ORIGINAL ARTICLE

Phylogeography of the harvestman genus *Metasiro* (Arthropoda, Arachnida, Opiliones) reveals a potential solution to the Pangean paradox

Ronald M. Clouse[1,2] · Prashant P. Sharma[1,3] · Jessie C. Stuart[4] · Lloyd R. Davis[5] · Gonzalo Giribet[6] · Sarah L. Boyer[7] · Ward C. Wheeler[1]

Abstract At both global and local scales, mite harvestmen (Opiliones, Cyphophthalmi) have been shown to have achieved their current global distribution strictly through vicariance. However, the implicit low dispersal capability of this group does not explain how they expand their ranges and come to occupy enormous landmasses prior to rifting. To investigate at the population level the limited vagility that characterizes the suborder generally, and how its dispersal capacity determines diversification dynamics, range expansion, and historical biogeography, we examined as a test case the phylogeography of the genus *Metasiro*. This genus consists of three widely separated, morphologically cryptic species that inhabit the Southeastern United States. Distances between sampling sites spanned a range of geographic scales, from 4 m to over 500 km. Population structure was inferred from fragments of six loci (three mitochondrial, three nuclear) amplified from 221 specimens. We tested for population structure and gene flow, constructed a dated phylogeny of the genus, and developed a program for estimating the effective population size with confidence intervals. Individuals of *Metasiro americanus* demonstrate remarkable population structure at scales of less than 25 m, but populations vary in their haplotypic diversity, and some exhibit evidence of historical gene flow. The estimated timing of cladogenesis within the genus accords closely with the geological history of the North American coastline, and the three species are at the endpoints of large watersheds. This suggests that mite harvestman lineages expand their ranges by hydrochory, providing for the first time a plausible mechanism whereby these animals dispersed across Pangea despite their low vagility in stable environments.

✉ Ronald M. Clouse
ronaldmclouse@gmail.com

[1] Division of Invertebrate Zoology, American Museum of Natural History, 200 Central Park West, New York, NY 10024, USA

[2] Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, 9201 University City Blvd., Bioinformatics, Room 224, Charlotte, NC 28223, USA

[3] Department of Zoology, University of Wisconsin–Madison, 351 Birge Hall, Madison, WI 53706, USA

[4] Harvard Medical School, 25 Shattuck Street, Boston, MA 02115, USA

[5] 3920 NW 36th Place, Gainesville, FL 32606, USA

[6] Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

[7] Biology Department, Macalester College, 1600 Grand Avenue, Saint Paul, MN 55105, USA

Introduction

The close correspondence between mite harvestman tree topologies, divergence times, and ancient landmass configurations has long implied their candidacy for being among the worst dispersers on Earth (Boyer et al. 2007b; Boyer and Giribet 2007; Clouse and Giribet 2010; Murienne and Giribet 2009; Giribet et al. 2012). Even before the advent of molecular phylogenetics, low vagility was suggested by their constrained species distributions (species with ranges of only a few kilometers) and high fidelity between higher taxonomic

ranks and formerly contiguous landmasses (Giribet 2000; Juberthie 1988). Fossil-calibrated, dated molecular phylogenies suggest that mite harvestmen arose over 400 million years ago in the Silurian or Devonian and began diversifying on the ancient supercontinent of Pangea around 330 million years ago. New lineages then continued to arise almost exclusively through vicariance, and the suborder became globally distributed through plate tectonics (Boyer et al. 2007b; Clouse and Giribet 2010; Giribet et al. 2012). There are two possible cases of trans-oceanic dispersal in Cyphophthalmi, but even these are on landmasses with complex and thoroughly debated histories (New Guinea and New Caledonia) (Clouse and Giribet 2007; Sharma and Giribet 2009).

Many other groups of organisms have large numbers of local endemics, like Cyphophthalmi. But unlike mite harvestmen, those endemics are often closely related to widespread species and appear to be part of an ongoing cycle of local isolation and long-distance dispersal. For example, ants tend to have a large number of endemic species throughout the Pacific islands, but these islands are often no older than 5 million years, and molecular phylogenies of *Camponotus*, *Pheidole*, *Nylanderia*, and *Paratrechina* repeatedly show close relationships between widespread species and those restricted to remote islands or specific habitats (Clouse et al. 2015; Economo et al. 2015; Matos-Maraví et al. 2015). Zalmoxid harvestmen, which inhabit south Pacific islands, likewise have many local endemics, but they also have been dispersing across the Pacific from the Americas and inhabit a number of relatively young, remote islands, like those in Micronesia (Sharma and Giribet 2012).

If Cyphophthalmi have always been poor dispersers, however, with even small streams and patches of dry habitat limiting species ranges for millions of years, how did they come to completely cover Pangea before it rifted apart? Reconstructions of the ancestral cyphophthalmid's morphology have led to the surprising conclusion that it was small and possibly blind (de Bivort et al. 2012); this morphology is opposite that of Stylocellidae, the modern Southeast Asian family that includes several large and large-eyed species, as well as some of the only possible transoceanic dispersers in the order (Clouse and Giribet 2007). A small ancestral cyphophthalmid would have had few options in the Paleozoic landscape but to live in the detritus along streams, and it would have likely lived in deeper layers of this debris where it was less coarse and at a consistent humidity (de Bivort et al. 2012). Lacking mechanisms to cope with xeric conditions, Silurian and Early Devonian land plants would have been in abundance only near coastlines and rivers, and the landscape would have had large regions lacking any significant vegetation and thus little suitable habitat for leaf-litter dwellers (Labandeira 2005). Moreover, and more recently, diversification of some Cyphophthalmi lineages on post-Pangean landmasses that underwent turbulent geological histories in the Cenozoic (e.g., landmass accretion in Sundaland; ophiolithic obduction in New Caledonia; marine transgression in New Zealand; Boyer and Giribet 2007, 2009; Sharma and Giribet 2009; Clouse and Giribet 2010) suggests that the mechanism behind Cyphophthalmi's expansion across Pangea continues to operate.

Clues to this puzzle might lie in the population genetics and dynamics of gene flow within harvestman species, and although the number of such studies is currently small, they can show evidence of high vagility and gene flow within populations. Harvestmen in general tend to show strong evidence of historical events in their phylogenies and divergence times (Giribet and Kury 2007; Boyer et al. 2007b; Sharma and Giribet 2011; but see Sharma and Giribet 2012; Schönhofer et al. 2013), and phylogeographic studies of harvestmen consistently reveal strong population structure. However, the scale at which this structure persists and other aspects of their population genetics can vary. The laniatorid harvestman *Fumontana deprehendor* in the southern Appalachians has been found to be fragmented into geographically and genetically distinct clades, but within those areas population structure is almost nonexistent (Thomas and Hedin 2008). That is, populations are well constrained by geographic barriers, but individuals are moving freely within them. In Argentina, the laniatorid harvestmen *Geraeocormobius sylvarum* has been found to have significant population structure but also possible cases of human-mediated dispersal (Vaschetto et al. 2015). Brazilian laniatorid species in the genus *Promitobates* and Japanese species in the family Epedanidae also show significant population structure but in addition there is evidence of historic and ongoing hybridization (Bragagnolo et al. 2015; Kumekawa et al. 2014). In large-bodied laniatorids like Gonyleptidae, high vagility within geographic barriers has likewise been inferred when complex mating systems are present, wherein heavily armored males of many species defend territories and establish harems during the breeding season, and beta males sneak copulations and move between territories (Zatz et al. 2011; Machado and Macías-Ordóñez 2007).

The only species complex of Cyphophthalmi investigated to date from the perspective of phylogeography is a New Zealand pettalid with a relatively broad distribution, *Aoraki denticulata* (Boyer et al. 2007a; Fernández and Giribet 2014). There was, as expected, significant structure in *A. denticulata* (no cases of shared haplotypes between populations, some as close as 10 km), but not every population was recovered as monophyletic, raising the possibilities of cryptic species, incomplete lineage sorting, or ancient genetic mixing. Whether the *A. denticulata* system is informative of mite harvestman population dynamics more broadly is questionable for two reasons. First, in contrast to nearly all other mite harvestman species, *A. denticulata* is broadly distributed over a relatively large territory, whereas most mite harvestmen are short-range

endemics. Second, a body of biogeographic literature continues to advocate the hypothesis that the entire biota of New Zealand has been founded by post-Oligocene dispersal events, subsequent to a complete and prolonged drowning of the archipelago (Landis et al. 2008; Crisp et al. 2011). While the evidence contrary to a complete drowning scenario is substantial (reviewed by Sharma and Wheeler 2013; Mildenhall et al. 2014), if a substantial proportion of the New Zealand landmass did undergo high and rapid biotic turnover, then the history of diversification potential through time experienced by the New Zealand mite harvestmen may not be directly comparable to that of Cyphophthalmi inhabiting more geologically stable regions of the world.

Toward investigating the interplay between low vagility, range expansion, and geologic history as reflected in population structure and speciation dynamics, we identified the North American genus *Metasiro* as an ideal study system due to (1) its distribution in a region of well-understood geological history; (2) a clear timeframe for lineage origins, evidenced by molecular dating and phylogenetic placement as sister group to a clade of northern South American and West African species (Giribet et al. 2012); and (3) the accessibility of specimens. The relationship of *Metasiro* to the Tropical West Gondwanan Cyphophthalmi has long been invoked as indicative of an ancient connection between SE USA and Tropical Gondwana (today tropical Africa and South America) that fragmented in the Mesozoic (Boyer et al. 2007b; Giribet et al. 2012). In addition, *Metasiro* has been inferred to have a morphology and, in many cases, habitat similar to the ancestor of all mite harvestmen (de Bivort et al. 2012).

Once considered monotypic, this genus was recently investigated by a subset of the authors, whereupon it was revealed that supposedly disjunct populations of *Metasiro americanus* (Davis 1933) had no shared COI haplotypes and genetic distances of around 15 %, despite no discernable morphological differences (Clouse and Wheeler 2014). Thus, two separate species were established: *Metasiro sassafrasensis* Clouse & Wheeler, 2014 from Sassafras Mt. in South Carolina (SC) and *Metasiro savannahensis* Clouse & Wheeler, 2014 from the Savannah River Delta, also in South Carolina. The nominal species *M. americanus* is therefore restricted to populations in the Florida (FL) panhandle and nearby areas of Georgia (GA). With a known range of 40–50 km, *M. americanus* is still not as restricted as are many other cyphophthalmids, including its congeners (Fig. 1).

