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Widespread ecomorphological convergence in multiple fish families spanning the marine–freshwater interface

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The theoretical definition and quantification of convergence is an increasingly topical focus in evolutionary research, with particular growing interest on study scales spanning deep phylogenetic divergences and broad geographical areas. While much progress has recently been made in understanding the role of convergence in driving terrestrial (e.g. anole lizards) and aquatic (e.g. cichlids) radiations, little is known about its macroevolutionary effects across environmental gradients. This study uses a suite of recently developed comparative approaches integrating diverse aspects of morphology, dietary data, habitat affiliation and phylogeny to assess convergence across several well-known tropical–temperate fish families in the percomorph suborder Terapontoidei, a clade with considerable phenotypic and ecological diversity radiating in both marine and freshwater environments. We demonstrate significant widespread convergence across many lineages occupying equivalent trophic niches, particularly feeding habits such as herbivory and biting of attached prey off hard substrates. These include several examples of convergent morphotypes evolving independently in marine and freshwater clades, separated by deep evolutionary divergences (tens of millions of years). The Terapontoidei present a new example of the macroevolutionary dynamics of morphological and ecological coevolution in relation to habitat and trophic preferences, at a greater phylogenetic and habitat scale than most well-studied adaptive radiations.

1. Introduction

The concept of diversification on a macroevolutionary adaptive landscape, a multivariate phenotype surface where species evolve to the same adaptive peaks, has a long-standing history in evolutionary biology [1,2]. Evolution of repeated, parallel sets of functional traits in response to similar environmental factors across phylogenetically independent lineages suggests predictable and deterministic responses to similar selective regimes [2–4]. The appreciation of convergence is enjoying a recent resurgence in terms of both theoretical definition [5,6], and development of an increasingly complex and sensitive suite of methodological approaches to detect and quantify its scale and nature [7,8].

Ecomorphological patterns within fish assemblages, particularly relating to trophic habits, have provided some of the strongest evidence of convergent evolution [9–11]. Dietary modes such as molluscivory [9], piscivory [12], zooplanktivory [13] and herbivory [14,15] are all considered to present specialized adaptive peaks among fishes. While most convergence studies have focused on clades of closely related species (often a single genus or family inhabiting the same general area or habitat), assessing the broader role of convergence as a

driver of macroevolution requires a more expansive comparison of phylogenetically distant radiations diversifying in different regions [16–18]. For example, while marine fish lineages that successfully colonize freshwater habitats are usually confronted with a variety of potentially novel niches, often catalysing adaptive radiations [19,20], evidence of convergent evolution across this most profound environmental transition for aquatic organisms is currently rare.

The percomorph fishes of the suborder Terapontoidei (Eupercaria: Centrarchiformes) are a compelling model clade for examination of evolutionary convergences across deep phylogenetic levels and geographically distinct habitat types. Here, multiple families have evolved across diverse temperate-tropical regions and macrohabitats, including several marine-to-freshwater transitions. Collective lineages exhibit comparable ecological roles (figure 1a) [15,21], with trophic groups including benthic invertivores, herbivores and carnivores that are frequently associated with distinctive morphotypes relating to body shape, intestinal length and oral anatomy [14,21,22]. If convergence has played a significant role in shaping the macroevolutionary history of the Terapontoidei, we predict ecologically similar species with respect to diet (otherwise phylogenetically distant and often geographically isolated) will converge into the same areas of morphological and dietary space. To examine these hypotheses, we use a suite of recently developed comparative approaches to identify the patterns and strengths of convergence among phenotypic traits and diets among species of the Terapontoidei.

2. Material and methods

(a) Taxonomic sampling, phylogeny and ancestral state

The phylogenetic component for this comparative study is based on the taxonomic sampling of 88 Terapontoidei (out of 126) valid species currently recognized, including 29 (out of 32) valid genera [23] and all families in the suborder (i.e. Dichistiidae, Kuhliidae, Oplegnathidae, Terapontidae and Kyphosidae; the latter further divided into Kyphosidae *sensu stricto*, Girellidae, Microcanthidae and Scorpididae [24–26]). The phylogenetic tree was inferred from a previous examination of grunter macroevolution [15], although that study only reported a single-family clade (Terapontidae). Here, we use the complete tree for Terapontoidei previously estimated [15] as the framework for the comparative analyses. In summary, the time tree was inferred using a concatenated dataset of three mitochondrial and four nuclear markers (8471 sites), and time-calibrated in BEAST [27] using a secondary calibration point for the root age (74 Myr). Some of the phylogenetic comparative analyses conducted (see below) used the consensus BEAST tree, while others used a collection of 500 trees evenly sampled from the posterior distribution. Five taxa for which no specimen data were available were pruned from trees prior to comparative analyses.

Ancestral diets (i.e. ‘carnivore’, ‘omnivore’ and ‘herbivore–detritivore’; see ‘Dietary classification and quantification’ section) and habitat preferences (i.e. ‘marine’, ‘euryhaline’ and ‘freshwater’) were reconstructed using stochastic character mapping (SIMMAP [28]). To integrate uncertainty in tree topology, branch lengths and the evolutionary history of diet and habitats, stochastic maps were estimated across the 500 Bayesian trees. Three hierarchical transition models—equal rates (ER), symmetrical rates (SYM) or all rates different (ARD)—were assessed by maximum likelihood with results averaged across all runs; the best-fitting model for SIMMAP was identified using likelihood ratio tests.

(b) Anatomical preparation and trait quantification

We quantified morphological variation in the Terapontoidei through a range of traits specifically chosen to capture major axes of functional variation (additional details provided in the electronic supplementary material). Fish specimens (sourced from surveys and museum collections spanning the Indo-Pacific and Australasian regions) were digitally photographed in a standardized manner, on their right side with a scale bar. External body shape was quantified by a single investigator via digitization of the Cartesian coordinates based on 17 landmarks placed on specimen photographs (electronic supplementary material, figure S1) using TpsDIG v. 2.17 [29]. A generalized least-squares Procrustes superimposition was conducted [30] in MORPHOJ v. 1.05c [31], which aligns the specimens to a common coordinate system, and removes positional, rotational and size information from the dataset, while retaining shape variation. The empirical distribution of species’ mean shapes was assessed using a principal component analysis (PCA) of the resulting shape coordinates.

