

A preliminary study of population structure of kawakawa, *Euthynnus affinis* (Cantor 1849) in the straits of Malacca

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Abstract

Kawakawa, *Euthynnus affinis*, small epipelagic, migratory, neritic tuna is one of the major commercial tuna species being caught in Malaysia. Therefore, its sustainability needs to be ensured by effective management. In this study, genetic variation was assessed using sequence analyses of mitochondrial DNA (mtDNA) cytochrome b (cyt *b*) gene. A 331 bp segment of cyt *b* gene was sequenced in 113 samples collected from 4 different sources (Kuala Perlis (KP), Bayan Baru (BB), Batu Lanchang (BL), and Jalan Tenggara (JT)). Seventy four haplotype sequences were homologous (99%) to each other while thirty nine were divergent (3%) indicating a single population along the straits of Malacca. The results obtained need to be supported by more individuals and gene studied, examination of historical aspects of population distribution and further analysis.

Keywords: Population genetics, neritic tuna, cyt *b*

Introduction

Kawakawa, also known as little tuna, belongs to the family Scombridae which includes the mackerels, tunas and bonitos. It is an epipelagic migratory tuna species that is widely distributed in the tropical and subtropical waters of the Indo-Pacific region. Although also inhabiting ocean waters, kawakawa prefer to stay close to the coast and juveniles are even found in bays and harbours. It is a highly migratory species and frequently forms a large schools which are often mixed with other scombrid species. In the western Pacific Ocean, this species is distributed along the Asian continent from Malaysia northeastward through Mainland China, Taiwan, and to the southern Japan (Taghavi Motlagh *et al.*, 2009).

The annual landings of neritic tuna in Malaysia between 2000 and 2010, ranging from 41,565 tonnes to 64,215 tonnes per year, and more than 90% of the landing comprises Kawakawa and *Thunnus tonggol*. Despite the economical importance of this species, little is known about the biology of this fish.

Different aspects of biological work of kawakawa have been done by different authors (Raja Bidin, 2002; Rita, 2006; Khoddami, 2012) but no work has been done on genetic population of this species especially in the straits of Malacca. Stock structures in tunas have been determined previously using different method including morphometrics, length frequency, meristics and genetic markers (allozyme, RAPD, AFLP, mitochondrial DNA and microsatellite). The mitochondrial DNA (mtDNA) can be an efficient tool for gathering information for population genetics analyses since it has unique characteristics due to its maternal transmission, rapid rate of evolutionary changes, transmission without recombination, and haploid inheritance (Martins, 2003). In this context, mtDNA variations can be extremely useful for identifying and managing stocks of fish species.

Material and methods

Sampling and DNA sequencing

Kawakawa samples were collected from commercial fishing vessels in Kuala Perlis and markets around Penang during June –October 2012 (Table 1; Fig.1). A part from Kuala Perlis, they were sampled from three different markets; two in the island (Batu Lanchang and Bayan Baru) and one in the mainland (Jalan Tenggara). Each market was sampled twice at different date except for Jalan Tenggara was done three times. All together there are eight groups of samples. Samples were fixed in 90% ethanol and frozen at -20°C until DNA extraction.

Total genomic DNA was obtained from fish caudal or anal fin tissue samples, in order to prevent the sacrifice of the animals using DNeasy Tissue Kit (Qiagen GmbH, Hilden). Amplifications were carried out in a 50 µl final volume of a PCR mix containing GoTaq colourless mastermix (Promega, USA). Two primers:

L14841 5'-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3'

H15149 5'-AAACTGCAGCCCCTCAGAATCATATTTGTCCTCA-3'

were used to amplify mtDNA fragment containing a small portion of the *cyt b* gene (Kocher *et al.* 1989). The PCR reaction was performed as following: an initial incubation at 95°C for 10 min, followed by 30 cycles of PCR (denaturation at 95°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min), and final extension at 72°C for 5 min. the reactions were carried out in a Super Cycler Thermal Cycler (Kyratex, Australia). PCR products were sequenced directly using Applied Biosystem genetic analyzer (1st Base) applying the PCR-primers as sequencing primers.

