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Polymorphic analysis of mitochondrial *12S rRNA* gene of common sun skink *Eutropis multifasciata* (Reptilia: Squamata: Scincidae) in Central Vietnam

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ABSTRACT

Analysis of 12S rRNA sequences of twenty specimens from Common Sun Skink Eutropis multifasciata in Central Vietnam showed genetic differences among specimens range from 0% (between the specimens H1, QB1, QB2 and DL6; or H2 and QT1; or H9, HT1, DN1 and NA2; or DN7 and DN8) to 1,79% (between the specimens DN2 and DL5). The mitochondrial tree generated from these sequences confirmed the monophyly of all specimens of E. multifasciata and the monophyly of the genus Eutropis. These mitochondrial 12S rRNA sequences of specimens from E. multifasciata (HI, H2, H3, H9, H10, DL5, DL6, QN1, QN2, QN8, NA2, NA3, QB1, QB2, QT1, HT1, DN1, DN7, DN8, and DN2) were deposited in GenBank with accession number KT350390-KT350409, respectively.

Keywords: *Eutropis multifasciata*, *Mabuya*, mitochondrial *12S rRNA* gene, Reptilia, Squamata

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INTRODUCTION

The genus *Eutropis* or the tropical Asian *Mabuya* belonging to the family Scincidae, ordo Squamata of Reptile, currently consist of around 30 described species distributed predominantly in the Indomalayan (The Indomalayan region is further divided into Indian, Indochinese and Sundaic subregion) [8] and several yet undescribed species occurring from the Middle East to Palau, Oceania [22]. The Common Sun Skink, *Eutropis multifasciata* (formerly *Mabuya multifasciata*), is a widely distributed species in India, Southern China (Yunnan, Guangdong, and Hainan), Taiwan, Myanmar, Thailand, Indochina, Philippines, Indonesia, Singapore, Malaysia, and New Guinea [25], [29]. About 470 species of reptiles are known from Vietnam and five of these are in the genus *Eutropis* [11], [25]. *E. multifasciata* is commonly distributed in open regions, villages, and occasionally in secondary forest at various temperature and humidity regimes. They generally feed on insects (such as Blattodea, Coleoptera, Diptera, Hemiptera, Isoptera…), insect larvae, plant materials etc... [6], [7], [20]. This explains for their important roles in ecosystem. Local people in Vietnam prefer to have it for their meals by different ways of cooking and soak it in alcohol for a tonic. Due to its great food and pharmaceutical value, in the future the population of this family might experience a sharp decline.

Studies of vertebrate species generally have shown that the sequence divergence accumulate more rapidly in mitochondrial than in nuclear DNA. This has been attributed to a faster mutation rate in mtDNA that may result from a lack of repair mechanisms during replication [19]. The sequence of *12S rRNA* gene has been widely used to estimate genetic relationships between specimens (altered nucleotide) or groups [2], [9], [10], [16], [18] and phylogenetic relationship of the species [3], [5], [8], [13], [21-25], [29]. In this work, we analyze of *12S ribosomal RNA* (*rRNA*) gene of *E. multifasciata* collected from Central Vietnam.

MATERIALS AND METHODS

Sample collection

E. multifasciata were caught from locality sites in Central Vietnam: Nghe An, Ha Tinh, Quang Binh, Quang Tri, Hue, Da Nang, Quang Nam and Dac Lak (Table 1 and Figure 1) by hand or in traps. Tail samples from captured specimens were stored in 98% alcohol.

Table 1. Specimens for this analysis with locality and voucher code

Locality	Number of specimens	Voucher code
Nghe An	3	NA1, NA2, NA3
Ha Tinh	1	HT1
Quang Binh	2	QB1, QB2
Quang Tri	1	QT1
Hue	20	H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18, H19, H20
Da Nang	10	DN1, DN2, DN3, DN4, DN5, DN6, DN7, DN8, DN9, DN10
Quang Nam	10	QN1, QN2, QN3, QN4, QN5, QN6, QN7, QN8, QN9, QN10
Dac Lak	6	DL1, DL2, DL3, DL4, DL5, DL6

PCR amplification

Genomic DNA was isolated from tail tissue using phenol-chloroform according to Thanh et al. (2006). The concentration and quality of extracted DNA was spectrophotometrically estimated.

