Molecular genetics of cirrhitoid fishes (Perciformes: Cirrhitoidea): phylogeny, taxonomy, biogeography, and stock structure

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Statements

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October 2000

Summary

The molecular phylogenetic relationships within three of the five cirrhitoid fish families were reconstructed from mitochondrial DNA cytochrome *b*, cytochrome oxidase I, and D-loop sequences.

Analysis of the Cheilodactylidae provided evidence that much taxonomic revision is required. The molecular data suggest that this family should be restricted to the two South African *Cheilodactylus*, as they are highly divergent from the other cheilodactylids and one member is the type species. The remaining 25 cheilodactylids should be transferred to the Latridae. Nine of the non South African *Cheilodactylus* can be allocated to three new genera; *Goniistius* (elevated to generic rank), *Zeodrius* (resurrected), and *Morwong* (resurrected), while the placement of three species is uncertain. The three South African *Chirodactylus* should revert to *Palunolepis*, as they are distinct from the South American type species *Chirodactylus variegatus*. *Acantholatris* clusters within *Nemadactylus*, and the former should be synonymised. Cryptic speciation has occurred within *Cheilodactylus (Goniistius) vittatus*.

The generic allocation of the four latrid species is sound, although this family should be expanded to encompass all but two cheilodactylids. Relative levels of genetic divergence within the Aplodactylidae support the most recent revision of this family, during which the monotypic genus *Crinodus* was synonymised with *Aplodactylus*.

Molecular phylogenetic relationships and estimates of divergence time obtained from molecular clock calibrations suggest a dominant role of long distance dispersal for the present distribution of cheilodactylid and aplodactylid fishes. Suggestions that ancestral taxa were vicariantly isolated during the fragmentation of Gondwana are rejected, as estimated divergence times appreciably postdate this event. Dispersal and radiation of *Nemadactylus* and *Acantholatris* throughout the Southern Ocean was particularly recent, occurring within the last 0.6-2.6 Myr. The waters of Australia and New Zealand represent a likely origin for this dispersal, and at least two events are identified, one eastward. Similarly, it appears that

aplodactylids also originated in the waters of Australia and New Zealand, but in this instance the majority of radiation was undertaken prior to colonisation of the southeastern Pacific. Ocean currents and long duration offshore pelagic larvae probably facilitated dispersal.

Phylogeographic analysis of the antitropically-distributed cheilodactylid subgenus *Goniistius* identified three transequatorial divergences, rather than a minimum of two as inferred from the distributions of individual taxa. The identified divergences also occurred during two distinct periods, the mid Miocene and mid to late Pliocene, and are best explained by chance dispersal or vicariance resulting from biotic interactions or temperature changes.

The levels of genetic separation for three cirrhitoid species pairs with east-west allopatric distributions across southern Australia reject the possibility that the members of each pair diverged simultaneously during a shared vicariance event. Although the levels of genetic separation were similar for *Goniistius* and *Aplodactylus* pairs, separate north and south coast vicariance events are invoked based on likely thermal tolerances. Speciation resulting from chance dispersal and the founding of new populations is rejected due to the absence of barriers sufficiently large to isolate taxa with such high dispersal capabilities. Estimated divergence times fall between the late Miocene and mid Pliocene, and fail to implicate recent Pleistocene glaciations.

Seven microsatellite loci were characterised for *Nemadactylus macropterus* in an effort to resolve its stock structure in Australian waters and to assess the resolving power of different molecular techniques. Microsatellites did not identify any stock structuring in the waters of southern Australia. Divergence was also absent between Australian and New Zealand populations, which contrasted the findings from allozyme and mitochondrial DNA studies. Homoplasy of alleles at highly polymorphic loci is offered as a possible explanation for the lower resolution of stock structure obtained with microsatellites.

The microsatellites characterised for *N. macropterus* were also employed to examine the taxonomic status of the morphologically similar South American species *N. bergi*. Separate status was supported by divergence at a single locus. Microsatellites also provided evidence for a recent bottleneck in the effective population size of *N. bergi*, but not *N. macropterus* or *A. monodactylus*. Based on this observation, the mitochondrial DNA lineage monophyly observed for *N. bergi*, but not *N. macropterus* or *A. monodactylus*, may reflect the influence of effective population size on the time required for complete sorting of mitochondrial lineages.

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CHAPTER 1: General introduction.

General biology

Cirrhitoids are a group of marine fishes comprising five families and approximately 76 species: Cirrhitidae, Chironemidae, Aplodactylidae, Cheilodactylidae, and Latridae. The Cirrhitidae is widely represented in tropical waters, particularly those of the Indo-Pacific (Randall, 1963), while the other cirrhitoid families predominantly occupy temperate waters of the Southern Hemisphere, with greatest diversity around Australia and New Zealand (Lamb, 1990; Meléndez, 1990; Russell, 2000). Most cirrhitoids are sedentary over nearshore reef substrates as adults (Randall, 1963; Last *et al.*, 1983; Sano and Moyer, 1985; Cappo 1995, Stepien, 1990; Lowry and Suthers, 1998), and feed on small benthic invertebrates (Andrew and Hecht, 1992; Wöhler and Sánchez, 1994; McCormick, 1998), although aplodactylids are almost completely herbivorous (Benavides *et al.*, 1994). Larvae occur pelagically in offshore waters for durations of up to 12 months (Annala, 1987; Andrew *et al.*, 1995). Maximum ages can exceed 25 years, with generation times typically of 2-10 years (Françis, 1981; Annala, 1987; Andrew *et al.*, 1995). Cheilodactylids and latrids are commonly exploited for human consumption, while cirrhitids are targeted for the aquarium market.

Taxonomy

There are a number of problems regarding cirrhitoid taxonomy, at the family, genus and species levels. The majority of problems involve generic allocation within the Cheilodactylidae, and these have not been resolved despite numerous morphological studies (Allen and Heemstra, 1976; Smith, 1980; Randall, 1983; Lamb, 1990).

The species composition of *Cheilodactylus* and *Chirodactylus* are questionable. These groups presently encompass 18 of the 27 recognised cheilodactylids. In the most recent review of cheilodactylid taxonomy, Lamb (1990) suggested that *Cheilodactylus* should be restricted to the South African members of this genus, as these were equally divergent from the remaining *Cheilodactylus* as any other genus. Regarding the generic placement of non South African *Cheilodactylus*, Lamb (1990) suggested elevating the subgenus *Goniistius* to generic rank,

and resurrecting *Morwong* for the remainder. However, the distinction of *Goniistius* and *Morwong* from *Chirodactylus* is dubious (Lamb, 1990).

The separate status of the South American cheilodactylid species *Nemadactylus bergi* is questionable. This species is morphologically similar to the Australian and New Zealand species *N. macropterus*, and distinguishing features are inconsistent among the holotype and paratypes of *N. bergi* (R.W.G. White, University of Tasmania, Australia, 1995, pers. comm.).

The taxonomy of the Latridae was considered sound, with three genera and four species recognised (Gon and Heemstra, 1987; Lamb, 1990). However, there has been a recent suggestion that the cheilodactylid genera *Nemadactylus* and *Acantholatris* should be transferred to the Latridae based on urohyal form (Greenwood, 1995). This is in conflict with the predominant external features used to distinguish the two families, namely the presence of thickened lower pectoral rays which are also produced beyond the fin membrane (Lamb, 1990).

The taxonomy of the Aplodactylidae has undergone considerable change despite comprising a small number of taxa. The most recent revision recognised five species and one genus, synonymising the monotypic *Crinodus* with *Aplodactylus* (Russell, 2000). The Chironemidae comprises six species and two genera, *Chironemus* and the monotypic *Threpterius*. The only recent change to this group was the transfer of *Cheilodactylus bicornis* from the Cheilodactylidae to *Chironemus* (Meléndez, 1990).

The most recent revision of the Cirrhitidae was conducted by Randall (1963), during which six new species and one new genus were described, and a total of 34 species and 10 genera were recognised. One monotypic genus was later transferred to the Serranidae (Randall and Heemstra, 1978), another two species were synonymised with existing cirrhitids (Randall, 1973, 1997), and three new species were described (Lavenberg and Yañez 1972; Kotthaus, 1976; Lubbock, 1977). The species identified by Lavenberg and Yañez (1972) from Easter

Island is problematic. It contains features in common with both *Cirrhitus* and *Amblycirrhitus*, and may comprise a new genus (J.E. Randall, B.P. Bishop Museum, Hawaii, 1996 pers. comm.). Additional questions of cirrhitid taxonomy include the specific status of colour morphs observed for *Paracirrhites forsteri*, *P. arcatus*, and *P. hemistictus*.

Historical biogeography

There are a number of interesting questions regarding the historical biogeography of cirrhitoid fishes. The majority of these involve possible explanations for widespread and disjunct distributions.

The four temperate cirrhitoid families are each widespread in the Southern Hemisphere, and their distributions may be explained by vicariant isolation accompanying the fragmentation of Gondwana, chance oceanic dispersal, or a combination of both. The occupation of multiple Southern Hemisphere continents is consistent with Gondwanan origins, and such distributions are observed for the Aplodactylidae and Cheilodactylidae. However, a role of dispersal in widespread cirrhitoid distributions is implicated by the occupation of isolated islands and seamounts, and high dispersal capabilities are suggested by the 7-12 month offshore pelagic larval stage observed for several taxa (Annala, 1987; Andrew *et al.*, 1995). As the sequence and timing of Gondwana fragmentation is well understood (Lawver *et al.*, 1992), dispersal and vicariance hypotheses may be discriminated by the reconstruction of species-area (phylogeographic) relationships and the estimation of lineage divergence times.

The Cheilodactylidae is unique among cirrhitoids in that this family is antitropically distributed (Randall, 1981, 1983). The subgenus *Goniistius* is represented in subtropical and temperate waters both north and south of the equator, yet not in the intervening tropical waters. Antitropical and other similar disjunct distributions have long intrigued biogeographers, and several dispersal and vicariance hypotheses have been offered as possible explanations (Hubbs, 1952; Ekman, 1953; Rehder, 1980; Valentine, 1984; Nelson, 1985). Several of these theories invoke climatic and geological events of known age, such as

glaciations and continental fragmentation. Consequently, such theories can be assessed as explanations for the antitropical distribution of *Goniistius* by the estimation of lineage divergence time. The antitropical distribution of *Goniistius* is also particularly interesting as multiple transequatorial divergences are suggested, but knowledge of the frequency and direction of these events requires a resolved species phylogeny.

Similar east-west allopatric and parapatric distributions are observed for several putatively geminate taxa in the marine waters of southern Australia (Knox, 1980; Edgar, 1986; Hutchins, 1987). It has been suggested that many of these distributions have a common vicariant origin, with ancestral taxa isolated by decreases in water temperature or sea level (Edgar, 1986; Hutchins, 1987). Alternatively, east-west allopatric and parapatric distributions of geminate taxa may reflect chance dispersal across large expanses of inhospitable habitat and the founding of new populations. There are four cirrhitoid species pairs with east-west allopatric distributions across southern Australia. Evidence for a shared vicariant history would comprise confirmed geminate status and similar estimates of divergence time for the members of each pair. Estimates of divergence time can also be compared to known climatic transitions, such as Pleistocene glaciations, which have been commonly implicated for vicariance along the south coast (Knox, 1980; Edgar, 1986; Hutchins, 1987).

Population genetics

Several cirrhitoids are commercially exploited, and as such they should be managed in an effort to maintain sustainable yields and genetic variation. As populations in different regions are often genetically and demographically independent, even in continuously distributed species, separate management of distinct populations or "stocks" is desirable for the maintenance of genetic variation and to avoid regional over-exploitation (Carvalho and Hauser, 1995).

Nemadactylus macropterus is a commercially exploited cheilodactylid of New Zealand and southern Australia. The stock structure of this species has been the subject of both genetic and

non-genetic studies for management purposes. Allozyme electromorph and mitochondrial DNA (mtDNA) characters did not detect stock structuring in Australian waters, and identified only slight but significant divergence between Australian and New Zealand populations (Elliott and Ward, 1994; Grewe *et al.*, 1994). In contrast, otolith microchemistry and larval advection studies proposed three stocks within the waters of southeast Australia alone (Thresher *et al.*, 1994; Bruce *et al.*, 1996). A comparatively new class of molecular characters, called microsatellites, have identified stock structure in marine species not detected by other molecular techniques (Bentzen *et al.*, 1996; Ruzzante *et al.*, 1996, 1998; Shaw *et al.*, 1999a, b). As both allozyme and mtDNA data have been collected for *N. macropterus*, the application of microsatellites to this species offers additional information on both stock structure and the relative resolving power of different molecular techniques.

Aims

The first aim of this study is to examine cirrhitoid taxonomy by reconstructing phylogenetic relationships from mitochondrial DNA sequence data. The majority of taxonomic studies conducted on cirrhitoids have been restricted to external features (Randall, 1963; Allen and Heemstra, 1976; Randall, 1983; Lamb, 1990; Russell, 2000), and they have often been somewhat reserved in their conclusions due to the low number of characters analysed and disagreement among them. A greater number of characters can be readily scored during molecular studies, and specific characters can be chosen which are more likely to be informative for the question under investigation (Meyer, 1994). Molecular studies can also test for reproduction isolation of taxa which differ only in subtle features, such as colouration (e.g. Smith *et al.*, 1996).

The second aim of this study is to address questions relating to the historical biogeography of cirrhitoids. Addressing questions of historical biogeography often requires information of phylogeographic relationships and lineage divergence times (Avise, 1992; Bowen and Grant, 1997; Waters and Burridge, 1999). In addition to the advantages of molecular data for the reconstruction of phylogenetic relationships outlined above, molecular variation can also be

used as a proxy for divergence time. If the accumulation of molecular variation is observed to be clock-like among lineages, the levels of variation can be considered relative measures of lineage divergence time (Vawter *et al.*, 1980). In addition, if molecular clock calibrations are available, the levels of genetic variation can be translated into geological dates (Vawter *et al.*, 1980; Bermingham *et al.*, 1997).

The third aim of this study is to examine the stock structure of *Nemadactylus macropterus* based on microsatellite characters, and to compare the resolving power of those molecular techniques applied to this species.

Due to the requirement of suitably preserved material for DNA analysis, the phylogenetic components of this study will be largely restricted to those groups for which fresh material is readily collectable. These groups comprise the Aplodactylidae, Cheilodactylidae, and Latridae.

CHAPTER 2: General molecular systematic methods

DNA extraction

Genomic DNA was extracted from frozen or ethanol preserved muscle tissue using a modified CTAB protocol (Hillis *et al.*, 1990). For each DNA extraction performed a small sample of tissue, approximately 40 mg, was isolated and partially macerated with a sterile scalpel blade. Samples were then incubated for 2h at 60°C in 600 µL of CTAB buffer (100 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 55 mM hexadecyltrimethylammonium bromide, 0.002% 2-mercaptoethanol [v/v]) with 5 µL of proteinase K (20 mg/mL). Samples were homogenised further during incubation using power pestles and occasional vortex-mixing.

DNA was purified by successive extraction. An initial extraction was performed with an equal volume of chloroform-iso-amyl alcohol (24:1 v/v) and centrifugation at 12 000 G for 20 min, followed by two extractions of the supernatant with equal volumes of phenol-chloroform-iso-amyl alcohol (25:24:1 v/v) at 12 000 G for 10 minutes. A brief extraction was performed again with chloroform-iso-amyl alcohol to remove any trace of phenol. The resultant supernatant was mixed with 1.5 volumes of iso-propanol and incubated overnight at -20°C. DNA was pelleted by centrifugation at 12 000 G for 20 min, washed twice with 500 µL of 70% ethanol, and then dried under vacuum. DNA was eluted in dH_2O to give a final concentration of 20-100 ng/µL.

PCR primers

The mitochondrial genome comprises a variety of different sequence types which many be employed for phylogenetic inference. These sequences differ in their length (base pairs) and rate of nucleotide substitution (variability). Consequently, specific regions within the mitochondrial genome are favoured for phylogenetic studies depending on the degree to which the subject taxa have diverged. During this study the cytochrome *b* region was first targeted for phylogenetic analysis. Within the mitochondrial genome, protein-coding genes such as cytochrome *b* exhibit median levels of variation. Consequently, if there was

insufficient variation detected with cytochrome *b*, the more variable non-coding D-loop region could be targeted. Alternatively, if there was too much variation within cytochrome *b*, resulting in extensive homoplasy from superimposed nucleotide substitutions, less variable rRNA or tRNA genes could be analysed. If the level of variability for cytochrome *b* was adequate, but more characters were required to reliably resolve phylogenetic relationships, other protein coding sequences could be analysed, such as cytochrome oxidase I.

During this study polymerase chain reaction (PCR) amplifications of mitochondrial DNA fragments employed the universal oligonucleotide primers L6586 and H7086, flanking a region of the cytochrome *c* oxidase subunit I gene (Palumbi *et al.*, 1991), and L14841 and H15149, flanking a region of the cytochrome *b* gene (Kocher *et al.*, 1989). Primer L14724 (Pääbo, 1990) was used to amplify cytochrome *b* sequences when there was inadequate amplification with L14841. Sequences corresponding to the left domain of the D-loop were obtained using the primers proline-light (5' AACTC TCACC CCTAR CTCCC AAAG 3') and D-loop-heavy (5' GGCCC TGAAR TAGGA ACCAR ATG 3').

PCR amplification and sequencing

Amplifications were conducted in 50 µL volumes, containing reaction buffer and 1.0 units of *Taq* DNA polymerase (Fisher Biotech), 200 µM dNTPs, 0.5 µM of each oligonucleotide primer, 1.5-2.5 mM $MgCl_2$, and 25-100 ng of genomic DNA. Thermal cycling conditions for cytochrome oxidase I were 10 cycles of 94°C/30 sec, 65°C/30 sec, and 72°C/60 sec, followed by 25 cycles with annealing at 55°C instead of 65°C. Conditions for the amplification of cytochrome *b* sequences were identical with the exception that annealing was conducted at 45°C for the first ten cycles, while D-loop sequences were amplified with all annealing at 60°C. An initial denaturation of 94°C/5 min and a final extension of 72°C/10 min were employed for all sequences.

The results of PCR were assessed by 1.0% agarose gel electrophoresis, with visualisatioin under UV radiation after ethidium bromide staining. Templates were gel purified using the

QIAquick Gel Extraction Kit (Qiagen). DNA sequences were determined using either the *fmol* DNA sequencing system (Promega) with [γ^{33}P]ATP end-labelled primers, or the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc.). Both light and heavy strands were sequenced for each template, enabling the verification of character-states.

Data analysis

The majority of analyses were conducted using PAUP 3.1.1 (Swofford, 1993), demonstration and beta versions of PAUP*4.0 (Swofford, 1999), and components of the PHYLIP 3.573 package (Felsenstein, 1993).

Where combined analysis of different gene sequences was desired, phylogenetic congruence of datasets was investigated using the partition homogeneity test of Farris *et al.* (1995), as implemented by PAUP*. Two-hundred partition replicates were analysed by the heuristic (100 random sequence addition replicates) or branch and bound search algorithm. Pairwise homogeneity chi-squared analysis was performed to identify any significant difference in the nucleotide composition of sites variable among taxa.

An approximate indication of phylogenetic information content was derived from the skewness of tree length-frequency distributions (Hillis and Huelsenbeck, 1992). Skewness was quantified by $g1$ values, which were either calculated for the entire tree-length frequency distibution (exhaustive parsimony search), or estimated from the construction of 1,000,000 random trees using the RANDOM TREES and GENERATE TREES options of PAUP 3.1.1 and PAUP* respectively.

Maximum parsimony analysis (Cavalli-Sforza and Edwards, 1967) was conducted using the exhaustive, branch and bound (Hendy and Penny, 1982), or heuristic search algorithms of PAUP, depending on the number of taxa analysed. Accelerated character-state optimisation

(ACCTRAN) and tree bisection-reconnection (TBR) branch swapping were employed. Four weighting schemes were used for transition (TI) and transversion (TV) nucleotide substitutions. These were equal weighting, exclusion of transitions, exclusion of transitions at third codon positions, and increased weighting of transversions in accordance with the reciprocal of the observed frequencies, the optimum TI/TV from maximum likelihood analysis (see below), or by an arbitrary value. Consistency (Kluge and Farris, 1969) and retention (Farris, 1989) indices were calculated based on all characters. Measures of support for tree nodes were obtained from nonparametric bootstrap analysis (Felsenstein, 1985a), based on 2000 replicate data sets and retaining groups compatible with the 50% majority-rule consensus.

Neighbour-joining analysis (Saitou and Nei, 1987) was performed using components of PHYLIP. DNADIST was used to calculate pairwise sequence distances, corrected for multiple substitutions by the Kimura (1980) two-parameter model using a TI/TV of 2.0 or that obtained from maximum likelihood analysis (see below). Clustering of pairwise distances was conducted using NEIGHBOR. Bootstrap analysis was performed on 2000 replicate data sets created by SEQBOOT, and consensus trees were calculated by CONSENSE.

Maximum likelihood analysis was conducted using DNAML of PHYLIP, with empirical base frequencies, one category of substitution rate, global rearrangements, and ten randomisations of sequence input order. The expected TI/TV nucleotide substitution ratio was either optimised to achieve a near-asymptotic likelihood value, or a value of 2.0 was used.

The number of additional steps required to achieve alternative topologies was determined using the CONSTRAINTS option of PAUP. Significantly different topologies were identified by the nonparametric two-tailed Wilcoxon signed ranks test (Templeton, 1983; Felsenstein, 1985b). When n was greater than 20 a normal approximation of the test statistic with correction for ties was calculated by SYSTAT 5.2 (SPSS Inc.). The parametric Kishino and

Hasegawa (1989) test implemented by DNAML was used to identify significant differences in the likelihoods of alternative topologies.

The two-cluster and branch-length tests of Takezaki *et al.* (1995) were employed to identify significant deviation from nucleotide substitution rate constancy, using programs distributed by the authors (iubio.bio.indiana.edu/soft/molbio/evolve/lintre). Tests were conducted on third codon positions only, using the Kimura (1980) two-parameter model. Branch lengths were also calculated under the assumption of clock-like evolution for the estimation of lineage divergence times.

Two-dimensional scaling was performed to enable the visualisation of relative genetic distances between sequences. The Kruskal loss function and monotonic regression were employed, implemented by SYSTAT 5.2.

CHAPTER 3: Molecular phylogeny of *Nemadactylus* and *Acantholatris* (Cheilodactylidae), with implications for taxonomy and biogeography.

Abstract

Mitochondrial DNA sequences were obtained from all five species of *Nemadactylus*, two of the three *Acantholatris* species, and several outgroup taxa. Analysis of cytochrome *b* sequences placed *A. monodactylus* and *A. gayi* within a clade otherwise composed of all *Nemadactylus* clade, suggesting that these genera are synonymous. The *Acantholatris* sequences were also very similar to those from three of the *Nemadactylus* species, despite their geographic separation. Analysis of D-loop sequences paralleled the cytochrome *b* results, but provided greater resolution of species relationships. *Nemadactylus* sp. and *A. gayi* are transoceanic sister taxa. Polytypic clades observed for *N. macropterus* and *A. monodactylus* most likely reflect incomplete sorting of mitochondrial DNA lineages. It is proposed that these taxa dispersed and radiated during the last 0.3-0.6 million years, and the possible mechanisms of this process are discussed.

Introduction

The species of *Nemadactylus* Richardson and *Acantholatris* Gill are marine perciforms that occur in subtropical to cool-temperate waters throughout the Southern Hemisphere (Figure 3.1). Juveniles and adults occur around reefs, at depths of 1-350 m (Annala, 1987; Wöhler and Sánchez, 1994; Andrew *et al.*, 1995). A common trait of these and related taxa is an offshore pelagic larval phase of 7-12 months in duration, which implies high dispersal capabilities (Annala, 1987; Andrew *et al.*, 1995). These species mature at 2-6 years, and serially spawn large numbers of small eggs (Annala, 1987; Andrew *et al.*, 1995; Jordan, 1997); maximum ages exceed 25 years (Andrew *et al.*, 1995; Jordan, 1997).

There are five recognised species of *Nemadactylus*. Four of these are restricted to the waters of Australia and New Zealand, and the remaining species occurs along the east coast of South America (Figure 3.1). One species, commonly called "king tarakihi", was recently proposed (Roberts, 1993; Smith *et al.*, 1996). The record of *N. macropterus* (Bloch and Schneider) at Saint Paul and Amsterdam Island by Agnot (1951) was a false identification according to Duhamel (1989). *Acantholatris* is not represented in the waters of Australia or New Zealand. Instead, its three members occur around isolated islands and seamounts such as Juan Fernández and the Desventuradas in the southeastern Pacific, and those which form a loose chain from Tristan da Cunha and Gough Island in the South Atlantic, to Saint Paul and Amsterdam Islands in the Indian Ocean (Figure 3.1).

The taxonomy of these species as based on external characteristics has been problematic. Although Allen and Heemstra (1976) synonymised *Acantholatris* with *Cheilodactylus* Lacépède, the characters described by Gill (1862) and Smith (1980), particularly the presence of a relatively long and narrow anal fin, distinguish *Acantholatris* from the cheilodactylid genera *Cheilodactylus*, *Chirodactylus* Gill and *Dactylophora* DeVis (Lamb, 1990). While the remaining cheilodactylid genus *Nemadactylus* shares the majority of characteristics which define *Acantholatris*, including the long narrow anal fin, these genera have been separated

primarily on the number of anal fin rays, with the former having 14-19, and the latter 10-12 (Gill, 1862; Lamb, 1990).

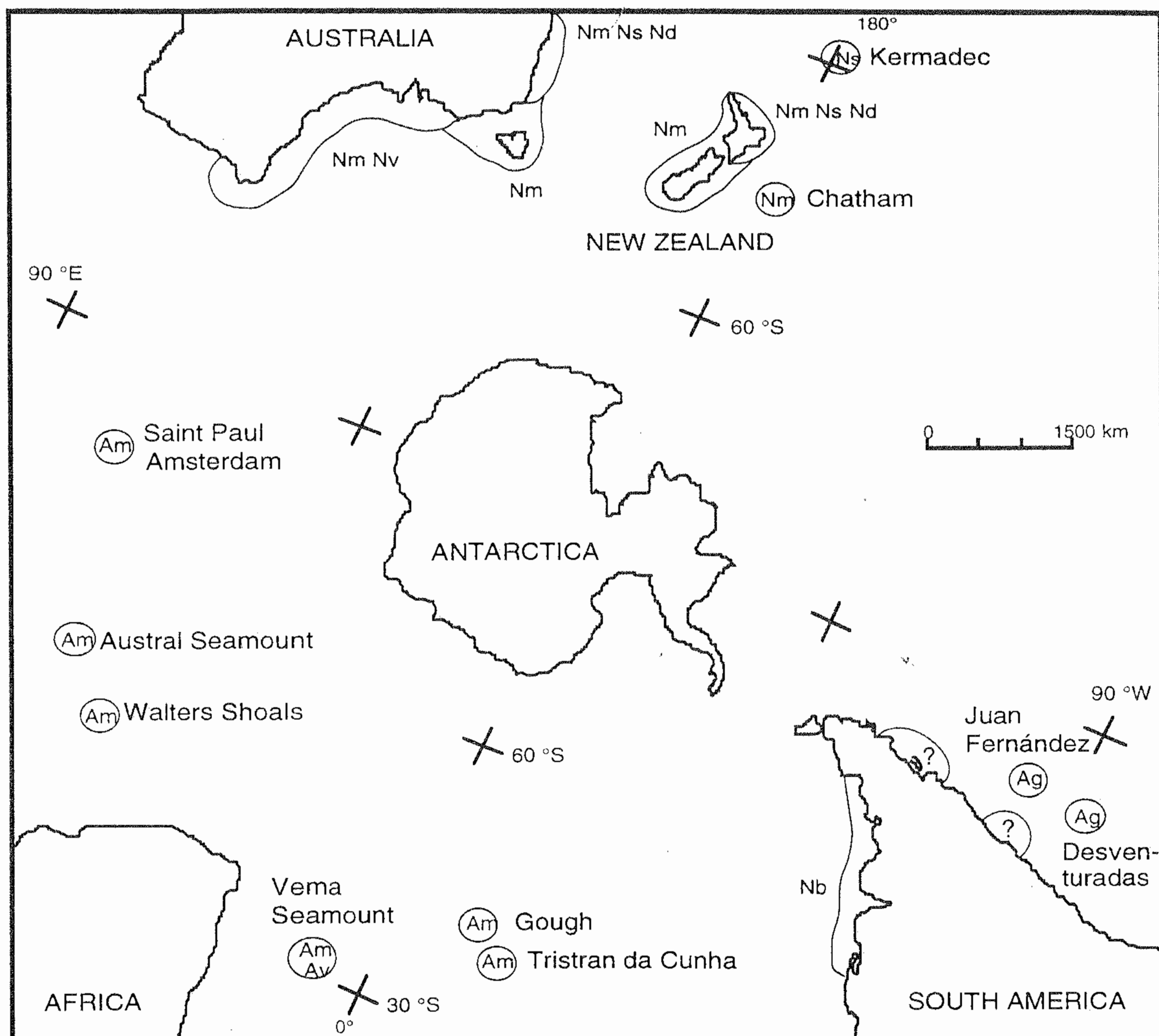

Figure 3.1. Distribution of *Nemadactylus* and *Acantholatris* species. Ns=*Nemadactylus* sp., Nm=*N. macropterus*, Nb=*N. bergi*, Nd=*N. douglasii*, Nv=*N. valenciennesi*, Am=*A. monodactylus*, Av=*A. vemae*, Ag=*A. gayi*; ?=possible records of *A. gayi* (R. Meléndez C., Museo Nacional de Historia Natural, Santiago, Chile, 1998, pers. comm.).

Nemadactylus bergi (Norman) and *A. gayi* (Kner) have been called *Cheilodactylus* in recent non-systematic literature (e.g. Meléndez and Villalba, 1992; Wöhler and Sánchez, 1994), and were also indexed as *Acantholatris* in the systematic work by Greenwood (1995). These species are obviously not members of *Cheilodactylus* given their relatively long and narrow

anal fins, and since they have anal fin ray counts of 14-15 and 12 they should be referred to as *Nemadactylus* and *Acantholatris* respectively (Lamb, 1990).

Nemadactylus bergi and *N. macropterus* are morphologically similar, but have been distinguished by the width of the supra-cleithrum relative to the diameter of the eye, the relative lengths of their thickened pectoral fin rays, and lateral line scale counts (Norman, 1937; Lamb, 1990). However, two of these differences are not consistent among the holotype and paratypes of *N. bergi* (R.W.G. White, University of Tasmania, Australia, 1995, pers. comm.), and the separate status of this species requires justification.

In addition to their taxonomic uncertainties, the distribution patterns and potentially high dispersal capabilities of *Nemadactylus* and *Acantholatris* makes them interesting subjects for a molecular phylogenetic study. The biogeography and radiation of these and similarly distributed fishes has attracted some attention (Eschmeyer and Hureau, 1971; Briggs, 1974; Wilson and Kaufmann, 1987; Collette and Parin, 1991; Andrew *et al.*, 1995), but no molecular studies have been conducted. Members of the rock lobster genus *Jasus* have distributions and dispersal capabilities similar to *Nemadactylus* and *Acantholatris*, and their radiation have, together with that of the related genus *Panulirus*, received much attention (Pollock, 1990, 1992, 1993; George, 1997), including two molecular phylogenetic studies (Brasher *et al.*, 1992; Ovenden *et al.*, 1997). The theories regarding the radiation of these rock lobsters may well be applicable for much of the Southern Hemisphere marine fauna with similar species distributions and dispersal capabilities.

The aim of this study was to obtain molecular data from species of *Nemadactylus* and *Acantholatris* that would clarify their taxonomy, and provide phylogenetic information about their radiation with respect to biogeographic processes and events.

Materials and methods

Frozen or ethanol preserved muscle and liver tissues were obtained from all species of *Nemadactylus* and *Acantholatris* with the exception of *A. vemae* Penrith, for which only formalin-fixed material was easily accessible (Table 3.1). The technique of Shedlock *et al.* (1997) was employed for formalin-fixed material. Tissues from representatives of the remaining cheilodactylid genera, and *Cirrhitus splendens* Ogilby (Cirrhitidae) were also obtained for use as outgroups. The Cirrhitidae is considered the most plesiomorphic of the five cirrhitoid families (Greenwood, 1995).

Table 3.1. Collection details of *Acantholatris* and *Nemadactylus* individuals analysed.

Species	Collection site	Month/Year	Individuals
A. monodactylus (Carmichael)	Tristan Island	/1994	3
A. monodactylus (Carmichael)	Gough Island	/1994	3
A. monodactylus (Carmichael)	Saint Paul Island	3/1997	9
A. monodactylus (Carmichael)	Amsterdam Island	2-3/1997	7
A. gayi (Kner)	Juan Fernández	2-3/1997	3
A. vemae Penrith	Vema Seamount[a]	6/1966	1
N. macropterus (Bloch & Schneider)	Albany, Australia	10/1991 - 2/1992	32
N. macropterus (Bloch & Schneider)	Tasman Island, Tasmania	9/1991	1
N. macropterus (Bloch & Schneider)	New Zealand	4/1992	1
Nemadactylus sp.	Kiama, Australia[b]	6/1994	1
Nemadactylus sp.	Three Kings Islands, N. Z.	4/1994	2
N. bergi (Norman)	Mar del Plata, Argentina	8/1996	51
N. douglasii (Hector)	Coffs Harbour, Australia[b]	6/1994	1
N. valenciennesi (Whitley)	Port Lincoln, Australia	5/1995	1

[a] South African Museum specimen 25030 (formalin-fixed).

[b] Australian Museum frozen tissue collection (*Nemadactylus* sp. I.34845-001, *N. douglasii* I.34844-001).

D-loop sequences were aligned using CLUSTALW 1.7 (Thompson *et al.*, 1994). Gaps were treated as missing data or analysed as a separate character-state. D-loop nucleotide diversities (π) and their standard deviations were calculated using DnaSP 2.52 (Rozas and Rozas, 1997), according to equations 10.5 and 10.7 of Nei (1987). D-loop haplotype diversities (h) were calculated using equation 8.5 of Nei (1987).

To test for the presence of certain character-states, PCR amplified D-loop fragments were digested with *Dpn* II and *Hinc* II restriction endonucleases. Digests were conducted in 10 μL volumes, containing 5 μL of amplified DNA, 0.5 units of either enzyme, and 1 μL of the appropriate concentrated buffer (New England Biolabs). Digests were incubated at 37 °C for 1 h, and the results were visualised as for PCR amplification, using a 100 bp increment size standard (Promega).

Results

The protocol of Shedlock *et al.* (1997) was successful in obtaining DNA fragments of up to 450 bp in length from formalin-fixed *A. vemae* material. However, attempts at the amplification of cytochrome *b* and D-loop sequences from this DNA were not successful.

Cytochrome *b*

With the exception of *A. vemae*, cytochrome *b* sequences were obtained from one individual of each *Nemadactylus* and *Acantholatris* species and several outgroup taxa (Genbank accession numbers AF067084 - AF067095). Among the species of *Nemadactylus* and *Acantholatris*, substitutions were observed at 27 of the 307 nucleotide positions analysed, and the variation was phylogenetically informative at 10 of these. The majority of the substitutions observed in the entire dataset were transitions at third codon positions, and this pattern, combined with the absence of length mutations, suggested that orthologous sequences were obtained. The estimated tree length-frequency distribution for these sequences was significantly skewed ($g1$=-0.61, P<0.01), suggesting the presence of phylogenetic signal.

Unweighted maximum parsimony analysis recovered a single most-parsimonious tree of 172 steps (Figure 3.2). The sequences from *A. monodactylus* and *A. gayi* clustered within an otherwise entirely *Nemadactylus* clade. The *Acantholatris* sequences were also very similar to those from *Nemadactylus* sp., *N. macropterus*, and *N. bergi*, forming a single clade with a maximum of only three transition substitutions, or 0.98% corrected sequence divergence,

between any of these five taxa (Table 3.2). The sister clade of this group comprised *N. douglasii* and *N. valenciennesi*. The sequences of these two clades differed by 14-18 transitions and two to three transversions, corresponding to 5.39-7.15% sequence divergence (Table 3.2). High bootstrap values (>70%) were observed for the entire *Nemadactylus-Acantholatris* clade, the *N. valenciennesi* and *N. douglasii* clade, and the clade containing the five most similar *Nemadactylus* and *Acantholatris* sequences (Figure 3.2). The low bootstrap value for the relationship between the *Nemadactylus -Acantholatris* clade and that containing other cheilodactylids reflects variation in the composition of the latter.

Both maximum likelihood and neighbour-joining analyses produced topologies identical to that from unweighted parsimony analysis. Parsimony analysis with increased weighting of transversions over transitions in accordance with the reciprocal of their observed frequencies also recovered the same topology. The bootstrap values from this weighted parsimony and the neighbour-joining analysis were similar to those from unweighted parsimony. Parsimony analysis restricted to transversion substitutions produced a slightly different topology, but this was probably due to the small number of informative characters.

The two-cluster test did not reveal third codon position nucleotide substitution rate heterogeneity when nodes were analysed individually (CP<95%), or when nodes were analysed simultaneously (χ^2=14.23, df=10, 0.50>P >0.25). The branch-length test also did not detect significant rate heterogeneity (CP<95%).

