

ORIGINAL ARTICLE

Molecular diagnosis and geographic distribution of species in the endangered whipray genus *Himantura*, with description of a new species from the Indo-Malay archipelago

Philippe Borsa^{1,*} , Udhi E. Hernawan², Régis Hocdé³  and Laurent Pouyaud⁴ 

¹ Institut de recherche pour le développement (IRD), UMR 250 Entropi, Montpellier, France

² National Research and Innovation Agency of the Republic of Indonesia (BRIN), Research Center for Oceanography (RCO), Jakarta, Indonesia

³ MARBEC, Univ Montpellier, Ctr nat rech sci, Ifremer, IRD, Montpellier, France

⁴ IRD, UMR 226 ISEM, Montpellier, France

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Abstract – The overfished and endangered reticulate whipray genus (*Himantura* spp.) comprises up to eight genetically separate lineages that are considered distinct species: *H. uarnak*, *H. undulata*, *H. leoparda*, *H. tutul*, *H. australis*, and three possibly undescribed species (*Himantura* spp. 1-3). Their geographic distributions so far have not been clearly determined, possibly because of persistent misidentification when using morphological criteria. To circumvent this problem, we provide diagnoses based on an updated nucleotide-sequence dataset at the *COI* locus for all seven species that have been sequenced at this locus. Species distribution maps based on genetically-validated records are presented. Notably, *H. uarnak* was confirmed as endemic to the Red Sea and the western part of the Indian Ocean while its sister species *H. leoparda* was endemic to the Indo-Malay archipelago. Two other sister-species, from the Indo-Malay-Australia region, *Himantura* sp. 1 and *H. australis*, were geographically distributed in a parapatric fashion. *Himantura tutul* was distributed from the Western Indian Ocean to the Sunda Shelf while *H. undulata*'s range extended from the Arabian Sea to the Sunda Shelf. The present molecular taxonomic revision of the threatened genus *Himantura* thus clarifies the identity of the sequences in public repositories and provides new information on their distribution that will be highly relevant to their conservation. *Himantura* sp. 1, which is endemic to the Indo-Malay archipelago, is described as a new species.

Keywords: Genetic markers / DNA barcodes / molecular taxonomy / geographic distribution / Dasyatidae / *Irma* S. Arlyza

1 Introduction

Recognizing cryptic species is important to marine fisheries management, biodiversity management, and conservation (Bickford et al., 2007; Krishnamurthy and Francis, 2012; Van Campenhout et al., 2014; Pante et al., 2015; Cahill et al., 2024). “Cryptic species” may include species previously confused with one another because they effectively lack morphological differences that would help to distinguish them, or because the distinctive characters or combinations of characters they may possess and that may be used for their diagnoses have not yet been adequately investigated (i.e., “pseudo-cryptic species”) (Knowlton 1993; Cahill et al., 2024).

Diagnosing a species consists of highlighting characters that distinguish it from related species: “When describing a new nominal taxon, an author should make clear his or her purpose to differentiate the taxon by including with it a diagnosis, that is to say, a summary of the characters that differentiate the new nominal taxon from related or similar taxa” (International Commission on Zoological Nomenclature 1999: 13A). As the diagnosis is the basis for unambiguous identification, we agree with Renner (2016) that diagnoses should be considered the core of species descriptions. Although for the last 250 yr, descriptions of species have mainly relied on morphological characters (Dunn, 2003), there is no reason that DNA sequences should not equally be accepted for this purpose, alongside morphological characters or on their own (Tautz et al., 2003; Vogler and Monaghan, 2006; Cook et al., 2010; Renner, 2016; Fedosov et al., 2022). Genetic characters, including allozymes and single-nucleotide

*Corresponding author: philippe.borsa@ird.fr;
philippeborsa@gmail.com

polymorphisms have indeed proven essential in delimitating, diagnosing, and identifying species (e.g., Manwell and Baker 1963; McDonald and Koehn 1988; Momigliano *et al.*, 2018; Neves *et al.*, 2021). Within the last two decades, DNA-barcoding, the identification of species based on nucleotide sequence variability in a fragment of, mainly, the *COI* gene, has proven a tool of choice for flagging cryptic species in animals (Ward *et al.*, 2009; Zemlak *et al.*, 2009; Hebert *et al.*, 2010; Collins and Cruickshank, 2014; Wang *et al.*, 2020) and for diagnosing and describing them (e.g., Brower, 2010; Baldwin *et al.*, 2011; Wang *et al.*, 2016).

In particular, sharks and rays (Elasmobranchs) have seen a relatively recent surge of new species descriptions consecutive to the pioneer genetic surveys of Ward *et al.* (2008) and Naylor *et al.* (2012) which had revealed cryptic lineages within a number of species. Applications of nucleotide sequence variation to the taxonomy of Elasmobranch species include the works of Yagishita *et al.* (2009), Poortvliet *et al.* (2015), Borsa *et al.* (2018), Fernando *et al.* (2019) and Cabebe-Barnuevo *et al.* (2025).

One of the yet poorly resolved taxonomic tangles in Elasmobranchs concerns the *Himantura uarnak* [Gmelin (ex-Forsskål), 1789] or *Himantura* spp. (reticulate whipray) group of species in the family Dasyatidae Jordan and Gilbert, 1879 (WoRMS Editorial Board, 2023). Searching for *Himantura* spp. in either the Barcode of Life Datasystem (BOLD) (<http://www.barcodinglife.com/>; Ratnasingham and Hebert, 2007) or GENBANK (<http://www.ncbi.nlm.nih.gov/>; Benson *et al.*, 2017) reference databases returns several different species names for any single genetic lineage (Borsa *et al.*, 2021). This may in part reflect the absence of clear diagnoses for those *Himantura* species which description is exclusively based on morphology, including the two recently-described *H. leoparda* Manjaji-Matsumoto and Last, 2008 and *H. australis* Last, White and Naylor, 2016. Indeed, the recent literature (e.g., Weigmann, 2016; Bogorodsky *et al.*, 2021) claims to rely on morphological characters to identify species in the genus, even though the available morphological diagnoses (Manjaji-Matsumoto and Last, 2008; Last *et al.*, 2016a, b) lack the necessary discriminative power (Borsa *et al.*, 2013b, 2021). For instance, up to 16 morphological characters have been used for tentatively diagnosing the four *Himantura* spp. species considered valid in recent reviews (Last *et al.*, 2016a; Weigmann, 2016) but none proved diagnostic (Tab. 1) and no diagnostic combination of characters is yet available either. A fifth species, *H. tutul* Borsa, Durand, Shen, Arlyza, Solihin and Berrebi, 2013 was revealed as a phylogenetically distinct lineage within, and reproductively isolated from, *H. leoparda* (Arlyza *et al.*, 2013; Borsa *et al.*, 2013b). However, *H. tutul* was ignored by Last *et al.* (2016a) and Weigmann (2016) because its morphological diagnosis was deemed insufficient (S. Weigmann, in litt.). This may indicate misconception about morphologically cryptic (or pseudo-cryptic) species. This may also reflect misinterpretation of DNA-based evidence despite the clear taxonomic relevance of the latter (Naylor *et al.*, 2012; Arlyza *et al.*, 2013; Borsa *et al.*, 2013b, 2021). To complicate matters, two other mitochondrial lineages (*Himantura* sp. 1, *Himantura* sp. 2) that may rank as distinct species have been identified from nucleotide

sequences at the *COI* locus (Borsa *et al.*, 2021). *Himantura* sp. 1 from the Indo-Malay archipelago (Borsa *et al.*, 2021; Cabebe-Barnuevo *et al.*, 2025) is reproductively isolated from the three other sympatric, congeneric species, namely *H. leoparda*, *H. tutul* and *H. undulata* (Arlyza *et al.*, 2013).

Accurate taxonomic definition of species in the genus *Himantura* would be necessary to map their geographic distribution. This would also be highly relevant to their conservation. Within the last decades, reticulate stingrays have declined abruptly throughout their range in the tropical Indo-West Pacific because of considerable fishing pressure. These large-sized stingrays (see Tab. 1) are taken as bycatch or targeted using various fishing gears including harpoon, dropline, bottom line, gillnet and trawl (Sherman *et al.*, 2021). They are exploited for their flesh and their leather, which is in high demand from the luxury goods industry (Sahubawa *et al.*, 2018; Sherman *et al.*, 2021, 2023; D'Alberto *et al.*, 2022). As a consequence of their over-exploitation, three species (*H. leoparda*, *H. uarnak*, *H. undulata*) have been assessed as “endangered” (Sherman *et al.*, 2020, 2021; Rigby *et al.*, 2024).

In a context where morphological diagnoses do not provide unequivocal distinction between species, as is currently the case with *Himantura* spp., DNA markers can at once provide the tools for species delineation, species description, and species identification (Vogler and Monaghan, 2006; Cook *et al.*, 2010). In this paper, we provide an updated nucleotide-sequence dataset at the *COI* locus and use it along with publicly available data at other genetic loci to (i) confirm the delineation of species in the endangered whipray genus *Himantura*; (ii) diagnose these species; (iii) refine their geographic distributions and provide genetically-validated distribution maps; and (iv) describe as a new species the lineage previously identified as *Himantura* sp. 1.

2 Methods

2.1 Nucleotide sequence datasets

Himantura spp. nucleotide sequences at the cytochrome-oxidase subunit 1 (*COI*), cytochrome b (*cytb*), and NADH dehydrogenase subunit 2 (*NADH2*) mitochondrial loci were obtained from the GENBANK and BOLD repositories, last accessed on 23 March 2025. These datasets were incremented with two unpublished partial *COI*-gene sequences (ZB01210333(W2)5440F and ZB01210334(W3)5440F, both from X. Chen's laboratory at South China Agricultural University, Guangzhou), and 15 partial *cytb*-gene sequences extracted from Manjaji (2004). For all nucleotide sequences included in these datasets, when available, sampling details, GENBANK accession numbers and BOLD registration numbers were collated (Suppl. Tabs. S1–S3). The *COI* dataset was an update of Borsa *et al.* (2021). Additional information including sex, disk width (DW) and specimen photographs was retrieved from the Elasmobranch Specimens section of the Global Cestode Database hosted by the University of Connecticut (<https://tapewormdb.uconn.edu/>; Caira *et al.*, 2020) including pictures of specimens.

Table 1. Morphological characters used in the diagnoses of the four *Himantura* spp. species considered valid under Last *et al.* (2016a). *N* number of specimens examined; *NA* not acknowledged.

Character	Species (<i>N</i>) <i>H. uarnak</i> (NA)	<i>H. undulata</i> (NA)	<i>H. leoparda</i> (12 ^c)	<i>H. australis</i> (29 ^d)
Disc width (DW)	'≤ 160 cm ^a	'≥ 130 cm ^a	'≥ 140 cm ^a	'≥ 113 cm ^a
DW/disc length	'1.00–1.10 ^a	'DW subequal to disc length ^{a, b}	'1.00–1.16 ^c	'1.00–1.10 ^a
Disc shape	'Broad, rhombic, robust ^a	'Robust, suboval ^a	'Robust, weakly rhombic ^a 'Rhomboidal ^c	'Weakly rhomboidal ^d 'Very broad, weakly rhombic, robust ^a
Snout shape	'Broadly triangular, tip pointed ^a	'Narrowly pointed, triangular, small pointed lobe at tip ^a	'Broadly triangular (more so in young) with small apical lobe, broadly pointed ^a 'With distinct apical lobe ^c	'Moderately short, rather broad, with distinct apical lobe ^d 'broadly triangular, tip pointed ^a
Snout length	NA	NA	'Moderately long ^c	'19–22% DW ^d
Angle at apex	NA	NA	'101–120 ^c	'117–127 ^{c, d}
Anterior margins	'Concave ^a	'Straight to convex ^a	'Straight or weakly concave ^a	'Straight (rather than concave) ^a
Lateral apices	'Weakly angular to narrowly rounded ^a	'Broadly rounded ^a	'Narrowly rounded (more angular in young) ^a 'Moderately angular to narrowly rounded ^c	'Narrowly rounded ^d 'Narrowly to broadly rounded ^b
Orbit	NA	NA	'Moderately large, protruded slightly ^c	'Moderately large, often strongly protruding (particularly in young) ^d
Suprascapular denticles	'1–3 small heart-shaped thorns, but no other enlarged thorns extending along mid-line of disc and tail ^a	'Large pearl thorn, followed by 2–3 slightly smaller similar thorns in adults, no other enlarged thorns extending along mid-line of disc and tail ^a	'Usually 2, broad heart-shaped thorns, preceded by row of up to 13 smaller denticles of similar shape and a row of smaller denticles posteriorly ^a '2 prominent, broad heart-shaped, 1 row of enlarged widely spaced, narrow, heart-shaped ^c	'1–2, mostly heart-shaped (not preceded before and after by row of smaller primary denticles) ^d 'usually with 2 small, broadly heart-shaped thorns, but no other enlarged thorns extending along mid-line of disc ^a
Nasal curtain	'Broad and short, posterior margin finely fringed ^a	'Skirt-shaped, broad and short, posterior margin finely fringed ^a	'Skirt-shaped, broad and short, posterior margin finely fringed ^a	'Skirt-shaped, broad and short, posterior margin finely fringed ^a
Pectoral-fin radials	NA	NA	'148–158 ^c	'146–152 ^d
Vertebral centra (incl. synarcual)	NA	NA	'117–123 (120–126) ^c	'123 (124) ^d
Tail shape	'Narrow-based, subcircular in cross-section, tapering evenly toward sting ^a	'Narrow-based, suboval in cross-section, tapering evenly toward caudal sting ^a	'Narrow-based, suboval in cross-section, tapering evenly toward caudal sting ^a NA. Note: holotype lacks tail	'Narrow-based and subcircular in cross-section, tapering evenly toward sting ^a
Tail squamation	'Small sharp thorns beyond caudal sting ^a	NA	NA	'Small prickly denticles beyond caudal sting ^a
Caudal sting(s)	'Usually one ^a	'Usually one ^a	'Usually one ^a	'Usually one ^a

^aFrom Last *et al.* (2016a); ^bThis follows Bleeker's (1852) diagnosis of *Trygon undulata*; ^cfrom Manjaji-Matsumoto and Last (2008); ^dfrom Last *et al.* (2016b).

