**Appendix S1** Supplementary methods and results

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**1. Supplementary Methods**

**1.1. Taxon Sampling, Molecular Data, and Outgroup Details**

Here, we provide additional information on sampling, DNA sequence data, and outgroup taxa used in our analyses that we were unable to give in the main text due to space limitations. First, the majority of our analyses did not include outgroup taxa; this majority includes the nucleotide diversity and % divergence calculations, estimating *t*MRCAs in BEAST, tests for simultaneous diversification in MTML-msBayes, and our IMa2 analyses of community divergence. However, it was necessary to specify outgroup taxa in our analyses evaluating whether the data were consistent with predictions of the neutral model of molecular evolution (Kimura, 1983). To this end, we conducted McDonald & Kreitman’s (1991; MK) tests, which test for deviations of the ratio of replacement (nonsynonymous fixed; RI) to synonymous fixed (SI) substitutions within species from that between species. Specifically, the SI:RI ratio from patterns of substitutions within one species (e.g. one of our focal population-pair datasets) is compared with that of the between-species patterns yielded from comparison to a close relative (e.g. a congeneric outgroup or sister species of one of our focal taxa). In the implementation of the MK test in DnaSP, substitution patterns in an intraspecific dataset are compared against the one or multiple sequences in an outgroup species dataset, and a non-significant difference between the two rations based on two-tailed Fisher’s exact tests is taken as evidence of neutrality. Next, we list outgroups used in MK tests for each population-pair dataset as well as references to studies used to determine the appropriate outgroups. We also provide GenBank accession numbers for outgroup sequences in parentheses, and all outgroup sequences were homologous to those of the focal taxon dataset. Based on phylogenetic analyses in Robertson *et al.* (2009) and Robertson & Zamudio (2009), we used one *16S-ND1* sequence of the congener *Agalychnis saltator* (GenBank GQ366296, Faivovich *et al.*,2010) in our MK test of the *A. callidryas* dataset. Based on phylogenetic analyses in Crawford *et al.* (2007), we used 12 composite cyt*b*-*cox1* sequences of the congener *Craugastor fitzingeri* (GenBank DQ350193–198, DQ350236–241, Crawford *et al.*,2007; EF635371, EF629419–423, EF629462, EF629458, EF629459, EF629455, EF629453, other studies cited in Crawford *et al.*) in our MK test of the *C. crassidigitus* dataset. Based on Robertson *et al.* (2009), we used one composite sequence of *16S*, *tRNA*-Leu, and *ND1* genes from the congener *Dendropsophus microcephalus* (GenBank AY819503, Wiens *et al.*,2005) as the outgroup in our MK test of the *D. ebraccatus* dataset. Based on Weigt *et al.* (2005), we used one congeneric *Engystomops petersi* *cox1* sequence from that same study (GenBank DQ120042) as the outgroup in our MK test of the *E. pustulosus* dataset. Based on McCafferty *et al.* (2012), we used eight *ATPase6/8* sequences from congener *Andinoacara rivulatus* (GenBank JX677777–784, Musilová *et al.* 2008, 2009) as the outgroup sequences in our MK tests of the *A. coeruleopunctatus* dataset. Based on results in Bermingham & Martin (1998) and Martin & Bermingham (2000), we used composite *ATP6/8*, *cox1* sequences from six individuals of the “type B” *Pimelodella chagresi* lineage (a cryptic species lineage discovered in that study; GenBank AF040388, AF040407–409, AF040412, AF040415, AF040484–489) as the outgroup sample in our MK test of the *P. chagresi* population-pair dataset. Last, based on Bermingham & Martin (1998), we used composite *ATP6/8*, *cox1* sequences from three individuals of the congener *Roeboides meeki* (GenBank AF040522–524 and AF040559–561, Bermingham & Martin, 1998) as the outgroup sample in our MK test of the *R. occidentalis–R. guatemalensis* population-pair dataset.

**2. Supplementary Results**

**2.1. Additional Genetic Diversity and Neutrality Results and Discussion**

Based on *P-*values from Fisher’s exact tests, our MK tests did not detect significant (*P* < 0.05) departures from mtDNA neutrality in any of the WPI break population-pair-to-outgroup comparisons: *A. callidryas P* = 0.271; *C. crassidigitus P* = 0.620; *D. ebraccatus* *P* = 0.449; *E. pustulosus P* = 0.116; *A. coeruleopunctatus P* = 1.000; *P. chagresi P* = 1.000; *R. occidentalis–R. guatemalensis* *P* = 0.104.

