DNA barcoding adjudicate two different morphs of *Bascanichthys deraniyagalai* (Anguilliformes: Ophichthidae): re-description and first record from Chilika lagoon, India

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**ABSTRACT**
Morphological characters and distribution pattern of the snake eel, *Bascanichthys deraniyagalai* is debatable during the past half-century. Thus, the species is re-described herewith by morphometry, vertebrae, and molecular data. In recent Chilika expedition in India, we have collected 10 specimens and observed a vertebral count dimorphism. The present study distinctly detected two vertebral groups of *B. deraniyagalai*: first group with 5 predorsal vertebrae, 81–83 preanal vertebrae, 190–196 total vertebrae in females; however, the sex of the second group with 5 predorsal vertebrae, 72–74 preanal vertebrae, 176–178 total vertebrae was not able to confirm. Further, the molecular data of morphologically identified two distinct groups of *B. deraniyagalai* shows negligible Kimura 2 parameter genetic divergence and cohesive clustering in Neighbor-Joining phylogeny proved to be the two different morphs of the same species. Hence, the present study indicated there might be vertebral count or sexual dimorphism in *B. deraniyagalai*, which need further sampling and taxonomic revision to unwrap the fact.

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**Introduction**
Snake eels or worm eels of family Ophichthidae are distributed worldwide from tropical to warm-temperate oceans, and they live in shallow water to deep water at a depth of 1300 m (McCosker 2010). The family Ophichthidae is represented by a total of 344 valid species worldwide containing two subfamilies Myrophinae (69 species) and Ophichthinae (275 species) worldwide (Eschmeyer and Fong 2018). The snake eel, *Bascanichthys deraniyagalai* Menon, is described under subfamily Ophichthinae, family Ophichthidae, and order Anguilliformes. The species was described with five specimens collected from Arasalar River in Tamil Nadu state of Southern India. However, during the original description of this species, the characteristics of vertebral pattern and key characters are being overlooked.

In recent past, the molecular data have gained evidence as supporting tool in morphology as well as in systematics research; that enables unequivocal species identification (Laskar et al. 2018). The short fragments of mitochondrial Cytochrome C oxidase subunit I gene has been proved to be effective in species identification of several other faunal groups including both freshwater and marine fishes (Mohapatra et al. 2013; Laskar et al. 2018). Despite the fact, many new Anguilliformes fishes have been recently discovered form India (Ray et al. 2015; Mohapatra et al. 2016, 2017a, 2017b, 2017c), but very few genetic information are available in both GenBank and BOLD database. The present study was shouted to re-examine the poorly described *B. deraniyagalai* by using morphology, meristic, vertebrae, and molecular data to re-describe the species and evidenced the major lineages originated between the end of the Cretaceous and Early Eocene (Santini et al. 2013). Further, the partial segment of mitochondrial Cytochrome C oxidase subunit I gene has been proved to be effective in species identification of several other faunal groups including both freshwater and marine fishes (Mohapatra et al. 2013; Laskar et al. 2018). Despite the fact, many new Anguilliformes fishes have been recently discovered form India (Ray et al. 2015; Mohapatra et al. 2016, 2017a, 2017b, 2017c), but very few genetic information are available in both GenBank and BOLD database. The present study was shouted to re-examine the poorly described *B. deraniyagalai* by using morphology, meristic, vertebrae, and molecular data to re-describe the species.

**Materials and methods**

**Sample collection and morphological data**
Total 10 specimens were collected using box trap net (locally known as Khanda) operated by the local fishermen in Chilika lagoon at the outside of the Nalban Bird Sanctuary (19.69 N 85.29 E). The specimens were collected from the scattered sea grasses in the box trap net, and immediately preserved...
in 70% ethanol. The specimens were deposited in Estuarine Biology Regional centre, Zoological Survey of India, Gopalpur-on-Sea (Registration No. EBRC/ZSI/F9312). Further, the specimens were compared with the original description as well as the holotype and paratypes (ZSI F1964/2, ZSI F1965/2) and confirmed as *B. deraniyagalai*. Vertebrae were counted from radiographs as explained by Bohlke (1982). The holotype of the species was also re-examined for the vertebral count, morphometric data, and meristic characters and thus, evaluated and compared. The tissue samples were collected into the 500 μl commercialized ATL buffer (Qiagen, Valencia, CA). The samples were stored at −20°C in Centre for DNA Taxonomy laboratory, ZSI, Kolkata for further molecular analysis.