Metasiro obviously has a mechanism to disperse throughout a large range, and three scenarios seem possible, each of which would leave different genetic signatures related to population connectivity and the age of species. One hypothesis is that within *Metasiro* species, or at least among geographically proximate populations, there is considerable gene flow, and regardless of the mechanism behind this gene flow (e.g., male

migration, mate-seeking behavior, kin avoidance, etc.), the result is that at times when connectivity of suitable habitat permitted dispersal, ranges steadily expanded. If the ages of *Metasiro*'s origin and diversification are very old, as has been demonstrated in other mite harvestman lineages, there could be ample time for the genus to disperse throughout its present range through small expansions every year. On the other hand, if there is local gene flow but the species arose recently, then there would be a second possibility, which is that these animals do disperse locally, but they also experience rare, long-distance dispersal events. Of course, these first two scenarios depend on a definition of "recent" and reconstructions of the historic ecology and availability of suitable habitat, both debatable points. A third hypothesis is that there is little or no gene flow among *Metasiro* populations, indicating a lack of dispersal under ordinary circumstances, but rare events cause long-distance dispersal, and the genus expands its range dramatically when these events establish new populations. In this third scenario, we should find population structure even at small scales, but some closely related haplotypes will be found in different populations, giving evidence of past long-distance dispersal.

We therefore sampled *Metasiro* populations throughout its range and at various distances to determine the absolute scale at which population structure becomes evident, as has been done in some other invertebrates (Calderon et al. 2007). To distinguish between the first two dispersal hypotheses, and to establish a temporal context for the divergence of the species complex, we inferred a dated phylogeny of the genus. Our prediction was that *M. americanus* would be the oldest species, for it is closer than *M. sassafrasensis* to related lineages in South America and Africa, it would not have been subjected to inundation like *M. savannahensis*, it has a large range, and it lives in an area known for an ice age relic tree, the Florida torreya (Burns and Honkala 1990).

Materials and methods

Sampling localities

Specimens were sampled throughout the documented range of *Metasiro* (Davis, 1933), beginning with the type locality in Torreya State Park, as well as other sites along the Apalachicola River. In addition, we collected in Florida Caverns State Park in Jackson County, FL (last collected in 1981), a few miles north of the Georgia border in Jasper Co., SC, now part of the Savannah River National Wildlife Refuge (last collected in 1974), and Sassafras Mountain (Fig. 2), 750 m high in Pickens Co., SC (collected once previously in 1969). Collections have been made in Georgia just north of the Florida border, in Grady Co. in 1965 and Decatur Co., but

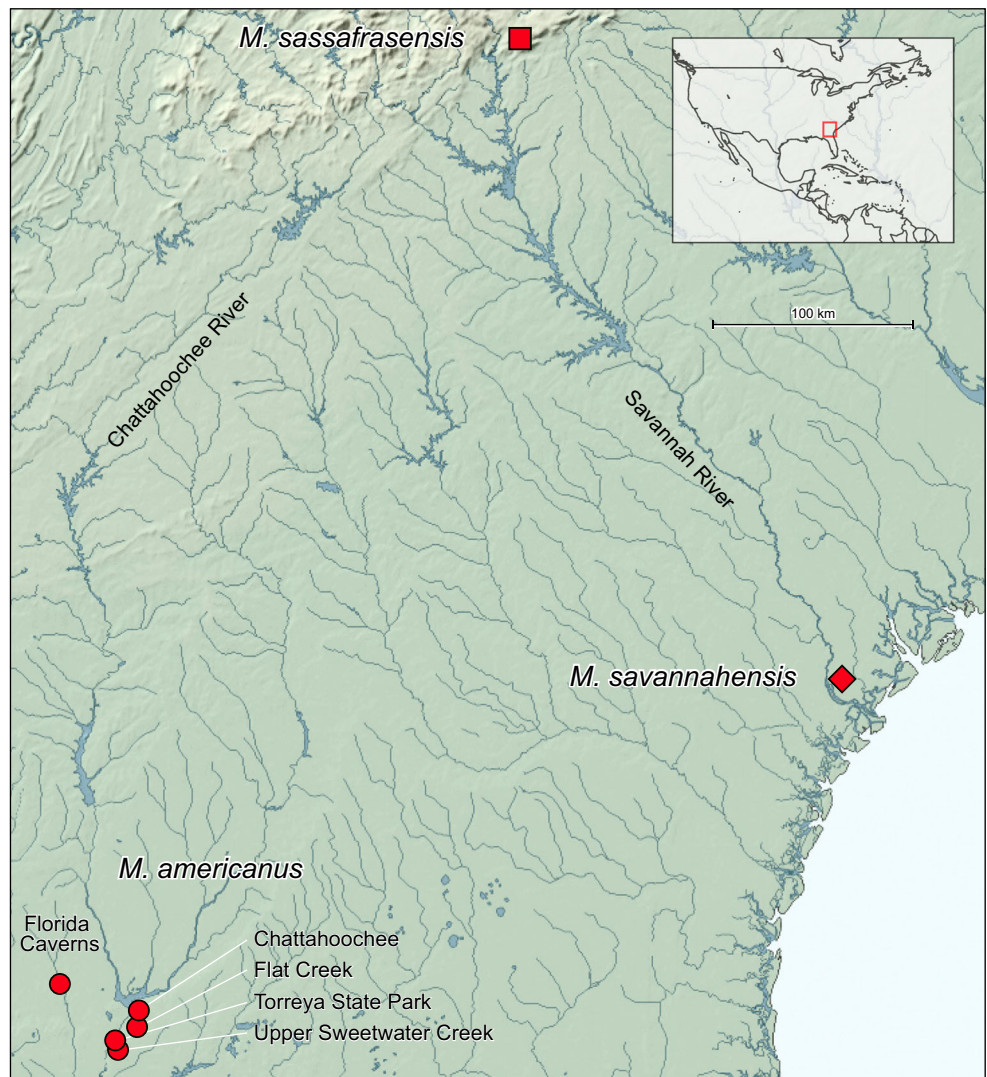**Fig. 1** Terrain of the Southeastern United States, showing *Metasiro* localities

Fig. 2 *Top*, *Metasiro sassafrasensis* male specimen 134535-7171 dorsal (*left*) and lateral (*right*) views. *Below*, a view of the Southern Appalachians from Mt. Sassafras (photo by Dennis Chastain)

due to the lack of sufficiently specific locality information, recollection sites in Georgia could not be identified.

Specimen collection

Specimens were collected by sifting leaf litter into white pans, which were then examined for live animals. These were then preserved in 95 % ethanol and kept cool. Specimens are deposited in the Museum of Comparative Zoology, Harvard University, and data are accessible in MCZbase (http://mczbase.mcz.harvard.edu/); each specimen was also entered into a Giribet Lab Biota database (Colwell 2004–2011) for Cyphophthalmi. Individual specimens are reported by the MCZbase code (which is assigned to collections) and then the individual Biota specimen number. Thus, "133808-7388" corresponds to MCZbase Invertebrate Zoology collection 133808 and Biota specimen SPM007388. All specimen data are available in Supplemental Table 1, where we also report the original MCZ DNA database codes for collections.

DNA sequencing

DNA was extracted using the Qiagen DNeasy tissue kit (Qiagen, Valencia, CA, USA) and amplified using illustra™ Ready-To-Go™ PCR Beads (GE Healthcare, Little Chalfont, UK). We sequenced markers that were well developed and informative for Opiliones, had potential to vary at the population level, and had more conserved regions which could resolve deeper relationships for *Metasiro*. Fragments of the mitochondrion together totaled 1667 to 1669 bp per specimen: 769 bp of cytochrome *c* oxidase subunit I (COI) and ~898 bp of the ribosomal genes 12S rRNA and 16S rRNA. In order to capture additional highly variable sequence, the front loop of 12S was sequenced separately by inverting the standard 12S forward primer (12Sai) into a reverse primer and developing new forward primers further upstream. Primers and temperature profiles are detailed in Supplemental Table 2. Nuclear fragments of histone H3 (327 bp), and the ribosomal rRNAs 18S (1763 bp) and 28S (2041 bp) were amplified and sequenced from representatives of four localities (Sassafras Mt., Savannah, Apalachicola, and Florida Caverns), chosen simply by the first samples to produce clean sequence data; one to three representative sequences were used per locality for the nuclear ribosomal markers, and two to five were used for histone H3, since it did show variation in a few third codon positions (Supplemental Table 1). We attempted to sequence the ribosomal internal transcribed spacer (ITS) region (containing ITS1, 5.8S rRNA, and ITS2, which lie between 18S and 28S), but it was difficult to amplify and obtain good sequence, and cloning revealed a considerable number of length-variable paralogs, so these data were discarded. All sequences have been deposited in GenBank under the accession numbers KT200610–KT201081 and KJ405955–KJ406175 (Supplemental Table 1).

Phylogenetic analysis

Sequences were proofread in Sequencher 5.0 (Gene Codes 2011) and inspected in BioEdit 7.0.5.3 (Ibis Biosciences 2007) and SeaView 4.3.2 (Gouy et al. 2010), which incorporates alignment algorithms MUSCLE (Edgar 2004) and ClustalW (Larkin et al. 2007). We aligned the data in MAFFT 6.847 (Katoh et al. 2002) under default settings, which was trivial for most of the sequence. These alignments were used to infer tree topologies under the maximum likelihood criterion in RAxML v.7.2.7 (Stamatakis et al. 2008) and using Bayesian inference in BEAST v. 1.7.2 (Drummond and Rambaut 2007), both as implemented on the CIPRES (Miller et al. 2010) computing cluster. In RAxML, rapid booootstrapping was conducted using partitioned data and the GTR+CAT model; the "-f a" command was used, in which the best tree on which bootstraps are mapped is evaluated under the GTR+Γ model, where rate heterogeneity follows a gamma model (Stamatakis et al. 2008).

We also analyzed the data under parsimony and dynamic homology in POY v.5 (Varón et al. 2009; Wheeler 1996) using two cost schemes: (1) all transformations equal and (2) indels costing 4, transversions costing 2, and transitions costing 1 (named "111" and "221," respectively, from the ratio of costs). These cost schemes were chosen to explore the effects of increasing tranversion and gap costs, but their resulting topologies were nearly the same, so further cost schemes were not tested. Bootstrap values were calculated in POY using 100 pseudoreplicates under dynamic homology and, to conserve memory, using the optimal tree as the starting tree for each search. Two fragments were analyzed as prealigned at all times in POY, COI and H3; the former had length variation among the outgroups, which was easily aligned using amino acid sequences, and the latter showed no length variation among our terminals. Trees and alignments have been deposited in TreeBASE at the following url: http://purl.org/phylo/treebase/phylows/study/TB2:S17839.