Fragment of the mitochondrial DNA (mtDNA) corresponding to the *12S rRNA* gene was amplified by using the polymerase chain reaction (PCR) specific primers 12SEM-F (5'-CTTAGCCCTTAACACAGACA-3') and 12SEM-R (5'-GGTGTGTGCGCGCTCCAGAG-3'). The PCR reactions were performed in a total volume of 25 µl containing 20 ng genomic DNA, 20 pmol of each primer (1.0 µl), 2.5 mM dNTP (2.0 µl), 25 mM $MgCl_2$ (1.5 µl), 10X PCR buffer (2.5 µl), sterile distilled H_2O and 1 unit *Taq* polymerase. The amplication was performed in a thermocycler programed at 95°C for 4 min; 94°C for 45 sec, 52°C for 1 min, 72°C for 1 min for 30 cycles with a final extention at 72°C for 10 min. PCR products were confirmed by 1% agarose gel electrophoresis.

12S rRNA gene sequencing

The PCR products of mitochondrial *12S rRNA* gene fragment of *E. multifasciata* were cut out from 1% agarose gel and purified by Wizard®SV Gel and PCR Clean Up System Kit (Promega), they were then ligated to a pGEM-T Easy vector. Components of ligation reaction comprising of 1 µl vector, 5 µl ligation buffer, 1 µl T4 DNA ligase, 1 µl PCR product, 2 µl distilled water were well mixed and incubated at 4°C overnight. Recombinant vectors from ligation reaction were then transformed into *E. coli* TOP 10 cells (5 µl ligation+40 µl cells) by the heat-shock method. After 45 sec of transformation at 45°C, the solution was put into 500 µl LB medium containing 50 mg/ml ampicillin and incubated at 37°C with shaking 200 rpm for 60 min. The cell biomass of transformants containing the recombinant plasmids was used to extract plasmid DNA for the identification of recombination by restriction digestion and the sequencing by the method of fluorescent dideoxy-terminator on 3130 ABI system (Applied Biosystem).

Phylogenenic analysis

The *12S rRNA* sequences were compared each other and with those held in the databases in the GenBank by using Mega 6.0 software. The sequences of *E. multifasciata china*, *E. multifasciata india*, *E. multifasciata myanmar*, *E. multifasciata philippines*, *E. multifasciata indonesia*, *E. longicaudata*, *E. macularia*, *M. stangeri*, *M. delalandii*, *M. capensis*, *M. aurata*, *M. vittata*, *M. frenata* and *M. agilis* were taken from GenBank.

