



Phylogeography and population structure of the Reeve's Butterfly Lizard (*Leiolepis reevesii*) inferred from mitochondrial DNA sequences

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ABSTRACT

Butterfly lizards of the genus *Leiolepis* (Agamidae) are widely distributed in coastal regions of Southeast Asia and South China, with the Reeve's Butterfly Lizard *Leiolepis reevesii* having a most northerly distribution that ranges from Vietnam to South China. To assess the genetic diversity within *L. reevesii*, and its population structure and evolutionary history, we sequenced 1004 bp of cytochrome *b* for 448 individuals collected from 28 localities covering almost the whole range of the lizard. One hundred and forty variable sites were observed, and 93 haplotypes were defined. We identified three genetically distinct clades, of which Clade A includes haplotypes mainly from southeastern Hainan, Clade B from Guangdong and northern Hainan, and Clade C from Vietnam and the other localities of China. Clade A was well distinguished and divergent from the other two. The Wuzhishan and Yinggeling mountain ranges were important barriers limiting gene exchange between populations on the both sides of the mountain series, whereas the Gulf of Tonkin and the Qiongzhou Strait were not. One plausible scenario to explain our genetic data is a historical dispersion of *L. reevesii* as proceeding from Vietnam to Hainan, followed by a second wave of dispersal from Hainan to Guangdong and Guangxi. Another equally plausible scenario is a historically widespread population that has been structured by vicariant factors such as the mountains in Hainan and sea level fluctuations.

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1. Introduction

The Indo-Burma region encompasses more than 2 million km² of tropical Asia west of Wallace's Line, and is one of the most threatened biodiversity hotspots in the world. The hotspot begins in eastern Bangladesh and extends across northeastern India, parts of Yunnan Province of China, Myanmar, Vietnam, Malaysia, Thailand, Cambodia and Laos (Fig. 1); it also covers the coastal lowlands of South China (Guangxi and Guangdong), as well as several offshore islands such as Hainan Island (of China) in the South China Sea and Andaman Islands (of India) in the Andaman Sea. One driver of biodiversity in this region is the repeated accretion of different microcontinents with a megacontinent such as Asia (Macey et al., 2000). Tectonic processes stimulate episodic restructuring of continental faunas in two ways: faunal diversity remains relatively stable during times of isolation, whereas rapid faunal turnover occurs during times of contact between tectonic plates (Macey et al., 2000). The Indo-Burma hotspot currently holds remarkable endemism in animal species including agamid lizards of the genus *Leiolepis* that are

threatened with local extinction due to over-harvesting, habitat loss, and habitat fragmentation.

Lizards of the genus *Leiolepis* are widely distributed in coastal lowlands of the Indo-Burma hotspot (Fig. 1; Jiang, 1999; Malysheva et al., 2006). The genus contains seven species of large-sized (up to 150 mm snout-vent length) oviparous lizards, with the Reeve's Butterfly Lizard *Leiolepis reevesii* having a most northerly distribution that ranges from Vietnam to South China (Guangdong, Guangxi and Hainan) (Jiang, 1999). *Leiolepis* lizards occur only on the Southeast Asian blocks that broke from the northern margin of the Australia–New Guinea plate hundreds of MYBP and accreted to Asia 120 MYBP (Richter and Fuller, 1996) or earlier (Metcalf, 1996). Macey et al. (2000) suggested that the Southeast Asian plates introduced *Leiolepis* species complex to Asia and South China. Only one species (*L. reevesii*) occurs in China, in the south.

Hainan Island, lying in the northeastern margin of the Indo-Burma hotspot, is influenced by glacially driven eustatic sea-level changes that have caused recent land connections and disconnections among Sumatra, Java, Borneo and the Asian mainland (Hall and Holloway, 1998). The Island is topographically diverse, with the Wuzhishan and Yinggeling Mountains approaching an elevation of 1800 m, rising steeply from the central and southern