Multidimensional tooth shape was quantified via measurements taken directly from anterior scanning electron microscope (SEM) or digital camera images of the largest, anteriormost tooth of the lower jaw. Tooth shape was digitized in TpsDIG with 27 points, including three homologous landmarks (two basal tooth points and tip) and 24 sliding semi-landmarks (electronic supplementary material, figure S1) [15]. This configuration of points was also subjected to a generalized Procrustes analysis and PCA. Additional variables relating to tooth size and arrangement typically associated with diet [14,15] were also generated from tooth imagery (electronic supplementary material, figure S1).

With intestinal length often a key correlate of dietary habits [22,32], specimen digestive tracts were also dissected out and intestinal length measured as the distance (in millimetres) from the pyloric outlet of the stomach to the rectum. Finally, to generate a simple biomechanical model of species’ potential bite force and jaw velocity [33], mouth-opening and mouth-closing lever ratios (electronic supplementary material, figure S1) were calculated using either direct distance measures (with calipers) from dissected specimens or coordinate data taken from digital X-ray images (using a KeveX–Varian digital X-ray system).

(c) Dietary classification and quantification

As a broad classification of diets for use in subsequent analyses, we assigned the terminal taxa in our phylogeny into commonly recognized trophic categories based on a comprehensive literature review of stomach content across the clade (electronic supplementary material, table S1). Following Price *et al.* [34], species’ proportionate dietary data were coded into a discrete variable describing each species’s degree of carnivory: ‘carnivore’ (greater than 80% animal prey), ‘omnivore’ (animal prey greater than 20% and less than 80%) and ‘herbivore–detritivore’ (less than 20% animal prey). While these broad trophic classifications conceal finer-scale dietary distinctions within categories (e.g. piscivores, invertivores and planktivores within ‘carnivores’), their use is analytically tractable and minimizes missing character data. Some downstream analyses requiring quantitative traits rather than discrete categorizations used logit-transformed proportion of animal prey as input.

(d) Principal component analyses

To minimize allometric biases, all linear variables (tooth length, spacing and intestinal length) were log-transformed followed by regressions against standard length using a phylogenetic size correction [35]. A combined dataset of geometric (the major axes of lateral body shape and anterior tooth shape variation) and orthogonal measurements (tooth length and spacing, intestinal length and jaw biomechanics) was then

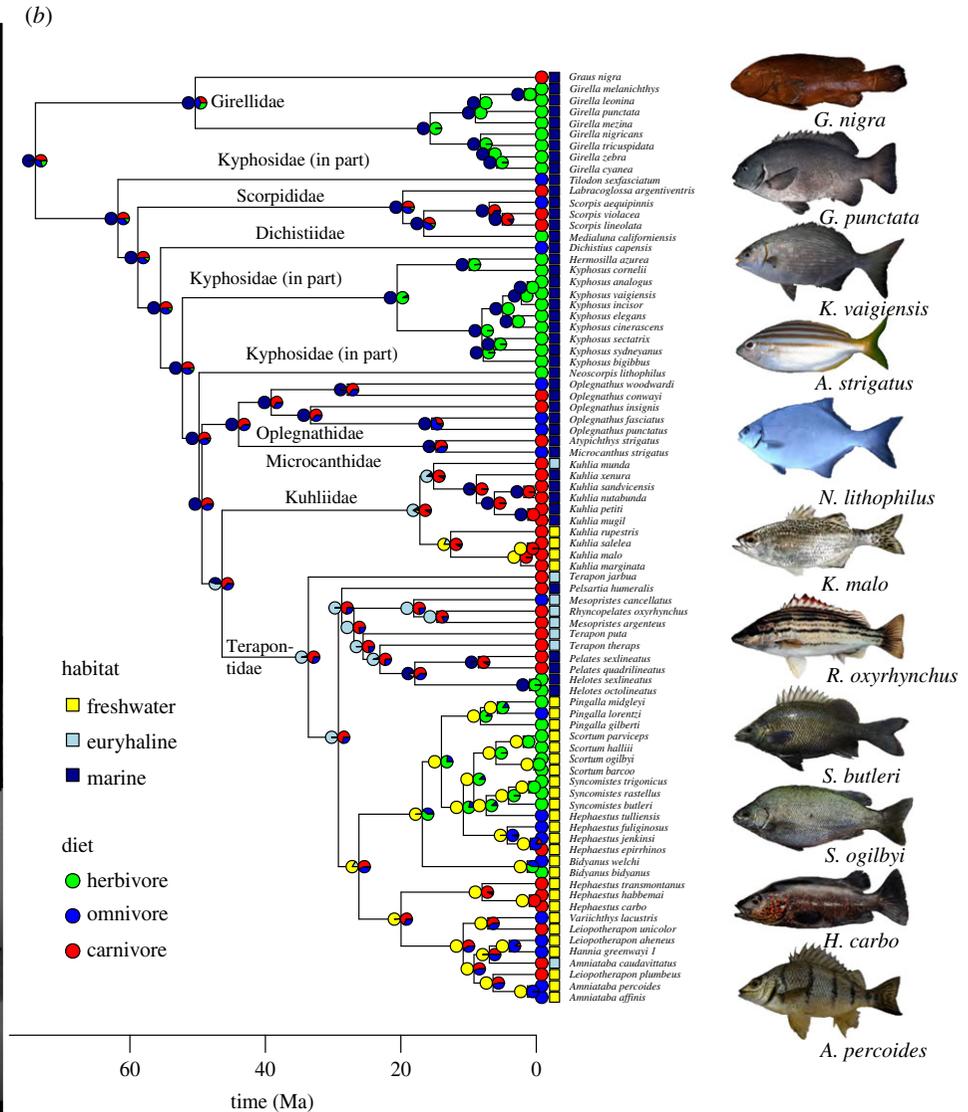
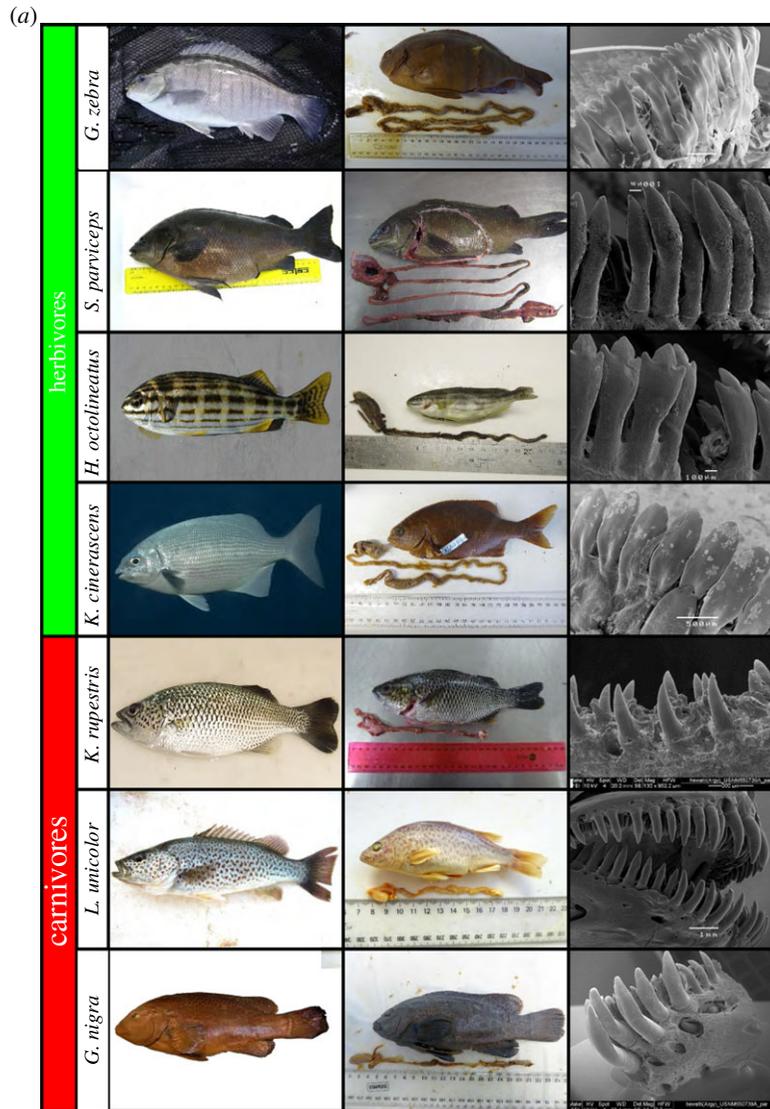


