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Species in Disguise: A New Species of Hornshark from Northern Australia (Heterodontiformes: Heterodontidae)

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Abstract: A new species of hornshark is described from northwestern Australia based on six whole specimens and a single egg case. *Heterodontus marshallae* n. sp. was previously considered to be conspecific with *H. zebra* from the Western Pacific. The new species differs from *H. zebra* in the sequence of its *NADH2* gene, several morphological characters, egg case morphology and key coloration features. Despite the coloration being similar between *H. marshallae* n. sp. and *H. zebra*, i.e., pale background with 22 dark brown bands and saddles, they differ consistently in two key aspects. Firstly, the snout of *H. marshallae* n. sp. has a dark semicircular bar, usually bifurcated for most of its length vs. a pointed, triangular shaped dark marking in *H. zebra*. Secondly, *H. zebra* has a dark bar originating below the posterior gill slits and extending onto anterior pectoral fin, which is absent in *H. marshallae* n. sp. The *Heterodontus marshallae* n. sp. is endemic to northwestern Australia and occurs in deeper waters (125–229 m) than *H. zebra* (0–143 m).

Keywords: *Heterodontus*; taxonomy; species complex; egg case; morphology; genetics

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1. Introduction

The Heterodontiformes is a unique shark order comprising a single family (Heterodontidae) and single extant genus (*Heterodontus* Blainville 1816 [1]). They are also one of the earliest identified modern elasmobranch lineages with distinctly recognisable isolated teeth recorded from the Lower Jurassic, ~175 mya [2]. These horn or bullhead sharks are characterised by their large, blunt heads with prominent crests above the orbits and a small, almost terminal mouth [3]. They also have a large spine in front of both dorsal fins and an anal fin. There are nine currently recognised nominal species of *Heterodontus* [4]: *H. francisci* (Girard, 1855) [5] from the Eastern Pacific; *H. galeatus* (Günther, 1870) [6] from eastern Australia; *H. japonicus* Miklouho-Maclay & Macleay, 1884 [7] from the Northwest Pacific; *H. mexicanus* Taylor & Castro-Aguirre, 1972 [8] from the Eastern Pacific; *H. omanensis* Baldwin, 2005 [9], from the northwestern Indian Ocean; *H. portusjacksoni* (Meyer, 1793) [10] from southern Australia; *H. quoyi* (Fréminville, 1840) [11] from the Eastern Pacific; *H. ramalheira* (Smith, 1949) [12] from the western Indian Ocean; and *H. zebra* (Gray, 1831) [13] ranging from northern Australia to Japan. No extant *Heterodontus* species occur in the Atlantic or Central Pacific oceans.

Australia is home to two endemic heterodontid species, i.e., *H. galeatus* and *H. portusjacksoni*, which occur in temperate waters. A third species, *H. zebra*, is found in the Western Central Pacific from tropical northern Australia extending further north to Japan. Naylor et al. [14] found that the sequence of the *NADH2* gene of *H. zebra* from northern Australia was different from *H. zebra* from Japan and Malaysian Borneo. They suggested that the northern Australian population is probably an undescribed species and is referred to as *H.* cf. *zebra*. The fact that *H.* cf. *zebra* was genetically closer to *H. portusjacksoni* (average pairwise difference of 13 nucleotides for *NADH2*) than it was to the true *H. zebra* samples (average pairwise difference of 24) provides compelling support for the separation of the northern Australian population as a distinct species. These two populations also occupy different habitats. In the Western Pacific, *H. zebra* is associated with the insular and continental shelves to depths of about 50 m, whilst the northern Australian population (*H.* cf. *zebra*) occur on the lower continental shelf at depths, ranging from 150 to 200 m [15]. This provides further evidence that *H. zebra* represents a species complex.

Centracion zebra was originally described by Gray (1831) [13] in the Sea of China, presumably a misspelling for the genus *Cestracion*, based on 'Les Cestracions' of Cuvier [16], a junior synonym of *Heterodontus* Blainville [1]. Bleeker [17] reported on a specimen of *H. zebra* from off Ambon, Indonesia, and comments on its occurrence in Manado. Günther [6] included *H. zebra* as synonym of *Cestracion philippi* Bloch & Schneider, 1801 [18], and stated its range as New Zealand, Australia, East Indies and Japan, thus including the Bleeker specimens from Indonesia in its distribution. The 580 mm TL Bleeker specimen deposited in the Natural History Museum, London, from Ambon was subsequently described as a new species, *Cestracion amboinensis* by the authors of [19]. Regan [19] differentiated it from *H. zebra* from China as its dorsal fins less elevated and dark crossbars broader and separated by narrower interspaces. However, Garman [20] subsequently placed *C. amboinensis* into the synonymy of *Cestracion zebra*, which was followed by subsequent authors (as *Heterodontus zebra*), e.g., [21,22].

In this paper, specimens of *H.* cf. *zebra* from northern Australia were compared to true *H. zebra* specimens from the Northwest Pacific and to the type of *Cestracion amboinensis* to determine whether they refer to a new species. This paper addresses a high priority knowledge gap for this species in the Action Plan for Australian Sharks and Rays 2021 [23].

2. Materials and Methods

2.1. Morphology

The morphometric measurements taken follow those for sharks in general, as detailed by Compagno (2001) [15], although we typically use direct (point-to-point) measurements rather than horizontal measurements, and for those used for sharks with fin spines, i.e., the dogshark genus *Squalus* by Last et al. [24]. For comparative purposes, we have included both direct and horizontal measurements for some key characters, e.g., pre-first dorsal length, head length, preorbital length and prenarial length. Dorsal fin origins were located using a finger or thumb against the midline to determine the location of the fin origin and a pin used to mark the position. Total length (TL) was taken horizontally from the tip of the snout to the tip of the caudal fin when lowered to be in line with snout, i.e., stretched total length. The holotype (WAM P.35408-007) and five paratypes (CSIRO CA 3286, CSIRO H 6581–01, NTM S.12929-001, WAM P.26193-010 and WAM P.30424-001) of the *H.* cf. *zebra* specimens were measured in full. For comparison, eight specimens of *H. zebra* (AMS I.1366, NMW 85504, NMMB-P 1825, NMMB-P 5040, NMMB-P 8845 (two specimens), NMMB-P 15867 and NMMB-P unregistered) and five specimens of *H. portusjacksoni* (CSIRO unregistered (two specimens), CSIRO H 6354-09, CSIRO H 6354-10 and CSIRO H 6340-02) were also measured. The holotype of *H. zebra* (BMNH 1953.5.10.4) is a dry skin and is thus not suitable for measuring or meristics. In the description, the first measurement proportion or ratio is that of the holotype followed in parentheses by the ranges of the paratypes.

2.2. Vertebral Meristics

Vertebral counts were obtained from radiographs of the holotype and five paratypes of the *H.* cf. *zebra* specimens and from two specimens of northwest Pacific *H. zebra* (AMS I.1366 and NMMB-P.5040). Counts were obtained separately for trunk (monospondylous precaudal centra), precaudal (monospondylous precaudal centra + diplospondylous precaudal centra to origin of the caudal fin upper lobe) and diplospondylous caudal centra (centra of the caudal fin) vertebrae following the methods used by Compagno (1988) [25] for carcharhiniform sharks.

2.3. Dentition

The dental formula used, including symphyseal (S), anterior (A), antero-lateral (AL) and (postero-) lateral teeth (L), follows Casier [26]. Tooth file counts were taken in situ from paratype CSIRO H 6581-01 by making short cuts from both mouth corners to expose the entire jaws. All ERB specimens (jaws) were collected following the protocol of Mollen et al. [27].

2.4. Denticles

Dorsal, lateral and ventrolateral skin patches and photographs were taken just posterior to first dorsal fin in *H.* cf. *zebra* and *H. zebra*, that correspond to topological codes B4–B6 of Reif [28].

2.5. Distribution

The distribution map was generated in QGIS 3.30.2 (www.qgis.org (accessed on 14 April 2023) using a Google Satellite layer. Records of the *H. zebra* complex were included in the distribution map. For *H.* cf. *zebra*, the locations of specimens were mapped, as well as additional records from CSIRO demersal trawl surveys (Atlas of Living Australia; www.ala.org.au (accessed on 10 March 2023). While the latter records do not have associated voucher specimens or images for validation, they are included since it is unlikely that the *H. zebra* complex would be misidentified off the Northwest Shelf of Australia. For *H. zebra*, the locations of specimens examined and other records with specific localities from reliable literature records were mapped. The literature records used for *H. zebra* are (from the north of range to the south): Japan [29–36]; Korea [36,37]; China [36,38–41]; Taiwan [42,43]; Disputed territory—Paracel Islands [44]; Vietnam [45,46]; Philippines [47]; Thailand [48]; Brunei Darussalam [44]; Malaysia [49,50]; and Indonesia [17,51].

2.6. Molecular Analyses

Specimens were sampled for liver or muscle tissue and temporarily stored in 95% alcohol. DNA was extracted using the phenol chloroform extraction [52] or using High Pure PCR Template Preparation Kit by Roche Diagnostics (Indianapolis, IN, USA). Extracted total DNA was stored at −20 °C until used for amplification via a polymerase chain reaction (PCR). Samples were amplified using Takara ExTaq (Clonetech, Mountain View, Ca) with primers designed to target the complete coding sequence for NADH dehydrogenase subunit 2 (*NADH2*). A single set of new universal primers (ILEM_LY: 5′-AAG GAY CAC TTT GAT AGA GT-3′; ASNM_LY: 5′-AAC RCT TAG CTG TTA AYT AAG AT-3′), designed to bind to the ASN and ILE tRNA regions of the mitochondrial genome, was used to amplify the target fragment. PCR reactions were generally carried out in 25 µL tubes by adding 14.775 µL of PCR grade water, 2.5 µL of PCR buffer, 2.0 µL of MgCL2 (25 mM), 2.0 µL of dNTP mix (2.5 mM each), 0.8 µL of each primer (10 µM), 0.125 µL of Takara ExTaq (5 U/µL) and 2 µL of DNA template. The reaction cocktail was denatured at 94 °C for 3 min, after which it was subjected to 35 cycles of denaturation at 94 °C for 30 s, annealing at 54 °C for 30 s and extension at 72 °C for 90 s. PCR products were sent off to commercial sequencing centres for purification and sequencing (Retrogen Inc., San Diego, CA, USA). Sequence trace files were evaluated for quality, translated to amino acids and aligned using the software package Geneious 11.0.5 (https://www.geneious.com (accessed 20 April 2023)). The aligned amino acid sequences were translated back, in the frame of their original nucleotide sequences, to yield a nucleotide alignment that was 1044 base pairs long.

Partitioned maximum likelihood (ML) analyses were conducted for the ND2 dataset using RAxML v.8.0.26 [53,54]. Based on the results from PartitionFinder v2.1.1 [55], the dataset was partitioned by the three codon positions and the model GTR + I + G model was chosen. A total of 200 distinct runs were performed based on 200 random starting trees.

The tree with the best likelihood score was chosen as the final tree. The bootstrap analyses (1000 replicates) were also conducted using RAxML [56,57] with the same partitioning strategy and nucleotide substitution model as above. PAUP 4.0.b10 [58] was then employed to obtain the 50% majority rule consensus tree and bootstrap values (BP). Sequences have been uploaded to GenBank (see Table S2 for accession numbers and collection details).

