RESEARCH NOTE

Phylogeny of endemic skinks of the genus *Lygosoma* (Squamata: Scincidae) from India suggests an *in situ* radiation

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Introduction

The Indian subcontinent is an interesting biogeographical entity as it is isolated from the rest of the Asian landmass by very high mountain ranges of the Himalayas in the north and is surrounded by ocean in the south. Furthermore, much of this land mass was part of a Gondwanan fragment that merged with Asia around 42–55 mya (Briggs 2003). Thus, the Indian subcontinent has witnessed prolonged period of isolation (Datta-Roy and Karanth 2009) and appears to be largely cut-off from much of Asia. In this regard, the subcontinent could be considered as an island separated from the mainland (Asia). One interesting feature of an isolated island is the presence of endemic radiations, i.e., unique clades of taxa whose members are endemic to the island. As a consequence, the island's biota tends to be unique and distinct from that of the mainland. Similarly, the Indian subcontinent harbours many endemic species and its biota is quite distinct from rest of Asia. This distinction is reflected in traditional zoogeographical classification wherein much of the Indian subcontinent is placed in a separate subregion (the Indian subregion) within the Indomalayan biogeographical region (formerly the Oriental realm) (Datta-Roy *et al.* 2012 and references therein). Given this scenario, it would be interesting to ascertain if these endemic taxa constitute endemic Indian radiations that were generated through *in situ* diversification or if they had multiple independent origins. Although there are genera where conspecifics are distributed both in the Indian subregion as well as Southeast Asia (e.g. in birds, butterflies, etc.), their evolutionary origins remain unknown owing to a lack of biogeographic studies in a phylogenetic framework. However, recent molecular phylogenetic studies suggest that India does harbour endemic radiations in a range of vertebrate groups including langurs (Karanth *et al.* 2008), lizards (Datta-Roy *et al.* 2012), frogs (Bossuyt and Milinkovitch 2001), toads (Bocxlaer *et al.* 2009) as well as invertebrates (Köhler and Glaubrecht 2007). In other groups, such as macaques (Tosi *et al.* 2000) and plants (Yuan *et al.* 2004), there is evidence of multiple origins.

In this regard, the skinks of the genus *Lygosoma* Hardwicke and Gray, 1827 (subfamily Lygosominae, also see Hedges and Conn (2012) (family Lygosomidae)) from India are of much interest, particularly because of the high endemicity seen: seven out of the nine species distributed in the Indian subregion are endemic. The genus *Lygosoma* (as defined by Greer 1977) is distributed predominantly in tropical Asia (Indomalayan region) which harbours 24 of the 39 species of this genus. The remaining species are distributed in Africa. *Lygosoma* along with the African genera *Mochlus* Gunther, 1864 and *Lepidothyris* Cope, 1892 and the Asian genus *Lamprolepis* Fitzinger, 1843 are placed in a larger *Lygosoma* group (Greer 1977; Honda *et al.* 2003). Recently, some molecular phylogenetic as well as morphological studies have been undertaken on skinks which included members of the *Lygosoma* group (Honda *et al.* 2003; Ziegler *et al.* 2007; Wagner *et al.* 2009; Skinner *et al.* 2011). These studies suggest that the genus *Lygosoma* may not be monophyletic, and related genera such as *Mochlus* and *Lepidothyris* appear to be nested within a larger *Lygosoma* clade (Wagner *et al.* 2009). However these studies were based on limited sampling of *Lygosoma* spp. from Asia.

The goal of the current study was to determine the phylogenetic position of the endemic *Lygosoma* spp. of the Indian subregion within the *Lygosoma* group. In particular, we were interested to determine if India harbours an endemic

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radiation of *Lygosoma* spp. To this end we sequenced two mitochondrial markers (12S rRNA and 16S rRNA) from seven out of the nine *Lygosoma* spp. (including five endemics). These sequences were combined with published sequences to derive a robust phylogeny of the *Lygosoma* group.

Materials and methods

The study included 18 members of the '*Lygosoma* group' (see Honda *et al.* 2003; Skinner *et al.* 2011), with representative members of the genera *Lamprolepis*, *Lepidothyris*, *Lygosoma* and *Mochlus*. The Indian subregion harbours around nine species of *Lygosoma* out of which seven were sampled including five endemics. *L. ashwamedhi*, an endemic from the Eastern Ghats was the only endemic from India that could not be included in the phylogeny as numerous attempts to collect this elusive species in the field remained unsuccessful. Another endemic, *L. singha*, from Sri Lanka was also not collected due to logistic reasons. The sampled specimens were identified using morphological keys provided in Smith (1935), and Sharma (2002). Tissue collected from the specimens was preserved in 95% ethanol and was later stored at −20°C. The sequences for the non-Indian species were obtained from GenBank (accession numbers are provided in table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/).

