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MOSAICISM IN A NEW EOCENE PUFFERFISH HIGHLIGHTS RAPID MORPHOLOGICAL INNOVATION NEAR THE ORIGIN OF CROWN TETRAODONTIFORMS

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Abstract: Tetraodontiformes (pufferfishes and kin) is a taxonomically and structurally diverse, widely-distributed clade of acanthomorphs, whose members often serve as models for genomics and, increasingly, macroevolutionary studies. Morphologically disparate Palaeogene fossils suggest considerable early experimentation, but these flattened specimens often preserve limited information. We present a three-dimensionally preserved beaked tetraodontiform from the early Eocene (c. 53 Ma) London Clay Formation, UK. Approximately coeval with the oldest crown tetraodontiforms, †Ctenoplectus williamsi gen. et sp. nov. presents an unprecedented combination of characters, pairing a fused beak-like dentition with prominent dorsal-fin spines that insert transversely-expanded pterygiophores roofing the skull. Bayesian total-evidence tip-dating analysis indicates that †Ctenoplectus represents the sister lineage of Triodontidae and highlights considerable levels of homoplasy in early tetraodontiform evolution. According to our dataset, rates of morphological character evolution were elevated at the origin of crown Tetraodontiformes, especially within gymnodonts, but declined after the principal body plans were established. Such 'early burst' patterns are regarded as a hallmark of adaptive radiations, but are typically associated with diversification at smaller spatiotemporal scales. However, denser sampling of Neogene and Recent taxa is needed to confirm this pattern.

Key words: Tetraodontiformes, London Clay, Eocene, Bayesian phylogenetics, rates of evolution.

Tetraodontiformes (430 species, 10 families) are globally distributed and inhabit a broad spectrum of environments, including coastal, reef, open-water pelagic, deep-sea, and freshwater habitats (Tyler 1980; Yamanoue et al. 2011; Matsuura 2014). Adult body masses span six orders of magnitude (Tyler 1980; Britz & Kottelat 1999), and the clade exhibits a remarkable variety of adaptations for defence, both mechanical and behavioural (Tyler 1980; Brainerd 1994). Because of their high diversity, wide environmental distribution, small, accessible genome and well-understood intrafamilial phylogenetic relationships, tetraodontiforms have been adopted as model organisms for genomic research (Brenner et al. 1993; Crno gorac-Jurcevic et al. 1997; Yamanoue et al. 2009), and are increasingly targeted by studies of macroevolution and biodiversity (Alfaro et al. 2007; Yamanoue et al. 2011; Santini et al. 2013).

Morphological classifications (Winterbottom 1974; Tyler 1980; Santini & Tyler 2003, 2004) divide tetraodontiforms into the Balistoidei or Sclerodermi (Cuvier 1817), which retain jaws with discrete, unfused teeth, and the Tetraodontoidei or Gymnodontes, in which teeth are fused with the jaws to form a parrot-like beak (Tyler 1980; Andreucci et al. 1982; Britski et al. 1985). Extant beaked families comprise the Triodontidae (three-tooth puffers), Tetraodontidae (four-tooth puffers), Diodontidae (porcupinefishes)
and Molidae (molas), while the Triacanthodidae (spikefishes), Triacanthidae (triplefin families), Balistidae (triggerfishes), Monacanthidae (filefishes), Aracanidae (deep-water boxfishes) and Ostraciidae (boxfishes or trunkfishes) represent the non-beaked families. This division has been challenged by recent molecular phylogenies presenting multiple conflicting topologies (Holcroft 2005; Alfaro et al. 2007; Yamanoue et al. 2007, 2008; Near et al. 2012, 2013; Santini et al. 2013). However, a recent total-evidence analysis (Arcila et al. 2015) has found that the inclusion of morphological character data resolves gymnodonts as a monophyletic group, albeit one nested within a paraphyletic ‘Sclerodermi’.

Disagreements about interfamilial relationships are partially attributable to the paucity of intermediate forms available to bridge morphological gaps between major lineages. Extant tetraodontiforms are highly specialized, and novel data from extinct species may prove informative. Fortunately, the tetraodontiform fossil record is rich and well-studied. The earliest unambiguous crown-group members are Thanetian–Ypresian in age (Paleocene–Eocene), but almost exclusively consist of highly-compressed body fossils and isolated dentitions (Tyler & Santini 2002; Gallo et al. 2009).

To date, only a single three-dimensionally preserved skull has been described from this important interval of tetraodontiform history: an incomplete specimen from the early Eocene (Ypresian, c. 53 Ma) London Clay Formation of England assigned to the extant genus Triodon, which delivers few insights beyond those provided by living taxa (Tyler & Patterson 1991). Here, we present the skull and paired-fin girdles of a new beak-toothed tetraodontiform from the London Clay Formation, found in 1992 at Aveley, Essex, by R. J. Williams (briefly noted, but not described, in Williams 2002). Although an initial attempt at preparation and study was made in 1992 by C. Patterson and one of us (JCT), insufficient anatomical information could be exposed. Now, however, x-ray micro-computed tomography (µCT) allows us to fully reveal its unique morphology. Unlike the similarly-preserved material assigned to Triodon, this fossil exhibits a remarkable combination of primitive and derived characters, as well as striking autapomorphies with no clear precedent among tetraodontiforms.

