African Journal of Biotechnology Vol. 9(21), pp. 3063-3067, 24 May, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Rastrelliger systematics inferred from mitochondrial cytochrome *b* sequences

Jamsari Amirul Firdaus Jamaluddin[1], Abu Talib Ahmad[3], Samsudin Basir[3], Masazurah Abdul Rahim[3] and Siti Azizah Mohd Nor[1,2*]

[1]School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia.
[2]Centre for Marine and Coastal Studies, Universiti Sains Malaysia, Penang, Malaysia.
[3]Fisheries Research Institute, Batu Maung, Penang, Malaysia.

Accepted 20 April, 2010

The fish genus *Rastrelliger* is composed of three morphologically recognized species; *Rastrelliger kanagurta, Rastrelliger brachysoma* and *Rastrelliger faughni.* In this study, cytochrome *b* gene sequencing was applied to address the systematics and phylogenetic relationships of these species. In agreement with previous morphological data, the results corroborate monophyletic discrimination between all the species. However, inconsistent bootstrap support (< 50 to 88%) between *R. kanagurta* and *R. brachysoma* was observed indicating limited divergence between these two species. *R. faughni* is recognized as the most basal species for this genus with high statistical support (99 and 100%). Diversification of *Rastrelliger* might have happen in two epochs, Miocene and early Pleistocene.

Key words: Molecular systematics, cytochrome *b*, genus *Rastrelliger*.

INTRODUCTION

Rastrelliger genus is comprised of three recognized species: *Rastrelliger kanagurta* (Indian mackerel), *Rastrelliger brachysoma* (Indo Pacific mackerel) and *Rastrelliger faughni* (island mackerel) (Matsui, 1967; Froese and Pauly, 2009). They are the most commercially important small pelagic fish in the tropical region providing cheap protein source and fish bait (FAO, 1987; Froese and Pauly, 2009). These tropical species are found in the Indo-West Pacific with *R. kanagurta* introduced into the Mediterranean waters through the Suez Canal. However, *R. brachysoma* and *R. faughni* distribution are restricted to central Indo-West Pacific region (Collette and Nauen, 1983; Froese and Pauly, 2009). In Malaysia, *R. brachysoma* is distributed in the more coastal near-shore areas while *R. kanagurta* and *R. faughni* are more oceanic (Chee, 2000). In the West Coast of Peninsular Malaysia, *Rastrelliger* landings make up a large portion of the total catch of small pelagic fishes. While *R. brachysoma* and *R. kanagurta* is widely exploited, *R. faughni* is not of high commercial importance (FAO, 1987; Chee, 2000).

Ten species have been described which are now considered as synonyms of the three mentioned species (Matsui, 1967; Froese and Pauly, 2009). They differ in a series of morphological characters including number and size of gill rakers, body depth and stripes along sides of the body (Matsui, 1967; Mansor et al., 1998; Moazzam et al., 2005). They were initially described as *Scomber* and subsequently reclassified as *Rastrelliger* genus by Jordan and Dickerson (1908). This was split into two species *R. kanagurta* and *R. brachysoma*, characterized by long gill rakers. Later, Matsui (1967) included *R. faughni,* a misidentified species of *Scomber australasicus* as a new member of *Rastrelliger.* Although this species has relatively short gill rakers, the study found many other closely related anatomical structures for example osteological characters to justify their inclusion into the *Rastrelliger* genus. Based on recent published literature,

*Corresponding author. E-mail: sazizah@usm.my. Tel: +604 653 4004. Fax: +604 6565125.

Abbreviations: PCR, Polymerase chain reaction; **SDS,** sodium dodecyl sulfate; **EDTA,** ethylene diamine tetraacetic acid; **NJ,** neighbour-joining; **MP,** maximum parsimony; **ML,** maximum likelihood; **hLRT,** hierarchical likelihod ratio tests; **CI,** consistency index; **mtDNA,** mitochondrial DNA.

studies of *Rastrelliger* species have been focused on population studies, fishery aspects, ecological, morphological characters and biological aspects mostly for *R. kanagurta* (example, Matsui, 1967; Rohde, 1989; Mwebaza-Ndawula, 1990; Jayasankar et al., 2004; Mohan et al., 2008; Siti Azizah et al., 2008) but to date no molecular and morphological phylogenetic inferences of *Rastrelliger* is available. Thus, to investigate the molecular systematics of the three *Rastrelliger* species; *R. kanagurta, R. brachysoma* and *R. faughni*, partial sequencing of the mtDNA cytochrome *b* (cyt *b*) gene was performed in the present study.

