



Molecular phylogeny and divergence of the map turtles (Emydidae: *Graptemys*)

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ARTICLE INFO

Keywords:

Rapid radiation
Deirochelyinae
Molecular clock
Multispecies coalescent
Southeastern United States
Turtle conservation

ABSTRACT

The map turtles (genus *Graptemys*) comprise a morphologically diverse clade that forms a major component of the southeastern US hotspot of chelonian diversity. Map turtles have experienced both recent and rapid diversification resulting in long-standing uncertainty regarding species boundaries and phylogenetic relationships within the genus as well as timing of their divergence. We present a phylogeny for the group that includes geographically representative sampling for all described species and subspecies. We make use of an empirical prior on rates of molecular evolution to estimate divergence times with a molecular clock under a coalescent framework. Together, the phylogeny and divergence time estimates suggest that diversification has been both more recent and more rapid than has so far been suspected. We provide a well-supported evolutionary framework for *Graptemys* that is necessary for understanding map turtle diversity, biogeography, and for conservation of this threatened clade of turtles.

1. Introduction

The southeastern United States is a well-recognized biodiversity hotspot for several groups of organisms, including molluscs, crayfish, freshwater fishes, and turtles (Lydeard and Mayden, 1995). Evolutionary radiations in this region are generally characterized by a high degree of endemism (often associated with river drainages), small ranges, and rapid and recent diversification. The region is particularly important as a global repository of freshwater turtle diversity. It supports 42 species of turtles and forms one of the two primary hotspots of turtle diversity globally (Mittermeier et al., 2015). The 13 or 14 currently recognized species of map turtles (*Graptemys*) make up roughly one third of this diversity, despite being generally recognized as a relatively recent radiation (Ernst and Lovich, 2009; Lindeman, 2013). Resolving both species boundaries and phylogenetic relationships in this genus has been one of the most persistent, challenging problems in turtle evolutionary biology, hampering comparative evolutionary, ecological, and conservation studies.

The map turtles are members of the Emydidae (subfamily: Deirochelyinae), the most diverse chelonian clade in North America (Turtle Taxonomy Working Group, 2017). As currently understood, *Graptemys* species fall into three major clades including the common map turtle (*Graptemys geographica*), a western group (7 species), and a

'megacephalic' or broad-headed group in which females, but not males, often develop greatly enlarged heads (5 species) (Lindeman, 2000; Lovich and McCoy, 1992). *Graptemys* range from southeastern Canada through the central United States and into the southern US from eastern Texas to the Florida panhandle (Fig. 1; Ernst and Lovich, 2009; Lindeman, 2013). Most species within the clade (including all megacephalic species and nearly all western group species) are typically found in medium to large rivers with moderate or fast flows (Lindeman, 2013). Unlike many other emydid turtles, most map turtles exhibit extreme specificity to their preferred lotic habitats. They are well known for their habit of basking on logs and fallen debris, but most species are rarely found on land (Lindeman, 2013; personal observations). A presumed consequence of this habitat specificity is that the genus exhibits a large amount of river drainage endemism. Both the western clade and megacephalic clades have radiated into allopatrically distributed species across the drainages of the Gulf of Mexico such that most drainages now support one or two unique species (Ernst and Lovich, 2009; McKown, 1972). Many authors have suggested that the map turtles have diversified recently (Lamb et al., 1994; Lindeman, 2013; Lovich and McCoy, 1992; Tinkle, 1958). McKown (1972) placed the crown age of the group in the Oligocene (23–33 million years before present), while more recent work has suggested the group is much younger. Lamb et al. (1994) estimated the crown age at six to eight

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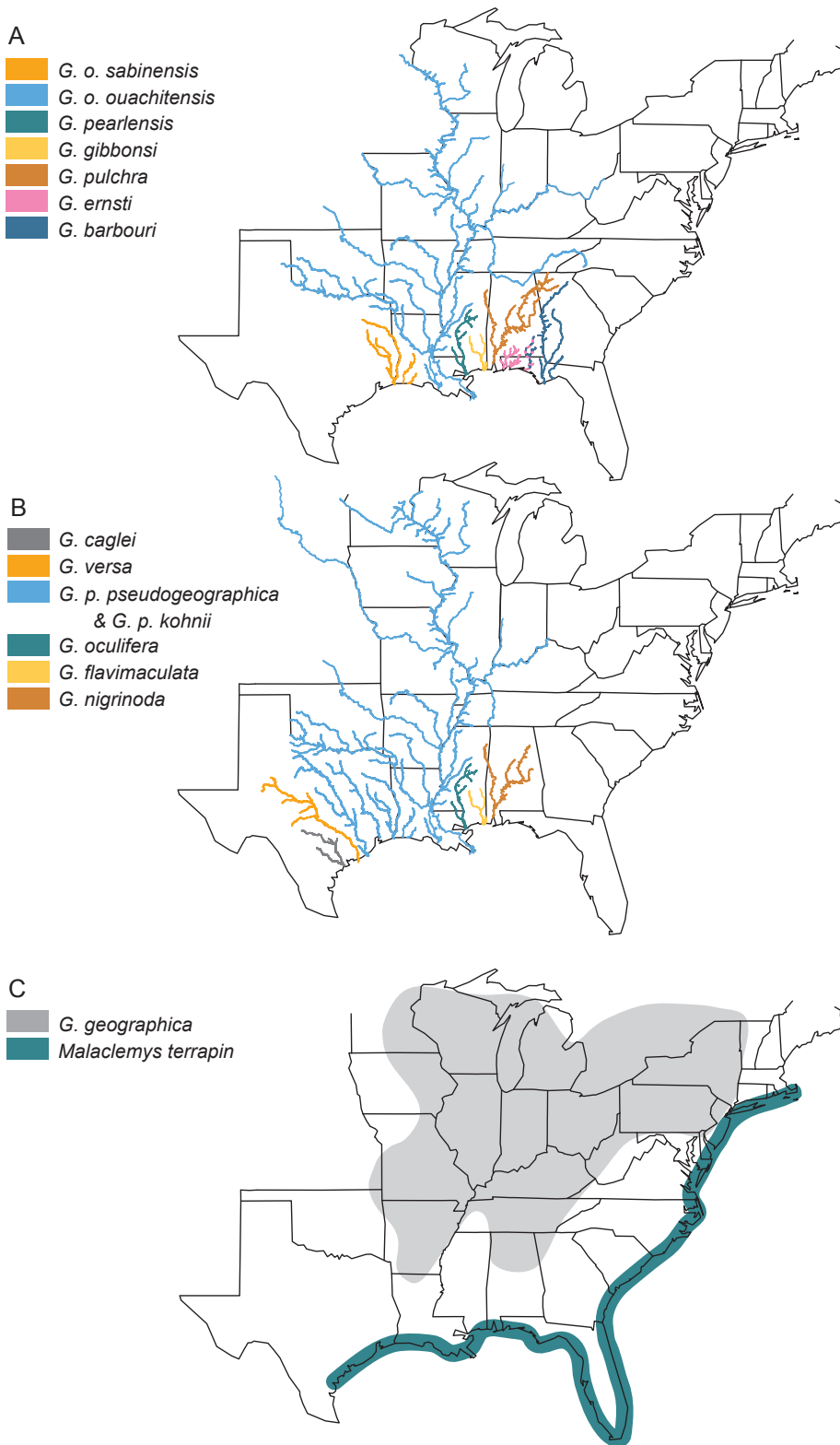


