAL HISTORY OF RECURRENT  
OVERWATER DISPERSAL EVENTS, SINGLE-ISLAND ENDEMICIS, AND NEW  
SPECIES OF CARIBBEAN *MICRATHENA* (ARANEAE: ARANEIDAE)

A Thesis Presented

by

Lily K. Shapiro

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements  
for the Degree of Master of Science  
Specializing in Biology

August, 2022

Defense Date: May 19, 2022  
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## ABSTRACT

Elucidating the processes responsible for the provenance of taxon biodiversity on islands can help us to understand macroevolutionary mechanics in addition to specific organismal histories. Current species distributions are a result of an admixture of both abiotic (i.e. geologic) and biotic (i.e. dispersal capability, speciation and extinction rates) factors. The heterogeneous tectonic histories and local ecological differentiation among islands within the Caribbean contribute to high levels of endemism and diversity. Differential historical biogeographic processes, namely vicariance (physical separation of populations by barriers) or long-distance dispersal, represent hypotheses to explain current distributions of species on islands resulting from colonization followed by radiation and extinction. In the Caribbean, the GAARlandia landbridge, connecting northern South America with the Greater Antilles (emergent 35-33 mya) presents an appealing vicariant explainer for diversity among various animal groups. Spiders epitomize excellent study organisms for phylogeographic analyses due to their high biodiversity, occupation of a wide variety of ecological niches, and high variation in their ability to disperse.

This study assesses the evolutionary and biogeographic history of the spider genus *Micrathena* within the Caribbean to discern the role of long-distance dispersal and vicariance in shaping their distribution and diversity. Through the process several new species were discovered that are here described, adding to our understanding of diversity within the group. Prior work on *Micrathena* has been limited by relatively poor specimen sampling and resulted in conflicting reports supporting opposing colonization processes and routes. A robust specimen set emphasizing increased sampling on continental mainland areas (Colombia, Florida, plus Genbank mining of data from Brazil and the largest Greater Antilles Islands (Hispaniola, Cuba) was included in our analysis. Multilocus sequence data, ancestral range reconstruction, and biogeographic model testing procedures were integrated to reconstruct an evolutionary history of *Micrathena* and test the roles of the alternative vicariant (GAARlandia) and long-distance dispersal hypotheses. The history of *Micrathena* in the Caribbean spans approximately 30 million years beginning in the mid- Oligocene. The genus originates in South America, but Caribbean groups have a newer North American origin and dispersed five times to the Caribbean, supporting a long-distance dispersal hypothesis for colonization and rejecting a GAARlandia colonization route. Our results suggest high endemism among Caribbean islands. The novel phylogeny highlighted previously uncovered species diversity, and species delimitation along with the primary phylogenetic dataset was used to develop new species hypotheses and descriptions. Three new species are herein described with representatives from Costa Rica, Mexico, and Colombia, and we resurrect *M. flavomaculata* (Keyserling, 1863) from Hispaniola. As so few specimens (<4) currently represent these species, future sampling will be necessarily undertaken at proximal collection localities to ensure diversity is adequately ascertained. These results spotlight Caribbean *Micrathena* among Caribbean arachnid groups in that they repeatedly dispersed to the Caribbean despite their profuse spininess, and as having originated in North America.

## CITATIONS

Material from this thesis has been published in the following form:

**Shapiro, L., Binford, G.J., & Agnarsson I.** (2022). Single-Island Endemism despite Repeated Dispersal in Caribbean *Micrathena* (Araneae: Araneidae): An Updated Phylogeographic Analysis. *Diversity*, 14(2), 128.

## ACKNOWLEDGEMENTS

I would like to first thank my advisor, Ingi Agnarsson, for supporting me as I moved from an undergraduate in his lab into the Biology Graduate program and in designing, conducting, synthesizing, and presenting my research. I have learned from him a great deal about not only how to conduct field and lab science in the proper manner, but how to navigate difficult times as a graduate student. Thank you for believing in my work and putting in the time to build me up as a growing scientist these last five years.

Thank you to my committee, David Barrington and Laura May-Collado, for assisting me in developing this thesis and being mentors to be through this process, your experience and knowledge has pushed this thesis and my work to be the best version possible.

I would also like to thank Bryan Ballif for serving as a secondary advisor, your guidance in time management, organization, and advice on structuring this thesis has been invaluable to me, I could not have completed nearly the work that is presented here without you.

I am extremely grateful to the former members of the Agnarsson Lab, in particular Lisa Chamberland and Laura Caicedo-Quiroga, for their input and advice as I was beginning my foray into *Micrathena* and arachnid systematics as an undergraduate and throughout my first year in the graduate program. Without your technical and personal advice (especially in phylogenetic methodology) there is no world in which my

research would be published or would I have any clue how to complete any of my analyses. I dearly miss collaborating with you both in the lab.

Deepest thanks to members of the Biology Department for providing insight and helping me become a better presenter during BioLunch and thank you to the Biology Department staff for being patient with me and my tendency to procrastinate handing in important paperwork.

Most importantly, thank you to my parents and sister Isabelle for allowing me to send them innumerable drafts and for letting me talk about this specific genus of spiders for hours on end. You believed in me and my work during the most difficult periods and allowed me to eventually believe in myself.

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## INTRODUCTION

How species biodiversity is assembled on global and regional scales and across taxonomic levels has always been of great import to biologists. How did the organisms we see today, with their modern ranges, get there? The myriad of interacting factors ranging from the tectonic to the physiologic play a role in crafting the unique assemblages of organisms that are present in modern day across varying habitats, regions, and biogeographic provinces. By synthesizing these influences, we can develop a story for how and when groups of taxa came to be where they are. Geologically old lineages in old tectonic systems have likely undergone immense shifts in taxonomic diversity along with range expansions and contractions over time, providing insight into long-term macroevolutionary dynamics if the structure and timing of these changes is to be deciphered. Phylogenetic analyses allow us to visualize and illuminate relationships, and coupled with biogeographic reconstruction, provide insight into the timing of colonization events and impetuses of speciation within a group.

Islands are of particular interest to phylogeography, as they represent distinct, isolated land masses where patterns of dispersal, speciation and extinction can be observed with particular clarity. Using molecular phylogenetics as a baseline architecture to study large scale patterns of radiation, dispersal and speciation is ideal in exploring the history of understudied, often hyperdiverse groups as we can glean information not available to us through morphology and specimen samples alone, and explore not only the contained capsule of evolutionary history available to us in a phylogeny, but this phylogeny in a spatial context as well. Illuminating the roles of vicariance (i.e. classic

allopatric speciation) and long-distance dispersal is integral in exploring what factors lead to modern species assemblages, particularly in islands where both processes may be occurring concurrently. The magnitude by which each impacts the evolutionary path of a lineage is dependent on not only the local geography at a particular time, but also the dispersal capability of a group (whether that is biophysically or ecologically relevant [1-5] and the role of stochastic events within the evolutionary timeline of the lineage. The impact of both vicariance and LDD is something that was necessarily highlighted in my exploration of the evolutionary history of a clade.

The addition of phylogenetically poorly assessed lineages to known groups in a biogeographic context, bolsters our understanding of these processes in the island systems they inhabit. In this thesis, I assess the phylogeographic history and taxonomy within a relatively old spider genus, *Micrathena*, in the Caribbean, a geologically heterogeneous system, to understand the genesis of the modern *Micrathena* distribution and of species diversity.

This thesis centers around the construction of the most comprehensive molecular phylogeny for the spider genus *Micrathena*, and the delineation and description of putative new species uncovered in the initial phylogeny construction. *Micrathena* is an araneid (orb-weaver) genus of spider inhabiting the Americas and Caribbean islands. They are an elaborately decorated, spiny, colorful, and extremely sexually dimorphic group of spiders of 119 described species with a diversity epicenter in northern South America (Colombia, Brazil) but ranging from Argentina into southern Canada [6,7]. In this thesis, I attempt to reconstruct the history of *Micrathena* in the Caribbean

region. While previous molecular phylogenetics studies on *Micrathena* have been conducted, a lack of taxon sampling from mainland sources meant that the phylogenies and biogeographic inferences developed upon them were not fully informed [8]. With a new phylogeny built, I reassess taxonomic relationships within *Micrathena* and describe species to correctly reflect those represented in our primary analysis.

