Northeast Journal of Contemporary Research, December 2014. Vol. 1 No. 2, pp 30-42
ISSN 2349-3089 ©NeJCR, 2014

Morphological and mitochondrial D-loop region based genetic characterization of two Synbranchid eels of genus *Monopterus*

Papari Devi* **and D.K. Sharma**
Department of Zoology, Gauhati University, Guwahati – 781014, Assam, India

ABSTRACT

Two synbranchid species of genus *Monopterus* found in the Northeast India, *Monopterus cuchia* (Hamilton) and *Monopterus albus* (Zuiew) are analyzed in the present study for both morphological and genetic characterization. Morphological observation followed by sequencing and molecular characterization has been performed on the mitochondrial D-loop region to identify the molecular differences of *M. cuchia* and *M. albus*. The study has revealed several interesting morphological and genetic differences between *M. albus* from *M. cuchia*, which clarifies their genetic distinctness rather than species complex as suggested by earlier studies. There are minor morphological but well defined genetic diversity between *M. cuchia* and *Monopterus albus*.

Key words: D-loop, eels, *Monopterus*, morphology, phylogeny

INTRODUCTION

Freshwater habitats provide the occurrence of various species of freshwater eel – a fish bearing elongated snake-like structure. The freshwater air-breathing mud eel- *Monopterus cuchia* (Hamilton, 1822) and swamp eel- *Monopterus albus* (Zuiew, 1793), are tentatively identified as belonging to the synbranchid genus *Monopterus* (Collins *et al*., 2002; Li *et al.,* 2007). They are regarded as species complex and require taxonomic revision (Dahanukar, 2010). Both *M. cuchia* and *M. albus* are economically important freshwater fishes, recorded from India, Bangladesh, Nepal, Pakistan and Myanmar (Menon, 1999; Mirza and Alam, 2002; Zhou *et al*., 2002).

Comparative examination of morphological characters is one of the traditional methods of distinguishing fish taxa and stocks (Hubbs and Lagler, 1947). The modern genetic analysis is helping the traditional morphometric observation for proper identification of organisms or species. The morphological analysis is essential in genetic identification of species to see the concordance of genetic data with

**Corresponding author's Email : papari.devi2013@gmail.com*

morphological data to draw a proper conclusion on speciation. Although, the freshwater air-breathing mud eel- *Monopterus cuchia* (Ham.) and swamp eel- *Monopterus albus* (Zuiew), are regarded as species complex (Dahanukar, 2010), this morphological and genetic aspects of these two species have not been so far reported together.

Systematic position of *Monopterus*:

Phylum-Chordata
Class-Actinopterygii (ray finned fish)
Order-Synbranchiformes
Sub order-Synbranchoidei
Family-Synbranchidae
Genus-*Monopterus*
Species- *M. cuchia, M. albus*

The development of DNA-based genetic markers has a revolutionary impact on animal genetics. It is theoretically possible to observe and exploit genetic variation in the entire genome of organisms with DNA markers. In recent years, mtDNA, because of its fast evolution i.e. 5 to 10 times faster than single copy nuclear genes (Avise, 2000), has been widely applied in systematics, population genetics and conservation biology of animals.

Although some 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. According to IUCN (2014), Taxonomic investigation is needed to clarify confusion between *M. cuchia* and *M. albus* within India, which could impact upon the species. Therefore, in the present investigation, an attempt has been made to study the both morphological and genetic variation between *Monopterus cuchia* and *Monopterus albus* of Northeast India based on morphological observation and mitochondrial DNA (D-loop region) based genetic analysis. The study represents the first hand information generation that includes field data collection, sample collection and analyses including DNA isolation, PCR, sequencing of D-loop region followed by study of sequence-based molecular genetic variation between *M. cuchia* and *M. albus*.

MATERIALS AND METHODS

Sample collection

Field work was carried out during July, 2011 to June, 2014 in different parts of Assam and Manipur in Northeast India (GPS location 26°10′22.79″- 27°39′32.79″N Latitude and 91°26′39.74″- 96°15′39.84″E Longitude) (**Table 1** and **Figure 1**). A total of 230 *Monopterus* individuals were sampled from their habitat for morphological observations. In the laboratory, each sample was measured for its body weight and standard body length. Length was measured to the nearest mm and weight to 0.1 g. Phenotypic variations were recorded and used for the analysis of molecular data. Most reliably measurable morphometric characters were considered for the study.

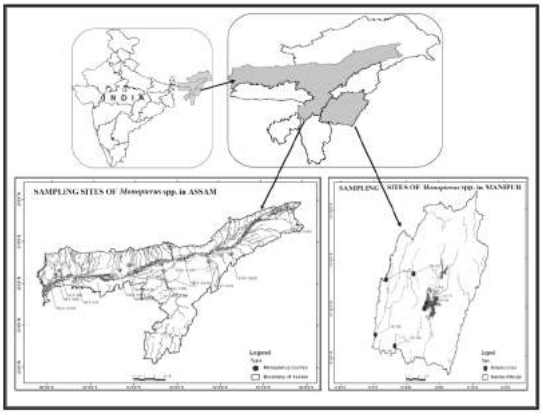


Figure 1. Map of study area showing the sampling sites.