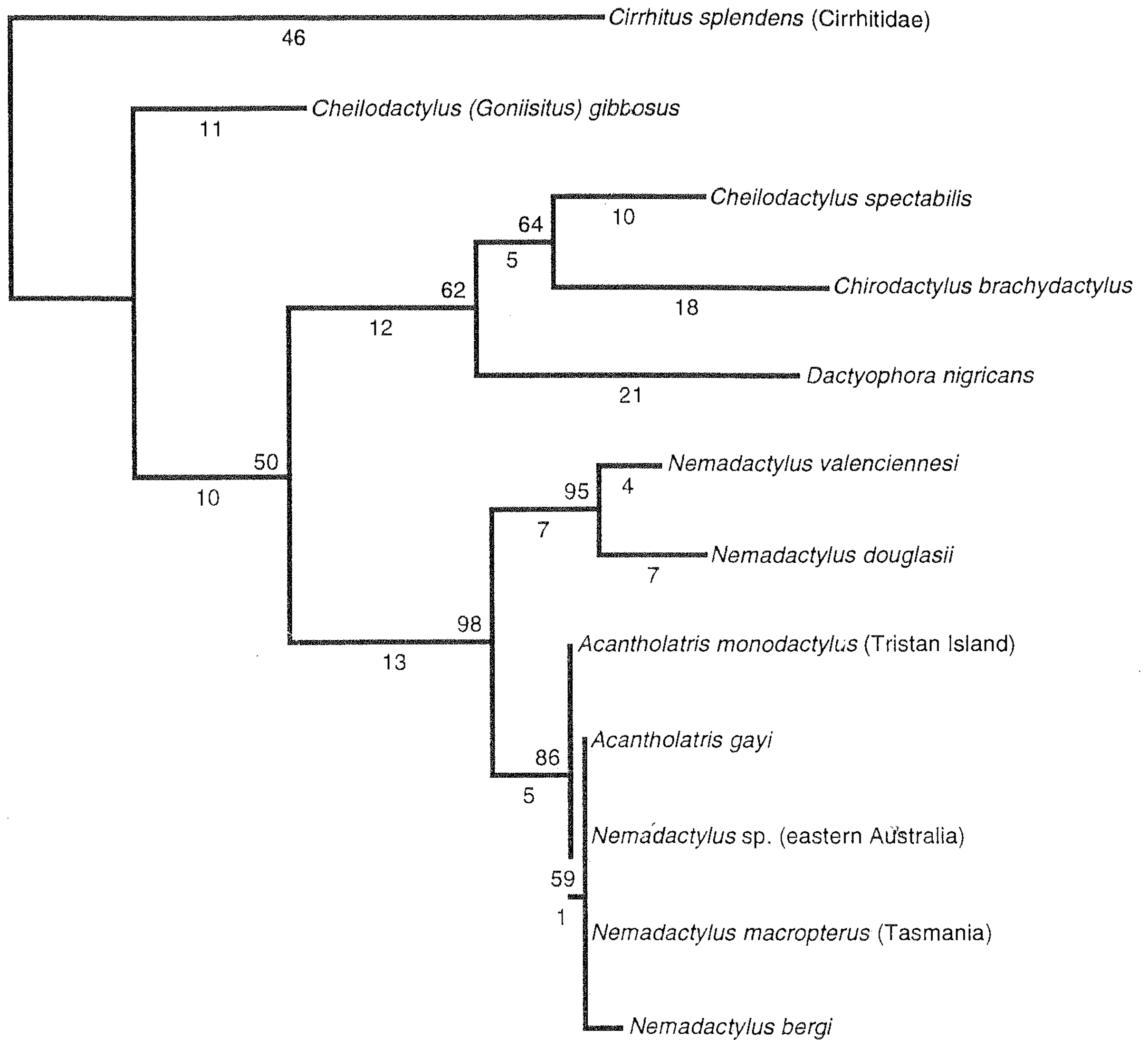

Figure 3.2. Single most-parsimonious cladogram from the analysis of 307 bp partial mitochondrial DNA cytochrome *b* sequences from *Nemadactylus* and *Acantholatris* species. *Cirrhitus splendens* (Cirrhitidae) and representatives of the other cheilodactylid genera were included as outgroups. The Branch and Bound algorithm was employed and all character-state changes were equally weighted. Branch lengths are proportional to the number of substitutions, quantified by the numbers below the branches. Bootstrap proportions for the taxa in each clade, as derived from 1000 replicates, are indicated by the numbers above the braches at each node. Tree length=172 steps, CI=0.744, RI=0.651.

D-loop

D-loop

Sequences representing the left domain of the D-loop were obtained from the outgroup *C. spectabilis* and 1-7 individuals per species of *Nemadactylus* and *Acantholatris*, excluding *A.*

vemae (Genbank accession numbers AF067096 - AF067120, AF072876 - AF072877). The sequences analysed varied in length between 360 and 366 base pairs, and the favoured alignment was 373 characters long (Figure 3.3). A total of 150 characters were variable among *Nemadactylus* and *Acantholatris*, and 91 of these were phylogenetically informative. Transition substitutions were observed at 125 of the variable sites, while transversions were observed at 62. Gaps were inserted at 19 positions for sequence alignment, with nine of these positions representing otherwise invariant sites. No identical sequences were identified for the D-loop region, and intraspecific nucleotide diversities ranged from 0.018 to 0.054 (Table 3.2). The estimated tree length-frequency distribution of all sequences was significantly skewed ($g1$=-1.25, P<0.01), suggesting the presence of phylogenetic signal. The amplified fragments also contained 28 bp of the tRNA proline gene and a putative termination associated sequence (TAS). These were invariant among taxa, suggesting that orthologous sequences were obtained.

Unweighted maximum parsimony analysis of sequences, as aligned in Figure 3.3 with gaps treated as missing data, produced a single most-parsimonious tree of 311 (Figure 3.4a). As observed from the analysis of cytochrome *b*, *A. monodactylus* and *A. gayi* sequences clustered within an otherwise entirely *Nemadactylus* clade. The *Acantholatris* sequences were again closest to those of *Nemadactylus* sp., *N. macropterus*, and *N. bergi*, but the D-loop sequences provided greater resolution amongst these taxa.

Nemadactylus sp. and *A. gayi* sequences were structured as sister clades, with predominantly high bootstrap values for their relationships (Figure 3.4a). The sequences from *N. macropterus*, *N. bergi*, and *A. monodactylus* were structured into four clades. Three of these were polytypic, individually containing both *N. macropterus* and *A. monodactylus* sequences, while the remaining clade was monotypic for *N. bergi*. The *N. macropterus* (Western Australia) and *A. monodactylus* (Amsterdam Island 1) sequence clade was distinguished due to its distance from other sequences. Although the bootstrap values representing three of these four clades were quite high, the inferred relationships between these clades received only moderate

(50-70%) bootstrap values. The sequences from *N. douglasii* and *N. valenciennesi* again formed the sister clade to the other *Nemadactylus* and *Acantholatris* sequences.

Table 3.2. Interspecific variation of partial cytochrome *b* and D-loop sequences and measures of intraspecific partial D-loop sequence variation. Values above the diagonal represent observed substitutions, while those below the diagonal are Kimura (1980) corrected percentage sequence divergences obtained with a transition/transversion ratio of 2.0. For each pairwise comparison the upper value represents cytochrome *b*, and the lower value is D-loop. Nucleotide diversities (π), their standard deviations, and haplotype diversities (*h*) were calculated using equations 10.5, 10.7, and 8.5 of Nei (1987) respectively.

	1	2	3	4	5	6	7	8
INTERSPECIFIC VARIATION								
1 *Acantholatris monodactylus*		1	1	1	3	19	16	35
		30	22	31	20	90	86	82
2 *Acantholatris gayi*	0.33		0	0	2	19	16	36
	8.76		24	17	23	92	81	75
3 *Nemadactylus macropterus*	0.33	0.00		0	2	19	16	36
	6.36	6.97		28	19	94	88	82
4 *Nemadactylus* sp.	0.33	0.00	0.00		2	19	16	36
	9.10	4.84	8.18		29	91	83	81
5 *Nemadactylus bergi*	0.98	0.65	0.65	0.65		21	18	36
	5.71	6.62	5.44	8.44		87	82	78
6 *Nemadactylus douglasii*	6.45	6.45	6.45	6.45	7.15		11	38
	31.74	32.78	33.51	32.44	30.43		67	101
7 *Nemadactylus valenciennesi*	5.39	5.39	5.39	5.39	6.08	3.66		36
	29.32	27.58	30.19	28.43	27.69	21.43		100
8 *Cheilodactylus spectabilis*	12.32	12.70	12.70	12.70	12.70	13.55	12.76	
	28.33	25.68	28.37	27.9	26.74	38.01	36.92	
INTRASPECIFIC D-LOOP VARIATION								
Haplotypes discerned (n)	6	3	5	3	7	1	1	1
Maximum sequence divergence (%)	10.33	3.38	6.96	2.24	2.8	-	-	-
Minimum sequence divergence (%)	0.83	0.83	2.79	1.39	1.38	-	-	-
Haplotype diversity (*h*)	1.00	1.00	1.00	1.00	1.00	-	-	-
Nucleotide diversity (π)	0.054	0.024	0.050	0.018	0.022	-	-	-
Standard deviation of π	0.012	0.008	0.008	0.005	0.003	-	-	-

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A. monodactylus (Tristan Is.)              CATAAAT_TT T_TAGTGCAT ATATGTAATT TCAACATATT ATGTTTTTAA CCATAATATT CAGAGTATAA  70
A. monodactylus (Gough Is.)                ......A... .T........ .......... .......... .......... .......... ..........
A. monodactylus (Saint Paul Is.)           .....__... .T........ .......... .......... .......... .......... ..........
A. monodactylus (Amsterdam Is. 1)          ......A... .T........ .......... .......... .......... ......C... ..........
A. monodactylus (Amsterdam Is. 2)          ......A... .T........ .......... .......... .......... ......C... ..A.......
A. monodactylus (Amsterdam Is. 3)          ..C...A... .T........ .......... .......... .......... ......C... ..A.......
A. gayi 1                                  ......_... .T..A..... .......... .......... .......... ......C... .........C
A. gayi 2                                  ......A... .G..A..... .......... .......... .......... ......C... .........C
A. gayi 3                                  ......A... .G..A..... .......... .......... .......... ......C... .........C
N. macropterus (Tasmania)                  ....._AA.. .T........ .....C.... .......... .......... ......C... ..........
N. macropterus (West Australia 1)          ......A... .T........ .......... .......... .......... ......C... ..........
N. macropterus (West Australia 2)          ....._AA.. .T........ .......... .......... .......... ......C... ..........
N. macropterus (West Australia 3)          ....._AA.. .T........ .......... .......... .......... ......CG.. ..G.......
N. macropterus (New Zealand)               ......A... .T........ .......... .......... .......... .......... ..........
Nemadactylus sp. (east. Australia)         ....._A... .T..A..... .......... .......... .......... ......C... .........C
Nemadactylus sp. (New Zealand 1)           ......_... .T..A..... .......... .......... .......... ......C... .........C
Nemadactylus sp. (New Zealand 2)           ......_... .T..A..... .......... .......... .......... ......C... .........C
N. bergi 1                                 ......A... .T........ .......... .......... .......... ......C... ..........
N. bergi 2                                 ....._AA.. .T........ .......... .......... .......... ......C... ..........
N. bergi 3                                 ....._AA.. .T........ .......... .......... .......... ......C... ..........
N. bergi 4                                 ....._AA.. .T........ .......... .......... .......... ......C... ..........
N. bergi 5                                 .....__A.. .T........ .......... .......... .......... ......C... ..........
N. bergi 6                                 ....._AA.. .T........ .......... .......... .......... ......C... ..........
N. bergi 7                                 ....._AA.. .T........ .......... .......... .......... ......C... ..........
N. douglasii                               ..C..__... .T........ .......... .T.......C ......._.. .......CCC .C.G....._
N. valenciennesi                           ..C..__... .T........ .......... .......... ..A....... ......C..C ..........
Cheilodactylus spectabilis                 T...GCAT.. .AC.A.A... .......... ...C...... ...A.A.... .........C ..AG..T.._

A. monodactylus (Tristan Is.)              _GCGCATACT TATGTTTTAT CACCATATAT GAAACTAGTC ACTATCAGG  CCACCATAAT ACTAAGGTAT  140
A. monodactylus (Gough Is.)                .......... .......... .......... .......... .......... .......... ..........
A. monodactylus (Saint Paul Is.)           .......... .......... .......... .......... G......... .......... ..........
A. monodactylus (Amsterdam Is. 1)          .......... .......... .......... A......... .......... T..T...... ......A...
A. monodactylus (Amsterdam Is. 2)          .......... .......... .......... .......... .......A.. .T........ ..........
A. monodactylus (Amsterdam Is. 3)          .......... .......... .......... .......... .......A.. .T........ ..........
A. gayi 1                                  .........C .......... .......... A......... .......A.. TT......G. ..........
A. gayi 2                                  .........C .......... .......... A......... .......A.. .T.....G.. ..C.......
A. gayi 3                                  .........C .......... .......... A......... .......A.. .T.....G.. ......A...
N. macropterus (Tasmania)                  .......... .......... .......... A......... .......A.. .T........ ..........
N. macropterus (West Australia 1)          .......... .......... .......... A......... .......... .T.T....G. ..........
N. macropterus (West Australia 2)          .......... .......... .......... .......... .......A.. .T........ ..........
N. macropterus (West Australia 3)          .......... .......... .......... .......... .......A.. .T........ ..........
N. macropterus (New Zealand)               .......... .......... .......... .......... .......... .......... ..........
Nemadactylus sp. (east. Australia)         .........C .......... .......... .......... .......A.. T......... .....A....
Nemadactylus sp. (New Zealand 1)           .........C .......... .......... .......... .......A.. .......... ...G.A....
Nemadactylus sp. (New Zealand 2)           .........C .......... .......... A......... .......A.. .T........ .....A....
N. bergi 1                                 .......... .......... .......... A......... .......A.. .T........ ........G.
N. bergi 2                                 .......... .......... .......... .......... .......A.. .T........ ......A...
N. bergi 3                                 ..A....... .......... .........A A......... .......A.. .T........ ..C.......
N. bergi 4                                 .......... .......... .......... A......... .......A.. .T........ ..........
N. bergi 5                                 .......... .......... .......... A......... .......A.. .T........ ..........
N. bergi 6                                 .......... .......... .......... A......... .......A.. .T........ ..........
N. bergi 7                                 .......... .......... .......... .......... .......A.. .T........ .........C
N. douglasii                               T.TA....T_ .......A.. ......TCTC A...T..AA. .......A.T .A..ATACC. C.......G.
N. valenciennesi                           T.TA....._ C......A.C ......GAC. AG..T..AGT ..C....A.G .A..ATA.G. C.......G.
Cheilodactylus spectabilis                 ...A....T. .....A.A.. ..G....... TG......C. ....C..A.T .A.TT..TG. ..........
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                                                                         ↓                                            ↓ Dpn II
A. monodactylus (Tristan Is.)            ACATAAACC_ AAACTCTA_C ATAA_TTGAA TACACTA TT ACTTCGACCT GGTCGAAATA TTAAGACCTA  210
A. monodactylus (Gough Is.)              .......... .......... .......... .......... .......... .......... ..........
A. monodactylus (Saint Paul Is.)         .......... .......... .......... .......... .......... .......... ..........
A. monodactylus (Amsterdam Is. 1)        .......... ...T...... .......A.. C..GTAT... .......... ..G....... .....TT...
A. monodactylus (Amsterdam Is. 2)        .......... .......... .......AT. C.......A. .T........ .......... ........G.
A. monodactylus (Amsterdam Is. 3)        .......... .......... .......A.. C.......A. .T........ .......... ........G.
A. gayi 1                                C......... .......... .......A.. C.......A. .T.......C .......... .....G..G.
A. gayi 2                                C......... .......... ....A..A.. C.......A. .T.......C .......... ........G.
A. gayi 3                                C......... .......... ....A..A.. C.......A. .T.......G .......... ........G.
N. macropterus (Tasmania)                .......... .......... ..G....T.. C....C.C.. .T.......A .......... ........G.
N. macropterus (West Australia 1)        .......... .......... ...G...T.. C..GT..... .......... .......... .....T....
N. macropterus (West Australia 2)        .......... .......... .......A.. .......C.. .T........ ..C....... ......T.G.
N. macropterus (West Australia 3)        .......... .......... .......A.. .......C.. .T.......A .......... C.......G.
N. macropterus (New Zealand)             .......... .......... ......CA.. ........C. .......... .......... ..........
Nemadactylus sp. (east Australia)        T....T.... ....C...A. .....C.A.. C...A...A. .T.......C .......... ........G.
Nemadactylus sp. (New Zealand 1)         T....T.... ....C...A. .......A.. C...A...A. .T........ .A........ ........G.
Nemadactylus sp. (New Zealand 2)         T....T.... ....C..... .......A.. C...G...A. .T.......C .A........ ........G.
N. bergi 1                               .........A .........T .......A.. C......A.. .......... .......... ......T.G.
N. bergi 2                               .........A .........T .......A.. C......A.. .......... .......... ........G.
N. bergi 3                               .......... .......... .......A.. C......A.. .........A .......... ......T.G.
N. bergi 4                               .......... .......... ..G....A.. C......A.. .......... .......... ......T.G.
N. bergi 5                               .........A .........T .......A.. C.....GA.. .......T.. .......... ......T.G.
N. bergi 6                               .......... .........G .......A.. C......AC. .......... .......... ......T.G.
N. bergi 7                               ........T. .......... .......A.. C...G..A.. .......... .......... ......T.G.
N. douglasii                             .T.CT...T. CC.A.A.G.T .C.....C.. ..ATT..A.. .T..AA...C AA.......T .AT..TT...
N. valenciennesi                         .T.CT..... TC.GCA.G.T G......T.. C.ATT.GA.. .T.CTA..T. A.......CT .CT..C....
Cheilodactylus spectabilis               .........C .C...A.TTT ...T..CA.. C..TAA..A. .TC.TA...A ......G... ......T...