MATERIALS AND METHODS

Eighteen specimens (nine *R. kanagurta*, five *R. brachysoma* and four *R. faughni*) were collected along the Northern to the Central West Coast of Peninsular Malaysia. DNA from finclip or muscle tissue stored in TNES-Urea (100 mM Tris-HCl pH 7.5, 125 mM NaCl, 10 mM EDTA pH 7.5, 1% SDS, 3 M Urea) and 95% ethanol was extracted using AquaGenomic™ DNA isolation Kit (BioSyntech, Salt Lake City, Utah, USA) following the manufacturer's manual. The universal primers L14841 and H15149 (Kocher et al., 1989) were used to amplify partial cyt *b* gene by PCR. Amplification was carried out in 25 µl reaction mixture containing 1 µl template DNA, 1.0X PCR buffer, 3.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.02 µM each Primer and 0.08 U *Taq* DNA polymerase. PCR was performed with the following profile: initial denaturation at 98°C for 1 min followed by 35 cycles consisting of 95°C for 1 min, 55°C for 1 min, 72°C for 2 min and final extension at 72°C for another 2 min. PCR products were purified using QIAquick PCR purification (Qiagen, Valencia, CA, USA) and sequenced on an ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). As the closest relative of the genus *Rastrelliger* (Matsui, 1967; Collette and Nauen, 1983), four species of *Scomber* (*S. scombrus* - EF439581*, S. japonicus* - EF141177*, S. australasicus* - DQ497864 and *S. colias* - EF439576) were selected as outgroups to root the phylogenetic trees. All sequences were viewed, edited and aligned using MEGA version 4 software (Tamura et al., 2007). Substitution saturation tests were performed using DAMBE (Xia and Xie, 2001) by plotting the number of observed transitions (Ti) relative to that of transversions (Tv) against genetic distance values (Kimura, 1980) to evaluate the usefulness of these sequences for phylogenetic analysis. The alignment sequences were imported into PAUP* beta version 4.0b10 (Swofford, 2003) for phylogenetic analyses using neighbour-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML). Prior to NJ and ML analyses, ModelTest 3.8 (Posada and Crandall, 1998) was employed to select the best substitution model and was then used to calculate pairwise sequence distances and to construct the trees among the three investigated species. The best-fit score was chosen using Hierarchical Likelihod Ratio Tests (hLRT). Confidence limits were assessed using bootstrap procedure with 1000 replicates for NJ and MP and 500 replicates for ML.

RESULTS AND DISCUSSION

Excluding the outgroups, the combined sequence data resulted in 305 characters, of which 272 were constant, 33 were variable sites with 29 being parsimony informative. Thirty two of the variable sites were in the third codon position with the remaining one at the first codon position. Thirty three transitions and a single transversion (Table 1) were observed. The unambiguous alignment showed no indels (insertions/deletions) and no amino acid replacement. MtDNA amino acid replacements in fishes is known to be slower compared to mammals and birds (Kocher et al., 1989). A total of ten haplotypes were revealed (Table 1 and Figure 1). Sequences were mono-typic in *R. brachysoma* and almost conserved in other taxa. All haplotypes have been deposited in Gen Bank under accession numbers EU170508, EU170509, EU170511, FJ375338, FJ375339 and GU003972 to GU003976. The substitution saturation tests showed that the sequences have not reached the saturation zone thus, validating their use for phylogenetic inference. ModelTest output indicated that the Hasegawa-Kishino-Yano model (Hasegawa et al., 1985) with gamma correction (HKY85 + G) was the most appropriate model of evolution for this data set.

