THE TAXONOMY AND PHYLOGENETIC RELATIONSHIPS OF AUSTRALIAN SCINCID
LIZARDS (SCINCIDAE : LYGOSOMINAE)

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had satisfied requirements for admission to this degree. This thesis represents
a major part of the prescribed program of study.

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ABSTRACT

The taxonomic and phylogenetic relationships of *Lampropholis* skinks were investigated using molecular criteria. Although allozyme evolution was identified as occurring at a relatively slow rate, genetic diversity among taxa is substantial and of an order of magnitude not generally contemplated previously.

Using allozyme data, several described species were identified as consisting of species' complexes, while relationships within *Lampropholis* are considered sufficient to identify four taxa considered worthy of generic rank.

The immunological data supports the concept of three monophyletic groups within the Australian Lygosominae. Dichotomy of one of these groups (the **Eugongylus**), based on fusion of the atlantal bones is supported, while the dichotomy of the genus *Leiolopisma*, on the basis of reproductive mode, is also supported.

Due to the observed diversity among Australian Lygosominae and between Australian taxa and those of New Guinea, New Zealand and South East Asian, a Gondwanan radiation is hypothesized.

CERTIFICATE

Expect where otherwise stated, all work presented in this thesis is the original work of the author and has not been presented for a degree award at this or any other University.

Shelley Burgin

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ACKNOWLEDGMENTS.

Of the numerous people who have contributed to this thesis, I would first like to pay tribute to Dr Michael Sabath. Unfortunately he won't read these lines since he died of cancer some years before this thesis was begun. However, in the mid-1970s when I entered University (one of the new wave of "mature students", without scientific background, unsure of myself and with crushing external pressures) Mike "knew" I would make it in the field of biology and refused to consider any other eventuality. In his last days, extremely ill and in severe pain, he wrote a reference which landed me my first scientific appointment. I am proud to say that I was one of Sabath's students.

I have also been extremely fortunate to have worked with, and been guided by, Assoc. Prof. John Pernetta. Like Mike he had no direct involvement with this thesis but throughout my graduate years he has been a friend and mentor.

Since arriving at Macquarie University, I have had the constant support, encouragement and friendship of my supervisors (Dr David Briscoe and Dr Jean Joss) throughout all aspects of my career, both as a student and as a member of academic staff. In addition, many other staff have supported and guided my progress. Dr George McKay has been of tremendous help, having aided with the analyses

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LIST OF ABBREVIATIONS

Abbreviation	Definition
my/mya	millions years (ago)
ID/IDs	immunological distance (s)
MC'F	micro-complement fixation
genus	specific / generic level
complex	grouping within genus
subgroup	within suprageneric groups
group	suprageneric groups

PREFACE.

0.01: INTRODUCTION TO THE GARDEN OR PENNY SKINKS, *LAMPROPHOLIS*.

Representatives of *Lampropholis* scincid lizards are widely distributed in populated areas on the eastern coast of Australia. Due to some species predilection for disturbed areas, they are generally abundant throughout regions of human habitation including parkland and urban gardens. Despite this widespread association with humans, the group has not been extensively studied. Thus although they are sufficiently abundant to gain common names such as garden or penny skinks, little is known of even their basic biology or natural history.

This preface is therefore a summary of the general biology of the group as it has been recorded. It is also augmented by my own observations in the field while collecting animals for molecular taxonomic study. Subsequently it represents a series of 'thumb-nail' sketches of the species within the genus *Lampropholis*. Information is included on several taxa as yet not formally described, but recognized as distinct populations (Cogger, quoted in Baker, 1979; Ingram, 1986). It is typical of this group that such taxa exist. Although long accepted as representing separate species, 'good' morphological characters have not always been forthcoming (Greer, pers. comm.). Consequently, while each group

is treated as a separate taxon, it is not my intention to regard this work as constituting formal descriptions or indeed to claim recognition as having 'discovered' them. Dr. Glen Ingram (Queensland Museum) initially indicated that these and other populations may be of specific status and has more recently published information to this effect (Ingram, 1986). Donnellan (in press) has undertaken a karyotypic study of these taxa and Mather (1986) has investigated the status of *L. delicata* and its congeners in southeast Queensland. This work has confirmed that several of the populations are genetically distinct. They are therefore treated here as separate taxa.

0.02: HABITAT OVERLAP AMONG TAXA.

Skinks of the genus *Lampropholis* are relatively small, terrestrial, diurnal and oviparous. All are superficially similar in appearance, the majority possess pentadactyle limbs. The one known exception has tetradactyle forelimbs. In general they are found in moist habitats on the east coast of Australia[1]. Frequently species are restricted to typical refugia areas such as the snowgrass areas of wet sclerophyll forests on Barrington Tops and the northern rainforest blocks of Queensland. Conversely other taxa are widespread, invading disturbed habitat or openings in forested areas throughout their range (Cogger, 1983).

Within their range all species have been observed to occur, if

not in overlap, at least in close proximity to at least one other taxon, while up to four *Lampropholis* species may be observed in close association in many habitats. Other superficially similar taxa such as those of the genera *Leiolopisma* and *Carlia* also frequently share habitat and are encountered when searching for *Lampropholis*. However, within these areas of overlap, species may utilise different microhabitats, for example *L. challengeri* complex animals tend to be restricted to more mesic environments than their sister complex, *L. delicata.* Spellerberg (1972) and Greer (1980) demonstrated that species with a preference for shaded areas had a lower maximum critical temperature (measured cloacally) than species from more open habitats.

(1) Pertinent Australian geographical information referred to in this text are included in a map (Fig. 0.1)
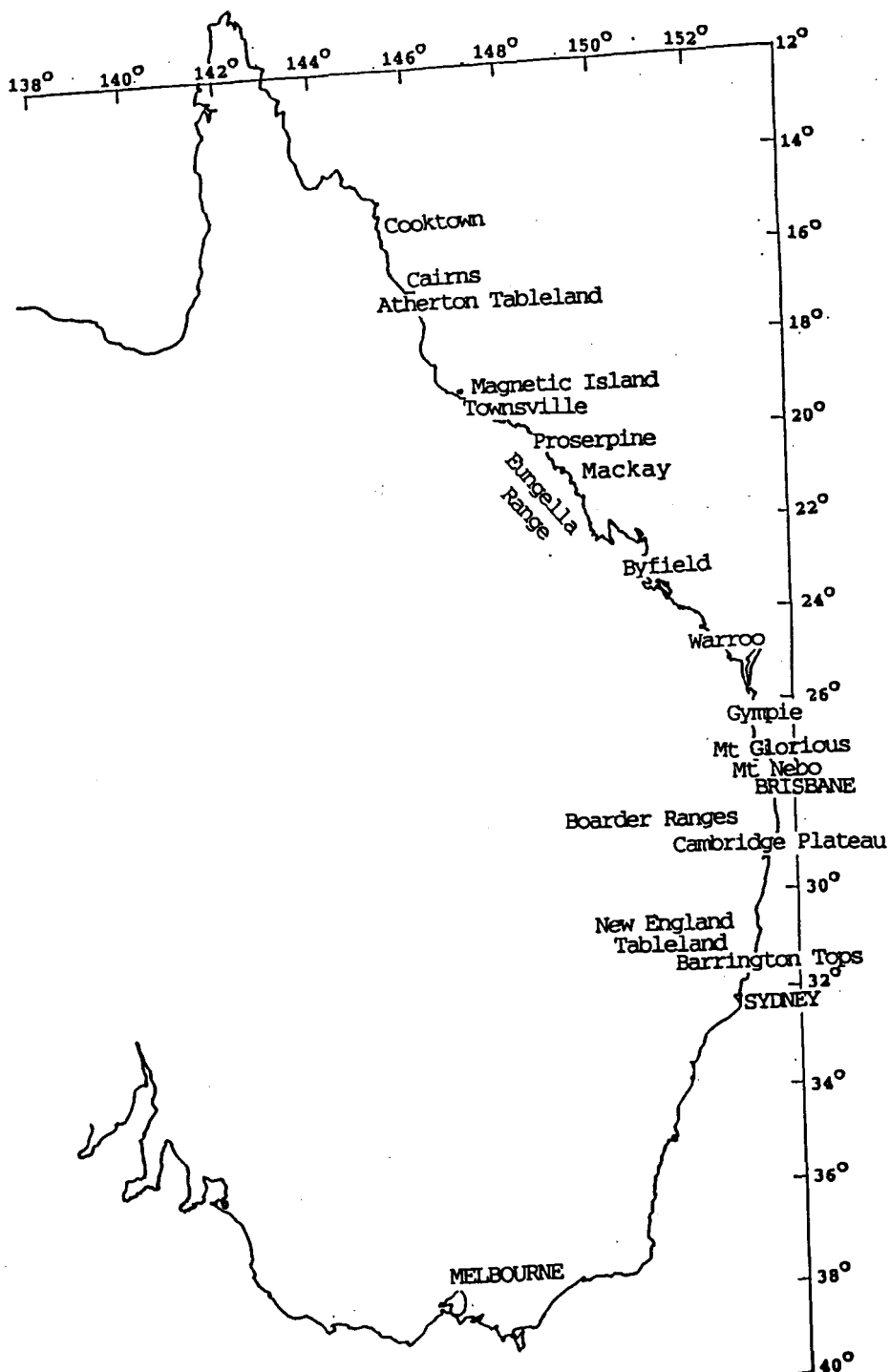
Figure 0.01: Pertinent Australian geographical information referred to in the text (supplementary information on collecting localities is contained in Appendix, Table A.01-A.02).

Plate 0.01: *Lampropholis amicula* (Ingram & Rawlinson, 1981)

0.03:   *LAMPROPHOLIS* BIOLOGY.

0.03.01: *Lampropholis amicula* (Ingram & Rawlinson, 1981)

Lampropholis amicula is the smallest member of the genus   (snout-vent length 22-34 mm) and is frequently mistaken for juvenile *L. delicata* because it has very similar coloration and body proportions. The species is little known, having been first collected in 1973 (Ingram & Rawlinson, 1981).

Its range is bounded by the Burnett Range south to Daisy Hill (13 km south of Brisbane) and west to the Great Dividing Range (Ingram & Rawlinson, 1981). It is a ground dwelling heliothermic species which may be observed foraging in open forest or open heath, frequently along track verges. When disturbed it generally disappears into the base of grass clumps or leaf litter. At least in the Rainbow Beach area, this species lives in a reptile-rich environment, with various *Ctenotus, Sphenomorphus, Carlia* and other *Lampropholis* species sharing the same macrohabitats.

Plate 0.02: *Lampropholis basiliscus* (Ingram & Rawlinson, 1981)

0.03.02: *Lampropholis basiliscus* (Ingram & Rawlinson, 1981).

Lampropholis basiliscus is relatively larger than *L. amicula* (snout-vent length 18-47mm). It has a disjunct distribution in the two major rainforest blocks of Northern Queensland. In the north *L. basiliscus* has been collected in areas from Mt. Webb (50 km north of Cooktown) to the Paluma Range, west of Townsville. Some 290 km south, it has been collected from Proserpine south to the Eungella range, west of Mackay (Cogger, 1983; Greer & Kluge, 1980; Ingram & Rawlinson, 1981).

Animals were observed in a variety of habitats. In the Crystal Cascades area, near Cairns, *L. basiliscus* was seen in areas of unconsolidated rock scattered among mature closed canopy rainforest trees with little understory growth. This habitat was very similar to some *L. challengeri* habitat in the Lamington Plateau area (Southern Queensland). The species has also been observed in large numbers in very disturbed habitat in a small coppice of trees at the edge of rainforest on the Paluma Range. Whenever *L. basiliscus* were observed however, it was in filtered sunlight or shaded areas of relatively high humidity. Although never seen basking, individuals tend to retreat under cover when the sun disappears behind a cloud, in the same manner observed in the shuttling heliotherms such as *L. delicata* and *L. guichenoti*.

Lampropholis basiliscus has been observed to forage sympatrically with *L. czechurai*, *L. tetradactyla* and, to a lesser extent, *L.*

sp. 1. It tends to be a microhabitat generalist which forages widely on the forest floor often in areas of unconsolidated rock, 'lawyer vine' or unconsolidated leaf litter. Sometimes this species will move into open areas when chased. It is unusual among species of this genus, since it tends to 'bolt' rather than 'go to ground' as its congeners generally behave when disturbed. Other unusual attributes of this species are that it forages at night and will forage and rest well above ground level. In Danbulla State Forest near Tinaroo Lake (Northern Queensland) one individual was observed to fall, apparently from a bird's nest fern over two metres up a tree at approximately 9.00 p.m. *Lampropholis basiliscus* has also been observed 'resting' on the lower leaves of trees at night.

Sexually dimorphic colour patterns allow observation of some niche separation between sexes, at least during the period immediately preceding and during oviposition (December/January). As with all species, home range behaviour was observed, although this home range shifts as the day progresses and the sun flecks move on the forest floor. Males were frequently observed to be in association with logs, prop roots, and other 'high' vantage points while females tended to retain a home range with lower vantage points.

Plate 0.03: *Lampropholis caligula* (Ingram & Rawlinson, 1981)

0.03.03: *Lampropholis caligula* (Ingram & Rawlinson, 1981).

Lampropholis caligula has a snout-vent length of 35-48 mm. Its short limbs and relatively long body give the impression of an elongate *L. delicata* (Cogger, 1983; Ingram & Rawlinson, 1981). This species has a restricted distribution, only being recorded from the woodlands and tall wet sclerophyll forests of Barrington Tops, Central New South Wales. It has been observed in association with leaf litter and snowgrass in upland areas and also on the edge of *Nothofagus moorei* (Antarctic Beech) forests along creeks in the region. This animal has a novel locomotion pattern which gives the appearance of 'swimming' through the snow grass. *Lampropholis caligula* is also unusual in that it has only ever been found in low abundance. However, within this habitat it occurs at least seasonally, with extremely high numbers of *Leiolopisma entrecasteauxii*.

Plate 0.04: *Lampropholis challengeri* (Boulenger, 1887)

0.03.04: *Lampropholis challengeri* (Boulenger, 1887).

Lampropholis challengeri, as with most members of the genus, is little known despite its early description, relatively large body size (snout-vent length approximately 50 mm) and relatively slow movement. This species inhabits rainforest and adjacent wet sclerophyll forests in coastal areas and ranges from Mideastern New South Wales to far Northern Queensland (Cogger, 1983). As with *L. basiliscus*, this species has been observed foraging in a variety of different microhabitats, including the lower branches of trees. On Cambridge Plateau (Northern New South Wales) the species was active in leaf litter and around buttress roots of rainforest trees. It was also observed in association with fallen logs. Dale (1973) recorded that the species was 'quite common' in mountainous areas near Brisbane. Within the Sydney region, the species is frequently very common in association with sandstone cliffs. However, after wet weather the animals appear to disperse widely having been observed in gardens several kilometers from 'typical' habitat.

Domrow (1974) confirmed the presence of mites parasitising this species while a single individual was observed to be parasitised by a tick. This latter observation is unusual in such small reptiles.

Plate 0.05: *Lampropholis czechurai* (Ingram & Rawlinson, 1981)

0.03.05: *Lampropholis czechurai* (Ingram & Rawlinson, 1981).

Lampropholis czechurai is a relatively small (snout-vent length 20-34mm) member of the group from North Queensland. Its presence has been confirmed in rainforests from Shipton's Flat (30 km south of Cooktown) to Charmillin Creek in the southern region of the Atherton Tableland (Ingram & Rawlinson, 1981). During the fieldwork for this project, the species was also observed in the more southerly rainforest block in the Eungella area of North Queensland. *Lampropholis czechurai* has been observed in overlap with *L. tetradactyla*, *L. sp.3*, and *L. basiliscus*, all being observed in the same fern bank at the side of a track but at different times of the day. However, despite some close association with its congeners in dry areas, *L. czechurai* was more commonly observed to forage or rest in moist areas of closed canopy. It was also frequently observed in very moist areas such as fern banks beside creeks in the rainforest, in contrast to *L. sp.3* which was generally observed in relatively drier microhabitats. In common with *L. basiliscus* and *L. czechurai* this species was seen foraging relatively late in the day, frequently at dusk.

Plate 0.06: *Lampropholis delicata* (De Vis, 1888)

0.03.06: *Lampropholis delicata* (De Vis, 1888).

Lampropholis delicata is a widely distributed species occurring in open forest types from South Eastern South Australia through Southern Victoria and Eastern New South Wales to North Eastern Queensland (Cogger, 1983). In addition the species occurs in Eastern Tasmania (Green, 1981) and has been introduced to Hawaii (Baker, 1979; Loveridge, 1934; Oliver & Shaw, 1953) and New Zealand (Hardy, 1977).

As observed with many species of *Lampropholis*, the preferred microhabitat varies with prevailing weather conditions. In general this species tends to be associated with sun flecks in forested areas with relatively uniform, although open, canopy cover. However in upland areas such as the *N. moorei* forests of the Boarder Ranges (Northern New South Wales) the species is more common in relatively open areas along road and track verges. This behaviour is also common during the cooler months of the year in other parts of the range of *L. delicata*. Baker (1979) commented both on this species being widely distributed in urban areas and its importance in the Hawaiian zoogeography, being distributed over habitats from the dry lowlands to wet upland regions. Outside of closed forest habitat, *L. delicata* tends to be restricted to areas of permanent cover representing relatively moist microhabitats, such as logs and rock piles (Belmont, 1977) and is frequently observed in more open habitat (including urban

gardens) in association with relatively deep leaf litter such as at the bases of mature eucalypts and other appropriately moist areas.

Clarke (1965) recorded growth patterns in *L. delicata* and observed that young hatch in summer and reach maturity in the following year. Joss and Minard (1985) also studied the Sydney *L. delicata* population. They confirmed that oviposition occurred in summer with ovaries being quiescent during autumn and winter (i.e. February to July). Between August and October follicles grew, with one or two per ovary becoming vitellogenic and being ovulated in October or November. After eggs were laid (approximately one month later) a second wave of vitellogenic follicles occurred, subsequently these were observed to regress after mating in late summer. Males produced mature sperm during October and November and again in February. Testes are depleted of sperm by the end of autumn. The females thus carry the sperm over winter, with developing eggs being fertilized in the spring.

While small individuals occasionally have a single egg, two is more common. Most commonly females carry three eggs, with large individuals occasionally producing up to five eggs. In Hawaii Baker (1979) reported that females may produce as many as seven eggs (mean clutch size on Hawaii being 4.7; range being 3-7) although egg number per individual varied among islands.

Clutch size in oviparous species is known to be correlated with female size. Baker (1979) suggested that the smaller size attained on Oahu Island (Hawaiian group) may have been a result of greater competition (both intraspecific and interspecific) than on Kauai or Hawaii. However, since on some islands the species apparently grows to a significantly larger body size than has been recorded anywhere in Australia this phenomenon may be attributed to reduced predation pressure, allowing individuals to live longer and therefore attain a greater body size and consequently achieve larger litter size. Observations of predation give some basis for the prediction that large females are at great predatory risk immediately preceding oviposition.

Plate 0.07: *Lampropholis guichenoti* (Duméril & Bibron, 1839)

0.03.07: *Lampropholis guichenoti* (Duméril & Bibron, 1839)

Lampropholis guichenoti and *L. delicata* are relatively more better known than other species in the genus. This is undoubtedly due to their abundance in urban areas of the Australian east coast. The range of *L. guichenoti* extends from South Eastern South Australia through most of Victoria and Eastern New South Wales to South Eastern Queensland. Within this range the species is broadly in overlap with *L. delicata* although *L. guichenoti* tends to tolerate drier areas (Cogger, 1983). Both achieve similar body temperatures in the field despite *L. guichenoti* preferring air temperatures approximately one degree higher (Graham, 1987). Thus *L. guichenoti* tends to be in open areas, frequently in association with leaf litter at the base of trees or in rockpiles. Where the habitats of the two species overlap, *L. guichenoti* is invariably in more open areas, although frequently both species are associated with disturbed habitat.

Joss and Minard (1985) observed that *L. guichenoti* had a similar reproductive cycle to *L. delicata*. However, in seasons of high rainfall, *L. guichenoti* may produce two clutches of eggs in a single season in the Sydney region. Elsewhere indications are that this is generally the case (Milton, 1980, Brisbane area; Pengilley, 1972, Southern Highlands of New South Wales) or, alternatively, Milton (1980) suggested that the species may breed later in South Eastern Queensland. In the New England Tableland

area (Northern New South Wales), however, the species apparently produces only one clutch annually (Simbotwe, 1985). Simbotwe (1985) recorded a clutch size of 1 - 3 eggs (mean=2.26) and suggested that recruitment began in February. Juvenile growth rate is rapid, both sexes reach sexual maturity in eight to nine months. Subsequently growth rates decrease (Heatwole, 1976; Simbotwe, 1985). Egg incubation requirements of the two species, *L. delicata* and *L. guichenoti*, are similar since communal nesting occurs (Mitchell, 1959; Wells, 1979; 1981).

Some sexual dimorphism has been identified in *L. guichenoti* with both head width and height being significantly larger in males than in females (Simbotwe, 1985). Colour pattern varies between sexes as it does in various taxa including *L. basiliscus* and some populations of *L. delicata*.

Plate 0.08: *Lampropholis mirabilis* (Ingram & Rawlinson, 1981)

0.03.08: *Lampropholis mirabilis* (Ingram & Rawlinson, 1981).

Lampropholis mirabilis is a relatively large member of the genus, typically reaching 50 mm snout-vent length. It has long limbs and long toes reflecting its preference for a partially saxicoline lifestyle on large granite boulders (Ingram & Rawlinson, 1981). This species is restricted to such boulders on Magnetic Island and the nearby mainland of Mt. Elloit and Cape Cleveland (North Queensland). Although considered rare due to its restricted distribution (Covacivich *et al*, 1982), within its range the species is abundant. Although not in rainforest areas, *L. mirabilis* is nevertheless restricted to relatively cool areas along creeks and gullies and was never observed in full sunlight.

Plate 0.09: *Lampropholis mustelina* (O'Shaughnessy, 1874)

0.03.09: *Lampropholis mustelina* (O'Shaughnessy, 1874).

Lampropholis mustelina is little known despite its large size (snout-vent length approximately 55 mm) and early description. This is undoubtedly due to its secretive nature rather than its distribution since it is known from the coast and ranges of New South Wales and Eastern and Southern Victoria (Cogger, 1983).

Lampropholis mustelina prefers habitat which is moist and with lower temperatures than its heliothermic relatives. Generally it is seen foraging in the late afternoon, often in association with large rotting logs or other substantial shelter. Its unusual gait has earned it the common name of weasel skink.

Plate 0.10: *Lampropholis sp.1*

0.03.10: *Lampropholis sp.1.*

Lampropholis sp.1 (= Race A of Donnellan, 1985; = L.d. Northern
of Mather, 1986; also known under the sobriquet of "coggeri"
among herpetologists) occurs in rainforest areas between Cooktown
and Townsville (North Queensland). As with *L. sp.3,* it is a
common inhabitant of the leaf litter at the margins of the forest
or in more open areas within the forest.

As observed with other heliothermic members of the genus, *L. sp.1*
appears immediately full sun is available, for example when the
sun emerges from behind a cloud. The animal will then typically
(although not always) head-bob. Generally three head-bobs occur,
movement at this time always being an up and down motion. The
individual then proceeds to forage. Head bobbing continues to
occur at regular intervals during foraging and the animal will
also stop at brief intervals to bask in areas of full sunlight.
When reaching a high spot in the individual's range, for example
the top of a rock or log, the animal will stop, head-bob and look
around. This time the head-bob involves the animal tipping its
head first on one side, hesitating, and then tipping its head in
the opposite direction. After this activity the animal will
generally move off to continue foraging. This head-bob behaviour
is assumed to be to assess safety since it was never observed as
part of social interaction.

To bask, an individual will enter a patch of full sunlight and
orient its body to receive maximum warmth. Frequently one leg,
although sometimes both, are flattened against the body. If one
leg only is flattened, the individual will frequently turn and
repeat the behaviour, sunning both sides. Always the individual
presents the greatest amount of dark pigmented skin possible to
the sun. Early in the day relatively longer periods (some
minutes) are spent basking. However, as the temperature increases
basking behaviour is reduced to shorter periods, of as little as
ten seconds. These behaviour patterns have also been observed in
other species including *L. delicata* and *L. guichenoti*.

As with all species observed, adult *L. sp.1* has a definite,
although ever changing home range. That is the animals tend to
follow sun flecks such that the range changes diurnally.
Individuals move through this home range along predetermined
routes. Generally an individual will work its way along the most
protected path available. Thus it will move along high points
that are protected by overhanging vegetation but will move around
or under unprotected projections. In this way open areas are
generally not utilized.

There was a definite overlap in home range of numerous adults,
with an individual's range often being encroached upon by up to
four adults. Young juveniles appear to move in a much more random

manner and are ignored by the adults at all times. This is in contrast to *L. guichenoti* which is much less tolerant of juveniles.

In areas where *Carlia romboidalis* was abundant, *L. sp.1* were frequently observed following these animals, either close behind for short distances or, for considerable periods, at distances approximating 0.5 metre. This was always tolerated by the larger species and appeared to act as mutual protection. Occasionally however, *L. sp.1* would be distracted and forage under leaves that the larger animal had disturbed. This behaviour was not observed in any other species of *Lampropholis*.

Plate 0.11: *Lampropholis sp.2*

0.03.11: *Lampropholis sp.2.*

Lampropholis sp.2 (≡ Race D of Donnellan, 1985; ≡ L.d. Nebo of Mather, 1986; also currently known under the sobriquet of "neboensis" among herpetologists) is known from a restricted area in South Eastern Queensland including Mt. Nebo, Mt. Glorious and the Conondale Ranges.

The only observations of this species during the present study were at a locality on Mt. Glorious where it was observed foraging in ferns in relatively open areas of the rainforest in association with *L. challengeri*. As with other heliothermic animals of this group, it tended to be in more open habitat where some full sunlight was available in contrast to *L. challengeri* which tended to remain in areas of complete canopy cover. Both were observed diurnally active within preferred microhabitats at the same time.

Plate 0.12: *Lampropholis sp.3*

0.03.12: *Lampropholis sp.3.*

Lampropholis sp.3 (= Race C of Donnellan, 1985; = L.d. portcurtis of Mather, 1986; also currently known under the sobriquet of "portcurtisi" among herpetologists) has an apparently fragmented distribution. It is known from the rainforest areas of the Atherton Tablelands and Eungella region in the north, the creek line areas of wet sclerophyll forest in Central Queensland and from the Conondale Ranges area in the south.

In rainforest areas the species was observed to be abundant along paths, roadways and creeks where the overstory was open. However, further south the species was found in sheltered areas associated with small coppices of trees in the vicinity of creeks. In this area, habitat is very restricted due to past large scale clearing and the introduction of 'improved pastures' for grazing. No obvious separation of resources occurred between *L. delicata* and *L. sp.3* in this latter area, with the two species apparently sharing habitat as do *L. guichenoti* and *L. delicata* in some regions. In addition species of the genera *Ctenotus*, *Sphenomorphus* and *Carlia* were typically observed in the same habitat as *L. sp.3* in Central Queensland. The reptiles observed in this area are typical of dry land fauna, whereas the habitat and associated species in the north tended to be those more typical of rainforest fauna.

Plate 0.13: *Lampropholis tetradactyla* (Greer & Kluge, 1980)

0.03.13: *Lampropholis tetradactyla* (Greer & Kluge, 1980).

Lampropholis tetradactyla is the only recognized species of the genus with four toes on the front limbs rather than five and is one of the smaller members of the genus (snout-vent length of 30 mm). It is known from the coast and adjacent ranges of North Eastern Queensland between Townsville and Cairns (Greer & Kluge, 1980).

Greer and Kluge (1980) reported that this species was the most mesic of the group, preferring very moist areas. *Lampropholis tetradactyla* was certainly observed in such habitat, for example in the spray zone of the Millaa Millaa Falls (Atherton Tablelands). However it was occasionally observed in drier areas, the extreme being on the rainforest floor on a hot, dry December day where it was seen foraging among rock between 2.30 - 4 p.m. Humidity at that time was relatively low, the area being quite dry. In general, however, members of the species were found in areas of high humidity close to creeks in forested areas. Frequently the species was in association with a well developed, undisturbed horizon of humus under leaf litter and in association with rocks. In such areas they appeared to forage mainly within the leaf litter zone and were not seen to move more than 80 cm across the top of the litter. Nowhere were they seen in areas of disturbance and the species, like *L. caligula*, appears to 'swim' through the litter.

0.04: COMPARATIVE NATURAL HISTORY OF *LAMPROPHOLIS*.

Species of *Lampropholis* occur in overlap with sister taxa throughout their ranges although diurnal and microhabitat separation is frequently observed. The extent of overlap appears to vary significantly depending on prevailing climatic conditions. The major area of habitat segregation would appear to be based on differences in preferred temperatures and humidity, indicating variation in physiological responses to temperature and moisture.

The thermo-regulatory behaviour patterns described for *L. sp.1* were widely observed among heliothermic members of the genus (e.g. *L. delicata* and *L. guichenoti*). However, species which avoid full sun, relying instead on radiated heat, may also share habitat with these species. Frequently this results in a distinct diurnal separation within the same habitat, although micro-habitat separation also occurs. Thus, for example, although *L. mustelina* is frequently observed in the same habitat as *L. delicata*, *L. guichenoti* and *L. challengeri* there is generally a diurnal separation. *Lampropholis mustelina* avoids full sunlight and tends to be more commonly seen in the late afternoon, frequently after sunset during appropriate weather conditions. *Lampropholis delicata* and *L. guichenoti* are most commonly found foraging in full sun, mid-morning. *L. challengeri* tends to seek cooler microenvironments and display the same diurnal activity as

L. delicata and *L. guichenoti,* and not, as may be expected the same as *L. mustelina.* Such habitat segregation was observed widely in all areas of overlap.

This separation of habitat not only occurs among species but also between sexes at some times of the year. This habitat separation was observed in sexually dimorphic species such as *L. basiliscus* and *L. guichenoti.* Although not obvious in species which are monomorphic, these also demonstrate separation of microhabitat at some times of the year. This is assumed since frequently when collections are made in an area, one sex will be collected to the near total exclusion of the other.

Fighting occurs constantly during the mating season, in all species observed at that time of year. Throughout the rest of the year individuals appear to ignore other members of the genus, both individuals of their species and congeners.

In northern areas no large aggregations of eggs were observed. This was despite disclosing numerous 'clutches' (1-3 eggs). These eggs were assumed to belong to *Lampropholis* species since no other reptiles of the same relative size as *Lampropholis* were observed in these areas and it was an appropriate time for this species to be laying eggs. Most females collected within this region during December and January had either recently laid or laid soon after capture. Obviously gravid females were also

frequently seen in the field. However, south of the northern rainforest blocks, communal egg-laying was extensive. This phenomenon was widely observed in the field between *L. delicata* and *L. guichenoti* species and has also been noted for *L. basiliscus* and *L. sp.1*. Consequently all species south of the northern rainforest region appear to lay communally while in areas such as the Atherton Tableland, only single clutches were found.

Belmont (1977) and Crome (1981) compared dietary preferences of *L. delicata* and *L. guichenoti* and all dietary information on the group is restricted to these species. However field observation of other *Lampropholis* confirms that feeding strategies are similar and Brown (1983) has reported that the feeding mode of most Australian skinks studied was common. Animals, in general, forage over a range of substrates, feeding opportunistically on a wide range of arthropods (significantly insects and ants), both aerial and arboreal. Both 'sit-and-wait' and 'active foraging' strategies are involved.

Lampropholis challengeri, L. delicata and *L. guichenoti* were seen to be actively predated upon by birds in the Sydney region. It was apparent that larger animals, particularly gravid females, were preferentially selected, probably due to their obviously slower movements. The birds, particularly currawongs and

kookaburras, have been observed sitting on convenient vantage points, for example in front of sandstone cliffs in the case of *L. challengeri*, picking animals from the cliff face.

Several species including *L. basiliscus*, *L. czechurai* and *L. challengeri* (i.e. most closed forest species) were observed carrying mites while some 20 - 30% of *L. challengeri* collected in the Sydney region were infected. Species preferring more open habitat were not observed to carry mites. Jones (1985) described a species of tapeworm, *Baerietta hichmani*, which he found was common to *L. challengeri*, *L. delicata*, *L. guichenoti* and *L. mustelina*.

Therefore despite the paucity of data it may be gleaned that reproductive cycles of *Lampropholis* are similar, all are oviparous and communal nesting is widely practiced both within and among taxa. Dietary preferences are not restrictive. Species have been observed to take a wide range of prey items from a variety of sources and occur broadly in overlap with sister species throughout their range.

CHAPTER 1: THE ORIGINS AND CLASSIFICATION OF THE SCINCIDAE.

1.01: ORIGINS AND TIME OF ENTRY OF THE AUSTRALIAN SCINCIDAE.

Immigration of reptiles into Australia has occurred over a vast time frame. Extant forms are represented by a range in taxonomic divergence with some species indistinguishable from the original stock (frequently recent immigrants), while others exhibit significant taxonomic divergence (Storr, 1964). Although many of these groups are considered to have entered Australia from South East Asia, it is possible that some ancestral forms were present on the Australian plate at the time of the Gondwana break-up. Tyler (1979) for example, has stated that there is no reason to exclude Gondwana as an origin for at least some modern families of herpetofauna. On the other hand Greer (1979) has argued that there is no apparent distribution pattern among lizards to indicate this.

Cogger and Heatwole (1984) summarized the major events to be considered in a biogeographical treatment of Australian reptiles. Their salient points are as follows:

* some 130 million years ago (mya), early Cretaceous, the Indian plate broke from Gondwana and began its movement northward;

* approximately 53 mya (late Paleocene) Australia began separating from Antarctica;

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* New Zealand and Australia have had an oceanic separation for less than 83 my and have been totally separated for at least 50 - 60 mya;

* Australia probably reached its present latitudes, thereby closely approaching with South East Asia, in late Eocene (less than 40 mya);

* a series of Quaternary climatic fluctuations have occurred within the last 2 - 3 my;

* Pleistocene glaciations, accompanied by sea-level changes, have resulted in 'land bridges' between Australia and New Guinea, as well as between the mainland and Tasmania, enabling dispersal of reptiles in recent geological time. The associated climatic variation and glaciations restructured major biotypes.

It is commonly accepted that reptile migrations have occurred in a series of discontinuous events from the north (e.g. Cogger & Heatwole, 1984; Greer, 1976; Molnar, 1984a; Tyler, 1979) with a 'reasonably reliable' (Cogger & Heatwole, 1984) documentable sequence, although chronological order is more elusive. For convenience Cogger and Heatwole (1984) have recognized three broad groupings based on probable time of arrival and origin of the founder population. These founder groups were:

    (1) the 'old' Mesozoic or Gondwana fauna;

    (2) the 'intermediate', post-Gondwanan or Tertiary fauna;

    (3) 'modern' or Quaternary fauna.

Since Australia is generally believed to have begun its northward drift between 52-55 mya, the herpetological element of this 'old' group should be easily distinguished from more recent forms. This is because Gondwanan stock would generally be expected to be morphologically distinct and to have restricted distributions outside Australia. The 'intermediate' and 'modern' faunas are usually perceived to have an Oriental origin (Tyler, 1979) .

Cogger and Heatwole (1984) suggest wide acceptance of the idea that the 'intermediate' fauna entered Australia in the Tertiary (<40 mya). The Elapidae, Diplodactylinae, Pygopodidae and major segments of the Agamidae, Varanidae and Scincidae are among groups derived from ancestral forms which arrived in Australia no later than this period. These groups would have evolved in isolation until the later Quaternary influx occurred, allowing some 30 - 35 million years of virtual isolation. The most recent invasion, however, probably included secondary invasions of representatives of those families which had entered Australia previously during the Tertiary invasions. When Australia reached its present latitudes, immigration from the north apparently occurred, although there appears to be little evidence of emigration. On the basis of 'negative' evidence (Cogger & Heatwole, 1984) concluded that most of Australia's modern families of reptiles are Asian immigrants, post-Gondwana.

With reservations, Cogger and Heatwole (1984) used the following

groupings to categorize the various families in terms of their relative ages and regional affinities:

(1) Families of Gondwana origin; high endemism at the generic level in the Australian - New Guinea region with the New Guinea forms probably Australian derivatives.

(2) Families probably of Australian origin, terrestrial forms generally remaining endemic to Australia.

(3) Families with origins elsewhere, although probably represented in Australia since mid-Tertiary. Such families would generally be represented by endemism at all levels although some may demonstrate considerable generic, and little specific, level sharing with New Guinea.

(4) Families with old, nearly globally distributed genera demonstrating either high endemism and adaptive radiation at the species level within Australia or considerable sharing with New Guinea; Australian forms probably of Tertiary, Asian origin.

(5) Families, or major familial elements, which are new arrivals in Australia, perhaps Pleistocene or more recent; many generic affinities with New Guinea. These groups have undergone very little adaptive radiation within Australia and exhibit low endemism.

(6) Aquatic species with little if any endemism; area of origin either obscure or Indo-Australasian waters.

Tyler (1979) concluded that Oriental influences predominated among Australian reptiles, groups such as Typhlopidae and

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Varanidae having arrived in Australia when a northern 'collision'
occurred. However, he considered that the Scincidae colonised
Australia before this time, probably before mid-Miocene and
by over-water dispersal. Tyler (1979) recognized that this
hypothesis presented a major problem in explaining the large
degree of diversity and adaptive radiation associated with the
quite recent postulated time of entry.

Thus, there has been general acceptance that the Scincidae
entered Australia in a series of invasions from the north,
although the estimated time of entry varies among authors (e.g.
see Cogger, 1961; Cogger & Heatwole, 1984; Greer, 1974, 1976,
1979; King & King, 1975; Rawlinson, 1974; Tyler, 1972, 1979).
After successful establishment on the continent, radiation
southward ensured (Horton, 1972). Most recent interpretations of
past tectonic movement, however, suggest that except for
Quaternary contact, Australia has been isolated for at least 65
my and a previously proposed migration route from the north
probably never existed (Brunnschweiler, 1984).

Although it has been suggested at various times that some groups
have been derived from Gondwanan stock (e.g. Cogger & Heatwole,
1984; King, 1987; Savage, 1973; Tyler, 1979), Schuster (1981,
1981a) is alone in suggesting such a derivation for members of
the Scincidae. He hypothesized that the ancestral stock of
Anotis, *Lampropholis* and *Leiolopisma* were represented in the

Eastern Australia/New Caledonia cool temperate rainforests during the late Cretaceous. He based this conclusion largely on present day distributions and the assumption that these genera were closely related. Conversely Greer (1974, 1976, 1979) has suggested a series of mid-Tertiary invasions from the Indo-Malay region and is quoted by Tyler (1979) as having said that the present day distribution of Australian skinks may be explained without reference to continental drift.

Greer (1976) argues that 'primitive skinks' are restricted to Northern and Central America, Asia, Africa and Madagascar together with some Western Indian Ocean islands. The distribution of these species is greatest in Madagascar and Southern Africa while in Southern Asia the distribution becomes fragmented with most 'primitive' species occupying more marginal niches. This distribution is explained in terms of competition, the derived stock having evolved in Southern Asia and displaced the more 'primitive' group of that region. After they asserted dominance in the region, outward migration became possible with dispersal along major land routes. Ultimately there was an invasion of the Indonesian Archipelago and thence Australia. On the Australian continent radiation was enhanced due to the variety of available niches.

1.02: RELATIONSHIPS WITHIN THE SCINCIDAE.

The Scincidae (*sensu* Greer, 1970) comprises the largest, most diverse family of lizards, with more than 1,275 described species in eighty-five genera and four sub-families (Cogger *et al*, 1983). Although represented worldwide, the diversity of skinks is variable. Extensive adaptive radiations have occurred in the Oriental, Ethiopian and Australian regions with low diversity in the Palaeartic and Nearcatic regions and the group is poorly represented in the Neotropical region (Hutchinson, 1983).

Within Australia the reptile fauna is dominated by scincid lizards. Some 250 species are currently recognized spanning twenty-two genera. All Australian skinks belong to one sub-family, the Lygosominae (Cogger *et al*, 1983). The ultimate size of this subfamily is uncertain however, since new species are continually being described and there are frequent changes in generic arrangement (Hutchinson, 1983).

1.03: HISTORICAL TAXONOMIC TREATMENT OF THE SCINCIDAE.

Although Cuvier introduced the concept of a family for scincid lizards in 1817, the taxonomy of the group has remained unstable until quite recently. As a result many papers have been generated and these are scattered throughout hundreds of publications. Much

of this work is included in obscure exploration reports, zoological treatises and miscellaneous papers and monographs (Mittleman, 1952). No attempt has been made to review the literature as a whole and much of this section relies on the review of the historical aspects of scincid systematics presented by Hutchinson (1983). He concluded that by 1845 there was 'general concordance' among major taxonomists concerning scincid lizards. At that time skinks were recognized as a diverse group, prone to limb reduction. Typical characters were uniform, overlapping cycloid scales, flat and scaled tongue with a terminal notch, together with a range of eyelid structures. In general, osteological (including cranial) features, together with other internal anatomy, were not considered by early taxonomists.

