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Morphological and Genetic Diversity of *Monopterus cuchia* (Hamilton) and *Monopterus albus* (Zuiew) in Northeast India

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ABSTRACT

Samples of morphologically identified *Monopterus albus* (50 nos.) and *Monopterus cuchia* (180 nos.) were collected from Manipur and Assam, were allocated into four populations based on the geographical proximity of the water bodies. Mitochondrial COI gene sequence was used to sort out genetic diversity and population differentiation of *Monopterus species* complex of Northeast India. Out of all morphological measurements, 8 measurements showed significant differences between males and females of *M. cuchia*. The morphometric measurements Pre anal length (PAL), Length of lateral line (LAL), Upper jaw length (UJL), Eye diameter (ED), Head width (HW), Pre orbital length (POL), Greatest width of body (GWB), Highest body diameter (HBD) showed significant differences at $P<0.05$ in t-test. The COI gene sequences were found to be A:T rich. The study based on mitochondrial COI gene clearly revealed that *M. cuchia* and *M. albus* are two distinct species. However, both the species *M. cuchia* and *M. albus* might have two subspecies within each species.

Keywords: coi gene, eels, *Monopterus*, Northeast India

INTRODUCTION

The freshwater air-breathing mud eel, *Monopterus cuchia* (Hamilton, 1822), locally known as cuchia (Order-synbranchiformes) often exhibit differences within and among population from different parts of its range (Dahanukar, 2010). *Monopterus albus* (Zuiew, 1793), swamp eel, tentatively identified as belonging to the synbranchid genus *Monopterus* (Collins *et al*., 2002; Li *et al.,* 2007) termed as species complex demands taxonomic revision (IUCN, 2017). *Monopterus albus* and *Monopterus cuchia* are regarded as species complex and require taxonomic revision (Dahanukar, 2010). Both *M. cuchia* and *M.albus* have ecological importance and high nutritional components, which can play a unique role for the development of socio-economic status of fishermen as well as culture practice (Quddus *et al*., 2000). However, these valued fishery resources have declined in recent years due to overfishing and environmental pollution (He *et al*., 2004; Yin *et al*., 2005).

Although, some earlier research has investigated population differentiation in *M. albus* population using RAPD (Liu *et al*., 2005) and isozymes (Yang *et al*., 2005), yet, little is known about the genetic diversity of *M. albus* and *M. cuchia* in northeast India. Recently, it has been emphasized to clarify confusion between *M. albus* and *M. cuchia* within India, which could impact upon the species after taxonomic evaluation (IUCN, 2014). Therefore, the present study has been made for the first time to address the question of genetic differentiation of *Monopterus cuchia* and *Monopterus albus* together.

MATERIALS AND METHODS

The study area

The present study focuses on the molecular characterization of the freshwater eel species of the genus *Monopterus* in Northeast India (Assam and Manipur) including bordering area of Assam-Meghalaya, part of the two hotspot region, viz. Indo-Burma and Himalayan hotspots (CEPF, 2005) (Figure 1). The GPS location of the study area is 26°10'22.79"- 27°39'32.79"N Latitude and 91°26'39.74"- 96°15'39.84"E Longitude.

Sample collection

Field work was carried out during January, 2014 to June, 2016 in different parts of Assam and Manipur in Northeast India in certain suitable habitats like paddy-field and swamps in order to collect the *M. cuchia* and *M. albus* samples (Figure 1). A total of 230 *Monopterus* individuals were sampled from 3 water bodies of Manipur and 18 water bodies of Assam with varying geographical co-ordinates. *Monopterus* individuals were morphologically identified, based on twenty three (23) reliably measurable morphometric characters. The individuals were geographically allocated into four populations based on the proximity of

