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*Invertebrate Systematics*, 2014, **28**, 337–349
http://dx.doi.org/10.1071/IS13051

# Molecular phylogenetics of Caribbean *Micrathena* (Araneae : Araneidae) suggests multiple colonisation events and single island endemism

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**Abstract.** The terrestrial biota of the Caribbean islands includes many lineages, some whose presence on the islands dates back some 35–40 million years ago, when land bridges are thought to have linked islands to continents, and others that have colonised more recently via dispersal. The New World spiny orb-weavers (*Micrathena* Sundevall, 1833) are a diverse group of mostly Neotropical spiders. Eight species have been described on the Greater Antilles islands: three widespread and five single island endemics. Here, using three molecular markers (16S rRNA, ITS-2 and COI) we provide a preliminary phylogenetic test of the taxonomy and biogeography of Caribbean *Micrathena* through the first molecular phylogeny of the genus. Our analyses support monophyly of the genus, but not that of Caribbean *Micrathena* with at least 3–4 colonisations from South America. We sampled six of the eight nominal Caribbean species (*M. banksi*, *M. cubana*, *M. similis*, *M. forcipata*, *M. horrida*, *M. militaris*), but demark eight divergent genetic lineages that all are single island endemics, and morphologically distinct. Thus a revision of the taxonomy of Caribbean *Micrathena* is needed. Our results function foremost to guide more thorough taxon sampling of *Micrathena* that enable more rigorous assessments of its diversity and biogeography in the Caribbean.

**Additional keywords:** biogeography, Cenozoic dispersal, phylogeography, spiny orb-weavers, vicariance.

Received 18 October 2013, accepted 3 February 2014, published online 12 September 2014

## Introduction

The Caribbean archipelago is a hotspot of biological diversity, with large numbers of endemic birds, reptiles, plants, fish and arthropods (Myers *et al*. 2000; Ricklefs and Bermingham 2008). Some of the Caribbean islands have remained emergent during approximately the past 40 million years, at times potentially connected to South America through land bridges (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006). Modern Caribbean biodiversity is thus the product of a long history of vicariance, dispersal and subsequent speciation. Our understanding of the processes underlying the formation of biodiversity in the Caribbean is advancing rapidly (Dávalos 2004; Ricklefs and Bermingham 2008; Říčan *et al*. 2013). These advances are mainly due to the use of molecular methods in taxonomy, phylogenetics and biogeography (Ricklefs and Bermingham 2008; Agnarsson and Kuntner 2012). Such methods often reveal greater diversity and finer scale biogeographical patterns than earlier work based on morphological data alone (e.g. Losos and Schluter 2000; Losos *et al*. 2006).

*Micrathena* is a genus of highly decorative, spiny orb-weaving spiders (Araneidae) containing some 115 species distributed throughout South and Central America, southern and eastern United States, and the Caribbean (Levi 1985; Magalhães and Santos 2012). The bulk of the diversity is in South America with 55 endemic and many widespread species (Platnick 2013). Eight species are known in the Caribbean, 33 in Central America, and four in North America. Of the Caribbean taxa, three are currently considered widespread, that is, found on more than one island or on continents (Levi 1985). The remaining five are thought to be single island endemics, three from Cuba and one each from Jamaica and Hispaniola (Levi 1985; Platnick 2013). Levi's (1985) taxonomy of *Micrathena* has been recently revised (Magalhães and Santos 2012). These authors define 12 species-groups. Caribbean species are distributed in three of these groups, which also contain three out of the four North American species. Conclusions of this revision are consistent with Levi's (1985) assessment that Caribbean *Micrathena* are not monophyletic, implying multiple colonisation events, and that the genus represents a mixture of widespread and narrow endemic species in the region (Levi 1985; Magalhães and Santos 2012). Other spider taxa also show multiple colonisations and the number of colonisation events seems to correlate roughly with dispersal ability (e.g. Crews and Gillespie 2010; Zhang and Maddison 2013). While Magalhães and Santos (2012) present a robust morphological phylogeny, our current

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study specifically targets Caribbean taxa using molecular markers.

Here, we present the first molecular phylogeny of the genus *Micrathena* with a focus on the Caribbean species. We test molecular support for: (1) the proposed species-groups, suggesting non-monophyly of Caribbean *Micrathena*, and (2) morphologically delimited species. We also preliminarily assess biogeographic patterns by exploring support for endemicity of nominal species and divergent genetic lineages, and by conducting molecular dating analyses to see if divergence dates are consistent with Caribbean geological history. A central goal is to guide future research effort and propose new hypotheses that can be tested with additional data.

## Materials and methods

Specimens were collected using standard aerial searching and beating methods in Cuba, Hispaniola, Puerto Rico, Colombia and North America and fixed in 95% ethanol (Fig. 1). To test the origin of the Caribbean groups, COI sequences of additional *Micrathena* specimens from South and Central America were kindly made available by Ivan L. F. Magalhães (Santos laboratory, Brazil). As the primary outgroup we used the theridiid genus *Achaearanea*, and then included six araneid species of the genera *Zygiella*, *Argiope* and three *Gasteracantha*. Caribbean voucher specimens will be deposited at the National Museum of Natural History, Smithsonian Institution. Taxon sample information is included in Table 1.

DNA was isolated from 119 individuals, with the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA, USA). We sequenced fragments of two mitochondrial (cytochrome c oxidase subunit 1, COI, and 16S rRNA) and one nuclear (internal transcribed spacer 2-ITS2) loci previously demonstrated to be effective phylogenetic markers at low taxonomic levels for spiders (Agnarsson *et al*. 2007; Agnarsson 2010; Kuntner and Agnarsson 2011). We amplified COI with LCO1490 (Folmer *et al*. 1994) and C1-N-2776 (Hedin and Maddison 2001). We used 16SA and B primers (Simon *et al*. 1994) to amplify the 16S rRNA marker, and the ITS-5.8S (FITS) and ITS-28S (RITS, or ITS 4) primers for ITS2 (White *et al*. 1990). Polymerase chain reaction (PCR) conditions are included in Table 2. Sequencing was done at the University of Arizona. Sequences were submitted to GenBank (accession numbers: COI: KJ157211–KJ157320; 16S: KJ156988–KJ157102; ITS2: KJ157103–KJ157210).

Sequences were interpreted from chomatograms using Phred and Phrap (Green and Ewing 2002; Green 2009) using the Chromaseq module (Maddison and Maddison 2011*a*) in the evolutionary analysis program Mesquite 2.75 (Maddison and Maddison 2011*b*) with default parameters. The sequences were then proofread by examining chromatograms by eye. Alignments were done in MAFFT (Katoh 2013) through the online portal EMBL-EBI, using default settings except increasing the tree rebuilding and maxiterate settings to 100. Gaps were treated as missing characters, a maximum likelihood analysis including gaps as fifth bases gave identical results. For Bayesian analyses, the appropriate substitution model was selected with jModeltest 2.1.4 (Darriba *et al*. 2012) using the Akaike information criterion (Posada and Buckley 2004) to select among the 24 models implemented in MrBayes. The best model for COI and 16S was GTR+G+I, and for ITS2 was SYM+G+I. We ran a Bayesian analysis of each locus separately using the CIPRES online portal (Altekar *et al*. 2004; Miller *et al*. 2010) and the three loci concatenated locally using MrBayes V3.1.2 (Huelsenbeck *et al*. 2001; Ronquist and Huelsenbeck 2003). The concatenated analysis was partitioned by locus. We ran the Markov chain Monte Carlo with four chains for 30 000 000 generations, sampling the Markov chain every 1000 generations. The results were examined in Tracer 1.5 (Rambaut and Drummond 2007) to verify proper mixing of chains and that stationarity had been reached, and to determine adequate burn-in. Maximum likelihood analysis of the concatenated matrix was done using Garli (Zwickl 2006) using the same partitioning scheme and models.

