**VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY**
**VIỆN HÀN LÂM KHOA HỌC VÀ CÔNG NGHỆ VIỆT NAM**

ISSN 0866-7160

**Volume 39, Number 4** **December 2017**

**Ha Noi**

# TAP CHI SINH HOC

*http://vjs.ac.vn/index.php/vjbio*

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VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

JOURNAL OF BIOLOGY 2017, VOLUME 39, NUMBER 4

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**TAP CHI SINH HOC** 2017, 39(4): 463-466
DOI: 10.15625/0866-7160/v39n4.8964

# MOLECULAR IDENTIFICATION OF MINNOW *Opsariichthys hainanensis* (Teleostei: Cyprinidae) FROM THE LAKE PHU NINH, QUANG NAM PROVINCE

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**ABSTRACTS:** The fish of genus *Opsariichthys* was collected from the Lake Phu Ninh (Quang Nam Province) and its taxonomic relation to other species in the same genus was analyzed based on the molecular data of mitochondrial DNA D-Loop region. The result showed that the fish from Quang Nam Province is closely related to its congener, *Opsariichthys hainanensis*, from Hainan Island (China), but differed from *Opsariichthys bidens*, from mainland China or from *Opsariichthys uncirostris*, from Japan.

*Keywords*: Cyprinidae, *Opsariichthys*, taxonomy, Vietnam.

*Citation:* Huynh Quang Thien, Nguyen Van Khanh, Chen I-Shiung, 2017. Molecular identification of minnow *Opsariichthys hainanensis* (Teleostei: Cyprinidae) from the lake Phu Ninh, Quang Nam province. Tap chi Sinh hoc, 39(4): 463-466. DOI: 10.15625/0866-7160/v39n4.8964.

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*Received 5 December 2016, accepted 12 August 2017*

## INTRODUCTION

There were several research articles dealt with taxonomy and nomenclature of the genus *Opsariichthys* from Japan, Taiwan and China (Chen et al., 2008; Chen et al., 2009). In Vietnam, since Chevey & Lemasson (1937) first described this genus, several articles related to the morphology-based taxonomy of this genus have been published (Nguyen 1987; Nguyen & Nguyen 2000; Nguyen et al. 2007; Huynh & Chen 2013; 2015; Vu 2004). In the world database of fishes (Froese & Pauly 2016), a total of 24 nominal species are listed this genus, in which 10 of them are considered as valid species. In Vietnam, 7 nominal species, namely *Opsariichthys uncirostris*, *O. elegans*, *O. bea*, *O. hieni*, *O. bidens*, *O. dienbienensis* and *O. songmaensis* have been listed. Most recently, Huynh & Chen (2013) added a new species in this genus as *Opsariichthys duchuunguyeni* from northern Vietnam.

At least two studies on this genus and other fish have been done for the Lake Phu Ninh (Quang Nam Province) (Vu 2004; Nguyen et al., 2007). In these two studies, the authors mentioned the name *Opsariichthys uncirostris* as the species described in preceded works.

Chen et al. (2008, 2009) from Taiwan and Huynh & Chen (2013) from Vietnam revealed that the species distributed in the Hainan Island and northern Vietnam are actually *Opsariichthys hainanesis,* which has been misidentified as *O. bidens* and/or *O. uncirostris* for a long time in those areas. The aim of this study is to clarify the taxonomic status of the fish of the genus *Opsariichthys* from the Lake Phu Ninh, Quang Nam Province, central Vietnam using molecular data analysis.

## MATERIALS AND METHODS

Totally six fish individuals were collected using cast net from a stream at Tam Tra Commune, Nui Thanh District, Quang Nam Province (15°22'N 108°32E) on February 02, 2013. Specimens are cataloged as NTOU P-2013-10-160 (6 ex., 69.39-76.61mm SL.) at Fish collection of the Institute of Marine Biology, National Taiwan Ocean University (Keelung, Taiwan).

