

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

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**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; Eleotridae; 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 distinct from existing cases.

We present such a system, a radiation of *Gobiomorphus* (Eleotridae) 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-environment 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. Glor (2010)'s phylogenetic perspective delineates three key features of adaptive radiation that overlap with Schluter (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 summary 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  $\gamma$  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 macrostomus*, *Hypseleotris compressa*, *Hypseleotris klunzingeri*, *Mogurnda adspersa*, and *Giurus margaritacea*; all species known from Australian freshwaters), Butidae (*Oxyeleotris lineolata*) and Odontobutidae (*Perccottus glenni*). 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 for the 75% complete taxon coverage data set included 530,304 bp for 416 loci, and the 95% complete alignment included 116,160 bp for 89 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 GTRGAMMA 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

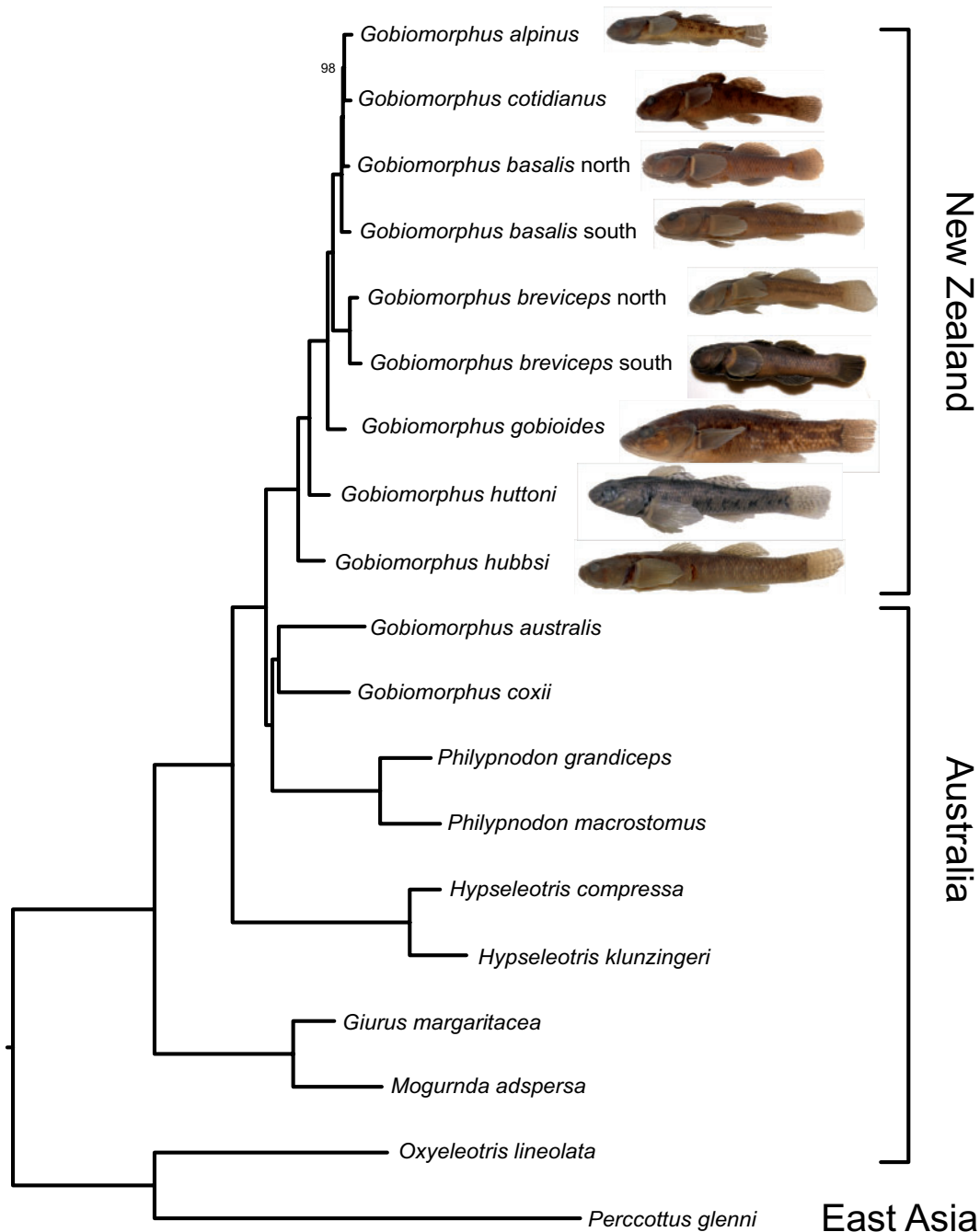


FIGURE 1. Consistent phylogenomic topology of relationships among *Gobiomorphus* species and outgroups. This topology was obtained in all analyses: RAxML, ExaBayes, and ASTRAL multispecies coalescent. All nodes were supported at 100% bootstrap in the RAxML analysis except those among *G. basalis* north, *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.037510), *G. gobioides* (NMNZ P.056515), *G. breviceps* south (NMNZ P.004596), *G. breviceps* north (NMNZ P. 004441), *G. basalis* south (NMNZ P.058915), *G. basalis* north (NMNZ P.058870), *G. cotidianus* (NMNZ P.012637), and *G. alpinus* (NMNZ P.003109). 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).

lengths, utilizing a root age between 15.1 and 35.1 Ma. This date range corresponds to the 95% highest posterior density of the age estimate for the clade of *Gobiomorphus* + *Philypnodon*, resolved in the only available calibrated Eleotridae phylogeny that contains those taxa (Thacker

2017). We tested a total of three models in RevBayes: a typical one-rate model of speciation and extinction rate, a BiSSE model, and a hidden state speciation and 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 15.1–35.1 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; Schwarzhan 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 *Philypnodon*, based on information in Allen et al. (2002), McDowall (1975), 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 Phylogenetic Diversification Analyses

