Journal of Marine Science and Technology

Volume 21
Issue 7 *Vol 22, Supplemental*

Article 18

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Recommended Citation

Huynh, Thien Quang and Chen, I-Shiung (2013) "A NEW SPECIES OF CYPRINID FISH OF GENUS Opsariichthys FROM KY CUNG-BANG GIANG RIVER BASIN, NORTHERN VIETNAM WITH NOTES ON THE TAXONOMIC STATUS OF THE GENUS FROM NORTHERN VIETNAM AND SOUTHERN CHINA," *Journal of Marine Science and Technology*: Vol. 21 : Iss. 7 , Article 18.
DOI: 10.6119/JMST-013-1219-12
Available at: https://jmstt.ntou.edu.tw/journal/vol21/iss7/18

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Acknowledgements

NCBI accession numbers of mtDNA D-loop sequences of cyprinid fishes used in this study: O. acutipinnis (OPACHK1-2: KF785753-KF785754); Opsariichthys duchuunguyeni (OPDUCB1: KF753239); O. dienbienensis (OPDBNR1: KF785757); O. minutus (OPMIFJ1-2: KF785755-KF785756); O. songmaensis OPSMDB1-2: KF785758-KF785759 and OPSMTH1: KF785760); other sequences were cited in Chen et al. [6-8].

Journal of Marine Science and Technology, Vol. 21, Suppl., pp. 135-145 (*2013*)
DOI: 10.6119/JMST-013-1219-12

A NEW SPECIES OF CYPRINID FISH OF GENUS *Opsariichthys* FROM KY CUNG – BANG GIANG RIVER BASIN, NORTHERN VIETNAM WITH NOTES ON THE TAXONOMIC STATUS OF THE GENUS FROM NORTHERN VIETNAM AND SOUTHERN CHINA

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Key words: *Opsariichthys*, new species, Ky Cung, Bang Giang, Vietnam, mtDNA, D-loop.

ABSTRACT

Opsariichthys duchuunguyeni, a new species of cyprinid fish from Ky Cung – Bang Giang river basin, northern Vietnam, is described. It is characterized by the following unique combination of features: short maxillary extending; 12-15 rounded tubercles on lower jaw; 11-12 crossbars on body; pectoral fin reaching the pelvic fin origin in adult male; lateral-line scales comprising 41; scales above lateral-line 8; scales below lateral-line 3. Moreover, the genetic differentiation and a diagnostic key of the new species and other congeners are given in this paper for discussion about taxonomic status of the genus *Opsariichthys* from Vietnam and nearby geographical regions, southern China and Taiwan.

I. INTRODUCTION

The freshwater fishes genus *Opsariichthys* Bleeker, 1863 was initially described as *Leuciscus uncirostris* Temminck & Schlegel, 1864. This genus is distributed ranging from Japan, via China, Korea, Taiwan to Vietnam (Chen [9], Nguyen & Ngo [18], Kawanabe *et al*. [12]). At present time, the genus *Opsariichthys* comprises 11 species, including *O. uncirostris* from Japan, Korea and Russia (Okazaki *et al*. [21]), *O. bidens* from the east China, *O. minutus* and *O. acutipinnis* from southern China (Chen *et al*. [8]), *O. evolans* from southern China and Taiwan, *O. pachycephalus* and *O. kaopingensis* from Taiwan (Chen *et al*. [8]), "*O. hieni*" and "*O. bea*" from north central Vietnam (Nguyen [16]), *O. songmaensis* and *O. dienbienensis* from northern Vietnam (Nguyen & Nguyen [17]).

In a field trip on June 2013, five individuals of opsariichthine were collected from Ky Cung – Bang Giang river basin, northern Vietnam by the first author. These specimens are significantly different from all congeneric species in the genus by morphological characters and confirmed by mitogenetic evidence. Herein we describe a distinct species from northern Vietnam, *Opsariichthys duchuunguyeni*.

In fact, the Chinese fish name *Opsariichthys bidens* has been used commonly in Vietnamese literatures (Mai [15], Nguyen & Nguyen [17], Nguyen & Ngo [18], Kottelat [13], Serov [24]) without examining any specimens from its true distribution range. Besides, several new species have been described since 1987 but they seem to be having some problems of lacking careful identification. Some of them were found that named into the wrong genus. Therefore, all available specimens were compared to clarify this confusion.

II. MATERIALS AND METHODS

Fish collecting and specimen storage: fish samples were collected by cast netting or picking from local fish markets. Fin tissues used for molecular analysis were directly preserved in 95% ethanol when caught and transferred into refrigerator in laboratory. Specimens used for morphological studies were fixed by 10% formalin and later being transferred in 70% ethanol for long-term preservation.

Morphological studies: meristic counts and measurements followed Hosoya *et al*. [11], Chen *et al*. [6] and Chen *et al*. [8] with adding of scales surrounding caudal peduncle. The head illustrations were made by camera lucida in the the dissecting microscope as Leica MZ 75. Type specimens and other comparative materials are deposited in the Institute of Marine

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Biology, National Taiwan Ocean University (NTOU) and the types of new species will be deposited in the Vietnam National Museum of Nature (VNMN) and the University of Science, Vietnam National University Ho Chi Minh City (UNS). Meristic abbreviations: D, dorsal fin; A, anal fins; P1, pectoral fin; P2, pelvic fin; LL, lateral-line scales; LLa, scales above lateral-line; LLb, scales below lateral-line; PreD, pre-dorsal scales; VC, vertebral count; CPS, scales surrounding caudal peduncle. All fish lengths are standard length (SL).

Molecular phylogenetic analysis:

Crude DNA extraction followed the methods of Sambrook *et al*. [23], Chen *et al*. [3-8]. The DNA fragments of D-loop region were amplified by polymerase chain reaction (PCR) using primers based on the flanking region (P-THRA: 5'-AAA GCA TCG GTC TTG TAA TCC GAA G-3'; CB-Phe-SR1: 5'-CAT CTT CAG TGC TAT GCT TT-3') that were designed from the conserved sequences of tRNA-THR and tRNA-Phe. PCRs were performed in 50 μL reaction volumes and done in a Model 2700 or 9700 thermal cycler (Perkin-Elmer) and 30-40 cycles were carried out. PCR products were run on a 1.0% L 03 agarose gel (Takara) and stained with ethidium bromide for band characterization under ultraviolet trans-illumination.

Double-strand PCR products were purified using a kit (High Pure Product Purification kit, Roche), before undergoing direct cycle sequencing with dye-labeled terminators (ABI Big-Dye kit). The sequencing primers used were either same as those for PCR or following two primers: CYP-DLMF3: 5'-AAT CAG GGA CAC AAA ATG TGG GGG-3' and CYP-DLMR3: 5'-GCT CGG CAT GTT GGG TAA-3'. All sequencing reactions were performed according to the manual of producer. Labeled fragments were analyzed using as ABI PRISM Model 377-64 DNA Automated Sequencer (ABI).

Nucleotide sequence alignment was verified manually after through CLUSTAL W (Thompson *et al*. [25]) and BIOEDIT version 5.9 (Hall [10]). The analysis of aligned mutation sites were conducted using Molecular Evolutionary Genetics Analysis (MEGA) version 3 (Kumar *et al*. [14]) for aligned mutation sites analysis.

For the Bayesian (BI) analysis, the best-fitting model for sequence evolution was determined for mtDNA D-loop sequences using MrMODELTEST version 2.2 (Nylander [20]). The BI analysis was performed using MrBayes 3.0 (Ronquist & Huelsenbeck [22]). The posterior probabilities of each node were computed from remaining 75% of all sampled tree.

III. SYSTEMATICS

***Opsariichthys duchuunguyeni* Huynh & Chen, new species**
(Figs. 1 and 2)

Zacco platypus Vang & Hwang, 1964: 46 in Nguyen & Nguyen 2001: 77 (Lao Cai and Lang Son province, Vietnam).

