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Updated distribution of the endangered freshwater stingray *Urogymnus polylepis* in Malaysia, with notes on biology and genetics

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Abstract. The giant freshwater stingray *Urogymnus polylepis* (Bleeker, 1852) (family Dasyatidae) is an endemic species in the Southeast Asian region, but comprehensive records of the distribution of this species within local river systems and their biology are still lacking. We reviewed current information for this species based on media reports, published literature as well as field observations. Two direct encounters of this species in Mukah, Sarawak, and in Sandakan, Sabah, confirmed recent occurrences of this species in Malaysian Borneo with the finding of an adult female with four pups. Public wildlife sighting information also provided additional 29 novel sightings of this species in Malaysia, out of which four were first records at the locations of Kukup and Sungai Sembrong in Johor, Pulau Bruit in Sarawak, and Tanjung Batu Laut in Sabah. These records suggested relatively high capture rates by trawlers in coastal areas and that the animals are closely associated with clean river systems. Genetic analysis showed paraphyly in *U. polylepis* with the formation of two major clades; one from the Gulf of Thailand and Malaysian Borneo, and the other from the Andaman Sea region and Indonesia. Threats to *U. polylepis* due to their own biological uniqueness, rarity of their occurrence, increasing level of river pollution, and potentially isolated populations highlight the urgent need for formal protection of the species.

Key words. giant freshwater stingray, social media, occurrence, river, Borneo, Peninsular Malaysia

INTRODUCTION

Urogymnus polylepis (Bleeker, 1852) is a large species of freshwater stingray in the family Dasyatidae. Previously known as *Himantura chaophraya* and *H. polylepis*, this species has undergone recent taxonomic revisions upon comparison of the holotype of *Trygon polylepis* in Java, Indonesia with specimens from various countries (including Malaysia), supported by additional molecular evidence (Last & Manjaji-Matsumoto, 2008; Last et al., 2016a). *Urogymnus* was previously a monotypic genus with a single species (*U. asperrimus*, porcupine ray). The genus currently has five additional valid species, namely *U. acanthobothrium*, *U. dalyensis*, *U. granulatus*, *U. lobistoma*, and *U. polylepis* (Last et al., 2016a; Last et al., 2016b). Four of these species (*U. asperrimus*, *U. granulatus*, *U. lobistoma*, and *U. polylepis*) have been recorded in Malaysian waters (Kottelat, 1998; Yano et al., 2005; Manjaji-Matsumoto & Last, 2006; Last et al., 2010). These species are mainly found in marine environments except *U. dalyensis* and *U. polylepis* (Chin & Compagno, 2016; Kyne, 2016; Manjaji-Matsumoto et al., 2020; Sherman et al., 2020; Grant et al., 2021; Rigby et al., 2021).

The giant freshwater stingray appears to be restricted in distribution to major rivers in South Asia and Southeast Asia (Vidthayanon et al., 2016; Sen et al., 2020; Grant et al., 2021). Published records indicate that this species occurs in at least seven countries in the South and Southeast Asia region, ranging from India to eastern Indonesia (Last et al., 2010). Recent distributional records confirmed the occurrence of this species in Sumatra, Indonesia, an island located across the Malacca Straits from Peninsular Malaysia with at least 12 sightings from 2008 to 2016 (Iqbal & Yustian, 2016). The ray was reported to occur both in river mouths as well as all the way up to 170 km upstream (Iqbal & Yustian, 2016). In addition, this species has also been reported in Indonesian Borneo in Kalimantan rivers with at least 16 sightings within the same period (Iqbal et al., 2020a). The name 'freshwater stingray' is somewhat of a misnomer as *U. polylepis* is euryhaline in nature and uses freshwater, brackish and inshore marine environments (Grant et al., 2021).

The species is listed as Endangered in the 2021 International Union for Conservation of Nature (IUCN) Red List of Threatened Species (Grant et al., 2021). Genetic information on the species is highly limited but distinct molecular differences between specimens from India and Thailand suggest that subpopulations are likely geographically isolated with very limited exchange, if any, between the subpopulations (Sezaki et al., 1999). Given the freshwater

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association of the species, *U. polylepis* is likely to be highly vulnerable to land use changes that impact riverine systems. Major threats noted for this species include riverine system modification, water pollution from domestic, industrial and agricultural sources, as well as fishing exploitation (Vidthayanon et al., 2016; Grant et al., 2021). Other anthropogenic threats to the animals are construction of hydroelectric and reservoir dams that potentially impede their movement upstream (Chong et al., 2010). Clarifying precise distribution information of the species within river drainages is important for accurate species inventory, especially in the context of river-related Environmental Impact Assessments (EIAs), and to inform targeted conservation and river management efforts.

Presently, it is known that this species measures about 30 cm disc width (DW) at birth and can grow up to the size of 250 cm DW, weighing about 600 kg (Phomikong et al., 2019; Campbell et al., 2020). The age of sexual maturity of the species was not well documented but male *U. polylepis* attained maturity at about 110 cm DW (Last et al., 2010). Examination of the vertebrae of *U. polylepis* populations in Thailand indicated that their current lifespan is about 12 years with a maximum DW of 250 cm recorded (Phomikong et al., 2019). However, caution has been raised about this estimate due to the lack of age validation, and it is likely that the maximum age is considerably older (Grant et al., 2021). A study on the ray population in Cambodia documented a drastic decrease (largest at about 300 cm DW) in the size of captured rays within the last 20 years which was mainly caused by overexploitation from multiple fishing techniques as well as habitat degradation due to large dam construction and pollution driven by rapid human population growth (Campbell et al., 2020). Based on an extensive search, there is no formally reported number of pup(s) per female, although litter sizes of one to two pups with the possibility of up to three pups have been observed (see Grant et al., 2021).

The rarity of encounters with *U. polylepis* during formal scientific surveys posed a challenge for thorough evaluation of the biology and distribution of the species. In Malaysia, *U. polylepis* has been formally recorded only in the Kinabatangan and Padas rivers located in Sabah's eastern and western drainages respectively (Compagno, 2002; Manjaji, 2002; Last et al., 2010, DOFM, 2014) but recent findings based on citizen science sources revealed 17 new sightings at multiple locations in Malaysian Borneo (Windusari et al., 2020) and seven new sightings at multiple locations in Peninsular Malaysia (Iqbal et al., 2020b). In addition, two sightings at local fish markets were reported from a three-year extensive elasmobranch survey in Kuching, Sarawak (Booth et al., 2021). From a conservation standpoint, *U. polylepis* is not protected legally in Malaysia despite its endangered status.

Against this background, this research sought to address existing knowledge gaps for this species via updated collation of available occurrence information of the species across Malaysia with biological and genetic notes using data derived from direct field surveys, published literature, and from media reports. The findings and implications for conservation action for this species are discussed with biodiversity considerations within river management plans in Malaysia.

