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A phylogenomic analysis of turtles

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ABSTRACT

Molecular analyses of turtle relationships have overturned prevailing morphological hypotheses and prompted the development of a new taxonomy. Here we provide the first genome-scale analysis of turtle phylogeny. We sequenced 2381 ultraconserved element (UCE) loci representing a total of 1,718,154 bp of aligned sequence. Our sampling includes 32 turtle taxa representing all 14 recognized turtle families and an additional six outgroups. Maximum likelihood, Bayesian, and species tree methods produce a single resolved phylogeny. This robust phylogeny shows that proposed phylogenetic names correspond to well-supported clades, and this topology is more consistent with the temporal appearance of clades and paleobiogeography. Future studies of turtle phylogeny using fossil turtles should use this topology as a scaffold for their morphological phylogenetic analyses.

1. Introduction

The evolutionary relationships of turtles (Testudines) are contentious. Until recently, the placement of turtles within Amniota was uncertain (Hedges and Poling, 1999). Genome-scale and whole genome analyses have confirmed the phylogenetic position of turtles as the sister group to archosaurs (Crawford et al., 2012; Field et al., 2014; Fong et al., 2012; Shaffer et al., 2013; Wang et al., 2013), rejecting a putative relationship between turtles and lepidosaurs (Lyson et al., 2012). However, relationships among turtles have not been studied using phylogenomic techniques. Similar to the placement of turtles relative to their amniote ancestors, molecular studies within Testudines (Shaffer et al., 1997; Fujita et al., 2004; Krenz et al., 2005; Parham et al., 2006a; Barley et al., 2010) have challenged prevailing phylogenetic hypotheses based on cladistic analyses of morphological data (e.g., Gaffney and Meylan, 1988; Gaffney et al., 1991).

One example of the discrepancies among previous phylogenetic approaches involves the position of trionychians (Fig. 1a), a group of turtles that have lost their scales and developed a fleshy snorkel-like proboscis. In the morphology-based hypothesis, the morphologically bizarre trionychians are nested high in one of two fundamental branches of the turtle tree, the diverse clade Cryptodira (Fig. 1b). Molecular studies disagree with this placement but are equivocal on the alternate position of trionychians. Some studies remove trionychians from their highly nested position within Cryptodira and place them as the sister taxon of all other cryptodires (Fig. 1c). Other studies place Trionychia as sister taxon to Pleurodira, the other branch of the turtle tree (Fig. 1d), or as the sister taxon to both Cryptodira and Pleurodira (Fig. 1e). As molecular phylogenies changed the position of Trionychia and other branches of the turtle tree of life, it prompted the simultaneous development of new nomenclature, and phylogenetically defined clade names were created for several higher-level nodes (Joyce et al., 2004; Danilov and Parham, 2006; Knauss et al., 2011; Joyce et al., 2013) in the turtle phylogeny.

Here, we use sequence data collected from thousands of ultraconserved elements (UCEs; sensu Faircloth et al., 2012) to infer a genome-scale phylogeny of turtles. We use this phylogeny to assess and update the phylogenetic nomenclature and to compare the evolutionary relationships of turtles to broad temporal and spatial patterns from the fossil record.

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2. Materials and methods

2.1. Materials

We selected 32 turtle/ingroup operational taxonomic units (OTUs) and six outgroups (three lepidosaurs [*Sphenodon* and two squamates], two archosaurs [a crocodilian and a bird], and one mammal [human]; Table 1) for analysis. Our 32 ingroup OTUs represent all of the 'major lineages' of turtles; we define a 'major lineage' as an established, uncontroversial, monophyletic group of extant species. Although the inferred relationships among these lineages can vary (Fig. 1), all recent studies accept the monophyly of six major lineages: Pleurodira, Trionychia, Testudinoidea, Chelonioidea, Chelydridae, and Kinosternoidea. In addition to sampling these six lineages we also included samples from OTUs representing all 14 traditionally accepted families (Turtle Taxonomy Working Group, 2014).

With the exception of *Pelodiscus sinensis* and *Chelonia mydas,* which have sequenced genomes, we sampled tissues for all ingroup OTUs from vouchered specimens kept at the California Academy of Sciences and the Museum of Vertebrate Zoology (Table 1). We used the phylogenetic nomenclature from Joyce et al. (2004), except where otherwise noted. For the sake of simplicity we refer to OTUs/specimens in the text and figures by their assigned genera according to a recent checklist (Turtle Taxonomy Working Group, 2014). As parts of a species binomial can be unstable and/or controversial, Table 1 includes full species names that can be compared to the aforementioned annotated checklist. We also use this checklist for the counts of species given in the text.

