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Phylogeny of the Asian *Eutropis* (Squamata: Scincidae) reveals an ‘into India’ endemic Indian radiation

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ABSTRACT

Recent generic rearrangement of the circumtropical distributed skink genus ‘*Mabuya*’ has raised a lot of debate. According to this molecular phylogeny based rearrangement, the tropical Asian members of this genus have been assigned to *Eutropis*. However, in these studies the Asian members of ‘*Mabuya*’ were largely sampled from Southeast (SE) Asia with very few species from Indian subcontinent. To test the validity of this assignment and to determine the evolutionary origin of Indian members of this group we sequenced one nuclear and two mitochondrial genes from most of the species from the Indian subregion. The nuclear and mitochondrial trees generated from these sequences confirmed the monophyly of the tropical Asian *Eutropis*. Furthermore, in the tree based on the combined mitochondrial and nuclear dataset an endemic Indian radiation was revealed that was nested within a larger Asian clade. Results of dispersal-vicariance analysis and molecular dating suggested an initial dispersal of *Eutropis* from SE Asia into India around 5.5–17 million years ago, giving rise to the extant members of the endemic Indian radiation. This initial dispersal was followed by two back dispersals from India into SE Asia. We also discuss the relationships within the endemic Indian radiation and its taxonomic implications.

1. Introduction

The skink genus *Mabuya* Fitzinger, 1826 (Scincidae: Subfamily Lygosominae) is one of the most widely distributed skink genera with circumtropical distribution. Recently, based on molecular data, this genus was putatively divided into four genera (Mausfeld et al., 2000, 2002; Mausfeld and Schmitz, 2003). The Asian species of ‘*Mabuya*’ are now under the genus *Eutropis* Fitzinger, 1843. The Afro-Malagasy species were assigned to the genus *Euprepis* Wagler, 1830 but have since been reassigned to *Trachylepis* Fitzinger, 1843 by Bauer (2003). The species from Cape Verde islands are now under the resurrected genus *Chioninia* Gray, 1845 and the South American species retained the name *Mabuya* Fitzinger, 1826. Work done by Honda et al. (2003) and Whiting et al. (2006) also supported the monophyly of tropical Asian, Afro-Malagasy, and South American *Mabuya* thereby confirming Mausfeld’s taxonomic scheme. However, Whiting et al. (2006) noted that more complete sampling of the Africa and Asia *Mabuya* will be necessary to resolve this taxonomic issue.

The genus *Eutropis* or the tropical Asian ‘*Mabuya*’ currently consists of around 30 described species distributed predominantly in the Indomalayan region (formerly Oriental realm; Fig. 1). The Indomalayan region is further divided into Indian, Indochinese and Sundaic subregions (Corbet and Hill, 1992). In their study Mausfeld et al. (2002), Mausfeld and Schmitz (2003) and Honda et al. (2003) sampled predominantly from Southeast (SE) Asia (Indochinese + Sundaic subregion) with only two species from the Indian subregion, both of which are widely distributed across the subcontinent (*E. carinata* from southern India and *E. macularia* from Pakistan). More importantly, none of the eight endemic species from Indian subregion were included. Thus, for a rigorous test of the monophyly of Asian *Mabuya* species, it is clear that the Indian endemics need to be added to the phylogeny.

Another interesting outcome of the work done by Mausfeld and Schmitz (2003) was the phylogenetic position of *Eutropis dissimilis*. This species is distributed over much of northern India as well as SE Asia (Mausfeld and Schmitz, 2003), with the voucher specimen used in their study collected from Myanmar. Rather than being nested within the *Eutropis* clade, *Eutropis dissimilis* was sister to the genus *Dasia* and this subclade was sister to the rest of the tropical Asian *Eutropis* clade in the Maximum Parsimony tree, while its position was not resolved in the maximum likelihood tree. Mausfeld and Schmitz (2003) had tentatively placed this species under *Euprepis* (now called *Trachylepis* as per Bauer, 2003). One of the characters that all the *Trachylepis* share is the presence of a transparent eyelid in contrast to a scaly eyelid in almost all the *Eutropis* from Asia. *Eutropis dissimilis* is known to have a transparent

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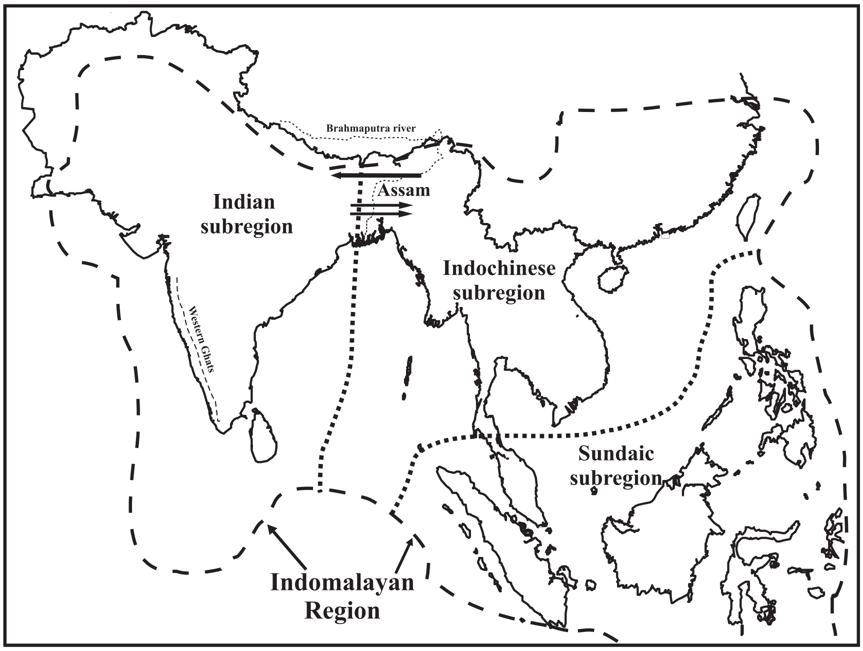

Fig. 1. Map of the Indomalayan biogeographic region. The three subdivisions of the Indomalayan region are depicted as Indian subregion, Indochinese subregion and Sundaic subregion according to Corbet and Hill (1992). Assam in the Northeast India served as a 'gateway' for passage of SE Asian elements (Indochinese + Sundaic subregion) into India. Brahmaputra river which passes through Assam is known to be a barrier for geneflow. The large arrow refers to one initial dispersal into India from SE Asia and the smaller arrows two back dispersals.

eyelid which Mausfeld and Schmitz (2003) consider as a synapomorphy and thus put *dissimilis* in the genus (*Euprepis*) *Trachylepis*. However, Bauer et al. (2008) tentatively allocated *Trachylepis dissimilis* to *Eutropis* with a disclaimer that further phylogenetic studies are needed in order to investigate its actual generic position in addition to its relatedness to the arboreal genus, *Dasia*. It is interesting to note that among the peninsular Indian endemics two species (*Eutropis bibronii* and *Eutropis innotata*) also possess a similar transparent window in the eyelid.