Node ages were estimated using BEAST 1.7.5. under a relaxed clock model (Drummond *et al*. 2012). As we only have sequence data for South American taxa for COI, the BEAST analysis was run only on this alignment. Because of the absence of a fossil record for *Micrathena* and closely related spider lineages we calibrated the phylogeny based on the estimated age of Araneidae and of the most recent common ancestor, including Theridiidae and Araneidae from the fossil calibrated study of Kuntner *et al*. (2013). The age of Araneidae was set as a normal prior with mean 70 million years and s.d. = 3. Similarly, the ancestor of Theridiidae plus Araneidae was set at mean 100 million years and s.d. = 9. In both cases distribution covered 95% confidence intervals from Kuntner *et al*. (2013). For this analysis we pruned terminal taxa eliminating only 'redundant' taxa (species represented by more than one individual) with less than 80% of the COI sequence data. We also used average and standard deviation of substitution rates of COI that have been estimated and have been found to be similar across several spider lineages, and can thus be used to approximately estimate divergence times (Bidegaray-Batista and Arnedo 2011; Kuntner *et al*. 2013). Thus, we set the COI mitochondrial substitution rate parameter (ucld.mean) as a normal prior with mean = 0.0112 and s.d. = 0.001. Only COI data were used for the dating analysis as other markers are not yet available for the majority of the South American taxa. For the BEAST analysis the monophyly of *Micrathena* was constrained, based on the results of our concatenated analysis. The analysis was run for 30 000 000 generations with a Yule tree prior, chosen as most species in that matrix are represented only by a single terminal taxon (Drummond *et al*. 2012). The results were again examined in Tracer 1.5 (Rambaut and Drummond 2007) to determine burn-in and to check for stationarity.

We ran an analysis of ancestral ranges in LaGrange (Ree and Smith 2008). For this analysis, each terminal (each species was reduced to a single terminal taxon for this analysis) was scored based on collection locality, with areas demarked as Cuba, Hispaniola, Puerto Rico, North America, South America and Central America. In LaGrange we assumed the root ancestral area for *Micrathena* to be South America as most of the missing *Micrathena* species in this analysis were South American and some were hypothesised to be basal to our ingroup based on Magalhães and Santos (2012). No dispersal constraints were enforced in LaGrange, but each node was

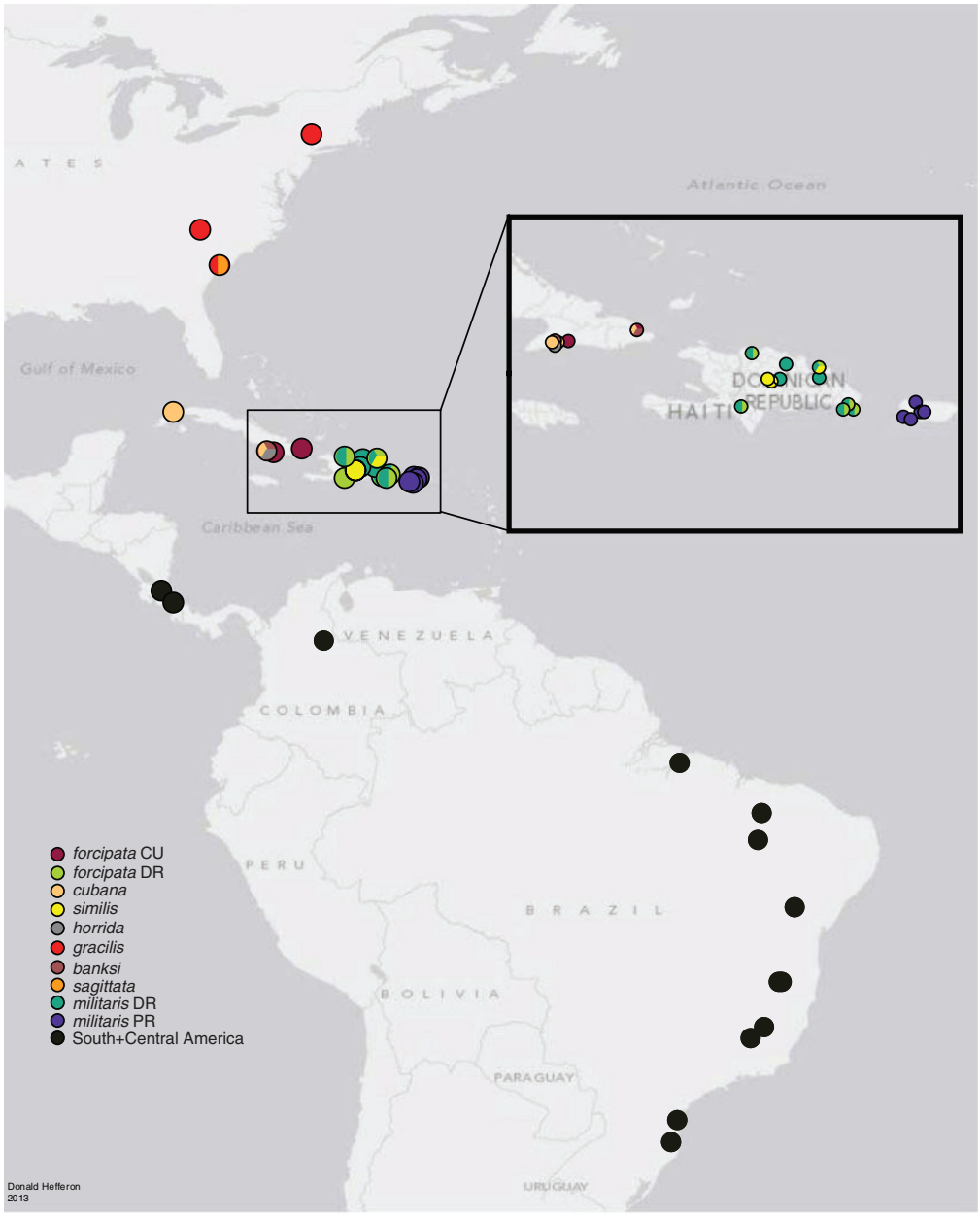


**Fig. 1.** Map of collecting localities of all specimens used for the molecular analysis. Localities are coloured by species name.

constrained to two putative ancestral areas. The data were configured in Lagrange configurator online (www.reelab.net/LaGrange/configurator).

Mitochondrial uncorrected genetic distances were calculated within and among lineages using Mega 5.2 (Table 3), with nominal species defined as groups, and nominal species that span more than one island divided into groups by island.

Photographs were taken using the Visionary Digital BK laboratory system, equipped with a Canon 5D camera and a 65 mm macro 5× zoom lens. Successive images were combined with Helicon Focus 5.3 and thereafter minimally processed with Photoshop CS6 to adjust for both contrast and brightness and to remove background blemishes. For photography, anatomical preparations were temporarily mounted in alcohol-based hand sanitiser (65% ethanol), and the specimen then covered with 95% ethanol. Trees were edited in Mesquite and then exported as PDF files and all figures were compiled and finalised in Adobe Illustrator.

**Table 1. Taxon sample with specific collection information and accession numbers**

**O**, blank. Ivan Magalhães donated 14 COI sequences and Matjaž Kuntner donated the outgroup sequence data for COI, ITS2 and 16S for *Zygiella atrica*, *Argiope lobata* and *Argiope agbru*