The modern higher taxonomy of lizards has been based largely on refinements of the work of Cope (1864) who, in addition to using external characters, pioneered the use of internal morphological characters. Hutchinson (1983) has pointed out, however, that many of Cope's taxonomic decisions regarding the skinks appear to be based more on convenience than on evolutionary relationships. In 1887 Boulenger attempted to classify the scincid lizards into monophyletic genera, but failed to do so. He was therefore forced, due to lack of suitable diagnostic characters, to classify a large diverse group as subgroups of the genus *Lygosoma*. Boulenger's (1885-1887) taxonomic decisions were based

on a combination of internal and external morphological characters. The most significant of these characters was the condition of the secondary palate. Unfortunately Boulenger did not remove the mucosa covering the palatal bones and as a result he frequently misinterpreted the underlying bone configurations. Despite this the primary characters used in his generic key have remained in vogue and have been used in more recent subfamilial schemes (Hutchinson, 1983).

Although Boulenger (1885-1887) did not formalize subfamilial or suprageneric groups he did achieve a degree of stability in the nomenclature. Despite this stability, his scheme produced two unwieldy polyphyletic genera, *Ablepharus* and *Lygosoma*. The only character clearly separating the two is the presence of an immovable transparent lower eyelid in *Ablepharus* not present in *Lygosoma*.

Many workers have either accepted Boulenger's arrangements *per se* or alternatively elevated some of his subsections to generic rank (e.g. Smith, 1937; Mittleman, 1952; Storr, 1964; Greer, 1974, 1977).

Boulenger's (1885-1887) work was closely followed by that of Siebenrock (1892) who did not draw any taxonomic conclusions, but further developed the use of cranial osteology. In doing so he

demonstrated that Boulenger's (1885-1887) interpretation of the pterygoid bones was incorrect. Siebenrock (1892) also pioneered the use of other osteological features in skink morphology. These included the posterior projections of the palatine bones and variable development of both the postorbital bone and the anteriorly directed palatal process of the ectopterygoid.

No comprehensive attempt was made to deal with Boulenger's *Lygosoma* for several decades after his catalogues appeared in 1887 (Hutchinson, 1983). The work of Camp (1923) dealt mainly with relationships above the family level. Within scincid lizards, he generally retained Boulenger's concept of the group. In 1937, Smith reviewed the genus relying heavily on the techniques used by Boulenger (1885-1887). In general, Smith accepted the former's concept of the genus although some refinements resulted. These changes included the erection of several readily definable genera, although he retained some sections of *Lygosoma* (*Sphenomorphus* and *Leiolopisma*) as a single group. He was clearly not satisfied with this arrangement and commented that he included in the section *Leiolopisma* some species which could not be clearly assigned to any other genus. He noted that all were united by a 'worthless' character from a generic viewpoint. This character, "an undivided, more or less transparent disc in the lower eyelid" (Smith, 1937) is present in several other genera including *Mabuya*, *Riopa*, *Scelotes* and

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Sepsina. In Smith's opinion some *Leiolopisma* were derived from *Lygosoma* and others from *Emoia*. His arrangement of the Section was therefore based both on conveniently determined characters and geographical distribution.

The next taxonomist to attempt to resolve the problem of a satisfactory generic arrangement of the Scincidae was Mittleman (1952). His classification in many respects paralleled the work of Smith (1937). However, he subdivided *Lygosoma* and some related genera into four subfamilies within the Scincidae. These were Lygosominae, Mabuyinae, Scincinae and Chalcidinae. With the exception of the genera *Tiliqua*, *Egernia* and *Trachydosaurus* all Australian scincid lizards were included in the Lygosominae. These exceptions were included in the Scincinae. Mittleman (1952) envisaged the Lygosominae alone as comprising at least thirty-three genera in contrast to Smith's (1937) recognition of ten genera. In reality Mittleman (1952) fragmented the group, raising Smith's (1937) unnamed subsections to genera.

Some of these classifications have been recognized by others although there has been reluctance to accept them all, due mainly to the weak diagnoses and the lack of a phylogenetic framework (Greer, 1974). Mittleman (1952) relied heavily on external morphological characters, particularly scalation, without discussion of their taxonomic significance. Unfortunately many of

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these characters are known to vary among closely related species (Hutchinson, 1983).

As with Boulenger (1885-1887) and Smith (1937), Mittleman (1952) did not remove the mucosa from the palate thereby often deducing the configuration of the underlying bones incorrectly. He judged the condition of the pterygoid bones by determining the point at which the posterior portion of the secondary palate terminated. Since most of the Lygosominae do not have pterygoid contact, the subfamily lacked its diagnostic character.

Despite his poor choice of characters, most of Mittleman's (1952) generic arrangements have been retained, indicating an acceptable intuitive knowledge of the group. Recent concepts of his genera have been based on more rigorous science utilizing various systematic and phylogenetic attributes (Pengilley, 1972).

Hutchinson (1983) observed that systematics based on external or buccal morphology had reached saturation point with the work of Mittleman. This was because characters were unavailable to effectively break up groups which were widely believed to be polyphyletic. The work of Mitchell (1950) on the Australian genera *Egernia* and *Tiliqua* pioneered the modern use of palatal morphological techniques. Hutchinson (1983) reported that in the late 1960's these techniques were used to address the genera

Ablepharus, Leiolopisma and *Sphenomorphus.* Greer (1967) was the first to publish work on the Scincidae using palatal osteology when he revised the Australian genus *Rhodona.*

1.04:   MODERN TAXONOMIC STATUS OF AUSTRALIAN SCINCIDAE.

Relying heavily on osteological criteria Greer (1970) went on to revise the whole of the Australian Scincidae. He recognized only one Australian subfamily, Lygosominae, instead of the two (Lygosominae and Scincinae) proposed by Mittleman (1952). Both recognized four subfamilies within the Scincidae world wide. Detailed comparisons cannot be made of the two classifications, however, since Mittleman (1952) only published his interpretation of the subfamily Lygosominae. From the information available, Mittleman's type genera of the subfamilies Mabuyinae and Chalcidinae have been submerged in Greer's (1970) definition of the Scincinae (Pengilley, 1972; Figure 1.1).

Greer (1968) diagnosed the Lygosominae as those with:
* Fused frontal bones,
* Secondary palatine bones which meet ventrally along the midline except in some *Egernia* and *Corucia zebrata;*
* Supratemporal arch always complete;
* External nares in discrete nasal scales;
* External evidence of limbs.