This new fossil joins a series of other peculiar and approximately coeval crown tetraodontiforms from the Bolca Lagerstätte and the former USSR (Tyler & Santini 2002) that cannot be placed within extant families. Interpreted at face value, these highly disparate early forms suggest an explosive morphological divergence mirroring that hypothesized for Acanthomorpha as a whole (Friedman 2010). Younger molecular age-estimates for the origin of crown tetraodontiforms (c. 59 Ma; Alfaro et al. 2007) are consistent with rapid early diversification, but more ancient estimates (as early as 185 Ma; Arcila et al. 2015) imply long intervals of missing tetraodontiform history over which anatomical innovations could have slowly accumulated. Here, we use a Bayesian total-evidence tip-dating analysis incorporating a sampled-ancestor fossilized birth–death (SA-FBD) model of tree evolution and fossil sampling (a methodological innovation that may address the tendency for tip-dating methods to yield implausibly ancient divergence dates) to simultaneously estimate rates of both molecular and morphological character evolution across the tetraodontiform tree.

MATERIAL AND METHOD

Geological setting

The material presented here was collected from the London Clay Formation, Division B2 (King 1981; Williams 2002), ‘No. 2 pit’ of ‘Greenways’ landfill site at Sandy Lane, Aveley, Essex RM15, UK, OS grid reference TQ 556 809; Ypresian, based on a variety of microfossil and macrofossil assemblages (King 1981; Ali & Jolley 1996). The deposits represent a subtropical deeper-water, low-energy marine environment that lay at least 80 km from the shoreline (Friedman et al. 2015).

Initial preparation and study of specimen

The specimen was donated to the then British Museum (Natural History), where Colin Patterson oversaw sufficient mechanical preparation to realize that it represented a gymnodont with a complex mosaic of primitive and advanced characters. In late 1992, Patterson brought the specimen to the Smithsonian Institution in Washington to work on it with one of us (JCT). Additional mechanical preparation was performed, but was curtailed for fear of damaging the skull bones. Ordinary radiographs were taken, but only the more superficial features of the specimen were revealed. The decision was then taken to put this interesting taxon aside until more could be determined about its morphology via additional material or technological advances.

X-ray tomography

NHMUK P63336 was scanned using micro computed tomography (µCT) at the Imaging and Analysis Centre (IAC) of the Natural History Museum, London, UK. Two scans were made, one of the whole specimen (energy: 200 kV; current: 200 mA; voxel-size: 50.4 µm; filter: 2.5 mm Cu; dimensions: 1550 × 1072 × 1919 pixels) and
one higher-resolution scan of the jaws (energy: 205 kV; current: 165 mA; filter: 0.5 mm Sn; voxel-size: 17.8 μm; dimensions: 1401 × 2000 × 1960). Tomographic segmentation was carried out in MIMICS 15.01 (Materialise, Leuven, Belgium), while volume-rendered visualisations were performed in DRESiTTI 2.0 (Limaye 2012). Surface models were rendered in BLENDER 2.7 (http://www.blender.org).

Phylogenetic analyses

Morphological data. NHMUK P63336 was included in the most complete morphological character matrix for Tetraodontiformes published to date, that of Santini & Tyler (2003; also used by Arcila et al. 2015). Fossil taxa described after the publication of Santini & Tyler's (2003) morphological matrix are currently being incorporated into a revised matrix by one of us (JCT), but this dataset is not yet available for downstream analyses. The matrix comprises 219 characters coded for 59 taxa, including †Ctenoplectus (Close et al. 2016). However, characters 211–219 were excluded as they were deemed to be overly ambiguous by Santini & Tyler (2003). The zeiform Cyttus novaezelandiae and caproid Antigonia capros are included as outgroups (though see note about Cyttus under Molecular Data, below).

Molecular data. With the exception of Zeus faber and Kentrocapros rosapinto, every extant taxon in our total-evidence analysis was paired with sequence data for 20 nuclear and 2 mitochondrial loci. This dataset is very similar to that previously analysed by Santini et al. (2013) and Arcila et al. (2015). With the two exceptions noted above, sequences were originally generated by Santini et al. (2013) and were downloaded from GenBank (see Santini et al. 2013, table 1, for details and accession numbers). A subset of eight of these loci were available for Kentrocapros rosapinto, a taxon not sampled by Santini et al. (2013), and as adequate sequence data for Cyttus was not available on GenBank, we instead used sequence data representing 18 loci from Zeus faber, such that this outgroup represents a composite zeiform taxon (additional sequences also downloaded from GenBank; for accession numbers, see Close et al. 2016, table 1). Sequences were aligned manually using se-al v. 2.0 (http://tree.bio.ed.ac.uk/software/seal/).

Bayesian MCMC phylogenetic analysis. We performed a Bayesian relaxed-clock phylogenetic analysis using BEAST 2.3.0 (Bouckaert et al. 2014). We used a total-evidence tip-dating approach that combines morphological and molecular data with stratigraphical information (in the form of tip-age distributions for terminal taxa) to jointly estimate topology, branch lengths, lineage-specific rates of morphological and molecular evolution and divergence dates under a sampled-ancestor fossilized birth–death (SA-FBD) tree prior. The SA-FBD tree prior explicitly models the diversification, fossilisation and sampling processes, and allows fossil taxa to be placed either as terminals or on branches as direct ancestors (Heath et al. 2014; Gavryushkina et al. 2015). We elected to use the SA-FBD tree model in our tip-dating analysis in order to examine the effect that it might have on the antiquity of the inferred divergence dates (the recent tip-dating study of Arcila et al. (2015), employing a simple birth–death tree model, yielded implausibly ancient origins for the group). Preliminary results from total-evidence tip-dating analyses employing the SA-FBD tree model (Gavryushkina et al. 2015) suggest that it may yield considerably younger divergence estimates.

