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Genetic Diversity and Population Structure of the Oriental Garden Lizard, *Calotes versicolor* Daudin, 1802 (Squamata: Agamidae) along the Mekong River in Thailand and Lao PDR

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Abstract *Calotes versicolor* Daudin, 1802, is geographically widespread along the Mekong River basin. The Mekong River is play important role as a significant natural barrier to several terrestrial animals living on different sides. This study aims to analyze the genetic diversity and population structure of *C. versicolor* populations collected from different sides of Mekong River using mitochondrial cytochrome *c* oxidase subunit 1 (*CO1*) sequences. We obtained sequences of 200 individuals from 18 sampling localities from left and right sides of the Mekong River in Lao PDR and Thailand respectively. Overall, 91 haplotypes were detected, which reflect high levels of genetic diversity in this species at the study areas. Haplotype network and phylogenetic analyses revealed that there were six major lineages (lineage C – lineage H) of *C. versicolor* populations within the Mekong River, whereas lineages A and B have previously been found from China and Vietnam. The genetic distance among *C. versicolor* was significantly related to spatial distance, however, the Mekong River had no significant effect on genetic distance. Our findings, together with previous studies, suggests that *C. versicolor* in Asia is a species complex with other cryptic lineages being likely but there is a need for further exploration. Thus, comprehensive genetic, biological and ecological studies of *C. versicolor* should be conducted throughout its entire distribution range.

Keywords Agamid, *CO1*, haplotype, lizard, phylogeny, reptile

1. Introduction

The Oriental garden lizard, *Calotes versicolor* Daudin, 1802, is one of a few geographically widespread tropical lizards (Radder, 2006). *Calotes versicolor* is also known as the "changeable crested lizard", due to its wide variation in coloration and ability to change colors significantly during the breeding season (Ji *et al.*, 2002). Recent field surveys confirm its distribution stretching from Oman in the West, across Southern and South-East Asia to the East, the Maldives, Réunion, Mauritius, the Seychelles, and more recently it has been introduced to Florida in the United States of America (Radder, 2006). This lizard is naturally found in open forest and shrub but has adapted tremendously well to urban environments and can be found in agricultural areas, parks, empty lots and gardens where it is usually seen off the ground in low vegetation (Radder, 2006). It helps to control insect populations, occasionally feeding on

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small lizards, mollusks, crustaceans, baby birds and rodents, or seeds (Sudasinghe and Somaweera, 2015). It is also a prey item of snakes and birds (Matyot, 2004), as well as being used for cooking in some localities in Southeast Asia (unpublished data).

In Thailand, the local people, especially in the northeastern region (I-san) often cook and eat several lizards in the genera *Leiolepis*, *Varanus* and *Calotes*. It is a famous dish for I-san people called "Koi Kapom", which is 'spicy lizard salad' (unpublished data). It is hunted massively in its breeding season (around March–April). Therefore, populations of *C. versicolor* may be dramatically reduced in the near future. It is important to gather information relating to its habitat, biology, and ecology, including genetic diversity, in order to facilitate the conservation of this species. A comprehensive genetic investigation of *C. versicolor* was previously conducted in Hainan Island and adjacent mainland China, including a locality in northern Vietnam and found high genetic variation with two major lineages, lineage A and B (Huang *et al.*, 2013). But there has been no study investigating the genetic diversity within *C. versicolor* in Southeast Asia. There are, however, morphometric studies comparing *C. versicolor* in the Lower Mekong Basin in Thailand and Lao PDR (Thongnetr *et al.*, 2015), and southern and northern Thailand (Prakobkarn *et al.*, 2016), which show some significant differences in characteristics between different populations. This suggests that *C. versicolor* in Thailand may comprise a number of cryptic lineages within a species complex.

The Mekong River flows 4800 km from Tibet through China, Myanmar, Lao PDR, Thailand, Cambodia, to its delta in Vietnam, and then into the South China Sea, draining an area of 795 000 km^2 (Mekong River Commission, 2011). The river forms and extensive sections of the border between Thailand from Lao PDR, except in the northern region. The Mekong River is also known as a significant natural barrier to gene flow for some freshwater and terrestrial animals living on different sides of it (Saijuntha *et al.*, 2019; Tantrawatpan *et al.*, 2020). This study aims to test whether the Mekong River plays an important role as a natural barrier to gene flow among populations of the lizard. Thus, *C. versicolor* populations living on the left and right sides of the Mekong River in Lao PDR and Thailand were collected and examined to address these goals. The genetic diversity and genetic structure of *C. versicolor* populations were also examined. In order to make meaningful comparison of our work with the previous study of Huang *et al.*, (2013), the same DNA region, namely mitochondrial cytochrome c oxidase subunit 1 (*CO1*), was chosen as a marker. This maternally inherited marker has proven to be useful in population genetic studies, including the definition of cryptic species of the *Calotes* (Huang *et al.*, 2013; Saijuntha *et al.*, 2017; Saijuntha *et al.*, 2020).