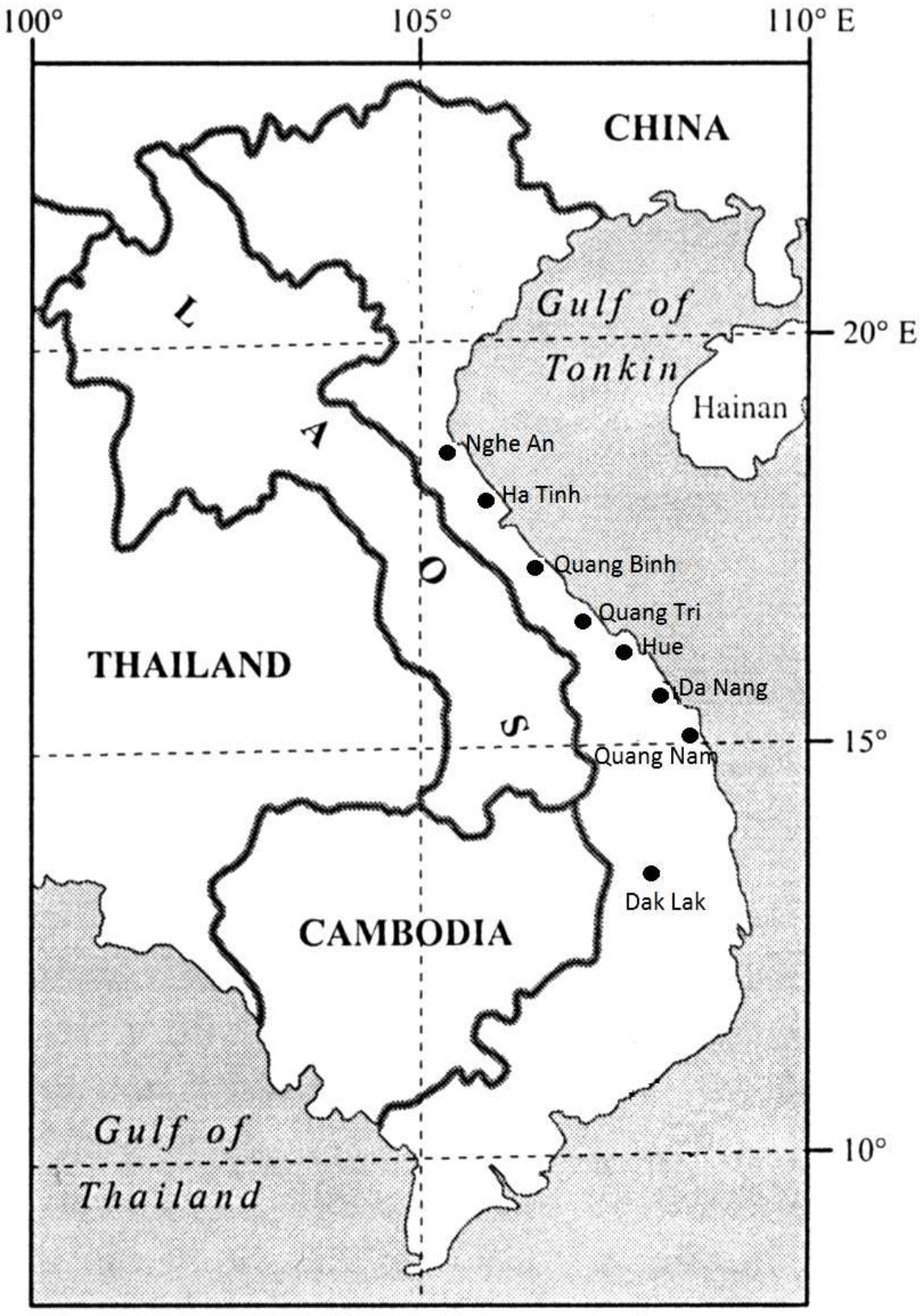

Figure 1. Map of sampling localities for specimens *E. multifasciata* used in this study

RESULTS AND DISCUSSION

Twenty mitochondrial *12S rRNA* gene fragments were analyzed. The nucleotide sequences of *12S rRNA* gene fragment are shown in Figure. 2. The *12S rRNA* gene fragment consisted of 393 total sites, 10 of which were

variable. Analysis of mitochondrial *12S rRNA* gene fragments of specimens from *E. multifasciata* showed genetic differences among specimens range from 0% (between the specimens H1, QB1, QB2 and DL6; or H2 and QT1; or H9, HT1, DN1 and NA2; or DN7 and DN8) to 1,79% (between the specimens DN2 and DL5).

