Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Phylogenetics and Evolution 39 (2006) 503–511
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

Using ancient and recent DNA to explore relationships of extinct and endangered *Leiolopisma* skinks (Reptilia: Scincidae) in the Mascarene islands

J.J. Austin [1], E.N. Arnold *

Department of Zoology, Natural History Museum, Cromwell Rd., London SW7 5BD, UK

Received 29 August 2005; revised 15 December 2005; accepted 15 December 2005
Available online 10 February 2006

Abstract

Phylogenetic analysis, using 1455 bp of recent mtDNA (cytochrome *b* 714 bp, 12S rRNA 376 bp) and nuclear (c-*mos* 365 bp) sequence from 42 species and 33 genera of Scincidae, confirms *Leiolopisma telfairii*, now confined to Round island off Mauritius, is a member of the mainly Australasian *Eugongylus* group of the Lygosominae. Ancient mtDNA (cytochrome *b* 307 bp, 12S rRNA 376 bp) was also extracted from subfossils of two other Mascarene taxa that are now extinct: the giant *L. mauritiana* from Mauritius and *Leiolopisma* sp., known only from fragmentary remains from Réunion. Sequence divergences of 4.2–5.7% show that all three forms were distinct and form a clade. There is restricted evidence that *L. mauritiana* and *L.* sp. from Réunion were sister species. Monophyly and relationships suggest *Leiolopisma* arose from a single transmarine invasion of the oceanic Mascarene islands from Australasia, 5600–7000 km away. This origin is similar to that of *Cryptoblepharus* skinks and *Nactus* geckos in the archipelago but contrasts with *Phelsuma* day geckos, which appear to have arrived from Madagascar where Mascarene *Cylindraspis* tortoises may also have originated. Diversification of the known species of *Leiolopisma* occurred from about 2.3–3.4 Mya, probably beginning on Mauritius with later invasion of Réunion. The initial coloniser may have had a relatively large body-size, but *L. mauritiana* is likely to have become gigantic within the Mascarenes. Other relationships supported by this investigation include the following. Scincines: *Pamelaescincus* + *Janetaescincus*, and *Androngo* (*Amphiglossus*, *Paracontias*). Lygosomines: *Sphenomorphus* group—(*Sphenomorphus*, *Lipinia* (*Ctenotus*, *Anomalopus* (*Eulamprus* and *Gnypetoscincus*))): *Egernia* group—*Egernia* (*Cyclodomorphus*, *Tiliqua*); *Eugongylus* group—(*Oligosoma*, *Bassiana*. (*Lampropholis* (*Niveoscincus*, *Carlia*))).

Keywords: *Leiolopisma* skinks; Island colonisation; Island gigantism; Mascarene islands; Ancient DNA; Mitochondrial DNA; c-*mos*

1. Introduction

The Mascarene islands in the Southwest Indian Ocean (Fig. 1) had the richest oceanic island reptile fauna in the World, but this was devastated after the arrival of people and the animals they introduced 500 years ago. Of the 33 reptile species known to have been present, 15 (46%) are extinct and 11 (33%) reduced to small relicts, leaving only 7 (23%) that retain substantial ranges (Arnold, 2000). Among the forms affected were three large-bodied skinks that are referred to *Leiolopisma* Duméril and Bibron, 1839; a genus in which many Australasian species were once included, but which is now restricted to the Mascarene taxa (Hutchinson et al., 1990). Unlike the Australasian species, the Mascarene forms frequently have pterygoid teeth (Arnold, 1980; Hutchinson et al., 1990) and in *L. telfairii*, the one species in which they can be checked, there are distinctive features of head scalation (Hutchinson et al., 1990). Only one of the species, *L. telfairii* Desjardins, 1831; survives and is now confined to the 150 ha offshore Round Island, although it was once also

* Corresponding author. Fax: +44 0 20 7 942 5054.
E-mail address: ena@nhm.ac.uk (E.N. Arnold).
[1] Present address: School of Earth and Environmental Sciences, University of Adelaide, North Terrace, Adelaide, SA 5005, Australia.