| Genus | Species | Barcode | Country | Latitude | Longitude | 16S | COI | ITS2 |
|---|---|---|---|---|---|---|---|---|
| *Micrathena* | *annulata* | MIC007 | Brazil | 26.08933 S | 48.64006 W | **O** | KJ157272 | **O** |
| *Micrathena* | *aureola* | MIC009 | Brazil | 4.904167 S | 42.79083 W | **O** | KJ157249 | **O** |
| *Micrathena* | *banksi* | 784750 | Cuba | 20.05269 N | 76.50296 W | KJ156991 | KJ157215 | KJ157104 |
| *Micrathena* | *banksi* | 784760 | Cuba | 20.0107 N | 76.8843 W | KJ156992 | KJ157216 | **O** |
| *Micrathena* | *banksi* | 784976 | Cuba | 20.00939 N | 76.89402 W | KJ156993 | KJ157217 | KJ157105 |
| *Micrathena* | *banksi* | 785101 | Cuba | 20.00939 N | 76.89402 W | KJ156994 | KJ157220 | KJ157106 |
| *Micrathena* | *banksi* | 785175 | Cuba | 20.33178 N | 74.56919 W | KJ156995 | KJ157219 | KJ157107 |
| *Micrathena* | *banksi* | 787933 | Cuba | 20.01742 N | 76.89781 W | KJ156996 | KJ157218 | KJ157108 |
| *Micrathena* | *bimucronata* | MIC123 | Costa Rica | 10.233518 N | 84.075411 W | **O** | KJ157236 | **O** |
| *Micrathena* | *brevipes* | MIC121 | Costa Rica | 09.552960 N | 83.112910 W | **O** | KJ157223 | **O** |
| *Micrathena* | *cubana* | 784355 | Cuba | 20.01309 N | 76.83400 W | KJ156997 | KJ157224 | KJ157109 |
| *Micrathena* | *cubana* | 784820 | Cuba | 20.00874 N | 76.88777 W | KJ156998 | KJ157225 | KJ157110 |
| *Micrathena* | *cubana* | 785048 | Cuba | 22.65707 N | 83.70161 W | KJ156999 | KJ157226 | KJ157111 |
| *Micrathena* | *cubana* | 787840 | Cuba | 20.33178 N | 74.56919 W | KJ157000 | KJ157227 | **O** |
| *Micrathena* | *digitata* | MIC017 | Brazil | 11.39983 S | 40.52206 W | **O** | KJ157238 | **O** |
| *Micrathena* | *forcipata* | 784425 | Cuba | 20.00939 N | 76.89402 W | KJ157002 | KJ157256 | KJ157113 |
| *Micrathena* | *forcipata* | 787842 | Cuba | 20.33178 N | 74.56919 W | KJ157003 | KJ157257 | **O** |
| *Micrathena* | *forcipata* | 782311 | Hispaniola | 18.355536 N | 068.61825W | KJ157004 | KJ157258 | **O** |
| *Micrathena* | *forcipata* | 782434 | Hispaniola | 19.34405 N | 069.46635 W | KJ157005 | KJ157260 | KJ157114 |
| *Micrathena* | *forcipata* | 784362 | Hispaniola | 18.32902 N | 068.80995 W | KJ157006 | KJ157264 | KJ157115 |
| *Micrathena* | *forcipata* | 784366 | Hispaniola | 18.32902 N | 068.80995 W | **O** | KJ157271 | KJ157116 |
| *Micrathena* | *forcipata* | 784447 | Hispaniola | 18.2205360 N | 68.4806070 W | KJ157007 | KJ157261 | KJ157117 |
| *Micrathena* | *forcipata* | 785054 | Hispaniola | 19.746175 N | 71.257726 W | KJ157008 | KJ157263 | KJ157118 |
| *Micrathena* | *forcipata* | 785282 | Hispaniola | 18.355536 N | 068.61825 W | KJ157009 | KJ157259 | KJ157119 |
| *Micrathena* | *forcipata* | 785682 | Hispaniola | 18.2205360 N | 68.4806070 W | KJ157010 | KJ157262 | KJ157120 |
| *Micrathena* | *forcipata* | 787132 | Hispaniola | 18.310010 N | 71.6000 W | **O** | KJ157265 | **O** |
| *Micrathena* | *forcipata* | 787135 | Hispaniola | 18.310010 N | 71.6000 W | KJ157011 | KJ157266 | **O** |
| *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 |
| *Micrathena* | *furcata* | MIC037 | Brazil | 27.66667 S | 49.01667 W | **O** | KJ157242 | **O** |
| *Micrathena* | *gracilis* | 00000804A | NC, USA | 35.44842 N | 81.58694 W | **O** | KJ157250 | KJ157188 |
| *Micrathena* | *gracilis* | 00002487A | NY, USA | 42.01807 N | 73.91707 W | KJ157088 | **O** | KJ157196 |
| *Micrathena* | *gracilis* | 00002501A | NY, USA | 42.01807 N | 73.91707 W | KJ157089 | **O** | KJ157197 |
| *Micrathena* | *gracilis* | 00000889A | SC, USA | 33.03913 N | 79.56459 W | KJ157082 | KJ157251 | KJ157190 |
| *Micrathena* | *gracilis* | 00000935A | SC, USA | 33.03913 N | 79.56459 W | KJ157083 | KJ157254 | KJ157191 |
| *Micrathena* | *gracilis* | 00000954A | SC, USA | 33.03913 N | 79.56459 W | KJ157084 | KJ157252 | KJ157192 |
| *Micrathena* | *gracilis* | 00000976A | SC, USA | 33.03913 N | 79.56459 W | KJ157085 | **O** | KJ157193 |
| *Micrathena* | *gracilis* | 00000984A | SC, USA | 33.03913 N | 79.56459 W | KJ157086 | KJ157253 | KJ157194 |
| *Micrathena* | *gracilis* | 00000988A | SC, USA | 33.03913 N | 79.56459 W | KJ157087 | KJ157255 | KJ157195 |
| *Micrathena* | *horrida* | MIC042 | Brazil | 16.59553 S | 41.57925 W | **O** | KJ157248 | **O** |
| *Micrathena* | *horrida* | MIC122 | Costa Rica | 10.233518 N | 84.075411 W | **O** | KJ157245 | **O** |
| *Micrathena* | *horrida* | 784351 | Cuba | 20.00939 N | 76.89402 W | KJ157016 | KJ157243 | KJ157125 |
| *Micrathena* | *horrida* | 784751 | Cuba | 20.00939 N | 76.89402 W | KJ157017 | KJ157246 | KJ157126 |
| *Micrathena* | *horrida* | 787913 | Cuba | 20.00939 N | 76.89402 W | KJ157018 | KJ157247 | KJ157127 |
| *Micrathena* | *horrida* | 787919 | Cuba | 20.00939 N | 76.89402 W | KJ157019 | KJ157244 | KJ157128 |
| *Micrathena* | *macfarlanei* | MIC054 | Brazil | 19.65000 S | 42.56667 W | **O** | KJ157241 | **O** |
| *Micrathena* | *militaris* | 782365 | Hispaniola | 18.355536 N | 068.61825 W | KJ157020 | **O** | KJ157129 |
| *Micrathena* | *militaris* | 784338 | Hispaniola | 18.32902 N | 068.80995 W | KJ157021 | KJ157273 | **O** |
| *Micrathena* | *militaris* | 784363 | Hispaniola | 18.32902 N | 068.80995 W | KJ157022 | KJ157293 | KJ157130 |
| *Micrathena* | *militaris* | 784403 | Hispaniola | 18.32902 N | 068.80995 W | KJ157023 | KJ157298 | KJ157131 |
| *Micrathena* | *militaris* | 784430 | Hispaniola | 18.32902 N | 068.80995 W | KJ157024 | **O** | KJ157132 |
| *Micrathena* | *militaris* | 784448 | Hispaniola | 18.32902 N | 068.80995 W | KJ157025 | KJ157294 | KJ157133 |
| *Micrathena* | *militaris* | 784458 | Hispaniola | 18.32902 N | 068.80995 W | KJ157026 | **O** | KJ157134 |
| *Micrathena* | *militaris* | 784503 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157027 | KJ157300 | KJ157135 |
| *Micrathena* | *militaris* | 784531 | Hispaniola | 18.355536 N | 068.61825 W | KJ157028 | **O** | KJ157136 |

(*continued next page*)

