10.1071/MF22048

Marine and Freshwater Research

Supplementary Material

Using integrative taxonomy to distinguish cryptic halfbeak species and interpret distribution patterns, fisheries landings, and speciation

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Supplemental detailed sample preparation

All whole fish were thawed and labelled before tissues samples were taken, which were then stored frozen in 90% ethanol. Duplicate tissue samples were also taken and preserved in the Australian Museum's frozen tissue collection for long term storage at -80°C (voucher numbers AMS I.50005.002–I.500012.071). Whole fish were photographed with a Nikon D90 camera before being X-rayed with an Eresco AS2 (Model number EXR 150-23 BW, License number 5079378) and developed with a Fujifilm Digital Console (Model number CR-IR 368).

Sagittal otoliths were then extracted with fine-tipped forceps while leaving the gill rakers intact and head semi-attached to the body, then cleaned, dried, accessioned into the Australian Museum's collection (voucher numbers AMS I.50005.003–I.50012.072), and stored at room temperature for later analysis. Gill rakers on the first and second arches were counted with a dissecting microscope. Rakers were separated one by one with a dissecting probe and all counts were made by one person (I. J. Riley) to ensure consistency.

Sex and gonad stage were determined macroscopically with reference to stages identified for *H. australis* (Hughes and Stewart, 2006). Whole fish specimens were then preserved in 10% formalin for 2 weeks before being stepped up (25 and 50%) into a final storage solution of 70% ethanol and registered in the Australian Museum's Ichthyology collection (voucher numbers AMS I.50005-001–I.50012-070).

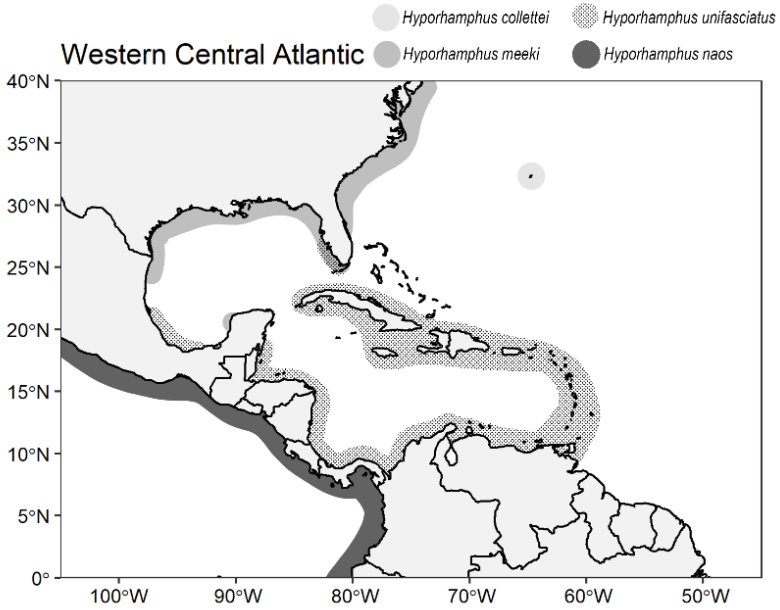
Supplemental detailed genetic methods

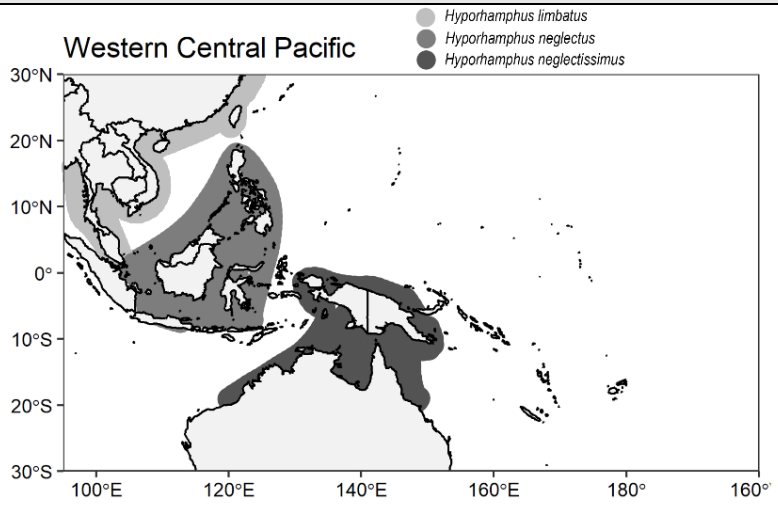
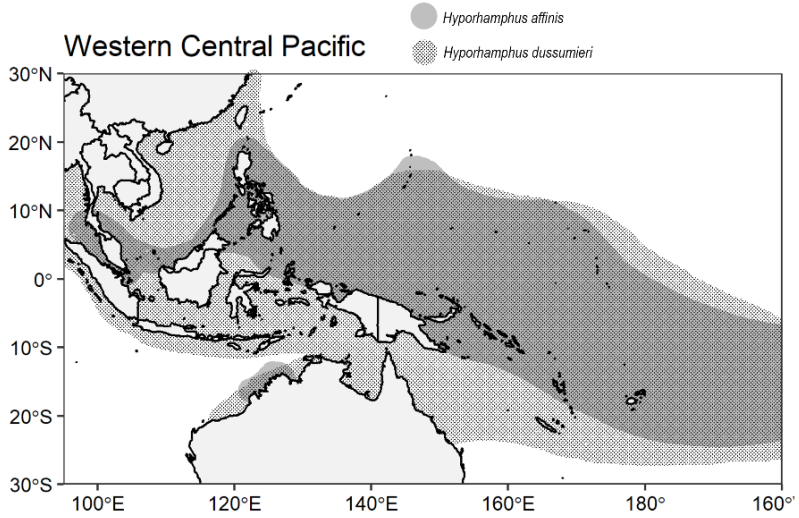
Initial primer optimisation performed by the Ramaciotti Centre for Genomics failed to amplify the RAG2 nuclear region using the modified primers RAG2 2F and RAG2 2R (DiBattista *et al*., 2012). Owing to time and budget constraints, we performed further testing of other primers for both the RAG2 and TMO-4C4 nuclear regions in-house at the Australian Centre for Wildlife Genomics laboratory at the Australian Museum in Sydney, Australia. Inspection of TMO-4C4 sequences downloaded from GenBank for other *Hyporhamphus* species showed considerable variability among congeners, making it a viable alternative to the *RAG2* gene.

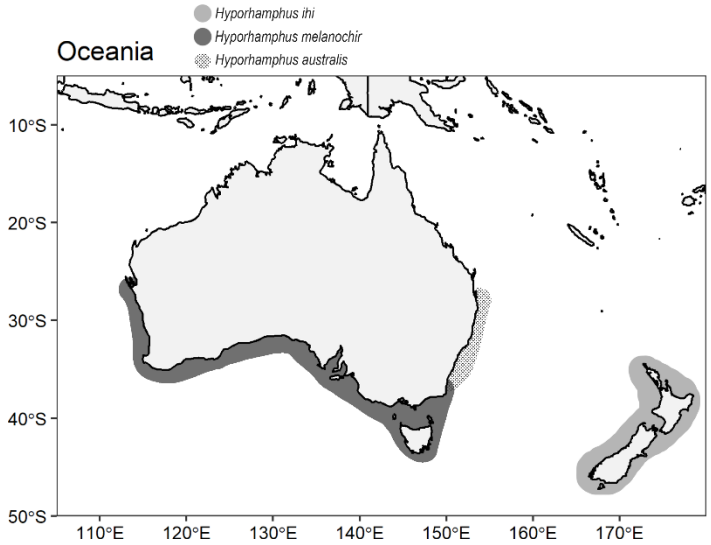
Primers for fragments of the nuclear RAG2 (Lovejoy and Collette, 2001) and TMO-4C4 (Streelman and Karl, 1997) genes were tested on a subset of six samples to determine their success at amplifying these regions in these two species (sequences and PCR conditions in Table S3). PCR mixes for these reactions in a total volume of 25 µL of water included - 5 µL of Bioline MyTaq Buffer (Meridian Bioscience, Cincinnati, USA), 0.2 µL of Bioline MyTaq Polymerase (Meridian Bioscience, Cincinnati, USA), 0.5 µL of each primer (10 µL), 2 µL of DNA (neat), and the remainder ultrapure water.

