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EXECUTIVE SUMMARY

Lavery, S.; Clements, K.; Hickey, A. (2006). Molecular identification of cryptogenic/invasive gobies in New Zealand. ***New Zealand Aquatic Environment and Biodiversity Report No. 5.*** **48 p.**

Molecular species description, characterisation and discrimination
DNA sequences were determined for four species of gobies recorded from New Zealand waters: *Acentrogobius bifrenatus*, *A. pflaumii*, *Favonigobius exquisitus* and the endemic *F. lentiginosus*. The first two are considered invasive, and the third is considered to have colonised New Zealand from Australia through natural mechanisms of dispersal. Three mitochondrial sequence fragments (from the 16s rRNA gene, the COI gene, and the mitochondrial control region) have been used to investigate the molecular distinctions between these species. All four species can be easily and unambiguously distinguished from each other using these gene sequences.

Molecular species identification
Our results highlighted two categories of problems with these gobies: (a) taxonomic confusion, i.e uncertainty over the correct name for a species, and (b) misidentification of specimens, i.e an error in the labelling of a specimen. We appear to have resolved the second of these issues for the specimens we sequenced. However, the first issue indicates the need for taxonomic revision in light of our results.

DNA sequences from New Zealand specimens of *Acentrogobius bifrenatus*, *A. pflaumii*, and *F. exquisitus* closely match those from conspecific Australian specimens. *Acentrogobius pflaumii* collected in New Zealand and Australian waters most closely match one of the two forms of this species found in its native distribution in the Northwest Pacific. However, a number of specimens of "*A. pflaumii*" collected in New Zealand waters were molecularly identified as *F. exquisitus*.

DNA sequences from New Zealand specimens of *F. lentiginosus* are very similar to that from one specimen of "*Favonigobius tamarensis*" collected in Sydney Harbour. This may be a misidentified specimen of *F. lateralis* (east coast form), which has been recently considered to be conspecific to *F. lentiginosus*. This may indicate that *F. lentiginosus* is conspecific to populations that also occur in Australian waters. *F. lateralis* (south coast form) from Australia appears to be a closely related sister species of *F. lentiginosus*.

Patterns of colonisation determined from molecular diversity
Acentrogobius pflaumii appears to exhibit a classic pattern of recent colonisation, as mitochondrial diversity declines progressively from Tokyo (haplotype diversity, H = 1.0) to Sydney (H = 0.78) to New Zealand (H = 0.57). This suggests a progressive settlement of adventive *A. pflaumi*. Within New Zealand, Waitemata specimens (H = 0.57) had greater diversity than those from Whangapoua (H = 0.0). Additional evidence suggests a colonisation from Tokyo to Sydney and New Zealand, as one common mitochondrial haplotype is found in all three locations. Sydney is implicated as the source of New Zealand's *A. pflaumii* by the presence of two shared haplotypes. Whangapoua's single haplotype is one of four found in the Waitemata, suggesting a spreading colonisation within New Zealand rather than an independent colonisation from overseas. These preliminary conclusions require further sampling for confirmation.

Acentrogobius bifrenatus in New Zealand waters appears to have similar molecular diversity to conspecific populations in New South Wales and South Australia. This suggests that this species has been present in New Zealand waters for a considerable period of time, has resulted from multiple introductions, or that the founding population was large and diverse. Although the New Zealand population is most similar to the Sydney population, no haplotype is shared between New Zealand,

New South Wales, and South Australia. However, all New Zealand locations sampled share haplotypes. These preliminary conclusions require further sampling for confirmation.

Favonigobius exquisitus in New Zealand waters has a slightly lower diversity (H = 0.90) than that found in New South Wales (H = 1.0), but current samples do not share any haplotypes across the Tasman. There is no apparent strong population structure in this species within New Zealand waters.

Favonigobius lentiginosus was believed to be an endemic species, but our data now suggest it is very closely related, if not conspecific, to a form found in Sydney at least. Australian specimens have been difficult to acquire, and are required for more comprehensive comparison with New Zealand *F. lentiginosus*. In New Zealand waters, this species does not appear to have any strong population structure.

Rapid molecular identification of invasive or cryptogenic gobioid and blennioid fish

Molecular criteria (both DNA sequences and RFLP patterns) have been established to rapidly identify all four native or introduced species that form the focus of this study. In addition, such criteria have also been established for all other native marine goby species, plus a series of other gobioid and blennioid fish from the Australasian region that are potential invasives. These include *Thalasseleotris* sp. (no common name – an endemic eleotrid), *Gobiopsis atrata* (black goby – endemic), *Parachaeturichthys polynema* (tail-eyed goby – wide distribution in Indo-West Pacific), *Oxyurichthys tentacularis* (no common name – wide distribution in Indo-West Pacific), *Myersina filifer* (no common name – wide distribution in Indo-West Pacific) and *Acentrogobius caninus* (tropical sand goby – wide distribution in Indo-West Pacific). This will permit rapid identification of any life stage (larvae, juvenile, or adult) or sex of any of these potentially invasive species, and discrimination from natives.

1. INTRODUCTION

1.1 Invasive marine fishes

The global impact and threat of invasive alien marine species has grown dramatically in recent decades. With an estimated 10 000 species in transit around the world in ballast water, invasive species can threaten biodiversity, marine industries (including fishing and tourism), and even human health (Bax et al. 2003). The appearance of adventitious marine species globally has resulted in some documented cases of significantly affected endemic species distributions and ecosystems (Carlton & Geller 1993). The Pacific region is no exception, with numerous invertebrates and fish now listed as invasive species (Lockett & Gomon 2001). In Australian waters, invasive invertebrate species, such as the North Pacific seastar (*Asterias amurensis*) and the European fan-worm (*Sabella spallanzannii*) have had a significant impact on endemics and ecosystems (Furlani 1996). Several small invasive benthic fishes (members of the families Blenniidae, Eleotridae, and Gobiidae) have also been recorded (Furlani 1996). The most notable group of adventives have been gobies, and in particular *Acentrogobius pflaumii*, which is assumed to have an East Asian origin (Lockett & Gomon 2001, Francis et al. 2003). Gobies have invaded New Zealand waters, and at least two species have colonised via ships (Willis et al. 1999; Francis et al. 2003).

The most important unintentional mechanism for introduction of invasive marine species is through ballast water and sea chests of commercial vessels (Wonham et al. 2000). Water taken on board in one port and discharged in another provides a mechanism for small planktonic organisms to be transported over large distances. Transport by ship is the only possible vector for many fish invasions. Members of Gobiidae and Blenniidae appear to dominate ballast water mediated introductions, and account for over half of the marine fish introductions reported worldwide (Wonham et al. 2000). As gobies and blennies naturally occur in and around ports (e.g on wharf piles, in bottom debris), spawn in crevices, and have extended pelagic larval phases, these fishes are predisposed to ballast intakes and can survive lengthy transport periods to similar habitats at distant locations. As these small fishes are common to the Indo-Pacific region, they pose an obvious biosecurity threat to New Zealand.

At least two goby species have been introduced to New Zealand marine waters. Willis et al. (1999) reported the Australian bridled goby, *Acentrogobius bifrenatus*, from the Hauraki Gulf and the Waitemata Harbour. Francis et al. (2003) showed that this species is more widespread in northeastern New Zealand than first thought and appears well established. They also reported the presence of the Asian goby, *Acentrogobius pflaumii*, from the Waitemata and Whangapoua Harbours. These two invasive populations have most likely been transported in ship water collected in Australian ports, where *A. bifrenatus* occurs naturally and *A. pflaumii* has been introduced (Francis et al. 2003). A third species (not marine), the dart goby (*Parioglossus marginalis*), was recorded by McDowall (2001) in wetlands on Great Barrier Island and at Tom Bowling Bay, North Cape, in 2000. To complicate matters, there has been considerable taxonomic confusion over the two *Favonigobius* species that are found in New Zealand. The names of these two species have changed several times (e.g *F. exquisitus* was formerly known as *A. lentiginosus*, and *F. lentiginosus* as *F. lateralis*), and the relationship of *F. lentiginosus* to the Australian species *F. lateralis* is uncertain. It should also be noted that three species of triplefin fishes (F. Tripterygiidae) have been reciprocally introduced to Australian ports from New Zealand (Clements et al. 2000). Two of these species (*Grahamina gymnota* and *Forsterygion varium*) were probably transported with shipments of live oysters in the early 20th century (Clements et al. 2000, Hickey et al. 2004), but *F. lapillum* is thought to have been introduced via ballast water or sea chests (Lockett & Gomon 2001). New Zealand and Australia share similar environmental conditions and significant international shipping trade, thereby increasing the chance of transport of marine adventive species between them. Given the history of trans-Tasman fish introductions, it seems likely that more are possible, if not probable.

Identification of adventive species demands an accurate taxonomy. Historically, identifications have relied on morphology for taxonomic classification, which is practical, rapid, and cost-effective. However, morphological taxonomy can be problematic, particularly when specimens vary through life

history, have been damaged, or have degraded, or existing taxonomies are absent or confused. While the adults of most of the target fish species are identifiable from external morphology by experts (if all possible specimens are on hand), field keys do not contain descriptions of all possible invasive species, and larvae are extremely hard to identify to species. With gobioid and blennioid fishes (blennies and triplefins are both blennioid fishes), larvae of exotic species may be almost impossible to distinguish from larvae of endemic species. Since larvae of these species are easily transported by ship, identification of potential invasive species may be extremely difficult. Furthermore, if adventive species are reported at multiple locations (for example Japanese, Australian, and New Zealand ports), morphological data can not provide adequate information to elucidate the origins of adventives and transport routes. Such information would be important to determine which transport routes, modes, or potentially vessels, require the most control and management.

Genetic techniques can resolve some of these problems, providing suitable loci and a reference database are available. DNA sequence data generated using the polymerase chain reaction (PCR) and cycle sequencing has been used for forensic detection of endangered or exploited marine fauna (Baker et al. 1996, Smith & McVeagh 2000) and monitoring marine biodiversity (Feral 2002). The same molecular techniques can be applied to detect invasive species, and potentially determine the origins and therefore direction of transport (Dawson et al. 2001). Reference gene sequences provide an extremely valuable resource for identifying larval gobioid and blennioid fishes (Lindstrom 1999) and, potentially, for identifying source populations of invasive species. For example, our previous molecular work on New Zealand triplefin fishes allowed us to identify to species 84 triplefin larvae collected from light traps in the Hauraki Gulf (Hickey et al. unpublished results). Similarly, we were able to use gene sequences to show that what was thought to be the Tasmanian endemic triplefin *G. gymnota* was in fact an introduced New Zealand species (Hickey et al. 2004).

In this project we proposed to extend our previous work on triplefins by adding sequences from known invasive species (e.g *A. bifrenatus* and *A. pflaumii*) and from potential invasive gobioid and blennioid species in the Australasian region to our reference database. We focused on gobioids and blennioids because they are the major problem in fish introductions worldwide, and have been involved in trans-Tasman introductions in the past.

For gobies and blennies, it is very difficult to specify or quantify the nature of the problem facing New Zealand due to the inability to quickly and easily differentiate them (Francis et al. 2003). Potential problems are easily envisaged, given the serious impacts observed elsewhere. Some introductions have profoundly affected the community structure of coastal ecosystems (Baltz 1991). *Tridentiger bifasciatus*, a goby from Japan and China that was discovered in San Francisco Bay in 1985, was found to prey on and compete with the native goby *Eucyclogobius newberryi* (Matern & Fleming 1995). The spread of the introduced Japanese goby *Acanthogobius flavimanus* in the same waters has been described as "explosive" (Brittan et al. 1970). *Acentrogobius pflaumii* has become abundant and widely distributed in Port Philip Bay and elsewhere in Australia in less than a decade (Lockett & Gomon 1999, 2001). Environmental conditions in New Zealand estuaries are presumably conducive to the expansion of the range of *A. pflaumii*. The possible ecological impact of *A. bifrenatus*, which is more widespread than previously thought, is also unknown. Studies on the ecology of this species in New Zealand are under way, and should shed light on its ecological role (T. Willis, University of Auckland, pers. comm.). Both *A. pflaumii* and *A. bifrenatus* overlap in range and habitat with two other New Zealand gobies, *Favonigobius lentiginosus* and *F. exquisitus* (McKenzie 1984, Francis et al. 2003). The first of these is currently considered to be endemic, although it has been suggested that it is conspecific to (and the senior synonym of) the Australian *F. lateralis* east coast form (D. Hoese, Australian Museum, pers. comm.). *Favonigobius exquisitus* also occurs in Australia (Kuiter 1993), but is thought on the basis of historical distribution records to have arrived in New Zealand by natural dispersal. Both introduced species grow substantially larger than the two native species.

In summary, although the specific problems are difficult to predict, there is substantial anecdotal evidence to suggest that introduced gobies and blennies represent a substantial biosecurity threat to New Zealand's marine fish biodiversity.

1.2 Molecular and analytical approaches

There are three main requirements for molecular approaches to assist in detecting invasive species. First, the native fauna of the region must be known and sampled, providing a baseline against which potentially invasive species can be compared. Second, samples of the potential invasive species (or its DNA sequences) must be available from its native distribution. Third, a genetic locus must be available for analysis that exhibits sufficient variation to enable clear discrimination between native and invasive species. In general, molecular techniques are required for species identification only when there is strong morphological similarity. However, the geographic origins of an invasive species can often be investigated only through molecular methods. Additional requirements to achieve this are sufficient population-level divergence in DNA sequences, as well as, of course, adequate sampling of the original distribution.

In general, the molecular identification of cryptogenic or invasive species can be undertaken at two fundamentally different levels. At the most basic level, one can determine the likely species identification of an unknown by sequencing one to a few individuals of the potential invader, one to a few individuals of the morphologically similar natives, and one to a few individuals from the possible invader's original distribution. Then, simply, if the unknown samples are far more similar to the offshore species than the natives, then they are likely to belong to an invasive species. But, usually this is only a crude beginning to answering the question, as the species identification is not definitive (only likely, at an indeterminate probability level), and it does not take into account two important sources of variability. One source is the possibly extreme geographic variation within each species, and the other is the set of alternative possible related species that are unsampled. This is a substantial problem in the accurate identification of marine species. Therefore, in order to adequately identify marine invasive species, there is a need to undertake molecular work from a more substantial sampling base, that is, from different geographic regions within a species' known distribution, and from all the possible alternative (related) species. However, once this more thorough strategy has been undertaken, the data are of far more use than simply being able to answer the simple question "Is this one sample more likely to belong to species X or species Y?". Apart from answering this question far more definitively with the additional data, we can also answer questions such as: 1) what are the likely locations of origin of the invaders? (and thus the likely danger routes for the future), 2) how long has the invader been present?, 3) how many separate invasions have taken place?, and 4) has there been secondary transport of the invader to increase its new range, or is it simply natural expansion?

The present project covered both these options in the analysis of invasive / cryptogenic species in this group. The first, simple and basic strategy is covered under objective one. The more thorough extension to this strategy is covered under objective two. Objective three is common to both strategies.

1.3 Molecular techniques

For over 30 years, molecular markers have proven to be highly valuable in discriminating and identifying species. Firstly allozyme loci, then DNA RFLP (restriction fragment length polymorphism) markers, and more recently DNA sequences have revealed that genetic diversity among species is usually much greater than that within species, thus making it feasible to correctly distinguish among species using such markers. With the aid of reference individuals, species can therefore also be correctly identified using such techniques. The main qualifying factor has been that sufficient genetic variation must be screened before confident identification to species can be achieved. In the same way that not all species can be identified using a limited set of morphological characters, sufficient variable genetic characters must also be screened to allow species discrimination. Also, it is clear that species cannot be confidently identified using molecular markers until one or more reference specimens have been typed.

Particularly in marine organisms, molecular markers have proven to be invaluable additions to the taxonomist's tools, as they have frequently permitted the identification of "cryptic" species. That is,

molecular markers have often revealed species-level distinctions between otherwise identical or nearly identical morphological forms (e.g., Knowlton 2000). Of course, it can be problematic to allocate species status on the basis of genetic criteria alone, but if the different forms also inhabit the same locations, this is strong evidence that interbreeding does not occur. Such cryptic species are relatively common among marine organisms, particularly invertebrates, perhaps because of strong selective forces maintaining conservative morphological body forms.

There is now a very wide range of molecular methods available that can be used to screen genetic variation at either the protein or DNA level. They all have their advantages and disadvantages, depending on the exact nature of the questions, the available expertise and equipment, and the need for wide comparison with other laboratories' results. Some of the most crucial aspects of this project were firstly to use the highest level of discriminatory power for any given locus, secondly to be able to use the genetic data already available on related marine species, and thirdly to make our data available to future workers in the most usable form. These criteria strongly weighed in favour of collecting DNA sequence data.