Figure 1. Morphological, ecological and phylogenetic diversity in Terapontoidei. (a) Potentially convergent ecological guilds in several unrelated genera with similar trophic preferences and phenotypically similar traits (body shape, dentition and intestinal length). (b) Maximum clade credibility phylogeny with species coded by discrete trophic and habitat designations. Ancestral states are shown as proportion of discrete dietary (right pies) and habitat (left pies) (reconstructed from 500 BEAST trees subjected to SIMMAP). Note that several KuhlIID species are primarily freshwater residents, but are thought to spawn in estuarine–coastal waters. Image credits: *K. cinerascens* (Graham Edgar), *S. butleri* (Mark Kennard), *K. malo* (Jeff Williams).

subjected to a phylogenetically corrected principal component analysis (PPCA) [35] to eliminate multicollinearity and to synthesize the main axes of integrated terapontoid morphological variation. The PPCA used the evolutionary correlation matrix of the maximum clade credibility tree, with species' scores on the main principal component (PC) axes used as character values in subsequent analyses.

(e) Testing for ecomorphological correlations

We used several approaches to assess whether morphological traits were associated with published trophic habits (electronic supplementary material, table S1). We firstly used the recently developed partial least-squares (PLS) correlation [36] to assess the level of integration between Terapontoidei morphology and diet. This approach can accommodate complex, multidimensional traits (e.g. shape), is relatively robust to differing sample and variable sizes, and provides a comparison of relative covariation-integration across different phenotypic datasets under BM. Covariation between specific morphological matrices and logit-transformed proportion of animal prey in diet was first characterized using r_{PLS} correlations and statistically evaluated using 1000 permutations. Standard deviates of trait r_{PLS} (z-scores) were then obtained and the standardized 'effect sizes' of the relative levels of correlation between specific morphological variables and diet were compared using the *compare.pls* function in *geomorph* [37]. Morphological traits compared were all linear variables (tooth length, spacing and intestinal length), jaw lever ratios, body and tooth shape (all PC axes), and synthesized PC axes 1–3. While lacking scope to explicitly compare relative levels of covariation, to assess robustness of results across methods we also ran phylogenetic generalized least-squares (PGLS) regressions (using *caper* [38]) under Brownian motion (BM) to examine phylogenetically independent correlations between morphological traits and diet. Examined traits were essentially the same as preceding PLS analyses, including individual traits (but tooth and body shape assessments were limited to major PCA axes explaining variation) and the first three morphological PPCA axes. PLS and PGLS analyses were run on the consensus tree and the 500 trees sampled from the posterior distribution.

(f) Convergence analyses

Given that no single metric can capture all relevant aspects of convergent evolution [8], we used several recent measurements in concert to assess the nature and scale of convergence. Because of potential for PCA to introduce undesirable statistical artefacts into evolutionary model fitting [39], we also ran (wherever possible) most analyses on raw traits as well as synthesized PC axes.

