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Phylogenetic Analysis of *Channa* Genus based on Morphological Characters and X-Ray Imaging

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Key words
***Channa* species, Morphological analysis, Phylogenetic relationship, X-ray photography**

ABSTRACT

Classification of the dispute is in existence among the *Channa* genus. In present study, we measured 11 countable characteristics and 29 measurable characteristics of 89 individuals and performed morphological analysis among five *Channa* species. The principal component analysis showed that the cumulative contribution rate of five principal components reached 78.928%, and tail shank (TS) and head length (HL) were the main contributors to the first principal component (35.435% contribution rate). The scatter diagram of the principal component analysis showed that white type and *Channa argus* had common overlap, while the other three *Channa* species were grouped together. Cluster analysis showed that all *Channa* species can be completely separated, and *O. argus* var Kimnra and *C. argus* clustered together. The discriminant analysis showed that *O. argus* var Kimnra and *C. argus* were 41.7% and 58.3% similar to each other, respectively. X-ray photography revealed that *O. argus* var Kimnra and *C. argus* have similar forms, but they are far from *C. asiatica*. Therefore, *O. argus* var Kimnra and *C. argus* have a close relationship with no significant morphological differences.

INTRODUCTION

Morphological analysis is a convenient way to study genetic variation because the markers are visible, specific external features. There have been many morphology studies of fish species (Mir *et al*., 2014; Hammami *et al*., 2016; Song *et al*., 2015) and morphological identification is one of the most direct methods for observing and identifying fish phenotype traits. Advantages include the ease of experimentation and minimal damage to animals. The study of species classification, resource identification, and biological evolution have been based on morphological markers. The *Channa* genus includes 33 Species, the *C. argus, C. maculata, C. asiatica and C. maculata x C. argus* (Perciformes, Channoidei, Channidae) are widely distributed in China. While the white type C. argus is only discovered in the Jialing River in Sichuan (105.05E, 29.58N) in China, which is white without any blotches, the size and appearance are very similar with the biocolor one (Zhou *et al*., 2015).

In order to comprehensively understand interspecific differences and identify *Channa* species, we used morphological methods. However, preliminary identification of *O. argus* var Kimnra based on appearance can provide some theoretical guidance for standardizing *Channa* species breeding and production. By comparing the morphological characteristics of different *Channa* species, we can understand their genetic relationship and provide information for resource evaluation, protection, and utilization of *O. argus* var Kimnra.

MATERIALS AND METHODS

Experimental animal collection

The samples used in this study were collected from wild in the non breeding season; the body weight and length ranges were 29.23-273.89 g and 13.59-30.84 cm, respectively. *Channa* species information is provided in Table I.

Measurement and data collection

The experimental *Channa* species were weighed on

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Table I. Sample information of five *Channa* species.

Sample name	Sample size	Location	Date
C. argus	20	Neijiang, Chongqing	2015, 2016
O. argus var Kimnra	20	Neijiang, Chongqing	2015, 2016
C. maculata	16	Qingyuan, Guangdong Province	2016
C. maculata x C. argus	18	Zhongshan, Guangdong Province	2016
C. asiatica	15	Guangzhou, Guangdong Province	2016

Table II. Meristic characters of five *Channa* species.

