Delayed Adaptive Radiation among New Zealand Stream Fishes: Joint Estimation of Divergence Time and Trait Evolution in a Newly Delineated Island Species Flock

Christine E. Thacker1,2,*, James J. Shelley3, W. Tyler McCraney4, Peter J. Unmack5 and Matthew D. McGinn6

1Vertebrate Zoology, Santa Barbara Museum of Natural History, 2559 Puesta del Sol, Santa Barbara, CA 93105, USA; 2Research and Collections, Section of Ichthyology, Natural History Museum of Los Angeles County, 900 Exposition Boulevard, Los Angeles, CA 90007, USA; 3National Institute of Water and Atmospheric Research, Gate 10 Silverdale Road Hillcrest, 3216 Hamilton, New Zealand; 4Department of Ecology and Evolutionary Biology, University of California, Los Angeles, 625 Charles E. Young Drive South, Box 957246, Los Angeles, CA 90095-7246, USA; 5Centre for Applied Water Science, Institute for Applied Ecology, University of Canberra, Canberra, ACT 2601, Australia; and 6School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

*Correspondence to be sent to: Vertebrate Zoology, Santa Barbara Museum of Natural History, 2559 Puesta del Sol, Santa Barbara, CA 93105, USA; E-mail: christine.e.thacker@gmail.com.

Received 21 May 2020; reviews returned 23 February 2021; accepted 1 March 2021

Abstract—Adaptive radiations are generally thought to occur soon after a lineage invades a region offering high levels of ecological opportunity. However, few adaptive radiations beyond a handful of exceptional examples are known, so a comprehensive understanding of their dynamics is still lacking. Here, we present a novel case of an island species flock of freshwater fishes with a radically different tempo of adaptive history than that found in many popular evolutionary model systems. Using a phylogenomic data set combined with simultaneous Bayesian estimation of divergence times and trait-based speciation and extinction models, we show that the New Zealand Gobiomorphus gudgeons comprise a monophyletic assemblage, but surprisingly, the radiation did not fully occupy freshwater habitats and explosively speciate until more than 10 myr after the lineage invaded the islands. This shift in speciation rate was not accompanied by an acceleration in the rate of morphological evolution in the freshwater crown clade relative to the other species, but is correlated with a reduction in head pores and scales as well as an increase in egg size. Our results challenge the notion that clades always rapidly exploit ecological opportunities in the absence of competing lineages. Instead, we demonstrate that adaptive radiation can experience a slow start before undergoing accelerated diversification and that lineage and phenotypic diversification may be uncoupled in young radiations. [Adaptive radiation; Electrotridae; freshwater; Gobiomorphus; New Zealand.]

Adaptive radiations have repeatedly shaped the history of life on Earth, but we are only beginning to understand the general dynamics of the process. Historically, studies of adaptive radiation have focused on exceptional examples, such as Galapagos finches (Grant 1999), Caribbean anolis lizards (Rabosky and Glor 2010; Losos 2011), and the young species-rich radiations of East African cichlid fishes (Seehausen 2006). However, the total number of adaptive radiation systems that have been intensively studied is quite small, particularly if closely related adaptive radiations are not as evolutionarily independent as originally thought due to shared ancestral segregating variation (Meier et al. 2017). The number shrinks further once we consider radiations evolving in the same geographic area, such as the many species flocks occupying the Hawaiian Islands (Freed et al. 1987; Gillespie 2004) or the radiations of both invertebrate and vertebrate groups within Lake Tanganyika in eastern Africa (Salzburger et al. 2014). If we seek a general understanding of the process of adaptive radiation, it is imperative to sample lineages both geographically and evolutionarily and distinctively from existing cases.

We present such a system, a radiation of Gobiomorphus (Electrotridae) gudgeons in the freshwaters of New Zealand that possesses many similarities but also some critical differences to existing evolutionary model systems. Gobiomorphus species occur across a range of tectonically active postglacial freshwater and saltwater environments that vary considerably in depth and flow gradients (Bell and Foster 1994; McDowall 2010). One species is predominantly confined to estuaries and the lower reaches of streams (Gobiomorphus gobioides), two species (Gobiomorphus hubbsi and Gobiomorphus huttoni) occur in high-flow coastal streams, one is facultatively amphidromous (Gobiomorphus cotidianus), and one species is limited to a series of small alpine lakes on the South Island (Gobiomorphus alpinus; McDowall 2010). The remaining species (Gobiomorphus basalis and Gobiomorphus breviceps) occur across a range of freshwater habitats and likely contain a range of ecomorphs comparable to threespine stickleback (Gasterosteus aculeatus) in the Northern Hemisphere (Smith et al. 2005; McDowall and Stevens 2007; Craw et al. 2016; Shelley et al. 2020). Crucially, three of the species (G. gobioides, G. hubbsi, and G. huttoni) lack the ability to establish freshwater resident populations due to their planktonic larval period in nearshore marine waters.