We used PARTITIONFINDER 1.1.1 (Lanfear et al. 2012) to optimally partition the molecular sequence data and select appropriate settings for nucleotide substitution models, invariant sites and gamma-distributed rate variation (Close et al. 2016, table 2). We partitioned the morphological data according to number of character states present (the preferred strategy for partitioning morphological data in BEAST 2) and specified the Mk + Gamma model (Lewis Mk model conditioned on the absence of invariant or autapomorphic characters) for unordered characters for all partitions. The treelikelihood was set to treat character-state ambiguities as equally-likely states for morphological data only; for sequence data, ambiguities were treated as unknowns (the default in BEAST 2, as treating ambiguities as equally-likely states makes the calculations twice as slow). Clock models were unlinked between morphological and molecular partition blocks, with separate uncorrelated lognormal relaxed-clock models specified for each. This allowed rates of morphological and molecular character change to be independently estimated. The number of discrete rate categories was set to equal the number of branches in each tree. Although we performed an additional set of analyses using a single clock for all partitions and data types, they did not reach stationarity for key parameters linked to rates, most likely due to substantial conflict in rate patterns between morphological and molecular datasets. The SA-FBD model was conditioned on rho sampling (i.e. that there would be at least one extant taxon present), with the parameter rho (probability of sampling extant taxa) being set to 0.066 to reflect the proportion of all known extant tetraodontiform taxa included in the analysis. In order to avoid unidentifiability issues when inferring the clock rate, the mean substitution rate was separately fixed within all morphological and all molecular partitions.

Tip ages for fossils were sampled from uniform distributions defined by first and last occurrence dates obtained from the literature (Close et al. 2016, table 3). A starting
tree was obtained by analysing the data in MRBAYES 3.2.6 without node-age constraints or tip ages (starting tree embedded in BEAST XML file; see Close et al. 2016, data file 1). In order to hasten convergence of the analysis, we created taxon sets to enforce the monophyly of uncontroversial groupings, such as crown Tetraodontiformes, and various familial memberships within the crown (Close et al. 2016, table 4). A taxon set for Gymnodontes was also specified, but monophyly not enforced. This permitted us to observe the proportion of trees in the posterior in which beak-toothed puffers were found to be monophyletic. In accordance with the recommendations of O’Reilly et al. (2015), we incorporated some node-age calibrations into our tip-dating analysis. Firstly, we specified a node-age prior on the root corresponding to the mean of means drawn from all available published divergence-date estimates for Acanthomorpha (Close et al. 2016, table 5). Secondly, the node-age prior for crown Tetraodontiformes was specified as the mean of means drawn from all available published divergence-date estimates for Tetraodontiformes (Close et al. 2016, table 6).

To ensure that the analysis had reached stationarity, four replicates of the analysis were run (400 million generations, sampling every 100 000 generations) and the resulting posteriors checked for convergence using TRACER 1.6 (Rambaut et al. 2014) and AWTY (Nylander et al. 2008). The maximum sampled-ancestor clade credibility tree (MSACC) tree was calculated using TREE ANNOTATOR using mean node heights.

Institutional abbreviation. NHMUK, Natural History Museum, London, UK.

SYSTEMATIC PALAEONTOLOGY

This published work, and the nomenclatural acts it contains, have been registered in ZooBank: http://zoobank.org/References/8A087529-208D-4BA3-A1A1-B52BD57F0378

Acanthomorpha Rosen, 1973
Tetraodontiformes Berg, 1940
Tetraodontoidei Tyler, 1980
Genus †CTENOPLECTUS nov.

LSID. urn:lsid:zoobank.org:act:9FFBD06D-87D6-465B-A3B0-7E30A4A240A6

Derivation of name. The generic name combines the Greek root Cteno-, meaning ‘comb,’ and the Greek root plectos, meaning plaited or twisted, in reference to the firm, inflexible articulation of the jaw bones by which Cuvier diagnosed ‘Plectognathi,’ the original name for Tetraodontiformes.

Type species. †Ctenoplectus williamsi sp. nov.

Diagnosis. Tetraodontiform fish displaying the following unique combination of characters: series of greatly enlarged, broad, plate-like dorsal-fin spines and expanded, flattened supporting proximal radials inserting far anteriorly along the skull roof; proximal radials with posterodorsal shaft overlapped by previous proximal radial; paired premaxillae and dentaries fully fused across their midlines, lacking sutures, anteriorly projecting bulge of teeth associated with fused premaxillae; tooth roots elongate with pointed tips; post-temporals present; well-developed, rodlike pelves; pleural ribs present; anterior abdominal vertebras with prominent transverse processes.

†Ctenoplectus williamsi gen. et sp. nov.

Figures 1–3

LSID. urn:lsid:zoobank.org:act:11ACD1DC-37C5-4D6B-AA63-1DC51628CEAB

Derivation of name. The specific name honours R. J. Williams, who discovered the specimen.

Holotype. NHMUK PV P. 63336 (Fig. 1), Natural History Museum, London, UK.

Diagnosis. As for genus.

Description

NHMUK PV P. 63336 (preserved in clay nodule, broken into two halves along cleithrum) preserves a partial skull in articulation with pectoral and pelvic girdles, three vertebrae and one rib (Figs 1, 2; Close et al. 2016, fig. 1). The head is bilaterally compressed, reflecting genuine proportions rather than taphonomic distortion; material from the London Clay is generally uncompressed. The skull is slightly deeper than it is long. Most bones have a striated appearance resulting from weak ossification, a character reminiscent of molids (Tyler 1980).