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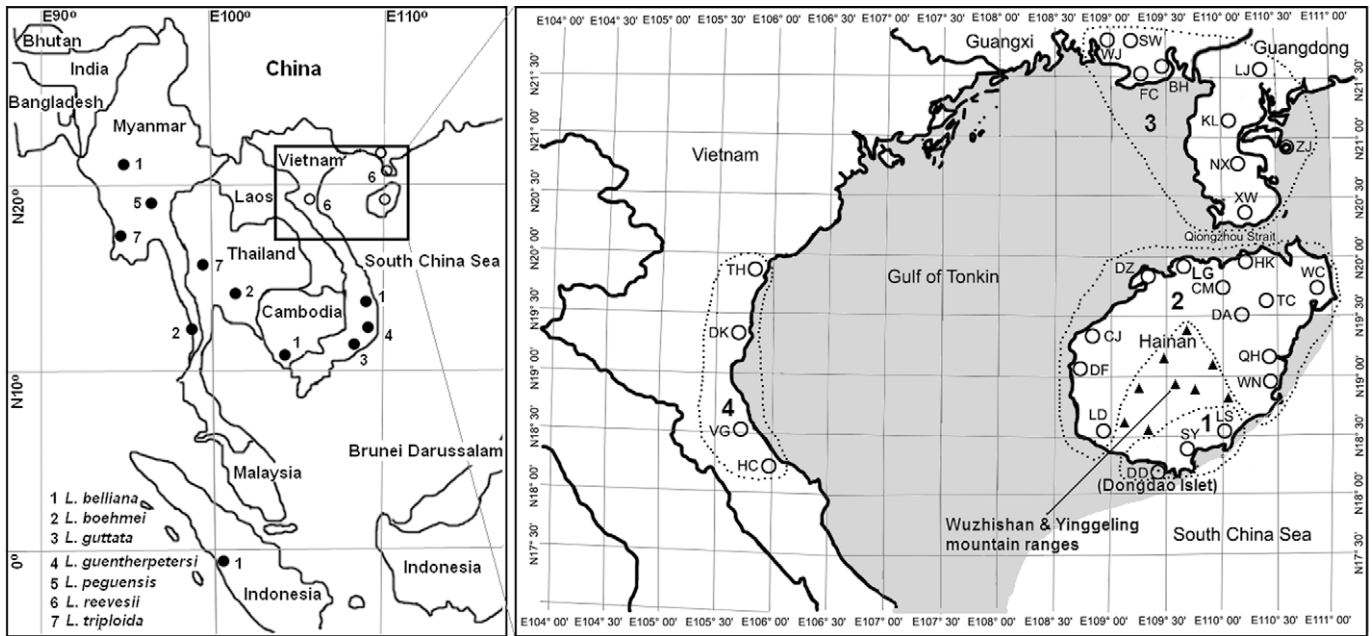


Fig. 1. Map of the distribution of the genus *Leiolepis* (left) and sampling locations of *L. reevesii* in this study (right). The Arabic numerals in the right plot represent populations grouped according to geographical areas. Shading indicates shoreline limits at times of Pleistocene maximum glaciation. See Table 1 for abbreviation of sample sites. ▲: The mountain series in Hainan.

regions and giving way to a broad northern plain. However, whether these mountains play an important role in influencing gene exchange among populations of organisms such as *L. reevesii* remains unclear.

The quantification of genetic variability and population genetic structure is crucial for improved management and conservation of natural populations (Avice, 1989; Marmi et al., 2006), yet genetic variation in *Leiolepis* has received little empirical scrutiny. Malysheva et al. (2006) examined intraspecific genetic variation of *L. reevesii* by using multilocus DNA fingerprinting with several microsatellite probes, but their samples were localized in central Vietnam. Until now, there are no comparable sequence data on *L. reevesii* from geographically separated populations, although such data are urgently needed to elucidate the evolutionary history of the species and contemporary population genetic structure and to guide the development of appropriate management and conservation strategy for the lizard.

The effects of historical events on faunal movements outlined by Macey et al. (2000) and the range of *L. reevesii* in South China raise several questions that form the basis of this study: (1) How did *Leiolepis* extend its historical range into China? Did the dispersal route proceed from Vietnam in a northerly direction to Chinese mainland and then southerly from Chinese mainland to Hainan, or easterly from Vietnam to Hainan and then northerly from Hainan to Chinese mainland? (2) How important were the Wuzhishan and Yinggeling mountain ranges and the sea barrier in the Gulf of Tonkin and the Qiongzhou Strait to gene flow (Fig. 1)? (3) What is the demographic history of *L. reevesii* and the events that shaped its population demography? (4) What is the level of genetic variability among populations of *L. reevesii* and how is the variation partitioned within populations?