In the first chapter of this thesis, we describe the construction of a novel molecular phylogeny and undertake a biogeographic reconstruction for *Micrathena* using the CO1, ITS-2 and 16rRNA loci and with the introduction of several new species from regions such as Colombia and Florida. The inclusion of additional (mostly continental) species increases the statistical robustness of tree topology and the multitudinous specimens from varying regions sources allow us to predict the path of these species more confidently as they colonized the Caribbean region (either originally from island or continental sources). We present a historical analysis for the colonization of the group over a span of ~30 million years from the Oligocene to the Holocene and demonstrate subsequent radiations and specific taxonomic structuring as a result of the interaction between long-distance dispersal and vicariance before and after reaching Caribbean islands [9]. We additionally specifically test the hypothesis that an overwater landbridge aided in Caribbean colonization within the group within the context of vicariance and use model testing software to compare range expansion models to identify the model that best describes biogeographic histories within *Micrathena* [9].

With the construction of an updated phylogeny, we opportunistically describe new species suggested in that analysis. Taxonomy is the fundamental architecture by

which we add and categorize organisms within the scope of life. Adding to our knowledge of local and regional diversity not only allows us to conduct corresponding studies on that known organism but may save it from blurring into obscurity or extinction had it not been known to science. Known spider diversity recently reached a significant taxonomic milestone: 50,000 known species spanning 4000 genera [10] although estimates for true species number fall between 120,000 and 200,000 [11]; diagnosing species via molecular means may reveal cryptic lineages where morphology alone did not indicate division. The integration of both molecular and morphological data in taxonomic work can serve to fill gaps in the flaws of each technique (i.e. crypticism and convergence in phenotypic characters), as suggested by Bond et al. 2022 [12], although integrative taxonomic methodologies are debated [13]. Overall, by describing novel species on a molecular framework in addition to incorporating high resolution imagery, we can more accurately assign species.

In the second chapter of this thesis, we describe three new *Micrathena* species from Colombia, Costa Rica and Mexico and reinstate *M. flavomaculata* from Hispaniola. All three described species were hypothesized as distinct from sister species or close relatives based on the full Bayesian inference tree constructed for Chapter 1 [9]. We describe *M. izzyae* from Colombia, sister to *M. nigrichelis*, for which we have three female specimens, *M. sp\_2* from Costa Rica, previously considered a lineage of *M. schreibersi* but that is obviously genetically and morphologically distinct for which we have one female specimen, and *M. sp\_4* from Mexico, formerly considered as a member of *M. sagittata*, for which we have one female specimen. In addition to providing

photographic evidence and measurements, we conduct species delimitation analyses using the species delimitation plugin in Geneious and the bPTP server (<https://species.hits.org/>), a Bayesian implementation of the Poisson Tree Process. These analyses confirm the existence of these new species, which are named and described.

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**CHAPTER 1: SINGLE ISLAND ENDEMISM DESPITE REPEATED DISPERSAL  
IN CARIBBEAN *MICRATHENA* (ARANEAE: ARANEIDAE): AN UPDATED  
PHYLOGEOGRAPHIC ANALYSIS**

*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.

### *1.1 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 differentially isolated from continents, the dispersal ability of a selected lineage is especially significant in understanding its historical colonization of the Caribbean.

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 *Micrathena gracilis*) [55]. Ballooning behavior has only been formally observed in the juveniles of *Micrathena 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 *Micrathena* 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, *Micrathena forcipata* from Cuba and Hispaniola, and *Micrathena 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 *Micrathena* 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 *Micrathena*, indicating that Caribbean *Micrathena* 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 *Micrathena* 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 *Micrathena* 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 *Micrathena* within the region.

## 1.2 Materials and Methods

### 1.2.1 Specimen and taxon sampling

*Micrathena* specimens were collected in the field from 1997–2015 (Table 1, Figure 1). Specimens were stored at  $-20\text{ }^{\circ}\text{C}$  in 95% ethanol at the University of Vermont. In this

work, we added 50 individuals, representing 14 additional *Micrathena* species, to the previous McHugh et al. [51] *Micrathena* 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 *Achaeearanea 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 *Micrathena* [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
<i>Micrathena</i>	<i>annulata</i>	MIC007	Brazil	26.08933S	48.64006W		KJ15727 2	
<i>Micrathena</i>	<i>aureola</i>	MIC009	Brazil	4.904167S	42.79083W		KJ15724 9	
<i>Micrathena</i>	<i>banksi</i>	784750	Cuba	20.05269N	76.50296W	KJ15699 1	KJ15721 5	KJ15710 4
<i>Micrathena</i>	<i>banksi</i>	784760	Cuba	20.0107N	76.8843W	KJ15699 2	KJ15721 6	
<i>Micrathena</i>	<i>banksi</i>	784976	Cuba	20.00939N	76.89402W	KJ15699 3	KJ15721 7	KJ15710 5
<i>Micrathena</i>	<i>banksi</i>	785101	Cuba	20.00939N	76.89402W	KJ15699 4	KJ15722 0	KJ15710 6
<i>Micrathena</i>	<i>banksi</i>	785175	Cuba	20.33178N	74.56919W	KJ15699 5	KJ15721 9	KJ15710 7
<i>Micrathena</i>	<i>banksi</i>	787933	Cuba	20.01742N	76.89781W	KJ15699 6	KJ15721 8	KJ15710 8

<i>Micrathena</i>	<i>beta</i>	MIC238	Peru	4.5674444S	73.45925W				KX6873 06
<i>Micrathena</i>	<i>bimucronata</i>	MIC123	Costa Rica	10.233518 N	84.075411 W				KJ15723 6
<i>Micrathena</i>	<i>brevipes</i>	MIC121	Costa Rica	9.552960N	83.112910 W				KJ15722 3
<i>Micrathena</i>	<i>cornuta</i>	MIC199	Peru	12.8088056 S	69.30175W				KX6873 09
<i>Micrathena</i>	<i>cubana</i>	784355	Cuba	20.01309N	76.83400W	KJ15699	KJ15722	KJ15710	7 4 9
<i>Micrathena</i>	<i>cubana</i>	784820	Cuba	20.00874N	76.88777W	KJ15699	KJ15722	KJ15711	8 5 0
<i>Micrathena</i>	<i>cubana</i>	785048	Cuba	22.65707N	83.70161W	KJ15699	KJ15722	KJ15711	9 6 1
<i>Micrathena</i>	<i>cubana</i>	787840	Cuba	20.33178N	74.56919W	KJ15700	KJ15722		0 7
<i>Micrathena</i>	<i>digitata</i>	MIC017	Brazil	11.39983S	40.52206W				KJ15723 8
<i>Micrathena</i>	<i>duodecimspinos a</i>	00004833 A	Costa Rica	San Antonio de Escazú					x x
<i>Micrathena</i>	<i>embira</i>	MIC182	Brazil	9.642419S	41.446727 W				KX6873 11
<i>Micrathena</i>	<i>exlinae</i>	MIC147	Brazil	0.99185S	62.15915W				KX6873 13
<i>Micrathena</i>	<i>forcipata</i>	00002846 A	Cuba	Juan Gonzalez, Guamá					x x
<i>Micrathena</i>	<i>forcipata</i>	00002848 A	Cuba	20.01309N	76.83400W				x x
<i>Micrathena</i>	<i>forcipata</i>	00002845 A	Cuba	20.01309N	76.83400W				x x
<i>Micrathena</i>	<i>forcipata</i>	784425	Cuba	20.00939N	76.89402W	KJ15700	KJ15725	KJ15711	2 6 3
<i>Micrathena</i>	<i>forcipata</i>	787842	Cuba	20.33178N	74.56919W	KJ15700	KJ15725		3 7
<i>Micrathena</i>	<i>forcipata</i>	782311	Hispaniola	18.355536 N	68.61825W	KJ15700	KJ15725		4 8
<i>Micrathena</i>	<i>forcipata</i>	782434	Hispaniola	19.34405N	69.46635W	KJ15700	KJ15726	KJ15711	5 0 4
<i>Micrathena</i>	<i>forcipata</i>	784362	Hispaniola	18.32902N	68.80995W	KJ15700	KJ15726	KJ15711	6 4 5
<i>Micrathena</i>	<i>forcipata</i>	784366	Hispaniola	18.32902N	68.80995W		KJ15727	KJ15711	1 6
<i>Micrathena</i>	<i>forcipata</i>	784447	Hispaniola	18.2205360 N	68.480607 W	KJ15700	KJ15726	KJ15711	7 1 7
<i>Micrathena</i>	<i>forcipata</i>	785054	Hispaniola	19.746175 N	71.257726 W	KJ15700	KJ15726	KJ15711	8 3 8
<i>Micrathena</i>	<i>forcipata</i>	785282	Hispaniola	18.355536 N	68.6185W	KJ15700	KJ15725	KJ15711	9 9 9
<i>Micrathena</i>	<i>forcipata</i>	785682	Hispaniola	18.2205360 N	68.480607 W	KJ15701	KJ157		0

