Short Communication

The Species Identity of the Two Color Morphs of Northern Snakehead (Channa argus) Based on mtDNA Control Region Sequences

Aiguo Zhou 1,2, Di Sun 1,2, Shulin Liu 1,2, Yongyong Feng 1,2, Yue Zhang 3, Yanfeng Chen 4, Shaolin Xie 1,2 * and Jixing Zou 1,2 *

1Joint Laboratory of Guangdong Province and Hong Kong Region on Marine Bioresource Conservation and Exploitation, College of Marine Sciences, South China Agricultural University, Guangzhou 510642, China
2Guangdong Laboratory for Lingnan Modern Agriculture, South China Agricultural University, Guangzhou 510642, China
3Departments of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA USA 90089
4School of Life Science and Engineering, Foshan University, Foshan 528231, Guangdong, China

ABSTRACT

The control region for mitochondrial DNA (mtDNA) has been considered as one of the most effective molecular markers in the study of identification for fish species, which has a fast evolutionary rate. And thus, in the present study, investigation of genetic comparison was performed based on the complete sequences of mtDNA control region for “Bicolor” and “White” types of northern snakehead (Channa argus) due to an uncertain classification of them. The results showed that the genetic distance for the inter-species ranged from 0.000 to 0.492 for the Channidae family. And the analysis of sequences showed that there were nine haplotypes in 60 individuals, which were eight unique and one shared haplotypes. In addition, the genetic distance for inter-species of all the haplotypes ranged from 0.000 to 0.004. And the mean pair-wise genetic distance between the two color morphs were estimated to be 0.001. This indicated that the “Bicolor” and “White” types of northern snakehead belong to the same species at the molecular level. Moreover, all the haplotypes were gathered together by the Neighbour-Joining (NJ) tree, further confirming that the two kinds of snakehead morphs belong to one species.

One characteristic of the great northern snakehead is gradient variation from north to south of China, which is usually caused by the difference of temperature, light density, and etc. In other words, some areas show a relatively steep performance, but some areas show gentle performance (Wang et al., 1992; Zhou et al., 2015). Accordingly, there are two distinct color morphs of northern snakehead Channa argus, the “bicolor” type that was widely distributed in China, and the monochromatic “white type” that was white without any blotches and only found in Jialing waters (Ding, 1994). Some researchers have classified them as two distinct species (Kimura, 1934), however, others have treated them as color varieties of one single species; these judgments were based solely on the morphological aspects (Wang et al., 1992).

In order to elucidate the genetic relationship between the two color morphs and clarify genetic relatedness among the family Channidae, we herein determined the level of sequence divergence between the two colored morphs based on the complete sequences of mtDNA control region.

MATERIALS AND METHODS

Northern snakeheads were collected in 2014 and 2015 from three locations, in Jialing river systems in China. Basic characteristics of the sites for sampling were given in Table I. Fish were captured using lift and seine nets. All samples were identified according to morphological characters (Courtenay and Williams, 2004). Fin clips were taken as tissue samples and preserved in 95% ethanol. Total genomic DNA was extracted from the caudal fin using a standard extraction kit (DNAsy tissue kit, Baitaike Biotech Co., Ltd, China). The genes for mitochondrial control region and partial adjacent regions were amplified using the following primers:
F: 5′-ATCGGACAAGTCGCTCTTTCCTCT-3′ and
R: 5′-TGCGGATACTTGCATGTGTAAGT-3′ (Zhou et al., 2016). The PCR amplification was performed in a PE 9700 thermocycler (PerkinElmer Co. Ltd., USA). The amplification reaction was carried out in 50μl volumes consisting of 25μl of 2× PCR mix buffer, 0.5μl of 2.5 U/μl Taq DNA polymerase, 2μl of 100 ng/μl DNA template, 2μl of 10 mM of each primer, and 20.5μl of sterile ultrapure water (Dongsheng Biotech Co., Ltd, China). Thermal cycling condition were 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 60 sec for annealing, and 72°C for 90 sec for extension, and then followed by 72°C for 10 min for a final extension. The PCR product was purified using the QIAquick PCR purification kit (Qiagen, Hilden). Sequencing was conducted on an ABI377 automatic sequencer with both forward and reverse primers.

