

DNA Barcoding of fishes in South China Sea

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INTRODUCTION

- Fishes are the main animal protein source for human beings and they play a major role in the aquatic ecosystem. Species delimitation and eventual recognition of them is not only of interest for taxonomy and systematics, but also is tightly related with authentication of food products and studies of natural history and ecology such as fishery management, egg & larval dispersal and recruitment and spawn area estimates.
- Recently, DNA barcoding has been proposed as a universal method to identify species and uncover biological diversity. Hebert (2004) demonstrated that sequence divergences for 5' region of the mitochondrial DNA gene cytochrome oxidase subunit I (COI) were generally much greater between species than within them for many species and in turn suggests that the approach is widely applicable across phylogenetically distant animal groups.
- South China Sea belongs to Indo-west Pacific Ocean, which is regarded as the center of the world's marine biodiversity. The species-rich biota of this area is particularly attractive to test the reliability and efficiency of DNA barcoding.

METHODS

- All specimens were preserved in 70% ethanol. Tissue samples were dissected from the dorsal muscle and genomic DNA extracted according to the protocol in Barcode of Life (Ivanova et al., 2006). Firstly, fragments of 5' region of mitochondrial COI gene were amplified using C_FishF111/ C_FishR111 and C_VF1LF11/ C_VR1LR11 primer combinations (Ivanova et al., 2007).
- Amplicons were visualized on 2% agarose E-Gel® 96-well system (Invitrogen), and only for plates, which held >70% positive amplicons, were selected for sequencing PCR.
- Final PCR products were directly sequenced using an ABI 3730 capillary sequencer according to manufacturer's instructions.
- DNA sequences were aligned with SeqScape v.2.1.1 software (Applied Biosystems, Inc.). Sequence divergences were calculated using the Kimura two parameter (K2P) distance model, and unrooted NJ trees based on K2P distances were created in MEGA software.
- Distribution of these categories of K2P distances: intraspecific distances (S), inter-species within the congener (G) and inter-species from different genus but within intra-family (F) were plotted using the boxplot. Boxplots describe median (central bar), interquartile range (IQR: between upper [Q3] and low [Q1] quartile), values lying within 1.5 × IQR beneath Q1 or 1.5 × above Q3 ("whiskers") and extreme values (outliers). Mann-Whitney tests were performed between S, G and F distributions to estimate the overlap among taxonomic ranks.

RESULTS

- 321 sequences (all >500 bp) belong to 122 species (one species was identified to the genus level) were ultimately obtained. Specific primer combinations and electropherogram trace files for each specimen are available at FSCS project in BOLD (Ratnasingham.&Hebert, 2007).
- The average K2P distance of intraspecies was 0.319% and it increased sharply to 15.742% among individuals of congeneric species. Overall, the average of genetic distance among congeneric species is nearly 50 fold than that of intraspecies. For species within high taxonomic ranks such as families, orders and classes, pairwise genetic divergences increased gently and reached 20.199%, 24.656% and 25.225%, respectively (Table 1).
- The interspecific hybridization between *Pampus cinereus* and *Pampus argenteus* was detected in this study. For several specimens, they possessed combinations of morphological characteristics from *Pampus cinereus* and *Pampus argenteus* (Fig.1).

comparisons within	taxa	number of comparisons	mean	median	minimum	maximum	s.e.#
species	121	453	0.319	0.150	0	2.021*	0.018
genus	85	397	15.742	16.490	0.154**	25.189	0.292
family	55	848	20.199	19.850	11.532	34.333	0.134
order	15	17881	24.656	—	12.923	39.627	0.024
class	2	29262	25.225	—	15.730	40.800	0.016

RESULTS

- In case the suspicious congeneric K2P distances in the genera *Pampus* are excluded, the pairwise genetic divergences for the other individuals among congeneric species are all above 10%. Actually, there are no overlaps between intraspecific K2P distances and those among congeneric species within the same family (Fig.2).
- Values of G (interspecific genetic distance within the congener) fluctuated within a narrow range, but values of F (interspecific genetic distance from different genus but within intra-family) were different between families (Fig.3).
- In this study, for individuals from different localities, some intraspecific genetic variations reduced to zero within families Carangidae, Sciaenidae and Mullidae. However, some pairwise K2P distances exceeded 1.00% within the coastal species such as *Acentrogobius caninus*, *Scomber japonicus*, *Terapon jarbua*, *Upeneus sulphureus*, *Elops hawaiiensis*, *Gymnothorax pseudothyrsoides* and *Dendrophysa russelli*.

DISCUSSION

- Except for the introgression for genus *Pampus*, there are no overlaps between genetic variations of S (intraspecific genetic distance) and G, and even so, intraspecific specimens formed monophyletic units and could be definitely differentiated from other species.
- Complex causes were responsible for deviations from taxonomic monophyly, and one potential cause of polyphyly for species is the occasional mating between distinct species and resulting in gene transfer to hybrid offspring. Mitochondrial genes are generally subjected to introgression more frequently than nuclear ones and introgression also causes the phylogenetic paraphyly, like the hybridization between *Pampus argenteus* and *Pampus cinereus* in this study.
- Biological mechanisms or the complex water currents may play the main contribution to the fluctuation of intraspecific divergences, in particular, for minitypic species lacking the gene flow.
- An assumed threshold is helpful to expedite discovering new species and biodiversity, especially in facing of biotas involving with few studies.

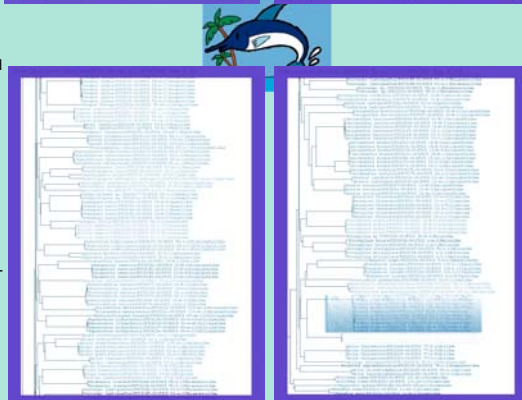


Fig. 1
Above - Neighbor-joining (NJ) tree of COI sequences. Scale: 5% K2P distance.
The front numbers on branches are the process ID and the latter are the sample ID in Project Barcode of Life for specimens. The shadow indicates interspecific hybridization between *Pampus cinereus* and *Pampus argenteus*.

Table 1
Above - Genetic divergences (percentage, K2P distance) within various taxonomic levels. Data are based on 321 sequences (>500 bp) from 122 species. ** between *Pampus cinereus* and *Pampus argenteus*, discussed in the text.

Fig. 3
Right - Boxplot distributions of S, G and F.
Intra-species (S), inter-species among congeneric species (G), and inter-genera but intra-family (F) K2P distances for different families.

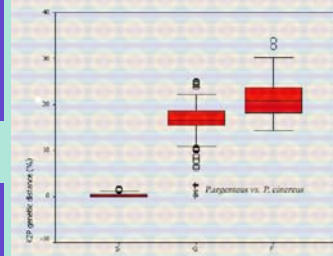


Fig. 2
Above - Box plots of K2P distances.
IQR: interval into which the "central" 50% of the data fall. Black bar in the box indicates the median. Circle: "Mild outlier" and asterisks: "extreme outliers". Extreme outliers are discussed in the text.

CONCLUSIONS

Effectiveness of species Identification

- These results suggest DNA barcoding is definitely effective for species identification of fishes from South China Sea, and some phylogenetic information was also revealed. The Barcode of Life provided a powerful platform for molecular taxonomy.

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