Phylogeny and conservation status of the Indian egg-eater snake, *Elachistodon westermanni* Reinhardt, 1863 (Serpentes, Colubridae)

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Abstract. The Indian egg-eater (*Elachistodon westermanni*) is a monotypic species of the Genus *Elachistodon* distributed across the Indian sub-continent. In Africa, there are 13 species of egg-eating snakes of the Genus *Dasypeltis*. These two genera, *Elachistodon* and *Dasypeltis* were thought to be closely related due to similar diet specialization, and shared biogeographic history between the Indian sub-continent and the continent of Africa. In our study, we amplified three mitochondrial genes and one nuclear gene from *E. westermanni* and reconstructed molecular phylogeny utilizing published sequences to understand the evolutionary relationships between the African, and the Indian egg-eating snakes. We used morphological characters to reinforce our inferences on phylogenetic relationships. We show that the Indian egg-eater is sister to cat snakes of the Genus *Boiga*, and it does not share recent ancestry with the African egg-eating snakes. Morphological character states point at similarities between *Elachistodon* and *Dasypeltis* only in characters associated with their feeding behaviour. *Elachistodon westermanni* was similar to the *Boiga* spp. in several other morphological characters, and we provisionally assign *E. westermanni* under the genus *Boiga*. Compilation of records of *E. westermanni* across the Indian sub-continent over the years revealed a positive "Lazarus" effect. We conclude that, the egg-eating behaviour and the associated morphological characters in the snake genera *Dasypeltis* and *Elachistodon* are a result of convergent evolution. Based on the conservation status of *E. westermanni*, it could serve as a flagship species to conserve important wildlife habitats that are being lost rapidly in India.

Keywords: Boiga, conservation, convergent evolution, dietary niche, morphology, oophagy, taxonomy.

Introduction

The Indian egg-eater (*Elachistodon westermanni*) is the sole representative of the Genus *Elachistodon*. It was known from only three specimens since its description in 1863 until 2005, and it was thought to be at the verge of extinction (Sharma, 2004). It was rediscovered from central India by Captain et al. (2005), who published colour photographs of the species for the first time. Since then a number of reports have emerged (Khandal, Sahu and Sharma, 2016). Re-discovery of a species, after it is thought to be extinct, attracts media attention and motivates public opinion in favour of conservation of rare and endangered species,

and this is referred to as Lazarus effect (Dawson et al., 2006). Reports of *E. westermanni* have grown exponentially in the last decade and it deserves to be examined in the context of the Lazarus effect.

Several snakes are known to feed on eggs opportunistically, but *Dasypeltis* spp. and *E. westermanni* are the most notable obligatory egg-eating snakes from the Old world (Gans and Williams, 1954). These two genera, *Elachistodon* and *Dasypeltis* were considered closely related to one another, due to similar diet specialization and shared biogeographic history between the Indian sub-continent and the continent of Africa. Morphological adaptations imposed by dietary niche specialization in *Dasypeltis* spp. and *E. westermanni* were compared, and they were hypothesized to be closely related to each other (Gans and Williams, 1954). The evolutionary relationship between the two groups of egg-eater snakes remains unknown (Vyas, 2006, 2010).

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In this study, i) we tested the phylogenetic hypothesis that genera *Dasypeltis* and *Elachistodon* are sister lineages, by constructing a molecular phylogenetic tree using four gene sequences, ii) we compared morphological and ecological traits of species of the genera *Boiga* and *Dasypeltis* with *Elachistodon* and make taxonomic decision, iii) we analyzed the records of *E. westermanni* accumulated through time to examine the Lazarus effect. We discuss the implications of these findings for conservation of the Indian egg-eater and its habitat.

Materials and methods

DNA isolation

We obtained shed skin sample from a female *E. westermanni* (fig. 1) rescued at Patancheru, Medak District, Telangana (GPS: 17°30′16″N, 78°17′18″E; 556 m a.s.l.) by the Friends of Snakes Society, Hyderabad. The occurrence of the snake and associated natural history observations were recorded before relocation (Visvanathan, 2015). We extracted DNA from the tissue sample using standard Phenol-Chloroform method (Sambrook, Fritsch and Maniatis, 1989). We amplified three mitochondrial gene regions: 16S ribosomal RNA, Cytochrome B (Cytochrome B oxidase), and Nuclear Dehydrogenase subunit 4 (ND4), and one nuclear gene region: C-mos (Serine/threonine kinase). We selected these gene regions as they have been extensively used in squamate phylogenies (e.g. Pyron, Burbrink and Wiens, 2013). We used the primers L-1926 (Knight and Mindell, 1993) and H-2456 (Heise et al., 1995) to amplify 16S region, GLUDG and ATRCB3 to amplify CytB region (Parkinson, Campbell and Chippindale, 2002), ND4 and Leu to amplify ND4 region (Arèvalo et al., 1994) and S77 and S78 to amplify C-mos region (Lawson et al., 2005). We amplified the DNA for 35 cycles with annealing temperatures of 52°C (16S), 47°C (CytB), 53.3° (ND4) and 48°C (C-mos). Amplified DNA was sequenced using the Sanger (Dideoxyribonucleotide chain termination) method (Sanger, Nicklen and Coulson, 1977). All nucleotide sequences used in the study were deposited in the Genbank database (Supplementary table S1).