Statistical phylogeography

Statistical phylogeography was conducted on the POY implied alignment of the mitochondrial haplotypes; POY separates homoplastic changes in the alignment, allowing convergent mutations to be treated differently. Measures were made using total mutations, which numbered 189 across all populations, as opposed to 180 segregating sites. Haplotypes were identified in DNAsp v. 5 (Librado and Rozas 2009), where we also measured the haplotype diversity (h), the average pairwise nucleotide differences (k), nucleotide diversity (π, calculated as k divided by the number of sites excluding gaps,

which was 1652–1669), theta per site calculated from η (θ), Watterson's (1975) estimator of theta per site using the number of segregating sites (S), and Tajima's D (Table 1). Populations where the number of mutations (η) and segregating sites (S) are equal are calculated using η only. Measures of population structure (Φ_{ST}), gene flow, and Mantel tests were completed in GenAlEx 6.41 (Peakall and Smouse 2006) (Table 2). Population structure measures used only those populations with nine or more individuals (i.e., not the two smallest collections, from Flat Creek and the Florida Caverns hillside, which had seven specimens each). For populations that did not share any mitochondrial haplotypes, only the COI fragment was used for gene flow estimates. Haplotype networks were constructed using the same mitochondrial haplotype alignment in TCS v. 1.21 (Clement et al. 2000), with network connections limited to 100 steps (using the 95 % probability of homoplasy criterion produced a large number of unconnected networks with little visualization utility).

In the program Geneland v. 0.7 (Guillot et al. 2005), we performed a Bayesian population analysis for the "hillside log" collections (a series of collections made along a decomposing fallen tree) in Florida Caverns, as our first analysis of population structure (Φ_{ST}) among these collections gave ambiguous results. Geneland estimates the number of populations across a landscape using basic models of population structure and a Markov Chain Monte-Carlo MCMC searching technique. After inputting locality and full mitochondrial haplotype data (12S, front loop of 12S, 16S, and COI) for the three collections that had more than nine specimens sequenced, we ran ten independent runs of 500,000 iterations each, setting the possible number of populations at 1–3. We did this using the correlated model, which assumes that the haplotype frequencies will be more similar in more proximal populations. We report the results from the run that

had the highest average log posterior probability after a burnin of 10,000.

We explored the program LAMARC v. 2.1.6 (Kuhner 2006) to estimate θ and gene flow, but it became clear that our data did not meet its relevant assumptions (constant migration and steady exponential population growth or decline) (Excoffier and Heckel 2006), and these are likely why its initial results for θ differed from those obtained from DNAsp by an order of magnitude. There is evidence that F_{ST} (and presumably its counterpart for haplotypes with several variants, Φ_{ST}) is superior to a gene-tree method when migration rates are low (Hudson et al. 1992) and remains an effective tool to understand and compare gene flow (Neigel 2002; Pearse and Crandall 2004). Indeed, a clear initial signature of micro-scale population structure, recurrent bottlenecks, and incomplete lineage sorting or admixture made our dataset inappropriate for many MCMC or coalescent programs.

Molecular dating

The origin and diversification of *Metasiro* have previously been dated by Giribet et al. (2012), who used several fossil calibrations and culled missing data and indel positions (since BEAST treats both as missing data, which can influence branch lengths). This effort represents the most comprehensively dated cyphophthalmid phylogeny to date. In that study, the separation of *Metasiro* from the remainder of Neogoveidae coincides well with the ~200 Ma breakup of Pangea (218±34.27 Ma [95 % confidence interval]), and the diversification happened after the Southeastern United States was exposed by a retreating Atlantic 66 Ma (35.94±15.81 Ma). However, that study recovered a different relationship among the three populations than we recovered here, likely caused by the previous study (1) having only one exemplar from each of the three *Metasiro* populations, (2) using a separate set of partially overlapping markers, and (3)

Table 1 Measures of diversity, tests of population size stability, and estimates of effective population size for all populations and subpopulations of *Metasiro*

	n	h	k	π	η	θ	D	p	S	θ_W	D	p	N_e median	N_e mean	95 % CI
Sassafras	12	0.924±0.057	6.29	0.004	15	0.003	1.14	>0.10					0.7	0.8	0.5–1.3
Savannah	85	0.923±0.025	2.15	0.001	60	0.007	−2.68	<0.001**	57	0.007	−2.64	<0.001**	1.6	1.8	1.1–3.1
Florida	124	0.893±0.021	47.86	0.029	189	0.021	1.20	>0.10	180	0.020	1.42	>0.10	4.9	5.3	3.3–9.3
Apalachicola	55	0.985±0.009	21.84	0.013	149	0.020	−1.17	>0.10	144	0.019	−1.09	>0.10	4.6	5.1	3.1–8.8
Torreya	29	0.983±0.015	5.96	0.004	37	0.006	−1.36	>0.10					1.4	1.5	0.9–2.6
Caverns	69	0.663±0.043	20.99	0.013	101	0.013	−0.01	>0.10					3.0	3.3	2–5.7
Hillside log	47	0.530±0.074	28.74	0.017	101	0.014	0.92	>0.10					3.2	3.6	2.2–6.2
Upland glade	22	0.312±0.106	0.31	0.0002	1	0.0002	0.24	>0.10					0.05	0.05	0.03–0.09

Column headings are as follows: n, the number of sequences used; h, the haplotype diversity (±SD); k, the average number of pairwise nucleotide differences; π, nucleotide diversity; η, the number of mutations; θ, theta per site calculated from η; θ_W, Watterson's estimator of theta per site (Watterson 1975); S, the number of segregating sites; Tajima's D; and N_e, the effective population size (in millions). Significant (<0.05) and marginally significant (<0.10) p values for Tajima's D are marked with asterisks

Table 2 Measures of population structure (Φ_{ST}), gene flow (N_m), and isolation by distance (Mantel) for all populations and subpopulations of *M. americanus*

	Mitochondria						COI fragment only			
	Φ_{ST}	Φ_{ST} p	N_m	Within pop. var.	Mantel R_{xy}	Mantel p	Φ_{ST}	Φ_{ST} p	N_m	Within pop. var.
Savannah	0.002	0.382	216.058	1.00	−0.035	0.220	−0.017	0.737	Inf.	1.00
Florida	–	–	–	–	0.725	0.010**	0.375	0.001**	0.834	0.63
Florida[†]	–	–	–	–	−0.140	0.016**	–	–	–	–
Apalachicola	–	–	–	–	0.001	0.266	0.176	0.001**	2.334	0.82
Torreya	–	–	–	–	−0.034	0.481	0.038	0.184	12.598	0.96
Caverns	0.387	0.001**	0.791	0.61	−0.130	0.040**	0.430	0.001**	0.662	0.57
Caverns[†]	–	–	–	–	0.000	1.000	–	–	–	–
Hillside log	0.084	0.080*	5.472	0.92	0.154	0.010**	0.111	0.060*	4.023	0.89
Hillside log[†]	–	–	–	–	0.000	1.000	–	–	–	–
Upland glade	−0.097	1.000	–	1.00	–	–	−0.097	1.000	–	1.00

Population structure could not be measured where no subpopulations had overlapping haplotypes, so in those cases we used only COI. Migration rates were not applicable in cases of a complete lack of population structure. Populations in Florida were analyzed with and without (†) the ancient haplotype (no. 103) found at the hillside log in Florida Caverns State Park. The Mantel test was not possible between the upland glade localities, as both samples had the same two haplotypes. Significant (<0.05) and marginally significant (<0.10) p values are marked by asterisks

including single errant base-pair calls in each of the Sassafras Mt. 18S and 28S rRNA sequences, corrected in this study after re-sequencing. We therefore aimed here to date independently the history of *Metasiro* in more detail than before.

The tree recovered from the aligned nuclear and mitochondrial sequences in RAxML was dated in PATHd8 (Britton et al. 2007) by fixing the diversification of Opiliones at 432.9 Ma at the root and using the date range 246.6–325.9 Ma for the MRCA (most recent common ancestor) of Sternophthalmi, the Infraorder to which *Metasiro* belongs; both dates come from Giribet et al. (2012). Confidence intervals for mean path lengths from PATHd8 were computed after inputting the length of the alignment, 6051 bp. Although the use of the tree found using nuclear markers has branch lengths calculated while analyzing a large amount of missing data (we only sequenced them for exemplars of each species since they showed no variation in *Metasiro*), we needed the information from these markers to resolve the deep nodes among the outgroups where our calibration point was positioned. The use of transitive dates in molecular dating (using dates output by one analysis as input for another) has been criticized (Graur and Martin 2004; Shaul and Graur 2002), but in this case the root date was little different from what we would have used directly from fossils (see also Sharma and Giribet 2012 for an empirical case where transitive use of dates with error intervals resulted in nearly identical age estimates). Given the occurrence of one fossil Opiliones from the Early Devonian (Dunlop 2010; Dunlop et al. 2004), we have previously fixed the root of an Opiliones phylogeny at 425 Ma (Clouse and Giribet 2010). Additionally, that analysis recovered the diversification of Sternophthalmi within the large, 79.3-Myr range of Sternophthalmi dates input here.

Older dates for the origin of Opiliones (up to a soft bound of 500 Ma) have been inferred from molecular dating (Hedin et al. 2012), but molecular dating algorithms are inclined to extend the depth of the root, a nuisance parameter, if it is not fixed (often conflicting with the fossil record of the lineage of interest) (Dunlop 2010; Graur and Martin 2004), and we have observed earlier that the main determinant of nodal ages of interest are constraints on the most recent common ancestor (here the range on Sternophthalmi) (Clouse and Giribet 2010). Hedin et al.'s (2012) dating is also limited by the interpretation of the Devonian *Eophalangiun sheari* being a stem group Eupnoi and constraining Palpatores to 410 Ma, a phylogenetic position that is now contested (Garwood et al. 2014; Sharma and Giribet 2014).

BEAST (Drummond and Rambaut 2007) found our data set difficult to resolve, for it consistently placed outgroups within *Metasiro*. Using monophyly or swapping constraints, as well as more, fewer, or different date priors and starting trees, caused runs on the full nuclear and mitochondrial data set to remain unstable. After four runs of 50 million generations each, around half of the parameters in our most promising search failed to reach ESS >100.

Calculating mutation rate and effective population size

The effective population size (N_e) was estimated by using θ (Table 1) and a mutation rate (μ) calculated in two ways. First, using just the *Metasiro* portion of the phylogeny recovered in RAxML (with the outgroups removed) and the COI partition of the alignment (769 bp), we optimized branch lengths in BEAST. This was done by using a strict clock without calibrations, run four times for 10 million generations, which

quickly stabilized. The resulting node depths, which are a per site probability of mutation, were divided by date estimates from geologic events—66 Ma for the diversification of *Metasiro*, 40 Ma for the split between Florida and Savannah populations, and 14 Ma for the diversification of the Florida population—to estimate the per-site mutation rate per Ma.

We also calculated the mutation rate of the entire mitochondrial fragment from the average number of mutations since the most recent common ancestor of *M. americanus* (using the dates from PATHd8). Parsimony branch lengths were calculated by reading into the program TNT (Goloboff et al. 2008) the tree and haplotype implied alignment recovered under the 221 cost scheme in POY. The average parsimony branch length distance between all Sassafras specimens and all other *M. americanus* terminals (paths which pass through the most recent common ancestor of all *M. americanus*) was divided by two, giving the average number of mutations that any one lineage would have experienced. We chose the tree and implied alignment found using the cost scheme 221 because of its general applicability, although alignments and trees differed only trivially between cost schemes 111 and 221; the implied alignment ensured that homoplastic nucleotides would be counted as different mutations. Branch lengths were measured in APE (Analysis of Phylogenetics and Evolution, implemented in R) (Paradis et al. 2004; R Development Core Team 2012) using the "cophenetic.phylo" command.