2.2 Analysis of nucleotide sequences at the *CO1* locus

Nucleotide sequences were aligned visually under BIOEDIT v. 7.1.3.0 (Hall, 1999). Variable nucleotide sites were selected using the highlighting tool of MEGA12 (Kumar *et al.*, 2024). Nucleotides diagnostic or quasi-diagnostic to a given species were screened by eye. “Quasi-diagnostic” applied to a nucleotide that was >95% private to one of the seven *Himantura* spp. lineages, i.e., observed in <5% of individuals in the rest of the dataset. Nucleotide sites were numbered with reference to the full-mitogenome sequence of *H. leoparda* (GENBANK no. NC_028325; Shen *et al.*, 2016).

Species were delimited based on the *CO1* sequence dataset using Puillandre *et al.*'s (2021) ‘Assemble Species by Automatic Partitioning’ (ASAP) algorithm. ASAP is a distance-based method that analyses single-locus sequence alignments and identifies the barcode gaps that separate distinct mitochondrial lineages (‘species’). Goulpeau *et al.* (2022) recently proved ASAP to be not only a particularly fast-processing species delimitation algorithm, but also a particularly reliable one when compared to other commonly used approaches. The FASTA-format (<https://www.ncbi.nlm.nih.gov/genbank/fastafomat/>) file containing the sequences was uploaded to the ASAP webserver (<https://bioinfo.mnhn.fr/abi/public/asap/>; accessed 23 March 2025). The Kimura-2 parameter substitution model (Kimura 1980) was chosen, with a transition-to-transversion ratio set to 2. The other options were set as default. Simultaneously, a phylogenetic tree was built with BIONJ (<http://www.atgc-montpellier.fr/bionj/>; Gascuel 1997). This algorithm, which uses genetic distances between individual nucleotide sequences, has better topological accuracy than the Neighbor-Joining algorithm (Gascuel 1997). In the present work, the ASAP analysis was intended as complementary to previous species-delimitation that also employed a coalescence-based algorithm (Borsa *et al.*, 2021).

Nucleotide sequences of reference at the *CO1* locus included GenBank nos. MW178188 for *H. uarnak* (neotype; Borsa *et al.*, 2021), NC_028325 for *H. leoparda* (Shen *et al.*, 2016) and JX263335 for *H. tutul* (holotype; Borsa *et al.*, 2013b). Sequence GENBANK no. NC_028325 was identified as *H. leoparda* as it clustered with *CO1*- and *cytb*-gene sequences from specimens assumed to belong to this species by their spot patterns, identical to those of *H. leoparda*'s holotype (Borsa *et al.*, 2013b; Shen *et al.*, 2016). *Himantura undulata* individuals were identified from their clustering with specimen no. ir007 (Arlyza *et al.*, 2013) itself ascertained as *H. undulata* from its clustering with reference *H. undulata* specimens vouchered at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) and examined by Manjaji (2004) (see next sub-section). *Himantura australis* sequences were identified by their distinctness as a sub-clade sister to the *Himantura* sp. 1 sub-clade in phylogenies (respectively, the ‘NWA’ and ‘IMR’ subclades of Borsa, 2017: Fig. 2c). The BOLD datasystems distinguishes clusters of *CO1*-gene sequences that qualify as operational taxonomic units (OTUs) using a private algorithm that groups sequences with high similarity and connectivity (Ratnasingham and Hebert, 2013). Each OTU thus identified was also allocated a barcode index number (BIN) in BOLD.

Nucleotide distances within and between species were estimated under MEGA12, using as nucleotide-substitution model the one found to have the lowest Bayesian information criterion score.

2.3 Analysis of nucleotide sequences at the *cytb* and *NADH2* loci

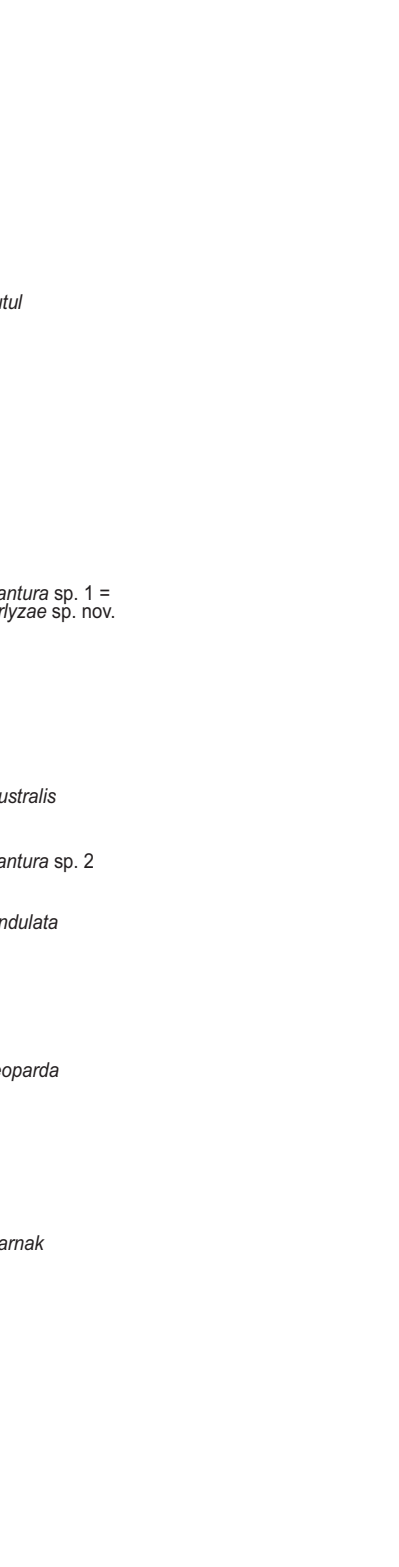
These two datasets were collated with the aim to provide additional genetically-validated records for building point maps. Nucleotide sequences at the *cytb* locus were available from GENBANK and from Manjaji (2004). All sequences were aligned and subjected to phylogenetic analysis using MEGA12, to identify clusters representing distinct *Himantura* spp. The *cytb* nucleotide sequences of reference for *H. undulata* were from two voucher specimens, CSIRO nos. H5481.01 and H5483.01 originating from, respectively, the Sulu Sea and the South China Sea (Manjaji, 2004). Those for *H. leoparda* were from CSIRO specimen no. H5478.01 (Manjaji, 2004) and GENBANK no. NC_028325 (Shen *et al.*, 2016). That for *H. tutul* was GENBANK no. JX274333 (holotype; Borsa *et al.*, 2013b).

The nucleotide sequences at the *NADH2* locus available from GENBANK were similarly compiled and analyzed. *NADH2* nucleotide sequences of reference for *H. undulata* were nos. JQ518803 and MK335291 (Naylor *et al.*, 2012; Fernando *et al.*, 2019). That for *H. leoparda* was no. NC_028325 (Shen *et al.*, 2016). That for *H. australis* was no. JQ518807 (then labelled ‘uarnak 2’; Naylor *et al.*, 2012). Nucleotide sequences nos. KM073044 and KM073045 of two specimens (HLEO1, HLEO2) previously identified as *H. tutul* (Borsa *et al.*, 2021) albeit erroneously labelled ‘*H. leoparda*’ in Lim *et al.* (2015) were used as references for *H. tutul*. Consequently, nucleotide sequences nos. JQ518800, PP313931 and PP313930 labelled ‘uarnak 3’ or ‘*H. cf. uarnak 3*’ (Naylor *et al.*, 2012; Henderson *et al.*, 2016) were confirmed to be *H. tutul* as assumed previously (Borsa, 2017). Sequences of reference for *Himantura* sp. 1 and *Himantura* sp. 3 (resp. ‘uarnak 1’, ‘uarnak 4’ of Naylor *et al.*, 2012) were, respectively, no. JQ518805 and no. JQ518791.

For both *cytb*- and *NADH2*-gene sequence datasets, nucleotide substitution models, maximum-likelihood phylogenies and nucleotide distances were computed under MEGA12.

2.4 Nuclear markers

Allelic frequencies at two actin (*Act-2 A, B*) and three calmodulin (*Cam-3 A-C*) length-polymorphic intron loci (Atarhouch *et al.*, 2003; Borsa *et al.*, 2012) were derived from the individual multi-locus genotypes produced by Arlyza *et al.* (2013). This concerned 112 *Himantura* spp. individuals collected from the Indo-Malay archipelago including fishing harbours in the Java Sea, the Bali Sea, Makassar Strait, the Flores Sea and the Banda Sea. The four reproductively isolated lineages that were then identified, then provisionally labelled ‘*H. undulata*’, ‘*H. leoparda* cluster 1’, ‘*H. leoparda* cluster 4’ and ‘*H. uarnak*’ (Arlyza *et al.*, 2013), were considered.



Distance-based BIONJ tree of nucleotide sites (in%, from 500 Neighbor-Joining; [Miralles et al., 2022](#)); number on top of a distinct colour. Partition scores were, identification of the seven species. This

Fig. 2. Reticulate whipray, *Himantura* spp. Informative nucleotide sites at the *COI* locus. Nucleotides diagnostic to a species are in bold capitals and highlighted blue; quasi-diagnostic nucleotides are in capitals; nucleotides that are shared between *Himantura* sp. 1 (= *H. arlyzae* sp. nov.) and *Himantura* sp. 2 and differentiate them from the other species are in bold letters and highlighted pink. Nucleotide site numbering starts at first nucleotide of the *COI* gene, i.e., at homologous position no. 5518 of the mitogenome sequence of *H. leoparda* (Shen et al., 2016). The sequences compared were 613 bp long, beginning at nucleotide position no. 80 of the *COI* gene. Dots indicate nucleotides identical to sequence chosen as reference (*H. uarnak*). R=A or G; Y=C or T; K=G or T.

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Sampling locations were placed on point maps to visualize the geographic distributions of species. All the specimens used to this effect were identified to species from their placement in the *COI*, *cytb* and *NADH2* gene phylogenies. Equivalence between clades at different loci was based on Arlyza et al. (2013), Borsa (2017) and present work. Geographic records were obtained from the Global Cestode Database, the GENBANK nucleotide-sequence database, the BOLD database, and the original articles that used the *Himantura* spp. material entered into the databases. Geographic details are reported in Suppl. Tables S1–S3. Names of marine regions where samples were located followed the International Hydrographic Organization, Monaco (<https://iho.int/>; International Hydrographic Organization, 1953).

2.6.1 Rationale

2.6.2 Holotype

The International Commission on Zoological Nomenclature (1999: 73) defines the holotype as “*the single specimen upon which a new nominal species is based in the original [i.e., this] publication*” and requires that the holotype be designated “*in a way that will facilitate its subsequent recognition*”.

2.6.3 Language

The International Commission on Zoological Nomenclature (1999: 13B) recommends that diagnoses be provided in widely used international languages and also in languages used in the regions relevant to the taxa diagnosed. We considered the following: (i) the new species is described from a specimen collected in Indonesia; (ii) the distribution of the species is confined to the central part of the Indo-Malay archipelago; and (iii) most of the languages spoken in this region belong to the Malay language group. Therefore, in addition to English we chose Indonesian for the diagnosis of the new species. Indeed, Indonesian is currently the most widely spoken Malay language (Seddon, 2004) and it is also the official language in Indonesia.

3.1 Species delimitation

The alignment of *COI*-gene sequences ($N=249$) was trimmed to a core length of 613 base pairs (bp) beginning at nucleotide position no. 80 and ending at nucleotide position no. 692 of the gene. The first nine partitions provided by ASAP had partition scores less than 10, indicating three to 16 species (Fig. 1). The three-species hypothesis was deemed too conservative, as it failed to distinguish *H. undulata* from *H. australis* and *Himantura* sp. 1, and *H. uarnak* from

Table 2. Reticulate whipray *Himantura* spp. Results of Puillandre *et al.*'s (2021) ASAP partitioning into species, based on the *COI*-gene sequence dataset. *N*, size of the subset of individuals.