The African and Asian '*Mabuya*' are also interesting with respect to the biogeography of peninsular India (PI). The PI plate was part of the supercontinent Gondwanaland around 200 million years ago (mya). During the Jurassic period, around 160 mya the PI plate along with Madagascar and Seychelles separated from Africa. Subsequently, the PI plate separated from Madagascar around 80 mya and drifted across Indian Ocean before colliding with the Eurasian plate around 55–42 mya (Briggs, 2003). After PI plate merged with Eurasian plate, there was exchange of life forms between the two landmasses (Mani, 1974). Thus Gondwanan forms dispersed out of India (Datta-Roy and Karanth, 2009), while Asian and African forms dispersed into India (Renner, 2004; Köhler and Glaubrecht, 2007). Carranza and Arnold (2003) suggested that *Mabuya* originated in the Asian mainland and dispersed to others parts of the world. Given this scenario the phylogenetic position of Indian '*Mabuya*' is of much interest. For example, do *Eutropis dissimilis*, *E. bibronii* and *E. innotata*, that share a character with the African *Trachylepis*, represent African elements in India? Are the remaining members of the Indian *Eutropis* monophyletic? In other words, has there been only one dispersal of *Eutropis* from SE Asia into India?

To address these issues we sampled endemic as well widespread '*Mabuya*' from across India and generated sequences for two mitochondrial and one nuclear marker. The resulting phylogeny was used to: (1) test the monophyly of the Tropical Asian *Eutropis* taxa after inclusion of Indian congenerics. (2) determine the phylogenetic position of the Indian *Eutropis* species with respect to the rest of the Asian species as well as Afro-Malagasy, Neotropical and Cape Verdean species. (3) determine the phylogenetic position of the endemics among the widespread species. Further, we explored the biogeographical implications of the resulting phylogeny.

2. Materials and methods

2.1. Taxon sampling, DNA extraction, PCR amplification and sequencing

The present study contains 44 taxa which includes representatives from South and SE Asian as well as, Afro-Malagasy, Neotropical and Cape Verdean species. Sequences of the SE Asian, Afro-Malagasy, Neotropical and Cape Verdean representatives for this study have been taken from the Genbank (accession numbers in Table 1). A total of 9 out of the 13 species of *Eutropis* found in the Indian subregion were sampled. The sampling included 8 out of the 12 endemics and 6 widespread species that have conspecifics in SE Asia. The sampled specimens were identified using morphological keys provided by Smith (1935), Inger et al. (1984) and Sharma (2002).

Total genomic DNA was extracted from tail clips stored in 95% alcohol by following standard Phenyl–Chloroform–Isoamyl alcohol protocol as described in Sambrook and Russell (2001). In order to compare the Indian species with the Southeast Asian congenerics and the Afro-Malagasy and Neotropical species, a section of the mitochondrial 12S rRNA and 16S rRNA genes were sequenced using primers as in Mausfeld and Schmitz (2003). These mitochondrial markers have been proven to be informative in resolving the relationships within squamates (Reeder and Wiens, 1996; Pellegrino et al., 2001; Whiting et al., 2003, 2006). Nuclear DNA markers are known to be useful for resolution at the higher level relationships (Saint et al., 1998; Bansal and Karanth, 2010), therefore a fragment of C-*mos* was also sequenced using primers as in Saint et al. (1998). C-*mos* is a proto oncogene involved in the arrest of oocyte maturation and has been used extensively in many studies to resolve relationships among squamates especially because it gives divergence information up to 400 mya (Saint et al., 1998; Harris

Table 1
List of samples used for this study with accession numbers. The catalogue numbers for the samples for which the sequences were generated are shown. 'CES' refers to Centre for Ecological Sciences and 'NHM. OU' stands for Natural history museum, Osmania University where the reference samples are stored.