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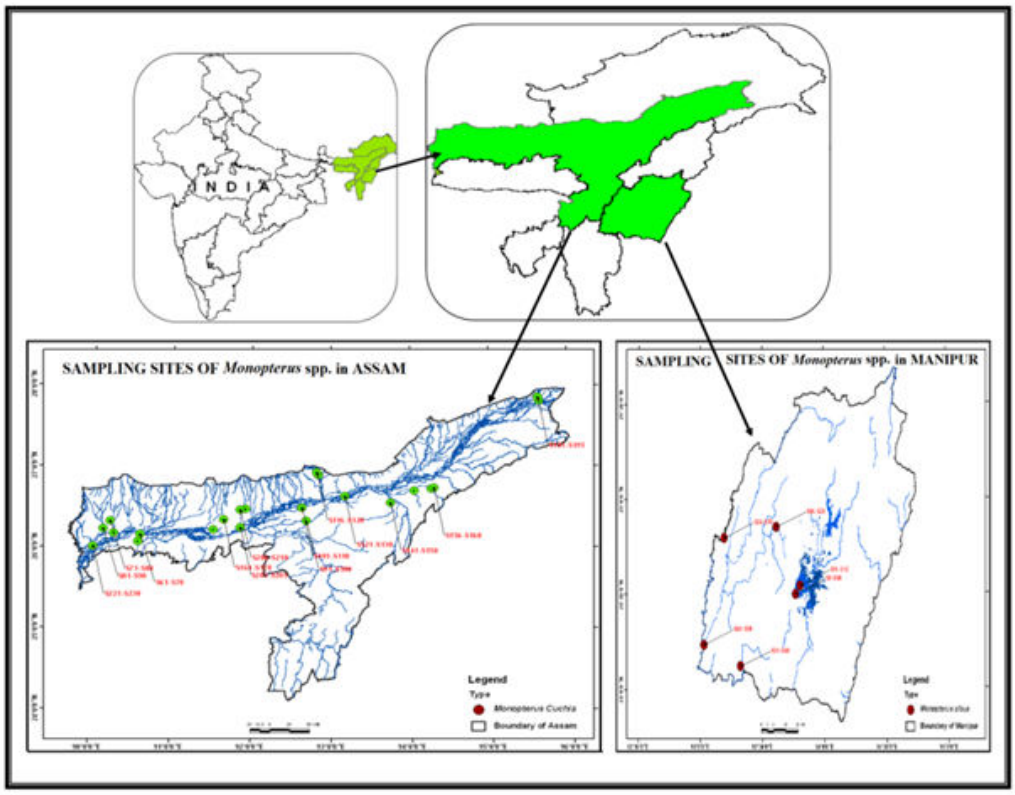

Figure 1. Map of study area showing the sampling sites.

the water bodies i.e. each *Monopterus* population was sampled at gap of about 100-400 km away from any other population (Figure 2).

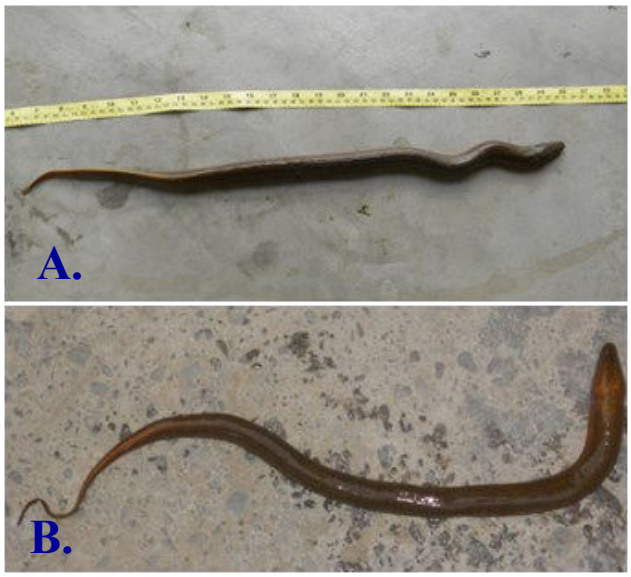

Figure 2. Photographs (Dorsal view) of A. *M. cuchia* collected from Assam, B. *M. albus* collected from Manipur.