Table 1. GPS coordinates of the sampling sites of *Monopterus cuchia* and *Monopterus albus*

Sampling area	Sampling Site(s)	Coordinates
Manipur	Loktak Lake	24.3°N, 93.5°E
	Ijei River, Longmai, Tamenglong	24.5°N, 93.4°E
	Tuivel River, Manipur	24.08°N , 93.2°E
	Wetlands, Hallawgaon, Sadiya, Tinsukia	27.50°N, 95.45°E
	Rice field, Mariani, Jorhat	27.52°N, 95.37°E
	Bhogdoi River, Jorhat	26.77°N, 94.22°E
	Kakodonga River, Golaghat	26.43°N, 94.3 °E
	Dhansiri River, Golaghat	26.35°N, 93.35°E
	Kolong River, Nagaon	26.36°N, 92.69°E
	Jia Bhorali River, Sonitpur	26.69°N, 92.87°E
Assam	Bishwanath Ghat, Sonitpur	26.66°N, 93.17°E
	Chandubi beel, Kamrup	25.51°N; 91.21° E
	Kulshi river, Kamrup	26.03°N; 91.26° E
	Rice field, Nalbari	26.14°N, 91.08°E
	Puthimari river, Kamrup	26.22°N, 91.4°E
	Rice field, Hajo, Kamrup	26.14°N, 91.32°E
	Wetland, Manikpur, Bangaigaon	26.45°N, 90.80°E
	Rice field, Bilaishipara, Dhubri	26.11°N, 90.16°E
	Rice field, Dhubri	26.01°N, 89.6°E
	Urpad Beel, Goalpara	26.06°N, 90.35°E
	Rice field, Dudhnoi, Goalpara	25.98°N, 90.79°E

DNA extraction, PCR amplification and sequencing of D-loop region:

Selected samples (6 each from *M. cuchia* and *M. albus*) were used simultaneously for genomic DNA extraction, sequencing of targeted regions of mitochondrial genome (D-Loop). DNA was isolated from the tissue using the Chloroforme-Octanol method (Salah and Iciar, 1997; Cabe *et al*., 2007). The informative regions of D-loop region were PCR amplified using primer pairs F-TTCCAATGGAGG GATGGTGC and R- AACCACCGAAAAG-CGAAAGC.

All PCR amplifications were carried out in 25 µL reaction volume, with 1.5 units of Taq DNA Polymerase (Bangalore Genei, Bangalore, India), 0.25 mM of dNTPs (Bangalore Genei), 2.0 mM of $MgCl_2$, 0.1 M (Sigma) of each primer and 20 ng of genomic DNA.The condition for amplification was an initial denaturation temperature 94 °C for 5 min, followed by 35 cycles of 94 °C denaturing

temperature for 50 sec , then by 45 sec at appropriate annealing temperature followed by extension temperature of 72°C for 90 sec and then by a final extension at 72°C for 10 min. The amplified PCR products were separated in 2% agarose gel by electrophoresis at 100 V. The results of electrophoresis was observed and recorded in the UVIdoc gel documentation system. Using the QIA quick PCR Purification kit (Qiagen), the amplified PCR products were purified. Sequencing of D-loop region was carried out in ABI PRISM® 377 DNA sequencer (at BioAxis DNA Research Centre, Hyderabad).

Sequence analysis

The sequences were simultaneously aligned using aligned using ClustalW 1.6 (Thompson *et al*., 1994) integrated in software MEGA6 (Tamura *et al*., 2013). The nucleotide sequence analyses were performed in the CLC Genomics Workbench 7.0.3 (CLC Bio, Hyderabad).

Molecular Phylogenetic analysis

Additional sequence of *Danio rerio* was retrieved from the GenBank (NCBI) (Boeckmann *et al*., 2003) to include as out-group in the phylogenetic analysis. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013). The evolutionary history was inferred by using the Maximum Parsimony (Eck and Dayhoff, 1966**)** and Maximum Likelihood methods (Jones *et al.,* 1992).

RESULTS

A total of 230 *Monopterus* individuals (150 *M. cuchia* and 80 *M. albus*) ranging in size from 18.0 to 86.5 cm in total length (TL) and weighing 9.2 to 840 g were collected and studied morphologically (Figure 2).

A. B.

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

Morphological observation:

The major morphological similarities and differences of *M. cuchia* and *M. albus* have been listed in Table 2.

