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

A thesis

presented in partial fulfilment

of the requirements for the Degree

of

Master of Science in Ecology

at

Massey University, Palmerston North

New Zealand

by

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2006

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.

PREFACE and ACKNOWLEDGEMENTS

Each chapter has its own acknowledgements section. Here I thank those people whose assistance was not specific to any one chapter of this thesis.

First I would like to thank my supervisor, Dr Michael Joy for providing excellent guidance throughout the long and often difficult journey that a thesis can often be. I would also like to thank Mike for his humour (although often very dry) and for the 'counselling' sessions that myself and other students made full use of! Furthermore, I would like to thank my co-supervisors Dr Mark Stevens and Dr Russell Death. Thank-you Mark for your excellent tutoring and crash course in genetic analysis; from the laboratory work to the write-up. Without you I do not think my thesis would have been so interesting! Russell you have my gratitude for the help that you gave me early on during my undergraduate study. You are the one that facilitated my interests in freshwater ecology and helped me plan my future career, which is turning out to be rather fun! Without you I may not have realised my aims in life.

I would also like to acknowledge the support given to me by the Julie Alley bursary, which gave me some much needed financial aid.

I am also very grateful to the other post-graduate students around the ecology department (as well as a few in the Allan Wilson Centre) who provided many hours of entertainment, especially during lunch breaks that may have at times gone on for a little too long! Having people in the same boat as you also helps you stay sane and focussed. Angela, thank-you for your guidance and providing for me with an outlet when things got a little frustrating! I also really enjoyed those coffee breaks (which often went a little longer than planned) even when we didn't actually get coffee, and I hope we will always find the time to sit down together every once in awhile. I also must thank Richard Seaton for sharing his office with me, especially as I gave him so much cheek! One day Richard you'll be able to get me back (how does the score stand? 100 to me and 1 to you I think!).

The support provided by the rest of the staff in Ecology has been phenomenal. A big thank-you goes to all the technicians for helping with field work and anything else I needed, and also to the office staff who provided so much help on so many levels. I would also like to thank the rest of the lecturers in the department for many helpful comments on my project and for giving me the encouragement I often needed. During

my time in Ecology I also really enjoyed the morning tea shouts (at which all post-graduate students could be found!), which provided me with much sustenance. Long live the morning tea shouts!

To my Mum and Dad; thank-you so much for everything you have done for me, without you I could not have achieved what I have. Your love, support and confidence in my ability has meant the world to me. My achievements are yours to be proud of too. Thank-you also to my siblings (James, Melissa, Claire and Benjamin) for just being there and for fun chats on the phone. Nanna, Grandad, and my many Aunties and Uncles have also contributed; they have always provided me with support and shown a strong interest in what I am up to, so for that I thank-you. My cat Mia has also helped in her own way. Her constant need for my attention and loud purrs have reminded me to think beyond my own problems and needs.

Last, but certainly not least a big thanks goes to my partner Mike Ushaw, who has been infinitely patient with me and provided me with much love and support, which is essential when completing a task such as a thesis. Cheers for sticking around, especially as I may not have always been in the best mood! I would also like to thank Mike's family who have treated me as one of their own and taken me into the fold. This has been especially wonderful, as my own family is in the South Island, so it is nice to know I have support in the North!

If I have forgotten anyone, my apologies; many people contribute in positive ways to your life, but it is very difficult to thank everyone, but I think I have covered the main ones.

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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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CHAPTER I

PHYLOGENY OF NEW ZEALAND AND AUSTRALIAN ELEOTRIDAE WITH A FOCUS ON *GOBIOMORPHUS*[*]

Key Words: Gobiomorphus, mitochondrial DNA, origin, diadromy, morphology

[*]to be published under the same title as: Emily P. Atkinson, Mark I. Stevens, Michael K. Joy, Russell G. Death

ABSTRACT

Gobiomorphus Gill (1863) is the only representative Eleotridae genus in New Zealand freshwaters and is comprised of seven species, of which four are diadromous. The only other *Gobiomorphus* species are *G. coxii* and *G. australis*, which are found around the Victorian south-eastern coast of Australia in freshwaters and estuaries.
The New Zealand *Gobiomorphus* were originally described as *Eleotris* and two species (*G. hubbsi* and *G. breviceps*) were described as *Philypnodon*. Furthermore, there was some confusion with the recognition of species in the early literature, resulting in most species being classified as *Gobiomorphus gobioides* (giant bully) at some point.

We used molecular data to examine the phylogenetic relationships within the genus, as well as looking at the relationship between *Gobiomorphus, Philypnodon* and *Eleotris*. This study aimed to test the origins of New Zealand *Gobiomorphus* and explore the evolution of diadromy. The hypotheses were: 1) *Gobiomorphus* dispersed from Australia to New Zealand in a single event; and 2) diadromy in *Gobiomorphus* is an ancestral character.

The monophyly of New Zealand *Gobiomorphus* was strongly reinforced, with the Australian species forming a polyphyletic group basal to the New Zealand *Gobiomorphus* species. The data suggested that the two *Gobiomorphus* groups have been isolated between 6 to 30 million years, indicating that *Gobiomorphus* may have dispersed to New Zealand from Australia since the break-up of Gondwana about 80 million years ago. *Gobiomorphus hubbsi* is a diadromous species that formed a sister group to all the other New Zealand *Gobiomorphus* suggesting that diadromy is an ancestral state at least for the New Zealand radiation of bullies. The colonisation of New Zealand via oceanic dispersal requires a marine phase in the lifecycle of a species, which was further supported by the basal position of the migratory species on the phylogenetic trees. In New Zealand there were three major radiations, where *Gobiomorphus cotidianus, Gobiomorphus basalis* and *Gobiomorphus alpinus* formed a species complex (species not easily differentiated) in the most recent radiation. *Gobiomorphus breviceps*, a non-diadromous species formed two distinct North and South monophyletic groups, suggesting that the two groups are actually separate species.

INTRODUCTION

The family Eleotridae (Teleostei: Perciformes: Gobioidei) is comprised of approximately 35 genera containing about 150 species (Allen *et al.*, 2002). Members of the family are characterised by their long slender bodies, two dorsal fins, lack of a lateral line, benthic life-style and relatively small size (Allen *et al.*, 2002). The family is widely distributed around the world with the majority of species found in the vicinity of the Indo-Pacific (Allen *et al.*, 2002). They are present in a variety of habitats; from inland freshwaters, brackish estuarine waters, to coastal seas with some species adopting diadromous life strategies utilising both fresh and salt waters as part of their reproductive cycles. This strategy involves larvae migrating out to sea for a feeding phase before returning as juveniles to freshwater. The presence of coastal species and saltwater tolerance of some larvae has led to the suggestion that the Eleotridae are derived from marine origins and that the freshwater fauna is composed of secondary freshwater species (Allen *et al.*, 2002).

Although New Zealand freshwaters have just one eleotrid genus (*Gobiomorphus*), Australia has approximately 14 genera. For example, *Mogurnda* and *Philypnodon* are endemic to Australia, whereas members of *Hypseleotris* occupy a broad geographic range encompassing South Africa, Japan, Southeast Asia and Australia (Thacker & Unmack, 2005). Thacker and Unmack (2005) examined the origins of the Australian *Hypseleotris* species, and suggested that they dispersed from Southeast Asia via marine environments before invading freshwater in the southeast (the majority of the eleotrids in Australia are distributed around the north- to south-eastern coasts). Potentially, similar dispersals could explain the origins of the eleotrid species in New Zealand and Australia. For example, McDowall (1975) suggested that New Zealand freshwater fish are derived from Australia via prevailing ocean currents. He thought that *Gobiomorphus* may have reached New Zealand through this route, and that *Gobiomorphus coxii* is ancestral to or has common ancestry with New Zealand *Gobiomorphus*. Further, he noted that this colonisation may have occurred through either a single event, or repeated dispersal events. However, the origin of *Gobiomorphus* may alternatively be vicariant - fitting a Gondwanan model, as has been shown for other southern temperate biota. For example, galaxiid fish, southern beech trees and ratite birds have all been postulated to fit a vicariant Gondwanan distribution

(Waters *et al.*, 2000a), where current distributions can be explained by plate tectonics. However, it is now known that some of the southern New Zealand biota obtained their current distributions by means of dispersal. For example, long-distance dispersal of southern beech is supported by molecular clock evidence where divergence dates post-date continental drift (Knapp *et al.*, 2005).

Gobiomorphus Gill (1863) in New Zealand freshwaters is comprised of seven species, of which four are diadromous. The species are widespread around the lowland streams and coasts of New Zealand (non-diadromous species penetrate further inland), and are characterised by several morphological characters, including 27-30 vertebrae, partly scaled cheeks, 5-8 rays on the first dorsal fin and 7-10 rays on the second dorsal fin (Stokell, 1941). The only other *Gobiomorphus* species (*G. coxii* (Cox's gudgeon) and *Gobiomorphus australis* (striped gudgeon)), are found in fresh water bodies and estuaries around the Victorian south-eastern coast of Australia.

New Zealand *Gobiomorphus* has had a particularly turbulent taxonomic history in the literature, spanning approximately 150 years from Gill (1863) to the present. Originally part of the *Eleotris* genus; the *Gobiomorphus* genus was proposed by Gill (1863), and then recognised again by Bleeker (1874). Following this, the Australian *Eleotris coxii* was moved to *Gobiomorphus* by McCulloch and Ogilby (1921). Meanwhile, Stokell (1941) identified the presence of four species; *Gobiomorphus gobioides, Gobiomorphus radiata, Gobiomorphus basalis* and *Gobiomorphus huttoni*. He described *G. huttoni* (redfin bully) as a "species of questionable validity known from a single specimen...Affinities with *G. radiata*, and possibly identical with that species" (Stokell, 1941 p.274). *G. radiata* was eventually synonymised with *G. huttoni* (McDowall, 1962). Two of the New Zealand *Gobiomorphus* species (*Gobiomorphus breviceps* (upland bully) and *Gobiomorphus hubbsi* (bluegill bully)) were originally described as *Philypnodon*, and left out of the early *Gobiomorphus* taxonomy. This was due to the fact that the heads of these species lack scales (Stokell, 1940, 1941, 1959, 1962). Stokell (1959) expressed doubt over this decision to keep them separate from *Gobiomorphus*. He compared them to the Australian *Philypnodon*, and said that they differed in that they had a broad isthmus and narrow gill openings, unlike the Australian forms and more like the New Zealand *Gobiomorphus*. The genus *Philypnodon* was later discarded in New Zealand by Whitley (1968) and the two species moved to *Gobiomorphus*.

There has been some confusion regarding the recognition of various species, which runs from the early literature to current times. Recently, there has been significant controversy concerning *Gobiomorphus alpinus* (Tarndale bully), *Gobiomorphus cotidianus* (common bully) and *G. basalis* (Cran's bully). Two of these species (*G. cotidianus* and *G. alpinus*) have been shown to be indistinguishable using mitochondrial DNA, however, are distinct morphologically (Smith *et al.*, 2003). *Gobiomorphus alpinus* was first described in 1962 by Stokell, as it was found at high altitudes that; "exceeds the recorded maximum of *Philypnodon breviceps*" (Stokell, 1962 p. 31). All other *Gobiomorphus* species were considered lowland species. McDowall (1975) returned *G. alpinus* to *G. basalis* and named it as a junior synonym. He also described a new species *G. cotidianus* that had originally been synonymised with *G. gobioides* until 1941 later put with *G. basalis*. Cranfield (1962) discovered that the syntypes of *G. basalis* were different species. *Gobiomorphus alpinus* was then re-described by McDowall (1994), who suggested that their small eggs cast doubt on the relationship with *G. basalis* and recommended that they be reinstated as a separate species that are derived from *G. cotidianus*. More recently, Smith *et al.* (2003) rejected *G. alpinus* as a separate species on the basis of mitochondrial DNA that failed to differentiate the species from *G. cotidianus*. They suggested that *G. alpinus* be considered an ecophenotype of *G. cotidianus*.

The belief that amphidromous species (i.e. those that spawn in freshwater, with larvae migrating out to sea for a feeding phase, before returning to freshwaters) have 'marine ancestry' stems mostly from the presence in these species of a saltwater tolerant phase. This view has been challenged recently by McDowall (2004), who argues that amphidromy implies an ancestry to other amphidromous species elsewhere; or ancestry amongst other freshwater taxa as they spawn in freshwater, and rules out marine as the ancestral condition. Indeed, Thacker and Hardman (2005) found that basal taxa of gobioid fishes are usually found in fresh or brackish water and the genus *Rhyacichthys* (basal to the Eleotridae) is exclusively freshwater dwelling. It is believed that gobioids speciated in freshwater from marine ancestors, and returned to marine habitats one or more times (e.g. Allen *et al.*, 2002; Thacker & Hardman, 2005).If this is true of *Gobiomorphus*, then the non-diadromous bullies would potentially occupy a more basal position on a phylogenetic tree than the diadromous species. However, most of the basal gobioids examined by Thacker & Hardman (2005) exhibited at least partial saltwater tolerance. Alternatively, diadromy may well be an ancestral character. Waters and

Wallis (2001) examined loss of diadromy in the *Galaxias vulgaris* complex (approximately 10 non-migratory lineages) and found that three species had independently derived non-migratory life histories, indicating that non-migratory species were of a more recent origin, at least in *Galaxias*. Meanwhile, Allibone *et al.* (1996) found that speciation within *Galaxias* species has been promoted by the loss of diadromy, and data collected by Waters *et al.* (2000a) on galaxiid fishes further supported this hypothesis that migratory ability is a primitive characteristic.

Here, we used two mitochondrial (mt) genes (cytochrome *c* oxidase *b* and the control region) to provide further understanding of the origins of Australasian *Gobiomorphus*. In particular, we examined the phylogenetic relationships within the genus, as well we looked at the relationship among *Gobiomorphus*, *Philypnodon,* and *Eleotris*. This is the first phylogenetic study to include all New Zealand and Australian *Gobiomorphus* species. The present study aimed to test the hypotheses: 1) that *Gobiomorphus* dispersed from Australia to New Zealand in a single event; and 2) that diadromy in *Gobiomorphus* is an ancestral character.

MATERIALS and METHODS

Specimen collection

A total of 91 Eleotridae specimens were collected from New Zealand (including the Chatham Islands) and Australia (Fig. 1) for the purpose of this study. Specimens were identified to species using diagnostic colour patterns (when possible) as well as pectoral and dorsal fin ray counts. Both males and females were used. Blood samples were taken from a total of 79 fish in New Zealand. Fish were anaesthetized using Phenoxy-2-ethanol and then 5-50 µL of blood was taken (depending on the size of the fish) from the caudal vein at the base of the anal fin using a fine gauge needle (29 gauge x ½") primed with approximately 50 µL of anticoagulant (sterilised EDTA (100mM)) (Gleeson *et al.*, 1999). Fish were then exposed to a lethal dose of Phenoxy-2-ethanol and preserved in 80% ethanol. Fin clips were taken from the pectoral fin of all samples for possible DNA extraction as necessary. Blood samples and fin clips were frozen at -80°C until required. Fin clips or muscle tissue were taken from the 12 Australian and Chatham Island fish and stored in 80% ethanol (Table 1).

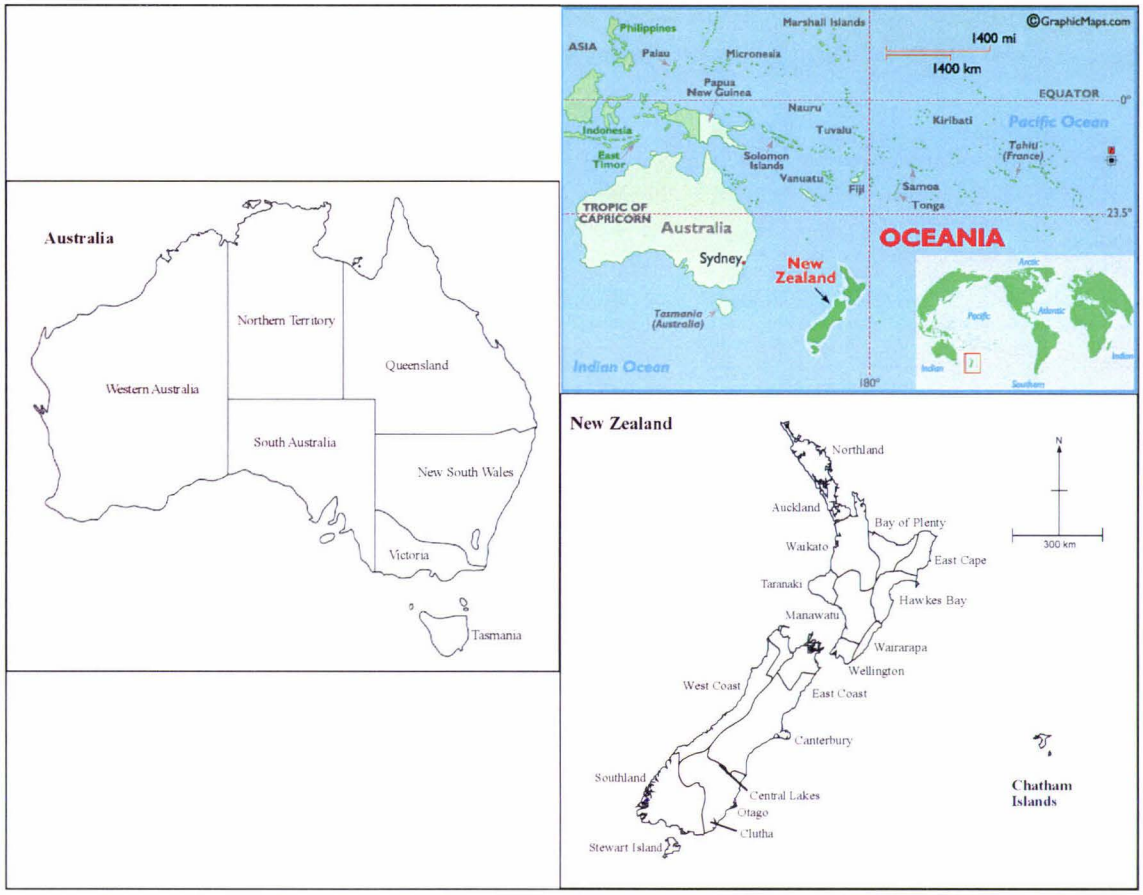


FIGURE 1. Map showing relative locations of regions in New Zealand and Australia.

TABLE 1. Sampling locations for all specimens throughout New Zealand and Australia (see also Smith *et al.*, 2005: Akihito *et al.*, 2000 and Thacker & Hardman, 2005). [ ] indicate sequences yet to be placed on GenBank, numbers indicate number of sequences for each mtDNA region, and (-) no sequences for the region. * - sequenced by M. Stevens and B. Hicks (unpublished data), all others collected for the purpose of this study. Sequences with GenBank accession numbers: AY644 – Smith *et al.* (2005); AY722 – Thacker & Hardman (2005); ABO212 – Akihito *et al.* (2000).

Region	Site name	Species	Code	Genbank accession CR	cyt b	Collected by§
Northland	Kerikeri River	*G. huttoni*	NKR (3)	-	[3]*	
		G. cotidianus	NKR (3)	-	[3]*	
		G. gobioides	NKR (2)	[1]*	[2]*	
	Lake Ngatu	*G. cotidianus*	NLN (1)	-	[1]*	
		G. basalis	NLN (2)	-	[2]*	
	Stony Stream	*G. babaq*	NSS (4)	[3]*	[4]*	
	Kaihu River	*G. basalis*	NK (4)	[1]*	[4]*	
		G. cotidianus	NK (1)		[1]*	
	Waikanamu Stream	*G. cotidianus*	NWS (3)		[3]*	
	Unnamed Stream	*G. huttoni*	NUS (3)		[3]*	
	Lake Waiporeia	*G. cotidianus*	NLW (3)		[3]*	
	Lake Ngakeketa	*G. cotidianus*	NN (2)	[2]*	[2]*	
		G. basalis	NN (1)		[1]*	
	Mangamuka River	*G. huttoni*	NM (2)		[2]*	
	Mimiwhangata Stream	*G. huttoni*	NMi (3)		[3]*	
	Waipoua River	*G. cotidianus*	NWR (5)		[5]*	
		G. huttoni	NWR (1)		[1]*	
	Lake Waikaka	*G. cotidianus*	NW (2)		[2]*	
Waikato	Waikato River	*G. cotidianus*	WWR (1)		[1]*	
	Mangaotaua Stream	*G. basalis*	WMS (5)		[5]*	
	Martin's Stream	*G. basalis*	WM (2)		[2]*	
		G. huttoni	WM (3)	[3]*	[3]*	
		G. cotidianus	WM (3)		[3]*	
Taranaki	Waitanga Stream	*G. breviceps*	TWS (5)	[1]*	[5]*	
Bay of Plenty	Rangataki River	*G. cotidianus*	BOP RR (4)		[4]*	
	Kaituna River	*G. cotidianus*	BOP KR (2)		[2]*	
	Lake Tarawera	*G. cotidianus*	BOP TL (3)		[3]*	
East Cape	Gisborne	*G. cotidianus*	EC G (3)		[3]*	N. Tang. 2-vi-2
Wairarapa	Kopuaranga Stream	*G. basalis*	WaKS (11)	[11]	[11]	M.Joy 2005
		G. breviceps	WaKS (9)	[9]	[9]	M.Joy 2005
	Kaiwhakapapa	*G. breviceps*	WaK (14)	[14]	[14]	Z.Dawson & A.James 2-ii-5
	Mangaroatua	*G. breviceps*	WaM (2)	AY644528,529	AY644586,587	
	Huangaru	*G. breviceps*	WaH (4)	AY644546,547 564,565	AY644614,615 622,623	
	Waipoua River	*G. breviceps*	WaWR (2)	AY644548,552	AY644616,619	
Manawatu	Tuitea Stream	*G. basalis*	MTS (1)	[1]	[1]	
		G. breviceps	MTS (9)	[9]	[9]	
	Kahuterawa	*G. breviceps*	MK (10)	[10]	[10]	
		G. breviceps	MK (2)	AY644534,552	AY644592,610	
	Tokomaru	*G. breviceps*	MT (9)	[8]	[9]	
	Ngaruroa	*G. basalis*	MN (9)	[9]	[9]	
		G. breviceps	MN (1)	[1]	[1]	
	Otaki River	*G. breviceps*	MOR (4)	AY644527,549 553,563	AY644585,607 611,621	
	Mangapapa	*G. breviceps*	MM (2)	AY644530,532	AY644589,596	
Wellington	Makara	*G. breviceps*	WeM (10)	[10]	[10]	
	Fraki Street	*G. breviceps*	WeFS (2)	AY644525,526	AY644583,584	
	Karori	*G. breviceps*	WeK (2)	AY644575,576	AY644633,634	

TABLE 1. (continued)

Region	Site name	Species	Code	Genbank accession CR	cyt b	Collected by§
North East (SI)	Marlborough	*G. huttoni*	NE/M (3)	_	[3]*	M. Stevens (2003)
		G. cotidianus	NE/M (3)	_	[3]*	M. Stevens (2003)
	Takaka	*G. breviceps*	NE/T (2)	AY644531,573	AY644589,631	
	Branch	*G. breviceps*	NE/B (5)	AY644533,535 570, 571,572	AY644591,593 628,629,630	
	Motueka	*G. breviceps*	NE/Mo (2)	AY644544,545	AY644602,603	
	Maitai	*G. breviceps*	NE/Mt (1)	AY644538	AY644596	
West Coast (SI)	Crooked River	*G. cotidianus*	WC/CR (2)	[1]*	[2]*	M. Stevens (2003)
	Lake Brunner outlet	*G. cotidianus*	WC/LB (3)	[1]*	[3]*	M. Stevens (2003)
	Ruffe Creek	*G. breviceps*	WC/R (1)	AY644557	AY644615	
	Stony Creek	*G. breviceps*	WC/S (1)	AY644558	AY644616	
	Lester Creek	*G. breviceps*	WC/L (2)	AY644566,567	AY644624,625	
	Flagstaff Creek	*G. breviceps*	WC/F (2)	AY644568,569	AY644626,627	
East Coast (SI)	Clarence River trib.	*G. alpinus*	Eco/C (1)	[1]*	[1]*	M. Stevens (2003)
	Kowai	*G. breviceps*	Eco/K (2)	AY644536,537	AY644594,595	
	Dry Street	*G. breviceps*	Eco/D (1)	AY644559	AY644617	
	Conway	*G. breviceps*	Eco/CW (2)	AY644560,561	AY644618,619	
	Clarence River	*G. breviceps*	Eco/CR (1)	AY644562	AY644620	
Canterbury	Ashley River	*G. hubbsi*	C/AR (1)	_	AB021239	
		G. breviceps	C/AR (1)	_	AY722224	
	Unknown location	*G. hubbsi*	C/U (3)	_	AY722227,28,30	
North Otago	Kakanui	*G. breviceps*	NO/K (2)	AY644519/539	AY644577/597	
Central Lakes	Ahuriri	*G. breviceps*	CL/A (1)	AY644554	AY644612	
	Fraser Street	*G. breviceps*	CL/F (1)	AY644555	AY644613	
	Tasman River	*G. breviceps*	CL/TR (1)	AY644556	AY644614	
Central Otago	Linnburn	*G. breviceps*	Ce/L (4)	AY644520,521 522,574	AY644578,579 580,632	
Coastal Otago	Waikouiati	*G. breviceps*	CO/W (2)	AY644523,524	AY644581,582	
Clutha	Manuherika	*G. breviceps*	Cl/M (2)	AY644542,543	AY644600,601	
Southland	Mossburn	*G. breviceps*	S/M (2)	AY644540,541	AY644598,599	
	Centre Burn	*G. breviceps*	S/CB (1)	AY644550	AY644608	
Chatam Islands	Waipaua Stream	*G. huttoni*	CI/W (1)	[1]	[1]	M. Joy (2005)
Australia	Burringbar Creek	*G. coxii*	*G. coxii* (3)	[1]	[1]	T. Fowler (2003)
	Lachlan Catchment	*P. grandiceps*	*P.grandiceps* (5)	_	[5]	K. Pitman (2003)
	Clarence Catchment	*P.sp*	*P.sp* (3)	_	[1]	D. Gilligan (2003)
	Gascoyne River, WA	*H. aurea*	*H. aurea* (1)	_	AY722187	
	Ross River, QLD	*H. compressa*	*H. compressa* (1)	_	AY722188	
	Barcoo River, QLD	*H. klunzingen*	*H. klunzingen* (1)	_	AY722189	
	Ross River, QLD	*M. mogurnda*	*M. mogurnda* (2)	_	AY722192,210	
	Ross River, QLD	*M. adspersa*	*M. adspersa* (1)	_	AY722184	
	East coast (unknown location)	*G. australis*	*G. australis* (5)	[1]*	AY722216,18 20,23	
		M. thermophila	*M. thermophila* (2)	_	[2]*	L. Faulks & D. Gilligan (2003)
		M. larapintae	*M. larapintae* (2)	[1]*	[2]*	L. Faulks & D. Gilligan (2003)
		P. grandiceps	*P.grandiceps* (1)	_	[1]*	L. Faulks & D. Gilligan (2003)
		P.sp	*P.sp* (1)	_	[1]*	L. Faulks & D. Gilligan (2003)
		H. compressa	*H. compressa* (1)	_	[1]*	L. Faulks & D. Gilligan (2003)
		H. galii	*H. galii* (1)	_	[1]*	L. Faulks & D. Gilligan (2003)
		M.adspersa	*M.adspersa* (1)	_	[1]*	L. Faulks & D. Gilligan (2003)
		M. clivicola	*M. clivicola* (1)	_	[1]*	L. Faulks & D. Gilligan (2003)
Japan	Yoshida-cho, Shizuoka	*E. oxycephala*	*E. oxycephala* (2)	_	ABO21235,238	
	Iriomote Island, Okinawa	*E. fusca*	*E. fusca* (1)	_	ABO21236	
	Ishigaki Island, Okinawa	*E. melanosoma*	*E. melanosoma* (1)	_	ABO21237	
Panama	Rio Caimito	*E. picta*	*E. picta* (2)	_	AY722204,19	
	Rio San Lorenzo & Punto del Medio	*E. amblyopsis*	*E. amblyopsis* (3)	_	AY722205,12,26	
Hawaii	Kaaawa, Oahu	*E. sandwicensis*	*E. sandwicensis* (1)	_	AY722186	
Mexico	Ounta de Mita	*E. pisonis*	*E. pisonis* (1)	_	AY722229	
Sulawesi	Unknown location	*E. fusca*	*E. fusca* (1)	_	AY722245	
aquarium	Unknown location	*M. mogurnda*	*M. mogurnda* (1)	_	ABO21242	

§: If specimens collected by persons other than who sequenced them.
Genera abbreviated as: *G – Gobiomorphus, P – Philypnodon, H – Hypseleotris, M – Morgurnda, E – Eleotris.*

DNA Analyses

mtDNA extraction, amplification and sequencing

Total genomic DNA was extracted from blood and fin/muscle tissue by homogenisation and digestion with proteinase-K and high salt buffer at 57°C for four hours (modified from; Sunnucks & Hales, 1996); then purified by sodium chloride extraction and ethanol precipitation techniques. Two regions of the mitochondrial genome were amplified for each individual using the polymerase chain reaction (PCR). An approximate 500 base pair fragment of the mitochondrial (mt) cytochrome *c* oxidase *b* gene was amplified using the universal primers Cyb 2 (modified) (5′ – ccc tca gaa tga tat ttg tcc t – 3′) (Kocher *et al.*, 1989) and tGludg (5′ – tga ctt gaa raa cca ycg ttg – 3′) (Palumbi *et al.*, 1991), and the primer pairs H-16498 (5′ – cct gaa cta gga acc aga tg – 3′) (Shields & Kocher, 1991) and L-15995 (5′ – aac tct cac ccc tag ctc cca aag – 3′) (Meyer *et al.*, 1994) were used to amplify approximately 400 base pairs of the control region. Amplifications for each specimen used a 10 µl reaction volume containing 0.75 µl of the extracted DNA (unquantified), 1 x PCR buffer (Roche) 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer-Mannheim), 0.2 µl of each primer (tGludg/Cyb 2 and H-16498/L-15995), 0.5µl of BSA (Bovine Serum Albumin), and 0.1 unit of *Taq* DNA polymerase (Roche). The thermal cycles for both genes (carried out on a T1 thermocycler Whatman Biometra) were: 2 min of initial denaturation at 94°C followed by 35 cycles of denaturation and polymerase amplification (94°C for 30 s, 55°C for 45 s and then 72°C for 30 s), followed by 5 min at 72°C.

PCR samples were purified using SAPEXO (USB Corp.) following the manufacturers guidelines. Products were sequenced (BigDye™ Terminator chemistry (Perkin-Elmer Applied Biosystems)) using the tGludg and H-16498 primers. Sequencing reactions were cleaned using CleanSEQ (Agencourt Bioscience Corp.), before capillary separation on an AB13730 genetic analyser (Applied Biosystems Inc.) at the Allan Wilson Centre Genome Service, Massey University, Palmerston North.

Data Analyses

In addition to the 91 specimens obtained in this study, sequences from 99 specimens were obtained from M. Stevens and B. Hicks (unpubl. data); and further sequences from 86 specimens were taken from GenBank, giving sequences from a total of 276 specimens (Table 1). Individual DNA sequences were verified as being derived from the relevant taxa using GenBank™ BlastN search, and were aligned using SEQUENCHER (Gene Codes ver. 4.2) sequence editor. Sequence data were analysed with PAUP* ver. 4.0b10 (Swofford, 2002). Distance matrices of pairwise nucleotide sequence divergence (based on uncorrected distance and maximum likelihood model; Appendices I & II) were calculated using PAUP*.

Phylogenetic analyses were performed separately for the cytochrome *b* and control region sequences; the cytochrome *b* alignment included 271 sequences and the control region alignment included 150 sequences. Initial phylogenies were explored with PAUP* using neighbour-joining (NJ) and used as initial configurations for heuristic searches of Maximum likelihood (ML) trees. *Mogurnda, Philypnodon* and *Hypseleotris* were included as outgroup taxa (after Akihito *et al.*, 2000). Maximum likelihood was used for the final phylogenies. Modeltest ver. 3.7 (Posada & Crandall, 1998) was used to determine the appropriate substitution model for maximum likelihood (ML) heuristic searches (using all unique sequences). The model selected for the cytochrome *b* region was GTR+I+γ (-InL = 4408.8896; rate matrix: A-C = 1.0949, A-G = 13.5963, A-T = 0.5297, C-G = 1.5282, C-T = 5.8474, G-T = 1.0000; proportion of invariable sites (I) = 0.4925, (γ) = 1.0071; with base frequencies set to A = 0.2951, C = 0.3741, G = 0.0970, T = 0.2338); and for control region was HKY+I+γ (-InL = 2123.9351; ti/tv = 0.8299, I = 0.3245, (γ) = 0.5847; with base frequencies set to A = 0.3922, C = 0.1775, G = 0.1356, T = 0.2947). The robustness of each phylogeny was assessed by implementing bootstrap analysis consisting of 500 replicates (Felsenstein, 1985). Comparisons of log likelihood scores (using X^2 tests) for trees with and without a molecular clock enforced, indicated that these sequences were evolving in a "clock-like" manner. Ages among lineages were estimated using the common clock calibration of 0.8 - 2.5 % divergence per million years derived from cytochrome *b* mitochondrial data (Meyer *et al.*, 1990; Orti *et al.*, 1994; Rocha-Olivares *et al.*, 1999), and 4% - 15% derived from control region mitochondrial data (Rocha-Olivares *et al.*, 1999).

RESULTS

Cytochrome b

The *cytochrome b* region alignment provided a 377 bp fragment. Within this alignment, 175 bp were variable. Analysis revealed 107 haplotypes (Table 2), of which many were region specific. However, the 258 sequences sampled from across Australia and New Zealand fell into several groups (Fig. 2). The New Zealand species formed five groups with the Australian *Gobiomorphus* forming another two groups that were basal to the New Zealand *Gobiomorphus*. The ML tree (Fig. 2) suggested that the two Australian *Gobiomorphus* species were not monophyletic but polyphyletic, as *G. australis* was found outside of the *Gobiomorphus* group and formed a sister group to *Mogurnda*. The average divergence between the two Australian *Gobiomorphus* species was observed at 13.7% uncorrected distance (UD) and 25.3% ML (Appendix I). *Gobiomorphus australis* occupied the most basal position in the phylogeny.

The New Zealand *Gobiomorphus* species formed a monophyletic group, within which *G. hubbsi* was the most basal species. Average sequence divergence between *G. hubbsi* and *G. coxii* was 15.6% UD and 29.7% ML. Average sequence divergence between *G. coxii* and the rest of the New Zealand taxa were similar (Table 3). Divergence within the New Zealand *Gobiomorphus* ranged from 4.6 % to 25 % ML and 0.4 % to 14.9 % UD. The lowest average sequence divergence was observed between *G. breviceps*, and the *G. cotidianus, G. basalis* and *G. alpinus* complex (CBAC), which formed a sister group to all other *Gobiomorphus* (Fig. 2). The highest average divergence (11.2 % UD and 16.0 %) was detected between *G. hubbsi* and CBAC. *Gobiomorphus huttoni* and *G. gobioides* formed a monophyletic group (4.6 % UD and 4.8 % ML average divergence) on the ML tree (Fig. 2). Average divergence between and *G. gobioides, G. huttoni*, and *G. hubbsi* was 14.3 % UD and 23.7 % ML The Chatham Island *G. huttoni* (CI/W) was a unique haplotype that is sister to the other *G. huttoni* from the North Island (0.5 % divergence UD & ML). CBAC and *G. breviceps* formed two sister groups but with low bootstrap support suggesting that we did not have enough information to resolve these groups.

TABLE 2. The 375 variable sites for cytochrome *b* region for *Gobiomorphus*. Locations are indicated using location codes from Table 1. Identical character states are indicated by dots. A – *Gobiomorphus gobioides*.

