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MOLECULAR PHYLOGENY OF COMMON SUN SKINK, *EUTROPIS MULTIFASCIATA* (SQUAMATA: SCINCIDAE), OF NORTHWESTERN PART OF BANGLADESH

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Abstract: A study on the phylogenetic relationship of Bangladeshi Skink confirmed species as *Eutropis multifasciata* by Bayesian inference (BI) (100%) and 98% node support to maximum likelihood (ML) tree topology, respectively. The sequence divergences between *Eutropis multifasciata* and other congeneric species were significant, ranging from 0.1 to 13.6% for *16S rRNA*. Intraspecific genetic divergence within *E. multifasciata* was estimated 10.3%. *E. multifasciata* formed a distinct clade with high posterior probability support. This maiden study on Bangladeshi Skink results support that one monophyletic subgroup containing all specimens of *E. multifasciata* and all recently recognized *E. multifasciata* populations have evolved from one common ancestor.

Key words: Scincidae, *Eutropis multifasciata*, phylogeny, *16S* rRNA gene

INTRODUCTION

The genus *Eutropis* representing Asian radiation of Scincid lizards have 31 described species (Mausfeld *et al.* 2002) and many yet to undescribed species occurring from South Asian countries. The phylogenetic relationships of Asian *Eutropis* are far from being understood and therefore remain largely unclear and speculative. However, some major works provide quite comprehensive data on the *Eutropis* taxa recognized. Many more additional species have been described by researchers (Boulenger 1887, Rooij 1915, Smith 1935, Tikader and Sharma 1992, Mahony and Reza 2008). We have collected two specimens of robust form of *Eutropis* that closely resemble *Eutropis* (25.636574°N, 88.636322°E). Because of limited size of sample and *mutifasciata* one from Lalpur (24.1833°N, 88.9750°E) and one from Dinajpur observational differences between specimens from India and those from South Asia, we report these specimens as *Eutropis multifasciata* pending a more thorough morphological and genetic analysis. Both the specimens were collected during the day while foraging in grassy vegetation.

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Although, it is well documented from north-east India (Smith 1935, Das 1996, Sharma 2002, Das 2008), there is no substantial data on *E. multifasciata* in Bangladesh. Even Bangladesh has no scientifically maintained natural history museum or other recognized national repository for biological specimens, further adding difficulty when trying to confirm the presence or absence of presumed extant species. According to IUCN (2015) in Bangladesh have 13 species of skinks, and only four under genus *Eutropis* (i.e. *E. carinata, E. dissimilis, E. multifasciata, E. macularia*) that are described morphologically, or photograph compared elsewhere. However, it raises some contradiction of species description, distribution, and even systematic position. Most recently Khan (2007) described two new country records of skinks found in Chittagong, namely *Scincella reevesi* and *Sphenomorpha indicus*. The record (and photograph) of *Scincella reevesi* and *Sphenomorpha indicus*, based on photographs and general descriptions. Although both *Scincella reevesi* and *Sphenomorphus indicus* are likely to be present in Bangladesh, none of the above species have molecular or genetic databases in Bangladesh. Therefore, the present study has undertaken a step morphologically similar species of *Eutropis multifasciata* to confirm its genus and species by mitochondrial DNA analysis first time in this study.

MATERIAL AND METHODS

The specimens were collected from two localities, namely Lalpur (24.1833°N, 88.9750°E) and Dinajpur (25.636574°N, 88.636322°E) in the north-western part of Bangladesh (Figs 1 and 4) between February and April, 2016 and stored in 95% ethanol. For the molecular analysis, small amount of livers/muscles were removed from anesthetized or dead specimens and stored at – 80°C.

Ethical permission: Tissues of the studied specimens were carried to China for Research and study with the permission of Chief Conservator of Forest (Original permit/Certificate No.06/2017, BD 9118468, 30 March 2017) under the Forest Department and Ministry of Environment and Forest, Bangladesh. The voucher specimen's tissues were deposited in the Forest Department and ethical rules were followed Wildlife Preservation and Security Acts, 2012. Collected tissues from the specimens are not threatened species and not listed in IUCN Red list or CITES. All specimens' tissues were collected from non-protected areas.

Morphological description: The following characteristics were followed for proper identification of the specimens as described by Boulenger (1887): (1) Snout moderate and obtuse, (2) lower eyelid scaly, (3) nostril behind vertical of the suture between rostral and first tibial, (4) ear opening roundish or oval, (5)

dorsal scale more or less distinctly tri-carinate (nuchals and laterally keeled), (6) the hind limbs reaches the wrist and sub-digital lamellae smooth, (7) tail larger than body and tapering to the end, (8) body color brown to olive and ventral surface light yellowish and (9) limbs pentadactylies.