There are a large number of possible genetic loci from which to choose, for use in such a project as this. However, there were many criteria that quickly whittled down the number of feasible candidates. We wished to amplify and sequence species-specific loci from diverse taxa, and compare them, where possible, to previous reference sequences. As such, we required robust PCR amplifications of relatively commonly used loci exhibiting either high among-species variability or high within-species variability, preferably using a limited number of PCR primers that would work on a variety of taxa. We also aimed, if possible, to collect data from both mitochondrial (mt) and nuclear (n) DNA loci, which aids in species discrimination. To meet these criteria, we trialled a number of loci, including mitochondrial 12s and 16s ribosomal RNA, cytochrome oxidase I (COI) and cytochrome b (cytB) genes, and nuclear ribosomal (external transcribed spacer, ETS2; 18s and 28s rRNA), creatine kinase (CKA7), lactate dehydrogenase (LDH6), and recombination activating gene (RAG-2) genes.

2. OBJECTIVES

2.1 Overall objective

The overall aim of this project was to use molecular methods to discriminate, identify, and characterise the invasive gobies in New Zealand waters. In addition, by describing the genetic diversity within each species both within New Zealand and within potential source regions, we aimed to shed light on the routes and timing of colonisation.

2.2 Specific objectives

2.2.1 Objective 1

To provide a molecular description and characterisation of gobies that are introduced (*Acentrogobius bifrenatus* and *A. pflaumii*) or native (e.g., *Favonigobius lentiginosus* and *F. exquisitus*).

2.2.2 Objective 2

To describe the molecular diversity of the above species throughout their native and introduced distributions, and characterise a range of potential invasive gobioid and blennioid species from the Australasian region.

2.2.3 Objective 3

To develop molecular criteria to rapidly identify invasive or cryptogenic gobioid and blennioid fish.

3. METHODS

3.1 Sample collections

3.1.1 Sample design

Sample collection was designed to achieve the two major goals of the project: (i) acquire representative specimens from each introduced and native target species, including all morphologically similar species where possible, plus suitable reference outgroup species for phylogenetic comparison – the "descriptive" samples, and (ii) for each target species, acquire the maximum number of specimens possible (up to a maximum of about 25 per location) from the maximum number of locations possible, both within and outside New Zealand, within the time-frame of the project – the "diversity" samples. Representatives of all known reference samples of target species were acquired from both New Zealand and Australia, although the difficulties in taxonomic naming made this a difficult task. International researchers with access to samples from all known locations of the target species were contacted. There are no known suitable museum specimens of these species that have not been sampled.

3.1.2 Overseas sampling

All overseas samples were acquired through personal scientific contacts: Mark McGrouther (Australian Museum), Di Bray and Martin Gomon (Museum of Victoria), Tom Winstanley (Hong Kong), Yuji Ikeda (Biological Laboratory, Imperial Household, Japan), Tomoki Sunobe (Natural History Museum, Chiba, Japan), and Michael Hammer (University of Adelaide). A summary of the overseas samples acquired is provided in Tables 1 and 2, and collectors of individual samples are provided in Appendix 2.

3.1.3 New Zealand sampling

New Zealand samples were acquired either through collaborative collections made by NIWA staff (Malcolm Francis and Mark Morrison), previous collections made by K. Clements and A. Hickey in conjunction with studies on New Zealand triplefins, or collection trips specially organised for this project and undertaken by Natalie Usmar (see Usmar (2003) for detailed descriptions of standard collection protocols). A detailed summary of the samples acquired and their locations and dates is provided in Tables 1 and 2, with collectors of individual samples provided in Appendix 2.

3.2 Molecular analysis

A considerable number of alternative methodologies were trialled during this project. Only the final, optimal, methods implemented are reported here.

3.2.1 DNA extraction

DNA was extracted from muscle tissue of all specimens acquired using at least one of the following protocols.

Phenol/Chloroform DNA extraction

Whole genomic DNA extraction was undertaken using standard proteinase-K, phenol/chloroform techniques, and checked for quality and concentration by electrophoresis on 0.6% agarose gels. Final purified DNA was stored in TE buffer (10 mM Tris, 1 mM EDTA) at –20 °C. Reference DNA is archived at –20 °C. Genomic DNA extractions were diluted 1:10 or 1:100 in 1/10 TE (10 mM Tris, 1 mM EDTA) (depending on DNA concentration) for use as template in PCR reactions. 1 µl of one or

both of these dilutions was used in a single 25 µl PCR reaction. Specimens extracted using this method (n = 61) are indicated in their codes in Appendix 2 using a "G" prefix.

Chelex DNA extraction

Using flamed forceps and scalpel, 1 mm^3 of muscle tissue was placed in a tube with 200 µl of a 20 % solution of Chelex-100 (BioRad). Samples were then incubated at 94 °C for 30 min, and cooled to 4 °C for 1 min. Each sample tube was then frozen either at –20 °C overnight or at –70 °C for 10 min, before again being incubated at 94 °C for 10 min. About 2 µl of supernatant was used in each PCR reaction. Specimens extracted using this method (n = 188) are indicated in their codes in Appendix 2 using a "GC" prefix.

3.2.2 Polymerase Chain Reaction (PCR)

A variety of genes was trialled for amplification, including mitochondrial 12s and 16s ribosomal RNA, cytochrome oxidase I (COI), and cytochrome b (cytB) genes, and nuclear ribosomal (external transcribed spacer, ETS2; 18s and 28s rRNA, Palumbi 1996), creatine kinase (CKA7), lactate dehydrogenase (LDH6), and recombination activating gene (RAG-2) (Willet et al. 1997, Peixoto et al. 2000) genes. The gene products that were most successfully amplified and sequenced, and thus used in the final analyses, were from the mt large subunit 16s rRNA (16s), cytochrome oxidase subunit I (COI), and a segment of the mitochondrial control region. The final PCR primers used are listed in Table 3.

Final PCR reaction volumes were 25 µl, and contained 1 µl of DNA template, 25 mM Tris-HCl pH 8.3, 2.5 mM $MgCl^2$, 20µM premixed deoxynucleotides, 0.5 unit of Amplitaq-Gold polymerase (Applied Biosystems Inc.), and 0.1 µmole of each oligonucleotide primer. The amplification profiles for all loci consisted of an initial denaturation step at 94 °C for 10 min, followed by 40 cycles at 92 °C for 30 s, annealing at 50 °C for 30 s, and extension at 74 °C with a final extension at 74 °C for 5 min.

3.2.3 DNA sequencing

Primers were enzymatically removed from PCR products following Werle et al. (1994), by incubation at 37 °C for 30 min with 20 units ml^{-1} of exonuclease 1 and nucleotides phosphorylated by addition of 10 units ml^{-1} shrimp alkaline phosphatase. Exonulease 1 and shrimp alkaline phosphatase activities were then stopped by incubation at 80 °C for 15 min. This removes carry-over dNTPs and oligonucleotides from the sequencing reaction. Direct cycle sequencing was performed using ABI BigDye Terminator Cycle Sequencing Ready Reaction Kit reagents and one of the original PCR primers. Products were analysed on an ABI 3100 automated sequencer.

3.2.4 Bioinformatics

All sequences were initially screened for quality and erroneous base calls using Sequencher software. Resulting sequences were edited and aligned using Sequencher, and then by eye in MacClade. Sample comparisons and identification were then carried out in a variety of ways. Initially, sequences were BLASTed against the Genbank DNA database to check for exact or close matches with species already in the public databases. Using those preliminary results, sequences were then aligned (using ClustalX) with other sequences of the same locus. Phylogenetic reconstructions were made using the Neighbour Joining method with Kimura's 2-parameter model using the software packages MEGA 2.1 (Kumar et al. 2001) and Paup* (Swofford 2000). Bootstrap support values for each clade were calculated from 1000 replicates. Comparative measures of molecular diversity were calculated using DnaSP 3.5.3 (Rozas & Rozas 1999), and include nucleotide diversity (π) and haplotype diversity (H).

Nucleotide diversity is the average divergence between sequences. Haplotype diversity ranges from 0.0 (all individuals have the same haplotype) to 1.0 (all individuals have different haplotypes).

3.2.5 Databases

The DNA sequences are provided as electronic appendices (see Appendix 1 for details). All unique sequences are in the process of being submitted to Genbank (accession numbers DQ437117-DQ437328).

4. RESULTS

4.1 Descriptive sample collection

The initial "descriptive" sample collection was assembled to address objective 1, that is, to provide a molecular description and characterisation of the New Zealand goby species, either native or introduced. The descriptive samples are listed in Table 1, and their locations shown in Figure 1.

4.2 Diversity sample collection

After preliminary analysis of the descriptive sample collection, the "diversity" sample collection was assembled to address objective 2, that is, to describe the molecular diversity of the above species throughout their native and introduced distributions, and to characterise a range of potential invasive gobioid and blennioid species from the Australasian region. After considerable efforts to track down sources of suitable material, and with invaluable assistance from colleagues in both New Zealand and overseas, we assembled a suitable collection of individuals of all required species and outgroups, from representative locations within New Zealand (based on Willis et al. (1999) and Francis et al. (2003)) and in the native overseas distributions. The descriptive samples are listed in Table 2, and their locations shown in Figure 1 and 2.

4.3 DNA sequence analysis

About 250 specimens have been sequenced for at least one gene (177 specimens were sequenced for a single locus, 23 specimens for 2 loci, and 21 specimens for 3 loci). Details of which gene have been sequenced for which individuals are provided in Appendix 2. The 16s and COI genes were sequenced for all individuals from the descriptive sample collection (where possible), while the control region was sequenced from as many individuals of the diversity collection as possible (n=42, 35 & 247 sequences for 16S, CO1 and D-loop, respectively). All individuals on the phylogenetic trees can be identified by the unique codes given in Appendix 2, allowing comparison of which specimens have been sequenced for more than one gene.

4.3.1 16s

DNA sequences from the 16s rRNA gene for 42 individuals from the descriptive sample collection were aligned, and a phylogenetic tree constructed to reveal the relationships among species (Figure 3). This tree shows that each of the four target species (*A. pflaumii*, *A. bifrenatus*, *F. exquisitus* and *F. lentiginosus*) are very clearly distinguished genetically using this locus, and that there is maximum (100%) bootstrap support for the reciprocal monophyletic nature of each clade of sequences from each species. Importantly, this is also the case for the variety of related species that we have genotyped. These include the closely related species (which could potentially cause morphological taxonomic confusion) *F. lateralis* south coast form and *A. frenatus*, as well as the only other true goby present in New Zealand, the endemic *Gobiopsis atrata*. In brief, this shows that that there are very clear

molecular distinctions between each of the species for this locus, with low levels of intra-specific diversity, and high levels of inter-specific divergence. These are exactly the type of molecular patterns that are required for easy discrimination and identification of species, and thus this gene will prove to be a valuable marker for future species discrimination and identification of this group. It is particularly useful because the gene is relatively conserved among species, making it easy to amplify, and few assumptions are required to align the sequences.

For the purposes of future rapid species identification of these gobies, the 16s sequences were analysed to identify restriction endonuclease recognition sites that could be used in rapid RFLP (restriction fragment length polymorphism) screening of this locus. Unique, species-specific RFLP patterns were determined, and are detailed in Appendix 3. A combination of two restriction enzymes (*MseI* and *MaeI*) successfully discriminate all species examined except the closely related *A. bifrenatus* and *A. frenatus*. An additional restriction enzyme (*MaeIII*) will discriminate these two species (see Appendix 3.)

Although most molecular species identifications concurred with their initial morphological species identifications, there were some exceptions. One important finding evident in the 16s results concerns *F. lateralis* (east coast form) and *F. lentiginosus*. We had managed to procure material identified as *F. lateralis* (east coast form) from Australia. This species is now considered to be a junior synonym of *F. lentiginosus* by Doug Hoese (Australian Museum, unpublished). However, one of these specimens (F.(len)exqBWBNSWG37) could be sequenced for 16s, and it is clear (Figure 3 – name in bold) that it falls within the range of diversity found within the species *F. exquisitus*, rather than within *F. lentiginosus*. It thus appears that some specimens of Australian *F. exquisitus* have previously been misidentified as *F. lateralis* (east coast form).

Some close phylogenetic relationships are clear from this data. It can be seen from Figure 3 that *F. lateralis* (south coast form) is most closely related to *F. lentiginosus*, as expected, and similarly, *A. frenatus* is most closely related to *A. bifrenatus*.

This tree (Figure 3) is not intended for estimating higher-level (e.g., generic) phylogenetic relationships among these taxa, as this was not the aim of the study. Indeed, we expect that some of the higher-level relationships depicted will be inaccurate, given the rate of molecular evolution of the various domains in this gene, and the type of phylogenetic analysis to which the sequence data have been subjected. On the other hand, we strongly expect the species and sister-species relationships depicted to be accurate, given the high levels of inter-specific divergence and low levels of intra-specific diversity. These comments are likely to also apply to the other loci we have examined in this study.

4.3.2 COI

DNA sequences from the cytochrome oxidase subunit I (COI) gene for 35 individuals from the descriptive sample collection were aligned, and a phylogenetic tree constructed to reveal the relationships among species (Figure 4). Once again, this tree shows that all four target species are very clearly distinguished genetically using this locus, and that there is maximum (100%) bootstrap support for the reciprocal monophyletic nature of each clade of sequences from each species. Along with the 16s data, this confirms the species distinctiveness of all the species we have examined in this study.

Once again, one of the individuals from Sydney, NSW, described as *F. lateralis* (east coast form) (F.exqBWBNSWG37) could be sequenced for COI, and it is clear (Figure 4) that it falls within the range of diversity found within the species *F. exquisitus*.

An important finding evident in the COI data concerns the identity of some individuals of the Asian goby *A. pflaumi* collected in New Zealand waters, but this will be dealt with below.

As in the 16s tree, it can be seen from Figure 4 that *F. lateralis* (south coast form) is most closely related to *F. lentiginosus*, and *A. frenatus* is most closely related to *A. bifrenatus*.

It may be seen in Figure 4 that there is less informative data about the higher-level relationships among species from COI than there is in the 16s tree (Figure 3). There are relatively long branches leading to most species, while there are only very short (or zero-length) inter-node branches between groups of species. This is to be expected from the COI locus at the DNA level. More informative higher-level relationships may be depicted from COI protein sequences, but at the expense of inter-specific resolution.

4.3.3 Control region (D-loop)

The mitochondrial DNA control region (commonly referred to as the D-loop region in vertebrates) is rapidly evolving in comparison to other mtDNA loci, largely because of its less specific functionality and lowered levels of purifying selection. As such, it provides the finest level resolution between taxonomic or population units. However, this means that it may provide poor information about the true phylogenetic relationships among species or higher taxa, due to the increased chances of homoplasy, multiple substitutions per site, considerable insertions and deletions, and potential difficulties with alignment.

DNA sequences from the control region were acquired from 247 individuals (Appendix 2). The relationships among all species examined in the descriptive sample collection, as depicted by the D-loop sequences, are shown in Figure 5. This tree is shown merely to indicate that, as shown for both 16s and COI loci, all species examined show clear species distinctiveness, as well to provide a broader map for the following trees, which portray more detailed information about each of the target species. Higher-level relationships should not be overly interpreted from this tree. For example, it can be seen in Figure 5 that although, once again, *A. frenatus* is most closely related to *A. bifrenatus*, the relationships among *F. lateralis* (south coast form), *F. lentiginosus*, and *F. exquisitus* are not as clear as in the other two loci.

A tree showing more detail of the D-loop relationships among the *Favonigobius* species, based on the diversity sample collection, is shown in Figure 6. Of most importance to note here is the position of the *F. lateralis* (south coast form) specimens from Australia. Figure 6 shows that all specimens of this species fall into one clade of sequences, regardless of location. Specimens from southwest Australia (Perth), South Australia (Adelaide), and Port Phillip Bay (Victoria) are all closely related. This species is clearly more closely related to *F. lentiginosus* than to *F. exquisitus*. These two species are dealt with in more detail below.

