

INTRODUCTION

1. INTRODUCTION

Recent years have witnessed that both Aquaculture and Fisheries have developed into potential industries by providing nutrition for uplifting livelihood through foreign exchange. Aquaculture and Fisheries are both industries serving not only as a source of providing essential nutrition but also, helping in the upliftment of livelihood of the greater human population in the form of earning foreign exchange. India is the second largest country in global fish production with aquaculture contributing about 5.68% (30,213 crores) (**Goswami and Zade, 2015**). The total fish production during 2013-14 was 9.58 million metric tonnes with a contribution of 6.14 million metric tonnes from Inland sector and 3.44 million metric tonnes from Marine sector, respectively. The overall growth in fish production in 2013-14 was 5.9%, being mainly due to 7.3% growth in inland and 3.7% in marine fish production (**Handbook on Fisheries Statistics, 2014**).

The ornamental aquatic industry dealing with live animals and plants for aquaria or ponds, its associated equipment and feed is a worldwide business with a trade of approximately 15 million US \$ (**FAO, 2013**) retail value. India's share in ornamental fish trade is estimated to be less than 1% of the global trade (0.008%) and a domestic market of Rupees 10 crores with the state of West Bengal in the forefront with a share of around 90 percent of the export earnings from ornamental fish (**Dholakia, 2009**). In terms of money it comes to 23 million or two crores and thirty lakhs rupees.

Ornamental fishes are called 'living jewels' for their beautiful colour and playful behaviour and are typically small sized; attractive and bizarre shaped in appearance (**Dey, 1996**). Ornamental fish keeping being one of the most popular hobbies in the world is triggering a hike in aquarium fish trade globally. They are usually kept in aquarium and popularly known as aquarium fishes. It is a source of attraction for fish lovers and

aquarists all over the world. According to **Angami (2012)**, Indian ornamental fishes with their brilliant colours and unique features need no introduction to the world market. The tropical ornamental fishes from North Eastern and Southern provinces of India are in great demand in the hobbyists market. Many attractive loaches, barbs, badis, zebra fishes, catfishes and glass fishes are indigenous to the lentic and lotic water ecosystems of India. Terai and Dooars regions of Eastern Himalaya are considered as “Hot Spots” for fresh water fish biodiversity (**Dey et al., 2015f**). In these regions, ornamental fishes are dominant over the food fishes. Almost 85 % of the exportable ornamental fish are contributed by the North Eastern region of India (**Swain, 2004**). A great number of species have been reported from Cooch Behar district on fish biodiversity and 10 species of loaches are available in Cooch Behar district (**Dey et al., 2015a**). Among all the rivers flowing through the district of Cooch Behar, Kaljani is the richest in fresh water fish biodiversity. This river which is about 96 Km long originates from Gabaur Bachhra forest, lying in the borders of Bhutan and West Bengal, and outfalls into Shiltorsa in Cooch Behar. Biodiversity is essential for balancing ecosystem but it is drastically reduced by anthropogenic activity. The river water is used for agriculture, fisheries, residential and industrial developments, mining activity, navigation, power generation and variety of other activities including sand digging and disposal of industrial and domestic wastes and as such natural breeding is hampered.

The fishes of the family Cobitidae and Balitoridae are popularly known as ‘Loach’. Balitorinae fishes are known as ‘hill stream loaches’ whilst, Cobitidae fishes are popularly known as ‘Loaches’. These loaches are mainly found both in lentic and lotic water bodies. *Botia* species (subfamily Botinae) are less abundant, Endangered and Vulnerable whereas, other subfamily (Nemacheilinae, Cobitinae and Balitorinae) of loaches are abundant and Least Concern. Therefore, *Botia* species were found in the river

Kaljani selected as experiment fish for the present study. The *Botia* loaches are high demanding species having both ornamental and economical important food value and contribute to a major share of the world market for beautiful coloured indigenous ornamental fish. Among the loaches, *Botia dario* (Hamilton-Buchanan) commonly known as “Queen loach” or “Rani Mach”, *Botia rostrata* (Gunther), commonly known as “Ladder loach”, are vulnerable fishes (IUCN, 2010) whereas, *Botia almorhae* (Grey), commonly known as “Almorha loach” and *Botia lohachata* (Chaudhuri), popularly known as “Y-loach” or “Tiger loach” or “Lohachata”, are endangered species (IUCN, 2010) are distributed widely in North-East India and Bangladesh. These *Botia* species are high demanding species of Terai region of West Bengal for aquarium fish. They lead a nocturnal life and remain buried in sand or silt for most of the time. The fishes are very colourful with bright bands, peaceful nature, lesser scales and barbels. Loaches are omnivores, and usually prefer *Daphnia*, earthworms, bloodworms, snails and animal proteins. The “Y-loach” is a scavenger but does not eat fish wastes.