To evaluate diversification dynamics across the *Gobiomorphus* phylogeny, we first used the  $\gamma$  statistic, calculated with the R package *ape* (version 5.4-1; Paradis and Schliep 2019). We calculated  $\gamma$  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 ( $\lambda$ ), extinction rate ( $\mu$ ), 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  $\chi^2$  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* and *G. breviceps*). We measured a total of 177 undistorted, adult individuals (20 from each species or population except for 17 individuals of *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 *phyl.resid* 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 7 different single and multiple-rate models to the first four PC axes (accounting for 95% 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 *G. basalis*, *G. cotidianus*, and *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 *G. alpinus* + *G. cotidianus* node, which was supported at 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 *Gobiomorphus* + *Philypnodon* as sister to the New Zealand *Gobiomorphus*. *Gobiomorphus breviceps* was recovered as monophyletic, with separate northern and southern lineages, but *G. basalis* comprised two distinct clades.

Our stepping-stone analysis indicated that the BiSSE model had the highest log-likelihood (−25,161.09) in comparison to the one-rate model (−25,165.62), with the HiSSE model having the lowest (−25,165.31). Transformation of these log-likelihoods to Bayes factors (BF) indicates strong support for the BiSSE model (BF = 68.71) and does not support the HiSSE model (BF = 1.36). In the BiSSE model, freshwater resident taxa had a speciation rate nearly an order of magnitude above the background rate (0.3180 vs. 0.0428) and a similarly elevated extinction rate (0.0693 vs. 0.0047). All models suggested that the evolution of the rapidly evolving freshwater crown clade of *G. alpinus*, *cotidianus*, *basalis*, and *breviceps* occurred between 1 and 6 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  $\gamma$  statistic for the phylogeny including all New Zealand *Gobiomorphus* as well as just the freshwater resident clade were not significant based on a two-tailed test (all NZ *Gobiomorphus*:  $\gamma=0.335$ ,  $P=0.738$ ; FW clade only:  $\gamma=-0.636$ ,  $P=0.525$ ), 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 0.197 for freshwater resident lineages vs. 0.033 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 0.150, and for the amphidromous lineages of 0.024. Extinction rates were also elevated in the freshwater resident lineages, although not extremely, with a rate of 0.670 versus 0.041 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 82.0% of the variation (59.0% on PC1 and 23.0% 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 *G. hubbsi*, 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 95% of the total variation, for analysis of evolutionary dynamic models with *mvMORPH*. We found that the most highly favored model was a single rate BM model, with no shifts favored (AICc weight 0.567; Supplementary Table S4 available on Dryad), with some support for the BM → Early Burst model (AICc weight 0.283) and somewhat less support for the Early Burst → BM model (AICc weight 0.197). 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  $t=0.301$ ,  $P=0.774$ ; PC2  $t=-0.102$ ,  $P=0.922$ ; PC3  $t=-0.527$ ,  $P=0.617$ ; PC4  $t=-0.753$ ,  $P=0.480$ ).