1. *M. cuchia* bears smooth, tiny cycloid scales embedded in the skin but *M. albus* is scale less.
2. *M. cuchia* is pale red in ventral side but *M. albus has* white, orange, light brown ventral side.
3. In *M. cuchia* mature females are larger than males but in *M. albus* males are larger than female.
4. Fin formula of *M. cuchia* is: $D._{\text{very rudimentary}}$; P_1; V_2; A. and C. Absent .
5. Fin formula of *M. albus* is: $D._{\text{vestigial}}$; P_0; V_0; $A_{\text{vestigial}}$. and C_0

Table 2. Morphological Characters of two *Monopterus* species

Character	*M. albus*	*M. cuchia*
Caudal fin	Absent	Absent
Gill slit	gill opening is confined to a single slit, which is ventral; narrow	gill opening is confined to a single slit, which is ventral; wide
Posterior nares	Between eyes	Between eyes
Soft tissue around upper jaw	Jaw-like flap over upper jaw	Jaw-like flap over upper jaw
Branchiostegal membrane	Attached to isthmus	Attached to isthmus
Branchiostegal rays	six	six
Holobranchs	Reduced or modified on first 3 arches. Absent from 4th arch	Reduced or modified on first 3 arches. Absent from 4th arch
Suprapharyngeal pouches	Present but incomplete	present
Scale	none	present on tail

The total length is highest (69.68±1.92) in *M. cuchia* females of population-2 and minimum in *Monopterus cuchia* males (42.93± 1.55) of the population-2. The total length of *M. cuchia* males ranged between 41 cm and 63cm. The total length of *M. cuchia* females ranged between 58-74 cm. However, the total length of *M. albus* males ranged between 51-67 cm and the females ranged between 40-52cm (Table 3). This data on total lengths on both sexes indicates that females are larger than males in *M. cuchia.* On the other hand males are larger than females in *M. albus.*

Table 3. Data on total length and weight between both sexes of *M. cuchia* and *M. albus* specimens from Assam and Manipur.

Species identified	Popula-tion code	N	Sex	Ns	Mean TL ± SD (cm)	*t*-statistics of TL	p value of TL	Mean weight ± SD (g)	*t*-statistics of weight	p value of weight
M. albus	1	50	M	23	62.91 ± 5.2 (51-67)	15.8744	< 0.0001	215.43 ±30.5 (140-250)	8.9778	< 0.0001
			F	27	43.22 ± 3.13 (40-52)			142.62±26.15 (75-195)		
M. cuchia	2	45	M	29	42.93± 1.55 (41-45)	-47.7949	< 0.0001	184.65±57.27 (110-325)	-9.7745	< 0.0001
			F	16	69.68±1.92 (67-73)			567.81±150.92 (370-750)		
	3	40	M	21	56.33± 4.47 (51-63)	-12.2342	< 0.0001	237.61±48.17 (200-450)	-9.456	< 0.0001
			F	19	69.53±2.01 (66-74)			470.47±97.07 (400-850)		
	4	95	M	56	55.37±2.97 (52-63)	-12.9367	< 0.0001	152.76±28.95 (125-350)	-15.1797	< 0.0001
			F	39	64.89±3.87 (58-69)			252.82±33.33 (210-450)		

M: Male; F: Female; SD = standard deviation; TL: Total Length; Ns: Sample size collected from a site. N: Total samples from each population; **P<0.05*

Identification key of *Monopterus cuchia:*

The present study has confirmed the identified 150 *Monopterus cuchia* individuals based on following keys- (i) Skin of branchial region of ventral side of head drawn into deep longitudinal folds; in gill arch skeleton, epibran-

chial two short and wide-based, broadly triangular, epibranchial three a stout rod as robust as that of fourth arch, (ii) Teeth on palate and laterally on jaws uniserial; with 99 to 112 abdominal and 55 to 70 caudal vertebrae. A rudimentary dorsal fin originates a little anterior to vertical from anus. Dorsal fin is continuous with caudal fin.

Identification key of *Monopterus albus:* The present study has confirmed the identified 80 nos. of *Monopterus albus* individuals based on following keys- (i) Body robust, not whiplike; ventral gill opening triangular; 88 to 102 abdominal and 45 to 74 caudal vertebrae; epigean. (ii) Without pelvic or pectoral fins; all other fins greatly reduced or not evident. Body eel-like, to 70 cm (24 in) long, tapering at hind end to a point. Anguilliform body; no scales; no pectoral and pelvic fins; dorsal, caudal and anal fins confluent and reduced to a skin fold; gill openings merged into single slit underneath the head. Rice paddy eels are red to brown with a sprinkling of dark flecks across their backs; large mouths and small eyes.

PCR amplification and sequencing:

The DNA samples of purified PCR product gave ~ 600 bp D-loop regions, amplified in separate reaction set for each gene by using the specific primer pairs. The representative gel images for the PCR amplification profile for mitochondrial and nuclear genes are shown in Figure 3.

