

RESEARCH ARTICLE

Genome-wide analysis resolves the radiation of New Zealand's freshwater *Galaxias vulgaris* complex and reveals a candidate species obscured by mitochondrial capture

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Abstract

Aim: Freshwater fish radiations are often characterized by multiple closely related species in close proximity, which can lead to introgression and associated discordance of mitochondrial and nuclear characterizations of species diversity. As a case in point, single locus nuclear versus mitochondrial analyses of New Zealand's stream-resident *Galaxias vulgaris* complex have yielded conflicting phylogenies. Our goal is to use genome-wide divergence patterns among these fishes to evaluate the potential role of mitochondrial capture in obscuring species diversity, and to understand how ancient and anthropogenic drainage modification explain this diversity.

Location: Freshwater ecosystems of New Zealand.

Methods: We generate and analyse a genome-wide dataset comprising 52,352 SNPs across 187 *Galaxias* specimens to resolve the phylogeny of this recent fish radiation. We conduct phylogenetic, PCA, STRUCTURE, and ABBA-BABA analyses to evaluate the evolutionary relationships of lineages in the context of natural and anthropogenic river drainage alterations.

Results: In addition to the 11 previously recognized stream-resident lineages, genome-wide data reveal a twelfth candidate species (G. 'Pomahaka'), apparently obscured by introgressive mitochondrial capture. We identify additional examples of mito-nuclear discordance and putative mitochondrial capture, likely mediated by geological and anthropogenic modification of drainage boundaries.

Main conclusions: Our study highlights the need for genome-wide approaches for delimiting freshwater biodiversity. Genetic data also reveal the influence of drainage history on freshwater biodiversity, including the rapid divergence of recently fragmented fish populations, and the conservation genetic risks of anthropogenic translocations events.

KEYWORDS

biodiversity, biogeography, conservation, cryptic species, fish, freshwater, genomic, hybridisation

1 | INTRODUCTION

As anthropogenic pressures on freshwater ecosystems increase, reliable delimitation of species is increasingly important for the conservation of freshwater biological diversity (Allendorf et al., 2022; Closs et al., 2016; Olden et al., 2010). To this end, the analysis of genome-wide data is transforming our understanding of freshwater biodiversity across a range of spatial and evolutionary scales (Melo et al., 2021; Ronco et al., 2021). Indeed, freshwater fish assemblages often exhibit astonishing levels of diversity considering the restricted nature of habitats they occupy (Adams et al., 2014; Raadik, 2014; Ronco et al., 2021; Shelley et al., 2018). This high diversity may be partly explained by divergence of isolated freshwater populations in the absence of marine links through salinity tolerance or migratory (diadromous) life history (Burridge & Waters, 2020; Delgado et al., 2020; Ward et al., 1994; Waters et al., 2020a). In addition to these effects of geographic isolation per se (Waters et al., 2020a), dynamic geological processes (Craw et al., 2015; Waters et al., 2001a, 2020b) and rapid adaptive shifts can also contribute substantially to freshwater biological diversification (Barluenga et al., 2006; Melo et al., 2021; Ronco et al., 2021).

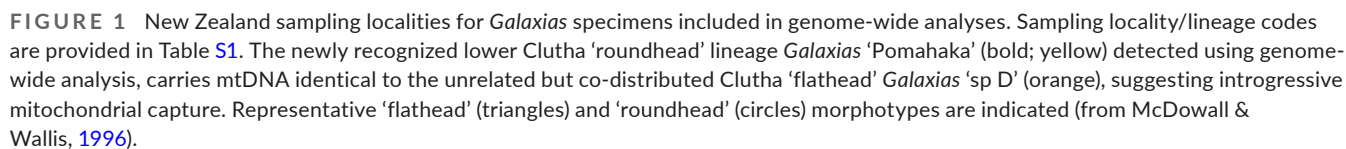
Freshwater-limited fish radiations are often characterized by multiple closely related species in close proximity, and hence are particularly prone to hybridization and introgression (Irisarri et al., 2018; MacGuigan & Near, 2019; Wallis et al., 2017; Waters et al., 2010). This mixing may be exacerbated via human-driven range shifts (Blackwell et al., 2021; Esa et al., 2000), further complicating the estimation of phylogenetic relationships and systematics. Given that distinct genomic regions can vary in their propensities for introgression, freshwater lineages are particularly prone to mitonuclear discordance (Ford et al., 2019; Wallis et al., 2017). As a case in point, introgressive *mitochondrial capture* (Perea et al., 2016; Unmack et al., 2011; Willis et al., 2014) – whereby the mitochondrial DNA (mtDNA) of one lineage is completely replaced by that of another – represents the most extreme form of such discordance and has potential to severely compromise the detection and conservation of biodiversity. However, the recent application of genome-wide data (e.g. Elshire et al., 2011) to freshwater systematics has begun to resolve such discordance (Buckley et al., 2018; Perea et al., 2016; Unmack et al., 2017), enhancing the recognition and conservation of freshwater biodiversity.