MATERIALS AND METHODS

We searched for reported occurrences of *U. polylepis* from online Malaysian-based news portals and social media platforms that were accompanied by photographic or video evidence. We then personally confirmed these individual records and excluded records that were not definitive, specifically those without visual confirmation. Photographic confirmation of species identity was possible due to the large size and almost circular disc profile with a long pointed anterior tip, and distinctive broad blackish border along lateral margins of the ventral side of the disc (Last et al., 2010). The species can be distinguished from another similar looking species, *U. dalyensis*, by being larger (maximum recorded disc width 250 cm (Phomikong et al., 2019), vs 124 cm for *U. dalyensis* (Last & Manjaji-Matsumoto, 2008)), and in possessing "a less truncate anterior disc margin, more acute snout (angle 112–117°, vs. 120–121° in *U. dalyensis*) with a relatively larger apical lobe" (Last & Manjaji-Matsumoto, 2008). We collated accompanying information of reported size (weight and disc width) where available. Where possible, we also determined sex from the photographs. For completeness, we also included reported citizen sightings that had been collated by recently published studies (Windusari et al., 2019; Iqbal et al., 2020a).

We also collated available biological information from direct encounters of the ray during our site surveys conducted from the years 2015 to 2018 that covered most major ray-fishing states in Peninsular Malaysia, Sarawak and Sabah. Disc width, weight, and sex were recorded when possible, and tissue samples were collected for molecular analysis. The studied ray species is not legally protected in Malaysia. All rays were dead when encountered at the landing sites. DNA extraction was performed using 10% Chelex resin based on the modified protocol of Hyde et al. (2005), under incubation period of 2 min at 60 °C then 25 min at 103 °C. The Cytochrome Oxidase Subunit I (COI) gene was amplified by polymerase chain reaction (PCR) using the universal primer FishF2 (5′ TCG ACT AAT CAT AAA GAT ATC GGC AC 3′) and FishR2 (5′ACT TCA GGG TGA CCG AAG AAT CAG AA 3′) (Ward et al., 2008). The PCR amplification was performed using a 20 µL reaction mix containing 2 µL of 10x PCR buffer, 0.5 µL of dNTPs mixture (2.5mM each), 1 µL of 10 pmol primer (both primers), 1.25 unit of Taq DNA polymerase (iNtRON Biotechnology, INC., Korea), 1 µL of 50 pg to 1.0 µg DNA templates, and molecular-grade water. The PCR cycles for the COI gene consisted of 2 min initial denaturation at 94°C, followed by 30 cycles of 20 sec at 94 °C, 20 sec at 50 °C, 1 min at 72 °C and with final extension of 5 min at 72 °C. The PCR products were examined using 1% agarose in TAE buffer prior to Sanger sequencing service at Apical Scientific Sdn Bhd (Selangor, Malaysia).

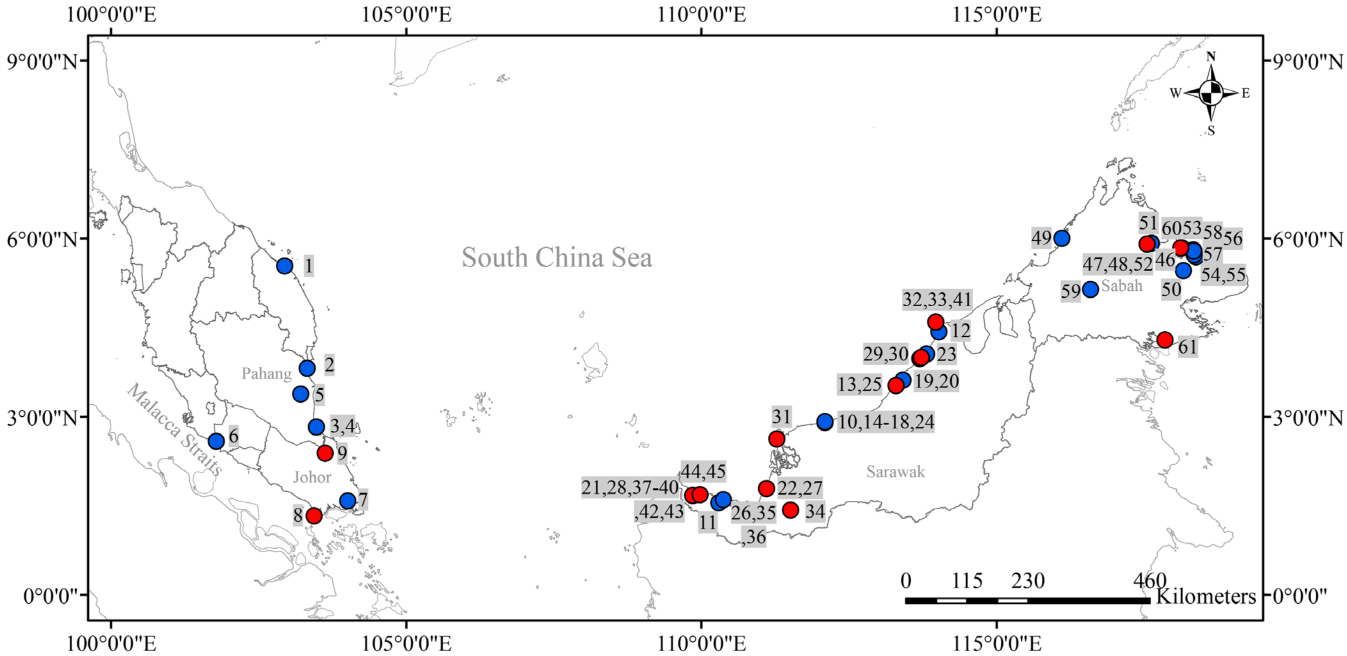

Fig. 1. Updated distribution map of *Urogymnus polylepis* in Malaysia based on citizen reports and direct sightings between 2011–2021). Blue = published sightings, red = unpublished sightings (see Appendices 3 and 4 for detailed information on individual sightings and river location).

Available sequences of *U. polylepis* and its sister species *U. dalyensis* and *U. granulatus* (as outgroup) in the NCBI Genbank were included in the following analysis (Appendix 1). Sequences were reviewed manually using BioEdit (Hall, 1999), aligned using ClustalX (Thompson et al., 1997) and finally trimmed using BioEdit (Hall, 1999). The aligned sequences were subjected to the best model search based on Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) for Maximum Likelihood (ML) and Bayesian Inference (BI) analysis respectively using Kakusan v.3 (Tanabe, 2007). ML analysis was performed under TIM + Gamma model with 1000 bootstrap replicates using Treefinder (Jobb et al., 2004). BI analysis under HKY85 model was performed using MrBayes (Huelsenbeck & Ronquist, 2001). This analysis was initiated with a random starting tree and two parallel runs, each of which consisted of running four chains of Markov chain Monte Carlo (MCMC) iterations for 2,000,000 generations (sampled every 100th generation each chain). Both the parameters "sump burnin" and "sumt burnin" were set as 5000 and the remaining tree samples after burn-in were used to calculate posterior probabilities. Uncorrected p-distance was calculated using PAUP* 40b10 software (Swofford, 2002) to assess the genetic divergence among compared sequences.

Length-weight relationship for the ray was determined following the equation $W = aL^b$, where W is body weight and L is the disc width. Media reports of sightings accompanied by disc width and weight information were uncommon in the records we collated. Therefore, we expanded our search to other regions for additional length-weight information (Appendix 2).