Despite the presence of an ecologically diverse range of endemic species evolving in a dynamic island environment, Gobiomorphus have not previously been considered in studies of adaptive radiation. Schluter (2000) listed four primary criteria as necessary to demonstrate adaptive radiation: monophyly of a lineage, rapid speciation, phenotype-environmental correlation,
and adaptation. Confirmation of at least two of these criteria depends heavily on a quality time-calibrated phylogeny, hampering our ability to establish a case for adaptive radiation without such a tree. Gloor (2010)’s phylogenetic perspective delineates three key features of adaptive radiation that overlap with Schröder (2000): monophyly, accelerated diversification, and adaptation (trait utility), and also requires a calibrated phylogeny. In the case of Gobiomorphus, the single comprehensive phylogenetic study of the group used a small fragment of the cytochrome b gene, but the resulting mitochondrial phylogeny was only able to provide strong support for the monophyly of each species, not the relationships among them (Stevens and Hicks 2009).

In this study, we seek to determine whether or not the freshwater resident species of Gobiomorphus constitute an adaptive radiation. We use the novel approach of jointly estimating divergence times along with speciation rates and identifying rate shifts coupled with trait evolution using RevBayes (Höhna et al. 2016). In principle, joint estimation of divergence times and speciation rates can improve upon the common practice of searching for diversification rate shifts on a phylogeny calibrated with the assumption of a single clade-wide rate. We first use phylogenomic methods to produce a species tree topology in order to test whether New Zealand’s endemic Gobiomorphus are monophyletic, and then examine the tempo of speciation, extinction, and freshwater adaptation within New Zealand Gobiomorphus and their close relatives. Our phylogeny is based on hundreds of independent nuclear ultraconserved element (UCE) loci, and the trait we postulate is coupled with a speciation rate increase is the ability to form resident populations in freshwater (loss of amphidromy). We complement our joint RevBayes analysis with the standard sequential approach of phylogeny calibration followed by detection of character-dependent diversification rate shifts with binary-state speciation and extinction (BiSSE; Fitzjohn et al. 2009), as well as evaluating diversification dynamics with the γ statistic and lineage through time (LTT) plots.

To investigate potential phenotypic correlates of diversification rate shifts among New Zealand Gobiomorphus, we use a data set of linear morphometric measurements that capture overall body shape. We analyze these trait data in conjunction with our phylogeny using comparative methods that fit models of evolutionary tempo and mode, in order to determine whether or not the rate or pattern of phenotypic evolution has shifted concordant with the switch to freshwater resident ecology, and evaluate changes in morphological disparity using disparity through time (DTT) analyses. Finally, we discuss possible environmental adaptations for life history characteristics such as egg size and larval morphology. Our analyses confirm that the New Zealand Gobiomorphus constitute an adaptive radiation, one with markedly different dynamics than found in those previously known.

**Materials and Methods**

**Sample Collection, UCE Sequencing, and Phylogenetic Analysis**

We assembled UCE sequence data for 19 individuals, 11 Gobiomorphus from New Zealand and Australia, and 8 outgroup taxa. The 11 Gobiomorphus included all 9 described species, plus 2 additional individuals of both G. basalis and G. breviceps representing northern and southern subpopulations within those species thought to potentially represent divergent species. Gobiomorphus samples were collected using seine nets and backpack electrofisher, and preserved in 99% ethanol. We included outgroup taxa from Eleotridae (Philypnodon grandiceps, Philypnodon macrotomus, Hypseleotris compressa, Hypseleotris kunzingeri, Mogunnda adspersa, and Giurus marginates; all species known from Australian freshwaters), Butidae (Oxyeleotris linolata) and Odontobutidae (Philypnodon griffithisi). Taxa utilized in this study, localities, and voucher information are listed in Supplementary Table S1 (available on Dryad at https://doi.org/10.5061/dryad.xd2547ddw), UCE sequencing methods follow Alfaro et al. (2018) and are available as Supplementary methods on Dryad. Our UCE alignment included 503,008 bp for 427 loci, and the UCE alignment for the 75% complete taxon coverage data set included 503,008 bp for 427 loci, and the 95% complete alignment included 115,236 bp for 93 loci. We estimated the phylogeny for both alignments using maximum likelihood and Bayesian methods, and constructed a species tree using ASTRAL (complete details of analyses are available on Dryad).