The widespread ‘Gondwanan’ galaxiid fishes represent a key component of the Southern Hemisphere's freshwater fish fauna, comprising a combination of diadromous and freshwater-limited taxa (Burridge et al., 2012; McDowall, 1970, 1990). In recent decades, genetic analyses have detected substantial cryptic species diversity, particularly within various freshwater-limited galaxiid lineages, leading to the recognition of several distinct species-rich complexes (Adams et al., 2014; Allibone et al., 1996; Chakona et al., 2013; Waters & Wallis, 2001a, 2001b). These findings have led to the description of numerous new taxa (e.g. McDowall, 1997; McDowall & Wallis, 1996; Raadik, 2014). New Zealand's (NZ's) *Galaxias vulgaris*

complex, for example, was once thought to comprise just a single stream-resident taxon (McDowall, 1970, 1990), but is now recognized as comprising 11 lineages (including six formally described species along with five additional undescribed lineages; Figure 1; Burridge et al., 2012; Waters et al., 2010). Many of these distinctive freshwater lineages differ genetically, morphologically and ecologically (e.g. Allibone & Townsend, 1997; Crow et al., 2009), with distinctive ‘flathead’ versus ‘roundhead’ lineages (Figure 1) often co-occurring, facilitating tests for reproductive isolation (e.g. Crow et al., 2009; McDowall & Wallis, 1996; Waters, Esa, & Wallis, 2001). In the context of widespread habitat alteration and the introduction of salmonid predators, conserving this endemic freshwater biodiversity presents a major conservation challenge (Dunn et al., 2018; McDowall, 2006).

New Zealand's *G. vulgaris* complex shares a common ancestry with the widespread diadromous taxon *G. brevipinnis* (shared by Australia and NZ; McDowall, 1990). This distinctive freshwater-limited assemblage was initially suggested to have evolved via numerous independent transitions from diadromous to stream-resident life history (Allibone & Wallis, 1993; Waters & Wallis, 2001b; Figure 2), as is the case with several freshwater-limited fish complexes elsewhere (Colosimo et al., 2005; Delgado et al., 2019, 2020; Fang et al., 2020; Veale & Russello, 2017). However, a subsequent multilocus phylogeny found the freshwater-limited *G. vulgaris* complex to be monophyletic (Figure 2), and thus concluded that the radiation can be explained by a single loss of diadromy (Waters et al., 2010). Multidisciplinary studies combining geological and biological data (predominantly mtDNA) suggest that geological processes such as mountain building and river capture (Burridge et al., 2008a; Craw et al., 2015) have been highly influential in the diversification and spread of these stream-resident lineages (e.g. Waters et al., 2001a, 2020b).

In biogeographic terms, the diversity of the *G. vulgaris* complex is centred on the Otago region of southern South Island (Figure 1), with the Taieri and Clutha river systems each housing numerous taxa (Waters et al., 2001a; Waters et al., 2010; Waters et al., 2015a). In addition to the 11 currently recognized lineages (Dunn et al., 2018), a preliminary multilocus sequence analysis (Waters et al., 2010) detected an additional nuclear DNA lineage that remained cryptic at the mtDNA level (Burridge et al., 2007; Waters et al., 2001a). Specifically, a few individuals sampled from the Pomahaka R (a major tributary of Otago's Clutha system; Figure 1) have yielded a distinctive nuclear DNA clade (which we here provisionally name *G. ‘Pomahaka’*), apparently phylogenetically affiliated to ‘roundhead’ taxa *G. anomalus* and *G. gollumoides* (Waters et al., 2010; Figure 2). By contrast, extensive mtDNA analyses from this region have consistently allocated all Pomahaka *Galaxias* samples (and most other lower Clutha samples) to the widespread Clutha ‘flathead’ lineage *G. ‘sp D’* (Burridge et al., 2007; Waters et al., 2001a). Interestingly, subsequent morphological assessment has suggested the presence of both ‘roundhead’ and ‘flathead’ morphotypes within the Pomahaka system (D. Jack, pers. comm.). However, extensive mtDNA sequencing throughout the Pomahaka system has continued to yield just a



