Pertanika J. Sci. & Technol. 31 (4): 1911 - 1920 (2023)

PERTANIKA JOURNALS

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Short Communication

Genetic Diversity of Asian Seabass (*Lates calcarifer*) in Captive Populations

Athirah Mohd Bakri[1] and Yuzine Esa[2]*

[1]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Selangor, Malaysia*
[2]*International Institute of Aquaculture and Aquatic Sciences, Universiti Putra Malaysia, Lot 960 Jalan Kemang 6, 71050 Port Dickson, Negeri Sembilan, Malaysia*

ABSTRACT

This study examined the genetic diversity of Asian seabass (*Lates calcarifer*) captive populations using sequencing of the mitochondrial DNA cytochrome c oxidase I (COI) fragment. The phylogenetic analyses of the 609 base pair regions of the COI fragment from 146 samples identified 15 haplotypes and divided them into two clades with a genetic divergence of 10%. Thus, phylogenetic results supported two genetic groups (the Australia/Southeast Asia group and the India/Myanmar group) within the captive populations under study. Mixed levels of genetic diversity were observed among captive populations, which indicated a certain degree of inbreeding depression. The findings would be useful for future aquaculture management of captive Asian seabass in Malaysia.

Keywords: captive, CO1 mtDNA, genetic distance, *L. calcarifer*

INTRODUCTION

Asian seabass or scientifically known as *Lates calcarifer,* is a marine teleost fish belonging to the order of Carangiformes. This prominent species had a widegeographical range across northern Australia and southward to southern Papua New Guinea, southern Japan, and Taiwan, including the Indo-West Pacific and the eastern edge of the Persian Gulf to China (Froese & Pauly, 2022). It is a remarkable aquaculture species worldwide, including Malaysia (Zhu et al., 2006). COI is the best marker compared to other mitochondrial genes as it retains more phylogenetic signal (Hebert et al., 2003). Genetic studies of Asian seabass in Malaysia's farm population are still lacking. Nevertheless, there are several studies on wildpopulations of Asian seabass using mitochondrial DNA markers (e.g.,

ARTICLE INFO

Article history:
Received: 01 June 2022
Accepted: 16 August 2022
Published: 25 May 2023

DOI: https://doi.org/10.47836/pjst.31.4.18

E-mail addresses:
athirahbakri17@gmail.com (Athirah Mohd Bakri)
yuzine@upm.edu.my (Yuzine Esa)
*Corresponding author

ISSN: 0128-7680
e-ISSN: 2231-8526

COI or cytochrome b), including Norfatimah et al. (2009). This study's mitochondrial DNA (mtDNA) analysis clarifies the genetic diversity of captive *L. calcarifer* in Peninsular Malaysia.

METHODOLOGY

Lates calcarifer juveniles (n = 146) from five commercial hatcheries were collected throughout Peninsular Malaysia, including the West coast (Selangor, n = 30), East coast (Terengganu, n = 30; Kelantan, n = 27), Southern territory (Johor, n = 30) and Northern Territory (Perak, n = 29) (Table 1). Alcohol of 95% concentration (ethanol) was used to preserve muscle tissues of the abdominal part. The ReliaPrep gDNA Tissue Miniprep System (Promega Corp, Madison, USA) standard extraction protocol was referred for DNA genomic extraction. Universal primers FishF1 (5'-TCAACCAACCA CAAAGACATTGGCAC-3'), and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') were used following Ward et al. (2005). The 25 µl total PCR mix's reaction contains 14.3 µl sterile distilled water, 5 µl Taq buffer 5×, 2.0 µl of 25 Mm MgCl, 0.5 µl of 10Mm dNTP, 0.5 µl of 10 µM of each primer, 0.2 µl of 5 µ μ^{-1} of Taq DNA polymerase and 2 µl of DNA template using Mastercycler Gradient PCR system (Eppendorf, Hamburg, Germany). PCR protocol was conducted according to the following profile: 2 min at 94°C, 30 cycles of 2 min at 94°C for denaturation, 1 min at 53°C (*COI*) for annealing, and 1 min and 30 s at 72°C for extension followed by a final step of 2 min at 72°C for the complete fragment extension. The PCR result was electrophoresed using a 1% agarose gel matrix from Fisher Scientific in New Jersey, USA. The GelRed (Thermo Fisher Scientific, USA) of 1 µl was used to stain the gel. Under UV light, the stained gel was visualized using AlphaImager HP (Biotechne, USA). The molecular weight (MV) standard used in this study was BenchTop 1kb DNA Ladder (Promega Corp, Madison, USA). The PCR products were sent for sequencing in only forward direction on an ABI 377 automated sequencer (Applied Biosystems) to the service supplier, First BASE Laboratories Sdn. Bhd. The phylogenetic analysis also included five COI sequences of *L. calcarifer* from GenBank. Three sequences originated from Southeast Asian waters (KU496228, DQ108026, and FJ237999), while another two were from Indian waters (EF60937 and JF919828). Kimura 2-parameter evolution model was used to calculate sequence divergence, grouped by the Neighbor-Joiningmethod (Figure 1), and bootstrapped with 1000 replications using MEGA7 (Kumar et al., 2016).