<i>Micrathena forcipata</i>	787132	Hispaniola	18.310010 N	71.6000 W	KJ15726 5		
<i>Micrathena forcipata</i>	787135	Hispaniola	18.310010 N	71.6000 W	KJ15701 1	KJ15726 6	
<i>Micrathena forcipata</i>	787150	Hispaniola	18.310010 N	71.6000 W	KJ15701 2	KJ15726 7	KJ15712 1
<i>Micrathena forcipata</i>	787153	Hispaniola	18.310010 N	71.6000 W	KJ15701 3	KJ15726 9	KJ15712 2
<i>Micrathena forcipata</i>	787210	Hispaniola	18.310010 N	71.6000 W	KJ15701 4	KJ15726 8	KJ15712 3
<i>Micrathena forcipata</i>	787243	Hispaniola	18.310010 N	71.6000 W	KJ15701 5	KJ15727 0	KJ15712 4
<i>Micrathena furcata</i>	MIC037	Brazil	27.66667 S	49.01667W		KJ15724 2	
<i>Micrathena gracilis</i>	10000619 A	Florida, USA	29.4776N	82.5627W		x	x
<i>Micrathena gracilis</i>	10000629 A	Florida, USA	29.62986N	82.29880W		x	
<i>Micrathena gracilis</i>	10000627 A	Florida, USA	29.62986N	82.29880W		x	
<i>Micrathena gracilis</i>	10000638 A	Florida, USA	29.63680N	82.23961W		x	x
<i>Micrathena gracilis</i>	10000644 A	Florida, USA	29.46368N	82.52898W		x	
<i>Micrathena gracilis</i>	10000642 A	Florida, USA	29.62688N	82.29878W		x	
<i>Micrathena gracilis</i>	10000643 A	Florida, USA	29.62688N	82.29878W		x	
<i>Micrathena gracilis</i>	00000804 A	NC, USA	35.44842N	81.58694W		KJ15725 0	KJ15718 8
<i>Micrathena gracilis</i>	00000954 A	SC, USA	33.03913N	79.56459W	KJ15708 4	KJ15725 2	KJ15719 2
<i>Micrathena gracilis</i>	00000935 A	SC, USA	33.03913N	79.56459W	KJ15708 3	KJ15725 4	KJ15719 1
<i>Micrathena gracilis</i>	00000889 A	SC, USA	33.03913N	79.56459W	KJ15708 2	KJ15725 1	KJ15719 0
<i>Micrathena gracilis</i>	00000984 A	SC, USA	33.03913N	79.56459W	KJ15708 6	KJ15725 3	KJ15719 4
<i>Micrathena gracilis</i>	00000988 A	SC, USA	33.03913N	79.56459W	KJ15708 7	KJ15725 5	KJ15719 5
<i>Micrathena gracilis</i>	00002487 A	NY, USA	42.01807N	73.91707W	KJ15708 8		KJ15719 6
<i>Micrathena gracilis</i>	00002501 A	NY, USA	42.01807N	73.91707W	KJ15708 9		KJ15719 7
<i>Micrathena gracilis</i>	00000976 A	SC, USA	33.03913N	79.56459W	KJ15708 5		KJ15719 3
<i>Micrathena horrida</i>	MIC042	Brazil	16.59553S	41.57925W		KJ15724 8	
<i>Micrathena horrida</i>	MIC122	Costa Rica	10.233518 N	84.075411 W		KJ15724 5	
<i>Micrathena horrida</i>	00003552 A	Jamaica	18.1635N	77.39410W		x	x



<i>Micrathena</i>	<i>horrida</i>	784351	Cuba	20.00939N	76.89402W	KJ15701 6	KJ15724 3	KJ15712 5
<i>Micrathena</i>	<i>horrida</i>	784751	Cuba	20.00939N	76.89402W	KJ15701 7	KJ15724 6	KJ15712 6
<i>Micrathena</i>	<i>horrida</i>	787913	Cuba	20.00939N	76.89402W	KJ15701 8	KJ15724 7	KJ15712 7
<i>Micrathena</i>	<i>horrida</i>	787919	Cuba	20.00939N	76.89402W	KJ15701 9	KJ15724 4	KJ15712 8
<i>Micrathena</i>	<i>lucasi</i>	00004785 A	Costa Rica	San Antonio de Escazú				
<i>Micrathena</i>	<i>macfarlanei</i>	MIC054	Brazil	19.65000S	42.56667W		KJ15724 1	
<i>Micrathena</i>	<i>miles</i>	MIC142	Peru	3.82975S	73.375333 W		KX6873 17	
<i>Micrathena</i>	<i>militaris</i>	10000526 A	Dominica	15.32710N	61.3381W		x	x
<i>Micrathena</i>	<i>militaris</i>	10000528 A	Dominica	15.32710N	61.3381W		x	x
<i>Micrathena</i>	<i>militaris</i>	782365	Hispaniola	18.355536 N	068.61825 W	KJ15702 0		KJ15712 9
<i>Micrathena</i>	<i>militaris</i>	784338	Hispaniola	18.32902N	068.80995 W	KJ15702 1	KJ15727 3	
<i>Micrathena</i>	<i>militaris</i>	784363	Hispaniola	18.32902N	068.80995 W	KJ15702 2	KJ15729 3	KJ15713 0
<i>Micrathena</i>	<i>militaris</i>	784403	Hispaniola	18.32902N	068.80995 W	KJ15702 3	KJ15729 8	KJ15713 1
<i>Micrathena</i>	<i>militaris</i>	784430	Hispaniola	18.32902N	068.80995 W	KJ15702 4		KJ15713 2
<i>Micrathena</i>	<i>militaris</i>	784448	Hispaniola	18.32902N	068.80995 W	KJ15702 5	KJ15729 4	KJ15713 3
<i>Micrathena</i>	<i>militaris</i>	784458	Hispaniola	18.32902N	068.80995 W	KJ15702 6		KJ15713 4
<i>Micrathena</i>	<i>militaris</i>	784503	Hispaniola	18.3150011 N	71.580556 W	KJ15702 7	KJ15730 0	KJ15713 5
<i>Micrathena</i>	<i>militaris</i>	784531	Hispaniola	18.355536 N	068.61825 W	KJ15702 8		KJ15713 6
<i>Micrathena</i>	<i>militaris</i>	784566	Hispaniola	18.32902N	068.80995 W	KJ15702 9	KJ15729 6	KJ15713 7
<i>Micrathena</i>	<i>militaris</i>	784671	Hispaniola	19.06707N	069.46355 W	KJ15703 0		KJ15713 8
<i>Micrathena</i>	<i>militaris</i>	784721	Hispaniola	18.32902N	068.80995 W	KJ15703 1	KJ15731 0	KJ15713 9
<i>Micrathena</i>	<i>militaris</i>	784759	Hispaniola	18.355536 N	068.61825 W	KJ15703 2	KJ15727 7	KJ15714 0
<i>Micrathena</i>	<i>militaris</i>	784762	Hispaniola	18.2205360 N	68.4806070 W	KJ15703 3		KJ15714 1
<i>Micrathena</i>	<i>militaris</i>	784772	Hispaniola	18.32902N	068.80995 W	KJ15703 4	KJ15728 7	KJ15714 2
<i>Micrathena</i>	<i>militaris</i>	784806	Hispaniola			KJ15703 5		KJ15714 3
<i>Micrathena</i>	<i>militaris</i>	784926	Hispaniola			KJ15703 6		KJ15714 4