**DNA isolation, PCR and sequencing**

The total genomic DNA was extracted followed by QIAamp DNA Mini Kit (Qiagen, Valencia, CA) standard protocol. The published primer pair, FishF1-5' TCAACCAACCACAAAGACA CTTGGCAC3', and FishR1-5' TAGACTTCTGGGTGGCCAAAGAA TCA3' (Ward et al. 2005) was used for amplification of partial mitochondrial cytochrome c oxidase subunit I (mtCOI) (~650 bp) gene segment in a Veriti® Thermal Cycler (Applied Biosystems, Foster City, CA). The 25 μl PCR mixture contains 10 pmol of each primer, 100 ng of DNA template, 1× PCR buffer, 1.0–1.5 mM of MgCl2, 0.25 mM of each dNTPs, and 0.25 U of Platinum Taq DNA Polymerase High fidelity (Invitrogen, Life Science Technologies). PCR conditions were initial denaturation at 94°C (2 min) followed by 30 cycles at
were aligned by ClustalX program (Thompson et al. 1997) to GenBank database. The generated and acquired sequences species (family Ophichthidae) were retrieved from the search and taxonomic hierarchy, 10 reference sequences of 5 search in the GenBank database. Based on the similarity generated sequences was performed through nucleotide BLASTn ing the accession numbers. The homology search of the gen-
sequences were submitted to GenBank database for acquir-
grating the quality value of bases were discarded from the generated chromatograms, calculated using the Kimura 2 parameter (K2P) by MEGA6.0 make a comprehensive dataset with equal length and com-
mon start position. The mean genetic divergences were cal-
culated using the Kimura 2 parameter (K2P) by MEGA6.0 (Tamura et al. 2013). Phylogenetic analysis was performed under the optimality criteria of Neighbor-Joining (NJ) by using PAUP* 4.0b10 (Swofford 2002) with 1000 bootstrap support. A sequence of Anguilla rostrata (Family Anguillidae) was also retrieved from GenBank and used as out-group in the dataset.

Sequence quality control measures, dataset preparation and analysis

The generated chromatograms that represent sequences of both DNA strands were obtained for each sample. The noisy sequences were trimmed at both end and >=2% ambiguous bases were discarded from the generated chromatograms, using the quality value of >=40 for bidirectional reads. The sequences were submitted to GenBank database for acquiring the accession numbers. The homology search of the generated sequences was performed through nucleotide BLASTn search in the GenBank database. Based on the similarity search and taxonomic hierarchy, 10 reference sequences of 5 Myrophinae species and 34 sequences of 19 Ophichthinae species (family Ophichthidae) were retrieved from the GenBank database. The generated and acquired sequences were aligned by ClustalX program (Thompson et al. 1997) to make a comprehensive dataset with equal length and common start position. The mean genetic divergences were calculated using the Kimura 2 parameter (K2P) by MEGA6.0 (Tamura et al. 2013). Phylogenetic analysis was performed under the optimality criteria of Neighbor-Joining (NJ) by using PAUP* 4.0b10 (Swofford 2002) with 1000 bootstrap support. A sequence of Anguilla rostrata (Family Anguillidae) was also retrieved from GenBank and used as out-group in the dataset.

Morphological data

The studied B. deraniyagalai was showed below mentioned morphological characters, body cylindrical, elongated with anus slightly before mid-body (preanal length 2.1–2.2 in total length). Dorsal fin origin on the head just before the gill opening and far behind to the rictus; Teeth small, slender, and blunt tipped. No premaxillary teeth, uniserial on maxilla and dentary. Two uniserial prevomerine teeth, vomerine teeth uniserial but placed irregularly. Pectoral fin reduced to a minute flap. There exist clearly two vertebral groups of the species: the first one with 5/81-83/190-196 and the second group with 5/72-74/177-178. Colour olive brown dorsally and dull yellow ventrally with a dark spot on ventral surface of the body, situated nearly one head length from gill opening.