PCR products were visualised on 1% agarose gels by electrophoresis, which suggested successful amplification using the TMO-4C4 primers but not the RAG2 primers. The six test samples that amplified using TMO-4C4 primers were then cleaned using ExoSAP (New England Biolabs, Massachusetts, USA) and sent to the Ramaciotti Centre for Genomics for Sanger sequencing. The above methods for PCR amplification of the TMO region were then repeated for 271 samples selected from the total pool of 307 DNA extractions. Gel-checks were performed on a random subset of these PCR products (n = 56) prior to cleaning with ExoSAP and Sanger sequencing with the Ramaciotti Centre for Genomics.

Table S1: Examples of cryptic garfish species from the genus *Hyporhamphus*.

Species	Distribution	Distinguishing features	References
Hyporhamphus unifasciatus		**Gill Rakers**: usually 28 – 32 on the first arch, usually 19 - 25 on the second arch Ratio of **preorbital length** to orbit diameter: usually <0.70 Ratio of **body depths** to standard length: usually >0.12	(Collette, 2002) (Collette *et al*., 2019)
Hyporhamphus naos		**Gill Rakers**: usually 32 – 36 on first arch, usually 23 – 27 on second arch Ratio of **preorbital length** to orbit diameter: usually >0.70 (but diagnostically smaller than *H. meeki*)	(Banford & Collette, 2001) (Collette, 2002)
Hyporhamphus meeki		**Gill Rakers**: usually 33 – 39 on the first arch, usually 25 – 30 on the second arch Ratio of **preorbital length** to orbit diameter: usually >0.70	(Banford & Collette, 1993) (Collette, 2002)
Hyporhamphus collettei		Gill Rakers: usually 29 – 30 on the first arch, usually 21-22 on second arch Ratio of **preorbital length** to orbit diameter: usually <0.70 Ratio of **body depths** to standard length: usually <0.12	(Collette, 2002) (Banford, 2010)
Hyporhamphus limbatus		**Lower jaw** usually longer than head in adults: contained 0.7 to 1.3 times in head length	(Collette, 1999)
Hyporhamphus neglectus		**Lower jaw** usually not as long as head in adults: contained 0.9 to 1.8 times in head length **Preorbital distance** smaller: contained 1.4 to 2 times in orbital diameter, contained 0.8 to 1.35 times in upper-jaw length	(Collette, 1999)

Species	Distribution	Distinguishing features	References
Hyporhamphus neglectissimus		**Lower jaw** usually not as long as head in adults: contained 0.9 to 1.8 times in head length **Preorbital distance** larger: contained 1.05 to 1.35 times in orbital diameter, contained 0.6 to 0.8 times in upper jaw length	(Collette, 1974) (Collette, 1999)
Hyporhamphus affinis		**Gill rakers:** usually 35 or less on first arch, usually 24 or less on second arch **Preorbital distance** larger**:** contained 1.35 to 1.9 times in orbit diameter, usually greater than upper jaw length	(Collette, 1974) (Collette, 1999)
Hyporhamphus dussumieri		**Gill rakers:** usually 36 or more on first arch, usually 26 or more on second arch **Preorbital distance** larger**:** contained 1.7 to 2.15 times in orbit diameter, usually less than upper jaw length	(Collette, 1974) (Collette, 1999)

Species	Distribution	Distinguishing features	References
Hyporhamphus australis		**Gill rakers:** usually 34 or more on first arch, usually 27 or more on second arch	(Collette, 1974)
Hyporhamphus melanochir		**Gill rakers**: usually 33 or less on first arch, usually 26 or less on second arch **Pelvic fins** further posterior	(Collette, 1974)
Hyporhamphus ihi		**Gill rakers**: usually 33 or less on first arch, usually 26 or less on second arch **Pelvic fins** further anterior	(Collette, 1974)

All species chosen as examples of species with only small morphological differences in their number of gill rakers, morphological measurements or both. Compiled distribution maps created in R (ver. 4.0.4) and Adobe Illustrator with reference to Food and Agriculture Organization of the United Nations Regional Guides and past studies by B. B. Collette.

Table S2: Growth, life history and diet of *Hyporhamphus australis* and *Hyporhamphus melanochir*.

Trait	*Hyporhamphus australis*	*Hyporhamphus melanochir*
Diet	Omnivore • Algae, *Zostera* seagrass, and crustaceans • No studies into potential diurnal feeding patterns (Thomson, 1959; State Pollution Control Commission, 1981; Parsons, 2002)	Omnivore • Diurnal feeders that selectively consume *Zostera* seagrass species during the day and night-emergent hyperbenthic crustacea at night (Klumpp & Nichols, 1983; Earl *et al*., 2011)

Mean Size (FL)	Smaller
	• 22-24 cm
	  ○ Larger in late summer
	  ○ Smaller in winter
	(Stewart *et al*., 2005)
		Larger
		• Victoria: 24.7 cm
		  ○ Smaller in summer
		  ○ Larger in winter
		(Jones *et al*., 2002)

Growth Rate	Faster • 230 mm FL at 1 year of age (Stewart & Hughes, 2007)	Slower • 160 – 180 mm FL at 1 year of age (Jones *et al*., 2002)
Size at 50% maturity (FL)	Smaller • 20.1 cm (Stewart *et al*., 2005)	Larger • Victoria: 22.8 cm (Jones *et al*., 2002)
Age at 50% maturity	Younger • 10 months (Stewart *et al*., 2005)	Older • Victoria: 19.3 months (Jones *et al*., 2002)
Peak GSI	Overlap Nov - Jan • Northern New South Wales: Peak in Jun - Sep • Southern New South Wales: Peak between Nov – Dec (Stewart & Hughes, 2007)	Overlap Nov - Jan • Victoria: Peak between Sep – Jan (Jones *et al*., 2002)

Information from the closest geographic population of *H. melanochir* (Victoria) used where possible. Fork Length (FL) size for *H. melanochir* converted from Total Length (TL) reported in fisheries reports using the formula provided in Smith *et al*. (2007): FL=(0.9452 ×TL)+0.1954

Table S3: Summary of the number, size and sex of all whole fish from each location.

Site Number	Location		Sampling date	Latitude & Longitude	Number of Fish	Size (Standard Length, mm)			Sex (n)		
						Mean	*Min*	*Max*	*Male*	*Female*	*Unknown*
1	Forster	NSW	27/02/2020	32.18°S 152.51°E	10	246	228	279	8	2	0
3	Nelson Bay	NSW	15/08/2019	32.72°S 152.15°E	10	254	238	281	4	5	1
8	Eden	NSW	17/05/2018 and 25/07/2019	37.07°S 149.90°E	25	244	183	294	7	4	11
9	Corner Inlet	VIC	26/08/2019	38.78°S 146.33°E	31	228	190	258	7	17	4
10	Adelaide	SA	19/02/2020	34.93°S 138.60°E	20	234	224	251	16	1	0
11	Perth	WA	22/04/2019	31.95°S 115.86°E	24	239	206	289	11	8	5

Site numbers corresponds with the numbers in Figure 1. NSW = New South Wales, VIC = Victoria, SA = South Australia, WA = Western Australia.

