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Research Article

Identification of Tuna Species in Xincun Harbour, Hainan Province, China Using Mitochondrial DNA Control Region as the Marker

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Abstract

The tuna species from Xincun Harbour, Hainan Province, China were identified in this study. The mitochondrial DNA control region (mtDNA CR) fragments of the tuna samples were amplified and sequenced. The phylogenetic trees were constructed using the amplified control region sequences to identify species of the samples, included *Thunnus* and *Euthynnus* genera. The result showed that using mitochondrial DNA control region as marker accurately distinguish tuna species. Our results indicate that fish of *Thunnus* and *Euthynnus* genera from the sea area near Xincun Harbour were mainly longtail tuna (*Thunnus tonggol*) and Kawakawa (*Euthynuus affinis*). The identification of tuna species in the sea near Xincun Harbour with an accurate and efficient method could provide a good basis for standardizing and promoting the local trade of tuna.

Key words: Species Identification; Tuna; Mitochondrial DNA; Control Region.

Introduction

Fish of Scombridae family, which are the basis of worldwide commercial and recreational fisheries [1], is one of the most important groups of commercial fishes, including genera of *Thunnus*, *Euthynnus*, and Bonito [2]. *Thunnus*, the largest genera in the Scombridae family [3], is one of the most important and most commercially valuable trade fish in the world [4]. There are eight species of the *Thunnus*, including Atlantic bluefin tuna (*Thunnus thynnus*), Pacific bluefin tuna (*Thunnus orientalis*), Southern bluefin tuna (*Thunnus maccoyii*), yellowfin tuna (*Thunnus albacares*), albacore (*Thunnus alalunga*), bigeye tuna (*Thunnus obesus*), blackfin tuna (*Thunnus atlanticus*) and longtail tuna (*Thunnus tonggol*). The individual species various from geographic distribution, in terms of their commercial value, especially albacore is greatly popular among consumers [1]. Although morphological features can be used to identify different tunas, the morphologies of some tuna species are similar that most of people cannot identify these different tuna species clearly in trading. Moreover, identification of commercial processed tuna products which have not kept enough characteristic morphological features is difficult [2,5].

Identification of species is playing a significant role in the food safety [2,6], and molecular identification methods provide an efficient tool for the species identification. For tuna species, it is essential to choose appropriate genes as molecular markers to make an identification. Previous studies have shown that mitochondrial genes have been preferred over nuclear genes as molecular markers of tuna species as the mitochondrial DNA (mtDNA) is maternally

inherited and has a relatively fast mutation rate [2,7,8]. While the mitochondrial genome which is haploid, it is present in a high copy number in the cell. Usually, the mitochondrial cytochrome oxidase subunit I (COI) gene, cytochrome b (Cyt b) gene, 16S rRNA and D-loop region (control region, CR) are used in tuna species identification. However, using Cyt b as genetic marker cannot distinguish some albacore and Pacific bluefin tuna because they are so close genetically [9]. Although COI has been proved that it is a good choice for species identification, some studies have shown that COI cannot successfully differentiate all tuna species [5,10]. The mitochondrial DNA control region, which is a non-coding stretch of DNA that shows high levels of genetic variation, has been proved that it is more accurate than other markers. It has found that the differences in CR gene fragments were the highest when these mitochondrial DNA genes are used as markers to identify tuna species [7]. Using CR genes as markers can distinguish most tuna or tuna-like fishes, except the occurrence of gene introgression [2,5,11,12].

Xincun Harbour, located in the southeast of Xincun town, Lingshui county, Hainan province, is a natural harbor with unique advantages. There are 45 kinds of commercial fish, while tuna is one of the most important trad fish in Xincun Harbour. In recent years, some studies on tuna in the south China sea have been conducted [13,14], but there are few reports on tuna in the sea area around Xincun Harbour, such as the identification of tuna species. Therefore, this experiment is designed for identification of tuna species in Xincun Harbour, Hainan province, China, using mitochondrial DNA control region as markers, in order to make sure the main tuna species in the sea near Xincun Harbour and to provide a good basis for regulating and promoting the local trade of tuna.

Materials and Methods

Sample collection

A total of 15 *Thunnus* and 15 *Euthynnus* were collected from the sea area (18°22'55.35'' N, 109°58'20.23''E, (Figure 1) near Xincun Harbour, Xincun town, Lingshui county, Hainan province, while the preliminary morphological identification was performed to distinguish genera. The tail fins of three *Thunnus* and three *Euthynnus* were collected respectively as samples for DNA extraction, storing in 95% alcohol and at 4°C.