RESULTS AND DISCUSSION

The 609 bp of the COI sequences were retrieved after alignment and were characterized by 64 (10.5%) variable sites, including 63 parsimoniously informative sites and 545 (89.5%) conserved sites. The haplotypes contained 230 substitutions (190 transitions and 41 transversions). The mean total nucleotide composition was 21.9, 29.1, 30.7, and 18.3% for A, T, C, and G, respectively. In total, 15 haplotypes were identified from the 146

Table 1

Distribution of 15 observed haplotypes, nucleotide diversity, number of haplotypes, haplotype diversity, and number of polymorphic sites among populations of Lates calcarifer

		Populations				
Haplotypes	GenBank Accession Numbers	Kelantan (n = 27)	Terengganu (n = 30)	Perak (n = 29)	Selangor (n = 30)	Johor (n = 30)
LC1	MZ540093	0.4814	0.9666	0.4827	0.8333	0.5000
LC2	MZ540094	0.3703	-	0.2413	0.0666	0.1000
LC3	MZ540095	0.0370	-	-	-	-
LC4	MZ540096	0.0370	-	-	-	-
LC5	MZ540097	0.0370	-	-	-	-
LC6	MZ540098	0.0370	-	-	-	-
LC7	MZ540099	-	0.0333	-	-	0.0666
LC8	MZ540100	-	-	0.1379	-	0.2333
LC9	MZ540101	-	-	0.0344	-	-
LC10	MZ540102			0.0344	-	-
LC11	OK184465	-	-	0.0344	-	-
LC12	MZ540103	-	-	0.0344	-	-
LC13	MZ540104	-	-	-	0.1000	-
LC14	MZ540105	-		-	-	0.0666
LC15	MZ540106	-	-	-	-	0.0333
Nucleotide diversity (PiJC)		0.0475	0.0001	0.0389	0.0125	0.0237
Number of haplotypes		6	2	7	3	6
Haplotype diversity (Hd)		0.06496	0.0667	0.7094	0.3012	0.6989
Number of polymorphic sites		57	1	60	55	57

samples and were deposited to GenBank under accession numbers ranging from MZ540093 - MZ540106, OK184465, and ON920310 - ON920440 (Appendix).

Phylogenetic analysis using ML showed that the Asian seabass sampled could be divided into two clades in the tree. The first clade (L1) contains 10 haplotypes (120 samples) together with GenBank sequences from Australia and Southeast Asia, while the second clade (L2) contains 5 haplotypes (26 samples) together with GenBank sequences from India and Myanmar (Figure 1). Thus, based on phylogenetic analyses, the Asian seabass samples could be recognized into two genetic groups: the Australia/Southeast Asia (group 1) and the India/Myanmar region (group 2), with a genetic divergence value of 10% between them (Table 2).