Upper and lower jaws are well preserved and largely complete (Fig. 2). Paired premaxillae and dentaries are completely fused with their associated dentitions to form a parrot-like beak. Both beaks are slightly recurved. The dentary terminates in a sharp point, but the tip of the upper beak is damaged. An anteriorly-projecting bulge reinforces the premaxillary symphysis (Fig. 3A). There does not appear to be any evidence for tritural pads. †Ctenoplectus shares coalescent dentition with extant gymnodont families and a range of fossil taxa, including the Palaeogene genera †Eoplectus (Tyler 1973), †Zignoichthys (Tyler 1973), †Uranoplectus (Tyler et al. 2006) and †Eotetraodon (Tyler 1980), which feature numerous primitive features relative to extinct
**FIG. 1.** †Ctenoplectus williamsi gen. et sp. nov. A, anterior view of holotype (NHMUK PV P. 63336). B, dorsal view. C, right-lateral view. D, left-lateral view. Scale bar represents 10 mm. **Abbreviations:** af, articulation facets; bst, branchiostegals; bu, burrow; ch, ceratohyal; cl, cleithrum; cor, coracoid; d.pcl, dorsal postcleithrum; den, dentary; dfs, dorsal-fin spine; fr, frontal; Leth, lateral ethmoid; mpg, meso/metapterygoid; mx, maxilla; ns, neural spine; op, opercle; pal, palatine; pel, pelvis; pfr, pectoral-fin rays; pmx, premaxillae; pop, preopercle; psptg, posterior shaft of the pterygiophore; ptg, pterygiophore (= proximal radial); ptt, post-temporal; qu, quadrate; rb, rib; sca, scale; scl, supracleithrum; sop, subopercle; tp, transverse process of the vertebra; v.pcl, ventral postcleithrum; ver, vertebra.
gymnodonts. Among these, only †Eoplectus bears a well-developed spiny dorsal-fin, but this occupies a more typical postcranial position. In terms of gross morphology, the beak of †Ctenoplectus most closely resembles that of the extant Triodon and, to a lesser extent, tetraodontids: both jaws are similarly-proportioned, being approximately equal in length and width, and form acute arrow-head shapes in dorsal/ventral views. By contrast, the beaks of molids and diodontids are proportionately wider, forming obtuse triangles in dorsal/ventral views, and often have straight cutting or crushing edges that sometimes taper anteromedially to a sharp point. In †Eoplectus (Tyler 1973), a primitive postcranial anatomy resembling that of triacanthodids is combined with teeth that are fully incorporated into the bony matrix to form a beak, though as in tetraodontids, neither the upper nor lower paired jaw-elements are fused. In triodontids, only the dentaries are indistinguishably fused, while in diodontids and, with occasional individual exceptions, in molids, the paired premaxillae and dentaries are fully fused (Tyler 1980). Although lost in certain tetraodontids and diodontids (Tyler 1980), tritural pads are found in all extant gymnodont families and, barring their post-mortem loss, their absence in †Ctenoplectus appears to be yet another departure from the dentition of other beak-toothed puffers.

μCT resolves fine internal structure of the jaws of †Ctenoplectus. Batteries of individual teeth, fully incorporated into the outer layer of osteodentine, line the interiors of the upper and lower beaks, but are only visible at the masticatory surface and the base of the jaw (Fig. 3). The exterior of the beak is composed of a very thin layer of this smooth, high-density, compact osteodentine, while internally and at the jaw base, fibrous osteodentine is aligned with the direction of jaw growth. Teeth are moderately anteroposteriorly flattened and arranged in an overlapping, shingle-like pattern. Cusps are rounded, but roots are elongate and pointed, being longer in teeth closer to the midline and shorter laterally. At least four tooth-rows are present in the upper jaw and six in the lower, increasing to eight or more rows within the hooked part of the beak, where teeth are stacked directly along the midline, contributing to the anteriorly projecting bulge. No median hiatus exists between left and right tooth-rows.

The structure and arrangement of the internal teeth and osteodentine layers vary substantially between gymnodont families (Andreucci et al. 1982; Britski et al. 1985; Fraser et al. 2012). The dental structure of †Ctenoplectus most closely resembles that of triodontids (simple, rounded elements arranged in a stacked, shingle-like pattern) and, to a lesser extent, diodontids (upper and lower jaw fusion, teeth arranged in shingle-like pattern that continues uninterrupted across the symphyseal region). However, the elongate teeth of †Ctenoplectus contrast with the short, lenticular elements of triodontids and diodontids: the dorsoventrally-elongate tooth roots with pointed tips appear to be unique. The occlusal tips are also considerably more rounded, and tooth rows more tightly stacked (Fig. 3B, C). Only a single layer of teeth (viewed in sagittal section) is present in triodontids and (discounting the symphyseal region) in †Ctenoplectus. The dentition of the tetraodontid Sphoeroides, by contrast, contains several layers of teeth stacked anteroposteriorly (Andreucci et al. 1982). Further variation exists in the extent of osteodentine within the jaws of extant gymnodonts (Britski et al. 1985). As in triodontids (Fraser et al. 2012), the jaws of †Ctenoplectus (Close

FIG. 2. Upper and lower jaws of †Ctenoplectus williamsi (NHMUK PV P. 63336) virtually isolated to reveal hidden morphology. A–C, upper jaws in left-lateral, dorsal and ventral (occlusal) views. D–F, lower jaws in left-lateral, ventral and dorsal (occlusal) views. Scale bar represents 10 mm.
et al. 2016, fig. 2) bear osteodentinic posterointernal walls but lack anteroexternal walls, while teeth formed in cavities near the jaw base distal to the outer layer of compact osteodentine, and are visible near the surface at the bases of the upper and lower beaks (Fig. 3B). Thus, except for midline fusion of the premaxillae and probable lack of tritural plates, the dentition of †Ctenoplectus shares more with triodontids than with any other extant tetraodontiforms.