Traits	*Channa* species				
	***O. argus* var Kimnra**	***C. argus***	***C. maculata***	***C. maculata x C. argus***	***C. asiatica***
Soft ray of dorsal fin	47-50(48.25±1.14)^{c}	47-51(48.8±1.47)^{c}	43-49(45.73±2.05)^{b}	42-49(45.53±2.39)^{b}	42-46(43.82±1.33)^{a}
Soft ray of pectoral fin	16-19(17.17±1.27)^{b}	16-18(16.92±0.90)^{b}	15-18(16.45±1.37)^{b}	13-18(14.93±1.58)^{a}	14-16(14.91±0.83)^{a}
Soft ray of pelvic fin	6(6.00±0.00)^{b}	5-6(5.50±0.52)^{a}	6(6.00±0.00)^{b}	5-6(5.47±0.52)^{a}	0
Soft ray of anal fin	29-34(31.33±1.87)^{b}	30-34(32.58±1.38)^{b}	26-32(29.18±2.23)^{a}	27-32(29.80±1.90)^{a}	29-30(29.64±0.50)^{a}
Soft ray of tail fin	16-19(17.42±2.15)^{c}	16-20(18.00±1.48)^{c}	15-17(15.91±0.83)^{b}	13-18(15.53±2.00)^{b}	12-16(14.09±1.64)^{a}
Lateral line scales	61-65(62.67±1.56)^{b}	57-65(60.83±2.82)^{b}	51-60(54.82±3.03)^{a}	56-68(63.00±3.74)^{b}	58-64(61.09±2.26)^{b}
Scales above lateral line	8-12(10.33±1.50)^{c}	8-10(8.92±0.90)^{b}	6-8(7.00±0.89)^{a}	7-10(8.80±1.21)^{b}	8-9(8.55±0.52)^{b}
Scales below lateral line	17-19(18.08±0.90)^{b}	16-19(17.42±1.08)^{b}	14-18(15.45±1.37)^{a}	16-19(17.60±1.06)^{b}	15-17(15.82±0.98)^{a}
Gill rakers	10-13(11.08±1.16)^{b}	10-12(11.25±0.87)^{b}	9-13(11.09±1.51)^{b}	9-14(11.33±1.63)^{b}	8-12(9.82±1.54)^{a}
Vertebra	56-60(57.08±1.16)^{b}	55-60(57.67±1.97)^{b}	53-55(53.91±0.83)^{a}	53-56(54.53±1.30)^{a}	54-55(54.55±0.52)^{a}
Rib	51-55(52.75±1.48)^{b}	50-55(52.92±1.73)^{b}	48-50(48.73±0.79)^{a}	48-51(49.60±1.24)^{a}	49-50(49.45±0.52)^{a}

an electronic scale (accurate to 0.01 g), and a digital vernier caliper was used to measure traditional morphological and frame data. The traditional morphological data included 10 countable traits (soft ray of dorsal fin number, soft ray of pectoral fin number, soft ray of pelvic fin number, soft ray of anal fin number, soft ray of tail fin number, lateral line scales number, scales above lateral line number, scales below lateral line number, gill rakers number, vertebra number and rib number) and 10 measurable traits (accurate to 0.01 cm: full length (FL), body length (BL), body height (BH), body width (BW), caudal peduncle length (CPL), caudal peduncle depth (CPD), head length (HL), snout length (SnL), head length behind the eyes, eye diameter (EL) and eye interval (IW)). Frame parameters (accurate to 0.01 cm) included 21 items**.** Measurement features are as follows: BL*,* Distance from the snout front to the tail vertebrae. BW*,* Maximum distance between the two body sides. BH*,* Maximum vertical distance from the top of the trunk to the abdomen. HL*,* Distance from the snout front to the external edge of the preopercle. SnL*,* Distance from the front of the snout to the eye leading edge. EL*,* Maximum distance between the front and back edges of the eye. IW*,* Distance between the upper edge of the head on both sides of the eyes. CPL, Horizontal distance from the end of the anal fin base to the front end of the caudal fins. CPD, Shortest vertical distance between the dorsal and ventral edges of the caudal handle.

Statistical analysis

In order to eliminate the effect caused by the different sample size, the ratio of original data and BL or HL are used as the correction value, and 29 morphological characters were included as a parameter for least significant difference (LSD) testing using Excel 2016 and SPSS19.0 software. We carried out cluster analysis and principal component analysis and then calculated the Euclidean Distance of each group, as well as the principal component eigenvalue and contribution rate. The principal component scores were used to generate a scatter diagram. Principal Component Analysis: SPSS19.0 software was used to analyze morphological data, and then we calculated the principal component eigenvalue and contribution rate, which were used to generate a scatter diagram. Clustering Analysis: We used the Analyze-Classify-Hierarchical-Cluster method in SPSS19.0 software to perform cluster analysis for the five *Channa* species.

Table III. Morphological data of five *Channa* species.