1055-7903/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2005.12.011

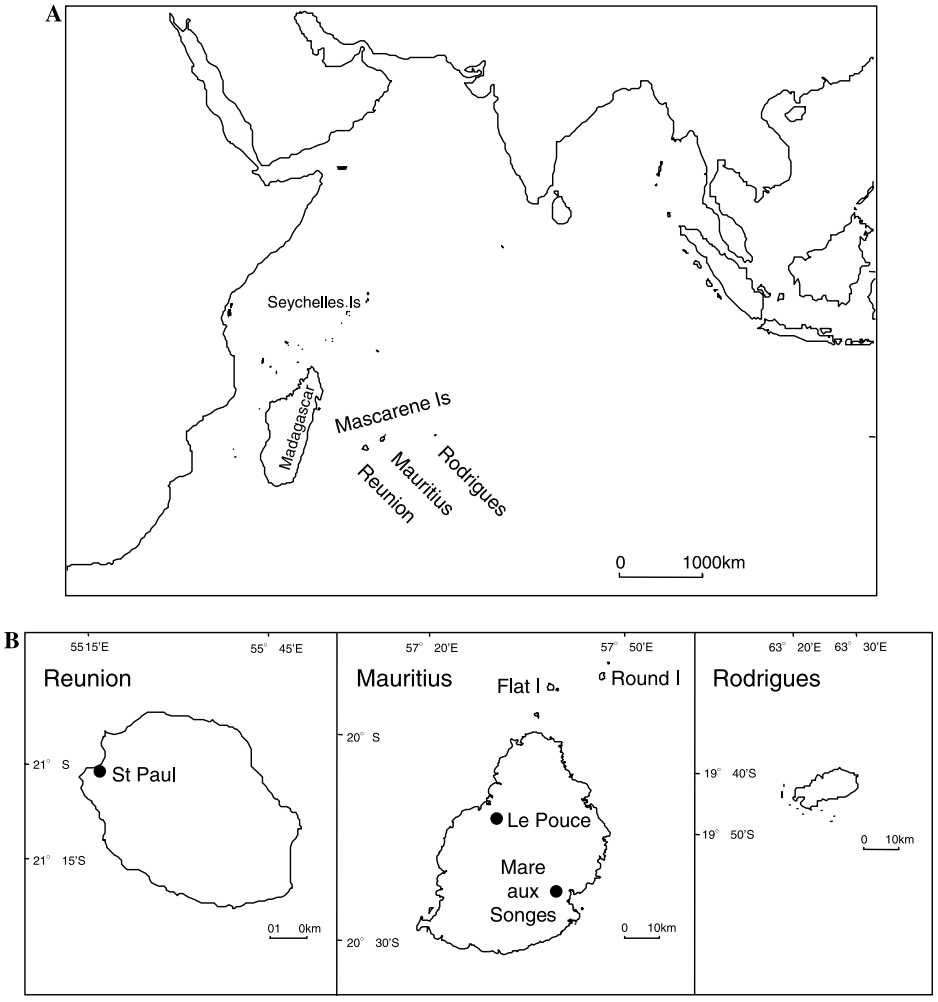

Fig. 1. (A) Map of the Indian Ocean and surrounding landmasses showing the location of the Mascarane islands. (B) Mascarane islands showing collection localities for *Leiolopisma* skinks.

present on neighbouring Flat island and subfossil material shows that it used to occur widely on the main island of Mauritius as well (Arnold, 1980; Fig. 1). A second species found on Mauritius, *L. mauritiana* (Günther, 1877) is extinct and was one of the largest skinks known. It reached an estimated snout-vent length of around 340 mm, compared with 170 mm for the largest Round Island *L. telfairii* and an estimated 200 mm for Mauritian sub-fossils of this species (Arnold, 1980). *L. mauritiana* was originally assigned its own genus, *Didosaurus* Günther, 1877, its apparent affinity to *L. telfairii* being noted later (Arnold, 1980). A third, as yet unnamed, taxon occurred on Réunion island 145 km southwest of Mauritius and is also extinct, being known only from fragments (isolated post-cranial and skull bones, including dentaries). This form was similar to *L. telfairii* but was more robust and had coarser dentition (Arnold, 1980; Arnold and Bour, Submitted for publication).

Leiolopisma telfairii and *L. mauritiana* are clearly members of the Lygosominae, having the characteristic features of this group (fused frontal bones, and a secondary palate—Greer, 1970). Within this assemblage, their elevated number of premaxillary teeth (11 instead of the 9 usually present in other lygosomines), lack of a separate postorbital bone and a covered Meckel's canal in the dentary bone suggests they are members of the mainly Australasian *Eugongylus* group (Greer, 1979). Other typical features of this assemblage not checkable in *L. mauritiana* can be discerned in *L. telfairii*, including parietal scales in contact behind the interparietal, 28 presacral vertebrae and a diploid chromosome number of 30 (Hardy, 1979; Donnellan, 1985). In contrast, the fragmentary nature of available material of the Réunion skink makes it impossible to be sure of its affinities, and at the same time it is not clear how distinct this taxon is from *L. telfairii*. To test hypotheses about the relationships of these Mascarene skinks and their relative status, they are investigated here, using recent mitochondrial (cytochrome *b* and 12S rRNA) and nuclear (c-*mos*) DNA from *L. telfairii*, and ancient mtDNA (cytochrome *b* and 12S rRNA) from the two extinct forms. The results are then used to consider the history and biogeography of *Leiolopisma*. The investigation also incidentally tests other hypothesised relationships within the Lygosominae.

2. Materials and methods

2.1. Material

Tissue samples from members of the following assemblages are included (numbers are given in brackets). Lygosominae—*Eugongylus* group (13), *Egernia* group (3), *Sphenomorphus* group (6) and *Mabuya* (2); Scincinae (14); Feylininae (1) and Acontinae (1). Three taxa, *Zonosaurus* sp. (Gerrhosauridae), *Pseudocordylus capensis* and *Cordylus cordylus* (Cordylidae) were included as outgroups. Samples of Mascarene *Leiolopisma* comprise four individuals from the extant population of *L. telfairii* on Round Island, one old alcohol-preserved *L. telfairii* from the extinct population on Flat Island, two subfossil bones of the extinct *L. mauritiana* collected from different localities on Mauritius, and two subfossil bones of the extinct *L.* sp. population from Réunion (Fig. 1). All species and samples included in the study are listed in Table 1.

2.2. DNA extraction, PCR amplification and sequencing

DNA sources included ethanol-preserved tissue (tail tips and liver), genomic DNA extracts and subfossil bones (extinct *L. mauritiana* from Mauritius and *L.* sp. from Réunion). For extant taxa, DNA was extracted from 2 to 3 mm^3 of preserved tissue samples using standard Proteinase K digestion and phenol:chloroform protocols (Carranza et al., 1999). Fragments of two mitochondrial genes, 12S rRNA (~400 bp) and cytochrome *b* (714 bp), and one fragment of the nuclear gene, c-*mos* (374 bp), were amplified via PCR using universal oligonucleotide primers 12Sa, 12Sb, L14841, CB3H, G73 and G74 (Kocher et al., 1989; Palumbi, 1996; Saint et al., 1998) and PCR conditions described by Austin et al. (2004). PCR products were purified and directly sequenced as described by Austin et al. (2002, 2004).