ASAP subset no.	(<i>N</i>)	Sequence accession no. ^a	Barcode identification ^b
1	(28)	GU673383, JF493651, JF493652, KF899500-KF899502, KY176708, MW178184-MW178196, FOAI180-08, FOAN974-11, OR284662, FEE001-16, FOAI181-08, FOAN972-11, FOAN973-11, FOAN975-11	<i>H. uarnak</i>
2	(19)	DQ108167, JX263336, KF899503-KF899506, KM073001, MG792127, MH235646, MH235647, MN013427, MZ363897, OP860901, OP860910, OQ385101, OQ628398, OQ628404, IRREK806-08, ZB01210333_W2_5440F	<i>H. undulata</i>
3	(68)	JX263361-JX263416, JX263417, JX263418, KR019776 (=NC_028325), MK422130, FOAG126-07, FOAO1113-18, FOAO1135-18, FOAO1142-18, IRREK038-08, IRREK068-08, KAM104-08, KERRI482-08	<i>H. leoparda</i>
4	(73)	JX263306-JX263335, KC508509, KF899353, KF899507-KF899511, KM072996-KM072998, MG774902, MG774915, MG774922, MG774903, MG774913, MG792078, MG792101, MG792112, MG792113, MG792124, MG792125, MN897011, MN897012, MT308608, OQ385099, OQ628402, OR284663, PQ272502, PV361869, PV361873, IRREK171-08, IRREK662-08, IRREK678-08, IRREK679-08, IRREK687-08, IRREK741-08, IRREK829-08, JTFR167-20, SAIAB712-08, SAIAB713-08, SAIAB714-08, SAIAB715-08, ZB01210334_W3_5440F	<i>H. tutul</i>
5	(23)	GU673414, GU673416-GU673419, JQ765509, JQ765519-JQ765530, JQ815394, JQ815395, JQ929047, AMS045-06, FOAO1107-18	<i>H. australis</i>
6	(35)	GU673716, JX263337-JX263360, KC970332, KM072999, MG792110, MG792123, OQ385100, OR614479, JTFR099-17, JTFR112-17, IRREK193-08, IRREK863-08	<i>Himantura</i> sp. 1 = <i>H. arlyzae</i> sp. nov.
7	(2)	KM073000, IRREK752-08	<i>Himantura</i> sp. 2

^a As in GENBANK or BOLD except ZB01210333_W2_5440F and ZB01210334_W3_5440F (X. Chen, unpubl.).

^b Following Borsa *et al.* (2021).

H. leoparda. The four-species hypothesis similarly failed to distinguish *H. australis* from *Himantura* sp. 1 and *H. uarnak* from *H. leoparda*. The next most-conservative hypothesis was a six-species partition that distinguished all the foregoing but embedded *Himantura* sp. 2 within *Himantura* sp. 1 despite the topology of the BIONJ tree which indicated that *Himantura* sp. 2 forms a clade external to both *H. australis* and *Himantura* sp. 1. The seven-species hypothesis, which had a partition score (8.5) similar to the six-species hypothesis (8.0) was eventually retained, as it enabled the distinction of *Himantura* sp. 2. Partitions with a larger number of species were not retained, because doing so would require as of today unnecessary splitting of currently accepted species; for example, the nine-species partition would require that each *H. undulata* and *H. tutul* be split in two. The 613-bp fragment of the *COI* gene a posteriori offered a total of 38 taxonomically informative nucleotide sites that enabled us to distinguish all seven *Himantura* spp. species from one another (Fig. 2). Publicly available *COI*-gene sequences are listed by species in Table 2.

The above results were confirmed by the topology of the *cytb*-gene tree (Fig. 3), which featured *H. undulata*, *H. leoparda*, *H. tutul* and *Himantura* sp. 1 as separate clusters. The sister-clade to *Himantura* sp. 1 on this tree was represented by two

individuals sampled in West Papua. The latter could be *H. australis* or, perhaps, *Himantura* sp. 2 or, perhaps, another species for which no *COI*-gene sequence is available yet.

The above results were also confirmed by the topology of the *NADH2*-gene tree (Fig. 4), which featured *H. undulata*, *H. leoparda*, *H. australis* and *H. tutul* as separate clusters. The Indian-Ocean sister-clade to *H. leoparda* was assumed to represent *H. uarnak*. It differed from *H. leoparda* by several nucleotide substitutions: nucleotide C in this clade was replaced by T in *H. leoparda* at nucleotide sites nos. 201, 378, 492, 543, 822 and 913 of the gene; T was similarly replaced by C at sites nos. 61, 66, 343, 406 and 657; A was similarly replaced by G at sites nos. 52 and 789; and G was similarly replaced by A at sites nos. 78, 475 and 694. *Himantura* sp. 1 and *Himantura* sp. 3 were represented by single sequences, distinct from each other and both distinct from the other clusters. *Himantura* sp. 2 was not represented in the *NADH2* dataset.

3.2 Molecular diagnosis of species

All *Himantura* species except *Himantura* sp. 1 were diagnosed by a fixed nucleotide substitution at at least one nucleotide site (Fig. 2). *Himantura* sp. 1 was diagnosed by

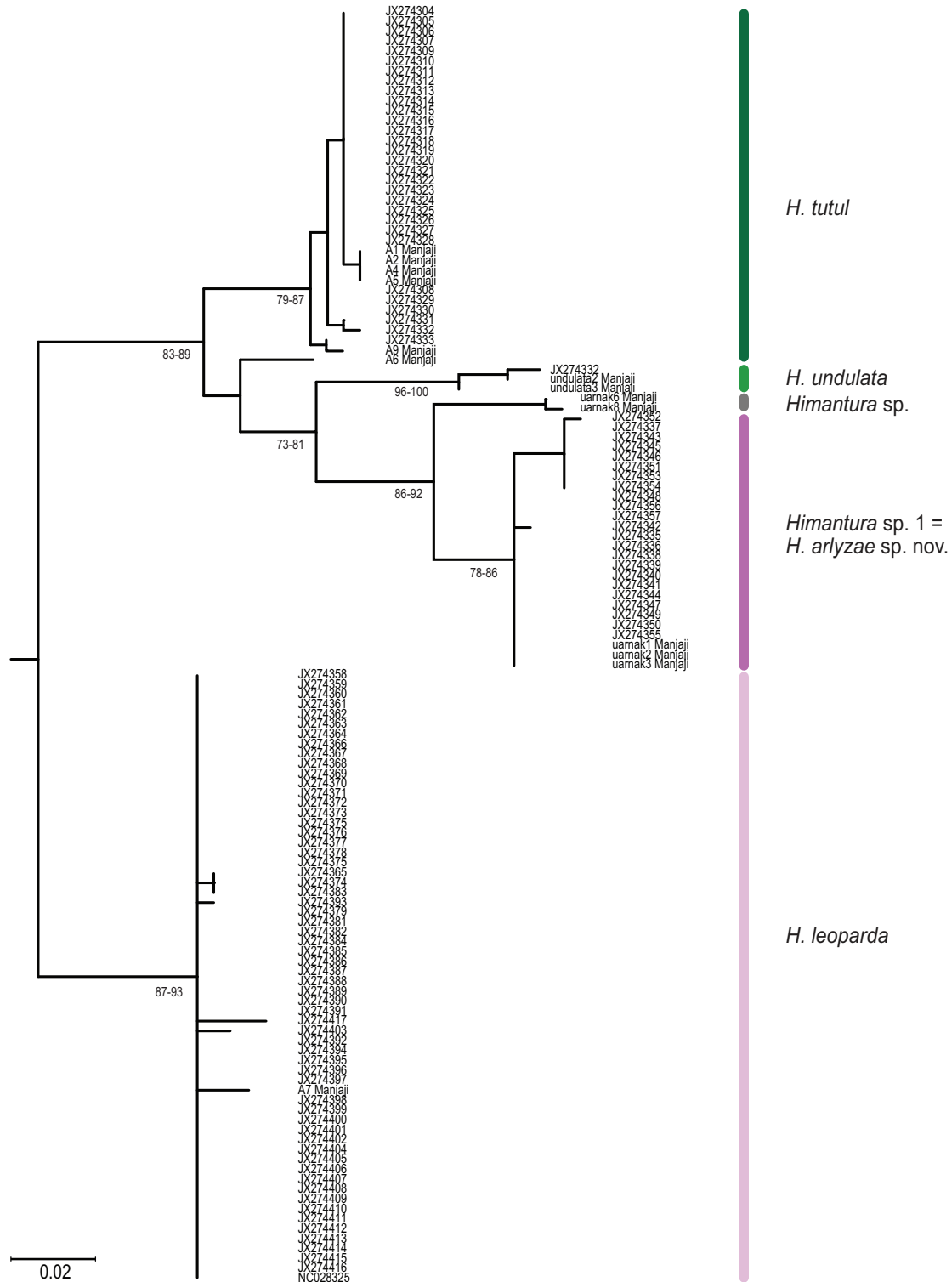


Fig. 3. Reticulate whiplay *Himantura* spp. Maximum-likelihood tree (T92+G+I substitution model, selected according to Bayesian information criterium) of nucleotide sequences at the *cytb* locus. Sequences were those publicly available in GENBANK (Benson et al., 2017) and from Manjaji (2004). The tree was constructed under MEGA12 (Kumar et al., 2024). Test of phylogeny was made by bootstrap resampling of nucleotide sites, using the ‘Adaptive Bootstrap’ option of MEGA12. Numbers at a node are the bootstrap scores (in%). Tree rooted by homologous sequences of *Maculabatis gerrardi*, GENBANK nos. AB021498 and AF110637. “*Himantura* sp.” was assigned to two unidentified individuals from West Papua (Manjaji, 2004); it is possible that the species they belong to is not yet represented in the available *COI* gene sequence dataset. For the other species, the colour codes are the same as Figure 1.

Table 3. Allelic frequencies at five intron loci for four reticulate whipray *Himantura* spp. species sympatrically occurring in the Indo-Malay archipelago. Alleles are designated by their size (in base pairs). Protocols for scoring genotypes have been presented previously (Atarhouch et al., 2003; Borsa et al., 2012; Arlyza et al., 2013). Individual genotypes are presented in supplementary table S1 of Arlyza et al. (2013). *N* sample size.

Locus	Species			
	<i>H. undulata</i>	<i>H. leoparda</i>	<i>H. tutul</i>	<i>H. arlyzae</i> sp. nov.
<i>Act-2 A</i>				
192	0.50	0.01	—	—
190	—	0.99	—	—
187	0.50	—	—	—
186	—	—	1.00	1.00
(<i>N</i>)	(1)	(57)	(27)	(23)
<i>Act-2 B</i>				
100	0.50	0.97	0.95	0.98
098	—	0.03	0.05	0.02
097	0.50	—	—	—
(<i>N</i>)	(1)	(60)	(30)	(23)
<i>Cam-3 A</i>				
178	—	0.01	0.89	—
175	1.00	0.99	0.11	1.00
(<i>N</i>)	(1)	(58)	(28)	(23)
<i>Cam-3 B</i>				
165	1.00	0.99	0.11	1.00
163	—	0.01	0.89	—
(<i>N</i>)	(1)	(55)	(28)	(23)
<i>Cam-3 C</i>				
144	0.50	0.82	—	—
129	0.50	0.18	0.11	—
125	—	—	0.89	1.00
(<i>N</i>)	(1)	(57)	(28)	(23)

combined nucleotide substitutions at six pairs of nucleotide sites (Fig. 2). *Himantura* sp. 1 was also distinguished from three other sympatric species (*H. leoparda*, *H. tutul* and *H. undulata*) by its allelic composition at five intron loci (Tab. 3).

3.3 Nucleotide distances

At locus *COI*, mean nucleotide distance between pairs of species varied from 0.016 to 0.122 (Tab. 4). Notably, *Himantura* sp. 1 was separated from its sister-species *H. australis* by 0.022 nucleotide distance. Mean within-species nucleotide distance was comprised between 0.002 and 0.009, significantly below inter-specific estimates. The histogram of individual-pairwise nucleotide distances (*p*-distance) drawn under ASAP showed a clear barcode gap between 0.020 and 0.048. Similar patterns of between- vs. within-species nucleotide distances were observed at loci *cytb* and *NADH2* (Tab. 4).

3.4 Species distributions

Nucleotide sequences at each of the three marker loci, grouped by species, are listed as entries into Suppl. Tables S1–S3 along with geographic information. Point maps based on the geographic records for each genetically-validated species are presented in Figure 5. The geographic distribution inferred

from these maps is detailed for each species in the Taxonomy section. The main results were the following: (i) *Himantura* sp. 1 and its sister-species *H. australis* appeared to be parapatric (Fig. 5A). (ii) *H. uarnak* and its sister-species *H. leoparda* had disjunct Indian vs. Pacific distributions (Fig. 5B). (iii) The distributions of *H. undulata* and *H. tutul* widely overlapped (Fig. 5C).