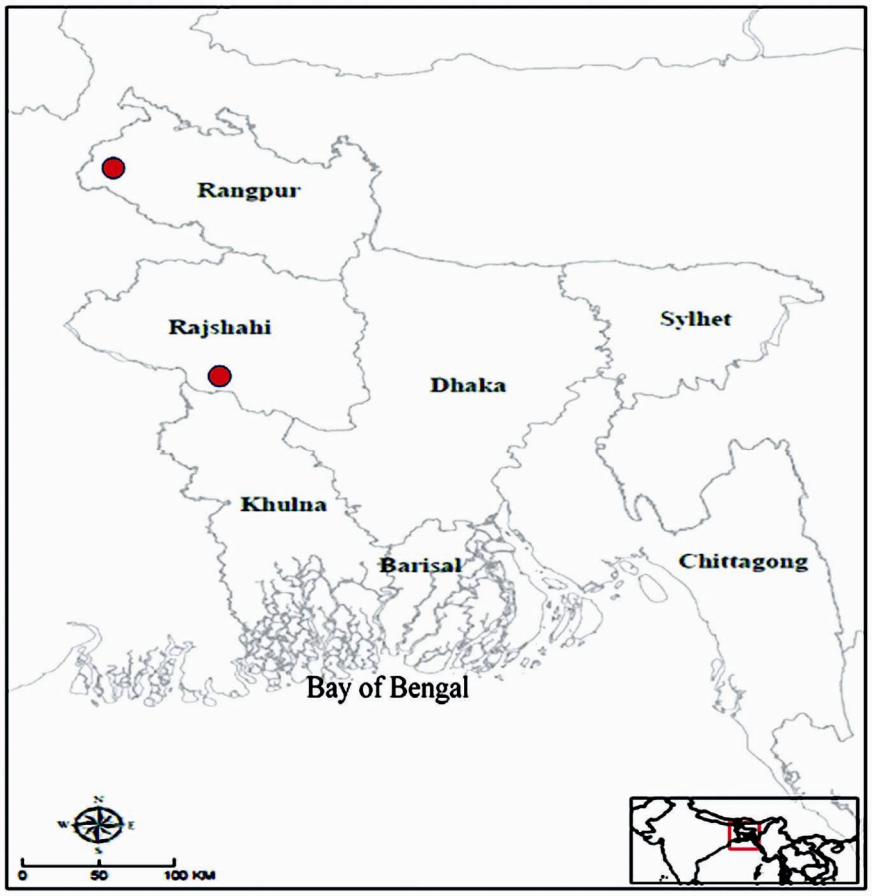

Fig. 1. Sites (color filled circle) of sampling specimens, northwestern part of Bangladesh.

DNA extraction and PCR protocol: Total genomic DNA was extracted from the muscle or liver tissues with three steps of standard phenol-chloroform methods of Sambrook *et al.* (1989) with some modifications. The tissues were homogenized in 0.6 ml of STE buffer containing 10 mM tris/HCl (pH 8.0), 0.10 ml SDS (10%) and added 0.30 ml proteinase K (0.1 mg/ml) for digesting protein for 4 - 12 hrs at 56ºC. The solution was treated with phenol and chloroform /isoamyl alcohol, finally DNA was precipitated with ethanol. The DNA

precipitates were dried and then resuspended in 0.5 ml of TE (10 mM Tris/HCl, 1 mM EDTA, pH 8.0), and quantity was determined by measuring the absorbance at 260 nm and the concentration, purity and quality were determined by measuring the absorbance at 260/280 nm and 230/260 ratios using a NanoDrop TM 1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) and quality was checked by 1.2% agarose gel. Extracted DNA were appropriately labeled and stored at –20°C for analysis, however 1.5 µl of working DNA was used for polymerase chain reaction (PCR).

Fig. 1. *Eutropis multifasciata* in natural habitat found at Lalpure, Natore, Bangladesh.

One pair of primers (2215 and 2216) was used for the amplification of *Eutropis* DNA. The primer sequences as 2215 (16SAR 5' CGCCTGTTTAYCAAAAA CAT-3') forward and 2216 (16SBR 5'-CCGGTYTGAACTCAGATCAYGT-3') reverse designed according to Bossuyt and Milinkovitch (2000). The PCR mixture was prepared from the company in a final volume of 25 µl containing PCR buffer 2.5 µl, forward and reverse primer 1 µl each, dNTPs 1 µl, *Taq* polymerase 0.25 µl and remaining was sterile water. In PCR, the *16S* rRNA gene was amplified for 35 cycles, each consisting of denaturing at 94ºC for 1 min, annealing for 40 sec at 55ºC and extension for 1 min at 72ºC. The PCR product was checked in 2% agarose gel under UV immunofluorescence before sequencing.

Sequencing and phylogenetic analysis: The purified PCR products were directly sequenced with an ABI automated DNA sequencer in both the directions for each species DNA. Sequence data obtained for each sample were adjusted manually by eye using DNASTARv.7.1 (DNASTAR Inc., Madison, WI, USA). The sequences of each gene region were aligned using the ClustalW option of Bioedit (Hall 1999). They were submitted for BLAST search in GenBank to ensure the

target sequences had been properly amplified. The obtained sequences were deposited in National Centre for Biotechnology Information (NCBI) for accession numbers (MK524576). Available sequences for these genus and species were downloaded from GenBank and an out group (Table 1).

