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RESEARCH ARTICLE

Sexual dimorphism in structures, size and shape of the cyprinid Nilgiri melon barb, *Haludaria fasciata*

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Abstract. The morphological differences between the sexes of a species is relevant to its captive breeding, ethology, and eco-biology and are studied extensively in fishes. In this paper, we analyzed the sexual dimorphism of the cyprinid Nilgiri melon barb, *Haludaria fasciata* (Jerdon), in their external structures, size, and shape. Physical examination, microscopical analysis, and morphometric and geometrical analyses of both sexes of the fish were conducted. The presence of tubercle pits and protuberant tubercles on the snout and orbital region were observed in males, while these were absent in females. Smaller-sized tubercles were arranged like a serration on the pectoral fin of males. Body size analysis indicated significant sexual size differences (P < 0.05) in body depth, fork length, eye diameter, pectoral fin length, and caudal fin length. Shape analysis indicated that the coordinate lengths L8 and L1 were the two that were significantly different (P < 0.05) between the sexes. In global redundancy trace analysis of vertices, the males in the population had more substantial variations in their shapes than did females. The paper discusses the possible functions linked with the dimorphic traits identified in *H. fasciata*.

Keywords: Tubercles, sexual size difference, shape analysis

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Introduction

Most fishes are bisexual and exhibit different sexually dimorphic traits in their secondary sexual characteristics (Nikolsky 1978). In many teleosts, discriminating features are reported for morphological traits such as body color (Baube 1997, Dijkstra et al. 2007, Mieno and Karino 2017), ornamental markings (Beeching et al. 1998), breeding tubercles on the snout (Wiley and Collette 1970), pectoral fins (McMillan et al. 2013) and scales (Ahnelt and Keckeis 1994, Hussain and Bordoloi 2016), fin hooks (Wiley and Collette 1970, Longoni et al. 2018), body size (Selander 1965, Shine 1989) and shape (Ostrand et al. 2001), and fin shape (Basolo 1990) and size (Karino et al. 2011). The causes suggested for developing dimorphic traits are competition within and among species for natural selection and sexual selection (Darwin 1981, Fernandes 1998). Mating competition, intrasexual competition, sexual roles, and territoriality on spawning grounds are the sexual selection factors that drive the evolution of sexually dimorphic traits (Selander 1965, Shine 1989, Forsgren et al. 2002). Sexual size dimorphism is an indicator in determining many North American cyprinids' mating systems, i.e., whether they pair or group spawn (Pyron et al. 2013).

Diverse sexual dimorphic traits such as the presence of special structures, and color, size and shape differences are described in the Cyprinid family. Among these structures, tubercles are typically found in males of Cyprinidae (Wiley and Collette 1970). Tubercles were observed on the snout of *Bangana dero* (Hamilton) (Hora and Misra 1936) and *Pimephales promelas* Raf. (Mayden 1992), in the opercula and pectoral fins of *Cyprinus carpio* L. (Swee and McCrimmon 1966) and *Carassius auratus* (L.) (Thompson et al. 2004), and on the skin of *Rutilus rutilus* (L.) (Kortet et al. 2004). McMillan et al. (2013) reported clusters of tubercles in pectoral fin rays of male *Danio rerio* (Hamilton). In *Barilius bendelisis* (Hamilton), males are distinguished from females by the presence of tubercles studded on scales (Hussain and Bordoloi 2016). In *Paedocypris progenetica* Kottelat, Britz, Tan & Witte modified pelvic fins with hypertrophied muscles and a keratinised pad in front of the pelvic girdle is a distinguishing characteristic of males (Kottelat et al. 2006). Sexual dimorphism in body and fin sizes has been widely studied in fishes and reported in cyprinids such as *Cyprinella spiloptera* (Cope) (Pyron et al. 2007), *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi (Radojković et al. 2018), *Salaria fluviatilis* (Asso) (Laporte et al. 2018), *Schizothorax raraensis* Terashima (Terashima 1984), and *Schizothorax macrophthalmus* Terashima (Terashima 1984). Larger fin sizes in males are sexually dimorphic traits in *Poecilia reticulate* Peters (Bischoff et al. 1985), *Xiphophorus helleri* Heckel (Basolo 1990), *Gila atraria* (Girard) (Belk et al. 2014), and *Puntius titteya* Deraniyagala (Mieno and Karino 2017). Body shape is a sexually dimorphic character in *Barbodes binotatus* (Val.) (Dorado et al. 2012) and *Salaria fluviatilis* (Laporte et al. 2018).