Fig. 1. Overview of high order rivers in which map turtles occur. (A) River endemics in the megacephalic clade and part of the 'false map turtle complex' (*G. o. ouachitensis* and *G. o. sabinensis*). Hatched pink and blue lines indicate a zone of sympatry in the Choctawhatchee River of western Florida. (B) River endemics from the western clade (except those in panel A). (C) Range of the common map turtle (*Graptemys geographica*), and the diamond-backed terrapin (*Malaclemys terrapin*, the sister genus to *Graptemys*). See Appendix 1 for specific localities used in this study and Lindeman (2013) for detailed range maps.

million years, based partly on estimates of molecular rates of evolution in the mitochondrial DNA. Spinks et al. (2016) estimated the crown age for the group to be between 6.3 and 20 million years based on a relaxed clock analysis of nuclear and mitochondrial data, although this analysis focused on deeper emydid relationships and therefore contained limited sampling within genera. In addition, the *Graptemys* radiation appears to have occurred rapidly, complicating previous efforts to resolve a phylogeny for the group (Lamb et al., 1994; McKown, 1972; Prashag et al.,

2017; Stephens and Wiens, 2003; Wiens et al., 2010).

The monophyly of the map turtles has only ever seriously been questioned by Wood (1977) who suggested a scenario where each riverine endemic *Graptemys* was derived independently from that region's coastal population of the Diamondback Terrapin (*Malaclemys terrapin*, Fig. 1). This hypothesis never garnered much support, was tested and clearly rejected by Lamb and Osentoski (1997), and the monophyly of the group has not been seriously questioned since.

Several morphological and molecular analyses agree that the genus is made up of the three contained clades (western, megacephalic, and *G. geographica*) and that *G. geographica* is sister to the remaining lineages. However, the relationships within these three lineages are far less certain. Several previous analyses using both molecular and morphological data recovered either very little resolution (Lamb et al., 1994; Prasad et al., 2017), or phylogenies that were in strong conflict with each other or the likely biogeographic history of the group (Lamb and Osentoski, 1997; Stephens and Wiens, 2003; Wiens et al., 2010). In addition, most analyses to date have contained limited sampling within species, and phylogenetic analyses among closely-related groups based on such sampling can lead to highly misleading results (Shaw and Small, 2005; Spinks et al., 2013).

Other aspects of *Graptemys* evolutionary history have never been resolved. Primary among these is the relationships and species boundaries among the 'false map turtle complex' of the western clade, which includes *Graptemys pseudogeographica pseudogeographica*, *Graptemys pseudogeographica kohnii*, *Graptemys ouachitensis ouachitensis*, and *Graptemys ouachitensis sabinensis*. Taxonomy within this group has been extremely fluid, with authors variously considering *G. p. kohnii* its own species, *G. ouachitensis* a subspecies of *G. pseudogeographica*, and/or *G. o. sabinensis* its own species (reviewed by Turtle Taxonomy Working Group, 2017). No clear consensus exists on species boundaries in these turtles, and much of the shifting taxonomy has occurred in the absence of data and formal analysis. Taken together, the previous literature suggests that our understanding of *Graptemys* phylogeny is still incomplete and in need of further study.

Here we present a new phylogenetic analysis of *Graptemys* that samples multiple representatives of all species and subspecies currently recognized within the genus. We provide a well-supported estimate of most evolutionary relationships, identify areas that will require additional work to resolve, and discuss the taxonomic implications of this phylogeny. We also estimate divergence times and find that the clade may be much younger than previously suspected.

2. Materials and methods

2.1. Taxon and locus sampling

We collected data for 55 individuals comprising all named taxa within *Graptemys* plus samples of three outgroup taxa (*Chrysemys picta*, *Emys marmorata*, and *Malaclemys terrapin*). We assembled geographically representative sampling within all wide-ranging species (e.g. *Graptemys geographica*) and several individuals of each narrow ranging species (Appendix 1). We sequenced each individual for the following panel of 18 nuclear genes: *AHR*, *BDNF*, *BMP2*, *HNFL*, *NB17483*, *NB17637*, *NB22519*, *PER*, *RAG1*, *RELN*, *TB01*, *TB02*, *TB49*, *TB53*, *TB73*, *TB82*, *TB86*, *VIM*, and two mitochondrial genes: *COI*, *ND4* (Backström et al., 2008; Kimball et al., 2009; Krenz et al., 2005; Noonan and Chippindale, 2006; Primmer et al., 2002; Spinks et al., 2010; Spinks and Shaffer, 2007; Stuart and Parham, 2004; Thomson et al., 2008; Townsend et al., 2008).