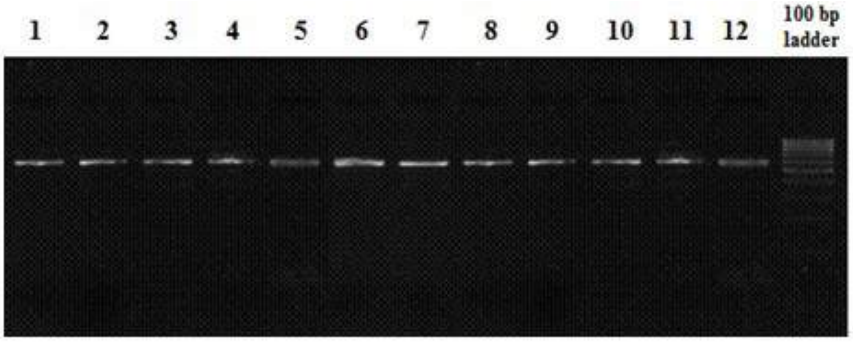


Figure 3. PCR amplification profile of D-loop region (~600 bp); Lane 1-6: *M. cuchia*, Lane 7-12: *M. albus*, 13. 100 base pair ladder (1kb).

Comparative sequence analysis D-loop region:

The D-Loop region sequence of the present study ranged from 610(in both the *Monopterus* species) to 950 (in *Danio rerio*) nucleotide long and with molecular weights of 195.84 kDa (in *M. albus*), 195.95 kDa (in *M. cuchia*) and 304.272 kDa (in *Danio rerio*) respectively. The melting temperature was found to be 78.06 (in *Danio rerio*), 81.70 (in *M. cuchia*) and 82.04 (in *M. albus*) at 0.1M salt concentration (Table 4). The frequency of AT in D-loop region (cDNA) sequence ranged from 0.582 (in *M. albus*) to 0.679 (in *Danio rerio*). On the other hand frequency of GC ranged from 0.321 (in *Danio rerio*) to 0.418 (in *M. albus*) (Table 4). The D-loop region was found to A:T rich (Table 4; Figure 4). The transition/ transversion frequency for the nucleotides of the D-loop region are- A=>T = 0.06, A=>C = 0.04, A=>G= 0.11, T=>A = 0.06, T=>C= 0.11, T=>G= 0.04, C=>A= 0.06, C=>T= 0.17, C=>G=0.04, G=>A= 0.17, G=>T=0.06, G=>C=0.04. Multiple sequence alignment of D-loop region in *M. cuchia* and *M. albus* has been presented in Figure 5.

Table 3. Comparative Nucleotide sequence statistics of the D-loop region cDNA sequence *M. cuchia M. albus* and *Danio rerio*.

Statistical parameter	***M. cuchia***	***M. albus***	***Danio rerio***
Sequence source/GenBank Accession numbers	This study	This study	AC024175
Length (bp)	610bp	610bp	950bp
MW in single stranded condition (kDa)	195.95 kDa	195.84 kDa	304.272 kDa
Melting temperature (^{0}C) [salt] = 0.1M	81.70	82.04	78.06
Frequency of A + T	0.590	0.582	0.679
Frequency of C + G	0.410	0.418	0.321

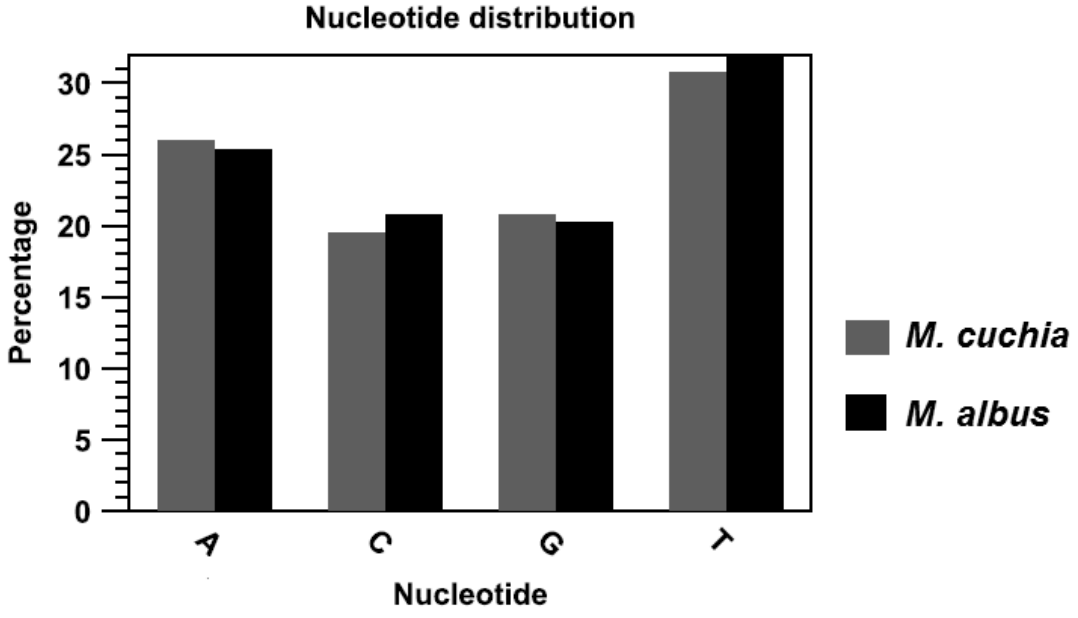


Figure 4. Comparative nucleotide composition (% in average) in the D-loop region cDNA sequence of *M. cuchia* and *M. albus*.