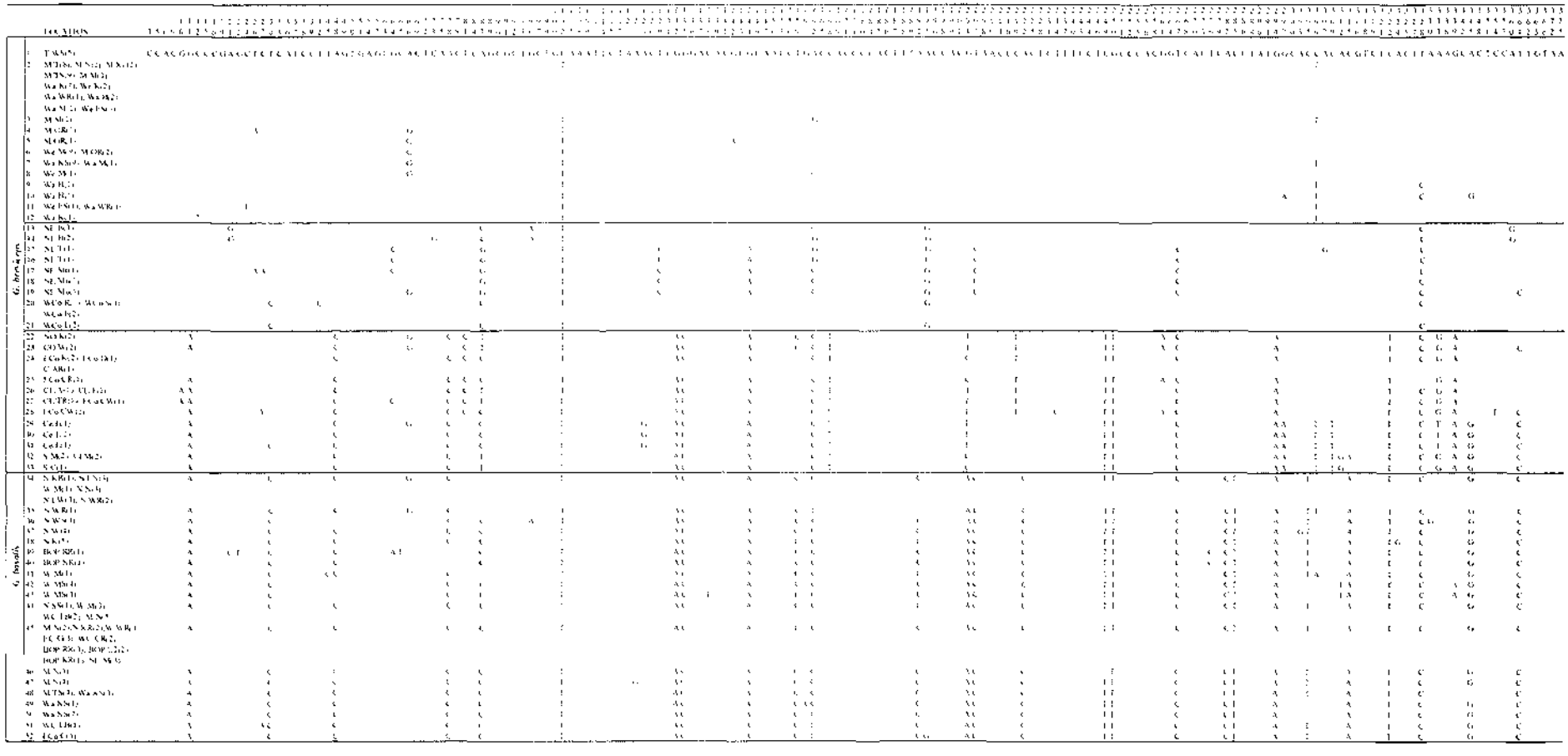


TABLE 2. (continued)

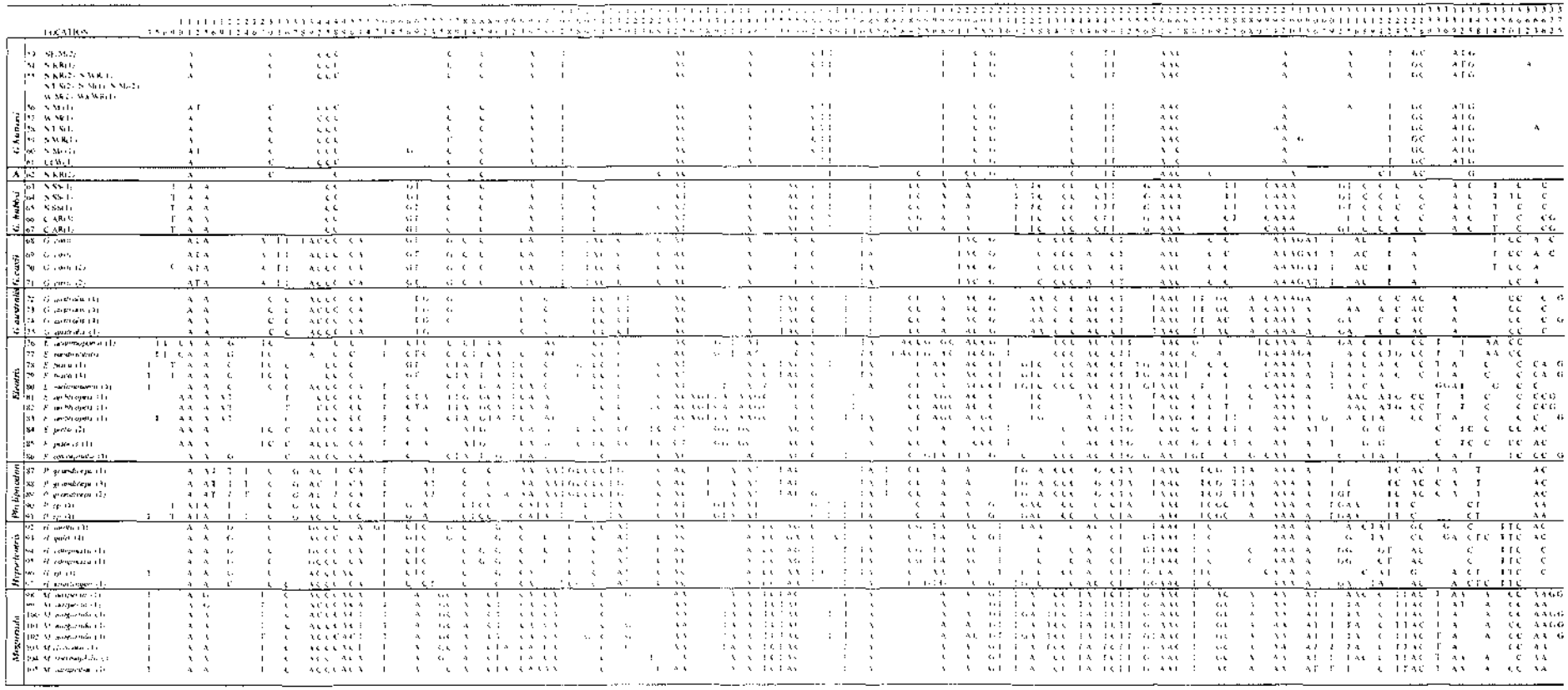


TABLE 3. Sequence divergence (both regions of mtDNA) between *G. coxii* and the extant New Zealand *Gobiomorphus* taxa.

Species	Cytochrome b		Control Region	
	Maximum Likelihood (ML) (%)	Uncorrected Distance (UD) (%)	Maximum Likelihood (ML) (%)	Uncorrected Distance (UD) (%)
G. hubbsi	29.7	15.6	10.7	8.6
G. huttoni	24.2	14.3	19.2	12.8
G. gobioides	25.1	14.5	21.7	13.9
G. breviceps	27.6	15.9	18.3	12.3
CBAC	26.9	14.8	15.5	10.9

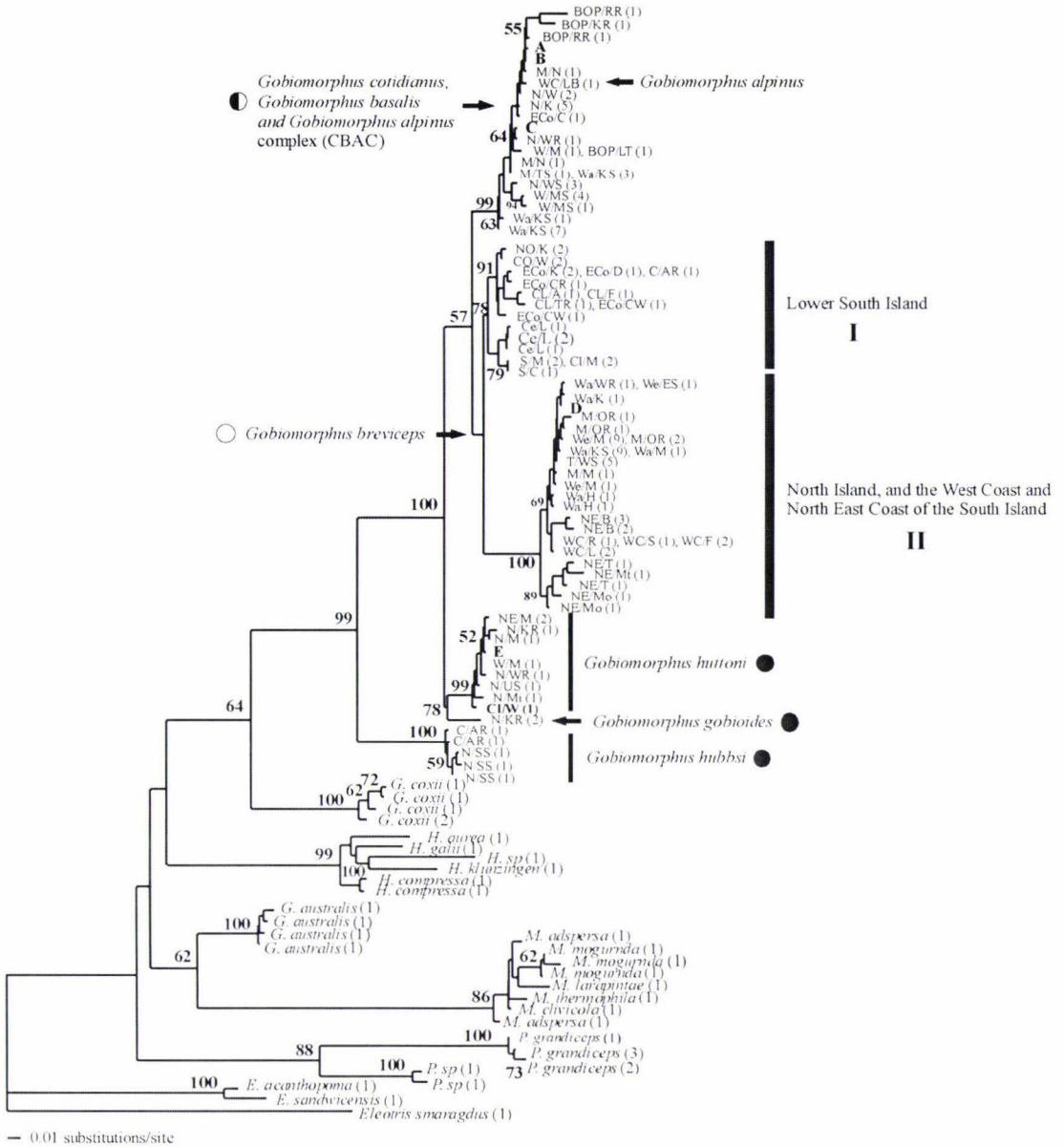


FIGURE 2. Maximum likelihood phylogram based on the substitution model GTR+ I +G (-InL = 4408.8896; base frequencies set to A = 0.2951, C = 0.3741, G = 0.0970, T = 0.2338) derived from Modeltest vers. 3.7 (see methods), using a 377-bp fragment of the mitochondrial DNA (cytochrome *b*) gene using only unique sequences. Bootstrap confidence limits (500 replicates) shown above nodes. Codes are those used in Table 1. Number of identical haplotypes present at any site is given in parentheses. Symbols indicate life history patterns; open circle = non-diadromous, closed circle = diadromous, half circle = presence of both life history patterns. *G. huttoni* species in bold is Chatham Island haplotype. A – N/KR(2), W/WR(1), BOP/LT(2), BOP/RR(3), BOP/KR(1), NE/M(3), EC/G(3), M/N(2), WC/CR(2). B – N/SS(1), W/M(3), M/N(5), WC/LB(2). C – N/KR(1), N/LN(3), N/N(3), N/LW(3), N/WR(2), W/M(1). D – M/T(8), M/W(1), M/TS(9), M/K(12), M/M(1), Wa/K(7), Wa/WR(1), Wa/H(2), Wa/M(1), We/K(2), We/ES(1). E – N/KR(2), N/WR(2), N/Mi(2), N/M(1), N/US(2), W/M(2).

Average sequence divergence ranged from 0.3 % to 7.2 % within-species for ML and 0.2 % to 6.4 % for UD. The lowest average divergence (1 % for both UD and ML) was within the CBAC, with strong bootstrap support that these species should be grouped together. The three species could not be differentiated using this region of mitochondrial DNA. This was reflected by the ML tree where the species did not form discrete groups (only unique haplotypes are presented). However, Manawatu and Wairarapa *G. basalis* populations formed separate groups. Bay of Plenty populations of *G. cotidianus* also formed a distinct group. Meanwhile, the greatest within-species divergence was within *G. breviceps*; which formed two distinct clusters that are sister groups; a lower South Island group (I) and a Northern group (II) that included the specimens from the upper South Island. Average divergence detected between the two groups was 7.2 % ML and 6.4 % UD.

Control Region

The control region provided a 384 bp alignment for analysis. Within this fragment, 131 bp were variable. There were 56 unique haplotypes from 161 sequences (Table 4). The control region sequences resulted in an ML tree (Fig. 3) with a similar topology to the cytochrome *b* region (Fig. 2). The individual haplotypes separated into seven groups. *Gobiomorphus breviceps* formed two groups, with the rest of the species separating into distinct groups (Fig. 3). The New Zealand *Gobiomorphus* formed a monophyletic group. Average divergence between the two Australian species was 12.4% UD and 18.2% ML. In agreement with the *cytochrome b* analyses, the most basal New Zealand species was *G. hubbsi*. The average divergence between *G. hubbsi* and *G. coxii* was observed at 8.6 % UD and 10.7 % ML (Table 4). The monophyletic group consisting of both *G. huttoni* and *G. gobioides* (average divergence observed at 4.9 % UD and 5.5 % M) was the next most basal group, with average divergence from *G. coxii* observed at 13.2 % UD and 20.0 % ML and from *G. hubbsi* at 9.9 % UD and 13.8 % ML. The Chatham Island *G. huttoni* (CI/W) was a unique haplotype that was sister to *G. huttoni* from the

TABLE 4. The 368 variable nucleotide sites for control region sequences for *Gobiomorphus*. Locations are indicated using codes from Table 1. Identical character states are indicated by dots. A = *G. gobioides*

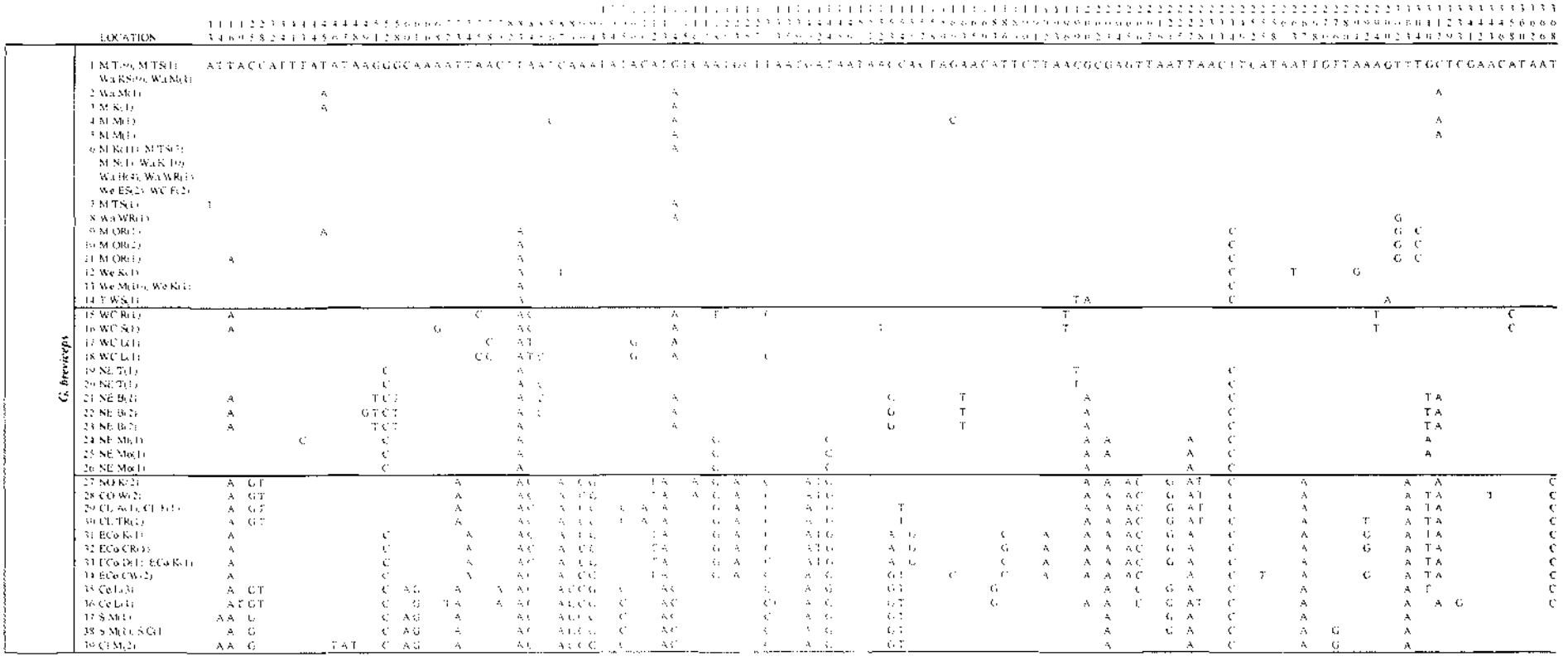
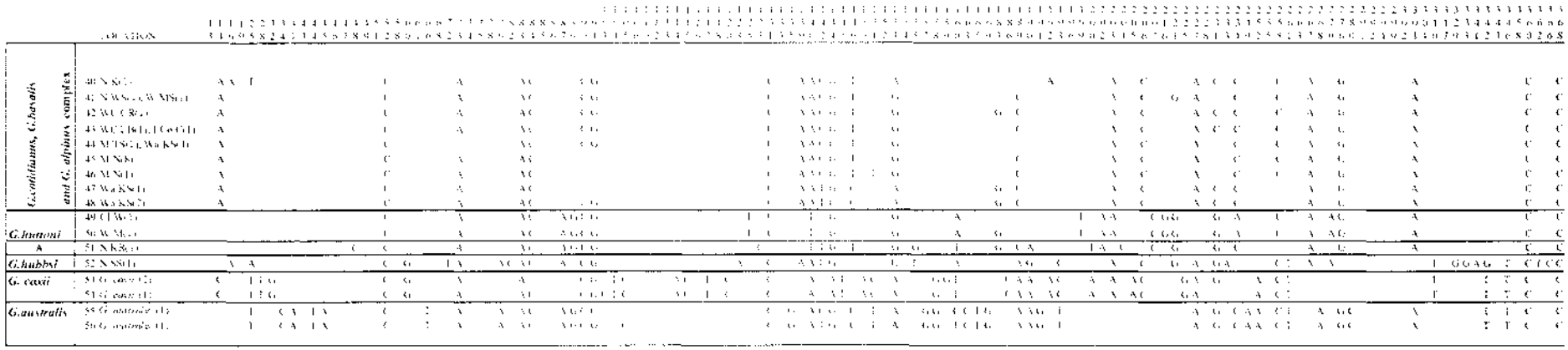


TABLE 4. (continued)



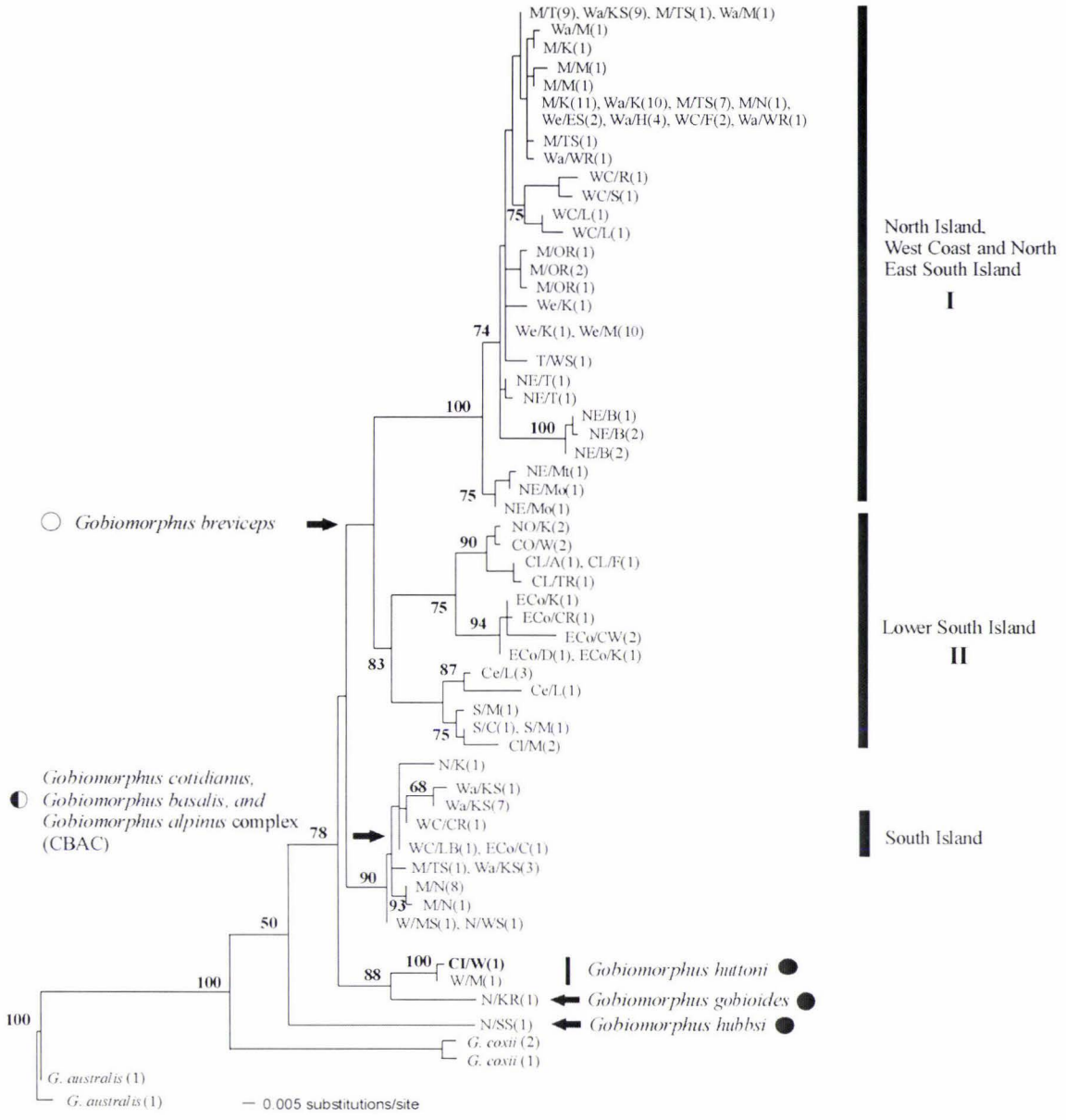


FIGURE 3. Maximum likelihood phylogram based on the substitution model HKY+I+G (-InL = 2123.9351; base frequencies set to A = 0.3922, C = 0.1775, G = 0.1356, T = 0.2947) derived from Modeltest vers. 3.7 (see methods), using a 384-bp fragment of the mitochondrial DNA (control region) gene using only unique sequences. Bootstrap confidence limits (500 replicates) shown above nodes. Codes are those used in Table 1. Number of identical haplotypes present at any site is given in parentheses. Symbols indicate life history; open circle = non-diadromous species, closed circle = diadromous species, half circle = presence of both life history patterns. *G. huttoni* in bold is the Chatham Island haplotype.

Waikato region (0.3 % UD and ML divergence). The most recently derived lineages were *G. breviceps* and CBAC. The ML tree (Fig. 3) indicated that *G. cotidianus, G. basalis* and *G. alpinus* were indistinguishable using mtDNA, although again some Wairarapa and Manawatu populations of *G. basalis* formed separate groups. This species complex formed a sister group to *G. breviceps*. The divergence within CBAC ranged from 0.3 – 2.8 % ML and 0.3 – 11.6 % UD. *Gobiomorphus alpinus* shared the same haplotype as a *G. cotidianus* specimen. *Gobiomorphus breviceps* separated into two groups (Fig. 3) that formed sister groups. Group I was formed by the North Island and west coast South Island species. The greatest average within-species divergence was within *G. breviceps* at 8.6 % UD and 10.7 % ML. The greatest average divergence between any two New Zealand *Gobiomorphus* species was detected between *G. breviceps* and *G. hubbsi* (most basal species) at 11.6 % UD and 17.1 % ML. The lowest average divergence between species was observed between *G. breviceps* and CBAC at 6.9 % UD and 8.5 % ML.

DISCUSSION

The analysis showed *Gobiomorphus* as polyphyletic. However, excluding *G. australis*, *Gobiomorphus* is supported as a monophyletic group. *Gobiomorphus australis* was found outside of the *Gobiomorphus* group and as a sister group to *Mogurnda*. Furthermore, this study indicated that the smallest monophyletic group to include all *Gobiomorphus* species also included *Hypseleotris* and *Mogurnda*. *Eleotris* and *Philypnodon* were basal to the *Gobiomorphus* group with *Hypseleotris* sister to the New Zealand *Gobiomorphus* and *G. coxii*. Thacker and Hardman (2005) examined the phylogeny of basal gobioid fishes and found that all of *Gobiomorphus,* including *G. australis,* formed a monophyletic group. Further, they found that *Mogurnda* and *Philypnodon* were basal to *Gobiomorphus*, and that *Gobiomorphus* was basal to *Hypseleotris* and *Eleotris*. *Eleotris* is widespread worldwide and represents a major radiation into the new world tropics. Additionally, their results revealed the Australian *Gobiomorphus* as a polyphyletic group basal to the New Zealand radiation of *Gobiomorphus*. Although the sequences of the coding and non-coding regions evolve at a rate that has been proven to be useful for resolving ancient and recent events in many

vertebrates (e.g. Kocher *et al.*, 1989), the lack of resolution in the present study for the 'deeper' taxonomic questions may be due in part to the short gene fragment (< 400 base pairs). There is limited variation in first and second codon positions resulting in little phylogenetic information for 'deep' evolutionary hypotheses (Meyer, 1994) hence the need for a longer fragment in future studies. Indeed, the phylogeny examined by Thacker and Hardman (2005) was an analysis of four regions of approximately 1000 base pairs, and this most likely allowed for the greater resolution revealed in their study.

Gobiomorphus coxii formed a sister group to all New Zealand taxa, with *G. hubbsi* being the most basal species. Molecular clock calibrations indicated that *G. coxii* and extant New Zealand taxa have probably been isolated for about 6 to 20 million years using a non-conservative rate of divergence and 10 to 30 million years using a conservative rate of divergence for the cytochrome *b* region. New Zealand has been physically separated from Australia by at least 1,200 km for the last 60 million years (Cooper & Milliner, 1993), indicating that there has been dispersal and gene flow between the two continents since the break-up of Gondwana. Although the divergence of *Gobiomorphus* is more indicative of a dispersal event from Australia to New Zealand (Australian taxa basal to New Zealand taxa), a vicariant theory can not be ruled out, as divergence merely indicates the period of isolation between the Australian and New Zealand *Gobiomorphus* i.e. dispersal "stopped" about 30 million years ago (Mya). Further, changes in oceanic circulation patterns (after the geological separation of New Zealand from Australia) could have been the cause of the isolation and subsequent divergence of New Zealand *Gobiomorphus*.

The popular approach is that New Zealand's flora and fauna is Gondwanan because New Zealand's underlying substrate is Gondwanan in origin (e.g. Stevens, 1980). This concept reached its peak with the panbiogeographical school that stressed the ancientness of New Zealand's biota and used it to attribute broad southern distributions of taxa to former connections of lands, with distributions becoming fragmented by continental drift (Croizat *et al.*, 1974; Rosen, 1974; Craw *et al.*, 1999). However, during the Palaeocene (65-23 Mya), New Zealand was progressively submerged beneath a marine transgression until the late Oligocene epoch (34-23 Mya). In this period there is little indication of the presence of land in the marine sediments, although it is under continual debate whether New Zealand was completely submerged during this time (Pole, 1994). If New Zealand was completely submerged then much of the fauna and flora of New Zealand would have arrived after this period via trans-

oceanic dispersal. Pole (1994) indicated that a trans-Tasman dispersal is likely and that it is possible that the entire forest-flora of New Zealand arrived by long distance dispersal rather than the popular Gondwanan origin theory (the predominant theory used by early biogeographers, and still used by some). Therefore, it seems plausible that New Zealand *Gobiomorphus* may have arrived via oceanic dispersal after the Oligocene epoch, which is well matched by the molecular data. Fossil records indicate the presence of *Gobiomorphus* in New Zealand since the early Miocene epoch (16-20 Mya) (McDowall, *pers. comm.*), which is supported by the genetic data and indicates a minimum time *Gobiomorphus* has been present in New Zealand. However, there could have been other dispersal events where the taxa became extinct and are missing from the fossil record. Furthermore, the oldest fossil record of a taxon only provides a minimum estimate of its age.

Other freshwater fish species in New Zealand have been postulated as having achieved their current distributions by means of oceanic dispersal. Molecular studies support the importance of marine dispersal in *Galaxias maculatus* (Berra *et al.*, 1996; Waters & Burridge, 1999; Waters *et al.*, 2000b), as the sister relationship of Australian and New Zealand haplotypes conflicts with the popular idea of plate tectonics being responsible for their observed distributions (Waters *et al.*, 2000a). This is further supported by the high dispersal ability of juveniles, which have been found as far as 700 km from continental land (McDowall *et al.*, 1975). Furthermore, *Neochanna,* and *G. brevipinnis* (Galaxiidae species) have divergence rates that post-date the Gondwanan break-up. Additional evidence for oceanic dispersal comes from the recent dispersal of the Australian anguilliid (*Anguilla reinhardtii*) to northern New Zealand that was previously only known from eastern Australia, New Caledonia, Norfolk Island and Lord Howe Island (McDowall *et al.*, 1998).

Therefore, although a vicariant origin for *Gobiomorphus* cannot be completely ruled out, evidence appears to favour marine dispersal. Oceanic dispersal requires a marine phase to be present in the lifecycle of a species (i.e. a species must be diadromous). *Gobiomorphus hubbsi* is a diadromous species that is a sister species to the rest of the New Zealand *Gobiomorphus*, indicating that diadromy is an ancestral characteristic in the *Gobiomorphus* species. Diadromy has also been considered to be a primitive feature of the galaxiidae (McDowall, 1970; 1978), with species that do not exhibit diadromy being the result of land-locking. The loss of diadromous behaviour has been postulated as an important mechanism in speciation (the result of a decrease in

gene flow, where genes are no longer transferred from one population to another), especially within *Galaxias* species (Allibone *et al.*, 1996). *Galaxias vulgaris* have diverged into 10 lineages of which three species have independently derived non-migratory life histories (Waters and Wallis, 2001). The recently derived species in *Gobiomorphus* are non-diadromous species e.g. *G. breviceps, G. basalis* and *G. alpinus*. *Gobiomorphus alpinus* has been suggested as a lake-derived form of *G. cotidianus*, which perhaps is in the early stages of speciation (Smith *et al.*, 2003). *Gobiomorphus basalis* may also have been derived from the diadromous *G. cotidianus* (although diadromy is not obligatory in this species). This indicates that the loss of diadromy may also play an important role in the speciation of New Zealand *Gobiomorphus*.

The molecular data suggest that New Zealand *Gobiomorphus* dispersed to New Zealand from Australia in a single event (i.e. presence of monophyly). Once in New Zealand there was then a series of radiations. The first radiation produced *G. hubbsi* and the monophyletic group that included *G. huttoni* and *G. gobioides* from which later the two species diverged. The second radiation produced the very diverse (large genetic variation among individuals) group that makes up *G. breviceps*, with the final radiation producing the *G. cotidianus, G. basalis* and *G. alpinus* complex.

Gobiomorphus huttoni is the only *Gobiomorphus* species from New Zealand to have dispersed to the Chatham Islands, which have been regularly colonized by both vertebrates and invertebrates via oceanic currents. Animal taxa on Chatham Islands are generally affiliated with taxa from both the South Island and North Island (Stevens & Hogg, 2004). This has been supported by the findings of McGaughran *et al.* (2005), who examined the genetic divergence of freshwater amphipods and found that Chatham Island species had affinities with South Island taxa. They postulated that the Southland current along the subtropical front provided a plausible mechanism for dispersal events to the Chatham Islands. This is further supported by Stevens and Hogg (2004) who found that corophiid amphipods have affinities with mainland New Zealand taxa. Much of Chatham Islands terrestrial fauna are also postulated to have arrived via dispersal. The taxonomy of large flightless insects, avifauna and flora is in keeping with more recent dispersal events rather than vicariance as a mechanism of colonisation (Trewick, 2000). The *G. huttoni* Chatham Island haplotype is affiliated with haplotypes from the Waikato region, which appear to have become isolated between 20,000-75,000 years ago (control region) and 200,000-400,000 years ago (cytochrome *b* region) based on molecular clock calibrations. The Chatham Islands have been identified as remnants of

late Cretaceous volcanic islands and have been oceanic islands for at least 70 million years (Campbell *et al.*, 1993). Thus, the time frames involved indicate that *G. huttoni* dispersed from New Zealand to Chatham Islands rather than having an older vicariant distribution. The low divergence observed between the Chatham Island haplotype and the New Zealand haplotypes suggests that dispersal occurs on a regular basis, and is perhaps supplying the Chatham Island populations (juveniles going out to sea are unlikely to return to the Chathams). However, as *G. huttoni* is the only *Gobiomorphus* species on the Islands, it seems unusual that no other migratory bullies have populated the Islands. Perhaps *G. huttoni* is the only species to reach the Islands due purely to numbers, as they are the most common obligatory diadromous *Gobiomorphus* (*G. cotidianus* is more common in lakes) (Joy *pers. comm.*). To resolve this issue more sampling would be required.

The *G. cotidianus, G. basalis* and *G. alpinus* complex had the lowest amount of between-species divergence, and were not easily differentiated using mtDNA. Although, two populations of *G. basalis* from around the Manawatu and Wairarapa regions had some support for their separate species status. However, not all *G. basalis* populations grouped together. For example, *G. alpinus* had a unique haplotype for the cytochrome *b* region but not the control region; where it had an identical haplotype to a *G. cotidianus* species from the South Island. Smith *et al.* (2003) were able to differentiate one *G. basalis* population from *G. cotidianus*, and *G. alpinus*, but could not differentiate *G. alpinus* from *G. cotidianus* as they shared identical haplotypes. Subsequently, they suggested that *G. alpinus* is an eco-phenotype of *G. cotidianus*, and suggested that they were either a population in the early stages of speciation or that the meristic differences were an adaptation to a high-altitude environment. C. Michel, M.I. Stevens and M. van den Heuvel (unpublished data) examined amplified fragment length polymorphisms (AFLPs) of the three species and found that they could be differentiated, and that *G. basalis* and *G. alpinus* each formed monophyletic groups, indicating that *G. alpinus* should retain its species status. According to the rules of nomenclature *G. alpinus* should never have lost its species status, rather *G. cotidianus* should have been re-described as *G. alpinus* as the senior synonym (described by Stokell in 1962; *G. cotidianus* wasn't described until 1975 by McDowall).

Akihito *et al.* (2000) found that morphological differences or similarities in fishes were not always correlated with genetic patterns. Weins and Penkrot (2002) compared morphology and mtDNA with regard to examining the phylogeny of newly

diverged species and found that mtDNA was preferred because it changed at a faster rate (Thacker, 2004), and genetic variation is not always expressed phenotypically (Vrijenhoek, 1998). The *G. cotidianus, G. basalis* and *G. alpinus* complex indicate that this may not always be the case, as morphology may be diverging at a faster rate than mtDNA. Some factors however, may have accelerated morphological differentiation; namely habitat or sexual selection (both *G. alpinus* and *G. basalis* are non-diadromous and sexually dimorphic). The extent of sexual dimorphism exhibited by species indicates the importance of female choice (Ritchie *et al.*, 2005) which then accelerates speciation by altering male characteristics. In fact sexual selection (mate choice) has been referred to as the 'engine of speciation' (Ritchie *et al.*, 2005). D'Anatro and Loureiro (2005) examined the geographic variation in *Austolebias luteoflamulatus* and found that the differences between the sexes may be a result of the forces of sexual selection. However, "over evolutionary time scale[s], biogeography and ecological specialisation may swamp the more subtle phylogenetic signal due to sexual selection." (Ritchie *et al.*, 2005 p.928). Within *Gobiomorphus* we are looking at a more recent situation where phylogenetic patterns are unlikely to have been obscured over time. Signals due to sexual selection may have instead been swamped by the cost to males of elaborate colours. Environmental influences may also play a role in the observed morphological divergence, where there are adaptive roles for morphological differences (Langerhans *et al.*, 2003). Smith *et al.* (2003) suggested this as a reason why *G. alpinus* is morphologically different to *G. cotidianus*, as they are exposed to high altitude conditions. However, *G. cotidianus* is found in similar environments as both *G. basalis* and *G. alpinus*, suggesting that the environment has little influence on the expressed phenotypes.