4 Discussion

4.1 Pigmentation-pattern and morphological variation in *Himantura* spp

The pigmentation patterns of *H. uarnak* have been described as “brown to blackish with white to yellow lines on dorsal surface forming a marbled or reticulated pattern” (Smith and Heemstra 1986), or said to consist of “numerous, round to oblong dark brown spots” (Randall 1995), or “in young with leopard pattern, honey-comb or reticulate pattern in adults” (Carpenter et al. 1997), or “a highly ornamented pattern of dark brown vermiculations/reticulations covering disc” (Allen and Erdmann, 2012), or “finely reticulate or spotted colour pattern on upper surface in adults (finely spotted or densely reticulate in young)” (Last et al., 2016a). The foregoing patterns may also be present in e.g., *H. leoparda* [“characteristic leopard-like rings on the dorsal surface of adults” (Manjaji-Matsumoto and Last, 2008), later changed to

Table 4. Reticulate whipray *Himantura* spp. Estimates (mean $\pm$ SD) of nucleotide distance between pairs of species (below diagonal), and within species (diagonal, highlighted grey) at loci *COI*, *cytb* and *NADH2*. Nucleotide substitution models were, respectively, T92+I, K2+G, and TN93+I.

Marker	Species						
Species	<i>H. uarnak</i>	<i>H. undulata</i>	<i>H. leoparda</i>	<i>H. tutul</i>	<i>H. australis</i>	<i>H. arlyzae</i> s.n.	<i>H. sp.2</i>
<i>COI</i>							
<i>H. uarnak</i>	0.004 $\pm$ 0.002				–		
<i>H. undulata</i>	0.105 $\pm$ 0.015	0.009 $\pm$ 0.002					
<i>H. leoparda</i>	0.018 $\pm$ 0.005	0.090 $\pm$ 0.014	0.003 $\pm$ 0.001				
<i>H. tutul</i>	0.106 $\pm$ 0.015	0.115 $\pm$ 0.016	0.100 $\pm$ 0.014	0.005 $\pm$ 0.002			
<i>H. australis</i>	0.105 $\pm$ 0.016	0.052 $\pm$ 0.010	0.092 $\pm$ 0.015	0.122 $\pm$ 0.018	0.002 $\pm$ 0.001		
<i>H. arlyzae</i> s.n.	0.100 $\pm$ 0.015	0.059 $\pm$ 0.010	0.086 $\pm$ 0.014	0.114 $\pm$ 0.016	0.022 $\pm$ 0.006	0.004 $\pm$ 0.001	
<i>H. sp. 2</i>	0.091 $\pm$ 0.015	0.063 $\pm$ 0.011	0.078 $\pm$ 0.013	0.111 $\pm$ 0.016	0.037 $\pm$ 0.008	0.016 $\pm$ 0.005	0.000
<i>cytb</i>							
<i>H. uarnak</i>	–				–		
<i>H. undulata</i>	–	0.014 $\pm$ 0.006					
<i>H. leoparda</i>	–	0.132 $\pm$ 0.031	0.002 $\pm$ 0.001				
<i>H. tutul</i>	–	0.102 $\pm$ 0.025	0.115 $\pm$ 0.026	0.004 $\pm$ 0.002			
<i>H. australis</i>	–	–	–	–	–		
<i>H. arlyzae</i> s.n.	–	0.100 $\pm$ 0.026	0.135 $\pm$ 0.035	0.090 $\pm$ 0.023	–	0.006 $\pm$ 0.003	
<i>H. sp.^a</i>	–	0.085 $\pm$ 0.025	0.141 $\pm$ 0.035	0.092 $\pm$ 0.022	–	0.053 $\pm$ 0.017	0.004 $\pm$ 0.004
<i>NADH2</i>							
<i>H. uarnak</i>	0.004 $\pm$ 0.002				–		
<i>H. undulata</i>	0.116 $\pm$ 0.012	0.014 $\pm$ 0.004					
<i>H. leoparda</i>	0.020 $\pm$ 0.004	0.122 $\pm$ 0.012	0.000 $\pm$ 0.000				
<i>H. tutul</i>	0.113 $\pm$ 0.011	0.124 $\pm$ 0.011	0.116 $\pm$ 0.011	0.007 $\pm$ 0.002			
<i>H. australis</i>	0.115 $\pm$ 0.012	0.067 $\pm$ 0.008	0.123 $\pm$ 0.013	0.132 $\pm$ 0.012	0.002 $\pm$ 0.001		
<i>H. arlyzae</i> s.n.	0.122 $\pm$ 0.013	0.073 $\pm$ 0.008	0.128 $\pm$ 0.013	0.132 $\pm$ 0.012	0.055 $\pm$ 0.008	–	
<i>H. sp. 3</i>	–	–	–	–	–	–	–

^a From two yet-unidentified individuals sampled in West Papua (Manjaji, 2004).

“dense pattern of dark medium-sized rings in adults” (Last et al., 2016a)], or in *H. undulata* (“ornamented pattern of leopard-like dark spots covering disc”; Allen and Erdmann, 2012). Bleeker’s (1852) description of *H. undulata* soberly characterized pigmentation patterns as “*fusco undulatum et nebulatum*” but only three juvenile specimens were examined. Specimens of other *Himantura* species have been reported with similar undulate patterns (Manjaji, 2004). In addition, dorsal pigmentation patterns of *H. australis* are described as “darker brown narrow rings, speckles or lines, finest around disc margins” (Last et al., 2016a) which may apply to other species (Fernando et al., 2019; Caira et al., 2020). In fact, Forsskål’s (1775) original description of the pigmentation patterns of *H. uarnak* (“*tota maculata*”) could apply to any species of the genus (Fernando et al., 2019). To effectively assess the extent of variation in pigmentation patterns within and between species in the genus *Himantura*, it is necessary to observe the pigmentation of many individuals genetically identified to species. Pigmentation patterns in adults were found to statistically differ, albeit with substantial overlap, between the two genetically divergent clusters initially placed under *H. leoparda* by Manjaji-Matsumoto and Last (2008): *H. leoparda* stricto sensu, and *H. tutul* (Borsa et al., 2013b). To the best of our knowledge, there has been no other such attempt to analyse differences in pigmentation patterns between species in the genus *Himantura*. From the available evidence (Borsa et al., 2013b; Caira et al., 2020), it is unlikely

that pigmentation patterns could fully discriminate among species. Moreover, spot patterns are thought to change in an individual during its growth (Manjaji-Matsumoto and Last, 2008; Borsa et al., 2021). Nevertheless, further investigation may help uncover trends in species-specific pigmentation patterns.

In addition to pigmentation patterns, morphological diagnoses have been attempted for four species in the genus *Himantura*, namely *H. uarnak*, *H. undulata*, *H. leoparda* and *H. australis* (Last et al., 2016a). None of the morphological characters examined (Tab. 1) is diagnostic of any of the four species considered. However, in *H. undulata*, the disc-length to disc-width ratio is said to be higher than in *H. uarnak*, despite apparent overlap (Bleeker 1852; Allen and Erdmann, 2012; Last et al., 2016a). For the other species under comparison, the proposed diagnoses are based on unknown sample sizes. Moreover, insufficient or no information has been provided regarding infra-specific variation.

Thus, identifying *Himantura* spp. species on the basis of colour patterns or morphology leads to enduring confusion. Weigmann’s (2016) hasty claim that “*H. tutul* is a junior synonym of *H. uarnak* due to strong morphological resemblance” stemmed from such confusion. Also, it is not possible to synonymize *H. tutul* with *H. uarnak* if simultaneously confusing the former with *H. leoparda*, while recognizing the latter as distinct from *H. uarnak*. As confirmed by the present DNA-based revision, *H. tutul* is clearly genetically different from both *H. uarnak*, with which it has been erroneously

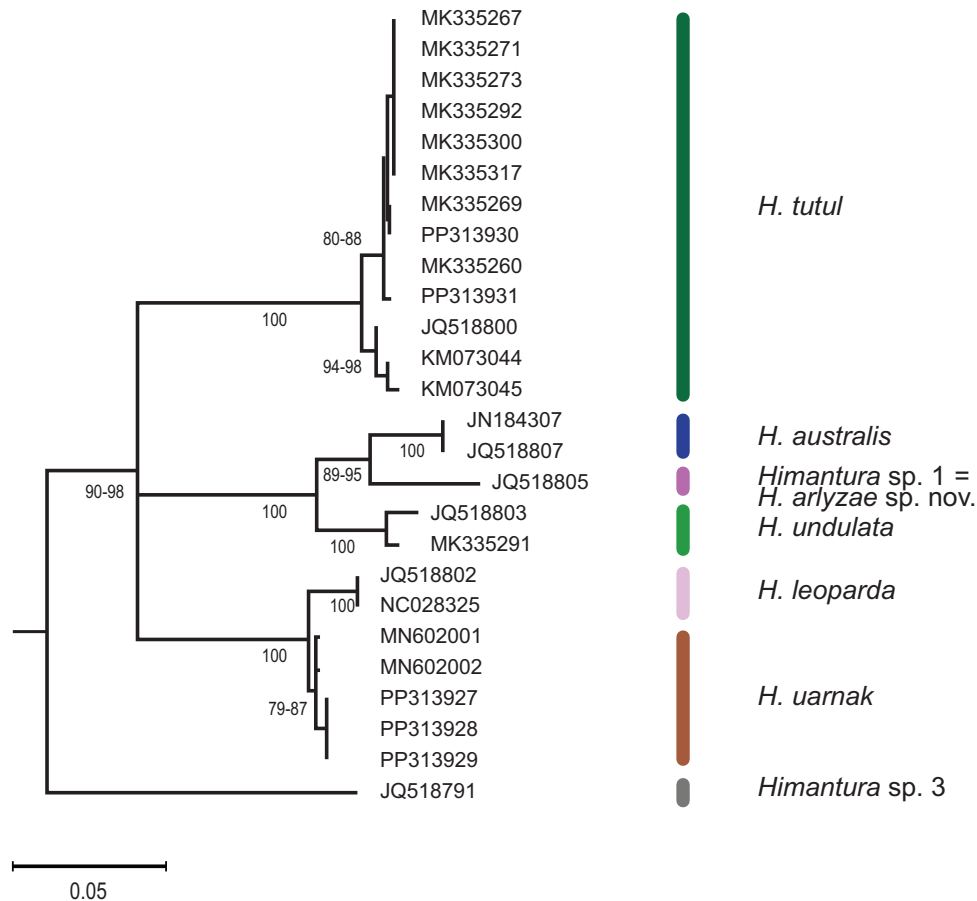


Fig. 4. Reticulate whiplay *Himantura* spp. Maximum-likelihood tree (TN93+I substitution model, selected according to Bayesian information criterium) of nucleotide sequences at the *NADH2* locus. Sequences were those publicly available in GENBANK (Benson *et al.*, 2017). The tree was constructed under MEGA12 (Kumar *et al.*, 2024). Test of phylogeny was made by bootstrap resampling of nucleotide sites, using the 'Adaptive Bootstrap' option of MEGA12. Numbers at a node are the bootstrap scores (in%). Tree rooted by homologous sequences of *Maculabatis gerrardi*, GENBANK nos. JN184304, JQ518792, JQ518809, JQ518824, JQ519070, JQ519087, KF927895-KF927897, KM396931 and NC_026208. Same colour codes as Figure 1.

synonymized by Weigmann (2016) and *H. leoparda*, under which it has been implicitly included by Manjaji-Matsumoto and Last (2008) and Last *et al.* (2016a).

Unless one arbitrarily imposed morphological traits as the sole acceptable operational criteria (*sensu* de Queiroz, 2007) to distinguish species, morphology should be considered as a mere possible source of taxonomic characters among other potential sources (Cook *et al.*, 2010). In the particular case of *Himantura* spp., morphology-based only descriptions do not offer diagnoses that would make identifications based on these morphological traits possible. It is therefore sensible to base species descriptions in the genus *Himantura* primarily on diagnostic molecular markers. It will be only after all species of the genus have been delineated and diagnosed that a search for eventual diagnostic morphological characters will be possible.

4.2 Relevance of molecular markers

In contrast to morphological characters, molecular markers including the mitochondrial marker offer clear-cut diagnoses for species in the genus *Himantura* (Borsa *et al.*, 2021).

Nevertheless, the potential for mitochondrial introgression, i.e. the occurrence of sp. A-type mitochondria in a related but distinct species (sp. B), should be considered when using the mitochondrial marker for identification to species. In species with uniparental mitochondrial inheritance, mitochondrial introgression may result from a past hybridization event (sp. A female x sp. B male), followed by successful backcrosses to sp. B individuals. It is commonly assumed that hybridization is a rare phenomenon in sharks and rays, because of internal fertilisation and pre-mating behaviour (Pierce *et al.*, 2009; Marshall and Bennett, 2010) which both help prevent inter-specific crosses. The only study of genetic variation known to us, that employed both nuclear and mitochondrial markers to distinguish species in the genus *Himantura* (Arlyza *et al.*, 2013), found no evidence of species-level mitochondrial paraphyly. This result confirms that the mitochondrial marker and, in particular, the *COI* barcode, is a highly reliable genetic marker for identifying individuals to species in the genus *Himantura*, as in other Elasmobranch genera (Ward *et al.*, 2008). Having confirmed the reliability of the genetic markers employed for the purpose of identifying *Himantura* spp.

