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PHYLOGENETICS, DIVERGENCE AND MORPHOLOGY OF NEW
ZEALAND ELEOTRIDAE (*GOBIOMORPHUS* GILL)

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ABSTRACT

The genus *Gobiomorphus* Gill (1863) is the only representative freshwater Eleotridae in New Zealand and is comprised of seven species, of which four are diadromous. The species are endemic to New Zealand and are widespread around lowland streams and coasts (with non-diadromous species penetrating further inland). The only other *Gobiomorphus* species are *G. coxii* and *G. australis*, which are endemic to Australia. Eleotridae are stocky fishes of small size (up to 150 mm in length) (McDowall, 1990) and are characterised by two dorsal fins, large pectoral fins, separate thoracic pelvic fins (fused in gobies) and the absence of a lateral line (McDowall, 1990; Allen *et al.*, 2002).

Gobiomorphus has had a particularly turbulent taxonomic history in the literature, spanning approximately 150 years from Gill (1863) to the present, where many species have been synonymised with one another (particularly, most *Gobiomorphus* species were synonymised with *G. gobioides*) due the plasticity of many morphological characters. Additionally, similar morphologies have led to identification difficulties. Phenotypic plasticity can also make cladistic approaches difficult (e.g. Vrijenhoek, 1998; Orti *et al.*, 1994), for example there has been a lot of controversy surrounding *G. alpinus* and its species status. Furthermore, studies of evolution using morphological characters often lack an accurate perspective on relationships and origins of fish species, in particular, little information exists on the evolutionary origins of the *Gobiomorphus* genus. Genetic studies have contributed to resolving problems with taxonomically difficult groups by detecting diversity between morphologically similar species (where DNA variation is often not expressed phenotypically), and examining geographical divergence within species (e.g. Vrijenhoek, 1998; Kocher *et al.*, 1989). Thus, this thesis employed two regions of mitochondrial DNA (cytochrome *b* and control region) to resolve issues surrounding species identification, morphological variation, phylogenetic relationships (including divergence), origins and the evolution of diadromy within the *Gobiomorphus* group.

Mitochondrial DNA sequences were obtained from all seven *Gobiomorphus* species in New Zealand, as well as from both Australian *Gobiomorphus*. The morphology of both *G. basalis* and *G. breviceps* in the lower North Island was also examined. The results suggested that the Australasian *Gobiomorphus* are a polyphyletic group, although with the exclusion of *G. australis* the rest of the species formed a

monophyletic group. The Australian group formed a polyphyletic group basal to the New Zealand monophyletic group. *Gobiomorphus hubbsi*, a diadromous species was found to be a sister group to the New Zealand *Gobiomorphus*. Clock calibrations indicated that the New Zealand and Australian groups have been isolated for about 6-37 Myr, suggesting that the New Zealand species dispersed here (in a single event) from Australia post-Gondwana break-up. These results are discussed in terms of New Zealand's geological history. Once in New Zealand there was a series of radiations; the most recent radiation produced the non-diadromous species (*G. breviceps*, and a *G. basalis*, *G. cotidianus* (although not all populations are diadromous) and *G. alpinus* species complex). Furthermore, *G. huttoni* and *G. gobioides* (both diadromous) formed a monophyletic group that is part of the first radiation, indicating that diadromy is a primitive feature of *Gobiomorphus*.

Mitochondrial DNA accurately distinguished between *G. breviceps* and *G. basalis* (suggesting a genetic basis to morphological variation), and coupled with morphological data, identified pectoral fin ray counts as the best quantitative character for differentiating the species. However, within species high morphological variation was observed that did not fit expected patterns of geographical divergence. Limited time periods may have obscured subtle morphological divergence between catchments. Mitochondrial DNA revealed some unique haplotypes within both catchments, whereas some catchments shared identical haplotypes. The lack of divergence between catchments may have been due in part to connections during the Pleistocene, whereas populations with unique haplotypes may have been isolated for a greater length of time.

Collectively, these studies highlighted the usefulness of mitochondrial DNA for exploring; phylogenetic relationships (including divergence) and solving problems with taxonomically difficult groups, and origins of fish species. Furthermore, the use of molecular data coupled with morphological data can be used to aid in the improvement of identification of morphologically similar species.