DNA extraction

From each captured specimen, approximately 1 cm of tail tissue was removed with forceps and was placed it in a sterile 1.5 ml microtube containing 95% ethanol and was stored at – 20 o C until processing. The eels were released at their points of capture. The samples were placed in an ice chest during transport to the laboratory. Genomic DNA was isolated from the tissue using the Chloroform-Octanol method (Salah and Iciar, 1997; Cabe et al., 2007).

Sequencing, analysis and Phylogeny of Mitochondrial COI gene

Sequencing of mitochondrial COI gene was performed by ABI PRISM® 377 DNA sequencer (at BioAxis DNA Research Centre, Hyderabad). After verification the nucleotide sequences were deposited to GenBank (NCBI) public sequence repository (Benson *et al*., 2013). Data mining and sequence analyses of COI gene was performed using the CLC Genomics Workbench 7.0.3 (CLC Bio, Hydarabad, India). The nucleotide sequences were aligned using CLUSTAL-W (Higgins *et al*., 1994).

For COI gene-based phylogeny, the nucleotide sequence of COI of other eel shaped fishes belonging to the families Anguillidae (*Anguilla bengalensis*), Mastacembelidae (*Mastacembelus armatus, Macrognathus pancalus, Macrognathus aral*, *Macrognathus aculeatus*) were included to establish the evolutionary relationships of *Monopterus albus* and *Monopterus cuchia* with other eel species. The evolutionary history was inferred by using Maximum Parsimony (Eck and Dayhoff, 1966**)** method using the Close-Neighbor-Interchange algorithm (Nei and Kumar, 2000). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) (Felsenstein, 1985).

RESULTS AND DISCUSSION

Morphometric variation

The data on morphological measurements of the observed specimens are listed Table 1. Out of all

Table 1. Comparison of morphological measurements between both sexes of *M. cuchia* (N=180)

Sl No.	Characters	*M. cuchia* (N= 180) (in cm)		*t*-statistics	Significance or Probability (p value)
		M (Ns=106)	F (Ns=74)		
1	Pre dorsal length (PDL)	46.83±4.26	47.12±3.58	-0.4942	0.6218
2	Post dorsal length (PoDL)	13.54±1.62	13.16±1.78	1.4618	0.1459
3	Pre anal length (PAL)	44.42±1.11	45.24±1.27	-4.4855	**< 0.0001***
4	Post anal length (PoAL)	14.24±2.24	13.86±1.32	1.4273	0.1553
5	Length of lateral line (LAL)	53.82±3.32	55.26±1.18	-4.1092	**< 0.0001***
6	Head length (HL)	4.13±0.68	4.19±0.28	-0.8149	0.4164
7	Snout length (SnL)	1.19±0.18	1.22±0.24	-0.9112	0.3639
8	Upper jaw length (UJL)	2.43±0.21	2.53±0.33	-2.3016	**0.0232***
9	Lower jaw length (LJL)	2.38±0.28	2.48±0.42	-1.7893	0.0761
10	Mouth gape (MG)	1.85±0.48	1.84±0.45	0.1427	0.8867
11	Eye diameter (ED)	0.52±0.14	0.58±0.15	-2.7134	**0.0074***
12	Head depth (HD)	2.02±0.24	2.06±0.32	-0.9112	0.3639
13	Head width (HW)	2.11±0.15	2.20±0.20	-3.2802	**0.0013***
14	Pre orbital length (POL)	1.02±0.16	1.23±0.36	-4.7041	**< 0.0001***
15	Post orbital length (PoOrL)	2.94±0.35	2.92±0.25	0.4472	0.6553
16	Greatest body depth (GBD)	2.51±0.32	2.46±0.32	1.0315	0.3039
17	Least body depth (LBD)	2.06±0.12	2.04±0.14	0.9991	0.3195
18	Greatest width of body (GWB)	1.98±0.20	2.08±0.17	-3.6087	**0.0004***
19	Highest body diameter (HBD)	7.95±0.70	8.16±0.68	-2.0141	**0.0457***
20	Width of body at vent (WBV)	1.45±0.32	1.62±1.05	-1.3497	0.1808
21	Depth of body at vent (DBV)	2.26±1.06	2.12±0.38	1.2496	0.2135
22	Distance between vent and commencement of dorsal fin (DBCB)	2.32±0.73	2.28±0.43	0.4611	0.6453