We extracted DNA from tissue samples following a salt extraction protocol (Sambrook and Russell, 2001), generated sequences by amplifying each locus in a 20 µl PCR reaction that was incubated for 60 s at 95 °C as an initial denaturation, then thermocycled through 35 cycles of denaturation (95 °C for 30 s), annealing (60–65 °C for 45 s), and extension (60 s at 72 °C), with a final extension of 72 °C for 10 min. PCR products were checked on 1% agarose gels and sequenced in both directions using the same primers by Beckman Coulter Genomics (<http://www.beckmangenomics.com>) or the UC Davis DNA sequencing facility (dnaseq.ucdavis.edu). Sequences were then assembled, trimmed, and aligned using the Geneious software package (Kearse et al., 2012).

2.2. Phylogenetic analysis

We partitioned the alignment by locus and by codon position (for protein coding genes) using PartitionFinder v 1.1.1 to identify a suitable partitioning strategy and substitution models (Lanfear et al., 2012). We searched among partitions using the greedy search algorithm and selected models from the set implemented in MrBayes using AICc. Using MrBayes v 3.2.5, we carried out a preliminary set of analyses to assess MCMC performance under the best scoring partition and set of models (Ronquist et al., 2012). Based on initial runs, we simplified the model for two partitions from HKY to JC based on poor MCMC mixing for the κ parameter. We then ran six replicated analyses for 50 million generations, recording the state of the chain every 5000 generations. Each analysis used one cold chain and three incrementally heated chains (temperature setting 0.05). We discarded the first 10% of samples from each run as burn-in.

We assessed MCMC convergence and mixing using the diagnostics provided in MrBayes v 3.2.5, Tracer v 1.6, and AWTY (Nylander et al., 2008; Rambaut and Drummond, 2013; Ronquist et al., 2012). Specifically, we checked that the potential scale reduction factor approached 1.0 and that the average standard deviation of split frequencies approached zero. We visually inspected the samples for continuous parameters of all six analyses in Tracer to confirm that: (1) no apparent trends were present in the MCMC traces, (2) the marginal distribution of each parameter had adequate samples to form a smooth distribution, and (3) all ESS values were much greater than 1000. In AWTY, we used sliding window plots and compare plots to visually inspect the topology samples, ensuring that no apparent trends in node probabilities were present within each of the six analyses, and that the node probabilities were nearly identical among the six runs. Following MCMC diagnosis, all runs were combined and summarized. We carried out these analyses on the primary alignment using all the data, as well as alignments using the nuclear data alone, the mitochondrial data alone, and each gene alignment independently. To further characterize phylogenetic uncertainty among map turtle lineages, we used the NeighborNet algorithm implemented in Splitstree v. 4.14 to visualize the distribution of genetic distances among individuals (Huson, 1998).

2.3. Divergence time and coalescent analysis

The fossil record for the map turtles is sparse, which severely limits our ability to construct informative node age priors for divergence dating. All fossil material known from map turtles is relatively recent, with estimated ages only within the last few hundred thousand years (reviewed by Lindeman, 2013). Most fossils are also difficult to place in the phylogeny with any certainty, further complicating their use for divergence time analysis. As an alternative, we could potentially employ a minimum and/or maximum age prior on the Deirochelyinae (the clade that contains *Chrysemys*, *Deirochelys*, *Graptemys*, *Malaclemys*, *Pseudemys*, and *Trachemys*). Using the fossil record, Joyce et al. (2013) empirically justified a uniform and wide prior for this clade spanning 3–34 million years. This time interval is very wide relative to the suspected age of *Graptemys* and, therefore it is largely uninformative on node ages within *Graptemys*.

While suitable fossil material for the clade is sparse, rate information is well characterized for turtles as a whole and for the Emydidae specifically. Many studies have identified relatively slow rates of molecular substitution for turtles, as well as relatively little among lineage rate variation (Hugall et al., 2007; Lourenço et al., 2013, 2012; Shaffer et al., 2013). We elected to employ an empirical prior on rate motivated by our ongoing broad-scale molecular phylogenetic work in turtles. We extracted the estimated rate of evolution for every lineage in a chronogram containing nearly all turtle species (Thomson et al., unpublished data) and used these to fit a lognormal distribution using the *fitdistrplus* package v 1.0 in R v 3.3.0 (Delignette-Muller and Dutang, 2015), which we then applied as a lognormal prior (mean = −7.861, standard

deviation = 0.316) on clock rate for each gene. This prior implies a 95% highest probability density from 2.2×10^{-4} to 6.48×10^{-4} , which is similar to other molecular evolutionary rate estimates for turtles available from the literature (Hugall et al., 2007; Lourenço et al., 2013; Shaffer et al., 2013).

Using this empirical rate prior, we estimated divergence times, species phylogeny, and underlying gene genealogies using *BEAST v. 2.4.0 (Bouckaert et al., 2014; Heled and Drummond, 2008). We treated all individuals from each described taxon as a separate lineage and used a demographic model that allowed for linear changes in population size along branches but constrained the root population to be constant. We used independent substitution and strict clock models for each locus (except for the two mitochondrial genes which we treated as linked), applying the empirical rate prior to each of the clock rates. We conducted a series of preliminary analyses to check MCMC performance and adjust settings of the model and MCMC as needed. We also carried out several additional preliminary analyses to check the impact of alternative demographic, substitution, and clock models, the use of the multispecies coalescent model itself, as well as an alternative log normal prior (mean = -6.145, standard deviation = 0.6) for the rate of the mitochondrial clock, which is known to be faster than rates in the nuclear genome for vertebrates. As a final set of preliminary analyses, we checked sensitivity to inclusion or exclusion of outgroups, as well as members of the poorly resolved false map turtle complex. We then ran six independent analyses each for 350 million generations, recording the state of the chain every 35,000 generations and assessing convergence as previously. We trimmed burn-in (10%) from each analysis, combined the samples from each analysis, and summarized the results.