Total genomic DNA was extracted from tissues using a phenol–chloroform–isoamyl alcohol protocol (Sambrook and Russell 2001). Two mitochondrial genes, 12S rRNA (12S) and 16S rRNA (16S), were PCR amplified from these samples using the primers and protocol published in Mausfeld and Schmitz (2003). These markers have been used extensively in molecular phylogenetic studies in lizards (Mausfeld *et al.* 2003; Datta-Roy *et al.* 2012) and are known to be variable and informative in resolving the relationships within squamates. A QIAquick PCR purification kit (Qiagen, Germany) was used to purify the PCR products and the sequences were obtained commercially from Eurofins Biotech (Bangalore, India).

The sequences obtained from Indian species were aligned with the downloaded sequences (table 1 in electronic supplementary material) using MUSCLE (Edgar 2004) which is incorporated in the software Mega 5.05 (Tamura *et al.* 2011) using default parameters. First, two separate datasets corresponding to two markers (12S and 16S) were assembled. These datasets were subjected to parsimony analyses as described below. The resulting trees were very similar, therefore all subsequent analyses were undertaken on the combined dataset. The individual genes were then combined to generate the mitochondrial dataset which was subjected to various phylogenetic analyses. The maximum parsimony (MP) tree was generated through a heuristic search with 10 random sequence addition and TBR branch swapping options in PAUP* ver. 4.0b 10 (Swofford 2002). The program ModelTest ver. 3.7 (Posada and Crandall 1998) in conjunction with PAUP* was used to choose the DNA substitution model that best fits the combined dataset as well as the individual datasets. The DNA substitution models chosen using the Akaike information criterion (AIC) in ModelTest ver. 3.7 were GTR+G+I for the combined dataset (12S+16S rRNA) and GTR+G and GTR+G+I for 12S and 16S datasets, respectively. The chosen model for the combined dataset along the respective likelihood parameters obtained from ModelTest ver. 3.7 was used to generate a maximum likelihood (ML) tree using the same search options as in the MP analysis in PAUP*. Consequently, we also generated a ML tree with partitioned dataset where each partition was assigned its own respective DNA substitution model in RAxML ver. 7.0.3 (Stamatakis 2006). The ML trees generated by both PAUP* and RAxML with the combined and partitioned datasets, respectively, were identical. The bootstrap supports for the MP tree were determined for 1000 pseudoreplicates using a simple heuristic search option in PAUP*. For the ML tree, the bootstrap support was determined for 1000 pseudoreplicates with the partitioned dataset using rapid bootstrapping method in RAxML-HPC ver. 7.0.3. The Bayesian analysis was carried out in the program MrBayes ver. 3.1 (Ronquist and Huelsenbeck 2003) using default priors. For this analysis, the dataset was partitioned by gene. The DNA substitution model for each partition was specified based on the ModelTest ver. 3.7 results and the partitions were unlinked. The program was run for eight million generations with two separate runs, and the tree sampling was made after every 100 generations. Run length was based on the standard deviation of split frequencies, when the value of this diagnostics fell below 0.01 the run was stopped. At the end of the Bayesian run, the log-likelihood scores of the saved trees were loaded onto the software Tracer ver. 1.5. The likelihood scores were plotted against generation time to determine stationarity in the log-likelihood parameters. The first 25% of the total number of saved trees were discarded as burn-in. The saved trees were then used to generate a majority rule consensus tree using the *sumt* command. The trees were rooted using *Corucia zebrata* and *Eutropis carinata* which are known to be sisters to the *Lygosoma* group (based on Honda *et al.* 2003) and *Eugongylus* group (*Oligosoma lichenigera* and *Eugongylus rufescens*) as per Skinner *et al.* (2011).

