

Primary and Secondary Sexual Characteristics of Kuhli Loach (*Pangio kuhlii*)

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Abstract

Kuhli loach (*Pangio kuhlii*) a popular ornamental freshwater fish species with economic potential, originating from Sumatra, Kalimantan, Java, and Malaysia. The kuhli loach's appeal lies in its vibrant colors and distinctive eel-like body. This study aims to explore the sexual primary and secondary characteristics of the kuhli loach. Conducted at Universitas Airlangga in Banyuwangi, Indonesia, the study involved a thorough analysis of 50 kuhli loaches, evenly split between males and females. Morphological observations revealed significant differences ($p < 0.05$), such as the elongated body and expanded abdominal region in females, suggesting adaptations for reproduction. Internal examinations, including peritoneal dissections and histological analysis, unveiled distinct stages of spermatogenesis in males and ovarian development in females, providing insights into the reproductive cycle. Meristic and morphometric analyses identified noteworthy differences in pectoral fin rays, suggesting potential roles in reproductive behavior, while other traits exhibited similarity between genders. This study contributes to the understanding of kuhli loach ecology and reproductive biology, highlighting the importance of addressing sustainability concerns arising from the continuous exploitation of wild populations. This study serves as a foundational step towards advancing reproductive improvement in fish and supports the conservation of this unique ornamental fish species, with implications for aquaculture practices and economic considerations.

Keywords: aquaculture, female, fish species, male, reproductive behavior

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INTRODUCTION

Ornamental fish, characterized by their vibrant colors and unique patterns, have gained widespread popularity among enthusiasts (Ulkhayq *et al.*, 2019). Among them, kuhli loach (*Pangio kuhlii*) stands out as a notable freshwater species, known for its elongated eel-like body and distinctive coloration. Indigenous to the regions of Sumatra, Kalimantan, Java, and Malaysia, kuhli loach inhabits shallow and calm waters, such as swamps, reservoirs, and dam peripheries, with a preference for slow-flowing sandy riverbeds (Radkhah *et al.*, 2021). Belonging to the family Cobitidae, kuhli loach is part of the broader category of "loaches," recognized for their small size, bright patterns, attractive

behaviors, and robust health, making them suitable for aquarium keeping (Acharjee and Barat, 2014). The economic value of kuhli loach is further emphasized by its role as an active aquarium cleaner, contributing to its popularity among hobbyists (Ferdiansyah *et al.*, 2020). With prices ranging from IDR 7,000 to 15,000 per individual, kuhli loach holds significant economic potential, making it a suitable candidate for aquaculture (Gao *et al.*, 2023).

However, the continuous and excessive exploitation of wild populations poses a threat to the sustainability of kuhli loach. To address this issue, cultivation efforts, including breeding, are essential (Budi *et al.*, 2020; Budi *et al.*, 2023; Budi *et al.*, 2024; Syafariyah *et al.*, 2023). To ensure the sustainability of kuhli loach breeding



programs, understanding their basic biology, especially reproductive aspects, is crucial (Cahyanti *et al.*, 2021). Fish captive breeding, whether in a natural or artificial setting, requires knowledge of the characteristics that differentiate between male and female fish (Priyadi *et al.*, 2024). This knowledge is crucial for the success of the breeding process. Additionally, understanding the characteristics that distinguish between the sexes in fish is valuable for advancing reproductive technology in fish. This information can serve as a foundation for the development of reproductive engineering in fish.

The sexual characteristics of fish can be classified into primary and secondary sexual characteristics. Primary sexual characteristics involve organs directly associated with reproduction, such as gonads and their ducts (Greven, 2019). The primary sexual characteristics are the testes (sperm) in males and ovaries (eggs) in females. Secondary sexual characteristics can be determined by observing the morphological differences in the fish's body, known as sexual dimorphism and sexual dichromatism. Sexual dimorphism involves differences in body parts such as tail fin shape, abdominal body shape, and overall body shape, while sexual dichromatism is observed through color variations, including the fins. Sexual dimorphism can be identified by examining meristic and morphometric characteristics of the body to distinguish between male and female fish (Acharjee and Barat, 2014; Endrueit, 2016; Manoharan *et al.*, 2019; Passos *et al.*, 2015; Sharma *et al.*, 2014; Taugbøl *et al.*, 2020).