**Joint Bayesian Estimation of Divergence Times, Character States, and Diversification Rates**

We used RevBayes v1.0.13 (Höhna et al. 2016) to simultaneously date our ASTRAL topology and examine the dynamics of freshwater adaptation in New Zealand Gobiomorphus and their Australian sister clade (Fig. 1). First, we reduced our alignment to the clade Gobiomorphus + Philypnodon (n = 13) and reduced our UCE loci set to only those with complete sampling for our focal taxa (n = 46), in order to facilitate the computationally intensive RevBayes analysis. We divided each locus into 10 equal partitions and retained only the second and ninth partitions. The partitions in these positions strike a balance between avoiding close proximity to the UCE whereas also avoiding the regions at the very beginning and end of the locus for reasons of both alignment and error rate increases at paired read ends.

To generate a starting tree for RevBayes, we passed the alignment to RAxML v8.2.12 (Stamatakis 2014), treating the front and back portion of each UCE locus as its own partition, then used a GTR+Gamma model and fixed topology from ASTRAL v5.6.3 (Mirarab and Warnow 2015; Sayyari and Mirarab 2016) to estimate branch lengths. We used TreePL (Smith and O’Meara 2012) to create an ultrametric tree from our estimated branch
FIGURE 1. Consistent phylogenomic topology of relationships among Gobiomorphus species and outgroups. This topology was obtained in all analyses: RAxML, ExaBayes, and ASTRAL multispecies coalescent. All nodes were supported at 100% bootstrap in the RAxML analysis except those among G. basalis north, G. cotidianus, and G. alpinus, as shown. All nodes in ExaBayes and ASTRAL analyses were supported with a posterior probability of 1. Images of Gobiomorphus species are specimens from the collection of Te Papa Tongarewa, the National Museum of New Zealand: G. hubbsi (NMNZ P.058616), G. huttoni (NMNZ P.057310), G. gobioides (NMNZ P.056515), G. breviceps south (NMNZ P.004596), G. breviceps north (NMNZ P.004441), G. basalis south (NMNZ P.059959), G. basalis north (NMNZ P.058870), G. cotidianus (NMNZ.F.012657), and G. alpinus (NMNZ P.053309). Images are not scaled to actual size; G. gobioides is the largest species, reaching lengths of 24.0 cm, whereas the other species range from 7.5 to 15.8 cm in length (McDowall 2000).
extinction (HiSSE) model. For all models, we utilized a relaxed clock and GTR + G model. Our prior choices reflect default options suggested for previous RevBayes examples with the addition of the previously described 15.1–35 Ma uniform prior on the root and an exponential prior and clamped fossil date of 20 Ma on the node subtending New Zealand Gobiomorphus, in accordance with abundant skeletal and otolith Gobiomorphus fossils known from the early Miocene of New Zealand, indicating that Gobiomorphus was well established in New Zealand by that time (McDowall et al. 2006; Schwarzhans et al. 2012).

For our BiSSE and HiSSE models, we coded each species by whether or not they were able to establish freshwater resident populations for all Gobiomorphus species as well as the two species of Phylipnodon, based on information in Allen et al. (2002), McDowall (1979), and McDowall (2000). The root state was fixed for “non-freshwater resident.” We ran the MCMC sampler for 200,000 generations with a burn-in of 20%, then used Tracer 1.71 to verify ESS >200 for all key parameters. We then calculated model support using 50 stepping-stones with 1000 states sampled for each step (Xie et al. 2011).

**Sequential Phylegetic Diversification Analyses**

To evaluate diversification dynamics across the Gobiomorphus phylogeny, we first used the γ statistic, calculated with the R package ape (version 5.4-1; Paradis and Schliep 2019). We calculated γ across the phylogeny of New Zealand Gobiomorphus as well as just for the freshwater resident clade and assessed significance with a two-tailed test. We also constructed LTT plots to compare the lineage diversification pattern for the phylogeny using phytools (version 0.7-20; Revell 2012). We calculated LTT for the RevBayes tree as well as a random sample of 1000 trees from the posterior distribution, to account for topological uncertainty. As a check on the joint estimation method, we also use a more traditional sequential approach to detecting trait-dependent diversification shifts. We analyzed the ultrametric treePL phylogeny and ecological data independently with BiSSE using the R package diversitree (version 0.9-13; Fitzjohn 2012), fitting a series of models that varied in their constraints on speciation rate (s), extinction rate (u), and probability of transition between ecological states (q). First, as a baseline, we fit a full model with unconstrained speciation, extinction, and transition rates. Then, we fit several constrained models, imposing equal speciation, extinction, and/or transition rates (and their various combinations) between amphidromy and freshwater resident ecology. We compared fits of models with Akaike information criteria (AIC) and assessed significance using a χ² test. Results of the BiSSE model fitting are presented in Supplementary Table S2 available on Dryad.