Data analyses

Nucleic acid sequences were subjected to BLASTn (Altschul *et al.*, 1990) searches at the National Center for *Biotechnology* Information (NCBI), website (<http://www.ncbi.nlm.nih.gov/blast>) and they were aligned using ClustalW software (Thompson *et al.*, 1994). Kimura –2 parameter genetic distances (Kimura, 1980) were determined using the computer program Molecular Evolutionary Genetics Analysis (MEGA Version 5) (Kumar *et al.* 2011). Unique haplotype sequences were obtained using Collapse-1.2. Minimum spanning network was drawn using Network 4.6.0.0.

Table 1 Kawakawa sampling sites; number of samples, and number of haplotypes

Samples	Date of collection	No. of sample	No. of Haplotype
Kuala Perlis (KP)	July 2012	20	3
Batu Lanchang 1 (BLf)	1 st week Oct 2012	16	2
Batu Lanchang 2 (BLs)	2 nd week Sept 2012	18	3
Bayan Baru 1 (BBf)	1 st week Sept 2012	15	5
Bayan Baru 2 (BBs)	4 th week Sept 2012	18	5
Jalan Tenggara 1 (JTf)	1 st week Oct 2012	10	2
Jalan Tenggara 2 (JT _s)	2 nd week Oct 2012	10	1
Jalan Tenggara 3 (JT _t)	3 rd week Oct 2012	6	2

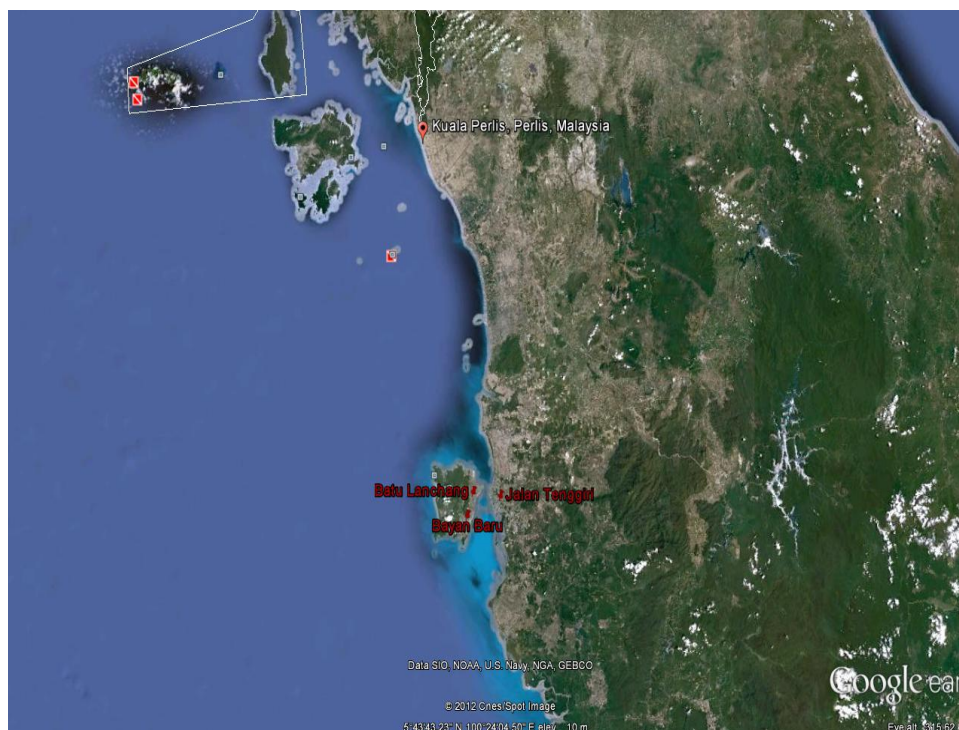


Fig. 1 Map showing the sampling sites of kawakawa

Results

A total of 113 kawakawa were investigated (Table 1). All samples were subjected to PCR and sequencing. PCR primers L14841 and H15149 amplified a 331 bp fragment sequence of *cyt b* gene. Sequence analysis of *cyt b* gene revealed only 6 haplotypes with the most abundant haplotype was KP1 (frequency 65.49%), followed by BBf7