Table S4: Summary of the number, size and sex of all fish corresponding with tissue and otolith samples from each location.

Site No.	Location		Latitude & Longitude	n – Tissue & Otolith	Size (Standard Length, mm)			Sex (n)			Gonadosomatic Index (GSI)		
					Mean	*Min*	*Max*	*Male*	*Female*	*Unknown*	*Mean*	*Min*	*Max*
1	Forster	NSW	32.18° S 152.51° E	20	232	199	276	8	11	1	1.55	0	6.64
2	Tea Garden	NSW	32.66° S 152.15° E	20	259	235	294	13	7	0	3.46	0.07	10.21
3	Nelson Bay	NSW	32.72° S 152.15° E	30	244	202	327	11	18	1	1.68	0.33	8.68
4	Sydney	NSW	33.87° S 151.21° E	20	265	216	360	12	8	0	1.29	0.08	3.17
5	Wollongong	NSW	34.43° S 150.8931° E	26	255	205	300	8	17	1	1.53	0.14	7.21
6	Kiama	NSW	34.67° S 150.84° E	25	257	199	321	7	17	1	2.54	0	8.70
7	Ulladulla	NSW	35.36° S 150.46° E	26	245	205	285	12	10	4	3.14	0.35	16.10
8	Eden	NSW	37.07°S 149.90° E	20	266	239	322	4	11	5	0.99	0.12	3.45

Site numbers corresponds with the numbers in Figure 1. NSW; New South Wales, VIC, Victoria; SA, South Australia; WA, Western Australia.

Table S5: Details of all primer sequences and PCR protocols.

Marker	Direction	Primer Name	Sequence (5'–3')	PCR protocol	Reference
COI	Forward	FishF1	TCAACCAACCACAAAGACATTGGCAC	Initial denaturing step at 95 °C for 2 min 35 cycles of amplification (94 °C for 30 sec, 54 °C for 30 sec, 72 °C for 60 sec) Final extension at 72 °C for 10 min	Ward *et al*. (2005)
	Forward	FishF2	TCGACTAATCATAAAGATATCGGCAC		
	Reverse	FishR2	ACTTCAGGGTGACCGAAGAATCAGAA		
TMO	Forward	TMO_f1_5	CCTCCGGCCTTCCTAAAACCTCTC	Initial denaturing step at 95 °C for 2 min 30 cycles of amplification (95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 60 sec) Final extension at 72 °C for 7 min	Streelman and Karl (1997)
	Reverse	TMO_r1_3	CATCGTGCTCCTGGGTGACAAAGT		
RAG2	Forward	RAG2_f1	TTTGGRCARAAGGGCTGGCC	Initial denaturing step at 95 °C for 30 sec "Touchdown" protocol of annealing for 60 sec at 58 °C, 56 °C, 54 °C and 52 °C, repeated for two cycles at each temperature, followed by 72 °C for 90 sec 27 cycles of amplification (30 sec at 95 °C, 60 sec at 50 °C, 90 sec at 72 °C) Final extension at 72 °C for 5 min	Lovejoy and Collette (2001)
	Reverse	RAG2_r4	GTRGARTAGTAGGGCTCCCA		

Table S6: Estimates of the percent pairwise difference in the mitochondrial DNA COI sequence of other *Hyporhamphus* species.

	affinis	***australis***	***dussumieri***	***ihi***	***limbatus***	***meeki***	***melanochir***	***quoyi***	***regularis***	***sajori***	***unifasciatus***	***xanthopterus***
affinis	*											
australis	8.34 (7.0)	*										
dussumieri	9.92 (8.3)	12.51 (10.4)	*									
ihi	8.36 (7.0)	**0.52** **(0.4)**	13.08 (10.9)	*								
limbatus	8.11 (6.8)	12.23 (10.2)	10.14 (8.4)	12.44 (10.4)	*							
meeki	14.92 (12.4)	17.80 (14.8)	15.06 (12.5)	18.27 (15.2)	14.45 (12.0)	*						
melanochir	8.39 (7.0)	**0.70** **(0.6)**	13.00 (10.8)	**0.52** **(0.4)**	12.48 (10.4)	18.27 (15.2)	*					
quoyi	4.23 (3.5)	9.14 (7.6)	9.47 (7.9)	9.09 (7.6)	3.55 (3.0)	14.31 (11.9)	9.33 (7.8)	*				
regularis	11.58 (9.7)	14.86 (12.4)	12.98 (10.8)	14.18 (11.8)	11.36 (9.5)	18.50 (15.4)	14.41 (12.0)	10.92 (9.1)	*			
sajori	17.15 (14.3)	17.49 (14.6)	13.90 (11.6)	18.22 (15.2)	15.94 (13.3)	19.97 (16.6)	18.22 (15.2)	15.81 (13.2)	20.08 (16.7)	*		
unifasciatus	9.60 (8.0)	12.58 (10.5)	9.93 (8.3)	12.95 (10.8)	8.98 (7.5)	2.91 (2.4)	12.86 (10.7)	8.41 (7.0)	12.54 (10.5)	16.54 (13.8)	*	
xanthopterus	6.22 (5.2)	12.94 (10.8)	14.10 (11.8)	12.73 (10.6)	14.09 (11.7)	19.99 (16.7)	12.95 (10.8)	10.61 (8.8)	16.54 (13.8)	21.13 (17.6)	14.42 (12.0)	*

The top value in each cell represents the Tamura and Nei (TrN) percent difference between the sequences (%), and the bottom values in parentheses are estimates of the time since divergence (Ma) based on an estimated COI mutation rate of 1.2% per million years. Names are shortened to species. Bold values indicate differences between *Hyporhamphus australis*, *Hyporhamphus melanochir* and *Hyporhamphus ihi*. All sequences used to construct the table are taken from GenBank and BOLD (see Table S7).

Table S7: List of all Hyporhamphus sequences used in K2P comparison from GenBank and BOLDsystems.

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus sajori	KP112263	ihb201306979	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	Shen, Y. DNA barcoding of common commercial fish species in Central China – Unpublished. Direct Submission
Hyporhamphus sajori	KP112264	ihb201306965	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112265	ihb201306967	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112266	ihb201306969	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112267	ihb201306970	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112268	ihb201306971	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112269	ihb201306972	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112270	ihb201306974	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112271	ihb201306975	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112272	ihb201306976	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112273	ihb201306977	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	KP112274	ihb201306978	China: Hubei, Wuhan, Maidelong supermarket	17-Jul-2013	
Hyporhamphus sajori	JF952762	SDLG1	Japan: Kanto, Yokosuka, Arasaki	09-Dec-2005	Zhang, J. B and Hanner, R. (2011) DNA barcoding is a useful too for the identification of marine fishes from