                                                                                      ↓ Hinc II   ↓ Hinc II   ↓
A. monodactylus (Tristan Is.)            GCTAAA_CAC TAAAGTGTCT AGTTATACGA AAATC ACCA TCCCGCAACT TTAATAATTC TTAATGTAGT  280
A. monodactylus (Gough Is.)              .......... .......... .......... .......... .......... .......... ..........
A. monodactylus (Saint Paul Is.)         .......... .......... .......... G......... .......... .......... ..........
A. monodactylus (Amsterdam Is. 1)        .......T.. ........TA ........A. .......... .....T..TC ....C...C. ..........
A. monodactylus (Amsterdam Is. 2)        ......A.GT .......... .......... G......... .....T..TC .......C.. ..........
A. monodactylus (Amsterdam Is. 3)        .......T.. .......... .......... G......... .....T..TC .......C.. ..........
A. gayi 1                                .........T .......... .......... GG....C... .....T..T. .C...G.... ..........
A. gayi 2                                ......A... .......... .......... G......... .....T..T. .C...G.... ..........
A. gayi 3                                .......... .......... .......... G......... .....T..T. .C...G.... ..........
N. macropterus (Tasmania)                ......A.C. .......... ........A. .......... .....T..TC .......C.. ..........
N. macropterus (West Australia 1)        .......T.. .........A .......... G......... .....T..TC .C......C. ..........
N. macropterus (West Australia 2)        .......... .......... .......... G......... .....T..TC .......C.. ........A.
N. macropterus (West Australia 3)        .......... .......... .......... GG........ .....T..TC .......C.. ..........
N. macropterus (New Zealand)             .......... .......... .......... G......... .....T.... .......... ..........
Nemadactylus sp. (east Australia)        .......... .......... .......... G......... ...TAT..T. .C........ ..........
Nemadactylus sp. (New Zealand 1)         .......... .......... .......... G......... ...TAT..T. .C........ ..........
Nemadactylus sp. (New Zealand 2)         .......... .......... .......... G......... .....T..T. .C........ ..........
N. bergi 1                               .......... .......... .......... G......... .....T..TG ....CG...T ..........
N. bergi 2                               .......... .......... .......... G......... .....T..TG ....C....T ..........
N. bergi 3                               .......... .......... .......... G......... .....T..TG ....C....T ..........
N. bergi 4                               .......... .......... .......... G......... .....T..TA ....C....T ..........
N. bergi 5                               .......... .......... .......... G......... .....T..TA ..G.C....T ..........
N. bergi 6                               .T........ .......... .......... GG........ .....T..TC ....C....T ..........
N. bergi 7                               .......... .......... .......... G......... .....T..TG ....C....T ..........
N. douglasii                             A.....TA.T ....A...AC .A.C.C...T G......... .TTTATG.T. C.T....CCG ..T...C.A.
N. valenciennesi                         A.....CA.T ........T. .....C...T GG........ .T.TATGGT. CC.G...... CCG.......
Cheilodactylus spectabilis               T.AC..TA.T C...TG..AA .......... GG.C.C.... .TTA.T.GT. C.GCAG.... ..........
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```
A. monodactylus (Tristan Is.)            AAGAACCGAC CAACAGTTGA TCCCTTAATG CATACTCTTA TTGAAGGTGA GGGACAACTA TTGTGGGGGT 350
A. monodactylus (Gough Is.)              .......... .......... .......... .......... .......... .......... ..........
A. monodactylus (Saint Paul Is.)         .......... .......... .......T.. .......... .......... .......... ..........
A. monodactylus (Amsterdam Is. 1)        .......... .......... .......... .......... .......... .......T.. ..........
A. monodactylus (Amsterdam Is. 2)        .......... .......... .TT....... .......... .......... .......... ..........
A. monodactylus (Amsterdam Is. 3)        .......... .......... .......... .......... .......... ........C. ..........
A. gayi 1                                .......... .......... .T........ .......... ......A... ........C. ..........
A. gayi 2                                .......... .......... .TT....... .......... ......A... ........C. C.........
A. gayi 3                                .......... .......... .TT....... .......... ......A... ........C. C.........
N. macropterus (Tasmania)                .......... .......... .T........ .......... .......... ........C. ..........
N. macropterus (West. Australia 1)       .......... .......... .T........ .......... .......... .......T.. ..........
N. macropterus (West. Australia 2)       .......... .......... .T........ .......... .......... ........C. ..........
N. macropterus (West. Australia 3)       .......... .......... .T........ .......... .......... ........C. ..........
N. macropterus (New Zealand)             .......... .......... .T........ .......... .......... .......... ..........
Nemadactylus sp. (east. Australia)       .......... .......... .T........ .......... ......A... .......TC. ..........
Nemadactylus sp. (New Zealand 1)         .......... .......... .T........ .......... ......A... .......TC. ..........
Nemadactylus sp. (New Zealand 2)         .......... .......... .T........ .......... ......A... .......TC. ..........
N. bergi 1                               .......... .......... .......... .......... .......... ........C. ..........
N. bergi 2                               .......... .......... .......... .......... .......... ........C. ..........
N. bergi 3                               .......... .......... .T........ .......... .......... .......... ..........
N. bergi 4                               .......... .......... .T........ .......... .......... .......... ..........
N. bergi 5                               .......... .......... .T........ .......... .......... .......T.. ..........
N. bergi 6                               .......... .......... .......... .......... .......... .......... ..........
N. bergi 7                               .......... .......... .T........ .......... .......... .......... ..........
N. douglasii                             .......... .......... .T........ .......... .......... .......T.. C.........
N. valenciennesi                         .......... .......... .TT....... ......G... .......... ........C. C.........
Cheilodactylus spectabilis               .......... ....TTA... .T........ ......A... .....A.... ........C. ..........
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```
A. monodactylus (Tristan Is.)            TTCACCCCGT GAATTATTCC TGG 373
A. monodactylus (Gough Is.)              .......T.. ...C...... ...
A. monodactylus (Saint Paul Is.)         .......T.. .......... ...
A. monodactylus (Amsterdam Is. 1)        .......... .......... ...
A. monodactylus (Amsterdam Is. 2)        .......T.. .......... ...
A. monodactylus (Amsterdam Is. 3)        ......TT.. .......... ...
A. gayi 1                                ......TT.. .......... ...
A. gayi 2                                ......TT.. .......... ...
A. gayi 3                                ......TT.. .......... ...
N. macropterus (Tasmania)                .......T.. .......... ...
N. macropterus (West. Australia 1)       .......T.. .......... ...
N. macropterus (West. Australia 2)       ......TT.. .......... ...
N. macropterus (West. Australia 3)       .......TA. .......... ...
N. macropterus (New Zealand)             .......... .......... ...
Nemadactylus sp. (east. Australia)       ......TT.. .......... ...
Nemadactylus sp. (New Zealand 1)         ......TT.. .......... ...
Nemadactylus sp. (New Zealand 2)         ......TT.. .......... ...
N. bergi 1                               .......T.. .......... ...
N. bergi 2                               .......T.. .......... ...
N. bergi 3                               .......T.. .......... ...
N. bergi 4                               .......T.. .......... ...
N. bergi 5                               .......T.. .......... ...
N. bergi 6                               .......T.. .......... ...
N. bergi 7                               .......T.. .......... ...
N. douglasii                             CA...TTTA. .......... ...
N. valenciennesi                         CG...TT.T. .......... ...
Cheilodactylus spectabilis               .....TTA.. .......... ...
```

The trees recovered by neighbour-joining (Figure 3.4b) and maximum likelihood (not shown) analyses differed slightly in topology from each other, and also in comparison to that of unweighted parsimony. Although the same polytypic *N. macropterus-A. monodactylus* and monotypic *N. bergi* clades were produced by all three methods, there were differences in the inferred relationships between these clades, and also relative to the *Nemadactylus* sp.-*A. gayi* clade. It is these same relationships which do not receive high bootstrap values from parsimony and neighbour-joining analyses. Similar differences in topology and low bootstrap values were also observed during parsimony analysis when including gaps as a character-state, when differentially weighting character-state changes, and during the analysis of alternative sequence alignments (not shown).

The tree lengths obtained during the enforcement of maximum likelihood and neighbour-joining topologies during parsimony analysis were only one and two steps longer than that of unconstrained parsimony (311 steps) respectively. These three topologies were not significantly different as determined by the Templeton and Kishino-Hasegawa tests ($P>0.05$). Simultaneous enforcement of *N. macropterus* and *A. monodactylus* sequence monophyly during parsimony analysis produced 15 trees of 330 steps in length, 19 steps longer than the most parsimonious tree. Individual monophyly of either *N. macropterus* (11 trees) or *A. monodactylus* (5 trees) sequences required 327 steps. These enforced monophyly topologies were significantly inferior than the maximum parsimony, neighbour-joining, and maximum likelihood topologies ($P<0.05$). Basal placement of the *N. bergi* clade relative to the polytypic *N. macropterus-A. monodactylus* clades increased tree length by two steps (313 steps) relative to the most parsimonious topology, but this tree was not significantly inferior than the maximum parsimony, neighbour-joining, or maximum likelihood topologies ($P>0.05$).

Two dimensional scaling of pairwise genetic distances provided another representation of the relationships between D-loop sequences (Figure 3.5). *Nemadactylus* sp. and *A. gayi* sequences were represented as distinct clusters. The *N. bergi* sequences also formed a tight cluster, which was surrounded, but not interspersed, by *N. macropterus* and *A. monodactylus* sequences. The

intraspecific distances observed for *N. macropterus* and *A. monodactylus* often exceeded interspecific distances (see also Table 3.2).

The restriction enzymes *Dpn* II and *Hinc* II were used to identify any divergent D-loop sequences within *N. bergi*, or alternatively, *N. bergi*-like sequences within *N. macropterus* or *A. monodactylus*. These two enzymes had recognition sites diagnostic for three of the five character-states associated with the *N. bergi* clade (Figure 3.3). All of 48 additional *N. bergi* individuals screened possessed the *Dpn* II site, but nine lacked the *Hinc* II site. Four of these nine individuals were sequenced (*N. bergi* 4-7, Figure 3.3), as the *Dpn* II site may not have been restricted to *N. bergi*-like sequences. However, in each instance at least four of the five defining character-states of the *N. bergi* clade were present. Thirty-one individuals of *N. macropterus* and 16 individuals of *A. monodactylus* were similarly screened for *N. bergi*-like sequences. Each *A. monodactylus* individual lacked both the *Dpn* II and *Hinc* II diagnostic restriction sites. All of the *N. macropterus* individuals also lacked the *Hinc* II site, but five individuals were digested by *Dpn* II. Two of these individuals were sequenced (Western Australia 2 and 3, Figure 3.3), but neither possessed *N. bergi*-like character-states at more than one of the five *N. bergi* clade defining positions.

Discussion

Phylogenetic analysis of cytochrome *b* sequences clustered *Acantholatris monodactylus* and *A. gayi* within a clade otherwise comprising exclusively *Nemadactylus*. The *Acantholatris* sequences were also very similar to those from *Nemadactylus* sp., *N. macropterus*, and *N. bergi*, forming a single clade with a maximum of only three transition substitutions, or 0.98% corrected sequence divergence, between any of these five taxa. The sequences from *N. valenciennesi* and *N. douglasii* formed the sister clade to these five taxa.

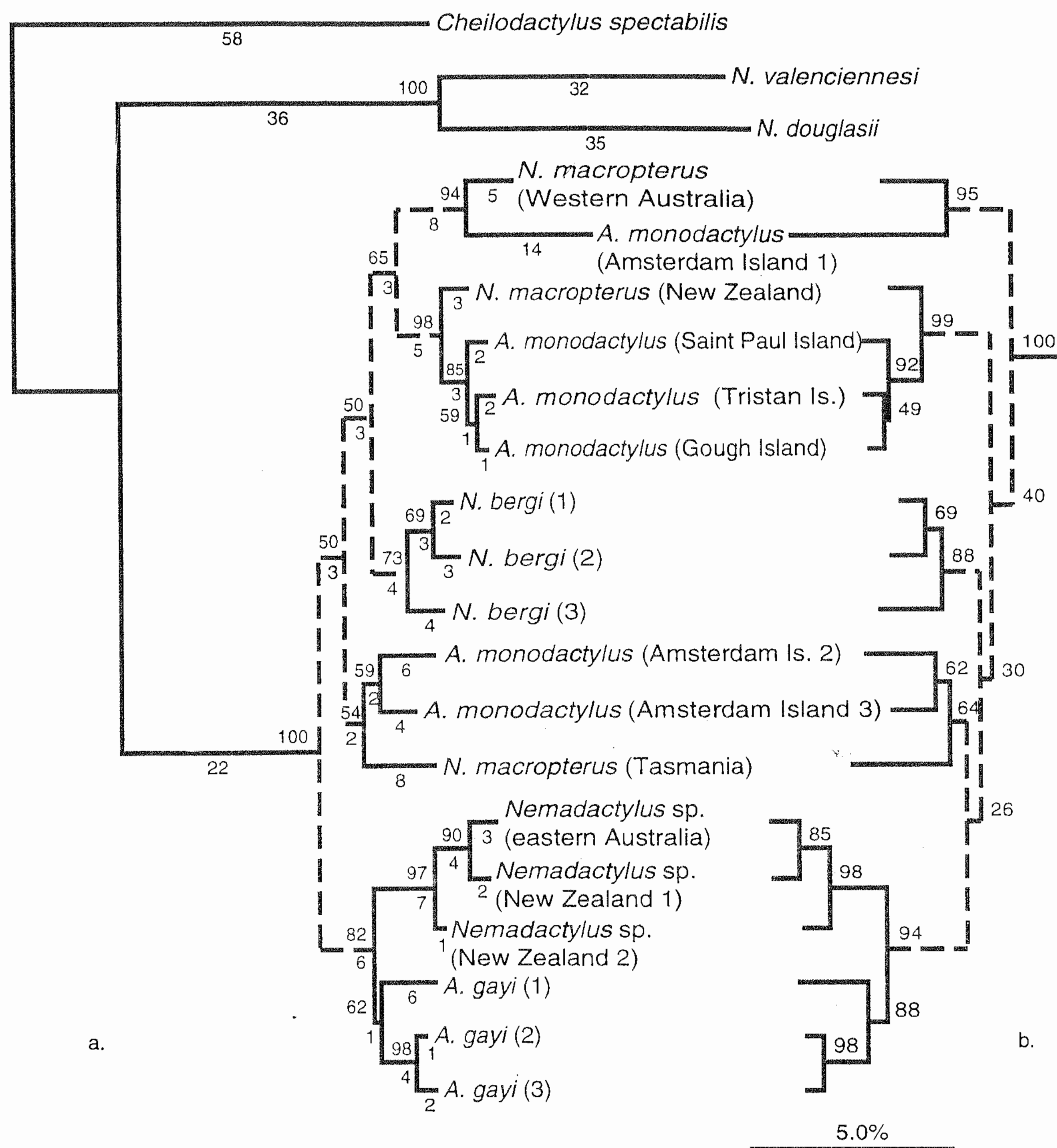


Figure 3.4. (**a**) Single most-parsimonious cladogram from the analysis of partial mitochondrial DNA D-loop sequences from *Acantholatris* and *Nemadactylus* species. *Cheilodactylus spectabilis* was employed as the outgroup. The Heuristic search algorithm was used (100 random sequence stepwise additions), all character-state changes were equally weighted, and characters containing gaps were ignored. Branch lengths are proportional to the number of substitutions, quantified by the numbers below the branches. Tree length=311 steps, CI=0.740, RI=0.704. (**b**) Corresponding neighbour joining phenogram. Branch lengths are proportional to Kimura (1980) genetic distance, measured relative to the scale bar. Regions of topological difference between the two trees are indicated by broken lines. In both trees the bootstrap proportions for the taxa in each clade, as derived from 1000 replicates, are indicated by the numbers above the braches at each node. Branches leading to *C. spectabilis*, *N. valenciennesi*, and *N. douglasii* have been omitted from the neighbour joining phenogram, but do not differ in topology or support from (a).

Phylogenetic analysis of D-loop sequences produced similar results to that of cytochrome *b*, but with greater resolution of the relationships between the five closest taxa. The sequences from *Nemadactylus* sp. and *A. gayi* clustered as sister clades. Those from *N. macropterus*, *N. bergi*, and *A. monodactylus* were structured into four clades, three of which were polytypic, individually containing both *N. macropterus* and *A. monodactylus* sequences, while the fourth was monotypic for *N. bergi*. Consistent relationships between these four clades, and relative to the *Nemadactylus* sp.-*A. gayi* clade, were not recovered, although the topologies obtained were not significantly different. In contrast, significantly poorer topologies were observed when enforcing the monophyly of *N. macropterus* and *A. monodactylus* sequences. The levels of intraspecific D-loop sequence divergence for *N. macropterus* and *A. monodactylus* were as large as some of the interspecific sequence divergences within *Nemadactylus* and *Acantholatris*.

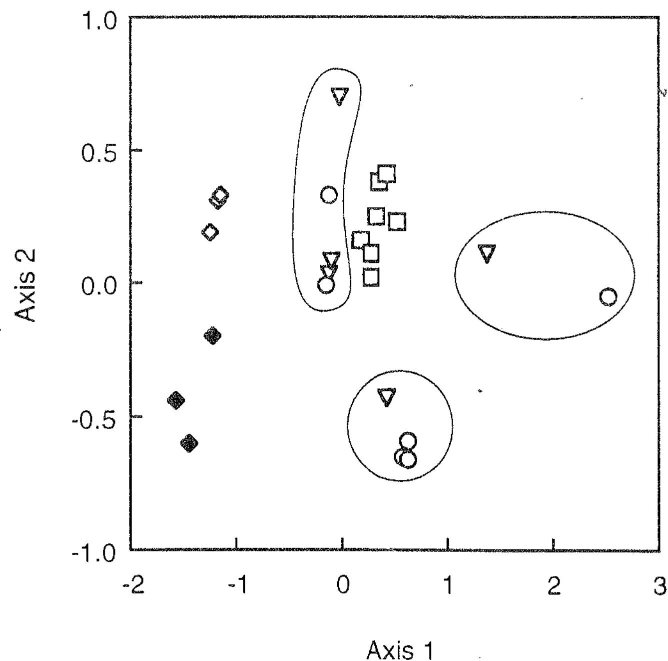


Figure 3.5. Two-dimensional scaling of Kimura (1980) genetic distances between *Acantholatris* and *Nemadactylus* partial mitochondrial DNA D-loop sequences, using the Kruskal loss function and monotonic scaling. Circles=*A. monodactylus*, open diamonds=*A. gayi*, closed diamonds=*Nemadactylus* sp., triangles=*N. macropterus*, squares=*N. bergi*. The polytypic clades observed during phylogenetic analysis are circled. Stress of configuration=0.088. *Nemadactylus douglasii* and *N. valenciennesi* were excluded from scaling so as to facilitate maximum resolution of the remaining sequences.

Taxonomy

Analysis of cytochrome *b* and D-loop sequences placed *A. monodactylus* and *A. gayi* within clades otherwise composed entirely of *Nemadactylus* clades (Figures 3.2 and 3.4). These results suggest that *Nemadactylus* and *Acantholatris* are synonymous, with the name *Nemadactylus* having priority. Although there are no molecular data for the third species of *Acantholatris*, *A. vemae*, it is most likely that this species would cluster within *Nemadactylus*. *Acantholatris vemae* is similar in morphology to *A. monodactylus*, and these species are sympatric at Vema Seamount (Penrith, 1967; Lamb, 1990).

The suggestion of synonymy for *Nemadactylus* and *Acantholatris* is supported by morphological data. The distinction of *Nemadactylus* and *Acantholatris* is based predominantly on the number of anal fin rays, with *Nemadactylus* possessing 14-19, and *Acantholatris* having 10-12 (Gill, 1862; Lamb, 1990). However, the recently identified "king tarakihi" species, considered to be *Nemadactylus* because of its sympatry and almost identical appearance with *N. macropterus*, possesses only 12 anal fin rays (Roberts, 1993). Therefore, conflict exists between the expected placement of this new species and the dominant characteristic used to separate *Nemadactylus* and *Acantholatris*.

The analysis of D-loop sequences indicates that *Nemadactylus* sp. is most closely related to *A. gayi* from Juan Fernández (Figure 3.4). This accords with their identical anal fin ray counts (Lamb, 1990; Roberts, 1993). Because Roberts (1993) and Smith *et al.* (1996) only compared their specimens of *Nemadactylus* sp. with *N. macropterus*, it is possible that *Nemadactylus* sp. may simply represent the first record of *A. gayi* in the waters of Australia and New Zealand; it may not comprise a new species. There are insufficient morphological data available to distinguish these two forms, but the genetic results from this study suggest that separation at some level is warranted (Figures 3.4 and 3.5). However, more material should be analysed.

It is possible that *N. bergi* may be a junior synonym of *N. macropterus*, as the characters used to distinguish these taxa are not consistent among the holotype and paratypes of *N. bergi*

(R.W.G. White, University of Tasmania, Australia, 1995, pers. comm.). The D-loop sequences obtained from three *N. bergi* individuals formed a monotypic clade, distinct from the *N. macropterus* sequences (Figure 3.4). However, given the high level of intraspecific divergence within *N. macropterus* (Table 3.2, Figure 3.5), restriction enzyme analysis was employed to test for the presence of *N. macropterus*-like sequences within additional *N. bergi* individuals, and *vice versa*, but no such instances were identified. Although the *N. bergi* clade was not placed basal to the polytypic *N. macropterus-A. monodactylus* clades, the enforcement of such a topology required only two more steps than the most parsimonious tree, and was not significantly inferior than the maximum parsimony, neighbour-joining, or maximum likelihood topologies. These results suggest that some distinction of *N. bergi* is warranted.

The species *A. monodactylus*, *A. gayi*, *Nemadactylus* sp., *N. macropterus*, and *N. bergi* appear to be very closely related, and it is expected that sequence data would place *A. vemae* close to these taxa as well. In contrast, *N. douglasii* and *N. valenciennesi* form a divergent sister clade to this group (Figures 3.2 and 3.4). These results suggest that some systematic modification may be warranted in addition to synonymising *Acantholatris* with *Nemadactylus*. The two groups could be distinguished by allocation to separate subgenera within the expanded *Nemadactylus*. However, the overall degree of genetic divergence between these groups is low compared to that observed within other cirrhitoid genera (Chapters 3, 4, and 5; Burridge, unpubl. data). The very close relationships between at least five *Nemadactylus* and *Acantholatris* species, and in some cases their questionable separate status, may be better emphasised by reallocating these taxa, and probably *A. vemae*, as variants of a single species. A morphological revision of *Nemadactylus* is being conducted by C.D. Roberts (Museum of New Zealand), which will hopefully resolve the taxonomic questions outstanding.

Divergence time

The levels of cytochrome *b* sequence variation among the five most closely related species of *Nemadactylus* and *Acantholatris* suggest that they diverged within the last 0.3-0.6 million years, based on mitochondrial protein four-fold degenerate site molecular clock calibrations of

2.3% and 3.3% sequence divergence Myr^{-1} (Martin *et al.*, 1992; Bermingham *et al.*, 1997). These estimates of divergence time must be treated cautiously, given the assumptions made when applying molecular clock calibrations (Rand, 1994). Faster and slower calibrations have been reported for other taxa, and those employed here are considered "median" values. However, a similar estimate of divergence time, 0.5 Myr ago, has been proposed for some members of the rock lobster genus *Jasus*, which overlap in distribution with *Nemadactylus* and *Acantholatris* (Ovenden *et al.*, 1997). In addition, none of these divergence estimates predate the ages of the oldest islands and seamounts occupied by each species (Miller, 1964; McDougall and Ollier, 1982; Stuessy *et al.*, 1984).

Zoogeography

It appears that *Nemadactylus* and *Acantholatris* have dispersed throughout the temperate Southern Hemisphere from the waters of Australia and New Zealand. Both of the main clades observed for these species have representatives in these waters, and those taxa which occur elsewhere exhibited only limited genetic divergence from *Nemadactylus* sp. and *N. macropterus*. Dispersal from Australia or New Zealand may have proceeded in either an easterly or westerly direction, and presumably occured during their 7-12 month pelagic larval stages (Annala, 1987; Andrew *et al.*, 1995).

Any dispersal in an easterly direction would most likely have been mediated by the West Wind Drift Current. Dispersal by this current has been proposed for several fishes, including cheilodactylids (Eschmeyer and Hureau, 1971; Briggs, 1974; Andrew *et al.*, 1995), and a number of invertebrates (Fell, 1962; Newman, 1979; Lutjeharms and Heydorn, 1981; Pollock, 1990). In a stepwise manner, the West Wind Drift could have transported *Nemadactylus* and *Acantholatris* from Australia or New Zealand to the southeastern Pacific and the east coast of South America, and then along the chain of islands and seamounts from Tristan da Cunha and Gough Island in the South Atlantic, to Saint Paul and Amsterdam Island in the Indian Ocean. During the Pleistocene glaciations this current also flowed faster and at slightly lower latitudes (CLIMAP members, 1976; Howard and Prell, 1992), which would have made dispersal by this

mechanism easier. As the glacial periods were approximately ten times longer in duration than the interglacials, the former are most likely to have regulated these species (Pollock, 1990).

Westward dispersal of temperate fishes in the Southern Hemisphere is most likely to have been facilitated by the northern components of anticyclonic current gyres (Kensley, 1981; Collette and Parin, 1991; Pollock, 1993), but such movement of *Nemadactylus* and *Acantholatris* from Australia may have been limited. Recruitment from southwestern Australia into the Southern Indian Ocean Anticyclonic Gyre would have been impeded during interglacial periods by the south-flowing Leeuwin current and its associated offshore eddies (Figure 3.6a; CLIMAP members, 1976; Pollock, 1993). Although recruitment into this gyre would have been easier during glacial periods, transport around southern Africa at these times would have been hindered by the retroflective effects of stronger westerly winds and the shoaling of the Southern Madagascar Ridge (Figure 3.6b; CLIMAP members, 1976; Pollock, 1993). Therefore, only Indian Ocean locations may have been colonised by westward movement from Australia. If larvae did pass into the South Atlantic and establish a population, perhaps at Vema Seamount, dispersal throughout this basin could have been facilitated by the corresponding anticyclonic gyre. However, any dispersal into the Pacific would have been against the prevailing currents and most difficult. The use of these anticyclonic gyres is also questionable, as it would require that larvae could survive the warm temperatures at the northern extents of these systems. The temperatures at these low latitudes would have been only slightly abated, if at all, during Pleistocene glaciations (CLIMAP members, 1976; Prell *et al.*, 1980).

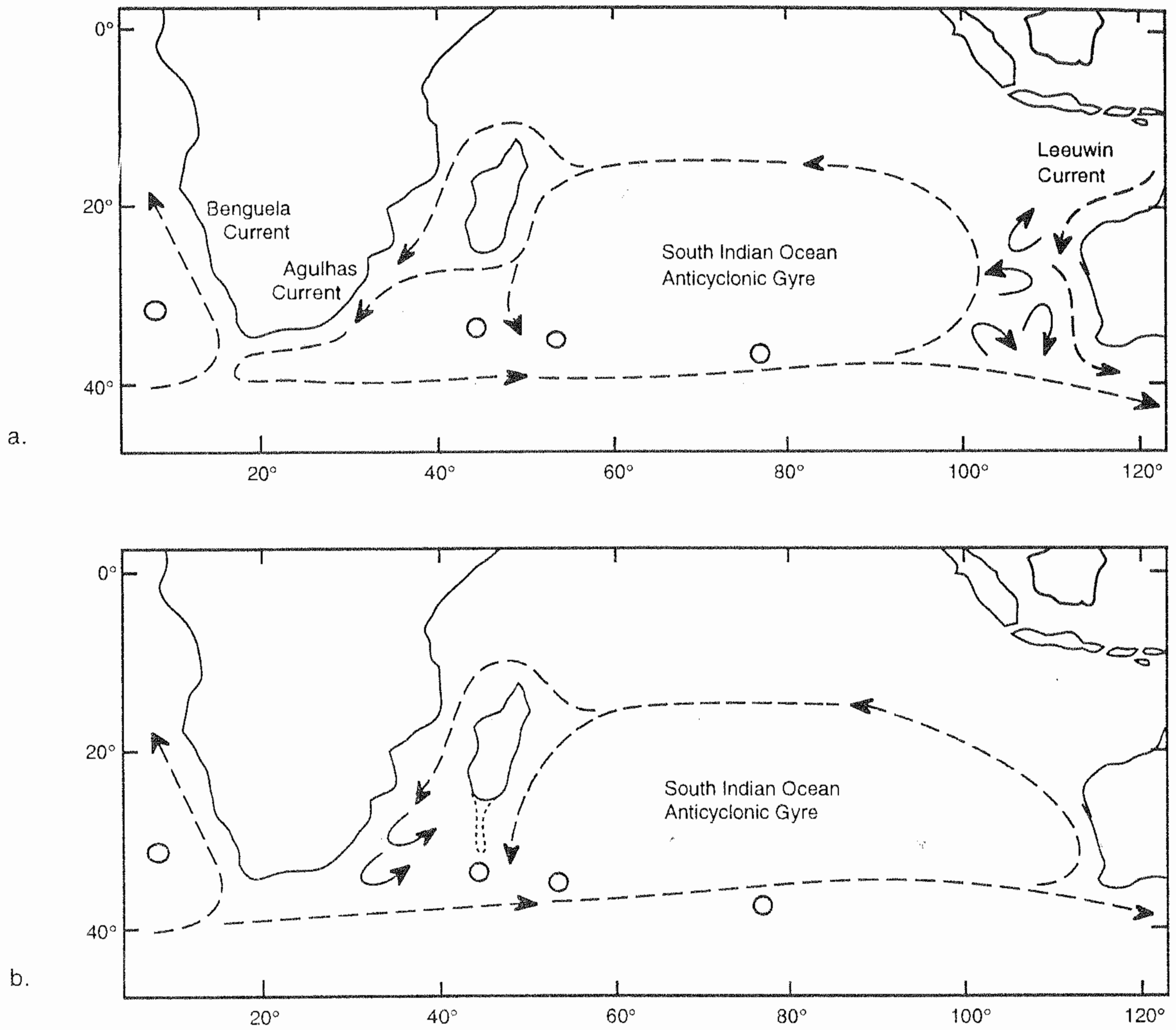


Figure 3.6. Approximate oceanographic conditions at present (**a**) and during a Pleistocene glacial maxima (**b**). Major differences during glaciations comprise the breakdown of the Leeuwin current and its associated offshore eddies, and the retroflective effects in the western Indian Ocean of the stronger westerly winds and the shoaling of the Southern Madagascar Ridge. Circles indicate islands and seamounts occupied by *Acantholatris*.

Given the observed sister-taxa relationship between *Nemadactylus* sp. and *A. gayi*, and the prevailing currents, it appears that dispersal eastward from Australia or New Zealand was responsible for the founding of *A. gayi* in the southeastern Pacific. However, as *Nemadactylus* sp. and *A. gayi* formed a monophyletic clade, it does not appear that Juan Fernández or Desventuradas populations acted as a source for any movement into the South Atlantic. The north-flowing Humboldt current also prevails around these islands. Therefore, the South Atlantic and Indian Ocean populations of *Nemadactylus* and *Acantholatris* were either founded

by a separate movement east from Australia or New Zealand, a movement west from Australia, or a combination of both. The phylogenetic information obtained from this study is insufficient to suggest by which method the populations of *N. bergi*, *A. monodactylus*, and *A. vemae* were founded. However, dispersal east from Australia and New Zealand is favoured as it was presumably faster and more direct.

Although dispersal of *Nemadactylus* and *Acantholatris* from Australia or New Zealand probably proceeded in a stepwise manner, speciation may not have immediately accompanied the founding of populations. Gene flow among even the most isolated populations may have been sufficient to prevent speciation, and therefore it is possible that individual ancestral species had widespread distributions in the temperate Southern Hemisphere. Speciation may have then resulted from a change in dispersal capabilities or oceanographic conditions that increased the isolation of populations. The transition to decreased West Wind Drift flow when proceeding from glacial to interglacial periods may represent such an isolating mechanism. The widespread distributions of the latrids *Mendosoma lineatum* and *Latris lineata* suggest that a similar distribution was possible for ancestral species of *Nemadactylus* or *Acantholatris*, given the cognate dispersal capabilities of these families (Andrew *et al.*, 1995).

Polytypic clades

The presence of polytypic *N. macropterus* and *A. monodactylus* clades (Figure 3.4) may be explained in terms of introgressive hybridisation or incomplete lineage sorting. The morphological separation of these taxa (Lamb, 1990) does not favour an alternate suggestion that they may be synonymous.

Although the spawning periods of *N. macropterus* and *A. monodactylus* overlap (Annala, 1987; Andrew *et al.*, 1995), there are no records of sympatry for these two species or natural hybridisation between any cirrhitoids. Therefore, mitochondrial DNA sequence exchange between *N. macropterus* and *A. monodactylus* appears unlikely. In addition, if introgressive hybridisation did take place between these two species it might be expected that a maximum of

two polytypic clades would be observed (introgression in both directions). Analysis of D-loop sequences suggested that three such clades exist (Figures 3.4 and 3.5), although the arbitrary distinction of two of these would be rejected if individuals of intermediate relatedness were identified.

Incomplete lineage sorting is the favoured explanation for the observed polytypic clades. During speciation, ancestral mitochondrial DNA lineages may not sort congruently with respect to species boundaries, and individuals can possess haplotypes more similar to those of non-conspecifics than individuals of the same species (Avise, 1986). That is, the gene tree for sister taxa may not be reciprocally monophyletic. The simulations of Neigel and Avise (1986) suggest that the probability of sister taxa possessing reciprocally monophyletic mitochondrial DNA lineages (complete lineage sorting) is only high after $4N$ generations of genetic isolation, where N is the number of females. Assuming female population sizes in excess of 50,000 individuals and an average generation time of 3 years, the minimum divergence time suggested for *N. macropterus* and *A. monodactylus* is too recent for a high probability of reciprocal lineage monophyly. The female population sizes of these species undoubtably exceed 50,000, given their total annual catches and average fish weights (Annala, 1987; Andrew *et al.*, 1995; Jordan, 1997). However, sweepstakes recruitment and bottlenecks in effective population size will reduce the amount of time required to achieve lineage monophyly, although the high levels of nucleotide and haplotype diversity observed within these two species (see also Grewe *et al.*, 1994) suggest that the latter has not occurred (Avise, 1989).

Despite its similarly recent divergence and undoubtably large female population size, lineage monophyly and a comparatively small level nucleotide diversity were observed for *N. bergi* in comparison with *N. macropterus* and *A. monodactylus* (Figure 3.4, Table 3.2). These could be the result of one or more bottlenecks in *N. bergi* female population size, such as the dramatic decline in the stocks of this species resulting from over-fishing during the 1960's (Cotrina, 1971; see Chapter 8 for supporting evidence). Similarly, this species may have been founded by only a small range of mitochondrial DNA lineages, or there could be a high variance in

reproductive success (Hedgecock, 1994). Because of their linked inheritance, selection for particular coding sequences within the mitochondrial genome can also reduce the nucleotide diversity of D-loop sequences (Chenoweth *et al.*, 1998). However, the degree of coding sequence non-synonymous variation within *N. bergi* would not be large, and therefore is unlikely to have influenced fitness. The lineage monophyly and similarly small levels of nucleotide diversity observed for *Nemadactylus* sp. and *A. gayi* could also be explained in terms of population size.

Conclusions

The molecular data obtained suggest that *Acantholatris* and *Nemadactylus* should be synonymised, with *Nemadactylus* having priority. At least five of these species are very closely related, and have probably dispersed and radiated throughout the Southern Hemisphere within the last 0.3-0.6 Myr, facilitated by their long larval durations. Sorting of mitochondrial DNA lineages among two of these taxa appears incomplete. Further studies are required to determine some of the dispersal directions, and to resolve questions of specific status.

CHAPTER 4: Molecular phylogeny of the antitropical subgenus *Goniistius* (Cheilodactylidae: *Cheilodactylus*): evidence for multiple transequatorial divergences and non-monophyly.

Abstract

The subgenus *Goniistius* comprises eight species of marine nearshore fishes that are antitropically distributed. The molecular phylogeny of these and other cheilodactylids was reconstructed from mitochondrial DNA cytochrome oxidase I and cytochrome *b* sequences. The placement within *Goniistius* of the morphologically divergent species *Cheilodactylus (G.) nigripes* was not supported. The remaining seven species are sufficiently divergent from other cheilodactylids to be designated as a separate genus. The antitropical distribution of *Goniistius* is the result of three transequatorial divergences, which occurred during two periods. Based on molecular clock calibrations, these periods are suggested to be the mid Miocene, and late Miocene to early Pliocene. Hypotheses of transequatorial dispersal, or vicariance resulting from interspecific interactions or climate change are favoured for the antitropical distribution of *Goniistius*, and hypotheses of Mesozoic or Pleistocene separations can be discounted. The degree of genetic divergence between North and South Pacific populations of *C. (G.) vittatus* Garrett indicates that they have undergone cryptic speciation.

Introduction

Several hypotheses have been offered for antitropical distributions of marine taxa. Species intolerant of tropical temperatures may have dispersed across equatorial waters at shallow depths during periods of climatic cooling, such as Pleistocene glaciations, or by isothermal submergence at any time (Darwin, 1859; Hubbs, 1952; Ekman, 1953; Randall, 1981; Lindberg, 1991). The islands occupied by antitropical taxa may have also moved across equatorial waters by ancient tectonic processes (Rotondo *et al.*, 1981). Alternatively, a mid Miocene rise in equatorial temperatures (Valentine, 1984; White, 1986), competition with younger, superior tropical species (Briggs, 1987a), or the submergence of equatorial islands (Rehder, 1980; Springer, 1982) may have vicariantly isolated Northern and Southern Hemisphere populations. More ancient vicariant events proposed include the fragmentation of Pangea or Pacifica (Nelson, 1985; Crame, 1993).

The application of molecular genetic techniques is attractive to studies of antitropical taxa. These techniques may better resolve phylogenetic relationships, particularly when taxa are morphologically conserved, so that the location, frequency, and direction of transequatorial divergences may be determined (Bowen and Grant, 1997; Grant and Bowen, 1998; Koufopanou *et al.*, 1999). In addition, levels of molecular variation can provide relative and absolute estimates of divergence time (Stepien and Rosenblatt, 1996; Bowen and Grant, 1997; Koufopanou *et al.*, 1999), further facilitating the evaluation of specific dispersal and vicariance hypotheses.