(frequency 9.73%) and BBf4 (frequency 7.96%). These haplotypes were present in all the sampling sites (source of samples) (Table 2). The overall sequence transition/transversion ratio was 1.76 while nucleotide composition exhibited TC bias (T = 31.1%, G = 19.6%, C = 25.1 and A = 24.2%).

The phylogenetic analyses using neighbor-joining method revealed two clades (Fig. 2). Clade 1 is the major lineage which contains most specimens in all 8 sources and strongly supported with bootstrap value of 97%. In contrast, clade 2 is a minor lineage which is weakly supported with bootstrap value smaller than 50%. It contains only twenty three specimens and none from Kuala Perlis.

To unravel and delineate the evolutionary relationships between the haplotypes of kawakawa, a minimum spanning network was constructed. Two haplotype groups separated by 10 mutation steps were observed and were nested into two different lineage (Fig. 3)

Table 2. Distribution of 6 observed mtDNA Cyt *b* haplotypes among population of Kawakawa

haplotype	KP	BLf	BLs	BBf	BBs	JTf	JTs	JTt	total
1	15	11	15	4	6	8	10	5	74
2	2	5	1						8
3	3			1	1			1	6
4				2	3				5
5				5	4				9
6			2	3	4	2			11
total	20	16	18	15	18	10	10	6	113