2. Materials and methods

2.1. Sample collection and DNA extraction

To answer the above questions, we sequenced the mitochondrial cytochrome *b* gene from 448 individuals. The study sample

represents 16 adults of *L. reevesii* collected between 2007 and 2008 from each of 28 sampling localities almost covering the species' distribution range from Chinese mainland (Guangdong and Guangxi) to Hainan (the largest island second to Taiwan in China) and Vietnam (Fig. 1). During our collecting trip to the coastal region of Vietnam and Guangxi (a province of China), we did not find any *L. reevesii* in the north of Vietnam and in the west of Guangxi, close to Vietnam. This is the region where the microcontinental blocks of Southeast Asia and the megacontinent of Asia contacted (Fig. 1), currently having no habitat suitable for *L. reevesii*.

The most distal 15 mm of the tail tip of each lizard was excised using a sterilized scalpel. Individual lizards were released at their site of capture after tissue sampling. Tissue samples were preserved in 95% ethanol before they were deposited at Hangzhou Normal University and assigned voucher numbers identified by locality-haplotype numbers. Total DNA was extracted using standard phenol–chloroform methods (Sambrook et al., 1989), and stored at -80°C until ready for use.

2.2. Mitochondrial DNA amplification and sequencing

We used primers L14910 (5'-GAC CTG TGA TMT GAA AAA CCA YCG TTG T-3') (de Queiroz et al., 2002) and H16064 (5'-CTT TGG TTT ACA AGA ACA ATG CTT TA-3') (Burbrink et al., 2000) to amplify the cytochrome *b* gene. Mitochondrial DNA was amplified from template DNA in 100 μl reactions using a hot-start method in a thermal cycler with a 7-min denaturing step at 94°C followed by 40 cycles of denaturing for 40 s at 94°C , primer annealing for 30 s at 46°C and elongation for one min at 72°C with a final 7-min elongation step at 72°C . Cycle sequencing was conducted with primer L14919 (Burbrink et al., 2000), L15584 (de Queiroz et al., 2002), H15149 (Kocher et al., 1989), and H15715 (Slowinski and Lawson, 2005).

2.3. Data analysis

The sequences were translated to amino acids with the program squnt (www.cebl.auckland.ac.nz/index.php) to verify if a func-

tional mitochondrial DNA sequence was obtained and that nuclear pseudogenes are not being amplified. We compiled and aligned sequences using mega version 3.1 (Kumar et al., 2004). We used dnasp version 4.0 (Rozas et al., 2003) to identify haplotypes and estimate genetic diversity within populations by haplotype (h) and nucleotide diversities (π) (Nei, 1987).

We reconstructed a phylogenetic tree based on the maximum likelihood (ML) and a Bayesian method, using the Chinese Water Dragon *Physignathus cocincinus* (GenBank accession no. AB263945) as the outgroup. ML analysis was carried out by a heuristic search of 10 random addition analyses with tree-bisection-reconnection (TBR) branch swapping using paup version 4.0 beta (Swofford, 2003). The TIM + I + G substitution model (Tamura and Nei, 1993) was selected by modeltest version 3.7 (Posada and Crandall, 1998) based on the Akaike information criterion (AIC; Akaike, 1974). The confidence level of the nodes in the ML tree was estimated using 1000 bootstrap pseudoreplicates. Bayesian analysis was carried out using mrbayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). We performed two Metropolis-coupled Markov chain Monte Carlo runs, started from a random tree and ran the analyses for 1×10^7 generations in mrbayes. The chains were sampled every 1000 generations. A substitution model was selected for each codon position using kakusan version 2.1 (Tanabe, 2007) with paup version 4.0 beta. After discarding the first 4200 trees as burn-in, we obtained the 50% majority rule consensus tree and the posterior probabilities of nodes in the tree. To confirm the tree topology, the MCMC analysis was repeated in triplicate. We also conducted a median-joining network (MJN) approach (Bandelt et al., 1999) to depict relationships among the haplotypes. This approach has been shown to yield the best-resolved genealogies relative to other rooting and network

procedures (Cassens et al., 2003). The MJN was estimated using network 4.5.0.0 software (Bandelt et al., 1999).

