Author for correspondence:
Ronald M. Bonett
e-mail: ron-bonett@utulsa.edu

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Biogeography and body size shuffling of aquatic salamander communities on a shifting refuge

Ronald M. Bonett[1], Ana Lilia Trujano-Alvarez[1], Michael J. Williams[2] and Elizabeth K. Timpe[1,3]

[1]Department of Biological Science, University of Tulsa, Tulsa, OK 74104, USA
[2]Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803, USA
[3]Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT 06269, USA

Freshwater habitats of coastal plains are refugia for many divergent vertebrate lineages, yet these environments are highly vulnerable to sea-level fluctuations, which suggest that resident communities have endured dynamic histories. Using the fossil record and a multi-locus nuclear phylogeny, we examine divergence times, biogeography, body size evolution and patterns of community assembly of aquatic salamanders from North American coastal plains since the Late Cretaceous. At least five salamander families occurred on the extensive Western Interior Coastal Plain (WICP), which existed from the Late Cretaceous through the Eocene. Four of these families subsequently colonized the emergent Southeastern Coastal Plain (SECP) by the Early Oligocene to Late Miocene. Three families ultimately survived and underwent extensive body size evolution *in situ* on the SECP. This included at least two major size reversals in recent taxa that are convergent with confamilial WICP ancestors. Dynamics of the coastal plain, major lineage extinctions and frequent extreme changes in body size have resulted in significant shuffling of the size structure of aquatic salamander communities on this shifting refuge since the Cretaceous.

1. Introduction

The age, size and isolation of habitat islands can have profound effects on the diversity and evolution of the communities they support [1–5]. At the same time, organisms are subject to historical biological contingencies (both intrinsic and extrinsic) that can strongly influence patterns of colonization, extinction, diversification and trait evolution, which are key processes that drive the course of community evolution [6,7]. Furthermore, extreme environmental changes that result in extinctions or significant population reductions of standing community members (incumbents) can provide opportunities for the colonization and radiation of alternative lineages [8–10]. The impacts of such phenomena have ranged from minor replacements of close relatives to some of the most dramatic episodes of community remodelling in history [9,10].

Freshwater coastal plain habitats occur on most continents and include several biodiversity hotspots. However, the persistence of these habitats can be highly influenced by only slight fluctuations in sea level and precipitation, which suggests that resident communities experienced a tumultuous past. Given the dynamics of this habitat type and the fact that lowlands can produce relatively comprehensive fossil records (compared with mountains), this is an ideal environment to examine patterns of community reassembly through space and time.

