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A Molecular Phylogenetic Study of the *Eugongylus* Group of Skinks

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Abstract

This project is a molecular phylogenetic investigation of the *Eugongylus* group of skinks. This group is of particular interest from a reproductive biology viewpoint because they are the best understood of the five reptilian lineages that have evolved complex placentation, and from a biogeographic perspective because of their distribution in the Australian and Pacific region.

Chapter 2 describes the genes used to obtain sequence data for reconstructing phylogenies. Mitochondrial DNA has proven to be more informative than either intron or exon sequences from the nuclear genome, however there were substantial problems with nuclear paralogues in two of three mitochondrial genes investigated. Exon sequences seem to be more easily amplified and are as informative as introns.

Chapter 3 is a phylogenetic analysis of representatives of the *Eugongylus* group from Australia, New Zealand, New Caledonia, several wide-spread Pacific lineages and Africa. The phylogeny derived from nucleotide sequence data from *c-mos* and *rag-1* exons, and *β-fibrinogen and myosin-2* introns, suggests that the earliest split within the group was between the African taxa, and a group including the remaining *Eugongylus* group taxa. The *Eugongylus* group may have diversified quickly throughout the Indo-Pacific, since each geographic region (except Australia) forms a lineage, but the relationships between these lineages were not resolved. Within Australia, Hutchinson *et al.*'s (1990) *Niveoscincus-Carlia-Lampropholis* lineage is supported, but their *Bassiana-Morethia-Pseudemoia* lineage is not.

Chapter 4 is a phylogenetic analysis of the placental lizard genus *Pseudemoia* based on allozymes and intron and mitochondrial sequence data. The phylogenetic relationships among species of *Pseudemoia* are recovered with high support. Chapter 5 is a phylogenetic analysis of New Zealand's endemic *Eugongylus* group skinks. My nuclear sequence data conflicts with the previously published *12S rRNA* data (Hickson *et al.*, 2000). The results presented here suggest that the phylogeny of New Zealand's endemic skinks does not include a hard polytomy as suggested by Hickson *et al.* (2000). My results also suggest that the only oviparous spices in New Zealand, *O. suteri*, is not

the sister taxon to the remaining species and, therefore, that reproductive mode in New Zealand's skink does not have a simple evolutionary history.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Dr Steve Cooper, Dr Remko Leijs and Dr David Morris shared in many useful discussions, of my work and their own. All members of the Evolutionary Biology Unit contributed to my study through discussion groups and through their friendship while sharing lab and office space. Kathy Saint, Leanne Haigh and Jan Armstrong helped with primer design and expert technical advice. Ralph Foster helped with sequence alignment. Chapter drafts were improved by the helpful comments of Dr Steve Cooper, Dr Remko Leijs, Dr Mike Lee and Dr Chris Austin. Ted Townsend provided unpublished primers and *Eumeces* sequence for *Rag-1*.

The work of Dr Jim Stewart and Mike Thompson on the physiology and morphology of placentae in skinks provides the context for this study, and they have shared their time and expertise freely.

Chapter 1 - Introduction

The order Squamata is the most diverse and speciose living clade of reptiles, and includes all lizards and snakes. "Lizards" is a paraphyletic group (snakes are actually a clade nested within lizards) including 4900 species occurring on all continents except Antarctica, and on many of the world's oceanic islands (Uetz, 2001; March 2001 update). More than a quarter of all lizards are skinks (family Scincidae including 1254 spp, Uetz, 2001), and the family occurs almost world-wide and in a correspondingly wide range of habitats and ecological niches.

Affinities of the Scincidae

The Scincidae belong to the infraorder Scincomorpha. This infraorder also includes the Cordylidae (South African Spinytail Lizards), Gerrhosauridae (African Plated Lizards [sometimes regarded as a subfamily of the Cordylidae]), Teiidae (North & South American Whiptails and Tegus), Gymnophthalmidae (South America Spectacled Lizards [sometimes regarded as a subfamily of the Teiidae]), Lacertidae (African, European and Asian Wall Lizards) and the Xantusiidae (South America Night Lizards). The affinities of the limbless blind lizards of the family Dibamidae are still unclear but there is some suggestion that they also belong to the Scincomorpha, or indeed that they are members of the family Scincidae (Camp, 1923; Rieppel, 1984).

The relationships of the six families making up the Scincomorpha have been approached using traditional morphological methods on different datasets by a number of authors (Camp, 1923; Estes and Pregill, 1988; Greer, 1970; Presch, 1988), and recently using limited molecular (gene sequence) data (Harris *et al.*, 2001; Harris *et al.*, 1999). The phylograms resulting from each of these analyses differ significantly from each other, but all have low statistical support for the relationships they suggest (Macey *et al.*, 1997 and M. Hutchinson unpublished re-analysis). The relationships of the Scincidae inferred by these analyses are usually with either the cordylid / gerrhosaurid clade (Estes *et al.*, 1988; Greer, 1970; Harris *et al.*, 1999) or the with Xantusiidae

(Camp, 1923; Presch, 1988). A larger amount of appropriately variable molecular data, or a simultaneous analysis of the morphological characters used in each of the separate analyses published previously may provide a robust estimate of Scincomorphan relationships.

Relationships among the Scincidae

The Scincidae is made up of four morphologically defined subfamilies, the Acontinae, Feyliniae, Lygosominae and Scincinae (Greer, 1970). The Acontinae and Feyliniae are small lineages (17 and six species respectively) restricted to Africa. The Scincinae is a paraphyletic assemblage including 210 species in 30 genera, distributed through north America and Africa, southwest Asia and the Philippines. The largest and most geographically widespread scincid subfamily is the Lygosominae, including 750+ species with centres of diversity in Asia, Australasia, New Guinea, and central and southern Africa. Lygosominae also occur in the Caribbean, southwest North America and central South America, and the Pacific and western Indian Ocean islands including the Seychelles and Madagascar (Greer, 1970; Zug *et al.*, 2001).

Relationships among the Lygosominae

The Lygosominae is thought to comprise five distinct lineages: the *Egernia*, *Eugongylus*, *Lygosoma*, *Mabuya*, and *Sphenomorphus* groups (Greer, 1974; Greer, 1979; Honda *et al.*, 1999; Honda *et al.*, 1999; Honda *et al.*, 2000; Hutchinson, 1981). The most recent molecular study of the Lygosominae suggests the relationships (*Sphenomorphus*, (*Lygosoma*, (*Egernia*, (*Mabuya, Eugongylus*)))) (Figure 1.1, Honda *et al.*, 2000). In a recent study of morphological evolution, Greer (2001) referred without further comment to the Eugongylini, Sphenomorphini and the *Mabuya* assemblage; the reasons for giving the *Eugongylus* and *Sphenomorphus* groups tribal status were not given. The skink fauna of Australia includes genera from the *Egernia*, *Eugongylus*, and *Sphenomorphus* groups. The *Eugongylus* group is the focus of this thesis, and is described further in Chapter 3.

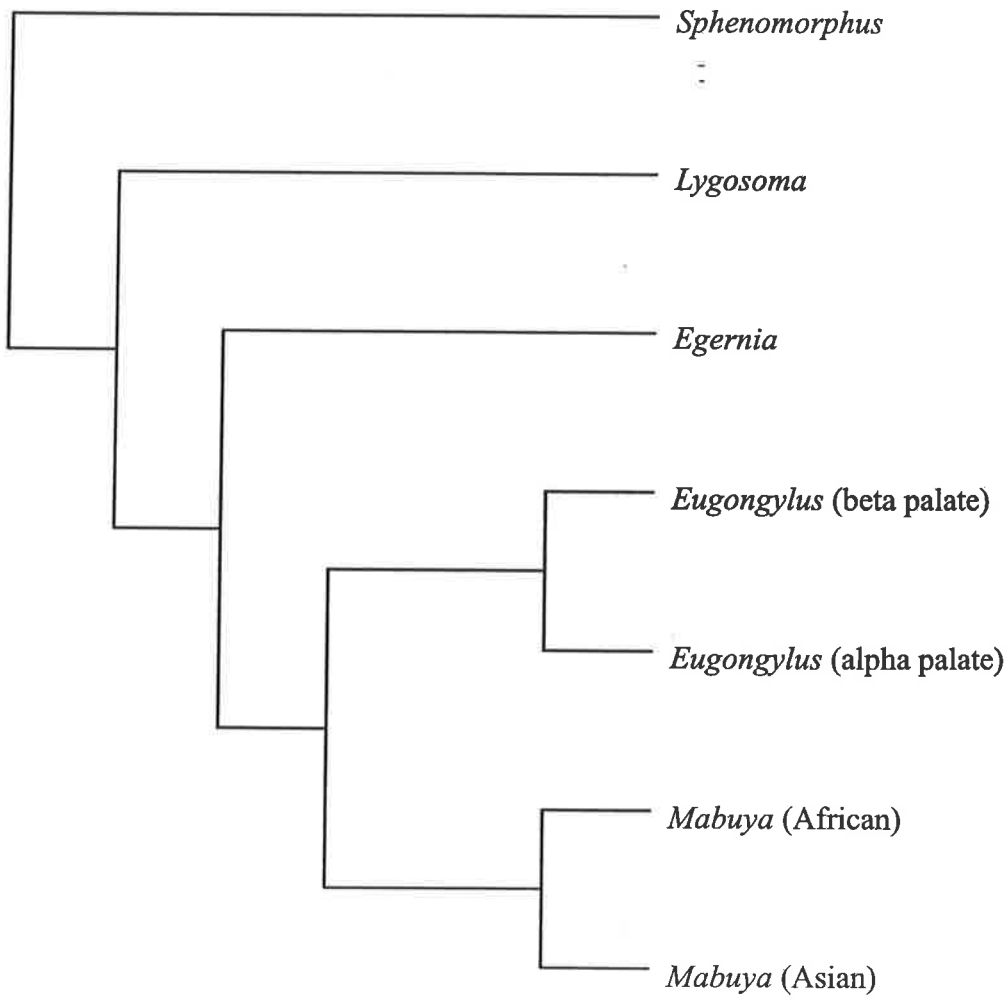


Figure 1.1: The phylogeny of the major groups of Lygosomine skinks recovered by Honda *et al.*, 2000.

Variation in reproductive mode in reptiles

Reptiles are the only terrestrial vertebrate group to have evolved live-bearing reproduction (viviparity) multiple times (Blackburn, 1981). Reptiles therefore provide a unique opportunity to examine the evolution of viviparity by comparing lineages which have independently acquired the same method of reproduction, or by comparing closely related egg-laying (oviparous) and live-bearing (viviparous) taxa. Comparative studies have established that live-bearing in reptiles frequently evolves in cold climates, a pattern that is repeated across the reptiles as a whole (Shine, 1985; Tinkle and Gibbons, 1977), within genera (Guillette *et al.*, 1980), and even within species (Smith, 1996; Smith *et al.*, 2001; Smith and Shine, 1997).

In contrast to viviparity, which has evolved over 100 times and in all reptile lineages except tuataras, crocodiles and turtles, complex placentation occurs in only five genera of scincid lizards (Blackburn, 2000; Thompson *et al.*, unpub ms). Most viviparous reptile embryos develop within a membranous sac, and derive nutrients for their development from a yolk. In these cases physiological transfer between mother and foetus is limited to exchange of water and some gases. In some reptiles however, the egg-shell membranes and the oviduct are modified to allow transfer of nutrients, gases and water between the mother and foetus (Blackburn, 1992). Although the yolk sac placenta has been found to be of importance in placentation in reptiles, the structure is little understood, and most research has focused on the chorio-allantoic placenta. The degree of modification of maternal and foetal tissues, and the relative importance of the nutrients provided by the chorio-allantoic placenta vary between placental lineages (Blackburn, 1993; Thompson *et al.*, 2000). Placental structure also varies during gestation within species. Using the scheme proposed by Weekes (1935) and modified by Blackburn (1992; 1993) reptilian placentae are categorised into four types on the basis of their complexity (Figure 1.2). Simple type I placentae occur in all viviparous reptiles so far examined. Type I placentae also occur during early gestation in species with more complex placentae later in development. Type II placentae occur in the

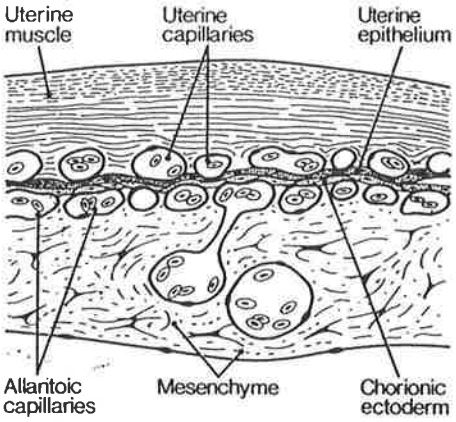


Fig. 5. Maternal–fetal interface of the Type I allantoplacenta. Uterine and chorionic epithelia can be so thin as to be difficult to see histologically. Thickness of the allantoic connective tissue varies according to species, but is usually much thinner than shown. Based on Weekes ('30).

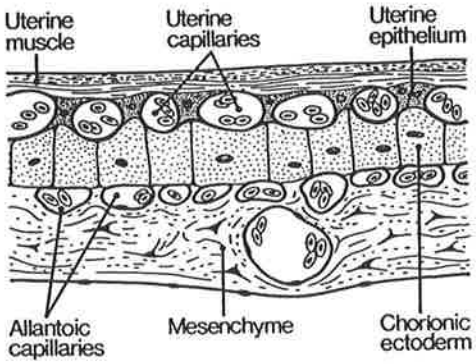


Fig. 8. Maternal–fetal interface of the Type II allantoplacenta. From Blackburn ('85b); based on Weekes ('30).

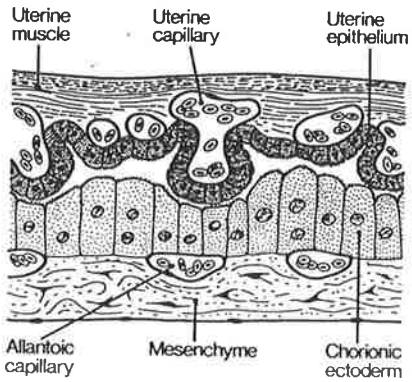


Fig. 10. Type III allantoplacenta in *Pseudemoia entrecasteauxii*, redrawn from Weekes ('27). Identity of the apical specializations of the uterine and chorionic epithelia is uncertain; however, microvilli are present in *Chalcides chalcides*.

Figure 1.2: Morphology of Type I, II, and III placentae. Figures from Blackburn (1993).

Australian genus *Niveoscincus* (Stewart and Thompson, 1994; Stewart and Thompson, 1998). Type III placentae occur in the Australian genus *Pseudemoia* (Harrison and Weekes, 1925; Stewart and Thompson, 1996; Stewart and Thompson, 1998) and the most complex type IV placenta has been described in some species of the genera *Chalcides* and *Mabuya* (Blackburn, 1992; Blackburn, 1993; Corso *et al.*, 2000; Jerez and Ramírez-Pinilla, 2001). A recent study suggests that at least one species of the African genus *Eumecia* many also have type IV placentae (Flemming and Branch, 2001).

The morphological complexity of placentae was thought by early authors to correlate directly with their physiological importance. Thus it was thought that type I placentae function in water exchange, type II in exchange of water and some gases and type III in exchange of water, gases and nutrients (Weekes, 1935). It is now possible to measure the contribution of the placenta to the overall energy requirements and specific mineral makeup of developing embryos (e.g. Swain and Jones, 1997; Thompson *et al.*, 2001; Thompson *et al.*, 2001). Studies of this type have shown that morphological complexity is a predictor of physiological importance of the placenta on a broad scale, but there is a great deal of variation not accounted for by structural differences. Within the genus *Pseudemoia*, three species with type III placentae show two-fold variation in the proportion of the energy for development which is derived from the placenta, and wide variation in the minerals provided (Stewart and Thompson, 1993; Thompson *et al.*, 1999; Thompson *et al.*, 1999). In at least one species (*P. entrecasteauxii*) the amount of energy derived from the placenta varies from clutch to clutch. These data have begun to demonstrate that there isn't a simple relationship between structure and function in reptilian placentae, and have complicated attempts to understand their evolution.

The *Eugongylus* group and placental evolution

The *Eugongylus* group is a particularly important lineage for studies of the evolution of placentation. Unlike genera with type IV placentae, it is thought that the

Eugongylus group genera with type II and III placentae (*Niveoscincus* and *Pseudemoia*) are each monophyletic lineages, and their affinity with the rest of the *Eugongylus* group is clear (Hutchinson *et al.*, 1990). These genera are thus the only placental genera (or indeed species) that could confidently be placed in a phylogenetic context with each other and with their oviparous relatives. Thus the *Eugongylus* group will allow comparative analyses of the evolution of placental characteristics (Harvey and Pagel, 1991). However, before such analyses can be undertaken a more robust and detailed analysis of the relationships of the *Eugongylus* group is needed.

Phylogenetic issues & approaches

Gene trees and species trees

Phylogenetic analyses based on data from a single genetic locus, or multiple linked loci will recover the evolutionary history of the gene or linkage group, which may not represent the evolutionary history of the organism (Pamilo and Nei, 1988). Gene phylogenies may differ from those of organisms for a number of reasons, including incomplete lineage sorting of ancestral polymorphisms (e.g. figure 1.3), horizontal gene transfer or selection. Reconstructing phylogeny from a number of independent loci (or independent non-genetic datasets), and testing for incongruence between these loci allows gene trees which are different from the organismal history to be identified. This issue is particularly important for phylogenetic studies utilising only mitochondrial genes which are linked and therefore represent a single gene history (Doyle, 1992). I have used a number of independent loci in each phylogenetic analysis presented here, and have tested for incongruence using the methods outlined below.

Combining data and identifying incongruence

The availability of data from a number of different sources for many taxa has lead to debate over the most appropriate approach to analysing independent data sets. The "total evidence" approach suggests that all data should always be analysed simultaneously because from a philosophical standpoint any hypothesis should be able

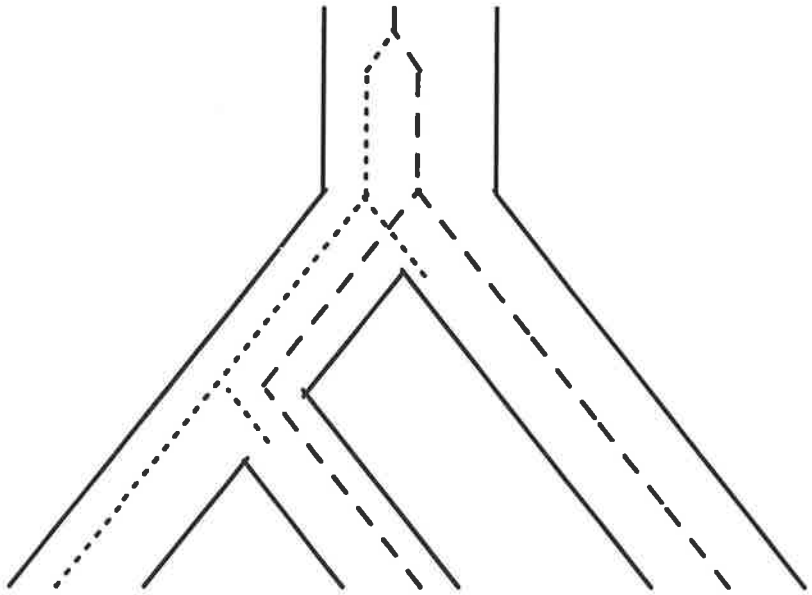


Figure 1.3: An example of gene evolution which would lead to reconstruction of an incorrect species tree.

to explain all the available data simultaneously, and because from an operational standpoint, the additivity of signal means that signal to noise ratio can be increased by combining data (Kluge, 1989; Kluge, 1998). However, when independent datasets evolved under different evolutionary rules, combining partitions obscures the phylogenetic signal in each dataset. Alternatively, there are authors who advocate a "consensus" approach, that is that each dataset should be analysed independently and the resulting trees compared (Miyamoto and Fitch, 1995). This approach results in strong corroborating support for results that are in common between analyses, but increases sampling error by using small datasets, and relies on an arbitrary choice of consensus method (see De Queiroz *et al.*, 1995 for a review of these approaches). More recently the "conditional combination" approach - where datasets are tested for signal heterogeneity before being analysed, has been widely accepted (e.g., Cannatella *et al.*, 1998; Messanger and McGuire, 1998; Olmstead and Sweere, 1994; Omland, 1994).

Debate currently focuses on the methods used to identify conflict between datasets. The most frequently used statistical test for heterogeneity is the ILD test of Farris (Farris, 1995 #138, also called the partition homogeneity test, Swofford, 1999). The ILD test is known to give false positive results in some cases of low heterogeneity. Uninformative characters in either data set, or heterogeneity of noise levels between data partitions, have been proposed as explanations for falsely positive ILD test results (Dolphin *et al.*, 2000; Lee, 2001). The partitioned Bremer Index (PBI, Baker and Desalle, 1997) is an alternative, non-statistical approach to evaluating heterogeneity. Although the PBI does not provide a statistical cut-off point for combining partitions, the distribution of conflict among clades and partitions is apparent. This can be particularly useful for understanding causes of heterogeneity. I have used a combination of ILD tests and PBIs to assess incongruence among data in the following chapters.

Sequence alignment

Alignment infers the homology of base positions among sequences. When all the sequences in the dataset are of approximately the same length, alignment can be trivial.

However, when the length or sequence of nucleotides varies significantly, homology of positions may become difficult to judge. In general, protein coding sequences are relatively easy to align, while non-coding regions are freer to vary in length and sequence. All sequence alignments use an implicit or explicit system of penalties for specific events in the alignment, these events include aligning non-matching bases, opening new gaps and extending existing gaps. These costs can be part of an explicit algorithm (e.g. Higgins *et al.*, 1991), or, in the case of manual alignment, are implicit. The use of an explicit alignment algorithm allows the cost system to be defined and justified, while manual alignment necessarily involves implicit penalties, which are rarely addressed. Explicit alignment algorithms however, are limited to a single set of penalties across the entire sequence, while manual alignment allows variation of penalties across the length of sequence. For these reasons, I have used manual alignment of sequences.

Including gaps in phylogenetic analyses

Gaps in aligned data matrices occur because of the insertion or deletion of bases in some sequences. The treatment of gaps in phylogenetic analysis of molecular data is of particular importance in analysis of non-coding nucleotide sequences because these sequences are more likely to be length variable and contain gaps with ambiguous alignment. There are a number of methods for treating gaps in analysis of aligned nucleotide data, the simplest of which is to treat gaps as unknown character states. This approach minimises the risk of incorrectly inferring homology of indels, but potentially disregards information contained in the distribution of gaps among sequences. Simmons and Ochoterena (2000) propose a simple and conservative approach to gap coding and a more complex method which weights transitions between gaps proportionally to the number of changes required for transformation of one gap to another. I have used several of these approaches and compared their applicability to different gene sequences.

Optimality criteria for phylogenetic analysis

Phylogenetic analysis is fundamentally a process of finding the tree which best explains the data under a set criterion of optimality. The two primary optimality criteria used in molecular studies are maximum parsimony and maximum likelihood. The maximum parsimony approach effectively assumes that "evolution is simple" and can be applied to any non-continuous data. Maximum parsimony is the mainstay of morphological systematic analysis. Maximum likelihood approaches to phylogenetic reconstruction have become widely used as nucleotide sequence data has become more common (Edwards, 1972; Felsenstein, 1981). Nucleotide sequence data allowed the use of likelihood analysis because, unlike most morphological characters, nucleotide sequence evolution can be mathematically modelled. Likelihood analyses rely on such models to evaluate alternative trees. There has been much debate over which, if either, of these methods is superior. Both methods have been shown to converge with confidence on the wrong tree (to be positively misleading) under particular conditions. Both parsimony and likelihood approaches are used here, and their results are compared.

Likelihood models

Likelihood analysis relies on the use of an appropriate model of sequence evolution. A number of models are available (such as those used by modeltest Posada and Crandall, 1998), that incorporate different parameters the values of which are estimated from the data (tree topology is also a parameter of the model). Choosing an appropriate model is a matter of finding a model which sufficiently explains the data, without including non-essential parameters (each additional parameter exponentially increases error). This is most simply done by using likelihood ratio tests (it is a property of likelihood that scores are directly comparable, and in nested analysis fit the chi-squared distribution), to evaluate the contribution of each additional parameter as it is added to the model. When the addition of a parameter does not significantly increase the likelihood of the tree, the previous model is used. Ultimately this model is fixed, and all

parameters including tree topology are estimated simultaneously. However, likelihood analyses are computationally intensive and time consuming, in addition increasing the number of parameters included in the model increases computation time exponentially. Therefore, full simultaneous estimation of all parameters is rarely possible. A number of alternative approaches to estimating parameters exist. Single parameters can be estimated in turn with all other parameters fixed for several iterations (i.e., all parameters bar one are fixed, the parameter of interest is estimated, then all parameters bar the next are fixed, etc.) (Swofford *et al.*, 1996). Alternatively, all parameters except tree topology can be estimated using the topology obtained from an alternative optimisation method (either parsimony, or distance analysis which can be undertaken using the appropriate model of evolution) (Swofford *et al.*, 1996). I have used full simultaneous estimate of parameters where possible (Chapter 4), and have estimated parameters using a distance tree topology in all other cases.

Indices of support

Decay indices, bootstrap resampling, and hypothesis tests are commonly used criteria for measuring support for the optimal tree. Each of these methods has limitations, but when used together they can give an indication of which areas of a phylogenetic hypothesis are robust. The most frequently used decay index, the Bremer index (Bremer, 1988) is simple and intuitively appealing, and, as previously mentioned, can be modified to assess incongruence between datasets (Baker and Desalle, 1997). However, the Bremer index does not provide an objective statistical measure of support. Bootstrap resampling indicates precision rather than accuracy and is often incorrectly interpreted as an absolute measure allowing comparisons between trees and datasets. Hypothesis tests are useful when a specific alternative tree topology can be proposed. Tests including Templeton's (1983) implementation of the Wilcoxon rank sum statistic, the Kishino-Hasegawa test (Kishino and Hasegawa, 1989) and the Shimodaira-Hasegawa test (Shimodaira and Hasegawa, 1999) determine whether the support for the

optimal tree is significantly greater than that for a specific alternative topology, but do not give general measures of overall support.

Summary

In this thesis I will examine the relationships among *Eugongylus* group taxa with particular focus on lineages important for understanding the evolution of live-birth and placentation in the group. I will address 1) nuclear DNA as a source of phylogenetic information, 2) the relationships among *Eugongylus* group lineages throughout the Pacific, 3) the relationships between species in the placental genus *Pseudemoia* and 4) the affinities of the single endemic egg-laying skink in New Zealand.

Chapter 2 - Genes for Phylogenetic Analysis

Traditional sources of data

There are few basic requirements that make data appropriate for systematic studies, despite the debate that has occurred over this issue (particularly the "molecules vs. morphology" debate, see Hillis, 1987 for a review). Any character which is heritable, and can be unambiguously identified and scored for the taxa in question can provide information about relationships. From this viewpoint, all characters, be they molecular, morphological, behavioural or chemical, are equally valid sources of information. How useful characters prove to be in practice is determined largely by their level of variation. Too little variation provides insufficient informative characters (phylogenetic signal) to fully resolve relationships, and too much variation obscures the phylogenetic signal with spurious variation (noise).

Morphological characters have proven useful for distinguishing broad taxonomic groups within skinks, but are relatively conserved compared to genetic characters (e.g. Austin, 1995; Austin and Zug, 1999; Bruna *et al.*, 1996; Daugherty *et al.*, 1990; Donnellan and Hutchinson, 1990). The morphological characteristics that have been used to define subgroups within the *Eugongylus* group tend not to be corroborated by other characters, and often show parallel variation in other groups of skinks (Greer, 1989; Hutchinson *et al.*, 1990). For example beta-palate morphology, used to divide the *Eugongylus* group (see Chapter 3), is thought to occur independently in some members of the genus *Emoia*, and widely in the *Sphenomorphus* group (Greer, 1979). A major new set of morphological characteristics would be required to resolve a phylogeny of the *Eugongylus* group using morphology alone.

Allozyme electrophoresis and immunological comparisons have also been used to address relationships within the *Eugongylus* group (Daugherty *et al.*, 1990; Donnellan and Hutchinson, 1990; Hickson *et al.*, 1992; Hutchinson and Donnellan, 1992; Hutchinson and Schwaner, 1991; Patterson and Daugherty, 1994). Immunological comparisons are, by their nature, distance measures, and allozyme electrophoretic data

are also most commonly analysed in a distance framework. These distance-based analyses are phenetic rather than phylogenetic in their approach, and recover similarity rather than relatedness of taxa. Previously published allozyme data can be re-coded and included in parsimony analysis, although alleles are scored relative to each other and additional taxa cannot usually be added. Distance matrices from immunological studies cannot be re-coded or included in non-distance analysis. Immunological and protein electrophoresis approaches have relatively low limits of resolution and are better at addressing relationships between relatively closely related taxa.

The most promising direction for furthering our understanding of skink phylogenies, and hence the approach I have taken, is the use of nucleotide sequence data. The genes used in this study are discussed below.

Mitochondrial DNA sequence data for phylogenetic analysis

The advent of the polymerase chain reaction (PCR) has allowed large amounts of nucleotide sequence data to be accumulated much more quickly than traditional phylogenetic characters. Mitochondrial DNA (mtDNA) has been much more prevalent in systematics than sequences derived from the nuclear genome for a number of reasons. Firstly, mtDNA has a higher evolutionary rate than other DNA types, and therefore may contain more informative variation. Secondly, there are multiple mitochondria in each cell, allowing ready amplification of mt genes from relatively small amounts of tissue, or under non-optimal PCR conditions. Thirdly, with rare exceptions, the genes on the mitochondrial genome occur as a single copy, meaning that homology is easily established. Finally the conserved structure of the mitochondrial genome across a wide range of taxa allows the use of "universal" primers. These primers are designed to amplify a locus across a wide spectrum of taxa, and their availability vastly reduces the developmental phase of systematic studies (Kocher *et al.*, 1989; Simon *et al.*, 1994).

Although mitochondrial DNA sequence data have been exceptionally useful in a wide range of systematic studies, like all data, mtDNA has its limitations. The utility of mitochondrial DNA is limited by 1) its susceptibility to saturation, 2) the non-

independence of loci and 3) the occurrence of nuclear paralogues. Saturation occurs when sites in a DNA sequence undergo multiple consecutive substitutions, and phylogenetic signal is obscured by a subsequent random change to the same site. The relatively fast evolutionary rate of mtDNA, which allows informative characters to be quickly accumulated, also makes mtDNA particularly prone to saturation and makes resolution of deep divergences using mtDNA problematic (Meyer, 1994). Saturation is a particular problem for third codon positions, which, due to redundancy in the genetic code, can often change without altering the gene product.

The non-independence of mitochondrial loci is important because multiple mitochondrial genes cannot be used to give independent estimates of a species phylogeny. The importance of sampling multiple gene trees when estimating a species phylogeny was outlined in Chapter 1. Because the mitochondrial genome is inherited as a single unit without recombination (but see Dowton and Campbell, 2001; Hagelberg *et al.*, 1999 for evidence of mitochondrial recombination), sequences from multiple mitochondrial loci increase confidence in the mitochondrial gene tree, without independently supporting the organismal phylogeny. To ensure that a mitochondrial gene tree correctly estimates the species tree, mtDNA data should be combined with data from independent sources – either non-mitochondrial DNA or non-sequence data.

Nuclear paralogues also pose a serious obstacle to the use of mtDNA for phylogenetic studies. Copies of mtDNA which have been transferred to the nucleus are especially problematic because they may be inadvertently amplified by PCR with primers designed to mitochondrial sequences. In the worst case this can lead to a dataset consisting of sequences from two or more loci. Nuclear paralogues were originally thought to occur infrequently, and to be readily identifiable by the lack of an open reading frame or by their position in the gene tree. Recent studies of animals (cats, passerine birds, snapping shrimp, grasshoppers and primates) have described paralogues which are large (up to 14.6 kb), frequent (up to 24 different copies in a single individual), have open reading frames, possibly recombine with each other or have exceedingly variable (0.2 - 18.8 %) divergence from mitochondrial ancestors

(Bensasson *et al.*, 2001; Bensasson *et al.*, 2000; Lopez *et al.*, 1996; Lopez *et al.*, 1994; Mourier *et al.*, 2001; Mundy *et al.*, 2000; Nielsen and Arctander, 2001; Williams and Knowlton, 2001; Zhang and Hewitt, 1996). In addition, a comparison of the working draft of the human nuclear genome with the complete mitochondrial genome (Mourier *et al.*, 2001) found that all parts of the mitochondrial DNA are represented in the nuclear genome. Data on paralogues from a number of published and unpublished studies have been compiled by Bensasson *et al.* (2001) at www.pseudogene.net. The data which have been accumulated in recent years suggest that nuclear copies of mt genes occur much more frequently and are more difficult to identify than originally thought. There are several pre-amplification techniques that may increase the chances that only mtDNA is available as template for PCR amplification. However none of these methods is guaranteed to result in sequence derived solely from the mitochondria.

The mitochondrial genes used in this study are described below and further information about the utility of these genes for phylogenetic studies is given in the following chapters.

Methods for mitochondrial DNA

To minimise the chance of amplifying nuclear paralogues, Welter *et al.*'s (1989) sucrose gradient method was used to obtain mt enriched DNA from specimens where good quality frozen tissue was available. The method uses a density gradient generated by centrifugation to separate intact membrane-bound mitochondria from other cellular components, and can thus have a low yield when mitochondrial membranes have been disrupted before preparation. The effectiveness of each enrichment was determined by amplifying serial dilutions (from 1/10 to 1/1,000,000) of each sample with the mitochondrial primers of interest and a control nuclear primer pair (either *c-mos* or *aldolase*). The difference in concentration of DNA which can be successfully amplified by nuclear and mitochondrial primers indicates how effective enrichment has been. Enriched DNA at least 10^3 times more dilute than was amplified by nuclear primers was sequenced wherever possible, but less dilute template was used in a few cases where

necessary. As a test for the existence of paralogues for each gene, amplification products from concentrated nuclear enriched DNA (produced during the mt enrichment process and used to maximise the chance of amplifying any nuclear sequence) and dilute mtDNA for each gene were compared for three species. Different sequences from mt and nuclear DNA would indicate the presence of at least one paralogue.

12S ribosomal RNA

Sequences of *12S ribosomal RNA* have been used in many phylogenetic and phylogeographic analyses in a range of reptile taxa, including lacertid lizards (Fu, 1998; Harris *et al.*, 1998; Harris *et al.*, 1998) and skinks (Brehm *et al.*, 2001; Hickson *et al.*, 2000; Honda *et al.*, 1999; Honda *et al.*, 2000; Mausfeld *et al.*, 2000). The variability of the commonly used region of *12S rRNA* is known to be relatively low compared with other mitochondrial regions (Hillis and Dixon, 1991). I sequenced *12S rRNA* from six *Eugongylus* group species to assess variability and phylogenetic information content in this group. There were 66 variable sites and 25 parsimony informative sites from a total of aligned 306 sites. *12S rRNA* was therefore considered unlikely to be efficient at recovering the phylogenetic history of the *Eugongylus* group skinks. *12S rRNA* sequences from the New Zealand *Eugongylus* group skinks previously published by Hickson *et al.* (2000) were included in Chapter 5, but *12S rRNA* was not used for other studies.

NADH dehydrogenase subunit 2 (ND2)

The *ND2* gene encodes subunit two of the NADH dehydrogenase protein. *ND2* nucleotide sequence data have been used in phylogenetic studies of a large number of reptile groups including Australian *Sphenomorphus* group skinks (Smith *et al.*, 2001). The reptile *ND2* primers, M136/M177 and M167/M178 (K. Saint unpublished) were used to amplify overlapping 420 and 480bp products respectively. However, in four taxa the two amplification products are not identical where they overlap. Figure 2.1 shows overlapping regions of *Carlia longipes*, *Lampropholis guichenoti*, *Saproscincus*

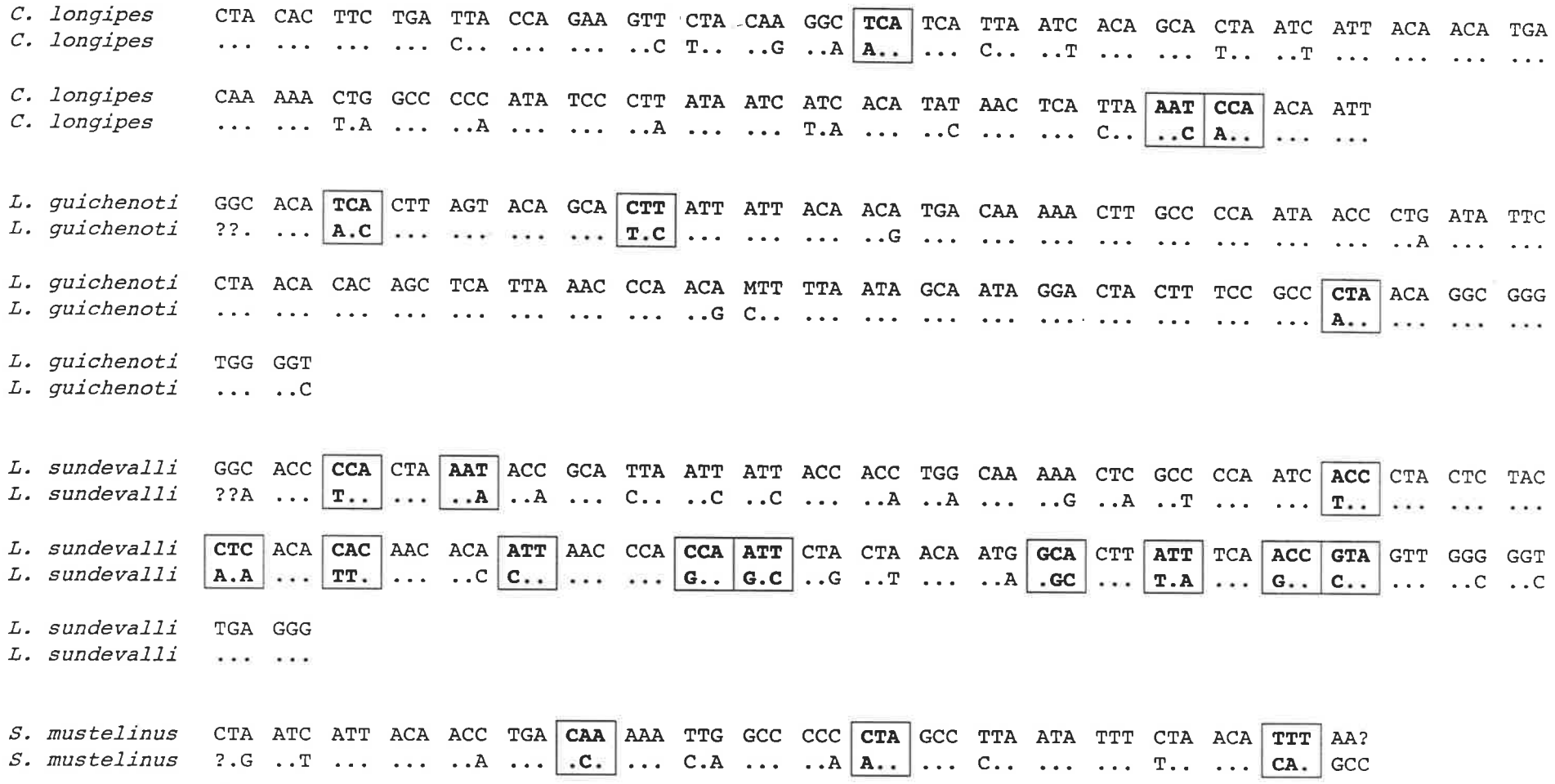


Figure 2.1: Paralogues of ND2 from *Carlia longipes*, *Lampropholis guichenoti*, *Saproscincus mustelinus*, and *Lygosoma sundevalli*. Nucleotide substitutions in bold, amino acid substitutions in boxes.

mustelinus, and *Lygosoma sundevalli* (a non-*Eugongylus* group skink). The *Lampropholis guichenoti* sequences were amplified from dilute mitochondrial DNA. *Saproscincus mustelinus* and *C. longipes* sequences were amplified from low-yield mt enrichments and *Lygosoma sundevalli* was amplified from a total cellular DNA sample. Although test sequences amplified from nuclear and dilute mitochondrial DNA of four other species do not differ, at least one of each pair of non-identical sequences may be a nuclear paralogue. The nucleotide sequences from the same individual were 6 - 25% different (Table 2.1). All sequences have an open reading frame but amino acid sequences differ (Figure 2.1, Table 2.1). Phylogenetic analysis of PCR fragments does not result in obvious groupings of mitochondrial and other sequences. It is impossible using only the sequences to determine which, if any, are mitochondrial in origin. The dataset may also contain other nuclear derived sequences which were not recognised because both PCR fragments are from the same locus. On the basis of the occurrence of at least two loci which were amplified from enriched mtDNA in one case, *ND2* was not included in any phylogenetic studies of the *Eugongylus* group.

NADH dehydrogenase subunit 4 (ND4)

The *ND4* gene encodes subunit four of the NADH dehydrogenase protein. *ND4* nucleotide sequence data have been used in phylogenetic studies of iguanids (Arévalo *et al.*, 1994; Malone *et al.*, 2000; Zamudio *et al.*, 1997), colubroid snakes (Kraus and Brown, 1998), sea turtles (Dutton *et al.*, 1996), pitvipers (Parkinson *et al.*, 2000) and scincid lizards in the genus *Mabuya* (Mausfeld *et al.*, 2000). The reptile *ND4* primers Leu and ND4L of Arévalo *et al.* (1994) and M256 and M257 designed specifically for skinks, were used to amplify two overlapping products with a total length of ~950 bp including *ND4* and tRNAs *His*, *Ser* and *Leu*. *ND4* did not amplify consistently in all *Eugongylus* group taxa, but was used for a phylogenetic study of the species belonging to the genus *Pseudemoia* (Chapter 4). There was no evidence of any paralogous sequences from *ND4*.

Sequence		# of copies	Nucleotide divergence (P)	Amino acid divergence (%)
ND2:				
Carlia longipes		2	0.155	7
Lampropholis guichenoti		2	0.064	6
Lygosoma sundervalli		2	0.233	25
Saproscincus mustelinus		2	0.256	17
ND5:				
Oligosoma fallai	1 *vs* 2	3	0.094	4
	1 *vs* 3		0.021	3
	2 *vs* 3		0.128	10
Oligosoma zealandicum		2	0.141	8

Table 2.1: Number of copies and amount of divergence in nucleotides and proteins between paralogous sequences of ND2 and ND5.

NADH dehydrogenase subunit 5 (ND5)

The *ND5* gene encodes subunit five of the NADH dehydrogenase protein. *ND5* nucleotide sequence data have not previously been used in phylogenetic studies of reptiles. Primers were designed in the neighbouring *ND4* and *Cytb* regions of the mitochondrial genome to amplify a fragment including partial *ND4*, tRNAs *His*, *Ser* and *Leu*, *ND5*, *ND6* and partial *Cytb* (Figure 2.2). Both *ND5* and *ND6* were sequenced from a subset of four *Eugongylus* group species (*Bassiana duperreyi*, *Emoia longicauda*, *Lampropholis guichenoti*, *Oligosoma smithi*) and outgroup *Tiliqua scincoides*. *ND5* was found to be more variable (and is longer) than *ND6* and was therefore chosen for further development. In an attempt to avoid amplifying paralogues, the 5.5kb fragment from M257 to M308 was amplified using long range PCR (using eLONGase enzyme, Gibco BRL following manufacturers instructions), purified from agarose gel and used as a template for amplification of *ND5* in three PCR fragments (Figure 2.3). The rationale behind this procedure was the assumption that large insertions of mitochondrial DNA into the nucleus are expected to occur less frequently than insertions of smaller fragments. In spite of these precautions, two non-identical sequences of the first PCR fragment were amplified from a single individual of *Oligosoma zelandicum* (Figure 2.4) and three sequences of the first, and two of the second PCR fragments were amplified from *O. fallai* (Figure 2.5). In many species, the 5' end fragment was amplified using primers M310/M324 and therefore sequences did not overlap with the first two fragments (see Figure 2.2), making anomalous sequences difficult to recognise. There were also some cases of PCR contamination for this fragment - identical sequences were obtained from 1) *O. microlepis* and *O. stenotis*, 2) *O. zelandicum* and *O. stenotis*, 3) *O. smithi* and *O. suteri*, and 4) *O. stenotis* and *O. fallai*. The third section of *ND5* was amplified later than the other sections, and earlier fragments show no signs of any PCR contamination. Although there were some multiple sequences which do not seem to be explained by PCR contamination, they are excluded from further discussion. The

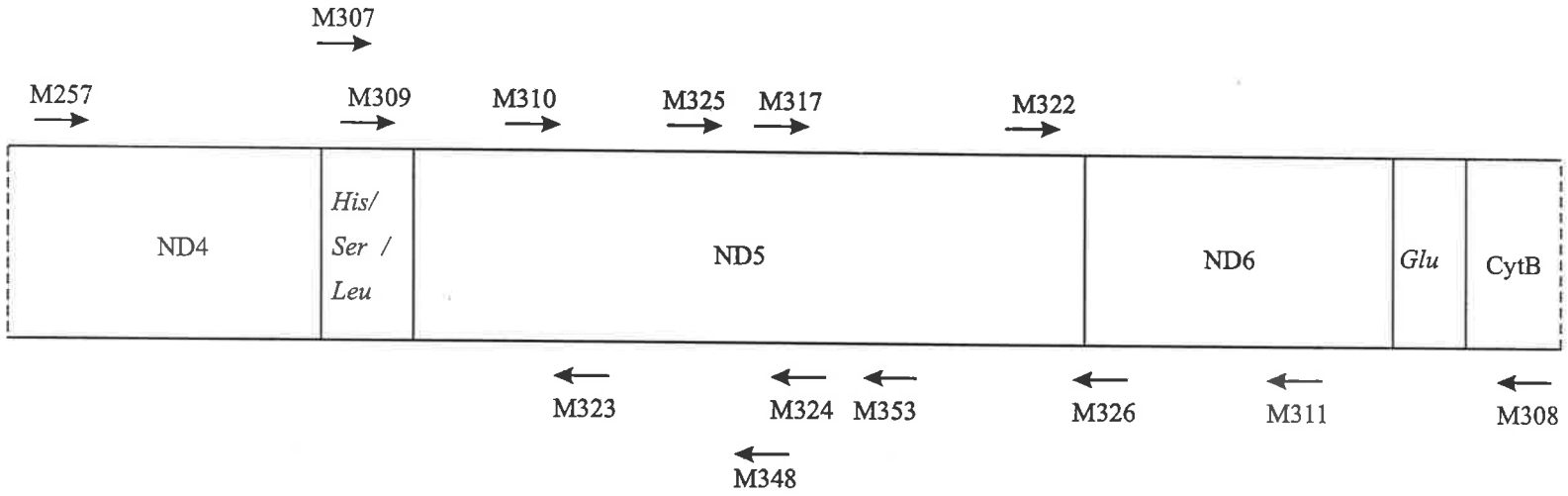


Figure2.2: Primers used to amplify and sequence all of ND5 and ND6.

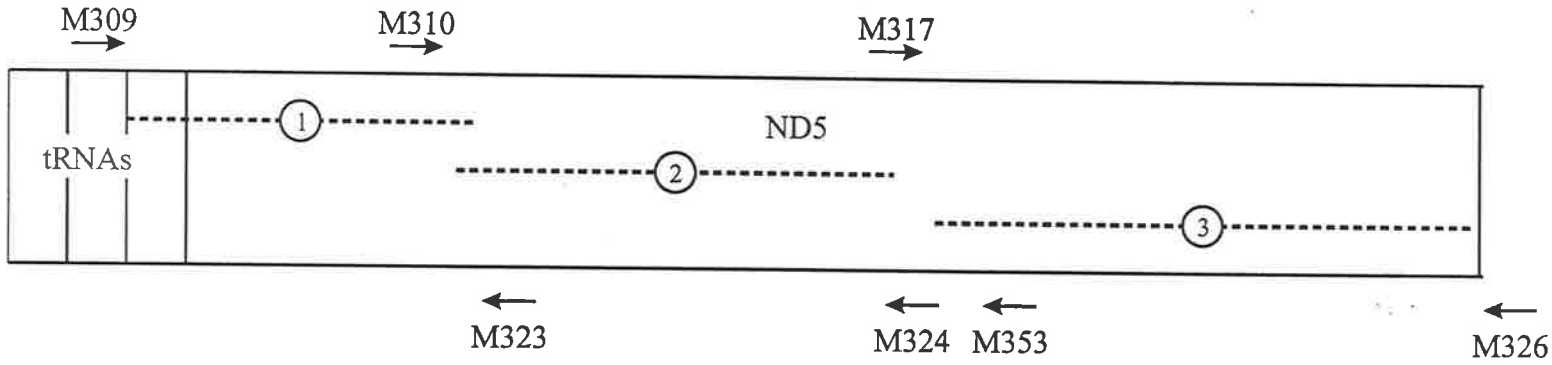
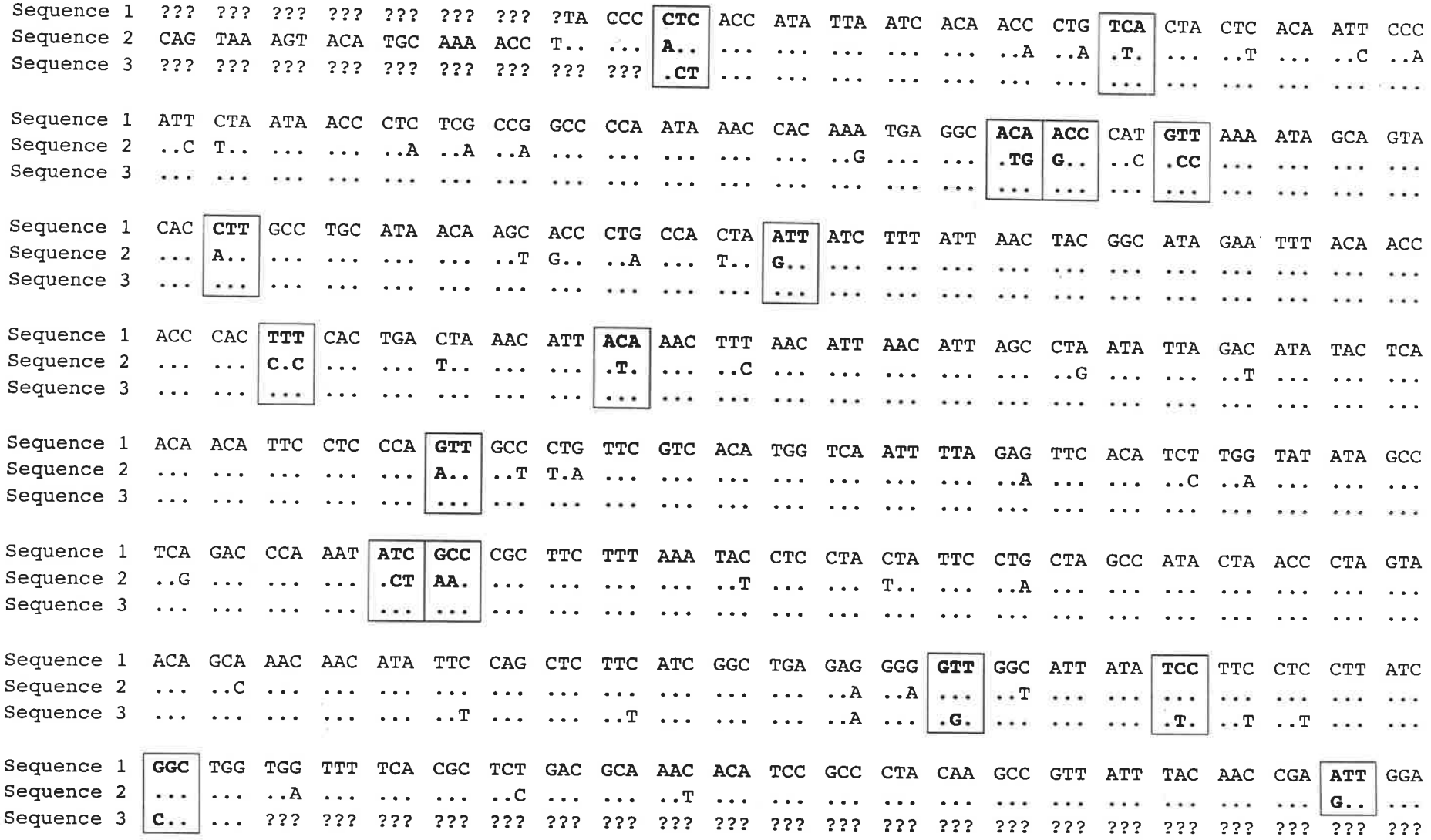


Figure 2.3: Most commonly used ND5 primers. There is an ~15 bp gap between fragments 2 and 3, unless primer M353 is used in place of M324.

```
Sequence 1    ?TG CCC CTT ACC ATA CTA GTC ACA ACC TTA TCA CTA CTT ACA ATT CCA ATC CTA ATA TCC CTT TCA CCA
Sequence 2    ??? ??? ??? ... ... ... A.. ... ..A C.. ..G ... ... ... ..C ..T ..T T.. ... A.. ..C ... ...

Sequence 1    GCC CCA ATA AGC CTT AAA TGG GGC CTA GCC CAT GTT AAA ATA ACA GTC CAC ATT GCC TGC ATA ACA AGC
Sequence 2    ... ... ... .A. .A. ... ..A ..G A.. A.. T.. .CC ... ..G G.. ..A ... ... ... ... ... ... ..T

Sequence 1    ACC CTA CCT TTA ATT ATC TTT ATT AAC TAC GGC ATA GAA TTT TCG GCC ACC CAC CTT CAC TGG TTA AAC
Sequence 2    ... T.. ... ... ... ..T ... ... ... ... ... ... ... ... A.A A.. ... ... ... ... ... ... ...

Sequence 1    ATT ATA AAC TTC AAC ATT AAC ATT AGC CTA ATA CTA GAC ATG TAC TCA ACA ACA TTC CTC CCA ATC GCC
Sequence 2    ... ... ... ... ... ..C ... ..C ... ... ... T.G ... ..A ... ... ... ..G ... ... ... ... ...

Sequence 1    CTG TTC GTC ACA TGA TCA ATT CTA GAA TTT ACA TCT TGA TAC ATG GMC TCA GAC CCA GAC ATC AAC CGC
Sequence 2    ... ... ..T ... ... ... ..C T.. ..G ... ... ..C ... ..T ..A .C. ... ... ... A.T ..T ... ...

Sequence 1    TTC TTT AAA TAC CT? CTA TTA TT? CTA CTA ?CC ATG CTA ?CA CTA GTG ACA GCA AAC AAC ATA TTC CAG
Sequence 2    ..T ..C ..G ... ..C ... C.. ..C ..G ... G.. ... ..C A.. ... ..A ... ..G ... ... ... ..T ..A

Sequence 1    CTT TTT ATT GGC TGA RAA GGA G??
Sequence 2    ..C ..C ..C ... ... G.. ..G .TT
```

Figure 2.4: Paralogous sequences of ND5 from *Oligosoma zealandicum*, both sequences from primer M309. Nucleotide substitutions in bold, amino acid substitutions in boxes.


Sequence 1 ??? ??? ??? ??? ??? ??? ??? ?TA CCC CTC ACC ATA TTA ATC ACA ACC CTG TCA CTA CTC ACA ATT CCC
Sequence 2 CAG TAA AGT ACA TGC AAA ACC T.. ... A.. ... ...A ..A .T. ... ..T ... ..C ..A
Sequence 3 ??? ??? ??? ??? ??? ??? ??? ??? ??? .CT ... ...
Sequence 1 ATT CTA ATA ACC CTC TCG CCG GCC CCA ATA AAC CAC AAA TGA GGC ACA ACC CAT GTT AAA ATA GCA GTA
Sequence 2 ..C T.. ... .A ..A ..A ... ...G ... ... .TG G.. ..C .CC ... ...
Sequence 3 ... ...
Sequence 1 CAC CTT GCC TGC ATA ACA AGC ACC CTG CCA CTA ATT ATC TTT ATT AAC TAC GGC ATA GAA TTT ACA ACC
Sequence 2 ... A.. ... ...T G.. ..A ... T.. G.. ... ...
Sequence 3 ... ...
Sequence 1 ACC CAC TTT CAC TGA CTA AAC ATT ACA AAC TTT AAC ATT AAC ATT AGC CTA ATA TTA GAC ATA TAC TCA
Sequence 2 ... ... C.C ... ... T.. ... ... .T. ... ..C ... ...G ... .T ... ...
Sequence 3 ... ...
Sequence 1 ACA ACA TTC CTC CCA GTT GCC CTG TTC GTC ACA TGG TCA ATT TTA GAG TTC ACA TCT TGG TAT ATA GCC
Sequence 2 ... ... A.. ..T T.A ... ...A ... .C ..A ... ...
Sequence 3 ... ...
Sequence 1 TCA GAC CCA AAT ATC GCC CGC TTC TTT AAA TAC CTC CTA CTA TTC CTG CTA GCC ATA CTA ACC CTA GTA
Sequence 2 ..G ... ... .CT AA. ... ...T ... ... T.. ... ..A ... ...
Sequence 3 ... ...
Sequence 1 ACA GCA AAC AAC ATA TTC CAG CTC TTC ATC GGC TGA GAG GGG GTT GGC ATT ATA TCC TTC CTC CTT ATC
Sequence 2 ... ..C ... ...A ..A ... ..T ... ...
Sequence 3 ... ...T ... .T ... ...A ... .G. ... ... .T. ..T ..T ... ...
Sequence 1 GGC TGG TGG TTT TCA CGC TCT GAC GCA AAC ACA TCC GCC CTA CAA GCC GTT ATT TAC AAC CGA ATT GGA
Sequence 2 ... .A ... ...C ... .T ... ... G.. ...
Sequence 3 C.. ... ???

```
Sequence 1  GAC ATT GGC CTA ATC CTT TTC ATA GCA TGA CTA GCC ATA AAC ATA AAC ACC TGA GAA ATA CAA CAA CTA
Sequence 2  ..T ..C ..A ... ... ..C ... ... ... ... ..G ... ... ... ... ... ... ... ... ..G ... T..
Sequence 3  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???

Sequence 1  TTC TAC AAC CAC AAC ACG ATA ACC CTT ATC CCC CTG CTT GGC CTA ATC CTA GCC GCA ACA GGA AAA TCC
Sequence 2  ... ... ... ... ... ..A ... ... ... ..T ... ... ... ... ..G ... T.G ... ... ... ... ... ...
Sequence 3  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???

Sequence 1  GCC CAA TTT GGC CTT CAC CCC TGA CTG CCA GCA GCC ATA GAA GGG CCC ACC CCC GTC TCC GCC CTA TTA
Sequence 2  ... ... ... ... ... ... ... ... ..A ... ... ... ... ... ... ... ... ..G ..T ... ... ... ...
Sequence 3  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???

Sequence 1  CAC TCT AGC ACA ATA GTT GTA GCT GGG GTA TTT CTA CTA ATT CGA CTC CAC CCC CTC CTA GAA ACA AAT
Sequence 2  ... ... ... ... ... ... ..G ... ... ... ... ... ... ... ... ..T ... ... ..A ..T TT. ??? ???
Sequence 3  ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
```

Figure 2.5: Paralogous sequences of ND5 from *Oligosoma fallai*, Sequence 1 was amplified and sequenced using primers M309/M323 and M310/353, sequence 2 was amplified and sequenced from primers M309/M323 twice and sequence 3 was amplified and sequenced using primer M309. Nucleotide substitutions in bold, amino acid substitutions in boxes.

Oligosoma fallai sequences were all amplified from dilute purified long-range PCR product from amplification of dilute mtDNA. The *O. zelandicum* sequences were amplified from dilute purified PCR product from amplification of total cellular DNA. All sequences had open reading frames, but amino acid as well as nucleotide sequences differ (Table 2.1). Phylogenetic analysis of all sequences does not result in a clade including all or most of the duplicate sequences. As was seen in *ND2*, these data are insufficient to determine which, if any, are mitochondrial in origin. Again, the dataset may also contain other nuclear derived sequences which were not recognised because both PCR fragments are from the same locus. Since the cause of multiple non-identical sequences in *ND5* could not be determined, it was not used for phylogenetic analysis.

An alternative explanation of multiple sequences from mt genes

Amplification of multiple sequences of single copy mt genes could be explained by nuclear paralogues as already discussed, or by mitochondrial heteroplasmy. The occurrence of two mitochondrial linegaes within an individual has been recorded in three genera of iguanine lizards (Densmore *et al.*, 1985; Haenel, 1997; Passoni *et al.*, 2000). In all of these studies, length variability of the non-coding part of the control region was detected by restriction fragment length differences. Mitochondrial length heteroplasmy was observed in 20 – 50% of individuals from each population in two of these studies (Densmore *et al.*, 1985; Haenel, 1997). In the third study, length variants were observed in 91% and 100 % of individuals from two populations of *Tropidurus divaricatus* (Passoni *et al.*, 2000). It is unknown whether the mitochondria in these species differ in any aspect other than control region length. However, the observed variability may indicate the presence of two mitochondrial lineages with different sequences for a number of genes (as occurs in mussels; see Ladoukakis and Zouros, 2001). This scenario could explain the amplification of two different sequences (although probably not three) of a mitochondrial gene from dilute mitochondrial DNA. The rarity of mitochondrial heteroplasmy means that this is a less likely explanation than nuclear paralogues, but it should not be discounted.

Nuclear DNA as an alternative source of phylogenetic data

Both the coding and non-coding regions of the nuclear genome provide alternative sources of sequence data for phylogenetic studies in animals and plants. However, the technical challenges of working with nuclear markers are much greater than those involved with mtDNA. The technical challenges of working with nuclear DNA are due to 1) the lack of universal primers for vertebrate nuclear genes; 2) gene families and duplicates in the nuclear genome; and 3) the rarity of long intronless genes. Much of the coding portion of the nuclear genome is made up of multiple copies of genes, or of closely related gene families. Generally only single copy genes are useful sources of sequence data because they can be unambiguously targeted in PCR. Protein encoding regions of the nuclear genome are known to evolve more slowly than mtDNA equivalents (Brown *et al.*, 1979). The relatively low rate of nuclear DNA evolution means that saturation is less likely to be a significant problem, however it also means that less information will be obtained than from an equivalent length of mtDNA. Long regions of coding nuclear DNA from single genes are therefore desirable. However, such long coding sequences can only be amplified from genomic DNA template from intronless genes or the few long exons. Identifying intronless genes from published data is made difficult because many published nuclear sequences do not specify if or where introns occur, and when introns are specified, their length and location is not generally conserved between taxa.