These *12S rRNA* gene fragments are compared with some *12S rRNA* sequences of species belonging to genus *Eutropis* in GenBank. The outcomes show genetic differences among specimens range from 0% (between the specimens *E. multifasciata* (H9, DN1) and *E. multifasciata china*) to 3,57% (between the specimens *E. multifasciata* (HI, QB2 and DL6) and *E. multifasciata philippines* or *E. multifasciata indonesia*).

```
                ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                10        20        30        40        50        60        70
DL5         CTTAGCCCTT AACACAGACA ACAGACATAC AATGCTGTCC GCCAGAGAAC
TACAAGTGAA AAACTTAAAA
DL6         .......... .......... .......... .......... .......... .......... ..........
DN1         .......... .......... .......... .......... .......... .......... ..........
DN2         .......... .......... ...A...... .......... .......... .......... ..........
DN7         .......... .......... .......... .......... .......... .......... ..........
DN8         .......... .......... .......... .......... .......... .......... ..........
H1          .......... .......... .......... .......... .......... .......... ..........
H2          .......... .......... .......... .......... .......... .......... ..........
H3          .......... .......... .......... .......... .......... .......... ..........
H9          .......... .......... .......... .......... .......... .......... ..........
H10         .......... .......... ...A...... .......... .......... .......... ..........
HT1         .......... .......... .......... .......... .......... .......... ..........
NA2         .......... .......... .......... .......... .......... .......... ..........
NA3         .......... .......... .......... .......... .......... .......... ..........
QB1         .......... .......... .......... .......... .......... .......... ..........
QB2         .......... .......... .......... .......... .......... .......... ..........
QN1         .......... .......... .......... .......... .......... .......... ..........
QN2         .......... .......... .......... .......... .......... .......... ..........
QN8         .......... .......... .......... .......... .......... .......... ..........
QT1         .......... .......... .......... .......... .......... .......... ..........
E.C         .......... .......... .......... .......... .......... .......... ..........
E.I         ---------- ---------- ---------- ---------- ---------- -----..... ..........
E.ID        .......... .......... G......... .......... .......... .......... .....C....
E.M         .......... .......... .......... .......... .......... .......... ..........
E.P         .......... .......... .......... ....T..... .......... .......... ..........
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                80        90        100       110       120       130       140
DL5         CTCCAAGGAC TTGGCGGTGC TCCATACCGT CCTAGAGGAG CCTGTCCTAT AATCGATACC
CCACGTTATA
DL6         .......... .......... .......... .......... .......... .......... ..........
DN1         .......... .......... .......... .......... .......... .......... ..........
DN2         .......... .......... .......... .......... .......... .......... ..........
DN7         .......... .......... .......... .......... .......... .......... ..........
DN8         .......... .......... .......... .......... .......... .......... ..........
H1          .......... .......... .......... .......... .......... .......... ..........
H2          .......... .......... .......... .......... .......... .......... ..........
H3          .......... .......... .......... .......... .......... .......... ..........
H9          .......... .......... .......... .......... .......... .......... ..........
H10         .......... .......... .......... .......... .......... .......... ..........
HT1         .......... .......... .......... .......... .......... .......... ..........
NA2         .......... .......... .......... .......... .......... .......... ..........
NA3         .......... .......... .......... .......... .......... .......... ..........
QB1         .......... .......... .......... .......... .......... .......... ..........
QB2         .......... .......... .......... .......... .......... .......... ..........
QN1         .......... .......... .......... .......... .......... .......... ..........
QN2         .......... .......... .......... .......... .......... .......... ..........
QN8         .......... .......... .......... .......... .......... .......... ..........
QT1         .......... .......... .......... .......... .......... .......... ..........
```

E.C ....
E.I ....
E.ID ....C.
E.M ....
E.P ....

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
150 160 170 180 190 200 210

DL5 CCCGACCATT TTTTGCAACC TCAGCCTATA TACCGCCGTC GTCAGCCTAC CTTATGAAGG CCAAAAAGTA
DL6 ....C..... .....
DN1 ....
DN2 ....G.. T.....
DN7 ....G.. .....
DN8 ....G.. .....
H1 ....C..... .....
H2 ....
H3 ....
H9 ....
H10 ....G.. .....
HT1 ....
NA2 ....
NA3 ....A. .....
QB1 ....C..... .....
QB2 ....C..... .....
QN1 ....
QN2 ....G.. .....
QN8 ....
QT1 ....
E.C ....
E.I ....
E.ID ....T. ....A. A.....
E.M ....
E.P ....

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
220 230 240 250 260 270 280

DL5 GGCAAAATAG TTATTCAACT AACACGTCAG GTCAAGGTGT AGCACATAAA ATGGAAGAGA TGGGCTACAT
DL6 ....
DN1 ....
DN2 ....
DN7 ....
DN8 ....
H1 ....
H2 ....
H3 .... .G..... ....
H9 ....
H10 ....
HT1 ....
NA2 ....
NA3 ....
QB1 ....
QB2 ....
QN1 ....
QN2 ....
QN8 A..... ....
QT1 ....
E.C ....
E.I ....

E.ID ....
E.M ....
E.P ....