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus sajori	JF952763	SUJL1	Japan: Kanto, Yokosuka, Arasaki	09-Dec-2005	*Japan. Biochem. Syst. Ecol. 39* (1), 31-42.
Hyporhamphus dussumieri	JX983320	NFX02	India: Gujarat, Bharuch, Estuary	20-Oct-2011	Khedkar GD, Jamdade R, Naik S, David L, Haymer D. (2014). DNA barcodes for the fishes of the Narmada, one of India's longest rivers. *PLoS One.* 9(7):e101460. doi: 10.1371/journal.pone.0101460.
Hyporhamphus dussumieri	EF607401	GD 9086051	China	18-Aug-2006	Zhang J. (2011). Species identification of marine fishes in china with DNA barcoding. *Evid Based Complement Alternat Med*. 2011:978253. doi: 10.1155/2011/978253.
Hyporhamphus dussumieri	EF607400	GD 9086052	China	18-Aug-2006	
Hyporhamphus xanthopterus	FJ237601	NBFGR: 1044B	India: Bay of Bengal	-	Lakra WS, Verma MS, Goswami M, Lal KK, Mohindra V, Punia P, Gopalakrishnan A, Singh KV, Ward RD, Hebert P. (2011). DNA barcoding Indian marine fishes. *Mol Ecol Resour.* 11(1):60-71. doi: 10.1111/j.1755-0998.2010.02894.x.
Hyporhamphus xanthopterus	FJ237602	NBFGR: 1044C	India: Bay of Bengal	-	
Hyporhamphus xanthopterus	EU148544	WL-M583	India	-	
Hyporhamphus xanthopterus	EU148545	WL-M586	India	-	
Hyporhamphus affinis	KU176401	ADC2013 115.3 #5	South Africa: KwaZulu-Natal, Sodwana Bay	09-Feb-2013	Dirk Steinke, Allan D. Connell, and Paul D.N. Hebert. (2016). Linking adults and immatures of South African marine fishes. *Genome*. **59**(11): 959-967. Doi: 10.1139/gen-2015-0212
Hyporhamphus affinis	KU176356	ADC2013 115.3 #4	South Africa: KwaZulu-Natal, Sodwana Bay	09-Feb-2013	
Hyporhamphus affinis	JF493670	ADC08 Smith 115.2 #2	Mozambique: Pomene	01-May-2008	
Hyporhamphus affinis	JF493671	ADC08 Smith 115.3 #1	Mozambique: Pomene	01-May-2008	
Hyporhamphus affinis	JF493672	ADC08 Smith 115.3 #3	Mozambique: Pomene	01-May-2008	
Hyporhamphus affinis	KJ013045	DB 23.3	Philippines: Luzon, Central Luon, Manila Bay, Bulacan	15-Dec-2012	Marucot,M.A.R., Alcantara,S.G. and Yambot,A.V. DNA barcoding of diadromous fish species from Bulacan, Philippines – Unpublished. Direct Submission

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus affinis	HQ654711	Haff1	Philippines: Batangas, Calabarzon, Taal Lake, Talisay	27-Apr-2010	Aquilino SV, Tango JM, Fontanilla IK, Pagulayan RC, Basiao ZU, Ong PS, Quilang JP. (2011). DNA barcoding of the ichthyofauna of Taal Lake, Philippines. *Mol Ecol Resour*. 2011 Jul;11(4):612-9. doi: 10.1111/j.1755-0998.2011.03000.x.
Hyporhamphus affinis	HQ654710	Haff2	Philippines: Batangas, Calabarzon, Taal Lake, Talisay	04-Jun-2010	
Hyporhamphus affinis	HQ654709	Haff3	Philippines: Batangas, Calabarzon, Taal Lake, Talisay	05-Jun-2010	
Hyporhamphus affinis	HQ654708	Haff4	Philippines: Batangas, Calabarzon, Taal Lake, Talisay	19-Jun-2010	
Hyporhamphus affinis	HQ654707	Haff5	Philippines: Batangas, Calabarzon, Taal Lake, Talisay	20-Jun-2010	
Hyporhamphus melanochir	HQ956059	BW-A9468	Australia: Tasmania, Eaglehawk Bay	23-Apr-2010	International Barcode of Life (iBOL). Direct Submission
Hyporhamphus melanochir	HQ956051	BW-A9459	Australia: Tasmania, Eaglehawk Bay	23-Apr-2010	
Hyporhamphus australis	KX781932	SGS233_2016	Australia	-	Mitchell,A., Rothbart,A., Frankham,G., Johnson,R.N. and Neaves,L.E. (2019). Could do better! A high school market survey of fish labelling in Sydney, Australia, using DNA barcodes. *PeerJ* 7, e7138 (2019)

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus ihi	MN123385	NMNZ P.044460	New Zealand: Eastern side of Mill Bay	-	Eme,D., Anderson,M.J., Struthers,C.D., Roberts,C.D. and Liggins,L. (2019). An integrated pathway for building regional phylogenies for ecological studies. *Glob. Ecol. Biogeogr.* 28 (12), 1899-1911
Hyporhamphus regularis	KJ669475	CSIRO:H 4313-09	Australia: Seafood Trade, Sydney Fish Markets, NSW	-	Hardy, C. M. Direct Submission.
Hyporhamphus quoyi	KP194350	UG0054	Australia: Queensland: Lizard Island: I.44707	05-Sep-2008	Steinke,D., Ward,R.D., Gomon,M.F., Hay,A., Reader,S.R., Johnson,J., Last,P., Moore,G., Dewaard,J., Hardie,D., Lucanus,O. and Cossey,J. Coral reef fish of Lizard Island – Unpublished. Direct Submission.
Hyporhamphus quoyi	GU674305	BW-A7141	Indonesia: East Java, Banyuwangi, Kalipuro	27-Feb-2009	International Barcode of Life (iBOL). Direct Submission
Hyporhamphus quoyi	GU674306	BW-A7142	Indonesia: East Java, Banyuwangi, Kalipuro	27-Feb-2009	
Hyporhamphus quoyi	GU674377	BW-A7613	Indonesia: West Nusa Tenggara, East Lombok, Menceh	05-Oct-2009	
Hyporhamphus quoyi	GU674376	BW-A7612	Indonesia: West Nusa Tenggara, East Lombok, Menceh	05-Oct-2009	
Hyporhamphus quoyi	MN200468	DUZM_MF_130B4	Bangladesh: Kuakata	05-Oct-2018	Ahmed,M.S., Datta,S.K., Saha,T., Susmita,U.M. and Haque,A.K. DNA barcoding of freshwater fishes of Bangladesh -Unpublished. Direct Submission
Hyporhamphus quoyi	MN083114	DUZM_MF_130B	Bangladesh: Moheshkhali	22-Oct-2015	
Hyporhamphus quoyi	MK988540	DUZM_MF_130B.3	Bangladesh: Cox's Bazar	22-Jun-2018	
Hyporhamphus quoyi	MK988533	DUZM_MF_130B.2	Bangladesh: Cox's Bazar	22-Jun-2018	

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus quoyi	MH673896	-	Malaysia	17-Mar-2016	Chu C, Loh KH, Ng CC, Ooi AL, Konishi Y, Huang SP, Chong VC. (2019). Using DNA Barcodes to Aid the Identification of Larval Fishes in Tropical Estuarine Waters (Malacca Straits, Malaysia). *Zool Stud.* 58:e30. doi: 10.6620/ZS.2019.58-30
Hyporhamphus quoyi	EU595153	MBCSC: Z711117	China: South China Sea	30-Oct-2007	Zhang, J. and Hanner, R. DNA Barcoding of fishes in the South China Sea – Unpublished. Direct Submission.
Hyporhamphus quoyi	FJ237994	MBCSC:ZC I07338	China: South China Sea	28-Oct-2007	
Hyporhamphus limbatus	MK572266	11640	Bangladesh: Dhaka Division, Khishoreganj District	20-Mar-2016	Rahman MM, Norén M, Mollah AR, Kullander SO. (2019). Building a DNA barcode library for the freshwater fishes of Bangladesh. *Sci Rep*. 9(1):9382. doi: 10.1038/s41598-019-45379-6.
Hyporhamphus limbatus	MK572267	10259	Bangladesh: Chittagong Division, Rangamati	28-Nov-2014	
Hyporhamphus limbatus	MK572268	10206	Bangladesh: Chittagong Division, Rangamati	27-Nov-2014	
Hyporhamphus limbatus	MK572269	11321	Bangladesh: Chittagong Division, Rangamati	28-Nov-2014	
Hyporhamphus limbatus	KJ013046	DB 23.2	Philippines: Luzon, Central Luzon, Manila Bay, Bulacan	15-Dec-2012	Marucot, M.A.R., Alcantara, S.G. and Yambot, A.V. DNA barcoding of diadromous fish species from Bulacan, Philippines – Unpublished. Direct Submission
Hyporhamphus limbatus	EF607402	GD 9081025	China	15-Aug-2006	Zhang J. (2011). Species identification of marine fishes in china with DNA barcoding. Evid Based Complement Alternat Med. 2011:978253. doi: 10.1155/2011/978253
Hyporhamphus limbatus	EU595151	MBCSC:Z711263	China: South China Sea	28-Oct-2007	Zhang, J. and Hanner, R. DNA Barcoding of fishes in the South China Sea – Unpublished. Direct Submission.
Hyporhamphus limbatus	EU595149	MBCSC:Z711265	China: South China Sea	28-Oct-2007	
Hyporhamphus limbatus	EU595148	MBCSC:Z711266	China: South China Sea	28-Oct-2007	
Hyporhamphus limbatus	EU595147	MBCSC:Z711267	China: South China Sea	28-Oct-2007	
Hyporhamphus limbatus	EU595152	MBCSC:Z711262	China: South China Sea	28-Oct-2007	