**2.2. Additional MTML-msBayes Results and Discussion**

The posterior distribution of the divergence time parameter showed relatively small amounts of variance, or *Var*[*τ*], in both analyses of the frogs (*M*2 mode *Var*[*τ*] = 3.89 × 10−4; model-averaging *Var*[*τ*] = 3.85 × 10−4, estimated from hyper-parameter modes) and freshwater fishes (*M*2 mode *Var*[*τ*] = 0.0015; model-averaging *Var*[*τ*] = 9.63 × 10−5, estimated from hyper-parameter modes). However, variance of the modal *τ* values from model averaging was greater than that from the best-supported models.

**3. Potential Limitations and Caveats**

Our study makes several novel contributions, for example by providing the first hABC modeling study illuminating comparative phylogeography of both terrestrial and freshwater species assemblages from the Isthmus of Panama. There are limitations to an approach based solely on mtDNA (as also discussed in the Introduction). Therefore, we have provided a cautious interpretation of our results with the following limitations and caveats in mind.

First, our hABC modeling analyses in MTML-msBayes accounted for coalescent stochasticity of the genetic data by allowing rates of lineage sorting to vary among population-pairs, but if multiple unlinked nuclear loci had been available to us, these may have allowed us to improve our accounting for the stochastic variance of coalescent processes and arrive at more accurate estimates of divergence times or other parameters (e.g. Edwards & Beerli, 2000; Arbogast *et al.*, 2002; Nielsen & Beaumont, 2009). Even so, simulation tests of MTML-msBayes using pseudo-observed datasets from five taxon-pairs show that a single locus outperforms smaller multilocus datasets. Significant improvements (i.e. lower root mean square error) in estimating the hyper-parameters *Ω* and *E*(*τ*) only come when the total number of unlinked loci reaches ≥16, and additional increases in estimator accuracy require >32 loci (Huang *et al.*, 2011). As a result, given we have analyzed mtDNA collected in studies conducted over a 14 year period (Table 1), it will likely take several more years before phylogeographic datasets from multiple Panamanian frog and freshwater fish taxa are sufficiently large to improve upon our results by scaling available datasets up to 16–32+ loci. Moreover, if nuclear loci indicate substantial recombination, incomplete lineage sorting, or admixture across the WPI break, then these will pose additional analytical challenges and, particularly in the case of including migration parameters (Hickerson *et al.*, 2007; Huang *et al.*, 2011), are likely to require many more nuclear loci to develop models that perform well. One reason for this is that substantial migration can hinder the msBayes model from accurately inferring or rejecting simultaneous diversification (e.g. Hickerson *et al.*, 2007).

A second caveat to our results is that they may have been influenced by errors in the mutation rates supplied to the software programs. We conservatively identified ranges of plausible mutation rates for protein-coding mtDNA genes in frogs and fishes, and used these to set uniform priors on *μ* parameters in BEAST and MTML-msBayes. In each case, we allowed the software to estimate *μ*, through ABC or MCMC inference, and we allowed *μ* values to vary among population-pairs. Thus, our analyses accounted for potential differences in *μ* among species/lineages, especially when estimating gene-tree depths in BEAST. However, had more accurate estimates of mtDNA divergence rates been available, our estimates of species/lineage and assemblage divergence times would have been more accurate, and we would have been able to reduce the number of parameters in our models by fixing the mutation rates to accurate estimates for each species/lineage, mainly in the case of BEAST. If, however, mutation rates are broadly similar among taxa (as our *μ* priors assume) but the prior ranges were off by some particular factor (e.g. by half, or two-fold), then we would have obtained similar results during our tests for simultaneous diversification under different *μ* priors correcting by that factor. We also note that the original studies that produced the datasets we analyzed (see Table 1, main text) estimated lineage divergence times using methods that infer gene divergence times and single loci. Their results are thus more likely to have overestimated the timing of lineage divergences across the WPI break than our methods, which only estimated population divergence times (e.g. Edwards & Beerli, 2000).

A third caveat is that, while all of our datasets met the assumption of mtDNA neutrality (Section 2.1 above), we have made assumptions about species demographic histories in each of our analyses that may have introduced error into the results. For example, in BEAST, we set constant population size tree priors assuming the entire sample (both daughter populations) for each population-pair evolved under the neutral coalescent with constant population size through time (Hudson, 1990). However, in MTML-msBayes, ancestral population size segments that evolved within each daughter population were assumed to experience exponential population growth to present. Additional demographic information and more flexible modeling approaches allowing different population-size transitions in different parts of the population trees for each population-pair, had they been available, may have permitted more accurate demographic models yielding more accurate parameter estimates. Although we allowed population size changes in our models, our main goal was not to infer historical demography of each species, at large.

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