Boulenger (1887)   Mittleman (1952)   Greer (1970)

```
Scincidae Lygosoma ┐
                   ├──────── Lygosominae ┐
          Ablepharus ┘                    ├──────── Lygosominae
                                          │
                      Scincinae           │
                                          │
                        Tiliqua           │
                        Egernia           │
                        Trachydosaurus ┘


                      Mabuyinae ┐
                                 ├──────── Scincinae
                        Mabuya   │
                                 │
                      Chalcidinae │
                                 │
                        Chalcides ┘
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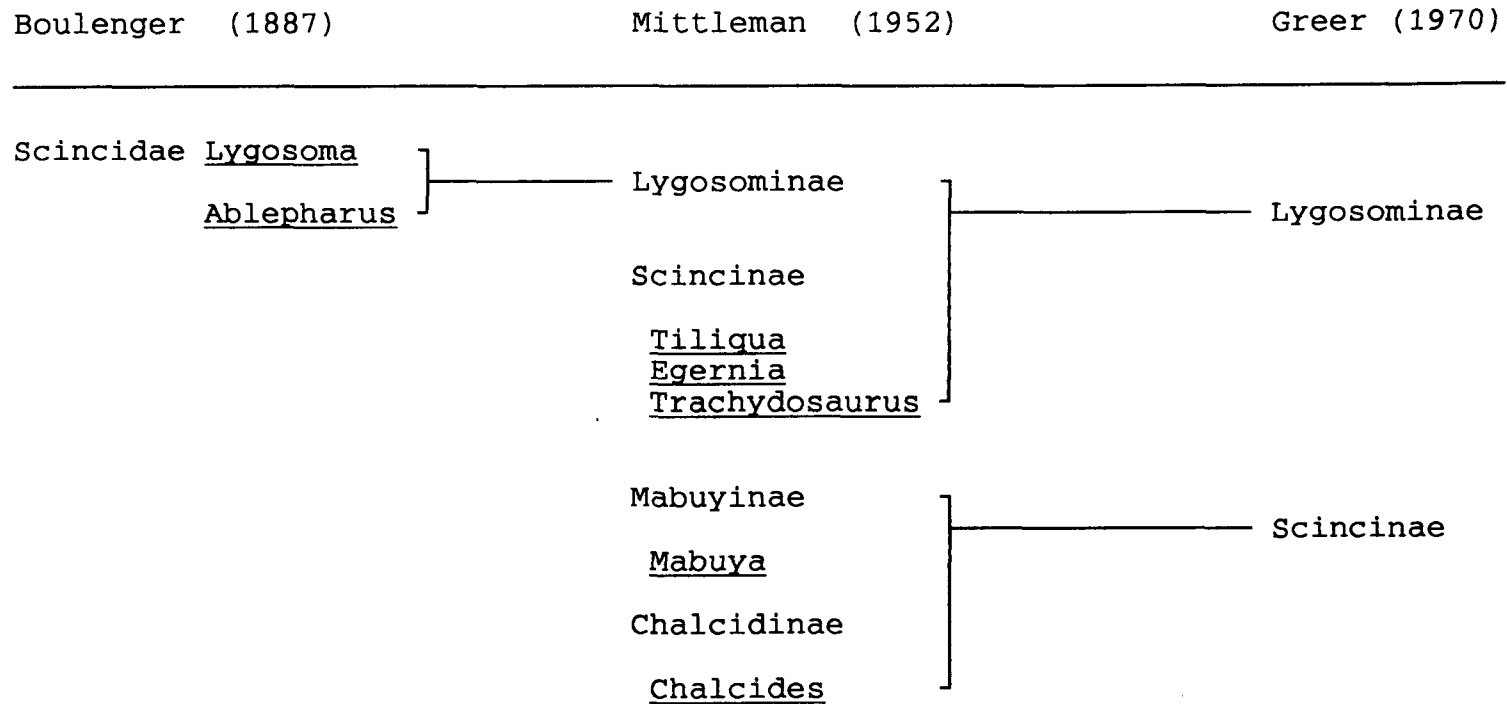
Figure 1.1: Classification of Australian Scincidae (after Pengilley, 1972).

The Lygosominae were considered an advanced group of scincid lizards, probably descended from Scincine stock. Greer (1968) based this conclusion on skull morphology together with present day geographical distribution. Advanced character states in scincid lizards were considered to be:

* Fusion and/or loss of skull bones;

* Reduction and loss of limbs;

* Viviparity.

Within the Lygosominae Greer (1979) recognized three distinct Australian groups; **Sphenomorphus**, **Egernia** and **Eugongylus** on the basis of eight characters:

* Total number of premaxillary teeth;

* Condition of Meckel's groove;

* Condition of parietal and interparietal scales;

* Scales in touch along the posterolateral edge of the parietal scale;

* Size of medial pair of preanal scales;

* Supradigital scale numbers;

* Iris colour;

* Morphology of everted hemipenis.

Hutchinson (1981) generally supported this classification but incorporated the remaining lygosome genera into an informal convenience group which he referred to as the **Mabuya** group. Later

he allocated a formal ranking to this group (Hutchinson, 1983; Table 1.1).

The **Sphenomorphus** group encompasses the Australian genera *Anomalopus*, *Ctenotus*, *Eremiascincus*, *Hemiergis*, *Lerista*, *Notoscincus*, *Saiphos*, *Sphenomorphus* and the species *Tropidophorus queenslandiae*. Non-Australian genera include *Lipinia*, *Lobulia* and *Prasinohaema* from the South West Pacific. Greer (1979) also suggested that *Tribolonotus* may belong in this group and not to the **Egernia** group. In addition he thought that *Ablepharus*, *Ateuchosaurus*, *Isopachys*, *Scincella* and *Tropidophorus*, all primarily Asian species, were 'probably' associates of this group (Greer, 1979; Table 1.1).

The **Egernia** group included the Australian genera *Egernia* and *Tiliqua* (encompassing *Omolepida* and *Trachydosaurus*). The latter genus included one member (*T. gigas*) known from New Guinea, west through the Sunda Islands to Sumatra. Greer (1979) concluded that *Corucia zebrata* from the Solomon Islands belonged to the group and remained equivocal about *Tribolonotus* from New Guinea, Bismarck Archipelago and the Solomon Islands (cf. **Sphenomorphus** group; Table 1.1).

The **Eugongylus** group was subdivided into two subgroups, **Eugongylus** and **Lampropholis**, based on the shape of the palatal

Table 1.1: Subdivision of the Lygosominae (after Greer, 1977; 1979; Hutchinson, 1981).

Eugongylus group	Egernia group	Sphenomorphus group	Mabuya group
* Carlia	* Corucia	Ablepharus	Apterygodon
* Cryptoblepharus	* Egernia	* Anomalopus	Dasia
* Emoia	* Tiliqua	Ateuchosaurus	Eumecia
* Eugongylus	? Tribolonotus	* Calyptotis	Lamprolepis
Geomyersia		* Ctenotus	Lygosoma
* Lampropholis		* Eremiascincus	Mabuya
* Leiolopisma		Fojia	Macroscincus
* Menetia		Hemiergis	
* Morethia		Isopachys	
* Nannoscincus		* Lerista	
* Problepharus		Lipinia	
* Pseudemoia		Lobulia	
* Sphenomorphus #		* Notoscincus	
? Copholsciniopus		Phoboscincus	
? Panaspis		Prasinohaema	
? Ristella		* Saiphos	
		Scincella	
		* Sphenomorphus #	
		Tachygyia	
		* Tropidorphorus	
		? Tribolonotus	

* Australian genera
# Segment of genus included in group, see text for species details
? Inclusion questioned in original paper

rami of their pterygoids. The **Eugongylus** subgroup, considered the more primitive of the two subdivisions, have palatal rami which diverge smoothly along their medial edges. This condition is referred to as an 'alpha palate' (Greer, 1979). This group includes the Australian genera *Cryptoblepharus*, *Emoia*, *Eugongylus*, *Leiolopisma* (including *Pseudemoia*), *Morethia* and *Proablepharus*, and the non-Australian genera *Phoboscincus* (New Caledonia and the Loyalty Islands), *Tachygyia* (Tonga Islands) and four species currently included with the genus *Sphenomorphus*, *S. aignanus* , *S. bignelli*, *S. louisiadensis* and *S. minutus*. These latter four species are known from New Guinea and the Solomon Islands (Table 1.2).

The palatal rami of the second subgroup, **Lampropholis**, "have a deep posterior emargination that gives them a distinctly hooked appearance" (Greer, 1979). This condition was referred to as a 'beta palate' and was considered to be more advanced than the 'alpha palate' of the **Eugongylus** subgroup. The **Lampropholis** subgroup included the Australian genera *Carlia*, *Lampropholis* and *Menetia*. Greer and Parker (1967; Greer, 1968, 1979) considered that the only non-Australian genus in the South West Pacific which belonged to this group is *Geomyersia*, a rare endemic of the Solomon Islands. In addition Greer (1979) believed there may be a number of species from sub-Saharan Africa, which could be placed in this subgroup if the palatal rami of the ptergoids is not a

Table 1.2: Alternative arrangements of the *Eugongylus* group.

Greer, 1979

Lampropholis subgroup | *Eugongylus* subgroup

* *Carlia*
* *Geomyersia*
* *Lamrpopholis*
* *Menetia*
? *Copholsciniopus*
? *Panaspis*
? *Ristella*

* *Cryptoblepharus*
* *Emoia*
* *Eugongylus*
* *Leiolopisma* #
* *Morethia*
  Phoboscincus
* *Proablepharus*
* *Pseudemoia*
* *Sphenomorphus* #
  Tachygyia

Greer & Rounsevell, 1986

Pseudemoia subgroup | *Eugongylus* subgroup

* *Carlia*
* *Cryptoblepharus*
  Geomyersia
* *Lampropholis*
* *Leiolopisma* #
* *Menetia*
* *Nannoscincus*
* *Pseudemoia*
* *Sphenomorphus* #

* *Eugongylus*
* *Emoia*
* *Leiolopisma*
* *Morethia*
  Problepharus

* Australian genera
# Segment of genus only included, see text for
  details
? Inclusion questioned in original paper

convergent character. These reptiles are currently placed in the genus *Copholsciniopus* and the four subgenera of *Panaspis*. He also considered that four Indian species currently allocated to the genus *Ristella* may belong to this grouping.

In a discussion on the relationships of *Pseudemoia palfreymani*, Greer and Rounsevell (1986) have recently erected a new grouping within **Eugongylus** based on a unique character state, the fusion of the atlantal intercentrum to the two atlantal arches in adults. Geographical distinctiveness also supports the concept of this group. In addition to the two described species of *Pseudemoia*, the taxa *Carlia*, *Cryptoblepharus*, *Geomyersia*, *Lampropholis*, one complex of **Leiolopisma** (*Le. atropunctatum*, *Le. aurestes*, *Le. austrocaledonicum*, *Le. coventryi*, *Le. deplanchei Le. festivum*, *Le. zia*, *Le. entrecasteauxi*, and *Le. jigurru*), *Menetia*, *Nannoscincus* and a small complex presently included in *Sphenomorphus* (*S. aignanus*, *S. bignelli*, *S. louisiadensis*, *S. minutus*) share this trait. The **Eugongylus** subgroup is therefore now reconstituted and apparently the **Pseudemoia** subgroup has replaced the **Lampropholis** subgroup (Table 1.2). All Australian species previously allocated to the **Lampropholis** subgroup as well as some island species are included in the new grouping, together with several taxa previously allocated to the **Eugongylus** subgroup. Greer and Rounsevell (1986) make no mention of other taxa discussed in association with the **Lampropholis** subgroup,

although it is implied that they do not belong since the new taxon, with the exception of *Cryptoblepharus* (which is known to be an efficient colonizer), is said to be centred on the "circum-Tasman/Coral Sea area" (Greer & Rounsevell, 1986).

In inferring phylogenies for the Australian skinks, Greer (1979), in addition to the eight character states above, also included two others:

* Presence/absence of pterygoid teeth; and

* Presence/absence of distinct postorbital bone.

Assuming that his phyletic analysis was correct and that no character states had demonstrated convergence, Greer (1979) proposed three phylogenies shown in Figure 1.2. Of these he concluded that 'C' was the most acceptable working hypothesis. He also suggested that within the line of the common ancestors of the **Eugongylus** and **Egernia** groups and the common ancestor of all three groups, there could have been a Lygosominae form similar to the most primitive extant *Mabuya*.

Based on distributional evidence Horton (1972) hypothesized that the Mabuyinae (*sensu* Mittleman = *Mabuya*, Central and South Eastern Asia, Africa and South America; *Tiliqua*, Australia; *Corucia*, Solomon Islands; *Macroscincus*, Cape Verde Islands and the extinct genus *Didosaurous*) occupied an intermediate position

Eugongylus group   Egernia group   Sphenomporhus group

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   |                    |                    |
   |                    |                    |
   |                    |                    |
   |                    |                    |
   |                    |                    |
A  |_____  Primitive Lygosominae _____|
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Eugongylus group   Sphenomorphus group   Egernia group

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   |                    |                      |
   |                    |                      |
   |_____|                      |
   |                                           |
   |                                           |
B  |_____  Primitive Lygosominae _____|
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Eugongylus group   Egernia group   Sphenomorphus group

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   |                    |                    |
   |                    |                    |
   |_____|                    |
   |                                         |
   |                                         |
C  |_____  Primitive Lygosominae _____|
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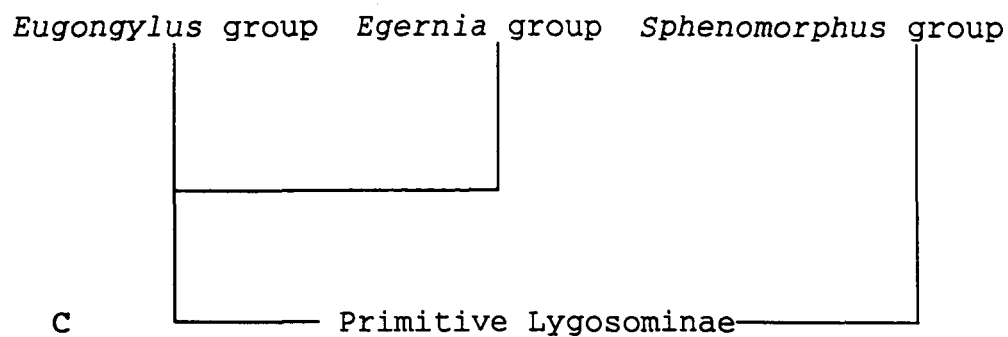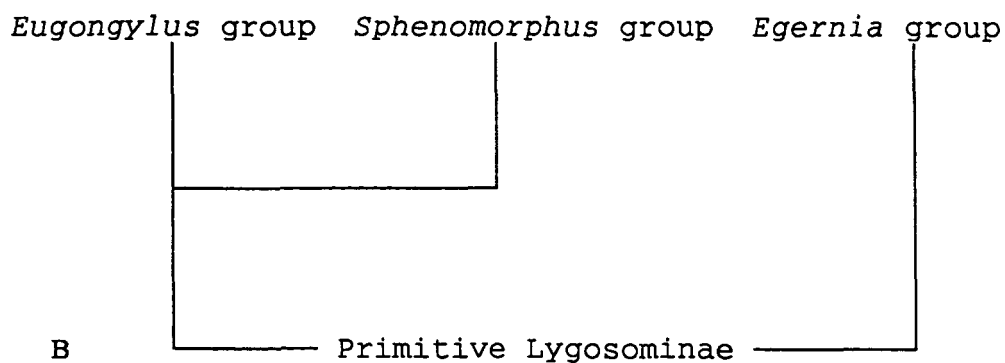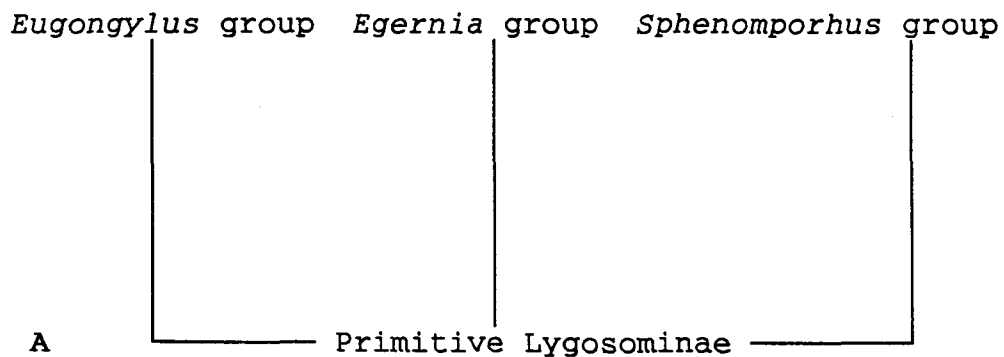
Figure 1.2: Greer's (1979) most parsimonious
           phylogenies based on osteological
           and morphological criteria.

phylogenetically between the 'primitive' subfamily Scincinae and the 'modern' Lygosominae. He further suggested that **Egernia** was the 'modern derivative' of the Scincidae with the closest extant relatives of **Mabuya** lying within **Egernia**.

Hutchinson (1983) reviewed the phylogeny of Australian skinks and criticised the basis of Greer's (1979) work. He claimed that several of the derived character states used as the basis of Greer's classification had arisen independently within at least one of the groups. Hutchinson (1983) ignored apomorphic character states and used only seven criteria to construct a phylogeny. Unfortunately use of these characters resulted in an unresolved trichotomy. The **Eugongylus**, **Sphenomorphus** and **Mabuya** groups all have apposed palatal rami of the palatines, while the **Egernia**, **Eugongylus** and **Mabuya** groups all have a closed Meckel's groove. Both these conditions were hypothesized to require an equivalent number of evolutionary events and one situation of parallel evolution. Hutchinson (1983) therefore suggested that both situations were equally likely to have occurred. He further claimed that the **Mabuya** group shared all its derived character states with the **Eugongylus** group and was thus only able to be separated on primitive character states.

Using micro immunoelectrophoresis techniques (IEP) Hutchinson (1981) investigated the systematic relationships of *Egernia* and

Tiliqua. His results supported Greer's concept of the **Egernia** group. His work further indicated that at least three taxa including the genera *Mabuya* and *Tribolonotus* and the group **Eugongylus** were clearly separated from each other, but were of approximately equivalent distance from *Egernia* and *Tiliqua*. The monotypic genus *Corucia* was observed to be antigenically closest to these two genera, although *Egernia* and *Tiliqua* were clearly most closely related.

The genus *Tribolonotus* did not fall into any of these groups and was also 'very distinct' from the **Sphenomorphus** group. It was, however, observed to be closer to the **Egernia** and **Eugongylus** groups than to **Sphenomorphus**, although the reactivity was a 'little stronger' with the **Eugongylus** group anti-serum. Hutchinson (1981) therefore concluded, on the basis of a reassessment of Greer's morphological work and the immunological data, that *Tribolonotus* is 'probably' closest to members of the **Eugongylus** group.

Hutchinson (1983) hypothesized that the **Sphenomorphus** group branched near the base of the lineage leading to the **Egernia** and **Eugongylus** groups. However, the dichotomy was expected to have occurred closer to the origin of the **Eugongylus** group rather than the **Egernia** group. He therefore concluded that his immunological work on the phylogenetic position of **Eugongylus** was consistent

with Greer's (1979) hypothesis, in that it is the closest relative of the **Egernia** group. Hutchinson (1983) further demonstrated that the **Eugongylus** group (including *Leiolopisma*, *Morethia*, *Cryptoblepharus* and *Lampropholis*) were the closest non-**Egernia** group to *Egernia* and *Tiliqua* and that the **Sphenomorphus** group was no more closely related to **Egernia** and **Eugongylus** than to the subfamily Acontinae. On this basis he suggested that the currently accepted taxon of Lygosominae needed re-examination.

Hutchinson (1983), while broadly accepting Greer's (1979) concepts, made two relevant changes to the previous classification. The **Sphenomorphus** group was given subfamilial status (Sphenomorphinae) and the polyphyletic **Mabuya** and **Eugongylus** groups were submerged into a single tribe (Lygosomini).

Rawlinson (1974) also had some disagreement with Greer's revision of the genera *Egernia* and *Tiliqua*. He claimed that, on the basis of Greer's (1970) criteria, they should have been referred to the subfamily Scincinae. This was because they conformed with Greer's diagnosis of the subfamily in all but one respect, that is, the frontal bones were not completely divided. Rawlinson (1974) pointed out that they were intermediate, not being completely fused and suggested that Greer should have separated the subfamilies on the character "presence of fully or partially divided frontal bones" (Rawlinson, 1974). More recently Greer

(1986) pointed out that Rawlinson's objections were largely semantic and also presented a third character to support his previous classification of Lygosominae.

The most recent work to emerge is that of Donnellan (1985) who undertook an extensive karyotypic survey of the Australian Scincidae. He concluded that each of Greer's (1979) groupings within the Australian subfamily, had a unique karyotype format (Table 1.3). Donnellan's work thus lends support to the monophyletic nature of Greer's three taxa within the Lygosominae. Thus Greer's phylogeny was accepted as a basis for diagnosis of the group for this study.

1.05:   DIAGNOSIS OF THE SUBGROUPS OF *EUGONGYLUS*.

Greer (1979) considered **Eugongylus** to be a monophyletic group. The work of Hutchinson (1983) and Donnellan (1985) support this supposition. Greer (1979) concluded that within this group there were two major subdivisions (see Section 1.04 above for discussion; Table 1.2). Although many of the genera within these lineages have been defined there are still problems that need to be addressed (Donnellan, 1985). For example, the genus *Leiolopisma* and its relatives have historically been problematic (Greer, 1974). The genus *Lampropholis* was erected from the

Table 1.3: Generalized karyotypes of the Australian
Scincidae groups (after Donnellan, 1985).

Group	Complement	Macro-chromosomes	Micro-chromosomes
Egernia	2n=32	9 pairs	7 pairs
Eugongylus	2n=30	9 pairs	6 pairs
Sphenomorphus	2n=30	8 pairs	7 pairs

synonymy of *Leiolopisma* by Fitzinger (1843) to describe a number of small Australian skinks. *Lygosoma guichenoti* (Duméril and Bibron 1839) was designated the type species. Subsequently Gray (1845) included the taxon in the genus *Mocoa*, although Boulenger (1885-1887) followed by Loveridge (1934), Smith (1935) and McCann (1955) have all commented on the significant overlap in the quantitative features used to separate the genera *Lampropholis* and *Leiolopisma*.

Smith (1937) in reviewing *Lygosoma* separated the Australian *Leiolopisma* into two complexes, those with fused frontoparietal scales and those with two distinct frontoparietal scales. Mittleman (1952) formalized this division by designating the first complex to <u>*Lampropholis*</u> (*L. ocellata*, *L. metallica*, *L. pretiosa*, *L. trilineata* and *L. guichenoti*) and the latter group to *Leiolopisma* (*L. entrecasteauxi*, *L. mustelina* and *L. challengeri*). Most subsequent workers have ignored these divisions, regarding all these species as *Leiolopisma* (see e.g. Clarke, 1965; Pengilley, 1972; Greer, 1970; Cogger, 1983). Thus the generic boundaries of this taxon are poorly defined. The genus serves as a 'catch-all' and has been diagnosed on the basis of plesiomorphic characters.

In an attempt to rectify this situation, Greer (1974) reinvestigated the genus *Leiolopisma* using osteological and

morphological characters. He resurrected and redefined the genus *Lampropholis* as follows:

"small (snout-vent length up to 62 mm) Australian beta skinks with five toes on the front foot, prefrontals and a movable lower eyelid with a clear transparent window; clutch size variable, up to 10. Frontoparietals distinct (2 species) or fused (2 species)" (Greer, 1974; pp. 25).

The species *Lampropholis challengeri* (Boulenger, 1887), *Lampropholis delicata* (De Vis, 1888), *Lampropholis guichenoti* (Duméril and Bibron, 1839) and *Lampropholis mustelina* (O'Shaughnessy, 1874) were assigned to the genus. In a more recent paper Greer (1979) recorded that Rawlinson was in the process of revising the species within the genus. Although at that time there were only four described species in the group, Greer (1979) claimed to know of some 11 or 12 additional undescribed species. More recently, Ingram and Rawlinson (1981) have described five species in the genus, *Lampropholis amicula*, *Lampropholis basiliscus*, *Lampropholis caligula* and *Lampropholis mirabilis*. In addition Greer and Kluge (1980) have described *Lampropholis tetradactyla* and Schuster (1981a) has discussed a species which he called *Lampropholis mongan*, a description of which has not yet been published.

1.06:   DIAGNOSES OF COMPLEXES WITHIN *LAMPROPHOLIS*.

Greer and Kluge (1980) have gone on to suggest that the genus consists of two subgroups or complexes, the L. *challengeri* and L. *delicata* complexes. *Lampropholis challengeri*, *L. mustelina* and the newly described *L. tetradactyla* were assigned to the former complex and *L. delicata* and *L. guichenoti* to the latter. Since this revision, Ingram and Rawlinson (1981) have suggested the inclusion of two of the five species they described (*L. czechurai* and *L. basiliscus*) in the L. *challengeri* complex, another two (*L. mirabilis* and *L. amicula*) in the L. *delicata* complex and the fifth species, *L. caligula*, they considered to be an aberrant member of the L. *delicata* complex.

Greer (1980) proceeded to suggest that these two complexes were worthy of generic rank. In 1983, Wells and Wellington elevated the two *Lampropholis* complexes to generic status. The L. *challengeri* complex was assigned to the genus *Saproscincus* and the genus *Lampropholis* was restricted to the L. *delicata* complex. Hutchinson (1983) using immunoelectrophoretic (IEP) comparisons, showed that *Lampropholis* (*sensu* Wells & Wellington) were allied with *Carlia* and *Menetia* (i.e. Greer's **Pseudemoia** subgroup) while *Saproscincus* species were observed to be more closely allied with the *Leiolopisma* - *Morethia* radiation (i.e. Greer's **Eugongylus** subgroup). Thus it would appear that, on the

basis of molecular data, support is given for the separation of the two groups at the generic level.

The work of Joss (1985) also supports the supposition that at least one of the species currently placed in the _L_. _challengeri_ complex may not be closely allied to the other members of _Lampropholis_. She worked on ovarian steroid production in _Lampropholis_ (_sensu_ Greer, 1974) from the Sydney area and observed that there was a similar pattern of steroid hormone synthesis in _L. guichenoti_, _L. delicata_ (both _L_. _delicata_ complex) and _L. mustelina_ (_L. challengeri_ complex). These species were observed to synthesize steroids in thecal glands prior to ovulation, with _L. mustelina_ having higher plasma concentrations of estradiol -17β and a greater abundance of thecal gland tissue than either of the other species. _Lampropholis challengeri_ demonstrated a very different pattern of steroid hormone synthesis. This species was not observed to have thecal gland tissue and steroid levels were low and confined to vitellogenic follicles. Joss (pers. comm.) suggested that these differences were of such magnitude that she expected that _L. challengeri_ may prove to be quite a distant relative of the other three species which she had investigated. Lack of data on other skink taxa precludes further discussion of the taxonomic significance of this work.

Likewise a discussion of phylogenies based on karyotypic data is difficult due to paucity of information (Donnellan, in press). Distinctive karyotypes have been observed for several taxa of *Lampropholis*. Generally chromosome number within the group is 2n=30, although two taxa investigated within the *L. delicata* complex had 2n=28. Deviation from the former complement has proved to be unusual among the **Eugongylus** group with only these two taxa and two species of *Carlia* having been observed to deviate from the common chromosome complement. In addition, *L. delicata* has a variable chromosome format at the intraspecific level with individuals from the core of its range heterozygous for five different chromosome variants. These variants are distinct from each other and from those of other *Lampropholis* taxa (Donnellan, in press).

Intra-generic relationships of *Lampropholis* have proved difficult to resolve, due to the lack of differences in suitable morphological characters. It is generally conceded that at least one of the described taxon (*L. delicata*) may be a species complex, but quantification has proved elusive. The work of Donnellan (1985; in press) has proved useful in delineating chromosomal races within *L. delicata*. He observed four unique karyotypic races and no hybrid individuals and considered this strong evidence for specific status for these races. Karyotype analysis, however, is not necessarily a universal means of species determination.

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There is therefore a need for a revision of species level
taxonomy in this group. Little published information exists for
many of these species, most of which are known from their type
description only. The genus has an East Coast distribution with
disjunct populations of some species recorded from Eastern
Tasmania (*L. delicata*), South Australia (*L. delicata*, *L.
guichenoti*) and Western Victoria (*L. delicata*). In addition *L.
guichenoti* is known from Kangaroo Island (South Australia) and *L.
mirabilis* from Magnetic Island (North Queensland). Some members
of the group have a wide distribution (e.g. *L. delicata*, *L.
guichenoti*, *L. mustelina*) while others (e.g. *L. caligula*, *L.
mirabilis*, *L. amicula*) are known only from restricted, refugia
areas. Thus within what is considered a closely related group,
there are extremes in distribution patterns.

1.07:   OBJECTIVES OF THE PROJECT.

The primary aim of this project is to determine whether two
genetic groups of *Lampropholis* can be distinguished. To this end
inter- and intra-generic relationships have been probed. Beyond
this level of analysis a diagnosis of the relationships of
Lampropholis to other **Eugongylus** group species is presented.
Relationships between *Lampropholis* and other Australian
Lygosominae are also addressed. Finally available data are
evaluated in an attempt to propose an hypothesis on the age and
origin of the Australian Lygosominae.

1.08:   SEQUENCE OF ARGUMENT.

In this thesis I address the problems of taxonomic and phylogenetic relationships of *Lampropholis*. In the preface I introduced the taxa within *Lampropholis* using information gleaned from published works and augmented by field observations. The current chapter introduces the history and current status of the group's taxonomy and phylogeny together with pertinent information to enable consideration of biogeographical implications. In Chapter two I present biochemical classification techniques and put them into perspective. In Chapter three allozyme electrophoretic techniques are used to resolve problems of species level taxonomy. Morphological and meristic data are assessed in Chapter four. I have then approached phylogenetic relationships of the group using micro-complement fixation techniques in chapter five. Having determined species boundaries and phylogenetic relationships I discuss, in Chapter six, the implications of my findings and propose an hypothesis on the origin of the group.

CHAPTER 2: RATIONALE FOR TECHNIQUES.

2.01:   THE INTRODUCTION OF MOLECULAR TECHNIQUES.

Traditionally taxonomic and phylogenetic studies of Australian reptiles have relied predominantly on morphological criteria, frequently supplemented by distributional, ecological, behavioural and fossil data. In the last two decades several intensive morphological and osteological studies have been undertaken on the Australian Scincidae, the most significant of these is the work of Greer (1967; 1970; 1974; 1977; 1979; Greer & Rounsevell, 1986) on taxonomic and phylogenetic relationships. The study of Clarke (1965) on the intrageneric relationships of some *Leiolopisma* (at that stage encompassing *Lampropholis*) is also pertinent to the present work. Although valuable in elucidating taxonomic boundaries, these studies do not always give clear answers to the problems of evolutionary origins and relationships among the group. Therefore alternative approaches are necessary to address these problems.

In recent years chromosomal and molecular techniques have proved useful in elucidating phylogenetic relationships. Since differentiation at the genomic level begins immediately reproductive isolation occurs, the data obtained from such studies can help delineate natural groups at any taxonomic level.

Similarities in molecular structure may indicate common ancestry while alterations in the structure can detect divergent evolutionary pathways, often concealed by morphological convergence or conservatism (Sarich, 1977).

2.02: THE SCOPE OF MOLECULAR TECHNIQUES IN TAXONOMY.

There are various techniques available to measure the degree of differentiation among taxa. These include immunological, electrophoretic, DNA and sequencing techniques, all of which are broadly comparable in terms of time, effort and money per unit of information obtained (Sarich, 1977a). Most current biochemical taxonomy relies heavily on electrophoresis and immunological comparisons of proteins (Sarich, 1977). Sequencing has not gained widespread acceptance as a routine taxonomic tool, due largely to time constraints. Very recently, however, refinements of these techniques have enhanced efficiency, so that they are now becoming more commonly used tools in taxonomy.

Within the limits of resolution for any of the biochemical techniques, that is "the number of visible substitutions per unit time", the cladistic and relative genetic distances are generally congruent. There is a close relationship between protein structure and gene structure, so that a comparison of homologous

protein properties among taxa is essentially a comparison of their genes (Gorman *et al*, 1971).

The limits of a particular technique may vary widely depending on the taxa being investigated and the taxonomic or phylogenetic level of interest. Basically the more distant the relationship between two taxa, the more emphasis should be placed on selecting slow evolving characters, whether morphological or biochemical. Investigating tooth structure in mammals or beak shape in birds may be useful for delineating species boundaries, but at higher taxonomic levels the utility of these characters diminishes rapidly. This is because they are prone to modification in response to environmental factors. If all taxa within a group have unique characters, the data contribute nothing above the species level, and, due to environmental plasticity, it is frequently impossible to determine whether the variation is a result of convergence or retention of ancestral states. In many respects the limits of biochemical techniques are more readily identified than those of techniques employing morphological characters. However it may be difficult to determine, without *a priori* knowledge, the most appropriate technique for a specific problem.

2.03: KARYOTYPE DATA IN REPTILE TAXONOMY.

Few biochemical studies have been undertaken on Australian

reptiles. However, in the past decade, several karyotype studies addressing both evolutionary and specific level taxonomy, have been undertaken. These include studies of varanids (King & King, 1975; King *et al*, 1982a), gekkonids (King, 1977; 1979; 1982; 1987; King & King, 1977; King *et al*, 1982; King & Rofe, 1976), elapids (Mengden, 1985; 1985a; Covacivich *et al*, 1981; Baverstock & Schwaner, 1985) and skinks (King, 1973; Donnellan, 1985; in press). The most pertinent of these studies to the present work is that of Donnellan on the Lygosominae. Although primarily an investigation of the evolution of sex chromosomes, it is also taxonomically useful. This work supports the study by Greer (1979) and shows that the Lygosominae contain at least three groups, the karyotype within each lineage being "highly conserved" (Table 1.3). Donnellan's data set is also useful at the specific level, since he was the first to demonstrate quantitative variability among 'races' of *L. delicata*, which was previously hypothesized to be a species complex (Donnellan, 1985).

Although karyotype data is not useful in dating times of divergence (Baverstock & Schwaner, 1985), this technique can be a useful adjunct to molecular studies (e.g. King, 1987). In this study the use of karyotype data is restricted to a discussion of the work of Donnellan. Additional studies were not undertaken due to that researcher's continued interest and involvement in the area.

2.04:   ALLOZYME ELECTROPHORESIS IN TAXONOMY.

Since enzyme polymorphism was first described by Porter and co-
workers in 1962, many studies of genetic variability in natural
populations have been undertaken (Graur, 1986). Over the last two
decades increasing use has been made of molecular techniques in
an attempt to quantitify single locus genetic variation.

Allozyme   electrophoresis is   one   such   general   biochemical
technique which has been widely used in this area of research. It
involves the separation of soluble proteins in an electrical
field on the basis of their net charge, and to a lesser extent,
by   their   shape and size (Ayala, 1975).   Proteins   which
differentially migrate usually differ by at least one amino acid
(Avise, 1974). Since this implies variation in at least one
nucleotide base pair of the DNA sequence, the observed variation
is accepted to be genetic. This genetic variation is discrete and
quantifiable, in marked contrast to the majority of conventional
data, such as morphology, which may be heavily influenced by
environment.

Although most early use of the allozyme electrophoretic technique
was in the field of population genetics, its taxonomic value was
first demonstrated in studies of *Drosophila* by Hubby and
Throckmorton (1965). Consequently many papers have included a

discussion of taxonomic implications (Webster, *et al*, 1972).
Powell (1975) and Nevo (1978) summarized much of the resulting
data on heterozygosity while the more recent taxonomic literature
has been extensively reviewed (Avise, 1974; Avise & Aquadro,
1982; Nei, 1975) and Richardson *et al* (1986) have presented the
current applications of the technique for both systematic and
population genetic studies.

Avise and Aquadro (1982) summarized much of the published
vertebrate electrophoretic data, some 3,800 pair wise comparisons
of species. Means, ranges and Nei genetic distances (D) were
calculated for all pairs of species investigated within each
genus. The results are reproduced in Figure 2.1.

Despite expectations to the contrary, Avise and Aquadro (1982)
observed "some strong and consistent patterns of genetic
differentiation" particularly along taxonomic lines. All avian
congeners exhibit extreme conservatism, with closely related
species frequently not varying electrophoretically (Avise &
Aquadro, 1982; Avise, *et al*, 1982; Adams, *et al*, 1984; Richardson
et al, 1986). Consequently the technique is unlikely to be useful
taxonomically at the intraspecific level for this group.
Electrophoretic studies are generally useful, however, in
elucidating relationships at higher taxonomic levels among birds
(Richardson *et al*, 1986).

Conversely, amphibians have extremely high levels of genetic variability. Avise and Aquadro (1982) observed that six of the seven genera assayed demonstrated larger mean D values than any of the non-amphibian genera. The genus *Taricha*, the least variable of all amphibians assayed (Fig. 2.1), showed a mean D value greater than 75% of all non-amphibian genera. Therefore, in general terms, electrophoretic studies of amphibians would necessarily need to be restricted to intra- and interspecific levels. The technique is not generally useful for elucidating boundaries at higher taxonomic levels.

Invertebrate groups have not been widely studied when compared to the total number of genera. Richardson *et al* (1986) suggest that no generalizations among this group are possible at present. It would appear that the entire spectrum of conceivable genetic distances may occur. For example, numerous species of Hymenoptera have shown no intraspecific variation but very high interspecific variation (Wagner & Briscoe, 1983). *Speyeria* species of butterflies have relatively low mean genetic distances at the species level, D=0.14 and D=0.18 respectively (Brittnacher *et al*, 1978). Conversely some studies have reported high levels of genetic variability, for example in *Drosophila* (D=1.06; Ayala, 1975). Other species such as spiders (Gray, 1986) and starfish (Ayala, 1975) have intermediate levels of genetic variability.
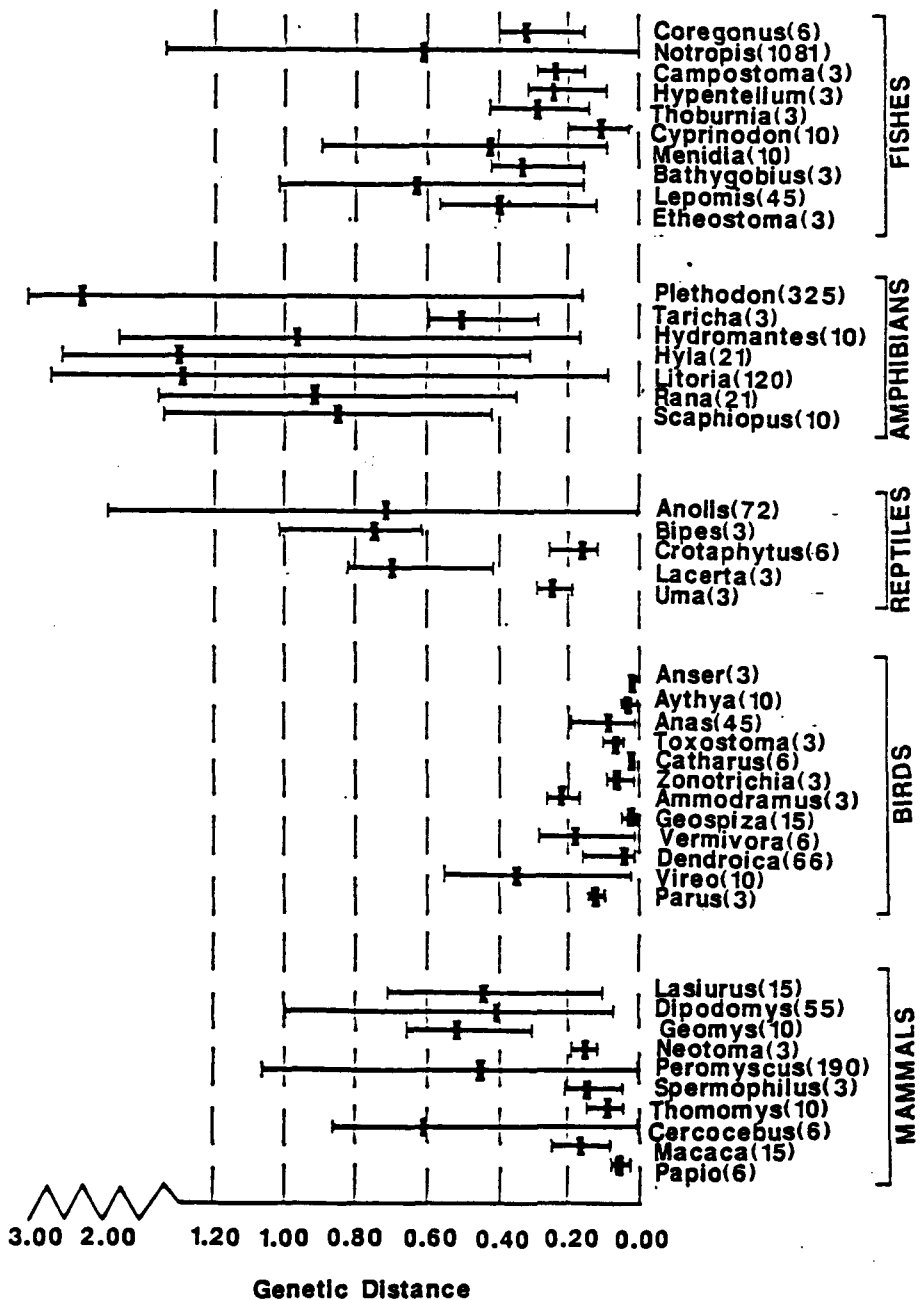
Figure 2.1: Means (and ranges) of electrophoretic distances between assayed species within each of 44 vertebrate genera. (Numbers in parentheses)(After Avise & Aquadro, 1982).

Fish and mammalian genera assayed are, in general, intermediate in genetic diversity between birds and amphibians (Fig. 2.1). Reptiles have not been widely studied as a group. However, three of the five genera examined by Avise and Aquadro (1982), *Anolis*, *Bipes* and *Lacerta*, demonstrated larger mean D values than recorded for any non-amphibian genera. The other two genera reported in this study, *Crophytus* and *Uma* demonstrated considerably less genetic variability than other group members assayed (Fig. 2.1). Thus the scope of allozyme electrophoresis as a general taxonomic tool for reptiles is largely unknown.

The utility of the technique therefore varies considerably depending on the group being analyzed. In many closely related animal species, differentiation typically occurs at 10% of loci. At this level of differentiation the technique is useful for delineating species boundaries and elucidating relationships. Traditionally the upper limit of utility has been considered to be 60 - 70% divergence (Richardson *et al*, 1986).

Traditionally the method of data retrieval used assesses the proportion of loci that are electrophoretically different without cognisance to the amount of difference observed on the gel for a specific protein. Recently Briscoe *et al* (1986) introduced a method which addresses this variation and claim to have extended the upper limit of the technique significantly. Their approach is

to measure the distance that electromorphs have migrated on the gel from the origin. To justify this approach, they assume that amino acid mutations approach random occurrence and that most point mutations for a specific isozyme tend to be reflected by an equivalent migration distance on the gel. If these assumptions are accepted, the relative migration distance gives a reflection of divergence time. Analysis of data collected in this manner will incorporate additional information, not available from traditional scoring techniques. Although largely untried, simulation models have been undertaken and are considered supportive of the technique (Briscoe, pers. comm.).

