Morphological variation in *Acrossocheilus hemispinus* (Teleostei: Cyprinidae: Barbinae), with comments on its taxonomic status

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Abstract

Differences in coloration and morphology between two subspecies of *Acrossocheilus hemispinus* were investigated based on museum-stored and freshly-caught specimens. There are marked differences in the coloration of either juveniles or adults, and in sexual dimorphism, between *A. h. hemispinus* and *A. h. cinctus*. Multivariate analysis of morphometric data too, shows the two taxa to be distinguishable from each other. Differences in body coloration and morphometric characters coincide with those of the mouthpart structure and the coiling pattern of the intestine in *A. h. hemispinus* and *A. h. cinctus*. Morphological distinction, coupled with different habitat and food preferences, supports the taxonomic elevation of the two hitherto subspecific populations of *A. hemispinus* to species.

Key words: *Acrossocheilus hemispinus*, *Acrossocheilus cinctus*, subspecific populations, China, taxonomy

Introduction

The taxonomic distinctions in some species of the cyprinid genus *Acrossocheilus* are still confusing, despite recent clarification of the misidentifications of the species identified in this genus by Shan et al. (2000) (Kottelat, 1998, 2000; Zhang, 2005; Yuan et al., 2006). A case of such confusion is represented by the uncertain status of *A. hemispinus cinctus* (Lin). It was first described as *Barbus hemispinus cincta* by Lin (1931). Later, Lin (1933) recognized it as conspecific with *Barbus* (*Lissochilichthys*) *hemispinus*, which was originally described in *Lissochilus* by Nichols (1925) from Yenping (Nanping), Fukien (Fujian) Province, South China. Wu et al. (1977) treated *cincta* as a subspecies of Nichols (1925) and placed it in *Acrossocheilus* subgenus *Lissochilichthys*. Some of succeeding Chinese authors accepted Wu et al.’s generic classification, but regarded it as identical to *A. hemispinus* (Fang in Zheng, 1981; Lin in Zheng, 1989; Chen in Pan, 1991). In the recent monograph of Chinese freshwater fishes, Shan et al. (2000) regarded *A. hemispinus* including two subspecies: *A. h. hemispinus* restricted to the Min Jiang drainage in Fujian Province and *A. h. cinctus* known from the Pearl River drainage in Guangxi and Guangdong Provinces, South China. Our recent surveys indicated that *A. h. cinctus* also occurs in the Ling Jiang of Zhejiang Province, Qiupu He (flowing to the lower Yangtze River drainage) of Anhui Province and Xin Jiang (flowing to the Poyang Lake) of Jiangxi Province. Yuan et al. (2006), in describing *Acrossocheilus spinifer*, noted that the two subspecific populations of *A. hemispinus* deserve specific status, but without elaboration. The only difference noted by them between the two subspecies was in body coloration. *Acrossocheilus cinctus* has a longitudinal black stripe extending along the lateral line and black blotches on the membranes between the branched dorsal-fin rays; both these are absent in *A. h. hemispinus*. Body coloration is of taxonomic significance for species identification in *Acrossocheilus* (Shan et al., 2000). Based on coloration, species of this genus can be tentatively divided into two groups: a barred one, including those species with several vertical black bars on each side of the body, and a striped one, comprising those species with a longitudinal black stripe along the lateral line on each side of the body (Kottelat, 1998, 2001). The number and width of vertical bars vary greatly among some species of this genus can be tentatively divided into two groups: a barred one, including those species with a longitudinal black stripe extending along the lateral line and black blotches on the membranes between the branched dorsal-fin rays; both these are absent in *A. h. hemispinus*. Morphological distinction, coupled with different habitat and food preferences, supports the taxonomic elevation of the two hitherto subspecific populations of *A. hemispinus* to species.
The present study aims to investigate morphological differences between *A. h. hemispinus* and *A. h. cinctus*, and then assess their taxonomic status.