Extreme sea-level fluctuations and global temperature shifts since the Late Cretaceous have had major impacts on the freshwater coastal plain habitats of North America [11,12]. The most drastic effects include the complete regional shift from the ancestral Western Interior Coastal Plain (WICP; Late Cretaceous to Eocene) to the modern Southeastern Coastal Plain (SECP; Oligocene to present; figure 1 map). There have subsequently been several less extensive marine
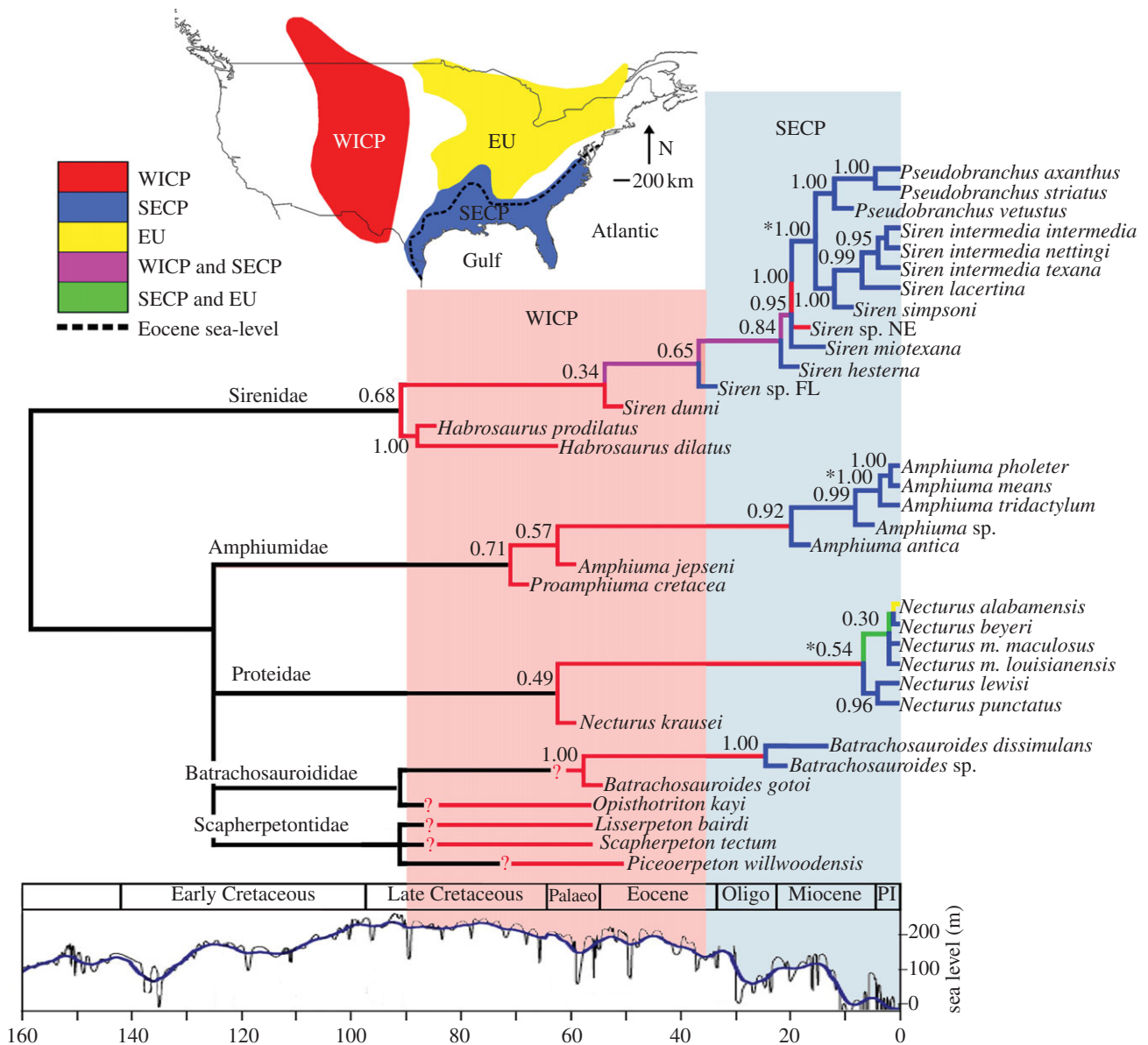
Figure 1. Distribution and biogeographic reconstruction of aquatic coastal plain salamanders of North America. Reconstructions based on LAGRANGE indicate the highest split probability for the ancestral area of each node on the consensus chronogram (see the electronic supplementary material, methods S2, table S9, figures S1 and S4); pruned to show relevant taxa. Internal branches are coloured according to their distribution. The most recent common ancestor of each extant group is indicated with an asterisk. Sea level is based on Miller *et al.* [13] and indicates a substantial decline from the end of the Cretaceous through the Palaeocene, unveiling the ancestral WICP, followed by exposing the modern SECP after the Eocene. The height of the Eocene sea level (dashed line) is based on Galloway [11].

transgressions, but the majority of the SECP has persisted since the Early Oligocene, providing the first opportunity for the colonization of modern freshwater communities. The SECP harbours a tremendous diversity of freshwater vertebrates [14–16] and is a refuge for many divergent lineages that were more broadly distributed in the Mesozoic and Early Cenozoic. Even though the SECP has been the subject of many classic phylogeographic studies, these largely document Pliocene/Pleistocene-levels of divergence [17,18]. The source of endemic SECP freshwater vertebrates, their timing of colonization and early patterns of evolution and diversification have not been well tested. Based on fossil distributions, the aquatic 'lower vertebrate fauna' are noted to have affinities to either the WICP or the neotropics, or they are too ubiquitous to assess their origin [19–21], but the patterns and timing of colonizations have yet to be analysed in a phylogenetic context.

Most or all of the modern representatives of the salamander families Amphiumidae, Proteidae (genus *Necturus* only) and Sirenidae are distributed on the SECP, and each group

diverged from their nearest living relatives in the Mesozoic [22–24]. These families exhibit a suite of aquatic features throughout life (e.g. gill openings) that tie them to freshwater environments, and make them ideal candidates for examining the biogeography and evolution of coastal plain communities. The extant and fossil salamander communities of the coastal plains of North America include multiple related species that span a wide range of body sizes. If body size variation represents niche differences (e.g. trophic niches) then the rich fossil record of aquatic salamanders from the WICP and SECP, as well as the extant diversity on the SECP, also offers the opportunity to examine community structure and reassembly throughout the history of this shifting habitat.

Phenotypic evolution, diversification and historical patterns of community assembly are typically analysed with molecular phylogenies of extant species or the fossil record, but these two data types are rarely merged in single studies. While the utility of fossil taxa (when available) may be limited by incomplete information on diversity and ecology,

they can provide refined estimates of ancestral trait reconstructions, and direct observations of coarse lineage composition and trait variation of ancestral communities [25–27]. In this study, we present a multi-locus nuclear phylogeny and divergence time estimates for all recognized extant species of amphiumids, proteids and sirenids. We placed the North American fossil taxa from these three families and two extinct families (Batrachosauroididae and Scapherpetontidae) on a time calibrated phylogeny, and used it to first reconstruct the origin and diversity of the SECP aquatic salamanders. We then used vertebral and body sizes of modern species to estimate the sizes of extinct species. Body size evolution (as a proxy for niche evolution) was then reconstructed to test the degree of conservation, liability or convergence of these traits between ancestral and modern species, and also to test if size diversification of modern species occurred before or after colonization of the SECP. Finally, we analysed the size structure, lineage composition and patterns of community reassembly during the evolution of this dynamic habitat through time.