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus limbatus	EU595150	MBCSC:Z711264	China: South China Sea	28-Oct-2007	
Hyporhamphus limbatus	FJ237993	MBCSC:ZC I07389	China: South China Sea	30-Oct-2007	
Hyporhamphus meeki	MT456231	USNM:FISH:423969	USA: Virginia, Northampton County, Lower Chesapeake Bay, Kiptopeke State Park	13-Aug-2012	Aguilar,R., Ogburn,M.B., Weigt,L.A., Driskell,A.C., Macdonald,K.S. and Hines,A.H. Chesapeake Bay Barcode Initiative (CBBI): Fishes of the greater Chesapeake Bay – Unpublished. Direct Submission.
Hyporhamphus meeki	MT456098	USNM:FISH:423982	USA: Virginia, Northampton County, Lower Chesapeake Bay, Kiptopeke State Park	13-Aug-2012	
Hyporhamphus meeki	MT456001	USNM:FISH:425012	USA: Virginia, Northampton County, Lower Chesapeake Bay, Kiptopeke State Park	13-Aug-2012	
Hyporhamphus meeki	MT455971	USNM:FISH:425018	USA: Virginia, Northampton County, Lower Chesapeake Bay, Kiptopeke State Park	13-Aug-2012	
Hyporhamphus meeki	MT455355	USNM:FISH:425014	USA: Virginia, Northampton County, Lower Chesapeake Bay, Kiptopeke State Park	13-Aug-2012	
Hyporhamphus unifasciatus	GU225337	Victor Garcia	Mexico: Quintana Roo, Holbox, Playa cerca I. Pasion	02-Feb-2006	Valdez-Moreno,M., Vasquez-Yeomans,L., Elias-Gutierrez,M., Ivanova,N.V. and Hebert,P.D.N. (2010). Using DNA barcodes to connect adults and early life stages of marine fishes from the Yucatan Peninsula, Mexico: potential in fisheries management. *Marine and Freshwater Research,* 61, 665-671
Hyporhamphus unifasciatus [A]	JQ842525	SMSA7442	USA: Florida, St. Lucie County, sea grass bed off A1A	-	Weigt LA, Baldwin CC, Driskell A, Smith DG, Ormos A, Reyier EA. (2012). Using DNA barcoding to assess Caribbean reef fish biodiversity: expanding taxonomic and geographic coverage. PLoS One. 7(7):e41059. doi: 10.1371/journal.pone.0041059
Hyporhamphus unifasciatus [A]	JQ842524	SMSA7247	USA: Florida, St. Lucie County, IRL zone J1, grid 512	-	

Species	GenBank Accession Number	Voucher Number	Location	Date Collected	Publication
Hyporhamphus unifasciatus BOLD ID: MFIV326-10.COI-5P	-	ECOCH7100	Mexico: Campeche, Carme, Candelaria river	24-Mar-2010	International Barcode of Life (iBOL). Direct Submission
Hyporhamphus unifasciatus BOLD ID: MFIV327-10.COI-5P	-	ECOCH7100	Mexico: Campeche, Carme, Candelaria river	24-Mar-2010	
Hyporhamphus unifasciatus BOLD ID: MXV795-15.COI-5P	-	ECO-CH P 7652	Mexico: Campeche, Sabancuy, An Antonio birdge. Mundo	24-Mar-2015	Martha Valdez-Moreno. Unpublished. Direct Submission.
Hyporhamphus unifasciatus BOLD ID: MXV796-15.COI-5P	-	ECO-CH P 7652	Mexico: Campeche, Sabancuy, An Antonio birdge. Mundo	24-Mar-2015	
Hyporhamphus unifasciatus BOLD ID: MXV797-15.COI-5P	-	ECO-CH P 7652	Mexico: Campeche, Sabancuy, An Antonio birdge. Mundo	24-Mar-2015	

[A]Two *H. unifasciatus* sequences likely misidentified and were excluded from analysis.

Table S8: Results of PERMANOVA analysis of length-standardised otolith wavelet coefficients comparing photoshopped and non-photoshopped *Hyporhamphus australis* otoliths at each sampling location.

Location		d.f.	Sum of Squares	Mean square	*F* model	R^2	Pr(>F)
Forster	Photoshop	1	0.3539	0.35395	0.84596	0.09563	0.593
	Residuals	8	3.3472	0.37975		0.90437	
	Total	9	3.7011			1.00000	
Tea Gardens	Photoshop	1	0.2557	0.25574	0.67344	0.04039	0.713
	Residuals	16	6.0760	0.011292		0.95961	
	Total	17	6.3318			1.00000	
Nelson Bay	Photoshop	1	0.4604	0.46039	0.92902	0.04583	0.409
	Residuals	28	13.8758	0.49556		0.95417	
	Total	29	14.3362			1.00000	
Sydney	Photoshop	1	0.2903	0.29031	0.68665	0.04675	0.711
	Residuals	14	5.9190	0.42279		0.95325	
	Total	15	6.2093			1.00000	
Wollongong	Photoshop	1	0.4934	0.49340	1.2941	0.04741	0.236
	Residuals	26	9.9133	0.38128		0.95259	
	Total	27	10.4067			1.00000	
Kiama	Photoshop	1	0.6848	0.68484	1.7141	0.06416	0.093
	Residuals	25	9.9887	0.39955		0.93584	
	Total	26	10.6735			1.00000	
Ulladulla	Photoshop	1	0.5099	0.50992	1.3256	0.0452	0.228
	Residuals	28	10.7707	0.38467		0.9548	
	Total	29	11.2806			1.0000	

Locations with no photoshopped otoliths were excluded from analysis.

Table S9: Results of the analysis of molecular variance (AMOVA) based on mitochondrial DNA COI data for *Hyporhamphus australis* and *Hyporhamphus melanochir*.

	Source of Variation	d.f.	Sum of Squares	Variance of Components	Percentage of Variation	F_{ST}	*P*-value
[A] All Samples, Species	Among Species	1	259.77	2.02	95.2	0.95	**<0.001**
	Within Species	289	29.45	0.1	4.8		
	Total	290	289.22	2.12			
[B] All Samples, Locations	Among Locations	10	312.55	1.13	69.01	0.69	**<0.001**
	Within Locations	292	147.6	0.51	30.99		
	Total	302	460.15	1.63			
[C] *H. australis*, Locations	Among Locations	7	0.73	0.00054	0.59	0.005	0.153
	Within Locations	187	17.17	0.09	99.41		
	Total	194	17.91	0.09			
[D] *H. melanochir*, Locations	Among Locations	4	0.83	0.005	4.02	0.04	0.057
	Within Locations	91	10.71	0.12	95.98		
	Total	95	11.54	0.12			

Groups used for analysis were A) Species *v*. Species, B) Location *v*. Location, C) H. australis within-species Location *v*. Location, and D) *H. melanochir* within-species Location *v*. Location. Significant differences indicated in bold.