For extinct taxa, all pre-PCR work was carried out in a dedicated ancient DNA laboratory, physically isolated from other DNA facilities, and using rigorous anti-contamination and authentication procedures appropriate for ancient DNA (Austin et al., 1997a,b). Genomic DNA was extracted from small pieces of subfossil bone as follows. Surface contamination was eliminated by physical removal with a Dremel drill fitted with a grinding wheel, or by soaking in a 5% bleach solution for 5 min and washing in DNA-free water. Sub-samples of each bone were placed inside 1.5 ml microcentrifuge tubes, the tube dipped in liquid nitrogen, and the bone crushed to a coarse powder using a disposable plastic pestle. Approximately 10–50 mg of bone powder was decalcified in 10 volumes of 0.5 M EDTA (pH 8.0) on a rotary mixer at room temperature for 24 h. The decalcified bone powder was washed once with 1 ml of 10 mM Tris (pH 8.0) to remove excess EDTA. DNA was extracted using a DNeasy tissue kit (Qiagen) according to the manufacturer's instructions. The final DNA eluate (200 µl) was concentrated to ~20 µl final volume using Microcon-30 centrifugal filter units (Millipore). DNA extraction attempts from alcohol-preserved museum tissues involved air drying small, 2–3 mm^3, pieces of muscle and washing once in 1 ml of 10 mM Tris–HCl (pH 8.0), followed by digestion and extraction using the DNeasy tissue kit (Qiagen).

For the ancient DNA, short (103–178 bp) overlapping segments of the 12S rRNA (~400 bp total) and cytochrome *b* (307 bp total) genes were amplified via two rounds of PCR as described by Austin et al. (2002, 2004) using combinations of the following primers: 12S rRNA, 12Sa (Kocher et al., 1989), 12SH1269 (5′-TTTCTTTCATAAGGTAGGCTGAC-3′), 12SL1266L (5′-GAAACTCAGCCTATATACCGCCG-3′), 12SH1382L (5′-GTTTCATTGTGCTGTTCGTGTTC-3′), 12SL1357L (5′-GTGTAGCAYATAAAGCGGAAGAG-3′), 12Sb (Kocher et al., 1989), cytochrome *b*, L14841 (Kocher et al., 1989), CBH14957 (5′-AAGTCATCCGTATTGTACGTCTCG-3′), CBH14953L (5′-GCCGTATTGGACATCCCGGGT-3′), CBL14936L (5′-CAGCAGACATTTCATCCGCATTCA-3′), CBH15039L (5′-GCCGTAATAAAGGCCCCGACCA-3′), CBL15030L (5′-GCCTCAATATTCTTYATCTGCMTCTA-3′), H15149 (Kocher et al., 1989). Negative extraction and PCR controls (no tissue and no DNA extract, respectively) were included alongside all extract and PCR amplification attempts. PCR products were purified and sequenced directly as described by Austin et al. (2002, 2004). All sequences have been deposited in GenBank (Accession Nos.: AF280114, AF280115, AF280117–AF280120, AF280122–AF280124, AF280129, AF280130, AF280133–AF280135, AY818735–AY818823).

2.3. Phylogenetic analyses

DNA sequences were aligned manually using translated amino acid sequences (cytochrome *b* and c-*mos*) and a secondary structure model (12S rRNA, Hickson et al., 1996) to guide alignment. Twenty-four nucleotide positions in the 12S rRNA gene containing gaps and adjacent sites of ambiguous alignment were excluded due to uncertain positional homology. Nine nucleotide positions in the c-*mos* gene containing indels were also excluded.

Phylogenetic relationships were estimated from the full dataset by maximum parsimony (MP), maximum likelihood (ML) and Bayesian (BML) methods using computer programs PAUP*4.0 (MP and ML Swofford, 2000) and MRBAYES v3.1 (BML, Huelsenbeck and Ronquist, 2001). MP analyses used equal character weighting and heuristic searches with 100 random sequence addition replicates and TBR branch swapping. Branch support was estimated using non-parametric bootstrapping (Felsenstein, 1985) with 1000 pseudo-replicates. ML searches used the GTR + I + Γ model of nucleotide substitution selected via hierarchical likelihood ratio tests implemented in ModelTest v3.06 (Posada and Crandall, 1998). Heuristic searches were used with 10 random sequence addition replicates and TBR branch swapping. The same model of nucleotide

Table 1
Skink samples, collection localities, and gene sequences obtained for specimens used in this study