3. Results

3.1. Data matrix and availability

Our data matrix consisted of 55 taxa (52 ingroup, 3 outgroup) and 13,041 basepairs (bp), which was nearly complete (1.7% missing data). PCR products could not be obtained for a small number of locus-by-taxon combinations despite repeated attempts under relaxed reaction conditions. We substituted sequences from a different individual of the same species in two of the outgroups (two genes for *E. marmorata*, and one gene for *C. picta*). All new sequences generated in this study were added to Genbank (Accession Numbers MG728119-MG729205; see Appendix 1 for additional detail). The complete alignment and gene trees are available in the Mendeley Data repository associated with this study (doi:<http://dx.doi.org/10.17632/fspsrbcctx.1>).

3.2. Phylogeny

For our concatenated, partitioned, 20-gene analysis, we recovered a well-resolved phylogeny that is congruent with expected results at deep levels, but contains several novel relationships at more shallow levels of divergence (Fig. 2). We recovered the three major lineages of the map turtles (*G. geographica*, megacephalic clade, western clade) as reciprocally monophyletic with strong statistical support (estimated posterior probability [PP] = 1.0), and the diamondback terrapin, *M. terrapin* as the sister group to *Graptemys*. Consistent with previous work, the *G. geographica* lineage was recovered as sister to a monophyletic (megacephalic + western) clade, again with strong support (PP = 1.0). As in previous studies, analysis of the mitochondrial DNA alone recovered a tree that is consistent with the nuclear estimate, but provides almost no resolving power (Fig. S1). Analyses of the combined nuclear data recovered a relatively well resolved phylogeny that was largely congruent with results from analysis of the full dataset (i.e. combined mtDNA and nuDNA) except for the position of *Malaclemys* (Fig. S2), while analyses of individual genes recovered phylogenies that were not well supported (trees available in Dryad repository). We therefore focus our results and discussion on the full dataset (i.e. analyses of combined

mtDNA and nuDNA), referring to the alternative analyses when relevant.

The phylogeny recovers several clades that are concordant with expectations based on biogeography as well as morphology. Aside from the three major lineages, we recovered the 'sawback' clade endemic to the geographically adjacent Pearl (*G. oculifera*), Pascagoula (*G. flavimaculata*), and Alabama (*G. nigrinoda*) drainages. We also recovered a clade of turtles endemic to the adjacent Guadalupe (*G. caglei*) and Colorado (*G. versa*) rivers of central Texas. Our analysis failed to recover monophyly of several species in the false map turtle complex, a group of morphologically similar species that occur in the Mississippi and adjacent drainages. The NeighborNet analysis similarly recovered mostly well-differentiated lineages on long branches with relatively little uncertainty, but with the false map turtle complex scattered throughout the base of the western clade part of the network (Fig. 3).

3.3. Divergence times and coalescent tree

The divergence time and species tree analysis recovered an essentially identical topology as the concatenated analysis (Fig. 4). All of the same major lineages and their interrelationships were recovered. The multispecies coalescent analysis also recovered a clade containing three of the four lineages of the false map turtle complex (*G. o. sabinensis*, *G. o. ouachitensis*, *G. p. pseudogeographica*), suggesting that *G. p. kohnii* is not a member of this complex, but rather is sister to the sawbacks of Mississippi and Alabama (Fig. 4). In this analysis, the remaining members of the false map turtle complex formed a sister clade to the rest of the western group (Fig. 4).

Our analyses suggest that the map turtles are considerably younger than previous estimates. The 95% highest posterior density for the crown age of the map turtles is 0.9224–2.41 million years (median = 1.54 million years). This date represents node ages of the species tree, which are necessarily younger than node ages of the underlying gene trees, and this may partly explain why these dates are younger than previous estimates (all of which are estimates of gene divergence times, not species divergence times). The median crown age of the group in the underlying gene trees is 5.24 million years (ranging from 3.97 to 8.91 million years; standard deviation = 1.0; mtDNA age estimate = 4.23 million years) which is closer to, but still younger than, previous estimates.

The megacephalic and western clades have virtually identical crown ages. The megacephalic clade was estimated to be 0.89 million years old (95% HPD: 0.624–1.20 mya), while the western clade estimate was 0.87 million years (95% HPD: 0.603–1.16 mya), suggesting that these lineages began to diversify shortly after they became isolated from one another. Estimates of divergence times were qualitatively similar across alternative demographic, clock, and substitution models; as well as the alternative prior that placed additional prior probability on faster rates of mitochondrial evolution (not shown).

4. Discussion

4.1. Phylogeny and species boundaries of the map turtles

Our results provide several insights into the evolution, biogeography, and timing of diversification within *Graptemys*, and highlight the rapid diversification that has occurred in this important biodiversity hotspot. Our phylogenetic results confirm the three major lineages of map turtles, as well as relationships among them. Morphology, ecology, and some phylogenetic results have all suggested the existence of these clades (Lamb et al., 1994; Prashag et al., 2017; Wiens et al., 2010), although several phylogenetic analyses have suggested otherwise (McKown, 1972; Stephens and Wiens, 2003; Wiens et al., 2010). Based on our relatively extensive individual and gene sampling, we are confident that this primary structure of the map turtle tree is now well established. We discuss each of these lineages in turn below.