2. Materials and Methods

2.1. Specimen collection and DNA extraction A total of 200 specimens of *C. versicolor* were sampled from 18 different localities along the lower Mekong River in Thailand and Lao PDR (Figure 1 and Table 1). Adult *C. versicolor* were caught using the fishing pole method (Saijuntha *et al.*, 2017). Their tails were excised around 5 mm from the end, soaked in 80% alcohol and kept at room temperature until DNA extraction. After collection of the apical portion, the lizard's tails were cleaned with 70% alcohol and the animals released back into their natural habitat. Total genomic DNA was extracted using E.Z.N.A.® Tissue DNA kit (Omega bio-tek, USA) following the manufacture's protocol. All DNA samples were kept at –20 °C until used.

2.2. Polymerase Chain Reaction and DNA sequencing A partial region of the mitochondrial cytochrome *c* oxidase subunit 1 (*CO1*) gene was amplified with the primers L5037 (5'-GAG TAG ACC CAG GAA CCR AAG TTC-3') and H6448 (5'-GTA TAC CGG CTA ATC CAA GCA TGT-3') (Huang *et al.*, 2013). Standard polymerase chain reactions (PCR) were performed in a total of 25 μL, including approximately 100 ng of template DNA, 1 μL of each primer (each 10 pmol/μL), 2.5 μL

Figure 1 A map of the 18 collection localities of *Calotes versicolor* aside the Mekong River in Thailand and Lao PDR.

of 10x *Ex-Taq* buffer (Mg^{2+} plus), 2 μL dNTPs (each 2.5 mmol/L), 0.125 μL of *Ex-Taq* DNA polymerase (5 U/μL), and deionized water. PCR was conducted with the following conditions: an initial denaturing step at 95 °C for 4 min; 35 cycles of denaturing at 94 °C for 35 s, annealing at 65 °C for 45 s, and extending at 72 °C for 90 s; and a final extending step of 72°C for 8 min. PCR products were electrophoresed in 1% agarose gels, visualized with GelRed™ Nucleic Acid Gel Stain (Biotium, Inc., Hayward, CA). The amplified band was cut and purified by using E.Z.N.A.® Gel Extraction kit (Omega bio-tek, USA). The purified PCR products were cycle-sequenced at Eurofins Genomics Company, Japan. All new sequences were deposited in GenBank under accession numbers MT438484 to MT438683.

2.3. Sequence and haplotype analyses All sequences generated in this study were checked and edited using the software program ABI sequence scanner v1.0. Multiple sequence alignment was performed using a BioEdit version 7.2.6 (Hall, 1999). Molecular variation indices and population structure patterns based on global AMOVA were calculated in Arlequin ver 3.5.1.3 (Excoffier and Lischer 2010). Genetic distance (*P*-distance) was calculated using MEGA X (Kumar *et al.*, 2018). Nei's genetic distance (Nei, 1978), isolation-by-distance (IBD), and isolation-by-barrier (IBB) were calculated using a randomized Mantel test (Mantel, 1976) of ade4 package (Bougeard and Dray, 2018) in the R program (R Core Team, 2013). Haplotype data was generated using the DnaSp v5 program (Librado and Rozas 2009). A maximum parsimony haplotype network was constructed in the Network 5.0.0.0 program based on a median-joining network (Bandelt *et al.*, 1999).

2.4. Phylogenetic analyses We performed a suite of analyses to infer the phylogenetic relationship of *C. versicolor* from 18 different localities along the Mekong River in Thailand and Lao PDR, as well as the 11 sequences of *C. versicolor* representing lineage A and the 24 sequences representing lineage B from China and Vietnam that were available in GenBank. For Bayesian Inference (BI) and Maximum Likelihood (ML) analysis, the best-fitting substitution model for the *CO1* data set was the general time reversibility with the gamma distribution model (GTR+G) selected by MrModeltest ver 2.2 (Nylander, 2008). We constructed a ML tree using MEGA X (Kumar *et al.*, 2018) with nodal support estimated using 1000 bootstrap re-samples. BI was performed using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). The number of generations used in BI was 20 000 000, sampling every 100 generations. The first 10 000 sampled trees were discarded as burn-in until the average standard deviation values of the run dipped below 0.01 and the potential scale reduction factor (PSRF) value approached 1.0. The consensus tree and posterior probability values were calculated using the remaining 100 000 trees.

3. Results

3.1. Sequences analyses Comparison between the 1254 bp of *CO1* gene of 200 samples from 18 different localities along both sides of the Mekong River revealed high genetic variation with

Table 1 Details of sampling localities of *Calotes versicolor* collected in this study.