Materials examined

Fig. 1 ***Opsariichthys duchuunguyeni*, new species: a, alive male and b, preserved specimen, NTOU P-2013-10-186, holotype, 68.8 mm SL, Ky Cung river, Ky Cung – Bang Giang river basin, Chi Lang commune, Trang Dinh district, Lang Son province, Vietnam; c, female, NTOU P-2013-10-185, paratype, 58.6 mm SL, Cun stream, Bang Giang river, Ky Cung – Bang Giang river basin, Ngu Lao commune, Hoa An district, Cao Bang province, Vietnam.**

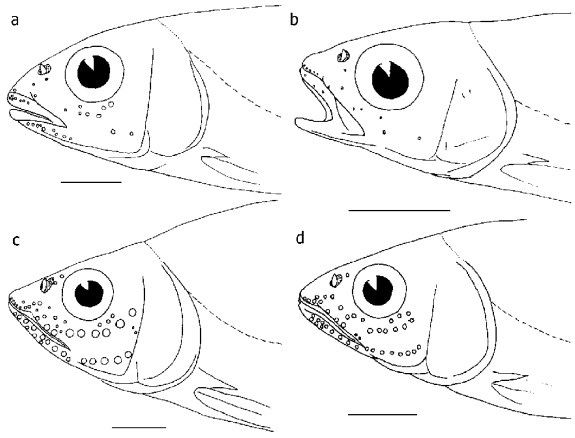

Fig. 2. Head illustrations of three "small-mouth" form of *Opsariichthys* species: a, *Opsariichthys evolans*, male, NTOU P-2008-11-460, 72 mm SL, Kong-liao, Taipei, Taiwan; b, *O. acutipinnis*, male, NTOU P-2013-10-197, 55.1 mm SL, Hongkong, China; c, *O. duchuunguyeni* new species, male, NTOU P-2013-10-186, 68.8 mm SL, holotype, Ky Cung river, Ky Cung – Bang Giang river basin, Chi Lang commune, Trang Dinh district, Lang Son province, Vietnam; d, *O. duchuunguyeni*, female, NTOU P-2013-10-185, 58.6 mm SL, paratype, Cun stream, Bang Giang river, Ky Cung – Bang Giang river basin, Ngu Lao commune, Hoa An district, Cao Bang province, Vietnam. Bar = 5 mm.

Holotype. NTOU P-2013-10-186, 68.8 mm SL, Ky Cung river, Ky Cung – Bang Giang river basin, Chi Lang commune, Trang Dinh district, Lang Son province, Vietnam; 22°17'20.7"N 106°27'30.0"E; T. Q. Huynh, 04 Jul 2013.

Table 1. Morphometric measurements of *Opsariichthys evolans*, *O. acutipinnis* and *O. duchuunguyeni*.

	Opsariichthys evolans						*Opsariichthys acutipinnis*						*Opsariichthys duchuunguyeni*, new species						
	Male			Female			Male			Female			Holotype	Male			Female		
N	5			5			8			2			1	2			3		
Standard length (mm)	70.3~89.9			52.4~69.7			44.7~65.4			49.5~52.6			68.8	68.8~72.5			52.4~61.0		
% in SL	min	max	mean	min	max	mean	min	max	mean	min	max	mean		min	max	mean	min	max	mean
Head length	21.2	24.1	22.8	21.7	23.1	22.4	26.7	29.4	28.3	27.9	28.3	28.1	27.2	25.1	27.2	26.2	25.1	26.7	25.9
Body depth	25.4	28.8	26.8	23.0	25.4	24.5	22.0	27.1	24.4	20.9	22.9	21.9	27.4	27.4	27.7	27.5	24.1	27.1	25.6
Body width at dorsal origin	10.0	13.5	12.1	11.6	12.6	12.0	9.4	11.1	10.3	9.7	10.3	10.0	11.2	11.2	12.7	12.0	10.2	11.0	10.6
Body width at anal origin	8.4	11.1	9.7	8.8	9.8	9.2	6.5	8.3	7.0	6.7	7.3	7.0	8.7	8.7	9.8	9.2	7.3	8.3	7.7
Depth of caudal peduncle	9.1	10.9	9.9	9.3	10.3	9.7	9.3	10.1	9.7	9.7	9.8	9.8	10.0	9.8	10.0	9.9	9.3	10.2	9.7
Length of caudal peduncle	18.6	20.8	19.8	19.3	21.0	20.1	19.1	21.6	20.0	19.7	21.6	20.7	20.7	20.7	22.4	21.6	21.8	22.5	22.2
Predorsal length	47.8	51.4	49.6	47.2	50.4	49.5	48.7	52.1	49.8	48.4	49.6	49.0	49.7	49.6	49.7	49.7	40.7	54.5	47.8
Preanal length	65.3	69.0	67.0	68.4	69.8	69.1	68.3	72.1	70.1	69.5	71.9	70.7	66.5	66.5	67.2	66.8	66.4	70.5	68.3
Preventral length	48.4	51.6	49.7	48.6	51.3	49.9	49.1	53.7	51.6	49.6	52.0	50.8	49.0	47.4	49.0	48.2	48.4	51.4	49.9
Dorsal origin to caudal base	51.8	53.9	52.6	51.5	53.6	52.4	50.1	53.5	51.6	51.8	53.5	52.6	53.3	53.3	54.2	53.7	53.6	54.5	54.0
Pectoral origin to pelvic insertion	24.1	25.6	24.8	24.2	24.5	24.4	23.4	27.6	25.3	23.3	27.0	25.1	23.8	23.8	24.0	23.9	23.8	25.1	24.3
Length of longest dorsal ray	19.4	21.0	20.0	17.8	19.3	18.4	18.3	20.5	19.5	18.8	19.8	19.3	20.2	19.8	20.2	20.0	18.2	19.7	19.1
Length of longest anal ray	34.3	38.2	36.5	19.1	29.7	23.8	17.9	32.6	22.2	18.4	19.4	18.9	28.3	23.5	28.3	25.9	17.8	20.2	19.1
Length of longest pectoral ray	25.2	28.8	27.0	19.6	21.6	20.2	18.6	22.0	20.4	19.9	20.1	20.0	24.4	21.1	24.4	22.8	20.2	21.3	20.9
Length of dorsal fin base	12.2	14.3	13.6	10.5	11.8	11.2	10.1	12.7	11.1	10.0	11.0	10.5	13.3	11.5	13.3	12.4	9.6	11.1	10.5
Length of anal fin base	17.2	20.1	18.9	12.0	15.9	13.7	11.4	15.1	12.9	12.4	12.7	12.5	17.4	14.4	17.4	15.9	12.5	13.1	12.9
Length of pelvic fin ray	17.3	19.9	18.6	14.1	15.7	14.9	13.9	22.1	15.5	13.0	14.7	13.8	17.3	16.2	17.3	16.8	13.2	15.5	14.7
% in HL																			
Head width at nasal section	27.0	32.9	30.3	26.1	31.8	29.3	27.2	31.3	29.4	7.2	28.0	17.6	30.4	30.4	30.8	30.6	29.4	33.0	30.7
Snout length	34.9	38.8	36.2	33.2	36.5	35.5	27.0	28.1	27.6	7.1	25.7	16.4	27.7	27.7	29.5	28.6	26.3	30.2	28.6
Inter-orbital width	36.6	40.6	38.6	34.4	37.3	36.1	27.3	31.4	29.2	8.1	29.4	18.7	29.0	29.0	32.0	30.5	30.3	32.8	31.5
Orbit diameter	29.2	32.8	31.0	32.1	35.4	33.5	30.6	37.9	35.3	9.4	35.2	22.3	28.7	28.7	29.0	28.9	28.7	32.1	30.6
Upper jaw length	40.2	43.7	41.9	43.0	46.2	44.8	37.9	40.9	39.5	10.3	34.2	22.3	40.9	40.8	40.9	40.9	36.4	39.0	37.6
Head depth at midline of orbit	53.5	61.0	58.9	53.7	60.3	57.2	49.9	56.4	53.6	13.7	50.2	32.0	50.9	50.9	53.7	52.3	52.7	61.3	57.5

Paratypes. NTOU P-2013-10-185, 3 ex., 52.4-72.5 mm SL, Cun stream, Bang Giang river, Ky Cung - Bang Giang river basin, Ngu Lao commune, Hoa An district, Cao Bang province, Vietnam; 22°42'15.9"N 106°16'23.4"E; T. Q. Huynh, 04 Jul 2013; NTOU P-2013-10-187, 1 ex., 61.0 mm SL, Ky Cung river, Ky Cung - Bang Giang river basin, Chi Lang commune, Trang Dinh district, Lang Son province, Vietnam; 22°17'20.7"N 106°27'30.0"E; T. Q. Huynh, 04 Jul 2013.