Gobiomorphus breviceps has two distinct Northern and Southern monophyletic groups and has the largest within-species divergence. The North Island group is made up of North Island populations as well as upper South Island populations. The South Island group is made up of populations from the East Coast of the South Island down through to Southland. The disjunction between the two groups is postulated by Smith *et al.* (2005) to be due to Pleistocene glacial periods 1,810,000-11,550 years before present (bp) when sea levels were lower than the present day and the northern South Island was connected to the lower North Island. Molecular clock calibrations agree with this theory, as they suggest the two *G. breviceps* groups have been isolated for around 2 million years. The presence of two monophyletic groups in *G. breviceps* suggests that

they are actually two separate species (phylogenetic species concept), which was further supported by the ML tree, where they could be compared to the other species in the genus. Turner (1999) believed that (taken from Cracraft, 1989; Mayden & Wood, 1995); "A species should be the smallest monophyletic group (all units contained are descended from a single common ancestor and no such units are excluded) that is supported by characteristics shared by all members of the group". However, defining a species purely on genetic criteria has been criticised, with many believing that species should be defined on the basis of common patterns of variation across multiple characters sets such as mtDNA and morphology (Grady & Quattro, 1999). Extensive morphological examination of *G. breviceps* was beyond the scope of this study, but perhaps should be examined in the future to confirm if they are separate species under the above definition. Furthermore, many believe that to define a species as a separate unit, they must be reproductively isolated (biological species concept) (Turner, 1999), which is difficult to determine in the case of *G. breviceps* as the populations are allopatric. Therefore, it is suggested that the two *G. breviceps* groups be defined as separate entities, however, further research is necessary to properly define these two "species".

CONCLUSIONS

The Australian *G. coxii* is a sister species to the New Zealand *Gobiomorphus* and has been isolated from the New Zealand group for 6-37 Mya, suggesting that *Gobiomorphus* dispersed to New Zealand from Australia via oceanic dispersal after Gondwana broke-up (~ 80 Mya), this is further supported by the fossil record that indicates that the oldest fossil is from the Miocene epoch (16-20 Mya), with no evidence of *Gobiomorphus* being in New Zealand before the Oligocene (period of marine transgression).

Gobiomorphus hubbsi is a diadromous species that formed a sister group to all other New Zealand *Gobiomorphus* species, indicating that diadromy is a primitive feature at least for the New Zealand radiation of *Gobiomorphus* (oceanic dispersal requires a marine phase to be present in the lifecycle of a species). Loss of diadromy (a

derived feature) has been postulated as an important mechanism for speciation, and appears to be important in the speciation of the New Zealand *Gobiomorphus* where the recently derived species are non-diadromous (e.g. *G. basalis, G. alpinus* and *G. breviceps*).

The presence of monophyly suggests that *Gobiomorphus* colonised New Zealand from Australia in a single event followed by a series of radiations: (1) *G. huttoni* and *G. gobioides* (which later diverged), (2) *G. breviceps*, which has two distinct North and South monophyletic clades. The Northern group is formed by populations from the North Island as well as some populations from the north-east of the South Island. The Southern group is formed by populations of the lower South Island. The two groups were potentially isolated by the Pleistocene glacial period (1,810,000 – 11,500 years BP) when the northern South Island was connected to the lower North Island. (3) *G. cotidianus, G. basalis* and *G. alpinus* complex, which had the lowest between species divergence rates, and are not easily differentiated using mtDNA. However, they are easily differentiated using AFLPs (C. Michel, M.I. Stevens and M. van den Heuvel, *unpubl. data*). The morphological divergence observed (despite the lack of mtDNA divergence) may have been accelerated by sexual selection, where certain traits are selected for, or by environmental influences, where there are adaptive roles for morphological divergence. However, environmental influences on morphology, seems an unlikely reason for the observed morphological divergence between *G. cotidianus* and *G. basalis* as they are found in the same environment.

ACKNOWLEDGEMENTS

This work was possible owing to the support from a Julie Alley Bursary, Massey University. We are grateful to Jessica Costall, Shaun Neilson, Logan Brown, Kiryn Weaver, Jono Tonkin, Carol Nicholson, Zoe Dewson and Alex James for their help in the field. We are also grateful to Angela McGaughran for help in the laboratory and for helpful comments on this manuscript. This study was carried out under approval from the Massey University ethics committee, protocol number 04/125.

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CHAPTER II

GENES AND MORPHOLOGY: A COMPARATIVE EXAMINATION OF *G. BREVICEPS* AND *G. BASALIS* IN THE LOWER NORTH ISLAND, NEW ZEALAND*

Key Words: *Gobiomorphus breviceps*, *G. basalis*, mitochondrial DNA, morphology, divergence

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ABSTRACT

Gobiomorphus breviceps (Upland bully) and *Gobiomorphus basalis* (Cran's bully) have an overlapping distribution in the lower North Island and can be difficult to differentiate due to similar morphologies. We examined mitochondrial DNA (mtDNA) and morphological characteristics to identify diagnostic characters that could clearly differentiate these two species. As *Gobiomorphus cotidianus* (common bully) is also found in the lower North Island and is known to often be confused with *G. basalis*, specimens were included in the morphological analysis. Furthermore, we examined whether these morphological characters could resolve any geographical patterns, and if these patterns were congruent between mtDNA and morphology. MtDNA proved to be an effective tool both for identifying fish species and for examining genetic divergence between populations and catchments. Populations in the Manawatu (Manawatu catchment) and Wairarapa (Ruamahanga catchment) regions shared a number of identical haplotypes, as well as harbouring some catchment specific haplotypes. However, the Wellington (Makara) populations had only unique haplotypes. These results are discussed in terms of catchment connections during the Pleistocene. The most effective diagnostic morphological characteristic for differentiating between species was found to be the number of pectoral fin rays. The morphological data showed relatively little divergence between populations or catchments, despite the characters being highly polymorphic. We conclude that the two species can be differentiated using the pectoral fin rays (although some error is unavoidable as there is some overlap), as well as mtDNA. In addition, we found that the mtDNA revealed geographic structuring between populations and catchments indicating the presence of isolating barriers to dispersal.

INTRODUCTION

The Upland and Cran's bullies are two of the four of New Zealand's endemic non-diadromous *Gobiomorphus* species (family Eleotridae). The upland bully (*Gobiomorphus breviceps*) occurs commonly at low altitudes in a variety of habitats, for example; wetlands, small streams, farm drains and large gravely rivers, anywhere where the flow is gentle (McDowall, 2000). *Gobiomorphus breviceps* is common and widespread in South Island; in Nelson/Marlborough region, on the west coast as far south as Hokitika and throughout eastern South Island (McDowall, 2000). However, it is also known in the lower North Island in parts of the Manawatu and Ruamahanga (Wairarapa) river systems, and around Wellington region (McDowall, 1990). The Cran's bully (*Gobiomorphus basalis*), like *G. breviceps* is found in a variety of habitats at low altitudes. It has a widespread yet intermittent distribution from Northland (throughout most of the North Island) to its southern limit at Wellington (McDowall, 2000).

The distribution of the two species shows that they have a broadly overlapping zone in the lower North Island, with both species having been found in the Manawatu and Ruamahanga River systems (McDowall, 1975). McDowall (1975) suggested that the two species evolved in the separate islands and were able to become sympatric during the Pleistocene when Cook Strait was bridged by land (Fleming, 1962), and that *G. breviceps* populations in lower North Island are remaining populations from this period (McDowall, 1975). This broad overlap in distribution has led to confusion in the identification of the two species in the lower North Island. Both species exhibit sexual dimorphism, where adult males can possess distinct colour patterns that allow for identification. However, juveniles and females lack the bright and distinct colouration of the males, thus females of both species are often confused (McDowall, 2000). Furthermore, like the non-migratory populations of common bullies (*Gobiomorphus cotidianus*) Cran's and upland bullies lack open pores on the head and have reduced scale cover on the back of the head and back (McDowall, 1990). In areas where non-diadromous bullies and *G. cotidianus* overlap in distribution it can become difficult to distinguish between the species. This has added to the confusion and has been attributed to why *G. basalis* did not reappear in the literature until 1962 when it was re-described

by H.J.Cranfield (it was thought to be one and the same with *G. cotidianus*), even though it was first discovered in 1842 (Cranfield, 1962).

The elucidation of fixed diagnostic morphological characters allows for species to be identified and distinguished. Morphological characters including body ratios and counts of serially repeated structures e.g. fin ray counts have a genetic basis (Christiansen *et al.*, 1988), therefore can be used for identification purposes. Although they have a genetic basis, they are phenotypically plastic and may be strongly influenced by environmental factors, resulting in population differences (Smith *et al.*, 2003; King *et al.*, 2003). This has been observed in both *G. breviceps* and *G. basalis*, where there is considerable variation between populations, although McDowall (1990) stated that there was little evidence of morphological variation in *G. breviceps*. McDowall (1975) however, reported a southward decline in the first dorsal spine counts in *G. basalis*. Additionally, he reported that counts tend to be stable between and within populations in other eleotrids. Ling *et al.* (2001) found that meristic variables such as numbers of vertebrae and gill rakers were strongly influenced by environmental factors in both *Galaxias maculatus* and *Galaxias gracilis* (Dwarf inanga), and the loss of diadromy resulted in fewer vertebrae and more gill rakers. Interestingly, non-diadromous *Gobiomorphus* species have more vertebrae (28-29) than the diadromous species (27) (although *G. cotidianus* a non-obligatory migratory species has 29 vertebrae). This variation in morphological variables has made it difficult to distinguish between *G. basalis* and *G. breviceps*, i.e. there is considerable overlap in the measurements of these features between the two species. For example, the first dorsal fin spine count of *G. breviceps* is usually six, but at times can also be five or seven, whereas for *G. basalis* the first dorsal fin spine count is usually seven or eight, but at times can be six.

Smith *et al.* (2003) provided a molecular analysis of the genus *Gobiomorphus* in New Zealand. They established that the two species (*G. basalis* and *G. breviceps*) could be identified using genetic information. Two mitochondrial DNA (mtDNA) regions; the cytochrome *b* gene (non-coding) and the control region (coding) are subject to different ecological constraints and have been used extensively to examine phylogenetic questions in fish (Smith *et al.*, 2005). The cytochrome *b* gene is variable enough to answer population questions, but the rate of evolution is slow enough to allow for deeper phylogenetic questions to be examined (Meyer, 1994).

Mitochondrial DNA allows for the two described species (*G. basalis* and *G. breviceps*) to be correctly identified and further distinguished by the presence of fixed diagnostic morphological characters. Here we examine two regions of the mitochondrial (mt) genome, the cytochrome *b* gene and the control region. These genes can accurately identify the two described species, to which we can then apply meristic information to identify diagnostic characters that can be used in the field to distinguish between the species. Further, as considerable morphological variation between populations has been observed in both; *G. breviceps* and *G. basalis*, we compared population differences in the mtDNA with morphological data to examine geographical patterns.

The two hypotheses used were: 1) that the lack of gene flow between catchments will result in genetic and morphological divergence; and 2) there will be no genetic or morphological divergence within catchments where gene flow is possible.

MATERIALS and METHODS

Specimen collection

Gobiomorphus specimens were collected from around the lower North Island in the region of Wairarapa, Manawatu and Wellington by hand netting and electric fishing. Specimens were identified (using colour patterns on large fish and first dorsal fin spine counts on all) in the field and returned to the laboratory for further identification as well as meristic measurement and blood sampling. Blood samples were taken from a total of 80 fish. Fish were anaesthetized using 2 Phenoxy-ethanol then 5-50 µL of blood was taken (depending on the size of the fish) from the caudal vein at the base of the anal fin using a fine gauge needle (29 gauge x ½") and primed with 50 µL of anticoagulant (sterilised EDTA (100mM) (Gleeson *et al.*, 1999). Fish were then exposed to a lethal dose of 2 phenoxy-ethanol and preserved in 80% ethanol. Fin clips were taken from the pectoral fin (right fin) on all the fish as a backup. Blood samples and fin clips were frozen at -80°C until required.

Ten *G. breviceps* specimens were collected from Makara, Wellington region in the upper reaches of the Makara stream, 10 from the Wairarapa region in the Kiriwhakapapa stream, 10 from Manawatu region in the lower reaches of the

Kahuterawa (a tributary of the Manawatu River), and 10 from Tokomaru River (Fig. 1). Ten *G. basalis* specimens were collected from Manawatu region in the Nguturoa stream, 10 from the Turitea stream (one specimen was correctly identified as *G. breviceps* by molecular analysis) and 20 from Wairarapa region in the Kopuaranga stream (Fig. 1) (nine specimens from this site were wrongly identified as *G. basalis*, but correctly identified by molecular analysis as *G. breviceps*). *Gobiomorphus cotidianus* was included in the morphological analysis, which were collected from the Marlborough region (9) and Lake Kohangatera in Wellington region (10).

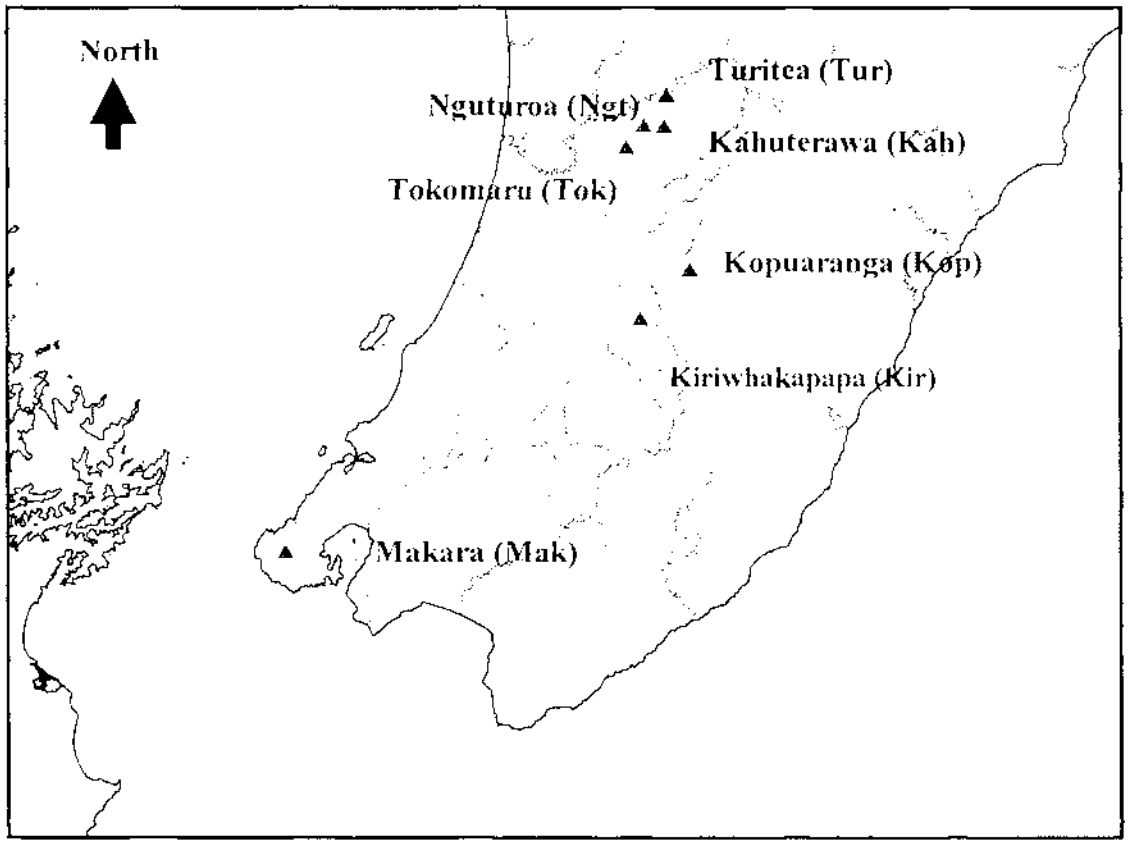


FIGURE 1. The location of the sites sampled for bullies in the Wellington, Manawatu and Wairarapa regions, North Island, New Zealand.

DNA Analyses

mtDNA extraction, amplification and sequencing

Total genomic DNA was extracted from blood and fin/muscle tissue by homogenisation and digestion with proteinase-K and high salt buffer at 57°C for four hours (modified from; Sunnucks & Hales, 1996); then purified by sodium chloride extraction and ethanol precipitation techniques. Two regions of the mitochondrial genome were amplified for each individual using the polymerase chain reaction (PCR). An approximate 500 base pair fragment of the mitochondrial (mt) cytochrome c oxidase *b* gene was amplified using the universal primers Cyb 2 (modified) (5′ – ccc tca gaa tga tat ttg tcc t – 3′) (Kocher *et al.*, 1989) and tGludg (5′ – tga ctt gaa raa cca ycg ttg – 3′) (Palumbi *et al.*, 1991), and the primer pairs H-16498 (5′ – cct gaa cta gga acc aga tg – 3′) (Shields & Kocher, 1991) and L-15995 (5′ – aac tct cac ccc tag ctc cca aag – 3′) (Meyer *et al.*, 1994) were used to amplify approximately 400 base pairs of the control region. Amplifications for each specimen used a 10 µl reaction volume containing 0.75 µl of the extracted DNA (unquantified), 1 x PCR buffer (Roche) 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer-Mannheim), 0.2 µl of each primer (tGludg/Cyb 2 and H-16498/L-15995), 0.5µl of BSA (Bovine Serum Albumin), and 0.1 unit of *Taq* DNA polymerase (Roche). The thermal cycles for both genes (carried out on a T1 thermocycler Whatman Biometra) were: 2 min of initial denaturation at 94°C followed by 35 cycles of denaturation and polymerase amplification (94°C for 30 s, 55°C for 45 s and then 72°C for 30 s), followed by 5 min at 72°C.

PCR samples were purified using SAPEXO (USB Corp.) following the manufacturers guidelines. Products were sequenced (BigDye™ Terminator chemistry (Perkin-Elmer Applied Biosystems)) using the tGludg and H-16498 primers. Sequencing reactions were cleaned using CleanSEQ (Agencourt Bioscience Corp.), before capillary separation on an AB13730 genetic analyser (Applied Biosystems Inc.) at the Allan Wilson Centre Genome Service, Massey University, Palmerston North.

Morphological Analyses

External meristic counts were made on all specimens (specimens left side). The spines in the first and second dorsal fins, and the pectoral, anal and pelvic fin rays were counted using multiple photos (see figure 2 for example photos) taken before the fish were killed and using the whole fish. Multiple measurements (3) were taken (one from photos and two from whole fish) for each characteristic measured to allow averages to be calculated.

Data Analysis

Individual DNA sequences were verified as being derived from the relevant taxa using GenBank™ BlastN search, and were aligned using SEQUENCHER (Gene Codes ver. 4.2) sequence editor (NB: Unable to get cytochrome *b* sequence for specimen Tok(4) so it was removed from genetic analysis). Sequence data were analysed with PAUP* ver. 4.0b10 (Swofford, 2002). Distance matrices of pairwise nucleotide sequence divergence (uncorrected 'p' distance method) were calculated using PAUP. Separate analyses of the cytochrome *b* and control region sequences, produced comparable results. We then examined the congruence of the two data sets with a partition homogeneity test (Farris *et al.*, 1994). One hundred partition replicates were analysed under maximum parsimony (MP) using heuristic searches in PAUP. The two datasets were combined and analysed as composite sequences. Bootstrapping (100 runs) was performed using splitstree ver. 4.1 (Huson & Bryant, 2006). Canonical discriminant analysis was carried out on the morphological data and the distance matrices with SAS system ver. 8.02 (SAS Institute Inc. 2001). MANOVA SAS tests were used to test the strength of associations in the analysis.

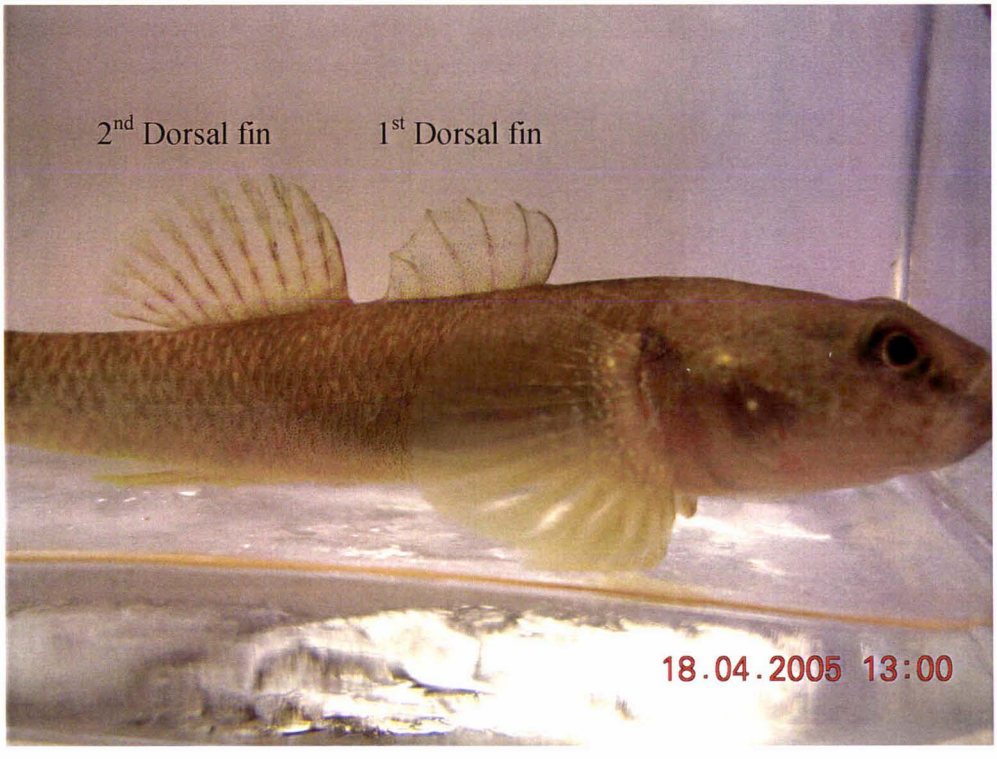


FIGURE 2. Examples of photos used to count fin rays.

RESULTS

Molecular Analysis

Molecular analyses of the specimens correctly identified the two species. As a result some specimens that had been collected and misidentified in the field were subsequently analysed as different species. In particular, one of the Turitea specimens and nine of the Kopuaranga specimens were wrongly identified as *G. basalis*.

Analyses used 382 basepairs (bp) of the control region and 411 bp of the cytochrome *b* gene. Of the cytochrome *b* sequences 33 of the 411 bases, and 31 of the 382 bases for the control region were variable (Table 1). Genetic divergences ranged from 0.13% to 1.6% for *G. basalis* (Appendix III), and 0.13% to 0.9% for *G. breviceps* (Appendix IV). The highest sequence divergences (0.4% - 0.9%) in *G. breviceps* were between the Makara population, and the Manawatu and Wairarapa populations. The lowest sequence divergence (0.13% - 0.3%) was between the Manawatu and Wairarapa populations (Appendix IV). The highest sequence divergences (0.6% - 1.6%) in *G. basalis* were between the Manawatu and Wairarapa populations. However, these populations also showed individuals with identical haplotypes for both mtDNA regions. Sequence divergence was generally low within populations (0.13% - 0.5%) (Appendix III).

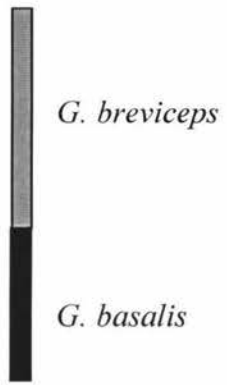
For the combined sequence data (combined based on partition homogeneity test), a total of 15 haplotypes were found; seven *G. breviceps* haplotypes and eight *G. basalis* haplotypes (Fig. 3). One identical haplotype was found between the two river catchments (Manawatu and Ruamahanga) for both species (Fig. 3). However, some haplotypes occurred only within particular catchments but not between them. For example, the Makara stream contained two haplotypes that were unique to that catchment (Fig. 3). They were also the most dissimilar haplotypes for the *G. breviceps* populations, where the Ruamahanga and Manawatu haplotypes were most similar. The *G. basalis* haplotypes in the Manawatu and Ruamahanga were genetically distant from each other (apart from the one identical haplotype) (Fig. 3). However, haplotypes from both catchments were found at each extreme of the network. For both *Gobiomorphus* species there was very little geographical divergence between populations (Fig. 3), with the populations in the lower North Island appearing genetically homogeneous. In particular, for *G. breviceps* the Wellington populations were distinct from the Manawatu and Wairarapa populations.

TABLE 1. Variable sites. The first set of variable sites is the cytochrome *b* sequences and the second is the control region sequences. Locations where each haplotype was found are indicated using location codes from fig. 3 and haplotypes are given a single letter code. Identical character states are indicated by dots. The (–) indicates a deletion.

Haplotype	LOCATION	6	18	32	33	51	69	87	99	108	142	153	156	165	180	181	183	213	225	228	243	270	271	288	303	306	315	326	327	336	343	349	366	382
A	Tok(1-3,5-9), Kah(1-10), Ngt(4), Tur(2-10), Kir(2,3,5,7-10)	A	C	G	C	T	A	A	A	A	A	G	G	G	T	G	A	A	G	T	A	C	G	T	A	C	G	C	T	C	C	T	A	T
B	Kir(1,4,6)	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	Kop(3,10,11,13,14,16,17,19,20)	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
D	Mak(1-2,4-10)	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
E	Mak(3)	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
F	Ngt(1,3,6,7,8,9,10)	G	.	A	.	C	C	.	C	C	.	A	C	A	C	.	C	C	A	C	C	T	T	C	C	T	A	T	C	A	T	C	G	C
G	Ngt(2)	G	.	A	.	C	C	.	C	C	G	A	C	A	C	.	C	C	A	C	C	T	T	C	C	T	A	T	C	A	T	C	G	C
H	Ngt(5)	G	.	A	.	C	C	.	C	C	.	A	C	A	C	.	C	C	A	C	C	.	T	C	C	T	A	T	C	A	T	C	G	C
I	Kop(4,6,15), Tur(1)	G	.	A	.	C	C	.	C	C	.	A	C	A	C	.	C	C	A	C	C	T	T	C	C	T	A	T	C	A	T	C	.	C
J	Kop(2,5,7,8,9,12,18)	G	A	A	.	C	C	.	C	C	.	A	C	A	C	.	C	C	A	C	C	T	T	C	C	T	A	.	C	A	T	C	G	C
K	Kop(1)	G	A	A	.	C	C	.	C	C	.	A	C	A	C	A	C	C	A	C	C	T	T	C	C	T	A	.	C	A	T	C	G	C

TABLE 1. (cont.)

Haplotype	LOCATION	13	14	52	65	73	83	84	90	94	114	131	139	140	142	144	149	151	153	183	189	203	206	226	230	233	257	265	274	301	358	366
A	Tur(3-8,10), Ngt(4), Kir(1-10), Kah(1-10)	A	T	G	_	T	T	A	A	A	A	T	T	G	A	T	A	A	C	A	T	G	T	T	A	T	T	T	T	T	T	T
B	Tok(1-3,5-9), Kop(3,10,11,13,14,16,17,19,20), Tur(9)	.	.	.	_	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	Mak(1-10)	.	.	.	_	.	A	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.
D	Tur(2)	T	.	.	_	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
E	Ngt(1-3,5-8,10)	.	A	C	A	A	A	C	.	.	G	C	A	A	C	G	T	.	G	.	C	A	C	A	.	C	C	A	G	A	C	C
F	Ngt(9)	.	A	C	A	A	A	C	.	.	G	C	A	A	C	G	T	T	G	.	C	A	C	A	.	C	C	A	G	A	C	C
G	Kop(2)	.	A	C	A	A	A	C	.	.	G	C	A	A	T	G	C	.	A	G	C	A	C	A	C	C	.	A	G	A	C	C
H	Tur(1), Kop(4,6,15)	.	A	C	A	.	A	C	C	G	G	C	A	A	C	G	T	.	G	.	.	A	C	A	.	C	C	A	G	A	C	C
I	Kop(1,5,7,8,9,12,18)	.	A	C	A	A	A	C	C	G	G	C	A	A	T	G	C	.	A	G	C	A	C	A	C	C	.	A	G	A	C	C



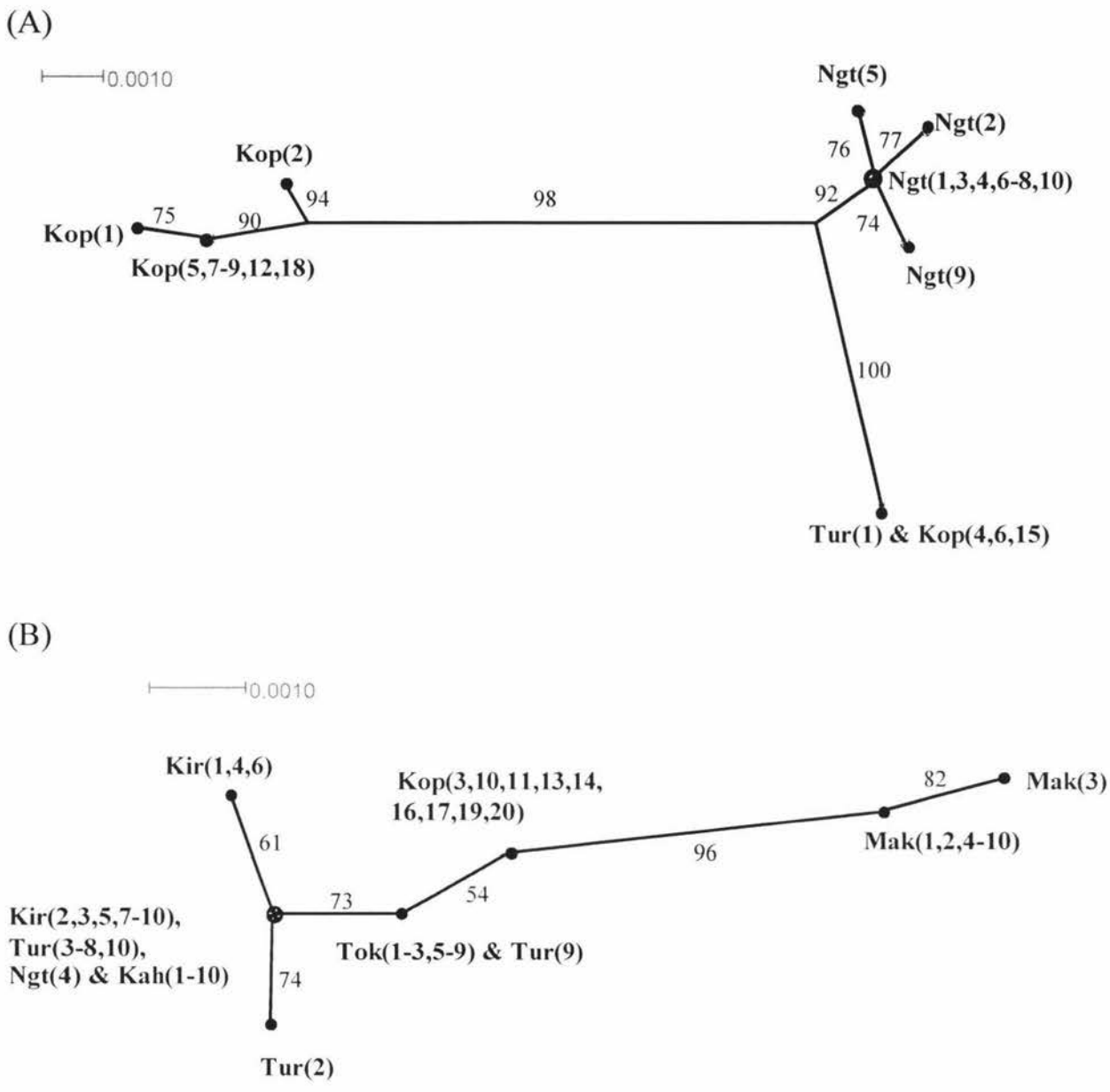


FIGURE 3. Haplotype network (neighbour joining with 100 bootstraps) showing mtDNA haplotypes and their relative connections, with bootstrap support. Data from: (A) *G. basalis* (B) *G. breviceps*.

Morphological Analysis

We examined external morphology for all individuals used for DNA analyses in addition to 19 (10 Lake and 9 riverine specimens) *Gobiomorphus cotidianus* (common bully). Canonical discriminant analyses were carried out using all five morphological characters (1st and 2nd dorsal spine counts, pectoral, anal and pelvic fin rays) to examine phenotypic differences between the species. The analysis showed that the morphological characters for all three species differed significantly (see Fig. 4). A MANOVA test rejected the null hypothesis that species had no overall effect (< 0.0001), indicating that species assignments had a significant relationship to the morphology of the individuals. The discriminant analysis had a correct classification success rate of 95 %. With cross-validation, this success rate became 74 %. The first canonical discriminant function (CDF) explained 97% of the variation seen between the three species, whereas the second CDF only explained 3% of the variation observed in the data. Therefore, only the canonical coefficients from the first CDF were interpreted.

The canonical coefficients from the first CDF showed that the pectoral fin ray counts had the highest influence of the variables in separating the species, followed by the anal fin ray counts and first dorsal spine count (see Table 2). This indicated that the species could be discriminated (both male and female) by using pectoral, anal and first dorsal spine counts. The rules for classifying the species are; pectoral fin rays ≤ 16 = upland, pectoral fin rays 17 or 18 AND anal fin rays < 11 = Cran's, pectoral fin rays $\geq$ 18 AND anal fin rays ≥ 11 = common. The classification success rate of this rule for this data set is 95 %. By comparing the canonical coefficients with figure 4 it can be seen that *G. basalis* and *G. cotidianus* had greater fin ray counts for both pectoral and anal fins as well as a greater number of first dorsal spine counts than found for *G. breviceps*. This is clearly seen in figure 5, showing pectoral fin rays against anal fin rays (two most defining characteristics). *Gobiomorphus basalis* and *G. cotidianus* could be discriminated from *G. breviceps* by the presence of more than 16 pectoral fin rays. This was the case most of the time; however, there was still some overlap with one *G. breviceps* specimen having 17 fin rays and one *G. basalis* specimen having only 15 fin rays. *G. cotidianus* could be discriminated to some extent from *G. basalis* by the presence of more than 18 pectoral fin rays, and also by slightly more anal fin rays (occasionally > 11). This separation of *G. basalis* from *G. cotidianus* suggests that *G.*

basalis caught and identified in the field, were *G. basalis* rather than *G. cotidianus*, although there is still potentially error here as there was some overlap.

TABLE 2. Canonical coefficients for each morphological character showing relative importance of each variable for discriminating the three species.

Variable (morphological character)	**Canonical coefficient** Canonical axis 1	Canonical axis 2
1st Dorsal fin	0.260	0.633
2nd Dorsal fin	-0.196	0.846
Pectoral fin	0.933	-0.095
Pelvic fin	0.000	0.000
Anal fin	0.378	-0.482

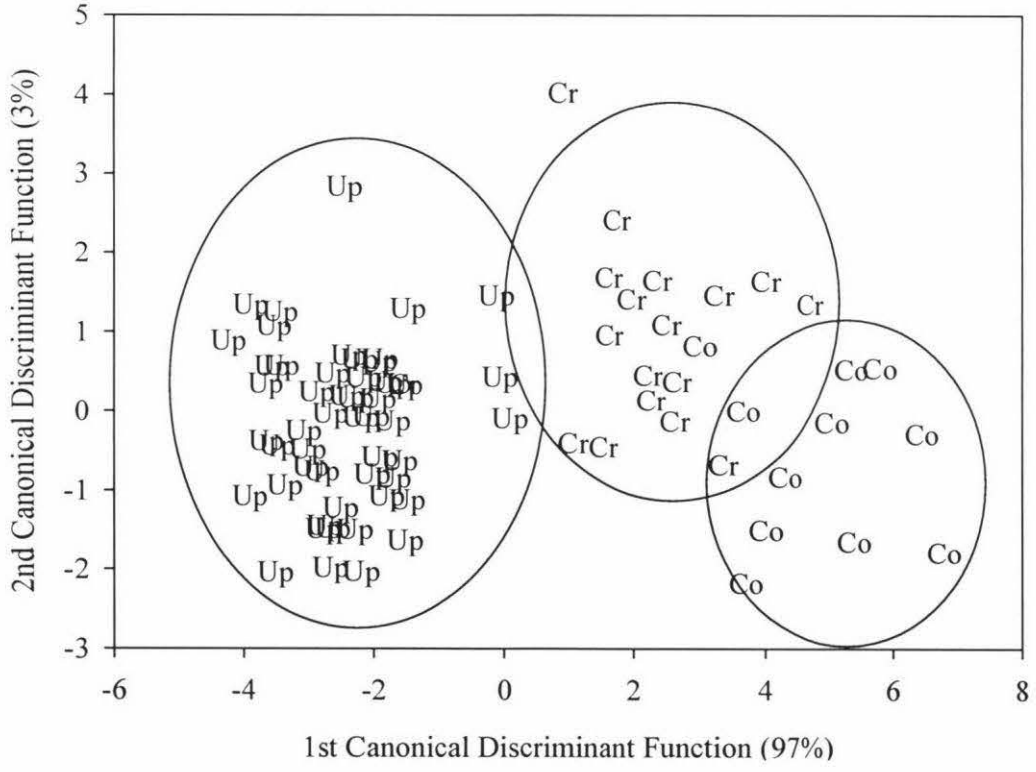


FIGURE 4. Discriminant analysis of all three species based on morphological data. The species form three distinct clusters with some overlap. Up= *G. breviceps*, Cr= *G. basalis* and Co= *G. cotidianus*.