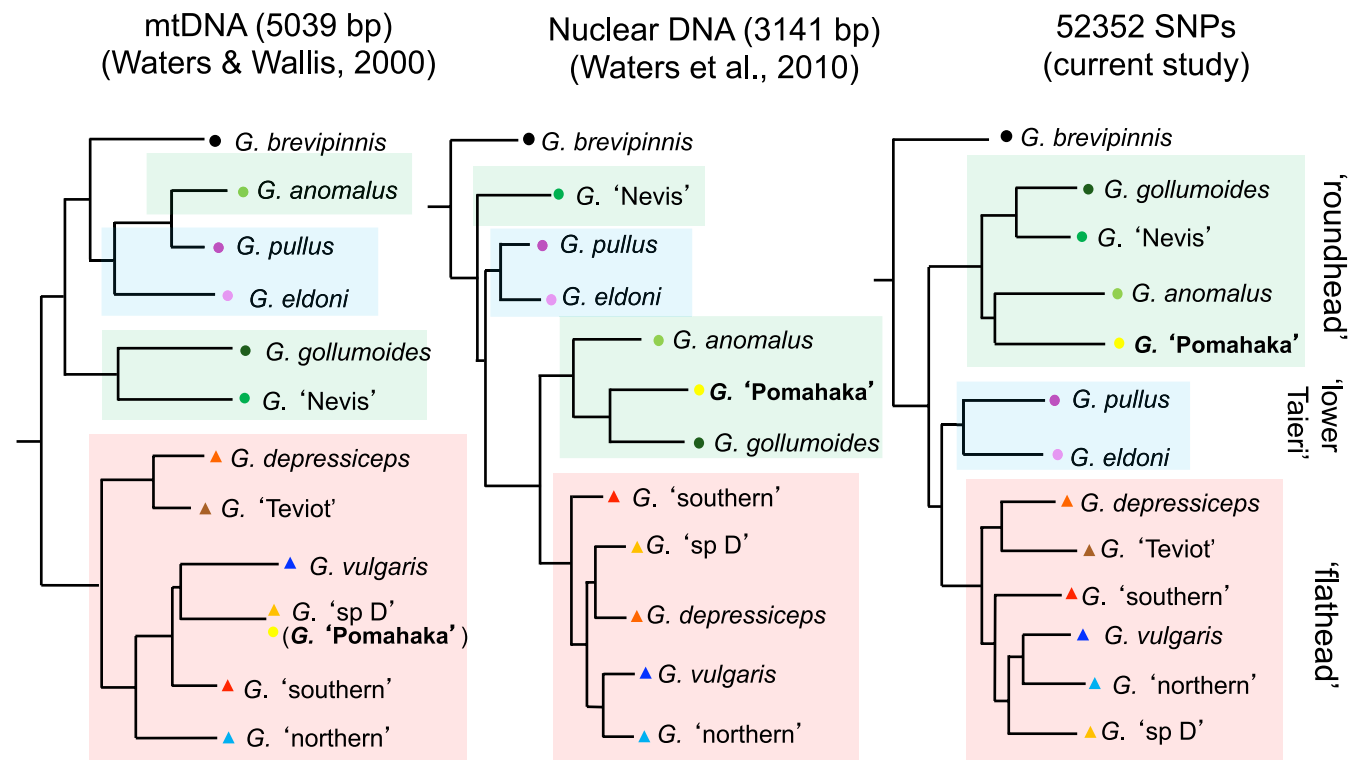


FIGURE 2 Discordant mito-nuclear phylogenetic relationships of New Zealand's *Galaxias vulgaris* complex based on mtDNA (left; redrawn from Waters & Wallis, 2001a) versus concatenated nuclear sequence data (centre; redrawn from Waters et al., 2010) and genome-wide SNPs (right; full details in Figure 3). Distinctive 'flathead' (triangles) versus 'roundhead' (circles) assemblages broadly characterized by morphological, life-history and ecological variation (e.g. Crow et al., 2009; Jones et al., 2016; Jones & Closs, 2016; McDowall, 1997; McDowall & Wallis, 1996) are highlighted by coloured boxes. The newly recognized 'roundhead' *Galaxias* 'Pomahaka' (bold) carries mtDNA identical to that of the sympatric 'flathead' *Galaxias* 'sp D' (left), but these two lineages are otherwise phylogenetically unrelated (centre; right). Similarly, *Galaxias anomalous* has strong mtDNA similarity with *Galaxias pullus* (left), but these two taxa are otherwise phylogenomically unrelated to each other (centre; right).

single mitochondrial clade, attributable to the widespread *G. 'sp D'* (Waters et al., 2015b).

Here we use Genotyping-by-Sequencing (GBS; Baird et al., 2008; Elshire et al., 2011) to undertake genome-wide analyses of NZ's species-rich *G. vulgaris* complex to fully characterize its diversity and infer the role of river-drainage alteration in its formation. In particular, we test the hypothesis that the lower Clutha system contains unrecognized species diversity. We use this system as a model for assessing the effects of introgressive mitochondrial capture on freshwater fish systematics and conservation. Our study also tests for phylogeographic divergence among recently fragmented fish populations, and assesses the genetic implications of anthropogenic drainage modification.

2 | METHODS

2.1 | Sampling and library preparation

We retrieved 227 ethanol-preserved *Galaxias* specimens from the University of Otago galaxiid fish collection where they are stored at -20°C . The vast majority of these samples had been collected by

NZ's Department of Conservation over the last two decades. We typically selected two specimens per locality, incorporating multiple locations for each of 11 recognized *G. vulgaris* complex lineages (Dunn et al., 2018; Waters et al., 2010), along with NZ and Australian representatives of the diadromous sister taxon *G. brevipinnis*. In addition to representing recognized stream-resident diversity, our sampling effort focused particularly on the Pomahaka R and surrounding catchments of the lower Clutha system (southern South Island), where previous studies have detected nuclear genetic diversity not evident using mitochondrial markers (Waters et al., 2010).

High-molecular-weight DNA was extracted from tissue samples using a Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's protocols. The concentration and quality of eluted DNA was assessed using a DeNovix DS-11 spectrophotometer and an Invitrogen Qubit 3.0 fluorometer. DNA degradation was assessed visually using agarose gel electrophoresis. Based on concentrations, purities and visual degradation assessments, 192 samples were selected (from the original 227 samples of extracted DNA) for library preparation. These samples had DNA concentrations greater than $10\text{ ng}/\mu\text{L}$, received no purity alerts, and had suffered minimal visual degradation within the 300–600bp range, crucial for GBS (Graham et al., 2015).

Library preparation for GBS followed methods described by Elshire et al. (2011) with modifications described in Dussex et al. (2015). The pooled library was size-selected (300–600bp) and sequenced at the Biomolecular Resource Facility, Australian National University using an Illumina NextSeq 500 (75bp paired-end).

2.2 | Bioinformatics

Quality scores and adapter content were investigated using FastQC v0.11.9 (Andrews et al., 2016). SNPs were called from sequence data using the *Stacks* pipeline v2.55 (Catchen et al., 2013). Briefly, the *process_radtags* module in *Stacks* was used to demultiplex sequence data, remove sequences with uncalled bases (–c) and discard low-quality reads (–q). *Process_radtags* removed sequences with no barcode, no cut-site, and low-quality sequences.