M: Male; F: Female; Ns: Sample size of *M. cuchia* male or female; N: Total samples ; **P<0.05*

morphological measurements, 8 measurements showed significant differences between males and females of *M. cuchia*. The morphometric measurements Pre anal length (PAL), Length of lateral line (LAL), Upper jaw length (UJL), Eye diameter (ED), Head width (HW), Pre orbital length (POL), Greatest width of body (GWB), Highest body diameter (HBD) showed significant differences at *P<0.05* in t-test (Table 1). On the other hand 9 morphometric measurements in *M. albus* showed significant difference in male and females at *P<0.05 in the t-test*. These are Length of lateral line (LAL), Upper jaw length (UJL), Lower jaw length (LJL), Mouth gape (MG), Eye diameter (ED), Head depth (HD), Head width (HW), greatest body depth (GBD) and Greatest width of body (GWB) (Table 1)

coi-based genetic variation

The COI genes of the present study ranged from 605 (COI of *Anguilla bengalensis*) to 655 (COI of *Macrognathus pancalus* and *Macrognathus aral*) nucleotide long with molecular weight of 185.602 kDa (in *A. bengalensis*) to 200.758 kDa (in *Macrognathus aral*) respectively. The melting temperature ranged from 83.40 (COI of *A. bengalensis*) to 84.49 (COI of *M. albus*) at 0.1M salt concentration (Table 2). The frequency of AT in COI mRNA (cDNA) sequence in different fishes of the present study ranged between 0.522 (in COI of *M. albus*) to 0.615 (in COI of *Monopterus cuchia*). On the other hand frequency of GC ranged from 0.385 (in COI gene of *Monopterus cuchia*) to 0.478 (in COI gene of *Monopterus albus*) . The COI gene sequences were found to be A:T rich (Table 2). The transition/ transversion frequency for the nucleotides of the COI gene are- A=>T = 0.05, A=>C = 0.04, A=>G= 0.1, T=>A = 0.04, T=>C= 0.19, T=>G= 0.2, C=>A= 0.04, C=>T= 0.2, C=>G=0.02, G=>A= 0.19, G=>T=0.05, G=>C=0.04.

Table 2. Statistics of COI cDNA sequence

Statistical parameter	*M. cuchia*	*M. albus*
Sequence source/ GenBank Accession numbers	KR705867 (This study)	KR705873 (This study)
Length (bp)	652	655
MW in single stranded condition (kDa)	200.473	200.571
Melting temperature (^{0}C) [salt] = 0.1M	80.68	84.49
Frequency of A + T	0.615	0.522
Frequency of G + C	0.385	0.478