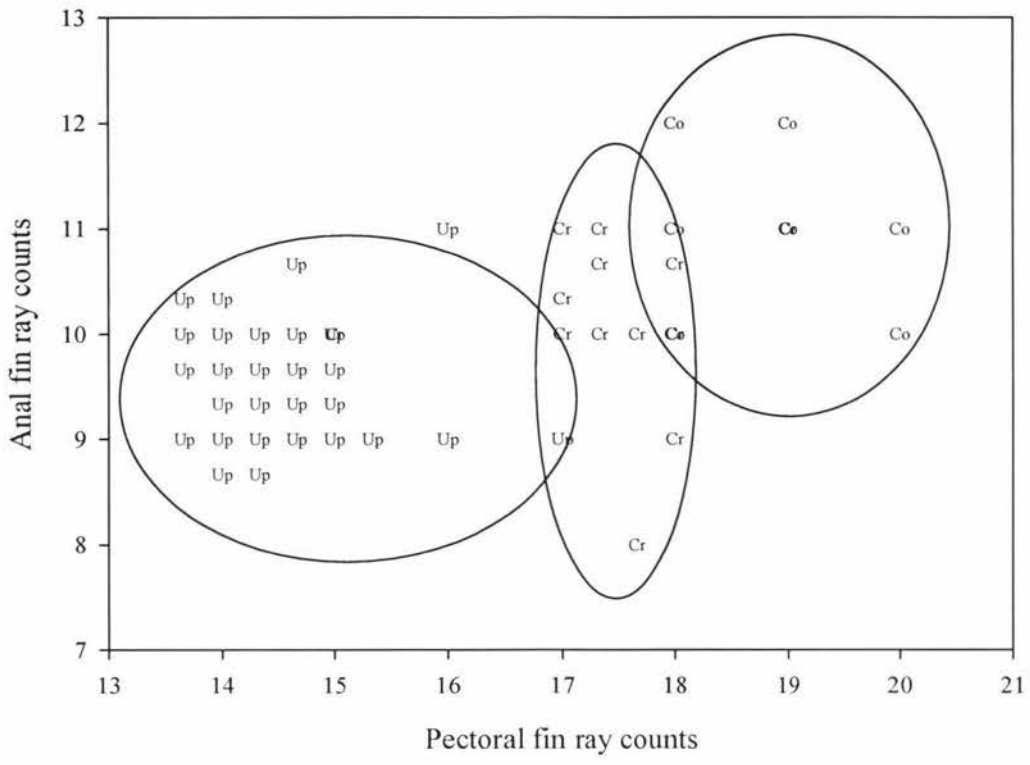


FIGURE 5. Scatter plot using the two best discriminating features (pectoral fin ray counts and anal fin ray counts) from canonical discriminant analysis, showing three fairly distinct species groups.

Canonical discriminant analysis was also used to examine differences between the populations. The location groups overlapped considerably with both species, indicating that morphology did not separate out the populations i.e. there is little divergence between populations morphologically (Figs. 6a & b). MANOVA tests showed that the null hypothesis that there is no overall location effect was marginally acceptable for the *G. basalis* populations ($p = 0.06$), whereas it could be rejected for the *G. breviceps* populations ($p = 0.02$) as there was a small effect of location on morphology. The eigenvectors from the first CDF, showed that the pectoral fin ray counts had the greatest influence (0.963) in separating the populations.

As there is the potential for dispersal between populations, morphological divergence may be more likely between catchments rather than populations. A canonical discriminant analysis was carried out on the morphological data of both *Gobiomorphus* species, looking at the difference between catchments. Figure 7 showed

that there is some clustering within catchments for *G. breviceps*, but there was a large amount of overlap between catchments. A MANOVA ($p = 0.008$) indicated that there is some catchment relationship with the morphology of the species. However, for *G. basalis* (MANOVA; $p = 0.510$), there is no significant catchment relationship to the morphology of the species. The large morphological variation both between catchments, populations and individuals indicated that morphology may be highly plastic.

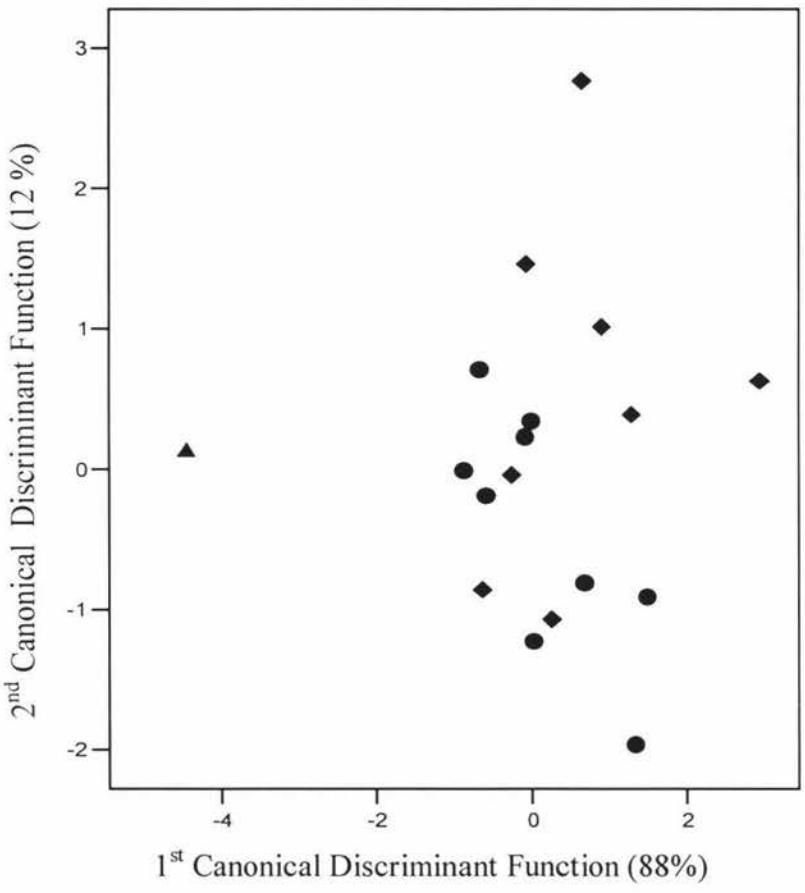


FIGURE 6a. Canonical discriminant analysis of the three *G. basalis* locations using morphological data (all 5 variables). Each point represents individual specimens. Location: Circle = Ngturoa; triangle = Turitea; diamond = Kopuaranga.

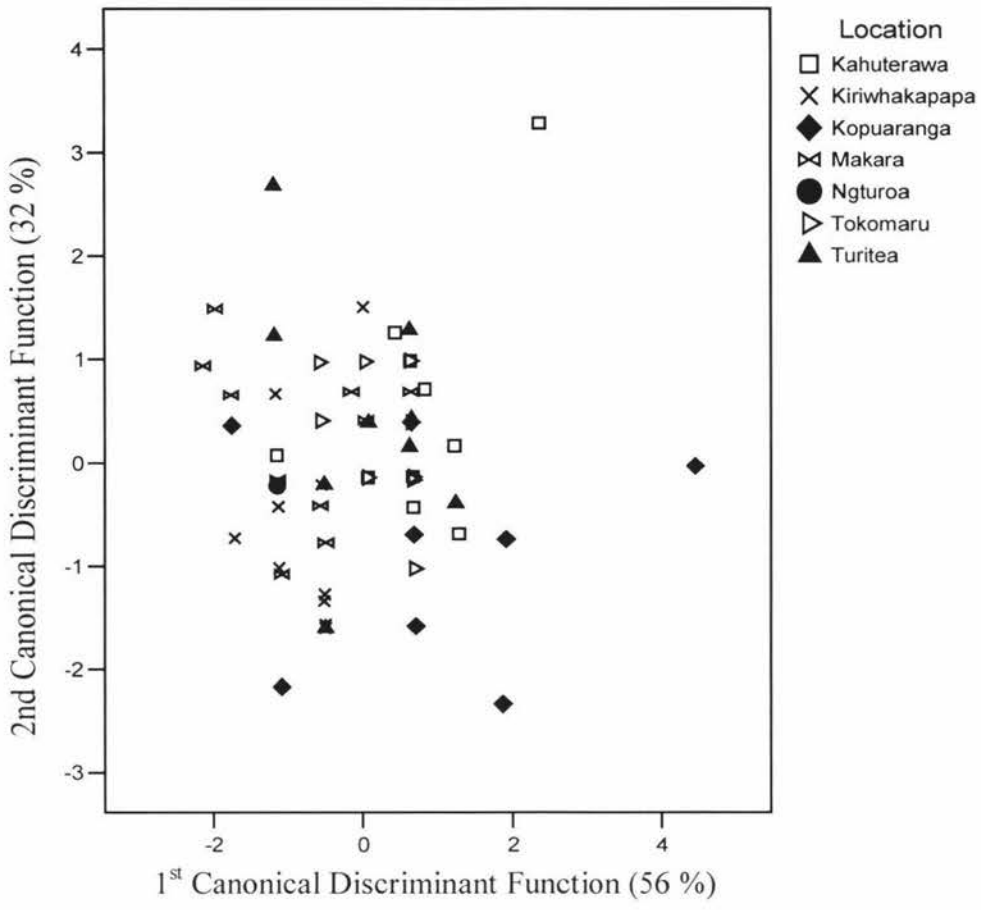


FIGURE 6b. Canonical discriminant analysis of the seven locations using *G. breviceps* morphological data (all 5 variables). Each point represents individual specimens.

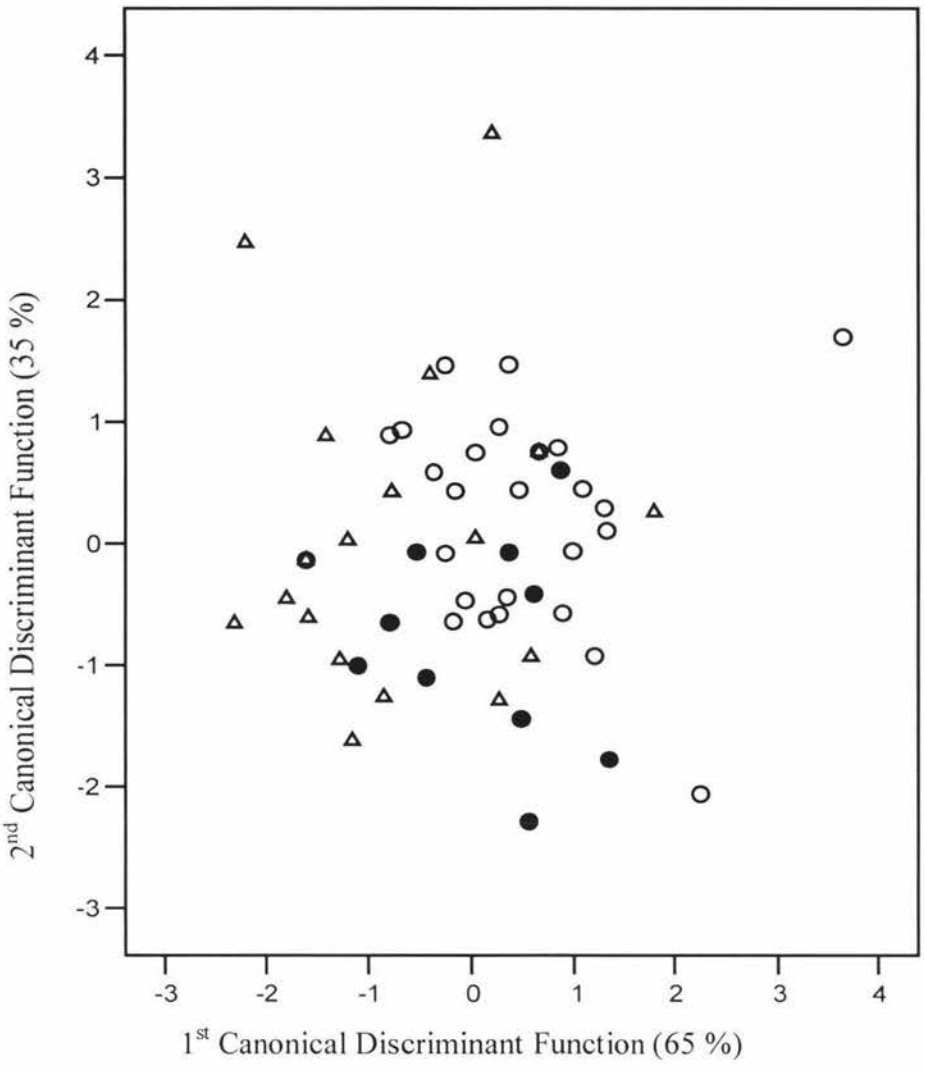


FIGURE 7. Canonical Discriminant analysis of the three catchments based on *G. breviceps* morphological data (all 5 variables). The three catchments overlap considerably. Catchment: Open circle = Manawatu; closed circle = Wellington; triangle = Wairarapa.

DISCUSSION

The mtDNA provided clear species discrimination, and demonstrated the geographic divergence between populations of both *G. breviceps* and *G. basalis*. Populations of non-diadromous fish are restricted to separate catchments, as they spend their whole life cycle in freshwater, this allows for these populations to evolve separately (Allibone, 2002). Therefore, we might expect these non-diadromous *Gobiomorphus* species to have diverged to some extent between catchments.

The North Island populations of *G. breviceps* have been described as relic populations from the last glaciation when there were land connections between the North and South Island due to lower sea levels (Smith *et al.*, 2005). At the end of the last glaciation, around 17,000 years ago (Allibone, 2002), the sea levels rose and thus for non-diadromous species, isolation between the North and South Island populations was likely. Due to this time-scale, the divergence between populations would be expected to be low between lower North Island and upper South Island, and even lower between populations in the North Island. This pattern is reflected by the genetic structure of dwarf Galaxias (*Galaxias divergens*) in the upper South Island and lower North Island (Allibone, 2002). The low divergence (0.4% – 0.9%) of *G. breviceps* reflects the fairly recent colonisation of the North Island, and relatively short time for evolution. Alternatively, these low divergence levels could reflect the time since *G. breviceps* stopped being diadromous. In comparison, *G. basalis*, which has divergence levels of (0.6% – 1.6%) is typically a North Island species and has potentially been present in isolated catchments for a greater length of time. However, once again divergence could merely be a reflection of life history i.e. the time since they were a diadromous species.

The variable sites (Table 1) showed that there are few haplotypes specific to certain catchments, as well as some identical haplotypes found between the Manawatu and Ruamahanga catchments. The Makara stream in Wellington has haplotypes (D and E; cytochrome *b* region, and C; control region) that are unique to that catchment. As both *G. breviceps* and *G. basalis* are non-diadromous species it is expected that there would be little mixing of haplotypes between catchments, with haplotypes exhibiting divergence between catchments. Smith *et al.* (2005) examined the genetic divergence of *G. breviceps*, and found that haplotypes were catchment specific both in the North and

South Island. In particular, they found that haplotypes were only shared between the Manawatu and Ruamahanga catchments, and proposed that it was due to the Pleistocene connections (geological evidence suggests that the two catchments were interconnected) (Stevens, 1980), this was also reflected by the molecular clock data. The Kopuaranga site in the Wairarapa drains into the Ruamahanga catchment, but is approximately 200 metres away from a river that drains into the Manawatu catchment. Potentially the Kopuaranga could have originally been part of the Manawatu river system and been 'captured' by the Ruamahanga river system through various means e.g. an earthquake or landslide. The Wairarapa fault line lies near the Wairarapa sites, and has been active in the last 1000 – 10 000 years (Schermer *et al.*, 2004), therefore, could potentially have caused an offset, allowing gene flow across catchments. Jackson *et al.* (1998) examined anticlines (a type of fold that contributes to mountain building) in the Manawatu, and found that there was a clear relationship between the active faults and drainage patterns. Streams and rivers may be captured or diverted by these vertical uplifts, affecting dispersal among of populations.

The divergence of the *G. breviceps* Makara population indicates that gene flow has been low enough to enable fine-scale divergence. Allibone (2002) found that lower south-west North Island populations of dwarf Galaxias (*Galaxias divergens*) were genetically distinct from those in the Manawatu and Ruamahanga. Smith *et al.* (2005) also found that *G. breviceps* in the Ohau River (south-west North Island) were genetically distinct. This suggests that there has been relatively little mixing between these populations that do not form part of the Manawatu or Ruamahanga river systems. The Makara population is well separated from the Manawatu catchment by the Rimutaka range and approximately 90 km of land, and separated from the Ruamahanga by the Tararua ranges as well as approximately 30 km of land. This may be responsible for the divergence patterns observed.

Despite the large within species morphological variation, all three species could be discriminated using multivariate analysis (with minor overlap). The ability to be able to differentiate *G. cotidianus* from *G. basalis* morphologically is of some importance, as they cannot always be differentiated using mtDNA. Smith *et al.* (2003) found a similar pattern between *G. cotidianus* and *G. alpinus*. Although the two species were morphologically distinct they were not differentiable using mtDNA. The quantitative character that most clearly separated the three species was the number of pectoral fin rays. The next best discriminator was the number of rays in the anal fin, which helped to

further clarify *G. cotidianus* from *G. basalis*. Pectoral fin ray counts as the best distinguishing feature between *G. breviceps* and *G. basalis* has also been found by Fenaughty (unpublished thesis, 1986) and McDowall (1990), who further described *G. basalis* as being distinguishable from *G. cotidianus* by the small and widely spaced scales on the back of the head and fewer scales on the opercula. In the present study only characters easily examinable in the field were looked at.

The ability to identify species based on fixed diagnostic morphological characters fairly accurately indicates that they have a genetic basis. Despite this they are also highly plastic, and may be strongly influenced by environmental factors. Lindsey (1988) found that the number of vertebrae and fin rays in two *Galaxias* species correlated with water temperatures. Population differences may then be a result of environmental influence rather than genetic variation (Smith *et al.* 2003), which has been postulated as the cause of the meristic differences in *G. alpinus*. Smith *et al.* (2003) suggested that it might be a response to the extreme high-altitude environment they live in. However, *G. cotidianus* found at similar altitudes express similar phenotypic characteristics to lowland *G. cotidianus*, and there is little evidence available to suggest that Eleotrids modify their morphology in response to environmental conditions (McDowall, *pers. comm.*). It is expected that if there is a strong genetic basis to morphological characters, then genetically diverse populations within a species may have diverged morphologically as well.

The morphology of the individual populations overlapped considerably; there was a large amount of variation that did not fit the patterns of genetic divergence. Location had no overall relationship with morphology. This could in part be due to the continual gene flow between populations within a river system, indicating that divergence may in fact be at the level of the catchment. However, there was a considerable amount of morphological overlap between catchments. The MANOVA test indicated that there is a weak catchment relationship with morphology, which may be due to several factors, namely; (1) that there has potentially been gene flow between the Manawatu and Ruamahanga in the recent past; (2) morphological divergence may closely reflect mtDNA divergence patterns; (3) environmental influences on morphology may disrupt divergence patterns. However, it would be expected that individual populations would have similar morphologies as they would be exposed to similar environmental influences, which was not the case in the present study (high variation within populations).

D'Anatro and Loureiro (2005) examined the geographic variation in *Austrolebias luteoflamulatus*, an annual killifish in Uruguay, where gene flow interruption between populations may occur on a regular basis. The results of their study showed that populations are structured according to geographical patterns corresponding to different drainage systems; this effect was greater in the males than females. It was thought that sexual selection and to some extent environmental variables played an important role in the differentiation of the populations in this species. As both *Gobiomorphus* species are sexually dimorphic, it is possible that sexual selection may have some impact on the morphology of the populations. Neves and Monterio (2003), in contrast discovered that there was an environmental selection pressure on the genotype expressed when looking at the morphological variation between populations of livebearing fish *Poecilia vivipara* in lagoons of Grussai and Iquipari in Brazil.

CONCLUSIONS

Molecular analysis provides a useful tool for the identification of fish to enable definition of diagnostic characters. It also allows for population differences to be examined and compared with morphological data to study geological differences. In this case, the Manawatu and Ruamahanga river systems shared identical haplotypes as well as having some unique haplotypes. The lack of divergence between the two catchments has been postulated to be due to catchment connection during the Pleistocene (although the possibility that divergence levels merely reflect life history i.e. time since the species were diadromous can not be ruled out without further examination). The spatial proximity of the two catchments could have led to a stream being "captured" by one river system from the other, thus allowing gene flow to occur. The Makara population of *G. breviceps* was the most divergent population probably due to lack of possible connection with the other two catchments.

Gobiomorphus breviceps, G. basalis and *G. cotidianus* in the lower North Island can be differentiated using morphological information i.e. fin ray counts (although there is some error involved i.e. with cross-validation there was 26 % error). The most effective diagnostic character is the pectoral fin ray followed by the anal fin rays. The

lack of morphological divergence and high variation between the populations and catchments can potentially be contributed to environmental influence on morphology, or the high gene flow between the populations, which may limit evolution. Sexual selection is unlikely to be effecting these populations, as it should enhance morphological divergence.

More extensive examination of morphology and genetics of the *Gobiomorphus* species throughout New Zealand may provide further insights to the divergence patterns between catchments and populations.

ACKNOWLEDGEMENTS

This work was possible owing to the support from a Julie Alley Bursary, Massey University. We are grateful to Jessica Costall, Shaun Neilson, Logan Brown, Kiryn Weaver, Jono Tonkin, Carol Nicholson, Zoe Dewson and Alex James for their help in the field. We are also grateful to Angela McGaughran for help in the laboratory and for helpful comments on this manuscript. This study was carried out under approval from the Massey University ethics committee, protocol number 04/125.

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THESIS CONCLUSION

Genetic studies have contributed to resolving problems with taxonomically difficult groups, detecting diversity between morphologically similar species, as well as examining geographical divergence within species that previously have not been well resolved by morphological studies alone (Vrijenhoek, 1998; Orti *et al.* 1994). Within the *Gobiomorphus* genus, cladistics have typically been carried out using morphological characters that have not allowed for the examination of deeper questions such as; the analysis of origins, evolution of diadromy and relationships within the taxa as well as relationships with other families. Molecular data has allowed for the high resolution analyses of population-level questions in many species of fish (Orti *et al.* 1994), as well as accurate identification of fish species. In New Zealand, mtDNA has been used extensively to provide insights into the origins, evolution and phylogenetics of many fishes including Galaxiidae and *Gobiomorphus*.

In this thesis, I used mtDNA to examine phylogenetic relationships (including divergence), the origins and evolution of diadromy within *Gobiomorphus*, species identification, and morphological variation.

Examination of phylogenetic relationships within the Australasian *Gobiomorphus* (Chapter I) revealed that the genus is indeed a monophyletic group if the species *G. australis* is removed. However, Thacker and Hardman (2005) found strong support for the monophyly of the group inclusive of *G. australis*. Furthermore, the Australian *Gobiomorphus* form a polyphyletic group basal to the New Zealand radiation. Molecular clock calibrations indicate that the New Zealand and Australian *Gobiomorphus* have been isolated for 6-37 Myr, suggesting that the New Zealand species dispersed here post- Gondwanan break-up from Australia. Interestingly, the molecular clock dates match the late Oligocene epoch (34-23 Mya), the end of the period in which New Zealand was progressively submerged beneath a marine transgression. Pole (1994) believes that much of the present fauna and flora of New Zealand arrived via trans-oceanic dispersal after this period. Furthermore, fossil records indicate the presence of *Gobiomorphus* since the early Miocene epoch (16-20 Mya) (McDowall, *pers. comm.*). The importance of marine dispersal in Galaxiidae species has also been supported by molecular studies (Berra *et al.*, 1996; Waters & Burridge, 1999; Waters *et al.*, 2000).

The molecular data suggest that New Zealand *Gobiomorphus* dispersed to New Zealand from Australia in a single event (i.e. the presence of monophyly). Once in New Zealand there was then a series of radiations. The most recent radiation produced the *G. cotidianus, G. basalis* and *G. alpinus* species complex. The species are not easily differentiated by mtDNA, which is reflected by the low between-species divergence rates. Smith *et al.* (2003) were able to differentiate *G. basalis* from *G. cotidianus* and *G. alpinus*, but not *G. alpinus* from *G. cotidianus*. Subsequently, they suggested that *G. alpinus* is an eco-phenotype of *G. cotidianus*. *Gobiomorphus breviceps* is also part of the later radiation of *Gobiomorphus*, and has the largest within-species divergence. The species forms two distinct Southern and Northern monophyletic groups, suggesting the presence of two species. The disjunction between the two groups has been postulated by Smith *et al.* (2005) to be due to Pleistocene glacial periods (1,810,000-11,550 bp) where the sea levels were lower than the present day, which is supported by molecular calibrations. It is suggested that the northern and southern groups be considered separate entities, however, further research is necessary to properly define these two putative species.

The later radiation of *Gobiomorphus* in New Zealand is of non-diadromous *Gobiomorphus*, suggesting that loss of diadromy (derived feature) is important in speciation. Diadromy appears to be a primitive characteristic as *G. hubbsi* a diadromous species, forms a sister group to all other New Zealand *Gobiomorphus*. Furthermore, *G. huttoni* and *G. gobioides* (both diadromous species) form a monophyletic group that is part of the first radiation. Diadromy has also been considered to be a primitive feature of the galaxiidae (McDowall, 1970; 1978), where the non-diadromous species have speciated from diadromous stock. The ability to disperse via marine currents has allowed *G. huttoni* to reach the Chatham Islands where no other *Gobiomorphus* species are found. The Chatham Island species affiliate with specimens from Waikato, with divergence rates measuring between 20,000 to 400,000 years ago. The Chatham Islands have been identified as remnants of late Cretaceous volcanic islands, and have been oceanic islands for at least 70 million years (Campbell *et al.* (1993). Thus, the presence of *G. huttoni* on the Chatham Islands suggests that dispersal is important mechanism in colonisation.

Chapter II aimed to use mtDNA to accurately distinguish between *G. breviceps* and *G. basalis* in order to identify diagnostic morphological characters. Additionally, population divergence and geographical patterns were examined. The mtDNA revealed

some unique haplotypes both within and between catchments for both *G. basalis* and *G. breviceps*. However, the Manawatu and Ruamahanga catchments shared identical haplotypes, suggesting that there has been some dispersal (gene flow) between the two catchments in the past. Smith *et al.* (2005) examined divergence of *G. breviceps* between catchments, and found that haplotypes were catchment specific with the exception of the Manawatu and Ruamahanga catchments. They suggested that the lack of divergence between the two catchments may be due to connections during the Pleistocene. Additionally, the proximity of the two catchments could have led to river "capture", thus allowing gene flow to occur. The Makara population of *G. breviceps* was the most divergent population, which may be due to the lack of connections with the other two catchments (separated by the Rimutaka and Tararua ranges, as well as approximately 90 km of land).

The mtDNA provided clear species discrimination between the two species, which allowed for the identification of a diagnostic morphological character. It was possible to discriminate between all there species (*G. breviceps, G. basalis* and *G. cotidianus*) using all morphological characters, although there was some overlap, particularly between *G. basalis* and *G. cotidianus*. The quantitative character that most clearly separated the three species was the number of pectoral fin rays, followed by the number of rays in the anal fin, which helped to further clarify *G. basalis* from *G. cotidianus* However, using the classification rules there was still 5 % classification error in the data set. Fenaughty (1986) and McDowall (1990) also found pectoral fin ray counts to be the best discriminatory feature between these species.

The ability to identify species based on fixed diagnostic morphological characters indicates that they have a genetic basis. However, within species and between populations high morphological variation was observed. It has been suggested that morphology is strongly influenced by environmental factors (e.g. Lindsey, 1988; Smith *et al.* 2003; Neves & Monterio, 2003; D'Anatro & Loureiro, 2005). Furthermore, sexual selection (both *G. breviceps* and *G. basalis* are sexually dimorphic) has been found to play an important role in the differentiation of populations in some fish species such as *Austrolebias luteoflamulatus* (an annual killifish in Uruquay) (D'Anatro & Loureiro, 2005), and may have some impact on the morphology of the *Gobiomorphus* populations i.e. morphological divergence between catchments should be enhanced. The lack of morphological divergence between catchments (where dispersal is limited) despite high morphological variation could potentially be due to these influences. However, all the

species are exposed to similar environmental conditions, and sexual selection should result in males exhibiting the same characters within each catchment. Furthermore, the lack of morphological divergence could be a reflection of the genetic homogeneity of the populations.

The issues examined in this thesis highlight the usefulness of mitochondrial DNA for exploring, phylogenetic relationships (including divergence) and solving problems with taxonomically difficult groups, origins of fish species, and evolution of diadromy. Furthermore, molecular data coupled with morphological data can be used to improve identification techniques when dealing with morphologically similar species, as well as defining species and population 'conservation units'. Genetic data, and in particular mtDNA has allowed for high resolution analyses of population-level questions, and has already provided insights into the origins and phylogenetics of New Zealand fishes including Galaxiidae and *Gobiomorphus*.

Future Research

Historically research on New Zealand freshwater fish has focussed on morphology characteristics for systematics, which often lack an accurate perspective on the relationships and origins of fish species (Orti *et al.*, 1994). However, more recently genetic data has had a positive contribution to what we know of relationships and origins of fish species in New Zealand. Typically genetic analysis in New Zealand has focussed on short fragments of mtDNA for examining 'deep' evolutionary hypotheses, however, there is limited variation in the first and second codon positions of these shorter fragments (Meyer, 1994). It is suggested that for future research longer fragments (of approximately 1000 base pairs) are used when looking for high resolution in taxonomic studies.

Genetic data has been coupled with morphological data to some extent to distinguish new species, for example, morphological analyses of the *Galaxias vulgaris* complex has led to a taxonomic revision and identification of four new species (McDowall, 1997). The presence of two distinct *G. breviceps* groups, as indicated by mtDNA, suggests the presence of two species. Morphological analyses of this species would perhaps aid in the description of this species.

Furthermore, the presence of only one *Gobiomorphus* species (*G. huttoni*) in the Chatham Islands is rather puzzling as *G. gobioides, G. hubbsi* and *G. cotidianus* all have dispersive capabilities. This warrants further investigation.

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APPENDIX I. Genetic distance based on sequence variation in the 105 unique mtDNA cytochrome *b* sequences for *Gobiomorphus*. Lower triangle is uncorrected distances and upper triangle is maximum likelihood distances. Location codes refer to those used in Table 1. A – *Gobiomorphus gobioides*.

