An addition to the endemic Indian radiation of *Eutropis*: Phylogenetic position of *Eutropis dissimilis* Hallowell (Squamata: Scincidae)

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Abstract. Skinks of the genus *Eutropis* represent one of the most widespread and speciose lizard groups in tropical Asia. Numerous recent studies have utilized a variety of genes and methods to reconstruct the phylogeny of these lizards, however these studies have not resolved the placement of one of the widely distributed *Eutropis* Fitzinger, *E. dissimilis*. We have sequenced a specimen of *E. dissimilis* from the type locality and our result suggests that it is part of the Indian radiation of *Eutropis* and not related to African *Trachylepis* Fitzinger or Southeast Asian *Dasia* Gray as previously suggested. Furthermore, we report that the sequence of *E. dissimilis* used in an earlier study of the once cosmopolitan genus ‘*Mabuya*’ may have been erroneously identified and appears to be a sequence of *E. novemcarinata*. We also demonstrate that the evolution of a clear lower eyelid, which was considered a synapomorphy for the sister genus *Trachylepis*, has arisen multiple times in *Eutropis*.

Key words: *Eutropis*, Indian radiation, Scincidae, systematics

Introduction

Skinks of the genus *Eutropis* represent a seemingly monophyletic clade split out from the genus *Mabuya* Fitzinger which possesses a circumtropical distribution (Mausfeld & Schmitz 2003). The genus *Eutropis* is one of the most speciose genera of the family Scincidae in tropical Asia with over 31 species. Recent molecular phylogenetic studies (Datta-Roy et al. 2012; Barley et al. 2014) found strong support for two clades within the Asian *Eutropis* radiation: one largely confined to India and the other in the Philippines (see Figure 4 in Barley et al. 2014). Relationships among the remaining Southeast Asian species have been more difficult to resolve, and some of the deeper nodes in the phylogeny require further investigation (Barley et al. 2014).

The phylogenetic position of *E. dissimilis* (one of the most widely distributed species of *Eutropis*) has remained particularly enigmatic (Mausfeld & Schmitz 2003; Bauer et al. 2008; Datta-Roy et al. 2012). The distribution of *E. dissimilis* ranges from Afghanistan in the west (Clarke et al. 1969) and continues throughout northern India including the Nepal Terai (Sceleich & Kastle 2002), while the southernmost population is known from Madhya Pradesh state (Murthy 1990). The eastern limits of its range remain poorly understood, although it has been reported from Bengal (Smith 1935; Tikader & Sharma 1992), Bangladesh (Husain 1974; Khan 1982), Arunachal Pradesh, Assam (Bauer et al. 2008), and Myanmar (Zug et al. 1998). Thus, it appears that the Brahmaputra River, a major biogeographic boundary in Northeast India (Vidya et al. 2005; Pawar et al. 2006; Veron et al. 2006) has not restricted the dispersal of *E. dissimilis*.

*Eutropis dissimilis* possesses a transparent window in the lower eyelid rather than a scaly eyelid which is present in most other *Eutropis* (Broadley 2000; Mausfeld & Schmitz 2003; Datta-Roy et al. 2012). On the other hand, all species of *Trachylepis* (the Afro-Malagasy clade) possess a transparent window in the lower eyelid (Broadley 2000; Mausfeld & Schmitz 2003). In a phylogenetic study of *Eutropis* by Mausfeld & Schmitz (2003), *E. dissimilis* collected from Myanmar was sister to the arboreal genus *Dasia* Gray (albeit with low node support) rather than to *Eutropis*. Although the clade consisting of *E. dissimilis* and *Dasia* was sister to the *Eutropis* clade, Mausfeld & Schmitz (2003) placed *E. dissimilis* in the genus ‘Euprepis’ (now *Trachylepis*). They further mentioned that the transparent lower eyelid in...
‘Euprepis’ (Trachylepis) is a synapomorphous character and can be useful in distinguishing between the Tropical Asian Eutropis and the Afro-Malagasy Trachylepis. This statement is untenable given recent studies that have shown that the Asian species Eutropis bibronii, which also possesses transparent lower eyelid, is a member of the Asian radiation of Eutropis (Datta-Roy et al. 2012). Additionally there are two other Asian Eutropis (E. innotata and E. novemcarinata) that possess transparent lower eyelids, however, their phylogenetic position is not well understood. Therefore, presence of a transparent lower eyelid appears to be an evolutionarily labile trait, and rather than being diagnostic of the Afro-Malagasy clade, may have evolved convergently. Although the study by Datta-Roy et al. (2012) included the published sequence of ‘E. dissimilis’ from Mausfeld & Schmitz (2003), its phylogenetic position was still largely unresolved, as it remained sister to a clade consisting of Dasia, albeit with low support in both Maximum likelihood (ML) and Maximum parsimony analysis. Bauer (2003) and Bauer et al. (2008) pointed out that ‘Euprepis’ was a junior synonym of the South American clade ‘Mabuya’. The species dissimilis was not phylogenetically related to either Mabuya or the African Trachylepis (Mausfeld & Schmitz 2003). Bauer et al. (2008) therefore recommended that the species dissimilis be placed back in the genus Eutropis with a disclaimer that further studies were needed in order to clarify its phylogenetic position. In order to provide some clarity regarding the placement of Eutropis dissimilis, we sampled the species from its type locality (Bengal) and generated sequences for one nuclear and two mitochondrial markers. This data was combined with previously published genetic data and phylogenetic relationships were reconstructed. In addition to Eutropis dissimilis, we included data from species in the genus Dasia, Trachylepis, and Emoia Gray (a more distantly related outgroup, Mausfeld & Schmitz 2003; Datta-Roy et al. 2012). Furthermore, we also included another Asian species with transparent lower eyelid, E. novemcarinata, in the analysis.