2.05: TAXONOMIC VALUE OF ALLOZYME ELECTROPHORESIS

2.05.01: Differences in approach between population genetics and systematics

Since population genetic studies probe the extent of variation within populations and the systematist is interested in the delineation of biological species, and frequently, phylogenetic reconstruction, there is a fundamental difference in approach depending on the aim of the study. Questions concerning population genetics require an investigation of allelic frequency data. Conversely when emphasis is placed on inter-species

relationships, the number of loci showing fixed allelic frequencies among species is important (Richardson et al, 1986).

2.05.02: Investigation of biological species.

In general it is considered that the basic unit of evolution in outbreeding species is the population, since a population shares a common gene pool. The selection imposed on this gene pool by the random fixation of mutations constitutes the basis of evolution. A single such population, or group of populations, constitute a species. In general sexually reproducing, diploid individuals of one species do not inter-breed with other species (Ferguson, 1980).

2.05.03: Detection of sympatric species.

Since by definition a species maintains a separate gene pool, the demonstration that there are two different electromorphs, with no heterozygotes, at a single locality, is evidence of two non-interbreeding species. When it is suspected that two species exist in sympatry, this hypothesis can be explored. Richardson et al (1986) suggest that this is best achieved by addressing the null hypothesis, i.e. that random mating occurs at the locality. To investigate this it is necessary to determine the expected number of heterozygotes at a single locus. This is achieved by calculating deviation from Hardy Wienberg Equilibrium. The

probability of not obtaining heterozygotes in a given sample, is given by calculating the equation:

$$1-2pq$$

where p & q = allele frequency.

If the population is in Hardy Wienberg Equilibrium, the probability of one of two alleles, p and q, occurring in the population is 0.5. The expected probability of finding a heterozygote is 2pq = 0.5. Thus the likelihood of not finding a heterozygote in a sample of 10 individuals drawn from a single population is $(0.5)^{10}$ or 0.1%. Although Richardson *et al* (1986) point out that the real allele frequency in a population may not be 0.5, they show that the proportion of heterozygotes to allele frequency is relatively similar between p=0.3 and p=0.7 and therefore an error of such magnitude does not significantly distort the data. When incorporating information from additional loci which present the same trend, increasing confidence may be placed in the observation. When no heterozygotes are observed in a sample, but two different electromorphs are present, there is evidence for rejection of the null hypothesis and thereby acceptance that two species exist at the locality.

Frequently it is expected that two species exist at a single locality due to morphological, ecological or behavioural clues.

If, however, two species are not hypothesized the observation can only serve as the basis of hypotheses and not to conclude specific status. To reject the null hypothesis it is necessary to either collect further at the locality, and confirm the hypothesis, or alternatively to investigate more loci. Richardson et al (1986) propose the latter course of action as appropriate, both in terms of conservation and scientific rigor. Relying on the observation made on a single locus, may give erroneous results since it is possible that either non-genetic variation or some form of complex genetic control of that particular locus may be responsible for the observed electrophoretic pattern.

As implied above, only a small sample size is required for such a study. When it is hypothesized that two species exist, a fixed difference in a sample of ten individuals (five of each morph) is ample to reject the null hypothesis of a single species. This is because, as explained above, the chances of identifying no heterozygotes at a particular locus, but five individuals homozygous for one allele and five for an alternative allele is 0.1%. The probability of finding such a result at two loci is $(0.1\%)^2$ or 0.00001%. However, since the determination of cryptic species is based on observed fixed difference between taxa, it is desirable to maximize the number of loci screened and the sample size is therefore less significant (Richardson et al, 1986).

2.05.04: Detection of allopatric species.

As with the detection of sympatric species, allozyme electrophoresis is a powerful tool for determining allopatric speciation. A major advantage of the technique is that the extent of genetic divergence observed is quantitifable and each character (locus) is independent. In addition, allozyme electrophoresis measures a fixed proportion of the genome. This is in contrast to morphological data which is generally quantitative since these characters are not necessarily independent and it is frequently difficult to determine to what extent they represent a fixed proportion of the genome (Ayala, 1976).

Unlike the relative ease of determining the genetic divergence required to satisfy the hypothesis of distinct species in sympatry, the extent of genetic divergence required to satisfy the hypothesis of specific status in allopatry is more difficult. Richardson *et al* (1986) suggest once again that the most appropriate approach is to concentrate on the null hypothesis. In this way taxa are retained as a single species until sufficient genetic divergence is obtained to demonstrate otherwise. Marginal cases would therefore be retained as a single unit unless alternative data was available to support the hypothesis of two species.

As with use of any single technique, it is frequently unwise to reach a decision on the basis of allozyme electrophoretic data alone. Thus if two populations show no electrophoretic divergence, it does not necessarily mean that they belong to the same species. The conclusion should be that the information available failed to reject the null hypothesis of a single species. Alternatively where it is demonstrated, on the basis of allozyme electrophoretic data, that two taxa show extensive taxonomic divergence, a case may be made for allocating full species status to each. This is because populations of the same species differ mostly by less than 10% and seldom by more than 15%. Always, however, the accuracy of the percent fixed difference estimated will be influenced by the number of loci sampled. The greater the number of loci investigated, the more reliable the result. Richardson et al (1986) also suggest that more valid decisions are possible when the data are placed in the context of genetic differentiation between conspecific populations of taxa across their geographic range and between species of congeners.

2.06: PHYLOGENETIC RECONSTRUCTION.

For a technique to be of use for phylogenetic reconstruction, it is desirable for characters to be independent and to have a genetic basis. Allozyme data fulfills both these criteria,

therefore it is superior to the more traditionally used morphological criteria for such reconstruction.

Before investigating hypotheses concerning phylogenies, however, it is necessary to determine that the level of genetic differentiation for the group under study is appropriate. Bush and Kitto (1978) investigated several molecular techniques and concluded that allozyme electrophoresis was most useful for comparisons of races, species and closely related genera.

Whatever approach to systematics is taken it is necessary to infer relationships on the basis of shared characters (Buth, 1984), however, where no allozyme differentiation is detected, the technique is inappropriate and an alternative, for example analysis of mitochondria DNA may be implemented. Problems also occur where extensive genetic differentiation is identified and again alternative techniques need to be implemented although in the first instance measurement of mobilities as advocated by Briscoe *et al* (1986) may extend the technique but beyond this level techniques such as micro-complement fixation need to be introduced. Even when two species share electromorphs at few loci, apparent similarities due to convergence occur. Where relatively large numbers of enzymes share the same mobility, this problem is exacerbated. For example, if four bands have been identified at a specific locus, the residual chance of two taxa sharing the same electromorph is 25%. If these two species share

electromorphs at 50% of their loci, half the observed differences may be due to convergence.

As discussed in Section 2.04 differentiation occurs at different levels in different groups. Genetic distance among genera of birds are generally low, while amphibians demonstrate a significant degree of genetic differentiation (see Fig. 2.1). The restricted number of reptile genera investigated to date give little indication of specific trends. However, in general, with the exception of birds which may share most loci at the specific level, closely related animal species tend to differ by approximately 10% of their loci (Avise & Aquadro, 1982) and seldom by more than 15% (Baverstock et al, 1977). Richardson et al (1986) consider that the upper limit of allozyme electrophoresis is 60-70% of divergence due to the problems of convergence demonstrated above. Briscoe et al (1987) consider this limit may be extended using their data retrieval techniques (see Section 2.04). With these guidelines in mind, assessment of the utility of the technique for a specific study may be readily achieved by undertaking a preliminary study to determine the range of genetic divergence within the group of interest.

As with studies involving delineation of species boundaries, large numbers of individuals from a single locality are unnecessary for phylogenetic investigations. Genetic distance measurements are more severely affected by the number of loci

investigated than by the sample size (Nei, 1978; Gorman & Renzi, 1979). Richardson *et al* (1986) suggest that as few as two or three representatives of a single population are generally sufficient. However, collection of samples over the full geographic range of the species and maximisation of the number of loci investigated is desirable.

2.07: ANALYSIS OF ELECTROPHORETIC DATA

As discussed previously there is a fundamental difference in approach depending on whether the aims of the study are concerned with inter- or intra-specific problems. Likewise the approach to analysis differs.

Studies which involve population structure, require detailed study of a large number of individuals using some parameter which is variable among individuals. Gene frequency data is the essential tool at this level. It is therefore desirable to investigate relatively large numbers of individuals for fixed and polymorphic loci. Invariant loci do not contribute any information (Richardson *et al*, 1986).

The most appropriate approach to analysis of such data is to test the null hypothesis that the observed frequencies are not significantly different among samples. For a specific locus this can be achieved with the aid of a chi-squared test of homogeneity

which measures the probability that observed frequencies deviate from expected frequencies and thus gives a measure of divergence at a specific locus (Siegel, 1956).

When such deviation is detected at a number of loci, a measure of genetic distance or similarity may be calculated between species pairs. Various measures have been proposed (e.g. Nei, 1972; Morton et al, 1973; Rogers, 1972). Buth (1984) suggests that most are highly correlated despite varying mathematical and biological assumptions. The most widely used coefficients of variation are those of Nei (1972) and Rogers (1972).

When inter-specific relationships are of interest, the extent of genetic differentiation is of concern. All loci, whether variant or not, contribute to the interpretation. The amount of information contributed by polymorphic loci will depend on the level of polymorphism. When a taxon is monomorphic for all loci investigated, a single individual will represent the taxon. With increasing levels of polymorphism, a larger contribution to the interpretation will be made by gene frequency data. In general, however, most vertebrates demonstrate relatively low levels of polymorphism and although variable this is frequently around fifteen percent (Richardson et al, 1986).

Historically measures of similarity, particularly Nei (1972) and Rogers (1972), which were developed to probe population sub-

structure, were used in the analysis of systematic data. As previously discussed these measures rely heavily on the use of gene frequency data. In reality, however, gene frequency data contributes little to the study of genetic divergence. This is because most loci tend to have allele frequencies which are very similar, or alternatively fixed differently. Few demonstrate intermediate levels of polymorphism (Avise *et al*, 1975). This results in allele frequency data generally contributing very little additional information over that obtained from a study of fixed allelic data (Richardson *et al*, 1986).

Richardson *et al* (1986) demonstrate this point with the hypothetical example of two populations having fixed gene differences at 20% of their loci and a further 16% which are polymorphic with an average genetic similarity of 0.5. Under these circumstances, Nei's D is calculated to be 0.25 and Rogers' R at 0.28. In this latter statistic 0.2 is contributed by the number of fixed differences and 0.08 from gene frequency data. Therefore gene frequency data contributes relatively less information but at a much greater cost, since a single individual from each population enables a reasonable calculation of fixed differences while a large sample size is desirable to calculate frequency data accurately. Only when the test group show extreme polymorphism does frequency data contribute significantly to the measure of genetic divergence.

2.08: AIMS OF GENETIC ANALYSIS

2.08.01: Conceptual basis of analysis

Analysis of genetic divergence for classification has two possible aims. One is to categorize into groups for ease of identification, the other aims to base some predictive value on such relationships (Funk, 1983). Thus there are basically two alternative conceptual approaches to the study of systematics. At the polarized extremes argument rages over whether systematics should have a phenetic or phylogenetic basis, however, in reality the whole spectrum of possibilities between the two extremes are covered.

Three major schools of thought exist (Cracraft, 1983). Those advocating a phenetic approach (e.g. Sokal & Sneath, 1963; Sneath & Sokal, 1973) effectively synonomize the study of systematics with taxonomy. Such categorization is based solely on similarities among living organisms (Fergusion, 1980). At the alternative extreme the phylogenetic approach based primarily on the work of Hennig (1950; 1966) demands that phylogenetic relationships should be the basis of classification. Between these two extremes the evolutionary systematists (e.g. Mayr, 1969; 1981; Simpson, 1961) conceive systematics as incorporating both the study of diversity and evolutionary considerations. Cracraft (1983) explains that the latter group attempt to balance

the two alternative concepts (phenetic and phylogenetics). When conflict arises between the extremes, however, results of measures of phenetics are considered more acceptable since similarity is considered to reflect both the branching patterns of phylogenies together with evolutionary divergence along different lineages.

Despite protracted argument over these various possible methods of analysis, there has not emerged a clearly definable, universally acceptable method (Richardson *et al*, 1986). Although different in conceptual approach, intuitively it may be argued that grouping taxa on the basis of their phenetic similarities would reflect phylogenetic grouping. Early authors such as Yang *et al* (1974) assumed that such was the case. Under certain conditions this may occur, however, only under those relating to equal rates of evolution in all lineages and therefore a lack of convergent and parallel evolution. When such homoplasy exists, conclusions based on phenetics will be incorrect for phylogenetic reconstruction (Richardson *et al*, 1986).

2.08.02: Phenetic analyses.

Although it is frequently accepted that the structure of natural systems is the ideal basis for classification, phenetists argue that phylogenies are generally not known due to a lack of fossil

data and therefore advocate phenetic resemblances as the basis for groupings (Sokal & Sneath, 1963).

In general early numerical statistics with possible application to systematics (e.g. Rao, 1948; Anderson & Whitaker, 1934) were generally inadequate, although Sokal and Sneath (1963) recognized some as having similar aims to modern numerical techniques. Most, however, were formulated as discriminant function analyses primarily to allocate individuals to a pre-existing group. Emphasis was placed on the identification of a small number of characters which clearly differentiated among the groups under investigation. As a result, the extension of such analyses into systematics resulted in an undesirable emphasis on low numbers of characters. Sokal and Sneath (1963) suggest that major problems associated with most early techniques were the result of this undue emphasis on a small number of characters. The development of alternative methods, based on cluster analysis, are considered to have lead to major advances in numerical taxonomy.

The change in emphasis has resulted in phenetic similarity among taxa being based on a large number of characters without intentional emphasis on character weighting. As a result of the absence of criteria for character selection, it is implicitly assumed that evolutionary rates are constant among lineages. Thus no allowance is made to overcome the effects of homoplasy or character reversals. For these reasons phenetic analyses can only

be viewed as a first approximation of monophyletic grouping. Additional sources of information are therefore required to provide confirmation (Thockmorton, 1968).

2.08.03: Limitations of phenetic analysis for phylogenetic
        reconstruction

That such phenograms, constructed on the basis of similarities, reflect correctly evolutionary relationships may frequently be true but is seldom able to be demonstrated (Heywood, 1964). Intuitively one would expect that the more genetically similar two taxa are, the more closely related the two would be. This is only true if evolution has proceeded at the same rate in both lineages. If it has not, then a phylogenetic interpretation will be erroneous.

It is not possible, therefore, in such an analysis to determine to what extent observed similarities are phyletic (i.e. what proportion of the similarity of character states are due to commonly derived characters) and what proportion are due to evolutionary 'noise' (Cain & Harrison, 1960). Sokal and Sneath (1963) claim that no infallible criteria exists to determine convergence when analyses are restricted to extant organisms since it is necessary to determine features which accurately reflect phylogeny. They argue repeatedly that this is unknowable without a knowledge of the phylogeny and that therefore the

problem is insoluble in a logical framework, due to lack of independent evidence.

The distribution of shared character states enables hypotheses to be tested concerning relationships. When a character state is common to all taxa, interpretation is consistent with any hypothesized relationship but when a range of characters is not universally distributed within the group of interest, consideration of internal relationships are possible. However, this frequently leads to conflicts among alternative hypotheses. Since logically only one true phylogeny is possible, some at least will be incorrect (Wiley, 1981).

In such a system no attempt can be made to determine whether character states are derived (apomorphic) or ancestral (plesiomorphic) since the analysis is based on similarities alone and is therefore a closed system. Any attempt at designating a status to a particular character would be invalid in the empirical sense since it would necessarily be based on an *ad hoc* decision (Wiley, 1981).

To minimize internal conflicts involved in choosing the most appropriate hypothesis, it is necessary to invoke a parsimony criterion. However, when analyses are restricted to a closed system, no criterion exists to determine which of the conflicting hypotheses are a valid test and which are not. Therefore the most

parsimonious phylogeny achievable within this restricted context may not be the most appropriate when viewed in the broader context of higher level phylogeny (Wiley, 1981).

Thus when analyses are restricted to a closed system, no criterion exists to determine which of the conflicting hypotheses are phylogenetically valid (Wiley, 1981). Felsenstein (1982) suggested that leading on from the development of clustering techniques for classification, interest was stimulated in developing explicit numerical and algorithmic approaches to systematics. The expansion of the use of molecular data in systematics stimulated interest in inferring phylogenies (Felsenstein, 1982). Various procedures now exist to tackle the concepts of evolutionary 'noise' and despite differences in approach, most are due to the manner in which internal conflicts are addressed (Richardson et al, 1986).

2.08.04: Phylogenetic analyses

To overcome the problems of homoplasy and differences in evolutionary rates, phylogenetists use the most acceptable higher level phylogeny as correct in the context of the test taxon. An outgroup (or sister group) from this taxon may then be introduced to resolve the status of characters within the group and thus enable evolutionary considerations (Wiley, 1981). Ideally such an outgroup will be distinct from the in-group, although closely

related since it is desirable for both a significant overlap in character states and similar mutation rates between the outgroup taxon and the test group (Richardson *et al*, 1986). Although other techniques for assessing polarity states are available, notably the use of the fossil record or changes with ontogeny (Cogger, 1988) these are not appropriate to scincid analyses at this time.

An early advocate of the phylogenetic (or cladistic) approach was Hennig (1950: 1966). In contrast to the conceptual approach of phenetics, he considered that taxonomy should reflect phylogeny alone with classification restricted to monophyletic groups (or clades). Within the constraints of various assumptions, the most significant of which are that each character state has arisen once and no reversal of state occurs, Hennig (1966) considered a character may provide evidence of monophyly. Characters which are derived and held in common, less than universally (synapomorphic characters) are considered to give meaningful information of phylogenetic relationships. Autapomorphy (uniquely derived characters) or symplesiomorphy (ancestral characters held in common are considered to contain no useful information on phylogeny.

Richardson *et al* (1986) suggest that these formal processes of determining phylogenetic relationships have been widely accepted but an insistence on defining taxonomic rank for each dichotomy in a specific phylogeny has not. They present the essential

elements for a purely cladistic analysis; i.e. initially all character states are assessed and classified as either ancestral or derived, all symplesiomorphic and autapomorphic characters are discarded and a cladogram is constructed on the basis of synapomorphic characters. The characters upon which the cladogram is based are therefore derived characters shared among taxa, but not universally shared. Although conceptually sound Hennig (1966) largely ignored problems of internal contradictions, implying that when conflicts occurred a mistake had been made and therefore a re-evaluation of characters was required when such conflicts were identified.

Felsenstein (1982) suggested that there are two alternatives to resolving such conflicts. One approach is to expand the algorithm's underlying assumptions to incorporate reversal of character states and/or to allow characters to arise more than once, although phylogenies should be restricted to a minimum number of such changes. An alternative approach is to base a phylogeny on the largest subset of characters that are mutually compatible. The former approach requires a compromise among character states which may result in the situation where no individual character is completely compatible with the phylogeny. The latter situation will identify a suite of characters that are perfectly compatible while those excluded may be totally contradictory (Felsenstein, 1982). As an alternative to the purely cladistic methodology, these two approaches form the basis

of most modern phylogenetic analyses. A major variation among such algorithms is the manner in which internal conflicts are addressed although all rely on the outgroup criterion to identify anomalies in evolutionary rates (Richardson et al, 1986).

2.08.05: Compatibility methods

The compatibility approach to resolving phylogenies is based on identifying the sub-set of characters which are perfectly compatible. This is considered to occur when two characters complement each other in a particular phylogeny without recourse to the characters having arisen uniquely. Early proponents of this approach (e.g. Wilson, 1965) required both that the whole data set was compatible and a knowledge of ancestral states. Although Le Quesne (1969) extended the approach to include imperfect compatibility, Felsenstein (1982) suggested that the method was not placed on a 'firm logical foundation' until a series of papers by Estabrook and McMorris. In recent years alternative approaches have been formulated to deal with unknown ancestral states, for example McMorris (1977) showed that when the ancestral state was unknown, allocation of the most common character state as primitive was acceptable.

Another problem that needed to be addressed was that the largest group of compatible characters may exclude relevant information omitted from this clique and therefore not used to determine the

topology of the tree. Estabrook *et al* (1977) modified methods such that although the largest clique determines the initial configuration of the tree, taxa are subsequently divided into two groups still consistent with the original configuration and the analysis repeated. In this way characters that were originally excluded from the largest grouping may be reconsidered in the context of the new grouping. By repeating this procedure within the new hierarchy a number of times, most of the information excluded with the initial division can be incorporated.

Felsenstein (1982) identified two other approaches of overcoming the lack of information in the largest clique. He suggested that many accept compatibility in an advisory sense only and in practice may not always use the largest group *persu* but use external criteria to determine the most appropriate grouping on which to base a phylogeny. Alternatively a taxonomist may restrict their search to characters occurring over a number of the largest cliques.

2.08.06: Parsimony techniques.

Parsimony methods, first introduced by Edwards and Cavalli-Sforza (1963; 1964), strive to produce a phylogeny with the minimum number of evolutionary steps. Although originally used for gene frequency analysis, it has been modified to cope with discrete character data (Felsenstein, 1982). Such analyses, including

minimum-length Wagner networks and trees (Farris, 1970; 1972) together with those of Camin and Sokal (1965) and Fitch and Margoliash (1967) make no assumptions concerning evolutionary rates or the possibilities of errors resulting from similarities due to homoplasy, since the use of parsimony criterion is considered to reduce the impact of such errors. Although conceptually the same, different parsimony programs make different assumptions about evolutionary rates, for example the Wagner technique (Farris, 1970: 1972) aims to produce a minimum length tree while the Fitch and Margoliash (1967) method aims to minimize the difference between input and output.

Having decided upon a method of approach, and having ensured that the technique chosen has appropriate underlying assumptions, it is necessary to address the problem of searching for the most parsimonious topology. No computation is available which ensures that the topology chosen is the most parsimonious of all those potentially available unless all possible alternatives are assessed. This approach is, however, generally unrealistic due to the large number of possibilities for even a modest data set. Despite improvement in the efficiency of search procedures (e.g. Estabrook, 1968 & Nastansky et al, 1973), it remains impractical for all but the smallest of data sets. To maximize the chances of obtaining the most parsimonious tree, it is desirable to rearrange the data such that the construction of the tree begins at different points in the data set (e.g. a shuffling process),

thereby producing multiple data runs. This increases the chance of uncovering the most parsimonious tree (Felsenstein, 1982).

2.08.07: The appropriate approach.

Analysis of data for phylogenetic reconstruction is therefore not routine and requires a decision on the most appropriate analytical approach. Richardson et al (1986) warn of the highly controversial nature of such analysis and maintain that no one approach nears universal acceptance. If all characters were perfectly compatible, it would seem logical to use a purely cladistic approach as the basis of classification. In reality, however, it is unlikely that such a situation will arise, therefore alternative analyses generally need to be considered.

Under certain conditions of constant rate of evolution and no homoplasy, phenetic relationships will reflect evolutionary relationships. However, it is not possible to separate the effect of such environmental 'noise' from the phyletic component of an analysis based on phenetic similarities. Therefore, although being a useful adjunct to determining genetic relatedness, it is not an appropriate tool to use in isolation.

Parsimony approaches also have problems since it is intuitively difficult to accept that a taxon evolves in the most step-wise conservative manner, since evolution is accepted to occur as a

result of the random fixation of genes. This conceptual approach, however, is largely limited to this dimension since it is difficult to envisage criteria that allow for more than minimum evolution without chaos ensuring. Relying on compatibility techniques may also present problems since this approach may ignore a large amount of conflicting information.

Felsenstein (1982) investigated the grounds used to justify use of one or other of the various methods and concludes that none are available to choose one technique above another. All have limitations. He suggests that each method should be approached with 'skepticism'. In general then, it is prudent to approach the process of producing a phylogeny by analysing the data (with reference to the algorithms underlying assumptions) in a number of ways and thereby identify trends which are mutual to various approaches.

2.09: IMMUNOLOGICAL TECHNIQUES IN SYSTEMATICS

Within this context (outlined above) allozyme electrophoresis is a useful molecular tool for determining phenetic and phylogenetic relationships. As discussed above, the taxonomic value varies with the level of differentiation that has occurred within the group of interest. When differentiation approaches 60-70% divergence, the technique is nearing the limits of it resolution.

Apart from allozyme electrophoresis, the most commonly used biochemical criteria for genetic distance estimates are derived by immunological methods (Sarich, 1977a). Such techniques have been available for many decades (e.g. Nuttall, 1904) but most have relied on the serological analysis of a suite of proteins and polyvalent antisera (e.g. Cohen, 1955; Lewis, 1965; Frair, 1969; Hutchinson, 1981; 1983). The results of such studies are, however, difficult to interpret phylogenetically because of the complications implicit in assays involving multiple protein mixtures (Gorman et al, 1971) since interpretation of precipitin lines in double diffusion plates and on immunoelectrophoretic slides is problematical since it is not easy to distinguish between variation in antigen concentration and homology in mixed proteins. Phylogenetic analysis based upon such techniques may have some value among closely related species but this methodology has been largely superceded (Champion et al, 1974). In recent years improved techniques have allowed for more rigor in isolating single proteins. This has resulted in greater use of monovalent antisera and consequently greater accuracy in the determination of evolutionary relationships (e.g. Baverstock, 1984; Chen et al, 1980; Collier & O'Brien, 1985; Scanlan & Maxson, 1979).

2.10: MICRO-COMPLEMENT FIXATION: INTRODUCTION AND PRINCIPLE OF THE TECHNIQUE.

Outstanding among recent immunological methods is the technique of micro-complement fixation (Mc'F; Wilson *et al*, 1977) which assesses the degree of cross-reactivity of polyclonal antibodies, directed against a specific protein from one species, with an equivalent protein from other species. Interpretation of results is independent of concentration of the protein in the various samples.

The technique, micro-complement fixation, is a quantitative measure of the extent of the immunological reaction between antibody and antigen at low concentrations. When antibody molecules are immunologically active against a specific antigen molecule, the antibodies bind to specific portions of the antigen at recognized sites. When the complement system of guinea pig serum is added to the antibody/antigen mixture under appropriate conditions, it binds irreversibly to the antibody/antigen complexes. After this reaction has occurred, if sensitized sheep red blood cells are added to the solution, the available complement will lyse the cells, releasing hemoglobin. After removal of the unlysed cells by centrifugation, a measure of the optical density of the amount of hemoglobin remaining in the solution, gives a quantitative measure of the amount of

complement bound and therefore a measure of antibody/antigen binding (Champion *et al*, 1974).

The amount of cross-reactivity that occurs is dependent on the proportions of antigen and antibody available in solution. If one or the other are not optimal, the resulting complexes will tend to be linear and not three-dimensional. They will therefore not be in the appropriate configuration to bind available complement. When conditions are optimal, the 'point of equivalence' will be reached (i.e. when the maximum number of binding sites on both the antibody and antigen are occupied and thus the maximum amount of cross-linking between complexes occurs) (Champion *et al*, 1974).

It is necessary to find a series of dilutions across the experiment from a low antigen concentration through the 'point of equivalence' to an excess of antigen. An appropriate series of antibody/antigen concentrations plotted as a percentage of complement fixed versus antigen concentration will fit a bell-shaped curve. Varying the antibody concentration in the serial experiment effects both percent fixation and consequently the peak of the curve and its slope, but not its amplitude. The 'point of equivalence' will ideally represent the titre at which approximately eighty percent of antiserum is fixed. When at lysis less than eighty percent occurs in the controls, experimental variation increases, while at higher than ninety percent lysis,

an apparent drop in percent fixation occurs due to differential rates of lysis (Champion et al, 1974).

When the peak of the curves at different levels of fixation are plotted against the logarithm of the antiserum concentration, a straight line will result, the slope of which is defined by the equation:

$$Y = m\log X + b.$$

Where Y = complement fixed at the peak of the slope

X = antiserum concentration, and

b = the Y intercept.

Generally the angle of the line is characteristic of a particular antiserum. The amount by which the observed antiserum concentration of a specific heterologous antigen has to be increased to obtain a complement fixation curve (now converted to a straight line equivalent to that obtained for the homologous antigen) gives a quantitative immunological comparison (i.e. index of dissimilarity) (Champion et al, 1974). Wyles and Gorman (1978) gave a measure of cross-reactivity which can convert such data to immunological distance units by the following equation:

100 x log of index of dissimilarity

Each such immunological distance corresponds approximately to a single amino acid substitution between the two albumins being investigated (Wilson *et al*, 1977) and is therefore justifiable as the basis of phylogenetic analysis.

2.11:   MOLECULAR DIFFERENTIATION AND EVOLUTION