Nilgiri melon barb, *Haludaria fasciata* (Jerdon), is a cyprinid fish found in freshwater ecosystems of southern India (Jayaram 1981, 1991, Chakraborty et al. 2017). It is a commercially valuable ornamental fish exported from India under different trade names such as ember barb, melon barb, and panda barb (Daniels 2002, Sekharan 2006, Sekharan and Ramachandran 2006, Satyani and Subamia 2009). Its sex

identification characteristics have applications in brooder segregation in hatchery production, field-level biological studies, and phylogenetics. Mercy et al. (2007) and Daniels (2002) reported that *H. fasciata* exhibits temporary dichromatism and becomes reddish during the spawning season in natural habitats. They also observed the presence of breeding tubercles on the *H. fasciata* snout. In this work, we conducted morphological examinations to study different dimorphic characteristics other than dichromatism. The body and fin sizes and body shape were determined, and external body structures were examined to describe the characters of a *H. fasciata* sample collected from the Western Ghat region of India.

Methodology

A sample (n=116) of *H. fasciata* of total lengths above 3.5 cm were collected from a freshwater stream in Eruvessy Village in Kerala, India (12°05'19.5 N 75°34'24.3 E) in September 2019. The specimens were identified taxonomically based on identification keys by Jayaram (1991) and Pethiyagoda (2013). The samples were euthanized using 2-phenoxy ethanol at a dose of 3 ml l^{-1}.

Thirteen morphometric variables (Fig. 1 A), i.e., total length (TL), standard length (SL), fork length (FL), head length (HL), body depth (BD), pectoral fin length (PecL), dorsal fin height (DorH), caudal fin length (CaudL), pelvic fin length (PevL), anal fin length (AnalL), eye diameter (ED), and snout length (SnL), were measured using a digital caliper to the nearest 0.1 mm. Weights were measured to the nearest 1 mg using a calibrated digital balance (Contech, India). Fifty-eight fish were selected from the sample and photographed on a platform with a white background. Photographs were taken using a DSLR camera (Canon 700 D) fixed on a tripod perpendicular to the fish and the platform. The digital image file names and measurements were denoted by either male or female according to the sex identified. The sexes of the fishes were confirmed by dissecting and examining the gonads. Fifty-three males and 63
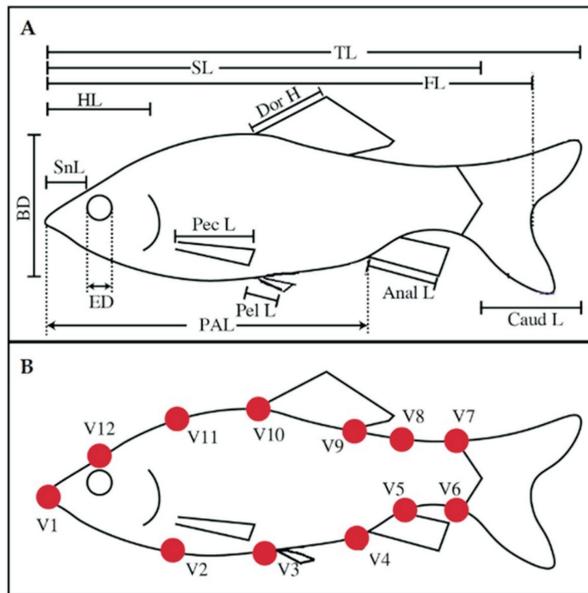
Figure 1. Selected morphological traits for size analysis (A) and geometrical landmarks for morphological shape analysis (B).

a scanning electron microscope (SEM). Scales were collected from the head region above the lateral line. All parts were cleaned superficially with 70% alcohol and cleaned with a fine brush to remove foreign particles before observation. The microscope was coupled with a CMOS camera (MD 500) to take photomicrographs. ImageJ software was used to measure the structures identified in the photomicrographs.

