CONGRUENCE OF FOLDSCOPE AND DNA BARCODING IN TAXONOMIC REVIEW OF LESSER KNOWN RICE-PADDY EEL PISODONOPHIS BORO

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Abstract Rice-paddy eel, *Pisodonophis boro* is sampled from the Brahmaputra valley, Assam, India and the species is tagged with the developed DNA barcode for the first time utilising mitochondrial partial COI gene. This is in complementary with the conventional morphotaxonomy to make good use for ichthyo taxonomists and conservation biologists in regard of error free authentic but fast identification of the species and population estimation. Foldscope, a novel paper microscope has been incorporated in morphotaxonomy component of the study. The fish is characterized with the diagnostic keys such as origin of dorsal fin far behind the pectoral fin, presence of sensory pores and granular teeth, number of vertebrae and pectoral fin rays. Comparative study of conspecific and related barcodes has made a straightforward delineation of the eel. Simultaneous intervention of morphotaxonomy and DNA barcode would complement each other leading to expansion of scientific exploration of this lesser known ichthyofauna.
Index Terms: Ichthyology, fish, Assam

1. INTRODUCTION

Eels have always been a point of attraction for their charismatic shape, size and movement and used as nutritive food in different parts of the globe. *Pisodonophis boro*, a teleost fish, is one of the nine cogeneric species those come under the order Anguilliformes and family Ophichthidae. The Ophichthidae is represented by the snake eels having gill opening situated in the pharynx in the form of wide slits. The species *pisodonophis boro* is diagnosed by the keys [1] such as origin of the dorsal fin far behind the pectoral, presence of sensory pores, granular teeth and 170-177 numbers of vertebrae. Most of the cogeneric species are marine with reports of entering estuaries and rivers. In this study *P. boro* is collected from river Brahmaputra, far away from the estuary in the Kamrup district of Assam, India which is part of Himalaya and Indo-Burma biodiversity hot-spot. This fish is less explored in scientific study and till this DNA barcode, there was no barcode information on this species. Quick but error free identification of lesser known and threatened species is always vital as these are the indicators of status of biodiversity and its degradation in a particular area. DNA barcoding utilising mitochondrial cytochrome oxidase subunit I (COI) gene near its 5′ end has become successful, specifically in case of animals. Development of DNA barcode would rotate the wheel of molecular characterization of this data dearth species in understanding the threshold value for species level discrimination and barcode gap among related species around the world. In addition, characterization of COI sequence in terms of nucleotide composition provides evolutionary indications such as substitution bias. The genus *Pisodonophis* contains 9 congeners, *viz., Pisodonophis boro, Pisodonophis cancrivorus*, *Pisodonophis copelandi*, *Pisodonophis daspilotus*, *Pisodonophis hijala*, *Pisodonophis hoeveni*, *Pisodonophis hypselopterus*, *Pisodonophis sangjuensis*, *Pisodonophis semicinctus*, Among these, *Pisodonophis boro* is the only congeners found in Assam. In this current study DNA barcode of *P. boro* is developed for the first time using universal primers to generate baseline data for ichthyotaxonomists, conservation biologists and population genesists. DNA barcoding is regarded as complementary to conventional taxonomy [2]. Thus, in this study before entering into the arena of molecular biology, the fish is characterised with conventional morphotaxonomy. Assessment of variation with body form is the basic in mophometry for evaluation of discreteness and relationship among taxa and stock [3]. Simultaneous intervention of the conventional taxonomy and the DNA barcoding has tagged the species sufficiently for further scientific exploration.

Figure 1: Preserved specimen of *Pisodonophis boro*

Figure 2: Live specimens of *Pisodonophis boro*

2. MATERIALS AND METHODS

2.1 Morphotaxonomy: 14 individuals of *P. boro* were collected at commercial fishing ground near river Brahmaputra in the state of Assam, India. After sorting out using diagnostic keys, fish were photographs. Seven sex independent body parameters were considered and measurements were taken parallel to anterior posterior axis except body depth that was considered in perpendicular to the anterior posterior axis. Continuous vernier calliper was used for obtaining the body measurements. Mean value of the parameters were standardised with either total length or head length. Vertebral count was performed by treating specimens with 2% KOH solution to obtain skeleton. An origami based paper microscope, Foldscope [4] has been incorporated in this study for study of skin pattern, detection of sensory pores and fin ray count.