Table 1. List of all specimens, locality and accession number used in this study

Genus	Species	Locality	Accession No.
Eutropis	*multifasciata*	Lalpur, Bangladesh	This study
Eutropis	*multifasciata*	Dinajpur, Bangladesh	This study
Eutropis	*multifasciata*	Philippines	JF497984
Eutropis	*multifasciata*	China	AY159083
Eutropis	*multifasciata*	Indonesia	AY159082
Eutropis	*multifasciata*	Laos	DQ238897
Eutropis	*multifasciata*	Cambodia	AY151459
Eutropis	*multifasciata*	Thailand	AB028788
Eutropis	*multifasciata*	Kaziranga, Assam, India	JQ767964
Eutropis	*macularia*	Satkosa, Orissa, India	JQ767961
Eutropis	*macularia*	Saravati valley, Karnataka, India	JQ767960
Eutropis	*macularia*	Bondla, Goa, India	JQ767959
Eutropis	*macularia*	Ponmudi, Kerala, India	JQ767958
Eutropis	*macularia*	Bagdogra, West Bengal, India	JQ767957
Eutropis	*rufigera*	Mt. Harriat, Andaman Is.□India	AY159079
Eutropis	*quadricarinata*	Chattin, Myanmar	AY159089
Eutropis	*macrophthalma*	Java, Indonesia	AY159077
Eutropis	*indreprensa*	NW Panay, Philippined	AY159076
Eutropis	*tytleri*	Mt. Harriat, Andaman island, India	AY159074
Eutropis	*beddomii*	Deomali, Orissa, India	JQ767965
Eutropis	*bibronii*	Rushikulya, Orissa, India	JQ767963
Eutropis	*clivicola*	Parambikulam, Kerala, India	JQ767956
Eutropis	*trivittata*	Satara, Maharashtra, India	JQ767951
Eutropis	*nagarjuni*	Nagarjunasgor, Andra Pradesh, India	JQ767952
Eutropis	*dissimilis*	Myanmar	AY159075
Eutropis	*macularia*	Myanmar	AY159078
Eutropis	*madaraszi*	Sri Lanka	AY159080
Eutropis	*multicarinata*	Luzon, Philippines	AY159081
Eutropis	*cumingi*	Luzon, Philippines	DQ238896
Eutropis	*carinata*	Kutch, Gujarat, India	JQ767955
Eutropis	*carinata*	Karnataka, India	JQ767953
Eutropis	*macularia*	Pakistan	AY070353
Eutropis	*longicaudata*	Phong Nha-Ke Bang, Vietnam	AY070359
Eutropis	*rufigera*	Nicobar, India	JQ767962
Scelotes	*caffer*	Brandberg, South Africa	AY217985

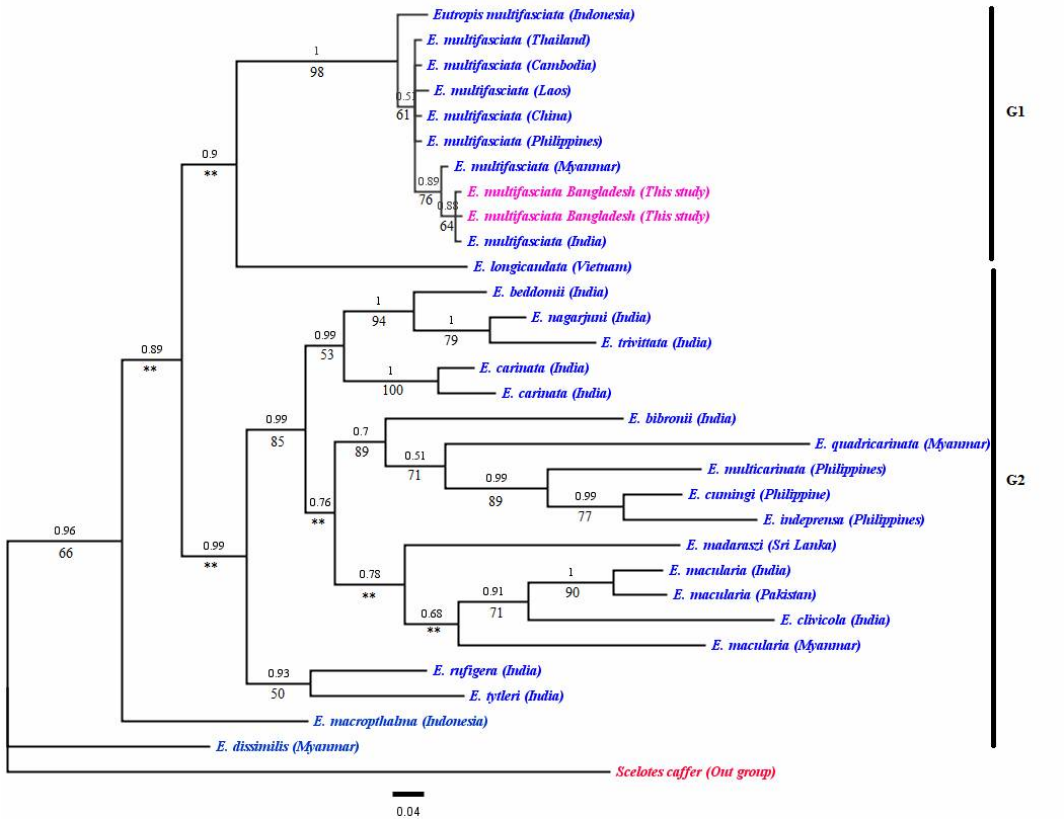

Fig. 2. Bayesian Inference (BI) tree based on mitochondrial DNA (*16S* rRNA gene) using the GTR+G+I substitution model from 30 *Eutropis* haplotypes with *Scelotes caffer* as an out group. The bootstrap values are given above the branches (BI) and below the branches (ML) are based on 1000 replication in both analyzed. The node support values <50% are denoted by '**'for maximum likelihood. The scale bar represents 0.04 nucleotide substitution per site for this tree.