2.2. UCE methods

We extracted DNA from approximately 25 mg of tissue using Qiagen DNeasy Tissue kits following the manufacturer's protocols, and we ran all genomic DNA extractions on an agarose gel to assess quality. We then sheared 1–2 μg of DNA to 400–600 bps in length using a Diagenode Bioruptor® Standard (UCD 200) with 6–8 cycles of sonication (depending on DNA quality). We prepared sequencing libraries from DNA extracts using KAPA library prep kits (Kapa Biosystems) following the library preparation protocols available at <http://ultraconserved.org/#protocols>. We attached sequence tags, designed by Faircloth (2012), to each library using individually barcoded primers during the library amplification step. After library amplification, we quantified 2 μL of each library using fluorometry (Qubit, Life Technologies), and we prepared six pools of eight libraries totaling 500 ng per pool (62.5 ng each library). We concentrated library pools using a Savant ISS110 SpeedVac Concentrator (Thermo Fisher) and rehydrated each library in 3.4 μL of ddH2O.

We enriched these pooled libraries using a synthesis of 2560 RNA probes (Mycroarray, Inc.) targeting 2386 ultraconserved elements (UCEs) and their flanking sequence (Faircloth et al., 2012). Detailed methods of library enrichment, post-enrichment PCR and validation using relative qPCR may be found at <http://ultraconserved.org/#protocols>. We generated sequences for each enriched library using paired-end 150 base-pair sequencing on an Illumina HiSeq 2500 in "rapid-run" mode. After using scythe (http://github.com/vsbuffalo/scythe) to remove adapter contamination and sickle to quality-trim sequence reads (version 1.210) (Joshi and Fass, 2011), we assembled reads into contigs using Velvet (version 1.2.10) (Zerbino and Birney, 2008). We used *VelvetOptimiser.pl* (version 2.2.5) to find the kmer value that produced the most contigs. Because this method is computationally expensive, we limited the search range to kmer values between 89 and 121.

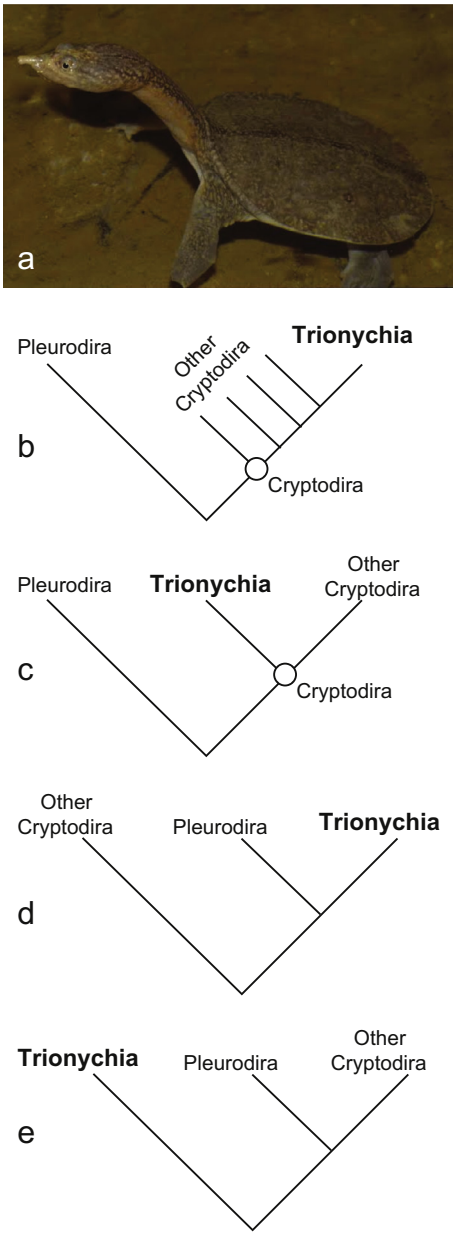

Fig. 1. (a) An extant trionychian showing some of the bizarre diagnostic characters for the group such as the lack of scales and a fleshy proboscis; (b) prevailing morphological hypothesis (Gaffney and Meylan, 1988; Gaffney et al., 1991; Gaffney, 1996); (c) molecular hypothesis with trionychians as the sister taxon to all other cryptodires (Shaffer et al., 1997 [mtDNA]; Fujita et al., (2004) [intron]; Krenz et al., (2005) [intron]); (d, e) Topologies showing alternative roots for the crown group Testudines (Barley et al., 2010 [nuDNA]; Sterli, 2010 [morphology, mtDNA, intron]; Field et al., 2014 [miRNAs]). Photo credit: *Dogania subplana* from Indonesia taken by Peter Paul van Dijk.

We used phyluce (Faircloth et al., 2012) to identify those contigs that were UCE loci, remove putatively duplicate UCE loci, create a database of UCE loci recovered, and prepare FASTA files for sequence alignment. We generated alignments from this monolithic FASTA file using MAFFT (version 7.130b) (Katoh, 2002; Katoh and Standley, 2013), and we trimmed resulting alignments using the trimming algorithm implemented by the *seqcap_align2.py* script within phyluce. From the trimmed alignments, we created two datasets: one where each locus contained all 36 taxa (100% complete), and one where we allowed up to 25% missing taxa per locus (i.e., we required data from a minimum of 29 taxa per locus). We estimated the appropriate finite-sites substitu-

Table 1
Newly sequenced samples are from museum specimens housed at either the California Academy of Science (CAS), the Museum of Vertebrate Zoology at Berkeley (MVZ). For each of the newly sequenced samples, a Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) number follows the museum specimen number. For samples from previously published papers the reference and genome builds in which UCE loci were identified (see Section 2.4).