**Materials and methods**

A total of 120 sexually mature (> 60.0 mm SL) individuals were utilized for studies of morphometric characters and comparisons of external morphology between subspecific populations or between the sexes. Sex is determined based on a combination of the following three characters: tuberculation, pelvic-fin length and anal-fin length (Mao and Xu, 1991). Adult males were recognized by the possession of more marked tubercles on the snout tip and the anterior part of the lachrymal, the pelvic fin extending slightly beyond the anus, and the anal fin shorter than that of the females. The material comprises 58 individuals (26 males and 32 females) of *A. h. hemispinus* from the Min Jiang drainage in Fujian Province and 62 (37 males and 25 females) of *A. h. cinctus* from the Ling Jiang drainage in Zhejiang Province, the Xi Jiang of the Pearl River drainage in Guangxi Province, and the Le’an Jiang, Chang Jiang and Xin Jiang, all of them draining to the Poyang Lake connected to the lower Yangtze River in Jiangxi Province (Fig. 1). A total of 13 fresh individuals of *A. h. cinctus* were collected during May, 2006 from the Ling Jiang drainage in Zhejiang Province; and 35 of *A. h. hemispinus* and 8 of *A. h. cinctus* were captured during May, 2007 from the Min Jiang drainage in Fujian Province and Xin Jiang drainage in Jiangxi Province, respectively. Juvenile individuals (< 60 mm SL), which are used for comparison of body coloration, were freshly collected from Zhejiang, Fujian and Jiangxi provinces. They were initially fixed in 10% formalin, then preserved in 75% alcohol, and deposited in the collection of the Museum of Aquatic Organisms at the Institute of Hydrobiology (IHB), Chinese Academy of Sciences, Wuhan. The remaining individuals examined here were museum-stored specimens of IHB collected prior to 1990. Specimens examined for comparison of the body coloration, mouthpart structure and intestinal coiling pattern were excluded in morphometric analysis.

For each individual, 18 morphometric characters were measured on the left side whenever possible. Measurements and abbreviations of these characters were shown in Table 1. Most morphometric measurements were made as described by Hubbs and Lagler (1958), with an electronic digital caliper connected directly into a computer database; data were recorded to the nearest 0.1 mm. Pre-dorsal, pre-pectoral, pre-pelvic and pre-anal lengths were measured from the anteriormost tip of the snout to the dorsal-, pectoral-, pelvic- and anal-fin origins, respectively.
Morphometric data were explored using multivariate analysis. Males and females were analyzed separately in order to avoid the bias caused by sexual dimorphism. Size-free canonical variate analysis (CVA) was performed on the covariance matrix of log10-transformed original measurements to assess morphometric variation between samples. This technique consists of removing the effect of allometric growth by Burnaby’s size correction method (Dos Reis et al., 1990; Bookstein, 1991). Analysis of variance (ANOVA) was utilized to test the significance of the difference in morphometric characters between samples for each sex. All analyses were performed on morphometric measurements using the software PAST v 1.78 (Hammer et al., 2001).
Results

Coloration. There are considerable ontogenetic alterations in body coloration between the two subspecies. In juvenile individuals of less than 60.0 mm SL, the two subspecies exhibit a similar color pattern that includes six vertical bars extending beneath the lateral line on each side of the body. This is entirely in agreement with the former observations by Huang in Chu (1984) and Chen et al. in Pan (1991) for \( A. h. \) hemispinus and \( A. h. \) cinctus, respectively. The two subspecies differ, however, in vertical-bar width: in \( A. h. \) hemispinus, each bar covers about two scales, instead of three or four in \( A. h. \) cinctus. This difference has never been reported before.

