Hidden in Plain Sight: freshwater whiprays of Southeast Asia and their ecological significance

JTF 6-2 Pillar II.
Promotion of sustainable aquaculture and resources enhancement in Southeast Asia

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Freshwater whiprays, belonging to the families Dasyatidae and Potamotrygonidae, are unique stingrays thriving in low-salinity environments such as rivers and lakes. Southeast Asia is the habitat of at least five whipray species belonging to the Dasyatidae family, namely: *Fluvitrygon signifer, F. kittipongi, F. oxyrhynchus, Urogymnus polylepis,* and *U. dalyensis*. SEAFDEC Marine Fishery Resources Development and Management Department (MFRDMD) explored the distribution patterns and conservation status of freshwater whiprays in Malaysia and Southeast Asia, highlighting the significant ecological roles and threats. Through DNA barcoding, the genetic diversity and phylogenetic relationships were examined among whipray populations, revealing distinct geographical clusters and considerable conservation concerns. The findings underscore the need for targeted conservation measures, including habitat protection, pollution control, and sustainable fishing practices, to ensure the survival and ecological integrity of freshwater whiprays. Continuous research and monitoring are essential to address the gaps in biodiversity knowledge and to implement effective conservation strategies in Southeast Asia.

Whiprays are primarily associated with marine ecosystems, favoring coastal and oceanic waters characterized by varying levels of salinity. They typically inhabit reefs, sandy bottoms, and seagrass beds. However, it is less commonly known that certain whipray species thrive in freshwater environments with low salinity, residing in rivers, lakes, and other bodies of freshwater throughout their lives. The Dasyatidae and Potamotrygonidae are the two families to which the freshwater whiprays belongs. Nonetheless, rather than whiprays, members of family Potamotrygonidae are more commonly referred to as freshwater stingrays. Within the family Dasyatidae, there are five identified species of freshwater whiprays (*Fluvitrygon signifer, F. kittipongi, F. oxyrhynchus, Urogymnus polylepis*, and *U. dalyensis*) which are primarily found in Southeast Asia and neighboring countries. In contrast, family Potamotrygonidae comprises 20 species that are known to exist in South America (Last *et al*., 2016a; Oldfield, 2005).

Freshwater whiprays are well-adapted to freshwater environments, possessing specialized respiratory systems designed to thrive in low-oxygen habitats common to slow-flowing rivers. Freshwater whiprays possess sophisticated osmoregulation mechanisms that enable them to manage the influx of water and maintain their internal salt balance, as the surrounding water has a lower salt concentration (hypotonic) compared to their bodily fluids (Ferreira & Silva, 2015). If these species were to inhabit marine environments with higher salt concentrations (hypertonic), they would struggle to maintain their osmotic balance, risking dehydration and potentially severe electrolyte imbalances. While marine whiprays exhibit varied movement and migration patterns in response to ocean currents, seasonal changes, and reproductive cycles, freshwater whiprays generally remain localized to their freshwater habitats (Charvet-Almeida & Almeida, 2018). Freshwater whiprays inhabit a range of depths from shallow waters to deeper pools, and they are known to migrate to deeper areas to escape desiccation and elevated temperatures during the dry season (Campbell *et al*., 2023).

As part of the aquatic ecosystems, the existence of freshwater whiprays contributes to biodiversity and helps preserve ecological functions. As predators, they help maintain the balance of species within their habitats by controlling populations of prey such as small fish and invertebrates. It helps prevent any single species from dominating the ecosystem, thereby promoting a more diverse and stable community. Additionally, freshwater whiprays are involved in nutrient cycling within aquatic ecosystems. Their feeding activities disturb sediments, which can release nutrients trapped in the substrate back into the water column, making them available for other organisms. This process is essential for maintaining the productivity of the ecosystem, particularly in nutrient-poor environments. This bioturbation can make small habitats for other species, which increases the complexity of the environment and supports biodiversity (Tocker, 2021).