2.7. Institutional Acronyms

Museum acronyms used: AMS, Australian Museum, Sydney; BMNH, Natural History Museum, London; CSIRO, Australian National Fish Collection, Hobart; ERB, Elasmobranch Research Belgium, Bonheiden; KAUM, Kagoshima University Museum, Korimoto; NMMB-P, National Museum of Marine Biology and Aquarium (Fishes), Checheng; NMW, Naturhistorisches Museum, Vienna; NTM, Museums and Art Galleries of the Northern Territory, Darwin; QM, Queensland Museum, Brisbane; RBINS, Royal Belgian Institute of Natural Sciences, Brussels; RMNH, Naturalis Biodiversity Center, Leiden; WAM, Western Australian Museum, Perth; ZMH, Zoologisches Museum, Universität Hamburg, Hamburg.

2.8. Comparative Material Examined

Heterodontus francisci: CSIRO H 8931-01, juvenile male, 311 mm TL; CSIRO H 8931-02, female 360 mm TL, San Clemente, CA, USA, 19 November 2021; ERB 1172 (jaws only; other skeletal remains as RBINS 25325, female, 388 mm TL, CA, USA, May 2017.

Heterodontus galeatus: AMS I.4781, egg case and embryo (wet), Coogee Bay, New South Wales, 33°55′47′′ S, 151°16′12′′ E; CSIRO CA 3938, female, 410 mm TL, no location data, 29 November 1904; CSIRO H 904-01, female, 325 mm TL, between Long Reef and Port Hacking, New South Wales, Australia, ~34° S, ~151° E, March 1987; CSIRO H 6354-08, adult male, 700 mm TL, east of Broken Bay, New South Wales, 33°32.8′ S, 151°27′ E, 40–49 m depth, 14 May 2006; QM I 13003, northwest of Cape Moreton, Queensland, 26°55′ S, 153°25′ E, 110 m depth, 27 February 1975.

Heterodontus japonicus: ERB 1151 (jaws only; other skeletal remains as RBINS 25176; tissue accession GN 19549), male, 440 mm TL, Japan, April 2016.

Heterodontus portusjacksoni: CSIRO H 6340-01, adult male; CSIRO H 6340-02, female, 462 mm TL, northwest of Rottnest Island, Western Australia, 31°54.27′ S, 151°38.47′ E, 100–101 m depth, 7 April 2006; CSIRO H 6354-09, juvenile male, 553 mm TL; CSIRO H 6354-10, juvenile male 362 mm TL; CSIRO H 6354-11, juvenile male, 368 mm TL; CSIRO H 6354-12, female, 347 mm TL, east of Broken Bay, New South Wales, Australia, 33°32.8′ S, 151°27′ E, 40–49 m depth, 14 May 2006; CSIRO H 8732-02, egg case from captive female, Seahorse World, Beauty Point, Tasmania, 10 January 2009; CSIRO unregistered, adult male, 630 mm TL, northwest of Rottnest Island, Western Australia, 149–151 m depth, 7 April 2006; CSIRO unregistered, adolescent male, 686 mm TL, south of Betsey Island, Tasmania, 25 m depth; NTM S.00043-001, female, 237 mm TL, York Sound, Western Australia, ~14°50′ S, ~125°05′ E, June 1975; ERB 0500 (jaws only), female, 845 mm TL, off Geraldton, Western Australia, June 2008; ERB 0904 (jaws only), female, 950 mm TL, off Bunbury, Western Australia, August 2010; ERB 1226 (jaws only), male, 670 mm TL; ERB 1227 (jaws only), female 352 mm TL, off Perth, Western Australia, November 2018.

Heterodontus zebra: AMS I.1366, female, 454 mm TL, off Shantou, China, 1887; BMNH 1867.11.28.183 (holotype of *Cestracion amboinensis*), female ~630 mm TL, Ambon, Moluccas, Indonesia, 1855; ERB 1147 (jaws only; other skeletal remains as RBINS 25173; tissue accession GN 19551), female, 778 mm TL; ERB 1148 (jaws and skin only; other skeletal remains and skin as RBINS 25174), female, 796 mm TL; ERB 1149 (jaws only), female, 666 mm TL; ERB 1150 (jaws only; other skeletal remains as RBINS 25175), male, 630 mm TL, Taiwan, April 2016; KAUM-I. 69456, egg case, off Oton, Panay Island, Philippines, 10°37′ N, 122°14′ E, 1 March 2005; NMMB-P 1825, female, 569 mm TL; NMMB-P 5040 (ex THUP 02957), juvenile male, 360 mm TL, Kaohsiung, Taiwan, 11 October 1965; NMMB-P 8845 (2 specimens), female, 220 mm TL, and juvenile male, 212 mm TL, Penghu, Taiwan, 30 August 2005; NMMB-P 15867, female, 1005 mm TL, no collection data, presumably

Taiwan; NMMB-P unregistered, 536 mm TL, no collection data, presumably Taiwan; NMW 85504, juvenile male, 457 mm TL, Asia?, no other data; RMNH PISC.7412 (2 specimens), adult male, 650 mm TL, and juvenile male, 434 mm TL, Indonesia, purchased from Bleeker auction in 1879; not retained, field code BOD-038 (GenBank accession JQ518723), adolescent male, 580 mm TL, Tanjung Manis, Mukah District, Sarawak, Malaysian Borneo, 02°07′07.04″ N, 111°19′37.16″ E, 6 July 2002; ZMH 10104, male, 457 mm TL, Fuzhou, Fujian Province, China, 4 April 1905; ZMH 10105, female, 165 mm TL, Fuzhou, Fujian Province, China, 24 August 1906; ZMH 10106, male, 434 mm TL, Fuzhou, Fujian Province, China, 1 June 1911.

3. Results

3.1. Comparison with Congeners

The *Heterodontus zebra* complex is easily distinguished from all other *Heterodontus* species due to the striking colour pattern of dark, narrow bands on a pale background. Despite the northern Australian *H.* cf. *zebra* and the *H. zebra* from Japan and Malaysian Borneo differing on a molecular level [14], their colour patterns are very similar. However, they do have some key differences, i.e., different dark markings on the snout and dorsal surface of pectoral fin. They also have different egg case morphology and vertebral counts (see Section 3.5 for detailed comparisons). The difference in dark markings on the snout and dorsal pectoral fin was consistent across the different size classes and are thus considered a reliable characteristic for separating the two species. An examination of the holotype of *Cestracion amboinensis* (BMNH 1867.11.28.183) found that it has the same shaped dark snout markings and pectoral fin markings as *H. zebra* from Japan, Taiwan and Malaysian Borneo. Therefore, *Cestracion amboinensis* remains in the synonymy of *H. zebra*, and the northern Australian specimens (*H.* cf. *zebra*) are herein formally named and described as a new species.

3.2. Systematic Account

Order Heterodontiformes Berg, 1937 [59].
Family Heterodontidae Gray, 1851 [60].
Heterodontus marshallae n. sp.
urn:lsid:zoobank.org:act:76D37CD9-AB48-4171-BF97-ED268114AFA7.

3.2.1. Synonymy

Heterodontus zebra—[22] (in part): p. 164 (northern Australian range); [61]: p. 22, Figure (northwestern Australia); [62] (in part): p. 115, figs, pl. 2 (Figure 11.3) (northern Australian range); [63]: p. 143 (Western Australia); [64]: p. 11 (Northern Territory); [65]: p. 13 (Western Australia); [15] (in part): p. 48 (northwestern Australian range); [66]: p. 23 (northwestern Australia); [67] (in part): p. 154 (northwestern Australia range); [3]: p. 120, fig., pl. 2 (northern Australian range); [68]: p. 6 (Northern Territory).

Heterodontus cf. *zebra*—[14]: p. 56, Figure 40 (northern Australia); [69]: 5 (northwestern Australia).

3.2.2. Type Material

Holotype: WAM P.35408-007, adolescent male, 541 mm TL, west of Exmouth Peninsula, Ningaloo Marine Park (Commonwealth waters), Western Australia, 22°22.946′ S, 113°40.308′ E, 210–212 m depth, 23 November 2022.

Paratypes (n = 6): CSIRO CA 3286, juvenile male, 432 mm TL, north of Port Hedland, Western Australia, 18°33′ S, 118°22.3′ E, 150 m depth, 28 March 1982; CSIRO H 6581–01, female, 580 mm TL, northwest of Cape Leveque, Western Australia, 14°58.69′ S, 121°40.18′ E, 191–202 m depth, 28 June 2007; NTM S.12929-001, adult male, 575 mm TL, north of Bathurst Island, Arafura Sea, Northern Territory, 10°10.02′ S, 130°04.02′ E, 125 m depth, 16 November 1990; NTM S.18275-001, egg case (wet), southeast of Evans Shoal, Arafura Sea, Northern Territory, 10°08′ S, 130°05′ E, 131 m depth, 1 October 1998; WAM P.26193-

010, female, 355 mm TL, 150 km north-northeast of Rosemary Island, Western Australia, 22°22′ S, 113°29′ E, 170–172 m depth, 16 May 1978; WAM P.30424-001, adult male, 601 mm TL, east of Exmouth Peninsula, Western Australia, 22°22′ S, 113°40′ E, 221–229 m depth, 29 September 1990.

3.2.3. Representative DNA Sequences

OR078587 (holotype—WAM P.35408-007); JQ519052 (paratype—CSIRO H 6581–01).

3.2.4. Etymology

The specific name is in honour of Dr. Lindsay Marshall (www.stickfigurefish.com.au (accessed 10 May 2023)), a scientific illustrator and elasmobranch scientist who expertly painted all the sharks and rays of the world for the Chondrichthyan Tree of Life Project.

The vernacular name proposed is painted hornshark, in allusion to not only the beautiful coloration of the species but also to its namesake, who has painted all the hornsharks in amazing detail.

3.2.5. Diagnosis

A small species of hornshark with the following combination of characters: colour pattern consisting of 22 dark bands and saddles; snout with a semicircular dark bar, usually bifurcated for most of its length; no dark bar below posterior gill slits extending onto anterior pectoral fin; anal fin well separated from caudal fin (anal-caudal space 11.0–13.5% TL); ventral lobe of caudal fin prominent (lower postventral margin 4.7–6.1% TL); dorsal spines long (exposed first dorsal spine length 3.9–4.5% TL); dorsal fins taller in juveniles than adults; symphyseal and anterior teeth pointed, lateral teeth molariform with a longitudinal keel; 20–22 tooth files in upper jaw, 17–19 in lower jaw; total vertebral centra 106–112, precaudal centra 70–76, monospondylous centra 33–37; egg case with narrow, curved, screw-like keels with 1.5 rotations from anterior to posterior margins.

3.2.6. Description

Proportional measurements of the holotype and ranges for the paratypes are provided in Table 1.

Table 1. Morphometrical measurements of the holotype (WAM P.35408-007) and ranges for the five measured paratypes of *Heterodontus marshallae* n. sp., expressed as a percentage of total length. Mean and standard deviation provided for all six type specimens of *H. marshallae* n. sp. Ranges, means and standard deviations also provided for seven specimens of *H. zebra* for comparison.