Taxon	Location	12S	16S	C-*mos*	Voucher number
*Eutropis carinata*1	B.R.T WLS, Karnataka, India	JQ767966	JQ767953	–	CES08/809
*Eutropis carinata*2	Chamrajnagar, Karnataka, India	JQ767967	JQ767950	–	CES08/808
*Eutropis carinata*3	Sitakund, Orissa, India	JQ767968	JQ767954	JQ767941	CES09/849
*Eutropis carinata*4	Kutch, Gujarat, India	JQ767969	JQ767955	–	CES09/879
Eutropis beddomii	Deomali, Orissa, India	JQ767970	JQ767965	JQ767946	CES09/943
Eutropis trivittata	Satara, Maharashtra, India	JQ767971	JQ767951	JQ767948	CES09/976
Eutropis nagarjuni	Nagarjunasagar-Srisailam Tiger Reserve, Andhra Pradesh, India	JQ767972	JQ767952	JQ767949	NHM.OU.REP.3-2009
Eutropis macularia 1 (Bengal)	Bagdogra, W. Bengal, India	JQ767973	JQ767957	–	CES08/805
Eutropis macularia 2 (W. Ghats)	Ponmudi, Kerala, India	JQ767974	JQ767958	–	CES08/813
Eutropis macularia 3 (Pakistan)	Pakistan	AY070335	AY070353	–	–
Eutropis macularia 4 (Myanmar)	Mwe Hauk village, Myanmar	AY159049	AY159078	–	–
Eutropis macularia 5	Thailand	–	–	DQ238976	–
*Eutropis allapallensis*1	Bondla WLS, Goa, India	JQ767975	JQ767959	JQ767942	CES09/834
*Eutropis allapallensis*2	Sharavathi valley WLS, Karnataka, India	JQ767976	JQ767960	–	CES09/844
*Eutropis allapallensis*3	Satkosia WLS, Orissa, India	JQ767977	JQ767961	JQ767943	CES09/851
Eutropis clivicola	Parambikulam WLS, Kerala, India	JQ767978	JQ767956	JQ767945	CESL 089
Eutropis madaraszi	Gammaduwa, SW Sri Lanka	AY159051	AY159080	–	–
Eutropis quadricarinata	Chattin WLS, Myanmar	AY159060	AY159089	–	–
Eutropis bibronii	Rushikulya, Orissa, India	JQ767979	JQ767963	JQ767947	CES09/949
Eutropis cumingi	Luzon, Philippines	DQ239218	DQ238896	DQ238977	–
Eutropis indeprensa	NW Panay, Philippines	AY159047	AY159076	–	–
Eutropis multicarinata	Luzon, Philippines	AY159052	AY159081	–	–
Eutropis rugifera 2 (Nicobar)	Navy Dera, Nicobar, India	JQ767980	JQ767962	–	CES09/897
Eutropis rugifera 1	Indonesia	AY159050	AY159079	–	–
Eutropis tytleri	Mt. Harriet, Andaman Is., India	AY159045	AY159074	–	–
Eutropis rudis	Bogani Nani Wartabone NP, Sulawesi	AB028779	AB028790	DQ238975	–
Eutropis macrophthalma	Java, Indonesia	AY159048	AY159077	–	–
Eutropis multifasciata 1	Mwe Hauk village, Myanmar	AY159059	AY159088	DQ238978	–
Eutropis multifasciata 2 (Assam)	Kaziranga WLS, Assam, India	JQ767981	JQ767964	JQ767944	CES09/925
Eutropis longicaudata	Phong Nha-Ke Bang, Vietnam	AY070341	AY070359	–	–
Trachylepis hoeschi	Namibia	AY218013	AY217963	AY217860	–
Eutropis dissimilis	Shwe Set Taw WLS, Myanmar	AY159046	AY159075	–	–
Dasia grisea	Gombak, Malaysia	AB028773	AB028828	–	–
Dasia vittata	Matang, Borneo	AB028771	AB028782	–	–
Dasia olivacea	Matang, Borneo	AB028772	AB028829	–	–
Trachylepis spilogaster	Erongo region, Namibia	AY218009	AY217959	AY217856	–
Trachylepis striata	Kunene region, Namibia	AY218016	AY217966	AY217863	–
Trachylepis perrotetii	USFWS, Ghana	DQ239227	DQ238905	DQ238982	–
Trachylepis quinquetaeniata	USFWS, Ghana	DQ239264	DQ238942	DQ238955	–
Trachylepis albilabris	Uganda	AB057381	AB057396	–	–
Trachylepis aureopunctata	Toliara, Madagascar	AB057389	AB057404	DQ238959	–
Trachylepis elegans	Toliara, Madagascar	DQ239203	DQ238881	DQ238962	–
Trachylepis maculilabris	Amani, Usambara Mts., Tanzania	AY070338	AY070356	–	–
Trachylepis polytropis	Cameroon	AB057385	AB057400	–	–
Trachylepis wrightii	Fregate Is., Seychelles	AF280124	AY151472	AY818788	–
Mabuya nigropunctata	French Guiana	AB057384	AB057399	–	–
Mabuya unimarginata	Honduras	AB057378	AB057393	–	–
Mabuya agilis	Prado, Bahia, Brazil	AY070326	AY070347		–
Mabuya agilis	Brazil: ES, UHE Rosal	–	–	DQ238995	–
Mabuya heathi	Res. Serra das Almas, Ceará, Brazil	AY070330	AY070349	–	–
Mabuya heathi	Brazil: PI, Serra das Confusões	–	–	DQ239009	–
Mabuya mabouya	Tobago	AY070339	AY070357	–	–
Mabuya dorsivittata	Cunha, São Paulo, Brazil	AY070346	AY070363	–	–
Mabuya dorsivittata	Brazil: SP, São Paulo	–	–	DQ239015	–
Mabuya macrorhyncha	Trancoso, Bahia, Brazil	AY070333	AY070351	–	–
Mabuya macrorhyncha	Brazil: SP, Itanhaém, Ilha da Queimada Grande Brazil: SP, Itanhaém, Ilha da Queimada Grande	–	–	DQ239001	–
Chioninia spinalis	Sal Island, Cape Verde	AY070327	AY070348	–	–
Chioninia spinalis	Maio, Cape Verde	–	–	AF335083	–
Chioninia stangeri	Branco, Cape Verde	AF280170	AY151479	–	–
Chioninia stangeri	Ilhéu Raso, Cape Verde	–	–	AF335086	–
Chioninia delalandii	São Thiago Island, Cape Verde	AY070344	AY070361	–	–
Chioninia delalandii	Fogo, Cape Verde	–	–	AF335081	–
Emoia caeruleocauda	Palau Islands	AB028801	AB028813	AY217859	–
Emoia cyanura	Fiji: Sigatoka	AY218018	AY217968	AY217865	–

et al., 1999; Whiting et al., 2003). The PCR conditions for the mitochondrial DNA primers was the same as the publication from which they have been taken from. For the nuclear primer C-*mos* although, the PCR conditions were changed (54 min–94°, 45 s–57°, 60 s–59°, 45 s–94°, 45 s–55°, 60 s–72°, 35 cycles, 6 min 72°) The PCR product was purified using a QIAquick PCR purification kit (Qiagen) and the sequences were obtained commercially from Eurofins Biotech Pvt. Ltd. (Bangalore, India). The sequences obtained were submitted in Genbank (See Table 1 for accession numbers and specimen voucher details).