Species	Locality	DNA source	Gene sequenced
Eugongylus group			
L. telfairii	Round I, Mauritius	Tail tip	12S rRNA, cyt. *b*, c-*mos*
L. telfairii	Round I, Mauritius	Tail tip	12S rRNA, cyt. *b*
L. telfairii	Round I, Mauritius	Tail tip	12S rRNA, cyt. *b*
L. telfairii	Round I, Mauritius	Tail tip	12S rRNA, cyt. *b*
L. telfairii, MNHN 2958	Flat I, Mauritius	Muscle, spirit specimen	—
L. mauritiana, BMNH R4691	La Pouce, Mauritius	femur, sub-fossil	12S rRNA, cyt. *b*
L. mauritiana, BMNH R9444	Mare aux Songes, Mauritius	Parietal, sub-fossil	12S rRNA, cyt. *b*
Leiolopisma sp.	St Paul, Réunion	Right pelvis, sub-fossil	—
Leiolopisma sp.	St Paul, Réunion	Left dentary, sub-fossil	12S rRNA, cyt. *b*
Cryptoblepharus boutoni	Flat I, Mauritius	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Cryptoblepharus carnabyi	New South Wales, Australia	Liver	12S rRNA, cyt. *b*, c-*mos*
Emoia impar	Roratonga, Cook Is	DNA extract	12S rRNA, cyt. *b*, c-*mos*
Emoia physicae	Papua New Guinea	DNA extract	12S rRNA, cyt. *b*, c-*mos*
Bassiana duperreyi	Tasmania, Australia	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Oligosoma zelandicum	Stephens I, New Zealand	DNA extract	12S rRNA, cyt. *b*, c-*mos*
Niveoscincus pretiosus	Tasmania, Australia	Tail tip	12SrRNA, cyt. *b*, c-*mos*
Niveoscincus ocellatus	Tasmania, Australia	Tail tip	12SrRNA, cyt. *b*, c-*mos*
Carlia rubrigularis	Queensland, Australia	DNA extract	12SrRNA, c-*mos*
Lampropholis delicata	New South Wales, Australia	DNA extract	12SrRNA, cyt. *b*, c-*mos*
Lampropholis coggeri	Queensland, Australia	DNA extract	12SrRNA, c-*mos*
Eugongylus rufescens	Papua New Guinea	DNA extract	12SrRNA, cyt. *b*, c-*mos*
Egernia group			
Egernia whitii	Tasmania, Australia	Tail tip	12SrRNA, cyt. *b*, c-*mos*
Tiliqua scincoides	GenBank	AF090187, AF039462	12SrRNA, c-*mos*
Cyclodomorphus casuarinae	Tasmania, Australia	Tail tip	12SrRNA, cyt. *b*, c-*mos*
Mabuya group			
Mabuya wrightii	Fregate, Seychelles	Tail tip	12SrRNA, cyt. *b*, c-*mos*
Mabuya sechellensis	Mahé, Seychelles	Tail tip	12SrRNA, cyt. *b*, c-*mos*
Sphenomorphus group			
Eulamprus amplus	Queensland, Australia	Tail tip	12S rRNA, c-*mos*
Gnypetoscincus queenslandiae	Queensland, Australia	Tail tip	12S rRNA, c-*mos*
Ctenotus taeniolatus	Queensland, Australia	Tail tip	12S rRNA, c-*mos*
Anomalopus verreauxii	Queensland, Australia	Tail tip	12S rRNA, c-*mos*
Sphenomorphus sp.	GenBank	AB028808, AF039464	12S rRNA, c-*mos*
Lipinia sp.	GenBank	AB028804, AF039465	12S rRNA, c-*mos*
Scincinae			
Ateuchosaurus pellopleurus	Okinawa, Japan	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Janetaescincus veseyfitzgeraldi	Fregate, Seychelles	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Janetaescincus braueri	Silhouette, Seychelles	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Pamelaescincus gardineri	Silhouette, Seychelles	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Chalcides ocellatus	Jeddah, Arabia	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Hakaria simonyi	Sokotra	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Amphiglossus igneocaudatus	Amboasary, Madagascar	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Paracontias holomelas	Antsiranana, Madagascar	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Androngo trivittatus	Amboasary, Madagascar	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Gongylomorphus bojeri	Gunner's Quoin, Mauritius	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Gongylomorphus fontenayi	Flat I, Mauritius	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Gongylomorphus fontenayi	Mare Longue, Mauritius	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Eumeces sp.	GenBank	NC000888, AF315396	12S rRNA, cyt. *b*, c-*mos*
Scincus mitranus	United Arab Emirates	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Brachymeles bicolor	Phillipines	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Feylininae			
Feylinia polylepis	Principe, Gulf of Guinea	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Acontinae			
Acontias meleagris	Port Elizabeth, South Africa	Tail tip	12S rRNA, cyt. *b*, c-*mos*
Gerrhosauridae			
Zonosaurus sp.	GenBank	AJ416928, AF315395	12S rRNA, c-*mos*

Table 1 (*continued*)

Species	Locality	DNA source	Gene sequenced
Cordylidae			
Cordylus cordylus	GenBank	AF236027, AF148711	12S rRNA, c-*mos*
Pseudocordylus capensis	Englemanskloof, South Africa	Tail tip	12S rRNA, cyt. *b*, c-*mos*

BMNH—Natural History Museum, London; MNHN—Museum national d'Histoire naturelle, Paris.

substitution was applied to BML analyses using three partitions of the dataset, 12S rRNA, cytochrome *b* and c-*mos*, to account for gene specific evolutionary rates. Model parameters for each partition were estimated separately during the MCMC process. BML analyses started from random trees and were run for 5×10^6 generations using four separate incrementally heated chains run simultaneously, sampling at intervals of 50 generations to produce 100,000 sampled trees. Trees sampled before stationarity was reached (burnin = generation 10,000) were discarded and a 50% majority rule consensus tree was generated from the remaining trees. Branch support was assessed from estimates of clade posterior probabilities.

3. Results

We obtained 1455 bp of aligned DNA sequence from 42 Scincidae taxa and three outgroups for the mitochondrial 12S rRNA, cytochrome *b* and nuclear c-*mos* genes. Cytochrome *b* sequences could not be obtained for 11 extant taxa (see Table 1). In the case of the subfossil bone samples, 12S rRNA sequence and partial (307 bp) cytochrome *b* sequence were obtained from one *L. mauritiana* and one *L.* sp. from Réunion. The second *L. mauritiana* subfossil bone yielded only 200 bp of 12S rRNA and 307 bp of cytochrome *b* sequence. No amplifiable DNA could be recovered from the alcohol-preserved *L. telfairii* from Flat Island or from a second *L.* sp. bone from Réunion. No subfossil bones yielded PCR product for the c-*mos* gene.