Although much of the neurocranium of †Ctenoplectus has been lost, a section of skull roof is preserved that extends from the region above the upper jaw to a point just short of the post-temporal. Much of this likely represents the frontal, and forms a broad supraorbital vault. The posterior-most extremity of the skull roof, directly behind the orbital vault, is thickened.

The palatine is relatively large. The quadrate and symplectic are approximately half the length of the preopercle. The opercle is twice as deep as high; the anterior margin is slightly concave, and the posterior margin more strongly convex. The anterior margin of the preopercle is only slightly concave, with a depth approximately one-third of its length. Unlike Triodon, but like non-triodontid gymnodonts (Tyler 1980; Santini & Tyler 2003; Santini et al. 2013), the anterior ceratohyal of †Ctenoplectus lacks a beryciform foramen.

The bases of two dorsal-fin spines are preserved. At least four (and possibly five) prominent dorsal-fin spines, supported by four greatly enlarged and dorsoventrally flattened proximal radials (basal pterygiophores), extended far forward to cover the most anteriorly preserved region of the skull roof. The first of the two preserved spines inserts onto the anterior margin of the second proximal radial, directly over the orbit, while the second preserved spine inserts onto the anterior margin of the third proximal radial. However, the anterior-most proximal radial preserves facets for the articulation of two additional dorsal-fin spines that would insert even closer to the front of the skull (Fig. 1B–D), and it is likely that a fifth spine inserted on the posterior margin of the third proximal radial, although damage precluded identification of an associated articular facet. Points of articulation of the dorsal-fin spines on the proximal radials

**FIG. 3.** Jaws of †Ctenoplectus williamsi (NHMUK PV P. 63336) rendered in various degrees of transparency to reveal internal structure. A, anterior view, no transparency. B, anterior view, moderate transparency. C, anterior view, greater transparency. D, left-lateral view, no transparency. E, right-lateral view, no transparency. F, right-lateral view, outer layer rendered completely transparent to show batteries of internal teeth. Scale bars represent 10 mm.
coincide with stellate patterns representing centres of growth. The reduced fourth proximal radial also bears this stellate pattern, and its degree of development suggests the possibility of a sixth dorsal-fin spine inserted on its posterior margin. Intact regions of the two preserved dorsal spines are similar in size and shape. They are wide at their bases, taper distally, and exhibit heavy anteroposterior compression. Stellate, rose-thorn-shaped scales are affixed to the sides of the dorsal-fin spines (Fig. 1B–D), though no body scales are apparent. Stellate, cone-like scales are also found in small extant molids. There is no evidence of a locking mechanism; the base of each dorsal-fin spine bears a midline notch to receive a small protuberance on the proximal radial, without evidence of the ring-link articulation seen in balistoids. The four preserved proximal radials (cf. 1–2 in balistoids) are dorsoventrally flattened and bilaterally expanded, covering the entire skull roof. The posterior portion of each proximal radial develops into a long, posteroventrally-directed shaft with a rounded cross-section terminating in a point, underlying the subsequent element in the series (Fig. 1C, D). The posteroventral shaft of the first radial is sufficiently long to extend past the position of the base of the second-preserved dorsal spine. In many tetraodontiform lineages, including triacanthoids and eoplecids, the ventral shaft of the first radial of the spiny dorsal-fin articulates with the open neural arches of an anterior vertebra (often the first vertebra, though this depends on the anteroposterior placement of the dorsal-fin radials; Tyler 1980). However, we cannot determine if this was the case in Ctenopectus as vertebrae are not in their original positions. The last posterior shaft extends considerably posterior to the cleithrum, and supports a smaller but still flattened and expanded distal face that lacks clear dorsal-spine articulation facets.

Prominent dorsal-fin spines, though primitive for tetraodontiforms, are more commonly associated with the ’scleroderm’ families (with the exception of aracanids and ostracids), whereas dorsal spines of any kind are not known from any other gymnodonts except Eoplectus, where they broadly resemble the triacanthoid condition (Tyler 1980), and Triodon, in which they are rudimentary (and often absent) and supported by small proximal radials confined to the abdominal region. Dorsal-fin spines of extinct triacanthoids and triacanthids resemble the ancestral acanthomorph condition. However, those of balistids and monacanthids are more derived, and resemble Ctenopectus by inserting further anteriorly along the skull roof. Among fossil taxa, anteriorly-placed dorsal spines are found in the protobalistids Protobalistum and Spinacanthus from Bolca (Tyler & Santini 2002). However, the dorsal spines of protobalistids and other ’scleroderms’ lack the marked anteroposterior compression and lateral expansion seen in Ctenopectus (this condition may be reflected in the broad, but more rounded, basal regions of the second and third dorsal-fin spines of the bolcabalistid Eosinus; Tyler & Bannikov 1992). Although in balistids and monacanthids the shafts that underlie each successive proximal radial are oriented ventrally, in Ctenopectus these shafts are directed posteroventrally.

