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Sexual dimorphism in species of *Schistura* (Teleostei: Nemacheilidae) from the Mae Khlong basin and peninsular Thailand

RUNGTHIP PLONGSESTHEE[1], F. WILLIAM H. BEAMISH[1] & LAWRENCE M. PAGE[2]
[1]*Environment Science Program, Faculty of Science, Burapha University, Bangsaen, Chonburi, 20131, Thailand.*
E-mails: kae.plongsesthee@gmail.com, billbeamish@hotmail.com
[2]*Florida Museum of Natural History, Gainesville, Florida, 32611, USA.*
E-mail: lpage1@ufl.edu

Abstract

Sexually dimorphic characteristics are described for four species of *Schistura* from the Mae Khlong basin and peninsular Thailand. Males of *S. mahnerti* have a suborbital flap and rows of unculi on the upper surfaces of the pectoral-fin rays. Females of *S. mahnerti* have a suborbital groove. None of the morphological measurements differ significantly between males and females of *S. mahnerti*. In the other three species, *S. aurantiaca*, *S.* cf. *aurantiaca*, and *S. sexcauda*, all individuals lack the flap or groove, but most males have a conspicuous black botch on the procurrent rays of the upper lobe of the caudal fin, a feature absent in most females. Some morphometric characteristics vary between sexes of these species. Sexually dimorphic traits presumably have a function related to reproduction; however, little is known about reproduction in *Schistura*, and variation in morphology in relation to habitat or other environmental factors has not been studied.

Key words: loaches, suborbital flap, suborbital groove

Introduction

Sexually dimorphic characters are common in many animals including fishes. Ornamentations such as bright colors and tubercles develop on males of many species during the breeding season and are associated with reproductive behavior. Dimorphic traits can be affected by both sexual and natural selection (Lande 1980). Many experimental and theoretical studies on sexual selection have sought to understand the origin of male traits and the basis of their attraction to females during reproduction (Wiens 2001; Kitano *et al*. 2007; Aguirre & Akinpelu 2010). Sexual selection can lead to the evolution of ornamentations perceived as indicators of fitness to potential mates and competitors, but they may be reduced or lost through natural selection when associated costs significantly outweigh advantages (Casselman & Schulte-Hostedde 2004).

Schistura is a diverse genus of about 180 nemacheilid loach species broadly distributed in low-order streams in Southern and Southeastern Asia (Eschmeyer 2012). Sexually dimorphic traits have been reported for some species (e.g., Kottelat 1990; Freyhof & Serov 2001); however, many species lack obvious dimorphism, and interspecific variation in these traits has been used for taxonomic recognition of species that are, or almost are, otherwise morphologically indistinguishable (Kottelat 1990). This study reports new information on traits that vary between sexes in four species of *Schistura* indigenous to the Mae Khlong basin and peninsular Thailand, including one trait that is previously unreported as sexually dimorphic for any species of *Schistura* and which provides an easy and nonlethal method to discriminate sex in field studies.

Materials and methods

Schistura aurantiaca, *S. mahnerti*, and *S. sexcauda* were collected in first- to third-order tributaries in the Mae Khlong basin. *Schistura* cf. *aurantiaca* and *S. mahnerti* were collected in first- to third-order tributaries in the

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Khanan River in Prachuap Khiri Khan Province, peninsular Thailand. *Schistura aurantiaca* and *S. sexcauda* were collected in March, July, and November, 2011, *S.* cf. *aurantiaca* in November 2011, and *S. mahnerti* in June 2011.

Fishes were captured with the aid of an Advance Backpack AbP-2 Electrofisher and seines. After capture, specimens were euthanized with an overdose of methane tricaine sulfonate (>150 mg/ l), fixed in 10% formalin for 7 days, and then preserved in 70% ethanol for permanent preservation. Gender was assigned in the laboratory by internal macro- and microscopic examination of the gonads (Goncalves *et al*. 2006; Gomes *et al*. 2011; Shinkafi *et al*. 2011).