The two subspecies diverge in coloration when specimens reach 60.0–100.0 mm SL. For \( A. h. \) hemispinus, vertical black bars on the flank are completely missing in males, and disappear in females with the increase in body size. Six vertical black bars on each side of the body, each about three scales in width, are still retained in the two sexes of \( A. h. \) cinctus, but do not extend beneath the lateral line. Besides, both sexes of \( A. h. \) cinctus in this size range gain a longitudinal black stripe extending along the lateral line on each side of the body. In adult specimens of more than 100.0 mm SL, there are striking differences in body coloration between \( A. h. \) hemispinus and \( A. h. \) cinctus. A longitudinal black stripe extending along the lateral line on each side of the body is retained in the two sexes of \( A. h. \) cinctus, and six indistinct vertical bars not extending beneath the lateral line on the flank are present only in females of this subspecies. No vertical bars and longitudinal stripe is found in \( A. h. \) hemispinus. Membranes between branched dorsal-fin rays have obscure black blotches in \( A. h. \) cinctus, but they are hyaline in \( A. h. \) hemispinus. These observed differences in body coloration agree with the previous observations by Huang in Chu (1984), Lin in Zheng (1989) and Chen et al. in Pan (1991). However, the present results differ from Lin in Zheng’s (1989) and Chen et al. in Pan’s (1991) observations. Acrossochilus h. cinctus is sexually dichromatic: adult females possess indistinct vertical bars on the flank, these bars being absent in adult males.
Morphology. Morphometric characters. Multivariate analysis shows the two subspecific populations of *A. hemispinus* to be distinguishable on the basis of morphometric characters. The size-free CVA of two subspecific populations of *A. hemispinus* revealed that the first two canonical variates (CV I and CV II) explained, respectively, 36.5% and 15.9% of the total variance for females, and 45.3% and 13.7% for males. In the plot of individual scores (Fig. 3A) for males, the first CV I separates *A. h. cinctus* from *A. h. hemispinus*. The highest canonical coefficients (Table 2) for head length and head depth indicate the two characters as being the best to discriminate the two subspecies along CV I. There exists a small amount of overlap in the graph of individual scores (Fig. 3B) for females between *A. h. hemispinus* and *A. h. cinctus*, but they are still mostly distinguished from each other along CV I. The highest canonical coefficients (Table 2) for head length and anal-fin length indicate these two characters as being the best to separate the two subspecies along CV I. ANOVA were conducted for these measurements, taken as proportions of SL. All showed significantly differences (*p* < 0.05) for each sex, but overlapped in their distributions of indexes between the two subspecies. Even so, the mean values of the index for head length and head depth are higher in *A. h. hemispinus* than in *A. h. cinctus* for both sexes, and those for anal-fin length lower in *A. h. hemispinus* than in *A. h. cinctus* for females (Table 1). This means that while *A. h. hemispinus* has a longer and deeper head than *A. h. cinctus* in both sex, it has a shorter anal fin than *A. h. cinctus* in females.
FIGURE 3. Scatter plots representing variation in shape between groups using Burnaby size-adjusted multivariate shape information, bounded by minimum convex polygons. (A) males of *A. h. hemispinus* (n=26, cross) and *A. h. cinctus* (n=37, open square); (B) females of *A. h. hemispinus* (n=32, cross) and *A. h. cinctus* (n=25, open square).

Qualitative characters. *Acrossocheilus hemispinus* exhibits marked differences in the mouthpart structure between two subspecific populations. *hemispinus* has a lower jaw entirely covered by the lower lip so that it is invisible when viewed ventrally (Fig. 4A, B), exposed or uncovered by the lower lip in *cinctus*, sometimes with a horny anterior margin (Fig. 4C, D).

*Acrossocheilus h. hemispinus* shows sexual dimorphism in the structure of the lower lip, a character not noted in previous studies. In females, the lower lip is longitudinally bisected by a shallow groove at the dentary symphysis into two lateral lobes, which are closely contacted without interruption (Fig. 4C). In males, these two lateral lobes are separated by a median interruption, its width being about 1/5–1/3 of the gape width (Fig. 4D). This sexual dimorphism is not exhibited by *A. h. cinctus* (Fig. 4A, B).
There also exist differences in the intestinal coiling pattern and length between the two subspecific populations of *A. hemispinus*. In order to facilitate description and comparison, each point of bending was numbered from 1 to 6 (Fig. 5). In *A. h. hemispinus*, the anterior part of the intestine, which is from the oesophagus to the 1st point of bending, straightly extends backwards, and its middle and posterior parts form a large loop extending forwards in an S-bend and covering the anterior part. *Acrossocheilus h. cinctus* has a more complex coiling pattern of the intestine and a greater intestine length compared with *A. h. hemispinus*. The loop protrudes anteriorly at the 2nd and 6th points of bending on the one side, and also at the 3rd and 5th points of bending in the reverse direction on the other side. As a consequence, two small loops are formed, namely the loop 1-2-3 and the 5-6-anus. The loop 3-4-5 in *A. h. cinctus* is more elongated than the corresponding one in *A. h. hemispinus*, with the 4th bending point protruded posterolaterally.
TABLE 2. Variable morphometric scores for the canonical axis for each sex of A. hemispinus.