Diagnosis

Opsariichthys duchuunguyeni can be distinguished from the congeneric species by the following combination of morphological features: (1) no maxillary barbels; (2) no anterior notch on tip of upper lip; (3) lateral-line scales 41; (4) pre-dorsal scales 13-14; (5) narrow body width; (6) maxillary extending to or slightly beyond vertical of anterior margin of orbit; (7) a series of two rows with 12-15 rounded tubercles on lower jaw; (8) pectoral fin extending to origin of ventral fin in adult male; and (9) snout and ventral side of head orange red in adult male in life.

Description

Body proportions listed in Table 1. Body elongated and compressed laterally, body depth slightly longer than head length. Body width rather narrow. No maxillary barbels. Mouth oblique, maxillary extending to or slightly beyond vertical of anterior margin of orbit. Eyes rather large. Upper lip slightly concave but not notched. Breeding tubercles distinct on head and anal rays of adult male, the area below lower jaw with two rows of totally 12-15 rounded tubercles, cheek with 3 longitudinal rows of tubercles which has the lower row separated and located at below of cheek while the rest with two rows positioned just below margin of orbit and the upper row of tubercles interrupted. Gill rakers 2+6 (5 specimens). Pharyngeal teeth arranged into two to three rows: 5-4-1; 1-4-4 or 4-4-1; 1-4-4 or 5-4; 4-4. Vertebrae count 4+16+20-4+16+21 (40-41).

Fins

Dorsal fin rays iii,7; anal fin rays iii,9; pectoral fin rays i,14-15; pelvic fin rays i,7-8. Pelvic fin origin vertical dorsal

Table 2. Frequency distribution of meristic features of *Opsariichthys* from Japan, central and southern China, Taiwan and northern Vietnam.

Species	D iii			A iii				P1 i				P2 i			CPS					
	7	8	M	8	9	10	M	13	14	15	M	7	8	M	16	17	18	19	20	M
Opsariichthys duchuunguyeni, new species	5	-	7.0	-	5	-	9.0	-	4	1	14.2	1	4	7.9	-	4	1	-	-	17.2
Opsariichthys acutipinnis	9	-	7.0	-	9	-	9.0	-	1	8	14.9	-	8	8.0	-	-	2	6	1	18.9
Opsariichthys bidens	15	-	7.0	1	14	-	8.9	1	5	9	14.5	-	15	8.0	-	4	10	1	-	17.8
Opsariichthys dienbienensis	10	-	7.0	-	10	-	9.0	3	6	1	13.8	2	8	7.8	1	4	3	2	-	17.6
Opsariichthys evolans	6	-	7.0	-	6	-	9.0	2	3	1	13.8	1	5	7.8	5	1	-	-	-	16.2
Opsariichthys hainanensis	14	2	7.1	-	14	2	9.1	2	11	3	14.0	1	15	7.9	4	7	5	-	-	17.1
*Opsariichthys kaopingensis**	118	-	7.0	3	115	-	9.0	5	147	72	14.3	210	14	8.1	-	1	1	1	1	18.5
Opsariichthys minutus	12	-	7.0	-	12	-	9.0	3	10	3	14.0	-	16	8.0	-	1	3	8	-	18.6
*Opsariichthys pachycephalus**	421	2	7.0	17	398	12	9.0	251	251	38	13.6	251	123	8.3	-	1	4	3	5	18.9
Opsariichthys songmaensis	10	-	7.0	-	9	1	9.1	7	3	-	13.3	4	6	7.6	-	2	5	3	-	18.1
Opsariichthys uncirostris	1	-	7.0	-	1	-	9.0	-	-	1	15.0	-	1	8.0	-	-	-	-	1	20.0

Species	LLa					LLb					PreD											
	8	9	10	11	M	3	4	5	6	M	13	14	15	16	17	18	19	20	21	22	23	M
Opsariichthys duchuunguyeni, new species	5	-	-	-	8.0	5	-	-	-	3.0	1	4	-	-	-	-	-	-	-	-	-	13.8
Opsariichthys acutipinnis	2	7	-	-	8.8	1	8	-	-	3.9	-	-	1	5	3	-	-	-	-	-	-	16.2
Opsariichthys bidens	1	14	-	-	8.9	14	1	-	-	3.1	-	-	-	-	-	-	2	8	5	-	-	20.2
Opsariichthys dienbienensis	2	8	-	-	8.8	-	10	-	-	4.0	-	-	1	4	3	2	-	-	-	-	-	16.6
Opsariichthys evolans	5	1	-	-	8.2	1	5	-	-	3.8	-	-	1	5	-	-	-	-	-	-	-	15.8
Opsariichthys hainanensis	1	15	-	-	8.9	2	14	-	-	3.8	-	-	-	4	9	3	-	-	-	-	-	16.9
*Opsariichthys kaopingensis**	-	95	17	-	9.2	104	7	-	-	3.1	-	-	-	-	7	47	60	-	-	-	-	18.6
Opsariichthys minutus	10	2	-	-	9.2	-	7	5	-	4.4	-	-	-	5	8	3	-	-	-	-	-	16.9
*Opsariichthys pachycephalus**	-	-	251	38	10.1	251	20	-	-	3.1	-	-	-	-	-	-	-	90	139	89	25	21.1
Opsariichthys songmaensis	2	8	-	-	8.8	-	10	-	-	4.0	-	-	6	2	1	1	-	-	-	-	-	15.7
Opsariichthys uncirostris	-	-	-	1	11.0	-	-	-	1	6.0	-	-	-	-	-	-	-	-	-	-	1	23.0

Species	LL																VC						
	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	M	38	39	40	41	42	43	M
Opsariichthys duchuunguyeni, new species	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	41.0	-	-	1	4	-	-	40.8
Opsariichthys acutipinnis	-	-	7	2	-	-	-	-	-	-	-	-	-	-	-	42.2	-	2	4	3	-	-	40.1
Opsariichthys bidens	-	-	-	-	1	7	4	3	-	-	-	-	-	-	-	45.6	-	-	-	3	3	-	41.5
Opsariichthys dienbienensis	2	3	5	-	-	-	-	-	-	-	-	-	-	-	-	41.3	-	3	5	2	-	-	39.9
Opsariichthys evolans	-	-	5	1	-	-	-	-	-	-	-	-	-	-	-	42.2	-	-	2	4	-	-	40.7
Opsariichthys hainanensis	9	7	-	-	-	-	-	-	-	-	-	-	-	-	-	40.4	-	2	14	-	-	-	39.9
*Opsariichthys kaopingensis**	-	-	-	-	13	84	66	57	2	-	-	-	-	-	-	45.8	8	77	33	-	-	-	39.2
Opsariichthys minutus	-	-	-	-	-	4	4	4	-	-	-	-	-	-	-	46.0	-	-	2	10	1	-	40.9
*Opsariichthys pachycephalus**	-	-	-	-	-	-	-	-	2	59	121	129	137	120	108	51.7	-	57	260	108	-	-	40.1
Opsariichthys songmaensis	-	2	7	1	-	-	-	-	-	-	-	-	-	-	-	41.9	-	1	7	2	-	-	40.1
Opsariichthys uncirostris	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	53.0	-	-	-	-	-	1	43.0

M: mean of all listed values
*: data from Chen *et al*. [8]

fin origin. Pectoral fin almost reaching pelvic fin in adult male, shorter in juvenile male and female. Pelvic fin margin roundish and extending to anal fin origin in adult male. Anal fin with first four elongated branched rays. Caudal fin forked, lower lobe slightly longer than upper one. Frequency distribution of meristic counts is provided in Table 2.