Linear model analysis was performed between standard length and morphometric variables. Assuming a near-normal SL distribution, we performed Student's t-test between the males and females. The data were analyzed with MANCOVA. Including SL as a covariate, we performed MANCOVA to improve the sex-based separation. For shape analysis, the images were analyzed using ImageJ software. The parts described in Table 1 were marked manually on the images. A peripheral outline of the body was done with ImageJ software to generate lengths L1, L2 to L12 (Table 1, Fig. 5A) as segments joining two vertices. We took twelve vertices (V1, V2 toV12) aligned with selected landmark coordinates on the body of the fish in each image (Fig.1B). Using custom scripts in Python, the length and vertex measurements were tested for significance using R software for shape analysis. Normalization avoided any influence SL

females were identified, and their morphometry data were sorted separately based on sex.

Topographical differences in fins, snout, orbital region, and scale of the two sexes (2n=40) were determined with external and microscopic examinations. The snout, orbital region, fins, and scales were dissected and wet mounts were under a light microscope (Esaw, India) to determine surface topography, and the structures identified were analyzed using

Table 1

Morphological landmarks and lengths selected for the study and their morphological description

No.	Lengths (Segments)	Vertices (End points)	Description of each segment
1.	L1	V1-V2	Snout tip to the point perpendicular to pectoral fin base at ventral region
2.	L2	V2-V3	Point perpendicular to pectoral fin base at ventral region to anterior end of pelvic fin base
3.	L3	V3-V4	Anterior end of pelvic fin base to the anus
4.	L4	V4-V5	Anus to the posterior end of anal fin base
5.	L5	V5-V6	Posterior end of anal fin base to ventral end of caudal peduncle
6.	L6	V6-V7	Ventral end of caudal peduncle to dorsal end of caudal peduncle
7.	L7	V7-V8	Dorsal end of caudal peduncle to perpendicular point to the posterior end of anal fin
8.	L8	V8-V9	Perpendicular point to the posterior end of anal fin to posterior end of dorsal fin base
9.	L9	V9-V10	Posterior end of dorsal fin base to dorsal fin base anterior end
10.	L10	V10-V11	Dorsal fin base to a point perpendicular to pectoral fin base at nape region
11.	L11	V11-V12	Point perpendicular to pectoral fin base at dorsal region to a point perpendicular to eye at supraorbital region
12.	L12	V12 -V1	Point perpendicular to eye at supraorbital region to the snout

Table 2
MANCOVA analysis of the morphological traits measured. Separation of sexes using SL as a covariate in MANCOVA analysis

Parameters	Dependent Variable	F	P
Standard morphometric variables			
	Total length	151.09	< 0.05
	Fork length	124.237	< 0.05
	Preanal length	33.307	< 0.05
	Body depth	53.361	< 0.05
	Head length	20.093	< 0.05
	Snout length	0.324	0.57
	Eye diameter	9.204	< 0.05
	Weight	6.371	< 0.05
Fin size			
	Pectoral fin length	27.793	< 0.05
	Dorsal fin height	14.042	< 0.05
	Caudal fin length	17.246	< 0.05
	Pelvic fin length	2.099	0.15
	Anal fin height	5.949	< 0.05

had on the samples. We employed a global redundancy trace and made a heatmap of the landmark coordinates. The significance of differences was 0.05 for all analyses.