Non-coding regions of the nuclear genome are also a potential source of phylogenetic nucleotide sequence data. Many of the non-coding regions in the nuclear genome, such as transcription regulating regions, are highly conserved. However, introns, which are excised from DNA as it is transcribed, are thought to be free of strong functional constraints and therefore may be free to vary at faster rate. The relatively fast rate of sequence evolution in introns relative to exons should allow phylogenetic information to be obtained from relatively short lengths of sequence. However, because the length and position of introns vary between taxa, it is difficult to

identify potentially useful loci from published information. Length variability also means that amplification products cannot always be confidently identified before they are sequenced. However, introns are often amplified using primers anchored in relatively conserved flanking exon sequence – exon primed intron crossing (EPIC) primers. This primer positioning also allows products to be identified using the amplified flanking exon sequence. The usefulness of EPIC primers is limited when exon regions that flank introns are conserved between introns either within or between loci. When empirical data are lacking (as is the case for many non-mammalian vertebrate groups), the occurrence, position and size of a given intron throughout a study group can thus only be established by conducting intensive pilot studies.

Methods for identifying and screening nuclear genes

Candidate intronless genes and introns were identified from the literature, commercially available primer kits, and public access sequence databases including GenBank and the European molecular biology laboratory (EMBL) database. Intronless genes were used if they were >500 bp in length. Primers were designed using reptile sequences where possible or at least two other vertebrate families where no reptile sequences exist. Primers were located in areas with the highest sequence similarity among all available sequences. Introns were sequenced if PCR amplification resulted in a single amplification product, or if a product of consistent length could be isolated by gel separation. Amplification products from EPIC primers were not sequenced if their length was either that expected from the exon flanking sequences only (i.e., there was no intron) or was greater than ~1500 bp, because sequencing of products greater than this length was impractical for the present study. Amplification products were identified using BLAST searches (Altschul *et al.*, 1997) of GenBank, or alignment with the sequences used to generate primers. Unidentified products were not used.

Table 2.2 summarises the progress made with each gene studied. Successful loci are described below, and unsuccessful loci are described in appendix 1. All primer

Table 2.2: Summary of results using trial phylogenetic markers. 65 unsuccessful pairs from the CATs primer kit are not listed. Columns indicate: whether amplification was successful under any conditions; whether amplification resulted in a single product; whether a product of the expected length could be isolated; whether any sequencing reactions were successful; whether the product was identified as the target loci; and whether the primers successfully amplify in additional taxa. * size within expected range, with intron >2KB, ** size expected without intron 3, *** expected size – but not long enough to be informative.

Gene	Primers	Amp.	Single product	Expected length	Sequence	Correct product	Additional taxa
Successful Genes, Exons:							
C-mos	G74/303	✓	✓	✓	✓	✓	✓
Rag-1	G396/425	✓	✓	✓	✓	✓	✓
Introns:							
β-fib	G359/360	✓	✓	✓	✓	✓	✓
β-fib	G375/376	✓	✓	✓	✓	✓	✓
Myh-2	G240/315	✓	✓	✓	✓	✓	✓
Myh-2	G240/241	✓	✗	✓	✓	✓	✓
Unsuccessful Genes, Exons:							
Brain-4	G362/373	✓	✓	✓	✓	✗	
Brain-4	G363/374	✗					
Brain-4	G362/363	✓	✗	✓	✗		
Rp-1 ex4	G364/365	✗					
Rh-1 ex	G153/154	✓	✓	✓***			
Introns:							
Ald	G25/26	✓	✗	✓	✓	✓	✗
Amel	G355/356	✗					
Amel	G357/358	✗					
Hbb	G414/415	✓	✗	✗			
Hbb	G414/416	✓	✗	✗			
Lam-A	G144/145	✓	✗	NA	✓	?	✗
Lam-A	G331/332	✓	✗	NA	✓	?	✗
Rh-1 int	G153/154	✓	✓	✓	✓	✓	✗
Ttr In 1	G340/341	✓	✓	✓	✓	??	✗
Ttr In 2	G341/342	✓	✓	✓*			
Ttr In 2+3	G341/345	✓	✓	✓**			

sequences are given in appendix 2. Further information about the utility of these genes for phylogenetic studies is provided in the following chapters.

Successful nuclear coding loci

C-mos

C-mos is a proto-oncogene proposed as a candidate for phylogenetic research by Graybeal (Graybeal, 1994). *C-mos* has been used in phylogenetic studies of Australian gekkonids and turtles (Donnellan *et al.*, 1999; Georges *et al.*, 1999), the scincid genus *Mabuya* (Brehm *et al.*, 2001) and in broader analyses of relationships within squamates (Harris *et al.*, 2001; Harris *et al.*, 1999; Saint *et al.*, 1998). Conserved primers (G73/G74) amplifying a 420 bp region in reptiles were described by Saint *et al.* (Saint *et al.*, 1998). I used these published primers, with G303 (developed but unpublished by Saint) and *Eugongylus* group specific primers G336/G337 to amplify a 700 bp region of *c-mos* in the *Eugongylus* group (Figure 2.6). *C-mos* sequences are used and discussed further in Chapter 3.

Recombinase activating protein 1 (RAG-1)

The gene for the recombinase activating protein 1 (*Rag-1*) has been used for systematic studies in birds (usually in combination with *c-myc*) (Groth and Barrowclough, 1999; Irestedt *et al.*, 2001; Johansson *et al.*, 2001) and in fish (Venkatesh *et al.*, 1999). I used the primers published by Groth & Barrowclough (1999)(G396/G397), unpublished primers provided by Ted Townsend of the Biology Department, Washington University Saint Louis (G398/G399), unpublished primers provided by Kathy Saint of the South Australian Museum (G292/G293 and G325/G326) and primers I designed from skink sequences (G432, G425) to sequence ~3200 bp of *rag-1* in a subset of five taxa (Figure 2.6). Analysis of these sequences indicated that most of the variable nucleotide positions in *rag-1* occur in the first 900 bp, therefore only this region was sequenced (using primers G396/G425) in all *Eugongylus* group taxa of interest. *Rag-1* sequences are used and discussed further in Chapter 3.

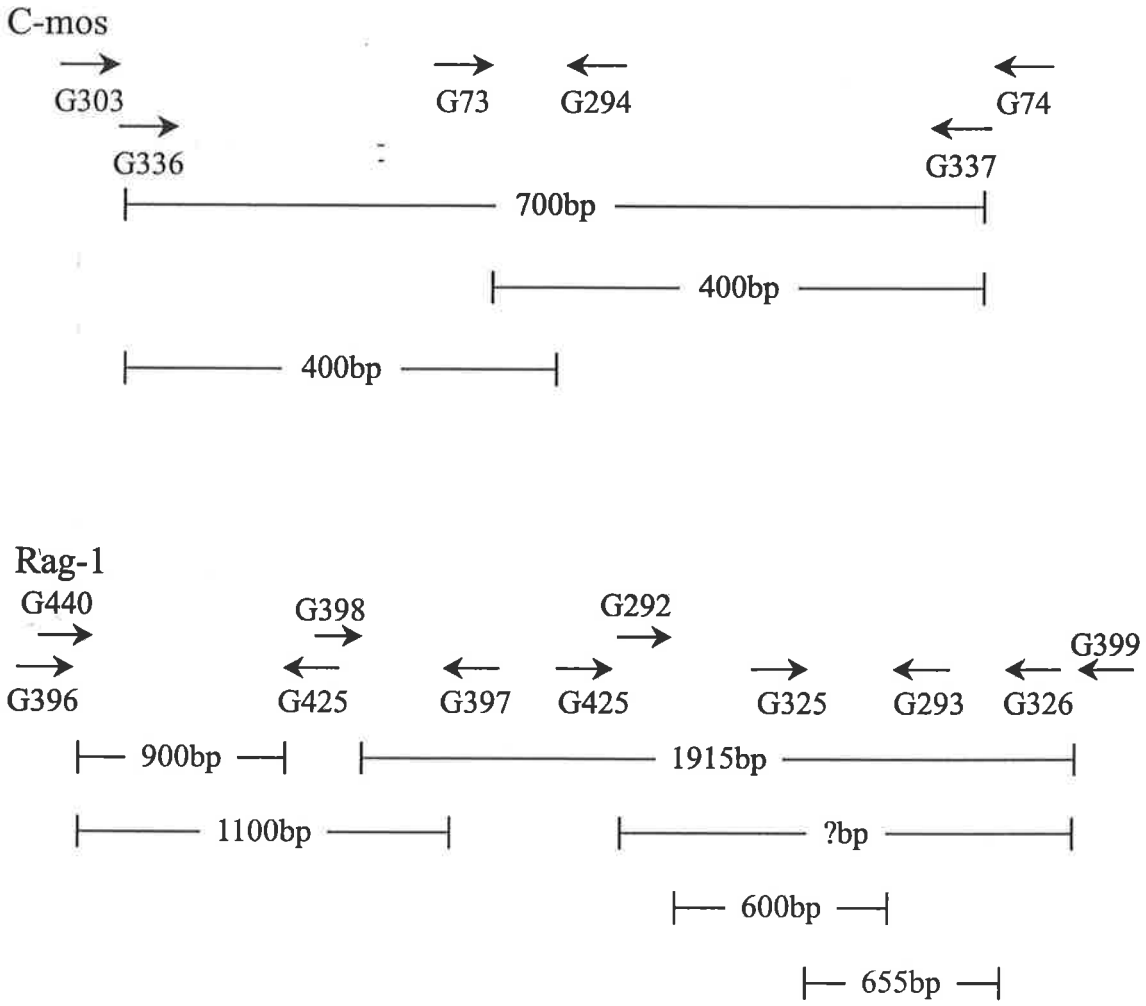


Figure 2.6: Primers used to amplify coding nuclear genes *c-mos* and *rag-1*.

Successful intron loci

β-fibrinogen (β-FIB)

Fibrinogen proteins control blood clotting and viscosity. The gene coding the beta chain is known to include eight exons and seven introns in mammals (Chung *et al.*, 1983). Intron seven of *β-fibrinogen* has been used as a phylogenetic tool in studies of birds, and comparisons with *cytochrome b* sequences for the same taxa show that although *β-fibrinogen* evolves much more slowly than *cyt b* (2.8 times more slowly overall and 14 times more slowly than *cyt b* third codon positions) it is equally phylogenetically informative (Johnson and Clayton, 2000; Prychitko and Moore, 1997; Prychitko and Moore, 2000). After it's development in birds, *β-fibrinogen* has been used in systematic studies of snakes (Giannasi *et al.*, 2001) and mammals (Springer *et al.*, 2001). The fragment amplified by Prychitko and Moore (1997) includes 40 bp of exon seven, 70 bp of exon eight and ~870 bp of intron seven. I used sequences generated with Prychitko and Moore's (1997) primers (FIB-BI7U and FIB-BI7L) to design skink specific primers (G375/G376). These primers consistently amplify a ~1000 bp product in *Eugongylus* group skinks. *β-fibrinogen* sequences are used and discussed further in Chapters 3, 4 and 5.

Heavy chain myosin 2 (alpha?) (MYH-2)

Myosin is a highly conserved protein which is active in cytokinesis, phagocytosis and muscle contraction in eukaryotic cells (Weiss and Leinwand, 1996). Class two myosins are made up of two heavy chain (*MYH*) and two pairs of light chain (*MYL*) subunits. The *MYH* intron used here was identified by a broad scale survey (all classes of vertebrates including four skink species were tested) of the loci proposed for use in mammals by Lyons *et al.* (1997). Primers used by Lyons *et al.* (1997) amplify two bands (~300 bp and ~400 bp) in *Eugongylus* group skinks. The 400bp product was sequenced and included 125bp of exon sequence. A reverse primer in the intron (G315) allowed amplification of a single product. Orthologous loci were amplified from all

Eugongylus group skinks, however because there is no reptile myosin sequence published, the exact identity of the locus has not been verified. *Myh-2* sequences are used and discussed further in Chapters 3, 4 and 5.

Summary

Nuclear paralogues of mitochondrial genes appear to be a significant problem within the *Eugongylus* group of skinks. Two of the four mitochondrial genes sequenced show evidence of nuclear copies. Paralogues were unlikely to be detected in *12S rRNA* because it was amplified as a single PCR fragment, and sequences of overlapping regions amplified with different primer pairs proved most useful for identifying paralogues. The paralogues identified here are particularly problematic because they have open reading frames, may be large (at least 5.5 kb), can be amplified from enriched mitochondrial DNA, and do not cluster in phylogenetic or distance analyses. These characteristics are not unusual among paralogues described in other taxa (see Chapter 1) but make the identification of true mitochondrial sequences difficult.

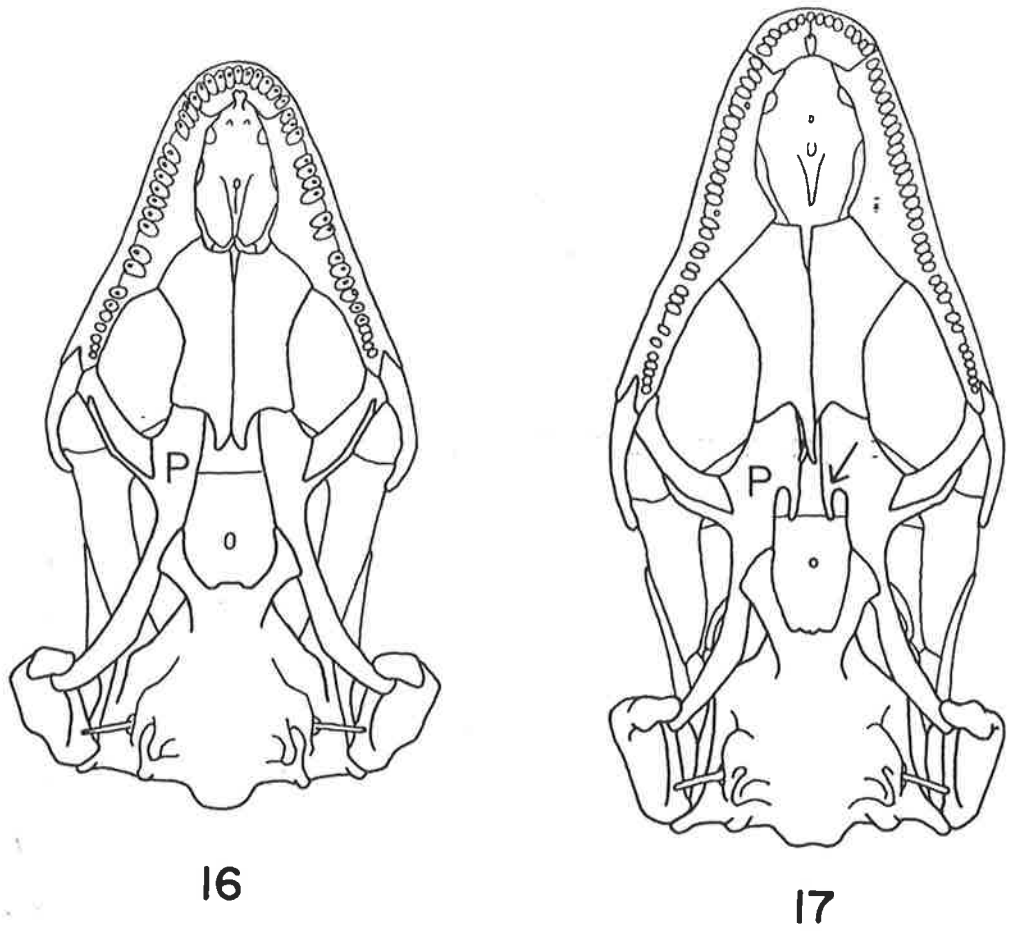
Although it has been widely recognised that nuclear DNA sequences are potentially very useful phylogenetic markers, the development of these markers has proven far more difficult than the development of mtDNA markers. Of the 128 primer pairs I attempted to utilise (including the CATS and UBC-SFU kits, see appendix 1), four (or 3%) have ultimately been demonstrably useful, and one, TTR (see appendix 1) may be useful among closely related groups, where primers inside the intron could be used to sequence shorter pieces.

Chapter 3 - Phylogenetic analysis of the *Eugongylus* group

Introduction

The *Eugongylus* group is one of five linegaes of scincid lizards that are the only reptiles to have evolved complex placentation. Two of the four placental types described in reptiles only occur in the *Eugongylus* group. The morphology and physiology of these placentae are far better documented than those of any other taxa (Blackburn, 2000; Thompson *et al.*, unpub ms). The *Eugongylus* group therefore offers the best available opportunity for evolutionary analysis of placental structure and function in a group of reptiles. Such evolutionary analyses however, rely on a robust estimate of the phylogenetic relationships among taxa, particularly the relationships among *Pseudemoia* and *Niveoscincus*, and related oviparous taxa .

The *Eugongylus* group includes ~40 genera distributed throughout the southern hemisphere (Greer, 1970). The taxonomy and relationships of these genera have previously been addressed using morphological, allozyme, chromosome, and immunological data (Donnellan and Hutchinson, 1990; Greer, 1974; Greer, 1980; Greer, 1982; Greer, 1979; Hardy, 1977; Hardy, 1982; Hutchinson *et al.*, 1990). The *Eugongylus* group was divided into the *Lampropholis* (beta palate) and *Eugongylus* (alpha palate) subgroups on the basis of secondary palate structure (Figure 3.1, Greer, 1979). The *Lampropholis* subgroup is geographically disjunct and includes five Australian and New Guinean genera, and the African genera *Panaspis* and *Cophoscincopus*. The remaining members of the *Eugongylus* group are assigned to the *Eugongylus* subgroup. Hutchinson *et al.* (1990) used immunological, karotypic and morphological data to divide the Australian species of *Leiolopisma* (a genus which was originally included most of the *Eugongylus* group genera occurring in the Indo-Pacific including Mauritius, New Caledonia, New Zealand and Australia) into five Australian-endemic genera. The relationships estimated by Hutchinson *et al.* (1990) from their immunological distance data are shown in figure 3.2. These results suggest that the



Figs. 16-17. The two types of secondary palate in the *Eugongylus* group: 16, the alpha palate of the *Eugongylus* subgroup *(Leiolopisma pretiosum)*; 17, the beta palate of the *Lampropholis* subgroup *(Carlia longipes)*.

Figure 3.1: Alpha and beta secondary palate structure. Figure from Greer (1979)

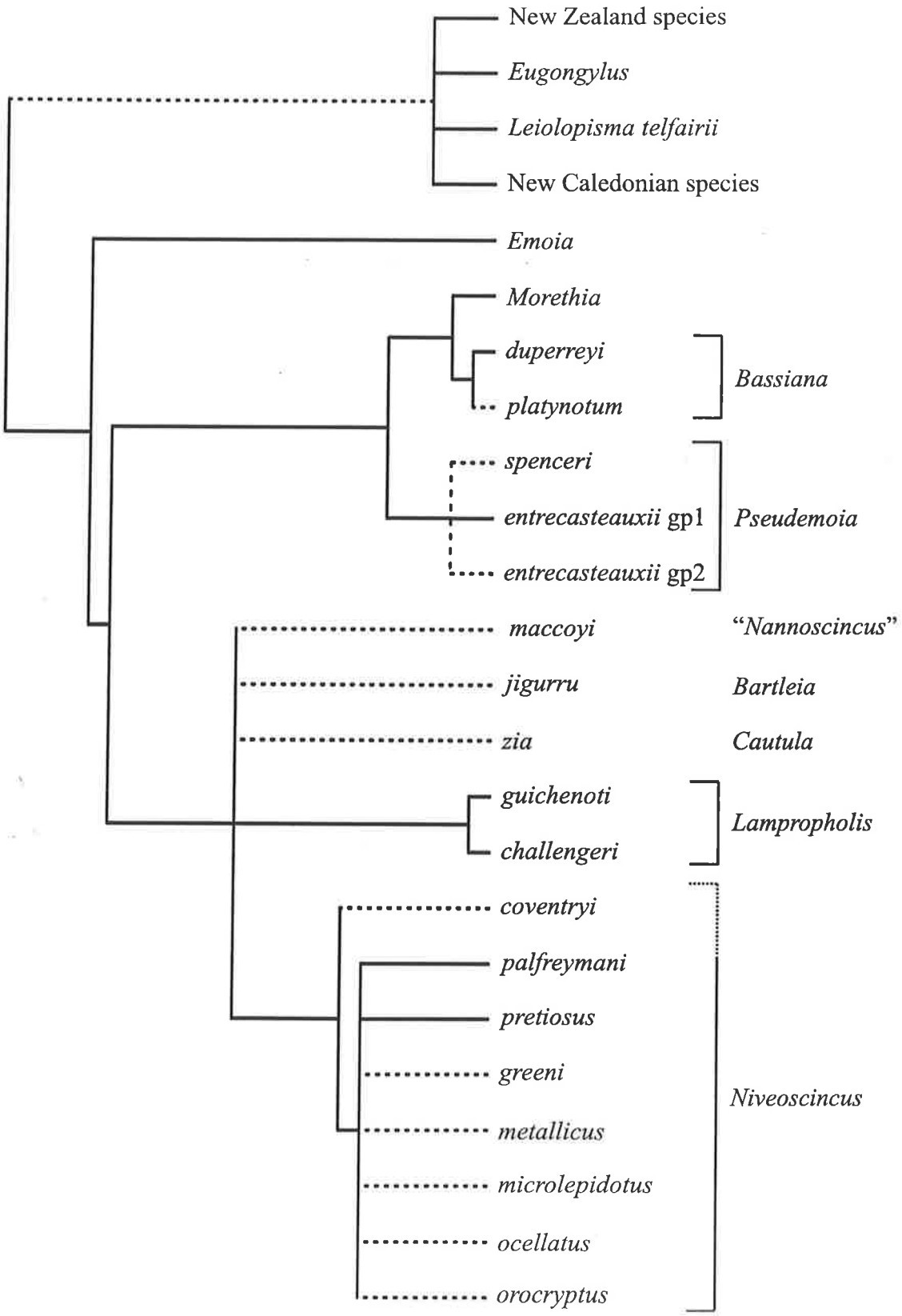


Figure 3.2: Phylogenetic relationships among Australian *Eugongylus* group skinks suggested by Hutchinson *et al*. (1990). Dotted lines represent taxa with single direction immunological comparisons, solid lines are taxa involved in reciprocal comparisons.

Australian *Eugongylus* group taxa form a monophyletic group and the New Zealand, New Caledonian, and Lord Howe Island *Eugongylus* taxa form the monophyletic sister lineage to the Australian species. Hutchinson *et al.*'s (1990) results also suggest that the Australian *Eugongylus* group is divided into two main lineages which do not correspond to the previously described *Lampropholis* and *Eugongylus* subgroups, and each include one of the placental genera. Although analysis of the immunological data resulted in an almost fully resolved tree, there is no estimate of the robustness of any of its lineages, and some rely on one-way comparisons of very dissimilar taxa. A nucleotide sequence based phylogeny of the *Eugongylus* group would allow the relationships between genera in Australia, Africa, New Zealand, and New Caledonia to be reconstructed with greater confidence. This information will contribute to studies of the biogeography of Gondwanan reptiles (Chambers *et al.*, 2001).

Coding nuclear DNA sequence data (from *c-mos*) have been used to recover relationships among squamate lineages (Harris *et al.*, 2001; Harris *et al.*, 1999; Saint *et al.*, 1998). Divergences among the *Eugongylus* group will be much shallower than divergences among squamates as a whole, and therefore may not be efficiently recovered using sequences from protein coding nuclear loci. Introns in nuclear genes are not under the same functional constraints as coding loci and are therefore expected to be more variable and more informative than coding regions for shallow divergences. Intron data may therefore be able to recover relationships among the *Eugongylus* group. I have used sequence date from *c-mos* and *rag-1*, and *β-fibrinogen* and *cardiac myosin* introns to recover the phylogenetic relationships between members of the *Eugongylus* group from throughout their geographic range. The two aims of this study are to 1) recover a phylogeny of the *Eugongylus* group skinks; and 2) to assess the usefulness of nuclear DNA for phylogenetic studies of reptiles.

Materials & Methods

Specimens and genes

The ingroup for this analysis comprises 22 *Eugongylus* group taxa from Australia, New Zealand, New Caledonia, the Pacific and Africa (Table 3.1). Australian and New Zealand live-bearing lineages (*Pseudemoia, Niveoscincus, Cyclodina* and *Oligosoma*) are represented by multiple species. There are at least two live-bearing species known from New Caledonia (*Lioscincus tillieri* and *Marmorosphax tricolor*), although the reproductive mode of many species remains unknown (Table 3.2). Tissues from live-bearing species from New Caledonia were not available, however an oviparous species of *Lioscincus*, a genus that includes one live-bearing and four egg-laying species, was used. Two specimens of *Emoia cyanura* were sequenced to verify the identity of this species because of its unexpected position in the tree and sequences from these individuals were identical. These sequences were also compared, and did not match, with published sequences from *Lipinia* and *Lamprolepis*, two skinks which could potentially have been misidentified as *E. cyanura*. Trees were rooted using the non-*Eugongylus* group lygosomine skinks *Mabuya longicaudata* and *Tiliqua scincoides* and the scincine *Eumeces skiltonianus* (*Rag-1* sequence from *E. inexpectatus* provided by T. Townsend, Washington University, St Louis, Missouri; was used). I used nucleotide sequences from *β-fibrinogen* intron 7, *c-mos*, *heavy chain myosin 2*, and *rag-1*. Primers are listed in Appendix 2 and are discussed in Chapter 2.

DNA extraction, amplification & sequencing

Amplification was conducted using TaqGold hot-start polymerase. After initial denaturation at 94 °C for 9 min, each cycle consisted of denaturation at 94 °C for 45s, annealing at 50-55 °C for 45s, and extension at 72 °C for 1 min, for 34 cycles. Negative controls were run for all amplifications. Amplified products were purified using UltraClean PCR cleanup columns (Mo Bio Laboratories). Products were sequenced

Taxon	Spec. Number	Location	Distribution	Reprod. Mode	Placenta Type
Bassiana trilineata	SAMA R22893	Pink Lake, Esperance, WA	Australia	Oviparous	
Caledoniscincus austrocaledonicum	AMS R110289	Redika Is, New Caledonia	New Caledonia	Oviparous	
Carlia longipes	QM J57111	Heathlands, Qld	Australia	Oviparous	
Cryptoblepharus plagiocephalus	SAMA R52942	Gammon Rages, SA	Australia, genus Pacific	Oviparous	
Cyclodina aenea	FT 5253		New Zealand	Viviparous	unknown
Emoia cyanura	AMS R136314 AMS R136317	Mole Is, Solomon Is	Broadly across Pacific	Oviparous	
Emoia nigra	AMS R136376	Honiara, Guadalcanal, Solomon Is	Solomon Islands, Vanuatu, eastward through Fiji to Tonga and Western Samoa, Papua New Guinea	Oviparous	
Eugongylus rufescens	AMS R122480	Bobole SHP, Papua New Guinea	Australia, Admiralty Islands, Indonesia, New Guinea, Solomon Islands	Oviparous	

Taxon	Spec. Number	Location	Distribution	Reprod. Mode	Placenta Type
Eumeces skiltonianus	ABTC66122	No data	Canada, USA, Mexico	Oviparous	
Lampropholis guichenoti	SAMA R39155	Goulbourn, NSW	Australia	Oviparous	
Leiolopisma telfairii		Round Is	Round Is, Mauritius	Oviparous	
Lioscincus nigrofasciolatum	AMS R125820	New Caledonia	New Caledonia	Oviparous	
Mabuya longicaudata					
Menetia greyii	SAMA R51563	Amata, SA	Australia	Oviparous	
Morethia ruficauda	SAMA R34102	Undoolya Station, NT	Australia	Oviparous	
Nannoscincus maccoyi	NMV D62103	Bonang tip, Vic	Australia	Oviparous	
"Niveoscincus" coventryi	NMV D62072	Beech Forest, Vic	Australia	Viviparous	type II (lecithotrophic)
Niveoscincus greeni	TMH C569	Mt Rufus, Tas	Australia	Viviparous	type II
Niveoscincus metallicus	NMV D60517	Westernport Bay, Vic	Australia	Viviparous	type II
"Oligosoma" lichenigera	AMS R93465	Balls Pyramid, Lord Howe Island	Lord Howe Island	Oviparous	

Taxon	Spec. Number	Location	Distribution	Reprod. Mode	Placenta Type
Oligosoma smithi	FT 193		New Zealand	Viviparous	unknown
Panaspis wahlbergi	ABTC367122	Mozambique	Sub-Saharan Africa	Oviparous	
Pseudemoia entrecasteauxii	SAMA R49571	Carpenter Rocks, SA	Australia	Viviparous	type III
Pseudemoia spenceri	AMS R104774	Kanangra-Boyd NP, NSW	Australia	Viviparous	type III
Tiliqua scincoides	SAMA R28511	120K NE Minnipa, SA	Australia, Genus New Guinea	Viviparous	type I

Table 3.1: Specimen number, location, species distribution and reproductive mode of species included in phylogenetic analysis of *Eugongylus* group skinks. AMS: Australian Museum, Sydney; SAMA: South Australian Museum, Adelaide; ABTC: Australian Biological Tissue Collection, South Australian Museum, Adelaide; FT: collection of Dr C Daugherty Victoria University, Wellington New Zealand; NMV: National Museum Victoria, Melbourne; TMH: Tasmanian Museum, Hobart; QM: Queensland Museum, Brisbane.

Species	Reprod. Mode of Genus
Caledoniscincus cryptos	Oviparous
Geoscincus haraldmeieri	Monotypic
Graciliscincus shonae	Monotypic
Lacertoides pardalis	Monotypic
Marmorosphax montana	Oviparous (2 spp)
Nannoscincus exos	Oviparous (2 spp)
Nannoscincus greeri	Oviparous (2 spp)
Nannoscincus hanchisteus	Oviparous (2 spp)
Nannoscincus humectus	Oviparous (2 spp)
Nannoscincus rankini	Oviparous (2 spp)
Phoboscincus boucourti	Unknown
Phoboscincus garnieri	Unknown
Simiscincus aurantiacus	Oviparous
Tropidoscincus aubrianus	Unknown
Tropidoscincus boreus	Unknown
Tropidoscincus variabilis	Unknown

Table 3.2: New Caledonian skink species for which reproductive mode is unknown. Reproductive mode of other species within the genus is indicated where known. All data taken from Bauer and Sadlier (2001).

using the ABI BigDye sequencing kit, and sequence products were electrophoresed on an ABI 3700 capillary sequencer.

Sequence interpretation and phylogenetic analysis

Sequences were edited using SeqEd (ABI) and aligned by eye using Se-Al v1.0 a1 (Rambaut, 1995). Complete alignments are given in appendix 3. Sites for which all taxa except one have gaps (autapomorphic indels) were excluded in all further analyses. Coding of gaps for parsimony analysis was discussed in Chapter one. I applied the simple and complex coding methods of Simmons and Ochoterena (2000) and the effectiveness of these methods is discussed below. All characters were weighted equally. Phylogenetic analyses were done using PAUP* v 4.0 b8 (Swofford, 1999) on power Macintosh G3 and G4 computers (G4 analyses used the altivec version of PAUP*). Each gene was treated as a separate partition and combinability of partitions was assessed using the incongruence length difference (ILD) test (Farris *et al.*, 1995; Mickevich and Farris, 1981) implemented in PAUP*, and the partitioned Bremer index (Baker and Desalle, 1997) (described in Chapter 1) implemented using Treerot v 2a (Sorenson, 1999) and PAUP*. Tree searches were conducted using an heuristic algorithm (with 500 random addition sequence replicates for maximum parsimony (MP) analysis and 'as-is' sequence addition for maximum likelihood (ML)). Maximum likelihood (ML) analysis was carried out under a model determined by hierarchical likelihood ratio tests implemented using Modeltest v 3.1 (Posada and Crandall, 1998). Model parameters for the dataset were estimated from the most parsimonious trees. Bootstrap resampling was used to gauge support for optimal trees, with 200 heuristic pseudoreplicates (with 10 random addition sequences each) for MP, and 100 pseudoreplicates for ML (using 'as-is' sequence addition). The optimal trees resulting from MP and ML analysis were tested against alternative topologies using Templeton's implementation of the Wilcoxon signed rank (Templeton, 1983) and Shimodaira-Hasegawa tests (Shimodaira and Hasegawa, 1999) respectively. The alternative

topologies tested were those that allowed more parsimonious explanations of the evolution of reproductive mode than the best tree (Table 3.7).

Resolving polytomies

Polytomies can occur either because of insufficient data (soft polytomies) or because the true species phylogeny includes a polytomy (hard polytomies). Hard polytomies are usually defined as "simultaneous" speciation events, but more practically represent rapid speciation. Distinguishing between hard and soft polytomies is complicated by the fact that all methods of phylogenetic reconstruction have a polytomy (where all species are equally related) as their null hypothesis, and this therefore cannot be tested. Despite the difficulty of these analyses, it is useful to know whether a polytomy is soft and therefore could be resolved with additional data, or hard and cannot be resolved with additional data. Several approaches have been used distinguish hard and soft polytomies. Broadly, approaches for understanding polytomies rely on one of three methods – simulation, statistical power tests, or likelihood ratio tests.

Hulsenbeck and Hillis (1996) suggest that the parametric bootstrap can be used to determine the amount of data required to resolve a tree, and therefore to determine whether inadequate sampling could be the cause of polytomies. In general the parametric bootstrap is implemented by the following steps: starting with a tree and model of evolution, simulate new character matrices assuming this model, then analyse the replicate simulated data sets. To determine the amount of data required to resolve a tree, the model and its parameters are determined from the pilot data, then datasets of varying sizes are simulated on a tree. The frequency with which the tree is recovered by simulated datasets of different size allows the minimum dataset size to be calculated. The two main limitations of this method are that simulation of datasets and analysis of replicates requires large amounts of computer power and time, and that the tree on which the data are simulated cannot be objectively specified. A fully resolved tree must be the basis of simulation to determine how much data would be required to recover

such a tree if it is the true species tree. But, if the preliminary data result in a polytomy, the resolution and branch length of the nodes in the simulation tree cannot be determined from the data. Jackman *et al.* (1999) also utilised simulation as part of a multifaceted approach to polytomy testing. Jackman *et al.* tested the support for branches (measured using the parametric bootstrap) obtained from subsets of their data against the resolution of simulated random data. This test was based on the expectation that multiple branching events which are not recovered when many taxa are analysed will be resolved by subsets of taxa. However, this expectation does not have any firm theoretical support, and the method has not been used by other authors.

Statistical power tests can also be used to determine whether polytomies are hard or soft. Walsh *et al.* (1999) used power tests (difference of a proportion and difference of a mean tests) to estimate the amount of sequence needed to distinguish an internode of 100,000 yr or more from a zero length internode for five species of auklet. The authors regard internodes of less than 100,000 yr, where divergence occurs over successive glacial / interglacial periods, as hard polytomies. The introduction of a biologically determined definition a soft polytomy is a particularly appealing aspect of Walsh *et al.*'s analysis. Braun and Kimball (2001) modified Walsh *et al.*'s method by modelling nucleotide substitution as a Poisson process. This allows much easier calculation and gives almost identical results (Walsh and Friesen, 2001). An alternative use of Braun and Kimball's method has not previously been noted. Using this analysis, it is possible to determine the shortest internode that can be distinguished with a dataset. This method is computationally simple and gives an estimate of the "softness" of a polytomy even in the absence of a specific definition of a polytomy for the species in question. Braun and Kimball's equation (3) (modified so β=0.20 as suggested in Walsh and Friesen, 2001) states

$$L = \frac{-\log \text{normal}(0.20)}{\lambda t}$$

where L is sequence length (in base pairs), λ is the rate of sequence evolution (in substitutions per site per year) and t is the time period considered in years. This

equation can be rearranged to give the minimum internode length which can be distinguished using the data:

$$t = \frac{-\ln(0.02)}{\lambda L}$$

The usefulness of this method is limited by the difficulty of calculating λ, the number of substitutions per site per year for a given dataset. In some cases, where no node on the tree can be dated, it may be impossible to make a realistic estimate of λ. I have applied the method to the four nuclear genes used here, with a hypothetical time scale.

In reviewing other methods for examining polytomies, Slowinski (2001) correctly noted that when applied to trees generated from a set of non-independent nucleotides they test for gene polytomies rather than species polytomies. Testing for gene polytomies can most simply be done using likelihood ratio tests (LRTs). Each node in turn is constrained to a length of zero and this tree is tested against the best unconstrained tree using an likelihood ratio test. Slowinski also suggested how this method can be extended to determine whether a species polytomy underlies a set of gene trees. Pamilo and Nei's (1988) equations show that when a species polytomy occurs, each gene tree resolution is expected to occur equally frequently. However, if the underlying species phylogeny is bifurcating, the true resolution of the gene tree should be observed more frequently than other alternatives. Under these conditions, a null distribution of gene tree topologies can be generated under the multinomial distribution. The distribution of real gene trees can then be tested against this expected distribution using a chi squared test. A limitation of this approach is that the power of the test is dependent upon the number of resolved gene trees that are used to evaluate each node. The number of resolved gene trees that can be used for this test is limited by the finite number of nucleotides in each gene (which limits the resolution of each gene tree), and the practical limitations on how many genes can be sequenced. Implementing this method successfully will require a large amount of sequence data, and significant computation time.

Results

Information content and congruence of partitions

Alignment of sequences resulted in a total of 2865 characters excluding autapomorphic insertions (4109 including all sites); of these characters 417 were parsimony informative. Table 3.3 lists the taxa for which sequences of some genes could not be obtained. Both intron loci were more difficult to amplify than the coding sequences, and *myh* was not successfully amplified from any outgroup taxon. A non-significant result of the ILD test (P = 0.06) suggests that all data should be combined in further analysis. Positive or low negative values of the partitioned Bremer index (the lowest being -2.5 for node 12) for each gene on the most parsimonious trees (Table 3.4) also suggest that there is little or no conflict between genes and they should therefore be combined.

The relative variability and information content of genes can be indirectly assessed in a number of ways. Table 3.5 shows the total number of characters, number of characters excluding autapomorphic indels, the number of variable characters, the number of parsimony informative characters, the percent of characters that are informative, and the rate of evolution (determined by ML from the most likely tree by applying site specific rates) for each gene. Figure 3.3 shows the relationship between ML distance and uncorrected sequence divergence for each pairwise comparison between taxa for each gene. The slope of each gene in figure 3.3 is approximately the site specific likelihood rate, however the vertical spread of points for each gene shows that some genes accumulate substitutions more predictably than others. *Myosin* in particular has points spread across a wide vertical range on this figure. Overall these data suggest that the introns accumulate substitutions more quickly than coding sequences (introns have greater slopes in figure 3.3 and higher SSR estimates), even when autapomorphic indels are excluded, but that this does not directly correlate with their information content. The highest proportion of informative characters occurs in *rag-1*, despite its moderate rate of evolution, presumably because the level of homoplasy in this gene is low.

Gene	Species	Putative explanation
β-fib	*Nannoscincus maccoyii*	Incomplete sequence
	Niveoscincus coventryi	2kb product failed to sequence
myh	*Carlia longipes*	Wrong locus amplified
	Eumeces skiltonianus	
	Mabuya longicauda	Wrong locus amplified
	Niveoscincus metallicus	
	Panaspis wahlbergi	Wrong locus amplified
	Tiliqua scincoides	
rag-1	*Leiolopisma telfairii*	No amplification

Table 3.3: Missing data.

Table 3.4: Bremer and bootstrap support for the MP consensus tree (Figure. 3.6). Bremer and bootstrap values apply to the branch proceeding each node. The number of parsimony informative characters for each partition are at the top of each column.

Node	Bootstrap Proportion	Bremer Index	Partitioned Bremer Index			
			β-fib	*c-mos*	*myh-2*	*rag-1*
			138	65	43	171
Ingroup:						
1	-	1	0	0	1	0
2	76	5	-0.3	3.5	0.2	1.6
3	95	7	0	5	2	0
4	-	1	0	0	1	0
5	97	5	0	2	2	1
6	99	8	0	6	2	0
7	60	4	0	2.6	0.4	1
8	-	4	1	0	0	0
9	-	2	0	0.8	1.4	-0.2
10	100	8	1	0	0	7
11	58	1	1	1.2	0.2	-1.5
12	65	6	0.5	-2.5	3.5	4.5
13	-	1	0	0.9	0.4	-0.3
14	52	1	-2.0	1	-0.6	2.6
15	-	1	-0.4	0.8	0.6	0.1
16	100	11	0	3	1	7
17	-	1	-2	1	-0.8	2.8
18	100	21	2	6.4	0.3	12.3
19	97	12	5	5	1	1
Outgroups:						
20	-	50	18.7	11.8	0.2	19.3
21	74	4	1	2	0	1

Gene	Length 1	Length 2	Variable Characters	PI Characters	% PI a	% PI b	CI	SSR
β-fib	2269	1073	422	138	13	33	0.59	1.330
c-mos	553	553	164 (91)	65 (39)	12 (21)	40 (43)	0.61	0.472 (0.87)
myh	417	369	162	43	12	27	0.59	1.203
rag-1	870	870	395 (186)	171 (87)	20 (30)	43 (47)	0.55	0.837 (1.23)
Total:	4109	2865		417		-	0.77	-

Table 3.2: Estimates of the information content of each gene. Data from third codon positions of coding genes are given in brackets. Length 1 = total length of sequence, Length 2 = length of sequence excluding autoapomorphic indels, Variable Characters = number of variable characters, PI Characters = number of parsimony informative characters, % PI = percent of (a) total characters and (b) variable characters from this gene (or codon position) which are parsimony informative, CI = consistency index on the most parsimonious trees from the combined data, SSR = rate estimated from ML.

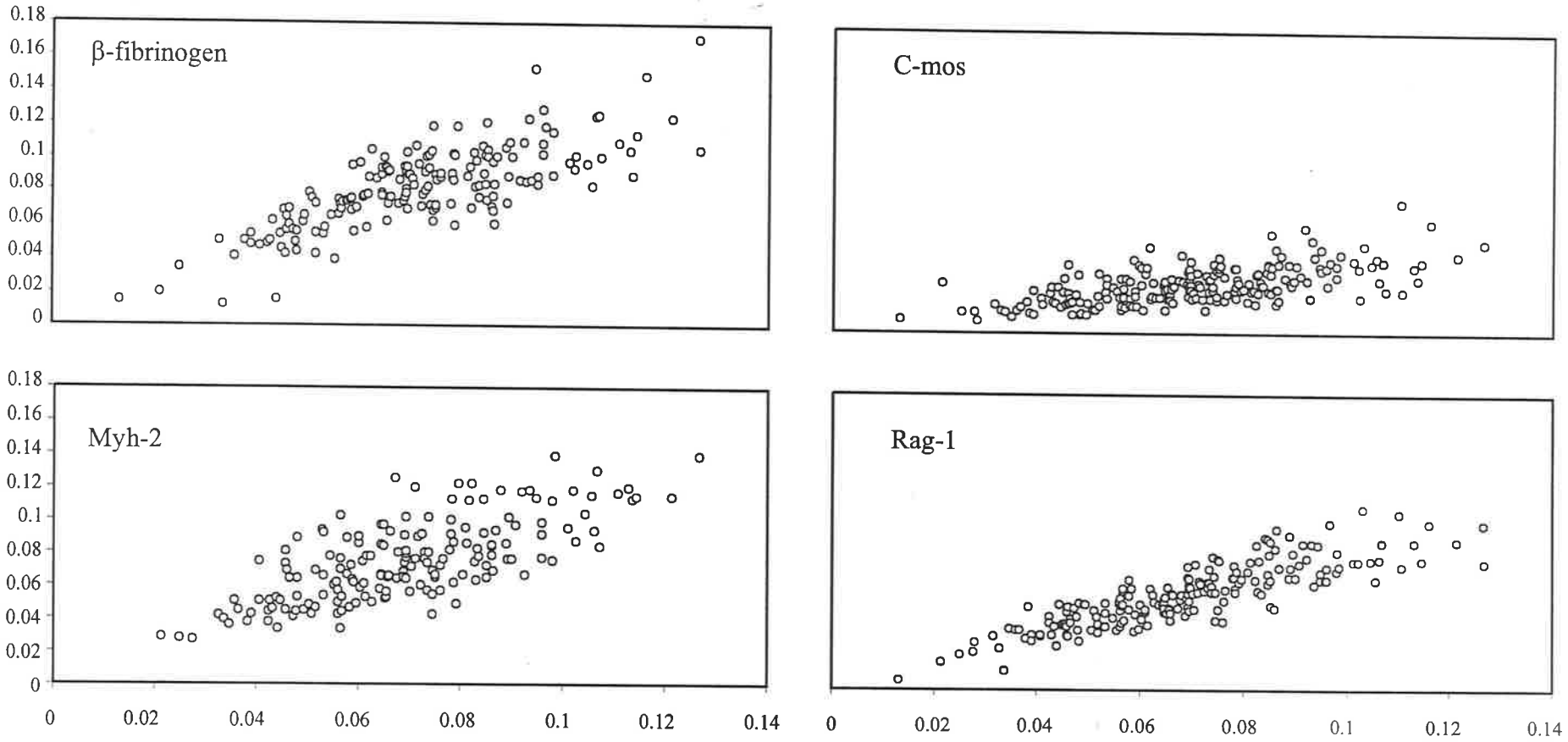


Figure 3.3: Pairwise comparisons of ML distance estimated from combined data (x-axis) *versus* uncorrected p from each gene (y-axis), for *Eugongylus* group taxa.

Gap coding

Gaps occur in the aligned sequences of all genes used (Appendix 3). Gaps in coding genes occur in groups of three bases, as expected when an entire codon is inserted or deleted from a sequence. Gaps in intron sequences are more common, length variable and occur in more complex arrangements. All gaps have been coded using Simmons and Ochoterena's (2000) simple and complex gap coding methods. *β-fibrinogen* has by far the most complex gap pattern of any of the genes used. The first 150bp of the *β-fibrinogen* alignment are used as an example of how simple and complex codings are applied. Excluding the autapomorphic gaps in bases 1-20, there are 15 gaps in the first 150 bp of *β-fibrinogen* (Figure 3.4). Using the simple gap coding method, each gap is a separate character scored as present, absent or inapplicable. Characters are inapplicable when the gap in question is entirely a subset of another gap – for example gap three cannot be scored in *M. longicaudata* because it is a subset of gap two and its absence is impossible to infer. Using simple gap coding, ten of the 15 gap characters are autoapomorphies and are therefore uninformative about phylogenetic relationships. Eleven characters have uncertainties caused by overlapping gaps; for example, gap four can only be scored for two taxa because the remaining taxa all have gap three which overlaps gap four. This introduction of missing data is one of the authors' criticisms of their simple method. The other criticism of simple coding is that it can infer changes between character states via fewer steps than is biologically possible. For example it costs one step to change from a sequence with gap two to a sequence with gap three, whereas at least two evolutionary transitions would be required - either an insertion at each end of gap two, or a single insertion of the whole of gap two followed by the deletion of gap three.

The complex gap coding method is proposed as an alternative to simple coding, and does not introduce missing data. The complex method involves the implementation of six rules based on explicit assumptions about sequence evolution (Simmons and Ochoterena, 2000, Table 1). Using complex coding, overlapping gaps are included in

```
B. trilineata          TGCATTTTAT GCATTTGAAG ---------- -TTAYGTGGA A-TGGAGATA AATTACAGCT TTATAACTCT TAATATTTGC
C. austrocaledonicus   .......... .......... ---------- -...CA.... .-........ .....A.... .A........ .......GCA
C. longipes            G......-.. .....-..CC ---------- -..-CA.... .-........ .......... .......C.. ...C...GCA
C. plagiocephalus      .......... .......... ---------- -...CA.... .-........ ..G....... .......... ..G....GCA
C. aenea               .......... .......... ---------- -...CA.... .-........ .......... .......... .......GCA
E. cyanura             ????...... .......... -----[1]---- -A..CA.... .-........ ...C...... .......... G......GCA
E. nigra               ?????????? ?????..... ---------- -...CA.... .-........ ...-G..... ...G...... ..C....GAA
E. rufescens           .......... ..T....... ---------- -G..CA.... .-........ .......... .......... .......GCA
L. guichenoti          G.......-- -------... ---------- -...CA.... .-........ .......... ....G..... .......GCA
L. telfairii           .......... ........G. ---------- -...CA.... .-........ .......... ....C..... .......GCA
L. nigrofascolatum     .......... .......... ---------- -...CA.... .-........ .....A.... .A........ .......GCA
M. greyii              ?????..... .........A ---------- -...TA.... .-........ .......... .......... ....G..GCA
M. ruficauda           .......... .......... ---------- -...CA.... .-........ .......... .......... .......GCA
N. greeni              .......... .......... ---------- -...CA.... .-........ .......... .......... ...C...GCA
N. metallicus          .......... .......... ---------- -...CA.... .-........ .......... .......... ...C...GCA
O. lichenigera         .......... .......... ---------- -...CA.... .-........ .......... .......... .......GTA
O. smithi              .......... .......... ---------- -...CA.... .-........ .......... ........G. .......GCA
P. entrecasteauxii     ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. spenceri            .......... .........C ---------- -...CA.... .-........ .......... .......... .......GCA
E. skiltonianus        .....C..G. .......... CATATGATTC T...C...T. .C......C. ....G.T... ...C.G.... .......GCA
M. longicaudata        ....CC..G. .......... CATATGATTT G...CA.... .A......G. ....G.C... ...A...... .......---[2]-
P. wahlbergi           ?????????? ?????????? ?????????? ????????.. --........ ..CC...... .......... ...C..CGCA
T. scincoides          .A..CC.... C....A.... CATATGATTG T...CA.... .A......C. ....G.T... ...C...... .......GCA
```

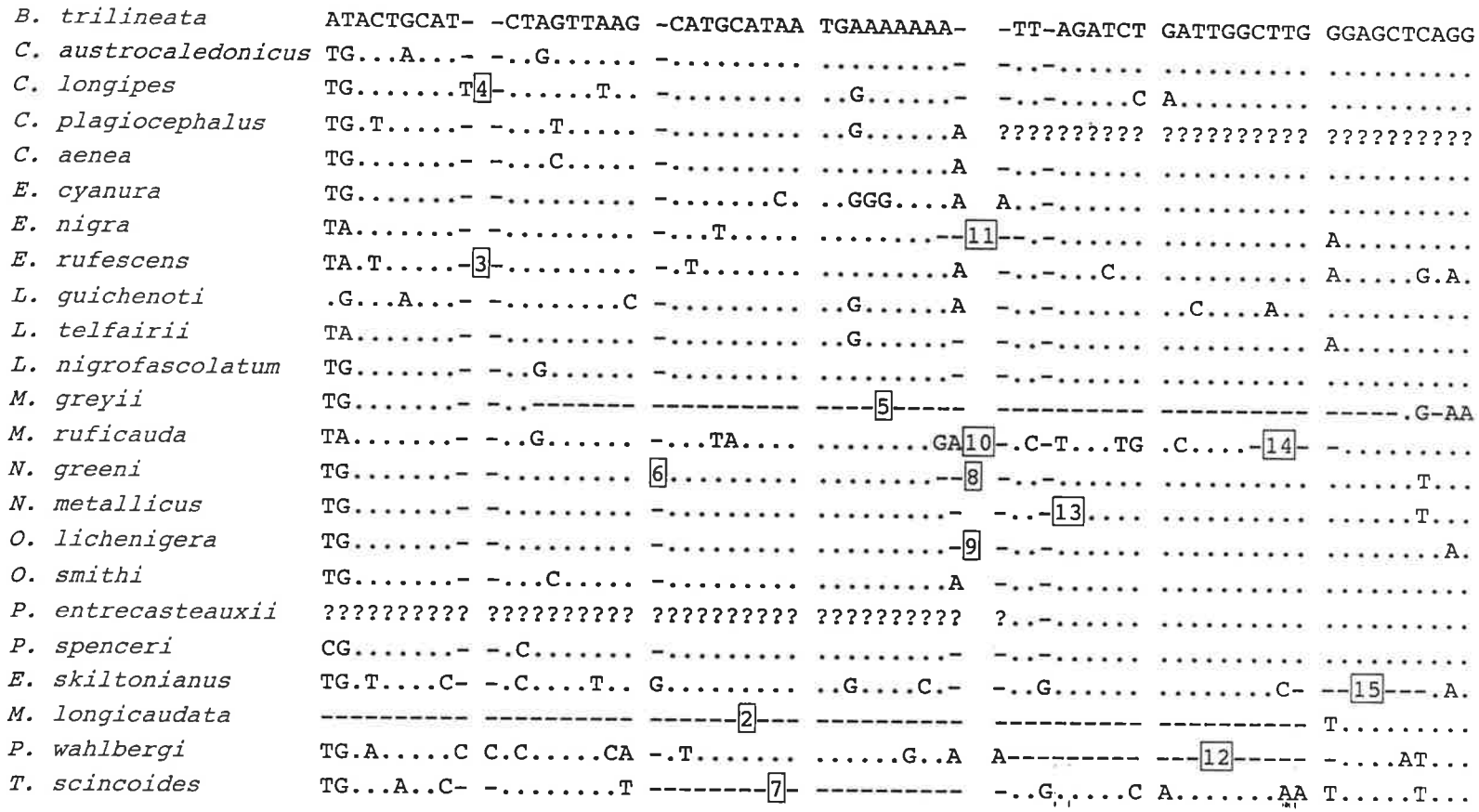


Figure 3.4: Distribution of gaps in 5' 150bp of *β-fibrinogen*. See Appendix 3 for coding of these gaps using simple and complex methods.

multistate characters with step matrices applied according to the assumptions about sequence evolution. For example, rule four states that an asymmetric step matrix should be implemented for gaps which are a subset of other gaps (with one step from short to long gap, and two steps from long to short gap). This weighting is justified by the model, which suggests that the short gap can be changed to the long gap in one step (one deletion) while two evolutionary events are required to change from a long gap to a shorter subset of the gap (either an insertion at each end of the existing gap, or a single insertion of the whole gap followed by the deletion of the shorter gap). The 15 gaps in the 5' 150bp of *β-fibrinogen*, which were 15 characters using simple coding, are combined into three characters under the complex gap coding rules. Character one is a simple character coding presence or absence of gap one, character three is the presence or absence of gap 15. Character two is a complex character with a step matrix of 24 states (Appendix 3) resolved using all six rules. Nine of the 12 states of character two that occur in the dataset (there are 12 states that are the presence of a single gap that do not occur in the dataset) are autapomorphic although these states are not necessarily uninformative when the step matrix is applied. Simple and complex coding of gaps in *c-mos*, *myh* and *rag-1* are shown at the end of sequences in appendix 3. Table 3.6 lists the total number of characters, and the number of autapomorphies from gaps in each gene.

Parsimony analysis of the data sets including gaps (maximum likelihood analysis incorporating gaps requires a likelihood model incorporating transitions between gaps and other characters, and is not practical at present) is not possible using complex coding because the step matrices generated are too large to be implemented using PAUP*; trees resulting from simple coding are discussed below.

Optimal trees

Maximum parsimony analysis resulted in two equally most parsimonious trees (1821 steps; Figure 3.5, consensus tree Figure 3.6). Hierarchical likelihood ratio tests of models suggest that the appropriate model of sequence evolution for these data is the

Gene	Simple Coding		Complex Coding	
	# Characters	# autap.	# Characters	# autap.
*β-fib***	14	10	3	1
c-mos	4	2	4	2
myh	8	8	5	3
rag-1	9	5	9	5

Table 3.6: Characters resulting from Simmons and Ochoterena's (2000) simple and complex gap coding methods. ** *β-fibrinogen* figures include only 150bp of the 5' end of sequences, see text for explanation.

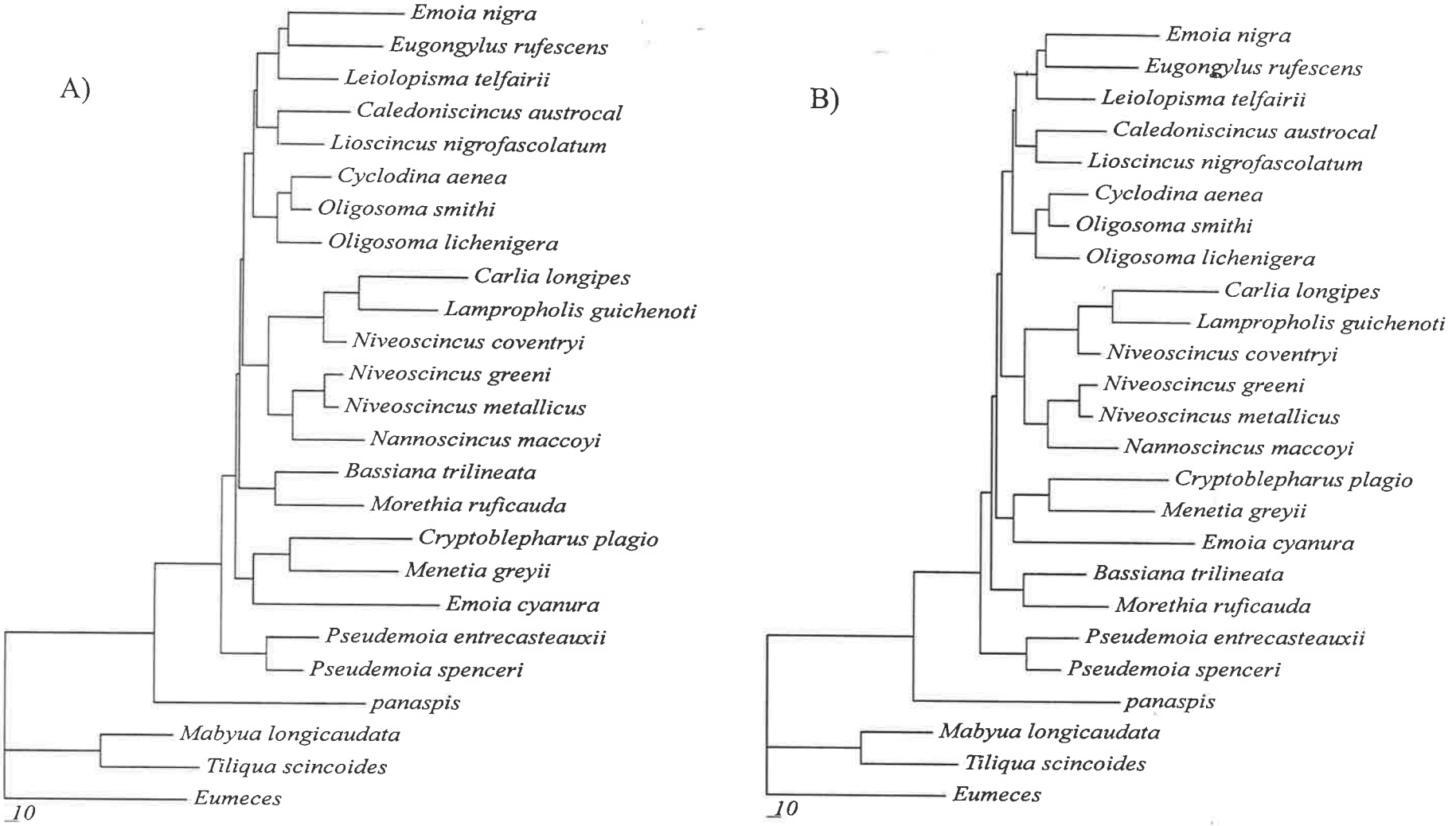


Figure 3.5: Two equally most parsimonious trees. Tree A is also the single most parsimonious tree when simple gaps are included.

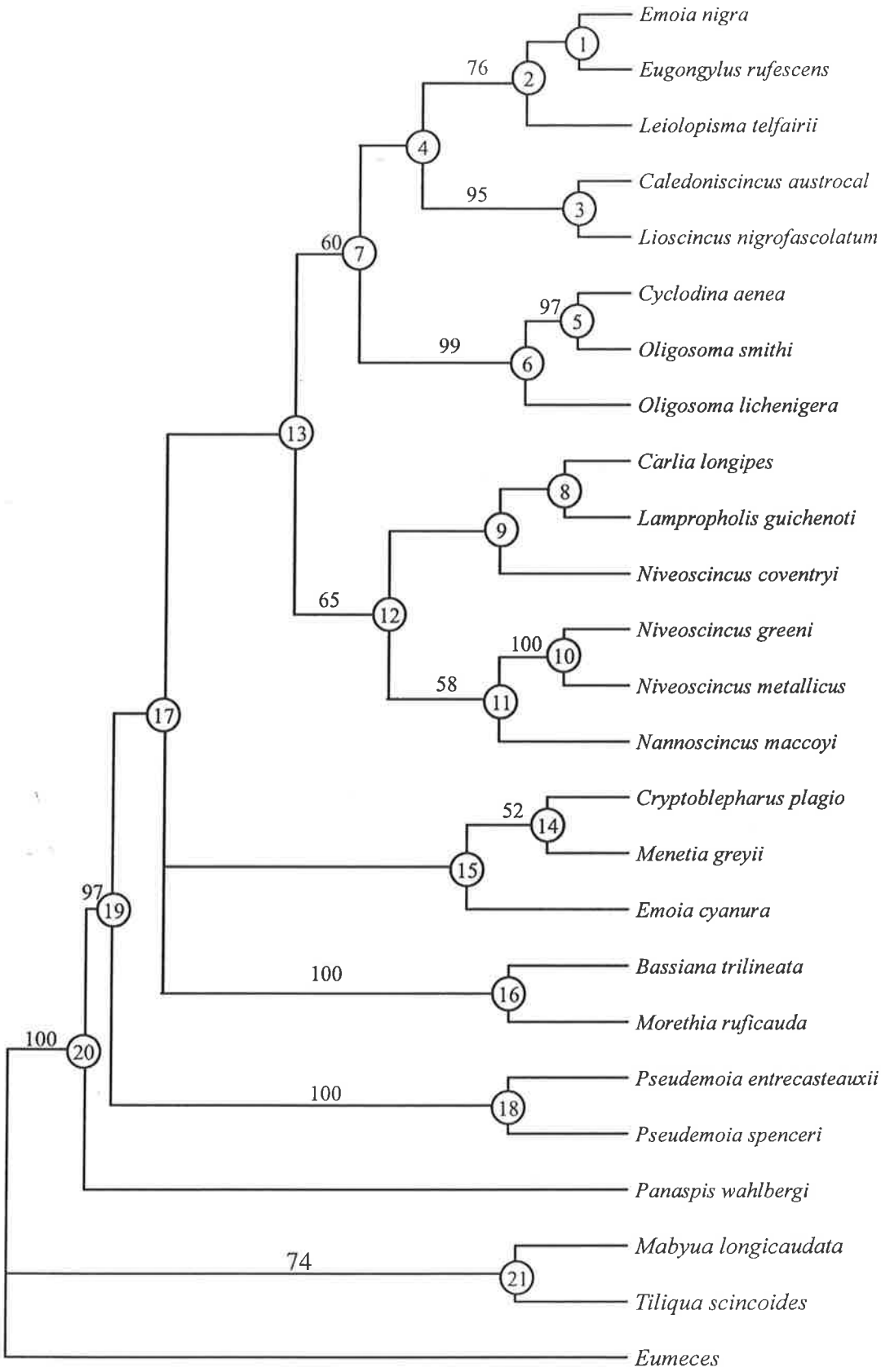


Figure 3.6: Consensus of two most parsimonious trees. Numbers above branches are bootstrap proportions >50. Node numbers refer to table 3.4 of partitioned bremer support from each gene.

general time reversible model (Rodríguez *et al.*, 1990) with three substitution rates and an estimate gamma shape parameter (the K81uf + g of Posada and Crandall, 1998) (base frequencies A:0.3029, C: 0.1949, G: 0.2226, T: 0.2796; substitution rate A↔C, G↔T: 1, A↔G C↔T: 3.0464, A↔T C↔G: 0.7352; gamma shape parameter: 0.9028). Maximum likelihood analysis resulted in a single optimal tree (-ln likelihood = 13462.68099, Figure 3.7). Parsimony analysis including gaps coded using the simple method results in a single optimal tree (1858 steps) sharing topology with tree A (Figure 3.5) from MP analysis without gaps. Maximum parsimony tree B (Figure 3.5) is a single step longer than tree one when gaps are included in the analysis. All trees have very short internodes with low bootstrap support. The nodes in common between all three trees from the data without gaps (Figure 3.8) are supported by bootstrap proportions greater than 70% in most cases. Monophyly of the *Eugongylus* group is supported by all trees, and the African *Panapsis wahlbergi* is supported as the sister taxon to the remaining *Eugongylus* group. Lineages within the *Eugongylus* group each include taxa from a single geographic region. The New Zealand and New Caledonian taxa each occur in a single monophyletic lineage, and a third geographic lineage includes taxa from throughout the Indo-Pacific. The Australian taxa form a monophyletic group in the ML tree, but this lineage is poorly supported, and does not occur in the parsimony trees. Live-bearing and placental taxa occur in at least four separate lineages in the *Eugongylus* group, although the relationships between these lineages are unclear.