The distribution of *Goniistius* Gill is antitropical distribution (Figure 4.1; Randall, 1981; Randall, 1983). Three species are restricted to the waters of southern Japan, southern Korea, China, and Taiwan: *Cheilodactylus (G.) zonatus* Cuvier and Valenciennes, *C. (G.) quadricornis* Günther, *C. (G.) zebra* Döderlein. Another three species occur around Australia and New Zealand: *C. (G.) gibbosus* Richardson, *C. (G.) vestitus* Castelnau, *C. (G.) nigripes* Richardson. A single species, *C. (G.) plessisi* Randall is endemic to Easter Island and Rapa. The remaining species, *C. (G.) vittatus* Garrett is itself antitropical, occurring between Midway

and the Hawaiian Islands in the North Pacific, and at Lord Howe, New Caledonia, and the Kermadecs in the South Pacific. This distribution implies at least two transequatorial divergences within *Goniistius*, one between the antitropical populations of *C. (G.) vittatus*, and another for the remaining Northern and Southern Hemisphere taxa respectively.

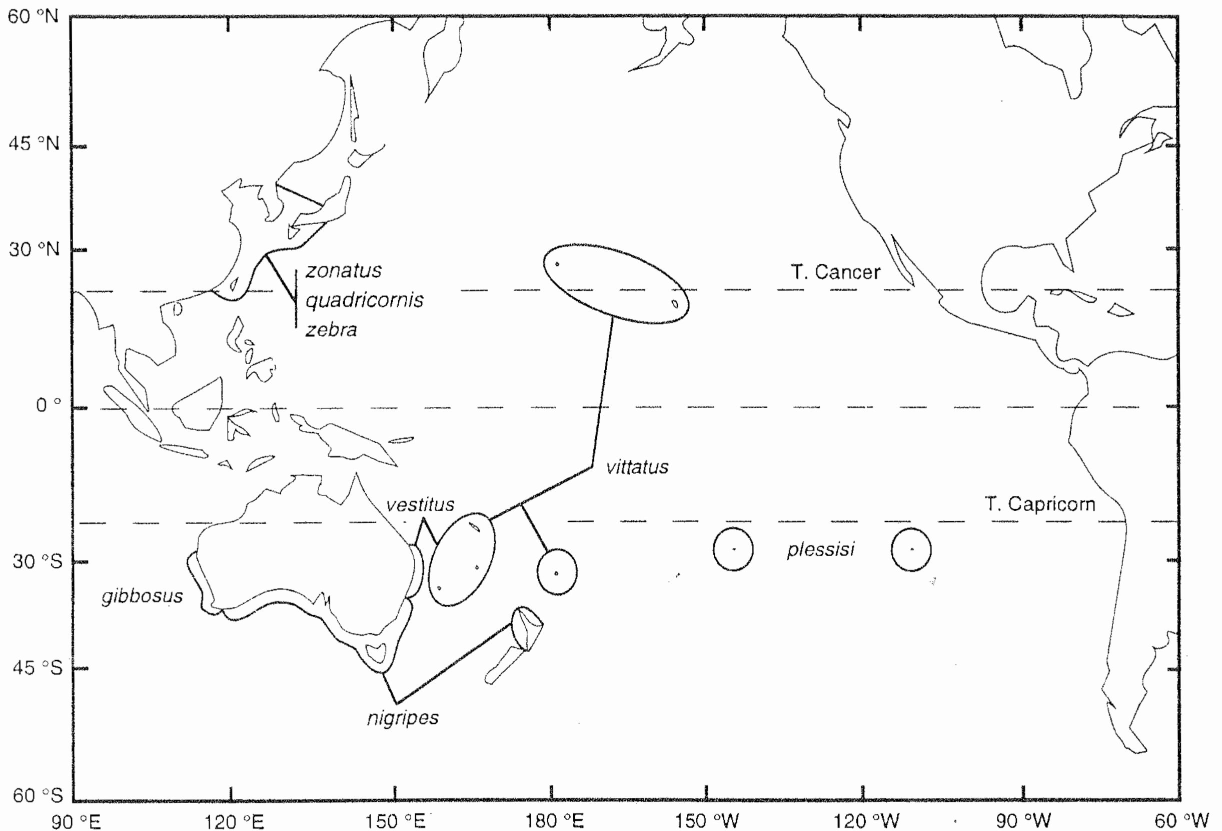


Figure 4.1. The antitropical distribution of *Goniistius*, based on Randall (1983) and Francis (1993).

The taxonomic rank of *Goniistius* is questionable. Initially erected at the generic level by Gill (1862), *Goniistius* was synonymised with *Cheilodactylus* by Allen and Heemstra (1976). Randall (1983) recognised *Goniistius* for eight species, including those previously allocated, based on the presence of a dark brown or black banding pattern on the head and body, a highly arched nape, and a pronounced fourth dorsal spine. However, he followed Allen and Heemstra (1976) in designating *Goniistius* as a subgenus within *Cheilodactylus*, but noted that generic rank would be warranted were it not for *C. fuscus* Castelnau and, in particular, *C. ephippium* McCulloch and Waite, which link the species of *Goniistius* to more typical cheilodactylids. The

rank of *Goniistius* is one of several problematic aspects of cheilodactylid taxonomy (Allen and Heemstra, 1976; Smith, 1980; Lamb, 1990; Greenwood, 1995; Chapters 3 and 5).

The objective of this study was to address questions relating to the taxonomy and antitropical biogeography of the cheilodactylid subgenus *Goniistius*. Specifically, what were the frequency, direction, timing, and possible mechanisms of transequatorial divergences, and does *Goniistius* warrant generic rather than subgeneric ranking? The analysis of mitochondrial DNA sequence data was adopted as external characters among cheilodactylids are insufficient to provide a conclusive phylogeny (Lamb, 1990).

Materials and methods

Genomic DNA was extracted from one individual of each species in Table 4.1 with the exception of *C. (G.) vittatus*, for which North and South Pacific individuals were sampled. Representatives of other cirrhitoid families were also analysed as potential outgroups.

Table 4.1. Species of *Goniistius*, *Cheilodactylus*, *Nemadactylus*, and the three potential outgroups analysed.

Species	Family	Collection site
C. (G.) nigripes Richardson	Cheilodactylidae	Horseshoe Island, Tasmania
C. (G.) zonatus Cuvier and Valenciennes	Cheilodactylidae	Shimoda Bay, Japan
C. (G.) quadricornis Günther	Cheilodactylidae	Misaki, Japan
C. (G.) zebra Döderlein	Cheilodactylidae	Misaki, Japan
C. (G.) plessisi Randall	Cheilodactylidae	Easter Island
C. (G.) vittatus Garrett	Cheilodactylidae	Lord Howe and Midway Islands
C. (G.) vestitus (Castelnau)	Cheilodactylidae	southern Queensland, Australia
C. (G.) gibbosus Richardson	Cheilodactylidae	Two Rocks, Western Australia
C. rubrolabiatus Allen and Heemstra	Cheilodactylidae	Two Rocks, Western Australia
C. spectabilis (Hutton)	Cheilodactylidae	southeastern Tasmania
C. fuscus Castelnau	Cheilodactylidae	Sydney Harbour, Australia
C. ephippium McCulloch and Waite	Cheilodactylidae	Norfolk Island
N. macropterus (Bloch and Schneider)	Cheilodactylidae	Tasman Island, Tasmania
N. valenciennesi (Whitley)	Cheilodactylidae	Port Lincoln, Australia
Aplodactylus arctidens Richardson	Aplodactylidae	Maria Island, Tasmania
Chironemus marmoratus Günther	Chironemidae	Diamond Head, NSW, Australia [a]
Cirrhitus splendens (Ogilby)	Cirrhitidae	Lord Howe Island

[a] Australian Museum frozen tissue collection (I.31253048).

Table 4.2. Genetic distances for partial mitochondrial DNA cytochrome *c* oxidase subunit I and cytochrome *b* sequences when combined. Values are Kimura (1980) two-parameter corrected percentage sequence divergences, obtained using a transition-transversion nucleotide substitution ratio of 5.5. The suffixes LH and MW correspond to Lord Howe and Midway Island respectively.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	*Cirrhitus splendens*																	
2	*Chironemus marmoratus*	25.0																
3	*Aplodactylus arctidens*	23.3	20.9															
4	*Cheilodactylus (G.) vittatus* LH	22.5	20.9	19.7														
5	*Cheilodactylus (G.) vittatus* MW	22.4	22.4	18.0	5.8													
6	*Cheilodactylus (G.) plessisi*	21.2	22.3	19.0	6.1	5.4												
7	*Cheilodactylus (G.) zebra*	22.0	22.1	18.2	5.3	4.8	4.5											
8	*Cheilodactylus (G.) gibbosus*	22.6	21.1	19.8	9.5	10.2	9.4	8.9										
9	*Cheilodactylus (G.) vestitus*	24.6	21.4	19.2	9. 7	10.7	10.4	9.8	7.2									
10	*Cheilodactylus (G.) quadricornis*	23.9	20.8	19.0	11.8	12.6	12.1	11.0	11.7	12.8								
11	*Cheilodactylus (G.) zonatus*	23.9	19.3	19.2	11.6	13.3	12.5	11.6	11.6	12.4	5.9							
12	*Cheilodactylus (G.) nigripes*	24.0	21.1	17.2	13.2	11.9	12.2	11.0	10.3	11.6	10.3	11.2						
13	*Cheilodactylus spectabilis*	23.2	20.2	19.1	12.1	12.7	11.2	10.6	10.3	11.5	11.3	10.6	8.0					
14	*Cheilodactylus fuscus*	22.7	20.8	19.9	12.5	11.1	11.0	9.8	11.5	12.4	11.8	11.3	9.1	8.0				
15	*Cheilodactylus ephippium*	22.9	19.7	17.6	10.7	10.1	10.1	9.4	11.0	12.4	11.4	10.7	8.4	8.4	6.1			
16	*Cheilodactylus rubrolabiatus*	22.8	19.4	19.3	12.8	12.3	12.3	11.1	11.8	13.5	11.0	11.4	12.0	12.9	12.3	13.7		
17	*Nemadactylus macropterus*	23.1	21.4	18.6	14.1	13.9	14.0	13.5	12.1	12.0	12. 7	12.1	10.0	10.6	11.1	12.5	12.3	
18	*Nemadactylus valenciennesi*	25.2	22.2	19.6	15.0	14.3	14.3	13.7	12. 8	13.2	13.0	12.6	10.1	11.2	12.2	12.4	14.0	4.9

Results

All sequences were deposited in GenBank (accession numbers AF067084-AF067086, AF067089, AF067091, AF092140-AF092167, AF092909, AF10251-AF102512). The cytochrome *c* oxidase subunit I and cytochrome *b* sequences analysed were 499 and 307 bp in length respectively. Among the cheilodactylids, 220 characters were variable, of which 178 were phylogenetically informative. Transition nucleotide substitutions were observed at 211 sites, while transversions were observed at 45. Twelve of the variable sites represented first or second codon positions, and five codons exhibited amino acid variation amongst the cheilodactylids. This pattern of variation, combined with the absence of length mutations, suggested that orthologous sequences were obtained.

Kimura (1980) two-parameter genetic distances among *Goniistius* species ranged from 4.5 to 13.3% sequence divergence, and distances between *Goniistius* and other cheilodactylids ranged from 8.0 to 15.0% (Table 4.2). Distances between the cheilodactylids and potential outgroups *Cirrhitus splendens*, *Chironemus marmoratus*, and *Aplodactylus arctidens*, ranged between 17.2 and 25.2% (Table 4.2). The cytochrome *b* region analysed was approximately 1.2 times more variable than the cytochrome oxidase I region, although the latter contributed the greater amount of variation to the study due to the larger number of characters analysed.

As the familial relationships between the Cheilodactylidae and the other four cirrhitoid families are not well resolved (Greenwood, 1995), outgroup selection was based on nucleotide composition and observed substitution pattern, in order to minimise random root placement (Wheeler, 1990; Lockhart *et al.*, 1994). A latrid was not assessed as the separation of this family from the majority of cheilodactylids is questionable (Chapter 5). During pairwise comparisons of each potential outgroup with the cheilodactylids, significant heterogeneity of variable site nucleotide composition was observed only for *Chironemus marmoratus* with *C. (G.) zebra* and *C. (G.) plessisi* (χ^2=9.614 and 8.881 respectively, d.f.=3, $P<0.05$). Saturation of sequence evolution was evidenced by significantly lower TI/TV ratios than that derived from

comparisons among the cheilodactylids (Figure 4.2; $P<0.001$, d.f.=3, one-way ANOVA with Tukey's HSD post hoc tests). The degree of saturation was smallest for *Aplodactylus arctidens*, and consequently this species was chosen as the outgroup for phylogenetic analyses.

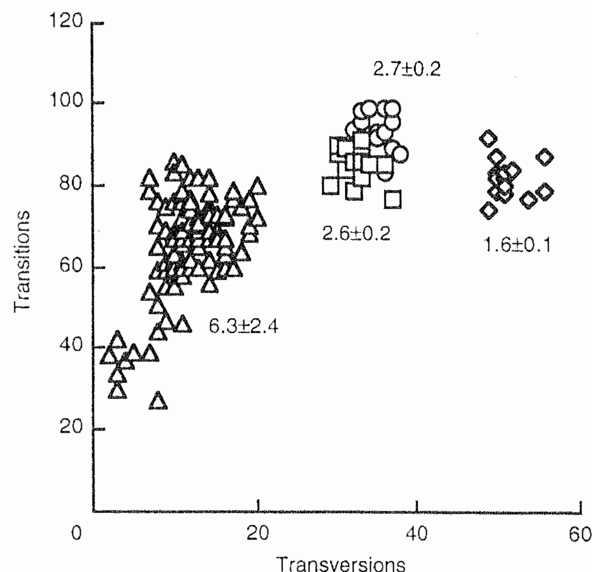
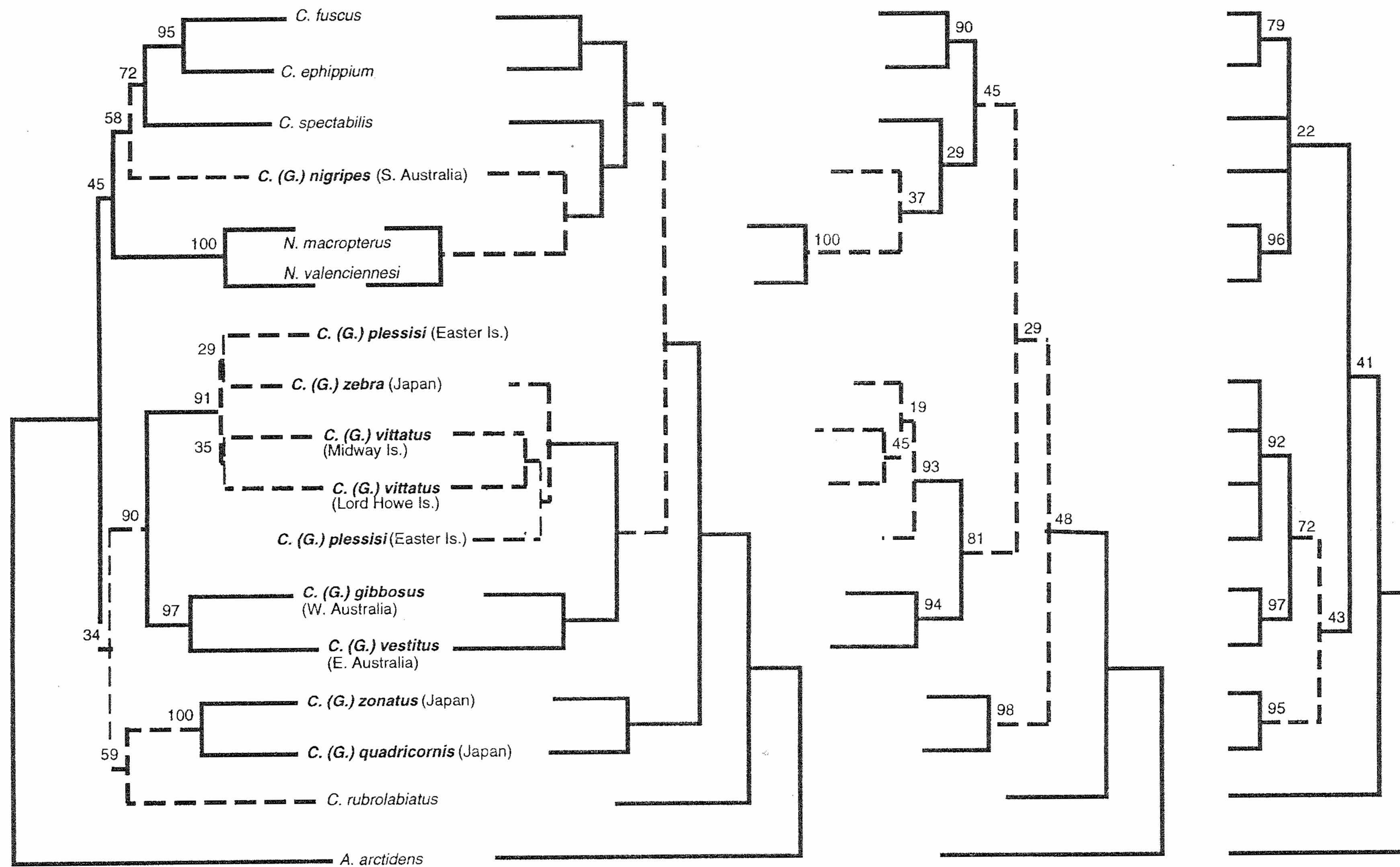


Figure 4.2. The pattern of observed nucleotide substitution accumulation at third codon positions for partial mitochondrial DNA cytochrome *c* oxidase subunit I and cytochrome *b* sequences when combined. Triangles represent pairwise comparisons among cheilodactylids. Squares, circles and diamonds represent comparisons of *Aplodactylus arctidens* (Aplodactylidae), *Chironemus marmoratus* (Chironemidae), and *Cirrhitus splendens* (Cirrhitidae) against the cheilodactylids respectively. The mean observed transition-transversion nucleotide substitution ratio and its standard deviation are listed for each set of comparisons.

The partition homogeneity test (200 partition replicates, Heuristic search algorithm with 100 replicates of random sequence stepwise addition) indicated phylogenetic congruence between cytochrome oxidase I and cytochrome *b* sequences, both for all characters (P=0.90) and third codon positions alone (P=0.97), allowing the combination of genes during phylogenetic analyses. Estimated tree length-frequency distributions were significantly skewed, both when all sequences ($g1$=-0.659, $P<0.01$) and those of *Goniistius* alone ($g1$=-0.890, $P<0.01$) were analysed, suggesting the presence of phylogenetic signal.



C. fuscus
C. ephippium
C. spectabilis
C. (G.) nigripes (S. Australia)
N. macropterus
N. valenciennesi
C. (G.) plessisi (Easter Is.)
C. (G.) zebra (Japan)
C. (G.) vittatus (Midway Is.)
C. (G.) vittatus (Lord Howe Is.)
C. (G.) plessisi (Easter Is.)
C. (G.) gibbosus (W. Australia)
C. (G.) vestitus (E. Australia)
C. (G.) zonatus (Japan)
C. (G.) quadricornis (Japan)
C. rubrolabiatus
A. arctidens
0 2 4 6 8% seq divergence
a
0.00 0.02 0.04 0.06 0.08 0.10 exp subs./site
b
0 20 40 60 80 100 steps
c
d

Figure 4.3 (previous page). Results from the phylogenetic analysis of cheilodactylid mitochondrial partial DNA cytochrome *c* oxidase subunit I and cytochrome *b* sequences when combined. Broken lines indicate regions of topological difference between the trees with respect to *Goniistius* species. The numbers above the branches at each node represent the bootstrap proportions for the taxa in each clade, as derived from 2000 replicate data sets. (**a**) Neighbour-joining analysis; (**b**) Maximum likelihood analysis, 10 randomisations of sequence input order, ml=-4195.6, optimum (near asymptotic) expected TI/TV nucleotide substitution ratio was 5.5, all branch lengths significantly positive ($P<0.05$); (**c**) One of the two equally most-parsimonious trees obtained from unweighted parsimony analysis (Heuristic search algorithm, 100 random stepwise sequence additions). Tree length=641 steps, CI=0.482, RI=0.467. The alternative topology was identical to **b**; (**d**) Strict consensus of five equally most-parsimonious trees obtained when giving transversion nucleotide substitutions increased weight in accordance to the optimum TI/TV ratio from maximum likelihood analysis. Tree length=2159 steps.

With respect to *Goniistius*, the topologies from neighbour-joining, maximum likelihood, and maximum parsimony analyses differed only at nodes that received less than 60% bootstrap support (Figure 4.3). *Goniistius* was not clustered as a monophyletic group in each of these analyses. Five species formed one clade that received high (>70%) bootstrap support: *C. (G.) plessisi*, *C. (G.) zebra*, *C. (G.) vittatus*, *C. (G.) gibbosus*, *C. (G.) vestitus*. Strong bootstrap support was observed for a sister relationship between *C. (G.) zonatus* and *C. (G.) quadricornis*, but the placement of this clade varied between analyses and did not receive high bootstrap support. The remaining *Goniistius* species, *C. (G.) nigripes*, consistently clustered with three non-*Goniistius* species of *Cheilodactylus* and two representatives of *Nemadactylus*. The relationships among these six taxa varied among analyses, and the bootstrap support for this clade and the placement of *C. (G.) nigripes* were low. There were insufficient transversions for the majority of relationships to be resolved when parsimony analysis was restricted to these character state changes.

The maximum likelihood, unweighted maximum parsimony, and neighbour-joining topologies were not significantly different according to the Templeton (1983) and Kishino and Hasegawa (1989) tests (Table 4.3). The most parsimonious topology obtained when enforcing *Goniistius* monophyly was significantly inferior to the maximum likelihood and unweighted maximum parsimony topologies, but not the neighbour-joining topology (Table 4.3). The topology obtained when enforcing monophyly of all *Goniistius* species except *C. (G.) nigripes* was not

significantly inferior to any alternative (Table 4.3). Enforcing monophyly of the Asian species, *C. (G.) quadricornis*, *C. (G.) zonatus*, and *C. (G.) zebra*, produced a topology significantly inferior than the majority of alternatives (Table 4.3).

Table 4.3. Probabilities that alternative cheilodactylid topologies are not significantly different. For each comparison the upper value represents the Templeton (1983) test, while the lower value represents the Kishino and Hasegawa (1989) test. The topologies compared were those obtained from maximum likelihood, unweighted maximum parsimony, and neighbour-joining analyses (Figure 4.3), and also three topologies obtained from the enforcement of topological constraints: *Goniistius* monophyly, *Goniistius* without *C. (G.) nigripes* monophyly, monophyly of the Asian species *C. (G.) quadricornis*, *C. (G.) zonatus* and *C. (G.) zebra*. Asterisks denote $P<0.05$.

Topology	1.	2.	3.	4.	5.
1. First unweighted parsimony and maximum likelihood; 641 steps, ml=-4195.6 (Figure 4.3b)					
2. Second unweighted parsimony;	1.000				
641 steps, ml=-4196.2 (Figure 4.3c)	0.865				
3. *Goniistius* without *nigripes* monophyly	>0.500	0.058			
(similar to 2:11 TI/TV weighted parsimony,	0.238	0.379			
Figure 4.3d); 647 steps, ml=-4202.5					
4. Neighbour joining;	0.086	0.086	0.493		
651 steps, ml=-4209.5 (Figure 4.3a)	0.271	0.294	0.596		
5. *Goniistius* monophyly;	0.011*	0.016*	0.102	0.546	
655 steps, ml=-4227.5	0.026*	0.035*	0.042*	0.263	
6. Monophyly of *C. (G.) quadricornis, zonatus,*	0.000*	0.000*	0.000*	0.057	0.140
and *zebra*; 665 steps, ml=-4265.1	0.000*	0.000*	0.000*	0.006*	0.066

The two-cluster test revealed third codon position nucleotide substitution rate heterogeneity at the 5% level amongst *C. (G.) zebra*, *C. (G.) plessisi*, and *C. (G.) vittatus* when nodes were analysed individually (CP=96.7%), but no heterogeneity was observed for all nodes when analysed simultaneously ($0.50>P>0.25$). The branch-length test did not detect significant rate heterogeneity.

Discussion

Non-monophyly of *Goniistius*

The results suggest that taxonomic modification of *Goniistius* is required. *Cheilodactylus (G.) nigripes* should be excluded from *Goniistius*, since it is not the type species and the topologies obtained do not favour its inclusion within this group (Figure 4.3; Table 4.3). It is readily distinguished from the other *Goniistius* by dorsal and anal fin ray counts, a more anterior position of the mouth, and broader black bars on the body (Randall, 1983). Rather than *C. (G.) nigripes* representing the most plesiomorphic member of *Goniistius* (Randall, 1983), it appears to be a member of a divergent cheilodactylid group. For the purpose of this discussion, it will be considered that *Goniistius* consists of only the remaining seven species.

Although only weighted parsimony analysis clustered the remaining seven *Goniistius* as monophyletic, the levels of bootstrap support for alternative relationships were low, and the topology recovered when enforcing monophyly was not significantly inferior to any alternative (Figure 4.3, Table 4.3). Therefore, the hypothesis of monophyly for these taxa cannot be rejected.

Rank of *Goniistius*

The levels of genetic divergence between *Goniistius* and non-*Goniistius* species of *Cheilodactylus* (8.0-13.5%) are of similar magnitude to that observed during comparisons between cheilodactylid genera (10.0-15.0%, Table 4.2; see also Chapter 5). This suggests that *Goniistius* warrants generic rather than subgeneric rank. In addition, none of the seven *Goniistius* appear closely related to *C. fuscus* or *C. ephippium*, the species that prevented *Goniistius* (with the inclusion of *nigripes*) attaining generic rank according to Randall (1983). The levels of divergence among *C. (G.) zonatus*, *C. (G.) quadricornis*, and the other five *Goniistius* (11.0-13.3%) are also of similar magnitude to that observed between any *Goniistius* and the other cheilodactylids (Table 4.2; Chapter 5). Therefore, these two divergent and well-supported *Goniistius* groups may warrant individual generic distinction. This separation would also highlight the non-monophyly of the three Asian cheilodactylids (Figure 4.3; Table 4.3).

Cryptic speciation

Levels of genetic divergence suggest that speciation has occurred within *C. (G.) vittatus* without the development of obvious morphological differentiation (cryptic speciation). The Lord Howe and Midway Island individuals of *C. (G.) vittatus* differed by 5.8% sequence divergence, while the interspecific divergences among these individuals, *C. (G.) zebra,* and *C. (G.) plessisi* ranged from 4.7 to 6.1% (Table 4.2). Each of these three species can be clearly distinguished morphologically, yet the divergence observed between northern and southern *C. (G.) vittatus* individuals is of similar magnitude to values of interspecfic divergence. Due to the difficulty of obtaining material from *C. (G.) vittatus*, it was not possible to assess the level of variability within North and South Pacific populations, and therefore reject the possibility of one single, highly variable species. However, high genetic variation is considered unlikely for *C. (G.) vittatus* given the presently small and geographically restricted nature of its populations.

Randall (1981, 1983) provisionally recognised *C. (G.) vittatus* from the South Pacific, based on live photographs taken at Lord Howe Island and New Caledonia. Kermadec and Lord Howe Island specimens were later identified as *C. (G.) vittatus* following Randall's (1983) classification (Francis *et al.* 1987; M. P. Francis, NIWA, New Zealand 1998 pers. comm.; M.A. McGrouther, Australian Museum 1998 pers. comm.), but further morphological examination of specimens may identify differences across the tropics.

Frequency, direction, and timing of transequatorial divergences

The distribution of *Goniistius* suggests that two transequatorial divergences have occurred, one between the populations of *C. (G.) vittatus*, and another for the remaining Northern and Southern Hemisphere taxa. However, the molecular phylogeny of *Goniistius* indicates three transequatorial divergences, as the Asian species are not monophyletic (Figure 4.3; Table 4.3). The first divergence involved the separation of the *C. (G.) zonatus-C. (G.) quadricornis* lineage from that of a Southern Hemisphere cheilodactylid, most likely the ancestor of the remaining *Goniistius*. Two transequatorial divergences are then evident for *C. (G.) zebra, C. (G.)*

plessisi, and the antitropical populations of *C. (G.) vittatus*, given that a Northern or Southern Hemisphere monophyletic group was not observed among these taxa (Figure 4.3).

If the antitropical distribution of *Goniistius* is entirely the result of dispersal (see below), the direction of two of the three transequatorial events is known. Firstly, the divergence of *C. (G.) zonatus* and *C. (G.) quadricornis* from a Southern Hemisphere cheilodactylid represents a south-to-north event. Movement in the same direction is required to account for *C. (G.) zebra* in the other *Goniistius* clade, but as this clade is not fully resolved it is not known whether the divergence of *C. (G.) vittatus* populations was a south-to-north or north-to-south event.

Levels of genetic divergence suggest that the three transequatorial divergences of *Goniistius* occurred during two distinct periods. Genetic divergences among *C. (G.) vittatus*, *C. (G.) zebra*, and *C. (G.) plessisi* were similar, ranging from 4.5-6.1%, while those between *C. (G.) zonatus* or *C. (G.) quadricornis* and any other cheilodactylid were distinctly larger, ranging from 10.3-13.3% (Table 4.2). Estimated times for these periods of equatorial divergence are 4.8-6.9 Myr ago (late Miocene to early Pliocene) and 12.7 - 18.3 Myr ago (mid Miocene), based on mitochondrial protein four-fold degenerate site molecular clock calibrations of 2.3% and 3.3% sequence divergence Myr^{-1} (Martin *et al.*, 1992; Bermingham *et al.*, 1997). These absolute estimates of divergence time should be considered with caution, given the number of assumptions made when employing molecular clock calibrations (Rand, 1994), and as marginal substitution rate heterogeneity was observed among some lineages. Faster and slower calibrations have been reported for other taxa, and those employed here are considered "median" values.

Mechanisms for transequatorial divergences

Hypotheses involving Mesozoic biogeographic events for the development of antitropical distributions, such as island integration (Rotondo *et al.*, 1981) and the fragmentation of Pangea or Pacifica (Nelson, 1985; Crame, 1993), can be rejected for *Goniistius* based on the estimated divergence times. These and other similarly ancient events have been discounted for the

majority of marine antitropical taxa (Briggs, 1987a; Cox, 1990; Lindberg, 1991). Consequently, the transequatorial divergences within *Goniistius* may be explained by chance dispersal across equatorial waters, or by vicariant isolation caused by interspecific competition or a mid Miocene rise in temperatures.

A proposed mid Miocene equatorial warming event, and other climatic changes of that period, may represent a common vicariant origin for many antitropical distributions (Valentine, 1984; White, 1986). The earliest transequatorial divergence involving *Goniistius* species is suggested to have occurred during the mid Miocene, and therefore equatorial warming may be invoked in this case. However, there is ongoing debate as to whether the required climatic transitions for this method of vicariance did in fact occur (Springer, 1982; Briggs, 1987a, b; White, 1989; Adams *et al.*, 1990), and the mid Miocene is too early to explain the other transequatorial divergences within *Goniistius* (but see Crame 1993 for more recent phases of global warming).

Briggs (1987a) suggested a vicariance mechanism with extinction in equatorial waters resulting from interspecific interactions, predominantly competition with younger taxa. This mechanism can be widely applied but is difficult to test, and hence remains plausible for most antitropical taxa unless equatorial habitat is not available.

Chance dispersal across equatorial waters, particularly during periods of climatic cooling such as the Pleistocene glaciations, has been suggested by several authors for the establishment of antitropical distributions (Darwin, 1859; Hubbs, 1952; Ekman, 1953; Randall, 1981; Lindberg, 1991). Although the juveniles and adults of *Goniistius* are predominantly sedentary around nearshore reefs (Randall, 1983; Sano and Moyer, 1985; Cappo, 1995), their larvae may have high dispersal capabilities given the 9-12 month offshore pelagic phase observed for other cheilodactylids (Annala, 1987; Andrew *et al.*, 1995), and evidence of recent gene flow between geographically distant populations of *Nemadactylus* and *Acantholatris* (Elliott and Ward, 1994; Grewe *et al.*, 1994; Chapter 3). The occupation of the isolated Hawaiian and Easter Island regions by *Goniistius* also suggests high dispersal capabilities for this group. Divergence time

estimates within *Goniistius* are not sufficiently recent to be allied with Pleistocene glaciations, but this does not discount chance dispersal during other epochs. The habits of these taxa make them unlikely candidates for isothermal submergence.

While the exact mechanisms responsible for the antitropical distribution of *Goniistius* have not been identified, such is often difficult to achieve (Stepien and Rosenblatt, 1996; Bowen and Grant, 1997; Koufopanou *et al.*, 1999). However, with respect to *Goniistius* several mechanisms and epochs can be discounted based on the results of this study.

CHAPTER 5: Molecular phylogeny of the Cheilodactylidae and Latridae.

Abstract

Cheilodactylid and latrid fishes are nearshore species, which are widely distributed in the Southern Hemisphere. The phylogenetic relationships of these taxa were reconstructed from mitochondrial DNA sequences and their taxonomy and biogeography was examined. The classification of cheilodactylids requires much modification. The two South African *Cheilodactylus* warrant familial distinction from all other cheilodactylids. As one of the South African *Cheilodactylus* is the type species, the remaining 25 cheilodactylids require familial reassignment, and the 12 non-African *Cheilodactylus* require generic reassignment. At the familial level, transfer to the Latridae is suggested for these 25 cheilodactylids. At the generic level, *Goniistius*, *Zeodrius*, and *Morwong* can be resurrected for at least nine species of *Cheilodactylus*. In addition, the South African members of *Chirodactylus* should revert to *Palunolepis*, and *Acantholatris* is synonymous with *Nemadactylus*. Divergence time estimates indicate that chance dispersal rather than vicariance accompanying Gondwana fragmentation best explains the present distribution of cheilodactylids.

Introduction

The Cheilodactylidae and Latridae comprise conspicuous components of the temperate coastal reef fish faunas of the Southern Hemisphere. They have greatest diversity in the waters of Australia and New Zealand, but also occur around South Africa, South America, and several oceanic islands (Figure 5.1). The Cheilodactylidae is also represented in the North Pacific; and is thus an antitropically-distributed taxon (Randall, 1981). The majority of species are solitary, occurring demersally over inshore rocky reefs and feeding on small benthic invertebrates (Sano and Moyer, 1985; Annala, 1987; Cappo, 1995; McCormick, 1998).

There are five genera and 27 species recognised in the Family Cheilodactylidae (Table 5.1). A number of problems have been identified regarding their taxonomy. It appears that the South African *Cheilodactylus* Lacépède merits generic distinction from the remaining members of this genus (Allen and Heemstra, 1976; Lamb, 1990). There are also questions regarding the status of *Chirodactylus* Gill (Lamb, 1990). Further, it has been suggested that *Nemadactylus* Richardson and *Acantholatris* Gill should be re-allocated in the Latridae (Greenwood, 1995). In contrast, only three genera and four species of latrids are recognised (Table 5.1), and their taxonomy is considered robust (Lamb, 1990).

There are a number of interesting questions regarding cheilodactylid and latrid biogeography. Cheilodactylid distribution is typically Gondwanan, with representation around Australia, New Zealand, South America, and Africa, suggesting vicariant isolation of taxa during continent fragmentation. However, the occupation of isolated islands and seamounts by *Nemadactylus* and *Acantholatris* indicates some chance dispersal for their present distribution. Dispersal and vicariance hypotheses may be discriminated in this case by species-area relationships and estimates of lineage divergence times, as the order and timing of Gondwana fragmentation is well understood (Lawver *et al.* 1992).

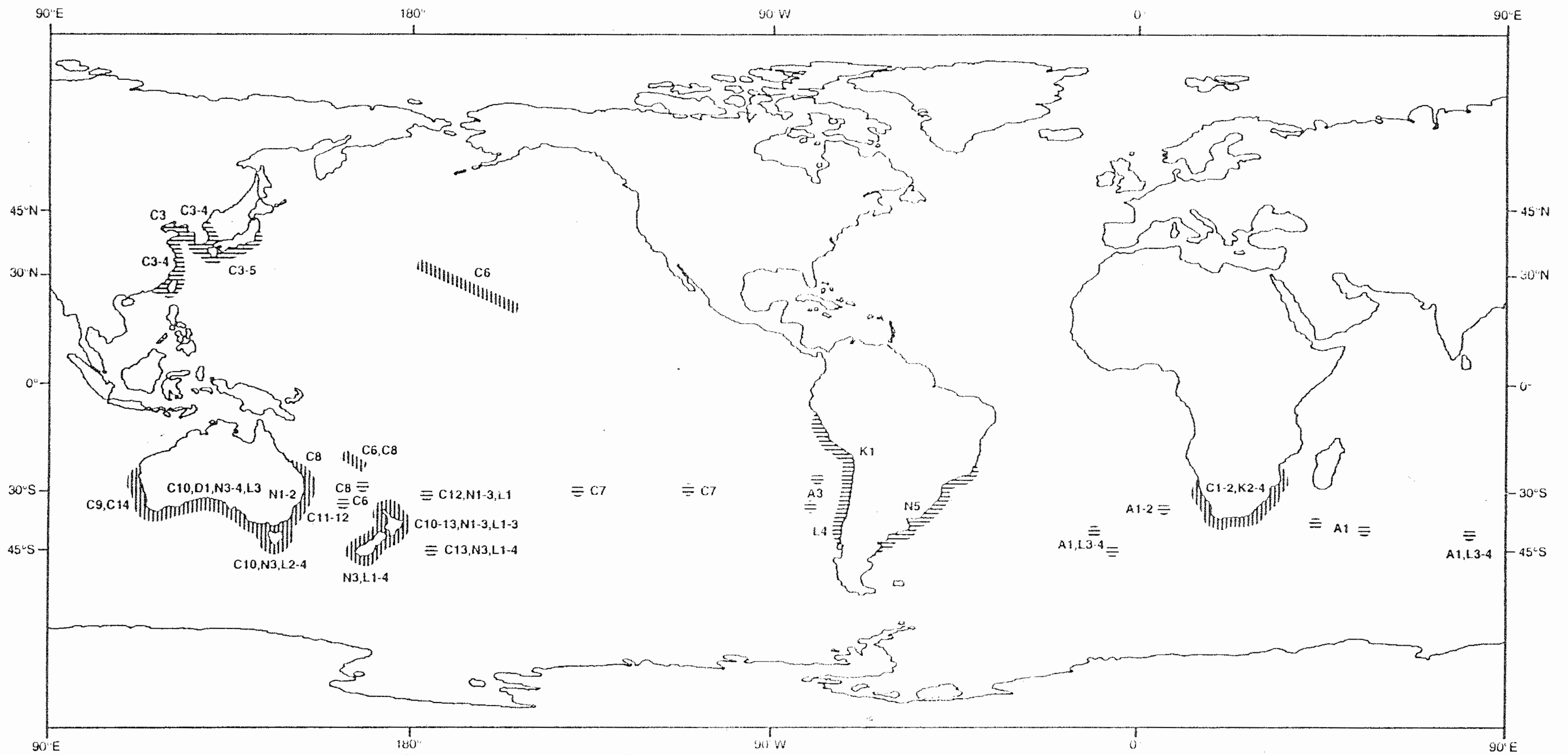


Figure 5.1. Distribution of cheilodactylid and latrid species. Hatched areas mark the overall distribution of both groups, with numbers corresponding to individual taxa as listed in Table 5.1.

Table 5.1. Present taxonomy of the Cheilodactylidae and Latridae, proposed changes, and sources of material analysed. Species are numbered in accordance to their distribution in Figure 5.1.

	Present allocation	Proposed change	Additional reference	Source material
	Cheilodactylidae			
1	*Cheilodactylus fasciatus* Lacépède	–		Tsitsikamma, South Africa
2	*Cheilodactylus pixi* Smith	–		Port Elizabeth, South Africa
		Latridae		
3	*Cheilodactylus (Goniistius) zonatus* Cuvier	*Goniistius zonatus*	Chapter 4	Shimoda Bay, Japan
4	*Cheilodactylus (Goniistius) quadricornis* (Günther)	*Goniistius quadricornis*	Chapter 4	Misaki, Japan
5	*Cheilodactylus (Goniistius) zebra* (Döderlein)	*Zeodrius zebra*	Chapter 4	Misaki, Japan
6	*Cheilodactylus (Goniistius) vittatus* Garrett[1]	*Zeodrius vittatus*	Chapter 4	Midway Island
7	*Cheilodactylus (Goniistius) plessisi* Randall	*Zeodrius plessisi*	Chapter 4	Easter Island
8	*Cheilodactylus (Goniistius) vestitus* (Castelnau)	*Zeodrius vestitus*	Chapter 4	southern Queensland, Australia
9	*Cheilodactylus (Goniistius) gibbosus* Richardson	*Zeodrius gibbosus*	Chapter 4	Two Rocks, Western Australia
10	*Cheilodactylus (Goniistius) nigripes* Richardson	generic re-allocation required	Chapter 4	Horseshoe Island, Tasmania
11	*Cheilodactylus fuscus* Castelnau	*Morwong fuscus*		Sydney Harbour, Australia
12	*Cheilodactylus ephippium* McCulloch and Waite	*Morwong ephippium*		Norfolk Island
13	*Cheilodactylus spectabilis* Hutton	generic re-allocation required		southeastern Tasmania
14	*Cheilodactylus rubrolabiatus* Allen and Heemstra	generic re-allocation required		Two Rocks, Western Australia
15	*Chirodactylus variegatus* (Valenciennes)	–		Las Cruces, Chile
16	*Chirodactylus grandis* (Günther)	*Palunolepis grandis*		Cape Seal, South Africa

	Present allocation	Proposed change	Additional reference	Source material
17	*Chirodactylus brachydactylus* (Cuvier)	*Palunolepis brachydactylus*		Kenton-on-sea, South Africa
18	*Chirodactylus jessicalenorum* Smith	*Palunolepis jessicalenorum*		Durban, South Africa
19	*Dactylophora nigricans* (Richardson)	_		Port Phillip Bay, Australia
20	*Nemadactylus macropterus* (Bloch and Schneider)	_	Chapter 3	Tasman Island, Tasmania
21	*Nemadactylus* sp.[2,3]	_	Chapter 3	Kiama, Australia[4]
22	*Nemadactylus bergi* (Norman)[3]	_	Chapter 3	Mar del Plata, Argentina
23	*Nemadactylus douglasii* (Hector)	_	Chapter 3	Coffs Harbour, Australia[4]
24	*Nemadactylus valenciennesi* (Whitley)	_	Chapter 3	Port Lincoln, South Australia
25	*Acantholatris monodactylus* (Carmichael)	*Nemadactylus monodactylus*	Chapter 3	Tristan Island, Tristan da Cunha
26	*Acantholatris vemae* Penrith	*Nemadactylus vemae*	Chapter 3	not analysed
27	*Acantholatris gayi* (Kner)[3]	*Nemadactylus gayi*	Chapter 3	Juan Fernández
	Latridae			
28	*Latridopsis ciliaris* (Bloch and Schneider)	_		Portobello, New Zealand
29	*Latridopsis forsteri* (Castelnau)	_		Derwent Estuary, Tasmania
30	*Latris lineata* (Bloch and Schneider)	_		southeastern Tasmania
31	*Mendosoma lineatum* Guichenot	_		Gough Island

[1] South Pacific *C. (G.) vittatus* appears worthy of separate specific status (Chapter 4)

[2] Roberts (1993), Smith *et al.* (1996)

[3] Excluded from study, but see Chapter 3 for their analysis

[4] Australian Museum frozen tissue collection (*Nemadactylus* sp. I.34845-001, *N. douglasii* I.34844-001)

The aims of this study are to reconstruct the phylogeny of cheilodactylids and latrids, address their taxonomic uncertainties, and test biogeographical hypotheses. As external characters are variable but largely phylogenetically uninformative among these taxa (Lamb, 1990), the analysis of molecular data is employed. Molecular approaches to systematic studies are often successful in addressing problems left unresolved by morphological analyses (Avise, 1994). In addition, the application of a molecular clock calibration enables the estimation of lineage divergence time (Vawter *et al.* 1980), facilitating the examination of alternative biogeographical hypotheses.

Materials and methods

DNA was extracted from one individual of each cheilodactylid and latrid species listed in Table 5.1, with exception of several *Nemadactylus* and *Acantholatris* which were analysed previously (Chapter 3), and *A. vemae* which was not available. DNA also was extracted from representatives of the other three cirrhitoid families, for use as outgroups; *Cirrhitus splendens* (Ogilby) (Cirrhitidae), *Chironemus marmoratus* Günther (Chironemidae), and *Aplodactylus arctidens* Richardson (Aplodactylidae).

Homogeneity chi-squared analysis was used to identify significant difference in the nucleotide composition of variable sites during comparisons of taxa. Nucleotide composition pairwise distances also were calculated according to Gillespie (1986), and subjected to minimum evolution analysis (see below) for comparison against reconstructed phylogenies. The presence of nucleotide substitution saturation was examined from the relative accumulation of observed transition (TI) and transversion (TV) substitutions during pairwise comparisons of taxa.

Minimum evolution analysis of DNA sequences was performed by PAUP* using the heuristic search algorithm (200 replicates). Starting trees were obtained by neighbour-joining, and pairwise distances were calculated under the Kimura (1980) two-parameter model.

Results

All sequences are deposited in GenBank (accession nos. AF067084-AF067091, AF067094, AF092140-AF092149, AF092151-AF092160, AF092162-AF092167, AF092909, AF102511-AF102512, AF133064, AF133069, AF136267, AF156229-AF156247). The cytochrome *c* oxidase subunit I and cytochrome *b* sequences analysed were 499 and 307 bp in length respectively. Among the cheilodactylids and latrids 273 characters were variable, of which 250 were phylogenetically informative. Transition nucleotide substitutions were observed at 257 sites, while TVs occurred at 101. Twenty-nine of the variable sites represented first or second codon positions, and 14 codons exhibited amino acid variation. Length mutations were absent. This pattern of variation, also observed for the outgroups, suggested that orthologous sequences were obtained.