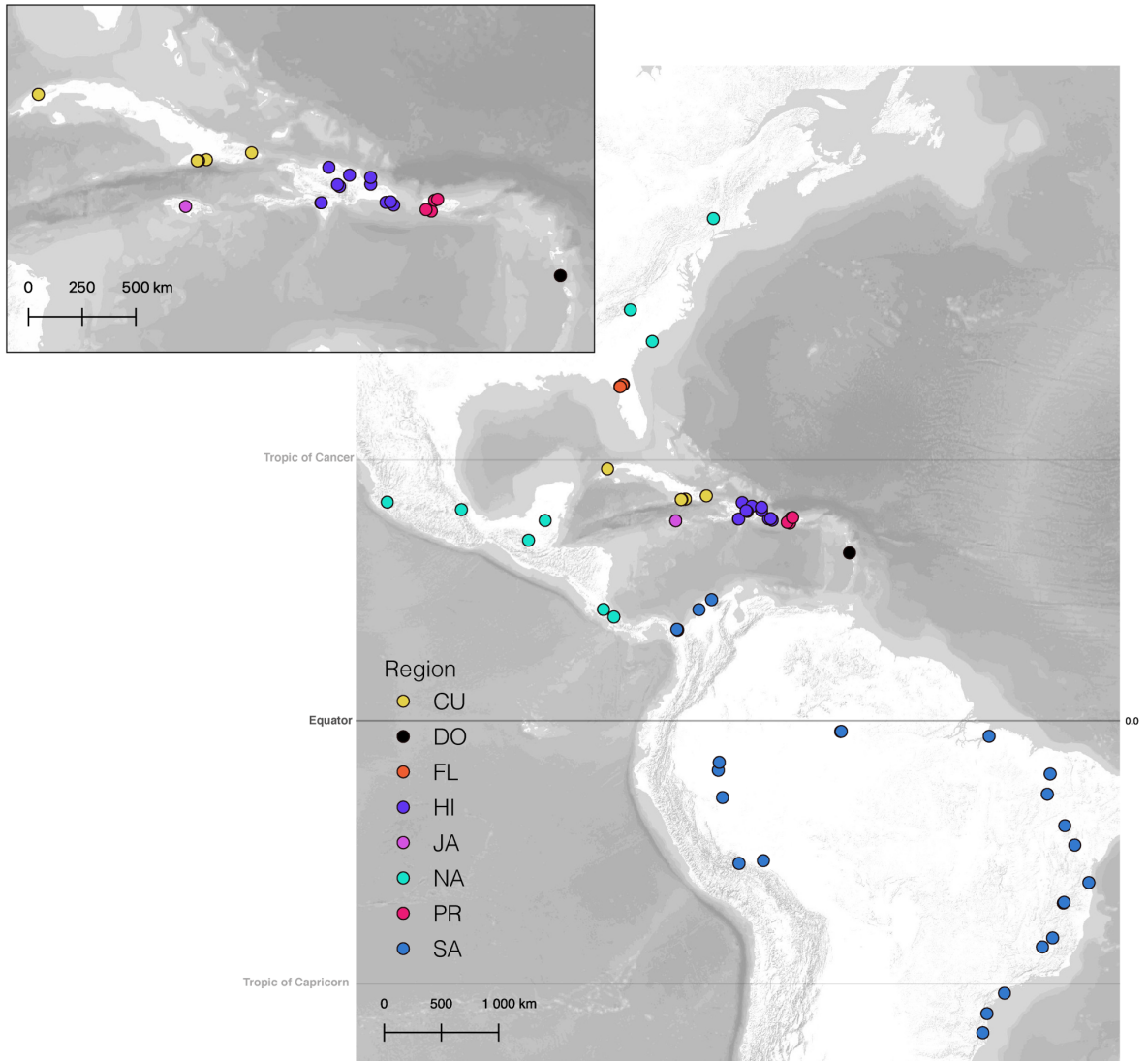
<i>Micrathena</i>	<i>militaris</i>	785066	Hispaniola	19.06707N	069.46355	KJ15703		KJ15714
					W	7		5
<i>Micrathena</i>	<i>militaris</i>	785080	Hispaniola	18.32902N	068.80995	KJ15703	KJ15727	KJ15714
					W	8	4	6
<i>Micrathena</i>	<i>militaris</i>	785099	Hispaniola	18.32902N	068.80995		KJ15731	
					W		3	
<i>Micrathena</i>	<i>militaris</i>	785128	Hispaniola	18.355536	068.61825	KJ15703		KJ15714
				N	W	9		7
<i>Micrathena</i>	<i>militaris</i>	785144	Hispaniola	19.746175	71.257726	KJ15704		KJ15714
				N	W	0		8
<i>Micrathena</i>	<i>militaris</i>	785169	Hispaniola	18.355536	068.61825	KJ15704	KJ15729	KJ15714
				N	W	1	0	9
<i>Micrathena</i>	<i>militaris</i>	785173	Hispaniola	19.06707N	069.46355	KJ15704	KJ15731	KJ15715
					W	2	4	0
<i>Micrathena</i>	<i>militaris</i>	785174	Hispaniola	19.06707N	069.46355	KJ15704	KJ15729	KJ15715
					W	3	2	1
<i>Micrathena</i>	<i>militaris</i>	785194	Hispaniola	18.355536	068.61825	KJ15704		
				N	W	4		
<i>Micrathena</i>	<i>militaris</i>	785208	Hispaniola	18.2205360	68.4806070	KJ15704	KJ15729	KJ15715
				N	W	5	7	2
<i>Micrathena</i>	<i>militaris</i>	785219	Hispaniola	18.355536	068.61825	KJ15704	KJ15728	KJ15715
				N	W	6	6	3
<i>Micrathena</i>	<i>militaris</i>	785263	Hispaniola	18.355536	068.61825	KJ15704		KJ15715
				N	W	7		4
<i>Micrathena</i>	<i>militaris</i>	785273	Hispaniola	19.432213	070.371412	KJ15704	KJ15727	KJ15715
				N	W	8	5	5
<i>Micrathena</i>	<i>militaris</i>	785280	Hispaniola	18.32902N	068.80995	KJ15704	KJ15731	KJ15715
					W	9	5	6
<i>Micrathena</i>	<i>militaris</i>	785312	Hispaniola	19.34405N	069.46635	KJ15705	KJ15728	KJ15715
					W	0	0	7
<i>Micrathena</i>	<i>militaris</i>	785401	Hispaniola	19.06707N	069.46355	KJ15705	KJ15727	KJ15715
					W	1	6	8
<i>Micrathena</i>	<i>militaris</i>	785402	Hispaniola	19.34405N	069.46635	KJ15705	KJ15728	KJ15715
					W	2	5	9
<i>Micrathena</i>	<i>militaris</i>	785423	Hispaniola	18.355536	068.61825	KJ15705		KJ15716
				N	W	3		0
<i>Micrathena</i>	<i>militaris</i>	785461	Hispaniola	19.06707N	069.46355	KJ15705	KJ15728	
					W	4	1	
<i>Micrathena</i>	<i>militaris</i>	785502	Hispaniola	19.06707N	069.46355	KJ15705	KJ15730	KJ15716
					W	5	1	1
<i>Micrathena</i>	<i>militaris</i>	785512	Hispaniola	19.06707N	069.46355	KJ15705	KJ15731	KJ15716
					W	6	6	2
<i>Micrathena</i>	<i>militaris</i>	785524	Hispaniola	18.355536	068.61825	KJ15705	KJ15731	KJ15716
				N	W	7	1	3
<i>Micrathena</i>	<i>militaris</i>	785527	Hispaniola	19.34405N	069.46635	KJ15705	KJ15727	KJ15716
					W	8	9	4
<i>Micrathena</i>	<i>militaris</i>	785563	Hispaniola	19.06707N	069.46355	KJ15705	KJ15729	KJ15716
					W	9	5	5
<i>Micrathena</i>	<i>militaris</i>	785604	Hispaniola	19.06707N	069.46355	KJ15706	KJ15728	KJ15716
					W	0	8	6
<i>Micrathena</i>	<i>militaris</i>	785706	Hispaniola	19.06707N	069.46355	KJ15706	KJ15727	KJ15716
					W	1	8	7