4.3.3.1 *Favonigobius lentiginosus*

Figures 5 and 6 show that for the mt control region (D-loop), all *F. lentiginosus* form a very distinct clade from their close relatives *F. lateralis* (south coast form) and *F. exquisitus*. DNA sequences from *F. lentiginosus* are very similar to that from one specimen identified as *Favonigobius* (now *Afurcagobius*, Gill (1993)) *tamarensis* that was collected in Sydney Harbour in 1998 (Figures 6 and 7) . This result suggests that this *A. tamarensis* specimen may actually be a misidentified *F. lateralis* east coast form, considered to be a junior synonym of *F. lentiginosus* by Doug Hoese (Australian Museum, unpublished). On further morphological examination, this specimen appears to have the opercular morphology characteristic of *Favonigobius*, rather than that of *A. tamarensis*. This is the first clear molecular evidence to suggest that *F. lentiginosus*, like *F. exquisitus*, also has a trans-Tasman distribution. It has been very difficult to acquire specimens of *F. lateralis* (east coast form), and obviously more specimens must be examined before final conclusions are drawn. However, if ours is an accurate interpretation, then (a) the Australian east and south coast forms of *F. lateralis* are

separate species, and (b) either *F. lentiginosus* originated in Australia before colonising New Zealand, or (less likely) it is a New Zealand species that invaded Australia.

There is considerable intra-specific diversity within the New Zealand samples from five different North Island locations. Mean nucleotide diversity within *F. lentiginosus* was 0.0065 ± 0.0016. Sequences from all individuals from the diversity sample are shown in Figure 7. There are over 30 distinct mtDNA haplotypes among the 60 individuals sequenced from throughout northern New Zealand. It is apparent that there is no major phylogeographic structuring within the sampled range of this species, as no particular clade is dominated by individuals from one geographic location. Although the sample sizes are sufficient to conclude that there is no major phylogeographic structure, a larger and more detailed study would be required to determine if there were restrictions to gene flow evident from geographic differences in haplotype frequency.

4.3.3.2 *Favonigobius exquisitus*

DNA sequences from the control region were acquired from 64 *F. exquisitus* individuals (Appendix 2). It is apparent from Figures 5 and 6 that, for the mt control region, all *F. exquisitus* form a very distinct clade from their closest relatives *F. lentiginosus* and *F. lateralis* (south coast form). New Zealand *F. exquisitus* are clearly conspecific with *F. exquisitus* from New South Wales. It is also clear that there has been some misidentification of Australian *Favonigobius* material. Some specimens identified by the Australian Museum as *F. lateralis* (east coast form), subsequently were identified from their DNA sequence as being *F. exquisitus*. One specimen of *F. lateralis* (east coast form) from Sydney is slightly divergent from the remaining *F. lateralis* (east coast form) and *F. exquisitus* (Figures 6 and 8), and may be a distinct species or population, but this requires additional sampling for confirmation.

Considerable mt control region diversity was also found within *F. exquisitus* (Figure 8). The diversity represented within the Australian (NSW) sample encompasses a slightly greater diversity than that found within all the New Zealand samples collected from 12 northern North Island locations (Appendix 2). The mean nucleotide diversity within New Zealand *F. exquisitus* is 0.0046 ± 0.00143, and after including Australian specimens is 0.0069 ± 0.00143. There is a similar level of mtDNA haplotype diversity within *F. exquisitus* as was found within *F. lentiginosus*. There are 29 distinct mtDNA haplotypes among the 63 individuals sequenced from throughout northern New Zealand and Australia. *F. exquisitus* in New Zealand waters has a slightly lower haplotype diversity (H = 0.90) than that found in New South Wales (H = 1.0), but current samples do not share any haplotypes across the Tasman.

The New Zealand haplotypes fall into two major clades. However, once again it is apparent that there is no major phylogeographic structuring evident within the sampled range of this species, as no particular clade is dominated by individuals from one geographic location. As described above, although the sample sizes are sufficient to conclude that there is no major phylogeographic structure, a larger and more detailed study would be required to determine if there were restrictions to gene flow evident from geographic differences in haplotype frequency.

A number of individual specimens that were originally identified as *A. pflaumii* from their morphology have D-loop genotypes that fall within the range of variation shown by *F. exquisitus*. These individuals come from both Waitemata (two separate collections) and from Whangapoua (one collection). There is considerable diversity within these sequences, with a number of these individuals having unique haplotypes.

4.3.3.3 *Acentrogobius bifrenatus*

DNA sequences from the control region were acquired from 31 *A. bifrenatus* individuals (Appendix 2). It is clear that both the Australian and New Zealand specimens of this species are very similar genetically, and fall together as a monophyletic clade that is distinct from the closely related *A. frenatus* from Australia (Figures 5 & 9).

Within *A. bifrenatus* (Figure 9) there does appear to be significant phylogeographic population structure in the mt control region diversity. Each of the three Australian sampling locations (Sydney, Yamba, and Adelaide) appears to have a distinct clade of haplotypes, although this may be influenced by the sample sizes. The New Zealand population is marginally more similar to the Sydney population than others in Australia, but none of the sampled specimens share haplotypes among regions. However, all four North Island New Zealand locations sampled share haplotypes.

A. bifrenatus in New Zealand waters appears to have a lower level of mt control region diversity (H = 0.71) than that observed in New Zealand populations of either *Favonigobius* species. However, *A. bifrenatus* appears to have diversity similar to conspecific populations in New South Wales (H = 0.78) and South Australia (H = 0.58) (Figure 9). This suggests that either this species has been present in New Zealand waters for a considerable period of time, or, if this species has been recently introduced, it has done so multiple times.

4.3.3.4 *Acentrogobius pflaumii*

DNA sequences from the control region were acquired from 38 *A. pflaumii* individuals (Appendix 2). In *A. pflaumii*, (Figures 5 & 10) the D-loop sequences show that the specimens from Japan (the native range of this species) fall into two very distinct geographic clades. Specimens from Australia and New Zealand belong to the clade of *A. pflaumii* from Tokyo. These results are totally supported by the sequences from both 16s and COI.

A complicating factor in unravelling the patterns of *A. pflaumii* diversity is that a number of specimens of "*A. pflaumii*" collected in New Zealand waters (from both Waitemata and Whangapoua) have subsequently been molecularly identified as belonging to *F. exquisitus* (Figure 8, Appendix 2). This was further complicated by the fact that a number of the specimens from these collections could not be molecularly identified due to poor preservation. Reassuringly, all *F. exquisitus* from Whangapoua that were originally identified as *A. pflaumii* were subsequently and independently re-identified correctly (M. Francis, NIWA, pers. comm.), although the Waitemata misidentifications were not detected. This highlights some of the considerable difficulties in morphological identification of many of these gobies, particularly of juvenile or immature specimens. Further examination of these specimens may help future field identification, although since all were frozen at point of capture they are in poor condition.

There is no major population structure apparent among any of the locations from which true *A. pflaumii* were sampled, despite the great distance separating some of them across the Pacific. This is an indication that the spread of this species has been recent in comparison to, for example, *A. bifrenatus*.

The mt control region diversity in *A. pflaumii* appears to exhibit a classic pattern of recent colonisation. *A. pflaumii* exhibits a pattern of reducing mitochondrial diversity as we compare Tokyo specimens (haplotype diversity, H = 1.0) to Sydney (H = 0.78) to New Zealand specimens (H = 0.57). Within New Zealand, Waitemata specimens (H = 0.57) had greater diversity than those from Whangapoua (H = 0.0). Furthermore, there is direct evidence to suggest a colonisation from Tokyo to Sydney and New Zealand as one common mitochondrial haplotype is found in all locations. This pattern of haplotype sharing was not found in any of the other three goby species examined in detail. Sydney is implicated as the source of New Zealand's *A. pflaumii* by the presence of two shared

haplotypes, again, a pattern not seen in the other gobies. Whangapoua's single haplotype is one of four found in the Waitemata, suggesting a spreading colonisation within New Zealand rather than an independent colonisation from overseas. These preliminary conclusions require further sampling for confirmation.

5. DISCUSSION

Given the somewhat confusing taxonomy and re-naming of species in the past, a brief summary of the background situation will assist in interpreting the discussion to follow. At least two species of gobies have been introduced to New Zealand waters. The Australian bridled goby, *Acentrogobius bifrenatus*, was reported from the Hauraki Gulf and the Waitemata Harbour by Willis et al. (1999). More recently, Francis et al. (2003) showed that *A. bifrenatus* is more widespread in northeastern New Zealand than first thought and appears well established, and that the Asian goby, "*Acentrogobius pflaumii*", was also present in the Waitemata and Whangapoua Harbours. These two invasive populations are most likely to have been transported by ships from Australian ports, where *A. bifrenatus* occurs naturally and "*A. pflaumii*" has been introduced. Two other goby species, *Favonigobius exquisitus* and *F. lentiginosus*, are also well established in New Zealand. The former species was considered to have conspecific populations in Australia, while the taxonomic relationships of *F. lentiginosus* were uncertain. The taxonomic identity of "*A. pflaumii*" was also uncertain, as this name applies to as many as three species in the Northwest Pacific (R. Kuiter, Museum Victoria, pers. comm.). At present, the endemic *Gobiopsis atrata* is the only New Zealand goby species that is certainly indigenous. In this study, we have produced reference gene sequences to (a) investigate the systematic relationships of New Zealand goby populations, (b) determine whether the level of genetic variation in these populations is suggestive of recent introduction, and (c) potentially identify source populations of invasive species.

5.1 Objective 1

- To provide a molecular description and characterisation of gobies that are introduced (*Acentrogobius bifrenatus* and *Acentrogobius pflaumii*) or native (e.g., *Favonigobius lentiginosus* and *F. exquisitus*).

Both invasive species (*Acentrogobius bifrenatus* and *A. pflaumii*) and both *Favonigobius* species (*F. lentiginosus* and *F. exquisitus*) can be easily and unambiguously distinguished and identified using the molecular sequences we have compiled. All three regions we have sequenced (16s, COI, and D-loop) have clearly shown the genetic differentiation among these four species, and all other related species examined.

There is potential for morphological misidentification of *A. pflaumii* because some specimens collected in New Zealand that were originally identified as this species were subsequently shown to be *F. exquisitus*.

5.2 Objective 2

- To describe the molecular diversity of the above species throughout their native and introduced distributions, and characterise a range of the greatest potential invasive gobioid and blennioid species from the Australasian region

From the patterns of genetic diversity present within the species in New Zealand, it appears that *F. lentiginosus* has been in these waters for a considerable time, and exhibits at least moderate levels of gene flow among northern New Zealand populations. The numbers of mitochondrial haplotypes present, and the levels of diversity within the species, are certainly not indicative of a recently arrived species. The lack of major phylogeographic structure in the New Zealand populations also suggests

that population mixing has been occurring to some extent for a considerable time. There is still some doubt about the presence of this species in Australian waters. The Australian species previously considered most likely to be conspecific with *F. lentiginosus* was *F. lateralis* (east coast form). However, all specimens of this form acquired and sequenced had mitochondrial haplotypes very similar to those of *F. exquisitus* (from both Australia and New Zealand). Instead, one specimen from the Australian Museum previously identified as *F. tamarensis*, and believed by current curators to probably be *F. exquisitus*, has ultimately been found to have a mitochondrial haplotype very similar (and probably conspecific) to *F. lentiginosus*. Unfortunately, additional Australian specimens of these species have been difficult to acquire in the time-frame of this project. This confusing pattern of morphological similarities is not unusual within this group of species, and indicates that more work is required to finally sort out the taxonomy. The one specimen with a mitochondrial type very similar to that of *F. lentiginosus* suggests that this species also occurs in Australian waters, and thus raises the question of origins and dispersal. Has this species also colonised New Zealand waters from Australia, or vice versa, and could this have been a recent event? This cannot be answered without a number of additional Australian specimens from a range of locations. However, it is clear that there is substantial diversity of *F. lentiginosus* within New Zealand waters, and that this has not been a very recent and isolated introduction.

Of the three species known to have conspecific populations in Australia, DNA sequences from local New Zealand-caught specimens of *A. pflaumii, A. bifrenatus*, and *F. exquisitus* match those from specimens collected in Australian waters, and thus clearly belong to those species. *F. exquisitus* from New Zealand waters appears to have a slightly lower genetic diversity than that found in Australian populations, as might be expected from a species that has potentially colonised from west to east. However, the existence of at least 24 haplotypes in New Zealand indicates that, if so, this has not been a very recent or restricted invasion. Historical museum specimens indicate that *F. exquisitus* has been present in New Zealand for at least 50 years (M. Francis, pers. comm.). It is also clear that there is no strong phylogeographic structure within *F. exquisitus* in New Zealand, further suggesting that any invasion was not recent or restricted. If an invasion had been recent, the genetic pattern expected would show relatively few haplotypes, with potentially major genetic differences between multiple sites of introduction, exaggerated by the effects of small founding populations and subsequent genetic bottlenecks. Instead, the pattern we see within New Zealand *F. exquisitus* is relatively high levels of diversity, with low levels of population differentiation among northern New Zealand populations. In other words, it appears that *F. exquisitus* has also been present in New Zealand for a substantial time, and that there is at least moderate gene flow among northern New Zealand populations. More accurate interpretations of just how long this species has been present in New Zealand could be provided by more detailed sampling in both New Zealand and Australia.

Similar interpretations can be made about *A. bifrenatus*, although our conclusions must be somewhat more cautious due to the smaller sample sizes that could be analysed, despite our efforts to acquire more samples. The samples in hand show for certain that the New Zealand individuals identified as this species do belong to the same species as the Australian *A. bifrenatus*. Although there is a small genetic divergence between the samples from both countries (and among regions), this is well within the usual level of diversity seen within any one fish species. The New Zealand *A. bifrenatus* are slightly more closely related to those from Sydney Harbour than to those from other Australian locations, but as there are no mitochondrial haplotypes directly shared between Australian and New Zealand specimens, the possible source of colonisation cannot be unambiguously identified. It is clear that *A. bifrenatus* is distinct from, although closely related to, the species *A. frenatus*, collected from Port Phillip Bay in Victoria.

The patterns of molecular diversity in *A. pflaumii* appear to show a clear case of colonisation. *A. pflaumii* in its native northwest Pacific range has been suspected to have more than one form. This has been confirmed by DNA sequencing here, which shows that populations from northern and southern Japan are phylogenetically distinct. This may suggest either that these represent two phylogeographically distinct populations of the one species, or two closelyrelated species. This will require further detailed morphological and genetic examination of additional specimens to determine.

Further sampling in that region may also reveal additional forms of this 'species'. Of great interest is the fact that the sampled specimens found in NSW and New Zealand all belong to the northern Japan form of *A. pflaumii*. It appears that this form is the original source of colonisation for both Australia and New Zealand. This is perhaps not surprising, given its presence in Tokyo, a major port of departure for vessels heading to Australian and New Zealand ports. It will require additional sampling from Australian waters, of course, to confirm that this is the only source of this species in Australia.

In the two New Zealand locations where *A. pflaumii* has been found (Waitemata and Whangapoua), this species exhibits a lower diversity than that found in the NSW sample, and shares two of its four mitochondrial haplotypes. The existence of multiple haplotypes within New Zealand shows that more than one initial breeding pair have successfully colonised, but the diversity is much lower than that seen in the other goby species, indicating that this has been a very restricted colonisation. Furthermore, only one haplotype has so far been found in Whangapoua, and this is one of the types found in the Waitemata.

Thus, in summary, *A. pflaumii* appears to exhibit a pattern of recent colonisation, with reducing mitochondrial diversity as we compare Tokyo to Sydney to New Zealand specimens, and within New Zealand, Waitemata to Whangapoua. The data suggest a colonisation from Tokyo to Sydney and then to New Zealand, and also suggest a single spreading colonisation within New Zealand rather than independent colonisation events from overseas. Of course, these conclusions are based on the current limited samples possible in the time frame of the present study. A more comprehensive sampling regime may provide additional information.

The potential for morphological misidentification of *A. pflaumii* has been shown by the fact that some specimens collected in New Zealand that were originally identified as being *A. pflaumii* were subsequently shown to be *F. exquisitus*.

5.3 Objective 3

- To develop molecular criteria to rapidly identify invasive or cryptogenic gobioid and blennioid fish

In the course of this project we have developed a set of sampling and laboratory protocols that will enable the accurate discrimination of all known local, invasive, related, and potentially invasive goby species. We now have DNA sequences that will also enable the accurate identification of all known local, invasive, and related goby species, and the identification of a range of other potential invasive goby species. We have determined that all three loci we chose can be used to discriminate these species. One reason that COI is a useful reference gene sequence for these species is that it is now being used much more broadly as a common "DNA barcode" for a wide diversity of animals (Hebert et al. 2003). However, given the relative difficulties of amplifying and analysing each of the loci, as well as the relative benefits for other applications such as phylogeny reconstruction, we believe that the 16s locus is likely to prove the most productive for future genetic analyses among species of this group.