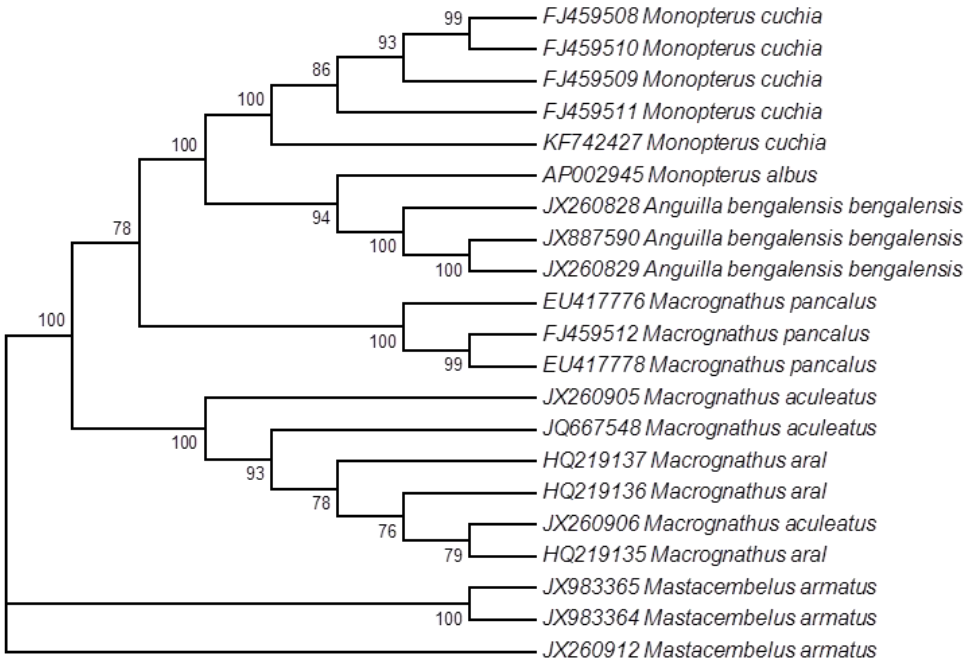

Figure 3. COI gene-based phylogenetic profile of *M. cuchia* and *M. albus* among seven eel species. A. Maximum Parsimony tree. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985).

The COI gene MP tree formed two distinct clades and revealed that *M. cuchia* is a sister taxa of *A. bengalenis* plus *M. albus* with 100% bootstrap support.*Macrognatus pancalus* in their successive sister taxa for ming a distinct clade. The second clade is formed by *Magrognathus aculeatus* and *Macrognathus aral* (boot strap support 100%) plus *Mastacembalus aramatus* as their successive sister taxa (100% bootstrap support) (Figure 3).

The data on morphological measurements (Table 1) revealed that in *M. cuchia*, females are larger than males. On the other hand in *M. albus*, males are larger than females. The process of sex reversal in synbranchid fish like *M. albus* has been studied by various researchers with a series of studies (Tao *et al*., 1993). The morphometric measurements having significant differences between males and females (at *P<0.05)* could be used as key for identification of *M. cuchia* and *M. albus.*

Both in *M. cuchia* and *M. albus* there were five (5) common morphometric measurements namely Length of lateral line (LAL), Upper jaw length (UJL), Eye diameter (ED), Head width (HW), Greatest width of body (GWB), which showed significant differences in their males and females at *P<0.05* in t-test (Table 1).

However, out of the morphometric measurements having significant differences between males and females (at *P<0.05)*, there were seven (7) measurements which are not common in *M. cuchia* and *M. albus.* These measurements namely Pre anal length (PAL), Pre orbital length (POL), Highest body diameter (HBD), Greatest body depth (GBD), Lower jaw length (LJL), Mouth gape (MG), Head depth (HD) revealed that *M. cuchia* and *M. albus* are two distinct species (Table 1).

Moderate levels of genetic diversity and differentiation were observed in our SSR studies (in communication). *M. albus* and *M. cuchia* were placed in separate genetic clusters, but admixture was observed. Population structure analysis placed *M. albus* and *M. cuchia* in two distinct clusters.Efficient identification of the two Synbranchid eel species of the present study is critical for aquaculture management as well as for eel conservation (Dudu *et al*., 2010). Thus, identification of *M. cuchia* and *M. albus* has been supported by molecular characterization in the present study instead of conventional methods (Huang, *et al*., 2010). The present study has revealed an interesting point of difference for identification of the two Synbranchid species that the cDNA sequence COI-gene of *M. cuchia* is more A: T rich than that of *M. albus* (Table 2).

CONCLUSION

The present study expands information in understanding morphological variation between *M. cuchia* and *M. albus* which in turn clarifies the taxonomic uncertainties (Dahanukar, 2010). Between the two species of *Monopterus* of the present study, there are minor morphological differences. Therefore, to clarify taxonomic confusion between *M. albus* and *M. cuchia,* development of DNA-based marker is very essential. The study based on mitochondrial COI gene clearly revealed that *M. cuchia* and *M. albus* are two distinct species. However, both the species *M. cuchia* and *M. albus* might have two sub-species within each species. Further, phylogeographic study based on sampling in large geographic area along their distribution ranges will help to establish such sub-speciation. The present study will certainly be helpful in understanding genetic variation between *M. cuchia* and *M. albus.*

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