2.2. Phylogenetic analyses

The sequences obtained for each gene were aligned with the published sequences using MUSCLE (Edgar, 2004) which is integrated in the software MEGA 5.05 (Tamura et al., 2011) using default parameters. Individual genes were concatenated to generate a combined nuclear and mitochondrial dataset. This combined dataset was subjected to various phylogenetics analyses. Maximum parsimony (MP) tree was built through a heuristic search with random sequence addition and TBR branch swapping options in PAUP* version 4.0b 10 (Swofford, 2002). The substitution models for individual mitochondrial and nuclear gene datasets and the combined dataset were chosen using the Akaike information criterion in MODELTEST version 3.7 (Posada and Crandall, 1998) in conjunction with PAUP*. The chosen model along with the estimated parameters was then used to build a maximum likelihood (ML) tree through the heuristic search options as in MP analysis. The MP and ML bootstrap support was determined for 1000 replicates in PAUP*. The Bayesian analyses was carried out in MrBayes version 3.1 (Ronquist and Huelsenbeck, 2003). The combined mitochondrial and nuclear DNA dataset was partitioned according to genes (12S, 16S and C-*mos*). The program was run for 10^8 million generations and the sampling was made for every 100 generations. After the Bayesian run, the log file containing 80,000 trees were loaded onto Tracer version 1.5 and the stage at which a stable likelihood value was obtained, was determined. The first 20,000 trees (25%) were discarded as burn-in. Gaps were treated as missing data. The trees were rooted using two species of *Emoia* (*E. cyanura* and *E. caeruleocauda*) of the *Eugongylus* group, which are known to be sister to '*Mabuya*' (Honda et al., 2000).

2.3. Biogeographic analysis

In order to better understand the evolutionary origin and biogeography of the Indian *Eutropis*, we subjected the combined dataset tree to dispersal vicariance analysis using the software S-DIVA (Statistical Dispersal-Vicariance analysis) (Yan Yu et al., 2010). The program S-DIVA is an improvement over DIVA 1.2 (Ronquist, 2001) as it statistically evaluates the alternative ancestral ranges at each node in a tree accounting for phylogenetic uncertainty and uncertainty in DIVA optimization (Yan Yu et al., 2010) using a method described by Nylander et al. (2008), and Harris and Xiang (2009). To this end, the Bayesian output file of the combined mitochondrial and nuclear DNA dataset consisting of 80,000 trees was loaded onto S-DIVA with the first 20,000 trees discarded as burn-in. From the remaining trees, every 100th tree was sampled to compute the condensed tree and the terminal nodes were assigned to various biogeographical regions/subregions. In the case of *Eutropis* that are distributed in tropical Asia, the assigned areas were: Indian subregion and Southeast Asia (which included Indochinese and Sundaic subregion) as per Corbet and Hill (1992) (Fig. 1). The non-*Eutropis* species were assigned to Afro-Malagasy, Neotropical and Cape Verdean regions. The max areas was set at default value of 4 and no constrain was imposed with respect to various combinations of ancestral areas. The program was then run using the default parameters to derive ancestral area reconstructions for various internal nodes as well as to determine number of dispersal events.

3. Results

The mitochondrial dataset included two genes, 12S rRNA (339 bp) and 16S rRNA (446 bp) in total consisted of 785 bp and the nuclear dataset included C-*mos* (340 bp). The mitochondrial dataset was more exhaustive with respect to taxon sampling as most studies in the past have used these genes in their phylogenetic analyses. This was not the case with the nuclear dataset as C-*mos* sequences were not available for most of the Southeast Asian and American species (see Table 1 for details). The Bayesian tree based on the combined mitochondrial and nuclear dataset is shown in Fig. 2. Similar tree topology was generated by both MP and ML approaches. In these trees a distinct tropical Asian clade was retrieved with high support. Separate analyses of nuclear and mitochondrial datasets also retrieved a tropical Asian clade. Nested within the tropical Asian clade was a group that consisted of species distributed predominantly in India. Thus the phylogeny retrieved a unique Indian radiation, with the SE Asian species being sister to this group.

The Indian radiation consisted of two distinct subclades. In the first subclade the endemics, *E. beddomii, E. trivittata and E. nagarjuni* branched together and this group was sister to the widespread species *E. carinata*. In the second subclade the widespread *E. macularia* from the Indian subregion was not monophyletic in the Bayesian and ML (not shown) tree, but was monophyletic in the MP tree (not shown). In all the three tree building methods, *E. allapallensis* was retrieved as monophyletic and sister to *E. clivicola*. *E. macularia* from Myanmar was sister to *E. macularia*(from Indian subregion) + *allapallensis* + *clivicola* group followed by *E. madaraszi* in the Bayesian and ML tree. In the MP tree although, *E. madaraszi* was sister to a clade consisting of *E. allapallensis* + *E. clivicola*. *E. bibronii*, which is endemic from Indian subregion and *E. quadricarinata* which is found in Northeast India and Myanmar (Indochinese subregion) were sisters to each other and together they were sister to *E. macularia* + *E. allapallensis* + *E. clivicola* + *E. madaraszi* group.

The Asian *Eutropis* clade was sister to a clade consisting of Afro-Malagasy, Cape Verdean, Neotropical species as well as *Dasia* and *E. dissimilis*. The Afro-Malagasy group also included *Trachylepis wrightii* from Seychelles.

Result from the dispersal-vicariance analysis on the Bayesian output of the combined dataset is shown in Fig. 3. This tree shows only the tropical Asian clade and for the complete tree see Supplementary Fig. 1. In this tree the ancestral area of the root node of the *Eutropis* clade was in SE Asia and there have been three dispersal events within this clade (Fig. 3). The earliest dispersal event was from SE Asia into India, this lineage constituted the Indian radiation. Following the radiation of this lineage in peninsular India were two independent back dispersals from India into Southeast Asia. It has been pointed out that ancestral area optimizations in DIVA become less reliable as we approach the root node (Ronquist, 1997) and is sensitive towards exclusion of outgroup taxa (Kodandaramaiah, 2009). Adding additional outgroup taxa to the analysis has been suggested to address this issue (Ronquist, 1997). As the focus of this paper was the *Eutropis* clade of Asia, we were interested in the reliability of the ancestral area reconstruction for the root node of this clade. In this regard, it must be pointed out that our S-DIVA analysis consisted of a large outgroup spanning species from Afro-Malagasy to the Neotropical regions.