Phylogenetic analysis

All *E. westermanni* gene sequences were manually checked for ambiguous sites and were excluded from further analyses. We used a total of 3034bp sequence data for molecular phylogenetic analyses. We aligned the sequences using Muscle algorithm (Edgar, 2004) incorporated in Mega 5.2 (Tamura et al., 2011). We obtained gene sequence data from all species of the clade consisting of *Dasypeltis* spp. and *Boiga* spp., representatives of the genera *Toxicodryas* and *Telescopus* and *Coelonathus radiatus* were

Figure 1. *Elachistodon westermanni* rescued at Patancheru, Medak District, Telangana, India by the Friends of Snakes Society, Hyderabad. Photographed by Karthikeyan Vasudevan.

used as outgroups (Figueroa et al., 2016), from published records in NCBI repository (Supplementary table S2). The three protein coding sequences (cmos, cytB, and ND4) were translated into amino acid sequences using MEGA 5.2 to correct the reading frame. The four gene sequences were concatenated using Sequence Matrix version 1.7.8 (Vaidya, Lohman and Meier, 2011). The concatenated sequences were analyzed for suitable models of evolution after partitioning them into ten partitions (16S, cmos_pos1, cmos_pos2, cmos_pos3, cytb_pos1, cytb_pos2, cytb_pos3, ND4_pos1, ND4_pos2 and ND4_pos3) using Akaike Information Criteria (AIC) applied in Partionfinder V1.1.1 (Lanfear *et al.*, 2012) (see partitions and models of evolution in Supplementary tables S1 and S2). We built a Maximum Likelihood (ML) tree using thorough bootstrap algorithm as applied in RAxMLGui 1.3 (Silvestro and Michalak, 2012). The ML tree was built with 10 runs and 1000 repetitions with GTR+Gamma+I model set as the model of evolution. The tree was then viewed using Fig Tree version 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

In order to reconstruct Bayesian phylogeny, we utilized partitioned Bayesian analysis (Nylander et al., 2004) in Mr.Bayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). We ran two searches for 10 000 000 generations with four chains each and trees were sampled every 200 generations. Priors were set to variable rates of evolution, topology was set to uniform and branch lengths were unconstrained (for all partitions). Burn-in was set to 0.25% (a quarter of Markov chain Monte Carlo (MCMC) chains were burned in). We visualized the log-likelihood values across time plot in Tracer version 1.6 (Rambaut et al., 2014) to ensure sufficient burn-in period.