No.	Code	N*	District	Province	Country	Region	Mekong side	Latitude / longitude
1	CRI	6	Chiang Khong	Chiang Rai	Thailand	North	Right	20.2498° N / 100.4106° E
2	LXB	10	Kenthao	Xayaburi	Lao PDR	North	Right	17.8336° N / 101.5555° E
3	LEI	8	Tha Li	Lei	Thailand	Northeast	Right	17.8153° N / 101.5538° E
4	NKI	7	Tha Bo	Nong Khai	Thailand	Northeast	Right	17.893° N / 102.6011° E
5	BKN	11	Mueang Bueng Kan	Bueng Kan	Thailand	Northeast	Right	18.379° N / 103.6347° E
6	NPM	16	Mueang Nakhon Phanom	Nakhon Phanom	Thailand	Northeast	Right	17.4448° N / 104.7461° E
7	MDH	15	Mueang Mukdahan	Mukdahan	Thailand	Northeast	Right	16.5144° N / 104.706° E
8	ARC	8	Chanuman	Amnat Charoen	Thailand	Northeast	Right	16.2342° N / 104.9991° E
9	UBN	15	Khemmarat	Ubon Ratchathani	Thailand	Northeast	Right	16.0861° N / 105.0686° E
10	UBK	6	Khong Chiam	Ubon Ratchathani	Thailand	Northeast	Right	15.3189° N / 105.4956° E
11	LHX	6	Huay Xai	Bokeo	Lao PDR	North	Left	20.2631° N / 100.4336° E
12	LPB	8	Nakhon Luang Prabang	Luang Prabang	Lao PDR	North	Left	19.8703° N / 102.1147° E
13	LSK	19	Sanakham	Vaintaine	Lao PDR	Central	Left	18.0334° N / 102.6255° E
14	LVT	13	Hadxayfong	Vaintaine	Lao PDR	Central	Left	17.8983° N / 102.6217° E
15	LPS	7	Pakxon	Bolikhamsai	Lao PDR	Central	Left	18.3964° N / 103.6558° E
16	LKM	11	Thakhek	Khammouane	Lao PDR	Central	Left	17.4553° N / 104.7871° E
17	LSV	14	Kaysone Phomvihane	Savannakhet	Lao PDR	Central	Left	16.6055° N / 104.7715° E
18	LCS	20	Pakse	Champasak	Lao PDR	South	Left	15.1008° N / 105.8523° E

* Number of samples.

191 variable sites consisting of 15 singleton variable sites with two variants and 176 parsimony informative sites including 156, 19 and one informative sites with two, three and four variants, respectively. Nucleotide and haplotype diversity within populations ranged between 0.000 to 1.000 ± 0.096 and 0.0000 to 0.0286 ± 0.0042, respectively (Table 2). The number of haplotypes (N) within each population ranged between 1 and 14 haplotypes and a total of 91 haplotypes were found and classified. Five haplotypes were shared between different populations, whereas the others were uniquely found in a particular locality (Table 2). Genetic (*p*) distance ranged between 0.0003 and 0.0492 (Table 3). The genetic distance between populations within lineages ranged between 0.0003 and 0.0153, whereas genetic distance between populations of different lineages ranged between 0.0313 and 0.0492 (Table 3).

3.2. Haplotype analyses Based on haplotype analyses, the 91 haplotypes generated in this study were grouped into six lineages based on mutational steps ≥ 20, i.e. lineage C – lineage H. Linage A and lineage B, which were previously classified by Huang *et al.* (2013), contained the haplotypes from China and Vietnam (Figure 2). Lineage C contained the three haplotypes from UBK in northeastern Thailand and 14 haplotypes from LCS in southern Lao PDR. Lineage D consisted of eight and four haplotypes from LSV and LKM in central Lao PDR, respectively. Lineage E consisted of the six haplotypes from CRI in north Thailand, as well as the two and one haplotypes from LHX and LSK in northern and central Lao PDR, respectively. Lineage E also included the three haplotypes from LEI in northeastern Thailand. Linage F contained ten and four haplotypes of UBN and ACR in northeastern Thailand, and also three and one haplotypes from MDH and BKN in northeastern Thailand, respectively. Lineage G contained all haplotypes of NKI in northeastern Thailand, as well as LVT, LXB, and LPB in Lao PDR, including three haplotypes from LEI. Lineage H contained three, three and six haplotypes from MDH, BKN and NPM in northeastern Thailand, and included two haplotypes from LPS in central Lao PDR (Figure 2).

3.3. Genetic structure analyses The genetic structure of the populations was examined by AMOVA (Table 4). This revealed that *C. versicolor* populations were genetically sub-structured and correlated with the six major lineages (lineage C – lineage H) classification (F_{CT} = 0.0639, P < 0.05), similar to the classification from haplotype network analysis. The isolation-by-distance (IBD) test also showed that genetic distance was significantly correlated with R^2 = 0.3894 (P < 0.001). However, it was not significantly related to the groups divided by the Mekong River (F_{CT} =0.0182, P > 0.05). The isolation-by-barrier (IBB) test also show that the Mekong River had no significant effect on genetic distance of *C. versicolor* with R^2 = 0.0113 (P > 0.05) (Figure 3).

3.4. Phylogenetic analyses The phylogenetic analyses of *C.*

Table 2 Summary statistics of molecular variation in 18 populations/localities of *Calotes versicolor* in Thailand and Lao PDR.