The partition homogeneity test indicated phylogenetic congruence between genes, both for all characters (P=0.070) and third codon positions (P=0.320), allowing their combination during phylogenetic analyses. The estimated tree length-frequency distribution of the dataset was significantly skewed ($g1$=-0.96, P<0.01), suggesting the presence of phylogenetic signal. Heterogeneity of nucleotide composition at variable sites was observed from 22 of the 435 pairwise comparisons of taxa (P<0.05). Eighteen of these comparisons involved *Latris lineata*, *Latridopsis ciliaris*, and *Latridopsis forsteri*, three were between the outgroups and cheilodactylids, and one was within the cheilodactylids. The topology obtained from clustering of nucleotide composition distances had little resemblance to those from phylogenetic analysis, indicating minimal influence of nucleotide composition on phylogenetic reconstruction. Saturation of transition substitutions was indicated by a reduction in their observed rate of accumulation relative to transversions (Figure 5.2).

The results from minimum evolution analysis and the consensus of four minimum length trees (1267 steps) from unweighted maximum parsimony analysis are depicted in Figure 5.3. The topologies differ only where bootstrap support values are less than 63%. Largely congruent topologies and levels of bootstrap support were obtained from 3:1 TV/TI weighted parsimony

analysis (three trees, 1731 steps), but relationships were poorly defined from parsimony analysis when excluding transitions at all (11 trees, 238 steps) or third (97 trees, 308 steps) codon positions, due to the large reduction of informative character state changes.

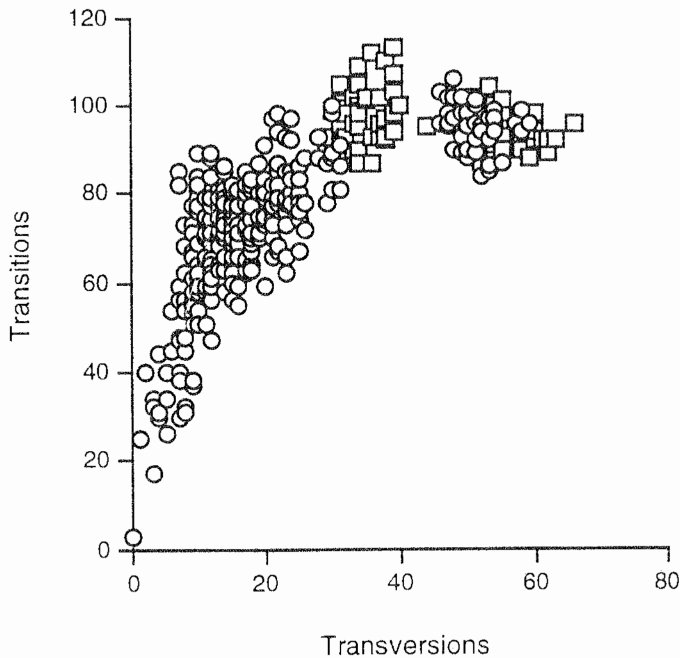


Figure 5.2. Frequency of observed transition and transversion nucleotide substitutions during pairwise comparisons of partial cytochrome *c* oxidase subunit I and cytochrome *b* mitochondrial DNA sequences Comparisons were performed among cheilodactylids and latrids (circles), and between these taxa and the outgroups *Cirrhitus splendens* (Cirrhitidae), *Chironemus marmoratus* (Chironemidae), and *Aplodactylus arctidens* (Aplodactylidae) (squares).

The two South African *Cheilodactylus* were clustered as sister taxa with high bootstrap support, but these species were divergent from the non-African *Cheilodactylus* and other cheilodactylids (Figure 5.3). The levels of sequence divergence between the South African and non-African *Cheilodactylus* were appreciably greater than those between the non-African *Cheilodactylus* and other cheilodactylid genera, and were more similar to the those observed during comparisons with other cirrhitoid families (Table 5.2). Minimum length trees recovered when enforcing *Cheilodactylus* monophyly were significantly inferior to those from unconstrained analysis (1301 steps, P=0.002), but not when excluding the South African *Cheilodactylus* (1276 steps, P=0.107). Cheilodactylid monophyly minimum length trees were

also significantly inferior to those from unconstrained analysis (1293 steps, P=0.019), but not without the South African *Cheilodactylus* (1271 steps, P=0.366).

Table 5.2. Range of pairwise Kimura (1980) percentage genetic distances among cheilodactylid species groups and against the outgroup taxa.

Species group	1.	2.	3.
1. South African *Cheilodactylus*			
2. non South African *Cheilodactylus*	19.49-22.28		
3. *Nemadactylus, Acantholatris, Chirodactylus, Dactylophora*	20.59-22.34	4.74-14.40	
4. Aplodactylidae, Chironemidae, Cirrhitidae	20.31-22.80	16.32-22.91	16.85-23.23

Ten of the 12 non-African *Cheilodactylus* were structured into four moderate (50-70% bootstrap) to highly (>70% bootstrap) supported clades (Figure 5.3). The largest of these contained five of the eight members of the subgenus *Goniistius*. *Cheilodactylus (G.) zonatus* and *C. (G.) quadricornis* were clustered as another clade with high support, while the placement of the remaining *Goniistius*, *C. (G.) nigripes*, was variable between analyses and received low support. Enforced *Goniistius* monophyly produced trees which were not significantly inferior to those from unconstrained analysis (1277 steps, P=0.371). *Cheilodactylus fuscus* and *C. ephippium* were placed as sister-taxa with high support. A sister relationship between *C. spectabilis* and *Chirodactylus variegatus* received moderate and high support from unweighted parsimony and minimum evolution analysis respectively, but such a relationship was not observed from 3:1 TV/TI weighted parsimony analysis. *Cheilodactylus rubrolabiatus* was clustered with the majority of *Goniistius*, although its placement varied and received only moderate support at best.

The South African *Chirodactylus* were clustered as a monophyletic clade with high support, but the genus was not monophyletic given placement of the South American *Chirodactylus variegatus*. Enforced *Chirodactylus* monophyly produced trees not significantly inferior to those from unconstrained analysis (1270 steps, P=0.683).

With the exception of the South African *Cheilodactylus*, *Dactylophora nigricans* was placed most basal of the cheilodactylids in minimum evolution and unweighted parsimony analysis (Figure 5.3), and also in two of the three trees obtained from 3:1 TV/TI weighted parsimony analysis. Representatives of *Nemadactylus* and *Acantholatris* formed a highly supported clade, with *Nemadactylus* paraphyletic relative to *Acantholatris*.

The latrids were placed in a highly supported clade containing all cheilodactylids with exception of the South African *Cheilodactylus*. *Latris lineata* and the two species of *Latridopsis* formed a similarly supported clade, but placement of the remaining latrid, *Mendosoma lineatum*, varied between analyses and did not receive high bootstrap support. Enforced latrid monophyly produced trees not significantly inferior to those from unconstrained analysis (1268 steps, P=1.000). Enforcing monophyly of *Nemadactylus*, *Acantholatris*, and the Latridae also produced trees not significantly inferior to those from unconstrained analysis (1272 steps, P=0.461).

The two-cluster test revealed third codon position nucleotide substitution rate heterogeneity among taxa when nodes were analysed simultaneously (χ^2=42.156, df=28, P<0.05). When analysed individually, rate heterogeneity was observed at five nodes, encompassing 16 taxa (CP>95%). The branch-length test also revealed rate heterogeneity above the 95% level for four of these taxa.

Discussion

South African *Cheilodactylus*

This study supports previous suggestions that the South African species of *Cheilodactylus*, *C. fasciatus* and *C. pixi*, are as divergent from the non-African *Cheilodactylus* as they are from members of the other cheilodactylid genera (Table 5.2; Allen and Heemstra 1976; Lamb 1990). However, these two South African species are actually as divergent from the other cheilodactylids as members of the Cirrhitidae, Chironemidae, and Aplodactylidae (Table 5.2,

Figure 5.3). Therefore, rather than the South African *Cheilodactylus* simply requiring generic distinction from the non-African *Cheilodactylus* as suggested by Lamb (1990), it appears that these two South African species also require familial distinction from all other cheilodactylids. The South African *Cheilodactylus* are distinguished from all other cheilodactylids by a higher lateral line scale count, the lack of a swim bladder, and the presence of scales on the postcleithrum (Lamb, 1990).

Familial reassignment

Based on the suggestion that *Cheilodactylus fasciatus* and *C. pixi* be given familial distinction from all other cirrhitoids, and as *C. fasciatus* is the type species, the remaining 25 cheilodactylids require familial re-assignment. These remaining cheilodactylids were clustered with the latrids at high bootstrap support, and no major division was observed among them (Figure 5.3, Figure 5.4). Therefore, it is suggested those cheilodactylids requiring familial reassignment should be transferred to the Latridae. This approach will obscure the common phyletic relationships of *Nemadactylus*, *Acantholatris* and the latrids proposed by Greenwood (1995) based on urohyal morphology, although this could be rectified, if warranted, by subfamilial distinction. While *Nemadactylus*, *Acantholatris*, and the Latridae did not cluster together as suggested by Greenwood (1995), enforced monophyly failed to produce significantly inferior minimum length topologies.

Figure 5.3 (next page). Results from phylogenetic analysis of cheilodactylid and latrid partial cytochrome *c* oxidase subunit I and cytochrome *b* mitochondrial DNA sequences. (a) Minimum evolution analysis. Branch lengths are proportional to Kimura (1980) two parameter distances, as measured relative to the scale bar. Tree length=1.45732. (b) Strict consensus of four equally-most parsimonious trees of 1267 steps (CI=0.362, RI=0.505). *Cirrhitus splendens* was employed as the basal outgroup, as the Cirrhitidae appears most plesiomorphic of the five cirrhitoid families (Greenwood 1995). Bootstrap values at each node are indicated when greater than 50%.

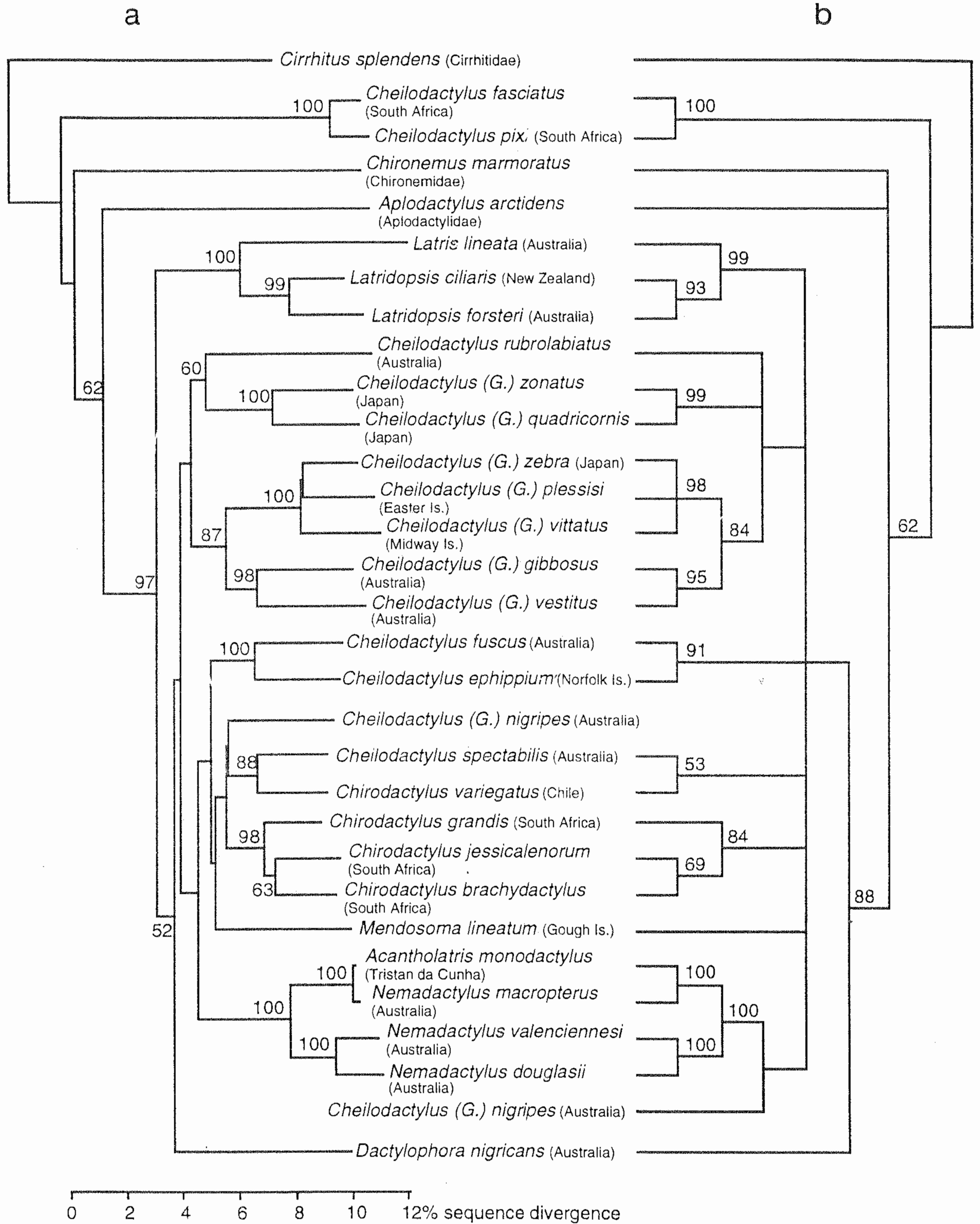


Figure 5.3.

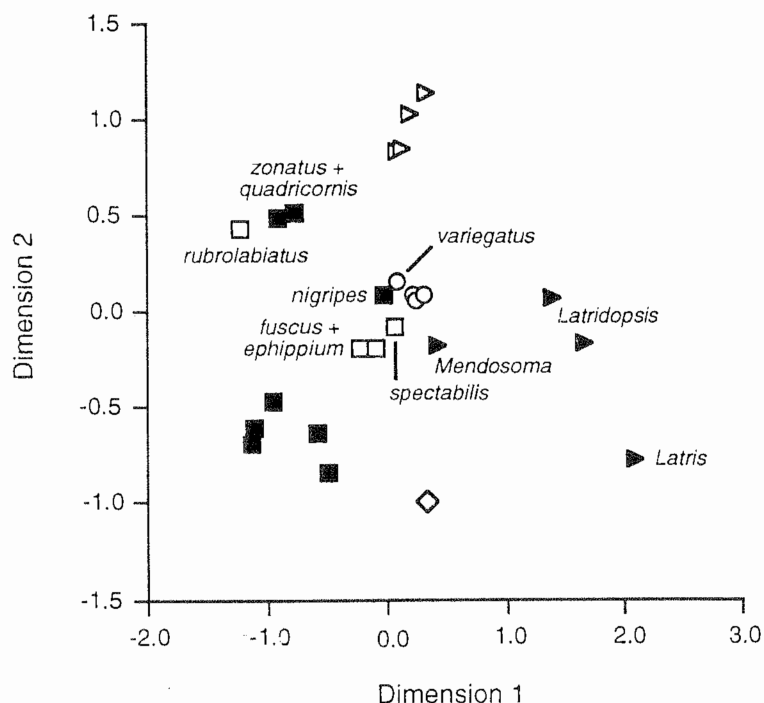


Figure 5.4. Two-dimensional scaling of Kimura (1980) genetic distances between cheilodactylid and latrid partial cytochrome *c* oxidase subunit I and cytochrome *b* mitochondrial DNA sequences. The Kruskal loss function and monotonic scaling were employed. Stress of configuration=0.148. The South African *Cheilodactylus* were excluded from scaling to facilitate maximum resolution of the remaining sequences. Closed squares, subgenus *Goniistius*; open square, remaining *Cheilodactylus*; circles, *Chirodactylus*; diamond, *Dactylophora*; open triangles, *Nemadactylus* and *Acantholatris*; closed triangles, Latridae.

Re-allocation of non-African *Cheilodactylus*

Anticipating generic distinction of the South African *Cheilodactylus*, Lamb (1990) identified two groups among the non-African *Cheilodactylus*, which could be given generic status, although he was unable to distinguish them from *Chirodactylus*. These two groups were the eight species which Randall (1983) allocated to the subgenus *Goniistius*, and the remaining four species which would be called *Morwong*. While the majority of non-African *Cheilodactylus* are divergent from *Chirodactylus* spp. (Figure 5.3, Figure 5.4), it appears that some modification of Lamb's (1990) proposed generic allocation is required.

The eight species of *Goniistius* were not clustered as a monophyletic clade (Figure 5.3), but enforced monophyly did not produce significantly inferior minimum length topologies. This result contradicts that obtained from a subset of taxa, which suggested that *C. (G.) nigripes* be

excluded from *Goniistius* (Chapter 4). This species is the most morphologically divergent member of *Goniistius*, distinguished by dorsal and anal fin ray counts, a more anterior position of the mouth, and broader black bars on the body (Randall, 1983). With exclusion of *C. (G.) nigripes*, the remaining *Goniistius* are distinct from *C. ephippium*, *C. fuscus*, and *Chirodactylus* (Figure 5.3, Figure 5.4), the species which have prevented *Goniistius* attaining generic rank (Randall, 1983; Lamb, 1990). However, another species of *Cheilodactylus*, *C. rubrolabiatus*, appears to have affinities with these *Goniistius* (Figure 5.3, Figure 5.4). *Cheilodactylus rubrolabiatus* shares only one of three features used by Randall (1983) to define *Goniistius*, namely the presence of dark brown to black bars or diagonal bands on the body. The characters of a highly arched nape and pronounced fourth dorsal spine are absent.

Given the need for generic reassignment of the subgenus *Goniistius*, but in light of potential errors associated with elevating the entire group to the genus level, a conservative and temporary assignment is suggested. As two distinct and highly supported monophyletic clades are observed for seven of the *Goniistius* (Figure 5.3, Figure 5.4), it would be conservative to nominate these as separate genera until the placement of *C. (G.) nigripes* and *C. rubrolabiatus* are resolved. *Cheilodactylus (G.) zonatus* and *C. (G.) quadricornis* would retain the name *Goniistius*, as the former is the type species, while *Zeodrius* Castelnau has priority for the remaining five species. The only character observed at present to distinguish these two groups is a higher dorsal spine count for *C. (G.) zonatus* and *C. (G.) quadricornis*.

The species which Lamb (1990) suggested for the genus *Morwong*, *C. fuscus*, *C. ephippium*, *C. rubrolabiatus*, and *C. spectabilis*, did not cluster as a monophyletic clade (Figure 5.3), and enforced monophyly produced a significantly inferior minimum length topology (1288 steps, P=0.001). *Cheilodactylus fuscus* and *C. ephippium* clustered as sister taxa with high support, although they were not particularly divergent from other cheilodactylids (Figure 5.3, Figure 5.4). As *C. fuscus* is the type for *Morwong*, it is suggested that this name be restricted to *C. fuscus* and *C. ephippium* until placement of remaining taxa is resolved. The combined presence

of six lower thickened pectoral rays and four prominent bony protuberances in front of the eyes and on the snout can be used to define *Morwong*.

Status of *Chirodactylus*

Lamb (1990) was unable to distinguish *Chirodactylus* from non-African *Cheilodactylus* using external features. A highly supported monophyletic clade was observed for the three South African members of *Chirodactylus*, but the South American *C. variegatus* predominantly clustered with *Cheilodactylus spectabilis* at moderate to high support (Figure 5.3). Although enforced monophyly of *Chirodactylus* did not produce significantly inferior minimum length topologies, the same result is likely for the inclusion of a non-African *Cheilodactylus* instead of *C. variegatus*. It is suggested that the South African *Chirodactylus* be distinguished from *C. variegatus* until placement of the latter is resolved. As *C. variegatus* is the type species, the South African *Chirodactylus* revert to *Palunolepis* Barnard, distinguished by relatively longer ventral rays of the pectoral fin and a lower lateral line scale count (Smith, 1980). *Cheilodactylus spectabilis* may require congeneric placement with *Chirodactylus variegatus*; these species are closest relatives based on meristic and genetic data.

Dactylophora, *Nemadactylus* and *Acantholatris*

The divergent and predominantly basal placement of *Dactylophora nigricans* (Figure 5.3) agrees with the morphological distinctiveness and monotypic status of this genus (Lamb, 1990). Representatives of *Nemadactylus* and *Acantholatris* clustered together with high bootstrap support, but *Nemadactylus* was paraphyletic with respect to *Acantholatris*. Synonymy of *Acantholatris* with *Nemadactylus* was identified during a more detailed study of these taxa (Chapter 5.3).

Taxonomic summary

A summary of the suggested taxonomic changes is given in Table 5.1. The proposed scheme is conservative, as only those taxa clustered in well supported monophyletic clades have undergone generic reallocation. The most important questions remaining to be addressed are the

placement of *Cheilodactylus rubrolabiatus*, *C. spectabilis*, *C. (G.) nigripes*, and *Chirodactylus variegatus*.

Cirrhitoid familial relationships

Greenwood (1995) produced a provisional scheme of cirrhitoid family relationships in which the Cirrhitidae was most plesiomorphic, followed by the Chironemidae, and then an unresolved trichotomy containing the Aplodactylidae, Cheilodactylidae, and Latridae. Although not conducted as a study of familial relationships, this work indicates that the latrids are more closely related to the majority of cheilodactylids than the aplodactylids, and that distinct placement of the South African *Cheilodactylus* in such a scheme is required (Figure 5.3). The remaining familial relationships inferred from this study were inconsistent and not supported, even when restricting analysis to less frequent character state changes.

Non resolution of higher-level cheilodactylid and latrid relationships

The lack of consistent and supported higher-level relationships among cheilodactylids and latrids may be explained by a period of more rapid species radiation and saturation of transition nucleotide substitutions (Avise *et al.*, 1994). The lengths of unsupported internodes were relatively short (Figure 5.3a), indicating a period of more rapid species radiation. Consequently, there was comparatively little phylogenetic signal present for the resolution of higher level relationships among cheilodactylids and latrids. Saturation of transitions was also evident during comparisons among cheilodactylids and latrids at these levels (Figure 5.2). Although increased weighting of transversions can reduce any influence of saturated transitions on phylogeny reconstruction, transversions were too infrequent to confidently resolve branching order. Analysis of more characters may facilitate better resolution of branching order at short internodes. If saturation is problematic, analysis of a large number of low variability characters will be required. Such characters are unlikely to become saturated, but a large number will need to be scored as only a small proportion will have undergone informative changes during the periods of interest (Avise *et al.*, 1994).

Zoogeography

Vicariant isolation of cheilodactylids accompanying Gondwana fragmentation could not be assessed by species-area relationships, as the majority of higher-level relationships were poorly supported (Figure 5.3). However, it appears that Gondwanan continental land masses became isolated prior to the divergence of corresponding cheilodactylids, based on mitochondrial protein four-fold degenerate site molecular clock calibrations of 2.3% and 3.3% sequence divergence per million years (Martin *et al.*, 1992; Bermingham *et al.*, 1997). The South African *Cheilodactylus* diverged from other cirrhitoids approximately 24-39 Myr ago, while the corresponding divergence of South African *Chirodactylus* from other members of the cheilodactylid-latrid clade (excludes South African *Cheilodactylus*) occurred 7-21 Myr ago. In contrast, Africa was isolated from the other Gondwanan continents at least 90 Myr ago (Lawver *et al.*, 1992). Similarly, the South American *Chirodactylus variegatus* diverged from its probable sister taxon, the Australian-New Zealand *Cheilodactylus spectabilis*, approximately 4-7 Myr ago, yet their corresponding continents were isolated 30-40 Myr ago (Lawver *et al.*, 1992).

Absolute estimates of divergence time based on molecular clock calibrations should be treated cautiously, given the number of untested assumptions (Rand, 1994). Substitution rate heterogeneity was also observed among some lineages in this study. Faster and slower calibrations have also been reported for other fish taxa, and those employed here are considered "median" values. Regardless, the divergence time estimates obtained are sufficiently recent to discount vicariant isolation during Gondwana fragmentation. Therefore, the distribution of continent-inhabiting cheilodactylids is best explained by chance dispersal. This mechanism has been previously invoked from genetic studies of other fishes with similar distributions, namely populations of *Sardinops* (Grant and Leslie 1996; Bowen and Grant, 1997), the diadromous *Galaxias maculatus* (Waters and Burridge, 1999), and the aplodactylids (Chapter 6).

Chance dispersal of cheilodactylids across large expanses of open water probably occurred during their pelagic larval phase, as juveniles and adults are predominantly demersal in

nearshore waters (Sano and Moyer, 1985; Annala, 1987; Cappo, 1995; McCormick, 1998). High dispersal capabilities of cheilodactylid larvae are suggested by the offshore larval phase of 9-12 months duration in *N. macropterus* and *A. monodactylus* (Annala, 1987; Andrew *et al.*, 1995). Molecular genetic studies also suggest larval gene flow among geographically isolated cheilodactylid populations (Elliott and Ward, 1994; Grewe *et al.*, 1994; Chapter 3). As the majority of higher-level relationships among cheilodactylids and latrids were poorly defined it is difficult to determine the direction of chance dispersal events. However, an origin in the waters of Southern Australia appears likely for these and other temperate cirrhitoid groups (Briggs, 1974; Chapter 3; Chapter 6).

CHAPTER 6: Molecular phylogeny of the Aplodactylidae.

Abstract

Aplodactylids are temperate marine nearshore fishes of New Zealand, southern Australia, several southwest Pacific islands, and western South America. The molecular phylogeny of the five aplodactylid species was reconstructed from mitochondrial DNA sequences. The observed relationships and levels of genetic variation supports the most recent review of the Aplodactylidae in which the monotypic *Crinodus* is synonymised with *Aplodactylus*, the only recognised genus. Phylogenetic relationships indicate that the Aplodactylidae originated in the approximate region of Australia and New Zealand, with the majority of radiation occurring prior to this family reaching South America. The disjunct trans-Pacific distribution of this family was the resulted from chance dispersal rather than vicariance accompanying Gondwana fragmentation. Such dispersal most likely occurred during the larval phases, via the West Wind Drift current.

Introduction

The Aplodactylidae comprises five primarily herbivorous Southern Hemisphere temperate marine fishes. These species occupy shallow nearshore reefs, and often dominate numerical and in biomass where macroalgae are abundant (Hutchins and Swainston, 1986; Stepien, 1990; Cole *et al.*, 1992; Francis, 1996). The distribution of the Aplodactylidae is similar to that of the other temperate cirrhitoid families (Cheilodactylidae, Chironemidae, Latridae), in that diversity centres in Australia and New Zealand, and they also are represented in the eastern Pacific (Figure 6.1). Members of this family are commonly known as "jerguilla" in South America and "marblefish" in Australia and New Zealand.

The taxonomy of the Aplodactylidae has undergone much change, as summarised in the recent review by Russell (2000). Only five species and a single genus were recognised by Russell (2000). *Crinodus* Gill, monotypic for *C. lophodon* (Günther) from eastern Australia, was synonymised with *Aplodactylus* Valenciennes as only scale size and the presence of vomerine teeth distinguish these two groups. The remaining species are *A. arctidens* Richardson from New Zealand and southeastern Australia, *A. westralis* Russell from southwestern Australia, *A. etheridgii* (Ogilby) from northern New Zealand, Lord Howe, Norfolk, and the Kermadec Islands, and the type species *A. punctatus* Richardson from the west coast of South America.

There are three questions about aplodactylid biogeography. First, where did this group originate, South America or in the vicinity of Australia and New Zealand? Second, when did aplodactylids radiate to both sides of the Pacific? Third, did their disjunct trans-Pacific distribution result from chance dispersal or vicariance accompanying Gondwana fragmentation? The reconstruction of aplodactylid phylogeny and the estimation of lineage divergence times can address these questions.

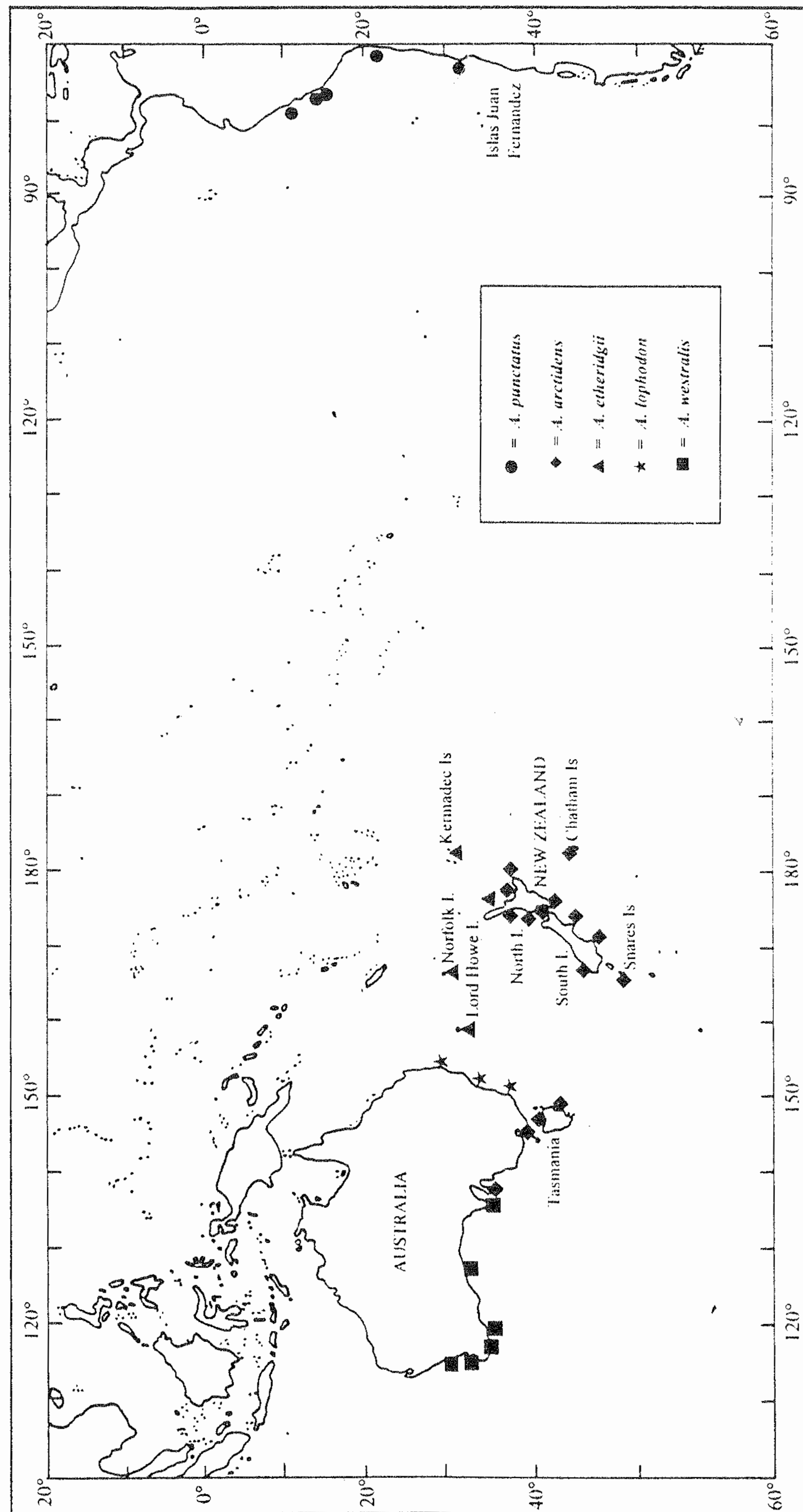


Figure 6.1. Distribution of Aplodactylus species. Obtained from Russell (2000).

The objectives of this study were to reconstruct the phylogeny of the Aplodactylidae and to address the questions relating to the taxonomy and biogeography of this family. Phylogenetic analysis of mitochondrial DNA sequences was undertaken to complement the recent morphological analysis of these taxa by Russell (2000). As there is no fossil record of the Aplodactylidae, this molecular approach also enables the estimation of lineage divergence times by applying molecular clock calibrations (Vawter *et al.*, 1980).

Materials and methods

Genomic DNA was extracted from one individual of each aplodactylid species recognised by Russell (2000) and representative taxa from three other cirrhitoid families (Table 6.1).

Table 6.1. Species of aplodactylids and outgroups from other cirrhitoid families analysed. Names of aplodactylids follow the revision by Russell (2000).

Species	Family	Collection site
Aplodactylus arctidens Richardson	Aplodactylidae	Maria Island, Tasmania
Aplodactylus etheridgii (Ogilby)	Aplodactylidae	Norfolk Island
Aplodactylus lophodon (Günther)	Aplodactylidae	Camden Head, NSW, Australia [a]
Aplodactylus punctatus Valenciennes	Aplodactylidae	Punta de Tralca, Chile
Aplodactylus westralis Russell	Aplodactylidae	Rottnest Island, Western Australia
Chironemus marmoratus Günther	Chironemidae	Diamond Head, NSW, Australia [a]
Cheilodactylus fasciatus Lacépède	Cheilodactylidae	Tsitsikamma National Park, South Africa
Cirrhitus splendens (Ogilby)	Cirrhitidae	Lord Howe Island

[a] Australian Museum frozen tissue collection (*A. lophodon* I 31252023 NI 252; *C. marmoratus* I 31253048 NI 330).

Results

DNA sequences analysed were deposited in GenBank (AF067084, AF092140, AF092155-AF092157, AF092167, AF133060-AF133069). The mitochondrial partial cytochrome *c* oxidase subunit I and cytochrome *b* sequences analysed were 499 and 402 bp in length respectively. Among the aplodactylids 164 characters were variable, and 82 of these were phylogenetically informative. Transition nucleotide substitutions were observed at 153 sites, while transversions were observed at 25. Only six of the variable sites were not third codon positions. Length mutations were absent. This pattern of sequence evolution, also observed for

the outgroup taxa, indicates that orthologous protein coding sequences were obtained. The cytochrome *b* region was approximately 1.2 times more variable than the cytochrome oxidase I region, but both fragments contributed similar amounts of variation to the study due to the larger number of cytochrome oxidase I characters analysed.

The partition homogeneity test (200 partition replicates, Branch and Bound search algorithm) indicated phylogenetic congruence between cytochrome oxidase I and cytochrome *b* sequences, both for all characters (P=0.36) and only third codon positions (P=0.65), allowing the combination of genes during phylogenetic analyses. Tree length-frequency distributions were significantly skewed for all taxa ($g1$=-0.60, P<0.01) and the aplodactylids alone ($g1$=-1.09, P<0.01), suggesting the presence of phylogenetic signal. Visual inspection did not reveal any nucleotide substitution saturation amongst the aplodactylids, evidenced by the linear accumulation of transitions relative to transversions (Figure 6.2). The transition-transversion ratio observed during comparisons between the aplodactylids and each of the three outgroups were significantly lower than that observed during comparisons among the aplodactylids (Figure 6.2, P<0.001, d.f.=3, one-way ANOVA with Tukey HSD post hoc tests), indicating substitution saturation during the former.

Kimura (1980) two-parameter genetic distances among the aplodactylids ranged from 6.1 to 12.4% sequence divergence (Table 6.2). Distances between the aplodactylids and the three cirrhitoid outgroups, and among these outgroups, ranged from 18.3 to 23.1% (Table 6.2). The levels of intraspecific variation were not assessed, but are typically less than 1.0% for protein coding genes in other cirrhitoids (Chapter 3; Burridge, unpubl.).

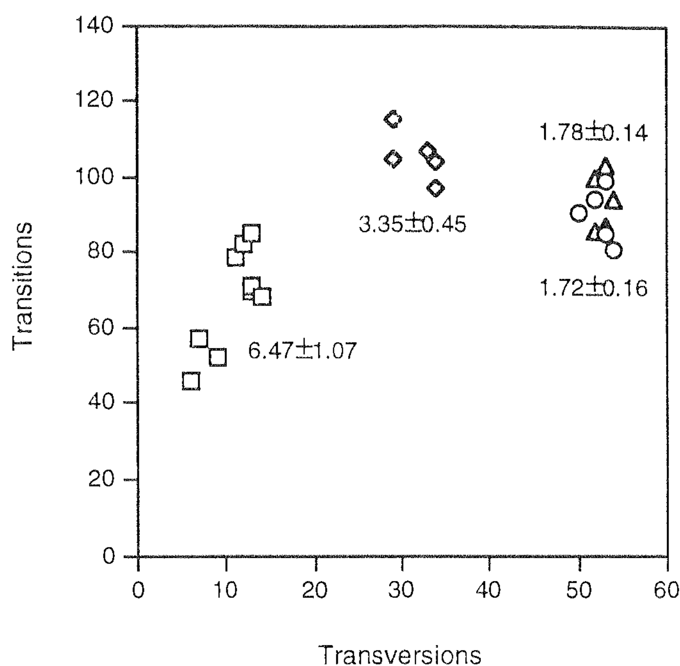


Figure 6.2. The pattern of observed transition and transversion nucleotide substitution accumulation at third codon positions for mitochondrial DNA partial cytochrome *c* oxidase subunit I and cytochrome *b* sequences when combined. Squares represent pairwise comparisons among aplodactylids. Diamonds, circles and triangles represent comparisons of *Chironemus marmoratus* (Chironemidae), *Cheilodactylus fasciatus* (Cheilodactylidae), and *Cirrhitus splendens* (Cirrhitidae) against the aplodactylids respectively. The mean observed transition-transversion nucleotide substitution ratio and its standard deviation are listed for each set of comparisons.

The neighbour-joining and unweighted maximum parsimony topologies clustered the aplodactylids as monophyletic, with high (>70%) bootstrap support (Figure 6.3a). *Aplodactylus arctidens* and *A. punctatus* were clustered together, with *A. westralis* as sister taxon to this clade. *Aplodactylus lophodon* and then *A. etheridgii* were successively removed. These relationships received high bootstrap support from neighbour-joining analysis, but moderate support (70-50%) from unweighted parsimony analysis. Increased weighting of transversions during parsimony analysis, according to the optimum TI:TV of 3.0 from maximum likelihood analysis, produced the same topology as unweighted analysis, with similar bootstrap values (not shown). Monophyly of the aplodactylids was also supported by parsimony analysis restricted to transversion substitutions, but the relationships among these taxa were not well defined due to the small number of informative character-state changes (not shown).

Table 6.2. Genetic distances for mitochondrial DNA partial cytochrome *c* oxidase subunit I and cytochrome *b* sequences when combined. Values are Kimura (1980) two-parameter percentage sequence divergences, obtained when using the optimum expected transition-transversion nucleotide substitution ratio of 3.0 from maximum likelihood analysis (Figure 6.3).

	1	2	3	4	5	6	7
1 *Aplodactylus arctidens*							
2 *Aplodactylus punctatus*	6.1						
3 *Aplodactylus westralis*	7.8	7.6					
4 *Aplodactylus etheridgii*	10.0	10.3	10.0				
5 *Aplodactylus lophodon*	11.8	11.9	12.4	11.1			
6 *Chironemus marmoratus*	20.0	18.7	18.3	19.3	19.5		
7 *Cheilodactylus fasciatus*	21.8	21.0	20.5	22.6	20.2	21.2	
8 *Cirrhitus splendens*	22.6	20.7	21.0	23.1	22.0	23.1	22.8

The maximum likelihood topology differed from the neighbour-joining and unweighted maximum parsimony topology in root placement among the aplodactylids (Figure 6.3b). The root was placed on the branch leading to *A. lophodon* and *A. etheridgii*, rather than that leading to *A. lophodon* alone. Enforcing the maximum likelihood topology during parsimony analysis produced a minimum length tree only one step longer than the most parsimonious unconstrained topology. Neither topology was significantly superior according to the Templeton (P=0.86) or Kishino and Hasegawa (P=0.75) tests. Choosing *Chironemus marmoratus* or *Cheilodactylus fasciatus* as the most basal outgroup had no effect on the inferred relationships among aplodactylids or the levels of bootstrap support from any method of analysis. Removing one or two outgroup taxa occasionally altered aplodactylid relationships inferred by a given method of analysis, but only in such a manner as observed for different methods of analysis when all outgroups were analysed.

The two-cluster and branch-length tests did not reveal significant nucleotide substitution rate heterogeneity for third codon positions at the 5% level, when nodes and branches were

analysed individually. Significant rate heterogeneity was also not observed when nodes were analysed simultaneously by the two-cluster test (χ^2=8.03, df=6, P>0.10).

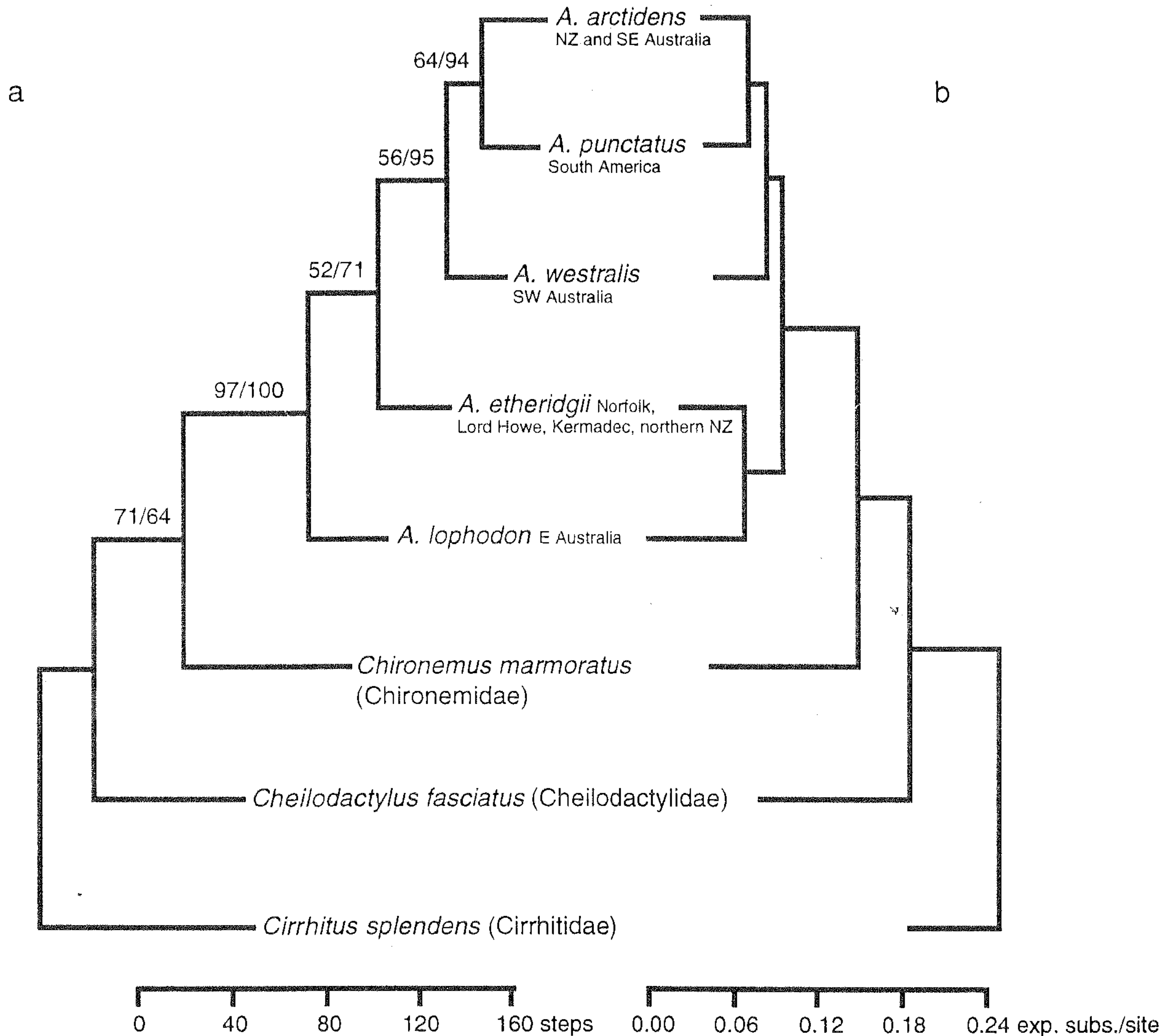


Figure 6.3. Results from the phylogenetic analysis of aplodactylid and outgroup mitochondrial DNA partial cytochrome *c* oxidase subunit I and cytochrome *b* sequences when combined. *Cirrhitus splendens* was used to root all phylogenies, as the Cirrhitidae appears the most plesiomorphic of the five cirrhitoid families (Greenwood, 1995). (**a**) Single most-parsimonious topology obtained from unweighted parsimony analysis (exhaustive search). Tree length=558 steps, CI=0.685, RI=0.413, based on all characters. The topology recovered from neighbour-joining analysis is identical to **a**. The numbers above the branches at each node represent the bootstrap proportions for the taxa in each clade, as derived from 2000 replicate data sets (unweighted maximum parsimony/neighbour-joining). (**b**) Maximum likelihood topology from 20 replicates of random sequence input order using the optimum expected TI:TV of 3.0. ml=-3691.3. All branches are significantly positive (P<0.01).

Discussion

Two topologies were recovered from phylogenetic analysis of aplodactylid and outgroup mitochondrial DNA cytochrome oxidase I and cytochrome *b* sequences. These varied in the placement of the root among the aplodactylids, and neither was significantly superior. The New Zealand and southeastern Australian species *Aplodactylus arctidens* and the western South American species *A. punctatus* were placed as sister taxa, in a monophyletic clade with the southwest Australian species *A. westralis*. The inconsistency in root placement influenced the relationships among the remaining aplodactylids, *A. lophodon* from eastern Australia and *A. etheridgii* from northern New Zealand and several southwest Pacific islands. These species were either clustered as sister taxa, or were successively removed from the other three aplodactylids with *A. lophodon* located basally. Bootstrap support for aplodactylid monophyly and the inferred relationships were moderate to high. There was no evidence of nucleotide substitution saturation among the aplodactylids, although saturation was present during comparisons with the outgroups. This may explain the variability in root placement (Smith, 1994).

Taxonomy