Molecular method followed to those described by Chen et al. (2008; 2009) and Huynh & Chen (2013). In brief, the right side of the pectoral fins of fish was stored in 95 percent alcohol, and then total DNA was extracted using GeneMark Pro Kit. Total DNA extracted was later used for PCR reactions using referenced primers in Chen et al. (2008) as forward primer: CYP-THRA

(5'-AAAGCATCGGTCTTGTAATCCG AAG-3') and backward primer: CYP-12SB (5' -CATGCGGAGTTTCTTAGGTC-3'). PCRs were performed in 50µL reaction volumes and followed as thermo cycling condition: 94$^{o}$C in 7 minutes, 35 cycles for combination of 94$^{o}$C in 0.5 minutes; 55$^{o}$C in 0.5 minute; 72$^{o}$C in 1 minute, 72$^{o}$C in 7 minutes. PCR products were run on a 1.0% L 03 agarose gel and stained with ethidium bromide for band characterization under ultraviolet trans-illumination, PCR products were under purification using Roche High Pure PCR Purification Kit before being sequenced at Academia Sinica (Taiwan) followed manual for producers. Labeled fragments were analyzed using as ABI PRISM Model 377-64 DNA Automated Sequencer (ABI).

Phylogenetic analysis: D-loop sequences of our samples were compared with available data on Genbank and previously published papers by Chen et al. (2008, 2009) for phylogenetic tree constructed using Maximum Likelihood (ML) analysis with Molecular Evolutionary Genetics Analysis (MEGA) version 3 (Kumar et al., 2004).

## RESULTS AND DISCUSSION

Vu (2004) used the name "*Opsarichthys uncirostris*" for the minnow from the Lake Phu Ninh. Three years later, Nguyen et al. (2007) included this name in their research for the fish from the Lake Phu Ninh. These researchers mis-written the spelling of the genus lacking an "*i*" in between r and c, being "*Opsar**i**chthys*" instead of "*Opsar**ii**chthys*". On the other hand, most of other Vietnam articles described this species under the name *O. uncirostris* from the north-central by Nguyen (1987) and *O. bidens* for the fish from the north-west by Nguyen & Nguyen (2000). This erroneous description may probably be caused because the main resource of reference is Chinese books and articles and real specimen comparison from the locality of description or at least from the same river basin has not been done.

*Table 1.* Pairwise distances among OTUs in this study

| | OHAND2 | OHAWC3 | OVQNA3 | OPQNA4 | OBZHO1 | OBZHA1 | OUJPA1 | OPJPA2 | ZAPLT1 | ZAPLC1 |
|---|---|---|---|---|---|---|---|---|---|---|
| OHAND2 | | | | | | | | | | |
| OHAWC3 | 0.001 | | | | | | | | | |
| OVQNA3 | 0.023 | 0.022 | | | | | | | | |
| OPQNA4 | 0.023 | 0.022 | 0.000 | | | | | | | |
| OBZH01 | 0.072 | 0.071 | 0.065 | 0.065 | | | | | | |
| OBZHA1 | 0.074 | 0.072 | 0.066 | 0.066 | 0.001 | | | | | |
| OUJPA1 | 0.062 | 0.060 | 0.061 | 0.061 | 0.034 | 0.035 | | | | |
| OPJPA2 | 0.062 | 0.060 | 0.061 | 0.061 | 0.034 | 0.035 | 0.000 | | | |
| ZAPLT1 | 0.073 | 0.072 | 0.064 | 0.064 | 0.075 | 0.076 | 0.066 | 0.066 | | |
| ZAPLC1 | 0.067 | 0.066 | 0.067 | 0.067 | 0.071 | 0.073 | 0.060 | 0.060 | 0.019 | |

From the molecular phylogenetic analysis, the minnow from the Lake Phu Ninh was considered as a geographical isolate of *O. hainanensis* and surely different from *O. uncirostris* and *O. bidens*. Based on the pairwise distance comparison, the genetic differentiation

between *O. hainanensis* populations from the Lake Phu Ninh and those from the Hainan Island is just 2.2-2.3%, while it was 6.1% and 6.5-6.6% when compared with the *O. uncirostris* species from Japan and *O. bidens* from China, respectively (table 1).