Population code	N	S	H	Uh	Hd	Nd
CRI	6	21	6	6	1.000 ± 0.096	0.0082 ± 0.0012
LEI	8	73	6	5	0.929 ± 0.007	0.0286 ± 0.0042
NKI	7	3	4	2	0.714 ± 0.181	0.0009 ± 0.0003
BKN	11	50	4	2	0.691 ± 0.016	0.0095 ± 0.0045
NPM	16	8	6	4	0.767 ± 0.084	0.0027 ± 0.0002
MDH	15	50	9	9	0.800 ± 0.108	0.0126 ± 0.0042
ARC	8	14	4	4	0.750 ± 0.019	0.0055 ± 0.0008
UBN	15	17	10	10	0.943 ± 0.002	0.0034 ± 0.0005
UBK	6	6	3	3	0.733 ± 0.155	0.0026 ± 0.0005
LXB	10	25	7	7	0.911 ± 0.077	0.0068 ± 0.0012
LHX	6	1	2	1	0.533 ± 0.172	0.0004 ± 0.0001
LPB	8	4	3	3	0.464 ± 0.200	0.0009 ± 0.0005
LSK	19	0	1	0	0.000 ± 0.000	0.0000 ± 0.0000
LVT	13	3	4	2	0.654 ± 0.106	0.0009 ± 0.0002
LPS	7	1	2	2	0.571 ± 0.119	0.0005 ± 0.0001
LKM	11	10	4	4	0.673 ± 0.123	0.0023 ± 0.0006
LSV	14	13	8	8	0.901 ± 0.058	0.0028 ± 0.0005
LCS	20	18	14	14	0.889 ± 0.068	0.0029 ± 0.0005
All populations	200	191	91	21	0.973 ± 0.005	0.0353 ± 0.0004

* N = number of specimens, S = segregation sites, H = number of observed haplotypes, Uh = unique haplotype, Hd = haplotype diversity, Nd = nucleotide diversity.

versicolor based on *CO1* sequences using *Calotes amma alticristatus* as out-group based on ML and BI methods were reciprocally demonstrated that eight well-supported lineages were observed (Figure 4). Lineage A and B contained the 37 sequences from China and Vietnam available in GenBank. Our samples were classified into six lineages, namely lineage C to H in concordance with the haplotype network analysis. Lineage C and D generated in the current study were closely clustered with lineage A and B reported by a previous study. Lineage E and F were subdivided into three and two sub-lineages, namely sub-lineage E1 – E3 and F1 – F2, respectively. Sub-lineage E1 and E2 contained the sequences from LEI and CRI, respectively, whereas sub-lineage E3 consisted of the sequences from LEI, LHX and LSK (Figure 4). Sub-lineage F1 contained the sequences from UBN and ACR, whereas sub-lineage F2 consisted of the sequences from MDH and BKN (Table 3 and Figure 4). Lineage E and G were found on both sides of the Mekong River, *i.e.* the northernmost (CRI) to northeast (NKI) of Thailand and the northernmost (LHX) to central (LVT) Lao PDR. Lineage F and H were found on the Thailand side from BKN to UBN, whereas the LPS in Lao PDR belonged to lineage H. Lineage D was found in the Lao PDR at LKM and LSV, whereas lineage C was found on both sides, UBK in Thailand and LCS in Lao PDR (Figure 4).

4. Discussion

To our knowledge, this is the first report of comprehensive genetic variation and genetic structure analyses of *C. versicolor* in the Lower Mekong Basin in Southeast Asia. We found high levels of genetic variation in the *C. versicolor* populations along the Mekong River from Thailand and Lao PDR, as indicated by the 91 haplotypes of *CO1*. The significant genetic distance among populations indicates that the *C. versicolor* populations examined in this study can be subdivided into at least six lineages. Each linage may contain several cryptic groups, such as