The mutation rate used to calculate N_e from θ ($N_e=\theta/2\mu$) is expressed in the number of mutations per generation, but since we were unsure of the generation time (i.e., the average age of reproduction), and the age of the common ancestor has variance as calculated in PATHd8, we wrote a program Ne_CALCULATOR (source code available in Supporting Information, and OSX binary and source code available from the authors upon request) to calculate N_e from a range of values and distributions of the age of the species and their generation times. The age of the most recent common ancestor was set as a normal distribution with a mean of 58.7 Ma (from the PATHd8 tree) and a variance of 6.25 Ma (derived from the 95 % CI of ±4.9). The age of reproduction was treated as uniformly distributed between 2 and 6 years, based on our own observations and those made by Juberthie (1964); a uniform distribution reflects our uncertainty of when *M. metasiro* adults have the highest reproductive success, while also not allowing reproduction during ages when we considered it very unlikely to happen.

Results

Specimen number and sex

A total of 438 recently collected specimens were available for sequencing, of which 221 were successfully sequenced for complete concatenations of COI, the front loop and central amplicon of 12S rRNA, and 16S rRNA. The adult sex ratio (152 males to 181 females) did not differ significantly from 1:1 (χ^2, $p=0.11$, $df=1$), and most of the variance was caused by the samples from the Apalachicola region (52 males vs. 72 females, $p=0.07$, $df=1$), which were collected at different times from the other samples. All the specimens collected by R.M.C. and P.P.S. in March 2010 were collected without regard to age and sex, and they were approximately evenly distributed among males, females, and juveniles (100:110:92, respectively). Juveniles, which could not be sexed, were given priority for DNA extraction and sequencing.
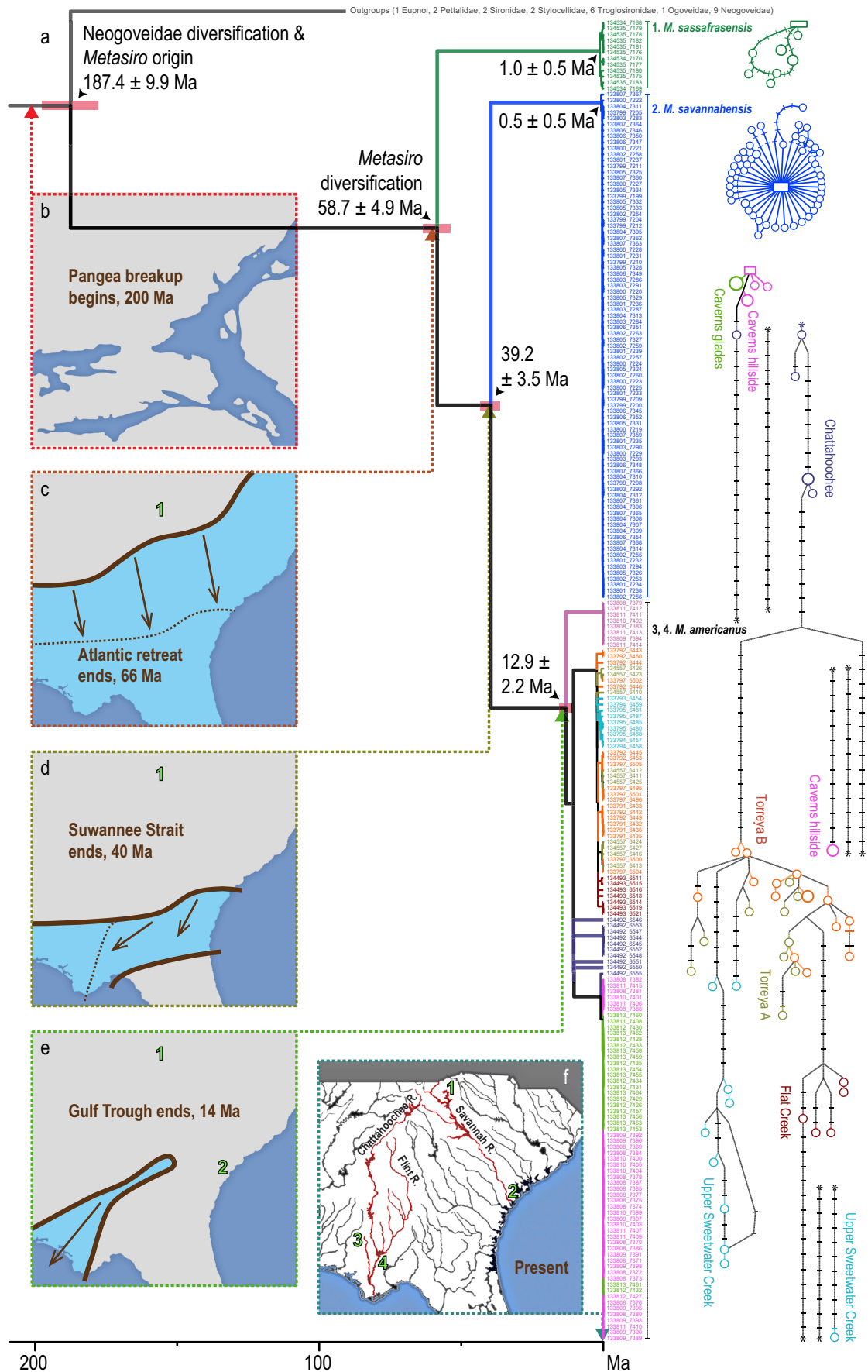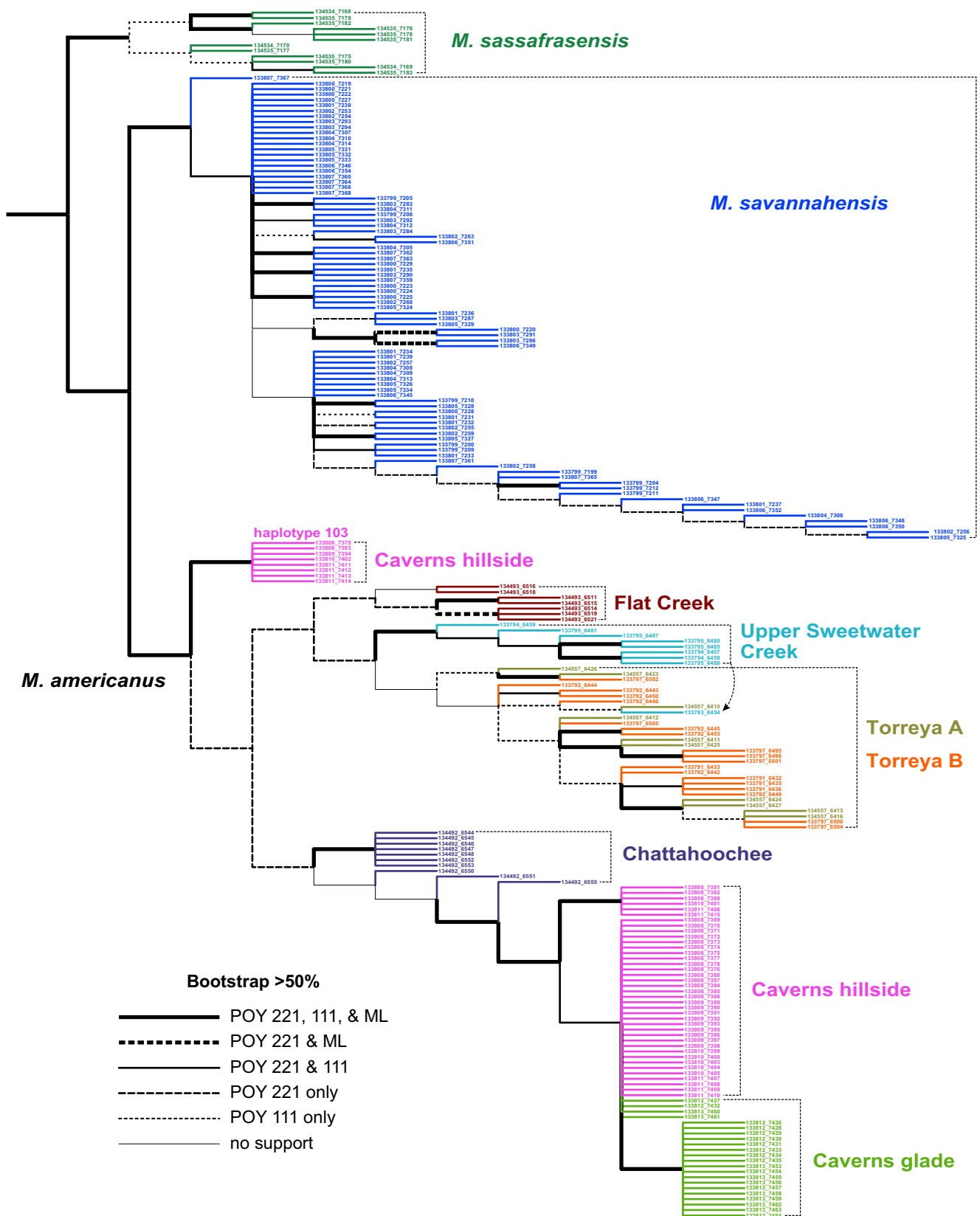
Sequence statistics

Of the 1667–1669 base pairs of concatenated mitochondrial sequence we obtained from 221 specimens, 426 non-gap sites (25.7 %) were variable when aligned. This was a slight underestimate since 17 bp of indel positions were not counted by the program DNAsp (Librado and Rozas 2009). For the 769 base pairs of COI (which did not have indels), 182 sites (23.6 %) were variable. COI had, on average, 1.27 variants per position and a G-C content of 0.36. For the front loop of 12S (~113 bp), the values were 1.55 and 0.28, respectively; for 12S (~322 bp), they were 1.36 and 0.29; and for 16S (~463 bp), they were 1.24 and 0.30. We found no variation in over 3800 base pairs of nuclear ribosomal sequence data (18S and 28S rRNA).