Examination of specimens for sexually dimorphic characteristics included assessments of those described in earlier studies on *Schistura* and other nemacheilids, including the presence or absence of a suborbital flap or groove, variation in color patterns, and variation in fin lengths (Kottlat 1990, 1998; Freyhof & Serov 2001; Bohlen & Ŝlechtová 2009, 2011). Measurements (±0.1 mm) were made point-to-point following Kottelat (1990), and included standard length; head depth; head width at two locations, one at the nares, the other at the point of maximum head width—measured at the extremity of the opercle; body maximum width and depth, both immediately in front of the dorsal fin; pectoral-fin length from the base of the first fin ray to the end of the longest ray; and caudal-fin length from the caudal-fin base to the end of the uppermost lobe. A difference in maximum head width between males and females was attributable to cheek thickness (cheek "inflation") related to reproductive condition.

Significance in differences between sexes in morphological features, each expressed relative to standard length, was examined using *t*-test (SPSS version 11.5). Photographs were taken of live and freshly preserved specimens with a Nikon COOLPIX P5100 camera to compare colors.

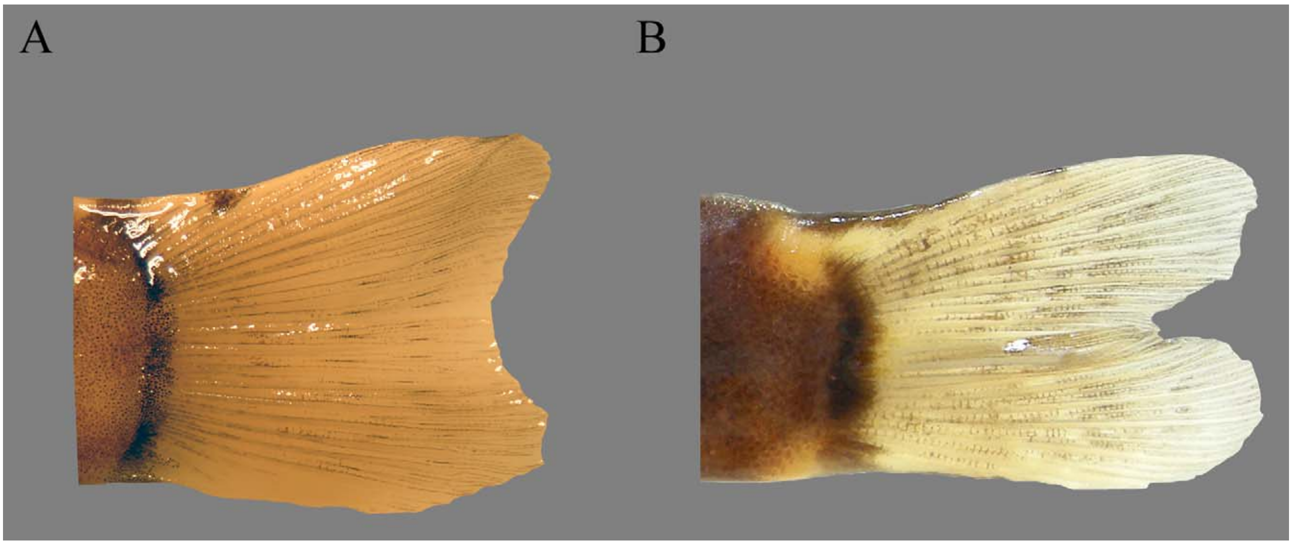

FIGURE 1. Black botch on procurrent rays of upper lobe of caudal fin of *Schistura* from Mae Khlong River basin. (a) Male *S. aurantiaca*, 48 mm TL; (b) Male *S. sexcauda*, 76 mm TL.

Results

A suborbital flap was present on all males of *S. mahnerti*, and a suborbital groove was present on all females of *S. mahnerti*. In the other three species (Table 1), all individuals lacked a flap or groove, but most males (80–88%) had a conspicuous black botch on the procurrent rays of the upper lobe of the caudal fin (Fig. 1a). Most females (90–94%) of these species lacked the black blotch present on males. The blotch on males of *S. sexcauda* was much darker and longer than that on males of *S. aurantiaca* or *S.* cf. *aurantiaca* (Fig. 1b).

Standard length of male and female *S. aurantiaca*, *S.* cf. *aurantiaca*, and *S. mahnerti* did not differ significantly (p<0.05). Standard length of *S. sexcauda* was significantly greater in males than in females (Table 2). Head width and maximum head width of *S. aurantiaca* were significantly greater in males than in females (p<0.05). Pectoral-fin length of *S.* cf. *aurantiaca* was significantly greater in males than in females (p<0.05). Body depth and maximum head width in male *S. sexcauda* were significantly less and greater, respectively, than that in females (p<0.05).] Other morphometric characteristics expressed relative to standard length did not vary between sexes. None of the morphological measurements for *S. mahnerti* differed significantly (p<0.05) between males and females.