Previous study has addressed sexual characteristics in various ornamental fish species, such as Kelabau (*Osteochilus melanopleurus*) (Asiah *et al.*, 2018), silver arowana (*Sclerophages macrocephalus*) (Chumaidi *et al.*, 2012), kissing gouramy (*Helostoma temminckii*) (Arifin *et al.*, 2017), and *Canthophrys gongota* (Akter *et al.*, 2019). However, to date, no studies have focused on the sexual characteristics of kuhli loach. There is still a gap in knowledge regarding the sexual characteristics and differences between male and female kuhli loaches. Therefore, it is necessary to conduct study on the sexual characteristics of

male and female kuhli loaches to provide valuable information for future cultivation efforts.

This study purposes to elucidate the primary and secondary sexual characteristics in Kuhli Loach. The findings aim to contribute valuable information to support sustainable aquaculture practices and conservation efforts for this unique ornamental fish species.

MATERIALS AND METHODS

Ethical Approval

This study was conducted in compliance with the Law of the Republic of Indonesia No. 18 of 2002 concerning the National System of Research, Development, and Application of Science and Technology.

Study Period and Location

This study was conducted at the laboratory of the Faculty of Health, Medicine, and Life Sciences, Universitas Airlangga, Banyuwangi, Indonesia; spanning from March to April 2023.

Experimental Fish

A batch of 50 kuhli loach, designated as test fish, comprised 25 males (averaging 5.754 ± 0.243 cm in total length and 0.556 ± 0.081 g in total body weight) and 25 females (averaging 5.741 ± 0.32 cm in total length and 0.584 ± 0.122 g in total body weight). These fish were obtained from their natural habitat in Riau, Indonesia, and transported to the research station in Banyuwangi, Indonesia, utilizing a combination of land and air transportation (flight from Riau to Jakarta then continued from Jakarta to Banyuwangi). Prior to the observation period, the male and female kuhli loach were kept in separate aquariums with dimensions of $40 \times 25 \times 30$ cm³.

Evaluation of Primary Sexual Characteristics

The primary sexual characteristics examined included the macroscopic and microscopic observation of male and female gonads. Macroscopic assessments involved the dissection of gonads, allowing direct observation of male testes and female ovaries extracted from the peritoneal cavity of the fish. Meanwhile,

microscopic observations were carried out by histological analysis of the testes and ovaries.

Gonads taken from the peritoneal cavity were fixed in Davidson's solution for a minimum of 24 hours and subsequently immersed in 70% ethanol before histological analysis. Dehydration involved a gradual increase in alcohol concentration (70, 80, 90, 95, and 100%). The samples underwent xylene treatments (I, II, III) and were embedded in paraffin, followed by the generation of 5–7- μ m transverse and longitudinal sections using a microtome (Thermo Scientific, HM 325). These sections were placed on glass slides, subjected to hematoxylin and eosin (H&E) staining, and then dehydrated in escalating alcohol solutions, followed by cleaning with xylene. A slip-cover, using Entellan mounting medium, was applied over the specimen. The microscopic analysis, conducted at 100 \times magnification.

Evaluation of Secondary Sexual Characteristics

To measure meristic characteristics, the fish was positioned under a stereo microscope, and specific body parts were counted using a hand counter. Meristic characteristics were then calculated following the guideline (Figure 1). Yellow strip (YS) and brown strip (BS) are characteristics of dichromatism which are calculated/measured meristically.

The examination of secondary sexual characteristics included morphometric and meristic traits. Morphometric measurements were conducted by photographing the fish and measuring the length of specific body parts using the ImageJ application. The fish, positioned on a laminated millimeter paper block with the left side facing forward, was measured following the guidelines for morphometric characteristics (Chumaidi *et al.*, 2012). The morphometric parameters measured can be seen in Figure 2.