To better understand characteristics of the riverine systems that are reportedly used by the ray, we collated available published information of length, width (minimum and maximum), and bottom substrate of these rivers and supplemented missing information on the first two variables by measuring them using the calibrated ruler function on Google Earth. Although these measurements using the Google Earth application are relatively crude, they can provide reasonable estimates of widths of rivers that may support viable populations of the giant stingrays. Assessment of water quality index and resulting river cleanliness categories as defined by the Department of Environment Malaysia (DOE) were collated as well (DOE, 2017, 2021).

RESULTS

The search from online news and social media sources revealed 29 novel reported captures of *U. polylepis* from 2014 to March 2022 in four major states in Malaysia, namely Pahang and Johor in Peninsular Malaysia, and Sarawak and Sabah in Malaysian Borneo (Fig. 1 & Appendix 3). Our field surveys across Malaysia since the year 2015 yielded two direct encounters of the rays in Sarawak and Sabah. Inclusion of captures reported by Manjaji-Matsumoto et al. (2017), Iqbal et al. (2020b), Windusari et al. (2020), and Booth et al. (2021) gave a total record of 61 captures of *Urogymnus polylepis* in Malaysia in the past decade from July 2011 to March 2022 (Appendix 4).

All recorded captures were of single individuals, with the exception of two records in 2016 — one direct field encounter of a female with four pups caught in Mukah, Sarawak, and a news report of two animals from Kuala Nyalau, Sarawak. Available reported individual weights ranged from 16 to 500 kg (Appendix 4). Where available, the fishing gear reportedly used to capture these animals were mainly bottom trawlers

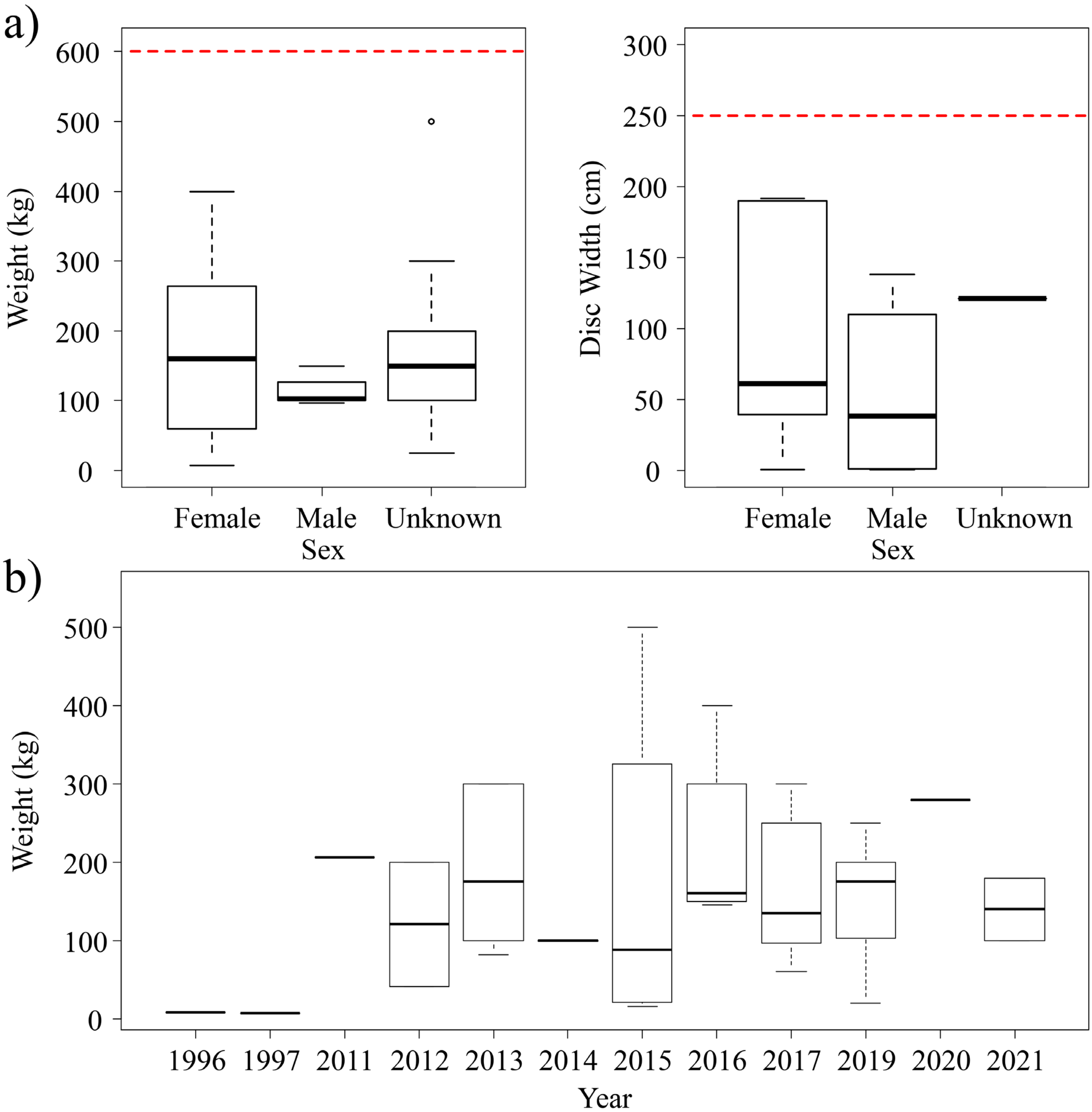

Fig. 2. a, Size range (left—weight in kg, right—disc width in cm) by sex for *Urogymnus polylepis* sighted in Malaysia in comparison with maximum sizes reported from specimens in Thailand (Weight of 600 kg in Monkolprasit & Roberts, 1990; Disc width of 250 cm in Phomikong et al., 2019). b, Number of sightings over period of time and size range of animals sighted in Malaysia.

(n = 13, 43%), as well as hook and line (n = 12, 40%), although longlines also captured these fish (n = 5, 17%).

The sex of 44% (n = 27) of the records could not be determined, while 36% (n = 22) were females and 20% (n = 12) were males. Based on available information for sexed animals, the females caught were generally larger than the males in both weight and disc width (Fig. 2a). Reported capture sizes in the 1990s from scientific surveys were relatively small, i.e. 0.4–1.2 kg (Manjaji, 2002); however, the reported sizes of animals caught from 2012 onwards that came mainly from social media platforms were generally much larger with an average weight of 176 kg (Fig. 2b). The largest reported stingray was a female from Kampung Kolapis, Sandakan (Sabah) weighing 500 kg. Based on available data (n = 9, Appendix 2), estimated length-weight relation relationship was $W = 1.16 \times 10^{-4} L^{2.76}$ (Fig. 3).

