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Research Article

Genetic Diversity and Phylogenetic Relationship of Catfish Order Siluriformes Inferred from Mitochondrial Gene Sequence Variation

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Abstract

Catfishes order siluriformes includes many commercially and economically important species throughout the world. Here, we investigated genetic relationships and diversity in this order. Sequence comparisons and phylogenetic analyses revealed considerable variations between mitochondrial CO1 genes among twenty four siluriformes species. The frequencies of nucleotide are 23.80% A, 29.62% T/U, 26.63% C and 19.95% G. Used for calculating ML values, a user-specified topology was used. The highest Log chance for this calculation was -9413.645. Evolutionary diversity over all sequencing average estimated numbers of base substitutions per location sequence pairs were 4.984. Our results suggest that *Batasio travancoria* formed a single clade; *R. rita, W. attu, M. montanus* and *B. bagarius* comprised a single separate family; and *M. vittatus, M. horai, B. tengana, M. malabaricus, M. bacourti, M. singaringan, M. bleekeri, M. gulio, M. multiradiatus, M. rhegma, M. cavasius, M. tengara, S. aor, S. seenghala, B. bajad, B. filamentosus, B. macracanthus, P. siamensis, E. vacha* and *B. travancoria* formed single subfamily.

Keywords: COI; Siluriformes; Genetic Diversity; Phylogeny

Introduction

Order Siluriformes belongs to large group of catfishes [1] which are dispersed in every part of world and have valid species about 3,088 spread along with 477 genera and 36 families [2-4]. Catfish are easy to culture in temperate climates, leading to economical and safe food at local grocers. Cultures of catfishes in domestic tanks or channels are considered as protected for the atmosphere. In Asia, many catfish species are significantly very important as food. Most of commercially important catfish species are cultured in Asia. The beneficial industry developed in the southern USA inside the unique variety of the species. Most catfishes have been studied by researchers; the majority of complete mitochondrial genome sequencing efforts has paying attention on aquaculture species of catfish [5-7]. Usually, phylogenetic interaction between the 33 to 37 existing families and 438 genera, 2753 species of siluriformes stay behind unsure [8]. All Catfish Species Inventory (ACSI) is a collective endeavor to resolve, name and categorize all siluriformes species. Adding together to its innermost every one of species target, ACSI is advancing the finding of the phylogenetic associations amongst family-level and superior catfish groups that are necessary for carrying out the nomenclature and a predictive classification of siluriformes. Due to their wide-reaching, generally freshwater allocation and diversity, catfishes are of big concentration to evolutionary biologists and ecologists. Siluriformes are main subjects in biogeography on all scales from local to international [9]. Such type of phylogenetic approaches in widespread use sequences from either a single gene or the complete mitochondrial genome. The majority of accepted sequences in phylogenetic studies are the..... (Cytb) and .....(COI) genes, which are utilized for a species and family level assessment [10-12]. The mitochondrial gene region COI has been used to discover the phylogenetic interaction of catfishes [13] reported the phylogenetic relationships of nine catfish species of families Clariidae and Pangasiidae using mitochondrial COI gene (Guo., *et al.* 2004). Aims and objectives of the current study were to offer encouraging in order on siluriformes and investigated the phylogenetic interaction within siluriformes by conducting phylogenetic investigation based on the mitochondrial gene sequence information.

Materials and Methods

Sample collection and DNA extraction

Total of five species of catfishes *Rita rita, Sperata seenghala, Bagarius bagarius, Wallago attu* and *Eutropiichthys vacha*, fin clip samples from Indus Rivers were collected. Fin clips were preserved in 99.9% ethanol and transported to the labora¬tory. Whole DNA was extracted by means of a (AxyPrep Multisource Genomic DNA MiniPrep Kit 50-Prep, USA). DNA was extracted as suggested by manufacturer protocol accordingly. The genomic DNA was extract-

ed and kept at 20°C until PCR analysis. Fish fin clip samples then were digested with 0.5 µg/µl proteinase K at 55ºC for the night. Later than centrifuge the solution, the supernatant precipitated in ethanol and dissolved in 1 ml 1× TE buffer. The quality and concentration of DNA from both sources was assessed by 1% agarose gel electrophoreses then sample were stored at 4ºC until use. The mitochondrial CO1 gene sequences of nineteen species order siluriformes were extracted from NCBI (www.ncbi.nlm.nih.gov).

PCR amplification and sequencing

Deoxyribonucleic acid (DNA) sequencing and Polymerase Chain Reaction(PCR) samples were cleaned by using QIAquick© purification PCR Kits. A 5 µM solution of every one primer and the PCR product were sent to company (Zhang Mei Tai He, Beijing). The program Codon Code Aligner was used to produce consensus sequences for each fish species. DNA sequences have been edited using BioEdit, associated using (ClustalW), with default data and 1000 bootstraps and observed by eye.