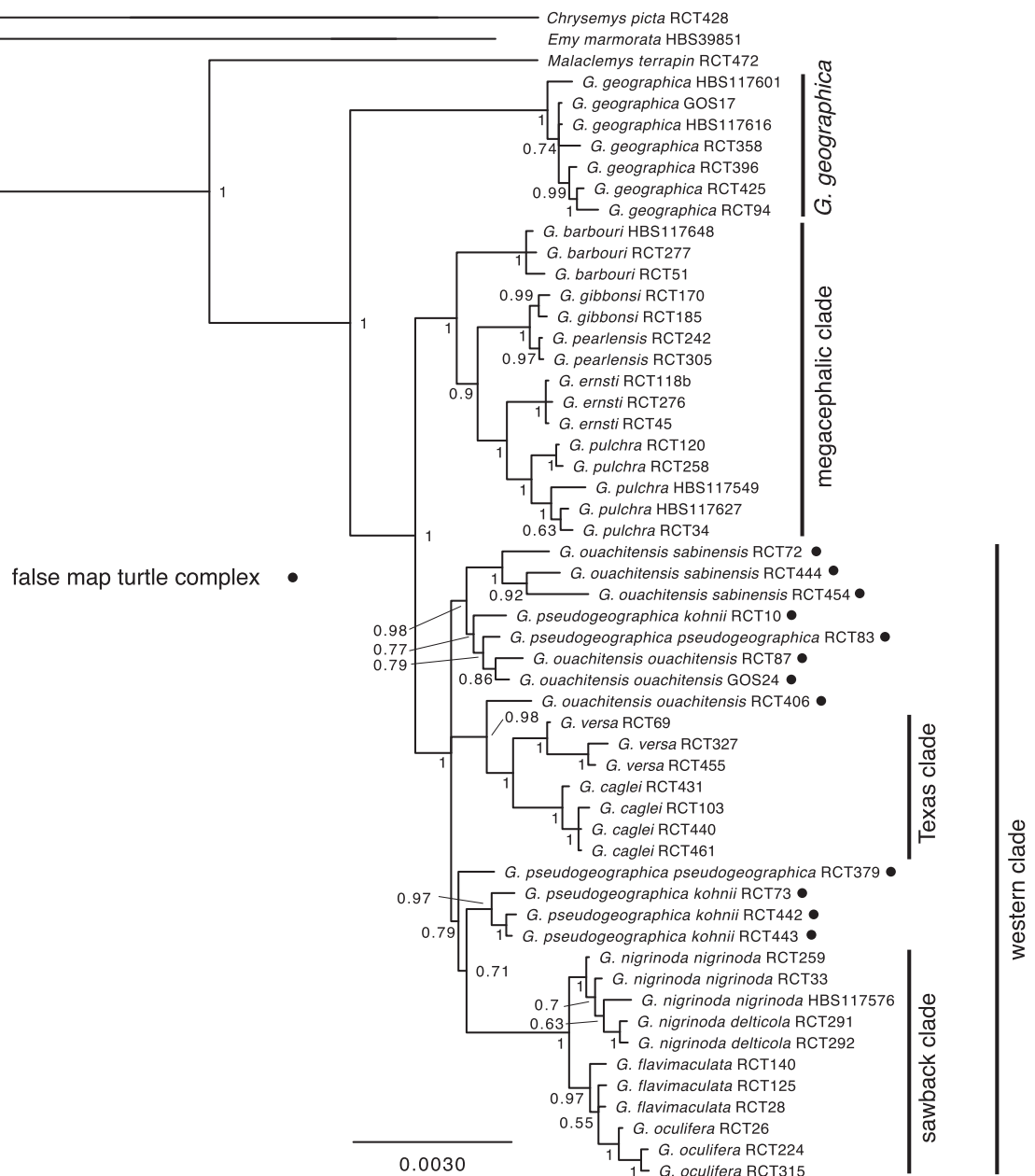
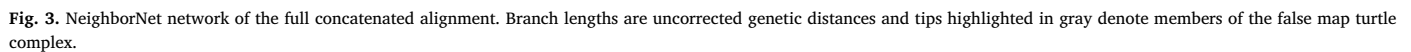


Fig. 2. Phylogram based on analyses of the concatenated mtDNA and nuDNA alignment, with major clades labeled. Numbers on nodes represent estimated posterior probabilities. Black dots denote members of the false map turtle complex. Branch lengths depict expected substitutions per site.

Graptemys geographica.—Our geographic sampling for the *G. geographica* lineage includes seven individuals that broadly encompass the species' geographic distribution. No apparent geographic structure exists across samples ranging from the northern edges of the range in Quebec and Minnesota (RCT94 and RCT 358), to the southern parts of the range in Alabama and Missouri (HBS117601, HBS117616, and RCT 425). Our sampling does not include individuals from the disjunct populations in eastern New York and Pennsylvania, and we provisionally assume that these populations have recent origins following the retreat of Pleistocene glaciation. Based on these results, it appears that *Graptemys geographica* is a single widely distributed species that occurs in a large number of drainage systems across midwestern and eastern North America (Ernst and Lovich, 2009). This pattern is starkly different than the single-drainage (or near single drainage) endemism seen in the rest of *Graptemys* (Fig. 1), and may reflect a combination of cold tolerance and post-glacial recolonization over much of the species range (Baker et al., 2003; Nagle et al., 2004).

Megacephalic clade.—Within the megacephalic clade, our sampling includes at least two individuals of all described species and represents all major river systems in which each occurs. Our results generally confirm the current view of diversity within this clade. Although our sampling was not exhaustive, our tree is concordant with the recently proposed elevation of the Pearl River population of *G. gibbonsi* to *G. pearlensis* (Ennen et al., 2010), with each recovered as reciprocally monophyletic. Prasad et al. (2017) found reciprocal monophyly of all the megacephalic species, except *G. gibbonsi* and *G. pearlensis*, in a phylogenetic analysis of mitochondrial DNA, which largely agrees with our own mitochondrial results (Fig. S1). In a principle components analysis of nuclear DNA haplotypes, they found that the megacephalic species are tightly clustered and concluded that all megacephalic species, with the exception of *G. barbouri*, are conspecific. By contrast, our results indicate that all species in this clade are reciprocally monophyletic with strong support (PP > 0.97), suggesting that current taxonomy accurately reflects the lineage diversity.



sampled from central and eastern Alabama (the Tallapoosa and Cahaba Drainages of the Alabama River drainage; RCT120, RCT258). Although the Tombigbee and Alabama ultimately join shortly before emptying into Mobile Bay, they are hydrologically separate for most of their drainages, and may be relatively independent in terms of *Graptemys* gene flow. Our current sampling is far too incomplete to speculate further on the significance of this pattern, and highlights this as an area in need of additional sampling and analysis.