The nucleotide sequences of the *16S* gene were aligned with sequences for other *Eutropis* species available from GenBank with ClustalW built into Bioedit (Thomson *et al.* 1994, Hall 1999) using the default parameters. The final sequence length used for the further phylogenetic analyses was 401 bp aligned in MUSCLE (Edgar 2004). The phylogenetic analyses were performed using Bayesian inference method. The GTR+G substitution model was selected as the optimal nucleotide substitution and bootstrap value 1000 used as replications implemented in Mega v.6.06. (Tamura *et al.* 2013).

For Bayesian analysis, ten million generations were run (Markov chain Monte Carlo method) with a sampling frequency of 1000, as implemented in Mr. Bayes v3.1.2. (Ronquist and Huelsenbeck 2003). Convergence of the runs was assessed by the average split frequency of standard deviations (<0.01) and by

checking the potential scale reduction factors (~1.0) for all model parameters. 30% of the trees were discarded as burn in and the remaining trees were used to generate the 50% majority rule consensus tree and to estimate the Bayesian posterior probabilities. Genetic pairwise distance of all taxa was calculated using Kimura-2 parameter model in Mega v.6.06. (Tamura *et al.* 2013, Kimura 1980). The matrilineal genealogy was assumed to reflect the phylogenetic relationships of the species.

In the present trees a distinct Indian and Asian clade was retrieved with high support. The sequence divergences between *Eutropis multifasciata* and other congeneric species were significant, ranging from 0.1 to 13.6% for *16S rRNA* (Table 2). Intraspecific genetic divergence within *E. multifasciata* was estimated 10.3% (Thailand population). *E. multifasciata* formed a distinct clade in the phylogenetic analyses with high posterior probability support both in BI (100%) and ML (98%) (Fig. 2). Interestingly it clade with Indian Assam and Myanmar *multifasciata* species. An aligned figure was drawn with our mtDNA sequences with others Asian *E. multifasciatus* from different countries compared by MEGA was downloaded from GenBank (Fig. 3).

RESULTS AND DISCUSSION

The molecular data presented here demonstrate the monophyly of the Asian *Eutropis multifasciata* and further support the generic assignment of Asian population of this large genus to *Eutropis*. Bangladeshi *Eutropis* was nested within the Indian *Eutropis* clade as well as sister clade with other Asian *multifasciata*. For the phylogenetic relationship 31 mitochondrial *16S rRNA* gene fragments were analyzed. The nucleotide sequences of *16S rRNA* gene fragment are shown in Fig. 2. The total *16S rRNA* gene fragment consists of 401 sites, 153 of which were variables. Phylogenetic tree with *E. multifascita* population from Asian countries based on *16S rRNA* gene fragment is shown in Fig. 3.

In this phylogenetic tree, specimens from Asia are well supported as a distinct clade (G1) that include all specimens of *E. multifasciata*. The other major groups are from available species clade under the genus *Eutropis* (G2). In clade G1, present *E. multifasciata* sequences clade with Indian species, and the remaining South-East Asian *multifasciata* clade in a sister group having high support (BI =100%, ML = 98%). Although, Indonesian species has separate sister clade. Mausfeld *et al.* (2002) used mitochondrial *16S* and *12S rRNA* gene to study phylogenetic affinities of *Mabuya* reflect four independent origins as Southern American, Asian, Afro-Malagacy and Cape Verdean: *Mabuya* Fitzinger 1926, *Eutropis* Fitzinger 1843; *Euprepis* Wagner 1830 and *Chioninia* Gray 1945

Table 2. Pairwise genetic divergence (number of base substitution per site) among Asian *Eutropis* based on 401 bp mtDNA *(16S* gene) sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses of 30 nucleotide sequences congeneric species were conducted in MEGA6