**Table 1.** (*continued*)

| Genus | Species | Barcode | Country | Latitude | Longitude | 16S | COI | ITS2 |
|---|---|---|---|---|---|---|---|---|
| *Micrathena* | *militaris* | 784566 | Hispaniola | 18.32902 N | 068.80995 W | KJ157029 | KJ157296 | KJ157137 |
| *Micrathena* | *militaris* | 784671 | Hispaniola | 19.06707 N | 069.46355 W | KJ157030 | **O** | KJ157138 |
| *Micrathena* | *militaris* | 784721 | Hispaniola | 18.32902 N | 068.80995 W | KJ157031 | KJ157310 | KJ157139 |
| *Micrathena* | *militaris* | 784759 | Hispaniola | 18.355536 N | 068.61825 W | KJ157032 | KJ157277 | KJ157140 |
| *Micrathena* | *militaris* | 784762 | Hispaniola | 18.2205360 N | 68.4806070 W | KJ157033 | **O** | KJ157141 |
| *Micrathena* | *militaris* | 784772 | Hispaniola | 18.32902 N | 068.80995 W | KJ157034 | KJ157287 | KJ157142 |
| *Micrathena* | *militaris* | 784806 | Hispaniola | | | KJ157035 | **O** | KJ157143 |
| *Micrathena* | *Militaris* | 784926 | Hispaniola | | | KJ157036 | **O** | KJ157144 |
| *Micrathena* | *militaris* | 785066 | Hispaniola | 19.06707 N | 069.46355 W | KJ157037 | **O** | KJ157145 |
| *Micrathena* | *militaris* | 785080 | Hispaniola | 18.32902 N | 068.80995 W | KJ157038 | KJ157274 | KJ157146 |
| *Micrathena* | *militaris* | 785099 | Hispaniola | 18.32902 N | 068.80995 W | **O** | KJ157313 | **O** |
| *Micrathena* | *militaris* | 785128 | Hispaniola | 18.355536 N | 068.61825 W | KJ157039 | **O** | KJ157147 |
| *Micrathena* | *militaris* | 785144 | Hispaniola | 19.746175 N | 71.257726 W | KJ157040 | **O** | KJ157148 |
| *Micrathena* | *militaris* | 785169 | Hispaniola | 18.355536 N | 068.61825 W | KJ157041 | KJ157290 | KJ157149 |
| *Micrathena* | *militaris* | 785173 | Hispaniola | 19.06707 N | 069.46355 W | KJ157042 | KJ157314 | KJ157150 |
| *Micrathena* | *militaris* | 785174 | Hispaniola | 19.06707 N | 069.46355 W | KJ157043 | KJ157292 | KJ157151 |
| *Micrathena* | *militaris* | 785194 | Hispaniola | 18.355536 N | 068.61825 W | KJ157044 | **O** | **O** |
| *Micrathena* | *militaris* | 785208 | Hispaniola | 18.2205360 N | 68.4806070 W | KJ157045 | KJ157297 | KJ157152 |
| *Micrathena* | *militaris* | 785219 | Hispaniola | 18.355536 N | 068.61825 W | KJ157046 | KJ157286 | KJ157153 |
| *Micrathena* | *militaris* | 785263 | Hispaniola | 18.355536 N | 068.61825 W | KJ157047 | **O** | KJ157154 |
| *Micrathena* | *militaris* | 785273 | Hispaniola | 19.432213 N | 070.371412 W | KJ157048 | KJ157275 | KJ157155 |
| *Micrathena* | *militaris* | 785280 | Hispaniola | 18.32902 N | 068.80995 W | KJ157049 | KJ157315 | KJ157156 |
| *Micrathena* | *militaris* | 785312 | Hispaniola | 19.34405 N | 069.46635 W | KJ157050 | KJ157280 | KJ157157 |
| *Micrathena* | *militaris* | 785401 | Hispaniola | 19.06707 N | 069.46355 W | KJ157051 | KJ157276 | KJ157158 |
| *Micrathena* | *militaris* | 785402 | Hispaniola | 19.34405 N | 069.46635 W | KJ157052 | KJ157285 | KJ157159 |
| *Micrathena* | *militaris* | 785423 | Hispaniola | 18.355536 N | 068.61825 W | KJ157053 | **O** | KJ157160 |
| *Micrathena* | *militaris* | 785461 | Hispaniola | 19.06707 N | 069.46355 W | KJ157054 | KJ157281 | **O** |
| *Micrathena* | *militaris* | 785502 | Hispaniola | 19.06707 N | 069.46355 W | KJ157055 | KJ157301 | KJ157161 |
| *Micrathena* | *militaris* | 785512 | Hispaniola | 19.06707 N | 069.46355 W | KJ157056 | KJ157316 | KJ157162 |
| *Micrathena* | *militaris* | 785524 | Hispaniola | 18.355536 N | 068.61825 W | KJ157057 | KJ157311 | KJ157163 |
| *Micrathena* | *militaris* | 785527 | Hispaniola | 19.34405 N | 069.46635 W | KJ157058 | KJ157279 | KJ157164 |
| *Micrathena* | *militaris* | 785563 | Hispaniola | 19.06707 N | 069.46355 W | KJ157059 | KJ157295 | KJ157165 |
| *Micrathena* | *militaris* | 785604 | Hispaniola | 19.06707 N | 069.46355 W | KJ157060 | KJ157288 | KJ157166 |
| *Micrathena* | *militaris* | 785706 | Hispaniola | 19.06707 N | 069.46355 W | KJ157061 | KJ157278 | KJ157167 |
| *Micrathena* | *militaris* | 785709 | Hispaniola | 19.06707 N | 069.46355 W | **O** | KJ157312 | KJ157168 |
| *Micrathena* | *militaris* | 785722 | Hispaniola | 19.06707 N | 069.46355 W | KJ157062 | KJ157283 | KJ157169 |
| *Micrathena* | *militaris* | 785729 | Hispaniola | 19.34405 N | 069.46635 W | KJ157063 | KJ157284 | KJ157170 |
| *Micrathena* | *militaris* | 785743 | Hispaniola | 19.06707 N | 069.46355 W | KJ157064 | KJ157282 | KJ157171 |
| *Micrathena* | *militaris* | 785769 | Hispaniola | 19.06707 N | 069.46355 W | KJ157065 | **O** | KJ157172 |
| *Micrathena* | *militaris* | 787068 | Hispaniola | 18.980122 N | 70.798425 W | KJ157066 | KJ157299 | KJ157172 |
| *Micrathena* | *militaris* | 787106 | Hispaniola | 18.980122 N | 70.798425 W | KJ157067 | KJ157289 | KJ157174 |
| *Micrathena* | *militaris* | 787148 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157068 | KJ157291 | KJ157175 |
| *Micrathena* | *militaris* | 787152 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157069 | **O** | KJ157176 |
| *Micrathena* | *militaris* | 787166 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157070 | **O** | KJ157177 |
| *Micrathena* | *militaris* | 787190 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157071 | **O** | KJ157178 |
| *Micrathena* | *militaris* | 787208 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157072 | **O** | KJ157179 |
| *Micrathena* | *militaris* | 787212 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157073 | **O** | KJ157180 |
| *Micrathena* | *militaris* | 787214 | Hispaniola | 18.3150011 N | 71.580556 W | KJ157001 | **O** | KJ157112 |
| *Micrathena* | *militaris* | 392672 | Puerto Rico | 17.971472 N | 66.867958 W | KJ157074 | KJ157302 | KJ157181 |
| *Micrathena* | *militaris* | 392677 | Puerto Rico | 17.971472 N | 66.867958 W | KJ157075 | KJ157303 | KJ157182 |
| *Micrathena* | *militaris* | 782048 | Puerto Rico | 18.414373 N | 66.728722 W | KJ157076 | KJ157307 | KJ157183 |
| *Micrathena* | *militaris* | 782126 | Puerto Rico | 18.173264 N | 66.590149 W | KJ157077 | KJ157308 | KJ157184 |
| *Micrathena* | *militaris* | 782153 | Puerto Rico | 18.414373 N | 66.728722 W | KJ157078 | KJ157306 | KJ157185 |
| *Micrathena* | *militaris* | 782174 | Puerto Rico | 18.414373 N | 66.728722 W | KJ157079 | KJ157304 | KJ157186 |
| *Micrathena* | *militaris* | 782201 | Puerto Rico | 18.032518 N | 67.094653 W | KJ157080 | KJ157305 | KJ157187 |
| *Micrathena* | *militaris* | 783400 | Puerto Rico | 18.45226 N | 66.59711 W | **O** | KJ157309 | **O** |
| *Micrathena* | *nigrichelis* | MIC056 | Brazil | 20.43481 S | 43.50906 W | **O** | KJ157239 | **O** |
| *Micrathena* | *plana* | MIC062 | Brazil | 16.53294 S | 41.51042 W | **O** | KJ157240 | **O** |
| *Micrathena* | *saccata* | MIC076 | Brazil | 1.424828 S | 48.43802 W | **O** | KJ157237 | **O** |

(*continued next page*)