4. Discussion

The molecular data presented here demonstrates the monophyly of the tropical Asian '*Mabuya*' and thus supports the generic

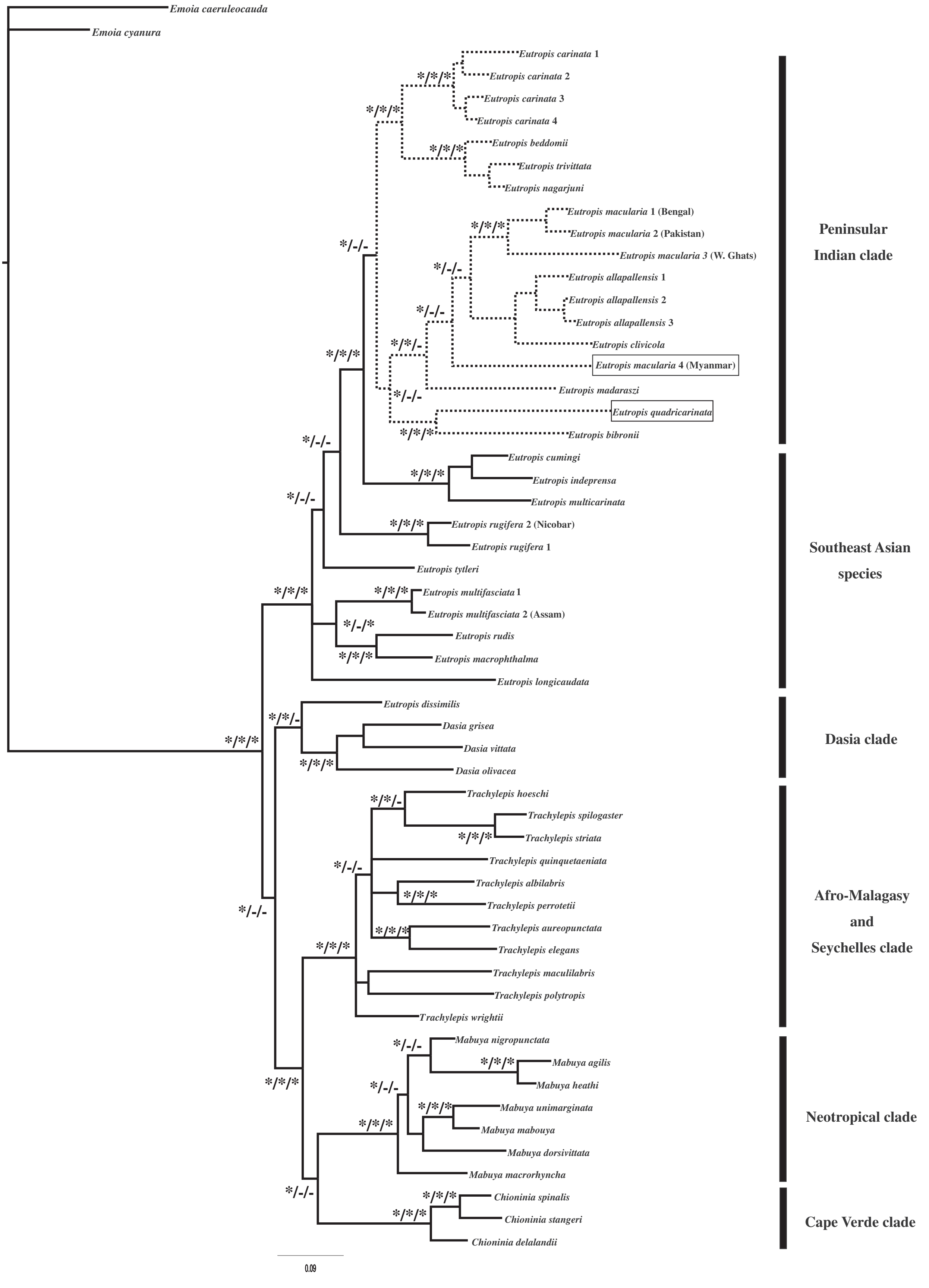

Fig. 2. Bayesian inference (BI) tree based on 12S + 16S + C-*mos* combined partitioned dataset. Stars represent bootstrap (bs) values of > 50% and posterior probability (pp) > 0.5 (pp/ML-bs/MP-bs/). Node support values < 50%/0.5 are denoted by '-'. Dotted clade indicates the Indian radiation.