Ward et al. (2008) claimed that Asian seabass originated from two different geographical regions: Australia and Myanmar. Rather than conspecific, it was identified

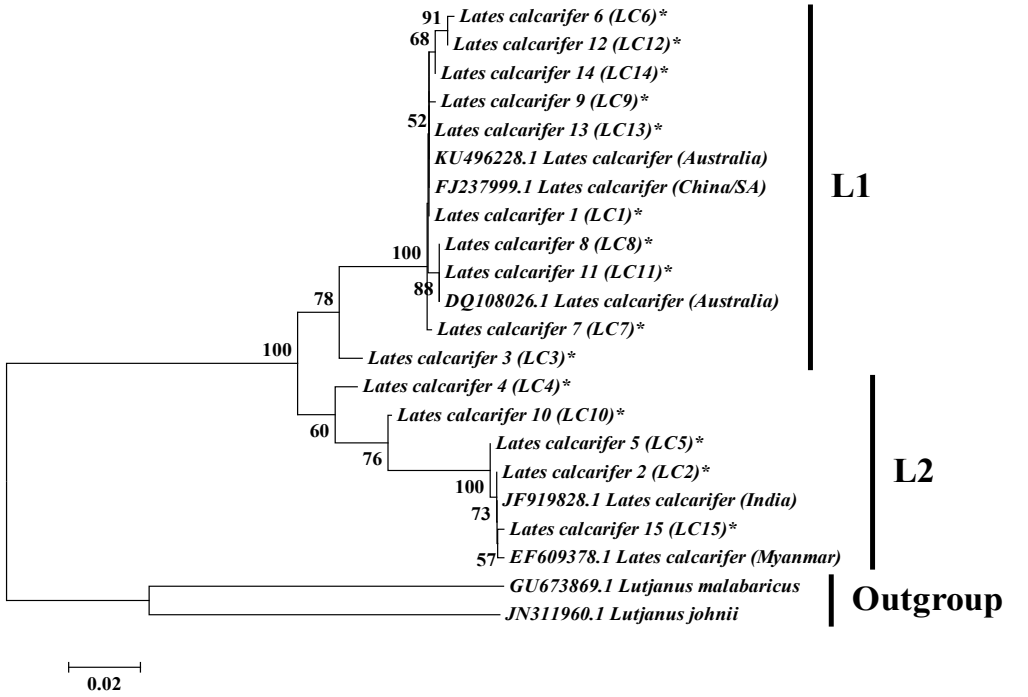

Figure 1. Neighbor-joining (nj) tree showing relationships among the seabasses. Samples were marked with an asterisk (*) at the end of the names. The number at each node represents the bootstrap value (%) based on the 1000 pseudoreplication of the dataset.

Note. SA = Southeast Asia

as congeneric. The proposition was further supported and confirmed by Vij et al. (2014) using comprehensive molecular and morphological analyses; plus, the Australian and Southeast Asia sequences were genetically close to each other (0.9% divergence); thus, they were not considered separate species. The current study also found similar results using captive or farm samples from different locations throughout Peninsular Malaysia. Thus, two different lineages in captive populations of Asian seabass might happen due to anthropogenic activities (Zhang et al., 2020) by exchanging fish stocks or seedlings between hatcheries or translocation across the country to obtain better breeds through importation. For example, fish farmers from Kelantan and even the Fisheries Research Institute (FRI), Department of Fisheries Malaysia (DOF), bought parental stocks of Asian seabass from neighboring farms located in Thailand (Idris et al., 2022).

The basic requirement of a successful selective breeding program is an appropriate base population. Starting from the production traits, an artificial population should harbor sufficient genetic diversity with selectable and desirable characteristics (Senanan et al., 2015). Two key indicators of genetic variation are haplotype (h) and nucleotide (π) diversity. A group with bigger h and π will have higher genetic variation and diversity (Falush et

Table 2

Pairwise Tamura-Nei genetic distance among 15 haplotypes of Lates calcarifer from five different farms in Malaysia

Haplotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
LC1	-																					
LC2	0.097	-																				
LC3	0.031	0.062	-																			
LC4	0.053	0.040	0.021	-																		
LC5	0.095	0.002	0.060	0.038	-																	
LC6	0.007	0.105	0.038	0.060	0.103	-																
LC7	0.002	0.099	0.033	0.055	0.097	0.008	-															
LC8	0.003	0.097	0.035	0.057	0.095	0.010	0.005	-														
LC9	0.002	0.099	0.033	0.055	0.097	0.008	0.003	0.005	-													
LC10	0.062	0.031	0.033	0.033	0.029	0.070	0.064	0.066	0.064	-												
LC11	0.003	0.097	0.035	0.057	0.095	0.010	0.005	0.000	0.005	0.066	-											
LC12	0.005	0.103	0.036	0.058	0.101	0.002	0.007	0.008	0.007	0.068	0.008	-										
LC13	0.000	0.097	0.031	0.053	0.095	0.007	0.002	0.003	0.002	0.062	0.003	0.005	-									
LC14	0.002	0.099	0.033	0.055	0.097	0.005	0.003	0.005	0.003	0.064	0.005	0.003	0.002	-								
LC15	0.099	0.002	0.064	0.042	0.003	0.107	0.101	0.099	0.101	0.033	0.099	0.105	0.099	0.101	-							
KU496228 LCAustralia	0.000	0.097	0.031	0.053	0.095	0.007	0.002	0.003	0.002	0.062	0.003	0.005	0.000	0.002	0.099	-						
DQ108026 LCAustralia	0.003	0.097	0.035	0.057	0.095	0.010	0.005	0.000	0.005	0.066	0.000	0.008	0.003	0.005	0.099	0.003	-					
FJ237999 LCChina/SA	0.000	0.097	0.031	0.053	0.095	0.007	0.002	0.003	0.002	0.062	0.003	0.005	0.000	0.002	0.099	0.000	0.003	-				
EF609378 LCMyanmar	0.099	0.002	0.064	0.042	0.003	0.107	0.101	0.099	0.101	0.033	0.099	0.105	0.099	0.101	0.003	0.099	0.099	0.099	-			
JF919828 LCIndia	0.097	0.000	0.062	0.040	0.002	0.105	0.099	0.097	0.099	0.031	0.097	0.103	0.097	0.099	0.002	0.097	0.097	0.097	0.002	-		
GU673869 *Lutjanusmalabaricus*	0.262	0.230	0.249	0.244	0.232	0.271	0.259	0.262	0.264	0.245	0.262	0.269	0.262	0.264	0.232	0.262	0.262	0.262	0.232	0.230	-	
JN311960 *Lutjanusjohnii*	0.249	0.254	0.251	0.256	0.252	0.258	0.246	0.249	0.251	0.251	0.249	0.256	0.249	0.251	0.257	0.249	0.249	0.249	0.257	0.254	0.196	-

Note. SA = Southeast Asia

al., 2003). Overall, *L. calcarifer* samples from Perak exhibited high haplotype diversity with low nucleotide diversity (h = 0.7094; π = 0.0389), while *L. calcarifer* samples from Terengganu displayed low haplotype and nucleotide diversity (h = 0.0667; π = 0.0001). According to Grant and Bowen (1998), *L. calcarifer* samples from Terengganu and Perak fall into the first and second categories. The first category (i.e., Terengganu) had a founder event by a single or a few mtDNA lineages or a recent population bottleneck. In contrast, the second category (i.e., Perak) indicates rapid population development and increased mutations after a population bottleneck. In addition, Perak haplotypes are mixed lineages of L1 and L2, while Terengganu is only from L1, which may cause the following result.

LC1 was shared among all Asian seabass populations signifying it as the ancestral haplotype. As the seed for Asian seabass came from either hatcheries or natural resources, the same origin of ancestral and the succeeding gene flow may be the main reason for the occurrence of sharing haplotypes among populations (Das et al., 2018). With only two haplotypes, the population in Terengganu possibly came from a small enclosed aquatic environment, resulting in inbreeding or collected samples from the same family (Zhang et al., 2020). High numbers (9 out of 15) of unique or private haplotypes were obtained across populations. Through mutation, the independent origin of haplotypes may produce a high percentage of unique haplotypes (Das et al., 2018). These population-specific haplotypes could also be used as an indicator for stock documentation.

CONCLUSION

The information on the genetic diversity of aquatic organisms is essential for the sustainable management of genetic resources, achieving productive aquaculture, and sustaining harvesting farm populations. In conclusion, this study has sorted out the species identification and genetic diversity of the important Asian seabass *L. calcarifer.* Two genetic groups (L1 and L2) were detected from 146 samples across five captive populations. Group L1 samples clustered with GenBank sequences from the South China Sea and Australia regions, whereas L2 clustered remaining samples with the GenBank sequences from India and Myanmar. The existence of two different mtDNA lineages in captive or farm populations in Peninsular Malaysia was possibly caused by translocation activities between hatcheries beyond countries rather than historical events. Identifying mixed genetic stocks or groups (South China Sea/Australia vs. India/Myanmar) in the captive populations highlighted the utility of the mitochondrial DNA COI marker for accurate genetic identification and selection of individual fish breeds for the breeding program. A mixed level of genetic diversity was observed across populations, with the Terengganu population harboring the lowest diversity level (h = 0.0667; π = 0.0001).