The genetic divergence values (Table 2) among taxa within this genus varied from 2.35 (between *R. kanagurta* and *R. brachysoma*) to 9.71% (between *R. faughni* and *R. kanagurta*) while within species ranged from 0 (*R. brachysoma*) to 0.99% (*R. kanagurta*). Each phylogenetic approach (NJ, MP and ML) indicated similar pattern of tree topologies (Figure 1) and resolved the relationships within *Rastrelliger* species. Parsimony analysis revealed a single most parsimonious tree with a length of 103, a consistency index (CI) of 0.864 and a retention index (RI) of 0.938. This analysis supported the monophyly of the three *Rastrelliger* species which clustered in two main groups. The basal-most member was *R. faughni* with strong statistical support (99 to 100%). The nucleotide divergence between this species to the sister lineage species; *R. kanagurta* and *R. brachysoma* ranged from 8.31 to 9.71%. The remaining two *Rastrelliger* species clustered into two distinct monophletic groups, however, without consistent bootstrap support (< 50 to 88%) and exhibited lower between cluster genetic divergence ranging from 2.35 to 2.69%. However, a detailed base character investigation revealed six autapomorphic sites which discriminated *R. kanagurta* from *R. brachysoma* (Table 1). Further studies utilizing other markers or longer sequences is recommended for better resolution of these closely related taxa (Faria et al., 2006; Infante et al., 2007). A crude molecular clock estimated based on 1 to 1.2% substitution per MY (conservative molecular clock for marine fishes) (Bermingham et al., 1997), suggested that the divergence times of *Rastrelliger* occurring around 2.35 MYA (*R. kanagurta- R. brachysoma*) and 9.71 MYA (*R. faughni- R. kanagurta/R. brachysoma*), were equivalent to the Tortonian (Miocene) and Gelasian (early Pleistocene) period, respectively. Based on these two distinct diver-gences time, it was suggested that two periods of diversification in the genus *Rastrelliger*, most probably influenced by vicariant processes (that is, ecological changes leading to geographical changes), resulted in the divergence of this genus before expanding to the present

Table 1. Substitutional variations in mtDNA haplotypes of *Rastrelliger* species. Dots indicate nucleotides identical with those of the top sequence and vertical numbers indicating the position of variable nucleotides within the 305-bp sequence. Character based autapomorphic sites distinguishing *R. kanagurta* and *R. brachysoma* are highlighted.

				3	3	4	5	6	7	7	0	1	2	2	3	3	4	5	5	6	6	7	7	8	8	8	9	9	9	2	3	6	6
	1	4	7	1	7	3	8	7	3	6	9	2	4	7	0	3	8	1	7	0	3	2	5	1	4	7	0	3	6	0	3	5	8
R. kanagurta 01	A	C	C	C	T	T	C	C	C	C	T	C	T	C	T	T	C	C	G	C	C	C	T	C	A	C	C	C	T	T	T	C	T
R. kanagurta 02	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	T	.	.	.	.	.	.	.	.	C	.	.	.
R. kanagurta 03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. kanagurta 04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. kanagurta 05	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.
R. kanagurta 06	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	.	.
R. kanagurta 07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	.	.	.	C	.	.	.
R. kanagurta 08	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	T	.	C	.	.	.
R. kanagurta 09	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
R. brachysoma 01	.	.	.	.	.	.	.	.	**T**	.	**C**	.	.	.	**C**	.	.	.	.	.	T	.	**C**	.	**G**	.	.	.	**C**	.	.	.	.
R. brachysoma 02	.	.	.	.	.	.	.	.	**T**	.	**C**	.	.	.	**C**	.	.	.	.	.	T	.	**C**	.	**G**	.	.	.	**C**	.	.	.	.
R. brachysoma 03	.	.	.	.	.	.	.	.	**T**	.	**C**	.	.	.	**C**	.	.	.	.	.	T	.	**C**	.	**G**	.	.	.	**C**	.	.	.	.
R. brachysoma 04	.	.	.	.	.	.	.	.	**T**	.	**C**	.	.	.	**C**	.	.	.	.	.	T	.	**C**	.	**G**	.	.	.	**C**	.	.	.	.
R. brachysoma 05	.	.	.	.	.	.	.	.	**T**	.	**C**	.	.	.	**C**	.	.	.	.	.	T	.	**C**	.	**G**	.	.	.	**C**	.	.	.	.
R. faughni 01	G	T	T	.	C	C	T	T	T	T	.	T	C	T	C	C	T	.	.	.	T	T	.	T	C	T	T	T	.	.	C	T	C
R. faughni 02	G	T	T	.	C	C	T	T	T	T	.	T	C	T	C	C	T	.	.	.	T	T	.	T	C	T	T	T	.	.	C	T	C
R. faughni 03	G	T	T	T	C	C	T	T	T	T	.	T	C	T	C	C	T	.	.	.	T	T	.	T	C	T	T	T	.	.	C	T	C
R. faughni 04	G	T	T	.	C	C	T	T	T	T	.	T	C	T	C	C	T	T	.	.	T	T	.	T	C	T	T	T	.	.	C	T	C

Table 2. Percentage of pair-wise sequence divergences (HKY + G model) derived from cytochrome *b* gene sequence data.