Material and methods

We sampled one specimen of E. dissimilis from West Bengal (23.12287°N, 87.02338°E, alt. 227 m asl; datum WGS 84). The liver tissue was collected in field and stored in 95% ethanol. A tissue sample for E. novemcarinata (CAS 215714) was obtained from the California Academy of Sciences (CAS) Museum. The total genomic DNA was extracted from these tissue samples using a DNeasy Blood & Tissue kit (Qiagen™). The extracted DNA was quantified and diluted accordingly for a PCR reaction. We amplified partial regions of two mitochondrial markers, 12S rRNA and 16S rRNA (12S and 16S), and one nuclear gene, the RNA fingerprint protein 35 (R35), using published primers (Kocher et al. 1989; Palumbi et al. 1991; Leaché 2009). The PCR products were purified using a QiAquick PCR Purification kit (Qiagen™) and the sequences were obtained commercially from Amnion Biosciences, Bangalore, India (see Appendix 1 for GenBank accession numbers). In addition to generating sequence data for E. dissimilis and E. novemcarinata, we included published sequence data from species in the genus Dasia (D. johnsinghii & D. vittata), Trachylepis (T. hoeschi & T. spilogaster) and Emoia (E. caeruleocauda & E. cyanura) (a more distantly related outgroup; Mausfeld & Schmitz 2003; Datta-Roy et al. 2012). The individual gene sequences we generated for E. dissimilis and E. novemcarinata were aligned with published sequences obtained from GenBank (see Appendix 1). This alignment consisting of 28 taxa included the aforementioned genera (Dasia, Trachylepis & Emoia) along with other members of the genus Eutropis across India and Southeast Asia (Mausfeld & Schmitz 2003; Datta-Roy et al. 2012; Barley et al. 2014). We also included the published sequence of the Myanmar ‘dissimilis’ population from Mausfeld & Schmitz (2003).

The alignment was performed with default parameters in MUSCLE (Edgar 2004) using the program MEGA v5.05 (Tamura et al. 2011). The best-fit partitioning scheme along with their respective models of sequence evolution was selected using PartitionFinder v1.1.0 (Lanfear et al. 2012), which suggested the data be partitioned by gene (12S + 16S + R35) and within R35, by codons. The selected DNA substitution model for 12S, 16S and the third position of the R35 codon were GTR+Γ+I. For the first and second positions of R35, HKY+Γ+I was selected. A partitioned, concatenated, maximum likelihood (ML) phylogenetic analysis of the combined nuclear and mitochondrial data was performed using raxmlGUI v1.3 (Silvestro & Michalak 2012). We ran 20 ML searches to identify the global ML tree estimate, with each partition being assigned a GTR+Γ+I model. Nodal support values were obtained by performing thorough bootstrapping analyses with 1000 pseudoreplicates.

We performed two additional ML analyses using constrained trees to test several phylogenetic hypotheses. In the first analysis the Eutropis species with a transparent lower eyelid, E. bibronii, E. dissimilis (from type locality as well as from Myanmar) and E. novemcarinata, were constrained to be monophyletic. In the second ML analysis all species in our dataset with transparent lower eyelids were constrained to be monophyletic. Here E. bibronii, E. dissimilis and E. novemcarinata were constrained to form a clade with Afro-Malagasy Trachylepis (represented by T. hoeschi and T. spilogaster). An Approximately Unbiased (AU) test (Shimodaira 2002) was performed to compare these two trees with the best tree. The AU test was performed using PAUP v4.0 b10 (Swofford 2002).
A Bayesian phylogenetic inference analysis (BI) was performed using MrBayes v3.1 (Ronquist & Huelsenbeck 2003). The dataset was partitioned based on the results obtained from PartitionFinder. The partitions were unlinked, allowing the model parameters for each partition to be estimated independently. The program was run for four million generations utilizing two separate runs, each with four chains (with default heating values) and sampling every hundred generations. We assessed convergence by confirming that the average standard deviation of split frequencies was < 0.01, and that all the parameters had reached stationarity and had attained sufficient effective sample sizes (>200) using TRACER v1.6 (Rambaut et al. 2014). Convergence of topology was assessed using AWTY (Wilgenbusch et al. 2004). We discarded 25% (10000) initial trees from the saved trees as burnin and a majority rule consensus tree was obtained for the remaining trees in MrBayes using the sumt command. The ML and the BI analysis trees were rooted using the two aforementioned species of Emoia.