To assist in the rapid and cost-effective identification of gobies in New Zealand, we have also developed RFLP (restriction fragment length polymorphism) markers that can identify all species that we have examined (Appendix 3). It is expected that these markers would also identify any additional species, but as is usually the case with such less-sensitive markers, this cannot be guaranteed. However, RFLPs may be more cost-effective for high-volume analyses. As the costs of DNA sequencing continue to drop, sequencing could eventually be used for these high-volume analyses.

5.4 General discussion

This study has shown that molecular techniques can resolve marine biosecurity problems in New Zealand. Holland (2000) indicated that molecular genetic techniques would have considerable scope in addressing fundamental questions about marine bioinvasions, and also could provide data useful in the development of predictive models. Along with similar work undertaken by Gust et al. (2003) on the invasive crab *Charybdis japonica*, this study indicates that this will be a productive direction in research into New Zealand's marine biosecurity issues.

This molecular approach has wide applications in the marine sphere, and is not restricted to the species groups examined here. Although the specific methods, such as loci used, may vary among different taxonomic groups to be examined, the fundamental methodology is largely the same. We believe that this approach could form an integral part of an overall marine biosecurity strategy for New Zealand. Hewitt et al. (2004) outlined the general directions and requirements necessary for a successful programme to deal with marine invasives, and recognised the potential role that molecular approaches may take. The primary role for this methodology is likely to be as part of "border protection" through the monitoring of species present in our harbours and those being introduced by incoming vessels in their ballast water, sea chests, and as hull fouling.

The importance of baseline monitoring in the success of such border protection programmes has been highlighted by Wotton & Hewitt (2004). The first step in recognising the introduction of new invasives is to have a knowledge of the species (native, cryptogenic, or invasive) already present. Therefore research targeted at biosecurity is inevitably closely aligned to, and highly dependent on, research describing our current biodiversity. Previous work (e.g., Read 2002) has indicated the minimum extent of the problem of marine invasions in New Zealand, and the harbour monitoring programme being set up by the Ministry of Agriculture and Forestry (MAF) should prove valuable in detecting new species as they arrive. However, this will be successful only if the species can be identified accurately, and identified in sufficient time to allow an appropriately rapid response of elimination or containment. It is in this task that molecular methods may prove extremely useful, particularly for larval specimens.

There is a need for on-going biosecurity research to be undertaken within New Zealand, without relying totally on research and development of techniques in other parts of the world. Not only are the individual species and issues of most concern to New Zealand largely different from those of other countries, but it appears that the whole suite of invasive organisms reaching and colonising southern shores is significantly different from those in the northern hemisphere (Hewitt et al. 2004). However, there is obviously a need for close collaboration with Australian researchers and authorities, as Australia is clearly implicated as an intermediary port for the entry into New Zealand of cosmopolitan marine invasives.

Understanding the colonisation routes of invasive organisms is another area where molecular techniques will be able to substantially contribute. A number of previous studies (e.g., Patti & Gambi 2001, Roy & Sponer 2002, Stepien et al. 2002, and Wares et al. 2002) have shown the value of molecular methods in determining the origins and likely routes of colonisation of a variety of marine species. This study also indicates that this approach can provide useful indications of source populations and routes. It will require more extensive sampling over several years to provide more definitive answers; however, the ability to identify the major sources and routes could prove invaluable in predicting and limiting future invasions (Kolar 2004).

It will also be important to adequately identify the extent and routes of colonisation of invasives within New Zealand waters. As Wasson et al. (2001) have shown, even locations distant from ports of entry can be quickly invaded through either natural or anthropogenic dispersal. Molecular methods could provide a powerful tool for potentially determining the direction, extent, and frequency of invasive dispersal within New Zealand, and the current study has already begun to elucidate these patterns. Such knowledge will be invaluable in allowing the determination of the existence, location,

and permeability of "internal borders", and their potential for use in controlling the spread of successful invasives.

Finally, an extensive range of developments in molecular techniques is currently under way (e.g., see Dale & Schantz 2002). The future possibilities for application of these techniques in New Zealand marine biosecurity is perhaps limited more by the specific identification and definition of short- and long-term goals than by any technical limitations. Potential applications include the use of micro-arrays and other "gene chips" in rapid identification of a range of species, the ability to rapidly screen all sampled organisms for those that belong to potentially threatening groups, and even the ability to detect any viable living organisms in ballast water after treatment. It is certain that we need to investigate all possible techniques in the ever-increasing battle against marine invasives, as it is becoming increasingly clear that the ecological and evolutionary consequences of such invasions are much greater than we have previously realised (Grosholz 2002).

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Table 1: Goby descriptive sample collection details. (These are the initial representative specimens from each introduced and native target species, collected for species description purposes.)

Species	Location*	Collection date	# specimens
Acentrogobius bifrenatus	Waitemata	9/2/01	3
A. pflaumii	Waitemata ARA0101	8/2/01	5
Favonigobius exquisitus	Waitemata LAN0102	9/2/01	4
F. lentiginosus	Whangarei ARA0118	20/3/01	7
Thalasseleotris sp.	Mokohinau Is.	7/4/03	3
Gobiopsis atrata	Mokohinau Is.	9/4/03	2

* Codes are original NIWA sample collection codes to enable cross-reference to stored collection.

Table 2: Goby diversity sample collection details. (This is the complete collection of all specimens from each species, for the purpose of evaluating the diversity within species.)

Species	Location*	Collection date	# specimens
Thalasseleotris sp.	Mokohinaus	14/2/03	1
Thalasseleotris sp.	Mokohinaus	7/4/03	3
	Total		**4**
Gobiopsis atrata	Mokohinaus	7/4/03	1
G. atrata	Mokohinaus	8/4/03	1
G. atrata	Mokohinaus	9/4/03	2
	Total		**4**
Parachaeturichthys polynema	Hong Kong	4/3/03	4
Oxyurichthys tentacularis	Hong Kong	4/3/03	3
Myersina filifer	Hong Kong	4/3/03	1
Acentrogobius caninus	Hong Kong	4/3/03	3
Acentrogobius pflaumii	Kagoshima, Japan	20/2/03	4
A. pflaumii	Tokyo Bay, Japan	6/3/03	5
A. pflaumii	White Bay, Sydney Hbr	17/5/01	6
A. pflaumii	White Bay, Sydney Hbr	17/5/01	1
A. pflaumii	Glebe I, Sydney Hbr	17/5/01	6
A. pflaumii?	Waitemata ARA0101	8/2/01	5
A. pflaumii	Waitemata ARA0102	9/2/01	1
A. pflaumii	Waitemata ARA0102	9/2/01	15
A. pflaumii	Waitemata LAN0102	9/2/01	14
A. pflaumii	Whangapoua HAK0203	23/3/02	4
A. pflaumii	Whangapoua Hbr	16/4/02	8
	Total		**69**
Acentrogobius bifrenatus	Yamba, NSW	25/3/02	4
A. bifrenatus	Rozelle Bay, Sydney Hbr	4/5/01	1
A. bifrenatus	Black Wattle Bay , Sydney Hbr	17/5/01	1
A. bifrenatus	Fullerton Cove, Newcastle NSW	12/11/97	1
A. bifrenatus	Adelaide SA	7/2/04	11
A. bifrenatus	Kangaroo I. SA	?	2
A. bifrenatus	Waitemata ARA0102	9/2/01	1
A. bifrenatus	Waitemata LAN0102	9/2/01	1
A. bifrenatus	Waitemata LAN0101	8/2/01	1
A. bifrenatus	Whangarei LAN0119	9/3/01	1
A. bifrenatus	Whangateau	2/5/03	7
A. bifrenatus	Matapouri	7/5/03	2
A. bifrenatus	Waitemata	21/11/03	1
	Total		**34**
Acentrogobius frenatus	Port Phillip Bay, Vic	7/2/03	1
Favonigobius lateralis (south coast form)	Port Phillip Bay, Vic	7/2/03	1
F. lateralis(south coast form)	Port R. Adelaide SA	7/2/04	1
	Total		**2**

Species	Location*	Collection date	# specimens
F. tamarensis	Sydney Hbr	8/10/98	11
Favonigobius lentiginosus / lateralis (east coast form)	Black Wattle Bay, NSW	17/5/01	2
F. lentiginosus / lateralis (east coast form)	Sydney Hbr, NSW	3/5/01	1
F. lentiginosus	Whangarei ARA0118	20/3/01	7
F. lentiginosus	Whangarei ARA0118	20/3/01	48
F. lentiginosus	Whangapoua HAK0203	23/3/02	27
F. lentiginosus	Mangawhai	3/1/02	18
F. lentiginosus	Whangateau	30/4/03 & 1/5/03	20
F. lentiginosus	Matapouri	7/5/03	20
	Total		**143**
Favonigobius exquisitus	Myall Quays estate, NSW	22/1/98	3
F. exquisitus	Kaipara Harbour ARA0122	30/3/01	1
F. exquisitus	Kaipara Harbour LAN0118	15/3/01	1
F. exquisitus	Kaipara Harbour LAN0117	14/3/01	1
F. exquisitus	Manukau ARA0105	14/2/01	1
F. exquisitus	Manukau LAN0103	12/2/01	1
F. exquisitus	Kawhia Harbour LAN0116	8/3/01	1
F. exquisitus	Kawhia Harbour LAN0116	8/3/01	1
F. exquisitus	Whangarei ARA0118	20/3/01	1
F. exquisitus	Waitemata LAN0102	9/2/01	4
F. exquisitus	Waitemata ARA0102	9/2/01	2
F. exquisitus	Waitemata LAN0102	9/2/01	2
F. exquisitus	Waitemata ARA0101	8/2/01	4
F. exquisitus	Waitemata LAN0102	9/2/01	3
F. exquisitus	Coromandel Har. LAN0108	20/2/01	1
F. exquisitus	Coromandel LAN0108	20/2/01	2
F. exquisitus	Whangapoua HAK0201	21/3/02	3
F. exquisitus	Whangapoua HAK0203	23/3/02	19
F. exquisitus	Whitianga Har. LAN0110	22/2/01	1
F. exquisitus	Whitianga LAN0110	22/2/02	1
F. exquisitus	Whitianga LAN0110	22/2/01	1
F. exquisitus	Tauranga ARA0111	23/2/01	2
F. exquisitus	Tauranga ARA0111	23/2/01	1
F. exquisitus	Tauranga LAN0111	23/2/01	10
F. exquisitus	Ohiwa Hbr ARA0112	24/2/01	3
F. exquisitus	Whangateau	30/4/03 & 1/5/03	3
	Total		**73**

* Codes are original NIWA sample collection codes, to enable cross-reference to stored collection.

Table 3: PCR primers used in goby analyses.

Mitochondrial 16s rRNA			
16SAR	5'-CGCCTGTTTATCAAAAACAT-3'	forward	(Palumbi 1996)
16SBR (reverse)	5'-CCGGTCTGAACTCAGATCACGT-3'	reverse	(Palumbi 1996)
Mitochondrial control region (Dloop)			
L-pro	5'-AACTCTCACCCCTAGCTCCCAAAG-3'	forward	(Meyer et al. 1994)
H16498	5'-CCTGAAGTAGGAACCAGATG-3'	reverse	(Meyer et al. 1994)
GOBHi-H	5'-GCGCTGCACTCTGAAATGC-3'	reverse	(Lee et al. 1995)
Mitochondrial CO1			
L6468	5'-GCTCAGCCATTTTACCTGTG-3'	forward	(Sorenson et al. 1999)
H7217	5'-ACYTCTGGGTGACCAAAGAATC-3'	reverse	(Sorenson et al. 1999)

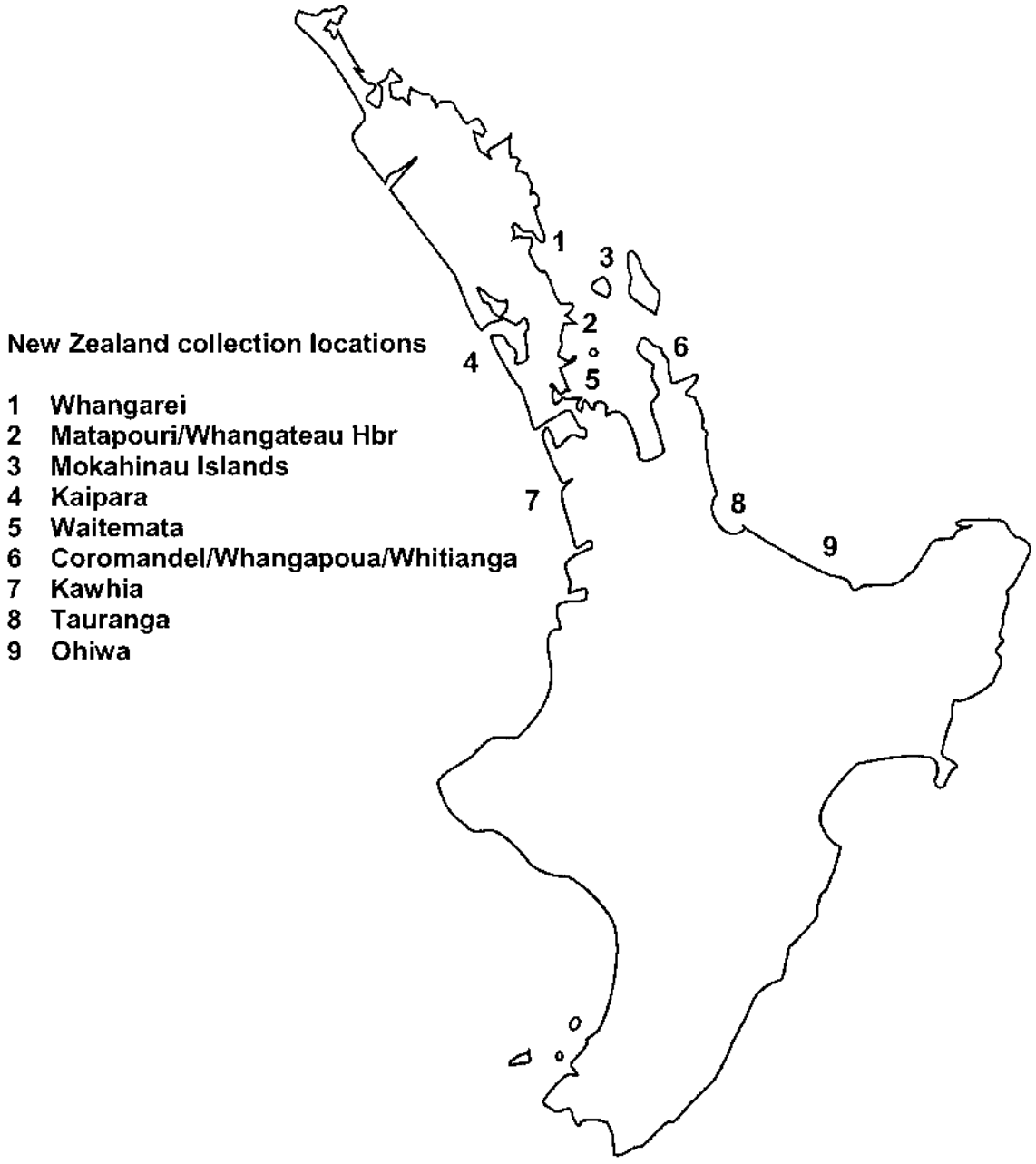


Figure 1. New Zealand collection locations. Location code numbers are used in phylogenetic trees following.