Body notably elongated and almost cylindrical (Figure 1(A)). Depth at gill opening and anus nearly equal 66.4–88.2 in total length. Anus at about little before the midpoint of body with preanal length 2.1–2.2 in total length. Head about 15.0–19.8 in total length. Dorsal fin origin on head, just before gill opening and far behind the rictus; predorsal distance about 20.4–28.6 in total length. Greatest depth 3.81–5.5; snout 5.6–8.3; predorsal length 1.2–1.5 in head length. Eye small, located slightly closer to rictus than tip of snout, its diameter 3–4 in snout length. Upper jaw slightly longer than the lower jaw; upper jaw about 4.1–5 in head length and lower jaw about 5.0–6.6 in head length. Anterior nostril tubular, not reaching tip of snout; posterior nostril flap like open in upper lip. Gill opening below lateral line. Head pores are depicted as in Figure 1(C). Dorsal fin and anal fin extending towards tip of tail but not confluent around tail, leaving tail tip exposed and hard (characteristic of subfamily Ophichthinae). Pectoral fin reduced to a minute flap, restricted to upper part of gill opening and difficult to distinguish. Lateral line pores 73–84 before vent and 10 or 11 before branchial opening (wide range more closely related to dimorphism in vertebral count). Teeth small, slender and blunt tipped. Premaxillary teeth absent; teeth uniserial on maxilla and dentary. Two uniserial prevomerine teeth present; vomerine teeth uniserial but placed irregularly (Figure 1(D)).

There exist clearly two vertebral groups of the species: the first one with five predorsal vertebrae, 81–83 preanal vertebrae and 190–196 total vertebrae (ZSI_CEF1) and the second group with five predorsal vertebrae, 72–74 preanal vertebrae and 176–178 total vertebrae (ZSI_CEF2 and ZSI_CEF4). The holotype is having the vertebrae 5/82/190. Colour-Body olive brown dorsally and dull yellow ventrally. A dark spot on ventral surface of the body; situated nearly one head length from gill opening. Lateral line pores pale and distinct in preserved specimens. The sexual dimorphism has been detected in Moringua species (Anguilliformes, Moringuidae), in which the male and female have different vertebral counts. However, the differences in vertebral are uncommon in the members of Ophichthidae so far. To review the fact, two more fresh specimens were examined from the same group with higher vertebral (5/81-83/190-196), which were with eggs thus confirmed females. Thus, we confirmed that the specimen (ZSI_CEF1) with higher vertebral count was female.
However, the sex determination could not confirm for the specimens (ZSI_CEF2 and ZSI_CEF4) with low vertebral count (136). Based on morphology and molecular data, the study effect-ively identified B. deraniyagalei species from Chilika Lake. Earlier this species might have been reported as Lamnostoma orientalis from Chilika lagoon as after extensive survey of two years no specimen of Lamnostoma orientalis have been collected.

The genus Bascanichthys comprises 17 species worldwide (Eschmeyer and Fong 2018). All these species are compared following original descriptions, as it was not possible to access type specimens at present. Of these, B. bascanium have pectoral fin as long as snout; B. bascanoides with pectoral slightly longer than eye; B. gaira with pectoral fin three to six times in its base and B. paulensis with a small, rounded pectoral fin, while in B. deraniyagalei, like rest of the species pectoral fin extremely reduced. B. ceciliae and B. inopinatus have higher vertebral count (225–226 and 198–205) and B. scuticaris with lesser count (159–167), verses 176–196 in B. deraniyagalei. B. pusillus is distinct in having biserial maxillary and vomerine teeth (uniserial in B. deraniyagalei); B. myersi and B. ceciliae have three to five intermaxillary teeth (absent in B. deraniyagalei). Tail length is distinctly shorter than the preanal length in B. cylindricus and B. panamensis. Bascanichthys sibogae can be distinguished in having gill pinnas.