Morphological and ecological data

We compared available and accessible morphological and ecological characters between different species included in the phylogenetic tree to attempt to identify any characters which associate with the species' evolutionary relationships (Supplementary table S3). Gans and Williams
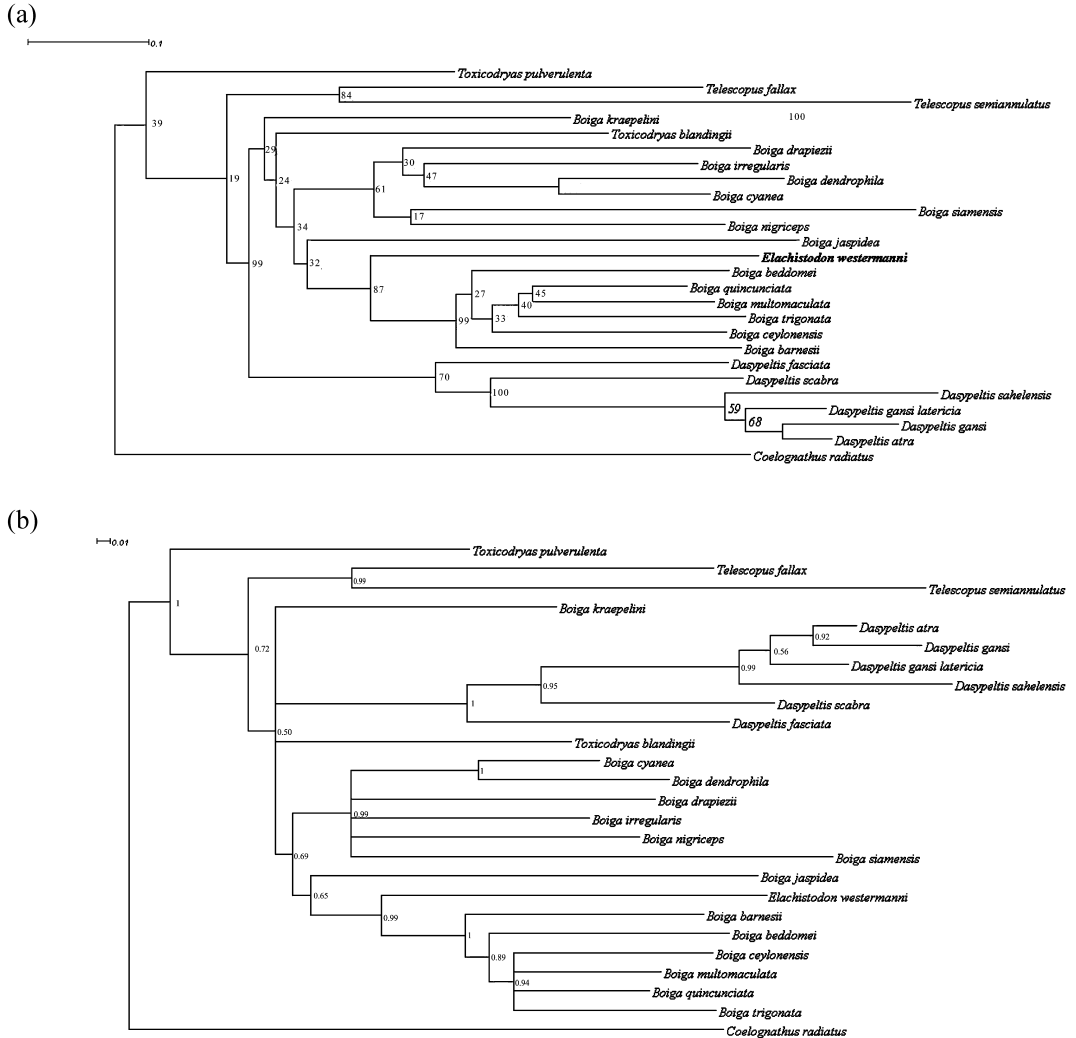
(a)

(b)

Figure 2. Molecular phylogeny tree with species of the genera: *Dasypeltis*, *Elachistodon* and *Boiga* derived from (a) Maximum Likelihood method; (b) Bayesian method.

(1954) tabulated morphological character differences between the genera *Dasypeltis* and *Elachistodon*, these included both external morphological characters such as scale shapes, presence/absence of keels on scales and internal osteological characters such as developed/moderately developed hypapophyses and structure of the jaws. In this study, we add the Genus *Boiga* to this comparison based on the results of our molecular phylogenetic tree. In addition, we listed morphological characters of species included in the phylogeny and additional species of the genera *Boiga*, *Dasypeltis* and *Elachistodon*. Data on characters such as body size (total length), occurring habitats, prey consumed were obtained from published records and literature (Supplementary table S4) and we compared them qualitatively. All published records of *E. westermanni* were aggregated and analysed for pattern in detection (Supplementary table S5).

Results

Molecular phylogenetic tree placed *E. westermanni* basal to a clade containing Old World cat snakes belonging to the genus *Boiga* with a bootstrap support of 87 and a posterior probability of 0.99 (fig. 2). The species in this clade are distributed in India, Sri Lanka and Myanmar. Two clades belonging to members of the Genus *Boiga* were retrieved in the phylogenetic tree. However, the distinctiveness of the clades was not supported by bootstrap values. A qualitative morphological character state

comparison between the genera *Elachistodon*, *Boiga*, and *Dasypeltis* revealed that five character states are shared between *Elachistodon* and *Boiga*, two were common to all three genera, and six were shared between *Elachistodon* and *Dasypeltis* (table 1). Three character states exhibited differences in all the three genera. The character states of head size in relation to the neck, vertebral scales, gular scales, body scale texture (keeled vs smooth scales) and dentition were similar among the genera *Boiga* and *Elachistodon*. Sublingual scales and median groove, presence of hypapophyses, apical pits on scales, feeding habits, size of harderian gland and skull morphology were similar among the genera *Elachistodon* and *Dasypeltis*. *E. westermanni* was unique in having of a pair of pits in the posterior region of the nasal shield. All three genera were oviparous and have paired sub-caudal scales. The *Boiga* spp. sister to *E. westermanni* are smaller in adult body size (range: 595-1550 mm); arboreal and terrestrial; and distributed in South Asia. In contrast, rest of the *Boiga* spp. have large adult body size (range: 1700-3100 mm); are arboreal and are distributed largely in Southeast Asia (Supplementary table S4). Based on phylogenetic and morphological data from representatives of the genera *Boiga*, *Dasypeltis* and *Elachistodon*, we provisionally assign *Elachistodon westermanni* Reinhardt, 1863 to the genus *Boiga* Fitzinger, 1826 under the new combination name: *Boiga westermanni* (Reinhardt, 1863).