Combined mtDNA sequences (1090 bp of the 12S rRNA and cytochrome *b* genes) from the four extant Round Island *L. telfairii* were identical except for a single, first codon position A–G transition substitution that distinguished one individual from the remaining three. Similarly, sequences from the two *L. mauritiana* (507 bp of 12S rRNA and cytochrome *b*) differed by only a single A–G transition substitution in the 12S rRNA gene. For 683 bp of mtDNA sequence available from the two extinct species, the majority *L. telfairii* sequence differed from that of *L. mauritiana* by 30 substitutions (4.4% uncorrected divergence) and from the extinct Réunion *L.* sp. sequence by 39 nucleotide substitutions (5.7% uncorrected divergence). The latter two sequences differed by 29 substitutions (4.2% uncorrected divergence). The three species of *Leiolopisma* differ from all other lygosomine skinks by 13–21% uncorrected sequence divergence.

The maximum likelihood phylogeny of the Scincidae is presented in Fig. 2. Across all three analyses, relationships among the Scincinae are largely unresolved, except that the Seychelles genera, *Pamelaescincus* and *Janetaescincus*, form a well supported clade, as do the members of three Madagascan genera, *Amphiglossus*, *Androngo* and *Paracontias*. The single representatives of the Felyininae and Acontinae group within the Scincinae. Both ML and BML analyses support the monophyly of the Lygosominae. Within the Lygosominae, members of the recognised assemblages included here form well substantiated clades, namely the *Sphenomorphus*, *Egernia*, *Mabuya* and *Eugongylus* groups. The *Sphenomorphus* group is sister to the others, in agreement with other molecular (Honda et al., 2000; Reeder, 2003; Whiting et al., 2003) and morphological (Greer, 1979) investigations, but other group relationships vary among recent studies. The present analysis places *Leiolopisma* firmly within the *Eugongylus* group but without well-substantiated relationships to other members. However, it forms a clade with *Emoia*, *Cryptoblepharus* and a unit formed by species of *Niveoscincus*, *Carlia*, *Lampropholis*, *Oligosoma* and *Bassiana*. *Eugongylus* is placed outside this assemblage. Other relationships supported within the Lygosominae are as follows. In the *Sphenomorphus* group, *Eulamprus* and *Gnypetoscincus* form a sister pair related to *Ctenotus* and *Anomalopus*, with *Sphenomorphus* and *Lipinia* outside this assemblage. In the *Egernia* group *Cyclodomorphus* and *Tiliqua* are a sister pair. In the *Eugongylus* group, *Niveoscincus* and *Carlia* are a sister pair related successively to *Lampropholis*, and then *Oligosoma* and *Bassiana*.

The phylogeny clearly shows that the two extinct and one extant species of *Leiolopisma* form a well-supported clade. Within this, *L. mauritiana* is placed as sister to *L.* sp. from Réunion, with MP bootstrap support of 78% and Bayesian clade support of 91%. When all three possible topological arrangements within the *Leiolopisma* clade are considered, Shimodaira–Hasegawa tests (Shimodaira and Hasegawa, 1999) do not reject any of the alternative topologies (SH-test implemented in PAUP* with RELL optimisation and 1000 bootstrap replicates. ML tree—*L. telfairii* (*L. mauritiana*, *L.* sp. Réunion), $-\ln L = 4544.06$; tree 2—*L.* sp. Réunion (*L. mauritiana*, *L. telfairii*) $\Delta\ln L = 1.65$, $P = 0.30$; tree 3—*L. mauritiana* (*L. telfairii*, *L.* sp. Réunion) $\Delta\ln L = 1.10$, $P = 0.39$).

4. Discussion

4.1. Phylogeny of the Scincidae and relationships of Mascarene Leiolopisma

Our estimate of phylogenetic relationships within the Scincidae, based on 1455 bp of combined mtDNA and nuclear gene sequence, is broadly congruent with previous studies that have focussed on the Scincinae (Brandley et al., 2005; Whiting et al., 2003) and Lygosominae (Honda et al., 2000). In