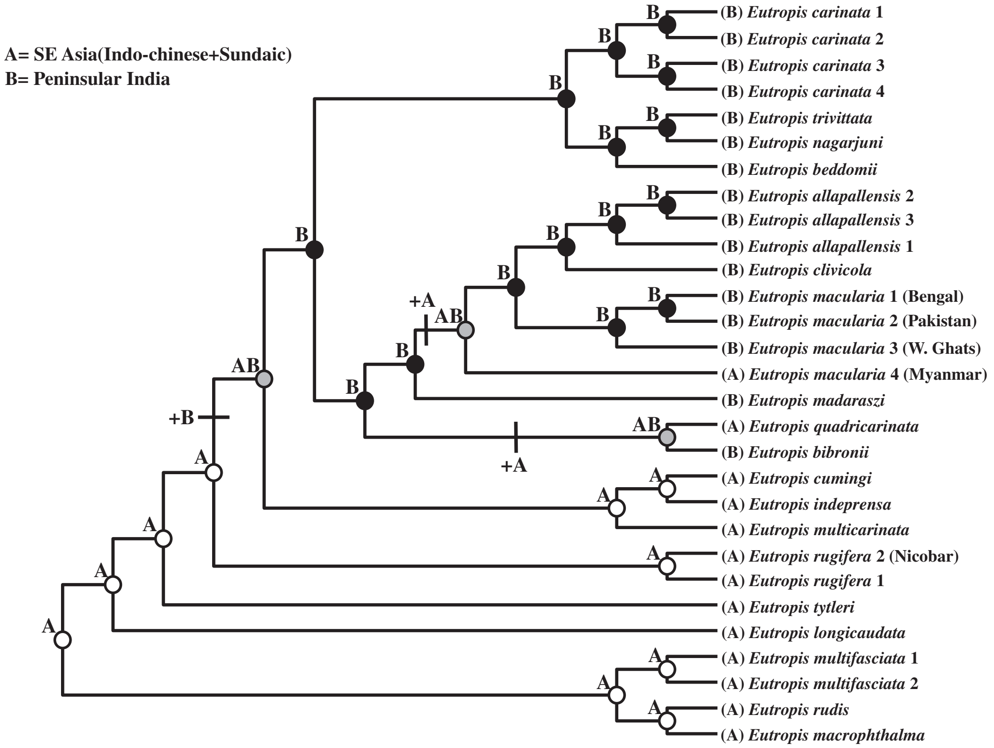

Fig. 3. Results of dispersal – vicariance analysis (S-DIVA) based on the Bayesian output of tree files obtained from 12S + 16S + C-*mos* dataset. The ancestral area reconstructions at each of the nodes were 100%. Only tropical Asian part of the tree shown here (see *Supplementary* Fig. 1 for complete tree). White, black and gray circles represent ancestral areas A (SE Asia), B (India) and AB (SE Asia + India).

assignment of Asian members of this large genus to *Eutropis*. The Indian *Eutropis* were nested within the larger Asian *Eutropis* clade. These results suggested that none of the Indian *Eutropis* species sampled here had an African origin as they are not related to the Afro-Malagasy genus *Trachylepis*. Thus, the character that the Indian endemic *E. bibronii* shares with *Trachylepis* (transparent lower eyelid) might have evolved independently (convergence) in the Indian species.

4.1. Phylogenetic position of the Indian radiation with respect to SE Asian counterparts

The Indian members of *Eutropis* formed a distinct clade that was nested within the larger Asian *Eutropis* clade. Furthermore, Southeast Asia *Eutropis* were sister to this Indian clade. These results suggested that the Indian *Eutropis* constituted an endemic Indian radiation and that this lineage was derived from Southeast Asia. The S-DIVA analysis supported this scenario, wherein the earliest dispersal within the *Eutropis* clade was from Southeast Asia into India (Fig. 3). Following this dispersal event this lineage radiated to give rise to the extant Indian species. The subclade immediately sister to the Indian radiation consisted of *E. cumingi*, *E. indeprensa* and *E. multicarinata* from Southeast Asia. The average percentage sequence divergence between this subclade and the members of the Indian radiation was around 11%, which suggested that this ‘into India’ dispersal might have occurred around 5.5–17 mya in the Miocene. This age was based on the molecular rate of 0.65–2% per million year for lizard mitochondrial DNA (see Macey et al. 1998; Carranza and Arnold, 2003; Honda et al., 2003). This dispersal event occurred much after peninsular India was completely sutured with the Asian mainland (around 42 mya; Briggs, 2003). Thus the ‘into India’ dispersal is most likely to have been over land (geodispersal) as opposed to trans-marine dispersal which has been reported in Afro-Malagasy and Neotropical *Mabuya* (Carranza and Arnold, 2003).

In this regard, Mani (1974) had suggested that the Assam region of Northeast India might have served as a ‘gateway’ through which the Southeast Asian elements reached peninsular India. North of Assam, the Himalayan chain of mountains acted as a barrier and south was covered by sea, which deemed Assam as a possible route facilitating the exchange of biota between the two landmasses (Fig. 1). Such Southeast Asian origin hypothesis has been invoked for numerous Indian taxa that have related forms in Southeast Asia (Hora, 1949; Mani, 1974; Bande and Prakash, 1986; Bande, 1992; Clyde et al., 2003; Köhler and Glaubrecht, 2007). However, none of these studies, except Köhler and Glaubrecht (2007), have used molecular phylogenetic tools to test this hypothesis. Köhler and Glaubrecht (2007) based on molecular phylogeny of fresh water molluscs (Pachychylids) of tropical Asia established the SE Asian origin of Indian members of this group. Our work is a further advancement towards understanding this pattern as we have used an analytical method (S-DIVA analysis) for a more robust test of this hypothesis and our taxon sampling from India is more complete. Interestingly our phylogenetic and DIVA analyses also suggested that there were two back dispersals into SE Asia (Figs. 1 and 3). The first dispersal was along the lineage leading to the common ancestor of *E. quadricarinata* and *E. bibronii* and the second dispersal was along the *E. macularia* lineage. Thus, the Indian subregion not only served as a sink for dispersals from SE Asia but was also source for some of its species.

4.2. Phylogenetic position of the Indian endemics within the Indian radiation

Within the Indian radiation, the first subclade consisted of the widespread *E. carinata* which was sister to the three endemics; *E. beddomii*, *E. trivittata* and *E. nagarjuni*. All the members of this subclade have relatively large sized bodies with a pattern of dorsal stripes. *E. carinata* is widespread and is found in a range of habitats from wet tropics to drier habitats. Additionally, *E. carinata* has not been reported from south of the Brahmaputra river in northeast India (Abhijit Das pers. comm.) or in SE Asia. This river has long been recognized as a barrier for dispersal in many species (Meijaard and Groves, 2006; Sengupta et al., 2009) and might represent the borderline between Indian and Indochinese subregions (Fig. 1). We therefore consider *E. carinata* an Indian endemic as it is confined largely to Indian subregion. *E. carinata* was sympatric with the three sister endemics in almost all the localities where they have been reported. Among the three endemics, *E. beddomii* and *E. trivittata* are also found in Sri Lanka, and although *E. nagarjuni* has not been reported from Sri Lanka, it is morphologically quite close to *E. beddomii* (Srinivasulu et al., 2005).