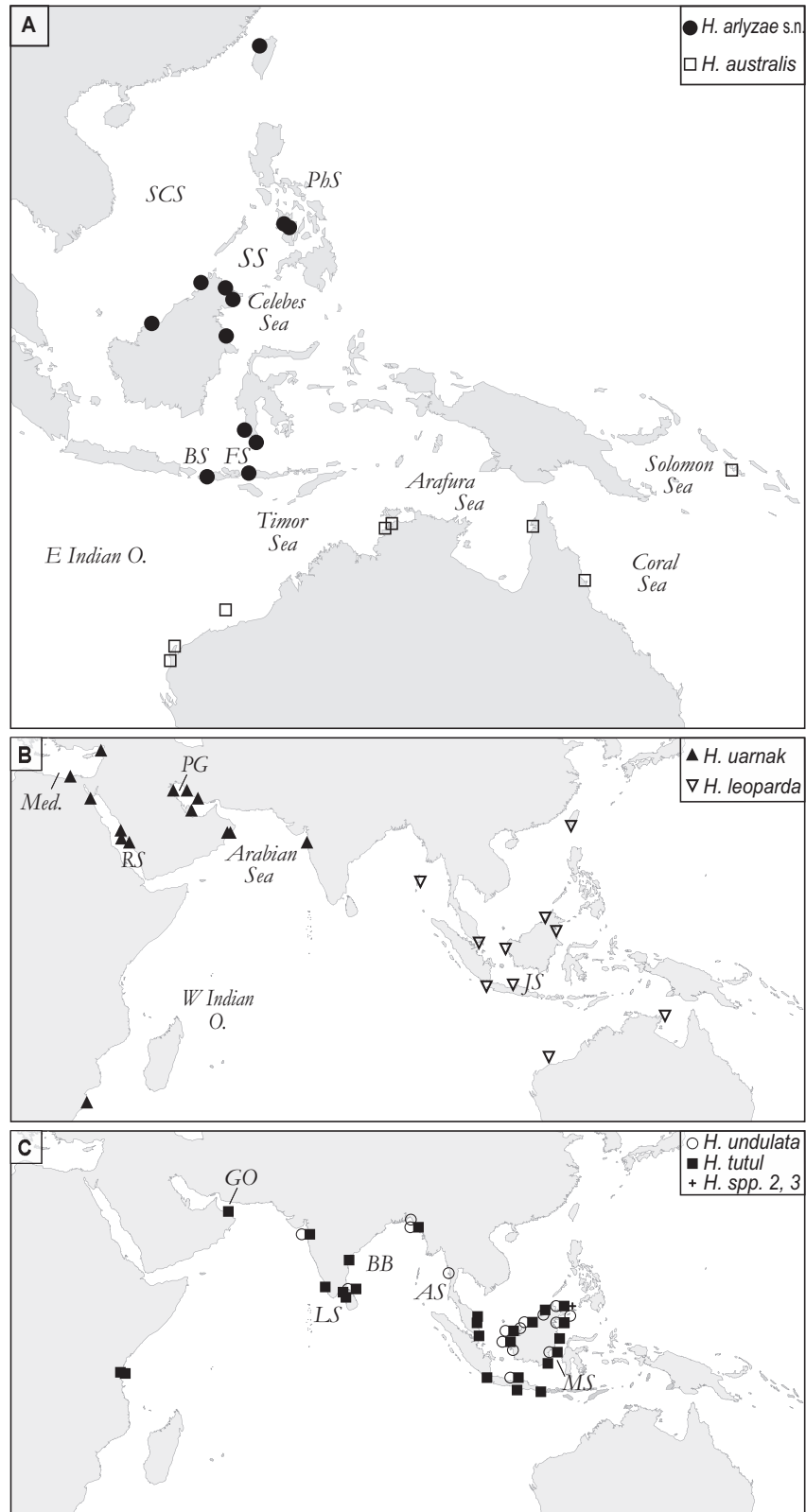


Fig. 5. Reticulate whipray *Himantura* spp. Geographic records of specimens genetically-identified to species (from Suppl. Tables S1-S3). *Abbreviations:* AS Andaman Sea; BB Bay of Bengal; BS Bali Sea; E Indian O. East Indian Ocean; FS Flores Sea; GO Gulf of Oman; JS Java Sea; LS Laccadive Sea; Med. Mediterranean Sea – Eastern Basin; MS Makassar Strait; PG Persian Gulf; PhS Philippine Sea; RS Red Sea; SCS South China Sea; Sol. S. Solomon Sea; SS Sulu Sea; W Indian O. West Indian Ocean. (A) *H. arlyzae* sp. nov. and sister species *H. australis*; (B) *H. uarnak* and sister species *H. leoparda*. (C) *H. tutul*, *H. undulata*, and the two yet-undescribed *Himantura* sp. 2 and *Himantura* sp. 3.

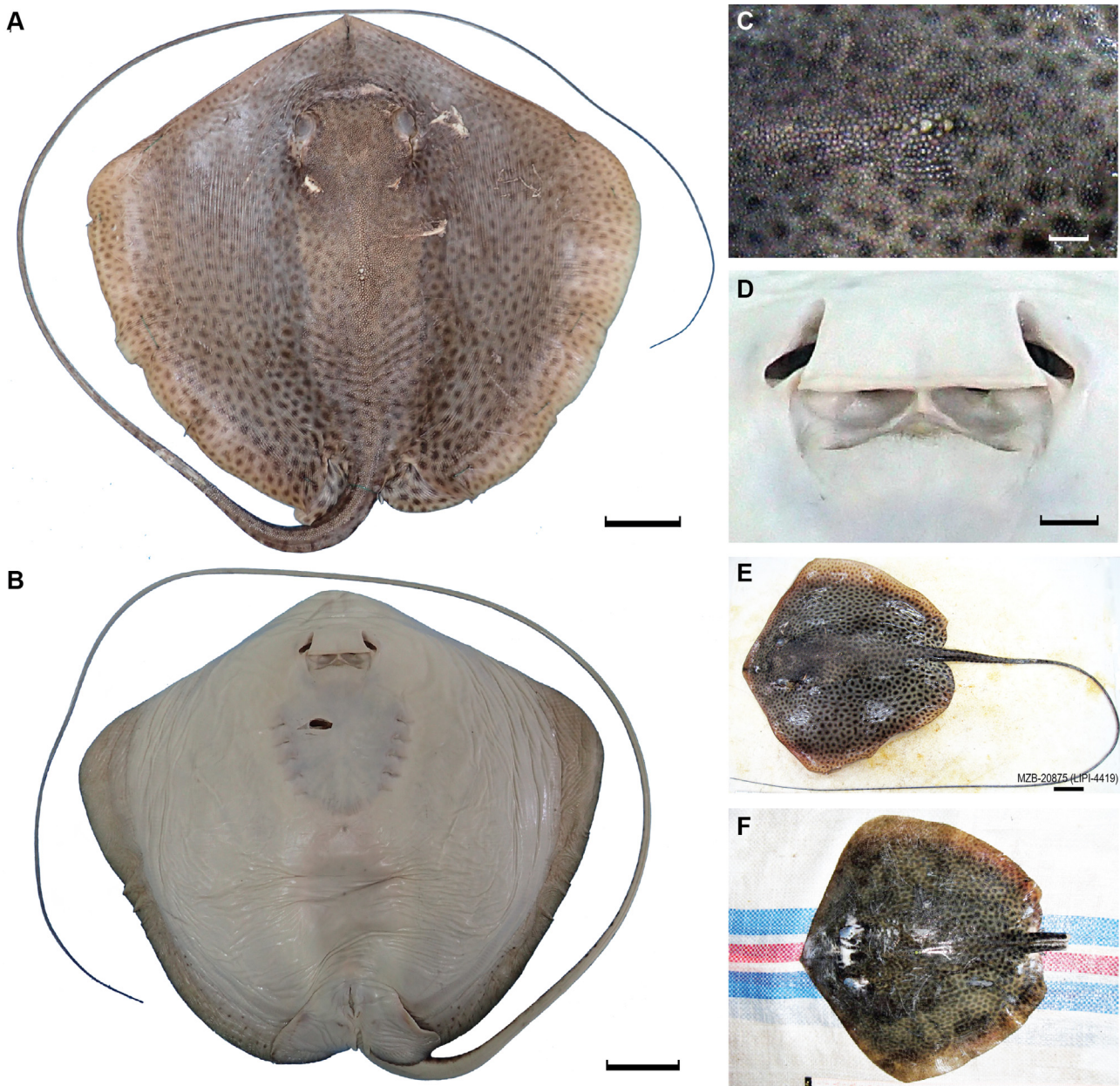


Fig. 6. External morphology and pigmentation patterns of Indo-Malay reticulate whipray *Himantura arlyzae* sp. nov. (A) Dorsal side of holotype (no. MZB-20875). Scale bar 5 cm. (B) Ventral side, idem. (C) Detail of squamation in interscapular region. Scale bar 1 cm. (D) Detail of mouth. Scale bar 1 cm. (E) Holotype, when freshly caught (credit: I.S. Arlyza/LIPI). Scale bar 5 cm. (F) Male specimen (30 cm disk width) from Paotere, Makassar Strait, 02 Nov. 2009.

species, one can proceed to the next step, which concerns the geographic distribution of the different species in this Indo-West Pacific genus.

4.3 Genetically-validated distribution maps and their utility in conservation

Batoids, including stingrays are among the most seriously threatened groups of marine animals (Dulvy *et al.*, 2021), and research on the management and conservation of these species should be a high priority. To help in this task, it is not only

necessary to correctly identify species, but also to correctly map their distribution. Indeed, mapping species ranges has benefits to conservation management (Guisan *et al.*, 2013; Suzzi and Devarajan, 2023; Salimi *et al.*, 2025). For example, maps may help to detect eventual gaps in distribution; to estimate areas of occupancy and fragmentation rate (IUCN, 2012); or to evaluate threats by superimposing other layers of geographic information such as fishing effort, habitat use, and other anthropogenic threats (e.g., Brooker *et al.*, 2020).

The present work provides the first genetically-validated point maps of species in the genus *Himantura*. The

distributions of two endangered species, *H. uarnak* and *H. leoparda*, considerably differ from those presented in the recent literature. For instance, it has been claimed that the geographic distribution of *H. uarnak* is continuously distributed from the Red Sea to southern Africa and all along the southern Arabic and Asian shores east to the Indo-Malay archipelago, including the western half of this archipelago, Sulawesi island, and the Philippines (Last *et al.*, 2016a; Sherman *et al.*, 2021). The geographic distribution of *H. leoparda* as claimed by the same sources (Last *et al.*, 2016a; Rigby *et al.*, 2024) was similar, except that the latter's range was reported to extend as far to the East as the Ryukyu Islands, the southern Coral Sea and the Louisiades archipelago (Solomon Sea). In contrast with the foregoing, the present maps confirm that *H. uarnak* and *H. leoparda* have disjoint distributions (as previously observed by Borsa *et al.*, 2021), the easternmost record of *H. uarnak* being the Arabian Sea while the westernmost record of *H. leoparda* was the Andaman Sea, that is, thousands of kilometres apart. The present maps also enable us to place the line of demarcation between *H. australis* and *Himantura* sp. 1 as the Timor Sea, a stretch of ocean reaching over 3000 m deep that separates the Sahul Shelf from the Indo-Malay archipelago (Ryan *et al.*, 2009).

4.4 Systematics and conservation

In de Queiroz's (2007) unified species concept, species are defined as separately evolving metapopulation lineages. Despite the unavailability of morphological criteria to distinguish species in the genus *Himantura*, such independently evolving metapopulation lineages were identified using three lines of evidence or operational criteria: phylogenetics, reproductive isolation, and geography.

An estimate of the divergence time between *H. australis* and *Himantura* sp. 1 can be derived from their genetic distance at the *COI* locus (2.2%). Using as a yardstick the rate of nucleotide substitution that has been previously estimated for the Dasyatidae at this locus [0.23% per million year (My); Puckridge *et al.*, 2013] leads to estimating the date of the last common ancestor to the two species at 4.78 My ago. Thus, the two species may well have been genetically isolated since the mid-Pliocene. This amount of genetic isolation indeed characterizes separate species rather than populations or even sub-species (Puckridge *et al.*, 2013; Poortvliet *et al.*, 2015). Whereas genetically distant species may overlap geographically (e.g., *H. tutul* and *H. undulata*), more-closely related geminate species pairs [(*H. uarnak*, *H. leoparda*) and (*H. australis*, *Himantura* sp. 1)] had separate geographic distributions. The apparently parapatric distribution of *H. australis* and *Himantura* sp. 1 could indicate both reproductive isolation and unachieved ecological compatibility, preventing one species to colonize the habitat of the other (Bull 1991). The present DNA-based study might thus constitute a new step in our understanding of the evolutionary biogeography of Indo-West Pacific stingrays, in addition to contributing to clarify the tangled taxonomy of species in the genus *Himantura*.

