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Research Article

Testing hypotheses of diversification in Panamanian frogs and freshwater fishes using hierarchical approximate Bayesian computation with model averaging

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Running head: Frog & fish diversification in Panama (37 characters w/spaces)

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Abstract

Most Neotropical frog and freshwater fish species sampled to date show phylogeographic breaks along the Pacific coast of the Isthmus of Panama, with lineages in Costa Rica and western Panama isolated from central Panama. We examine temporal patterns of diversification of taxa across this 'western Panama isthmus' (WPI) break to test hypotheses about the origin of species geographical distributions and genetic structuring in this region. We tested for synchronous diversification of four codistributed frog taxon-pairs and three fish taxon-pairs sharing the WPI break using hierarchical approximate Bayesian computation with model averaging based on mitochondrial DNA sequences. We also estimated lineage divergence times using full-Bayesian models. Several of our results supported synchronous divergences within the frog and freshwater fish assemblages; however, Bayes factor support was equivocal for or against synchronous or asynchronous diversification. Nevertheless, we infer that frog populations were likely isolated by one or multiple Pliocene–Pleistocene events more recently than predicted by previous models, while fish genetic diversity was structured by Pleistocene events. By integrating our results with external information from geology and elevational sea level modeling, we discuss the implications of our findings for understanding the biogeographical scenario of the diversification of Panamanian frogs and fishes. Consistent with the 'Bermingham/Martin model' (*Mol. Ecol.* 1998, 7: 499-517), we conclude that the regional fish assemblage was fractured by processes shaping isthmian landscapes during the Pleistocene glaciations, including drainage basin isolation during lowered sea levels.

Introduction

By revealing the geographical histories of genetic lineages within species, phylogeography helps identify processes that influence the divergence, spread, and spatial-demographic fluctuations of lineages (Avise *et al.*, 1987; Avise, 2000; Kidd & Ritchie, 2006). Single-species phylogeography surveys often reveal cryptic refugia and speciation events (e.g. Avise, 2000; Soltis *et al.*, 2006; Bagley & Johnson, 2014a). By contrast, 'comparative phylogeography' presents a stronger approach for testing the generality of evolutionary patterns recovered within single species, often uncovering historical processes shaping species richness and genetic diversity (Arbogast & Kenagy, 2001; Hickerson *et al.*, 2010). Comparative phylogeography infers the histories of regional assemblages by testing for spatial and temporal phylogeographic congruence across codistributed species (Bermingham & Avise, 1986; Avise *et al.*, 1987; Bermingham & Martin, 1998). Congruent spatiotemporal divergences across taxa can indicate a shared history of responses to historical events (Bermingham & Martin, 1998; Sullivan *et al.*, 2000).

Most comparative surveys in phylogeography are based on mitochondrial DNA (mtDNA) and are consequently subject to the caveats of using a single locus. Phylogeographic histories within individual species may be confounded by effects of stochastic variance in coalescent and mutational processes, natural selection, sex-biased dispersal, or reproductive success on genetic data (Avise, 1989; Edwards & Beerli, 2000; Hey & Machado, 2003; Kuo & Avise, 2005; Irwin, 2012). Comparative studies guard against effects of such stochastic processes on inferences by searching for phylogeographic patterns replicated across multiple lineages (Avise, 2000; Zink, 2002). This is analogous to improving evolutionary inferences through using multiple loci to estimate population parameters, multilocus models, or population/species trees across multiple gene genealogies (Edwards & Beerli, 2000; Kubatko *et al.*, 2009; Heled & Drummond, 2010). Still, comparative approaches face major challenges to distinguish among potential scenarios that

63 may have led to the observed patterns. Parameter estimates from mtDNA can suffer from low
64 resolution, yielding wide confidence intervals that limit hypotheses testing (Edwards & Beerli,
65 2000). The critical task of understanding the temporal framework of diversification to infer the
66 historical biogeographical scenario underpinning spatial-genetic patterns can also be
67 problematic, even if spatially congruent breaks are identified. Because variance in past effective
68 population sizes (N_e), mutation rate, and generation time create high variation in gene tree
69 topologies and their depths (correlated with timing of their divergences; e.g. Avise, 1989;
70 Hudson, 1990), what appear as multiple divergence events may actually reflect the obscured
71 signal of a single event (Riddle & Hafner, 2006). Thus, comparing independent gene-tree depths
72 and divergence time estimates can lead to erroneous inferences of multiple vicariance events.
73 To guard against potential misinterpretations of biogeographical history, it is imperative
74 to rigorously evaluate the timing of population divergences across species. Approximate
75 Bayesian computation (ABC) methods for phylogeography allow accounting for potentially
76 confounding stochastic coalescent effects while estimating parameters of phylogeographic
77 datasets in such a way that sidesteps the need to calculate explicit likelihood functions. These
78 "likelihood-free" methods permit simultaneously estimating demographic parameters and
79 divergence times across populations by replacing the data with summary statistics, resulting in
80 more efficient extraction of information (e.g. Beaumont *et al.*, 2002; Hickerson *et al.*, 2007). One
81 broadly validated ABC method for comparative phylogeography, the MTML-msBayes pipeline
82 (Hickerson *et al.*, 2006, 2007, 2014; Huang *et al.*, 2011), permits comparing hypotheses in a
83 parameter-rich hierarchical ABC (hABC) model using data from multiple codistributed
84 population-pairs. This method has become widely used for testing explicit hypotheses about the
85 timing of genetic divergences that have arisen during the assembly and diversification of

regional species assemblages (Leaché *et al.*, 2007; Barber & Klicka, 2010; Bell *et al.*, 2012; Dolman & Joseph, 2012; Bagley & Johnson, 2014b; Smith *et al.*, 2014). Based on simulations, some have cautioned that overly broad priors can result in low power to detect multiple divergences using MTML-msBayes (Oaks *et al.*, 2013). However, this pitfall can be avoided by graphical sampling checks as well as comparing multiple MTML-msBayes model classes (priors) using ABC model averaging to account for uncertainty in model selection (Hickerson *et al.*, 2014; Bagley, 2014). Other workers have also advocated leaving the summary statistics vector unsorted, as well as employing a Dirichlet-process prior as the hyperprior for the hyperparameter on the number of co-divergence events (Ψ) in the MTML-msBayes model and relying on this hyperparameter for inference (Oaks, 2014; Oaks *et al.*, 2014; Papadopoulou & Knowles, 2015). However, our recent study using a cross-validation approach applied to simulated and empirical data, including the Panamanian datasets analyzed in the current paper, shows conclusively that choice of prior on Ψ has limited impact on inference, while re-sorting of summary statistics yields considerably more reliable results (Overcast *et al.*, 2017).

The Central American Isthmus has been an active area of phylogeographic research for nearly 20 years, with most studies focusing on inferring how, when, and why lineages colonized the isthmian landscape, diversified, and assembled into local communities (Bagley & Johnson, 2014a). A growing catalogue of species is being identified with common phylogeographic divergences across the Pacific-coastal lowlands of the Isthmus of Panama, with lineages in Costa Rica's Golfo Dulce region and western Panama isolated from lineages in central Panama (Figs 1 and 2; reviewed by Bagley & Johnson, 2014a). This pattern has become known as the 'western Panama isthmus' (WPI) break (Bagley & Johnson, 2014a). Biogeographers interpret WPI breaks in frogs as resulting from multiple dispersals of different lineages into Central America across a

continuous Miocene–Pliocene wet forest corridor, from both the north and the south (Savage, 1966, 1982, 2002; Vanzolini & Heyer, 1985), followed by vicariance caused by the development of tropical dry forest 12–4 million years ago (Ma; 'dry forest hypothesis'; Crawford *et al.*, 2007; Wang *et al.*, 2008). While frog fossils from the region are lacking, this scenario is supported by plant microfossils showing that modern dry forest species began appearing in central Panama up to mid-Pliocene (Graham & Dilcher, 1995; Piperno & Pearsall, 1998). Among freshwater fishes, the WPI break appears to reflect the effectiveness of rugged terrains at Soná and Azuero peninsulas (Fig. 1) as biogeographical barriers to fish lineages, which arrived in multiple northwestward waves from South America since the Neogene. Bermingham & Martin (1998) hypothesized that a corridor formed by low sea level and tectonic uplift of the isthmus as the Cocos Ridge collided with the Panama microplate by ~7 Ma (Coates & Obando, 1996) permitted 'early' emplacement of freshwater fish species since Miocene–Pliocene. Subsequently, they argued that high eustatic sea levels of the mid-late Pliocene ~3.5–3 Ma (Cronin & Dowsett, 1991) recreated a seaway over the isthmus, causing freshwater fishes to go extinct in central Panama ~3 Ma, before final isthmus emergence (Bermingham & Martin, 1998). They hypothesized that the WPI break in fishes resulted from fragmentation due to drainage basin isolation during Pleistocene glacial periods over 2–0 Ma (Bermingham & Martin, 1998). This 'Bermingham/Martin model' has also been invoked to explain phylogeographic patterns in pseudoscorpions, eels, catfishes, frogs, and other taxa (e.g. Zeh *et al.*, 2003; Perdices *et al.*, 2002, 2005; Weigt *et al.*, 2005; Bagley & Johnson, 2014a).