The nucleotide sequences of mtDNA control region and adjacent regions were aligned using Clustal-X (Thompson et al., 1997) and then were edited and connected using BioEdit (Hall, 1999). The number of haplotypes and its frequencies, number of polymorphic sites, nucleotide composition, as well haplotype diversity and nucleotide diversity were estimated by Arlequin 3.0 (Excoffier et al., 2005). The dendrogram of nine populations was constructed using Kimura 2-parameter model in MEGA 6.0 based on the pairwise genetic distance (Tamura et al., 2013).

All of the procedures and animal handling were performed in accordance with the guide for the Chinese Association for Laboratory Animal Sciences. Approval of the study was obtained from the Animal Ethics Committee of South China Agricultural University.

Results and discussion

A total of 1060 bp was sequenced for 75 individuals of mtDNA control region. The complete sequences were aligned unambiguously with 907bp. The variation sites (510), parsimony informative polymorphic sites (282) and conversion/transversion ratio (0.90) were obtained.

Six haplotypes (WtcaHD1–D6) of mtDNA control region for “white” type and four haplotypes (BtcaHD1–D4) of “bicolor” type northern snakeheads that were defined from 60 nucleotide sequences were obtained (Table II). The nucleotide sequences of all the haplotypes were submitted in GenBank (KU852448-KU852457). From the haplotypes, the sequences of WtcaHD1 and BtcaHD2 were the common haplotypes, which have the largest number in terms of the distribution.

For genetic distance analysis Lateolabrax maculatus and Epinephelus coioides were used as outgroups. Based on DNA sequence of the mtDNA control region of Channa species in NCBI, sequences alignment via MEGA6.0 showed that all the haplotypes were gathered together as a branch. Genetic distance was calculated using Kimura 2-parameter model. The inter-specific genetic distances between nine kinds of Channidae were 0.000-0.496. And the maximum of genetic distance between the “white” and “bicolor” type C. argus was 0.004. The intra-species genetic distance based on haplotype sequences was 0.001. All the genetic distances and standard errors were shown in Table III.

The molecular phylogenetic trees were composed from the NJ tree. The phylogenetic tree showed that all haplotypes were clustered into a single group with a high confidence value between the “white” and “bicolor” type C. argus. And the fact that the progenies of C. argus x C. maculate was also clustered into one group provides a piece of evidence that the mtDNA belongs to maternal inheritance (Fig. 1).

The control region for mtDNA acts as a noncoding sequence between the tRNA⁰⁰ pros and tRNA⁰⁰ pros genes on the mitochondrion. The control region has the largest and fastest growing region of variations in the vertebrate mitochondrial sequences. And it generally used for phylogenetic analysis within and between populations (Nikolic et al., 2016). The analysis of sequences analysis showed that all the Channidae family have rich mtDNA polymorphism. And the ratio of transition and transversion was 0.90, which means that the base variation in the Channidae family is high and the base substitution tends to saturation. This result was consistent with the characteristics how the sequence for the control region of mtDNA evolves.
Table I. Basic information of sampling sites and size (n) of *Channa* species.

| Species                  | Location name                          | GPS coordinate             | Altitude (m) | Date (Size) |
|--------------------------|----------------------------------------|----------------------------|--------------|-------------|
| **Biocolor type** *C. argus* | Neijiang city, Sichuan province, China | 29°35'3.16"N 105°2'49.95"E | 332          | 2015(30)    |
| **White type** *C. argus*  | Neijiang city, Sichuan province, China | 29°34'14.64"N 105°4'1.95"E | 332          | 2014(19)    |
| *C. maculata*            | Ziyang city, Sichuan province, China   | 30°1'628.77"N 104°38.39.46"E | 357          | 2014(11)    |
| *C. striata*             | San Jiaozen, Zhongshan city, Guangdong province, China | 22°39'14.31"N 113°25'43.95"E | 1            | 2015(3)     |
| *C. maculata x C. argus* | Guangzhou city, Guangdong province, China | 23°5'43.82"N 113°14'19.60"E | 3            | 2015(3)     |
| *C. asiatica*            | Guangzhou city, Guangdong province, China | 23°5'48.06"N 113°14'5.13"E | 3            | 2015(3)     |
| *C. micropeltes*         | Guangzhou city, Guangdong province, China | 23°5'28.31"N 113°14'1.34"E | 3            | 2015(2)     |
| *C. striata*             | Vientiane city, Laos                   | 17°57'52.27"N 102°35'17.97"E | 167          | 2014(2)     |
| *C. lucius*              | Ha noi City, Vietnam                   | 21°141.45"N 105°51'48.94"E | 12           | 2014(2)     |

