



Environmental DNA approach complements social media reports to detect an endangered freshwater stingray species in the wild

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ABSTRACT: Environmental DNA (eDNA) barcoding has emerged as an important non-invasive sampling technique for the detection of rare and endangered species that can be difficult to sample. Our objective was to develop a low-cost eDNA barcoding approach for the detection of an Endangered freshwater stingray species *Fluviatrygon kittipongi* in a tropical river system in Peninsular Malaysia. We designed a species-specific primer for a fragment of *F. kittipongi* cytochrome oxidase subunit I mtDNA (244 bp). The effectiveness of this primer to detect the stingray was evaluated using water samples taken from the upper and middle reaches of the Pahang River coupled with social media reports on sightings of *F. kittipongi*. Five of 14 water samples tested showed positive PCR amplification for the targeted species. These results represent the first successful application of eDNA to detect freshwater stingrays in Malaysia. Using a combination of freshly obtained carcass samples, social media reporting and target species eDNA detection, this study provides formal occurrence records of *F. kittipongi* in Malaysia in 3 major watersheds: the Perak, Pahang and Kelantan Rivers.

KEY WORDS: *Fluviatrygon kittipongi* · eDNA · Distribution · Malaysia · Pahang River · Non-invasive sampling

1. INTRODUCTION

A review of available studies suggest that there are at least 10 dasyatid stingray species known to inhabit or enter freshwater habitats of the geopolitical region of Southeast Asia (both mainland and islands): *Breviatrygon heterura* (Bleeker, 1852), *B. imbricata* (Bloch & Schneider, 1801), *Fluviatrygon kittipongi* (Vidthayanon & Roberts, 2005), *F. oxyrhyncha* (Sauvage, 1878), *F. signifer* (Compagno & Roberts, 1982), *Hemiatrygon laosensis* (Roberts & Karnasuta, 1987), *Makararaja chindwinensis* Roberts, 2007, *Pastinachus ater* (Macleay, 1883), *P. stellurostris* Last, Fahmi & Naylor, 2010 and *Urogymnus polylepis* (Bleeker, 1852) (Kottelat 2013, Last et al. 2016). Among these, the genera of *Fluvi-*

atrygon and *Makararaja* inhabit freshwater and occasionally explore brackish water or estuaries (Kottelat 2013, Last et al. 2016). Three *Fluviatrygon* species had been reported in Malaysian rivers (Yano et al. 2005, Last et al. 2010, Hasan et al. 2021a) and currently, *F. signifer* and *F. oxyrhyncha* are known from the Pahang River (Yano et al. 2005, Hasan et al. 2021a). Furthermore, captures of *F. cf. kittipongi* have recently been reported from the Pahang, Kelantan and Perak Rivers by local fisher groups on various social media platforms (Hasan et al. 2021b, K. C. Lim unpubl. data).

All 3 *Fluviatrygon* species occurring in Malaysia are listed as Endangered on the IUCN Red List (Compagno 2016a,b, Vidthayanon & Manjaji 2016) due to geographic range reduction to less than 5000 km²

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