Statistical Analysis

Primary sexual characteristics underwent descriptive analysis, while secondary sexual characteristics, including morphometrics and meristics were subjected to analyze using the

independent T-test within the SPSS 25 program, maintaining a confidence level of 95%.

RESULTS AND DISCUSSION

Based on Figure 3, it was presented distinguishable differences between male and female kuhli loach fish. A quick observation reveals that the female fish possesses a more elongated body structure, notably taller, and a larger abdominal region compared to the male. Figures 4 and 5 were reflected peritoneal dissections of male and female kuhli loach, respectively, revealing the internal reproductive organs. Histological examination of testicles in males revealed distinct stages of spermatogenesis, including spermatogonia, spermatocytes, spermatids, and spermatozoa. Females exhibited ovaries containing previtellogenic and late-vitellogenic oocytes.

The meristic characters of male and female kuhli loaches were analyzed to discern potential sexual dimorphism. The study encompassed a sample size of 25 individuals, and the differences in various morphological traits between the two sexes were assessed. The results are summarized in Table 1. The pectoral fin rays (PeFR) exhibited a significant difference between males and females, with males displaying a range of P.I.7-P.I.8 and a mean of 8.80 ± 0.40 , while females had a range of P.8-P.10 and a mean of 9.44 ± 0.87 ($P = 0.002$, Sig. = S), indicating sexual dimorphism in this characteristic.

Pelvic fin rays (PFR) showed no statistically significant difference between the sexes, with males having a mean of 6.32 ± 0.47 and females 6.20 ± 0.40 ($P = 0.344$, Sig. = NS). Similarly, dorsal fin rays (DFR) ($P = 0.071$, Sig. = NS), anal fin rays (AFR) ($P = 0.086$, Sig. = NS), caudal fin rays (CFR) (Sig. = NS), barbel count (BrB) (Sig. = NS), and the presence of right and left yellow strips (YS) and brown strips (BS) on the body, respectively, did not show statistically significant differences between male and female kuhli loaches.

The morphometric characteristics of male and female kuhli loach were investigated in a sample size of 25 individuals. The measured

parameters and their respective ranges, mean values, and standard deviations are presented in Table 2. The total length (TL) ranged from 5.33 to 6.13 cm in males, with a mean \pm standard deviation of 5.75 ± 0.24 cm, and from 5.20 to 6.22 cm in females, with a mean \pm standard deviation of 5.74 ± 0.32 cm. No significant difference was

observed between male and female TL ($p = 0.869$, NS). Similar results were obtained for fork length (FL) and standard length (SL), with no statistically significant differences between genders ($p = 0.912$, NS; $p = 0.802$, NS, respectively).

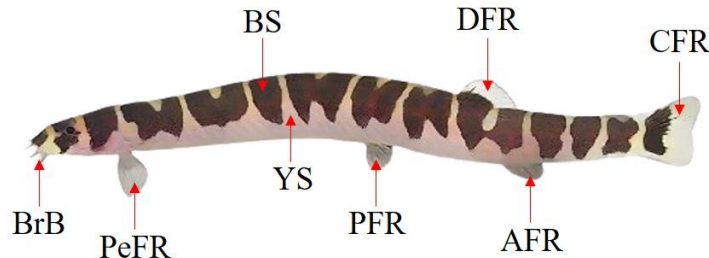


Figure 1. Measurement of meristic characteristics of kuhli loach. PeFR= pectoral fin rays, PFR= pelvic fin rays, DFR= dorsal fins rays, AFR= anal fin rays, CFR= caudal fin rays, BrB= barbell, YS= yellow strip, BS= brown strip. DOI: 10.6084/m9.figshare.26946424.

