Morphometric studies of genus *Placocheilus* (Teleostei: Cypriniformes) from Red River, China

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ABSTRACT

It is practically difficult to differentiate *Placocheilus robustus* and *Placocheilus caudofasciatus* from Red River drainage of China. Without stated reasons, *P. robustus* has been assumed as the synonyms of *P. caudofasciatus*. The present study aims to decipher the morphological differences between two species so as to provide reliable clues for their classification by multivariate morphometry. A total of 72 specimens of two species in genus *Placocheilus* were examined. Besides morphological character comparisons, 10 anatomic landmarks were utilized and 23 frame structures and 15 general characters measured. The scatter plot results of principal component analysis showed that all specimens were clustered together and could not be defined as two distinct species. No significant morphological differences existed in four diagnostic characters between *P. robustus* and *P. caudofasciatus*. Thus the results of the present study fail to support *P. robustus* as a valid and independent species.

Keywords: *Placocheilus caudofasciatus*; *Placocheilus robustus*; Mophomemetrics; Principal component analysis; Evidence for taxonomy

INTRODUCTION

The genus *Placocheilus* was established by Wu (1977) on the basis of the description of type species *Discognathus caudofasciatus* Pellegrin et Chevey. *Placocheilus* was originally described as of order Cypriniformes, subfamily Barbininae and later re-classified into subfamily Labeoninae. The genus *Placocheilus* is distributed in Dulong River, Nujiang River and Yuanjiang River-Honghe River (Red River) drainage, Yunnan Province, China; Nam Na Basin in Lai Chau, Vietnam and Nam Ma Basin in Laos (Chen et al, 2012; Chu & Cui, 1989; Cui & Li, 1984; Kottelat, 2001; Wu, 1977; Zhang et al, 2002). The species of genus *Placocheilus* is rheophilic and its lower lip has been modified into a mental adhesive disc (Chu & Cui, 1989). So far, the genus contains four species: *P. caudofasciatus* Pellegrin & Chevey, *P. cryponemus* Cui & Li, *P. robustus* Zhang et al and *P. dulongensis* Chen et al.

For quite some time, the *Placocheilus* from Yuanjiang River-Honghe River drainage was deemed as *P. caudofasciatus*. Zhang et al (2002) re-evaluated the structures of scaleless midventral belly region, ratio of depth/length of caudal peduncle, and length/width of mental adhesive disc. Herein the *Placocheilus* from Yuanjiang River-Honghe River drainage was re-classified into two independent species: *P. caudofasciatus* from Lixian River with its branches in Yunnan, China as well Tuojiang River (Heishui River or Black River, lower Lixian River Basin), Vietnam and Nam Ma Basin in Laos, all belong to the tributaries of Honghe drainage; and a new species, *P. robustus*, from Yuanjiang River and its tributaries of Red River Basin. However, due to the difficulties of discriminating *P. robustus* and *P. caudofasciatus*, the classification has been usually based on collecting sites. As stated in *Checklist of Fishes of Yunnan*, *P. robustus* was considered to be the synonym of *P. caudofasciatus* without explanations (Chen, 2013). The taxonomic status of these two species should be further clarified. [1]

The conventional morphological measurements have limitations in comprehensiveness and accuracy. That is to say, the measuring distance extends along both horizontal and vertical coordinates and it is rather restricted to head and caudal peduncle areas. As a result, it is impossible to cover the entire body surface (Xie et al, 2003). The multivariate morphometry overcomes the above shortcomings (Bookstein et al, 1985) so that it has been successfully applied for population measurements. In other words, determining the validity of existing species or conjecture unknown species by evaluating the morphological differences among congeners (Cai et al, 2001; Xie et al, 2003; Yang et al, 2003). Through multivariate

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morphometry, Li et al (2008) reported that one single fish species *Pseudecheneis sulcata* from different drainages actually belonged to several different species. The findings of Yang et al (2011) and Yang et al (2013) accorded with the previous results of *Discogobio yunnanensis* and *Garra orientalis,* and no intraspecies difference was detected. And Min et al (2009) revealed no morphological differentiations among different populations of *Sinocyclocheilus grahami* from the same drainage and confirmed the validity of the species.

In the present study, by adopting multivariate morphometry and principal component analysis, through measuring external morphological characters, including longitudinal, lateral and oblique distances, the major morphological differentiations and morphometric differences between *P. robustus* and *P. caudofasciatus* were compared. These findings provide evidence for clarifying the taxonomic status of these two species.