Accurate identification to species is necessary for effective management and conservation. Given the endangered status of reticulate whiprays (*Himantura* spp.), the present DNA-based taxonomic revision has important conservation implications.

First, the fact that the genus consists of up to eight valid species, and not four as currently reported in the International Union for the Conservation of Nature (IUCN) Red List (IUCN, 2025), could mean that the current population estimates of reticulate whipray species are overestimated and demographic trends are biased, particularly in the Indo-Malay archipelago. Each *H. uarnak* and *H. leoparda* as currently understood in the IUCN Red List sheets actually consists of several species. As seen in this study, *H. uarnak* and *H. leoparda* have smaller geographic ranges than assumed by the IUCN and as a consequence, they necessarily have smaller population sizes. As conservation efforts and legal protections often apply at the species level, the recognition of *Himantura* sp. 1 as distinct from the species with which it is presently confused in the IUCN Red List means that conservation strategies must be adjusted to account for its specific ecological needs, threats, and population dynamics. Another species that still awaits assessment is *H. tutul*.

To summarize, the approximations in the distributions of *H. uarnak* and *H. leoparda* in the IUCN Red List and the coincident lack of proper diagnoses available for these two species (Sherman *et al.*, 2021; Rigby *et al.*, 2024) call for their reassessment. This reassessment has to be done together with assessments of *H. tutul* and *Himantura* sp. 1 which so far have been ignored by the IUCN and with which *H. uarnak* and *H. leoparda* likely have been confused. A taxonomic update is warranted. Overall, our findings emphasize the need for more detailed ecological assessment for each species in the genus *Himantura*, reclassification of conservation priorities, and new protective measures to ensure their survival.

5 Taxonomy

Many Elasmobranch species face unsustainable fishing pressure, including, in particular, the coastal species of the Indo-Malay archipelago (Blaber *et al.*, 2009; White and Kyne, 2010; Dulvy *et al.*, 2014, 2021; D'Alberto *et al.*, 2022). *Himantura* spp. whiprays are in need of strict protection (Dulvy *et al.* 2017, 2021). For this, it is important that significant evolutionary lineages in this genus be properly diagnosed. This is the purpose of the present section, which also includes the formal description of *Himantura* sp. 1.

- Family Dasyatidae Jordan and Gilbert, 1879
- Genus *Himantura* Müller and Henle, 1837
- Type species *H. uarnak* [Gmelin (ex-Forsskål), 1789]

5.1 *Himantura arlyzae* sp. nov

ZooBank (<http://zoobank.org>): urn:lsid:zoobank.org:act:7D2E28C0-A048-485B-A949-5FE195967358.

5.1.1 Notice

This article and the nomenclatural act it contains have been registered in the online registration system for the International Commission on Zoological Nomenclature, ZooBank, under life science identifier (LSID) no. urn:lsid:zoobank.org:pub:F37722D0-2FBE-442B-AD30-4A67953FFDB8.

5.1.2 Previous references

'*H. uarnak*' (Manjaji, 2004; Compagno *et al.*, 2005; Arlyza *et al.*, 2013; Hanner, 2020); '*uarnak* 1' (Naylor *et al.*, 2012); 'Indo-Malay region' sub-clade under *H. uarnak* (Borsa *et al.*, 2013b; Borsa, 2017); '*H. uarnak* 1' (Caira *et al.*, 2020); '*H. uarnak* i' (Hanner, 2020); '*Himantura* sp. 1' (Borsa *et al.*, 2021, Cabebe-Barnuevo *et al.*, 2025). Also BIN number BOLD:AAB7832 in BOLD (<https://v4.boldsystems.org/>; consulted 22 March 2025).

5.1.3 Material examined

Includes specimen no. MZB-20875 (=LIPI 4419) deposited at the Museum Zoologicum Bogoriense in Bogor, here designated holotype, specimen no. UPVMI-3111 deposited at the University of the Philippines Visayas in Miagao (here designated paratype), and specimen no. NMMB-P015784 deposited at the Chondrichthyan collection of the National Museum of Marine Biology and Aquarium in Pingtung (voucher). Also includes vouchers H5476.03, H5477.01 and H5484.01 deposited in the Australian Fish Collection at CSIRO, Hobart. Also includes tissue samples nos. BW-A7728 (CSIRO, Hobart), GN2238 (University of Florida, Gainesville FL), BO-82 (=GN3119; U. Connecticut, Mansfield CT) and PSK-11 (Southeast Asian Fisheries Development Center/ Marine Fishery Resources Development and Management Department, Kuala Lumpur).

5.1.4 Etymology

The new species is named after Dr Irma S. Arlyza (1974-2019), who was a researcher interested in the genetics of Elasmobranchs at LIPI-P2O, Jakarta (Borsa *et al.*, 2019). One of her contributions to the taxonomy of the genus *Himantura* was a comprehensive DNA survey of a collection of specimens from the Indonesia-Papua archipelago as part of her PhD project (Arlyza, 2013). Nuclear and mitochondrial markers helped her score four distinct, reproductively isolated species in this region, including a cryptic species (now *H. tutul*) within the then-newly described *H. leoparda* (Arlyza *et al.*, 2013). I.S. Arlyza co-authored the description of five new stingray species (Borsa *et al.*, 2013b, 2018) and the resurrection of another (Borsa *et al.*, 2013a). We chose to name this species after her as an acknowledgement of both her contribution to the genetics and taxonomy of *Himantura* spp. and her efforts to promote molecular genetics into studies of Elasmobranchs in the Indonesian archipelago. Epithet *arlyzae* is the non-capitalized latin genitive singular form of feminine noun "Arlyza". The following vernacular names are proposed in relation to the geographic distribution of *H. arlyzae* sp. nov. (Fig. 6A): Indo-Malay reticulate whipray (English); pari cambuk bertutul Indo-Melayu (Indonesian); pastenague à fouet d'Indo-Malaisie (French).

5.1.5 Diagnosis

Based on the alignment of 249 partial *COI*-gene sequences (Fig. 2), *H. arlyzae* sp. nov. ($N=35$) differs from all other species in the genus *Himantura* that were sequenced at the *COI* locus (except *Himantura* sp. 2), by possessing the following private nucleotides: T at nucleotide site no. 192 and

G at nucleotide sites nos. 249 and 576 of the gene. In addition, *H. arlyzae* sp. nov. differs from *Himantura* sp. 2 ($N=2$) by possessing nucleotide A (instead of G) at site no. 111 and nucleotide G (instead of A) at site no. 363. Based on allelic frequencies at five intron loci (Tab. 3), *H. arlyzae* sp. nov. individuals ($N=23$) differ from *H. leoparda* ($N=57$) by possessing size allele 186 (vs. allele 190) at actin intron locus *Act-2 A*; and by possessing allele 125 (vs. alleles 129 and 144) at calmodulin intron locus *Cam-3 C*. Also, while *H. arlyzae* sp. nov. individuals ($N=23$) are all homozygous for allele 165 at locus *Cam-3 B* and allele 125 at locus *Cam-3 C*, the probability of encountering an *H. tutul* individual homozygous for these two alleles is approximately 0.1% (based on a sample of 28 individuals, assuming Hardy-Weiberg equilibrium at both loci, and assuming the latter are independent).

Diagnosis in Indonesian language: Berdasarkan penjabaran 249 sekuens parsial gen *COI* yang sebagian besar diambil dari database GenBank dan BOLD (Gbr. 2), *H. arlyzae* sp. nov. ($N=35$) berbeda dengan semua spesies valid lainnya pada genus *Himantura* (kecuali *Himantura* sp. 2), karena terdapat nukleotida privat pada lokus gen *COI*, yaitu T pada situs nukleotida no. 192 dan G pada situs nukleotida no. 249 dan 576. Selain itu, *H. arlyzae* sp. nov. juga berbeda dengan *Himantura* sp. 2 ($N=2$) karena terdapat nukleotida A (bukan G) pada situs nukleotida no. 111 dan nukleotida G (bukan A) pada situs nukleotida no. 363. Berdasarkan frekuensi alel pada lima lokus intron (Tab. 4), individu dari *Himantura* sp. nov. ($N=23$) berbeda dengan *H. leoparda* ($N=57$) karena memiliki alel ukuran 186 (vs. alel 190) pada lokus intron aktin *Act-2 A*; dan alel 125 (vs. alel 129 dan 144) pada lokus intron kalmodulin *Cam-3 C*. Selain itu, semua individu *Himantura* sp. nov. ($N=23$) bersifat homozigot untuk alel 165 pada lokus *Cam-3 B* dan alel 125 pada lokus *Cam-3 C*, dan kemungkinan untuk menemukan individu *H. tutul* yang homozigot untuk kedua alel ini adalah sekitar 0,1% (jumlah sampel: 28 individu, dengan asumsi kesetimbangan Hardy-Weiberg berlaku pada kedua lokus, dan kedua lokus tersebut bersifat independen).

5.1.6 Description

The holotype (Fig. 6A-E) is a juvenile male specimen, 38.2 cm DW and 142.0 cm total length, collected by I.S. Arlyza in November, 2009 from local fishermen on the western coast of Selayar Island in the Flores Sea (6°06'S; 120°26'E). It was captured in shallow water using a harpoon. It is registered at the Museum Zoologicum Bogoriense under no. MZB-20875; it is also registered at BRIN-RCO in Ancol, Jakarta under no. LIPI-4419. The specimen has been preserved in 70% alcohol and 4% formalin solution. A piece of ca. 0.5x1.5 cm² of fresh skin and muscle was excised from the ventral side in the interbranchial region (Fig. 6C). A subsample of tissue, labelled 'H.spp-FS-JS1' [encoded 'IR102' in Arlyza *et al.* (2013)] is preserved in molecular-biology grade alcohol at -20°C in the freezers of the genetics laboratory at RCO-BRIN, Jakarta.

Morphological description of holotype: disc pentagonal, snout with pointed apex; anterior margins of disc convex, lateral apices rounded; posterior margin convex, free rear tip rounded; tail whip-like, tapering gently toward sting; two large, heart-shaped thorns in mid-scapular region in posterior half of a line of 16 slightly enlarged thorns. The following

measurements are expressed as percentages of DW: disc length 91.4; distance from tip of snout to pectoral fin insertion 80.4; distance from tip of snout to eye 19.6; mouth width 8.0; nostril length 5.2; minimal distance between nostrils 8.1; head length (from tip of snout to rear tip of fifth gill slit) 34.9; eye length 4.2; orbit diameter 5.8; inter-orbital width 12.1; inter-ocular width 11.8; spiracle length 6.6; interspiracular width 18.0; orbit and spiracle length 9.6; distance of orbit to insertion of pectoral fin 57.3; length of nasal curtain 10.6; width of nasal curtain 5.2; width of first, third and fifth gill slits respectively 3.6, 3.7 and 2.6; distance between first and fifth gill slits, respectively left and right: 13.2 and 12.6; minimum distance between left and right first gill slits: 18.8; minimum distance between left and right fifth gill slits: 11.6; pre-nasal length of snout: 14.8; distance from tip of snout to cloaca origin: 74.9; distance from pectoral fin insertion to base of sting: 38.2; distance from origin of cloaca to base of sting: 45.0; cloaca length: 5.6; tail width at axil of pelvic fin: 5.9; tail width at base of sting: 2.3; tail height at axil of pelvic fin: 4.4; tail height at base of sting: 2.3; pelvic fin (embedded) length: 18.6; width across pelvic fin base: 9.6; greatest width across pelvic fin: 10.7; clasper length from pelvic axil: 5.5; post-cloacal clasper length: 8.5.

Dorsal pigmentation patterns consisted of roughly rounded spots densely covering the disk, which size varied from 0.5% to 3.3% DW. The number of spots crossed by transect of the entire disk width was 35. Their size was $2.1 \pm 0.4\%$ DW in the squamosed central part of the body, $2.4 \pm 0.3\%$ DW in the thick part of the disk, and $1.4 \pm 0.5\%$ DW in the external margins of the pectoral fins. Ventral side has a uniform, creamy colour.