Hypothesis tests

The alternative topologies that combine lineages of viviparous taxa into fewer clades were tested against the best trees. These alternative topologies allow more parsimonious resolution of the evolution of reproductive mode across the tree. The first alternative hypothesis tested was that the three species of *Niveoscincus* (*N. greeni*, *N. metallicus* and *N. coventryi*) belong to a single lineage. Secondly I tested whether the two Australian viviparous genera (*Niveoscincus* and *Pseudemoia*) belong to a single lineage, and thirdly, whether the New Zealand genera (*Cyclodina* and *Oligosoma* - excluding *O.*

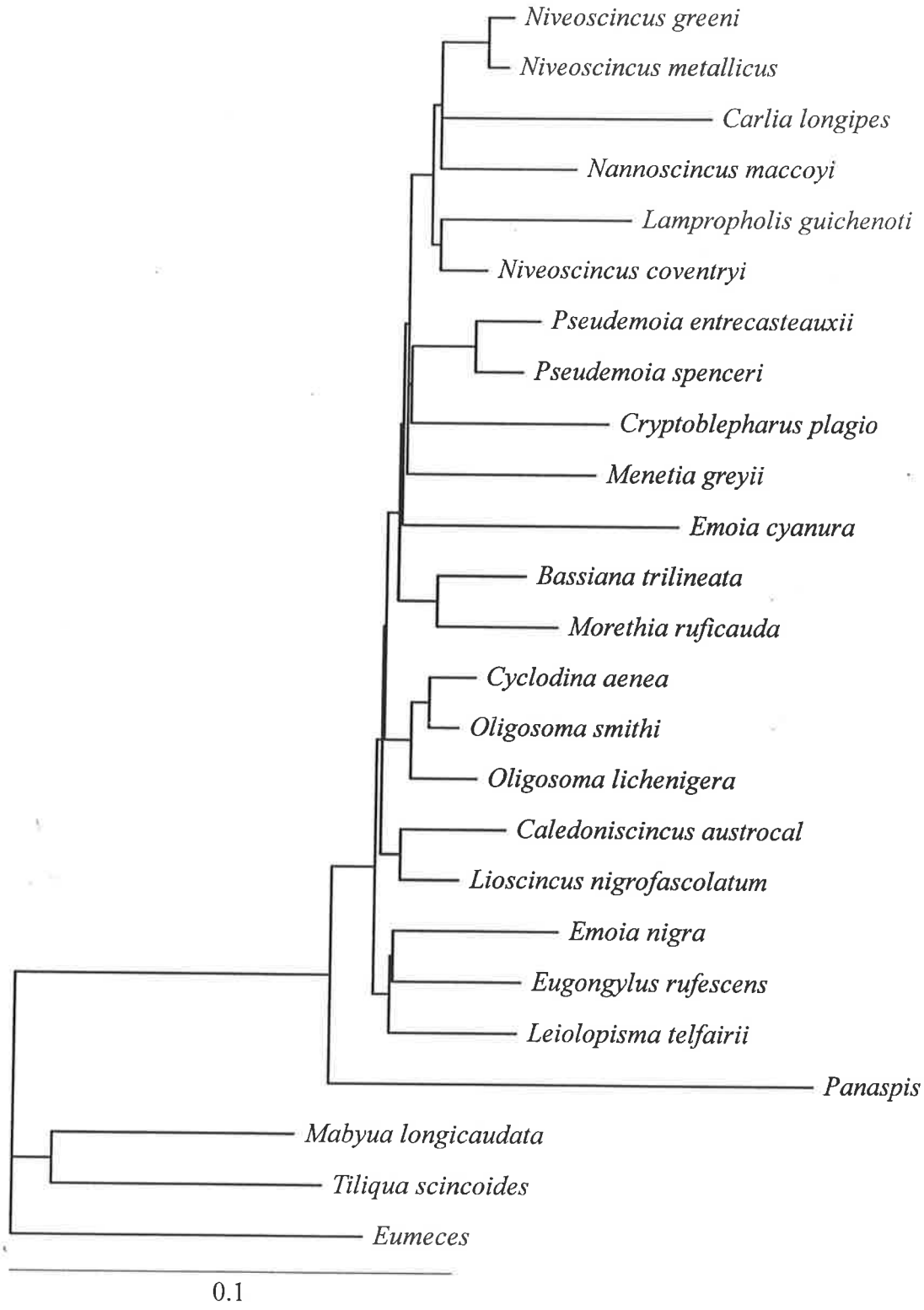


Figure 3.7: Optimal tree of phylogenetic relationships among the *Eugongylus* group under maximum likelihood criterion.

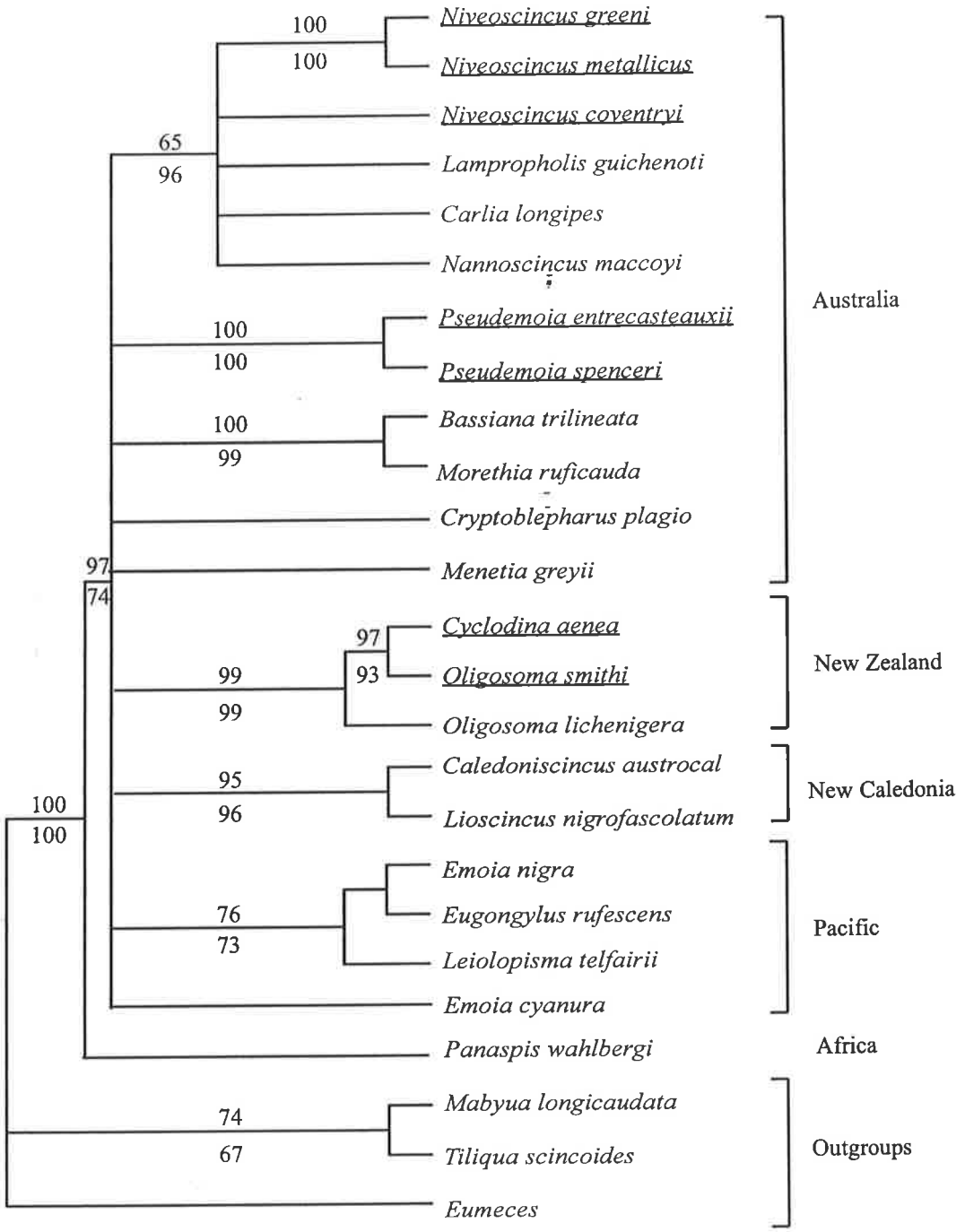


Figure 3.8: Strict consensus of most likely and two most parsimonious trees, showing geographic groups. Bootstrap proportions from MP shown above branches, ML values below. Viviparous taxa are underlined.

lichenigera) form a monophyletic group with either *Niveoscincus* or *Pseudemoia*. Results of Templeton tests of parsimony trees and Shimodaira-Hasegawa tests of likelihood trees are shown in tables 3.7 and 3.8. Monophyly of *Niveoscincus* was the only alternative hypothesis which could not be statistically rejected by either Templeton or Shimodaira-Hasegawa tests.

Resolving polytomies

Likelihood ratio tests were used to determine which internodes in the optimal tree under likelihood are significantly longer than zero (Slowinski, 2001). Significance levels were corrected for split degrees of freedom (Goldman and Whelan, 2000; Slowinski, 2001) and a Bonferroni correction for multiple comparisons applied (Table 3.9, Figure 3.9). Nine internodes in the tree are not significantly longer than zero with the Bonferroni correction applied (the most stringent conditions), including most of the short nodes occurring between well supported geographic groups (Figure 3.9). Shimodaira-Hasegawa tests were used to determine whether trees with several nodes collapsed simultaneously are worse than the most likely tree. Trees with all non-significant nodes, non-significant nodes under the least stringent conditions, or nodes between geographically distinct groups were tested (Figure 3.8). The tree with all non-significant nodes collapsed was the only alternative that could be rejected.

The modification of Braun and Kimball's (2001) equation to allow an estimate of the "hardness" of a polytomy relies on having an estimate of the rate of substitution, λ. Although there is no realistic rate estimate available for the genes used and no known date in the phylogeny generated to calibrate a clock, I have used a very rough clock estimate to demonstrate this method. The rate of evolution of gene sequences was calculated using the *Sphendon punctatus c-mos* sequence of Saint *et al.* (Saint *et al.*, 1998), and 200 MYA as the date of the *Sphenodon* – squamate split (Evans and Barbadillo, 1997). This very rough divergence time estimate implies a sequence evolution rate of 1.3×10^{-9} substitutions/site/year. The calculation of minimum branch–

Hypothesis	Alt. Tree Length	Templeton test statistics			
		T	n	z	P
Monophyletic *Niveoscincus*	1824	3	5	-1.3416	>0.0625
	13 trees	8	7	-1.1339	>0.0547
		15	9	-1.0000	>0.0645
		24	11	-0.9045	>0.0508
		35	13	-0.8321	0.4054
		48	15	-0.7746	0.4386
		63	17	-0.7276	>0.0544
		80	19	-0.6882	0.4913
		99	21	-0.6547	>0.0516
		182	28	-0.5388	0.5900
		210	30	-0.5222	0.6015
Monophyletic *Niveoscincus*	1836	144	32	-2.5355	**0.0112**
+ *Pseudemoia*	7 trees	153	33	-2.6112	**0.0090**
		160	33	-2.4042	**0.0162**
		170	34	-2.4660	**0.0137**
		260	40	-2.2875	**0.0222**
		280	41	-2.1906	**0.0285**
		294	42	-2.2361	**0.0253**
		315	43	-2.1455	**0.0319**
		330	44	-2.1880	**0.0287**
Monophyletic *Niveoscincus*	1849	49.5	33	-4.6667	**<0.0001**
+ *Oligosoma* / *Cyclodina*	4 trees	66.5	34	-4.4137	**<0.0001**
		70	35	-4.5422	**<0.0001**
		88	36	-4.3087	**<0.0001**

Hypothesis	Alt. Tree Length	Templeton test statistics			
		T	n	z	P
Monophyletic *Pseudemoia* + *Oligosoma* / *Cyclodina*	1838 10 trees	198	37	-2.5942	**0.0095**
		228	39	-2.5361	**0.0112**
		260	41	-2.4817	**0.0131**
		294	43	-2.4306	**0.0151**
		552	39	-2.5361	**0.0112**

Table 3.7: Templeton's phylogenetic hypothesis tests of alternative hypotheses of relationships among live-bearing *Eugongylus* group lineages. Each of the two best unconstrained trees were compared to each of the best trees under each constraint. Where n is less than 25, critical values of the Wilcoxon rank sum were obtained from statistical tables in Rohlf and Sokal (1981).

Hypothesis	Alt tree L	Diff –ln L	P
Monophyly of:			
Niveoscincus	13462.68	3.57	0.314
Niveoscincus + *Pseudemoia*	13513.99	51.30	**0.001**
Niveoscincus + *Oligosoma* / *Cyclodina*	13572.82	110.14	**0.000**
Pseudemoia + *Oligosoma* / *Cyclodina*	13521.46	58.78	**0.000**
Collapsed Nodes:			
all non-sig. (3, 6, 7, 8, 13, 15, 16, 17)	13504.79	42.11	**0.006**
sig. without Bonferoni (6, 8, 13, 15, 16)	13469.32	6.643	0.491
geographic groups (7, 8, 13, 15)	13475.87	13.19	0.199

Table 3.8 – Results of Shimodaira-Hasegawa tests of alternate hypotheses of relationships among live-bearing *Eugongylus* group lineages, and of multiple collapsed nodes in the ML tree, see Figure 3.9 for node numbers.

Node 1	Node 2	Branch length	Standard error	lnL diff	P
B. trilineata	9	0.020	0.003	115.611	<0.001
C. aenea	11	0.01	0.002	87.395	<0.001
C. austrocaledonicum	14	0.024	0.003	172.065	<0.001
C. longipes	2	0.062	0.007	492.762	<0.001
C. plagiocephalus	6	0.045	0.005	337.756	<0.001
E. cyanura	8	0.063	0.006	514.632	<0.001
E. nigra	16	0.037	0.004	242.051	<0.001
E. rufescens	16	0.029	0.004	175.563	<0.001
E. skiltonianus	root	0.080	0.008	172.363	<0.001
L. guichenoti	3	0.044	0.005	220.774	<0.001
L. nigrofasciolatum	14	0.013	0.002	85.207	<0.001
L. telfairii	17	0.029	0.005	183.02	<0.001
M. greyii	7	0.043	0.005	705.867	<0.001
M. longicaudata	20	0.055	0.008	98.944	<0.001
M. ruficauda	9	0.028	0.004	187.086	<0.001
N. coventryi	3	0.011	0.003	44.499	<0.001
N. greeni	1	0.006	0.002	26.577	<0.001
N. maccoyi	2	0.031	0.005	259.242	<0.001
N. metallicus	1	0.005	0.002	25.648	<0.001
O. lichenigera	12	0.015	0.003	121.55	<0.001
O. smithi	11	0.007	0.002	50.585	<0.001
P. entrecasteauxii	5	0.015	0.003	84.518	<0.001
P. spenceri	5	0.011	0.002	59.558	<0.001
P. wahlbergi	19	0.110	0.009	268.408	<0.001
T. scincoides	20	0.061	0.008	123.246	<0.001
1	2	0.011	0.002	61.742	<0.001
2	4	0.002	0.001	6.997	<0.001

Node 1	Node 2	Branch length	Standard error	lnL diff	P
3	4	0.002	0.001	3.129	0.012*
4	7	0.005	0.002	48.894	<0.001
5	6	0.014	0.003	76.847	<0.001
6	7	0.001	0.001	1.506	0.083**
7	8	0.001	0.001	2.994	0.014*
8	10	0.001	0.001	0.682	0.243**
9	10	0.009	0.002	32.611	<0.001
10	13	0.003	0.001	21.15	<0.001
11	12	0.004	0.001	19.697	<0.001
12	13	0.006	0.002	33.014	<0.001
13	15	0.000	0.001	1.61	0.073**
14	15	0.005	0.002	20.727	<0.001
15	18	0.001	0.001	1.107	0.137**
16	17	0.001	0.001	0.176	0.553**
17	18	0.004	0.002	6.169	<0.001*
18	19	0.010	0.004	4.92	0.002
19	32	0.071	0.008	121.135	<0.001
20	root	0.009	0.005	2.86	0.017*

Table 3.9: Likelihood ratio tests of branch length. P values are from the chi squared test (output from PAUP*), values marked with ** are significant at $\alpha = 0.05$ with 0/1 split degrees of freedom (see Goldman and Whelan, 2000), values marked with * are significant at $\alpha = 0.05$ with split degrees of freedom only when the Bonferroni correction for multiple tests is applied. See figure 3.9 for node numbers.

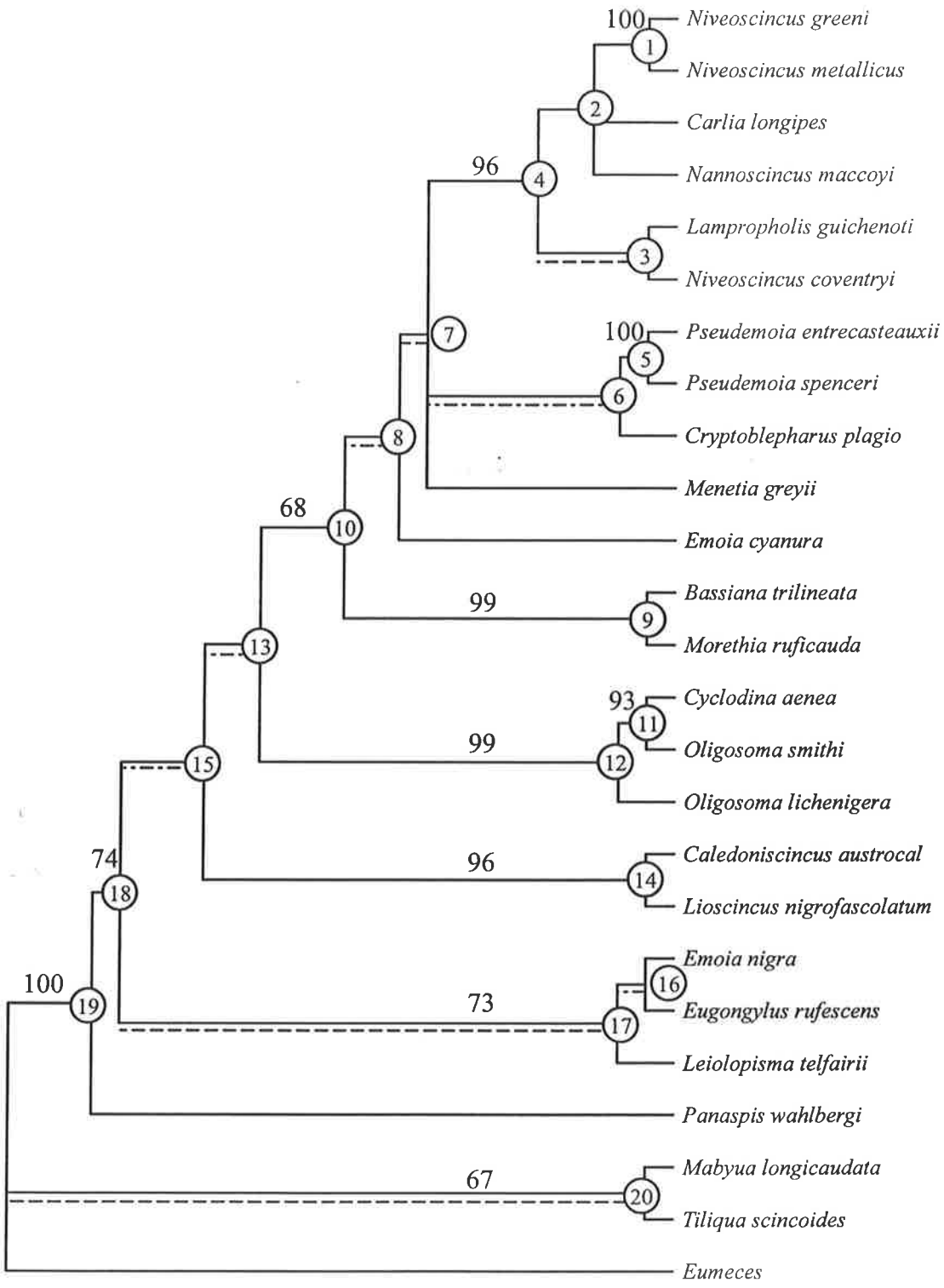


Figure 3.9: Maximum likelihood cladogram. Node numbers refer to table 3.9, branches not significantly longer than zero are dashed (Bonferroni correction and split degrees of freedom) or dash-dot (with split degrees of freedom only). Numbers above branches are bootstrap support values.

length [t = -ln (0.20) / λ L] therefore gives an estimate of ~1 million years. The general lack of evidence for the origins and early diversification of skinks makes this estimate difficult to evaluate, however it does not conflict with expectations of the speed of radiation of *Eugongylus* group skinks throughout the poorly resolved part of the tree.

Discussion

Nuclear data

Nuclear sequence data failed to resolved many of the nodes in the *Eugongylus* group phylogeny. Nonetheless, these new genes may prove useful for further systematic studies of reptiles at a number of levels. *C-mos* is the only gene used here that has previously been used widely in systematic studies of reptiles. The three newer genes have equal or higher proportions of informative characters and higher rates of sequence evolution than *c-mos*. These genes, particularly *rag-1*, may therefore be useful for addressing broad squamate relationships which are not fully resolved by *c-mos* (Harris *et al.*, 2001; Harris *et al.*, 1999; Saint *et al.*, 1998). *β-fibrinogen* and *myosin* are among the first intron sequences to have been used to reconstruct phylogenetic relationships within reptiles. These loci have higher rates of evolution than coding genes (SSR, Table 3.5), but are not more phylogenetically informative. This is at least in part due to homoplasy and missing data introduced by length variability in sequences. The introns used here were also more difficult to amplify and align than coding genes. Despite their lack of functional constraint, introns do not provide more phylogenetic information than coding loci, and are technically more challenging. Intron sequences are therefore not as good candidates for phylogenetic studies at this level of divergence, as coding nuclear genes.

Phylogenetic conclusions

The relationships among *Eugongylus* group taxa that have been resolved by nuclear DNA sequences have implications for hypotheses of biogeography, taxonomy and the evolution of reproductive mode among the group. The monophyly of Greer's

morphologically defined *Eugongylus* group (Greer, 1970), relative to the *Mabuya* and *Egernia* group taxa included as outgroups, is supported. Nuclear DNA data suggest that the earliest divergence within the *Eugongylus* group was between an African lineage and a lineage which then diversified throughout the Indo-Pacific. Within the *Eugongylus* group, each geographic region (with the exception of Australia) forms a single lineage. This pattern broadly suggests that the *Eugongylus* group radiated quickly throughout the Pacific, and diversified further within each geographic region. These data are not conclusive about the monophyly of the Australian taxa; the ML tree includes a monophyletic Australian lineage while the MP trees suggest that the Australian *Eugongylus* group is made up of several lineages. It is therefore not clear whether Australia may have acted as a centre for diversification throughout the rest of the distribution, or whether several invading lineages diversified separately within Australia. Within Australia, one of the lineages suggested by the immunological data of Hutchinson *et al.* (1990) (including *Niveoscincus*, *Nannoscincus*, and *Lampropholis*) is supported. The other lineage suggested by Hutchinson *et al.* (1990) including *Bassiana*, *Morethia* and *Pseudemoia*, was not recovered by either MP or ML analysis. Thus, the affinities of the placental genus *Niveoscincus* suggested by Hutchinson *et al.* (1990) were supported, but the sister group relationship of *Pseudemoia* with *Bassiana* + *Morethia* was not supported by my analysis. The alpha and beta palate subgroups (Greer, 1979) were not supported by analysis of the nuclear sequence data.

The monophyly of the genera *Emoia* and *Niveoscincus* is challenged by the nuclear DNA results presented here. *Niveoscincus greeni* and *N. metallicus* are sister taxa in both ML and MP analysis, but *N. coventryi* does not belong to this lineage. *Niveoscincus coventryi* is the sister taxon to *Lampropholis* in the most likely tree and to *Lampropholis* and *Carlia* in the most parsimonious tree. A previously published phylogenetic study of *Niveoscincus* based on the mitochondrial COI region also found that *N. coventryi* is not nested within the other *Niveoscincus* species (Melville and Swain, 2000). Templeton and Shimodaira-Hasegawa tests conducted on the basis of reproductive mode failed to reject the hypothesis that *Niveoscincus* is monophyletic. In

the description of *Niveoscincus*, Hutchinson *et al.* (1990) tentatively included *N. coventryi* primarily on the basis of it reproductive mode. A recent study has shown, however, that *N. coventryi* is largely lecithotrophic, while most the remaining *Niveoscincus* are placentotrophic (Thompson *et al.*, 2001). *Niveoscincus coventryi* also has morphological, reproductive and distribution characteristics that are not shared with the remaining members of the genus. My finding that *N. coventryi* may not belong to a monophyletic *Niveoscincus* thus does not conflict with existing evidence. The >80 species of *Emoia*, distributed widely throughout the Pacific islands are known to belong to a number of species groups (Brown, 1991). The results presented here, where *E. nigra* and *E. cyanura* are not each other's closest relative, suggest that some of these species groups may in fact be separate lineages.

Summary

Nuclear coding and intron loci have been used to reconstruct the evolutionary relationships among the *Eugongylus* group of skinks. Introns have proved to be technically more difficult and no more informative than exon sequences. These data suggest that the *Eugongylus* group may have radiated quickly throughout the Pacific. Lineages occurring in New Caledonia and New Zealand are monophyletic. Hutchinson *et al.*'s (1990) *Niveoscincus*, *Nannoscincus*, *Lampropholis* clade is supported, but the relationships among the Australian taxa are broadly unresolved.

Chapter 4 - Phylogenetic analysis of the Genus *Pseudemoia*

Introduction

Pseudemoia is one of two Australian *Eugongylus* group skink genera with morphologically complex and physiologically important placentae (*Pseudemoia* and *Niveoscincus*, Thompson *et al.*, unpub ms). As it is currently recognised, *Pseudemoia* comprises six species distributed across southeastern mainland Australia and Tasmania (Hutchinson and Donnellan, 1992). Detailed studies of placental morphology and function have been carried out for three species; *P. pagenstecheri* (Stewart and Thompson, 1996, morphology from very late developmental stages only; Thompson *et al.*, 1999), *P. entrecasteauxii* (Stewart and Thompson, 1993; Stewart and Thompson, 1996) and *P. spenceri* (Stewart and Thompson, 1998; Thompson *et al.*, 1999). These studies have shown that the structure of the chorioallantoic placentae of these three species is virtually identical, and corresponds to Type III placentation in Weekes' (1935) classification. Despite their morphological similarity, there are significant differences in the importance and type of nutrients provided to the developing embryos via the placenta. *Pseudemoia* also differ in other reproductive characteristics including the number and size of offspring and male breeding coloration. In *P. cryodroma*, *P. pagenstecheri* and *P. entrecasteauxii*, the white or cream mid-lateral stripe of males becomes red or orange during the breeding season, male *P. entrecasteauxii* also have red ventral surfaces during the breeding season (Hutchinson and Donnellan, 1992). *Pseudemoia cryodroma*, *P. spenceri* and *P. baudini* do not have male breeding colours although female *P. baudini* can have reddish ventral coloration while gravid (M. Hutchinson, personal communication, 2001). The structural similarity and physiological variation in placentae in *Pseudemoia* calls into question the assumption of a correlation between placental structure and function in many early studies of reptilian placentation (Weekes, 1935). *Pseudemoia* could therefore provide a model system to elucidate structure-independent physiological variation in reptilian placentae. The phylogenetic

relationships between species in this genus provide an essential historical framework for these studies.

The species currently assigned to *Pseudemoia* have historically been distributed in a number of genera. *Pseudemoia* was originally thought to be monotypic, including only *P. spenceri* (Fuhn, 1967), although *Niveoscincus palfreymani* was included by Rawlinson (1974). *Pseudemoia entrecasteauxii*, and *P. baudini*, were assigned to *Leiolopisma* (Greer, 1982) and to *Claireascincus* (Wells and Wellington, 1985), although the later genus name was not widely adopted. *Pseudemoia rawlinsoni* was described as *Leiolopisma*. Its affinities with *P. entrecasteauxii* were noted, but the genus *Claireascincus* was not recognised by the authors (Hutchinson and Donnellan, 1988). Hutchinson and Donnellan (1990), according to their immunological and morphological data, included all of these species (except *N. palfreymani*) in *Pseudemoia*, and later recognised *P. cryodroma* (Hutchinson and Donnellan, 1992). The current definition of *Pseudemoia* is supported by morphological, karyotypic, allozyme and immunological evidence (Hutchinson and Donnellan, 1988; Hutchinson and Donnellan, 1992; Hutchinson *et al.*, 1990). The phylogenetic relationships among species of *Pseudemoia* have only been addressed on the basis of allozyme characters (Hutchinson and Donnellan, 1992), which have limited power to resolve phylogenetic relationships. Allozyme analysis suggests that *P. entrecasteauxii* is paraphyletic with *P. cryodroma*. In this chapter I use mitochondrial and nuclear sequence data in combination with previously published allozyme characters to determine the phylogenetic relationships between species of *Pseudemoia* (Hutchinson and Donnellan, 1992).

Materials & Methods

Specimens and allozyme data

Sequence data were obtained from two individuals from each of the six *Pseudemoia* species. Three populations of *P. entrecasteauxii* from Victoria, New South Wales and Tasmania were included to test the paraphyly of this species with *P. cryodroma* suggested by Hutchinson and Donnellan (1992). Specimen numbers and

collection locations are listed in Table 4.1. Trees were rooted using *Bassiana duperreyi, Morethia ruficauda, Lampropholis guichenoti* and a combination of *Niveoscincus metallicus* and *N. greeni* sequences.

I included the thirty one allozyme loci used in the previous study of *Pseudemoia* by Hutchinson and Donnellan (1992) in a parsimony analysis (Appendix 4). I used allozyme data from the populations that were included in sequence analysis; this means that where more than one individual from a given population was sequenced, identical allozyme data were used. *Pseudemoia pagenstecheri* from Nundle NSW (R112313) was not sampled by Hutchinson and Donnellan (1992), in this case allozyme data were taken from Donnellan and Hutchinson (1990); this resulted in 6 missing loci for this individual. *Niveoscincus metallicus* is the only outgroup taxon for which I have allozyme data. Loci were treated as characters with alleles as non-additive character states. All allozyme polymorphisms were treated as polymorphisms in the multistate taxa option in PAUP* regardless of the frequency of alternative alleles.

DNA extraction, amplification & sequencing

I used nucleotide sequences from *β-fibrinogen* intron 7, *heavy chain myosin* 2, and *ND4* including tRNAs *His* and *Ser*. Primers are listed in Appendix 2 and discussed in Chapter 2. Mitochondrial DNA (mtDNA) enriched extractions were prepared using a modification of the plasmid preparation procedure of Welter *et al.* (1989) from some specimens (see Table 4.1). These specimens provided mitochondrial reference sequences. The potential of the *ND4* primers to amplify nuclear paralogues rather than mitochondrial copies of the genes was assessed by sequencing products from amplifications of total cellular DNA and dilute mitochondrial enriched DNA from a single individual following the method of Donnellan *et al.* (1999). Indistinguishable sequences from total cellular and mt DNA indicate that the primers used are unlikely to amplify nuclear paralogues. All other genes were amplified from either nuclear DNA prepared during mt enrichment, or from total cellular DNA.

Species	Specimen no.	Location	ND4 Source
Pseudemoia baudini	ABTC 22957/64	Port Lincoln area, SA	mt/tc
P. cryodroma	MV D61886	Mt Arbuckle, Vic	tc
P. cryodroma	ABTC 40890	Mt Baw Baw, Vic	mt
P. entrecasteauxii	MV D61836	Mt Tamboritha, Vic	mt
P. entrecasteauxii	ABTC 23023	King Is, Tas	tc
P. entrecasteauxii	ABTC 40981	Charlotte's Pass, NSW	tc
P. pagenstecheri	AMS R112313	Nundle SF, NSW	mt
P. pagenstecheri	SAMA R45268	Tunbridge, Tas	mt
P. rawlinsoni	SAMA R49585/90	Beachport, SA	mt
P. spenceri	AMS R104773/74	Kanangra-Boyd NP, NSW	mt
Bassiana duperyii	MV D62253	Emita Flinders Is, Tas	mt
Lampropholis guichenoti	SAMA R39155	Goulburn, NSW	mt
Morethia ruficauda	SAMA R34102	Undoolya Stn, NT	mt
Niveoscincus metallicus (ND4, BF)	MV D60517	Western Port Bay, Vic	mt
N. greeni (myh)	TMH C569	Mt Rufus, Tas	N/A

Table 4.1 - Museum identification number and collection location for specimens used in this study. All locations and tissue collections are in Australia. AMS: Australian Museum, Sydney; SAMA: South Australian Museum, Adelaide; ABTC: Australian Biological tissue Collection, South Australian Museum, Adelaide; MV: Victorian Museum, Melbourne; TMH: Tasmanian Museum, Hobart. ND4 Source refers to the template used for amplification of ND4, mt: mt enriched, TC: total cellular.

Amplification was conducted using TaqGold hot-start polymerase, after initial denaturation at 94 °C for 9 min, each cycle consisted of denaturation at 94 °C for 45s, annealing at 50-55 °C for 45s, and extension at 72 °C for 1 min, for 34 cycles. Negative controls were run for all amplifications. Amplified products were purified using UltraClean PCR cleanup columns (Mo Bio Laboratories). Products were sequenced using the ABI BigDye sequencing kit on an ABI 3700 capillary sequencer.

Sequence interpretation and phylogenetic analysis

Sequences were edited using SeqEd (ABI) and aligned by eye using Se-Al v1.0 a1 (Rambaut, 1995), alignments are given in appendix X. The difficulty encountered in coding gaps in *β-fibrinogen* for the whole *Eugongylus* group in chapter 3 were also encountered within *Pseudemoia*; gaps were therefore not coded. Secondary structure was not necessary for unambiguous alignment of tRNAs. All characters were weighted equally. Phylogenetic analyses were undertaken using PAUP* v 4.0 b8 (Swofford, 1999) on power Macintosh G3 and G4 computers (G4 analyses used the altivec version of PAUP*). Separate partitions were defined for each gene and tRNA, the allozyme dataset was included as a partition in parsimony analyses. Combinability of partitions was assessed using the incongruence length difference (ILD) test (Farris *et al.*, 1995; Mickevich and Farris, 1981) implemented in PAUP*, and the partitioned Bremer index (Baker and Desalle, 1997) implemented using Treerot v 2a (Sorenson, 1999) and PAUP*. Maximum parsimony (MP) searches were conducted using a branch and bound algorithm. Maximum likelihood analysis (ML) was carried out under a model determined by hierarchical likelihood ratio tests implemented using Modeltest v 3.1 (Posada and Crandall, 1998), parameters including tree topology were determined simultaneously using an heuristic search algorithm. Bootstrap resampling was used to gauge support for optimal trees, with 500 heuristic pseudoreplicates (with 10 random addition sequences each) for MP, and 100 pseudoreplicates for ML (using 'as-is' sequence addition).

Results

Information content and congruence of partitions

In all cases where *ND4* was sequenced from mitochondrial DNA enriched extractions and total cellular DNA of the same individual, the sequences were indistinguishable, and the two PCR fragments match exactly in all cases. In addition there was no conflict between phylogenies recovered from mitochondrial and nuclear genes. This supports the assumption that all *ND4* sequences are mitochondrial in origin. I obtained 1932 nucleotides when autoapomorphic indels were removed (2236 sites in total), 353 of which were parsimony informative. The information content of each partition is shown in table 4.2. Figures 4.1 and 4.2 show the relationship between ML distance and uncorrected sequence divergence for each pairwise comparison between taxa for each gene.

The ILD test suggests that there is significant (P = 0.01) heterogeneity in the data, and that partitions should therefore not be combined. However, partitioned Bremer analyses, which allow the sources of incongruence to be determined, indicate that there is very little conflict between data partitions (Table 4.3 and Figure. 4.3). Recent studies have emphasised the effect that unequal levels of noise and uninformative characters between partitions can have on the significance of the ILD test (Dolphin *et al.*, 2000; Lee, 2001). Given the large differences between the proportion of characters which are informative in each partition (33% in *ND4 vs.* 6% in *myh*, 8% in *β-fibrinogen*), the noise effect may be responsible for the significance of incongruence between the data partitions. All data were combined for further analysis.

Optimal trees

Hierarchical likelihood ratio tests suggest that appropriate model of evolution for these data is the general time reversible model (Rodríguez *et al.*, 1990) with estimated gamma shape parameter (*g*) and proportion of invariable sites (*i*) (GTR+i+g) (base frequencies: A: 0.3077, C: 0.2266, G: 0.1890, T: 0.2765; substitution rates: A↔C: 2.0047 , A↔G: 4.9728 , A↔T: 0.9549, C↔G: 0.6322, C↔T: 6.9715; gamma shape

Data Partition	Length 1	Length 2	Variable Characters	PI Characters	% PI a	%PI b	CI	SSR
allozymes	-	31	28	16	52	57	0.94	-
β-fib	993	704	180	56	8	31	0.91	0.590
myh	391	376	65	24	6	37	0.67	0.459
ND4	-	685	299 (194)	225 (158)	33 (69)	75 (81)	0.59	1.884 (4.649)
tRNA His	-	69	19	11	16	58	0.63	0.977
tRNA Ser	-	67	33	21	31	63	0.84	1.213

Table 4.3: Estimates of the information content of each gene. Data from third codon positions of *ND4* are given in brackets. L1 = total length of sequence, L2 = length of sequence excluding autoapomorphic indels,, Variable Characters = number of variable characters, PI Characters = number of parsimony informative characters, % PI = percent of (a) total characters and (b) variable characters from this gene (or codon position) which are parsimony informative, CI = consistency index on the most parsimonious tree from combined data (all excluding 12s), or on the most parsimonious trees from the 12s data (12s), SSR = rate estimated from ML.

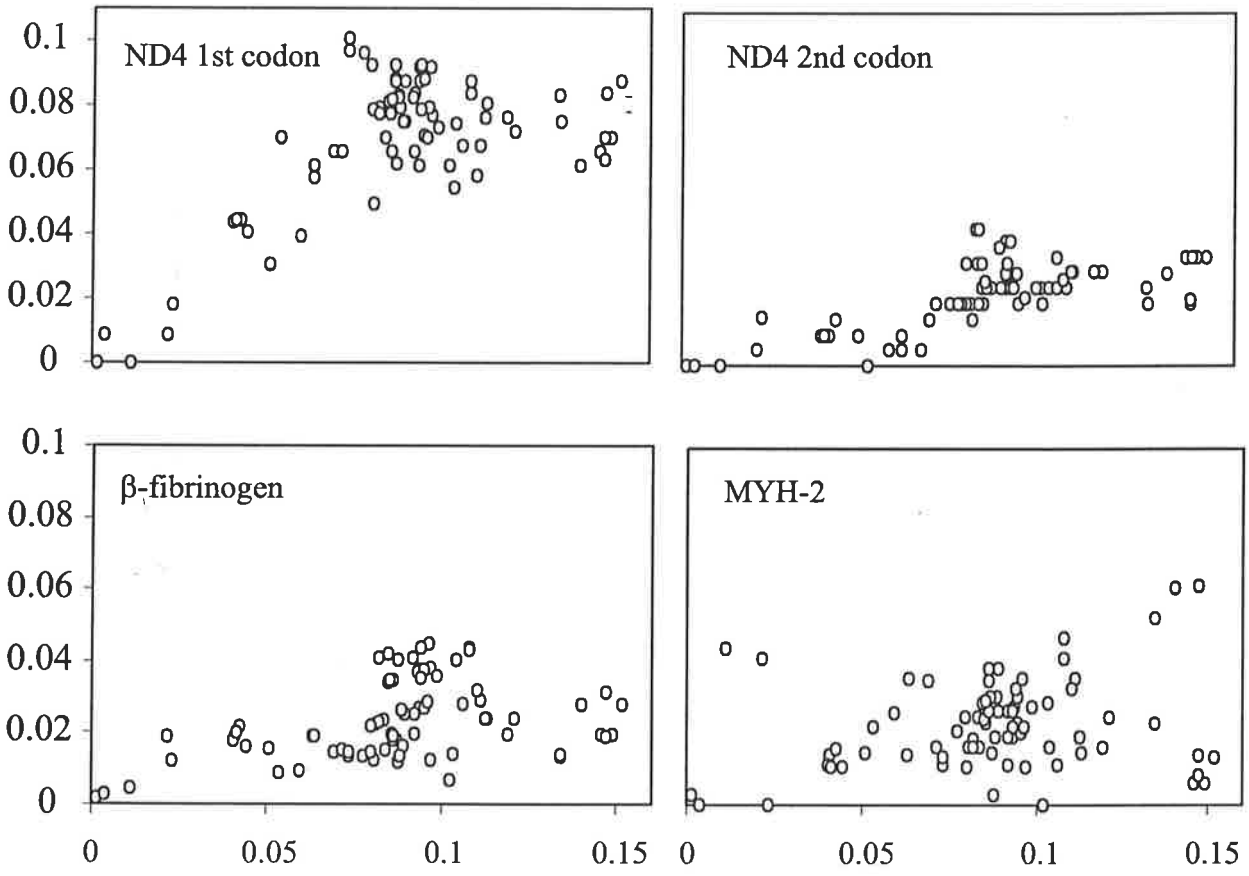


Figure 4.1: Pairwise comparisons of ML distance estimated from combined data (X-axis) *versus* uncorrected p from each gene, for *Pseudemoia* species.

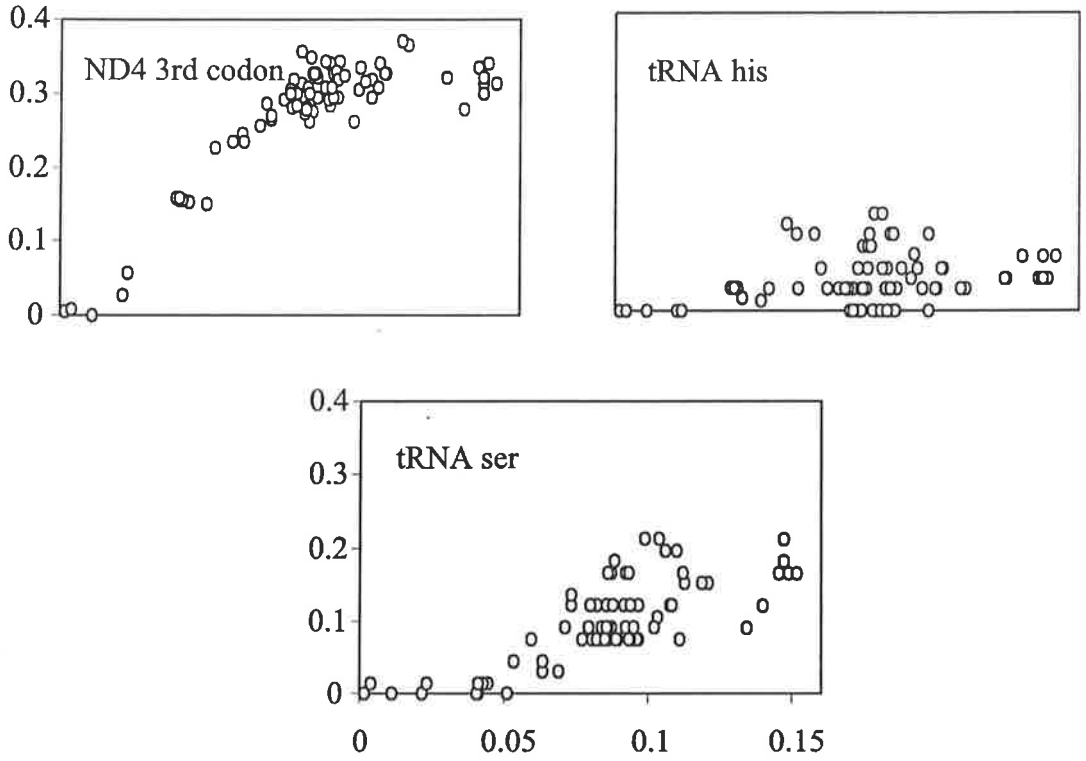


Figure 4.2: Pairwise comparisons of ML distance estimated from combined data (X-axis) *versus* uncorrected p from each gene, for *Pseudemoia* species.

Node	Bootstrap Proportion	Bremer Index	Partitioned Bremer Support					
			ND4	His	Ser	*β-fib*	*myh-2*	Allozyme
			225	11	21	56	24	16
Ingroup:								
1	100	32	24	0	0	8	0	0
2	100	25	14.5	2	0	1.5	3	4
3	100	24	20	0	1	0	0	3
4	100	25	26	0	0	-0.5	-0.5	0
5	72	4	0	0	0	2	0	2
6	77	4	5	-1	0	0	0	0
7	91	6	6	0	-1	0	1	0
8	100	25	16	0	9	0	0	0
9	90	8	4	0	4	0	-1	1
10	92	9	-1	-3	4	9	-1	1
11	100	34	21	0	4	7	-1	3
Outgroup:								
12	100	43	12	6	5	16	4	0
13	98	10	2	0	0	5	3	0
14	94	14	15.5	-1.5	-0.5	1	-0.5	0

Table 4.3 - Bremer and bootstrap support for the single best MP tree (Fig. 4.3). Bremer and bootstrap values apply to the branch proceeding each node. The number of parsimony informative characters for each partition are at the top of each column.

parameter: 0.6264, proportion of invariable sites: 0.3346). Maximum parsimony and ML phylogenetic analyses resulted in identical optimal tree topologies (Figure 4.3; trees scores are 1219 steps and -ln L = 8324), although branch lengths differ slightly between methods. All relationships are supported by bootstrap proportions >70%. These results show that *Pseudemoia* is monophyletic, with the species relationships (*P. spenceri*, ((*P. rawlinsoni*, *P. baudini*), (*P. entrecasteauxii,* (*P. cryodroma*, *P. pagenstecheri*))).

Discussion

Sequence data

In this study of the relationships among species of *Pseudemoia*, nuclear intron sequences are significantly less informative than mitochondrial DNA. Lack of information in intron sequences in Chapter 3 was attributed to homoplasy and missing data caused by length variability. These factors are much less significant in the shallower divergences within *Pseudemoia*. However, within *Pseudemoia*, introns contribute relatively little information because of a lack of variable characters. Thus introns appear to be compromised by length variability at deep divergences (Chapter 3), and by lack of variability among closely related taxa. The mitochondrial *ND4* sequences used here do not show any signs of nuclear paralogues and are much more informative than introns.

Phylogenetic conclusions

This study has provided a fully resolved and well-supported hypothesis of the species relationships within *Pseudemoia*. The relationships suggested by the data presented here are largely congruent with phenograms generated by previous studies (Donnellan and Hutchinson, 1990; Hutchinson and Donnellan, 1992). *Pseudemoia spenceri*, a specialised arboreal species, is the sister species to the remaining *Pseudemoia*. Although the sampling here is not sufficient to robustly test the hypothesis, I have not found evidence for a hybrid origin of *P. cryodroma* (Donnellan

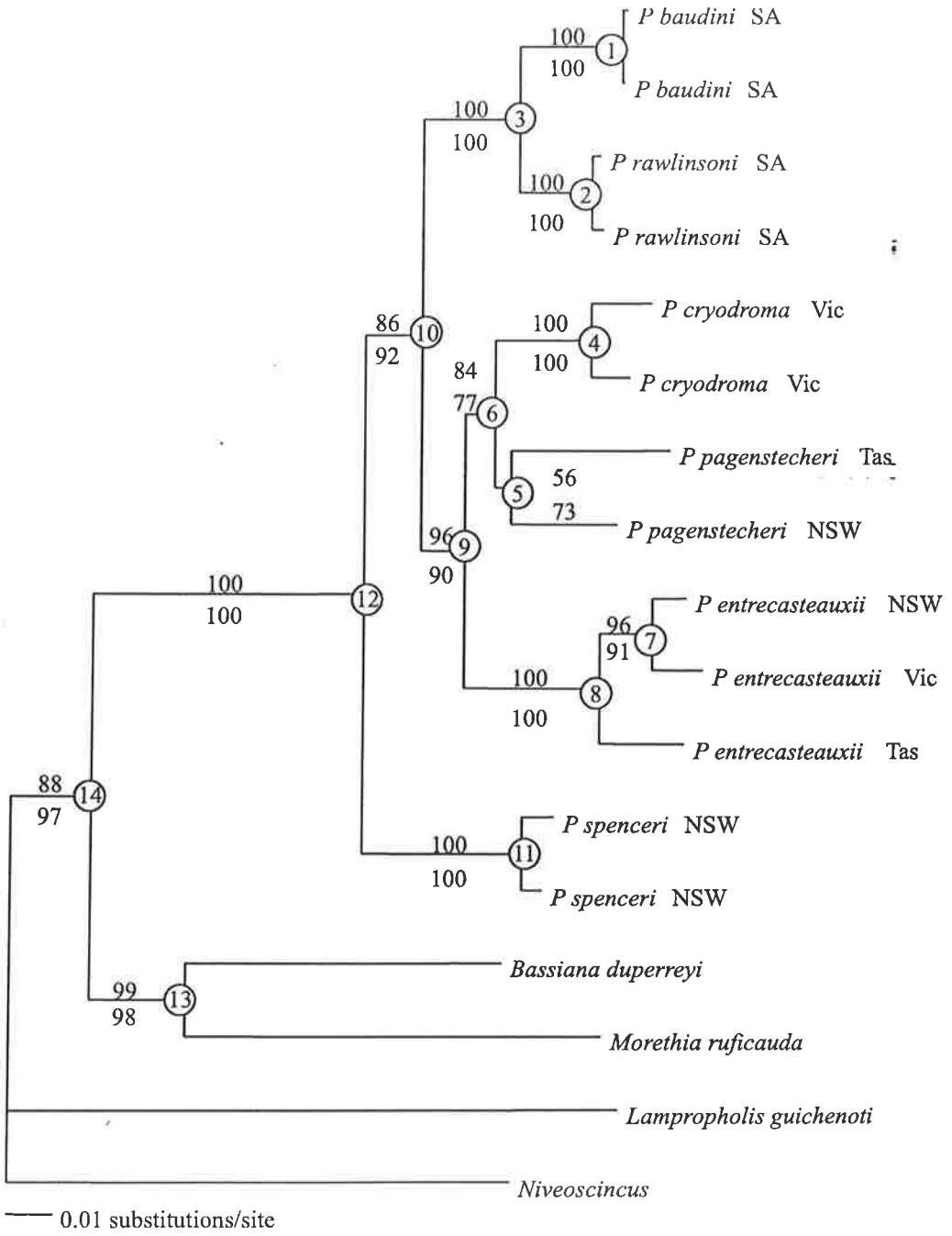
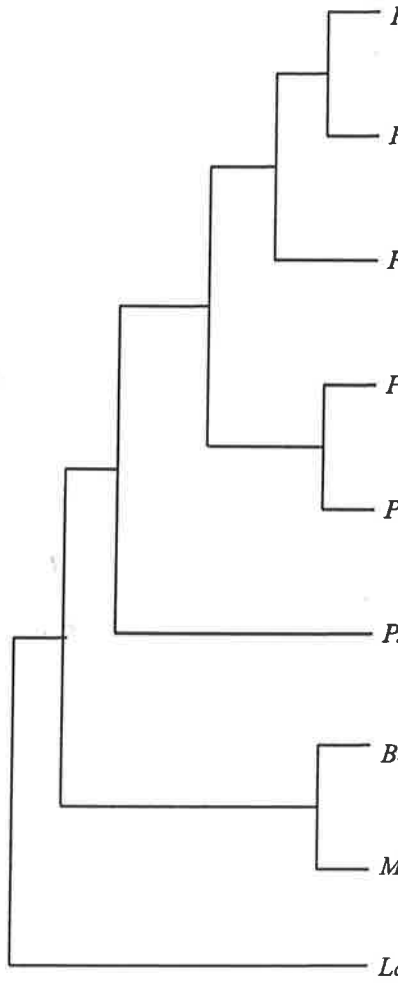


Figure 4.3 - Topology of most likely (-ln likelihood: 8323.6582) and most parsimonious (1219 steps) trees. Branch lengths are from ML analyses under the general time reversible model with estimated gamma shape parameter and proportion of invariable sites. Bootstrap proportions from ML analyses are above the branches MP analysis below. Node numbers refer to Table 4.3.

and Hutchinson, 1990; Hutchinson and Donnellan, 1992). The data presented here suggest that *P. cryodroma* is the sister species to *P. pagenstecheri*, rather than *P. entrecasteauxii*, and that *P. entrecasteauxii* is monophyletic. As suggested by Hutchinson and Donnellan (1992) the two species occurring in apparently relictual habitats (*P. baudini* and *P. rawlinsoni*) are sister species. Male breeding coloration appears to have evolved only once in *Pseudemoia*, in the ancestor of *P. cryodroma*, *P. pagenstecheri* and *P. entrecasteauxii*. This is particularly significant because male breeding coloration was one of the characteristics supporting the sister group relationship between *Pseudemoia* and *Bassiana* + *Morethia* recovered by Hutchinson *et al.* (1990). The analyses in Chapter 3 did not recover this grouping, and the results here suggest that, because breeding coloration is derived within *Pseudemoia*, it is not a synapomorphy of a lineage including the three genera. The other shared characters listed by Hutchinson *et al.* (1990) (elevated presacral count, virtual or complete closure of the temporal fenestra and male pair seven XY sex-chromosome system) still provide evidence of the relationship. However, the use of *Bassiana* to provide comparative data about reproductive characteristics from a close oviparous relative of *Pseudemoia* (Stewart and Thompson, 1993; Stewart and Thompson, 1996) may not be justified.

Few preliminary conclusions about the evolution of placentation within this genus can be drawn yet. The ratio of ovulated egg and neonate dry masses (a measure of relative yolk and placental contribution to development), and the make up of minerals transported across the placenta of the more commonly occurring species has been recorded (Figure 4.4, Thompson and Stewart, 1994; Thompson *et al.*, 1999; Thompson *et al.*, 1999). As yet however, there are no obvious ecological or morphological correlates of the variation in placental function. The availability of the phylogeny presented here means that when such covariates are identified, their statistical significance can be tested using statistical comparative methods. The independent contrasts method (Felsenstein, 1985), where differences in trait values between sister species or internal nodes are divided by the square root of the sum of their branch lengths, is one of the best understood and most prevalent of these methods.



	Litter size	Egg:embryo dry mass	Minerals
P. cryodroma	3		
P. pagenstecheri	5 - 7	2.16	Na^+, K^+, 90% Ca^{2+}
P. entrecasteauxii	4 - 5	1.66	Na^+, K^+, Mg^{2+} PO_4^-, 72% Ca^{2+}
P. rawlinsoni	5.6		
P. baudini	3.25		
P. spenceri	1.9	1.29	Na^+, K^+, 59% Ca^{2+}
Bassiana	5.5	0.85	none
Morethia	2.0	0.76	none
Lampropholis	2.5	0.75	none

Figure 4.4: Published reproductive data relative to phylogeny generated by this analysis. See text for details.

Summary

Nuclear intron and mitochondrial sequence data and allozymes have been used to reconstruct the evolutionary relationships among *Pseudemoia* species. Mitochondrial sequence data are much more phylogenetically informative than other types of data. The results of these analyses suggest that male breeding coloration has evolved once within *Pseudemoia*.

Chapter 5 -Phylogenetic analysis of New Zealand's skinks

Introduction

New Zealand has a large diversity of endemic reptiles, including the only extant members of the order Sphenodontida and endemic radiations of skinks and geckos. The thirty or so endemic skink species have been assigned to the genera *Oligosoma* and *Cyclodina*. *Oligosoma* generally have shallow, pointed heads, oval bodies and long limbs and toes, and are diurnally active and sun loving, *Cyclodina* have deep, blunt heads, squarish bodies and shorter limbs and toes, they also tend to be crepuscular (Hardy, 1977; Patterson and Daugherty, 1995). Morphological, immunological and allozyme studies suggest that the New Zealand skink radiation is monophyletic with respect to other *Eugongylus* group skinks (Hardy, 1977; Hardy, 1982; Hutchinson *et al.*, 1990, and Chapter 3; Patterson and Daugherty, 1995). Unusually, almost all of the skinks in New Zealand are live-bearing. The only egg-laying species are *Oligosoma suteri* and the introduced *Lampropholis delicata*. The predominance of live-bearing in New Zealand is consistent with the broadly supported "cold-climate" hypothesis which suggests that live-bearing in reptiles is an adaptive advantage in cold climates (Neill, 1964; Shine, 1985; Shine and Guillette, 1988). The presence of a single endemic oviparous species provides the opportunity to gain an insight into the evolution of reproductive mode in the history of the New Zealand skinks. If *Oligosoma suteri* is the sister taxon to a live-bearing lineage, viviparity is most simply explained by a single evolutionary transition in the ancestor of that lineage. If however, *O. suteri* is nested among the viviparous taxa, a more complex evolutionary scenario must be postulated.

Molecular approaches, including allozyme electrophoresis and nucleotide sequencing, have been widely applied to the identification of species within the New Zealand skinks (Daugherty *et al.*, 1990; Daugherty *et al.*, 1994; Hickson *et al.*, 1992; Patterson and Daugherty, 1994; Towns *et al.*, 1985). Recently Hickson *et al.* (2000) used mitochondrial *12S rRNA* sequences to address the relationships among species of *Oligosoma* and *Cyclodina*. Although the support and resolution of the tree generated

from *12S rRNA* data was very low, some surprising conclusions were suggested. Hickson *et al.* (2000) found that the New Zealand skink radiation does not form a monophyletic lineage, with the Australian oviparous species *Lampropholis guichenoti* nested within the New Zealand radiation. This result is in conflict with the findings of Chapter 3 in this thesis (and those of Hardy, 1977; Hardy, 1982; Hutchinson *et al.*, 1990; Patterson and Daugherty, 1995) that the New Zealand skinks form a monophyletic group, and *L. guichenoti* is nested within an Australian radiation. Hickson *et al.* (2000) also found that neither *Cyclodina* or *Oligosoma* are monophyletic, suggesting that the behavioural and morphological characteristics used to define the genera may be convergent. Hickson *et al.*'s (2000) analysis also suggests that *O. suteri* is not the sister group to the viviparous species, implying a complex evolutionary history of reproductive mode in the group. The strongest conclusion of the paper, however, was that the geological history of New Zealand which has involved a large amount of climatological and geological change, caused periods of rapid allopatric speciation and is therefore responsible for the lack of resolution in the skink phylogeny. In reaching this conclusion, Hickson *et al.* (2000) used a number of statistical methods to test for bias and saturation in the data. However, the possibility that the lack of resolution was simply the result of low information content in their dataset was not sufficiently addressed.

I have used the *12S rRNA* data published by Hickson *et al.* (2000) in combination with an unpublished allozyme dataset, and new sequences of *β-fibrinogen* and *myosin* introns to recover the phylogeny of the New Zealand skinks. This has allowed the key conclusions of Hickson *et al.*'s (2000) paper to be tested.

Materials & Methods

Specimens and allozyme data

The ingroup for this analysis comprises 16 species of *Oligosoma* and *Cyclodina* from New Zealand and Lord Howe Island (Table 5.1). Taxa were chosen to represent the major lineages identified in an unpublished allozyme analysis (C. H. Daugherty and

Species:	*12S* GenBank accession #	Tissue #
Cyclodina aenea	new sequence	FT 5253
Cyclodina alani	AF194089	FT 147
Cyclodina ornata	AF194093	FT 192
Cyclodina fallai	AF194096	CD 597
Oligosoma infrapunctatum	AF194099	CD 535
"Oligosoma" lichenigera	new sequence	AMS R93465
Oligosoma lineoocellatum	new sequence	CD 433
Oligosoma maccanni	AF194101	LE 2
Oligosoma microlepis	AF194101	CD 1299
Oligosoma moco	AF194103	FT 156
Oligosoma nigriplantare polychroma	AF194105	FT 5252
Oligosoma smithi	AF194108	FT 193
Oligosoma stenotis	AF194109	FT 2
Oligosoma suteri	AF194110	FT 148
Oligosoma waimatense	new sequence	CD 1214
Oligosoma zelandicum	AF194111	CD 336
Lampropholis guichenoti	new sequence	SAMA R39155
Tiliqua scincoides	new sequence	SAMA R28511

Table 4.1: Specimen number of species. All *Cyclodina* and *Oligosoma* except "*O.*" *lichenigera* are from the collection of Dr C.H. Daugherty Victoria University, Wellington New Zealand. Other collection abbreviations AMS: Australian Museum, Sydney; SAMA: South Australian Museum, Adelaide.