Elachistodon westermanni is now known from 113 records spread across the Indian subcontinent (fig. 3), and several of them are clustered, because they originate from snake rescuers that operate in human dominated areas (Supplementary table S5). The spurt in records coincides with the publication of the first colour photograph of the species in 2005. Since then, it has been reported at a mean rate of 1.64, SE ± 0.49, records per year.

Discussion

Based on the phylogenetic relationship presented, we infer that the exclusive egg-feeding habit and the associated morphological specialisation have evolved in the African egg-eaters (*Dasypeltis* spp.) and the Indian egg-eater (*E. westermanni*) are a result of convergent evolution. Our study confirms the phylogenetic relationship of *E. westermanni* with the cat snakes belonging to the genus *Boiga*. However, it did not resolve the relationship between *Dasypeltis* spp. and *Boiga* spp., and it did not support monophyly of the genus *Boiga*. We attribute this to insufficient taxon sampling, and lack molecular data, as the genus *Boiga* is monophyletic (Figueroa et al., 2016).

The genus *Boiga* is known to be sister to a clade consisting snakes of the genera *Toxicodryas* and *Dasypeltis* (Figueroa et al., 2016). They represent radiations of snakes that are exclusive to the African continent, whereas the genus *Boiga* is represented in the Oriental biogeographic realm. It consists of 33 recognized species (Uetz, 2010) distributed across south and south-east Asian continents; the genus was previously considered to be paraphyletic (Pyron, Burbrink and Wiens, 2013). Molecular phylogeny has been able to resolve the paraphyly (Figueroa, 2016). However, only 16 out of the 33 species known from the genus *Boiga* have molecular data (Supplementary table S2). Therefore, taxon sampling might have influenced some of the phylogenetic inferences. The species contained in the clade of *Boiga* spp. related to *E. westermanni* were also morphologically distinct from rest of the *Boiga* spp. (Supplementary table S5). The phylogenetic relationship and the position of *E. westermanni* within the large clade containing all *Boiga* spp., suggests the need for a thorough review of the genus *Boiga*. In our phylogenetic tree, *E. westermanni* was retrieved as a basal member to a group of *Boiga* spp., which have their geographic distribution in the Indian subcontinent. Based on available information, we assign *E.*

Table 1. Morphological character state comparison of the genera: *Dasypeltis*, *Elachistodon* and *Boiga*.