	RB01	RK01	RK02	RK05	RK06	RK07	RK08	RF01	RF03	RF04
RB01										
RK01	2.35									
RK02	2.69	0.99								
RK05	2.35	0.66	0.33							
RK06	2.35	0.66	0.99	0.66						
RK07	2.69	0.99	0.66	0.33	0.99					
RK08	2.69	0.99	0.66	0.33	0.99	0.66				
RF01	8.92	8.92	9.31	8.92	8.15	9.31	8.53			
RF03	9.31	9.31	9.71	9.31	8.53	9.71	8.92	0.33		
RF04	9.31	9.31	9.71	9.31	8.53	9.71	8.92	0.33	0.66	

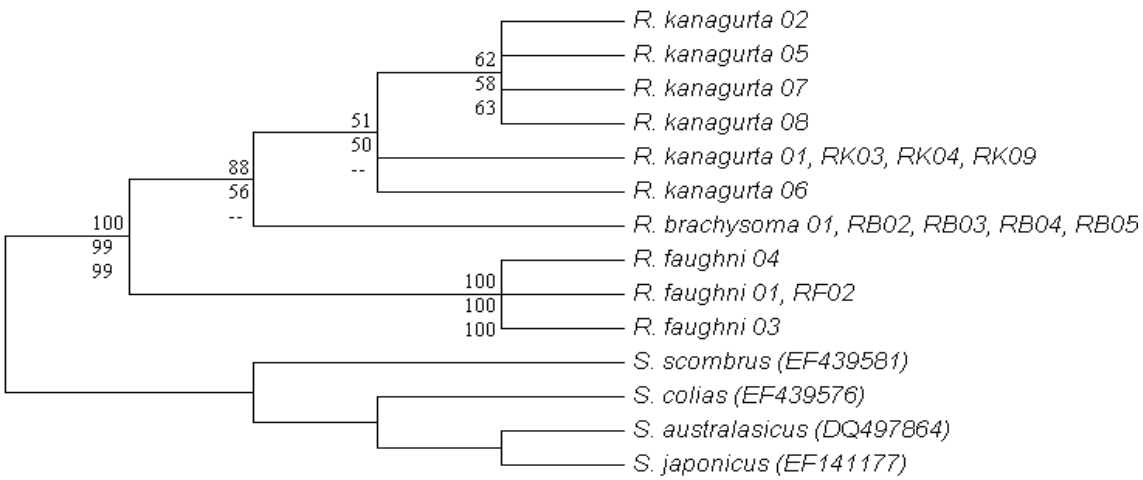

Figure 1. The 50% majority-rule consensus phylogenetic tree (NJ, MP and ML analyses) based on 305 bp cytochrome *b* sequences between three species of *Rastrelliger* rooted by the *Scomber* sequences. Numbers above branches represent bootstrap confident level for NJ (1000 replicates); numbers below branches correspond to bootstrap values for MP and ML (1000 and 500 replicates).

distribution upon reconnection of the barriers.

The present data resolved the phylogenetic relationships and confirmed the morphological classification of *Rastrelliger* species in particular supporting the taxonomic status of *R. faughni* by Matsui (1967) despite not conforming to the typical gill raker length observed in other members namely *R. kanagurta* and *R. brachysoma*. Correct species identification and phylogenetic relationships of species is very important especially in the case of morphologically close related taxa for the establishment of adequate fisheries management and conservation for biodiversity studies and for population dynamics (Casey et al., 2004; Faria et al., 2006; Persis et al., 2008). This study confirms that cyt *b* can be efficiently used as a marker for taxonomic identification of this genus as has been similarly reported in other organisms (Johns and Avise, 1998; Nikoletta et al., 2003; Casey et al., 2004) and again highlights that genetic analysis should be an integral part of the validation process to complement morphological traits which is the starting point of any taxonomic investigation.

REFERENCES

Bermingham E, McCafferty SS, Martin AP (1997). Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In: Molecular Systematics of Fishes. Kocher TD, Stepien CA (Eds). N.Y. Academic Press. pp. 113-128.

Casey SP, Hall HJ, Stanley HF, Vincent ACJ (2004). The origin and evolution of seahorses (genus *Hippocampus*): a phylogenetic study using the cytochrome b gene of mitochondrial DNA. Mol. Phylogenet. Evol. 30: 261-272.