(g) Evolutionary trajectories of principal axes

To assess different underlying processes potentially shaping morphological evolution and phenotypic distributions in relation to dietary habits, several evolutionary models were assessed to find the best fit explaining evolution of species' scores on important morphological PPCA axes. We used an *a priori* assessment of selective regimes, building on the stochastic character-mapped reconstructions of foraging behaviour from the three predefined trophic groups ('carnivore', 'omnivore' and 'herbivore–detritivore'). Using *Ouwie* [40] and *geiger* [41], we fitted a range of 'early burst' (EB), single- and multiple-rate BM, and single- and multiple-rate and multiple-optima Ornstein–Uhlenbeck (OU) models (details provided in the electronic supplementary material). While not strictly testing convergence, *Ouwie* allows comparison of the appropriateness of different evolutionary models underlying clade evolution on specific PC axes. If our hypotheses of trophically similar lineages evolving onto similar adaptive peaks is true, the best-fitting models for any diet-related PC axes should correspond to

multi-peak OU models (i.e. OUM or OUMV) with distinct adaptive optima for specific dietary modes. Evolutionary model testing of PC axes and specific traits also provides insights as to whether our comparative analyses were unduly affected by PCA (e.g. use of PCs can be often biased towards early burst patterns of trait evolution [39]).

(h) Estimates of convergence using *convevol*

We also calculated the degree of independently evolved phenotypic similarity within collective PC axis scores on the maximum clade credibility tree using both distance- and frequency-based measures of convergence [8] in *convevol* [42]. Distance-based measures (C1–C4) are calculated between two lineages relative to their distance at the point in evolutionary history where the two lineages were maximally dissimilar. C1 specifically measures the proportion of phenotypic distance closed by evolution, ranging from 0 to 1 (where 1 indicates complete convergence). Similarly, the raw value of the difference between the maximum and extant distance between the two lineages (C2) can be scaled by either the total evolution (sum of squared ancestor-to-descendant changes) between the two lineages (C3) or the total evolution in the whole clade (C4). While contingent upon ancestral state reconstruction of phenotypic characters, these measures can reliably quantify incomplete convergence in multidimensional space. The frequency-based measure (C5) quantifies and reports the number of convergent events where lineages evolve into a specific region of morphospace (crossing it from outside). To test significance of our measures of C1–C5, we ran 1000 simulations for each comparison using BM on a variance–covariance matrix based on data-derived parameters, with convergence measures for each simulation calculated to determine if the observed C value is greater than expected by chance. *A priori* focal groups forming the basis of convergence tests were the same trophic categorizations used in *Ouwie* analyses.

(i) Estimates of convergence using *ℓ1ou*

To identify potential convergence onto phenotypic optima without *a priori* designation of selective regimes we used the *ℓ1ou* package [43]. A model-based approach to detecting convergence, *ℓ1ou* employs a regularized least absolute shrinkage and selection operator (LASSO) method to determine the optimal number of selective regimes in a phylogeny, and can accommodate single or multiple traits. *ℓ1ou* paints a phylogeny with different OU models to determine how many different selective regimes are needed to describe trait evolution and attempts to collapse regimes together. Convergence with *ℓ1ou* is indicated by either identical (collapsed) or very similar sets of OU parameters in distantly related taxa. We assessed the combined three PC axes used in previous *Ouwie* and *convevol* analyses (multivariate), individual PC axes (univariate) and several raw traits (univariate) using random root covariance and the maximum clade credibility tree. A benefit of this entirely data-driven method is avoidance of the data bias and circularity inherent to *a priori* trophic designations. Note that *ℓ1ou* is similar to (but more conservative than) the popular SURFACE method [7], which has recently been shown to suffer from model overfitting [43,44]. We also ran the SURFACE package for comparison and find overall similar results (see electronic supplementary material).

As the specific method for inferring trait optima on trees can substantially affect results, we applied two methods to select the number of model shifts in *ℓ1ou* (and assess the robustness of any identified convergent regimes): the widely used Akaike information criterion (AIC_c), and the more conservative Bayesian information criterion (pBIC) [43]. To determine to what extent any identified convergence in the Terapontoidei adaptive landscape could have occurred by chance under a non-convergent process [4,7], null distributions were generated by simulating two simpler evolutionary models 99 times; the first was a constant-rate

BM model, and the second a single-regime OU process. The observed value of the best-supported ℓ_{1ou} model provides a significance test (p -value) of whether the observed value exceeded the null model expectation. All phylogenetic analyses were performed using R v. 2.15.0 [45], with data manipulations conducted using functions from *phytools* [35] and *ape* [46].

3. Results

(a) Terapontoidei phylogeny and ancestral states

We report the complete multi-locus, time-calibrated phylogeny of Terapontoidei, estimated for our recent study on grunter diversification (figure 1*b*) [15]. The relationships among families in the suborder are similar to those obtained by other recent molecular studies (e.g. [24–26,47,48]), but most of those included more limited taxonomic sampling for species in the group. Relative to a recent analysis [26], the most extensive in terms of taxonomic coverage, the main topological discrepancy is that the family Kyphosidae (as delineated by [26]) is not monophyletic in our tree (figure 1*b*). Ancestral character reconstructions of discrete dietary and habitat types (electronic supplementary material, table S6) highlight the repeated and independent evolutionary origins of specialized herbivorous diets across both marine and freshwater clades from a predominantly carnivorous–omnivorous ancestral state (figure 1*b*) [15].

(b) Morphometric analyses

The Terapontoidei display considerable morphological diversity in both body shape (relating primarily to body depth, mouth positioning, eye size, head and maxilla lengths) and tooth shape (contrasting mammiliform, spatulate, incisiform and caniniform dentition; figure 1; electronic supplementary material, figures S2 and S3). The PPCA of the final combined dataset (body form and tooth shape PC axes, tooth length and spacing, and intestinal length) yielded three significant axes responsible for approximately 55% of the variance within the morphological dataset (electronic supplementary material, table S2). The largest axis of variation in morphology (PC1; approx. 27% variance) corresponded to a gradient between carnivores (longer head and jaw lengths, wider-spaced, caniniform dentition and short intestines) and herbivores (closer-packed teeth, longer intestinal lengths, higher jaw-closing ratios, and shorter head and maxilla lengths; figure 2). PC2 (approx. 17% variance) showed broader overlap between diet regimes, and was most positively correlated with PC1 body shape (ovate versus fusiform body shapes) and tooth height. PC3 (approx. 12% variation) was most strongly associated with mouth orientation (sub-terminal versus supra-terminal), jaw-opening lever ratio and tooth shape.