		G. cotidianus, G. basalis and *G. alpinus* complex																			*G. breviceps*																																
	LOCATION*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
1	BOP/RR(1)		0.010	0.015	0.015	0.015	0.018	0.018	0.018	0.018	0.018	0.018	0.021	0.027	0.024	0.024	0.021	0.018	0.027	0.030	0.079	0.071	0.075	0.079	0.080	0.081	0.080	0.077	0.073	0.070	0.071	0.075	0.110	0.110	0.105	0.105	0.111	0.109	0.105	0.109	0.104	0.105	0.101	0.101	0.102	0.107	0.095	0.091	0.097	0.093	0.105	0.092	0.091
2	BOP/KR(1)	0.011		0.005	0.005	0.005	0.007	0.007	0.007	0.007	0.007	0.007	0.010	0.015	0.013	0.012	0.010	0.007	0.015	0.018	0.062	0.055	0.058	0.062	0.063	0.067	0.063	0.060	0.057	0.053	0.054	0.058	0.089	0.089	0.085	0.085	0.090	0.088	0.085	0.089	0.084	0.085	0.081	0.081	0.086	0.091	0.076	0.072	0.081	0.077	0.088	0.073	0.073
3	A	0.016	0.005		0.002	0.000	0.002	0.002	0.002	0.002	0.002	0.002	0.005	0.010	0.007	0.007	0.005	0.002	0.010	0.013	0.055	0.055	0.051	0.055	0.055	0.059	0.055	0.052	0.049	0.046	0.047	0.051	0.090	0.090	0.086	0.086	0.091	0.089	0.085	0.089	0.085	0.086	0.082	0.082	0.087	0.091	0.077	0.073	0.082	0.078	0.089	0.074	0.073
4	M/N(1)	0.019	0.008	0.003		0.002	0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.012	0.010	0.010	0.007	0.005	0.012	0.015	0.058	0.058	0.054	0.058	0.058	0.062	0.058	0.055	0.052	0.049	0.050	0.054	0.094	0.094	0.089	0.089	0.095	0.093	0.089	0.093	0.089	0.090	0.085	0.085	0.090	0.095	0.080	0.076	0.085	0.082	0.092	0.077	0.077
5	B	0.016	0.005	0.000	0.003		0.002	0.002	0.002	0.002	0.002	0.002	0.005	0.010	0.007	0.007	0.005	0.002	0.010	0.013	0.055	0.055	0.051	0.055	0.055	0.059	0.055	0.052	0.049	0.046	0.047	0.051	0.090	0.090	0.086	0.086	0.091	0.089	0.085	0.089	0.085	0.086	0.082	0.082	0.087	0.091	0.077	0.073	0.082	0.078	0.089	0.074	0.073
6	N/W(2)	0.019	0.008	0.003	0.005	0.003		0.005	0.005	0.005	0.005	0.005	0.007	0.012	0.010	0.010	0.007	0.005	0.012	0.015	0.058	0.058	0.054	0.058	0.058	0.062	0.058	0.055	0.052	0.049	0.050	0.054	0.094	0.094	0.089	0.089	0.095	0.093	0.089	0.093	0.089	0.090	0.085	0.085	0.090	0.095	0.080	0.076	0.085	0.082	0.092	0.077	0.077
7	N/K(5)	0.019	0.008	0.003	0.005	0.003	0.005		0.005	0.005	0.005	0.005	0.007	0.012	0.010	0.010	0.007	0.005	0.012	0.015	0.058	0.058	0.054	0.058	0.058	0.062	0.058	0.055	0.052	0.049	0.050	0.054	0.094	0.094	0.089	0.089	0.095	0.093	0.089	0.093	0.089	0.090	0.085	0.085	0.090	0.095	0.080	0.076	0.085	0.082	0.092	0.077	0.077
8	WC/LB(1)	0.019	0.008	0.003	0.005	0.003	0.005	0.005		0.005	0.005	0.005	0.007	0.012	0.010	0.010	0.007	0.005	0.013	0.015	0.059	0.059	0.055	0.059	0.059	0.063	0.059	0.056	0.053	0.050	0.051	0.055	0.095	0.095	0.091	0.091	0.096	0.094	0.090	0.095	0.090	0.091	0.087	0.087	0.092	0.097	0.081	0.078	0.086	0.083	0.084	0.078	0.078
9	ECo/C(1)	0.019	0.008	0.003	0.005	0.003	0.005	0.005	0.005		0.005	0.005	0.007	0.012	0.010	0.010	0.007	0.005	0.012	0.015	0.058	0.058	0.054	0.058	0.058	0.062	0.058	0.055	0.052	0.049	0.050	0.054	0.094	0.094	0.089	0.089	0.095	0.093	0.089	0.093	0.089	0.090	0.085	0.085	0.083	0.088	0.073	0.069	0.078	0.074	0.085	0.070	0.070
10	M/N(1)	0.019	0.008	0.003	0.005	0.003	0.005	0.005	0.005	0.005		0.005	0.007	0.012	0.010	0.010	0.007	0.005	0.013	0.015	0.058	0.059	0.054	0.058	0.059	0.063	0.059	0.056	0.053	0.049	0.051	0.054	0.086	0.086	0.082	0.082	0.087	0.085	0.081	0.085	0.081	0.082	0.078	0.078	0.083	0.087	0.073	0.069	0.078	0.074	0.084	0.070	0.069
11	M/TS(1), Wa/KS(3)	0.019	0.008	0.003	0.005	0.003	0.005	0.005	0.005	0.005	0.005		0.007	0.012	0.010	0.010	0.007	0.005	0.012	0.015	0.052	0.052	0.048	0.052	0.052	0.056	0.052	0.056	0.052	0.049	0.050	0.054	0.086	0.086	0.082	0.082	0.088	0.086	0.082	0.086	0.082	0.082	0.078	0.086	0.084	0.088	0.073	0.070	0.078	0.075	0.085	0.070	0.070
12	C	0.021	0.011	0.005	0.008	0.005	0.008	0.008	0.008	0.008	0.008	0.008		0.005	0.007	0.012	0.010	0.007	0.015	0.018	0.052	0.053	0.058	0.062	0.059	0.063	0.062	0.053	0.056	0.053	0.051	0.055	0.089	0.089	0.085	0.084	0.082	0.081	0.077	0.081	0.077	0.085	0.081	0.081	0.095	0.100	0.085	0.081	0.084	0.081	0.091	0.076	0.069
13	N/WR(1)	0.027	0.016	0.011	0.013	0.011	0.013	0.013	0.013	0.013	0.013	0.013	0.005		0.010	0.018	0.015	0.012	0.021	0.024	0.052	0.052	0.058	0.061	0.059	0.063	0.062	0.046	0.049	0.046	0.044	0.048	0.080	0.080	0.076	0.076	0.082	0.080	0.076	0.072	0.076	0.084	0.072	0.072	0.094	0.099	0.084	0.080	0.083	0.080	0.090	0.075	0.068
14	W/M(1)	0.024	0.013	0.008	0.011	0.008	0.011	0.011	0.011	0.011	0.011	0.011	0.008	0.011		0.015	0.012	0.010	0.018	0.021	0.063	0.064	0.062	0.066	0.064	0.068	0.067	0.061	0.058	0.054	0.052	0.056	0.091	0.091	0.087	0.086	0.096	0.093	0.090	0.090	0.089	0.090	0.083	0.083	0.101	0.106	0.090	0.086	0.090	0.086	0.097	0.081	0.081
15	N/WS(3)	0.024	0.013	0.008	0.011	0.008	0.011	0.011	0.011	0.011	0.011	0.011	0.013	0.019	0.016		0.012	0.010	0.012	0.015	0.065	0.066	0.061	0.065	0.066	0.070	0.066	0.063	0.060	0.056	0.057	0.061	0.092	0.092	0.088	0.088	0.094	0.092	0.088	0.092	0.088	0.088	0.084	0.084	0.082	0.087	0.079	0.075	0.084	0.081	0.091	0.076	0.076
16	Wa/KS(1)	0.021	0.011	0.005	0.008	0.005	0.008	0.008	0.008	0.008	0.008	0.008	0.011	0.016	0.013	0.013		0.002	0.010	0.012	0.055	0.055	0.051	0.054	0.055	0.059	0.055	0.052	0.049	0.046	0.047	0.051	0.090	0.090	0.086	0.085	0.091	0.089	0.085	0.089	0.085	0.086	0.082	0.082	0.087	0.091	0.077	0.073	0.081	0.078	0.088	0.073	0.073
17	Wa/KS(7)	0.019	0.008	0.003	0.005	0.003	0.005	0.005	0.005	0.005	0.005	0.005	0.008	0.013	0.011	0.011	0.003		0.007	0.010	0.051	0.052	0.048	0.051	0.052	0.055	0.052	0.049	0.046	0.043	0.044	0.048	0.086	0.086	0.082	0.082	0.087	0.085	0.081	0.085	0.081	0.082	0.078	0.078	0.083	0.087	0.073	0.069	0.078	0.074	0.084	0.070	0.069
18	W/MS(4)	0.027	0.016	0.011	0.013	0.011	0.013	0.013	0.013	0.013	0.013	0.013	0.016	0.021	0.019	0.013	0.011	0.008		0.002	0.056	0.057	0.053	0.056	0.057	0.061	0.057	0.054	0.051	0.047	0.045	0.049	0.091	0.091	0.086	0.086	0.092	0.090	0.086	0.090	0.086	0.087	0.082	0.082	0.087	0.092	0.077	0.073	0.082	0.079	0.089	0.074	0.074
19	W/MS(1)	0.029	0.019	0.013	0.016	0.013	0.016	0.016	0.016	0.016	0.016	0.016	0.019	0.024	0.021	0.016	0.013	0.011	0.003		0.060	0.061	0.056	0.060	0.060	0.065	0.061	0.058	0.054	0.051	0.049	0.052	0.095	0.095	0.091	0.090	0.096	0.094	0.090	0.094	0.090	0.091	0.086	0.086	0.091	0.096	0.081	0.077	0.086	0.083	0.093	0.078	0.077
20	NO/K(2)	0.066	0.056	0.050	0.053	0.050	0.053	0.053	0.053	0.053	0.053	0.048	0.048	0.048	0.056	0.058	0.050	0.048	0.050	0.053		0.005	0.015	0.012	0.010	0.012	0.015	0.031	0.034	0.037	0.034	0.031	0.076	0.076	0.072	0.072	0.070	0.069	0.065	0.069	0.065	0.072	0.069	0.075	0.077	0.081	0.074	0.071	0.075	0.072	0.082	0.068	0.068
21	CO/W(2)	0.061	0.050	0.050	0.053	0.050	0.053	0.053	0.053	0.053	0.053	0.048	0.048	0.048	0.056	0.058	0.050	0.048	0.050	0.053	0.005		0.020	0.018	0.015	0.018	0.015	0.031	0.034	0.037	0.034	0.031	0.075	0.075	0.072	0.071	0.069	0.068	0.065	0.068	0.064	0.072	0.068	0.075	0.076	0.080	0.074	0.070	0.075	0.072	0.081	0.067	0.060
22	D	0.064	0.053	0.048	0.050	0.048	0.050	0.050	0.050	0.050	0.050	0.045	0.053	0.053	0.056	0.056	0.048	0.045	0.048	0.050	0.016	0.021		0.007	0.010	0.012	0.015	0.031	0.028	0.031	0.028	0.025	0.061	0.061	0.058	0.058	0.062	0.061	0.057	0.061	0.057	0.058	0.054	0.061	0.059	0.063	0.057	0.054	0.061	0.058	0.067	0.054	0.061
23	ECo/CR(1)	0.066	0.056	0.050	0.053	0.050	0.053	0.053	0.053	0.053	0.053	0.048	0.056	0.056	0.058	0.058	0.050	0.048	0.050	0.053	0.013	0.019	0.008		0.012	0.015	0.012	0.034	0.031	0.034	0.031	0.028	0.064	0.064	0.061	0.061	0.065	0.064	0.061	0.064	0.060	0.061	0.065	0.071	0.069	0.073	0.067	0.064	0.072	0.068	0.078	0.064	0.071
24	CL/A(1), CL/F(1)	0.066	0.056	0.050	0.053	0.050	0.053	0.053	0.053	0.053	0.053	0.048	0.053	0.053	0.056	0.058	0.050	0.048	0.050	0.053	0.011	0.016	0.011	0.013		0.002	0.015	0.031	0.028	0.031	0.028	0.025	0.069	0.069	0.065	0.065	0.070	0.068	0.065	0.068	0.065	0.065	0.062	0.068	0.070	0.074	0.067	0.064	0.068	0.065	0.074	0.061	0.068
25	CL/TR(1), ECo/CW(1)	0.066	0.058	0.053	0.056	0.053	0.056	0.056	0.056	0.056	0.056	0.050	0.056	0.056	0.058	0.061	0.053	0.050	0.053	0.056	0.013	0.019	0.013	0.016	0.003		0.018	0.034	0.031	0.034	0.031	0.028	0.073	0.073	0.069	0.069	0.074	0.073	0.069	0.073	0.069	0.070	0.066	0.073	0.074	0.078	0.072	0.068	0.064	0.061	0.070	0.065	0.072
26	ECo/CW(1)	0.066	0.056	0.051	0.053	0.051	0.053	0.053	0.053	0.053	0.053	0.048	0.056	0.056	0.059	0.059	0.051	0.048	0.051	0.053	0.016	0.016	0.016	0.013	0.016	0.019		0.031	0.028	0.031	0.034	0.031	0.080	0.080	0.076	0.076	0.081	0.079	0.076	0.079	0.075	0.076	0.072	0.079	0.077	0.081	0.075	0.071	0.080	0.076	0.086	0.072	0.071
27	Co/L(1)	0.064	0.053	0.048	0.050	0.048	0.050	0.050	0.050	0.050	0.050	0.050	0.048	0.042	0.053	0.056	0.048	0.045	0.048	0.050	0.032	0.032	0.032	0.034	0.032	0.034	0.032		0.002	0.005	0.018	0.015	0.076	0.076	0.072	0.072	0.078	0.076	0.073	0.069	0.072	0.080	0.069	0.062	0.081	0.085	0.079	0.075	0.084	0.080	0.091	0.076	0.068
28	Co/L(2)	0.061	0.050	0.045	0.048	0.045	0.048	0.048	0.048	0.048	0.048	0.048	0.050	0.045	0.050	0.053	0.045	0.042	0.045	0.048	0.034	0.034	0.029	0.032	0.029	0.032	0.029	0.003		0.002	0.015	0.012	0.073	0.073	0.069	0.069	0.081	0.080	0.076	0.072	0.076	0.077	0.065	0.059	0.078	0.082	0.076	0.072	0.080	0.077	0.087	0.072	0.072
29	Co/L(1)	0.058	0.048	0.042	0.045	0.042	0.045	0.045	0.045	0.045	0.045	0.045	0.048	0.042	0.048	0.050	0.042	0.040	0.042	0.045	0.037	0.037	0.032	0.034	0.032	0.034	0.032	0.005	0.003		0.018	0.015	0.077	0.077	0.073	0.073	0.085	0.084	0.080	0.076	0.080	0.081	0.069	0.062	0.082	0.086	0.072	0.068	0.084	0.081	0.091	0.076	0.076
30	S/M(2), Cl/M(2)	0.061	0.050	0.045	0.048	0.045	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.042	0.048	0.053	0.045	0.042	0.042	0.045	0.034	0.034	0.029	0.032	0.029	0.032	0.035	0.019	0.016	0.019		0.002	0.076	0.076	0.072	0.072	0.085	0.083	0.079	0.075	0.079	0.080	0.068	0.062	0.084	0.088	0.082	0.078	0.083	0.080	0.090	0.075	0.075
31	S/C(1)	0.064	0.053	0.048	0.050	0.048	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.045	0.050	0.056	0.048	0.045	0.045	0.048	0.032	0.032	0.027	0.029	0.027	0.029	0.032	0.016	0.013	0.016	0.003		0.071	0.071	0.068	0.068	0.080	0.078	0.075	0.071	0.074	0.075	0.064	0.058	0.079	0.084	0.077	0.074	0.078	0.075	0.085	0.071	0.070
32	Wo/ES(1), Wa/WR(1)	0.085	0.074	0.074	0.077	0.074	0.077	0.077	0.077	0.077	0.072	0.072	0.074	0.069	0.074	0.077	0.074	0.072	0.074	0.077	0.066	0.066	0.056	0.058	0.061	0.064	0.069	0.066	0.064	0.066	0.066	0.064		0.005	0.002	0.005	0.010	0.009	0.007	0.005	0.009	0.007	0.005	0.009	0.022	0.025	0.017	0.015	0.030	0.028	0.034	0.025	0.030
33	Wa/K(3)	0.085	0.074	0.074	0.077	0.074	0.077	0.077	0.077	0.077	0.072	0.072	0.074	0.069	0.074	0.077	0.074	0.072	0.074	0.077	0.066	0.066	0.056	0.058	0.061	0.064	0.069	0.066	0.064	0.066	0.066	0.064	0.005		0.002	0.005	0.010	0.009	0.007	0.005	0.009	0.007	0.005	0.009	0.022	0.025	0.017	0.015	0.030	0.028	0.034	0.025	0.030
34	E	0.082	0.072	0.072	0.074	0.072	0.074	0.074	0.074	0.074	0.069	0.069	0.072	0.066	0.072	0.074	0.072	0.069	0.072	0.074	0.064	0.064	0.053	0.056	0.058	0.061	0.066	0.064	0.061	0.064	0.064	0.061	0.003	0.003		0.002	0.007	0.007	0.005	0.002	0.007	0.005	0.002	0.007	0.020	0.022	0.015	0.012	0.028	0.025	0.031	0.022	0.027
35	M/M(1)	0.082	0.072	0.072	0.074	0.072	0.074	0.074	0.074	0.074	0.069	0.069	0.072	0.066	0.072	0.074	0.072	0.069	0.072	0.074	0.064	0.064	0.053	0.056	0.058	0.061	0.066	0.064	0.061	0.064	0.064	0.061	0.005	0.005	0.003		0.010	0.009	0.007	0.005	0.005	0.007	0.005	0.009	0.017	0.020	0.017	0.015	0.025	0.022	0.028	0.020	0.025
36	M/OR(1)	0.085	0.074	0.074	0.077	0.074	0.077	0.077	0.077	0.077	0.072	0.072	0.069	0.069	0.077	0.077	0.074	0.072	0.074	0.077	0.061	0.061	0.056	0.058	0.061	0.064	0.069	0.066	0.069	0.072	0.072	0.069	0.011	0.011	0.008	0.011		0.005	0.002	0.005	0.005	0.007	0.010	0.015	0.022	0.025	0.017	0.015	0.031	0.028	0.028	0.025	0.025
37	M/OR(1)	0.085	0.074	0.074	0.077	0.074	0.077	0.077	0.077	0.077	0.072	0.072	0.069	0.069	0.077	0.077	0.074	0.072	0.074	0.077	0.061	0.061	0.056	0.058	0.061	0.064	0.069	0.066	0.069	0.072	0.072	0.069	0.011	0.011	0.008	0.011	0.005		0.002	0.005	0.005	0.007	0.009	0.014	0.022	0.025	0.017	0.015	0.030	0.028	0.034	0.025	0.025
38	Wo/M(9), M/OR(2)	0.082	0.072	0.072	0.074	0.072	0.074	0.074	0.074	0.074	0.069	0.069	0.066	0.066	0.074	0.074	0.072	0.069	0.072	0.074	0.058	0.058	0.053	0.056	0.058	0.061	0.066	0.064	0.066	0.069	0.069	0.066	0.008	0.008	0.005	0.008	0.003	0.003		0.002	0.002	0.005	0.007	0.012	0.019	0.022	0.015	0.012	0.027	0.025	0.031	0.022	0.022
39	Wa/KS(9), Wa/M(1)	0.085	0.074	0.074	0.077	0.074	0.077	0.077	0.077	0.077	0.072	0.072	0.069	0.064	0.074	0.077	0.074	0.072	0.074	0.077	0.061	0.061	0.056	0.058	0.061	0.064	0.069	0.061	0.064	0.066	0.066	0.064	0.005	0.005	0.003	0.005	0.005	0.005	0.003		0.005	0.007	0.005	0.009	0.022	0.025	0.017	0.015	0.030	0.028	0.034	0.025	0.025
40	Wo/M(1)	0.082	0.072	0.072	0.074	0.072	0.074	0.074	0.074	0.074	0.069	0.069	0.066	0.066	0.074	0.074	0.072	0.069	0.072	0.074	0.058	0.058	0.053	0.056	0.058	0.061	0.066	0.064	0.066	0.069	0.069	0.066	0.011	0.011	0.008	0.005	0.005	0.005	0.003	0.005		0.007	0.009	0.014	0.017	0.020	0.017	0.014	0.025	0.022	0.028	0.019	0.020
41	T/WS(5)	0.082	0.072	0.072	0.074	0.072	0.074	0.074	0.074	0.074	0.069	0.069	0.072	0.072	0.074	0.074	0.072	0.069	0.072	0.074	0.064	0.064	0.053	0.056	0.058	0.061	0.066	0.069	0.066	0.069	0.069	0.066	0.008	0.008	0.005	0.008	0.008	0.008	0.005	0.008	0.008		0.007	0.012	0.020	0.022	0.015	0.012	0.028	0.025	0.031	0.022	0.027
42	Wa/H(1)	0.080	0.069	0.069	0.072	0.069	0.072	0.072	0.072	0.072	0.066	0.066	0.069	0.064	0.069	0.072	0.069	0.066	0.069	0.072	0.061	0.061	0.050	0.058	0.056	0.058	0.064	0.061	0.058	0.061	0.061	0.058	0.005	0.005	0.003	0.005	0.011	0.011	0.008	0.005	0.011	0.008		0.005	0.017	0.020	0.012	0.010	0.025	0.022	0.028	0.020	0.025
43	Wa/H(1)	0.080	0.069	0.069	0.072	0.069	0.072	0.072	0.072	0.072	0.066	0.072	0.069	0.064	0.069	0.072	0.069	0.066	0.069	0.072	0.066	0.066	0.056	0.064	0.061	0.064	0.069	0.056	0.053	0.056	0.056	0.053	0.011	0.011	0.008	0.011	0.016	0.016	0.013	0.011	0.016	0.013	0.005		0.022	0.025	0.017	0.015	0.030	0.028	0.034	0.025	0.030
44	NE/B(3)	0.082	0.074	0.074	0.077	0.074	0.077	0.077	0.077	0.072	0.072	0.072	0.080	0.080	0.082	0.072	0.074	0.072	0.074	0.077	0.069	0.069	0.056	0.064	0.064	0.066	0.069	0.072	0.069	0.072	0.074	0.072	0.024	0.024	0.021	0.019	0.024	0.024	0.021	0.024	0.019	0.021	0.019	0.024		0.002	0.014	0.012	0.025	0.022	0.028	0.020	0.025
45	NE/B(2)	0.085	0.077	0.077	0.080	0.077	0.080	0.080	0.080	0.074	0.074	0.074	0.082	0.082	0.085	0.074	0.077	0.074	0.077	0.080	0.072	0.072	0.058	0.066	0.066	0.069	0.072	0.074	0.072	0.074	0.077	0.074	0.027	0.027	0.024	0.021	0.027	0.027	0.024	0.027	0.021	0.024	0.021	0.027	0.003		0.017	0.014	0.028	0.025	0.031	0.022	0.028
46	F	0.077	0.066	0.066	0.069	0.066	0.069	0.069	0.069	0.064	0.064	0.064	0.072	0.072	0.074	0.069	0.066	0.064	0.066	0.069	0.066	0.066	0.053	0.061	0.061	0.064	0.066	0.069	0.066	0.064	0.072	0.069	0.019	0.019	0.016	0.019	0.019	0.019	0.016	0.019	0.019	0.016	0.013	0.019	0.016	0.019		0.002	0.025	0.023	0.029	0.020	0.025
47	WCo/L(2)	0.074	0.064	0.064	0.066	0.064	0.066	0.066	0.066	0.061	0.061	0.061	0.069	0.069	0.072	0.066	0.064	0.061	0.064	0.066	0.064	0.064	0.050	0.058	0.058	0.061	0.064	0.066	0.064	0.061	0.069	0.066	0.016	0.016	0.013	0.016	0.016	0.016	0.013	0.016	0.016	0.013	0.011	0.016	0.013	0.016	0.003		0.022	0.020	0.026	0.017	0.022
48	NE/T(1)	0.077	0.069	0.069	0.072	0.069	0.072	0.072	0.072	0.066	0.066	0.066	0.072	0.072	0.074	0.072	0.069	0.066	0.069	0.072	0.066	0.066	0.056	0.064	0.061	0.058	0.069	0.072	0.069	0.072	0.072	0.069	0.032	0.032	0.029	0.027	0.032	0.032	0.029	0.032	0.027	0.029	0.027	0.032	0.027	0.029	0.027	0.024		0.002	0.007	0.005	0.009
49	NE/T(1)	0.074	0.066	0.066	0.069	0.066	0.069	0.069	0.069	0.064	0.064	0.064	0.069	0.069	0.072	0.069	0.066	0.064	0.066	0.069	0.064	0.064	0.053	0.061	0.058	0.056	0.066	0.069	0.066	0.069	0.069	0.066	0.029	0.029	0.027	0.024	0.029	0.029	0.027	0.029	0.024	0.027	0.024	0.029	0.024	0.027	0.024	0.021	0.003		0.005	0.002	0.007
50	NE/Mt(1)	0.080	0.072	0.072	0.074	0.072	0.074	0.074	0.069	0.069	0.069	0.069	0.074	0.074	0.077	0.074	0.072	0.069	0.072	0.074	0.069	0.069	0.058	0.066	0.064	0.061	0.072	0.074	0.072	0.074	0.074	0.072	0.034	0.034	0.032	0.029	0.029	0.034	0.032	0.034	0.029	0.032	0.029	0.034	0.029	0.032	0.029	0.027	0.008	0.005		0.007	0.012
51	NE/Mo(1)	0.074	0.064	0.064	0.066	0.064	0.066	0.066	0.066	0.061	0.061	0.061	0.066	0.066	0.069	0.066	0.064	0.061	0.064	0.066	0.061	0.061	0.050	0.058	0.056	0.058	0.064	0.066	0.064	0.066	0.066	0.064	0.027	0.027	0.024	0.021	0.027	0.027	0.024	0.027	0.021	0.024	0.021	0.027	0.021	0.024	0.021	0.019	0.005	0.003	0.008		0.005
52	NE/Mo(1)	0.074	0.064	0.064	0.066	0.064	0.066	0.066	0.066	0.061	0.061	0.061	0.061	0.061	0.069	0.066	0.064	0.061	0.064	0.066	0.061	0.056	0.056	0.064	0.061	0.064	0.064	0.061	0.064	0.066	0.066	0.064	0.032	0.032	0.029	0.027	0.027	0.027	0.024	0.027	0.021	0.029	0.027	0.032	0.027	0.029	0.027	0.024	0.011	0.008	0.013	0.005	

APPENDIX I. (continued)

	G. huttoni									A	G. hubbsi				
	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67
1	0.093	0.099	0.093	0.097	0.098	0.099	0.102	0.102	0.100	0.085	0.195	0.198	0.193	0.203	0.193
2	0.075	0.080	0.075	0.079	0.079	0.080	0.083	0.083	0.080	0.067	0.165	0.168	0.163	0.172	0.163
3	0.067	0.072	0.067	0.071	0.071	0.072	0.075	0.075	0.072	0.066	0.152	0.155	0.151	0.159	0.151
4	0.070	0.075	0.070	0.074	0.075	0.075	0.078	0.078	0.075	0.070	0.156	0.159	0.155	0.163	0.155
5	0.067	0.072	0.067	0.071	0.071	0.072	0.075	0.075	0.072	0.066	0.152	0.155	0.151	0.159	0.151
6	0.070	0.075	0.070	0.074	0.075	0.075	0.071	0.078	0.075	0.070	0.156	0.159	0.155	0.163	0.155
7	0.070	0.075	0.070	0.074	0.075	0.075	0.078	0.078	0.075	0.070	0.151	0.155	0.150	0.159	0.150
8	0.071	0.076	0.071	0.075	0.076	0.076	0.079	0.079	0.077	0.071	0.159	0.162	0.158	0.166	0.158
9	0.070	0.075	0.070	0.074	0.075	0.075	0.078	0.078	0.075	0.070	0.156	0.159	0.155	0.163	0.155
10	0.071	0.076	0.071	0.074	0.075	0.075	0.078	0.079	0.076	0.070	0.157	0.160	0.156	0.165	0.156
11	0.071	0.075	0.071	0.074	0.075	0.075	0.078	0.078	0.076	0.070	0.153	0.156	0.151	0.160	0.152
12	0.075	0.080	0.075	0.078	0.079	0.079	0.082	0.076	0.080	0.074	0.154	0.157	0.153	0.162	0.153
13	0.074	0.079	0.074	0.078	0.078	0.079	0.082	0.075	0.079	0.083	0.157	0.170	0.166	0.175	0.166
14	0.072	0.077	0.072	0.076	0.076	0.077	0.080	0.080	0.077	0.079	0.161	0.164	0.160	0.168	0.160
15	0.071	0.076	0.071	0.075	0.076	0.076	0.079	0.079	0.077	0.078	0.159	0.162	0.158	0.166	0.158
16	0.067	0.072	0.067	0.071	0.071	0.071	0.074	0.075	0.072	0.066	0.152	0.155	0.150	0.159	0.151
17	0.064	0.068	0.064	0.067	0.068	0.068	0.071	0.071	0.068	0.063	0.147	0.150	0.146	0.154	0.146
18	0.069	0.074	0.069	0.073	0.074	0.074	0.077	0.077	0.074	0.076	0.158	0.156	0.151	0.160	0.151
19	0.073	0.078	0.073	0.077	0.077	0.078	0.081	0.081	0.078	0.080	0.163	0.161	0.157	0.165	0.157
20	0.067	0.071	0.067	0.070	0.063	0.063	0.066	0.060	0.063	0.071	0.142	0.143	0.145	0.154	0.149
21	0.075	0.080	0.075	0.078	0.070	0.071	0.073	0.067	0.071	0.079	0.143	0.145	0.147	0.155	0.151
22	0.063	0.067	0.063	0.066	0.059	0.059	0.062	0.062	0.059	0.064	0.153	0.155	0.157	0.166	0.161
23	0.060	0.064	0.060	0.063	0.056	0.056	0.059	0.059	0.057	0.061	0.150	0.152	0.154	0.162	0.158
24	0.066	0.071	0.066	0.070	0.062	0.063	0.066	0.066	0.063	0.071	0.160	0.162	0.164	0.173	0.168
25	0.071	0.076	0.071	0.074	0.066	0.067	0.070	0.070	0.067	0.075	0.167	0.169	0.171	0.181	0.176
26	0.067	0.072	0.067	0.071	0.063	0.063	0.066	0.066	0.064	0.072	0.141	0.143	0.145	0.153	0.149
27	0.064	0.069	0.064	0.068	0.060	0.061	0.063	0.057	0.061	0.075	0.154	0.156	0.158	0.167	0.163
28	0.061	0.066	0.061	0.065	0.057	0.057	0.060	0.060	0.058	0.072	0.159	0.161	0.163	0.172	0.167
29	0.058	0.062	0.058	0.061	0.054	0.054	0.057	0.057	0.054	0.069	0.164	0.166	0.169	0.178	0.173
30	0.059	0.064	0.059	0.063	0.063	0.063	0.066	0.066	0.064	0.075	0.162	0.155	0.157	0.166	0.161
31	0.063	0.068	0.063	0.067	0.059	0.059	0.062	0.062	0.060	0.071	0.159	0.152	0.154	0.162	0.158
32	0.108	0.114	0.108	0.112	0.103	0.111	0.106	0.107	0.094	0.094	0.230	0.233	0.237	0.249	0.242
33	0.108	0.114	0.108	0.104	0.103	0.111	0.106	0.098	0.094	0.094	0.230	0.233	0.237	0.249	0.242
34	0.104	0.110	0.104	0.108	0.098	0.106	0.102	0.102	0.090	0.090	0.224	0.227	0.230	0.242	0.235
35	0.103	0.110	0.103	0.108	0.098	0.106	0.102	0.102	0.093	0.093	0.223	0.226	0.229	0.241	0.234
36	0.109	0.116	0.109	0.114	0.104	0.112	0.108	0.100	0.095	0.095	0.224	0.227	0.230	0.242	0.235
37	0.107	0.113	0.107	0.111	0.102	0.110	0.105	0.098	0.093	0.093	0.218	0.221	0.224	0.236	0.229
38	0.103	0.109	0.103	0.107	0.098	0.106	0.101	0.094	0.089	0.089	0.212	0.215	0.219	0.230	0.223
39	0.107	0.114	0.107	0.112	0.102	0.110	0.106	0.098	0.093	0.093	0.218	0.221	0.225	0.236	0.230
40	0.103	0.109	0.103	0.107	0.098	0.105	0.101	0.094	0.093	0.093	0.212	0.214	0.218	0.229	0.222
41	0.104	0.110	0.104	0.108	0.099	0.106	0.102	0.102	0.090	0.090	0.224	0.227	0.231	0.242	0.235
42	0.099	0.106	0.099	0.104	0.094	0.102	0.098	0.098	0.086	0.086	0.218	0.221	0.224	0.236	0.229
43	0.092	0.098	0.092	0.096	0.087	0.095	0.091	0.091	0.079	0.086	0.212	0.215	0.218	0.230	0.223
44	0.097	0.103	0.097	0.101	0.092	0.100	0.095	0.096	0.087	0.095	0.211	0.214	0.217	0.222	0.222
45	0.102	0.108	0.102	0.106	0.097	0.104	0.100	0.100	0.092	0.099	0.219	0.222	0.225	0.230	0.230
46	0.086	0.092	0.086	0.090	0.081	0.089	0.085	0.085	0.073	0.081	0.220	0.223	0.226	0.238	0.231
47	0.090	0.096	0.090	0.094	0.085	0.092	0.089	0.089	0.077	0.077	0.214	0.216	0.220	0.231	0.224
48	0.099	0.105	0.099	0.103	0.094	0.102	0.098	0.098	0.089	0.088	0.235	0.238	0.241	0.254	0.233
49	0.096	0.102	0.096	0.100	0.091	0.098	0.094	0.094	0.086	0.085	0.230	0.233	0.237	0.249	0.228
50	0.107	0.114	0.107	0.112	0.102	0.110	0.105	0.106	0.097	0.096	0.252	0.256	0.260	0.273	0.251
51	0.091	0.097	0.091	0.095	0.086	0.093	0.089	0.090	0.081	0.080	0.221	0.224	0.227	0.239	0.219
52	0.098	0.104	0.098	0.102	0.093	0.101	0.097	0.090	0.089	0.088	0.209	0.212	0.216	0.227	0.208

	G. caxii				G. australis				Eleotris									
	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
1	0.308	0.301	0.289	0.281	0.360	0.355	0.366	0.345	0.525	0.589	0.407	0.398	0.432	0.541	0.537	0.527	0.531	0.519
2	0.265	0.258	0.247	0.241	0.307	0.304	0.313	0.294	0.458	0.514	0.381	0.373	0.406	0.502	0.498	0.487	0.453	0.443
3	0.277	0.270	0.259	0.252	0.305	0.302	0.291	0.298	0.429	0.482	0.375	0.366	0.382	0.498	0.494	0.476	0.484	0.474
4	0.282	0.275	0.264	0.257	0.311	0.307	0.297	0.304	0.435	0.488	0.380	0.372	0.387	0.504	0.500	0.483	0.491	0.480
5	0.277	0.270	0.259	0.252	0.305	0.302	0.291	0.298	0.429	0.482	0.375	0.366	0.382	0.498	0.494	0.476	0.484	0.474
6	0.271	0.264	0.253	0.246	0.311	0.295	0.297	0.304	0.435	0.474	0.380	0.372	0.387	0.504	0.500	0.483	0.491	0.480
7	0.282	0.275	0.264	0.257	0.304	0.300	0.290	0.297	0.435	0.474	0.373	0.365	0.387	0.490	0.486	0.483	0.491	0.480
8	0.266	0.259	0.248	0.241	0.319	0.315	0.304	0.312	0.441	0.496	0.384	0.376	0.397	0.518	0.514	0.497	0.500	0.489
9	0.282	0.275	0.264	0.257	0.311	0.307	0.297	0.304	0.421	0.474	0.380	0.372	0.387	0.504	0.500	0.483	0.491	0.480
10	0.270	0.262	0.252	0.245	0.297	0.294	0.283	0.290	0.419	0.471	0.366	0.358	0.373	0.488	0.483	0.488	0.474	0.463
11	0.272	0.264	0.254	0.247	0.299	0.296	0.286	0.293	0.422	0.475	0.369	0.361	0.376	0.492	0.488	0.470	0.486	0.476
12	0.282	0.275	0.264	0.257	0.297	0.294	0.284	0.291	0.409	0.460	0.378	0.369	0.372	0.484	0.480	0.463	0.470	0.460
13	0.263	0.256	0.246	0.239	0.319	0.315	0.305	0.312	0.437	0.490	0.402	0.393	0.395	0.514	0.510	0.493	0.479	0.491
14	0.288	0.281	0.269	0.262	0.297	0.294	0.283	0.291	0.439	0.493	0.391	0.382	0.380	0.495	0.491	0.474	0.476	0.471
15	0.288	0.281	0.270	0.263	0.331	0.327	0.316	0.324	0.461	0.516	0.402	0.393	0.397	0.518	0.514	0.511	0.506	0.495
16	0.280	0.273	0.262	0.255	0.309	0.305	0.295	0.302	0.432	0.485	0.378	0.370	0.385	0.501	0.497	0.480	0.488	0.477
17	0.274	0.267	0.256	0.250	0.302	0.299	0.289	0.296	0.425	0.477	0.372	0.363	0.378	0.494	0.489	0.472	0.480	0.469
18	0.307	0.299	0.287	0.280	0.327	0.323	0.304	0.312	0.465	0.532	0.400	0.391	0.407	0.547	0.543	0.513	0.537	0.526
19	0.315	0.307	0.295	0.287	0.336	0.332	0.312	0.320	0.475	0.543	0.409	0.400	0.416	0.536	0.532	0.501	0.548	0.537
20	0.274	0.267	0.257	0.250	0.392	0.386	0.373	0.383	0.472	0.529	0.407	0.417	0.416	0.566	0.575	0.531	0.571	0.560
21	0.262	0.256	0.245	0.239	0.374	0.369	0.380	0.359	0.482	0.541	0.415	0.425	0.424	0.572	0.581	0.543	0.536	0.525
22	0.288	0.281	0.270	0.263	0.401	0.395	0.382	0.392	0.488	0.546	0.441	0.451	0.432	0.583	0.578	0.550	0.582	0.570
23	0.271	0.264	0.253	0.246	0.398	0.392	0.379	0.389	0.486	0.544	0.439	0.449	0.411	0.582	0.590	0.548	0.565	0.554
24	0.298	0.291	0.280	0.273	0.409	0.403	0.389	0.400	0.492	0.551	0.436	0.446	0.439	0.579	0.574	0.545	0.576	0.565
25	0.310	0.303	0.291	0.284	0.426	0.419	0.405	0.417	0.512	0.573	0.453	0.463	0.457	0.603	0.597	0.569	0.600	0.588
26	0.260	0.253	0.242	0.249	0.384	0.378	0.365	0.375	0.454	0.511	0.435	0.444	0.409	0.589	0.597	0.555	0.583	0.572
27	0.271	0.264	0.253	0.246	0.415	0.409	0.395	0.406	0.479	0.535	0.404	0.414	0.463	0.564	0.561	0.531	0.523	0.535
28	0.277	0.270	0.259	0.252	0.409	0.403	0.389	0.400	0.481	0.537	0.411	0.421	0.465	0.566	0.563	0.534	0.525	0.537
29	0.284	0.277	0.266	0.259	0.399	0.393	0.380	0.390	0.471	0.526	0.402	0.412	0.455	0.578	0.574	0.546	0.514	0.526
30	0.285	0.277	0.266	0.260	0.378	0.372	0.347	0.357	0.469	0.538	0.400	0.409	0.427	0.567	0.563	0.534	0.558	0.570
31	0.273	0.266	0.256	0.249	0.394	0.388	0.363	0.372	0.489	0.561	0.416	0.426	0.444	0.590	0.586	0.558	0.535	0.547
32	0.323	0.315	0.303	0.296	0.427	0.420	0.433	0.410	0.591	0.657	0.568	0.557	0.519	0.690	0.687	0.692	0.545	0.557
33	0.307	0.300	0.288	0.281	0.427	0.420	0.433	0.410	0.591	0.657	0.568	0.557	0.519	0.690	0.687	0.692	0.545	0.557
34	0.315	0.308	0.296	0.288	0.418	0.411	0.424	0.401	0.580	0.645	0.557	0.547	0.510	0.679	0.675	0.680	0.534	0.546
35	0.314	0.306	0.294	0.287	0.416	0.409	0.422	0.399	0.578	0.643	0.555	0.545	0.508	0.676	0.673	0.677	0.532	0.544
36	0.333	0.325	0.312	0.305	0.436	0.429	0.442	0.419	0.596	0.664	0.567	0.556	0.522	0.698	0.695	0.700	0.574	0.562
37	0.323	0.315	0.303	0.296	0.421	0.415	0.427	0.405	0.570	0.635	0.547	0.537	0.505	0.672	0.669	0.673	0.552	0.540
38	0.317	0.309	0.297	0.290	0.414	0.408	0.420	0.398	0.567	0.632	0.540	0.530	0.498	0.665	0.662	0.665	0.544	0.533
39	0.309	0.301	0.290	0.282	0.423	0.417	0.430	0.407	0.578	0.643	0.550	0.540	0.508	0.676	0.673	0.677	0.532	0.544
40	0.316	0.308	0.296	0.289	0.413	0.406	0.419	0.396	0.565	0.629	0.538	0.528	0.496	0.662	0.659	0.663	0.542	0.530
41	0.331	0.323	0.311	0.304	0.399	0.393	0.405	0.383	0.558	0.622	0.537	0.527	0.490	0.656	0.652	0.656	0.535	0.524
42	0.323	0.315	0.303	0.296	0.408	0.402	0.414	0.392	0.569	0.634	0.547	0.537	0.520	0.667	0.664	0.668	0.546	0.558
43	0.323	0.315	0.303	0.296	0.408	0.402	0.414	0.392	0.569	0.634	0.547	0.537	0.520	0.667	0.664	0.668	0.537	0.550
44	0.321	0.314	0.302	0.295	0.432	0.425	0.438	0.415	0.570	0.636	0.537	0.527	0.524	0.674	0.670	0.688	0.576	0.564
45	0.333	0.325	0.313	0.305	0.448	0.441	0.455	0.431	0.591	0.661	0.555	0.545	0.543	0.698	0.695	0.714	0.599	0.587
46	0.324	0.316	0.304	0.297	0.403	0.397	0.410	0.387	0.575	0.642	0.529	0.519	0.509	0.681	0.678	0.682	0.557	0.546
47	0.331	0.324	0.312	0.304	0.413	0.406	0.419	0.396	0.563	0.629	0.518	0.508	0.518	0.693	0.689	0.695	0.569	0.557
48	0.323	0.316	0.304	0.296	0.417	0.410	0.423	0.400	0.567	0.633	0.527	0.517	0.517	0.643	0.639	0.688	0.552	0.541
49	0.318	0.311	0.298	0.291	0.411	0.405	0.417	0.394	0.561	0.627	0.521	0.511	0.511	0.637	0.633	0.695	0.546	0.534
50	0.321	0.314	0.301	0.294	0.452	0.444	0.459	0.434	0.609	0.681	0.564	0.553	0.558	0.697	0.694	0.763	0.595	0.583
51	0.306	0.299	0.287	0.280	0.395	0.389	0.401	0.379	0.539	0.602	0.501	0.491	0.493	0.612	0.608	0.668	0.523	0.512
52	0.293	0.285	0.274	0.267	0.391	0.386	0.398	0.376	0.526	0.589	0.484	0.474	0.481	0.599	0.595	0.653	0.510	0.499