<i>Micrathena</i>	<i>militaris</i>	785709	Hispaniola	19.06707N	069.46355 W		KJ15731	KJ15716 2 8
<i>Micrathena</i>	<i>militaris</i>	785722	Hispaniola	19.06707N	069.46355 W	KJ15706	KJ15728	KJ15716 2 3 9
<i>Micrathena</i>	<i>militaris</i>	785729	Hispaniola	19.34405N	069.46635 W	KJ15706	KJ15728	KJ15717 3 4 0
<i>Micrathena</i>	<i>militaris</i>	785743	Hispaniola	19.06707N	069.46355 W	KJ15706	KJ15728	KJ15717 4 2 1
<i>Micrathena</i>	<i>militaris</i>	785769	Hispaniola	19.06707N	069.46355 W	KJ15706		KJ15717 5 2
<i>Micrathena</i>	<i>militaris</i>	787068	Hispaniola	18.980122 N	70.798425 W	KJ15706	KJ15729	KJ15717 6 9 3
<i>Micrathena</i>	<i>militaris</i>	787106	Hispaniola	18.980122 N	70.798425 W	KJ15706	KJ15728	KJ15717 7 9 4
<i>Micrathena</i>	<i>militaris</i>	787148	Hispaniola	18.3150011 N	71.580556 W	KJ15706	KJ15729	KJ15717 8 1 5
<i>Micrathena</i>	<i>militaris</i>	787152	Hispaniola	18.3150011 N	71.580556 W	KJ15706		KJ15717 9 6
<i>Micrathena</i>	<i>militaris</i>	787166	Hispaniola	18.3150011 N	71.580556 W	KJ15707		KJ15717 0 7
<i>Micrathena</i>	<i>militaris</i>	787190	Hispaniola	18.3150011 N	71.580556 W	KJ15707		KJ15717 1 8
<i>Micrathena</i>	<i>militaris</i>	787208	Hispaniola	18.3150011 N	71.580556 W	KJ15707		KJ15717 2 9
<i>Micrathena</i>	<i>militaris</i>	787212	Hispaniola	18.3150011 N	71.580556 W	KJ15707		KJ15718 3 0
<i>Micrathena</i>	<i>militaris</i>	787214	Hispaniola	18.3150011 N	71.580556 W	KJ15700		KJ15711 1 2
<i>Micrathena</i>	<i>militaris</i>	392672	Puerto Rico	17.971472 N	66.867958 W	KJ15707	KJ15730	KJ15718 4 2 1
<i>Micrathena</i>	<i>militaris</i>	392677	Puerto Rico	17.971472 N	66.867958 W	KJ15707	KJ15730	KJ15718 5 3 2
<i>Micrathena</i>	<i>militaris</i>	782048	Puerto Rico	18.414373 N	66.728722 W	KJ15707	KJ15730	KJ15718 6 7 3
<i>Micrathena</i>	<i>militaris</i>	782126	Puerto Rico	18.173264 N	66.590149 W	KJ15707	KJ15730	KJ15718 7 8 4
<i>Micrathena</i>	<i>militaris</i>	782153	Puerto Rico	18.414373 N	66.728722 W	KJ15707	KJ15730	KJ15718 8 6 5
<i>Micrathena</i>	<i>militaris</i>	782174	Puerto Rico	18.414373 N	66.728722 W	KJ15707	KJ15730	KJ15718 9 4 6
<i>Micrathena</i>	<i>militaris</i>	782201	Puerto Rico	18.032518 N	67.094653 W	KJ15708	KJ15730	KJ15718 0 5 7
<i>Micrathena</i>	<i>militaris</i>	783400	Puerto Rico	18.45226N	66.59711W		KJ15730	9
<i>Micrathena</i>	<i>mitrata</i>	10000679 A	Mexico	19.79357N	104.0554W			x x
<i>Micrathena</i>	<i>mitrata</i>	00002849 A	Mexico	19.79357N	104.0554W			x x
<i>Micrathena</i>	<i>nigrichelis</i>	MIC056	Brazil	20.43481S	43.50906W		KJ15723	9
<i>Micrathena</i>	<i>perfida</i>	MIC026	Brazil	24.387111S	47.017583 W		KX6873	18

<i>Micrathena</i>	<i>plana</i>	MIC062	Brazil	16.53294S	41.51042W		KJ15724 0		
<i>Micrathena</i>	<i>reimoseri</i>	MIC072	Brazil	11.399833S	40.522056 W		KX6873 21		
<i>Micrathena</i>	<i>saccata</i>	MIC076	Brazil	1.424828S	48.43802W		KJ15723 7		
<i>Micrathena</i>	<i>sagittata</i>	10000618 A	Florida, USA	29.4776N	082.5627W		x		
<i>Micrathena</i>	<i>sagittata</i>	10000621 A	Florida, USA	29.63703N	082.23976 W		x		
<i>Micrathena</i>	<i>sagittata</i>	10000631 A	Florida, USA	29.62986N	082.29880 W		x	x	
<i>Micrathena</i>	<i>sagittata</i>	10000633 A	Florida, USA	29.62986N	082.29880 W		x		
<i>Micrathena</i>	<i>sagittata</i>	10000636 A	Florida, USA	29.63680N	082.23961 W		x	x	
<i>Micrathena</i>	<i>sagittata</i>	10000634 A	Florida, USA	29.46397N	082.55285 W		x	x	
<i>Micrathena</i>	<i>sagittata</i>	10000639 A	Florida, USA	29.63680N	082.23961 W		x		
<i>Micrathena</i>	<i>sagittata</i>	10000640 A	Florida, USA	29.62688N	082.29878 W		x		
<i>Micrathena</i>	<i>sagittata</i>	00002847 A	Mexico	18.18963N	89.46333W		x		
<i>Micrathena</i>	<i>sagittata</i>	00000833 A	SC, USA	33.03913 N	79.56459W	KJ15708 1	KJ15722 1	KJ15718 9	
<i>Micrathena</i>	<i>schreibersi</i>	00002357 A	Colombia	Bucaraman ga			x		
<i>Micrathena</i>	<i>schreibersi</i>	10000650 A	Colombia	8.39104N	77.21548W		x		
<i>Micrathena</i>	<i>schreibersi</i>	10000652 A	Colombia	8.39104N	77.21548W		x		
<i>Micrathena</i>	<i>schreibersi</i>	10000653 A	Colombia	8.39104N	77.21548W		x	x	
<i>Micrathena</i>	<i>schreibersi</i>	10000664 A	Colombia	8.424N	77.29216W		x		
<i>Micrathena</i>	<i>schreibersi</i>	10000673 A	Colombia	8.39104N	77.21548W		x		
<i>Micrathena</i>	<i>schreibersi</i>	10000658 A	Colombia	8.39104N	77.21548W		x		
<i>Micrathena</i>	<i>schreibersi</i>	10000651 A	Colombia	8.39104N	77.21548W		x	x	
<i>Micrathena</i>	<i>schreibersi</i>	10000663 A	Colombia	8.424N	77.29216W		x		
<i>Micrathena</i>	<i>schreibersi</i>	10000665 A	Colombia	8.424N	77.29216W		x	x	
<i>Micrathena</i>	<i>schreibersi</i>	00004787 A	Colombia	10.21192N	75.25403W		x	x	
<i>Micrathena</i>	<i>schreibersi</i>	00004818 A	Trinidad				x	x	
<i>Micrathena</i>	<i>schreibersi</i>	00002900 A	Costa Rica	10.430686 N	84.007089 W		x	x	

<i>Micrathena</i>	<i>schreibersi</i>	00000936 A	Colombia	7.062695N	73.073058 W	KJ15709 0	KJ15731 8	KJ15719 8
<i>Micrathena</i>	<i>schreibersi</i>	00002357 A	Colombia	7.062695N	73.073058 W	KJ15709 2	KJ15731 9	KJ15719 9
<i>Micrathena</i>	<i>sexspinosa</i>	10000690 A	Colombia	8.35249N	77.22118W		x	
<i>Micrathena</i>	<i>sexspinosa</i>	10000659 A	Colombia	8.35249N	77.22118W		x	
<i>Micrathena</i>	<i>sexspinosa</i>	10000674 A	Colombia	8.35249N	77.22118W		x	x
<i>Micrathena</i>	<i>sexspinosa</i>	10000677 A	Colombia	11.120083 N	74.082805 W		x	
<i>Micrathena</i>	<i>sexspinosa</i>	10000683 A	Colombia	11.120083 N	74.082805 W		x	
<i>Micrathena</i>	<i>sexspinosa</i>	10000669 A	Colombia	8.39104N	77.21548W		x	x
<i>Micrathena</i>	<i>sexspinosa</i>	10000670 A	Colombia	8.39104N	77.21548W		x	x
<i>Micrathena</i>	<i>sexspinosa</i>	10000681 A	Colombia	8.35249N	77.22118W		x	
<i>Micrathena</i>	<i>sexspinosa</i>	10000678 A	Colombia	8.35249N	77.22118W		x	
<i>Micrathena</i>	<i>sexspinosa</i>	00000987 A	Colombia	7.062695N	73.073058 W	KJ15709 1	KJ15722 2	
<i>Micrathena</i>	<i>similis</i>	785024	Hispaniola	19.34405N	69.46635W	KJ15709 3	KJ15722 8	KJ15720 0
<i>Micrathena</i>	<i>similis</i>	785496	Hispaniola	19.34405N	69.46635W	KJ15709 4	KJ15723 2	KJ15720 1
<i>Micrathena</i>	<i>similis</i>	787265	Hispaniola	19.05116N	70.88866W	KJ15709 5	KJ15723 3	KJ15720 2
<i>Micrathena</i>	<i>similis</i>	787297	Hispaniola	19.05116N	70.88866W	KJ15709 6		KJ15720 3
<i>Micrathena</i>	<i>similis</i>	787308	Hispaniola	19.03627N	70.54337W	KJ15709 7	KJ15722 9	KJ15720 4
<i>Micrathena</i>	<i>similis</i>	787309	Hispaniola	19.05116N	70.88866W	KJ15709 8		KJ15720 5
<i>Micrathena</i>	<i>similis</i>	787311	Hispaniola	19.05116N	70.88866W		KJ15723 5	KJ15720 6
<i>Micrathena</i>	<i>similis</i>	787318	Hispaniola	19.03627N	70.54337W	KJ15709 9	KJ15723 4	KJ15720 7
<i>Micrathena</i>	<i>similis</i>	787320	Hispaniola	19.05116N	70.88866W	KJ15710 0	KJ15723 0	KJ15720 8
<i>Micrathena</i>	<i>similis</i>	787322	Hispaniola	19.05116N	70.88866W	KJ15710 1	KJ15723 1	KJ15720 9
<i>Micrathena</i>	<i>sp.</i>	10000656 A	Colombia	11.120083 N	74.082805 W		x	
<i>Micrathena</i>	<i>sp.</i>	10000671 A	Colombia	11.120083 N	74.082805 W		x	x
<i>Micrathena</i>	<i>sp.</i>	00006693 A	Colombia	11.120083 N	74.082805 W		x	x
<i>Micrathena</i>	<i>spinulata</i>	MIC205	Mexico	19.1381667 N	97.2045W		KX6873 24	