(c) Ecomorphological correlations

Except for tooth height, PLS analyses (electronic supplementary material, table S3) revealed significant r_{PLS} scores between all morphological traits and diet. Pairwise comparison of standardized effects sizes across all traits (z -scores), however, revealed dietary habits were most strongly correlated with intestinal length and body shape, which had significantly higher levels of covariation with diet than other variables. All PC axes synthesizing the majority of morphological variation across the group were similarly correlated with diet, but standardized effect sizes of PC1 and PC2 explained similar

(though significantly higher) dietary variation than PC3. PGLS analyses were broadly consistent with PLS results (figure 2; electronic supplementary material, tables S4 and S5), and the PLS–PGLS relationships evident on the consensus tree were also largely consistent on analyses run across the 500 trees from the Bayesian posterior distribution (electronic supplementary material, table S5). Collective ecomorphological analyses revealed highly significant diet–morphology associations in the form of negative correlations between species scores for several traits and the proportion of animal prey in diet. For instance, relative to carnivores, species with herbivorous habits often have longer intestines, deeper bodies, ventral mouth orientation, higher jaw-closing ratios, and shorter heads and maxillas (figure 2*e–g*). The two diet types also tend to differ in dental morphology (e.g. narrow-based, often spatulate or multicuspidate dentition in herbivores versus caniniform dentition in carnivores; figure 1*a*).

(d) Adaptive peak-based estimates of convergence

Results from PC model fitting are summarized in electronic supplementary material, table S7, which provides information regarding fit (AIC_C and AIC_W values, the latter being model-averaged Akaike weights) as well as estimates of diet optima for each of the six evolutionary models averaged over 500 stochastic reconstructions. Three-peak OU models (particularly OUMV) were substantially favoured for PC1–PC2, generating the highest AIC_W in all reconstructions. The most favoured OUMV model indicates separate phenotypic optima and differing rates of stochastic evolutionary change towards these adaptive peaks across the three diet regimes. Plotting of trophic strategy and habitat affiliation onto morphological SIMMAP-based traitgrams highlighted the evolution of dietary strategies onto separate adaptive peaks for PC1–PC2 (figure 2*b–d*). The phenotypic extent occupied by these optima were, however, broad and covered large areas of morphospace (figure 2*a*; electronic supplementary material, figure S4). PC3 models favoured an OU1 model of evolution, with a single adaptive peak across all species. Analyses run on selected individual traits loading strongly on different PC axes also produced similar results (electronic supplementary material, table S8), and simulations conducted using our best-fit model parameters largely supported modelling with real data (electronic supplementary material, table S9 and figure S5).

(e) *Convevol* analyses

Pattern-based estimates of convergence in collective PC scores revealed $C1$ values ranging between 0.41 and 0.56 for the three alternative trophic categorizations, all with highly significant p -values (table 1). This indicates that evolution has closed (on average) around half the phenotypic distance between clades sharing similar dietary habits. Convergence also accounted for 2–3% of the total evolution in the clades containing all the purportedly convergent taxa ($C4$ values; $p < 0.001$ for all values). The frequency-based measure of convergence ($C5$; number of lineages evolving into the region of interest) reported statistically non-significant values for PC data in all trophic classifications ($p > 0.05$).

Results from *convevol* distance-based measures of convergence computed with selected trait data generally yielded similar $C1$ – $C4$ values, explaining comparable levels of convergent evolution. Unlike PC1–PC3 axes, however, frequency-based measures of convergence based on raw traits