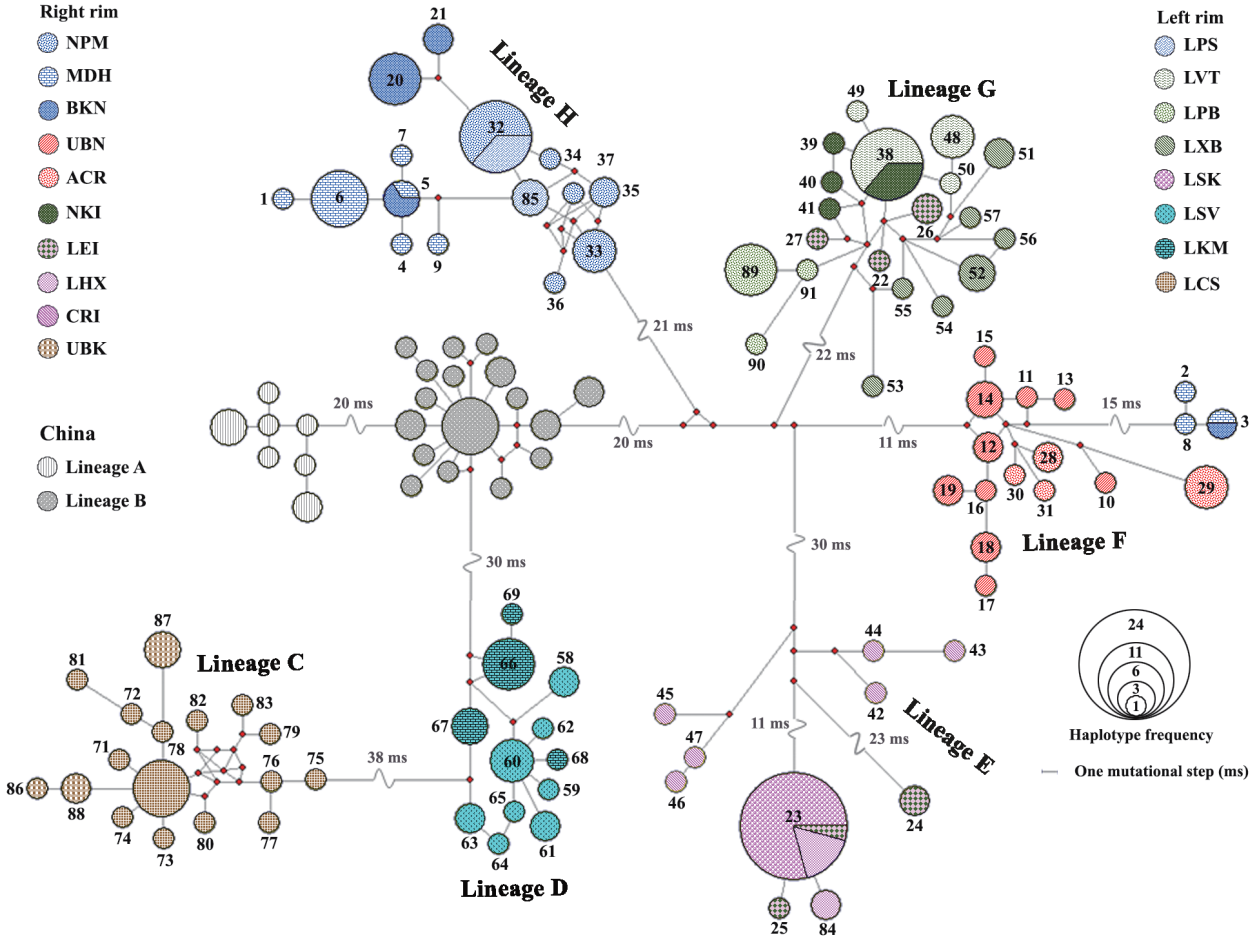

Figure 2 Minimum spanning haplotype network of *Calotes versicolor* generated based on partial *CO1* sequences corresponds to their geographical localities separated into 18 different localities along the Lower Mekong River in Thailand and Lao PDR, including 34 sequences of *C. versicolor* from China retrieved from GenBank. The area of the circles represents the proportion of specimen number found in each haplotype.

Table 3 Pairwise genetic distance (*p*) of 18 populations of *Calotes versicolor* aside the Mekong River in Thailand and Lao PDR. * Code of sampling localities (see detial in Table 1).

Lineage	Local*	MDH	NPM	BKN	LPS	NKI	LXB	LVT	LPB	LEI	CRI	LHX	LSK	UBN	ACR	LSV	LKM	UBK	LCS
H/F	MDH	–																	
H/F	NPM	**0.0150**	–																
H	BKN	**0.0150**	**0.0079**	–															
H	LPS	**0.0143**	**0.0014**	**0.0070**	–														
G	NKI	0.0347	0.0337	0.0351	0.0346	–													
G	LXB	0.0381	0.0374	0.0385	0.0383	**0.0058**	–												
G	LVT	0.0352	0.0342	0.0356	0.0351	**0.0008**	**0.0058**	–											
G	LPB	0.0359	0.0348	0.0362	0.0357	**0.0067**	**0.0096**	**0.0072**	–										
G/E	LEI	0.0362	0.0360	0.0367	0.0363	0.0213	0.0245	0.0216	0.0237	–									
E	CRI	0.0409	0.0423	0.0409	0.0414	0.0401	0.0425	0.0406	0.0410	0.0292	–								
E	LHX	0.0402	0.0412	0.0399	0.0403	0.0444	0.0465	0.0449	0.0446	0.0280	**0.0153**	–							
E	LSK	0.0405	0.0415	0.0402	0.0406	0.0447	0.0469	0.0452	0.0450	0.0280	**0.0149**	**0.0003**	–						
F	UBN	0.0314	0.0327	0.0332	0.0337	0.0319	0.0342	0.0324	0.0313	0.0340	0.0388	0.0428	0.0439	–					
F	ACR	0.0320	0.0335	0.0341	0.0345	0.0329	0.0352	0.0334	0.0332	0.0347	0.0399	0.0425	0.0435	**0.0036**	–				
D	LSV	0.0365	0.0379	0.0378	0.0370	0.0344	0.0378	0.0349	0.0337	0.0368	0.0393	0.0446	0.0442	0.0343	0.0358	–			
D	LKM	0.0351	0.0367	0.0366	0.0359	0.0352	0.0383	0.0357	0.0362	0.0362	0.0377	0.0426	0.0423	0.0337	0.0343	**0.0046**	–		
C	UBK	0.0448	0.0460	0.0438	0.0449	0.0473	0.0493	0.0478	0.0456	0.0475	0.0461	0.0510	0.0507	0.0434	0.0449	0.0414	0.0413	–	
C	LCS	0.0438	0.0454	0.0439	0.0445	0.0469	0.0492	0.0474	0.0462	0.0455	0.0438	0.0482	0.0482	0.0417	0.0429	0.0393	0.0387	**0.0039**	–