MATERIALS AND METHODS

Materials

The specimens (*n*=72) of *P. caudofasciatus* and *P. robustus* were deposited in Museum of Animal Section of Southwest Forestry University (SWFU) and Museum of Fish Section of Kunming Institute of Zoology (KIZ) (Table 1, Figure 1). The specimens Zhang et al (2002) used for describing *P. robustus* as a new species are currently conserved at KIZ. And 53 specimens at SWFU collected from Phona Tho at Lai Chau, Noire [Song Da] River at Lai Chau, Black River, Vietnam, were utilized for observing scale coverage only.

Study methods

Conventional measuring methods and multivariate morphometric studies were combined for specimen measurements. Multiple variable statistics (principal component analysis) was used for statistical analysis.

Morphological character measurements

A total of 10 anatomical coordinates were selected (Figure 2). With the left side of body taken as the bench mark, 23 frame characters and 15 general characters were measured. The measurements of countable and general characters were performed per Kottelat (2001); disc width and length per Zhang et al (2002). The scale coverage was observed under binocular microscope (Nikon, SMZ645). The length of scaleless region (distance from the origin line of pectoral fins to scaleless midventral belly region) was determined and its percentage in total length from the origin of pectoral fin to ventral fin calculated. Lineal distances between anatomical coordinates were obtained by an electronic digital caliper (accuracy=0.1 mm).

Statistical analysis

Logarithmic (log10) transformation of all morphological character data were processed with Microsoft Excel 2003 for eliminating the variations caused by size differences among the specimens (Xie et al, 2003; Li et al, 2008). Principal component analysis was conducted by SPSS 17.0 for Windows. Standard

Table 1 List of examined specimens

Species	Specimen origin	Collection site	Collection date	Numbers of specimens (*n*)
P. robustus	KIZ	Lvshuihe hydrostation (upstream), Pingbian	2012.04	2
		Honghe	1960.05	12
		Yuanjiang	2012.04	1
	SWUF	Biaheqiao, Pingbian	1997.12	1
		Yuanyang	1999.02	1
		Huayudong, Hekou	2014.07	3
		Biaheqiao, Pingbian	2013.07	3
		Huayudong, Hekou	2013.07	5
		Yuanjiang Town, Yuanjiang	2006.05	1
P. caudofasciatus	KIZ	Nama River (upstream), Vietnam	2008.11	20
		New food market, Yuanyang, Lvchun	2009.06	3
	SWUF	Bashahe River, Daheishan, Lvchun	2009.11	5
		Bashahe River, Daheishan, Lvchun	2005.06	8
		Jinshuihe Town	2003.01	2
		Daheishan Village, Lvchun	2014.07	2
		Bashahe River, Daheishan, Lvchun	2013.11	3
Total				72

KIZ: Museum of Fish Section of Zoology Kunming Institute of Zoology, Chinese Academy of Sciences; SWFU: Museum of Animal Section of Southwest Forestry of University Southwest Forestry University