Table 10: Matrix of population pairwise FST values (associated *P* values in parentheses) based on mitochondrial DNA COI sequence data for *Hyporhamphus australis* and *Hyporhamphus melanochir* sampled at locations throughout their mainland coastal distribution in Australia.

Location	Forster	Tea Gardens	Nelson Bay	Sydney	Wollongong	Kiama	Ulladulla	Eden	Victoria	South Australia	Western Australia
Forster	*										
Tea Gardens	0.029 (0.072)	*									
Nelson Bay	0.006 (0.236)	0.005 (0.344)	*								
Sydney	-0.007 (0.608)	0.023 (0.488)	-0.016 (0.794)	*							
Wollongong	0.014 (0.122)	0.022 (0.150)	0.013 (0.110)	0.001 (0.497)	*						
Kiama	0.006 (0.284)	-0.020 (0.684)	-0.015 (0.645)	-0.012 (0.999)	0.008 (0.283)	*					
Ulladulla	0.006 (0.341)	-0.022 (0.595)	-0.021 (0.770)	-0.011 (0.999)	0.009 (0.367)	-0.033 (0.999)	*				
Eden	**0.460** (<0.001)	**0.340** (<0.001)	**0.470** (<0.001)	**0.440** (<0.001)	**0.450** (<0.001)	**0.400** (<0.001)	**0.410** (<0.001)	*			
Victoria	**0.867** (<0.001)	**0.805** (<0.001)	**0.872** (<0.001)	**0.890** (<0.001)	**0.867** (<0.001)	**0.845** (<0.001)	**0.861** (<0.001)	**0.220** (<0.001)	*		
South Australia	**0.867** (<0.001)	**0.792** (<0.001)	**0.874** (<0.001)	**0.899** (<0.001)	**0.866** (<0.001)	**0.842** (<0.001)	**0.862** (<0.001)	**0.190** (0.003)	-0.029 (0.736)	*	
Western Australia	**0.948** (<0.001)	**0.907** (<0.001)	**0.946** (<0.001)	**1** (<0.001)	**0.953** (<0.001)	**0.938** (<0.001)	**0.957** (<0.001)	**0.290** (<0.001)	0.057 (0.125)	0.058 (0.031)	*

Colours indicate groups of locations that do not significantly differ from one another, but which differ from other colour groups. Numbers in bold are significant, $P \leq 0.003$ (corrected for multiple pairwise tests as per Narum, 2006).

Table S11: Molecular diversity indices for *Hyporhamphus australis* and *Hyporhamphus melanochir*, pooled as species and as separate locations, based on A) mitochondrial DNA (*CO1*) sequence data, and B) nuclear DNA (TMO-4C4).

Collection Locality	N	Hn	Hu	Haplotype Diversity (h +/- SD)	Nucleotide Diversity (phi +/- SD)	Fu's Fs
All *H. australis*	195	13	13	0.1575 +/- 0.0358	0.000302 +/- 0.000433	**-22.51**
Forster	30	6	4	0.3103 +/- 0.1092	0.000649 +/- 0.000693	**-4.81**
Tea Gardens	18	2	0	0.1111 +/- 0.0964	0.000182 +/- 0.000346	-0.79
Nelson Bay	39	3	1	0.1484 +/- 0.0753	0.000248 +/- 0.000397	**-1.99**
Sydney	19	1	0	0.0000 +/- 0.0000	0.0000 +/- 0.0000	NA
Wollongong	26	5	3	0.2892 +/- 0.1147	0.000621 +/- 0.000679	**-3.48**
Kiama	24	2	1	0.0833 +/- 0.0749	0.000137 +/- 0.000293	-1.03
Ulladulla	24	1	0	0.0000 +/- 0.0000	0.0000 +/- 0.0000	NA
Eden	15	3	1	0.2571 +/- 0.1416	0.000438 +/- 0.000569	-1.55
All *H. melanochir*	96	6	6	0.2316 +/- 0.0563	0.000398 +/- 0.000508	**-5.3**
NSW	4	2	0	0.5000 +/- 0.2652	0.000821 +/- 0.001018	0.17
Eden	23	4	1	0.4387 +/-0.1140	0.000779 +/- 0.000783	-1.56*
Victoria	28	3	0	0.2037 +/- 0.0978	0.000343 +/- 0.000480	-1.59
South Australia	17	3	2	0.2279 +/- 0.1295	0.000386 +/- 0.000525	-1.68
Western Australia	24	1	0	0.0000 +/- 0.0000	0.0000 +/- 0.0000	NA

Collection Locality	N	Hn	Hu	Haplotype Diversity (h +/- SD)	Nucleotide Diversity (phi +/- SD)	Fu's Fs
All *H. australis*	270	8	0	0.5220 +/- 0.0177	0.001248 +/- 0.001132	-3.42
Forster	50	5	0	0.5224 +/- 0.0528	0.001254 +/- 0.001152	-1.66
Tea Gardens	24	3	0	0.4891 +/- 0.0843	0.001216 +/- 0.001157	0.08
Nelson Bay	54	4	0	0.5346 +/- 0.0337	0.001258 +/- 0.001154	-0.49
Sydney	32	2	0	0.4657 +/- 0.0563	0.001035 +/- 0.001034	1.54
Wollongong	34	5	0	0.5775 +/- 0.0578	0.001438 +/- 0.001272	-1.65
Kiama	28	3	1	0.5529 +/- 0.0406	0.001305 +/- 0.001203	0.34
Ulladulla	18	2	0	0.5033 +/- 0.0639	0.001118 +/- 0.001113	1.33
Eden	30	4	0	0.5609 +/- 0.0581	0.001400 +/- 0.001255	-0.69
All *H. melanochir*	152	14	6	0.5505 +/- 0.0362	0.001490 +/- 0.001272	**-11.68**
NSW	8	2	0	0.2500 +/- 0.1802	0.000556 +/- 0.000788	-0.18
Eden	38	7	3	0.6216 +/- 0.0646	0.001751 +/- 0.001445	**-3.24**
Victoria	48	6	0	0.5709 +/- 0.0552	0.001460 +/- 0.001273	-2.39
South Australia	22	5	0	0.6407 +/- 0.0702	0.001712 +/- 0.001452	-1.67
Western Australia	36	6	3	0.4270 +/- 0.0964	0.001245 +/- 0.001158	**-3.28**

Significant F_s values are in bold ($P < 0.02$)

Table 12: Results of the analysis of molecular variance (AMOVA) based on nuclear DNA TMO-4C4 data for *Hyporhamphus australis* and *Hyporhamphus melanochir*.

	Source of Variation	d.f.	Sum of Squares	Variance of Components	Percentage of Variation	F_{ST}	*P*-value
[A] All Samples, Species	Among Species	1	0.73	0.002	0.72	0.007	0.09
	Within Species	420	126.94	0.3	99.28		
	Total	421	127.66	0.3			
[B] All Samples, Locations	Among Locations	10	2.93	-0.0002	0	0	0.47
	Within Locations	431	130.09	0.3	100		
	Total	441	133.02	0.3			
[C] *H. australis*, Locations	Among Locations	7	1.22	-0.003	0	0	0.8
	Within Locations	262	74.77	0.29	100		
	Total	269	75.99	0.29			
[D] *H. melanochir*, Locations	Among Locations	4	1.87	0.005	1.36	0.013	0.17
	Within Locations	147	49.08	0.33	98.64		
	Total	151	50.95	0.34			

Groups used for analysis were A) Species *v*. Species, B) Location *v*. Location, C) H. australis within-species Location *v*. Location, and D) *H. melanochir* within-species Location *v*. Location.