Results

Examinations on fins, tubercles, and scales

Numerous small tubercles were observed on the snout and anterior infraorbital region of males (Fig. 2A). The tubercles were white, the base was circular and cornified with a pointed tip (Fig. 2C), and was epidermal in origin. The epidermis of the snout in males had pits filled with keratin-like substances to form the tubercles (Figs. 2D, E).

White tubercles were protuberant in some males, and these tubercles has a pointed cap or a thorn-like structure (Fig. 2C). We observed 85 ± 36 tubercles in males (n=10) with a total length range of 4.2–6.5 mm. The tubercles had a mean height of 100.42 ± 99.5 µm, and the diameter of the tubercle pit was 162.42 ± 60.09 µm. White tubercles were not found during physical examination in 52% of male specimens (n=13) examined, but tubercle pits were seen under microscopic examination. Neither tubercles nor pits were seen on the snouts of females (Fig.2 B).

Small tubercles were found on the upper side of the pectoral fins of males. They were arranged like a serration on the epidermal layer of the first 3-4 pectoral fin rays. Three rows were noted on each fin ray (Fig. 3A). In wet mounted micrographs, we observed each tubercle to be cornified (Fig. 3C). This presented as a layer of a keratin-like substance above the epidermis (Fig. 3D). The height (n = 10) of the tubercle from the epidermal layer was 12.1 ± 0.7 µm and the base width was 25.01 ± 1.1 µm. Developed and underdeveloped forms of tubercles on the pectoral fin were observed in the males. Tubercles were not present on the pectoral fins of females. Tubercles or other dimorphic structures were not present on other fins or scales. In surface topographical observation, the scales were similar in both sexes.
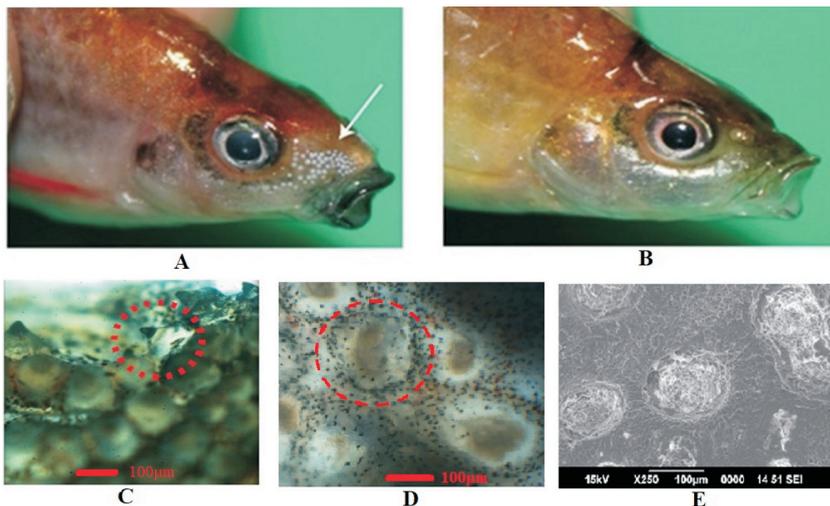

Figure 2 . Tubercles found on snout. A - Males with white tubercles, B - Females without tubercles; C - Tubercles with cornified cap (Wet mounted); D - Tubercle pits (wet mounted); E - Tubercle pits (SEM).
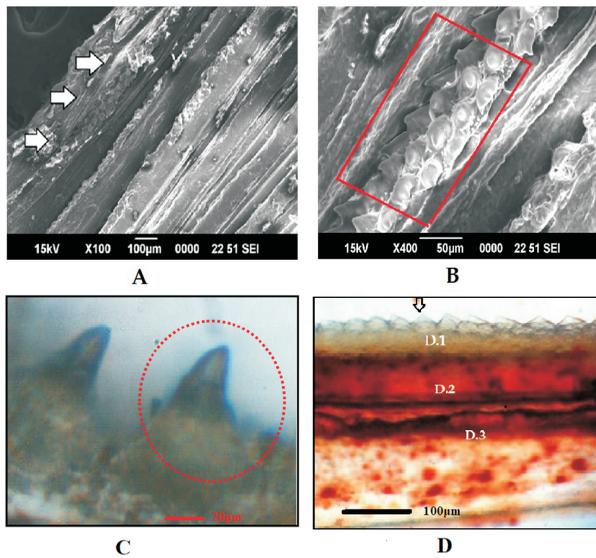
Figure 3. Micrograph of the tubercles on the pectoral fin of males. A. Tubercles (White arrow) on pectoral fin of males (SEM, 100x); B. Three rows of tubercles on a pectoral fin ray (SEM, 400x); C. Cornified pointed tubercle (wet mounted); D. Layer of keratin-like substance (D1) on the vascular epidermis (D2) of the fin ray (D3) (wet mounted).