The upper surfaces of pectoral-fin rays of adult males of *S. mahnerti* were covered by rows of small unculi throughout the year; unculi were especially dense during the reproductive season (Plongsesthee, pers. obs.).

TABLE 1. Occurrence of black blotches at base of upper lobe of caudal fin. N_p indicates presence on males, and N_a absence on females. Total numbers of specimens examined in parentheses.

Species	Standard length (mm)	Male; blotch present		Female; blotch absent	
		N_p (N)	Percentage (%)	N_a (N)	Percentage (%)
S. aurantiaca (a)	22–44	13 (16)	81	36 (40)	90
S. cf. *aurantiaca* (b)	25–45	7 (8)	88	14 (15)	93
S. sexcauda (a)	36–67	24 (30)	80	32 (34)	94
S. mahnerti (a, b)	38–67	0 (8)	0	0 (11)	0

TABLE 2. Morphometric features examined for sexual dimorphism in *Schistura aurantiaca*, *S.* cf. *aurantiaca*, *S. mahnerti*, and *S. sexcuada.* All values except standard length (SL) are proportions of standard length. Statistically significant differences between sexes (*p*<0.05) are indicated by asterisk.

Species	Measurement	Male		Female	
		Range (min–max)	Mean±S.D.	Range (min–max)	Mean±S.D.
S. aurantiaca	No. specimens	16		40	
	SL, mm	29–44	36.4±4.3	22–44	34.1±4.4
	Head width*	0.08–0.15	0.11±0.02	0.08–0.13	0.10±0.01
	Maximum head width*	0.14–0.18	0.16±0.01	0.12–0.17	0.15±0.01
	Head depth	0.07–0.12	0.09±0.01	0.07–0.12	0.09±0.01
	Pectoral-fin length	0.18–0.21	0.19±0.01	0.16–0.21	0.19±0.01
	Body width	0.12–0.15	0.14±0.01	0.11–0.15	0.13±0.01
	Body depth	0.13–0.17	0.15±0.01	0.14–0.17	0.16±0.01
	Caudal-fin length	0.18–0.26	0.22±0.02	0.20–0.24	0.22±0.01
S. cf. aurantiaca	No. specimens	8		15	
	SL, mm	25–44	36.0±7.6	26–45	38.5±5.5
	Head width	0.10–0.16	0.13±0.02	0.10–0.15	0.12±0.02
	Maximum head width	0.13–0.19	0.16±0.02	0.13–0.18	0.15±0.01
	Head depth	0.07–0.10	0.09±0.01	0.08–0.11	0.09±0.01
	Pectoral-fin length*	0.17–0.21	0.19±0.02	0.16–0.19	0.18±0.01
	Body width	0.11–0.14	0.13±0.01	0.12–0.15	0.14±0.01
	Body depth	0.13–0.16	0.15±0.01	0.14–0.18	0.15±0.01
	Caudal-fin length	0.18–0.24	0.21±0.02	0.16–0.24	0.20±0.02
S. mahnerti	No. specimens	8		11	
	SL, mm	38–63	52.5±10.2	44–65	56.3±6.1
	Head width	0.12–0.15	0.13±0.01	0.13–0.16	0.13±0.01
	Maximum head width	0.15–0.18	0.16±0.01	0.15–0.18	0.16±0.01
	Head depth	0.10–0.13	0.11±0.01	0.10–0.13	0.11±0.01
	Pectoral-fin length	0.23–0.24	0.23±0.01	0.20–0.24	0.23±0.01
	Body width	0.13–0.16	0.14±0.01	0.14–0.16	0.14±0.01
	Body depth	0.17–0.21	0.19±0.01	0.17–0.20	0.19±0.01
	Caudal-fin length	0.20–0.24	0.23±0.01	0.21–0.25	0.23±0.01
S. sexcauda	No. specimens	30		34	
	SL, mm*	36–67	56.4±7.9	36–59	48.3±6.6
	Head width	0.09–0.14	0.11±0.02	0.06–0.15	0.10±0.02
	Maximum head width*	0.14–0.18	0.16±0.01	0.09–0.22	0.15±0.02
	Head depth	0.09–0.12	0.10±0.01	0.08–0.14	0.10±0.01
	Pectoral-fin length	0.18–0.22	0.20±0.01	0.13–0.28	0.20±0.02
	Body width	0.10–0.23	0.14±0.02	0.08–0.21	0.14±0.02
	Body depth*	0.15–0.19	0.16±0.01	0.11–0.27	0.18±0.02
	Caudal-fin length	0.18–0.25	0.22±0.02	0.16–0.30	0.22±0.02