**Table 1.** (*continued*)

| Genus | Species | Barcode | Country | Latitude | Longitude | 16S | COI | ITS2 |
|---|---|---|---|---|---|---|---|---|
| *Micrathena* | *sagittata* | 00000833A | SC, USA | 33.03913 N | 79.56459 W | KJ157081 | KJ157221 | KJ157189 |
| *Micrathena* | *schreibersi* | MIC078 | Brazil | 14.77297 S | 39.2205 W | **O** | KJ157317 | **O** |
| *Micrathena* | *schreibersi* | 00000936A | Colombia | 7.062695 S | 73.073058 W | KJ157090 | KJ157318 | KJ157198 |
| *Micrathena* | *schreibersi* | 00002357A | Colombia | 7.062695 S | 73.073058 W | KJ157092 | KJ157319 | KJ157199 |
| *Micrathena* | *sexspinosa* | 00000987A | Colombia | 7.062695 S | 73.073058 W | KJ157091 | KJ157222 | **O** |
| *Micrathena* | *similis* | 785024 | Hispaniola | 19.34405 N | 069.46635 W | KJ157093 | KJ157228 | KJ157200 |
| *Micrathena* | *similis* | 785496 | Hispaniola | 19.34405 N | 069.46635 W | KJ157094 | KJ157232 | KJ157201 |
| *Micrathena* | *similis* | 787265 | Hispaniola | 19.05116 N | 70.88866 W | KJ157095 | KJ157233 | KJ157202 |
| *Micrathena* | *similis* | 787297 | Hispaniola | 19.05116 N | 70.88866 W | KJ157096 | **O** | KJ157203 |
| *Micrathena* | *similis* | 787308 | Hispaniola | 19.03627 N | 070.54337 W | KJ157097 | KJ157229 | KJ157204 |
| *Micrathena* | *similis* | 787309 | Hispaniola | 19.05116 N | 70.88866 W | KJ157098 | **O** | KJ157205 |
| *Micrathena* | *similis* | 787311 | Hispaniola | 19.05116 N | 70.88866 W | **O** | KJ157235 | KJ157205 |
| *Micrathena* | *similis* | 787318 | Hispaniola | 19.03627 N | 070.54337 W | KJ157099 | KJ157234 | KJ157207 |
| *Micrathena* | *similis* | 787320 | Hispaniola | 19.05116 N | 70.88866 W | KJ157100 | KJ157230 | KJ157208 |
| *Micrathena* | *similis* | 787322 | Hispaniola | 19.05116 N | 70.88866 W | KJ157101 | KJ157231 | KJ157209 |
| *Micrathena* | *swainsoni* | MIC090 | Brazil | 6.761528 S | 43.05458 W | **O** | KJ157320 | **O** |
| Outgroups | | | | | | | | |
| *Gasteracantha* | *cancriformis* | 787198 | Hispaniola | 18.3150011 N | 71.580556 W | KJ156989 | KJ157212 | **O** |
| *Gasteracantha* | *cancriformis* | 782149 | Puerto Rico | 18.172979 N | 66.491798 W | KJ156990 | KJ157214 | **O** |
| *Gasteracantha* | *cancriformis* | 784515 | Hispaniola | 18.2205260 N | 68.4806070 W | **O** | KJ157213 | **O** |
| *Achaearanea* | | 784841 | Cuba | 21.59166 N | 77.78822 W | **O** | KJ157211 | **O** |
| *Zygiella* | *atrica* | ZYG318 | Czech Republic | 50.034303 N | 15.781696 E | KJ157102 | **O** | KJ157210 |
| *Argiope* | *lobata* | Arg0160 | Spain | Missing GPS data | | KJ156988 | **O** | KJ157103 |
| *Argiope* | *agbru* | 36 | Slovenia | 46 13 06.0 N | 14 56 34.1E | KC849106 | KC849062 | **O** |

**Table 2. Polymerase chain reaction conditions for the three genes included in this study**

| Gene | Primer 1 | Primer 2 | Annealing temp. (°C) | Fragment length (bp) |
|---|---|---|---|---|
| Cytochrome oxidase 1 (COI) | LCOI1490 | C1-N-2776 | 48 | 1250 |
| 16S | 16SA/12261 | 16SB | 48 | 400–500 |
| Internal transcribed spacer 2 | ITS5.8 | ITS4 | 47 | 350–500 |

## Results

Our results support the monophyly of *Micrathena* (Figs 2–4, S1) regardless of method (Bayesian inference and maximum likelihood) or data partition (concatenated matrix, COI, 16S and ITS2). The overall tree topology, to the extent that it is comparable, is largely congruent with the recent morphological phylogeny of Magalhães and Santos (2012). Hence, our findings reject the monophyly of Caribbean *Micrathena*, while approximately recovering the three species-groups containing Caribbean taxa: the *militaris*-group including *M. banksi*, *M. militaris*, *M. sagittata* and *M. sexspinosa*; the *furcula*-group including *M. cubana* and *M. similis*; and the *gracilis*-group, in part, including *M. horrida* and *M. gracilis*, but not *M. forcipata*, which groups sister to the *furcula*-group (Fig. 4).

Each named *Micrathena* species is supported as monophyletic in all analyses (Figs 2–4). Moreover, the 'widespread' *M. forcipata* from Cuba and Hispaniola and *M. militaris* from Hispaniola and Puerto Rico show evidence of single island endemism, with reciprocal monophyly of islands, much higher between than within island population genetic distances, and conspicuous morphological differences between island populations (Fig. 5, Table 3). *Micrathena horrida* from Cuba is genetically distinct from South American *horrida* (genetic distance between clades: 11.3%), which is similar to or greater than the genetic distance between various other species pairs, but is not genetically distinct from the Central American *horrida* (Fig. 3, Table 3).

Overall, the phylogeny is well supported at the tips with all species and island populations receiving strong support in the concatenated analyses (Figs 2, S1). The variable mitochondrial genes also strongly support the monophyly of each unique species on single islands (Fig. 2). The less variable and short nuclear ITS2 is also congruent with most groups, but places *M. banksi* inside Puerto Rican *M. militaris*, and *M. sagittata* inside Hispaniolan *militaris* (ITS gene tree available from authors). It furthermore places Cuban *M. forcipata* inside the Hispaniolan clade. ITS2 data were available only for a single specimen of Cuban *M. forcipata* and of *M. sagittata*. Several deeper nodes are also well supported: *Micrathena*, the *forcipata*-group, the *similis* +*cubana*-group, the *militaris*-group, and Caribbean plus North American members of the *militaris*-group (Figs 2, S1). Other deep clades are poorly supported (Figs 2, S1) and many of these are not recovered in the maximum likelihood bootstrap analysis (Fig. S1).

Results from the LaGrange ancestral area reconstruction suggest that South/Central America is ancestral to all clades containing Caribbean and North American *Micrathena* (Fig. 4). It also minimally supports four separate Caribbean colonisation events from South America (*militaris* clade,

**Table 3. Uncorrected genetic distances in COI data between and within clades**
Calculated in MEGA ver. 5.2.1

| | *milit*_DR | *milit*_PR | *banksi* | *cubana* | *forci*_CU | *forci*_DR | *gracilis* | *horr*_CU | *horr*_CA | *horr*_SA | *sexspin* | *sagitta* | *schreibe* | *similis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *militaris*_DR | 0.012 | | | | | | | | | | | | | |
| *militaris*_PR | 0.049 | 0.004 | | | | | | | | | | | | |
| *banksi* | 0.147 | 0.149 | 0.003 | | | | | | | | | | | |
| *cubana* | 0.189 | 0.194 | 0.212 | 0.002 | | | | | | | | | | |
| *forcipata*_CU | 0.175 | 0.184 | 0.195 | 0.130 | 0.005 | | | | | | | | | |
| *forcipata*_DR | 0.175 | 0.181 | 0.199 | 0.125 | 0.034 | 0.006 | | | | | | | | |
| *gracilis* | 0.170 | 0.168 | 0.190 | 0.162 | 0.120 | 0.133 | 0.003 | | | | | | | |
| *horrida*_CU | 0.161 | 0.168 | 0.176 | 0.172 | 0.140 | 0.141 | 0.138 | 0.001 | | | | | | |
| *horrida*_CA | 0.161 | 0.167 | 0.173 | 0.168 | 0.143 | 0.143 | 0.136 | 0.007 | n/a | | | | | |
| *horrida*_SA | 0.177 | 0.173 | 0.185 | 0.163 | 0.145 | 0.147 | 0.132 | 0.113 | 0.106 | n/a | | | | |
| *sexspinosa* | 0.142 | 0.136 | 0.171 | 0.171 | 0.166 | 0.166 | 0.164 | 0.166 | 0.163 | 0.161 | n/a | | | |
| *sagittata* | 0.122 | 0.130 | 0.154 | 0.163 | 0.158 | 0.166 | 0.172 | 0.168 | 0.169 | 0.170 | 0.150 | n/a | | |
| *schreibersi* | 0.211 | 0.218 | 0.253 | 0.177 | 0.187 | 0.190 | 0.190 | 0.188 | 0.189 | 0.209 | 0.236 | 0.246 | 0.000 | |
| *similis* | 0.188 | 0.187 | 0.191 | 0.069 | 0.130 | 0.138 | 0.155 | 0.165 | 0.160 | 0.161 | 0.179 | 0.155 | 0.186 | 0.001 |

*similis/cubana* clade, *horrida* clade and *forcipata* clade) (Fig. 4). Raw LaGrange output is available from authors.