Binomial	Specimen/Genome	Reference
Outgroup OTUs:		
Anolis carolinensis	anoCar1	Alföldi et al. (2011)
Crocodylus porosus	croPor1	St. John et al. (2012)
Gallus gallus	galGal3	Cons ICGS (2004)
Homo sapiens	hg19	Lander et al. (2001)
Python molurus	pytMol1	Castoe et al. (2013)
Sphenodon punctatus	UMFS 10956	Crawford et al. (2012)
Ingroup OTUs:		
Agrionemys horsfieldii	CAS 228637	SAMN02900523
Apalone ferox	CAS 202549	SAMN02900524
Carettochelys insculpta	MVZ 238114	SAMN02900525
Chelydra serpentina	MVZ 265668	SAMN02900526
Chelonia mydas	cheMyd1	Wang et al. (2013)
Chrysemys picta	H2662	Crawford et al. (2012)
Cyclemys dentata	CAS 243787	SAMN02900527
Deirochelys reticularia	MVZ 204282	SAMN02900528
Dermatemys mawii	MVZ 269552	SAMN02900529
Dermochelys coriacea	MVZ 149844	SAMN02900530
Emys marmorata	CAS 224202	SAMN02900531
Erymnochelys madagascariensis	MVZ 238759	SAMN02900532
Geoemyda spengleri	MVZ 208234	SAMN02900533
Gopherus berlandieri	MVZ 250594	SAMN02900534
Graptemys pseudogeographica	MVZ 250644	SAMN02900535
Kinosternon arizonense	CAS 228101	SAMN02900536
Lepidochelys olivacea	CAS180267	SAMN02900537
Lissemys punctata	CAS 232082	SAMN02900538
Mesoclemmys nasuta	MVZ 247578	SAMN02900539
Nilssonia formosa	CAS 246283	SAMN02900540
Pelodiscus sinensis	pelSin1	Wang et al. (2013)
Pelomedusa subrufa	MVZ 236628	SAMN02900541
Pelusios castaneus	CAS 219222	SAMN02900542
Platemys platycephala	MVZ 247579	SAMN02900543
Platysternon megacephalum	MVZ 230486	SAMN02900544
Podocnemis erythracephala	MVZ 269553	SAMN02900545
Rhinoclemmys punctularia	MVZ 247582	SAMN02900546
Staurotypus triporcatus	MVZ 263984	SAMN02900547
Sternotherus minor	CAS 221865	SAMN02900548
Stigmochelys pardalis	MVZ 241333	SAMN02900549
Terrapene ornata	MVZ 230553	SAMN02900550
Trachemys scripta	CAS 252979	SAMN03102956

tion model for each locus in all datasets using CloudForest (Crawford and Faircloth, 2014), and we prepared a concatenated dataset for subsequent analyses by grouping together loci having the same substitution model into a partition.

We performed Bayesian analysis of the concatenated alignment data using two runs of MrBayes version 3.2.2 (r879) (Ronquist et al., 2012) for 500,000 iterations (4 chains; burn-in: 25%; thinning: 500). We assessed convergence of the runs using TRACER (http://tree.bio.ed.ac.uk/software/tracer/). We performed maximum likelihood analyses of the concatenated data using RAxML version 7.2.6 (Stamatakis, 2006) with the "GTRGAMMA" option and 10,000 bootstrap replicates. We also performed gene-tree species-tree analysis by estimating gene trees for each UCE locus incorporating 100 multi-locus bootstrap replicates, which we integrated into STEAC and STAR species trees (Liu and Yu, 2010; Liu et al., 2009). *A posteriori* bootstrapping analysis conducted with RAxML's autoMRE tool indicated that trees converged after 50 replicates.

We root our tree with the mammals following the approach of recent analyses that confirm the archosaur affinities of Testudines (Crawford et al., 2012; Fong et al., 2012; Field et al., 2014). Lyson et al. (2012) phylogenetically defined Ankylopoda for an alternative placement for turtles, the crown clade of turtles and lepidosaurs, but the crown clade of turtles and archosaurs is an unnamed amniote lineage. We fill this important nomenclatural gap, and phylogenetically define the name 'Archelosauria' to refer to the clade that originated from the most recent common ancestor of *Crocodylus niloticus* Laurenti, 1768 and *Testudo graeca* Linnaeus, 1758. The name was chosen to evoke the two included lineages, archosaurs and chelonians (Testudines).