Clearly, previous studies generated or tested biogeographical models that provide convincing evidence for the WPI break. However, none of these studies explicitly accounted for genetic variance associated with coalescent processes, or among-lineage differences in

demography or mutational rates, that may have influenced the variable patterns of genetic divergences of taxa associated with the WPI break (Bermingham & Martin, 1998; Crawford *et al.*, 2007; see Results). Thus, the temporal pattern and underlying mechanisms of diversification across the WPI break have not yet been fully explored. Additional studies are needed to evaluate whether the pattern of genetic structuring shared among frogs and freshwater fishes in the western Panama isthmus arose synchronously or asynchronously, and to determine the timing of historical events that have shaped community assembly and genetic divergence in this region.

The goal of this study is to examine the temporal patterns of diversification of frogs and freshwater fishes across the WPI break to test existing hypotheses about the origin of species distributions and genetic structuring in west-Pacific Panama. We use hABC simulations with ABC model averaging, and full-Bayesian divergence time estimation, to test the alternative hypotheses of a single, synchronous pulse of diversification, versus multiple pulses of diversification, in the frog and fish assemblages across this region. In doing so, we are able to directly test the hypotheses discussed above. Support for synchronous diversification correlated to Pliocene high seas ~3.5–3 Ma would agree with frog and fish WPI divergences arising from a common history of Neogene dispersal into western Panama followed by isolation wrought by fluctuating Plio–Pleistocene sea levels, and possibly other factors (e.g. dry forests isolating the frog populations). This would support the Bermingham/Martin model as a general model of diversification applicable to frogs and freshwater fishes. By contrast, an inference of one or multiple pulses of frog diversification <4 Ma would reject the dry forest hypothesis, whereas a pulse of fish vicariance events >2 Ma would reject the Bermingham/Martin model as an explanation for fish assemblage genetic structure across the western Isthmus of Panama.

Materials and methods

155 **Taxon sampling and molecular data**

156 We considered all phylogeographic studies of lower Central American (Costa Rica–Panama)
157 taxa, including studies summarized in a recent comprehensive review (Bagley & Johnson,
158 2014a), and subsequent publications. Taxa were included in this study if they met minimum
159 criteria for our analyses: frogs or freshwater fish species/lineages codistributed across the study
160 area; genetic divergence at the WPI break, with at least two individuals sampled on either side;
161 and mtDNA gene sequences available from GenBank. We focused on the mtDNA locus because
162 it presents a rapidly evolving and coalescing genomic region that often reflects population
163 history (Zink & Barrowclough, 2008). Also, protein-coding mtDNA genes have been widely
164 studied in Central American taxa, permitting interspecific comparisons.

165 We obtained seven population-pair comparisons from eight taxa from our literature
166 search (Table 1). Taxa that met our criteria included the following four lineages of frogs: the red-
167 eyed treefrog, *Agalychnis callidryas* (Robertson & Zamudio, 2009); the dirt frog, *Craugastor*
168 *crassidigitus* (Crawford *et al.*, 2007); the hourglass treefrog, *Dendropsophus ebraccatus*
169 (Robertson *et al.*, 2009), and the túngara frog, *Engystomops* (*Physalaemus*) *pustulosus* (Weigt *et*
170 *al.*, 2005). The following three freshwater fish lineages also met our criteria: the blue acara,
171 *Andinoacara coeruleopunctatus* (McCafferty *et al*., 2012); the Chagres catfish, *Pimelodella*
172 *chagresi* (Bermingham & Martin, 1998; Martin & Bermingham, 2000); and headstander tetras,
173 *Roeboides occidentalis–R. guatemalensis* (Bermingham & Martin, 1998). While their broader
174 geographical distributions differ to varying degrees, all species/lineages are codistributed in the
175 study area and exhibit the WPI break (Fig. 2), and most are thought to have expanded their
176 ranges into Central America from southern source populations in eastern Panama or northern
177 South America (Bussing, 1976, 1998; Savage, 1982, 2002; Bermingham & Martin, 1998). An

178 exception to this is *C. crassidigitus*, which more likely colonized the region from an ancestral
179 population in the north, i.e. Nuclear Central America (Vanzolini & Heyer, 1985; Crawford &
180 Smith, 2005). We excluded from our analyses two taxa inferred to exhibit the WPI break in
181 previous studies, *Brachyhypopomus occidentalis* (Bermingham & Martin, 1998) and
182 *Synbranchus marmoratus* (Perdices *et al*., 2005). In the case of *B. occidentalis*, available mtDNA
183 sequences had insufficient sample sizes for estimating population parameters and coalescent
184 modeling, which require ≥2 individuals from daughter lineages on either side of the break. Also,
185 two studies conflicted as to whether this taxon showed the WPI break (Bermingham & Martin,
186 1998; Picq *et al.*, 2014). In *S. marmoratus*, the geographical location of the WPI break included a
187 major north–south component of isolation across the Central Cordillera, thus the break was not
188 as well confined to the Pacific coast as in other taxa we analyzed, making comparisons difficult.
189 A summary of sampling intensity and molecular characteristics of each dataset, and their
190 published sources, is provided in Table 1. Our final database consisted of 109 sequences
191 representing 10–28 individuals sampled from each of these seven species (2–16 individuals
192 sampled from the west or east side of the break), permitting us to address the evolution of frog
193 and freshwater fish species assemblages. Final mtDNA alignments for each species/lineage
194 ranged from 564 to 1877 bp in length, and most (4/7) datasets contained cytochrome oxidase
195 subunit 1 (*cox1*) 'DNA barcode' sequences. GenBank numbers and locality data for the samples
196 used in each dataset are provided in Table S1. Sequence data were grouped *a priori* into
197 populations east and west of the WPI break. We assumed HKY (Hasegawa *et al.*, 1985) models
198 with gamma-distributed rate variation (Γ) and an estimated proportion of invariant sites (*I*)
199 wherever possible 1) because this model provides a more appropriate description of mtDNA
200 sequence evolution (e.g. allowing recurrent mutations) than other nucleotide substitution models

201 available in the MTML-msBayes pipeline (e.g. Jukes-Cantor or equal-input), and 2) to make
202 results comparable across analyses.

203 **Genetic diversity and neutrality**

204 We examined genetic diversity by calculating nucleotide diversity (π; Nei, 1987) for each dataset
205 using the 'obsSumStats.pl' Perl script available in the latest version of the MTML-msBayes
206 pipeline (Huang *et al.*, 2011). To further evaluate patterns of genetic divergence across the WPI
207 break, we estimated average % divergence between clades for all population-pairs using
208 distances corrected using the HKY+Γ+*I* substitution model in PAUP* 4.0b10 (Swofford, 2002).
209 In PAUP*, we specified Γ and *I* parameter estimates derived from Bayesian coalescent gene-tree
210 analyses of each population-pair (below). Although selective neutrality is often a valid
211 assumption for vertebrate organellar mtDNA, this was a key assumption of our analyses; thus,
212 we tested for mtDNA selective neutrality using McDonald & Kreitman's (1991; MK) tests
213 conducted in DnaSP 5 (Librado & Rozas, 2009). Closely related, congeneric outgroup taxa were
214 included in the MK tests for each population-pair based on phylogenies in the original studies, or
215 large-scale phylogenetic studies (see Appendix S1 for details).

