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Genetic assessment of ornamental fish species from North East India

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ABSTRACT

Ornamental fishes are traded with multiple names from various parts around the world, including North East India. Most are collected from the wild, due to lack of species-specific culture or breeding, and therefore, such unmanaged collection of the wild and endemic species could lead to severe threats to biodiversity. Despite many regulatory policies, trade of threatened species, including the IUCN listed species have been largely uncontrolled, due to species identification problems arising from the utilization of multiple trade names. So, the development of species-specific DNA marker is indispensable where DNA Barcoding is proved to be helpful in species identification. Here, we investigated, through DNA Barcoding and morphological assessment, the identification of 128 ornamental fish specimens exported from NE India from different exporters. The generated sequences were subjected to similarity match in BOLD-IDS as well as BLASTN, and analysed using MEGA5.2 for species identification through Neighbour-Joining (NJ) clustering, and K2P distance based approach. The analysis revealed straightforward identification of 84 specimens into 35 species, while 44 specimens were difficult to distinguish based on CO1 barcode alone. However, these cases were resolved through morphology, NJ and distanced based method and found to be belonging to 16 species. Among the 51 identified species, 14 species represented multiple trade names; 17 species belonged to threatened category. Species-level identification through DNA Barcoding along with traditional morphotaxonomy reflects its efficacy in regulating ornamental fish trade and therefore, appeals for their conservation in nature. The use of trade names rather than the zoological name created the passage for trafficking of the threatened species and demands immediate attention for sustaining wildlife conservation.

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1. Introduction

Aquarium fish keeping has become one of the most popular hobbies and fishes suitable for keeping in the aquarium fetch a high economic value across the globe contributing to increased growth in international ornamental fish trade. The worldwide export has increased from US\$ 176 million to US\$ 251 million, and the import has increased from US\$ 257 million to US\$ 303 million during the year 1998 to 2004 (FAO Fisheries and Aquaculture Dept. Fishery and Aquaculture Information and Statistics Service, 2008). The Indian ornamental fish export represents a small portion of the global share but it is increasing with 20% rise per annum with annual income of US\$ 1.2 million (Marine Products Export Development Authority, MPEDA). The North-eastern (NE) region of India contributes the lion's share of Indian aquarium

fish trade (Bhattacharya and Choudhury, 2004). This region of the country is important in view of large-scale production from capture fishery underlined by the existence of innumerable rivers, rivulets and lentic water bodies, which harbour a diverse fish fauna. There are about 267 species belonging to 136 genera of fresh water fishes inhabiting in NE India (Ponniah and Sarkar, 2000). Out of which, 54.32% possess either of the three values as food for human, component in angling tourism or aquarium fish trade, and are thus potential resources for the growth of economy. About 80% of the total ornamental fish trade is rooted from wild catch and is contributed by this region of India via Kolkata Airport (Das and Biswas, 2009; Kalita and Deka, 2013). Aquarium fishes are categorized based on vivid colour pattern (colourful), morphological uniqueness (special), and behaviourally charismatic (semi-aggressive, community, non-community) (Ponniah and Sarkar, 2000). Besides, ornamental fish traders always intend to publicize uncommon or unseen species in the trade in order to attract hobbyists. In the process, some traders adopt an unfair practice, like, use of synthetic dyes to develop colour in those species which are originally colourless thus claiming them to be ornamental and traded by different names to the clients. On the other hand, many endemic fish species are being traded from the wild harvest, due to lack of established species-specific culture or breeding, and serve as a threat to biodiversity from harvest pressure. Despite several regulatory enactments in India, aquarium fishes are

Abbreviations: K2P, Kimura 2 parameter; COI, cytochrome C oxidase 1; NJ, Neighbour Joining; ML, maximum likelihood; S.E., standard error; NUMTs, nuclear DNA originating from mitochondrial DNA sequences; EDTA, ethylenediaminetetraacetic acid; PCR, polymerase chain reaction; dNTPs, deoxynucleotide triphosphates; MEGA, Molecular Evolutionary Genetic Analysis; DNA, deoxyribonucleic acid; BOLD, Barcode of Life Datasystem; BLAST, Basic Local Alignment Search Tool; IUCN, International Union for Conservation of Nature.

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Table 1

Straight forward identification of the studied ornamental fishes based on similarity match with BOLD Identification System and GenBank. The match is exclusively based on similarity of developed sequences with database sequences. 84 traded fishes identified as 35 species, i.e. in many cases; same species is traded with multiple trade names.