The genus *Crinodus*, monotypic for *C. lophodon*, is only distinguished from *Aplodactylus* by larger scales and the absence of vomerine teeth, and has been relegated to the synonymy of *Aplodactylus* by Russell (2000). Genetic data provides support for this revision. *Crinodus lophodon* does not appear sufficiently divergent from species of *Aplodactylus* to be given distinct generic status; it is only slightly more divergent from *A. punctatus*, *A. arctidens*, and *A. westralis* than is *A. etheridgii* (Table 6.2). The placement of *C. lophodon* is also uncertain, as two topologies were recovered and neither was significantly superior. If *C. lophodon* is the sister taxon to *Aplodactylus* (Figure 6.3a), retention of *Crinodus* at the generic level could be argued despite of the limited molecular and morphological divergence. However, if *C. lophodon* and *A. etheridgii* are sister taxa (Figure 6.3b), then *Aplodactylus* is paraphyletic. Given the absence of marked genetic distinction, and the possibility that *Aplodactylus* is

paraphyletic, Russell's (2000) hypothesis in which *Crinodus* is synonymised with *Aplodactylus* is supported.

Most aplodactylids recognised by Russell (2000) have widespread distributions and encompass several previously described species. While Russell (2000) examined many specimens throughout the ranges of the taxa he recognised, the possibility exists that speciation has occurred in some of these without the development of readily apparent morphologic features. While genetic studies often identify such instances of cryptic speciation (Knowlton, 1993), this study has not been sufficiently intensive to test the status of species recognised by Russell (2000).

Zoogeography

The recovered phylogenies suggest that the Aplodactylidae originated in the vicinity of Australia and New Zealand, with the majority of radiation occurring prior to this family achieving representation in South America. This is evidenced by the basal positions of the Australian, New Zealand, and southwest Pacific island aplodactylids relative to the South American *A. punctatus* (Figure 6.3). An Australian-New Zealand origin and subsequent movement east has also been proposed for the cheilodactylids of *Nemadactylus* and *Acantholatris* (Chapter 3).

Mitochondrial third codon position molecular clock calibrations of 2.3% and 3.3% sequence divergence My^{-1} (Mxartin *et al.*, 1992; Bermingham *et al.*, 1997) suggest that the aplodactylids shared a common ancestor 12.8 to 18.5 Myr ago. Similarly, the disjunct trans-Pacific species pair *A. arctidens* and *A. punctatus* diverged 6.2 to 9.0 Myr ago. While estimates of divergence time from molecular clock calibrations should be treated cautiously given the number of untested assumptions and the range of calibrations available (Rand, 1994), these values appreciably post-date the isolation of Australia and South America from Antarctica during the fragmentation of Gondwana (40-30 Myr ago; Lawver *et al.*, 1992). In addition, the presence of conspecific populations in Australia and New Zealand (*A. arctidens*), but the location of their sister species in South America (*A. punctatus*), also argues against a distribution based entirely

on vicariance accompanying Gondwana fragmentation, as New Zealand was the first of these land masses to become isolated (Lawver *et al.*, 1992). Therefore, the disjunct transoceanic distribution of aplodactylids is best explained by chance dispersal. Chance dispersal rather than Gondwanan vicariance was also inferred for the similarly distributed diadromous species *Galaxias maculatus* (Jenyns 1842), based on intraspecific relationships and levels of molecular divergence (Waters and Burridge, 1999).

Chance dispersal of aplodactylids across the Pacific was most likely undertaken during their larval phase, as juveniles and adults are restricted to nearshore habitats (Stepien, 1990; Cole *et al.*, 1992; Hutchins and Swainston, 1986; Francis, 1996). Although little is known of aplodactylid larval dispersal capabilities (B. Bruce, CSIRO Marine Laboratories, Hobart, pers. comm.), high dispersal capabilities have been suggested for other cirrhitoid larvae. The cheilodactylids *N. macropterus* and *A. monodactylus* possess a 9-12 month offshore pelagic larval phase (Annala, 1987; Andrew *et al.*, 1995), and molecular genetic studies on members of these genera suggest gene flow across distances in excess of 1000 km (Elliott and Ward, 1994; Grewe *et al.*, 1994; Chapter 3). Particularly relevant is the species pair *Nemadactylus* sp. and *A. gayi*, which are similar in distribution to *A. arctidens* and *A. punctatus* and exhibit negligible cytochrome *b* sequence variation (Chapter 3).

CHAPTER 7: Biogeographic history of geminate cirrhitoids with east-west allopatric distributions across southern Australia, based on molecular data

Abstract

The biogeographic history of three cirrhitoid species pairs with east-west allopatric distributions across southern Australia was examined by determining levels of mitochondrial DNA sequence divergence and applying molecular clock calibrations. Similar levels of genetic divergence were observed for *Aplodactylus* Valenciennes and *Goniistius* Gill species pairs, but these were more than twice that observed for a *Nemadactylus* Richardson pair. Molecular clock calibrations suggested divergences occurred during the late Miocene and mid Pliocene, respectively. Given evidence of high dispersal capabilities, the habitat and climatic barriers of the Australian south coast appear too small to have facilitated speciation of the cirrhitoids examined. A mechanism is proposed by which ancestral cirrhitoids were vicariantly isolated into east and west coast populations during periods of climate change. Although *Aplodactylus* and *Goniistius* divergences occurred during the same period, separate vicariant events across the Australian north and south coasts are invoked.

Introduction

East-west allopatric and parapatric distributions across southern Australia have been observed for several marine and putatively geminate populations and species (Dartnall, 1974; Knox, 1980; Edgar, 1986; Hutchins, 1987). Such distributions may have resulted from chance movement across a pre-existing barrier and the founding of a new population (dispersal). Large expanses of the southern Australian coast are devoid of reef substrate, and these represent barriers to obligate reef-dwelling taxa (Edgar, 1986; J. B. Hutchins, personal communication). Similarly, water temperatures in Bass Strait are also lower than those that may be tolerated by taxa occurring to the east and west (J. B. Hutchins, personal communication). Alternatively, east-west allopatric and parapatric distributions of geminate taxa may have resulted from the development of a barrier between populations not previously isolated (vicariance). During periods of climatic cooling, such as the Pleistocene glaciations, species previously widespread across the south coast may have been forced north and vicariantly isolated into east and west coast populations (Knox, 1980; Edgar, 1986; Hutchins, 1987; Gomon and Johnson, 1999). The emergence of Bass Strait during periods of lower sea level may have also vicariantly isolated eastern and western populations (Collette, 1974; Dartnall, 1974; Knox, 1980; Hutchins, 1987). Such vicariant events may have influenced several taxa simultaneously.

Previous studies have compared levels of DNA sequence divergence of geminate taxa to identify shared biogeographic histories (Avise, 1992; MacMillan and Palumbi, 1995; Bermingham and Martin, 1998; Knowlton and Weigt, 1998; Schneider *et al.*, 1998; Taberlet *et al.*, 1998; Tringali *et al.*, 1999). Levels of DNA sequence difference between taxa can be considered measures of divergence time if mutations have accumulated in a clock-like manner (Vawter *et al.*, 1980), and similar levels of genetic divergence provide support for a shared biogeographic history. Although mutation rate can vary between lineages (Martin *et al.*, 1992), the extent is likely to be small in taxonomically, ecologically, and physiologically similar taxa (Martin and Palumbi, 1993; Rand, 1994). By applying a mutation rate calibration it is possible to estimate the geological period of lineage divergence (Vawter *et al.*, 1980;

Martin *et al.*, 1992; Bermingham *et al.*, 1997), which may correspond to past events such as the Pleistocene glaciations.

East-west allopatric distributions across southern Australia are observed for four ecologically similar reef-dwelling cirrhitoid species pairs. These are the aplodactylids *Aplodactylus arctidens* Richardson and *A. westralis* Russell (Russell, 2000), the chironemids *Chironemus marmoratus* Günther and *C. georgianus* Cuvier (Gomon *et al.*, 1994), and the cheilodactylids *Nemadactylus douglasii* (Hector) and *N. valenciennesi* (Whitley) (Lamb, 1990; Gomon *et al.*, 1994), and *Cheilodactylus (Goniistius) vestitus* (Castelnau) and *C. (G.) gibbosus* Richardson (Randall, 1983; Lamb, 1990) (Figure 7.1). The members of each pair are morphologically distinguished as listed in Table 7.1, and their relationships with congeners have been investigated previously (Chapters 3,4, and 6).

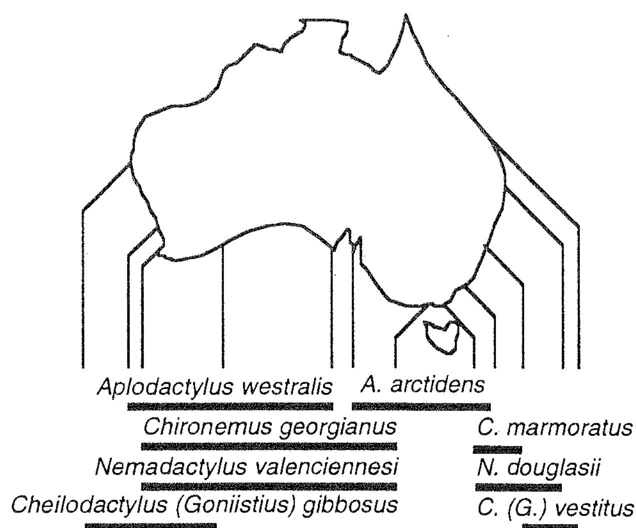


Figure 7.1. East-west allopatric distributions of cirrhitoid species pairs across southern Australia. Species ranges are measured relative to the solid bars below the map, and comprise the nearshore waters bordered by the lines intersecting the coast.

The aim of this study is to examine the biogeographic history of cirrhitoid species pairs with east-west allopatric distributions across southern Australia. Questions to be addressed include at what period did divergences occur, was dispersal or vicariance responsible, and is

biogeographic history shared among these similar taxa? Molecular data were employed to provide relative and absolute estimates of divergence time for the members of each allopatric pair, and information on dispersal capabilities was used to examine whether habitat and climatic barriers of the Australian south coast were sufficiently large to facilitate speciation.

Table 7.1. Morphological distinction of cirrhitoid species with east-west allopatric distributions across southern Australia.

Genus, character	Western species	Eastern species	Reference
Aplodactylus	*A. westralis*	*A. arctidens*	Russell (2000)
Vomerine teeth	Absent	Present	
Lateral line scales	72-82	100-120	
Cheilodactylus (Goniistius)	*C. (G.) gibbosus*	*C. (G.) vestitus*	Randall (1983), Lamb (1990)
Dorsal fin	XVI-XVII, 34-37	XVI-XVII, 32-35	
Anal fin	III, 8-9	III, 8-9 (usually 8)	
Lateral line scales	61-65	58-65	
Nemadactylus	*N. valenciennesi*	*N. douglasii*	Lamb (1990), Gomon *et al.* (1994)
Dorsal fin	XVI-XVIII, 30-32	XVII-XVIII, 27-28	
Anal fin	III, 17-19	III, 16-17	
Lateral line scales	62-68	53-62	
Chironemus	*C. georgianus*	*C. marmoratus*	Last *et al.* (1983), Gomon *et al.* (1994)
Dorsal fin	XV-XVI, 14-18	XIV, 19-20	
Dorsal spine cirri	Usually present	Usually absent	
Lateral line scales	46-48	55-58	

Materials and methods

Mitochondrial DNA partial cytochrome *c* oxidase subunit I and cytochrome *b* sequence data were obtained for three of the four east-west allopatric cirrhitoid species pairs during wider phylogenetic studies of *Nemadactylus*, *Goniistius*, and *Aplodactylus* (Chapters 3, 4, and 6). Sequences from New Zealand individuals of *N. douglasii* and *A. arctidens* were obtained specifically for this study. Appropriately preserved tissues from *Chironemus georgianus* were

not available for DNA sequence analysis, and therefore the remaining east-west allopatric cirrhitoid species pair could not be included in this study. Logical outgroups were chosen for each allopatric species pair based on the phylogenetic studies listed above.

Results

All DNA sequences analysed are deposited in GenBank (AF067084-AF067085, AF067089-AF067091, AF092140, AF092145-AF092147, AF092149, AF092151, AF092153-AF092155, AF092157, AF092160, AF092162, AF092164, AF092166, AF133060-AF133063, AF133065-AF133068, AF136267, AF202545-AF202548). The cytochrome *c* oxidase subunit I and cytochrome *b* sequences analysed were 499 and 402 bp in length respectively. The partition homogeneity test indicated phylogenetic congruence between cytochrome oxidase I and cytochrome *b* sequences, for all characters (P=0.89) and third codon positions (P=0.78), and therefore the genes were combined during subsequent analyses.

As observed during previous studies (Chapters 3, 4, and 6), sister taxa relationships were evident from neighbour-joining analysis for the *Nemadactylus* and *Goniistius* species pairs with east-west allopatric distributions across southern Australia (Figure 7.2). Although *Aplodactylus westralis* and *A. arctidens* were not clustered as immediate sister taxa, they can be considered sister taxa for the purpose of this southern Australian biogeographic analysis as the only other derivative of their common ancestor is endemic to South America (Figure 7.2). The inferred relationships for the east-west allopatric species pairs each received high (>88%) bootstrap support. New Zealand individuals of *N. douglasii* and *A. arctidens* exhibited only slight sequence divergence from southeast Australian conspecifics (<0.5%).

Neither the two-cluster or branch-length test revealed significant nucleotide substitution rate heterogeneity for third codon positions among species pairs with east-west allopatric distributions across southern Australia, when nodes and branches were analysed individually (CP<95%). Rate heterogeneity was also not observed from the two-cluster test when analysing nodes simultaneously (χ^2=15.46, df=14, P>0.05). The only evidence of substitution

rate heterogeneity among taxa was for the branches leading to *N. macropterus* and the *N. douglasii-N. valenciennesi* clade (CP=98.64%, two-cluster test).

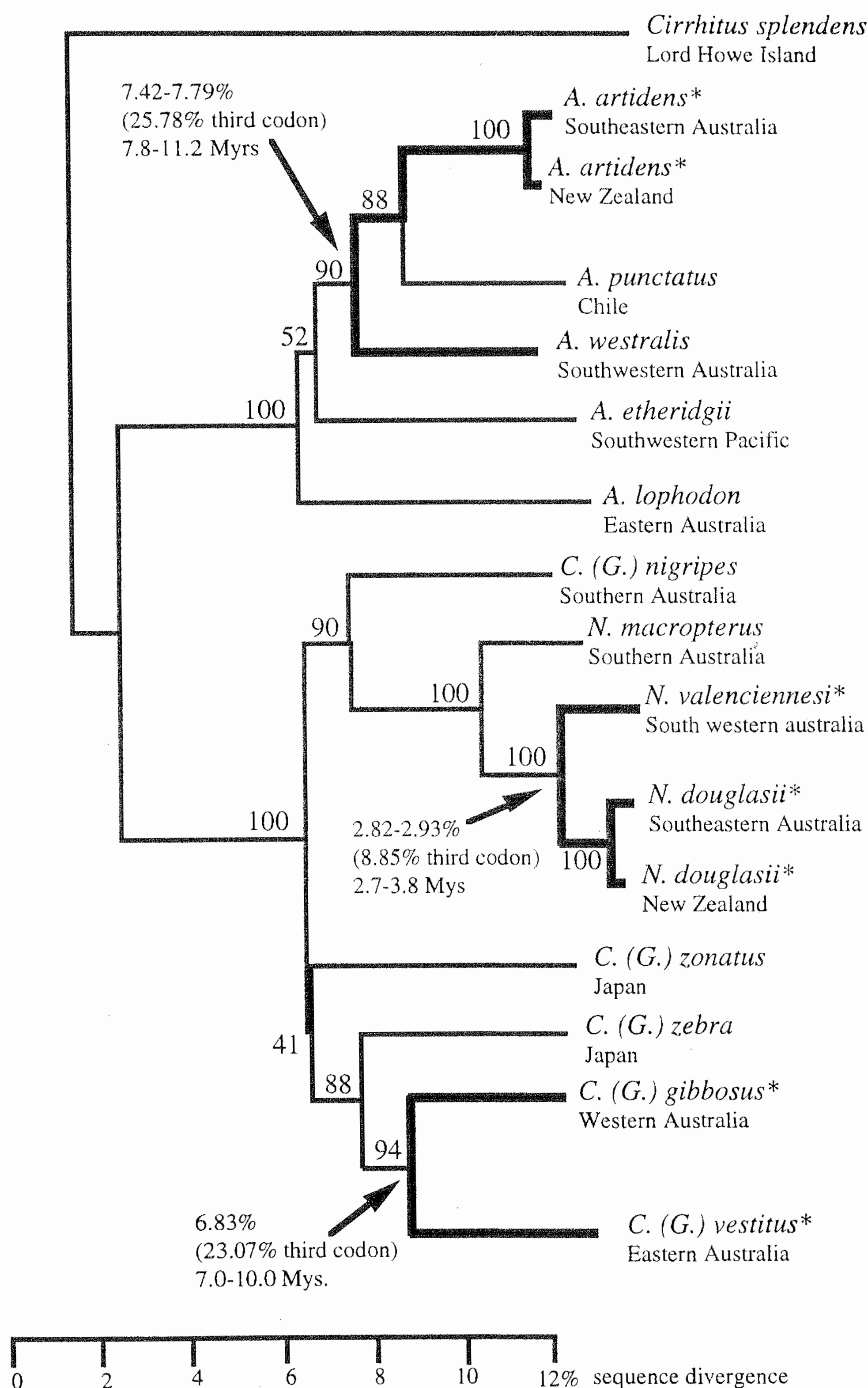


Figure 7.2

Figure 7.2 (previous page). Neighbour-joining topology from the analysis of mitochondrial partial cytochrome *c* oxidase subunit I and cytochrome *b* DNA sequences when combined. Branch lengths are proportional to Kimura (1980) two-parameter sequence divergence TI/TV=2.0), as measured relative to the scale bar. The numbers above the branches at each node represent bootstrap proportions for the taxa in each clade, as derived from 2000 replicate data sets. *Cirrhitus splendens* was chosen as the outgroup, as the Cirrhitidae appears the most plesiomorphic of the five cirrhitoid families (Greenwood, 1995). Species pairs with east-west allopatric distributions across southern Australia are indicated by asterisks, and linked by thickened branches. For each east-west allopatric species pair the levels of genetic separation and estimated divergence times are also indicated (see text).

Kimura (1980) two-parameter corrected genetic distances were similar for the *Goniistius* and *Aplodactylus* east-west allopatric species pairs, 6.83 and 7.42-7.79% respectively, while that for the *Nemadactylus* pair was 2.82-2.93% (Figure 7.2). Corresponding levels of sequence divergence at third codon positions, calculated under the constraint of clock-like sequence evolution, were 23.07% for the *Goniistius* pair, 25.78% for the *Aplodactylus* pair, and 8.85% for the *Nemadactylus* pair (Figure 7.2).

Discussion

Mitochondrial DNA partial cytochrome *c* oxidase subunit I and cytochrome *b* sequences were obtained from three cirrhitoid species pairs with east-west allopatric distributions across southern Australia, and logical outgroup taxa. Well supported sister taxa relationships were observed for *Nemadactylus* and *Goniistius* species pairs (Figure 7.2). Although *Aplodactylus arctidens* and *A. westralis* were not placed as sister taxa, it is apparent that they diverged from a common ancestor in the waters of Australia as the only additional species derived from their common ancestor is endemic to South America (Figure 7.2). Levels of DNA sequence divergence based on all codon positions were similar for *Goniistius* (6.83%) and *Aplodactylus* (7.42-7.79%) species pairs, but these were more than twice as large as that for the corresponding *Nemadactylus* species pair (2.82-2.93%). There was no evidence of nucleotide substitution rate heterogeneity at third codons among these three species pairs, and corresponding levels of divergence calculated when enforcing clock-like sequence evolution were 23.07% for *Goniistius*, 25.78% for *Aplodactylus*, and 8.85% for *Nemadactylus*.

East-west allopatric distributions of geminate populations and species along the southern Australian coast may have resulted from chance dispersal across habitat and climatic barriers. Although the habitat and climatic barriers along the Australian south coast are expansive, particularly the reef-devoid areas of the Coorong and the Nullarbor (Edgar, 1986; J. B. Hutchins, personal communication), they are probably insufficient to restrict gene flow in cirrhitoids below the levels required for speciation. Cirrhitoids are predominantly sedentary as adults (Sano and Moyer, 1985; Annala, 1987; Cappo, 1995; Francis, 1996), but high dispersal capabilities are suggested for their larvae. The cheilodactylids *N. macropterus* (Bloch and Schneider) and *Acantholatris monodactylus* (Carmichael) possess a 9-12 month offshore pelagic larval phase (Annala 1987; Andrew *et al.*, 1995), and both cheilodactylid distribution and the results from molecular genetic studies suggest high dispersal capabilities for these taxa (Elliott and Ward, 1994; Grewe, Smolenski and Ward, 1994; Chapters 3 and 4). High larval dispersal capabilities are also suggested for aplodactylids, as the divergence of the Australian-New Zealand *A. arctidens* from the South American *A. punctatus* postdates the geographic isolation of these continents (Chapter 6). Ocean currents would promote larval dispersal throughout southern Australia, particularly the Leeuwin Current that flows from west to east. Consequently, it is more likely that the differentiation of east and west coast cirrhitoids resulted from vicariant isolation during periods of climate or sea level change.

While vicariance is favoured to explain cirrhitoid east-west allopatric distributions, it is unlikely that these taxa share a biogeographic history. Clock-like DNA sequence evolution was observed for the three species pairs examined, and therefore the levels of sequence difference can be considered relative measures of divergence time. Only two of the three cirrhitoid species pairs exhibited similar levels of genetic divergence, *Aplodactylus* and *Goniistius*, and therefore these must have a history distinct from the *Nemadactylus* pair. However, it is also unlikely that *Aplodactylus* and *Goniistius* species pairs share a biogeographic history, as their ancestral taxa would not have possessed broadly overlapping distributions if contemporary latitudinal ranges are assumed.

A mechanism by which the east-west allopatric distributions of *Aplodactylus* and *Goniistius* species pairs could have arisen vicariantly at approximately the same period, but across the Australian south and north coasts respectively, is depicted in Figure 7.3. Under climatic conditions similar to the present an ancestral *Aplodactylus* taxon inhabited the south coast, and an ancestral *Goniistius* was found on either the east or the west coast (Figure 7.3a). During climatic cooling, the *Aplodactylus* ancestral taxon was vicariantly isolated into east (*arctidens-punctatus*) and west (*westralis*) coast lineages, while the ancestral *Goniistius* relocated to northern Australia (Figure 7.3b). During subsequent warming to conditions similar to the present day, the ancestral *Goniistius* became vicariantly isolated into east (*vestitus*) and west (*gibbosus*) coast populations, while the *Aplodactylus* species attained contemporary-like distributions (Figure 7.3c). This hypothesis suggests that the *Aplodactylus* lineage diverged prior to *Goniistius*, which agrees with the levels of genetic variation observed (Figure 7.2). It is more likely that the *Nemadactylus* and *Chironemus* Cuvier species pair divergences proceeded by the mechanism proposed for the *Aplodactylus* pair rather than that for the *Goniistius* pair, based on contemporary distributions. As the divergence of *Nemadactylus* species was somewhat more recent, a second event of climatic cooling is invoked. A similar mechanism combining north and south coast vicariance events has been proposed for the stargazer genus *Ichthyscopus* Swainston (Gomon and Johnson, 1999).

It is unlikely that the emergence of Bass Strait appreciably contributed to the isolation of eastern and western cirrhitoid populations. Receding sea level was commonly associated with periods of climatic cooling, and therefore cirrhitoids occupying the south coast would have been forced north and isolated prior to the emergence of Bass Strait (Edgar, 1986). Tectonic processes capable of isolating eastern and western populations are also unlikely in Bass Strait, as this region has been geologically stable for the last 60 Myr (Quilty, 1994).

Lamb (1990) previously suggested a northern Australian divergence for *C. (G.) vestitus* and *C. (G.) gibbosus*. The subgenus *Goniistius* is also antitropical in distribution, and three

transequatorial divergence events have been identified for this group (Chapter 4), indicating that ancestral taxa have occupied equatorial latitudes at certain periods. Hutchins (1987) also raised the possibility of a northern divergence for members of *Paraplesiops* Bleeker similar in distribution to *C. (G.) vestitus* and *C. (G.) gibbosus*. Vicariance across northern Australia has been commonly suggested for tropical taxa, but predominantly as the result of Pleistocene sea level fluctuation in Torres Strait rather than temperature changes (Chenoweth *et al.*, 1998; Duke *et al.*, 1998; and references therein).

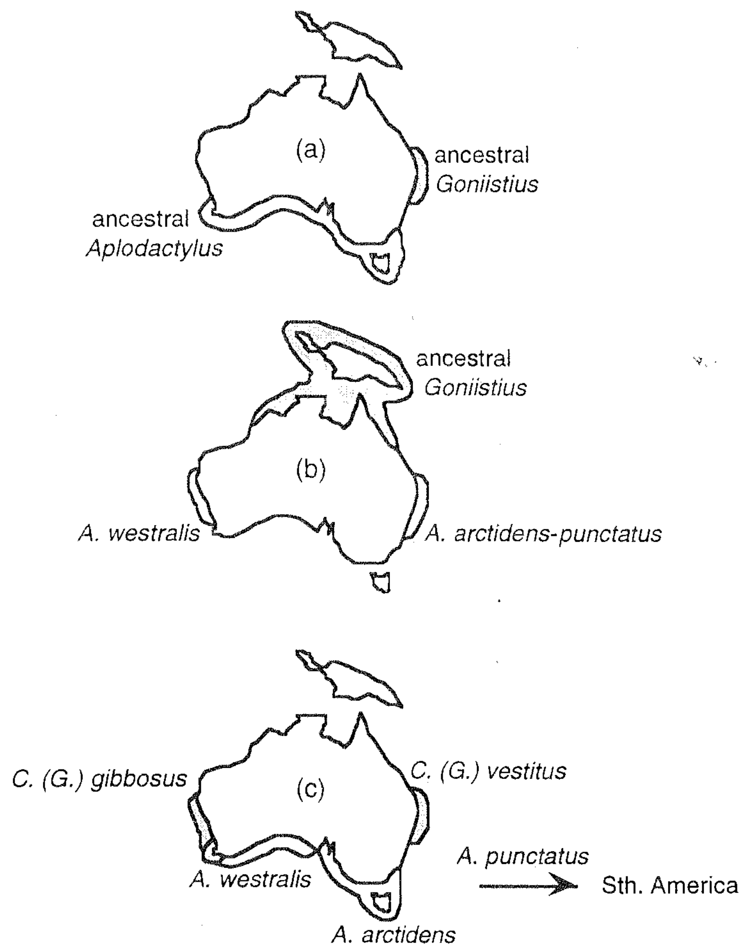


Figure 7.3. Possible mechanism for the vicariant isolation of *Aplodactylus* and *Goniistius* ancestral populations. (a) Distribution of ancestral taxa during conditions similar to present. The ancestral *Goniistius* may have been distributed on either the east or west coast. (b) Northward movement of ancestral taxa with climatic cooling, resulting in the vicariant isolation of *Aplodactylus* populations. (c) Southward movement during climatic warming, resulting in the vicariant isolation of *Goniistius* populations. It is suggested that the divergences of *Nemadactylus* and *Chironemus* species followed that depicted for *Aplodactylus*.

Pleistocene glacial-interglacial changes in temperature and sea level have been implicated for the vicariant formation of east-west allopatric and parapatric distributions of geminate populations and species across Australia (Dartnall, 1974; Knox, 1980; Edgar, 1986; Hutchins, 1987; Chenoweth *et al.*, 1998; Duke *et al.*, 1998; and references therein). However, when employing mitochondrial third codon substitution calibrations of 2.3% divergence Myr^{-1} (Martin *et al.*, 1992) and 3.3% divergence Myr^{-1} (Bermingham *et al.*, 1997), separation time estimates for both the *Goniistius* and *Aplodactylus* species pairs range from 7.8 to 11.2 Myr ago (late Miocene), while those for *Nemadactylus* are 2.7-3.8 Myr ago (mid Pliocene) (Figure 7.2). Although absolute estimates of divergence time obtained from molecular data should be treated cautiously (Rand, 1994), the values for these taxa suggest that glaciations and climatic transitions prior to the Pleistocene should also be considered for the development of east-west allopatric and parapatric distributions of geminate populations and species across Australia.

The proposed biogeographic history of east-west allopatric cirrhitoid species pairs differs from that suggested for another marine fish, *Pomatomus saltatrix* Linnaeus. Populations of *P. saltatrix* are similar in distribution to the *Goniistius* species pair, and have high dispersal potentials during both adult and early life history stages (Goodbred and Graves, 1996). Genetic studies revealed low but significant genetic divergence between east and west coast populations, and suggested recent separation, during the last 0.2-1.1 Myr (Nurthen *et al.* 1992; Goodbred and Graves, 1996). Lower genetic variability of the east coast population is consistent with founding from the west, and it has been proposed that dispersal occurred across the south coast during a period of elevated water temperature (Graves, 1998).

The results of this study indicate that three cirrhitoid species pairs with east-west allopatric distributions across southern Australia each diverged from common ancestors during two distinct periods, the late Miocene and mid Pliocene. In the instance where divergences occurred during the same period (*Aplodactylus* and *Goniistius*, 7.8-11.2 Myr ago, late Miocene), different vicariance mechanisms are suggested by likely ancestral distributions. Although this study finds little evidence of a shared biogeographic history for geminate taxa

with east-west allopatric or parapatric distributions across southern Australia, it is hoped that it will encourage comprehensive studies of this topic. Such studies would examine genetic variation in taxa with different distributional ranges, habitat requirements, and dispersal capabilities. Other geminate taxa may also exhibit levels of genetic variation more consistent with isolation during Pleistocene glaciations.

CHAPTER 8: Microsatellite analysis of *Nemadactylus macropterus* and related taxa (Cheilodactylidae).

Abstract

Variation at seven microsatellite loci was scored for the marine fish *Nemadactylus macropterus* in an effort to resolve population stock structure and provide information on the performance of this technique. Stock structuring was not detected among Australian populations, or between Australian and New Zealand populations. The latter is incongruent with allozyme and mitochondrial DNA studies, which detected slight but significant divergence across the Tasman Sea. The highly polymorphic nature of the microsatellite loci analysed may have hindered the resolution of population structure. Microsatellites were also scored for a morphologically similar South American species, *N. bergi*, and differentiation from *N. macropterus* was observed at one locus. A morphologically distinct species, *Acantholatris* (=*Nemadactylus*) *monodactylus*, differed from both *N. macropterus* and *N. bergi* at all loci. Analysis of expected heterozygosities suggested a recent reduction in the effective population size of *N. bergi* but not *N. macropterus* or *A. monodactylus*, consistent with observations from mitochondrial DNA.

Introduction

Measures of population divergence are useful for the management of fisheries. Populations in different regions are often demographically and genetically independent, even in continuously distributed species. Consequently, separate management of distinct populations, or "stocks", is desirable for the maintenance of genetic variation and avoidance of regional over-exploitation. Measures of population divergence may be provided by a variety of molecular, morphological, and ecological characters.

Two classes of molecular genetic characters have been commonly employed during studies of population divergence and fisheries management, allozyme electromorphs and mitochondrial DNA (mtDNA). Studies of marine taxa employing these characters have generally observed only limited population divergence (Gyllensten, 1985; Ward *et al.*, 1994). Recently, microsatellite characters have been increasingly used, which exhibit more variation than either allozymes or mtDNA (O'Connell and Wright, 1997). Consequently, microsatellite analyses conducted on marine taxa have often identified population divergence not evident from other molecular characters (Bentzen *et al.*, 1996; O'Connell *et al.*, 1998; Shaw *et al.*, 1999a, b).

The morwong or tarakihi, *Nemadactylus macropterus* Bloch and Schneider (Cheilodactylidae), is an abundant and commercially important marine fish of New Zealand and southern Australia (Figure 8.1). This species occurs demersally in nearshore and continental shelf waters at depths of 10-200 m (Annala, 1987). Maximum ages exceed 35 yr, yet maturity is attained within 3-6 yr (Annala, 1987). Fecundity is high, and spawning occurs serially during late summer and autumn (Annala, 1987; Jordan, 1997). This species has potentially high dispersal capabilities, indicated by an offshore pelagic larval stage of 8-12 months in duration (Annala, 1987; Bruce *et al.*, 1996). Adult movements of up to 300 km have also been observed (Annala, 1987, 1993; Smith 1989).

Four population genetic studies have been conducted on *N. macropterus* in an effort to resolve its stock structure for management purposes, using the techniques of allozyme electrophoresis (Gauldie and Johnston, 1980; Richardson, 1982; Elliott and Ward, 1994), and mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP; Grewe *et al.*, 1994). The studies of Richardson (1982), Elliott and Ward (1994), and Grewe *et al.* (1994) did not identify population divergence in southern Australia despite its range of over 3000 km of coastline. Small but significant divergence were detected between Australia and New Zealand. The first find is in contrast with the results of non-genetic studies based on otolith microchemistry (Thresher *et al.*, 1994) and larval advection (Bruce *et al.*, 1996), which

proposed that up to three stocks exist within southeast Australian waters alone. Gauldie and Johnston (1980) identified seven genetically defined stocks in New Zealand waters, although differences in allelic frequencies appeared attributable to selection rather than genetic isolation.

Nemadactylus macropterus resembles *N. bergi*, and the latter occurs along the temperate east coast of South America. These taxa differ in lateral line scale counts, the width of the supracleithrum relative to the diameter of the eye, and the relative lengths of thickened pectoral fin rays (Norman, 1937; Lamb, 1990). However, these characters vary among the holotype and paratypes of *N. bergi* (R.W.G. White, University of Tasmania, 1995, pers. comm.), and its separate status requires justification. Although *Acantholatris monodactylus* (=*N. monodactylus*) and *N. macropterus* can be readily identified, they do not possess separate monophyletic mtDNA lineages (Chapter 3).

The aim of this project was to develop microsatellite markers for *Nemadactylus macropterus* and assess the levels of genetic divergence among Australian and New Zealand samples relative to those observed from allozyme and mtDNA RFLP studies. Individuals from the investigations of Elliott and Ward (1994) and Grewe *et al.* (1994) were analysed, facilitating comparison of the three molecular techniques in resolving stock structure. As microsatellite loci developed for one taxon may be applied to other closely related species (McConnell *et al.*, 1995), *N. bergi* and *A. monodactylus* were also analysed.

Materials and methods

Partial genomic library construction

Total genomic DNA was extracted from frozen liver tissue of *Nemadactylus macropterus*. A standard CTAB phenol-chloroform protocol (Hillis *et al.* 1990) was employed, and extracted DNA was tested for the presence of high molecular weight (>20 kb) fragments by agarose gel electrophoresis. Approximately 20 μg of genomic DNA was digested with 50 units of *Dpn* II

and *Hae* III at 37°C for 16 h. The entire digest was subjected to agarose gel electrophoresis, and 400-600 bp fragments were excised and purified using the GENECLEAN Spin Kit (BIO 101, Inc.). pUC 19 vector was similarly digested with *Bam*H I and *Hinc* II, dephosphorylated with 10 units of calf intestinal alkaline phosphatase at 37°C for 1 h, and gel purified. The ligation of genomic DNA fragments into digested vector was performed in approximately equal molar ratios (400 ng vector, 67 ng insert) with 120 units of T4 DNA ligase at 14°C for 16 h. Approximately 100 ng of ligated DNA was transformed into XL1-Blue heat competent cells (Stratagene). Cells were grown on LB plates containing 50 µg/mL ampicillin, 40 µg/mL X-gal, and 120 µg/mL IPTG.

Microsatellite characterisation

$(AC)_{16}T$ oligonucleotide was 3' end-labelled with digoxygenin-11-ddUTP using the DIG Oligonucleotide 3' End Labelling Kit (Boehringer Mannheim). Colony lifts were performed using Hybond-N nylon membranes (Amersham), and treated following the instructions for the DIG Luminescent Detection Kit (Boehringer Mannheim). Hybridisation was conducted at 50°C for 6-12 h with a probe concentration of 10 pmol/mL, and chemiluminescent detection employed CSPD substrate. Plasmids from positive colonies were purified using a miniprep protocol (Sambrook *et al.*, 1989). Insert sequences were PCR amplified using M13 universal primers, gel purified with the GENECLEAN Spin Kit, and sequenced with the ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kit (Perkin Elmer).

Microsatellite amplification and electrophoresis

Oligonucleotide primers for the PCR amplification of microsatellites were designed using the PrimerSelect program of the Lasergene package (DNASTAR). One primer for each locus was 5' end-labelled with either FAM or HEX dye. Amplifications were conducted in 20 µL volumes comprising 67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/mL gelatin, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 units *Taq* F1 DNA polymerase (Fisher Biotech), 0.15-0.4 mM of each oligonucleotide primer, and approximately 20 ng DNA. Thermal cycling conditions for each locus comprised 35 cycles of 94°C/30 sec,

62.5°C/30 sec, and 72°C/60 sec. An initial denaturation of 94°C/5 min and a final extension of 72°C/10 min were employed. PCR products for each locus were mixed in appropriate ratios to achieve even peak heights when multiplexed on an ABI 377. Alleles were scored relative to the GS 500 size standard.

Material analysed

Nemadactylus macropterus DNA and frozen tissue samples from the studies of Elliott and Ward (1994) and Grewe *et al.* (1994) were analysed. Only four of the nine abundantly sampled populations were included in this study, although those chosen demarcate the majority of the species range (Figure 8.1). DNA was also extracted from ethanol preserved muscle tissues of 51 *Nemadactylus bergi* individuals (Mar del Plata, Argentina) and 29 *Acantholatris monodactylus* individuals (Saint Paul and Amsterdam Islands, Indian Ocean, Figure 8.1). Total genomic DNA was extracted as for *N. macropterus* library construction. The ages of individuals were not known.

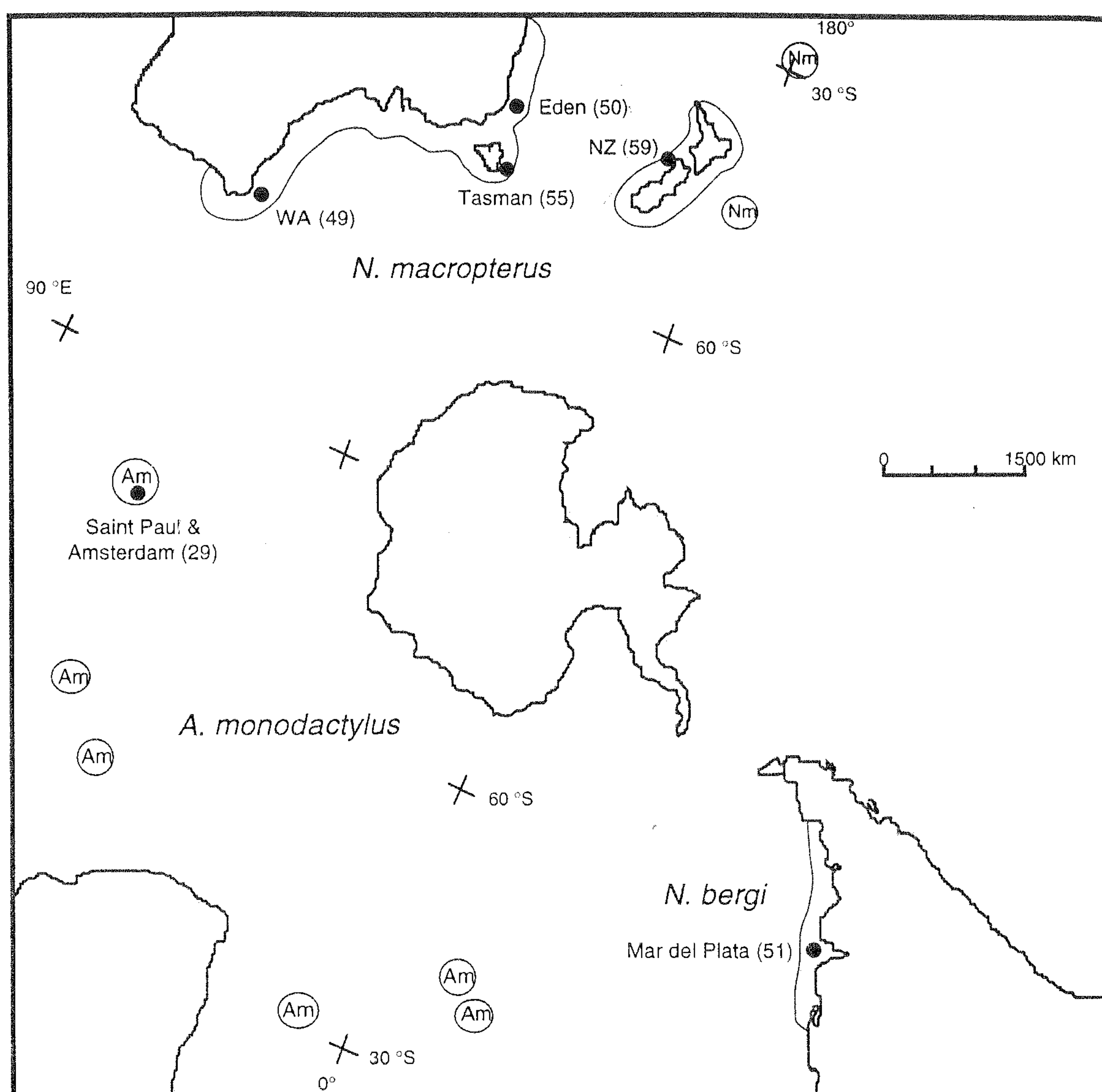