Phylogenetic analysis

Phylogenetic analyses consistently recovered the relationship (*M. sassafrasensis*, (*M. savannahensis*, *M. americanus*)) (Figs. 3, 4, and S1). Nuclear markers, which had the potential to change outgroup relationships and up-pass optimizations of mtDNA sequence, had no effect on the rooting of *Metasiro*. Using both nuclear and mitochondrial markers, the best tree recovered under maximum likelihood in RAxML had a log likelihood of −37,859.36 (Fig. S1, dated in Fig. 3), and the best tree recovered under parsimony in POY had a length of

Fig. 3 Phylogeny recovered under maximum likelihood in RAxML ▶ using mitochondrial and nuclear markers, then dated in PATHd8 using a fossil-based calibration for the root and 79.3 Ma range for the origin of *Metasiro*'s infraorder (**a**). The origin and diversification of *Metasiro* and its main clades are shown with dates in millions of years and 95 % confidence intervals (*red/gray bar*=CI, not shown for values less than 0.5). Significant events in the history of the southeastern United States (**b**)–(**e**) are shown below. The present-day distribution of *M. americanus* populations, and land, sea, and rivers are shown in the *bottom right* (**f**). The Savannah and Chattahoochee watersheds are colored and labeled, and population localities are *1* Sassafras Mt., *2* Savannah River delta, *3* Florida Caverns, and *4* east of the Apalachicola River. Haplotype networks for each species are shown to the *right*

M. sassafrasensis

M. savannahensis

haplotype 103

Caverns hillside

Flat Creek

Upper Sweetwater Creek

Torreya A

Torreya B

M. americanus

Chattahoochee

Caverns hillside

Caverns glade

Bootstrap >50%

▬▬▬	POY 221, 111, & ML
▪▪▪▪	POY 221 & ML
———	POY 221 & 111
– – –	POY 221 only
········	POY 111 only
———	no support

12,304 weighted steps under the "221" cost scheme and 7242 steps under the "111" cost scheme (Fig. 4). *Metasiro*

americanus specimens generally separated by collection locality, especially under parsimony, but sequences from the two

◄ **Fig. 4** Phylogenetic hypothesis of *Metasiro* recovered under the parsimony criterion in POY from unaligned mitochondrial and nuclear data, under a cost scheme where indels cost 4, transversions 2, and transitions 1 (12,304 steps), with zero-length branches collapsed. *Thick* branches received bootstrap support in POY as well as under likelihood in RAxML; *medium* branches received bootstrap support in POY only. *Dashed lines* indicate clades that were not recovered under a cost scheme where all transformation costs were equal ("111")

Torreya State Park localities did not show reciprocal monophyly and were closely related to one haplotype in the Upper Sweetwater Creek population (Figs. 2 and 4). Additionally, Florida Caverns State Park had one haplotype that was usually recovered as sister to all other *M. americanus* haplotypes (Fig. 4).

Statistical phylogeography

Statistical analyses of 221 mitochondrial haplotypes showed a strong trend for populations to be structured, except among *M. savannahensis* collections (which were all collected within 100 m) (Fig. 5 and Supplemental Table 1). *Metasiro savannahensis* did show a significant signature of expansion (Table 1 and Fig. 6). Isolation by distance was significant for most *M. americanus* populations, but this was driven mostly by the highly divergent haplotype (103) found on the hillside in Florida Caverns (Fig. 4); when this haplotype was not considered, *M. americanus* populations had a significant relationship between distance and divergence, but this was weak and negative (Mantel $R_{xy}=-0.14$, Table 2). Population structure was significant among populations along the Apalachicola River and in Florida Caverns. Along the Apalachicola River, no two localities shared entire mitochondrial sequences, so Φ_{ST} was calculated from just the COI fragment, and for the region as a whole it was significantly structured. Among the Apalachicola populations, the two collections from Torreya State Park (359 m apart) did not show significant structure, likely an effect of the large number of different haplotypes (24 from 29 individuals, none of which were shared between the two localities); even when focusing on only the COI region, no structure was detected, likely from the same effect of a large number of haplotypes (ten different haplotypes, two of which were shared). In Florida Caverns, the two collections in the upland glade were significantly differentiated from those along the hillside log (768 m away), and the hillside log ones (7–21 m apart) were structured to a degree that was marginally significant. A Bayesian analysis of the hillside log collections in Geneland found two populations in all ten independent runs, with P and Q in one population, and collection S in another (Fig. 7).

Molecular dating

Dating the phylogeny recovered under maximum likelihood using PATHd8 gave dates which are concordant with geologic events (Fig. 3): 187.4±9.9 Ma for the divergence of *Metasiro* from other neogoveids inhabiting tropical South America and West Africa (expected soon after the rifting of Pangea, 200 Ma), diversification of *Metasiro* at 58.7±4.9 Ma (soon after the retreat of the Atlantic at the end of the Mesozoic, 66 Ma), origin of *M. savannahensis* and *M. americanus* at 39.2±3.5 Ma (soon after the northern retreat of the Suwannee Strait at 40 Ma), and diversification of *M. americanus* at 12.9±2.2 Ma (immediately after the proposed end date for the Gulf Trough, which had inundated the entire Apalachicola and lower Chattahoochee region until about 14 Ma) (Dallmeyer 1989; Popenoe et al. 1987; Randazzo 1997; Rowley and Pindell 1989; Smith and Lord 1997). Using the breakup of Pangea at 200 Ma as a fixed date in PATHd8 made little difference in the recovered dates relative to the original dating using a fixed root and date range for Sternophthalmi: 62.6 Ma for *Metasiro* diversification, 41.9 Ma for the *M. savannahensis*–*M. americanus* split, and 13.7 Ma for diversification of *M. americanus*. The general behavior of PATHd8 dating is shown in Fig. S2.

Mutation rate and effective population size

The per-site mutation rate (N.B., not the divergence rate) of COI, as estimated from optimizing branch lengths only for COI in BEAST and dividing by possible node times, was 0.12 % per Ma using the diversification of *Metasiro*, 0.19 % per Ma at the split between Florida and Savannah, and 0.10 % per Ma using the node of Florida diversification; these averaged to a COI mutation rate of 0.13 % per Ma. This is slower than was found in an earlier study of just the COI data when describing *M. sassafrasensis* and *M. savannahensis* (Clouse and Wheeler 2014); that study gave an estimate of ~0.26 % per Ma, but it used the dates from Giribet et al. (2012) directly, with no new dating estimates.

The mutation rate of the entire mitochondrial fragment estimated from the average parsimony branch length in *M. americanus*, the age of the species from our dated phylogeny, and the number of sites in the mitochondrial sequences (105.7 mutations for 1667 bp over 58.7 Ma) was 0.11 % per Ma, very similar to the rate calculated for COI in BEAST. We used this number of mutations and clade age in combination with ranges of values for generation time and clade age to estimate the effective population size since this was the same fragment used to calculate θ. The resulting values (N_e±95 % CI, doubled to account for both males and females, in millions) were 5.3±3.0 for all of *M. americanus*, 5.1±2.9 for Apalachicola, 3.3±1.9 for Florida Caverns, 0.8±0.4 for *M. sassafrasensis*, and 1.8±1.0 for *M. savannahensis*
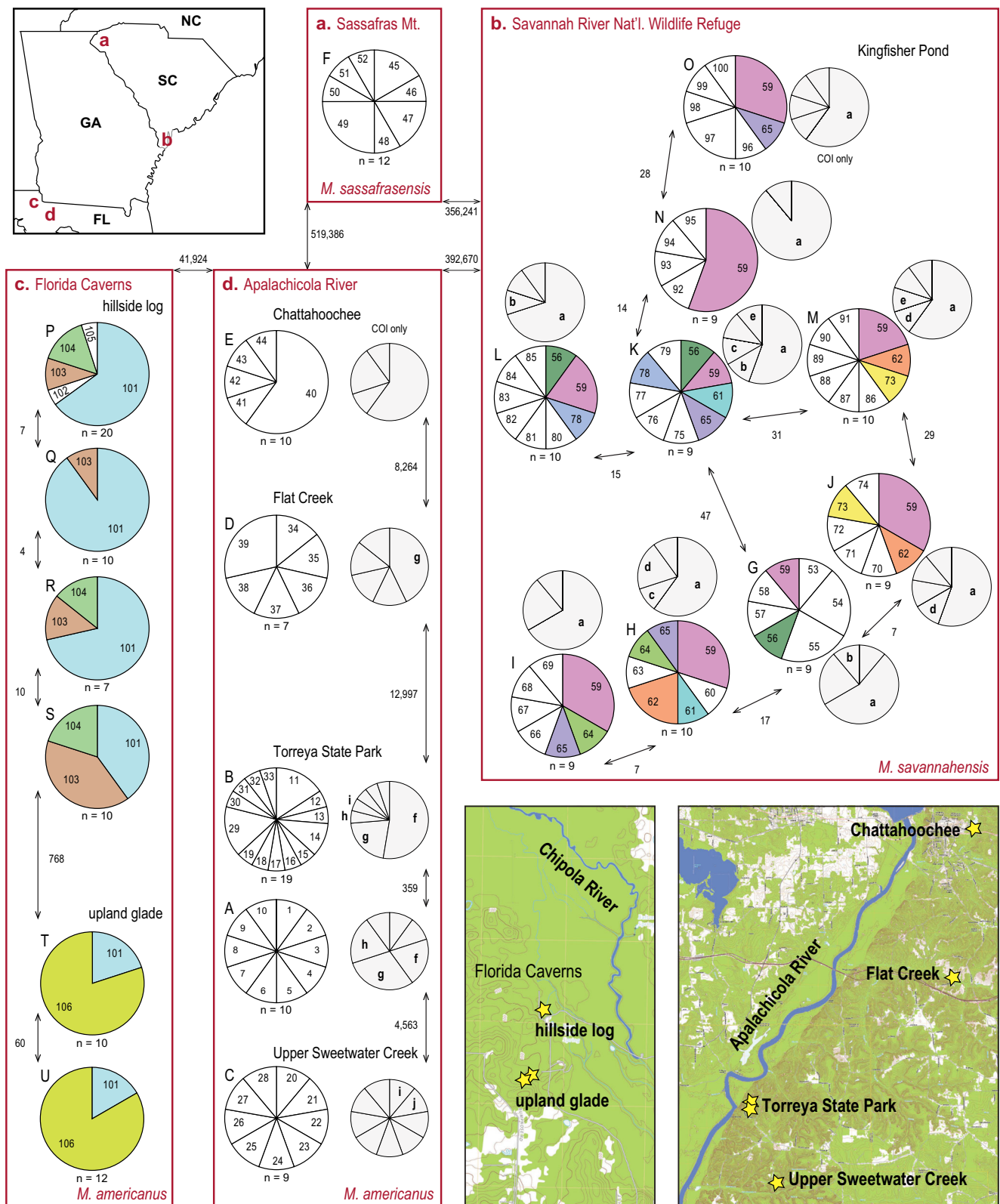
Fig. 5 Mitochondrial haplotype distributions for all collections of *Metasiro*. Localities *a–d* shown on the map in the *upper left* correspond to the sets of haplotype pie charts, each chart representing a single collection. The *lower right* shows the locations of Florida collections (Florida Caverns, *left*, and the Apalachicola River region, *right*). Pie charts are lettered in the order they were collected, and underneath each is the number of sequences obtained from that collection. All slices are numbered by haplotype, and only shared haplotypes are *colored/gray*; unique haplotypes are *white*. Smaller pie charts are for the COI region only, with shared haplotypes indicated by *small letters*. Distances between collections are noted in meters
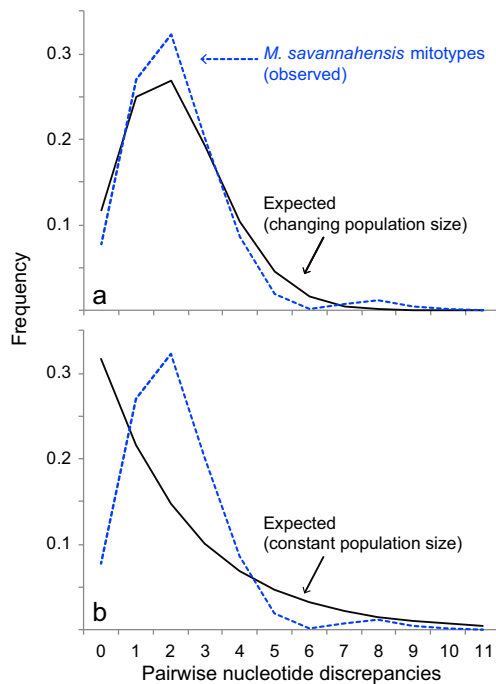
Fig. 6 The distribution of nucleotide discrepancies expected in the Savannah population if the population is fluctuating (**a**) or remaining constant (**b**). Expected values are shown with the *solid, black line*, and actual values are shown by the *dashed, blue line*

