











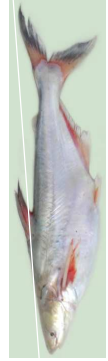



Appendix:








Appendix A: List of specimen details of 27 freshwater fish species used in this study.

No.	Voucher details		Image	Collection data			
	Species	Sample ID		Institution storing	Collection sites	Latitude	Longitude
1	<i>Barbonymus schwanenfeldtii</i>	SLM-BS(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
		SLM-BS(PH)-02				3.5101406	102.7738548
		SLM-BS(PH)-03				3.5101406	102.7738548
		SLM-BS(PH)-04				3.5101406	102.7738548
		SLM-BS(PH)-05				3.5101406	102.7738548
2	<i>Amblyrhynchichthys truncatus</i>	SLM-AT(PH)-04		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
		SLM-AT(PH)-05				3.5101406	102.7738548
		SLM-AT(PH)-06				3.5101406	102.7738548
		SLM-AT(PH)-07				3.5101406	102.7738548
		SLM-AT(PH)-08				3.5101406	102.7738548
3	<i>Hypsibarbus waemorei</i>	SLM-HWE(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
		SLM-HWE(PH)-02				3.5101406	102.7738548
		SLM-HWE(PH)-03				3.5101406	102.7738548
		SLM-HWE(PH)-06				3.5101406	102.7738548
		SLM-HWE(PH)-07				3.5101406	102.7738548
4	<i>Pseudolais micronemus</i>	SLM-PM(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-PM(PH)-02				3.7736179	102.3664341
		SLM-PM(PH)-03				3.7736179	102.3664341
		SLM-PM(PH)-04				3.7736179	102.3664341
		SLM-PM(PH)-05				3.7736179	102.3664341
5	<i>Osteochilus vittatus</i>	SLM-OH(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
		SLM-OH(PH)-02				3.5101406	102.7738548
		SLM-OH(PH)-03				3.5101406	102.7738548
		SLM-OH(PH)-04				3.5101406	102.7738548
		SLM-OH(PH)-05				3.5101406	102.7738548
6	<i>Barbichthys laevis</i>	SLM-BL(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
		SLM-BL(PH)-02				3.5101406	102.7738548
		SLM-BL(PH)-04				3.5101406	102.7738548
		SLM-BL(PH)-05				3.5101406	102.7738548
		SLM-BL(PH)-05				3.5101406	102.7738548
7	<i>Bagarius yarrelli</i>	SLM-BY(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-BY(PH)-02				3.7736179	102.3664341
		SLM-BY(PH)-04				3.7736179	102.3664341
		SLM-BY(PH)-05				3.7736179	102.3664341
		SLM-BY(PH)-05				3.7736179	102.3664341


Appendix A continued'

No.	Voucher details		Image	Collection data			
	Species	Sample ID		Institution storing	Collection sites	Latitude	Longitude
8	<i>Puntiplites proctozystron</i>	SLM-PP(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
		SLM-PP(PH)-02				3.5101406	102.7738548
		SLM-PP(PH)-03				3.5101406	102.7738548
		SLM-PP(PH)-04				3.5101406	102.7738548
9	<i>Rasbora sumatrana</i>	SLM-RS(PH)-02		Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia	Lubuk Paku, Kuala Mai (Pahang)	3.5101406	102.7738548
		SLM-RS(PH)-04				3.7736179	102.3664341
		SLM-RS(PH)-05				3.7736179	102.3664341
10	<i>Luciosoma setigerum</i>	SLM-LS(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-LS(PH)-02				3.7736179	102.3664341
11	<i>Hampala macrolepidota</i>	SLM-HM(PH)-10		(1) Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia (2) University of Malaya	Lubuk Paku (Pahang) Temenggor (Perak)	3.5101406	102.7738548
		SLM-HM(PR)-02				5.51	101.36
		SLM-HM(PR)-03				5.51	101.36
12	<i>Probarbus jullieni</i>	SLM-PJ(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia	Lubuk Paku (Pahang)	3.5101406	102.7738548
13	<i>Pangasius nasutus</i>	SLM-PN(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-PN(PH)-02				3.7736179	102.3664341
		SLM-PN(PH)-03				3.7736179	102.3664341
		SLM-PN(PH)-04				3.7736179	102.3664341
14	<i>Thynnichthys thynnoides</i>	SLM-TT(PH)-01 SLM-TT(PH)-02		Department of Fisheries, Freshwater Fisheries Research Division, Jejebu, Malaysia	Kuala Mai (Pahang)	3.7736179 3.7736179	102.3664341 102.3664341

Appendix A continued'