## DISCUSSION

*New Zealand Freshwater Resident Gobiomorphus as an Adaptive Radiation*

Our results indicate that the freshwater resident *Gobiomorphus* species of New Zealand satisfy the

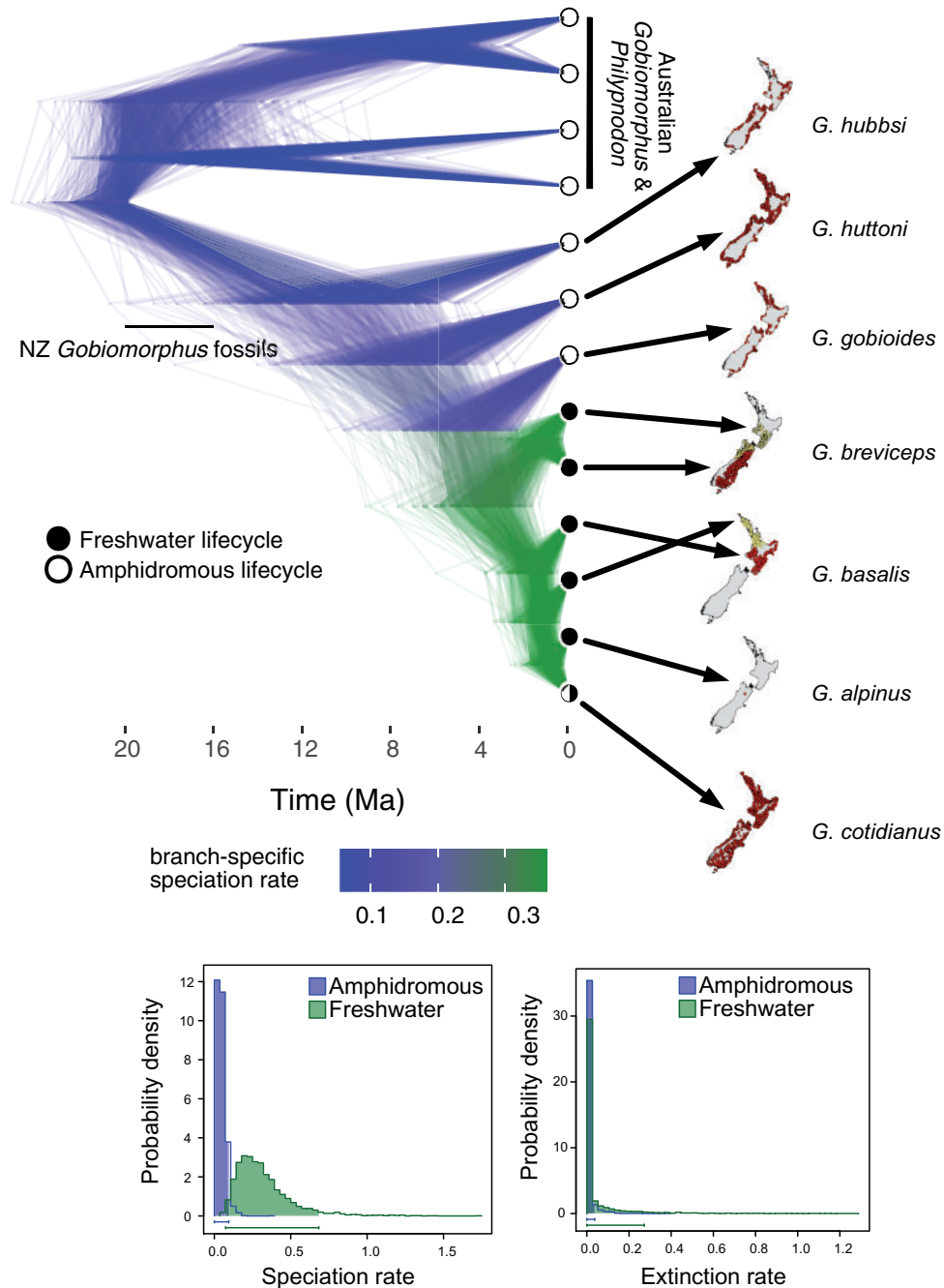


FIGURE 2. Joint estimation of divergence time and diversification rates in New Zealand *Gobiomorphus* and close relatives. DensiTree visualization shows 500 ultrametric trees sampled from the posterior of our BiSSE model. A bar indicates the age range of Miocene *Gobiomorphus* fossils from New Zealand. Lower panels depict probability density distributions of speciation rate (left) and extinction rate (right) for amphidromous (blue) and freshwater resident (green) lineages. Tip symbols indicate a freshwater or amphidromous lifecycle; note that one freshwater species, *G. cotidianus*, can also utilize an amphidromous lifecycle in some populations. Each species' occurrence data are superimposed on a map of New Zealand, showing predominantly coastal populations of the amphidromous species not able to form freshwater resident populations but full invasion New Zealand's freshwater habitats by the species that can.

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

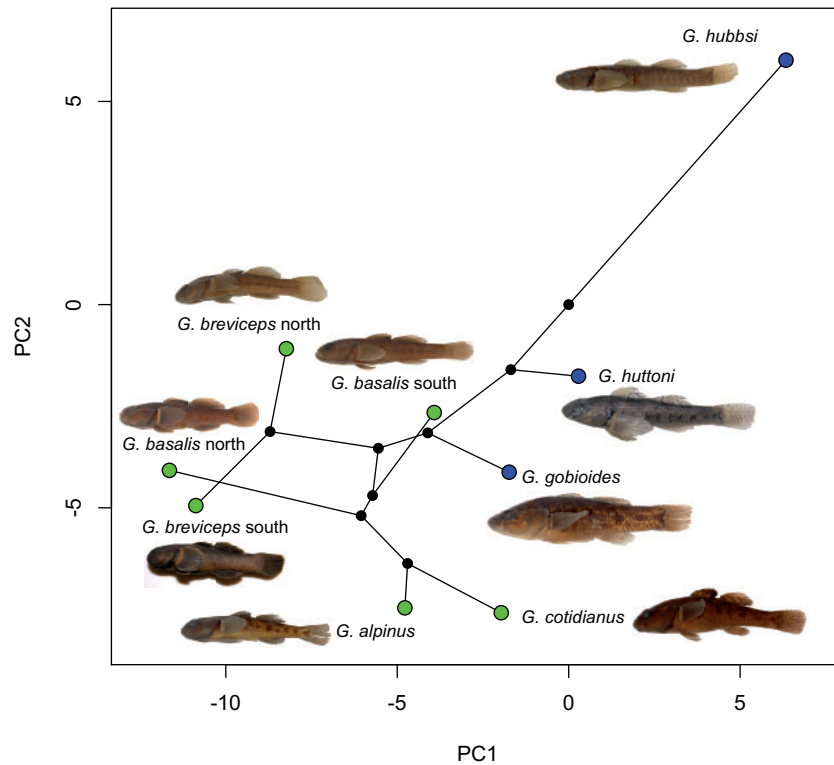


FIGURE 3. Phylomorphospace (plot of PC1 vs. PC2 with phylogeny superimposed) for *Gobiomorphus* species. Fish images are the same as those used in Figure 1. The species that can establish resident freshwater populations are highlighted in green; amphidromous species are highlighted in blue. The amphidromous species are arrayed to the upper right of the phylomorphospace, with the most elongate and slender species (*G. hubbsi*) the most divergent from the remainder. Freshwater resident species are generally more stout and compact and are clustered in the lower-left portion of the phylomorphospace.

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  $\gamma$  statistic did not indicate an early burst of speciation, although the value for the freshwater resident clade was slightly negative at  $-0.636$ . 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 *mvMorph* and DTT analyses did not indicate a change in the rate or mode of shape evolution at that transition, but as with the  $\gamma$  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; Burrell 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  $\rightarrow$  early burst. These metrics are consistent with the earliest stages of