<i>Micrathena</i>	<i>triangularispinosa</i>	MIC156	Brazil	0.97799S	62.10292W			KX6873 27
<i>Micrathena</i>	<i>yanomami</i>	MIC193	Peru	13.055639S	71.546194 W			KX6873 32
Outgroups								
<i>Achaearanea</i>	<i>sp.</i>	784841	Cuba	21.59166N	77.78822W			KJ15721 1
<i>Argiope</i>	<i>lobata</i>	Arg0160	Spain	Missing GPS data		KJ15698 8		KJ15710 3
<i>Gasteracantha</i>	<i>cancriformis</i>	787198	Hispaniola	18.3150011 N	71.580556 W	KJ15698 9	KJ15721 2	
<i>Gasteracantha</i>	<i>cancriformis</i>	784515	Hispaniola	18.2205260 N	68.480607 W		KJ15721 3	
<i>Gasteracantha</i>	<i>cancriformis</i>	782149	Puerto Rico	18. 172979N	66.491798 W	KJ15699 0	KJ15721 4	



**Figure 1:** Map of collection localities of all specimens included in analysis. Points are colored by biogeographic area assigned for BioGeoBEARS analysis.

### 1.2.2 Tissue Extraction and PCR

Tissue samples were taken from the right legs, and DNA was isolated using the QIAGEN 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 ITS4S [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

### 1.2.3 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.

#### *1.2.4 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 +  $\Gamma$  for position 1, TVM + I +  $\Gamma$  for position 2, and TRN +  $\Gamma$  for position 3), along with an unpartitioned analysis, which was run using the best-fit model for CO1 overall (GTR + I +  $\Gamma$ ). 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 (ucl.d.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). 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]. 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 explainer 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 ( $\Delta\text{AICc}$  of 2 was considered significant) [84].

### *1.2.5 Specimen Photography*

Specimen photographs, depicting morphological variation between the populations or species, were taken using a Canon 5D camera with a 65 mm macro 5x 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.

## *1.3 Results*

### *1.3.1 Sequence Alignment*

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

### 1.3.2 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*.

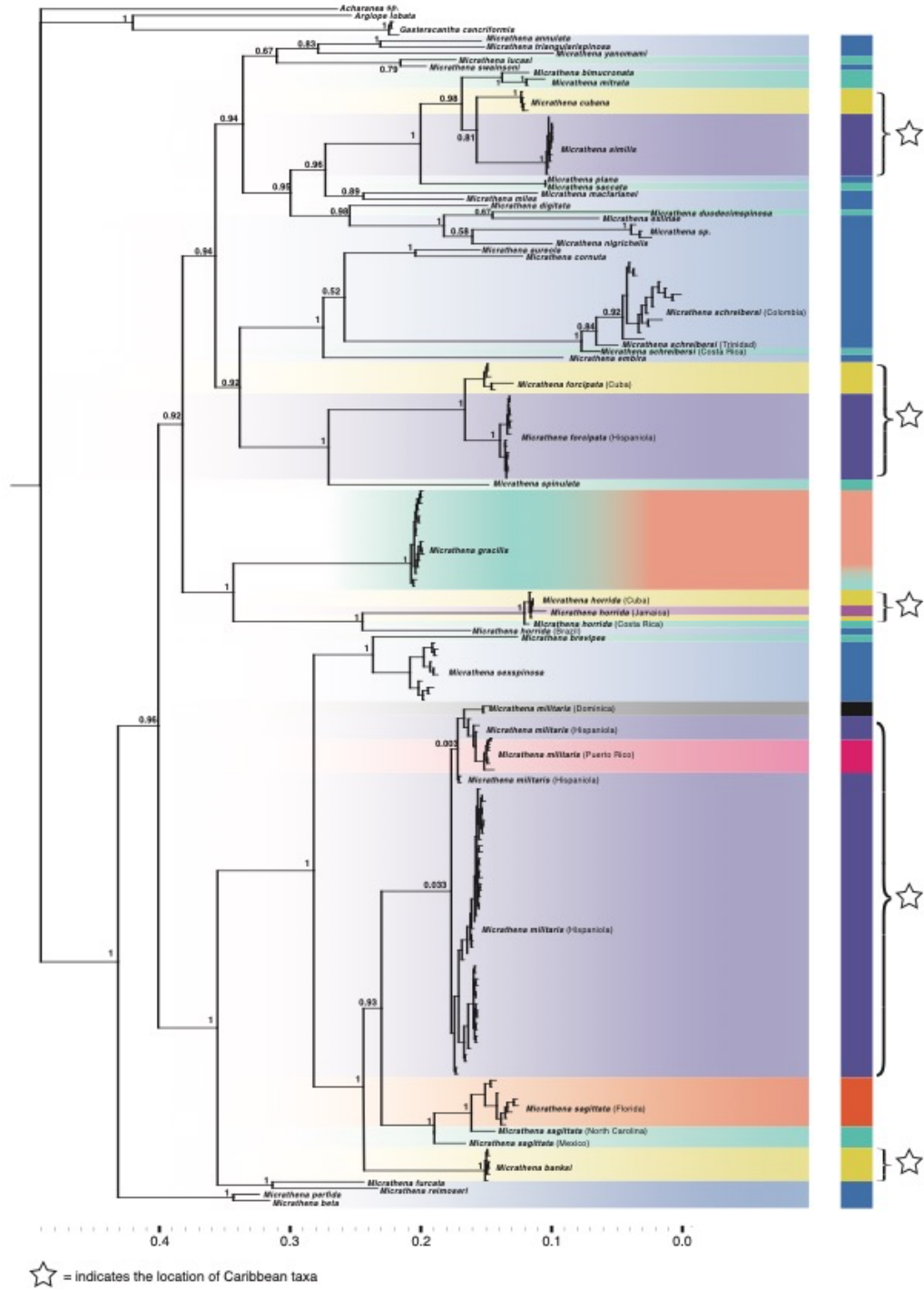
The *gracilis*-group, including *M. gracilis* and *M. horrida*, was additionally delineated but did not include *M. forcipata* in our multilocus analysis (Figure 3). Instead, we found that *Micrathena forcipata* was located as a sister to *M. schreibersi*, together forming the sister group to the *furcula* 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).