Table II. Haplotype information of mtDNA control region among white and biocolor type *C. argus*.

| Haplotype          | Populations (number of the haplotypes and its frequencies) | Common haplotype |
|--------------------|------------------------------------------------------------|------------------|
|                    | white type frequencies | biocolor type frequencies | mtDNA control |
| WtcaHD1            | 23 | 0.77 | BtcaHD1 | WtcaHD1, |
| WtcaHD2            | 2  | 0.067|
| WtcaHD3            | 1  | 0.035|
| WtcaHD4            | 2  | 0.067|
| WtcaHD5            | 1  | 0.035|
| WtcaHD6            | 1  | 0.035|
| BtcaHD1            | 25 | 0.83 |
| BtcaHD2            | 1  | 0.033|
| BtcaHD3            | 2  | 0.067|

WtcaH and BtcaH represent the Haplotype of white and biocolor type *C. argus*. D is mtDNA control region.

Table III. Pairwise distances calculated using Kimura 2-parameter model for mtDNA control region.

| Wtca HD1 | Wtca HD2 | Wtca HD5 | Wtca HD6 | Wtca HD3 | Btca HD1 | Btca HD2 | C. argus x C. maculata | C. maculata x C. argus | C. asiatica | C. striata | C. maculata x C. argus | P. insignis | E. coioides | L. maculatus |
|---------|---------|---------|---------|---------|---------|---------|----------------------------|--------------------------|-------------|-----------|------------------------|------------|------------|--------------|
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
| 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.000   | 0.012                      | 0.012                    | 0.012       | 0.012     | 0.012                  | 0.012      | 0.012      | 0.012         |
For different species in family or genus category, a larger inter-specific distance is a prerequisite for accurate identification of species (Peng et al., 2009). Hebert et al. (2003) analyzed 13320 species of 11 phylums in the animal kingdom, indicating that the genetic distances within the species were generally less than 0.020 and that most of the genetic distances for intra-specific were less than 0.010. The results of this study showed that the average genetic distance within two color morphs of northern snakeheads was 0.001. The maximum genetic distance between different haplotypes is 0.004, which was less than 0.010, these results indicated that they belonged to the same species and they are not subspecies at the molecular level. And compared with other Channidae species, the genetic distances among them ranged from 0.092 to 0.483. Previous studies showed that many subspecies were at intermediate values as expected, but several had levels of divergence equivalent to populations, resulting in classifying errors (Rosel et al., 2017). The control region of mtDNA has been used successfully to determine the phylogeography and structure of populations within species (Craig et al., 2016). From NJ tree, all the Channidae species clustered together and all the haplotypes clustered in a clade solely with C. argus x C. maculate, which is consistent with maternal inheritance. These results indicated that the “white” type is probably an albino of “bicolor” type C. argus. This conclusion is consistent with that of our previous studies (Zhou et al., 2016, 2017, 2018). In summary, these results have certain application value in phylogenetic and germplasm analysis.