Analysis of size differences of body and fins

The mean SL was 40.79 ± 6.36 mm for males and 39.26 ± 5.01 mm for females. No significant differences in SL were noted between the sexes, but significant differences between them were noted for FL, BD, ED, PecL, and CaudL (P ≤ 0.05). Except for anal fin height, the mean value of all other variables was higher in males than in females. The correlation analysis of SL with other variables showed a positive correlation and growth was proportional to SL in both sexes. The results of the student t-test indicated that CaudL was a highly significant separable parameter (P = 0.0013), followed by PecL (P = 0.002), BD (P = 0.011), ED (P = 0.019), and FL (P=0.04). Other parameters were not significant, and snout length was the least significant. The general distribution is presented as a Manhattan plot (Fig. 4).

Analysis of body shape differences

Six lengths among the morphological coordinates differed significantly (P<0.05) between the sexes. They were identified, separated, and shown as significant (Fig. 5A). ANOVA indicated that L8 and L1 were two significant lengths that can separate the sexes. The males had higher means and variation for L4 and L5 than did females. Variation in the lengths of the peripheral structure was visualized using a plot that shows the mean and standard deviation
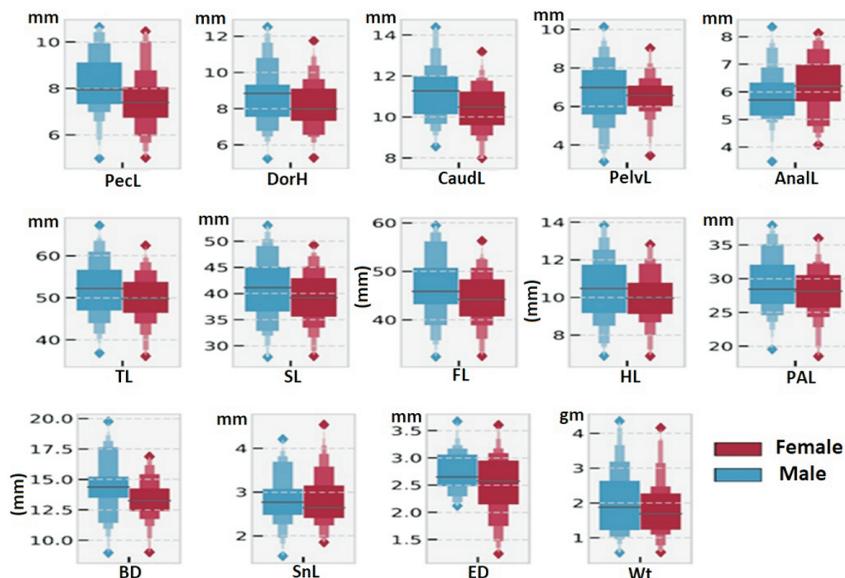