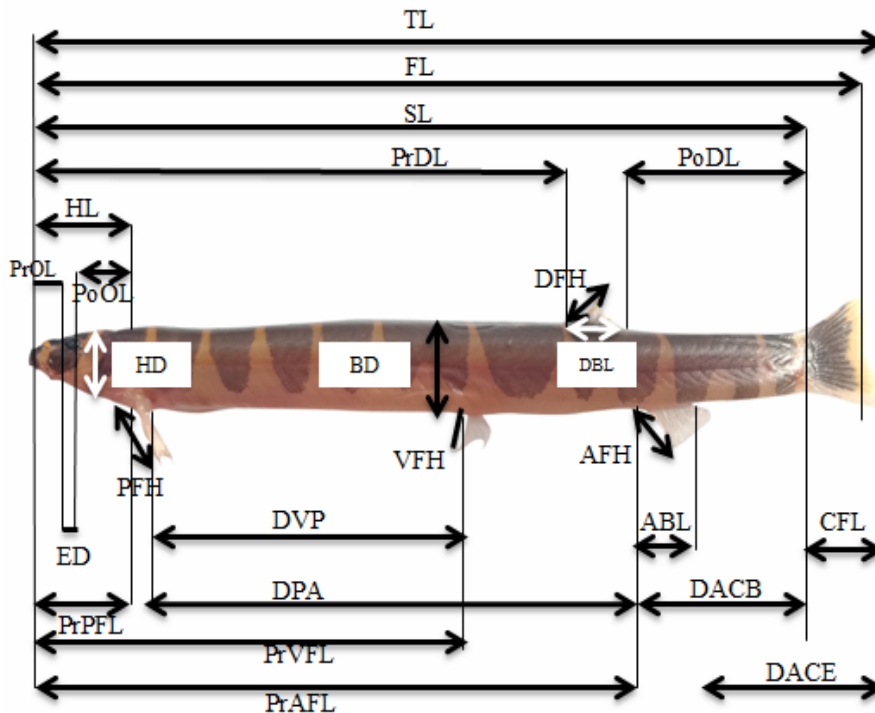


Figure 2. Measurement of morphometric characteristics of kuhli loach. TL (total length), FL (fork length), SL (standard length), ED (eye diameter), PrOL (pre-orbital length), PoOL (post-orbital length), HD (head depth), HL (head length), PrDL (pre-dorsal length), PoDL (post-dorsal length), PrVFL (pre-ventral fin length), PrPFL (pre-pectoral fin length), PrAFL (pre-anal fin length), DACE (distance from anal fin to caudal fin end), DACB (distance from anal fin to caudal fin base), DPA (distance between pectoral and anal fin), DVP (distance between ventral and pectoral fin), DFH (dorsal fin height), DBL (dorsal fin base length), AFH (anal fin height), ABL (anal fin base length), PFH (pectoral fin height), VFH (ventral fin height), CFL (caudal fin length), and BD (body depth).

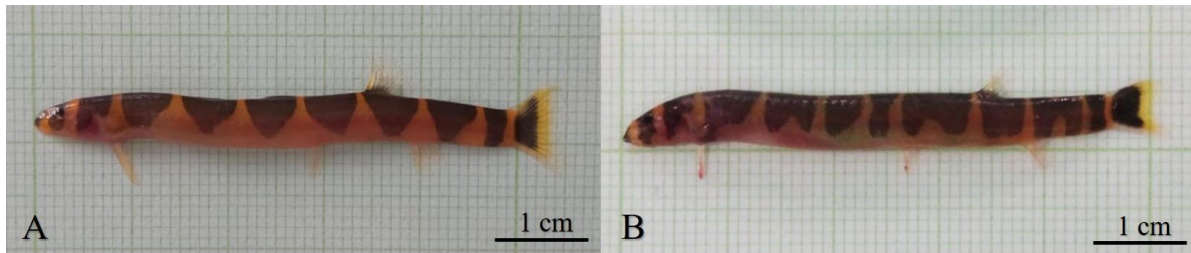


Figure 3. (A) Male and (B) female of kuhli loach.