2.3. Trachemys whole genome sequencing, assembly, and UCE identification

Whole genome sequencing libraries were prepared using Illumina's Nextera library preparation kit, following manufacturer protocols, with the following modifications: after library preparation, a 600–700 bp size selection was performed using the BluePippin size selection system (Sage Science). Size selected products were amplified in a 7 cycle PCR using the KAPA Real Time Library Amplification kit (KAPA Biosystems) following manufacturer's instructions. PCR products were cleaned using the standard Ampure XP (Beckman Coulter Inc.) bead clean-up method with a 0.8:1 bead to PCR product ratio. Libraries were validated by running 1 μl of product on an Agilent 2100 Bioanalyzer (Agilent Technologies) and quantified using a Qubit fluorometer (Life Technologies). Final libraries were sequenced on a HiSeq2500 (Illumina). Two lanes of 150 bp paired-end sequencing were run using the rapid run output mode, with each lane containing two libraries pooled in equimolar amounts. These same libraries were also sequenced on two runs of an Illumina MiSeq with 600 cycle v3 kits and 300 bp paired-end sequencing mode.

After adapter trimming and quality filtering using Trimmomatic version 0.32 (Bolger et al., 2014), we assembled 12,731,817 *Trachemys scripta elegans* contigs from 138,894,454 HiSeq and 53,677,903 MiSeq reads with Soapdenovo2 (Luo et al., 2012) using its multi-kmer method. Kmer sizes ranged from 23 to 127. To identify those contigs that contained UCE loci we used standalone BLAT version 35 (Kent, 2002) to match a 2560 UCE probe set (obtained from http://ultraconserved.org) to the assembled contigs. BLAT was run with default parameters on contigs greater than 300 bp. Then a custom PYTHON script was used to extract those contigs matching the UCE probes with reported *E*-value scores of 1e-1 or lower. These 2926 contigs ranged in size from 301 bp to 33,369 bp. This final set of 2926 *Trachemys scripta elegans* contigs enriched for UCE sequences was used in all subsequent phylogenetic analyses.

2.4. Data availability

With the exception for *Pelodiscus sinensis* for which a published genome is available, all ingroup OTUs have specimen vouchers, and all tissues and specimens are available to qualified researchers. The data we included for all outgroup taxa and *P. sinensis* are publicly available at: <https://github.com/faircloth-lab/uce-probe-sets> (Faircloth et al., 2012). Additional details concerning UCE sequence capture methods and phylogenetic methods are described in Faircloth et al. (2012) and detailed protocols are available at <http://ultraconserved.org>. Sequenced reads are available in the short read archive (PRJNA254176) and alignments and trees at data dryad (doi:10.5061/dryad.t77q4).

3. Results

We sequenced a total of 86 million read pairs with a mean of 3,083,947 per sample from 28 taxa (Table 1). We assembled a mean of 5377.86 contigs per sample (95CI, min = 1919, max = 12,511) (Supp. S1). We also incorporated an average of

2939.9 UCEs drawn from eight taxa with published genomes. Combining the UCEs identified in published genomes with the contigs assembled from the 28 UCE enriched genomic libraries and running the matrix generation procedures produced: (1) a 100% complete matrix containing 233 alignments having a mean length of 820.26 bp (±48.58 CI) per alignment, totaling 191,121 bp of aligned sequence and (2) a 75% complete matrix containing 2381 alignments having a mean length of 721.61 bp (±15.65 CI) per alignment, totaling 1,718,154 bp of aligned sequence.

We recover a phylogeny of Testudines that is identical across Bayesian, maximum likelihood approaches and where every node is fully resolved (e.g., 100% bootstrap support or posterior probabilities of 1.0). The topologies of the STEAC and STAR species trees contain a few inconsistencies when compared to the ML and Bayesian trees. These include the position of *Trachemys scripta* within Emydidae and the position of Chelonioidea within Durocryptodira. This is likely caused by both incomplete lineage sorting, and poorly resolved gene trees due to the small amount of DNA sequence used to infer individual trees (mean = 820.26 bp) (McCormack et al., 2013). Alternately the high support of these groups in the ML and Bayesian trees may be from systematic biases in inferring trees from concatenated datasets (Mossel and Vigoda, 2005; Kubatko and Degnan, 2007).

Our analysis of the UCE data recovers a monophyletic Pleurodira and Cryptodira. Within Pleurodira, we recover the traditional families based on inclusion of two representatives, each, of Chelidae, Pelomedusidae, and Podocnemididae. We also recover a monophyletic Pelomedusoides (Pelomedusidae + Podocnemididae), a long-recognized group. Within Cryptodira, Trionychia is the sister group to all of the other cryptodire lineages (Durocryptodira). Within durocryptodires, Testudinoidea is the sister group of a clade including all of the other lineages. *Platysternon megacephalum* is resolved as the sister taxon to Emydidae as in Parham et al. (2006a). The phylogeny places emydid OTUs consistent with their subfamilial designations, with the two emydine taxa (*Emys* and *Terrapene*) forming a monophyletic group. On the deirochelyine side, *Deirochelys* is the sister taxon of a clade that includes *Chrysemys*, *Trachemys*, and *Graptemys*. This result differs from that of Spinks et al. (2009), which placed *Chrysemys* outside of a clade that includes *Deirochelys* and *Graptemys*. We also recovered the Testuguria clade (Geoemydidae + Testudinidae), and within each of those included clades the topology of the tree matches previous phylogenetic analyses (Spinks et al., 2004; Parham et al., 2006b). A monophyletic Kinosternoidea is the sister taxon of Chelydridae, thereby affirming the Chelydroidea clade codified by Knauss et al. (2011). The chelonioids are the sister taxon of the chelydroids, which together form the recently named Americhelydia (Joyce et al., 2013). The ability for UCEs to reconstruct relatively recent divergences (e.g., within the traditional families) was previously demonstrated by Smith et al. (2014) and is supported here.