No.	Voucher details		Image	Institution storing	Collection data		
	Species	Sample ID			Collection sites	Latitude	Longitude
15	<i>Hemibagrus wyckii</i>	SLM-HW(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-HW(PH)-02				3.7736179	102.3664341
16	<i>Cirrhinus Caudimaculatus</i>	SLM-CC(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-CC(PH)-02				3.7736179	102.3664341
17	<i>Cylocheilichthys apogon</i>	SLM-CA(PH)-02		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-CA(PH)-03				3.7736179	102.3664341
		SLM-CA(PH)-05				3.7736179	102.3664341
18	<i>Anabas testudineus</i>	SLM-ATE(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		#					
19	<i>Belodontichthys dinema</i>	SLM-BD(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-BD(PH)-02				3.7736179	102.3664341
20	<i>Puntipolites bulu</i>	SLM-PB(PH)-01		Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia	Kuala Mai (Pahang)	3.7736179	102.3664341
		SLM-PB(PH)-02				3.7736179	102.3664341
		SLM-PB(PH)-03				3.7736179	102.3664341
		SLM-PB(PH)-04				3.7736179	102.3664341
		SLM-PB(PH)-05				3.7736179	102.3664341
21	<i>Channa striata</i>	SLM-CS(JH)-08		(1) Department of Fisheries, Freshwater Fisheries Research Division, Jelebu, Malaysia (2) University of Malaya	Batu Pahat dam (Johor)	1.8586	102.93777
		SLM-CS(JH)-11				1.8586	102.93777
		SLM-CS(JH)-15				1.8586	102.93777
		SLM-CS(JH)-25				1.8586	102.93777
		SLM-CS(JH)-30				1.8586	102.93777

Appendix A continued'

No.	Voucher details		Collection data				
	Species	Sample ID	Image	Institution storing	Collection sites	Latitude	Longitude
		SLM-CS(PP)-01			Permatang Kuang,	5.40665	100.36783
		SLM-CS(PP)-02			Paddy Fields	5.40665	100.36783
		SLM-CS(PP)-03			(Pulau Pinang)	5.40665	100.36783
		SLM-CS(PP)-04				5.40665	100.36783
		SLM-CS(PP)-06				5.40665	100.36783
		SLM-CS(TG)-05			Terengganu River	5.3795	103.08884
		SLM-CS(TG)-06			(Terengganu)	5.3795	103.08884
		SLM-CS(TG)-08				5.3795	103.08884
		SLM-CS(TG)-16				5.3795	103.08884
		SLM-CS(PH)-06			Pahang River	3.69172	102.89516
		SLM-CS(PH)-07			(Pahang)	3.69172	102.89516
		SLM-CS(PH)-09				3.69172	102.89516
		SLM-CS(PH)-10				3.69172	102.89516
		SLM-CS(PH)-12				3.69172	102.89516
		SLM-CS(KD)-01			Kubur Panjang,	6.11427	100.54928
		SLM-CS(KD)-02			Paddy Fields	6.11427	100.54928
		SLM-CS(KD)-03			(Kedah)	6.11427	100.54928
		SLM-CS(KD)-04				6.11427	100.54928
		SLM-CS(NS)-06			Sikamat,	2.75925	101.94901
		SLM-CS(NS)-11			Paddy Fields	2.75925	101.94901
		SLM-CS(NS)-13			(Negeri Sembilan)	2.75925	101.94901
		SLM-CS(NS)-14				2.75925	101.94901
		SLM-CS(NS)-15				2.75925	101.94901
		SLM-CS(SL)-16			Tanjung Karang,	3.44285	101.1834
		SLM-CS(SL)-18			small stream	3.44285	101.1834
		SLM-CS(SL)-20			(Selangor)	3.44285	101.1834
		SLM-CS(SL)-26				3.44285	101.1834
		SLM-CS(SL)-27				3.44285	101.1834
		@ CS(SRW)-1,2			Miri River	4.6134368	114.3129719
		CS(SRW)-3,4			(Sarawak)	4.6134368	114.3129719
		CS(SRW)-5,6				4.6134368	114.3129719
		CS(SRW)-7,8				4.6134368	114.3129719
		CS(SRW)-9,10				4.6134368	114.3129719
22	<i>Rasbora paviana</i>	SLM-RP(PR)-01		University of Malaya	Temenggor	5.51	101.36
		SLM-RP(SL)-02			(Perak)	3.230603	101.634806
					Kepong		
					(Selangor)		

Appendix A continued'






No.	Voucher details		Image	Institution storing	Collection data		
	Species	Sample ID			Collection sites	Latitude	Longitude
23	<i>Hemibagrus nemurus</i>	SLM-HN(PR)-01		University of Malaya	Temenggong (Perak)	5.51	101.36
		SLM-HN(PR)-02				5.51	101.36
		SLM-HN(PR)-03				5.51	101.36
24	<i>Devario regina</i>	SLM-DR(PR)-01		University of Malaya	Temenggong (Perak)	5.51	101.36
		SLM-DR(PR)-02				5.51	101.36
		SLM-DR(PR)-03				5.51	101.36
25	<i>Channa lucius</i>	SLM-CL(PR)-01		University of Malaya	Temenggong (Perak)	5.51	101.36
26	<i>Puntius banksi</i>	SLM-PBA(SL)-01		University of Malaya	Kepong (Selangor)	3.230603	101.634806
		SLM-PBA(SL)-02				3.230603	101.634806
		SLM-PBA(SL)-03				3.230603	101.634806
27	<i>Poropuntius smedleyi</i>	SLM-PS(SL)-02		University of Malaya	Kepong (Selangor)	3.230603	101.634806
		SLM-PS(SL)-03				3.230603	101.634806
		SLM-PS(SL)-04				3.230603	101.634806
		SLM-PS(SL)-10				3.230603	101.634806
		SLM-PS(SL)-11				3.230603	101.634806