Discussion

A previously unknown method of determining the gender of individuals in some species of *Schistura* was discovered during this study. Most males (at least 80%) of three species examined have a black botch on the procurrent rays of the upper lobe of the caudal fin. The blotch is absent on most females (at least 90%). Although the number of species examined is small and restricted to one basin, this sexually dimorphic characteristic may be more widespread in the genus and useful in sex determination of living, as well as preserved, individuals. This character provides a method for nonlethal sex determination in species of *Schistura*, and possibly other nemacheilid loaches.

The black blotch may serve as a conspecific signal to attract females or to warn competing males, or it may be the effect of a sex-linked gene and have no function. The discovery of this characteristic is interesting given that many species of *Schistura* are brightly colored but are not obviously dimorphic in color. This is in contrast to many other freshwater fishes in which males are much more brightly colored than females (e.g., North American darters, South American and African cichlids—Page 1983; Barlow 2000). In other fishes, brightly colored males often hold territories, a behavior not recorded for *Schistura*.

The presence of a suborbital flap in adult males is a more conspicuously dimorphic trait in *Schistura*. As the name implies, the suborbital flap lies directly below the eye and is flap-like in that it is attached anteriorly and free posteriorly. Suborbital flaps are absent on most species of *Schistura* (e.g., Kottelat 1990; Freyhof & Serov 2001), but are present on a variety of species that are otherwise morphologically quite distinct from one another in body shape, color pattern or other characteristics. Many of the species with flaps are almost certainly only distantly related to one another, and groups of morphologically similar and presumably closely related species vary interspecifically in the presence/absence of flaps. Bohlen and Ŝlechtová (2009) differentiated *S. udomritthiruji* from the closely related *S. vinciguerrae* by the presence of a suborbital flap, and Kottelat (1990) distinguished *S. poculi* from the similar *S. mahnerti* and *S. bella* by the absence of a suborbital flap.

The presence of suborbital flaps in species that are only distantly related may indicate independent origins of the flap in separate lineages. Alternatively, the flap may have been lost in most species of *Schistura* due to costs associated with hydrodynamic drag or another factor outweighing potential benefits that led to sexual selection earlier in the ancestor of the group. Given its presence on males but not females, the flap presumably has a function related to reproduction; however, interspecific variation in presence/absence of the flap in relation to habitat or other environmental factors appears not to have been studied. Males of *S. mahnerti* have a well-developed flap, and females have a less conspicuous groove in place of the flap (Fig. 2). The groove on females may have a function, or it may be only a pleiotropic effect of the presence of the flap on males. As sister-group relationships among species of *Schistura* and related genera are documented, the evolution of these sexually dimorphic features can be better understood in relation to behavior or environmental factors.