Traits	*Channa* species				
	***O. argus* var Kimnra**	***C. argus***	***C. maculata***	***C. maculata* x *C. argus***	***C. asiatica***
BL/FL	0.84±0.02^{a}	0.86±0.03^{b}	0.90±0.01^{c}	0.86±0.01^{b}	0.85±0.01^{ab}
BH/BL	0.19±0.01^{b}	0.16±0.01^{a}	0.20±0.02^{c}	0.19±0.02^{b}	0.18±0.01^{b}
CPD/CPL	1.41±0.05^{d}	1.29±0.13^{c}	0.85±0.09^{a}	1.18±0.09^{b}	2.06±0.18^{e}
HL/BL	0.31±0.02^{b}	0.31±0.01^{b}	0.33±0.02^{c}	0.33±0.03^{c}	0.24±0.01^{a}
SnL/HL	0.16±0.02^{bc}	0.16±0.04^{cd}	0.14±0.01^{ab}	0.14±0.03^{a}	0.17±0.01^{d}
HLBE/HL	0.70±0.08^{b}	0.70±0.11^{b}	0.72±0.01^{c}	0.72±0.11^{c}	0.68±0.02^{a}
EL/HL	0.14±0.01^{b}	0.15±0.02^{c}	0.13±0.01^{b}	0.13±0.03^{b}	0.11±0.01^{a}
IW/HL	0.32±0.03^{ab}	0.33±0.03^{bc}	0.34±0.01^{c}	0.32±0.04^{a}	0.47±0.02^{d}
D1-2/BL	0.24±0.03^{c}	0.24±0.02^{c}	0.28±0.02^{d}	0.22±0.02^{b}	0.18±0.01^{a}
D1-3/BL	0.17±0.03	0.18±0.02	0.17±0.01	0.17±0.01	/
D1-4/BL	0.12±0.01^{a}	0.14±0.01^{c}	0.16±0.02^{e}	0.15±0.01^{d}	0.13±0.01^{b}
D2-3/BL	0.40±0.02^{b}	0.40±0.03^{b}	0.39±0.05^{b}	0.38±0.04^{a}	/
D2-4/BL	0.26±0.05^{c}	0.25±0.02^{b}	0.28±0.02^{d}	0.24±0.01^{b}	0.18±0.03^{a}
D3-4/BL	0.20±0.01^{a}	0.22±0.02^{b}	0.24±0.03^{c}	0.21±0.01^{b}	/
D3-5/BL	0.15±0.01^{a}	0.14±0.01^{a}	0.17±0.02^{c}	0.15±0.01^{b}	/
D3-6/BL	0.17±0.02^{b}	0.16±0.01^{a}	0.19±0.02^{c}	0.17±0.01^{b}	/
D4-5/BL	0.32±0.06^{a}	0.33±0.01^{b}	0.35±0.01^{c}	0.33±0.01^{b}	0.37±0.01^{d}
D4-6/BL	0.08±0.01^{a}	0.12±0.02^{c}	0.11±0.02^{b}	0.13±0.02^{c}	0.11±0.02^{b}
D5-6/BL	0.26±0.04^{b}	0.24±0.01^{a}	0.26±0.01^{b}	0.24±0.02^{a}	0.26±0.01^{b}
D5-7/BL	0.40±0.05^{b}	0.39±0.03^{b}	0.38±0.01^{a}	0.38±0.02^{a}	0.42±0.03^{c}
D5-8/BL	0.43±0.03^{bc}	0.44±0.02^{c}	0.41±0.02^{a}	0.42±0.02^{ab}	0.46±0.03^{d}
D6-7/BL	0.60±0.05^{cd}	0.58±0.02^{bc}	0.57±0.01^{ab}	0.56±0.02^{a}	0.61±0.02^{d}
D6-8/BL	0.60±0.07^{b}	0.61±0.01^{b}	0.58±0.01^{a}	0.58±0.03^{a}	0.66±0.03^{c}
D7-8/BL	0.09±0.02^{a}	0.09±0.02^{ab}	0.10±0.01^{cd}	0.10±0.01^{bc}	0.11±0.01^{d}
D7-9/BL	0.06±0.02^{b}	0.07±0.02^{b}	0.12±0.02^{d}	0.08±0.01^{c}	0.06±0.01^{a}
D7-10/BL	0.11±0.02^{a}	0.11±0.01^{a}	0.13±0.01^{c}	0.12±0.01^{b}	0.12±0.02^{c}
D8-9/BL	0.10±0.02^{ab}	0.10±0.01^{a}	0.13±0.02^{d}	0.11±0.01^{bc}	0.11±0.01^{c}
D8-10/BL	0.04±0.01^{a}	0.05±0.01^{a}	0.06±0.01^{b}	0.05±0.01^{a}	0.05±0.01^{a}
D9-10/BL	0.09±0.02^{a}	0.08±0.01^{a}	0.11±0.01^{b}	0.10±0.01^{b}	0.10±0.01^{b}