### Molecular data

The five species of Myrophinae and 20 species of Ophichthinae, including the studied species B. deraniyagalei, shows distinct clustering in the NJ phylogeny (Figure 1B). The two morpho-groups, ZSI_CEF1 (MH117907) and ZSI_CEF2, ZSI_CEF4 (MH117908, MH117909) depicts cohesive clustering and cladded as sister species of Yirrkala misolensis, Lamnostoma mindora, and Lamnostoma kampeni in the NJ tree. The overall mean genetic divergence of the eel data set was 17.8%. The within-species genetic divergence of the studied dataset was revealed ranging from 0% to 1.09%. Further, the genetic divergence among the species of Ophichthinae subfamily ranging from 3.1% to 21.6% with mean genetic divergence 16.5%. The genetic divergence among the species of Myrophinae subfamily ranging from 11.9% to 27.7% with mean genetic divergence 15.9%. The mean genetic divergence between two subfamilies of Ophichthidae resulted 20.4% in the current dataset. The B. deraniyagalei shows 0% K2P genetic divergence between two morpho-groups. The studied species was morphologically confused with the Lamnostoma species; however, sufficient genetic divergence (9.9–10.9%) was observed (Table 1). Based on both morphology and molecular data, the study effectively identified B. deraniyagalei species from Chilika Lake. Earlier this species might have been reported as Lamnostoma orientalis from Chilika lagoon as after extensive survey of two years no specimen of Lamnostoma orientalis have been collected.

### Table 1. The between and within-species K2P genetic distance of the studied eel dataset.

| Species               | Between species (%) | Within species (%) |
|-----------------------|---------------------|--------------------|
| B. deraniyagalei      |                     | 0                  |
| Lamnostoma mindora    | 9.9                 | n/c                |
| Lamnostoma kampeni    | 10.9                | 3.1                |
| Yirrkala misolensis   | 11.9                | 5.7                |
| Callechelys muraena   | 16.1                | 15.3                |
| Brachysamophis heshaw  | 17.3                | 13.7                |
| Ophiusurus macrorynchos | 17.8                | 14.1                |
| Gordichthys randalli  | 18.0                | 14.4                |
| Apterichus australis  | 18.1                | 17.6                |
| Ophichthys brevicaudatus | 18.4               | 17.9                |
| Myrichthys melas     | 18.5                | 18.0                |
| Echelus uroraphter    | 18.7                | 17.3                |
| Calechelys ceciliae   | 19.3                | 17.4                |
| Pisodonophis cancrivorous | 19.4             | 16.7                |
| Apterichus kloisingalian | 19.5             | 15.0                |
| Leuiranus semicinctus | 19.6                | 17.2                |
| Echelus punctifcr     | 19.7                | 15.7                |
| Myrichthys colubrinus | 19.9                | 17.8                |
| Pisodonophis cancrivorous | 19.9             | 17.8                |
| Apterichus kloisingalian | 19.5            | 15.0                |
| Leuiranus semicinctus | 19.6                | 17.2                |
| Echelus punctifcr     | 19.7                | 15.7                |
| Myrichthys colubrinus | 19.9                | 17.8                |
| Pisodonophis cancrivorous | 19.9             | 17.8                |
| Apterichus kloisingalian | 19.5            | 15.0                |
| Leuiranus semicinctus | 19.6                | 17.2                |
| Echelus punctifcr     | 19.7                | 15.7                |

n/c: not able to calculate due to single sequence. The genetic divergence between the species of subfamily Myrophinae are indicated by grey colour.
very closely similar to *B. deraniyagalei*. However, as observed by Menon (1961), all these four species have anus about mid-point of body but *B. deraniyagalea*is having anus nearer to snout than the tip of the tail. Head length of *B. deraniyagalea*is longer than other four species (8.0–10.0 times in trunk versus 6.2–8.0 in *B. deraniyagalei*). Additionally, *B. kirkii* and *B. longipinnis* have a stouter body, body-depth about 3 times in head length (versus body depth 3.8–5.5 in the head for *B. deraniyagalea*). Further, *B. filaria* has biserial posterior maxillary teeth and pectoral fin developed into a minute filament. Although the taxonomic characters are available previously for this species, the present study added new morphometric characters, and variations recorded within *B. deraniyagalea* population in the same eco-system along with vertebral or sexual dimorphism. These data might be helpful for further taxonomic research on the particular group and helps to reduce the species misidentification. Further, a wide range of survey of the same and related taxa and generation of more nucleo-mitochondrial molecular data would render more authentic scenario of eel phylogeny and systematics research. This study also reports the species for the first time from Chilika lagoon.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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