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
290 300 310 320 330 340 350

DL5 TTTCTACTTC AGAAGACACG GACTGCACAA TGAAACACGT GCAAGAAGGT GGATTTAGCT GTAAGACAAA
DL6 .... A..... .....T.... ....
DN1 .... A..... .....T.... ....
DN2 .... A..... .....T.... ....
DN7 .... A..... .....T.... ....
DN8 .... A..... .....T.... ....
H1 .... A..... .....T.... ....
H2 .... A..... ....
H3 .... A..... .....T.... ....
H9 .... A..... .....T.... ....
H10 .... A..... .....T.... ....
HT1 .... A..... .....T.... ....
NA2 .... A..... .....T.... ....
NA3 .... A..... .....T.... ....G.
QB1 .... A..... .....T.... ....
QB2 .... A..... .....T.... ....
QN1 .... A..... .....T.... ....
QN2 .... A..... .....T.... ....
QN8 .... A..... .....T.... ....
QT1 .... A..... ....
E.C .... A..... .....T.... ....
E.I ....A..... A..... .....T.... ....
E.ID .....T... ....A..... A....T.... ..... A..... ....
E.M ....A..... A..... .....T.... ....
E.P ....A..... A..... .....T.... ....

....|....| ....|....| ....|....| ....|....| ....|....| ....|..
360 370 380 390 400

DL5 CAAGAGTGTT TGCCTAAACC TCGCTCTGGA GCGCGCACAC ACC------- -------
DL6 ....T..... ....------- -------
DN1 ....T..... ....------- -------
DN2 .....A.... ..T..... ....------- -------
DN7 .....A.... ..T..... ....------- -------
DN8 .....A.... ..T..... ....------- -------
H1 ....T..... ....------- -------
H2 .....A.... ....------- -------
H3 .....A.... ..T..... ....------- -------
H9 ....T..... ....------- -------
H10 .....A.... ..T..... ....------- -------
HT1 ....T..... ....------- -------
NA2 ....T..... ....------- -------
NA3 ....T..... ....------- -------
QB1 ....T..... ....------- -------
QB2 ....T..... ....------- -------
QN1 .....A.... ..T..... ....------- -------
QN2 .....A.... ..T..... ....------- -------
QN8 ....T..... ....------- -------
QT1 .....A.... ....------- -------
E.C ....T..... ....------- -------
E.I .....A.... ..T..... ....---- ---------- -------
E.ID .....AA... ..T..... ....GCCCGTC ACCCTCA

E.M .....A.... ..T..... ....GCCCGTC A------
E.P .....A.... ..T..... ....------- -------

Figure 2. *12S rRNA* sequences of species belonging to genus *Eutropis*: *E. multifasciata* (HI, H2, H3, H9, H10, DL5, DL6, QN1, QN2, QN8, NA2, NA3, QB1, QB2, QT1, HT1, DN1, DN7, DN8, and DN2), *E. multifasciata china* AY159054.1 (E.C), *E. multifasciata india* JQ767981.1 (E.I), *E. multifasciata myanmar* AY159059.1 (E.M), *E. multifasciata philippines* AY159055.1 (E.P), *E. multifasciata indonesia* AY159056.1 (E.ID)

Phylogenetic tree base on mitochondrial *12S rRNA* sequences is shown in Figure 3. In this tree, specimens from Asia are well supported as a distinct clade (clade A) that include all specimens of *E. multifasciata*. The other major groups are species from Cape Verde islands and South Africa (clade B), those from Turkey (clade C) and Brazil (clade D).

Genetic relationships between specimens within this species also are estimated (Figure 4). Two distinct groups are supported; group I includes specimens from Nghe An, Ha Tinh, Quang Binh, Quang Tri, Hue, Da Nang, Quang Nam and Dac Lak and group II includes specimens from Hue, Da Nang, and Quang Nam. Specimens in group I relate closely to *E. multifasciata china* AY159054.1 and specimens in group II relate closely to *E. multifasciata india* JQ767981.1.

The mitochondrial *12S rRNA* sequences of specimens from *E. multifasciata* HI, H2, H3, H9, H10, DL5, DL6, QN1, QN2, QN8, NA2, NA3, QB1, QB2, QT1, HT1, DN1, DN7, DN8, and DN2 were deposited in GenBank with accession number KT350390-KT350409, respectively.