Our BEAST dating analysis of the COI data reached stationarity after 30 000 0000 generations. Tracer suggested that the burn-in should be 3 million generations, which was implemented in TreeAnnotator. The dating analysis resulted in the following divergence times (Fig. 4). *Micrathena cubana* and *similis* split 14 (5–26) million years ago (mya) and this clade diverged from their South American ancestors 23 (10–36) mya. The *forcipata* from Hispaniola and Cuba diverged 21 (7–38) mya and *forcipata* diverged from their South American ancestors 47 (29–64) mya. The South American *horrida* diverged from the Cuban and Central American *horrida* 20 (5–39) mya. *Micrathena militaris* from Hispaniola and Puerto Rico split 9 (1–22) mya. *Micrathena militaris* and *banksi* split 21 (9–33) mya. *Micrathena sagittata* from North America split from the *banksi/militaris* 25 (12–40) mya, and the *sagittata/banksi/militaris* clade split from South American taxa 32 (18–50) mya. The dates for the age of Araneidae and the Theridiidae/Araneidae split are consistent with those found in Kuntner *et al*. (2013) (70 (65–77) mya and 92 (76–109) mya, respectively). Raw BEAST output trees are available from the authors.

## Discussion

We conducted the first phylogenetic analysis of *Micrathena* based on molecular data, and found support for the monophyly of *Micrathena* (Figs 2–4, S1). Overall, our results are congruent with a recent analysis based on morphological data (Magalhães and Santos 2012) (Figs 2–4, S1). Magalhães and Santos (2012) defined 12 species-groups, four of which contain Caribbean taxa. All of the species-groups containing Caribbean taxa also contain *Micrathena* from North, South and/or Central America. This suggests that Caribbean *Micrathena* are not monophyletic and therefore must have colonised the region multiple times. In agreement with Magalhães and Santos (2012), our findings approximately recover the three species-groups containing Caribbean taxa (*militaris*, *furcula* and *gracilis*-group minus *M. forcipata*), and hence support the hypothesis that Caribbean *Micrathena* are not monophyletic (Figs 2–4, S1).

Our results suggest four independent colonisations of the Caribbean from South and Central America (Fig. 4) based on a LaGrange ancestral area reconstruction and the structure of the tree supporting the placement of Caribbean *Micrathena* in 3 to 4 species-groups. We dated these colonisation events using a BEAST analysis with a relaxed molecular clock (Bidegaray-Batista and Arnedo 2011) and with two calibration points derived from the fossil calibrated tree presented by Kuntner *et al*. (2013) (Fig. 4). Estimated dates suggest that these are not very recent events, and are reconcilable with the geological history of the Caribbean, with some splits possibly dating back to the GAARlandia land bridge (Iturralde-Vinent and MacPhee 1999; Iturralde-Vinent 2006; Crews and Gillespie 2010; Říčan *et al*. 2013).

Our results also support the monophyly of all named *Micrathena* species. Furthermore, our phylogeny suggests that two of the 'widespread' taxa (*M. forcipata*, Cuba-Hispaniola; *M. militaris*, Hispaniola-Puerto Rico) are in fact single island endemics (Figs 2–4). Each species shows reciprocal monophyly of islands in all analyses (Fig. 2). In comparing genetic distances across reciprocally monophyletic clades and within single island clades, it is clear that divergence among *M. militaris* from Puerto Rico and the Dominican Republic and among *M. forcipata* from the Dominican Republic and Cuba has occurred (Table 3). Genetic isolation of these taxa is supported by conspicuous morphological differences between island populations (Fig. 5). In contrast to *M. forcipata* and *M. militaris*, *Micrathena horrida* from Cuba is genetically distinct from South American *horrida*, but not from Central American *horrida* (Fig. 4, Table 3). However, with only a single specimen available from each mainland population, further data are necessary to test the endemicity of the Cuban *M. horrida*.

One of the more intriguing patterns in our phylogeny is the close, repeated sister clade relationship between Cuba and Hispaniola taxa. We find two separate Cuba-Hispaniola sister clades, and an additional clade with Cuba sister to a clade containing Hispaniola and Puerto Rico (*militaris*-group). Close relationships between Cuban and Hispaniolan taxa are predicted based on geology (Iturralde-Vinent 2006), and future work will

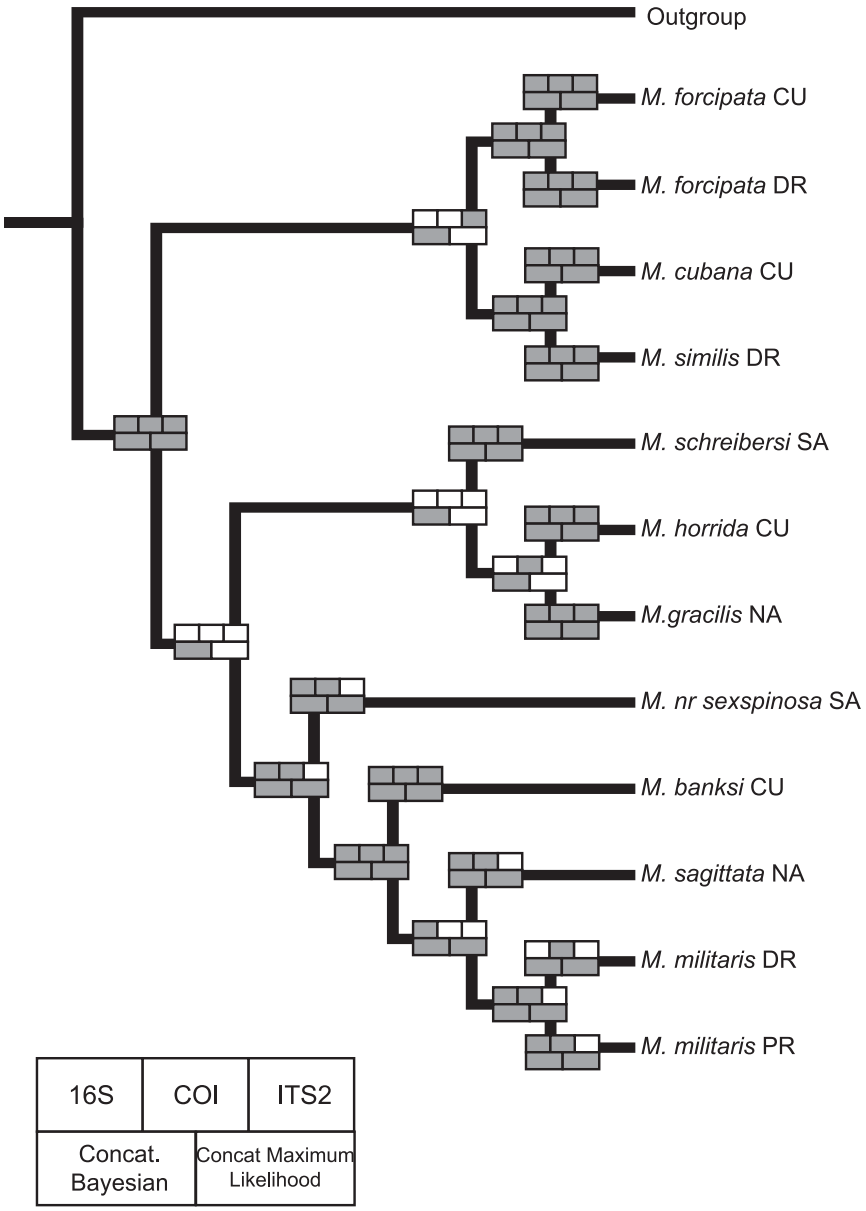


**Fig. 2.** A summary tree based on Bayesian inference of the concatenated matrix, illustrating support for major clades across all analyses. Shaded boxes represent support for the clade in the three single-gene Bayesian analyses and the concatenated analyses using Bayesian and maximum likelihood methods. Terminal taxa were trimmed to represent species clades.

establish whether there is a clear link between phylogeny and geology in *Micrathena*.

Interestingly, *M. sagittata* from North America nests within a Caribbean clade in most analyses, consistent with colonisation of North America from the Caribbean as also seen in some other spiders (Binford *et al*. 2008). However, while our sample is from North America, current taxonomy suggests the species *M. sagittata* is found also in Central America. Additional sampling of this nominal species from its entire range is necessary to establish how it arrived in North America and to establish whether it is possible that a Caribbean to mainland colonisation occurred in the biogeography of *M. sagittata*.