2. Methods

(a) Sampling, phylogeny and divergence time estimates

Phylogenetic analyses were based on portions of four nuclear genes: recombination activating gene-1 (*Rag1*, 1410 bp), proopiomelanocortin (*Pomc*, 535 bp), sodium–calcium exchanger 1 (*Ncx1*, 541 bp) and solute carrier family 8 member 3 (*Slc8a3*, 568 bp). Our sampling of 27 taxa included all the recognized species (and most subspecies) of amphiumid, proteid and sirenid salamanders, as well as representatives of the other seven extant salamander families, three divergent frogs and one caecilian. We sequenced nearly all of the proteids, sirenids and several outgroups, but most amphiumid and other outgroup sequences were taken from Genbank [22,28–31] (see the electronic supplementary material, methods S1, tables S1 and S2).

MRMODELTEST v. 2.2 [32] was used to determine the best model of nucleotide substitution for each codon position of each gene (see the electronic supplementary material, table S3). Bayesian estimation of the phylogeny and divergence times based on the four concatenated genes was implemented in BEAST v. 1.6.2 [33]. Divergence time estimation was based on an uncorrelated lognormal molecular clock, a Yule speciation prior and several node calibrations (based on fossils and/or prior age estimates, not mutation rate; electronic supplementary material, methods S2, table S4 and figure S1). We implemented three calibration schemes to test the robustness of our estimates, which were highly consistent across all analyses. These are the first complete species-level divergence time estimates for sirenids and *Necturus*.

Twenty-one pre-Pleistocene fossil taxa (four amphiumids, four batrachosauroidids, one proteid, three scapherpetontids and nine sirenids) were placed (based on their taxonomy and age) onto the chronograms from all three of the divergence time analyses described earlier (see the electronic supplementary material, table S5). Age ranges were based on Paleobiology Database [34] with exceptions noted in the electronic supplementary material, table S6. The Batrachosauroididae and Scapherpetontidae were placed as part of a basal polytomy with salamandroids [35]. We set cladogenetic events between each fossil taxon and its respective stem group at one, three and five million years prior to the age of the earliest specimens on each of the three chronograms. We performed all analyses on the nine alternative chronograms and a consensus chronogram, and all results were highly consistent across trees.

(b) Ancestral area reconstructions

The ancestral distributions of the three extant salamander families were reconstructed using likelihood-based inference in LAGRANGE [36,37]. The analyses were performed on the topology and branch lengths of the nine alternative chronograms that included extant and fossil species (described above), and a consensus of these chronograms. The geographical regions used for our reconstruction were (i) WICP, (ii) SECP and (iii) Eastern Uplands of North America (EU). LAGRANGE also allows taxa and ancestors to be distributed across multiple regions (see figure 1 and electronic supplementary material, table S5).

Our divergence time and biogeographic analyses were primarily used to test between two alternative scenarios for the origin and evolution of extant groups (amphiumids, sirenids and *Necturus*). (i) If within-group divergence occurred after the Eocene (approx. 34 Ma) and the most recent common ancestor of extant species was probably on the SECP, then this would suggest that *in situ* diversification and size evolution gave rise to extant SECP diversity. Alternatively, (ii) if within-group divergences occurred prior to the Oligocene, and/or the most recent common ancestor was probably outside the SECP, then this would indicate that extant SECP diversity of the group (and perhaps size evolution) result from a collection of confamilial lineages that independently colonized the SECP.

(c) Body size estimates and reconstructions

Salamanders are roughly cylindrical from snout to vent, so we estimated body volume ($\pi \times$ axial width/2 $\times$ axial height/2 $\times$ snout to vent length) as a proxy for overall size for all extant species of amphiumids, sirenids and *Necturus*. We partitioned body volumes into three size categories: small (less than 100 ml), large (101–500 ml) and gigantic (greater than 500 ml). This categorization was based on extant species that fit into one of these three size categories, and because discretely small, large and/or gigantic confamilials occur in sympatry (see §3). Most fossil salamanders are only known from isolated vertebrae, so to estimate their body sizes (body volumes), we compared the centrum volumes of more than 200 fossil trunk vertebrae to those dissected from modern species (see the electronic supplementary material, methods S3 and table S7). The body volumes of fossil amphiumids, *Necturus* and sirenids were interpolated from confamilial regression lines. The trunk vertebral counts of the extant families we examined encompasses the variation found in all salamanders (*Necturus* approx. 17, sirenids approx. 40 and amphiumids approx. 60), yet centrum volume is still highly correlated with body volume across these families ($r^2 = 0.90$). Therefore, we interpolated (and extrapolated) the body volumes of extinct families (batrachosauroidids and scapherpetonids, in which trunk vertebral numbers are unknown) from the collective regression line (see the electronic supplementary material, figures S2 and S3). Using the regression lines of any of the three families did not change our categorical estimates of the body sizes of fossil species.