G. B. Patterson, Victoria University, Wellington, New Zealand; Figure 5.1). I included the 17 allozyme loci used in this study in a parsimony analysis (Appendix 5). Loci were treated as characters with alleles as non-additive character states. All allozyme polymorphisms were treated as polymorphisms in the multistate taxa option in PAUP* regardless of the frequency of alternative alleles. Trees were rooted using the Australian *Eugongylus* group skink *Lampropholis guichenoti* and the *Egernia* group skink *Tiliqua scincoides*.

DNA extraction, amplification & sequencing

I used nucleotide sequences from *β-fibrinogen* intron 7, *heavy chain myosin* 2, and previously published sequences of mitochondrial *12S rRNA* (Hickson *et al.*, 2000). *12S rRNA* sequences were also obtained for species that were not sequenced by Hickson *et al.*, and for an additional specimen of *Lampropholis guichenoti* (specimen SAMA R39155 used in Chapter 3) because of the unexpected position of this species in the phylogeny generated in the previous study. *Cyclodina aenea* was represented by two non-monophyletic populations in Hickson *et al.*'s (2000) analysis, therefore I sequenced *12S rRNA* from the individual used for nuclear genes. Primers are listed in Appendix 1 and discussed in Chapter 2. Amplification was conducted using TaqGold hot-start polymerase, after initial denaturation at 94 °C for 9 min; each cycle consisted of denaturation at 94 °C for 45s, annealing at 50-55 °C for 45s, and extension at 72 °C for 1 min, for 34 cycles. Negative controls were run for all amplifications. Amplified products were purified using UltraClean PCR cleanup columns (Mo Bio Laboratories). Products were sequenced using the ABI BigDye sequencing kit on an ABI 3700 capillary sequencer.

Sequence interpretation and phylogenetic analysis

Sequences were edited using SeqEd (ABI) and aligned by eye using Se-Al v1.0 a1 (Rambaut, 1995). Complete alignments are given in appendix 5. Sites for which all taxa except one have gaps (autoapomorphic indels) were excluded in all further analyses.

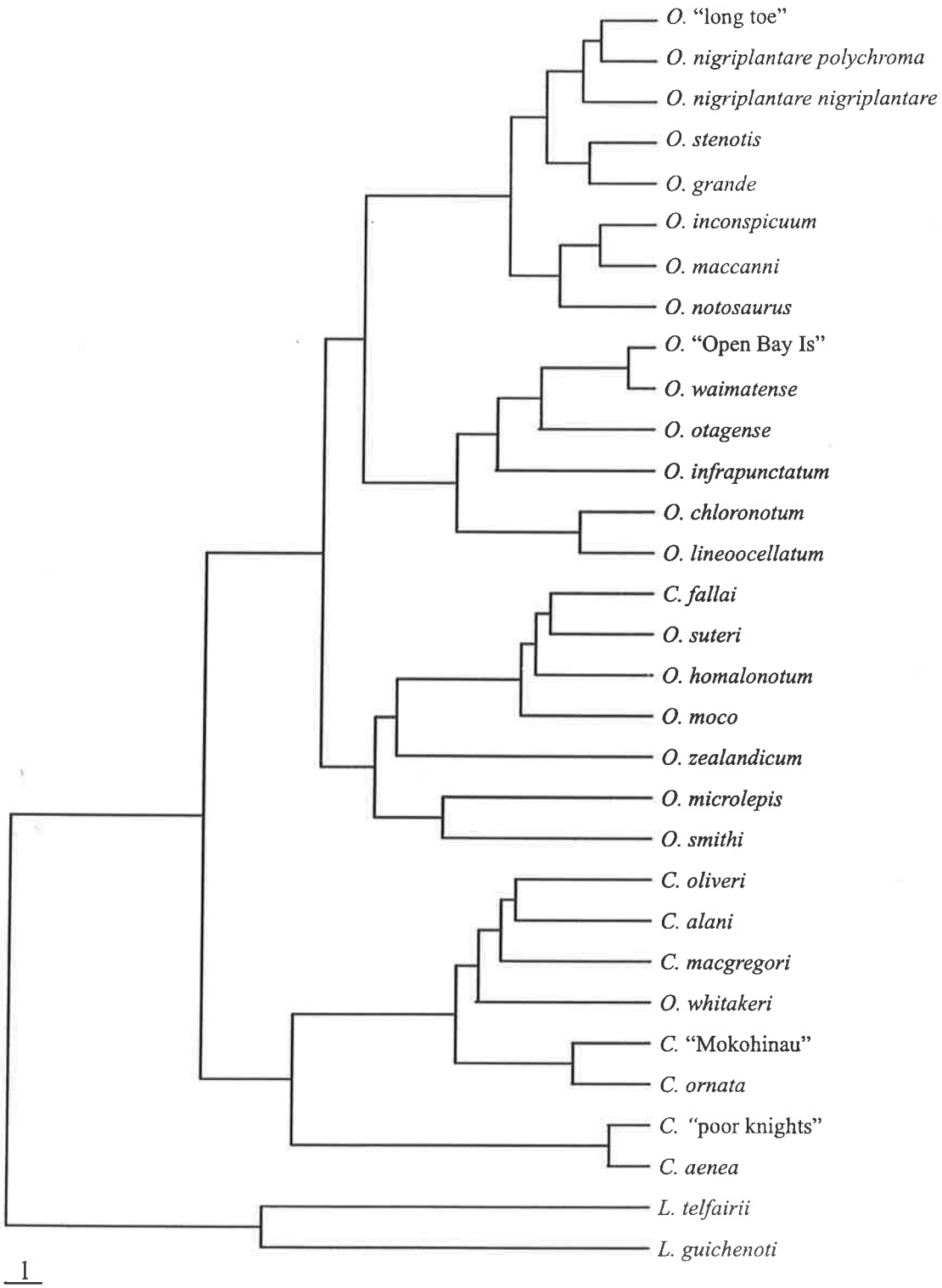


Figure 5.1: Unpublished allozyme tree from C. H. Daugherty and G. B. Patterson, Victoria University, Wellington, New Zealand. Based on WGPMA tree from 17 loci, distances measure as Nei's unbiased D.

Gaps were not coded as characters for analysis. All characters were weighted equally. Phylogenetic analyses were done using PAUP* v 4.0 b8 (Swofford, 1999) on power Macintosh G3 and G4 computers (G4 analyses used the altivec version of PAUP*). Separate partitions were defined for each gene and the allozyme dataset and combinability of partitions was assessed using the incongruence length difference (ILD) test (Farris *et al.*, 1995; Mickevich and Farris, 1981) implemented in PAUP*, and the partitioned Bremer index (Baker and Desalle, 1997) (described in Chapter 1) implemented using Treerot v 2a (Sorenson, 1999) and PAUP*. Tree searches were conducted using an heuristic algorithm (with 500 random addition sequence replicates for maximum parsimony (MP) analysis and 'as-is' sequence addition for maximum likelihood (ML)). Maximum likelihood analysis was carried out under a model determined by hierarchical likelihood ratio tests implemented using Modeltest v 3.1 (Posada and Crandall, 1998). Parameters including tree topology were determined simultaneously using an heuristic search algorithm. Bootstrap resampling was used to gauge support for optimal trees, with 200 heuristic pseudoreplicates (with 10 random addition sequences each) for MP, and 100 pseudoreplicates for ML (using 'as-is' sequence addition with model parameters except topology fixed). The optimal trees resulting from MP and ML analysis were tested against an alternative topology where the viviparous *Oligosoma* and *Cyclodina* are constrained to form a monophyletic group, using Templeton and Shimodaira-Hasegawa tests.

Hickson *et al.* (2000) suggest that the phylogenetic history of the New Zealand skinks includes a hard polytomy. I tested trees for a hard polytomy using the likelihood ratio test suggested by Slowinski (Slowinski, 2001) and the modification of Braun and Kimball's (Braun and Kimball, 2001) method described in Chapter 3.

Results

Information content and congruence of partitions

Alignment of sequences resulted in a total of 1048 characters excluding autoapomorphic insertions (3145 including all sites). 112 characters were parsimony

informative. The published *Lampropholis guichenoti* sequence was 12% divergent from the new sequence generated here (47 of 384 bp differ). The previously published sequence is more similar to *12S rRNA* sequences of New Zealand skinks (data not shown). Two large insertions, one in *β-fibrinogen* and one in *myosin*, were identified from GenBank as short interspersed repetitive element (SINE) insertions; both of these insertions belong to a single SINE family. These retrotransposable elements of the genome have been proposed as powerful phylogenetic markers (Shedlock and Okada, 2000). A fully resolved SINE phylogeny requires at least one insertion for each node, and usually involves SINEs from a number of families. The insertion in *β-fibrinogen* is 482 bp and occurs in a single species, *O. moco*, and the insertion in myosin is 370 bp and occurs in *O. n. polychroma* and *O. maccanni*. The information content of each partition is shown in Table 5.2. Figure 5.2 shows the relationship between ML distance and uncorrected sequence divergence for each pairwise ingroup comparison between taxa for the nuclear genes. In contrast to previous chapters, *myh* has a higher proportion of informative characters and accumulated substitutions faster in the ingroup than *β-fibrinogen*, (Figure 5.2).

The ILD test of all partitions was significant and Bremer indices for the most parsimonious tree from the combined data (Figure 5.3, Table 5.3) suggest that the incongruence is largely between *12S rRNA* and the remaining nuclear sequence and allozyme data. The ILD test of *12S rRNA versus* a partition including the remaining data was also significant (P = 0.03), suggesting that the *12S rRNA* data should be analysed separately from the nuclear and allozyme data. Bremer indices for the nuclear sequence and allozyme data do not suggest that there is any conflict among these data partitions.

Optimal trees

12S rRNA data

The neighbour-joining tree published by Hickson *et al.* (2000) is shown in figure 5.4. My analysis of some of Hickson *et al.*'s (2000) sequences with some additional taxa (Table 5.1) using parsimony resulted in eight equally most parsimonious trees (296

Data Partition	Length 1	Length 2	Variable Characters	PI Characters	% PI a	% PI b	CI	SSR
allozymes	-	17	15	15	88	100	0.64, 0.65	-
12S rRNA	-	386	140	64	17	64	0.61	-
β-fib	1878	655	139	11	1.5	8	0.98, 0.97	1.031
myh	864	376	77	22	6	29	0.84	0.934

Table 5.2: Estimates of the information content of each gene. Length 1 = total length of sequence, Length 2 = length of sequence excluding autoapomorphic indels, Variable Characters = number of variable characters, PI Characters = number of parsimony informative characters, % PI = percent of (a) total characters and (b) variable characters from this gene (or codon position) which are parsimony informative, CI = consistency index on the most parsimonious trees from the combined data (*12S rRNA* on separate tree), SSR = rate estimated from ML.

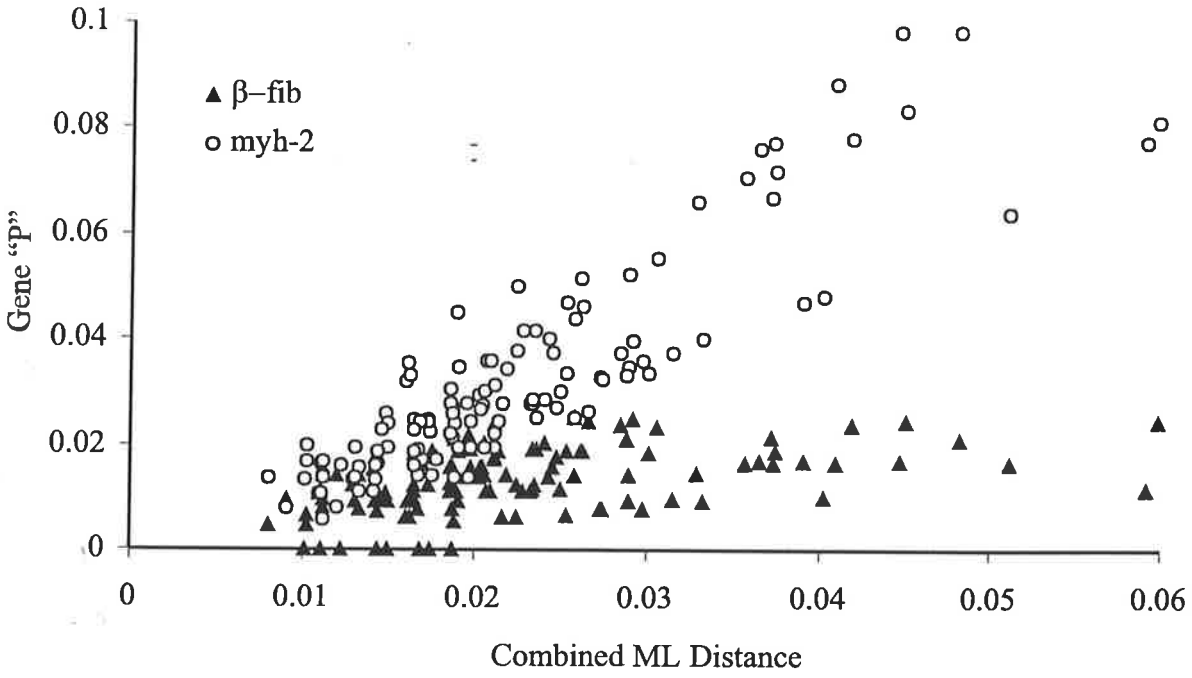


Figure 5.2: Pairwise comparisons of ML distance estimated from combined data (x-axis) *versus* uncorrected p from each gene (y-axis), for *Oligosoma* and *Cyclodina*.

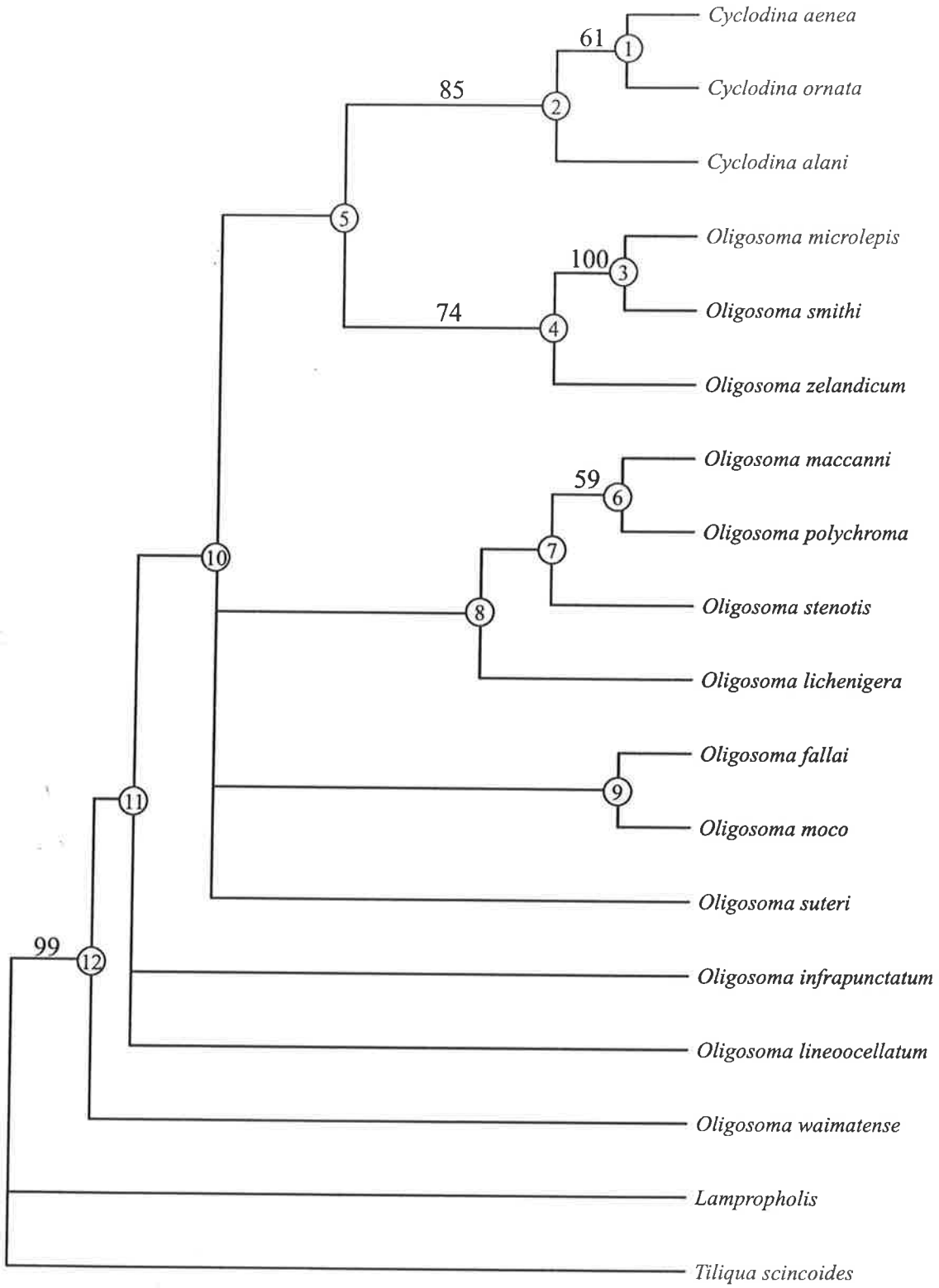


Figure 5.3: Consensus of two equally most parsimonious trees from mitochondrial and nuclear sequence data and allozymes.

Node	Bootstrap Proportion	Bremer Index	Partitioned Bremer Support			
			12s rRNA 64	*β-fib* 11	*myh-2* 22	Allozyme 15
Ingroup:						
1	61	1	-0.7	0.7	1.2	-0.2
2	85	4	0	0	4	0
3	100	9	6.4	1.2	-0.2	1.4
4	74	2	-1	0.2	1.5	1.3
5	-	1	-2.4	2.6	1.1	-0.3
6	59	2	3	-1	0.5	-0.5
7	-	1	0.7	0.3	0.5	-0.5
8	-	1	2	-0.7	0	-0.3
9	-	1	-0.7	1	0.7	0
10	-	1	3.8	-1.2	-1.1	-0.7
11	-	1	5.5	-1.5	-2.5	-0.5
Outgroup:						
12	99	9	8	1	1.5	-0.5

Table 5.3 - Bremer and bootstrap support for the two equally most parsimonious trees resulting from analysis of combined mitochondrial, nuclear and allozyme data (Fig. 5.3). Bremer and bootstrap values apply to the branch proceeding each node. The number of parsimony informative characters for each partition are at the top of each column.

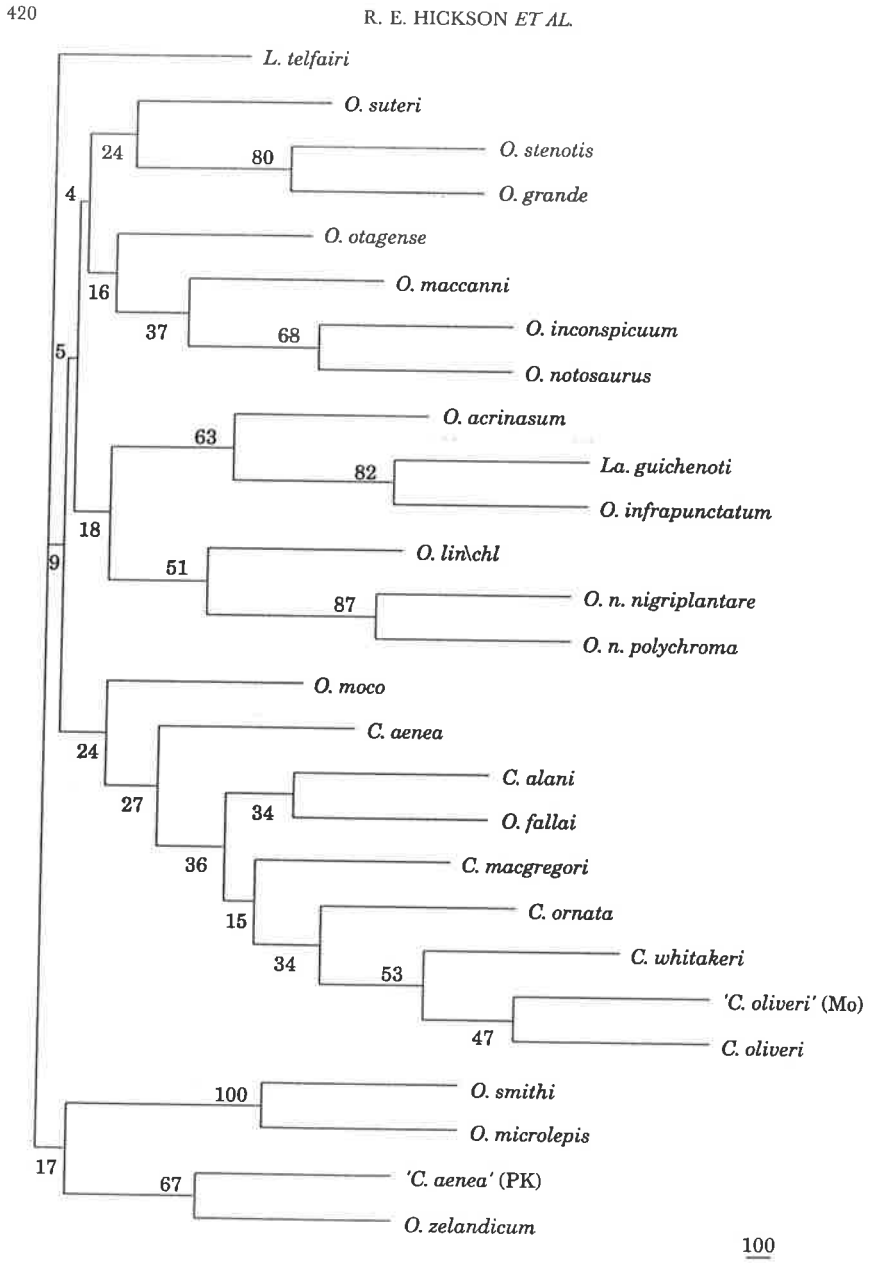


Figure 1. Neighbour-joining phylogeny for the skink taxa using the Kimura two-parameter correction. Transversions were weighted four-fold higher than transitions. Percentage bootstrap values from 1000 replicates are shown. Branch lengths are drawn proportional to their probability of change. Note the long branches leading to individual taxa, the short internal branches, and low bootstrap values. *L. telfairi* was used as the outgroup. The scale bar at the lower right indicates a probability of change of 0.01. Phylogenies derived from other tree reconstruction programs were essentially the same.

Figure 5.4: Hickson *et al.*'s (2000) phylogeny of the New Zealand's skinks from their *12S rRNA* data.

steps) with only two nodes in common. A 70% majority rule consensus of these eight trees (Figure 5.5), reveals a monophyletic *Cyclodina* with *O. fallai* as the sister taxon; a clade including ((*O. microlepis*, *O. smithi*), *O. zelandicum*); and *O. n. polychroma* and *O. suteri* as sister taxa. Maximum likelihood analysis of these data using the TrN93 + *i* + *g* model (base frequencies A: 0.3891, C: 0.2488, G: 0.1745, T: 0.1876; substitution rate A↔C, A↔T, C↔G, G↔T: 1, A↔G: 7.5710, C↔T: 13.9992; gamma shape parameter: 0.4203; proportion of invariable sites: 0.3517) resulted in a single optimal tree (Figure 5.6). The ML tree surprisingly does not include a monophyletic *Cyclodina* / *Oligosoma* lineage and instead includes the *Lampropholis* + *O. infrapunctatum* grouping recovered in Hickson *et al.*'s neighbour joining tree (even though this sequence is not from their misidentified sample). Slowinki's (2001) likelihood ratio test of branch lengths on the best tree is the most appropriate measure of polytomy "hardness" for phylogenies based on a single genetic locus. Likelihood ratio tests of the *12S rRNA* ML tree show that only one of the internodes of this trees is significantly longer than zero (Figure 5.7, Table 5.4).

Nuclear and allozyme data

Maximum parsimony analysis of *β-fibrinogen*, *myosin* and allozyme data resulted in 17 equally most parsimonious trees (320 steps, consensus tree figure 5.8), with several nodes that have >70% bootstrap support. Maximum likelihood analysis using the Hasegawa-Kishino-Yano (HKY, Hasegawa *et al.*, 1985) model with estimated gamma shape parameter (base frequencies A:0.2943, C:0.1848, G:0.2257, T:0.2952; ti/tv ratio: 1.6081, gamma shape parameter: 1.4418) resulted in a single tree (-ln likelihood = 2817.5307; Figure 5.9). Likelihood ratio tests were used to determine which internodes in the most likely tree are significantly longer than zero (Slowinski, 2001). Significance levels were corrected for split degrees of freedom (Goldman and Whelan, 2000; Slowinski, 2001) and a Bonferroni correction for multiple comparisons applied. Only three internodes are significantly longer than zero under the most stringent conditions, and all but two nodes are longer than zero without a Bonferroni

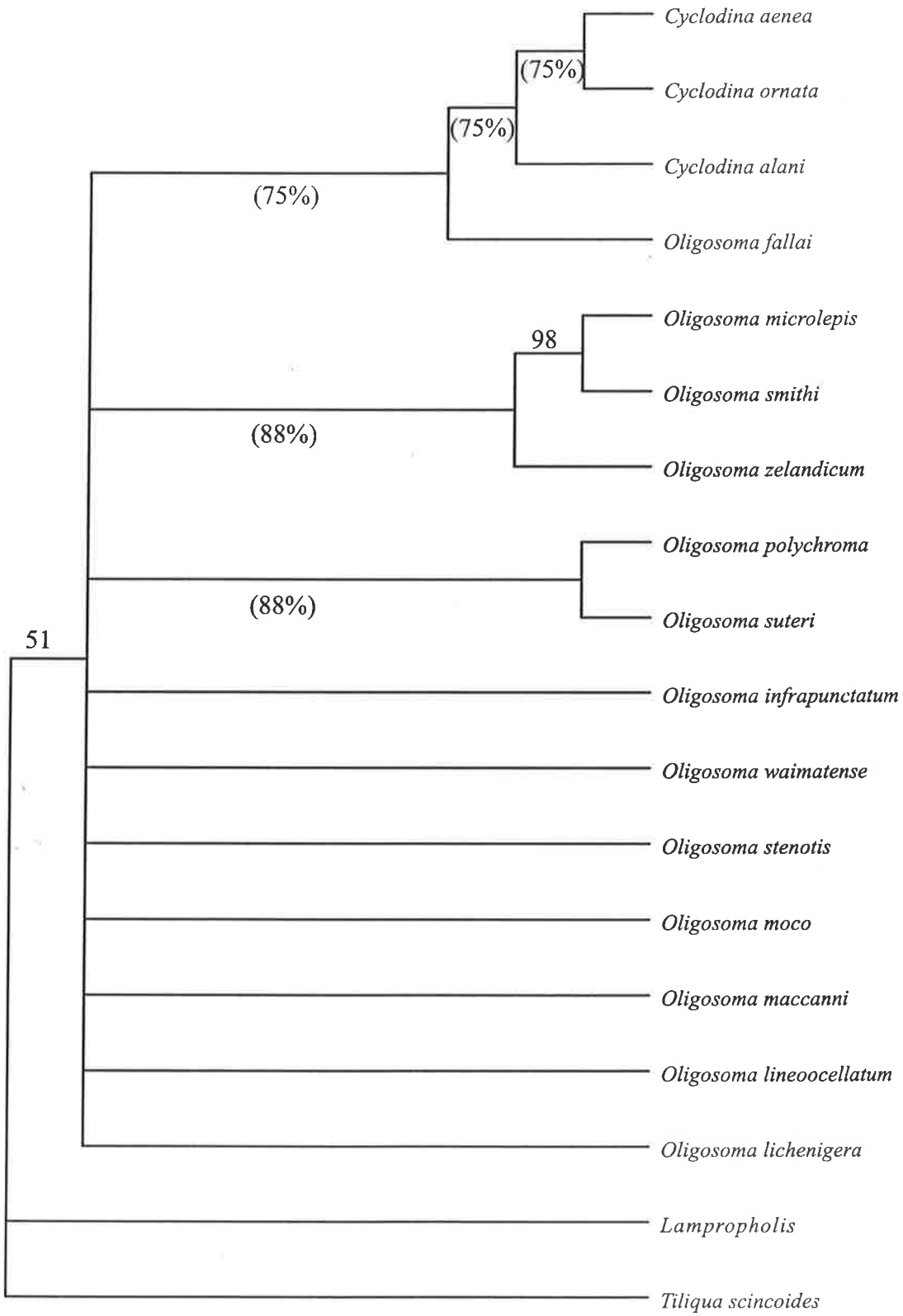


Figure 5.5: 70% majority rule consensus of 8 most parsimonious trees resulting from analysis of *12S rRNA* nucleotide sequence data. Frequency of nodes occurring in less than 100% of the most parsimonious trees are given in brackets. Bootstrap proportions >50% are shown above branches.

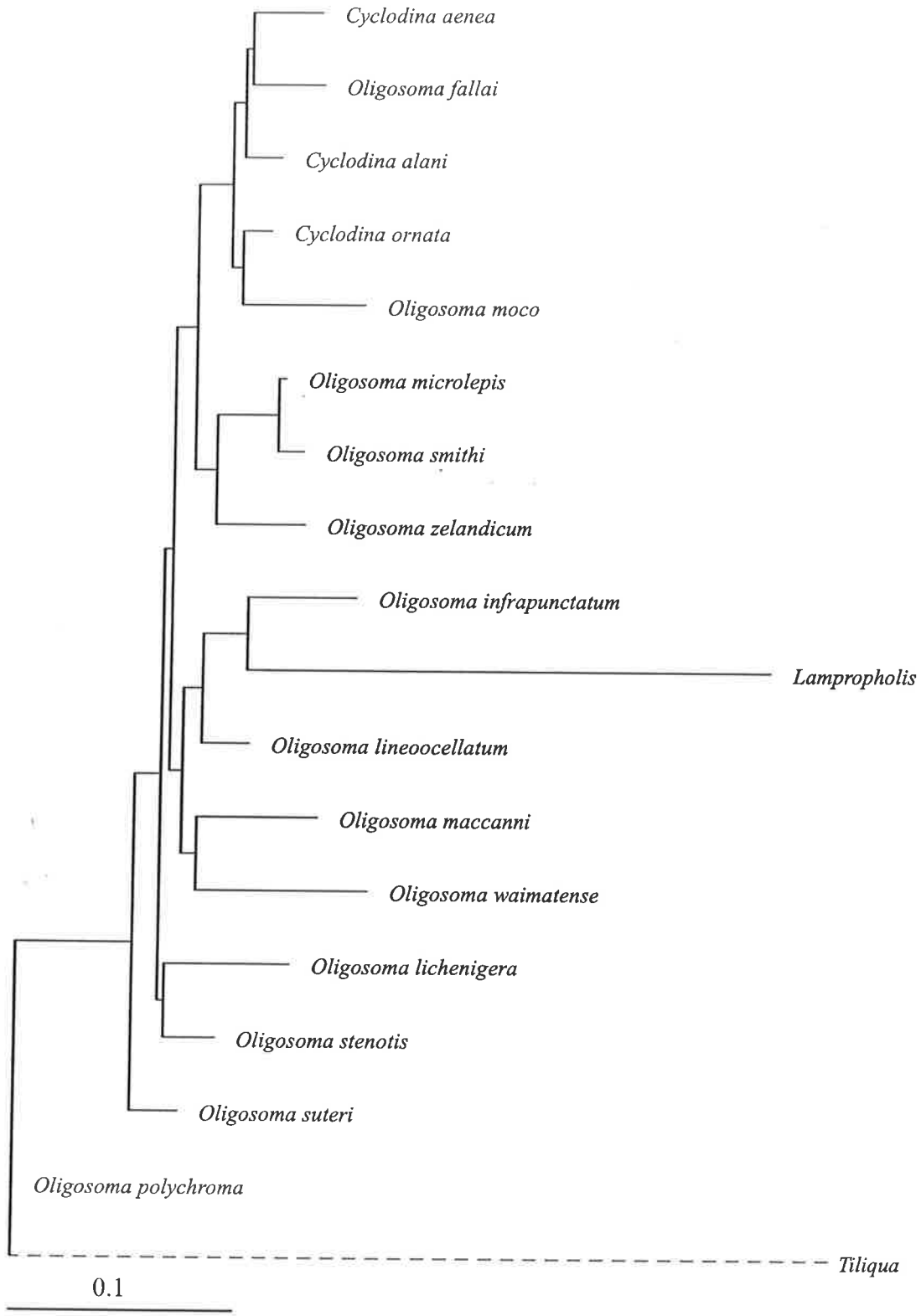


Figure 5.6: Maximum likelihood tree from analysis of *12s rRNA* data for New Zealand skinks. *Tiliqua scincoides* branch has been shortened by ~50% to fit on page.

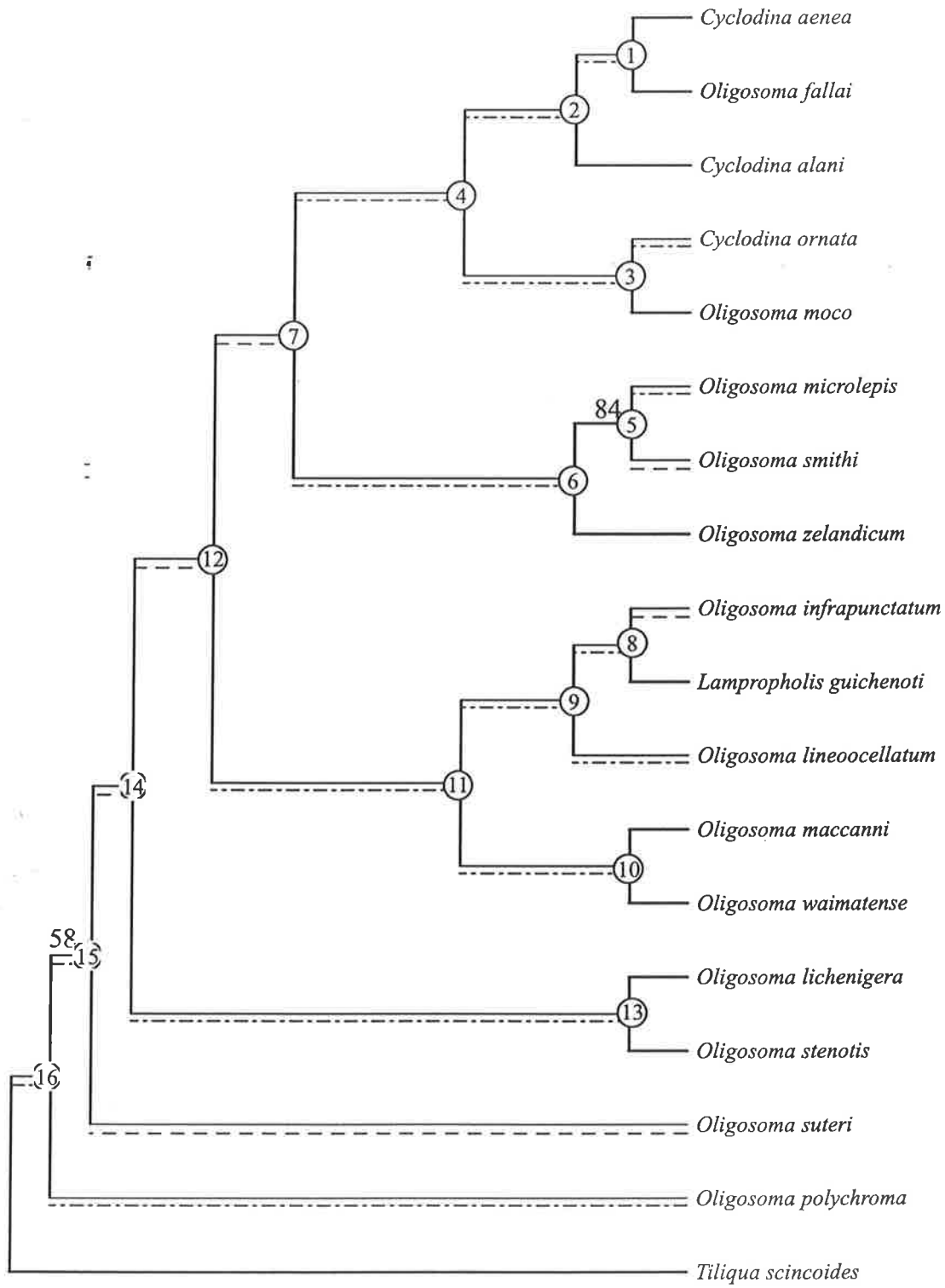


Figure 5.7: Maximum likelihood cladogram from analysis of *12S rRNA* data. Bootstrap proportions are shown above branches. Branches not significantly longer than zero are dashed (Bonferroni correction and split degrees of freedom) or dash-dot (with split degrees of freedom only) see table 5.4. Numbers above branches are bootstrap support values.

Node 1	Node 2	Branch length	Standard error	lnL diff	P
Cyclodina aenea	1	0.031	0.011	12.64	<0.001
Cyclodina alani	2	0.017	0.008	19.34	<0.001
Cyclodina ornata	3	0.013	0.008	2.15	0.038**
Oligosoma fallai	1	0.032	0.012	21.60	<0.001
Oligosoma infrapunctatum	8	0.049	0.018	6.33	<0.001*
Oligosoma lichenigera	13	0.057	0.017	31.82	<0.001
Oligosoma lineoocellatum	9	0.021	0.010	5.03	0.002*
Oligosoma maccanni	10	0.054	0.016	13.21	<0.001
Oligosoma microlepis	5	0.003	0.004	0.33	0.415**
Oligosoma moco	3	0.056	0.016	27.88	<0.001
Oligosoma n. polychroma	16	0.000	0.017	<0.001	1**
Oligosoma smithi	5	0.012	0.007	5.48	<0.001*
Oligosoma stenotis	13	0.023	0.010	10.27	<0.001
Oligosoma suteri	15	0.021	0.010	6.44	<0.001*
Oligosoma waimatense	10	0.077	0.021	25.38	<0.001
Oligosoma zelandicum	6	0.040	0.013	14.06	<0.001
Lampropholis guichenoti	8	0.236	0.053	62.93	<0.001
Tiliqua scincoides	16	0.721	0.150	116.47	<0.001
1	2	0.003	0.004	0.61	0.27**
2	4	0.006	0.005	1.78	0.059**
3	4	0.005	0.006	0.35	0.401**
4	7	0.016	0.009	6.15	<0.001*
5	6	0.027	0.011	8.62	<0.001
6	7	0.010	0.008	2.23	0.035**
7	12	0.009	0.007	2.70	0.02*
8	9	0.020	0.015	1.02	0.153**
9	11	0.008	0.007	1.54	0.079**

Node 1	Node 2	Branch length	Standard error	lnL diff	P
10	11	0.007	0.008	0.49	0.322**
11	12	0.006	0.006	2.90	0.016*
12	14	0.004	0.005	0.23	0.502**
13	14	0.003	0.005	0.17	0.562**
14	15	0.012	0.008	4.94	0.002*
15	16	0.052	0.016	2.62	0.022**

Table 5.4: Likelihood ratio tests of branch length. P values are from the chi squared test (output from PAUP*), values marked with ** are significant at $\alpha = 0.05$ with 0/1 split degrees of freedom (see Goldman, 2000), values marked with * are significant at $\alpha = 0.05$ with split degrees of freedom only when the Bonferroni correction for multiple tests is applied. See figure 5.7 for node numbers.

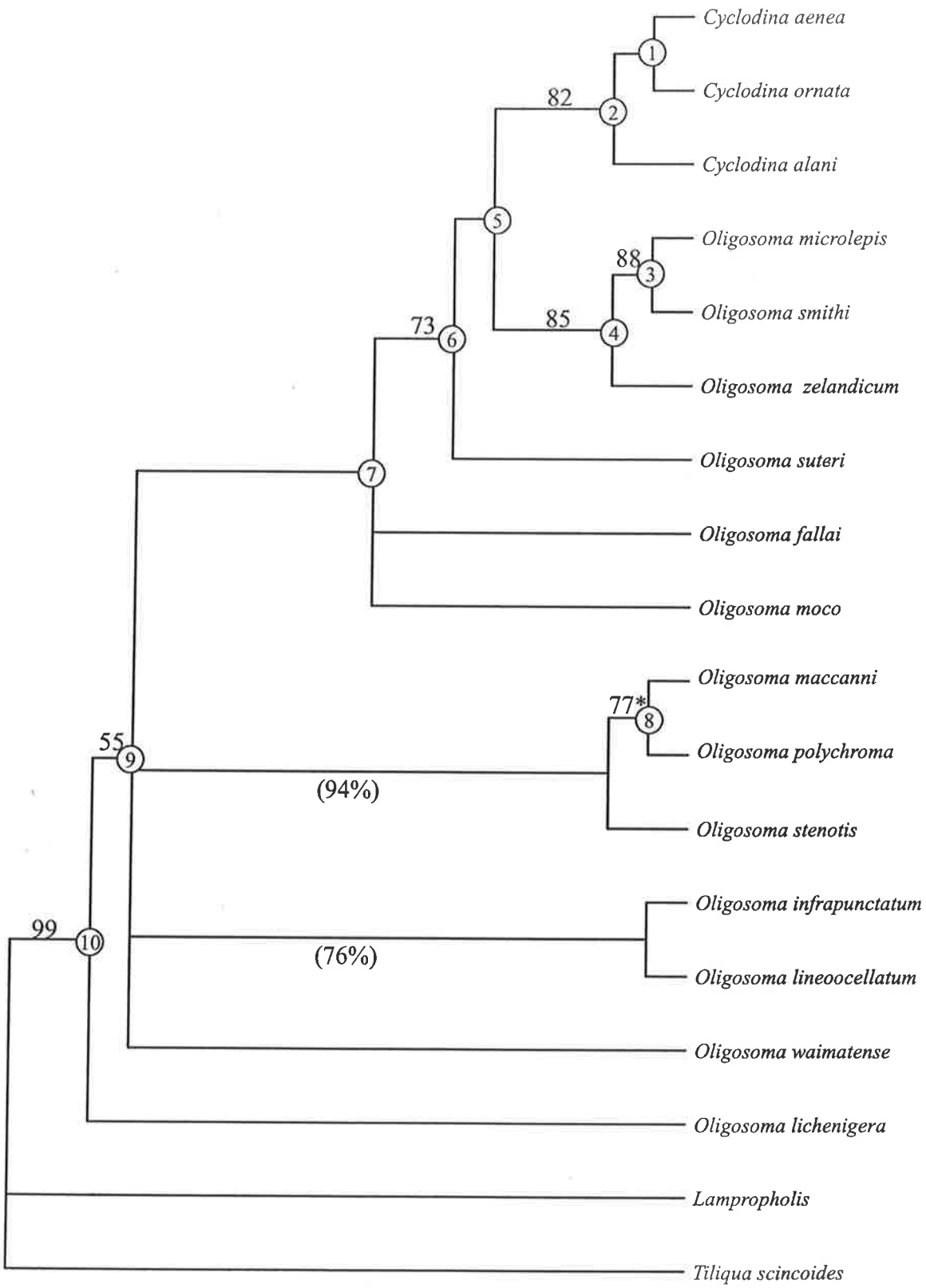


Figure 5.8: 70% majority rule consensus of 17 most parsimonious trees resulting from analysis of allozymes, and β-*fibrinogen* and *myh-2* nucleotide sequence data. Frequency of nodes occurring in less than 100% of the most parsimonious trees are given in brackets. Bootstrap proportions >50% are shown above branches. * The sister relationship of *O. n. polychroma* and *O. maccanni* is also supported by the presence of a ~480bp SINE in these species.

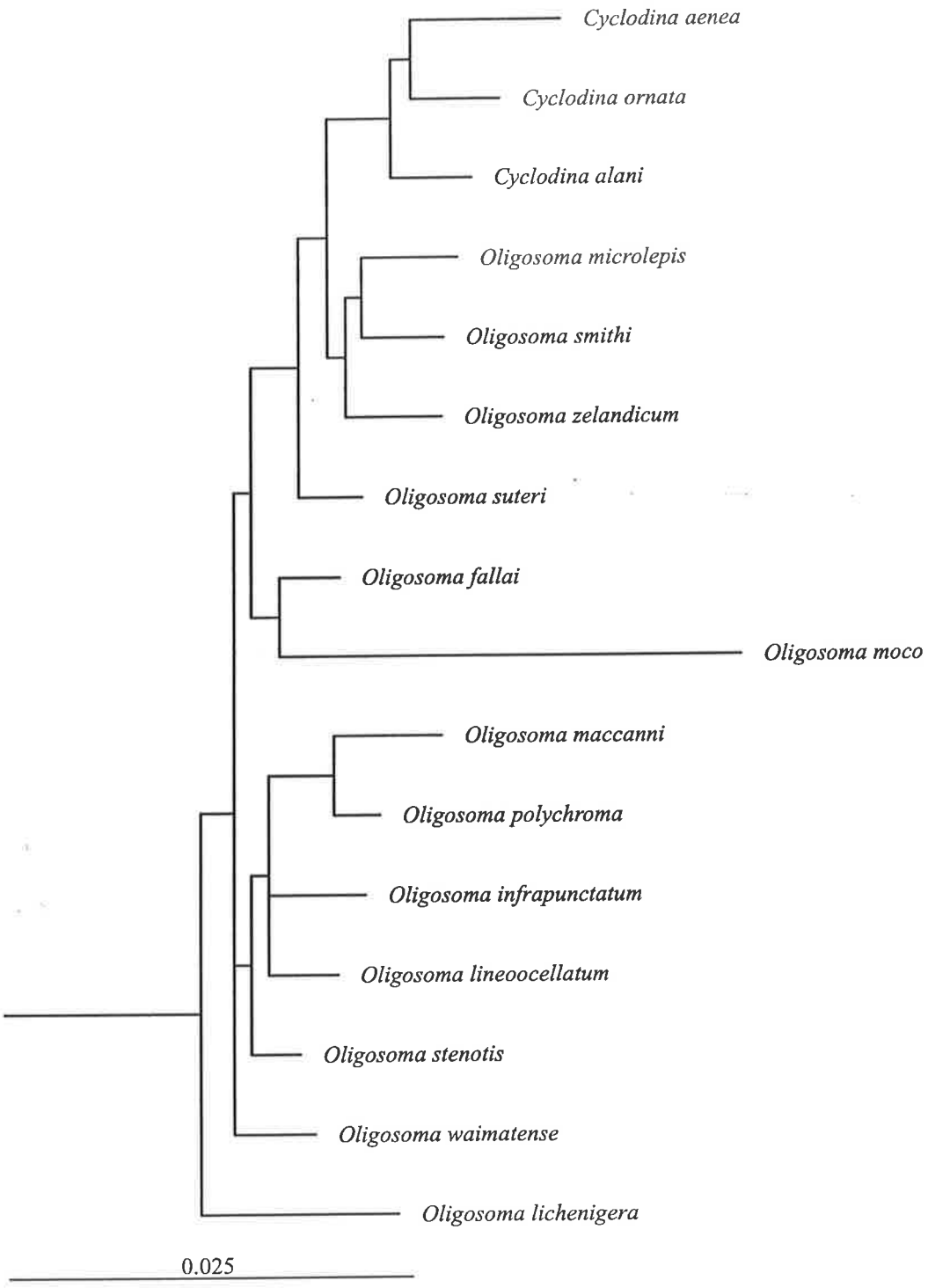


Figure 5.9: Optimal tree from nuclear sequence data under maximum likelihood. Outgroups are not included.

correction (Table 5.5, Figure 5.10). The topology of the ML and MP analyses are largely consistent. Both MP and ML analyses suggest that the New Zealand skinks form a monophyletic group, which is the sister group to "*Oligosoma*" *lichenigera*. MP and ML analyses recovered a monophyletic group consisting of the three *Cyclodina* species sampled. The sister group to *Cyclodina* in both analyses is a clade including *Oligosoma microlepis*, *O. smithi* and *O. zelandicum*. *Oligosoma suteri* is the sister group to these clades. MP and ML analyses also support the affinity of *O. fallai* and *O. moco* with this lineage, and the sister relationship between *O. maccanni* and *O. nigriplantare polychroma*. The hypothesis that the viviparous members of the New Zealand skink radiation form a monophyletic group excluding *O. suteri* was not statistically rejected by either Templeton or Shimodaira-Hasegawa tests (Table 5.6).

The increased resolution and support of branches resulting from nuclear data compared to the less informative *12S rRNA* data suggests that the lack of resolution among the New Zealand skinks is the result of insufficient data rather than rapid diversification. The modification of Braun and Kimball's (2001) equation to allow an estimate of the "hardness" of a polytomy described in Chapter three relies on having an estimate of the rate of substitution, λ. Although there are no fossil calibration points for the time of divergence of skinks in New Zealand, Hickson *et al.* (2000) used comparisons of *12S rRNA* divergence within *Oligosoma* and *Cyclodina* with divergence within bovid and ratite groups to estimate the divergence time of *Oligosoma* and *Cyclodina* as 23-24 million years ago (MYA). Using 24 MY as the calibration for the *"O." lichenigera* – NZ taxa split (probably an underestimate), the divergence between *"O." lichenigera* and the remaining *Cyclodina* and *Oligosoma* give an estimation of the rate of evolution of nuclear genes (λ) of 5.315×10^{-4} substitutions/site/million years. The calculation of minimum branch length ($t = -\ln(0.2) / \lambda L$) gives an estimate of 2.94 million years. This minimum branch length is much less than the estimate from the *12S rRNA* data alone, but in the context of the New Zealand fauna is not sufficient to consider the relationships unresolvable.

Node 1	Node 2	Branch length	Standard error	lnL diff	P
Cyclodina aenea	1	0.009	0.003	29.52	<0.001
Cyclodina alani	2	0.005	0.003	18.90	<0.001
Cyclodina ornata	1	0.006	0.003	11.38	<0.001
Oligosoma fallai	7	0.004	0.002	5.33	0.001*
Oligosoma infrapunctatum	10	0.006	0.004	27.88	<0.001
Oligosoma lichenigera	13	0.012	0.004	34.32	<0.001
Oligosoma lineoocellatum	10	0.004	0.002	29.70	<0.001
Oligosoma maccanni	8	0.007	0.003	14.12	<0.001
Oligosoma microlepis	3	0.006	0.002	28.66	<0.001
Oligosoma moco	7	0.029	0.007	90.51	<0.001
Oligosoma n. polychroma	9	0.003	0.003	3.71	0.006*
Oligosoma smithi	3	0.005	0.002	25.55	<0.001
Oligosoma stenotis	11	0.003	0.017	17.47	<0.001
Oligosoma suteri	6	0.004	0.002	17.40	<0.001
Oligosoma waimatense	12	0.005	0.002	61.39	<0.001
Oligosoma zelandicum	4	0.006	0.002	31.23	<0.001
Lampropholis guichenoti	root	0.058	0.009	66.43	<0.001
Tiliqua scincoides	root	0.102	0.013	162.73	<0.001
1	2	0.001	0.001	1.76	0.061**
2	5	0.005	0.002	19.99	<0.001
3	4	0.001	0.001	3.30	0.010*
4	5	0.001	0.001	4.76	0.002*
5	6	0.002	0.001	4.88	0.002*
6	8	0.003	0.002	13.74	<0.001
7	8	0.002	0.017	1.69	0.066**
8	12	0.001	0.001	5.44	<0.001*
9	10	0.004	0.002	7.07	<0.001

Node 1	Node 2	Branch length	Standard error	lnL diff	P
10	11	0.001	0.001	3.84	0.006*
11	12	0.001	0.001	4.41	0.003*
12	13	0.002	0.002	3.89	0.005*
13	root	0.012	0.005	4.74	0.002*

Table 5.5: Likelihood ratio tests of branch length from nuclear gene sequnces. P values are from the chi squared test (output from PAUP*), values marked with ** are significant at $\alpha = 0.05$ with 0/1 split degrees of freedom (see Goldman, 2000), values marked with * are significant at $\alpha = 0.05$ with split degrees of freedom only when the Bonferroni correction for multiple tests is applied. See figure 5.10 for node numbers.

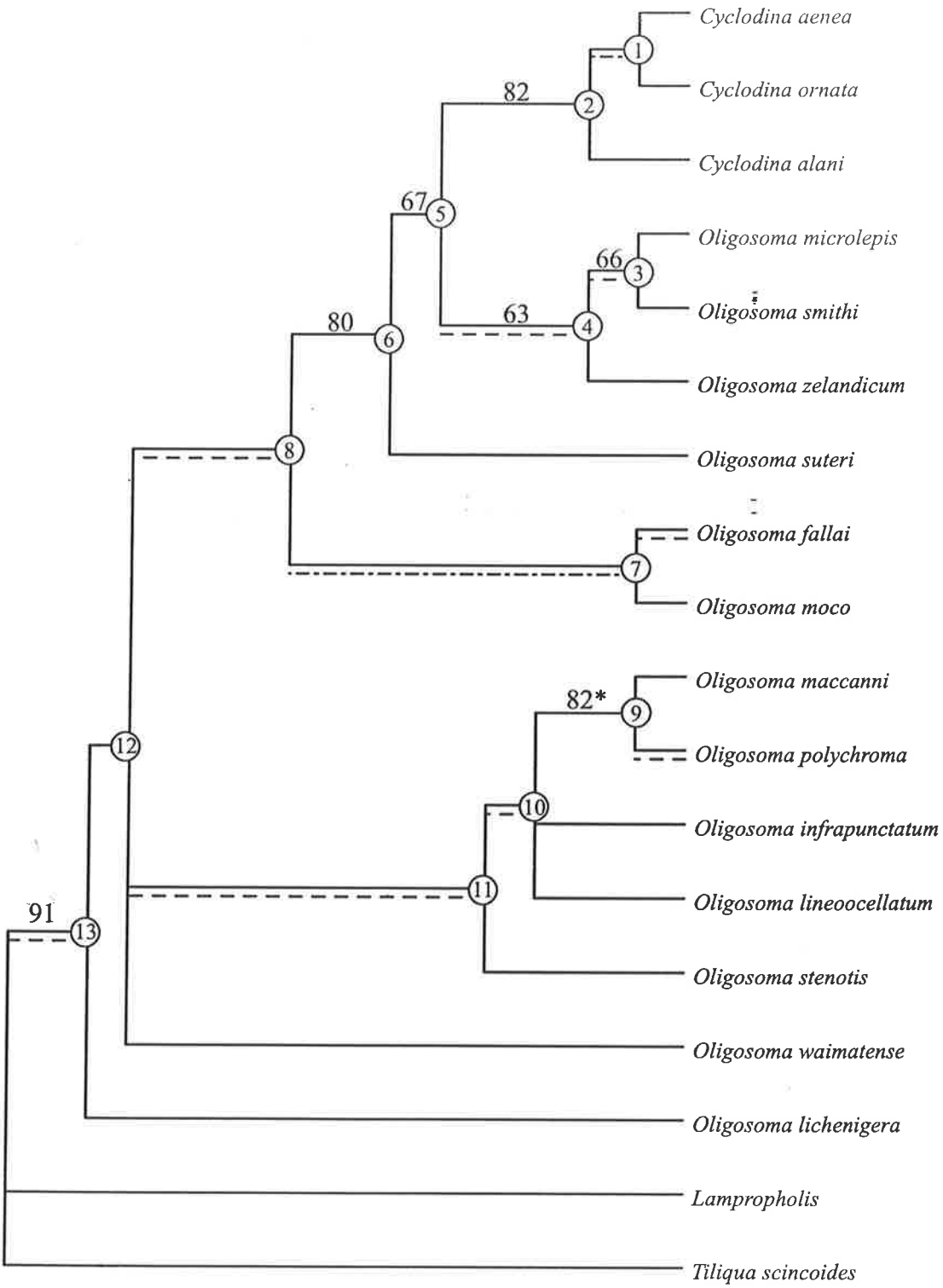


Figure 5.10: Maximum likelihood tree from nuclear sequence data. Bootstrap proportions >50% are shown above branches. Node numbers refer to table 5.5, branches not significantly longer than zero are dashed (Bonferroni correction and split degrees of freedom) or dash-dot (with split degrees of freedom only). * The sister relationship of *O. n. polychroma* and *O. maccanni* is also supported by the presence of a ~480bp SINE in these species.

Alt. Tree Score	Number of Alt. Trees	T	n	z	P
323 steps	9	0	3	-1.7321	0.1
		3	5	-1.3416	0.2
		8	7	-1.1339	0.3
		15	9	-1	0.3
		24	11	-0.9045	0.4
		35	13	-0.8321	0.4
-ln(L) = -2817.531	1				0.063

Table 5.6: Results of a Templeton test of the hypothesis that *Oligosoma* and *Cyclodina* form a monophyletic group excluding *O. suteri* and *O. lichenigera*. Nine alternative trees of 323 steps were tested against the 17 most parsimonious trees of 320 steps.

Discussion

Molecular data

As seen in other chapters, the information content of intron loci is lower than that of mitochondrial DNA. In contrast to previous chapters, however, the more variable mitochondrial DNA is less informative about the phylogeny (the consensus tree from *12S rRNA* data is much less resolved and poorly supported). Also in contrast to previous chapters, *myosin* is much more informative than *β-fibrinogen* within the New Zealand skinks. The reason for this improved relative performance of *myh* is unclear. If variability in information content between lineages is a general characteristic of introns, it will be difficult to predict which intron loci are likely to be informative for a particular study.

The incongruence between the mtDNA and nuclear and allozyme data, and the lack of information contained in the relatively variable mtDNA, deserves further attention, particularly in the context of the multiple sequences of *ND5* amplified from New Zealand skinks (discussed in Chapter 2). Although both Hickson (2000) and this study included mtDNA enriched extractions as controls for *12S rRNA* amplification, unidentified nuclear paralogues for some sequences are still possible.

Phylogenetic conclusions

The results presented here conflict with the major conclusions of Hickson *et al.* (2000) that the New Zealand skinks are not monophyletic, and that the phylogenetic history of the New Zealand skinks includes a hard polytomy. Although the new nuclear sequence data do not provide a fully-resolved and strongly supported phylogeny, it provides greater resolution and support (>50% bootstraps and a greater number of branches with significant length) for the phylogeny than the *12S rRNA* data. This increase in resolution with new data is in contrast to the expectations of a phylogeny that includes a hard polytomy. The "softness" of the polytomy recovered is also supported by the estimate of the shortest branch that could be recovered by the nuclear

data at ~3 million years. A much more resolved and strongly supported phylogeny of the New Zealand skinks should be achievable with a larger dataset. The topology of the nuclear DNA and allozyme phylogeny also suggest that the New Zealand skinks belong to a monophyletic lineage. The inclusion of *Lampropholis guichenoti* in the New Zealand lineage by Hickson *et al.* (2000), was the result of a misidentified tissue sample; our new *L. guichenoti* sample is outside of the New Zealand lineage in all analyses (including those in Chapter 3) except for the *12S rRNA* ML tree. The nuclear DNA phylogeny also suggests that the sister taxon to the New Zealand skink radiation is the endemic Lord Howe Island skink *"O." lichenigera*. This relationship establishes the closest oviparous sister lineage to the largely viviparous New Zealand radiation. The nuclear DNA and allozyme data suggest that *Cyclodina* may be monophyletic (although further sampling is required), and that *Oligosoma* is composed of a number of lineages whose relationships are likely to be resolved by further data.

The nuclear and allozyme data, and to a lesser extent *12S rRNA* data, suggest that *O. suteri*, the only oviparous species in the endemic radiation, is not the sister taxon to the viviparous species. This result is not statistically significant because of the lack of support for any of the nodes in the tree, and needs to be tested with further data. If the result is upheld, it suggests that the evolution of reproductive mode among the New Zealand skinks was not simple, and potentially includes secondarily derived oviparity in *O. suteri*.

Summary

Results of a phylogenetic analysis of a relatively small amount of nuclear DNA sequence data are not compatible with the previous (mtDNA based) conclusions about the phylogenetic history of the New Zealand skinks. Although a resolved phylogeny was not obtained from these data, results suggest that the problem is due to low information content in the dataset rather than the evolutionary history of the organisms.

Chapter 6 - Discussion

Usefulness of nucleotide sequence data

Mitochondrial nucleotide sequence data suffer from extensive problems with nuclear paralogues in the *Eugongylus* group. The paralogues occurring in *ND2* and *ND5* in *Eugongylus* group skinks have open reading frames and are not most similar to each other. These paralogues have, in some cases, been amplified from dilute mitochondria-enriched DNA template. These characteristics together mean that it is difficult to confidently assign the origin of any putatively mitochondrial sequence from these taxa. Mitochondrial paralogues in other taxa have been subject to more detailed investigation. A combination of amplification from cDNA and cloning of total cellular PCR product can allow paralogues to be sequenced and their divergence from the mitochondrial copy evaluated. Amplification of mitochondrial genes from cDNA template has been found, in at least one case, to result in a single product, when an identical amplification from total cellular DNA resulted in multiple sequences (Williams and Knowlton, 2001). The single gene copy amplified from cDNA was assumed to be mitochondrial in origin, although there is no evidence to support the assumption that nuclear paralogues of mitochondrial genes are not transcribed. Cloning of PCR product allows multiple paralogue sequences to be isolated (Nielsen and Arctander, 2001; Williams and Knowlton, 2001) and the combination of these approaches allows the comparative evolution of mitochondrial and nuclear copies of the genes to be investigated. Such a study of the *ND5* paralogues identified here would be particularly interesting because there are at least three copies of the gene in some taxa, and the insertions are at least 5.5kb long. Mitochondrial heteroplasmy is an alternative, although probably less likely, explanation for the multiple products I obtained from mitochondrial genes.

Nuclear sequences from coding and non-coding loci have been less phylogenetically informative than those of mitochondrial loci, but are nonetheless an important independent source of data. Coding nuclear loci proved to be easier to identify, amplify and align than introns, and are equally or more informative. The

relative information content of the two intron loci, *β-fibrinogen* and *myh-2*, varied between lineages within the *Eugongylus* group. This variability could make the selection of an appropriate intron for phylogenetic studies difficult. Overall, coding loci seem to offer much more potential as phylogenetic tools than introns. Although intronless genes long enough to make sequencing worthwhile are rare, amplification and sequencing from cDNA template may be a productive approach.

Incongruence between nuclear and mitochondrial sequence data in my analysis of New Zealand's skinks emphasises the importance of testing for gene tree / species tree conflict with independent datasets. The occurrence of multiple nuclear paralogues of *ND5* in these skinks raises the possibility that incongruence is due to undetected paralogues in the *12S rRNA* dataset, or possibly to mitochondrial heteroplasmy as discussed in Chapter 2.