2.2 DNA barcoding: Total genomic DNA was isolated from white muscle tissue following the procedure developed by Miller *et al.* [5] with minor alterations. Genomic DNA, so obtained, was passed through quantitative and qualitative checking using 0.8% agarose gel in horizontal electrophoresis. Partial mitochondrial cytochrome c oxidase I (COI) was amplified using genomic DNA as template in polymerase chain reactions (PCR). A set of universal primers [6], viz. FishF1: 5′-TCAACCAACCACAAAGACATTGGCAC-3′ and FishR1: 5′-TAGACTTCTGGGTGGCCAAAGAATCA-3′ was used in amplification of around 650bp partial COI gene. Partial gene was amplified in 25 µl reaction mixture containing 1X PCR buffer, 2mM MgCl2, 10 pmol each primer, 1mM dNTPs and 1U Taq polymerase. The thermal condition for polymerase chain reactions was as follows: an initial denaturation at 95°C for 2 minutes, 35 cycles at denaturation temperature of 95°C for 30 seconds, annealing temperature of 55°C for 30 seconds and extension temperature of 68°C for 1 minute and concluded with a final extension step at 68°C for 10 minutes followed by a hold at 4°C. The amplified products were purified and sequenced through commercial sequencing. The raw sequences, so obtained, were aligned and edited using BIOEDIT. Genetic divergence is calculated within the species and with primarily obtained DNA barcode of native eel species *Anguilla bengalensis*.

3. RESULTS

3.1 Taxonomic Review

Order: Anguilliformes
Family: Ophichthidae
Genus: *Pisodonophis*
Species: *P. boro*
Current valid name: *Pisodonophis boro* (Hamilton, 1822) (Type locality: Hoogly estuary near Kolkata) (Fig.1 and Fig.2)
Taxonomic History: *Ophisurus boro* Hamtlton-Buchanan, 1822; *Ophichthys boro* Day, 1889; *Pisodonophis boro* Smith,1962.
English name: Rice-paddy eel
Vernacular Assamese name: Nal Bami/ Xaap maach

3.2 Distribution range: India (Brahmaputra and Barak drainage, Southern India), East Indian coasts through Indonesia to Polynesia, Indo-West Pacific.

3.3 Diagnostic features: Dorsal fin originates behind the pectoral fin; dorsal and anal fin are low and fin rays are embedded into groove making it difficult to count; lateral line is inconspicuous; body colour olive yellow, brown spots are present in upper region, lower shows bleak; vertebrae 172 in total, sensory pores present; 1+4 supraorbital pores, 1+4 supratemporal pores, 4+2 infraorbital pores and 6+3 preoperculomandibular pores; brancheostageal rays 34. Certain features observed using Foldscope are shown in Fig 3, 4 and 5.

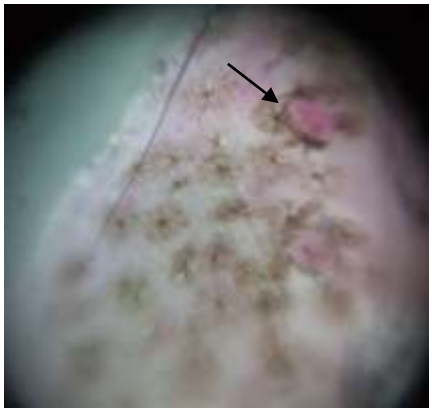

Figure 3: Body pattern with sensory pores

Figure 4: Fin rays of pectoral fin

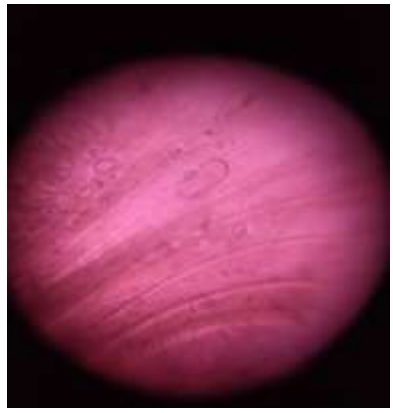

Figure 5: Brancheostageal rays

3.4 Biology

P. boro is found in lagoons and estuaries and enters in rivers and pools. Spawning in paddy fields during monsoon season have been reported [7]. It lives generally in bottom holes and forages on small fishes nocturnally. Diminutive sound production has been noticed during study from the fish in stressed condition. Morphometric data are shown in Table 1.