**Acquisition and Analysis of Morphometric Trait Data**

In order to determine whether or not morphological evolution co-varied with the change from amphidromy to freshwater resident ecology, we examined preserved specimens of the seven New Zealand Gobiomorphus species (including separate groups of northern and southern individuals for G. basalis, G. breviceps, and G. alpinus; Supplementary Table S3 available on Dryad), for a suite of 12 linear morphological distances plus standard length, as shown in Supplementary Figure S1 available on Dryad. We computed means for each species, natural log transformed those data and corrected for size variation by regressing each measurement against standard length and computing the residuals with the phyRes function, and then performed phylogenetic principal components analysis (PCA) using phyL.pca in the R (version 3.6.2; R Core Team 2019) package phytools. To visualize these results, we superimposed the phylogeny onto a plot of PC1 versus PC2 to create a phylomorphospace, again using phytools.

To evaluate whether or not the phenotypic trait data underwent any rate or mode shifts across the phylogeny, we fitted 10 different single and multiple-rate models to the first four PC axes (accounting for 94% of the trait variance) on the RevBayes tree using the R package mvMORPH (version 1.1.3; Clavel et al. 2015). We incorporated intraspecific standard error of the measurements and compared the fits, using AIC (corrected for small sample size as AICc) and relative AICc weights, of models including single and multiple-rate parameters. We evaluated Brownian motion (BM: trait variances increase over time without restraint), and Ornstein–Uhlenbeck (OU: trait variance is constrained around a mean, consistent with selection) single and multiple-rate models, a model of OU to BM shift, and models incorporating a BM pattern preceding or following a period of early burst dynamics, in which the rate of evolution slows exponentially, consistent with an adaptive radiation. A list of the models is given in Supplementary Table S4 available on Dryad.

We then used geiger (version 2.0.6.4; Pennell et al. 2014) to construct morphological DTT plots based on the first four PC axes and calculate the morphological disparity index (MDI). We plotted the observed DTT for each PC based on the phylogeny, superimposed on a simulated range of 1000 trees generated under a BM model. The MDI represents the difference between the observed and simulated trajectories, with strongly negative MDI values consistent with an early burst of trait evolution. We also performed node height tests individually for PC1-4 to gauge whether or not the independent contrast of each trait was correlated with subtending node height. A significant correlation indicates non-Brownian evolution, with an early burst pattern manifesting as a positive slope and a late burst (niche-filling model) indicated by a negative slope (Freckleton and Harvey 2006).
RESULTS
Phylogenetic and Joint Bayesian Analyses of Divergence and Diversification

The Maximum Likelihood (ML) and Bayesian topologies for both the 95% and 75% complete alignments were identical, as were the node support values (bootstrap and posterior probability), except for slight differences in the bootstrap support for nodes subtending \textit{G. basalis}, \textit{G. cotidianus}, and \textit{G. alpinus}. All nodes were supported with posterior probability of one in the Bayesian analyses, and all obtained 100% bootstrap support in the ML analysis, except the \textit{G alpinus} + \textit{G. cotidianus} node and the node subtending \textit{G. alpinus}, \textit{G. cotidianus}, and the northern population of \textit{G. basalis}, supported at 96–98%-bootstrap. Our ASTRAL topology was identical to the ML and Bayesian topologies (Fig. 1), with posterior probability of 1 for all nodes. In all analyses, we recovered the Australian \textit{Gobiomorphus + Philipnodon} as sister to the New Zealand \textit{Gobiomorphus}. \textit{Gobiomorphus breviceps} was recovered as monophyletic, with separate northern and southern lineages, but \textit{G. basalis} comprised two distinct clades.