Previous phylogenetic concepts and biogeography

The *Eugongylus* group, as morphologically defined, is monophyletic relative to the representatives of the *Mabuya* and *Egernia* groups used as outgroups in this study. The early split between the African and Indo-Pacific *Eugongylus* group taxa suggests the possibility that the group may have originated in Africa rather than in south-east Asia. The south-east Asian origin of the *Eugongylus* group was proposed by Greer (1979) on the basis that the "primitive" *Mabuya* species, which he thought were the ancestor of all Australian lineages, have their centre of diversity in this region. This sister group relationship between the *Eugongylus* and *Mabuya* groups was supported by Honda *et al.* (fig 1.1, Honda *et al.*, 2000), although the *Eugongylus* group was equally close to the African and Asian *Mabuya*. A thorough understanding of the relationships among all lygosomine lineages will provide the framework within which hypotheses these about the origins of the *Eugongylus* group can be distinguished.

The lack of resolution among the non-African lineages of the *Eugongylus* group is in conflict with several other studies. I did not find evidence for a monophyletic Australian lineage as suggested by the immunological data of Hutchinson *et al.* (1990).

Hutchinson *et al.*'s conclusion, however, was based on one-way immunological comparisons of relatively divergent taxa, and is therefore not necessarily a robust result. Why the Australian taxa may not form a monophyletic group when the representatives from New Zealand and New Caledonia do, is unclear. It may be that Australia was independently invaded by several lineages, or that Australia acted as a centre of diversification for the whole region. The latter explanation would contrast with the biogeographic history of diplodactyline geckos throughout Australia, New Zealand and New Caledonia (Chambers *et al.*, 2001). Mitochondrial and nuclear ribosomal RNA nucleotide sequence data suggest that New Zealand's two endemic gecko genera form a monophyletic group, which is the sister lineage to a clade including the New Caledonian geckos and one tropical Australian genus (*Pesudothecadactylus*) (Chambers *et al.*, 2001). The remaining Australian diplodactyline genera belong to the sister lineage of the NZ + NC clade. Sister group relationships between New Zealand and New Caledonia are also found in parakeets and cicadas (Chambers *et al.*, 2001).

Nuclear DNA sequence data suggest that the New Zealand genus *Cyclodina* is nested within the genus *Oligosoma*. *Oligosoma* should thus be split into a number of monophyletic genera, although a more strongly supported phylogeny, including all the species of *Oligosoma* should form the basis of any such taxonomic change. The relationship among the endemic skinks parallels the finding that New Zealand's endemic green gecko genus *Naultinus* is nested within the brown gecko genus *Hoplodactylus* (Chambers *et al.*, 2001). My phylogeny of New Zealand's skinks conflicts with the hard polytomy, and thus the biogeographic scenario, of Hickson *et al.* (2000). I do not agree that there are sufficient data to suggest that New Zealand's skinks underwent rapid allopatric speciation, however the incongruence between nuclear and mitochondrial sequence data from these taxa should be investigated.

Evolution of reproductive mode and placentation in the *Eugongylus* group

This study has established that complex placentae have evolved separately in *Niveoscincus* and *Pseudemoia*. A fully resolved and robust intraspecfic phylogeny of

Pseudemoia has been developed which will act as a framework for statistical comparative analysis of the evolution of placentation in this genus. My results did not support the suggestion by Hutchinson *et al.* (1990) that *Bassiana* and *Morethia* form the oviparous sister lineage to *Pseudemoia*. The affinities of *Niveoscincus coventryi*, the only *Niveoscincus* restricted to mainland Australia and the only primarily lecithotrophic *Niveoscincus* described, remain unclear. Nuclear DNA sequence data suggest that *N. coventryi* is more closely related to *Lampropholis* or *Carlia*, than to the remaining *Niveoscincus*. A focused study, with more thorough sampling of taxa from this part of the tree should be able to firmly establish the relationships of *N. coventryi* and to determine whether this species represents a third origin of placentation in the Australian *Eugongylus* group.

My study of New Zealand's skinks suggests that the only endemic oviparous species, *Oligosoma suteri*, is not the sister species all of the viviparous species. This means that the evolutionary history of reproductive mode in New Zealand must have involved several evolutionary transitions. A more robust phylogeny of *Cyclodina* and *Oligosoma* is required to fully understand the evolutionary history of reproductive mode in New Zealand's skinks.

Before the evolution of reproductive mode in the *Eugongylus* group can be fully understood, the morphology and physiology of placentae (if any) in New Zealand and New Caledonia members of the group must be documented.

Future *Eugongylus* group phylogenetic research

There are many issues unresolved by this study. Further studies incorporating more nuclear gene sequences, and more thorough taxon sampling should address the following issues: 1) What are the sister lineages of the *Eugongylus* group, and what is the likely geographic origin of the group?; 2) Is the polytomy recovered by this study in fact a hard polytomy reflecting rapid diversification of the *Eugongylus* group? More focused studies are also need to determine exactly where *Oligosoma suteri* fits in the

phylogeny of New Zealand's skinks to identify origins of live-bearing in New Zealand's skinks.

Appendix 1 – Genes unsuccessfully trialed for phylogenetic analysis of *Eugongylus* group skinks

Nuclear coding DNA

Brain-4

Brain-4 has been included in studies of the evolution of single amino acid repeats in POU transcription factors in mammals (Nakachi *et al.*, 1997; Sumiyama *et al.*, 1996). The 2260 bp intronless gene has not been used in any phylogenetic studies. I designed two pairs of primers (G362/G373, G363/G374, Figure A1.1) to amplify overlapping fragments using sequence from *Anolis* and *Mus*. Primers were located in regions with high similarity between *Anolis* and *Mus* but designed to match the *Anolis* sequence exactly. Primers G362/G363 amplified multiple products of different lengths for some taxa at high stringency. A product ~1 kb long was purified from *Lampropholis guichenoti*, but failed to sequence in either direction in two attempts. A strong 500 bp product from *Niveoscincus greeni* from these primers was also sequenced, and failed to show similarity to any known sequence in a BLAST search (Altschul *et al.*, 1997) of sequences on GenBank. Primers G362/G373 failed to amplify under any conditions. Primers G374/G363 amplified a 1 kb product in a number of species, but when sequenced from *L. guichenoti*, this product also failed to show similarity to any GenBank sequences.

Retinitis pigmentosa (RP1)

The autosomal dominant allele of retinitis pigmentosa (*RP1*) is responsible for the blindness causing disease retinitis pigmentosa. In *Mus* and *Homo* the gene includes four exons of 136, 172, 627, and 6,165 bp. I designed primers from *Mus* and *Homo* sequences from GenBank to amplify a 900 bp region at the 3' end of exon 4 (G364/G365). These primers amplified a faint 650bp product in *Mus* but failed to amplify in skinks under any conditions.

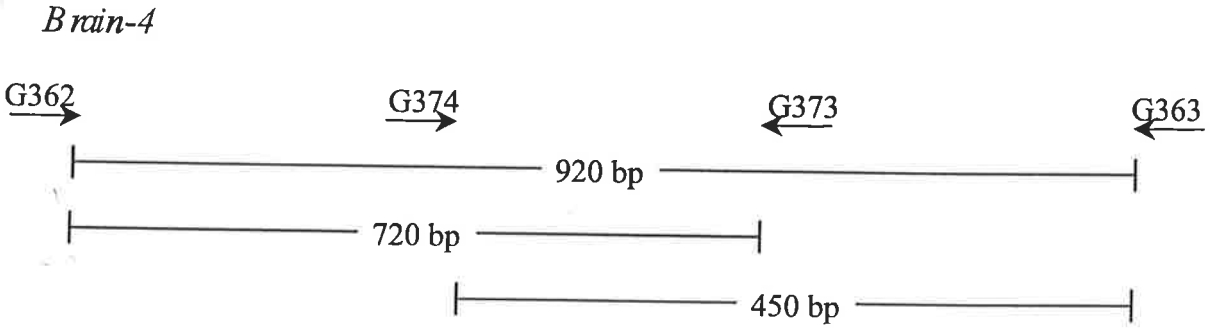


Figure A1.1: Primers used to amplify coding nuclear gene *brain-4*.

Nuclear non-coding DNA

CATS primer set

The comparative anchor tagged sequence (CATS) primer kit (Lyons *et al.*, 1997) was developed based on the description of reference loci for mammalian genome mapping (O'Brein *et al.*, 1993). The kit contains 318 primer pairs designed to amplify single introns in mammals. 66 pairs of primers from the kit were the focus of a broad scale study carried out by the South Australian Museum, Southern Cross University and the University of Queensland, assessing their applicability to other taxa. Four *Eugongylus* group skink species were included in this study. *MYH2* was the only locus from this kit subject to further investigation (see Chapter 2).

SFU-UBC nuclear primer set

The Simon Fraser University and University of British Colombia produce the SFU-UBC invertebrate nuclear primer kit. The kit includes 50 primer pairs for eight intron and seven coding nuclear loci. Although the transferability of these primers to vertebrates was expected to be low in many cases, I trialed 10 pairs of primers for six intron loci on three *Eugongylus* group species. *Cytochrome c* and *calmodulin* failed to amplify in all cases, *creatine kinase*, *actin*, *elongation factor 1α* and *tubulin* primers amplified products <500 bp which were not expected in include informative introns.

Aldolase (ALD-1, ALD-2)

The glycolytic aldolase enzymes are encoded by a small gene family. Lessa and Applebaum (1993), designed primers to amplify introns from the three mammalian aldolases A, B and C. In the skink *Gnypetoscincus queenslandiae* these primers amplify two aldolase products, 900 bp and 410 bp in length, although it could not be determined whether they were *ALD-A* or *ALD-C*, hence they are referred to as *ALD-1* and *ALD-2* respectively (Slade *et al.*, 1993). In *Eugongylus* group skinks, a 300 bp *ALD-2* product amplified consistently, and a 900 bp *ALD-1* was amplified in a few taxa. *Aldolase-2* was considered likely to have too few informative sites to justify the sequencing effort

involved. *Aldolase-1* amplified consistently within the genus *Pseudemoia* and was sequenced for five species. Among five species of *Pseudemoia* the 90 bp of intron and 810 bp of exon sequence include nine parsimony informative characters, and show 1 - 2% uncorrected sequence divergence. This level of variation was too low for use within the genus *Pseudemoia*.

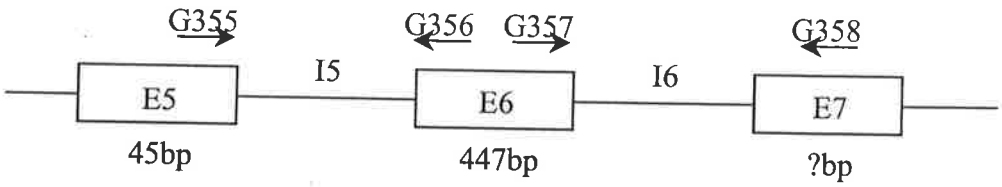
Amelogenin (Amel)

Amelogenin is a matrix protein which is a precursor to enamel in the early stages of tooth formation in jawed vertebrates. Sequences of reptile *amelogenin* are available from a study of the evolution of amelogenin genes (Toyosawa *et al.*, 1998) and a description of tooth formation in the snake (Ishiyama *et al.*, 1998). These studies suggest that there is a single copy of the gene in reptiles and that the structure is roughly analogous to that in mammals (although there is no exon 4 in reptiles). Published cDNA sequences of the Caiman *Paleosuchus palpebrosus* and the colubrid snake *Elaphe quadrivirgata* include the region from mammalian exon 5 to exon 7. I designed primers from these sequences to amplify the introns between exons 5 and 6 (G355/G356) and exons 6 and 7 (G257/G358 Figure A1.2). Both primer pairs failed to amplify under all conditions.

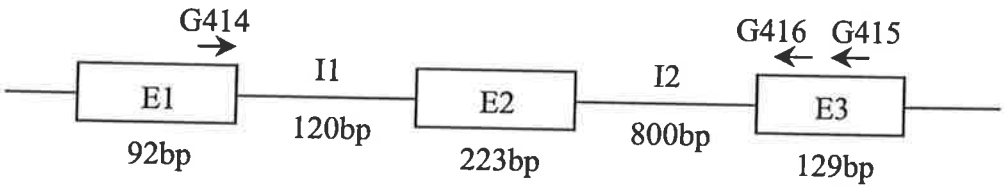
β-haemoglobin (Hbb)

β-haemoglobins belong to a mulitgene family with at least two members in reptiles (Gorr *et al.*, 1998). Haemoglobin gene coding sequences have been used for molecular systematics in reptiles (Gorr *et al.*, 1998) and mammals (Porter *et al.*, 1997). I obtained cDNA sequences of the *Egernia* group skink *Tiliqua rugosa* from Scott Spargo of the Molecular Biosciences Department of the University of Adelaide. Primers (G414/G415, G414/G416) were designed to amplify across introns one and two (~1200 bp product expected, figure A1.2). These primers either failed to amplify, or resulted in a smear of multiple products under all conditions.

Amelogenin



β-*haemoglobin*



Rhodopsin

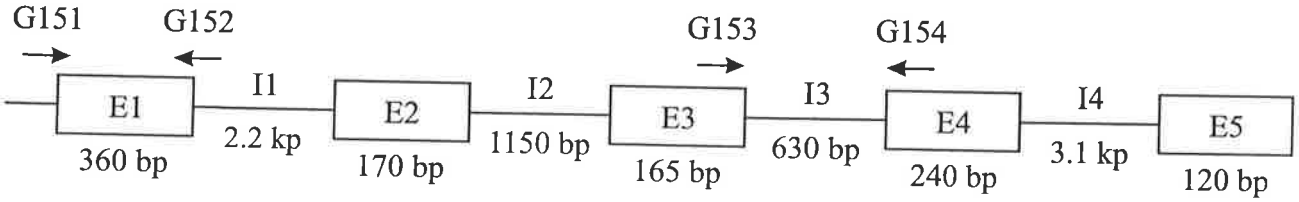


Figure A1.2: Primers used to amplify introns in nuclear genes *amelogenin*, β-*haemoglobin* and *rhodopsin*.

Lamin-A (LAM-A)

Lamins are a cytoskeletal element comprising the nuclear lamina - a meshwork of filaments lining the inner surface of the nuclear membrane in eukaryotic cells. Birds are known to have one A-type and two B-type lamins (Peter *et al.*, 1989; Vorburger *et al.*, 1989), while mammals have two B-type (B_1 and B_2) and two A-type lamins (A, and C_1) expressed in all cells, in addition to germline specific B_3 and C_2 (Höger *et al.*, 1990; Nakajima and Abe, 1995). In human and in mouse all three A-type lamins (A, C_1 and C_2) are coded in a single locus and transcribed by specific splicing (Lin and Worman, 1993; Nakajima and Abe, 1995). Lamin gene structure has not been described for reptiles. *Lamin-A* intron three was included in the single strand conformation polymorphism (SSCP) study of intron variation in coastal seabirds of Freisen *et al.* (1997). Freisen's primers (L724/H792) amplified a ~450 bp product in some *Eugongylus* group skinks, however this product failed to sequence in most cases. Reptile specific primers designed from these sequences, but failed to amplify a single band in most cases.

Rhodopsin (Rh 1)

Rhodopsin is a light absorbing transmembrane protein, which in non-mammalian vertebrates occurs in the retina, pineal complex and in the deep brain (Foster *et al.*, 1993). *Rhodopsin* in *Anolis carolinensis* is a single copy 8.1 kb gene which shares gene structure with *Rh* genes from other vertebrates (figure 1, Kawamura and Yokoyama, 1994). *Rhodopsin* is similar to but easily distinguished from the 18.6 kb *Rhodopsinlike opsin* (Rh2) in *Anolis* (Kawamura and Yokoyama, 1995). *Anolis* and *Alligator mississippiensis* (Smith *et al.*, 1995) sequences were used to design primers for intron three (G153/G154, K. Saint unpublished, figure A1.2). These primers consistently amplify a 900bp product, primer G154 sequences efficiently for the fragment, but G153 failed to sequence in all cases. Sequences from G154 were used to design a *Eugongylus* group specific primer pair G456/G457, these primers did not amplify across all taxa.

Transthyretin (TTR)

Transthyretin is a thyroid hormone binding protein synthesized and secreted by the liver and brain in mammals and birds, and by the brain only in reptiles (Achen *et al.*, 1993; Schreiber *et al.*, 1993). I downloaded published cDNA sequences from *Tiliqua rugosa*, *Crocodylus porosus*, and *Gallus gallus* from GenBank. The position of three introns was inferred from the *Gallus* sequence. Since short exons limited the range of available primer sites, initially I designed primers to amplify intron one (G340/G354), intron two (G341/G342) and introns two and three (G341/G345, Figure A1.3). Several of these primer pairs were too close to intron/exon boundaries to allow identification of the products. Amplification of intron one resulted in a product of ~700 bp in *Lampropholis guichenoti* but failed to amplify in all other species. Amplification of intron two (G341/G342) resulted in a product of approximately 2000 bp, in *Lampropholis guichenoti* and *Tiliqua rugosa* this product was not further investigated, as it is considered too long to be sequenced in a single reaction. Amplification of introns two and three resulted in a product of the size expected with at most a very short (<100 bp) intron three.

Transthyretin

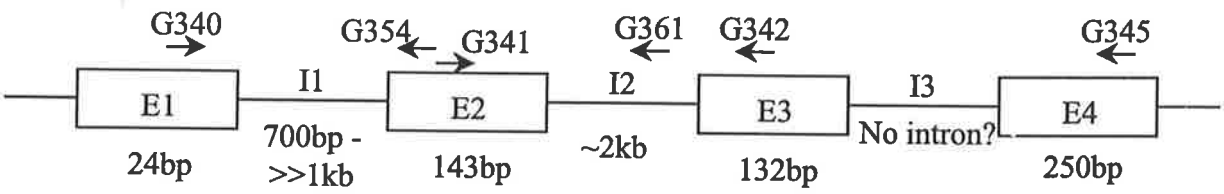


Figure A1.3: Primers used to amplify introns one, two and three in nuclear gene *transthyretin.*

Appendix 2: Primer Sequences

Gene	Primer Name	Author	Direction	Sequence 5' to 3'
Mitochondrial Genes:				
12S	H1478	Kocher *et. al* 1989	F	TGA CTG CAG AGG GTG ACG GGC GGT GTG T
12S	L1091	Kocher *et. al* 1989	R	AAA AAG CTT CAA ACT GGG ATT AGA TAG CCC ACT AT
ND2	M136	Saint, unpublished	F	CAC TGA CTT CTT GCC TGA WTY GG
ND2	M167	Saint, unpublished	R	GCT GTY TGT GTY TGG TTT ADK CC
ND2	M177	Saint, unpublished	R	GTK CGT AGG TAG AAD AWT AGG CT
ND2	M178	Saint, unpublished	F	CAK ARK CCG CRA CAA AAT ACT TC
ND4	ND4	Arevalo *et. al* 1994	F	TGA CTA CCA AAA GCT CAT GTA GAA GC
ND4	LEU	Arevalo *et. al* 1994	R	TTT TAC TTG GAT TTG CAC CA
ND4	M256	presented here	F	CCT TTT ATR TTR ATT GTK GGK GG
ND4	M257	presented here	R	CGT ACM CAY AGC CGA AC
ND5	M307	presented here	F	CAA AGG ATM AAA GTT AAT C
ND5	M308	presented here	R	AAR GTC TGC TGT GTA GTG
ND5	M309	presented here	F	GCA AYT CCA AGT AAA AG
ND5	M310	presented here	F	CAA ACT CAT CCG CCT ACA RG
ND5	M311	presented here	R	CAT GTC TCA GGA TAR GG

Gene	Primer Name	Author	Direction	Sequence 5' to 3'
ND5	M317	presented here	F	CTG AGC CCT AAC ACT MAC
ND5	M355	presented here	F	ATC TGA TGC AAA CWC CTC
ND5	M353	presented here	R	CGY ATR ATT GGG YGT GTT A
ND5	M348	presented here	R	TYA GGG CAA GGC TKC
ND5	M322	presented here	F	GAC CAA CTA TGR TAY GAA A
ND5	M323	presented here	R	GTT TCT AGT AGG GGG TG
ND5	M324	presented here	R	AGG TAT GAG GTG TTT ATT GTT TC
ND5	M325	presented here	F	GAA AAA TAG GSG GMA TYC
ND5	M326	presented here	R	GGT AGG YTT TAA TKA GRC
Sucessful Exons:				
C-mos	G73	Saint *et. al* 1998	F	GCG GTA AAG CAG GTG AAG AAA
C-mos	G74	Saint *et. al* 1998	R	GAT TGG AGA CTT TGG ATG CTC A
C-mos	G294	Saint unpublished	R	CTG GGC AGA ACT AAA TGT GG
C-mos	G303	Saint unpublished	F	ATT ATG CCA TCM CCT MTT CC
C-mos	G336	presented here	F	CAC GAC CAT GTA GCA GTC CC
C-mos	G337	presented here	R	GCA GGT TTT ATA TCA AGA TGC AC
Rag-1	G292	Saint unpublished	F	TGG GAG ATG TSA GTG AGA A
Rag-1	G293	Saint unpublished	R	TCM CCW ATC TCT AKC TGG AA
Rag-1	G325	Saint unpublished	F	TCT TGT CTT CCA CTC CAT MAC

Gene	Primer Name	Author	Direction	Sequence 5' to 3'
Rag-1	G326	Saint unpublished	R	TCA TTT CCY TCA CTT GCC CA
Rag-1	G396 (R13)	Groth *et. al* 1999	F	TCT GAA TGG AAA TTC AAG CTG TT
Rag-1	G397 (R18)	Groth *et. al* 1999	R	AAA GGT GGC CGA CCG AGG CAG CAT C
Rag-1	G398 (R27)	Townsend unpublished	F	
Rag-1	G399 (R29)	Townsend unpublished	R	
Rag-1	G423	Presented here	F	CAT GCT CTC CGA ACT GCT GAA
Rag-1	G425	Presented here	R	AAA GCA AGG ATA GCG ACA AGA G
Rag-1	G440	Presented here	F	CAT GAT AAA GGG AGA GTR GAA GA
Sucessful Introns:				
β-fib	G359 (BI7U)	Prychitko *et. al* 1997	F	GGA GAA AAC AGG ACA ATG ACA ATT CAC
β-fib	G360 (BI7L)	Prychitko *et. al* 1997	R	TCC CCA GTA GTA TCT GCC ATT AGG GTT
β-fib	G375	Presented here	F	GAC AGA GAC AAT GAT GGA
β-fib	G376	Presented here	R	GTG AGG AAT AAT RCA CAA AG
β-fib	G439	Presented here	F	GAA GTT ACA TGG AAT GGA G
MYH2	G240 (MYH2F)	Lyons *et. al* 1997	F	TCA GAG TCA CAA TAA GGA CA
MYH2	G241 (MYH2R)	Lyons *et. al* 1997	R	TGG TGT CCT GCT CCT TCT TC
MYH2	G315	Presented here	R	TCA GAG TCA CAA TAA GGA CAC
MYH2	G378	Presented here	R	CAT CAG ART ACA AGC AAG G

Gene	Primer Name	Author	Direction	Sequence 5' to 3'
Unsuccessful Exons:				
Brain-4	G362	Presented here	F	GCG TCC AAC CCG TAC AGC
Brain-4	G363	Presented here	R	GGC TTG GGG CAC TTG AGG
Brain-4	G373	Presented here	R	CTT GCA CAT GTT CTT GAA GC
Brain-4	G374	Presented here	F	ATG CTG GAG CAC GGS GGA
Rp-1	G364	Presented here	F	AGA ACT TCA AAA CTT ATG G
Rp-1	G365	Presented here	R	TAG CCT CAG GAA AAT CAG
Unsuccessful Introns:				
Ald	G25 (L724)	Lessa *et. al* 1993	F	GAA CGC TGT GCC CAG TAT AAG AAG GAT GG
Ald	G26 (H892)	Lessa *et. al* 1993	R	CCC ATC AGG GAG AAT TTC AGG CTC CAC AA
Amel	G355	Presented here	F	GTT CCA GAG CMT AAT KGG AC
Amel	G356	Presented here	R	GTG TAK CCA TCC TCC CAT KG
Amel	G357	Presented here	F	CCT CAG CTA CCT GGG ART GC
Amel	G358	Presented here	R	TGG AYT YTT GAA AGC
HBB	G414	Presented here	F	GAA ATT GGT GGC GAG TGC CTG G
HBB	G415	Presented here	R	GAC CTC CGG GGT GAA CTC CTT G
HBB	G416	Presented here	R	GTG GGC TGC CTA GCC GAT GAC G
Lam-A	G144	Friesen *et. al 1997*	F	CCA AGA AGC AGC TGC AGG ATG AGA TGC
Lam-A	G145	Friesen *et. al 1997*	R	CTG CCG CCC GTT GTC GAT CTC CAC CAG

Gene	Primer Name	Author	Direction	Sequence 5' to 3'
Lam-A	G331	Presented here	F	AGA CCC TGC GTG AAG AGC TG
Lam-A	G332	Presented here	R	GCC CGT TGT CGA TCT CCA CC
Rh-1	G153	Saint unpublished	F	GCT CAG CCA TCT ACA ATC C
Rh-1	G154	Saint unpublished	R	CAT GAT CAT ACA GTT ACG G
Rh-1	G456	Presented here	F	TAA CTA CAT AAC ACT TCT GAG
Rh-1	G457	Presented here	R	ATT GTC ATA GGT TCA ACA G
TTR	G340	Presented here	F	TGG TGT ACC TCA CTG AAG CTG C
TTR	G354	Presented here	R	CAT AAG AGG ACA CTT GGA ATC A
TTR	G341	Presented here	F	TAT GGT GAA AGT CCT GGA TGC
TTR	G342	Presented here	R	TTG TTC ATC TGT TGT AAG CTC
TTR	G345	Presented here	R	GAC GTT GAC CAG AAT CAT TAG C

Appendix 3 – *Eugongylus* group alignments

3.1 - *β-fibrinogen* alignment 137

3.2 - Step matrix for gap character 2 166

3.3 - *β-fibrinogen* gap characters 168

3.4 - *C-mos* alignment 170

3.5 - *Myh-2* alignment 179

3.6 - *Rag-1* alignment 185

Appendix 3.1 - *β-fibrinogen* alignment of ***Eugongylus*** **group taxa** (see table 3.1)

```
B. trilineata          TGCATTTTAT GCATTTGAAG ---------- -TTAYGTGGA A-TGGAG-AT AAATTACAGC TTTATAACTC TTAATATTTG
C. austrocaledonicus   .......... .......... ---------- -...CA.... .-.....-.. ......A... ..A....... ........GC
C. longipes            G......-.. .....-..CC ---------- -..-CA.... .-.....-.. .......... ........C. ....C...GC
C. plagiocephalus      .......... .......... ---------- -...CA.... .-.....-.. ...G...... .......... ...G....GC
C. aenea               .......... .......... ---------- -...CA.... .-.....-.. .......... .......... ........GC
E. cyanura             ????...... .......... ---------- -A..CA.... .-.....-.. ....C..... .......... .G......GC
E. nigra               ?????????? ?????..... ---------- -...CA.... .-.....-.. .....-G.... ....G..... ...C....GA
E. rufescens           .......... ..T....... ---------- -G..CA.... .-.....-.. .......... .......... ........GC
L. guichenoti          G.......?? ???????... ---------- -...CA.... .-.....-.. .......... .....G.... ........GC
L. telfairii           .......... ........G. ---------- -...CA.... .-.....-.. .......... .....C.... ........GC
L. nigrofascolatum     .......... .......... ---------- -...CA.... .-.....-.. ......A... ..A....... ........GC
M. greyii              ?????..... .........A ---------- -...TA.... .-.....-.. .......... .......... .....G..GC
M. ruficauda           .......... .......... ---------- -...CA.... .-.....T.. .......... .......... ........GC
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              .......... .......... ---------- -...CA.... .-.....-.. .......... .......... ....C...GC
N. metallicus          .......... .......... ---------- -...CA.... .-.....-.. .......... .......... ....C...GC
O. lichenigera         .......... .......... ---------- -...CA.... .-.....-.. .......... .......... ........GT
O. smithi              .......... .......... ---------- -...CA.... .-.....-.. .......... .........G ........GC
P. entrecasteauxii     ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. spenceri            .......... .........C ---------- -...CA.... .-.....-.. .......... .......... ........GC
E. skiltonianus        .....C..G. .......... CATATGATTC T...C...T. .C.....-.C .....G.T.. ....C.G... ........GC
M. logicaudata         ....CC..G. .......... CATATGATTT G...CA.... .A.....-.G .....G.C.. ....A..... ......----
P. wahlbergi           ?????????? ?????????? ?????????? ????????.. --.....-.. ...CC..... .......... ....C..CGC
T. scincoides          .A..CC.... C....A.... CATATGATTG T...CA.... .A.....-.C .....G.T.. ....C..... ........GC
```

Appendix 3.1

```
B. trilineata          CATACTGCAT --CTAGTTAA G-CATGCATA ATGAAAAAAA ---------- ---------- ---------- ----------
C. austrocaledonicus   ATG...A... --..G..... .-......... .......... ---------- ---------- ---------- ----------
C. longipes            ATG....... T-......T. .-......... ...G...... ---------- ---------- ---------- ----------
C. plagiocephalus      ATG.T..... --...T.... .-......... ...G...... A?--??---- ---------- ---------- ----------
C. aenea               ATG....... --...C.... .-......... .......... A--------- ---------- ---------- ----------
E. cyanura             ATG....... --......... .-.......C ...GGG.... AAAA------ ---------- ---------- ----------
E. nigra               ATA....... --......... .-...T.... .........- ---------- ---------- ---------- ----------
E. rufescens           ATA.T..... --......... .-.T...... .......... A--------- ---------- ---------- ----------
L. guichenoti          A.G...A... --......... C-......... ...G...... A--------- ---------- ---------- ----------
L. telfairii           ATA....... --......... .-......... ...G...... ---------- ---------- ---------- ----------
L. nigrofascolatum     ATG....... --..G..... .-......... .......... ---------- ---------- ---------- ----------
M. greyii              ATG....... --..------ ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ATA....... --..G..... .-...TA... .........G A--------- ---------- ---------- ----------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ATG....... --......... .-......... .........- ---------- ---------- ---------- ----------
N. metallicus          ATG....... --......... .-......... .......... ---------- ---------- ---------- ----------
O. lichenigera         ATG....... --......... .-......... .......... ---------- ---------- ---------- ----------
O. smithi              ATG....... --...C.... .-......... .......... A--------- ---------- ---------- ----------
P. entrecasteauxii     ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. spenceri            ACG....... --.C...... .-......... .......... ---------- ---------- ---------- ----------
E. skiltonianus        ATG.T....C --.C....T. .G........ ...G....C. ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ----CTTAGT TAAACATGCT AATGGAAAAA TTGAGATCCA
P. wahlbergi           ATG.A..... CC.C.....C A-.T...... .......G.. AA--------- ---------- ---------- ----------
T. scincoides          ATG...A..C --......... T--------- ---------- ---------- ---------- ---------- ----------
```

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B. trilineata       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. greeni           ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. metallicus       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri         ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
E. skiltonianus     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi        ATTAGGATT- AGGTAAGATC CTAA------ ---------- ---------- ---------- ---------- ----------
T. scincoides       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
                    ---------- ---------- -----CCATT TTTTGCCCAG CCAACAGGTG TACACATTTG GTCCCTGTTG CATATATGCA
```

Appendix 3.1

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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ------TT-A GATCTGATTG
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
C. longipes            ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ....CA....
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ------???? ??????????
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ------.-. ..........
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..C.......
L. guichenoti          ---------- ---------- ---------- ---------- ---------- ---------- ------..-. .......C..
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ------.C-T ...TG.C...
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
P. entrecasteauxii     ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????..-. ..........
P. spenceri            ---------- ---------- ---------- ---------- ---------- ---------- ------..-. ..........
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ------..G. ..........
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- TAGACCTGAT TACCTGATTA TCTACA---- ----------
T. scincoides          ACATTGGTCA GAAATGGCTT AAGCATGCAT AATAGAATGA ---------- ---------- ------..G. ....CA....
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```
B. trilineata          GCTTGGGAGC -TCAGGATTT TTGCCTGTAG AGATTTTTT- ---------- ----AAAGG- ---------- ----CCACAG
C. austrocaledonicus   .......... -......... .-........ ..-...C..T ---------- ----.....- ---------- ----......
C. longipes            .......... -......... .-........ ..-......T ---------- ----....A- ---------- ----.T....
C. plagiocephalus      ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. aenea               .......... -......... .-........ ..-......T ---------- ----.....- ---------- ----......
E. cyanura             .......... C......... .-........ ---......T TTT------- ----.....- ---------- ----......
E. nigra               .....A.... -......... .-........ .A-......T T--------- ----.....- ---------- ----.....T
E. rufescens           .....A.... -.G.A..... .-........ ..-......T ---------- ----.....- ---------- ----......
L. guichenoti          ..A....... -......... .-..T..... ........-- ---------- ---A....A- TCTTGACCAA TGGA......
L. telfairii           .....A.... -......... .-........ ..-.....-- ---------- ----.....T T--------- ------...-
L. nigrofascolatum     .......... -......... .-....A... ..-......T ---------- ----.....- ---------- ----......
M. greyii              ---------- -.G-AA.... .-........ ..-......T T--------- -----....- ---------- ----......
M. ruficauda           .-----.... -......... .......... ..-......- ---------- ----...T.- ---------- ----....C.
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              .......... -.T....... .-........ ..-......T ---------- ----.....- ---------- ----...G..
N. metallicus          .......... -.T....... .-........ ..-......T T--------- ----.....- ---------- ----......
O. lichenigera         .......... -...A..... .-........ ..-......T ---------- ----.....- ---------- ----......
O. smithi              .......... -......... .-........ ..-.A....T ---------- ----.....- ---------- ----......
P. entrecasteauxii     .......... -......... .-........ ..G......T ---------- -----....- ---------- ----......
P. spenceri            .......... -......... .-..T..... ..-......T ---------- ----.....- ---------- ----......
E. skiltonianus        ...C------ ---.A.G... .-.A...... .--......-- ---------- ----.....- ---------- ----...T..
M. logicaudata         -----T.... -......... .-AT...... ..-......T TTAGTTTCTT TTT--..T.- ---------- ----..----
P. wahlbergi           ------.... -AT....... .-A...A... .........T AA-------- -----TG..- ---------- -----..T.A
T. scincoides          ...AAT.... -.T....... .-AA...... ..-......T GTTTTTTTT- ----....-T T--------- -----...T..
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Appendix 3.1

B. trilineata	TATGG-ACCA	TTTTATAAAA	A-CTATCCCA	TAAGTTAGTT	TAGTTATATT	ACCCTATTT-	----------	----------
C. austrocaledonicus	..C..-....	......C...	.-....T...	..G.......	....	...A.....-	----------	----------
C. longipes	.....-....	...C..C...	.-........	..GA......	....	...T.....-	----------	----------
C. plagiocephalus	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????
C. aenea	.....-....	......C...	.-........	C.G.......	....	...G.....-	----------	----------
E. cyanura	.....-....	......C...	.-........	..-----...	...C.G....	..AG.....-	----------	----------
E. nigra	.....-....	......C...	.-........	..-----.A.	....	.ATG.....-	----------	----------
E. rufescens	.....-....	......C...	.-........	..GA......	....	...AAT...-	----------	----------
L. guichenoti	.....-....	......C...	.-........	....	...C..CG..	...G.....-	----------	----------
L. telfairii	....AA....	....G.C...	.-........	..G.......	.---..--..	...A.....-	----------	----------
L. nigrofascolatum	.---------	----------	----------	ATG.......	....	...G.....-	----------	----------
M. greyii	.....-...G	..A.T.CC..	.-.....T..	..G.......	.G.....T..	...A.....-	----------	----------
M. ruficauda	...T.-....	....	.AT.......	....	..C.......	.........-	----------	----------
N. maccoyi	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????
N. greeni	.....-....	......C...	M-......A.	..G.......	....	...G.....-	----------	----------
N. metallicus	.....-....	......C...	.-......A.	..G.......	....	...G.....-	----------	----------
O. lichenigera	.....-....	......C...	.-........	..G.......	....	...A.....-	----------	----------
O. smithi	.....-G...	......C...	.-........	C.G.......	....	...G.....-	----------	----------
P. entrecasteauxii	.....-....	...A..C...	.-........	..G.......	........C.	..T.....-	----------	----------
P. spenceri	.....-M...	...A..C...	M-........	..G.......	....	..T.....-	----------	----------
E. skiltonianus	.C...-.A..	......C...	.-........	----------	----------	----------	----------	----------
M. logicaudata	--...-.A..	...G..C...	T-........	G.G.------	----------	----------	----------	----------
P. wahlbergi	.....-..T.	....	.AA-..T...	..G.----..	...C......	..T.A..G.-	----------	----------
T. scincoides	.C...-.A..	......C...	.-........	G.G.------	----------	----------	---TTTCTTT	AAAAGCAATA

Appendix 3.1

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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. greeni              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          TTAATTGTAT GGAAAGCTAT GGAGCAATCT CATGGGATAG AAAGATTGGT TAATAGGGCT GGTCTTCTCC AAGCAAGGCA
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```
B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus      ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. longipes            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          TCAGTGTTTC TGGATTCACA GTTACATGTA TACTCCAGGT AAAAAGGCCC TGTTCTCCCT TTGCATCTCC AGGTCAAACT
```

B. trilineata	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus	?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. longipes	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum	---------- ---------- ---------- -CCCATAGGT TATTTCCCAT AACCTATATC CCATAGGTTA GTTTAGTTAT
M. greyii	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi	?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi	---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides	GGAAAAGACC CCTGTGTGAA ACCCTTGAAA G--------- ---------- ---------- ---------- ----------

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B. trilineata          ---------- --ACACAAAT ACAATG-CAC CATCTACTCT AATGTGCACC TTGA-TTTTG GAGA------ ----------
C. austrocaledonicus   ---------- --.TT..... ......-... ....G.A... ........YG C...-..... ....CT---- ----------
C. longipes            ---------- --..T..... ...T..-... ....G.A... .......... C...-..... ....CT---- ----------
C. plagiocephalus      ?????????? ?????????? ?????????? ????ATA... .......... C...-..A.. .G..CT---- ----------
C. aenea               ---------- --..T..... ....-CA... ......A... .......... C...-..... ....CT---- ----------
E. cyanura             ---------- --..T..... .T....-... ...TG.A... .......... C...-..... ....CT---- ----------
E. nigra               ---------- --..T..... ....A.-... ....G.A... .......... C...-..... ....CT---- ----------
E. rufescens           ---------- --..T..... ......-... ....G.A... .......... C...-..... ....YT---- ----------
L. guichenoti          ---------- --.TT..... ......-..A ....A.A... ...A...... C...-..... ....CT---- ----------
L. telfairii           ---------- --..T..... ......-... ....A.A... .......... C...-..... ....CT---- ----------
L. nigrofascolatum     ATTACCGTAT TT..T..... ......-... ....G.A... .......... C...-..... ....CT---- ----------
M. greyii              ---------- --G.T..... .A....-... ....A.A... .......... -...-..... ....CTTACC CTCCAAGCCA
M. ruficauda           ---------- --........ .AG...-G.. ...AG.A.TG ......G... CA..-..... ....CT---- ----------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ---------- --........ ......-... ....A.A... .......... C...-..... ....CT---- ----------
N. metallicus          ---------- --........ .A.G..-... ....A.A... .......... C...-..... ....CT---- ----------
O. lichenigera         ---------- --..T..... ....CA-... ......A... .......... C...-..... ....CT---- ----------
O. smithi              ---------- --..T..... ....CA-... ......A... .......... C...-..... ....CT---- ----------
P. entrecasteauxii     ---------- --..G..... ......-... ....G.A... ...C...... C.A.-..... ....TT---- ----------
P. spenceri            ---------- --..G..... .A....-... ....A.A... ...C...... C.AW-..... R...TT---- ----------
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- --------.. T...G.A.T. .....T..T. CA.TC..... ....CT---- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
```

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B. trilineata	----------	----------	----------	----------	----------	----------	----------	----------
C. austrocaledonicus	----------	----------	----------	----------	----------	----------	----------	----------
C. longipes	----------	----------	----------	----------	----------	----------	----------	----------
C. plagiocephalus	----------	----------	----------	----------	----------	----------	----------	----------
C. aenea	----------	----------	----------	----------	----------	----------	----------	----------
E. cyanura	----------	----------	----------	----------	----------	----------	----------	----------
E. nigra	----------	----------	----------	----------	----------	----------	----------	----------
E. rufescens	----------	----------	----------	----------	----------	----------	----------	----------
L. guichenoti	----------	----------	----------	----------	----------	----------	----------	----------
L. telfairii	----------	----------	----------	----------	----------	----------	----------	----------
L. nigrofascolatum	----------	----------	----------	----------	----------	----------	----------	----------
M. greyii	GAGTAACTAC	GGGAAGACAT	AATAGGCACT	TACTCTGGAT	TGCTCTATGC	ATTTCCTATA	GTGGCACTGT	AGCTATGTCA
M. ruficauda	----------	----------	----------	----------	----------	----------	----------	----------
N. maccoyi	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????
N. greeni	----------	----------	----------	----------	----------	----------	----------	----------
N. metallicus	----------	----------	----------	----------	----------	----------	----------	----------
O. lichenigera	----------	----------	----------	----------	----------	----------	----------	----------
O. smithi	----------	----------	----------	----------	----------	----------	----------	----------
P. entrecasteauxii	----------	----------	----------	----------	----------	----------	----------	----------
P. spenceri	----------	----------	----------	----------	----------	----------	----------	----------
E. skiltonianus	----------	----------	----------	----------	----------	----------	----------	----------
M. logicaudata	----------	----------	----------	----------	----------	----------	----------	----------
P. wahlbergi	----------	----------	----------	----------	----------	----------	----------	----------
T. scincoides	----------	----------	----------	----------	----------	----------	----------	----------

B. trilineata	----------	----------	----------	----------	----------	----------	----------	----------	----------
C. austrocaledonicus	----------	----------	----------	----------	----------	----------	----------	----------	----------
C. longipes	----------	----------	----------	----------	----------	----------	----------	----------	----------
C. plagiocephalus	----------	----------	----------	----------	----------	----------	----------	----------	----------
C. aenea	----------	----------	----------	----------	----------	----------	----------	----------	----------
E. cyanura	----------	----------	----------	----------	----------	----------	----------	----------	----------
E. nigra	----------	----------	----------	----------	----------	----------	----------	----------	----------
E. rufescens	----------	----------	----------	----------	----------	----------	----------	----------	----------
L. guichenoti	----------	----------	----------	----------	----------	----------	----------	----------	----------
L. telfairii	----------	----------	----------	----------	----------	----------	----------	----------	----------
L. nigrofascolatum	----------	----------	----------	----------	----------	----------	----------	----------	----------
M. greyii	GTGTAAAAGG	TCCCATAGTT	ATTCTGGATT	GTAGCCTAAG	GCTGCAATCC	TAAAGACACT	TTCCTGGGAA	TAAGACCCAT	
M. ruficauda	----------	----------	----------	----------	----------	----------	----------	----------	----------
N. maccoyi	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????	??????????
N. greeni	----------	----------	----------	----------	----------	----------	----------	----------	----------
N. metallicus	----------	----------	----------	----------	----------	----------	----------	----------	----------
O. lichenigera	----------	----------	----------	----------	----------	----------	----------	----------	----------
O. smithi	----------	----------	----------	----------	----------	----------	----------	----------	----------
P. entrecasteauxii	----------	----------	----------	----------	----------	----------	----------	----------	----------
P. spenceri	----------	----------	----------	----------	----------	----------	----------	----------	----------
E. skiltonianus	----------	----------	----------	----------	----------	----------	----------	----------	----------
M. logicaudata	----------	----------	----------	----------	----------	----------	----------	----------	----------
P. wahlbergi	----------	----------	----------	----------	----------	----------	----------	----------	----------
T. scincoides	----------	----------	----------	----------	----------	----------	----------	----------	----------

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B. trilineata           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea                ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra                ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii            ---------- ---------- ---------- ---------- ---------- --AAGAGCAA AATCCAGAGC AGATTCGAGT
L. nigrofascolatum      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii               TAAAAACAAT GGGGTTTACT TTGGAGGAGA CATGGTTAGG AAAAGCTCTA AA-------- ---------- ----------
M. ruficauda            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi              ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Appendix 3.1

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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          CCATACAATT ACAGGACCCT CTAGTGTTAT CTTATGGAAA GTCACCTTAA CTGCTCCAAC GCAAAGGGCT TAATTTTATT
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          TTAGGATATG GGCCCAAATT TTACAGGAAA ---------- ---------- ---------- ---------- ----------
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- --------TT GTAGACGATG GGCAAAATCC AGAGTAACTA
P. spenceri            ---------- ---------- ---------- ---------- --------TT GTAGACGATG GGCAAAATCC AGAGTAACTA
E. skiltonianus        ---------- ---------- ---------- GCGGTTTCTC TATAAGCA-- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. greeni              ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri            CGGGGAGTCC TTGCTCCGGT GTAAGAACAA CTTACTCTGG ATTTG-ATAT GAATTTCCCA TAGGTTTTCA CTAGGTGGTG
E. skiltonianus        CAGGGAGTCC TTGCTCCGGT GTAAGAACAA CTCACTCTGG ATTGCAATAT GAATTTCCCA TAGGTTTTCA CTAGGTGGTG
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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B. trilineata           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes             ---------- ---------- ---------- --AAGGGCGC AATCCAGAGT AACTACGGGG AGTCCTTGCT CCGGTGTAAG
C. plagiocephalus       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea                ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra                ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi              ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni               ---------- ---------- ---------- --AAAGGCAC AATCCAGAAC AGTTACGGC- AGTTCTTGCG TCAAYGCACG
N. metallicus           ---------- ---------- ---------- --AAAGGCAC AATCCAGAAC AGTTACGGC- AGTTCTTGCG TCGACGCACG
O. lichenigera          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii      CAGTAACTAC GGGTGCACGT AAAGTCTCTT AC-------- ---------- ---------- ---------- ----------
P. spenceri             CAGTAACTAC AGGTGCACGT AAAGTCTCTT AT-------- ---------- ---------- ---------- ----------
E. skiltonianus         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes            AACAATTTAC TCTGGATTGC AATATGAATT TCCCATAGGT TTTCACTGGG TGGTGCAGTA ACTACGGG?? ??????????
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              TAAAATTTAC TCTGGATTGC ACTATGAATT TCCCATAGGT TTC------- ---------- --------TA TTAAGTGGCG
N. metallicus          TAAAATTTAC TCTGGATTGC ACTATGAATT TCCCATAGGT TTC------- ---------- --------TA TTAAGTGGCG
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Appendix 3.1

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B. trilineata       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes         ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. telfairii        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi          ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??ATGGCCCT
N. greeni           CCGTAACTGC GTCAACGCAA ATCTCTCGTG TGATGACGCA AGAGCTGCCG TAACTGCTCT GTATTGTGCC CT--------
N. metallicus       CCGTAACTGC GTCAACGCAA ATCTCTCGTG TGTTGACGCA AGAGCTGCCA TAACTGCTCT GGATTGTGCC CT--------
O. lichenigera      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Appendix 3.1

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B. trilineata       ---------- ---------- ---------- ------GTAA ATTT-GACTG GAAAAGGTGC ACATTATATT -TGAGCAAAT
C. austrocaledonicus ---------- ---------- ---------- --------G. ....-..... A.......A. ......C... -C....T...
C. longipes         ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus   ---------- ---------- ---------- --------.. ....-..... .......... .......... TC...T....
C. aenea            ---------- ---------- ---------- -----AAA.G ....T..... .......... ......C... -C.......
E. cyanura          ---------- ---------- ---------- --------.T ....-..... .......... ......C... -.........
E. nigra            ---------- ---------- ---------- --------.. ....-.G..T .........T .......... -C.......
E. rufescens        ---------- ---------- ---------- --------.. ..G.-.G... ........A. .......... -C.......
L. guichenoti       ---------- ---------- ---------- ---------- ---------- ---------- ---...C... -C.......
L. telfairii        ---------- ---------- ---------- --------.. ....-.T... .......... ..G....... -C......C.
L. nigrofascolatum  ---------- ---------- ---------- --------G. ....-..... ........A. ......CC.. -C....T...
M. greyii           ---------- ---------- ---------- ---------- -...-..... ......A... ......C... -......TG.
M. ruficauda        ---------- ---------- ---------- --------.. ....-..... .......... .......... -.........
N. maccoyi          ---------- ---------- ---------- --------.. ....-..... .G....T... ......C... -CA.......
N. greeni           ---------- ---------- ---------- --------.. ....-..... .......... ......C... -.........
N. metallicus       ---------- ---------- ---------- --------.. ....-..... .......... ......C... -.........
O. lichenigera      ---------- ---------- ---------- -----AAA.G ....T..... .......... ......C... -C.......
O. smithi           ---------- ---------- ---------- -----AAA.G ....T..... .......... ......C... -C.......
P. entrecasteauxii  GTGCTCCCAT AGTTACTCTG GATTTGGGGG TATA------ -...-..... .........T ....A.C... -C.......
P. spenceri         GTGCTCCCGT AGTTACTCTG GATTTGGGGG TATA------ -...-..... .........T ..G...C... -C.......
E. skiltonianus     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata      ---------- ---------- ---------- ---------- -...-CTTCA T..GCA..AT TA...G...- ----------
P. wahlbergi        ---------- ---------- ---------- ----ACAGG. .A..TAG... ........A. .....GC.A. -.........
T. scincoides       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Appendix 3.1

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B. trilineata          ACNGTAATTG TAT------- ---------- -------GGA GAGTTATATT AATTATACGG AGAGCTATGG AACAA-----
C. austrocaledonicus   ..G.......  ...------- ---------- -------... ..........  .......T..  ..........  -----GATTA
C. longipes            ??????????? ??????????? ??????????? ??????????? ??????????? ??????????? ??????????? ???????????
C. plagiocephalus      ..A.......  ...------- ---------- -------... ..........  .......T..  .....C.A..  .....-----
C. aenea               ..C.......  ...------- ---------- -------... ..........  .......T..  ..........  .....-----
E. cyanura             T.A.C.....  ...------- ---------- -------... ..A...--..  .......T..  ....T....A  ..--.-----
E. nigra               .TG.......  ...------- ---------- -------... ..A.......  .......T..  ..........  .....-----
E. rufescens           ..G.......  ...------- ---------- -------... ........C.  .......T..  ..........  .....-----
L. guichenoti          ..A.......  ...------- ---------- -------... .......G..  .......T..  .A........  .....-----
L. telfairii           .TG.......  G..------- ---------- -------... ..........  .......T..  ..........  .....-----
L. nigrofascolatum     ..G.......  ...------- ---------- -------... ..........  .......T..  ......C...  G....-----
M. greyii              ..AT......  ..A------- ---------- -------... A.........  .......T..  .....CT...  .....-----
M. ruficauda           ..G.......  ...------- ---------- -------... ..........  .......T..  ..........  ..T..-----
N. maccoyi             ..G.......  ...------- ---------- -------... ...AG.....  .......T..  ..........  .....-----
N. greeni              ..G.......  ...------- ---------- -------... ...AG.....  ....T..T..  GA........  .....-----
N. metallicus          ..G.......  ...------- ---------- -------... ...AG.....  ....T..T..  G.........  .....-----
O. lichenigera         ..C.......  ...------- ---------- -------... A.........  .......T..  ..........  .....-----
O. smithi              ..C.......  ...------- ---------- -------... ..........  .......T..  ..........  .....-----
P. entrecasteauxii     .TG.......  ...------- ---------- -------... ..........  .......TA.  .A........  .....-----
P. spenceri            .TG.......  ...------- ---------- -------... ..........  .......T..  ..........  .....-----
E. skiltonianus        ----------  ---------- ---------- ---------- ----------  ....G..T..  .........A  .G...-----
M. logicaudata         ----------  ---GGGGAAC TATGGAGCAA TCTCCTG... T..AA.G.W.  GG.....A--  ----------  ----------
P. wahlbergi           .TG.......  ...------- ---------- -------... ..........  ....G..A..  ......T..A  ..T..-----
T. scincoides          ----------  ---------- ---------- ---------- ----------  ----------  ----------  ----------
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B. trilineata          ---------- -TTTCCTGGG ATAGAATGAT T--------- ------AGAT AATAG----- ---------- ----------
C. austrocaledonicus   ATTATATGGA G......... .......... .--------- ------.... .....----- ---------- ----------
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus      ---------- -......... .......... .--------- ------.... T....----- ---------- ----------
C. aenea               ---------- -......... ...A...... .--------- ------.... .....----- ---------- ----------
E. cyanura             ---------- -.A..T.... .......A.. .--------- ------.... ....A----- ---------- ----------
E. nigra               ---------- -......... .......... N--------- ------.... ....A----- ---------- ----------
E. rufescens           ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
L. guichenoti          ---------- -......... .C........ .--------- ------.... ..A..GCTTG GAGCACAATC CTAACCATGT
L. telfairii           ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
L. nigrofascolatum     ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
M. greyii              ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
M. ruficauda           ---------- -......... .......--. .--------- ------.... .....----- ---------- ----------
N. maccoyi             ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
N. greeni              ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
N. metallicus          ---------- -C........ .......... .--------- ------.... .....----- ---------- ----------
O. lichenigera         ---------- -......... ...A...... .--------- ------.... .....----- ---------- ----------
O. smithi              ---------- -......... ...A...... .--------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
P. spenceri            ---------- -......... .......... .--------- ------.... .....----- ---------- ----------
E. skiltonianus        ---------- -.C.G...A. ......A... .--------- ------G.C. ..C..----- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ---------- -...T..... .C........ .GGTCTGGTC TTAATAG... .....----- ---------- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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B. trilineata          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. austrocaledonicus   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ----------
C. plagiocephalus      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. aenea               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. cyanura             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. nigra               ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. rufescens           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti          CTACTCAAAA GTAAGCTCCA TTGAATTCAA TGGGTCTTAC TCCCATGAAA GTGTCTTTAG GATTGCA--- ----------
L. telfairii           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. nigrofascolatum     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. greyii              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. maccoyi             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. greeni              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. smithi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecasteauxii     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
E. skiltonianus        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. logicaudata         ---------- ---------- ---------- ---------- ---------- ---------- -------AGG CTGGGTTTCT
P. wahlbergi           ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Appendix 3.1

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B. trilineata          ----GCC-TG GGCTTCTCCA ATGAAGGTAT A--------- ---------- ---------- -----AGTAT TTCTGTATAT
C. austrocaledonicus   ----...-.. .T........ .G......G. .--------- ---------- ---------- -----..... .-........
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... ??????????
C. plagiocephalus      -----..-.. .T....C... .G........ .--------- ---------- ---------- -----..... ..........
C. aenea               ----...-.. .T........ .G........ .--------- ---------- ---------- -----..... C..C......
E. cyanura             ----...-.. .A..C..... .G....A... .--------- ---------- ---------- -----.T... ..........
E. nigra               ----...-.. .T...T.... .G...CA... .--------- ---------- ---------- -----.A... ..A.......
E. rufescens           ----...-.. .T........ .G........ .--------- ---------- ---------- -----.A... ..A.......
L. guichenoti          ----...T.. .T......TG .G........ .--------- ---------- ---------- -----..... ..........
L. telfairii           ----...-.. .TT..G.... .G....A... .--------- ---------- ---------- ---------. .GA.......
L. nigrofascolatum     ----...-.. .T........ .G........ .--------- ---------- ---------- -----..... ..........
M. greyii              ----...-.A .T........ TGA..... .--------- ---------- ---------- -----..... ....T.....
M. ruficauda           ----...-.. .T........ G........ .--------- ---------- ---------- -----TT... ..........
N. maccoyi             ----...-.. .T.......G .G...????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ----...-.. .T........ .G........ .--------- ---------- ---------- -----..... ..........
N. metallicus          ----...-.. .T........ .G........ .--------- ---------- ---------- -----..... ..........
O. lichenigera         ----...-.. .T........ .G........ .--------- ---------- ---------- -----..... C.........
O. smithi              ---------- .T........ .G........ .--------- ---------- ---------- -----..... C.........
P. entrecasteauxii     ----...-.. .T........ .G........ .--------- ---------- ---------- -----..... ....A.....
P. spenceri            ----C..-.. .T........ .G..R..K.. .--------- ---------- ---------- -----..... ....A.....
E. skiltonianus        ----.G.-.A .C........ .GC...A... .CCGGTGTTT CTGGATTCAC AGTTGCAGGT AAACT----- ----------
M. logicaudata         CAAA------ -------... .--------- ---TGTGTTT CTGGATTCAC AGCTACA--- ---------- ---.A....C
P. wahlbergi           ----.A.-.. .C......T. .G........ .--------- ---------- ---------- -----T..G. .....G...C
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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Appendix 3.1

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B. trilineata          TCCAG-AAGA AAGTCCCA-G TTCTCCTG-- ---------- ---------- -TTGCATCTC CAGAT----C AGATTGGAAA
C. austrocaledonicus   ..A..-..A. .........-. .........-- ---------- ---------- -......... ..........-. ...C......
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus      .....-.... ..A.....-. .......A-- ---------- ---------- -......... .....----T ...C......
C. aenea               .....-.... .........-. .........-- ---------- ---------- -......... ..........-. ...C......
E. cyanura             .....-.... ....T..C-A .........-- ---------- ---------- -......... ..........-. ...C......
E. nigra               .....-.... .........-. .........-- ---------- ---------- -......... ..........-. ..........
E. rufescens           .....-.... .........-. .........-- ---------- ---------- -.........T ..........-. .A........
L. guichenoti          .....-.R.. .........-. .........-- ---------- ---------- -......... ..........-. ...C......
L. telfairii           .....-.... .........-. .........-- ---------- ---------- -.A....... .T........-. ..CC......
L. nigrofascolatum     ..A..-.... .........-. .........-- ---------- ---------- -......... T.........-. ...C......
M. greyii              .....-.... ...G.....-. .........-- ---------- ---------- -......... ..........-. ...C......
M. ruficauda           .....-.... .........-. .........-- ---------- ---------- -......... ..........-. ...C......
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              .....-.... ....T...-. .........-- ---------- ---------- -......... ..........-. ...C......
N. metallicus          .....-.... ....T...-. .........-- ---------- ---------- -......... ..........-. ...C......
O. lichenigera         .....-.... .........-. .........-- ---------- ---------- -......... .....GCAA. ...C......
O. smithi              .....-.... .........-. .........-- ---------- ---------- -....T.... ..........-. ..........
P. entrecasteauxii     .....-.... .........A. .........-- ---------- ---------- -......... .C........-. ...C......
P. spenceri            .....-.... .........-. .....Y..-- ---------- ---------- -......... .M........-. ...C......
E. skiltonianus        -....G.... .........-. ...------CT ACCCT----- ---------- -......... ...G.....-. ...C...G..
M. logicaudata         .....GC... G.....T.-. ..--------- -----TAGAT CTACAGGACC T..AG..... ...G.....-. ...C......
P. wahlbergi           ...G.-.... .........-. .....TGT-- ---------- ---------- -.........T ...G.....-. G..C....T.
T. scincoides          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
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B. trilineata          AGGT--CGCT CTGTGAAACT CTGGAAAGTT TC-------- ---------- ---------- ---------- GGTGTCA-GT
C. austrocaledonicus   .....--TA.. .....C.... .......... ..-------- ---------- ---------- ---------- A......-..
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus      ....--.A.. .......... -..-...... .T-------- ---------- ---------- ---------- .......-..
C. aenea               ....--.A.. .......... .......... ..-------- ---------- ---------- ---------- .......-..
E. cyanura             ....--.A.. .......... .......... ..-------- ---------- ---------- ---------- A......-..
E. nigra               ....--.A.. ..C....... .......... .T-------- ---------- ---------- ---------- .......-..
E. rufescens           ....--.A.. .........C .......... .T-------- ---------- ---------- ---------- .......-..
L. guichenoti          ....AA.AT. ..A....... .......... ..-------- ---------- ---------- ---------- .......-..
L. telfairii           ....--.A.. .......... ......T... ..-------- ---------- ---------- ---------- ...A...-..
L. nigrofascolatum     ..T.--.A.. .......... .......... ..-------- ---------- ---------- ---------- .-------..
M. greyii              .T..--.A.. .......... .......... ..-------- ---------- ---------- ---------- A......-..
M. ruficauda           ....--.T.. .......... ..A....... .T-------- ---------- ---------- ---------- ..--------
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ....--.AT. .......... .......... ..-------- ---------- ---------- ---------- .......-..
N. metallicus          ....--.A.. ....A..... .......... ..-------- ---------- ---------- ---------- .......-..
O. lichenigera         ....--.A.. .......... .......... ..-------- ---------- ---------- ---------- .......-..
O. smithi              ....--.A.. .......... .......... ..-------- ---------- ---------- ---------- .......-..
P. entrecasteauxii     ....--.A.. A......... ...T.....G ..-------- ---------- ---------- ---------- A......-..
P. spenceri            .R..--.A.. .......... .......... ..-------- ---------- ---------- ---------- .......-..
E. skiltonianus        ..AC--.C.. G........TC .........-- --CCATTGTC AATCAGTGT- --CAGTCATA TT-------- ----------
M. logicaudata         ..A.--.C.. G.A....GA. A.........-- --CCATTGTC AATCATTGTA TG-------- ---------- ----------
P. wahlbergi           GT..--.A.. .......... ..A.....GC A--------- ---------- ---------- -TAGTCAATC A....A.-A.
T. scincoides          ---------- ---------- ---------- --CCATT--- ---------- ---------- ---------- ---....-..
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Appendix 3.1

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B. trilineata          TATACT-GAC TGAGAY---- ---------- ---------- --------GG ATCAATTATC TGAGA----- ----------
C. austrocaledonicus   ......-..T .....T---- ---------- ---------- --------.. .......... .....----- ----------
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus      ......---- ---------- ---------- ---------- --------.. ..A....... .....----- ----------
C. aenea               ......-..T C....T---- ---------- ---------- --------.. .......... .....----- ----------
E. cyanura             ......-..T .....T---- ---------- ---------- --------.. .......... .....----- ----------
E. nigra               G.....-..T CA...T---- ---------- ---------- --------.. .......C.T ...CT----- ----------
E. rufescens           ......-..T CA...T---- ---------- ---------- --------.. .......... .....TCAAT TACCTAGCTG
L. guichenoti          ...G..-..T C....T---- ---------- GGGTCAATTA TCTGAGAT.. .......... .....----- ----------
L. telfairii           ......-..T C....T---- ---------- ---------- --------.. .......... .....----- ----------
L. nigrofascolatum     ....T.T.CT CA...T---- ---------- ---------- --------.. .......... .....----- ----------
M. greyii              ....T.-..T .....T---- ---------- ---------- --------.. .........A .....----- ----------
M. ruficauda           ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. maccoyi             ......-..T C....T---- ---------- ---------- --------.. .......... .....----- ----------
N. greeni              ......-..T C....T---- ---------- ---------- --------.. .......... .....----- ----------
N. metallicus          ......-..T C....T---- ---------- ---------- --------.. T......... .....----- ----------
O. lichenigera         ......-..T C....T---- ---------- ---------- --------.. .......... .....----- ----------
O. smithi              ......-..T C....T---- ---------- ---------- --------.. .........C. .....----- ----------
P. entrecasteauxii     ......-..T C....T---- ---------- ---------- --------.. .........C. .....----- ----------
P. spenceri            --------..T CT...T---- ---------- ---------- --------A. ....G.G... ....-TCA-- ----------
E. skiltonianus        --------..T CT...T---- ---------- ---------- --------.T ......G... ....-TCC-- ----------
M. logicaudata         ......-AGT CA...TATAG CAATGATTAA ---------- ---------- ---------- ---------- ----------
P. wahlbergi           ....T.-..T GT...T---- ---------- ---------- --------.T ....G.G... C...-TCA-- ----------
T. scincoides
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Appendix 3.1

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B. trilineata          GTATAAGATA GGCTCATATG TTTATT---- ---------- ---------- ---------C ACAGAT-GT- ----------
C. austrocaledonicus   .......... ..T....... ......---- ---------- ---------- ---------. ......A..G TATCT----A
C. longipes            ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
C. plagiocephalus      .......... ..T......C ......---- ---------- ---------- ---------. .T.A..A..G TATCT----A
C. aenea               .......... ..T....... ......---- ---------- ---------- ---------. ......A..G TATCT----A
E. cyanura             ......T... ..T....... ......---- ---------- ---------- ---------. ......A..A TATCT----A
E. nigra               .......... ..T....... ......---- ---------- ---------- ---------. ......A..G TATCTACTGA
E. rufescens           .......... .-T.M..... ......---- ---------- ---------- ---------. ......A..G TATCTACTAA
L. guichenoti          ----...... ..T....... ......---- ---------- ---------- ---------. ..C...A..G TGCCT----A
L. telfairii           .........-- -.T....... ......---- ---------- ---------- ---------. ......A..G TATCTACTAA
L. nigrofascolatum     .......... ..T.....G. G..---CTGG GTTCATACGT TCATATT--- ---------. ......A..G TATCT----A
M. greyii              ........C. ..T....... ......---- ---------- ---------- ---------- ......G..G TATCT----A
M. ruficauda           .......... ..T....... ......???? ?????????? ?????????? ?????????? ?????????? ??????????
N. maccoyi             ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
N. greeni              ....C..... ..T....... ......---- ---------- ---------- ---------. ......A..G TATCT----A
N. metallicus          ....C..... ..T....... ......---- ---------- ---------- ---------. ......A..G TATCT----A
O. lichenigera         .......... ..T....... ......---- ---------- ---------- ---------. ......A..G T---------
O. smithi              .......... ..T....... ......---- ---------- ---------- ---------. ......A..G TATCT----A
P. entrecasteauxii     ...GNNNN.. ..T....... ......---- ---------- ---------- ---------. ......A.CA TATCT----A
P. spenceri            ...G...... ..T....... ......---- ---------- ---------- ---------T ......A..G TATCT----A
E. skiltonianus        ...G....C. .CT..T.... C.....---- ---------- ---------- ---------. ......GA.G CATCT----A
M. logicaudata         A.....A.C. .CT....... ......---- ---------- ---------- ---------. ......GACG GATCT----A
P. wahlbergi           ........C. ..T.....-- ---------- ---------- -------CTA TTCATACTA- ---------- ---CT----A
T. scincoides          ......A.C. .CT....... .....---- ---------- ---------- ---------. T.....AACC TATCT----A
```

B. trilineata	-TATTTCCTC	AGAGTGCAGA	CATCTTTGA
C. austrocaledonicus	C.....	....CA..T.	.....G
C. longipes	??????????	??????????	?????????
C. plagiocephalus	C.....	.....TC	TT.GAC...
C. aenea	C.T.....C.	.....T.	.....
E. cyanura	CC.-.C....	.....TGT.	T....A...
E. nigra	C.....T	.....T.	.....?
E. rufescens	C.....	.....T.	.....?
L. guichenoti	C.....	.T.....T.	.....G??
L. telfairii	C.....	.....T.TG	.....????
L. nigrofascolatum	C.G.....	.....T.	.....
M. greyii	C.....-..	...-....T.	....?????
M. ruficauda	??????????	??????????	?????????
N. maccoyi	??????????	??????????	?????????
N. greeni	C.G.....	.....T.	.....
N. metallicus	C.G.....	.....A.T.	.....
O. lichenigera	-----.....	.....T.	.....
O. smithi	C.T.....	.....T.	.....
P. entrecasteauxii	C...??????	??????????	?????????
P. spenceri	C.....	.....T.	.....
E. skiltonianus	C.C???????	??????????	?????????
M. logicaudata	C.????????	??????????	?????????
P. wahlbergi	---.....	...T..T.T.	....A....
T. scincoides	C.C.G..A..	.AT.....T.	TG....-..