	Philypnodon					Hypseleotris						Eleotris	Mogurnda							
	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
1	0.562	0.562	0.573	0.428	0.447	0.408	0.455	0.407	0.399	0.549	0.456	0.443	0.639	0.640	0.691	0.688	0.697	0.662	0.655	0.709
2	0.521	0.521	0.532	0.396	0.413	0.354	0.399	0.354	0.347	0.477	0.425	0.389	0.557	0.557	0.603	0.600	0.608	0.577	0.570	0.615
3	0.554	0.554	0.565	0.421	0.439	0.372	0.417	0.379	0.371	0.510	0.454	0.365	0.524	0.524	0.568	0.565	0.574	0.544	0.582	0.578
4	0.560	0.560	0.571	0.427	0.445	0.378	0.423	0.385	0.377	0.516	0.460	0.371	0.530	0.530	0.574	0.571	0.580	0.550	0.587	0.584
5	0.554	0.554	0.565	0.421	0.439	0.372	0.417	0.379	0.371	0.510	0.454	0.365	0.524	0.524	0.568	0.565	0.574	0.544	0.582	0.578
6	0.560	0.560	0.571	0.427	0.445	0.378	0.423	0.385	0.377	0.516	0.460	0.371	0.530	0.530	0.574	0.571	0.580	0.550	0.587	0.584
7	0.552	0.552	0.563	0.418	0.437	0.378	0.423	0.385	0.377	0.516	0.460	0.363	0.521	0.521	0.565	0.562	0.571	0.541	0.578	0.574
8	0.577	0.577	0.588	0.438	0.457	0.389	0.435	0.396	0.388	0.532	0.474	0.380	0.539	0.539	0.585	0.582	0.591	0.560	0.599	0.596
9	0.560	0.560	0.571	0.427	0.445	0.378	0.423	0.385	0.377	0.516	0.447	0.359	0.530	0.530	0.574	0.571	0.580	0.550	0.587	0.584
10	0.543	0.543	0.554	0.412	0.430	0.362	0.408	0.370	0.362	0.499	0.444	0.357	0.535	0.535	0.579	0.577	0.585	0.555	0.593	0.590
11	0.548	0.548	0.559	0.423	0.441	0.373	0.419	0.381	0.373	0.512	0.456	0.368	0.518	0.518	0.562	0.559	0.568	0.538	0.576	0.572
12	0.538	0.538	0.549	0.431	0.450	0.354	0.393	0.369	0.361	0.486	0.433	0.373	0.510	0.510	0.552	0.549	0.558	0.529	0.565	0.561
13	0.571	0.571	0.582	0.459	0.479	0.355	0.386	0.369	0.362	0.476	0.425	0.367	0.480	0.479	0.520	0.518	0.526	0.498	0.532	0.527
14	0.566	0.566	0.577	0.467	0.486	0.362	0.408	0.377	0.370	0.499	0.444	0.381	0.508	0.508	0.551	0.549	0.557	0.528	0.564	0.560
15	0.571	0.571	0.582	0.438	0.457	0.402	0.448	0.409	0.401	0.532	0.474	0.380	0.545	0.546	0.591	0.588	0.597	0.566	0.559	0.602
16	0.557	0.557	0.568	0.424	0.443	0.375	0.421	0.383	0.375	0.513	0.458	0.369	0.527	0.527	0.571	0.568	0.576	0.547	0.584	0.581
17	0.549	0.549	0.561	0.418	0.436	0.368	0.414	0.376	0.368	0.505	0.450	0.362	0.520	0.520	0.564	0.561	0.569	0.540	0.577	0.573
18	0.589	0.589	0.590	0.455	0.474	0.414	0.448	0.412	0.403	0.549	0.479	0.365	0.562	0.562	0.609	0.606	0.615	0.583	0.576	0.622
19	0.577	0.577	0.578	0.464	0.484	0.424	0.457	0.422	0.413	0.561	0.489	0.373	0.572	0.573	0.620	0.617	0.626	0.594	0.586	0.634
20	0.517	0.524	0.535	0.463	0.483	0.437	0.404	0.400	0.392	0.471	0.441	0.355	0.507	0.464	0.501	0.512	0.528	0.471	0.526	0.521
21	0.507	0.514	0.525	0.455	0.474	0.417	0.386	0.374	0.367	0.442	0.413	0.362	0.518	0.473	0.511	0.522	0.539	0.481	0.495	0.532
22	0.525	0.532	0.543	0.458	0.478	0.447	0.421	0.410	0.401	0.473	0.452	0.383	0.515	0.473	0.509	0.519	0.535	0.479	0.534	0.529
23	0.523	0.530	0.542	0.436	0.456	0.444	0.418	0.407	0.399	0.458	0.450	0.362	0.513	0.471	0.507	0.518	0.534	0.477	0.533	0.527
24	0.535	0.542	0.553	0.473	0.492	0.456	0.428	0.417	0.408	0.483	0.460	0.378	0.525	0.481	0.518	0.529	0.545	0.488	0.544	0.539
25	0.556	0.564	0.575	0.492	0.511	0.475	0.445	0.434	0.425	0.502	0.479	0.393	0.546	0.500	0.538	0.550	0.566	0.508	0.565	0.561
26	0.529	0.537	0.548	0.482	0.502	0.450	0.422	0.412	0.404	0.485	0.454	0.367	0.488	0.445	0.482	0.492	0.509	0.453	0.507	0.500
27	0.544	0.565	0.576	0.474	0.494	0.468	0.433	0.429	0.420	0.510	0.494	0.374	0.507	0.445	0.480	0.491	0.507	0.453	0.505	0.499
28	0.538	0.559	0.570	0.481	0.501	0.470	0.440	0.430	0.421	0.512	0.496	0.376	0.501	0.439	0.475	0.485	0.501	0.447	0.499	0.492
29	0.527	0.549	0.560	0.471	0.491	0.460	0.431	0.421	0.412	0.501	0.486	0.385	0.512	0.449	0.485	0.496	0.512	0.457	0.510	0.504
30	0.585	0.586	0.584	0.439	0.458	0.434	0.416	0.392	0.384	0.513	0.446	0.377	0.503	0.474	0.510	0.521	0.538	0.480	0.537	0.531
31	0.562	0.569	0.567	0.457	0.477	0.452	0.426	0.401	0.393	0.493	0.466	0.361	0.524	0.460	0.496	0.507	0.523	0.467	0.521	0.516
32	0.547	0.553	0.571	0.505	0.504	0.502	0.460	0.456	0.446	0.487	0.502	0.444	0.545	0.499	0.559	0.549	0.584	0.530	0.474	0.560
33	0.568	0.574	0.592	0.485	0.504	0.502	0.460	0.456	0.446	0.487	0.502	0.444	0.545	0.499	0.559	0.549	0.584	0.530	0.474	0.560
34	0.558	0.564	0.582	0.495	0.515	0.492	0.451	0.446	0.437	0.477	0.491	0.435	0.535	0.489	0.549	0.539	0.575	0.520	0.464	0.549
35	0.564	0.569	0.575	0.488	0.508	0.490	0.449	0.443	0.434	0.473	0.488	0.433	0.541	0.495	0.554	0.545	0.580	0.525	0.470	0.555
36	0.580	0.586	0.604	0.503	0.523	0.505	0.457	0.458	0.448	0.489	0.504	0.446	0.577	0.529	0.591	0.581	0.617	0.561	0.502	0.594
37	0.548	0.554	0.572	0.472	0.492	0.487	0.442	0.442	0.433	0.472	0.487	0.431	0.546	0.499	0.559	0.550	0.585	0.530	0.474	0.560
38	0.554	0.559	0.577	0.478	0.498	0.480	0.435	0.435	0.426	0.465	0.479	0.424	0.551	0.505	0.565	0.555	0.591	0.536	0.480	0.566
39	0.564	0.569	0.587	0.488	0.508	0.490	0.444	0.444	0.435	0.475	0.490	0.433	0.541	0.495	0.554	0.545	0.580	0.525	0.470	0.555
40	0.559	0.565	0.571	0.471	0.491	0.478	0.433	0.432	0.423	0.462	0.476	0.422	0.557	0.511	0.570	0.561	0.596	0.541	0.485	0.572
41	0.559	0.565	0.582	0.496	0.515	0.472	0.432	0.427	0.419	0.457	0.492	0.416	0.536	0.490	0.550	0.540	0.575	0.521	0.465	0.550
42	0.548	0.554	0.572	0.506	0.525	0.482	0.442	0.437	0.428	0.487	0.481	0.445	0.525	0.480	0.539	0.529	0.565	0.510	0.455	0.539
43	0.548	0.554	0.572	0.498	0.518	0.474	0.434	0.429	0.420	0.493	0.473	0.436	0.525	0.480	0.539	0.529	0.565	0.510	0.455	0.539
44	0.567	0.573	0.579	0.473	0.492	0.493	0.451	0.438	0.429	0.484	0.469	0.400	0.566	0.519	0.580	0.570	0.605	0.550	0.500	0.582
45	0.580	0.586	0.592	0.490	0.509	0.511	0.468	0.454	0.445	0.502	0.480	0.415	0.585	0.537	0.599	0.589	0.625	0.568	0.517	0.602
46	0.544	0.550	0.568	0.463	0.482	0.477	0.437	0.424	0.416	0.483	0.463	0.417	0.563	0.516	0.577	0.567	0.603	0.547	0.489	0.579
47	0.555	0.560	0.579	0.473	0.492	0.488	0.446	0.434	0.425	0.493	0.474	0.426	0.573	0.525	0.587	0.577	0.613	0.557	0.499	0.589
48	0.566	0.572	0.577	0.503	0.523	0.492	0.431	0.412	0.404	0.517	0.456	0.452	0.584	0.537	0.597	0.588	0.603	0.567	0.510	0.579
49	0.560	0.566	0.572	0.497	0.517	0.486	0.425	0.406	0.398	0.511	0.449	0.446	0.579	0.531	0.592	0.582	0.597	0.562	0.504	0.573
50	0.610	0.616	0.622	0.543	0.565	0.533	0.466	0.447	0.437	0.560	0.495	0.489	0.622	0.572	0.636	0.626	0.642	0.604	0.542	0.619
51	0.540	0.546	0.551	0.478	0.497	0.467	0.409	0.390	0.382	0.492	0.431	0.428	0.558	0.512	0.572	0.562	0.577	0.543	0.486	0.552
52	0.556	0.562	0.568	0.481	0.501	0.455	0.393	0.379	0.371	0.480	0.420	0.417	0.554	0.508	0.567	0.558	0.573	0.538	0.483	0.548

APPENDIX I. (continued)

	LOCATION*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
		G. cotidianus, G. basalis and G. alpinus complex																			G. breviceps						
53	NE/M(2)	0.077	0.066	0.061	0.064	0.061	0.064	0.064	0.064	0.064	0.064	0.064	0.066	0.066	0.064	0.064	0.061	0.058	0.061	0.064	0.061	0.066	0.058	0.056	0.061	0.064	0.061
54	N/KR(1)	0.080	0.069	0.064	0.066	0.064	0.066	0.066	0.066	0.066	0.066	0.066	0.069	0.069	0.066	0.066	0.064	0.061	0.064	0.066	0.064	0.069	0.061	0.058	0.064	0.066	0.064
55	G	0.077	0.066	0.061	0.064	0.061	0.064	0.064	0.064	0.064	0.064	0.064	0.066	0.066	0.064	0.064	0.061	0.058	0.061	0.064	0.061	0.066	0.058	0.056	0.061	0.064	0.061
56	N/M (1)	0.080	0.069	0.064	0.066	0.064	0.066	0.066	0.066	0.066	0.066	0.066	0.069	0.069	0.066	0.066	0.064	0.061	0.064	0.066	0.064	0.069	0.061	0.058	0.064	0.066	0.064
57	W/M(1)	0.080	0.069	0.064	0.066	0.064	0.066	0.066	0.066	0.066	0.066	0.066	0.069	0.069	0.066	0.066	0.064	0.061	0.064	0.066	0.058	0.064	0.056	0.053	0.058	0.061	0.059
58	N/US(1)	0.080	0.069	0.064	0.066	0.064	0.066	0.066	0.066	0.066	0.066	0.066	0.069	0.069	0.066	0.066	0.064	0.061	0.064	0.066	0.058	0.064	0.056	0.053	0.058	0.061	0.059
59	N/WR(1)	0.082	0.072	0.066	0.069	0.066	0.064	0.069	0.069	0.069	0.069	0.069	0.072	0.072	0.069	0.069	0.066	0.064	0.066	0.069	0.061	0.066	0.058	0.056	0.061	0.064	0.061
60	N/Mi (1)	0.082	0.072	0.066	0.069	0.066	0.069	0.069	0.069	0.069	0.069	0.069	0.066	0.066	0.069	0.069	0.066	0.064	0.066	0.069	0.056	0.061	0.058	0.056	0.061	0.064	0.061
61	Cl/W(1)	0.080	0.069	0.064	0.066	0.064	0.066	0.066	0.066	0.066	0.066	0.066	0.069	0.069	0.066	0.066	0.064	0.061	0.064	0.066	0.058	0.064	0.056	0.053	0.058	0.061	0.059
62	N/KR(2)	0.069	0.058	0.058	0.061	0.058	0.061	0.061	0.061	0.061	0.061	0.061	0.064	0.069	0.066	0.066	0.058	0.056	0.064	0.066	0.064	0.069	0.058	0.056	0.064	0.066	0.064
63	C/AR(3)	0.125	0.114	0.109	0.111	0.109	0.111	0.109	0.111	0.111	0.111	0.109	0.109	0.111	0.111	0.111	0.109	0.106	0.109	0.111	0.103	0.103	0.111	0.109	0.114	0.117	0.104
64	C/AR(1)	0.127	0.117	0.111	0.114	0.111	0.114	0.111	0.114	0.114	0.114	0.111	0.111	0.117	0.114	0.114	0.111	0.109	0.109	0.111	0.103	0.103	0.111	0.109	0.114	0.117	0.104
65	N/SS(1)	0.125	0.114	0.109	0.111	0.109	0.111	0.109	0.111	0.111	0.111	0.109	0.109	0.114	0.111	0.111	0.109	0.106	0.106	0.109	0.103	0.103	0.111	0.109	0.114	0.117	0.104
66	N/SS(1)	0.127	0.117	0.111	0.114	0.111	0.114	0.111	0.114	0.114	0.114	0.111	0.111	0.117	0.114	0.114	0.111	0.109	0.109	0.111	0.106	0.106	0.114	0.111	0.117	0.119	0.106
67	N/SS(1)	0.125	0.114	0.109	0.111	0.109	0.111	0.109	0.111	0.111	0.111	0.109	0.109	0.114	0.111	0.111	0.109	0.106	0.106	0.109	0.106	0.106	0.114	0.111	0.117	0.119	0.106
68	*G. eatti*	0.159	0.149	0.151	0.154	0.151	0.149	0.154	0.149	0.154	0.149	0.149	0.151	0.146	0.151	0.154	0.154	0.151	0.159	0.162	0.151	0.149	0.159	0.151	0.162	0.164	0.146
69	*G. eatti*	0.156	0.146	0.149	0.151	0.149	0.146	0.151	0.146	0.151	0.146	0.146	0.149	0.143	0.149	0.151	0.151	0.149	0.156	0.159	0.149	0.146	0.156	0.149	0.159	0.162	0.143
70	*G. eatti* (2)	0.154	0.143	0.146	0.149	0.146	0.143	0.149	0.143	0.149	0.143	0.143	0.146	0.141	0.146	0.149	0.149	0.146	0.154	0.156	0.146	0.143	0.154	0.146	0.156	0.159	0.141
71	*G. eatti* (2)	0.151	0.141	0.143	0.146	0.143	0.141	0.146	0.141	0.146	0.141	0.141	0.143	0.138	0.143	0.146	0.146	0.143	0.151	0.154	0.143	0.141	0.151	0.143	0.154	0.156	0.143
72	*G. australis* (1)	0.162	0.151	0.149	0.151	0.149	0.151	0.149	0.151	0.151	0.146	0.146	0.149	0.154	0.146	0.156	0.151	0.149	0.151	0.154	0.167	0.164	0.172	0.170	0.172	0.175	0.165
73	*G. australis* (1)	0.164	0.154	0.151	0.154	0.151	0.149	0.151	0.154	0.154	0.149	0.149	0.151	0.156	0.149	0.159	0.154	0.151	0.154	0.156	0.170	0.167	0.175	0.172	0.175	0.178	0.168
74	*G. australis* (1)	0.164	0.154	0.149	0.151	0.149	0.151	0.149	0.151	0.151	0.146	0.146	0.149	0.154	0.146	0.156	0.151	0.149	0.149	0.151	0.167	0.167	0.172	0.170	0.172	0.175	0.165
75	*G. australis* (1)	0.159	0.149	0.149	0.151	0.149	0.151	0.149	0.151	0.151	0.146	0.146	0.149	0.154	0.146	0.156	0.151	0.149	0.149	0.151	0.167	0.162	0.172	0.170	0.172	0.175	0.165
76	*E. acanthopoma* (1)	0.191	0.183	0.178	0.180	0.178	0.180	0.180	0.178	0.175	0.175	0.175	0.175	0.180	0.180	0.186	0.180	0.178	0.183	0.186	0.186	0.186	0.194	0.191	0.191	0.194	0.181
77	*E. sandwicensis*	0.199	0.191	0.186	0.188	0.186	0.183	0.183	0.186	0.183	0.183	0.183	0.183	0.188	0.188	0.194	0.188	0.186	0.194	0.196	0.194	0.194	0.202	0.199	0.199	0.202	0.189
78	*E. fusca* (1)	0.183	0.178	0.178	0.180	0.178	0.180	0.178	0.178	0.180	0.175	0.175	0.175	0.180	0.178	0.186	0.180	0.178	0.180	0.183	0.180	0.180	0.194	0.191	0.191	0.194	0.189
79	*E. fusca* (1)	0.180	0.175	0.175	0.178	0.175	0.178	0.175	0.175	0.178	0.172	0.172	0.172	0.178	0.175	0.183	0.178	0.175	0.178	0.180	0.183	0.183	0.196	0.194	0.194	0.196	0.191
80	*E. melanosoma* (1)	0.194	0.188	0.183	0.186	0.183	0.186	0.186	0.186	0.186	0.180	0.180	0.183	0.188	0.183	0.186	0.186	0.183	0.186	0.188	0.183	0.183	0.191	0.183	0.191	0.194	0.181
81	*E. amblyopsis* (1)	0.202	0.194	0.196	0.199	0.196	0.199	0.194	0.199	0.199	0.194	0.194	0.196	0.202	0.196	0.199	0.199	0.196	0.204	0.202	0.204	0.202	0.212	0.210	0.207	0.210	0.210
82	*E. amblyopsis* (1)	0.199	0.191	0.194	0.196	0.194	0.196	0.191	0.196	0.196	0.191	0.191	0.194	0.199	0.194	0.196	0.196	0.194	0.202	0.199	0.207	0.204	0.210	0.212	0.204	0.207	0.213
83	*E. amblyopsis* (1)	0.194	0.186	0.186	0.188	0.186	0.188	0.188	0.188	0.188	0.188	0.183	0.186	0.191	0.186	0.194	0.188	0.186	0.188	0.186	0.188	0.188	0.196	0.194	0.191	0.194	0.194
84	*E. picta* (2)	0.191	0.180	0.186	0.188	0.186	0.188	0.188	0.186	0.188	0.183	0.186	0.186	0.186	0.183	0.188	0.188	0.186	0.194	0.196	0.204	0.199	0.210	0.204	0.204	0.207	0.207
85	*E. pisonis* (1)	0.188	0.178	0.183	0.186	0.183	0.186	0.186	0.183	0.186	0.180	0.183	0.183	0.188	0.183	0.186	0.186	0.183	0.191	0.194	0.202	0.196	0.207	0.202	0.202	0.204	0.205
86	*P. grandiceps* (1)	0.202	0.194	0.199	0.202	0.199	0.202	0.199	0.202	0.202	0.196	0.196	0.199	0.204	0.202	0.199	0.202	0.199	0.199	0.196	0.191	0.191	0.196	0.194	0.196	0.199	0.194
87	*P. grandiceps* (3)	0.202	0.194	0.199	0.202	0.199	0.202	0.199	0.202	0.202	0.196	0.196	0.199	0.204	0.202	0.199	0.202	0.199	0.199	0.196	0.194	0.194	0.199	0.196	0.199	0.202	0.197
88	*P. grandiceps* (2)	0.207	0.199	0.204	0.207	0.204	0.207	0.204	0.207	0.207	0.202	0.202	0.204	0.210	0.207	0.204	0.207	0.204	0.202	0.199	0.199	0.199	0.204	0.202	0.204	0.207	0.202
89	*P. sp* (1)	0.186	0.178	0.183	0.186	0.183	0.186	0.183	0.186	0.186	0.180	0.183	0.183	0.188	0.191	0.186	0.186	0.183	0.188	0.191	0.188	0.188	0.191	0.183	0.194	0.196	0.194
90	*P. sp* (1)	0.191	0.183	0.188	0.191	0.188	0.191	0.188	0.191	0.191	0.186	0.188	0.188	0.194	0.196	0.191	0.191	0.188	0.194	0.196	0.194	0.194	0.196	0.188	0.199	0.202	0.199
91	*H. aurea* (1)	0.167	0.159	0.162	0.164	0.162	0.164	0.164	0.164	0.164	0.159	0.162	0.159	0.162	0.159	0.170	0.164	0.162	0.170	0.172	0.178	0.175	0.183	0.180	0.183	0.186	0.181
92	*H. galii* (1)	0.186	0.178	0.180	0.183	0.180	0.183	0.183	0.183	0.183	0.178	0.180	0.175	0.175	0.178	0.188	0.183	0.180	0.183	0.186	0.175	0.172	0.183	0.180	0.183	0.186	0.181
93	*H. compressa* (1)	0.170	0.162	0.167	0.170	0.167	0.170	0.170	0.170	0.170	0.164	0.167	0.167	0.170	0.167	0.175	0.170	0.167	0.172	0.175	0.175	0.170	0.180	0.178	0.180	0.183	0.178
94	*H. compressa* (1)	0.170	0.162	0.167	0.170	0.167	0.170	0.170	0.170	0.170	0.164	0.167	0.167	0.170	0.167	0.175	0.170	0.167	0.172	0.175	0.175	0.170	0.180	0.178	0.180	0.183	0.178
95	*H. sp* (1)	0.191	0.183	0.188	0.191	0.188	0.191	0.191	0.191	0.191	0.186	0.188	0.186	0.186	0.186	0.191	0.191	0.188	0.191	0.194	0.186	0.180	0.188	0.183	0.188	0.191	0.189
96	*H. kluzingeri* (1)	0.183	0.178	0.183	0.186	0.183	0.186	0.186	0.186	0.180	0.180	0.183	0.180	0.180	0.180	0.186	0.186	0.183	0.183	0.186	0.180	0.175	0.186	0.183	0.186	0.188	0.184
97	*E. oxycephala* (1)	0.188	0.180	0.175	0.178	0.175	0.178	0.175	0.178	0.172	0.172	0.175	0.175	0.175	0.175	0.178	0.178	0.175	0.175	0.178	0.170	0.170	0.180	0.172	0.178	0.180	0.173
98	*M. adspersa* (1)	0.218	0.207	0.202	0.204	0.202	0.204	0.202	0.202	0.204	0.204	0.199	0.202	0.196	0.196	0.204	0.204	0.202	0.204	0.207	0.194	0.194	0.199	0.196	0.199	0.202	0.189
99	*M. adspersa* (1)	0.215	0.204	0.199	0.202	0.199	0.202	0.199	0.199	0.202	0.202	0.196	0.199	0.194	0.194	0.202	0.202	0.199	0.202	0.204	0.186	0.186	0.191	0.188	0.191	0.194	0.181
100	*M. mogurnda* (1)	0.223	0.212	0.207	0.210	0.207	0.210	0.207	0.207	0.210	0.210	0.204	0.207	0.202	0.202	0.210	0.210	0.207	0.210	0.212	0.194	0.194	0.199	0.196	0.199	0.202	0.189
101	*M. mogurnda* (1)	0.223	0.212	0.207	0.210	0.207	0.210	0.207	0.207	0.210	0.210	0.204	0.207	0.202	0.202	0.210	0.210	0.207	0.210	0.212	0.196	0.196	0.202	0.199	0.202	0.204	0.191
102	*M. mogurnda* (1)	0.225	0.215	0.210	0.212	0.210	0.212	0.210	0.210	0.212	0.212	0.207	0.210	0.204	0.204	0.212	0.212	0.210	0.212	0.215	0.202	0.202	0.207	0.204	0.207	0.210	0.197
103	*M. elevicola* (1)	0.220	0.210	0.204	0.207	0.204	0.207	0.204	0.204	0.207	0.207	0.202	0.204	0.199	0.199	0.207	0.207	0.204	0.207	0.210	0.188	0.188	0.194	0.191	0.194	0.196	0.183
104	*M. thermophila* (1)	0.218	0.207	0.207	0.210	0.207	0.210	0.207	0.207	0.210	0.210	0.204	0.207	0.202	0.202	0.204	0.210	0.207	0.204	0.207	0.196	0.191	0.202	0.199	0.202	0.204	0.191
105	*M. larapintae* (1)	0.215	0.204	0.199	0.202	0.199	0.202	0.199	0.199	0.202	0.202	0.196	0.199	0.194	0.194	0.202	0.202	0.199	0.202	0.204	0.188	0.188	0.194	0.191	0.194	0.196	0.183

	LOCATION*	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52
		G. breviceps																									
53	NE/M(2)	0.058	0.056	0.053	0.056	0.058	0.088	0.088	0.085	0.085	0.088	0.088	0.085	0.088	0.085	0.085	0.082	0.077	0.082	0.085	0.074	0.077	0.082	0.080	0.085	0.077	0.082
54	N/KR(1)	0.061	0.058	0.056	0.058	0.061	0.090	0.090	0.088	0.088	0.090	0.090	0.088	0.090	0.088	0.088	0.085	0.080	0.085	0.088	0.077	0.080	0.085	0.082	0.088	0.080	0.085
55	G	0.058	0.056	0.053	0.056	0.058	0.088	0.088	0.085	0.085	0.088	0.088	0.085	0.088	0.085	0.085	0.082	0.077	0.082	0.085	0.074	0.077	0.082	0.080	0.085	0.077	0.082
56	N/M (1)	0.061	0.058	0.056	0.058	0.061	0.090	0.085	0.088	0.088	0.090	0.090	0.088	0.090	0.088	0.088	0.085	0.080	0.085	0.088	0.077	0.080	0.085	0.082	0.088	0.080	0.085
57	W/M(1)	0.056	0.053	0.050	0.058	0.056	0.085	0.085	0.082	0.082	0.085	0.085	0.082	0.085	0.082	0.082	0.080	0.074	0.080	0.082	0.072	0.074	0.080	0.077	0.082	0.074	0.080
58	N/US(1)	0.056	0.053	0.050	0.058	0.056	0.090	0.090	0.088	0.088	0.090	0.090	0.088	0.090	0.088	0.088	0.085	0.080	0.085	0.088	0.077	0.080	0.085	0.082	0.088	0.080	0.085
59	N/WR(1)	0.058	0.056	0.053	0.061	0.058	0.088	0.088	0.085	0.085	0.088	0.088	0.085	0.088	0.085	0.085	0.082	0.077	0.082	0.085	0.074	0.077	0.082	0.080	0.085	0.077	0.082
60	N/Mi (1)	0.053	0.056	0.053	0.061	0.058	0.088	0.082	0.085	0.085	0.082	0.082	0.080	0.082	0.080	0.085	0.082	0.077	0.082	0.085	0.074	0.077	0.082	0.080	0.085	0.077	0.077
61	Cl/W(1)	0.056	0.053	0.050	0.058	0.056	0.080	0.080	0.077	0.080	0.080	0.080	0.077	0.080	0.080	0.077	0.074	0.069	0.077	0.080	0.066	0.069	0.077	0.074	0.080	0.072	0.077
62	N/KR(2)	0.066	0.064	0.061	0.066	0.064	0.077	0.077	0.074	0.077	0.077	0.077	0.074	0.077	0.077	0.074	0.072	0.072	0.080	0.082	0.069	0.066	0.074	0.072	0.077	0.069	0.074
63	C/AR(3)	0.109	0.111	0.114	0.114	0.114	0.143	0.143	0.141	0.141	0.138	0.138	0.135	0.138	0.135	0.141	0.138	0.135	0.138	0.141	0.141	0.138	0.146	0.143	0.149	0.141	0.135
64	C/AR(1)	0.109	0.111	0.114	0.109	0.109	0.143	0.143	0.141	0.141	0.138	0.138	0.135	0.138	0.135	0.141	0.138	0.135	0.138	0.141	0.141	0.138	0.146	0.143	0.149	0.141	0.135
65	N/SS(1)	0.109	0.111	0.114	0.109	0.109	0.143	0.143	0.141	0.141	0.138	0.138	0.135	0.138	0.135	0.141	0.138	0.135	0.138	0.141	0.141	0.138	0.146	0.143	0.149	0.141	0.135
66	N/SS(1)	0.111	0.114	0.117	0.111	0.111	0.146	0.146	0.143	0.143	0.141	0.141	0.138	0.141	0.138	0.143	0.141	0.138	0.138	0.141	0.143	0.141	0.149	0.146	0.151	0.143	0.138
67	N/SS(1)	0.111	0.114	0.117	0.111	0.111	0.146	0.146	0.143	0.143	0.141	0.141	0.138	0.141	0.138	0.143	0.141	0.138	0.141	0.143	0.143	0.141	0.143	0.141	0.146	0.138	0.133
68	*G. eatti*	0.149	0.151	0.154	0.156	0.154	0.170	0.164	0.167	0.167	0.170	0.170	0.167	0.164	0.167	0.172	0.170	0.170	0.172	0.175	0.172	0.175	0.170	0.167	0.167	0.164	0.159
69	*G. eatti*	0.146	0.149	0.151	0.154	0.151	0.167	0.162	0.164	0.164	0.167	0.167	0.164	0.162	0.164	0.170	0.167	0.167	0.170	0.172	0.170	0.172	0.167	0.164	0.164	0.162	0.156
70	*G. eatti* (2)	0.143	0.146	0.149	0.151	0.149	0.164	0.159	0.162	0.162	0.164	0.164	0.162	0.159	0.162	0.167	0.164	0.164	0.167	0.170	0.167	0.170	0.164	0.162	0.162	0.159	0.154
71	*G. eatti* (2)	0.141	0.143	0.146	0.149	0.146	0.162	0.156	0.159	0.159	0.162	0.162	0.159	0.156	0.159	0.164	0.162	0.162	0.164	0.167	0.164	0.167	0.162	0.159	0.159	0.156	0.151
72	*G. australis* (1)	0.172	0.170	0.167	0.167	0.170	0.183	0.183	0.180	0.180	0.183	0.183	0.180	0.183	0.180	0.175	0.178	0.178	0.186	0.188	0.175	0.178	0.180	0.178	0.183	0.175	0.175
73	*G. australis* (1)	0.175	0.172	0.170	0.170	0.172	0.186	0.186	0.183	0.183	0.186	0.186	0.183	0.186	0.183	0.178	0.180	0.180	0.188	0.191	0.178	0.180	0.183	0.180	0.186	0.178	0.178
74	*G. australis* (1)	0.172	0.170	0.167	0.162	0.164	0.186	0.186	0.183	0.183	0.186	0.186	0.183	0.186	0.183	0.178	0.180	0.180	0.188	0.191	0.178	0.180	0.183	0.180	0.186	0.178	0.178
75	*G. australis* (1)	0.172	0.170	0.167	0.162	0.164	0.180	0.180	0.178	0.178	0.180	0.180	0.178	0.180	0.178	0.172	0.175	0.175	0.183	0.186	0.172	0.175	0.178	0.175	0.180	0.172	0.172
76	*E. acanthopoma* (1)	0.194	0.194	0.191	0.188	0.191	0.215	0.215	0.212	0.212	0.212	0.210	0.210	0.212	0.210	0.207	0.210	0.210	0.207	0.210	0.207	0.204	0.207	0.204	0.207	0.202	0.199
77	*E. sandwicensis*	0.202	0.202	0.199	0.202	0.204	0.223	0.223	0.220	0.220	0.220	0.218	0.218	0.220	0.218	0.215	0.218	0.218	0.215	0.218	0.215	0.212	0.215	0.212	0.215	0.210	0.207
78	*E. fusca* (1)	0.183	0.186	0.183	0.183	0.186	0.218	0.218	0.215	0.215	0.212	0.212	0.210	0.212	0.210	0.210	0.212	0.212	0.218	0.220	0.212	0.210	0.207	0.204	0.207	0.202	0.196
79	*E. fusca* (1)	0.186	0.188	0.186	0.186	0.188	0.215	0.215	0.212	0.212	0.210	0.210	0.207	0.210	0.207	0.207	0.210	0.210	0.215	0.218	0.210	0.207	0.204	0.202	0.204	0.199	0.194
80	*E. melanosoma* (1)	0.196	0.196	0.194	0.191	0.194	0.215	0.215	0.212	0.212	0.212	0.212	0.210	0.212	0.210	0.207	0.215	0.215	0.215	0.218	0.210	0.212	0.212	0.210	0.215	0.207	0.204
81	*E. amblyopsis* (1)	0.210	0.210	0.212	0.210	0.212	0.231	0.231	0.228	0.228	0.228	0.228	0.225	0.228	0.225	0.223	0.225	0.225	0.225	0.228	0.225	0.228	0.218	0.215	0.220	0.212	0.210
82	*E. amblyopsis* (1)	0.207	0.207	0.210	0.207	0.210	0.228	0.228	0.225	0.225	0.225	0.225	0.223	0.225	0.223	0.220	0.223	0.223	0.223	0.225	0.223	0.225	0.215	0.212	0.218	0.210	0.207
83	*E. amblyopsis* (1)	0.194	0.194	0.196	0.194	0.196	0.223	0.223	0.220	0.220	0.220	0.220	0.218	0.220	0.218	0.215	0.218	0.218	0.223	0.225	0.218	0.220	0.218	0.220	0.225	0.218	0.215
84	*E. picta* (2)	0.196	0.196	0.194	0.199	0.196	0.199	0.199	0.196	0.196	0.202	0.202	0.199	0.196	0.199	0.196	0.199	0.196	0.204	0.207	0.199	0.202	0.199	0.196	0.199	0.194	0.191
85	*E. pisonis* (1)	0.199	0.199	0.196	0.202	0.199	0.202	0.202	0.199	0.199	0.199	0.199	0.196	0.199	0.196	0.194	0.202	0.199	0.202	0.204	0.196	0.199	0.196	0.194	0.196	0.191	0.188
86	*P. grandiceps* (1)	0.204	0.202	0.199	0.207	0.204	0.210	0.215	0.212	0.215	0.215	0.210	0.212	0.215	0.215	0.212	0.210	0.210	0.215	0.215	0.207	0.210	0.215	0.212	0.218	0.210	0.215
87	*P. grandiceps* (3)	0.210	0.207	0.204	0.207	0.207	0.212	0.218	0.215	0.218	0.218	0.212	0.215	0.218	0.218	0.215	0.212	0.212	0.218	0.218	0.210	0.212	0.218	0.215	0.220	0.212	0.218
88	*P. grandiceps* (2)	0.215	0.212	0.210	0.207	0.207	0.220	0.225	0.223	0.220	0.225	0.220	0.223	0.225	0.220	0.223	0.220	0.220	0.220	0.220	0.218	0.220	0.220	0.218	0.223	0.215	0.220
89	*P. sp* (1)	0.188	0.191	0.188	0.186	0.188	0.204	0.199	0.202	0.199	0.199	0.194	0.196	0.199	0.194	0.202	0.204	0.202	0.199	0.202	0.196	0.199	0.202	0.199	0.204	0.196	0.196
90	*P. sp* (1)	0.194	0.196	0.194	0.191	0.194	0.204	0.204	0.207	0.204	0.204	0.199	0.202	0.204	0.199	0.207	0.210	0.207	0.204	0.207	0.202	0.204	0.207	0.204	0.210	0.202	0.202
91	*H. aurea* (1)	0.183	0.183	0.180	0.180	0.183	0.199	0.199	0.196	0.196	0.196	0.196	0.194	0.196	0.194	0.191	0.194	0.191	0.196	0.199	0.191	0.194	0.196	0.194	0.199	0.191	0.188
92	*H. galii* (1)	0.180	0.183	0.180	0.183	0.183	0.196	0.196	0.194	0.194	0.191	0.191	0.188	0.191	0.188	0.188	0.191	0.188	0.194	0.196	0.188	0.191	0.188	0.186	0.191	0.183	0.178
93	*H. compressa* (1)	0.180	0.180	0.178	0.175	0.175	0.194	0.194	0.191	0.191	0.191	0.191	0.188	0.191	0.188	0.186	0.188	0.186	0.188	0.191	0.183	0.186	0.180	0.178	0.183	0.175	0.172
94	*H. compressa* (1)	0.180	0.180	0.178	0.175	0.175	0.194	0.194	0.191	0.191	0.191	0.191	0.188	0.191	0.188	0.186	0.188	0.186	0.188	0.191	0.183	0.186	0.180	0.178	0.183	0.175	0.172
95	*H. sp* (1)	0.194	0.194	0.191	0.196	0.194	0.194	0.194	0.191	0.191	0.191	0.191	0.188	0.191	0.188	0.186	0.194	0.196	0.191	0.194	0.191	0.194	0.202	0.199	0.204	0.196	0.194
96	*H. kluzingeri* (1)	0.191	0.191	0.188	0.183	0.186	0.199	0.199	0.196	0.196	0.196	0.196	0.194	0.196	0.194	0.196	0.194	0.191	0.188	0.188	0.186	0.188	0.186	0.183	0.188	0.180	0.178
97	*E. oxycephala* (1)	0.178	0.178	0.180	0.175	0.172	0.191	0.191	0.188	0.188	0.188	0.188	0.186	0.188	0.186	0.183	0.191	0.188	0.180	0.183	0.186	0.188	0.191	0.188	0.194	0.186	0.183
98	*M. adspersa* (1)	0.199	0.196	0.199	0.196	0.199	0.215	0.215	0.212	0.215	0.220	0.215	0.218	0.215	0.220	0.212	0.210	0.210	0.220	0.223	0.218	0.220	0.225	0.223	0.225	0.220	0.220
99	*M. adspersa* (1)	0.186	0.183	0.186	0.186	0.186	0.204	0.204	0.202	0.204	0.210	0.204	0.207	0.204	0.210	0.202	0.199	0.199	0.210	0.212	0.207	0.210	0.215	0.212	0.215	0.210	0.210
100	*M. mogurnda* (1)	0.194	0.191	0.194	0.194	0.194	0.220	0.220	0.218	0.220	0.225	0.220	0.223	0.220	0.225	0.218	0.215	0.215	0.225	0.228	0.223	0.225	0.231	0.228	0.231	0.225	0.225
101	*M. mogurnda* (1)	0.196	0.194	0.196	0.196	0.196	0.218	0.218	0.215	0.218	0.223	0.218	0.220	0.218	0.223	0.215	0.212	0.212	0.223	0.225	0.220	0.223	0.228	0.225	0.228	0.223	0.223
102	*M. mogurnda* (1)	0.202	0.199	0.202	0.202	0.202	0.228	0.228	0.225	0.228	0.233	0.228	0.231	0.228	0.233	0.225	0.223	0.223	0.233	0.236	0.231	0.233	0.233	0.231	0.233	0.228	0.228
103	*M. elevicola* (1)	0.188	0.186	0.188	0.188	0.188	0.215	0.215	0.212	0.215	0.220	0.215	0.218	0.215	0.220	0.212	0.210	0.210	0.220	0.223	0.218	0.220	0.225	0.223	0.225	0.220	0.220
104	*M. thermophila* (1)	0.196	0.194	0.196	0.196	0.196	0.202	0.202	0.199	0.202	0.207	0.202	0.204	0.202	0.207	0.199	0.196	0.196	0.210	0.212	0.204	0.207	0.212	0.210	0.212	0.207	0.207
105	*M. larapintae* (1)	0.188	0.186	0.188	0.188	0.188	0.210	0.210	0.207	0.210	0.215	0.210	0.212	0.210	0.215	0.207	0.204	0.204	0.215	0.218	0.212	0.215	0.215	0.212	0.215	0.210	0.210