Species (Accession #)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
E. multifasciata (This study)	**1**																														
E. multifasciata (This study)	**2**	0.000																													
E. multifasciata (JQ767964)	**3**	0.000	0.000																												
E. multifasciata (AY159088)	**4**	0.010	0.010	0.010																											
E. multifasciata (JF497984)	**5**	0.010	0.010	0.010	0.000																										
E. multifasciata (AY159083)	**6**	0.020	0.020	0.020	0.010	0.010																									
E. multifasciata (AY159082)	**7**	0.013	0.013	0.013	0.003	0.003	0.013																								
E. multifasciata (DQ238897)	**8**	0.010	0.010	0.010	0.000	0.000	0.010	0.003																							
E. multifasciata (AY151459)	**9**	0.010	0.010	0.010	0.000	0.000	0.010	0.003	0.000																						
E. multifasciata (AB028788)	**10**	0.103	0.103	0.103	0.098	0.098	0.095	0.098	0.100	0.098																					
E. macularia (JQ767957)	**11**	0.136	0.136	0.136	0.141	0.141	0.138	0.141	0.141	0.141	0.126																				
E. quadracarinata (AY159089)	**12**	0.003	0.003	0.003	0.008	0.008	0.018	0.010	0.008	0.008	0.101	0.138																			
E. rufigera (AY159079)	**13**	0.069	0.069	0.069	0.074	0.074	0.082	0.074	0.074	0.074	0.110	0.107	0.072																		
E. macularia (AY159078)	**14**	0.127	0.127	0.127	0.129	0.129	0.132	0.129	0.132	0.129	0.100	0.125	0.129	0.116																	
E. macrophtalma (AY159077)	**15**	0.098	0.098	0.098	0.096	0.096	0.101	0.098	0.098	0.096	0.087	0.127	0.098	0.103	0.111																
E. indeprensa (AY159076)	**16**	0.112	0.112	0.112	0.110	0.110	0.105	0.110	0.109	0.110	0.111	0.120	0.115	0.098	0.120	0.129															
E. tytleri (AY159074)	**17**	0.101	0.101	0.101	0.101	0.101	0.106	0.101	0.105	0.101	0.110	0.132	0.103	0.067	0.126	0.115	0.107														
E. beddomii (JQ767965)	**18**	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.105	0.098	0.115	0.108	0.086	0.107	0.099	0.097	0.089													
E. bibronii (JQ767963)	**19**	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.113	0.093	0.108	0.116	0.092	0.092	0.110	0.101	0.117	0.103												
E. clivicola (JQ767956)	**20**	0.134	0.134	0.134	0.131	0.131	0.129	0.131	0.133	0.131	0.082	0.154	0.134	0.126	0.115	0.117	0.136	0.123	0.116	0.129											
E. nagarjuni (JQ767952)	**21**	0.103	0.103	0.103	0.103	0.103	0.103	0.103	0.106	0.103	0.101	0.119	0.106	0.081	0.100	0.105	0.108	0.074	0.046	0.104	0.114										
E. trivittata (JQ767951)	**22**	0.105	0.105	0.105	0.108	0.108	0.108	0.108	0.110	0.108	0.109	0.123	0.108	0.086	0.107	0.110	0.121	0.077	0.064	0.116	0.127	0.038									
E. multicarinata (AY159081)	**23**	0.115	0.115	0.115	0.120	0.120	0.118	0.120	0.120	0.120	0.124	0.113	0.118	0.085	0.120	0.132	0.086	0.109	0.103	0.103	0.147	0.098	0.095								
E madaraszi (AY159080)	**24**	0.109	0.109	0.109	0.107	0.107	0.109	0.107	0.111	0.107	0.085	0.138	0.112	0.093	0.122	0.103	0.117	0.109	0.094	0.115	0.105	0.111	0.113	0.125							
E. carinata (JQ767955)	**25**	0.092	0.092	0.092	0.097	0.097	0.097	0.097	0.097	0.097	0.093	0.113	0.095	0.073	0.099	0.099	0.095	0.084	0.066	0.082	0.108	0.069	0.082	0.087	0.105						
E. carinata (JQ767953)	**26**	0.102	0.102	0.102	0.102	0.102	0.102	0.102	0.102	0.102	0.095	0.122	0.105	0.078	0.104	0.109	0.094	0.089	0.061	0.087	0.111	0.074	0.089	0.095	0.104	0.025					
E. cumingi (DQ238896)	**27**	0.102	0.102	0.102	0.105	0.105	0.099	0.105	0.104	0.105	0.119	0.120	0.105	0.098	0.117	0.119	0.053	0.114	0.113	0.090	0.139	0.106	0.108	0.061	0.120	0.087	0.094				
E. dissimilis (AY159075)	**28**	0.095	0.095	0.095	0.096	0.096	0.098	0.098	0.095	0.096	0.127	0.146	0.098	0.082	0.142	0.108	0.120	0.118	0.121	0.124	0.139	0.119	0.118	0.115	0.125	0.108	0.115	0.118			
E. longicaudata (AY070359)	**29**	0.082	0.082	0.082	0.084	0.084	0.084	0.087	0.084	0.084	0.098	0.132	0.082	0.090	0.126	0.097	0.118	0.102	0.095	0.106	0.127	0.096	0.096	0.111	0.108	0.105	0.105	0.103	0.117		
E. macularia (AY070353)	**30**	0.108	0.108	0.108	0.105	0.105	0.103	0.105	0.108	0.105	0.028	0.129	0.108	0.115	0.095	0.082	0.113	0.115	0.098	0.096	0.082	0.101	0.113	0.121	0.092	0.095	0.103	0.121	0.129	0.101	