Appendix 3.2: Character state definition and asymmetric step matrix for transformations between the states of gap 2 occurring in the first 150bp of *β-fibrinogen* in the *Eugongylus* group.

Gaps	Initial State ↓	Final State:																								
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
none	0	-	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	3	4	4	4	4	4	5
2	1	1	-	2	4	2	2	2	2	2	2	2	2	2	2	3	3	3	3	4	5	5	5	5	5	6
3	2	1	1	-	1	2	2	2	2	2	2	2	2	2	2	1	1	1	3	2	3	3	3	3	4	4
4	3	1	1	1	-	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	4	4	4	4	3	5
5	4	1	2	2	2	-	2	2	2	2	2	2	2	2	2	1	3	3	3	4	5	5	5	5	5	6
6	5	1	1	2	2	1	-	1	2	2	2	2	2	2	2	3	3	3	1	2	3	3	3	3	3	4
7	6	1	1	2	2	1	1	-	1	1	1	1	2	2	2	3	1	2	2	4	3	3	4	4	4	3
8	7	1	1	3	2	1	2	1	-	1	1	1	2	2	2	2	2	2	3	4	3	4	4	5	4	5
9	8	1	1	2	2	1	2	1	1	-	1	1	2	2	2	2	2	1	3	4	4	3	4	5	3	5
10	9	1	1	2	2	1	2	1	1	1	-	1	2	2	2	2	2	2	3	4	4	4	3	5	4	4
11	10	1	1	2	2	1	1	2	1	2	2	-	2	2	2	2	3	2	3	4	4	5	5	3	5	6
12	11	1	2	2	2	1	2	2	2	2	2	2	-	2	1	2	3	3	1	4	5	5	5	5	9	6

Appendix 3.2

Gaps	Initial State ↓	Final State:																								
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
13	12	1	1	2	2	1	2	2	2	2	2	2	1	-	2	3	3	3	2	2	3	3	3	3	3	4
14	13	1	2	2	2	1	2	2	2	2	2	2	1	2	-	3	3	3	2	4	5	5	5	5	5	4
3, 5	14	2	2	1	2	1	3	3	3	3	3	3	3	3	3	-	2	2	4	3	4	4	4	4	5	5
3, 7	15	2	1	1	2	2	2	1	2	2	2	3	3	3	3	1	-	1	3	2	3	3	2	3	4	4
3, 9	16	2	1	1	2	2	3	2	2	1	2	3	3	3	3	1	1	-	4	3	3	2	3	3	3	4
6, 12	17	2	2	3	3	1	1	2	3	3	3	3	1	3	2	2	3	4	-	3	4	4	4	4	4	5
3, 6, 13	18	3	1	2	3	2	2	3	4	4	4	4	3	2	4	1	2	3	2	-	1	1	1	1	2	2
3, 6, 8, 13	19	4	1	3	4	2	3	3	3	4	4	4	4	3	5	1	2	3	3	1	-	1	1	1	2	2
3, 6, 9, 13	20	4	1	3	4	2	3	3	4	3	4	5	4	3	5	1	2	2	3	1	1	-	1	2	1	3
3, 6, 10, 13	21	4	1	3	4	2	3	3	4	4	3	5	4	3	5	2	2	3	3	1	1	1	-	2	2	1
3, 6, 11, 13	22	4	1	3	4	2	3	4	4	5	5	3	4	3	5	1	3	4	3	1	1	2	2	-	3	3
4, 6, 9, 13	23	4	1	4	3	2	3	3	4	3	4	5	4	3	5	2	3	3	3	2	2	1	2	3	-	3
3, 6, 10, 13, 14	24	5	1	4	5	2	4	4	5	5	4	6	4	4	4	1	3	4	3	2	3	2	1	3	2	-

Appendix 3.3: Simple and complex gap characters from the initial 150bp of *β-fibrinogen*. State "0" = absence of gap, state "1" = presence of gap, and "-" = inapplicable for simple coding, "?" = not sequenced for all characters. See Appendix 3.2 for character state definitions and step matrix for complex coding.

Taxon Character:	Simple Coding															Complex		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3
B. trilineata	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	20	0
C. austrocaledonicus	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	19	0
C. longipes	1	0	0	1	0	1	0	0	1	-	0	0	1	0	0	1	23	0
C. plagiocephalus	1	0	1	-	0	1	0	0	0	-	0	0	?	0	0	1	?	0
C. aenea	1	0	1	-	0	1	0	0	0	1	0	0	1	0	0	1	21	0
E. cyanura	1	0	1	-	0	1	0	0	0	0	0	0	1	0	0	1	18	0
E. nigra	1	0	1	-	0	1	0	-	-	-	1	0	1	0	0	1	22	0
E. rufescens	1	0	1	-	0	1	0	0	0	1	0	0	1	0	0	1	21	0
L. guichenoti	1	0	1	-	0	1	0	0	0	1	0	0	1	0	0	1	21	0
L. telfairii	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	20	0
L. nigrofasciolatum	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	20	0
M. greyii	1	0	1	-	1	1	-	0	-	-	-	-	-	-	0	1	14	0
M. ruficauda	1	0	1	-	0	-	0	0	0	1	0	0	1	1	0	1	24	0
N. greeni	1	0	1	-	0	1	0	1	-	-	0	0	1	0	0	1	19	0
N. metallicus	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	20	0
O. lichenigera	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	20	0
O. smithi	1	0	1	-	0	1	0	0	0	1	0	0	1	0	0	1	21	0
P. entrecasteauxii	?	?	?	?	0	?	?	?	?	?	0	0	1	0	0	?	?	0
P. spenceri	1	0	1	-	0	1	0	0	1	-	0	0	1	0	0	1	20	0
E. skiltonianus	0	0	1	-	0	0	0	0	1	-	0	0	0	0	1	0	16	1
M. longicaudata	0	1	-	-	0	-	-	-	-	-	-	0	-	0	0	0	1	0
P. wahlbergi	?	0	0	0	0	1	0	0	0	0	0	1	-	-	0	?	17	0
T. scincoides	0	0	1	-	0	-	1	-	-	-	0	0	0	0	0	0	15	0

Appendix 3.4 – *c-mos* alignment of *Eugongylus* group taxa (see table 3.1)

	TCA	CGA	CCA	TGT	AGC	AGT	CCC	TTG	GTG	TTC	TCC	ACC	AAA	GGT	GGA	ATG	CTT	TTA	TTG	GGA	GAA	GGC	CCT
B. trilineata																							
C. austrocaledonicum	???	???	???	???	???	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	A..	...
C. longipes	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.A.	...
C. plagiocephalus	???	???	???	???	???	?..	...	...	...	...	...	...	..G	...	...	.A.	T..	...	...	...	...	.A.	...
C. aenea	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	.G.	...	...	...	...
E. cyanura	...	...	..T	...	..T	...	..A	C..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	T..
E. nigra	...	...	...	...	...	...	...	...	.Y.	...	...	...	...	...	...	...	...	...	...	...	...	..T	...
E. rufescens	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	T..
L. guichenoti	..G	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.CT	A..	...	...	...	...	...	...
L. telfairii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
M. greyii	???	???	???	???	???	???	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...	...
M. ruficauda	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. maccoyii	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
N. coventryi	???	???	???	???	???	???	???	???	???	???	???	?..	...	...	...	...	...	..G	...	...	...	...	...
N. greeni	???	???	???	???	???	???	???	??.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O. lichenigera	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	...	...	...
O. smithi	???	???	???	???	??.	...	...	.?.	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...
P. entrecastauxii	?..	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. spenceri	...	...	.A.	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	A..	..C	...	...	...	..C	...	...	...	.CT	...	...	...	...	...	...	.CA	...	...	...	...	...	...
M. longicaudata	...	...	T..	...	..T	...	...	...	..A	...	...	...	...	...	...	...	..A	...	...	...	...	...	...
P. wahlbergi	...	A..	...	..C	...	...	..T	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...
T. scincoides	...	...	T..	...	...	...	..T	...	...	..T	...	...	.C.	...	...	...	..G	...	...	...	...	...	...

Appendix 3.4

	TCC	AGC	AGG	TCT	CGT	CGG	CTG	CCA	CCT	CAC	TTG	GCT	TGG	TGC	TCT	ATT	GAC	TGG	GAA	CAG	TTA	TGT	CTC
B. trilineata																							
C. austrocaledonicum	...	.C.	...	...	...	...	...	...	..G	...	...	...	...	..T	...	...	...	...	...	...	..G	...	...
C. longipes	...	...	...	...	..G	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	..G	...	...
C. plagiocephalus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
C. aenea	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
E. cyanura	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
E. nigra	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
E. rufescens	...	...	...	...	...	...	T..	...	...	..T	...	...	...	...	...	...	...	...	...	...	C.G	...	...
L. guichenoti	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
L. telfairii	...	.C.	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	..G	...	...
L. nigrofasciolatum	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
M. greyii	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	.M.	...	...	...	...	A..	..G	Y..	...
M. ruficauda	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
N. maccoyii	?.T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
N. coventryi	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
N. greeni	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
N. metallicus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
O. lichenigera	...	.C.	.A.	...	G..	...	...	...	A..	...	...	...	.A.	...	...	...	..G	...	...	...	..G	...	...
O. smithi	...	.C.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
P. entrecastauxii	...	.A.	...	A..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
P. spenceri	...	...	...	A..	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	..G	...	...
E. skiltonianus	..T	CT.	...	G..	..C	...	...	...	...	...	C..	A..	...	...	...	...	...	...	..C	...	..G	...	..A
M. longicaudata	..T	GC.	...	A..	...	...	..A	...	...	...	C..	...	...	...	...	...	...	...	...	...	..G	...	..A
P. wahlbergi	..T	.T.	...	...	...	...	...	...	...	...	...	T..	...	...	...	...	...	...	..T	..T	..G	...	...
T. scincoides	..T	GCA	...	A..	...	...	...	...	...	...	C..	...	...	...	...	...	...	...	...	...	..G	...	..A

Appendix 3.4

	CAT	CAC	CCA	CTT	GGC	TCT	GGT	GGT	TTT	GGT	TCT	GTT	TAC	AAG	GCT	ACC	TAC	TAT	GGA	TCT	ACT	GTG	GCT
B. trilineata																							
C. austrocaledonicum	...	T..	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
C. longipes	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
C. plagiocephalus	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
C. aenea	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. cyanura	...	A..	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. nigra	...	...	...	..C	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...
E. rufescens	...	A..	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	A..	...	...	...
L. guichenoti	...	...	..T	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. telfairii	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
M. greyii	...	...	...	...	...	...	...	..C	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...
M. ruficauda	...	...	...	...	...	..A	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. maccoyii	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. coventryi	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	C..	...	...	...	...	...
N. greeni	...	...	.A.	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	.G.	...	...	...	...	...
O. lichenigera	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O. smithi	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. entrecastauxii	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	.G.	...	...	...	...	...
P. spenceri	...	...	...	G..	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	TG.	..T	..C	T.A	...	...	...	..C	...	...	...	..C	...	...	...	..T	...	...	...	A..	..A	...	...
M. longicaudata	...	..T	..C	..A	...	...	...	..C	...	...	...	..C	...	...	...	..T	...	...	...	A..	..A	...	...
P. wahlbergi	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	..C	...	A..	..A	...	...
T. scincoides	TG.	...	..T	..A	...	...	...	..C	...	...	...	..C	...	...	...	..T	...	...	...	A..	...	...	...

Appendix 3.4

	GAC	AGT	TTG	GGT	ACT	ATC	ATA	ATG	GAA	TAT	GTC	GGT	AAC	AGC	ACT	TTG	CAC	CAT	GTT	ATC	TAT	GGA	ACT
B. trilineata																							
C. austrocaledonicum	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	..C	...	...
C. longipes	...	...	...	...	..A	...	..T	...	...	...	..A	...	..T	...	...	...	...	...	...	...	...	...	...
C. plagiocephalus	...	...	...	...	...	G..	...	...	...	...	..G	...	...	...	...	...	...	...	...	...	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
E. cyanura	...	...	...	...	...	G..	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
E. nigra	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	A..	...	...	...	...
E. rufescens	...	...	...	...	...	G..	...	...	...	...	..A	...	...	...	...	...	...	..C	...	...	...	...	...
L. guichenoti	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
L. telfairii	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
M. greyii	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
M. ruficauda	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
N. maccoyii	...	...	...	..C	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	..A	...	...	...
N. coventryi	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
N. greeni	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
O. lichenigera	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
O. smithi	...	...	...	...	..C	...	...	...	...	...	..A	...	G..	...	...	...	...	...	...	...	...	...	...
P. entrecastauxii	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
P. spenceri	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	...	...	...	...	..C	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	..G	...
M. longicaudata	...	...	...	..A	..C	...	...	...	...	...	..A	...	...	..G	...	...	...	...	...	...	...	...	...
P. wahlbergi	...	...	..A	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...
T. scincoides	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...

Appendix 3.4

	GGA	AGT	ATG	ATA	ACA	AAA	AGG	AAA	GAT	AGT	---	TTT	GTT	TGT	GGC	TAT	GAA	CCT	TTA	AGT	GTA	ATG	CAG
B. trilineata																							
C. austrocaledonicum	...	...	---	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
C. longipes	.A.	...	.CA	...	---	...	...	...	..G	...	---	...	...	...	...	...	...	...	...	...	...	...	...
C. plagiocephalus	...	...	...	...	---	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
E. cyanura	...	..C	...	...	..T	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
E. nigra	...	...	...	...	...	...	..A	...	..G	...	---	...	...	...	...	...	...	...	...	...	A..	...	..A
E. rufescens	...	.A.	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
L. guichenoti	...	...	.CT	---	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	.C.	A..	...	..A
L. telfairii	...	...	...	...	...	...	...	...	...	...	---	...	...	...	..G	...	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
M. greyii	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
M. ruficauda	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
N. maccoyii	...	...	...	...	...	...	...	...	...	...	---	...	...	...	.C.	...	...	...	...	...	...	...	...
N. coventryi	...	...	...	..C	..C	...	...	...	..G	...	---	...	...	...	...	...	...	...	...	...	...	...	...
N. greeni	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	...	...	..G	...	---	...	...	...	...	...	...	...	...	...	...	...	...
O. lichenigera	...	...	...	...	...	...	...	...	..G	...	---	...	...	...	...	...	...	...	...	...	...	...	...
O. smithi	...	...	...	..C	..T	C..	...	...	...	.??	???	???	???	???	???	???	???	???	???	???	???	???	???
P. entrecastauxii	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
P. spenceri	...	C..	...	...	...	...	...	...	...	.A.	---	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	...	C..	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
M. longicaudata	...	...	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...
P. wahlbergi	...	...	...	...	...	...	...	..G	...	GA.	GGG	C..	.G.	...	...	.G.	...	...	..G	...	A..	.CA	..A
T. scincoides	...	...	...	...	...	...	...	..G	...	.A.	GGG	C..	.G.	...	..A	...	...	...	..G	...	A..	.CA	...
	...	...	...	...	...	...	...	...	..C	...	---	...	...	...	...	.C.	...	...	...	...	...	.G.	...
	...	...	...	...	...	..G	...	..G	...	.A.	GAA	C..	.G.	.A.	...	...	...	...	..G	...	A..	.CA	...

Appendix 3.4

	TCT	TTG	AGC	TAC	TCG	TGT	GAC	ATT	GTT	GCA	GGC	TTG	GTC	TTT	CTC	CAT	TCA	CAA	TTC	ATT	GTG	CAT	CTT
B. trilineata																							
C. austrocaledonicum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
C. longipes	...	...	...	...	..C	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...
C. plagiocephalus	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C
E. cyanura	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...
E. nigra	.M.	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. rufescens	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...
L. guichenoti	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. telfairii	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..A
M. greyii	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	..?
M. ruficauda	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...
N. maccoyii	...	..?	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
N. coventryi	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..?	???	???	???
N. greeni	...	...	...	...	..A	...	...	..?	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
N. metallicus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O. lichenigera	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
O. smithi	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	..C
P. entrecastauxii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. spenceri	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	...	C..	...	...	...	...	...	...	..G	...	...	...	...	...	...	...	...	...	...	...	...	...	...
M. longicaudata	.G.	C..	...	...	..A	...	...	...	..G	...	...	...	...	...	...	...	...	...	..A	...	...	...	..G
P. wahlbergi	A..	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	..A	...	...	...	..G
T. scincoides	...	C..	...	...	..A	...	...	...	..G	...	..T	...	C..	...	...	...	...	...	..A	...	...	...	..G

Appendix 3.4

	Gap Characters			
	1	2	3	4
B. trilineata	0	0	0	1
C. austrocaledonicum	1	0	0	1
C. longipes	0	0	1	1
C. plagiocephalus	0	0	1	1
C. aenea	0	0	0	1
E. cyanura	0	0	0	1
E. nigra	0	0	0	1
E. rufescens	0	0	0	1
L. guichenoti	0	1	0	1
L. telfairii	0	0	0	1
L. nigrofasciolatum	0	0	0	1
M. greyii	0	0	0	1
M. ruficauda	0	0	0	1
N. maccoyii	0	0	0	1
N. coventryi	0	0	0	1
N. greeni	0	0	0	1
N. metallicus	0	0	0	1
O. lichenigera	0	0	0	?
O. smithi	0	0	0	1
P. entrecasteauxii	0	0	0	1
P. spenceri	0	0	0	1
E. skiltonianus	0	0	0	1
M. longicaudata	0	0	0	0
P. wahlbergi	0	0	0	1
T. scincoides	0	0	0	0

Appendix 3.5 – *myh-2* alignment of *Eugongylus* group taxa (see table 3.1)

```
B. trilineata          ?TGGATTC-G ACCTGTCCCA GCTCCAGACA GAGGTGGAGG AGGCAGTGCA GGAGTGCAGG AATGCGGAGG AGAAGGCCAA
C. austrocaledonicus A....G..C. .......... ...G.....C .......... ....G..T.. .......... ..C....... ..........
C. aenea             A....G..C. .......... A......T.G .......... ....G..T.. .......... .......... ..........
E. cyanura           ?????????? ??????.... .T.......G .......... ....G..... .......... ..C....... ..........
E. nigra             A....G..C. .......... .T.G.....C .......... ....G..T.. ......T... .....A.... ....A.....
E. rufescens         A....G..C. .......... ...G.....C .......... ....G..T.. .......... .......... ..........
L. guichenoti        A....G..C. .......... ...G...... .......... ....C..... A......... ..?....... ..........
L. telfairii         A....G..C. .......T.. ...G.....C .......... ....G..T.. .......... .......... ..........
L. nigrofasciolatum  A....G..C. .......... ...G.....C .......... ....C..T.. .......... ..?....... ..........
M. greyii            A....G..C. M......... ...G...... .......... .......... .......... .......... ..........
M. ruficauda         A....G..C. .......... ...G...... .......... .......... .......... .......... ..........
N. maccoyii          AGA.GA..C. .......... ...G..A... .......... .....T.... .......... .......... ..........
N. coventryi         A....G..C. .......... ...G...... .......... ....C..... .......... .......... ..........
N. greeni            A....G..C. .......... ...G...... .......... .......... .......... .......... ..........
O. lichenigera       A....G..C. .......... ...G.....C .......... ....G..T.. .......... .....A.... ..........
O. smithi            A....G..C. .......... ...G....GC .......... ....G..T.. .......... .......... ..........
P. entrecasteauxii   A....G..C. .......... ...G...... .......... .......... A......... .......... ..........
P. spenceri          A....G..C. .......... A......T.G .......... .......... .......... ..C..A.... ..........
```

B. trilineata	GAAAGCCATT	ACTGATGTGA	GCGGAATGGG	AAACATTCTT	AGTGAAGCAC	CAGAAATGAA	ACTTGAGTGG	GATTTATTTA
C. austrocaledonicus	...G.....C	..C.....	.T.....	...T.....	..------5-	---.....	T.....	.....
C. aenea	...G.....C	.....	.T.....C..	.....	.....	.....	T..C.....	.....
E. cyanura	A..G.....C	..C.....	.T...-----	------2---	------....	.G.....	T.....	.G.....
E. nigra	...G.....C	.....	.T....C...	.....	G.....	.....	TT.....	.....
E. rufescens	...G.....C	..C.....	.T.....	....G.....	G.....	.....	T.....	.....
L. guichenoti	...G.....C	.....C....	.T.....	....C....C	.....	.....G....	T..G.....	.....
L. telfairii	...G.....C	.....	.T.----1--	-.....C...	GA.....	.....	T.....	.....
L. nigrofasciolatum	...G.....C	..C.....	.T....C...	....G.....	.....	.....	T.....	.....
M. greyii	...G.....C	.....	.T.....	.C.....	C.....R...	YG.....	T....W....	.....C....
M. ruficauda	C	.....	.T.....	.....	..C.....	.....	.....	C
N. maccoyii	...G.....C	.....	.T.....	....C....G	.T.....	.....	T.....T...	.....
N. coventryi	...G.....C	.....	.T....G..T	....C....G	.....	.....	T.....	.....
N. greeni	...G.....C	.....	.T.....	....C..-3-	.....	.....	TA.....	......C...
O. lichenigera	...G.....C	.....	.T.....A..	....C.....	.....	.....	TA.....	.....
O. smithi	...G.....C	.....	.T.....	....------	-4------..	.....	T..C.....	.....
P. entrecasteauxii	...G.....C	.....	.T.....	.....	.....	.....	T.....	.....G..
P. spenceri	...G.....C	.....	.T.....	.....	.....	.....	T.....	.....

B. trilineata	GGATAGAGA-	CACTACAGAC	TTTCAATCCC	CTATTATTTT	CCCCCGCAAG	TAGGACAATA	GTCGGTGAAG	CTTACTTTTG
C. austrocaledonicus	.........T	......G...	..A.....	.........A	..T..AG...	....	..T.....	TCC.....
C. aenea	....C....C	....	..G.....	....	....TA....	....	.W.....	T.C.....
E. cyanura	..G..A...C	......G...	G.A...C...	T.....	.....A....	........C.	..T.....	T.C.....
E. nigra	........TC	........T.	..A..G....	....	.....A....	......G...	....	T.C.....
E. rufescens	C	.....T....	..A.....	....	.....A....	.....---8	---.....C.	T.C.....
L. guichenoti	...CG....C	....	..A.....	.........-	.....A----	----6-----	..T..A....	T.C.....-
L. telfairii	C	....	..A.....	....	TTT..A....	....	....	T.C.....A
L. nigrofasciolatum	.........T	....?....?	..A.....	....	.....A....	....	..T.....	T.C.....
M. greyii	...A...T.C	.......T..	..A.....	-.........	.....A.G..	....	..T.....	T.C.....
M. ruficauda	C	....	..A.....	....	.....A....	...----7--	..TA....-.	T.....
N. maccoyii	C	..T....A--	------...T	G.....	.....A....	....	..T.....C.	T.C.....
N. coventryi	C	....	..A.....	....	.....A....	....	..T.....	T.C.....
N. greeni	....T....C	....	..A.....	C	...T.A....	....	..T.....	T.C.....
O. lichenigera	C	....	..A.....	....	....TA....	....	..T.....	T.C.....
O. smithi	....C....C	.C.....	..A.....	....	....TA....	....	....	T.C.....
P. entrecasteauxii	C	....	..A.....	.........?	.?...A....	....	..T.....	T.C.....
P. spenceri	C	....	..A.....	....	.....A....	....	..T.....	T.C.....

Appendix 3.5

```
B. trilineata        TGTGTCCCAA AATGCTATTT TCCATTGAAG AAGTAGGCAT ACTCAATAGG GTGGACATGG GTATGAAGA- ----------
C. austrocaledonicus .......... ....T..... .......... .......... .G........ A......... ......G..- ----------
C. aenea             .......... .......... .......... .......... .....G.... .......... ..G.....G- ----------
E. cyanura           .......... ......?... .G........ .......AC. .......... .......A.. .....G...- ----------
E. nigra             .......... .......... ....C..... ..?.C..... ...T...... A..A...... ...C.....- ----------
E. rufescens         .......... .......C.. ..T....... .......... .......... A....G.... .........- ----------
L. guichenoti        ---------- ---------- -......... .......A.. .......... .......G... .........G- ----------
L. telfairii         .......... .......... .........T .......... .T...G.... A......... .........- ----------
L. nigrofasciolatum  .......... .....C.... ........C. .......... ......?... A......... .........- ----------
M. greyii            .......... .......... .......... .......... .A........ ......?... .......-.- ----------
M. ruficauda         .....A.... .......... .......... .......A.. .......... .........A .........- ----------
N. maccoyii          .......... .......... ...T...... T......... .......... ........T. ........T- ----------
N. coventryi         .......... .......... .......... ......A... .......... .......... .........- ----------
N. greeni            .......... .......... .......... .......... .......... .......... .........- ----------
O. lichenigera       .......... .......... .......... .......... ........A. .......... .........- ----------
O. smithi            .......... .......... .......... .......... .......... .......... .........- ----------
P. entrecasteauxii   .......... .......... .......... .......... G......... .......... .........- ----------
P. spenceri          .......... .......... .......... .......... .......... .......... .........- ----------
```

Appendix 3.5

```
B. trilineata          ---------- --------TG GCTATAGGGT GAAGAG---- ---------- -----GCCTT GCTTGTACTC CTGATGAGTT
C. austrocaledonicus   ---------- --------.. T......... ......---- ---------G GTCCT.???? ?????????? ??????????
C. aenea               ---------- --------.. .......... ......---- ---------G GCCCT..... .......... -.........
E. cyanura             ---------- --------C. A.....A... .....C---- ---------G GCCCC..T.. .T......C. -C........
E. nigra               ---------- --------.. .......A.. ......---- ---------G GCCCT..... .......T.. -.........
E. rufescens           ---------- --------.. .T.C...A.. ......---- ---------G GCCCT..... .......T.. -.........
L. guichenoti          ---------- --------.. .......... ...---TATA GGACCATATG GACCCA.... .......... -..T......
L. telfairii           ---------- --------C. .......... ......---- ---------G GCCCT....G .......T.. -.........
L. nigrofasciolatum    ---------- --------.. .......... ......---- ---------G GCCCT.???? ?????????? ??????????
M. greyii              ---------- --------.. .........- ......---- ---------G GTCTT-.... .......... -.........
M. ruficauda           ---------- --------.. .......... ......---- ---------G GCCCT..... .......... -..C......
N. maccoyii            ---------- --------.. ....AG.... ......---- ---------G GCCCT..... ......CA.. -.....C...
N. coventryi           ---------- --------.. .......... ......---- ---------G GCCCT...G. .......... -.........
N. greeni              ---------- --------.. .......... ......---- ---------C GCCCT...G. .......... -.........
O. lichenigera         ---------- --------.. ....C..... ......---- ---------G GCCCT..... .......T.. -.........G
O. smithi              ---------- --------.. .......... ......---- ---------G GCCCT..... .......T.. -.........G
P. entrecasteauxii     ---------- --------.. .......... ......---- ---------G ACCCT..... ..........  ----------...
P. spenceri            ---------- --------.. .......... ......---- ---------G ACCCT..... .......... -.........
```

			Simple	Complex
			12345678	12345
B. trilineata	ATCTCCAACC	CAAGAAC	00001000	00000
C. austrocaledonicus	??????????	???????	00000000	01000
C. aenea	....	T.T..T.	00000000	00000
E. cyanura	....	T.TA.T.	01-00000	00020
E. nigra	....	T.T..T.	00000000	00000
E. rufescens	....	T.T..C.	00000001	00100
L. guichenoti	....	A.T..T.	000001-0	00006
L. telfairii	.......G..	T.T..T.	10000000	10000
L. nigrofasciolatum	??????????	???????	00000000	00000
M. greyii	....	..T????	00000000	00000
M. ruficauda	.C........	T.T..T.	00000010	00007
N. maccoyii	....	T.?????	00000000	00000
N. coventryi	....	T.T..T.	00000000	00000
N. greeni	....	T.T..T.	00100000	00030
O. lichenigera	.......???	???????	00000000	00000
O. smithi	.........T	T.T..C.	00-10000	00040
P. entrecasteauxii	....	TGT..T.	00000000	00000
P. spenceri	....	T.T..T.	00000000	00000

Complex gap coding character definitions:

Ch1: presence/absence gap 1
Ch2: presence/absence gap 5
Ch3: presence/absence gap 8
Ch4: gap 2 / gap 3 / gap 4 / absence
Ch5: gap 6 / gap 7 / absence

Step matrix ch 4:

	A	2	3	4
A	-	1	1	1
2	1	-	2	2
3	1	1	-	1
4	1	2	2	-

Step matrix ch 5:

	A	6	7
A	-	1	1
6	1	-	1
7	1	1	-

Appendix 3.6 – *Rag-1* alignment of *Eugongylus* group taxa (see table 3.1)

	CCA	CTT	CCT	GAA	TGC	AGC	CAT	ATG	GCT	AAC	CAT	GAT	AAA	GGG	AGA	GTG	GAA	GAC	ACT	GTG	GAC	CAA	GTC
B. trilineata										..A													
C. austrocaledonicus	...	...	...	...	...	...	...	...	...		...	...	...	...	...	...	...	...	...	...	...	...	...
C. longipes	...	...	...	...	G..	...	...	...	.N.	...	.G.	...	...	...	...	...	...	...	...	...	...	..T	...
C. plagiocephalus	...	...	...	...	.A.	...	...	...	...	...	.G.	...	...	.AA	...	.A.	...	...	...	...	...	...	...
C. aenea	...	...	...	...	...	..T	...	G..	...	...	...	...	...	...	.T.	...	...	...	...	...	...	...	...
E. cyanura	...	...	...	..G	...	...	...	G..	...	...	...	...	G..	...	...	...	...	---	---	...	...	...	...
E. nigra	...	...	...	...	...	..T	...	...	...	...	...	..C	...	...	...	.CA	...	..T	G..	...	...	...	...
E. rufescens	A.C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. guichenoti	...	...	...	.G.	G..	...	...	...	..A	G..	...	...	...	.A.	..G	...	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...
M. greyii	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	.T.	..A	...	...	...
M. ruficauda	...	...	...	...	...	...	...	...	...	.M.	...	...	...	---	---	...	...	...	...	...	...	...	...
N. maccoyii	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	..C	.A.	...	...	...	...	..T	...	...
N. coventryi	..T	...	...	...	G..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. greeni	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	G..	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...
O. lichenigera	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O. smithi	...	...	...	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. wahlbergi	...	...	...	...	...	..T	.C.	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	...	...
P. entrecasteauxii	?.C	...	T..	...	...	.T.	..C	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. spenceri	...	...	...	.G.	...	.T.	..C	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	...	...	...	...	C..	...	...	G..	...	...	A..	...	...	...	.A.	...	.CG	...	T..	C.A	...	A..	...
M. longicauda	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
T. scincoides	...	...	...	..C	?..	...	...	...	...	...	...	...	..G	...	.A.	...	.G.	..G	T..	C..	...	A..	.C.

B. trilineata	TTT	GCG	GAA	AAG	GAA	AAT	---	---	---	---	---	---	---	GGT	GTG	GAC	CCA	TTT	CAG	---	---	---	---		
C. austrocaledonicus	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	A..	---	---	---	---		
C. longipes	...	.A.	A..	..A	..T	G..	---	---	---	---	---	---	---	...	...	...	T.C	..A	...	---	---	---	---		
C. plagiocephalus	A..	.A.	...	..A	...	G..	---	---	---	---	---	---	---	..G	...	...	.GC	...	...	---	---	---	---		
C. aenea	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
E. cyanura	...	.A.	.G.	...	...	...	---	---	---	---	---	---	---	...	...	...	..C	...	.T.	---	---	---	---		
E. nigra	...	.A.	...	...	...	G.C	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
E. rufescens	...	.G.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
L. guichenoti	..G	.A.	...	...	A..	G..	---	---	---	---	---	---	---	...	...	...	.TC	...	...	---	---	---	---		
L. nigrofasciolatum	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	..T	..C	...	...	---	---	---	---		
M. greyii	...	CA.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
M. ruficauda	...	.AA	...	...	A..	G..	---	---	---	---	---	---	---	...	A..	...	...	...	...	---	---	---	---		
N. maccoyii	..G	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	..T	..C	.C.	...	---	---	---	---		
N. coventryi	..G	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
N. greeni	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
N. metallicus	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
O. lichenigera	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
O. smithi	...	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
P. wahlbergi	...	AAT	...	...	...	GC.	---	---	---	---	---	---	---	...	...	...	..C	...	...	---	---	---	---		
P. entrecasteauxii	..G	.A.	...	...	...	G..	---	---	---	---	---	---	---	.T.	..A	...	TTT	...	...	---	---	---	---		
P. spenceri	..G	.A.	...	...	...	G..	---	---	---	---	---	---	---	...	.C.	...	..C	...	...	---	---	---	---		
E. skiltonianus	.C.	.A.	A..	G..	...	G.G	GCA	GTG	GGC	TTA	AGG	TCA	CAA	---	---	...	..C	...	...	TTA	GAC	ACA	GAA		
M. longicauda	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???		
T. scincoides	...	.A.	...	C..	...	G.C	GAA	GTG	GCC	TTA	ATG	CCA	CAA	---	---	...	..C	...	...	ATA	GAC	---	---		

B. trilineata	---	AGA	GAC	AAA	GAC	CAG	AAC	AAT	AGT	GTG	GAA	ACT	---	GAT	AAA	GAA	GCC	TTT	CGT	ATG	AAC	CAA	CAA
C. austrocaledonicus	---	...	.T.	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	...	.C.	...	...	.G.
C. longipes	---	...	...	R..	..T	...	...	..C	...	...	...	.G.	---	A..	...	..T	..Y	...	T..	G..	..Y	..G	..T
C. plagiocephalus	---	...	.G.	..C	...	.G.	...	...	...	...	...	..A	---	...	...	..T	...	...	...	...	...	G..	.GG
C. aenea	---	...	.G.	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	...	...	...	...	.G.
E. cyanura	---	...	...	...	...	...	...	...	...	...	...	...	---	...	...	..T	..T	...	...	...	...	...	.G.
E. nigra	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	T..	...	...	...	.G.
E. rufescens	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	A..	...	..T	...	...	...	...	...	...	.G.
L. guichenoti	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	T..	...	..T	...	AG.
L. nigrofasciolatum	---	...	...	...	..T	...	...	...	...	...	...	...	---	...	...	..T	...	...	T..	...	...	...	.G.
M. greyii	---	...	...	...	.T.	YG.	...	...	...	...	...	...	---	...	...	..T	...	...	.A.	..C	...	...	.G.
M. ruficauda	---	...	...	...	...	.GT	...	...	...	...	...	...	---	...	...	..T	A..	...	.A.	G..	...	...	.G.
N. maccoyii	---	...	...	...	..T	.G.	...	...	...	..A	...	...	---	...	C..	..T	..T	...	T..	...	...	...	...
N. coventryi	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	T..	...	...	..G	.C.
N. greeni	---	...	...	...	..T	YG.	..Y	...	...	...	...	...	---	...	...	..T	...	...	T..	...	.R.	...	AG.
N. metallicus	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	T..	...	...	...	AG.
O. lichenigera	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	.A.	...	...	...	.T.
O. smithi	---	...	...	...	..T	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	.A.	...	...	...	GG.
P. wahlbergi	---	.A.	...	...	..A	..A	...	...	T..	A..	...	.T.	---	...	.G.	..T	...	...	T..	...	..T	...	---
P. entrecasteauxii	---	...	...	...	...	.G.	...	...	...	...	...	...	---	...	...	..T	...	...	...	...	...	G..	.G.
P. spenceri	---	...	...	...	...	...	...	...	...	...	...	...	---	...	...	..T	...	...	...	...	...	G..	.G.
E. skiltonianus	CTG	---	---	---	---	---	...	...	C..	...	..G	..G	ATA	...	...	..T	...	...	...	G..	.C.	...	AG.
M. longicauda	???	???	???	???	???	???	???	...	...	...	.C.	..A	ACA	...	...	..T	.T.	...	T..	G..	...	...	AG.
T. scincoides	---	---	ACA	G..	C.G	A.C	..T	.G.	GTG	.CA	AC.	.TA	---	...	...	...	A..	...	.A.	GG.	..T	...	AG.

	GAG	ATT	GAA	GCT	CAC	CAA	GAA	AGG	CTG	CAG	AAC	CTC	TGC	CGG	ATC	TGT	GGA	GGA	TCA	TTT	AAA	GCT	GAC
B. trilineata																							
C. austrocaledonicus	...	...	...	...	...	...	.C.	..A	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
C. longipes	...	...	...	...	...	...	.C.	...	...	...	C..	..G	...	..A	C..	...	..T	...	...	...	...	...	...
C. plagiocephalus	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	...	..C	...	...	...
C. aenea	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	A.A	...	...	...	...	...	...	...	...	...
E. cyanura	...	...	...	...	...	...	.C.	.A.	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
E. nigra	...	...	...	...	...	...	.C.	...	...	...	C.T	...	...	..A	...	...	...	...	...	...	...	...	..G
E. rufescens	...	...	...	...	...	...	.C.	...	...	...	C.T	...	...	..A	...	...	...	...	...	...	...	AA.	...
L. guichenoti	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	...	C..	...	...	...	...	...	...	...	A..
L. nigrofasciolatum	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	C..
M. greyii	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
M. ruficauda	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
N. maccoyii	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	A.A	C..	...	...	...	...	...	...	...	...
N. coventryi	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	C.T	...	...	...	...	...	...	...	...
N. greeni	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	C..	...	...	...	...	...	...	..G	...
N. metallicus	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	C..	...	...	...	...	...	...	..G	...
O. lichenigera	...	...	...	...	...	...	.C.	...	T..	...	C..	...	...	..A	...	...	...	...	...	...	...	A..	...
O. smithi	...	...	...	...	...	...	.C.	...	T..	...	C..	...	...	A.A	...	...	...	...	...	...	...	...	...
P. wahlbergi	...	...	...	..A	...	...	.C.	.A.	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
P. entrecasteauxii	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
P. spenceri	...	...	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	...	...	...	...	...
E. skiltonianus	...	.A.	...	...	...	...	.C.	...	...	...	C..	...	...	..A	...	...	...	...	..T	..G	...	...	A..
M. longicauda	...	.C.	...	...	...	...	.C.	...	...	...	CR.	...	...	..A	...	...	...	...	...	...	...	...	...
T. scincoides	...	.C.	...	..A	...	...	.C.	...	...	...	C.G	...	...	..A	.,.	...	...	...	...	...	...	...	...

	CCT	TGC	AAG	AGA	AGT	CAC	CCA	GTG	CAT	GGA	CCG	GTG	GAT	AAT	GTG	ATG	CAG	GCC	CTA	TTG	AGA	AAG	AAA
B. trilineata																							
C. austrocaledonicus	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	..T	C..	...	...	...
C. longipes	..G	...	..A	...	...	...	...	...	...	...	..R	...	...	...	..T	...	...	...	...	...	...	...	...
C. plagiocephalus	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	..A	...	...	...	...	C.A	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	...	...
E. cyanura	AG.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	..A	...
E. nigra	...	...	G..	...	...	...	...	...	...	...	...	...	...	...	...	...	..A	...	...	C..	...	...	...
E. rufescens	...	...	...	...	...	...	...	...	...	...	..A	...	...	..C	...	...	..A	...	...	...	...	...	...
L. guichenoti	.T.	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	.Y.	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	...	...
M. greyii	...	...	...	...	.A.	...	...	...	...	...	...	...	...	...	...	...	A..	...	T..	C..	...	...	...
M. ruficauda	.G.	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	...	...	...	...
N. maccoyii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	...	C..	...	...	...
N. coventryi	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	..T	...	...	...	...	C..	...	...	...
N. greeni	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	..T	...	...	...	...	C..	...	...	...
N. metallicus	...	...	..A	...	...	..T	...	...	...	...	...	...	...	...	..T	...	...	...	...	C..	...	...	...
O. lichenigera	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	...	...
O. smithi	...	.A.	...	...	...	..T	..G	...	...	...	..A	...	...	...	..C	...	...	...	...	C..	...	...	...
P. wahlbergi	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	...	...
P. entrecasteauxii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	...	...
P. spenceri	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	C..	...	...	...
E. skiltonianus	...	.AT	...	...	..C	...	...	...	..C	...	..A	...	...	...	...	G..	...	...	...	C..	..G	...	...
M. longicauda	...	.AT	...	...	.TC	...	...	...	...	...	..A	...	...	...	..A	...	...	..T	G..	C..	...	...	...
T. scincoides	...	.AT	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	..A	...	...	C..	...	...	...

Appendix 3.6

	GAG	AGA	AGG	GCT	ACA	TCT	TGG	CCA	GAA	CTT	CTT	GCC	AAG	GTC	TTT	AAG	ACT	GAT	GTG	AGA	GGG	GAC	ATT
B. trilineata																							
C. austrocaledonicus	...	...	...	...	...	..A	...	...	...	...	...	...	...	A.T	...	...	.T.	...	...	...	...	...	...
C. longipes	...	...	...	...	...	..A	...	...	...	...	...	...	...	..T	..C	...	.T.	...	...	...	.K.	...	...
C. plagiocephalus	...	...	...	...	...	...	...	...	...	...	...	.T.	...	..T	...	...	.T.	...	...	...	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	.C.	...	...	...	..T	...	...	.T.	...	...	...	...	...	.A.
E. cyanura	...	...	...	...	...	..C	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
E. nigra	...	...	...	...	...	...	...	...	..G	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
E. rufescens	...	.A.	...	...	..G	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
L. guichenoti	...	...	...	.YC	...	...	...	...	...	...	...	A..	...	..T	...	...	.T.	...	...	...	...	...	..G
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	..T	...
M. greyii	...	...	...	.T.	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	..C
M. ruficauda	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.T.	..Y	...	...	...	...	...
N. maccoyii	...	...	...	...	...	...	...	...	...	...	...	A..	...	..T	...	...	.T.	...	...	...	...	...	...
N. coventryi	...	...	.A.	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
N. greeni	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
O. lichenigera	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
O. smithi	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
P. wahlbergi	...	...	.A.	..A	...	..A	...	...	...	...	..C	A.T	...	..T	...	...	.T.	...	..C	...	...	...	...
P. entrecasteauxii	...	...	C..	...	...	...	...	...	...	...	...	...	...	..T	...	...	.TG	...	...	...	...	...	...
P. spenceri	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	.T.	...	...	...	...	...	...
E. skiltonianus	...	.A.	...	...	..C	...	...	...	...	...	..C	T..	...	..T	...	...	.T.	...	...	...	..A	...	...
M. longicauda	...	.A.	...	...	...	...	...	...	...	...	...	...	...	..T	...	..A	.T.	..C	...	...	...	...	..G
T. scincoides	...	.A.	...	...	...	...	...	...	...	...	...	...	...	..G	...	...	.T.	..C	...	...	...	...	...

	GAC	ACA	ATC	CAT	CCT	ACT	AAG	TTT	TGT	CAC	AAC	TGC	TGG	AAC	TTT	ATT	CAG	AGG	AAA	TTC	AGC	AAT	GTC
B. trilineata																							
C. austrocaledonicus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...
C. longipes	...	T..	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	AG.
C. plagiocephalus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. cyanura	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...
E. nigra	..T	...	...	...	..G	...	..A	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...
E. rufescens	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. guichenoti	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
M. greyii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
M. ruficauda	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.A.	...	...
N. maccoyii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.G.	...	...	...
N. coventryi	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. greeni	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O. lichenigera	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
O. smithi	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. wahlbergi	...	...	...	...	...	..A	...	...	...	...	.C.	..T	...	...	...	..C	...	..A	...	...	...	...	...
P. entrecasteauxii	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...	.C.
P. spenceri	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
E. skiltonianus	..T	...	G..	...	...	...	..C	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...	...
M. longicauda	...	...	...	...	...	...	..C	...	...	...	...	...	...	C..	...	...	...	.A.	...	...	...	...	...
T. scincoides	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	..A	...	...

Appendix 3.6

	CCA	AGT	GAA	GTG	TAT	TTT	CCG	AGG	AAT	GGC	CCC	ATG	CAG	TGG	CAT	CCC	CAT	TCG	TCA	GGT	TGT	GAG	GTT
B. trilineata																							
C. austrocaledonicus	...	...	...	...	...	...	..A	...	...	..T	...	...	G..	...	...	...	...	...	...	...	...	...	...
C. longipes	...	...	...	...	...	...	..A	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	..R	...
C. plagiocephalus	...	...	...	...	...	...	..A	...	...	...	..T	.A.	G..	...	...	...	...	...	G..	...	..C	A..	...
C. aenea	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...
E. cyanura	...	...	...	...	...	...	..A	...	...	.A.	...	.A.	G..	...	...	...	...	...	...	...	...	...	...
E. nigra	...	...	...	...	...	...	...	...	..A	T..	...	..C	G..	...	...	.YG	...	...	...	...	...	...	...
E. rufescens	...	...	...	...	...	...	.T.	...	...	...	...	...	G..	...	...	...	...	...	..G	...	...	...	...
L. guichenoti	...	...	...	...	...	...	..A	...	...	...	...	...	G..	...	...	...	...	..A	A..	...	...	..A	...
L. nigrofasciolatum	...	...	..G	...	...	...	...	...	..C	...	...	...	G..	...	...	...	...	..A	...	...	...	...	...
M. greyii	...	...	...	...	...	..C	..A	...	...	...	G.T	...	G..	...	...	...	...	A..	...	...	...	...	...
M. ruficauda	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	...	..Y	...	...	...	...	...	...	...
N. maccoyii	...	...	...	..A	...	...	..A	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...
N. coventryi	...	...	...	...	...	...	..A	...	...	...	...	...	G..	...	..C	...	...	...	...	...	...	...	...
N. greeni	...	...	...	...	...	...	..A	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...
N. metallicus	...	...	...	...	...	...	..A	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...
O. lichenigera	...	...	...	..A	...	...	...	...	...	...	...	...	G..	...	...	...	...	..A	...	...	...	...	...
O. smithi	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	...	...	...	...	...	...	...	...	...
P. wahlbergi	...	T..	...	...	...	...	.T.	...	..A	...	...	.A.	G..	...	TC.	...	...	..A	...	CA.	...	...	...
P. entrecasteauxii	...	...	...	..T	...	...	..A	...	...	...	.T.	...	G..	...	...	...	...	A.A	...	...	...	A..	...
P. spenceri	...	...	...	...	...	...	...	...	...	...	.T.	...	G..	...	...	...	...	A.A	...	...	...	A..	...
E. skiltonianus	..C	T..	...	..A	...	...	..A	...	..A	...	...	.G.	G..	...	...	...	...	..A	...	AAC	...	..T	...
M. longicauda	..C	T..	...	...	...	...	..A	...	..A	...	..T	G..	G..	...	...	...	...	..A	...	A.C	..C	...	...
T. scincoides	.GC	T.C	...	...	...	...	..A	...	..A	...	A..	...	G..	...	...	...	...	..A	...	A.C	..C	...	...

Appendix 3.6

	TGT	GGC	ACT	TCG	GCC	CGT	GGG	GTG	AAG	AGA	AAG	CGG	CAA	GCC	TTA	AAG	CCA	CAA	GTG	AGA	AAA	AAA	CTC
B. trilineata																							
C. austrocaledonicus	...	...	...	...	A..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...
C. longipes	...	...	...	...	..T	...	..K	...	...	...	...	...	...	.T.	...	...	..T	...	...	..C	...	...	...
C. plagiocephalus	...	...	...	.T.	..T	...	...	..A	...	...	...	...	...	.M.	...	...	...	...	...	..C	...	...	...
C. aenea	...	...	...	...	...	...	...	...	...	...	...	...	...	.T.	...	...	...	...	.C.	..C	...	...	...
E. cyanura	...	...	...	.T.	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	A..	..C	...	...	..T
E. nigra	...	...	...	.T.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...
E. rufescens	...	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	..C	...	...	...
L. guichenoti	...	...	...	.T.	.T.	...	...	...	...	...	...	..T	...	.T.	...	...	..T	...	...	..C	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G..	...	..G	...	...	..C	...	...	...
M. greyii	...	...	...	.T.	...	T..	...	...	...	...	...	..T	A..	...	...	...	...	...	..A	..C	...	...	...
M. ruficauda	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...
N. maccoyii	...	...	...	...	...	...	...	...	...	...	...	...	...	.T.	...	...	..K	...	...	..C	...	...	...
N. coventryi	...	...	...	.T.	...	...	...	...	...	...	...	...	...	.T.	...	...	..T	...	...	..C	...	...	...
N. greeni	...	...	..C	.T.	...	...	...	...	...	...	...	...	...	.T.	...	...	..T	...	...	..C	...	...	...
N. metallicus	...	...	..C	...	...	...	...	...	...	...	...	...	...	.T.	...	...	..T	...	...	..C	...	...	...
O. lichenigera	...	...	...	.T.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..A	..C	...	...	...
O. smithi	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...
P. wahlbergi	...	..A	...	...	...	TA.	..A	...	...	...	..A	...	...	...	..G	...	...	...	...	..C	...	...	A..
P. entrecasteauxii	...	...	...	.T.	...	.R.	...	...	...	...	...	..A	...	...	...	...	...	...	...	..C	...	...	...
P. spenceri	...	...	...	...	...	...	...	...	...	...	...	..A	...	...	...	..T	...	...	...	..C	...	...	...
E. skiltonianus	..C	...	...	.TC	T..	T..	...	..C	...	...	...	AA.	...	...	C.G	..T	...	.C.	...	..C	...	...	...
M. longicauda	...	...	...	.T.	T..	...	...	..C	...	...	...	...	...	...	C.G	..T	...	...	...	..C	...	...	...
T. scincoides	..C	...	...	...	T..	..C	..A	..C	...	...	...	T..	...	...	C.G	..T	...	...	...	..C	...	...	...

Appendix 3.6

	AGG	ATC	ATG	GCA	AGA	TGT	GCT	AGC	CAA	ATA	AGG	CCA	GTA	GGG	AGC	TCC	CAA	AAA	GTG	CAC	AGC	AAA	AAC
B. trilineata																							
C. austrocaledonicus	...	...	G..	...	...	...	...	...	...	G..	...	...	...	A..	...	C..	A..	...	...	...	...	...	...
C. longipes	...	...	G..	..G	...	...	...	...	...	.G.	..A	...	A.G	A..	...	CT.	.C.	..R	...	...	...	...	..T
C. plagiocephalus	...	...	G..	...	...	...	...	...	...	...	...	...	...	A..	...	...	.C.	...	...	.T.	...	...	...
C. aenea	...	...	G..	..G	...	C..	...	...	...	...	...	...	...	A..	...	C..	...	...	...	...	...	...	...
E. cyanura	...	..T	G..	..G	...	G..	.G.	...	...	...	...	...	...	A..	G..	C..	...	...	..C	...	...	...	...
E. nigra	...	..T	G..	..G	...	...	...	.A.	...	...	...	...	...	A..	...	C..	A.G	...	...	...	...	...	..T
E. rufescens	...	..T	G..	..G	...	...	...	...	...	...	...	...	...	A..	...	C..	A..	...	...	...	...	...	..T
L. guichenoti	C.A	...	G..	..G	...	...	...	G..	...	.G.	...	...	...	A..	...	C..	.C.	...	..A	.C.	.A.	...	...
L. nigrofasciolatum	...	...	G..	..G	...	...	...	...	...	...	...	...	...	A..	...	C..	.G.	...	...	...	...	...	...
M. greyii	...	...	...	..G	..C	...	...	...	...	.A.	...	...	...	A..	...	C..	.C.	...	...	..A	...	...	..G
M. ruficauda	...	...	GY.	A..	...	...	.Y.	...	...	...	...	...	...	..N	...	...	...	...	...	...	...	...	...
N. maccoyii	...	...	G..	..R	...	...	...	...	...	...	...	...	...	A..	..T	C..	...	...	...	...	...	...	...
N. coventryi	...	...	G..	..G	...	...	...	...	...	...	...	...	...	A..	...	C..	.C.	...	...	...	..T	...	...
N. greeni	...	...	G..	..G	.A.	...	...	...	...	...	...	...	...	A..	...	C..	.C.	...	..A	..T	...	...	...
N. metallicus	...	...	G..	..G	.A.	...	...	...	...	...	...	...	...	A..	...	C..	...	...	..A	..T	...	...	...
O. lichenigera	...	G.T	G..	..G	...	C..	...	...	...	...	...	...	...	A..	...	C..	...	...	...	...	...	...	...
O. smithi	...	...	G..	..G	...	C..	...	...	...	...	...	...	...	A..	...	C..	...	...	...	...	...	...	...
P. wahlbergi	...	..T	G..	...	...	...	.A.	.A.	...	...	.AA	...	...	AA.	...	CA.	A..	...	...	...	...	...	...
P. entrecasteauxii	...	...	...	..G	...	...	...	..T	...	...	.A.	...	...	A..	...	C..	.C.	...	...	...	...	...	...
P. spenceri	...	...	...	.YG	...	...	...	..T	...	...	.A.	...	...	A..	..T	C..	TC.	...	...	...	...	...	...
E. skiltonianus	...	...	..T	..G	...	CA.	..G	..A	...	...	..A	.A.	..C	A.A	.A.	C..	A..	C..	A..	A..	..T	.G.	.GT
M. longicauda	...	...	...	A.G	...	CA.	...	..A	...	...	..A	...	..C	A..	.C.	C..	A..	C..	...	A..	...	...	.G.
T. scincoides	...	...	.C.	..G	...	CC.	...	..A	...	...	...	..G	...	A..	...	C..	AG.	C..	...	A..	...	...	.G.

B. trilineata	CTC	ATG	AAG	AAG	ATT	GTC	AGC	TGC	AAC	AAG	ATA	CAT	CTC	AGC	ACA	AAA	AGC	TTT	GTG	GTG	GAC	TAT	CCT
C. austrocaledonicus	...	...	...	...	...	...	.A.	...	...	...	...	...	G..	...	...	...	...	...	.C.	...	...	...	...
C. longipes	...	...	...	...	...	...	.A.	...	...	..A	...	...	...	...	..C	...	.A.	...	.CT	...	...	...	...
C. plagiocephalus	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	...	...	...	.C.	...	...	...	...
C. aenea	...	...	...	...	...	...	.A.	...	...	...	...	...	A..	...	...	...	..G	...	.C.	...	..T	...	...
E. cyanura	T..	...	...	...	...	...	.A.	..T	...	...	...	...	...	...	...	...	.A.	...	.CA	...	...	...	...
E. nigra	...	...	...	...	...	...	.A.	...	...	...	...	...	A..	...	...	...	..A	...	.CA	...	...	...	...
E. rufescens	G..	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
L. guichenoti	...	...	...	...	...	.C.	.AT	...	...	..A	..T	...	...	...	...	...	...	...	.C.	T..	...	...	...
L. nigrofasciolatum	...	...	...	...	...	...	.A.	...	...	...	...	...	G..	...	...	...	...	...	.C.	...	...	...	...
M. greyii	...	...	...	...	...	...	.A.	...	...	...	...	..C	...	...	...	G..	...	...	.C.	...	...	...	...
M. ruficauda	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	...	...	...	.C.	...	...	...	...
N. maccoyii	...	...	...	...	...	...	.A.	...	G..	..A	...	...	...	...	...	...	...	...	.C.	...	...	...	...
N. coventryi	...	...	...	...	...	...	.A.	...	...	..A	...	...	...	...	...	...	..T	...	.C.	...	...	...	...
N. greeni	...	...	...	...	...	...	.A.	...	...	.GA	...	...	...	...	...	...	...	..W	.C.	...	...	...	...
N. metallicus	...	...	...	...	...	...	.A.	...	...	.GA	...	...	...	...	...	...	...	...	.C.	...	...	...	...
O. lichenigera	...	...	...	...	...	...	.A.	...	...	...	...	...	A..	...	...	...	...	...	.C.	...	...	...	...
O. smithi	...	...	...	...	...	...	.A.	...	...	...	...	...	A..	...	...	...	..G	...	.C.	...	..T	...	...
P. wahlbergi	...	...	...	...	...	...	.A.	...	..T	...	...	...	...	...	...	...	..T	...	.CA	..T	...	...	..G
P. entrecasteauxii	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	...	...	...	.C.	...	..T	...	...
P. spenceri	...	...	...	...	...	...	.A.	...	...	...	...	...	...	...	...	T..	...	...	.C.	...	...	...	...
E. skiltonianus	..T	...	..A	...	...	...	.A.	...	..G	...	...	...	...	..T	...	..G	...	C..	.C.	...	...	...	...
M. longicauda	..T	...	..A	...	...	...	.A.	...	C..	...	...	...	...	...	...	...	GA.	...	.C.	...	...	...	...
T. scincoides	...	...	..A	...	...	...	.AT	...	...	...	...	...	...	...	...	..G	.A.	...	.C.	..A	...	...	...