The second subclade consists of widely distributed *E. macularia*, along with endemics from India and Sri Lanka. *E. macularia* is distributed in most parts of the Indian subcontinent as well as in SE Asia. Interestingly in an earlier study *E. macularia* was paraphyletic with respect to *E. tammanna* (Ota et al., 2001; Mausfeld and Schmitz, 2003; Das et al., 2008). In our analysis as well, *E. macularia* was not monophyletic, instead *E. macularia* from Myanmar was sister to a clade that consisted of *E. macularia* from Indian subregion, *E. allapallensis* and *E. clivicola* (in the Bayesian and ML tree but not MP). Mausfeld and Schmitz (2003) also noted very high sequence divergence between *E. macularia* from Myanmar and Pakistan (8.2%). Thus our results support Mausfeld and Schmitz (2003) proposal that the *E. macularia* from Myanmar represents a new species. The *E. macularia* from Pakistan is genetically very close to the specimen collected from Bengal. However, both these specimens are genetically divergent from the *E. macularia* from Western Ghats, by 6.7–6.8% (based on only the mitochondrial dataset). Thus the South Indian lineage of *E. macularia* may possibly represent a different subspecies/species. This population is also morphologically distinct from the rest of the Indian population.

E. macularia from the Indian subregion was sister *E. allapallensis + clivicola*, which are endemics from PI. Interestingly, *E. allapallensis* has been considered as a morphological variant of *E. macularia* (Smith, 1935). We confirm the specific status of *E. allapallensis* with respect to *E. macularia* as the genetic distance between them is quite high (around 9%). *E. clivicola* is restricted to the Southern Western Ghats and has been reported in habitats which are moist deciduous to wet evergreen, whereas *E. allapallensis* is confined to dryer parts of PI. The Sri Lankan endemic *E. madaraszi* was placed in synonymy with *E. macularia* by Smith (1935), but its species status was resurrected by Taylor (1950). In the mitochondrial tree generated by Mausfeld and Schmitz (2003) *E. madaraszi* was sister to *E. macularia* and *E. quadricarinata*. In our analysis *E. madaraszi* was sister to a clade consisting of *E. macularia + E. allapallensis + E. clivicola* in the Bayesian and ML trees. Thus in both studies *E. madaraszi* was genetically distinct from *E. macularia* supporting its validity.

The published sequence of *E. dissimilis* and *Dasia* were used here to assess whether their phylogenetic position could be resolved by adding more *Eutropis* representatives from India. In the work done by Mausfeld and Schmitz (2003), *Dasia-E. dissimilis* group either branched with SE Asian (*Eutropis*) clade (MP tree) or Afro-Malagasy and Middle East (*Trachylepis*) clade (ML tree). However, in our analyses, this group was sister to a large clade consisting of the Afro-Malagasy + Cape Verde + Neotropical species. Further sampling of both *Dasia* and *E. dissimilis* from the Indian subregion may help resolve their phylogenetics position.

While we have included most of the endemics from the Indian subcontinent, adding the remaining unsampled species from the Indian region might reveal additional interesting patterns. *Eutropis innotata*, for example, which is a central Indian endemic restricted to dry zone might be of particular interest. *E. innotata*, also has a clear eyelid similar to *E. bibronii*, *E. dissimilis* and *Trachylepis* from Africa and Madagascar and its phylogenetic position needs to be resolved. In the case of another endemic, *E. tammanna*, it has already been shown that it is morphologically and genetically closely related to *E. macularia* (Das et al., 2008), but this work was based on a different gene and therefore sequences of this species could not be included here. Although it is evident that *E. tammanna* would be part of the '*macularia* group', its phylogentic position within this group needs to be assessed.

5. Summary

Our result supports the monophyly of the Tropical Asian *Eutropis* and strengthens the generic re-arrangement proposed by Mausfeld et al. (2002), at least with respect to the Asian *Eutropis* species. The phylogeny of *Eutropis* from tropical Asia revealed an endemic Indian radiation that was derived from SE Asia. Our dating estimates suggested that this dispersal from SE Asia into India occurred in the Miocene around 5.5–17 million years ago, much after Indian plate had merged with Asian plate. Dispersal vicariance analysis also suggested two independent back dispersals leading to the lineages of *E. macularia* from Myanmar and *E. quadricarinata*. Within the Indian radiation, two large subclades were retrieved, one consisting of *E. carinata* with three endemic species from Indian subregion namely *E. beddomii*, *E. trivittata* and *E. nagarjuni* and the other subclade consisting of *E. macularia* with three more endemics namely *E. allapallensis*, *E. clivicola* and *E. madaraszi*. Indian endemic *E. bibronii* and SE Asia species *E. quadricarinata* were sister to this subclade.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2012.02.022.

References

Bande, M.B., 1992. The Palaeogene vegetation of peninsular India (megafossil evidence). Palaeobotanist 40, 275–284.

Bande, M.B., Prakash, U., 1986. The Tertiary flora of Southeast Asia with remarks on its palaeoenvironment and phytogeography of the Indomalayan region. Rev. Paleobot. Palyn. 49, 203–233.

Bansal, R., Karanth, K.P., 2010. Molecular phylogeny of *Hemidactylus* geckos (Squamata: Gekkonidae) of the Indian subcontinent reveals a unique Indian radiation and an Indian origin of Asian house geckos. Mol. Phylogenet. Evol. 57, 459–465.

Bauer, A., 2003. On the identity of *Lacerta punctata* Linnaeus, 1758, the type species of the genus *Euprepis* Wagler, 1830, and the generic assignment of Afro-Malagasy skinks. Afr. J. Herpetol. 52, 1–7.

Bauer, A., Firoz, A., Das, A.M., 2008. Notes on the distributions, taxonomy and biology of the skink*Eutropis dissimilis* (Reptilia: Squamata: Scincidae), with first records from Assam, India. Hamadryad 32, 76–80.

Briggs, J.C., 2003. The biogeographic and tectonic history of India. J. Biogeogr. 30, 381–388.

Carranza, S., Arnold, E.N., 2003. Investigating the origin of transoceanic distributions: mtDNA shows *Mabuya* lizards (Reptilia, Scincidae) crossed the Atlantic twice. Syst. Biodivers. 1, 275–282.

Clyde, W.C., Khan, I.H., Gingerich, P.D., 2003. Stratigraphic response and mammalian dispersal during initial India-Asia collision: Evidence from the Ghazij Formation, Balochistan. Pakistan. Geology 31 (12), 1097–1100.