Schistosomiasis, also known as “bilharzias”, “bilharziosis” or “snail fever” is a serious disease affecting human and domestic animals as well as wild animals (Dey *et al.*, 2015c). The intermediate host of the parasite which causes the disease is a species of freshwater snails. The snail eating loach is one of the many natural controls of the freshwater snail population and plays an important role in controlling the disease. *Botia* loach makes a cracking sound. This is either produced by forcing air through the gills and may be connected with feeding on the surface of the water or alternately produced by specialized teeth in the throat of the fish that appear to aid in the extraction of snails muscle from their shells.



Fig. 1. The *Botia* species (a) *Botia almorhae* (b) *Botia dario* (c) *Botia lohachata* (d) *Botia rostrata*

Loaches are considered as Endangered. The Endangered status of the loaches are mainly because of the deterioration of the environment particularly, water quality which may be due to agricultural run-offs or pesticidal effect of tea gardens in the Terai and Dooars regions, and big-water bodies being fragmented into small water bodies; thus drying up the water. *Botia* species are regarded an excellent ornamental fish and highly preferred among villagers for fish ornamental farming. In the Terai region *Botia almorhae*, *Botia lohachata* and *Botia rostrata* are very rare or their occurrence is low throughout the year. The habitats of fish fauna are rapidly shrinking due to human activity and drought. Immediate rehabilitation of *Botia species* is important from its extinction from the environment. Therefore, rearing, captive breeding and embryonic development of *Botia species* are the vital recodes to be taken without delay before their extinction from this region. Captive breeding of any species helps to reintroduce them in their natural habitat. This technology is very useful for Endangered species to survive in nature.

Studies of breeding behaviour, embryonic development and reproductive biology of any fish is essential for evaluating the commercial potentialities of its stock, life history, cultural practice and actual management of small indigenous fishes (**Lagler 1956; Doha and Hye, 1970**). The reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect of their gonadal condition (**Jhingran and Verma, 1972**). Knowledge of gonadal development and the spawning season of species allow subsequent studies on spawning frequency of its

population which is very important for its management. The histological studies help in detecting the breeding season and establishing phenotypic characters of mature brooders for successful artificial propagation of a species. Hence, it is very important to assess the yearly breeding cycle of loaches to ensure success in culture practice. Development of captive breeding and seed production techniques for the indigenous fish species is also an approach solution for conservation. This can be achieved by restocking their natural habitat with hatchery-reared individuals (**Philippart, 1995; Poncin & Philippart, 2002** and **Montchowui *et al.*, 2011**); a viable alternative to capture fisheries in providing a sustainable source of protein for fishing communities and local populations.

Mitochondrial DNA (mtDNA) is a valuable marker in population genetics or phylogeographic studies because it is maternally inherited and recombination is absent (**Singh *et al.*, 2014**). Mitochondrial DNA sequences being embedded in every cell are considered as genetic 'bar-codes'. The variation among DNA sequences is used to identify organisms (**Singh *et al.*, 2014**). DNA bar-coding is the use of a short DNA sequence or sequences from a standardized locus (loci) as a species identification tool (**Hebert *et al.*, 2003a**). The DNA barcode that is well established in animals is a sequence of a 655 base fragment of the 5' end of the mitochondrial Cytochrome C Oxidase 1 (COI or Cox1) gene. Fish mitochondrial DNA is small (16.5 kb approximately) double stranded, circular molecule composed of about thirty seven genes coding for twenty two tRNAs, two rRNAs and thirteen mRNAs. Mitochondrial DNA was used to examine the evolutionary and taxonomic relationships amongst taxa. The barcode sequence from each unknown specimen is then compared with a library of reference barcode sequences derived from individuals of known identity. A specimen is identified if its sequence closely matches one in the barcode library. Otherwise, the new record can lead to a novel barcode sequence for a given species (that is, new haplotype or

geographical variant), or it can suggest the existence of a newly encountered species (Hajibabaei *et al.*, 2007). The present study, therefore, also focussed to establish the evolutionary and taxonomic relationships amongst the species of genus *Botia* using mtDNA and to show the genetic distance between them. The study may thus contribute to some extent to the information database and conservation approach of the fish diversity in natural resources.

Therefore, a detailed survey of the Kaljani river of Cooch Behar district of North Bengal, West Bengal, India was carried out to select the *Botia* species and standardization of captive breeding, the barcoding of the selected species for understanding their taxonomic status and phylogenetic interrelationships among the species.

1.1. Objectives of the study

Based on the concept that *Botia spp* are Endangered, and to bring about its conservation a research project entitled “**Breeding behaviour, embryonic development and barcoding of the ornamental loaches (Cobitidae: Cypriniformes) of Terai Region of West Bengal, India**” was executed for a period of three years (August 2012 to July 2015) based on the following objectives.

1. To survey the water of river Kaljani; collect the loaches and estimate the limnochemistry of water.
2. To standardize breeding behavior and techniques in captivity and characterize embryonic development.
3. To standardize supplementary feed for growth of spawns.
4. To identify the species through molecular characterization using barcoding.