4. Discussion

4.1. The phylogeny of turtles based on UCEs

4.1.1. Pleurodira, Trionychia, and Durocryptodira

The UCE phylogeny supports the monophyly of Cryptodira, with Trionychia as the sister taxon to all other cryptodires (Figs. 2, 3a, c). The clade including non-trionychian cryptodires was phylogenetically defined as 'Durocryptodira' by Danilov and Parham (2006). The topology from ultraconserved elements and other molecular studies (Shaffer et al., 1997; Krenz et al., 2005; Barley et al., 2010) support the monophyly and recognition of Durocryptodira, which contrasts with the morphological hypothesis (Figs. 1, 3b).

A monophyletic Durocryptodira is consistent with the temporal appearance of lineages in the fossil record. Pan-trionychians (trionychians and their stem) are the most ancient cryptodire lineage. Therefore, the molecular phylogenetic placement of Trionychia as the sister taxon to all other cryptodires (Durocryptodira) is most consistent with their antiquity (Fig. 3a, b). In contrast, the morphological hypothesis requires significant missing time for several of the major lineages of turtles (Fig. 3b). These 'ghost lineages' (Norell, 1992) are hard to accept given the relatively rich fossil record of turtles. On the other hand, even though the phylogenetic position of many fossils at the base of Cryptodira is poorly constrained, there are several candidate taxa that could fill the ghost lineage on the stem of Durocryptodira (Fig. 3a).

4.1.2. Americhelydia

The UCE phylogeny supports the division of durocryptodires into the diverse and long recognized Testudinoidea (183 species) and the recently named Americhelydia (38 species, Joyce et al., 2013). Americhelydia is comprised of three major lineages that share a common ancestor in the Cretaceous of North America. Two of these lineages, the chelydroids and kinosternoids, are still North American endemics. The third lineage is the chelonioids, extant marine turtles, which have a cosmopolitan, oceanic distribution. Whereas the exclusive monophyly of extant marine turtles relative to extant, non-marine lineages is not controversial, the relationships of many fossil marine turtles are confounded by potential polyphyly (multiple origins of marine turtles) and parallel evolution (Joyce, 2007; Joyce et al., 2013). Given this confusion it is unclear whether the chelonioid lineage diverged from other Americhelydians in the Early or Late Cretaceous, but in either case their oldest fossils and presumed origins are in the Americas (Zangerl, 1953; Hirayama, 1998; Joyce, 2007). Therefore, just as the UCE phylogeny fits with the temporal appearance of clades in the fossil record, it also coincides well with biogeography by uniting some American durocryptodires into a monophyletic group.

4.1.3. Testudinoidea

Testudinoidea (183 species, more than half of turtle diversity) has deep fossil roots in Asia where it maintains a high diversity today. Within testudinoids, the sister taxon relationship between terrestrial tortoises (testudinids) and the geoemydids was phylogenetically defined as Testuguria by Joyce et al. (2004). At that time the phylogenetic position of the big-headed turtle (*Platysternon*) was not well established. Early molecular phylogenies placed it outside of Testudinoidea (Shaffer et al., 1997) or as the sister taxon to Testuguria (Krenz et al., 2005). Parham et al. (2006a) placed *Platysternon* as the sister group of the Emydidae based on an analysis of complete mitochondrial genomes, and this result has been confirmed by more comprehensive nuclear data sets (Barley et al., 2010; this study). The *Platysternon* – Emydidae node is the only node uniting two or more of the traditional families of turtles that does not have a name. We fill this important nomenclatural gap, and phylogenetically define the name 'Emysternia' to refer to the clade that originated from the most recent common ancestor of *Platysternon megacephalum* Gray, 1831 and *Emys orbicularis* (Linnaeus, 1758). The name was chosen to evoke the two included lineages Emydidae and *Platysternon*.