APPENDIX I. (continued)

	G. huttoni									A	G. hubbsi					G. coxii				G. australis				Eleotris										Philypnodon					Hypseleotris						Eleotris	Mogurnda							
	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105
53		0.002	0.000	0.002	0.002	0.007	0.005	0.010	0.007	0.048	0.161	0.163	0.165	0.174	0.169	0.259	0.253	0.242	0.236	0.305	0.301	0.291	0.298	0.447	0.503	0.417	0.426	0.388	0.523	0.532	0.548	0.553	0.542	0.540	0.540	0.551	0.411	0.430	0.362	0.371	0.344	0.337	0.442	0.358	0.382	0.486	0.458	0.520	0.531	0.526	0.490	0.540	0.535
54	0.003		0.002	0.005	0.005	0.010	0.007	0.012	0.010	0.052	0.170	0.172	0.175	0.184	0.179	0.273	0.266	0.255	0.248	0.322	0.318	0.307	0.315	0.471	0.529	0.438	0.448	0.408	0.550	0.560	0.578	0.584	0.572	0.567	0.567	0.578	0.433	0.453	0.382	0.391	0.362	0.355	0.466	0.378	0.402	0.511	0.482	0.546	0.557	0.552	0.515	0.567	0.563
55	0.000	0.003		0.002	0.002	0.007	0.005	0.010	0.007	0.048	0.161	0.163	0.165	0.174	0.169	0.259	0.253	0.242	0.236	0.305	0.301	0.291	0.298	0.447	0.503	0.417	0.426	0.388	0.523	0.532	0.548	0.553	0.542	0.540	0.540	0.551	0.411	0.430	0.362	0.371	0.344	0.337	0.442	0.358	0.382	0.486	0.458	0.520	0.531	0.526	0.490	0.540	0.535
56	0.003	0.005	0.003		0.005	0.010	0.007	0.007	0.010	0.051	0.166	0.168	0.171	0.180	0.175	0.252	0.246	0.236	0.229	0.313	0.310	0.299	0.306	0.457	0.513	0.426	0.436	0.396	0.534	0.543	0.559	0.565	0.553	0.550	0.550	0.561	0.401	0.420	0.371	0.380	0.352	0.345	0.452	0.367	0.391	0.496	0.468	0.530	0.541	0.536	0.500	0.550	0.545
57	0.003	0.005	0.003	0.005		0.005	0.002	0.007	0.005	0.044	0.158	0.159	0.162	0.171	0.166	0.249	0.242	0.233	0.226	0.319	0.315	0.304	0.312	0.466	0.524	0.434	0.444	0.404	0.545	0.555	0.572	0.530	0.518	0.519	0.525	0.536	0.429	0.448	0.378	0.380	0.351	0.344	0.424	0.374	0.366	0.506	0.445	0.505	0.516	0.511	0.477	0.524	0.519
58	0.008	0.011	0.008	0.011	0.005		0.007	0.012	0.010	0.050	0.158	0.160	0.162	0.171	0.167	0.239	0.233	0.223	0.217	0.320	0.316	0.305	0.313	0.468	0.525	0.430	0.440	0.400	0.541	0.551	0.568	0.516	0.505	0.507	0.513	0.524	0.417	0.436	0.366	0.368	0.352	0.345	0.425	0.375	0.362	0.494	0.433	0.494	0.504	0.500	0.465	0.513	0.507
59	0.005	0.008	0.005	0.008	0.003	0.008		0.010	0.007	0.047	0.162	0.164	0.166	0.175	0.170	0.243	0.237	0.227	0.221	0.324	0.308	0.310	0.317	0.472	0.517	0.440	0.449	0.409	0.551	0.561	0.579	0.536	0.525	0.525	0.531	0.541	0.435	0.454	0.384	0.386	0.357	0.350	0.430	0.380	0.372	0.512	0.451	0.511	0.522	0.517	0.483	0.530	0.525
60	0.011	0.013	0.011	0.008	0.008	0.013	0.011		0.007	0.053	0.162	0.164	0.167	0.176	0.171	0.241	0.235	0.225	0.219	0.339	0.334	0.323	0.331	0.481	0.539	0.442	0.452	0.417	0.560	0.569	0.588	0.545	0.534	0.541	0.547	0.557	0.417	0.437	0.391	0.388	0.364	0.357	0.438	0.388	0.379	0.528	0.467	0.527	0.538	0.533	0.498	0.546	0.542
61	0.008	0.011	0.008	0.011	0.005	0.011	0.008	0.008		0.043	0.169	0.171	0.174	0.183	0.178	0.264	0.258	0.247	0.241	0.339	0.335	0.323	0.332	0.493	0.553	0.457	0.467	0.426	0.574	0.584	0.604	0.560	0.548	0.505	0.510	0.529	0.425	0.444	0.401	0.403	0.366	0.359	0.442	0.391	0.388	0.492	0.433	0.492	0.502	0.497	0.464	0.510	0.504
62	0.045	0.048	0.045	0.048	0.042	0.048	0.045	0.050	0.042		0.176	0.168	0.171	0.180	0.164	0.263	0.256	0.245	0.239	0.333	0.323	0.312	0.320	0.450	0.507	0.401	0.410	0.458	0.548	0.558	0.576	0.581	0.569	0.462	0.468	0.486	0.381	0.399	0.400	0.403	0.335	0.321	0.449	0.397	0.451	0.578	0.533	0.571	0.582	0.576	0.540	0.598	0.572
63	0.111	0.114	0.111	0.114	0.111	0.111	0.114	0.114	0.117	0.119		0.007	0.010	0.013	0.010	0.296	0.288	0.283	0.290	0.263	0.256	0.247	0.253	0.505	0.548	0.251	0.259	0.296	0.563	0.572	0.530	0.482	0.472	0.514	0.499	0.510	0.421	0.439	0.367	0.396	0.360	0.347	0.379	0.412	0.291	0.484	0.470	0.476	0.487	0.515	0.461	0.493	0.508
64	0.111	0.114	0.111	0.114	0.111	0.111	0.114	0.114	0.117	0.117	0.008		0.007	0.010	0.007	0.300	0.293	0.287	0.295	0.251	0.244	0.225	0.230	0.469	0.523	0.240	0.247	0.282	0.538	0.547	0.528	0.481	0.471	0.512	0.497	0.496	0.391	0.409	0.380	0.403	0.359	0.346	0.386	0.407	0.289	0.494	0.479	0.486	0.497	0.526	0.470	0.503	0.519
65	0.111	0.114	0.111	0.114	0.111	0.111	0.114	0.114	0.117	0.117	0.011	0.008		0.002	0.005	0.317	0.309	0.297	0.305	0.260	0.253	0.233	0.239	0.477	0.532	0.249	0.256	0.277	0.544	0.553	0.499	0.474	0.464	0.500	0.485	0.483	0.403	0.421	0.387	0.409	0.365	0.351	0.393	0.414	0.298	0.522	0.493	0.513	0.524	0.540	0.484	0.517	0.534
66	0.114	0.117	0.114	0.117	0.114	0.114	0.117	0.117	0.119	0.119	0.013	0.011	0.003		0.007	0.312	0.304	0.292	0.300	0.256	0.250	0.230	0.235	0.467	0.521	0.262	0.270	0.291	0.571	0.580	0.525	0.465	0.455	0.524	0.509	0.507	0.423	0.441	0.366	0.389	0.346	0.333	0.373	0.392	0.284	0.511	0.484	0.503	0.514	0.530	0.475	0.543	0.523
67	0.114	0.117	0.114	0.117	0.114	0.114	0.117	0.117	0.119	0.114	0.011	0.008	0.005	0.008		0.301	0.294	0.282	0.290	0.261	0.254	0.234	0.239	0.484	0.539	0.235	0.242	0.293	0.545	0.554	0.521	0.454	0.444	0.486	0.472	0.470	0.386	0.403	0.393	0.397	0.353	0.340	0.380	0.401	0.283	0.523	0.494	0.514	0.525	0.541	0.485	0.518	0.513
68	0.149	0.151	0.149	0.146	0.146	0.141	0.143	0.143	0.151	0.149	0.156	0.156	0.162	0.162	0.156		0.002	0.005	0.007	0.258	0.256	0.281	0.270	0.431	0.457	0.347	0.339	0.318	0.498	0.507	0.601	0.377	0.387	0.453	0.459	0.470	0.355	0.374	0.345	0.353	0.305	0.300	0.351	0.353	0.325	0.438	0.415	0.453	0.444	0.458	0.434	0.443	0.440
69	0.146	0.149	0.146	0.143	0.143	0.138	0.141	0.141	0.149	0.146	0.154	0.154	0.159	0.159	0.154	0.003		0.002	0.005	0.251	0.249	0.273	0.263	0.421	0.446	0.339	0.330	0.310	0.487	0.495	0.589	0.367	0.377	0.443	0.449	0.460	0.346	0.365	0.337	0.345	0.298	0.292	0.343	0.344	0.317	0.428	0.406	0.444	0.434	0.449	0.425	0.433	0.430
70	0.143	0.146	0.143	0.141	0.141	0.135	0.138	0.138	0.146	0.143	0.154	0.154	0.156	0.156	0.151	0.005	0.003		0.002	0.240	0.238	0.261	0.251	0.403	0.426	0.324	0.316	0.297	0.473	0.482	0.565	0.351	0.360	0.425	0.430	0.442	0.331	0.349	0.322	0.330	0.285	0.280	0.329	0.331	0.303	0.418	0.389	0.434	0.424	0.431	0.408	0.415	0.412
71	0.141	0.143	0.141	0.138	0.138	0.133	0.135	0.135	0.143	0.141	0.156	0.156	0.159	0.159	0.154	0.008	0.005	0.003		0.233	0.231	0.254	0.244	0.399	0.422	0.316	0.308	0.295	0.462	0.471	0.554	0.341	0.351	0.415	0.421	0.432	0.322	0.340	0.314	0.322	0.278	0.273	0.321	0.322	0.295	0.409	0.380	0.424	0.415	0.421	0.399	0.406	0.403
72	0.149	0.151	0.149	0.151	0.151	0.151	0.154	0.159	0.156	0.156	0.138	0.135	0.138	0.138	0.138	0.135	0.133	0.130	0.127		0.010	0.015	0.012	0.381	0.381	0.280	0.272	0.219	0.399	0.408	0.493	0.340	0.331	0.325	0.325	0.336	0.373	0.390	0.172	0.194	0.195	0.199	0.293	0.237	0.236	0.274	0.274	0.310	0.294	0.291	0.282	0.308	0.315
73	0.151	0.154	0.151	0.154	0.154	0.154	0.151	0.162	0.159	0.156	0.138	0.135	0.138	0.138	0.138	0.138	0.135	0.133	0.130	0.011		0.010	0.012	0.369	0.355	0.285	0.277	0.234	0.435	0.444	0.505	0.352	0.343	0.322	0.322	0.332	0.380	0.397	0.185	0.208	0.202	0.194	0.310	0.252	0.240	0.266	0.278	0.301	0.286	0.283	0.286	0.312	0.306
74	0.149	0.151	0.149	0.151	0.151	0.151	0.154	0.159	0.156	0.154	0.135	0.127	0.130	0.130	0.130	0.146	0.143	0.141	0.138	0.016	0.011		0.005	0.334	0.348	0.275	0.267	0.225	0.428	0.437	0.488	0.364	0.354	0.336	0.336	0.335	0.384	0.401	0.194	0.218	0.197	0.188	0.319	0.247	0.224	0.251	0.273	0.296	0.281	0.279	0.281	0.326	0.288
75	0.149	0.151	0.149	0.151	0.151	0.151	0.154	0.159	0.156	0.154	0.135	0.127	0.130	0.130	0.130	0.143	0.141	0.138	0.135	0.013	0.013	0.005		0.344	0.358	0.295	0.286	0.230	0.434	0.443	0.517	0.342	0.333	0.317	0.317	0.316	0.363	0.380	0.186	0.209	0.183	0.175	0.300	0.231	0.242	0.268	0.280	0.315	0.300	0.297	0.288	0.307	0.296
76	0.183	0.186	0.183	0.186	0.186	0.186	0.188	0.191	0.191	0.180	0.191	0.183	0.183	0.183	0.186	0.172	0.170	0.167	0.167	0.156	0.156	0.146	0.146		0.020	0.447	0.437	0.317	0.447	0.455	0.447	0.435	0.424	0.558	0.574	0.572	0.546	0.568	0.434	0.477	0.404	0.389	0.494	0.489	0.501	0.529	0.543	0.611	0.600	0.617	0.636	0.639	0.558
77	0.191	0.194	0.191	0.194	0.194	0.194	0.191	0.199	0.199	0.188	0.194	0.191	0.191	0.191	0.194	0.172	0.170	0.167	0.167	0.156	0.151	0.151	0.151	0.021		0.471	0.460	0.338	0.448	0.456	0.461	0.475	0.464	0.551	0.568	0.579	0.550	0.573	0.412	0.454	0.399	0.384	0.469	0.491	0.535	0.520	0.535	0.603	0.592	0.609	0.628	0.630	0.549
78	0.186	0.188	0.186	0.188	0.188	0.186	0.191	0.191	0.194	0.180	0.138	0.135	0.138	0.141	0.133	0.162	0.159	0.156	0.154	0.138	0.138	0.135	0.141	0.180	0.180		0.002	0.157	0.299	0.299	0.287	0.382	0.373	0.566	0.583	0.594	0.455	0.455	0.383	0.359	0.347	0.357	0.370	0.370	0.257	0.388	0.399	0.390	0.413	0.396	0.404	0.418	0.415
79	0.188	0.191	0.188	0.191	0.191	0.188	0.194	0.194	0.196	0.183	0.141	0.138	0.141	0.143	0.135	0.159	0.156	0.154	0.151	0.135	0.135	0.133	0.138	0.178	0.178	0.003		0.152	0.292	0.292	0.280	0.373	0.364	0.555	0.572	0.583	0.466	0.465	0.373	0.350	0.339	0.348	0.361	0.361	0.250	0.379	0.390	0.400	0.404	0.406	0.413	0.408	0.406
80	0.178	0.180	0.178	0.180	0.180	0.178	0.183	0.186	0.186	0.199	0.151	0.149	0.146	0.149	0.151	0.156	0.154	0.151	0.151	0.122	0.127	0.125	0.125	0.159	0.162	0.109	0.106		0.296	0.302	0.321	0.275	0.268	0.482	0.483	0.495	0.463	0.462	0.282	0.314	0.279	0.286	0.297	0.280	0.240	0.386	0.371	0.404	0.408	0.390	0.391	0.404	0.422
81	0.196	0.199	0.196	0.199	0.199	0.196	0.202	0.204	0.204	0.202	0.204	0.202	0.204	0.207	0.204	0.178	0.175	0.175	0.172	0.170	0.180	0.180	0.178	0.178	0.178	0.167	0.164	0.159		0.010	0.121	0.376	0.367	0.625	0.642	0.653	0.535	0.556	0.462	0.456	0.404	0.416	0.556	0.494	0.439	0.588	0.608	0.627	0.616	0.623	0.622	0.652	0.625
82	0.199	0.202	0.199	0.202	0.202	0.199	0.204	0.207	0.207	0.204	0.207	0.204	0.207	0.210	0.207	0.180	0.178	0.178	0.175	0.172	0.183	0.183	0.180	0.180	0.180	0.167	0.164	0.162	0.011		0.121	0.351	0.343	0.633	0.650	0.661	0.543	0.565	0.471	0.464	0.412	0.424	0.556	0.502	0.439	0.611	0.632	0.650	0.639	0.645	0.644	0.652	0.649
83	0.196	0.199	0.196	0.199	0.199	0.196	0.202	0.204	0.204	0.202	0.199	0.202	0.194	0.196	0.199	0.207	0.204	0.202	0.199	0.186	0.186	0.183	0.188	0.180	0.186	0.159	0.156	0.167	0.095	0.095		0.474	0.464	0.691	0.709	0.719	0.640	0.663	0.483	0.441	0.474	0.488	0.581	0.475	0.464	0.601	0.639	0.634	0.623	0.640	0.629	0.673	0.650
84	0.194	0.196	0.194	0.196	0.191	0.186	0.194	0.196	0.196	0.199	0.191	0.194	0.191	0.191	0.186	0.162	0.159	0.156	0.154	0.159	0.162	0.162	0.156	0.172	0.178	0.172	0.170	0.143	0.175	0.167	0.196		0.002	0.531	0.531	0.542	0.514	0.536	0.427	0.396	0.367	0.383	0.428	0.401	0.315	0.543	0.475	0.560	0.540	0.557	0.534	0.509	0.496
85	0.191	0.194	0.191	0.194	0.188	0.183	0.191	0.194	0.194	0.196	0.188	0.191	0.188	0.188	0.183	0.164	0.162	0.159	0.156	0.156	0.159	0.159	0.154	0.170	0.175	0.170	0.167	0.141	0.172	0.164	0.194	0.003		0.520	0.520	0.532	0.502	0.525	0.417	0.386	0.358	0.374	0.419	0.391	0.307	0.555	0.487	0.572	0.552	0.569	0.546	0.521	0.508
86	0.202	0.204	0.202	0.204	0.199	0.194	0.202	0.207	0.199	0.191	0.199	0.202	0.196	0.199	0.194	0.183	0.180	0.178	0.175	0.156	0.159	0.162	0.156	0.199	0.196	0.204	0.202	0.180	0.212	0.215	0.225	0.199	0.196		0.005	0.012	0.213	0.213	0.518	0.512	0.455	0.445	0.518	0.481	0.583	0.569	0.506	0.545	0.548	0.543	0.527	0.505	0.495
87	0.202	0.204	0.202	0.204	0.202	0.196	0.204	0.210	0.202	0.194	0.196	0.199	0.194	0.196	0.191	0.186	0.183	0.180	0.178	0.156	0.159	0.162	0.156	0.202	0.199	0.207	0.204	0.180	0.215	0.218	0.228	0.199	0.196	0.005		0.007	0.213	0.213	0.518	0.511	0.454	0.444	0.525	0.481	0.583	0.585	0.506	0.545	0.548	0.543	0.527	0.505	0.495
88	0.207	0.210	0.207	0.210	0.207	0.202	0.210	0.215	0.210	0.202	0.202	0.199	0.194	0.196	0.191	0.191	0.188	0.186	0.183	0.162	0.164	0.162	0.156	0.202	0.204	0.212	0.210	0.186	0.220	0.223	0.233	0.204	0.202	0.013	0.008		0.207	0.207	0.530	0.522	0.451	0.441	0.535	0.477	0.586	0.604	0.525	0.564	0.567	0.562	0.533	0.524	0.501
89	0.175	0.178	0.175	0.172	0.178	0.172	0.180	0.175	0.180	0.170	0.186	0.178	0.180	0.183	0.175	0.156	0.154	0.151	0.149	0.172	0.180	0.178	0.172	0.191	0.194	0.188	0.191	0.183	0.196	0.199	0.218	0.188	0.186	0.133	0.133	0.130		0.005	0.517	0.501	0.434	0.424	0.506	0.484	0.504	0.558	0.533	0.494	0.517	0.513	0.478	0.538	0.529
90	0.180	0.183	0.180	0.178	0.183	0.178	0.186	0.180	0.186	0.175	0.191	0.183	0.186	0.188	0.180	0.162	0.159	0.156	0.154	0.178	0.186	0.183	0.178	0.196	0.199	0.188	0.191	0.183	0.202	0.204	0.223	0.194	0.191	0.133	0.133	0.130	0.005		0.539	0.522	0.454	0.444	0.505	0.505	0.525	0.557	0.532	0.493	0.517	0.512	0.478	0.537	0.529
91	0.164	0.167	0.164	0.167	0.167	0.162	0.170	0.172	0.172	0.170	0.164	0.167	0.167	0.164	0.170	0.162	0.159	0.156	0.154	0.111	0.117	0.119	0.117	0.172	0.172	0.164	0.162	0.146	0.175	0.178	0.186	0.178	0.175	0.191	0.191	0.196	0.191	0.196		0.074	0.066	0.066	0.125	0.120	0.320	0.448	0.475	0.516	0.505	0.493	0.486	0.500	0.513
92	0.167	0.170	0.167	0.170	0.167	0.162	0.170	0.170	0.172	0.172	0.180	0.180	0.180	0.178	0.178	0.164	0.162	0.159	0.156	0.119	0.125	0.127	0.125	0.188	0.188	0.164	0.162	0.159	0.180	0.183	0.180	0.172	0.170	0.194	0.194	0.199	0.191	0.196	0.066		0.072	0.073	0.123	0.086	0.334	0.434	0.420	0.481	0.462	0.458	0.458	0.477	0.453
93	0.167	0.170	0.167	0.170	0.167	0.167	0.170	0.172	0.170	0.156	0.167	0.164	0.164	0.162	0.162	0.156	0.154	0.151	0.149	0.122	0.125	0.119	0.114	0.172	0.178	0.162	0.159	0.151	0.170	0.172	0.188	0.167	0.164	0.180	0.180	0.180	0.175	0.180	0.061	0.066		0.002	0.092	0.094	0.320	0.435	0.434	0.480	0.462	0.458	0.445	0.469	0.453
94	0.167	0.170	0.167	0.170	0.167	0.167	0.170	0.172	0.170	0.154	0.164	0.162	0.162	0.159	0.159	0.156	0.154	0.151	0.149	0.122	0.122	0.117	0.111	0.170	0.175	0.162	0.159	0.151	0.170	0.172	0.188	0.170	0.167	0.180	0.180	0.180	0.175	0.180	0.061	0.066	0.003		0.093	0.095	0.329	0.420	0.419	0.464	0.446	0.442	0.429	0.452	0.437
95	0.180	0.183	0.180	0.183	0.178	0.178	0.180	0.183	0.180	0.183	0.167	0.167	0.167	0.164	0.164	0.167	0.164	0.162	0.159	0.151	0.156	0.156	0.151	0.186	0.186	0.162	0.159	0.149	0.202	0.202	0.207	0.186	0.183	0.183	0.186	0.191	0.178	0.178	0.098	0.095	0.080	0.080		0.127	0.303	0.482	0.478	0.506	0.500	0.524	0.480	0.495	0.495
96	0.162	0.164	0.162	0.164	0.164	0.164	0.167	0.170	0.167	0.170	0.178	0.172	0.172	0.170	0.170	0.170	0.167	0.164	0.162	0.135	0.141	0.135	0.130	0.186	0.196	0.167	0.164	0.151	0.196	0.199	0.191	0.175	0.172	0.180	0.180	0.180	0.180	0.186	0.098	0.074	0.082	0.082	0.098		0.313	0.465	0.460	0.509	0.503	0.499	0.498	0.511	0.489
97	0.172	0.175	0.172	0.175	0.170	0.167	0.172	0.175	0.175	0.188	0.156	0.154	0.156	0.154	0.151	0.156	0.154	0.151	0.149	0.127	0.127	0.122	0.127	0.186	0.188	0.143	0.141	0.133	0.191	0.191	0.188	0.159	0.156	0.204	0.204	0.207	0.196	0.202	0.154	0.154	0.156	0.156	0.143	0.149		0.340	0.345	0.366	0.364	0.390	0.362	0.386	0.356
98	0.194	0.196	0.194	0.196	0.196	0.191	0.199	0.204	0.196	0.212	0.188	0.188	0.196	0.196	0.196	0.178	0.175	0.175	0.172	0.143	0.143	0.138	0.143	0.196	0.194	0.175	0.172	0.164	0.207	0.212	0.210	0.194	0.196	0.207	0.210	0.218	0.212	0.212	0.191	0.183	0.191	0.188	0.188	0.194	0.159		0.024	0.033	0.024	0.042	0.040	0.033	0.036
99	0.183	0.186	0.183	0.186	0.183	0.178	0.186	0.191	0.183	0.204	0.191	0.191	0.194	0.194	0.194	0.175	0.172	0.170	0.167	0.141	0.146	0.146	0.146	0.199	0.196	0.178	0.175	0.159	0.212	0.218	0.218	0.180	0.183	0.194	0.194	0.202	0.202	0.202	0.196	0.175	0.188	0.186	0.191	0.188	0.164	0.024		0.026	0.023	0.035	0.027	0.024	0.027
100	0.199	0.202	0.199	0.202	0.199	0.194	0.202	0.207	0.199	0.212	0.188	0.188	0.196	0.196	0.196	0.183	0.180	0.180	0.178	0.151	0.151	0.151	0.156	0.212	0.210	0.170	0.172	0.164	0.218	0.223	0.218	0.199	0.202	0.199	0.199	0.207	0.194	0.194	0.204	0.191	0.199	0.196	0.194	0.199	0.167	0.032	0.027		0.007	0.012	0.015	0.039	0.042
101	0.202	0.204	0.202	0.204	0.202	0.196	0.204	0.210	0.202	0.215	0.191	0.191	0.199	0.199	0.199	0.180	0.178	0.178	0.175	0.146	0.146	0.146	0.151	0.210	0.207	0.178	0.175	0.167	0.215	0.220	0.215	0.194	0.196	0.202	0.202	0.210	0.202	0.202	0.202	0.186	0.194	0.191	0.194	0.199	0.167	0.024	0.024	0.008		0.015	0.013	0.036	0.039
102	0.202	0.204	0.202	0.204	0.202	0.196	0.204	0.210	0.202	0.215	0.202	0.202	0.204	0.204	0.204	0.186	0.183	0.180	0.178	0.146	0.146	0.146	0.151	0.215	0.212	0.172	0.175	0.162	0.220	0.225	0.220	0.199	0.202	0.199	0.199	0.207	0.199	0.199	0.199	0.186	0.194	0.191	0.199	0.196	0.175	0.040	0.034	0.013	0.016		0.018	0.049	0.052
103	0.194	0.196	0.194	0.196	0.194	0.188	0.196	0.202	0.194	0.207	0.188	0.188	0.191	0.191	0.191	0.180	0.178	0.175	0.172	0.143	0.149	0.149	0.149	0.212	0.210	0.178	0.180	0.164	0.220	0.225	0.220	0.194	0.196	0.199	0.199	0.202	0.194	0.194	0.199	0.186	0.191	0.188	0.191	0.199	0.170	0.037	0.027	0.016	0.013	0.019		0.040	0.037
104	0.199	0.202	0.199	0.202	0.199	0.194	0.202	0.207	0.199	0.215	0.191	0.191	0.194	0.196	0.194	0.178	0.175	0.172	0.170	0.149	0.154	0.156	0.151	0.207	0.204	0.178	0.175	0.162	0.212	0.212	0.218	0.183	0.186	0.191	0.191	0.199	0.202	0.202	0.196	0.186	0.191	0.188	0.188	0.196	0.167	0.032	0.024	0.037	0.034	0.045	0.037		0.036
105	0.191	0.194	0.191	0.194	0.191	0.186	0.194	0.199	0.191	0.202	0.188	0.188	0.191	0.191	0.186	0.172	0.170	0.167	0.164	0.149	0.149	0.143	0.143	0.194	0.191	0.172	0.170	0.162	0.210	0.215	0.212	0.180	0.183	0.186	0.186	0.188	0.196	0.196	0.194	0.175	0.183	0.180	0.183	0.186	0.159	0.034	0.027	0.040	0.037	0.048	0.034	0.034	

*A – M/N(2), N/K(2), W/WR(1), EC/G(3), WC/CR(2), BOP/RR(3), BOP/LT(2), BOP/KR(1), NE/M(3)

B – N/SS(1), W/M(3), WC/LB(2), M/N(5)

C – N/KR(1), N/LN(3), W/M(1), N/N(3), N/LW(3), N/WR(2)

D – ECo/K(2), ECo/D(1), C/AR(1)

E – M/T(8), M/N(1), M/TS(9), Wa/K(7)

F – WCo/R(1), WCo/S(1), WCo/F(2)

G – N/KR(2), N/WR(1), N/US(2), N/Mi(2), W/M(2), Wa/WR(1)

APPENDIX II. Genetic distance based on sequence variation in the 56 unique mtDNA control region sequences for *Gobiomorphus*. Lower triangle is uncorrected distances and upper triangle is maximum likelihood distances. Location codes refer to those used in Table 1. A = *G. gobioides*; B = *G. hubbsi*.