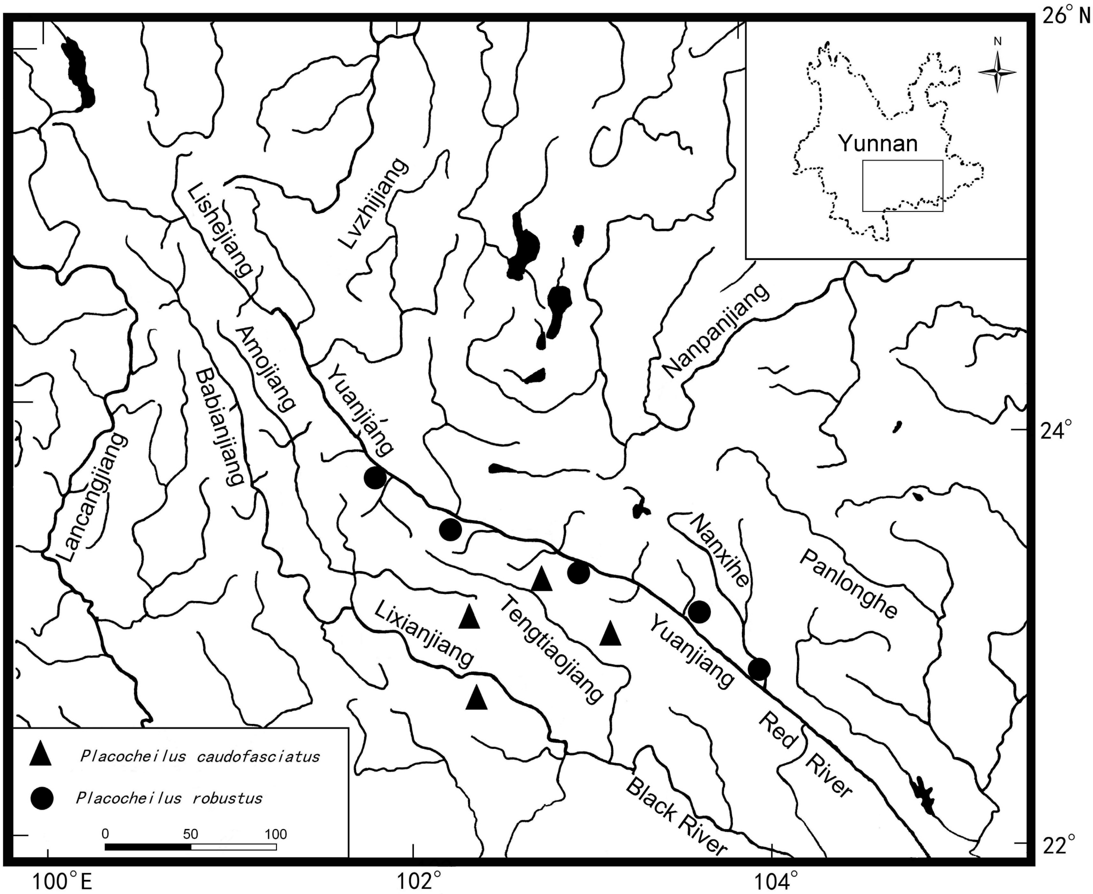

Figure 1 Distribution maps of *Placocheilus caudofasciatus* and *Placocheilus robustus*

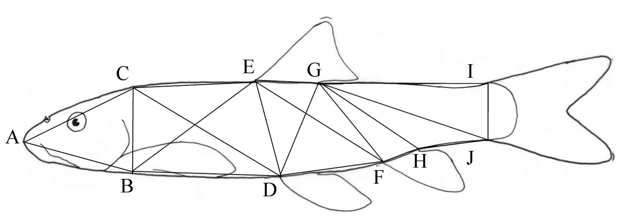

Figure 2 Morphometric frame characters of *Placocheilus caudofasciatus*

A: Snout tip; B: Pectoral fin origin; C: Supraoocipital end; D: Ventral fin origin; E: Dorsal fin origin: ; F: Anal fin origin; G: Dorsal fin base end; H: Anal fin base end; I: Caudal fin dorsal origin; J: Caudal fin ventral origin

data transformation was completed by the default settings for factorial analysis. Covariance matrix and Varimax were applied for factorial analysis. And scatter plots were constructed on the basis of the scores of principal components.

RESULTS

Comparison of morphological characters

Besides scale coverage of midventral belly region, caudal peduncle and length/width of mental adhesive disc were listed (Zhang et al, 2002). In the present study, the rays of fins, scales in lateral line and scales in peduncle were also compared between *P. caudofasciatus* and *P. robustus*. Yet no significant differences existed in none of the relevant characters (Table 2, Table 3).

Principal component analysis

The specimens were divided into two groups according to their collection sites: group from Yuanjiang River Basin and its tributaries (*P. robustus*) and group from Lixian River Basin (*P. caudofasciatus*). The principal component analysis of 23 frame characters and 15 general characters showed that the variances of PC1, PC2 and PC3 were 48.093%, 29.866% and 12.297% respectively with an accumulative variance of 90.257% (Table 4). Scatter plots regarding PC1 vs PC2 and PC2 vs PC3 were constructed (Figure 3). The scatter plots indicated that these two *Placocheilus* species were non-distinguishable.

DISCUSSION

Pellegrin (1936) described *Discognathus caudofasciatus* on the basis of one holotype of MNHN 1935-0327. And it was collected from Noire (Song Da) River at Lai Chau, Black River, Vietnam.

Table 2 Morphological character comparisons between *Placocheilus caudofasciatus* and *Placocheilus robustus*

Species	*P. caudofasciatus*		*P. robustus*	
	Present study	Zhang et al (2002)	Present study	Zhang et al (2002)
Number of specimens (*n*)	43	22	29	20
Standard length (mm)	32.3-81.7	48.0-76.5	31.7-116.8	48.6-117.2
Dorsal fin (D)	3, 8	3, 8	3, 8	3, 8
Anal fin (A)	3, 5	3, 5	3, 5	3, 5
Caudal fin (C)	i+8-7+i	–	i+8-7+i	–
Ventral fin (V)	1, 8	1, 7	1, 8	1, 8
Scales in lateral line	39-42	40-41	39-41	39-42
Scales in peduncle	12	12	12	12
Scale coverage of midventral belly region	–	1 / 4	–	3 / 4
	Range (mean±*SD*) (%)	Range (%)	Range (mean±*SD*) (%)	Range (%)
Disc length/disc width	74.0-92.6 (83.5±4.1)	44.5-49.2	70.1-91.7 (83.5±6.2)	37.0-43.1
Disc width/head length	41.2-63.2 (53.6±4.4)	54.8-66.2	44.3-65.0 (55.1±5.0)	58.9-65.2
Disc length/head length	33.0-53.4 (44.7±4.0)	44.5-49.2	35.1-52.9 (45.9±4.5)	37.0-43.1
Caudal peduncle depth/length	50.0-97.0 (72.1±10.5)	63.7-71.3	52.4-78.3 (67.7±8.9)	72.4-82.5