**Results**

The likelihood tree based on the combined dataset (12S + 16S + R35) is shown in Figure 1. The ML and the BI analyses recovered an endemic Indian radiation (Figure 1, Node C) as has been reported earlier (Datta-Roy et al. 2012; Barley et al. 2014). The representative Eutropis dissimilis from type locality was nested within the Indian radiation and was part of a clade consisting of E. carinata, E. trivittata, E. beddomii and E. nagarjuni, with the sister clade to E. dissimilis consisting of the latter three endemic species. The likelihood score of the best ML tree was significantly higher than the ML tree where E. dissimilis was constrained to be sister to E. bibronii and E. novemcarinata, or when E. bibronii + E. dissimilis + E. novemcarinata and the two species of Trachylepis (Figure 1, Node A) were constrained to be monophyletic (AU test, P < 0.05). The ‘E. dissimilis’ from Myanmar, included in the study of Mausfeld & Schmitz (2003) was highly genetically similar (~1% uncorrected pairwise sequence divergence) to E. novemcarinata, with the two samples forming a monophyletic group with 100% node support in both the ML and the BI trees. This clade was sister to a clade consisting of the two sampled species of Dasia with low node support (Figure 1, Node B).

**Discussion**

Our study suggests that E. dissimilis is a member of the endemic Indian radiation of Eutropis, adding another species to an already diverse species group. Our results also suggest that the specimen previously identified as ‘E. dissimilis’ in Mausfeld & Schmitz (2003) is likely E. novemcarinata. According to the museum record this specimen (voucher number CAS 213612) has subsequently been re-identified as ‘Mabuya novemcarinata’ collected from Myanmar: Magwe division: Shwe Set Taw Wildlife Sanctuary and therefore was mislabeled by Mausfeld & Schmitz (2003).

Based on their results, Mausfeld & Schmitz (2003) suggest that the clear transparent window in the lower eyelid can be used as a synapomorphy for the genus Trachylepis. Subsequently, Datta-Roy et al. (2012), Barley et al. (2014) and the current study have demonstrated that at least three Eutropis also possess clear lower eyelids. Our phylogeny suggests that this character has evolved independently in Trachylepis and multiple times within Eutropis, and therefore it appears to be an evolutionarily labile trait in this group of skinks. Thus, the clear lower eyelid trait is not a suitable diagnostic character for Trachylepis, and caution should be likely taken when interpreting this character as diagnostic more broadly across skink diversity given its propensity to evolve convergently. Additionally, we are missing one of the central Indian endemic species (E. innotata), which is known to have a clear eyelid (Smith 1935). It remains to be seen whether this species would be recovered within the Indian radiation of Eutropis.

The sister relationship of E. novemcarinata to the arboreal genus Dasia is surprising, however, this relationship is weakly supported. Barley et al. (2014) found E. novemcarinata to be sister to all other Eutropis species (although again, this relationship was weakly supported). Based on uncorrected pairwise sequence divergence, E. novemcarinata shows the lowest genetic difference to Dasia (10%) followed by the Southeast Asian Eutropis species (11–14%), Trachylepis (12%) and the Indian radiation of Eutropis (12–14%). Given the ambiguous results, it is clear that the relationships among Eutropis, Trachylepis, Dasia and Mabuya require further investigation, and that the splitting up of Mabuya into separate genera might have been premature. Many authors have pointed out that researchers should avoid making taxonomic changes until strong evidence is available that they are required, in favor of taxonomic stability (Pauley et al. 2009; Poe 2013; Vences et al. 2013). Although the ambiguities discussed here could be addressed by sinking all of the aforementioned genera (including those recently proposed by Hedges et al. 2012) into Mabuya, we refrain from proposing any additional changes so as not to contribute to the chaotic taxonomy in this group until phylogenetic relationships are better understood.
FIGURE 1. ML tree with highest likelihood score based on the concatenated dataset. The circles represent node support values above 95% for both ML bootstrap and Bayesian posterior probability (PP). The squares represent above 95% node support in case of Bayesian PP but not ML bootstrap. Nodes that lack significant support from both the analyses have been rendered as is. Node labels provided for three focal clades referenced within the manuscript.

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ADDITION TO THE ENDEMIC INDIAN "EUTROPIS" RADIATION

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APPENDIX 1. Accession numbers for specimens used in this study. ‘–’ indicates data unavailable.

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