Molecular data, being genetically derived, have advantages over analysis of many morphological character states since differences between a specific protein in two related species are not generally influenced by environmental factors and therefore indicate genetic differentiation among populations (Thorpe, 1982). Since Kimura (1968) hypothesized that the most significant cause of molecular evolution was the constant random fixation of selectively neutral mutations, numerous studies using a wide variety of organisms have attested to the generally time-dependent nature of divergence in structural proteins (e.g. Ayala, 1976; King & Jukes, 1969; Lewenton, 1974; Sarich, 1977; Thorpe, 1982; Wilson *et al*, 1977).

It has been demonstrated mathematically that such random fixation generally occurs at a constant rate equivalent to the neutral mutation rate (Kimura, 1968). Superimposed on these random substitutions will be those that are the result of selective effects and these may not occur at a constant rate (Gorman *et al*, 1971).

2.12: CORRELATION OF MOLECULAR EVOLUTION WITH TIME

Absolute rates of macromolecular evolution can be obtained by comparing amino acids of an homologous protein (i.e. the same macromolecule) from two or more taxa with known divergence times. The number of amino acid differences acquired since divergence in each lineage, represents an estimate of the absolute rate of molecular evolution in that lineage since divergence. However, a count of the observed number of amino acid differences in the different lineages will represent an underestimate of the true number of sequence differences that have occurred because sequential mutations at a single site will not be identified. The extent to which multiple mutations have occurred is most easily identified by reference to differences in phyletic distances (i,e, the number of differences that have occurred as estimated by phylogenetic topology construction) between the various taxa and the outgroup (Wilson et al, 1977).

Reproductive isolation of two taxa may take upwards of a million years, with subsequent independent divergence of proteins. Calculations of evolutionary rates are therefore generally restricted to species separated for in excess of one million years and a knowledge both of an estimate of evolutionary rates and time of divergence (as identified in the fossil record) are required (Wilson et al, 1977). For various reasons outlined in

Wilson et al (1977) calculation of divergence times is best restricted to taxa separated less than 100 million years.

Fitch and Langley (1976; Fitch, 1976) examined the available data on mammalian protein evolution. They observed approximately constant mutation rates over the last 120 million years in seven polypeptides (cytochrome c, myoglobin, hemoglobin α- & β-, insulin C peptide, fibrinopeptide A & B) although individual polypeptides had a characteristic mutation rate. Such analyses have not been carried out for other species groups due to both limited sequence data and generally poor fossil records (Wilson et al, 1977).

2.13: MOLECULAR CLOCK HYPOTHESIS

2.13.01: Time dependent evolution

The observation of time dependent mutation, first reported by Zuckerkandl and Pauling (1962), forms the basis of the molecular clock hypothesis. In addition to the mammalian data supporting the concept, further support is available from micro-complement fixation comparisons of proteins, particularly serum albumin and transferrin (e.g. Gorman et al, 1971). To calibrate these comparisons, homologous pure globular monomeric proteins with known amino acid sequences have been compared. Strong correlations resulted in the various groups tested including

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those of bird lysozymes, bacterial azurins, plant plastocyanins and mammalian ribonucleases (Wilson *et al*, 1977).

2.13.02: Serum albumin calibration of the molecular clock

Extensive use of albumin mirco-complement fixation in evolutionary studies (e.g. by 1977 over one thousand pairwise comparisons of vertebrate taxa had been assessed; Wilson *et al*, 1977) has demonstrated that, at least for two groups with good fossil records (i.e. carnivores and ungulates) correlations between albumin immunological distances and paleontological estimates of divergence time is strong (r=0.96). Based on this correlation Sarich (1985) revised an earlier calibration for albumin data such that he now postulates that 1.4 - 1.5% difference in the albumin cross-reaction occurs for every million years separation between two lineages. Non-mammalian groups including frogs, lizards and crocodiles yield comparable data, however, due to frequently inadequate fossil records, these divergence times have been supplemented with evidence of continental drift (Wilson *et al*, 1977).

2.13.03: Allozyme electrophoresis calibration of the molecular clock

Allozyme electrophoresis also theoretically allows for an examination of divergence time based on amino acid substitutions.

As previously discussed, the method is useful for discrimination of protein mutations among lineages. However, since scoring has been restricted to an observation of whether mobilities are equivalent or different, without reference to the absolute differences in mobilities. No estimation of the actual number of amino acid mutations can be made since multiple mutation events, together with sequential mutations at a single site, are considered as a single difference. Thus the estimation of amino acid substitution is less rigorous using allozyme electrophoresis data than it is for that obtained from micro-complement fixation data. A recent study by Briscoe *et al* (1977) addresses this problem and therefore studies which incorporate their approach could give a tighter correlation between electrophoretic data and albumin immunological distances. However, to date this approach is untried (see Sect. 2.04).

2.13.04: Correlation between allozyme electrophoretic and micro-
complement fixation data

Despite the limitations of allozyme data in estimating sequence divergence, various authors (e.g. Baverstock & Schwaner, 1985; Case *et al*, 1975; Highton & Larson, 1980; Maxson & Maxson, 1979; Sarich, 1977; Wyles & Gorman, 1980) have attested to the significant correlation between allozyme electrophoretic and albumin immunological distances. Sarich (1977; 1977a) compared Nei electrophoretic distances and albumin immunological distances

for 76 pairs of species, including iguanid lizards (*Anolis*) and several rodent genera. He observed a strong correlation (r=0.8) between the two genetic measures. Wyles and Gorman (1980) using the same genetic measures, observed an equivalent level of significance within *Anolis* alone (r=0.8).

Baverstock and Schwaner (1985) recognized problems with the preciseness of allozyme electrophoretic data. Although they were able to show a tight correlation between these data and albumin immunological data, they observed that there is a "considerable scatter of the points". They suggested, as do Wyles and Gorman (1980), that different loci evolve at very different rates and thus genetic distance may be affected by the choice of loci. Genetic factors, such as 'bottlenecks', are also expected to influence divergence, especially in the initial stages of isolation but by maximizing the number of loci investigated these problems are considerably reduced and the available evidence indicates that the two techniques, allozyme electrophoresis and micro-complement fixation, are complementary for reptile evolutionary studies.

2.14: ACCEPTANCE OF THE MOLECULAR CLOCK HYPOTHESIS

Despite strong evidence for the molecular clock concept, the idea is by no means universally accepted, its validity having been disputed since its inception over two decades ago (Thorpe, 1982).

Some authors go so far as to declare the whole concept a myth (e.g. Cain, 1983). Wilson et al (1977) reviewed the basis of these objections and observed that frequently anomalies, such as differences in divergence times among groups or the apparent influence of generation span on rates of mutation, can be explained in terms of poor knowledge of paleontological estimates. The introduction of an outgroup to test for relative rate differences among lineages, circumvents the need for precise knowledge of divergence time. Frequently when this approach has been taken, such as in the case of problems with divergence times in primates, apparent anomalies are resolved. Sometimes, however, anomalies in mutation rates remain despite overcoming the need for paleontological estimates. Although on occasions this may be the result of an inaccurate phylogenetic hypothesis, other factors such as gene duplication may be involved. Wilson et al (1977) suggest that this may be responsible for the apparent anomaly in the cytochrome c of rattlesnake when compared with birds and mammals. The classical taxonomic viewpoint, based on nonmolecular evidence, is that the divergence of snakes and birds is more recent than the divergence of mammals from the common ancestor of the group. Conversely, the cytochrome c sequences indicate that mammals and birds are monophyletic with respect to snakes. This data have been presented as evidence of acceleration in mutation rates (Jukes & Holmquist, 1972), however, Wilson et al (1977) have postulated that it may represent a paralogous

comparison of sequences; that is a comparison of a duplicated cytochrome c sequences (snake) with that of unduplicated ones. Another example where anomalies in evolutionary rates have not been resolved is that of guinea pig insulin. However, Wilson *et al* (1977) consider that an early duplication of the gene would not entirely account for the observed accelerated rate, neither does the concept of inaccurate phylogeny since other proteins investigated in the guinea pig conform with the classical taxonomic viewpoint. Thus although such problems exist, it is possible to identify such anomalies with reference to additional characteristics and treat the hypotheses accordingly.

Many studies have identified an apparent stochastic nature in protein divergence with time (e.g. Carlson *et al*, 1978; Nei, 1975; Sarich, 1977; Wilson *et al*, 1977) while Thorpe (1982) concluded that this aspect of the evolutionary clock hypothesis had caused most controversy.

This phenomenon may be identified in the form of a range of evolutionary distances in a phylogenetic tree. Since this variation is not reliant on paleontological divergence time (due to the inclusion of an outgroup), the variation results in apparent differences in evolutionary rates within equivalent time frames and therefore could represent an inherent error (Wilson *et al*, 1977). Nei (1977) investigated the standard error of immunological distances with time. He calculated that, the

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variance of recent divergences were twice the size of the mean (approximately 10 units) with the ratio of variance to mean increasing with increasing mean. This ratio of variance incorporates two sources of error, both the inherent error due to the stochastic variation in the clock and that associated with using immunological distances to represent sequence variation.

While mutation rates within a specific protein approximate constant evolutionary rates (although stochastically skewed), functionally different proteins evolve at quite different rates, despite assumed equivalent amino acid mutations in all sequences. These variations are thought to be associated with the probability of fixation of specific mutations (Wilson et al, 1977).

Hypotheses based on the idea of functional constraints appear to provide at least part of the explanation. Thus although recognizing that all amino acids in a sequence have a function, a specific site will be able to tolerate a different number of potential residues to fulfill the function of that site. Therefore at some sites only a single residue may be tolerated and any mutation would be disadvantageous, however, at other sites a range of mutations may be tolerated and the site still remain functional. Natural selection would thus act to suppress mutation rates in a sequence with a relatively large number of exclusive sites while those with relatively fewer exclusive sites

would approach a random distribution of mutational events (Wilson *et al*, 1977).

Analogous to this concept of site related constraints, is that of functionality of the sequence at the organismal level. Thus a sequence such as histone 4 would be expected to evolve very slowly since almost all of its sites, except those in the third (silent) position of the codon, affect its function. Fibrinopeptides are frequently thought to represent the alternative extreme since they would be expected to have relatively few active sites (Wilson *et al*, 1977). Wilson *et al* (1977) suggests, however, that such functional-constraint characteristics combined with 'dispensability' may provide greater influence on mutation rates than the former characteristic alone. Thus a peptide such as cytochrome c which is essential for energy transfer, is critical to an organism and therefore *any* specific mutation will potentially have a deleterious effect. Conversely a mutation in serum albumin will potentially present fewer problems, since other serum proteins are capable of the same function as albumin and it is possible (at least in humans) to reproduce with virtually no serum albumin. A specific mutation which mildly suppresses its function would therefore tend to have less impact on the organism than the essential peptide of cytochrome c. Adaptive changes in serum albumin would, therefore, be rare since most change would result from selectively neutral amino acid replacements since the

specific amino acid sequence of albumin is not critical to the organism. Under these circumstances albumin would be expected to evolve at a near constant rate (Gorman *et al*, 1971) in contrast to cytochrome c which is critical for energy transfer (Wilson et *al*, 1974).

Despite these observations of differential rates of fixation of mutations among different sequences, within a specific sequence mutations rates generally occur in a clock like manner, all be it stochastic. Therefore the existence of such variation does not invalidate the clock but rather serves to reinforce the concept of approaching interpretation with caution. Albumin, being an essentially unrequired protein, is considered to approach random mutation fixation. This characteristic, together with its extensive use in micro-complement fixation studies (which enables comparison across studies) makes the protein an ideal basis upon which to investigate divergence times within the Scincidae. Correlation between albumin micro-complement fixation and allozyme electrophoresis enable the latter to be utilized as an adjunct to the former.

CHAPTER 3: TAXONOMY OF *LAMPROPHOLIS* SKINKS: ELECTROPHORETIC VARIATION.

3.01:   TAXONOMIC UTILITY OF ALLOZYME ELECTROPHORESIS FOR REPTILE RESEARCH.

As discussed in the previous chapter (Section 2.04) allozyme electrophoresis has become one of the most widely employed techniques in the study of evolutionary biology while most early work concentrated on intrapopulation heterozygosity and geographic variation of alleles, however the utility of allozyme electrophoresis in systematic studies has been recognized for many years (see Gottlieb, 1971).

In contrast to its use in the taxonomy of many animal groups, the technique has not been widely applied in the area of reptile systematics. A notable exception is the North American Iguanidae, particularly the *Anolis* complex. Investigations of this group have included intraspecific variation (e.g. Wade *et al*, 1983) and species boundaries (e.g. Gorman *et al*, 1980; Webster *et al*, 1972) as well as phylogenetic relationships and biogeographical considerations (e.g. Gorman *et al*, 1978; Wade *et al*, 1983; Yang *et al*, 1974). Genetic relationships of other iguanid genera, for example *Uma* (Adest, 1977), *Uta* (Soule *et al*, 1973; Soule & Yang, 1973), *Sator* and *Sceloporus* have also been elucidated using allozyme electrophoresis (Wyles & Gorman, 1978).

Other reptile groups which have been investigated to some extent are snakes (Baverstock & Schwaner, 1985; Mengden, 1985; 1985a; Zweifel, 1981; Lawson & Dessauer, 1979), Gekkonidae (Murphy & Papenfuss, 1980) and Varanidae (Holmes et al, 1975).

Few researchers have applied allozyme techniques to the evolution, speciation and population genetics of the Scincidae and studies that have been undertaken tend to be limited in nature. Typical are the studies of Kim et al (1978) who investigated genetic differentiation in two species of *Typhlosaurus* and of Milton et al (1983) who confirmed specific distinctiveness for two species of *Egernia*. Of most significance to the present study is the work on *Lampropholis* by Harris and Johnston (1977) and Mather (1986). In the former investigation the authors concluded that sympatrically occurring species of *Lampropholis*, *L. delicata* and *L. guichenoti*, were taxonomically separate as they differed at two of the five loci investigated. Mather (1986) was interested in stabilising the nomenclature of *L. delicata* in South East Queensland. His taxonomic studies confirmed that there are at least four electrophoretically distinct taxa within the synonymy, *L. delicata*.

The work of the above authors indicated that allozyme studies would be useful in probing species distinctiveness within scincid lizards and lent support to its use in the current study of intrageneric relationships of *Lampropholis*.

3.02: MATERIALS AND METHODS FOR ALLOZYME ELECTROPHORESIS.

3.02.01: Limitations of the data

Although some of the species investigated, particularly *L. delicata* and *L. guichenoti* are widespread and abundant in appropriate microhabitats, others have never been found to be abundant, for example *L. caligula* and *L. amicula* (see Preface). Several of the species are known from relatively few individuals, collected by a few herpetologists. This resulted in many hours, and on some occasions, such as in the case of *L. caligula*, the equivalent of weeks of field work for very little return. In general, the lesser known and sparsely distributed animals inhabit relatively remote areas, requiring in the case of *L. tetradactyla* thousands of kilometers of travel before a search could be undertaken. Localities from which reptiles were collected are recorded in Table A.1 (Appendix 1).

The relatively small size of these animals, together with collections spread over three years has resulted in some species not being compared electrophoretically for all loci. Within the context of this postgraduate research this was impossible to achieve.

3.02.02: Laboratory techniques

Horizontal starch gel electrophoresis was performed using 12% Electrostarch (Otto Hiller, Madison, Wisconsin) and Cellogel (Chemetron, Milan). Preliminary screening of various enzymes for acceptable resolution resulted in the more intensive screening of 23 enzymes using four buffer systems on cellogel. The enzymes screened, together with buffer systems and other pertinent details are listed in Table 3.1.

Isozyme electrophoresis was carried out on an homogenate of liver, heart and head tissue or on a homogenate of liver tissue alone. Tissues were obtained from freshly killed specimens, weighed and homogenised on ice (1:1 w/v in a lysing solution consisting of 0.1% 2-mercaptoethanol, 0.1 mg/ml NADP in distilled water). Homogenates were used fresh or stored at $-20°C$. Immediately prior to electrophoresis the homogenates were centrifuged for three minutes to remove cellular debris. The supernatants were absorbed onto 5 mm X 5 mm squares of Whatman 3 MM paper for insertion into starch gels or applied directly to cellogel sheets using a draftsman's pen. Gels carried a sample of known mobility as a control for accurate scoring of isozymes and check gels were run to confirm allele identities.

All cellogels were run in a cold room at 4°C at 200 volts for one hour fifteen minutes - two hours depending on the mobility of the

Table 3.1: Enzymes screened and buffer systems utilized for allozyme electrophoretic analysis (all recipes are included in Richardson et al, 1986).

E.C. recommended name	Abbreviation	E.C. number	Buffer system
Aspartate aminotransferase	AAT	2.6.1.1	0.1M Tris-maleate (pH 7.8)
Aconitate hydratase	ACON	4.2.1.3	0.01M Citrate-phosphate (pH 6.4)
Adenosine deaminase	ADA	3.5.4.4	0.02M Phosphate (pH 7.0)
Adenylate kinase	AK	2.7.4.3	0.02M Phosphate (pH 7.0)
Aldolase	ALD	4.1.2.13	0.1M Tris-maleate (pH 7.8)
Enolase	ENOL	4.2.1.11	0.01M Citrate-phosphate (pH 6.4)
Fructose$_{1,6}$diphosphatase	$_{1,6}$DIPHOS	3.1.3.11	0.02M Phosphate (pH 7.0)
Fumarate hydratase	FUM	4.2.1.2	0.01M Citrate-phosphate (pH 6.4)
Glucose$_6$phosphate dehydrogenase	G$_6$PD	1.1.1.49	0.02M Phosphate (pH 7.0)
Isocitrate dehydrogenase	IDH	1.1.1.42	0.01M Citrate-Phosphate (pH 6.4)
Lactate dehydrogenase	LDH	1.1.1.27	0.02M Phosphate (pH 7.0)
Malate dehydrogenase	MDH	1.1.1.37	0.015M Tris-EDTA-borate-MgCl$_2$ (pH 7.8)
Mannose-phosphate isomerase	MPI	5.3.1.8	0.02M Phosphate (pH 7.0)
$_6$Phosphogluconate dehydrogenase	$_6$PG	1.1.1.44	0.02M Phosphate (pH 7.0)
Glucose-phosphate isomerase	PGI	5.3.1.9	0.1M Tris-maleate (pH 7.8)
Phosphoglucomutase	PGM	2.7.5.1	0.1M Tris-maleate (pH 7.8)

Pyruvate kinase	PK	2.7.1.40	0.01M Citrate-phosphate (pH 6.4)
Superoxide dismutase	SOD	1.15.1.1	0.02M Phosphate (pH 7.0)
Hexokinase	HK	2.7.1.1	0.02M Phosphate (pH 7.0)
Triosephosphate isomerase	TPI	5.3.1.1	0.1M Citrate-phosphate (pH 6.4)
Glycerol$_3$phosphate dehydrogenase	αGP	1.1.1.8	0.02M Phosphate (pH 7.0)
Nucleoside phosphorylase	NP	2.4.2.1	0.02M Phosphate (pH 7.0)
Malic enzyme	ME	1.1.1.40	0.02M Phosphate (pH 7.0)

enzymes. Starch gels were run under the same conditions but for fourteen - sixteen hours as appropriate. Following electrophoresis, starch gels were sliced horizontally into at least six slices. Each slice was then stained for a specific enzyme system by applying approximately 4 mls of the appropriate staining reagent to Whatman 1 MM filter paper overlaying the gel which was then sandwiched between two plastic sheets and incubated at 37°C until zymograms were visualized. The staining procedures for specific enzyme systems were either the established techniques, or minor modifications of techniques presented in Harris and Hopkins (1976), Shaw and Prasad (1970) and Richardson et al (1986).

Cellogels were treated in a similar manner to slices of starch gel. However it was necessary to administer only 2mls of the appropriate stain to these gels. No filterpaper overlay was necessary for cellogel.

3.03:   DATA ANALYSIS OF ALLOZYME ELECTROPHORESIS.

3.03.01: Scoring of electromorphs.

Traditionally measures used to score electromorphs countenance only that homologous enzymes differ in electrophoretic mobility i.e. they are either of the same mobility or different. This approach does not allow quantitation of the degree of

differentiation that has occurred since divergence. After determination of mobility variance at all loci, a matrix consisting of pairwise comparisons of all taxa was constructed.

In an attempt to measure the extent of differentiation, the distance each band had migrated from the origin was measured. The pairwise comparison of two taxa for any enzyme can then be taken as the absolute physical distance which separates the two electromorphs. A similarity index (based on % fixed difference) for each species pair was obtained by determining the absolute migration distance summed over all loci. Computer simulations and data analyses have demonstrated that this technique yields useful phylogenetic information even when genetic distances are large. It thus has the potential to extend the interpretation of electrophoretic studies and provides a partially independent data set (Briscoe *et al*, 1987).

3.04: ANALYSIS OF ALLOZYME ELECTROPHORESIS DATA

3.04.01: Justification for the analytical approach to allozyme data

As previously discussed (Sect. 2.08) there is no clear consensus on the most appropriate approach to analysis of electrophoretic data. In simplistic terms the phenetisists argue that, without fossil data, phylogenies are unknowable. At the other extreme the

cladists claim that classifications that are not based on phylogenetic considerations are meaningless. A recognized problem involved with allozyme data is that homoplasy is frequently a complicating factor. Problems also exist in determining the condition of character states for cladistic analysis.

In an attempt to account for these problems it was decided to undertake both phenetic and cladistic analyses together with the numerical approach of Fitch and Margoliash (1967). The rationale being that similar results from all three would indicate that homoplasy is not a significant factor in this data set and that character states have been determined appropriately whereas conflicting results would indicate that further investigations are necessary.

3.04.02: Sequence of analyses of phenetic data and results.

Preliminary phenetic analyses were run using both complete linkage and unweighted average linkage algorithms. Both these measures resulted in similar branching sequences for a given programme. Thus final analysis was restricted to unweighted average linkage.

Since the hypothesis and associated programme for the absolute distance measure (Briscoe et al, 1987) was formulated late in the project, a complete data set was not available for this analysis.

To avoid any associated bias in a comparison of methods, all analyses were initially undertaken on populations for which sufficient measurements of isozyme migration distances had been obtained. Hereafter these will be referred to as analyses of the restricted data set.

For the final phenetic analyses the data set was necessarily reduced due to computer limitations. The data set was therefore inspected and, where two populations had identical electromorphs, only one of the pair was included in the analysis. Each population thus eliminated, is listed in Table 3.2 along with its sister population.

The data were reduced to matrices of fixed gene differences among taxa, rather than to matrices of genetic similarities such as Nei (1972) or Rogers (1972) recommend. Thus percent fixed gene difference was used to express fixed differences among taxa and phenetic and phyletic analyses were undertaken.

The programs used were written, and modified where appropriate, by Dr. George McKay, Macquarie University. Computer analysis was achieved with his aid. The following approaches were used:

Two phenetic algorithms were utilized. Analysis of a distance measure of percent fixed differences (Richardson & McDermid, 1978) gives an estimate of the percentage of alleles which differ

Table 3.2: Populations incorporated into allozyme electrophoretic
analysis to represent identical taxa omitted.

Populations omitted   Populations incorporated

L. challengeri (Styx R.)   *L. challengeri* (Coffs H.)
L. mustelina (Chatswood)   *L. mustelina* (Pymble)
L. mustelina (Hunters Hill)   *L. mustelina* (Pymble)
L. mustelina (Lane Cove)   *L. mustelina* (Pymble)
L. mustelina (Ryde)   *L. mustelina* (Pymble)
L. mustelina (Wentworth Falls)   *L. mustelina* (Pymble)
L. mustelina (Jenolan)   *L. mustelina* (Bellevue Hill)
L. mustelina (Greenwich)   *L. mustelina* (Pymble)
L. mustelina (Barry)   *L. mustelina* (Bellevue Hill)
L. mustelina (Carrai S.F.)   *L. mustelina* (Pymble)
L. mustelina (Hampton)   *L. mustelina* (Jenolan)
L. mustelina (Barrington Tops)   *L. mustelina* (Pymble)
L. mustelina (Walcha)   *L. mustelina* (Pymble)
L. mustelina (Mt.Tomah)   *L. mustelina* (Bellevue Hill)
L. mustelina (King L)   *L. mustelina* (Bellevue Hill)
L. mustelina (Mt.Wilson)   *L. mustelina* (Pymble)
L. basiliscus (Danbulla S.F.)   *L. basiliscus* (Cairns)
L. basiliscus (Curtin Fig)   *L. basiliscus* (Cairns)
L. basiliscus (Mt. Spec)   *L. basiliscus* (Cairns)
L. basiliscus (Kuranda)   *L. basiliscus* (Cairns)
L. basiliscus (L. Eacham)   *L. basiliscus* (Cairns)
L. basiliscus (Palmston)   *L. basiliscus* (Cairns)
L. basiliscus (Ravenshoe)   *L. basiliscus* (Cairns)
L. mirabilis (Mt. Lewis)   *L. mirabilis* (C. Cleveland)
L. czechurai (Millaa)   *L. czechurai* (Kuranda)
L. czechurai (Ravenshoe)   *L. czechurai* (Kuranda)
L. czechurai (Finch Hatton)   *L. czechurai* (Kuranda)
L. czechurai (Eungella)   *L. czechurai* (Kuranda)
L.tetradactyla (Palmston Ck)   *L.tetradactyla* (Cairns)
L.tetradactyla (Kuranda)   *L.tetradactyla* (Cairns)
L. delicata (Pymble)   *L. delicata* (Ryde)
L. delicata (Bellevue Hill)   *L. delicata* (Ryde)
L. delicata (Boudi S.F.)   *L. delicata* (Eltham)
L. delicata (Wiangarie S.F.)   *L. delicata* (Eltham)
L. delicata (Scarborough)   *L. delicata* (Armidale)
L. delicata (Coffs Harbour)   *L. delicata* (Ryde)
L. delicata (Dorrigo)   *L. delicata* (Eltham)
L. delicata (Warroo)   *L. delicata* (Eltham)
L. delicata (Byfield)   *L. delicata* (Eltham)
L. sp.3 (Eungella)   *L. sp.3* (Danbulla S.F.)
L. sp.1 (L. Eacham)   *L. sp.1* (Ravenshoe)
L. guichenoti (Hampton)   *L. guichenoti* (Ryde)
L. guichenoti (Bellevue)   *L. guichenoti* (Hampton)
L. guichenoti (Chatswood)   *L. guichenoti* (Hampton)
L. guichenoti (Hunters Hill)   *L. guichenoti* (Hampton)
L. guichenoti (Pymble)   *L. guichenoti* (Hampton)
L. guichenoti (Cnr Monora/Snowy Hwy) *L. guichenoti* (Ryde)
L. guichenoti (Nimmitabel)   *L. guichenoti* (Bathurst)
L. guichenoti (Moona Plains)   *L. guichenoti* (Walcha)

between species pairs. A dissimilarity state for each species pair is calculated and tested in sequential order beginning with the most divergent taxa. A non-parametric test is used to make comparisons at each level of divergence. The second procedure, an unweighted pair-group method using arithmetic averages (Sneath & Sokal, 1973) initially clusters by finding reciprocally the most similar groups. Successive taxa are added by comparison with the mean of the preceding clusters, regardless of the cluster's internal structure.

Alternatively, complete linkage clustering (Sneath & Sokal, 1973) assesses a taxon for admission to a cluster on the basis of its similarity with the most distant member within the extant cluster. Subsequently when clusters join, their similarity is equivalent to that existing between the two most distantly related taxa, one from each cluster.

These two algorithms were used to produce phenograms (Figs 3.1-3.2) based on the traditionally derived, restricted data set (Table 3.3-3.4). The former algorithm was used to derive a phenogram (Fig 3.3) from the matrix (Table 3.5) generated from measured data. Subsequently, these same algorithms (Richardson & McDermid, 1978; Sneath & Sokal, 1977) were implemented to analyse the full data set (after identical populations had been removed; see Table 3.2). These phenograms are presented in Figures 3.4 - 3.5 (matrices Tables 3.6-3.7).

All these phenograms (Figs 3.1-3.5) give a pictorial view of the distance measures and are not intended to demonstrate phylogenetic relationships among taxa *per se* since no attempt is made to identify distortion due to homoplasy. However, they are frequently employed as a useful first approximation of relationships and are therefore used alongside phylogenetic results as a guide to their accuracy.

3.04.03: Phyletic analysis and results

In contrast to phenetic analyses which ignore homoplasy, phyletic techniques strive, in various ways, to identify and account for this problem. A tree based on the numerical 'goodness-of-fit' approach (Fitch & Margoliash, 1967) is presented in Figure 3.6 based on the matrix of fixed differences presented in Table 3.6. This approach attempts to produce a phylogeny based on a 'goodness-of-fit' criterion. As previously discussed (Section 2.08.06) it aims to combine the criterion of parsimony with the minimum distortion of the data. To ensure that the configuration of the phenogram was not influenced by the sequence of data input, the analysis was re-run three times using the 'shuffle' program of Phylip 2.9. These resultant phylograms were essentially the same and therefore only one is presented here (Fig 3.6).

A cladistic approach, initially formalized by Hennig (1966), relies on synapomorphies to construct a phylogeny, while similarities due to symplesiomorphs, or differences based on autapomorphs, provide no phylogenetic information. This analysis was undertaken by hand, identifying groups based on numbers of synapomorphs in common. Three such analyses were undertaken, one utilized _L_. _challengeri_ complex species as the outgroup (Fig. 3.7a), the second analysis relied on _L_. _delicata_ complex species as an outgroup (Fig. 3.7b) and finally this information together with _Le. zia_ was used to determine a consensus cladogram (Fig. 3.8).

3.04.04: Summary of results.

In summary, the phenograms constructed from analysis of the restricted data sets are presented in Figures 3.1-3.3 while the matrices from which these were derived appear in Tables 3.3-3.5. Phenograms constructed from the more extensive data sets are presented in Figures 3.4-3.5. Percent fixed differences upon which these latter figures were based are presented in matrices, Tables 3.6-3.7. A phylogram generated from the percent fixed differences of Table 3.6 is presented in Figure 3.6, while the cladograms are presented in Figures 3.7 and 3.8.
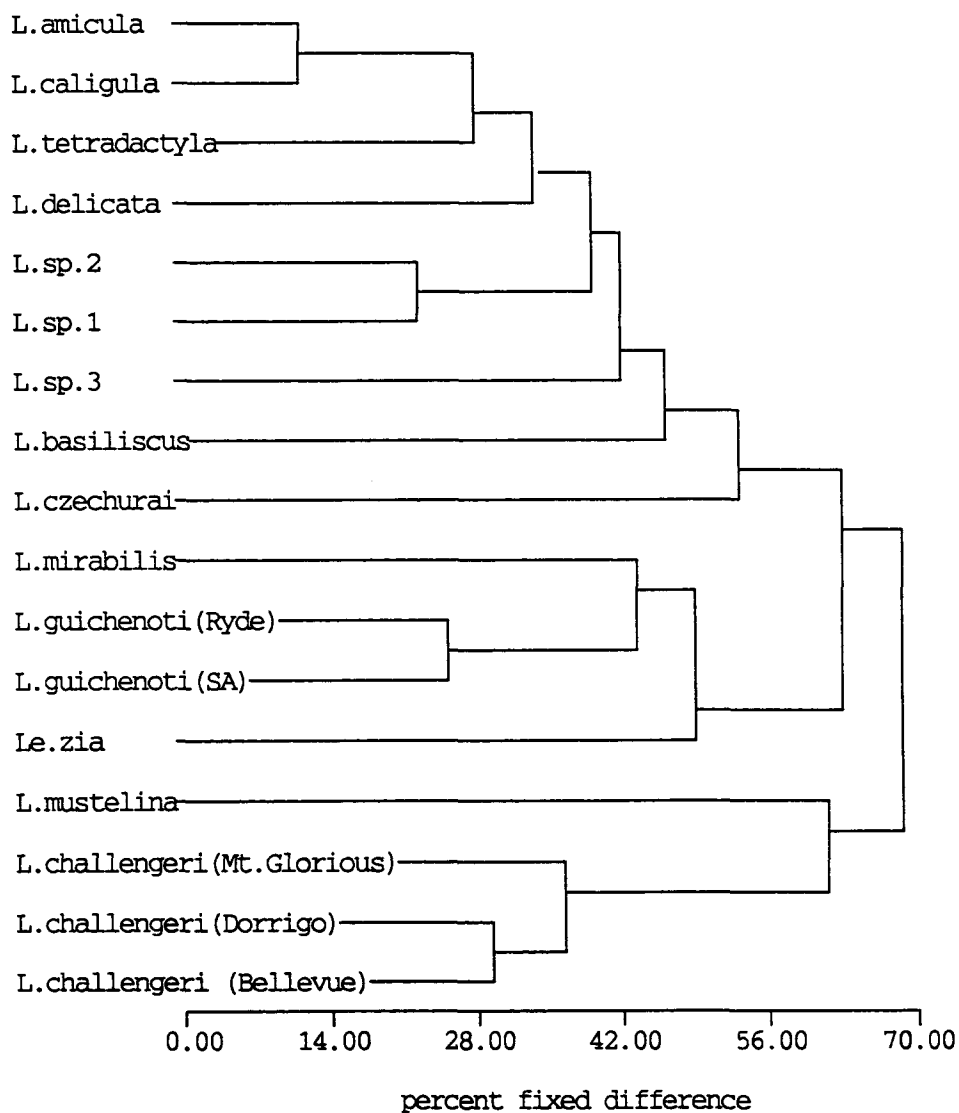
Figure 3.1: Phenogram of smaller electrophoretic data set using distance measure of percent fixed difference, Richardson & McDermid, 1978.

Table 3.3: Matrix of percent fixed difference (reduced data set), Richardson & McDermid, 1978.
(AMI=L.amicula; BAS=L.basiliscus; CZE=L.czechurai; MUS=L.mustelina; MTG=L.challengeri,
Mt.Glorious; NNS=L.challengeri,Dorrigo; SYD=L.challengeri,Sydney; MIR=L.mirabilis;
DEL=L.delicata,Sydney; PCU=L.sp.3; NEB=L.sp.2; COG=L.sp.1; GUI=L.guichenoti,Sydney;
CAL=L.caligula; TET=L.tetradactyla; LZI=Le.zia).

	AMI	BAS	CZE	MUS	MTG	NNS	SYD	MIR	DEL	PCU	NEB	COG	GUI	CAL	TET	LZI
BAS	28															
CZE	54	48														
MUS	59	61	61													
MTG	60	53	70	57												
NNS	49	58	65	52	36											
SYD	44	51	59	63	30	29										
MIR	52	59	66	70	78	73	63									
DEL	31	38	47	66	59	55	51	52								
PCU	32	42	52	58	62	60	51	60	47							
NEB	32	49	60	67	63	63	58	59	33	40						
COG	29	45	57	64	59	64	54	52	33	33	22					
GUI	37	47	59	72	73	68	63	34	37	57	47	44				
CAL	10	42	64	66	74	60	52	49	38	34	33	29	42			
TET	18	30	26	65	67	59	44	48	29	38	46	40	44	33		
LZI	65	64	53	71	81	68	70	46	64	68	67	73	47	64	50	
GUS	50	55	70	75	85	79	72	49	58	66	62	64	24	49	55	43
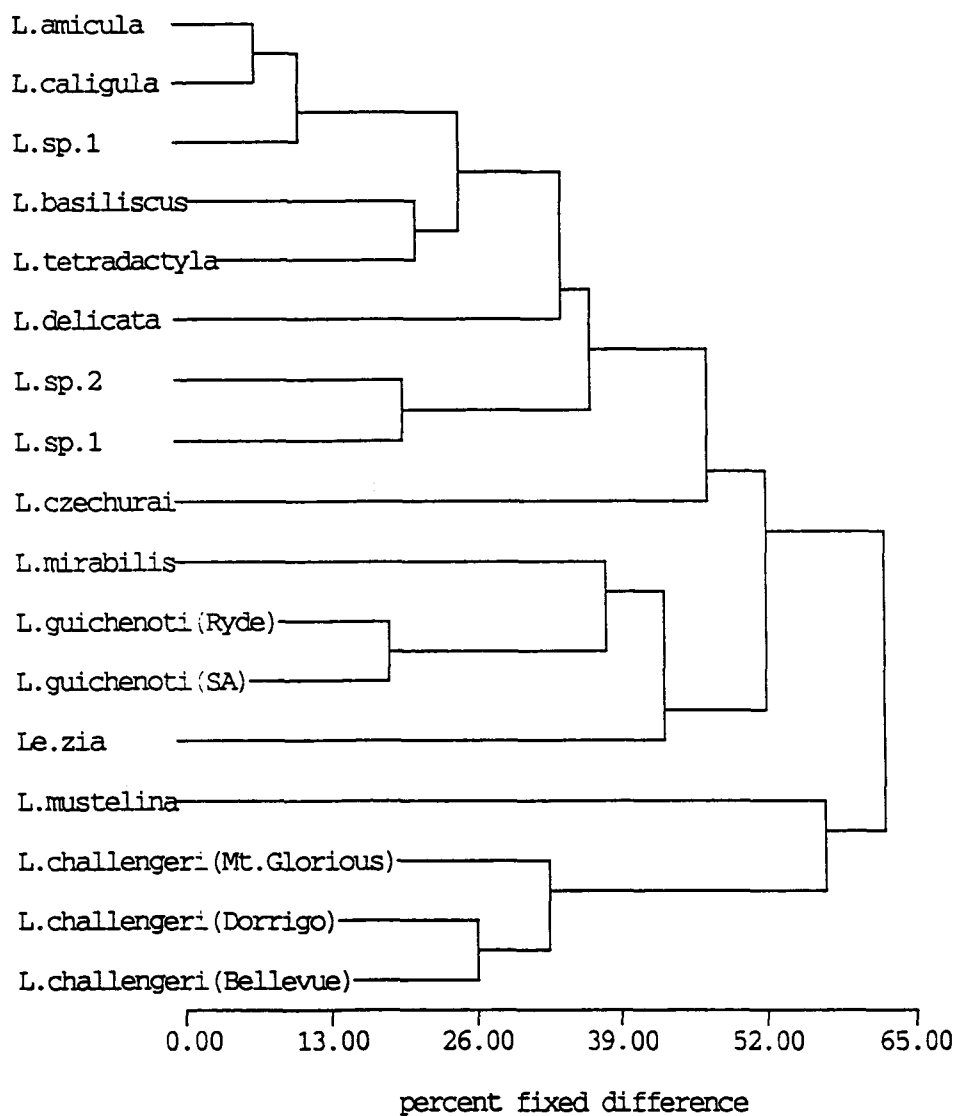
Figure 3.2: Phenogram of smaller electrophoretic data set using
distance measure of percent fixed difference, Sneath
& Sokal, 1973.

Table 3.4: Matrix of percent fixed difference (reduced data set), Sneath & Sokal, 1973.
(AMI=L.amicula; BAS=L.basiliscus; CZE=L.czechurai; MUS=L.mustelina; MTG=L.challengeri,
Mt.Glorious; NNS=L.challengeri,Dorrigo; SYD=L.challengeri,Sydney; MIR=L.mirabilis;
DEL=L.delicata,Sydney; PCU=L.sp.3; NEB=L.sp.2; COG=L.sp.1; GUI=L.guichenoti,Sydney;
CAL=L.caligula; TET=L.tetradactyla; LZI=Le.zia).

	AMI	BAS	CZE	MUS	MTG	NNS	SYD	MIR	DEL	PCU	NEB	COG	GUI	CAL	TET	LZI
BAS	16															
CZE	47	42														
MUS	50	55	55													
MTG	53	50	64	55												
NNS	42	55	58	50	30											
SYD	35	48	50	61	30	26										
MIR	45	50	60	62	75	70	57									
DEL	25	30	40	61	57	52	46	43								
PCU	10	32	40	50	60	55	43	48	39							
NEB	26	40	53	62	60	60	53	50	33	29						
COG	20	36	50	59	59	64	53	43	30	27	19					
GUI	26	35	53	67	70	65	57	25	29	43	40	38				
CAL	7	29	60	53	64	50	40	40	33	7	29	20	33			
TET	15	21	21	57	62	54	36	43	29	21	46	40	44	33		
LZI	56	56	47	63	73	60	63	38	57	57	63	69	47	58	42	
GUS	36	42	63	65	81	75	65	41	47	47	50	53	18	36	47	43
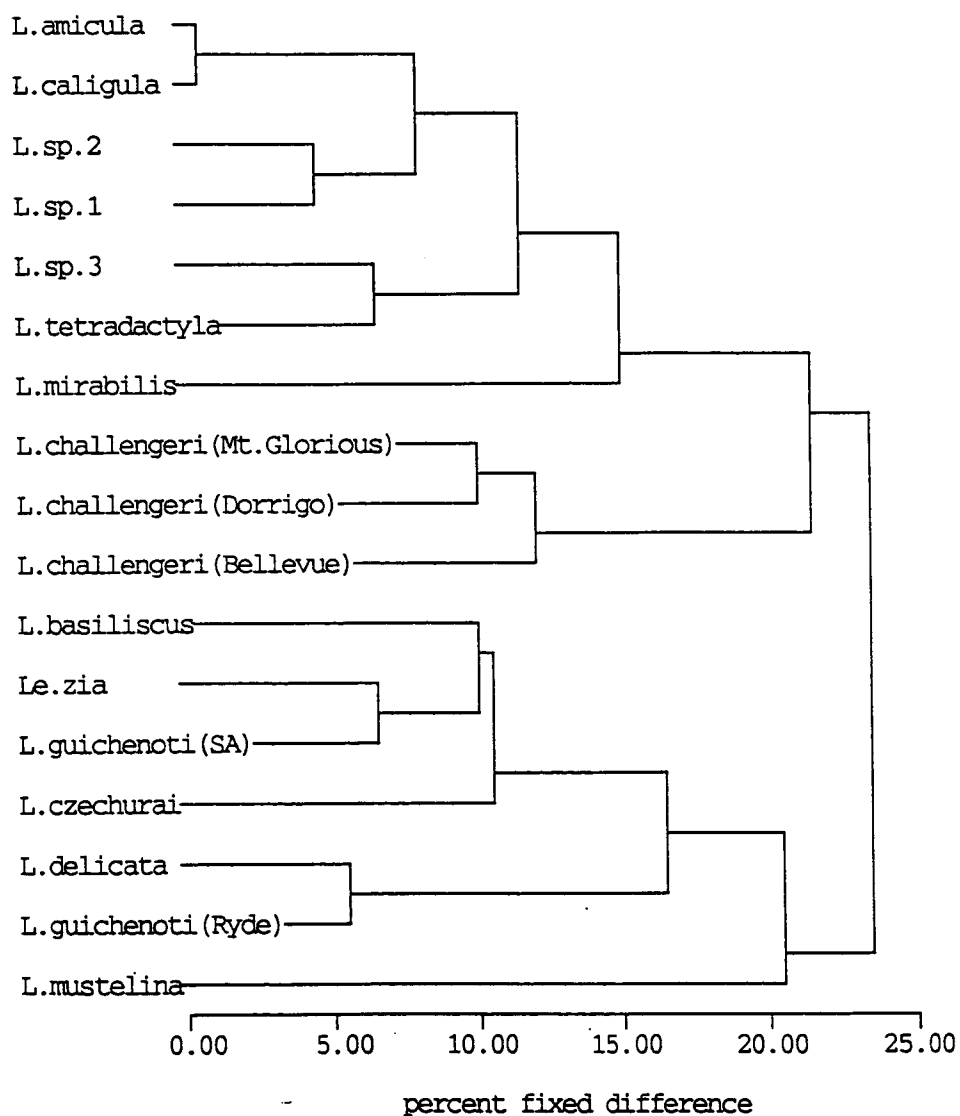
Figure 3.3: Phenogram of electrophoretic data using distance measure of percent fixed difference, Briscoe et al, 1987.

Table 3.5: Matrix of percent fixed difference, Briscoe *et al*, 1987
(AMI=L.amicula; BAS=L.basiliscus; CZE=L.czechurai; MUS=L.mustelina; MTG=L.challengeri,
Mt.Glorious; NNS=L.challengeri,Dorrigo; SYD=L.challengeri,Sydney; MIR=L.mirabilis;
DEL=L.delicata,Sydney; PCU=L.sp.3; NEB=L.sp.2; COG=L.sp.1; GUI=L.guichenoti,Sydney;
CAL=L.caligula; TET=L.tetradactyla; LZI=Le.zia).

|	AMI	BAS	CZE	MUS	MTG	NNS	SYD	MIR	DEL	PCU	NEB	COG	GUI	CAL	TET	LZI	GUS
BAS	15																
CZE	29	10															
MUS	17	16	22														
MTG	19	13	27	22													
NNS	16	17	28	18	9												
SYD	10	15	29	25	11	10											
MIR	15	20	34	28	28	25	20										
DEL	14	15	19	21	26	24	29	19									
PCU	12	19	22	25	26	23	17	17	22								
NEB	11	16	24	24	25	22	19	11	10	10							
COG	9	17	25	25	26	24	18	12	11	14	4						
GUI	12	12	18	20	22	24	25	15	6	17	9	11					
CAL	1	12	27	15	16	14	8	8	12	8	6	6	12				
TET	8	8	9	16	16	14	8	13	15	7	10	10	15	9			
LZI	22	9	8	12	16	18	18	19	16	15	16	19	11	17	12		
GUS	15	9	11	10	14	16	16	19	11	15	13	16	7	13	15	6	
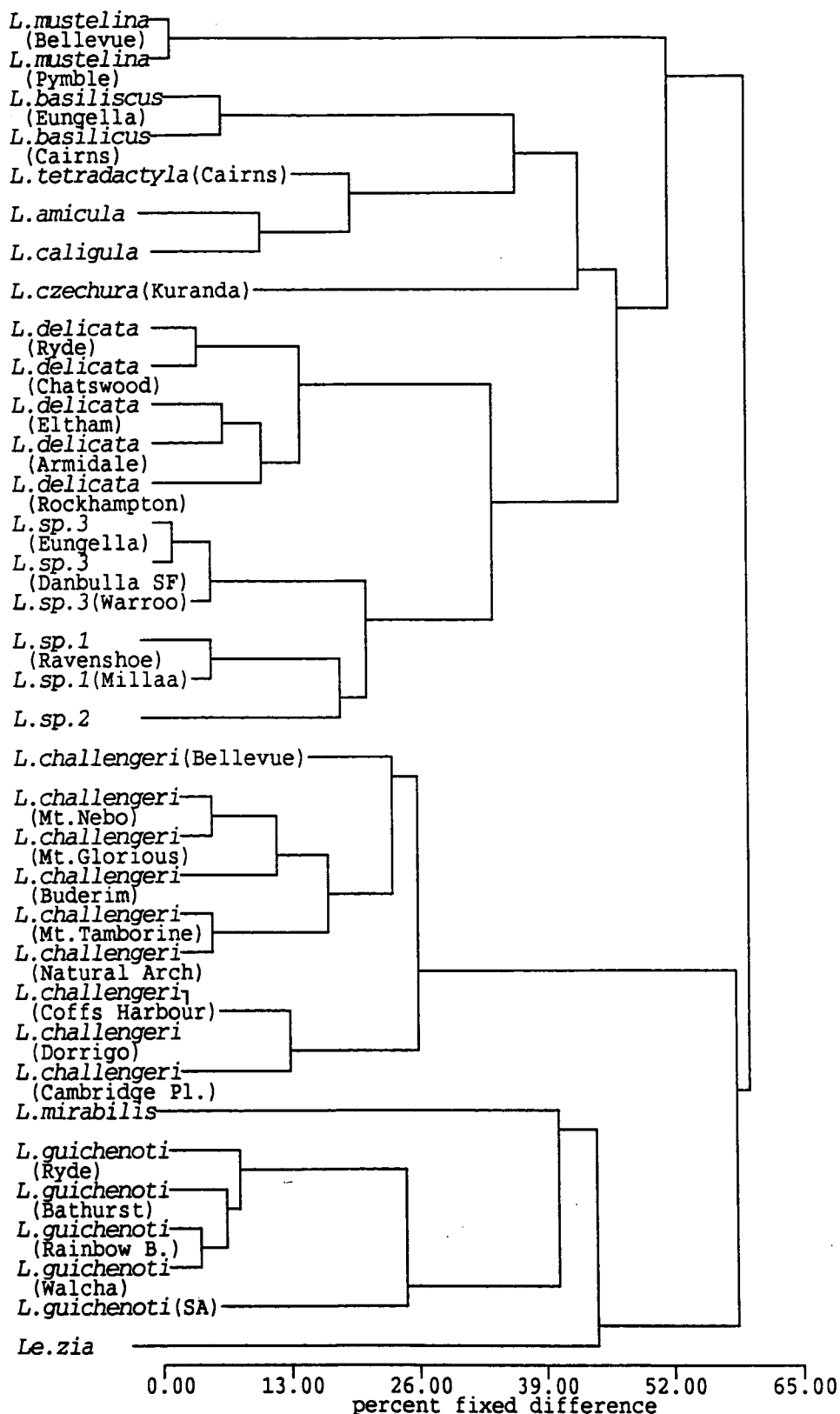
Figure 3.4: Phenogram of electrophoretic data using distance
measure of percent fixed difference, Richardson &
McDermid, 1978.

Table 3.6: Matrix of percent fixed difference, Richardson & McDermid, 1978 (CHS=L.challengeri,Bellevue Hill;CHN=L.challengeri,Mt.Nebo;CHT=L.challengeri, Mt.Tamborine;CHN=L.challengeri,Natural Arch;CHM=L.challengeri,Mt.Glorious;CHC=L.challengeri,Coffs Harbour;CHD=L.challengeri,Dorrigo;CHC=L. challengeri,Cambridge Plat.;CHB=L.challengeri,Buderim;MIC=L.mustelina,Bellevue Hill;MUP=L.mustelina,Pymble;MUG=L.mustelina,Greenwich;BAE=L. basiliscus,Eungella;BAC=L.challengeri,Cairns;MIC=L.mirabilis,Cape Cleveland;CZR=L.czechurai,Kuranda;CZM=L.czechurai,Millaa;TEC=L.tetradactyla, Cairns;AMC=L.amicula,Rainbow Beach;CAB=L.caligula,Barrington Tops;DER=L.delicata,Ryde;DEC=L.delicata,Chatswood;DEE=L.delicata,Eltham;DEB=L. delicata,Boudi S.F.;DFA=L.delicata,Armidale;DER=L.delicata,Rockhampton;RCE=L.sp.3,Eungella;RCD=L.sp.3,Danbulla S.F.;RCW=L.sp.3,Warroo;CCC=L. sp.1,Ravenshoe;CCM=L.sp.1,Millaa;NEM=L.sp.2,Mt.Glorious;GUR=L.guichenoti,Ryde;GUC=L.guichenoti,Bellevue Hill;GUB=L.guichenoti,Barrington Tops; GUL=L.guichenoti,Rainbow Beach;GUW=L.guichenoti,Walcha;GUN=L.guichenoti,Nimmitabel;GUM=L.guichenoti,Moora Plains).