Polymerase Chain Reaction (PCR) was analyzed with an Eppendorf Thermal Cycler, manufactured by (Eppendorf Company, Germany) contained by a reaction combination of 25 ng/µl containing 01 unit of polymerase Taq DNA, 2.5 µl PCR buffer products (Tiangen China), 2 µl template DNA (50 ng/µl), 4.0 µl dNTP mix (0.4 mM), 01 µl primers (0.2 lM each), and 16.25 µl taped water. Denatured effect was performed at 95°C for 5 minutes, with 32 cycles at 95°C for 30s, 56°C for 30s and 72°C for 8 minutes; the final addition step was started at 72°C for 4 minutes. The elongated PCR was carried out with following protocol of LATAK PCRTM Kit (Takara made in Japan). Amplified PCR samples were purified with the 3S Spin PCR samples refinement Kit (Biocolor Inc, made by China) subsequent the manufactures directions. Electrophoresis of 3 µl of the product of PCR was analyzed in 1× TBE buffer for 1 hour at 150V, in 1.5% agarose gel having 0.5 µgml-1 Ethidium bromide. PCR product volume was checked against a 5kbp ladder of DNA. After ending DNA fragment result of electrophoresis was visualized by the help of UV transillumination and photographed.

S. No	Species	Fragment	Accession No	Reference
1	*Rita rita*	CO1	NC023376	Punhal., *et al*. 2014
2	*Sperata seenghala*	CO1	AB907556	Punhal., *et al*. 2014
3	*Bagarius bagarius*	CO1	KJ204285	Punhal., *et al*. 2014
4	*Eutropiichthys vacha*	CO1	AB919123	Punhal., *et al*. 2014
5	*Wallago attu*	CO1	KJ410746	Punhal., *et al*. 2014
6	*Bagrichthys acracanthus*	CO1	JQ248065	Supiwong., *et al*. 2011
7	*Batasio travancoria*	CO1	HQ009500	Lekshmi., *et al*. 2010
8	*Mystus gulio*	CO1	KF574792	Edward., *et al*. 2013
9	*Mystus multiradiatus*	CO1	JX177677	Supiwong., *et al*. 2012
10	*Mystus vittatus*	CO1	NC032082	Das., *et al*. 2016
11	*Bagrus bajad*	CO1	HM882795	Nwani., *et al*. 2011
12	*Mystus bleekeri*	CO1	KT896741	Singh., *et al*. 2015
13	*Mystus horai*	CO1	FJ170791	Lakra., *et al*. 2008
14	*Mystus rhegma*	CO1	JX177678	Supiwong., *et al*. 2012
15	*Pseudomystus siamensis*	CO1	JQ289150	Supiwong., *et al*. 2011
16	*Bagrus filamentosus*	CO1	HM882794	Nwani., *et al*. 2011
17	*Mystusbocourti*	CO1	EU490863	Sullivan., *et al*. 2008
18	*Mystus malabaricus*	CO1	HQ219113	Lakra., *et al*. 2010
19	*Mystus singaringan*	CO1	KU692660	Dahruddin., *et al.* 2016
20	*Sperata aor*	CO1	KY290093	Laskar., *et al.* 2016
21	*Batasiotengana*	CO1	JX260836	Kalyankar., *et al.* 2012
22	*Mystus cavasius*	CO1	KY290113	Laskar., *et al.* 2016
23	*Mystus montanus*	CO1	HQ219128	Lakra., *et al*. 2010
24	*Mystus tengara*	CO1	KT896742	Singh., *et al*. 2015

Sequence and phylogenetic analyses

We used Bioedit to edit the nearby, overlapped sequencing. The prime DNA sequence records were categorized via BLAST searches. We used *Channa marulius* as the out-group. All nucleotides were combined using Clustal X all the way through default settings (Thompson., *et al.* 1997), and the sequences were observed and accustomed physically. On behalf of these information sets the model GTR+I+G were selected for neighbor joining (NJ)analysis using Model Test 3.7 (Posada and Crandall 1998). The analysis of NJ were managing using PhyML 3.0 by Guindon and Gascuel (2003), and 1000 bootstraps were used to calculate approximately the tree consistency method by Felsenstein (1985).