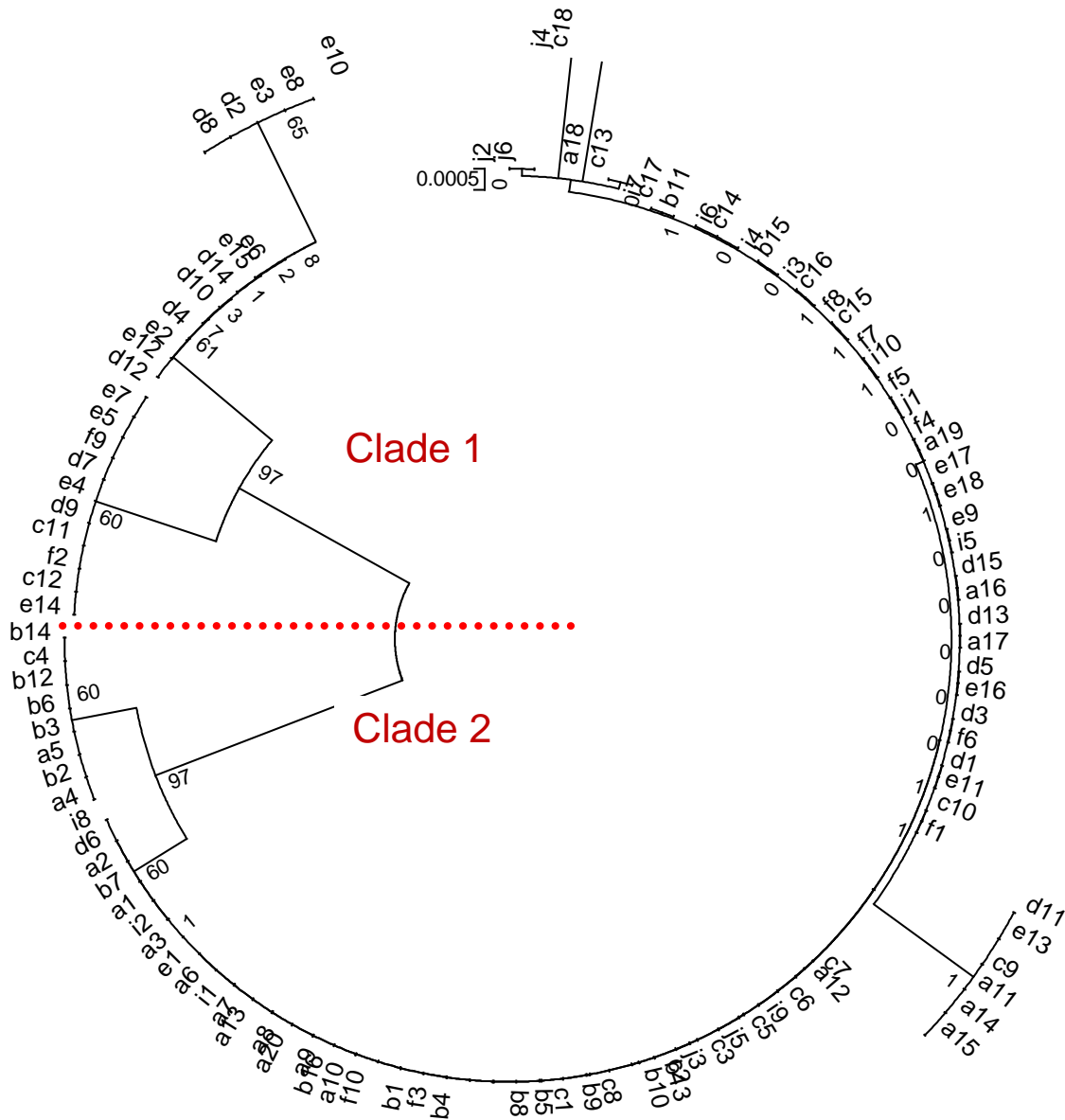


Fig. 2 Neighbor-joining tree estimated from Kimura two-parameter distances among mtDNA lineage of kawakawa. This bootstrap consensus tree inferred from 500 replicates. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. (a-Kuala Perlis; b-Batu Lanchang 1; c-Batu Lanchang 2; d-Bayan Baru 1; e-Bayan Baru 2, f-Jalan Tenggara 1; i-Jalan Tenggara 2; j-Jalan Tenggara 3)

Discussion

Several recent studies focused on tunas and related scombroid species (Carlsson *et al.*, 2004, Vinas *et al.*, 2004) reveal that large marine pelagic fishes show significant genetic

structuring, which may eventually accelerate collapse of stocks if not properly taken into account by fishery management policies. To date, no detailed studies of kawakawa genetic stock structure have been conducted in the straits of Malacca.

The observed homogeneity in the population of kawakawa in the straits of Malacca is similar to kawakawa caught from different oceans (Santos *et al.*, 2010). Marine species are generally characterized by large population sizes, high dispersion capacity during pelagic larval stages, and wide biogeographical distribution. The apparent lack of barriers to dispersal in the marine environment effectively reduces heterogeneity among populations, often making it difficult to differentiate discreet populations (Palumbi, 1992). Population genetic structure of kawakawa in this study conforms to this pattern. Chiou and Lee in 2004 found that MtDNA sequence analyses of five populations of kawakawa in waters of Taiwan showed no genetic heterogeneity among samples. Kawakawa in the Philippines and in Southeast Asia was found to be “panmixia” or mixing (Santos *et al.*, 2010).