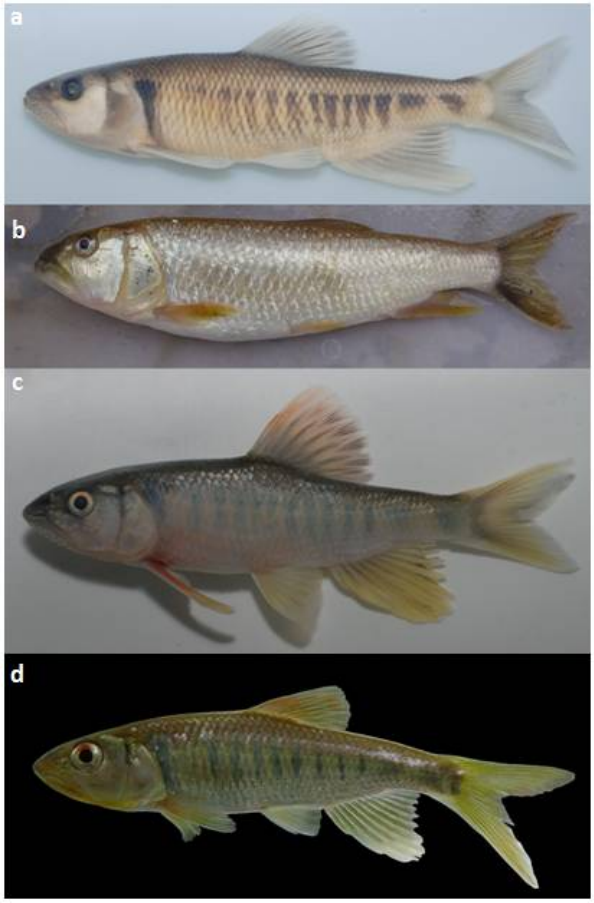


*Figure 1.* Morphological appearance of some species of *Opsariichthys*

a. *O. uncirostris* from the Lake Biwa, Japan, 153.56 mm SL; b. *O. bidens* from Mainland China, 112.9 mm SL (Photo by S.P. Huang); c. *O. hainanensis* from the Hainan Island, China, 71.7mm SL; d. *O. hainanensis* from the Lake Phu Ninh, Vietnam, 76.61mm SL.

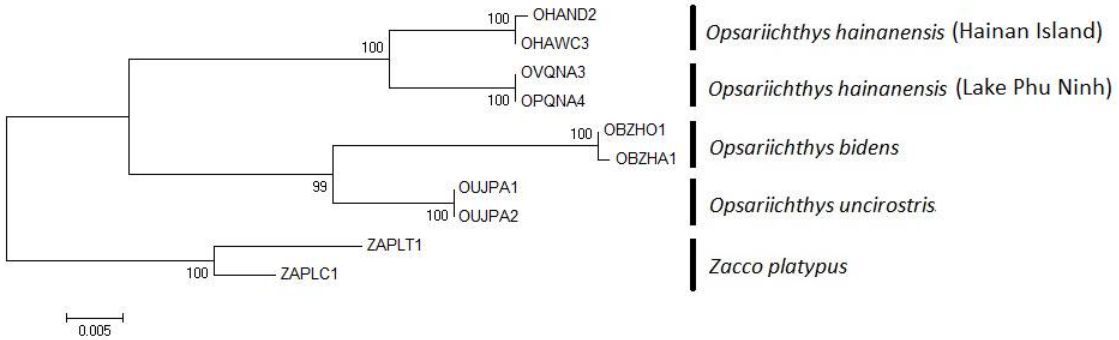


*Figure 2.* The Maximum Likelihood (ML) phylogenetic tree of *Opsariichthys* species based on the mtDNA D-loop control region: *Zacco platypus* is used as an out-group; *O. uncirostris* from Japan, *O. bidens* from mainland China, *O. hainanensis* from the Hainan Island (China) and the Lake Phu Ninh (Vietnam).

Based on the present results, the minnow from the Lake Phu Ninh (Quang Nam Province) is in fact *O. hainanensis* and this species is predicted to be distributing widely in northern and north-central Vietnam. A systematic study of combined morphological and molecular approaches is needed to clarify the actual status of this genus in Vietnam.

**CONCLUSION**

The present results clearly show that the minnow from the Lake Phu Ninh (Quang Ninh prov., Vietnam) is belonging to *Opsariicthys hainanensis* species with slight genetic variation ranging from 2.2 to 2.3% in D-loop region compared with that from Hainan Island. Thus, the correct name of this species in the Lake Phu Ninh should be *O. hainanensis* but not *O. uncirostris* or *O. bidens.* Our results suggest that new insights using molecular approach is needed for the taxonomy of freshwater fishes from Vietnam. Certainly, the combination of morphological and molecular characteristics is critically necessary to deal with those of morphologically similar species complex (fig. 1). More details will be presented in our coming paper of Vietnamese *Opsariichthys* review.

***Acknowledgements:*** This fieldwork was granted by Huynh Gia Group. Laboratory works were done at National Taiwan Ocean University, Taiwan. Logistical supports from Institute of Marine Biology (National Taiwan Ocean University, Taiwan) and Southern Institute of Ecology (Vietnam Academy of Science and Technology) are grateful. The authors thank Dr. Yukifumi Nawa for his critical comments that improved considerably the manuscript.

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