Results

The mitochondrial tree based on the ML method is shown in figure 1. Most of the relationships in this tree were similar to those obtained in MP and Bayesian trees (see figures in electronic supplementary material). However, relationships at the deeper nodes in the MP and Bayesian trees were unresolved. In these trees *Lepidothyris* and *Mochlus* were nested within a larger *Lygosoma* radiation, rendering *Lygosoma* paraphyletic. Further, all the tree building

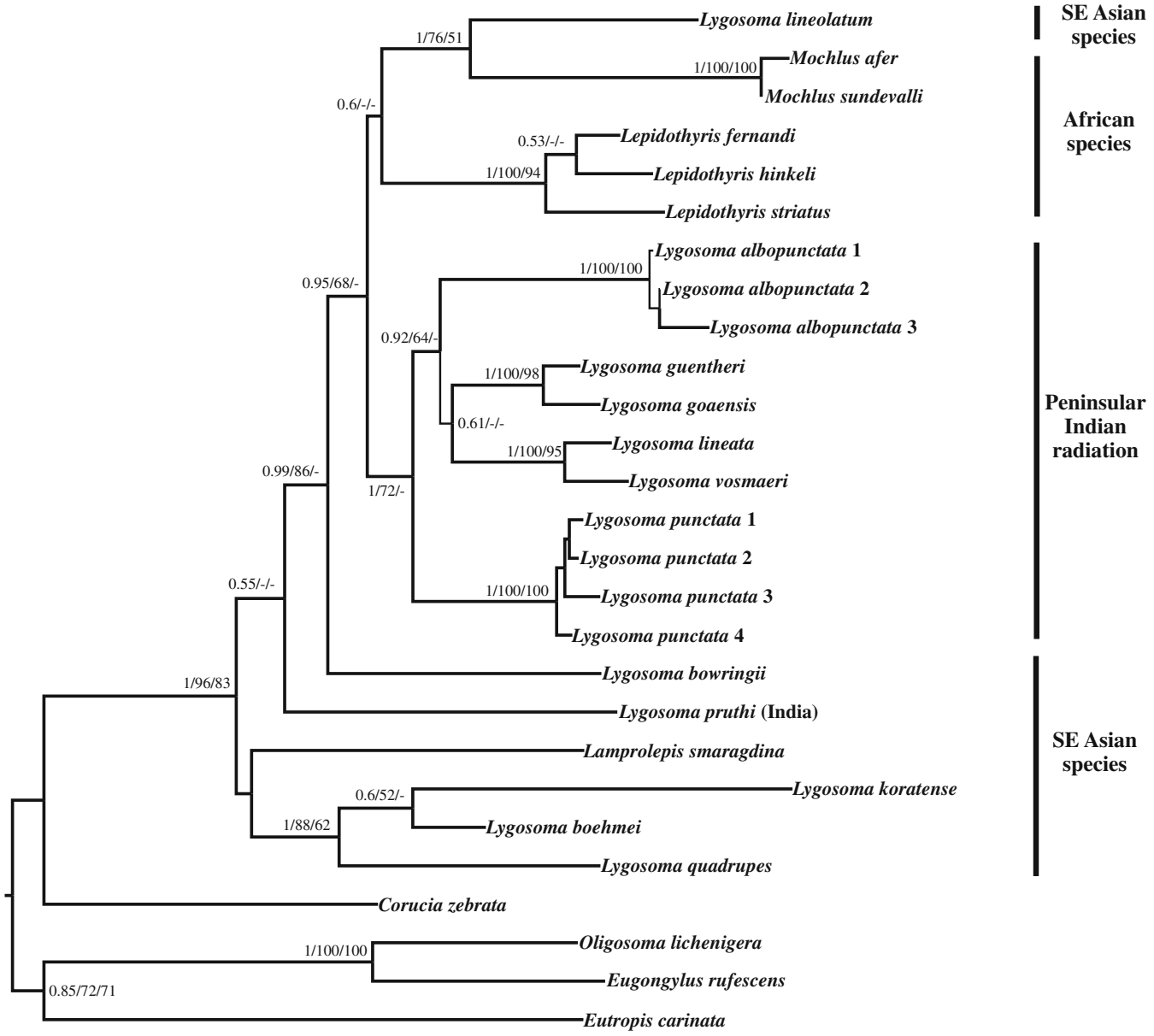

Figure 1. Maximum likelihood tree based on 12S+16S partitioned dataset. The values on each node (e.g. 1/16/51) are Bayesian posterior probability, ML bootstrap, MP bootstrap. Support values below 50 per cent have been denoted as '–'.