We assembled loci without a reference genome (i.e. de novo) by executing the *denovo_map.pl* script in *Stacks*. The minimum number of reads to create a locus was set at 3 (–m), and the maximum number of pairwise differences between loci was 2 (–M). To limit numbers of SNPs in linkage disequilibrium, only the first SNP in each locus was kept for subsequent analysis (–write-single-snp). We used VCFtools v0.1.15 (Danecek et al., 2011) to exclude SNP sites with greater than 10% missing data across individuals.

2.3 | Phylogenomic relationships and introgression

Principal Component Analysis (PCA) was undertaken in R v4.0.3 (R Core Team, 2020). The *vcfR* v1.10.0 package (Knaus & Grünwald, 2017) enabled R to read *Stacks* output files, and the PCA analysis was performed and visualized using *pcadapt* v4.3.1, *poppr* v2.8.5, *ade4* v2.1.2 and *ade4* v1.7 packages (Dray & Dufour, 2007; Jombart, 2008; Kamvar et al., 2014; Luu et al., 2017). Phylogenomic analyses were undertaken utilizing RAXML (Stamatakis, 2014) under a maximum likelihood framework using a GTR-GAMMA model with 100 rapid bootstraps. FigTree v1.4.4 (Rambaut, 2009) was used to visualize and root the tree with Australian *G. brevipinnis* specified as an outgroup (see Waters et al., 2010).

Phylogenomic relationships among lineages were also assessed under a coalescent framework using SNAPP (Bryant et al., 2012), implemented in BEAST v2.6.6 (Bouckaert et al., 2019). Specifically, we reduced the dataset to four haploid sequences of 5000 random SNPs for each candidate species as inferred from ML analyses (Table S1), and rooted the tree with Australian *G. brevipinnis*. The MCMC chain convergence was assessed using Tracer v1.7.1 and the output was visualized using DensiTree (Bouckaert, 2010). We tested for nuclear introgression among specific lineages using the ABBA-BABA approach (Durand et al., 2011; Green et al., 2010) as implemented in Dsuite v0.5. (Malinsky et al., 2021).

We used fastSTRUCTURE v1.0 (Raj et al., 2014) to assess genotypic subgroupings under a Bayesian framework. SNP data were exported and converted for fastSTRUCTURE analysis using

PGDSpider v2.1.1.5 (Lischer & Excoffier, 2012). The number of putative clusters *K* was allowed to vary between 2 and 14, and chooseK.py was used to infer an optimal number of clusters maximizing marginal likelihood.

3 | RESULTS

We obtained 354,543,964 75-bp paired-end reads across 187 *Galaxias* specimens (Figure 1; Table S1). After filtering, we retained a dataset of 52,352 SNPs present in at least 90% of samples. Phylogenetic analyses of these data, with Australian *G. brevipinnis* samples specified as outgroups, recovered a well-resolved ML tree, with most major nodes receiving 100% bootstrap support (Figures 2 and 3). The analysis provides support for the monophyly of NZ's freshwater-limited *Galaxias vulgaris* complex (Figures 2 and 3), with NZ *G. brevipinnis* (diadromous) placed sister to the stream-resident radiation. The analysis reveals substantial regional phylogenomic structure within the widespread *G. brevipinnis*, including individual monophyly of Tasmanian, mainland Australian, and NZ samples. The 'trans-Tasman' divergence between New Zealand and combined Australian *G. brevipinnis* samples is particularly strong (Figure 3). While no substantial phylogeographic structure is present among diadromous samples within these regions, fine-scale differentiation is evident for landlocked populations (e.g. subclades associated with different lakes: BT (Wakatipu), BO (Okareka), BZ (Great Lake)).

Phylogenetic analyses based on both ML (Figure 3) and SNAPP (Figure S2) support the individual monophyly of all 11 previously recognized stream-resident lineages (typically with 100% bootstrap support; Figure 3), with a few very local exceptions. Specifically, four samples of *G. 'sp D'* (DH, DN) are placed sister to *G. 'Teviot'*, likely reflecting hybridization among proximate 'flathead' lineages in secondary contact (Esa et al., 2000), and three northern samples of *G. vulgaris* (VL) are placed sister to the monophyletic *G. 'northern'*. In addition to supporting the monophyly of currently recognized lineages, our analyses reveal a distinctive clade of stream-resident 'roundhead' samples centred on the Pomahaka R and adjacent regions of the lower Clutha system (which we here refer to as *G. 'Pomahaka'*; Figures 1–3; Figure S2; see below). This clearly distinctive genomic lineage was previously unresolved by single-locus mtDNA analyses (Figure 2), as it carries mtDNA identical to that of the co-distributed 'flathead' *G. 'sp D'*.

In terms of interspecific relationships, both maximum likelihood phylogenetic (Figure 3) and coalescent SNAPP (Figure S2) analyses of GBS loci support the monophyly of broad 'flathead', 'roundhead', and 'lower Taieri' (McDowall, 1997; McDowall & Wallis, 1996) species groups of stream-resident taxa. PCA (Figure 4) and fastSTRUCTURE (Figure S1) analyses similarly provide evidence for these broad species groupings, with particularly clear differentiation of 'flathead' and 'roundhead' species groups along PC1 (explanatory capacity 19.3%; Figure 4). The 'flathead' lineage cluster is resolved by fastSTRUCTURE at *K* = 3–6, and the 'roundhead' cluster at *K* = 2–3. Despite their relatively narrow geographic ranges (Figure 1),