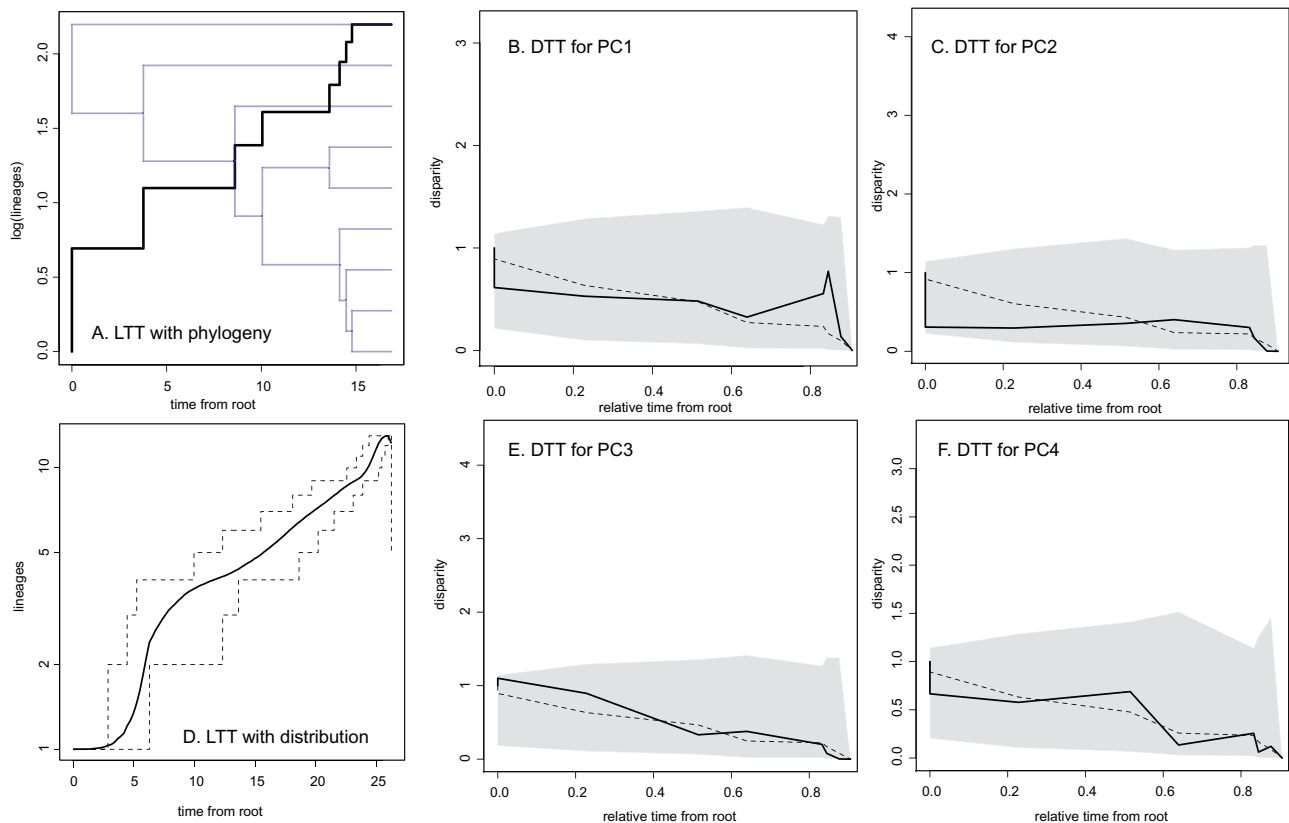


FIGURE 4. LTT and DTT plots for *Gobiomorphus*. LTT plots are shown in a and d. a) LTT curve for New Zealand species, superimposed on phylogeny. d) LTT curve shown over the range of results for 1000 randomly selected topologies from the RevBayes posterior distribution. DTT plots (b, c, e, and f) for PC axes show the observed DTT (solid line) superimposed on the DTT calculated on a set of 1000 trees (mean value represented by the dotted line, gray zone = 5% and 95% quantiles). b) DTT plot for PC1. c) DTT plot for PC2. e) DTT plot for PC3. f) DTT plot for PC4.

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 scalation and sensory pores (McDowall 1990; McDowall 2000; McDowall and Stevens 2007; Michel et al. 2008; Stevens and Hicks 2009; Closs et al. 2013; Vanderpham 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 Australasia 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



emergence of landforms following their Oligocene-early Miocene drowning, and took place during the same time period as the invasion (presumably by rafting) of terrestrial skinks (Chapple et al. 2009).

The most notable feature of the *Gobiomorphus* 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; Gleeson 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 *Gobiomorphus* 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 (Schluter and Conte 2009).

Another factor that is likely to have influenced the diversification of freshwater resident *Gobiomorphus* is the tectonic activity of their habitats. Our analyses indicate that rapid diversification in *Gobiomorphus* 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 recent timescales (McDowall 1996; Craw et al. 2016; Shelley et al. 2020). It is possible that a contributor 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>

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