Figure 4. Manhattan plot of morphological traits and descriptors of both sexes of *H. fasciata*. Total length (TL), standard length (SL), fork length (FL), head length (HL), body depth (BD), pectoral fin length (PecL), dorsal fin height (DorH), caudal fin length (CaudL), pelvic fin length (PevL), anal fin length (AnalL), eye diameter (ED), and snout length (SnL).
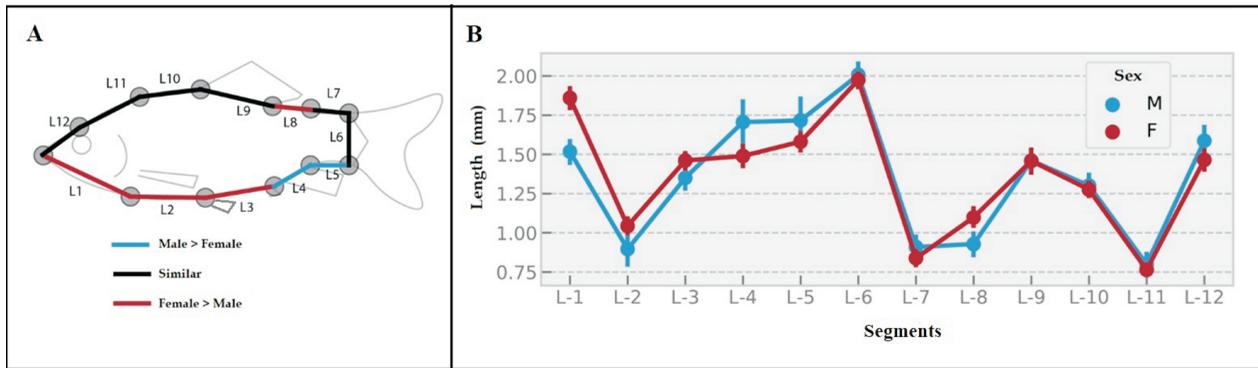
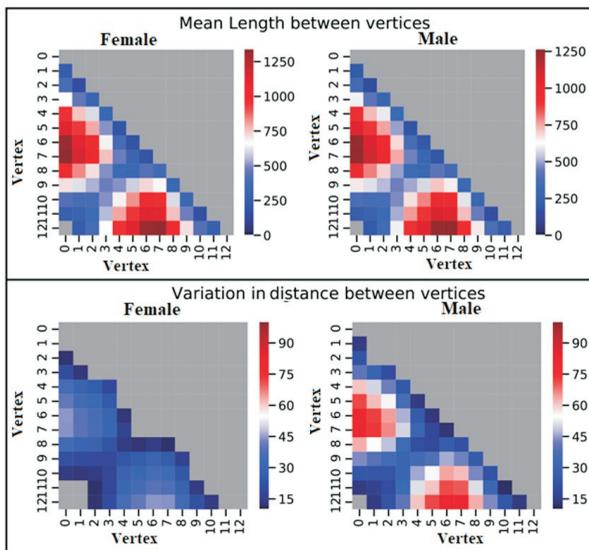Figure 5. Shape analysis by variation in lengths on fish peripheral structure.

Figure 6. Heatmap of 78 pairs of vertices of selected landmark coordinates on males and females.

shape. During physical examinations, tubercles or tubercle pits were noted on male snouts and orbital regions. Tubercles in other fishes are noted on different body parts like snouts, eyes, opercula, heads, fins, skin, and scales (Wiley and Collette 1970). Cyprinid fishes such as *Ictiobus cyprinellus* (Val.) (Morris and Burr 1982), *R. rutilus* (Kortet et al. 2003), *C. auratus* (Thompson et al. 2004), *Phoxinus phoxinus* (L.) (Jacob et al. 2009), *D. rerio* (McMillan et al. 2013), *B. bendelisis* (Hussain and Bordoloi 2016), and *Knodus nuptialis* Menezes & Marinho (Menezes and Marinho 2019) are a few examples of fishes with sexually dimorphic tubercles. The presence of tubercles and their intensification is a condition factor of health, breeding fitness, and dominant behavior (Kortet et al. 2003, 2004, Wiley and Collette 1970, Jacob et al. 2009).