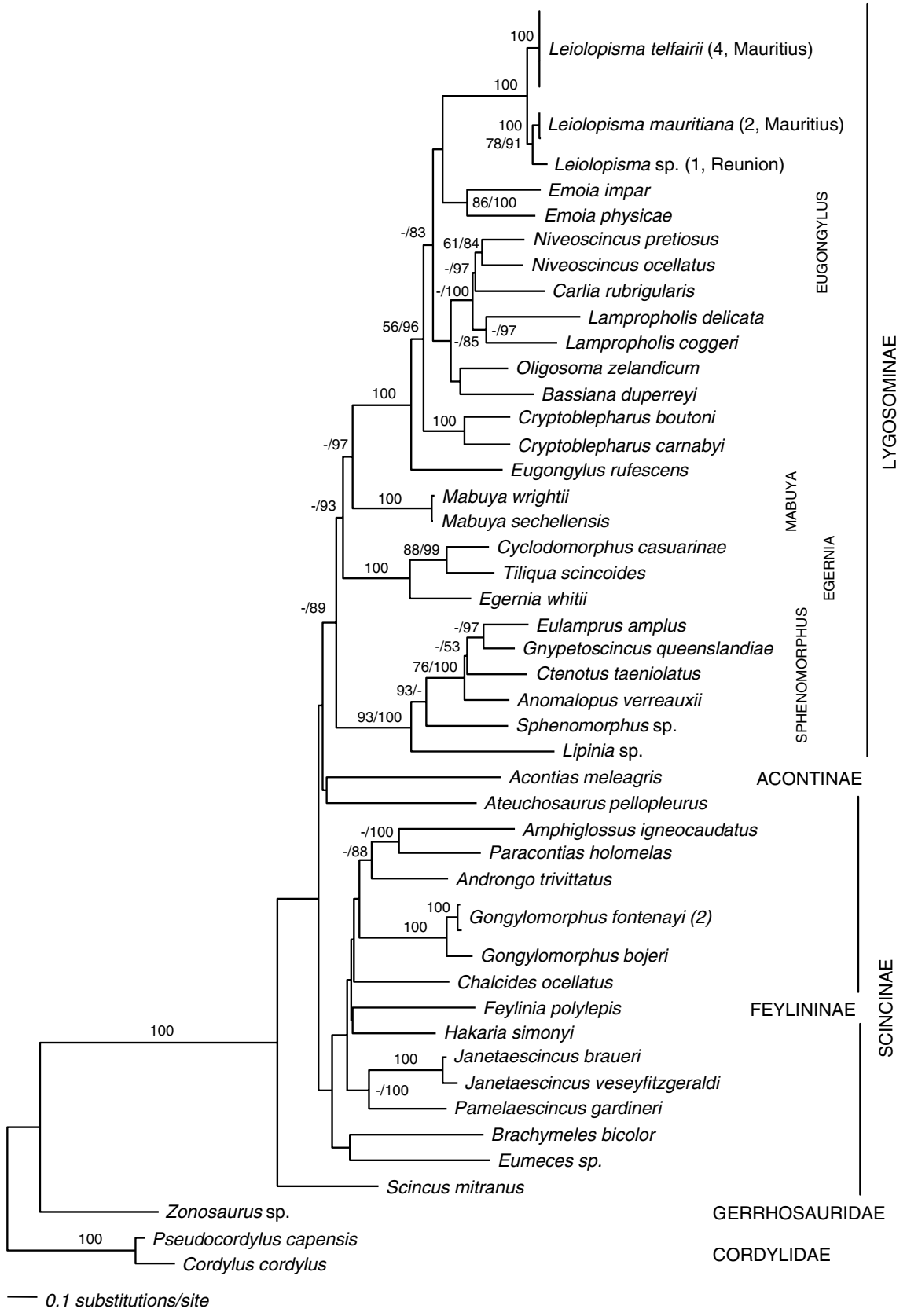

Fig. 2. Maximum likelihood phylogenetic tree for 42 taxa of extant skinks, including *Leiolopisma telfairii* (Scincidae), the extinct *L. mauritiana* and *L.* sp. from Réunion and gerrhosaurid and cordylid outgroups, based on 1455 bp of mitochondrial (12S rRNA, cytochrome *b*) and nuclear (c-*mos*) DNA. Numbers adjacent to nodes indicate MP bootstrap support/Bayesian posterior probability; where these are both 100, only a single figure is given. A '–' indicates support values less than 50%. Number of samples of *Leiolopisma* sequenced from particular islands are given in brackets.

particular, our phylogeny with a large taxon sampling of lygosomine skinks supports the monophyly of the Lygsominae and of the *Sphenomorphus*, *Egernia*, *Mabuya* and *Eugongylus* groups within the sub-family. In agreement with previous studies, the Scincinae are paraphyletic, including taxa from the sub-families Acontinae and Felylininae, and relationships among them are poorly resolved. The present phylogeny provides strong evidence that the Mascarane island *Leiolopisma* are monophyletic and are part of the largely Australian *Eugongylus* group of lygosomine skinks.

Leiolopisma telfairii and *L. mauritiana* are regarded as distinct species because they differed radically in adult size, and individuals of similar dimensions had different tooth counts (Arnold, 1980). They were also sympatric at least two localities on Mauritius where extensive subfossil material has been found: Mare aux Songes in the southeast and Le Pouce in the northwest. In agreement with this, the two show significant divergence in their mtDNA, differing by 4.4%. In contrast, the status of the third form, *L.* sp. from Réunion has been less certain as available subfossil material is generally similar to *L. telfairii*. However, the results presented here show it also differs markedly from the other two forms in its mtDNA, with a divergence of 5.7% from *L. telfairii* and 4.2% from *L. mauritiana*. All three forms are consequently best regarded as separate species.

As already noted, *Leiolopisma* lies outside a clade of five Australasian genera represented in the molecular analysis: *Niveoscincus*, *Carlia*, *Lampropholis*, *Oligosoma*, and *Bassiana*. These taxa are among a number of small-bodied genera characterised by a derived anatomical feature: complete fusion of the three units that form the atlas vertebra (Greer, 1990). This is absent in the remaining members of the *Eugongylus* group, including *Leiolopisma* and the mainly tropical *Eugongylus* and *Emoia*, all three of which are comparatively large bodied.

Relationships among the species of *Leiolopisma* are not strongly resolved, but the weakly supported sister relationship of *L. mauritiana* and *L.* sp. from Réunion receives some corroboration from the shared coarser dentition and more robust habitus of these forms compared with *L. telfairii*.

4.2. Biogeography

Mascarene *Leiolopisma* have a clear relationship to a group of Australasian genera which is paraphyletic with respect to them. As the Mascarenes are classic oceanic islands of volcanic origin that have never had subaerial connection with any other land masses, *Leiolopisma* must have reached them by a westward transmarine journey from Australasia. This was extremely long; the minimum distance from West Australia is 5600 km and a more tropical origin in New Guinea would involve a passage of at least 7000 km. Such a journey is in agreement with the prevailing currents and winds reaching the Mascarene area today, namely the Equatorial Current and the Southeast Trades. At least two other Mascarene groups appear to have made similar journeys: *Cryptoblepharus* skinks which also belong to the *Eugongylus* group, and *Nactus* geckos. These groups contrast with *Phelsuma* day geckos of the Mascarenes which originated in Madagascar just 700 km away (Austin et al., 2004), something that may also be true of the extinct *Cylindraspis* tortoises (Austin and Arnold, 2001; Austin et al., 2002).