(Table 2). We tested for a clocklike rate in PATHd8, and it was rejected for the whole of *M. americanus*, primarily because of the populations along the Apalachicola River in Florida, so we considered the influence of mutation rate on N_e in our program. Effective population sizes and critical values scaled linearly with mutation rate, so a faster mutation rate gives a proportionally smaller N_e. For example, if the mutation rate is 1.1 % per Ma, the population of *M. americanus* would be estimated as 0.53 ± 0.30 million individuals.
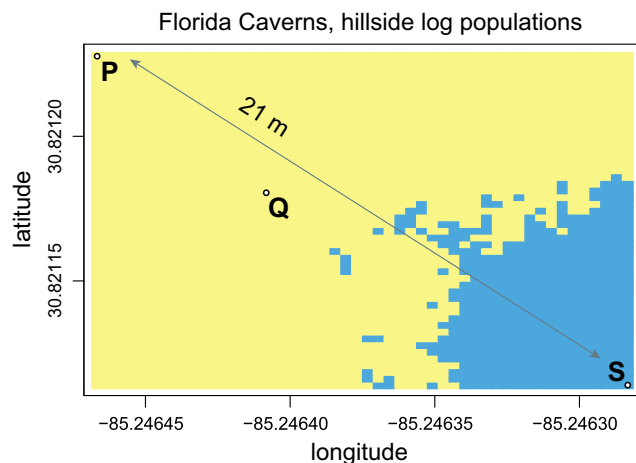
Fig. 7 Population assignments (*yellow/light* and *blue/dark*), as inferred from haplotype data by Geneland, for the three collections with (P, Q, and S) along the hillside log in Florida Caverns

Discussion

Metasiro dispersal and population histories

Our results are consistent with a scenario in which there is normally very little gene flow between populations, but rare long-distance dispersal events do occur. This is seen most clearly in *M. americanus* in Florida, whose history appears to include both (1) periods which may last many thousands of years when populations remain genetically isolated at scales of only a few dozen meters; and (2) episodes when individuals are passively carried across distances that, for a mite harvestman, are large. We are not aware of any other non-sessile, non-cloning, non-selfing animal where gene flow is restricted at such short distances, absent of cataclysmic events, and this is consistent with studies showing, with few exceptions, cyphophthalmid evolution and geologic history matching each other closely. The only possible similarity is with the effect of settlement of larvae in some marine invertebrates, showing a drastic decrease in genetic relatedness over the first 100 cm of distance (Calderon et al. 2007), but this is probably due to kin recognition effects.

Within this broad scenario, *Metasiro* populations do have different histories, as evidenced by their genetic diversity, haplotype relationships, and tests of population stability. In Florida Caverns State Park, we discovered a haplotype (no. 103) that is quite different from and distantly related to the other haplotypes in that locality. We also observed this in the Torreya populations, which include one haplotype that is most closely related to those in Upper Sweetwater Creek. Haplotype 101 in the Florida Caverns also gave evidence of past gene flow. This haplotype was collected at both the hillside log and the upland glade. However, it is not indicative of ongoing gene flow between the hillside and the glade, for no other haplotypes are shared between the two localities, not even the common haplotypes 106 and 103. The Florida Caverns populations are also distinctly less diverse than those along the Apalachicola River, and the common flooding in the Florida Caverns area (large areas had been inundated only days before our collections) may promote frequent population bottlenecks. Consistent with this, our estimate of the effective population size for the upland glade (30,000–90,000) is one to two orders of magnitude lower than other sites. This process would also be likely to happen in the lowland forests of the Savannah River delta, and indeed *M. savannahensis* shows clear evidence of a population bottleneck.

The habitat of *Metasiro* could be the cause of more than just bottlenecks, for flooding could move individuals in floating mats of debris. New populations could arise from mixtures of local survivors and individuals relocated from distant areas, creating the strange effect of unrelated haplotypes in the same population. *Metasiro* live deep in thick layers of fine detritus (the "duff" layer) where air pockets likely provide refuge

during brief floods and could aid in keeping debris afloat. An interesting line of inquiry would be to test experimentally whether flood waters have floating debris capable of carrying individual cyphophthalmids to new localities.

Flooding along the Apalachicola River can be caused by local storms but also by high water coming down the Chattahoochee River from the Southern Appalachians. The southern Appalachians and especially northern Georgia can experience heavy rains that increase the flow rate in local rivers in excess of 1000-fold in a few days (McCallum and Gotvald 2010), bringing debris to the lowlands. Moreover, the other widespread mite harvestman for which we have phylogeographic data, *Aoraki denticulata*, lives at a variety of elevations among the rivers of the Tasman and Nelson regions of New Zealand. This is also an area prone to flooding (NIWA 2010), which is generally one of New Zealand's most prominent hazards (Smart and McKerchar 2010). It was suggested that past geologic events isolated *A. denticulata* populations, causing the evolution of highly divergent COI haplotypes, and now the populations have merged and experience a small amount of gene flow between them (Boyer et al. 2007a). What we propose here for *M. americanus* is the opposite: isolated populations normally experience no gene flow among them, but gene flow happens on a rapid and massive scale among various subsets of populations during historical floods. We also notice that *M. sassafrasensis* lives at the head of both the Apalachicola and Savannah River watersheds. The three odd species of *Metasiro* are not as disjunct as they first appear, and these watersheds along with major floods could have been the conduit by which *Metasiro* expanded after ocean incursions. This demographic model may apply to *A. denticulata* as well, which has been reconstructed to have radiated from the mountains to the coast and which shows signs of haplotype mixture in local populations (Fernández and Giribet 2014).

Geologic history of the Southeastern United States and dating *Metasiro*

The dates we recovered for the history of *M. americanus* using a date-range calibration of Sternophthalmi from Giribet et al. (2012) in PATHd8 tightly match historical events in the region. Contrary to our expectations, *M. sassafrasensis* in the Southern Appalachians is sister to the other two species and appears to be the oldest of the three, and the variety of dates we recovered from tests in PATHd8 are consistent with a scenario in which *Metasiro* existed as a relict in the Southern Appalachians for many millions of years between the breakup of Pangea and the retreat of the Atlantic and Suwannee Strait. This relictual nature of *Metasiro* is reminiscent of the cyphophthalmid family Troglosironidae on New Caledonia (Sharma and Giribet 2009), which is also hypothesized to have expanded quickly once land exposure increased. The most recent event in the diversification of *Metasiro* of interest

here, and the one most removed from user-input calibration points, was the start of diversification of *M. americanus* in the western panhandle of Florida. We would expect this to match the retreat of the Gulf Trough 14 Ma, and we recovered mean dates of 12.9–15.9 Ma from different dating tests. Thus, fidelity between the dated phylogeny of harvestmen and geologic history appears to have remained remarkably unchanged since the Paleozoic. As independent tests of this hypothesis, future endeavors should add *Metasiro* specimens from Georgia, with the expectation that divergence of the Georgia lineages accords with the timing of exposure of the inhabited terranes.

Ecology and expansion in *Metasiro*

The population genetic data we present here are consistent with what we have inferred from mite harvestman biogeographic studies and species ranges, as well as the phylogeographic study of *A. denticulata*: mite harvestmen exhibit some of the lowest dispersal potential yet recorded. As expected, *Metasiro* shows evidence of major events in the region's geologic history, the riverine habitat and connecting watersheds of the Southeastern United States facilitating the establishment of lowland species as the coastline expanded. This ecology also allows for a small degree of genetic mixing, and since most mite harvestmen live in regions where such conditions do not exist, the local diversity and biogeographic signal of most cyphophthalmids is significant.

The location of the derived species at the ends of large rivers suggests they were founded by individuals displaced down the Savannah and Chattahoochee rivers from Sassafras Mt. This in turn supports a model of range expansion for *Metasiro* based on hydrochory (seed dispersal via water) (Merritt and Wohl 2002; Hyslop and Trowsdale 2012). When the vector of displacement is water, materials are dislodged, carried in fast-moving currents, and then deposited at slower velocities, such as inside river bends or on deltas; this fits *Metasiro*'s absence along most of the Savannah and Chattahoochee but abundance where the rivers level off and decelerate. The distribution of *M. americanus* also resembles certain Appalachian plants (Brooks 1965), and many plants have seeds that lie dormant until being carried by floods and deposited elsewhere (de Rouw et al. 2007). In addition, studies on the interaction between riparian habitats and leaf-litter production and movement (Xiong and Nilsson 1997) may be relevant to cyphophthalmid phylogeography.

In most cases, water-facilitated displacement is part of a larger suite of dispersal tactics that lead to widespread species that do not carry the signal of vicariance events, but if occurring only during major floods and with moderate survival, hydrochory might be a mechanism by which otherwise poor dispersers expand their ranges. Indeed, rare, extreme events that provide long-distance dispersal opportunities may hold more explanatory power in many biogeographical studies than

common processes (Nathan 2008). Dispersal down major rivers during floods could redress the persistent paradox of how such a poor disperser as Cyphophthalmi came to occupy all of Pangea and subsequently persist in post-Pangean landmasses—a prerequisite for retaining the signature of vicariance on various continental landmasses after supercontinental breakup.