216 **Estimating gene-tree depths using Bayesian dating**

217 To independently assess variation in gene-tree depths across WPI break taxa, we estimated times
218 to the most recent common ancestors (t_{MRCA}s) and their Bayesian credible intervals for each
219 species/lineage using BEAST 1.8.2 (Drummond *et al.*, 2012). BEAST runs (100 million
220 generations, sampled every 4000; 'burn-in' = 10%) started from UPGMA tree topologies,
221 employed HKY+Γ+*I* site models, and used coalescent constant size tree priors (Kingman, 1982).
222 We evaluated the fit of different molecular clock models and investigated clock-likeness of the
223 data by comparing results of strict-clock and relaxed-clock (uncorrelated lognormal model;

224 Drummond *et al.*, 2006) runs on each dataset. Due to uncertainty over frog mtDNA mutation
225 rates, we specified a range of 0.1–1.3% lineage^{-1} Myr^{-1} (per million years) in the frog runs
226 spanning rates of protein-coding mtDNA gene evolution documented in a broader mtDNA
227 dataset from one of our focal taxa (Weigt *et al.*, 2005). This 'frog rate' spans Macey *et al.*'s
228 (1998) Mongolian toad (*Bufo bufo*) rate of 0.69% lineage^{-1} Myr^{-1}, which is commonly used to
229 date patterns of herpetofaunal diversification (Weigt *et al*., 2005). Likewise, we specified a 'fish
230 rate' of 0.17–1.4% lineage^{-1} Myr^{-1} in fish runs, a range spanning mutation rates estimated in 15
231 previous studies of teleost freshwater fish mtDNA (refs. in Waters *et al.*, 1999; Burridge *et al*.,
232 2008). Rate ranges were supplied to the program as uniform priors. We summarized posterior
233 distributions and ensured convergence and adequate effective sample sizes (all ESS >> 200)
234 using Tracer v1.5 (available at: http://beast.bio.ed.ac.uk/Tracer). In TreeAnnotator 1.8.2, we
235 summarized the posterior distribution of trees from each run by calculating a maximum clade
236 credibility (MCC) tree annotated with median node ages from a sample of 5000 post-burn-in
237 trees obtained using the 'BEASTPostProc.sh' script in PIrANHA v0.1.4 (Bagley, 2017).

238 **Tests for synchronous diversification**

239 We tested the hypotheses of a single, synchronous pulse of diversification versus multiple pulses
240 of diversification of the seven population-pairs across the WPI break under a coalescent model,
241 using the MTML-msBayes pipeline (Huang *et al.*, 2011). Because hyperprior ABC samplers like
242 msBayes can inefficiently sample from the prior when overly broad prior bounds are used,
243 biasing them towards inferring simultaneous diversification (Oaks *et al.*, 2013), we used ABC
244 model averaging on candidate priors and visual checks for prior sampling efficiency to help
245 overcome this issue (Hickerson *et al.*, 2014). Also, following recommendations for MTML-
246 msBayes analyses of small numbers of taxon/population-pairs in an extensive simulation study

247 (Overcast *et al.*, 2017), we ran MTML-msBayes with the default summary statistics and sorting
248 algorithm, and we relied principally on the Ω hyperparameter (see below) for inference and
249 hypotheses tests. Our MTML-msBayes analysis consisted of a four-step procedure similar to
250 Hickerson *et al.* (2014). First, we developed four prior sets, or model classes (M_1–M_4), to
251 compare using model averaging. Each model class consisted of one of two uniformly distributed
252 priors on population divergence times (τ), ancestral population size (θ_A), and daughter population
253 size parameters (θ_D; Table 2). Second, we obtained 5 million random (simulated) samples from
254 each model class specified by a discrete uniform hyperprior distribution $\Pr(M_k)=1/4$. We visually
255 checked for efficient prior sampling by conducting principal components analysis on 1000 prior
256 draws from each model class in R. Third, we obtained the ABC joint posterior distribution using
257 the default summary statistic vector (D) from MTML-msBayes and rejection sampling to
258 identify the 1000 closest Euclidean distances between the observed summary statistics (D^*) for
259 the data and D_i calculated from 20 million random draws across all four priors (M_1–M_4).
260 However, prior to rejection sampling, we rescaled the dispersion index of population divergence
261 times [$\Omega = Var(\tau)/E(\tau)$; the ratio of variance to the mean of the divergence times] and the mean
262 assembly-wide divergence time, $E(\tau)$, from models with smaller upper θ_D prior bound values
263 (frog and fish M_1, M_2, and M_4) to have the same coalescent units as the other model, M_3
264 (Hickerson *et al.*, 2014). Resulting estimates of Ω and $E(\tau)$ were weighted by Bayesian model
265 averaging (Hickerson *et al.*, 2014). Last, we conducted hypothesis testing by comparing
266 hyperposterior probability distributions of Ω estimates, to determine whether the data supported
267 single or multiple diversification periods.

268 We conducted hypothesis testing by comparing posterior probabilities of the
269 hyperparameter estimates under a 'null' scenario of asynchronous diversification ($\Omega > 0.01$)

270 versus the alternative of synchronous diversification ($\Omega \leq 0.01$) (Hickerson *et al.*, 2006, 2007;
271 Bell *et al.*, 2012; Bagley & Johnson, 2014b) and subsequently calculating B_{10} Bayes factors
272 under the parameter thresholds above while accounting for prior support for the hypotheses,
273 using B_{10} "weight of evidence" criteria in Jeffreys (1961) and Kass & Raftery (1995). We
274 estimated mean assemblage-wide divergence times by converting model-averaged $E[\tau]$ estimates
275 (in coalescent units of $4N_{ave}$ generations) to absolute time (T_{div}) using the equation T_{div} =
276 $E[\tau]\times(\theta_{ave}/\mu)$, where μ is the assumed mutation rate per site per generation and θ_{ave} (per site) is
277 the mean of the upper θ prior. Conversions used mutation rates equivalent to 0.7% lineage^{-1} Myr^{-}
278 ^{1} and 0.785% lineage^{-1} Myr^{-1}, the median rates of uniform 'frog rate' and 'fish rate' priors used
279 in our BEAST analyses. We assumed an average generation time of 1 year, which was also used
280 in previous studies of our focal taxa (e.g. Weigt *et al.*, 2005; Robertson & Zamudio, 2009;
281 McCafferty *et al.*, 2012), and we acknowledge that divergence time estimates are sensitive to
282 generation times. Shell and R scripts used during our MTML-msBayes analyses are accessioned
283 in Mendeley Data (Bagley & Hickerson, 2018).

284 **Results**

285 **Genetic diversity and neutrality**

286 Clades examined here had considerably variable levels of genetic variation, with π values
287 ranging 6-fold from 0.0093 in *A. callidryas* to 0.0645 in *C. crassidigitus* (Table 1); these same
288 two taxa exhibited the lowest and highest levels of sequence divergence, respectively. There was
289 also more variation in nucleotide diversity among frogs than among freshwater fishes. Likewise,
290 genetic divergences based on HKY+Γ+I corrected distances between clades on either side of the
291 break ranged from 4.6% up to a maximum of 13.9% among frog taxa, whereas that among fish
292 species/lineages showed a more limited range from 2.4% to 6.4% (Table 1). Consistent with the

293 assumptions of our analyses, MK tests did not reveal departures from selective neutrality within
294 any of the mtDNA datasets (Fisher's exact test $P > 0.10$; Appendix S1).