Our stepping-stone analysis indicated that the BiSSE model had the highest log-likelihood (−24,106.72) in comparison to the one-rate model (−24,109.34), with the HISSE model having the lowest (−24,109.96). Transformation of these log-likelihoods to Bayes factors (BF) indicates strong support for the BiSSE model (BF = 13.73) and does not support the HISSE model (BF = 0.53). In the BiSSE model, freshwater resident taxa had a speciation rate approximately an order of magnitude above the background rate (0.03600 vs. 0.03989) and a similarly elevated extinction rate (0.07300 vs. 0.00922). All models suggested that the evolution of the rapidly diversifying freshwater crown clade of \textit{G. alpinus}, \textit{cotidianus}, \textit{basalis}, and \textit{breviceps} occurred between 2 and 7 Ma, more than 10 myr after fossils indicated the clade arrived in New Zealand (skeletal and otolith fossils are abundant in the early Miocene, 16–20 Ma). The calibrated hypothesis, shown as a DensiTree visualization of 500 ultrametric trees sampled from the posterior of our BiSSE model, is given in Figure 2.

Sequential Analyses of Phylogenetic Diversification

Values of the $y$ statistic for the phylogeny including all New Zealand \textit{Gobiomorphus} as well as just the freshwater resident clade were not significant based on a two-tailed test (all NZ \textit{Gobiomorphus}: $y$ = 0.403, $P = 0.629$; FW clade only: $y = −0.392$, $P = 0.695$), indicating no support for an early burst in lineage diversification. LTT plots confirm this pattern (Fig. 4), showing a nearly constant pattern of lineage accumulation over time, but with a slight deflection in the first half of the clade’s history, concordant with the lag in speciation prior to the establishment and radiation of the freshwater resident clade. Sequential state-dependent diversification analyses with BiSSE confirmed that transition to freshwater resident ecology was associated with an increase in speciation rate; models in which the habitat-specific speciation rates were constrained to be equal yielded significantly worse fits than the unconstrained model (Supplementary Table S2 available on Dryad). When speciation rates were unconstrained, speciation rates in the freshwater resident clade were consistently estimated to have undergone much higher rates of speciation (estimated at 0.343 for freshwater resident lineages vs. 0.014 for the amphidromous lineages). We additionally performed a Bayesian analysis of the full BiSSE model, with speciation, extinction, and transition rates all unconstrained. That analysis yielded estimates of speciation rate for the freshwater resident lineages of 0.152, and for the amphidromous lineages of 0.026. Extinction rates were also elevated in the freshwater resident lineages, although not extremely, with a rate of 0.057 versus 0.035 for amphidromous lineages. Graphs of the probability density distributions of these parameters are given in Supplementary Figures S2 and S3 available on Dryad.

PhyloMorphospace and Disparity Analyses of Morphometric Trait Data

Phylogenetic PCA of the morphometric data indicated that the first two PC axes collectively accounted for 81.2% of the variation (58.3% on PC1 and 22.9% on PC2); a phyloMorphospace plot of PC1 versus PC2 is shown in Figure 3. In this plot, the freshwater resident species are clustered in the lower-left portion of the morphospace, representing relatively shorter and squatter bodies and heads, in contrast to the amphidromous species, particularly \textit{G. huvbsi}, which are more elongate and slender. Separation on PC1 indicates change in body width and depth, whereas PC2 accounts for variation in head dimensions. We used data from PC1 to PC4, accounting for 94% of the total variation, for analysis of evolutionary dynamic models with \textit{mvMORPH}. We found that the most highly favored model was a single rate BM model, with no shifts favored (AICc weight 0.651; Supplementary Table S4 available on Dryad), with some support for the BM → Early Burst model (AICc weight 0.189) and somewhat less support for the Early Burst → BM model (AICc weight 0.159). We obtained similar results for the DTT plots for PC axes 1–4 (Fig. 4), with no significant deviations from a BM model for trait evolution across the history of the clade. Node height tests for each of the PC axes confirmed this pattern, with no values significantly deviating from a BM pattern (PC1 $t = 0.355$, $P = 0.735$; PC2 $t = −0.090$, $P = 0.931$; PC3 $t = −0.363$, $P = 0.729$; PC4 $t = −0.903$, $P = 0.402$).