Range expansion mechanisms have been examined with urgency in invasive exotics (Wang et al. 2011; Wilson et al. 2001), and the subject intersects with studies of habitat suitability (White et al. 2012), climate change (Davis and Shaw 2001), and behavior (Duckworth and Badyaev 2007). The issue has been addressed generally in vagile organisms whose distributional changes are of relevance to conservation, agriculture, or epidemiology (La Sorte 2006; Gotelli and Arnett 2000; Ricciardi et al. 1998; Medlock et al. 2012). However, for poorly dispersing organisms whose evolutionary histories retain the signal of ancient geological events, the mechanism of range expansion remains unknown, and passive mechanisms like water and wind have been discussed in much greater detail with plants than animals.

We expect expansion via hydrochory to be an explanatory phenomenon for various poor dispersers that carry clear signals of past land movements, such as velvet worms (Murienne et al. 2015), ricinuleids (Murienne et al. 2013), caecilians (Zhang and Wake 2009), and cypresses (Mao et al. 2012). First, the least vagile animals should be more prone to passive modes of displacement like being swept away by water, simply because they lack the mobility necessary to escape catastrophic flooding (unlike, say, *F. deprehendor*, which is more mobile but also not found outside the Appalachians). Second, flooding is the optimal vector for otherwise poor dispersers to carry the signal of plate tectonics, and so it should be more common in such lineages. Flood survivors have a natural stopping point at landmass edges, where floodwaters reach into level, lowland forests and deposit floating debris as waters recede, or drop heavy debris through slowing flow velocity. Expansion via hydrochory would also allow species to fill newly accreted, emerged, and deposited land on continental margins. Catastrophic floods may be especially survivable, carrying larger pieces of debris with refugia and large numbers of individuals, and carrying them more quickly to lowland areas where they could settle.

An important component of the expansion-via-hydrochory hypothesis is that lowland species must at some point attain high elevations from which to move downstream, and a standing question in the *Metasiro* system is how the population on Sassafras Mt. attained that location. During the breakup of Pangea 200 Ma, the region between tropical western Gondwana and what is today the home range of *Metasiro* was an expanse of waterways and islands (Smith and Lord 1997), and reconstructing Sassafras Mt. before then is difficult. Only about 50 Ma earlier the Alleghanian orogeny,

which formed the Appalachian Mountains as Laurentia and Gondwana collided, was ending, and the southern end of the chain underwent a complex series of sedimentation and uplift events (Sector et al. 1986). This tumult has left behind various oddities, such as the northward-flowing New River, and, it appears, *M. sassafrasensis* on Sassafras Mt. Future studies of species complexes within Cyphophthalmi and other candidate taxa exemplifying vicariance should test whether hydrochory is a generalizable mechanism for range expansion in organisms with low vagility.

References

Boyer, S. L., & Giribet, G. (2007). A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. *Cladistics, 23*(4), 337–361.

Boyer, S. L., & Giribet, G. (2009). Welcome back New Zealand: regional biogeography and Gondwanan origin of three endemic genera of mite harvestmen (Arachnida, Opiliones, Cyphophthalmi). *Journal of Biogeography, 36*, 1084–1099.

Boyer, S. L., Baker, J. M., & Giribet, G. (2007a). Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread 'mite harvestman' defies DNA taxonomy. *Molecular Ecology, 16*, 4999–5016.

Boyer, S. L., Clouse, R. M., Benavides, L. R., Sharma, P., Schwendinger, P. J., Karunarathna, I., et al. (2007b). Biogeography of the world: a case study from cyphophthalmid Opiliones, a globally distributed group of arachnids. *Journal of Biogeography, 34*, 2070–2085.

Bragagnolo, C., Pinto-da-Rocha, R., Manuel Antunes, J., & Clouse, R. M. (2015). Phylogenetics and phylogeography of a long-legged harvestman (Arachnida: Opiliones) in the Brazilian Atlantic Rain

Forest reveals poor dispersal, low diversity, and extensive mitochondrial introgression. *Invertebrate Systematics*, in press.

Britton, T., Anderson, C. L., Jacquet, D., Lundqvist, S., & Bremer, K. (2007). Estimating divergence times in large phylogenetic trees. *Systematic Biology, 56*(5), 741–752.

Brooks, M. (1965). *The Appalachians* (Vol. 1, The Naturalist's America). Boston: Houghton Mifflin Company.

Burns, R. M., & Honkala, B. H. (1990). *Silvics of North America: 1. Conifers; 2. Hardwoods. Agriculture Handbook 654* (Vol. 2). Washington, DC: U.S. Department of Agriculture, Forest Service.

Calderon, I., Ortega, N., Duran, S., Becerro, M., Pascual, M., & Turon, X. (2007). Finding the relevant scale: clonality and genetic structure in a marine invertebrate (*Crambe crambe*, Porifera). *Molecular Ecology, 16*(9), 1799–1810.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: a computer program to estimate gene genealogies. *Molecular Ecology, 9*(10), 1657–1659.

Clouse, R. M., & Giribet, G. (2007). Across Lydekker's Line—first report of mite harvestmen (Opiliones : Cyphophthalmi : Stylocellidae) from New Guinea. *Invertebrate Systematics, 21*(3), 207–227.

Clouse, R. M., & Giribet, G. (2010). When Thailand was an island—the phylogeny and biogeography of mite harvestmen (Opiliones, Cyphophthalmi, Stylocellidae) in Southeast Asia. *Journal of Biogeography, 37*(6), 1114–1130.

Clouse, R. M., & Wheeler, W. C. (2014). Descriptions of two new, cryptic species of *Metasiro* (Arachnida: Opiliones: Cyphophthalmi: Neogoveidae) from South Carolina, USA, including a discussion of mitochondrial mutation rates. *Zootaxa, 3814*(2), 177–201.

Clouse, R. M., Janda, M., Blanchard, B., Sharma, P., Hoffmann, B. D., Andersen, A. N., et al. (2015). Molecular phylogeny of Indo-Pacific carpenter ants (Hymenoptera: Formicidae, *Camponotus*) reveals waves of dispersal and colonization from diverse source areas. *Cladistics, 31*, 424–437.

Colwell, R. K. (2004–2011). *Biota* 2: *the biodiversity database manager.* Sunderland: Sinauer Associates.

Crisp, M. D., Trewick, S. A., & Cook, L. G. (2011). Hypothesis testing in biogeography. *Trends in Ecology and Evolution, 26*, 66–72.

Dallmeyer, R. D. (1989). Contrasting accreted terranes in the southern Appalachian Orogen, basement beneath the Atlantic and Gulf Coastal Plains, and West African orogens. *Precambrian Research, 42*, 387–409.

Davis, N. W. (1933). A new opilionid from Florida (Arachnida, Cyphophthalmi). *Journal of the New York Entomological Society, 41*(1/2), 49–53.

Davis, M. B., & Shaw, R. G. (2001). Range shifts and adaptive responses to Quaternary climate change. *Science, 292*(5517), 673–679.

de Bivort, B. L., Clouse, R., & Giribet, G. (2012). A cladistic reconstruction of the ancestral mite harvestman (Arachnida, Opiliones, Cyphophthalmi): portrait of a Paleozoic detritivore. *Cladistics, 28*(6), 582–597.

de Rouw, A., Douillet, M., Tjiantahosong, H., Ribolzi, O., & Thiébaux, J.-P. (2007). Dispersal of weed seeds by erosion and flow processes in upland fields. In L. Gebbie, A. Glendinning, R. Lefroy-Braun, & M. Victor (Eds.), *International Conference on Sustainable Sloping Lands and Watershed Management: Linking Research to Strengthen Upland Policies and Practices, Luang Prabang, Lao PDR, December 12–15, 2006* (pp. 156–166). Vientiane: National Agriculture and Forestry Research Institute.

Drummond, A. J., & Rambaut, A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology, 7*, 214.

Duckworth, R. A., & Badyaev, A. V. (2007). Coupling of dispersal and aggression facilitates the rapid range expansion of a passerine bird. *Proceedings of the National Academy of Sciences, 104*(38), 15017–15022.

Dunlop, J. A. (2010). Geological history and phylogeny of Chelicerata. *Arthropod Structure & Development, 39*, 124–142.

Dunlop, J. A., Anderson, L. I., Kerp, H., & Hass, H. (2004). A harvestman (Arachnida : Opiliones) from the Early Devonian Rhynie cherts, Aberdeenshire, Scotland. *Transactions of the Royal Society of Edinburgh: Earth Sciences, 94*, 341–354.

Economo, E. P., Sarnat, E. M., Janda, M., Clouse, R., Klimov, P., Fischer, G., et al. (2015). Breaking out of biogeographic modules: range expansion and taxon cycles in the hyperdiverse ant genus Pheidole. *Journal of Biogeography*, in press.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research, 32*(5), 1792–1797.

Excoffier, L., & Heckel, G. (2006). Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics, 7*, 745–758.

Fernández, R., & Giribet, G. (2014). Phylogeography and species delimitation in the New Zealand endemic, genetically hypervariable harvestman species, *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi). *Invertebrate Systematics, 28*, 401–414.

Garwood, R. J., Sharma, P. P., Dunlop, J. A., & Giribet, G. (2014). A Palaeozoic stem-group to mite harvestmen revealed through integration of phylogenetics and development. *Current Biology, 24*(9), 1017–1023.

Giribet, G. (2000). Catalogue of the Cyphophthalmi of the world (Arachnida, Opiliones). *Revista Iberica de Aracnologia, 2*, 49–76.

Giribet, G., & Kury, A. B. (2007). Phylogeny and biogeography. In R. Pinto-da-Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: the biology of Opiliones* (pp. 62–87). Cambridge: Harvard University Press.

Giribet, G., Sharma, P. P., Benavides, L., Boyer, S., Clouse, R., de Bivort, B. L., et al. (2012). Evolutionary and biogeographic history of the harvestman suborder Cyphophthalmi (Arachnida, Opiliones)—an ancient and global group of arachnids. *Biological Journal of the Linnean Society, 105*(1), 92–130.

Goloboff, P. A., Farris, J. S., & Nixon, K. C. (2008). TNT, a free program for phylogenetic analysis. *Cladistics, 24*, 774–786.

Gotelli, N. J., & Arnett, A. E. (2000). Biogeographic effects of red fire ant invasion. *Ecology Letters, 3*(4), 257–261.

Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution, 27*(2), 221–224.

Graur, D., & Martin, W. (2004). Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics, 20*(2), 80–86.

Guillot, G., Mortier, F., & Estoup, A. (2005). Geneland: a computer package for landscape genetics. *Molecular Ecology Notes, 5*(3), 712–715.

Hedin, M., Starrett, J., Akhter, S., Schönhofer, A. L., & Shultz, J. W. (2012). Phylogenomic resolution of Paleozoic divergences in harvestmen (Arachnida, Opiliones) via analysis of next-generation transcriptome data. *PLoS ONE, 7*(8), e42888.

Hudson, R. R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence data. *Genetics, 132*, 583–589.