295 **Estimating gene-tree depths using Bayesian dating**

296 Estimated t_{MRCA}s for each of the seven population-pairs were consistent across multiple BEAST
297 runs, which had ESS values >500 for nearly all parameters. Results also were similar across
298 strict-clock and relaxed-clock runs; however, we only present the results of the strict-clock
299 analyses because 95% highest posterior densities (HPDs) of 'ucld.stdev' (standard deviation of
300 the relaxed clock) abutted zero, indicating that the data could not reject strict clock models. The
301 mitochondrial MCC time trees had variable gene-tree depths (Fig. S1), and geometric mean
302 t_{MRCA} estimates (closer to peak likelihood values than the means) varied substantially across
303 species/lineages, ranging from 1.71 Ma in *A. coeruleopunctatus* to 10.79 Ma in *C. crassidigitus*
304 (Fig. 3; frog geometric mean t_{MRCA} range: 3.78–10.80 Ma; fish geometric mean t_{MRCA} range:
305 1.71–4.88 Ma). Consistent with patterns of DNA polymorphism above, t_{MRCA}s of the fish
306 lineages exhibited less variation with a much narrower region of overlap in their coalescence
307 times (1.92–5.57 Ma) as compared with that of the frog lineages (3.88–14.39 Ma), although the
308 frog t_{MRCA}s also overlapped substantially (Fig. 3).

309 **Tests for synchronous diversification**

310 Projecting the observed data into principal components calculated from summary statistics
311 randomly drawn from each model class showed that MTML-msBayes efficiently sampled our
312 prior distributions (Fig. S2). Observed data points also had similar positions across model
313 classes, suggesting different priors were similarly good samplers for the data. Below we report
314 results based on Ω posteriors derived from local linear regression (Beaumont *et al.*, 2002).

We found that the modes of model-averaged posterior estimates of hyperparameter Ω were consistent with synchronous divergences within the frog and fish assemblages (frog Ω mode = 0.0036; fish Ω mode = 0.0017; Table 2; Fig. 4). In agreement with this pattern, $\Omega = 0$ fell within the credible intervals (95% HPD) of each of the model-averaged Ω distributions. The best-supported models also indicated synchronous divergence of the frog and the fish lineages based on posterior modal Ω values of 0.0013 and 0.0047, respectively. Despite this, model comparisons using Bayes factors for the ABC model-averaging results indicated that the data provided equivocal support for or against either model. Bayes factors were around a value of 1 for both the synchronous diversification model (frog $B_{10} = 1.14$ for $\Omega < 0.01$ versus $\Omega > 0.01$; fish $B_{10} = 1.33$ for $\Omega < 0.01$ versus $\Omega > 0.01$) as well as the asynchronous diversification model (frog $B_{10} = 0.88$ for $\Omega > 0.01$ versus $\Omega < 0.01$; fish $B_{10} = 0.75$ for $\Omega > 0.01$ versus $\Omega < 0.01$). Bayes factors were technically less than 1 for two or more divergences, but at best this provides very weak negative evidence for asynchronous divergence (Jeffreys, 1961; Kass & Raftery, 1995). Likewise, posterior probabilities for the best-supported models were low (<0.5) and corresponding Bayes factors for synchronous diversification were weak, being approximately less than or equal to 1.

Based on model-averaging, the modes of assemblage $E(\tau)$ for the frog lineages and fish lineages were 0.106 (95% HPD = 0.056–0.139) and 0.054 (95% HPD = 0.022–0.079), respectively (Fig. 4). When converted to absolute time using the median frog and fish mutation rates above, these peak-likelihood estimates correspond to modal dates of 3.09 Ma (95% HPD = 1.62–4.03 Ma) in the Pliocene to early Pleistocene for the frog divergences and 1.36 Ma (95% HPD = 0.56–2.021 Ma) in the early-mid Pleistocene for the fish divergences (Fig. 4).

Discussion

338 Determining whether species assemblages experienced shared evolutionary responses to
339 historical geological and climate-change events is a central but challenging goal of comparative
340 phylogeography (Avise, 2000; Sullivan *et al.*, 2000; Arbogast & Kenagy, 2001). Multi-taxon
341 hABC methods for comparative phylogeography (e.g. Hickerson *et al.*, 2006, 2007, 2014; Huang
342 *et al.*, 2011) permit testing explicit hypotheses about the timing of genetic divergences that have
343 arisen during the assembly and diversification of regional species assemblages (Leaché *et al.*,
344 2007; Barber & Klicka, 2010; Bell *et al.*, 2012; Dolman & Joseph, 2012; Bagley & Johnson,
345 2014b; Bagley, 2014; Smith *et al.*, 2014). We used hABC coalescent models and full-Bayesian
346 divergence dating to investigate temporal patterns of diversification of frogs and freshwater
347 fishes across the WPI phylogeographic break (Figs 1 and 2; Bagley & Johnson, 2014a) to test
348 two *a priori* hypotheses about the origin of species distributions and genetic structuring along the
349 Pacific coast of Panama.

350 Several aspects of our results indicated that the frog and freshwater fish assemblages each
351 experienced a synchronous pulse of diversification in this region during the late Neogene to
352 Quaternary. This was supported by overlap in estimated t_{MRCA}s for the taxon-pairs (Fig. 3),
353 strong peaks in the Ω posteriors near zero (Fig. 4) and credible intervals including zero (Table 2),
354 and peak-likelihoods and credible intervals of assemblage $E(\tau)$. By contrast, Bayes factor model
355 selection indicated that the data provide only a marginal accumulation of evidence in favor of
356 synchronous diversification and against the null scenario of asynchronous diversification. The
357 msBayes approach has been shown to correctly reject synchronous diversification using only
358 mtDNA and summary statistics such as those used herein (e.g. Hickerson *et al.*, 2006; Huang *et*
359 *al.*, 2011; Hickerson *et al.*, 2014), and visual checks suggested that our priors were efficient
360 samplers of the data (Fig. S2). Moreover, we avoided the problem of overly broad or narrow τ

361 priors (e.g. in Leaché *et al.*, 2007; Oaks *et al.*, 2013), which can cause ABC samplers to explore
362 parameter space exceeding saturation effects on mitochondrial genes (Hickerson *et al.*, 2014), by
363 matching the upper bounds of these priors to empirical estimates from the data. As a result, we
364 conclude that additional genetic data from unlinked loci or additional species, or improved
365 methods for hABC or Bayes factor estimation, are needed to more confidently assess the timing
366 and number of events at the WPI break in these taxa. Nevertheless, the much narrower credible
367 intervals of our assemblage divergence times relative to the Bayesian gene-tree depths inferred in
368 BEAST (Fig. 3) indicate that accounting for coalescent processes and changes in population
369 sizes through time in our models yielded much more precise estimated divergence times across
370 the WPI break than were previously available. Assuming that peaks in Ω and $E(\tau)$ contain one or
371 multiple clusters of population divergence events, and acknowledging limitations and caveats of
372 our mtDNA data (see Introduction and Appendix S1 section 3), we use our Bayesian assemblage
373 $E(\tau)$ estimates to draw broad conclusions about the timing of diversification in these two
374 assemblages and conduct hypotheses tests. We also discuss implications of our results for
375 understanding the historical biogeography and diversification of Panamanian frog and freshwater
376 fish species assemblages.

377 The dry forest hypothesis predicts that the timing of diversification of frogs across the
378 WPI break was marked by the transition from wet tropical forest in the middle Cenozoic to drier
379 forest habitats along the Pacific coast of Panama by >4 Ma according to paleobotanical data
380 (Graham & Dilcher, 1995; Piperno & Pearsall, 1998), and up to 12 Ma based on mtDNA
381 phylogenetic dating analyses (Crawford *et al.*, 2007; Wang *et al.*, 2008). Against these
382 predictions, we inferred that frog diversification across the WPI break occurred more recently,
383 around 3.09 Ma (Figs 3 and 4). Credible intervals for model-averaged assemblage $E(\tau)$ from

MTML-msBayes slightly overlapped the predicted interval, by only ~31 thousand years (kyr); thus, we do not reject the dry forest hypothesis outright. However, modal Ω and assemblage $E(\tau)$ estimates favor the conclusion that the fracturing of western and eastern Pacific Panama frog assemblage coincided with one or multiple historical events occurring after 4 Ma, during the late Pliocene–early Pleistocene. Differences between frog divergence times inferred by earlier workers and this study likely owe in part to differences in sampling and methods used. Crawford *et al.* (2007) dated the isolation of Golfo Dulce *Craugastor* populations using analyses that combined interspecific samples and employed strict molecular-clock and nonparametric rate smoothing methods. Thus, unlike population divergence times we estimated in MTML-msBayes, earlier estimates of Golfo Dulce frog divergence times were based on gene divergences, which are more apt to overestimate the timing of diversification (e.g. Edwards & Beerli, 2000).