However, freshwater whiprays face numerous threats, including habitat destruction, deforestation, pollution, overfishing, and dam construction, and riverbank modification. Dams change the flow and sediment dynamics of rivers and impede migration routes, while pollutants introduced into aquatic systems by urban, agricultural, and industrial effluents adversely affect water quality. Moreover, pesticides, heavy metals, and other materials can accumulate in whiprays and harm the species health and ability to reproduce. Often caught as bycatch in fisheries targeting other species, they are also harvested for their meat and aquarium trade. Furthermore, overfishing reduces freshwater whipray populations and jeopardizes their genetic diversity (Dulvy *et al*., 2014).

For this reason, conservation efforts are crucial to protect freshwater whiprays and their habitats. While certain species in the Dasyatidae family are endangered or of least concern, all species in the Potamotrygonidae family are listed in CITES Appendix II. Establishing protected areas of this resource can help preserve critical environments. Pollution control is also important in ensuring safe habitats for their recruitment by implementing stricter regulations, especially on industrial and agricultural runoff to improve water quality. Furthermore, continuously promoting sustainable fishing practices such as reducing bycatch and releasing juveniles can help maintain populations of freshwater whiprays. In addition, studies aimed at improving our knowledge of the ecology, biology, and behavior of whiprays as well as population monitoring can be used to put conservation measures into practice.

Southeast Asia is well known for its rich biodiversity and unique freshwater environments which are habitats to at least five species of freshwater whiprays belonging to the Dasyatidae family. There is a need to investigate the distribution of freshwater whiprays to pinpoint significant habitats and hotspots for biodiversity that are necessary information for planning conservation efforts and managing the resources. Also, this information would shield these species from hazards like pollution and habitat loss. the SEAFDEC Marine Fishery Resources Development and Management Department (MFRDMD) investigated the geographical distribution, morphological characteristics, and genetic diversity of freshwater whipray species in Southeast Asia focusing on Malaysia under the project "Research for Enhancement of Sustainable Utilization and Management of Sharks and Rays in the Southeast Asian Region" implemented by SEAFDEC/MFRDMD in collaboration with Department of Fisheries Malaysia with support from the Japanese Trust Fund. The Project to the provisions of the Resolution and Plan of Action on Sustainable Fisheries for Food Security for the ASEAN Region Towards 2030 (RES&POA-2030) specifically RES No. 12 *Strengthen knowledge, including local knowledge, and science-based development and management of fisheries by enhancing the national capacity to collect, analyze, and share fisheries data and information;* POA No. 5 *Strengthen the collection of data and information, where relevant, on species under international concern, e.g. sharks and rays, sea turtles, catadromous eels, aquatic mammals, etc., and harmonize/standardize data collection methods among countries in the region; and* POA No. 82 *Strengthen cooperation and mechanism among AMSs to work towards common positions that could be reflected in international fish trade-related fora*. Since 2015, MFRDMD collaborated with stakeholders, especially local fishers, for data and sample collection from Malaysia particularly in Beluran, Perak, Pahang, Kelantan, Johor, and Terengganu.

Geographical distribution

In 2016, *F. kittipongi* was discovered in Perak, marking a new record in Malaysia. This discovery is significant because this species was only recorded in Indonesia and Thailand (Last *et al*., 2016a). All samples collected from Pahang and Kelantan were confirmed as *F. signifier*. The distribution of these five species of freshwater whipray in the Southeast Asian region is depicted in **Figure 1** and listed in **Table 1** with data supplemented by Windusari *et al*., (2019) for *U. dalyensis*, Oktaviyani and Fahmi (2024) for *U. cf. dalyensis*, and species occurrence records from the Global Biodiversity Information Facility database (GBIF, 2024).