	***H. marshallae* n. sp.**					***H. zebra***			
		Paratypes		**Mean**	**sd.**				
Character	**Holo**	**Min**	**Max**			**Min**	**Max**	**Mean**	**sd.**
Total length (mm)	541	355	601	514.0	98.4	212	1005	479.9	271.1
Precaudal length	73.9	73.2	78.4	75.8	2.0	67.3	81.1	73.7	4.6
Pre-second dorsal length	48.5	46.1	53.1	50.0	2.6	46.6	57.1	50.7	3.7
Pre-first dorsal length	26.0	23.9	29.5	26.9	1.9	23.4	28.6	25.1	1.8
Pre-first dorsal length (horizontal)	23.1	22.2	26.6	24.7	1.9	21.2	21.9	21.5	0.5
Head length	22.3	20.3	24.1	22.7	1.5	19.5	23.1	20.6	1.3
Head length (horizontal)	21.6	20.3	23.5	21.9	1.1	18.2	22.1	19.5	1.4
Prebranchial length	17.5	15.6	19.2	17.7	1.3	15.1	17.6	16.2	1.0
Prespiracular length	12.0	10.5	13.3	12.1	1.0	10.5	13.3	11.5	0.9
Preorbital length	9.6	8.1	10.5	9.4	0.9	7.6	11.1	8.5	1.2
Preorbital length (horizontal)	7.9	6.5	8.9	8.1	0.9	5.4	9.6	6.8	1.4
Prenarial length	3.8	3.1	4.4	3.9	0.5	3.3	3.7	3.5	0.2
Prenarial length (horizontal)	2.1	0.9	2.7	2.0	0.6	1.8	2.7	2.1	0.3
Preoral length	3.5	2.6	3.3	3.1	0.4	2.1	3.9	3.2	0.7
Prepectoral length	21.1	21.2	24.1	22.3	1.2	17.5	21.1	19.2	1.4
Prepelvic length	39.0	36.3	41.9	40.1	2.2	34.6	44.4	38.1	3.9
Pre-cloacal length	39.9	40.8	44.3	42.3	1.7	38.2	48.8	41.9	4.2
Preanal length	58.2	55.2	62.2	59.6	2.6	57.6	58.9	58.3	0.9

Table 1. *Cont.*

Character	Holo	Paratypes Min	Paratypes Max	*H. marshallae* n. sp. Mean	sd.	*H. zebra* Min	Max	Mean	sd.
Interdorsal space	17.3	15.3	17.8	16.8	0.9	15.6	20.9	18.5	2.0
Dorsal–caudal space	17.6	16.4	18.7	17.8	0.8	13.4	16.7	15.6	1.2
Pectoral–pelvic space	12.4	11.6	14.7	13.4	1.2	11.3	16.6	13.7	1.8
Pelvic–anal space	14.7	11.7	15.5	14.4	1.4	15.2	17.6	16.4	1.7
Anal–caudal space	13.2	11.0	13.5	12.5	1.0	9.4	10.5	10.0	0.8
Interorbital space	7.8	7.4	7.9	7.7	0.2	6.2	8.0	6.9	0.7
Eye length	3.7	3.7	3.8	3.7	0.0	2.6	3.7	3.3	0.3
Eye height	2.2	2.1	2.5	2.3	0.2	1.6	2.6	2.0	0.4
Spiracle length	0.7	0.4	0.7	0.6	0.1	0.2	0.6	0.4	0.2
Nostril width	1.9	1.4	2.1	1.9	0.2	1.1	1.6	1.2	0.2
Internarial space	4.9	4.9	5.9	5.4	0.4	3.9	5.8	5.2	0.8
Mouth width	9.1	8.1	10.2	9.3	0.8	7.2	9.1	8.3	0.7
Upper labial furrow length	1.5	1.3	1.7	1.5	0.2	0.8	1.4	1.1	0.2
Lower labial furrow length	3.0	3.2	3.9	3.5	0.3	3.5	3.9	3.7	0.2
Inner nostril to upper labial furrow	1.9	1.8	1.9	1.9	0.1	1.3	2.5	1.9	0.5
First gill slit height	4.4	4.1	5.3	4.6	0.5	3.2	5.0	3.9	0.6
Fifth gill slit height	2.6	2.0	3.3	2.6	0.4	1.8	3.4	2.4	0.6
Intergill length	4.9	4.7	5.8	5.3	0.4	4.2	5.2	4.7	0.4
Pectoral fin—anterior margin	20.1	18.5	22.4	20.5	1.7	18.8	26.1	22.1	2.7
Pectoral fin—height	19.0	15.1	18.3	17.4	1.4	17.2	19.3	18.3	1.5
Pectoral fin—inner margin	6.5	5.7	7.0	6.3	0.5	5.5	9.6	7.6	1.4
Pectoral fin—base	7.7	7.2	8.0	7.6	0.2	7.0	8.9	8.0	0.7
Pectoral fin—posterior margin	15.2	14.0	17.6	15.4	1.5	13.6	17.7	15.7	1.4
Dorsal caudal margin	25.2	21.4	25.6	23.5	1.8	17.9	28.0	24.4	3.3
Preventral caudal margin	14.9	13.1	14.5	13.9	0.7	12.1	14.5	13.2	1.0
Upper postventral caudal margin	5.2	3.8	5.3	4.7	0.6	4.2	6.5	5.4	1.6
Lower postventral caudal margin	5.2	4.7	6.1	5.2	0.5	3.2	3.5	3.3	0.3
Caudal fork width	8.5	7.5	9.3	8.1	0.7	8.8	8.9	8.8	0.1
Caudal fork length	12.0	10.6	13.3	11.5	1.0	12.4	12.5	12.5	0.1
Caudal terminal lobe length	11.5	10.2	13.5	11.1	1.3	11.9	12.6	12.2	0.5
Caudal terminal margin length	9.8	9.1	10.9	9.8	0.7	6.9	10.6	8.7	1.4
Caudal subterminal margin	4.5	4.2	6.2	4.9	0.7	5.2	6.6	5.9	1.0
First dorsal fin—length	13.4	13.7	14.6	14.1	0.5	12.0	15.4	13.5	1.1
First dorsal fin—anterior margin	16.9	14.0	20.0	16.3	2.2	11.7	29.5	19.5	5.8
First dorsal fin—base	9.0	9.1	9.8	9.4	0.3	8.2	9.6	8.8	0.5
First dorsal fin—height	12.5	9.2	15.6	12.0	2.3	8.8	22.3	15.1	5.2
First dorsal fin—inner margin	4.6	4.3	5.7	4.8	0.5	4.0	6.2	4.9	0.8
First dorsal fin—posterior margin	12.4	9.0	13.4	11.5	1.6	9.2	20.0	14.0	4.3
First dorsal fin—soft fin length	9.4	8.3	10.4	9.5	0.8	9.3	10.3	9.8	0.7
Exposed first dorsal spine length	4.5	3.9	4.3	4.2	0.3	1.0	3.7	2.3	1.0
Base first dorsal spine	1.1	1.2	1.4	1.3	0.1	0.8	1.6	1.2	0.3
Second dorsal fin—length	12.0	11.1	12.8	12.2	0.6	9.6	12.7	11.2	1.0
Second dorsal fin—anterior margin	12.8	10.1	14.5	12.4	1.5	8.9	20.1	14.3	3.8
Second dorsal fin—base	8.3	6.8	8.6	7.8	0.7	6.4	8.1	7.4	0.6
Second dorsal fin—height	8.8	6.7	11.1	8.5	1.5	6.8	16.4	10.4	3.8
Second dorsal fin—inner margin	4.0	3.8	5.3	4.4	0.5	3.4	4.3	3.9	0.4
Second dorsal fin—posterior margin	8.7	6.8	9.2	8.2	0.8	5.7	11.8	8.5	2.1
Second dorsal fin—soft fin length	6.8	6.5	7.3	6.9	0.3	6.2	6.8	6.5	0.5
Exposed second dorsal spine length	4.2	3.9	4.7	4.2	0.3	2.0	4.0	2.8	0.7
Base second dorsal spine	1.1	1.1	1.2	1.1	0.0	0.8	1.4	1.0	0.2
Pelvic fin—length	10.9	10.6	12.2	11.3	0.6	10.4	13.2	11.9	0.9
Pelvic fin—height	8.2	6.5	8.0	7.6	0.6	6.6	8.1	7.2	0.5
Pelvic fin—inner margin length	6.3	5.9	6.5	6.2	0.2	4.4	6.8	5.8	0.9

Table 1. *Cont.*

Character	*H. marshallae* n. sp.					*H. zebra*			
		Paratypes		Mean	sd.				
	Holo	Min	Max			Min	Max	Mean	sd.
Clasper outer length	8.3	3.1	9.9	7.6	3.1	2.5	2.7	2.6	0.2
Clasper inner length	11.7	5.9	13.6	10.8	3.4	5.0	6.1	5.6	0.8
Clasper base width	2.4	1.1	3.0	2.3	0.9	1.1	1.3	1.2	0.1
Anal fin—length	8.2	7.3	7.8	7.6	0.3	7.5	10.8	8.7	1.0
Anal fin—base	4.9	4.2	5.0	4.7	0.3	5.2	7.2	5.8	0.7
Anal fin—height	4.7	3.8	5.0	4.4	0.5	4.1	5.3	4.6	0.4
Anal fin—anterior margin	9.4	8.4	9.2	8.9	0.4	8.8	10.8	9.9	0.8
Anal fin—posterior margin	4.0	3.2	4.5	4.1	0.5	3.6	4.8	4.1	0.5
Anal fin—inner margin	3.1	2.2	4.0	3.0	0.6	2.6	3.7	3.2	0.5
Head height	12.9	12.0	13.8	13.0	0.6	11.1	12.0	11.6	0.3
Trunk height	13.5	12.8	15.0	13.9	0.8	10.6	13.6	12.4	1.1
Abdomen height	14.0	11.2	13.8	13.0	1.1	11.2	13.1	11.9	0.8
Tail height	7.6	7.1	8.5	7.8	0.5	6.5	8.6	7.6	0.7
Caudal peduncle height	3.1	3.2	3.5	3.3	0.2	2.7	3.7	3.4	0.4
Head width	12.2	11.1	13.8	12.7	0.9	11.8	14.1	12.8	0.9
Head width at anterior nostrils	7.8	6.5	7.8	7.2	0.6	7.3	9.2	8.2	0.8
Head width at anterior mouth	8.9	7.5	9.4	8.7	0.7	9.4	10.6	10.0	0.5
Trunk width	12.1	9.7	13.0	12.1	1.2	9.5	13.5	11.4	1.5
Abdomen width	11.5	9.5	13.1	11.6	1.2	8.3	12.6	10.8	1.5
Tail width	7.3	6.3	8.2	7.4	0.6	4.7	7.6	6.3	1.1
Caudal peduncle width	3.5	2.6	4.1	3.3	0.5	2.4	3.5	2.9	0.4
Second dorsal fin insertion to anal fin origin	2.2	1.5	2.9	2.2	0.5	0.5	2.7	1.3	0.8
Anal fin insertion to ventral caudal fin origin	12.4	10.4	14.0	12.4	1.2	10.3	11.9	11.0	0.6

Body moderately robust, nape slightly humped; deepest near first dorsal fin spine, trunk height 1.12 (1.07–1.31 in paratypes) times trunk width; tail height 1.05 (0.96–1.13) times tail width (Figures 1 and 2). Head robust, supraorbital ridges prominent but moderately low and broad; trapezoidal in cross-section at eyes, pear-shaped posteriorly; ventral surface of head mostly flat, slightly upturned anteriorly; head short, 22.3 (20.3–24.1)% LT (Figure 3). Supraorbital ridges broadest anteriorly, becoming relatively narrow posteriorly; originating about one eye length anterior to the eye and terminating about one eye length posterior to the eye; distance between ridges smallest anteriorly, widening posteriorly; interorbital space concave, depth less than one-third eye diameter; interorbital space 34.9 (30.7–36.7)% HL. Head width 1.01 (0.99–1.14) times trunk width, 1.06 (0.97–1.20) times abdomen width; length 1.79 (1.78–2.01) in pre-vent length; height 1.05 (0.95–1.08) times width.