Results

Base composition and genetic distance

Every one entrance is the possibility of changeover (r) from individual base (row) to a different base (column). Tariff of diverse intermediary substitutions are exposed in bold and those of trans-

versionsal substitutions are given in italics (Table 1). Comparative morals of instantaneous be supposed to be calculated while evaluate them. For easy, sum of (r) values are finished equivalent to 100, the CO1 gene sequences nucleotide composition was 23.80% A, 26.63.4% C, 19.95% G, and 29.62% T, the C+T ratio (65.5%) was higher than the A+G ratio. The expected conversion and transversion bias R is 1.96. The frequencies of nucleotides are 25.00% A, 25.00% T/U, 25.00% C and 25.00% G. The average genetic distance between the twenty four species was 0.455 and out group species was 0.452. The 24 nucleotide sequences were used for data analyzing. In the final data set there were total of 470 positions (Table 2). The evolutionary rate between sequences sameness was A (*Rita rita*) and B (*Wallago attu*), through sequence C (*Bagrichthys macracanthus*) used as an out-group. The $\chi 2$ test value was 0.03 (P = 0.86577 with one freedom degree). P-value less than 0.05 are frequently used to discard the null assumption of the same rates among lineages. The investigation concerned 3 nucleotide sequences. The total of 531 positions was shown in dataset (Table 3). Molecular clock assessment was accomplished by measuring the values of ML for the particular topology through and without the constraints of molecular clock. The null proposition of the same evolutionary time all over the tree was discarded at a 5% significance level (P < 0). In this research study 24 nucleotide sequences were involved. At present there were a total of 420 positions (Table 4).

Nucleotide	A	T/U	C	G
A	-	5.43	4.88	8.61
T/U	4.36	-	21.05	3.66
C	4.36	23.42	-	3.66
G	10.27	5.43	4.88	-

Table 1: Maximum calculate approximately substitution matrix.

Rita_rita	
Wallago_attu	0.043
Bagrichthys_macracanthus	0.481 0.464
Batasio_travancoria	0.474 0.470 0.509
Eutropiichthys_vacha	0.053 0.057 0.466 0.464
Bagarius_Bagarius	0.053 0.053 0.487 0.464 0.060
Mystus_gulio	0.477 0.472 0.523 0.096 0.466 0.466
Mystus_multiradiatus	0.472 0.460 0.434 0.521 0.470 0.466 0.519
Mystus_vittatus	0.513 0.504 0.517 0.464 0.511 0.528 0.457 0.500
Bagrus_bajad	0.509 0.504 0.479 0.523 0.519 0.511 0.509 0.432 0.464
Mystus_bleekeri	0.423 0.415 0.445 0.481 0.426 0.430 0.483 0.457 0.523 0.494
Mystus_horai	0.511 0.502 0.519 0.462 0.509 0.526 0.455 0.502 0.002 0.466 0.521
Mystus_rhegma	0.517 0.521 0.496 0.498 0.515 0.515 0.500 0.504 0.498 0.477 0.515 0.496
Pseudomystus_siamensis	0.550 0.543 0.483 0.481 0.533 0.545 0.510 0.488 0.512 0.483 0.510 0.510 0.512
Bagrus_filamentosus	0.509 0.504 0.479 0.523 0.519 0.511 0.509 0.432 0.464 0.000 0.494 0.466 0.477 0.483
Mystus_bocourti	0.474 0.453 0.436 0.502 0.455 0.460 0.509 0.457 0.506 0.455 0.451 0.509 0.489 0.498 0.455
Mystus_malabaricus	0.509 0.500 0.509 0.460 0.506 0.523 0.462 0.509 0.017 0.472 0.523 0.019 0.502 0.526 0.472 0.515
Mystus_singaringan	0.511 0.502 0.460 0.453 0.496 0.517 0.443 0.477 0.491 0.521 0.500 0.494 0.491 0.488 0.521 0.504 0.496
Sperata_aor	0.519 0.515 0.485 0.526 0.526 0.517 0.511 0.434 0.470 0.023 0.496 0.472 0.466 0.479 0.023 0.462 0.479 0.536
Batasio_tengana	0.477 0.468 0.468 0.504 0.466 0.457 0.494 0.494 0.513 0.513 0.411 0.511 0.500 0.464 0.513 0.449 0.521 0.506 0.506
Mystus_cavasius	0.489 0.477 0.515 0.504 0.479 0.487 0.515 0.477 0.517 0.517 0.500 0.519 0.479 0.452 0.517 0.526 0.521 0.494 0.528 0.523
Mystus_montanus	0.489 0.481 0.506 0.483 0.491 0.504 0.477 0.485 0.096 0.457 0.500 0.098 0.521 0.531 0.457 0.504 0.100 0.489 0.464 0.515 0.536
Mystus_tengara	0.506 0.498 0.515 0.470 0.504 0.521 0.477 0.511 0.045 0.474 0.534 0.047 0.513 0.514 0.474 0.504 0.040 0.502 0.481 0.528 0.519 0.102
Sperata_seenghala	0.504 0.504 0.487 0.528 0.511 0.506 0.513 0.432 0.460 0.026 0.485 0.462 0.472 0.495 0.026 0.460 0.468 0.530 0.019 0.513 0.521 0.453 0.470

Table 2: Hereditary distances of the mitochondrial gene COI between twenty four species of order siluriformes. The numbers of transversional differences per location between sequences are exposed. In the present study 24 nucleotide sequences were analyzed. Every part of positions by means of less than 95% position coverage was eliminated. That is, smaller quantity than % arrangement gaps, misplaced data, and indistinct bases were permissible at any location. Total of 470 positions were used in data analysis.