Table 3 Midventral region scale coverage of *Placocheilus caudofasciatus v.s. Placocheilus robustus*

	P. caudofasciatus		*P. robustus*
	Lixiang River & its tributaries (upper Black River)	Song Da River at Lai Chau, Vietnam	Yuanjiang River & its tributaries
Number of specimens (*n*)	43	53	29
Stand length (mm)	32.3-81.7	53.0-106.8	31.7-116.8
Length of scaleless region /P-V distance (%)	0.00-0.72 (0.28±0.20)	0.00-0.85 (0.40±0.27)	0.29-0.94 (0.62±0.20)

Table 4 Principal component analyses of *Placocheilus caudofasciatus v.s. Placocheilus robustus*

Characters	Results			Characters	Results		
	PC1	PC2	PC3		PC1	PC2	PC3
Ventral fin origin-dorsal fin origin (DE)	0.8467	0.4004	0.2344	Caudal peduncle depth	0.6900	0.4428	0.4204
Body depth	0.8414	0.3916	0.2489	Snout length	0.6820	0.5803	0.3692
Pectoral fin origin-supraoccipital end (BC)	0.8379	0.4490	0.0085	Caudal fin ventral origin-caudal fin dorsal origin (JI)	0.6816	0.4283	0.4192
Head depth	0.8303	0.4332	0.2781	Caudal fin dorsal origin-anal fin base end (IH)	0.6735	0.5576	0.4214
Ventral fin origin-dorsal fin base end (DG)	0.8301	0.4373	0.2199	Caudal fin ventral origin-dorsal fin base end (JG)	0.6587	0.2965	0.3419
Dorsal fin origin-anal fin origin (EF)	0.7990	0.5026	0.2338	Head length	0.6557	0.6102	0.3066
Dorsal fin base end-anal fin base end (GH)	0.7846	0.5097	0.3173	Pectoral fin origin-dorsal fin origin (BE)	0.6107	0.4793	0.3990
Anal fin origin-dorsal fin base end (FG)	0.7800	0.4992	0.3123	Caudal fin ventral origin-anal fin base end (JH)	0.5887	0.5219	0.5079
Supraoocipital end-dorsal fin origin (CE)	0.7767	0.3866	0.3632	Eye diameter	0.3401	0.8249	0.0984
Interorbital distance	0.7716	0.4604	0.3684	Anal fin length	0.3237	0.6925	0.4077
Body depth at anus	0.7690	0.4437	0.3247	Snout tip-supraoocipital end (AC)	0.6396	0.6788	0.2408
Snout tip-dorsal fin origin (AE)	0.7409	0.5603	0.3513	Ventral fin length	0.6416	0.6545	0.3436
Head width	0.7386	0.5648	0.3272	Pectoral fin length	0.6455	0.6513	0.3577
Snout tip-anal fin origin (AF)	0.7245	0.5879	0.3068	Snout tip-pectoral fin origin (AB)	0.5953	0.6488	0.2843
Snout tip-ventral fin origin (AD)	0.7245	0.5906	0.3095	Anal fin origin-anal fin base end (FH)	0.6172	0.6454	0.1985
Standard length	0.7236	0.5780	0.3450	Head length of posterior eye	0.5796	0.6364	0.3901
Dorsal fin base end-caudal fin dorsal origin (GI)	0.7140	0.5509	0.3669	Caudal peduncle length	0.1770	0.1601	0.8959
Ventral fin origin-anal fin origin (DF)	0.7083	0.5715	0.3349	Total	18.2756	11.3491	4.6730
Dorsal fin origin-dorsal fin base end (EG)	0.6937	0.6411	0.1702	Variance (%)	48.0936	29.8662	12.2974
Supraoocipital end-ventral fin origin (CD)	0.6911	0.5966	0.2302	Cumulative variance (%)	48.0936	77.9598	90.2572
Pectoral fin origin-ventral fin origin (BD)	0.6903	0.5880	0.2950				