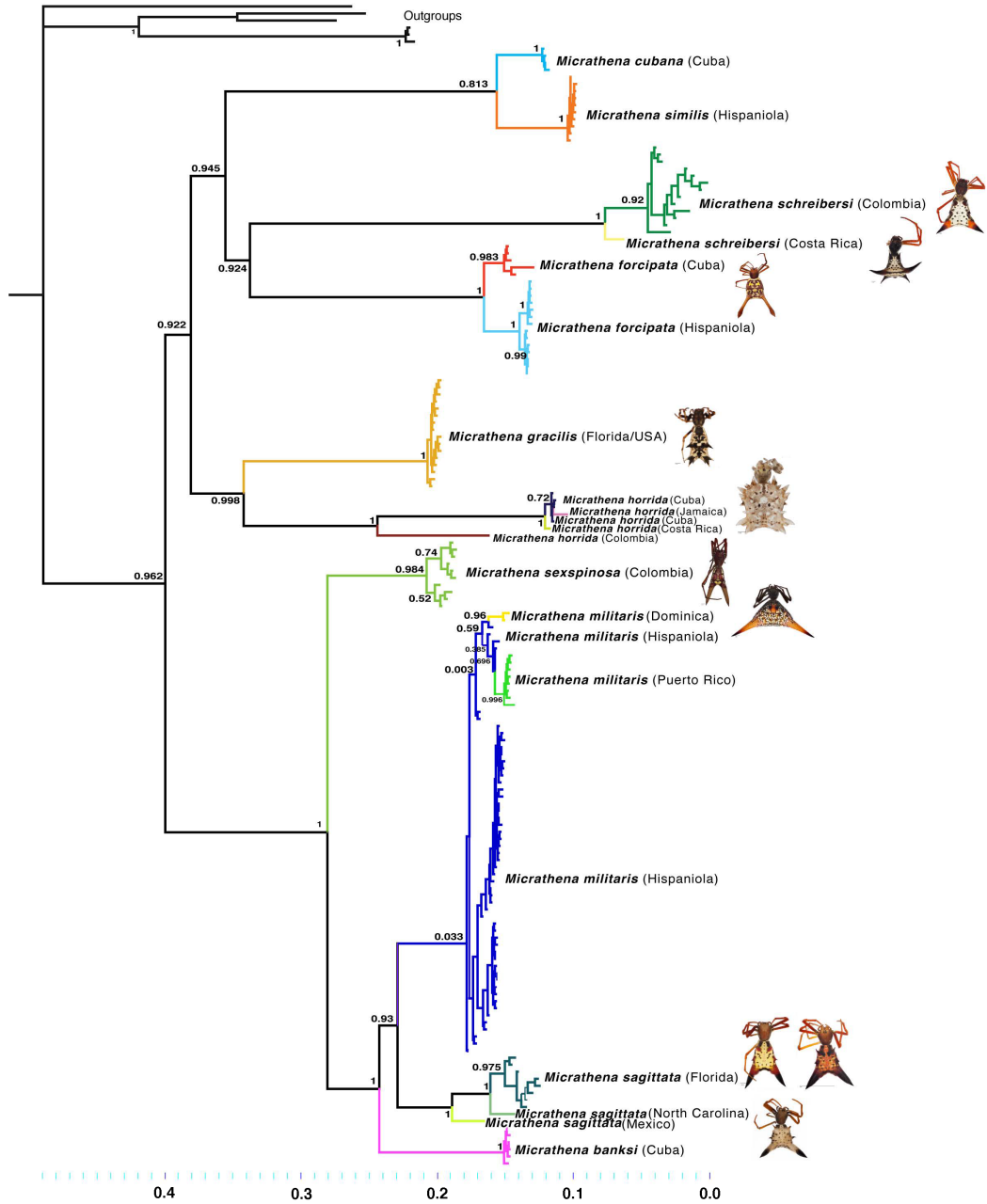
Our analysis also produced evidence in support of single-island endemism and island monophyly of *Micrathena forcipata*. High levels of island genetic structuring and relatively deep divergences were observed between *M. forcipata* from Cuba and *M. forcipata* from Hispaniola (Figures 2–5). At a finer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

### 1.3.3 Divergence Times



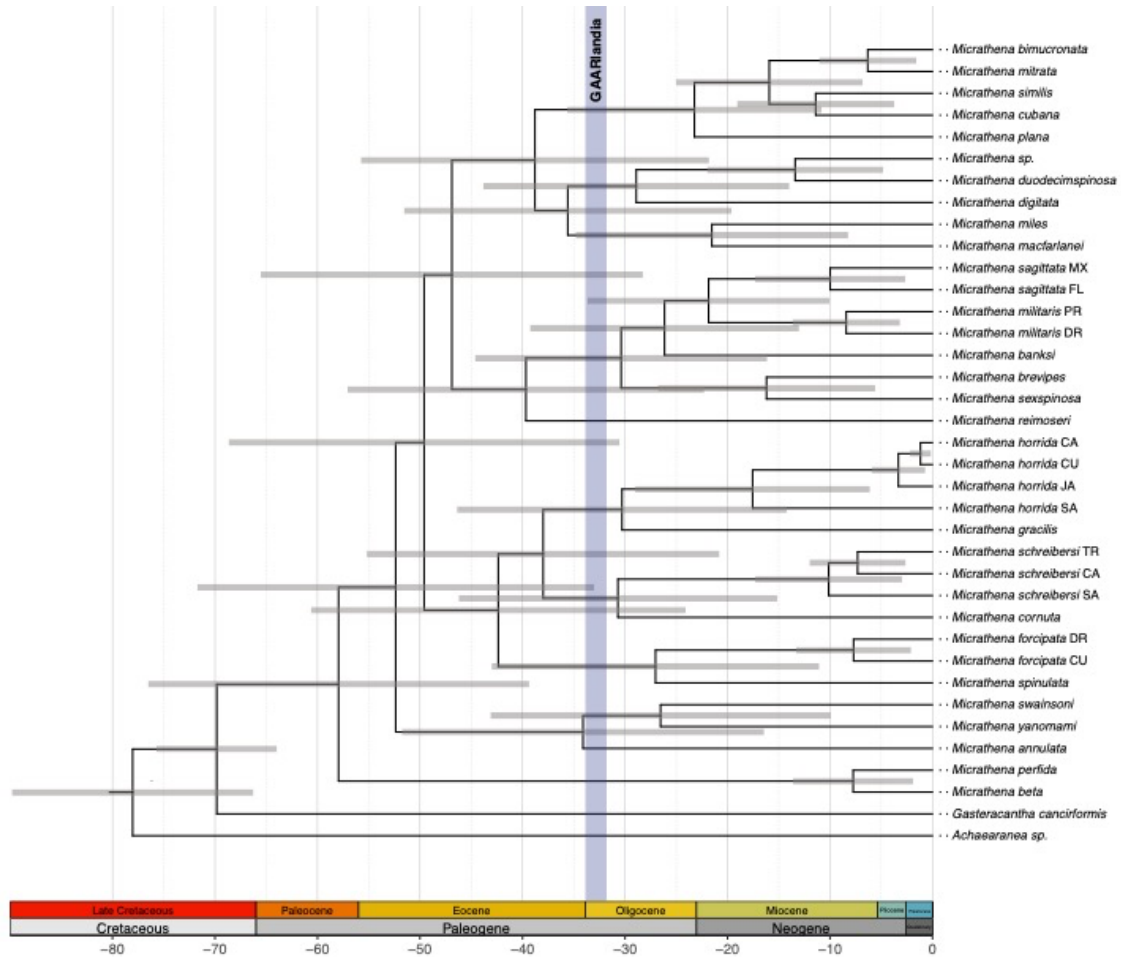
**Figure 2:** Complete consensus tree from MrBayes concatenated analysis depicting relationships among all sampled *Micrathena* species. Outgroups are located at the top of the phylogeny. Here, terminal individual labels have been replaced with species names along with locality. Overlaying colors are 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 distance.

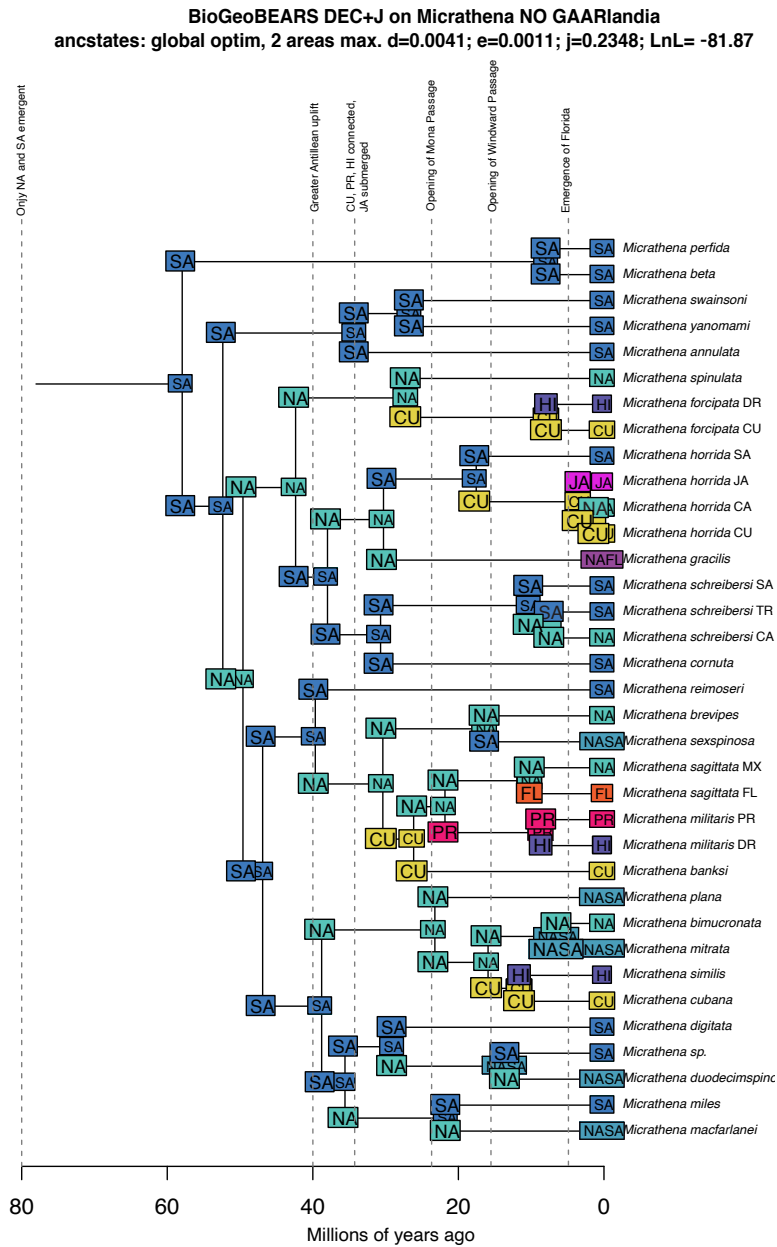
Only CO1 data were used to build our dated phylogeny, as sequences were available for various South American taxa for which data on other loci were absent.