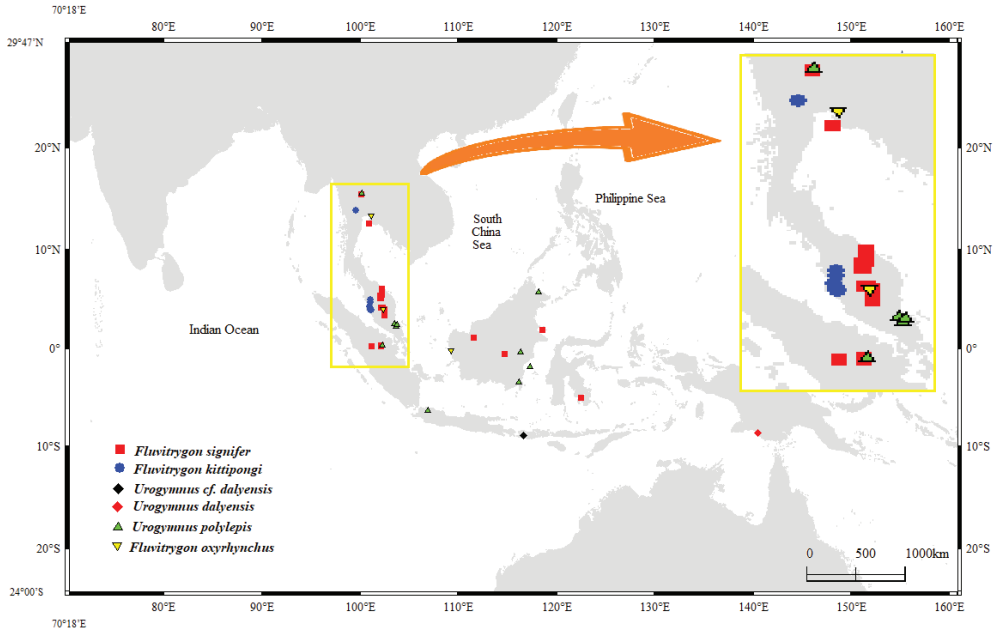

Figure 1. Distribution of freshwater whiprays in the Southeast Asian region (Indonesia, Malaysia, and Thailand)

Table 1. Distribution of freshwater whiprays in Malaysia

Species	Sampling site	No. of specimen	Year of sampling
Fluvitrygon signifer	Pahang • Sungai* Pahang (Temerloh) • Sungai Pahang (Kuala Lipis) • Sungai Pahang (Jerantut)	13	2018; 2022
	Kelantan • Sungai Kelantan (Pasir Mas) • Sungai Kelantan (Kuala Krai) • Sungai Nenggiri (Gua Musang) • Sungai Galas (Gua Musang) • Sungai Lebir (Gua Musang)	13	2021; 2022; 2023
Fluvitrygon kittipong	Perak • Sungai Perak	11	2016; 2021; 2022
Urogymnus polylepis	Sabah • Sungai. Kinabatangan, Sandakan • Sungai Beluran, Beluran	3	2015; 2023
	Johor • Sungai Sembrong (Kluang) • Sungai Endau Mersing (Mersing)	5	2021; 2022; 2023
	Terengganu • Sungai Kemaman, Kemaman	1	2024

*River

Morphological characteristics

Freshwater whiprays have a distinctive morphology well-adapted to their riverine habitats. They have a flattened, disc-shaped body that is wider than it is long. This shape helps them blend into the riverbed and remain undetected by predators and prey. The disc is formed by the expansion of the pectoral fins, which extend forward and fuse with the head, giving the whipray its flattened shape. The tail is elongated, slim, and like a whip, frequently reaching well beyond the disc. It is typically equipped with one or more venomous spines which are used for self-defence. Positioned on the top of the head, the eyes allow the whipray to see while remaining buried under sand or mud. Located just behind the eyes, spiracles are small openings that allow the whipray to draw water in for respiration without needing to expose its gills, which are located on the underside of the body. Freshwater whiprays can vary in size depending on the species, with disc widths ranging from about 30 cm to over 1 m in larger species. These morphological characteristics make them fascinating and unique among aquatic creatures, with adaptations that suit their specific ecological niches. The basic morphological measurements of whipray are shown in **Figure 2** (Last *et al*., 2016a).