FIGURE 3 Maximum likelihood phylogeny of the stream resident *Galaxias vulgaris* complex, based on 52,352 SNPs, rooted with Australian samples of the diadromous sister species *Galaxias brevipinnis*. Nodes receiving >95% bootstrap support are indicated by small asterisks. Blue stars represent potential hybrid populations where lineages may have come into secondary contact (e.g. Esa et al., 2000). Estimated ages (mya) of divergence linked to geological river capture events are indicated in bold (see Waters et al., 2020)

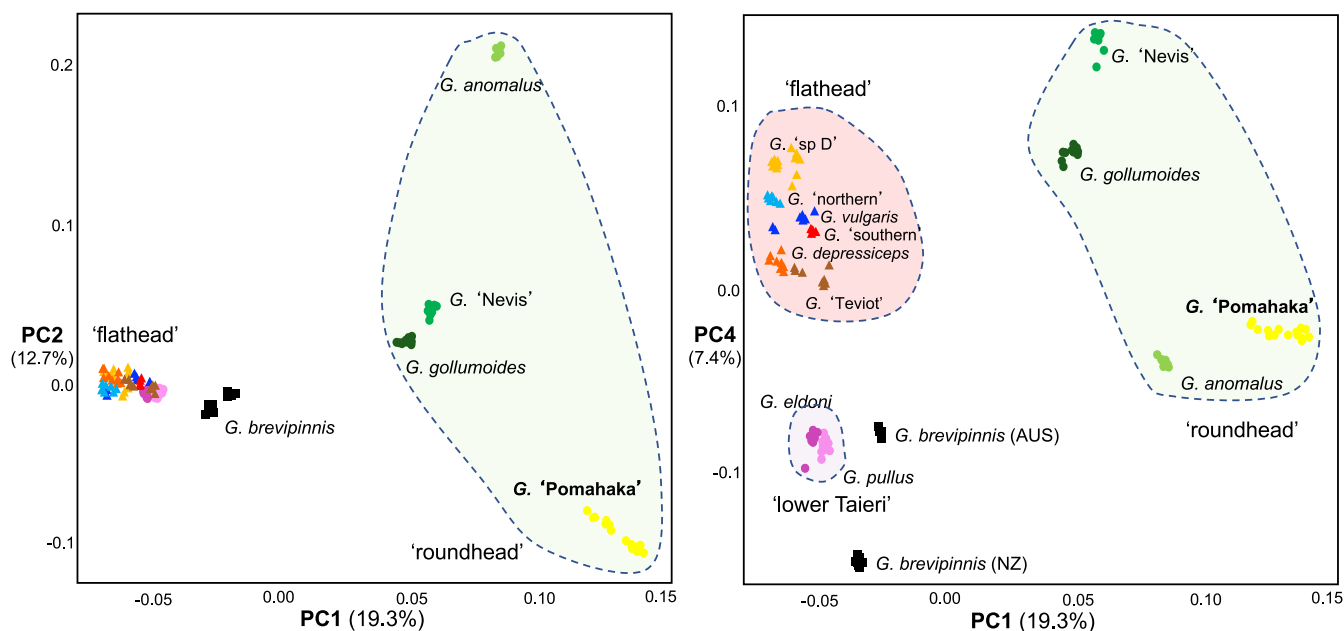


FIGURE 4 PCA plots based on 52,352 SNPs generated across 187 *Galaxias* specimens, showing relationships among 12 stream-resident lineages of the *Galaxias vulgaris* complex (restricted to South Island, NZ) and diadromous *Galaxias brevipinnis* (sampled from both NZ and Australia). Broad 'flathead' (triangles) versus 'roundhead' (circles) stream-resident species assemblages (indicated by large ellipses) are distinguishable on PC1. The newly recognized *Galaxias* 'Pomahaka' ('roundhead', lower Clutha system; shown in bold) was not resolved at the mtDNA level, but is clearly genomically distinct from the co-distributed and mitochondrially similar *Galaxias* 'sp D' (Clutha 'flathead').

'roundhead' lineages exhibit greater genetic divergence among one another than 'flathead' lineages (Figures 3 and 4). This strong differentiation is further emphasized by the finding of multiple distinct 'roundhead' (but not 'flathead') clusters at higher values of K (Figure S1; optimal K = 5).

Phylogeographic analysis of genome-wide data highlights that the Clutha system has substantial *Galaxias* species diversity: eight of the 12 *vulgaris*-complex lineages (Figure 1). Furthermore, the Clutha system contains six tributary-specific lineages that are more closely related to lineages from neighbouring river systems. Specifically, the Nevis R (a Clutha tributary; Figure 1) houses a distinctive clade (G. 'Nevis'; samples IA-IG) most closely related to Southland G. *gollumoides*, while the Teviot R (Figure 1) has a distinctive lineage (G. 'Teviot'; TV, TT, TL, TO) that is sister to Taieri R G. *depressiceps* (Figures 1 and 3). Similarly, the Von R (also a Clutha tributary) contains two species (GV, SV) otherwise broadly restricted to Southland rivers: G. *gollumoides* and G. 'southern' (Figure 1), while Clutha populations of G. *anomalous* (Manuherikia; AM, AP; Figure 1) and G. *pullus* (lower Clutha) have conspecific sister lineages in the Taieri R (Figure 3).