We performed hierarchical analysis of molecular variance (amova) in Arlequin 3.0 (Excoffier et al., 2005) with 10,000 permutations to examine partitioning of genetic diversity within and among populations. We used mismatch distributions to test demographic signatures of population expansions within mtDNA lineages (Rogers, 1995). To compare observed distributions with those expected under the expansion model, we calculated the sum of square deviation (SSD) and the Harpending's raggedness index (Harpending, 1994). Fu's F_s test (Fu, 1997) was used to test equilibrium of the populations. This statistics was expected to have large negative values under demographic expansion. The equation $\tau = 2ut$ (Rogers and Harpending, 1992) was used to estimate the approximate expansion time in generations (t), where τ is the date of the growth or decline measured in units of mutational time and u is the mutation rate per sequence and per generation. The value of u was calculated from $u = 2\mu k$, where μ is the mutation rate per nucleotide and k is the number of nucleotides of the analyzed fragment. Finally, the approximate time of expansion in years was calculated by multiplying t by the generation time of *L. reevesii*. The generation time for *L. reevesii* was estimated as three years based on approximate time at which animals become mature. The substitution rate of mtDNA cytochrome *b* estimated from various phylogenetic studies for a variety of lizards were in a range of 0.005–0.010 site⁻¹ myr⁻¹ (e.g. Macey et al., 1998; Carranza et al., 2001; Thorpe and Stenson, 2003; Jesus et al., 2005; Thorpe et al., 2005; Howes et al., 2006). We used the upper and lower values to estimate the overall range of potential dates. These statistics were estimated and tested for significance by random permutation (10,000 replicates).

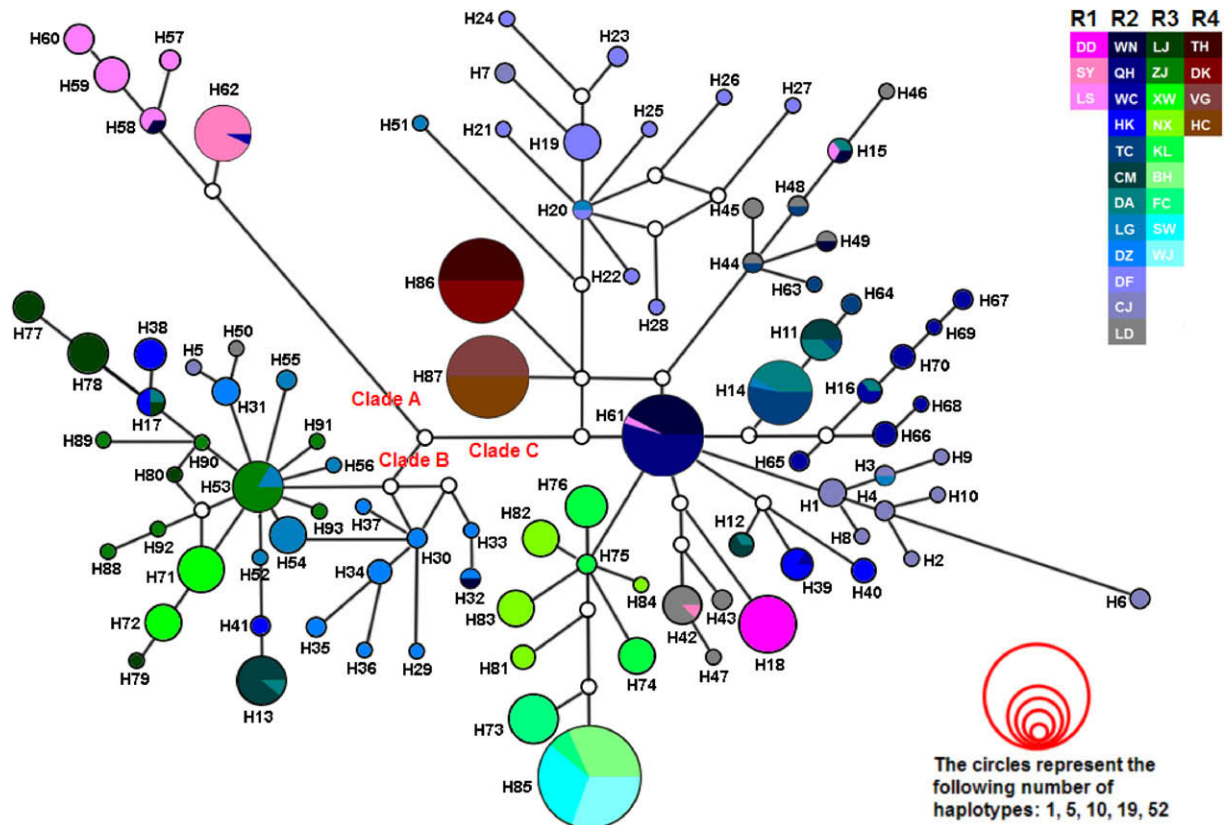


Fig. 2. Network of 93 mitochondrial cytochrome *b* haplotypes from 448 individuals of *L. reevesii*. The size of the circles is proportional to haplotype frequency; small open circles represent unsampled haplotypes. See Table 1 for abbreviation of sample sites. Locations from the same region had gradations of the same color(s). R1–4: Region 1–4.