**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

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 *Micrathena* 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



**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 are indicated by dotted lines.

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 *Micrathena* were ostensibly polyphyletic (Figures 2–5)

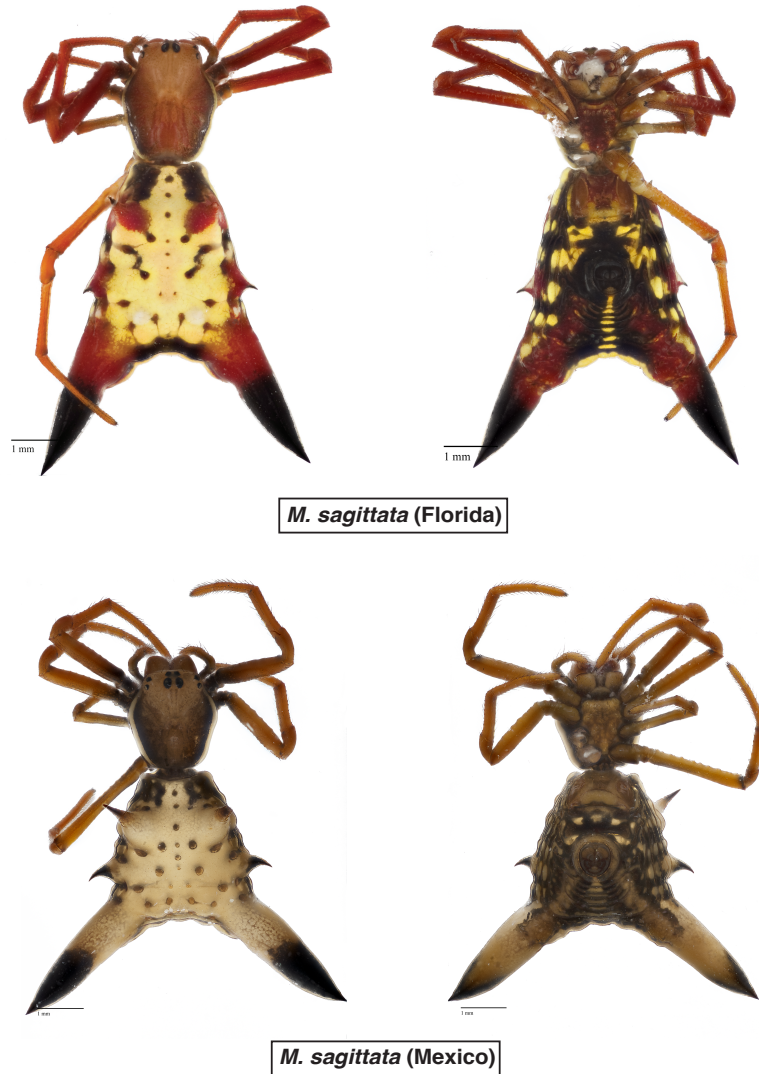
#### 1.3.4 Biogeographic Patterns

The ancestral range reconstruction in BioGeoBEARS suggested five independent colonizations of the Caribbean by *Micrathena* (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). *Micrathena* 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 *Micrathena* 52 million years ago and, subsequently, multiple

bifurcations between North/Central and South American *Micrathena* occurred thereafter (Figure 5). These results indicated that a fraction of *Micrathena*, other than the *swainsoni* and *perfidia* 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).

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



**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*.

*M. horrida*), 30 Ma (in the *M. militaris* clade), and 16 Ma (within the *M. similis*/*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).

**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  $\Delta AICc$  is AIC-min(AIC).

Model	LnL	Number of Parameters	d	e	j	AICc	AICc Weight	$\Delta AICc$
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	$2.9 \times 10^{-6}$	24.3
DEC + J GAARlandia	-94.48	3	0.025	$1.00 \times 10^{-12}$	2.4	195.7	$1.90 \times 10^{-6}$	25.2
DIVALIKE + J GAARlandia	-97.42	3	0.027	$1.00 \times 10^{-12}$	1.7	201.6	$9.90 \times 10^{-8}$	31.1
DEC no-GAARlandia	-99.69	2	0.013	0.0063	0	203.8	$3.40 \times 10^{-8}$	33.3
BAYAREALIKE no-GAARlandia	-107.9	2	0.017	0.025	0	220.2	$8.90 \times 10^{-12}$	49.7
BAYAREALIKE GAARlandia	-112	2	0.24	0.025	0	228.4	$1.50 \times 10^{-13}$	57.9
DIVALIKE GAARlandia	-112.8	2	0.11	0.0058	0	230	$6.90 \times 10^{-14}$	59.5
DEC GAARlandia	-112.9	2	0.16	0.01	0	230.2	$6.00 \times 10^{-14}$	59.7

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 BioGeoBEARS results are in agreement that colonization events are not tied to dispersal via the GAARlandia landbridge.

#### *1.4 Discussion*

Molecular analyses, with the expanded taxon sampling of *Micrathena*, 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 *Micrathena* 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 *Micrathena* 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 *Micrathena* 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 *Micrathena* across the Caribbean, as proposed for other arthropod groups [88–90]. Given that the Caribbean lineages of *Micrathena* 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 *Micrathena* to colonize the Caribbean reflect modern and paleoceanographic dynamics. Future investigations may consider integrating paleowind and paleocurrent data to better explain fine-scale dispersal



routes of Caribbean colonization that crisscross 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 *Micrathena*, 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 *Micrathena* and indicates that, although they are considered relatively poor dispersers due to their apparent bulkiness and elaborate spine coverage, *Micrathena* 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 *Micrathena*, and biogeographic investigations may benefit from increased physiological and behavioral analyses of the genus.

We recovered four distinct *Micrathena* 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 Magalhaães et al. [53], McHugh et al. [51], and this investigation (multilocus dataset, 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	Magalhaães et al., 2012	McHugh et al., 2014	Current <i>Micrathena</i> Study
<i>furcula</i>	<i>M. cubana</i> , <i>M. similis</i>	<i>M. cubana</i> , <i>M. similis</i>	<i>M. cubana</i> , <i>M. similis</i>
	<i>M. banksi</i> , <i>M. militaris</i> ,	<i>M. banksi</i> , <i>M. militaris</i> ,	<i>M. banksi</i> , <i>M. militaris</i> ,
<i>militaris</i>	<i>M. sagittata</i> , <i>M.</i>	<i>M. sagittata</i> , <i>M.</i>	<i>M. sagittata</i> , <i>M.</i>
	<i>sexspinosa</i>	<i>sexspinosa</i>	<i>sexspinosa</i>
<i>gracilis</i>	<i>M. horrida</i> , <i>M. gracilis</i> ,	<i>M. horrida</i> , <i>M. gracilis</i> <sup>1</sup>	<i>M. horrida</i> , <i>M. gracilis</i> <sup>2</sup>
	<i>M. forcipata</i>		

<sup>1</sup>*M. schreibersi* is the sister to the *gracilis* group; *M. forcipata* is the sister to the *furcula* group. <sup>2</sup>*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



**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.

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.

### *1.5 Conclusion*

We present a detailed molecular phylogenetic and biogeographic analysis of *Micrathena*, 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 *Micrathena*, 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.

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