Long transmarine journeys by reptiles, like that made by the ancestor of *Leiolopisma* are sometimes hypothesised to involve 'island hopping' (see for instance Mausfeld et al., 2002), but there is no indication of this in the present case. Intervening islands between Australasia and the Mascarenes lack any evidence that *Leiolopisma* ever reached them. This is true of Christmas and Cocos-Keeling islands, both around 1000 km from Australia, and of Rodrigues which lies 588 km east of Mauritius. Although extensive recent subfossils are known from Rodrigues that show it had a reptile fauna consisting of seven species of geckos, no skinks are included (Arnold et al., submitted for publication).

The history of *Leiolopisma* within the Mascarenes is not clear, partly because the internal phylogeny of the group is uncertain. If the weakly supported sister relationship of *L. mauritiana* and *L.* sp. from Réunion were accepted, a parsimonious interpretation would be a within-island speciation producing the *L. telfairii* and *L. mauritiana* lineages on Mauritius, followed by invasion of Réunion by a propagule of the latter to produce *L.* sp. An alternative scenario consists of a speciation event resulting from movement between the two islands followed by another resulting from invasion of Mauritius from Réunion. However, this would involve two inter-island journeys instead of one. In spite of spectacular exceptions like the arrival of *Leiolopisma* in the Mascarenes from Australia, lizards are not especially good transmarine colonisers. Because of this, minimising the number of 145 km journeys between Mauritius and Réunion seems appropriate and the first interpretation is preferred.

No molecular clock is presently available for lygosomine skinks. However, an estimated combined divergence rate for the same 12S rRNA and cytochrome *b* fragments used here is available for *Chalcides* scincines (Carranza and Arnold, unpublished data). It is based on the age of El Hierro in the Canary archipelago, on the assumption that this island was colonised, soon after its origin about 1 Ma, from nearby La Gomera, from the direction of which prevailing currents and winds come. The estimated rate for *Chalcides* is 2.05% per My and compares with 2.35% for *Tarentola* geckos (Carranza et al., 2000, 2002) and 1.5% for *Gallotia* lacertids (Maca-Meyer et al., 2003) based on the same method of calibration. If the *Chalcides* rate is used, the degree of divergence (average corrected GTR + I + Γ genetic distance of $35.8 \pm 5.6\%$) of Mascarene *Leiolopisma* from other *Eugongylus* group taxa considered here suggests *Leiolopisma* diverged a maximum of 17 Ma. More detailed sampling of *Eugongylus* group Lygosomine skinks is required to establish a minimum estimate of divergence time, however as Mauritius is about 8–10 My old

(McDougall and Chamalaun, 1969) and Réunion only around 2.1 My (McDougall, 1971), it is likely that the skinks arrived on Mauritius first and then spread to Réunion. Maximum likelihood corrected divergences between *Leiolopisma* species are: *L. telfairi* and *L. mauritiana*—5.1%; *L. mauritiana* and *L.* sp. from Réunion—4.7%; *L. telfairii* and *L.* sp. from Réunion—6.9%. If the first speciation event among the species of *Leiolopisma* separated *L. telfairii* on Mauritius, this would have occurred about 2.5–3.4 Ma. The separation of *L.* sp. from *L. mauritiana* resulting from invasion of Reunion would have been about 2.3 Ma soon after the island emerged from the sea.

As some other *Eugongylus* group taxa have relatively large body sizes, like *Leiolopisma telfairii* and *L.* sp. from Réunion, it is possible the ancestor of the genus was also comparatively large when it reached the Mascarenes. But the probably unique gigantic size of *L. mauritiana* must have arisen within the archipelago and presumably on Mauritius. This may possibly result from character displacement arising in sympatry with *L. telfairii.*

Acknowledgments

We are grateful to Roger Bour and Carl Jones for material and information, and to Salvador Carranza for reading the manuscript and for helpful advice. We thank anonymous referees for suggestions and comments that improved the manuscript. Roy Swain, Ross Sadlier, Sarah Smith, Ted Case, Gaynor Dolman and Conrad Hoskin kindly provided tissue samples or DNA extracts. This research was supported by NERC (UK) Grant GR3/10832. The Department of Palaeontology, Natural History Museum (London) generously provided space and dedicated facilities for the analysis of ancient DNA.

References

Arnold, E.N., 1980. Recently extinct reptile populations from Mauritius and Réunion, Indian Ocean. J. Zool. Lond. 191, 33–47.

Arnold, E.N., 2000. Using fossils and phylogenies to understand evolution of reptile communities on islands. Bonn. Zool. Monogr. 46, 309–323.

Arnold, E.N., Austin, J.J., Jones, C.G., Submitted for publication. A recently extinct fossil lizard community from Rodrigues island, Indian Ocean.

Arnold, E.N., Bour R., Submitted for publication. Fossil lizards from Réunion, Indian Ocean.

Austin, J.J., Arnold, E.N., 2001. Ancient mitochondrial DNA and morphology elucidate an extinct island radiation of Indian ocean giant tortoises (*Cylindraspis*). Proc. R. Soc. Lond. B 268, 2515–2523.

Austin, J.J., Arnold, E.N., Bour, R., 2002. The provenance of type specimens of extinct Mascarene Island giant tortoises (*Cylindraspis*) revealed by ancient mitochondrial DNA sequences. J. Herpet. 36, 280–285.

Austin, J.J., Arnold, E.N., Jones, C.G., 2004. Reconstructing an island radiation using ancient and recent DNA: the extinct and living day geckos (*Phelsuma*) of the Mascarene islands. Mol. Phylogenet. Evol. 31, 109–122.

Austin, J.J., Ross, A.J., Smith, A.B., Fortey, R.A., Thomas, R.H., 1997a. Problems of reproducibility—does geologically ancient DNA survive in amber preserved insects? Proc. R. Soc. Lond. B 264, 467–474.