3. Results

3.1. Genetic diversity

The 1004 bp mitochondrial cytochrome *b* gene sequences (deposited at GenBank under accession number EU304980–EU305052 and FJ599644–FJ599663) were obtained from 448 individuals after alignment. A total of 140 variable nucleotide sites (of which 75 were parsimony-informative), comprising 129 transitions (ts) and 13 transversions (tv) (both ts and tv at the 768th and the 828th site), defined 93 haplotypes.

The distribution of haplotypes among the 28 localities is shown in Fig. 2. The number of haplotypes within each locality ranged from 1 to 10. Haplotype diversity (*h*) ranges from 0 to 0.925, and nucleotide diversity (π) from 0 to 0.984%. For the whole sample, *h* was 1.000 and π was 1.338%, indicating high levels of haplotype and nucleotide diversity (Table 1).

3.2. Phylogenetic relationships between haplotypes and populations

The phylogenetic trees resulting from ML and the Bayesian methods were similar to each other, and revealed three clades (Fig. 3). This finding was also supported by the median-joining network (Fig. 2). Clade A includes haplotypes mainly from southeastern Hainan, Clade B from Guangdong and northern Hainan, and Clade C from Vietnam and the other localities of China (Fig. 2).

Clade A was divergent from the other two clades by at least 16 mutation steps (not shown in Fig. 2).

3.3. Population structure

Analysis of molecular variance indicated that a small but significant ($P < 0.001$) proportion (11.6%) of the total variation in the mitochondrial DNA data were attributable to differences among groups: [Region 1] [Region 2] [Region 3] [Region 4] (see Fig. 1 for the definition of each region). A significant ($P < 0.0001$) proportion of variation also occurred among populations within groups (42.5%) and within populations (45.9%). More than half (54.1%) of the total genetic variation occurred among the geographic regions and among populations within the regions.

3.4. Historical demography

Significantly large negative values of Fu's *F_s* test rejected the null hypothesis of neutral evolution of the cytochrome *b* marker for the population group in Region 2. This could indicate population expansion in Region 2, which is supported by the mismatch distribution analysis and Rogers test of sudden population expansion (Rogers, 1995; Table 1). The value of τ for the Region 2 population group was 13.966 (Table 1). For the substitution rate for a variety of lizards were in a range of 0.005–0.010 site⁻¹ myr⁻¹, the expansion time was estimated to be between 1.043 and 2.087 mya.

Table 1
Samples sites, number of haplotypes (*N*), haplotype diversity (*h*), nucleotide diversity (π), Fu's *F_s*, τ , sum of square deviation (SSD), and Harpending's raggedness index (HRI) for *Leiolepis reevesii*.