High numbers (9 out of 15) of unique or private haplotypes were also obtained across populations. The mixed levels of genetic diversity among the captive populations

indicated that evolutionary factors such as inbreeding might happen in populations with low genetic diversity due to the poor selection of stocks or breeds and poor maintenance of the population gene pool, which resulted in various growth development problems such as deformed opercula, slow growth, high vulnerability to diseases and many others. Upcoming research should concentrate on the complete phylogeographic and population structure of *L. calcarifer* across Malaysia's farm populations. Applying more sensitive markers, such as microsatellites, should be more informative in explaining the species' population structure. The findings of this pioneering study on captive *L. calcarifer* in Malaysia should be helpful for the selective breeding program of the species.

ACKNOWLEDGMENTS

We thank the Ministry of Higher Education, Malaysia for funding this project under the FRGS research grant (FRGS/1/2019/WAB01/UPM/02/26)—Genetic diversity and relatedness estimates for selective breeding. We also thank the Fish Genetic and Breeding Laboratory, Faculty of Agriculture, University Putra Malaysia, for laboratory facilities and everyone involved in sampling collection. The UPM-Kyutech International Symposium on Applied Engineering and Sciences 2021 (SAES2021) and Universiti Putra Malaysia were the main sponsor for the publication fee.

REFERENCES

Das, S. P., Swain, S., Jena, J., & Das, P. (2018). Genetic diversity and population structure of *Cirrhinus mrigala* revealed by mitochondrial ATPase 6 gene. *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, *29*(4), 495-500. https://doi.org/10.1080/24701394.2017.1310852

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, *164*(4), 1567-1587. https://doi.org/10.1093/genetics/164.4.1567

Froese, R., & Pauly, D. (Eds.). (2022). *FishBase*. World Wide Web electronic publication. http://www.fishbase.org

Grant, W. S., & Bowen, B. W. (1998). Obituary: Professor Michael Laskowski Sr. *Acta Biochimica Polonica*, *30*(2), 113-114.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1512), 313-321. https://doi.org/10.1098/rspb.2002.2218

Idris, S. M., Noordin, W. N. M., Manah, F. O., & Hamzah, A. (2022). Toward systematic breeding of asian sea bass, *Lates calcarifer* (Bloch, 1790), in Malaysia: status, challenges and prospects for future development. *Asian Fisheries Science*, *35*(1), 1-12. https://doi.org/10.33997/j.afs.2022.35.1.001

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870-1874. https://doi.org/10.1093/molbev/msw054

Norfatimah, M. Y., Azizah, M. N. S., Othman, A. S., Patimah, I., & Jamsari, A. F. J. (2009). Genetic variation of *Lates calcarifer* in Peninsular Malaysia based on the cytochrome b gene. *Aquaculture Research*, *40*(15), 1742-1749. https://doi.org/10.1111/j.1365-2109.2009.02279.x

Senanan, W., Pechsiri, J., Sonkaew, S., Na-Nakorn, U., Sean-In, N., & Yashiro, R. (2014). Genetic relatedness and differentiation of hatchery populations of Asian seabass (*Lates calcarifer*) (Bloch, 1790) broodstock in Thailand inferred from microsatellite genetic markers. *Aquaculture Research*, *46*(12), 2897-2912. https://doi.org/10.1111/are.12442

Vij, S., Purushothaman, K., Gopikrishna, G., Lau, D., Saju, J. M., Shamsudheen, K. V., Vinaya Kumar, K., Basheer, V. S., Gopalakrishnan, A., Hossain, M. S., Sivasubbu, S., Scaria, V., Jena, J. K., Ponniah, A. G., & Orbán, L. (2014). Barcoding of Asian seabass across its geographic range provides evidence for its bifurcation into two distinct species. *Frontiers in Marine Science*, *1*, Article 30. https://doi.org/10.3389/fmars.2014.00030

Ward, R. D., Holmes, B. H., & Yearsley, G. K. (2008). DNA barcoding reveals a likely second species of Asian sea bass (barramundi) (*Lates calcarifer*). *Journal of Fish Biology*, *72*(2), 458-463. https://doi.org/10.1111/j.1095-8649.2007.01703.x