methods also retrieved a distinct Indian clade consisting of species largely distributed in the Indian subregion which received high posterior probability but very low ML and MP bootstrap support. This Indian clade consisted of *L. albopunctata* and *L. punctata,* two widely distributed species, and the endemics *L. goaensis*, *L. lineata*, *L. vosmaeri* and *L. guentheri*. However *L. pruthi*, another Indian endemic, was not part of this clade. The likelihood score of the best ML tree was significantly higher than the score of the ML tree where *L. pruthi* was constrained to be part of the Indian clade (Shimodaira-Hasegawa (SH) test, $P < 0.05$). The Indian clade was sister to a clade consisting of the African genera *Mochlus* and *Lepidothyris*, and a single Asian species *L. lineolatum* (henceforth referred to as African clade). Sister to the Indian and African clades was *L. bowringii* which is a widespread species from Southeast Asia. In all the trees in our analyses *L. pruthi* was sister to this large clade consisting of *Lygosoma bowringii* + Indian clade + African clade. *Lamprolepis smaragdina* was sister to the Southeast Asian species *L. koratense*, *L. boehmei* and *L. quadrupes* in the ML tree whereas in the MP and Bayesian tree its position was unresolved. The Southeast Asian species formed a clade that was sister to the large clade consisting of *L. pruthi* + *L. bowringii* + Indian clade + African clade.

Discussion

Recent molecular phylogenetic studies on many tropical Asian taxa have revealed endemic Indian radiations (Köhler and Glaubrecht 2007; Datta-Roy *et al.* 2012). These studies suggest the Indian region harbours unique fauna that have, in some cases, been generated through *in situ* diversification. Our current work on the skink genus *Lygosoma* also retrieved what appears to be a distinct Indian radiation consisting of species distributed predominantly in India. However, one of Indian endemics, *Lygosoma pruthi*, was not part of this Indian clade. The Indian clade along with the African genera *Lepidothyris* and *Mochlus* as well as *Lygosoma pruthi* are nested within a larger phylogeny consisting of the remaining *Lygosoma* spp. and *Lamprolepis* from Southeast Asia. Thus the phylogenetic positions of the Indian *Lygosoma* suggest that they might have been derived from Southeast Asia through at least two dispersal events. The earlier dispersal event gave rise to *Lygosoma pruthi* and the later dispersal, which was probably followed by *in situ* radiation, might have generated much of the current diversity in India. All species of the Indian radiation are largely confined to the Indian subregion except *L. albopunctata* which is also distributed in mainland Southeast Asia. This species appears to have evolved in the Indian subregion and later dispersed into Southeast Asia. Such a biogeographical scenario has also been reported from the skink genus *Eutropis*, where India harbours an endemic radiation that was derived from Southeast Asia (Datta-Roy *et al.* 2012).

However it must be noted that the genus *Lygosoma* as defined by Greer (1977) consists of 39 species, most of which are distributed in tropical Asia. In our phylogeny we have included 12 out of the 24 Asian species and none of the African species. Thus, in the case of *Lygosoma*, for a robust support for the Indian radiation scenario additional sampling needs to be undertaken, particularly of the Southeast Asian species.

Taxonomic implications

In our phylogeny, we have included at least one representative of all the known members of the *Lygosoma* group, i.e., *Lygosoma*, *Mochlus*, *Lepidothyris* and *Lamprolepis*. Interestingly, the genus *Lygosoma* was paraphyletic with respect to the African genera *Mochlus* and *Lepidothyris*. Similar results were obtained in other studies (Ziegler *et al*. 2007; Wagner *et al*. 2009). Thus, the taxonomic status of the African genera need to be reconsidered given they are nested deep inside the *Lygosoma* radiation.

Another taxonomic issue in this group is the status of genus *Riopa* Gray, 1839. Greer (1977) conducted careful morphological analyses and synonymized *Lygosoma* and *Riopa* based on 'higher morphological and ecological similarities' when compared to the other closely related genera. Phylogenetic studies which included the genus '*Riopa*' have noted that members of the genus are nested within a much larger clade constituting species unambiguously assigned to the genus *Lygosoma* based on morphological characters (Ziegler *et al.* 2007; Wagner *et al.* 2009). Similarly, in our phylogeny *L. albopunctata*, which is often assigned to *Riopa*, is nested within *Lygosoma*. Thus, although some authors still use the genus name '*Riopa*' (Ziegler *et al.* 2007; Wagner *et al.* 2009), we have followed Greer's (1977) classification and consider *Riopa* a synonym of *Lygosoma*.

Conclusions

The phylogeny of *Lygosoma* and its allies from tropical Asia and Africa suggested an endemic Indian radiation which is likely to have been derived from Southeast Asia. The peninsular Indian endemic *L. pruthi* was not part of the Indian radiation. The African genera *Mochlus* and *Lepidothyris* were nested deep inside the *Lygosoma* radiation and thus the validity of these genera needs to be ascertained. Further, our results also question the validity of the genus *Riopa*. We recommend further sampling of *Lygosoma* species from the mainland Southeast Asia and Africa to resolve some of these issues.

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