Table 1: Morphological data of *Pisodonophis boro*.

Body measurements	**Mean in percentage of total length (% TL) with SD**
Standard Length	100±0.00
Head Length	7.00±0.52
Body Depth	3.44±0.23
Pre Dorsal Length	14.2±0.22
Pre Anal Length	36.41±1.53
Body measurements	**Mean in percentage of head length (% HL) with SD**
Eye diameter	5.46±0.54
Snout Length	16.57±2.0

3.5 DNA Barcoding

The partial COI sequence was obtained from *Pisodonophis boro* and visualised in 1% agarose gel as shown in Fig. 6. Sequencing was done in triplicate from both the directions and sequences were found totally conserved with no indels. No stop codons were detected within the sequences. All the sequences are more than 600 bp length, which indicates that there is no NUMTs (nuclear DNA sequences originating from mitochondrial DNA sequences) sequences; because, vertebrates NUMTs are typically smaller than 600 bp [8]. Three generated barcodes were submitted to BOLD (Barcode of life database) System and Barcode Index Number (BIN), BOLD:ACP1605 was obtained. The DNA barcodes developed in this research is the first enlisted barcode for *Pisodonophis boro* in BOLD system. The genetic distance analysis in BOLD system represented low mean intra-specific distance (0.43) in comparison to high distance with nearest neighbour (19.67), *Anguilla bengalensis*, the other freshwater eel found in this region. Among the other 8 congeners of *Pisodonophis*, only *Pisodonophis cancrivorus* has been mentioned as barcoded species in BOLD system.

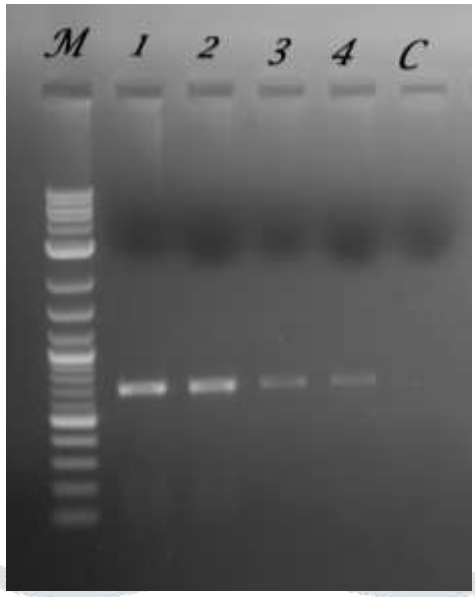

Figure 6: PCR amplified COI product (1-4), M: 100bp DNA marker, C: Negative control

4. DISCUSSION

Diagnostic information of *Pisodonophis boro* was found in accordance with earlier studies [9]. Body measurements showed slight difference with 'fishbase' [10]. *Pisodonophis assamensis* described by Sen (1985) [11] is a synonym of *Pisodonophis boro* [12]. *Pisodonophis boro* bears much similarity with *Pisodonophis cancrivorus* such as presence of 1+4 supraorbital pores, multiserial dentition with granular teeth type. Nevertheless, the two species can be differentiated with the origin of dorsal fin, which starts far behind pectoral fin in *Pisodonophis boro* and middle of pectoral fin in *Pisodonophis cancrivorus* [13]. *Pisodonophis boro* is the original name of the species which was later altered by Kaup with addition of extra 'o' making it *Pisoodonophis boro*. The first published spelling which is not thought to be invalid was used here.

Authentic identification of less studied species is vital for their conservation and management. Deficiency of data for *Pisodonophis boro* and related species all over the globe makes the overall analysis inconclusive. However, this study confirms efficiency of DNA barcoding in species level delineation. Combination of conventional morphology and DNA barcoding has tagged the species in a comprehensive way making it a good use for taxonomists and conservation biologists. Being a novel DNA barcode for *Pisodonophis boro*, it has plugged the void in the public database and accumulation of more barcodes would provide scopes for downstream analysis by retrieving the sequences.

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