The ancestral body sizes of amphiumids, *Necturus* and sirenids were reconstructed with BAYESTRAITS v. 1.0 (www.evolution.rdg.ac. uk) using 'BayesMulitState' [38] and a reversible-jump Markov Chain Monte Carlo (rjMCMC) model (see the electronic supplementary material, methods S3). The purpose of our body size analysis was threefold. (i) To test for size evolution within each clade with respect to the geographical shift of the coastal plain and patterns of colonization. If size evolution occurred after colonization of the SECP (in endemic SECP clades) then community re-assembly probably occurred through size diversification *in situ*. Whereas, if evolution of extant size diversity occurred prior to the origin or colonization of the SECP, then re-assembly was probably through evolutionary conserved dispersal to the SECP, followed by maintenance of ancestral body size patterns. (ii) Test for significant within clade reversals in body size between

WICP fossil taxa and extant SECP species. Bayes factors (BF) were used to determine whether ancestral state estimates from unconstrained analyses were significantly better than analyses in which ancestral states were 'fossilized' (fixed) at alternative states. BF > 2 is 'positive evidence' and BF > 5 is 'strong evidence' against the alternative hypothesis (state) [38]. This was performed for the most recent common ancestors of each extant species group, which occurred chronologically between each potential size reversal. (iii) To compare how each clade (family) contributed to local size variation through time, to determine whether clades show incumbency for a given body size (niche), whether major episodes of body size shuffling have occurred among clades and whether these patterns are related to the regional shift of the North American coastal plain.

3. Results

(a) Phylogeny, divergence time estimates and biogeography

Phylogenetic analyses based on four nuclear genes (*Rag1*, *Pomc*, *Ncx1* and *Slc8a3*) showed strong support for almost all nodes (Baysian Posterior Probabilities = 1) and divergence times were highly consistent among calibration strategies (see the electronic supplementary material, table S8 and figure S1). We estimate the deepest divergences within SECP endemic clades (amphiumids, sirenids and a subclade of *Necturus*) were no older than the Miocene, indicating that diversification of these groups occurred *in situ*.

The deepest divergence within extant amphiumids is less than 3.5 Ma. Therefore, the fossil amphiumids *Proamphiuma cretacea* (Late Cretaceous) and *Amphiuma jepseni* (Palaeocene) from the WICP, and *Amphiuma antica* (Early Miocene) and *Amphiuma* sp. (Early Pliocene, 4.5 Ma) from the SECP, probably diverged prior to the most recent common ancestor of the extant species. The distribution of Late Mesozoic and Early Cenozoic amphiumids was probably on the WICP of North America ($p = 0.71$), and the most recent common ancestor of all extant and extinct Neogene amphiumids occurred on the SECP by the Middle Miocene ($p \geq 0.92$, figure 1; electronic supplementary material, figure S4 and table S9).

We found strong support for two clades of extant sirenids (*Siren* and *Pseudobranchus*), which diverged in the Middle Miocene (approx. 15 Ma). *Habrosaurus* (Late Cretaceous to Palaeocene) and *Siren dunni* (Eocene) from the WICP, and *Siren* sp. (Early Oligocene of Florida) and *Siren hesterna* (Early Miocene) from the SECP existed well before the diversification of extant sirenids. The ancestral distribution of early fossil sirenids was probably the WICP ($p = 0.68$). Middle Miocene *Siren* from Texas and Louisiana (*Siren miotexana*), and Nebraska (tenuously identified as *Siren* sp.) occurred around the time of the divergence of extant species. Nevertheless, the common ancestor of extant sirenids probably occurred on the SECP ($p = 1.0$), and this result is highly robust regardless of the placement of Middle Miocene taxa. It is also notable that the presence of fossil *Siren* from the Eocene and Early Miocene shows that the genus *Pseudobranchus* (dwarf sirens) is phylogenetically nested within the genus *Siren*, which has major implications for understanding biogeography and size evolution of this family (see §4).

We found moderate to strong support for two clades of extant *Necturus* that diverged in the Late Miocene (approx. 5 Ma).

Therefore, *Necturus krausei* (Palaeocene) from the WICP predates the diversification of the clade, including modern species by at least 50 million years (figure 1). Based on the limited material for this group, we estimate the Early Cenozoic ancestral area of the genus was probably the WICP ($p = 0.49$) followed by EU and SECP by the Late Miocene. There is a high likelihood of at least one strictly endemic SECP clade that includes *Necturus lewisi* and *Necturus punctatus* ($p = 0.96$) on the Atlantic Coastal Plain.