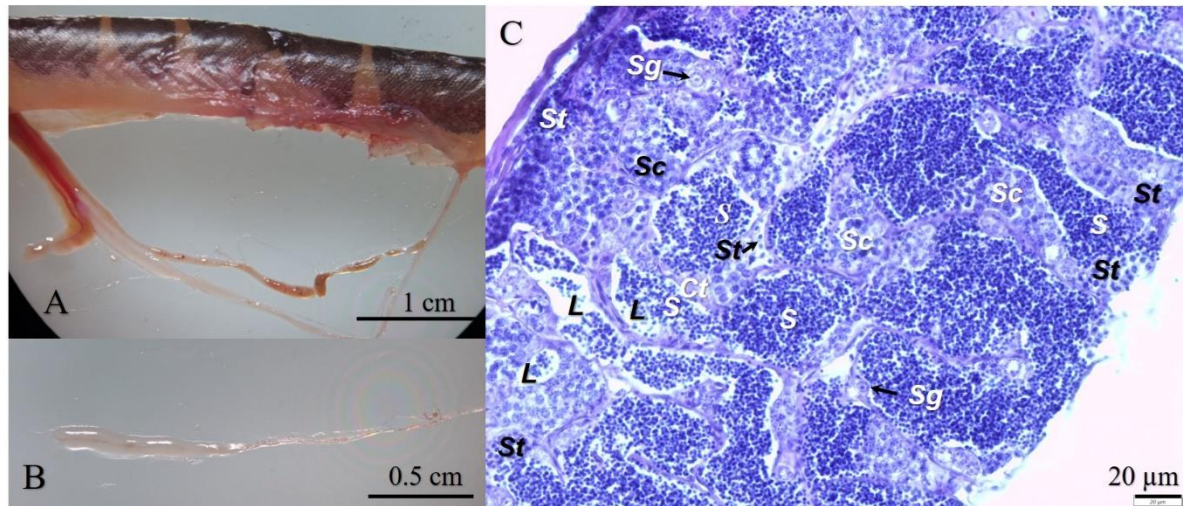


Figure 4. (A) The peritoneal dissected of male kuhli loach with visible testicles, (B) appearance of testicles after being removed from the peritoneal cavity, and (C) testicular histology consist of spermatogonium (Sg), spermatocytes (Sc), spermatid (St), and spermatozoa (S).