From our fisheries survey in Mukah, Sarawak, on 4 April 2016, we encountered a butchered adult female *U. polylepis* (DW length = 190 cm) in a local fish market which was landed at the adjacent fish landing site together with four fully developed pups (Fig. 4). Casual conversation with a fishmonger suggested that the rays were caught using trawl

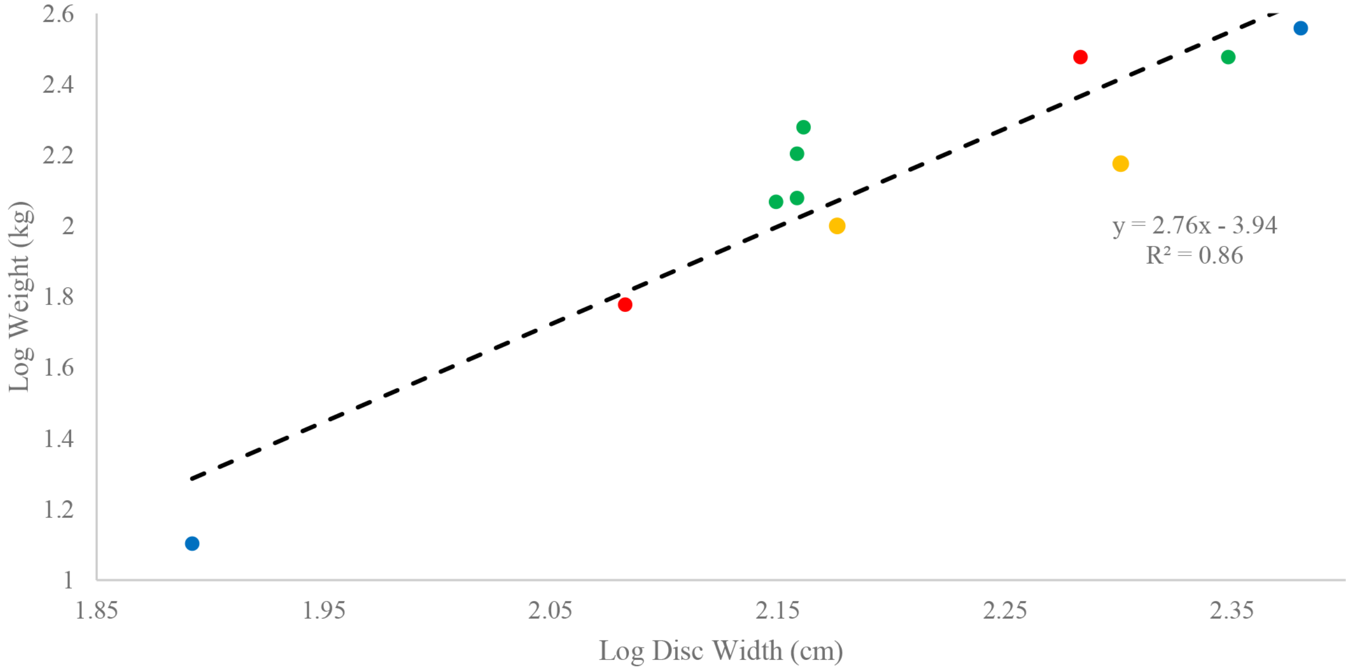

Fig. 3. Length-weight relationship for *Urogymnus polylepis*. Red = Malaysia, Blue = Thailand, Yellow = Indonesia, Green = India.

Table 1. Uncorrected p-distance for COI gene among *Urogymnus polylepis* clades and its sister species.

	Clade 1	Clade 2	*U. dalyensis*	*U. granulatus*
Clade 1	0.07			
Clade 2	2.86	0.50		
U. dalyensis	9.68	9.86	–	
U. granulatus	12.18	11.87	11.89	0.63

nets. The pups, one female and three males, had disc widths ranging from 37.0 to 39.5 cm and disc length from 41.5 to 43.0 cm (Appendix 4). A survey trip to the Sandakan landing site in Sabah on 26 August 2018 also encountered an adult male *U. polylepis* (DW length = 120 cm) (Appendix 4). It is important to note that these survey trips were focused on marine and not freshwater fisheries.

Using all available sequences, the ML and BI combined tree for the COI gene (Fig. 5) showed that *U. polylepis* can be divided into two clades: Clade 1 (Gulf of Thailand, Malaysian Borneo) and Clade 2 (Bangladesh, India, and Indonesia) with high and full support bootstrap value (ML/BI = 99%/1.00). The average inter-clade genetic distance was 2.86% while the average distance between *U. polylepis* and *U. dalyensis* was 9.72% (Table 1). Genetic sequences of the two adult individuals (one female and one male) as well as two of the pups (one female and one male) have been uploaded on NCBI GenBank (Appendix 1).

Based on collated information, the characteristics of the Malaysian rivers that *U. polylepis* have reportedly been caught in range from 11 to 560 km in length with an average width of 320 m (Appendix 3). Available water quality assessments have categorised these rivers as either clean or slightly polluted (DOE, 2017); for the latter category, the pollution level was considered to be low enough for the rivers to remain suitable for sensitive aquatic species. For rivers where bottom substrate information was available, the rays appeared to use those with sandy or sandy-silty bottoms.

DISCUSSION

Although many of the occurrences of the endangered giant freshwater stingray in Malaysia have been reported before, this study is the first to comprehensively document the distribution of this endangered ray across Malaysia using a combination of field surveys and publicly available media information. The latter, although often not regarded as the gold standard for formal species records, has proven to be particularly useful in determining and tracking occurrences of rare, endangered species that are not easily sampled during conventional scientific surveys (Windusari et al., 2019; Windusari et al., 2020; Hasan et al., 2021). We also confirmed that a mature female *U. polylepis* can give birth to up to four pups and, to the best of our knowledge, provided the first empirical estimate of the length-weight relationship for the species, which appears to indicate negative allometric growth.