Acknowledgements

This work was supported by the Science and Technology Planning Project of Guangdong Province (2017A020225035, 2016A020210141); Natural Science Foundation of Guangdong Province (2020A1515010367). Fund Fostering Talents for Young Scholars of South China Agricultural University (201707N025); and Talent introduction special funds of South China Agricultural University (201707N025, 2016A020210141); Natural Science Technology Planning Project of Guangdong Province (2015A020225035, 2016A020210141); Natural Science Technology Planning Project of Guangdong Province (2017A020225035, 2016A020210141); Natural Science Technology Planning Project of Guangdong Province (2015A020225035, 2016A020210141); Natural Science Technology Planning Project of Guangdong Province (2015A020225035, 2016A020210141); Natural Science Technology Planning Project of Guangdong Province (2015A020225035, 2016A020210141); Natural Science Technology Planning Project of Guangdong Province (2015A020225035, 2016A020210141). This work was supported by the Science and Technology Planning Project of Guangdong Province (2017A020225035, 2016A020210141); Natural Science Foundation of Guangdong Province (2020A1515010367). Fund Fostering Talents for Young Scholars of South China Agricultural University (201707N025); and Talent introduction special funds of South China Agricultural University (201707N025). We also wish to express our appreciation to the anonymous reviewers for providing valuable comments on the manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Courtenay, W.R. and Williams, J.D., 2004. Snakeheads (Pisces, Channidae): a biological synopsis and risk assessment. US Geological Survey. https://doi.org/10.3133/cir1251
Craig, E.H., Adams, J.R., Waits, L.P., Fuller, M.R. and Whittington, D.M., 2016. PLoS One, 11: e0164248. https://doi.org/10.1371/journal.pone.0164248
Ding, R.H., 1994. The fishes of Sichuan, China. 1st edition. Sichuan Science and Technology Press, Chengdu, pp. 554.
Excoffier, L., Laval, G. and Schneider, S., 2005. Evol. Bioinf., 1: 47. https://doi.org/10.1177/117693430500100003
Hall, T.A., 1999. Nucleic Acids Symp. Ser., 41: 95-98.
Hebert, P.D.N., Cywinska, A. and Ball, S.L., 2003. Proc. R. Soc. Lon. B. Biol. Sci., 270: 313-321. https://doi.org/10.1098/rspb.2002.2218
Kimura, S., 1934. J. Shanghai Sci. Inst., 3: 11-247. https://doi.org/10.1088/0950-7671/11/8/303
Nikolic, N., Jérôme, M., Fonteneau, A., Evano, H. and Verrez-Bagnis, V., 2016. Environ. Biol. Fishes, 99: 171-178. https://doi.org/10.1007/s10641-015-0464-7
Peng, J.L., Wang, X.Z., Wang, D. and He, S.P., 2009. Acta Hydrobiol. Sin., 2: 271-276.
Rosel, P.E., Hancock-hanser, B.L., Archer, F.I., Robertson, K.M., Martien, K.K., Leslie, M.S., Berta, A., Cipriano, F., Viricel, A., Viala-Martinez, K.A. and Taylor, B.L., 2017. Mar. Mamm. Sci., 33: 76-100. https://doi.org/10.1111/mms.12410
Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. Mol. Biol. Evol., 30: 2725-2729. https://doi.org/10.1093/molbev/mst197
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G., 1997. Nucl. Acids Res., 25: 4876-4882. https://doi.org/10.1093/nar/25.24.4876
Wang, J.X., Zhao, X.F., Zhuo, C.W. and Liao, Z.G., 1992. Trans. Oceanol. Limnol., 2: 51-57.
Zhou, A.G., Chen, J.T., Xie, S.L., Chen, Y.F. and Zou, J.X., 2017. Mitochondrial DNA Part A., 27: 1419-1420. https://doi.org/10.3109/19401736.2014.953073
Zhou, A.G., Wang, C., Jiang, W.Z., Li, Z.G., Chen, Y.F., Xie, S.L., Luo, J.Z. and Zou, J.X., 2016. Mitochondrial DNA Part A., 28: 971-973. https://doi.org/10.1080/24701394.2016.1186668
Zhou, A.G., Xie, S.L., Wang, Z.L., Fan, L.F., Wang, C., Ye, Q., Chen, Y.F. and Zou, J.X., 2017. Mitochondrial DNA Part B., 2: 283-286. https://doi.org/10.1080/23802359.2017.1325334
Zhou, A.G., Xie, S.L., Zhang, C.N., Wang, Z.L., Wang, C., Feng, Y.Y., Chen, Y.F. and Zou, J.X., 2018. Indian J. Fish., 65: 66-71. https://doi.org/10.21077/ijf.2018.65.2.57577-08