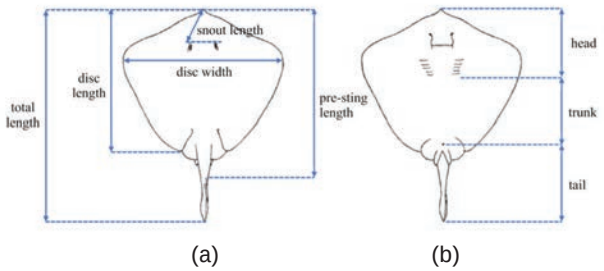

Figure 2. Morphological measurements (a) ventral view (b) dorsal view

Fluvitrygon signifer (Compagno & Roberts, 1982)

The white-edge whipray (*F. signifer*) (**Figure 3**), previously named *Himantura signifer*, is a small whipray with an oval to round disk with a disc width of 37 cm and features a pointed snout. The color of the dorsal surface is greyish brown with a distinct white marginal band. There are white spots anterior of orbits and posterior of spiracles. The tail behind the caudal sting is white and often features two stings. This species is found in sandy substrates in estuaries and rivers in Indonesia, Malaysia, and Thailand (Last *et al*., 2016a), and is categorized as "Endangered" in the Red List of Threatened Species by the International Union for Conservation of Nature (IUCN) (Grant *et al*., 2021a).

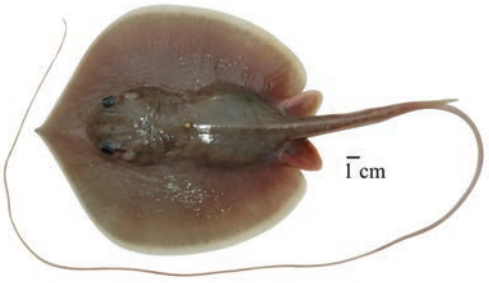

Figure 3. *Fluvitrygon signifer* (Compagno & Roberts, 1982)

***Fluvitrygon kittipongi* (Vidthayanon & Roberts, 2005)**

The roughback whipray (*F. kittipongi*) (**Figure 4**), formerly known as *Himantura kittipongi*, is a small species with a round or oval-shaped disk with smooth edges and a diameter of up to 60 cm. Its long, thin tail is equipped with venomous stings and densely covered with denticles behind the stings (Froese & Pauly, 2023). This species is inhabiting in the mainstream of the Maekhlong River in Thailand (Vidthayanon & Roberts, 2005) and Sungai Perak, Malaysia (Hamizah *et al*., 2021). This species has been assessed as "Endangered" by IUCN Red List of Threatened Species (Grant *et al*., 2021b).

Figure 4. *Fluvitrygon kittipongi* (Vidthayanon & Roberts, 2005)

***Fluvitrygon oxyrhynchus* (Sauvage, 1878)**

The marbled whipray (**Figure 5**), formerly recognized as *Himantura oxyrhyncha* and occasionally misidentified as *Himantura krempfi* (Chebanaund, 1923), is part of the Mekong freshwater whipray population, though its biology remains poorly understood. *F. oxyrhynchus* has been recorded from the Mekong River, Cambodia (Compagno Roberts, 1982); Chao Phraya River, Thailand (Cook & Compagno, 1994); Mahakam River, (Last *et al*., 2010); Musi River, Indonesia (Iqbal *et al*., 2017); Viet Nam (Last *et al*., 2016); and Kuala Lipis, Pahang, Malaysia (Hasan *et al*., 2021). This species has been assessed as "Endangered" in IUCN Red List of Threatened Species (Grant *et al*., 2021c).

Figure 5. *Fluvitrygon oxyrhynchus* (Sauvage, 1878)

***Urogymnus polylepis* (Bleeker, 1852)**

The giant freshwater whipray (*Urogymnus polylepis*) (**Figure 6**), initially classified as *Himantura chaophraya*, ranks among the largest freshwater fish globally, with specimens reaching diameters of up to 2.4 m and weights exceeding 600.0 kg. Equipped with one or more venomous stings on its tail for defense, this species inhabits various freshwater systems across Southeast Asia, including the Mekong, Chao Phraya, and other major river basins in Cambodia, Indonesia, Malaysia, Thailand, and Viet Nam, as well as parts of New Guinea and northern Australia (Froese & Pauly, 2023). Its status on the IUCN Red List of Threatened Species is 'Endangered' (Grant *et al*., 2021d).

