

## Genetic Variation of Hunga Fish Ecomorphs in Selected Provinces in Sri Lanka

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### Abstract

Hunga is one of the most important fresh water fish species in Sri Lanka and is widely spread in Thailand, Myanmar, Bangladesh, India, Nepal and Pakistan. This species is highly abundant in fresh water bodies throughout the country. Twelve fish samples from eight provinces were collected and analyzed to determine morphological and genetic variability. A barcoding approach was followed with universal fish primers for sequencing mitochondrial CO1. Results were compared with NCBI data base and a phylogenetic tree was developed. According to the phylogenetic analysis, samples were identified as *Heteropneustes fossilis* and *Plotosus anguillari*. Results revealed that *Heteropneustes fossilis* is unavailable in north province due to the lack of fresh water rivers and other fresh water bodies. However, *Plotosus anguillari* is rarely available in North Province.

**Keywords:** DNA Barcoding, Sri Lanka, Hunga Fish, *Plotosus anguillari*, *Heteropneustes fossilis*

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### Introduction

Catfishes, available as human food, Sporting fish and aquariums protective fish, are highly distributed in the world (Maloyjo *et al.*, 2012). In Sri Lanka there is a high abundance of catfishes throughout the natural water bodies. These species are widely distributed in Thailand, Burma, and Sri Lanka, in water bodies of different pH conditions (6.0-8.0 pH). The Asian Stinging Catfishes belong to the family Heteropneustidae of the order Siluriforms. There are over 2400 species of catfishes, divided into 344 families containing 422 genera. Members of the family Heteropneustidae are collectively known as air sac catfishes. The stinging cat fish (*Heteropneustes fossilis*), a member of the Heteropneustidae, often called "Hunga" is a commercially as well as economically important species in many Asian countries. The Stinging catfish is native to Sri Lanka, Bangladesh, Pakistan, India, Nepal, Burma and Laos.

The Asian Stinging Catfish also popular from nutritional and medical point of view. The muscles of the fish have been reported to have higher content of Iron (226mg/100g) and fairly high level of Calcium compared to many other fresh water fishes (fat content is only 2.57% on fresh basis) (Kamrussaman *et al.*, 2013). Due to the high nutritive value, the fish is recommended in the diet of sick and convalescents (Sultana *et al.*, 2010).

The Asian stinging catfish has been categorized as vulnerable red list in India by IUCN. Due to

that, it is a great concern whether the reduction in population size has had any impact on genetic variability of this species as there is an inverse relationship between the genetic variability and adaptability to environment changes (Sultana *et al.*, 2010). Genetic variability within and among population is essential for an evolutionary interpretation of interactions and for the management of endangered or commercially important taxa.

*Heteropneustes fossilis* popular for its high growth rate, fecundity, efficient feed utilization, and ability to survive in poorly oxygenated water (Sultana *et al.*, 2010). The Habitat degradation caused by natural and human interventions, release of industrial effluent have recently become great constraints for fish biodiversity in most aquatic eco-systems in Sri Lanka. As a result, there have been remarkable changes observed in natural *Heteropneustes fossilis* in different water bodies. In order to understand those variations, intensive study on genetic diversity of natural populations of *H. fossilis* in Sri Lanka is needed. Molecular markers are realistic and useful tools for the investigation and monitoring genetic conditions, both natural populations and in captive stocks.

The DNA barcoding technique is considered as a rapid, accurate, automatable, and globally accessible procedure for species identification. The most useful method for analyze the DNA partial sequencing of mtDNA. The effectiveness of this method relies on the relatively conserved stretch of approximately 650 nucleotides of the

mitochondrial Cytochrome oxidase c sub Unit-I (COI) gene. Based on the nucleotide sequences, accurate identification of organisms at the species level is reasonably straightforward and has been applied to numerous animal taxonomy (Maloyjo *et al.*, 2012).

#### Methodology

Eight native Catfish samples were collected in different natural water bodies from isolated geographical locations of eight provinces (Uva Province, Sabaragamuwa Province, Central Province, North Central Province, North Western Province, North Province and Eastern Province) of Sri Lanka. Samples were packed in a polythene bags and tagged separately and finally these samples were stored in 4°C in freezing conditions.

For the molecular analysis, 1mm of Muscle samples was separated from each fish samples aseptically and stored in the vials in -20°C. After that laboratory investigation followed by DNA extraction and PCR analysis. Results were analyzed with MEGA 5 computer package and phylogenetic was developed by using same program.

#### Results and discussion

According to the morphological observations three different fishes which change by the color and teeth were identified and all 3 species called same local name of "Le Hunga", "Kaha Hunga" and "Sunga". The first two fishes were belonging to fish species (*Heteropneustes fossilis*) and latter fish was identified as *Plotosus anguillaris*. According to the phylogenetic tree of the analyzed sequences, DNA samples of seven provincials show similarities to the single species of *Heteropneustes fossilis* except north province (Distance between 0.1054-0.1280). There have been significant variations in north province fish with other fishes It identified as another species of same fish order with similar morphological characters called as *Plotosus anguillaris*.

The fish sample which collected from north province is highly abundant in North coastal

areas of Sri Lanka. According to the phylogenetic tree of the analyzed sequences with out -groups, except all the Haplotypes of DNA one to five cluster together and out group of Accession number KT 364787 cluster separately.

#### Conclusion

The present results indicate that an identification system for animal life based on the COI gene will be highly effective. More importantly the present study has established that species level identifications are ordinarily achieved. COI analysis provides a taxonomic system that can be followed until final analysis of animal diversity is given. Further it can be concluded that *Plotosus anguillaris* is now become a very rare species and conservation activities of this species should be taken immediately.

#### Acknowledgement

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