Fossils of the batrachosauroidid *Opisthotriton* are common on the WICP from the Late Cretaceous through the Palaeocene [34]. A related genus, *Batrachosauroides*, is known from the Eocene of the WICP, and Miocene of the SECP, but not beyond. Batrachosauroidids probably dispersed from the WICP to the SECP by the Early Miocene. Three genera of scapherpetonids (*Lisserpeton*, *Piceoerpeton* and *Scapherpeton*) are known from the Late Cretaceous to Early Cenozoic of the WICP, and probably did not colonize the SECP.

(b) Body size evolution

Body size is highly variable within each extant family, including at least one significant size shift in endemic SECP clades (see figure 2 and electronic supplementary material, figure S4 and table S10). There is also evidence of at least one significant size reversal between members of each of the extant SECP clades with their WICP ancestors.

Late Cretaceous (*P. cretacea*) and Palaeocene (*A. jepseni*) amphiumids from the WICP were small, whereas *Amphiuma* from the Miocene and Early Pliocene of the SECP are large to gigantic. Two extant species (*Amphiuma tridactylum* and *Amphiuma means*) are more than 50 times larger than their WICP ancestors, while the third species (*Amphiuma pholeter*) is small. We estimate that the most recent common ancestor of extant amphiumids was gigantic ($p = 0.91$; figure 2) and demonstrate strong evidence of a recent reversal from gigantic to small size on the SECP (BF = 7.84 versus small; BF = 6.76 versus large). Sirenids exhibit an opposite trend, where WICP species from the Late Cretaceous and Palaeocene (*Habrosaurus*) are gigantic, but all known fossil *Siren* from the Eocene through the Late Miocene are small or only slightly large; up to 45-fold smaller than *Habrosaurus*. Likewise, we estimate that the most recent common ancestor of extant SECP sirenids was small ($p = 0.73$; BF = 8.74 versus gigantic; BF = 3.62 versus large), which indicates recent secondary size increases, including the re-evolution of gigantism. The pre-Pleistocene fossil record of *Necturus* is limited to two small but well-ossified Palaeocene vertebrae from the WICP [39]. We estimate that the Late Miocene ancestor of extant *Necturus* was large ($p = 0.68$; BF = 2.02 versus small; BF = 5.70 versus gigantic), and there was at least one significant body size shift among endemic SECP *Necturus* (size reduction in *N. punctatus*). If Early Cenozoic *Necturus* are indeed small then this would represent another, less extensive but significant, body size reversal from large to small size.

Several gigantic genera/families have gone extinct; especially batrachosauroidids and scapherpetonids. We estimate that the batrachosauroidid *Opisthotriton* (Late Cretaceous through Palaeocene) was marginally large. A related genus *Batrachosauroides* (Eocene through Miocene) was gigantic, but phylogentically nested among much smaller species (*Paleoproteus*, *Opisthotriton* and *Prodesmodon* [40]). Therefore, *Batrachosauroides* probably evolved gigantism on the WICP
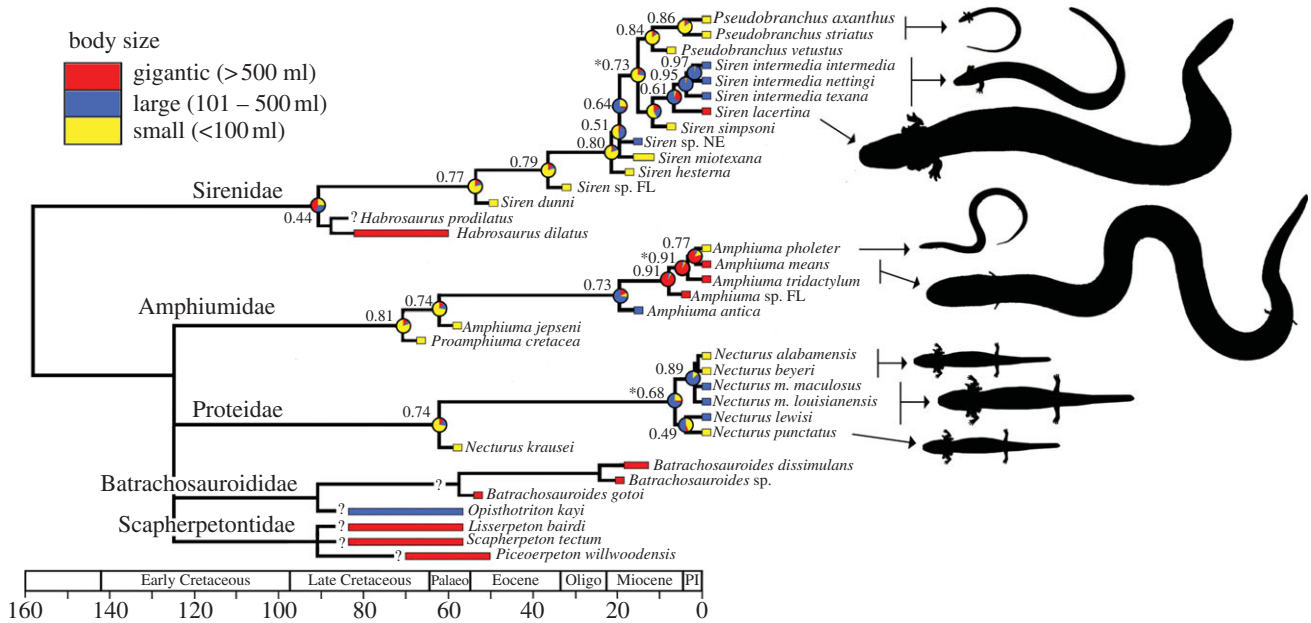
Figure 2. Body size reconstruction of aquatic coastal plain salamanders of North America. The ancestral body size reconstruction is based on *Bayemultistate* analysis in BAYESTRAITS for three categorical size classes (small, large and gigantic). Pie diagrams at each node show the probability of each state and the subtending number is the highest (see the electronic supplementary material, table S10). The consensus chronogram is the same as shown in figure 1. The most recent common ancestor of each extant group is indicated with an asterisk.