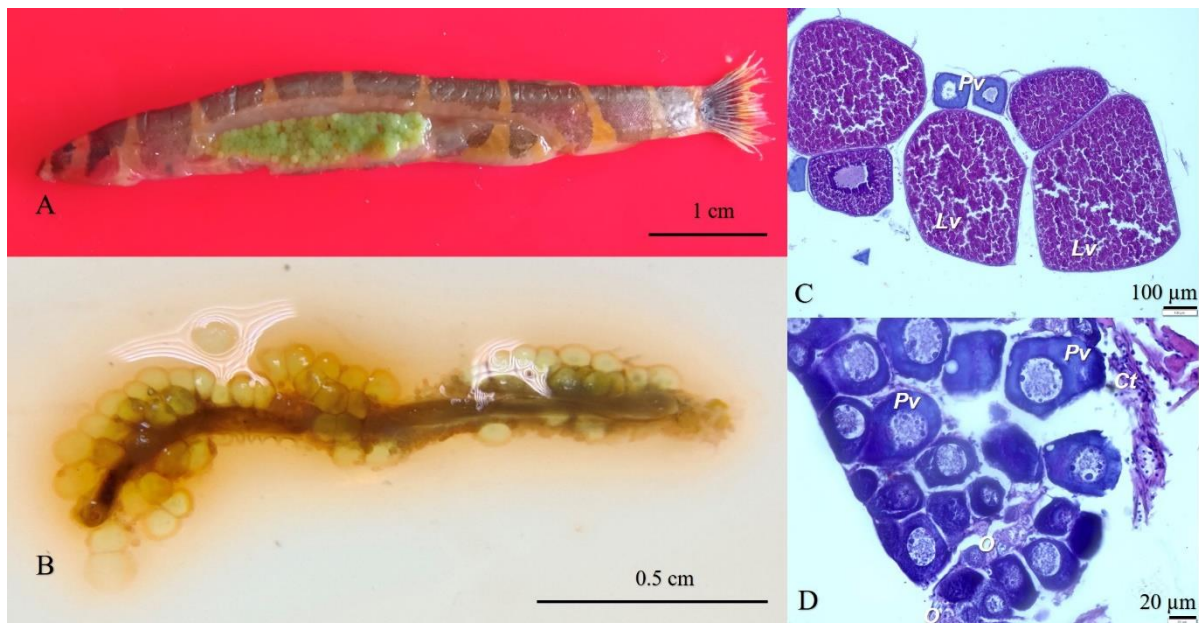


Figure 5. (A) The peritoneal dissected of female kuhli loach with visible ovaries, (B) appearance of ovaries after being removed from the peritoneal cavity, (C) late-vitellogenic, and (D) previtellogenic ovary histology. Ct= connective tissue, O= oogonium, Pv= previtellogenic oocyte, Lv= late-vitellogenic.

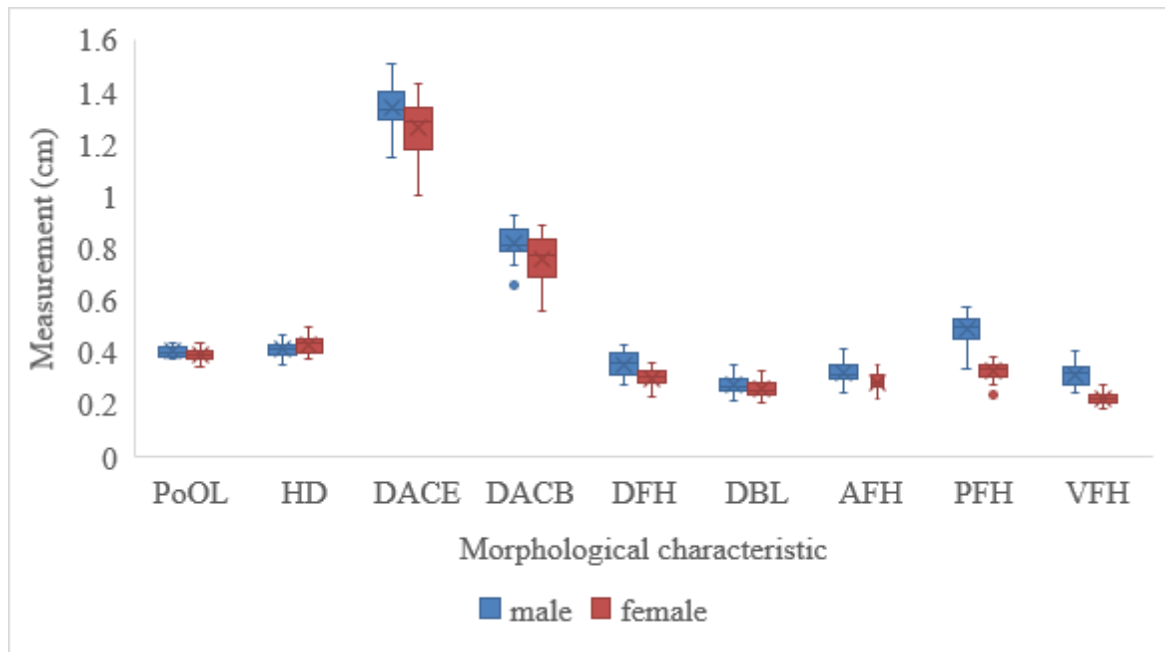


Figure 6. Summarized significant different morphometric characters of male and female kuhli loach by independent T-test (n = 25).