Note: Twenty-one truss parameter measurements are the distances between the two of 10 landmark points, e.g., $D_{1\text{-}2}$ denotes the distance between landmark point 1 and 2. 1. Most posterior of maxilla; 2. Tip of snout; 3. Origin of pelvic fin; 4. Terminus of head back; 5. Origin of anal fin ; 6. Origin of dorsal fin; 7. Terminus of anal fin; 8. Terminus of dorsal fin; 9. Ventral origin of caudal fin; 10. Dorsal origin of caudal fin.

Discriminant analysis: We used the Analyze-Classify-Discriminant-Analysis method in SPSS19.0 software to build a discriminant formula with contribution rate parameters with large differences for *O. argus* var Kimnra and *C. argus*. Differential Coefficient Analysis: Difference coefficient CD=(MB-MA)/(SDA+SDB), where MA and MB were the mean values of A, B population parameters and SDA and SDB were the standard deviations of A, B population parameters. If the difference coefficient <1.28, it indicates a geographical difference between populations (Mayr *et al*., 1953).

RESULTS

Characteristic analysis of countable traits

The analysis of countable traits showed that the major differences were gill rakers number, soft ray of dorsal fin number, soft ray of anal fin number, vertebrae number, and scales above lateral line number (Table II). Combining these with the changes in fish body pattern, which can distinguish between different fish types. Still, the body pattern is challenging to determine the precise fish type based solely on the countable traits mentioned above. It

needs to used in conjunction with other analytical methods to make provide more accurate identification.

Analysis of measurable traits

LSD significance test analysis of 8 measurable traits ratio and 21 frame correction data (Table III) showed that *O. argus* var Kimnra has similar measurable trait parameters with *C. argus,* but there were 24 significantly different measurable traits parameters with *C. maculata* and *C. asiatica* ($P < 0.05$). Compared with the female parent *C. Maculata*, morphological characters of *C. maculata x C. argus* had greater similarity with the male parent *C. argus.*

Principal component analysis

From principal component analysis, we can obtain the load value, contribution rate, and cumulative contribution rate from the first to the fifth principal component (Table IV). Morphological indexes that had a main effect on the first principal component loads value were CPD/CPL, HL/BL, D2-4/BL, D5-8/BL, D7-8/BL, and D7-10/BL, which mainly reflected the characteristics of tail shank and HL. The mainly effect on the second main component loads value was D8-9/ BL, which mainly reflected tail shank features. The HLBE/HL and D5-6/BL had large impacts on the third main component loads value, which mainly reflected the HL and BH features. However, the five principal components accumulated a 78.93% contribution rate, which indicates that there are differences among the five species.

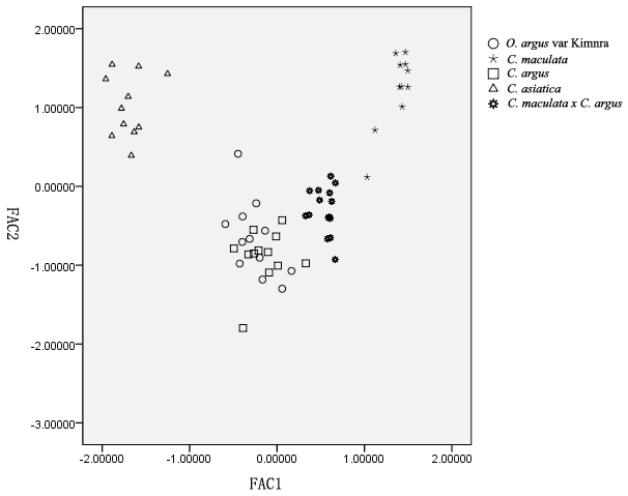

Fig. 1. Plot of the first and second principle components (FAC1 and FAC2) from PCA based on the morphological characteristics among five *Channa* species.

According to the first and second principal component scatter diagram (Fig. 1), The relationship of *C. maculata x C. argus* was between *C. argus* and *C. maculata*. *C. maculata x C. argus, C. maculata*, and *C. asiatica* can respectively form a group. This suggests that *O. argus* var Kimnra and *C. argus* have high morphologic similarity with each other, but there are certain morphological differences compared to the other three *Channa* species.