Figure 3 Graphs demonstrating the pairwise relationship between Euclidean geographic distance and Edward's genetic distance of every sample pair of *Calotes versicolor*, based on *CO1* sequences. The graph shows a proportional increase in genetic distance as the geographic distance increases, so called isolation by distance (IBD) (A). Another graph shows the difference between pairwise genetic distance categorizing whether the pairs were the same (Same) or different (Different) from the Mekong River's side, recognizing Mekong River as the natural barrier of gene flow, so called isolation by barrier (IBB) (B).

lineages E and F that can also be subdivided into sub-lineages. This evidence that there are other cryptic lineages needs to be explored in the future.

The IBD test revealed that there was a spatial distance effect on the genetic differences between *C. versicolor* populations examined in this study. This is likely to be due to limited *C. versicolor* movement/migration between spatial distance areas as previously evidenced by their related species *Calotes mystaceous* Duméril & Bibron, 1837 (Saijuntha *et al.*, 2017) and *Calotes emma* schmidt, 1925 (Saijuntha *et al.*, 2020). In addition, unique haplotypes were commonly found in all localities and high genetic distances (*p*-distance) between the populations from different lineages were observed. A similar finding was previously reported in *C. versicolor* populations from Hainan Island and adjacent mainland China, as well as Vietnam, where