Primer design

The control region (CR) gene was selected as the marker for species identification of tuna species of *Thunnus* genera and *Euthynnus* genera, primers referring to the design of Alvarado et al. (Forward primer: 5'-TACCCCAAACTCCCAAAGCTA -3' and Reverse primer: 5'-TACCCCAAACTCCCAAAGCTA -3') for PCR amplification [15].

DNA extraction and PCR amplifications

DNA of all samples was extracted from the tail fins using the TIANamp Genomic DNA Kit (TIANGEN BIOTECH, Beijing City, China). Their lengths were estimated by 2% agarose gel electrophoresis. The PCR amplification for the CR was carried out in 20 ml volumes, containing 1.0 μL DNA template, 10.0 μL Taq qPCR Probe Master Mix II, 0.5 μL forward primers and 0.5 μL reverse primers, and 8 μL ddH2O, and under the thermal cycles conditions: initial denaturation at 94 °C for 5 min; 30 cycles of denaturing at 94 °C for 45 s, annealing at 50 °C for 45 s, and extension at 72 °C for 1 minute.

Sequencing and phylogenetic analysis

The PCR products were checked on 2% agarose gel and then sequenced in one direction on an ABI 3730 automated sequencer after purifying. Then the phylogenetic tree based on CR sequences was constructed with MEGA 5.2 software, using the maximum likelihood (ML) method [16], while all sequences aligning with ClustalW, the Kimura two-parameter distance method for calculating the genetic divergence, non-parametric bootstrap analysis with 1000 iterations for evaluating the robustness of the tree [17]. In addition to the sample sequences obtained in this experiment, the CR sequences of *Thunnus thynnus* (GenBank Accession number

Figure 1: The sea area (18°22'55.35'' N, 109°58'20.23''E) near Xincun Harbour, Xincun town, Lingshui county, Hainan province, collected samples of tunas or tuna-like species.

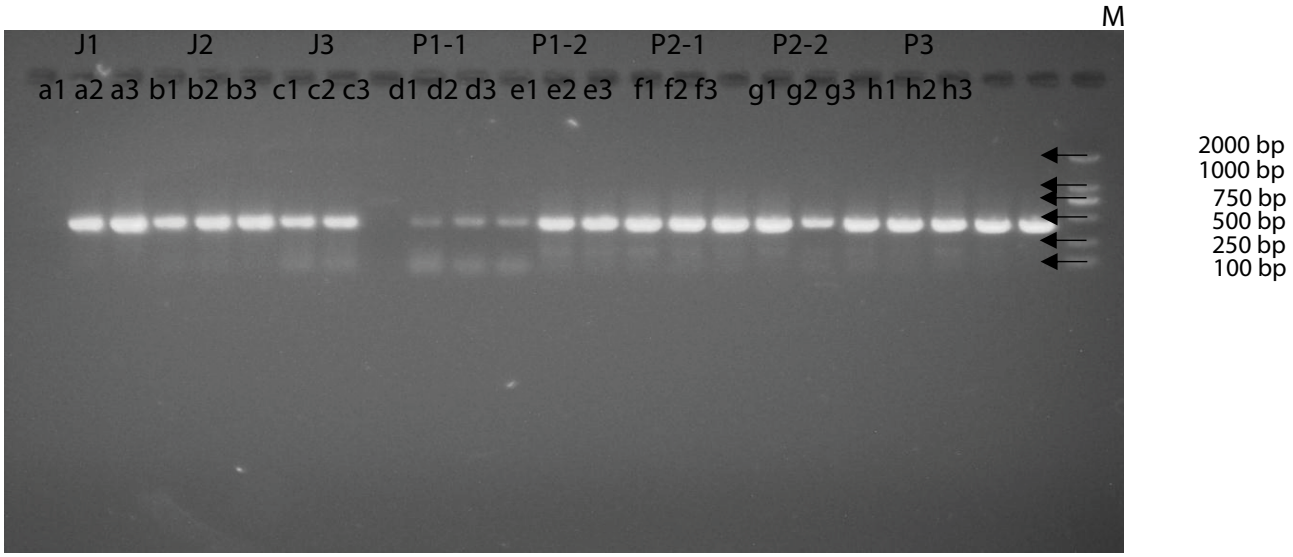

Figure 2: PCR amplification of control region (CR) from the sample of tuna or tuna-like species.