Hyslop, J., & Trowsdale, S. (2012). A review of hydrochory (seed dispersal by water) with implications for riparian rehabilitation. *Journal of Hydrology. New Zealand, 51*(2), 137–152.

Juberthie, C. (1964). *Recherches sur la biologie des Opilions*. Toulouse: Université de Toulouse.

Juberthie, C. (1988). Les Opilions Cyphophthalmes: biogéographie, vitesse d'évolution, périodes de colonisation du milieu souterrain. *TUB-Dokumentation Kongresse und Tagungen, Berlin, 38*, 303–308.

Katoh, K., Misawa, K., Kuma, K., & Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research, 30*(14), 3059–3066.

Kuhner, M. K. (2006). LAMARC 2.0: maximum likelihood and Bayesian estimation of population parameters. *Bioinformatics, 22*(6), 768–770.

Kumekawa, Y., Ito, K., Tsurusaki, N., Hayakawa, H., Ohga, K., Yokoyama, J., et al. (2014). Phylogeography of the laniatorid harvestman *Pseudobiantes japonicus* and its allied species (Arachnida: Opiliones: Laniatores: Epedanidae). *Annals of the Entomological Society of America, 107*(4), 756–772.

La Sorte, F. A. (2006). Geographical expansion and increased prevalence of common species in avian assemblages: implications for large-scale patterns of species richness. *Journal of Biogeography, 33*, 1183–1191.

Labandeira, C. C. (2005). Invasion of the continents: cyanobacterial crusts to tree-inhabiting arthropods. *Trends in Ecology & Evolution, 20*(5), 253–262.

Landis, C. A., Campbell, H. J., Begg, J. G., Mildenhall, D. C., Paterson, A. M., & Trewick, S. A. (2008). The Waipounamu Erosion Surface: questioning the antiquity of the New Zealand land surface and terrestrial fauna and flora. *Geological Magazine, 145*, 173–197.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23*(21), 2947–2948.

Librado, P., & Rozas, J. (2009). DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics, 25*, 1451–1452.

Machado, G., & Macías-Ordóñez, R. (2007). Reproduction. In R. Pinto-da-Rocha, G. Machado, & G. Giribet (Eds.), *Harvestmen: the biology of Opiliones* (pp. 414–454). Cambridge: Harvard University Press.

Mao, K., Milne, R. I., Zhang, L., Peng, Y., Liu, J., Thomas, P., et al. (2012). Distribution of living Cupressaceae reflects the breakup of Pangea. *Proceedings of the National Academy of Sciences, 109*(20), 7793–7798.

Matos-Maraví, P., Clouse, R. M., Sarnat, E. M., Economo, E. P., LaPolla, J. S., Borovanska, M., et al. (2015). An ant genus-group (*Prenolepis*) illuminates the drivers of insect diversification in the Indo-Pacific. *Journal of Biogeography*, in press.

McCallum, B. E., & Gotvald, A. J. (2010). Historic flooding in Northern Georgia, September 16–22, 2009. In Interior (Ed.), *USGS fact sheet* (July 2010 ed.). U.S. Department of the Interior, U.S. Geological Survey.

Medlock, J., Hansford, K., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., et al. (2012). A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. *Vector-Borne and Zoonotic Diseases, 12*(6), 435–447.

Merritt, D. M., & Wohl, E. E. (2002). Processes governing hydrochory along rivers: hydraulics, hydrology, and dispersal phenology. *Ecological Applications, 12*(4), 1071–1087.

Mildenhall, D. C., Mortimer, N., Bassett, K. N., & Kennedy, E. M. (2014). Oligocene paleogeography of New Zealand: maximum marine transgression. *New Zealand Journal of Geology and Geophysics, 57*(8), 107–109.

Miller, M. A., Pfeiffer, W., & Schwartz, T. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, Louisiana, November 14, 2010* (pp. 1–8)

Murienne, J., & Giribet, G. (2009). The Iberian Peninsula: ancient history of a hot spot of mite harvestmen (Arachnida: Opiliones: Cyphophthalmi: Sironidae) diversity. *Zoological Journal of the Linnean Society, 156*(4), 785–800.

Murienne, J., Benavides, L. R., Prendini, L., Hormiga, G., & Giribet, G. (2013). Forest refugia in Western and Central Africa as 'museums' of Mesozoic biodiversity. *Biology Letters, 9*, 20120932.

Murienne, J., Daniels, S. R., Buckley, T. R., Mayer, G., & Giribet, G. (2015). A living fossil tale of Pangaean biogeography. *Proceedings of the Royal Society of London B: Biological Sciences, 281*(1775), 20132648.

Nathan, R. (2008). Long-distance dispersal of plants. *Science, 313*(5788), 787–788.

Neigel, J. E. (2002). Is F_{ST} obsolete? *Conservation Genetics, 3*(2), 167–173.

NIWA. (2010). New Zealand Historic Weather Events Catalog. http://hwe.niwa.co.nz/. Accessed 11 Oct 2012.

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: analyses of phylogenetics and evolution in R language. *Bioinformatics, 20*, 289–290.

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes, 6*, 288–295.

Pearse, D. E., & Crandall, K. A. (2004). Beyond F_{ST}: analysis of population genetic data for conservation. *Conservation Genetics, 5*(5), 585–602.

Popenoe, P., Henry, V. J., & Idris, F. M. (1987). Gulf trough—the Atlantic connection. *Geology, 15*, 327–332.

R Development Core Team. (2012). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Randazzo, A. F. (1997). The sedimentary platform of Florida: Mesozoic to Cenozoic. In A. F. Randazzo & D. S. Jones (Eds.), *The geology of Florida* (pp. 39–56). Gainesville: University Press of Florida.

Ricciardi, A., Neves, R. J., & Rasmussen, J. B. (1998). Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology, 67*(4), 613–619.

Rowley, D. B., & Pindell, J. L. (1989). End Paleozoic–Early Mesozoic Western Pangean reconstruction and its implications for the distribution of Precambrian and Paleozoic rocks around Meso-America. *Precambrian Research, 42*, 411–444.

Schönhofer, A. L., McCormack, M., Tsurusaki, N., Martens, J., & Hedin, M. (2013). Molecular phylogeny of the harvestmen genus *Sabacon* (Arachnida: Opiliones: Dyspnoi) reveals multiple Eocene–Oligocene intercontinental dispersal events in the Holarctic. *Molecular Phylogenetics and Evolution, 66*, 303–315.

Sector, D. T., Snoke, A. W., & Dallmeyer, R. D. (1986). Character of the Alleghanian orogeny in the southern Appalachians: part III. Regional tectonic relations. *Geological Society of America Bulletin, 97*(11), 1345–1353.

Sharma, P., & Giribet, G. (2009). A relict in New Caledonia: phylogenetic relationships of the family Troglosironidae (Opiliones: Cyphophthalmi). *Cladistics, 25*(3), 279–294.

Sharma, P. P., & Giribet, G. (2011). The evolutionary and biogeographic history of the armoured harvestmen—Laniatores phylogeny based on ten molecular markers, with the description of two new families of Opiliones (Arachnida). *Invertebrate Systematics, 25*, 106–142.

Sharma, P. P., & Giribet, G. (2012). Out of the Neotropics: Late Cretaceous colonization of Australasia by American arthropods. *Proceedings of the Royal Society B: Biological Sciences, 279*, 3501–3509.

Sharma, P. P., & Wheeler, W. C. (2013). Revenant clades in historical biogeography: the geology of New Zealand predisposes endemic clades to root age shifts. *Journal of Biogeography, 40*, 1609–1618.

Sharma, P. P. & Giribet, G. (2014). A revised, dated phylogeny of the arachnid order Opiliones. *Frontiers in Genetics, 5*, 255.

Shaul, S., & Graur, D. (2002). Playing chicken (*Gallus gallus*): methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. *Gene, 300*, 59–61.

Smart, G. M., & McKerchar, A. I. (2010). More flood disasters in New Zealand. *Journal of Hydrology (NZ), 49*(2), 69–78.

Smith, D. L., & Lord, K. M. (1997). Tectonic evolution and geophysics of the Florida basement. In A. F. Randazzo & D. S. Jones (Eds.), *The geology of Florida* (pp. 13–26). Gainesville: University Press of Florida.

Stamatakis, A., Hoover, P., & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology, 57*(5), 758–771.

Thomas, S. M., & Hedin, M. (2008). Multigenic phylogeographic divergence in the paleoendemic southern Appalachian opilionid *Fumontana deprehendor* Shear (Opiliones, Laniatores,

Triaenonychidae). *Molecular Phylogenetics and Evolution, 46*(2), 645–658.

Varón, A., Sy Vinh, L., & Wheeler, W. C. (2009). POY version 4: phylogenetic analysis using dynamic homologies. *Cladistics, 26*(1), 72–85.

Vaschetto, L. M., González-Ittig, R. E., Vergara, J., & Acosta, L. E. (2015). High genetic diversity in the harvestman *Geraeocormobius sylvarum* (Arachnida, Opiliones, Gonyleptidae) from subtropical forests in north-eastern Argentina revealed by mitochondrial DNA sequences. *Journal of Zoological Systematics and Evolutionary Research, 53*(3), 211–218.

Wang, R., Wang, J.-F., Qiu, Z.-J., Meng, B., Wan, F.-H., & Wang, Y.-Z. (2011). Multiple mechanisms underlie rapid expansion of an invasive alien plant. *New Phytologist, 191*, 828–839.

Watterson, G. A. (1975). On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology, 7*(2), 256–276.

Wheeler, W. (1996). Optimization alignment: the end of multiple sequence alignment in phylogenetics? *Cladistics, 12*(1), 1–9.

White, T. A., Lundy, M. G., Montgomery, W. I., Montgomery, S., Perkins, S. E., Lawton, C., et al. (2012). Range expansion in an invasive small mammal: influence of life-history and habitat quality. *Biological Invasions, 14*, 2203–2215.

Wilson, T. B., Webb, R. H., & Thompson, T. L. (2001). Mechanisms of range expansion and removal of mesquite (*Prosopis* spp.) in desert grasslands in the southwestern United States, RMRS-GTR-81. *U.S. Forest Service General Technical Report, United States Department of Agriculture*, 23 pp.

Xiong, S., & Nilsson, C. (1997). Dynamics of leaf litter accumulation and its effects on riparian vegetation: a review. *The Botanical Review, 63*(3), 240–264.

Zatz, C., Werneck, R. M., Macías-Ordóñez, R., & Machado, G. (2011). Alternative mating tactics in dimorphic males of the harvestman *Longiperna concolor* (Arachnida: Opiliones). *Behavioral Ecology and Sociobiology, 65*(5), 995–1005.

Zhang, P., & Wake, M. H. (2009). A mitogenomic perspective on the phylogeny and biogeography of living caecilians (Amphibia: Gymnophiona). *Molecular Phylogenetics and Evolution, 53*, 479–491.