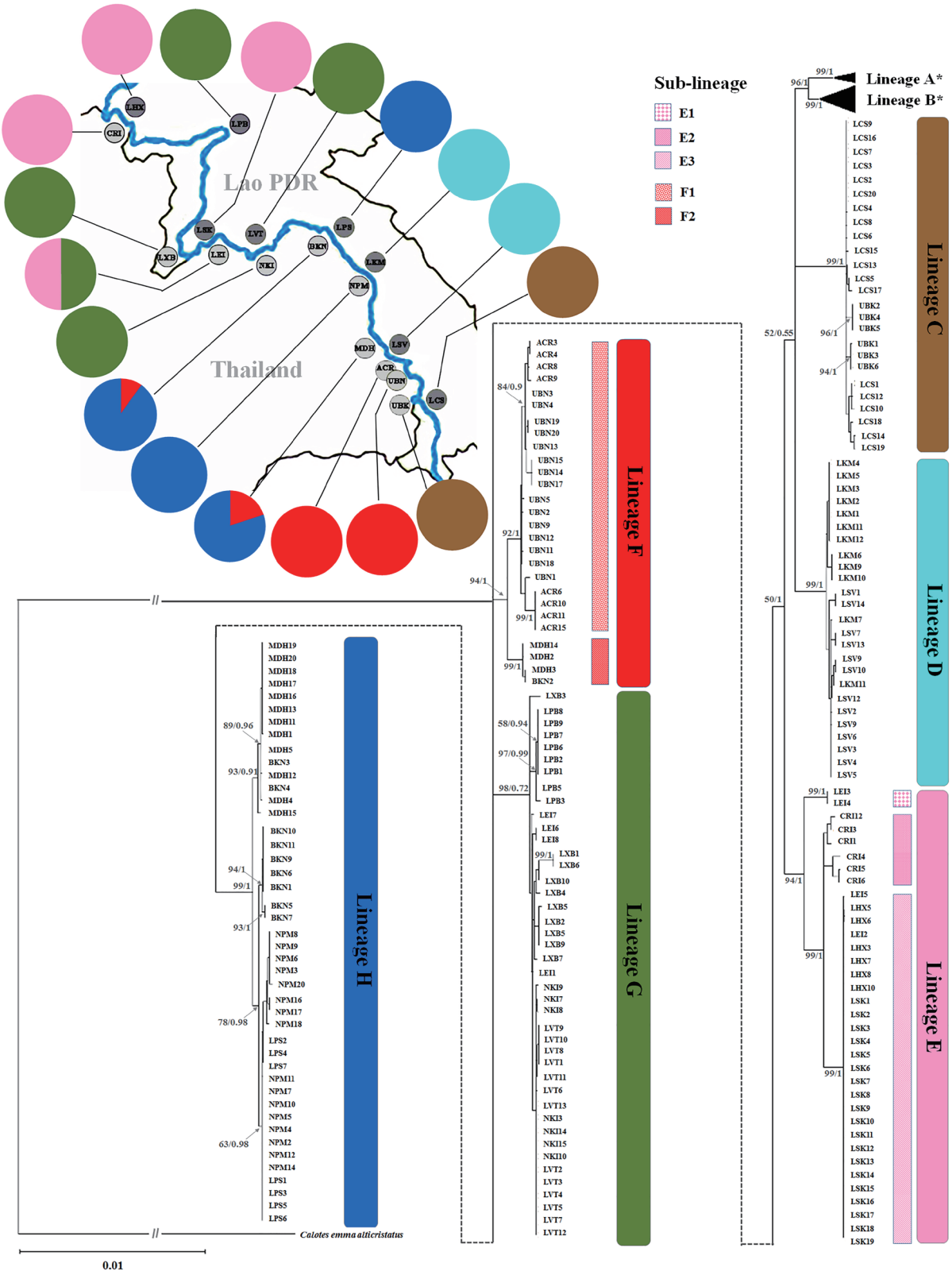

Figure 4 Phylogenetic tree constructed based on 1254 bp of *CO1* sequence of *Calotes versicolor* 91 haplotypes along the Mekong River in Thailand and Lao PDR including the 11 sequences lineage A, namely KC875637, KC875647–KC875649, KC875741–KC875744, KC875802–KC875804) and the 24 sequences of lineage B, namely KC875610, KC875660, KC875680, KC875687, KC875690, KC875695, KC875717, KC875747, KC875749, KC875751, KC875753, KC875754, KC875759, KC875762, KC875772, KC875773, KC875775, KC875780, KC875784, KC875786, KC875809, KC875810, KC875813, KC875815 from China, KC875765 and KC875771 from Vietnam.

Table 4 Analysis of molecular variance (AMOVA) of *Calotes versicolor* classified into two groups defined by the left and right side of the Mekong River, as well as the six groups defined by six lineages (lineage C – lineage H) classified in this study.

Source of variation	Hypothesized structure							
	Left and right groups of Mekong River's side				Six lineages groups			
	Ss	Vc	%Va	Fi	Ss	Vc	%Va	Fi
Among groups	1.481	0.0089	1.82	F_{CT} = 0.0182	14.615	0.0319	6.39	F_{CT} = 0.0639*
Among populations Within group	31.247	0.1473	30.01	F_{SC} = 0.2948**	20.368	0.1215	24.35	F_{SC} = 0.2601**
Within group	64.122	0.3523	68.17	F_{ST} = 0.2819**	61.868	0.3456	69.26	F_{ST} = 0.3074**

Ss = Sum of squares, Vc = Variance components, %Va = Percentage of variation, Fi = Fixation indices, * $P < 0.05$, ** $P < 0.001$.

two mitochondrial lineages were defined and separated by putative nonphysical or ecological barriers (Huang *et al.*, 2013). These reports and the current study support the hypothesis that geographical distance and ecological barriers play significant roles in the genetic differences in *Calotes* populations (Huang *et al.*, 2013; Saijuntha *et al.*, 2017, 2020).

The Mekong River can act as a significant natural barrier blocking gene flow between populations of several organisms on different banks of the river, including several freshwater and terrestrial animals, such as *Brachytrupes portentosus* (Lichtenstein 1796) (Tantrawatpan *et al.*, 2011) and *Bithynia siamensis goniomphalos* (Morelet, 1866) (Tantrawatpan *et al.*, 2020). However, several reptile species in this region such as *Gekko gecko* (Linneasus, 1758) (Saijuntha *et al.*, 2019) show no significant genetic difference between the populations from different sides of the Mekong River. Our results also indicate that the variation found in *C. versicolor* populations from different sides (left and right) of the river is not related to genetic distances indicated by the IBB test. These findings suggest that the Mekong River does not play a significant role as a natural barrier to block the gene flow of endemic reptiles.

In addition, morphological variation of *C. versicolor* from different localities along the Mekong River in Thailand and Lao PDR has been recorded, and the high variation observed by morphometric analyses has been used to define seven major groups (Thongnetr *et al.*, 2015). Thus, the results from present and previous studies suggest that cryptic lineages of *C. versicolor* exist in the Mekong River Basin in Thailand and Lao PDR that are genetically distinct from the Chinese and Vietnamese isolates. Interestingly haplotype 24 (two specimens) from LEI was genetically very distinct from the other F lineage observed in a haplotype network analysis. This finding revealed that another cryptic lineage of *C. versicolor* may exist in this geographical area. This needs to be further evaluated by expanding sampling sites from this locality.