Snout short, broadly triangular (moderately rounded in smaller paratypes) in lateral view, apex rounded; moderately rounded in dorsal view, horizontal length 2.13 (1.75–2.40) times eye length, 1.01 (0.88–1.21) times interorbital space; horizontal prenarial length 3.83 (3.28–7.03) in horizontal preorbital length; preoral length 2.24 (2.34–2.91) in horizontal preorbital length. Eye oval, moderately large, length 6.04 (5.44–6.45) in head length, 1.70 (1.52–1.80) times eye height. Spiracle very small, suboval, situated below posterior margin of eye; greatest diameter of spiracle 5.20 (5.53–9.62) in eye length. First gill opening almost vertical, second to fifth angled slightly posteroventrally; first and second gill openings longest, fifth shortest, height of first slit 4.4 (4.1–5.3)% LT, 1.71 (1.62–2.07) times height of fifth slit; gill openings more elevated posteriorly, lower margin of first gill opening slightly below plane of pectoral fin, lower margin of fifth gill opening three quarters eye length above pectoral fin base.

Mouth relatively large, short; lower jaw slightly concave, width 2.57 (2.77–3.53) times preoral length; labial furrows prominent, lower almost twice length of upper; labial flaps thick; no post-oral groove (Figure 4). Nostril incurrent aperture small, pear-shaped, surrounded by a prominent circumnarial groove; circumnarial groove J-shaped, hook wrapping around posterior end of nostril; deep nasoral groove connecting excurrent aperture with mouth; anterior nasal flap long, reaching mouth; internarial width 22.0 (23.0–25.1)% HL; internarial space 4.54 (3.98–4.34) times preoral length.

Figure 1. Holotype of *Heterodontus marshallae* n. sp. (WAM P.35408-007, adolescent male, 541 mm TL), fresh: (**a**) dorsal view; (**b**) lateral view.

Figure 2. Lateral view of female paratypes of *Heterodontus marshallae* n. sp., fresh: (**a**) WAM P.26193-010, juvenile, 355 mm TL; (**b**) CSIRO H 6581-01, 580 mm TL (image flipped, right side of specimen shown).

Figure 3. Ventral head and pectoral fins of the holotype of *Heterodontus marshallae* n. sp., fresh (WAM P.35408-007, adolescent male, 541 mm TL).

Figure 4. Oronasal region of the holotype of *Heterodontus marshallae* n. sp., preserved (WAM P.35408-007, adolescent male, 541 mm TL).

First dorsal fin large, upright (more so in smallest paratype), narrowly rounded apically; anterior margin strongly convex (moderately convex in smallest paratype) (Figures 1b and 2); posterior margin concave; free rear tip thick basally, moderately long; inner margin of fin almost straight; insertion of base well forward of pelvic fin origin, over posterior third of pectoral fin free rear tip (sometimes level with apex of free rear tip); fin spine origin slightly anterior to pectoral fin insertion; spine robust, broad-based, exposed anteriorly near junction of spine and soft portion of fin; soft portion of fin connected at about half total spine length; spine tapering distally, anterior margin weakly convex to nearly straight; exposed portion of spine almost vertical (directed posterodorsally in WAM P.30424-001), subequal in length to exposed portion of second dorsal fin spine; pre-first dorsal length 4.33

(3.76–4.51) times in TL; first dorsal fin length 1.07 (0.94–1.51) times its height, 1.12 (1.11–1.23) times second dorsal fin length; first dorsal fin height 1.42 (1.35–1.46) times second dorsal fin height; exposed first dorsal spine length 0.48 (0.25–0.45) times height of fin.

Second dorsal fin large, upright; anterior margin convex, apex narrowly rounded (Figures 1b and 2); posterior margin slightly to strongly concave; free rear tip thick basally, moderately long; second dorsal fin length 1.36 (1.14–1.86) times its height; exposed spine length 0.48 (0.35–0.62) in height of fin, 0.93 (0.96–1.06) times exposed first dorsal fin spine length; fin spine origin about level with or slightly anterior to free rear tip of pelvic fin, exposed at about level of junction with spine and soft portion of fin; spine robust, moderately broad-based, tapering distally; exposed portion of spine angled slightly posterodorsally; interdorsal space 1.34 (1.45–1.57) in pre-first dorsal length, 1.29 (1.05–1.31) times first dorsal fin length.

Anal fin relatively small, short based, anterior margin slightly to moderately convex, apex narrowly rounded, posterior margin slightly concave, free rear tip relatively long (Figures 1b and 2); apex well behind insertion, slightly posterior to free rear tip; anal fin origin about level with mid second dorsal fin free rear tip; pre-anal length 58.2 (55.2–62.2)% TL; anal fin base 2.68 (2.32–3.21) in anal–caudal space, anal fin length 0.68 (0.57–0.70) in second dorsal fin length, anal fin height 0.53 (0.34–0.63) in second dorsal fin height.

Pectoral fin large, subtriangular anterior margin slightly convex (moderately convex in smallest paratype), with a slight concavity midway (Figure 3); apex narrowly rounded; posterior margin weakly concave to nearly straight; inner margin moderately convex, free rear tip broadly rounded; anterior margin length 1.05 (0.94–1.25) in pre-pectoral length; base short, 2.60 (2.32–3.03) in anterior margin length.

Pelvic fins small, subtriangular; anterior margin weakly convex, apex subangular; posterior margin slightly concave; inner margin slightly convex, free rear tip subangular; pelvic fin height 2.32 (2.05–2.83) in pectoral fin height. Clasper moderately stout, extending well past pelvic fin free tip; clasper groove long, not enclosed; clasper glans with a large and deep pseudosiphon (clasper glans and pseudosiphon about 52% of clasper outer length), a cover rhipidion, a thin rhipidion and clasper spine; external surface of cover rhipidion with a short, narrow groove; outer clasper length in adolescent male holotype 8.3% TL and in two adult male paratypes 9.2 and 9.9% TL (Figure 5).

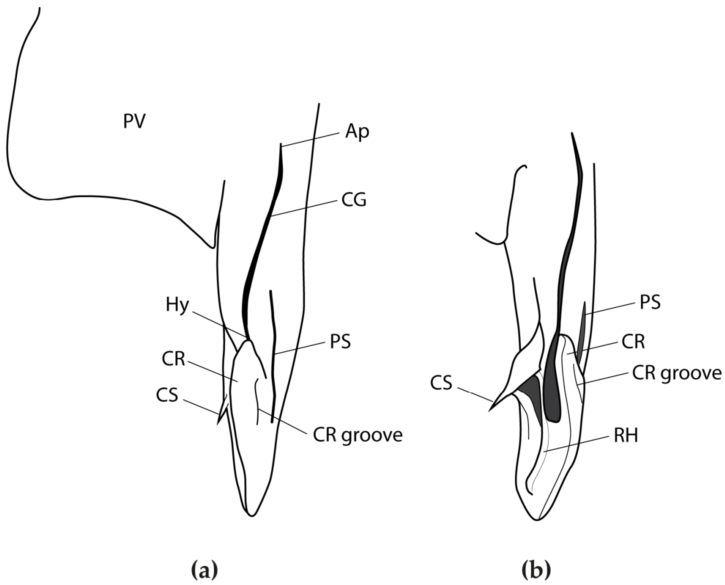
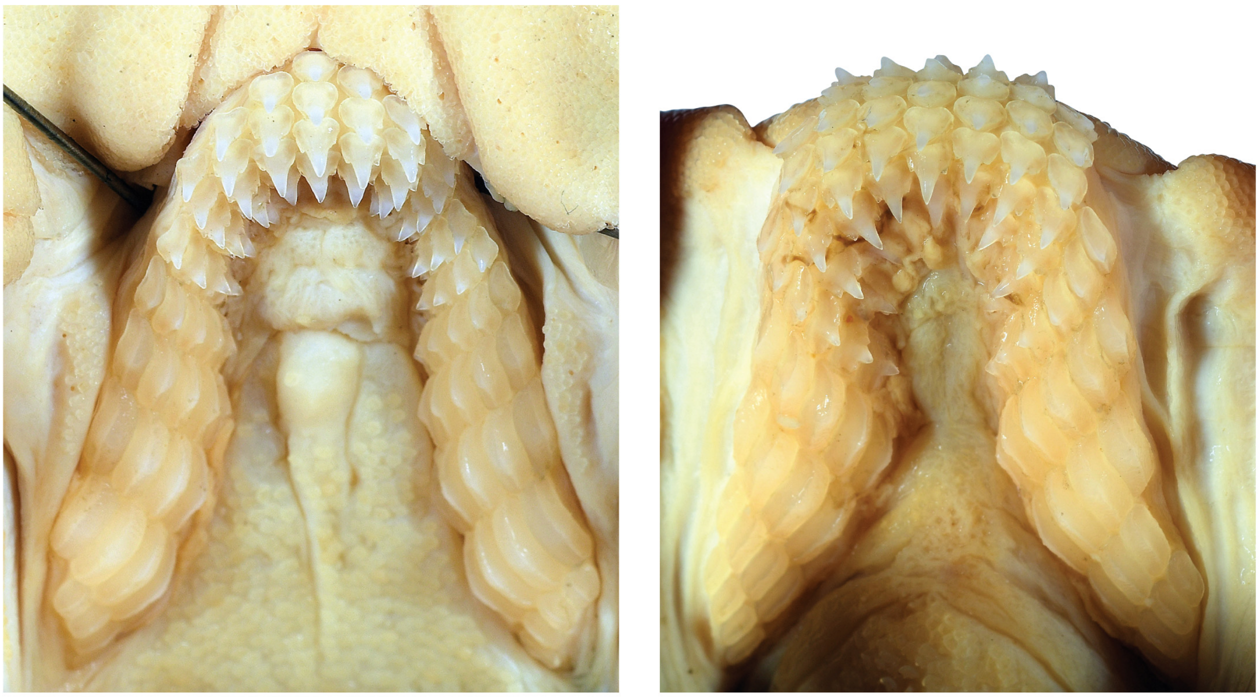


Figure 5. Clasper of *Heterodontus marshallae* n. sp., adult male paratype (NTM S.12929-001, 575 mm TL: (**a**) glans not dilated; (**b**) glans spread. Abbreviations: Ap, apopyle; CG, clasper groove; CR, cover rhipidion; CR groove, groove on external surface of cover rhipidion; CS, clasper spine; Hy, hypopyle; PV, pelvic fin; PS, pseudosiphon; RH, rhipidion.