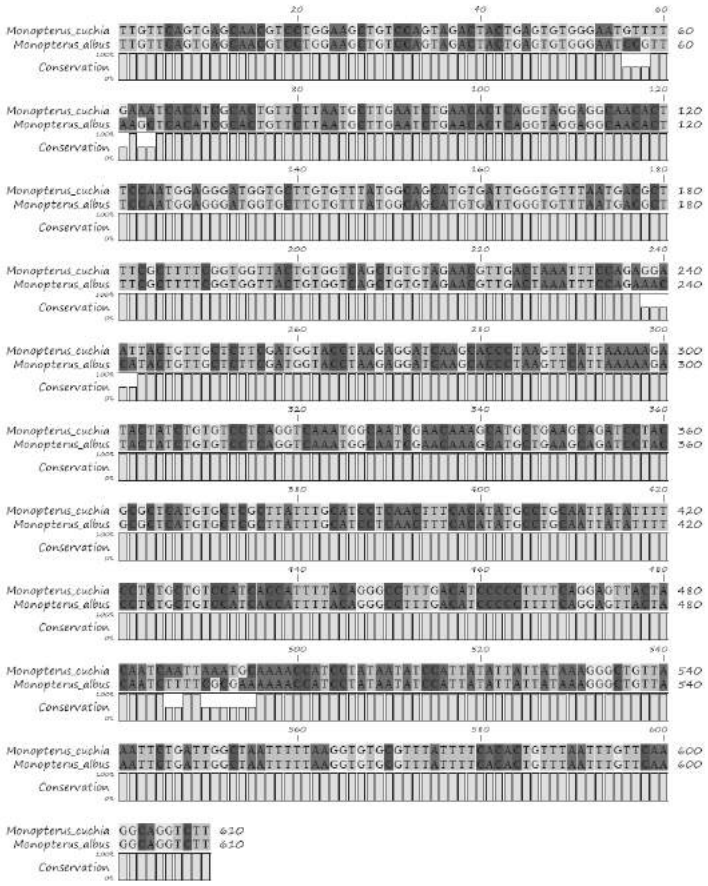


Figure 5. Multiple sequence alignment of D-loop region between *M. cuchia* and *M. albus.* The height of the bar diagram represent the level of conservation at each alignment position of respective amino acid(s).

Phylogeny of *Monopterus* based on D-loop sequence

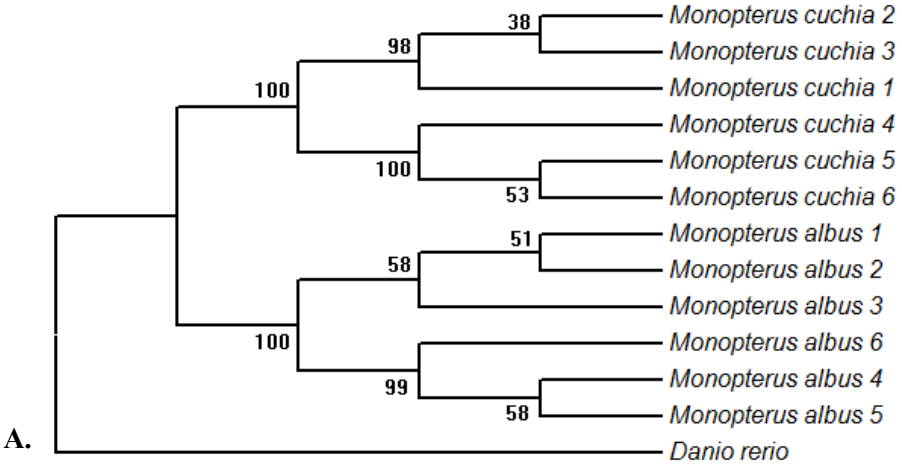
The Maximum-likelihood model T92 has been used based on Modeltest (Posada and Crandall, 1998). Pair-wise distances (P-distance) of D-loop region have been depicted in the Table 5. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985).