The pectoral girdle preserves the right post-temporal (absent in all other gymnodonts except Triodon; Tyler & Patterson 1991) and supracleithrum, both cleithra, postcleithra and coracoids (though not scapulae), radials and fin-rays. The well-developed post-temporal and supracleithrum (oriented obliquely to the axis of the skull) together form a strap-like unit that tapers to a point dorsally. Both elements are broader where they abut. At its articulation with the post-temporal, the supracleithrum is bifid, and bears a flattened flange that projects medially. The cleithra are gently curved, taper to points at both extremities, and are not posteriorly-deflected at their posterodorsal ends. The postcleithra are gracile, gently curved, and taper ventrally. The coracoid possesses a long, anteroventrally-directed shaft and a triangular dorsal region, which bears a posterodorsally-directed process on its posterior corner. The well-developed pelves consists of paired, rod-like elements with a gentle, posteriorly-directed curvature. Unfused but tightly united, these rods are of a uniform diameter throughout their lengths and terminate ventrally with flat broken surfaces. Triodon and Eoplectus are the only other beak-toothed puffers known with a pelvic girdle, which is absent in all other gymnodonts, and in aracanids and ostracids (Tyler 1980). The rod-like morphology of the pelves in Ctenopectus is distinctive, departing from that of T. macropterus, in which the contralateral rods are fused together and taper substantially at their extremities. Only the base of the pelves of †T. antiquus are preserved, precluding comparison. Rod-like pelves are not present in any extant members of the Tetraodontoidei.

Three disarticulated abdominal vertebrae are present. The shortest, probably the first abdominal vertebra, possesses a centrum one-half the length of the most closely-associated vertebra, and one-third the length of the more distant vertebra. The large, laterally-expanded lateral walls of the neural arch of the first and second vertebrae do not meet at the midline (Fig. 1B, D). The neural arches of the third vertebra are, proportionally, slightly shorter than those of the other two preserved vertebrae, and nearly meet at the midline to enclose the neural canal. The posterior-most vertebra (probably the third abdominal vertebra) has well-developed transverse processes under the centrum; these are anteroposteriorly compressed and almost as wide as the centrum. Within tetraodontiforms, abdominal vertebrae with prominent transverse processes like those in Ctenopectus are present in all of the ’scleroderm’ families (triacanthoids, triacanthids, balistids, monacanthids and, in a specialized form, as low haemal arches without haemal spines in aracanids and ostracids; Tyler 1980) but these are not present on the abdominal vertebrate in the families of gymnodonts. This suggests that transverse processes are plesiomorphic for tetraodontiforms, related to the presence of ribs (which often articulate to transverse vertebral processes). One short, curved and flattened pleural rib is preserved close to the third vertebra; Triodon, Eoplectus and Eotetraodon are the only other gymnodonts known to bear ribs (Tyler 1980).

RESULTS

Phylogenetic relationships

The maximum sampled-ancestor clade credibility (MSACC) tree of our Bayesian phylogenetic analysis
Within gymnodonts, directly on branches in ancestral positions. Our analysis lineage to Balistoidea and various fossil forms. Triacanthidae coid, and all other scleroderm; within the latter group, phyletic gymnodont clade, followed by relationships of crown tetraodontiform clades. A considerable uncertainty that still exists regarding the deep matically 72% of the posterior trees sampled, highlighting the however, gymnodonts were monophyletic in approxi-

We anticipated that employing an SA-FBD model would yield considerably younger divergence estimates than those previously inferred for tetraodontiforms using a tip-dating approach, as this was highlighted as a key result by Gavryushkina et al. (2015) in their dating analysis of penguins. Contrary to expectations, we recovered timings of deep divergences that are broadly in line with those obtained by Arcila et al. (2015) using a less sophisticated birth–death total-evidence tip-dating approach. Crown ages for all extant tetraodontiform families, however, lie within the Cenozoic (Close et al. 2016, fig. 3). A range of divergence-dating studies support Cenozoic origins for crown families, including balistids (Dornburg et al. 2011), tetraodontids (Santini et al. 2013) and monacanthids (McCord & Westneat 2016). One possible explanation for our divergence date estimates being more ancient than anticipated, and with greater uncertainty, is that both Arcila et al. (2015) and Gavryushkina et al. (2015) used fixed tip-calibrations for fossil taxa, while we used stratigraphic occurrence information to define minimum and maximum bounds of uniform distributions. As O’Reilly et al. (2015) have recently demonstrated, incorporating tip-age uncertainty yields less precise divergence-time estimates compared to analyses using fixed tip-ages (accommodating realistic tip-age uncertainty was also found to affect topology and rates). Nevertheless, the impact of this propagation of uncertainty may be partially mitigated by our use of node-age calibrations at the acanthomorph root and on the tetraodontiform crown. It is also worthy of note that no fossils exist to constrain the divergence of Tetraodontiformes between the earliest crown members in the earliest Eocene and the plecto-

Patterns of evolutionary rates

Patterns of branch-specific evolutionary rates were inferred independently for morphological and molecular data partitions, by assigning each an independent uncorrelated lognormal relaxed clock. Rates were inferred jointly with other parameters (e.g. topology and branch lengths) by our Bayesian phylogenetic analysis, and are thus inseparable from other key findings, such as relationships and divergence dates. Patterns of branch-specific rate variation in total-evidence tip-dating analyses are very rarely dissected by studies where the primary objective is the estimation of divergence dates. Nevertheless, it
is clear that these parameters must be understood in conjunction with one another, and so we devote special attention to these results. Temporal rate heterogeneity is much more pronounced for morphology than molecules, and the two sources of data show contrasting patterns across the tetraodontiform tree (Fig. 4A–B shows median rates painted onto the MSACC tree; Fig. 4C–D compares log-transformed median branch rates for the MSACC with branch rates for a sample of 150 trees from the posterior and LOESS lines to indicate overarching trends). The morphological data appear to show an ‘early burst’ pattern, decelerating towards the present, while molecular rates are broadly constant through time.