The partial *COI* gene sequence of the holotype, a fragment 620 bp long comprised between nucleotide sites nos. 80 and 699 of the gene, homologous to nos. 5597 and 6216 of the mitogenome of *H. leoparda* (NC_028325; Shen *et al.*, 2016) is 5'-CAGGGATAGTGGGTACTGGTCTTAGCC TACTAATTCGGACAGAGCTAAGCCAAACCA GGTGCATTACTGGGTGATGATCAAACTCA TAACGTGATTGTCACCTGCCCATGCGCTTTG TAATAATCTTTTTTCATGGTGATACCTATTA TAATTGGTGGTTTTTGGTAATTGACTGATT CCCCTAATAATCGGTGCCCCAGACATGGC CTTTCCTCGAATAAACAAACATAAGTTTTT GACTTCTTCCACCATCCTTTCTACTTCTCT TAGCCTCTGCTGGAGTAGAAGCGGGAGC TGGAACAGGCTGAACAGTCTATCCCCCAT TAGCTGGTAACCTAGCACACGCAGGGGC TTCAGTAGATTAGCAATCTTTTCGCTAC ATCTAGCTGGCGTATCTTCTATCTTTGGCCT CCATCAATTTTATTACTACAATTATTAACA TAAACCACAGCAATTTACAGTATCAA ACACCCCTCTTTGTCTGATCAATTCTTATT ACGGCCGTACTCCTCTTACTATCTCTTCCT GTCCTAGCAGCAGGTATTACAATACTTCT AACAGATCGCAACCTCAATACAACCTTCT TTGATCCTGCAGGAGGAGGTGACCCAATT CTTTATCA A-3'. This sequence has been deposited in GENBANK with accession no. JX263359. The holotype's nucleotide sequence of a fragment of the *cytb* gene 239 bp long comprised between nucleotide sites nos. 167 and 405 (nucleotide sites nos. 14 616 and 14 854 of the mitogenome of *H. leoparda*; Shen *et al.* 2016) is: 5'-ATACCGCAGACA

TCTCCTCGGCATTCTCCTCAGTCGCTCAT ATCTGTCGAGATGTAAACTATGGCTGACT AATCCGCAACATCCACGCCAATGGTGCTT CCATGTTCTTTATCTGCATTTATCTTCACA TTGCTCGAGGTTTTTACTATGGCTCCTATC TTTATAAAGAAACCTGAAATATCGGAGTA ATTATCCTAATACTACTAATAGCCACTGCC TTTGTAGGTTACGTTCTTCCA-3' (GenBank no. JX274348). In addition, the holotype is homozygous for alleles 186 at locus *Act-2A*, 100 at locus *Act-2B*, 175 at locus *Cam-3A*, 165 at locus *Cam-3B* and 125 at locus *Cam-3C* (Arlyza *et al.*, 2013).

The paratype is a male specimen 37.7 cm DW collected from the Carles fish market of Iloilo Province, Philippines in February 2022 and registered under no. UPVMI-3111 at the University of the Philippines Visayas, Miagao (Cabebe-Barnuevo *et al.*, 2025).

5.1.7 Distribution

The type locality of *H. arlyzae* sp. nov. is Selayar Island in the Flores Sea. Based on genetically-validated records, the geographic distribution of *H. arlyzae* sp. nov. spans part of the Indo-Malay archipelago including the South China Sea, the Sulu Sea, the Philippine Sea, Makassar Strait, the Bali Sea, the Flores Sea and the Banda Sea (Fig. 5A). Other *Himantura* species occurring in this region are *H. undulata*, *H. leoparda*, and *H. tutul* (Figs. 5B, and 5C). Two more lineages, *Himantura* sp. 2 and *Himantura* sp. 3, have so far been sampled from the southern Sulu Sea and northern Celebes Sea area (Fig. 5C).

5.1.8 Remarks

Borsa (2017: his figure 2c) assumed that 'uarnak 1', the clade sister to 'uarnak 2' in the tree of *NADH2* gene sequences produced by Naylor *et al.* (2012), was equivalent to the 'Indo-Malay region (IMR)' lineage (now *H. arlyzae* sp. nov.) sister to the 'NW Australia (NWA)' lineage (now *H. australis*) in his tree of partial *COI* gene sequences. This equivalence is now confirmed through the placement in the phylogenetic trees of two 'uarnak 1' individuals labelled GN2238 and GN3119 in Naylor *et al.* (2012) and sequenced at the *COI* locus as, respectively, IRREK193-08 and IRREK863-08 in BOLD (Hanner, 2020).

Both specimen MZB-20875 (Figs. 6A-6E) and a discarded specimen from the Makassar Strait (Fig. 6F) possess a pale outer dorsal margin reminiscent of Gray's (1834) figuration of *Trygon russellii*. However, a number of characters observable on these two specimens differ from *T. russellii*: both exhibit spots in the paler region (*T. russellii* as represented in drawing has none), two enlarged median denticles in the midscapular region (*T. russellii* has a single enlarged denticle) and three to four longitudinal rows of spots at the basis of the tail (only two rows in *T. russellii*). Based on these observations, there is no apparent reason to make *H. arlyzae* n. sp. a junior synonym of *T. russellii*. Also, the single interscapular thorn figured for *T. russellii* (Gray 1934) indicates *H. undulata* according to Manjaji-Matsumoto and Last (2008). Moreover, the distribution of *H. arlyzae* sp. nov. as inferred from genetically-validated records (Fig. 5A) does not reach India, where the holotype of *T. russellii* was collected (Gray, 1834).

Another species, *T. punctata* Günther, 1870 has been synonymized with *H. uarnak* (Manjaji, 2004) although this synonymy has been questioned (Kottelat, 2013). The type locality is not certain: it has been given as the “East-Indian archipelago” preceded by a question mark (Günther, 1870). The precision that “the disk is a little broader than long” seems to exclude that *T. punctata* be synonymous with *H. undulata*. The only known preserved specimen is the holotype, a juvenile male 28.1 cm DW. Juvenile pigmentation patterns cannot be distinguished among *Himantura* spp. except, perhaps, those of *H. undulata* (Bleeker 1852).

Pictures of specimens genetically identified as *H. arlyzae* sp. nov. also include Global Cestode Database nos. BO-82, BOD-67, BOD-73, HBO-109, KA-410 and KA-411, all from the Celebes Sea, and BO-500 from the South China Sea. It is not clear whether specimen no. KA-411 (a female, 99.8 cm DW) has been kept as voucher as mentioned by Naylor *et al.* (2012) or has been discarded as understood from its sheet in the Global Cestode Database (<https://tapewormdb.uconn.edu/>; consulted 09 April 2025). From the pictures available, dorsal spot patterns in *H. arlyzae* sp. nov. include roughly rounded spots densely covering the entire disk, which size decreases from center to extremity of disk (holotype, BO-82, BO-500, KA-410, MZB-20875), a similar pattern that also includes slightly polygonal and some rare dumbbell-shaped spots (UPVMI 3111) or slightly elongate spots, sometimes anastomosed (NMMB-P015784), and long spots arranged in a convoluted fashion on the posterior half of the disk (BOD-73, KA-411). The latter two specimens, one male (89.2 cm), one female (99.8 cm) are the largest of the genetically-validated specimens on which spot patterns have been documented. They may possess the adult patterns of at least a proportion of individuals in this species, although a dedicated study based on much larger sample sizes of adults would be needed for confirmation. These patterns somewhat differ from those observed on the other specimens mentioned above, all juveniles.

5.2 Molecular diagnoses and updated distributions of other *Himantura* species

In the following, species that represent distinct evolutionary lineages are arranged by chronological order, according to their date of description. Main reference follows species name.

5.2.1 *Himantura uarnak* [Gmelin (ex-Forsskål), 1789]

Material examined includes specimens nos. CAS-ICH 247241 (neotype; <https://zoobank.org/References/b2113697-5ebf-4364-b50c-63019a1a076a>) and IMSMETU-HL-01 (voucher). Also includes tissue samples ADC30.10-1 and Smith 30.10-2 (South African Institute for Aquatic Biodiversity, Makhanda), and BW-A6088, BW-A6343, BW-A6087 and BW-A11765 to BW-A11768 (CSIRO, Hobart). Genetically-validated references include Steinke *et al.* (2011), Caira *et al.* (2020), Appleyard (2021), and Borsa *et al.* (2021). This species has been misidentified as ‘*H. leoparda*’ (Bineesh *et al.*, 2017; Yucel *et al.*, 2017); and ‘*H. cf. leoparda*’ (International Barcode of Life, 2019).

Diagnosis: based on the alignment of the partial *COI*-gene sequences of a total of 249 *Himantura* spp. individuals, *H. uarnak* ($N=28$ including neotype) is distinguished from all other species in the genus *Himantura* by possessing nucleotides T, C or T, C, A, and G at, respectively, nucleotide sites 270, 366, 478, 480, and 624 of the *COI* gene; the other species in the genus *Himantura* possess nucleotide C at site 270, A or G at site 366, T at site 478, G or T at site 480, and A at site 624 (Fig. 2).

Distribution: The type locality of *H. uarnak* is the Red Sea (Fricke, 2008; Allen and Erdmann, 2012; Borsa *et al.*, 2021). Based on genetically validated records, its current geographic distribution includes the Eastern Basin of the Mediterranean Sea, the Red Sea, the Mozambique Channel, the West Indian Ocean, the Arabian Sea, and the Persian Gulf (Fig. 5B).

Remarks: The Global Cestode Database holds tissue samples and sequences of three specimens labelled EG-1, EG-2 and EG-38 collected in the northern Red Sea and in the eastern Mediterranean Sea off Egypt and identified as *H. uarnak*. Their *NADH2*-gene sequences cluster into a lineage sister to *H. leoparda* (Fernando *et al.*, 2019) labelled ‘species ID no. 62451’ in the Global Cestode Database, which is distinct from the other species of that database, including *H. undulata* (‘species ID no. 552’), *H. tutul* (‘species ID no. 1359’), *H. australis* (‘species ID no. 1358’) and *H. arlyzae* sp. nov. (‘species ID no. 1357’). To formally ascertain that these three specimens from off Egypt effectively represent Forsskål’s *H. uarnak*, their *NADH2*-gene sequences need to be compared to those of the series of specimens collected from off Jeddah by Borsa *et al.* (2021) including *H. uarnak*’s neotype. Alternatively, specimens EG-1, EG-2 and EG-38 could be compared to *H. uarnak*’s neotype using *COI* barcodes. Barcode identification no. BOLD:AAB7830 has so far been allocated to this species (<https://v4.boldsystems.org/>; consulted 23 March 2025).

5.2.2 *Himantura undulata* (Bleeker, 1852)

Junior synonym: *H. fava* (Annandale, 1909). Reference material includes voucher specimens nos. H5481.01 and H5483.01 of CSIRO, Hobart, and no. MMTW 2 190504 of Universiti Malaysia Sabah, Kota Kinabalu. Genetically-validated references include Manjaji (2004), Ward *et al.* (2005) (as “*H. fava*”), Naylor *et al.* (2012), Arlyza *et al.* (2013), Lim *et al.* (2015), Bineesh *et al.* (2017), Mohd-Arshaad and Jamaludin (2018), Segura-Garcia and Yain Tun (2018), Ahmed *et al.* (2019), Fernando *et al.* (2019), Caira *et al.* (2020), and Borsa *et al.* (2021). Misidentified as “*H. uarnak* i” (Hanner, 2021) and “*H. leoparda*” (Loh *et al.*, 2023).

Diagnosis: based on the alignment of the partial *COI*-gene sequences of 249 individuals, *H. undulata* ($N=19$) is distinguished from all other species in the genus *Himantura* by possessing nucleotides G, T, A, G, and C at, respectively, nucleotide sites 294, 457, 537, 627, and 681 of the *COI* gene; the other species in the genus *Himantura* possess nucleotide A at sites 294 and 627, C at site 457, G at site 537, and T at site 681 (Fig. 2).

Distribution: The type locality of *H. undulata* is the Java Sea (Bleeker, 1852). Based on available genetically-validated

records, its geographic distribution includes the Arabian Sea, the Bay of Bengal, the Andaman Sea, the South China Sea and the Java Sea, Sulu Sea, Celebes Sea and Makassar Strait coasts of Borneo (Fig. 5C).

Remarks: Bleeker (1852) diagnosed ‘*Trygon undulata* Blkr.’ based on three syntypes from the Java Sea, whose disk width ranged between 27.0 cm and 32.0 cm (Manjaji, 2004) against two individuals from the same region that he identified as ‘*T. uarnak* Rüpp.’ 24.0 cm and 31.5 cm DW. According to Bleeker (1852), the two species differed by their general disk shape: “*Discus corporis longior quam latus*” in *T. undulata* vs. “*Discus corporis latior quam longus*” in *T. uarnak* and by their dorsal pigmentation: “*Corpus superne fusco undulatum et nebulatum*” in *T. undulata* vs. “*Corpus superne maculis fuscis confertis*” in *T. uarnak*. The illustration of *T. undulata* in Bleeker (1883) represents an immature male specimen with an intact tail and a single sting, presumably the only immature male syntype described in Bleeker (1852). The intertwined large comma-like spots visible on this illustration seem to be characteristic of immature *H. undulata* (Caira *et al.*, 2020). Squamation in the dorsal interscapular region of adults often reveals a single, large pearl-shaped denticle, which is generally absent in other *Himantura* spp. (Manjaji, 2004). Barcode identification no. BOLD:AAF0692 has so far been allocated to this species (<https://v4.boldsystems.org/>; consulted 23 March 2025).