Discussion

The present study indicates that there are substantial morphological differences between subspecific populations of the currently recognized species A. hemispinus. In addition to the differences in body coloration of adults, the difference in the width of each vertical bar on the flank of juveniles is found between A. h. hemispinus and A. h. cinctus (Fig. 2). Multivariate analyses of morphometric characters show that either males or females between A. h. hemispinus and A. h. cinctus are distinguishable from each other (Figs. 3A, B). The statistically significant variations in several morphometric measurements between these two subspecies coincide with those in the mouthpart structure and coiling pattern of the intestine, which have currently been utilized for identification of some species of Acrossocheilus. For example, Zhao et al. (1997) considered intestinal coiling pattern as a diagnostic character to separate A. jishuoensis and A. parallens (Nichols). It has also been shown to be of taxonomic value in other groups of fishes (Kafuku, 1986; Rainboth, 1989).
The variations in the mouthpart structure and coiling pattern of the intestine between \( A. \ h. \ cinctus \) and \( A. \ h. \ hemispinus \) have some ecological implications. Unmatched by other families, the Cyprinidae show high morphological modifications in lips and associated structures (Roberts, 1982). These modifications consist of the degree of development and specialization of lips and the rostral cap, and the presence or absence of horny jaw sheaths on the jaws in addition to, or in place of, the normal lips (Roberts, 1982). The lips and associated structures (or mouthpart structures) in different groups of fishes are greatly modified in relation to the characteristic mode of feeding, food preference and the mode of life exhibited by the fish (Pinky et al., 2004). In \( A. \ h. \ cinctus \), the lower lip is anteriorly separated from the lower jaw, which is fully uncovered by the lower lip and usually bears a sharp horny sheath on its anterior cutting edge. Presumably, the lower jaw assists the fish in scraping food materials from the substratum. The lower jaw of \( A. \ h. \ hemispinus \) does not function as a food scraper as it is entirely covered by the lower lip. It is apparent that the difference in the mouthpart structure indicates different feeding strategies that the two subspecies utilize. The length of the intestine has been shown in the former investigations to be closely associated with feeding habits and food preference (Reinthal, 1989). Presumably, the differences in the coiling pattern and length of the intestine between \( A. \ h. \ cinctus \) and \( A. \ h. \ hemispinus \) too, is indicative of differences in their feeding ecology, which however, remains to be demonstrated.
It is surprising to find in the present investigation that *A. h. cinctus* is sexually dichromatic and dimorphic in lower-lip structure, neither of which has been documented previously. Adult females possess some indistinct vertical bars on the flank, but these bars are missing in adult males (Figs. 2 I, J). The two lateral lobes of the lower lip are closely in contact with each other, without interruption in adult females, while separated from each other by a median interruption, with its width being roughly 1/5–1/3 of the mouth width, in adult males (Figs. 4C, D). These sex-associated differences are not exhibited by *A. h. hemispinus*. Sexual dimorphism is commonly known to be the result of sexual selection during long-term evolution (Hedrick and Temeles, 1989; Andersson, 1994). Helbig (2002) held that characters used in the diagnosis of taxa must be the result of evolution: they must be genetically based and not caused purely by environmental factors. It is apparent that the presence or absence of the sex-associated differences in body coloration and the lower lip morphology can be used as diagnostic characters for distinguishing *A. h. cinctus* from *A. h. hemispinus*.