in the Early Cenozoic and this size persisted onto the SECP. The scaherpetonids, *Lisserpeton* and *Scapherpeton* (Late Cretaceous through Palaeocene) were also gigantic. *Piceoerpeton* (Late Cretaceous through Eocene) were 'super giants' and may have been one of the largest known salamanders; with a body volume of more than 7 l they were at least two times larger than the extant giants (e.g. *Siren lacertina* and *A. means*).

(c) Community composition and body size shuffling

The aquatic salamander diversity from the Late Cretaceous through Palaeocene of the WICP is composed of at least five families (Amphiumidae, Batrachosauroididae, Proteidae, Scapherpetonidae and Sirenidae). The deposit with the highest known diversity is the Bug Creek Anthills locality of Montana (Hell Creek Formation, Late Cretaceous) and includes at least one small (*Proamphiuma*), one slightly large (*Opisthotriton*) and four gigantic (*Habrosaurus*, *Scapherpeton*, *Lisserpeton*, *Piceoerpeton*) species (see figure 3 and electronic supplementary material, table S11) [34,41]. At least a portion of this assemblage (especially *Opisthotriton*, *Habrosaurus* and *Scapherpeton*) is common throughout the WICP from the Late Cretaceous through the Palaeocene [34]. Subsequently, amphiumids and batrachosauroidids show dramatic increases in body size, whereas sirenids shrunk. *Batrachosauroides* are the primary gigantic aquatic salamanders of the SECP during the Early and Middle Miocene, where they co-occur with small *Siren* [21,34]. *Batrachosauroides* are not known beyond the Middle Miocene, and body size diversification of the three surviving families (amphiumids, sirenids and proteids) occurs on the SECP from the Late Miocene through the Pleistocene. Major body size shifts, in at least batrachosauroidids and sirenids, did not immediately correspond to the regional shift of the WICP to the SECP (Eocene/Oligocene), but rather occurred much earlier (Paleoene/Eocene) or much later (Middle Miocene) within each of these regions (discussed below).

Current SECP communities vary regionally in lineage composition, and the contribution of each lineage to local size variation. A common assemblage includes at least one gigantic amphiumid, one small and/or one large proteid, and one large sirenid. *Necturus* are currently absent from peninsular Florida, which is largely dominated by communities, including three sirenids (small, large and gigantic) and one amphiumid (gigantic). The highest local diversity is five species in a community that also includes a small-bodied amphiumid (*A. pholeter*) on the northeastern Gulf Coastal Plain. The size distributions of modern SECP communities show similarities with those from the Late Cretaceous to Palaeocene from the WICP, but are assembled from either different families or through the re-evolution of body size within families (figure 3).

4. Discussion

(a) Cenozoic biogeography of North American coastal plains

We found that divergence times within three extant groups of aquatic salamanders (amphiumids, sirenids and *Necturus*) are no older than the Middle Miocene. We also show that the SECP was the probable ancestral area for the most recent common ancestors of extant amphiumids, extant sirenids and one subclade of *Necturus*, each of which colonized the SECP prior to the close of the Miocene and subsequently diversified *in situ*.