In order to show the differences between the five groups, the average value of the 24 eigenvalue groups was analyzed by cluster analysis. The results showed that the five populations could be divided into three groups: *O. argus* var Kimnra, C. *argus* and *C. maculata x C. argus* clustered into the one group, and *C. maculata* and *C. asiatica* clustered into the other two groups (Fig. 2). *O. argus* var Kimnra and C. *argus* had close genetic distances and similar forms. *C. maculata x C. argus* was more similar to the male parent *C. argus* compared with its female parent *C. maculata.*

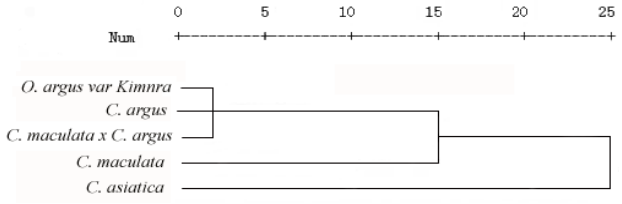

Fig. 2. Dendrogram showing the relationhip of five *Channa* species.

Discriminant analysis

Based on the above results, we performed discriminant analysis of *O. argus* var Kimnra and *C. argus* using 8 measurable parameters and 21 frame parameters, and the discriminant effect was highly significant ($P < 0.01$). In order to improve the practicality, we selected 9 higher contribution rate characteristics to distinguish species, and the *F* value is shown in Table V.

The discriminant equation was established using the selected nine morphological parameters as follows: *O. argus* var Kimnra: Y=1584.714 D3-5/BL +1721.382 BH / BL+1655.351 D1-4/ BL +6896.650D3-6/BL: +4724.734 EL/HL +551.480 CPD/ CPL+1747.480D5-6/BL -2577.771 D4-6/ BL +1555.991 IW/HL -1965.121

C. argus: Y=2463.010D3-5/BL+1547.279BH/ BL+7715.315D3-6/BL+2142.617D1-4/BL+ 5162.266 EL/ HL +579.297 CPD/ CPL +1569.328D5-6/BL -3046.163 D4-6/BL +1608.042 IW/HL -2253.068.

According to the above discriminant formula, we can distinguish the two *Channa* species. The method uses the morphological parameters corrected by FL into two above formulae, then the Y value is calculated.

Table IV. Factor loading value of 24 measurable characters principal component analysis among five *Channa* species.

Traits	Principal component				
	1	2	3	4	5
BL/FL	0.644	0.518	0.016	0.243	-0.242
BH/BL	0.387	0.510	0.031	-0.548	0.126
CPD/CPL	-0.953	0.088	-0.017	-0.095	-0.116
HL/BL	0.851	-0.246	-0.062	-0.114	0.325
SnL/HL	-0.542	-0.024	0.123	0.346	0.197
HLBE/HL	0.059	-0.013	0.763	0.194	-0.380
EL/HL	-0.095	-0.403	0.059	0.741	-0.004
IW/HL	-0.744	0.490	-0.137	0.127	-0.311
D1-2/BL	0.789	0.062	0.439	0.169	0.200
D1-4/BL	0.726	0.395	-0.299	0.233	0.128
D2-4/BL	0.807	-0.240	0.373	0.043	0.238
D4-5/BL	-0.331	0.737	-0.166	0.117	-0.065
D4-6/BL	0.160	0.002	-0.742	0.485	0.147
D5-6/BL	-0.159	0.617	0.640	-0.065	0.023
D5-7/BL	-0.776	0.222	0.253	0.133	0.251
D5-8/BL	-0.816	0.291	-0.099	0075	0.272
D6-7/BL	-0.588	0.312	0.432	-0.005	0.347
D6-8/BL	-0.837	0.250	0.159	0.191	0.184
D7-8/BL	-0.174	0.724	-0.170	-0.071	0.267
D7-9/BL	0.808	0.488	0.116	0.158	0.041
D7-10/BL	0.096	0.811	-0.181	-0.023	-0.023
D8-9/BL	0.440	0.731	0.064	0.153	0.014
D8-10/BL	0.527	0.654	0.119	0.300	-0.136
D9-10/BL	0.099	0.664	-0.208	-0.258	-0.114
Eigenvalue	8.504	5.261	2.456	1.711	1.009
Contribution rate	35.435	21.921	10.234	7.131	4.206
Cumulative contribution rate	35.435	57.356	67.590	74.722	78.928

The single factor variance analysis of *O. argus* var Kimnra and *C. argus* population identified seven extremely significant different characteristics ($P < 0.01$), and one significant feature between the two populations ($P < 0.05$). The mean value, variance, and difference coefficient are shown in Table VI. It can be seen that their difference coefficient is <1.28, the threshold value of subspecies classification, indicating that they belong to different geographic populations, but not up to the level of subspecies.