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THESIS INTRODUCTION

The freshwater bullies of New Zealand form part of the genus *Gobiomorphus* Gill, 1863 (Eleotridae), which consists of nine species to date. The name *Gobiomorphus* can be broken down into two parts, with *Gobio* recognising the similarity between them and the European gudgeon *Gobio gobio* and *morphus* taken from the Latin word representing form or shape (McDowall, 1990). They are stocky fishes of small size (up to 150 mm in length) and are characterised by two dorsal fins, large pectoral fins, separate thoracic pelvic fins (fused in gobies) and the absence of a lateral line (McDowall, 1990; Allen *et al.*, 2002). The bullies are widely distributed throughout New Zealand and two species (*G. coxii* (Cox's gudgeon) and *G. australis* (striped gudgeon)) are endemic to Australia and found on the Victorian south-eastern coast. The species occupy both brackish- and inland fresh-water habitats and are typically benthic, feeding on small aquatic invertebrates. While some members of the genus spend their entire life cycle in freshwater, four of the seven New Zealand species are diadromous, where part of their life cycle is spent in salt water. In particular, New Zealand *Gobiomorphus* are amphidromous (a form of diadromy) where the larvae migrate out to sea for a feeding phase before returning as juveniles to freshwater (McDowall, 1998).

Morphological characters have been used historically in systematics, however, several characteristics (e.g. fin ray numbers) that have been used to describe fish are often phenotypically plastic, where morphology tends to reflect the environmental conditions. This has made cladistic approaches difficult (Vrijenhoek, 1998; Orti *et al.*, 1994). Thus, in the early literature many species were not recognised (most species were originally described as the one species; *G. gobioides*) due to their similar morphology. Additionally, little information exists on the evolutionary origins of this genus, including explanations regarding how *Gobiomorphus* reached New Zealand, where the Eleotridae family is at southern-most limit (McDowall, 1975). However, many genetic studies have contributed to resolving problems with taxonomically difficult groups by detecting diversity between morphologically similar species (where DNA variation is often not expressed phenotypically), and examining geographical divergence within species (e.g. Vrijenhoek, 1998; Kocher *et al.*, 1989). In particular, mitochondrial DNA (mtDNA) has allowed for high resolution analyses of population-level questions in many species of fishes (Orti *et al.*, 1994), and has provided insights

into the origins, evolution and phylogenetics of many New Zealand fishes including Galaxiidae and *Gobiomorphus* (e.g. Waters *et al.*, 2000; Smith *et al.*, 2005). Thus, this thesis consists of two chapters dealing with genetic species identification, morphological variation, phylogenetic relationships (including divergence), origins and the evolution of diadromy within the *Gobiomorphus* group.

Chapter I utilised two regions of mitochondrial DNA (control and cytochrome *b* regions) to examine and resolve phylogenetic relationships within the *Gobiomorphus* genus and examine the relationship between *Gobiomorphus*, *Philypnodon* and *Eleotris*. Additionally, this chapter aimed to provide an understanding of the somewhat contentious view of species origins for this Australasian group, with a special focus on the New Zealand species and the evolution of diadromy.

Although five out of the seven currently recognised species were recognised in the early literature, there were some difficulties with identification. For example, *Gobiomorphus* was once considered part of the *Eleotris* and *Philypnodon* genera—*G. gobioides* (giant bully), *G. huttoni* (redfin bully), and *G. basalis* (Cran's bully) (*G. cotidianus* (common bully) was not described until 1975 by McDowall) were all part of the genus *Eleotris* before being moved to *Gobiomorphus* in the late 1800's; *G. hubbsi* (bluegill bully) and *G. breviceps* (upland bully) were part of the genus *Philypnodon* before being placed in *Gobiomorphus* in the 1960's. Chapter I aimed to address the confusion surrounding genetic species identification (and thus bully systematics) in New Zealand by applying phylogenetic analyses to this species group.

Additionally, while the origin of typically migratory New Zealand Galaxiidae has been well studied, with species distributions assigned as being either dispersal- or vicariance-driven (McDowall, 2002; Waters & Burridge, 1999), little is known of the origins of *Gobiomorphus* in New Zealand. However, some authors have suggested a role for dispersal in creating current *Gobiomorphus* distributions. For example, McDowall (1975) suggested that *Gobiomorphus* may have reached New Zealand via dispersal in prevailing ocean currents, and believes that *G. coxii* is ancestral to or has common ancestry with New Zealand *Gobiomorphus*. Meanwhile, Thacker and Unmack (2005) examined the origins of the Australian *Hypseleotris* species whose widespread distribution encompasses South Africa, Japan, Southeast Asia and Australia, and suggested that this species dispersed from Southeast Asia via marine environments before invading freshwaters in Australia. These findings highlight the dispersal potential of fish species within the Eleotridae family. However, *Gobiomorphus* origins may