4.2. Global paleobiogeography of turtles based on the UCE phylogeny

Combining the UCE phylogeny with the known fossil record of turtles allows us to reconstruct some global biogeographic patterns (Fig. 3c). Intercontinental dispersal of turtles is common, usually involving a limited number of species. For our discussion we focus primarily on the broad patterns of vicariance and dispersal events that generated significant turtle diversity (i.e., speak to geographic

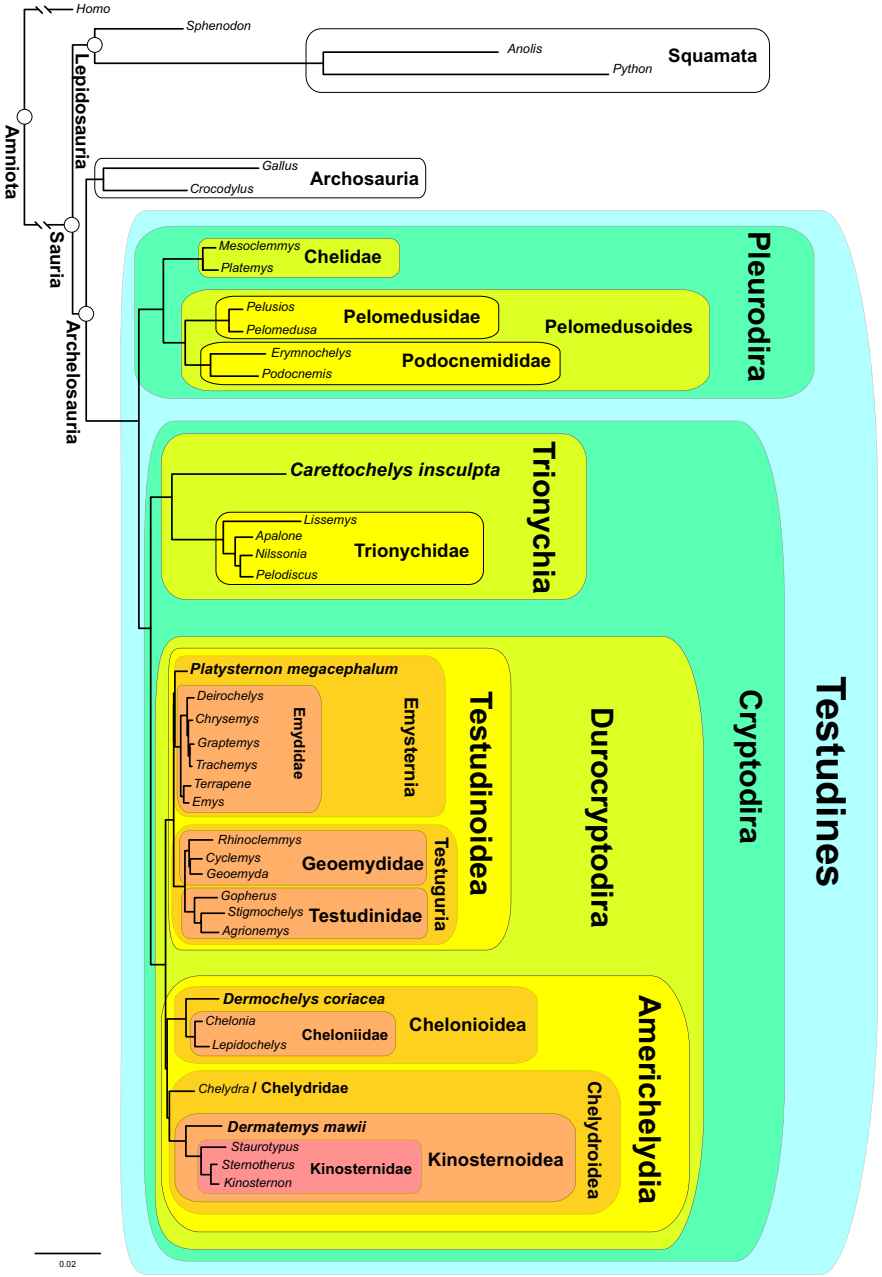

Fig. 2. Phylogenetic hypothesis based on RAxML analysis of UCE data showing phylogenetically defined crown clades of turtles (Testudines). All clades were supported by likelihood bootstrap percentages of 100 except for the position of *Chelydra* in the STAR species tree, which has a bootstrap support of 68. The scale bar is in units of substitutions per site.

origin of 'major lineages' and clades that have been recognized as families, especially in North America). We assign each lineage to a continent based on the their area of origin as shown by the fossil record (stem taxa). For the timing of events we use the simple appearance of lineages in the fossil record used to construct divergence-dating priors by Joyce et al. (2013). For the divergences discussed below, the fossil record of turtles is complete enough that there is no discrepancy between prior and posterior estimates (Joyce et al., 2013) and so molecular divergence dating of the UCE phylogeny would be superfluous.

The earliest fossils of stem testudinoids, stem trionychians, and stem cryptodires are from Eurasia (Danilov and Parham, 2006, 2008; Joyce et al., 2013; Pérez-García et al., 2014). Mapping these data onto the UCE phylogeny demonstrates that cryptodires have a Jurassic (>145 Ma) Eurasian origin (Fig. 3c). The emergence of cryptodires in Eurasia is complemented by the concurrent origin of pan-pleurodires in the Southern Hemisphere (Gondwana; Joyce et al., 2013). Given the distribution of the clades and the timing of their origin, the geography of the cryptodire-pleurodire split can be plausibly linked to the breakup of the supercontinent Pangaea (Scotese, 2001; Rogers and Santosh, 2003; Smith et al., 2004). In this way turtles demonstrate a pattern common to other terrestrial vertebrates (e.g., placental vs. marsupial mammals).