AY650409-AY650414), *Thunnus orientalis* (GenBank Accession number AB185022, KF906721, LC377898, NC008455), *Thunnus maccoyii* (GenBank Accession number GU256523, JN086150, KF925362, NC014101), *Thunnus albacares* (GenBank Accession number AY899520-AY899524), *Thunnus alalonga* (GenBank Accession number AF390331, AF390353-AF390354, HQ853210-HQ853210), *Thunnus obesus* (GenBank Accession number AY640302-AY640303), *Thunnus atlanticus* (GenBank Accession number KU955343-KU955344), *Thunnus tonggol* (GenBank Accession number MF593022, MF593043-MF593044, MF593048), *Euthynuus affinis* (GenBank Accession number AP012946, HQ630700, KM651783, NC025934) and *Euthynuus alletteratus* (GenBank Accession number AB099716, KY446419-KY446420, NC004530) were retrieved from the National Center for Biotechnology Information (NCBI) database.

Results and discussion

PCR amplification and sequencing results

Estimated by agarose gel electrophoresis, the CR fragment of all sample of tuna or tuna-like species was successfully amplified (Figure 2). The lengths of PCR products were among 400-450 bp, which was consistent with the expected results.

M, DNA Marker;

J1 (a1–a3), the first sample of genus *Thunnus*;

J2 (b1–b3), the second sample of genus *Thunnus*;

J3 (c1–c3), the third sample of genus *Thunnus*;

P1-1 (d1–d3) and P1-2 (e1–e3), the first sample of genus *Euthynnus*;

P2-1 (f1–f3) and P2-2 (g1–g3), the second sample of genus *Euthynnus*;

P3 (h1–h3), the third sample of genus *Euthynnus*.

Species identification

Species identification was performed for three samples of *Thunnus* and three sample of *Euthynnus*. Through the construction of phylogenetic tree, CR gene could accurately distinguish different species of tuna, with bootstrap supports of more than 75%. The result showed that the samples of *Thunnus*, collected from the sea area near Xincun Harbour, were clustered into the group of *Thunnus tonggol*, with bootstrap supports of 94% (Figure 3). And *Thunnus orientalis* and *Thunnus alalonga* could be accurately distinguished, with bootstrap supports of 90% and 94%, while they seemed to have a closely relationship [18]. The samples of *Euthynnus* were grouped together with *Euthynuus affinis* (Figure 4). The study of Zhang et al. has also proved that there is a high catch rate of *Euthynuus affinis* in the south China sea in the fishing ground of large-scale light falling-net fisheries [19].

However, some studies consider that the CR fragment was not suitable for distinguishing tuna at the genus level [7,20]. Using the ribosomal DNA first internal transcribed spacer (ITS1) for an additional analysis can be conducive to identify the tuna species which are closely related [5,11].

Conclusion

Using mitochondrial DNA to study the relationship between species and phylogenetic analysis, which can effectively avoid the errors caused by morphological characteristics observation and different geographic populations, is very necessary for species identification. For the identification of various tuna species, it is a good choice to construct phylogenetic tree with CR gene. The identification of tuna species in the sea near Xincun Harbour with an accurate and efficient method can provide a good basis for standardizing and promoting the local trade of tuna.