While we cannot reject the dry forest hypothesis outright, our results are more consistent with the interpretation that Plio–Pleistocene sea level highstands limited frog dispersion and gene flow across the region, as predicted by the Bermingham/Martin model (Bermingham & Martin, 1998). The Pliocene warm period was a time of reduced global ice volume, stable and warmer temperatures, and widely fluctuating sea levels 3.5–2.5 Ma (Cronin & Dowsett, 1991; Lambeck *et al.*, 2002). Geological evidence from deep-sea sediments and passive continental margins shows that a Pliocene sea-level highstand +35 ± 18 m above present sea levels (a.s.l.) occurred 3.5–3 Ma (Cronin & Dowsett, 1991; Miller *et al*., 2005) and swept over the emerging central Panama isthmus just prior to its final formation ~2.8 Ma (Cronin & Dowsett, 1996; Coates & Obando, 1996; O'Dea *et al.*, 2016; but see Montes *et al.*, 2015). Our divergence time estimates from MTML-msBayes place diversification in the frog assemblage around the time of this eustatic highstand (it falls within the Bayesian credible intervals completely) and match closest

407 to its lower boundary (Fig. 3).

408 To test whether a Pliocene eustatic highstand could have isolated frogs across the WPI
409 break, we developed a GIS (geographic information systems) model of sea-level rise over a
410 modern 250 m Digital Elevation Model using ArcMap (ESRI, Redlands, CA). Assuming the
411 upper limit of Cronin & Dowsett (1991), the GIS sea-level model suggests a highstand ~+50 m
412 a.s.l. would have inundated the coastal plain between Golfo Dulce and Soná peninsula, the valley
413 between Soná and Azuero peninsulas, and much of the east coast of Azuero peninsula, isolating
414 it from central Panama (Fig. S3). This model is conservative, given that lower Central American
415 lands approached their modern extent by mid-late Pliocene, and the Pliocene Panama landscape
416 was likely lower and more topographically homogeneous (reviewed by Graham & Dilcher, 1995;
417 Smith & Bermingham, 2005; Bagley & Johnson, 2014a). We hypothesize that the WPI break
418 was subsequently reinforced by the mid-Pleistocene eustatic highstand ~550–390 ka, when sea
419 levels were +22 m a.s.l. (Hearty *et al.*, 1999; Miller *et al.*, 2005). Relative to the dry forest
420 hypothesis, this 'marine vicariance hypothesis' presents a testable and non-mutually exclusive
421 hypothesis that explains how a eustatic highstand might have sparked multiple responses to a
422 single period of sea-level rise and maintained any previous genetic divergence at the WPI break
423 originally created by expansion of dry forests.

424 Within the Panamanian frog assemblage, lineage dispersal and persistence appear to be at
425 least partly controlled by ecological requirements and degree of specialization, as well as life-
426 history mode. For example, *E. pustulosus* is a generalist occurring in savannahs and open
427 environments as well as humid and dry forests (Savage, 2002). In our results, this species
428 exhibited the youngest divergence across the WPI (Fig. 3), possibly reflecting greater capacity to
429 disperse and establish populations in different habitats, or exchange genes with other

430 populations. That said, the degree of genetic admixture among Panamanian populations of *E.*
431 *pustulosus* is poorly understood, and has only previously been documented by nuclear allozymes
432 (Weigt *et al.*, 2005). *Dendropsophus ebraccatus* and *A. callidryas* are ecologically similar
433 species (Savage, 2002; Robertson *et al.*, 2009, refs. therein) and display broadly overlapping
434 t_{MRCA}s slightly older than that of *E. pustulosus* but younger than that of *C. crassidigitus* (Fig. 3).
435 In turn, *C. crassidigitus* (like *C. talamancae*) is considered more ecologically specialized,
436 preferring wet forest habitat more so than its dry-forest congeners (i.e. *C. fitzingeri*; Crawford *et*
437 *al.*, 2007). That the *C. crassidigitus* gene tree extends farthest backward in time thus seems to
438 suggest this species experienced long-term isolation and persistence in preferred habitats, rather
439 than an ability to tolerate climatic or vegetational shifts. However, whereas all the other frog
440 species are egg-layers, *Craugastor* dirt frogs are direct-developing species that readily
441 reproduce, and their local population sizes can therefore be notoriously large (Crawford, 2003).
442 Given the direct relationship between N_e and time to coalescence from coalescent theory, this
443 contrast in life-history strategies would suggest that on one hand *C. crassidigitus* may have been
444 superior at colonizing open niches or patches and spread throughout isthmian wet forest habitats
445 more easily than the other taxa, while on the other hand its t_{MRCA} estimate may also be inflated
446 due to large ancestral N_e (Hudson, 1990).
447 Freshwater fishes typically exhibit higher levels of mtDNA sequence divergence and
448 endemism in Atlantic relative to Pacific drainages of the Isthmus of Panama (Bermingham &
449 Martin, 1998). On the Pacific versant, the ~124 km-wide Gulf of Panama continental shelf (Fig.
450 1) would have been broadly exposed as eustatic sea levels lowered 110–135 m during the Last
451 Glacial Maximum (LGM, ~21 ka) and other glaciations (e.g. Lambeck *et al.*, 2002). The above
452 pattern likely owes to greater opportunities for dispersion and gene flow between drainages that

453 coalesced over Pacific shelf areas during Pleistocene glacial periods (Bermingham & Martin,
454 1998; Smith & Bermingham, 2005). Yet, this trend breaks down slightly in western Panama,
455 where freshwater fishes show distinct associations between biogeography and phylogeography.
456 Despite low endemism within fish provinces, geographical ranges of 14 fish species terminating
457 at Soná and Azuero peninsulas (Fig. 1) support a boundary between the Chiriquí and Santa Maria
458 fish biogeographical provinces at the Río Santa Maria, Soná peninsula (Smith & Bermingham,
459 2005). The WPI genetic break occurs in the same area in freshwater fishes (Fig. 2C, D). Thus,
460 historical processes importantly shaped Panamanian fish species turnover and diversification at
461 the WPI break zone. Yet what mechanism best explains the isolation of fishes across the WPI
462 break?

463 The Bermingham/Martin model predicts that WPI breaks in fishes have resulted from the
464 isolation of fish populations west of Soná peninsula from those between Soná peninsula and El
465 Valle volcano during glacial periods with sea-level lowstands since 2 Ma in the Pleistocene
466 (Bermingham & Martin, 1998). Consistent with this model, we found that the lower 95% HPDs
467 for t_{MRCA} estimates for all three fish species/lineages fell within the predicted interval of
468 diversification (Fig. 3). Peak posterior distributions from hABC model-averaging (e.g. Fig. 4)
469 also suggested that the focal fish species/lineages most likely diversified across the WPI break
470 1.36 Ma in the early Pleistocene, with Bayesian credible intervals ranging from early–late
471 Pleistocene. This time period correlates best to the 'Calabrian' age (1.806–0.781 Ma; Gibbard *et*
472 *al.*, 2010), a time of 41-kyr periodicity of Pleistocene glaciations, with drier and cooler-than-
473 present conditions but less extreme climatic oscillations than those of the last 800 ka (Lambeck
474 *et al.*, 2002; Gibbard & Kolfschoten, 2004). Nevertheless, glacial periods vastly dominated the
475 Calabrian to present, such that the Panama isthmus would have experienced many glacio-eustatic