We thus conclude that statistically significant differences in three morphometric characters such as head length, head depth and anal-fin length (Table 1), coupled with additional differences in both the mouthpart structure and intestinal coiling pattern, differences in the coloration of their juveniles or adults, and the presence or absence of the sex-specific differences in body coloration and the lower lip morphology, indicate that *A. h. hemispinus* and *A. h. cinctus* are morphologically diagnosable from each other. This diagnosability, in combination with differences in putative habitat and food preference, strengthens the taxonomic recognition of *A. hemispinus* and *A. cinctus* as distinct species. We thus conclude that statistically significant differences in three morphometric characters such as head length, head depth and anal-fin length (Table 1), coupled with additional differences in both the mouthpart structure and intestinal coiling pattern, differences in the coloration of their juveniles or adults, and the presence or absence of the sex-specific differences in body coloration and the lower lip morphology, indicate that *A. h. hemispinus* and *A. h. cinctus* are morphologically diagnosable from each other. This diagnosability, in combination with differences in putative habitat and food preference, strengthens the taxonomic recognition of *A. hemispinus* and *A. cinctus* as distinct species. It is worthwhile to point out that *A. hemispinus* and *A. cinctus* are not necessarily two closely related species, or sister species, although they were traditionally recognized as being only subspecifically distinct. Adult specimens of the two species have different color patterns. *Acrossocheilus cinctus* has a color pattern consisting of an indistinct longitudinal black stripe and six indistinct vertical black bars on the flank in females, which is typical of the barred group of *Acrossocheilus*. *Acrossocheilus hemispinus* lacks a longitudinal black stripe and vertical black bars on the flank. On the basis of this color pattern, Kottelat (1998: 123) considered it associated with the striped species of *Acrossocheilus*. However, juvenile specimens of *A. hemispinus* possess black vertical bars on the flank, by which they can be referred to the barred group of this genus. Apparently, this species has a color pattern atypical for the barred species. Moreover, it has no interruption between the two lateral lobes of the lower lip and no sexual dimorphism. These dissimilarities indicate that *A. hemispinus* is only distantly allied to the other barred species of this genus. Thus, its taxonomic position among the barred species group needs further investigation.

**Material examined**

*Acrossocheilus hemispinus*–Male: IHB 20070500144–46, 20070500153–54, 20070500159, 20070500161, 20070500165, 20070500168, 20070500175, 10 specimens, 84.2–107.9 mm SL, Dazhang Xi of the Min Jiang river in Yongtai, Fujian Province; IHB 20070500194, 20070500196, 20070500198, 20070500212, 4 specimens, 87.5–115.6 mm SL, Chongyang Xi (upper part of the Min Jiang) in Wuyishan, Fujian Province; IHB 20070500083–84, 2 specimens, 118.3–124.6 mm SL, Huotong Xi flowing to East China Sea) in Huotong, Fujian Province; IHB 20070500093–94, 2 specimens, 96.3–109.9 mm SL, Futun Xi (upper reach of the Min Jiang) in Shaowu, Fujian Province; IHB 74IV2501–03, 74IV2557, 74IV2562, 5 specimens, 88.0–146.1 mm SL, Jianxi river (upper part of the Min Jiang) in Jianyang, Fujian Province; IHB 74IV1372, 74IV1401, 74IV1404, 3 specimens, 96.8–141.1 mm SL, Jiujiang Xi (headwaters of the Min Jiang) in Ningsha, Fujian Province.