Where did the SECP diversity originate? The distributions of extant and fossil *Amphiuma*, *Siren*, *Necturus* and *Batrachosauroides* each provide direct congeneric connections between the Early Cenozoic WICP and the Late Cenozoic SECP. Our ancestral area reconstructions suggest that the ancestors of *Amphiuma*, *Siren* and *Batrachosauroides* dispersed from the WICP (before it became completely arid at the close of the Eocene [12,42]) to the emerging Oligocene/Miocene SECP [11]. It is also possible that undiscovered fossil taxa more closely related to extant lineages occurred on the
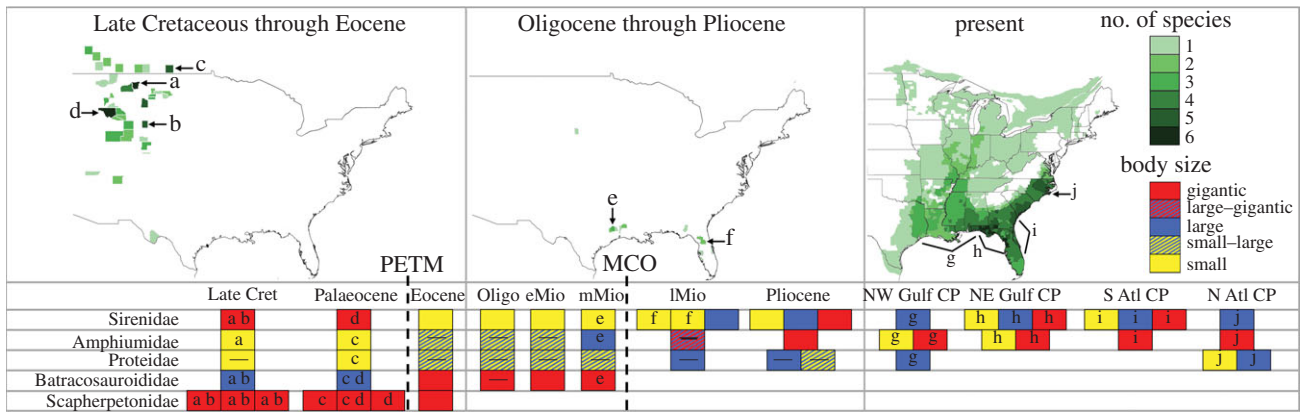
Figure 3. Aquatic coastal plain salamander diversity through time. Maps show levels of regional diversity for three broad time increments (Late Cretaceous through Eocene, Oligocene through Pliocene and Present). United States occurrences are based on county-level distributions, and Canadian occurrences are based on approx. 100 km sections (data are from Paleobiology Database [34] and IUCN Red List, with corrections). Nearly, all regional diversity pools are from the same age without repeats. Below, shows the size distributions and community composition through time for each family (one block per species). Letters in blocks signify occurrences from representative formations/locations for fossil taxa, or regions for extant species: (*a*) Hell Creek Form., Bug Creek Anthills; (*b*) Lance Form., Bushy Tailed Blowout; (*c*) Ravenscrag Form., Roche Percée; (*d*) Torrejonian., Rock Bench Quarry; (*e*) Fleming Form., Barringer Farm; (*f*) Alachua Form., Haile 6a; (*g*) northwestern Gulf Coastal Plain; (*h*) northeastern Gulf Coastal Plain; (*i*) southern Atlantic Coastal Plain; (*j*) Neuse River., northern Atlantic Coastal Plain. Details are provided in the electronic supplementary material, table S11. Hashed coloured blocks show transitions between sizes. Dashes in blocks signify that a species presence and size are inferred. Coloured, but empty (unlettered) blocks are based on fossil that occur at one or more sites, but without other known aquatic salamanders present. eMio, mMio and lMio refer to Early, Middle and Late Miocene. Dashed vertical lines indicate the Palaeocene–Eocene Thermal Maximum (PETM) and Miocene Climatic Optimum (MCO), which are coincident with major extinctions and episodes of size shuffling.

unsubmerged margins of the Palaeocene/Eocene SECP, or other proximal regions of eastern North America that are depauperate of Cenozoic strata. Nevertheless, extant SECP aquatic salamander communities include three endemic clades and are significantly younger than ancestral WICP communities.

(b) Body size evolution and shuffling of coastal plain salamander communities

Body size is often a primary axis of evolution in adaptive radiations [43]. This is probably because size can evolve at a rapid rate and can have direct effects on niche differentiation and reproductive compatibility among even close relatives [44–46]. Body size has been a major axis of change in amphiumids, batrachosauroidids, proteids and sirenids, as each family shows one or more distinct size shifts since the Early Cenozoic. These include well-supported secondary size reversals in recent amphiumids and sirenids, and potentially a third within *Necturus* (figure 2). The recent size shifts within all three extant families have resulted in sympatric confamilials that are divergent in size. Assuming that size differences correspond to divergence in niche parameters (e.g. trophic level) then each of the extant families show signatures of *in situ* adaptive divergence on the SECP. Furthermore, some environmental niche parameters (e.g. low elevation, aquatic habitat and warm temperature) may generally be conserved in these families, but the frequent size shifts suggest that size-related niche parameters have been highly dynamic and may underlie diversification. This is analogous to patterns seen in other salamanders (e.g. Appalachian plethodontids) in which many clades exhibit niche conservatism for climatic variables [47], but trophic level and habitat type are clearly dynamic traits in some groups [45,48].