476 cycles but spent the majority of time since 1.8 Ma under glacial conditions with lowered sea
477 levels and exposed continental shelf habitats. Eustatic sea-level curves give no convincing
478 evidence that the oceans reached modern sea levels for any substantial period of time (e.g. >10–
479 20 kyr) since the Calabrian, and the next eustatic sea-level highstand is not registered in the
480 geological record until ~550–390 ka (Hearty *et al.*, 1999; Miller *et al.*, 2005). Geological
481 patterns and processes are also consistent with decreased likelihood of fish dispersal across the
482 WPI break zone since the Calabrian. In the break zone, the Pacific continental shelf becomes
483 narrower (~0-40 km), tapering to the western Azuero peninsula coastline before being bisected
484 by Cébaco and Coiba islands at the nearby Gulf of Montijo draining Soná peninsula (Fig. 1). To
485 evaluate the impact of lowered sea levels during Pleistocene glaciations on drainage connectivity
486 in this area, we obtained a GIS model predicting paths of LGM paleo-drainages over modern
487 bathymetry using ArcMap (courtesy of Peter J. Unmack, University of Canberra). The GIS
488 model suggests that rivers draining to the west versus east of Soná peninsula did not anastomose
489 over the continental shelf during the LGM (Fig. 1), and possibly also preceding glaciations.
490 Overall, our results combined with external environmental data suggest that a relatively stable
491 geological setting at the Soná peninsula barrier has aided the historical isolation of drainage
492 basins, maintaining fish lineage divergences at the WPI break during lower seas of the Calabrian
493 to present.

494 While this study chiefly focuses on comparative phylogeographic modeling of
495 codistributed Panamanian frogs and freshwater fishes, these assemblages also provide a system
496 with ecological affinities (e.g. associations with freshwater pools and streams) and contrasts
497 (terrestrial versus freshwater habits) making them suitable for assessing possible influences of
498 the degree of ecological differentiation on phylogeographic structuring. One obvious ecological

499 pattern among our results is that, at the deepest phylogenetic and ecological division among taxa
500 in our study, frogs and fishes may have experienced largely independent pulses of diversification
501 in the WPI break zone. Divergence events in these assemblages are not entirely statistically
502 independent, given the overlapping credible intervals of their divergence times (Figs 3 and 4),
503 and our analyses treated these assemblages separately. However, the two-fold difference in the
504 peak-likelihood $E(\tau)$ estimates suggest that frogs and freshwater fishes may have responded
505 differently to the fluctuating paleogeographic, geologic, and climatic context of western Pacific
506 Panama (e.g. Sullivan *et al.*, 2000). We recommend that this hypothesis be tested using hABC
507 models for comparative phylogeography improved by additional sampling and methodological
508 improvements increasing the resolution of the models. However, under this scenario, dispersal of
509 different lineages would be more strongly controlled by differences in ecological requirements
510 among, rather than within, the two species assemblages. Thus, a potential biogeographical
511 explanation for why these assemblages might have failed to register the impact of the same
512 historical events is that the focal frog species/lineages, but not the fish lineages, colonized the
513 Pacific coast of Costa Rica and Panama prior to the Pliocene eustatic highstand event, which left
514 a greater signature on their genotypes than subsequent historical processes. This is supported by
515 phylogenetic dating analyses of one frog lineage that we evaluated, the *Craugastor fitzingeri*
516 group containing *C. crassidigitus*, which likely colonized the Central American Isthmus in the
517 Eocene–early Miocene from a South American source population (Crawford & Smith, 2005).

518 **Conclusions**

519 We conducted the first tests for simultaneous diversification of frogs and fishes at the WPI break
520 in western Panama (reviewed by Bagley & Johnson, 2014a) by analyzing mtDNA sequences
521 using ABC methods accounting for uncertainty in model selection using modeling averaging

522 (Huang *et al.*, 2011; Hickerson *et al.*, 2014). Current findings show that, despite good prior
523 selection and sampling properties (also supported for our datasets by simulations in Overcast *et*
524 *al.*, 2017), hABC tests for synchronous diversification using mtDNA datasets remain challenging
525 and difficulties can arise in conducting hypotheses tests based on Bayes factors when analyzing
526 small numbers of taxon/population-pairs. However, our study also demonstrates that, in such
527 cases, integrating assembly-wide divergence time estimates [$E(\tau)$] from hABC with external
528 information from geology and elevation data can still generate novel biogeographical insights
529 and hypotheses towards refining the existing biogeographical context for a region, even despite
530 the limitations of mtDNA (see Introduction and Appendix S1 section 3). Recent advances in
531 hABC analysis, including extensions to genome-wide single nucleotide polymorphism (SNP)
532 data (Xue & Hickerson, 2017) and better inference through buffering multi-taxon divergence
533 times (Overcast *et al.*, 2017), suggest an exciting period ahead for comparative studies of the
534 diversification of vertebrate species assemblages in Panama and other Neotropical 'hotspots'.
535 We encourage future phylogeographic studies of the frog and fish taxa analyzed herein, and
536 other Panamanian taxa, to build upon the present foundation by developing and analyzing
537 datasets with increased taxon and character sampling, while capitalizing on these computational
538 advances. A particularly fruitful way forward in future examinations of the WPI break would be
539 to develop SNP datasets, for example using ddRAD-seq (e.g. Peterson *et al.*, 2012), and then test
540 for co-demographic expansion and co-diversification using aggregate site frequency spectrum
541 methods (Xue & Hickerson, 2017). Such approaches should provide the increased power and
542 resolution needed to more confidently identify the number and timing of divergence events that
543 have shaped vertebrate diversification across the WPI break, and other multi-taxon genetic
544 breaks along the Isthmus of Panama.

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Table 1 (on next page)

List of taxa used to examine temporal diversification patterns across the western Panama isthmus.

For multi-gene datasets, total alignment length is given in nucleotide base pairs (bp) followed by length of each gene in parentheses, in the same order genes are listed at left. The transition/transversion ratios (Ti/Tv; i.e. kappa parameters) are HKY+Γ+*I* model estimates, and percent genetic divergences (% div) are given as average pairwise HKY+Γ+*I* distances between population-pairs. *π*, nucleotide diversity (Nei, 1987).

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	mtDNA genes	Length (bp)	n_{total} (west/east)	π	Ti/Tv	% div	Source
Frogs							
Agalychnis callidryas	*16S*, *ND1*	1149 (118, 1031)	28 (16/12)	0.0337	11.373	8.0	Robertson & Zamudio (2009)
Craugastor crassidigitus	cyt*b*, *cox1*	1353 (714, 639)	13 (5/8)	0.0645	14.55	13.9	Crawford *et al.* (2007)
Dendropsophus ebraccatus	*ND1*, *tRNA*s	1877	18 (11/7)	0.0245	12.04	5.9	Robertson *et al.* (2009)
Physalaemus pustulosus	*cox1*	564	15 (2/13)	0.0139	31.67	4.6	Weigt *et al.* (2005)
Freshwater fishes							
Andinoacara coeruleopunctatus	*ATP6/8*	842	10 (2/8)	0.0093	20.77	2.4	McCafferty *et al.* (2012)
Pimelodella chagresi	*ATP6/8*, *cox1*	1471 (842, 629)	14 (6/8)	0.0210	12.19	3.5	Bermingham & Martin (1998)
Roeboides occidentalis–R. guatemalensis	*ATP6/8*, *cox1*	1493 (842, 651)	11 (8/3)	0.0311	10.36	6.4	Bermingham & Martin (1998)

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Table 2 (on next page)

Prior model classes and results of tests for synchronous diversification using ABC model averaging in MTML-msBayes.

Parameters are shown for four prior model classes ran for each of two analyses of *Y* population-pairs used to test for synchronous diversification across the WPI break. Prior models had varying τ, θ_D, and θ_A prior distributions, $P(x)$, but assumed zero post-divergence migration. Approximate posterior probabilities $P(M_k|D)^{1000}$ of each model are given based on 1000 accepted simulated draws from 20 million random draws from the four prior models, with that of the best-supported model underlined. Modal Ω hyper-parameter estimates and their 95% highest posterior densities (HPDs) from model averaging over all four prior models are given in the first row of each section.