Scales

Body with moderately cycloid scales. Lateral-line complete, depressed downward above pectoral fin and extending along lower half of body to mid-lateral on caudal peduncle. Lateral-line scales 41 (5 specimens); scale series above lateral-line 8; scale series below lateral-line scale 3; pre-dorsal scales 13-14; anterior scales to pelvic fin 13-14; and scales surrounding caudal peduncle 17. Belly with tiny scales.

Coloration in life

Body sides silver gray and turning silver white from below lateral-line. In adult males, body with 11-12 greenish blue irregular bars, therein caudal peduncle with two wider bars. In female, body sides with relatively distinct bars. In which, a wider bar cover 3-4 anterior scales just behind gill-opening and elongated over auxiliary scale. Extending scales at the end of caudal peduncle with two fused dark blotches. Upper and lateral sides of head grayish, shiny at sides and turn to

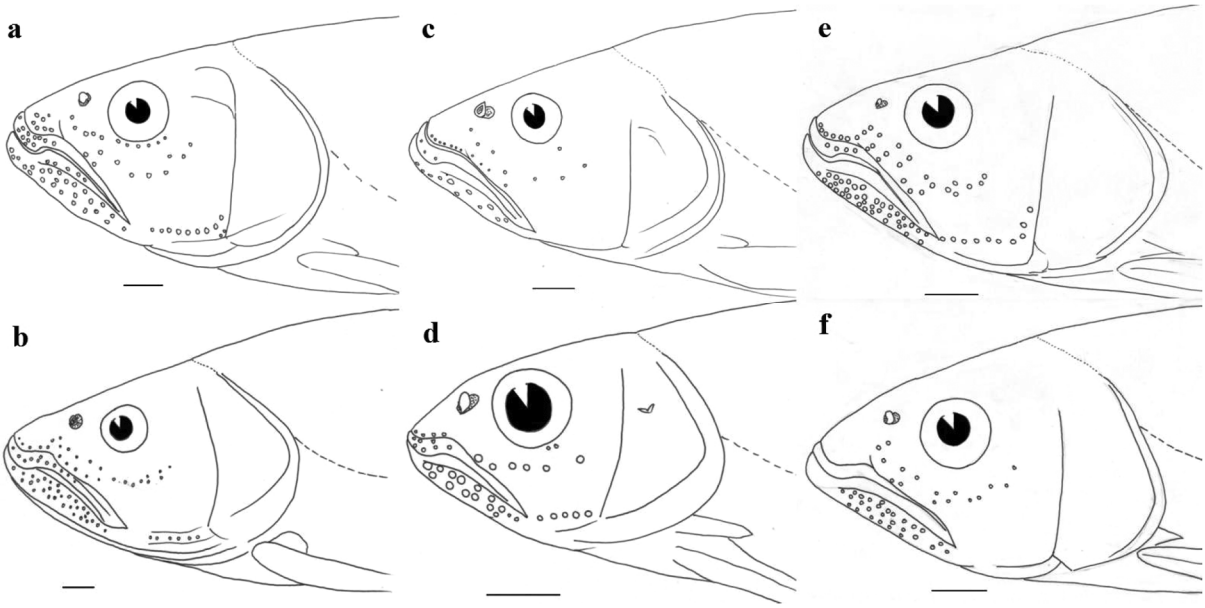

Fig. 3. Head illustrations of "large-mouth" form of *Opsariichthys* species: a, *O. uncirostris*, male, NTOU P-2013-10-155, Biwa lake, Japan, 153.6 mm SL; b, *O. bidens*, male, NTOU P-2008-06-289, Central market, Xianju county, Zhejiang province, China, 169.1 mm SL; c, *O. minutus*, male, NTOU P-2013-10-193, Fujian province, China, 93.3 mm SL; d, *O. hainanensis*, male, NTOU P-2008-6-386, Nankai, Nandujiang basin, Paishar county, Hainan province, China, 57.2 mm SL; e, *O. dienbienensis*, male, NTOU P-2013-10-181, Huy Ha commune, Phu Yen district, Son La province, Vietnam, 122.2 mm SL;f, *O. songmaensis*, male, NTOU P-2013-10-172, Cam Phong commune, Cam Thuy district, Thanh Hoa province, Vietnam, 105.1 mm SL. Bar = 5 mm.

orange red on ventral side and lower margin of cheek in male. Iris's margin yellow. Dorsal fin yellowish or white, each membrane between rays with a long, oblong dark bands. Anal fin yellowish with pale bands along membrane between rays. Pectoral fin's outer orange red, pelvic fins yellow with no remark on membranes. Caudal fin light yellow with somewhat dark mark between rays.

Etymology

The specific name, *duchuunguyeni*, honors ichthyologist, Prof. Nguyen Huu Duc, who has contributed for Vietnamese freshwater fishes research.

Distribution

The new species was found from the streams running to Bang Giang river (Cao Bang province) and Ky Cung river (Lang Son province) themselves are tributaries of Pearl river.

Habitat

This species lives in upper reach of stream with moderate velocity of clear water on the benthic substratum of small and medium pebbles and boulders.

Remarks

The new species, *Opsariichthys duchuunguyeni* can be well distinguished from its congeners: *O. uncirostris* from Japan, Korea and northeastern China; *O. bidens*, *O. minutus* and *O. hainanesis* from central and southern China; "*O. bea*", "*O. hieni*", *O. dienbienensis* and *O. songmaensis* from Vietnam by the absence of anterior notch on upper lip (*vs.* the presence of distinct anterior deep notch on that) (Fig. 3). This new species shared the morphological similarity of lacking the anterior notch on upper lip as *O. pachycephalus*, *O. kaopingensis*, *O. evolans* from Taiwan and *O. acutipinnis* from southern China (Fig. 2).

It can be well separated from *O. pachycephalus* by the following features: (1) lateral-line scales 41 (*vs.* 48-54); (2) pre-dorsal scales 13-14 (*vs.* 20-23); (3) maxillary extending to or slightly beyond the anterior vertical of orbit in adult male (*vs.* maxillary long, always extending to or beyond the middle vertical of orbit); (4) two rows with 12-15 rounded tubercles under lower jaw (*vs.* one row with 6-7 rounded tubercles on that).

Besides, the new species also can be distinguished from *O. kaopingensis* by having: (1) lateral-line scales 41 (*vs.* 44-48); (2) pre-dorsal scales 13-14 (*vs.* 17-19); (3) scales above lateral-line 8 (*vs.* 9-10); (4) scales surrounding caudal peduncle 17 (*vs.* modally 18-19); (5) pelvic fin rays modally 8 (*vs.* modally 7); (6) two rows with 12-15 rounded tubercles under lower jaw (*vs.* one row with 4-5 rounded tubercles on that).

In addition, *Opsariichthys duchuunguyeni* can also be recognized from *O. evolans* by the following combination of characters: (1) lateral-line scales 41 (*vs.* 42-43); (2) pre-dorsal scales 13-14 (*vs.* 15-17); (3) maxillary extending to or slightly beyond the anterior vertical of orbit in adult female (*vs.* maxillary never extending to or slightly beyond the anterior vertical of orbit in adult female); (4) Opercle and ventral side of head orange red in adult male (*vs.* snout with blackish purple background in adult male).

Table 3. The OTU codes of cyprinid fishes of *Opsariichthys* and related genera used for mitogenetic analysis.