Analysis of X-ray imaging in Channa *species*

Based on X-ray studies of the five *Channa* species, we observed developed girdle and pelvic fins in *O. argus* var Kimnra, *C. argus, C. maculata*, and *C. maculata* x *C. argus*, but *C. asiatica* had neither a girdle nor pelvic fin, and the spine and rib numbers were also significantly different. Skull imaging showed that *O. argus* var Kimnra and *C. argus* had similar snout tips, but *C. asiatica* had a blunt snout. Observed from the side, we noted that the rears of the heads of *O. argus* var Kimnra and *C. argus* are flat and slightly concave; the eyes are located in the upper part of the skull; and the skull was long, narrow, and higher. The back head margin of *C. asiatica* curved up, and the eyes were positioned slightly close to the outside of the skull, which was short, wide, and lower (Fig. 3).

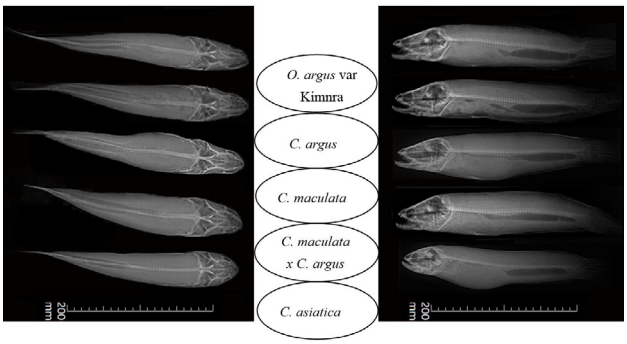

Fig. 3. X-ray transmission map of five *Channa* species. (Left: dorsal view; Right: lateral view).

DISCUSSION

Morphological markers application in fishes

Traditional morphological analysis is an intuitive method to identify distantly related fishes. At present, there are many reports on fish morphological differences (Ecoutin *et al*., 2005; Elliott *et al*., 1995; Mir *et al*, 2014; Ruiz-Campos *et al*., 2003; Tzeng, 2004; Yang *et al*., 2003). Our study results showed that the five populations could be divided into three groups through morphological markers, and there is a high level of overlap among the two color morphs of *C. argus* and *C. maculata* x *C. argus*, which is similar to the research of Sicily and Tunisia (Traina *et al*., 2011). Morphological analysis of lake and stream-dwelling rock bass and pumpkinseed populations suggests that smaller fins may be more common in stream-dwelling individuals (Brinsmead and Fox, 2002). Correlation of morphological characters and buoyancy were investigated in lake trout (Zimmerman *et al*., 2009), our results also showed that morphological markers can effectively distinguish species with large differences.

Morphological analysis among different Channa *species*

Channa species morphology are widely studies. Six species of snakehead fish in Malaysia were previously

Table V. Variables (ranged by *F* test values) with high contribution in discriminant analysis of *O. argus* var Kimnra and *C. argus*.

Parameter	D3-5/BL	BH/BL	D1-4/BL	D3-6/BL	EL/BL	CPD/CPL	D5-6/BL	D4-6/BL	IW/HL
F values	209.245	48.412	38.169	29.609	19.763	9.237	9.186	8.046	4.000

Table VI. Characters of high variance between two populations of *O. argus* var Kimnra and *C. argus*.