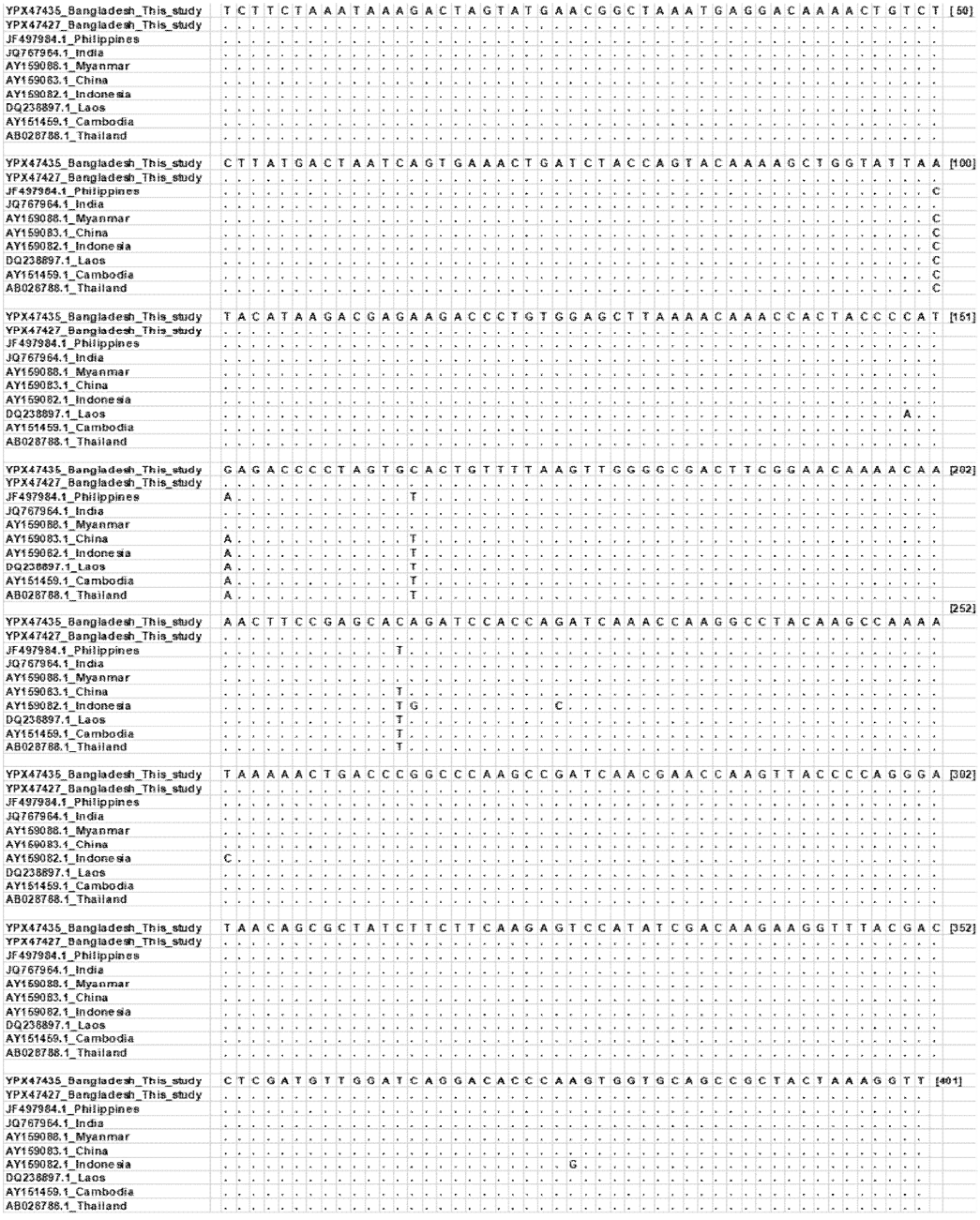

Fig. 3. Aligned sequences of 401 bp segment of 16S rRNA mitochondrial gene from the Asian *Eutropis multifasciata* population. Dots indicate identity to the Bangladeshi *Eutropis multifasciata* compared with other Asian countries.

clade four respectively (Mausfeld *et al.* 2002). However, in later, Mausfeld and Schmid (2003) again used *12S+16S rRNA* mitochondrial genes combined used to

resolve the previous problem, and found that all Asian Scincid lizard genus *Eutropis* clade in a monophyletic clade but the problem remains in *E. multifasciata*. To resolve this problem, they used seven *E. multifasciata* population from different geographic location and found that both BI and ML tree showed similar topology supporting monophyletic clade containing al South Asian *Eutropis* taxa.

Authors of this study also found the similar tree topology support that all South Asian *E. multifasciata* showed strong bootstrap support for monophyletic clade. However, South East Asian *Eutropis* clade two monophyletic subgroups are conspicuous; one containing Bangladesh and Indian *E. multifasciata* including Myanmar and other containing Indonesia, Philippines, China, Laos, Cambodia and Thailand population of *E. multifasciata.* In G2, several subgroups were formed; Myanmar *Eutropis* clade with Indian *bibrinii*, all Philippines *Eutropis* formed monophyletic clade (*E. multicarinata* + *E. indreprensa* + *E. cumingi*) in this study agree with previous studies (Crombie and Pregill 1999, Brown and Alcala 1980). Indian all main land species formed single clade (*E. carinata* + *beddomii* + *nagarjuni* + *trivittata*) with high support value (BI = 99%), however Andaman island species formed sister clade. Other island species like Sri Lanka, Java, Andaman clade with Pakistani *E. macularia* species. Only *E. longicaudata* from Vietnam formed individual clade with low support, and most dispersible species *E. dissimilis* of Myanmar clade G2 (ML = 66%), although previously it was identified as *Mabuya novencarinata* (Mausfeld and Schmitz 2003). In the second group, *E. macularia* which is widely distributed is endemic in India and Sri Lanka. *E. macularia* is distributed in most areas of Indian subcontinent as well as South East Asia, although it was shown paraphyletic with respect to *E. tammanna* (Ota *et al.* 2001, Mausfeld and Schmitz 2003, Mausfeld *et al.* 2000, Das *et al.* 2008).