Table 1. Differences in meristic characters between male and female kuhli loach (n = 25)

Characters	Male		Female		p*	Sig.**
	Range (%)	Mean ± SD (%)	Range (%)	Mean ± SD (%)		
PeFR	P.I.7–P.I.8	8.80 ± 0.40	P.8–P.10	9.44 ± 0.87	0.002	S
PFR	V.6–V.7	6.32 ± 0.47	V.6–V.7	6.20 ± 0.40	0.344	NS
DFR	D.7–D.8	7.56 ± 7.80	D.7–D.8	7.80 ± 0.40	0.071	NS
AFR	A.6–A.8	6.92 ± 0.75	A.6–A.8	7.32 ± 0.85	0.086	NS
CFR	C.16	6.00 ± 0.00	C.16	6.00 ± 0.00	-	-
BrB	6	6.00 ± 0.00	6	6.00 ± 0.00	-	-
Right YS	7–13	9.76 ± 1.73	7–14	10.48 ± 2.08	0.191	NS
Left YS	7–14	10.76 ± 1.78	7–16	10.72 ± 2.13	0.943	NS
Right BS	6–10	8.36 ± 1.15	6–14	8.96 ± 1.64	0.142	NS
Left BS	6–10	8.24 ± 1.30	6–12	8.44 ± 1.44	0.609	NS

PeFR= pectoral fin rays, PFR= pelvic fin rays, DFR= dorsal fins rays, AFR= anal fin rays, CFR= caudal fin rays, BrB= barbel, YS= yellow strip, BS= brown strip. *By independent T-test, **Significant (S) and not significant (NS).

Several morphometric characters, including eye diameter (ED), pre-orbital length (PrOL), and post-orbital length (PoOL), showed no significant differences between males and females (p = 0.792, NS; p = 0.326, NS; p = 0.035, S, respectively). However, head depth (HD) exhibited a significant difference (p = 0.048, S), suggesting variation in head morphology between the sexes.

Fin dimensions were also investigated, with pre-dorsal length (PrDL), post-dorsal length (PoDL), pre-ventral fin length (PrVFL), pre-pectoral fin length (PrPFL), and pre-anal fin length (PrAFL) showed no significant differences

between males and females (p = 0.650, NS; p = 0.780, NS; p = 0.908, NS; p = 0.605, NS; p = 0.496, NS, respectively). Significant differences were observed in the distances from anal fin to caudal fin end (DACE) and caudal fin base (DACB) (p = 0.006, S), as well as in dorsal fin height (DFH), dorsal fin base length (DBL), anal fin height (AFH), pectoral fin height (PFH), ventral fin height (VFH), and body depth (BD) (p = 0.006, S; p < 0.001, S; p = 0.001, S; p < 0.001, S; p < 0.001, S; p = 0.024, S, respectively). The distribution of data on morphometric parameters that show significance in Table 2. can be summarized in the boxplot diagram in Figure 6.

These findings suggest notable sexual dimorphism in these morphometric traits.

The findings presented in this study provide valuable insights into the sexual dimorphism and reproductive anatomy of kuhli loach fish. The observed differences in external morphology, as illustrated in Figure 3, indicate distinct features between male and female individuals. The

elongated body, heightened stature, and expanded abdominal region in females may indicate adaptations linked to reproductive functions, such as accommodating a greater number of eggs during spawning, aligning with findings from prior studies on *Botia birdi* (Sharma *et al.*, 2014) and *Lepidocephalus thermalis* (Manoharan *et al.*, 2019).

Table 2. Morphometric characters of male and female kuhli loach (n = 25)