We recovered a phylogeny that resolves several of these relationships and finds support for monophyly of most (but not all) species within the genus. For instance, the 'sawbacks' (*G. oculifera*, *G. flavimaculata* and *G. nigrinoda*) form a well-supported clade that likely represents allopatric isolation among these species in the Pearl, Pascagoula, and Alabama Drainages, respectively. We find support for monophyly of both *G. oculifera* and *G. nigrinoda*, but not *G. flavimaculata* which could potentially arise from low power or incomplete lineage sorting owing to the rapid isolation and divergence within the clade. Similarly, we recovered a central Texas clade of *G. versa* and *G. caglei* that occurs in adjacent river drainages (the Colorado and the Guadalupe, respectively), each of which are monophyletic and strongly supported. Our phylogeny excludes *G. o. sabinensis* as a part of the clade, again with strong support.

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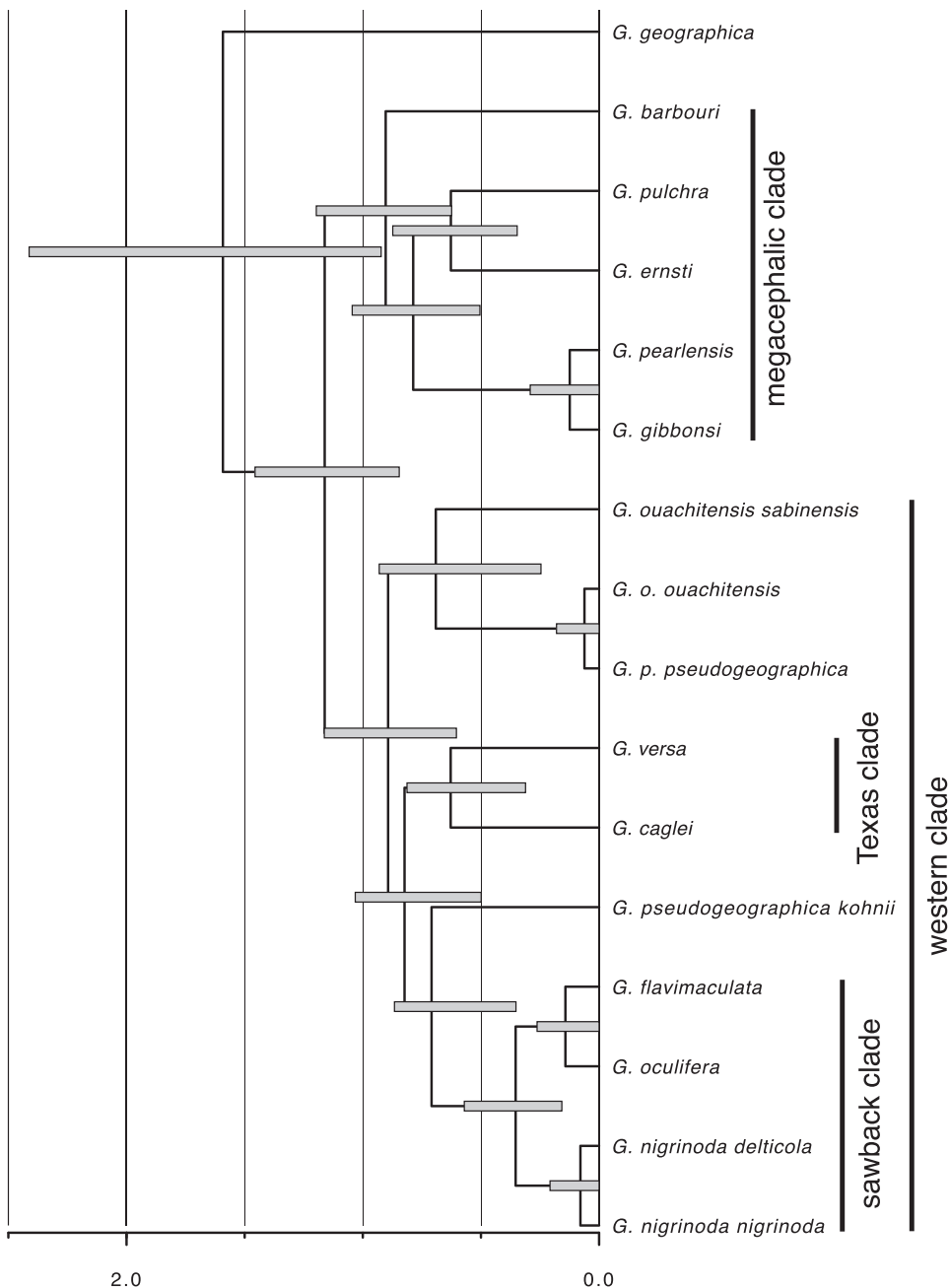


Fig. 4. Multispecies coalescent species tree of the map turtles. Gray bars depict the 95% highest posterior density of node ages and branch lengths are in units of time (millions of years).

ouachitensis, and *G. o. sabinensis*) remains largely unresolved. These two species were not recovered as monophyletic in our analyses, although this could potentially be explained by a lack of power. We recovered individuals belonging to this group in four lineages within the western clade part of the tree, but most of the relevant nodes were poorly supported. Only one individual conflicts strongly with monophyly of the false map turtle complex (*G. o. ouachitensis* RCT406 is sister to the Texas clade with PP = 0.98). Our phylogeny also does not recover monophyly of the described taxa within the complex. If we examine the four named lineages within the false map turtle complex, *G. o. sabinensis* forms a monophyletic group with strong support, which is consistent with recent suggestions to treat it as a full species (Lindeman, 2013). However, the remaining three taxa fail to correspond well with existing taxonomy or with geography. For instance, we recovered a single *G. o. ouachitensis* from Illinois (RCT406) as sister to the central Texas clade, whereas *G. o. ouachitensis* from nearby populations in Ohio (GOS24) and Minnesota (RCT87) are more closely related to *G. p. kohnii* lineages

from southern Louisiana (RCT10).