|	CHS	CHN	CHT	CHN	CHM	CHC	CHD	CHC	CHB	MIC	MUP	MUG	BAE	BAC	MIC	CZR	CZM	TEC	AMC	CAB	DER	DEC	DEE	DEB	DFA	DER	RCE	RCD	RCW	CCC	CCM	NEM	GUR	GUC	GUB	GUL	GUW	GUN
CHN	26																																					
CHT	20	16																																				
CHN	18	14	6																																			
CHM	24	5	10	17																																		
CHC	35	29	21	16	33																																	
CHD	32	32	22	17	35	2																																
CHC	31	27	19	17	28	10	9																															
CHB	29	9	12	15	9	36	33	20																														
MIC	55	58	57	61	53	60	62	59	63																													
MUP	62	63	64	69	57	63	64	64	70	2																												
MUG	57	61	64	70	51	68	68	68	65	3	0																											
BAE	56	60	60	57	63	64	67	60	69	48	48	51																										
BAC	45	47	47	45	53	53	56	50	69	46	48	58	6																									
MIC	63	82	70	67	83	68	68	68	73	70	67	67	61	61																								
CZR	57	72	60	64	62	69	72	69	77	55	58	51	45	43	71																							
CZM	80	76	77	77	79	78	79	80	79	87	88	91	97	83	73	95																						
TEC	38	62	51	48	58	55	55	51	72	55	59	70	42	26	52	27	82																					
AMC	51	68	61	51	70	58	61	57	71	64	68	71	40	31	44	57	81	19																				
CAB	48	65	61	51	68	58	58	54	71	61	65	71	47	37	51	51	87	15	10																			
DER	59	72	70	66	70	71	67	64	75	65	67	54	39	43	58	47	79	43	41	47																		
DEC	68	75	78	73	78	77	77	77	86	67	67	58	37	37	62	55	80	50	38	50	4																	
DEE	58	54	58	50	54	50	46	61	54	55	61	50	46	56	48	50	36	45	12	6																		
DEB	72	68	53	66	56	69	71	57	59	49	53	49	71	69	76	62	85	66	71	69	65	79	57															
DFA	50	61	56	56	61	56	50	50	64	56	52	45	56	50	36	64	78	57	50	57	11	17	6	59														
DER	52	60	61	56	59	62	62	59	73	51	52	52	38	33	58	52	77	39	33	43	13	10	9	65	7													
RCE	55	66	68	62	66	70	70	67	77	49	51	51	44	39	59	58	79	44	33	39	47	49	33	60	38	33												
RCD	53	66	65	58	64	67	68	65	77	46	48	51	42	37	59	60	78	44	41	42	44	43	33	62	46	33	2											
RCW	46	60	61	54	59	63	63	59	74	43	45	51	44	39	59	54	85	44	36	39	41	42	33	60	38	31	5	2										
CCC	59	67	70	64	67	73	73	70	77	55	57	51	51	45	58	61	85	48	32	31	40	38	27	62	38	29	25	23	14									
CCM	55	66	67	60	64	69	71	67	77	51	53	51	51	45	58	61	83	41	39	36	27	60	29	26	29	28	23	17	13										
NEM	58	64	67	63	63	69	69	66	75	51	53	51	48	42	64	59	83	50	30	33	36	36	27	60	29	26	29	28	23	17	13								
GUR	76	84	76	71	86	74	73	69	77	73	76	72	58	51	44	68	71	51	31	40	41	45	40	69	57	40	57	57	58	48	46	48							
GUC	74	82	74	69	83	75	71	68	74	77	81	76	58	54	44	66	72	55	36	44	42	49	45	69	57	47	62	63	64	54	52	54	3						
GUB	71	79	71	66	82	72	68	64	74	75	79	76	58	51	44	69	69	51	31	40	41	46	40	69	57	44	58	61	62	49	49	49	6	3					
GUL	71	79	71	66	82	72	68	64	74	75	79	76	58	51	44	69	69	51	31	40	41	46	40	69	57	44	58	61	62	49	49	49	6	3	4				
GUW	73	81	73	68	83	74	69	66	74	76	80	76	58	54	44	66	71	55	36	44	39	46	45	69	57	47	60	63	64	52	52	52	9	5	5	3			
GUN	68	78	68	62	79	70	66	62	70	72	76	73	55	51	46	63	73	56	33	40	37	45	45	69	57	46	59	62	59	49	49	49	7	3	0	4	6		
GUM	71	79	79	71	87	71	71	71	85	71	71	68	47	43	46	64	81	57	31	39	31	37	44	76	50	43	53	57	57	47	47	47	9	8	9	5	0	8
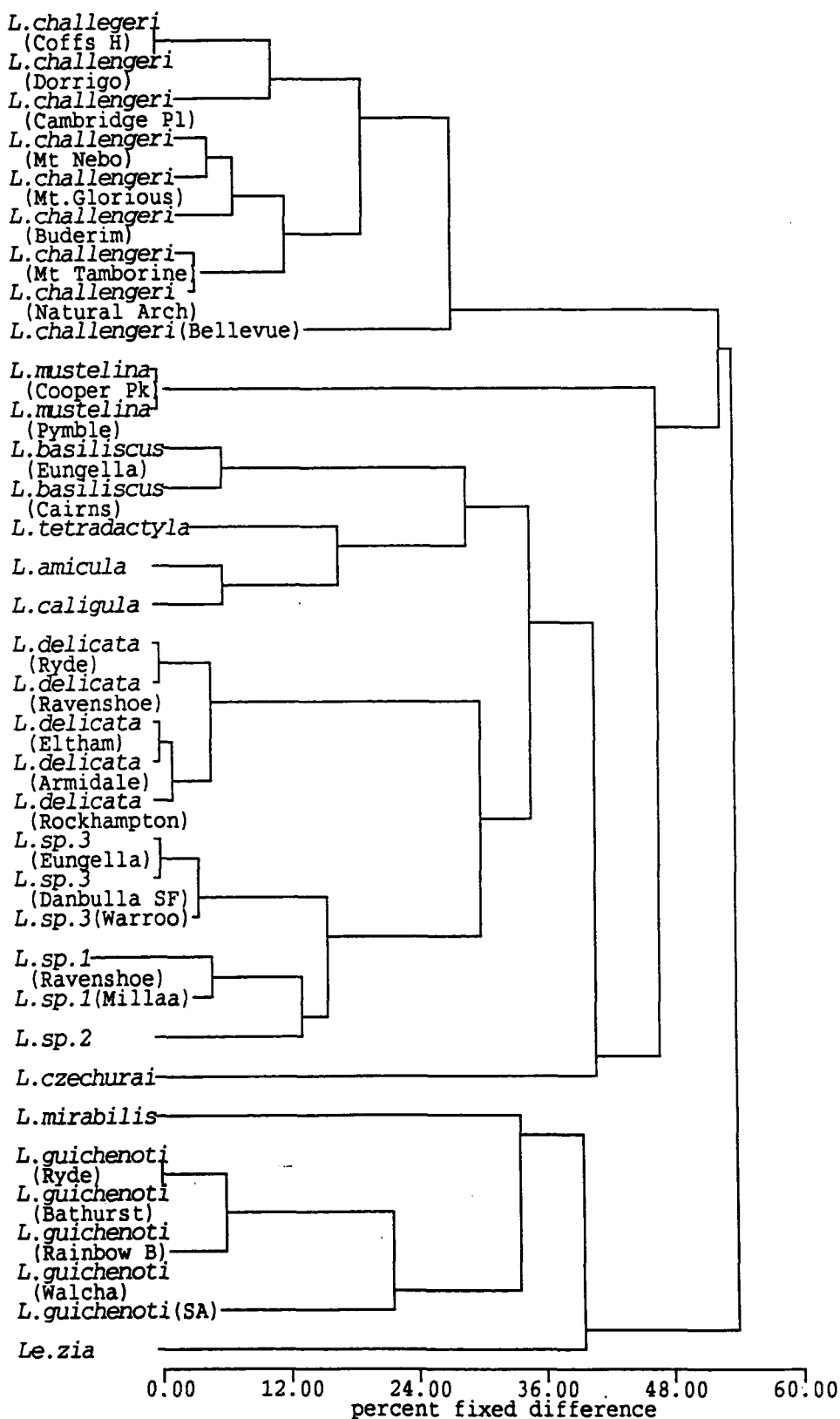
Figure 3.5: Phenogram of electrophoretic data using distance measure of percent fixed difference, Sneath & Sokal, 1973.

Table 3.7: Matrix of percent fixed difference, Sneath & Sokal, 1973. (CHS=L.challengeri,Bellevue Hill;CHN=L.challengeri,Mt.Nebo;CHT=L.challengeri, Mt.Tamborine;CHN=L.challengeri,Natural Arch;CHM=L.challengeri,Mt.Glorious;CHC=L.challengeri,Coffs Harbour;CHD=L.challengeri,Dorrigo;CHC=L. challengeri,Cambridge Plat.;CHB=L.challengeri,Buderim;MIC=L.mustelina,Bellevue Hill;MIP=L.mustelina,Pymble;MIG=L.mustelina,Greenwich;BAE=L. basiliscus,Bungella;BAC=L.challengeri,Cairns;MIC=L.mirabilis,Cape Cleveland;CZR=L.czechurai,Kuranda;CZM=L.czechurai,Millaa;TEC=L.tetradactyla, Cairns;AMC=L.amicula,Rainbow Beach;CAB=L.caligula,Barrington Tops;DER=L.delicata,Ryde;DEC=L.delicata,Chatswood;DEE=L.delicata,Eltham;DEB=L. delicata,Bondi S.F.;DFA=L.delicata,Armidale;DER=L.delicata,Rockhampton;FCE=L.sp.3,Bungella;FCD=L.sp.3,Danbulla S.F.;FCW=L.sp.3,Warroo;CCC=L. sp.1,Ravenshoe;COM=L.sp.1,Millaa;NEM=L.sp.2,Mt.Glorious;GLR=L.guicheroti,Ryde;GLC=L.guicheroti,Bellevue Hill;GLB=L.guicheroti,Barrington Tops; GLL=L.guicheroti,Rainbow Beach;GLW=L.guicheroti,Walcha;GLN=L.guicheroti,Nimmitabel;GLM=L.guicheroti,Moona Plains).

	CHS	CHN	CHT	CHN	CHM	CHC	CHD	CHC	CHB	MIC	MIP	MIG	BAE	BAC	MIC	CZR	CZM	TEC	AMC	CAB	DER	DEC	DEE	DEB	DFA	DER	FCE	FCD	FCW	CCC	COM	NEM	GLR	GLC	GLB	GLL	GLW	GLN
CHN	26																																					
CHT	20	14																																				
CHN	16	9	4																																			
CHM	24	5	8	13																																		
CHC	33	25	19	14	29																																	
CHD	30	27	18	14	30	0																																
CHC	29	23	17	13	20	10	9																															
CHB	29	6	12	12	6	33	31	18																														
MIC	52	55	54	58	50	60	59	57	59																													
MIP	57	57	59	64	52	60	59	59	63	0																												
MIG	50	53	56	63	44	64	60	60	57	0	0																											
BAE	56	60	60	53	63	64	67	60	69	43	44	47																										
BAC	45	47	47	42	53	53	56	50	69	42	44	53	6																									
MIC	60	79	67	60	80	64	64	69	67	60	60	53	53																									
CZR	52	67	55	59	57	63	67	64	71	50	52	44	38	37	67																							
CZM	61	57	59	59	64	67	65	67	63	77	80	87	93	78	57	95																						
TEC	33	57	47	40	53	50	50	47	67	50	54	64	36	21	45	27	77																					
AMC	44	60	53	40	63	50	53	50	62	56	60	62	29	20	38	50	67	15																				
CAB	40	57	53	40	60	50	50	47	62	53	57	62	38	29	46	47	79	15	7																			
DER	52	64	63	58	63	65	59	57	65	58	59	44	31	37	53	41	65	43	38	47																		
DEC	58	65	68	63	68	71	60	67	73	58	58	47	29	29	57	47	65	50	33	50	0																	
DEE	54	50	54	46	50	45	45	42	56	50	50	56	44	42	56	58	54	50	36	45	8	0																
DEB	46	42	33	42	33	50	45	33	40	20	27	30	45	42	50	33	69	44	42	45	31	44	44															
DFA	44	56	50	50	56	50	44	44	57	50	38	29	50	44	29	56	67	57	50	57	0	0	0	33														
DER	38	47	48	43	48	50	47	45	59	43	40	38	27	24	47	43	68	36	27	40	5	0	0	36	0													
FCE	48	60	61	52	61	65	62	59	71	44	45	44	33	29	47	52	70	36	13	20	36	37	27	36	29	24												
FCD	45	60	57	48	59	61	60	57	71	41	43	44	31	28	47	55	75	36	25	27	33	29	27	33	38	24	0											
FCW	37	53	53	42	53	56	53	50	67	37	39	44	33	29	47	47	82	36	20	27	28	27	21	27	27	21	0	0										
CCC	54	62	65	57	63	70	68	65	71	50	52	44	44	39	53	55	76	43	25	27	35	32	27	33	38	24	17	18	11									
COM	50	60	62	52	59	67	65	62	71	45	48	44	44	39	53	55	75	43	25	27	33	29	27	33	38	24	10	18	11	5								
NEM	50	55	59	55	55	63	60	57	65	45	48	44	40	35	60	52	70	50	27	33	33	33	27	36	29	24	18	19	16	14	10							
GLR	70	78	70	65	80	71	67	63	71	70	74	69	47	40	40	58	58	42	21	36	35	39	40	45	57	32	45	47	47	45	42	45						
GLC	71	79	71	67	81	72	68	65	71	71	75	69	47	44	40	60	55	46	27	40	33	39	45	45	57	35	48	50	50	48	45	48	0					
GLB	68	76	68	63	79	69	65	61	71	68	72	69	47	40	40	63	56	42	21	36	32	35	40	45	57	32	42	47	47	42	42	42	0	0				
GLL	68	76	68	63	79	69	65	61	71	68	72	69	47	40	40	63	56	42	21	36	32	35	40	45	57	32	42	47	47	42	42	42	0	0	0			
GLW	70	78	70	65	80	71	67	63	71	70	74	69	47	44	40	60	58	46	27	40	30	35	45	45	57	35	45	50	50	45	45	45	5	5	5	0		
GLN	65	75	65	59	76	67	63	59	67	65	69	64	46	43	46	59	59	50	29	36	29	36	45	45	57	35	41	47	44	41	41	41	0	0	0	0	6	
GLM	67	75	75	67	83	67	67	67	80	67	67	64	36	33	45	58	67	50	25	33	25	30	44	50	50	33	33	42	42	42	42	42	9	8	9	0	0	8
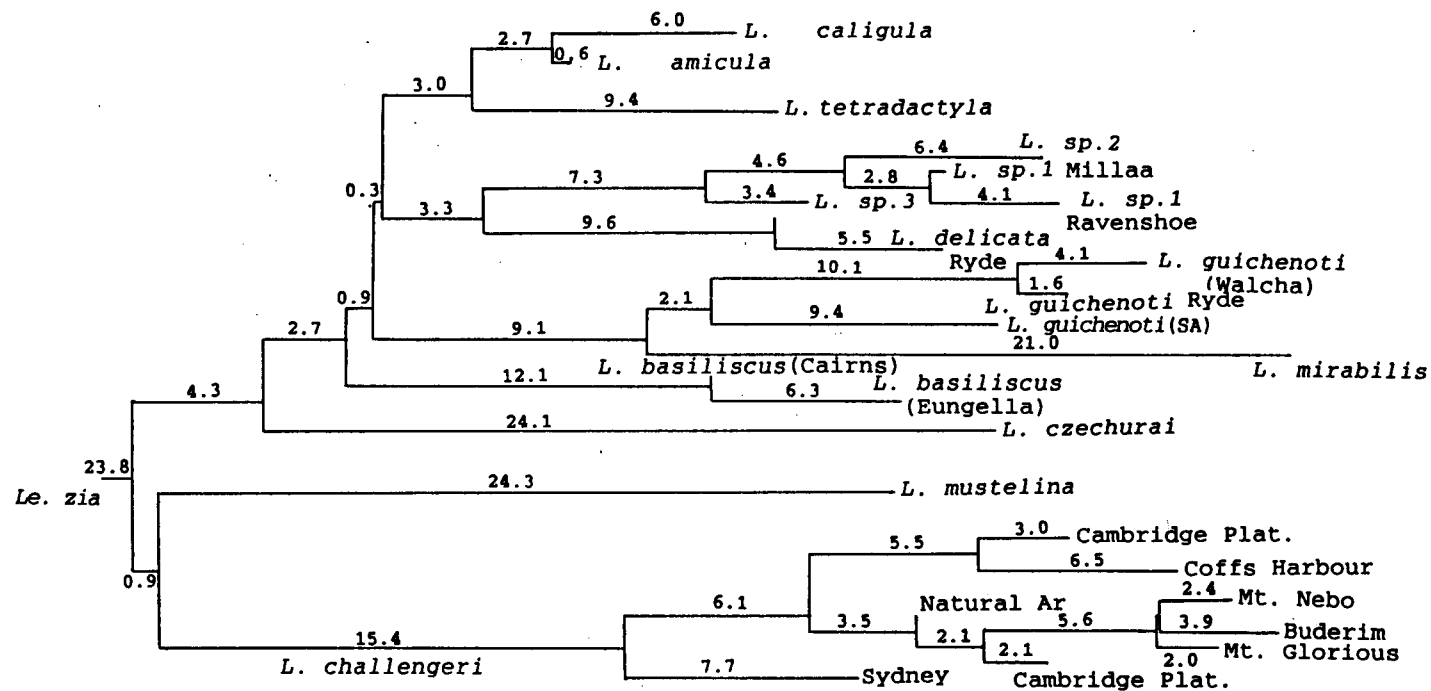
Figure 3.6: Phylogram of electrophoretic data using Fitch and Margoaliash (1967) method of tree construction (branch lengths are drawn to scale and therefore reflect allozyme divergence).
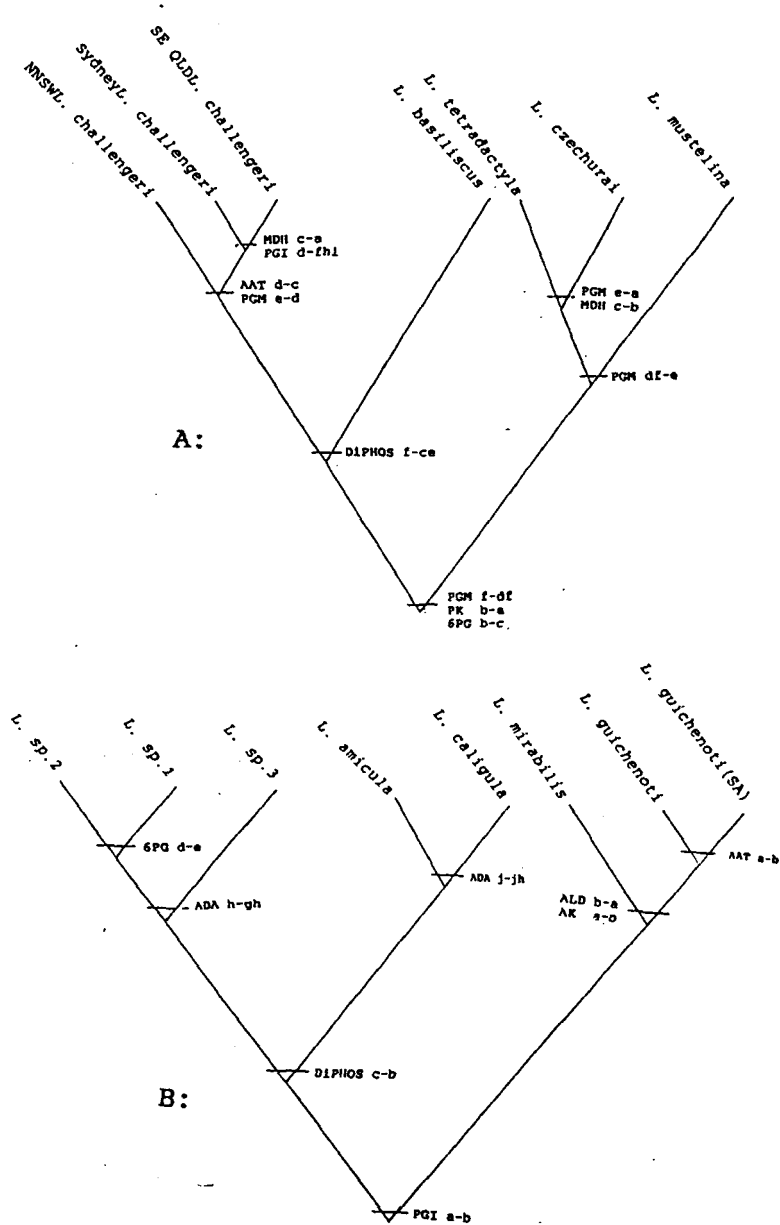
Figure 3.7: Cladistic analysis of electrophoretic data (A: *L. delicata* complex as outgroup; B: *L. challengeri* complex as outgroup; For enzyme abbreviations see Table 3.1).

Figure 3.8: Consensus cladogram, based on information gained from using *Le. zia* as an outgroup, supplemented by information from figure 3.7.
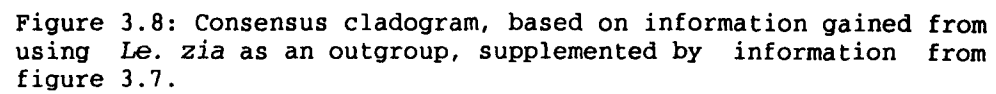
3.05: DISCUSSION OF ALLOZYME ELECTROPHORETIC DATA.

3.05.01: Aspects of *Lampropholis* taxonomy investigated.

The analyses were undertaken to investigate the following aspects of *Lampropholis* taxonomy:

(1) Does the genus, *Lampropholis*, consist of two monophyletic groups worthy of generic rank?

(2) If two genera are identifiable, to which does *L. caligula* belong?

(3) To what extent is it possible to delineate species boundaries?

3.05.02: Expected variation among topologies.

Before proceeding to address these questions it is first necessary to consider the accuracy and limitations of the data presented in Figures 3.1 - 3.8. That variation among topologies occurs is predictable since, for even a modest data set, there are a large number of configurations possible from a single analysis. Cavalli-Sforza and Edwards (1967) calculated the formula as:

$$1 \times 3 \times 5 \times 7 \ldots (2n-5)$$

Where n = the number of pairwise comparisons

This represents 6.2^{15} possibilities for the smaller of the two

data sets investigated here and many orders of magnitude more for the larger data set. As a result of this large number of potential topologies, computer analyses generally consider only a sub-set of all possibilities. It is therefore not prudent to rely on a single reconstruction from a data set nor to expect identical results when different algorithms or approaches to analysis are utilized. The data used for construction of the Fitch and Margoliash (1967) phylogram was shuffled three times to ensure that topologies did not change significantly with varying sequence of input.

There has been enormous controversy over the method of approach to analysis of data for taxonomy and phylogeny (see Section 2.08). In this instance, however, the various methods gave essentially the same picture. The minor variations among topologies may be expected given the potential number of results possible and the contrasting approaches taken.

According to the most recent classification of this group (Greer, 1980) the species should cluster into two groups, one comprising taxa associated with *L. challengeri, L. basiliscus, L. czechurai, L. tetradactyla* and *L. mustelina* and the other comprising the taxa related to *L. delicata, L. guichenoti, L. mirabilis* and *L. amicula*. The position of *L. caligula*, previously unknown, should be resolved. *Leiolopisma zia*, introduced as an outgroup for phylogenetic analysis, would be expected to be less related to

the two complexes within *Lampropholis* than these are to each other while the three groups would be expected to cluster discretely.

3.05.03: Generic status of *Lampropholis*

This was not the case (see Phenetic analyses, Fig. 3.1-3.5; Phyletic analysis, Fig. 3.6; Cladistic analyses, Fig. 3.7-3.8). All analyses resulted in topologies that were very similar and demonstrated that the genus does not constitute two genetically distinct groups as anticipated. Moreover *Le. zia* is no more distinct from the groupings that do appear than many are from each other.

Using phylogenetic relationships as a basis for grouping (= estimation of genetic relationships; Fig. 3.6), *L. challengeri*, *L. mustelina* and *L. czechurai* each separate discretely and all are approximately equidistant from *Le. zia*. Although not so distinctly separated, other groupings are also evident; *L. guichenoti* and *L. mirabilis* cluster to the exclusion of all other taxa, as do *L. delicata* taxa while a third cluster includes *L. caligula*, *L. amicula*, *L. tetradactyla* and probably *L. basiliscus*. Cladistic analysis (Fig. 3.7-3.8) does not contradict this interpretation, while the phenetic analysis generally supports the genetic relationships (Fig. 5.1-5.5).

All analyses suggest the distinctiveness of *L. mustelina*, *L. challengeri* and *L. czechurai* from each other and from all other species. Relationships within this latter group show some variation among topologies but this is not seen to detract from the overall picture. Intuitively the phenogram based on absolute distances (Briscoe *et al*, 1987) should present superior results (see Sect. 3.03.01). However, this was not the case. All other approaches grouped taxa of a specific species together, however, in this instance the two populations of *L. guichenoti* were substantially separated, one (Sydney population) clustered most closely with *L. delicata* and the South Australian population with *Le. zia* and hierarchically with *L. basiliscus* and *L. czechurai* (Fig. 5.3). In all other instances (Fig. 5.1,5.2,5.4,5.5) *Lampropholis guichenoti* populations cluster as sister taxa, thus although the relationship with *L. delicata* may be historically acceptable, separation of the South Australian taxon and its grouping with *Le. zia*, *L. basiliscus* and *L. czechurai* is not justifiable on the basis of other allozyme analysis or on distributional grounds. This would indicate that homoplasy was involved among absolute mobilites of electromorphs.

If it is accepted that *Le. zia* does indeed represent a separate genus, then all clusters of higher magnitude than that represented by *Le. zia* should necessarily be identified as distinct genera. Using this criterion, four genera are identifiable from the phyletic analysis. These are *L.*

challengeri, L. czechurai, L. mustelina and a fourth group which consists of the remaining taxa (Table 3.8). The sister taxa, *L. guichenoti* and *L. mirabilis* approach the level of diversity of *Le. zia* (Fig. 3.6). On a phenetic basis (Fig. 3.1-3.5) this species pair is sufficiently distinct from other taxa to also be considered as a separate genus, as are the other taxa identified by the phyletic analysis (Table 3.8).

Before accepting that these groupings are worthy of generic rank it is necessary to address the degree of genetic diversity between groups and confirm that this is a realistic conclusion.

Previous work undertaken on the genetics of reptiles, while limited, does show that the level of allozyme diversity varies greatly among groups examined. For example, studies of *Anolis* indicate that the level of genetic diversity is extremely high, and of a magnitude generally observed among amphibian genera (see Table 2.1). If this was a general trend among reptiles, then genetic diversity demonstrated in the present study would not necessarily be sufficient to constitute generic status. However, two other groups assessed electrophoretically, *Crotaphylus* and *Uma* are more closely alligned with data provided from studies of Aves. If this level of genetic diversity were more closely a reflection of the *Lampropholis* genome, then the level of diversity identified in the present study would confirm the existence of at least four genera within the presently described

Table   3.8:   Generic   arrangement   identified   by   allozyme
              electrophoretic analysis.

Genus 1	Genus 2	Genus 3	Genus 4
L.challengeri taxa	*L.mustelina*	*L.czechurai*	*L.basiliscus* *L.tetradactyla* *L.delicata* *L.sp.1* *L.sp.2* *L.sp.3* *L.caligula* *L.amicula*

one. Intermediate levels of genetic diversity have also been observed in reptiles (see Table 2.1).

Therefore in the absence of clear cut guidelines for the expected level of genetic differentiation necessary to identify genera, it is necessary to concentrate on the internal structure of the group and try to identify hierarchical groupings. Cladistic relationships contribute no information on the level of relatedness. Those species which cluster together are considered most closely related, but no quantitative measure is possible. To assess the degree of relatedness it is therefore necessary to use the level of phyletic diversity identified in Figure 5.6, although due to the close agreement between the phyletic and phenetic results these phenograms are also a useful adjunct in this investigation. As a broad rule of thumb, where two allopatric populations differ by more than 15% fixed gene difference they can usually be shown to represent two distinct species (see Richardson *et al*, 1986 for detailed discussion). The recognition of one or more fixed difference between populations occurring in close sympatry is sufficient to hypothesize specific status (see Sect. 2.05.03).

As an indication of the minimum level of diversity for species boundaries, *L. amicula* and *L. caligula* are considered to be an ideal 'bench mark' for the following reasons:

As discussed in the Preface, *L. amicula* is small in size (snout-vent length 22-34mm) and is known only from coastal areas in southern Queensland. *L. caligula* is a much larger, elongate species (snout-vent length 35-48mm) restricted to woodlands and wet sclerophyll forests of Barrington Tops, Central New South Wales. Substantial variation in chromosome morphology occurs between the two species with chromosomes seven to nine varying (Donnellan, 1985). This makes it unlikely that the two species could successfully interbreed.

Therefore, on morphological, chromosomal, habitat and distributional grounds, *L. amicula* and *L. caligula* are two quite distinct species. However, these two species differ only by ten percent of their loci. Isozyme evolution has therefore been slow indicating that the pattern in these reptiles is similar to that generally observed among Aves (Table 2.1).

Another indication that fixation of random mutations is conservative in this group, can be gained by reference to Table 3.2. This gives a list of populations observed to be identical to another population and therefore eliminated from analysis. *Lampropholis delicata* collected from areas as widely separated as Etham (urban Melbourne, Victoria) and Byfield in Central Queensland, were identical electrophoretically, Likewise *L. mustelina* populations and *L. guichenoti* populations collected

throughout much of their respective east coast ranges were identical.

Although *L. challengeri* and *L. mustelina* have been previously considered to be closely related, in this study they are demonstrated to be genetically very different and distinct (e.g. in excess of 50% fixed differences; see Table 3.6). As discussed above, *L. mustelina* shows minimal allozyme variation over a large portion of its range and where it is variable the level of genetic variation is minimal (e.g. % fixed difference 2-3%; Table 3.6). Conversely while *L. challengeri* is clearly distinct from *L. mustelina*, variation among taxa identified within *L. challengeri* may be as high as 35% (see Table 3.6). It is therefore not considered possible for these two taxa to be closely related since it is difficult to conceive a scenario in which *L. mustelina* could maintain its genetic integrity to such a degree and yet be capable of being so genetically distinct from its genetically heterogeneous closest congener. This lack of variation between populations of a single species indicates that the electrophoretic divergence observed within this group is not as extreme as shown for *Anolis* and amphibians in general. It is much more consistent with slower evolutionary rates. On the basis of these indications it is realistic to consider the differentiation observed to be of generic level. It is therefore hypothesized that four genera exist within the presently described genus *Lampropholis*.

3.05.04: Species boundaries within *Lampropholis* (*sensu* Greer, 1974)

As discussed above *L. caligula* and *L. amicula* are considered 'good' morphological species and therefore an argument could be given for using these as a 'bench mark' for determining species status. Taking this one step further, *L. tetradactyla* (undoubtedly separate from all other species in the group in having four instead of five toes) differ by approximately 15% fixed gene difference from these two species (Table 3.6). It is therefore considered than any taxon shown to be of greater distance from sister taxa than this, has grounds for being given species status.

Using the level of genetic differentiation determined for *L. caligula* and *L. amicula* as a guide to species boundaries, it is possible to fragment *L. challengeri* into at least five species, *L. mustelina* remains as a single species as does *L. basiliscus*, *L. tetradactyla*, *L. amicula*, *L. caligula*, *L. czechurai* and *L. mirabilis*. *L. delicata* is confirmed as consisting of four species and *L. guichenoti* represents two species.

If the more conservative approach of setting the level of species differentiation to that of the split between *L. tetradactyla* and *L. amicula/L. caligula*, then *L. challengeri* constitutes at least three species while *L. guichenoti* and *L. delicata* incorporate

only two species each. On the basis of the electrophoretic analysis this more conservative approach is considered inappropriate since *L. caligula* and *L. amicula* would then have to be considered as a single species and *L. delicata* taxa clearly identified as being chromosomally very different (e.g. chromosome formats among *L. delicata* taxa were unique while *L. sp.2* has 2n=28 compared with all other *L. delicata* taxa investigated having 2n=30; Donnellan, 1985) would be combined into two taxa. There is therefore ample evidence to indicate that the use of *L. amicula/L. caligula* as a 'bench mark' for species separation is appropriate.

At present, however, no data exists to support the fragmentation of the *L. challengeri* taxon. The taxa presently incorporated in *L. challengeri* appear to have a distribution at least encompassing numerous cool microhabitats in areas from Gympie south to Sydney and west into mountainous areas. It is indicative of the state of knowledge of many reptile species that little more is known of this group (see Preface). Dale (1973) recorded that *L. challengeri* were 'common' in the mountains near Brisbane and specimens were observed in appropriate weather conditions in this region. In the Sydney region it is possible to find areas with congregations of individuals under certain climatic conditions. However, at other times extensive searching will reveal few individuals. In other areas a full day's search by several experienced people in potentially appropriate habitat may

reveal none, or few individuals. Extensive searches on several visits to the type area (Gympie) yielded no individuals.

Collections were obtained from ten localities within the known range of *L. challengeri* and were sought in many other potential localities. However, due to the distribution of genetic diversity observed, it would be desirable to determine more precisely the distribution of this group, this would mean collecting more intensively which was beyond the scope of the present study.

Thus approaching the interpretation of the data with caution, three groups are clearly identifiable among *L. challengeri* taxa. The taxon of the Sydney area is most removed from the other taxa, while those from the northern segment of their range (Mt. Nebo, Mt. Glorious, Buderim, Mt. Tambourine and Natural Arch; all South Eastern Queensland localities) are separated from those investigated from Northern New South Wales (Coffs Harbour, Dorrigo, Cambridge Plateau and Styx River). However, there is also evidence that these various taxa may represent more regionally restricted groups. Clinal variation should be more intensively investigated before attempting further classification. Indications are, however, that each mountain range may have a specific species. In the absence of specimens from the type locality and the small number of localities from which specimens were obtained, a more definitive investigation is precluded in this study.

Chromosomal data supports the concept of five species within the presently described 'umbrella' species *L. delicata*. As previously discussed using *L. caligula* and *L. amicula* as an acceptable basis upon which to base species boundary decisions this study shows there are at least four species within *L. delicata* (one restricted chromosomal race known only from Mt Bartle Frere was not available for incorporation into this study).

There is also good evidence that *L. guichenoti* constitutes two species. One is widespread on the east coast, while the second was only identified in South Australia.

3.06 : CONCLUSIONS DRAWN FROM ALLOZYME ELECTROPHORETIC DATA

Thus, to return to the questions posed at the beginning of this study (Sect. 3.05.01), *Lampropholis* does not consist of two groups but rather constitutes four monophyletic taxa considered worthy of generic status (Table 3.9). *Lampropholis caligula* clustered within the largest composite (Table 3.11), most closely associated with *L. amicula*. This relationship was such that *L. caligula/L. amicula* were used as a 'bench mark' upon which to investigate species boundaries and is therefore clearly within this group. Using this criterion for evidence of specific status *L. delicata* was confirmed to consist of at least four species (previously hypothesized; see Preface for details), *L. guichenoti*

is considered to constitute two species (the South Australian taxon is delineated from all other populations) and *L. challengeri* is considered to represent in excess of three species. All other taxa investigated are considered to be discrete species.

CHAPTER 4: MORPHOLOGICAL AND MERISTIC CHARACTERIZATION OF
LAMPROPHOLIS SKINKS.

4.01:   INTRODUCTION: THE SCOPE OF THE TECHNIQUE

4.01.01: Utility of morphological and meristic characterization
        as a technique in reptile taxonomy.

Meristic and morphological characters generally provide the
simplest means of identification, both of the individual organism
and at higher taxonomic levels (Goin & Goin, 1971). However, many
such characters are the result of multi-gene and environment
interactions, the genetics of which are poorly understood. In
addition, most of these morphological characters are adaptive in
some way so that environmental selection may result in
convergence of form in unrelated taxa. While such homoplasy may
complicate interpretations of relationships among taxa, selection
may also act to exaggerate differences, thus causing additional
complications (Mather, 1986).

Many characters used for the classification of reptiles, such as
scale counts, body proportions, scale arrangement and colour
pattern, vary intraspecifically, frequently making interpretation
difficult (Clarke, 1965; Mather, 1986). Despite these recognized
problems, identification and classification of reptiles have
necessarily relied heavily upon external characters (Cogger,

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1983) and has generally been the method of choice for previous studies of *Lampropholis* species.

4.01.02: Morphological and meristic characterization of *Lampropholis* (*sensu* Greer, 1974).

Apart from the recent electrophoretic studies of Mather (1986), previous taxonomic studies of *Lampropholis* have centred around morphological differences. Clarke (1965), working on a group then considered to be *Leiolopisma* but which included the *Lampropholis* species, *L. delicata* and *L. guichenoti*, recognized that many external characters displayed marked intraspecific variation, with overlap between species (and by implication genera). However, the number of fourth toe lamellae and colour patterns were identified as being useful in separating species. Harris and Johnston (1977) also observed that colour was the most useful morphological feature for distinguishing between *L. delicata* and *L. guichenoti* from a single locality but the number of lamellae under the fourth toe was not a reliable marker.

More recently, Mather (1986) subjected a subset of *Lampropholis* species (*cf. L. delicata, L. sp.1, L. sp.2, L. sp.3* of this study, *L. mirabilis* and *L. tetradactyla*) together with *Le. zia*, to a series of univariate and multivariate analyses. As with previous studies (Clarke, 1965; Harris & Johnston, 1977) a variety of meristic, morphological and colour characteristics

were chosen. Difficulty was encountered in consistently separating the four taxa, *L. delicata*, *L. sp.1*, *L. sp.2* and *L. sp.3* on the basis of morphological and colour characteristics, using univariate analysis. However, the remaining three species, *L. mirabilis*, *L. tetradactyla* and *Le. zia* were separable using morphological characters alone. Mather (1986) observed that *L. mirabilis* possessed relatively high midbody row scale counts; *L. tetradactyla* had four fingers on the forelimb as opposed to five in other taxa; and *Le. zia* possessed a relatively low fourth toe lamellae count. Multivariate analysis discriminated *L. delicata*, *L. sp.2* and *L. sp.3* populations when both colour and morphology were included, and further discriminated within *L. delicata* populations, separating this taxon into two groups (Mather, 1986).

Greer and Kluge (1980), on the basis of five described *Lampropholis* species, recognized two complexes within the genus (Sect. 1.06). Greer (1980) considered that these two complexes merited generic status. The *L. challengeri* complex was identified on the basis of three morphological characters; distinct frontoparietal scales, a white spot (ocellus) at the posterior base of the thigh and the fourth supralabial scale placed beneath the centre of the eye. *Lampropholis delicata* complex animals were identified as possessing fused frontoparietal scales, no ocellus and the fifth supralabial scale below the eye. In addition, it was suggested that *L. delicata* complex skinks preferred drier,

open regions of sclerophyll forest and rainforest while _L._ _challengeri_ complex animals were typically restricted to more mesic habitats.

Clarke (1965) employed morphometrics in her study while, more recently, Ingram and Rawlinson (1981) also used ratios to depict variation in the five species they described. Mather (1986) restricted his analyses to absolute measurements without reference to allometric growth.

4.01.03:   Morphological and meristic characterization of _Lampropholis_ (_sensu_ Greer, 1974) in perspective

In general, morphometric analysis of reptiles has been used in an attempt to account for allometric growth. This technique, however, has been demonstrated to be invalid and tends to introduce spurious variation (e.g. see Albrecht, 1978; Atchley & Anderson, 1978; Atchley, 1978). The widespread introduction of computer analysis and proliferation of programmes that allow a multivariate approach to data analysis now make it possible to examine allometry and morphological variation on the basis of regression and multivariate analysis. Thus statistically invalid methods inherent in using ratios or ignoring allometric growth may be replaced by more appropriate techniques (Sneath & Sokal, 1973).

Since conflict was identified between previous morphological studies and the biochemical analyses presented in the previous chapter, it was deemed desirable to investigate these anomalies using appropriate morphological and meristic criteria previously implemented, together with statistically valid techniques, to investigate relationships among taxa.

Despite the apparent limitations of this data set it is nevertheless the most extensive recorded for *Lampropholis*. Previous work has generally either been restricted to descriptions of species (e.g. Greer & Kluge, 1980; Ingram & Rawlinson, 1981), comparisons of two species from single locality (Harris & Johnston, 1977) or regional relationships (Clarke, 1965; Mather, 1986) In the case of Greer's work (e.g. 1967; 1970; 1977; 1979) concentration has been at higher taxonomic levels. This work then is the first attempt to analyse the relationships among the majority of the species within *Lampropholis* over a wide range.

4.02: MATERIALS AND METHODS FOR MORPHOLOGICAL AND MERISTIC DATA.

4.02.01: Data collection for morphological and meristic data.

Animals were returned live to the laboratory, sacrificed and measurements recorded before the animals were processed for electrophoretic, and/or MC'F analyses. Collecting localities are

included in Table A.01 (Appendix 1). Frozen or chemically preserved specimens were not included. Morphological and meristic characters used, together with their definitions, are given in Table 4.1, while mean, standard deviation and sample sizes for populations investigated appear in Table 4.2. Measurements were obtained using dial calipers (Mitutoyo) to an accuracy of 0.05 mm.

4.02.02: Data analyses for morphological and meristic data.

Of the numerical procedures available for morphological and meristic data sets, a cluster analysis of cases and a multivariate discriminant function analysis were utilized from the BMD biomedical computer programs of Dixson (1974). The former was chosen because of the lack of weighting implicit in the alogrithm for the clustering analysis, the same basis of justification for its use in the phenetic analysis of electrophoretic data. Since the cluster analysis relied on a relatively small number of morphological and meristic characters thereby potentially biasing the results a multivariate discriminant function analysis was also undertaken. This technique is designed to identify a small number of the most appropriate distinguishing characters upon which to allocate taxa to pre-existing groups. The emphasis on a small number of distinguishing characters was considered appropriate since parameters utilized in these analyses were those generally used

Table 4.1: Definitions of morphological and meristic criteria

Character	Definition
Snout-vent length (SVL)	Distance from anterior of rostal to posterior edge of the preanal scales.
Head length (HL)	Distance from anterior of rostal to posterior edge of eye.
Head width (HW)	Maximum head width at the level of the quadrate.
Tail length (TL)	Distance from posterior edge of anal scales to tip of tail.
Forelimb length (FLMB)	Distance from anterior edge of the insertion of the forelimb to the tip of the longest toe, including claw.
Hindlimb length (HLMB)	Distance from anterior edge of the insertion of the hindlimb to the tip of the longest toe, including claw.
Subdigital lamellae (4TOE)	Number of transverse scales on the lower surface of the longest toe (4th) of left of left hindlimb.
Mid-body scales rows (MBDY)	Number of scales transversely around the middle of the body.
Midline scale number (MLNE)	Number of scales on the midline from chin to preanal scales.
Axilla-groin length (AXGN)	Distance from the posterior edge of the insertion of the forelimb to the anterior edge of the insertion of the hindlimb.
Nuchal number (NCHL)	Number of scales posteriorally in contact with parietal scales.
Supracilaries number (SCIL)	The number of small scales along the outer margin of the upper eyelid.
Supracoulars number (SOCL)	Series of scales lying above the eyes.
Supralabial number (LABL)	Series of scales immediately beneath the eye.

Table 4.2: Mean (and standard deviation) of all parameters utilized in morphormetric and meristic analyses (for abreviations see Table 4.1).

Locality	n	SVL	HL	HW	TL	FLMB	HLMB	4TOE	MBDY	MLNE	AXGN	NCHL	SCIL	SOCL	LABL
L. challengeri															
Bellevue H	31	45.6	5.9	6.0	67.6	13.1	17.7	20.7	20.8	54.7	23.7	4.0	4.8	7.4	6.0
		(4.83)	(0.53)	(0.67)	(11.30)	(1.42)	(1.88)	(1.49)	(1.28)	(3.02)	(2.92)	(0.18)	(0.37)	(0.66)	(0.52)
Mt. Glorious	6	46.6	6.2	5.7	71.6	13.0	17.7	21.0	24.7	55.3	24.1	4.3	5.0	6.5	5.7
		(5.63)	(0.52)	(0.50)	(12.40)	(1.53)	(1.50)	(2.00)	(1.37)	(1.21)	(3.01)	(0.52)	(0)	(0.55)	(0.52)
Dorrigo	2	50.5	6.5	6.3	73.0	14.6	19.2	19.5	23.5	56.5	25.1	4.0	5.0	7.0	6.0
		(7.78)	(0.85)	(0.64)	(23.62)	(0.42)	(3.46)	(0.71)	(4.95)	(7.00)	(0)	(0)	(0)	(0)	(0)
Cambridge Pl.	5	50.8	6.9	6.4	59.7	14.9	20.8	22.0	24.2	49.2	23.8	4.2	5.2	6.0	6.0
		(4.60)	(1.00)	(0.63)	(23.12)	(1.46)	(1.68)	(2.45)	(0.45)	(2.68)	(2.05)	(0.45)	(0.45)	(0)	(0)
L. mustelina															
Sydney	19	44.4	5.2	5.6	69.2	10.3	14.5	16.2	21.8	53.3	23.6	4.2	4.6	6.4	6.0
		(4.45)	(0.46)	(0.66)	(17.17)	(1.15)	(1.68)	(1.64)	(1.61)	(2.23)	(2.68)	(0.42)	(0.51)	(0.50)	(0)
Hampton	3	48.7	5.8	6.7	82.3	11.2	15.3	14.3	20.3	56.3	29.8	4.0	4.3	7.0	5.7
		(2.52)	(0.29)	(0.58)	(24.79)	(0.29)	(0.29)	(1.15)	(0.58)	(4.51)	(3.88)	(0)	(0.58)	(1.00)	(0.58)
L. delicata															
Sydney	10	37.0	5.1	5.0	59.0	9.7	13.6	21.7	23.2	51.1	19.0	4.0	4.7	6.9	7.0
		(3.81)	(0.42)	(0.30)	(21.68)	(0.42)	(0.97)	(2.79)	(1.93)	(3.11)	(2.51)	(0)	(0.48)	(0.32)	(0)
Rockhampton	6	32.6	4.4	4.7	49.4	8.9	11.9	21.5	24.0	52.0	16.7	4.3	4.3	6.7	6.0
		(5.60)	(0.74)	(0.61)	(5.48)	(1.80)	(2.18)	(1.87)	(2.00)	(1.79)	(3.71)	(0.82)	(0.52)	(0.52)	(0)
Wiangarie SF	13	41.0	4.9	5.8	47.2	11.4	15.0	21.6	26.2	52.7	20.5	4.0	4.8	6.6	6.8
		(4.01)	(0.58)	(0.61)	(12.66)	(1.17)	(1.53)	(1.61)	(2.05)	(4.63)	(2.57)	(0)	(0.44)	(0.51)	(0.38)
Coffs Harbour	6	33.0	4.4	4.8	38.2	9.3	12.8	20.3	21.7	50.8	17.6	4.0	4.0	7.0	7.0
		(6.01)	(0.66)	(0.68)	(11.21)	(1.60)	(2.68)	(2.07)	(1.21)	(2.32)	(4.27)	(0)	(0)	(0)	(0)
Byron Bay	3	35.7	4.5	5.2	54.8	10.8	14.8	19.3	24.7	52.0	18.3	4.0	4.3	7.0	7.0
		(6.83)	(0.50)	(0.29)	(2.36)	(1.04)	(1.44)	(1.53)	(1.53)	(1.00)	(4.48)	(0)	(0.58)	(0)	(0)
Dorrigo	9	38.1	5.1	5.7	56.8	11.2	15.2	22.0	24.1	49.0	20.1	4.0	4.0	7.0	7.0
		(4.81)	(0.81)	(0.75)	(11.21)	(1.80)	(1.84)	(1.80)	(2.20)	(2.18)	(2.00)	(0)	(0)	(0)	(0)