Caudal peduncle moderately long, relatively slender, slightly depressed; no lateral keels; pelvic–anal space 14.7 (11.7–15.5)% LT; no interdorsal groove; shallow post-dorsal and post-anal grooves; tapering slightly to caudal fin; broadly oval in cross-section; anal–caudal space 13.2 (11.0–13.5)% LT, 0.94 (0.86–1.34) in pectoral–pelvic space; dorsal–caudal space 1.02 (0.97–1.19) times interdorsal space; no dorsal or ventral caudal pits. Caudal fin short; dorsal caudal margin weakly convex to straight, dorsal apex narrowly rounded; ventral lobe well produced, ventral caudal margin slightly convex, ventral apex narrowly rounded; postdorsal margin moderately concave; terminal lobe large and deep, terminal margin slightly concave, subterminal margin almost straight, its apex angular; dorsal caudal margin 0.89 (0.79–1.09) in head length; length of lower caudal lobe 1.69 (1.51–1.83) in upper lobe length (add in CTR as proportion of DCM).

Dental formula (based on paratype CSIRO H 6581-01, Figure 6) from left to right, in upper jaw 4L–3AL–3A–1S–3A–2AL–4L (20 tooth files in total) and lower jaw 4L–1AL–3A–1S–3A–1AL–4L (17 tooth files in total). Holotype with about 22 files in upper jaw and 19 files in lower jaw; counts difficult to confirm on digital radiographs where jaws overlap. Strong monognathic heterodonty, including clutching and grinding type dentitions (*sensu* Cappetta [70]). Symphyseal and anterior teeth pointed, consisting of a principal cusp, flanked by one, or sometimes two pairs of cusplets that are hardly individualised. Lateral teeth molariform, lanceolate shaped, relatively broad but not extremely elongated, and with a longitudinal keel. Labial and lingual crown ornamentation present, showing a texture of transverse ridges running perpendicular to keel. Antero-laterals with intermediate tooth morphology. Dignathic heterodonty weak, with a higher number of antero-lateral teeth in the upper jaws, resulting in more upper tooth files compared to lower jaws. In contrast to lower posterior most tooth file, the upper file is much smaller and mesio-distally compressed compared to adjacent files. Ontogenetic dental shifts present and characterized transformation from clutching to grinding type dentition in lateral tooth positions during early maturity stages. The presence of sexual dimorphism was not examined.



(a) **(b)**

Figure 6. (**a**) Upper and (**b**) lower jaws of *Heterodontus marshallae* n. sp., preserved (CSIRO H 6581-01, female, 580 mm TL).

Dermal denticles (based on paratype CSIRO H 6581-01, Figure 7a,c,e) vary both in size and density along the shark's flank. Dermal denticles and squamation fairly large (>1 mm) and dense in the dorsal region (Figure 7a), moderate in the lateral region (Figure 7c), and small (<1 mm) and wide-spaced in the ventrolateral region (Figure 7e), as is also observed in *H. zebra* (Figure 7b,d,f). Dermal denticles star-shaped (i.e., the 'standard scale type of Reif [28]), the crown consisting of anterior, posterior and two lateral protrusions ('wings' of Reif [28]) that are ornamented with ridges. Anterior and posterior protrusions somewhat elongated and more pointed in the ventral region, resulting in denticles that are often longer than broad vs. broader than long in the dorsal region. Lateral protrusions are less developed in the ventral region, and somewhat rounded in the dorsal region.

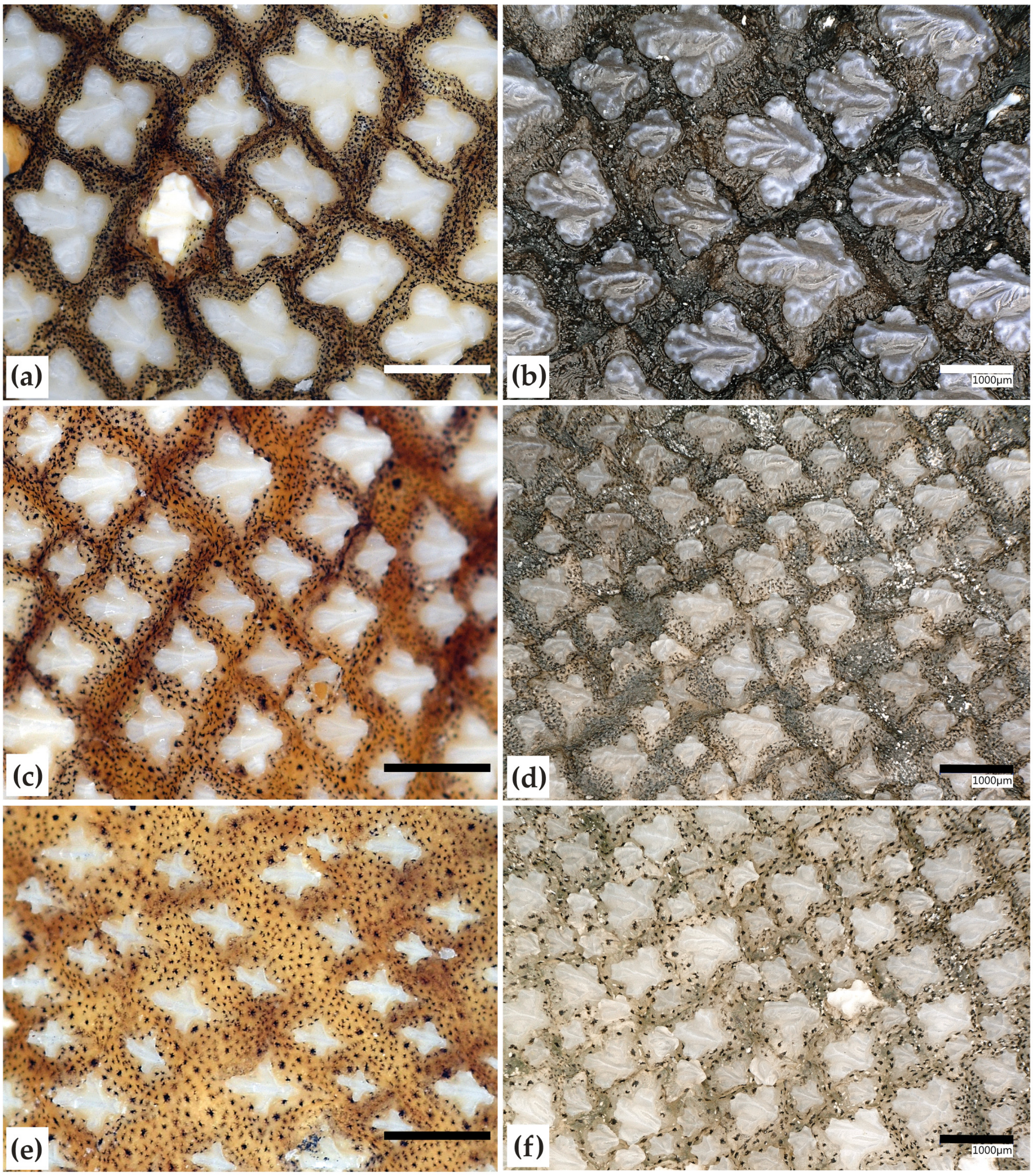


Figure 7. Lateral trunk denticles from: (**a**,**b**) dorsolateral; (**c**,**d**) lateral; (**e**,**f**) ventrolateral surfaces of: (**a**,**c**,**e**) *Heterodontus marshallae* n. sp. (CSIRO H 6581-01, female, 580 mm TL); (**b**,**d**,**f**) *Heterodontus zebra* (RBINS 25174, ex ERB 1148, female, 780 mm TL). Scale bars equal 1 mm.

Vertebral centra 109 (106–112), precaudal centra 70 (70–76), monospondylous 35 (33–37), diplospondylous trunk centra 35 (35–42) and diplospondylous caudal centra 39 (35–39).

3.2.7. Colour

Colour is light greyish brown with a series of 22 dark brown bars and saddles; an additional diffuse-edged, medium brown bar or saddle often present between main bifurcated or single dark brown bars and saddles (Figures 1 and 2). Snout with a dark brown blotch at tip; a semicircular bar extending from anterior of circumnarial grooves over anterior of snout, bifurcated for most of its length, interspace medium brown (Figure 8a,c,e); bar at origin of supraorbital crest, barely evident in holotype but distinct in paratypes (bar sometimes separated on dorsal midline). Head with five bars: two bars at level of eyes, sometimes bifurcated for part of their length, first bar on holotype with a subtriangular indentation on dorsal midline; third bar originating from anterior of first gill slit, sometimes bifurcated dorsally; fourth originating from above first or second gill slits, sometimes bifurcated dorsally; fifth originating from above interspace of third and fourth gill slit; interspaces of bifurcated bars medium brown. Body with four bars (defined hereon as extending down entire depth of sides); bifurcated bar at origin of first dorsal spine, merging lateroventrally just above pectoral fin base and extending onto pectoral fins, interspace usually medium brown (sometimes pale greyish brown); bar at first dorsal fin mid-base, extending onto pectoral fins; two bars below and just posterior to first dorsal fin free rear tip, interspace usually medium brown (in paratype WAM P.30424-001 bars bifurcated below lateral line). Body with nine saddles (defined hereon as extending only half body depth); single saddle above pelvic fin origin, sometimes bifurcated dorsally; two saddles above anterior pelvic fin free rear tip, interspace sometimes medium brown; single saddle at level of second dorsal fin origin; two saddles below posterior half of second dorsal fin, usually bifurcated for most or all of their length, interspace usually medium brown (in paratype WAM P.30424-001 saddles bifurcated for their entire length, appearing as two pairs of close-set saddles, interspaces paler); single saddle above anal fin free rear tip bifurcated dorsally; two close set saddles anterior to caudal fin origin, sometimes bifurcated dorsally. Caudal fin with three saddles anteriorly: first saddle is short, at dorsal origin of fin; second saddle less than an eye length behind first, usually bifurcated dorsally; third saddle less than an eye length behind second, usually more diffuse than anterior two saddles; apex of terminal lobe usually blackish or dusky. Pectoral fin dorsal surfaces with two dark bars; anterior bar an extension of the first bifurcated bar on body that extends to the anterior margin; posterior bar an extension of second dark bar on body below first dorsal fin, not extending to outer margin of fin, often becoming diffuse distally. Pelvic fin dorsal surface with a short dark bar anteriorly, sometimes diffuse. Ventral surfaces of head, body and fins pale, sometimes with numerous small, diffuse, greyish blotches; a diffuse dark blotch centrally on pectoral fins in holotype (not present in paratypes). Dorsal fins with distal third blackish in holotype, variably dusky-tipped in paratypes.

3.2.8. Egg Case

The egg case was collected through trawling, independent from a specimen. Although it is superficially similar to the egg cases of *H. zebra* as it has very narrow keels, it is confidently assigned to *H. marshallae* n. sp. since it differs in number of rotations along the length of the egg case. Furthermore, it was collected in the same general area as an adult specimen of the new species.