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Prior	$P(\tau)$	$P(\theta_D)$	$P(\theta_A)$	$P(M_k\|D)^{1000}$	Ω mode	Ω 95% HPDs
WPI frogs ($Y = 4$)					0.0036	[0.000, 0.0565]
M_1	~U(0, 1.75)	~U(0, 0.1)	~U(0, 0.25)	0.2666	–	–
M_2	~U(0, 1.75)	~U(0, 0.1)	~U(0, 0.5)	0.4990	–	–
M_3	~U(0, 1.75)	~U(0, 0.4)	~U(0, 0.25)	0.0000	–	–
M_4	~U(0, 0.875)	~U(0, 0.1)	~U(0, 0.25)	0.2344	–	–
WPI fishes ($Y = 3$)					0.0017	[0.000, 0.0423]
M_1	~U(0, 0.8)	~U(0, 0.1)	~U(0, 0.25)	0.3124	–	–
M_2	~U(0, 0.8)	~U(0, 0.1)	~U(0, 0.5)	0.3766	–	–
M_3	~U(0, 0.8)	~U(0, 0.4)	~U(0, 0.25)	0.0000	–	–
M_4	~U(0, 0.4)	~U(0, 0.1)	~U(0, 0.25)	0.3110	–	–

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Figure 1(on next page)

Map of the study area.

The western Panama isthmus (WPI) break zone is shaded gray, and major physiographic features including the continental divide, peninsulas, and mountain ranges are shown over a digital elevation layer; GC, Golfo de Chiriquí; GM, Golfo de Montijo. Paleo-bathymetric river paths modeled assuming a 135 m eustatic sea level drop during the Last Glacial Maximum using ArcMap (ESRI, Redlands, CA; courtesy of Peter J. Unmack) are shown with the −135 m bathymetric contour (dashed line) as a reference.

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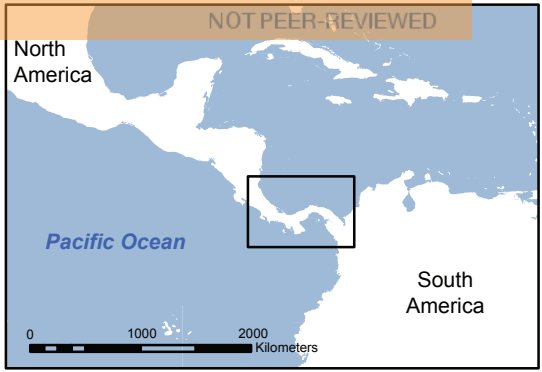

'WPI break' zone

Bathymetric rivers

Continental shelf

Continental divide

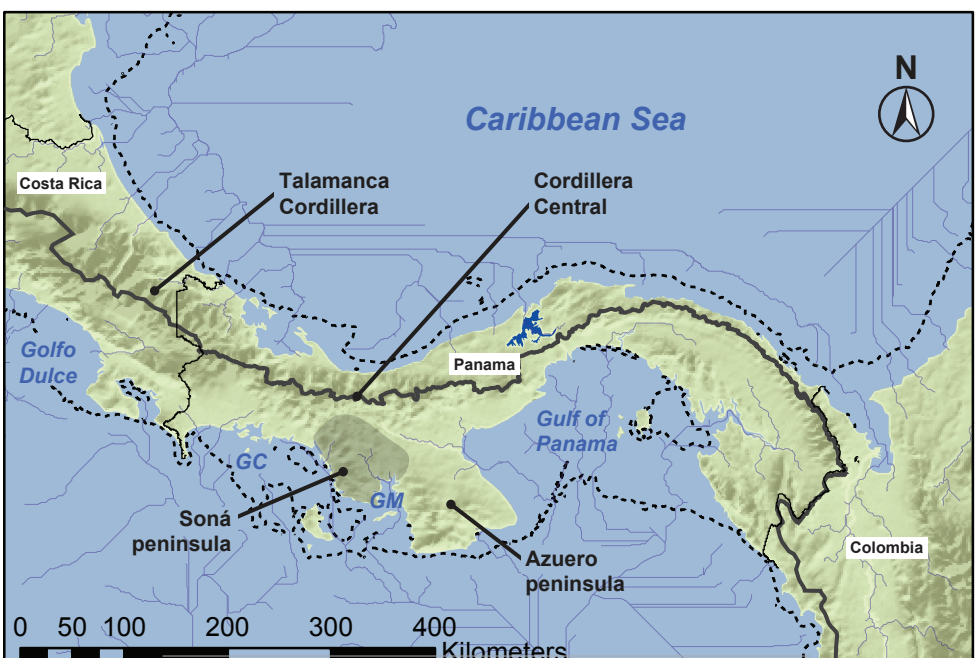

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Figure 2(on next page)

Geographical locations of WPI phylogeographic breaks registered in different species/lineages of Panamanian (A, B) frogs and (C, D) freshwater fishes evaluated in this study.

Map features and lines are identical to those in Fig. 1.

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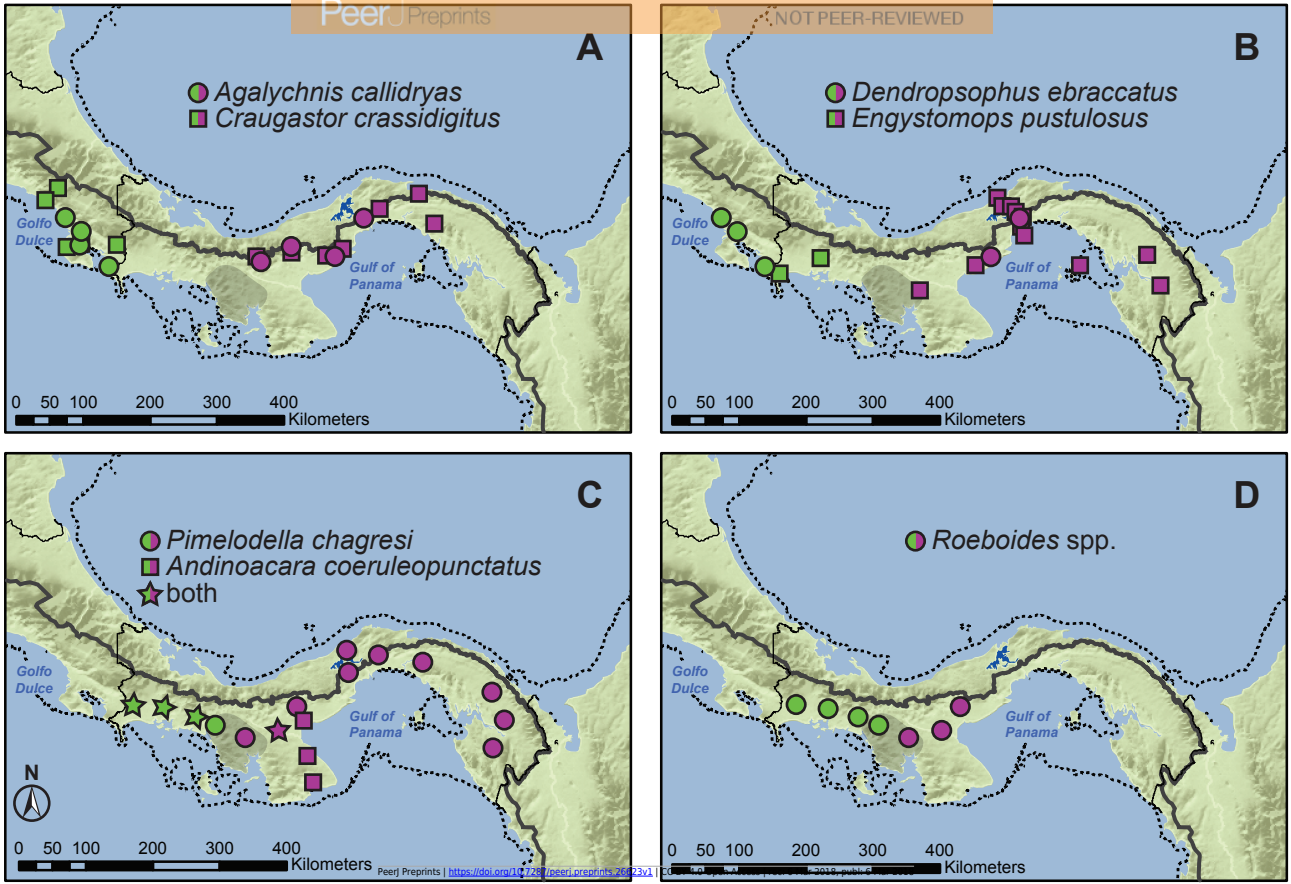

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Figure 3(on next page)

Comparison of divergence time estimates for species/lineages, and species assemblages, diverged across the WPI break.