Rates of morphological change early in the history of tetraodontiforms are generally substantially elevated, while low rates inferred for the vast majority of more recent branches. Many morphological character changes are concentrated on deep internal branches (particularly leading to Gymnodontes and beak-toothed families, ostracioids and balistoids), but a bimodal pattern is partially evident from the plot of branch rates versus time, as few changes occur on branches leading to the less morphologically-derived triacanthids and triacanthodids (Fig. 4B).

High rates are less commonly inferred for terminal than internal branches, perhaps due to the omission of a priori autapomorphies from our morphological character matrix (though many a posteriori autapomorphies are associated with terminal branches due to homoplasy). Independently visualising internal and terminal median branch-rate estimates (Close et al. 2016, fig. 4) demonstrates that both are characterized by higher early rates, suggesting that the omission of a priori autapomorphies does not strongly bias observed patterns. However, the true impact on morphological rate-patterns of autapomorphies, character sampling more generally, and taxonomic sampling (particularly within more shallower-diverging clades that have poorer fossil records, such as balistids and monacanthids) can only be rigorously tested by gathering new data.

Molecular data, by contrast, does not appear to show an early burst pattern; in fact, changes seem to be concentrated at shallower levels of the tree. However, consistent with the pattern for morphology, moderately elevated molecular rates are present on internal branches leading to tetraodontids + diodontids, and to balistoids. If, as seems likely, our analysis substantially overestimates the crown age of Tetraodontiformes, then the temporal rate heterogeneity we observe may conversely be underestimated.

**DISCUSSION**

With its unprecedented combination of scleroderm and gymnodont characters, †Ctenopectus highlights homoplasy in the skeleton of early diverging crown-group tetraodontiforms, as well as the potential for fossils to disentangle such convergences. Although †Ctenopectus is approximately coeval with the earliest crown-group tetraodontiforms, it is already highly apomorphic. The new taxon exhibits characteristics not only of triodontids (including a number of internal structural features of the beak-like dentition, plus a series of general tetraodontiform characters that, within beak-toothed representatives, are retained only in diodontids, including post-temporals, well-developed pelvic girdle and, along with †Eotetraodon, pleural ribs), diodontids (both upper and lower jaws fully fused along the midline) and molids (fully fused jaws as in diodontids, and striated, weakly-ossified bone), but also arguably with various ‘scleroderm’ families (greatly enlarged dorsal spines and associated proximal radials that extend down the dorsal surface of the skull).

In spite of this apparently conflicting suite of characters, Bayesian total-evidence analyses consistently resolve †Ctenopectus on the triodontid stem. This is a credible placement, as the dentitions of †Ctenopectus and Triodon share very similar patterns of internal structure and development, and these are the only gymnodonts aside from †Eoplectus not to have lost ribs, pelvis, post-temporals and dorsal fin-spines (of these, †Eotetraodon retains only ribs). Nevertheless, complete fusion of the dentition, combined with highly-developed dorsal-fin spines (the latter being a recurrent theme among early Palaeogene fossil tetraodontiforms) demands homoplasy at some level. Lastly, placement of †Ctenopectus on the triodontid stem suggests that the development of prominent, anteriorly-situated dorsal-fin spines along the roof of the skull may have been derived independently of similar structures in certain balistoid lineages. Dorsal fin-spines are retained in other early-diverging gymnodonts, although they do not encroach upon the skull. Most notably, anteriorly-inserting dorsal fin-spines are shared with †Balkaria histiopterygia, a newly-described gymnodont from the earliest Eocene of the northern Caucasus, Russia, that was published while this manuscript was in the final stages of peer-review (Bannikov et al. 2016). This taxon, which may prove to have a close affinity with †Ctenopectus, is placed by Bannikov et al. (2016) in the monotypic family †Balkaridae, but is resolved by their total-evidence phylogenetic analysis as an early-diverging member of the suborder Tetraodontoidae. The presence of prominent dorsal fin-spines in †Ctenopectus, †Balkaria and †Eoplectus strongly suggests that this condition may be primitive for gymnodonts.

To date, no phylogenetic hypothesis based exclusively on molecular evidence supports ‘gymnodont’ monophyly (Holcroft 2005; Alfaro et al. 2007; Yamanoue et al. 2008; Near et al. 2012; Santini et al. 2013). However, in
agreement with the recent study of Arcila et al. (2015; using a similar morphological matrix to ours and an overlapping set of sequence data) we find that the addition of even a comparatively small fraction of morphological character data has the power to recover the monophyly of all extant beak-toothed puffers, albeit with a comparatively low PP of 0.73.

Our Bayesian tip-dating analysis suggests that the origin of crown Tetraodontiformes, and particularly the early evolution of gymnodonts and ostracioids, saw elevated rates of morphological character evolution. This was followed by a decline in rates that began approximately in the early to mid-Eocene. However, undersampling of taxa in the later Neogene and Recent prohibits us from drawing more robust conclusions about morphological rates during these intervals (e.g. Plio-Pleistocene monacanthids described by Sorbini & Tyler 2004).