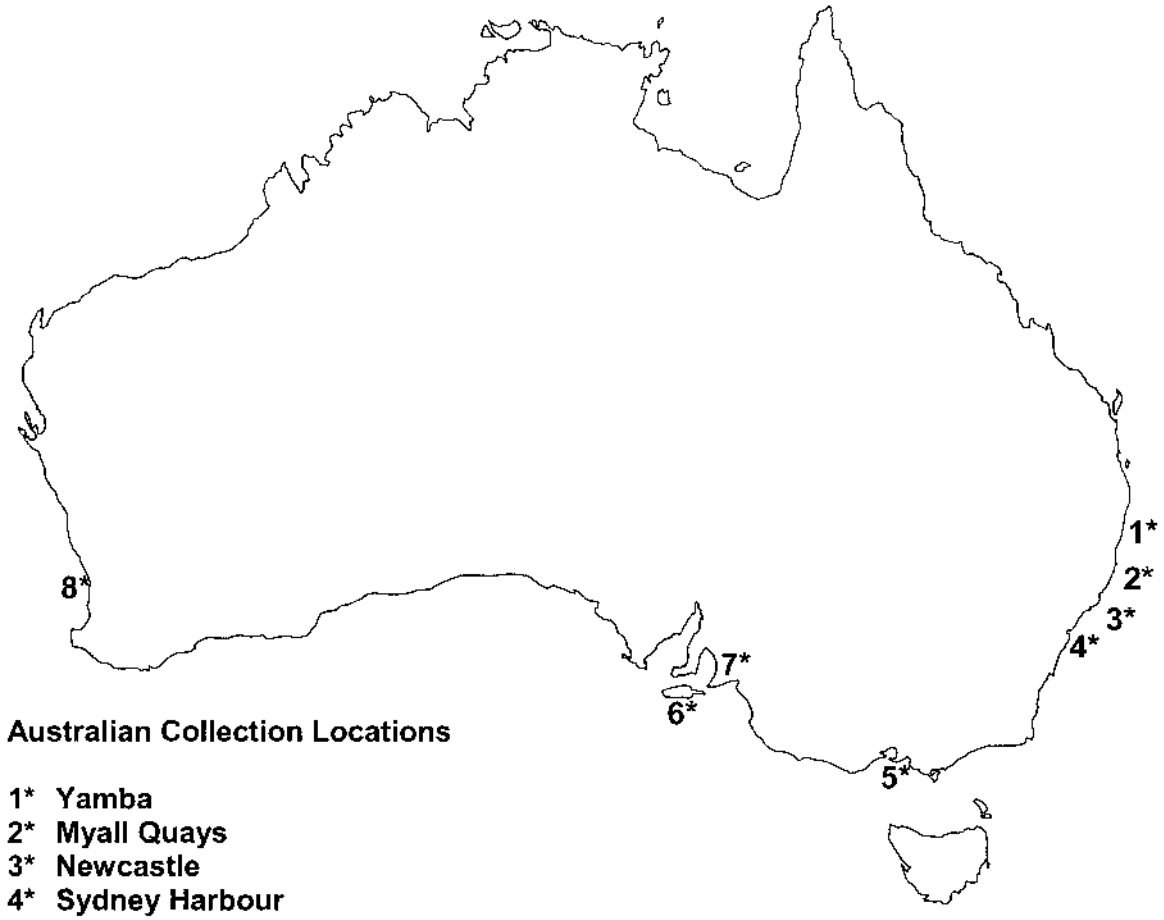


Figure 2. Australian collection locations. Location code numbers are used in phylogenetic trees following. Note that all Australian location numbers are followed by an asterisk.

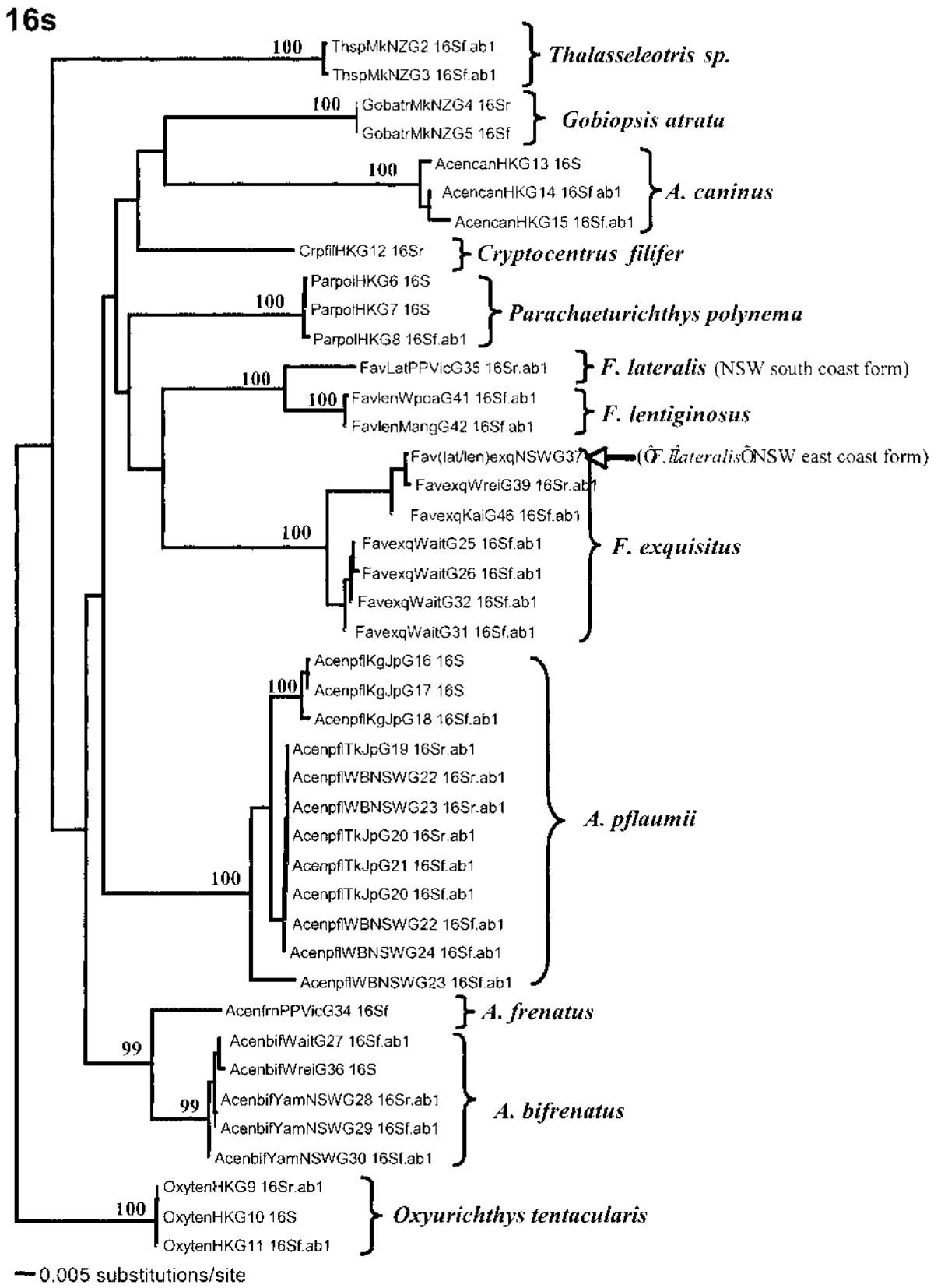


Figure 3. 16s neighbour-joining (NJ) phylogenetic tree of all species (n=42 sequences), with bootstrap support values. Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. Flagged specimens are those that have been referred to individually in the text

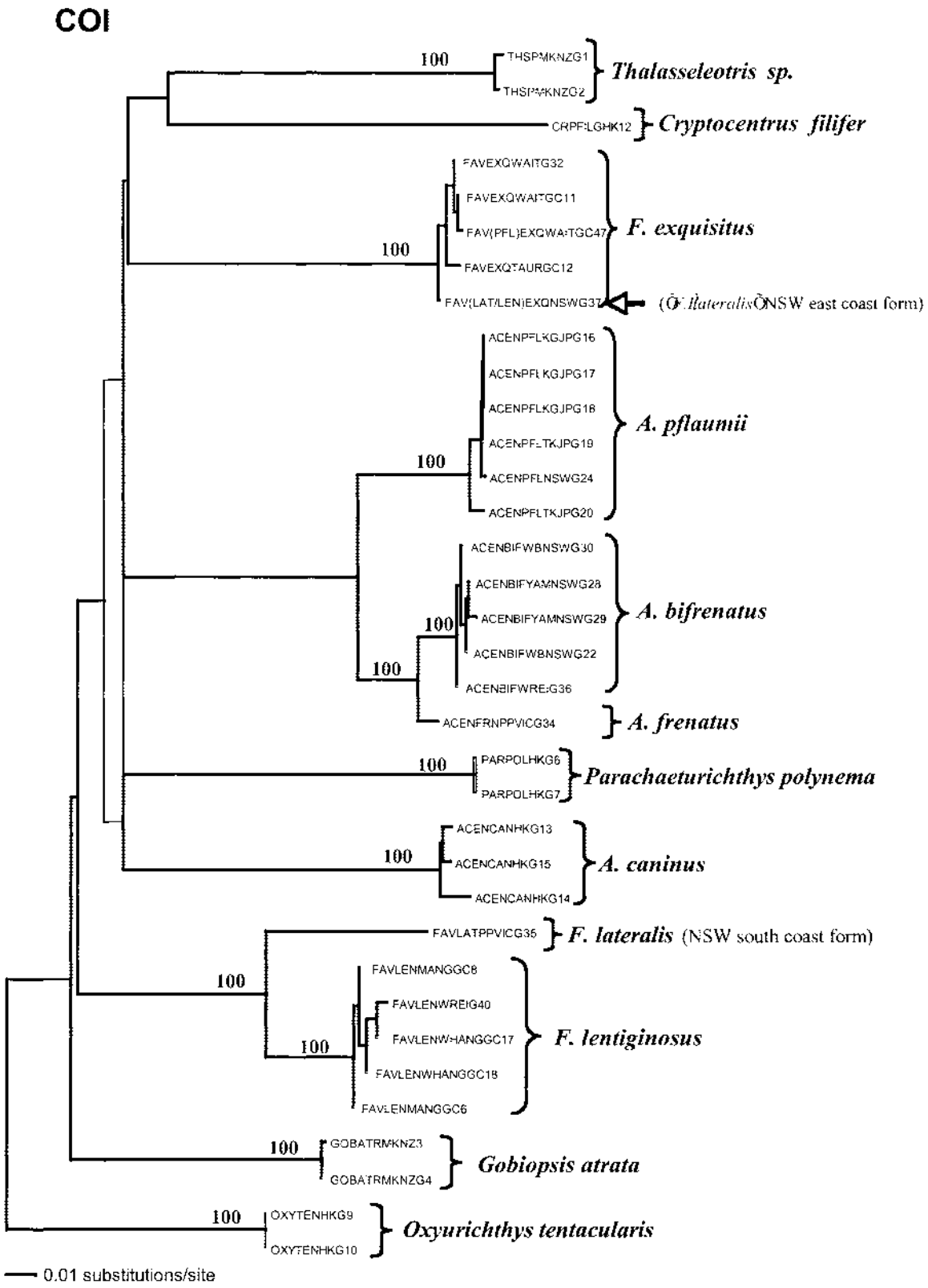


Figure 4. COI NJ phylogenetic tree of all species (n=35 sequences), with bootstrap support values. Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. Flagged specimens are those that have been referred to individually in the text.

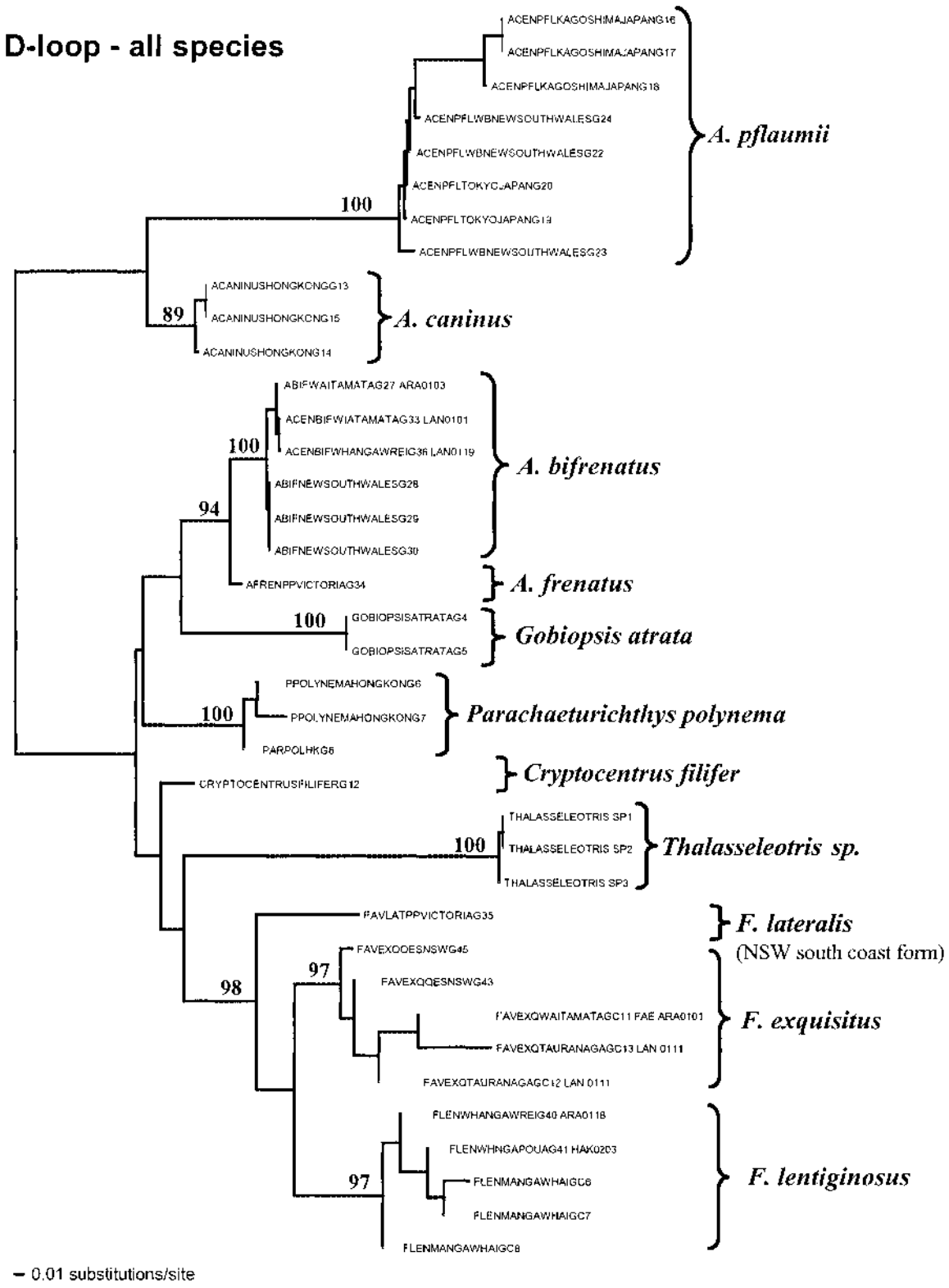


Figure 5. . D-loop neighbour-joining phylogenetic tree of all species (n=38 sequences), with bootstrap support values. Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. Flagged specimens are those that have been referred to individually in the text.