One of our future goals in researching *Micrathena* is to understand its historical biogeography. A key component of studying this is understanding whether *Micrathena* is able to disperse across sea water. *Micrathena* are generally large and

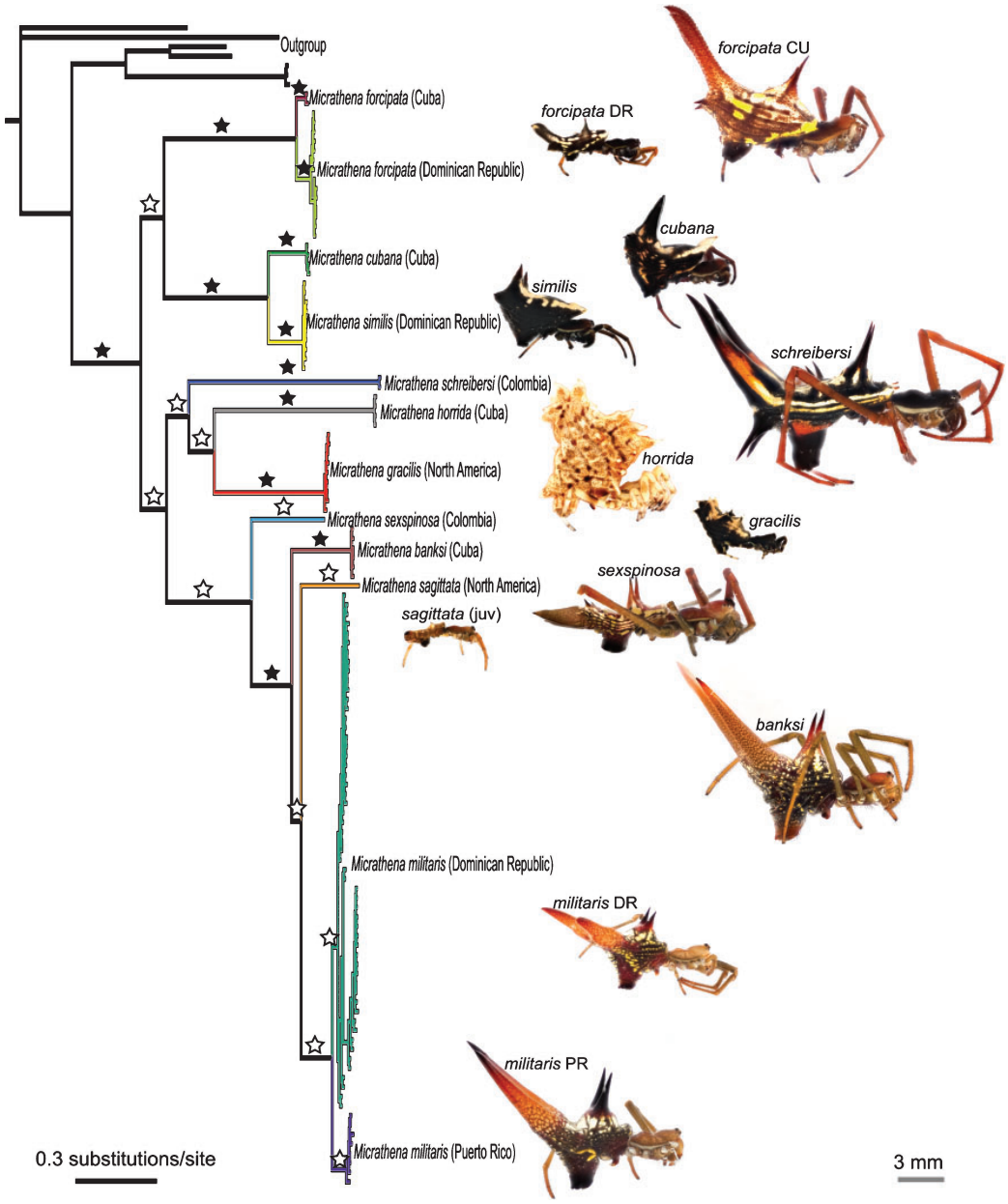


**Fig. 3.** A Bayesian inference tree illustrating relationships and morphological differences among species-groups. Individual terminal taxa have been replaced with species names, while full taxon clade structure is retained. Branches with a black star above are supported by all six of the analyses considered in Fig. 3. Branches with a white star above lacked support from at least one of the six analyses. The monophyly of *Micrathena*, three of its species-groups, and each species and island population is supported. Scale bar for tree represents 0.3 expected substitutions per site; scale bar = 3 mm. All images are high-resolution composite photographs of lateral views of female *Micrathena* species.

bulky spiders that dwell in webs, whose spines may perform anti-predatory functions (Cloudsley-Thompson 1995). Female *Micrathena* do not walk readily off web (personal observation). Thus, *Micrathena* biology suggests that these spiders are poor dispersers as adults. Furthermore, *Micrathena* are habitat specialists, preferring to live in shady, minimally disturbed forests (pers. comm. F. Cala Riquelme). There is also no evidence for ballooning in *Micrathena* (Decae 1987; Bell

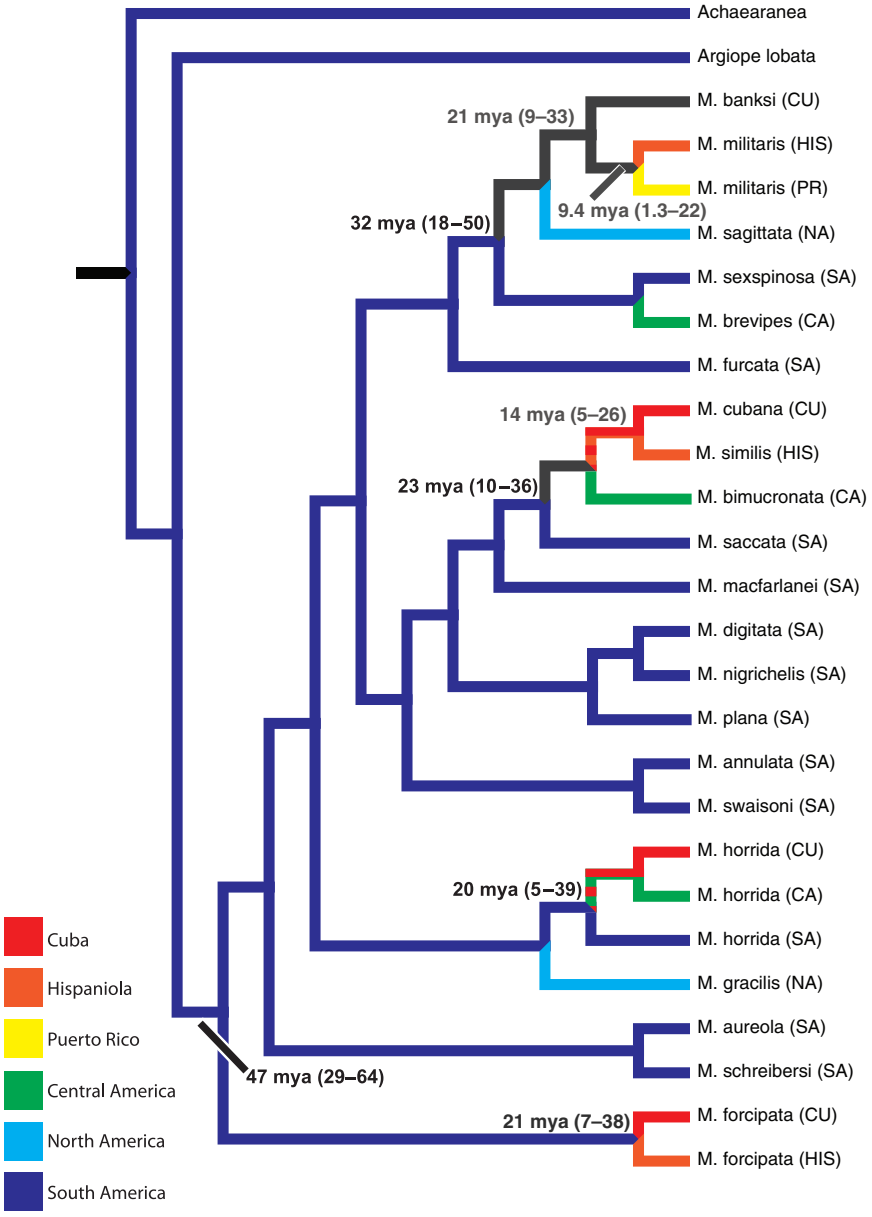


**Fig. 4.** LaGrange reconstruction of ancestral areas on COI Bayesian inference tree. Branches are coloured by ancestral area reconstructed with maximum likelihood. Key results of the BEAST dating analysis are displayed at appropriate nodes. Dates representing inferred colonisation from South America to the Caribbean are in black, dates representing divergences between Caribbean islands are in grey.