Figure 8.1. Species range, collection sites ("•"), and sample sizes for *Nemadactylus macropterus* (WA, Tasman, Eden, NZ), *N. bergi* (Mar del Plata), and *Acantholatris monodactylus* (Saint Paul and Amsterdam).

Data analysis

Genetic variability was measured in terms of the number of alleles per locus, the observed heterozygosity (H_o), and the Hardy-Weinberg expected heterozygosity (H_e, gene diversity), calculated by Genepop 3.1c (Raymond and Rousset, 1995). Genepop was also used to calculate F_{IS} for the quantification of heterozygosity deficiency or excess, and to perform tests for Hardy-Weinberg equilibrium, genotypic linkage disequilibrium, and sample divergence. Fisher's exact test was used when there were less than five alleles per locus,

otherwise an unbiased estimate of the exact test statistic was calculated using a Marchov chain procedure. Critical significance levels were adjusted for simultaneous tests using the sequential Bonferroni procedure (Rice, 1989).

Sample divergence was quantified by the calculation of F_{ST} (Wright, 1951) and R_{ST} (Slatkin, 1995), using the programs FSTAT (Goudet, 1995) and R_{ST}-calc (Goodman, 1997) respectively. These measures differ in their assumptions of allele mutation. F_{ST} assumes the infinite allele model (IAM), where mutations only produce new allelic states (Kimura and Crow, 1964), while R_{ST} assumes a stepwise mutation model (SMM), where mutation changes the state of an allele by one step forward or backward, with equal probability (Ohta and Kimura, 1973). Multilocus estimates of both measures were obtained by averaging the variance components across loci (Weir and Cockerham, 1984; Slatkin, 1995). One thousand replicates of permutation were used to determine whether divergence was significantly greater than zero. The presence of an isolation-by-distance relationship between genetic divergence and shortest sea distance between populations was assessed using Mantel's (1967) test. Estimates of migration per area per generation (N_em) were obtained from F_{ST} and R_{ST} according to the equation of Wright (1943), assuming an island model and migration-drift equilibrium.

Evidence for a recent bottleneck in effective population size (N_e) was assessed by determining the average heterozygosity excess across loci, using BOTTLENECK 1.1 (Piry *et al.*, 1999). This program calculates the difference between the Hardy-Weinberg expected heterozygosity (H_e, gene diversity) and the expected heterozygosity based on the number of alleles and sample size assuming mutation-drift equilibrium (H_{eq}). Three models of allele mutation were employed when calculating heterozygosity; IAM, SMM, and a two-phase model (TPM, DiRienzo *et al.*, 1994) incorporating 95% single-step mutations and a 12% variance of multiple step mutations.

The relative divergence of populations and species was visualised using components of the PHYLIP 3.573 package (Felsenstein, 1993). Cavalli-Sforza and Edwards (1967) chord distances were calculated using GENDIST, and clustered by the neighbour-joining algorithm (Saitou and Nei, 1987) implemented with NEIGHBOR. Support for tree nodes was assessed by bootstrapping (Felsenstein, 1985a). Allele frequencies were resampled 1000 times using SEQBOOT, trees were constructed for each replicate dataset as above, and the consensus topology and bootstrap proportions were calculated using CONSENSE.

Results

Approximately 5000 clones were screened for the presence of $(TG)_n$ microsatellite repeat sequences, and 105 positive colonies were identified. Thirty-one positive clones were sequenced, containing a total of 46 microsatellite repeats, mostly of the type $(TG)_n$. PCR primers were designed for seven loci, encompassing a range of microsatellite types (perfect, imperfect, compound; Weber, 1990), motifs, and number of repeats (Table 8.1). Two loci, *Nma 187* and *Nma 245*, were developed from the same clone, their repeat sequences separated by 95 bp and amplified in a non-overlapping manner. The 3' thymine-rich primer for locus *Nma 106* produced a non-specific product of approximately 180 bp in length, and therefore the opposite primer was chosen for dye-labelling. Other clone sequences that could be used for the design of microsatellite-amplifying PCR primers are deposited in GenBank (accession numbers AF125121-AF125138).

The PCR primers successfully amplified each locus in the three species analysed, and all were polymorphic (Table 8.2). *Nemadactylus macropterus* exhibited 8-42 alleles per locus, with a gene diversities (H_e, expected heterozygosity) of 0.56-0.93 (Table 8.2). *Nemadactylus bergi* exhibited similar levels of polymorphism as *N. macropterus* (6-27 alleles per locus, H_e = 0.56-0.94), while *A. monodactylus* exhibited less variation (4-14 alleles per locus, H_e = 0.10-0.77, Table 8.2). Allele size ranges for each locus overlapped between species and

among populations of *N. macropterus* (Table 8.2). A total of 30 private alleles were observed, but all were at low frequencies (<0.034, Appendix 8.1).

Table 8.1. Repeat sequence, GenBank accession, and PCR primers for each of the seven microsatellite loci developed for *Nemadactylus macropterus.* Locus names correspond to the species in which they were characterised and the length of the amplified product in the plasmid clone.

Locus	Repeat sequence	GenBank acc.	Primer sequences
Nma 106	$(AC)_{16}$	AF125115	GCACTTCATGTACATGCAGGGTTTT
			TAAGCGGCATCTTGAGTGTCTGG [1]
Nma 118	$(TCA)_{9}$	AF125116	CAAAAAGCAGCTCTACAGTGACAG [2]
			CAGAGACAGTTTAGGGAAGTGAAGAC
Nma 187	$(AC)_{13}(AG)_{4}$	AF125117	GCAACTTCCCCGAGCATCATTA
			AGAGCCTGCAATTAGAGTCAACCAA [2]
Nma 230	$(GT)_{15}$	AF125118	AGTTTCCCCCTGCCTACA
			CCTGAACCACTGCGACACTG [1]
Nma 245	$(GT)_{21}$	AF125117	TTCTTAAAGGGCGAGTGATGCTA [2]
			ATGAAAGATGAAGTGATGGAAACAGAC
Nma 305	$(GT)_{7}AT(GT)_{9}$	AF125119	GATCAGGCTCTTCCAGTTGTCATTCC [1]
			GTGTCGGCGTTCAGAGGCATCC
Nma 311	$(AT)_{8}$	AF125120	ACTCCGTCTGTACTCTTTGTTGA
			CTCAGGCTGCAGGTGGTC [2]

[1] 5' end-labelled with FAM dye

[2] 5' end-labelled with HEX dye

Within populations, significant deviation from Hardy-Weinberg expected genotype frequencies were observed in nine instances after applying sequential Bonferroni corrections (Table 8.2). Each deviation involved heterozygote deficiency (F_{IS}>0). Four significant deviations occurred at locus *Nma 106*, three at *Nma 311*, and two at *Nma 305* (Table 8.2). Significant deviation was also observed at these three loci when pooling *N. macropterus* populations. Significant genotypic linkage disequilibrium was only observed during one comparison of loci after Bonferroni correction, involving the adjacent loci *Nma 187* and *Nma 230* in *A. monodactylus* (P<0.001).

Table 8.2. Variation at seven microsatellite loci in populations of *Nemadactylus macropterus*, *N. bergi*, and *Acantholatris monodactylus*. Population sample size (n), number of alleles (A), size range, observed heterozygosity (H_o), Hardy-Weinberg expected heterozygosity(H_e, gene diversity), F_{IS} (Weir and Cockerham, 1984), and the probability of deviation from Hardy-Weinberg equilibrium based on exact tests.

	N. macropterus					*N. bergi*	*A. monodactylus*
	WA	Eden	Tasman	NZ	pooled		
n	49	50	55	59	213	51	29
Nma 106 $(AC)_{16}$							
A	25	30	33	29	42	27	10
range	91-147	93-190	91-185	91-177	91-190	95-195	93-153
H_o, H_e	0.61, 0.91	0.82, 0.94	0.65, 0.91	0.66, 0.95	0.69, 0.93	0.82, 0.94	0.24, 0.69
F_{IS}	0.331	0.125	0.283	0.309	0.263	0.126	0.655
P	0.0000*	0.0929	0.0006*	0.0000*	0.0041*	0.0748	0.0000*
Nma 118 $(TCA)_9$							
A	7	7	7	8	8	6	4
range	112-133	112-130	112-133	112-133	112-133	109-127	118-130
H_o, H_e	0.57, 0.61	0.50, 0.54	0.42, 0.50	0.56, 0.59	0.51, 0.56	0.55, 0.59	0.34, 0.35
F_{IS}	0.063	0.068	0.173	0.054	0.083	0.076	0.019
P	0.6573	0.4478	0.2056	0.6153	0.3599	0.1194	1.0000
Nma 187 $(CA)_{14}(GA)_4$							
A	20	16	19	21	24	18	10
range	179-223	185-215	181-219	177-227	177-227	179-223	181-211
H_o, H_e	0.92, 0.90	0.92, 0.93	0.96, 0.93	0.92, 0.92	0.93, 0.92	0.94, 0.93	0.76, 0.77
F_{IS}	-0.016	0.01	-0.035	0.011	-0.009	-0.016	0.012
P	0.6226	0.964	0.8989	0.2874	0.6275	0.6679	0.3142
Nma 230 $(GT)_{15}$							
A	21	19	21	20	25	19	13
range	221-265	229-265	219-267	215-263	215-267	229-273	233-273
H_o, H_e	0.92, 0.91	0.88, 0.92	0.91, 0.91	0.95, 0.94	0.92, 0.92	0.90, 0.91	0.69, 0.72
F_{IS}	0.049	0.08	0.143	0.089	0.089	0.101	0.28
P	0.6379	0.0938	0.1238	0.5153	0.5375	0.2504	0.2923
Nma 245 $(GT)_{21}$							
A	19	18	18	16	23	15	9
range	217-257	219-261	219-259	217-251	217-261	219-253	221-245
H_o, H_e	0.88, 0.92	0.84, 0.91	0.69, 0.80	0.83, 0.91	0.81, 0.89	0.75, 0.83	0.38, 0.52
F_{IS}	-0.006	0.04	0.005	-0.011	0.008	0.005	0.041
P	0.4171	0.8054	0.1047	0.6061	0.0187	0.0692	0.0451
Nma 305 $(GT)_7AT(GT)_9$							
A	21	19	23	21	27	17	14
range	291-345	289-345	289-343	291-357	289-357	295-327	291-347
H_o, H_e	0.78, 0.94	0.74, 0.85	0.84, 0.95	0.81, 0.92	0.79, 0.92	0.92, 0.92	0.28, 0.73
F_{IS}	0.387	0.315	0.193	0.12	0.242	0.231	-0.022
P	0.0000*	0.0141*	0.0064	0.0785	0.0000*	0.0233	1.0000
Nma 311 $(AT)_8$							
A	7	6	8	10	11	7	4
Range	297-313	297-307	297-328	297-328	297-328	291-313	301-311
H_o, H_e	0.37, 0.60	0.38, 0.55	0.45, 0.56	0.56, 0.63	0.45, 0.59	0.43, 0.56	0.10, 0.10
F_{IS}	0.181	0.136	0.118	0.113	0.135	-0.001	0.626
P	0.0425	0.0065*	0.0083*	0.0548	0.0000*	0.2679	0.0000*

*significant after sequential Bonferroni correction for simultaneous tests.

Significant allelic frequency differences were not observed during comparisons of *N. macropterus* populations at any locus following Bonferroni correction (Table 8.3). One locus, *Nma 245*, differentiated *N. bergi* from *N. macropterus*, while each locus differentiated *A. monodactylus* from *N. macropterus* and *N. bergi* (Table 8.3).

When excluding those loci shown to deviate from Hardy-Weinberg expectations (*Nma 106*, *Nma 305*, *Nma 311*), F_{ST} and R_{ST} for *N. macropterus* were -0.001 and 0.00095 respectively, and neither was significantly greater than zero (P>0.528). Values for pairwise comparisons of individual populations did not exceed 0.00943, and were not significantly greater than zero (P>0.099, Table 8.4). Larger F_{ST} and R_{ST} were observed for the comparison of *N. bergi* and *N. macropterus*, 0.0075 and 0.02992 respectively, and these were significantly greater than zero (P<0.001 Table 8.4). Pairwise F_{ST} and R_{ST} values involving *A. monodactylus* exceeded 0.0581 and 0.01331 respectively, and were significantly greater than zero (P<0.037) with exception of R_{ST} for the comparison against *N. bergi* (P<0.15100, Table 8.4).

There was no evidence of an isolation by distance relationship among *N. macropterus* populations based on Mantel's test (F_{ST} P=0.401, R_{ST} P=0.586, one-tailed Spearman rank correlation coefficient). Estimates of migration per area per generation (N_em) for *N. macropterus* were -250 individuals based on F_{ST}, and -264 based on R_{ST}. Negative sign of estimates indicates greater within population variance than between population variance, and effectively unlimited exchange.

Table 8.3. Probabilities of homogeneity in allelic frequencies between populations of *Nemadactylus macropterus*, and among *N. macropterus*, *N. bergi*, and *Acantholatris monodactylus*, based on exact tests. The Australia population comprises the WA, Eden, and Tasman populations combined.

	WA	Eden	Tasman	Australia		*N. macropterus*	*N. bergi*
Nma 106							
Eden	0.21728				*Nma 106*		
Tasman	0.70219	0.19998			*N. bergi*	0.07579	
NZ	0.87096	0.08239	0.92245	0.8255	*A. monodactylus*	0.00000*	0.00000*
Nma 118							
Eden	0.90176				*Nma 118*		
Tasman	0.90127	0.91157			*N. bergi*	0.10528	
NZ	0.60275	0.75697	0.78151	0.37778	*A. monodactylus*	0.00036*	0.00000*
Nma 187							
Eden	0.34003				*Nma 187*		
Tasman	0.38245	0.27945			*N. bergi*	0.17567	
NZ	0.79078	0.59866	0.73159	0.74278	*A. monodactylus*	0.00000*	0.00000*
Nma 230							
Eden	0.61966				*Nma 230*		
Tasman	0.87245	0.44222			*N. bergi*	0.0491	
NZ	0.28602	0.50699	0.03298	0.13198	*A. monodactylus*	0.00000*	0.00071*
Nma 245							
Eden	0.12551				*Nma 245*		
Tasman	0.67207	0.95392			*N. bergi*	0.00000*	
NZ	0.30034	0.48608	0.23884	0.167	*A. monodactylus*	0.00000*	0.00000*
Nma 305							
Eden	0.91083				*Nma 305*		
Tasman	0.03528	0.05086			*N. bergi*	0.05741	
NZ	0.07479	0.089	0.60376	0.06886	*A. monodactylus*	0.00000*	0.00000*
Nma 311							
Eden	0.52763				*Nma 311*		
Tasman	0.411	0.42766			*N. bergi*	0.14036	
NZ	0.03125	0.10875	0.45654	0.05654	*A. monodactylus*	0.00000*	0.00000*

*significant after sequential Bonferroni correction for simultaneous tests

Table 8.4. Pairwise measures of genetic divergence between populations of *Nemadactylus macropterus*, and among *N. macropterus*, *N. bergi*, and *Acantholatris monodactylus* (F_{ST} below diagonal, R_{ST} above diagonal) based on loci *Nma 118*, *Nma 187*, *Nma 230* and *Nma 245*. The probability that values are greater than zero was assessed by 1000 replicates of permutation.

N. macropterus	WA	Eden	Tasman	NZ	Australia
WA		-0.00783	-0.00537	-0.00006	
Eden	0.0013		-0.00118	-0.00329	
Tasman	-0.0034	-0.0009		0.00943	
NZ	-0.0016	-0.0022	-0.0007		0.00408
Australia				-0.001	
	A. monodactylus	*N. bergi*	*N. macropterus*		
A. monodactylus		0.01331	0.02257*		
N. bergi	0.0641*		0.02992*		
N. macropterus	0.0581*	0.0075*			

* $P<0.05$

A significant excess of Hardy-Weinberg expected heterozygosity was observed for *N. bergi* under the infinite allele model (P=0.03125), but no such excess was observed for any other population or mutation model (Table 8.5).

A representation of genetic divergence among samples and species was obtained by clustering Cavalli-Sforza and Edwards (1967) pairwise chord distances (Figure 8.2). *Acantholatris monodactylus* was divergent from the samples of *N. macropterus* and *N. bergi*. *Nemadactylus bergi* clustered immediately adjacent to samples of *N. macropterus*, although the level of divergence was only slightly greater than the interpopulation divergences for *N. macropterus*. The *N. macropterus* populations appeared equally divergent from one another. Low bootstrap support (<70%) was observed for the inferred relationships among *N. macropterus* sampling sites, while high support was observed for the placement of *N. bergi* and *A. monodactylus*.

Table 8.5. Probability that Hardy-Weinberg expected heterozygosity (H_e, gene diversity) does not exceed the heterozygosity expected from the number of alleles and sample size when assuming mutation-drift equilibrium (H_{eq}), averaged across loci. Wilcoxon signed-ranks tests were conducted for infinite allele (IAM), stepwise (SMM), and two-phase (TPM, 95% single step, 12% variance of multistep) mutation models. Those loci exhibiting significant deviation from Hardy-Weinberg expected genotype frequencies were excluded from analysis, leaving *Nma 118*, *Nma 187*, *Nma 230*, and *Nma 245*.

Species/population	IAM	TPM	SMM
A. monodactylus	0.43750	0.96875	0.96875
N. bergi	0.03125*	0.15625	0.56250
N. macropterus	0.06250	0.90625	1.00000
New Zealand	0.06250	0.56250	0.84375
Albany	0.06250	0.90625	1.00000
Eden	0.06250	0.90625	0.90625
Tasman	0.06250	0.90625	0.90625

* $P<0.05$

Discussion

Allelic divergence was not observed among sampling sites of *Nemadactylus macropterus* in the waters of New Zealand and southern Australia (Table 8.3). Measures of genetic divergence were small, and not significantly greater than zero (F_{ST} = -0.001, R_{ST} = -0.00095, P>0.528), as were the values obtained during pairwise comparisons of populations (F_{ST}<0.0014, R_{ST}<0.00944, P>0.099, Table 8.4). Estimates of migration per area per generation indicated unlimited exchange. The South American species *N. bergi* differed from *N. macropterus* only at *Nma 245* (P<0.00001, Table 8.3). Pairwise F_{ST} and R_{ST} for this comparison were small, 0.0075 and 0.02992 respectively, but significantly greater than zero (P<0.001, Table 8.4). *Acantholatris monodactylus* diverged from *N. macropterus* and *N. bergi*, with significantly different allele frequencies at all loci (P<0.00071, Table 8.3). Genetic divergence of *A. monodactylus* was also comparatively high (F_{ST} = 0.0581-0.0641,

R_{ST} = 0.01331-0.02257, Table 8.4), and significantly greater than zero (P<0.037) with exception of the R_{ST} with *N. bergi* (P=0.15100)

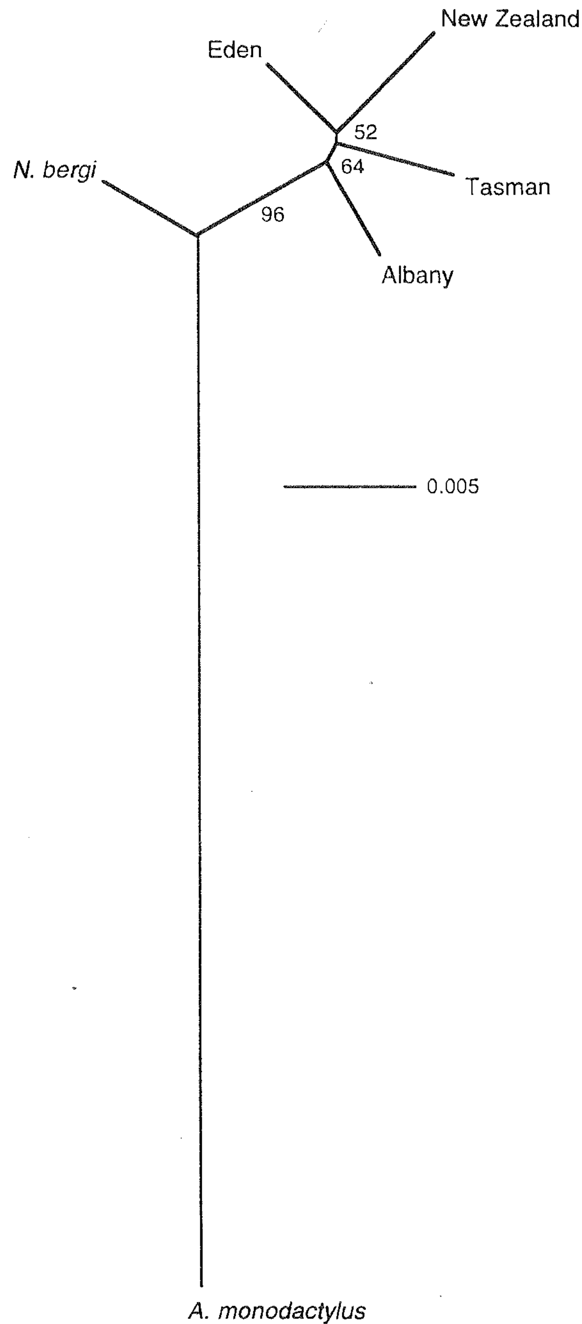


Figure 8.2. Neighbour-joining phenogram of pairwise Cavalli-Sforza and Edwards (1967) chord distances derived from microsatellite allele frequency data (Appendix 8.1). Support for tree nodes is indicated by bootstrap percentages, based on 1000 replicate datasets. Those loci exhibiting significant deviation from Hardy-Weinberg expected genotype frequencies were excluded from analysis, leaving *Nma 118*, *Nma 187*, *Nma 230*, and *Nma 245*.

Deviation from Hardy-Weinberg expectations

Significant deviation from Hardy-Weinberg expected genotype frequencies, involving heterozygote deficiency, were observed for nine population-locus combinations. Four of these involved *Nma 106*, three were for *Nma 311*, and two were at *Nma 305* (Table 8.2). Each of these loci was out of equilibrium for two or three samples of *N. macropterus* (Table 8.2). They were also out of equilibrium when all four *N. macropterus* samples were pooled, and for *A. monodactylus*, but not *N. bergi* (Table 8.2).

Interpretations of population divergences based on loci not conforming to Hardy-Weinberg expectations can be misleading, as any differences observed may not be entirely the result of genetic drift. Three instances of significant Hardy-Weinberg disequilibrium were observed at loci which differentiated populations or species, involving comparisons between *N. macropterus* and *A. monodactylus* at *Nma 106*, *Nma 305*, and *Nma 311*. However, these species diverged at the remaining four loci which were in Hardy-Weinberg equilibrium, and F_{ST} and R_{ST} values also were significantly greater than zero for this comparison when excluding the potentially problematic loci.

Deviation from Hardy-Weinberg expected frequencies in the form of heterozygote deficiency could be explained by several factors relating to the microsatellite markers themselves. The presence of non-amplifying (null) alleles is a commonly invoked explanation (García de León, 1997; Rico *et al.*, 1997). Allele size homoplasy, a situation where alleles of the same size differ in sequence composition, may be particularly predominant at highly variable loci. Scoring errors can also lead to the underestimation of heterozygote frequencies, especially as the shorter allele of microsatellite heterozygotes is often preferentially amplified, making the larger allele comparatively difficult to detect. Another alternative is non Mendelian inheritance, although the absence of significant heterozygote deficiency for every population at any single locus makes this unlikely for *N. macropterus* and *A. monodactylus* (Table 8.2).

Factors relating to the biology of the species can also cause heterozygote deficiencies, such as Wahlund effect, inbreeding, selection, and non-random mating. Wahlund effect is unlikely for *N. macropterus* and *A. monodactylus* given the low values of F_{ST}. Similarly, inbreeding is unlikely given the large population sizes for these species. Although microsatellites are considered to be non-coding sequences, they may be closely linked to loci which are under selection (Jarne and Lagoda, 1996). Little is known about the reproductive behaviour of these species, and therefore non-random mating is a possibility.

Comparison of microsatellites with allozyme and mtDNA RFLP studies of *Nemadactylus macropterus*

The results obtained from the analysis of microsatellite data concur with the previous allozyme and mtDNA RFLP studies (Richardson, 1982; Elliott and Ward, 1994; Grewe *et al.*, 1994), in that there was little suggestion of population divergences within southern Australia. The microsatellite values of F_{ST} and R_{ST} for the Australian populations were low and not significantly greater than zero (-0.001 and -0.00095 respectively, P>0.528), as was the homologous measure obtained from allozymes (G_{ST}=0.0052, P=0.269; Elliott and Ward, 1994; no homologous value was calculated for mtDNA).

The allozyme and mtDNA RFLP studies found slight but significant divergence between New Zealand and Australian populations (Elliott and Ward, 1994; Grewe *et al.*, 1994). No such differences were observed for any of seven microsatellite loci, even with pooling of the Australian samples (P>0.031, Table 8.3). Similarly, Trans-Tasman Sea microsatellite F_{ST} and R_{ST} were small and not significantly greater than zero, both during pairwise comparisons of individual samples (F_{ST}<-0.0007, P>0.584; R_{ST}<0.00943, P>0.099;, Table 8.4), or when pooling Australian samples (F_{ST}=-0.001, P=0.760; R_{ST}=0.00408, P=0.161). Trans-Tasman Sea values of G_{ST} from the allozyme and mtDNA RFLP studies were also small, but larger than the homologous measures obtained for microsatellites and significantly greater than that attributable to sampling error alone ($G_{ST\ allozymes}$=0.0046, P=0.002, Elliott and Ward, 1994; $G_{ST\ mtDNA}$=0.013, P=0.024, Grewe *et al.*, 1994).

Several microsatellite studies of marine species have identified small but significant population differences that were not revealed from the analysis of allozyme or mtDNA data (Bentzen *et al.*, 1996; O'Connell *et al.*, 1998; Shaw *et al.*, 1999a, b). In contrast, this study did not identify stock structuring in *N. macropterus* from southern Australia, and also failed to distinguish Australian and New Zealand populations despite divergence detected by other molecular techniques (Elliott and Ward, 1994; Grewe *et al.*, 1994). It has been suggested that reduced levels of population difference detected by microsatellites relative to allozymes and mtDNA might result from homoplasy in allele sizes at highly polymorphic microsatellite loci (Hauser and Ward, 1998; Shaw *et al.*, 1999a, b). This explanation may be particularly applicable to *N. macropterus*, as the effects of allelic homoplasy will be most pronounced in species with large effective population sizes due to the greater influence of mutation relative to drift (Nauta and Weissing, 1996; Shaw *et al.*, 1999b). Direct sequencing, single strand conformation polymorphism (SSCP), or heteroduplex analysis can detect allelic homoplasy.

Conclusions for *Nemadactylus macropterus*

The absence of genetically detectable stock structuring in *N. macropterus* of southern Australia has been previously ascribed to dispersal (Elliott and Ward, 1994; Grewe *et al.*, 1994). This species possesses an offshore pelagic larval phase of 8-12 months in duration, suggestive of high dispersal capabilities, and adult movements of up to 300 km within a year have been recorded (Annala, 1987, 1993; Smith 1989; Bruce *et al.*, 1996). Molecular phylogenetic analysis of *Nemadactylus* also suggests high dispersal capabilities within this group (Chapter 3). Larval dispersal of *N. macropterus* appears linked with surface circulation patterns (Bruce *et al.*, 1996), and the principal ocean currents operating within the Australian range of this species, the Leeuwin and East Australian Currents, could facilitate movement throughout much of this region.

Alternatively, dispersal may be low in *N. macropterus*, and stock structuring may not have existed long enough for any genetic signal to develop. A recent origin of *N. macropterus* is

inferred from the molecular phylogenetic analysis of *Nemadactylus* (Chapter 3), and the large population sizes of this species may result in slow genetic drift.

It is poignant to note that the levels of gene flow required to homogenise populations genetically are somewhat lower than the levels of migration at which managers would consider stocks distinct (Carvalho and Hauser, 1995). Although the estimates of migration provided by this and the previous genetic studies of *N. macropterus* are high from an evolutionary perspective, in management terms they suggest that recovery of populations after regional over-exploitation will be slow, although there should be little loss of genetic diversity. Otolith microchemistry and larval advection studies of *N. macropterus* suggested three stocks within southeast Australia alone, and as these techniques are less sensitive to the effects of dispersal their findings may be considered better representations of the true stock structure (Thresher *et al.* 1994; Bruce *et al.*, 1996). However, an appreciation of their sensitivity to fluctuations in environmental conditions is required.

Species-level comparisons

The separate status of *N. bergi* is questionable, as the morphological characters used to distinguish this species from *N. macropterus* are inconsistent. A previous mtDNA analysis revealed lineage monophyly for *N. bergi*, suggesting that separate status at some level was warranted (Chapter 3). In contrast, polytypic clades were observed for *N. macropterus* and *A. monodactylus* despite clear morphological difference (Chapter 3). Microsatellite allele frequencies distinguished *N. bergi* and *N. macropterus* at one locus, *Nma 245*, while *A. monodactylus* differed from both *N. macropterus* and *N. bergi* at all seven microsatellite loci (Table 8.3). Consequently, the separate taxonomic status of these species is supported.

Incomplete lineage sorting was offered as an explanation for the presence polytypic clades in *N. macropterus* and *A. monodactylus*, given the recent origins and large population sizes of these species (Chapter 3). Given a similarly recent origin and presently large population size, the absence of polytypic clades for *N. bergi* was explained in terms of a historical reduction

in effective population size, which decreased the number of generations required for complete lineage sorting (Chapter 3; Avise 1986). This hypothesis is supported by comparisons of Hardy-Weinberg expected heterozygosity (H_e) and mutation-drift equilibrium heterozygosity (H_{eq}), which suggest a recent reduction in the effective population size of *N. bergi*, but not in the other taxa (Table 8.5). Founder effect, population bottlenecks, and variance in reproductive success (Hedgecock, 1994) could each have reduced the effective population size of *N. bergi*. A candidate for a population bottleneck is the over-fishing of this species during the 1960's (Cotrina, 1971).

Appendix 8.1. Microsatellite allele frequencies at seven loci in populations of *Nemadactylus macropterus*, *N. bergi*, and *A. monodactylus*.

	N. macropterus				*N. bergi*	*A. monodactylus*
Locus/Allele	Albany	Tasman Is.	Eden	New Zealand		
n	49	55	50	59	51	29
Nma 106						
91	0.021	0.028	-	0.034	-	-
93	0.021	0.028	0.071	0.025	-	0.276
95	0.021	0.028	0.040	-	0.029	-
97	0.064	0.057	0.020	0.034	0.069	0.017
98	-	-	-	0.008	-	-
99	0.032	0.019	0.030	0.051	0.098	-
100	-	0.009	-	-	-	-
101	0.032	0.066	0.061	0.042	0.118	0.483
103	0.021	0.038	0.121	0.059	0.088	-
105	0.043	0.028	0.040	0.059	0.118	0.034
107	0.117	0.057	0.040	0.085	0.039	0.086
109	0.106	0.057	0.061	0.068	0.049	-
111	0.074	0.038	0.040	0.059	0.020	-
112	-	-	-	0.008	-	-
113	0.064	0.038	0.081	0.025	0.069	-
115	0.064	0.075	0.071	0.102	0.020	0.034
117	0.043	0.075	0.010	0.076	0.049	-
119	0.043	0.066	0.040	0.042	0.049	-
121	0.032	0.066	0.030	0.034	0.020	-
123	0.032	0.028	0.010	0.042	0.010	-
125	0.021	0.019	0.010	0.008	0.029	-
127	0.032	0.019	0.030	0.017	0.029	-
129	0.011	0.019	0.040	0.025	0.010	-
131	0.053	-	0.030	0.017	0.010	0.017
133	0.011	0.009	0.010	0.008	0.010	-
135	-	0.019	-	0.008	-	-
137	0.011	-	0.020	-	0.010	0.017
139	-	0.009	-	0.008	0.010	-
141	-	-	-	0.017	0.010	-
143	0.021	0.009	0.010	0.017	0.010	-
145	-	-	0.020	-	0.010	-
147	0.011	0.009	0.010	-	-	-
149	-	0.009	-	-	-	-
151	-	0.028	0.010	0.008	0.010	0.017
153	-	0.009	-	-	-	0.017
157	-	-	0.010	-	-	-
161	-	-	0.010	-	-	-
163	-	-	0.010	-	-	-
175	-	0.009	-	-	-	-
177	-	0.009	-	0.008	-	-
183	-	0.009	-	-	-	-
185	-	0.009	-	-	-	-

Locus/Allele	*N. macropterus* Albany	Tasman Is.	Eden	New Zealand	*N. bergi*	*A. monodactylus*
190	-	-	0.010	-	-	-
195	-	-	-	-	0.010	-
missing	0.041	0.036	0.010	-	-	0.362
Nma 118						
109	-	-	-	-	0.010	-
112	0.020	0.010	0.010	0.025	0.010	-
115	0.020	0.010	0.020	0.042	-	-
118	0.582	0.594	0.650	0.593	0.520	0.793
121	0.214	0.260	0.200	0.237	0.350	0.034
124	0.071	0.073	0.060	0.051	0.080	0.155
127	0.082	0.042	0.050	0.025	0.030	-
130	-	-	0.010	0.008	-	0.017
133	0.010	0.010	-	0.017	-	-
missing	-	0.127	-	-	0.020	-
Nma 187						
177	-	-	-	0.008	-	-
179	0.010	-	-	0.008	0.029	-
181	-	0.009	-	0.008	0.010	0.121
183	0.031	0.027	-	0.008	0.010	-
185	-	0.055	0.040	0.017	0.020	0.017
187	0.063	0.045	0.070	0.025	0.078	-
189	0.042	0.045	0.060	0.076	0.108	0.052
191	0.125	0.055	0.100	0.110	0.098	0.448
193	0.073	0.100	0.110	0.068	0.137	0.121
195	0.146	0.091	0.100	0.110	0.078	0.069
197	0.104	0.136	0.060	0.110	0.088	-
199	0.115	0.109	0.140	0.136	0.088	-
201	0.083	0.064	0.070	0.068	0.088	0.034
203	0.052	0.073	0.030	0.076	0.020	-
205	0.010	0.027	0.060	0.025	-	0.052
207	0.021	0.055	0.040	0.059	0.049	0.034
209	0.031	0.064	0.020	0.017	0.029	-
211	0.042	0.009	0.030	0.017	0.039	0.052
213	0.010	0.018	0.040	0.034	-	-
215	0.010	-	0.030	-	0.010	-
217	0.010	0.009	-	-	-	-
219	0.010	0.009	-	-	-	-
223	0.010	-	-	0.008	0.020	-
227	-	-	-	0.008	-	-
missing	0.020	-	-	-	-	-
Nma 230						
215	-	-	-	0.008	-	-
219	-	0.009	-	-	-	-
221	0.010	-	-	-	-	-

	N. macropterus				*N. bergi*	*A. monodactylus*
Locus/Allele	Albany	Tasman Is.	Eden	New Zealand		
225	0.010	-	-	-	-	-
227	0.021	0.009	-	0.034	-	-
229	-	0.009	0.010	0.017	0.051	-
231	0.021	0.009	0.020	0.068	-	-
233	0.052	0.027	0.040	0.034	0.010	0.167
235	0.073	0.055	0.050	0.085	0.051	-
237	0.052	0.064	0.090	0.102	0.082	0.271
239	0.042	0.082	0.130	0.102	0.071	0.146
241	0.135	0.164	0.080	0.076	0.092	0.042
243	0.115	0.145	0.090	0.085	0.031	0.021
245	0.094	0.091	0.110	0.051	0.051	-
247	0.073	0.127	0.170	0.093	0.061	-
249	0.104	0.045	0.030	0.042	0.041	0.021
251	0.031	0.055	0.070	0.042	0.092	-
253	0.021	0.027	0.030	0.059	0.102	0.063
255	0.063	0.009	0.010	0.042	0.051	0.063
257	0.042	0.027	0.030	0.017	0.041	0.125
259	0.010	0.018	0.010	0.008	0.041	-
261	0.010	0.009	0.010	0.017	0.071	0.021
263	0.010	0.009	0.010	0.017	0.020	-
265	0.010	-	0.010	-	0.031	-
267	-	0.009	-	-	-	0.021
271	-	-	-	-	-	0.021
273	-	-	-	-	0.010	0.021
missing	0.020	-	-	-	0.039	0.172
Nma 245						
217	0.010	-	-	0.008	-	-
219	0.020	0.031	0.010	0.008	0.011	-
221	0.010	0.021	0.040	0.008	0.011	0.135
223	0.051	0.010	0.050	0.085	0.128	0.135
225	0.071	0.062	0.080	0.085	0.085	0.054
227	0.102	0.052	0.070	0.076	0.053	0.027
229	0.092	0.113	0.200	0.161	0.032	-
231	0.163	0.155	0.080	0.127	0.191	0.189
233	0.092	0.082	0.110	0.110	0.170	-
235	0.092	0.144	0.100	0.068	0.085	0.054
237	0.102	0.072	0.080	0.068	0.053	0.027
239	0.051	0.072	0.050	0.102	0.064	-
241	0.031	0.041	0.050	0.042	0.032	0.081
243	0.041	0.031	0.010	-	0.043	0.135
245	0.020	0.021	0.010	-	0.032	0.162
247	-	-	-	0.034	-	-
249	0.020	0.031	0.020	0.008	-	-
251	0.010	0.031	0.010	0.008	-	-
253	-	0.010	-	-	0.011	-
255	-	-	0.020	-	-	-
257	0.010	-	-	-	-	-

	N. macropterus				*N. bergi*	*A. monodactylus*
Locus/Allele	Albany	Tasman Is.	Eden	New Zealand		
259	-	0.021	-	-	-	-
261	0.010	-	0.010	-	-	-
missing	-	0.118	-	-	0.078	0.229

	N. macropterus				*N. bergi*	*A. monodactylus*
Locus/Allele	Albany	Tasman Is.	Eden	New Zealand		
Nma 305						
289	-	0.018	0.011	-	-	-
291	0.010	-	-	0.009	-	0.365
293	-	0.009	0.011	0.043	-	0.019
295	0.082	0.064	0.063	0.034	0.010	-
297	0.102	0.045	0.116	0.026	0.127	0.115
299	0.041	0.027	0.074	0.026	0.098	0.058
301	0.061	0.091	0.063	0.095	0.098	-
303	0.102	0.091	0.158	0.112	0.088	0.077
305	0.041	0.100	0.074	0.112	0.108	-
307	0.061	0.082	0.084	0.129	0.108	-
309	0.092	0.055	0.021	0.078	0.039	-
311	0.071	0.045	0.053	0.069	0.069	0.058
313	0.041	0.036	0.063	0.060	0.108	0.038
315	0.071	0.036	0.042	0.043	0.020	0.038
317	0.031	0.036	0.063	0.043	0.020	-
319	0.031	0.073	0.042	0.017	0.029	0.077
321	0.020	0.055	0.032	0.017	0.029	0.058
323	0.041	0.045	0.011	0.026	0.010	-
325	-	0.009	-	0.017	0.029	-
327	0.020	0.009	0.011	-	0.010	-
329	0.010	0.009	-	-	-	0.038
331	0.020	0.027	-	-	-	0.019
333	-	-	-	-	-	0.019
335	-	0.018	-	0.017	-	-
341	0.020	-	-	-	-	-
343	-	0.018	-	0.009	-	-
345	0.031	-	0.011	-	-	-
347	-	-	-	-	-	0.019
357	-	-	-	0.017	-	-
missing	-	-	0.050	0.017	-	0.103
Nma 311						
291	-	-	-	-	0.010	-
297	0.020	0.037	0.010	0.026	-	-
299	0.010	-	0.010	0.009	-	-
301	-	0.019	-	0.026	-	0.020
303	0.061	0.102	0.070	0.068	0.020	-
305	0.602	0.639	0.640	0.564	0.627	0.020
307	0.163	0.074	0.160	0.145	0.127	0.940
309	0.122	0.074	0.110	0.077	0.186	-
311	-	0.037	-	0.068	0.020	0.020
313	0.020	-	-	-	0.010	-
315	-	-	-	0.009	-	-
328	-	0.019	-	0.009	-	-
missing	-	0.018	-	0.008	-	0.138

CHAPTER 9: General discussion

The use of molecular phylogenetic analysis to examine aspects of cirrhitoid taxonomy was largely successful. With respect to the Cheilodactylidae, the results from this study were in general agreement with the taxonomic revisions suggested by Lamb (1990) based on the analysis of morphological features. Distinct placement of the two South African *Cheilodactylus* was supported, but at the familial rather than generic level (Chapter 5). Remaining members of *Cheilodactylus* are sufficiently divergent from one another and *Chirodactylus* for separate generic allocation, although it appears that the composition of *Goniisitus* and *Morwong* should differ slightly from that suggested by Lamb (1990) (Chapters 4 and 5). In contrast, this study identified taxonomic problems not previously suggested by morphology. These include the synonymy of *Nemadactylus* and *Acantholatris* (Chapter 3), and the doubtful monophyly of *Chirodactylus* (Chapter 5). The ability to statistically compare alternative classifications by the enforcement of topological constraints during phylogenetic analysis was highly useful.

The advantages of molecular techniques for systematic studies of morphologically similar taxa were clearly evident during aspects of this study. Cryptic speciation was identified in *C. (G.) vittatus* based on relative levels of molecular divergence, and given the molecular data there can be confidence in the use of subtle external features to distinguish Northern and Southern Hemisphere groups as species (Chapter 4). Molecular phylogenetic analysis of *Nemadactylus* and *Acantholatris* suggested that formal recognition of the king tarakihi, *Nemadactylus* sp., requires morphological and perhaps molecular comparison with *A. gayi* (Chapter 3). Microsatellites clearly distinguished *N. bergi*, *N. macropterus*, and *A. monodactylus* (Chapter 8). Conflicting results for *N. macropterus* and *A. monodactylus* from the analysis of mtDNA demonstrate the need for thorough interpretation of molecular data (Chapter 3).