D-loop - all *Favonigobius* species

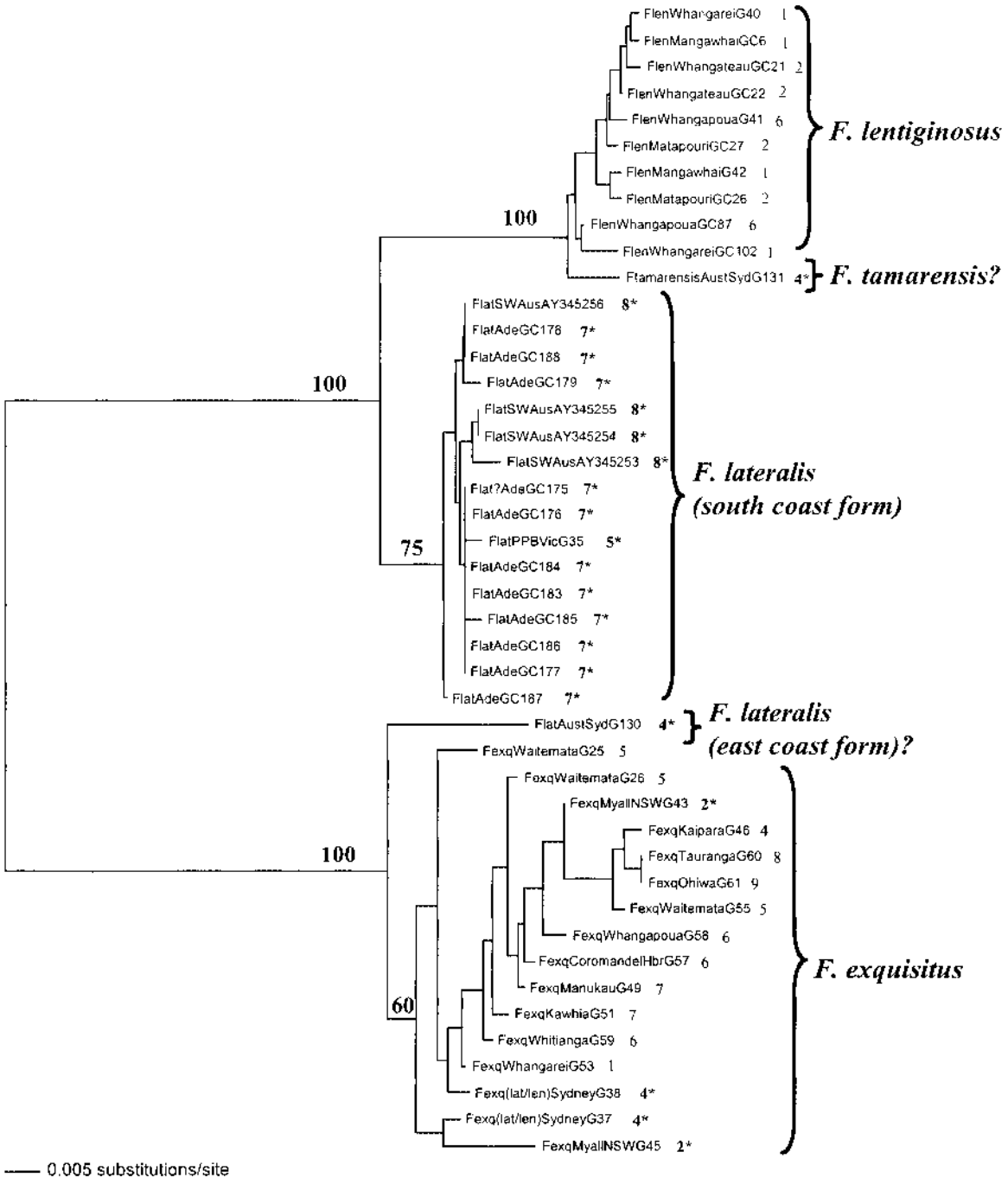


Figure 6. D-loop neighbour-joining phylogenetic tree of all *Favonigobius* species (n=44 sequences), with bootstrap support values. Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. They are followed by location codes as shown in figs 1 and 2. Note that all Australian location numbers are followed by an asterisk. Flagged specimens are those that have been referred to individually in the text

D-loop - *Favonigobius lentiginosus*

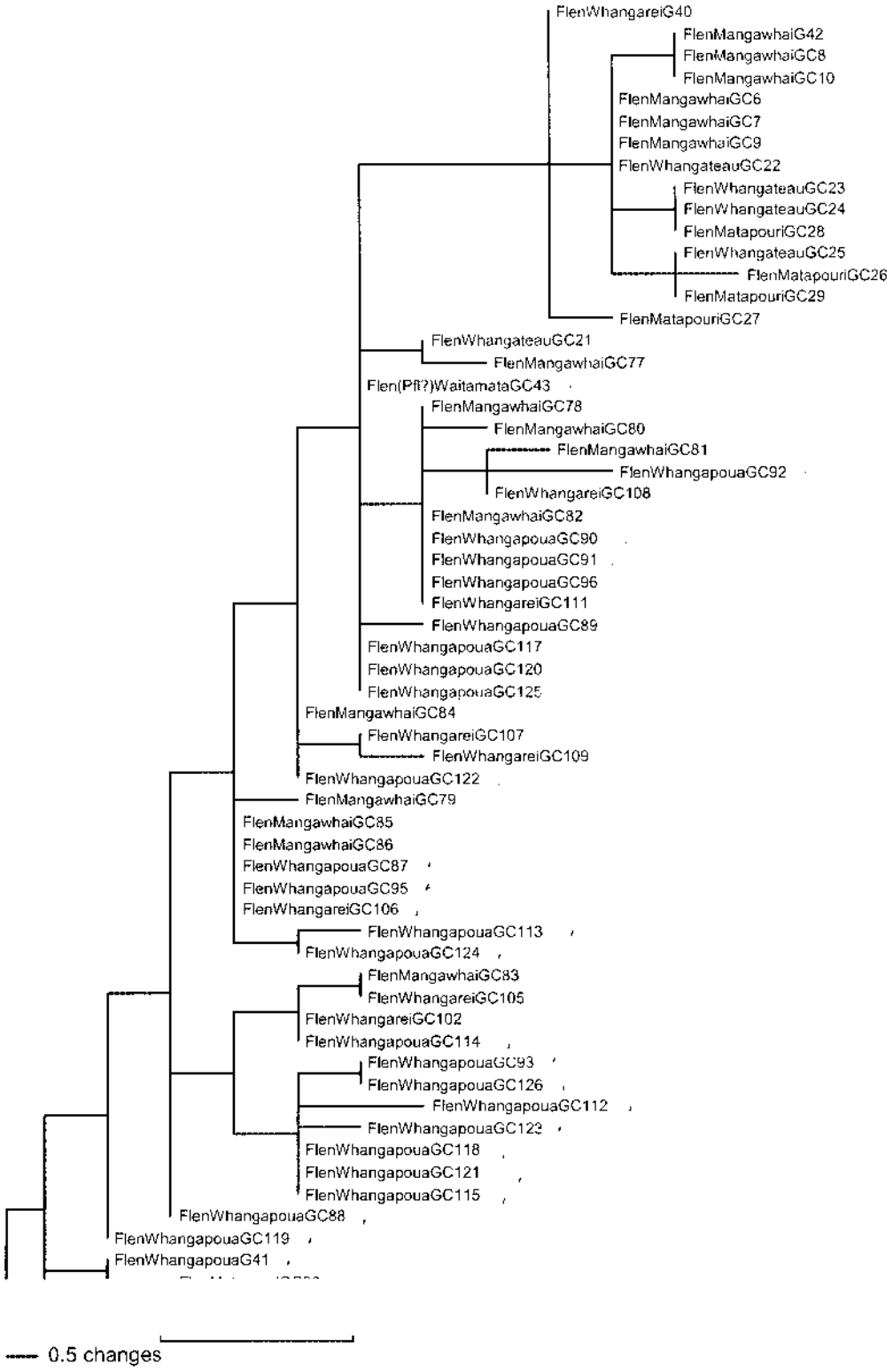


Figure 7. D-loop parsimony tree for *F. lentiginosus* (n=60 sequences). Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. They are followed by location codes as shown in figs 1 and 2.

D-loop - *Favonigobius exquisitus*

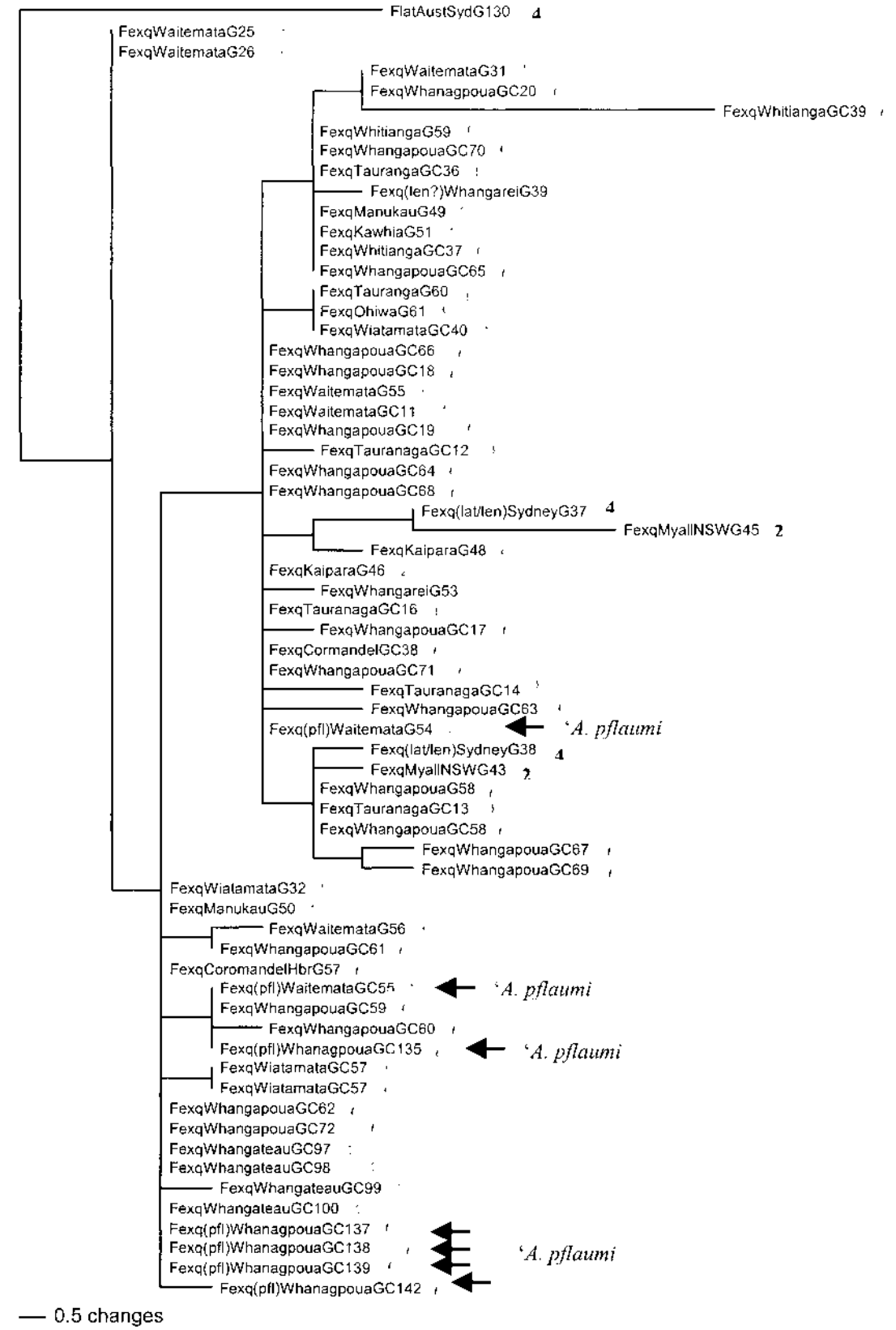


Figure 8. D-loop parsimony tree for *F. exquisitus* (n=64 sequences). Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations.

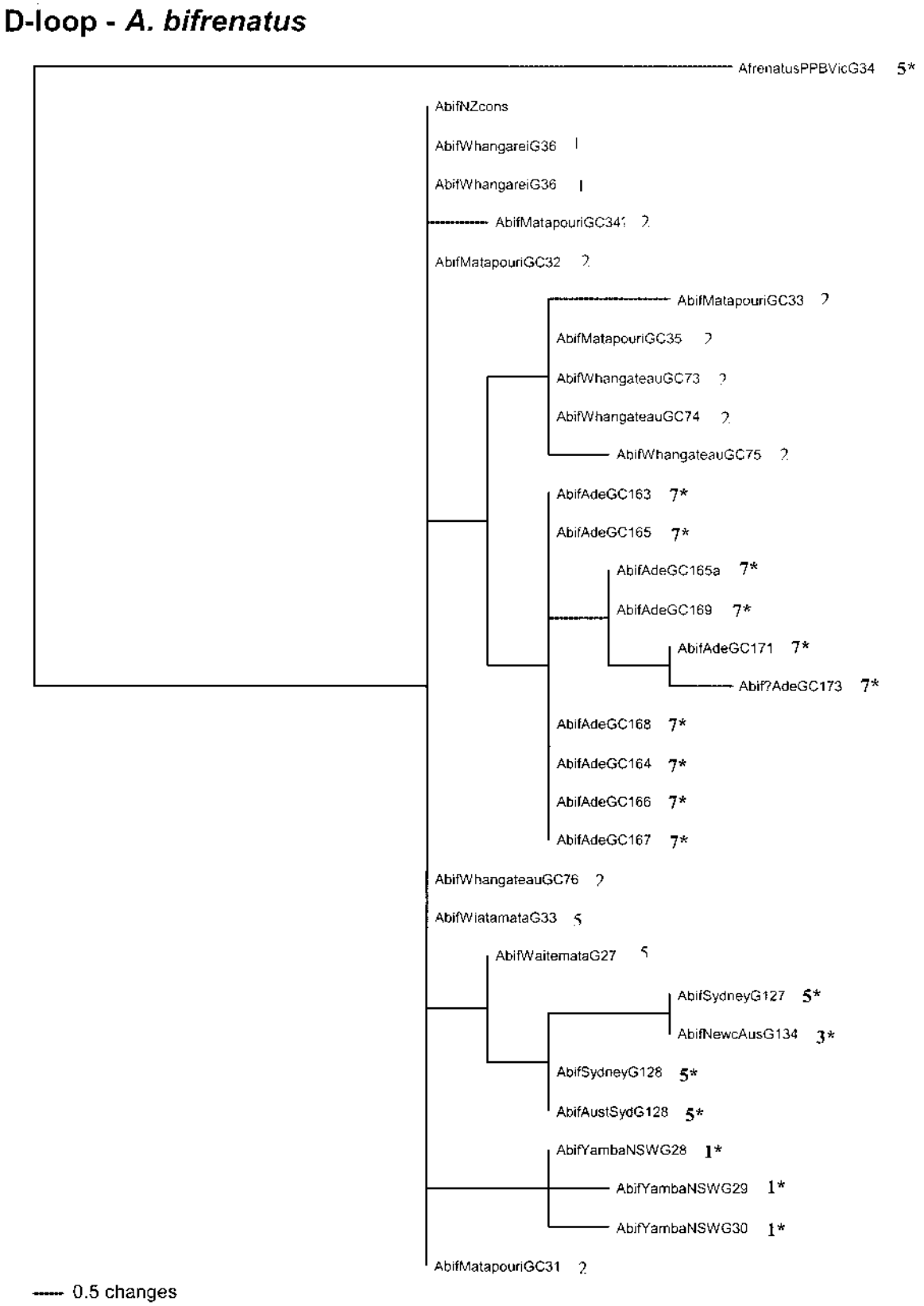


Figure 9. D-loop parsimony tree for *A. bifrenatus* (n=31 sequences). Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. They are followed by location codes as shown in figs 1 and 2. Note that all Australian location numbers are followed by an asterisk

D-loop - *A. pflaumii*

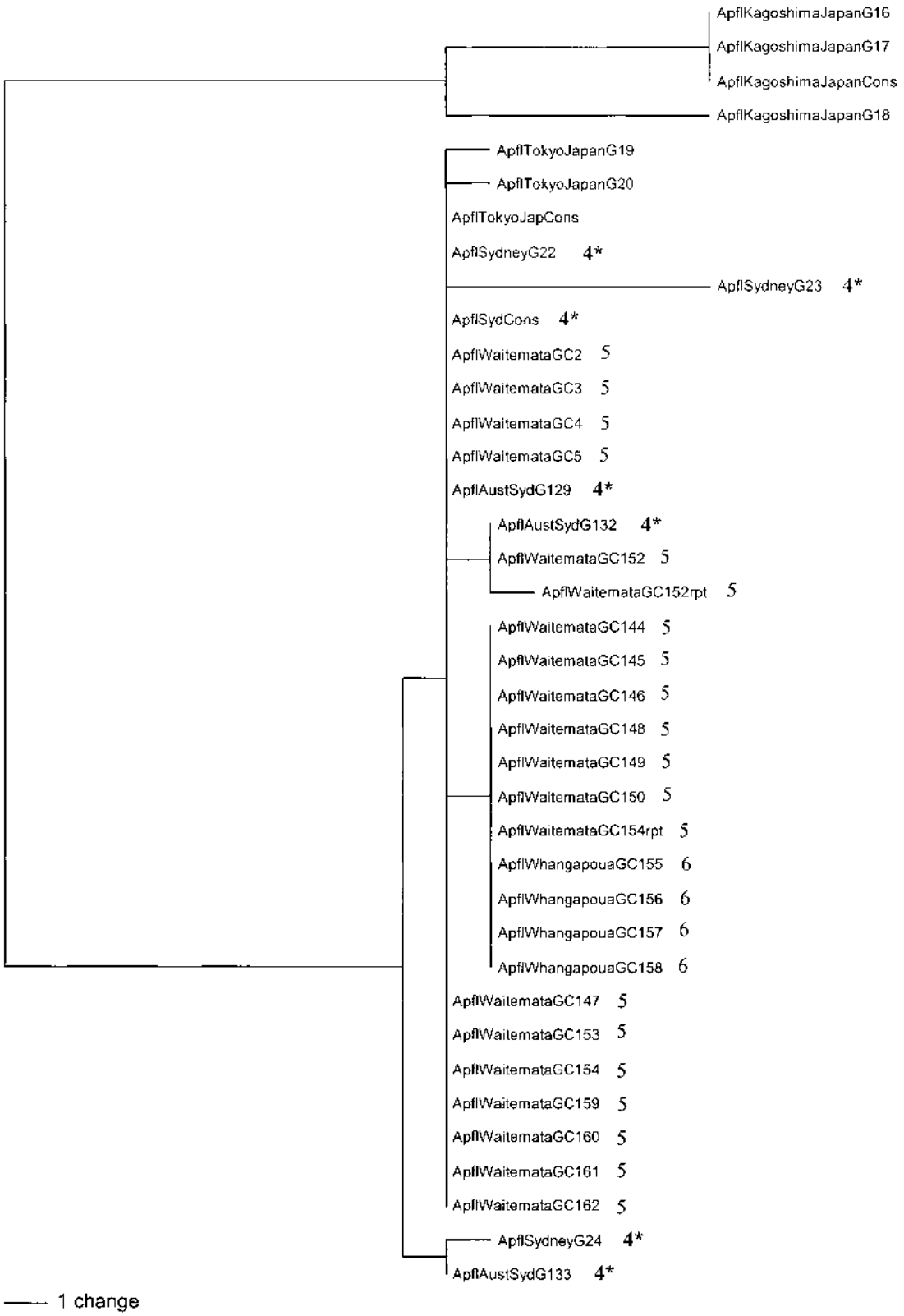


Figure 10. D-loop parsimony tree for *A. pflaumii* (n=38 sequences). Sequence codes refer to those listed in Appendix 2, and include species and location abbreviations. They are followed by location codes as shown in figs 1 and 2. Note that all Australian location numbers are followed by an asterisk. Note also that all A. pflaumii from Whangapoua have only one haplotype, which is shared with specimens from the Waitemata Harbour.