Code	Sample site	Genetic diversity			Neutrality test	Mismatch distribution		
		<i>N</i>	<i>h</i>	π (%)		Fu's <i>F_s</i>	SSD	HRI
SY	Sanya ¹	2	0.125	0.262	6.062 ^{NS}	0.023 ^{**}	0.797 ^{NS}	
LS	Lingshui ¹	6	0.808	0.618	2.742 ^{NS}	–	–	
DZ	Danzhou ²	9	0.908	0.524	–0.693 ^{NS}	0.018 ^{NS}	0.042 ^{NS}	
CM	Chengmai ²	3	0.567	0.887	10.570 ^{NS}	0.341 ^{***}	0.697 ^{NS}	
LG	Lingao ²	9	0.858	0.743	0.349 ^{NS}	0.052 ^{NS}	0.047 ^{NS}	
HK	Haikou ²	5	0.825	0.984	6.545 ^{NS}	0.087 ^{**}	0.151 [†]	
XW	Xuwen ³	2	0.500	0.050	1.247 ^{NS}	–	–	
ZJ	Zhanjiang ³	7	0.625	0.132	–3.068 ^{**}	0.011 ^{NS}	0.063 ^{NS}	2.909
LJ	Lianjiang ³	5	0.683	0.183	0.102 ^{NS}	0.055 ^{NS}	0.202 ^{NS}	
WC	Wenchang ²	9	0.925	0.518	–0.727 ^{NS}	0.011 ^{NS}	0.032 ^{NS}	
TC	Tunchang ²	6	0.617	0.287	0.300 ^{NS}	0.048 ^{NS}	0.123 ^{NS}	
DA	Dingan ²	7	0.742	0.571	1.376 ^{NS}	0.041 ^{NS}	0.069 ^{NS}	
QH	Qionghai ²	1	0	0	–	–	–	
WN	Wanning ²	5	0.450	0.472	2.973 ^{NS}	0.108 ^{NS}	0.380 ^{NS}	
DD	Dongdao Islet ¹	1	0	0	–	–	–	
LD	Ledong ²	9	0.858	0.745	0.359 ^{NS}	0.045 ^{NS}	0.076 ^{NS}	
DF	Dongfang ²	10	0.867	0.341	–3.213 [*]	0.016 ^{NS}	0.052 ^{NS}	4.235
CJ	Changjiang ²	10	0.925	0.771	–0.415 ^{NS}	0.039 ^{NS}	0.043 ^{NS}	
TH	Thanh Hoa ⁴	1	0	0	–	–	–	
DK	Dong Ky ⁴	1	0	0	–	–	–	
VG	Vinh Gia ⁴	1	0	0	–	–	–	
HC	Hau Con ⁴	1	0	0	–	–	–	
NX	Nanxing ³	4	0.725	0.259	2.188 ^{NS}	0.088 [*]	0.221 ^{NS}	
KL	Kelu ³	3	0.633	0.153	2.039 ^{NS}	0.092 ^{NS}	0.306 ^{NS}	
BH	Beihai ³	1	0	0	–	–	–	
FC	Fucheng ³	2	0.400	0.080	2.177 ^{NS}	0.147 [*]	0.680 ^{NS}	
SW	Shiwan ³	1	0	0	–	–	–	
WJ	Wujia ³	1	0	0	–	–	–	
Region 1	Southeastern Hainan	9	0.780	1.150	9.327 ^{NS}	0.093 ^{**}	0.132 ^{**}	
Region 2	The remaining part of Hainan	79	0.966	1.001	–24.210 ^{***}	0.002 ^{NS}	0.003 ^{NS}	13.966
Region 3	Guangxi and Guangdong	26	0.937	1.057	1.288 ^{NS}	0.037 ^{NS}	0.052 [*]	
Region 4	Vietnam	2	0.508	0.253	9.347 ^{NS}	0.324 ^{***}	0.758 [*]	

NS, not significant. Superscript denotes Region.

^{*} 0.05 $\geq P \geq$ 0.01.

^{**} 0.01 $> P \geq$ 0.001.

^{***} $P < 0.001$.