DISCUSSION
New Zealand Freshwater Resident Gobiomorphus as an Adaptive Radiation

Our results indicate that the freshwater resident \textit{Gobiomorphus} species of New Zealand satisfy the
criteria of an adaptive radiation. The first condition (monophyly) for adaptive radiation is met (Schluter 2000; Glor 2010), as shown in Figure 1. We find that the New Zealand Gobiomorphus are monophyletic, as are the crown clade of freshwater resident species. The Australian species Gobiomorphus australis and Gobiomorphus coxii, however, are most closely related to the Australian species of Philypnodon. Gobiomorphus have a similar overall morphology to Philypnodon, with commonalities including a shared general body shape and overlapping meristic counts, although Philypnodon species have notably larger mouths and opercular
openings (McDowall 1975; Allen et al. 2002). This result is not in conflict with the earlier phylogeny of Stevens and Hicks (2009) because Philypnodon is their only included outgroup and their hypothesis re-rooted yields the topology we obtained. Our results suggest that reallocation of Australian Gobiomorphus to Philypnodon is likely warranted. The second criterion for adaptive radiation is an acceleration in the rate of species diversification (early burst pattern) and sometimes a concordant burst in phenotypic trait diversification (Harmon et al. 2010; Yoder et al. 2010; Colombo et al. 2015; Puttick 2018). Both our joint estimation (RevBayes) and sequential calibration/BiSSE analyses detected a significant elevation in speciation rate for the freshwater resident Gobiomorphus. The power of BiSSE models for detecting rate shifts in clades this small has been questioned (Davis et al. 2013), but it is notable that all of the BiSSE analyses tried showed significant speciation rate increases, ranging from roughly 5 to 25 times those found in the amphidromous species. In contrast, the y statistic did not indicate an early burst of speciation, although the value for the freshwater resident clade was slightly negative at −0.392. This result may simply indicate that the freshwater Gobiomorphus radiation is young and still actively speciating.

The final criteria for adaptive radiation are phenotype–environment correlation and adaptation. Separation of the freshwater resident from the amphidromous species in morphospace supports the link between phenotype and novel environment, and indicates that a squatter, more robust overall shape is correlated with freshwater resident ecology. There is also a convergent pattern among the freshwater species in morphospace, and potentially a reduction in disparity as compared with the amphidromous taxa, although the representation of those species is low in this small clade. The moveMorph and DTT analyses did not indicate a change in the rate or mode of shape evolution at that transition, but as with the y statistic, it may be that a shift is not yet be apparent because the radiation is too young or that the clade is too small for the trait evolution models to detect a shift. Empirical studies on trait diversification in adaptive radiations have yielded mixed results, with rate shifts detected among some groups (López-Fernández et al. 2013; Colombo et al. 2015; Puttick 2018), but not others (Harmon et al. 2010; Derryberry et al. 2011; Burress and Wainwright 2018). It is possible that Gobiomorphus simply are not manifesting significant overall shape changes in the freshwater resident species, but it is also likely that phenotypic divergence is still ongoing. Although the most highly favored model for trait evolution was single rate BM, the next highest favored was BM → early burst. These metrics are consistent with the earliest stages of
both lineage and trait radiation among the freshwater resident *Gobiomorphus*.

The criterion of adaptation is also supported by more subtle morphological and life history traits. The freshwater species possess larger eggs and larvae (eggs 1.5–2.3 mm diameter in *G. breviceps*, *G. basalis*, and *G. alpinus* vs. 0.8–1.0 in *G. hubbsi*, *G. huttoni*, and migratory *G. cotidianus*), increased vertebral counts, and reductions in head scoliation and sensory pores (McDowall 1990; McDowall 2000; McDowall and Stevens 2007; Michel et al. 2008; Stevens and Hicks 2009; Closs et al. 2013). One species, *G. cotidianus*, includes both amphidromous and freshwater resident populations, which are divergent in these sensory and life history characters as well as in spawning time, likely adaptations to the loss of the migratory and marine larval development stages. Those populations also display partial genetic isolation and could be in the process of speciating (Michel et al. 2008). A similar pattern is seen among populations of the goby *Rhinogobius formosanus* in Taiwan, in which landlocked freshwater resident populations experience shorter larval durations than their amphidromous counterparts (Liao et al. 2020). If freshwater resident *Gobiomorphus* do represent a young adaptive radiation, it is possible that functional characters associated with freshwater residency, including fin ray morphology adaptations to high flow (Kane and Higham 2012), as well as characters associated with suction feeding performance such as gape size and epaxial musculature (McGee et al. 2013), may simply have not yet arisen.