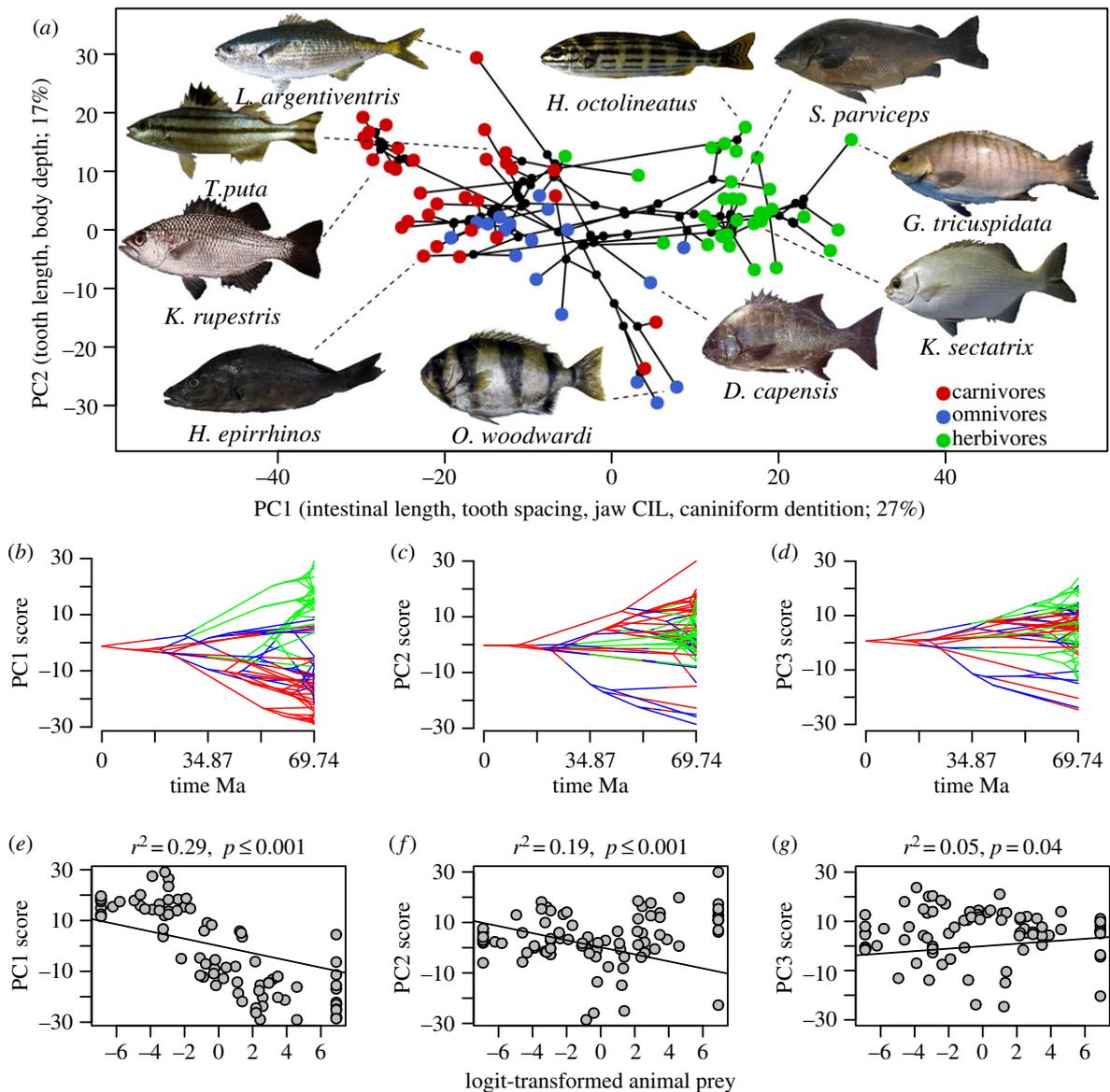


Figure 2. (a) A phylomorphospace plot of Terapontoidei (representatives depicted), with colour-coded trophic classification, superimposing the branching patterns of the phylogeny (black lines) onto the first two PC axes (phylogenetic PCA of combined morphological dataset). (b–d) Traitgram projections [35] of evolution of morphological PC axes through time, according to trophic categorization. The vertical position of each terminal branch on the y-axis represents PC score for that species; the x-axis signifies relative time since root. (e–g) PGLS correlations (fitted under BM) between PC1–PC3 scores and diet. Image credits: *T. puta* (CSIRO), *H. octolineatus* (Chris Dowling), *O. woodwardi* (Tom Trnski © Australian Museum).

reported highly significant C5 values for all trophic guilds ($p < 0.001$). Here, few lineages entered the carnivore morphospace, with higher clade numbers entering the morphospace of omnivorous and particularly herbivorous species. These results are essentially consistent with diet reconstructions showing an ancestral carnivorous condition from which herbivory–omnivory has evolved on multiple occasions (figure 1b). Such contrasting results between analyses based on PC axes versus a selection of individual traits may simply reflect the increased statistical power associated with inclusion of additional individual variables, but may also reflect convergence testing on a trait subset more directly associated with dietary habits.

(f) *ℓ1ou* analyses

The *ℓ1ou* model using AIC_C for shift detection on the full PPCA dataset (multivariate) identified 12 distinct adaptive shifts from mean trait values ($AIC_C = 1620.93$), which collapsed to seven distinct regimes, composed of eight shifts to convergent peaks and four unique non-convergent peak shifts

($AIC_C = 1604.02$). Several independent marine (*Helotes*) and freshwater (*Scortum*, *Syncomistes*) herbivore lineages were all identified as independently converging onto a similar phenotypic optimum (figure 3; electronic supplementary material, figure S6). Several mobile, fusiform carnivores, such as the marine and freshwater distributed Kuhliidae, marine–euuryhaline terapontids (*Pelates* and *Terapon* genera) and the marine *Labracoglossa argentiventris*, converged to similar morphotypes. Results also reveal a previously unidentified potential morphological convergence between the Oplegnathiidae and *Dichistius capensis* (Dichistiidae), clades sharing similar trophic habits (biting attached, hard-bodied invertebrates, sponges and algae off rock surfaces; electronic supplementary material, table S1) and morphologies (relatively deep, ovate bodies, high force transmission jaws and large, robust teeth; figure 2). Notably, the girellids—marine herbivores occupying one of the extremes of PC1 and representing the earliest-branching lineage of Terapontoidei—demonstrated non-convergent phenotypic optima. Additional distinct non-convergent optima were evident for several single species

Table 1. C1–C5 convergence measures and p -values for *convevol* analyses run on PC1–PC3 morphological dataset and a subset of selected traits correlating with dietary habits (table 1; electronic supplementary material, table S2; intestinal length, jaw opening-out lever ratio, jaw closing-in lever ratio, tooth spacing, body shape PC1–PC3 and tooth shape PC1–PC2). p -values were derived from 1000 simulations to test the hypothesis that the observed values are greater than random simulations based on Brownian motion. Asterisks denote statistical significance. * $p < 0.001$.

convergent regime	input data	C1	C2	C3	C4	C5
carnivores	PC1–PC3 axes	0.543 ($p < 0.001$)*	23.57 ($p < 0.001$)*	0.009 ($p < 0.001$)*	0.027 ($p < 0.001$)*	5 ($p = 0.63$)
	selected traits	0.595 ($p < 0.001$)*	1.124 ($p < 0.001$)*	0.011 ($p = 0.091$)	0.032 ($p < 0.001$)*	2 ($p < 0.001$)*
omnivores	PC1–PC3 axes	0.413 ($p < 0.001$)*	17.322 ($p < 0.001$)*	0.007 ($p < 0.001$)*	0.023 ($p < 0.001$)*	4 ($p = 0.69$)
	selected traits	0.400 ($p < 0.001$)*	0.845 ($p < 0.001$)*	0.008 ($p = 0.545$)	0.027 ($p < 0.001$)*	3 ($p < 0.001$)*
herbivores	PC1–PC3 axes	0.561 ($p < 0.001$)*	22.940 ($p < 0.001$)*	0.009 ($p = 0.091$)	0.027 ($p < 0.001$)*	6 ($p = 0.50$)
	selected traits	0.570 ($p < 0.001$)*	1.180 ($p < 0.001$)*	0.011 ($p = 0.363$)	0.033 ($p < 0.001$)*	7 ($p < 0.001$)*

(e.g. within Girellidae, *Scortum* and *Hephaestus*), but several of these occurred on short tree branches, and may represent model fitting issues rather than distinct morphological optima. Results using SURFACE were similar, but detected higher numbers of convergent herbivorous and carnivorous–omnivorous lineages (electronic supplementary material, figure S14). The ℓ_{1ou} results using the pBIC to detect shifts on multivariate PC data were much more conservative, identifying only four shifts in trait evolution across collective PC axes, none of which collapsed into convergent regimes (figure 3b; electronic supplementary material, figure S7). Such contrasts in criterion results are not entirely unexpected: AIC_C-based ℓ_{1ou} tends to be liberal (finding not only many true shifts but also some false positives), whereas ℓ_{1ou} coupled with pBIC tends to exhibit high precision (any detected shifts are typically true) but a low recall rate (i.e. false negatives) [43].