Table S13: Loading matrices for principal component values with meristic characters

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6
DOR	0.66760	-0.31892	-0.16886	0.03873	-0.64944	0.02851
ANA	0.43498	-0.62306	-0.38787	0.37741	0.36002	0.00991
P1	0.13571	0.69641	-0.69442	0.10937	-0.02155	-0.04424
VERT	0.64941	-0.11073	-0.20074	-0.69673	0.19710	0.03783
RGR1	-0.80302	-0.37916	-0.28935	-0.20236	-0.10665	-0.27450
RGR2	-0.87444	-0.17939	-0.31298	-0.09733	-0.07577	0.30001

Table S14: Loading matrices for principal component values with morphometric characters

	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10	Dim.11	Dim.12	Dim.13
ABASE	0.08549	-0.52609	0.60169	-0.19927	0.11620	-0.33718	0.20082	0.06659	0.03447	0.21685	0.25579	-0.10498	-0.13251
BD.P1	0.67182	0.56876	-0.10308	-0.20727	-0.06395	0.02287	0.01578	-0.05741	-0.05288	-0.14260	-0.06332	-0.12824	-0.34620
BD.P2	0.57438	0.22702	0.08703	-0.53125	0.14149	0.07354	-0.23192	-0.42643	0.02119	0.02923	0.16681	-0.04550	0.19103
DBASE	0.27076	-0.13752	0.66703	0.12559	0.39486	0.03418	-0.29162	0.11092	-0.29822	-0.28391	-0.11519	0.09919	-0.00107
HDL	0.60355	-0.34713	-0.30571	0.04022	-0.33012	-0.13177	0.06655	0.17489	-0.04791	-0.39360	0.30124	0.08804	0.05551
LJL	-0.17845	-0.29299	-0.10166	-0.75761	0.02465	0.32099	0.26687	0.26204	-0.18655	-0.00848	-0.11090	0.08490	0.00978
ORB	0.73932	-0.01762	-0.19386	0.09812	-0.05111	-0.33475	0.15557	0.08839	-0.35543	0.20667	-0.21436	-0.14712	0.15021
P1.P2	-0.31881	0.23736	-0.37955	-0.11555	0.68798	-0.26038	0.04994	0.21087	0.13747	-0.20738	0.04649	-0.17141	0.05558
P1L	0.43904	0.25343	0.34693	0.26114	0.16371	0.29806	0.61755	-0.09111	0.16704	-0.11227	-0.01385	-0.00958	0.09142
P2.C	-0.24673	0.37349	0.48846	-0.28305	-0.47309	-0.29669	-0.02441	0.14821	0.23328	-0.20175	-0.17877	-0.08458	0.11085
PREORB	0.07592	-0.73877	-0.17209	-0.09763	0.10075	-0.26745	0.11259	-0.41008	0.17751	-0.17566	-0.26914	0.09178	-0.05966
UJL	0.42692	-0.64619	-0.01365	0.07578	-0.04429	0.37482	-0.24357	0.18069	0.25711	0.00095	-0.08880	-0.29240	0.01835
UJW	0.75221	0.17081	-0.04919	-0.06762	0.17052	-0.10705	-0.11825	0.29437	0.35145	0.22189	-0.06905	0.28149	-0.00891

Table S15: Eigenvalues for principal component values with meristic characters

	eigenvalue	variance.percentage	cumulative.variance.percentage
Dim.1	2.48453	41.40885	41.40885
Dim.2	1.16309	19.38487	60.79373
Dim.3	0.88315	14.71921	75.51293
Dim.4	0.69176	11.52933	87.04226
Dim.5	0.60781	10.13020	97.17246
Dim.6	0.16965	2.82754	100

Table S16: Eigenvalues for principal component values with morphometric characters

	eigenvalue	variance.percentage	cumulative.variance.percentage
Dim.1	2.91371	22.41313	22.41313
Dim.2	2.12992	16.38403	38.79716
Dim.3	1.50176	11.55207	50.34922
Dim.4	1.14740	8.82614	59.17536
Dim.5	1.07085	8.23729	67.41266
Dim.6	0.82139	6.31836	73.73101
Dim.7	0.74971	5.76699	79.49801
Dim.8	0.67115	5.16268	84.66069
Dim.9	0.57914	4.45495	89.11564
Dim.10	0.52293	4.02252	93.13815
Dim.11	0.37893	2.91482	96.05298
Dim.12	0.28576	2.19814	98.25112
Dim.13	0.22736	1.74888	100

Table 17: Results of ANCOVA comparing size-adjusted means of each morphometric character between *Hyporhamphus australis* and *Hyporhamphus melanochir*.

Variable	Effect	DFn	DFd	F	p	P < 0.003	ges
UJL	SL	1	108	90.619	5.78E-16	LJL excluded	0.456
	Species	1	108	33.673	6.60E-08	LJL excluded	**0.238**
UJW	SL	1	108	127.85	4.97E-20	LJL excluded	0.542
	Species	1	108	0.037	8.48E-01		0.000343
HDL	SL	1	107	520.119	6.92E-43	LJL excluded	0.829
	Species	1	107	46.911	4.88E-10	LJL excluded	**0.305**
P1L	SL	1	108	115.931	8.36E-19	LJL excluded	0.518
	Species	1	108	9.99	2.00E-03	LJL excluded	**0.085**
DBASE	SL	1	108	376.027	5.75E-37	LJL excluded	0.777
	Species	1	108	0.608	4.37E-01		0.006
ABASE	SL	1	106	263.48	1.66E-30	LJL excluded	0.713
	Species	1	106	0.042	8.37E-01		0.0004
P1.P2	SL	1	107	966.912	2.11E-55	LJL excluded	0.9
	Species	1	107	1.642	2.03E-01		0.015
P2.C	SL	1	108	1012.159	1.12E-56	LJL excluded	0.904
	Species	1	108	3.999	4.80E-02		0.036
BD.P1	SL	1	108	308.289	2.02E-33	LJL excluded	0.741
	Species	1	108	6.29	1.40E-02		0.055
BD.P2	SL	1	108	253.127	4.48E-30	LJL excluded	7.01E-01
	Species	1	108	0.008	9.30E-01		7.26E-05
ORB	SL	1	106	105.2	1.47E-17	LJL excluded	0.498
	Species	1	106	0.504	4.79E-01		0.005
PREORB	SL	1	106	167.876	1.39E-23	LJL excluded	0.616
	Species	1	106	42.855	2.16E-09	LJL excluded	**0.288**
POSTORB	SL	1	107	110.69	3.36E-18	LJL excluded	0.508
	Species	1	107	3.074	8.20E-02		0.028

Significant variables ($P < 0.003$, Bonferroni adjustment for multiple testing) highlighted in grey; ges, effect size (generalised eta squared). LJL was excluded from some analyses as it violated statistical assumptions of homogeneity of variance and homogeneity of residuals.