Fish specimen with trade name	Sequences generated (Acc. no GenBank) (size in bp)	Closest match with species in BOLD-IDS		Close match in GenBank (BLASTN similarity in %)	Identified as species (number)
		Species level barcode records (process ID)	Public record barcode database (process ID)		
Gangetic latia	JN815299 (626 bp), JX105481 (618 bp), KF511500 (624 bp), KF511501 (624 bp), KF511502 (627 bp)	<i>Crossocheilus latius</i> (ANGBF9951-12, ANGBF9956-12)	<i>Crossocheilus latius</i> (ANGBF9951-12, ANGBF9956-12)	<i>Crossocheilus latius</i> (99)	<i>Crossocheilus latius</i> (1)
Neon Hatchet	JN815300 (631 bp), JN815301 (634 bp)	<i>Chela cachius</i>	<i>Chela cachius</i> (GBGC4871-08)	<i>Chela cachius</i> (97)	<i>Chela cachius</i> (2)
Tank goby	JN815293 (537 bp), JN815296 (623 bp), JQ713857 (632 bp), JN815294 (604 bp), JN815295 (619 bp)	<i>Glossogobius guiris</i>	No match	No match (99)	<i>Glossogobius guiris</i> (3)
Glass fish	JN815274 (534 bp), JN815275 (584 bp), JN815276 (607 bp), JN815283 (639 bp)	<i>Parambassis ranga</i>	No match	<i>Pseudoambassis ranga</i> (99)	<i>Parambassis ranga</i> (4)
Barred Spiny eel	JX105465 (602 bp), JN815289 (631 bp)	<i>Macrogathus pancalus</i>	<i>Macrogathus pancalus</i> (GBGC4234-08, GBGC4233-08)	<i>Macrogathus pancalus</i> (99)	<i>Macrogathus pancalus</i> (5)
Labeo	JQ713848 (624 bp)	<i>Labeo calbasu</i> (GBGC4252-08, ANGBF7333-12)	<i>Labeo calbasu</i> (GBGC4252-08, ANGBF7333-12)	<i>Labeo calbasu</i> (100)	<i>Labeo calbasu</i> (6)
Flying barb	JN673955 (655 bp), KF511504 (622 bp), KF511505 (622 bp), KF511506 (622 bp)	<i>Esomus danricus</i>	<i>Esomus danricus</i> (ANGBF6124-12, ANGBF6125-12)	<i>Esomus danricus</i> (99)	<i>Esomus danricus</i> (7)
Silver barb	JQ713846 (655 bp)	<i>Barbonymus gonionotus</i> (GBGC6590-09, GBGC6591-09, GBGC6592-09, ANGBF5730-12)	<i>Barbonymus gonionotus</i> (GBGC6590-09, GBGC6591-09, GBGC6592-09, ANGBF5730-12)	<i>Barbonymus gonionotus</i> (100)	<i>Barbonymus gonionotus</i> (8)
Corsula mullet	JX105471 (623 bp)	<i>Rhinomugil corsula</i>	<i>Rhinomugil corsula</i>	<i>Rhinomugil corsula</i> (98)	<i>Rhinomugil corsula</i> (9)
Giant Danio	KF511497 (628 bp), KF511498 (628 bp), KF511499 (626 bp)	<i>Devario aequipinnatus</i>	<i>Devario aequipinnatus</i> (RCYY279-11, RCYY280-11, RCYY334-11, ANGBF6126-12)	<i>Devario aequipinnatus</i> (98–99)	<i>Devario aequipinnatus</i> (10)
Clown knifefish	KF511511 (558 bp)	<i>Chitala chitala</i> (ANGBF6042-12, ANGBF6132-12, ANGBF6043-12)	<i>Chitala chitala</i> (ANGBF6042-12, ANGBF6132-12, ANGBF6043-12)	<i>Chitala chitala</i> (99–100)	<i>Chitala chitala</i> (11)
Suckerthroat	KF511525 (622 bp), KF511526 (622 bp), KF511527 (603 bp)	<i>Pseudecheneis sulcata</i>	<i>Pseudecheneis sulcata</i> (GBGC8471-09)	<i>Pseudecheneis sulcata</i> (99)	<i>Pseudecheneis sulcata</i> (12)
Indian Hill Trout	KF511547 (629 bp), JN815290 (661 bp), JN815291 (660 bp), KF511548 (629 bp), KF511549 (628 bp)	<i>Barilius bendelisis</i> (CYTC3711-12, CYTC4266-12)	<i>Barilius bendelisis</i> (CYTC3711-12, CYTC4266-12)	<i>Barilius bendelisis</i> (100)	<i>Barilius bendelisis</i> (13)
Indian Whiptail catfish	KF511561 (625 bp)	<i>Sisor rabdophorus</i>	<i>Sisor rabdophorus</i> (CFISH028-12, CFISH027-12)	<i>Sisor rabdophorus</i> (99)	<i>Sisor rabdophorus</i> (14)
Long Whiskers Catfish	KF511564 (620 bp)	<i>Mystus gulio</i>	<i>Mystus gulio</i> (GBGCA2888-13)	<i>Mystus gulio</i> (99)	<i>Mystus gulio</i> (15)
Striped dwarf catfish	KF511563 (620 bp)	<i>Mystus vittatus</i>	<i>Mystus vittatus</i> (CFISH009-12)	<i>Mystus vittatus</i> (99)	<i>Mystus vittatus</i> (16)
Butter catfish	KF511565 (620 bp)	<i>Ompok bimaculatus</i>	<i>Ompok bimaculatus</i>	<i>Ompok bimaculatus</i> (98)	<i>Ompok bimaculatus</i> (17)
Gangetic ailia	KF511566 (620 bp)	<i>Ailia coila</i> (ANGBF6053-12, ANGBF6054-12, GBGC4011-08)	<i>Ailia coila</i> (ANGBF6053-12, ANGBF6054-12, GBGC4011-08)	<i>Ailia coila</i> (100)	<i>Ailia coila</i> (18)

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