Despite their Jurassic (>145 Ma) origin, cryptodires did not dominate the northern continents for almost 100 million years (until the Cenozoic). Instead, stem turtles (especially the extinct clade Paracryptodira) were diverse and abundant in North America throughout the Cretaceous (145–66 Ma) and into the Cenozoic (<66 Ma; Lyson and Joyce, 2009; Lyson et al., 2011). In the Late Cretaceous (100–66 Ma), cryptodires (trionychians and durocryptodires) began to appear in North America, invading through high latitude dispersal routes (Hirayama et al., 2000; Parham and

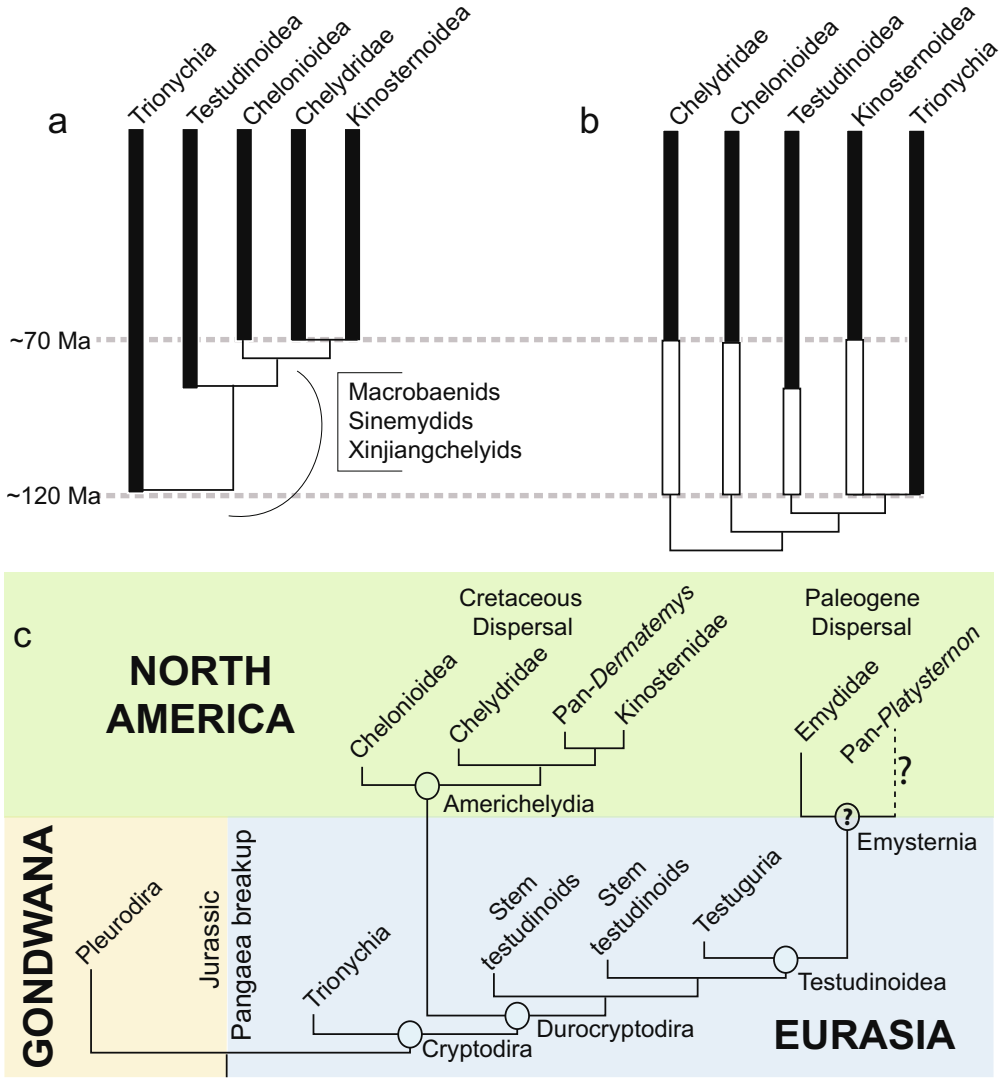

Fig. 3. Phylogenetic hypotheses for the major lineages of turtles mapped against the first appearance of clades in the fossil record and geography. (a) Based on UCE data. Some fossil taxa of basal cryptodires that cannot be attributed to any of the major lineages (i.e. potential stem durocryptodires; Rabi et al., 2013, 2014) are shown; (b) prevailing morphological hypothesis requiring multiple, long ghost lineages; (c) UCE phylogeny mapped against intercontinental paleobiogeography. Two dispersal events of durocryptodires into North America are shown. The geographic origin of Pan-*Platysternon* is uncertain so the Paleogene radiation may have included the common ancestor of Emysternia.

Hutchison, 2003; Brinkman and Tarduno, 2005; Vandermark et al., 2009). The UCE phylogeny confirms that one of the North American durocryptodire lineages (Americhelydia) underwent a modest radiation, accounting for three of the six 'major lineages' of extant turtles (38 extant species, Fig. 3c). The relatively short branches among the Americhelyidian lineages suggest this radiation was rapid.