The Pair-wise distance of D-loop region sequences among the different eel species of the present study revealed genetic distance exist (0.013) within the *Monopterus cuchia* samples collected from lower Assam and upper Assam populations (Table 5). Between *Monopterus cuchia* and *Monopterus albus* samples, highest genetic distance (0.062) exists between *M. cuchia 4,5,6* (upper Assam population) and *M. albus*-10,11,12 (Manipur) populations. The longest genetic distance (0.810) exists between the outgroup sequence *Danio rerio* and *M. cuchia 4,5,6* (middle-upper Assam population) (Table 5).

Table 4. Estimates of Evolutionary Divergence between Sequences of D-loop region

		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	*Monopterus cuchia 1*	-												
2	*Monopterus cuchia 2*	0.000												
3	*Monopterus cuchia 3*	0.000	0.000											
4	*Monopterus cuchia 4*	0.013	0.013	0.013										
5	*Monopterus cuchia 5*	0.013	0.013	0.013	0.000									
6	*Monopterus cuchia 6*	0.013	0.013	0.013	0.000	0.000								
7	*Monopterus albus 1*	0.032	0.032	0.032	0.046	0.046	0.046							
8	*Monopterus albus 2*	0.032	0.032	0.032	0.046	0.046	0.046	0.000						
9	*Monopterus albus 3*	0.032	0.032	0.032	0.046	0.046	0.046	0.000	0.000					
10	*Monopterus albus 4*	0.048	0.048	0.048	**0.062**	**0.062**	**0.062**	0.015	0.015	0.015				
11	*Monopterus albus 5*	0.048	0.048	0.048	**0.062**	**0.062**	**0.062**	0.015	0.015	0.015	0.000			
12	*Monopterus albus 6*	0.048	0.048	0.048	**0.062**	**0.062**	**0.062**	0.015	0.015	0.015	0.000	0.000		
13	*Danio rerio*	0.789	0.789	0.789	**0.810**	**0.810**	**0.810**	0.790	0.790	0.790	0.804	0.804	0.804	-

The evolutionary history of *Monopterus cuchia* and *Monopterus albus* based on D-loop region was inferred using the Maximum Parsimony method. Tree #1 out of 116 most parsimonious trees (length = 321) is shown (**Figure 6**). The consistency index is (0.980392), the retention index is (0.992308), and the composite index is 0.989216 (0.972851) for all sites and parsimony-informative sites (in parentheses). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The MP tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 13 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 608 positions in the final dataset.



A.

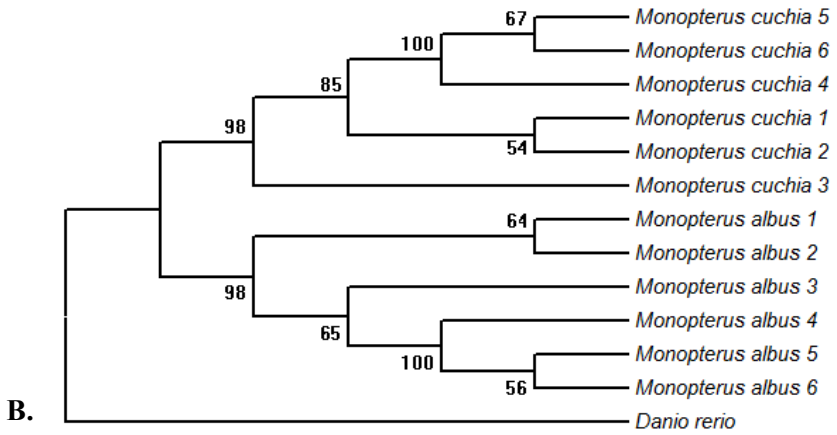


Figure 6. Molecular phylogenetic anaylsis of *Monnopterus* based on D-loop sequence. A. Maximum Parsimony analysis; B. Maximum Likelihood method based on the Tamura 3-parameter model (Tamura, 1992). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein,1985).

The D-loop region based MP phylogenetic tree (Figure 6A) reveled that *M. cuchia* and *M. albus* are two separate sister groups (bootstrap support 100%). The ML tree (Figure 6B) revealed that M. cuchia and M. albus are to phylogenetically distinct species (bootstrap separation 98%) having common ancestor. Both in *M. cuchia* and *M. albus* taxon in the tree showed sub-clades within the genus, indicating the possibility of existence of sub-species in each of the species (**Figure 6**). Within the *Monopterus cuchia* populations, the samples of lower Assam (*M. cuchia 1-3*) formed a distinct clade (bootstrap support 99%) from upper Assam population (*M. cuchia 5-6*) with bootstrap separation 100%, which indicates the possibility of existence of two subspecies in *Monopterus cuchia*. Within the genus *M. albus*, differences exists in evolutionary distance and two distinct sub-clades are observed in bootstrap value 100% (**Figure 6A**).

DISCUSSION

Morphometric study as well as its variation plays an important role in physiological, evolutionary and ecological implication. *Monopterus albus* as well as *Monopterus cuchia* are almost certainly a species complex, and taxonomic identity of the eastern Himalaya needs to be validated (IUCN, 2014). The study has generated necessary information for the support of recognition of two *Monopterus* species based on morphology and mitochondrial genetic variation, which will provide the information about the systematics and natural history of this genus.