Image data obtained from FishBase (<http://www.fishbase.org>)

@ Sample sequence not submitted to BOLD

* Refer BOLD (<http://www.barcodinglife.org/>) project code: FWFMM for more details on specimens.

Appendix B: A list of optimum PCR mixture used in this study.

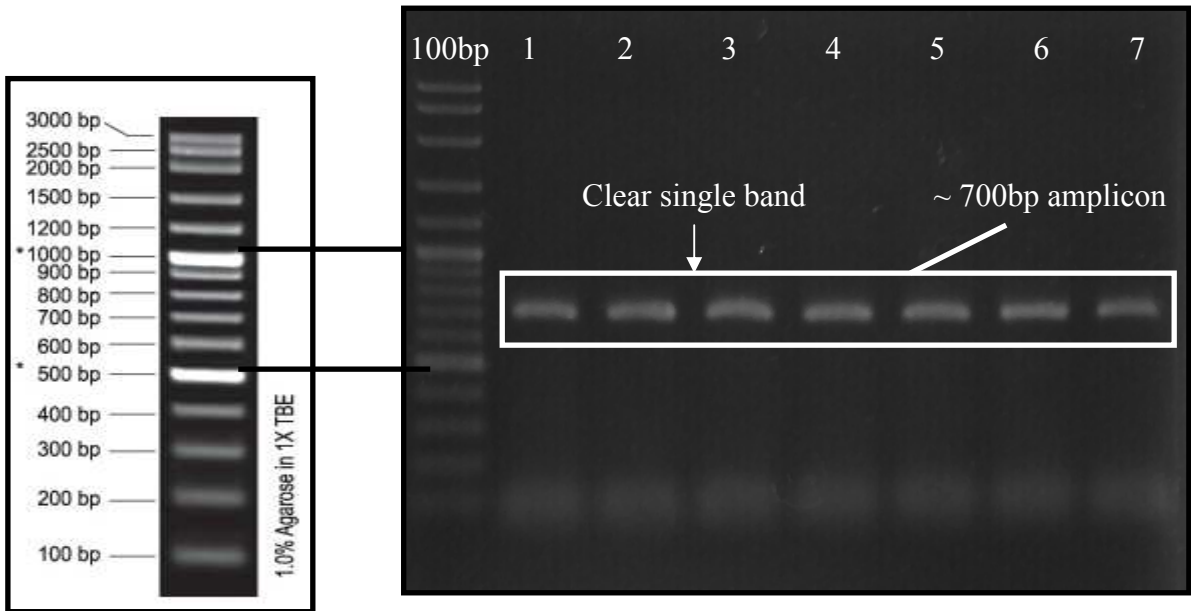
Component	Stock concentration	Working concentration	Working volume (μl)
5X Buffer (Promega)	5X	1X	6.0
MgCl₂ (Promega)	25mM	2.5mM	2.0
dNTPs	10mM	0.25mM	0.5 (each)
F. Primer (Fish F1)	10μM	0.25μM	0.5
R. Primer (Fish R1)	10μM	0.25μM	0.5
Taq Polymerase (Promega)	5U/μl	3U	0.6
Autoclaved dH₂O			6.8
DNA Template	50ng/μl	80ng	1.6
Total volume/tube			20.0

Appendix C: A list of optimum thermal cycling profile used in this study.

Thermal Cycle	Temperature (°C)	Time (min)
Initial denaturation	96	5
Denaturation	94	45sec
Annealing	45	45sec
Extension	72	30sec
Final extension	72	10
Hold	4	∞

Appendix D: List of components of 10X TBE buffer

Component	Volume
Tris Base	108 g
Boric Acid	55 g
EDTA	40 ml
dH ₂ O	Top up to 1 litre



Appendix E: Banding pattern of COI amplicon with optimized annealing temperature in the range of 45 – 55° and optimum amount of 25mM MgCl₂ of 2µl per reaction volume.

Note:

- (1) Lane 1: 45°C, Lane 2: 46.1°C, Lane 3: 47.4°C, Lane 4: 48.9°C, Lane 5: 51.0°C, Lane 6: 53.4°C and Lane 7: 54.7°C.
- (2) Marker: 100bp DNA Ladder (Vivantis).
- (3) PCR products run on 1% agarose gel at 80 volt for 35 minutes.