Appendix 1. List of electronic appendix databases

16s rRNA sequences file:`16sGobies7_2_04.nex`

COI sequences file:`COIGobies10_10_03.nex`

Mitochondrial control region (D-loop) sequences:`DlpGobies20_5_04ed.nex`

Appendix 2. Detailed list of specimens and sequences

1- "Genetic ID" indicates the species identification from DNA, where different from morphological ID. "?" indicates Genetic ID was not possible
2- Refer to Table 2 for full location details
3- Dashes (-) under Locus indicate that amplification and sequencing was attempted for this specimen, but was not successful.
4- "G" indicates a full genomic DNA extraction; "GC" indicates a chelex DNA extraction

	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC181	Acentrogobius	bifrenatus		Kangaroo.I. S.Aus	2/7/04	Michael Hammer	AbifKIAusGC181	dlp		
GC180	Acentrogobius	bifrenatus		Kangaroo.I. S.Aus	2/7/04	Michael Hammer	AbifKIAusGC180	dlp		
GC127	Acentrogobius	bifrenatus		Rozelle Bay-NSW	no date	Aust.Mus.	AbifRB-NSWGC127	dlp		
GC128	Acentrogobius	bifrenatus		BlackWattleB-NSW	17/5/01	Aust.Mus.	AbifBWB-NSWGC128	dlp		
GC134	Acentrogobius	bifrenatus		FullertonCove-NSW	12/11/97	Aust.Mus.	AbifFC-NSWGC134	dlp		
GC31	Acentrogobius	bifrenatus		Matapouri	7/5/03	Natalie Usmar	AbifMatapouriGC31	dlp		
GC32	Acentrogobius	bifrenatus		Matapouri	7/5/03	Natalie Usmar	AbifMatapouriGC32	dlp		
GC33	Acentrogobius	bifrenatus		Matapouri	7/5/03	Natalie Usmar	AbifMatapouriGC33	dlp		
GC34	Acentrogobius	bifrenatus		Matapouri	7/5/03	Natalie Usmar	AbifMatapouriGC34	dlp		
GC35	Acentrogobius	bifrenatus		Matapouri	7/5/03	Natalie Usmar	AbifMatapouriGC35	dlp		
GC163	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC163	dlp		
GC164	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC164	dlp		
GC165	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC165	dlp		
GC165a	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC165a	dlp		
GC166	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC166	dlp		
GC167	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC167	dlp		
GC168	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC168	dlp		
GC169	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC169	dlp		
GC170	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC170	dlp		
GC171	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC171	dlp		
GC172	Acentrogobius	bifrenatus		Adelaide Aus.	2/7/04	Michael Hammer	AbifAdeAusGC172	dlp		
GC143	Acentrogobius	bifrenatus	?	Waitemata	21/11/03	Natalie Usmar	AbifWaitemataGC143	-		
G27	Acentrogobius	bifrenatus		Waitemata	8/2/01	NIWA	AbifWaitG27	dlp		16S
G33	Acentrogobius	bifrenatus		Waitemata	8/2/01	NIWA	AbifWaitG33	dlp		
G36	Acentrogobius	bifrenatus		Whangarei	9/3/01	NIWA	AbifWreiG36	dlp	co1	

	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC73	Acentrogobius	bifrenatus		Whangateau	1/5/03	Natalie Usmar	AbifWhangateauGC73	dlp		
GC74	Acentrogobius	bifrenatus		Whangateau	1/5/03	Natalie Usmar	AbifWhangateauGC74	dlp		
GC75	Acentrogobius	bifrenatus		Whangateau	1/5/03	Natalie Usmar	AbifWhangateauGC75	dlp		
GC76	Acentrogobius	bifrenatus		Whangateau	1/5/03	Natalie Usmar	AbifWhangateauGC76	dlp		
G28	Acentrogobius	bifrenatus		Yamba NSW	25/3/02	Aust. Mus.	AbifYamNSWG28	dlp	co1	16S
G29	Acentrogobius	bifrenatus		Yamba NSW	25/3/02	Aust. Mus.	AbifYamNSWG29	dlp	co1	16S
G30	Acentrogobius	bifrenatus		Yamba NSW	25/3/02	Aust. Mus.	AbifYamNSWG30	dlp	co1	16S
G13	Acentrogobius	caninus		HongKong	4/3/03	Tom Millaochich	AcanHongKongG13	dlp	co1	16S
G14	Acentrogobius	caninus		HongKong	4/3/03	Tom Millaochich	AcanHongKongG14	dlp	co1	16S
G15	Acentrogobius	caninus		HongKong	4/3/03	Tom Millaochich	AcanHongKongG15	dlp	co1	16S
G34	Acentrogobius	frenatus		Port Philip B. Vic	7/2/03	Museum Victoria	AfrnPPVicG34	dlp	co1	16S
GC41	Acentrogobius	pflaumii	**exquisitus**	Waitemata	9/2/01	NIWA	F(pfl)exqWaitemataGC41	dlp		
GC42	Acentrogobius	pflaumii	**exquisitus**	Waitemata	9/2/01	NIWA	F(pfl)exqWaitemataGC42	dlp		
GC43	Acentrogobius	pflaumii	**exquisitus**	Waitemata	9/2/01	NIWA	F(pfl)exqWaitemataGC43	dlp		
GC44	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC44	-		
GC45	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC45	-		
GC46	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC46	-		
GC47	Acentrogobius	pflaumii	**exquisitus**	Waitemata	9/2/01	NIWA	F(pfl)exqWaitemataGC47	-	co1	
GC48	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC48	-		
GC49	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC49	-		
GC50	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC50	-		
GC51	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC51	-		
GC52	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC52	-		
GC53	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC53	-		
GC54	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC54	-		
GC55	Acentrogobius	pflaumii	**exquisitus**	Waitemata	9/2/01	NIWA	F(pfl)exqWaitemataGC55	dlp		
GC56	Acentrogobius	pflaumii	**?**	Waitemata	9/2/01	NIWA	F(pfl)WaitemataGC56	-		
GC155	Acentrogobius	pflaumii		Whangapoua	23/3/02	NIWA	ApflaumiWhangapouaGC155	dlp		
GC156	Acentrogobius	pflaumii		Whangapoua	23/3/02	NIWA	ApflaumiWhangapouaGC156	dlp		
GC157	Acentrogobius	pflaumii		Whangapoua	23/3/02	NIWA	ApflaumiWhangapouaGC157	dlp		
GC158	Acentrogobius	pflaumii		Whangapoua	23/3/02	NIWA	ApflaumiWhangapouaGC158	dlp		

	Original morphological ID		Genetic ID[1]			collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC144	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC144	dlp		
GC145	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC145	dlp		
GC146	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC146	dlp		
GC147	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC147	dlp		
GC148	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC148	dlp		
GC149	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC149	dlp		
GC150	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC150	dlp		
GC151	Acentrogobius	pflaumii	?	Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC151	-		
GC152	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC152	dlp		
GC153	Acentrogobius	pflaumii		Waitemata	9/2/01	NIWA	ApflaumiWaitemataGC153	dlp		
GC132	Acentrogobius	pflaumii		Glebe. I. -NSW	no date	Aust.Mus.	ApflG-NSWGC132	dlp		
GC133	Acentrogobius	pflaumii		Glebe. I. -NSW	no date	Aust.Mus.	ApflG-NSWGC133	dlp		
GC129	Acentrogobius	pflaumii		White. Bay-NSW	17/5/01	Aust.Mus.	ApflWB-NSWGC129	dlp		
G19	Acentrogobius	pflaumii		TokyoJapan	6/3/03	Yuji Ikeda	ApflTkJapanG19	dlp		16S
G20	Acentrogobius	pflaumii		TokyoJapan	6/3/03	Yuji Ikeda	ApflTkJapanG20	dlp	co1	16S
G21	Acentrogobius	pflaumii		TokyoJapan	6/3/03	Yuji Ikeda	ApflTkJapanG21	-		16S
G54	Acentrogobius	pflaumii	**exquisitus**	Waitemata	9/2/01	NIWA	F(pfl)exqWaitemataG54	dlp		
GC135	Acentrogobius	pflaumii	**exquisitus**	Whangapoua	16/4/02	NIWA	F(pfl)exqWhangapouaGC135	dlp		
GC136	Acentrogobius	pflaumii	**?**	Whangapoua	16/4/02	NIWA	F(pfl)WhangapouaGC136	-		
GC137	Acentrogobius	pflaumii	**exquisitus**	Whangapoua	16/4/02	NIWA	F(pfl)exqWhangapouaGC137	dlp		
GC138	Acentrogobius	pflaumii	**exquisitus**	Whangapoua	16/4/02	NIWA	F(pfl)exqWhangapouaGC138	dlp		
GC139	Acentrogobius	pflaumii	**exquisitus**	Whangapoua	16/4/02	NIWA	F(pfl)exqWhangapouaGC139	dlp		
GC140	Acentrogobius	pflaumii	**?**	Whangapoua	16/4/02	NIWA	F(pfl)WhangapouaGC140	-		
GC141	Acentrogobius	pflaumii	**?**	Whangapoua	16/4/02	NIWA	F(pfl)WhangapouaGC141	-		
GC142	Acentrogobius	pflaumii	**exquisitus**	Whangapoua	16/4/02	NIWA	F(pfl)exqWhangapouaGC142	dlp		
G22	Acentrogobius	pflaumii		Sydney	17/5/01	Aust. Mus.	ApflSydneyG22	dlp		16S
G23	Acentrogobius	pflaumii		Sydney	17/5/01	Aust. Mus.	ApflSydneyG23	dlp		16S
G24	Acentrogobius	pflaumii		Sydney	17/5/01	Aust. Mus.	ApflSydneyG24	dlp	co1	16S
G16	Acentrogobius	pflaumii		KagoshimaJapan	20/2/03	Tomoki Sunobe	ApflKgJapanG16	dlp	co1	16S
G17	Acentrogobius	pflaumii		KagoshimaJapan	20/2/03	Tomoki Sunobe	ApflKgJapanG17	dlp	co1	16S
G18	Acentrogobius	pflaumii		KagoshimaJapan	20/2/03	Tomoki Sunobe	ApflKgJapanG18	dlp	co1	16S

	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC173	Acentrogobius	tamarensis	?	Adel. S.Aus	2/7/04	Michael Hammer	AtamAdeAusGC173	dlp?		
GC174	Acentrogobius	tamarensis	?	Adel. S.Aus	2/7/04	Michael Hammer	AtamAdeAusGC174	dlp?		
GC154	Acentrogobius?	pflaumii?	pflaumi	Waitemata	8/2/01	NIWA	ApflaumiWaitemataGC154	dlp		
GC2	Acentrogobius?	pflaumii?	pflaumii	Waitemata	8/2/01	NIWA	F(pfl)exqWaitemataGC2	dlp		
GC3	Acentrogobius?	pflaumii?	pflaumii	Waitemata	8/2/01	NIWA	F(pfl)exqWaitemataGC3	dlp		
GC4	Acentrogobius?	pflaumii?	pflaumii	Waitemata	8/2/01	NIWA	F(pfl)exqWaitemataGC4	dlp		
GC5	Acentrogobius?	pflaumii?	pflaumii	Waitemata	8/2/01	NIWA	F(pfl)exqWaitemataGC5	dlp		
GC1	Acentrogobius??	pflaumii??	??	Waitemata	4/2/01	NIWA	Fpfl?WaitemataGC1	-?		
GC182	blank									
G12	*Myersina*	filifer		HongKong	4/3/03	Tom Millaochich	CrpfilHongKongG12	dlp	co1	16S
GC130	Favonigobius	lateralis		SydneyH-NSW	3/5/01	Aust.Mus.	FlatSH-NSWGC130	dlp		
GC87	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC87	dlp		
GC88	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC88	dlp		
GC89	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC89	dlp		
GC90	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC90	dlp		
GC91	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC91	dlp		
GC92	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC92	dlp		
GC93	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC93	dlp		
GC94	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC94	-		
GC95	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC95	dlp		
GC96	Favonigobius	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC96	dlp		
GC102	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC102	dlp		
GC103	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC103	-		
GC104	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC104	-		
GC105	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC105	dlp		
GC106	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC106	dlp		
GC107	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC107	dlp		
GC108	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC108	dlp		
GC109	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC109	dlp		
GC110	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC110	-		
GC111	Favonigobius	lentiginosus		Whangarei	20/3/01	NIWA	FlenWhangareiGC111	dlp		

	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC131	Favonigobius	tamarensis	**lentiginosus?**	SydneyH-NSW	8/10/98	Aust.Mus.	FtamSH-NSWGC131	dlp		
G57	Favonigobius.	exquisitus		Coromandel	20/2/01	NIWA	FexqCorG57	dlp		
GC38	Favonigobius.	exquisitus		Coromandel	20/2/01	NIWA	FexqCoromandelGC38	dlp		
GC58	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC58	dlp		
GC59	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC59	dlp		
GC60	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC60	dlp		
GC61	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC61	dlp		
GC62	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC62	dlp		
GC63	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC63	dlp		
GC64	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC64	dlp		
GC65	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC65	dlp		
GC66	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC66	dlp		
GC67	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC67	dlp		
GC68	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC68	dlp		
GC69	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC69	dlp		
GC70	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC70	dlp		
GC71	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC71	dlp		
GC72	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA	FexqWhangapouaGC72	dlp		
G46	Favonigobius.	exquisitus		Kaipara	30/3/01	NIWA	FexqKaiparaG46	dlp		
G48	Favonigobius.	exquisitus		Kaipara	14/3/01	NIWA	FexqKaiparaG48	dlp		
G47	Favonigobius.	exquisitus		Kaipara	15/3/01	NIWA	FexqKaiparaG47	-		
G51	Favonigobius.	exquisitus		Kawhia	8/3/01	NIWA	FexqKawhiaG51	dlp		
G52	Favonigobius.	exquisitus		Kawhia	8/3/01	NIWA	FexqKawhiaG52	-		
GC12	Favonigobius.	exquisitus		Tauranga	28/2/01	NIWA	FexqTaurangaGC12	dlp	co1	
GC13	Favonigobius.	exquisitus		Tauranga	28/2/01	NIWA	FexqTaurangaGC13	dlp		
GC14	Favonigobius.	exquisitus		Tauranga	28/2/01	NIWA	FexqTaurangaGC14	dlp		
GC15	Favonigobius.	exquisitus		Tauranga	28/2/01	NIWA	FexqTaurangaGC15	-		
GC16	Favonigobius.	exquisitus		Tauranga	28/2/01	NIWA	FexqTaurangaGC16	dlp		
G49	Favonigobius.	exquisitus		Manukau	14/2/01	NIWA	FexqManukauG49	dlp		
G50	Favonigobius.	exquisitus		Manukau	12/2/01	NIWA	FexqManukauG50	dlp		
G61	Favonigobius.	exquisitus		Ohiwa	24/2/01	NIWA	FexqOhiG61	dlp		