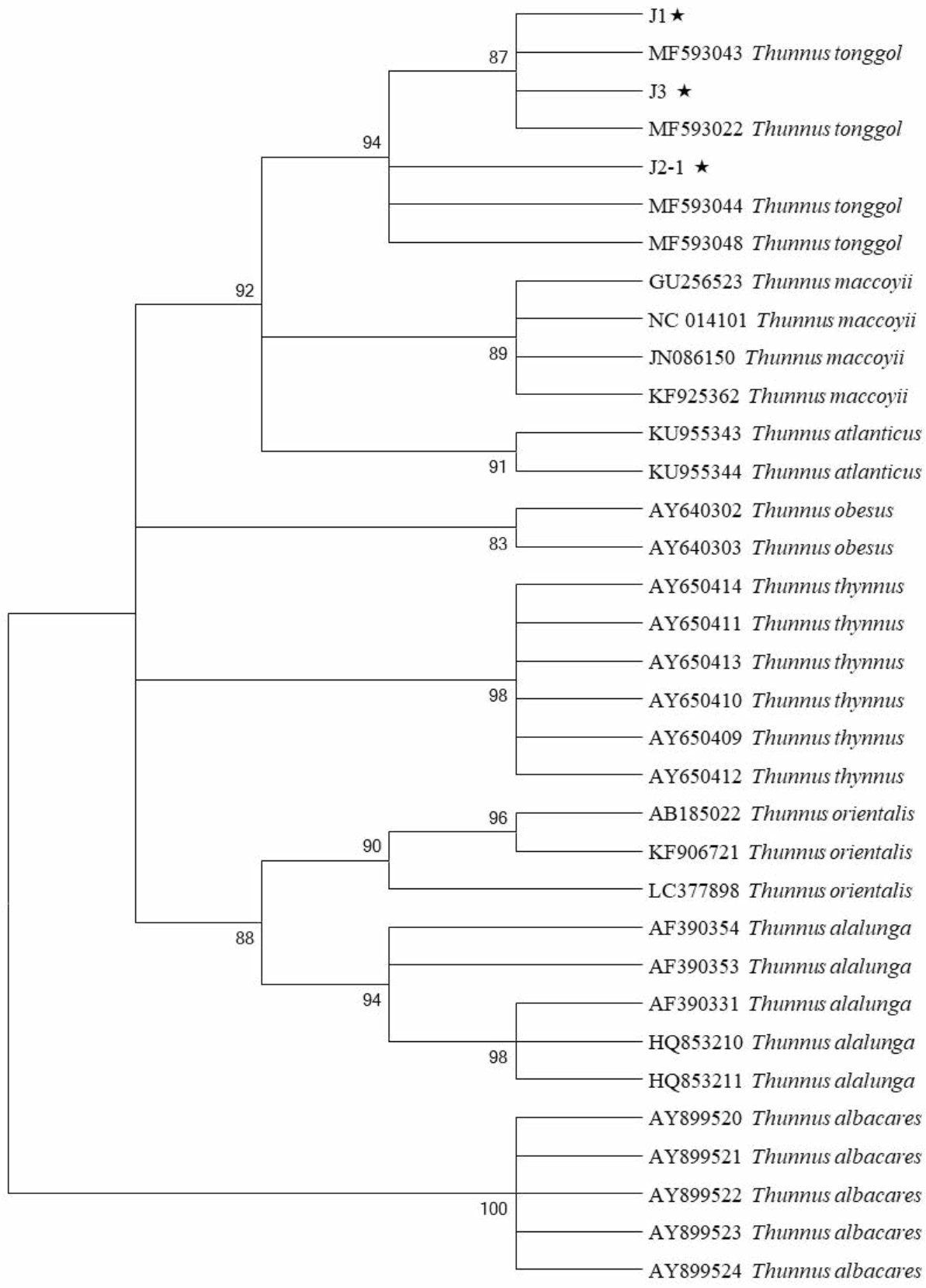

Figure 3. Maximum likelihood tree based on phylogenetic analysis of the fragments of CR genes, showing the relationships of tuna species of genus *Thunnus*. Numbers at nodes indicate bootstrap values. J1, J2, and J3, the sample of *Thunnus*, with mark "★".

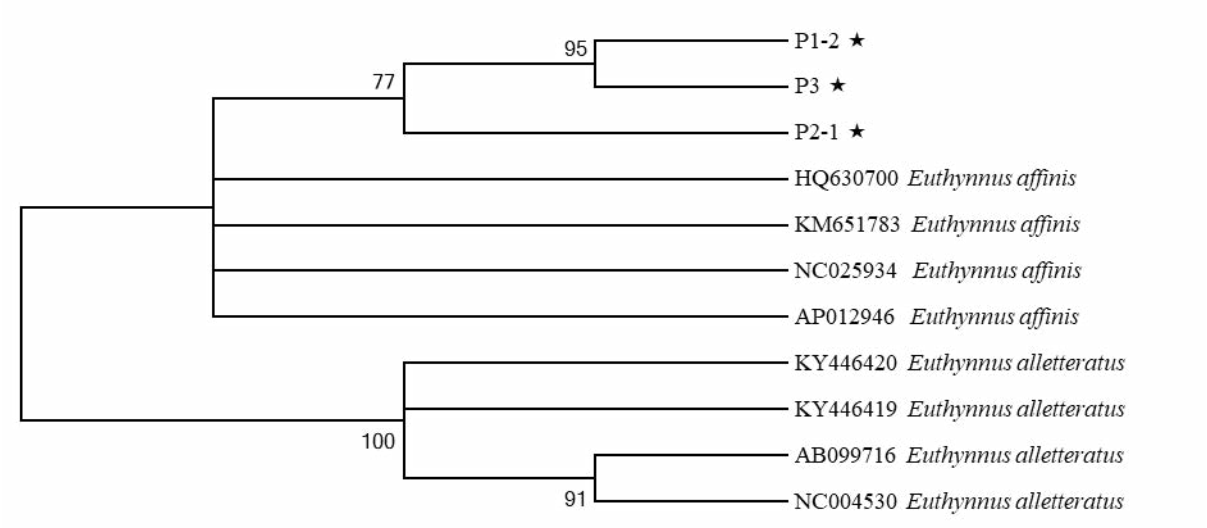

Figure 4 Maximum likelihood tree based on phylogenetic analysis of the fragments of CR genes, showing the relationships of tuna species of genus *Euthynnus*. Numbers at nodes indicate bootstrap values. P1-2, P2-1 (f1–f3) and P3, the sample of *Euthynnus*, with mark "★".