Figure 6. *Urogymnus polylepis* (Bleeker, 1852)

***Urogymnus dalyensis* (Last & Manjaji-Matsumoto, 2008)**

The freshwater whipray (*Urogymnus dalyensis*) (**Figure 7**), was previously described as *Himantura chaophraya* (Last & Stevens, 1994; Monkolprasit & Roberts, 1990). Specimens from Australia and Papua New Guinea were then split to become *Himantura dalyensis* (Last & Manjaji-Matsumoto, 2008). More recent revisions have been made within the Dasyatidae family and the species is now called *Urogymnus dalyensis* (Last *et al*., 2016a; Last *et al*., 2016b; Froese & Pauly, 2023). A single individual of *U. dalyensis* based on photographic evidence was recorded at Lampu Satu Beach, Merauke District, Papua Province, Indonesia (Windusari *et al*., 2019). While, a specimen of *U.* cf. *dalyensis* was recorded at East Lombok, West Nusa Tenggara Province, Indonesia in July 2020 (Oktaviyani & Fahmi, 2024). Its status on the IUCN Red List of Threatened Species is 'Least Concern' (Kyne, 2016).

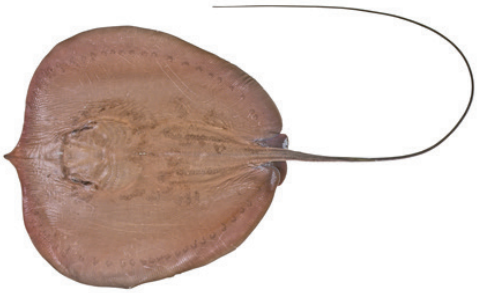

Figure 7. *Urogymnus dalyensis* (Photo: CSIRO)

Phylogenetic relationship and genetic diversity

DNA barcoding serves as a genetic marker for identifying, classifying, and studying the evolutionary traits of organisms. The mitochondrial cytochrome c oxidase 1 (CO1) gene is particularly effective as a DNA barcode for distinguishing between vertebrate and invertebrate species. These barcodes, which consist of short gene sequences from a standard part of the genome, provide unique identifiers for species recovery and characterization. Since many closely related shark and ray species exhibit similar morphological traits, differentiation often requires expert taxonomists. DNA-based identification has successfully identified numerous cryptic taxonomic forms. The reliability of DNA barcoding has been proven for ray identification across various regions. DNA barcoding has accurately identified over 95 % of aquatic species (Savolainen *et al*., 2005; Ward *et al*., 2009).

Since 2013 until now, MFRDMD has collected biological data on freshwater whiprays from fisher catch reports, surveys in local markets, and collaboration with local fishers. MFRDMD successfully DNA barcoded 49 individuals of freshwater whiprays. A phylogenetic tree was constructed utilizing the sequences identified by MFRDMD alongside those accessible in the National Center for Biotechnology Information (NCBI) GenBank. The neighbor-joining tree (**Figure 8**) demonstrates distinct phylogenetic and phylogeographic pattern. All 64 sequences, including the outgroup *Chiloscyllium punctatum*, were clearly categorized into their respective genera. The tree is predominantly split into three main clades: *F. signifer*, *F. kittipongi*, and *U. polylepis.* For *F. signifer* clade, samples from Pahang (PTM) and Kelantan (PSKN) were included. These samples were further classified into two geographical clusters: Pahang and Kelantan, with bootstrap values of 62 % (Pahang), 68 % (Kelantan), and a robust node at 98 % supporting the Kelantan cluster. *F. kittipongi* clade