Traits	*O. argus* var Kimnra	*C. argus*	Diversity factor
BH/BL	0.190±0.021^{b}	0.162±0.016^{a}	0.757
CPD/CPL	1.412±0.082^{b}	1.294±0.139^{a}	0.534
EL/HL	0.141±0.016^{a}	0.152±0.023^{b}	0.282
D1-4/BL	0.124±0.018^{a}	0.142±0.015^{b}	0.545
D3-5/BL	0.152±0.017^{b}	0.146±0.013^{a}	0.200
D3-6/BL	0.173±0.028^{b}	0.166±0.016^{a}	0.159
D4-6/BL	0.084±0.015^{A}	0.121±0.027^{B}	0.881
D5-6/BL	0.263±0.044^{b}	0.241±0.018^{a}	0.355

Note: a, b very significant difference (P<0.01), A, B significant difference($P < 0.05$).

subjected to morphological analysis (Tam *et al*., 2006). In addition, morphometric analysis revealed a close relationship between *C. striatus* and *C. marulius* among the five *Channa* species (Haniffa *et al*., 2014). The Malabar snakehead fish *C. diplogramma* was evaluated for its phylogenetic relationships and evolutionary biogeography using morphological and molecular genetic analyses (Benziger *et al*., 2011). The taxonomic statuses of *C. marulioides* and *C. melanoptera* were clarified using morphological analysis (Lee *et al*., 1994). Coefficient of morphometric variation data showed that the snakehead fish from Kalimantan was higher than that for Jawa and Sumatera (Oktaviani, 2013). A morphometric and genetic study was also conducted on six of the seven *Channa* species found in Peninsular Malaysia (Mohd Husin, 2007). Mayr believes that subspecies can be further divided into different geographical populations, and the critical value of the difference coefficient should be 1.28 (Mayr *et al*., 1953). In our study, the difference coefficient of *O. argus* var Kimnra and *C. argus* was <1.28. According to the theory, this did not reach the level of subspecies. Indeed, we can see that *O. argus* var Kimnra and *C. argus* had a large cross phenomenon based on the external shape measurable data, therefore, They have no significant morphological differences, and they are distantly related with *C. maculata*, *C. maculata* x *C. argus*, and *C. asiatica*. The scatter diagram demonstrated that the coincidence degree of *O. argus* var Kimnra and *C. argus* were the highest, and the cluster analysis showed similar results. Discriminant analysis and single factor analysis of variance showed that the differences between *O. argus* var Kimnra and *C. argus* did not reach the level of subspecies (Wang *et al*., 1992, 1993), and similar findings were obtained via our previous studies (Zhou *et al*., 2019). Based on these findings, we can preliminarily determine that *O. argus* var Kimnra should serve as a *C. argus* albino mutant.

Relationship between morphological differences and geographical environment

Biological evolution divides organisms into different populations based on geographical environments. However, some research shows that the morphological differences and geographical environments have some connection. *Channa* species are mainly distributed in the fresh water areas of tropical and subtropical Asia and Africa. An analysis carried out on seven anchovy samples in the northwestern Mediterranean revealed that morphological variation appeared to have a predominantly environmental basis (Tudela, 1999). The morphological and genetic variation of eight Tunisian sharp snout samples showed that the Siculo-Tunisian Strait does not seem to act as a barrier limiting connectivity (Hammami *et al*., 2016). The populations of *C. maruliussite* could be divided into four major clusters in Pakistan, and this was related to the impacts of changing environment and other possible factors (Bhatti *et al*., 2014). The geographic distribution of different *Channa* species in China is diverse. *C. argus* is mainly distributed in the Yangtze River basin and north to the Heilongjiang area. Currently, *O. argus* var Kimnra is only found in the Jialing River basin, overlapping with the geographic distribution of *C. argus*, especially in Sichuan Province. *C. maculata* is located in the south of the Yangtze River Valley, especially in southern China, and *C. maculata* x *C. argus* has high and low temperature resistance, so it can be farmed in both southern and northern China. Conversely, *C. asiatica* is mainly located in the south of the Yangtze River basin; it is especially popular in the Guangdong area. According to X-ray findings, *O. argus* var Kimnra and *C. argus* are very similar, having developed belt and pelvic fins, but there are also some differences. *C. asiatica* has neither belt nor pelvic fins, and that may be related to the different geographical environment.

In summary, morphological markers is an effective method to study the genetic diversity and phylogenetic relationship among five genus *Channa*. At the same time, X-ray can partly distinguish species with large differences. It is suggested that *O. argus* var Kimnra and *C. argus* have no significant morphological differences, and the the former is attributed to an albino variant of the latter.

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Declaration of conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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