5.2.3 *Himantura leoparda* Manjaji-Matsumoto and Last, 2008

Synonym: ‘Cluster 4’ / ‘clade IV’ (Arlyza *et al.*, 2013). Reference material includes specimen no. CSIRO H5478.01 (paratype). Genetically-validated references include Naylor *et al.* (2012), Borsa *et al.* (2013b, 2021), Shen *et al.* (2016), Appleyard (2017), Borsa (2017), Ravi *et al.* (2019). Misidentified as ‘*H. undulata*’ (Appleyard, 2020) and ‘*Himantura* sp. a’ (Hanner, 2021).

Diagnosis: based on the alignment of the partial *COI*-gene sequences of 249 individuals, *H. leoparda* ($N=68$) is distinguished from all other species in the genus *Himantura* by possessing nucleotide T at nucleotide site 145 of the *COI* gene. All other species in the genus *Himantura* possess nucleotide C at that nucleotide site (Fig. 2).

Distribution: The type locality of *H. leoparda* is the Gulf of Carpentaria in the Arafura Sea (Manjaji-Matsumoto and Last, 2008). Based on available genetically validated records, its geographic distribution also includes the Andaman Sea, the Java Sea, the East Indian Ocean off Western Australia, the South China Sea, and the Celebes Sea (Fig. 5C).

Remarks: Of all of Manjaji’s (2004) specimens labelled ‘*Himantura* sp. A’ (the name that she provisionally assigned to *H. leoparda* pending its formal description) that were sequenced at the *cytb* locus, only one (no. A7, also registered in the Australian National Fish Collection as CSIRO H5478.01) turned out to be *H. leoparda* while specimens nos. A1, A2, A4, A5 and A9 were identified as *H. tutul* (Borsa, 2017). The placement of the nucleotide sequence of Manjaji’s (2004) specimen no. A6, albeit uncertain (Fig. 3) is closer to *H. tutul* than to any other *Himantura* species. This specimen, also registered in the Australian National Fish Collection as no. CSIRO H.5284.05 has been hastily designated paratype of

H. leoparda (Manjaji-Matsumoto and Last, 2008). Barcode identification no. BOLD:AAB7830 has so far been allocated to this species (<https://v4.boldsystems.org/>; consulted 23 March 2025).

5.2.4 *Himantura tutul* Borsa *et al.*, 2013b

Previously referred to as ‘*uarnak* 3’ (Naylor *et al.*, 2012); and ‘Cluster 1’ / ‘clade I’ (Arlyza *et al.*, 2013) within what was then known as *H. leoparda*. Also ‘Species ID no. 1359’ in the Global Cestode Database. Has been misidentified as ‘*H. uarnak*’ (Weigmann, 2016; Bineesh *et al.*, 2017; Gouws, 2020; Bogorodsky *et al.*, 2021), ‘*H. leoparda*’ (Lim *et al.*, 2015; Bineesh *et al.*, 2017; Mohd-Arshaad and Jamaludin, 2018; Mohd Arshaad, 2020; Loh *et al.*, 2023) and ‘*H. uarnak* i-iii’ (Hanner, 2020). Genetically-validated references include Borsa *et al.* (2013b, 2021), Borsa (2017), Fernando *et al.* (2019), Caira *et al.* (2020) and Kumar *et al.* (2020). Reference material includes specimens nos. MNHN:ICTI5184 (holotype, Museum National d’Histoire Naturelle, Paris), UMS MMSK [c4] [Universiti Malaysia Sabah (UMS), Kota Kinabalu], and HBO-86 (UMS, Kota Kinabalu); and tissue samples nos. BO-47, BOD-49, BOD-65, BOD-66 and BOD-74 of the Global Cestode Database.

Diagnosis: based on the alignment of the partial *COI*-gene sequences of 249 individuals, *H. tutul* ($N=73$ including holotype) can be distinguished from all other species in the genus *Himantura* by possessing the following diagnostic nucleotides: T at nucleotide sites nos. 129, 264, 273, 282, 331, 378 and 631 of the *COI* gene; C at sites nos. 267, 309, 318, 321, 390, 570, 576 and 666, and G at sites nos. 312, 564 and 609. The other species in the genus *Himantura* possess nucleotide C at nucleotide sites nos. 129, 264, 282, 331, 378 and 631, T at nucleotide sites nos. 267, 309, 318, 390, 570 and 666, and A at nucleotide sites nos. 273, 312, 321, 564, 576 and 609 (Fig. 2).

Distribution: The type locality of *H. tutul* is Pemba Island in the West Indian Ocean (Borsa *et al.*, 2013b). Based on genetically-validated records, its distribution also includes the Mozambique Channel, the Gulf of Oman, the Arabian Sea, the Laccadive Sea, the Bay of Bengal, the South China Sea, the Java Sea, the East Indian Ocean off southern Java, the Sulu Sea, the Celebes Sea, the Bali Sea, and Makassar Strait (Fig. 5C).

Remarks: *H. tutul* is one of the two cryptic species previously under ‘*Himantura* sp. A’ (a precursor synonym of *H. leoparda*; Manjaji, 2004) and *H. leoparda* (Arlyza *et al.*, 2013; Borsa *et al.*, 2013b). Borsa (2017: his figure 2c) and Fernando *et al.* (2019) assumed that Naylor *et al.*’s (2012) ‘*uarnak* 3’ clade represented *H. tutul*. This equivalence is now confirmed through the placement in the phylogenetic trees of four ‘*uarnak* 3’ individual samples labelled GN2207, GN2916, GN2995 and GN3085 in Naylor *et al.* (2012) and sequenced at the *COI* locus as, respectively, IRREK171-08, IRREK662-08, IRREK741-08, and IRREK829-08 in BOLD (Hanner, 2020). Fernando *et al.* (2019) collected *H. tutul* specimens from the Laccadive-Sea and Bay-of-Bengal coasts of Sri Lanka, which identification to species was ascertained by their nucleotide sequences at the *NADH2* locus. Pictures of 17 genetically-validated *H. tutul* individuals sampled from the Indo-Malay archipelago have been published as supplementary plates to

Borsa *et al.* (2013b). Additional pictures of *H. tutul* specimens including nos. BO-47, BO-249, BO-422, BO-502, BOD-9, BOD-49, HBO-86, KA-48, KA-63, KA-119 and KA-392 have been posted on Global Cestode Database website. Barcode identification no. BOLD:AAB7831 has so far been allocated to this species (<https://v4.boldsystems.org/>; consulted 23 March, 2025).

5.2.5 *Himantura australis* Last *et al.*, 2016b

Synonym of ‘*uarnak* 2’ (Naylor *et al.*, 2012) and ‘Species ID no. 1358’ in the Global Cestode Database. Also previously referred to as ‘*H. uarnak*’ (Cerutti-Pereyra *et al.*, 2012), ‘NW Australia’ sub-clade under *H. uarnak* (Borsa *et al.*, 2013b; Borsa, 2017); and ‘*Himantura* sp. 4’ (Last *et al.*, 2016a). Misidentified as ‘*H. leoparda*’ (Cerutti-Pereyra *et al.*, 2012). Genetically-validated references include Appleyard (2020), Caira *et al.* (2020), and Borsa *et al.* (2021). Reference material includes voucher specimen AMS I.39533-003 (Australian Museum, Sydney); and tissue samples CSIRO BW-A6280 to BW-A62823, BW-A6285 and BW-A14305.

Diagnosis: based on the alignment of 249 partial *COI*-gene sequences, *H. australis* ($N=23$) is distinguished from all other species in the genus *Himantura* by possessing nucleotide C at nucleotide site 471 of the *COI* gene (Fig. 2). All other species in the genus *Himantura* possess nucleotide T at that nucleotide site.

Distribution: The type locality of *H. australis* is Daru (9°04.43’S, 143°08.53’E) in the Western Province of Papua New Guinea (Last *et al.*, 2016a). Its geographic distribution, based on available genetically validated records includes the East Indian Ocean off Western Australia, the Timor Sea, the Arafura Sea, the Coral Sea, and the Solomon Sea (Fig. 5A). The distribution of *H. australis* overlaps with that of *H. leoparda*.

Remarks: According to Last *et al.* (2016a, c), the distribution of *H. australis* is “confined to the Australasian plate” and the species is “known from off Papua New Guinea and northern Australia, from Shark Bay to Brisbane”. Based on this geographic delimitation, it is clear that Last *et al.* (2016a, b) did not consider Naylor *et al.*’s (2012) sister-lineages ‘*uarnak* 2’ (now *H. australis*) and ‘*uarnak* 1’ (now *H. arlyzae* sp. nov.), as belonging to the same species. The Global Cestode Database mentions one outlying record from Semporna, a fish landing place in the Celebes Sea. If confirmed by genetic evidence, this record would suggest that the distribution of *H. australis* is much more widespread than indicated by Last *et al.* (2016a). However, it cannot be excluded that this individual was captured by a long-range fishing vessel and landed far away from its site of capture. Barcode identification no. BOLD:ABZ0063 has been allocated to this species (<https://v4.boldsystems.org/>; consulted 23 March 2025).

5.2.6 *Himantura* sp. 2

Previously referred to as ‘*H. uarnak*’ (Lim *et al.*, 2015) and ‘*H. uarnak* ii’ (Hanner, 2020). Also BIN number BOLD:ACV9539 in BOLD (<https://v4.boldsystems.org/>; consulted 23 March 2025). Reference material includes tissue samples BOD-67 and HBO-109 of the Global Cestode Database.

Diagnosis: based on the alignment of two partial *COI*-gene sequences retrieved from the GenBank and BOLD databases, *Himantura* sp. 2 differs from all the foregoing ($N=247$) by possessing nucleotides G and A at nucleotide sites 111 and 363, respectively (Fig. 2). Collected from two fish landing places in the southern Sulu Sea and the adjacent northern Celebes Sea (Naylor *et al.*, 2012; Lim *et al.*, 2015).

5.2.7 *Himantura* sp. 3

Synonym: ‘*uarnak* 4’ (Naylor *et al.*, 2012). Known from the *NADH2* gene sequence of a single specimen from the Malaysian shores of the Sulu Sea. The specimen was discarded and apparently no photograph is available.

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Conflicts of interests

The authors declare no conflict of interests.

Data availability statement

The complete lists of nucleotide sequences identified by their GenBank accession numbers or their BOLD registration numbers are given along with sampling details in Suppl. Tables S1–S3. *Himantura arlyzae*, the new species, has been registered in ZooBank (<http://zoobank.org/>). Its holotype has been deposited at MZB in Cibinong, Indonesia.

Author contribution statement

The following terms are those of the contributor role taxonomy proposed by Brand *et al.* (2015). Conceptualization: PB; Validation: PB; Investigation: PB, LP; Resources: PB; Data curation: PB; Writing-original draft: PB; Writing-review and editing: PB, UH, RH, LP; Visualization: PB; Supervision: PB.

Supplementary material

Supplementary Table S1. Reticulate whipray, *Himantura* spp. List of nucleotide sequences at the *COI* locus used for the present work, with GENBANK (Benson *et al.*, 2017) and BOLD (Ratnasingham

and Hebert, 2007) identification nos., sampling location, and references. *Himantura* spp. sequences were retrieved on 23 March 2025 from the GENBANK and BOLD public databases ($N=247$) or donated by X. Chen (South China Agricultural University, Guangzhou) ($N=2$). Sequences are arranged by species and presented in alphabetical and arithmetical order. Type material highlighted red.

Supplementary Table S2. Reticulate whipray, *Himantura* spp. List of nucleotide sequences at the *cytb* locus used for the present work, with GENBANK (Benson *et al.*, 2017) identification nos., sampling locations, and references. Sequences are arranged by species and presented in alphabetical and arithmetical order. Type material highlighted red.

Supplementary Table S3. Reticulate whipray, *Himantura* spp. List of nucleotide sequences at the *NADH2* locus used for the present work, with GENBANK (Benson *et al.*, 2017) identification nos., sampling locations, and references. Sequences are arranged by species and presented in alphabetical and arithmetical order.

Supplementary Figure S1. Indo-Malay reticulate whipray *Himantura arlyzae* sp. nov. Three differently labelled pictures of what careful examination of spot patterns showed to represent a single and same specimen; injuries and other marks were also identical. **A.** Picture of the specimen as registered in the fish collections of the National Museum of Marine Biology and Aquarium (NMMBA), Pingtung as no. NMMB-P015784, a female specimen (82.0 cm DW) purchased in Taiwan (<https://helloocean.nmmba.gov.tw/nmmba-front/SpecimenDetail.aspx?id=49101>; accessed 25 May 2025; credit U. Kuan-Chen). **B.** Picture labelled “NMMBP015601” in Borsa *et al.* (2013: suppl. Fig. S1); credit H.-C. Ho (NMMBA). **C.** Picture posted on Wikimedia Commons (Wikimedia Foundation Inc., San Francisco CA), presented as a specimen from CSIRO, labelled “Reticulate whipray (*Himantura* sp.) CSIRO National Fish Collection - <http://www.fishesofaustralia.net.au/home/species/2026>” (https://commons.wikimedia.org/wiki/Category:Himantura_urnak; accessed 31 May 2025).

The Supplementary Material is available at <https://www.alr-journal.org/10.1051/alr/2026011/olm>

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