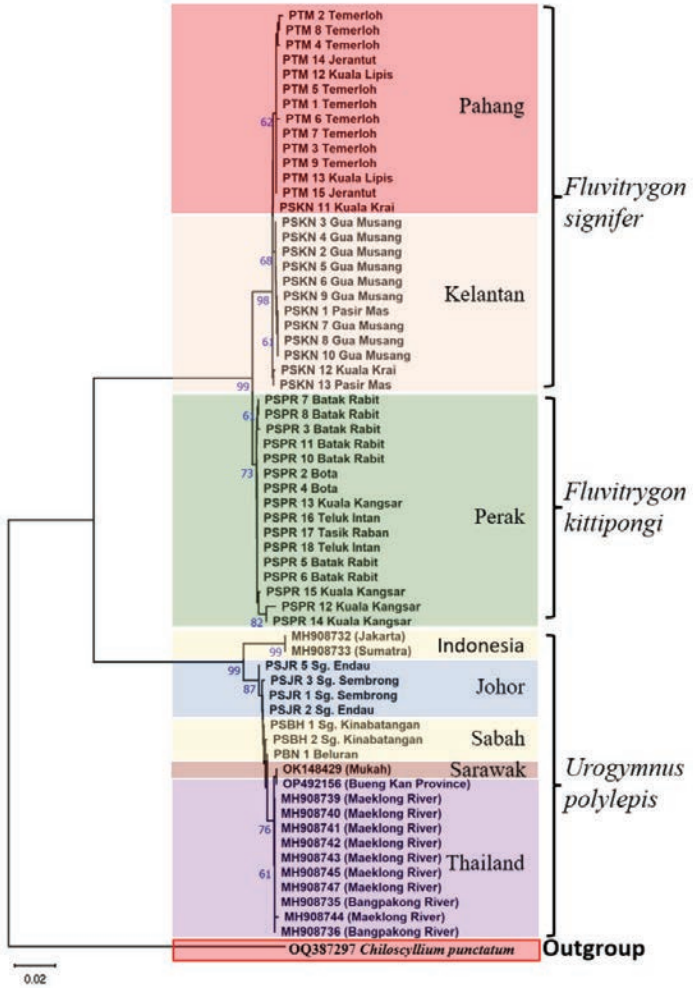

Figure 8. Neighbor-joining tree for freshwater whiprays including data from GenBank with *Chiloscyllium punctatum* as an outgroup

encompassed samples exclusively from Perak (PSPR), displaying three sub-clusters with bootstrap values of 61 %, 73 %, and 82 %. As for *U. polylepis*, this clade revealed several geographical clusters: Malaysia (Johor, Sabah, Sarawak), Thailand, and Indonesia. Indonesian samples exhibited the highest bootstrap value of 99 %. Thailand cluster showed significant support with bootstrap values of 76 % and 61 %, although a sample from Mukah (Malaysia) was also incorporated into this subclade.

The tree suggests a closer relationship between *F. signifer* and *F. kittipongi* than either is to *U. polylepis*. The findings indicate that all samples from Pahang and Kelantan are *F. signifer*; however, they are represented as different stocks within separate sub-clades. Conversely, all samples from Perak belong to *F. kittipongi*. The average genetic distance within species was very low at 0.39 % and the interspecies genetic distance measured was also low at 1.21 %. Genetic diversity is a cornerstone of effective conservation and management of freshwater ray populations. By preserving and enhancing genetic variation, conservationists can ensure that these species remain resilient and adaptable to future challenges, thereby securing their long-term survival and ecological roles in their natural habitats.

Way Forward

MFRDMD will continue the collection of specimens of freshwater whiprays and regular monitoring of their populations in major rivers and tributaries in Malaysia to obtain more knowledge of their ecology and behavior. Since the information on the biodiversity of this resource in Southeast Asia is limited, the SEAFDEC Member Countries are urged to conduct research and monitoring of freshwater whiprays. The implementation of science-based conservation measures for the protection and preservation of freshwater whiprays and their ecosystems in Southeast Asia are critical for their survival, particularly for giant freshwater whipray which is vulnerable due to its size and specific habitat requirements.

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