Corbet, G.B., Hill, J.E., 1992. The Mammals of the Indomalayan Region: A Systematic Review. Oxford University Press, New York, pp. 1–12.

Das, I., Silva, A.D., Austin, C.C., 2008. A new species of *Eutropis* (Squamata: Scincidae) from Sri Lanka. Zootaxa 1700, 35–52.

Datta-Roy, A., Karanth, K.P., 2009. The out-of-india hypothesis: what do molecules suggest? J. Biosci. 34 (5), 687–697.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids res. 32 (5), 1792–1797.

Harris, A.J., Xiang, Q., 2009. Estimating ancestral distributions of lineages with uncertain sister groups: a statistical approach to Dispersal–Vicariance Analysis and a case using *Aesculus* L. (Sapindaceae) including fossils. J. Syst. Evol. 47, 349–368.

Harris, D.J., Sinclair, E.O., Mercader, N.L., Marshall, J.C., Crandall, K.A., 1999. Squamate relationships based on C-*mos* nuclear DNA sequences. Herpetol. J. 9, 147–151.

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

Honda, M., Ota, H., Kohler, G., Eneich, I., Chirio, L., Chen, S., Hikida, T., 2003. Phylogeny of lizard subfamily Lygosominae (Reptilia: Scincidae), with special reference to the origin of new world taxa. Genes Genet. Syst. 78, 71–80.

Hora, S.L., 1949. Satpura hypothesis of the distribution of Malayan fauna and flora of peninsular India. Proc. Natl. Inst. Sci. India 15, 309–314.

Inger, R.F., Shaffer, H.B., Koshy, M., Bakde, R., 1984. A report on a collection of amphibians and reptiles from the Ponmudi, Kerala, South India. J. Bombay Natl. His. Soc. 81, 551–570.

Kodandaramaiah, U., 2009. Use of Dispersal– Vicariance analysis in biogeography – a critique. J. Biogeogr. 37, 3–11.

Köhler, F., Glaubrecht, M., 2007. Out of Asia and into India: on the molecular phylogeny and biogeography of the endemic freshwater gastropod *Paracrostoma* Cossmann, 1900 (Caenogastropoda: Pachychilidae). Biol. J. Linn. Soc. 91, 621–657.

Macey, J.R., Schulte II, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M., Papenfuss, T.J., 1998. Phylogenetic relationships among agamid lizards of the *Laudakia caucasia* species group: Testing hypotheses of biogeographic fragmentation and an area cladogram for the Iranian Plateau. Mol. Phylogenet. Evol. 10, 118–131.

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., 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., Vences, M., Schmitz, A., Veith, M., 2000. First data on the molecular phylogeography of scincid lizards of the genus *Mabuya*. Mol. Phylogenet. Evol. 17, 11–14.

Mausfeld, P., Schmitz, A., Böhme, W., Misof, B., Vrcibradic, D., Rocha, C.F.D., 2002. Phylogenetic Affinities of *Mabuya* Atlantica Schmidt, 1945, Endemic to the Atlantic Ocean Archipelago of Fernando de Noronha (Brazil): Necessity of Partitioning the Genus *Mabuya* Fitzinger, 1826 (Scincidae: Lygosominae). Zoolog. Anz. 241, 281–293.

Meijaard, E., 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).

Nylander, J.A.A., Olsson, U., Alstrom, P., In, I.S., 2008. Accounting for phylogenetic uncertainty in biogeography: a bayesian approach to dispersal – vicariance analysis of the thrushes (Aves: *Turdus*). Syst. Biol. 57, 257–268.

Ota, H., Hikida, T., Nabhitabhata, J., Panha, S., 2001. Cryptic Taxonomic diversity in two broadly distributed lizards of Thailand (*Mabuya macularia* and *Dixonius siamensis*) as revealed by chromosomal investigations (Reptilia: Lacertilia). Nat. Hist. J. Chulalongkorn Univ. 1, 1–7.

Pellegrino, K.C.M., Rodrigues, M.T., Yonenaga-Yassuda, Y., Sites, J.W.J., 2001. A molecular perspective on the evolution of microteiid lizards (Squamata: Gymnophthalmidae), and a new classification for the family. Biol. J. Linn. Soc. 74, 315–338.

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

Reeder, T.W., Wiens, J.J., 1996. Evolution of the lizard family Phrynosomatidae as inferred from diverse types of data. Herpetol. Monogr. 10, 43–84.

Renner, S.S., 2004. Multiple Miocene Melastomataceae dispersal between Madagascar, Africa and India. Philos. Trans. Biol. Sci. 359 (1450), 1485–1494.

Ronquist, F., 1997. Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. Syst. Biol. 46, 195–203.

Ronquist, F., 2001. DIVA version 1.2. Computer program for Mac OS and Win 32. Evolutionary Biology Centre, Uppsala University. <http://www.ebc.uu.se/systzoo/research/diva/diva.html>.

Ronquist, F., Huelsenbeck, J.P., 2003. MRBAYES 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.

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

Sambrook, J., Russell, D., 2001. Preparation of genomic DNA from mouse tails and other small samples. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 6.23–6.27.

Sengupta, S., Das, A., Das, S., Hussain, B., Choudhury, N.K., 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.

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

Srinivasulu, C., Srinivasulu, B., Rao, C.A.N., 2005. Present Status of *Eutropis nagarjuni* (Sharma, 1969) (Reptilia: Scincidae) – an Endemic Skink from Andhra Pradesh, India. Zoo's print J. 20, 1865–1866.

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

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739.

Taylor, E.H., 1950. Ceylonese lizards of the family Scincidae. Univ. Kansas Sci. Bull. 33, 481–518.

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

Whiting Jr., A.S., Sites, J.W., Pellegrino, K.C., Rodrigues, M.T., 2006. Comparing alignment methods for inferring the history of the new world lizard genus *Mabuya* (Squamata: Scincidae). Mol. Phylogenet. Evol. 38, 719–730.

Yu, Y., Harris, A.J., He, X.J., 2010. S-DIVA (Statistical Dispersal-Vicariance Analysis): a tool for inferring biogeographic histories. Mol. Phylogenet. Evol. 56, 848–850.