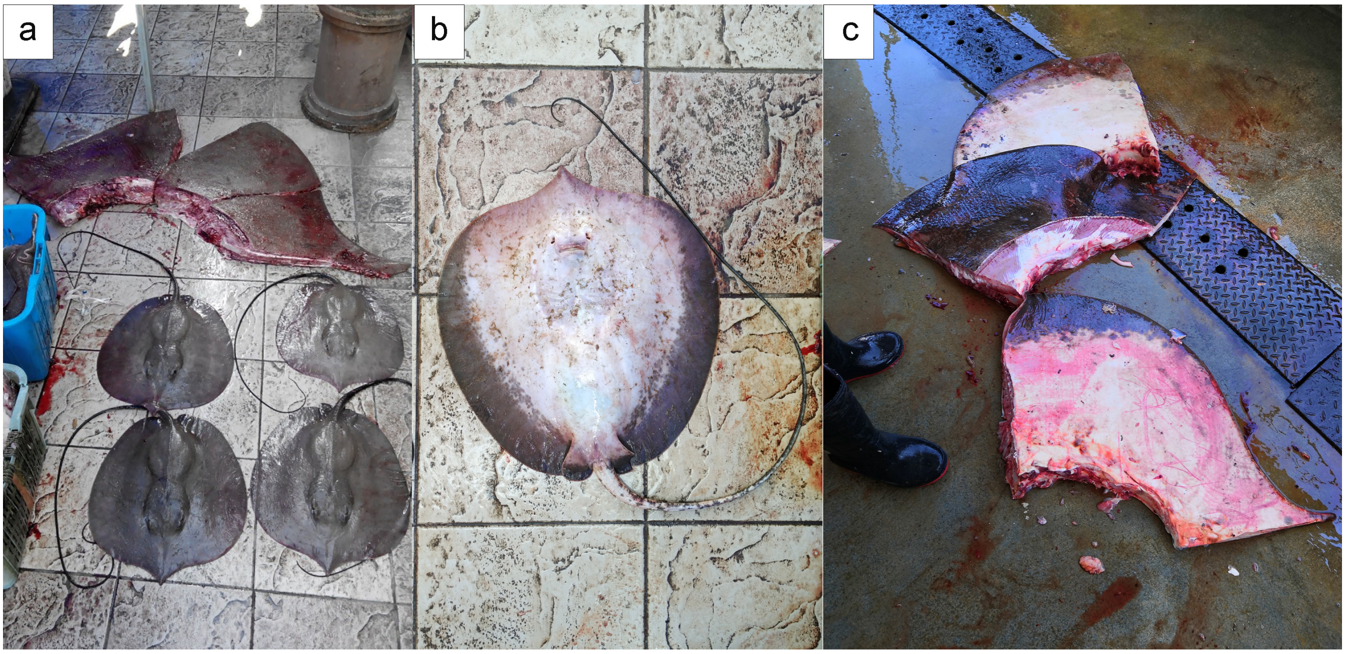

Fig. 4. a, Butchered female *Urogymnus polylepis* (disc width ca 190 cm) caught in the waters near Mukah, Sarawak in 2016 together with 4 fully developed pups. b, Underside of one of the pups. c, Butchered male *U. polylepis* (disc width ca 120 cm) caught in the waters near Sandakan, Sabah in 2018.

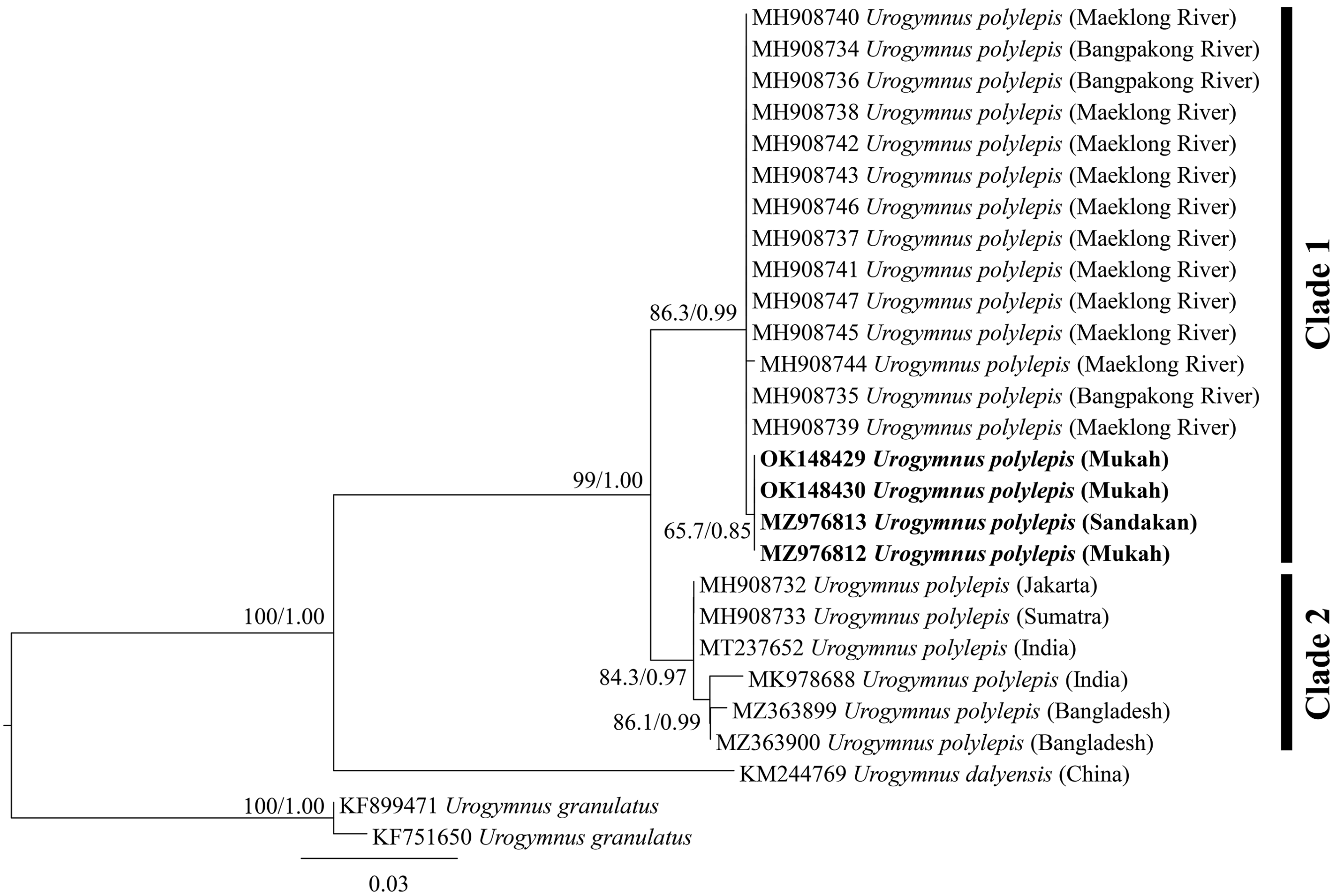

Fig. 5. COI gene phylogenetic relationships and genetic distance of *Urogymnus polylepis* and its sister species. The bootstrap values (ML/Bayesian Inference) are shown at the branches.

Compared to scientific surveys, social media and news reports are likely biased towards catches of larger animals for the species. Nevertheless, these information sources are still useful in estimating local maximum size, which could be a function of riverine size. Captured animals in Malaysia are comparable in size to those sampled from other more well-known river systems elsewhere, albeit of smaller maximum sizes than those reported from neighbouring Thailand (Monkolprasit & Roberts, 1990; Phomikong et al., 2019). To the best of our knowledge, there have been no reports of sexual dimorphism in growth for *U. polylepis* although sexual dimorphism is not uncommon for other closely related rays such as *Dasyatis hypostigma*, *Hemitrygon akajei*, and *Telatrygon biasa* that show sexual heterodontia (rounded teeth in female, sharp monocuspidate teeth in male) and *Brevitrygon heterura* that shows sexual dimorphism in the tail (filamentous tail in females, bulbous tail in males) (Nishida & Nakaya, 1988; Taniuchi & Shimizu, 1993; Last & Compagno, 1999; Rangel et al., 2014).