Preoccupation of a niche (incumbency) can provide a foothold that inhibits the colonization, or evolution of ecologically similar species in sympatry [8]. However, incumbent advantages can be overcome by environmental changes (even brief events) that result in extinctions or severe reductions in incumbent populations [9,10]. In the same way, the formation of a refuge in a new region can provide empty habitat for at least initial stochastic community reassembly [3–5]. Coastal plain aquatic salamander communities show at least two major episodes of size order shuffling since the Cretaceous (figure 3), but these appear to have occurred within the WICP and the SECP, rather than directly from the regional shift of the coastal plain. The first major size shifts occurred on the WICP across the Palaeocene–Eocene boundary when previously common gigantic sirenids (*Habrosaurus*), gigantic scapherpetonids (*Lisserpeton* and *Scapherpeton*) and somewhat large batrachosauroidids (*Opisthotriton*) disappear from the fossil record. This is followed by the appearance of gigantic batrachosauroidids and small sirenids on the WICP in the Eocene. This turnover is coincident with the initiation of the Palaeocene–Eocene Thermal Maximum (PETM), which was a major period of floral and faunal extinction and migration on the WICP [49,50]. Batrachosauroidids and sirenids probably maintained their respective sizes through colonization of the SECP and until at least the Early to Middle Miocene. Amphiumids and *Necturus* also increased in size between the Palaeocene and Miocene, but it is not clear whether these initial changes occurred before or after the colonization of the SECP. The second major episode of size shifts occurred on the SECP in the Middle Miocene and is coincident with the increased temperature during the Miocene Climatic Optimum (MCO) [49]. This period marks the last known gigantic *Batrachosauroides* followed by at least one significant size shift in all three of the remaining families from the Late Miocene through the Pleistocene (figure 3). The recent radiation of these families minimally included the reversal to gigantic (and large) sirenids, and secondary size reductions to small *Amphiuma* and *Necturus*. The frequent size evolution and shuffling of aquatic coastal plain salamanders suggests that any incumbent advantages for a given niche (size) were often overcome. This is most evident for

gigantic taxa that were frequently lost and replaced. The vulnerability of freshwater coastal plain habitats (to marine incursion or desiccation), combined with the potential for rapid size evolution (or migration), may have facilitated the body size shuffling and reorganization of aquatic coastal plain salamander communities.

Incumbency may have at times contributed to the local structure of aquatic coastal plain salamander communities, although we note that both extant and ancestral communities commonly show co-occurrence of similar-sized species (figure 3). Based on body size reconstructions of extant species, Kozak *et al*. [51] showed evidence of parallel size increases in sympatry for clades of stream-dwelling plethodontid salamanders in the Appalachian Mountains. They also reviewed the potential for parallel convergence occurring in sympatry with (and without) additional niche differences. Based on fossil distributions and ancestral size reconstructions, we show that large and gigantic *Siren* evolved on the SECP while either large or gigantic *Amphiuma* were already present in this region. Gigantic *S. lacertina* and *A. means* are predatory generalists that occur in broad sympatry. However, small isolated wetlands often include only one of the species [52], and habitat partitioning has been shown where they do co-occur [53]. Similarly, small sirenids have been continuously present on the SECP since the Oligocene, yet amphiumids and proteids both recently evolved small body sizes in this region. Even though some small species (*A. pholeter*, both *Pseudobranchus* and *N. punctatus*) occur in close proximity, none co-occur in the same type of aquatic habitat. In general, habitat characteristics (e.g. aquatic vegetation, substrate and flow) are more similar among confamilials [54], and recent body size evolution in most cases has produced scaled-up or scaled-down versions of otherwise morphologically and ecologically congruent organisms. The inherent ecological differences of each family may have allowed for multiple parallel instances of size evolution (gigantic and small) with limited spatial or ecological overlap.

(c) Integrating molecular phylogenies and fossils to study community evolution

Historical perspectives, viewed through a phylogeny and/or the fossil record, can be critical for understanding patterns of community assembly [5,25]. Ideally, the integration of fossil taxa onto molecular-based chronograms can improve such studies in a few important ways. (i) Chronograms based on molecular data can clarify the origin and evolution of some fossil taxa. For example, our molecular phylogeny shows that *Pseudobranchus* arose on the SECP in the Middle Miocene and is phylogenetically nested among *Siren* (fossil and extant). Based on fossils alone one would assume that these genera diverged minimally in the Early Eocene (the earliest fossil *Siren* from the WICP), which would then imply (incorrectly) that extant SECP sirenid diversity arose from multiple colonizations of the SECP rather than from a more recent *in situ* radiation. (ii) Fossil taxa can improve the estimation of ancestral states and rates of change for some traits [26,27]. The estimated size of the most recent common ancestor of extant sirenids is highly ambiguous without the incorporation of fossil taxa (see figure 2 and electronic supplementary material, table S10). (iii) Fossil taxa can also provide direct information on the origin and extinction of lineages, and the composition of lineages and trait distributions of ancestral communities. Such patterns may then be compared with data from extant species to understand the dynamics of trait and community evolution through space and time.

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