Not all male *H. fasciata* exhibited white coniferous tubercles, but tubercle pits were present in all males. This showed that *H. fasciata* can either generate or degenerate tubercles at tubercle pits based on their condition. The superficial cap of the tubercles was filled with keratin as a layer on the epidermis in *D. rerio* (McMillan et al. 2013). These keratinized layers are continuously renewed on the epidermis to maintain the tubercles (Kang et al. 2013). In *H. fasciata*, a similar layer of a white keratin-like substance was present in the tubercles at the snout, anterior orbital region, and epidermis of the pectoral fin ray. Androgenic hormones such as testosterone and 11-ketotestosterone are the possible inducers for generating tubercles (Kortet et al. 2003). The

(Fig. 5B). The heatmap (Fig. 6) shows the morphometric variation in males (~90 pixels), which was approximately three times larger than in females (~30 pixels), while the mean distances remained similar (~1250 pixels). The heatmap focuses on the distances among all combinations of vertices (78 pairs) and the mean length and standard error among lengths (n[male]=22; n[female]=36).

Discussion

The present study revealed that *H. fasciata* exhibited sexually dimorphic traits including breeding tubercles and differences in body and fin size and body

generation of tubercles can take weeks and, in some fishes, tubercles degenerate after the spawning season (Wiley and Collette 1970). Breeding tubercles act as contact organs for males during spawning and serve sexual roles such as rubbing and bumping to induce egg release in females (Marconato and Shapiro 1996, Kang et al. 2013). Territorial males at the spawning site exhibited more breeding tubercles than non-territorial males in common bream, *Abramis brama* (L.) (Poncin et al. 2011). During our field studies, we observed antagonist, territorial behavior among males at different collection sites. We hypothesized that the functions of territoriality and sexual roles of these tubercles might be present in *H. fasciata*.

Sexual size difference in fishes is a widely studied dimorphic trait. The size analysis of body and fins revealed there was size variation between the sexes in *H. fasciata* for eye diameter, body depth, fork length, pectoral fin length, and caudal fin length. Sexual dimorphism in size results from evolution in a fish's territorial nature, sexual competition, mate selection, and reproductive success (Darwin 1981, Fernandes 1998, Amundsen and Forsgren 2001, Pyron et al. 2013). Pyron et al. (2013) demonstrated that in many cyprinids males were larger in size than females in species with pair spawning mating systems, while females were larger than or the same size as males in species with group spawning mating systems. The genus Haludaria is a small brood pair spawner that is territorial in breeding grounds (Kortmulder 1982, McConnell and Lowe-McConnell 1987, Harikumar et al. 1994). The mean SL of males in the population was found to be comparatively higher than that of females, which confirmed Pyron's (2013) conclusions regarding links between mating systems and dimorphism; however, there were also small-sized males in the population.

Fishes can develop dimorphic traits based on the purposes of breeding behavior. Larger heads are thought to be adaptive for three-spined sticklebacks to collect spawning nest materials as a gender role (Wootton 1984, Kitano et al. 2007). Larger eye size has been observed in different animals (Land 1989, Ziemba and Rutowski 2000, Faiman et al. 2018) and

is thought to have an advantageous role in finding mates (Ziemba and Rutowski 2000) and foraging (Hutcheon et al. 2002). Caves et al. (2017) reported that larger eye size improved vision by providing higher acuity or sensitivity. Guppies with larger eye sizes were reported to have larger brains (Corral-López et al. 2017). In *H. fasciata*, the eye diameter is larger in males than in females and significantly differed from females even in their small size (SL). We assumed the male might have a developed brain and vision adaptive to the purpose of intra-sexual competition in foraging, territoriality, and mating.

In *H. fasciata*, the pectoral fin and caudal fin lengths were significantly longer in males than females at similar standard lengths. The size differences of caudal fins and pelvic, pectoral, and anal fins was reported in other fishes (Py-Daniel and Fernandes 2005). Fin size is a mate selection criterion for females in males of *X. helleri* and *P. reticulata* (Basolo 1990, Karino et al. 2011). In our observations, male pectoral fins had a red hue, while those of females had a hyaline color. We proposed a sexual or rival role for having a serration of tubercles on the dichromatic longer pectoral fins in *H. fasciata*.