Species/lineage gene-tree depths (t_{MRCA}s) from BEAST (Drummond *et al.*, 2012; corresponding to tree depths in Fig. S1) are shown as geometric means (dots) and 95% HPDs, with regions of overlap in coalescence times shaded gray. Estimated times of assemblage co-divergences are shown as modal/peak posterior estimates (diamonds) and 95% HPDs from ABC model averaging in MTML-msBayes.

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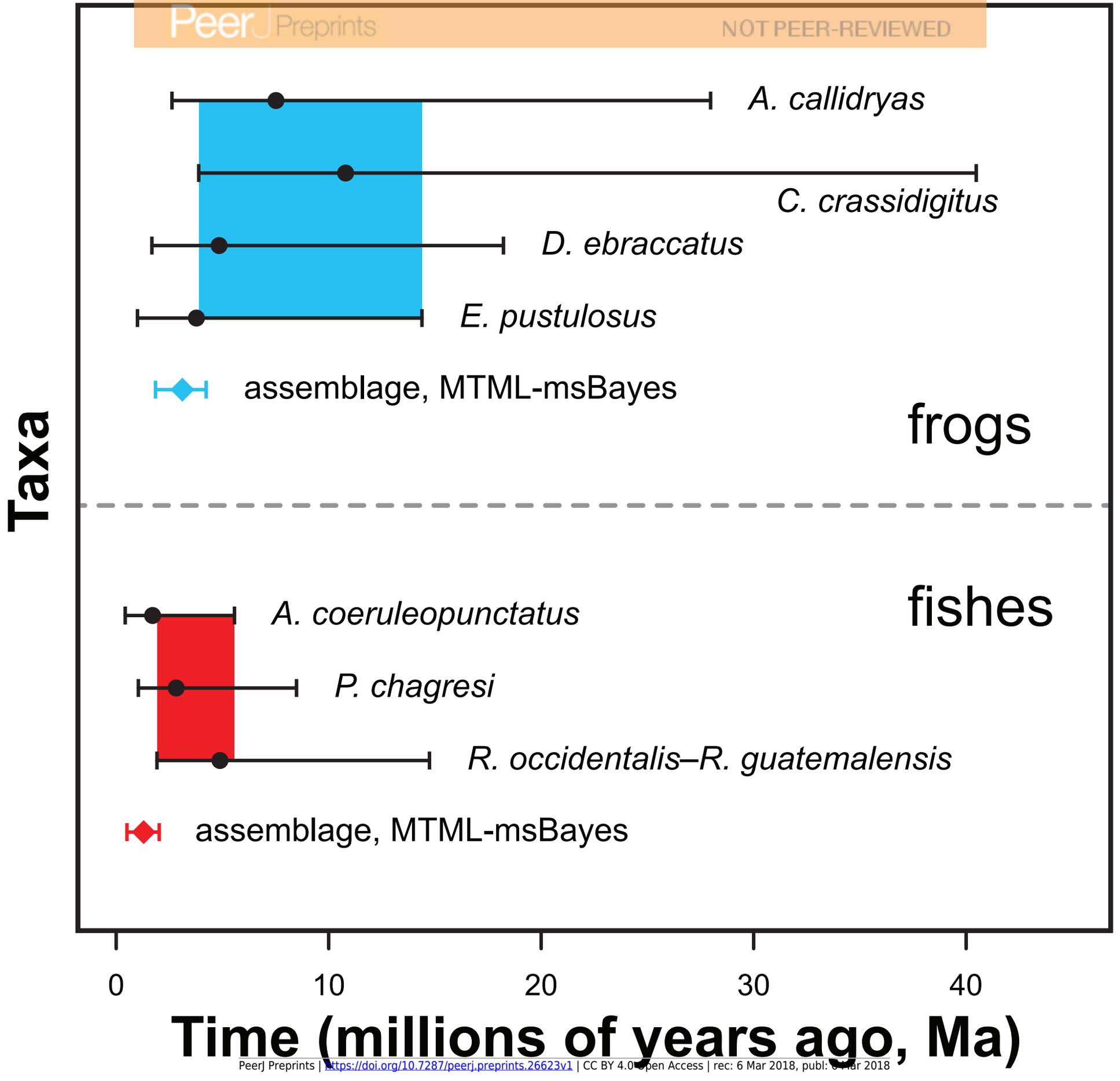

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Figure 4(on next page)

Hierarchical approximate Bayesian computation (hABC) results.

Joint hyper-posterior probability distributions of the mean divergence time, $E(\tau)$ (left *x*-axis, coalescent time; right *x*-axis, absolute time), and the dispersion index of divergence times, Ω, from MTML-msbayes (Huang *et al.*, 2011) are presented for (A) frogs and (B) freshwater fishes based on ABC model-averaging across model classes. Inset graphs show the posterior densities of Ω from each analysis.

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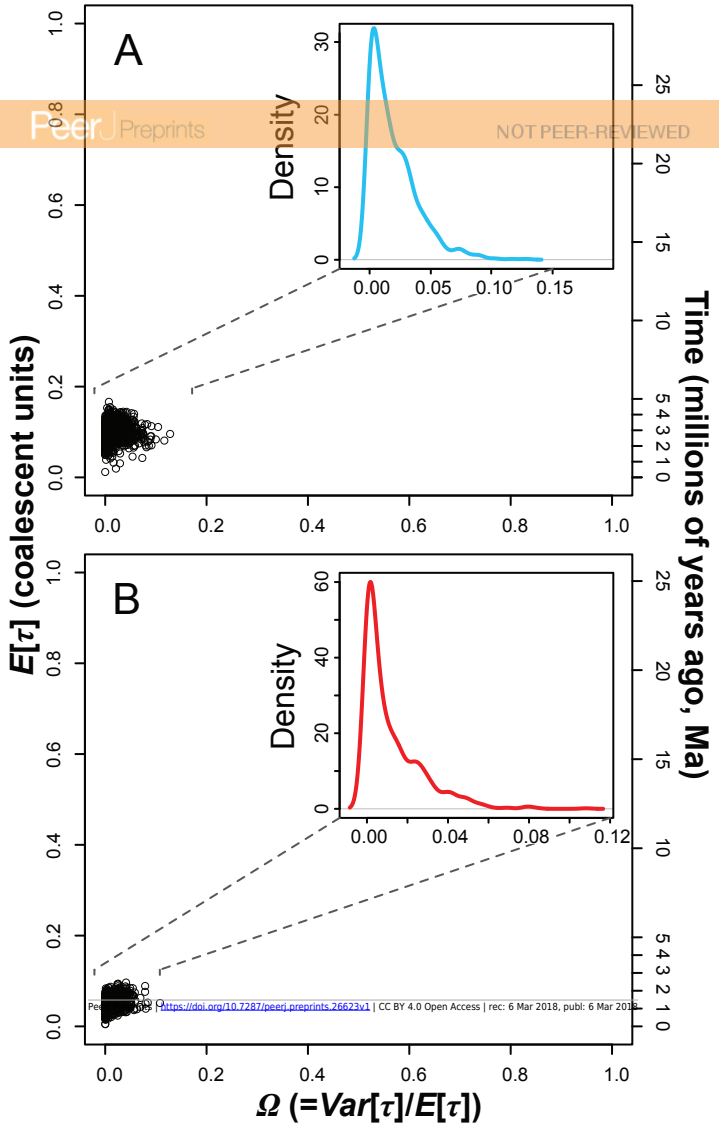

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