Female: IHB 20070500193, 20070500199, 20070500201, 20070500204, 20070500210, 5 specimens, 85.0–99.4 mm SL, Chongyang Xi in Wuyishan, Fujian Province; IHB 20070500167, 20070500151–52, 20070500155–57, 20070500159, 20070500160, 8 specimens, 90.5–107.9 mm SL, Dazhang Xi in Yongtai, Fujian Province; IHB 20070500081–82, 2 specimens, 93.1–120.3 mm SL, Huotong Xi in Huotong, Fujian Province; IHB 74IV2504–06, 74IV2552, 74IV2555, 74IV2559, 74IV2563, 74IV2740, 8 specimens, 91.3–151.3 mm SL, Jianxi river in Jianyang, Fujian Province; IHB 20070500092, 20070500097, 2 specimens, 80.9–97.9 mm SL, Futun Xi in Shaowu, Fujian Province; IHB 74IV1359, 74IV1370–71, 74IV1374, 74IV1403, 74IV1405–06, 74IV1372, 7 specimens, 87.9–157.3 mm SL, Jiujiang Xi in Ningsha, Fujian Province.
Acrossocheilus cinctus. –Male: IHB 86X111, 86X112, 86X116, 86X123–26, 8 specimens, 91.3–113.4 mm SL, Yu Jiang (a tributary of the Xi Jiang) in Nanning, Guangxi Province; IHB 539448, 539505, 539535, 53932, 53455, 53501, 6 specimens, 92.4–117.9 mm SL, Gui Jiang (a tributary of the Xi Jiang) in Guilin, Guangxi Province; IHB 810126, 814632–34, 4 specimens, 110.76–137.89 mm SL, Gui Jiang in Yangshuo, Guangxi Province; IHB 380242, 1 specimen, 121.7 mm SL, Gui Jiang in Zhaoping, Guangxi Province; IHB 75IV2615, 1 specimen, 116.9 mm SL, Gui Jiang in Lipu, Guangxi Province; IHB 0605431, 0605435–37, 0605442, 0605445–46, 7 specimens, 105.34–121.1 mm SL, Ling Jiang (a tributary flowing to the East China Sea) in Xianju, Zhejiang Province; IHB 902152–53, 2 specimens, 80.1–123.4 mm SL, Chang Jiang (not Yangtze River, but a stream draining to the Poyang Lake) in Jingdezhen, Jiangxi Province; IHB 90V1940, 90V1943, 90V1946–47, 4 specimens, 104.1–130.1 mm SL, Le’an Jiang (a stream draining to the Poyang Lake) in Wuyuan, Jiangxi Province; IHB 20070600296–99, 4 specimens, 69.2–105.2 mm SL, Xin Jiang (a stream draining to the Poyang Lake) in Shangrao, Jiangxi Province.

Female: IHB 86X110, 86X114, 86X118, 3 specimens, 104.9–125.3 mm SL, Yu Jiang river in Nanning, Guangxi Province; IHB 539431, 539534, 53420, 53502, 4 specimens, 95.7–131.6 mm SL, Gui Jiang in Guilin, Guangxi Province; IHB 814631, 814637, 144, 3 specimens, 100.7–143.7 mm SL, Gui Jiang in Yangshuo, Guangxi Province; IHB 380272, 1 specimen, 102.8 mm SL, Gui Jiang in Zhaoping, Guangxi Province; IHB 75IV1426, 1 specimen, 154.5 mm SL, Gui jiang in Lipu, Guangxi Province; IHB 0605429, 0605430, 0605438, 0605443, 0605448–49, 6 specimens, 126.4–141.8 mm SL, Ling Jiang in Jian’ou, Jiangxi Province; IHB 90V1940, 90V1944–45, 3 specimens, 120.7–145.3 mm SL, Le’an Jiang in Wuyuan, Jiangxi Province; IHB 20070600292–95, 4 specimens, 68.5–92.3 mm SL, Xin Jiang in Shangrao, Jiangxi Province.

Additional specimens used for examination of coloration: IHB 20070500086, juvenile, 56.1 mm SL, Huotong Xi in Huotong, Fujian Province; IHB 20070600187, female, 70.4 mm SL, Min Jiang in Jian’ou, Province; IHB 20070600188, male, 76.8 mm SL, Min Jiang in Jian’ou, Province; IHB 20070500083, female, 124.6 mm SL, Huotong Xi in Huotong, Fujian Province; IHB 20070500093, male, 109.9 mm SL, Min Jiang in Shaoowu, Fujian Province. IHB 75V2912, juvenile, 40.6 mm SL, Xi Jiang in Jinxiu, Guangxi Province; IHB 831X2203, female, 98.1 mm SL, Qiupu He in Shitai, Anhui Province; IHB 83IX2205, male, 99.1 mm SL, Qiupu He in Shitai, Anhui Province; IHB 83X110, female, 123.4 mm SL, Xi Jiang inNanning, Guangxi Province; IHB 90V1940, male, 104.3 mm SL, Le’an Jiang in Wuyuan, Jiangxi Province.

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References