		G. huttoni	A	*G. breviceps*																												
	LOCATION*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	CI/W(1)		0.003	0.057	0.096	0.109	0.104	0.109	0.105	0.100	0.104	0.104	0.114	0.118	0.103	0.108	0.104	0.100	0.104	0.100	0.092	0.092	0.083	0.084	0.123	0.127	0.118	0.095	0.091	0.091	0.104	0.113
2	W/M(1)	0.003		0.053	0.100	0.113	0.108	0.113	0.109	0.104	0.108	0.108	0.118	0.122	0.107	0.112	0.108	0.104	0.108	0.104	0.096	0.096	0.087	0.087	0.127	0.131	0.122	0.099	0.095	0.095	0.108	0.118
3	N/KR(1)	0.050	0.048		0.110	0.124	0.119	0.122	0.119	0.114	0.119	0.119	0.138	0.133	0.117	0.131	0.114	0.110	0.114	0.109	0.101	0.105	0.100	0.101	0.129	0.133	0.124	0.108	0.104	0.096	0.117	0.127
4	A	0.077	0.079	0.084		0.008	0.005	0.011	0.005	0.003	0.005	0.005	0.028	0.025	0.014	0.022	0.014	0.011	0.014	0.014	0.005	0.014	0.011	0.014	0.038	0.042	0.035	0.028	0.025	0.019	0.101	0.110
5	Wa/M(1)	0.085	0.087	0.092	0.008		0.003	0.008	0.003	0.005	0.008	0.008	0.032	0.029	0.017	0.025	0.016	0.019	0.022	0.022	0.014	0.022	0.019	0.022	0.035	0.038	0.032	0.038	0.035	0.029	0.105	0.115
6	M/K(1)	0.082	0.085	0.090	0.005	0.003		0.011	0.005	0.003	0.005	0.005	0.029	0.026	0.014	0.022	0.014	0.016	0.019	0.019	0.011	0.019	0.016	0.019	0.039	0.042	0.035	0.034	0.031	0.025	0.110	0.119
7	M/M(1)	0.085	0.087	0.092	0.010	0.008	0.011		0.005	0.008	0.011	0.011	0.035	0.032	0.019	0.028	0.025	0.022	0.025	0.025	0.016	0.025	0.022	0.025	0.038	0.042	0.035	0.041	0.038	0.032	0.109	0.119
8	M/M(1)	0.082	0.085	0.090	0.005	0.003	0.005	0.005		0.003	0.005	0.005	0.029	0.026	0.014	0.022	0.019	0.016	0.019	0.019	0.011	0.019	0.016	0.019	0.032	0.035	0.029	0.035	0.031	0.025	0.101	0.110
9	B	0.079	0.082	0.087	0.003	0.005	0.003	0.008	0.003		0.003	0.003	0.026	0.022	0.011	0.019	0.016	0.014	0.016	0.016	0.008	0.016	0.014	0.017	0.035	0.038	0.032	0.031	0.028	0.022	0.105	0.114
10	M/TS(1)	0.082	0.085	0.090	0.005	0.008	0.005	0.010	0.005	0.003		0.005	0.029	0.025	0.014	0.022	0.019	0.016	0.019	0.019	0.011	0.019	0.016	0.019	0.039	0.042	0.035	0.034	0.031	0.025	0.110	0.119
11	W/WR(1)	0.082	0.085	0.090	0.005	0.008	0.005	0.011	0.005	0.003	0.005		0.029	0.026	0.014	0.022	0.014	0.011	0.014	0.019	0.011	0.019	0.016	0.019	0.039	0.042	0.035	0.034	0.031	0.025	0.110	0.119
12	WC/R(1)	0.087	0.090	0.100	0.026	0.029	0.026	0.032	0.026	0.024	0.026	0.026		0.014	0.028	0.025	0.038	0.035	0.031	0.038	0.028	0.038	0.035	0.038	0.052	0.056	0.049	0.051	0.048	0.041	0.106	0.115
13	WC/S(1)	0.090	0.093	0.098	0.024	0.026	0.024	0.029	0.024	0.021	0.024	0.024	0.013		0.025	0.035	0.035	0.031	0.028	0.034	0.025	0.035	0.032	0.035	0.049	0.052	0.045	0.051	0.048	0.041	0.114	0.123
14	WC/L(1)	0.082	0.085	0.090	0.013	0.016	0.013	0.018	0.013	0.011	0.013	0.013	0.026	0.024		0.008	0.022	0.019	0.022	0.022	0.014	0.022	0.019	0.022	0.042	0.045	0.038	0.038	0.034	0.028	0.109	0.118
15	WC/L(1)	0.085	0.087	0.098	0.021	0.024	0.021	0.026	0.021	0.018	0.021	0.021	0.024	0.032	0.008		0.031	0.028	0.031	0.031	0.022	0.031	0.028	0.025	0.045	0.048	0.048	0.047	0.044	0.038	0.114	0.123
16	M/OR(1)	0.082	0.085	0.087	0.013	0.016	0.013	0.024	0.018	0.016	0.018	0.013	0.034	0.032	0.021	0.029		0.003	0.005	0.016	0.008	0.016	0.014	0.017	0.042	0.045	0.038	0.031	0.028	0.022	0.105	0.115
17	M/OR(2)	0.079	0.082	0.084	0.010	0.018	0.016	0.021	0.016	0.013	0.016	0.011	0.032	0.029	0.018	0.026	0.003		0.003	0.014	0.005	0.014	0.011	0.014	0.038	0.042	0.035	0.028	0.025	0.019	0.101	0.110
18	M/OR(1)	0.082	0.084	0.087	0.013	0.021	0.018	0.024	0.018	0.016	0.018	0.013	0.029	0.026	0.021	0.029	0.005	0.003		0.016	0.008	0.016	0.014	0.017	0.035	0.038	0.032	0.031	0.028	0.022	0.096	0.105
19	We/K(1)	0.079	0.082	0.084	0.013	0.021	0.018	0.024	0.018	0.016	0.018	0.018	0.034	0.032	0.021	0.029	0.016	0.013	0.016		0.008	0.016	0.014	0.016	0.042	0.045	0.038	0.031	0.028	0.022	0.101	0.110
20	We/M(10), We/K(1)	0.074	0.077	0.079	0.005	0.013	0.011	0.016	0.011	0.008	0.010	0.011	0.026	0.024	0.013	0.021	0.008	0.005	0.008	0.008		0.008	0.005	0.008	0.032	0.035	0.029	0.022	0.019	0.014	0.093	0.101
21	T/WS(1)	0.074	0.077	0.082	0.013	0.021	0.019	0.024	0.019	0.016	0.018	0.018	0.034	0.032	0.021	0.029	0.016	0.013	0.016	0.016	0.008		0.008	0.011	0.035	0.038	0.032	0.025	0.022	0.017	0.093	0.102
22	NE/T(1)	0.069	0.071	0.079	0.010	0.018	0.016	0.021	0.016	0.013	0.016	0.016	0.032	0.029	0.018	0.026	0.013	0.010	0.013	0.013	0.005	0.008		0.003	0.032	0.035	0.029	0.022	0.019	0.014	0.101	0.110
23	NE/T(1)	0.069	0.071	0.079	0.013	0.021	0.018	0.024	0.018	0.016	0.019	0.018	0.034	0.032	0.021	0.024	0.016	0.013	0.016	0.016	0.008	0.011	0.003		0.029	0.032	0.032	0.025	0.022	0.016	0.101	0.110
24	NE/B(1)	0.092	0.095	0.095	0.034	0.032	0.034	0.034	0.029	0.032	0.034	0.034	0.045	0.042	0.037	0.040	0.037	0.034	0.032	0.037	0.029	0.032	0.029	0.026		0.003	0.003	0.042	0.038	0.035	0.116	0.116
25	NE/B(2)	0.095	0.098	0.097	0.037	0.034	0.037	0.037	0.032	0.034	0.037	0.037	0.047	0.045	0.040	0.042	0.040	0.037	0.034	0.040	0.032	0.034	0.032	0.029	0.003		0.005	0.045	0.042	0.038	0.120	0.120
26	NE/B(2)	0.090	0.092	0.092	0.032	0.029	0.032	0.032	0.026	0.029	0.032	0.032	0.042	0.040	0.034	0.042	0.034	0.032	0.029	0.034	0.026	0.029	0.026	0.029	0.003	0.005		0.038	0.035	0.032	0.111	0.111
27	NE/Mt(1)	0.077	0.079	0.084	0.026	0.034	0.032	0.037	0.032	0.029	0.032	0.032	0.045	0.045	0.034	0.042	0.029	0.026	0.029	0.029	0.021	0.024	0.021	0.024	0.037	0.040	0.034		0.003	0.008	0.081	0.085
28	NE/Mo(1)	0.074	0.077	0.082	0.024	0.032	0.029	0.034	0.029	0.026	0.029	0.029	0.042	0.042	0.032	0.040	0.026	0.024	0.026	0.026	0.018	0.021	0.018	0.021	0.034	0.037	0.032	0.003		0.005	0.077	0.081
29	NE/Mo(1)	0.074	0.077	0.076	0.019	0.026	0.024	0.029	0.024	0.021	0.024	0.024	0.037	0.037	0.026	0.034	0.021	0.018	0.021	0.021	0.013	0.016	0.013	0.016	0.032	0.034	0.029	0.008	0.005		0.077	0.085
30	NO/K(2)	0.082	0.084	0.089	0.079	0.082	0.084	0.084	0.079	0.082	0.084	0.084	0.082	0.087	0.084	0.087	0.082	0.079	0.076	0.079	0.074	0.074	0.079	0.079	0.087	0.089	0.084	0.066	0.063	0.063		0.005
31	CO/W(2)	0.087	0.090	0.095	0.084	0.087	0.090	0.090	0.084	0.087	0.090	0.090	0.087	0.092	0.090	0.092	0.087	0.084	0.082	0.084	0.079	0.079	0.084	0.084	0.087	0.089	0.084	0.069	0.066	0.069	0.005	
32	CL/A(1), CL/F(1)	0.084	0.087	0.097	0.082	0.085	0.087	0.087	0.082	0.084	0.087	0.087	0.084	0.090	0.087	0.090	0.085	0.082	0.079	0.082	0.077	0.077	0.082	0.082	0.084	0.087	0.082	0.066	0.063	0.066	0.016	0.016
33	CL/TR(1)	0.087	0.090	0.100	0.084	0.087	0.090	0.090	0.085	0.087	0.090	0.090	0.087	0.092	0.090	0.092	0.087	0.085	0.082	0.085	0.079	0.080	0.084	0.085	0.087	0.090	0.084	0.069	0.066	0.069	0.018	0.018
34	ECo/K(1)	0.085	0.087	0.090	0.087	0.090	0.093	0.093	0.087	0.090	0.093	0.093	0.090	0.095	0.093	0.095	0.090	0.087	0.085	0.087	0.082	0.082	0.082	0.082	0.082	0.085	0.079	0.066	0.064	0.066	0.034	0.034
35	ECo/CR(1)	0.085	0.088	0.090	0.087	0.090	0.093	0.093	0.087	0.090	0.093	0.093	0.090	0.095	0.093	0.095	0.090	0.087	0.085	0.087	0.082	0.082	0.082	0.082	0.082	0.085	0.079	0.066	0.064	0.066	0.034	0.034
36	ECo/D(1), ECo/K(1)	0.082	0.085	0.087	0.085	0.087	0.090	0.090	0.085	0.087	0.090	0.090	0.087	0.093	0.090	0.093	0.087	0.085	0.082	0.085	0.079	0.080	0.079	0.080	0.079	0.082	0.077	0.064	0.061	0.064	0.032	0.032
37	ECo/CW(2)	0.085	0.087	0.100	0.087	0.090	0.093	0.087	0.087	0.090	0.093	0.093	0.090	0.095	0.093	0.095	0.090	0.087	0.085	0.087	0.082	0.082	0.082	0.082	0.079	0.082	0.077	0.066	0.064	0.066	0.045	0.045
38	Ce/L(3)	0.071	0.069	0.082	0.084	0.090	0.087	0.092	0.087	0.084	0.087	0.087	0.087	0.090	0.087	0.090	0.087	0.084	0.082	0.084	0.079	0.085	0.079	0.079	0.084	0.087	0.082	0.074	0.071	0.074	0.053	0.053
39	Ce/L(1)	0.084	0.082	0.092	0.098	0.098	0.100	0.100	0.095	0.098	0.100	0.100	0.100	0.103	0.100	0.103	0.100	0.098	0.095	0.098	0.092	0.092	0.092	0.092	0.092	0.095	0.089	0.084	0.082	0.082	0.050	0.055
40	S/M(1)	0.063	0.066	0.079	0.071	0.076	0.074	0.079	0.074	0.071	0.074	0.074	0.074	0.077	0.074	0.077	0.074	0.071	0.068	0.071	0.066	0.071	0.066	0.066	0.076	0.079	0.074	0.063	0.061	0.061	0.055	0.061
41	S/M(1), S/C(1)	0.058	0.061	0.074	0.071	0.077	0.074	0.079	0.074	0.071	0.074	0.074	0.074	0.077	0.074	0.077	0.074	0.071	0.069	0.071	0.066	0.072	0.066	0.066	0.077	0.079	0.074	0.063	0.061	0.061	0.055	0.061
42	CI/M(2)	0.071	0.074	0.084	0.079	0.084	0.082	0.087	0.082	0.079	0.082	0.082	0.082	0.084	0.082	0.084	0.082	0.079	0.076	0.079	0.074	0.079	0.074	0.074	0.084	0.087	0.082	0.071	0.069	0.069	0.068	0.074

APPENDIX II (continued)

	G. breviceps cont.											*G. cotidianus, G. basalis and G. alpinus* complex										B	*G.coxii*		*G. australis*	
	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	0.108	0.113	0.108	0.108	0.104	0.108	0.087	0.108	0.076	0.068	0.087	0.072	0.057	0.064	0.061	0.061	0.068	0.072	0.079	0.071	0.126	0.190	0.189	0.155	0.160
2	0.112	0.117	0.112	0.112	0.108	0.113	0.083	0.104	0.079	0.071	0.091	0.076	0.061	0.061	0.064	0.064	0.071	0.075	0.075	0.068	0.130	0.195	0.194	0.160	0.165
3	0.131	0.136	0.117	0.117	0.113	0.135	0.104	0.123	0.100	0.091	0.108	0.090	0.067	0.068	0.071	0.078	0.078	0.082	0.075	0.068	0.158	0.217	0.216	0.156	0.152
4	0.105	0.110	0.114	0.115	0.110	0.115	0.110	0.133	0.089	0.089	0.103	0.093	0.084	0.088	0.085	0.073	0.073	0.077	0.077	0.084	0.178	0.178	0.179	0.198	0.203
5	0.109	0.114	0.119	0.119	0.114	0.120	0.119	0.134	0.098	0.098	0.112	0.106	0.097	0.101	0.097	0.085	0.085	0.089	0.089	0.097	0.196	0.186	0.187	0.204	0.209
6	0.114	0.119	0.123	0.124	0.119	0.124	0.114	0.139	0.094	0.094	0.107	0.102	0.092	0.097	0.093	0.081	0.081	0.085	0.085	0.093	0.190	0.179	0.181	0.197	0.202
7	0.114	0.118	0.123	0.124	0.119	0.114	0.124	0.138	0.102	0.102	0.117	0.111	0.101	0.105	0.101	0.089	0.089	0.093	0.093	0.101	0.196	0.184	0.185	0.209	0.221
8	0.105	0.109	0.114	0.114	0.110	0.115	0.115	0.128	0.094	0.094	0.108	0.102	0.092	0.097	0.093	0.081	0.081	0.085	0.085	0.093	0.190	0.180	0.181	0.210	0.216
9	0.109	0.114	0.119	0.119	0.114	0.120	0.110	0.133	0.089	0.089	0.103	0.097	0.088	0.092	0.089	0.076	0.076	0.080	0.080	0.088	0.183	0.173	0.174	0.203	0.209
10	0.114	0.119	0.123	0.124	0.119	0.124	0.114	0.139	0.093	0.094	0.107	0.102	0.092	0.097	0.093	0.080	0.080	0.085	0.085	0.092	0.189	0.179	0.180	0.210	0.216
11	0.114	0.119	0.123	0.124	0.119	0.124	0.114	0.139	0.094	0.094	0.107	0.102	0.092	0.097	0.093	0.081	0.081	0.085	0.085	0.093	0.190	0.179	0.181	0.210	0.216
12	0.110	0.115	0.119	0.120	0.115	0.120	0.115	0.139	0.094	0.094	0.108	0.102	0.101	0.106	0.102	0.089	0.089	0.093	0.093	0.102	0.190	0.205	0.193	0.224	0.230
13	0.118	0.123	0.127	0.128	0.123	0.129	0.118	0.143	0.097	0.098	0.111	0.105	0.105	0.109	0.105	0.093	0.093	0.097	0.097	0.105	0.194	0.210	0.198	0.229	0.235
14	0.113	0.117	0.122	0.123	0.118	0.123	0.113	0.137	0.093	0.093	0.106	0.101	0.091	0.096	0.092	0.080	0.080	0.084	0.084	0.092	0.187	0.184	0.179	0.208	0.213
15	0.118	0.123	0.127	0.128	0.123	0.129	0.118	0.143	0.097	0.098	0.111	0.105	0.096	0.100	0.096	0.084	0.084	0.088	0.088	0.096	0.194	0.191	0.185	0.215	0.221
16	0.110	0.114	0.119	0.120	0.115	0.120	0.114	0.139	0.093	0.094	0.107	0.098	0.088	0.093	0.089	0.077	0.077	0.081	0.081	0.089	0.195	0.195	0.196	0.191	0.197
17	0.105	0.110	0.114	0.115	0.110	0.115	0.110	0.133	0.089	0.089	0.103	0.093	0.084	0.088	0.085	0.073	0.073	0.077	0.077	0.085	0.188	0.189	0.190	0.197	0.203
18	0.100	0.105	0.109	0.110	0.105	0.110	0.105	0.128	0.085	0.085	0.098	0.089	0.088	0.092	0.089	0.077	0.077	0.081	0.081	0.089	0.182	0.195	0.196	0.203	0.209
19	0.105	0.109	0.114	0.115	0.110	0.115	0.110	0.133	0.089	0.089	0.103	0.097	0.088	0.092	0.088	0.076	0.077	0.081	0.081	0.088	0.188	0.194	0.195	0.197	0.203
20	0.097	0.101	0.105	0.106	0.101	0.106	0.101	0.124	0.081	0.081	0.094	0.085	0.076	0.080	0.077	0.065	0.065	0.069	0.069	0.077	0.177	0.178	0.179	0.186	0.191
21	0.097	0.101	0.106	0.106	0.102	0.106	0.109	0.124	0.089	0.089	0.102	0.093	0.084	0.088	0.084	0.073	0.073	0.077	0.077	0.084	0.194	0.184	0.185	0.197	0.202
22	0.105	0.109	0.105	0.105	0.101	0.105	0.100	0.123	0.081	0.081	0.094	0.084	0.076	0.080	0.076	0.065	0.065	0.069	0.069	0.076	0.176	0.176	0.177	0.184	0.189
23	0.105	0.110	0.105	0.105	0.101	0.106	0.101	0.123	0.081	0.081	0.094	0.085	0.076	0.080	0.076	0.065	0.065	0.069	0.069	0.076	0.182	0.183	0.184	0.185	0.190
24	0.111	0.115	0.105	0.105	0.101	0.102	0.110	0.125	0.098	0.098	0.112	0.110	0.106	0.110	0.106	0.093	0.093	0.098	0.102	0.110	0.194	0.191	0.192	0.229	0.235
25	0.115	0.119	0.109	0.109	0.105	0.106	0.114	0.129	0.102	0.102	0.116	0.115	0.110	0.114	0.110	0.097	0.097	0.102	0.106	0.114	0.199	0.196	0.197	0.235	0.241
26	0.106	0.111	0.101	0.101	0.097	0.097	0.105	0.120	0.094	0.094	0.108	0.106	0.101	0.106	0.102	0.089	0.089	0.093	0.097	0.106	0.188	0.185	0.186	0.222	0.228
27	0.081	0.085	0.080	0.081	0.077	0.081	0.092	0.109	0.076	0.076	0.089	0.080	0.072	0.075	0.072	0.061	0.061	0.065	0.065	0.072	0.169	0.165	0.166	0.187	0.193
28	0.077	0.081	0.077	0.077	0.073	0.077	0.088	0.105	0.073	0.073	0.085	0.076	0.068	0.072	0.068	0.058	0.058	0.061	0.061	0.068	0.164	0.160	0.161	0.182	0.187
29	0.081	0.085	0.080	0.081	0.077	0.081	0.091	0.105	0.072	0.073	0.085	0.076	0.068	0.072	0.068	0.057	0.058	0.061	0.061	0.068	0.169	0.165	0.166	0.171	0.176
30	0.017	0.020	0.038	0.038	0.035	0.052	0.062	0.058	0.065	0.065	0.084	0.077	0.073	0.084	0.080	0.077	0.084	0.089	0.085	0.077	0.139	0.195	0.184	0.207	0.207
31	0.017	0.020	0.038	0.038	0.035	0.052	0.061	0.066	0.072	0.073	0.093	0.085	0.081	0.093	0.089	0.085	0.093	0.097	0.093	0.085	0.139	0.195	0.183	0.221	0.221
32		0.003	0.045	0.045	0.042	0.045	0.044	0.048	0.054	0.055	0.073	0.085	0.081	0.093	0.089	0.085	0.093	0.097	0.097	0.089	0.144	0.177	0.166	0.221	0.215
33	0.003		0.045	0.045	0.045	0.045	0.048	0.051	0.058	0.058	0.077	0.089	0.085	0.097	0.093	0.089	0.097	0.102	0.102	0.093	0.149	0.183	0.172	0.228	0.222
34	0.040	0.040		0.003	0.003	0.019	0.075	0.088	0.079	0.076	0.100	0.076	0.080	0.091	0.088	0.076	0.092	0.096	0.088	0.080	0.134	0.180	0.169	0.218	0.218
35	0.040	0.040	0.003		0.005	0.022	0.076	0.088	0.080	0.076	0.101	0.076	0.080	0.092	0.088	0.076	0.092	0.096	0.089	0.081	0.134	0.186	0.175	0.219	0.219
36	0.037	0.040	0.003	0.005		0.022	0.072	0.084	0.076	0.072	0.096	0.072	0.076	0.088	0.084	0.072	0.088	0.092	0.084	0.077	0.130	0.175	0.164	0.213	0.213
37	0.040	0.040	0.018	0.021	0.021		0.072	0.085	0.076	0.073	0.089	0.085	0.089	0.093	0.089	0.077	0.093	0.097	0.101	0.093	0.150	0.179	0.168	0.219	0.231
38	0.040	0.042	0.064	0.064	0.061	0.061		0.025	0.019	0.019	0.034	0.076	0.069	0.073	0.076	0.073	0.080	0.084	0.080	0.073	0.121	0.172	0.161	0.182	0.177
39	0.042	0.045	0.071	0.071	0.069	0.069	0.024		0.041	0.041	0.058	0.097	0.088	0.093	0.097	0.093	0.101	0.105	0.101	0.093	0.141	0.212	0.200	0.213	0.207
40	0.047	0.050	0.066	0.066	0.064	0.064	0.018	0.037		0.005	0.014	0.065	0.058	0.068	0.065	0.061	0.068	0.072	0.076	0.068	0.136	0.183	0.172	0.178	0.172
41	0.048	0.050	0.064	0.064	0.061	0.061	0.018	0.037	0.005		0.014	0.065	0.058	0.068	0.065	0.061	0.068	0.072	0.076	0.068	0.136	0.184	0.173	0.166	0.161
42	0.061	0.063	0.079	0.079	0.077	0.071	0.032	0.050	0.013	0.013		0.069	0.068	0.072	0.069	0.065	0.072	0.076	0.080	0.072	0.162	0.203	0.192	0.184	0.179

APPENDIX II (continued)

		G. huttoni	A	*G. breviceps*																												
	LOCATION*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
43	N/K(1)	0.061	0.063	0.074	0.074	0.082	0.079	0.084	0.079	0.076	0.079	0.079	0.079	0.082	0.079	0.082	0.076	0.074	0.071	0.076	0.068	0.074	0.068	0.069	0.084	0.087	0.082	0.066	0.063	0.063	0.063	0.068
44	N/WS(1), W/MS(1)	0.050	0.053	0.058	0.068	0.076	0.074	0.079	0.074	0.071	0.074	0.074	0.079	0.082	0.074	0.076	0.071	0.069	0.071	0.071	0.063	0.069	0.063	0.063	0.082	0.084	0.079	0.061	0.058	0.058	0.061	0.066
45	WC/CR(1)	0.055	0.053	0.058	0.071	0.079	0.076	0.082	0.076	0.074	0.076	0.076	0.082	0.084	0.076	0.079	0.074	0.071	0.074	0.074	0.066	0.071	0.066	0.066	0.084	0.087	0.082	0.063	0.061	0.061	0.068	0.074
46	WC/LB(1), ECo/C(1)	0.053	0.055	0.061	0.068	0.076	0.074	0.079	0.074	0.071	0.074	0.074	0.079	0.082	0.074	0.076	0.071	0.069	0.071	0.071	0.063	0.069	0.063	0.063	0.082	0.084	0.079	0.061	0.058	0.058	0.066	0.071
47	M/TS(1), Wa/KS(3)	0.053	0.055	0.066	0.060	0.069	0.066	0.071	0.066	0.063	0.066	0.066	0.071	0.074	0.066	0.069	0.063	0.061	0.063	0.063	0.055	0.061	0.055	0.055	0.074	0.076	0.071	0.053	0.050	0.050	0.063	0.068
48	M/N(8)	0.058	0.061	0.066	0.060	0.068	0.066	0.071	0.066	0.063	0.066	0.066	0.071	0.074	0.066	0.069	0.063	0.061	0.063	0.063	0.055	0.061	0.055	0.055	0.074	0.076	0.071	0.053	0.050	0.050	0.068	0.074
49	M/N(1)	0.061	0.063	0.068	0.063	0.071	0.069	0.074	0.069	0.066	0.068	0.068	0.074	0.076	0.069	0.071	0.066	0.063	0.066	0.066	0.058	0.063	0.058	0.058	0.076	0.079	0.074	0.055	0.053	0.053	0.071	0.076
50	Wa/KS(1)	0.066	0.063	0.063	0.063	0.071	0.069	0.074	0.069	0.066	0.068	0.069	0.074	0.077	0.069	0.071	0.066	0.063	0.066	0.066	0.058	0.063	0.058	0.058	0.079	0.082	0.076	0.055	0.053	0.053	0.068	0.074
51	Wa/KS(7)	0.061	0.058	0.058	0.068	0.076	0.074	0.079	0.074	0.071	0.074	0.074	0.079	0.082	0.074	0.077	0.071	0.069	0.071	0.071	0.063	0.069	0.063	0.063	0.084	0.087	0.082	0.061	0.058	0.058	0.063	0.068
52	N/SS(1)	0.092	0.095	0.111	0.119	0.127	0.125	0.127	0.125	0.122	0.124	0.124	0.125	0.127	0.124	0.127	0.127	0.124	0.122	0.124	0.119	0.127	0.119	0.122	0.127	0.130	0.124	0.116	0.114	0.116	0.100	0.100
53	*G. coxii* (2)	0.127	0.129	0.139	0.121	0.124	0.121	0.124	0.121	0.119	0.121	0.121	0.132	0.134	0.124	0.127	0.129	0.126	0.129	0.129	0.121	0.124	0.121	0.124	0.126	0.129	0.124	0.116	0.113	0.116	0.126	0.126
54	*G. coxii* (1)	0.127	0.129	0.139	0.121	0.124	0.121	0.124	0.121	0.118	0.121	0.121	0.126	0.129	0.121	0.124	0.129	0.126	0.129	0.129	0.121	0.124	0.121	0.124	0.126	0.129	0.124	0.116	0.113	0.116	0.121	0.121
55	*G. australis* (1)	0.108	0.111	0.110	0.127	0.129	0.127	0.132	0.132	0.129	0.132	0.132	0.137	0.140	0.132	0.135	0.124	0.127	0.129	0.127	0.121	0.127	0.121	0.121	0.140	0.142	0.137	0.124	0.121	0.116	0.132	0.137
56	*G. australis* (1)	0.111	0.113	0.108	0.129	0.132	0.129	0.137	0.135	0.132	0.135	0.135	0.140	0.143	0.135	0.137	0.127	0.129	0.132	0.129	0.124	0.130	0.124	0.124	0.142	0.145	0.140	0.127	0.124	0.119	0.132	0.137

APPENDIX II (continued)

	G. breviceps cont.											*G. cotidianus, G. basalis and G. alpinus* complex									B	*G.coxii*		*G. australis*	
	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
43	0.069	0.071	0.063	0.063	0.061	0.069	0.063	0.076	0.055	0.055	0.058		0.019	0.016	0.013	0.016	0.022	0.025	0.028	0.022	0.116	0.145	0.139	0.131	0.140
44	0.066	0.069	0.066	0.066	0.063	0.071	0.058	0.071	0.050	0.050	0.058	0.018		0.008	0.005	0.008	0.008	0.011	0.025	0.019	0.107	0.158	0.148	0.133	0.143
45	0.074	0.076	0.074	0.074	0.071	0.074	0.061	0.074	0.058	0.058	0.061	0.016	0.008		0.003	0.011	0.011	0.014	0.016	0.011	0.116	0.159	0.153	0.134	0.143
46	0.071	0.074	0.071	0.071	0.069	0.071	0.063	0.076	0.055	0.055	0.058	0.013	0.005	0.003		0.008	0.008	0.011	0.019	0.013	0.111	0.154	0.148	0.130	0.139
47	0.068	0.071	0.063	0.063	0.061	0.063	0.060	0.074	0.053	0.053	0.055	0.016	0.008	0.011	0.008		0.011	0.013	0.028	0.022	0.116	0.159	0.148	0.134	0.143
48	0.074	0.076	0.074	0.074	0.071	0.074	0.066	0.079	0.058	0.058	0.060	0.021	0.008	0.011	0.008	0.010		0.003	0.016	0.022	0.120	0.164	0.153	0.139	0.148
49	0.076	0.079	0.077	0.077	0.074	0.077	0.068	0.082	0.060	0.061	0.063	0.024	0.011	0.013	0.011	0.013	0.003		0.019	0.025	0.125	0.163	0.153	0.134	0.143
50	0.076	0.079	0.071	0.071	0.069	0.079	0.066	0.079	0.063	0.063	0.066	0.026	0.024	0.016	0.018	0.026	0.016	0.018		0.005	0.130	0.170	0.164	0.144	0.144
51	0.071	0.074	0.066	0.066	0.063	0.074	0.060	0.074	0.058	0.058	0.060	0.021	0.018	0.011	0.013	0.021	0.021	0.024	0.005		0.120	0.159	0.153	0.134	0.134
52	0.103	0.106	0.098	0.098	0.096	0.106	0.092	0.103	0.100	0.100	0.114	0.087	0.082	0.087	0.084	0.087	0.090	0.092	0.095	0.090		0.169	0.168	0.195	0.195
53	0.119	0.121	0.121	0.124	0.119	0.121	0.118	0.134	0.124	0.124	0.132	0.103	0.111	0.111	0.108	0.111	0.113	0.113	0.116	0.110	0.116		0.011	0.188	0.177
54	0.113	0.116	0.116	0.119	0.113	0.116	0.113	0.129	0.118	0.119	0.126	0.100	0.105	0.108	0.105	0.105	0.108	0.108	0.113	0.108	0.116	0.010		0.187	0.177
55	0.137	0.140	0.138	0.138	0.135	0.138	0.121	0.134	0.118	0.114	0.121	0.095	0.097	0.097	0.095	0.097	0.100	0.098	0.103	0.097	0.126	0.126	0.126		0.008
56	0.135	0.137	0.138	0.138	0.135	0.143	0.119	0.132	0.116	0.111	0.118	0.100	0.103	0.103	0.100	0.103	0.105	0.103	0.103	0.097	0.126	0.121	0.121	0.008	

* A – M/T(9), M/TS(1), Wa/KS(9), Wa/M(1)
B – M/K(11), M/TS(7), M/N(1), Wa/K(10), Wa/H(4), Wa/WR(1), We/ES(2), WC/F(2)

APPENDIX III. Uncorrected genetic distances based on sequence variation in 21 mtDNA sequences for *G. basalis*. Location codes refer to those used in Figure 1.

	LOCATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1	Ngt10																					
2	Ngt1	0.000																				
3	Ngt2	0.001	0.001																			
4	Ngt3	0.000	0.000	0.001																		
5	Ngt5	0.001	0.001	0.003	0.001																	
6	Ngt6	0.000	0.000	0.001	0.000	0.001																
7	Ngt7	0.000	0.000	0.001	0.000	0.001	0.000															
8	Ngt8	0.000	0.000	0.001	0.000	0.001	0.000	0.000														
9	Ngt9	0.001	0.001	0.003	0.001	0.003	0.001	0.001	0.001													
10	Tur1	0.006	0.006	0.008	0.006	0.008	0.006	0.006	0.006	0.008												
11	Kop12	0.013	0.013	0.014	0.013	0.014	0.013	0.013	0.013	0.014	0.014											
12	Kop15	0.006	0.006	0.008	0.006	0.008	0.006	0.006	0.006	0.008	0.000	0.014										
13	Kop18	0.013	0.013	0.014	0.013	0.014	0.013	0.013	0.013	0.014	0.014	0.000	0.014									
14	Kop1	0.014	0.014	0.015	0.014	0.015	0.014	0.014	0.014	0.015	0.015	0.001	0.015	0.001								
15	Kop2	0.010	0.010	0.011	0.010	0.011	0.010	0.010	0.010	0.011	0.016	0.003	0.016	0.003	0.004							
16	Kop4	0.006	0.006	0.008	0.006	0.008	0.006	0.006	0.006	0.008	0.000	0.014	0.000	0.014	0.015	0.016						
17	Kop5	0.013	0.013	0.014	0.013	0.014	0.013	0.013	0.013	0.014	0.014	0.000	0.014	0.000	0.001	0.003	0.014					
18	Kop6	0.006	0.006	0.008	0.006	0.008	0.006	0.006	0.006	0.008	0.000	0.014	0.000	0.014	0.015	0.016	0.000	0.014				
19	Kop7	0.013	0.013	0.014	0.013	0.014	0.013	0.013	0.013	0.014	0.014	0.000	0.014	0.000	0.001	0.003	0.014	0.000	0.014			
20	Kop8	0.013	0.013	0.014	0.013	0.014	0.013	0.013	0.013	0.014	0.014	0.000	0.014	0.000	0.001	0.003	0.014	0.000	0.014	0.000		
21	Kop9	0.013	0.013	0.014	0.013	0.014	0.013	0.013	0.013	0.014	0.014	0.000	0.014	0.000	0.001	0.003	0.014	0.000	0.014	0.000	0.000	

APPENDIX IV. Uncorrected genetic distance based on sequence variation in the 57 mtDNA sequences for *G. breviceps*. Location codes refer to those used in Figure 1.

	LOCATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
1	Tok1																																																								
2	Tok2	0.000																																																								
3	Tok3	0.000	0.000																																																						
4	Tok5	0.000	0.000	0.000																																																					
5	Tok6	0.000	0.000	0.000	0.000																																																					
6	Tok7	0.000	0.000	0.000	0.000	0.000																																																			
7	Tok8	0.000	0.000	0.000	0.000	0.000	0.000																																																		
8	Tok9	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																																	
9	Kah10	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001																																															
10	Kah1	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000																																														
11	Kah2	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000																																												
12	Kah3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000																																											
13	Kah4	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000																																										
14	Kah5	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000																																									
15	Kah6	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000																																									
16	Kah7	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																									
17	Kah8	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																								
18	Kah9	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																								
19	Ngu4	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																								
20	Mak10	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006																																							
21	Mak1	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000																																						
22	Mak2	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000																																					
23	Mak3	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.001	0.001	0.001																																					
24	Mak4	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000	0.000	0.001																																			
25	Mak5	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000	0.000	0.001	0.000																																			
26	Mak6	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000	0.000	0.001	0.000	0.000																																			
27	Mak7	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000	0.000	0.001	0.000	0.000	0.000																																			
28	Mak8	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000																																			
29	Mak9	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000																																			
30	Tur10	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006																																			
31	Tur2	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.008	0.008	0.008	0.009	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.001																																			
32	Tur3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001																																			
33	Tur4	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000																																			
34	Tur5	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000																																			
35	Tur6	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000																																			
36	Tur7	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000																																			
37	Tur8	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000																																			
38	Tur9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.005	0.005	0.005	0.006	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001																																			
39	Kop10	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001																																			
40	Kop11	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000																																			
41	Kop13	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000																																			
42	Kop14	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000																																			
43	Kop16	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000																																			
44	Kop17	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000																																			
45	Kop19	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000																																			
46	Kop20	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																			
47	Kop3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																																			
48	Kiz10	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003																																			
49	Kiz1	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.008	0.008	0.008	0.009	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.001																																			
50	Kiz2	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001																																			
51	Kiz3	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000																																			
52	Kiz4	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.008	0.008	0.008	0.009	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.001	0.000	0.001	0.001																																			
53	Kiz5	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001																																			
54	Kiz6	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.008	0.008	0.008	0.009	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.001	0.000	0.001	0.001	0.000	0.001																																			
55	Kiz7	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.001																																			
56	Kiz8	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.000																																			
57	Kiz9	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.006	0.006	0.008	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.000	0.000																																			

APPENDIX IV. Cont.

	LOCATION	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
1	Tok1																															
2	Tok2																															
3	Tok3																															
4	Tok5																															
5	Tok6																															
6	Tok7																															
7	Tok8																															
8	Tok9																															
9	Kah10																															
10	Kah1																															
11	Kah2																															
12	Kah3																															
13	Kah4																															
14	Kah5																															
15	Kah6																															
16	Kah7																															
17	Kah8																															
18	Kah9																															
19	Ngt4																															
20	Mak10																															
21	Mak1																															
22	Mak2																															
23	Mak3																															
24	Mak4																															
25	Mak5																															
26	Mak6																															
27	Mak7																															
28	Mak8	0.000																														
29	Mak9	0.000	0.000																													
30	Tur10	0.006	0.006	0.006																												
31	Tur2	0.008	0.008	0.008	0.001																											
32	Tur3	0.006	0.006	0.006	0.000	0.001																										
33	Tur4	0.006	0.006	0.006	0.000	0.001	0.000																									
34	Tur5	0.006	0.006	0.006	0.000	0.001	0.000	0.000																								
35	Tur6	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000																							
36	Tur7	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000																						
37	Tur8	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000																					
38	Tur9	0.005	0.005	0.005	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001																				
39	Kop10	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001																			
40	Kop11	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000																		
41	Kop13	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000																	
42	Kop14	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000																
43	Kop16	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000															
44	Kop17	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000														
45	Kop19	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000													
46	Kop20	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000												
47	Kop3	0.004	0.004	0.004	0.003	0.004	0.003	0.003	0.003	0.003	0.003	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000											
48	Kir10	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003										
49	Kir1	0.008	0.008	0.008	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.001									
50	Kir2	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001								
51	Kir3	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000							
52	Kir4	0.008	0.008	0.008	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.001	0.000	0.001	0.001						
53	Kir5	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001					
54	Kir6	0.008	0.008	0.008	0.001	0.003	0.001	0.001	0.001	0.001	0.001	0.001	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.001	0.000	0.001	0.001	0.000	0.001				
55	Kir7	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.001			
56	Kir8	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.000		
57	Kir9	0.006	0.006	0.006	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.000	0.001	0.000	0.000	0.001	0.000	0.001	0.000	0.000	