The network analysis shows a similar pattern (Fig. 3), and identifies relatively well-differentiated clades for the megacephalic species, *G. geographica*, and the 'sawbacks' within the western clade. The false map turtle complex, however, is scattered throughout the deep divergences within this clade, suggesting many possible arrangements and a high degree of uncertainty. Node support throughout much of these parts of the tree is weak (PP < 0.9) although this alone cannot explain the non-monophyly of each species, since they are scattered among multiple well supported clades. Prosaic explanations, such as sample misidentification or erroneously mixed samples does not explain this pattern. Nearly all field collections were undertaken by biologists with extensive experience in the identification of these species in the field, almost all tissue samples are supported with museum or photographic vouchers that show relevant characters (primarily head stripes), and after re-extracting several samples there was no indication of contamination in our lab work.

The prevalence of such extensive non-monophyly in the group could stem from several sources, none of which are mutually exclusive. First, the false map turtle complex might contain fewer evolutionary lineages than current taxonomy indicates. This is the hypothesis favored by [Praschag et al. \(2017\)](#), who conclude that several currently recognized species should be subsumed into one another. An alternative possibility is that incomplete lineage sorting and/or admixture has obscured phylogenetic signal for these species. The false map turtle complex inhabits several extremely large river drainages, most of which are much more extensive than those occupied by other species in the western clade. For example, the Mississippi drainage (a false map turtle complex river) forms a basin that encompasses over 2.9 million square km, whereas the adjacent Pearl drainage (a *G. oculifera* river) encompasses only 22,600 square km ([Seaber et al., 1987](#)). The difference in range size is perhaps the most conspicuous difference between most false map turtle complex species and all other members of the western clade. In addition, the only member of the false map turtle clade that is recovered as monophyletic is *G. o. sabinensis*, which inhabits a similarly small set of drainages (primarily the Sabine River [25,268 km²], plus the smaller drainages of the Neches, Calcasieu, and Mermentau). If river basin size is even partially correlated with population size via habitat availability, we might expect most members of the false map turtle group to have much larger ancestral populations than all other species in the clade, which consequently could allow for extensive incomplete coalescence driving the phylogenetic patterns seen here.

A final possibility is that these patterns may be driven by incomplete reproductive isolation among the false map turtle complex lineages. Ongoing or historic admixture could lead a set of gene trees that reflect both historical differentiation and more recent reticulation, confounding the concatenated analysis in this part of the tree. Unfortunately, individual gene trees are too poorly resolved in our dataset to clearly characterize the underlying distribution of gene trees. All species in this complex are sympatric with one other member in some part of their range, which means that admixture is at least plausible. During the extensive field collections involved in this project, we did occasionally (though rarely) capture individuals in this group that appeared to have morphologically intermediate diagnostic characters. If hybridization is occurring, the question arises of whether it is ongoing or historic, and what this implies about species boundaries within the false map turtle complex. One possibility is that these are distinct but nascent lineages that are experiencing a low rate of gene flow as reproductive isolation evolves to completion. An alternative is that these lineages underwent speciation during a period of historical isolation, but are now collapsing back together after secondary contact. Distinguishing among these scenarios will require more detailed analysis of population demographics and migration using more extensive population genetic data. Some combination of these latter two hypotheses (incomplete lineage sorting or hybridization) are implicitly assumed in the analysis of [Praschag et al. \(2017\)](#). Those authors elected not to undertake a phylogenetic analysis of their nuclear data owing to their assumption that reticulate patterns in this clade would 'be completely misleading for any bifurcating representation.' We agree that some reticulation is compatible with the data, but our analyses also clearly demonstrate that a history of evolutionary bifurcations are apparent for most of the map turtles, especially outside of the false map turtle complex.

In their analysis, [Praschag et al. \(2017\)](#) conclude that map turtle taxonomy is 'oversplit' and the authors recommend extensive taxonomic revision based on their mitochondrial phylogeny, nuclear DNA haplotype networks, and the similarity of nuclear DNA haplotypes in PCA space. Under a general metapopulation lineage view of species ([de Queiroz, 2007](#)), we disagree with their recommendation for several reasons. First, all of the species, except for the false map turtle complex and *G. flavimaculata*, are monophyletic with strong support, suggesting that these are distinct evolutionary lineages. Most species are easily recognizable using morphology alone, forming a second line of

evidence that evolutionary lineages exist within the clade. While not all aspects of evolutionary history are well resolved, here or in [Praschag et al. \(2017\)](#), it is clear that substantial lineage-level diversity exists and that this diversity appears to be largely structured by allopatric divergence of populations among adjacent river systems. The false map turtle complex is the major exception to this pattern and may well require taxonomic revision in the future. Several taxon pairs within the complex (e.g., *G. o. sabinensis* and *G. p. kohnii* in the Neches drainage) occur sympatrically and appear to remain distinct both morphologically and genetically, while in other areas (e.g., *G. pseudogeographica* and *G. ouachitensis* in the northern areas of the Mississippi drainage) distinctiveness is much less clear. In our view, the number of evolutionary lineages, their boundaries, and the evolutionary history within this complex are all in need of further work, but they do not appear to be consistent with a model of a single wide-ranging species.

