Short Note

Preliminary Study of Genetic Diversity in the Giant Freshwater Stingray, *Himantura chaophraya* (Batoidea: Dasyatidae) from the Remnant Populations in Thailand

JENJIT KHUDAMRONGSAWAT¹*, TUCKSAORN BHUMMAKASIKARA² AND NANTARIKA CHANSUE³

¹ Animal Systematics and Molecular Ecology Research Group, Department of Biology, Faculty of Science, Mahidol University, Rama 6 Road, Rajathewi District, Bangkok 10400, THAILAND
² Wildlife Conservation Office, Department of National Park, Wildlife and Plant Conservation, 61 Phaholyothin Road, Chatuchak District, Bangkok 10900, THAILAND
³ Veterinary Medical Aquatic Animal Research Center, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Road, Pathumwan District, Bangkok 10330, THAILAND

* Corresponding Author: Jenjit Khudamrongsawat (khudamrong@gmail.com)

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*Himantura chaophraya*, also known as the giant freshwater stingray, is one of the largest freshwater fishes in the world found in Thailand. The genetic distinctness of the *H. chaophraya* population in Thailand from the population of *H. chaophraya* in India was demonstrated¹, which is described and recognized the geographic uniqueness of each population. The presence of *H. chaophraya* in the central plain in Thailand has been known since 1983². The distribution of this specie from local people interview and literature search was compared with field surveys between 2009 and 2011. The result revealed the diminishing range as well as the decrease in abundance³. Nevertheless, the capture of this species is occasionally reported in the Mekong River near Nakhon Phanom Province and Mae Klong River near Amphawa Floating Market in Samut Songkram Province. Causes of the reduction in population abundance are thought to include overfishing and by catch fishers, but the actual causes have not been identified⁴,⁵. Water pollution evidently have caused several stingray deaths in the past few years as seen in the sugar barge accident on 31st May 2011 in the Chaophraya River resulting in death of over 20 individuals of the giant freshwater stingrays⁶ and a most recent incident in the Mak Klong River in October 2016 causing death of at least 50 individuals of the stingrays⁷. All losses urge the Thai government to enforce legal protection of the species.

Currently, the Thai Cabinet has passed the new regulation that will include *H. chaophraya* into the protected lists in the Wild Animal Reservation and Protection Act. In addition, proposed criteria for IUCN Red List have considered using genetic diversity information of a species⁸. Therefore, information regarding this species will be useful for conservation and management. This study aimed to evaluate fine scale genetic variation of the remnant populations of *H. chaophraya* in Thailand. This information may provide supporting evidence for the appropriate national conservation status and legal protection designation of the species and serve as baseline data for future conservation.
Samplings of *H. chaophraya* tissue were opportunistically collected from 2009 to 2011 (Fig. 1). Although these rivers drain into the Gulf of Thailand in nearby provinces within 100 km of each other, they constitute discrete river basins but occasionally connected through seasonal inundation and man-made canals. *Himantura chaophraya* was caught using hooks and line and tissue samples from the pectoral fin were obtained and preserved in 95% ethanol. After tissue sampling, the fish were labeled with microchip on the left side of the body for recapture identification, and released. Thirty-two tissue samples were collected from the lower Mae Klong River in Samut Songkhram Province (only 28 were used for cytochrome b sequencing), seven from the upper Chaophraya River in Nakhon Sawan Province (only six were used for microsatellite DNA analysis), and one sample from the lower Bangpakong River in Chachoengsao Province (Fig. 1).

The genomic DNA was extracted using the commercial kit (QIAGEN Valencia, CA, USA). Genetic diversity of microsatellite DNA and cytochrome b were investigated using eleven
published microsatellite DNA primers\(^9\) as well as those for cytochrome b\(^1\). All PCR reactions were repeated twice for result confirmation. PCR products were sent to MACROGEN (Korea) for fragment length analysis and sequencing. Genetic diversity as measured by the number of microsatellite alleles, heterozygosity, cytochrome b haplotype polymorphism, and nucleotide diversity was determined using ARLEQUIN version 3.\(^5\)\(^10\).

Samples from all locations were pooled for the analyses. The average microsatellite allele per locus was 8.9 alleles per locus. Observed heterozygosity for each locus ranged from 0.231 to 0.769. Half of the studied loci (Hcha-02, Hcha-03, Hcha-04, Hcha-05, Hcha-10, and Hcha-11) showed significant deviation from Hardy-Weinberg equilibrium in contrast to the results from a previous screening using only samples collected from the lower Mae Klong River\(^8\).

Of these polymorphic loci, some, such as loci Hcha-09 and Hcha-10, were highly variable and exhibited more than 10 alleles (Fig. 2). On the other hand, some loci, such as Hcha-06, were less variable. Most alleles were present in the samples from the lower Mae Klong River where the majority of the samples were obtained with a few exceptions. For example, allele 274 at locus Hcha-05 was observed in the sample from the upper Chaophraya River regardless of a small sample size. Additional samples from these rivers and other rivers that \(H.\) chaophraya is reported may provide an opportunity for further investigation of genetic variation and population structure of wild populations in Thailand. Although genetic diversity inferred from microsatellite DNA cannot be compared among species due to questionable evolution of the loci used in studies, the diversity of \(H.\) chaophraya quantified in this study appeared to similar to several threatened batoids\(^11,12,13\).

In addition to the microsatellite DNA analysis, fragments of 1,002 base pairs (bp) of cytochrome b from 36 samples (28 samples from Mae Klong River, seven samples from Chaophraya River with one additional sample obtained from Nakhon Sawan Provincial Administrative Organization after microsatellite DNA analysis, and one sample from Bangpakong River) were used for the analyses after correction and alignment. Variations of cytochrome b sequences were obtained (Table 1). Overall nucleotide diversity was 0.00039 ± 0.00043 with eight polymorphic sites. Three haplotypes were identified. One dominant haplotype (pattern 1) was found in all sampling locations and accounted for 88.9% (32 samples) of total samples. Moreover, the dominant haplotype was the precisely same sequence that was previously reported in 1999 using samples from the Chaophraya River\(^1\). The presence of a dominant haplotype, together with low haplotype diversity suggests recent or ongoing dispersal events. The very low level of nucleotide diversity in \(H.\) chaophraya indicated little genetic polymorphism at evolutionary scale, which likely influences the viability of natural populations\(^14\).

Because the great difference in numbers of samples from different sampling locations, the analysis of population structure could not be determined with statistic confidence. It is questionable about the natural population structure of \(H.\) chaophraya population since its divergence from the marine ancestor. The distribution of this species in large rivers that are not parts of the same basin, and its occurrence in a wide range of habitats from over a hundred kilometers inland freshwater to
estuaries suggests dispersal and movement through seasonal river connections in the Tertiary through Quaternary\(^{15}\) and use of the marine environment during sea level rise.

**FIGURE 2.** Microsatellite DNA allelic variation of ten polymorphic loci (a - j) from three sampling locations. White bars = samples from the lower Mae Klong River; grey bars = samples from the upper Chaophraya River; black bars = a sample from the lower Bangpakong River.
Man-made canals connecting rivers and introduction by humans could occasionally contribute to movement between rivers. Opportunistic capture of the giant freshwater stingrays in different rivers indirectly suggests that these rivers serve as habitats for this large fish, and the Mae Klong River likely harbors a large number of wild populations in Thailand. River pollutions have diminished wild population as well as exacerbated the viability of the species that has low level of genetic diversity as inferred from this study. Thus, genetic data that provide baseline evidence of pre-population reduction will facilitate future monitoring and restoration of wild populations.

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LITERATURE CITED


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