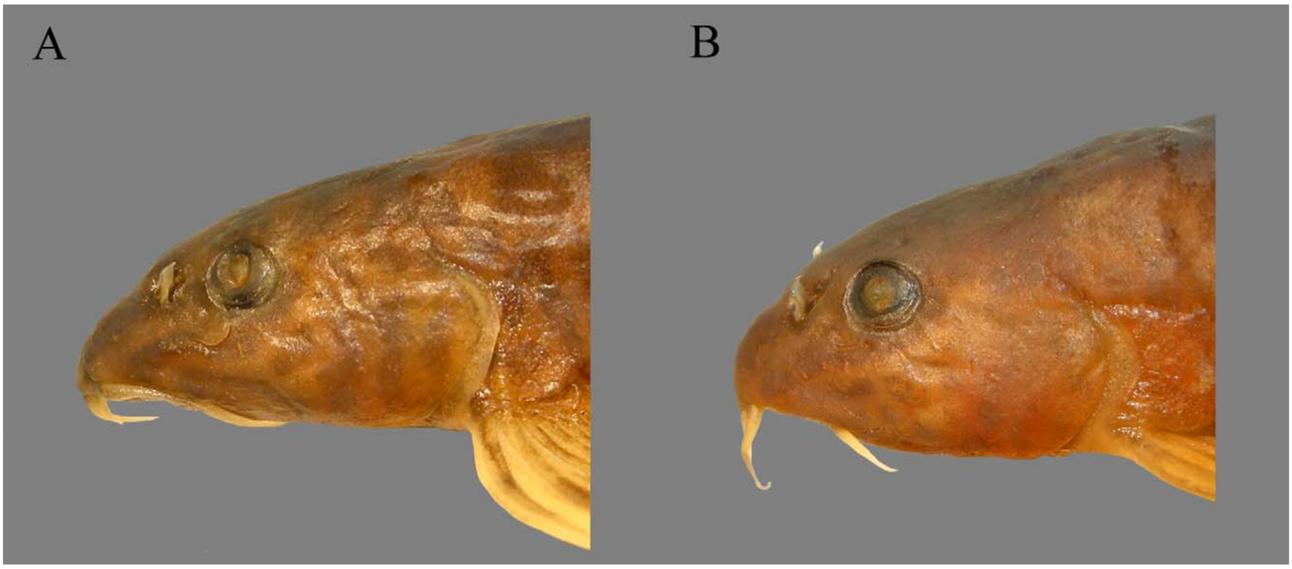

FIGURE 2. Sexual dimorphism in *Schistura mahnerti*, Mae Khlong River basin, Thailand. (A) male with suborbital flap, 78 mm TL; (B) female with suborbital groove, 82 mm TL.

Some morphometric measurements differed between the sexes of those species lacking a suborbital flap or groove. Head width and maximum head width were greater in males of *S. aurantiaca* than in females, and pectoral fins were longer in males of *S.* cf. *aurantiaca*. In *S. sexcauda*, maximum head width was greater in males and body depth was greater in females. These differences may relate to mate recognition or other selection pressures related to reproduction. Longer pectoral fins on males might facilitate nest site preparation or in maintaining proximity to females during egg release and fertilization. Deeper bodies in females may be an external manifestation of larger gonads.

The upper surfaces of pectoral fin rays of male *S. mahnerti* from the Mae Khlong and peninsular basins are covered by rows of small unculi throughout the year, but are most dense during the reproductive season. *Schistura vinciguerrae* also develops unculi on the upper surfaces of pectoral-fin rays 1–3 and pelvic-fin rays 1–2, and have unculi on the interior surface of all pectoral and pelvic-fin rays during what is presumed to be the reproductive season (Kottelat 1990). Kottelat (1990) also reported males of *S. kohchangensis* to have thickened pectoral rays 1–7, and the upper surfaces of the rays to be covered by several rows of small tubercles. Pads with tubercles and unculi on the pectoral and pelvic fins of benthic fishes are thought to facilitate station-holding in fast water (Hora 1930; Roberts 1982), where they may be particularly advantageous during reproduction.

Other sexually dimorphic characteristics have been reported in *Schistura*. Lokeshwor & Vishwanath (2011) noted that adult males of *S. fasciata* have a swollen anterior body, a triangular-shaped head with a straight dorsal surface, a prominent dorsal adipose crest, and vertical bars along the body from the middle of the dorsal fin to the base of the caudal fin. In the adult female, the anterior body is well arched, the adipose dorsal crest is less prominent, and vertical bars occur along the entire body. Freyhof & Serov (2001) found males of several species to have inflated cheeks in relation to those of females. No functional explanation was offered for the dimorphism, but it was recorded only for species lacking suborbital flaps. Perhaps the swollen cheeks have a function analogous to that of the suborbital flaps, such as the secretion of pheromones or other chemical stimuli. The greater maximum head width found in males of *S. aurantiaca* and *S. sexcauda* (Table 2) presumably is the same characteristic described as inflated cheeks by Freyhof & Serov.

In summary, sex-related characteristics are known for several species of *Schistura*, some of which provide a method for nonlethal sex determination and can be beneficial in demographic and reproduction studies. Sexually dimorphic traits are hypothesized to have a function related to reproduction; however, little is known about reproduction in *Schistura*, and variation in morphology in relation to habitat or other environmental factors has not been studied.

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FIGURE 3. *Schistura* cf. *aurantiaca*, Burapha University, uncatalogued, 37.7 mm SL, female; Thailand, Prachuap Khiri Khan Prov., Khanan River, 2 March 2010.

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