Monopterus is characterized by specializations of the dorsal gill arch skeleton; upper lip jowl-like, without a separate or swollen fold; gills, if present, reduced to single rows of filaments on the first three arches; gill membrane attached internally to the isthmus; and other modifications of the branchial circulatory system and skeleton (Rosen and Greenwood, 1976).

The process of sex reversal in synbranchids, on the other hand, has been

subject of a series of studies (Tao *et al*., 1993; Ravaglia *et al*., 1997). *Synbranchus marmoratus*, particularly, is known as a proto-gynous diandric fish (Lo Nostro and Guerrero, 1996), with two different kinds of males. Primary males develop directly as males while secondary males arise from the sex reversal of females (Sadovy and Shapiro, 1987; Lo Nostro and Guerrero, 1996).

The mitochondrial DNA control region, which includes the D-loop in vertebrates, is usually the fastest evolving region in the mitochondrial DNA of vertebrates and invertebrates and therefore more sensitive than protein loci as a marker to score intraspecific variations of many organisms (Avise, 2000). Direct sequencing of mtDNA D-loop (745 bp) and mtATPase6/8 (857 bp) regions was used to investigate genetic variation within common carp (*Cyprinus carpio*) and develop a global genealogy of common carp strains (Thai *et al*., 2004).

In the present study, although, the morphological data did not very strongly supported distinctness between *M. albus* and *M. cuchia*, the molecular investigation, based on mitochondrial (D-loop region) clearly revealed that *M. cuchia* and *M. albus* are two distinct species. D-loop and COI gene based phyologentic tree also revealed that both the specis *M. cuchia* and *M. albus* might have two sub-specis within each species. 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*., 2001).

The high bootstrap value in the D-loop region based phylogenetic tree also supports that *M. cuchia* and *M. albus* are two separate species (bootstrap support 100%) (Figure 6). Within the *Monopterus cuchia* populations, lower Assam (*M. cuchia 1-3*) and upper Assam population (*M. cuchia 5-6*) showed evolutionary distinctness by forming two distinct sub-clade (bootstrap separation 100%. This clarly supports the finding of COI gene based phylogeny that there is higher possibility of existence of two subspecies in *Monopterus cuchia* (Figure 6). The *M. albus* also shows evolutionary distance by forming two distinct sub-clades and the bootstrap value 100% indicates the possibility of existence of two subspecies within this species (Figure 6).

The study revealed minor morphological but well defined genetic distinctness between *Monopterus cuchia* and *Monopterus albus*. The sequencing and molecular characterization has been performed on the mitochondrial D-loop region and COI genes to identify the molecular differences of *Monopterus cuchia* (Hamilton) and *Monopterus albus* (Zuiew). The study has revealed several interesting morphological and genetic differences between *M. albus* from *M. cuchia*, which clarifies their genetic distinctness rather than species complex as suggested by earlier studies.

The present study will certainly be helpful in understanding genetic variation between *M. cuchia* and *M. albus* and will clarify taxonomic uncertainties mentioned by earlier workers. (Dahanukar, 2010). Further, D-loop region based phylogenetic study showed genetic distinctness of *M. cuchia* and *M. albus* rather than a species complex.

CONCLUSION

he present study based on morphological observation and mitochondrial D-loop

region revealed that *M. cuchia* and *M. albus* are two distinct species. The D-loop region based phyologentic tree revealed that both *M. cuchia* and *M.albus* might have two sub-specis 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* and will clarify taxonomic uncertainties mentioned by earlier workers. The study study is useful in modern molecular biology and fish biotechnology in sequence analysis, characterization two synbranchid fishes of genus *Monopterus* found in Northeast India.

ACKNOWLEDGEMENT

The authors are thankful to DBT-Govt. of India for the Bioinformatics Infrastructure Facility (BIF) at Gauhati University, which has been utilized in the present study.

REFERENCES

Avise, J.C. 2000. Phylogeography: the history and formation of species. Harvard University Press, USA.

Boeckmann B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, Martin MJ, Michoud K, O'Donovan C, Phan I, Pilbout S, Schneider M. 2003. The SWISS-PROT protein knowledgebase and its supplement TrEMBL in 2003. Nuc. Ac. Res. 31:365-370.

Cabe PR, Page RB, Hanlon TJ, Aldrich ME, Connors L, Marsh DM. 2007. Fine-scale population differentiation and gene flow in a terrestrial salamander (Plethodon cinereus) living in continuous habitat. *Heredity* 98:53–60.

Collins, T.M., Trexler, J., Nico, L., Rawlings, T.A. 2002. Genetic diversity in a morphologically conservative invasive taxon: multiple introductions of swamp eels to the southeastern United States. Conserv. Biol., 16: 1024-1035.