Appendix 3.6

	GCA	GAC	TTT	GTG	AAA	GCT	GTC	TCT	TGC	CAG	ATC	TGT	GAG	CAT	GTC	CTG	GCA	GAC	CCA	GTA	GAG	ACC	ACG
B. trilineata																							
C. austrocaledonicus	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
C. longipes	...	...	..C	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	..G	..G	...	...	..A
C. plagiocephalus	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	..T	...	...	...	..A
C. aenea	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
E. cyanura	...	...	...	...	...	..C	...	...	...	...	...	...	..A	..C	A..	...	...	...	...	..G	...	...	..A
E. nigra	...	...	...	...	...	..C	...	...	...	.G.	...	...	...	..C	...	...	...	...	...	...	...	..T	..A
E. rufescens	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	A..	...	...	...	...	...	...	...	..A
L. guichenoti	...	...	...	...	...	..C	...	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	..A
L. nigrofasciolatum	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
M. greyii	...	...	...	...	..G	...	...	...	...	...	...	...	...	...	...	T..	...	...	..T	...	...	...	..A
M. ruficauda	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
N. maccoyii	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	A..	...	...	...	...	...	...	...	..A
N. coventryi	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.TA
N. greeni	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
N. metallicus	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
O. lichenigera	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
O. smithi	...	...	...	...	...	..C	...	...	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	..A
P. wahlbergi	.TG	...	...	...	...	AG.	...	...	...	...	...	...	...	..C	A..	T..	...	...	...	..T	...	...	..A
P. entrecasteauxii	...	...	...	...	...	..C	...	...	...	...	...	...	..A	..C	A..	...	...	...	...	...	...	...	..A
P. spenceri	...	...	...	...	...	..C	...	...	...	...	...	...	..A	..C	A..	...	...	...	...	...	...	...	..A
E. skiltonianus	...	A..	...	...	...	T.G	..T	...	...	...	...	...	...	..C	A..	...	...	...	...	...	..A	..A	..A
M. longicauda	...	C..	...	.??	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???
T. scincoides	...	...	...	...	...	..A	...	...	...	...	...	...	...	..C	A..	...	...	...	..T	...	...	...	...

Appendix 3.6

															Simple/Complex gaps
															123456789
B. trilineata	TGC	AAG	CAC	TTA	TTY	TGC	AAA	GTT	TGC	ATC	CTG	AAA	TGC	CTC	001010010
C. austrocaledonicus	...	...	...	...	..C	...	.G.	...	...	G..	...	???	???	???	001010010
C. longipes	...	...	...	...	..C	...	.G.	...	...	..T	.CT	G..	ATG	.CT	001010010
C. plagiocephalus	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
C. aenea	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
E. cyanura	...	...	...	...	..C	...	.G.	...	...	G.T	.CT	G..	ATG	TC?	011010010
E. nigra	..T	...	...	...	..C	...	.G.	...	...	...	..?	???	???	???	001010010
E. rufescens	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
L. guichenoti	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	..G	001010010
L. nigrofasciolatum	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
M. greyii	...	..A	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
M. ruficauda	...	...	...	...	..C	...	...	..G	...	...	...	...	...	..T	101010010
N. maccoyii	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
N. coventryi	...	...	..T	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
N. greeni	...	...	..T	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
N. metallicus	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	.??	001010010
O. lichenigera	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
O. smithi	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010010
P. wahlbergi	...	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	001010011
P. entrecasteauxii	...	...	...	...	..C	...	.G.	.C.	...	...	T..	...	...	...	001010010
P. spenceri	...	...	...	...	..C	...	.G.	...	...	...	T..	...	...	...	001010010
E. skiltonianus	...	...	...	G.G	..C	...	.G.	A..	...	...	..T	...	...	..T	000100100
M. longicauda	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???????00
T. scincoides	..T	...	...	...	..C	...	.G.	...	...	...	...	...	...	...	000101010

Appendix 4 – *Pseudemoia* alignments

4.1 - Allozymes 199

4.2 – *ND4* alignment 201

4.3 – tRNA His and Ser alignment 205

4.4 - *β-fibrinogen* alignment 206

4.5 - *Myh-2* alignment 213

Appendix 4.1 – Allozyme characters for *Pseudemoia* species (see Chapter 4, and table 4.1)

	aat-2	acoh-1	acoh-2	ada	ak-1	ak-2	alb	eno	est	gda	g3pdh	gpi	hbdh	idh-1	idh-2	lap
P. baudini 22957	2, 3	3	34	25	2	2	2	2	2, 4	2, 3	1, 4	2	4, 6	1	2	2
P. baudini 22964	2, 3	3	34	25	2	2	2	2	2, 4	23	1, 4	2	4, 6	1	2	2
P. cryodroma 40890	3	3	34	3, 4, 5	2	2	2	2	2, 4	3	4	2	4, 5	1, 3	2	2
P. cryodroma 61886	3	3	2, 3, 4	1, 3, 5	2	2	2	2	4	3	4	2	4, 6	1	2	2
P. entrecasteauxii 40981	3	3	34	5	2	2	2	2	4	3	4	2, 4	4	1	2	2
P. entrecasteauxii 61836	3	3	4	5	2	2	2	2	4, 7	3	4	2	4	1	2	2
P. entrecasteauxii 23023	1, 3	3	2, 3, 4	7	2	2	2	2	2, 4	3	4	2	4	1	2	2
P. pagenstecheri 45268	3	3	3, 4	3	2	2	2	2	4	3	4	2	4	1	1, 2	2
P. pagenstecheri 112313	3	3	3, 4	3	?	?	2	2	4	?	4	2	4	1	?	2
P. rawlinsoni 49585	3	2	3, 4, 5	6	2	2	2	2	1	3	1	2	3	1	2	2
P. rawlinsoni 59590	3	2	3, 4, 5	6	2	2	2	2	1	3	1	2	3	1	2	2
P. spenceri 104773	3	3	2	3	3	2	2	2	4	3	1, 4	4	2	1, 3	2	2
P. spenceri 104774	3	3	2	3	3	2	2	2	4	3	1, 4	4	2	1, 3	2	2
N. metallicus	2	1	3	5, 6	3	2	3	2	1, 2	3	4	2	2, 4	3	2	2

Appendix 4.1

	lgl	mdh-1	mdh-2	mpi	pepa	pepb	pepd	pgam	pgdh	pgk	pgm-1	pgm-2	pnp	sod	tpi
P. baudini 22957	1	3	1	2	4	4, 5	6, 7	2	3	1	5	2	12	1	2
P. baudini 22964	1	3	1	2	4	4, 5	6, 7	2	3	1	5	2	12	1	2
P. cryodroma 40890	2	2	1	3, 5	2, 4	4, 5	7	2	3	1	5, 6	2	2	2	1, 5
P. cryodroma 61886	2	1, 2	1	3, 5	4	4	7	2	3	1	6	2	2	2	1
P. entrecasteauxii 40981	2	2	1	3	4	4	7	2	3	1	5	2	2	2	3, 7
P. entrecasteauxii 61836	2	1, 2	2, 3	4	4	7	2	3	1	5, 6	2	2	2	2	?
P. entrecasteauxii 23023	2	2	1	2, 3	4	4	7, 8	2	2, 3, 5	1	3	2, 4	2	2	2
P. pagenstecheri 45268	1	2	1	3	4	4	7	2	3, 4	1	2, 3, 5	2	2	1	2
P. pagenstecheri 112313	1	2	1	2, 3	4, 5	4	?	2	3	1	?	2, 3	2	1	1
P. rawlinsoni 49585	1	3	1	2	4	4, 5	1, 2, 5, 6	2	3	1	5	2	2	1	2
P. rawlinsoni 59590	1	3	1	2	4	4, 5	1, 2, 5, 6	2	3	1	5	2	2	1	2
P. spenceri 104773	2	3	1	3	4	1	7	1, 2	3	1	6	2	2	2	7
P. spenceri 104774	2	3	1	3	4	1	7	12	3	1	6	2	2	2	7
N. metallicus	2	4	1	3, 4	3	4	7, 8	2	3	3	1, 3	2	3, 4	2	8

Appendix 4.2: *Pseudemoia* ND4 alignment (see table 4.1)

```
P. baudini 22957          GCC GCA ATC TTA CTA AAA CTC GGA GGA TAT GGA ATT ATC CGT ATT ACC CCA ATT CTC AAC CCC ATT TCA
P. baudini 22964          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ...
P. cryodroma 40890        ... ... ... C.G ..G ... ..T ... ... ..C ... ... ... ..C ..C T.. .SG ..C ... ... ..A ... ...
P. cryodroma 61886        ... ... ... C.G ... ... ..T ... ..G ..C ... ... ... ..C ..C T.. ..G ..C ... ... ..A ... ...
P. entrecasteauxii 40981  ... ... G.. ..G T.. ... ..T ... ... ... ... ..C ... ..C ..C T.T ... ..C ... ... ..A ..C ...
P. entrecasteauxii 61836  ??? ??? ??? ??? T.. ... A.T ... ... ... ..G ..C ... ..C ..C T.T ... ..C ... ... ..A ..C ...
P. entrecasteauxii 23023  ... ... ... C.. ..G ... ..T ..T ... ..C ..G ..C ... ..C ... T.T ... ..C ... ... ..A ..C ...
P. pagenstecheri 45268    ... ... ... C.. T.. ... ... ..C ... ..C ... ... ... ..C ..C T.. ... ..C ..A ... ..A ..C ...
P. pagenstecheri 112313   ... ... ... C.. T.. ... ... ..C ... ... ... ... ... ..C ..C T.. ..C ..C ... ... ... ... ...
P. rawlinson 49585        ... ... ... C.. ... ... ..T ..G ... ... ... ... ... ..C ... ... ..G ... ... ... ... ... ...
P. rawlinson 49590        ... ... ... C.. ... ... ..T ..G ... ... ... ... ... ..C ... ... ..G ... ... ... ... ... ...
P. spenceri 104773        ..A ... ... ... ... ..G ... ... ... ..C ..G ... ... ... ..A T.G ..G ..C ... ... ..A C.. ...
P. spenceri 104774        ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ?.. ...
B. duperreyi              ..A ... ... C.. ... ... ..T ..C ..T ..C ..G ..C ..T ..C ... ... ..G ..A ..A ... ..A ..C ...
M. ruficauda              ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ???
L. guichenoti             ..A ... ..T C.. ..C ... ..T ... ... ... ..G ..C ..T ..C ..C ... ..T ..A ..T ... ..A ... ...
N. metallicus             ... ..C G.A C.. T.. ... ..T ..C ... ... ..C ... ..T ..C ... ..A ..C ..A ..A ... ..A GCC ...

P. baudini 22957          ACA AAA CTC TAC TAC CCA TTT ATT ATG TTA GCT ATC TGG GGC ATC CTT ATA ACA AGC TCC ATC TGC TTG
P. baudini 22964          ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
P. cryodroma 40890        ... ... A.. ... ... ... ... ... ... ... ..C ... ... ... ... A.. ... ... ... ... ... ..T ..A
P. cryodroma 61886        ... ... A.. ... ... ... ... ... ... ... ..C ... ... ... ... G.. ... ... ... ... ... ..T ..A
P. entrecasteauxii 40981  ... ... A.T ..T ... ..G ... ... ..A C.. ..C ... ... ..T ..T A.. ... ... ... ... ... ... C.A
P. entrecasteauxii 61836  ... ... A.T ..T ... ..G ... ..C ..A C.. ..C ... ... ..T ..T A.. ... ... ... .GT ... ... C.A
P. entrecasteauxii 23023  ... ... A.T ..T ... ... ... ..C ..A C.. ..C ... ..A ... ... A.. ... ... ... ... ... ... C.A
P. pagenstecheri 45268    ... ... A.T ... ... ... ... ... ... C.. ..C ..T ..A ... ... A.C ... ... ... ... ... ... ..A
P. pagenstecheri 112313   ... ... A.A ... ... ... ... ... ... C.. ..C ... ..A ... ... A.. ... ... ... ... ... ... ..A
P. rawlinson 49585        ... ... ... ... ... ..C ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ..A
P. rawlinson 49590        ... ... ... ... ... ..C ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ... ..A
P. spenceri 104773        ... ... A.T ... ... ... ... ... ... C.C ..C ... ..A ... ..T A.C ... ... ... ... ... ... ..A
P. spenceri 104774        ... ... A.T ... ... ... ... ... ... C.C ..C ... ..A ... ..T A.C ... ... ... ... ... ... ...
B. duperreyi              ... ... ..T ..T ... ..C ..C ..C ..A C.. ... GC. ..A ..A ..T A.. ... ... ... ... ... ... ...
M. ruficauda              ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ??? ..A
L. guichenoti             ... ... ..G ..T ..T ..G ..C ... ..C C.. ..C ... ..A ..A ..T A.. ... ... ... ... ... ... C.A
N. metallicus             ... ... ..A ..T ..T ... ... ... ..T ... ... ... ..A ..T ..T A.. ... ... ... ... ... ..T C.T
```

Appendix 4.2

	CGA	CAA	ACA	GAC	CTG	AAG	TCC	CTA	ATC	GCC	TAC	TCA	TCA	GTA	AGC	CAC	ATG	GGC	CTT	GTT	ATC	GCA	GCA
P. baudini 22957																							
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. cryodroma 40890	...	..G	...	...	...	...	...	...	...	...	...	...	..C	..C	...	...	..A	..A	...	...	...	...	...
P. cryodroma 61886	...	..G	G..	...	...	..A	...	...	...	...	...	...	..C	..C	...	...	..A	..A	...	...	...	...	...
P. entrecasteauxii 40981	...	...	...	...	..A	..A	...	...	...	..T	...	..G	..C	..C	...	...	...	...	...	...	..T	...	...
P. entrecasteauxii 61836	...	...	...	...	..A	..A	...	T..	...	..T	...	...	..C	..C	...	...	...	...	...	...	..T	...	...
P. entrecasteauxii 23023	...	...	...	...	..A	..A	..T	...	...	..T	...	...	..C	..C	...	...	...	...	...	...	..T	...	...
P. pagenstecheri 45268	...	...	...	...	...	..A	...	...	...	...	...	...	..C	..T	...	...	..A	...	...	...	...	...	...
P. pagenstecheri 112313	...	...	...	...	..A	..A	..T	T.G	...	...	...	...	...	..C	..T	...	..A	...	...	...	...	...	...
P. rawlinson 49585	...	...	...	...	...	..A	...	...	...	...	...	...	...	..C	...	..T	..A	...	...	...	...	...	...
P. rawlinson 49590	...	...	...	...	...	...	...	...	...	...	...	...	...	..C	...	..T	..A	...	...	...	..T	...	...
P. spenceri 104773	...	...	...	...	..A	..A	...	...	...	...	...	...	...	..C	...	..T	..A	...	...	...	..T	...	...
P. spenceri 104774	...	...	...	...	..A	..A	...	...	...	...	..T	...	..C	..C	...	...	..A	...	...	...	...	...	...
B. duperreyi	...	...	...	...	..A	..A	..A	...	...	..T	...	...	..C	..C	...	...	..A	...	...	...	...	...	...
M. ruficauda	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	T.A	..A	..T	...	..T
L. guichenoti	...	...	...	...	..A	...	G.A	T..	...	...	...	...	...	..G	...	...	..A	...	...	...	...	A.T	..C
N. metallicus	...	...	...	...	..A	..A	..A	...	...	..T	...	..C	...	...	...	...	..A	..A	..A	...	..T	...	T..

	ACC	TTA	ATC	CAA	ACC	CCA	TGA	AGC	CTG	ACC	GGA	GCA	ATA	ACA	CTA	ATA	ATT	GCC	CAC	GGC	CTT	ACA	TCC
P. baudini 22957																							
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. cryodroma 40890	...	C..	..T	...	...	...	...	..T	..A	...	..C	...	...	...	..G	...	G.C	...	..T	..T	...	...	...
P. cryodroma 61886	...	C..	..T	...	...	...	...	..T	..A	...	..C	...	...	...	..G	...	G..	...	..T	..T	...	...	...
P. entrecasteauxii 40981	..T	...	...	...	...	...	...	...	..A	...	..C	...	...	...	...	...	..C	...	...	...	..C	..G	...
P. entrecasteauxii 61836	..T	C..	...	...	...	...	...	...	..A	...	..C	...	...	...	Y..	...	..C	...	...	...	..C	..G	...
P. entrecasteauxii 23023	..T	...	...	...	...	...	...	...	..A	...	..C	...	...	...	...	...	...	...	...	...	..C	..G	...
P. pagenstecheri 45268	...	C..	...	...	...	...	...	..T	..A	...	..T	...	...	..G	...	...	..C	...	...	...	..C	..G	...
P. pagenstecheri 112313	...	A..	...	...	...	...	...	...	..A	...	..C	...	...	G..	...	...	...	...	...	..T	..C	...	...
P. rawlinson 49585	...	C..	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. rawlinson 49590	...	C..	...	...	...	...	...	...	..A	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. spenceri 104773	G..	C..	...	...	...	...	...	..T	T.A	...	..C	...	...	...	...	...	..C	...	...	...	..C	...	...
P. spenceri 104774	G..	C..	...	...	...	...	...	..T	T.A	...	..C	...	...	...	...	...	..C	...	...	...	..C	...	...
B. duperreyi	G.A	...	..T	...	...	...	...	...	...	..A	..G	...	...	...	...	...	..C	...	...	...	..C	...	...
M. ruficauda	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	..C	...	..T
L. guichenoti	...	C..	..T	...	...	..T	..G	G..	...	..A	..C	...	...	G..	...	...	..C	...	..T	..A	T.A	..T	...
N. metallicus	...	C.T	..T	...	...	...	...	G..	...	...	..C	T..	..C	GTT	...	...	...	...	...	..T	T.A	...	..A

Appendix 4.2

P. baudini 22957	TCC	GTC	CTC	TTC	TGC	CTA	GCA	AAC	ACA	AAT	TAT	GAA	CGA	ACC	CAT	AGC	CGA	ACT	ATG	CTG	CTT	GCA	CGA
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. cryodroma 40890	...	..T	...	..T	..T	..G	...	...	...	..C	..C	S.G	...	..A	Y.C	...	...	..A	..A	..A	..C	...	...
P. cryodroma 61886	...	..T	...	..T	..T	..G	...	...	...	..C	..C	..G	...	..A	..C	...	...	..A	...	..A	..C	...	...
P. entrecasteauxii 40981	...	..T	..A	...	...	T..	...	...	...	..C	...	...	...	..A	..C	...	...	..A	..A	...	...	...	...
P. entrecasteauxii 61836	...	..T	..A	...	..T	T..	...	...	...	..C	...	...	...	..A	..C	...	...	..A	..A	...	...	...	...
P. entrecasteauxii 23023	...	..T	...	...	..T	T..	...	...	...	..C	...	...	...	..A	..C	...	...	..A	..A	...	...	...	...
P. pagenstecheri 45268	...	..T	...	...	...	...	...	...	...	..C	...	..G	...	..G	..C	...	...	..A	..A	...	...	...	...
P. pagenstecheri 112313	...	..T	..A	...	..T	...	...	...	...	..C	...	...	...	..A	..C	...	...	..A	..A	...	...	...	...
P. rawlinson 49585	...	...	...	...	...	T..	...	...	...	...	...	...	...	...	...	...	...	..C	..A	...	...	...	...
P. rawlinson 49590	...	...	...	...	...	T..	...	...	...	...	...	...	...	...	...	...	...	..C	..A	...	...	...	...
P. spenceri 104773	...	..T	..T	...	...	T.G	T..	..T	...	..C	...	...	...	...	...	...	...	..C	..A	...	...	...	...
P. spenceri 104774	...	..T	..T	..S	...	T.G	T..	..T	...	..C	...	...	...	...	...	...	...	..C	..A	A..	...	...	...
B. duperreyi	...	A.A	..A	...	..T	...	...	..T	..C	..C	..C	...	...	...	...	...	...	..C	..A	A..	...	...	...
M. ruficauda	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	?..	..C
L. guichenoti	..T	.CA	..A	..T	...	...	...	...	...	..C	..C	...	..C	..A	..C	...	...	..C	..A	..A	..A	A..	..C
N. metallicus	...	ACA	..T	...	...	...	...	...	..C	..C	...	...	...	..A	..C	...	...	..C	..A	..T	..A	AT.	...

P. baudini 22957	GGC	TTA	CAG	CTT	GTT	CTC	CCC	CTG	ATA	ACA	ACA	TGA	TGA	CTA	CTA	GCC	AAC	CTA	ATA	AAC	ATA	GCC	CTA
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. cryodroma 40890	...	C..	..A	..A	.C.	...	...	T.A	...	...	...	...	..G	..C	...	..A	...	...	...	...	..G	...	..G
P. cryodroma 61886	...	C..	..A	..A	...	...	...	T.A	...	...	...	...	...	..C	...	..A	...	...	...	...	..G	...	..G
P. entrecasteauxii 40981	...	C..	..A	..C	ACC	..T	...	...	...	...	...	...	...	...	...	..A	...	..T	...	...	...	...	...
P. entrecasteauxii 61836	..T	C..	..A	..C	A.C	..T	..T	...	...	...	...	...	...	...	...	..A	...	...	...	...	...	...	...
P. entrecasteauxii 23023	...	C..	..A	..C	...	..T	...	...	...	...	...	...	..G	...	...	..A	...	...	...	...	...	...	...
P. pagenstecheri 45268	...	C..	..A	..A	..C	...	...	..A	...	...	...	..G	..G	..T	...	..A	...	..G	...	..T	...	...	..G
P. pagenstecheri 112313	...	C.G	..A	..A	..A	...	...	..A	...	...	...	...	...	..C	...	..A	...	...	...	...	...	...	..G
P. rawlinson 49585	...	...	...	...	A.C	...	..T	..A	...	...	G..	...	...	T..	T..	...	...	...	...	...	...	...	..C
P. rawlinson 49590	...	...	...	...	A.C	...	..T	..A	...	...	...	...	...	T..	T..	...	...	...	...	...	...	...	..C
P. spenceri 104773	...	C..	...	..C	A.C	..G	...	..A	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...
P. spenceri 104774	...	C..	...	..C	A.C	..G	...	..A	...	...	...	...	...	..C	...	...	...	...	...	...	...	...	...
B. duperreyi	...	C..	..A	T.A	A.C	T.A	...	..A	..G	..C	...	...	...	..G	...	..A	...	...	...	..T	...	..A	..T
M. ruficauda	...	C..	...	...	A.C	T.A	..A	..A	...	...	...	...	...	T..	...	..T	..T	...	...	...	...	..A	...
L. guichenoti	..T	C.C	..A	..A	A..	...	..T	..A	...	..C	G..	...	...	...	T..	ATT	...	..T	...	...	...	..T	..C
N. metallicus	..A	C..	..A	T.A	A.C	..A	...	..A	...	..T	...	...	..G	..C	T..	ATT	..T	T..	...	...	..G	...	...

Appendix 4.2

	CCA	CCA	ACA	ATC	AAC	CTA	GCA	GGA	GAA	CTT	TTA	ATT	ATC	ACC	TCC	CTA	TTT	GAC	TGA	TCA	CCA	ACA	ACC
P. baudini 22957																							
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. cryodroma 40890	..C	...	...	...	..Y	T..	A..	...	...	..C	...	...	...	..A	...	...	...	...	...	...	...	.T.	...
P. cryodroma 61886	..C	...	...	...	...	T..	A..	...	...	..C	...	...	...	..A	...	...	...	...	...	...	...	.T.	...
P. entrecasteauxii 40981	...	...	...	...	..T	T..	A..	..G	..G	...	...	..C	...	..A	...	...	...	...	...	...	...	.T.	...
P. entrecasteauxii 61836	...	...	...	...	..T	T..	A..	..G	..G	...	...	..C	...	..A	...	...	...	...	...	...	...	.T.	...
P. entrecasteauxii 23023	...	..G	...	...	...	T.G	A.G	..G	..G	...	...	..C	..T	..A	...	...	...	...	..G	...	...	.T.	...
P. pagenstecheri 45268	..C	...	...	..T	..T	T..	..C	..G	...	...	C..	...	..T	..A	...	...	...	A..	...	...	...	.T.	...
P. pagenstecheri 112313	..C	...	...	...	...	T..	A..	..T	...	..C	...	...	...	..A	...	...	...	...	...	...	...	.T.	...
P. rawlinson 49585	...	...	...	..T	...	...	AT.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.T.	...
P. rawlinson 49590	...	...	...	..T	...	Y..	AT.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.T.	...
P. spenceri 104773	...	...	...	...	...	..G	A..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.T.	...
P. spenceri 104774	...	...	...	...	...	..G	A..	...	...	...	...	...	...	...	...	T..	..C	...	...	...	..T	G..	...
B. duperreyi	...	...	...	..T	...	T..	A..	...	...	..C	...	..C	..T	T.A	..A	T..	..C	...	...	...	..T	G..	...
M. ruficauda	..T	...	...	...	...	..G	...	...	...	..C	A.G	..C	..A	..A	G..	...	...	A.T	...	..C	...	CT.	..T
L. guichenoti	...	...	...	...	...	..G	AT.	..G	...	...	..T	...	..T	..A	...	GC.	..C	A..	...	..T	TAC	.TT	..A
N. metallicus	...	..C	...	..T	...	...	AT.	...	...	..A	...	..C	..T	...	...	...	.AC	A..	...	..C	T..	CT.	..A

	ATC	ATC	ATA	ACA	GGC	CTG	GGC	ACC	CTA	CTA	ACA	GCA	ACT	TAC	TCT	TTA	TTT	ATG	TTT	CTA	ACC	ACA	CAG
P. baudini 22957																							
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
P. cryodroma 40890	...	..T	...	..T	..G	...	..G	...	S..	...	...	...	..C	...	..A	C..	CAC	..A	..C	..G	..T	...	..A
P. cryodroma 61886	...	..T	...	..T	..G	...	..G	...	...	...	...	...	..C	...	..A	C..	CAC	..A	..C	..G	..T	...	..A
P. entrecasteauxii 40981	...	..T	...	...	..A	T.A	..A	...	...	...	...	...	..C	..T	..A	..G	.AC	..A	...	..G	...	..T	..A
P. entrecasteauxii 61836	...	..T	...	...	..A	..A	..A	...	..G	...	...	...	..C	...	..A	..G	.AC	..A	...	..G	...	..T	..A
P. entrecasteauxii 23023	...	G.T	...	...	..A	T.A	..A	...	...	..C	...	...	...	...	..A	..G	CA.	..A	...	..G	...	..T	..A
P. pagenstecheri 45268	...	..T	...	..T	..G	...	..A	...	T..	...	...	...	G..	...	..A	C.C	.AC	..A	..C	...	...	...	..A
P. pagenstecheri 112313	C..	..T	...	...	..G	..T	..A	...	...	...	...	...	G..	..T	..A	C..	.A.	..A	..C	...	...	...	..A
P. rawlinson 49585	...	..T	...	...	...	...	...	...	T..	..G	...	..G	...	...	...	...	...	..A	..C	...	...	...	..A
P. rawlinson 49590	...	..T	...	...	...	..A	...	...	T..	..G	...	..G	...	...	...	...	...	..A	..C	...	...	...	..A
P. spenceri 104773	...	..T	...	...	..A	..T	..A	..A	..C	...	...	...	...	...	...	..A	...	CAC	...	..C	..C	...	...	..A
P. spenceri 104774	...	..T	...	...	..A	..T	..A	..A	..C	...	...	...	...	...	...	..A	...	CAC	...	..C	..C	...	...	..A
B. duperreyi	..T	GC.	...	..T	..A	A.T	..G	..G	...	...	...	...	G.C	...	..A	C.T	CAC	..A	..C	...	.TT	...	..A
M. ruficauda	...	...	...	..C	..G	G.C	...	...	...	...	...	...	..C	...	..C	C..	CAC	..A	..C	T..	..A	..T	..A
L. guichenoti	..T	GCT	...	..T	..A	A.A	..G	...	...	T..	...	..C	G.C	..T	..A	C..	CAC	...	...	A.T	..A	..C	..A
N. metallicus	..T	...	T..	...	...	..T	..G	...	T..	T..	...	..C	..C	..T	..C	C..	...	..A	...	T..	..A	...	..A

Appendix 4.2

P. baudini 22957	CGA	AAC	AAA	CTT	CCT	ACA	AAC	ATT	---	AAC	ACA	CAC	CCA	ACC	CAT	ACA	CGA	GAG	CAC	CTG	TTA	ATA	RCA
P. baudini 22964	...	...	...	...	...	...	...	...	---	...	...	...	...	...	...	...	...	...	...	...	...	...	G..
P. cryodroma 40890	...	...	..G	..C	...	...	...	...	---	...	..C	A..	...	...	A.C	...	...	..A	...	...	C..	...	GT.
P. cryodroma 61886	...	...	..G	..C	...	...	...	...	---	...	..C	A..	...	...	A.C	...	...	..A	...	...	C..	...	GT.
P. entrecasteauxii 40981	...	.G.	..G	..C	..C	...	...	...	---	...	..C	AC.	...	...	..C	...	...	..A	...	...	C..	...	GT.
P. entrecasteauxii 61836	...	...	..G	..C	..C	...	...	..C	---	...	..C	AC.	...	..T	..C	...	...	..A	...	..A	...	...	A.G
P. entrecasteauxii 23023	...	.GT	..G	..A	..C	...	...	...	---	...	..C	A.T	...	...	..C	...	...	..A	...	..A	...	...	A.G
P. pagenstecheri 45268	...	...	...	..C	..C	...	...	...	---	...	.TT	A..	...	...	..C	...	...	..A	...	..A	...	...	A.G
P. pagenstecheri 112313	...	...	..G	..C	..C	...	...	...	---	...	..C	A..	...	...	..C	...	...	..A	...	...	...	...	GT.
P. rawlinson 49585	...	...	..G	..C	...	...	...	G..	---	...	...	...	...	...	..C	...	...	..A	...	..A	...	..G	GTG
P. rawlinson 49590	...	...	..G	..C	...	...	...	G..	---	...	...	...	...	G..	..C	...	...	..A	...	...	...	...	G..
P. spenceri 104773	...	.GT	...	..C	...	...	...	...	---	...	.TC	.C.	...	..T	..C	...	...	..A	...	..T	...	...	G..
P. spenceri 104774	...	.GT	...	..C	...	...	...	...	---	...	.TC	.C.	...	..T	..C	...	...	..A	...	..T	...	...	G..
B. duperreyi	...	..T	...	..G	...	.A.	...	..C	ACA	...	..C	..G	...	...	..A	..C	..G	..A	..T	..T	C..	..G	G..
M. ruficauda	...	...	...	..A	..A	...	...	..C	ACA	.G.	.TT	.CT	...	...	..C	..C	...	..A	...	..A	C..	..C	CT.
L. guichenoti	...	...	...	..A	T.A	...	.GT	.C.	ACC	...	.TT	TCA	...	..T	A.A	...	...	..A	...	...	C.T	...	G.C
N. metallicus	...	..T	..G	..A	T..	...	...	..C	ACA	...	..C	..A	...	...	..C	...	...	..A	...	..C	C..	...	G.C

P. baudini 22957	CTT	CAT	ACA	ATC	CCC	ATA	CTG	CTT	CTT	CTA	ATA	AAA	CCA	GAG	CTT	ATT	ATG	GGC	CCC	TTC	ACA	T			
P. baudini 22964	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.			
P. cryodroma 40890	...	..C	...	GC.	..A	...	..C	...	..C	...	T..	...	..T	...	..C	..C	...	...	...	...	...	.			
P. cryodroma 61886	...	..C	...	GC.	..A	...	..C	...	..C	...	T..	...	..T	...	..C	..C	...	...	...	...	...	.			
P. entrecasteauxii 40981	..C	...	...	GC.	..T	...	..T	..C	..A	...	...	...	...	.CA	..C	...	...	...	...	...	...	.			
P. entrecasteauxii 61836	..C	..C	...	GC.	..T	...	..T	..C	..A	...	...	...	...	.CA	..C	...	...	...	...	...	...	.			
P. entrecasteauxii 23023	..C	...	...	.C.	..T	...	..T	...	..A	...	...	...	...	.CA	...	...	...	...	...	...	...	.			
P. pagenstecheri 45268	...	..C	...	GC.	..A	...	..A	..C	..C	...	...	...	...	..A	..C	..C	...	...	...	...	...	.			
P. pagenstecheri 112313	..C	..C	..C	GC.	..A	..G	..C	..C	..C	T..	...	...	...	...	..C	..C	...	...	...	...	..G	.			
P. rawlinson 49585	..C	..C	...	..T	..T	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.			
P. rawlinson 49590	..C	..C	...	..T	..T	...	..T	...	...	...	...	...	...	...	...	...	...	...	...	...	...	.			
P. spenceri 104773	..A	..C	...	GC.	..T	..G	..A	..A	..C	...	...	...	...	.G.	...	..C	...	...	...	...	...	.			
P. spenceri 104774	..A	..C	...	GC.	..T	..G	..A	..A	..C	...	...	...	...	.G.	...	..C	...	...	...	...	...	.			
B. duperreyi	..A	..C	.??	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	???	?			
M. ruficauda	...	...	.TT	GCA	..T	.C.	..A	..A	..C	...	.C.	...	...	.CC	T.A	..C	..A	...	..T	...	...	.			
L. guichenoti	..A	..C	..T	...	..A	C..	..C	...	...	T..	...	...	..G	.G.	..A	G.C	...	...	..A	..T	.TG	.			
N. metallicus	...	...	.TC	...	..A	...	..A	T.C	T.A	T..	...	...	...	...	..C	..C	...	...	..T	..T	...	.			

Appendix 4.3: *Pseudemoia* tRNA alignments (see table 4.1)

***Pseudemoia* tRNA his alignment**

```
                          GTCATTATAG TTTAACAAAA ATATTAGAAT GTGAGCCTAA AAATAGAAGT TTAACCCTTC TTAATGACC
P. baudini 22957          .......... .......... .......... .......... .......... .......... .........
P. baudini 22964          .......... .......... .......... .......... .......... .......... .........
P. cryodroma 40890        .......... .......... .......... .......... .......... .......... .........
P. cryodroma 61886        .......... .......... .......... .......... .......... .......... .........
P. entrecasteauxii 40981  ....C..... .......... .......... ....A..... .......... .......... ...G.....
P. entrecasteauxii 61836  ....C..... .......... .......... ....A..... .......... .......... ...R.....
P. entrecasteauxii 23023  ....C..... .......... ..G....... ....A..... .......... .......... ...G.....
P. pagenstecheri_45268    ....CC.... .......... .C.....GG. ....T..... .......... .......... ...G.....
P. pagenstecheri_112313   .......... .......... .......... ....A..... .......... .C........ .........
P. rawlinsoni_49585       .......... .......... .......... .......... .......... .C...T.... .........
P._rawlinsoni_49590       .......... .......... .......... .......... .......... .C...T.... .........
P. spenceri_104773        .......... .......... .......... .......... .......... .......... .........
P. spenceri 104774        .......... .......... .......... .......... .......... .......... .........
M. ruficauda              ....CC.... .....T.... .C........ ...GA..... .......... ....TT.... ..GG.....
L._guichenoti             ....CC.... .....T.... -C........ ...GA..... .......... ....TT.... ..GG.....
N._metallicus             .....C.... .....T.... .C........ ....A..... ....G.G... .G..TA.CC. C.G......
```

***Pseudemoia* tRNA ser alignment**

```
                          GAAGGGGGTA AGAA-CATGA AGAACTGCTA ATTCTCCACC CTGGGGCTAA ACTCCCCAGA CCCTTCA
P. baudini 22957          .......... ....-..... .......... .......... .......... .......... .......
P. baudini 22964          ........C. ...G-..... ..G....... .......... ...T..T... .......... .......
P. cryodroma 40890        ........C. ...G-..... ..G....... .......... ...T..T... .......... .......
P. cryodroma 61886        ..G.....C. ...G-..... ..G....... ..C....... ...TA.T... .TC..A.... .......
P. entrecasteauxii 40981  ........C. ...G-..... ..G....... ..C....... ...TA.T... .TC..A.... .......
P. entrecasteauxii 61836  ..G.....C. ...G-..... ..G....... ..C....... ...TA.T... .TC..A.... .......
P. entrecasteauxii 23023  ........C. G..G-..... ..G....... .......... ......T... .......... .......
P. pagenstecheri_45268    ........C. ...G-..... ..GG...... ..C....... ...T..T... .....T.... .......
P. pagenstecheri_112313   .......... ....-..... .......... .......... .......... .......... .......
P. rawlinsoni 49585       .......... ....-..... .......... .......G.. .......... .......... .......
P._rawlinsoni_49590       .......... ....-.G... .......... .C.......T .......T... .......... .T.....
P. spenceri_104773        .......... ....-.G... .......... .C.......T .......T... .......... .T.....
P. spenceri_104774        .........C ....-..CA. G......... ....CT.G.. ..A...T... ......T... .......
M. ruficauda              .........C ....-..CA. .......... ....CTAG.. ......T... .A........ .......
L._guichenoti             ..G....ACC TTC.A..CA. .A........ .....TTGT. ......T... .......... ...C...
N._metallicus
```

Appendix 4.4: *Pseudemoia β-fibrinogen* alignment (see table 4.1)

```
P. baudini 22957          TTAGATCTGA TTGGCTTGGG AGCTCAGGA- TTTTGCCTGT AGAGTGTTTT TT---AAGG- ---------- ----CCACAG
P. baudini 22964          .......... .......... .........- .......... .......... ..---..... ---------- ----......
P. cryodroma 40890        .......... .......... .........- .......... .K.K.T.... ..---R...- ---------- ----......
P. cryodroma 61886        .......... .......... .........- .........A. .....T.... .----..... ---------- ----......
P. entrecastauxii 10981   .......... .......... .........- .......... .....T.... ..---..... ---------- ----......
P. entrecastauxii 61836   ...A...... .......... .........- .......... .....T.... .----..... ---------- ----......
P. entrecastauxii 23023   .......... .......... .........- .......... ....GT.... ..---..... ---------- ----......
P. pagenstecheri 45268    ?????????? ?????????? ?????????? ?????????A .AN..T.... -----..... ---------- ----......
P. pagenstecheri 112313   .......... .......... .........- .......... .....T.... -----..... ---------- ----......
P. rawlinsoni 49585       .......... .........A .........- .......... ....G..... ..TT-..... ---------- -----...N..
P. rawlinsoni 49590       .......... .......... .........- .......... ....GT.... ..T-..... ---------- -----...T..
P. spenceri 104773        .......... .......... .........- ......T... .....T.... .---A..... ---------- ----......
P. spenceri 104774        .......... .......... .........- ......T... .....T.... .---A..... ---------- ----......
B. duperreyi              .......... .......... .........T A.......A.. .....T.A.. ..--A..... ---------- ----......
M. ruficauda              ?????..... .......... .........T .......... .....T.... ----A..T.- ---------- -----....C.
L. guichenoti             .......... C....A.... .........- ......T... ....AT.... ----A..A.A TCTTGACCAA TGGA......
N. metallicus             .......... .......... ....T....- .......... .....T.... ..--A..... ---------- ----......

P. baudini 22957          TATGGACCAT TTAATCAAAA CT-ATCCCAT AGGTTAGTTT AGTTATACTA CTGTATTTCC GCAAA-TACA ATGCACCATC
P. baudini 22964          .......... .......... ..-....... .......... .......... .......... .....-.... ..........
P. cryodroma 40890        .R........ .......... ..-....... .......... .......... ..N.....A. .....-.... .........T
P. cryodroma 61886        .......... .......... ..-....... .......... .......... ..C.....A. .....-.... ..........
P. entrecastauxii 10981   .......... ......-.... ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   .......... .......... ..-....... .......... .......... ..C.....A. .....-.... ..........
P. entrecastauxii 23023   .......... .......... ..-....... .......... .......... ..C.....A. .....-.... ..........
P. pagenstecheri 45268    .......... .......... ..-....... .......... .......... ..C.....A. .....A.... ..........
P. pagenstecheri 112313   .......... .........N ..-....... .......... .......... ..C.....A. .....-.... ..........
P. rawlinsoni 49585       .......... .......... ..-....... .......... .......... ..C.....A. .....A.... ..........
P. rawlinsoni 49590       .......... .......... ..-....... .......... .......... ..C.....A. .....-.... ..........
P. spenceri 104773        .......... .......... ..-....... .......... .......T.. ..C.....A. .....-..A. ..........
P. spenceri 104774        .....M.... .........M ..-....... .......... .......T.. ..C.....A. .....-..A. ..........
B. duperreyi              ........T. ..T..A.... ..-....... .A........ .......T.. .CC.....A. A....-.... ..A.......
M. ruficauda              ...T...... ..T..A.... A.T....... .A........ .C.....T.. .CC.....A. A....-..AG ...G.....A
L. guichenoti             .......... ..T....... ..-....... .A........ ..C..CGT.. .C......AT T....-.... .....A....
N. metallicus             .......... ..T....... ..-....A.. .......... .......T.. .C......A. A....-..A. G.........

P. baudini 22957          GAATCTAATC TGCACCCTAA TTTTGGAGAT TTTGTAGACA ATG-----GG CAAAATCCAG AGTAACTACG GGGAGTCCTT
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P. baudini 22964          .......... .......... .......... .........G ...-----.. .......... .......... ..........
P. cryodroma 40890        .......... .......... .......... .....N.... ...-----.. .......... ...N...... ..........
P. cryodroma 61886        .......... .......... .......... .........G ...-----.. .......... .......... ..........
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   .....G.... .......... .......... .........G ...-----.. .......... .......... ..........
P. entrecastauxii 23023   .......... .......... .......... .........G ...-----.. .......... .......... ..........
P. pagenstecheri 45268    .....G.... .......... .......... .......... ...-----.. .......... .......... ..........
P. pagenstecheri 112313   .......... .......... ....T..... .........G ...-----.. .......... .......... ..........
P. rawlinsoni 49585       .......... ....Y..... .......... .........Y ...-----.. .......... .......... ..........
P. rawlinsoni 49590       .......... .......... .......... .........T ...-----.. .......... .......... .........G
P. spenceri 104773        .......... .......... .......... .........G ...-----.. .......... .......... .........G
P. spenceri 104774        A......... .........W .....R.... .........G ...-----.. .......... .........A ..........
B. duperreyi              ..C......G ........G. .........- ---------- ---------- ---------- ---------- ----------
M. ruficauda              ....TG...G ..G....AG. .........- ---------- ---CTAAA-- ---------- ---------- ----------
L. guichenoti             A........G ........G. .........- ---------- ---CTAAGA. .......... ..C.GA.T.. A.---------
N. metallicus             A........G ........G. .........- ---------- ---CTAAA.. ..C....... .AC.GT.... .C-...T...

P. baudini 22957          GCTCCGGTGT AAGAACAACT TACTCTGGAT TCCAATATGA ATTTCCCATA GGTTTTCACT AGGTGGTGCA GTAACTACGG
P. baudini 22964          .......... .......... .......... .......... .......... .......... .......... ..........
P. cryodroma 40890        .......... ..N.....A. ........C. ..G-...... ....N..... .......... .......... .N........
P. cryodroma 61886        .......... .......... .......... .-........ .......... .......... .......... ..........
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   .......... .......... .......... .-.......? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .......... .......... .......... .TG-...... .......... .......... .......... ..........
P. pagenstecheri 45268    .......... .......... .......... .-........ .......... .......... .......... ..........
P. pagenstecheri 112313   ........R. .......... .......... .-........ .......... .......... .......... ..........
P. rawlinsoni 49585       .......... .......... .......... .......... .......... .......... .......... ..........
P. rawlinsoni 49590       .......... .......... .......... .......... .......... .......... .......... ..........
P. spenceri 104773        .......... .......... C......... .G........ .......... .......... .......... ..........
P. spenceri 104774        .......... .......... C......... .G........ .......... .......... .......... .........A.
B. duperreyi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- .........A.
M. ruficauda              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
N. metallicus             ..GT..AC.C .C.T.A..T. .......... .G..C..... .......... .....CT.T. .A....C..C .......----
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P. baudini 22957          GTGCA----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. baudini 22964          .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. cryodroma 40890        .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. cryodroma 61886        .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. pagenstecheri 45268    .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. pagenstecheri 112313   .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. rawlinsoni 49585       .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. rawlinsoni 49590       .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri 104773        .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri 104774        .....----- ---------- ---------- ---------- ---------- ---------- ---------- ----------
B. duperreyi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti             ---------- ---------- ---------- ---------- -TCCATACAA TTACAGGACC CTCTAGTGTT ATCTTATGGA
N. metallicus             -----GCGTC AACGCAAATC TCTCGTGTGT TGACGCAAGA G--------- ---------- ---------- ----------

P. baudini 22957          ---------- ---------- ---------- ---------- ---------- -------CGT AAAGTCTCTT -ATGTGCTCC
P. baudini 22964          ---------- ---------- ---------- ---------- ---------- -------... .......... -.........
P. cryodroma 40890        ---------- ---------- ---------- ---------- ---------- -------... .......... T.C.......
P. cryodroma 61886        ---------- ---------- ---------- ---------- ---------- -------... .......-.. -.C....A..
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   ---------- ---------- ---------- ---------- ---------- -------... .......... -.C.......
P. pagenstecheri 45268    ---------- ---------- ---------- ---------- ---------- -------... .......... -.CT.A....
P. pagenstecheri 112313   ---------- ---------- ---------- ---------- ---------- -------... .......... -.CT.A....
P. rawlinsoni 49585       ---------- ---------- ---------- ---------- ---------- -------.R. ...RN..... -.C.......
P. rawlinsoni 49590       ---------- ---------- ---------- ---------- ---------- -------.R. ...RK..... -.C.......
P. spenceri 104773        ---------- ---------- ---------- ---------- ---------- -------... .......... -.........
P. spenceri 104774        ---------- ---------- ---------- ---------- ---------- -------... .......... -.........
B. duperreyi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti             AAGTCACCTT AACTGCTCCA ACGCAAAGGG CTTAATTTTA TTTTAGGATA TGGGCCC--- ---------- ----------
N. metallicus             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------..G.
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P. baudini 22957          CATAGTTACT CTGGATTTGG GGGTATATTT GACTGGAAAA GGTGTACAA- ----GTATGC AAATATGGTA ATTGTATGGA
P. baudini 22964          .......... .......... .......... .......... .........- ----...... .......... ..........
P. cryodroma 40890        .......... .......... .......... .......... ........TA ACATTCGA.. .......... ..........
P. cryodroma 61886        .......... .......... .......... .......... ........TA ACATTCGA.. .......... ..........
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .......... .......... .......... .......... ........TA ACATTCGA.. .......... ..........
P. pagenstecheri 45268    .......... .......... .......... .......... ........TA ACATTCGA.. .......... ..........
P. pagenstecheri 112313   .R........ .......... .......... .......... ........TA ACATTCGA.. .......... ..........
P. rawlinsoni 49585       .R........ .......... .......... .......... ........TA ACATTCRA.. .......... ..........
P. rawlinsoni 49590       .G........ .......... .......... .......... .......GTT ACATTCGA.. .......... ..........
P. spenceri 104773        .G........ .......... .......... .......... .......GTT ACATTCGA.. .......... ..........
P. spenceri 104774        ---------- ---------- --...A.... .......... ....C...TT ATATT.GA.. .....C.... ..........
B. duperreyi              ---------- ---------- ----------.. .......... ....C...TT ATATT.GA.. .....C.... ..........
M. ruficauda              ---------- ---------- ----.A.... T..A....-- -------.TT ACATTCGA.. .....CA... ..........
L. guichenoti             ....AC.G.. .......GT. CCC..A.... .......... ....C...TT ACATT.GA.. .....C.... ..........

P. baudini 22957          GAGTTATATT AATTATATAG AAAGCTATGG AACAATTTCC TGGGATAGAA TGATTAGATA ATAGGCC--- ----------
P. baudini 22964          .......... .......... .......... .......... .......... .......... .......--- ----------
P. cryodroma 40890        .......... .......... .......... .........A .......... .......... .......--- ----------
P. cryodroma 61886        .......... .......... .......... .......... .......... .......... .......--- ----------
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .......... .......... .......... .......... .......... .......... .......--- ----------
P. pagenstecheri 45268    .......... .......... .......... .......... .......... .......... .......--- ----------
P. pagenstecheri 112313   .......... .......... .......... .......... .......... .......... .......--- ----------
P. rawlinsoni 49585       .......... .......... .......... .......... .......... .......... .......--- ----------
P. rawlinsoni 49590       .......... .......... .......... .......... .......... .......... .......--- ----------
P. spenceri 104773        .......... ........G. .G........ .......... .......... .......... ....CA.--- ----------
P. spenceri 104774        .......... ........G. .G........ .......... .......... .......... ....C..--- ----------
B. duperreyi              .......... ........G. .G........ .......... .......... .......... .......--- ----------
M. ruficauda              .......... ........G. .G........ ..T....... .......... ---....... .......--- ----------
L. guichenoti             .......G.. ........G. .......... .......... .....C.... .......... .A....TTGG AGCACAATCC
N. metallicus             ...AG..... ...AG...G. GG........ .....C.... .......... .......... .......--- ----------
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P. baudini 22957          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. baudini 22964          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. cryodroma 40890        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. cryodroma 61886        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. pagenstecheri 45268    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. pagenstecheri 112313   ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. rawlinsoni 49585       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. rawlinsoni 49590       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri 104773        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
P. spenceri 104774        ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
B. duperreyi              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
M. ruficauda              ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti             TAACCATGTC TACTCAAAAG TAAGCTCCAT TGAATTCAAT GGGTCTTACT CCCATGAAAG TGTCTTTAGG ATTGCAGCCT
N. metallicus             ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

P. baudini 22957          TGGTCTTCTC CAAGGAAGG- TATAAGTATT TCTATATATT CCAGAAGAAA GTCCCA-GTT CTCCTGTTGC ATCTCCAGAT
P. baudini 22964          .......... .........- .......... .......... .......... ......A... .......... ..........
P. cryodroma 40890        .......... .........- ......A... .......... .......... ......-... .......... ..........
P. cryodroma 61886        .......... .........- .......... .......... .......... ......A... ........T. ..........
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .......... .........- .......... .......... .......... ......A... .......... ......C...
P. pagenstecheri 45268    .......... .........G .......... .......... .......... ......-... ........T. ..........
P. pagenstecheri 112313   .......... .........- .......... .......... .......... ......-... ........T. ..........
P. rawlinsoni 49585       .......... .........- .......... .......... .......... ......-... ..---..... ..........
P. rawlinsoni 49590       .......... .........- .......... .......... .......... ......-... ..---..... ..........
P. spenceri 104773        .......... .........- .......... .T........ .......... ......-... .......... ..........
P. spenceri 104774        .......... ......R..- K......... .......... .......... ......-... ...Y...... ......M...
B. duperreyi              ...G...... ...T.....- .......T.. ...G...... .......... ......-... .......... ..........
M. ruficauda              .......... ..GT.....- ....TT.... ...G...... .......... ......-... .......... ..........
L. guichenoti             .......... TG.......- .......... ...G...... .....R.... ......-... .......A.. ......T...
N. metallicus             .......... .........- .......... ...G...... .......... ..T...-... .......... ..........
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P. baudini 22957          CAGACTGGAA AAGG------ ----TCACTA TGTGAAAGTC TGGAAAGTTT CAGTGTCACG -TTATACTGA TCGAGATGGA
P. baudini 22964          .......... ....------ ----...... .......... .......... ........-. -......... ..........
P. cryodroma 40890        .......... ....------ ----...... .......C.. .......... ........-. -......... ..........
P. cryodroma 61886        .......... ....------ ----...... .......T.. .......... ........-. -......... .T........
P. entrecastauxii 10981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .......... ....------ ----...... .......C.. ..T.....G. ........-. -......... ..........
P. pagenstecheri 45268    .......... ....------ ----...... .......C.. .......... ........-. G......... ..........
P. pagenstecheri 112313   .......... ....------ ----...... .......T.. .......... ........-. -......... ..........
P. rawlinsoni 49585       .......... ....------ ----...... .......C.. .......... ........-. -......... ..........
P. rawlinsoni 49590       .......... ....------ ----...... .......C.. .......... ........-. -......... ..........
P. spenceri 104773        .......... ..A.------ ----.....C .......C.. .......... .G......-. -......... ..........
P. spenceri 104774        .......... ..R.------ ----.....C .......C.. .......... .G......-. -......... ..........
B. duperreyi              ....T..... ....------ -----..G..C .......C.. .......... .G......-. -......... CT........
M. ruficauda              ....T..... ....------ ----..T..C .......C.. .A........ TG.------- --....T... .T........
L. guichenoti             ...C...... ....TAACAT TCTA------ --.....C.. .......... .G......-. -......... ..A......G
N. metallicus             .......... ....------ ----.....C ...A...C.. .......... .G......N. -......... ..........

P. baudini 22957          TCAATTAACT GAGA------ ---------- ---------- -------GTA GAAGATAGGT TCATATGTTT ATTCACAGAT
P. baudini 22964          .......... ....------ ---------- ---------- -------... .......... .......... ..........
P. cryodroma 40890        .......C.. ....------ ---------- ---------- -------... ........A. .......... ..........
P. cryodroma 61886        .......C.. ....------ ---------- ---------- -------... .......... .......... ..........
P. entrecastauxii 10981   ??????CC.. ....------ ---------- ---------- -------... .......... .......... ..........
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 23023   .......C.. ....------ ---------- ---------- -------... .----..... .......... ..........
P. pagenstecheri 45268    .......C.. ....------ ---------- ---------- -------... ........N. .......... ..........
P. pagenstecheri 112313   .......C.. ....------ ---------- ---------- -------... .......... .......... ..........
P. rawlinsoni 49585       .......C.. ....------ ---------- ---------- -------... .......... .......... ..........
P. rawlinsoni 49590       .......C.. ....------ ---------- ---------- -------... .......... .......... ..........
P. spenceri 104773        .......C.. ....------ ---------- ---------- -------... .......... .......... ..........
P. spenceri 104774        .......C.. ....------ ---------- ---------- -------... .......... .......... ...T......
B. duperreyi              .......T.. ....------ ---------- ---------- -------... .......... .......... ...T......
M. ruficauda              .......TA. ....------ ---------- ---------- -------... T......... .......... ..........
L. guichenoti             .......T.. ....TGGATC AATTATCTGA GATCAATTAC CTAGCTG--- -......... .......... ....??????
N. metallicus             .......T.. ....------ ---------- ---------- -------... TC........ .......... .......C...
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P. baudini 22957          AGCATATCTA CTATTTCCTC AGAGTGCATA CAT
P. baudini 22964          .......... .....-.... .......... ...
P. cryodroma 40890        .......... .......... .......... ...
P. cryodroma 61886        .......... .......... ...??????? ???
P. entrecastauxii 10981   .......... .......... .......... ..?
P. entrecastauxii 61836   ?????????? ?????????? ?????????? ???
P. entrecastauxii 23023   .......... ....?????? ?????????? ???
P. pagenstecheri 45268    .......... .....????? ?????????? ???
P. pagenstecheri 112313   .......... .......... .......... ...
P. rawlinsoni 49585       .......... .....-.... ..???????? ???
P. rawlinsoni 49590       .......... .......... .......... ...
P. spenceri 104773        ..TG...... .......... .......... ...
P. spenceri 104774        ..TG...... .......... .......... ...
B. duperreyi              ---------G T......... ........G. ...
M. ruficauda              ?????????? ?????????? ?????????? ???
L. guichenoti             ..TG.GC... .......... .T........ ...
N. metallicus             ..TG...... ..G....... ......A... ...
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Appendix 4.5: *Pseudemoia myh-2* alignment (see table 4.1)

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P. baudini 22957          TGGAGTCCGA CCTGTCCCAG CTGCAGACAG AGGTGGAGGA GGCAGTGCAG GAGTGCAGGA ATGCGGAGGA GAAGGCCAAG
P. baudini 22964          .......... .......... .......... .......... .......... .......... .......... ..........
P. cryodroma 40890        .......... .........A ..C...T.G. .......... .......... .......... .C........ ..........
P. cryodroma 61886        AT........ ...C...... .......... .......... .......... .......... .......... ..........
P. entrecastauxii 40981   .......... ...C...... .......... .......... .......... .......... .......... ..........
P. entrecastauxii 61836   .......... ...C...... .......... .......... .......... .......... .......... ..........
P. entrecastauxii 23023   .......... .......... .......... .......... .........A .......... .......... ..........
P. pagenstecheri 45268    .......... .........A ..C...T.G. .......... .......... .......... .C..A..... ..........
P. pagenstecheri 112313   .......... ...C...... .......... .......... .......... .......... .......... ..........
P. rawlinsoni 49585       .......-.. ...C...... T......... .......... .......... .......... .......... ..........
P. rawlinsoni 49590       .......... ...C...... T......... .......... .......... .......... .......... ..........
P. spenceri 104773        .......... ...C...... .......... .......... .......... .......... .......... ..........
P. spenceri 104774        .......... .........A ..C...T.G. .......... .......... .......... .C..A..... ..........
B. duperreyi              .......T.. .T........ .......... .......... .......... .......... .......... ..........
M. ruficauda              .......... .......... .......... .......... .......... .......... .......... ..........
L. guichenoti             .......... .......... .......... .......... ...C.....A .......... .N........ ..........
N. metallicus             .......... .......... .......... .......... .......... .......... .......... ..........

P. baudini 22957          AAGGCCATCA CTGATGTGAG TGGAATGGGA AACATTCTTA GTGAAGCACC AGAAATGAAT CTTGAGTGGG ATTTATTTAG
P. baudini 22964          .......... .......... .......... .......... .......... .......... .......... ..........
P. cryodroma 40890        .......... .......... .......... .......... .A........ .......... .......... ..........
P. cryodroma 61886        .......... .......... .......... .......... .......... .......... .......... ..........
P. entrecastauxii 40981   .......... .......... .......... ......???? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   .......... .......... .......... .......... .......... .......... .......... ..........
P. entrecastauxii 23023   .......... .......... .......... .......... .......... .......... .......... ......G...
P. pagenstecheri 45268    .......... .......... .......... .......... .......... .......... .......... ..........
P. pagenstecheri 112313   .......... .......... .......... .......... .......... .......... .......... ..........
P. rawlinsoni 49585       .......... .......... .......... .......... .......... .......... .......... ..........
P. rawlinsoni 49590       .......... .......... .......... .......... .......... .......... .......... ..........
P. spenceri 104773        .......... .......... .......... .......... .......... .......... .......... ..........
P. spenceri 104774        .......... .......... .......... .......... .......... .......... .......... ..........
B. duperreyi              ..A....... .......... .......... .......... .......... .........A .......A.. .........C.
M. ruficauda              ..A....... .......... .......... .......... .C........ .........A .......... .........C.
L. guichenoti             .......... ....C..... .......... ...C....C. .......... ....G..... ..G....... ..........
N. metallicus             .......... .......... .......... ...C..---. .......... .......... A......... .....C....
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P. baudini 22957          GATAGAGACC ACTACAGACT TACAATCCCC TATTATTTTC CCCCACAAGT AGGACAATAG TTGGTGAAGT TCAC-TTTTG
P. baudini 22964          .......... .......... .......... .......... .......... .......... .......... ....C.....
P. cryodroma 40890        .......... .......... .......... .......... ....G..... .......... .......... ....-.....
P. cryodroma 61886        .......... .......... .......... .........C A......... .......... .......... ....C.....
P. entrecastauxii 40981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   .......... .......... .......... .........C A......... .......... .....A.... ....-.....
P. entrecastauxii 23023   .......... .......... .......... .........Y M......... .......... .......... ....-.....
P. pagenstecheri 45268    .......... .......... .......... .......... .......... .......... .......... ....-.....
P. pagenstecheri 112313   ...G...... .......... .......... .......... .......... ....Y..... .......... ....-.....
P. rawlinsoni 49585       .......... .......... .......... .......... .......... .......... .......... ....-.....
P. rawlinsoni 49590       .......... .......... .......... .......... .......... .......... .......... ....-.....
P. spenceri 104773        ...G...... ....N..... .......... .........C A......... .......... .....A.... ....-.....
P. spenceri 104774        .......... .......... .......... .......... .......... .......... .......... ....-.....
B. duperreyi              .......... ...T...... .......... .........C .......... .......... .C.......C .T..-.....
M. ruficauda              .......... .......... .......... .......... .......... ..-------. ..A....-.. .T..-.....
L. guichenoti             ..CG...... .......... .......... .........-. .....----- ---------. ....A..... ....-....-
N. metallicus             ...T...... .......... .......... .........C ..T....... .......... .......... ....-.....

P. baudini 22957          TGTGTCCCAA AATGCTATTT TCCATTGAAG AAGTAGGCAT ACTCAATAGG ATGGACATGG GTATGAAGAT GGCTATAGGG
P. baudini 22964          .......... .......... .......... .......... .......... R......... ..N....... ..........
P. cryodroma 40890        .......T.. .......... .......... .......... G....G.... G......... .......... ..........
P. cryodroma 61886        .......... .......... .......... .......... .......... G......... .......... ..........
P. entrecastauxii 40981   ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
P. entrecastauxii 61836   .......... .......... .......... .......... G......... G......... .......... ..........
P. entrecastauxii 23023   .......... .......... .......... .......... G......... G......... .......... ..........
P. pagenstecheri 45268    .......... .......... .......... .......... G......... G......... .......... ..........
P. pagenstecheri 112313   .......... .......... .......... .......... G......... G......... .......... .A........
P. rawlinsoni 49585       ........C. .......... .......... .......... G......... G......... .......... ..........
P. rawlinsoni 49590       ........C. .......... .......... .......... G......... G......... .......... ..........
P. spenceri 104773        .......... ....T..... .......... .......... G......... GC........ .......... ..........
P. spenceri 104774        .......... .......... .......... .......... .......... G......... .......... ..........
B. duperreyi              .......... .......... .......... .......... .T........ G......C.. .......... ..........
M. ruficauda              .....A.... .......... .......... .......A.. .......... G........A .......... ..........
L. guichenoti             ---------- ---------- -......... .......A.. .......... G.....G... ........G. ..........
N. metallicus             .......... .......... .......... .......... .......... G......... .......... ..........
```

Appendix 4.5

									Simple Gaps
P. baudini 22957	TGAAGAGGA-	----------	-----CCCTG	CCTTGCTTGT	ACTCTGATGA	GTTGTCTCCA	ACCTACGATC	T	12345678
P. baudini 22964	.........-	----------	-----.....	.Y........	....	...R......	.....T....	.	00010000
P. cryodroma 40890	.........-	----------	-----.....	.........-	---------.	...A......	....GT....	.	00000000
P. cryodroma 61886	.........-	----------	-----.....	.........-	---------.	...A......	....GT....	.	00010001
P. entrecastauxii 40981	??????????	??????????	??????????	??????????	??????????	??????????	??????????	?	00000001
P. entrecastauxii 61836	.........-	----------	-----.....	.........-	---------.	...A......	....GT....	.	????000?
P. entrecastauxii 23023	.........-	----------	-----.....	.........-	---------.	...A......	....GT....	.	00010001
P. pagenstecheri 45268	.........-	----------	-----.....	.........?	??????????	??????????	??????????	?	00010001
P. pagenstecheri 112313	.........-	----------	-----.....	....	....	...A......	.....T....	.	0001000?
P. rawlinsoni 49585	.........-	----------	-----.....	....	...???????	??????????	??????????	?	00010000
P. rawlinsoni 49590	.........-	----------	-----.....	....	....	....	.....T....	.	00000000
P. spenceri 104773	.........-	----------	-----.....	.........-	---------.	...A......	....GT....	.	00010000
P. spenceri 104774	.........-	----------	-----.....	....	....	...A......	.....T....	.	00010001
B. duperreyi	.......---	----------	---------.	....	......T...	...A......	.....T....	.	00010010
M. ruficauda	........G-	----------	-----.....	....	......C...	...AC.....	.....T....	.	00110000
L. guichenoti	....-----T	ATAGGACCAT	ATGGA...A-	....	......T...	...A......	...A.T....	.	01-11100
N. metallicus	.......CG-	----------	-----.....	..G.......	....	...A......	.....T....	.	10010000

Appendix 5 – New Zealand skink alignments

5.1 - Allozymes 217

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Appendix 5.1 – Allozyme characters for New Zealand skink species (see Chapter 5, and table 5.1)

	cpk-1	cpk-3	gp-3	gpi-1	g3p-1	g6p-1	idh-2	ldh-1	mdh-1	mdh-2	mpi-2	np-1	pep-3	6pg-1	pgm-1	pgm-3	sod-1
C. aenea	2	1	1	2	2	5	5	3	1	1	4	2	3	5	6	1	3
C. alani	1	5	2	2	2	1	5	4	1	1	1,2	1	1	2	1,2	1,3	4
C. ornata	2	5	2	2	2	1	3	2	1	5	1	1	4	2	4	2	3,5
O. fallai	1	1	2	2	2	5	2,5	1	1	1	2,4	1	1	2	2	2	1
O. infrapunctatum	1	1	3	1	2	5	2	6	1	1	2	2	1	2	1	4	2
O. lineoocellatum	1	1	1	1	2	3	3	6	1	1	4	2	1	1,2	1	1	1
O. maccanni	1	2	1	1	1	1	1	1	1	1	1	3	1	1	2	1	1
O. microlepis	1	5	2	1	2	2,3	2	5	1	1	2	1	1	1	3,7	1	6
O. moco	1	1	2	2	3	5	2	2	1	1	2	1	1	2	7	1	1
O. n. polychroma	1	1	1	1	1	2	1	1	1	1	1,5	1	1	1	2	1	1
O. smithi	1	1	2	1	2	1	2	5	2	1	2	5	3	1	3	2	3
O. stenotis	1	1	2	2	1	1	5	1	1	1	1	1	1	1	2,3	1	1
O. suteri	1	1	2	2,3	2	5	2	2	1	1	4	1	1	2	2	1	3
O. waimatense	1	1	3	1	1	4	5	6	1	1	2	2	1	1	2	1	2
O. zelandicum	1	3	2	4	2	1	1,2	2	1	1	2	4	2	1	1	1	1

Appendix 5.2: New Zealand skink *12S rRNA* alignment (see table 4.1)

```
C. aenea          GCCCAGCCGT CAACAAAGAC AGTATAAA-C ACAACACTGT TCGCCAGAGA ACTACAAGC- TAAAGCTAAA AACCCCAAGG
C. alani          ..T.......  ..........  ........A.  ..........  ..........  .........T  ..........  ...T......
C. ornata         ..T.......  ..........  .......G-T  ....T.....  ..........  .........-  ..........  ...T......
O. fallai         ..T.......  ..........  ........AT  ..........  ..........  .T.......-  ..........  ..........
O. infrapunctatum ..........  A.........  ........A.  ..........  ..........  ........T-  ...CA..C..  ...T......
O. lichenigera    ..T.......  ..........  C...A.G.AT  ....T.....  ..........  .........-  A..GA..C..  ...T......
O. lineoocellatum ..T.......  A.........  ........A.  ....T...A.  ..........  .........-  ...T...C..  ...T.T....
O. maccanni       ..T.......  A......A..  ....-...AT  ...GT.....  ..........  .........-  ....A..C..  ...T......
O. microlepis     ..T.......  ..........  ....C...AT  ....TG....  ..........  .........-  A...A..C..  ...T......
O. moco           ..T.......  T.........  ..C.....A.  .....G....  ..........  .........-  .......G..  ...T......
O. n. polychroma  ..T.......  .........T  ........AT  ...GTG..A.  ..........  .........-  A..GA..C..  ...T.T....
O. smithi         ..T.......  ..........  ....C...AT  ....T.....  ..........  .........-  A...A..C..  ...T......
O. stenotis       ..T.......  ..........  ........A.  ....T.....  ..........  .........-  ....A..C..  ...T......
O. suteri         ..T.......  ..........  ....C...AT  ....T.....  ..........  .........-  ....A..C..  ...T......
O. waimatense     ..........  A.........  ..C.C...AT  .....G....  ..........  .........-  ....A..C..  ...T......
O. zelandicum     ..TT......  T......A..  .....G..C.  ..........  ..........  .........-  A......C..  ...T......
L. guichenoti     ??...A....  A......A..  ..C.CCCG-T  ....AG....  .........G  .....C..TA  C..CA..C..  ...T......
T. scincoides     ...T....C.  A....T...T  G..TA..CA.  ....A..CA.  ..........  .........G  A......CG.  ...T.A....
```

Appendix 5.2

C. aenea	ACTTGGCGGT	GCTCCACATC	ACCCTAGAGG	AGCCTGTCCT	ATAATCGATA	CCCCCCGATC	CACCTCACCA	CTTTTTGAAA
C. alani	....	....	C.........	....	....	....	....	T.........
C. ornata	....	....	....	....	....	....	T.........	T.........
O. fallai	....	....	....	....	....	....	....	T.........
O. infrapunctatum	....	........C.	.A........	....	....	....	T.........	T.........
O. lichenigera	....	....	.A........	....	....	....	T.........	....
O. lineoocellatum	....	........C.	GA........	....	....	....	....	T.........
O. maccanni	....	....	....	....	....	....	T.........	....
O. microlepis	....	....	.A........	....	....	....A.....	T........G	.........G
O. moco	....	....	....	....	....	....	T.........	....
O. n. polychroma	....	........C.	.A........	....	....	....	T.........	....
O. smithi	....	........C.	.A........	....	....	....A.....	T.........	....
O. stenotis	....	....	GT........	....	....	....	T.........	....
O. suteri	....	....	....	....	....	....	T.........	....
O. waimatense	....	..C...T...	.G........	....	....	.........A	T.........	.CCA......
O. zelandicum	....	....	.T........	....	....	....A..C.T	T...C.....	T.C....C..
L. guichenoti	....	....	....	....	....	....	....	....
T. scincoides	....	....	....	....	....	....	....	....