Ward, R. D, Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. N. (2005). DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1462), 1847-1857. https://doi.org/10.1098/rstb.2005.1716

Zhang, Q., Sun, C., Zhu, Y., Xu, N., & Liu, H. (2020). Genetic diversity and structure of the round-tailed paradise fish (*Macropodus ocellatus*): Implications for population management. *Global Ecology and Conservation*, *21*(159), Article e00876. https://doi.org/10.1016/j.gecco.2019.e00876

Zhu, Z. Y., Lin, G., Lo, L. C., Xu, Y. X., Feng, F., Chou, R., & Yue, G. H. (2006). Genetic analyses of Asian seabass stocks using novel polymorphic microsatellites. *Aquaculture*, *256*(1-4), 167-173. https://doi.org/10.1016/j.aquaculture.2006.02.033

APPENDIX

Accession No.	Kelantan	Accession No.	Terengganu	Accession No.	Perak	Acccession No.	Selangor	Accesion No.	Johor
MZ540093	K1	ON920331	B1	ON920360	SE1	ON920384	S1	ON920413	J1
MZ540094	K2	ON920332	B2	ON920361	SE2	ON920385	S2	ON920414	J2
ON920310	K3	ON920333	B3	ON920362	SE3	ON920386	S3	ON920415	J3
ON920311	K4	ON920334	B4	MZ540100	SE4	ON920387	S4	ON920416	J4
ON920312	K5	ON920335	B5	ON920363	SE6	ON920388	S5	ON920417	J5
MZ540095	K6	ON920336	B6	ON920364	SE7	ON920389	S6	ON920418	J6
ON920313	K7	ON920337	B7	ON920365	SE8	ON920390	S7	ON920419	J7
ON920314	K8	ON920338	B8	ON920366	SE9	ON920391	S8	ON920420	J8
ON920315	K9	ON920339	B9	ON920367	SE10	ON920392	S9	ON920421	J9
MZ540096	K11	ON920340	B10	ON920368	SE11	ON920393	S10	ON920422	J10
ON920316	K12	ON920341	B11	ON920369	SE12	ON920394	S11	ON920423	J11
ON920317	K13	ON920342	B12	MZ540101	SE13	ON920395	S12	ON920424	J12
ON920318	K14	ON920343	B13	ON920370	SE14	ON920396	S13	ON920425	J13
ON920319	K15	ON920344	B14	ON920371	SE15	ON920397	S14	ON920426	J14
ON920320	K16	ON920345	B15	MZ540102	SE16	MZ540104	S15	ON920427	J15
ON920321	K17	ON920346	B16	ON920372	SE17	ON920398	S16	ON920428	J16
MZ540097	K18	MZ540099	B17	ON920373	SE18	ON920399	S17	ON920429	J17
ON920322	K20	ON920347	B18	ON920374	SE19	ON920400	S18	ON920430	J18
ON920323	K21	ON920348	B19	ON920375	SE20	ON920401	S19	MZ540105	J19
ON920324	K22	ON920349	B20	OK184465	SE21	ON920402	S20	ON920431	J20
ON920325	K23	ON920350	B21	MZ540103	SE23	ON920403	S21	ON920432	J21
ON920326	K25	ON920351	B22	ON920376	SE22	ON920404	S22	ON920433	J22
ON920327	K26	ON920352	B23	ON920377	SE24	ON920405	S23	MZ540106	J23
ON920328	K27	ON920353	B24	ON920378	SE25	ON920406	S24	ON920434	J24

APPENDIX (Continue)

Accession No.	Kelantan	Accession No.	Terengganu	Accession No.	Perak	Accession No.	Selangor	Accession No.	Johor
ON920329	K28	ON920354	B25	ON920379	SE26	ON920407	S25	ON920435	J25
MZ540098	K29	ON920355	B26	ON920380	SE27	ON920408	S26	ON920436	J26
ON920330	K30	ON920356	B27	ON920381	SE28	ON920409	S27	ON920437	J27
		ON920357		ON920382	SE29	ON920410	S28	ON920438	J28
		ON920358		ON920383	SE30	ON920411	S29	ON920439	J29
		ON920359		B30		ON920412	S30	ON920440	J30