Mausfeld et al. (2002) used 859 bp of the mitochondrial *16S* and *12S rRNA* genes to study the phylogenetic affinities of *Mabuya atlantica* Schmidt, 1945 and partitioned the genus *Mabuya* into four genera to reflect the independent origins of the Southern American, Asian, Afro-Malagasy and Cape Verdian groups: *Mabuya* Fitzinger, 1826; *Eutropis* Fitzinger, 1843; *Euprepis* Wagler, 1830 and *Chioninia* Gray, 1845 for four clades [22]. Basing on a combined analysis of 564 bp and 408 bp fragments, respectively, of the mitochondrial *16S* and *12S rRNA* genes, Mausfeld and Schmitz (2003) examined phylogenetic relationships within the Asian scincid lizard genus *Eutropis* Fitzinger, 1843 and taxonomic questions concerning *E. multifasciata.* They included individuals from seven populations over the entire range of *E. multifasciata*. Both MP and ML methods were used. Both produced quite similar topologies. Both trees showed strong bootstrap support for a monophyletic clade containing all South East Asian *Eutropis* taxa. Within the strongly supported South East Asian *Eutropis* clade two monophyletic subgroups were conspicuous: one containing all specimens of *E. multifasciata*, and one containing all Philippine/Palau species. Genetic variation within the *E. multifasciata* clade varied from 0.4% (between the Kalimantan + Myanmar specimens and the specimens from Myanmar + China) to 2.0% (between the specimens from the Philippine and China). The populations from Myanmar and South China were considered to represent the basis-like stock of *E. multifasciata* and the populations of Java, Bali, Cream and the Philippines were considered to represent island populations. These studies indicated that sampling predominantly from Southeast Asia (Indochinese and Sundaic subregion) with only two species from the Indian subregion, both of which are widely distributed across the subcontinent [23].

To test of the monophyly of *Eutropis* species and to determine the evolutionary origin of Indian members of this group, Datta-Roy et al. (2012) sequenced one nuclear (*C-mos*) and two mitochondrial (*12S rRNA* and *16S rRNA*) genes from most of the species from the Indian subregion: a fragment of *C-mos* was sequenced using primer as in Saint et al. (1998) [27] and a fragment of mitochondrial *12S rRNA* and *16S rRNA* genes were sequenced using primers as in Mausfeld and Schmitz (2003) [23]. The nuclear and mitochondrial trees generated from these sequences demonstrated the monophyly of *Eutropis* and thus supported the generic assignment of Asian members of this large genus to *Eutropis*. The Indian *Eutropis* nested within the larger Asian *Eutropis* clade [8]. In the studies mentioned above, Mausfelt et al. (2002, 2003) and Datta-Roy et al. (2012) have used specimen *E. longicaudata* collected from Phong Nha-Ke Bang National Park, Quang Binh, Vietnam (accession number AY070359), but unfortunately does not have specimen *E. multifasciata* collected from Vietnam.

Work done by Whiting et al. (2006) also recovered a monophyletic *Eutropis* which is sister group to a monophyletic Afro-malagasy *Mabuya* and a monophyletic South American *Mabuya*. Magalasy *Mabuya* are monophyletic and sister to one clade of South African taxa, while *M. atlantica* is sister to the remaining African species [29].

Our results support for one monophyletic subgroup containing all specimens of *E. multifasciata* and all currently recognized *E. multifasciata* populations have evolved from one common ancestor.