The egg case was large (115 mm egg case length) and screw-like with 1.5 rotations from anterior to posterior margins, a pair of lateral keels, surfaces smooth; anterior margin broad and straight, abruptly tapering posteriorly, posterior margin closed with opposing keels meeting to touch; keels rigid, narrow, curved anteriorly; keels at posterior end extending into long curling tendrils (Figure 9a). Colour golden brown with slight greenish hue (preserved).

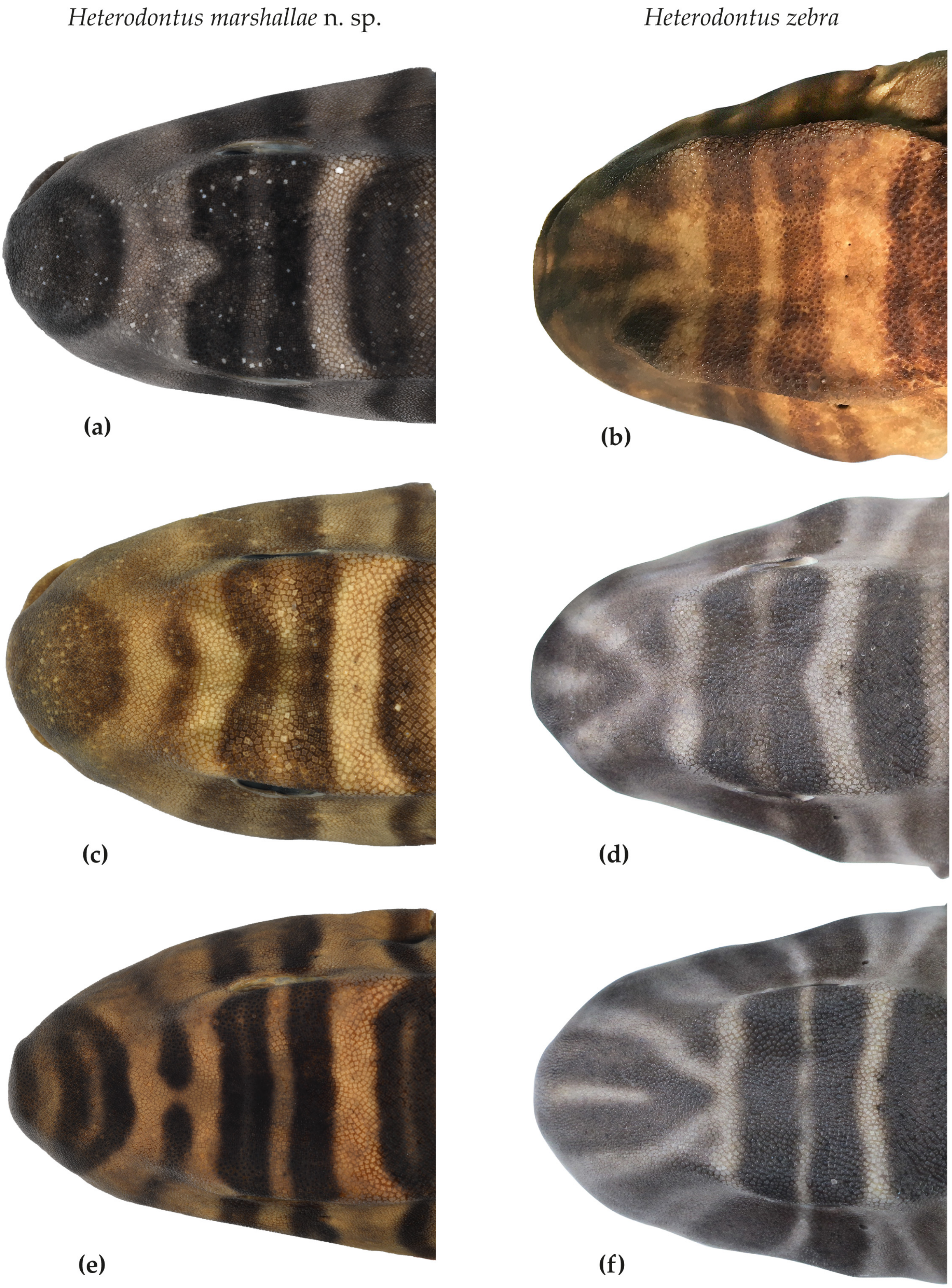


Figure 8. Dorsal head highlighting shape of dark marking extending from snout tip towards interorbital of: *Heterodontus marshallae* n. sp. (**a**,**c**,**e**) and *H. zebra* (**b**,**d**,**f**): (**a**) Adolescent male, 541 mm TL, preserved (holotype WAM P.35408-007); (**b**) Female, ~630 mm TL, preserved (holotype of *C. amboinensis* BMNH 1867.11.28.183); (**c**) Female, 580 mm TL, preserved (paratype CSIRO H 6581-01); (**d**) Female, 778 mm TL, fresh (ERB 1147); (**e**) Adult male, 601 mm TL, preserved (paratype WAM P.30424-001); (**f**) Female, 796 mm TL, fresh (ERB 1148).

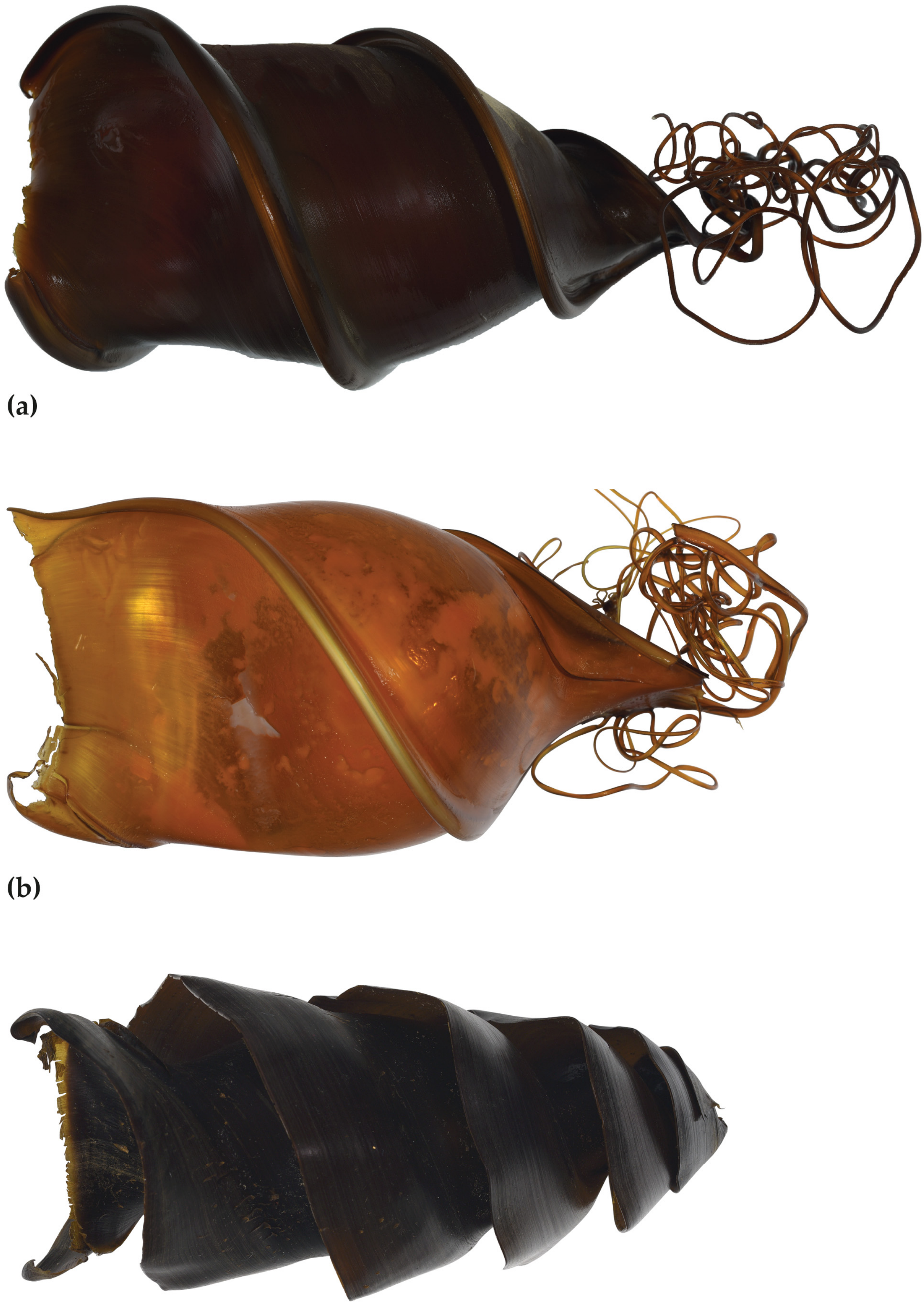


Figure 9. Egg case of: (**a**) *Heterodontus marshallae* n. sp., preserved (paratype, NTM S.18275-001); (**b**) *H. zebra*, preserved (KAUM-I. 69456); (**c**) *H. portusjacksoni*, preserved (CSIRO H 8732-02).

3.2.9. Size

Type specimens ranged from 355 to 601 mm TL. A 432 mm TL male was immature; three males of 555–601 mm TL were mature.

3.2.10. Distribution

The species occurs in northern Australia from west of Exmouth Peninsula (22°22.946′ S, 113°40.308′ E) east to at least off Bathurst Island in the Northern Territory (Figure 10), at depths of 125–229 m.