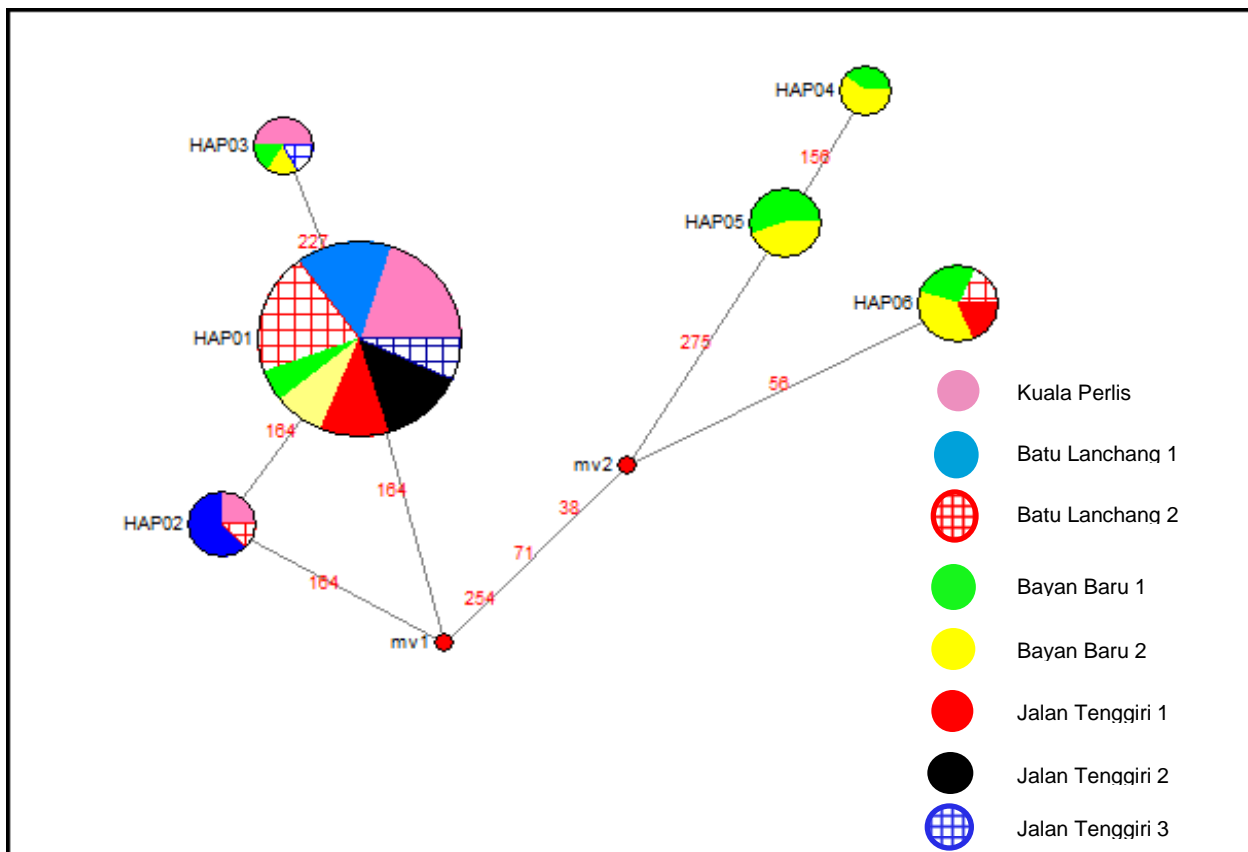


Fig. 3 Minimum spanning network showing relationships among six haplotypes of mtDNA Cyt b of Kawakawa

Analyses of mtDNA direct sequencing data exhibited very little divergent among the eight populations (sources) analyzed, suggesting the existence of single panmictic population of kawakawa in the straits of Malacca.

While the evidence for panmixia is strong, we could not ignore the absence of haplotype 1 and 2 in clade 1, haplotype 2 in the Bayan Baru (BB) and Jalan Tenggara (JT) samples, haplotype 5 in the Kuala Perlis (KP), Batu Lanchang (BL) and Jalan Tenggara (JT) samples. Is it possible that we could be seeing some evidence of a subpopulation in this area, which is not significantly detectable with the present numbers of samples in the study? Increasing sample size or using different class of marker with different population dynamic may reveal heterogeneity and restrictions on gene flow among regions once classed as homogeneous (Ward, 2000). Furthermore, there are limitations to the use of mtDNA in analyzing populations because it does not take into account for genetic consequences of male dispersal. Nevertheless, this study provides important initial information on kawakawa in the straits of Malacca.

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