4.2. Age and diversification of the map turtles

Many previous authors have noted that map turtles comprise a young clade that has diversified rapidly. The primary molecular clock analysis available to date ([Lamb et al., 1994](#)) relied on a fixed mitochondrial clock and estimated that the crown age of *Graptemys* was 6–8 million years. We recovered substantially younger estimates. There are several potential explanations for the younger ages estimated here. First, we estimated divergence times in a multispecies coalescent framework, which estimates divergence times of species that are necessarily younger than the divergence times of the underlying alleles that are sorting within those species. Thus, our approach is focused on recovering the time when lineages become isolated, as opposed to the time at which the alleles within that lineage last shared a common ancestor. However, the multispecies coalescent analysis jointly estimates gene tree divergence times along with species divergence times, and our gene tree divergences are still younger than those previously reported. Second, we calibrated our molecular clock using empirical rates estimated from other turtles. These rates are broadly consistent across the literature and suggest that turtles have experienced a slowdown in their rate of molecular evolution relative to other vertebrates ([Hugall et al., 2007](#); [Loureño et al., 2013, 2012](#); [Shaffer et al., 2013](#)). Nevertheless, they represent a source of uncertainty in our analysis (as would the age and phylogenetic placement of fossil calibrations, had we been able to use these) and could explain part of the age discrepancy. It is worth noting that all known map turtle fossils are also younger than the age we estimate for the group, which is required if this estimate is accurate. The sole possible exception to this, of which we are aware, are a set of Tertiary fossils (dating to approximately 34 mya) whose phylogenetic affinities are highly uncertain. These have been referred variously to the deirochelyine genera *Chrysemys*, *Graptemys*, or (most recently) the extinct genus *Pseudograptemys* ([Bjork, 1967](#); [Clark, 1937](#); [Hay, 1908](#); [Hutchison, 1996](#); [Loomis, 1904](#)). All authors that discuss these fossils refer them to a genus only tentatively, typically calling for further study. The fossils display no known characters that unambiguously place them within the crown group of *Graptemys*. [Hutchison \(1996\)](#) questioned whether they could even be assigned to the Emydidae. Further, the age of these fossils predates several molecular clock estimates for when the genera within the Deirochelyinae first diverged ([Loureño et al., 2012](#); [Near et al., 2005](#); [Spinks et al., 2016](#); [Wang et al., 2012](#)), suggesting that these fossils are likely members of a stem deirochelyine lineage, rather than a member of the *Graptemys* crown lineage.

A young origin for the map turtles make their rate of diversification and morphological evolution all the more surprising among turtles. Their bright, high-contrast, coloration coupled with dramatic keels and spines, suggest that a combination of sexual and natural selection has played a strong role in shaping their diversity. Map turtles employ elaborate head-to-head courtship behavior characteristic of other deirochelyine turtles (e.g., *Chrysemys*, *Pseudemys*, and *Trachemys*; [Cagle,](#)

1950; Ernst and Lovich, 2009; Ernst, 1971; Marchand, 1944), which at least provides the opportunity for female choice based on the bright, high contrast head stripe patterns. This suggests that sexual selection could play a role in driving rapid morphological divergence. Related genera that show this behavior frequently have elaborate and high contrast head markings, although other dirochelyine genera have not evolved the extreme morphological diversity that characterizes map turtles. While speculative, one potential explanation consistent with the pattern seen in *Graptemys* is that the high endemism (and the allopatry among lineages which results) allows sexual selection to run away toward different morphologies in different lineages, thereby generating high morphological diversity over a short period of time. Within other dirochelyine genera, less endemism (and consequently more gene flow across the range) would constrain the same sexual selection process to move toward only a single morphology that is shared among populations because of inter-drainage gene flow.

5. Conclusions

We provide a well-sampled and well-resolved phylogeny for the map turtles that clarifies and confirms most features of the taxonomy for the clade. Understanding the biogeographic history and diversification of the group ultimately rests on understanding its age, and we report an age for the map turtles that is considerably younger than any that has yet been proposed. The most compelling way to reject this new hypothesis would be through the identification of a fossil crown group map turtle that is unequivocally older than 2.5 million years. This depends on assigning new fossils to stem versus crown lineages, which can be a challenge in many radiations, this one included. Unfortunately, few fossils have been identified from the Gulf Coastal Plain where most map turtle diversity resides (Lindeman, 2013), and currently none predate our molecular age estimate. Whether this proposed young age of the map turtles is eventually corroborated or not, the group contains a striking level of morphological diversity and forms a primary component of the southeastern US global hotspot of turtle diversity. This diversity is also inherently vulnerable, owing to the clade's endemism and small ranges, habitat loss as rivers have been dammed and channelized, and a vulnerable life history that is characterized by long lives and slow reproductive output.

Acknowledgements

We thank Anthony Barley, John Carr, Becky Chong, Levi Gray, Dean Leavitt, Gregory Pauly, Will Selman, and Dick Vogt for their assistance during field collecting trips. For specimens and tissue samples, we thank Joel Babitzke, Jeff Brigler, Russ Burke, Josh Ennen, Jim Godwin, Fred Janzen, Jarrett Johnson, Brian Kreiser, Jim Lamer, Paul Moler, Erin Myers, Michael Pappas, Greg Pauly, Willem Roosenberg, Will Selman, and Nathalie Tessier. We appreciate permits and assistance from wildlife agencies in several states including Alabama, Florida, Georgia, Illinois, Louisiana, Michigan, Minnesota, Mississippi, Ohio, and Texas. Craig Moritz, Dick Vogt, Peter Wainwright, and Phil Ward all provided helpful advice during the early development of this project. Finally, we thank Anthony Barley, Amber Wright, Becky Chong, and two anonymous reviewers for comments that improved this manuscript.

Funding

This work was supported by funding from the National Science Foundation (DEB 0710380; DEB 0817042; DEB 1239961; DEB 1457832, DBI 1356796), fellowship and research funding from the UC Davis Center for Population Biology, and a graduate student award from the Society of Systematic Biologists.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2017.11.012>.

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