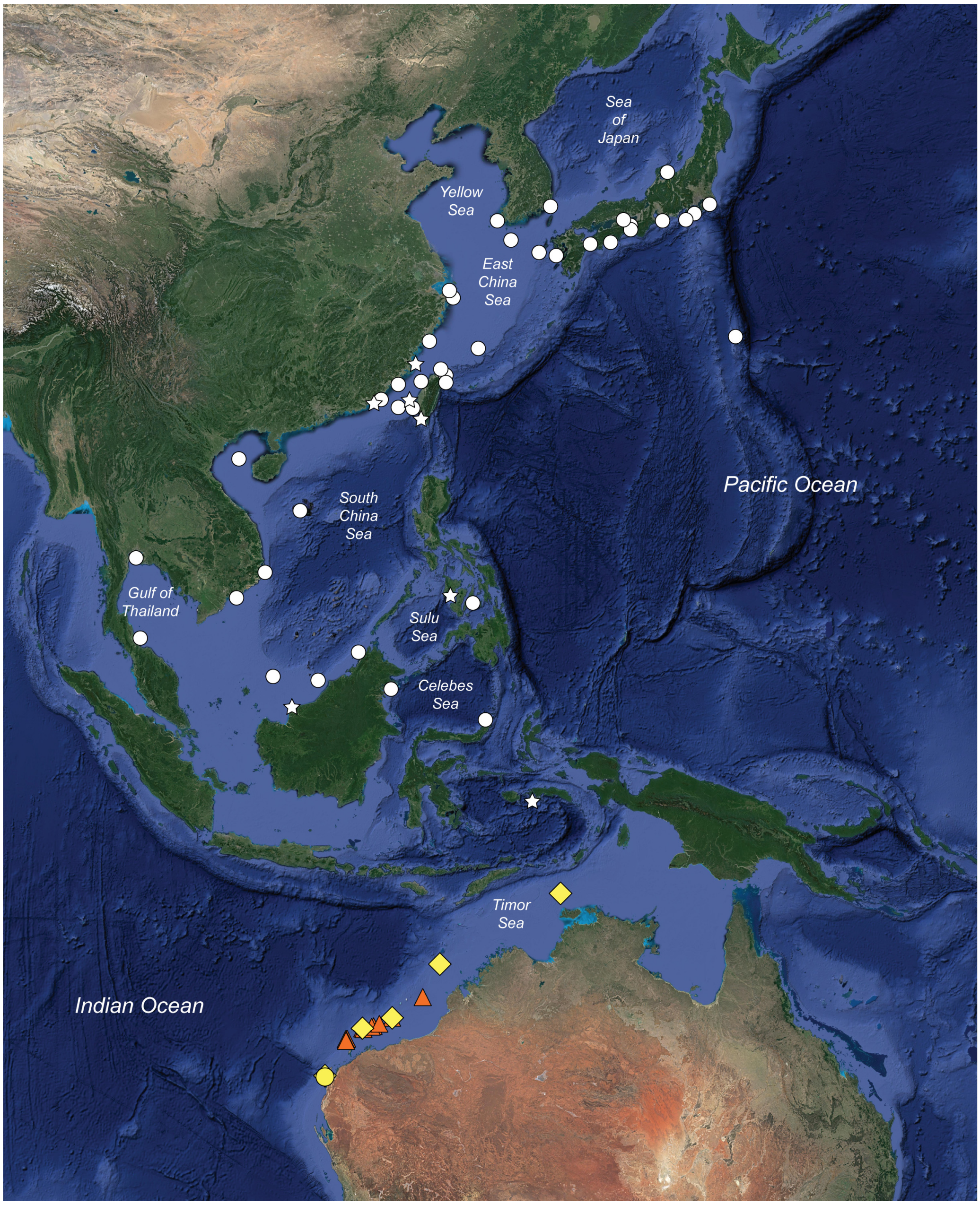


Figure 10. Distribution of *Heterodontus marshallae* n. sp. and *Heterodontus zebra* in the Indo–West Pacific. Yellow circle denotes holotype, yellow diamonds paratypes, orange triangles denote other records (specimens not retained) of *H. marshallae* n. sp. White stars denote specimens examined and white circles denote other records of *H. zebra* (Image © Google 2023, SIO, NOAA, U.S. Navy, NGA, GEBCO).

3.3. Molecular Analyses

The analysis of the *NADH2* data suggest that *Heterodontus marshallae* n. sp. represents a monophyletic lineage that is distinct from but most closely related to *H. portusjacksoni* from southern Australia and *H. zebra* from the Western Pacific (Figure 11). These three species are, in turn, sisters to *H. galeatus* from eastern Australia. The low node support for the sister relationships between *H. portusjacksoni* and *H. marshallae* n. sp. was due to their being only 13 sites variable among the 1044 bp NADH2 sequences. The node support will likely be higher when more genes are used in future studies.

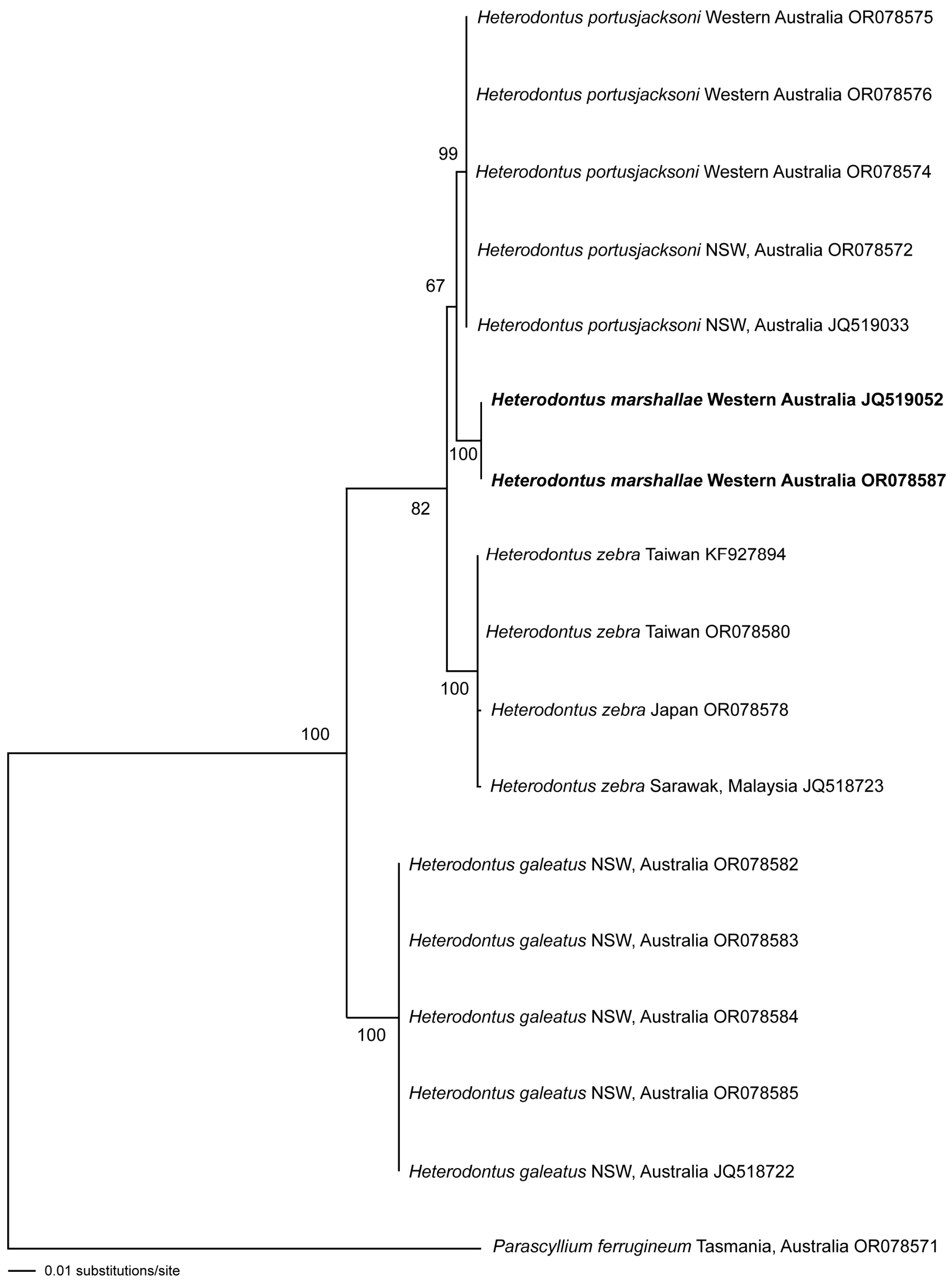


Figure 11. Maximum likelihood tree estimated under the general time reversible model (GTR) with model terms to accommodate both invariant site (I) and gamma distributed rates (G). Bootstrap support values are shown from a separate ML bootstrap analysis. GenBank accession numbers provided.

3.4. Intraspecific Variation

The morphometrics of the type series of *Heterodontus marshallae* n. sp. highlighted several ontogenetic differences between juveniles and adults. The two juveniles (355 and 432 mm TL) differed from the subadult and adult specimens (541–601 mm TL) in the following ways: taller dorsal fins (first dorsal fin height 13.1–15.6 vs. 9.2–12.5% TL; second dorsal fin height 9.0–11.1 vs. 6.7–8.8% TL); shorter snouts (preorbital length 8.1–8.7 vs. 9.6–10.5% TL); and smaller heads (horizontal head length 20.3–21.4 vs. 21.6–23.5% TL; head height 12.0–12.5 vs. 12.9–13.8% TL).

The colour pattern did not differ much between juveniles and adults, although juveniles typically had darker and more defined dark markings on the head, body and fins.

3.5. Comparison with Congeners

Heterodontus marshallae n. sp. and *H. zebra* have a distinctive colour pattern consisting of numerous dark bars and saddles, which easily distinguishes both species from their congeners. In contrast, the three Eastern Pacific species, *H. francisci*, *H. mexicanus* and *H. quoyi*, have a colour pattern of numerous dark spots on the body and fins; *H. ramalheira* has numerous small white spots; *H. galeatus* and *H. omanensis* have dark broad bands or saddles, without spots; *H. japonicus* has a pattern of about 12 irregular dark saddles and bands, sometimes indistinct; and *H. portusjacksoni* has a distinctive dark, harness-like marking on anterior body.

Although *H. marshallae* n. sp. and *H. zebra* have similar colour patterns, two key features were found to differ consistently between the two species. Firstly, the dark marking on the snout differs consistently between the two species regardless of size or sex for the specimens examined. In *H. marshallae* n. sp., the snout tip has a pair of dark brown, semicircular bars which extend posteriorly on the dorsal midline to about midway between snout tip and anterior eyes, and the second bar is sometimes bifurcated dorsally (Figure 8a,c,e). In contrast, *H. zebra* has a dark brown, triangular bar with the medial extension usually forming a sharp point and extending from just posterior of the midlength of the preorbit to level with anterior eyes (Figure 8b,d,f). Secondly, *H. zebra* has a dark stripe extending from the ventral margin of the fourth or fifth gill slits onto the anterior dorsal surface of the pectoral fins (Figures 12 and 13). *Heterodontus marshallae* n. sp. has no dark marking extending from the posterior gill slits onto the pectoral fin (Figures 1 and 2).

Figure 12. Dorsal view of *Heterodontus zebra*: ERB 1149, female, 666 mm TL, fresh. Arrows highlight the dark bar originating from posterior gill slits and extending onto the anterior of pectoral fins.

Figure 13. Lateral view of *Heterodontus zebra*, fresh: (**a**) juvenile female, 220 mm TL, preserved (NMMB-P 8845); (**b**) adolescent male, 580 mm TL, fresh (not retained, field code BO-038, GenBank accession JQ518723). Arrows highlight the dark bar below posterior gill slits extending onto anterior of pectoral fins.

Heterodontus marshallae n. sp. also differs from *H. zebra* in the following morphometric characters: anal fin slightly further from caudal fin (anal–caudal space 11.0–13.5 vs. 9.4–10.5% TL); more distinct ventral caudal fin lobe (lower postventral caudal margin 4.7–6.1 vs. 3.2–3.5% TL); and longer dorsal spines (exposed first dorsal fin spine length 3.9–4.5 vs. 1.0–3.7% TL; exposed second dorsal fin spine length 3.9–4.7 vs. 2.0–4.0% TL). *Heterodontus marshallae* n. sp. also has less vertebral centra than *H. zebra*, i.e., total centra 109–112 vs. 115–120.

Given the level of ontogenetic variation in certain morphometric characters of *Heterodontus marshallae* n. sp., interspecific variation is difficult to adequately highlight without making comparisons of the two species at different life stages. Juveniles (<460 mm TL) of *H. marshallae* n. sp. differed from juvenile of *H. zebra* in terms of: longer pre-pectoral length (21.2–21.5 vs. 17.5–20.1% TL); longer dorsal–caudal space (18.3–18.7 vs. 13.4–16.3% TL); smaller anal fin (anal fin length 7.3–7.5 vs. 8.4–10.8% TL; and anal fin anterior margin 8.7–8.9 vs. 9.4–10.8% TL). Larger specimens (>540 mm TL) of *H. marshallae* n. sp. differed from large *H. zebra* in the following: shorter pre-cloacal length (39.9–44.3 vs. 45.3–48.8%

TL); dorsal fins closer together (interdorsal space 16.9–17.8 vs. 18.6–20.9% TL); larger eyes (eye length 3.7–3.8 vs. 2.6–3.3% TL); much larger dorsal fin spines (exposed first dorsal fin spine length 4.2–4.5 vs. 1.0–1.8% TL; and exposed first dorsal fin spine length 4.0–4.7 vs. 2.0–2.2% TL).