In the present result, the *E. macularia* clade with Indian subcontinental *Eutropis* population of Myanmar and Pakistan that is similar with that of Dutta-Roy *et al.* (2012). The extensively distributed *E. multifasciata* recorded from India in the west of Philippines in the northeast (Brown and Alcala 1980, Tikader and Sharma 1992), Indonesia (Mertens 1930, Mausfeld and Böhme 2002) and other South East Asian (China, Cambodia, Laos) - forms a well-supported monophyletic clade (ML = 99%, BI = 100%). Thus, we concluded that all currently recognized *multifasciata* populations evidently evolve from one common ancestor. Biogeographically, Bangladesh is part of Oriental region, nested between Indo-Himalayan and Indo-Chinese sub-regions of the Orient (Nishat *et al.* 2002). In this regard, Mani (1974) suggested that the Assam region of Northeast India might have served a gateway through which Southeast Asian

elements reached Indian peninsula. North of Assam, the Himalayan chain of mountains acted as a barrier and south was covered by sea, therefore Assam as a possible route of which facilitated the exchange of biota.

Thus, the present study completely supports the hypothesis of Mani (1974) that Bangladeshi population of *E. multifasciata* clade with Assam *E. multifasciata* although the great dispersal ability of *E. multifasciata* via humans has already been indicated for Taiwan (Ota *et al.* 1994) and the Philippines (Brown and Alcala 1970). Although researchers (Meijaard and Grove 2006, Sengupta *et al.* 2009, Chen *et al.* 2018) have reported that the mighty Brahmaputra river has long been recognized as a barrier of many species in India and Indo-China sub-regions (Dutta-Roy *et al.* 2012), in case of *E. multifasciata* dispersal in Bangladesh such scenario was not effective. A recent survey (IUCN 2015) reported that this species is confined in Northeastern part of Bangladesh (Near Assam in India), however this first time reporting on molecular phylogenetic relationship of *Eutropis multifasciata* collected from the North-western part of Bangladesh needs further extensive survey throughout the country.

CONCLUSION

The molecular phylogenetic results of this study support one monophyletic subgroup containing all specimens of *E. multifasciata* and all recently recognized *E. multifasciata* populations have evolved from one common ancestor including Bangladesh. Habitat degradation is likely the cause of decline, but the species can adapt to some degree of habitat disturbances and the species categorized least concern to IUCN list Bangladesh.

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LITERATURE CITED

BOSSUYT F. and MILINKOVITCH M.C. 2000. Convergent adaptive radiations in Madagascan and Asian ranid frogs reveal covariation between larval and adult traits. *Proc. Natl. Acad. Sci. USA* **97**: 6585-6590.

BOULENGER G.A. 1887. Catalogue of the Lizards in the British Museum (Natural History). Volume III. Lacertidae, Gerrosauridae, Scincidae, Anelytropidae, Dibamidae, Chamaeleonidae. London.

BROWN W.C. and ALCALA A.C. 1970. The zoogeography of the herpetofauna of the Philippine Islands, a fringing archipelago. *Proc. Cal. Acad. Sci.* **38**: 105-130.

BROWN W.C. and ALCALA A.C.1980. Philippine lizards of the family Scincidae (with a description of a new species of *Tropidophorus* by James P. Bacon). *Sill. Univ. Nat. Sci. Monogr.* **2**: 1-264.

CHEN J.M., POYARKOV Jr. N.A., SUWANNAPOOM C., LATHROP A., Wu Y.H., ZHOU W.W., YUAN Z.Y., JIN J.Q., CHEN H.M., LIU H.Q. , NGUYEN T.Q, NGUYEN S.G., DUONG T.V., ETO K., NISHIKAWA K., MATSUI M., ORLOV N.L., STUART B.L., BROWN R.M., ROWLEY J.J.L., MURPHY R.W., WANG Y.Y. and CHE J. 2018. Large-scale phylogenetic analyses provide insights into unrecognized diversity and historical biogeography of Asian leaf-litter frogs, genus *Leptolalax* (Anura: Megophryidae). *Mol. Phyl. Evol*. **124**: 162-171.

CROMBIE R.I. and PREGILL G.K. 1999. A checklist of the herpetofauna of the Palau Islands (Republic of Belau), Oceania. *Herpetol. Monogr.* **13**: 29-80.

DAS A. 2008. Diversity and distribution of herpetofauna and evaluation of conservation status in Barail Hill Range (including Barail Wildlife Sanctuary), Assam, northeast India. Final Report Aaranyak. Guwahati. pp. 94.

DAS I., SILVA A.D. and AUSTIN C.C. 2008. A new species of *Eutropis* (Squamata: Scincidae) from Sri Lanka. *Zootaxa* **1700**: 35-52.