Austin, J.J., Smith, A.B., Thomas, R.H., 1997b. Palaeontology in a molecular world: the search for authentic ancient DNA. Trends Ecol. Evol. 12, 303–306.

Brandley, M.C., Schmitz, A., Reeder, T.W., 2005. Partitioned Bayesian analyses, partition choice, and the phylogenetic relationships of Scincid lizards. Syst. Biol. 54, 373–390.

Carranza, S., Arnold, E.N., Mateo, J.A., Geniez, P., 2002. Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial; and nuclear DNA sequences. Mol. Phylogenet. Evol. 23, 244–256.

Carranza, S., Arnold, E.N., Mateo, J.A., López-Jurado, L.F., 2000. Long-distance colonization and radiation in gekkonid lizards, *Tarentola* (Reptilia: Gekkonidae), revealed by mitochondrial DNA sequences. Proc. R. Soc. Lond. B 267, 637–649.

Carranza, S., Arnold, E.N., Thomas, R.H., Mateo, J.A., López-Jurado, L.F., 1999. Status of the extinct giant lacertid lizard *Gallotia simonyi simonyi* (Reptilia: Lacertidae) assessed using mtDNA sequences from museum specimens. Herpetol. J. 9, 83–86.

Desjardins, J., 1831. Sur trois espèces de lézard du genre Scinque qui habitent l'ı˙le Maurice (Ile de France). Ann. Sci. nat. 22, 292–299.

Donnellan, S.C., 1985. The evolution of sex chromosomes in scincid lizards. Unpublished Ph. D. Thesis, Macquarie University, Australia.

Duméril, A.M.C., Bibron, G., 1839. Erpétologie Génerale ou Histoire Naturelle complète des Reptiles, vol. 5. Librairie Encyclopédique de Roret, Paris.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Greer, A.E., 1970. A subfamilial classification of scincid lizards. Bull. Mus. Comp. Zool. Harv. 139, 151–183.

Greer, A.E., 1979. A phylogenetic subdivision of Australian skinks. Rec. Aust. Mus. 32, 339–371.

Greer, A.E., 1990. The Biology and Evolution of Australian Lizards. Surrey Beatty and Sons, Australia.

Günther, A., 1877. The extinct reptiles of Rodriguez. Phil. Trans. R. Soc. Lond. B 168 (extra volume), 452–456.

Hardy, G.S., 1979. The karyotypes of two scincid lizards, and their bearing on relationships in the genus *Leiolopisma* and its relatives (Scincidae: Lygosominae). New Zealand J. Zool. 6, 609–612.

Hickson, R.E., Simon, C., Cooper, A., Spicer, G.S., Sullivan, J., Penny, D., 1996. Conserved sequence motifs, alignment, and secondary structure for the third domain of animal 12S rRNA. Mol. Biol. Evol. 13, 150–169.

Honda, M., Ota, H., Kobayashi, M., Nabhitabhata, J., Yong, H.-S., Hikada, T., 2000. Phylogenetic relationships, character evolution, and biogeography of the subfamily Lygosominae (Reptilia: Scincidae) inferred from mitochondrial DNA sequences. Mol. Phylogenet. Evol. 15, 452–461.

Huelsenbeck, J.P., Ronquist, F.R., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Hutchinson, M.N., Donnelan, S.C., Baverstock, P.R., Krieg, M., Simms, S., Bergin, S., 1990. Immunological relationships and generic revision of the Australian lizards assigned to the genus *Leiolopisma* (Scincidae: Lygosominae). Aust. J. Zool. 38, 535–554.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.

Maca-Meyer, N., Carranza, S., Rando, J.C., Arnold, E.N., Cabrera, V.M., 2003. Status and relationships of the extinct giant Canary Island lizard Gallotia goliath (Reptilia: Lacertidae), assessed using ancient mtDNA from its mummified remains. Biol. J. Linn. Soc. 80, 659–670.

Mausfeld, P., Schmitz, A., Böhme, W., Misof, B., Vrcibradic, D., Rocha, C.F.D., 2002. Phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945, endemic to the Atlantic Ocean archipelago of Fernando de Noronha (Brazil): necessity of partitioning the genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). Zool. Anz. 241, 281–293.

McDougall, I., 1971. The geochronology and evolution of the young volcanic island of Réunion, Indian Ocean. Geochim. Cosmochim. Acta 35, 261–288.

McDougall, I., Chamalaun, F.H., 1969. Isotopic dating and geomagnetic polarity studies on volcanic rocks from Mauritius, Indian Ocean. Geol. Soc. Am. Bull. 80, 1419–1442.

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer, Sunderland, MA, pp. 205–247.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Reeder, T.W., 2003. A phylogeny of the Australian *Sphenomorphus* group (Scincidae: Squamata) and the phylogenetic placement of the crocodile skinks (*Triblonotus*): Bayesian approaches to assessing congruence and obtaining confidence in maximum likelihood inferred relationships. Mol. Phylogenet. Evol. 27, 384–397.

Saint, K.M., Austin, C.C., Donnellan, S.C., Hutchinson, M.N., 1998. C-*mos*, a nuclear marker useful for squamate phylogenetic analysis. Mol. Phylogenet. Evol. 10, 259–263.

Shimodaira, H., Hasegawa, M., 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16, 114–116.

Swofford, D.L., 2000. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4. Sinauer Associates, Sunderland, MA.

Whiting, A.S., Bauer, A.M., Sites, J.W., 2003. Phylogenetic relationships and limb loss in sub-Saharan African scincine lizards (Squamata: Scincidae). Mol. Phylogenet. Evol. 29, 582–598.