C. aenea	TTCAGCCTAT	ATACCGCCGT	CGTCAGCCTA	CCTTGTGAAA	GAAGCGTAGT	AAGCAAAATA	GTCACCAACT	AAAACGTCAG
C. alani	C.........	....	..A.......	....A.....	.....AC...	....	....	....
C. ornata	C.........	....	....	....	.....AC...	C	....	....
O. fallai	C.........	....	....	....A.....	...A.AC...	....	....	....
O. infrapunctatum	C.........	....	....	....A.....	...AAA....	....	....T.....	.G........
O. lichenigera	C.........	....	C	....A.....	...A.AA...	G.......C.	....	....
O. lineoocellatum	C.........	....	....	....A.....	.....A....	.G........	....	....
O. maccanni	....	....	..C.......	....	.G...AA...	....	....T.....	....
O. microlepis	C.........	....	....	....A.....	....TAC...	....	....	....
O. moco	.....T....	....	....	....A.....	..GC.A...C	C	....	....
O. n. polychroma	....	....	.......T..	....A.....	.....AA...	.G........	....	....
O. smithi	C.........	....	....	....A.....	.....AC...	....	....	....
O. stenotis	C.........	....	....	....A.....	....AAC...	....	....	......C...
O. suteri	C.........	....	....	....A.....	....TA....	...T......	....T.....	....
O. waimatense	....	....	....	....A...G.	..GCTA....	......G...	.....T....	....
O. zelandicum	C.........	....	....	....A.....	..GATAC...	....	....	....
L. guichenoti	C.........	....	....	....A.....	...A.AA...	....	.C..TA....	.G........
T. scincoides	C.........	....	.......T..	..C.......	...A.AA...	.G......A.	..TTA.....	..C.......

Appendix 5.2

```
C. aenea          GTCAAGGTGT AGCACATAAA GCGGAAGAGA TGGGCTACAC TCTCTCCCCC AGAGAACACG AACAGCACCA ATGAAACACT
C. alani          .......... .......... AT........ .......... ........A. .......... ..G....... ..........
C. ornata         .......... .......G.. AT........ .......... .......... .......... .......... ..........
O. fallai         .......... ....T..... AT........ .......... .......... .........T .......T.. ..........
O. infrapunctatum .......... .......... AT........ .......... ........T. .......... ..T....TAG ......TT..
O. lichenigera    .......... ....T..... .T........ .......... .....G.... ......T... .......T.. ......A...
O. lineoocellatum .......... .......... .T........ .......... .......... ......T... .......T.. ......T...
O. maccanni       .......... ....T..... AT........ .......... .......... .......... .......T.. ..........
O. microlepis     .......... .......GT. .T........ .......... ........T. .......... .......... ..........
O. moco           .......... .......... AT........ .......... .......... .......... .......T.. ..........
O. n. polychroma  .......... .......... .T........ .......... ........T. .......... .......T.. .......C..
O. smithi         .......... .......GT. .T........ .......... ........T. .......... .......... ..........
O. stenotis       .......... .......... .T........ .......... .....A..T. .......... .......T.. ..........
O. suteri         .......... .......... .T........ .......... .......... ....G..... .......T.. .......C..
O. waimatense     .......... .......... .T........ .......... .....A.... .......... ...G...T.. ..........
O. zelandicum     .......... .......... .T........ .......... .......... ......T... .......... ..........
L. guichenoti     .......... .......GGG .T..T..... .......... .....AG..T ......T..A G.......-.. .......C..
T. scincoides     .......... ....G...G. AT........ .........T .T...G..A. ...A..ATAC G.A..GTGT. .........A
```

```
C. aenea          GCTC-AAAGG CGGATTTAGT AGTAAGATAA ACAAGAGAAC TTATCTTAAA -CCAGCCCTG -AGCGC
C. alani          ....-..... T......... .......... .......... .......... A......... G.....
C. ornata         ....-..... .......... .......... .......... .......... -......... G.....
O. fallai         ....-..... T......... .......C.. ........G. ..G...A... -......... G.....
O. infrapunctatum ....-..... T......... .........G ........G. .A........ -......... G.....
O. lichenigera    ....-..... T......... .......... .......... ..G....... -......... G.....
O. lineoocellatum ....-..... .......... .......... ........G. .......... -......... G.....
O. maccanni       ...T-G.... T......... .........G ........G. .......... -......... G.....
O. microlepis     ....-..... T........C .......... G.......G. C......... -T........ G.....
O. moco           ....-G.... .......... .......... ..G....... .......... -......... G.....
O. n. polychroma  ....-..... T........C .......... .......... .......... -......... G.....
O. smithi         ....-..... T........C ........G. G.......G. CCG....... -T........ G.....
O. stenotis       ....-..... T......... .......... .......... ...C...... -.....T... G.....
O. suteri         ....-..... T........C .......... .......A... .......... -......... G.....
O. waimatense     ..CT-..... ..A....... .......... G.......G. C......... -......... G.....
O. zelandicum     ....-..... T......... .........G ........G. C......... -......... G.....
L. guichenoti     ....-..... .......... .......... ......A.GT .......... -......... GGA...
T. scincoides     CAC.C..... A........A ......C... .T.....TCT .......... -TTT..T... A?????
```

Appendix 5.3: New Zealand *β-fibrinogen* alignment (see table 5.1)

```
C. aenea          ACTTGGCTTG CTATTGTACT GCATTTTATG CATTTGAAG- ---------- TTACATGG-A ATGGAGATAA ATTACAGCTT
C. alani          .......... .......... .......... .........- ---------- .........- .......... ..........
C. ornata         .......... .......... .......... .........- ---------- .........- .......... ..........
O. fallai         ?????????? ?????..... .......... .........- ---------- .........- .......... ..........
O. lichenigera    .......... .......... .......... .........- ---------- .........- .......... ..........
O. lineoocellatum .......... .......... .......... .........- ---------- .........- .......... ..........
O. maccanni       .......... .......... .......... .........- ---------- .........- .......... ..........
O. microlepis     .......... .....A.... ..T....... .........- ---------- .........- .......... ..........
O. moco           .......... .......... .......... .........- ---------- .........- .......... ..C.......
O. infrapunctatum .......... .......... .......... .........- ---------- .........- .......... .K........
O. n. polychroma  .......... .......... .......... .........- ---------- .........- .......... ..........
O. smithi         .......... ..-....... .......... .........- ---------- .........- .......... ..........
O. stenotis       .......... .......... .......... .........- ---------- .........- .......... ....A.....
O. suteri         .......... .......... .......... .........- ---------- .........- .......... ..........
O. waimatense     .......... ....G..... .......... .......C.- ---------- .........- .......... ..........
O. zealandicum    .......... ..C....... .......... .........- ---------- .........-W .......... ..........
L. guichenoti     ????...... .........G .......--- ------...- ---------- .........- .......... ..........
T. scincoides     .....AT... T......... A..CC....C ...-A....C ATATGATTGT ........A. .......C.. ...G.T....
```

Appendix 5.3

```
C. aenea          TATAACTCTT AATATTGCAT GACTGCATCT ACTTAAGCAT GCATAATGAA AAAAAA--TT AGATCTGATT GGCTTGGGAG
C. alani          .......... .......... .......... .......... .......... ......--.. .......... ..........
C. ornata         .......... .......... .......... .......... .......... .....W--.. .......... ..........
O. fallai         .......... .......... .......... .G........ .......... ......--.. .......... ..........
O. lichenigera    .......... .......T.. .......... .G........ .......... .....---.. .......... ..........
O. lineoocellatum .......... .......... .......... .G........ .......... ......--.. .......... ..........
O. maccanni       .......... .......... .....TT... .G........ .......... ..G...AA.. .......... ..........
O. microlepis     .......... .......... .......... .......... .......... ......--.. .......... ..........
O. moco           .......... .......... .....A.... .G........ .......... ......--.. .......... ..........
O. infrapunctatum .......... .......... .......... .G........ .......... ......???? ?????????? ??????????
 O. n. polychroma .......... .......... .....Y.... .G........ .......... ..??????? ?????????? ??????????
O. smithi         .......G.. .......... .......... .......... .......... ......--.. .......... ..........
O. stenotis       .......... .......... .......... .G........ .......... ......--.. .......... ..........
O. suteri         .......... .......... .......... .......... .......... ......--.. .......... ..........
O. waimatense     .......... .......... .......... .G........ .......... ......A-.. .......... ..........
O. zealandicum    .......... .......... .......... .......... .......... ......--.. .......... ..........
L. guichenoti     ...G...... .........A ....A..... .G....C... ........G. ......--.. ........C. ...A......
T. scincoides     ..C....... .......... ....A..C.. .G....---- ---------- ---------- ---------- ----------

C. aenea          CTCAGGATTT TGCCTGTAGA G--------- ---------- ---------- ---------- ---------- ----------
C. alani          .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
C. ornata         .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. fallai         .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. lichenigera    ....A..... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. lineoocellatum .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. maccanni       .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. microlepis     .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. moco           .......... .......... ATCATAGAAT CATAGAATCC TAGAGTTGGA AGGGACCCAG TAGGTCATCT AGTCCAACCC
O. infrapunctatum ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
O. n. polychroma  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
O. smithi         .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. stenotis       .......... ....K..... .--------- ---------- ---------- ---------- ---------- ----------
O. suteri         .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. waimatense     .......... .......... .--------- ---------- ---------- ---------- ---------- ----------
O. zealandicum    .......... .......... R--------- ---------- ---------- ---------- ---------- ----------
L. guichenoti     .......... ...T...... .--------- ---------- ---------- ---------- ---------- ----------
T. scincoides     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
```

```
C. aenea          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. alani          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. ornata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. fallai         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lineoocellatum ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. maccanni       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. microlepis     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. moco           CCTGCCTGTA GCAGGAAATC CTCCTAGAGC ATCTCCAACA GGTGCTCGTC AAGCCTCTGC TTGAAGATCC CCAGTGAGGG
O. infrapunctatum ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
O. n. polychroma  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
O. smithi         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. stenotis       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. suteri         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. waimatense     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. zealandicum    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
T. scincoides     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

C. aenea          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. alani          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
C. ornata         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. fallai         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lichenigera    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. lineoocellatum ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. maccanni       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. microlepis     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. moco           AGAGTCCACC ACCTCCCTCG GCAATCTGTT CCACTGCCGA ACCGCCCTCA CCGTCAGGAA TTTTTTCCTG ATATCCAACC
O. infrapunctatum ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
O. n. polychroma  ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
O. smithi         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. stenotis       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. suteri         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. waimatense     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. zealandicum    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
L. guichenoti     ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
```

Appendix 5.4: New Zealand *myh-2* alignment (see table 5.1)

```
C. aenea          ATGGAGTCCG ACCTGTCCCA ACTCCAGTCG GAGGTGGAGG AGGCGGTTCA GGAGTGCAGG AATGCGGAGG AGAAGGCCAA
C. alani          .......... .......... .......... .......... .......... .......... .......... ..........
C. ornata         .......... .......... .......... .......... .......... A......... ..Y....... ..........
O. fallai         .......... .......... G..G...A.C .......... .......... .......... .......... ..........
O. infrapunctatum .......... .......... G..G...A.C .......... .......... .......... ..C....... ..........
O. lichenigera    .......... .......... G..G...A.C .......... .......... .......... .....A.... ..........
O. lineoocellatum --........ .......... G..G...A.A .......... .......... .......... .......... ..........
O. maccanni       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
O. microlepis     .......... .......... G..G...AGC .......... .......... .......... .......... ..........
O. moco           ---------- ----...... G..G...A.C .......... ...T....AS .AGTGCAT.. ..C....... ..........
O. n. polychroma  -......... .......... G..G...A.C .......... .......... .......... .......... ..........
O. smithi         .......... .......... G..G...AGC .......... .......... .......... .......... ..........
O. stenotis       .......... .......... G..G...A.C .......... .......... .......... .......... ..........
O. suteri         .......... .......... G..G...A.C .......... .......... .......... .......... ..........
O. waimatense     .......... .......... G..G...A.C .......... .......... .......... .......... ..........
O. zealandicum    .......... .......... G..G...AGC .......... .......... .......W.. ..C....... ..........
L. guichenoti     .......... .......... G..G...A.A .......... ....A..G.. .......... .......... ..........
T. scincoides     .......... .......... G..G...A.A .......... ....C..G.. .......... .......... ..........
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Appendix 5.4

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C. aenea          GAAGGCCATC ACTGATGTGA GTGGAATCGG AAACATTCTT AGTGAAGCAC CAGAAATGAA TCTCGAGTGG GATTTATTTA
C. alani          .......... .......... .......G.. .......... .......... .......... .......... ..........
C. ornata         .......... .....Y.... .......G.. .......... .......... .......... .......... ..........
O. fallai         .......... .......... .......G.. .......... .......... ...N...... .......... ..........
O. infrapunctatum .......... .......... .......G.. .......... ..C....... .......... .......... ..........
O. lichenigera    .......... .......... .......A.. ....C..... .......... .......... .A.T...... ..........
O. lineoocellatum .......... .......... .......G.. .......... .......... .......... .......... ..........
O. maccanni       ---------- ---------- ---------- ---------. .......... .......... ...T...... ..........
O. microlepis     .......... .......... .......G.. ....------ --------.. .......... .......... ..........
O. moco           .......... .......... .......G.. .......... .......... ........C. .......... .......K..
O. n. polychroma  .......... .......... .......G.. .......... .......... .......... .......... ..........
O. smithi         .......... .......... .......G.. ....------ --------.. .......... .......... ..........
O. stenotis       .......... .......... .......G.. .......... .A........ .......... .......... ..........
O. suteri         .......... .......... .......G.. .......... .......... .......... .......... ..........
O. waimatense     .......... .......... .......G.. .......... .......... .......... .......... ..........
O. zealandicum    .......... .......... .......G.. ....------ --------.. .........G .......... ..........
L. guichenoti     .......... ..G....... .....G.G.. ....C.C..C C.......... .......... G......... ..........
T. scincoides     .......... .......... ......GG.T ....C....G .......... .......... ...T...... ..........

C. aenea          GGATCGAGAC CACTACAGAC TTGCAATCCC CTATTATTTT -CCCCTACAA GTAGGACAAT AGWCGGTGAA GTTCACTTTT
C. alani          .......... .......... ..A....... .......... -.......... .......... ..T.A..... ..........
C. ornata         .......... .......... ..A....... .........C -.......... .......... ..T....... ..........
O. fallai         .......... .......... .......... .........C -.......... .......... ..T....... ..........
O. infrapunctatum ....A..... .......... ..A....... .........C -.......... .......... ..T....... ..........
O. lichenigera    ....A..... .......... ..A....... .......... -.......... .......... ..TT...... ..........
O. lineoocellatum ....A..... .......... ..A....... .........C -.......... .......... ..T....... ..........
O. maccanni       ....A..... .......... ..A..G.... .........C -..T....... .......... ..TT...... ..........
O. microlepis     .......... .......... ..A....... .......... -.......... .......... ..T....... ..........
O. moco           .......... .......... ..A....... .........C -....----- ---------- ---------- ----------
O. n. polychroma  ....A..... .......... ..A..G.... .........C -..T....... .......... ..T....... A.........
O. smithi         .......... .C........ ..A....... .......... -.......... .......... ..T....... ..........
O. stenotis       ....A..... .......... ..A....... .......... -.......... ........N. ..T.A..... ..........
O. suteri         .......... .......... ..A....... .......... -.......... .......... ..T....... ..........
O. waimatense     ....A..... .......... ..A....... .......... -.......... .......... ..T....... ..........
O. zealandicum    .......... .......... G.A....... .......... -.......... .......... ..T....... ..........
L. guichenoti     ....A..... .......... ..A....... .........- -....C.T.. .......... ..TT.....C ..........
T. scincoides     ....A..... .......... ..A....... .......... -....C.... .......... ..TT...... ..........
```
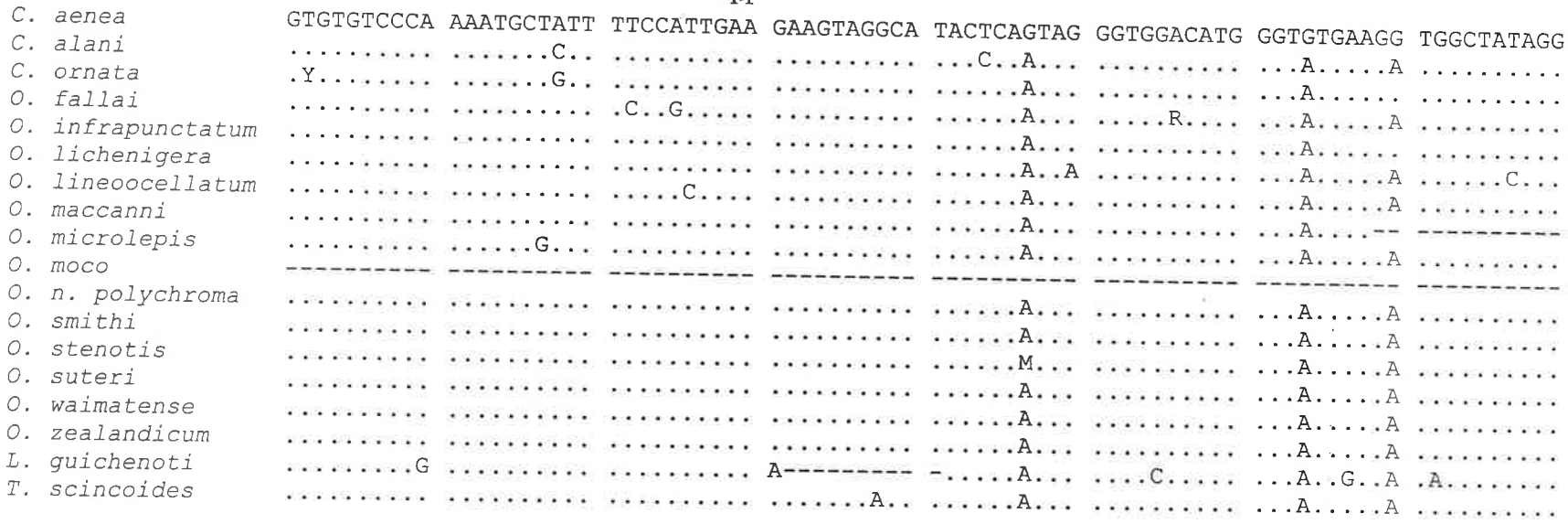

Appendix 5.4


C. aenea GTGTGTCCCA AAATGCTATT TTCCATTGAA GAAGTAGGCA TACTCAGTAG GGTGGACATG GGTGTGAAGG TGGCTATAGG
C. alani
C. ornata
O. fallai
O. infrapunctatum
O. lichenigera
O. lineoocellatum
O. maccanni
O. microlepis
O. moco
O. n. polychroma
O. smithi
O. stenotis
O. suteri
O. waimatense
O. zealandicum
L. guichenoti
T. scincoides
C. aenea GTGAAGAGGG CCCTGCCTTG CTTGTACTCT GATGAGTTAT CTCCAACCTA TGATCT
C. alani
C. ornata
O. fallai
O. infrapunctatum
O. lichenigera
O. lineoocellatum
O. maccanni
O. microlepis
O. moco
O. n. polychroma
O. smithi
O. stenotis
O. suteri
O. waimatense
O. zealandicum
L. guichenoti
T. scincoides

References

Achen, M.G., W. Duan, T.M. Pettersson, P.J. Harms, S.J. Richardson, M.C. Lawrence, R.E.H. Wettenshall, A.R. Aldred and G. Schreiber (1993). Transthyretin gene expression in choroid plexus first evolved in reptiles. American Journal of Physiology (Regulatory Integrative Comp. Physiology). 265(34): R982-R989.

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D.L. Lipman (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research.: 3389-3402.

Arévalo, E., S.K. Davis and J.W.J. Sites (1994). Mitochondrial DNA sequence divergence and phylogenetic relationships among eight chromosome races of the *Sceloporus grammicus* complex (Phrynosomatidae) in central Mexico. Systematic Biology. 43(3): 387-418.

Austin, C.C. (1995). Molecular and morphological evolution in south Pacific Scincid lizards: morphological conservatism and phylogenetic relationships of Papuan *Lipinia* (Scincidae). Herpetologica. 51(3): 291-300.

Austin, C.C. and G.R. Zug (1999). Molecular and morphological evolution in the south-central Pacific skink *Emoia tongana* (Reptilia : Squamata): uniformity and human-mediated dispersal. Australian Journal of Zoology. 47(5): 425-437.

Baker, R.H. and R. Desalle (1997). Multiple sources of character information and the phylogeny of Hawaiian Drosophilids. Systematic Biology. 46(4): 654-673.

Bauer, A.M. and R.A. Sadlier (2000). The herpetofauna of New Caledonia. Society for the Study of Amphibians and Reptiles.

Bensasson, D., D. Zhang, D.L. Hartl and G.M. Hewitt (2001). Mitochondrial pseudogenes: evolution's misplaced witnesses. Trends in Ecology and Evolution. 16(6): 314-321.

Bensasson, D., X. Zhang and G.M. Hewitt (2000). Frequent assimilation of mitochondrial DNA by grasshopper nuclear genomes. Molecular Biology and Evolution. 17(3): 406-415.

Blackburn, D.G. (1981). An evolutionary analysis of vertebrate viviparity. American Zoologist. 26(1): 57-71.

Blackburn, D.G. (1992). Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. American Zoologist. 32: 313-321.

Blackburn, D.G. (1993). Chorioallantoic placentation in squamate reptiles: structure, function, development, and evolution. Journal of Experimental Zoology. 266: 414-430.

Blackburn, D.G. (1993). Histology of the late-stage placentae in the matrotrophic skink *Chalcides chalcides* (Lacertilia; Scincidae). Journal of Morphology. 216: 179-195.

Blackburn, D.G. (2000). Reptilian viviparity: past research, future directions, and appropriate models. Comparative Biochemistry and Physiology Part A. 127(2000): 391-409.

Braun, E.L. and R.T. Kimball (2001). Polytomies, the power of phylogenetic inference, and the stochastic nature of molecular evolution: a comment on Walsh *et al.* (1999). Evolution. 55(6): 1261-1263.

Brehm, A., J. Jesus, M. Pinheiro and D.J. Harris (2001). Relationships of scincid lizards (*Mabuya* spp; Reptilia : Scincidae) from the Cape Verde islands based on mitochondrial and nuclear DNA sequences. Molecular Phylogenetics and Evolution. 19(2): 311-316.

Bremer, K. (1988). The limits of amino acid sequence data on angiosperm phylogenetic reconstruction. Evolution. 42: 795-803.

Brown, W.C. (1991). Lizards of the genus *Emoia* (Scincidae) with observations of their evolution and biogeography. Memoirs of the Californian Academy of Sciences. 15.

Brown, W.M., M. George and A.C. Wilson (1979). Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences, USA. 76(4): 1967-1971.

Bruna, E.M., R.N. Fisher and T.J. Case (1996). Morphological and genetic evolution appear decoupled in Pacific skinks (Squamata: Scincidae: *Emoia*). Proceedings of the Royal Society of London, B. 263(1971): 681-688.

Camp, C.L. (1923). Classification of the lizards. Bulletin of the American Museum of Natural History. 48: 289-482.

Cannatella, D.C., D.M. Hillis, P.T. Chippindale, L. Weigt, A.S. Rand and M.J. Ryan (1998). Phylogeny of frogs of the *Physalaemus pustulosus* species group, with an examination of data incongruence. Systematic biology. 47(2): 311-335.

Chambers, G.K., W.M. Boon, T.R. Buckley and R.A. Hitchmough (2001). Using molecular methods to understand the Gondwanan affinities of the New Zealand biota: three case studies. Australian Journal of Botany. 49: 377-387.

Chung, D.W., B.G. Que, M.W. Rixon, M.J. Mace and E.W. Davie (1983). Characterisation of complementary deoxyribonucleic acid and genomic deoxyribonucleic acid for the β chain human fibrinogen. Biochemistry. 22:3244-3250.

Corso, G., G.M. Delitala and M. Carcupino (2000). Uterine morphology during the annual cycle in *Chalcides ocellatus tiligugu* (Gmelin) (Squamata : Scincidae). Journal of Morphology. 243(2): 153-165.

Daugherty, C., G. Patterson, C. Thorn and D. French (1990). Differentiation of the members of the New Zealand *Leiolopisma nigriplantare* species complex (Lacertilia: Scincidae). Herpetological Monographs. 4: 61-76.

Daugherty, C., G.B. Patterson and R.A. Hitchmough (1994). Taxonomic and conservation review of the New Zealand herpetofauna. New Zealand Journal of Zoology. 21: 317-323.

De Queiroz, A., M.J. Donoghue and J. Kim (1995). Separate versus combined analysis of phylogenetic evidence. Annual Review of Ecology and Systematics. 26: 657-681.

Densmore, L.D., J.W. Wright and W.M. Brown (1985). Length variation and heteroplasmy are frequent in mitochondrial DNA from parthenogenetic and bisexual lizards (genus *Cnemidophorus*). Genetics. 110: 689-707.

Dolphin, K., R. Belshaw, C.D.L. Orme and D.L.J. Quicke (2000). Noise and incongruence: interpreting results of the incongruence length difference test. Molecular Phylogenetics & Evolution. 17(3): 401-406.

Donnellan, S.C. and M.N. Hutchinson (1990). Biochemical and morphological variation in the geographically widespread lizard *Leiolopisma entrecasteauxii* (Lacertilia: Scincidae). Herpetologica. 46(2): 149-159.

Donnellan, S.C., M.N. Hutchinson and K.M. Saint (1999). Molecular evidence for the phylogeny of Australian gekkonoid lizards. Biological Journal of the Linnean Society. 67(1): 97-118.

Dowton, M. and N.J.H. Campbell (2001). Intramitochondrial recombination - is it why some mitochondrial genes sleep around? Trends in Ecology & Evolution. 16(6): 269-271.

Doyle, J.J. (1992). Gene trees and species trees: molecular systematics as one-character taxonomy. Systematic Botany. 17: 144-163.

Dutton, P.H., S.K. Davis, T. Guerra and D. Owens (1996). Molecular phylogeny for marine turtles based on sequences of the ND4-leucine tRNA and control regions of mitochondrial DNA. Molecular Phylogenetics and Evolution. 5(3): 511-512.

Edwards, A.W.F. (1972). Likelihood. Cambridge, Cambridge University Press.

Estes, R., K. De Quiroz and J. Gauthier (1988). Phylogenetic relationships within the Squamata. Phylogenetic relationships of the lizard families. Essays commemorating Charles L. Camp. Eds R. Estes and G. Pregill. Stanford, Stanford University Press.

Estes, R. and G. Pregill, Eds. (1988). Phylogenetic relationships of the lizard families. Essays commemorating Charles L. Camp. Stanford, Stanford University Press.

Evans, S.E. and J. Barbadillo (1997). Early cretaceous lizards from Las Hoyas, Spain. Zoological Journal of the Linnean Society. 119: 23-49.

Farris, J.S., M. Kallersjo, A.G. Kluge and C. Bult (1995). Constructing a significance test for incongruence. Systematic Biology. 44(4): 570-572.

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

Felsenstein, J. (1985). Phylogenies and the comparative method. The American Naturalist. 125(1): 1-15.

Flemming, A.F. and W.R. Branch (2001). An extraordinary case of matrotrophy in the African skink *Eumecia anchietae*. Journal of Morphology.

Foster, R.G., J.M. Garcia-Fernandez, I. Provencio and W.J. DeGrip (1993). Opsin localization and chromophore retinoids identified within the basal brain of the lizard *Anolis carolinensis*. Journal of Comparative Physiology A. 172: 33-45.

Friesen, V.L., B.C. Congdon, H.E. Walsh and T.P. Birt (1997). Intron variation in marbled murrelets detected using analyses of single-stranded conformational polymorphisms. Molecular Ecology. 6(11): 1047-1058.

Fu, J. (1998). Toward the phylogeny of the family Lacertidae: Implications from mitochondrial DNA 12S and 16S gene sequences (Reptilia: Squamata). Molecular Phylogenetics and Evolution. 9(4): 118-130.

Fuhn, I.E. (1967). *Pseudemoia*, eine neue monotypische Gattung aus Sudostaustralien (*Ablepharus/Emoia spenceri* Lucas and Frost). Zoologischer Anzeiger. 179: 243-247.

Georges, A., J. Birrell, K.M. Saint, W. McCord and S.C. Donnellan (1999). A phylogeny for side-necked turtles (Chelonia : Pleurodira) based on mitochondrial and nuclear gene sequence variation. Biological Journal of the Linnean Society. 67(2): 213-246.

Giannasi, N., A. Malhotra and R.S. Thorpe (2001). Nuclear and mtDNA phylogenies of the *Trimeresurus* complex: Implications for the gene versus species tree debate. Molecular Phylogenetics & Evolution. 19(1): 57-66.

Goldman, N. and S. Whelan (2000). Statistical tests of gamma-distributed rate heterogeneity in models of sequences evolution in phylogenetics. Molecular Biology and Evolution. 17(6): 975-978.

Gorr, T.A., B.K. Mable and T. Kleinschmidt (1998). Phylogenetic analysis of reptilian haemoglobins: trees, rates and divergences. Journal of Molecular Evolution. 47: 471-485.

Graybeal, A. (1994). Evaluating the phylogenetic utility of genes: a search for genes informative about deep divergences among vertebrates. Systematic Biology. 43: 174-1983.

Greer, A.E. (1970). A subfamilial classification of scincid lizards. Bulletin of the Museum of Comparative Zoology, Harvard. 139(3): 151-184.

Greer, A.E. (1974). The generic relationships of the scincid lizard genus *Leiolopisma* and its relatives. Australian Journal of Zoology, Supplementary Series. 31: 1-67.

Greer, A.E. (1980). A new species of *Morethia* (Lacertilia: Scincidae) from northern Australia, with comments on the biology and relationships of the genus. Records of the Australian Museum. 33(2): 89-122.

Greer, A.E. (1982). A new species of *Leiolopisma* (Lacertilia: Scincidae) from Western Australia, with notes on the biology and relationships of other Australian species. Records of the Australian Museum. 34(12): 549-573.

Greer, A.E. (1989). The biology and evolution of Australian lizards. Chipping Norton, Surrey Beatty and Sons.

Greer, A.E. (2001). Distribution of maximum snout-vent length among species of scincid lizards. Journal of Herpetology. 35(3): 383-395.

Greer, A.G. (1979). A phylogenetic subdivision of Australian skinks. Records of the Australian Museum. 32(8): 339-371.

Groth, J.G. and G.F. Barrowclough (1999). Basal divergences in birds and the phylogenetic utility of the nuclear RAG-1 gene. Molecular Phylogenetics & Evolution. 12(2): 115-123.

Guillette, L.J.J., R.E. Jones, K.T. Fitzgerald and H.M. Smith (1980). Evolution of viviparity in the lizard genus *Sceloporus*. Herpetolgica. 36: 201-215.

Haenel, G.J. (1997). Mitochondrial DNA variation in populations of the tree lizard, *Urosaurus ornatus*. Copeia.(1): 174-178.

Hagelberg, E., N. Goldman, P. Lio, S. Whelan, W. Schiefenhovel, J.B. Clegg and D.K. Bowden (1999). Evidence for mitochondrial DNA recombination in a human population of island Melanesia. Proceedings of the Royal Society of London - Series B: Biological Sciences. 266(1418): 485-492.

Hardy, G.S. (1977). The New Zealand Scincidae (Reptilia: Lacertilia); a taxonomic and zoogeographic study. New Zealand Journal of Zoology. 4: 221-325.

Hardy, G.S. (1982). Review of some aspects of New Zealand skink taxonomy. New Zealand herpetology. Proceeding of a symposium held at Victoria University of Wellington January 1980. Eds D. G. Newman. Wellington, New Zealand Wildlife Service. Occasional publication No. 2.

Harris, D.J., E.N. Arnold and R.H. Thomas (1998). Rapid speciation, morphological evolution, and adaptation to extreme environments in South African sand lizards (*Meroles*) as revealed by mitochondrial gene sequences. Molecular Phylogenetics & Evolution. 10(1): 37-48.

Harris, D.J., E.N. Arnold and R.H. Thomas (1998). Relationships of lacertid lizards (Reptilia, Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proceedings of the Royal Society of London - Series B: Biological Sciences. 265(1409): 1939-1948.

Harris, D.J., J.C. Marshall and K.A. Crandall (2001). Squamate relationships based on c-mos nuclear DNA sequences: increased taxon sampling improves bootstrap support. Amphibia-Reptilia. 22: 235-242.

Harris, D.J., E.A. Sinclair, N.L. mercader, J.C. Marshall and K.A. Crandall (1999). Squamate relationships based on c-mos nuclear DNA sequences. Herpetological Journal. 9: 147-151.

Harrison, L. and H.C. Weekes (1925). On the occurrence of placentation in the scincid lizard *Lygosoma entrecasteauxii*. The Proceedings of the Linnean Society of New South Wales. IV (203): 470-486.

Harvey, P.H. and M.D. Pagel (1991). The comparative method in evolutionary biology. New York, Oxford University press.

Hasegawa, M., K. Kishino and T. Yano (1985). Dating the human-age splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution. 32: 443-445.

Hickson, R.E., D. Penny and D.B. Scott (1992). Molecular systematics and evolution in New Zealand: applications to cryptic skink species. New Zealand Journal of Zoology. 19: 33-44.

Hickson, R.E., K.E. Slack and P. Lockhart (2000). Phylogeny recapitulates geography, or why New Zealand has so many species of skinks. Biological Journal of the Linnean Society. 70(3): 415-433.

Higgins, D.G., A.J. Bleasby and R. Fuchs (1991). CLUSTAL V: Improved software for multiple sequence alignment. CABIOS. 8: 189-191.

Hillis, D.M. (1987). Molecular versus morphological approaches to systematics. Annual Review of Ecology and Systematics. 18: 23-42.

Hillis, D.M. and M.T. Dixon (1991). Ribosomal DNA: molecular evolution and phylogenetic inference. The Quarterly Review of Biology. 66(4): 411-453.

Höger, T.H., K. Zatloukal, I. Waizenegger and G. Krohne (1990). Characteristation of a second highly conserved B-type lamin present in cells previously thought to contain only a single B-type lamin. Chromosoma. 99(6): 379-390.

Honda, M., H. Ota, M. Kobayashi and T. Hikida (1999). Phylogenetic relationships of Australian skinks of the *Mabuya* group (Reptilia : Scincidae) inferred from mitochondrial DNA sequences. Genes & Genetic Systems. 74(4): 135-139.

Honda, M., H. Ota, M. Kobayashi, J. Nabhitabhata, H.S. Yong and T. Hikida (1999). Evolution of Asian and African Lygosomine skinks of the *Mabuya* group

(Reptilia : Scincidae): a molecular perspective. Zoological Science. 16(6): 979-984.

Honda, M., H. Ota, M. Kobayashi, J. Nabhitabhata, H.S. Yong and T. Hikida (2000). Phylogenetic relationships, character evolution, and biogeography of the subfamily Lygosominae (Reptilia: Scincidae) inferred from mitochondrial DNA sequences. Molecular Phylogenetics and Evolution. 15(3): 452-461.

Hulsenbeck, J.P. and D.M. Hillis (1996). Parametric bootstrapping in molecular phylogenetics: applications and performance. Molecular Zoology: advances, strategies and protocols. Eds J. D. Ferraris and S. R. Palumbi. New York, Wiley-Liss.

Hutchinson, M.N. (1981). The systematic relationships of the genera *Egernia* and *Tiliqua* (Lacertilia: Scincidae). A review and immunological reassessment. Proceedings of the Melbourne Herpetological Symposium. Eds C. B. Banks and A. A. Martin. Melbourne, Zoological Board of Victoria: 176-193.

Hutchinson, M.N. and S.C. Donnellan (1988). A new species of scincid lizard related to *Leiolopisma entrecasteauxii*, from southeastern Australia. Transactions Royal Society of South Australia. 112(4): 143-151.

Hutchinson, M.N. and S.C. Donnellan (1992). Taxonomy and genetic variation in the Australian lizards of the genus *Pseudemoia* (Scincidae: Lygosominae). Journal of Natural History. 26: 215-264.

Hutchinson, M.N., S.C. Donnellan, P.R. Baverstock, M. Kreig, S. Simms and S. Burgin (1990). Immunological relationships and generic revision of the Australian lizards assigned to the genus *Leiolopisma* (Scincidae: Lygosominae). Australian Journal of Zoology. 38: 535-554.

Hutchinson, M.N. and T.D. Schwaner (1991). Genetic relationships among the Tasmanian scincid lizards of the genus *Niveoscincus*. Journal of Herpetology. 25(1): 49-58.

Irestedt, M., U.S. Johansson, T.J. Parsons and P.G.P. Ericson (2001). Phylogeny of major lineages of suboscines (Passeriformes) analysed by nuclear DNA sequence data. Journal of Avian Biology. 32(1): 15-25.

Ishiyama, M., M. Mikami, H. Shimokawa and S. Oida (1998). Amelogenin protein in tooth germs of the snake *Elaphe quadrivirgata*, immunohistochemistry, cloning and cDNA sequence. 61(5): 467-74.

Jackman, T.R., A. Larson, K. de Queiroz and J.B. Losos (1999). Phylogenetic relationships and tempo of early diversification in *Anolis* lizards. Systematic Biology. 48(2): 254-285.

Jerez, A. and M.P. Ramírez-Pinilla (2001). The alloplacenta of *Mabuya mabouya* (Sauria, Scincidae). Journal of Morphology. 249: 132-146.

Johansson, U.S., T.J. Parsons, M. Irestedt and P.G.P. Ericson (2001). Clades within the 'higher land birds', evaluated by nuclear DNA sequences. Journal of Zoological Systematics & Evolutionary Research. 39(1-2): 37-51.

Johnson, K.P. and D.H. Clayton (2000). Nuclear and mitochondrial genes contain similar phylogenetic signal for pigeons and doves (Aves: Columbiformes). Molecular Phylogenetics and Evolution. 14(1): 141-151.

Kawamura, S. and S. Yokoyama (1994). Cloning of the rhodopsin-encoding gene from the rod-less lizard *Anolis carolinensis*. Gene. 149: 267-270.

Kawamura, S. and S. Yokoyama (1995). Paralogous origin of the rhodopsinlike opsin genes in lizards. Journal of Molecular Evolution. 40: 594-600.

Kishino, H. and M. Hasegawa (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, A.G. (1989). A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Systematic Zoology. 38(1): 7-25.

Kluge, A.G. (1998). Total evidence or taxonomic congruence: cladistics or consensus classification. Cladistics-The International Journal of the Willi Hennig Society. 14(2): 151-158.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca and A.C. Wilson (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.

Kraus, F. and W.M. Brown (1998). Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences. Zoological Journal of the Linnean Society. 122(3): 455-487.

Ladoukakis, E.D. and E. Zouros (2001). Direct evidence for homologous recombination in mussel (*Mytilus galloprovincialis*) mitochondrial DNA. Molecular Biology and Evolution. 18(7): 1168-1175.

Lee, M.S.Y. (2001). Uninformative characters and apparent conflict between molecules and morphology. Molecular Biology & Evolution. 18(4): 676-680.

Lessa, E.P. and G. Applebaum (1993). Screening techniques for detecting allelic variation in DNA sequences. Molecular Ecology. 2: 119-129.

Lin, F. and H.J. Worman (1993). Structural organization of the human gene encoding nuclear lamin A and nuclear lamin C. The Journal of Biological Chemistry. 268(22): 16321-16326.

Lopez, J.V., S. Cevario and S.J. O'Brein (1996). Complete nucleotide sequences of the domestic cat (*Felis catus*) mitochondrial genome and a transposed mtDNA tandem repeat (Numt) in the nuclear genome. Genomics. 33: 229-246.

Lopez, J.V., N. Yuhki, R. Masuda, W. Modi and S.J. Obrien (1994). Numt, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. Journal of Molecular Evolution. 39(2): 174-190.

Lyons, L.A., T.F. Laughlin, N.G. Copeland, N.A. Jenkins, J.E. Womack and S.J. O'Brien (1997). Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nature Genetics. 15(1): 47-56.

Macey, J.R., A. Larson, N.B. Ananjeva, Z. Fang and T.J. Papenfuss (1997). Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Molecular Biology and Evolution. 14(1): 91-104.

Malone, C.L., T. Wheeler, J.F. Taylor and S.K. Davis (2000). Phylogeography of the Caribbean rock iguana (*Cyclura*): implications for conservation and insights on the biogeographic history of the West Indies. Molecular Phylogenetics and Evolution. 17(2): 269.

Mausfeld, P., M. Vences, A. Schmitz and M. Veith (2000). First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. Molecular Phylogenetics & Evolution. 17(1): 11-14.

Melville, J. and R. Swain (2000). Mitochondrial DNA-sequence based phylogeny and biogeography of the snow skinks (Squamata: Scincidae: *Niveoscincus*) of Tasmania. Herpetologica. 56(2): 196-208.

Messanger, S.L. and J.A. McGuire (1998). Morphology, molecules and the phylogenetics of cetaceans. Systematic Biology. 47(1): 90-124.

Meyer, A. (1994). Shortcomings of the cytochome b gene as a molecular marker. Trends in Ecology and Evolution. 9(8): 278-280.

Mickevich, M.F. and J.S. Farris (1981). The implications of congruence in *Menidia*. Systematic Zoology. 30: 351-370.

Miyamoto, M.M. and W.M. Fitch (1995). Testing species phylogenies and phylogenetic methods with congruence. Systematic Biology. 44: 64-76.

Mourier, T., A.J. Hansen, E. Willerslev and P. Arctander (2001). The human genome project revels a continuous transfer of large mitochondrial fragments to the nucleus. Molecular Biology and Evolution. 18(9): 1833-1837.

Mundy, N.I., A. Pissinatti and D.S. Woodruff (2000). Multiple nuclear insertions of mitochondrial Cytochrome b sequences in Callitrichine primates. Molecular Biology and Evolution. 17(7): 1075-1080.

Nakachi, Y., T. Hayakawa, H. Oota, K. Sumiyama, L. Wang and S. Ueda (1997). Nucleotide compositional constraints on genomes generate alanine-, glycine-, and proline-rich structures in transcription factors. Molecular Biology and Evolution. 14(10): 1042-1049.

Nakajima, N. and K. Abe (1995). Genomic structure of the mouse A-type lamin gene locus encoding somatic and germ cell-specific lamins. FEBS Letters. 365: 108-114.

Neill, W.T. (1964). Viviparity in snakes: some ecological and zoogeographic considerations. The American Naturalist. 98: 35-55.

Nielsen, K.K. and P. Arctander (2001). Recombination among multiple mitochondrial pseudogenes from a passerine genus. Molecular Phylogenetics and Evolution. 18(3): 362-369.

O'Brein, S.J., J.E. Womack, L.A. Lyons, K.J. Moore, N.A. Jenkins and N.G. Copeland (1993). Anchored reference loci for comparative genome mapping in mammals. Nature Genetics. 3: 103-112.

Olmstead, R.G. and J.A. Sweere (1994). Combining data in phylogenetic systematics: an empirical approach using three molecular datasets in the Solanaceae. Systematic Biology. 43(4): 467-481.

Omland, K.E. (1994). Character congruence between a molecular and a morphological phylogeny for dabbling ducks (*Anas*). Systematic Biology. 43(3): 369-386.

Pamilo, P. and M. Nei (1988). Relationships between gene trees and species trees. Molecular Biology and Evolution. 5: 568-583.

Parkinson, C.L., K.R. Zamudio and H.W. Greene (2000). Phylogeography of the pitviper clade Agkistrodon: historical ecology, species status, and conservation of cantils. Molecular Ecology. 9(4): 411-420.

Passoni, J.C., M.L. Benozzati and M.T. Rodrigues (2000). Mitochondrial DNA polymorphism and heteroplasmy in populations of the three species of *Tropidurus* of the *nanuzae* group (Squamata, Tropiduridae). Genetics & Molecular Biology. 23(2): 351-356.

Patterson, G.B. and C.H. Daugherty (1994). *Leiolopisma stenotis*, m. sp. (Reptilia: Lacertidae: Scincidae) from Stewart Island, New Zealand. Journal of the Royal Society of New Zealand. 24: 125-132.

Patterson, G.B. and C.H. Daugherty (1995). Reinstatement of the genus *Oligosoma* (Reptilia: Lacertilia: Scincidae). Journal of the Royal Society of New Zealand. 25(3): 327-331.

Peter, M., G.T. Kitten, C.F. Lehner, K. Vorburger, S.M. Bailer, G. Maridor and E.A. Nigg (1989). Cloning and sequencing of the cDNA clones encoding chicken lamins A and B_1 and comparison of the primary structures of vertebrate A- and B-type lamins. Journal of Molecular Biology. 208(1): 393-404.

Porter, C.A., S.L. Page, J. Czelusniak, H. Schneider, M.P.C. Schneider and M. Goodman (1997). Phylogeny and evolution of selected primates as determined by sequences of the epsilon-globin locus and 5' flanking regions. International Journal of Primatology. 18(2): 261-295.

Posada, D. and K.A. Crandall (1998). Modeltest: testing the model of DNA substitution. Bioinformatics. 14(9): 817-818.

Presch, W. (1988). Phylogenetic relationships of the Scincomorpha. Phylogenetic relationships of the lizard families. Essays commemorating Charles L. Camp. Eds R. Estes and G. Pregill. Stanford, Stanford University Press.

Prychitko, T.M. and W.S. Moore (1997). The utility of DNA sequences of an intron from the β-fibrinogen gene in phylogenetic analysis of woodpeckers (Aves: Picidae). Molecular Phylogenetics and Evolution. 8(2): 193-204.

Prychitko, T.M. and W.S. Moore (2000). Comparative evolution of the mitochondrial Cytochrome b gene and nuclear β-fibrinogen intron 7 in woodpeckers. Molecular Biology and Evolution. 17(7): 1101-1111.

Rambaut, A. (1995). Se-Al. Sequence alignment editor. Evolutionary Biology Group, University of Oxford.

Rawlinson, P.A. (1974). Revision of the endemic southeastern Australian lizard genus *Pseudemoia* (Scincidae: Lygosominae). Memoirs of the National Museum of Victoria. 35: 87-96.

Rieppel, O. (1984). The cranial morphology of the fossorial lizard genus *Dibamus* with a consideration of its phylogenetic relationships. Journal of Zoology, London. 204: 289-327.

Rodríguez, F., J.F. Oliver, A. Marín and J.R. Medina (1990). The general stochastic model of nucleotide substitutions. Journal of Theoretical Biology. 142: 485-501.

Rohlf, F.J. and R.R. Sokal (1981). Statistical tables. San Fransisco, Freeman.

Saint, K.M., C.C. Austin, S.C. Donnellan and M.N. Hutchinson (1998). C-mos, a nuclear marker useful for squamate phylogenetic analysis. Molecular Phylogenetics and Evolution. 10: 259-263.

Schreiber, G., T.M. Pettersson, B.R. Southwell, A.R. Aldred, P.J. Harms, S.J. Richardson, R.E.H. Wettenshall, W. Duan and S.C. Nicol (1993). Transthyretin expression evolved more recently in liver than in brain. Comparative Biochemistry and Physiology. 105B(2): 317-325.

Shedlock, A.M. and N. Okada (2000). SINE insertions: powerful tools for molecular systematics. Bioessays. 22(2): 148-160.

Shimodaira, H. and M. Hasegawa (1999). Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Molecular Biology and Evolution. 16(8): 1114-1116.

Shine, R. (1985). The evolution of viviparity in reptiles: an ecological analysis. Biology of the Reptilia. Eds C. Gans and F. Billett. New York, John Wiley and Sons,. 15: 605 - 694.

Shine, R. and L.J. Guillette (1988). The evolution of viviparity in reptiles: a physiological model and its ecological consequences. Journal of Theoretical Biology. 132: 43-50.

Simmons, M.P. and H. Ochoterena (2000). Gaps as characters in sequence-based phylogenetic analysis. Systematic Biology. 49(2): 369-381.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu and P. Flook (1994). Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. entomol. Soc. Am. 87(6): 651-701.

Slade, R.W., C. Moritz, A. Heideman and P.T. Hale (1993). Rapid assessment of single-copy nuclear DNA variation in diverse species. Molecular Ecology. 2: 359-373.

Slowinski, J.B. (2001). Molecular polytomies. Molecular phylogenetics and evolution. 19(1): 114-120.

Smith, S.A. (1996). The evolution of viviparity in the Australia Scincid lizard *Saiphos equalis*. School of Biological Sciences. Sydney, Australia, University of Sydney: 97.

Smith, S.A., C.C. Austin and R. Shine (2001). A phylogenetic analysis of variation in reproductive mode within an Australian lizard (*Saiphos equalis*, Scincidae). Biological Journal of the Linnean Society. 74(2): 131-139.

Smith, S.A. and R. Shine (1997). Intraspecific variation in reproductive mode within the scincid lizard *Saiphos equalis*. Australian Journal of Zoology. 45: 435-445.

Smith, W.C., G. Adamus, ., H. Van Der Wel, A. Timmers, K. Palczewski, R.J. Ulshafer, P.A. Hargrave and J.H. McDowell (1995). Alligator rhodopsin: sequence and biochemical properties. Experimental eye research. 61(5): 569-678.

Sorenson, M.D. (1999). TreeRot. Boston, MA, Boston University.

Springer, M.S., R.W. DeBry, C. Douady, H.M. Amrine, O. Madsen, W.W. de Jong and M.J. Stanhope (2001). Mitochondrial versus nuclear gene sequences in deep-level mammalian phylogeny reconstruction. Molecular Biology & Evolution. 18(2): 132-143.

Stewart, J.R. and M.B. Thompson (1993). A novel pattern of embryonic nutrition in a viviparous reptile. Journal of Experimental Biology. 174: 97-108.

Stewart, J.R. and M.B. Thompson (1994). Placental structure of the Australian lizard, *Niveoscincus metallicus* (Squamata: Scincidae). Journal of Morphology. 220: 223-236.

Stewart, J.R. and M.B. Thompson (1996). Evolution of reptilian placentation: development of extraembryonic membranes of the Australian scincid lizards, *Bassiana duperreyi* (oviparous) and *Pseudemoia entrecasteauxii* (viviparous). Journal of Morphology. 227(3): 349-370.

Stewart, J.R. and M.B. Thompson (1998). Placental ontogeny of the Australian scincid lizards *Niveoscincus coventryi* and *Pseudemoia spenceri*. Journal of Experimental Zoology. 282(4-5): 535-559.

Sumiyama, K., K. Washio-Watanabe, N. Saiou, T. Hayakawa and S. Ueda (1996). Class III POU genes: generation of homopolymeric amino acid repeats under GC pressure in mammals. Journal of Molecular Evolution. 43: 170-178.

Swain, R. and S.M. Jones (1997). Maternal-fetal transfer of H-3-labelled leucine in the viviparous lizard *Niveoscincus metallicus* (Scincidae, Lygosominae). Journal of Experimental Zoology. 277(2): 139-145.

Swofford, D.L. (1999). PAUP*. Phylogenetic analysis using parsimony (* and other methods). . Sunderland, Massachusetts, Sinauer Associates.

Swofford, D.L., G.J. Olsen, P.J. Waddell and D.M. Hillis (1996). Phylogenetic inference. Molecular Systematics. Eds D. M. Hillis, C. Moritz and B. K. Mable. Sunderland, Massachusetts, Sinauer Associates.

Templeton, A.R. (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, M.B., B.K. Speake, K.J. Russell and R.J. McCartney (2001). Utilisation of lipids, proteins, ions and energy during embryonic development of Australian oviparous skinks in the genus *Lampropholis*. Comparative Biochemistry and Physiology Part A.: 313-326.

Thompson, M.B., B.K. Speake, J.R. Stewart, K.J. Russell and R.J. McCartney (2001). Placental nutrition in the Tasmanian skink, *Niveoscincus ocellatus*. Journal of Comparative Physiology - B, Biochemical, Systemic, & Environmental Physiology. 171(2): 155-160.

Thompson, M.B. and J.R. Stewart (1994). Egg and clutch size of the viviparous Australian skink, *Pseudemoia pagenstecheri* and the identity of a species with Type III allantoplacentae. Journal of herpetology. 24(4): 519-521.

Thompson, M.B., J.R. Stewart and B.K. Speake (2000). Comparison of nutrient transport across the placenta of lizards differing in placental complexity. Comparative Biochemistry and Physiology Part A. 127(2000): 469-479.

Thompson, M.B., J.R. Stewart, B.K. Speake, C.R. Murphy and M. Hosie (unpub ms). Evolution of viviparity: what can Australian reptiles tell us? .

Thompson, M.B., J.R. Stewart, B.K. Speake, K.J. Russell and R.J. McCartney (1999). Placental transfer of nutrients during gestation in the viviparous lizard, *Pseudemoia spenceri*. Journal of Comparative Physiology - B, Biochemical, Systemic, & Environmental Physiology. 169(4-5): 319-328.

Thompson, M.B., J.R. Stewart, B.K. Speake, K.J. Russell, R.J. McCartney and P.F. Surai (1999). Placental nutrition in a viviparous lizard (*Pseudemoia pagenstecheri*) with a complex placenta. Journal of Zoology. 248(Part 3): 295-305.

Thompson, M.B., J.R. Stewart, B.K. Speake, K.J. Russell and R.J. Mccartney (2001). Utilisation of nutrients by embryos of the enigmatic Australian viviparous skinks *Niveoscincus coventryi*. Journal of Experimental Zoology. 290: 291-298.

Tinkle, D.W. and J.W. Gibbons (1977). The distribution and evolution of viviparity in reptiles. Miscellaneous Publications of the Museum of Zoology, University of Michigan. 154: 1-55.

Towns, D.R., C.H. Daugherty and D.G. Newman (1985). An overview of the ecological biogeography of the New Zealand lizards (Gekkonidae, Scincidae). Biology of the Australasian frogs and reptiles. Eds G. Grigg, R. Shine and H. Ehmann. , Royal Zoological Society of New South Wales.

Toyosawa, S., C. Ohuigin, F. Figueroa, H. Tichy and J. Klein (1998). Identification and characterization of amelogenin genes in monotremes, reptiles, and amphibians.

Proceedings of the National Academy of Sciences of the United States of America. 95(22): 13056-13061.

Uetz, P. (2001). The EMBL reptile database: http://www.embl-heidelberg.de/~uetz/LivingReptiles.html. .

Venkatesh, B., Y. Ning and S. Brenner (1999). Late changes in spliceosomal introns define clades in vertebrate evolution. Proceedings of the National Academy of Sciences of the United States of America. 96: 10267-10271.

Vorburger, K., C.F. Lehner, G.T. Kitten, H.M. Eppenberger and E.A. Nigg (1989). A second higher vertebrate B-type lamin cDNA sequence determination and *in vitro* processiong of chicken lamin B_2. Journal of Molecular Biology. 208(1): 405-416.

Walsh, H.E. and V.L. Friesen (2001). Power and stochasticity in the resolution of soft polytomies: a reply to Braun *et al.* Evolution. 55(6): 1264-1266.

Walsh, H.E., M.G. Kidd, T. Moum and V.L. Friesen (1999). Polytomies and the power of phylogenetic inference. Evolution. 53(3): 932-937.

Weekes, H.C. (1935). A review of placentation among reptiles with particular regard to the function and evolution of the placenta. Proceedings of the Zoological Society, London. 2: 625-645.

Weiss, A. and L.A. Leinwand (1996). The mammalian myosin heavy chain gene family. Annual Review of Cellular and Developmental Biology. 12: 417-439.

Wells, R.W. and C.R. Wellington (1985). A classification of the amphibia and reptilia of Australia. Australian Journal of Herpetology, Supplementary Series. 1: 1-61.

Welter, C., S. Dooley and N. Blin (1989). A rapid protocol for the purification of mitochondrial DNA suitable for studying restriction length polymorphisms. Gene. 83: 169-172.

Williams, S.T. and N. Knowlton (2001). Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. Molecular Biology and Evolution. 18(8): 1484-1493.

Zamudio, K.R., K.B. Jones and R.H. Ward (1997). Molecular systematics of short-horned lizards - biogeography and taxonomy of a widespread species complex. Systematic Biology. 46(2): 284-305.

Zhang, D.X. and G.M. Hewitt (1996). Nuclear integrations: challenges for mitochondrial DNA markers. Trends in Ecology & Evolution. 11(6): 247-251.

Zug, G.R., L.J. Vitt and J.P. Caldwell (2001). Herpetology: an introductory biology of amphibians and reptiles. San Diego, California, Academic Press.