Code	Species name	Locality	Source
APKITH1	*Aphyocypris kikuchii*	Hualian river, Hualian, Taiwan	Chen *et al*. [7]
CABATT1	*Candidia barbatus*	Tanshuei river, Taipei, Taiwan	Chen *et al*. [7]
CAPITF1	*Candidia pingtungensis*	Fongkang river, Pingtung, Taiwan	Chen *et al*. [7]
NINOHK1	*Nicholsicypris normalis*	Hongkong, China	Chen *et al*. [7]
NPKOK1	*Nipponocypris koreanus*	Gwangcheon river, Kyong Sanbuk-Do, Korea	Chen *et al*. [7]
NPSIJ1-2	*Nipponocypris sieboldii*	Biwa lake, Otsu, Honshu, Japan	Chen *et al*. [7]
NPTEJ1	*Nipponocypris temminckii*	Biwa lake, Otsu, Honshu, Japan	Chen *et al*. [7]
OPDUCB1	*Opsariichthys duchuunguyeni*, new species	Bang Giang river, Cao Bang province, Vietnam	This study
OPACHK1-2	*Opsariichthys acutipinnis*	Hongkong, China	This study
OPBIZC1-2	*Opsariichthys bidens*	Changtanjiang, Zhejiang province, China	Chen *et al*. [7]
OPDBNR1	*Opsariichthys dienbienensis*	Nam Rom river, Dien Bien province, Vietnam	This study
OPEVFM1	*Opsariichthys evolans*	Mulan river, Fujian province, China	Chen *et al*. [7]
OPEVTT1	*Opsariichthys evolans*	Tanshuei river, Taipei, Taiwan	Chen *et al*. [7]
OPHAHN1-2	*Opsariichthys hainanensis*	Nandujiang, Hainan island, China	Chen *et al*. [6]
OPKAKA1-2	*Opsariichthys kaopingensis*	Kaoping river, Kaoshiung city, Taiwan	Chen *et al*. [8]
OPMIFJ1-2	*Opsariichthys minutus*	Ming river, Fujian province, China	This study
OPPAPA1-2	*Opsariichthys pachycephalus*	Parchan river, Chayi county, Taiwan	Chen *et al*. [8]
OPSMDB1-2	*Opsariichthys songmaensis*	Ma river, Dien Bien province, Vietnam	This study
OPSMTH1	*Opsariichthys songmaensis*	Ma river, Thanh Hoa province, Vietnam	This study
OPUNJB1-2	*Opsariichthys uncirostris*	Biwa lake, Otsu, Honshu, Japan	Chen *et al*. [7]
PASPHK1	*Parazacco spilurus*	Hongkong, China	Chen *et al*. [7]
ZAPLJK1	*Zacco platypus*	Kyoto, Honshu, Japan	Chen *et al*. [7]
ZAPLJK2	*Zacco platypus*	Kagoshima, Japan	Chen *et al*. [7]

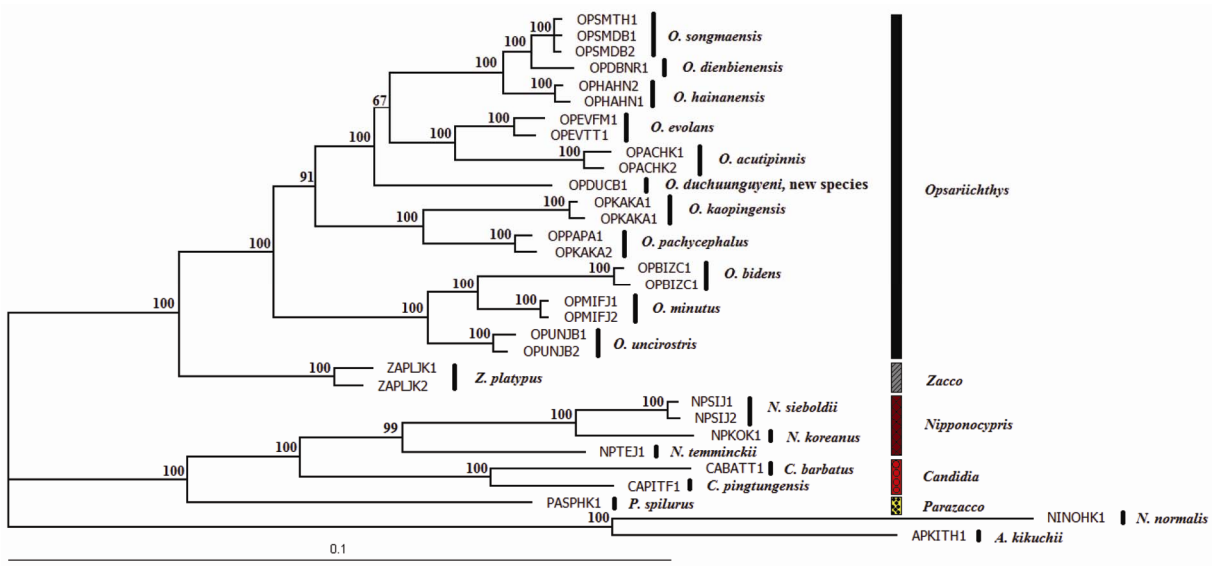

Fig. 4. Molecular phylogenetic tree reconstructed by Bayesian Inference method based on complete mtDNA D-loop sequences of *Opsariichthys* and related genera from Eastern Asiatic water. The posterior probability listed above the branch.

Lastly, the new species also can be detached from *O. acutipinnis* by showing: (1) lateral-line scale always 41 (*vs.* 42-43), (2) scales above lateral-line always 8 (*vs.* modally 9), (3) scales below lateral-line always 3 (*vs.* modally 4), (4) pre-dorsal scales 13-14 (*vs.* 15-17 modally 16), (5) scales surrounding caudal peduncle modally 17 (*vs.* modally 19), (6) vertical cross-bars always 11-12 (*vs.* 9-10), (7) tubercles on lower jaws arrange into two rows with 12-15 tubercles (*vs.* one row with less than 10 tubercles on that).

Molecular phylogenetic analysis

All taxa of *Opsariichthys* and related genera used in this

Table 4. Morphometric measurements of *Opsariichthys bidens* and *O. minutus*.

	Opsariichthys bidens						*Opsariichthys minutus*					
	Male			Female			Male			Female		
N	10			6			4			7		
Standard length (mm)	130.7~170.7			64.3~112.9			85.2~101.9			60.2~86.9		
% in SL	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Head length	28.5	30.7	29.8	23.4	25.4	24.4	30.7	31.8	31.1	27.9	32.7	30.5
Body depth	23.9	27.7	25.2	18.8	21.1	19.7	24.3	29.7	27.1	21.9	27.4	24.1
Body width at dorsal origin	11.3	14.6	13.3	10.5	11.7	11.2	11.7	14.0	12.7	10.4	12.8	11.5
Body width at anal origin	7.4	10.6	8.9	8.2	9.7	8.8	7.8	9.2	8.3	7.3	9.6	8.1
Depth of caudal peduncle	8.7	10.3	9.3	8.8	9.2	9.1	9.6	10.9	10.4	9.3	11.7	10.6
Length of caudal peduncle	17.8	21.6	19.5	18.7	21.0	19.9	18.4	20.9	19.4	18.3	20.3	19.3
Predorsal length	50.4	53.6	52.1	49.5	52.4	50.8	51.2	53.8	52.1	49.3	54.1	51.8
Preanal length	67.6	75.1	71.7	69.2	72.4	71.2	69.5	74.4	72.4	70.8	74.6	72.7
Preventral length	50.4	53.6	53.3	52.6	55.7	54.1	51.3	55.9	53.7	51.0	54.7	52.6
Dorsal origin to caudal base	48.2	53.1	50.7	48.3	51.0	49.7	50.1	52.9	51.6	50.7	52.2	51.5
Pectoral origin to pelvic insertion	24.0	29.0	27.1	25.3	27.9	26.3	25.0	29.4	26.8	23.5	27.4	25.4
Length of longest dorsal ray	16.4	18.2	17.3	18.0	19.0	18.6	16.6	17.7	17.1	15.7	18.5	17.0
Length of longest anal ray	19.6	29.9	25.8	18.6	23.7	21.3	21.7	24.4	22.5	14.6	18.1	16.3
Length of longest pectoral ray	18.9	21.5	20.5	18.0	20.3	19.0	19.1	21.7	19.9	17.7	21.4	19.2
Length of dorsal fin base	10.6	12.6	11.8	9.9	11.4	10.6	10.5	12.8	11.6	10.7	12.3	11.4
Length of anal fin base	10.1	12.9	11.5	10.8	11.8	11.3	11.5	12.5	12.1	11.1	12.7	11.9
Length of pelvic fin ray	13.8	16.7	15.5	11.4	13.8	12.8	13.4	21.7	16.3	13.6	15.5	14.4
% in HL												
Head width at nasal section	28.9	37.5	32.6	47.2	51.3	49.4	29.8	32.1	30.8	27.0	32.0	28.9
Snout length	30.2	37.7	34.3	38.5	40.8	39.8	27.3	30.8	29.3	27.4	30.6	28.6
Inter-orbital width	30.2	34.0	32.3	34.3	36.2	35.0	28.6	30.7	29.9	26.5	29.3	28.4
Orbit diameter	17.6	22.1	20.0	26.8	27.9	27.4	20.9	22.7	21.8	22.4	26.1	24.2
Upper jaw length	44.7	49.0	45.9	54.2	58.8	56.5	44.9	47.7	45.7	42.6	45.9	44.7
Head depth at midline of orbit	34.9	57.9	52.9	28.6	47.9	43.6	49.0	54.5	51.9	48.2	53.0	49.8

study are listed in Table 3. The complete mtDNA D-loop sequences have been amplified and sequenced for species belonging opsariichthine group. The total lengths of control region sequences of cyprinid fishes range from 920 bp to 937 bp. The shortest sequence, with 920 bp, was obtained from *Opsariichthys acutipinnis*, the longest sequence, 937 bp, was obtained from *Nicholsicypris normalis.* The sequence lengths of all members of fishes genus *Opsariichthys* group are from 920 to 928 bp.