ℓ_{1ou} analyses (using AIC) focusing on specific PC axes identified higher levels of convergence on PC1 (11 total shifts with nine collapses onto multiple convergent optima) aligning closely with dietary habits. All freshwater clades containing specialized herbivores (*Scortum*, *Pingalla*, *Syncomistes* and *Bidyanus*) converged onto similar optima as several marine herbivore clades (*Helotes* and *Kyphosidae*). Several carnivorous–omnivorous clades and the *Kuhlidae*, the epibenthic feeding *Oplegnathidae*, *Microcanthiidae*, *D. capensis* and *Medialuna californiensis* also collapsed onto multiple shared optima (figure 3c; electronic supplementary material, figure S8). While convergent events also occurred on PC2 (four shifts) and PC3 (five shifts), when assessed individually these tended to be of lower number and not clearly associated with diet (electronic supplementary material, figures S9 and S10). Of all the PC axes and several individual traits strongly associated with diet (intestinal length, jaw opening-out and closing-in lever ratios, tooth and body shape PCs), intestinal length (the trait explaining the greatest dietary variation; figure 3d) and PC2 (where the *Kuhlidae* and several elongate terapontid clades converged) were the only traits that demonstrated any significant convergence using the pBIC (electronic supplementary material, figures S11 and S12). Several marine and freshwater predominantly herbivorous–omnivorous clades (*Girellids*—*Pingalla*, *Bidyanus*, *Hephaestus* and the highly herbivorous *Kyphosids*—*Scortum*, *Syncomistes*) converged onto two similar optima with intestinal lengths being

significantly longer than mean trait values (figure 3d; electronic supplementary material, figure S12).

Comparisons of character histories simulated under both a single-peak OU model and a BM model (electronic supplementary material, figure S13) revealed that, while collective PCs and the dominant axis loading most strongly onto diet (PC1) had higher than average levels of convergence, there was little evidence of significantly greater numbers of convergent shifts than expected by chance under BM or OU1 simulated null distributions. Overall ℓ_{1ou} results suggest that convergence onto a complex of multiple adaptive peaks in trophic ecomorphology characterizes macroevolutionary trait changes in the Terapontoidei, particularly on the specific major PCs or traits relating strongly to diet. Evidence for convergence onto essentially identical phenotypes were, however, extremely rare and probably limited to specific traits.

4. Discussion

We found significant evidence supported by several analytical approaches that the Terapontoidei contains multiple replicated evolutionary radiations that produced parallel phenotypic adaptations to similar trophic strategies, often across phylogenetically and geographically distant lineages. While convergence towards carnivorous and omnivorous trophic modes were also evident, the repeated evolution of herbivory from ancestral carnivorous–omnivorous dietary habits seems a recurrent theme in the Terapontoidei macroevolutionary landscape, driving morphological divergence at finer phylogenetic scales, but broader convergence between many clades, often across both marine and freshwaters [49]. Herbivorous species frequently evolved towards a distinctive area of functional morphotype, aligning with previous studies where evolution of herbivorous–detritivorous diets prompted the expansion of fish clades into entirely new areas of phenotypic morphospace [14,50]. The adoption of herbivory places unique functional demands on fishes in terms of food procurement and processing. Similarities in functional adaptations across diverse herbivorous fishes [14,50,51] and our study results (longer intestines, higher-force-transmission jaws, closely packed flattened or multicuspidate dentition) suggest even broader convergence and the feasibility of predictive ‘niche schemes’ [5] may emerge in future comparative studies.