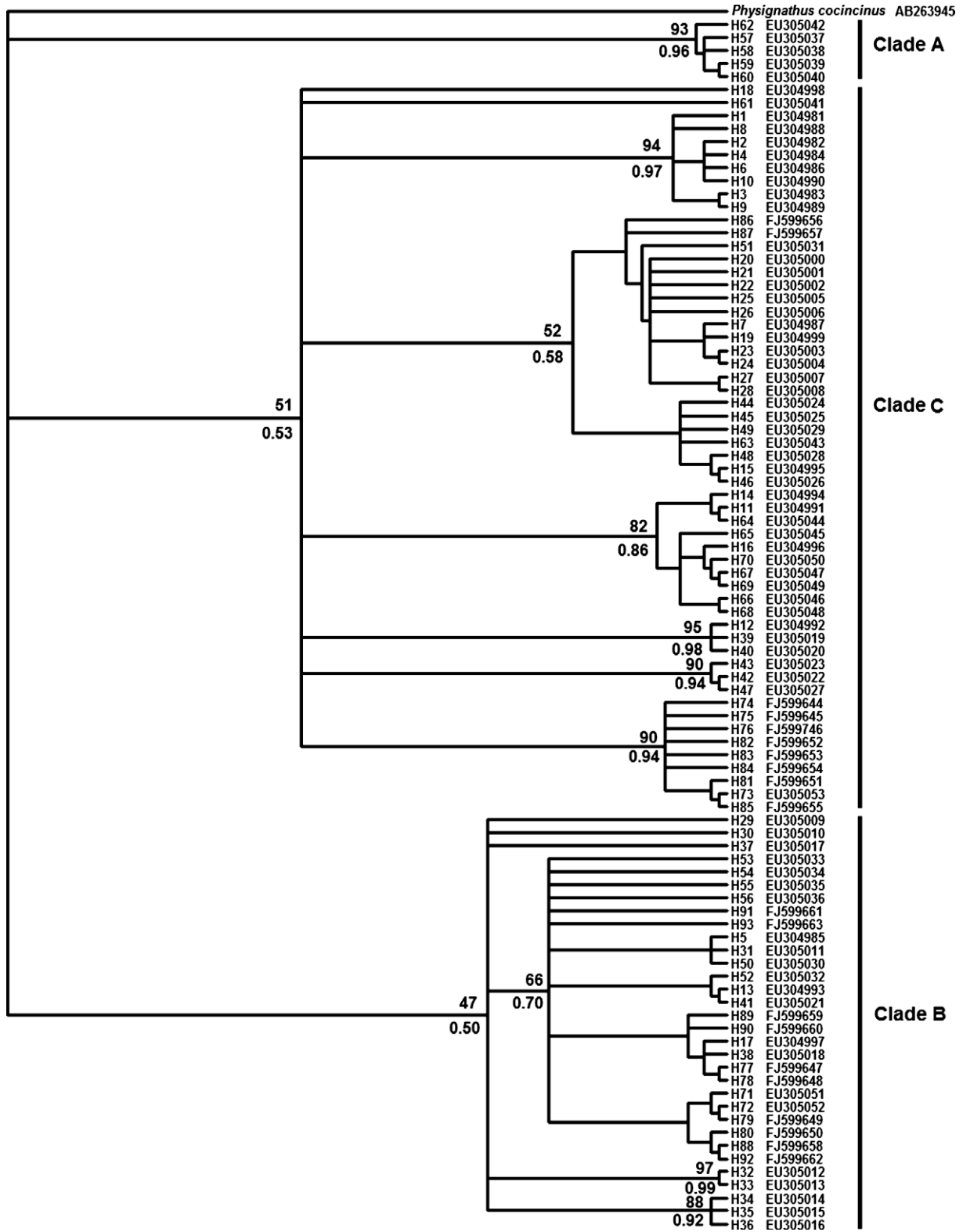


Fig. 3. Maximum likelihood tree of *L. reevesii*. Values above branches represent ML bootstrap values; values below branches represent Bayesian clade credibility values. GenBank accession numbers were given after each haplotype and the outgroup.

4. Discussion

4.1. Divergence between major clades

Clade A was well distinguished and divergent from the other two clades by at least 16 mutations steps. This result provides

strong evidence that the Wuzhishan and Yinggeling mountain ranges were important barriers limiting gene exchange between populations on the both sides of the mountain series.

The presence of common haplotypes among (Regions 2 and 3) populations from Dingan (DA), Haikou (HK), Lianjiang (LJ), Lingao (LG) and Zhanjiang (ZJ) on either the south or the north side of

the Qiongzhou Strait (Fig. 2) suggests that, historically, the Strait did not act as an important barrier to gene flow. In fact, Hainan and Guangdong had been connected and disconnected several times since the Qiongzhou Strait first appeared some 2.5 million years ago, with the last separation occurring some 10,000 years ago (Chen and Fan, 1988; Zhao et al., 1999). It is therefore not surprising that lizards such as *L. reevesii* could exchange genes between populations when Guangdong and Hainan contacted.

Phylogenetically, populations in Region 2 (the remaining part of Hainan) and Region 4 (Vietnam) are most closely related (Figs. 2 and 3). Region 2 is closest to Region 4, although the two regions are isolated by the Gulf of Tonkin (Fig. 1). This suggests the Gulf of Tonkin was not an important barrier to gene flow. This result can be explained by Pleistocene sea-level changes. According to the Pleistocene Sea Level Maps (Voris, 2000, updated at <http://www.fieldmuseum.org>), at times of maximum glaciation, the sea level was lower and the “Gulf” of Tonkin was not covered by water (Fig. 1). So the dispersal of these lizards and their range would not have been constrained by water body. Haplotypes from Vietnam (H86 and H87) were deeply embedded in the haplotype network (Fig. 2) and phylogenetic tree (Fig. 3) and so it was clear that they were relatively “old” haplotypes. This result supports the conclusion that *Leiolepis* originated in Southeast Asia (Macey et al., 2000). It is puzzling however that the genetic diversity was low despite the populations been ‘old’. One plausible explanation was that populations had gone through a recent bottleneck.