```
Warroo       5 35.6  4.7    5.2    52.1    9.2   12.5   20.0   24.0   49.8   18.0    4.0    5.0    8.0    6.0
             (0.80)(0.31)(0.51)(10.18)(0.48)(1.22)(1.00)(1.00)(2.05)(2.93)  (0)    (0)    (0)    (0)
```

<u>L. sp.3</u>
```
  Danbulla SF 3 36.0  5.3    5.7    46.2    9.7   13.3   19.0   25.7   51.0   19.5    4.0    6.0    6.0    6.0
             (2.60)(0.76)(0.58)( 4.31)(1.15)(1.15)(1.73)(4.73)(2.65)(1.50)  (0)  (0.58)  (0)    (0)
  Eungella  36 39.9  5.3    6.3    41.9   10.5   14.5   20.4   26.4   51.7   20.1    4.0    4.7    7.6    6.3
             (3.74)(0.58)(0.57)( 9.45)(1.10)(1.30)(1.98)(1.92)(2.82)(2.69)  (0)  (0.47)(0.56)(0.44)
  Finch Hatton 3 37.4  4.7    5.4    30.2    9.5   12.7   21.7   27.7   52.3   18.8    4.0    5.0    8.0    6.0
             (2.05)(0.46)(0.42)( 7.66)(0.87)(1.15)(1.15)(2.08)(3.06)(1.57)  (0)    (0)    (0)    (0)
  Warroo     2 43.9  5.8    6.9    39.1   11.5   15.5   21.0   27.5   53.0   20.6    4.0    5.0    7.5    6.0
             (4.07)(0.78)(0.78)( 4.81)(0.71)(0.71)  (0)  (0.71)(2.83)(2.19)  (0)    (0)  (0.71)  (0)
```

<u>L. sp.1</u>
```
  Ravenshoe  5 37.5  5.3    5.7    40.0   10.4   14.2   19.4   28.4   49.0   19.6    4.0    4.6    6.8    6.4
             (2.83)(0.27)(0.45)( 4.06)(1.14)(0.84)(1.67)(1.95)(2.12)(1.34)  (0)  (0.55)(0.45)(0.55)
  L. Eacham  3 35.2  4.5    4.8    32.5    9.0   12.8   19.3   27.0   52.7   17.7    4.0    5.0    7.3    6.0
             (1.26)(0.50)(1.04)(10.97)(0.50)(0.29)(1.53)  (0)  (1.53)(1.15)  (0)  (1.00)(0.58)  (0)
Millaa Millaa 5 32.2  4.7    4.2    32.8    9.2   12.1   17.2   24.8   49.8   17.9    4.0    5.0    7.0    6.0
             (3.56)(0.27)(0.57)(11.76)(1.15)(1.34)(0.84)(1.64)(3.03)(1.14)  (0)    (0)    (0)    (0)
```

<u>L. basiliscus</u>
```
  Danbulla SF 6 39.1  6.2    6.1    59.3   12.5   16.8   20.2   25.3   54.2   19.3    4.8    4.7    7.2    5.2
             (5.78)(1.21)(0.80)(15.00)(2.19)(2.36)(1.94)(2.07)(3.82)(1.60)(0.98)(0.82)(1.17)(0.52)
  Mt Spec   44 40.9  5.9    5.9    53.4   12.1   16.4   18.4   24.3   51.5   20.3    6.0    5.4    7.1    6.0
             (4.39)(0.43)(0.56)(11.09)(0.78)(1.05)(1.17)(1.37)(2.95)(1.69)(0.72)(0.62)(0.73)  (0)
  Kuranda    4 44.6  6.4    6.3    48.5   14.3   17.6   20.0   25.0   52.3   22.3    6.5    5.0    6.8    5.8
             (3.61)(0.48)(0.50)(21.49)(0.96)(1.26)(0.82)(2.16)(2.22)(1.71)(1.00)  (0)  (0.96)(0.5)
  L. Eacham  4 39.5  5.8    5.6    58.0   12.9   17.1   20.5   28.3   53.8   19.3    4.8    5.0    7.5    5.3
             (3.08)(0.29)(0.48)(15.17)(0.85)(0.85)(2.38)(0.96)(3.59)(1.85)(0.50)  (0)  (0.58)(0.50)
```

<u>L. sp.2</u>
```
  Mt Glorious 5 36.0  4.6    5.1    34.8    9.0   12.1   20.2   24.2   47.2   19.4    4.2    5.0    7.0    6.0
             (4.12)(0.42)(0.42)( 4.83)(0.71)(0.74)(0.84)(2.17)(3.56)(1.47)(0.45)  (0)  (0.71)  (0)
```

L. guichenoti

	N													
Ryde	12	37.4	5.2	5.5	48.4	10.4	14.6	19.0	24.9	53.8	17.0	4.0	4.1	6.3	6.9
		(4.00)	(0.40)	(0.48)	(11.16)	(0.90)	(1.00)	(1.62)	(1.24)	(3.64)	(4.51)	(0)	(0.29)	(0.40)	(0.29)
Rainbow Beach	3	45.7	6.2	5.0	55.2	9.7	14.3	19.0	23.3	53.0	17.0	4.0	6.0	4.0	7.0
		(3.51)	(0.29)	(0.20)	(5.52)	(1.15)	(0.58)	(1.00)	(2.08)	(2.65)	(0.70)	(0)	(0)	(0)	(0)
Walcha	8	40.1	4.9	5.5	44.3	9.9	13.8	17.5	23.5	54.0	18.5	4.0	5.6	4.7	6.8
		(5.27)	(0.60)	(0.46)	(12.87)	(1.13)	(1.28)	(2.07)	(1.60)	(1.31)	(2.29)	(0)	(0.52)	(1.04)	(0.46)

L. czechurai

Kuranda	10	38.1	5.1	5.4	42.0	10.0	13.5	21.5	25.6	52.2	19.3	4.1	4.9	7.7	5.8
		(1.75)	(0.43)	(0.46)	(9.70)	(1.00)	(1.07)	(2.68)	(2.37)	(4.37)	(1.14)	(0.32)	(0.32)	(0.48)	(0.42)
Millaa Millaa	2	30.5	4.5	4.8	36.5	7.8	9.5	14.0	22.5	53.0	16.0	6.0	5.0	7.0	5.0
		(1.41)	(0)	(0.35)	(3.54)	(0.35)	(0.71)	(0)	(0.71)	(0)	(0)	(0)	(0)	(0)	(0)
Eungella	5	32.9	5.1	4.9	36.1	8.8	12.4	17.2	20.8	46.6	14.6	5.0	5.2	7.0	5.0
		(3.66)	(0.38)	(0.47)	(10.42)	(1.30)	(0.89)	(1.64)	(0.45)	(3.05)	(2.76)	(1.00)	(0.45)	(0)	(0)

L. mirabilis

Mt Elliot	29	46.4	6.4	6.8	50.9	14.8	21.0	25.8	30.8	62.2	21.7	4.0	4.3	7.2	7.0
		(2.41)	(0.37)	(0.36)	(15.40)	(0.79)	(1.03)	(1.51)	(2.08)	(3.63)	(1.37)	(0)	(0.48)	(0.41)	(0)

Le. zia

Wiangarie SF	5	41.0	5.5	5.8	52.0	11.4	15.5	20.1	24.8	53.1	20.6	4.9	4.8	7.0	6.3
		(5.08)	(0.45)	(0.45)	(10.10)	(0.74)	(1.39)	(1.34)	(1.52)	(1.41)	(3.56)	(0)	(0)	(0)	(0)

for field identification (see also Sect. 2.08.02 for discussion).

Richardson *et al* (1986) suggest that the former algorithm is the most commonly used for cluster analysis while both have been demonstrated to be useful by several colleagues working on the taxonomy of fish (Crowley, 1983; 1988; Pavlov, 1984; Said, 1983) and spiders (Gray, 1986).

Before commencement of the discriminant analysis, the effects of allometric growth were determined by using regression of metric characters against snout-vent length. The data were then subjected to stepwise discriminant analysis (Dixson, 1974). The computations were carried out using the BMDP7M programme. As stated above this analysis serves to identify, from those available, a subset of characters which best discriminate among *a priori* populations. These are used to calculate canonical coefficients for each of the characters.

A one-way analysis of variance (F statistic) identifies characters displaying the greatest variation among population means. A hierarchical comparison of means then ensues until no further characters contribute to the discrimination process. Generally, it is only necessary to plot the first two canonical variates since they frequently incorporate a significant percentage of the variation.

A dendrogram based on BMDP2M cluster analysis (Dixson, 1974) was computed in order to determine phenetic relationships among *Lampropholis (sensu* Greer, 1974) species. As with electrophoretic analysis an unweighted average linkage algorithm was used as the basis for this analysis. Consequently the two cases which have the shortest distance between them are amalgamated and treated as a single case. This grouping is then, in turn, clustered successively. This process continues until all cases and clusters are amalgamated.

4.03:   RESULTS OF MORPHOLOGICAL AND MERISTIC ANALYSES

Sixteen characters, seven meristic and nine morphological were included in the analysis (see Table 4.1 for details of character states). One, the configuration of the parietal scales is discussed but not formally incorporated in the analysis. Two characters, the condition of the ocellus and which supralabial scale is beneath the centre of the eye, where exactly correlated for this data set, and therefore gave identical results when only one of these was incorporated (Fig. 5.1). All seven regression equations based on morphological characters were those of a straight line, so that adjustment for allometric growth was unnecessary.

The projection of the first two canonical variables on the data set is presented in Figure 4.1 while the projection of canonical

variables two and three on the remaining data set is presented in Figure 4.2. Figure 4.3 is a dendrogram constructed using the morphological and meristic attributes.
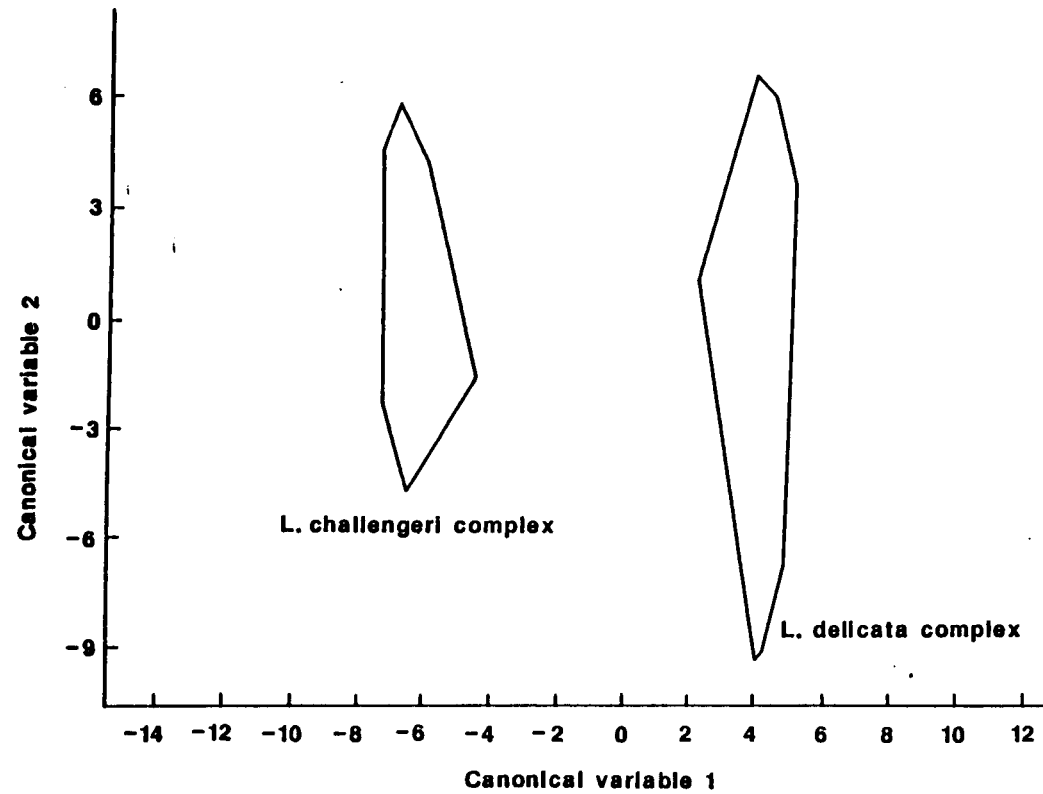
Figure 4.1: Preliminary discriminant function analysis of Lampropholis taxa.
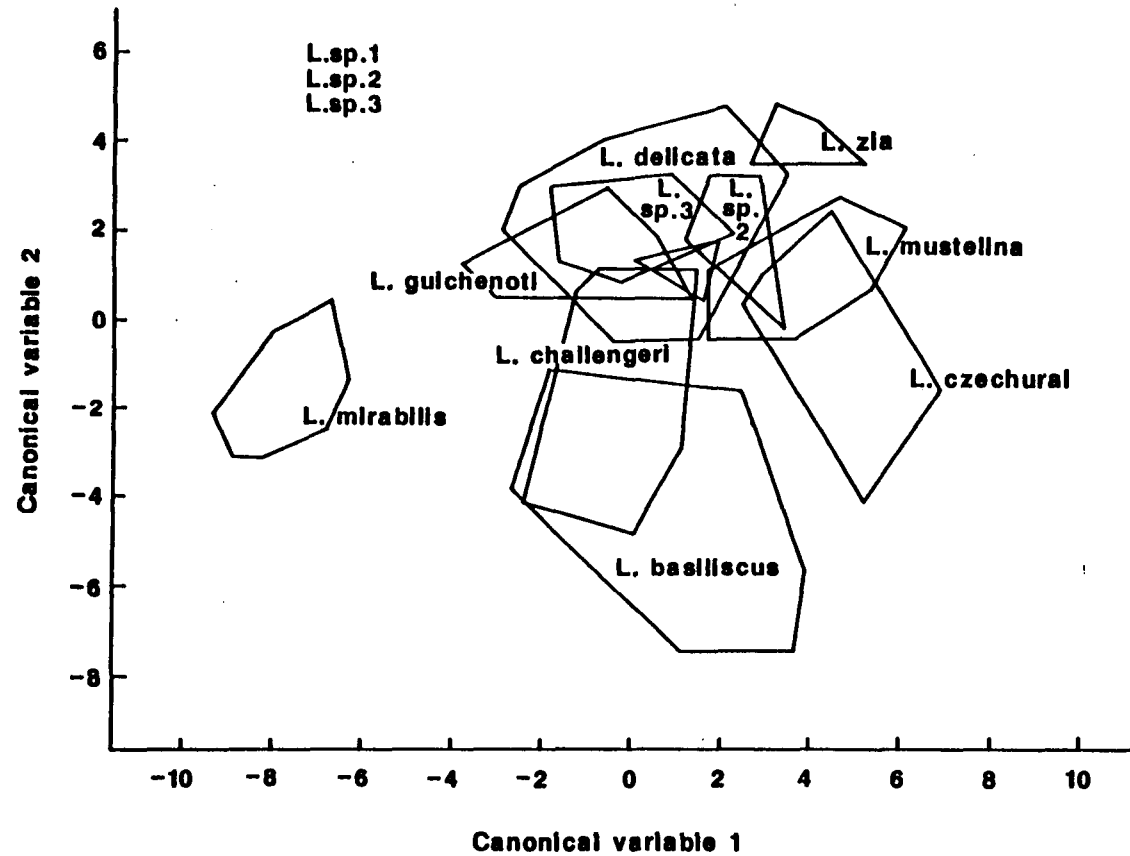
Figure 4.2: Discriminant function analysis of Lampropholis taxa.
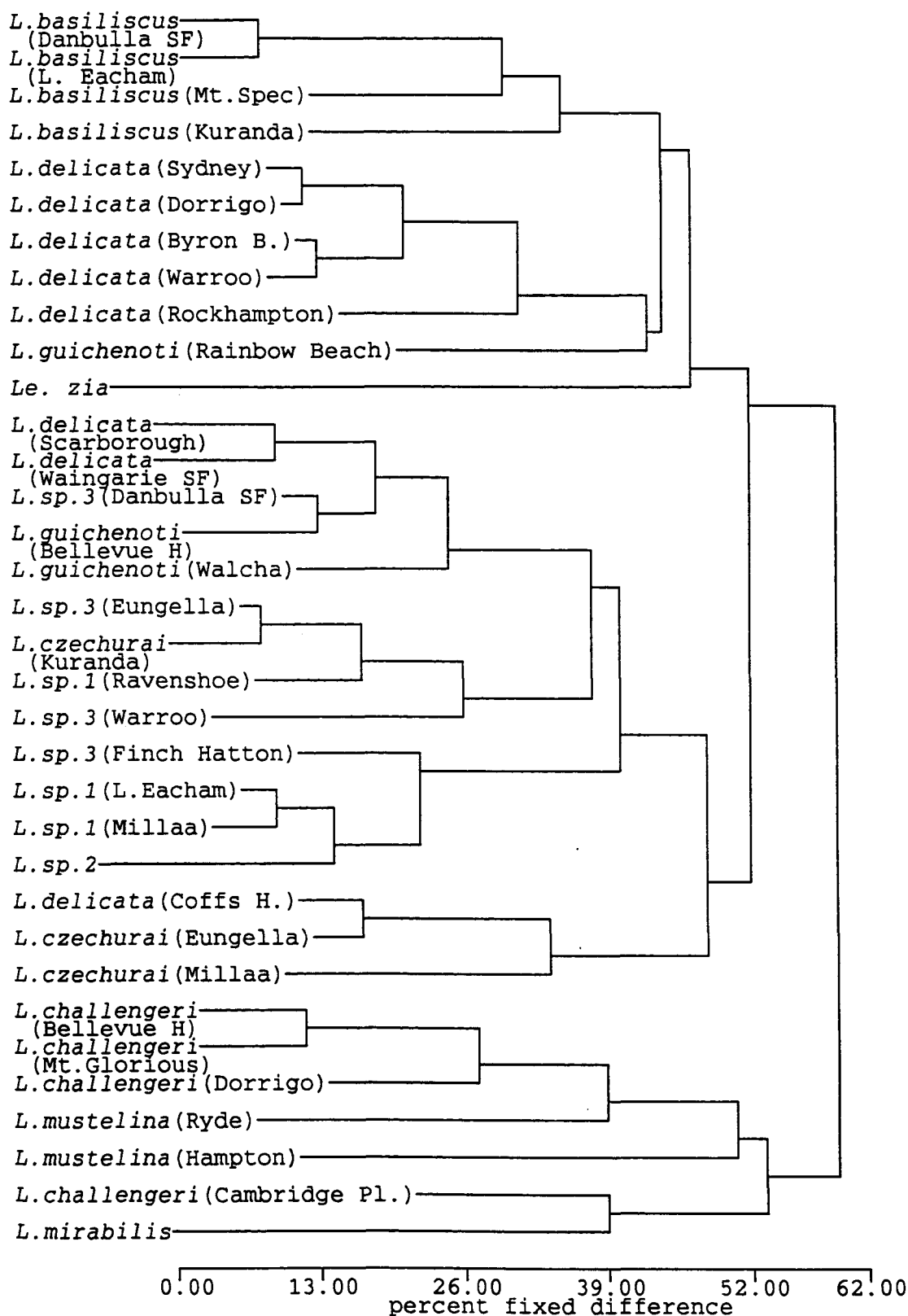
Figure 4.3: Cluster analysis based on *Lampropholis*
morphological and metistic data.

4.04:   DISCUSSION OF MORPHOLOGICAL AND MERISTIC RESULTS.

4.04.01: Aspects of *Lampropholis (sensu* Greer, 1974) taxonomy
investigated

Due to the apparent conflict between previous morphological work
and the biochemical taxonomy presented in the previous chapter,
confirmation of these conflicts, or their resolution were sought:

(1)   Confirmation   of higher   level   groupings   within
Lampropholis.

(2) Confirmation of relationships between *Le. zia* and
Lampropholis.

(3) Evidence for delineation of species boundaries.

4.04.02: Higher level groupings within *Lampropholis (sensu* Greer,
        1974)

In addressing question one, the electrophoretic data identifies
four groupings, and in no way supports the hypothesis, based on
previous morphological data, that two exist.

Previous research had identified three characters to distinguish
between the two *Lampropholis* complexes (Greer, 1974);   the
presence or absence of an ocellus at the posterior base of the
thigh, the supralabial scale below the centre of the eye and the

fusion or non-fusion of the frontoparietal scales (see Section 1.06).

The presence or absence of an ocellus was demonstrated to be useful in identifying two groups within _Lampropholis_. The _L. challengeri_ complex (_sensu_ Greer, 1980) taxa always have an ocellus at the posterior base of the thigh and it was never observed in any _L. delicata_ complex (_sensu_ Greer, 1980) taxa. Inclusion of this information into the data set results in the separation of taxa into two groups as in Figure 4.1. Unlike this character the remaining two characters used to distinguish groupings were less reliable. One of these characters (the supralabial scale occurring below the centre of the eye) clearly split the genus into two groups (Fig. 4.1). _Lampropholis challengeri_ complex (_sensu_ Greer, 1980) animals presented the fourth supralabial scale below the centre of the eye. Conversely, _L. delicata_ taxa (_sensu_ Greer, 1980) represented in this analysis had the fifth supralabial scale below the centre of the eye. However, this character is known to vary between alternative conditions in _L. caligula_. This species presents most frequently the fourth, although sometimes the fifth, supralabial scale below the centre of the eye (Ingram & Rawlinson, 1981). The elusiveness of this species has previously been discussed (Section 3.02.01) and therefore insufficient data were available for this species to be included in the analysis. The observed division between complexes (demonstrated in Fig. 4.1) is therefore not complicated

by this variation in character state although this intraspecific variation demonstrates that the character is not acceptable as sole basis for generic status.

The other character, the configuration of the frontoparietal scales did not always conform to a set pattern. Analysis of this character was not undertaken since it was noted that there was a great deal of variation in scale arrangement both within and among taxa. For example, in *L. sp.3* from Central Queensland (Warroo population), the interparietal scale had fused to the frontoparietal scales (which were also fused), forming a rhomboid shape. However other populations of this taxon had reduced, interparietal scales clearly distinct from the fused frontoparietal scale. In addition, the configurations of these scales in different taxa were observed to grade from the rhomboid condition in *L. sp.3*, to a much reduced interparietal in *L. mirabilis* through to a relatively large interparietal with distinct frontoparietal scales in *L. challengeri* taxa. Variability was also observed within taxa and recorded by Mather (1986). This character was therefore considered to be totally unacceptable as a generic marker.

Therefore since it was originally hypothesized that the genus was worthy of being divided into two genera (Greer, 1980), subsequent research and description of additional species has reduced the number of criteria which unequivocally separate the genus into

two groups phenetically from three to one. Phylogenetically, however, this character is unacceptable, any such analysis would necessarily identify one condition (using *Le. zia* as an outgroup this would be the lack of an ocellus) as a symplesiomorphic state and the alternative condition as synapomorphic. This combination would not constitute an acceptable criterion on which to base relationships, since common possession of a primitive condition (symplesiomorphic state) provides no phylogenetic information. Therefore none of the original criteria is seen as a completely appropriate basis on which to base generic decisions.

Including the information on presence or absence of an ocellus, dominated variation included in the first canonical variable and thereby suppressed separation inherent in the second and subsequent canonical variables (Fig. 4.1). The data was therefore reanalyzed to determine if alternative groupings could be identified (Fig. 4.2). The canonical variables used in the second analysis tended not to separate the taxa substantially. Despite distinct clusterings none, with the exception of *L. mirabilis*, were discretely clustered and therefore no clear groupings of taxa were identifiable. This analysis therefore, lent no additional information but rather reaffirmed a large amount of morphological variation within and between species.

Cluster analysis based on the data also identified substantial variation within and among species. At the upper limit, *L.*

challengeri, *L. mustelina* and *L. mirabilis* cluster to the exclusion of all other taxa investigated. This indicates that this group, comprising one *L. delicata* complex (*sensu* Greer, 1980) species (i.e. *L. mirabilis*) and two *L. challengeri* complex (*sensu* Greer, 1980) species, form a distinct group. Within this cluster, variation is substantial. For example, separation among *L. challengeri* taxa are greater than that observed among any other taxa. Beyond this grouping, two other major clusters may be identified. One includes *L. basiliscus* (*L. challengeri* complex, *sensu* Greer, 1980), most *L. delicata* populations, a single population of *L. guichenoti* (*L. delicata* complex, *sensu* Greer, 1980) and *Le. zia*. The second of these clusters comprises predominantly *L. delicata* complex (*sensu* Greer, 1980) taxa, including the remaining *L. delicata* and *L. guichenoti* populations, all populations *L. sp.1*, *L. sp.2* and *L. sp.3* together with all those of *L. czechurai* (*L. challengeri* complex, *sensu* Greer, 1980). None of the taxa represented by more than a single population, with the exception of *L. basiliscus*, are projected separately and even this latter species shows substantial variation among populations (Fig. 4.3).

On the basis of this data set, meristic and morphological characters for clear discrimination among taxa have not been identified. As with previous workers, these data also identified a large degree of overlap among species, although, when colour

characteristics are incorporated a degree of separation may be observed (e.g. Mather, 1986).

Coloration was not incorporated as a criterion in this study. This was due in part to difficulties inherent in quantification and because of its apparent environmental variation. However, in the field, general colour and colour patterns were recognized to be a good predictor for some taxa. For example, *L. sp.3* has a distinct pink wash on the ventral surface in life, while all other *Lampropholis* (*sensu* Greer, 1974) taxa encountered presented ventral washes ranging from white to bright yellow. Thus, when comparing only *Lampropholis* (*sensu* Greer, 1974) taxa, the pink ventral wash was an excellent predictor for this taxon. However, species of other genera which share *L. sp.3* habitat, particularly *Carlia* species, were also observed with this coloration. In addition, colour intensity varies dramatically within and among taxa as well as seasonally. This criterion is therefore an unsuitable predictor for *Lampropholis* (*sensu* Greer, 1974) species identification in general, despite being useful for identification of *L. sp.3* from other *Lampropholis* (*sensu* Greer, 1974) taxa in the field. Body colour patterning is also useful in some instances. For example, the phenomenon of white stripes on an otherwise dark or grey background has only limited use among *Lampropholis* (*sensu* Greer, 1974) and may vary considerably within species. One *L. delicata* morph has very distinctive lateral striping, which is sexually dimorphic, but this pattern is

completely absent throughout most of the species' range and not observed to constitute clinal variation. Other less dramatic variations in colour pattern among populations were also observed. Therefore, although it has been demonstrated to be generally useful in a regional study of *L. delicata* taxa (Mather, 1986) it is generally difficult to quantitate and needs to be incorporated with caution. It is considered more appropriate to approach the use of various colour criteria by identifying the full extent of variation throughout an individual species' range before attempting to compare such criteria across species boundaries. Mather's work (1986) provides a basis on which to build such a study for *L. delicata* taxa. The biochemical identification of various taxa within *L. challengeri* also provides an avenue of future studies in this area. However, a thorough and detailed investigation of the appropriateness of various colour criteria were considered outside of the limits of this study.

4.04.03: *Le. zia* as an outgroup to the *Lampropholis* genus (*sensu* Greer, 1974)

The data indicate that *Le. zia* is generally distinct from all taxa of *Lampropholis* (*sensu* Greer, 1974; Figs 4.2). However, phenetically it clusters most closely with a group comprising *L. basiliscus* and some populations of *L. delicata* and *L. guichenoti*. This indicates that it is morphologically more similar to these

species than some *Lampropholis* (*sensu* Greer, 1974) are to their congeners (Fig. 4.3).

4.05: CONCLUSIONS DRAWN FROM THE MORPHOLOGICAL AND MERISTIC DATA.

On the basis of the morphological and meristic data presented above there is no unequivocal support for either of the proposed groupings within the genus, *Lampropholis* (*sensu* Greer, 1974). The case for two genera within the taxon is significantly weakened by the identification of variation within two of the three meristic characters identified as supporting such a separation. No data supports a fragmentation of the genus into four genera as proposed on the basis of allozyme electrophoretic hypotheses.

The second apparent conflict between approaches was the choice of *Le. zia* as an outgroup for phylogenetic studies. This species was chosen as an outgroup on the basis of its close, although distinct, relationship to the genus *Lampropholis* (*sensu* Greer, 1974). While the genus *Leiolopisma* is considered heterogeneous, *Lampropholis* (*sensu* Greer, 1974) was removed from it on the basis of its distinctiveness from those species remaining, including *Le. zia* (Greer, 1974). However, if *Lampropholis* (*sensu* Greer, 1974) is a single group, *Le. zia* must be considered to be within that group both on morphological and allozyme electrophoretic criteria and this implies the possibility that

other *Leiolopisma* species are also within the group.

Finally, the morphological and meristic data indicated that there is substantial overlap among taxa (Figs 4.3). Only one species, *L. basiliscus*, presents a cohesive grouping. Inherent variation was observed to be significant, even among populations of this latter species. Conversely, the electrophoretic analysis grouped recognized species in discrete clusters, indicating that it is a more useful technique for discrimination at the species level.

Variation among taxa was found to be substantial, as it was with the biochemical data. In the absence of high level intraspecific variation this would indicate generic level differentiation, but this is not the case here. Intraspecific variation, for example among *L. delicata* and *L. guichenoti* populations, is in some instances substantial (see Fig. 4.3). In addition the large degree of overlap among taxa (Fig. 4.2) further complicates the identification of groups.

Ideally more attributes should have been investigated. Initially this phase of the work was seen as being a preliminary analysis using the most commonly used attributes from identification, as it would be just such characters which should demonstrate the most striking separation of species. However, even this preliminary work failed to discriminate species successfully.

As with previous workers (e.g. Clarke, 1965; Harris & Johnston, 1977; Mather, 1986), who used data on fewer species and with less representation within species, the present work illustrates that variation both within and among taxa is substantial and therefore this type of data are of limited value for the identification of discrete groups, at least within the *Lampropholis* genus (*sensu* Greer, 1974).

Thus in answer to the problems posed at the beginning of this chapter (Sect. 4.04.01), no confirmation of higher level groupings within *Lampropholis (sensu* Greer, 1974) were obtained either to support the previous views of Greer (1974; 1980) or the allozyme electrophoretic data. The relatively high level of morphological variation within recognized species precluded delineation either of species boundaries or higher level clusterings. *Leiolopisma zia* did not cluster discretely, as would be expected if it was a representative of a different genus. Instead it clustered within the group, as was observed in the results of the allozyme electrophoretic analyses (see previous Chapter).

CHAPTER 5: EVOLUTIONARY RELATIONSHIPS OF *LAMPROPHOLIS* SKINKS AND
THEIR RELATIVES: VARIATION IN THE MICRO-COMPLEMENT
FIXATION OF ALBUMIN.

5.01:   INTRODUCTION: TAXONOMIC UTILITY OF ALBUMIN MICRO-
COMPLEMENT FIXATION FOR REPTILE RESEARCH.

Since its introduction by Wasserman and Levine in 1961, micro-complement fixation has been widely used as a tool for assessing taxonomic and phylogenetic relationships among many extant taxa (Maxson *et al*, 1982).

As with allozyme electrophoresis the most extensive reptilian work has been carried out on North American taxa, most specifically iguanids and agamids (e.g. Gorman *et al*, 1971; 1980; Wyles & Gorman, 1978; 1980), while snakes have also received attention (e.g. Cadle, 1984; 1984a; Dowling *et al* ,1983). There is no published data on the Australian Scincidae although work is in progress (Baverstock, pers. comm.). Limited information is available on the Gekkonidae (King, 1987), while more extensive data on Australian amphibians has been published (e.g. Daugherty & Maxson, 1982; Heyer *et al*, 1982; Maxson *et al*, 1982; Maxson & Roberts, 1984; Maxson & Wilson, 1975; Roberts & Maxson, 1985).

To date most of the molecular information on Australian reptiles above species level has been obtained using immunoelectrophoretic

criteria (Hutchinson, 1981; 1983; Mao et al, 1983; Schwaner et al, 1985). Of these investigations the most pertinent to the present study is the work of Hutchinson (1981; 1983) on the Scincidae, genera *Tiliqua* and *Egernia* (see review in Section 1.04).

As previously discussed (Section 2.09), immunoelectrophoretic techniques are inappropriate if they are based on complex cross-reaction patterns, because the results are frequently difficult to interpret. Consequently, since it is now possible to undertake micro-complement fixation studies in Australia, it was deemed appropriate to use this technique to assess the taxonomic relationships of *Lampropholis* (*sensu* Greer, 1974) skinks beyond the level applicable for allozyme electrophoresis.

5.02:   MATERIALS AND METHODS FOR ALBUMIN MICRO-COMPLEMENT FIXATION.

5.02.01: Location of the study.

This aspect of the study was carried out at the Evolutionary Biology Unit of the South Australian Museum. The work was executed with the aid of staff within the Unit and under the direction and guidance of Dr. Peter Baverstock.

5.02.02: Extent of the research.

Antibodies were raised to four species of *Lampropholis* *(sensu* Greer, 1974): *L. guichenoti* and *L. sp.3* from the <u>*L*.</u> <u>*delicata*</u> complex *(sensu* Greer, 1980) and *L. basiliscus* and *L.sp.* (Sydney region *L. challengeri* taxon hereafter referred to for convenience by the pseudonym of Sydney *L. challengeri*) from the <u>*L*.</u> <u>*challengeri*</u> complex *(sensu* Greer, 1980). These taxa were reciprocally tested and a series of species within the subfamily Lygosominae were unidirectionally tested. The species, collection localities and other pertinent details appear in Table A.2 (Appendix 1).

5.02.03: Preparation of samples.

The chemicals and immunological reagents used were those in common usage at the Evolutionary Biology Unit. The procedures were similar to those described in Champion *et al* (1974) and Baverstock (1984).

To collect sufficient blood to raise antibodies to albumin of *Lampropholis* *(sensu* Greer, 1974) species, between thirty and forty individuals of each of the species *L. guichenoti*, *L. basiliscus*, Sydney *L. challengeri* and *L. sp.3.* were collected and bled. The carcasses were retained for morphological and electrophoretic analysis where appropriate and voucher specimens

will be lodged with The Australian Museum. Plasma was extracted by centrifugation and pooled to provide approximately 1 ml for each species. Care was taken to ensure that all animals of a species were collected from the same location so that homology of the albumin protein was maximized. In larger animals this could be tested (by allozyme electrophoresis) on a sample of plasma from each individual. In small skinks, however, this is impractical and it was assumed that by restricting the collection of each species to one locality, a single population was sampled.

Albumin of most non-*Lampropholis* skinks was supplied by Dr. Peter Baverstock. Serum albumin from whole blood of a single individual, or a small number of individuals, from each of the species investigated was used for the antigen reaction.

5.02.04: Antibody preparation.

Antibodies were raised to four species of *Lampropholis (sensu* Greer, 1974) and these were used to measure albumin sequence divergence in *Lampropholis (sensu* Greer, 1974). To purify albumin, approximately 1 ml of plasma was subjected to disc gel electrophoresis (Davis, 1964). The albumin containing region of the gel was removed and placed in buffer overnight at 4°C (Champion *et al*, 1974). The purity of the eluted albumin was confirmed by immunoelectrophoresis on SDS polyacrylamide gels.

Initially Freund's complete adjuvant, homogenized with the antigen preparation, was injected subcutaneously into the lumbar region of three rabbits. At week five, a second immunisation of antigen homogenized with Freund's incomplete adjuvant was administered in the same region of the rabbit. In weeks ten and eleven, pure antigen was injected into the ear vein of the same rabbits. The animals were bled in week twelve. The resulting antiserum was heated at 50°C for thirty minutes to inactivate the rabbit complement, centrifuged and the supernatant collected. Antisera from rabbits immunized with the same albumin were pooled, mixing them in inverse proportion to their titres to maximize reciprocity in heterologous reactions. The antisera were then stored at -15°C.

5.03:   MEASUREMENTS OF IMMUNOLOGICAL DISTANCES.

Methods used to determine immunological distances are discussed in Section 2.10 and were similar to those described in Champion et al (1974) and were routine within the laboratory. Reported immunological distances are the averages of two or more individual determinations.

5.04:   PHYLOGENETIC INFERENCES.

Reliability of immunological distances as a reflection of sequence divergence between albumins is discussed Section 2.12

but is also partly influenced by the level of reciprocity. Although the distance between the two taxa A and B are fixed, generally a measure of the immunological distance measured with an antisera of A to B and that of antisera B to A are not congruent. This anomaly is referred to as nonrecipocity. This variation between two taxa, intuitively expected to be equivalent, is considered to result from the influence of the animal (in this instance, rabbit) on antibody incubation. Since the albumins of the two species are divergent, the surface affinities of their respective albumin molecules will differ due to their sharing different segments of their sequence with the rabbit albumin. Thus the interaction of the rabbit albumin will effect sequence specificities and therefore the rabbit does not act as a totally unbiased intermediatery (Sarich, 1977a). Sarich (1977a) reported that it is necessary to identify this experimental 'noise' by estimating the degree of bias involved as follows:

$$\% \text{ nonreciprocity} = 100 \left[\frac{\text{anti-A with B} - \text{anti-B with A}}{\text{anti-A with B} + \text{anti-B with A}} \right]$$

In general the level of nonreciprocity is in the order of 5-12%. However, it has been observed that there is a significant nonrandomness to the distribution of the effect of nonreciprocity. To correct for this nonrandom effect, a matrix is produced of antigens and antibodies and the rows and columns of the matrix are summed. Each of the raw data in the columns is in

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turn multiplied by the corresponding row to column ratio beginning with the point of greatest discrepancy. Upon completion of this process, the ratios for the newly calculated matrix are evaluated and the procedure repeated until the rows and columns approach close agreement. In general such treatment of the data reduces nonreciprocity to 4-6%. This level of 'noise' is subsequently considered in the degree of probable error when conclusions on phylogenies are drawn (Sarich, 1977a).

The raising of antibodies is costly, in terms of time, expense and, in the case of small skinks, the number of donor individuals involved. It is therefore desirable to maximize information obtained from each antiserum produced. Unidirectional cross reactions are therefore valuable since they give an indication (at little extra cost) of relationships, as well as being informative in suggesting clusterings of taxa. When these clusterings conform to current taxonomic and phylogenetic groupings they may be considered to support the current philosophy. If not, they may be used as the basis for future hypotheses.

For the species on which reciprocal measurements were performed, the averages of the reciprocals were used as the basis of the analysis. This procedure gives an indication of how proficiently the albumin sequence differences between species pairs were estimated. The data matrix was subsequently corrected for

reciprocity following the suggestion of Sarich and Cronin (1976),

although a final scaling was applied such that the average of all

correction factors was 1.00. This allows for the identification

of significant non-randomness in the distribution of the data.

The standard deviation for reciprocity was calculated using the

method of Sarich and Cronin (1976). Since the data is genetically

derived, phylogenetic analyses are appropriate, therefore, the

Distance Wagner procedure (Farris, 1972) and Fitch and Margoliash

(1967) 'goodness-of-fit' procedures were adopted to infer

relationships.

5.05:   RESULTS OF ALBUMIN MICRO-COMPLEMENT FIXATION DATA.

Albumin immunological distances (IDs) were reciprocally tested

among four species of *Lampropholis*, *L. sp.3*, *L. guichenoti* (*L.*

delicata complex, *sensu* Greer, 1974), Sydney *L. challengeri*, *L.*

basiliscus (*L. challengeri* complex (*sensu* Greer, 1974). The

standard deviation from reciprocity for the raw data in Table 5.1

is 19.3% but after correction this was reduced to 5.8%.

The means of the reciprocal distances obtained from the corrected

data set (Table 5.2) were used to construct a Distance Wagner

tree (Fig. 5.1) and Fitch and Margoliash 'goodness-of-fit'

phylogram (Fig. 5.2). *Leiolopisma grande* was considered to be at

too great a distance from *Lampropholis* to root the dendrogram

accurately. The data are therefore reproduced as an unrooted

Wagner tree, although *Le. grande* was used to root the Fitch and Margoliash topology.

In addition to the reciprocal data set, the antibodies raised to Sydney *L. challengeri*, *L. basiliscus*, *L. sp.3* and *L. guichenoti* were cross-reacted unidirectionally with a number of other species within the subfamily Lygosominae. These data are presented in Table 5.3 (subgroups are clustered in this table on the basis of Greer & Rounsevell, 1986 and not according to Greer, 1974; see Table 1.2 for species' composition: the reason for this dichotomy will become apparent from the discussion in Sect. 5.06.03 and beyond).

Table 5.1: Immunological distances among representative members of both complexes of *Lampropholis*, together with outgroup *Leilopisma grande*. Data in parentheses have been corrected for reciprocity (Sarich & Cronin, 1976; CF = correction factor).

Antigen   Antibody

	L.challengeri	L.guichenoti	L.basiliscus	L.sp.3
CF:	0.82	0.92	1.50	0.88
L.challengeri	0	18(20)	29(19)	23(26)
L.guichenoti	16(20)	0	18(12)	20(20)
L.basiliscus	17(21)	13(14)	0	12(14)
L.sp.3	21(26)	19(21)	25(17)	0
Leiolopisma grande	45(55)	41(45)	44(29)	35(40)

Table 5.2: Average immunological distances among representative members of both complexes of *Lampropholis*, together with outgroup *Leiolopisma grande* (from corrected matrix).

Antibody   Antigen

            L.challengeri *L.guichenoti* *L.basiliscus* *L.sp.3*

	L.challengeri	*L.guichenoti*	*L.basiliscus*	*L.sp.3*
L.challengeri	0			
L.guichenoti	20	0		
L.basiliscus	20	13	0	
L.sp.3	26	23	16	0

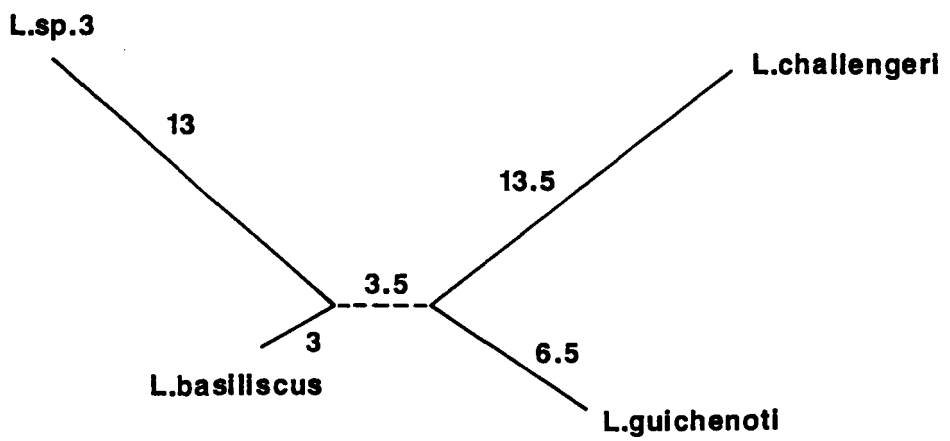|	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp.3*
Leiolopisma grande	55	45	29	40

Figure 5.1: Unrooted Wagner tree demonstrating relationships among *Lampropholis* using albumin immunological distances.
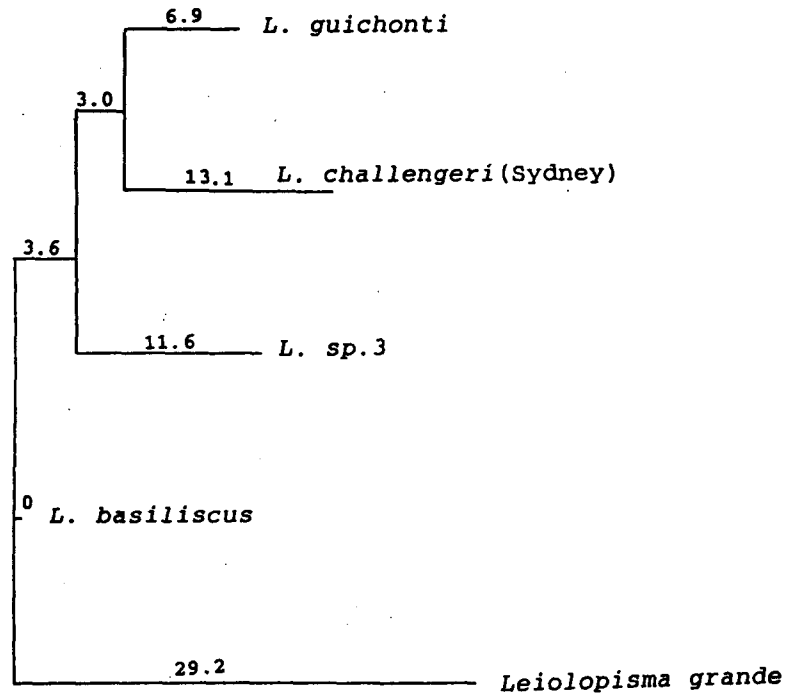
Figure 5.2: Fitch and Margoliash (1967) 'goodness-of-fit' phylogram demonstrating relationships among *Lampropholis* using albumin immunological distances.

Table 5.3: Unidirectional immunological distances between antibodies to four species of *Lampropholis* to antigens of a range of Lygosominae taxa (corrected data).

Antigen	Antibody			
	L.challengeri	*L.guichenoti*	*L.basiliscus*	*L.sp3*

Eugongylus group
Pseudemoia subgroup
Lampropholis chal*lengeri* complex

Antigen	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp3*
L. challengeri(Sydney)	0	20	19	26
L. challengeri(Mt.Nebo)	6	18	-	-
L. basiliscus	21	14	0	14
L. mustelina	22	17	14	15
L. tetradactyla	21	22	-	-
L. czechurai	18	16	15	11

Lampropholis delic*ata* complex

Antigen	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp3*
L. guichonti(Sydney)	20	0	12	23
L. guichenoti(SeymourCP)	20	0	-	-
L. guichenoti(KangarooIs)	21	1	-	-
L. sp.3	26	21	17	0
L. sp.2	37	18	19	16
L. sp.1	27	20	18	25
L. delicata(Sydney)	23	17	14	22
L. amicula	38	38	20	31
L. delicata(SA)	23	16	-	-
L. mirabilis	29	10	11	23
L. caligula	20	12	13	22
Leiolopisma jigurru	-	-	18	-
Leiolopisma zia	29	24	19	16
Leiolopisma metallicum	21	17	16	24
Leiolopisma entrecasteauxii	-	39	19	-
Pseudemoia paulfreyi	24	17	15	15
Calia rostralis	34	26	17	27
Menethia greyii	73	54	32	52

Eugongylus subgroup

Antigen	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp3*
Leiolopisma grande	55	46	29	40
Leiolopisma otagense	59	40	31	28
Leiolopisma duperreyi	82	55	-	53
Emoia longicauda	71	66	-	57
Eugongylus rufescus	65	59	-	63
Morethia adelaidensis	~72	67	-	52
Morethia boulengeri	67	50	32	56

Sphenomorphus group

Antigen	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp3*
Ctenotis grandis	134	127	-	-
Ctenotis robustus	120	136	-	-
Sphenomorphus murrayi	115	-	-	-
Sphenomorphus jobiensis	117	113	-	-
Sphenomorphus punctulatus	123	118	-	-

Egernia group

Antigen	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp3*
Tilqua rugosa	107	90	-	-
Egernia frerei	134	137	-	-

Unplaced Lygosominae species

Antigen	*L.challengeri*	*L.guichenoti*	*L.basiliscus*	*L.sp3*
Tribolonotus novaeguinae	~160	~125	-	-
Lamprolepis smaragdina	139	~133	-	-
Mabuya multifascata	-	65	-	-

5.06:   DISCUSSION OF ALBUMIN MICRO-COMPLEMENT FIXATION DATA.

5.06.01: Adequacy of the data set.

Sarich (1977a) considered that a range of 5 - 12% was an
acceptable 'noise' level and commented that 18% was "appalling"
for albumin data. In the context of this information, a reduction
of the standard deviation of reciprocity of the corrected data
set to 5.8% was within acceptable limits.

While only two-way comparisons give 'definitive' answers,
unidirectional data can be used to construct hypotheses and give
indications as to the likelihood of the correctness of other
proposals. In this context it is possible to use the present data
set to look at various hypotheses, suggestions, etc. In this
regard the IEP work of Hutchinson (1981; 1983) on the Australian
Scincidae can be incorporated to extend these findings.

5.06.02: Genetic diversity identified within *Lampropholis* *(sensu*
         Greer, 1974)

Cladograms generated by both the distance Wagner method (Farris,
1972) and Fitch and Margoliash (1967) present the same trends
with the data apparently confirming the electrophoretic evidence
of high level genetic diversity. Distances within groups (Sydney
L. challengeri to *L. basiliscus* ID=20: *L. guichenoti* to *L. sp.3*

ID=23) being as great as between groups (IDs 13-26; Table 5.2).

However, an examination of Figures 5.1. and 5.2 indicate apparently different rates of evolution among the various species. To further investigate this anomaly it is desirable to introduce an outgroup. To do this it is necessary to find a species that is phylogenetically outside of the group being investigated. If rates of evolution have been equal, then all species will be equidistant from the outgroup. Where slow rates of evolution have occurred, a taxon will appear to be more closely related to the outgroup than other members of the group, the converse will occur if rates of evolution have been unusually fast.

As discussed previously (Sect. 2.08.04) the outgroup must to be clearly outside the group under investigation but also needs to be close enough that mutation rates are similar and therefore evolutionary pathways to all species within the group are minimized. In the first instance (*Le. grande*), a representative of the **Eugongylus** subgroup (*sensu* Rounsevell, 1986) was utilized since this species fulfilled the criteria of being the most closely related group (i.e. within the **Eugongylus** group) while being clearly distinct from the **Pseudemoia** subgroup (Table 5.3).

Therefore by incorporating *Le. grande* as an outgroup, it is possible to investigate apparent differential rates of evolution (Fig. 5.1; 5.2). This approach confirmed that the albumin of

Lampropholis (sensu Greer, 1974) species demonstrate unequal rates of evolution. *L. basiliscus* has evolved at a substantially slower rate than the other species investigated (ID=29), while Sydney *L. challengeri* stands at the greatest distance (ID=55), therefore indicating relatively fast rates of mutation. *L. guichenoti* (ID=46) and *L. sp.3* (ID=40) are intermediate.

However, before these observations may be confirmed it is necessary to conclude that *Le. grande* is an appropriate outgroup. If this species is too distant from *Lampropholis (sensu* Greer, 1974), the outgroup may be contributing erroneous results. It is possible to determine the correctness of these results by investigating alternative outgroups. In an attempt to identify alternative outgroups, two things become apparent: firstly, none of the species within the **Pseudemoia** subgroup is clearly genetically outside of the genus *Lampropholis* with the exception of *Menetia greyii* (IDs 32-73) and therefore all are inappropriate choices as outgroups (subgroup, with the exception of *Menetia* IDs 15-39; genus IDs ≤38). This then leaves the alternative of investigating other representatives of the **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986), all of which are at least as distantly related to the genus *Lampropholis* as is *Le. grande* (*Le. grande* IDs 29-55; **Eugongylus** subgroup IDs 28-82). However, by examining other **Eugongylus** subgroup associations with the *Lampropholis* taxa, it is apparent that in all seven cases where **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986) species are

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cross-reacted with Sydney *L. challengeri*, IDs are larger (IDs 55-82) than in instances of cross-reactivity with other *Lampropholis* (IDs 28-67). In two of the three cases of cross-reactivity between *L. basiliscus* and **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986) taxa, IDs are comparatively low (IDs 29-32), the exception (i.e. the distance between *L. basiliscus* and *Le. otagense* (ID 33) is also at the lower end of the scale (*Lampropholis* to *Le. otagense* IDs 28-59) and therefore does not present a contradiction (see Table 5.3).

All information indicates therefore, that Sydney *L. challengeri* albumin has evolved at a relatively fast rate while that of *L. basiliscus* has evolved at a very much slower rate. *Lampropholis guichenoti* and *L. sp.3* are generally intermediate between the two. Therefore the apparent variation identified by using *Le. grande* as an outgroup is supported by all available evidence (Table 5.3). Any interpretation of the data should therefore be approached with this problem in mind.

Despite these difficulties, the data does support the electrophoretic hypothesis that a substantial level of genetic variation has occurred within the group and distances within and between *Lampropholis* complexes (*sensu* Greer, 1980) are in many instances equivalent (Table 5.3).

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Hutchinson (1983) concluded on the basis of IEP comparisons of four *Lampropholis* *(sensu* Greer, 1974) species, *L. challengeri*, *L. mustelina* *(L. challengeri* complex, *sensu* Greer, 1980), *L. delicata* and *L. guichenoti* (*L. delicata* complex, *sensu* Greer, 1980) that the two complexes represented separate genera. The present study appears to be in conflict with this conclusion. However, when only these four species from *Lampropholis* are considered, it is possible to come to this erroneous conclusion. The present, more in-depth study of this group, identifies all four of these species as belonging to separate genera (see Section 3.05.03). Sydney *L. challengeri* and *L. mustelina* are distantly related both to each other and to the rest of the genus *Lampropholis* *(sensu* Greer, 1974). The other taxa are more related to each other, although still quite separate. Without this prior knowledge it is not surprising that these conclusions were drawn on the basis of the species investigated and that the previous study was undertaken from the perspective of **Sphenomorphus**. The latter being a group that Hutchinson (1983) considered to be so distantly related from the rest of the Australian lygosomes that he considered it a separate subfamily.

5.06.03 *Leiolopisma* as a monophyletic group.

The genus *Leiolopisma* has been treated as a 'catch-all' group, with species amalgamated on the basis of inadequate character analyses (see Sect. 1.05 for discussion). Despite some species

being removed from the genus to form the genus *Lampropholis* by Greer (1974), those remaining are generally conceived as not being a monophyletic group due to its diagnosis based on plesiomorphic characters.

Despite its poor description, the genus is considered important since it is the typical genus of the **Eugongylus** group (Greer 1974). Past suggestions for splitting the group have been on the basis of small-scaled versus large-scaled individuals (Table 5.4) and, on the basis of immunoelectrophoretic data, viviparous species were separated from the oviparous, elongate group (Table 5.4). The present data set tends to support the latter hypothesis since IDs from various *Lampropholis (sensu* Greer, 1974) species to the small scaled species, *Le. palfreymani* (IDs 15-24) tend to be equidistant to those of *Lampropholis (sensu* Greer, 1974) to large scaled species, *Le. entrecasteauxii* (IDs 19-39), *Le. metallicum* (IDs 16-24) and *Le. zia* (IDs 16-29) whereas the large scaled *Le. deperreyi* is substantially outside this range (IDs 53-82; see Table 5.4). Separation on the basis of reproductive mode, however, implies more discrete clustering, i.e. the range of viviparous species IDs from the various *Lampropholis (sensu* Greer, 1974) species is between 11-39, while equivalent IDs for oviparous species was observed to be in the range of 28-82 (Table 5.4). Therefore oviparous species tend to be of similar distance from *Lampropholis (sensu* Greer, 1974) as are the **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986) taxa generally

Table 5.4: Hypothesized groupings of the genus *Leiolopisma*
(after Rawlinson, 1974a; 1975; Greer, 1982)

Le. baudini group 20-32 midbody scales (Large scaled)		*Le. spenceri* group 38-66 midbody scales (Small scaled)	
Le. baudini		*Le. greeni*	
Le. coventryi		*Le. ocellatum*	
Le. entrecasteauxii	(IDs 19-39)	*Le. palfreymani*	(IDs 15-25)
Le. deperreyi	(IDs 53-82)	*Le. pretiosum*	
Le. metallicum	(IDs 16-24)	*Le. spenceri*	
Le. platynotum			
Le. trilineatum			
Le. zia	(IDs 16-29)		

(after Rawlinson, 1974a; 1975; Greer, 1982)

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viviparous species		oviparous species	
Le. coventryi		*Le. duperreyi*	(IDs 53-82)
Le. entrecasteauxii	(IDs 11-39)	*Le. grande*	(IDs 29-55)
Le. greeni		*Le. otagense*	(IDs 28-59)
Le. jigurri	(ID =18)	*Le. platynotum*	
Le. metallicum	(IDs 16-24)	*Le. trilineatum*	
Le. ocellatum			
Le. paulfreymani	(IDs 15-24)		
Le. pretiosum			
Le. spenceri			
Le. zia	(IDs 16-29)		

(after Greer, 1982; Donnellan, pers. comm.)

(oviparous IDs 28-82; other **Eugongylus** subgroup IDs 32-71; Table 5.4). The viviparous group had IDs similar to those of **Pseudemoia** subgroup species (generally in the region of 20 IDs; see Fig. 5.4). Although overlap in IDs occur (i.e. viviparous group 15-39; oviparous group 29-82), in all instances when comparing a single antibody, the groups were separate (see Table 5.4). There are therefore grounds for hypothesizing that these two groups are separate monophyletic groups and that the small scaled versus large scaled dichotomy do not form monophyletic groups.

5.06.04: Alpha/beta palate dichotomy as a basis for groups.

As discussed in Section 1.05, Greer (1979) in his review of the Australian Lygosominae separated the **Eugongylus** radiation into two groups based on the shape of the palatal rami. In the more primitive group (the alpha condition) the palatal rami diverged smoothly along the medial edges. In the other group the palatal rami each had a deep posterior emargination giving it a hooked appearance (beta palate). This beta condition has apparently arisen more than once in the Scincidae since Greer (1979) pointed out that a group of African skinks have the same palatal configuration as do some species in the genus *Emoia*. No identified reason exists to believe that these taxa have a close relationship to **Lampropholis** subgroup species.

Table 5.5: Characters utilized by Greer to establish the phylogeny of the **Eugongylus** group (after Greer, 1974; 1980a; Hutchinson, 1983)

Plesiomorphic state   Apomorphic state

Alpha palate   Beta palate

Eyelids scaly, movable   Transparent disc present
                                        Eyelid immovable

Supranasal scales present   Supranasal scales absent

Frontoparietal scales paired   Frontoparietals fused
Interparietal scale distinct   Interparietal distinct

Prefrontal scales present   Prefrontals scales absent

Five toes on front limb   Four toes on front limb

Males without orange to red   Males without such colour
gular, ventral or lateral
pigmentation

Hutchinson (1983) suggested that the beta condition was easily derived from the alpha condition and recorded that in a series of skulls of *Le. entrecasteauxii* that he examined, variation between the two conditions existed, indicating that this character state could be variable within a single species. He concluded, on this basis, that its use as a sole indicator of monophyly was questionable.

Of the remaining characters used by Greer (1979), Hutchinson (1983) claimed all were "manifestly prone to parallel evolution" and therefore could not produce clear-cut relationships. Of these six characters (Table 5.5), three have been observed to be variable with the genus *Lampropholis* (*sensu* Greer, 1974); the condition of the parietal scales (Sect. 4.04.02), colouration (also discussed in Sect. 4.04.02) and variation in toe number (see Sect. 0.03.13). The remaining characters were not examined.

Hutchinson (1983) found that the alpha-beta palate dichotomy was not supported by his IEP data with the two **Eugongylus** group taxa being more closely related to each other than either were to *Emoia*. He further concluded that the *L. challengeri* complex was not the *L. delicata* complex (*sensu* Greer, 1974) species' closest relative and that the two groups represented a parallel evolution of the beta palate condition, although this latter conclusion is questioned by the more thorough analysis of *Lampropholis* (*sensu* Greer, 1974) relationships in this study (see Section 5.06.02).

The present study also does not support the alpha/beta palate dichotomy. As previously noted, *Leiolopisma* apparently represents at least two separate groups, one with IDs indicating it may be within the Pseudemoia subgroup (IDs 15-39), while the other grouping has IDs that would more suitably fit with the **Eugongylus** subgroup (*sensu* Greer, 1974; IDs 28-82) with no overlap occurring between IDs for a specific antibody (see Table 5.3). Therefore on the basis of the limited MC'F data and Hutchinson's (1983) IEP analysis, it is hypothesized that the alpha/beta dichotomy does not represent monophyletic groupings.

5.06.05: Fusion of the atlantal intercentrum to the atlantal arches as an indication of groups within the **Eugongylus** group

In a recent manuscript Greer and Rounsevell (1986) identified a character state which is unique among the Scincidae to some **Eugongylus** group taxa; the fusion of the atlantal intercentrum to the two atlantal arches in adults. On the basis of this synapomorphy he suggested an alternative grouping of these taxa (see Table 5.06). He thus re-affirmed his placement of *Menetia* in a grouping with *Carlia* and *Lampropholis* (*sensu* Greer, 1974) but expanded the group to include some *Leiolopisma* species as well as other genera (see Sect. 1.05 for discussion). This new classification was simply a reorganization of the subgroups within the **Eugongylus** group. The name **Eugongylus** was retained to

Table 5.6: Alternative arrangements of the *Eugongylus* group and comparison of immunological distances between hypotheses.