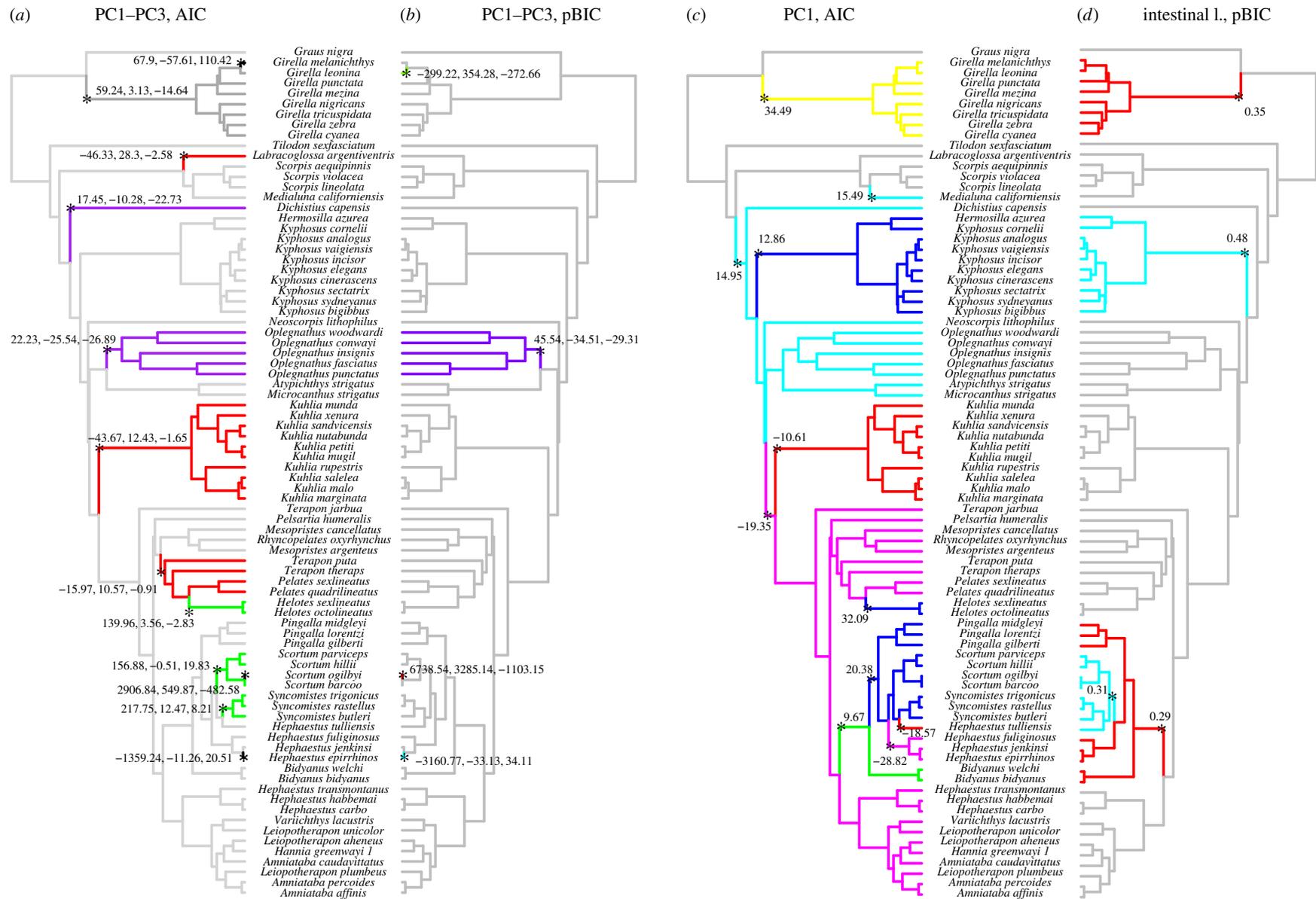


Figure 3. Convergent shifts in Terapontoidei optimum morphology (first three PC axes) with ℓ_{10u} using the AIC_c (a) and the pBIC (b) under a multi-peak Ornstein–Uhlenbeck (OU) process, and for PC1 using the AIC_c (c) and intestinal length using the pBIC (d). Asterisks indicate phenotypic shifts from mean trait values, and edges of the same colour are inferred to have converged to the same selection optimum (trait optima values for each axis indicated).

The collective results from analyses using both *a priori* and *a posteriori* methods, however, suggest the magnitude of convergence identified for all trophic categorizations, while significant, was rarely indicative of ‘complete’ convergence, where disparate taxa have evolved to be essentially phenotypically identical (e.g. as in placental and marsupial/Tasmanian wolves). While there was support for more precise convergence between certain clades, evidence also emerged of multiple optima in all trophic classifications. For example, even in the comparatively distinct region of herbivorous Terapontoidei morphospace, the phenotypic area occupied was still large. Some of these effects may be due to ‘imperfect morphological convergence’ [52], where phylogenetic constraints, or other aspects of ecology limit the strength of convergent evolution between clades. Subtle niche variations in otherwise broad ecological roles probably also increase the area of phenotypic morphospace occupied within broadly similar trophic designations. Differences in specific dietary targets and foraging behaviours of several closely related, nominally ‘herbivorous’ clades included in this study (Kyphosidae–Girellidae and *Scortum*, *Syncomistes*, *Pingalla*) are associated with notable differences in aspects of tooth shape and length and intestinal morphology [15,21,32]. That multiple adaptive peaks occur in functional morphology of such admittedly broadly classified dietary categories is not surprising.

Study results also demonstrate that a clear understanding of the specific ecomorphological convergence being investigated needs empirical testing using explicit analyses. Some of the lack of highly significant phenotypic convergence may be due to the general absence of extreme morphologies across the clade or missing taxa (43 of 132 species not examined). Several traits (such as high force closing-in lever ratios and closely packed dentition) may be functionally flexible, and relevant to procurement of diverse dietary items consumed across the group, such as dislodging attached prey (whether it be attached invertebrates or plant materials). Analyses conducted using individual PCs or specific traits relating specifically to diet, rather than PC axes synthesizing broader morphology, suggested more significant phenotypic shifts and greater strength of convergence in some cases. Intestinal length, for example, was one trait explaining the greatest dietary variation that also demonstrated highly significant convergence across both marine and freshwater environments. One of the most widely identified ecomorphological relationships between vertebrate morphology and ecology [22], it appears the trait most specifically relevant to the repeated evolution of herbivory from carnivory seen across the Terapontoidei. Overall results of significant but imperfect convergence provide further evidence for shared selection promoting diversification because

lineages often differ in phenotypic responses to similar selective demands [52].

5. Conclusion

Lineage convergence to shared adaptive peaks was a dominant mode of macroevolutionary trait change for the often-replicated adaptive radiations seen in the Terapontoidei. Convergent evolution in either entirely freshwater or marine habitats (and typically within single families; although see [11]) has been relatively well studied. The Terapontoidei, however, represents a clade with much deeper levels of convergence, often between different families separated by major phylogenetic gaps (greater than 30 Myr). Most remarkably, this is the first example (to our knowledge) of multiple convergence events occurring across broad salinity gradients spanning one of the most significant macrohabitat transitions available to aquatic organisms: the marine–freshwater interface [19]. While novel in some aspects, the macroevolutionary history of the Terapontoidei is in many ways analogous to the increasing evidence of convergent evolution operating at deeper time scales across different continental faunas [16,17,18]. The repeated evolution of herbivory from ancestral carnivory–omnivory in the Terapontoidei also represents a significant evolutionary step, and one that has had profound implications for the functioning of both marine and continental freshwater food webs [14,20].

Ethics. Animal collection for this study adhered to local guidelines under James Cook University Ethics licence A1787.

Data accessibility. The concatenated sequence data file, tree files and ecological data used in this study are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.4jk02> [53].

Authors’ contributions. R.B.-R performed phylogenetic analyses. A.M.D. collected data, performed comparative analyses and analysed output data. A.M.D. wrote the first draft of the manuscript, and both authors contributed substantially to revisions. Both authors gave their final approval for publication.

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