The lower Clutha system is dominated by two lineages: the 'flathead' lineage G. 'sp D' (throughout the Clutha system, including headwaters of the Pomahaka R: DP, DV, DT, DA), and G. 'Pomahaka', which is here resolved as a highly distinct 'roundhead' lineage

(Figures 2–4; detected at K = 5 and K = 6; Figure S1) that is sister to G. *anomalous*, and completely unrelated to the co-distributed G. 'sp D' (despite their identical mtDNA). This newly recognized 'roundhead' lineage is widespread across low-gradient streams of the Pomahaka R (Figure 1) and elsewhere in the lower Clutha system, with an additional isolated record from the adjacent Tokomairaro catchment (XT; Figures 1 and 3). ABBA-BABA tests provide further evidence for extensive introgression between these co-distributed lineages (D statistic 0.27; $p < 0.0001$; Table S2).

Phylogeographic structuring within species occurs at a wide range of spatial scales, with multiple samples from single localities almost inevitably represented by monophyletic subclades (Figure 3). Such a shallow structure occurs even among proximal locations within subcatchments (e.g. G. 'Pomahaka' samples XA, XB; G. 'Nevis' samples IA, IG, ID, IE, IF; G. *eldoni* samples EI, EP; G. *pullus* samples PO, PU; upper Clutha G. 'sp D' samples DC, DS, DK, DR, DO). Over broader spatial scales, the analysis frequently reveals substantial intraspecific phylogeographic structure among major subcatchments and drainages. Such intraspecific phylogenomic 'units' include major subdivision detected within the widespread Clutha 'flathead' G. 'sp D', with distinct 'upper Clutha' (DR, DO, DC, DS, DK), 'mid-Clutha' (DM), 'lower Clutha' (DB, DU, DP, DV, DT, DA), and 'Catlins' (DW, DD) lineages (Figure 3). Strong river-specific and/or regional differentiation is also evident within

G. anomalus (Taieri (AT, AH, AS, AD) versus Clutha (AM, AP)); for *G. depressiceps* (Taieri v Shag [FS]); *G. gollumoides* (Catlins (GW, GV) v Stewart Is (GR, GC) v Southland (GW, GV); and *G. vulgaris* (e.g. Waiakarua v Waitaki). By contrast, Taieri v Clutha populations of *G. pullus* are not reciprocally monophyletic.

4 | DISCUSSION

4.1 | Mitochondrial capture obscures cryptic diversity

Genome-wide analysis of the *G. vulgaris* complex provides support for 12 reciprocally monophyletic freshwater-limited lineages in southern NZ, broadly consistent with previous phylogenetic analyses based on mtDNA (Waters & Wallis, 2001a, 2001b) and nuclear markers (Waters et al., 2010; see Figure 2). However, one of these clades – *G. 'Pomahaka'* (lower Clutha roundhead) – was previously unrecognized, owing to the identical mtDNA lineage shared by it and the geographically co-distributed Clutha 'flathead' *G. 'sp D'* (Figure 2) (Burridge et al., 2007; Waters et al., 2001a; Waters et al., 2015b). While a preliminary nuclear DNA analysis alluded to a distinctive Pomahaka lineage (Waters et al., 2010), the current analysis highlights that *G. 'Pomahaka'* exhibits consistent genome-wide divergence relative to all other lineages and is widespread throughout the lower Clutha system (Figures 1 and 3).

While there are several potential explanations for mitochondrial discordance (e.g. selection; incomplete lineage sorting; introgression; Waters et al., 2010; Sunnucks et al., 2017; Wallis et al., 2017), introgression is a likely cause in the current system, given the extensive evidence for hybridization and introgression between lineages. In particular, the strong discordance between mtDNA versus genome-wide data for *G. 'Pomahaka'* (which carries mtDNA that is identical to that of the sympatric *G. 'sp D'*, a species to which it is otherwise completely unrelated; Figure 2) is most simply explained by introgressive mitochondrial capture (see also Perea et al., 2016; Willis et al., 2014). Specifically, we infer that the native mtDNA of *G. 'Pomahaka'* has been replaced by mtDNA of sympatric *G. 'sp D'*, due to hybridization (e.g. Perea et al., 2016), and this inference is further supported by ABBA-BABA tests for introgression between these co-distributed lineages (Table S2). Indeed, a growing number of freshwater phylogeographic studies have highlighted the remarkable potential for mtDNA introgression to generate extreme mito-nuclear discordance in such systems (Bisconti et al., 2018; Wallis et al., 2017). MtDNA can introgress among close sister taxa particularly readily, and while some such cases can be localized (e.g. Esa et al., 2000), others can lead to wholesale mtDNA replacement (Perea et al., 2016; Unmack et al., 2011; Willis et al., 2014). Consequently, such mtDNA capture has potential to substantially confound the accuracy of single-locus mtDNA barcoding analyses (approaches that have become increasingly popular in freshwater biodiversity studies; Bush et al., 2020; Hubert et al., 2008). In the current system,

for instance, past reliance on mtDNA has apparently obscured the presence of a unique freshwater fish lineage, and such issues have potential to severely hamper conservation efforts. In a related example, diversity within the *G. olidus* complex was underestimated by mtDNA relative to nuclear allozymes, with introgressive hybridization and/or incomplete lineage sorting implicated (Adams et al., 2014). Moving forward, it is clear that the application of genome-wide molecular approaches is needed to resolve such mitonuclear discordance and facilitate meaningful detection and preservation of freshwater biodiversity (Buckley et al., 2018; Unmack et al., 2017).