Cyprinids such as *S. fluviatilis* (Laporte et al. 2018) and *P. binotatus* (Dorado et al. 2012) were previously studied for sexual shape dimorphism using geometrical morphometrics. The present study determined shape by measuring the length between selected adjacent vertices and all lengths between the vertices and the shape differences between the sexes of *H .fasciata*. The males of *H. fasciata* had more length between the anus or genital pore to their caudal peduncle (L4). In females the length from the dorsal-fin base to the point perpendicular to the posterior proximal end of the anal fin was higher than in males (L8). Many cyprinids that pair spawn, including the genus Haludaria, were observed to have a mating pattern called holding, where the male clasps the female by bending its caudal peduncle over that of the female (Miller 1964, Kortmulder 1982, Jacob 2013). The higher L4 and caudal fin length in males and the higher L8 in females of *H. fasciata* could facilitate the clasping activity and keep

the genital pore intact during the holding act, thereby improving egg fertilization. The length from the snout to the anal fin base (L1, L2, and L3) was shorter in males than in females. This indicated that males were more torpedo-shaped than females, which allowed males to move faster in lotic systems. The better maneuvering in males might help in courtship behaviors such as chasing, darting, and hovering, which are generally observed in cyprinids during breeding (Miller 1964, Jacob 2013). All the trait-related functions hypothesized above can be validated by etho-ecobiological studies.

In the heatmap representation, we demonstrated that shape variation in males was approximately three times higher than in females. The main factor for such allometry was sexual selection (Pyron et al. 2013) and similar growth plasticity has been demonstrated in reef fishes (Walker and McCormick 2009). Apart from gender causes of dimorphism in fishes, their microhabitats might play a role in body shape and size. Shape differences can occur between two populations of the same species existing in lotic and lentic systems (Radojković et al. 2018). The more slender fish shape is typically found in lotic habitats, which require better swimming (Laporte et al. 2018), while fishes from lentic systems tend to be deeper-bodied. The habitats from which we collected samples of the *H. fasciata* population were streams that had both small dammed areas with moderate to higher water depth and very shallow regions with less hydrological connectivity. This also might have affected the size and shape of the samples collected for the study. Studies on landmark-based geometric morphometrics and geographical data of different populations are required to prove whether ecosystems affected the body shape of *H. fasciata*. However, this investigation gives preliminary information on the sexual dimorphic characteristics of *H. fasciata* from a population in south India. Even though these morphometric variables indicated significant differences between sexes, they were not visually notable characters that could be used in manual differentiation. Males can easily identified by examining snouts for tubercles or tubercle pits microscopically.

Conclusion

Sexual dimorphism in size, shape, and structures of the body of fishes evolved for functional causes by either natural or sexual selection. *H. fasciata* exhibited sexual dimorphic traits such as protuberant tubercles on the snout and a serration of tubercles on the pectoral fin, and there were differences in body shapes and sizes. Identifying the sex of *H. fasciata* can be done by examining these sexually dimorphic traits. For this study we hypothesized a few prospective functions of the dimorphic traits that were linked to reproduction and associated behaviors. The tubercles observed on the males fishes be breeding tubercles developed for inducing the breeding of females or for exhibiting a dominating signal in their territory. Pectoral fin length and the serration of tubercles on the fins might act as weapons for defending territory and during courtship. The holding act of mating could be supported by the caudal fin and sexual shape differences. Eye size and shape differences might facilitate the movement of males in water for chasing and darting behaviors. Male *H. fasciata* had a different shape from females, which could play a role in mate choice or sexual selection and the mating system. However, more ethological studies are required to confirm the roles of the dimorphic traits hypothesized in the study.

Author contributions. J.J.C. sampling and data collection, analyzing, writing, and revising the manuscript; M.S. supervision of the work and revising the manuscript.

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