



## REPLY COMMENT

# Population genetics of freshwater stingray require investigation to confirm DNA contamination: Reply to Alshari et al. (2023)

Kean Chong Lim<sup>1,\*</sup>, Amy Y. Then<sup>1,2</sup>

<sup>1</sup>Institute of Ocean and Earth Sciences, Universiti Malaya, Kuala Lumpur 50603, Malaysia

<sup>2</sup>Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia

**ABSTRACT:** Alshari et al. (2023; Endang Species Res 50:311–313) were able to collect 3 *Fluvi-trygon kittipongi* specimens from the Pahang River, which were not available to us at the time of our study (Lim & Then 2022; Endang Species Res 48:43–50). The cytochrome *c* oxidase I (COI) sequences (or haplotypes) of these 3 stingrays were identical to each other but differed from the haplotypes of our Perak River specimens. The result of 100% similarity between COI sequences of our Perak River rays and the eDNA water samples isolated from Pahang River in our study was suggested by Alshari et al. (2023) as DNA contamination in our study, which is plausible. However, further population genetics studies would be necessary to ascertain that the Perak River haplotype does not occur in stingrays of the Pahang River.

**KEY WORDS:** Haplotype · *Fluvi-trygon* · eDNA · Pahang River · Perak River · Malaysia

Alshari et al. (2023) presented additional evidence that was not available to us at the time our paper (Lim & Then 2022) was published, specifically the mitochondrial cytochrome *c* oxidase I (COI) sequences of 3 individuals of freshwater stingray *Fluvi-trygon kittipongi* from the Pahang River that our eDNA barcoding study intended to detect. The sequences of the 3 Pahang rays (Pahang haplotype) were shown to be identical to each other but displayed 4 nucleotide differences when compared against sequences from our Perak River rays (Perak haplotype) and the eDNA supposedly recovered from the Pahang water samples. This evidence was presented as a strong case for eDNA contamination in our study, specifically from the Perak River ray individuals during handling of the Pahang River water samples.

While the results presented by Alshari et al. (2023) are important and highly useful to highlight the presence of a different COI haplotype in Pahang River rays, the evidence for definite contamination in our study based on 3 individuals is not robust (T. Kajita pers. comm.). Without population genetic studies using multiple *F. kittipongi* samples across multiple rivers (in the context of our work, both Pahang and Perak Rivers), it is difficult to ascertain that the Perak haplotype does not occur in the Pahang River. A phylogeographic study on freshwater snakehead fish *Chana striata* showed multiple shared haplotypes of the ND5 mitochondrial gene between individuals sampled in rivers in both eastern and western Peninsular Malaysia (Tan et al. 2012). Such a possibility of shared COI haplotypes cannot be ruled out for *F. kittipongi* without in-depth studies. The finding of 1

\*Corresponding author: keanchonglim@gmail.com