Interestingly, our study also provides evidence of localized, asymmetric introgression of *G. 'sp D'* mtDNA into another 'roundhead' taxon, *G. anomalus*. This introgression is evident where these lineages co-occur in the Manuherikia R tributary of the Clutha (site AM/DM; Figure 1), and is supported also by ABBA-BABA analyses (Table S2). Specifically, two genomically *G. anomalus* specimens from this locality have yielded *G. 'sp D'* mtDNA (data not shown), whereas (as with *G. 'Pomahaka'*) we have no evidence of roundhead mtDNA introgressing into *G. 'sp D'*. We speculate that ecological preferences may render roundhead galaxiids particularly vulnerable to hybridization, with subsequent mtDNA introgression and capture, as their low-gradient habitats are more likely to experience disturbance, which can, in turn, promote hybridization (e.g. Grabenstein & Taylor, 2018). Such asymmetric mtDNA introgression may be enhanced by positive selection (e.g. Sunnucks et al., 2017) and/or genetic incompatibilities (Arntzen et al., 2009).

In addition to mtDNA replacement obscuring the diversity of taxa (above), our genome-wide analyses (Figures 2 and 3) provide another example of putative mitochondrial capture influencing inferred relationships (see also MacGuigan & Near, 2019), with implications for our understanding of processes creating species diversity. Specifically, the mtDNA placement of *G. anomalus* as a close sister species of *G. pullus* (Figure 2; Waters & Wallis, 2001a, 2001b) strongly conflicts with both morphology (McDowall, 1997; McDowall & Wallis, 1996) and phylogenetic evidence from across the nuclear genome (Waters et al., 2010; current study). This mito-nuclear discordance likely stems from a relatively ancient introgressive mtDNA capture event, reflected by the approximately 2.0% divergence between control region of *G. anomalus* and *G. pullus*; (Waters & Wallis, 2001b). Based on the phylogeographic history of these taxa (which currently co-occur in the Taieri R; Figure 1), we propose that historic introgressive replacement of *G. anomalus* mtDNA by *G. pullus* mtDNA occurred following the mid-late Pleistocene capture of the Kye Burn (formerly a tributary of the Clutha) by the proto-Taieri R (Craw et al., 2016; Waters et al., 2015a). Specifically, this composite formation of the Taieri likely mediated secondary contact and hybridization between *G. anomalus* (upper Taieri) and *G. pullus* (lower Taieri), apparently leading to introgressive mtDNA capture. Evidence for past introgression between these lineages is further provided by ABBA-BABA tests (Table S2). Hence, a mtDNA-centric assessment would invoke diversification of these taxa within the Taieri, as opposed to the invasion of one species from a neighbouring catchment.

4.2 | Population translocation, fragmentation and conservation

Human impacts have potential to erode and reshape freshwater biodiversity. As a case in point, artificial translocation events can promote hybridization and introgression between formerly isolated lineages (e.g. Blackwell et al., 2021; DeMarais et al., 1992; Echelle & Echelle, 1997), with potential implications for conservation (McDowall, 1990). Our genetic analyses have highlighted the potential impacts of man-made drainage alterations (e.g. water races, impoundments) on fish distributions in NZ (Esa et al., 2000) and elsewhere (Waters et al., 2002). In NZ, gold-mining operations in the 1800s often relied on construction of artificial water race connections between the headwaters of adjacent drainage systems (Esa et al., 2000), potentially connecting disjunct freshwater lineages. The hypothesis of anthropogenic secondary contact as a mediator of galaxiid hybridization is supported in the current study by the localized finding of an apparent hybrid genotype in the historic Poolburn water race (DN) which artificially connects adjacent Taieri (*G. depressiceps*) and Manuherikia (*G. 'sp D'*) 'flathead' populations otherwise separated by Rough Ridge (Figures 1 and 3; Esa et al., 2000). The current study provides additional evidence for similar anthropogenic translocation events elsewhere, including the distribution of a *G. 'Pomahaka'* subclade (XT, XG, XW; Figures 1 and 3) across the low Clutha-Tokomairaro divide (where an historic water race connected the adjacent Waitahuna (Clutha system; XG, XW) and Manuka (Tokomairaro R; XT) streams). Indeed, the Manuka Stream record represents the only known occurrence of *G. 'Pomahaka'* outside the Clutha system, and this local translocation scenario is supported by subcatchment phylogeographic structure within this lineage (Figure 3). Comparable artificial water race connections seem likely to also explain the genetic similarity *G. pullus* populations from adjacent headwaters of the Tuapeka (Clutha; PT, PR) and Waipori (Taieri; PB, PP) systems.

In addition to anthropogenic translocations and hybridization, NZ's stream-resident galaxiids are also threatened by population fragmentation, which has arisen via a combination of habitat modification (e.g. through impoundments) and invasive species introductions over the last 150 years (e.g. trout; McDowall, 1990, 2006). Such lasting fragmentation may lead to rapid genetic drift and even population extirpation. Notably, our genome-wide study detected strong phylogeographic structures within species, even among adjacent sites within single subcatchments (e.g. *G. 'Nevis'* samples IA, IG, ID, IE, IF; *G. eldoni* samples EI, EP). While the relatively deep phylogeographic structures we observe over broad spatial scales clearly have pre-human origins (e.g. based on deep mtDNA and genome-wide divergence between upper versus lower Clutha clades of *G. 'sp D'*; BurrIDGE et al., 2007; Waters et al., 2001a), we suspect that many shallow divergences (and reciprocal monophyly) detected among neighbouring sites within rivers at least partly reflect anthropogenic population fragmentation. For instance, reciprocally monophyletic samples of upper Clutha *G. 'sp D'* (DR, DO, DC, DS, DK) are isolated by both an impoundment