The result of BI analysis employed the "GTR + I + G model" suggested by Mr ModelTest with rather high posterior probability mostly 91-100 except one as 67 above inter-specific differentiation. The tree topology (Fig. 4) which the result clearly supports two distinct paraphyletic clades with the assigned outgroup based on cyprinid species with distinct ventral keel: both *Aphyocypris kikuchii* and *Nicholsicypris normalis*. The clade I is *Parazacco – Candidia – Nipponocypris* group with the basal group is *Parazacco spilurus*. The clade II is *Opsariichthys and Zacco* group, the basal group is *Zacco platypus.* Based on the result of BI tree and combined with morphological features, the *Parazacco – Candidia – Nipponocypris* is presented in having the lateral stripe on body. In contrast, the *Opsariichthys – Zacco* group exhibits the cross bars characteristics (Chen *et al*. [8]).

From the result of pairwise distance comparison, the uncorrected genetic distance among *O. evolans* species complex ranged from 2.5 to 5.4%. There in, the genetic divergence between *O. evolans* and *O. acutipinnis* is 2.5-2.7%, the differentiation between new species is 4.5-4.6% and 5.5-5.6% with *O. evolans* and *O. acutipinnis* respectively. This value even higher than the 3.5-3.6% between two endemic species of Taiwan, *Candidia barbatus* and *C. pingtungensis.* Through phylogenetic tree's result, the taxonomic status of several controversial species is also revealed such as the case of *O. bidens* and *O. minutus* with 2.7-2.8% with 100 posterior probability, supporting for morphological measurements differentiation (Table 4). The tree's topology shows the close relationship between *O. hainanensis* and the two Vietnamese fishes, *O. songmaensis* and *O. dienbienensis*. With genetic differentiation from 1.2-1.5% and meristic counts given in table 2, the Vietnamese species also can be distinguished from *O. hainanensis* by morphometric measurements (Table 5). Although the genetic differentiation between *O. songmaensis* and *O. dienbienensis* is just 0.7%, the two species still can be

Table 5. Morphometric measurements of *Opsariichthys hainanensis*, *O. songmaensis* and *O. dienbienensis*.

	Opsariichthys hainanensis						*Opsariichthys songmaensis*				*Opsariichthys dienbienensis*					
	Male			Female			Male	Female			Male			Female		
N	5			11			1	7			5			5		
Standard length (mm)	65.5~105.8			63.4~96.6			105	50.7~74.9			92.0~122.2			55.7~86.4		
% in SL	min	max	mean	min	max	mean		min	max	mean	min	max	mean	min	max	mean
Head length	30.1	32.9	31.9	30.4	33.0	31.8	29.2	27.8	29.5	28.7	28.4	31.3	30.1	28.2	30.3	29.0
Body depth	23.6	27.4	26.3	20.9	24.3	22.9	26.4	23.0	25.9	24.3	24.8	28.5	26.7	24.2	25.9	25.1
Body width at dorsal origin	10.8	14.4	12.8	10.6	13.6	11.9	12.3	9.6	12.4	10.5	9.3	12.0	11.1	8.9	11.6	9.8
Body width at anal origin	6.8	10.9	9.2	7.1	10.2	8.4	7.4	5.8	8.0	7.1	6.4	8.4	7.6	5.4	9.0	6.6
Depth of caudal peduncle	9.5	11.2	10.5	9.2	11.2	9.8	10.9	8.5	10.0	9.2	8.9	10.7	10.0	8.6	10.9	9.8
Length of caudal peduncle	18.4	19.8	19.0	17.0	20.3	18.6	17.6	18.3	19.6	19.3	16.4	18.3	17.5	18.7	20.0	19.4
Predorsal length	48.8	53.0	51.7	50.9	52.8	52.0	52.5	49.4	51.2	50.4	50.3	52.1	51.1	49.5	53.2	52.1
Preanal length	68.8	71.6	70.3	69.9	74.5	72.5	69.9	69.8	72.9	71.5	70.4	74.1	72.0	71.7	73.2	72.1
Preventral length	51.8	53.8	52.8	51.4	70.9	54.7	53.0	50.3	54.1	51.9	52.3	56.0	54.3	52.1	54.6	53.2
Dorsal origin to caudal base	50.4	53.0	51.8	48.9	51.9	50.2	52.6	50.1	53.0	51.3	49.8	52.4	50.8	49.7	52.4	51.0
Pectoral origin to pelvic insertion	23.0	26.4	25.0	22.9	25.8	24.7	25.0	24.4	28.2	25.7	25.7	28.4	26.8	25.6	28.5	26.8
Length of longest dorsal ray	17.5	22.8	19.6	15.2	18.4	17.3	22.0	16.2	21.2	18.2	18.5	22.3	20.9	17.6	20.1	19.2
Length of longest anal ray	17.5	28.2	21.0	14.2	17.8	16.0	25.4	13.0	18.2	15.1	23.5	27.6	25.4	16.2	21.6	20.0
Length of longest pectoral ray	20.1	24.2	21.8	12.7	20.9	18.9	22.6	18.1	19.5	19.0	19.6	21.7	20.7	19.1	20.2	19.8
Length of dorsal fin base	12.3	14.0	13.1	2.2	12.3	10.6	13.6	9.9	11.9	11.3	12.3	14.6	13.3	11.2	12.2	11.6
Length of anal fin base	12.2	14.5	13.5	10.1	12.4	11.7	14.8	11.4	12.7	12.1	13.0	15.7	14.4	11.1	12.6	12.0
Length of pelvic fin ray	14.7	18.0	15.9	12.8	14.9	13.8	16.9	12.6	14.5	13.5	14.6	16.8	15.5	14.2	15.5	14.8
% in HL																
Head width at nasal section	25.6	32.8	28.7	25.2	29.9	27.4	28.7	24.8	26.8	25.8	26.2	31.5	28.5	25.3	27.5	26.4
Snout length	28.3	35.1	32.2	29.8	37.2	32.6	32.5	24.3	30.4	28.0	29.2	30.7	30.1	24.6	30.7	27.3
Inter-orbital width	23.4	27.0	25.3	23.0	26.4	24.8	30.0	25.8	28.6	27.4	25.7	30.0	27.5	25.6	27.8	26.8
Orbit diameter	20.2	26.6	23.4	22.8	27.1	24.7	22.0	24.4	29.1	26.8	20.6	26.0	22.6	26.4	30.6	28.2
Upper jaw length	43.1	49.2	45.8	44.8	50.2	46.7	47.7	44.1	47.4	45.9	44.2	49.6	47.4	45.7	48.8	47.1
Head depth at midline of orbit	50.8	54.6	52.6	50.5	53.0	51.8	56.3	46.6	55.8	50.5	50.8	56.3	53.1	53.0	54.9	54.0

separated by morphometric data (Table 5), meristic counts (Table 2) and other morphological features presented in the diagnostic key to species of *Opsariichthys* in the next section.