The advantages of molecular phylogenetic analyses for investigations of historical biogeography were readily apparent during this study. Reconstructed phylogeographic

relationships provided information regarding the frequency, direction, and origins of dispersal events within *Nemadactylus*, *Acantholatris*, and *Aplodactylus*, and also the frequency of transequatorial divergences within *Goniisitus* (Chapters 3, 4, and 6). In the case of *Nemadactylus* and *Acantholatris*, at least two dispersal events from the region of Australia and New Zealand were identified, rather than a minimum of one suggested by distributions alone (Chapter 3). Similarly, three transequatorial divergences were identified for Goniistius, rather than a minimum of two (Chapter 4).

The presence of clock-like sequence evolution and the conservative application of rate calibrations also proved highly useful for the reconstruction of cirrhitoid historical biogeography. Levels of molecular variation indicated that the three transequatorial divergences within Goniistius occurred during two separate periods (Chapter 4). Similarly, different ages were inferred for the divergences of geminate cirrhitoids with similar east-west allopatric distributions across southern Australia, indicating separate biogeographic histories (Chapter 7). Absolute estimates of divergence time for members of the Cheilodactylidae and Aplodactylidae appreciably postdated periods of continental fragmentation, confidently rejecting this mechanism of vicariant isolation as an explanation for the observed distributions of these taxa (Chapters 4, 5 and 6).

Pleistocene glaciations have been widely promoted as causes for disjunct distribution patterns (Knox, 1980; Edgar, 1986; Hutchins, 1987; Lindberg, 1991). However, there was little evidence of divergence during this period for the antitropically distributed *Goniistius* (Chapter 4), or for those cirrhitoids with east-west allopatric distributions across southern Australia (Chapter 7). While Pleistocene glaciations have undoubtedly influenced the radiation and present distribution of taxa, they may be too readily applied as explanations in many instances, perhaps somewhat encouraged by the comparative paucity of climatic reconstructions for more ancient periods.

This study revealed synergistic benefit from the combined application of molecular systematic and population genetic analyses (Chapters 3 and 8). The recent origin of *N. macropterus* identified from molecular phylogenetic analysis suggested that the absence of genetically detectable stock structuring in the waters of southern Australia could be because there has been insufficient time for any genetic signal of structure to develop. Species ages may be estimated from DNA sequence divergence, and comprise useful information for population genetic studies in which stock structuring is not observed, yet such information is rarely collected. Analysis of microsatellites supported the suggestion that stochastic lineage sorting and differences in historical effective population sizes were responsible for the distribution of polytypic mtDNA clades among *N. macropterus*, *N. bergi*, and *A. monodactylus*, rather than interbreeding.

This study was not successful in achieving some of its goals, and there are several directions for future research. Relationships among *Nemadactylus* and *Acantholatris* were not completely resolved from the mtDNA sequences apparently due to incomplete lineage sorting (Chapter 3). Phylogenetic analysis based on microsatellite allele frequencies may resolve these relationships (Takezaki and Nei, 1996), and reveal the pathways of dispersal throughout the Southern Ocean. Higher-level relationships among the Cheilodactylidae also were poorly supported (Chapter 5). This may have been due to a rapid burst of radiation, obscuring phylogenetic signal. The analysis of large quantities of low variability characters may resolve higher-level cheilodactylid relationships (Avise et al., 1994), and this would assist the generic allocation of three species that could not be classified during this study (Chapter 5). The absence of *N. macropterus* stock structuring as suggested by microsatellites was unexpected given the results from previous genetic analyses (Chapter 8). The suggestion that microsatellite allelic homoplasy hindered stock divergence in this species requires investigation, to confirm the presence of homoplasy and quantify its consequences.

CHAPTER 10: References

Adams CG, Lee DE, Rosen BR. 1990. Conflicting isotopic and biotic evidence for tropical sea surface temperatures during the Tertiary. *Palaeogeography, Palaeoclimatology, Palaeoecology* **77**: 289-313.

Agnot M. 1951. Observations sur la faune marine et la pêche aux îles Saint-Paul et Amsterdam. *Mémoirs de la Institute Science, Madagascar* **6**: 1-51.

Allen GR, Heemstra PC. 1976. *Cheilodactylus rubrolabiatus*, a new species of morwong (Pisces: Cheilodactylidae) from Western Australia, with a key to the cheilodactylid fishes of Australia. *Records of the Western Australia Museum* **4**: 311-325.

Andrew TG, Hecht T. 1990. Feeding biology of *Acantholatris monodactylus* (Pisces: Cheilodactylidae) at Tristan da Cunha and Gough Island, South Atlantic. *South African Journal of Antarctic Research* **22**: 41-49.

Andrew TG, Hecht T, Heemstra PC, Lutjeharms JRE. 1995. Fishes of the Tristan da Cunha group and Gough Island, South Atlantic Ocean. *Ichthyological Bulletin of the J.L.B. Smith Institute of Ichthyology* **63**: 1-43.

Annala JH. 1987. The biology and fishery of tarakihi, *Nemadactylus macropterus*, in New Zealand waters. *New Zealand Fisheries Research Division Occasional Publication* **51**: 1-12.

Annala JH. 1993. Movements of tarakihi tagged at Kaikoura. *Seafood New Zealand* **1**: 48-49.

Avise JC. 1986. Mitochondrial DNA and the evolutionary genetics of higher animals. *Philosophical Transactions of the Royal Society, London* **312**: 325-342.

Avise JC. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. *Evolution* **43**: 1192-1208.

Avise JC. 1992. Molecular population structure and biogeographic history of a regional fauna: a case history with lessons for conservation. *Oikos* **63**: 62-76.

Avise JC. 1994. *Molecular Markers, Natural History and Evolution.* Chapman and Hall, New York.

Avise JC, Nelson WS, Sibley CG. 1994. Why one-kilobase sequences from mitochondrial DNA fail to solve the hoatzin phylogenetic enigma. *Molecular Phylogenetics and Evolution* **3**: 175-184.

Benavides AG, Cancino JM, Ojeda FP. 1994. Ontogenetic changes in gut dimensions and macroalgal digestibility in the marine herbivorous fish, *Aplodactylus punctatus*. *Functional Ecology* **8**: 46-51.

Bentzen P, Taggart CT, Ruzzante DE, Cook D. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 2706-2721.

Bermingham E, Martin AP. 1998. Comparative mtDNA phylogeography of neotropical freshwater fishes: testing shared history to infer the evolutionary landscape of lower Central America. *Molecular Ecology* **7**: 499-517.

Bermingham E, McCafferty SS, Martin AP. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In: Kocher TD, Stepien CA. (Eds). *Molecular Systematics of Fishes*. pp. 113-128. Academic Press, San Diego.

Bowen BW, Grant WS. 1997. Phylogeography of the sardines (*Sardinops* spp.): assessing biogeographical models and population histories in temperate upwelling zones. *Evolution* **51**: 1601-1610.

Brasher DJ, Ovenden JR, White RWG. 1992. Mitochondrial DNA variation and phylogenetic relationships of *Jasus* spp. (Decapoda: Palinuridae). *Journal of Zoology, London* **227**: 1-16.

Briggs JC. 1974. *Marine Zoogeography*. McGraw-Hill, New York.

Briggs JC. 1987a. Antitropical distribution and evolution in the Indo-West Pacific Ocean. *Systematic Zoology* **36**: 237-247.

Briggs JC. 1987b. Antitropicality and vicariance. *Systematic Zoology* **36**: 206-207.

Bruce BD, Sutton CA, Thresher RE. 1996. An ichthyoplankton based analysis of the spawning distribution and stock structure of temperate Australian finfish. *Final Report to Fisheries Research and Development Corporation* #92/019. CSIRO Division of Fisheries, Hobart.

Cantatore P, Roberti M, Pesole G, Ludovico A, Milella F, Gadaleta MN, Saccone C. 1994. Evolutionary analysis of cytochrome *b* sequences in some perciformes: evidence for a slower rate of evolution than in mammals. *Journal of Molecular Evolution* **39**: 589-597.

Cappo M. 1995. The population biology of the temperate reef fish *Cheilodactylus nigripes* in an artificial reef environment. *Transactions of the Royal Society of South Australia* **119**: 113-122.

Cavalli-Sforza LL, Edwards AWF. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* **32**: 550-570.

Chenoweth SF, Hughes JM, Keenan CP, Lavery S. 1998. When oceans meet: a teleost shows secondary intergradation at an Indian-Pacific interface. *Proceedings of the Royal Society, London* B**265**: 415-420.

CLIMAP project members 1976. The surface of the Ice-age Earth. *Science* **191**: 1131-1137.

Cole RG, Creese RG, Grave RV, Irving P, Jackson BR. 1992. Abundance and patterns of subtidal benthic invertebrates and fishes at the Kermadec Islands. *Marine Ecology Progress Series* **82**: 207-218.

Collette BB. 1974. The garfishes (Hemiramphidae) of Australia and New Zealand. *Records of the Australian Museum* **29**: 11-105.

Collette BB, Parin NV. 1991. Shallow-water fishes of Walters Shoals, Madagascar Ridge. *Bulletin of Marine Science* **48**: 1-22.

Cotrina CP. 1971. La pesca de la castañeta (*Cheilodactylus bergi*) en el área de Mar del Plata. Análisis de las estadísticas de captura y muestreos (1967-1970). *Instituto Biologia Marina, Mar del Plata, Contribution* **163**: 1-27.

Cox CB. 1990. New geological theories and old biogeographical problems. *Journal of Biogeography* **17**: 117-130.

Crame JA. 1993. Bipolar molluscs and their evolutionary implications. *Journal of Biogeography* **20**: 145-161.

Dartnall AJ. 1974. Littoral biogeography. In: Williams WD. (Ed). *Biogeography and Ecology in Tasmania*. pp 171-194. W. Junk, The Hague.

Darwin C. 1859. *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. J. Murray, London.

DiRienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB. (1994). Mutational processes of simple sequence repeat loci in human populations. *Proceedings of the National Academy of Sciences USA* **91**: 3166-3170.

Duhamel G. 1989. Ichthyofaune des les Saint-Paul et Amsterdam (océan Indien sud). *Mésogée. Bulletin of the Museum d'Historie Naturelle, Marseille* **49**: 21-47.

Duke NC, Benzie JAH, Goodall JA, Ballment ER. 1998. Genetic structure and evolution of species in the mangrove genus *Avicennia* (Avicenniaceae) in the Indo-west Pacific. *Evolution* **52**: 1612-1626.

Edgar GJ 1986. Biogeographical processes in the Southern Hemisphere marine environment. In: Westermeier R. (Ed). *Actas del Segundo Congreso sobre Algas Marinas Chilenas*. pp 29-46. Universidad Austral de Chile, Valdivia.

Ekman S. 1953. *Zoogeography of the sea*. Sidgwick and Jackson, London.

Elliott NG, Ward RD. 1994. Enzyme variation in jackass morwong, *Nemadactylus macropterus* (Schneider, 1801) (Teleostei: Cheilodactylidae), from Australian and New Zealand waters. *Australian Journal of Marine and Freshwater Research* **45**: 51-67.

Eschmeyer WN, Hureau JC. 1971. *Sebastes mouchezi*, a senior synonym of *Helicolenus tristanensis*, with comments on *Sebastes capensis* and zoogeographical considerations. *Copeia* **1971**: 576-579.

Farris JS. 1989. The retention index and the rescaled consistency index. *Cladistics* **5**: 417-419.

Farris JS, Källersjö M, Kluge AG, Bult C. 1995. Testing significance of incongruence. *Cladistics* **10**: 315-319.

Fell HB. 1962. West-Wind-Drift dispersal of echinoderms in the Southern Hemisphere. *Nature* **193**: 759-761.

Felsenstein J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution* **17**: 368-376.

Felsenstein J. 1985a. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.

Felsenstein J. 1985b. Confidence limits on phylogenies with a molecular clock. *Systematic Zoology* **34**: 152-161.

Felsenstein J. 1993. *PHYLIP: Phylogeny Inference Package*. Version 3.5c. Unpublished computer software. University of Washington, Seattle.

Francis MP. 1981. Age and growth of moki, *Latridopsis ciliaris* (Teleostei: Latridae). *New Zealand Journal of Marine and Freshwater Research* **15**: 47-49.

Francis MP. 1993. Checklist of the coastal fishes of Lord Howe, Norfolk, and Kermadec Islands, Southwest Pacific Ocean. *Pacific Science* **47**: 136-170.

Francis MP. 1996. *Coastal Fishes of New Zealand: An Identification Guide*. Reed Publishing, Auckland.

Francis MP, Grace RV, Paulin CD. 1987. Coastal fishes of the Kermadec Islands. *New Zealand Journal of Marine and Freshwater Research* **21**: 1-13.

García De León FL, Chikhi L, Bonhomme F. 1997. Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus, 1758). *Molecular Ecology* **6**: 51-62.

Gauldie RW, Johnston AJ. 1980. The geographical distribution of phosphoglucomutase and glucose phosphate isomerase alleles of some New Zealand fishes. *Comparative Physiology and Biochemistry* **66B**: 171-183.

George RW. 1997. Tectonic plate movements and the evolution of *Jasus* and *Panulirus* spiny lobsters (Palinuridae). *Marine and Freshwater Research* **48**: 1121-1130.

Gill TH. 1862. Synopsis of the family of cirrhitoids. *Proceedings of the Academy of Natural Sciences, Philadelphia* **14**: 102-124.

Gillespie JH. 1986. Variability of evolutionary rates of DNA. *Genetics* **113**: 1077-1091.

Gomon MF, Glover JCM, Kuiter RH. 1994. *Fishes of Australia's south coast.* State Press, Adelaide.

Gomon MF, Johnson JW. 1999. A new fringed stargazer (Uranoscopidae: *Ichthyscopus*) with descriptions of the other Australian species. *Memoirs of the Queensland Museum* **43**: 597-619.

Gon O, Heemstra PC. 1987. *Mendosoma lineatum* Guichenot 1848, first record in the Atlantic Ocean, with a re-evaluation of the taxonomic status of other species of the genus *Mendosoma* (Pisces, Latridae). *Cybium* **11**: 183-193.

Goodbred CO, Graves JE. 1996. Genetic relationships among geographically isolated populations of bluefish (*Pomatomus saltatrix*). *Marine and Freshwater Research* **47**: 347-355.

Goodman SJ. 1997. RST CALC: a collection of computer programs for calculating unbiased estimates of genetic differentiation and geneflow from microsatellite data and determining their significance. *Molecular Ecology* **6**: 881-885.

Goudet J. 1995. F-STAT (1.2), a program for IBM compatible PCs to calculate Weir and Cockerham's (1984) estimators of *F*-statistics. *Journal of Heredity* **139**: 463-471.

Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity* **89**, 415-426.

Graves JE. 1998. Molecular insights into the population structures of cosmopolitan marine fishes. *Journal of Heredity* **89**: 427-437.

Greenwood PH. 1995. A revised familial classification for certain cirrhitoid genera (Teleostei, Percoidei, Cirrhitoidea), with comments on the group's monophyly and taxonomic ranking. *Bulletin of the Natural History Museum, London* **61**: 1-10.

Grewe PM, Smolenski AJ, Ward RD. 1994. Mitochondrial DNA diversity in jackass morwong (*Nemadactylus macropterus*, Teleostei) from Australian and New Zealand waters. *Canadian Journal of Fisheries and Aquatic Sciences* **51**: 1101-1109.

Gyllensten U. 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *Journal of Fish Biology* **26**: 691-699.

Hauser L, Ward RD. 1998. Population identification in pelagic fish: the limits of molecular markers. In: Carvalho GR. (Ed). *Advances in Molecular Ecology*. pp. 191-224. IOS Press, Amsterdam.

Hedgecock D. 1994. Does variance in reproductive success limit effective population sizes of marine organisms? In: Beaumont AR. (Ed). *Genetics and the Evolution of Aquatic Organisms*. pp. 122-134. Chapman and Hall, London.

Hendy MD, Penny D. 1982. Branch and bound algorithms to determine minimal evolution trees. *Mathematical Biosciences* **59**: 277-290.

Hillis DM, Huelsenbeck JP. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* **83**: 189-195.

Hillis DM, Larson A, Davis SK, Kimmer EA. 1990. Nucleic acids III: Sequencing. In: Hillis DM, Moritz C. (Eds). *Molecular Systematics*. pp. 318-370. Sinauer, Sunderland.

Howard WR, Prell WL. 1992. Late Quaternary surface circulation of the southern Indian Ocean and its relationship to orbital variations. *Paleoceanography* **7**: 79-117.

Hubbs CL. 1952. Antitropical distribution of fishes and other organisms. Symposium on the problems of bipolarity and of pantemperate faunas. *Proceedings of the Seventh Pacific Science Congress (Pacific Science Association)* **3**: 324-329.

Hutchins JB. 1987. Description of a new plesiopid fish from south-western Australia, with a discussion of the zoogeography of *Paraplesiops*. *Records of the Western Australian Museum* **13**: 231-240.

Hutchins B, Swainston R. 1986. *Sea Fishes of Southern Australia*. Swainston Publishing, Perth.

Jarne P, Lagoda PJL. 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution* **11**: 424-429.

Jordan AR. 1997. Demersal trawl surveys of the continental shelf of southern and eastern Tasmania 1993-1995. *Technical Report, Marine Research Division, Department of Primary Industry and Fisheries, Tasmania* **50**: 1-129.

Kensley B. 1981. On the zoogeography of Southern African decapod crustacea, with a distributional checklist. *Smithsonian Contributions to Zoology* **338**: 1-64.

Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16**: 111-120.

Kimura M, Crow JF. 1964. The number of alleles that can be maintained in a finite population. *Genetics* **49**: 725-738.

Kishino H, Hasegawa M. 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *Journal of Molecular Evolution* **29**: 170-179.

Kluge AG, Farris JS. 1969. Quantitative phyletics and the evolution of anurans. *Systematic Zoology* **18**: 1-32.

Knowlton N. 1993. Sibling species in the sea. *Annual Reviews in Ecology and Systematics* **24**: 189-216.

Knowlton N, Weigt LA. 1998. New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society of London B* **265**: 2257-2263.

Knox GA. 1980. Plate tectonics and the evolution of intertidal and shallow-water benthic biotic distribution patterns of the southwest Pacific. *Palaeogeography, Palaeoclimatology, Palaeoecology* **31**: 267-297.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson, AC. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences USA* **86**: 6196-6200.

Kotthaus A. 1976. Fische des Indischen Ozeans. Ergebnisse der ichthyologischen Untersuchungen während der Expedition des Forschungsschiffes "Meteor" in den indischen Ozean, Oktober 1964 bis Mai 1965. A. Systematischer Teil, XVII. Percomorphi (7). *Meteor Forschungsergebnisse. Reihe D Bioogie.* **23**: 45-61.

Koufopanou K, Reid DG, Ridgway SA, Thomas RH. 1999. A molecular phylogeny of the patellid limpets (Gastropoda: Patellidae) and its implications for the origins of their antitropical distribution. *Molecular Phylogenetics and Evolution* **11**: 138-156.

Lamb TD. 1990. *The Taxonomy and Phylogeny of Cheilodactylid and Latrid Fishes.* BSc(Hons) Thesis. University of Tasmania, Hobart.

Last PR, Scott EOG, Talbot FH. 1983. *Fishes of Tasmania.* Tasmanian Fisheries Development Authority, Hobart.

Lavenberg RJ, Yañez ALA. 1972. A new species of *Cirrhitus* from Easter Island (Pisces, Cirrhitidae). *Gayana Zoologica* **21**: 3-11.

Lawver LA, Gahagan LM, Coffin MF. 1992. The development of paleoseaways around Antarctica. *Antarctic Research Series.* **56**: 7-30.

Lindberg DR. 1991. Marine biotic interchange between northern and southern hemispheres. *Paleobiology* **17**: 308-324.

Lockhart PJ, Steel MA, Hendy MD, Penny D. 1994. Recovering evolutionary trees under a more realistic model of sequence evolution. *Molecular Biology and Evolution* **11**: 605-612.

Lowry MB, Suthers IM. 1998. Home range, activity and distribution patterns of a temperate rocky-reef fish, *Cheilodactylus fuscus*. *Marine Biology* **132**: 569-578.

Lubbock R. 1978. A new hawkfish of the genus *Amblycirrhitus* Gill 1862, from Ascension Island/South Atlantic (Pisces: Perciformes: Percoidei: Cirrhitidae). *Senckenbergiana Biologica* **58**: 261-265.

Lutjeharms JR, Heydorn AE. 1981. The rock lobster *Jasus tristani* on Vema Seamount: drifting buoys suggest a possible recruiting mechanism. *Deep Sea Research* **28**: 631-636.

MacMillan WO, Palumbi SR. 1995. Concordant evolutionary patterns among Indo-West Pacific butterfly fishes. *Proceedings of the Royal Society of London B* **260**: 229-236.

Martin AP, Naylor GJP, Palumbi SR. 1992. Rates of mitochondrial DNA evolution in sharks are slow compared with mammals. *Nature* **357**: 153-155.

Martin AP, Palumbi SR. 1993. Body size, metabolic rate, generation time, and the molecular clock. *Proceedings of the National Academy of Sciences USA* **90**: 4087-4091.

Mantel N. 1967. The detection of disease clustering and a generalised regression approach. *Cancer Research* **27**: 209-220.

McConnell SK, O'Reilly P, Hamilton L, Wright JM, Bentzen P. (1995). Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 1863-1872.

McCormick MI. 1998. Ontogeny of diet shifts by a microcarnivorous fish, *Cheilodactylus spectabilis*: relationship between feeding mechanics, microhabitat selection and growth. *Marine Biology* **132**: 9-20.

McDougall I, Ollier CD. 1982. Potassium-argon ages for Tristan da Cunha, South Atlantic. *Geology Magazine* **119**: 87-93.

Meléndez RC. 1990. Chironemid fishes from Juan Fernandez Archipelago and Desventuradas Islands, Chile. (Perciformes: Chironemidae). *Revista de Biologia Marina* **25**: 83-92.

Meléndez RC, Villalba SC. 1992. New records and biological data for the icthyofauna from Juan Fernández archipelago, Chile. *Estudios Oceanographica* **11**: 3-29.

Meyer A. 1994. DNA technology and the phylogeny of fish. In: Beaumont AR. (Ed). *Genetics and the Evolution of Aquatic Organisms*. pp. 219-249. Chapman and Hall, London.

Miller JA. 1964. Appendix II. Age determinations made on samples of basalt from the Tristan da Cunha group and other parts of the mid-Atlantic ridge. In: Baker PE, Gass IG, Harris PG, Le Maitre RW. (Eds). *The Vulcanological Report of the Royal Society Expedition to Tristan da Cunha, 1962*. pp. 565-569. Royal Society of London.

Nauta MJ, Weissing FJ. 1996. Constraints on allele size at microsatellite loci: implications for genetic differentiation. *Genetics* **143**: 1021-1032.

Nei M. 1987. *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Neigel JE, Avise JC. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. In Nevo E, Karlin S. (Eds). *Evolutionary Processes and Theory*. pp. 515-534. Academic Press, New York.

Nelson GJ. 1985. A decade of challenge: the future of biogeography. *Earth Science History* **4**: 187-196.

Newman WA. 1979. On the biogeography of balanomorph barnacles of the Southern Ocean including new balanid taxa; a subfamily, two genera and three species. In: *Proceedings of the International Symposium on Marine Biogeography and Evolution in the Southern Hemisphere*. pp. 279-306. New Zealand Department of Scientific and Industrial Research.

Norman J. 1937. Coast fishes part II. The Patagonia region. *Discovery Reports* **16**: 1-150.

Nurthen RK, Cameron R, Briscoe DA. 1992. Population genetics of tailor, *Pomatomus saltatrix* (Linnaeus) (Pisces: Pomatomidae), in Australia. *Australian Journal of Marine and Freshwater Research*. **43**, 1481-1486.

O'Connell M, Dillon MC, Wright JM, Bentzen P, Merkouris S, Seeb J. 1998. Genetic structuring among Alaskan Pacific herring (*Clupea pallasi*) populations identified using microsatellite variability. *Journal of Fish Biology* **53**: 150-163.

O'Connell M, Wright JM. 1997. Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries* 7: 331-363.

Ohta T, Kimura M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research* **22**: 201-204.

Ovenden JR, Booth JD, Smolenski AJ. 1997. Mitochondrial DNA phylogeny of red and green rock lobsters (genus *Jasus*). *Marine and Freshwater Research* **48**: 1131-1136.

Pääbo S. 1990. Amplifying ancient DNA. In: Innes MA, Gelfand DH, Sninski JJ, White TJ. (Eds). *PCR Protocols: A guide to Methods and Applications*. pp. 159-166. Academic Press, San Diego.

Palumbi SR, Martin AP, Romano S, McMillan WO, Stice L, Grabowski G. 1991. *The Simple Fool's Guide to PCR*. Version 2.0. Unpublished manuscript. University of Hawaii.

Penrith MJ. 1967. The fishes of Tristan da Cunha, Gough Island, and the Vema Seamount. *Annals of the South African Museum* **48**: 523-548.

Piry S, Luikart G, Cornuet J-M. 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**: 502-503.

Pollock DE. 1990. Palaeoceanography and speciation in the spiny lobster genus *Jasus*. *Bulletin of Marine Science* **46**: 387-405.

Pollock DE. 1992. Palaeoceanography and speciation in the spiny lobster genus *Panulirus* in the Indo-Pacific. *Bulletin of Marine Science* **51**: 135-146.

Pollock DE. 1993. Speciation in spiny lobsters - clues to climatically-induced changes in ocean circulation patterns. *Bulletin of Marine Science* **53**: 937-944.

Prell WL, Hutson WH, Williams DF, Bé AWH, Geitzenauer K, Molfino B. 1980. Surface circulation of the Indian Ocean during the last glacial maximum, approximately 18 000 yr B.P. *Quaternary Research* **14**: 309-336.

Quilty PG. 1994. The background: 144 million years of Australian palaeoclimate and palaeogeography. In: Hill RS. (Ed). *History of the Australian Vegetation: Cretaceous to Recent*. pp 14-43. Cambridge University Press, Cambridge.

Rand DM. 1994. Thermal habit, metabolic rate and the evolution of mitochondrial DNA. *Trends in Ecology and Evolution* **9**: 125-131.

Randall JE. 1963. Review of the hawkfishes (family Cirrhitidae). *Proceedings of the U.S. National Museum* **114**(no. 3472): 389-451.

Randall JE. 1981. Examples of antitropical and antiequatorial distribution of Indo-West-Pacific fishes. *Pacific Science* **35**: 197-209.

Randall JE. 1983. A review of the fishes of the subgenus *Goniistius*, genus *Cheilodactylus*, with description of a new species from Easter Island and Rapa. *Occasional Papers of the B.P. Bishop Museum* **25**: 1-24

Randall JE. 1997. The hawkfish *Cirrhitichthys serratus* Randall, a junior synonym of *C. falco* Randall. *Micronesica* **30**: 199-203.

Randall JE, Heemstra PC. 1978. Reclassification of the Japanese cirrhitid fishes *Serranocirrhitus latus* and *Isobuna japonica* to the Anthiinae. *Japanese Journal of Ichthyology* **25**: 165-172.

Raymond M, Rousset F. 1995. GENEPOP (Version 1.2) - Population genetic software for exact tests and ecumenicism. *Journal of Heredity* **86**: 248-249.

Rehder HA. 1980. The marine mollusks of Easter Island (Isla de Pascua) and Sala y Gómez. *Smithsonian Contributions to Zoology* **289**: 1-167.

Regan CT. 1911. On the cirrhitoid percoids. *Annals and Magazine of Natural History* (8)**7**: 259-262.

Rice WR. 1989. Analysing tables of statistical tests. *Evolution* **43**: 223-225.

Richardson BJ. 1982. Geographical distribution of electrophoretically detected protein variation in Australian commercial fishes. II.* Jackass morwong, *Cheilodactylus macropterus* Bloch and Schneider. *Australian Journal of Marine and Freshwater Research* **33**: 927-931.

Rico C, Ibrahim KM, Hewitt GM. 1997. Stock composition in North Atlantic populations of whiting using microsatellite markers. *Journal of Fish Biology* **51**: 462-475.

Roberts CD. 1993. New fishes from the Kermadec Ridge. *Seafood New Zealand* **4**: 102-103.

Rotondo GM, Springer VG, Scott GAJ, Schlander SO. 1981. Plate movement and island integration - a possible mechanism in the formation of endemic biotas, with special reference to the Hawaiian Islands. *Systematic Zoology* **30**: 12-21.

Rozas J, Rozas R. 1997. DnaSP version 2.0: a novel software package for extensive molecular population genetic analysis. *Computer Applications for Biosciences* **13**: 307-311.

Russell BC. 2000. Review of the southern temperate fish family Aplodactylidae (Pisces: Perciformes). *Journal of Natural History* in press.

Saitou N, Nei M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**: 406-425.

Sambrook J, Fritsch EP, Maniatis T. 1989. *Molecular cloning. A laboratory manual*. Second edition. Cold Spring Harbour Laboratory Press, New York.

Sano M, Moyer JT. 1985. Bathymetric distribution and feeding habits of two sympatric cheilodactylid fishes at Miyake-jima, Japan. *Japanese Journal of Ichthyology* **32**: 239-247.

Schneider CJ, Cunningham M, Moritz C. 1998. Comparative phylogeography and the history of endemic vertebrates in the wet tropics rainforests of Australia. *Molecular Ecology* **7**: 487-498.

Shaw PW, Pierce GJ, Boyle PR. 1999a. Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. *Molecular Ecology* **8**: 407-417.

Shaw PW, Turan C, Wright JM, O'Connell M, Carvalho GR. 1999b. Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analyses. *Heredity* **83**: 490-499.

Shedlock AM, Haygood MG, Pietsch TW, Bentzen P. 1997. Enhanced DNA extraction and PCR amplification of mitochondrial genes from formalin-fixed museum specimens. *BioTechniques* **22**: 394-400.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457-462.

Smith AB. 1994. Rooting molecular trees: problems and strategies. *Biological Journal of the Linnean Society* **51**: 279-292.

Smith DC (1989). The Fisheries Biology of Jackass Morwong (*Nemadactylus macropterus* Bloch and Schneider) in South-eastern Australian Waters. PhD thesis. University of New South Wales, Sydney.

Smith MM. 1980. A review of the South African cheilodactylid fishes (Pisces: Perciformes), with a description of two new species. *Ichthyological Bulletin of the J.L.B. Smith Institute of Ichthyology* **42**: 1-14.

Smith PJ, Roberts CD, McVeagh SM, Benson PG. 1996. Genetic evidence for two species of tarakihi (Teleostei: Cheilodactylidae: *Nemadactylus*) in New Zealand waters. *New Zealand Journal of Marine and Freshwater Research* **30**: 209-220.

Springer VG. 1982. Pacific Plate biogeography, with special reference to shorefishes. *Smithsonian Contributions to Zoology* **367**: 1-182.

Stepien CA. 1990. Population structure, diets and biogeographic relationships of a rocky intertidal fish assemblage in central Chile: high levels of herbivory in a temperate system, *Bulletin of Marine Science* **47**: 598-612.

Stepien CA, Rosenblatt RH. 1996. Genetic divergence in antitropical pelagic marine fishes (*Trachurus*, *Merluccius*, and *Scomber*) between North and South America. *Copeia* **1996**: 586-598.

Stuessy TF, Foland KA, Sutter JF, Sanders RW, Silva O M. 1984. Botanical and geological significance of potassium-argon dates from the Juan Fernández islands. *Science* **225**: 49-50.

Swofford DL. 1993. *PAUP: Phylogenetic Analysis Using Parsimony.* Version 3.1. Unpublished computer software. Illinios Natural History Survey, Illinios.

Swofford DL. 1999. *PAUP**. *Phylogenetic Analysis Using Parsimony (*and Other Methods).* Version 4. Sinauer Associates, Sunderland.

Taberlet P, Fumagalli L, Wust-Saucy A, Cosson J. 1998. Comparative phylogeography and postglacial colonisation routes in Europe. *Molecular Ecology* **7**: 453-464.

Takezaki N, Nei M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics* **144**: 389-399.

Takezaki N, Rzhetsky A, Nei M. 1995. Phylogenetic test of the molecular clock and linearized trees. *Molecular Biology and Evolution* **12**: 823-833.

Templeton AR. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and the apes. *Evolution* **37**: 221-244.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. *Nuceic Acids Research* **22**: 4673-4680.

Thresher RE, Proctor CH, Gunn JS, Harrowfield IR. 1994. An evaluation of electron-probe microanalysis of otoliths for stock delineation and identification of nursery areas in a southern temperate groundfish, *Nemadactylus macropterus* (Cheilodactylidae). *Fishery Bulletin* **92**: 817-840.

Tringali MD, Bert TM, Seyoum S, Bermingham E, Bartolacci D. 1999. Molecular phylogenetics and ecological diversification of the transisthmian fish genus *Centropomus* (Perciformes: Centropomidae). *Molecular Phylogenetics and Evolution* **13**: 193-207.

Valentine JW. 1984. Neogene marine climate trends: implications for biogeography and evolution of shallow-sea biota. *Geology* **12**: 647-650.

Vawter AT, Rosenblatt R, Gorman GC. 1980. Genetic divergence among fishes of the eastern Pacific and the Caribbean: support for the molecular clock. *Evolution* **34**: 705-711.

Waters JM, Burridge CP. 1999. Extreme intraspecific mitochondrial DNA sequence divergence in *Galaxias maculatus* (Osteichthys: Galaxiidae), one of the world's most widespread freshwater fish. *Molecular Phylogenetics and Evolution* **11**: 1-12.

Weber JL. 1990. Informativeness of human (dC-dA)n.(dG-dT)n polymorphisms. *Genomics* **7**: 524-530.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

Wheeler WC. 1990. Nucleic acid sequence phylogeny and random outgroups. *Cladistics* **6**: 363-367.

White BN. 1986. The isthmian link, antitropicality and American biogeography: distributional history of the Atherinopsinae (Pisces: Atherinidae). *Systematic Zoology* **35**: 176-194.

White BN. 1989. Antitropicality and vicariance: a reply to Briggs. *Systematic Zoology* **38**: 77-79.

Whitley GP. 1957. Ichthyological illustrations. *Proceedings of the Royal Zoological Society of New South Wales* **1955-1956**: 56-71.

Wilson RR Jr, Kaufmann RS. 1987. Seamount biota and biogeography. In: Keating BH, Fryer P, Batiza R, Boehlert GW. (Eds). *Seamounts, Islands, and Atolls*. pp. 355-377. American Geophysical Union Geophysical Monograph No. 43.

Wöhler OC, Sánchez, F. 1994. Feeding ecology of castañeta (*Cheilodactylus bergi*; Pisces: Cheilodactylidae) in the south-western Atlantic (34-47 °S). *Australian Journal of Marine and Freshwater Research* **45**: 507-520.

Wright S. 1943. Isolation by distance. *Genetics* **28**: 114-138.

Wright S. 1951. The genetic structure of populations. *Annals of Eugenics* **15**: 323-354.