Dahanukar, N. 2010. *Monopterus cuchia*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2011.2. www.iucnredlist.org.

Dudu A., Georgescu S. E.,& Costache M. 2010. PCR-RFLP method to identify fish species of Importance. *Archiva Zootechnica*, 13(1):53-59.

Eck, R. V., Dayhoff, M. O. 1966. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, Maryland, USA.

Felsenstein J. 1985. Confidence limits on phytogenies: An approach using the bootstrap. Evol. 39: 783-791.

Hamilton, F.1822. An account of the fishes found in the river Ganges and its branches. Edinburgh & London. I-VII + 1-405, Pls. 1-39.

Huang J. P., Han Y. S., Tzeng W. N. 2001. Species identification of Anguillid eels by polemerase chain reaction/restriction fragment length polymorphism analysis of the gonadotrophin II-β subunit gene. Acta Zoologica Taiwanica, 12(2): 41-49.

Hubbs, C.L. and Lagler, K.F. 1947. Fishes of the Great Lakes region. Cranbrook Institute of Science, Bull. 26: 186 p.

IUCN, 2014. IUCN Red List of Threatened Species (ver. 2014.2). Available at: www.iucnredlist.org.

Jones DT, Taylor WR, Thornton JM. 1992. The rapid generation of mutation data matrices from protein sequences. Comput. Applic. Biosci. 8: 275-282.

L i W.T., Liao X.L.., Yu X.M.., Cheng L. and Tong J. 2007. Isolation and characterization of polymorphic microsatellites in a

sex-reversal fish, rice field eel (*Monopterus albus*) Molecular Ecology Notes 7: 705–707.

Liu L, Wang W, Zheng B, Luo Y. 2005. RAPD analysis of *Monopterus albus* (Zuiew) populations with three colors. *Fisheries Science*, 24, 22–25.

Lo Nostro, F. & G. Guerrero. 1996. Presence of primary and secondary males in a population of *Synbranchus marmoratus*, Bloch 1795, a protogynous fish (Teleostei -Synbranchiformes). Journal of Fish Biology, 49:788-800.

Menon, A.G.K. 1999. *Check list - fresh water fishes of India.* Records of the Zoological Survey of India, Occasional Paper No. 175.

Mirza, M.R., Alam, M.K. 2002. A checklist of the fishes of the Punjab, Pakistan. *Records of the Zoological Survey of Pakistan* 14, 31-35.

Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. Oxford University Press, Oxford.

Posada D, Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics. 14: 817–818.

Ravaglia, M. A., F. Lo Nostro, M. C. Maggese, G. A. Guerrero & G. M. Somoza. 1997. Characterization of molecular variants of GnRH, induction of spermiation and sex reversal using salmon GnRH-A and domperidone in the protogynous diandric fish, *Synbranchus marmoratus* Bloch (Teleostei, Synbranchidae). Fish Physiology and Biochemistry, 16:425-436.

Rosen, D. E., and Greenwood P.H.. 1976. A ibul-th Neotropical species of synbl-anchid eel ancl the phylogeny and systematics of synbranchiform fishes. Bull. American Mus.Nat. I-list. 157(1):1 -70.

Sadovy, I. & D. Shapiro. 1987. Criteria for the diagnosis of hermaphroditism in fishes. Copeia, 1987(1): 136-156.

Salah M, Iciar M. 1997. Universal and rapid salt -extraction of high genomic DNA for PCR-based techniques. *Nucleic Acids Research*,25, 4692–4693.

Tamura K., Stecher G., Peterson D., Filipski A., and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*30: 2725-2729.

Tao, Y., H. Lin, G. Van Der Kraak & R. Peter. 1993. Hormonal induction of precocious sex reversal in the rice field eel, *Monopterus albus*. Aquaculture, 118: 131-140.

Thai, B.T. Burridge, C.P., Pham, T.A, Austin, C.M. 2004. Using mitochondrial nucleotide sequences to investigate diversity and genealogical relationships within common carp (Cyprinus carpio L.). Anim Genet 36:23–28 SC. 2001. Polymorphisms of GA/GT Microsatellite Loci from Anguilla japonica. Mar. Biotechnol. 3, 275–280.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nuc. Ac. Res. 22: 4673–4680.

Yang F, Zhou Q, Zhang Y, Li X. 2005. Variation of esterase isoenzyme among three kinds of bodily color *Monopterus albus* in Poyang Lake Region. *Journal of Economic Animal*, 9 (2),110–113.

Zhou, R., Cheng, H., Zhang, Q., Guo, Y., Richard, R.C., Terrence, R.T. 2002. SRY-related genes in the genome of the rice field eel (Monopterus albus). Genet Sel Evol. 34,129–137.

Zuiew, B.1793. Biga muraenarum, novae species descriptae.Nova Acta Acad. Sci. Petropolitanae,vol. 7, pp. 296-301.