4.2. Historical demography

The populations from Region 2 showed the genetic imprints of a demographic expansion as inferred by a negative F_s test, which was also supported by the mismatch distribution analysis and Rogers test of sudden population expansion (Rogers, 1995; Table 1). The expansion time was estimated to be between 1.043 and 2.087 mya. However, the rise of the Tibet–Qinghai Plateau, which is believed to have a profound influence on the organisms on the Tibetan plateau itself and its neighboring areas including Hainan and Guangdong (Harrison et al., 1992; Molnar et al., 1993; Shi et al., 1998), occurred 0.60–1.10 million years ago (Zheng, 1981; Shi et al., 1998), later than the expansion time. Glaciation and deglaciation and the accompanying lowering and rising of sea levels during the Pleistocene is known to have greatly affected land mass configurations and plant and animal distributions in Southeast Asia (Voris, 2000). Given that these lizards are associated with sand at the seaside, sea-level changes are more important for these lizards than the rise of the Tibet–Qinghai Plateau. Depending on the size of a land mass and its connectivity to other land masses, sea level fluctuations could have led to local extinctions of some populations or cause populations to be isolated reproductively. Subsequent dispersal and accumulation of genetic differences over time are possible evolutionary mechanisms that account for the genetic divergence observed in this study.

4.3. Genetic diversity and dispersal

Low genetic diversity could be due to a number of factors, one of which is overexploitation (Kochzius and Nuryanto, 2008). Overexploitation of *L. reevesii* as a dietary item and for medicinal use is the main cause of population decline over the past three decades (Zhao, 1998). In Clade A [individuals from the two neighboring localities of Lingshui (LS) and Sanya (SY)], for example, high genetic diversity is found in LS (a less disturbed locality by human) and low genetic diversity is observed in disturbed and exploited populations in SY, a developed travel industry site where hunting pressure is extremely high.

The median-joining network shows that H85 on one tip of the network is a young haplotype (Fig. 2), shared by all individuals from Beihai (BH), Shiwan (SW) and Wujia (WJ), and four (25%) individuals from Fucheng (FC). This result provides evidence showing that BH, SW and WJ are most recently colonized by *L. reevesii* in Region 3. Clade C includes haplotypes from Region 3 that exhibit a trend of northwardly decline in nucleotide diversity with the value of 0 inferred from cytochrome *b* in the BH, WJ and SW populations (Fig. 2), showing a northward dispersal route. It is known that ancestral populations often lose their genetic diversities during their dispersal or expansion and contraction phases (e.g. Yang et al., 2004). Low genetic diversity in these populations presumably reflects the consequence of a founder effect. Genetic diversity is lower in Kelu (KL) than in Nanxing (NX) but higher in KL than in Fucheng (FC). This geographic trend highlights the importance of dispersal in shaping the spatial pattern of genetic diversities at that site.

Given that haplotypes from Vietnam is relatively “old” (ancient) and that haplotypes from Guangdong and Guangxi (Region 3) is relatively “new” (recent) (Fig. 2), one plausible scenario to explain our genetic data is a historical dispersion of *L. reevesii* as proceeding from Vietnam (Region 4) to Hainan (Region 1 and Region 2), followed by a second wave of dispersal from Hainan to Guangdong and Guangxi (Region 3). However, another equally plausible scenario is a historically widespread population that has been structured by vicariant factors such as the mountains in Hainan and sea level fluctuations. Isolation caused by the orogenesis and subsequent genetic drift, together with adaptation to local environments led to genetic differentiation and further speciation of *Leiolepis* (Macey et al., 2000). In this study, the phylogenetic tree indicates a three-way polytomy among the three major Clades (A, B and C), thus provides no evidence that any one clade is older than the other two (as would be expected in a dispersal scenario). The three-way polytomy might be the natural result of vicariant events subdividing widespread populations (though undoubtedly Clade A diverged from Clades B and C much earlier than B from C). AMOVA showed that more than half of the total genetic variation occurred among the regions and among population within the regions, indicating high levels of geographical structuring and restricted gene flow. This population genetic structure could be largely due to the fact that the lizard has a limited ability to disperse. Herpetiles including *L. reevesii* that are less likely to disperse are more prone to suffering substantial losses in genetic diversity resulting from habitat loss and fragmentation (Taylor et al., 1994; Bouzat et al., 1998; Wisely et al., 2002; Lin et al., 2007). This was a factor to be considered when evaluating dispersal versus vicariance scenarios. At this time, there is no overwhelming evidence supporting one scenario over another. Thus, it may have the benefit that alternative scenarios are evaluated in the future with additional genetic data (microsatellites might be very useful here) or by comparing similar taxa for genetically congruent patterns.

Acknowledgments

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