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References

1. Carrera E, Terni M, Montero A, García T, González I et al. (2013) ELISA-based detection of mislabeled albacore (*Thunnus alalunga*) fresh and frozen fish fillets. Food Agr Immunol 25, 569-577.
2. Liu S, Xu K, Wu Z, Xie X, and Feng J (2016) Identification of five highly priced tuna species by quantitative real-time polymerase chain reaction. Mitochondrial DNA A DNA Mapp Seq Anal 27, 3270-3279.
3. Kunal SP and Kumar G (2013) Cytochrome Oxidase I (COI) sequence conservation and variation patterns in the yellowfin and longtail tunas. Int J Bioinform Res Appl 9, 301-309.
4. Amaral CR L, Maciel V, Goldenberg Barbosa R, Silva DA, Amorim A et al. (2017) Tuna fish identification using mtDNA markers. Forensic Sci Int-Gen 6, e471-e473.
5. Vinas J and Tudela S (2009) A validated methodology for genetic identification of tuna species (genus *Thunnus*). PLoS One 4, e7606.
6. Lin Y, Yanhua J, Qingjiao L, Xiaochuan L, Wenjia Z et al. (2013) Progress and prospect of DNA-based techniques for processed aquatic product species identification. Chinese fishery quality and standards 3, 33-39.
7. Xu K, Feng J, Ma X, Wang X, Zhou D et al. (2015) Identification of tuna species (*Thunnini tribe*) by PCR-RFLP analysis of mitochondrial DNA fragments. Food Agr Immunol 27, 301-313.
8. Chow S, Nakagawa T, Suzuki N, Takeyama H and Matsunaga T (2006) Phylogenetic relationships among *Thunnus* species inferred from rDNA ITS1 sequence. J Fish Biol 68.
9. Chow S and Kishino H (1995) Phylogenetic relationships between tuna species of the genus *Thunnus* (Scombridae: Teleostei): Inconsistent implications from morphology, nuclear and mitochondrial genomes. J Mol Evol 41, 741-748.
10. Cawthorn DM, Steinman HA and Witthuhn RC (2011) Establishment of a mitochondrial DNA sequence database for the identification of fish species commercially available in South Africa. Mol Ecol Resour 11, 979-991.
11. Mitchell JK and Hellberg R S (2016) Use of the Mitochondrial Control Region as a Potential DNA Mini-Barcoding Target for the Identification of Canned Tuna Species. Food Anal Method 9, 2711-2720.
12. Pedrosa Gerasmio IR, Babaran RP and Santos MD (2012) Discrimination of Juvenile Yellowfin (*Thunnus albacares*) and Bigeye (T-obesus) Tunas using Mitochondrial DNA Control Region and Liver Morphology. Plos One 7, 7.
13. Bo F, Zhonglu L and Gang H (2014) Biology and distribution of *Thunnus obesus* and thunnus albacares in the south China sea. Oceanologia Et Limnologia Sinica 45, 886-894
14. Peng Z, Lin Y, Xufeng Z and Yongguang T (2010) The present status and prospect on explolition of tuna and squid fishery resources in south China sea. South China fishery science 6, 68-74.
15. Bremer JRA, Baker AJ and Mejuto J (1995) Mitochondrial DNA control region sequences indicate extensive mixing of swordfish(Xiphias gladius)populations in the Atlantic Ocean. Can J Fish Aquat Sci 52, 1720-1732.
16. Hall BG (2005) Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. Mol Biol Evol 22, 792-802.
17. Tamura K, Peterson D, Peterson N, Stecher G, Nei M et al. (2011) MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol 28, 2731-2739.
18. Abdullah A and Rehbein H (2016) The differentiation of tuna (family: Scombridae) products through the PCR-based analysis of the cytochrome b gene and parvalbumin introns. J Sci Food Agric 96, 456-464.
19. Heng Z, Zuli W, Weifeng Z, Shaofei J, Peng Z et al. (2016) Species composition, catch rate and occurrence peak time of Thunnidae family in the fishing ground of light falling-net fisheries in the Nansha Islands area of the South China Sea. Marine fishery 38, 140-148.
20. Collette BB, Reeb C and Block BA (2001) Systematics of the tunas and mackerels (Scombridae). Fish Physiol 19, 1-33.