The Paleogene experienced periods of extremely warm climate (e.g., the Late Paleocene Thermal Maximum and the Early Eocene Climatic Optimum) that are responsible for the dispersal of many organisms into North America through high latitude dispersal routes (Zachos et al., 2001), including a wave of testudinoids (Estes and Hutchison, 1980; Holroyd et al., 2001; Eberle et al., 2010; Hutchison, 2013). Three of these testudinoid lineages persist in North America into modern times. Two are modest radiations of testugurians (four species of *Gopherus* Testudinidae; nine species of *Rhinoclemmys*, Geoemydidae). Previous studies suggested that these genera are sister taxa to all of the Old World members of their respective clades (Parham et al., 2006b; Spinks et al., 2004). We sequenced *Gopherus*, *Rhinoclemmys*, and representative divergent members of geoemydids and testudinids; the UCE data confirm the basal position of these North American genera. This pattern links the overall diversification at the base of these clades with their intercontinental dispersal, which can logically be attributed to periods of warm climate. Similar to the Americhelydia, short branches within the testudinoids also suggest a rapid adaptive radiation that coincides with high latitude intercontinental dispersal events. This pattern suggests that global climate change has a major impact on the diversity and distribution of turtles.

The end of the Paleogene (~45–23 Ma) coincides with global environmental changes, with the climate becoming significantly cooler and drier (Zachos et al., 2001), i.e., much less favorable to turtles. Many turtle lineages that inhabited the Western Interior, including the last stem cryptodires in North America, go extinct at this time (Hutchison, 1982, 1992, 1998). One testudinoid lineage took advantage of the subtropical southeastern portions of the continent (Parmley et al., 2006), to radiate into the diverse clade Emydidae (53 species, Figs. 2, 3c). The recent description of a fossil taxon on the stem of *Platysternon megacephalum* from the Eocene

of North America (Hutchison, 2013) raises the possibility that the more inclusive Emysternia may also have an American origin. Depending on the resolution of that possibility, the UCE topology indicates that two dispersal events into North America led to the origin of 36 or 43% (5 or 6 of 14) of the recognized families (Fig. 3c).

4.3. Consilience in the turtle tree of life: a scaffold for paleontological studies

Because it is more consilient with temporal (stratigraphic, Fig. 3a, b) and spatial (biogeographic, Fig. 3c) patterns, we argue that the molecular phylogenetic topology is more plausible than the morphological topology. In this way, the genetic data from the modern turtle fauna provide an important window into the evolutionary history of turtles. However significant, this window is limited by extinction, and the living species represent only a fraction of past turtle diversity. Fortunately, by virtue of their aquatic tendencies, past abundance, and bony shell, turtles are one of the most common vertebrate fossils since the Late Jurassic (earlier fossils exist, but are rare). This rich fossil record of turtles provides crucial insights into their geographic origin (see Sections 4.1.2 and 4.2), temporal appearance (Joyce et al., 2013), and morphological evolution (Miyashita, 2013; Rabi et al., 2013, 2014). Consequently, any discussion of these patterns arising from molecular phylogenetic studies must consider fossil data. But it is also essential that paleontological studies take advantage of insights from molecular phylogenetics. In particular, paleontologists working on the systematics of lineages that include extant members must address and reconcile phylogenetic hypotheses based on DNA evidence (Parham et al., 2012).

Paleontological studies continue to generate phylogenies that unite Trionychia with the americhelyidian lineage Kinosternoidea (e.g., Bardet et al., 2013; Tong and Meylan, 2013). The conclusions of these studies are compromised because of the strong molecular signal rejecting that topology. Even studies that are not focused on trionychians or their putative close relatives suffer from the incorrect polarization of characters resulting from demonstrably incorrect topologies. The reasons that some paleontological studies do not incorporate information from molecular phylogenetics are usually not stated (but see Sterli, 2010). Explanations likely include a distrust of molecular data and/or the logistical hurdle associated with synthesizing these disparate data types. For the latter, combined analyses are understandably difficult because of non-overlapping taxa and unfamiliarity with analyzing molecular data sets.

The solution is to use a "molecular scaffold" (Springer et al., 2001), i.e., a backbone constraint tree for well-supported nodes involving extant lineages. Molecular scaffolds are useful because they do not require a statistical analysis of molecular data by paleontologists, just a determination of which nodes should be constrained. A molecular scaffold prevents incorrect morphological nodes, such as a highly nested Trionychia, from appearing in the tree, while allowing all fossil taxa to be placed anywhere in the topology. Danilov and Parham (2006) were the first to use this technique for turtles, and other workers have since adopted this method (e.g., Lyson and Joyce, 2010; Rabi et al., 2013; Rabi et al., 2014). We strongly recommend that all future phylogenetic studies of fossil turtles that include extant lineages use molecular scaffolds so that the resultant patterns and discussions can be more confidently interpreted.

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