alternatively fit the vicariance theory such that current distributions may be a result of Gondwanan plate tectonics and speciation. Therefore, Chapter I also aims to examine this ‘dispersal vs. vicariance’ issue by examining which of these methods resulted in *Gobiomorphus* colonising New Zealand, and determining whether this involved single, or multiple dispersal events (if *Gobiomorphus* distribution is dispersal-driven). Finally, Chapter I examines the evolution of diadromy in *Gobiomorphus*. The presence of a saltwater-tolerant phase in diadromous fish species that allows dispersal has led to the belief that ancestry for these species must be marine. This has been argued against by McDowall (2004) who believes that diadromy simply implies an ancestry to other diadromous species elsewhere rather than a purely marine ancestry. To further complicate the issue, the genus *Rhyacichthys* is exclusively freshwater and is basal to the Eleotridae (Akihito *et al.*, 2000; Thacker & Hardman, 2005), possibly indicating a freshwater ancestry for *Gobiomorphus*, and suggesting that non-diadromous bullies should have a more basal position within species groups than diadromous bullies. Alternatively, diadromy may be an ancestral character, which appears to be the case for galaxiid fishes, where the presence of a marine phase is found in the basal members of clades, but is absent in more derived species (Waters *et al.*, 2000). Potentially diadromous species in the New Zealand *Gobiomorphus* could belong in basal position within the *Gobiomorphus* group, with non-diadromous species being of more recent descent. Therefore, placement of species on phylogenetic trees will help to infer ancestry or derivation of diadromy in *Gobiomorphus*.

Chapter II focuses on the identification and divergence of two non-migratory, morphologically similar species (*G. breviceps* and *G. basalis*) in the lower North Island of New Zealand. *Gobiomorphus breviceps* has a widespread distribution in New Zealand, encompassing the South Island as well as lower North Island’s Manawatu, Wairarapa and Wellington regions (McDowall, 1990). Conversely, *G. basalis* has a widespread yet intermittent distribution in the North Island that reaches its southern limit at Wellington (McDowall, 2000). Both species occupy a variety of habitats at low altitudes (McDowall, 1975; 1990; 2000). The two species are sexually dimorphic; the adult males often have bright and distinct colouration that the females and juveniles lack. Colouration is often used as a diagnostic tool and is useful for identifying male bullies, but can cause complications when females and juveniles are being compared *in situ*. The overlap in distribution of the two species has led to confusion in the identification of females and juveniles in the lower North Island, which is further

confounded by the presence of *G. cotidianus* (common bully) a morphologically similar species.

Morphological characters including body ratios and serially repeated structures such as fin rays (Christiansen *et al.*, 1988) allow species to be further distinguished. However, they may be strongly influenced by environmental factors, causing variation in populations (Smith *et al.*, 2003; King *et al.*, 2003). For example, *G. alpinus* (Tarndale bully) occupies an extreme high-altitude environment and has the lowest number of first dorsal spines for *Gobiomorphus*, which Smith *et al.* (2003) suggest may be the result of the cold water that the species live in. The large variation in meristic counts (e.g. fin ray counts) observed between populations in *G. basalis* and *G. breviceps* may also be a result of environmental influence. For example, *G. breviceps* have 4-7 first dorsal spines and *G. basalis* have 6-8 first dorsal spines. This overlap in fin ray counts between *G. breviceps* and *G. basalis* can make identification of species difficult.

Therefore, this chapter aimed to utilise the control and cytochrome *b* regions of mtDNA to accurately distinguish between *G. breviceps* and *G. basalis*. Upon species identification, meristic information is applied to individuals in order to identify diagnostic morphological characters. Additionally, the genetic and morphological information for both species is used to examine divergence (both genetic and morphological) to ascertain geographical patterns. It is thought that there would be genetic and morphological divergence between catchments, as there will be no dispersal (non-diadromous species), and there would be no genetic or morphological divergence within catchments as dispersal would be possible.

Collectively, the two chapters of this thesis address the phylogenetic relationships, origins and evolution of diadromy in *Gobiomorphus*, as well as resolving issues surrounding identification and morphological variation. This work demonstrates how genetic data can be used to resolve difficult taxonomic questions, and clarify evolutionary patterns and origins in fish species. The thesis concludes with an overall summary, and then addresses priorities for future research.

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