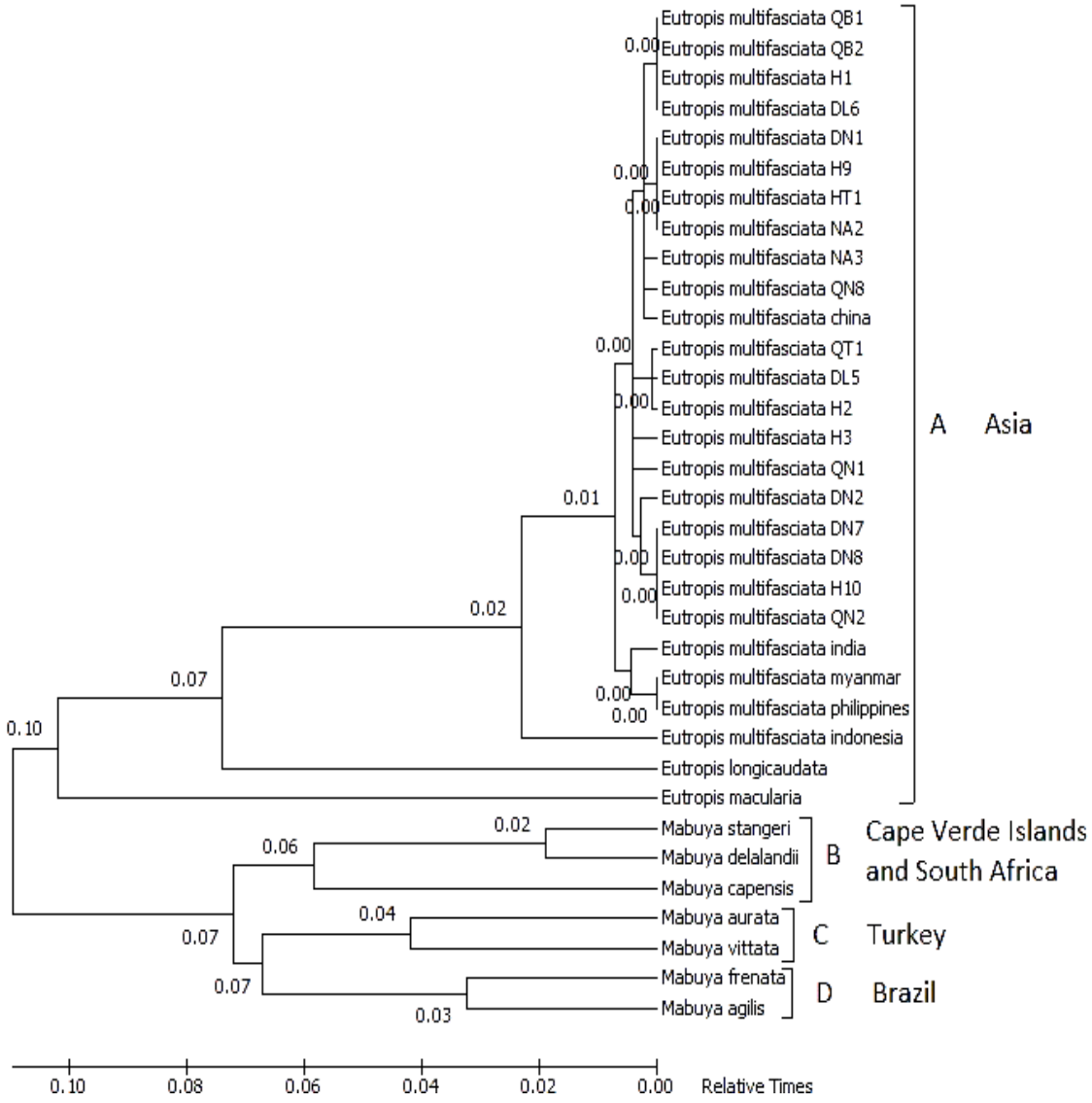

Figure 3. Phylogenetic tree of *E. multifasciata* (HI, H2, H3, H9, H10, DL5, DL6, QN1, QN2, QN8, NA2, NA3, QB1, QB2, QT1, HT1, DN1, DN7, DN8, and DN2) and species belonging to genus *Eutropis* and *Mabuya* in GenBank: *E. multifasciata china* (AY159054.1), *E. multifasciata india* (JQ767981.1), *E. multifasciata myanmar* (AY159059.1), *E. multifasciata philippines* (AY159055.1), *E. multifasciata indonesia* (AY159056.1), *E. longicaudata* (AY070341), *E. macularia* (AY159049), *M. stangeri* (AF280167), *M. delalandii* (AF280185), *M. capensis* (AF280139.2), *M. aurata* (AY151435), *M. vittata* (AY151423), *M. frenata* (AY151427), *M. agilis* (AY151434)