and by introduced salmonids; the situation is similar for Waipori R populations of *G. eldoni* (EI, EP) (Figures 1 and 3). Broadly, a combination of habitat fragmentation and predation by introduced salmonids (McDowall, 1990, 2006) has resulted in the widespread decline and/or extirpation of numerous *G. vulgaris* complex populations, which are now broadly restricted to isolated, trout-free habitats (Townsend & Crowl, 1991). This recent fragmentation, and lack of main-channel population connectivity, is highlighted here by the evolution of shallow genome-wide phylogeographic structures among proximate populations that were presumably connected prior to trout introduction. Given the isolating role of invasive salmonids (McDowall, 2006; Townsend & Crowl, 1991), there is an increasing need to locally remove trout, and to install trout barriers, to allow for recovery and re-connectivity of native galaxiid populations (e.g. Lintermans, 2000). Additionally, human-mediated galaxiid gene flow should be considered in areas where anthropogenically isolated populations are potentially experiencing inbreeding depression (i.e. 'genetic rescue'; BurrIDGE, 2019; Whiteley et al., 2015).

4.3 | River capture and fish evolution

Geological processes affecting river drainage connectivity are thought to have played important roles in the divergence of freshwater-limited lineages in many regions of the globe (e.g. Craw et al., 2015; Goodier et al., 2011; Kozak et al., 2006; Mayden, 1988). In several cases, the persistent genetic legacies of palaeodrainage features have been highlighted by interdisciplinary studies combining geological and biological data (e.g. Waters et al., 2020b). In the current study, we show that the Clutha River is home to a particularly diverse suite of *Galaxias vulgaris* complex clades (including eight of 12 currently recognized stream resident lineages; Figure 1), likely reflecting the river's complex, composite geological formation (Craw et al., 2012). Indeed, our galaxiid GBS analyses provide genome-wide support for several previously proposed links between fish biogeography and river capture. Specifically, several headwater tributaries of the Clutha River system harbour distinctive *Galaxias* lineages apparently 'captured' geologically from neighbouring drainage systems. In particular, genome-wide data support geologically proposed paleodrainage connections between the Nevis (*G. 'Nevis'*) and Mataura (Southland, *G. gollumoides*) (Waters et al., 2001a); between the Teviot (*G. 'Teviot'*) and Taieri (*G. depressiceps*) (Waters et al., 2015a); between the Von and Oreti (related populations of *G. gollumoides* and *G. 'southern'*; BurrIDGE et al., 2007); and between the Manuherikia and Taieri (divergent populations of *G. anomalus*; Craw et al., 2007).

The current study similarly finds support for dynamic river geological histories elsewhere in NZ. Notably, genome-wide analyses support a geologically composite origin for Otago's Taieri R (Craw et al., 2016; Waters et al., 2015), evidenced by divergent 'upper Taieri' (*G. anomalus*, *G. depressiceps*) versus 'lower Taieri' (*G. eldoni*, *G. pullus*; Figures 1–3) fish assemblages. Likewise, our genetic data

similarly support sister relationships between Taieri and Shag R *G. depressiceps* (Craw et al., 2016), a geologically derived capture hypothesis previously supported by mtDNA alone. Finally, the sister relationship observed for Wairau (NW) and Clarence (NC) populations of *G.* 'northern', relative to samples from the geographically intermediate Awatere R (NA, NB), strongly supports the proposed glacially mediated capture event involving the headwaters of the two former rivers (Burridge et al., 2006; McAlpin, 1992).

4.4 | Ecological and phylogeographic diversification

The phylogenetic detection of ecologically and morphologically distinct 'roundhead' (limnetic; pool-dwelling; surface feeding) versus 'flathead' (benthic; riffle-dwelling, midwater feeding) species groups (Figures 3 and 4) suggests that the broad divergence of these ecomorphs (e.g. Crow et al., 2009, 2010) was initiated relatively early in the radiation of the *G. vulgaris* complex. Additionally, the finding that genetic distances among 'roundhead' lineages exceed those detected among 'flathead' lineages (Figures 3 and 4) may partly reflect the relatively deep phylogenetic divergence of the former (despite their relatively narrow distributions). We also speculate that ecological preferences for slow-flowing pools and backwaters may increase rates of evolutionary divergence among 'roundhead' lineages (i.e. population fragmentation due to the absence of main-channel connectivity; Waters & Burridge, 2016). While the 'roundhead' clade has not achieved the dramatic northward biogeographic expansion evident in the more recent 'flathead' radiation (Figures 1 and 3), it nevertheless shows evidence of localized movement across low drainage divides (Burridge et al., 2008b).

5 | CONCLUSIONS

In summary, the current study highlights the power of high-throughput sequencing approaches to resolve the phylogenies of species-rich freshwater radiations, and for addressing long-standing systematic puzzles arising from mitonuclear discordance. Our study provides a key example of mtDNA capture which illustrates the need for multilocus approaches to accurately characterize freshwater biodiversity. Based on these data we also infer the role of natural and anthropogenic drainage alterations involved in the dispersal, mixing and diversification of freshwater-limited lineages (Allibone & Wallis, 1993; Waters et al., 2020a). Such hybridization and translocation events present potential threats to species conservation (Rhymer & Simberloff, 1996). Overall, it is clear that genome-wide approaches are crucial for resolving and conserving freshwater biodiversity.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All GBS data used in this study can be found in the NCBI Sequence Read Archive under project PRJNA869758. Scripts for SNP calling, and phylogenetic analyses are deposited on datadryad.org and can be found at <https://doi.org/10.5061/dryad.0k6djh38>. (<https://www.ncbi.nlm.nih.gov/sra>).

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BIOSKETCHES

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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