DATTA-ROY A., SINGH M., SRINIVASULU C. and KARANTH K.P. 2012. Phylogeny of the Asian *Eutropis* (Squamata: Scincidae) reveals an 'into India' endemic Indian radiation. *Mol. Phyl. Evol.* **63**: 817-824.

EDGAR R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**(5): 1792-1797.

FELSENSTEIN J. 2003. Confidence limits on phylogenies, an approach using bootstrap. *Evolution* **39:** 783-791.

HALL T.A.1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows95/98/NT. *Nucleic Acids Symp. Ser.* **41**: 95-98.

IUCN Bangladesh. 2015. *Red List of Bangladesh Volume 4: Reptiles and Amphibians*. IUCN, International Union for Conservation of Nature, Bangladesh Country Office, Dhaka, Bangladesh, xvi+320 pp.

KHAN M.M.H. 2007. First report of Reeve's ground skink *Scincella reevesi* and Himalayan litter skink *Sphenomorphus indicus* from Bangladesh. *Zoos' Print Journal* **22**(1): 2,545.

KIMURA M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111-120.

MAHONY S.and REZA A.H.M.A. 2008. A herpetofauna collection from the Chittagong Hill Tracts, Bangladesh, with two new species records for the country. *Hamadryad* **32**(1): 45-56.

MANI M.S. 1974. Biogeographical evolution in India. In: Mani, M.S. (Ed.), Ecology and Biogeography of India, Dr. W. Junk b.v. Publishers, The Hague, pp. 698-724.

MAUSFELD P. and BÖHME W. 2002. A new *Mabuya* from Java, Indonesia. *Salamandra* **38**: 135-144.

MAUSFELD P. and SCHMITZ A. 2003. Molecular phylogeography, intraspecific variation and speciation of the Asian scincid lizard genus *Eutropis* Fitzinger, 1843 (Squamata: Reptilia: Scincidae): taxonomic and biogeographic implications. *Org. Div. Evol*. **3**: 161-171.

MAUSFELD P., SCHMITZ A., BÖHME W., MISOF B., VRCIBRADIC D. and 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.

MAUSFELD P., VENCES M., SCHMITZ A. and VEITH M. 2000. First data on the molecular phylogeography of scincid lizards of the genus *Mabuya. Mol. Phyl. Evol.* **17**: 11-14.

MEIJAARD E. and GROVES C.P. 2006. The geography of mammals and rivers in mainland Southeast Asia, Springer Science + Business Media, LLC, 233 Spring Street, New York, NY 10013, USA, pp. 305-330 (Chapter 11).

MERTENS R.1930. Die Amphibien und Reptilien der Inseln Bali, Lombok, Sumbawa und Flores. *Abh Senckenberg Naturf Gesell*. **42**: 115-344.

NISHAT A., HUQ S.M.I., BARUA S.P., REZA A.H.M.A. and KHAN A.S.M. 2002. Bio-ecological Zones of Bangladesh. IUCN, The World Conservation Union, Bangladesh Country Office. XII+ 141 pp.

OTA H., CHANG H.W., LIU K.C. and HIKIDA T. 1994. A new record of the viviparous skink, M Mertens, R. (1930): Die Amphibien und Reptilien der Inseln Bali, Lombok, Sumbawa und Flores. Abh. Senckenberg. Naturf. Gesell. **42**: 115–344. *Mabuya multifasciata* (Kuhl, 1820) (Squamata: Reptilia), from Taiwan. *Zool Studies* **33**: 86-8.

POSADA D. and CRANDALL K.A. 1998. Model test: Testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

RONQUIST F. and HUELSENBECK J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.

ROOIJ N.D. 1915. The reptiles of Indo-Australian Archipelago. E.J. Brill. Ltd. Leiden. pp. 364.

SENGUPTA S., DAS A., DAS S., HUSSAIN B., CHOUDHURY N.K. and DUTTA S.K. 2009. Taxonomy and biogeography of Kaloula species of eastern India. *Nat. Hist. J. Chulalongkorn Univ.* **9**: 209-222.

SHARMA R.C. 2002. The fauna of India and the adjacent countries – Reptilia (Sauria). vol. II, Zoological Survey of India, Kolkata. pp. 1-430.

SMITH M.A. 1935. The Fauna of British India, Including Ceylon and Burma, Reptilia and Amphibia, vol. II – Sauria. Taylor and Francis, London.

STAMATAKIS A., HOOVER P., ROUGEMONT J. and RENNER S. 2008. A rapid bootstrap algorithm for the RAxML web servers. *Syst. Biol.* **57**(5): 758-771.

SWOFFORD D.L. 2002. PAUP: Phylogenetic Analysis Using Parsimony (and Other Methods), Version 4.10b. Sinauer Associates, Sunderland, MA.

TAJIMA F. and NEI M. 1984. Estimation of evolutionary distance between nucleotide sequences. *Mol. Bio. Evol.* **1**: 269-285.

TAMURA K., STECHER G., PETERSON D., FILIPSKI A. and KUMAR S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725-2729.

THOMPSON J.D., HIGGINS D.G. and GIBSON T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673-4680.

TIKADER B.K. and SHARMA R.C. 1992. Hand book of Indian Lizards. The Radiant Process Private Ltd., Kolkata. xv+250 pp.

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