Alphabet codes are the same as in Figure 2

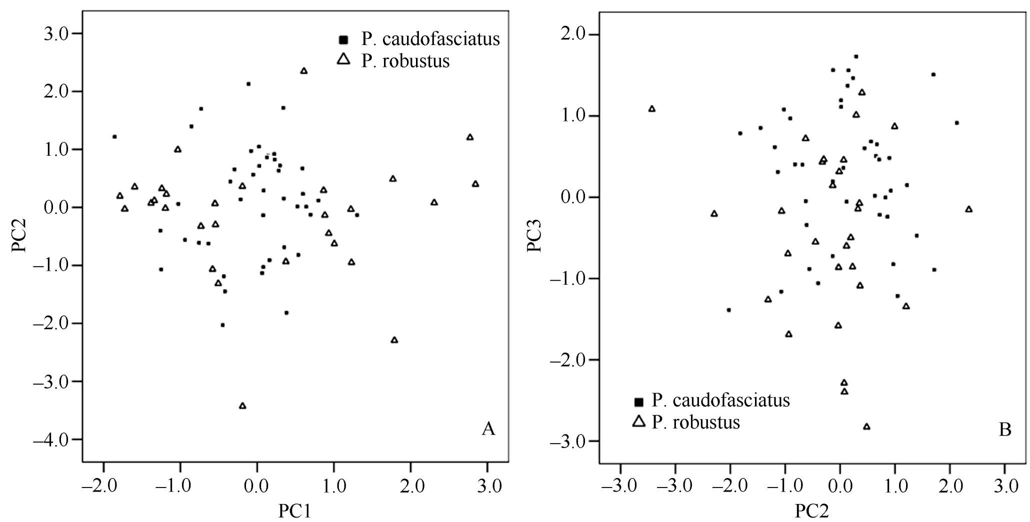

Figure 3 Scatter plots of principal component analysis

The specimens currently preserved at SWFU were also collected from Noire (Song Da) River at Lai Chau, Vietnam. Thus the above specimens are de facto topotypes.

As reported by Zhang et al (2002), comparing with *P. caudofasciatus*, *P. robustus* was characterized by stouter caudal peduncle, smaller mental adhesive disc, medium-sized scaleless midventral belly region extends slightly beyond halfway from pectoral fin to ventral fin origin, whereas scaleless midventral region in *P. caudofasciatus* is limited to basal area of pectoral fin. Significant variations of scale coverage existed in specimens from Phona Tho at Lai Chau, Noire [Song Da] River at Lai Chau, Black River, Vietnam, in fact, these scale coverage variations include all the midventral belly region scale coverage status in *P. caudofasciatus* and *P. robustus* specimens from Vietnam and China. Here the character data were different from those in Zhang et al (2002). Other than inevitable measurement variations from different experiments, we assume that the major reason for these differences was the body lengths of specimens fell into a wide range. Therefore, among various-sized individuals, significant variations in some body parts were observed due to allometry. Based upon the data here and Zhang et al (2002), most diagnostic character measurements from Zhang et al (2002) were lined up end-to-end between two species. Therefore some inter-species differences were implicated. However there were significant overlaps with the data here (Table 3). It is concluded that the diagnostic characters of Zhang et al (2002) for distinguishing *P. caudofasciatus* and *P. robustus* were not firmly supported.

Long-term geographic isolation blocks both genetic and unit communications among different populations. Thereafter, differentiations in morphology, ecology, physiology and biochemistry, or even reproductive isolation would occur as outcomes. When reproductive isolation happens, two populations are two independent species. However, in taxonomic studies, populations from different collection sites, but with similar or close physical forms are usually classified on the basis of their morphological characters. In the present study, the results of both external measurements and morphometry showed that no significant morphological differences existed between *P. caudofasciatus* and *P. robustus*, indicating that both were actually of one species and the latter was the synonym of the former. And it was in accordance with the results of Chen (2013). These conclusions are also supported by the results of muscular and skeletal anatomy (Li et al, 2016). The phylogenetic tree showed that *P. caudofasciatus* and *P. robustus* are gathered together. The genetic distance based on *Cyt b* showed that the distance among the effective specices of *Garra* and *Placocheilus* were 5% or higher; whereas, that between *P. caudofasciatus* and *P. robustus* was only 4.2%, suggesting that *P. robustus* was the synonym of *P. caudofasciatus* (Wang, 2012). Even though the morphological and molecular results were mutually supportive, the genetic distance between *P. caudofasciatus* and *P. robustus* (4.2%) was still higher than the standard criteria (3%) for discriminating species in DNA barcoding studies. Thus for clarifying the species effectiveness, further molecular phylogenetic studies are warranted.

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