Chee PE (2000). Fishcode management: Supplement to the report of a workshop on the fishery and management of a short mackerel (*Rastrelliger* spp.) on the West Coast of Peninsular Malaysia. FAO, Rome.

Collette BB, Nauen CE (1983). FAO species catalogue. Scombrids of the world. An annotated and illustrated catalogue of tunas, mackerels, bonitos and related species known to date. FAO Fish. Synopses. 125(2): 137.

FAO, United Nations (1987). Investigations of the mackerel and scad resources of the Malacca Straits. Available online at *http://www.fao.org*. Access date 02.10.2009.

Faria R, Weiss S, Alexandrino P (2006). A molecular phylogenetic perspective on the evolutionary history of *Alosa* spp. (Clupeidae). Mol. Phylogenet. Evol. 40(1): 298-304. http://www.answers.com/topic/digital-object-identifier

Froese R, Pauly D (Editors) (2009). FishBase. World Wide Web electronic publication. www.fishbase.org, version (07/2009).

Hasegawa M, Kishino H, Yano T (1985). Dating of the humanape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22: 160-174.

Infante C, Blanco E, Zuasti E, Crespo A, Manchado M (2007). Phylogenetic differentiation between Atlantic *Scomber colias* and Pacific *Scomber japonicus* based on nuclear DNA sequences. Genetic, 130: 1-8.

Jayasankar P, Thomas PC, Paulton, MP, Mathew J (2004). Morphometric and Genetic Analyzes of Indian Mackerel (*Rastrelliger kanagurta*) from Peninsular India. Asian Fish. Sci. 17: 201-215.

Johns GC, Avise JC (1998). A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. Mol. Biol. Evol. 15: 1481-1490.

Jordan DS, Dickerson MC (1908). On a collection of fishes from Fiji, with notes on certain Hawaiian fishes. Proc. US. Nat. Mus. 34: 603-617.

Kimura M (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111-120.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America, 86: 6196-6200.

Mansor MI, Kohno H, Ida H, Nakamura, HT, Aznan Z, Abdullah S (1998). Field Guide to Important Commercial Marine Fishes of the South China Sea. SEAFDEC MFRDMD/SP/2, Kuala Terengganu. 13(1287): 121.

Matsui T (1967). Review of the mackerel genera *Scomber* and *Rastrelliger* with description of a new species of *Rastrelliger*. Copeia, (1): 71-83.

Moazzam M, Osmany HB, Zohra K (2005). Indian Mackerel (*Rastrel*

liger kanagurta) from Pakistan-I. Some aspects ofbiology and fisheries. Rec. Zool. Surv. Pak. 16: 58-75.
Mohan M, Ramachandran D, Sankar TV, Anandan R (2008). Physicochemical characterization of muscle proteins from different regions of mackerel (*Rastrelliger kanagurta*). Food Chem. 106: 451-457.
Mwebaza-Ndawula L (1990). Seasonal variation in abundance of the Indian mackerel, *Rastrelliger kanagurta* Cuvier (Pisces: Scombridae) along the Zanzibar coast of East Africa. Hydrobiology, 190: 233-239.
Nikoletta K, Apostolidis AP, Triantafyllidis A, Kouvatsi A, Triantaphyllidis C (2003). Genetic identification and phylogeny of three species of the genus *Trachurus* based on mitochondrial DNA analysis. Mar. Biotechnol. 5: 493-504.
Persis M, Chandra SRA, Rao LM, Khedkar GD, Ravinder K, Nasruddin K (2009). COI (cytochrome oxidase-I) sequence based studies of Carangid fishes from Kakinada coast, Indian. Mol. Biol. Rep. 36: 1733-1740
Posada D, Crandall KA (1998). Modeltest: Testing the model of DNA substitution. Bioinform. 14: 817-818.
Rohde K (1989). Gill Monogenea of *Rastrelliger* spp. (Scombridae). Syst. Parasitol. 14: 79-91.
Siti Azizah MN, Abu Talib A, Mohd Ghows MA, Samsudin B (2008). A preliminary genetic investigation of *Rastrelliger kanagurta* based on RAPD and mitochondrial ND2 gene. Wetland Sci. 6(4): 518-525.
Swofford DL (2003). PAUP* Phylogenetic analysis using parsimony (* and other methods), version 4 beta 10. Sianuer, Sunderland, Massachusetts.
Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596-1599.
Xia X, Xie Z (2001). DAMBE: Data Anal. in Mol. Biol. Evol. J. Hered. 92: 371-373.