| Morphological character	Elachistodon	Dasypeltis	Boiga
Head size in relation to the neck	Head fairly distinct from neck	No such distinction	Head very distinct from neck
Eye size and pupil shape	Large eye, elliptical pupil	Large eye, elliptical pupil	Large eye, elliptical pupil
Pit in the nasal shield	A pit in the posterior region of the nasal shield	Absent	Absent
Subcaudal scales	paired subcaudals	paired subcaudals	paired subcaudals
Vertebral scales	Vertebral series of scales are elongated, hexagonal	Vertebral scales are same in size and shape as dorsal scales	Vertebral scales are more or less large in size and hexagonal
Sublingual scales and median groove	Two pairs of sublinguals closely joined, lack median groove	Two pairs of sublinguals closely joined, lack of median groove	Median groove present
Gular scales	One row of gular scales	No true gular scales	Gular scales present
Body scale texture	Smooth body scales	Keeled body scales	Smooth body scales
Presence of hypapophyses	Hypapophyses moderately developed, posterior blocks do not pierce oesophagus	Hypapophyses extensively developed, posterior spines pierce oesophagus	Hypapophyses present on posterior dorsal vertebrae in all the Asiatic species
Dentition	8-12 teeth diminishing in size posteriorly, enlarged groove teeth in posterior maxilla	Maxillary teeth minute and equal in size	Maxillary teeth 10 to 14, followed by 2 or 3 enlarged grooved fangs
Apical pits on scales	Apical pits absent	Apical pits absent	Apical pits present on body scale rows 19-29
Habitat	Terrestrial, semi-arboreal	Diverse	Semi-arboreal, arboreal
Feeding habits	Feeds on eggs	Feeds on eggs	Feeds on lizards, birds and eggs
Size of harderian gland	Harderian gland is notably enlarged	Harderian gland is notably enlarged	No notable enlargement of gland
Skull morphology	Palatal elements in the skull rigidly joined	Palatal elements in the skull rigidly joined	Palatal elements in the skull are not rigidly joined
Enamel on hypapophyses	No enamel on hypapophyses	Enamel present	Absent
Mode of birth	Oviparous	Oviparous	Oviparous
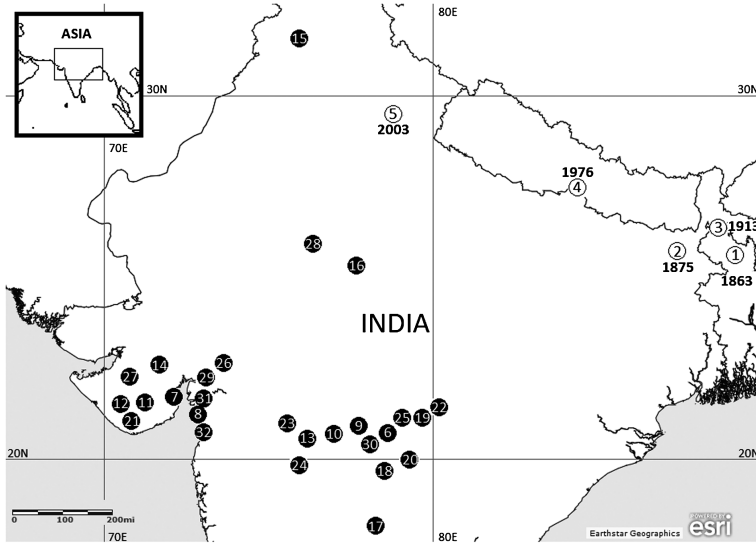
Figure 3. Locality records of *Elachistodon westermanni* (Reinhardt, 1863) – details of the records of occurrence are found in Supplementary table S4.

westermanni under the genus *Boiga*, and provide a new combination name *Boiga westermanni* (Reinhardt, 1863). Additional data on members of the genus *Boiga* would be essential to improve the present taxonomic assessment.

Gans and Williams (1954) had rightly speculated that the morphological similarities exhibited by the genera *Dasypeltis* and *Elachistodon* are associated with the feeding habits of the snakes; and that, the differences that exist in several morphological characters forms the basis for their taxonomic status (table 1). We found that several characters associated with feeding habits were similar in *E. westermanni* and *Dasypeltis* spp. The skull has evolved to accommodate the wide opening of the mouth and hypapophyses for crushing egg shells (Gans and Williams, 1954). Enlarged maxillary teeth in *E. westermanni* were not shared with members of the genus *Dasypeltis* (Gans and Williams, 1954).

After the description of *E. westermanni* in 1863, there were only three records in 142 years. The hiatus in reports of the species led to the speculation that it was at the verge of extinction (Sharma, 2004). Based on this information, it was listed in Schedule I of the Wildlife

Protection Act in 1972, in India. It accords the highest penalty under the Act, for any harm that might be caused to the species or its habitat in the country. Captain et al., (2005) reported *E. westermanni* from central India with colour photographs. Since then, several new localities have been added in peninsular India. Snake rescues that continually take place in human dominated areas in the country have played an important role in revealing the geographical range of *E. westermanni*. Colour photographs and detailed description of the snake have undoubtedly helped identification and subsequent reporting (Vyas, 2006). Apart from addressing knowledge gaps on the geographical distribution (Dandge and Tiple, 2016), a vibrant community of snake rescuers in India have made valuable contributions through observations on feeding habits of the species (Dandge, 2008; Visvanathan, 2015).

The Indian egg-eater presently occupies a wide range of habitats, ranging from coastal thorny forests to moist forests in the foothills of the Himalayas, covering an elevational range of 0-300 m (Vyas, 2013). This species is presently categorized under the 'Least Concern' category by the IUCN. Spurt in reports of such enigmatic species usually attract hobbyist collectors

and wildlife traders to exploit them. Conserving such species should involve providing disincentives for revealing locations of their occurrence (Meijaard and Nijman, 2014). With records of the snake emerging regularly, it is unlikely to trigger exploitation of the species, since the Act serves as a strong deterrent. On the contrary, *E. westermanni* could serve as a flagship species to conserve the last remaining wildlife habitats in the arid central and western India from a spate of disturbances that it currently faces.

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