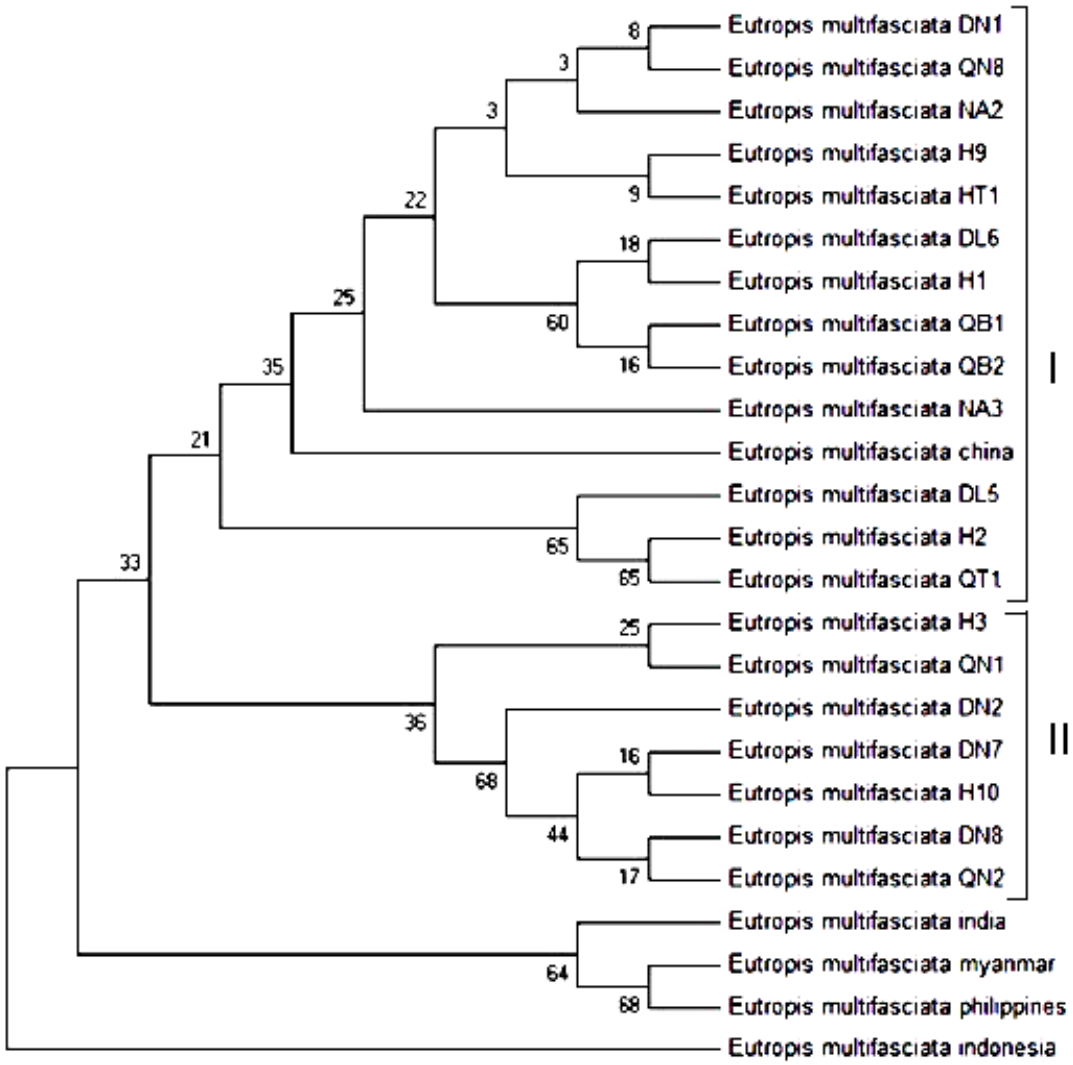

Figure 4. Phylogenetic tree of *E. multifasciata* HI, H2, H3, H9, H10, DL5, DL6, QN1, QN2, QN8, NA2, NA3, QB1, QB2, QT1, HT1, DN1, DN7, DN8, DN2, *E. multifasciata china* (AY159054.1), *E. multifasciata india* (JQ767981.1), *E. multifasciata myanmar* (AY159059.1), *E. multifasciata philippines* (AY159055.1) and *E. multifasciata indonesia* (AY159056.1)

CONCLUSION

Analysis of *12S rRNA* sequences of specimens from Common Sun Skink *Eutropis multifasciata* in Central Vietnam showed genetic differences among specimens range from 0% to 1,79%. The mitochondrial tree generated from these sequences confirmed the monophyly of all specimens of *E. multifasciata* and the monophyly of the genus *Eutropis.*

Acknowledgments
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