### Lag Time between Establishment in NZ and Full Colonization of Freshwater Habitat

The calibrated phylogenetic hypothesis shown in Figure 2 yields a timescale for *Gobiomorphus* diversification that corresponds well both with previous studies (Thacker 2017), and with the tectonic history of New Zealand (Smith et al. 2005; McDowall and Stevens 2007; Shelley et al. 2020). We infer that the earliest diverging New Zealand species are the widespread, amphidromous taxa *G. hubbsi*, *G. huttoni*, and *G. gobioides*. The high dispersal ability of amphidromous *Gobiomorphus* explains their transit from Australia to New Zealand during the Miocene, and also the presence of *Gobiomorphus huttoni* at the Chatham Islands 800 km to the east (Skrzynski 1967). Their estimated invasion of the islands in the Miocene by 16–20 Ma is just after the
The most notable feature of the *Gobiomorus* phylogeny is the lag in colonization of fully freshwater habitats. The amphidromous species diversified relatively slowly throughout the Miocene, followed by the origin of the freshwater crown clade over 10 myr later at the Miocene-Pliocene boundary. A similar pattern may exist in mudfish (*Neochanna* species), in which a radiation in New Zealand freshwaters (including cryptic species) followed the loss of diadromy after the likely invasion of a diadromous ancestor from Australia (McDowall 1997; Clineson et al. 1999; Waters and McDowall 2005). There are several possible causes for this dramatic lag time.

One possibility is that adaptation to freshwater was dependent on a range of traits not yet possessed by the invading lineage. Full freshwater adaptation necessitates abandonment of a planktonic marine larval stage, as well as a likely shift to lower fecundity with larger eggs and larvae (Closs et al. 2013), a pattern seen in *Gobiomorphus*. It is unclear why up to 10 myr were required to evolve these traits, though we note that *Gobiomorus* individuals colonizing a newly formed New Zealand would likely not have had access to the standing variation that would facilitate freshwater adaptation in the same manner as threespine stickleback (Schlüter and Conte 2009).

Another factor that is likely to have influenced the diversification of freshwater resident *Gobiomorus* is the tectonic activity of their habitats. Our analyses indicate that rapid diversification in *Gobiomorus* is associated with the evolution of species capable of forming freshwater resident populations (Smith et al. 2003; McDowall and Stevens 2007; Craw et al. 2016; Shelley et al. 2020). We also note that both speciation and extinction rates are elevated in freshwater *Gobiomorphus*. The increased extinction rate could be related to the colonization of unstable habitats subject to both glacial and tectonic activity. Extensive mountain building on the South Island has occurred over the past 5 Ma, and intense, widespread volcanism has taken place on the North Island, particularly over the last 2 Ma. The region has also experienced Pleistocene glacial cycles in which waterways have been fragmented and altered over fairly long timescales (McDowall 1996; Craw et al. 2016; Shelley et al. 2020). It is possible that a contributory factor to the observed lag time in full freshwater colonization is that early invaders were extirpated by landscape disruptions before the current established radiation was able to take hold. Once established, the restriction of gene flow among freshwater resident populations in allopatry, in contrast to the much higher gene flow resulting from oceanic mixing among populations of the amphidromous species, could potentially contribute to the increase in speciation, as seen in Australian *Galaxias* (Ovendon and White 1990) and Japanese freshwater gobies (Kano et al. 2012).

**CONCLUSIONS**

Our combination of phylogenomic species tree topology estimation combined with joint Bayesian estimation of divergence times, speciation, extinction, and trait evolution has resulted both in a methodological advance over previous sequential pipelines and evidence for a new island species flock available to evolutionary biologists. We confirm the results of the joint Bayesian analysis with sequential analyses of phylogenetic calibration followed by identification of trait-linked diversification shifts, show that amphidromous and freshwater resident *Gobiomorphus* occupy distinct regions of body shape morphospace, and identify adaptive life history traits such as egg and larval size that are correlated with the shift to a freshwater resident ecology. We postulate that the adaptive radiation of *Gobiomorphus* in the freshwaters of New Zealand represents a young, actively evolving lineage in the early stages of adaptive radiation, and thus an opportunity to track the genomic and adaptive patterns of speciation in progress.

**SUPPLEMENTARY MATERIAL**

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.xd2547ddw

**FUNDING**

This project was funded by the Strategic Science Investment Fund from the Ministry of Business Innovation and Employment administered through the National Institute for Water and Atmospheric Research, Hamilton. We also acknowledge funding from the Waikato Regional Council, NZ. All collections by the authors were made under Ministry for Primary Industries Special Permit 597.

**ACKNOWLEDGMENTS**

Daniel Geiger took the specimen photos presented in Figures 1 and 3 and Supplementary Figure S1 available on Dryad. Comments from Prosanta Chakrabarty and one anonymous reviewer greatly improved the manuscript. We are particularly grateful to Andrew Stewart (Te Papa Tongarewa, the National Museum of New Zealand) for processing and shipping a specimen loan of *Gobiomorphus* during a global pandemic.