Out of the 150 major rivers in Malaysia, *U. polylepis* has been recorded in 25 river systems, many of which are considered large river basin areas, including the Pahang (drainage area 29,300 km^2, length 430 km), the Kinabatangan (16,581 km^2, 365km), the Rajang (51,315 km^2, 560 km), and the Baram (22,325 km^2, 402 km) rivers. A relatively constant series of reported sightings in the past decade suggest that Malaysian riverine systems, especially in Borneo, still support viable populations of these giant stingrays. While the riverine characteristics of the waterways where they have been observed suggest that they may be able to tolerate a small amount of riverine pollution, clean rivers with a predominantly sandy-bottomed substrate are important for their habitat use. Their association with sandy rivers has also been reported elsewhere (Iqbal & Yustian, 2016; Vidthayanon et al., 2016; Windusari et al., 2019a; Grant et al., 2021).

A major constraint in the management of rivers in Malaysia is that under the Constitution of Malaysia, the management of rivers is a state prerogative (Yusoff et al., 2006), and federal policies may not necessarily be implemented at the state level. The majority of riverine areas and their biodiversity fall outside the terrestrial protected area system in Malaysia. Current river management objectives focus primarily on flood mitigation, water supply, and pollution control while conservation of biodiversity receives little attention (UNDP, 2015). One viable solution identified is to mainstream biodiversity conservation principles in the management of local river basins (UNDP, 2015), and we recommend the inclusion of the giant freshwater stingray within the fish species checklists of rivers where they occur as a starting point for biodiversity-focused river management.

As outlined in the current National Plan of Action for Sharks (second version NPOA2; an updated version is expected to be undergoing review at the time of writing), the management of freshwater fish is presently under the jurisdiction of individual states (DOFM, 2014). As of 1994, only five states had enacted Inland Fisheries Regulations (Ismail, 1992). The NPOA2 documentation had earmarked *U. polylepis* as listed for protection under the Sabah Aquaculture and Inland Fisheries Enactment 2003, although this had yet to be implemented at the time of writing (Kissol L., pers. comm.). It was also outlined in the NPOA2 that the federal government needs to inform the state governments where the rays can be found so that the management of freshwater sharks and rays can be included within the rules of individual state fisheries and be harmonised (DOFM, 2014). Our study identified the key states of Sarawak, Sabah, Pahang, and Johor as states that can play critical roles in the protection of the giant freshwater stingray through formal recognition as a species of conservation concern.

Through casual conversation with local fishers in Mukah, we learned that the meat of *U. polylepis* was of 'soft' quality and was therefore less desirable as a food fish compared to the other large-sized rays, consistent with what was reported in Vidthayanon et al. (2016). The butchered female was sold for RM2 per kg (approximately USD 0.50 per kg) back in 2016 in the fishing village of Mukah, Sarawak. A number of the reported sightings came from recreational fishers and recreational fishery (in both freshwater and marine environments) in Malaysia is currently not regulated. The multiple factors of having unpalatable meat coupled with their unique, easily identified morphological characteristics, as well as relatively high incidences of capture of animals by recreational fishers indicate that formal protection of these animals, as mentioned earlier, and implementation of regulations for recreational fishing, specifically on the catch and release of these giant stingrays, may help boost the conservation prospects of the giant freshwater stingray.

Our study found that the use of trawlers in local coastal waters is responsible for a significant number of reported catches, especially in the state of Sarawak on Borneo. This was also found to be the case for *U. polylepis* in Kalimantan (Grant et al., 2021). In Malaysia, trawl nets are banned from use in coastal areas within 5 nautical miles (8 nm in some states) from the shoreline to avoid destruction of nursery habitats and also encroachment into artisanal fishing designated areas. This suggests that there is a possibility that these animals may be using coastal waters that are beyond 5 nm offshore, but the likelihood of illegal trawling in inshore waters cannot be ruled out. Given that this species uses both the freshwater as well as the estuarine and coastal portions of major Malaysian rivers, protection accorded to the species should be comprehensive and extend to listings within both freshwater and marine lists of protected species.

The paraphyly seen in the COI gene tree of *U. polylepis* suggests two distinct clades, one of individuals found in the Gulf of Thailand and Malaysian Borneo (South China Sea region) and the other clade of Indonesia, Bangladesh and India within the Bay of Bengal and Andaman Sea region. The existence of the two clades could be partly explained by geographical barriers that prevent mixing between the clades. A similar division can also be found in another freshwater taxon, striped snakehead (*Channa striata*) (Tan et al., 2012). In addition, this barrier has also been shown to similarly affect many other marine elasmobranch species such as *Carcharhinus sorrah* (Giles et al., 2014), *Chiloscyllium punctatum* (Lim et al., 2021), and *Rhynchobatus australiae* (Giles et al., 2016), and speciation in *Scoliodon* 'species' (Lim et al. 2022) and *Neotrygon* species (Borsa et al., 2018). We recommend that additional morphometric and molecular analyses of *U. polylepis* be carried out in the future to clarify the phylogenetic relationships between different clades.

While direct site sampling and handling of actual animals remains important to further validate citizen science reports, successful capture of these freshwater stingrays can be challenging because of their benthic nature and preference for turbid habitats (Last et al., 2010). Moreover, their rare and endangered status further increases the difficulties of sampling which requires considerable labour, time and logistical planning (Davy et al., 2015). Clarifying the use of specific riverine systems and specific stretches within rivers by the rays would be important to inform any design of integrated management of river systems. The use of non-invasive approaches for detection of these rays, particularly environmental DNA barcoding, should be investigated further and developed for rapid species detection along river stretches and routine monitoring of health of local riverine biodiversity without lethal sampling —this approach would be highly beneficial not only for the rays, but also other rare endangered riverine species (Thomsen et al., 2012; Sigsgaard et al., 2015; Simpfendorfer et al., 2016; Wilson et al., 2018; Lim & Then, 2022).

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Appendix 1. NCBI Genbank sequences used in the phylogenetic analysis for *Urogymnus polylepis*.

Species	**Name used in NCBI**	**Location***	**Accession No.**
Urogymnus polylepis	*Himantura chaophraya*	Jakarta, Indonesia	MH908732
Urogymnus polylepis	*Himantura chaophraya*	Sumatra, Indonesia	MH908733
Urogymnus polylepis	*Himantura chaophraya*	Bangpakong River, Thailand	MH908734
Urogymnus polylepis	*Himantura chaophraya*	Bangpakong River,	MH908735
Urogymnus polylepis	*Himantura chaophraya*	Bangpakong River, Thailand	MH908736
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908737
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908738
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908739
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908740
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908741
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908742
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908743
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908744
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908745
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908746
Urogymnus polylepis	*Himantura chaophraya*	Maeklong River, Thailand	MH908747
Urogymnus polylepis	*Urogymnus polylepis*	India*	MK978688
Urogymnus polylepis	*Urogymnus polylepis*	India*	MT237652
Urogymnus polylepis	*Urogymnus polylepis*	Bangladesh*	MZ363899
Urogymnus polylepis	*Urogymnus polylepis*	Bangladesh*	MZ363900
Urogymnus dalyensis	*Himantura dalyensis*	China*	KM244769
Urogymnus granulatus	*Himantura granulata*	China*	KF751650
Urogymnus granulatus	*Himantura granulata*	China*	KF899471

*Location based on authors' affiliation, actual sample location not provided.