*et al.* 2005), although Eberhard (1987) has noted that four species, including *M. schreibersi* and *M. gracilis* produce a second 'airborne' line which is potentially a precursor to ballooning behaviour. These possible restrictions in dispersal ability are consistent with the single island structuring revealed by our phylogenetic analyses, the potentially old age of these groups in the Caribbean, and their general absence from the Lesser Antilles islands (Platnick 2013). All of these suggest limited, or no, over-water dispersal. Pulling our data together, we propose the testable hypothesis that *Micrathena* is a poor disperser whose origin in the Caribbean will best be explained through the GAARlandia hypothesis, and subsequent connections and splits among islands. We predict that geological history has played a prominent role in the

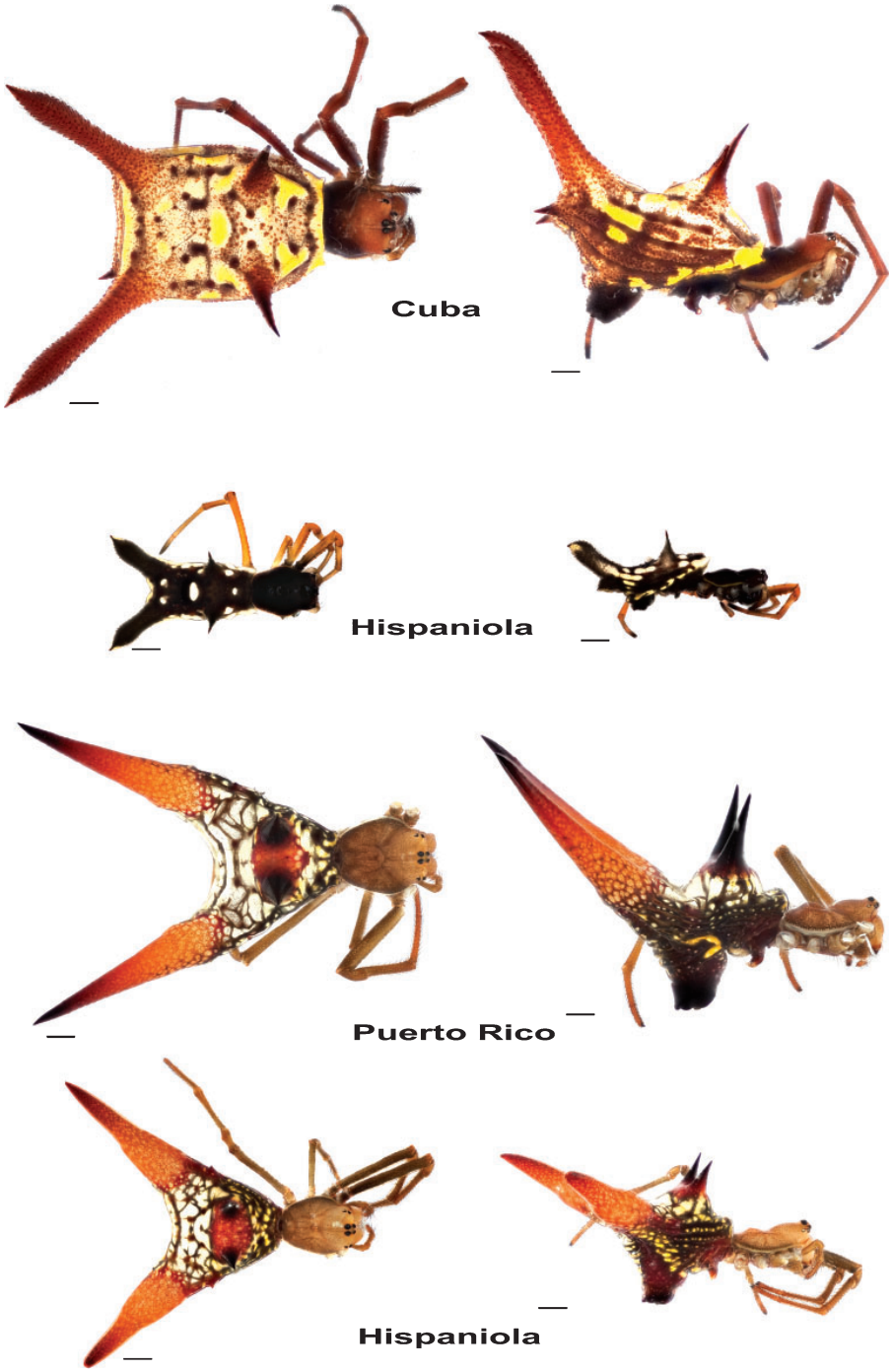


**Fig. 5.** High-resolution composite photographs of female *Micrathena forcipata* and *Micrathena militaris* comparing the morphology of genetically isolated island populations. Dorsal views are on the left-hand side of the figure and lateral views are on the right. Scale bars = 1 mm. In both species distinct morphological differences are evident. *Micrathena forcipata* is much smaller and darker on Hispaniola than Cuba, and *M. militaris* differs in the length and orientation of the abdominal spines between Hispaniola and Puerto Rico.

biogeography of this spider lineage, with a limited role of over-water dispersal.

In order to further our understanding of the biogeography of Caribbean *Micrathena*, current work aims to augment our taxon sample for both the mainland and Caribbean islands, and add related genera with fossil calibrations. We will also revise the taxonomy to reflect our findings on single island endemism in the Caribbean.

## Conclusions

We provide the first phylogenetic analysis of *Micrathena* based on molecular data. Our data support the monophyly of *Micrathena*. However, Caribbean *Micrathena* are not monophyletic. Our phylogenies suggest that Caribbean *Micrathena* have colonised from South America multiple times. Beyond this, we see strong patterns of single island endemism across *Micrathena* species *Micrathena* appears to be a poor disperser that does not readily move among islands, and may have utilised the GAARlandia land bridge to colonise the Caribbean. Both hypotheses require testing in future work.

## Acknowledgements

Thanks to Andy Austin, Mark Harvey and Gonzalo Giribet for inviting us to submit our paper to this special issue of *Invertebrate Systematics*. We would like to thank all the members of the CarBio team, especially those involved in expeditions in Puerto Rico (2011), the Dominican Republic (2012), Cuba (2012) and North America (2013). We are especially grateful to Alexander Sanchez for help with organising fieldwork in Cuba, and to Giraldo Alayon for his help on this project. We would also like to thank Sohath Zamira Yusseff Vanegas for supplying specimens from Colombia, and Federico Lopez Osorio for supplying specimens from North America. We thank Donald Hefferon, GIS specialist at GIS Consulting Services in Burlington, Vermont, for creating the map included in Fig. 1. Thanks to Gonzalo Giribet, two anonymous reviewers and members of the Agnarsson laboratory, Federico Lopez Osorio, Sohath Zamira Yusseff Vanegas, Heine Kiesbuy, Laura May–Collado and Lisa Chamberland for comments that improved this manuscript. Lauren Esposito and the Smithsonian Institute performed some of the DNA extraction. We are very grateful to Ivan Magalhães and the Santos Laboratory in Brazil for sharing unpublished *Micrathena* sequences. Matjaz Gregoric and Ren-Chun Cheng of the Kuntner laboratory in Slovenia provided sequence data for the outgroups *Zygiella* and *Argiope*. All material was collected under appropriate collection permits and approved guidelines. Funding for this work comes from National Science Foundation (DEB-1314749 and DEB-1050253) to I. Agnarsson and G. Binford. Additional funds came from the Smithsonian Institution 2013 SI Barcode Network to J. A. Coddington and I. Agnarsson. Development of this project was furthermore supported by a student grant from the organising committee of the 19th International Congress of Arachnology (2013, Taiwan), a UVM Ronald Suiter Prize, and a UVM Mini grant, all to A. McHugh.

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