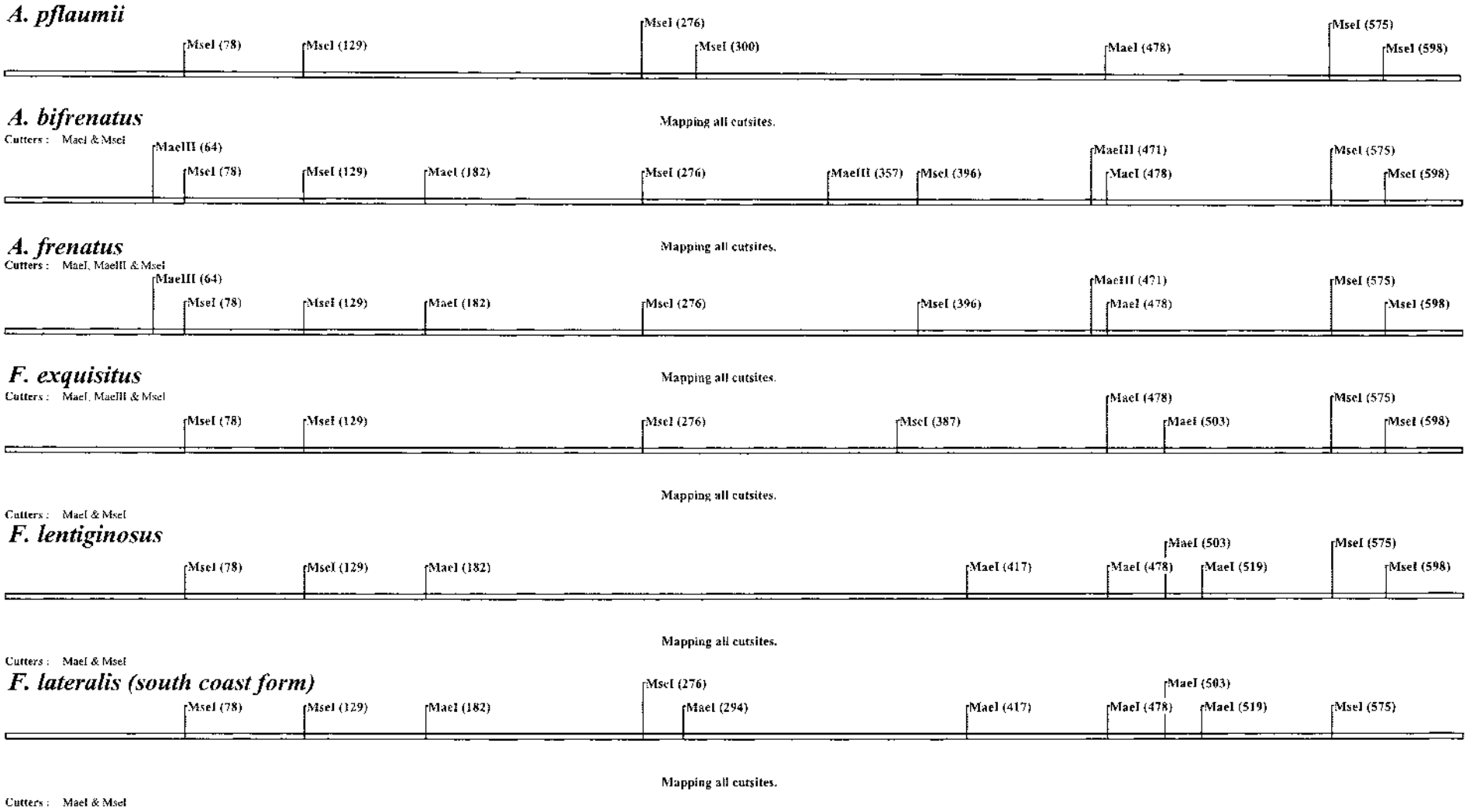
	Original morphological ID		Genetic ID[1]		collection				Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date		Source	Specimen code in figs.	Dlp	CO1	16s
G60	Favonigobius.	exquisitus		Tauranga	23/2/01	NIWA		FexqTaurG60	dlp		
GC36	Favonigobius.	exquisitus		Tauranga	23/2/01	NIWA		FexqTaurangaGC36	dlp		
GC11	Favonigobius.	exquisitus		Waitemata	8/2/01	NIWA		FexqWaitemataGC11	dlp	co1	
G55	Favonigobius.	exquisitus		Waitemata	9/2/01	NIWA		FexqWaitemataG55	dlp		
G56	Favonigobius.	exquisitus		Waitemata	9/2/01	NIWA		FexqWaitemataG56	dlp		
GC40	Favonigobius.	exquisitus		Waitemata	9/2/01	NIWA		FexqWaitemataGC40	dlp		
GC17	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA		FexqCorWhangGC17	dlp	co1	
GC18	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA		FexqCorWhangGC18	dlp	co1	
GC19	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA		FexqCorWhangGC19	dlp		
GC20	Favonigobius.	exquisitus		Whangapoua	23/3/02	NIWA		FexqCorWhangGC20	dlp		
G58	Favonigobius.	exquisitus		Whangapoua	22/2/01	NIWA		FexqCorWhangG58	dlp		
G53	Favonigobius.	exquisitus		Whangarei	20/3/01	NIWA		FexqWhangareiG53	dlp		
G59	Favonigobius.	exquisitus		Whitianga	23/3/02	NIWA		FexqCorWhitG59	dlp		
GC39	Favonigobius.	exquisitus		Whitianga	22/2/01	NIWA		FexqWhitiangaGC39	dlp		
GC37	Favonigobius.	exquisitus		Whitianga	22/2/01	NIWA		FexqWhitiangaGC37	dlp		
GC175	Favonogobius	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC175	dlp		
GC176	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC176	dlp		
GC177	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC177	dlp		
GC178	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC178	dlp		
GC179	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC179	dlp		
GC183	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC183	dlp		
GC184	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC184	dlp		
GC185	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC185	dlp		
GC186	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC186	dlp		
GC187	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC187	dlp		
GC188	Favonigobius.	lateralis	lat.(S coast)	Adel. S.Aus	2/7/04	Michael	Hammer	FlatAdeAusGC188	dlp		
G42	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey		FlenMangG42	dlp		16S
GC6	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey		FlenMangawhaiGC6	dlp	co1	
GC7	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey		FlenMangawhaiGC7	dlp		
GC8	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey		FlenMangawhaiGC8	dlp	co1	
GC9	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey		FlenMangawhaiGC9	dlp		

	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC10	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC10	dlp		
GC77	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC77	dlp		
GC78	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC78	dlp		
GC79	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC79	dlp		
GC80	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC80	dlp		
GC81	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC81	dlp		
GC82	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC82	dlp		
GC83	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC83	dlp		
GC84	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC84	dlp		
GC85	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC85	dlp		
GC86	Favonigobius.	lentiginosus		Mangawhai	3/1/02	A.Hickey	FlenMangawhaiGC86	dlp		
GC26	Favonigobius.	lentiginosus		Matapouri	7/5/03	Natalie Usmar	FlenMatapouriGC26	dlp		
GC27	Favonigobius.	lentiginosus		Matapouri	7/5/03	Natalie Usmar	FlenMatapouriGC27	dlp		
GC28	Favonigobius.	lentiginosus		Matapouri	7/5/03	Natalie Usmar	FlenMatapouriGC28	dlp		
GC29	Favonigobius.	lentiginosus		Matapouri	7/5/03	Natalie Usmar	FlenMatapouriGC29	dlp		
GC30	Favonigobius.	lentiginosus		Matapouri	7/5/03	Natalie Usmar	FlenMatapouriGC30	dlp		
G41	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWpoaG41	dlp		16S
G40	Favonigobius.	lentiginosus		Whangarei	20/3/01	NIWA	FlenWreiG40	dlp	co1	
GC21	Favonigobius.	lentiginosus		Whangateau	1/5/03	Natalie Usmar	FlenWhangateauGC21	dlp		
GC22	Favonigobius.	lentiginosus		Whangateau	1/5/03	Natalie Usmar	FlenWhangateauGC22	dlp		
GC23	Favonigobius.	lentiginosus		Whangateau	1/5/03	Natalie Usmar	FlenWhangateauGC23	dlp		
GC24	Favonigobius.	lentiginosus		Whangateau	1/5/03	Natalie Usmar	FlenWhangateauGC24	dlp		
GC25	Favonigobius.	lentiginosus		Whangateau	1/5/03	Natalie Usmar	FlenWhangateauGC25	dlp		
GC112	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC112	dlp		
GC113	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC113	dlp		
GC114	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC114	dlp		
GC115	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC115	dlp		
GC116	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC116	dlp		
GC117	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC117	dlp		
GC118	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC118	dlp		
GC119	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC119	dlp		

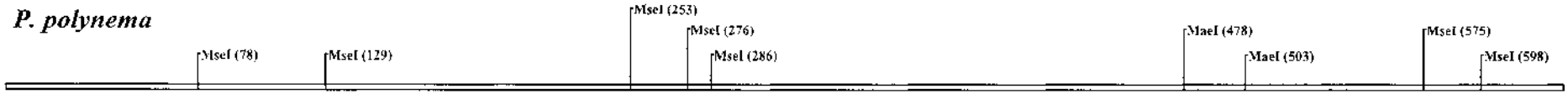
	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
GC120	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC120	dlp		
GC121	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC121	dlp		
GC122	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC122	dlp		
GC123	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC123	dlp		
GC124	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC124	dlp		
GC125	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC125	dlp		
GC126	Favonigobius.	lentiginosus		Whangapoua	23/3/02	NIWA	FlenWhangapouaGC126	dlp		
G39	Favonigobius.	lentiginosus??	**exquisitus**	Wrei	20/3/01	NIWA	F(len)exqWreiG39	dlp		16S
GC159	Favonigobius.	pflaumii		Waitamata	9/2/01	NIWA	ApflaumiWaitamataGC159	dlp		
GC160	Favonigobius.	pflaumii		Waitamata	9/2/01	NIWA	ApflaumiWaitamataGC160	dlp		
GC161	Favonigobius.	pflaumii		Waitamata	9/2/01	NIWA	ApflaumiWaitamataGC161	dlp		
GC162	Favonigobius.	pflaumii		Waitamata	9/2/01	NIWA	ApflaumiWaitamataGC162	dlp		
GC97	Favonigobius.	exquisitus		Whangateau	1/5/03	Natalie Usmar	FexqWhangateauGC97	dlp		
GC98	Favonigobius.	exquisitus		Whangateau	1/5/03	Natalie Usmar	FexqWhangateauGC98	dlp		
GC99	Favonigobius.	exquisitus		Whangateau	1/5/03	Natalie Usmar	FexqWhangateauGC99	dlp		
GC100	Favonigobius.	exquisitus		Whangateau	1/5/03	Natalie Usmar	FexqWhangateauGC100	dlp		
GC101	Favonigobius.	exquisitus		Whangateau	1/5/03	Natalie Usmar	FexqWhangateauGC101	-		
GC57	Favonigobius	exquisitus		Waitemata	8/2/01	NIWA	FexqWaitemataGC57	dlp		
G43	Favonigobius	exquisitus		MyallNSW	22/1/98	Aust. Mus.	FexqMyallNSWG43	dlp		
G44	Favonigobius	exquisitus		MyallNSW	23/1/98	Aust. Mus.	FexqMyallNSWG44	-		
G45	Favonigobius	exquisitus		MyallNSW	24/1/98	Aust. Mus.	FexqMyallNSWG45	dlp		
G25	Favonigobius	exquisitus		Wait	8/2/01	NIWA	FexqWaitG25	dlp		16S
G26	Favonigobius	exquisitus		Wait	8/2/01	NIWA	FexqWaitG26	dlp		16S
G31	Favonigobius	exquisitus		Wait	9/2/01	NIWA	FexqWaitG31	dlp		16S
G32	Favonigobius	exquisitus		Wait	9/2/01	NIWA	FexqWaitG32	dlp	co1	16S
G35	Favonigobius	lateralis		PortPhilipBVic	7/2/03	Museum Victoria	FlatPPVicG35	dlp	co1	16S
G37	Favonigobius	lentiginosus(lateralis)	**exquisitus**	Sydney	17/5/01	Aust. Mus.	F(lat/len)exqSydneyG37	dlp	co1	16S
G38	Favonigobius	lentiginosus(lateralis)	**exquisitus**	Sydney	17/5/01	Aust. Mus.	F(lat/len)exqSydneyG38	dlp	co1	
G4	Gobiopsis	atrata		Mokohinaus NZ	7/4/03	K.Clements	GobatrMkNZG4	dlp	co1	16S
G5	Gobiopsis	atrata		Mokohinaus NZ	8/4/03	K.Clements	GobatrMkNZG5	dlp		16S
G9	Oxyurichthys	tentacularis		HongKong	4/3/03	Tom Millaochich	OxytenHongKongG9	-	co1	16S

	Original morphological ID		Genetic ID[1]		collection			Locus[3]		
Extract#[4]	Genus	sp.	sp.	Location[2]	date	Source	Specimen code in figs.	Dlp	CO1	16s
G10	Oxyurichthys	tentacularis		HongKong	5/3/03	Tom Millaochich	OxytenHongKongG10	-	co1	16S
G11	Oxyurichthys	tentacularis		HongKong	6/3/03	Tom Millaochich	OxytenHongKongG11	-		16S
G6	Parachaeturichthys	polynema		HongKong	4/3/03	Tom Millaochich	ParpolHongKongG6	dlp	co1	16S
G7	Parachaeturichthys	polynema		HongKong	4/3/03	Tom Millaochich	ParpolHongKongG7	dlp	co1	16S
G8	Parachaeturichthys	polynema		HongKong	4/3/03	Tom Millaochich	ParpolHongKongG8	-		16S
G1	Thalasseleotris	sp.		Mokohinaus NZ	14/2/03	K.Clements	ThspMkNZG1	dlp	co1	
G2	Thalasseleotris	sp.		Mokohinaus NZ	7/4/03	K.Clements	ThspMkNZG2	dlp	co1	16S
G3	Thalasseleotris	sp.		Mokohinaus NZ	7/4/03	K.Clements	Thsp MkNZG3	dlp	co1	16S

Appendix 3. Diagnostic 16s RFLP patterns for all species.
Restriction enzyme sites on the 16s fragment are shown for each species



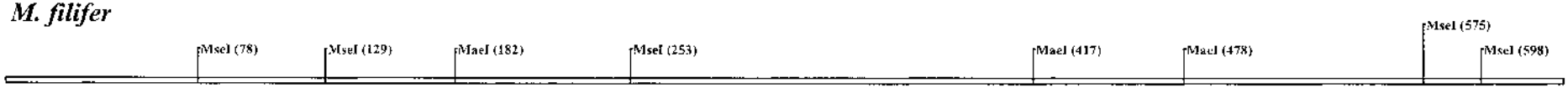
P. polynema



Mapping all cutsites.

Cutters : MaeI & MseI

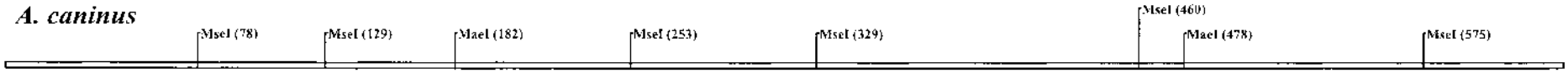
M. filifer



Mapping all cutsites.

Cutters : MaeI & MseI

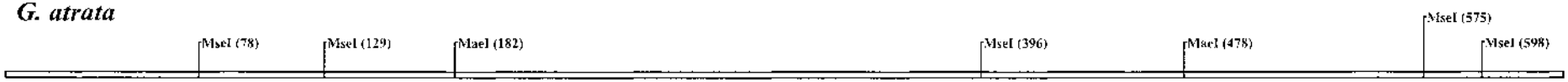
A. caninus



Mapping all cutsites.

Cutters : MaeI & MseI

G. atrata



Mapping all cutsites.

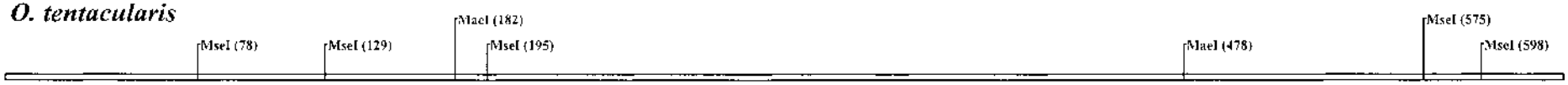
Cutters : MaeI & MseI

***Thalasseleotris* sp.**

Mapping all cutsites.

Cutters : MaeI & MseI

O. tentacularis



Mapping all cutsites.

Cutters : MaeI & MseI