Appendix 2. Sightings of *Urogymnus polylepis* with disc width and weight data for length-weight relationship analysis.

Site	Country	Date of reported sighting	Disc Width (cm)	Weight (kg)	Reference
Miri-Bekenu	Malaysia	8 Dec 2016	192	300	Windusari
Pekan Lundu	Malaysia	2017	121	60	E-jad
Mae Klong River	Thailand	13 Mar 2015	240	362	Utusan
Batanghari River	Indonesia	9 Nov 2008	200	150	I & Y
Musi River	Indonesia	20 Aug 2012	150	100	I & Y
Hoogly River	India	14 Dec 2018	144	120	Sen
Hoogly River	India	22 Jan 2019	141	117	Sen
Hoogly River	India	12 Feb 2019	144	160	Sen
Hoogly River	India	7 Mar 2019	145	190	Sen
Hoogly River	India	19 Mar 2019	223	300	Sen

References: Windusari = Windusari et al., 2020; E-jad = E-jad Soulfly Facebook; I & Y = Iqbal & Yustian, 2016; Sen = Sen et al., 2020.

Appendix 3. Characteristics of the Malaysian rivers where *Urogymnus polylepis* was previously encountered.

Sites	Sighting	River	Water quality index			Substrate	Length (km)	Width (m)	Basin area (km^2)
			2015	2016	2017				
Merang	1	Merang river	67, SP, III	71, SP, III	68, SP, III	Sand	35	18–185	1035
Tanjung Pahang	2, 5	Kuantan river	86, C, II	77, SP, II	79, SP, II	Sand	86	48–350	2025
Kuala Rompin	3, 4	Rompin river	82, C, II	79, SP, II	80, SP, II	Sand	175	38–1000	4285
Kuala Lukut, Port Dickson	6	Lukut Besar river	Not listed	Not listed	Not listed	–	18	9–113	–
Kampung Johor Lama, Kota Tinggi	7	Johor river	80, SP, II	83, C, II	81, C, II	–	68	34–3260	3250
Kukup, Pontian	8	Pulai river	73, SP, III	68, SP, III	64, SP, III	Mud sand	22	–	2660
Sungai Sembrong	9	Semberong river	77, SP, II	59, P, III	77, SP, II	–	85	25–83	–
Kampung Litong, Mukah	10, 14–18, 24	Mukah river	82, C, II	76, SP, II	76, SP, II	Sand	91	50–175	2275
Batu Kawa, Kuching	11	Sarawak river	84, C, II	80, SP, II	81, C, II	–	120	80–1000	2375
Pujut, Kuala Baram	12	Miri river	74, SP, III	78, SP, II	75, SP, III	Fine sand, silt	27	35–162	22930
Similajau, Bintulu	13, 25	Similajau river	82, C, II	78, SP, II	79, SP, II	–	40	9–80	660
Kuala Nyalau, Bintulu	19, 20	Nyalau river	Not listed	Not listed	Not listed	–	35	3–45	–
Kampung Semunin, Lundu	21, 28, 37–40, 42, 43	Kayan river	84, C, II	80, SP, II	79, SP, II	–	65	32–570	1645
Kabong, Saratok	22, 27	Krian river	Not listed	Not listed	Not listed	–	105	32–1250	1500
Tanjung Bungai, Bekenu	23	Sibuti river	83, C, II	82, C, II	80, SP, II	–	19	33–74	1020
Kuching	26	Santubong river	Not listed	Not listed	Not listed	–	24	182–322	–
Kuala Niah, Miri	29	Niah river	85, C, II	81, C, II	80, SP, II	–	81	16–126	1280
Kuala Sibuti, Miri	30	Sibuti river	83, C, II	82, C, II	80, SP, II	–	18	33–90	–
Pulau Bruit	31	no river	–	–	–	–	–	–	–
Sungai Kuala Baram, Miri	32, 33, 41	Baram river	83, C, II	79, SP, II	79, SP, II	Mud, silt, sand	402	65–466	22930
Batang Lupar	34	Padeh river	82, C, II	81, C, II	78, SP, II	–	125	6–1800	–
Kampung Sungai Limo Sampadi	44, 45	Sampadi river	Not listed	Not listed	Not listed	–	11	16–350	–
Sandakan	46, 51, 52, 60	NA	–	–	–	–	–	–	–
Beluran, Sandakan	47, 48	Sapi river	76, SP, III	79, SP, II	81, C, II	–	68	27–2000	–
Kampung Likas, Kota Kinabalu	49	Likas river	73, SP, III	69, SP, III	72, SP, III	Silt, clay	12	9–76	–
Orico estate, Kinabatangan	50, 53–59	Kinabatangan river	78, SP, II	81, C, II	82, C, II	–	560	60–316	16581

Water quality index = Water quality index, Category (C, SP, P), Class (I-V). C = clean, SP = slightly polluted, P = polluted. Refer to DOE (2021) for complete classification standard.

Appendix 4. Recent records of *Urogymnus polylepis* in Malaysia (2011-2021).

No	Sites	State	Date of reported sighting	Gear	Sex	Maturity	Weight (kg)	Disc Width (cm)	Sources	References
1	Merang	Terengganu	23 Jul 2013	–	–	–	300	–	Ayahchik Penarik[F]	Iqbal
2	Tanjung Pahang	Pahang	11 Jun 2012	–	–	–	41	–	Muhammad Amilin Ruslan[F]	Iqbal
3	Kuala Rompin	Pahang	24 Feb 2014	–	–	–	100	–	Nabila[F]	Iqbal
4	Kuala Rompin	Pahang	11 Aug 2017[P]	Long	F	–	250	–	Muhammad Azizi[F], Sinar Harian[N]	Iqbal
5	Tanjung Pahang	Pahang	9 Mar 2019	–	–	–	200	–	Profishganda[F]	Iqbal
6	Kuala Lukut, Port Dickson	Negeri Sembilan	19 Nov 2017	–	–	–	120	–	Zakaria[N]	Iqbal
7	Kampung Johor Lama, Kota Tinggi	Johor	3 Jul 2015[P]	Long	F	–	150	–	Friend of BN Barisan National[F], BH Online[N]	Iqbal
8	Kukup, Pontian	Johor	Dec 2017[C]	–	F	–	300	–	Burn Exc[F]	–
9	Sungai Sembrong	Johor	10 Aug 2019[P]	–	F	–	-	61	Halimi Hanip[F]	–
10	Kampung Litong, Mukah	Sarawak	26 Nov 2012	–	–	–	200	–	–	Windusari
11	Batu Kawa, Kuching	Sarawak	24 Feb 2013	–	–	–	175	–	–	Windusari
12	Pujut, Kuala Baram	Sarawak	4 Jul 2013	–	–	–	82	–	–	Windusari
13	Similajau, Bintulu	Sarawak	2014[C]	–	–	–	–	–	Ader Shahrizan[F]	–
14	Mukah fish market	Sarawak	4 Apr 2016[C]	Traw	F	–	–	190	Survey finding	Accession MZ976812
15	Mukah fish market	Sarawak	4 Apr 2016[C]	Traw	F	–	–	39.5	Survey finding	Accession OK148430
16	Mukah fish market	Sarawak	4 Apr 2016[C]	Traw	M	–	–	38.5	Survey finding	Accession OK148429
17	Mukah fish market	Sarawak	4 Apr 2016[C]	Traw	M	–	–	37	Survey finding	–
18	Mukah fish market	Sarawak	4 Apr 2016[C]	Traw	M	–	–	38	Survey finding	–
19	Kuala Nyalau, Bintulu	Sarawak	19 Apr 2016[C]	Traw	F	–	160	–	Sarawak Voice[N], My News Hub[N]	Windusari
20	Kuala Nyalau. Bintulu	Sarawak	19 Apr 2016[C]	Traw	-	–	145	–	Sarawak Voice[N], My News Hub[N]	Windusari