Diagnostic key to species of *Opsariichthys* from southern China (Fujian to Guangxi provinces), Taiwan and northern Vietnam:

1. Anterior lateral of upper jaw with a very distinct deep notch ····· 2
– Absence of anterior notch on upper lip ····· 6
2. Lateral-line scales 45-47, rounded tubercles on lower jaw rather small and arranging as 3 rows in male ····· 3
– Lateral-line scales 40-43, rounded tubercles on lower jaw rather large and arranging as 2-3 rows in male ····· 4
3. Pre-dorsal scales 19-21, scales above lateral-line 9, scales below lateral-line 3, caudal peduncle scales modally 18, (northern and central east China) ····· *O. bidens*
– Pre-dorsal scales 16-18, scales above lateral-line modally 8, scales below lateral-line 4-5, caudal peduncle scales modally 19, (southern China) ····· *O. minutus*
4. Lateral-line scales 41-43 (modally 42), pre-dorsal scales modally 15-16, rounded tubercles rather large or small and arranging as 2-3 rows, head rather small, body strongly laterally compressed at position of anal origin ····· 5
– Lateral-line scales 40-41 (modally 41), pre-dorsal scales modally 17, rounded tubercles on lower jaw rather large and arranging as 2 rows, head rather large, body rather wide at anal orgin, (Hainan island) ····· *O. hainanensis*
5. Pectoral fin rays i, 13-15 (modally 14), caudal peduncle scales 16-19 (modally 17), pre-dorsal scales 15-18 (modally 16), anterior scales before pelvic origin 14-16 (modally 15), rounded tubercles on lower jaw rather large, as 3 rows in male, body with 14 greenish blue cross-bars in male, maxillary extending to vertical of midline of orbit in female, snout length about 32-33% in male, inter-orbital width about 30% in male, (northern Vietnam) ····· *O. dienbienensis*
– Pectoral fin rays i, 13-14 (modally 13), caudal peduncle scales modally 18, pre-dorsal scales 15-18 (modally 15), anterior scales before pelvic origin 13-15 (modally 14), rounded tubercles on lower jaw rather small, as 2-3 rows in male, body with 13 greenish blue cross-bars in male, maxillary never extending to the vertical of midline of orbit in female, snout length about 30% in male, inter-orbital

width about 27-28% in male, (Ma river, Vietnam) ····· ·····*O. songmaensis*

6. Lateral-line scales 41; pre-dorsal scales 13-14; scales below lateral-line 3, very narrow body width; two rows with 12-15 rather large rounded tubercles on lower jaw in adult male, (northern Vietnam) ····· *O. duchuunguyeni* new species
– Lateral-line scales more than 42; pre-dorsal scales 15-17; scales below lateral-line modally 4, rather narrow to thick body width; a series of 4-7 rounded tubercles on lower jaw in adult male ····· 7
7. Lateral-line scales 42-44; pre-dorsal scales 15-17; rather narrow body width; maxillary not extending to or slightly reaching the vertical of anterior margin of orbit; pectoral fin reaching to or far beyond origin of ventral fin ····· 8
– Lateral-line scales more than 45; pre-dorsal scales 18-23; rather thick body width; maxillary extending to or far beyond the vertical of anterior margin of orbit; pectoral fin not extending beyond origin of ventral fin ····· 9
8. Scales surrounding caudal peduncle 18-20, pectoral fin rays modally 15, maxillary extending to vertical of anterior margin of orbit; pectoral fin never extending to origin or ventral fin, scales above lateral-line modally 9, (southern China) ····· *O. acutipinnis*
– Scales surrounding caudal peduncle 16-17, pectoral fin rays modally 14; maxillary never extending to vertical of anterior margin of orbit; pectoral fin extending far beyond origin of ventral fin, scales above lateral-line modally 8, (northern Taiwan, eastern China) ····· *O. evolans*
9. Lateral-line scales 45-48 (modally 45-47); pre-dorsal scales 18-19; vertebral count 4+35 (= 39); maxillary extending to or slightly beyond vertical of anterior margin of orbit in female; opercle and ventral side of head bright yellow in adult male, (southern Taiwan). ····· *O. kaopingensis*
– Lateral-line scales more than 48 (modally 50-54); pre-dorsal scales 20-23; vertebral count 4+36-37 (= 40-41); maxillary extending to or beyond vertical midline of orbit in female; opercle and ventral side of head orange-red to pink-red in adult male, (northern, middle and westen Taiwan) ····· *O. pachycephalus*

Notes on identifications of *"Opsariichthys hieni"* and *"O. bea"* (Nguyen 1987) from north of central Vietnam; *O. acutipinnis* (Guichenot in Bleeker 1871) and *O. minutus* (Nichols 1926) and from central and southern China:

Nguyen [16] described two new species of *Opsariichthys* from the Lam river basin, Nghe An province, Vietnam. The first species is named "*O. hieni*" with the diagnostic features are anal fin rays (A) 1.6 (indicated one soft ray and six branched rays, i, 6); lateral-line scales (LL) 33-34 and presence of dark stripe which runs from the end of caudal peduncle to end of gill opening. Besides, the note on habitat mentioned that the fish live in a static water environment. This description shows some characters that may refer to genus *Rasbora* (Fig. 5) instead of *Opsariichthys* (A iii, 8-10; LL 40-59 and having cross-bars on body side) at least until present time.

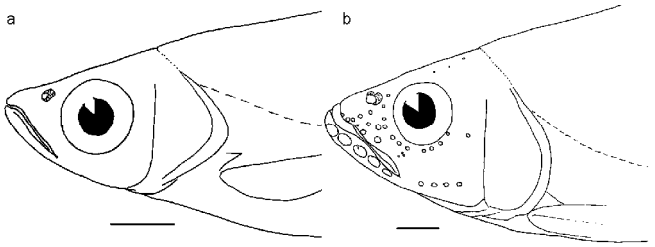

Fig. 5. Illustration of heads of two *"Opsariichthys"* from Nguyen (1987): a, *"O. hieni"* [= *Rasbora* cf. *steineri*] and b, *"O. bea"* [= *Parazacco* cf. *vuquangensis*], collected from Vu Quang district, Ha Tinh province, Vietnam. Bar = 5 mm.

The second species was described as "*O. bea*" appeared in description with much more anal fin ray couting than *Opsariichthys* (2.12 *vs.* iii, 8-10) and having an indistinct laterally stripe (*vs.* presence of cross-bars). The author also mentioned the number of lateral-line scales is from 50-54 and living at running water of upper stream. These features may indicate the characters of genus *Parazacco* (Fig. 5).

Through personal contact with Dr. Nguyen Thai Tu from Vinh University (the author of "*O. hieni*" and "*O. bea*"), we had his confirmation for these identifications and he was also willing to see the change of these names. This view met our opinion about these species based on the distinct characters of the fishes in either original descriptions or our specimens from the locality. The specimens collected from this sampling site will be presented in the other paper.

Based on the combination of morphological differentiations and molecular divergences from this study, *O. acutipinnis* and *O. minutus* are considered as valid nomenclature of fishes from central and southern China which have been ignored in long time. Also followed the result of this study, the name *O. bidens* can not be used for any *Opsariichthys* collected from Vietnamese waters.

IV. DISCUSSION

The generic status of fishes species belong to opsariichthine group including *Parazacco*, *Candidia*, *Zacco* and *Opsariichthys* has been confused in the long time. Recently, Chen and his team conducted several morphological and molecular combination studies to see the real species number and phylogeny of *Opsariichthys* and *Candidia* genus from Taiwan and China, and proposed a new genus *Nipponocypris* from "*Zacco temmincki*", "*Z. koreanus*" and "*Z. sieboldi*" from Japan and Korea (Chen *et al*. [6-8]). The results yielded from above combination traditional and modern technique may bring the suitable tools for fishes species identification.

The present study combines morphological and molecular methods indicating the validity of four fish names, including *Opsariichthys acutipinnis*, *O.minutus* from central and southern China; *O. songmaensis* and *O. dienbienensis* from northern Vietnam; and describes a new species of "short mouth"

form of *Opsariichthys* from Ky Cung – Bang Giang river basin, northern Vietnam as *O. duchuunguyeni*. The remaining groups are recommended for further studies, including *Zacco* from the northern China; *Parazacco* from southern China, northern and central Vietnam; *Opsariichthys* from central Vietnam.