Our findings, together with previous studies, suggest that *C. versicolor* in Asia is a species complex, which may contain more genetic structuring than that determined in this and previous studies (Huang *et al.*, 2013). More specimens from different distribution areas should be collected for a more comprehensive study of the genetic diversity, genetic structure and morphometric analyses of *C. versicolor* throughout Southeast Asia, as well as their other distribution localities worldwide.

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References

Bandelt H. J., Forster P., Rohl A. 1999 Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol, 16: 37–48

Bougeard S., Dray S. 2018. Supervised multiblock analysis in R with the ade4 package. J Stat Softw, 86(1): 1–17

Excoffier L., Lischer H. E. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Res, 10(3): 564–567

Hall T. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser, 41: 95–98

Huang Y., Guo X., Ho S. Y. W., Shi H., Li J., Li J., Cai B., Wang Y. 2013. Diversification and demography of the Oriental garden lizard (*Calotes versicolor*) on Hainan Island and the adjacent mainland. PLoS One, 8: e64754

Ji X., Qiu Q. B., Diong C. H. 2002. Sexual dimorphism and female reproductive characteristics in the Oriental garden lizard, *Calotes versicolor*, from Hainan, Southern China. J Herpetol, 36(1): 1–8

Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol, 35(6): 1547–1549

Librado P., Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25(11): 1451–1452

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res, 27: 209–220

Matyot P. 2004. The establishment of the crested tree lizard, *Calotes versicolor* (Daudin, 1802) (Squamata: Agamidae), in Seychelles. Phelsuma, 12: 35–47

Mekong River Commission, 2011. Atlas of the Lower Mekiong River Basin. Retrieved from www.mrcmekong.org

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 23: 341–369

Nylander J. A. A. 2008. MrModeltest v2.3 Program distributed by the author. Evolutionary Biology Centre. Uppsala University

Prakobkarn A., Thirakhupt K., Ngamprasertwong T. 2016. Sexual dimorphism and geographic variation of *Calotes versicolor* (Squamata: Agamidae) in northern and southern Thailand. Agri Nat Resour, 50(6): 474–482

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Radder R. S. 2006. An overview of geographic variation in the life history traits of the tropical agamid lizard, Calotes versicolor. Curr Sci, 91: 1354–1363

Ronquist F., Huelsenbeck J. P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19(12): 1572–1574

Saijuntha W., Khumkratok S., Wongpakam K., Thanonkeo S., Senakhun C., Appamaraka S., Yodsiri S., Thongnetr W., Pilap W., Kongbuntad W., Tawong W., Agatsuma T., Petney T. N., Tantrawatpan C. 2017. Genetic diversity and population structure of Blue-crested lizard, *Calotes mystaceus* Duméril & Bibron, 1837 (Squamata: Agamidae) in Thailand. J Genet, 96(2): 377–382

Saijuntha W., Sedlak S., Agatsuma T., Jongsomchai K., Pilap W., Kongbuntad W., Tawong W., Suksavate W., Petney T. N., Tantrawatpan C. 2019. Genetic structure of the red-spotted tokay gecko, *Gekko gecko* Linnaeus, 1758 (Squamata: Gekkonidae) in mainland Southeast Asia. Asian Herpetol Res, 10(2): 69–78

Saijuntha W., Tantrawatpan C., Pilap W., Sedlak S., Sakdakham K., Sripirom P., Suksavate W., Kongbuntad W., Tawong W. 2020. Genetic differentiation of the forest crested lizards, *Calotes emma alticristatus* Schmidt, 1925 and *C. emma emma* Gray, 1845 (Squamata: Gekkonidae) relating to climate zones in Thailand. Asian Herpetol Res, 11(1): 19–27

Sudasinghe H., Somaweera R. 2015. *Calotes versicolor* (Oriental Garden Lizard). Diet. Herpetol Rev, 46(4): 625–629

Tantrawatpan C., Saijuntha W., Pilab W., Sakdakham K., Pasorn P., Thanonkeo S., Thiha S. R., Petney T. N. 2011. Genetic differentiation among populations of *Brachytrypes portentosus* (Lichtenstein 1796) (Orthoptera: Gryllidae) in Thailand and the Lao PDR: The Mekong River as a biogeographic barrier. Bull Entomol Res, 101(6): 687–696

Tantrawatpan C., Saijuntha W., Bunchom N., Suksavate W., Pilap W., Walalite T., Agatsuma T., Tawong W., Sithithaworn P., Andrews R. H., Petney T. N. 2020. Genetic structure and geographical variation of *Bithynia siamensis goniomphalos* sensu lato (Gastropoda: Bithyniidae), the snail intermediate host of *Opisthorchis viverrini* sensu lato (Digenea: Opisthorchiidae) in the Lower Mekong Basin revealed by mitochondrial DNA sequences. Int J Parasitol, 50(1): 55–62

Thongnetr W., Saijuntha W., Satrawaha R. 2015. Morphological relations of Red headed lizard [*Calotes versicolor* (Daudin, 1802)] along the lower Mekong River [in Thai]. The 11th Mahasarakham University Research Conference, 145–155

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