The egg case of *Heterodontus marshallae* n. sp. is similar to that of *H. zebra* but differs in the 1.5 rotations from anterior to posterior ends vs. less than 1 rotation in *H. zebra* (Figure 9).

4. Discussion

4.1. Comparison between Species

Heterodontus marshallae n. sp. has a remarkably similar colour pattern to *H. zebra*, thus is not surprising that it has been considered conspecific with this species until now. The first indication of *H. zebra* being a species complex was through the molecular results reported by Naylor et al. [14]. In this study, the sequence of the *NADH2* gene was found to differ between the northwest Australian and Western Pacific samples. Furthermore, the northwest Australian sample appeared to be genetically closer (based solely on the *NADH2* gene) to *H. portusjacksoni* than to the true *H. zebra*. These molecular differences combined with the different depth preferences, i.e., *H. zebra* occurs in 0–143 m vs. 125–229 m in the northwest Australian specimens, provided strong evidence that *H. zebra* was a species complex. This study also found key differences in colour and morphology, enabling the formal description of the northwest Australian specimens as a new species, *H. marshallae* n. sp.

Colour pattern is an important characteristic in hornsharks. The southern Australian species, *H. galeatus* and *H. portusjacksoni* are most readily distinguished from each other in their colour pattern, with the former species lacking the blackish, harness-like marking on the side that the latter species has [3]. Likewise, *H. francisci*, *H. mexicanus* and *H. quoyi* from the Eastern Pacific all have a colour pattern of dark spots over a pale background which vary by number and size in each species [71]. While *H. marshallae* n. sp. and *H. zebra* have similar colour patterns, two key colour characteristics were found to consistently differ between the two species regardless of size and sex. These are the shape of the dark marking on the preorbital snout (semicircular in *H. marshallae* n. sp., triangular in *H. zebra*, Figure 8) and the presence of a dark bar below fifth gill slit extending onto anterior pectoral fin dorsal surface (absent in *H. marshallae* n. sp., present in *H. zebra*).

Despite *H. marshallae* n. sp. being most similar to *H. zebra* in both morphology and coloration, the molecular data suggested that it was closest, based on *NADH2* sequences, to *H. portusjacksoni*. As previously mentioned, these two species are clearly separated based on their coloration. Furthermore, the egg cases of *H. portusjacksoni* are very different from those of *H. marshallae* n. sp. in that they lack tendrils at the posterior end (vs. long tendrils present) and have very broad keels (vs. very narrow keels) (Figure 9a,c). *Heterodontus marshallae* n. sp. also differs from *H. portusjacksoni* as they have a shorter pectoral fin base (7.2–8.0 vs. 8.7–9.2% TL); larger dorsal fin spines (exposed first dorsal spine length 3.9–4.5 vs. 1.7–3.1% TL, its base 1.1–1.4 vs. 0.6–0.9% TL; exposed second dorsal spine length 3.9–4.7 vs. 2.0–3.1% TL, its base 1.1–1.2 vs. 0.7–0.9% TL); smaller anal fin (anal fin length 7.3–8.2 vs. 8.2–10.1% TL, its height 3.8–5.0 vs. 5.3–6.0% TL); and slightly less vertebrae (total centra 106–112 vs. 114–118).

4.2. Dentition and Denticles

Shark teeth are often diagnostic, thus allowing species identification [72], but this is not the case in hornsharks. Hornsharks show increasing tooth file counts due to allometric growth, and significant monognathic, ontogenetic and even gynandric heterodonties [73,74], hence the name *Heterodontus*. However, Reif [75] was able to distinguish two dental types in the genus, the so-called '*portusjacksoni* type' (also including *H. zebra* and *H. japonicus*) vs. the '*francisci* type' (also including *H. mexicanus*, *H. quoyi*, *H. galeatus* and *H. ramalheira*), which corresponds to '*Morphotype 1*′ and '*Morphotype 2*′ of Hovestadt [74], respectively, and who allocated the more recently described *H. omanensis* to the *francisci* type. In the latter type, molariform teeth are slenderer and more elongate (vs. broad and swollen), displaying a higher number of functional tooth rows and forming a wider occlusal bite surface compared to the '*portusjacksoni* type'. The dental morphology of the molariform teeth of *H. marshallae* n. sp. corresponds to the '*portusjacksoni* type', but individual teeth thus cannot be differentiated from its members such as *H. zebra*.

The dermal denticles of *Heterodontus* that are heterosquamous [28,76,77] are also difficult to differentiate between species. Small differences can be observed between specimens of *H. marshallae* n. sp. and *H. zebra* examined, e.g., in the general appearance of the denticles, which are more arrowhead-shaped in the dorsal region of *H. zebra* and in the margins of all four protrusions that are more rounded in the same region of this latter species (Figure 7b).

4.3. Distribution

Heterodontus zebra is a primarily shallow water species occurring from close inshore to depths of at least 143 m [35]. In contrast, *H. marshallae* n. sp. has only been recorded between 125 and 229 m off northwestern Australia. There have been numerous research trawls in shallower areas of the North West Shelf within the range of the new species, including, most recently, an intensive biodiversity survey in 2017 that trawled depths of 30 to 111 m [78]. Thus, it is unlikely that *H. marshallae* n. sp. occurs at depths shallower than 120 m. This contrasts with *H. zebra*, which typically occurs at depths less than 100 m throughout its range. Most species of *Heterodontus* inhabit shallow waters usually to depths of 150 m or less. The depth range of *H. marshallae* n. sp. is closest to that of *H. ramalheira* from southern Africa, which occurs at depths of 40–305 m, mostly below 100 m [71].

A specimen of '*H. zebra*' from the Marion Plateau in the Coral Sea, Queensland, was reported by Last et al. [79], at a depth of 398 m. This represents the deepest record for any *Heterodontus* species. However, no images or specimens are available to corroborate this record, which is surprising given the large range extension in Australia and new depth record. Since such verification is not available, we treat this record as questionable and do not consider it in the range of the new species.

4.4. Egg Cases

The egg case of *H. marshallae* n. sp. (paratype NTM S.18275-001, Figure 9a) is most similar to those of *H. zebra* (Figure 9b) and *H. omanensis* [9] as it has very short, curled keels. In contrast, the egg cases of *H. francisci*, *H. galeatus*, *H. japonicus*, *H. mexicanus*, *H. portusjacksoni* and *H. quoyi* all have much broader, non-curled keels. The *H. marshallae* n. sp. egg case also possesses long tendrils at the posterior end (Figure 9a). Such tendrils are also present in egg cases of *H. galeatus*, *H. mexicanus*, *H. omanensis* and *H. zebra*, but tendrils are absent in *H. francisci*, *H. japonicus*, *H. portusjacksoni* and *H. quoyi* egg cases.

The presence or absence of tendrils provides some insight into the locations that hornshark egg cases are deposited. It is well documented that *Heterodontus portusjacksoni* and *H. francisci* lay their eggs in shallow waters where they are deposited in rocky crevices and under ledges [80,81]. In contrast, those with tendrils presumably attach their egg cases to substratum, e.g., corals, kelp or sponges, although little information is available concerning the reproduction of the hornshark species with tendrilled egg cases. A thorough review of egg case morphology in the *Heterodontus* species is currently in progress by two of the authors (W.T.W. and H.L.O.).

4.5. Key to Species of Heterodontus

A key to the species of extant *Heterodontus* species is provided below.

1. Body and fins: spotted 2
   Body and fins with stripes, bars or bands: not spotted 5
2. Body and fins with white spots in adults and subadults; hatchlings with thin curved parallel lines on body . . . . *Heterodontus ramalheira* (Western Indian Ocean)
   Body and fins with dark spots and darker saddles (most evident in juveniles) 3
3. First dorsal fin origin behind pectoral fin bases . . . *Heterodontus quoyi* (Eastern Pacific)
   First dorsal fin origin over pectoral fin bases 4
4. Back and sides with small dark spots less than a third of eye diameter in size; no light-coloured bar on interorbital surface of head . . . . *Heterodontus francisci* (Eastern Pacific)
   Back and sides with larger dark spots at least half eye diameter in size; a light-coloured bar on interorbital surface of head. . . . . *Heterodontus mexicanus* (Eastern Pacific)
5. Supraorbital crests: very high . . . . *Heterodontus galeatus* (eastern Australia)
   Supraorbital crests: moderate to low 6
6. Body with a distinctive harness-like pattern of dark stripes extending from in front of dorsal fin on the dorsal midline to the pectoral and pelvic fin bases . . . . *Heterodontus portusjacksoni* (southern Australia)
   Body with vertical dark bands or saddles not arranged in a harness pattern 7
7. Body with 22–36 narrow dark bars and saddles from snout to origin of caudal fin 8
   Body with 5–14 broad or narrow, diffuse-edged markings from snout to origin of caudal fin 9
8. Narrow triangular dark marking extending from snout to midline of head in front of eyes; dark bar extending onto pectoral fin from posterior gills . . . . *Heterodontus zebra* (Northwest Pacific)
   Dark marking on snout semicircular, not narrowly triangular; no dark bar extending from onto pectoral fin from posterior gills . . . . *Heterodontus marshallae* n. sp. (northern Australia)
9. Dorsal, pectoral and caudal fins without abruptly black tips or white apical spots; body with 11–14 dark markings from snout to origin of caudal fin . . . . *Heterodontus japonicus* (Northwest Pacific)
   Dorsal and pectoral fins and ventral caudal fin lobe with abruptly black tips, dorsal fins with white apical spots; body with four or five broad dark saddles from snout tip to origin of caudal fin . . . . *Heterodontus omanensis* (Western Indian Ocean)

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15070849/s1, Table S1: Raw morphometric and vertebral count data for all Heterodontus marshallae n. sp. and Heterodontus zebra measured and radiographed; Table S2: Specimen and collection data for tissue samples used in this study. GN number refers to the genetic codes assigned by Gavin Naylor's team at Florida Museum of Natural History.

Author Contributions: W.T.W. conceived and designed the study; H.L.O. and W.T.W. carried out field work; L.Y. and G.J.P.N. performed molecular analyses and interpretation of results; F.H.M. provided dentition and squamation descriptions. W.T.W., H.L.O., F.H.M. and L.Y. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Most specimens examined in this study are from natural history museum collections and were not collected specifically for this study. The holotype of the new species was collected on a recent survey of the Gascoyne Marine Park off the Exmouth Peninsula off Western Australia and was caught using a demersal fish trawl. These samples were collected under approval from the Australian Animal Ethics Committee, application AEC 2021-25, allowing CSIRO to collect fishes in the Gascoyne and Ningaloo Marine Parks.

Data Availability Statement: All data used in this study are available in Table 1 and in the Supplementary Materials.

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