Table S18: Results of PERMANOVA analysis of length-standardised otolith wavelet coefficients comparing *Hyporhamphus australis* and *Hyporhamphus melanochir* otoliths.

	d.f.	Sum of Squares	Mean square	*F* model	R^2	Pr(>F)
Species	1	11.379	11.3790	40.535	0.13488	**0.001**
Residuals	260	72.986	0.2807		0.86512	
Total	261	84.365			1.00000	00

Table S19: Results of Random Forest Analysis, reclassifying *Hyporhamphus australis* and *Hyporhamphus melanochir* samples to their species groups based on their otolith wavelet coefficients

Random Forest	**Ntrees**	**Variables tried at each split**	**OOB estimate of error rate**
	500	7	12.45%
Confusion Matrix	***australis*** (actual)	***melanochir*** (actual)	**Class error**
H. australis (predicted)	155	9	0.05
H. melanochir (predicted)	20	49	0.29

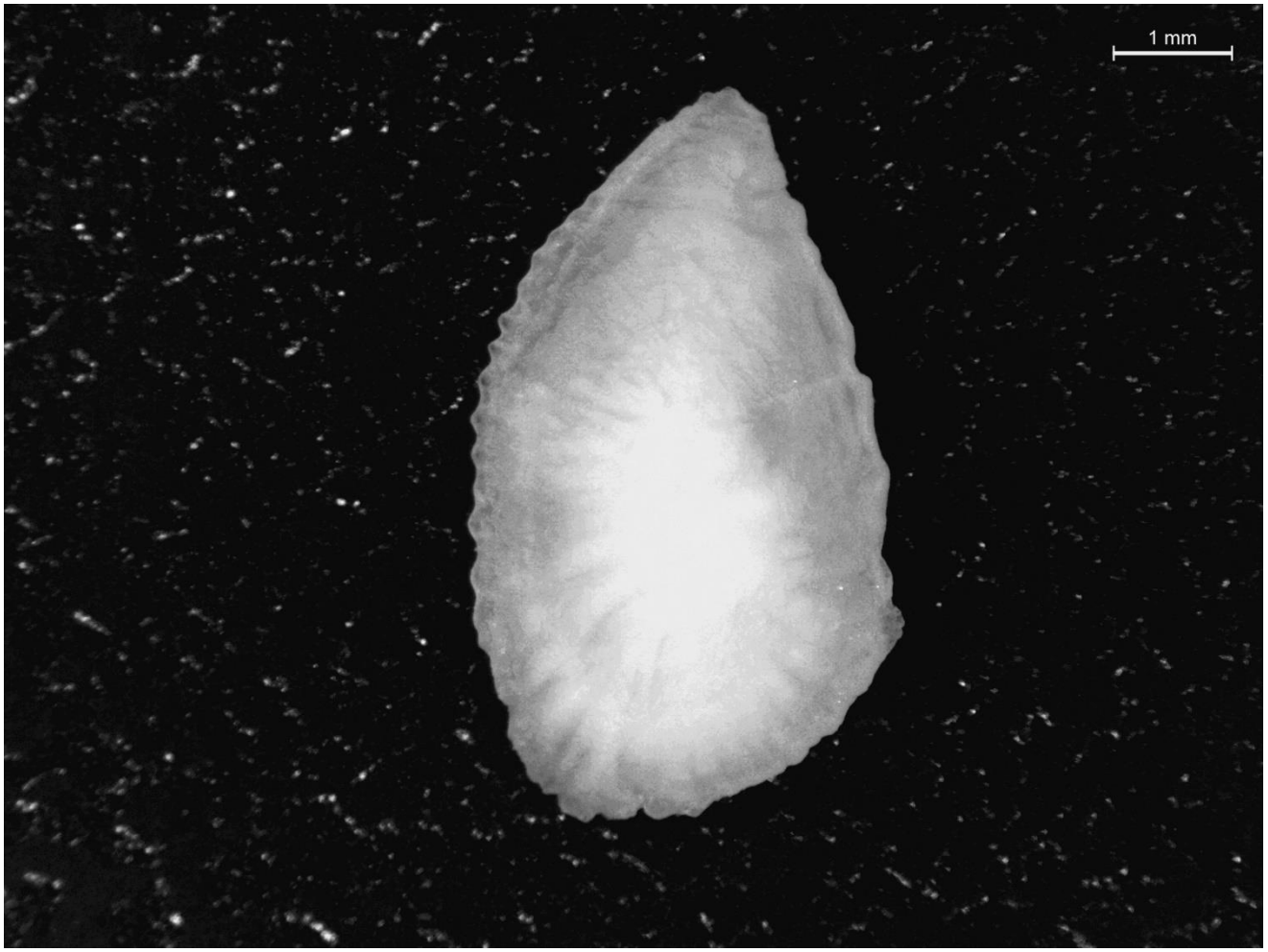


Figure S1*:* Image of the left-side sagittal otolith of a *Hyporhamphus australis* specimen captured using the Leica IC80 HD camera and M125 dissecting microscope. Otolith is positioned distal side up in the centre of the frame with the rostrum horizontally aligned, and contrast was slightly boosted to ensure clear distinction between the otolith outline and the background.

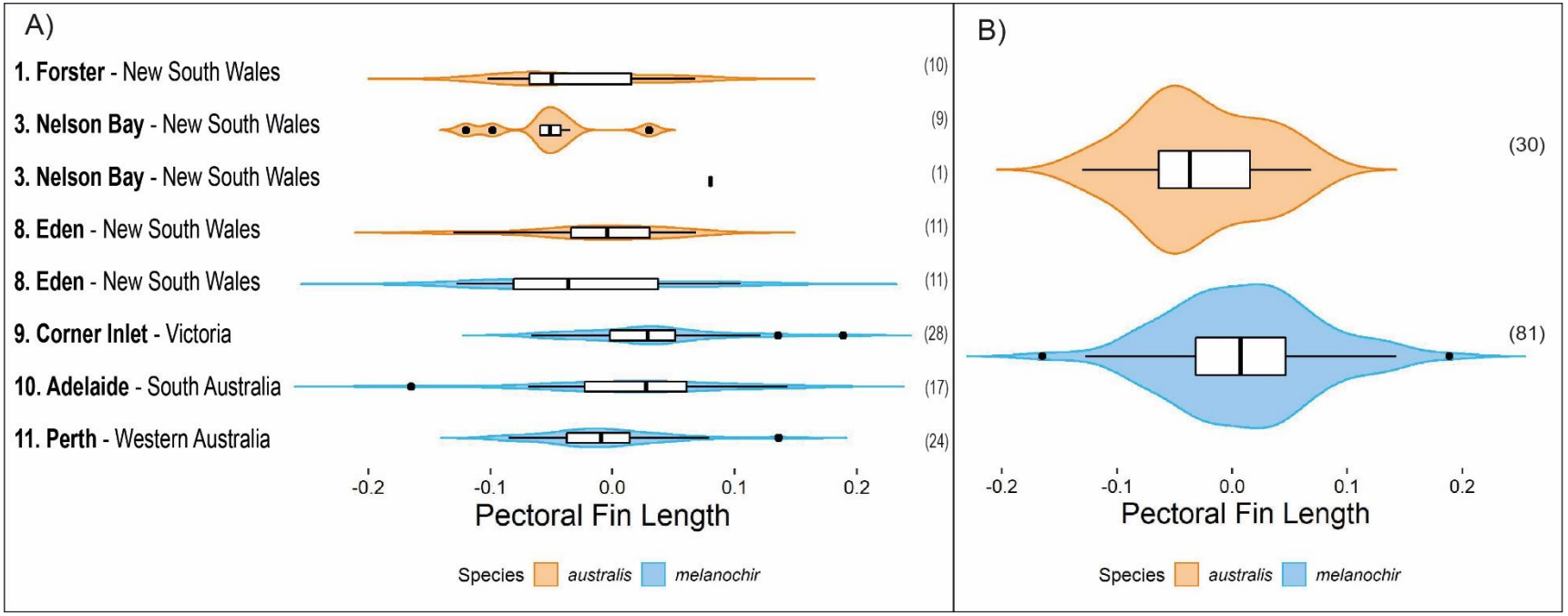


Figure S2: Violin plots showing the difference between the pectoral fin length of *Hyporhamphus australis* and *Hyporhamphus melanochir*, grouped by location (A) and pooled in species groups (B), standardised to account for differences in standard length. The x-axis displays the residuals of log-measurements plotted on log-length, not raw measurements.

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