No	Sites	State	Date of reported sighting	Gear	Sex	Maturity	Weight (kg)	Disc Width (cm)	Sources	References
21	Kampung Semunin, Lundu	Sarawak	21 Jun 2016[P]	–	F	–	150	–	Shelly Liew[F]	Windusari
22	Kabong, Saratok	Sarawak	23 Aug 2016[P]	–	F	–	400	–	Azizul Amin[Y]	Windusari
23	Tanjung Bungai, Bekenu	Sarawak	8 Dec 2016[C]	Long	F	–	300	192	Hazlina lena[F], Cari[N], myMetro[N]	Windusari
24	Mukah fish market	Sarawak	10 Mar 2017[C]	Traw	–	–	150	–	Kamek Miak Sarawak[F], Projekmm[N]	Windusari
25	Samalaju, Bintulu	Sarawak	26 Aug 2017[C]	–	–	–	–	–	Rosman Ramli[F]	–
26	Kuching	Sarawak	8 Sep 2017	–	–	–	–	–	–	Windusari
27	Sungai Krian, Kabong	Sarawak	1 Oct 2017[P]	Long	M	–	97	–	Riduan Wan[F]	–
28	Kampung Semunin, Lundu	Sarawak	2017[P]	H&L	–	–	60	121	E-jad Soulfly[F]	–
29	Kuala Niah, Miri	Sarawak	17 Mar 2019[C]	Traw	F	–	250	–	Borneo Post[N], BH Online[N]	–
30	Kuala Sibuti, Miri	Sarawak	21 Mar 2019[C]	Traw	M	–	150	–	Sarawak Aritok[F], Utusan Borneo Online[N], mStar[N]	–
31	Pulau Bruit	Sarawak	15 Apr 2019[C]	Traw	F	–	200	–	Kamek Miak Sarawak[F], Sarawak Voice[N]	–
32	Sungai Kuala Baram, Miri	Sarawak	16 Apr 2019[P]	-	F	–	–	–	Will Hunter[F]	–
33	Sungai Kuala Baram, Miri	Sarawak	22 Jun 2019[C]	-	M	–	103	–	Lak Samana Lak Samana[F], Umpan[N]	–
34	Batang Lupar	Sarawak	18 Jul 2019[C]	Traw	F	–	20	–	Sri Aman Mansang Maju[F]	–
35	Kuching	Sarawak	Unspecified, 2017–2019	–	–	–	–	–	–	Booth
36	Kuching	Sarawak	Unspecified, 2017–2019	–	–	–	–	–	–	Booth
37	Lundu	Sarawak	15 Mar 2020[C]	H&L	–	–	–	–	Haikal A-kal[F]	–
38	Lundu	Sarawak	1 Apr 2020[C]	H&L	F	–	–	–	Haikal A-kal[F]	–
39	Lundu	Sarawak	7 Apr 2020[C]	H&L	F	–	–	–	Haikal A-kal[F]	–
40	Kampung Stunggang, Lundu	Sarawak	14 May 2020[C]	Traw	F	–	280	–	Sarawak Aritok[F], astro AWANI[N], BH Online[N]	–
41	Sungai Kuala Baram, Miri	Sarawak	30 Jan 2021[P]	-	M	Juv	–	–	Ada Ada saja[Y]	–

No	Sites	State	Date of reported sighting	Gear	Sex	Maturity	Weight (kg)	Disc Width (cm)	Sources	References
42	Lundu	Sarawak	8 May 2021[C]	H&L	F	–	100	–	Sarawak Edition[F]	–
43	Lundu	Sarawak	12 May 2021[C]	H&L	F	–	-	–	Haikal A-kal[F]	–
44	Sungai Sampadi	Sarawak	2 Jul 2021[C]	H&L	–	–	180	–	Oohhaa Sarawak[F]	–
45	Sungai Sampadi	Sarawak	29 Mar 2022[C]	–	–	–	-	–	Amazing Sarawak[F]	–
46	Sandakan	Sabah	25 Jul 2011	–	–	–	206	–	–	Windusari
47	Beluran, Sandakan	Sabah	10 Jul 2012	–	–	–	-	–	–	Windusari
48	Beluran, Sandakan	Sabah	25 Aug 2013	–	–	–	300	–	–	Windusari
49	Kampung Likas, Kota Kinabalu	Sabah	6 Oct 2013	–	–	–	100	–	–	Windusari
50	Orico estate, Kinabatangan	Sabah	2 Aug 2014	–	–	–	–	–	–	Windusari
51	Kampung Kolapis, Sandakan	Sabah	13 Aug 2015	–	–	–	500	–	–	Windusari
52	Beluran, Sandakan	Sabah	27 Jan 2015[P]	–	–	–	–	–	Sabah Viral[F], Wan Azry[F]	–
53	Kinabatangan	Sabah	19 Sep 2015	Long	M	–	–	110	–	Manjaji
54	Kampung Abai, Kinabatangan	Sabah	9 Dec 2015	H&L	F	–	16	–	–	Manjaji
55	Kampung Abai, Kinabatangan	Sabah	9 Dec 2015	H&L	-	–	25	–	–	Manjaji
56	Sungai Kurapu	Sabah	Aug 2016	H&L	M	Juv	–	–	–	Manjaji
57	Sungai Watar	Sabah	Aug 2016	H&L	M	Adu	–	–	–	Manjaji
58	Sungai Kurapu	Sabah	Aug 2016	H&L	M	Juv	–	–	–	Manjaji
59	Sungai Milian, Labau	Sabah	21 Aug 2016	–	–	–	–	–	–	Windusari
60	Sandakan fish market	Sabah	26 Aug 2018[C]	–	M	–	–	120	Survey finding	Accession MZ976813
61	Tanjung Batu Laut	Sabah	21 Oct 2022[C]	–	F	Adu	–	–	Arjuna Keramat[F]	–

Date: C = captured date, P = posted date.
H&L = hook and line, Long = longline, Traw = trawler, F = female, M = male, Juv = juvenile, Adu = adult, DW = disc width.
Sources: F = Facebook post, N = Online news, Y = Youtube.
References: Manjaji = Manjaji-Matsumoto et al., 2017; Iqbal = Iqbal et al., 2019; Windusari = Windusari et al., 2020; Booth = Booth et al., 2021.