Configuration	Count
Identical sites in all three sequences	117
Divergent sites in all three sequences	45
Unique differences in Sequence A	18
Unique differences in Sequence B	17
Unique differences in Sequence C	334

Table 3: The equality of evolutionary rate among sequences A (*Rita rita*) and B (*Wallago attu*), with sequence C (*Bagrichthys Macracanthus*) used as an out-group in the relative rate test of Tajima.

	ln*L*	Parameters	(+G)	(+I)
With Clock	-13864.953	28	n/a	n/a
Without Clock	-12218.773	50	n/a	n/a

Table 4: Investigation of molecular clock was studied by matching the ML values. The null proposition of evolutionary equal rate all over the tree was discarded at a 5% level of significance (P< 0). The 24 species nucleotide sequences were used for examination. Total of 34 positions were finally recorded in the dataset.

Phylogenetic trees

During the phylogenetic tree analysis based on the COI, sequences resulted in unresolved trees, supported by 100% bootstrap probabilities. In the phylogenetic tree based on the COI sequences, catfish order siluriformes were divided into three major lineages (Figure 1). *Batasio travancoria* formed a single clade; *Rita rita, Wallago attu, Mystus montanus* and *Bagarius bagarius* comprised a single separate family; and *M. vittatus, M. horai, B. tengana, M. malabaricus, M. bacourti, M. singaringan, M. bleekeri, M. gulio, M. multiradiatus, M. rhegma, M. cavasius, M. tengara, S. aor, S. seenghala, B. bajad, B. filamentosus, B. macracanthus, P. siamensis, E. vacha* and *B. travancoria* formed single subfamily.

Figure 1: Evolutionary record was inferred using the Neighbor Joining (NJ) protocol. The best possible tree with the summation of branch length = 5.43549567. Evolutionary distances were computed using the technique is in the units of the figure of base differences per location. The 25 nucleotide sequences were investigation. Each and every one position with less than 95% place coverage was removed. With the intention of smaller quantity than % configuration gaps, missing information, and confusing bases were acceptable at any location. Total of 470 positions were involved in analysis.

Discussion

The relations within the diverse families of siluriformes have been investigated in the last few decades for the reason that of the reformed momentum provided as a result of the introduction of cladistic in the end of the 20th century [7]. Every single one cladistic mechanism presented up to now on phylogeny of catfishes, the immense larger part were committed to the study of the intra relationships of each part or the total of an exacting catfish family unique, precise cladograms on the interfamilial associations of both a significant part or the whole of the order siluriformes were discussed [2,3,6-8]. The DNA based mitochondrial gene COI has characteristically been used as the barcode for fish species categorization. In present study, the genetic distance between siluriformes based on the COI gene was 0.455; this result was inconsistent with the morphology of these species. As there is no previous DNA study for catfish from Indus River Sindh province region, our results of present study provides initial data for possible mitochondrial genome and some phylogenetic research. It is essential to understandable that further DNA research studies on commercially important catfish from the whole area of Indus River are requisite, as understanding of the genetic resources obtainable between the catfish species could prove helpful for scheming future artificial propagation and genetic breeding programs [14-18].

Conclusion

In present study, the genetic distance between siluriformes based on the COI gene was 0.455; this result was inconsistent with the morphology of these species. As there is no previous DNA study for catfish from Indus River Sindh province region, our results of present study provides initial data for possible mitochondrial genome and some phylogenetic research. It is essential to understandable that further DNA research studies on commercially important catfish from the whole area of Indus River are requisite, as understanding of the genetic resources obtainable between the catfish species could prove helpful for scheming future artificial propagation and genetic breeding programs.

It is concluded based on the COI, sequences that, catfish order siluriformes are divided into three major lineages. Batasio travancoria formed a single clade; *Rita rita, Wallago attu, Mystus montanus* and *Bagarius bagarius* comprised a single separate family; and *M. vittatus, M. horai, B. tengana, M. malabaricus, M. bacourti, M. singaringan, M. bleekeri, M. gulio, M. multiradiatus, M. rhegma, M. cavasius, M. tengara, S. aor, S. seenghala, B. bajad, B. filamentosus, B. macracanthus, P. siamensis, E. vacha* and *B. travancoria* formed single subfamily.

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Volume 2 Issue 5 May 2018