**REFERENCES**

Allard M.E., Faircloth B.C., Harrington R.C., Sorenson L., Friedman M., Thacker C.E., Oliveros C.H., Cerrity D., Near T.J. 2018. Explosive diversification of marine fishes at the Cretaceous-Palaeogene boundary. Nature Ecol. Evol. 2:688-696.

Allen G.R., Midgley S.H., Allen M. 2002. Field guide to the freshwater fishes of Australia. Perth: Western Australian Museum. 394 p.

Bell M.A., Foster S.A. 1994. Introduction: the evolutionary biology of the threespine stickleback. In: Bell M.A., Foster S.A., editors. The evolutionary biology of the threespine stickleback. Oxford UK: Oxford University Press. 1-27.
Burress E.D., Wainwright P.C. 2018. Adaptive radiation in lified fishes: a central role for functional innovations during 65 My of relentless diversification. Evolution. 72:346–359.

Chapple D.G., Ritchie P.A., Daugherty C.H. 2009. Origin, diversification, and systematics of the New Zealand skink clade (Reptilia: Scincidae). Mol. Phylogenet. Evol. 52:470–477.

Clavel J., Escarguel G., Mecereon G. 2015. mvMORPH: An R package for fitting multivariate evolutionary models to morphometric data. Methods Evol. Syst. 6:1311–1319.

Closs G.P., Hicks A.S., Jemison J.V., F. 2006. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Davis M.P., Milford F., C. 2013. Exploring power and parameter estimation of the BIStE method for analyzing species diversification. BMC Evol. Biol. 13:38.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Davis M.P., Milford F., C. 2013. Exploring power and parameter estimation of the BIStE method for analyzing species diversification. BMC Evol. Biol. 13:38.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.

Fitzjohn R.G., Maddison W.P., Otto S.P. 2009. Estimating trait-adapted dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fitzjohn R.G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

Freckleton R.P., Harvey P.H. 2006. Detecting non-Brownian trait evolution in R. Methods Ecol. Evol. 7:36–37.

Craw D.P., Upton P., Burridge C.P., Waters J.M. 2016. Rapid biological speciation driven by tectonic evolution in New Zealand. Nat. Geosci. 9:140.

Derryberry E.P., Clark L., D.H., Cracraft J., Alho A., Pérez-Emán J., REMO. 2015. Lineage diversification and morphological evolution in a large-scale continental radiation: The Neotropical ovenbirds and woodcreepers (Aves: Furnariidae). Evolution. 65:2973–2986.
Smith P.J., McVeagh S.M., Allibone R. 2003. The Tarndale bully revisited with molecular markers: an ecophenotype of the common bully Gobiomorphus cotidianus (Pisces: Gobiidae). J. R. Soc. N. Z. 33:663–673.

Smith P.J., McVeagh S.M., Allibone R. 2005. Extensive genetic differentiation in Gobiomorphus breviceps from New Zealand. J. Fish Biol. 67:627–639.

Smith S.A., O’Meara B.C. 2012. treePL: divergence time estimation using penalized likelihood for large phylogenies. Bioinformatics. 28:2699–2700.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30:1312–1313.

Stevens M.I., Hicks B.J. 2009. Mitochondrial DNA reveals monophyly of New Zealand’s Gobiomorphus (Teleostei: Eleotridae) amongst a morphological complex. Evol. Ecol. Res. 11:109–123.

Thacker C.E. 2017. Patterns of diversification in fish species separated by the Isthmus of Panama. BMC Evol. Biol. 17:111.

Vanderpham J.P., Nakagava S., Claus G.P. 2013. Habitat-related patterns in phenotypic variation in a New Zealand freshwater generalist fish, and comparisons with a closely related specialist. FW Biol. 58:396–408.

Waters J.M., McDowall R.M. 2005. Phylogenetics of the Australasian mudfishes: evolution of an eel-like body plan. Mol. Phyl. Evol. 37:417–425.

Xie W., Lewis P.O., Fan Y., Kuo L., Chen M.H. 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. Syst. Biol. 60:150–160.

Yoder J.B., Clancy E., Des Roches S., Eastman J.M., Gentry L., Godsoe W., Hagey T.J., Jochimsen D., Oswald B.P., Robertson J., Sarver B.A.J., Scheck J., Spear S.F., Harmon L.J. 2010. Ecological opportunity and the origin of adaptive radiations. J. Evol. Biol. 23:1581–1596.