The timescale we recover is only partially consistent with a post-K–Pg origin for the crown group: while every extant tetraodontiform family is estimated to have originated during the Cenozoic, deeper divergences (including beaked and non-beaked lineages) are estimated to have much more ancient origins in the mid-Cretaceous. A post-K–Pg diversification scenario, by contrast, would dramatically compress the morphological changes early in the tetraodontiform tree, resulting in a much more pronounced peak in rates. A substantial component of tetraodontiform disparity seems to have been established very early in their evolutionary history; familial/suprafamilial divergences involved considerable morphological innovation and all extant lineages, along with many bizarre lineages not represented today, were established by the Eocene at the very latest. This pattern is consistent with the finding that rapid early diversifications result in morphological disparity being partitioned among, rather than within, subclades (Harmon et al. 2003).

These results, therefore, appear to qualitatively show an ‘early burst’ of morphological evolution associated with the origin of the crown group, a key feature predicted by ecological models of adaptive radiation (Simpson 1953; Schluter 2000). Early-burst signatures appear to be rare in comparative phenotypic traits using extant datasets (Harmon et al. 2010; with notable exceptions, such as rates of body-size evolution in exceptionally speciose orders of mammals (Venditti et al. 2011) but are more commonly recovered by palaeontological studies, including those that analyse discrete morphological character data incorporating fossil taxa (Brusatte et al. 2008; Lloyd et al. 2012; Lee et al. 2013). However, some degree of caution must be adopted when interpreting these results, given potential acquisition bias in sampling morphological characters for systematic analysis. More specifically, the pattern we observe may partially stem from the use of a morphological dataset that was assembled with the aim of resolving deeper branches within tetraodontiform phylogeny. Such a dataset preferentially samples characters pertaining to the establishment of major tetraodontiform body plans. Conversely, such a dataset is likely less effective at capturing the finer morphological differences needed to disentangle lower-level relationships (e.g. meristic characters or more subtle variations in individual elements). In an analogous manner to third-codon positions in molecular sequences, such characters may evolve rapidly and be subject to weaker constraint than overall body plans. Ultimately, the results we report reflect the subset of phenotypic traits and lineages sampled, and denser sampling of characters and taxa will provide a critical test of the generalities of the patterns recovered here.

While the term ‘adaptive radiation’ was originally coined (Osborn 1902; Simpson 1953) to describe large-scale diversification occurring over large temporal and spatial scales, more recently (Schluter 2000) it has been argued that it should be restricted to monophyletic radiations occurring in restricted geographical areas. Classic examples of adaptive radiations, such as Galapagos finches, Caribbean Anolis lizards or East African cichlid fishes, tend to represent isolated systems with shallow temporal scales. However, some studies (Dornburg et al. 2011; Arbour & Lopez-Fernandez 2013; Moen & Morlon 2014) suggest that (particularly for the marine realm) the current paradigm could be expanded or adapted to encompass larger-scale radiations displaying early bursts of morphological evolution together with elevated speciation rates and ecological divergence (Friedman 2010; Slater et al. 2010; Derryberry et al. 2011; Monteiro & Nogueira 2011; Arbour & Lopez-Fernandez 2013), many

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**FIG. 4.** Dated maximum sampled-ancestor clade credibility (MSACC) tree of tetraodontiform relationships resulting from total-evidence analysis of morphological and molecular sequence data using BEAST 2.3.0 (†Cenoplatectus williamsi gen. et sp. nov. rendered in bold). Encircled numbers denote: 1, Crown Tetradontiformes; 2, Gymnodontes; 3, Sclerodermi. Blue-to-red shading of branches represents rate of character evolution (%/myr) inferred by BEAST for: A, morphological; and C, molecular data partitions using independent clocks. Posterior probability for selected clades indicated on lower left sides of nodes. B, D, time series plots of per-branch rates for: B, morphological; and D, molecular data partitions; more distinct horizontal lines represent median rates for each branch, while thinner, transparent clouds of lines depict the posterior distribution of rates from a random sample of 100 trees output by BEAST. A moving average (LOESS) is given by the solid trend line.
of which have contributed profoundly to present-day biodiversity. Regardless, the phenomena captured by the conceptual framework of adaptive radiations doubtless represents only one facet of a complex continuum of macroevolutionary processes.

Although it is tempting to link this rapid initial flourishing of form in tetraodontiforms to the invasion of abundant ecospace following the K–Pg extinction (cf. Friedman 2010), and perhaps to the two waves of reef invasions that occurred during the latest Cretaceous and early Palaeogene (Price et al. 2014), the timescale recovered in our study suggests that much of the morphological innovation in crown tetraodontiforms preceded this event. However, published ages for the tetraodontiform crown vary substantially (mean estimates range from 59 Ma (Alfaro et al. 2007) to 185.2 Ma (Arcila et al. 2015)), such that any hypothesized links between elevated rates of evolution and external drivers must be viewed, at present, with caution.

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Author contributions. RC and MF drafted the anatomical description and figures; JT reviewed the anatomical description; RH collated and formatted the sequence data; RC performed the phylogenetic analyses, and drafted the manuscript; MF, ZJ, JT and RH helped draft the manuscript; and MF and RC conceived the study. All authors gave final approval for publication.

DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.304n7

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