Alpha/beta dichotomy (after Greer, 1979)

Lampropholis subgroup   *Eugongylus* subgroup

Carlia	(IDs 17-34)	*Cryptoblepharus*	
Geomyersia		*Emoia*	(IDs 57-71)
Lamrpopholis	(IDs ≤38)	*Eugongylus*	(IDs 59-65)
Menetia	(IDs 32-73)	*Leiolopisma* #	(IDs 15-82)
? *Copholsciniopus*		*Morethia*	(IDs 32-72)
? *Panaspis*		*Phoboscincus*	
? *Ristella*		*Proablepharus*	
		Pseudemoia	
		Sphenomorphus #	
		Tachygyia	

Fusion/non-fusion of atlantal bones (after Greer & Rounsevell, 1986)

Pseudemoia subgroup   *Eugongylus* subgroup

Carlia	(IDs 17-34)	*Eugongylus*	(IDs 59-65)
Cryptoblepharus		*Emoia*	(IDs 57-71)
Geomyersia		*Leiolopisma*	(IDs 28-82)
Lampropholis	(IDS ≤38)	*Morethia*	(IDS 32-72)
Leiolopisma #	(IDs 15-39)	*Problepharus*	
Menetia	(IDS 32-73)		
Nannoscincus			
Pseudemoia			
Sphenomorphus #			

#Segment of genus only included, see taxt for details
?Inclusion questioned in original paper

refer to the previous subgroup, although now reduced in the number of genera represented, while **Pseudemoia** was used to describe the now expanded **Lampropholis** subgroup (Table 5.6).

The taxa investigated within the **Pseudemoia** subgroup (*Lampropholis, sensu* Greer, 1974; *Carlia*, one *Leiolopisma* complex & *Menetia*) all had IDs ≤38 with the exception of *Menetia* (IDs 32-73) which always had substantially higher IDs than those of other species to a specific antibody (Table 5.6). The **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986) had IDs in the range of 28 - 82 from *Lampropholis (sensu* Greer, 1974) species. The data therefore appears to fit better with the reformed groupings within the Eugongylus group (i.e. the **Pseudemoia/Eugongylus** dichotomy, *sensu* Greer & Rounsevell, 1986) than it does with that of the previously hypothesized alpha/beta palate dichotomy (i.e. **Lampropholis/Eugongylus** subgroupings, *sensu* Greer, 1974). It is therefore hypothesized that the fusion of the atlantal bones represents a genetically cohesive group. Once again, however, *Menetia* does not clearly fit in with its grouping (Table 5.6).

5.06.06: Placement of *Menetia* within the Eugongylus group.

Two alternative explanations are possible to account for the aberrant *Menetia*. The albumin of this taxon (or genus) may be evolving rapidly, as has been identified in Sydney *L. challengeri*. Alternatively *Menetia* may be genetically outside the

group, indicating that the atlantal fusion in this group represents parallel evolution. Since the fusion is unique to the **Pseudemoia** subgroup while albumin, at least in the instance of Sydney *L. challengeri*, has been demonstrated to evolve rapidly, it is more reasonable to assume that this is causing a distortion of the data.

The work of Hutchinson (1983) does not help to resolve the problem. As discussed previously, he grouped *Menetia* (also represented by *Menetia greyii*) with *Carlia* and the <u>*L.* *delicata*</u> complex (*sensu* Greer, 1980; represented by *L. delicata* and *L. guichenoti*). Neither study incorporates enough data to resolve the anomaly. To overcome these inconsistencies requires further investigation. To do this it would be necessary to raise antibodies to *M. greyii*, together with at least one other species in the genus.

5.06.07: Evidence for monophyletic groups within Lygosominae

As discussed in Section 1.02, Greer (1979) reviewed the Australian Scincidae and concluded that there were three monophyletic groups; **Egernia, Sphenomorphus** and **Eugongylus** (see Table 1.1). After formal subdivision of the Lygosominae into three subfamilies, he amalgamated various genera into an informal grouping on the basis of their common origin from *Mabuya* (Greer, 1977; 1979). This assemblage was later referred to as the **Mabuya**

group (Hutchinson, 1981). On the basis of IEP comparisons, Hutchinson (1983) used *Mabuya* and *Lamprolepis* as representatives of **Mabuya** and concluded that the group was more closely related to **Eugongylus** than to the **Egernia** but all were equidistant from **Sphenomorphus**. Based on this work he merged the **Mabuya** and **Eugongylus** groups into a formal taxonomic unit, the tribe Lygosomini.

Greer (1979) had concluded that the genus *Mabuya* was the most primitive extant ancestor of the Australian Lygosominae. The work of Hutchinson (1983) showed, however, that this genus was most closely aligned to **Eugongylus** while the differences between **Sphenomorphus** and other Lygosominae were sufficient to consider **Sphenomorphus** as a separate subfamily.

The MC'F data indicates that both **Egernia** and **Sphenomorphus** are equidistant from *Lampropholis (sensu* Greer, 1974; IDs 90-137) and therefore implies equidistant from **Eugongylus** (**Egernia** IDs 90-137; **Sphenomorphus** IDs 113-136), the *Mabuya* genus, however, is less distantly related to *Lampropholis (sensu* Greer, 1974; i.e. ID 65 units from *L. guichenoti)* than are **Sphenomorphus** and **Egernia**. Therefore if **Sphenomorphus** is considered to be sufficiently distinct to be of subfamilial status it is appropriate to hypothesize that the **Egernia** and **Eugongylus** groups are also worthy of subfamilial status.

Greer (1979) did not place the genus *Tribolonotus* in any of his suprageneric groups but implied it belonged to either the **Sphenomorphus** or **Egernia** groups, although no indication was given for his reasoning. Hutchinson (1983) examined *Tribolonotus schmidti* on the basis of the ten characters which Greer (1979) had investigated and concluded that it was more closely aligned to the **Eugongylus** group than to either **Sphenomorphus** or **Egernia**. Hutchinson's (1981; 1983) IEP evidence placed the genus closest to, but distinct from, this **Eugongylus** taxon, however, he considered that it was the only Lygosominae with membership that was indeterminate among the four groups.

The MC'F distances between *Lampropholis* *(sensu* Greer, 1974; **Eugongylus** group) and *Tribolonotus* are substantial. Immunological distances obtained were both approximate, and nearing the limits of resolution for the technique. Despite the limitations of the data *Tribolonotus* is clearly not genetically close to **Eugongylus** (i.e. IDs from *Lampropholis* *(sensu* Greer, 1974 to **Eugongylus** IDs<82; *Tribolonotus* IDs 125-160; Table 5.3). It is therefore hypothesized that this genus does not have a close association with any of the Australian Lygosominae.

The IDs from *Lampropholis* *(sensu* Greer, 1974) to two genera of the **Mabuya** group were calculated; *Mabuya* 65 units, *Lamprolepis* 133-139 units. The magnitude of these differences is such that it is unlikely that they are part of a single monophyletic group. *Mabuya* is equidistant from *Lampropholis* *(sensu* Greer, 1974) as

are members of the **Eugongylus** subgroup (*sensu* Greer &
Rounsevell, 1986) while *Lamprolepis* is equidistant to the **Egernia**
and **Sphenomorphus** groups from *Lampropholis* (*sensu* Greer, 1974;
Table 5.3).

It would therefore appear that the **Mabuya** group is, as Hutchinson
(1983) suggested, an assemblage of diverse forms and not a
cohesive group as his IEP results may have later implied.

Hutchinson (1983) in a discussion on the origin and relationships
of Australian Lygosominae comments on the "relative similarity"
of some Papuan and Indonesian genera with Australian taxa of the
Eugongylus group and uses *Lamprolepis* and *Tribolonotus* as
examples. In the light of the current study these relationships,
based on morphological similarity, appear quite erroneous.
Immunological distances to
a representative of each of these genera from *Lampropholis* (*sensu*
Greer, 1974; **Eugongylus** group) are at least 125 units, whereas
all **Eugongylus** taxa were <82 units. These groups, therefore, are
apparently genetically quite dissimilar and their "relative
similarity" is probably based on convergence in morphological
evolution.

Other species from New Guinea that are presently placed in the
Eugongylus group (i.e. *Emoia* IDs 66-71 and *Eugongylus* IDs 59-65)
were observed to have IDs that were equidistant from those of

Lampropholis (*sensu* Greer, 1974) as were Australian representatives of the group (IDs 28 - 82; Table 5.3), implying that these taxa may represent part of the same radiation as members of the Australian **Eugongylus** group. New Zealand members (i.e. *Le. grande* IDs 29-55; *Le. otagense* IDs 28-59) also conform to this pattern (see Table 5.3).

The current study, therefore, does not contradict the ideas of Greer (1979) that three monophyletic groups exist within the Australian Lygosominae and the **Mabuya** group is a loose assemblage of taxa. If, however, as Hutchinson (1983) suggested **Sphenomorphus** is worthy of subfamilial status, **Egernia** and **Eugongylus** are also. On the basis of immunological distances, the genus *Mabuya* can not constitute the most primitive extant relative of the Australian radiation, it may however, be part of the **Eugongylus** radiation, as are the New Zealand *Leiolopisma* and the New Guinea genera *Emoia* and *Eugongylus* although *Lamprolepis* and *Tribolonotus* are not.

5.07: CONCLUSIONS DRAWN THE MICRO-COMPLEMENT FIXATION DATA

As pointed out at the beginning of this discussion, unidirectional data does not supply 'definitive' answers and therefore is only useful as a basis upon which to hypothesize relationships among taxa, despite the demonstrated low standard deviation of reciprocity identified within *Lampropholis* (*sensu*

Greer, 1974) taxa tested by two-way analysis. However, despite limitations, the combination of molecular data (i.e. that of the present study, together with that of Hutchinson (1981; 1983) has made it possible to hypothesize the following relationships among Australian Lygosomes:

(1) Genetic diversity among the Australian Scincidae taxa is substantial and of an order not generally contemplated previously. This has been widely demonstrated in this study by immunological distances identified among *Lampropholis (sensu* Greer, 1974) species and their relatives and confirmed by the reciprocally analyzed data of the *Lampropholis (sensu* Greer, 1974) genus.

(2) Genetic relationships based on the fusion of the atlantal bones is a more appropriate grouping than any based on an alpha/beta palate dichotomy.

(3) The genus *Leiolopisma* consists of at least two taxa, one apparently more closely aligned with the **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986) while the other group appears closer to the Pseudemoia.

(4) Three monophyletic groups exist within the Australian Lygosominae, however, the genus *Mabuya* is not the most closely

related extant relative to this group but has genetic affinities with the **Eugongylus** group.

(5) The **Mabuya** group is a loose assemblage of genera without close genetic relationships.

(6) New Zealand *Leiolopisma* is part of the Australian **Eugongylus** subgroup, as are the New Guinea genera *Emoia* and *Eugongylus*, however *Lamprolepis* and *Tribolonotus* are not closely related to the Australian **Eugongylus**.

(7) If **Sphenomorphus** is considered of subfamilial status **Egernia** and **Eugongylus** are also worthy of subfamily status.

On the basis of this work, there is clearly a need to further investigate the genetic relationships of the family Scincidae so that convergent evolutionary events and parallel evolution can be isolated and true genetic relationships identified.

CHAPTER 6: EVOLUTIONARY RELATIONSHIPS OF *LAMPROPHOLIS* SCINCID LIZARDS AND THEIR RELATIVES: CONCLUDING REMARKS.

6.01: ORIGINS OF THE AUSTRALIAN SCINCIDAE

As discussed at the beginning of this thesis, Australia has an extremely diverse reptile fauna, dominated by scincid lizards. The large amount of diversity, endemism and the degree of adaptive radiation encountered among the group, together with the lack of evidence for a gradual transition from an Oriental to an Australian fauna, is incongruous with the widely held view of quite recent, multiple, northern invasions for the subfamily.

Generally ideas of relationships have been based on morphological data and are, in part, reliant on the view that Australian radiations are derived from primitive South-East Asian stock. Gondwana origins have generally not been seriously considered for the scincid fauna, although there seems to be no plausible argument to substantiate apparently older invasions except for 'over water' dispersal.

6.02: LIMITATIONS OF MOLECULAR TAXONOMY

The introduction of molecular data allows for a largely independent testing of ideas on relationships, and although, as repeatedly spelt out in this volume, there is a necessity to

approach with caution any extrapolation of the molecular data to the time of origin of such radiations, this information does provide better clues than are presently available from morphological criteria alone.

6.03: CORRELATION OF MORPHOLOGICAL AND MOLECULAR DATA

A major revelation of the molecular information presented in this thesis is the identification of a significant degree of genetic diversity at all levels investigated, despite a large amount of morphological overlap.

Morphological overlap is such among *Lampropholis (sensu,* Greer, 1974) that the use of criteria commonly employed for species identification, is of no value in separating species generally. Conversely allozyme electrophoresis, not only distinguished between recognized species but allowed for the identification of a number of additional taxa. That evolution of proteins may proceed at a relatively constant rate while phenotypes are frequently heavily influenced by environmental factors or adaptive shifts is well documented (e.g. primates, King & Wilson, 1975; snakes, Mao & Dessauer, 1971; fish, Avise *et al,* 1975; Turner, 1974; Wallace *et al,* 1973; amphibians, Maxson & Wilson, 1975). Thus while this variation may be the result of differing rates of protein evolution, this is not considered to be greatly distorting the data in this study. Convergent

evolution is therefore seen to be a major factor in the observed
overlap among species.

6.04: RATES OF MOLECULAR EVOLUTION

Information obtained from electrophoretic criteria indicated that
rates of evolution, in some instances, are very slow. As
discussed Section 3.05.04, *L. caligula* and *L. amicula* were
demonstrated to be genetically close, despite all available
evidence indicating that they have been separated for a
substantial period, thereby indicating a slow rate of protein
evolution generally.

Albumin evolution, however, does not always imply this trend.
Immunological distances to *L. caligula* and *L. amicula* from all
four *Lampropholis* (*sensu* Greer, 1974) antibodies tested imply
substantial separation (*L. caligula* IDs 12-22; *L. amicula* IDs 20-
38; Table 5.3). This constitutes additional confirmation of the
distinctiveness of these species (i.e. *L. caligula* and *L.
amicula*) and thus reinforces the observation that allozyme
evolution has occurred at a relatively slow rate in this group.

Differential rates of albumin evolution have been identified
among *Lampropholis* (*sensu* Greer, 1974); Sydney *L. challengeri*
demonstrates relatively fast rates while *L. basiliscus* has a much
slower rate, *L. guichenoti* and *L. sp.3* are intermediate between

the two. If this was a reflection of protein mutation in general, the situation would be reflected in allozyme mutation rates. Since the Fitch and Margoliash (1967) algorithm produces a phylogram with minimum distortion of the data (and branch lengths are drawn to scale), all taxa with the same mutation rates would have equal branch lengths in Figure 6.1. Although mutation rates have varied, those of the four taxa used as representatives of the group in immunological experiments were all intermediate (* marks taxa in Figure 6.1).

This revelation, together with the observation that trends in relationships from the various *Lampropholis (sensu* Greer, 1974) species to a specific taxon are similar, support the view that the data sets provide a true reflection of relationships between taxa despite some variation.

All evidence therefore points to the conclusion that reproductive isolation is well advanced. The reason for differential rates in protein evolution among taxa, however, is not clear. It could be argued that species with a restricted distribution may be more genetically homogeneous than those with a widespread distribution due to genetic interbreeding. There are no indications that such mechanisms are affecting the rates of allozyme evolution in *Lampropholis (sensu* Greer, 1974). For example *L. mustelina* and *L. czechurai* have similar rates of protein mutation (Fig 6.1) but *L. mustelina* has a widespread distribution while *L. czechurai* has a

substantially more restricted range (see Preface for

distributions). Moreover no indication of differences on the

basis of climatic conditions is evident. *Lampropholis caligula*,

L. tetradactyla and *L. amicula* all have restricted distributions

in very different geographical areas but all have a relatively

slow rate of protein evolution. Conversely *L. mirabilis*, which

also has a restricted distribution within the geographical range

intermediate between those of *L. tetradactyla* and *L. amicula* (see

Preface for distributions) has a relatively fast rate of allozyme

evolution. In addition this protein variation was not found to be

consistent within lineages since variable rates were observed

within *L. challengeri* taxa (see Fig 6.1).

6.05: EVIDENCE FOR SPECIATION WITHIN *LAMPROPHOLIS (SENSU* GREER,

    1974)

The identification of differential rates of evolution, together

with the lack of a mechanism influencing this variation (other

than homoplasy), makes it desirable to reassess taxonomic

decisions based on allozyme data alone. This is particularly

relevant to the *L. challengeri* group due to differential rates of

allozyme evolution within this taxon and the level of diversity

observed. Under these circumstances, and in the absence (at this

point) of substantiating evidence, it is necessary to approach

conclusions drawn about this group with caution. However, despite

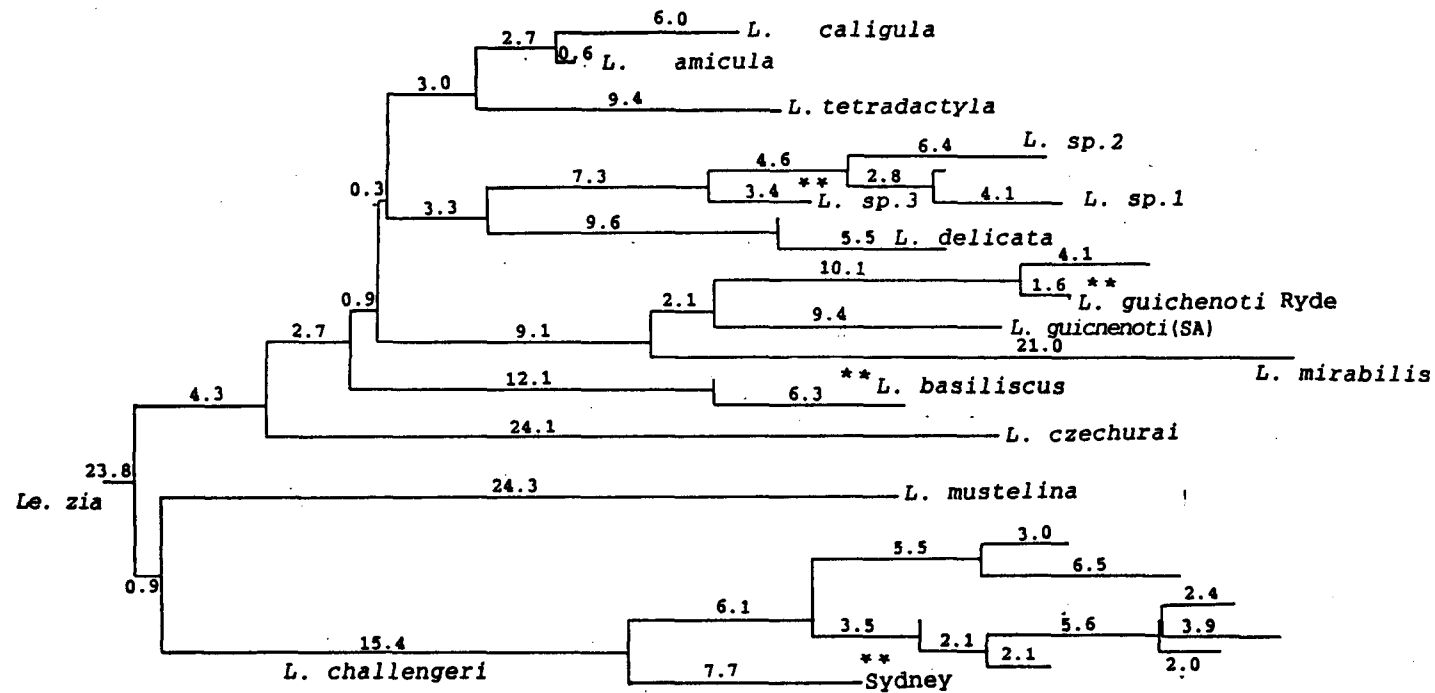this, it is considered that *L. challengeri* is a species complex

Figure 6.1: Comparison of allozyme and albumin evolution (redrawn
from phylogram based on allozyme data appearing in Fig. 3.6; **
indicates taxa utilized in immunological study.

with at least three genetically distinct groups. The separation of two of these was confirmed by the immunological data (Sydney taxon - Mt. Nebo population ID=6). Despite the recognized accelerated rates of albumin evolution in this taxon, this is considered to represent real differences since detection of immunological variability between species is generally not identifiable until substantial electrophoretic evolution has occurred and may not be observable for upwards of one million years of reproductive isolation (Sect. 2.12). It is therefore concluded that extensive speciation has occurred in this group and that the electrophoretic evidence is a valid basis upon which to hypothesize that *L. challengeri* represents a relatively large species complex centred around three regional groups.

If reluctance is expressed in accepting this electrophoretic evidence for speciation in *L. challengeri*, the evidence of slow evolutionary rates of albumin in *L. basiliscus* needs to be addressed. In the original description, Ingram & Rawlinson (1981) suggested that this species may represent two taxa on the basis of morphological differences. These differences were largely based on the character states investigated in this study. This work also suggests that variation has occurred between these two populations, however, in the context of the identified overlap among species within *Lampropholis (sensu* Greer, 1974) generally (Fig 4.3), this observation is considered meaningless. Electrophoretic analysis identified 6% fixed gene differences

(Table 3.6), as opposed to the 10% level used in this study as an indication of speciation (see Sect. 3.05.04 for justification) thus the level of genetic diversity, together with present morphological knowledge, are not considered sufficient to recognize *L. basiliscus* as two species.

On the basis of all evidence collected, i.e. electrophoretic, immunological and morphological, there is no reason to modify the conclusions drawn on the species relationships suggested from electrophoretic evidence alone.

6.06: THE GENERIC BOUNDARY OF *LAMPROPHOLIS* *(SENSU* GREER, 1974)

Immunological distances determined among *Lampropholis* *(sensu* Greer, 1974) species and between *Lampropholis* and its relatives, indicate a substantial level of divergence. Genetically there is no evidence for retaining the genus *Lampropholis* in its present context since genetic variation within the genus was frequently observed to be higher than between *Lampropholis* and its relatives. Two alternative approaches are available for reassessment of the genus. One alternative is to amalgamate all taxa of the Pseudemoia subgroup into a single genus. This would necessarily result in a large, genetically diverse and unwieldy conglomeration of species and is not seen as an appropriate approach on genetic criteria. The alternative, more appropriate, approach requires a reassessment of the Pseudemoia subgroup as a

whole. The present study has identified four groupings within *Lampropholis* which are more genetically diverse than *Le. zia*. The immunological data, while not able to directly confirm this evidence, gives no indication that *Le. zia* is unique in its relationship to *Lampropholis* (*sensu* Greer, 1974; excluding *Menetia*, Pseudemoia subgroup IDs <40; *Le. zia* IDs 16-29; the Pseudemoia *Leiolopisma* group IDs 15-39).

6.07: RELATIONSHIPS OF *LAMPROPHOLIS* (*SENSU* GREER, 1974) TO THE EUGONGYLUS GROUP

Fusion of the atlantal bones appears to be an acceptable synapomorphic character state on which to base a monophyletic grouping (i.e. Pseudemoia *sensu* Greer & Rounsevell, 1986). The osteological structure of the palate, however, is not. With the exception of *Menetia greyii*, all taxa investigated in the Pseudemoia subgroup have IDs which are approximately equidistant from *Lampropholis* (*sensu* Greer, 1974; IDs <40).

The acceptance of Pseudemoia as a discrete grouping, requires a dichotomy of the genus *Leiolopisma*. Micro-complement fixation data supports the molecular work of Hutchinson (1983) in hypothesizing that the most appropriate dichotomy of *Leiolopisma* is based on reproductive mode, viviparous species are therefore retained in the Pseudemoia subgroup while the oviparous species

are apparently aligned with the **Eugongylus** subgroup (*sensu* Greer, 1986; Table 5.4).

Reassessment of the group as a whole to determine more appropriate generic groupings would necessarily require reciprocal data with specific emphasis on an investigation of *Menetia* to determine if it should be included in the group.

6.08: RELATIONSHIPS OF *LAMPROPHOLIS (SENSU* GREER, 1986) TO THE LYGOSOMINAE

With the exception of *Menetia*, indications are that the **Pseudemoia** subgroup is a separate radiation within the **Eugongylus** group and there is no apparent contradiction to the **Eugongylus** subgroup (*sensu* Greer, 1986) also being a separate radiation within this group. **Sphenomorphus** and **Egernia** groups are less closely related to the genus *Lampropholis (sensu* Greer, 1974) than is any taxon within the **Eugongylus** group. Therefore the immunological evidence does not contradict the proposals of three monophyletic groups within the Australian subfamily, Lygosominae. One of these, the **Eugongylus** group, represents two separate monophyletic radiations. However, if as has been previously proposed (see Sect. 5.06.07), **Sphenomorphus** is worthy of subfamilial status, combined evidence derived from this work and that of Hutchinson (1983) indicate that **Egernia** and **Eugongylus** are also worthy of this treatment since **Egernia** is equidistant

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from *Lampropholis* (**Eugongylus** group) and Hutchinson (1983) has shown that the two groups were clearly distinct from each other. A reciprocal treatment of the data would be necessary to confirm such an hypothesis.

One area of conflict identified between this work and most other proposals of relationships among Australian scincids is that of the South-East Asian genus, *Mabuya*. This genus has frequently been accepted as the most primitive extant relative of the Australian radiation (Greer, 1974). Immunological evidence, however, indicates that this can not be the case. No scenario is apparent that would enable a taxon (i.e. genus *Mabuya*), observed to be equidistant from *Lampropholis* (*sensu* Greer, 1974) as is the **Eugongylus** subgroup, to also be the ancestor of the whole radiation.

Other relationships assumed on the basis of morphological similarity were also found to be erroneous. Both *Tribolonotus* and *Lamprolepis* have been considered to be **Eugongylus** group taxa, however, the magnitude of the immunological differences (**Eugongylus** IDs ≤38; *Tribolonotus* IDs ≥125; *Lamprolepis* IDs ≥133) indicate that the relationships previously assumed are based on evolutionary convergence and do not have a genetic basis.

6.09: THE AGE OF THE AUSTRALIAN SCINCID RADIATION

In summary, past ideas of relationships have generally been based on the view that the Australian scincid radiation is more recent than the molecular data indicates and, although, in general, relationships among groups apparently have a firm genetic basis, conservatism of morphological form has resulted in some erroneous conclusions. In the light of this new information it is useful to reassess the possible origins of the Australian radiation.

As discussed in Section 2.13, the molecular clock is highly controversial, partly due to the lack of fossil data to calibrate it for most groups and differential rates of albumin evolution. The former problem is, at present, unsolveable since there is only a single appropriate Australian fossil, representing *Egernia* from mid-Miocene deposits (≈16 mya; Hutchinson, 1983). However, correlation of the data with other appropriate groups will be discussed in Section 6.10. The problem of differential rates of albumin evolution has been addressed (Sect 2.14) and the conclusions to be drawn here are not considered to be distorted by the problem.

The radiation of the **Pseudemoia** subgroup has occurred within the last 15 my, while taxa within the **Eugongylus** group, including the New Zealand species of *Leiolopisma* (*Le. grande* & *Le. otagense*) and the New Guinea genera, *Emoia* and *Eugongylus*, have separated

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from *Lampropholis* (*sensu* Greer, 1974) within the last 26 my. The

genus *Mabuya* also fits into this category. **Sphenomorphus** and

Egernia together with the genera *Tribolonotus* and *Lamprolepis*

(New Guinea genera) have been separated from *Lampropholis* (*sensu*

Greer, 1974) for at least 50 million years.

On this basis, using even the most conservative scenario

possible, it is unrealistic to advocate a recent radiation of the

Australian Lygosominae. As stated at the outset, the level of

diversity is difficult to explain as a recent radiation, while

many of the species are represented by typically relic

distributions. The evidence for this restriction of taxa to

remnant pockets of vegetation is widely evident within

Lampropholis (*sensu* Greer, 1974), many species tend to be

confined to upland habitats. Where these habitats are fragmented,

speciation is apparently occurring, in contrast to the generally

more homogeneous widespread species (e.g. *L. mustelina*). The

limited genetic divergence identified between *L. basiliscus* taxa

from two adjacent rainforest blocks in Northern Queensland may be

a direct reflection of their isolation in the recent past.

Likewise genetic diversity identified in the *L. challengeri* taxon

is consistent with the concept of gradual isolation over

geological time, with initial isolation of the ancestor into

three separate regions and progressive isolation occurring as

Eastern Australia became drier. The dichotomy in *L. guichenoti*

may also be viewed as a reflection of a fragment of the range

(i.e. South Australian population) becoming isolated by intervening inhospitable habitat and consequently the taxon has diverged. The relatively older dichotomy of *L. guichenoti* and *L. mirabilis* and the fragmentation of species associated with *L. delicata* taxon presumably represent earlier isolating events.

Mather (1986), without the benefit of immunological evidence, considered that species restricted to the synonymy of *L. delicata* (including *cf. L. delicata, L. sp1, L. sp2, L. sp.3*) had evolved within the last million years (most of his discussion is in terms of thousands of years). In contrast to the observed results (*L. sp3* - other *L. delicata* taxa IDs 16-25), such recent speciation events would not be expected to be identifiable immunologically.

The widespread substantial overlap in distribution of numerous taxa of similar size, living in the same microhabitat (e.g. *L. tetradactyla* & *L. czechurai*) also implies a substantial period of reproductive isolation. Speciation occurs as a result of either behavioural or spatial isolation. Although little is known of the ecology of *Lampropholis* (*sensu* Greer, 1974), all indications are that life cycles of species living in a specific region (including reproductive behaviour and oviposition sites) are very similar and therefore there are no apparent widespread mechanisms influencing sympatric speciation. A more appropriate scenario for speciation would therefore be isolation of populations due to contraction of habitat, speciation and subsequent expansion of

habitat allowing for sympatry. Such a sequence, particularly in species with a demonstrated relatively slow rate of allozyme mutation, such as observed in *L. tetradactyla*, implies a relatively lengthy time frame.

6.10: ORIGIN OF AUSTRALIAN SCINCID RADIATION

Within *Lampropholis (sensu* Greer, 1974), there are indications of a graduation of taxonomic divergence from widespread homogeneous populations, through grades of speciation to clearly distinct taxa substantially divergent from other species. These relationships are frequently as distinct from other species within the genus as are those between *Lampropholis (sensu* Greer, 1974) and other genera within the subgroup **Pseudemoia.** The subgroup **Eugongylus** (*sensu* Greer & Rounsevell, 1986) has immunological distances from *Lampropholis (sensu* Greer, 1974) intermediate between those of **Pseudemoia** and **Egernia** and **Sphenomorphus,** the latter two being equidistant from *Lampropholis (sensu* Greer, 1974). There is therefore no contradiction to the view of a gradation in relationships across the Australian *Lygosominae.* No such gradation exists, however, between Oriental species and those of the Australian region (Auffenbery, 1980).

Fossil evidence supports the view that all three families of Australian lizards (Gekkonidae, Agamidae & Scincidae) originated in Pangea (Molnar, 1983; 1984). However, as with the Australian scincid radiation, the origins of the modern Australian

Gekkonidae have often been assumed to be in South-East Asia (e.g. Cogger & Heatwole, 1984; Storr, 1964). Recently, however, Heatwole (1988) reported that although the Gekkonidae may have survived in Australia (and thus the modern fauna would necessarily be of Gondwanan origin), the extensive radiations of both Agamidae and Scincidae were the result of subsequent introductions at the time of contact of the Asian and Australian plates. He concludes, however, that no 'firm decision' can be made due to the paucity of fossil data (Heatwole, 1988). However, Witten (quoted in Heatwole, 1988) believes that the Agamidae may be of Gondwanan origin and King (1987) has provided recent evidence for a Gondwanan origin of the Gekkonidae subfamily Diplodactylinae. Several parallels between this latter group and the Australian scincids may be identified from a comparison of this study and King's (1987) work:

(1) Both groups have a distribution which includes Australia, New Zealand, Loyality Islands and New Caledonia;

(2) Both groups demonstrate high diversity and extensive adaptive radiation within Australia;

(3) Switches in reproductive mode occur in both groups, indicating a relatively long period of reproductive isolation;

(4) Similar trends in immunological distances (i.e. within *Phyllurus* IDs 39,43; between *Phyllurus* & *Nephurus* ID=11; between tribes Diplodactylini & Carphodactylini IDs >110);

(5) Distinctive chromosomal formats within lineages in the both groups;

(6) Convergent organismal evolution.

King (1987) argues cogently that the Diplodactylinae represents a Gondwanan distribution and on the basis of the parallels identified above, this work is considered to support the contention that the lygosomines are also a Gondwanan radiation, and not a result of invasions from the north after ancestral stock had previously become extinct in Australia.

The snake families, Elapidae, Typhlopidae and Boidae, also demonstrate extensive adaptive radiation within Australia and are generally considered to represent a largely tertiary radiation originating from South-East Asia (Heatwole, 1988). Mao *et al* (1983), in a discussion of the affinity of the Elapidae to both the *Hydrophis* and *Laticauda* groups of sea snakes, reported albumin immunological distances within the Australian elapids (i.e. *Pseudonaja* *textilis* to a representative of each of nine genera) of between 19 - 60 units. This is within the range observed · for the <u>Eugongylus</u> group, and therefore indicates simultaneous radiation of these groups within Australia although, in this instance, not necessarily of the same origin.

On the basis of affinities with South America and extensive adaptive radiation throughout Australia, the Australian amphibian

families Hylidae and Myobatrachidae are generally thought to be
of Gondwanan origin (Heatwole, 1988). Albumin immunological
distances within the family Myobatrachidae (i.e. within the genus
Ranidella IDs ≤53; between *Ranidella signifera* & eleven
representative general IDs 29-160; Daugherty & Maxson, 1982;
Heyer *et al*, 1982) and Hylidae (i.e. within genus *Litoria* IDs
≤83; within genus *Cyclorana* IDs ≤54; between *Litoria* & *Cyclorana*
IDs 9-85; Maxson *et al*, 1982) are typical of the work undertaken
to date. Although the overall trends are similar within the
reptiles and amphibians reported here, the marginally larger
immunological distances identified among Myobatrachidae genera
(IDs ≤160) compared with the Lygosominae (between *Lampropholis*,
sensu Greer, 1974 & **Sphenomorphus** IDs ≤134; *Lampropholis*, *sensu*
Greer, 1974 & **Egernia** IDs ≤137) may be a reflection of general
differences in protein evolution (see Sect. 2.04). The
differences involved, whether a reflection of evolutionary rates
or not, do not greatly affect support for simultaneous radiations
in the two groups due to the relative difference and the
conservative approach taken in determining radiation timing.

In addition to amphibians, numerous other groups including
marsupials (Archer, 1984b; Kirsch, 1984), side-necked turtles
(Gaffney, 1977), ratite birds (Sibley & Ahlquist, 1981; Stapel *et*
al, 1984) and various invertebrate groups including various
spiders (China, 1962; Platnick, 1976) and snails (China, 1962)

have affinities with South America, thereby implying Gondwanan origins.

The identification of numerous groups with supposed Gondwanan affinities, evidence of simultaneous evolution within such groups with the Australian Lygosominae, together with the lack of a clear mechanism to account for various invasions from South-East Asia (other than long distance over water dispersal, followed by a long period of isolation and little indication of a renewed influx upon Australia reaching its present latitudes), are considered to support a Gondwanan origin for the Australian Scincidae generally.

The genetic associations identified among the Australian taxa and those from elsewhere further support this view. Species within the **Eugongylus** subgroup (*sensu* Greer & Rounsevell, 1986) from Australia, New Zealand and New Guinea were observed to be equidistant from the Australian genus *Lampropholis (sensu* Greer, 1974), as was the South-East Asian genus *Mabuya* (Table 5.3).

For such relationships to have occurred would have required entry of ancestral stock into Australia and New Guinea (or alternatively into New Guinea and then into Australia). These early invasions are generally considered to have occurred by mid-Tertiary (*Cogger* & Heatwole, 1984) but most recent evidence indicates that Australia was still closely associated with

Antartica thirty millions years ago. Therefore such an invasion would have required rafting significant distances. Times obtained from immunological data place these early radiations at in excess of fifty million years ago, approximately the time of inception of Australia's slow separation from Antarctica (see Table 6.1).

To account for the genetic relationships identified among the **Eugongylus** group required the establishment of ancestral stock in both Australia and New Guinea, at approximately the time of separation of Australia from Antarctica (Table 6.1). Dispersal, at least along the east coast of Australia, was necessary to enable over water dispersal to New Zealand at this time.

To have achieved this scenario would have required the establishment of founder populations in appropriate niches on two islands. On the Australian continent this available niche would have had to have been widespread, to allow rapid dispersal southward, followed by another over water dispersal event and establishment in an appropriate niche. Subsequently speciation would have occurred.

This scenario would necessarily have occurred quickly, to retain the relationships identified genetically. It ignores, however, the existence of other **Eugongylus** taxa whose genetic relationships were not assessed. *Leiolopisma*, for example, in addition to having a southern and eastern Australian distribution

Table 6.1:   Continental drift patterns potentially influencing
              Australian Scincid radiation

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pre-160 mya   South America and Africa (West Gondwana) linked
                through Madagascar with India, Artarctica and
                Australian/Lord Rise/New Zealand (East Gondwana)

160 mya   Break-up of Gondwana (east from west)

128 mya   India separated from East Gondwana

128-95 mya   India drifted north west with respect to Australia

95 mya   Two new plates, Antarctica and Lord Howe Rise/New
                Zealand formed with the inception of spreading
                from Australia.
                Oceanic separation of India and Australia

95-82 mya   Early spreading of above

85 mya   Major continental blocks of South East Asia were
                in their present position

65.7 mya   Separation of Papuan Peninsula Plate from Australia

54 mya   Inception of separation of Australia from
                Antarctica - slowly

30 mya   Tasmania and South Tasman rise separated from
                Antarctica by ocean (last separation of Aust. from
                Antarctica)

15 mya   Australia narrowly missed collision with west-ward
                moving South East Asia (i.e. Sundaland block)

5mya   Australia reached Sunda Arc system (vicinity of
                Timor Sea)

Today   Indo-Australian plate coalescenced
                Antarctica separate plate

                                        (after Johnson & Veever, 1984)

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is also known from islands which include Lord Howe, New Caledonia, Vanuatu, Loyality and New Hebrides (Greer, 1974). If it is assumed that at least the majority of these species are genetically associated with the Australian **Eugongylus**, the scenario would require additional rafting events.

A more realistic hypothesis is considered to be that of a Gondwanan origin for the group. To account for such an origin the following scenario is postulated:

Ancestral stock was present in Gondwana at the time of its fragmentation (fossil evidence predates this event) and were therefore already on the Australian, New Guinea and Lord Howe rise/New Zealand plates at that time. This scenario complies with observed genetic relationships, while the Pacific distribution of some species associated with islands of the Lord Howe rise/New Zealand plate and with New Guinea is not incongruous with this hypothesis. One possible association which may conflict with these proposals, however, is that of *Mabuya*.

The immunological distances for the **Eugongylus** group were unidirectional. Therefore while New Zealand, South-East Asia and New Guinea are all equidistant from *Lampropholis (sensu* Greer, 1974; i.e. an Australian taxon) it does not confirm relationships among taxa generally. Thus while *Mabuya* and New Zealand *Leiolopisma* have separated from Australian taxa at the same time,

it does not necessarily imply the same relationships between South-East Asia and New Zealand or New Guinea and does not necessarily imply a close association among all ancestral stock (reciprocal relationships would be necessary to identify these relationships more precisely).

The dispersion and relative positions through time of plates originally associated with Eastern Gondwana have been clearly established on the basis of palaeomagnetic and ocean floor data (see Table 6.1 for pertinent details). The relative position of these plates to Sundaland (i.e. South-East Asia) is, however, not so clear. Movement of Sundaland is complicated and conflicting models exist with insufficient data to resolve the problem. A further complication exists in that it is not clear if Sundaland constitutes a continental block for the purpose of reconstruction of drift patterns. It is known, however, that Australia was in close contact with the Sundaland block fifteen million years ago (Table 6.1; Johnson & Veevers, 1984). On present evidence then it is not possible to present a precise scenario for reptile migration in this region. However, if this required over water dispersal, a single instance resulting in colonization is a more parsimonious explanation than that needed to be invoked for the alternative sequence of migration. At this point in time, therefore, a Gondwanan origin is hypothesized for the Australian Lygosominae.

6.11: SIGNIGICANCE OF THE AUSTRALIAN SCINCID LIZARD RADIATION

In recent years much detailed information has been published on the history of the Australian continent (e.g. Archer & Clayton, 1984; Dyne, 1988; Veevers, 1984). Much of the basis of this information relies on the extrapolation of information from a few geological sites, with the overall jigsaw being pieced together using a variety of different techniques (Hill & MacPhail, 1983). However, the area is being actively researched and associated refinement of knowledge will allow for more detailed historical interpretation in the future.

At the same time, ongoing molecular taxonomy, will enable the genetic relationships of reptiles to be more fully elucidated. Such molecular work, combined with other taxonomic and ecological studies, will ultimately identify the full extent of diversity among the Scincidae and thus enable a greater understanding of relationships among the group. Concurrently, the improved understanding of phanerozoic history and paleoecology will allow for a more accurate resolution of global history. The study of the genetic relationships of a group such as the Australian Scincidae will lend an avenue of independent support to test hypotheses on global history.

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APPENDIX 1: SOURCE OF MATERIAL.

1.01: COLLECTION LOCALITIES

The collecting localities of Scincid lizards used in allozyme electrophoretic analyses are recorded in Table A.1. Details concerning the source of material for albumin micro-complement fixation analyses appear in Table A.2. Data for morphological and meristic analyses were drawn from these same groups of animals.

1.02: EXTENT OF COLLECTIONS.

Wherever possible species were collected throughout their known range. However, during the collection phase of the project, moratoriums were imposed on collecting by Fisheries and Wildlife Service, Victoria and National Parks and Wildlife Service, New South Wales. Collections were undertaken subsequently in New South Wales but undertaking Victorian collections at a later stage proved impractical.

Few individuals of some species were collected despite the investment of a significant amount of time and effort. *Lampropholis caligula* proved to be particularly elusive, despite three trips to known localities and searching by experienced collectors for a considerable number of man-hours, only two individuals were obtained.

1.03: NOMENCLATURE AND IDENTIFICATION.

Nomenclature follows that of Cogger *et al* (1983) and not Wells and Wellington (1983) since the validity of the latter work is presently facing a challenge before the International Code of Zoological Nomenclature. Species identifications were determined by reference to Cogger (1983), appropriate type descriptions (e.g. Ingram & Rawlinson, 1981; Greer & Kluge, 1980) and where necessary, verified by Dr. S. Donnellan (Evolutionary Biology Unit, South Australian Museum, Adelaide). Reference specimens will be deposited at The Australian Museum, Sydney.

1.04: TREATMENT OF TISSUES.

Tissues were obtained from a variety of sources. However, in all cases skinks were collected and returned to the laboratory alive. After processing, tissues were retained frozen until required. When this material was transported between Macquarie University and the South Australian Museum the tissues were packed in dry ice and air freighted.

Table A.1: Collecting localities of taxa incorporated in the allozyme electrophoretic analyses.

Species	Location	Latitude & Longitude
L.amicula	Rainbow Beach	25°55'x153°06'
L.basiliscus	Kuranda	16°49'x145°38'
	Cairns	16°55'x145°46'
	Danbulla S.F.	17°10'x145°38'
	Eungella	21°12'x148°32'
	Mt Spec	18°57'x146°11'
	L.Eacham	17°17'x145°37'
	Palmerston Ck	16°42'x145°22'
	Ravenshoe	17°43'x145°31'
L. caligula	Barrington Tops	32°03'x151°28'
L. challengeri	Bellevue Hill (Sydney)	33°51'x151°15'
	Mt. Nebo	27°24'x152°45'
	Mt. Tambourine	27°58'x153°11'
	Natural Arch	28°10'x153°14'
	Mt. Glorious	27°20'x152°45'
	Coffs Harbour	30°18'x153°08'
	Dorrigo	31°17'x149°38'
	Styx R.	30°36'x152°08'
	Cambridge Plat.	28°45'x152°45'
	Buderim	26°41'x153°03'
L. czechurai	Millaa Millaa	17°31'x145°37'
	Ravenshoe	17°43'x145°31'
	Kuranda	16°49'x145°38'
	Finch Hatton	21°07'x148°38'
	Eungella	21°12'x148°32'
L. delicata	Pymble	33°45'x151°08'
	Bellevue Hill	33°51'x151°15'
	Eltham	37°43'x145°09'
	Wiangarie S.F.	29°08'x153°07'
	Scarborough	27°12'x153°07'
	Rockhampton	23°22'x150°32'
	Byfield	22°51'x150°39'
	Ryde	33°47'x151°08'
	Boudi S.F.	37°08'x149°09'
	Armidale	30°31'x151°40'
	Coffs Harbour	30°18'x153°08'
	Byron Bay	28°38'x153°37'
	Dorrigo	31°17'x149°38'
	Cambridge Plat.	28°45'x152°45'
	Warroo	24°30'x151°40'
	Walcha	30°60'x151°35'
	Buderim	26°04'x153°03'

L. guichenoti	Pymble	33°45'x151°08'
	Ryde	33°47'x151°08'
	Hampton	33°39'x150°03'
	Bellevue Hill	33°51'x151°15'
	Chatswood	33°48'x151°11'
	Bathurst	33°25'x149°35'
	Hunters Hill	33°51'x151°10'
	Seymour C.P.(S.A.)	37°12'x140°42'
	Rainbow Beach	25°55'x153°06'
	Walcha	30°60'x151°35'
	Cnr Monara/Snowy Hwy	
	Nimmitabel	36°39'x149°19'
	Moona Plains	30°59'x151°36'
L. mirabilis	Mt Lewis	16°35'x145°18'
	Cape Cleveland	19°11'x147°01'
L. mustelina	Bellevue Hill	33°05'x151°15'
	Wentworth Falls	33°43'x150°22'
	Hampton	33°45'x150°02'
	Ryde	33°47'x151°08'
	Pymble	33°45'x151°08'
	Chatswood	33°48'x151°11'
	Lane Cove	33°50'x151°11'
	Greenwich	34°36'x149°56'
	Barry	31°50'x151°21'
	Carrai S.F.	31°10'x152°20'
	Jenolan	33°52'x149°57'
	Hunters Hill	33°51'x151°10'
	Barrington Tops	32°03'x151°28'
	Walcha	30°60'x151°35'
	King L	37°51'x147°45'
	Mt Tomah	33°33'x150°25'
	Mt Wilson	32°43'x150°50'
L.sp.1	Ravenshoe	17°41'x145°37'
	L.Eacham	17°17'x145°37'
	Millaa Millaa	17°31'x145°37'
L.sp.2	Mt. Glorious	27°20'x152°45'
L.sp.3	Eungella	21°12'x148°32'
	Danbulla S.F.	17°10'x145°38'
	Finch Hatton	20°07'x148°38'
	Warroo	24°30'x151°40'
L. tetradactyla	Palmerston Ck	16°42'x145°22'
	Kuranda	16°49'x145°38'
	Cairns	16°55'x145°46'
	Danbulla S.F.	37°10'x145°38'
Leiolopisma zia	Wiangarie S.F.	29°08'x153°07'

Table A.2: Collecting localities of taxa incorporated in the albumin micro-complement fixation analyses.

Species	Location	Latitude & Longitude
Carlia rostralis	Harvey Range	19°25'x146°30'
Ctenotus		
Ct.grandis	Granites	20°34'x130°21'
Ct.robustus	Adelaide Hills	34°55'x138°35'
Egernia frerei	Bunya Mtns	26°55'x151°37'
Emoia longicauda	Yuro	06°32'x144°51'
Eugongylus rufescus	Wau	07°20'x146°45'
Lampropholis		
L.amicula	Rainbow Beach	25°55'x153°06'
L.basiliscus	Mt. Spec	18°57'x146°11'
L.caligula	Barrington Tops	32°03'x151°28'
L.challengeri	Mt. Glorious	27°20'x152°45'
	Bellevue Hill(Sydney)	33°51'x151°15'
L.czechurai	Ravenshoe	17°43'x145°31'
	Kuranda	16°49'x145°38'
L.delicata	Warroo	24°30'x151°40'
	Sydney	33°51'x151°08'
L.guichenoti	Seymour C.P.	37°12'x140°42'
	Kangaroo Island	35°49'x137°57'
	Sydney	33°47'x151°08'
L.mirabilis	Mt. Lewis	16°35'x145°18'
L.mustelina	Hampton	33°45'x150°02'
L.sp.1	Ravenshoe	17°43'x145°31'
L.sp.2	Mt. Glorious	27°24'x152°45'
L.sp.3	Warroo	24°30'x151°40'
	Eungella	21°12'x148°32'
L.tetradactyla	Kuranda	16°49'x145°38'
Lamprolepis smaragdina	Yapsiei	04°40'x141°05'
Leiolopisma		
Le.entrecasteauxii	Reevesby	34°32'x136°17'
Le.grande	New Zealand	
Le.jigurru	Mt.bartle Frere	17°24'x145°49'
Le.metallicum	Pine Lake	41°45'x146°42'
Le.otagense	New Zealand	
Le.duperreyi	Collector	34°56'x149°21'
Le.zia	Wiangarie S.F.	29°08'x153°07'
Mabuya multifasciata	Unknown	
Menetia greyii	Cape Banks lighthouse	37°54'x140°22'

Morethia
  Mo.adelaidensis   Wingfield   31°21'x147°48'
  Mo.boulengeri   Kandos   32°52'x149°58'

Pseudoemoia palfreymani   Pedro Branca Rock   43°52'x146°58'

Sphenomorphus
  S.murrayi   Mt. Glorious   27°20'x152°45'
  S.punctulatus   Mt. Morgan   23°28'x150°23'
  S.jobiensis   Bobao   07°45'x154°25'

Tiliqua rugosa   Mt. Mary   34°06'x139°26'

Tribolonotus novaeguinae   Karkar Island   04°40'x146°00'