Beside that, the similarity of freshwater fish fauna among Hainan island, southern China and northern, central Vietnam needs to be paid attention to recognize the species dispersal history in nearby region. Most of northern Vietnamese scientific names need to be revised carefully from the name used in Chinese literatures, herein, the *O. bidens* can be considered as a typical case. This species should be restricted in the northern and the east central China. By doing these research, a widely picture of real species diversity of this group will be provided, which will be able to contribute for complete understanding of cyprinid, the enormous and important fishes component of the world.

ACKNOWLEDGMENTS

The first author (TQH) is very grateful for the master studentship from NTOU. Field work was financially supported by Huynh Gia Foundation for the first author. We also thank Prof. Nguyen Huu Duc, Mr. Nguyen Van Hao and Dr. Nguyen Thai Tu for their comments, and staffs of Research Institute of Aquaculture No. 1 (Bac Ninh, Vietnam) for access specimens under their care. The corresponding author (ISC) expresses his thanks to the grant support of NSC, Taipei, ROC.

Comparative materials

Opsariichthys acutipinnis: NTOU P-2013-10-197, 5 specimens, 51.58-78.35 mm SL., Hongkong, China, Coll. I-S. Chen, 2012; NTOU P-2013-10-198, 1 specimen, 61.3 mm SL., Changjiang, Zhejiang province, China; NTOU P-2013-10-199, 2 specimen, 52.6-65.4 mm SL., Xijiang, Guangdong province, China; NTOU P-2013-10-200, 1 specimen, 54.4 mm SL., Xijiang, Guangdong province, China.

Opsariichthys bidens: NTOU P-2008-06-381, 6 specimens, 69.7-84.8 mm SL., Tachiao market, Fenghua city, Zhejiang province, China, Coll. J.H. Wu & S.P. Huang, 10 Jan 2008; NTOU P-2008-06-389, 15 specimens, 112.9-163.2 mm SL., Central market, Xianju county, Zhejiang province, China, Coll. J.H. Wu & S.P. Huang, 08 Jan 2008.

Opsariichthys dienbienensis: NTOU P-2013-10-174, 4 specimens, 55.7-75.2 mm SL., Na Nhan commune, Dien Bien district, Dien Bien province, Vietnam; NTOU P-2013-10-181, 6 specimens, 86.4-122.2 mm SL., Huy Ha commune, Phu Yen district, Son La province, Vietnam.

Opsariichthys evolans: NTOU P-2006-3-457, 3 specimens, 61.6-89.9 mm SL., Yu-Rui stream, Keelung river, Tanshuei river basin, Keelung city, Taiwan, Coll. I-S. Chen *et al*., 17 Mar 2006; NTOU P-2008-11-459, 2 specimens, 84.8-89.6 mm SL., Rui-fang, Keelung river, Tanshuei river basin, Taipei county, Taiwan, Coll. S.P. Huang, 10 Jul 2008; NTOU P-2008-11-460, 70.2 mm SL., Kong-liao, Shi-ting stream, Shungshi river basin, Taipei county, Taiwan, Coll. S.P. Huang, 12 Aug 2008; NTOU P-2008-11-461, 79.3 mm SL., Keelung river, Tanshuei river basin, Taipei county, Taiwan, Coll. S.H. Su, 19 Jun 2008; NTOU P-2008-11-462, 3 specimens, 52.4-59.7 mm SL, Pau-Chong Bridge, Fongshan River, Shinchu County, Taiwan, Coll. S.H. Su, 26 Dec 2007.

Opsariichthys hainanensis: NTOU P-2008-6-382, 10 specimens, 39.7-71.7 mm SL., Tengchiaotung river, Pouting county, Hainan province, China, Coll. I-S. Chen & S.P. Huang, 09 Sep 2005; NTOU P-2008-6-383, 2 specimens, 46.8-54.3 mm SL., Partsun, Linshui river basin, Pouting county, Hainan province, China, Coll. I-S. Chen & S.P. Huang, 10 Sep 2005; NTOU P-2008-6-384, 2 specimens, 54.4-71.7 mm SL., Linshui river basin, Linshui county, Hainan province, China, Coll. I-S. Chen & S.P. Huang, 10 Sep 2005; NTOU P-2008-6-385, 2 specimens, 54.1-65.5 mm SL., Leunan, Nandujiang basin, Chungjung county, Hainan province, China, Coll. I-S. Chen & S.P. Huang, 11 Sep 2005; NTOU P-2008-6-386, 57.2 mm SL., Nankai, Nandujiang basin, Paishar county, Hainan province, China, Coll. I-S. Chen & S.P. Huang, 12 Sep 2005; NTOU P-2008-6-387, 8 specimens, 54.8-63.2 mm SL., Wangchuang river basin, Chungjung county, Hainan province, China, Coll. I-S. Chen & S.P. Huang, 11 Sep 2005.

Opsariichthys kaopingensis: NTOU P-2009-02-05, 2 specimens, 48.7-49.5 mm SL., Fengshan river, Pingtung county, Taiwan, Coll. S.P. Huang, 05 Feb 2009.

Opsariichthys minutus: NTOU P-2013-10-189, 2 specimens, 64.24-93.29 mm SL., Hanjiang, Guangdong province, China; NTOU P-2013-10-190, 2 specimens, 72.7-85.1 mm SL., Gaowu, Fujian province, China; NTOU P-2013-10-191, 1 specimen, 86.6 mm SL., Nanshan, Fujian province, China; NTOU P-2013-10-192, 2 specimens, 79.5-86.7 mm SL., Xinquan, Fujian province, China; NTOU P-2013-10-193, 3 specimens, 60.2-70.8 mm SL., Mingjiang, Fujian province, China; NTOU P-2013-10-194, 2 specimens, 77.4-101.9 mm SL., Zhongcun, Fujian province, China; NTOU P-2013-10-195, 1 specimen, 153.55 mm SL., Ming river, China, Coll. K-T. Shao; NTOU P-2013-10-196, 4 specimens, 77.9-126.7 mm SL., Baisha town, Fujian, China.

Opsariichthys pachycephalus: NTOU P-2013-10-150, 1 specimen, 96.3 mm SL., Lanyanxi, Yilan county, Taiwan; NTOU P-2013-10-151, 2 specimens, 85.8-86.7 mm SL., Annongxi, Yilan county, Taiwan; NTOU P-2013-10-152, 3 specimens, 53.5-86.2 mm SL., Dahanxi, Taoyuan county, Taiwan; NTOU P-2013-10-153, 3 specimens, 61.8-67.2 mm SL., Wuxi, Taichung county, Taiwan; NTOU P-2013-10-154, 5 specimens, 60.7-66.9 mm SL., Fengshanxi, Hsinchu county, Taiwan.

Opsariichthys songmaensis: NTOU P-2013-10-172, 1 specimen, 105.1 mm SL., Cam Phong commune, Cam Thuy district, Thanh Hoa province, Vietnam; NTOU P-2013-10-175, 6 specimens, 57.0-74.9 mm SL., Bung Lao commune, Muong Ang district, Dien Bien province, Vietnam; NTOU P-2013-10-176, 3 specimens, 50.7-57.4 mm SL., Chieng Sinh commune, Tuan Giao district, Dien Bien province, Vietnam.

Opsariichthys uncirostris: NTOU P-2013-10-155, 1 specimen, 153.56 mm SL., Biwa lake, Japan, Coll. I-S., Chen.

APPENDIX

NCBI accession numbers of mtDNA D-loop sequences of cyprinid fishes used in this study: *O. acutipinnis* (OPACHK1-2: KF785753-KF785754); *Opsariichthys duchuunguyeni* (OPDUCB1: KF753239); *O. dienbienensis* (OPDBNR1: KF785757); *O. minutus* (OPMIFJ1-2: KF785755-KF785756); *O. songmaensis* OPSMDB1-2: KF785758-KF785759 and OPSMTH1: KF785760); other sequences were cited in Chen *et al*. [6-8].

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