Characters	Male		Female		p*	Sig.**
	Range (cm)	Mean ± SD (cm)	Range (cm)	Mean ± SD (cm)		
TL	5.33–6.13	5.75 ± 0.24	5.20–6.22	5.74 ± 0.32	0.869	NS
FL	5.28–6.06	5.61 ± 0.24	5.02–6.12	5.60 ± 0.33	0.912	NS
SL	4.86–5.61	5.21 ± 0.24	4.65–5.64	5.19 ± 0.30	0.802	NS
ED	0.04–0.08	0.06 ± 0.01	0.04–0.10	0.06 ± 0.02	0.792	NS
PrOL	0.17–0.27	0.23 ± 0.04	0.17–0.29	0.22 ± 0.04	0.326	NS
PoOL	0.37–0.44	0.40 ± 0.02	0.35–0.44	0.39 ± 0.02	0.035	S
HD	0.35–0.64	0.41 ± 0.03	0.38–0.5	0.43 ± 0.03	0.048	S
HL	0.64–0.79	0.72 ± 0.04	0.62–0.78	0.72 ± 0.04	0.627	NS
PrDL	3.35–3.97	3.64 ± 0.19	3.27–4.03	3.67 ± 0.23	0.650	NS
PoDL	1.52–1.83	1.72 ± 0.09	1.48–1.92	1.71 ± 0.12	0.780	NS
PrVFL	2.73–3.22	2.94 ± 0.15	2.60–3.21	2.92 ± 0.19	0.908	NS
PrPFL	0.64–0.88	0.74 ± 0.05	0.63–0.90	0.75 ± 0.07	0.605	NS
PrAFL	3.84–4.55	4.11 ± 0.20	3.74–4.61	4.13 ± 0.27	0.496	NS
DACE	1.15–1.51	1.34 ± 0.08	1.00–1.43	1.26 ± 0.10	0.006	S
DACB	0.66–0.93	0.82 ± 0.06	0.56–0.89	0.76 ± 0.09	0.006	S
DPA	2.98–3.60	3.24 ± 0.18	2.93–3.70	3.31 ± 0.23	0.274	NS
DVP	1.88–2.24	2.08 ± 0.13	1.81–2.33	2.08 ± 0.15	0.859	NS
DFH	0.28–0.43	0.35 ± 0.04	0.23–0.36	0.30 ± 0.03	<0.001	S
DBL	0.22–0.35	0.28 ± 0.03	0.21–0.33	0.26 ± 0.03	0.042	S
AFH	0.25–0.42	0.32 ± 0.04	0.22–0.35	0.29 ± 0.03	0.001	S
ABL	0.24–0.29	0.27 ± 0.01	0.25–0.28	0.26 ± 0.01	0.520	NS
PFH	0.34–0.57	0.49 ± 0.05	0.24–0.38	0.33 ± 0.03	<0.001	S
VFH	0.25–0.40	0.32 ± 0.04	0.18–0.28	0.22 ± 0.03	<0.001	S
CFL	0.43–0.63	0.51 ± 0.05	0.44–0.56	0.50 ± 0.03	0.391	NS
BD	0.43–0.57	0.51 ± 0.03	0.46–0.71	0.54 ± 0.05	0.024	S

TL= total length, FL= fork length, SL= standard length, ED= eye diameter, PrOL= pre-orbital length, PoOL= post-orbital length, HD= head depth, HL= head length, PrDL= pre-dorsal length, PoDL= post-dorsal length, PrVFL= pre-ventral fin length, PrPFL= pre-pectoral fin length, PrAFL= pre-anal fin length, DACE= distance from anal fin to caudal fin end, DACB= distance from anal fin to caudal fin base, DPA= distance between pectoral and anal fin, DVP= distance between ventral and pectoral fin, DFH= dorsal fin height, DBL= dorsal fin base length, AFH= anal fin height, ABL= anal fin base length, PFH= pectoral fin height, VFH= ventral fin height, CFL= caudal fin length, and BD= body depth.

The peritoneal dissections depicted in Figures 4 and 5 offer a detailed examination of the internal reproductive organs of male and female kuhli loach, respectively. The ovarian structure in Cobitidae is relatively simple when compared to other fish species. It is distinguished by a hanging curtain-like arrangement, and after the eggs are released into the body cavity, they are expelled

from the body. This specific type of ovary is known as gymnovarian (Ganguly, 2013). On the other hand, the testes in Cobitidae exhibit an unconfined lobular structure, with spermatogonia potentially distributed along the entire length of the lobules (Uribe *et al.*, 2014).

The histological analysis of male testicles revealed various stages of spermatogenesis,

providing valuable information about the reproductive cycle in males. The presence of spermatogonia, spermatocytes, spermatids, and spermatozoa indicates a well-defined and active reproductive process (Snake *et al.*, 2020). This comprehensive examination enhances our understanding of the reproductive biology of male kuhli loach. In contrast, the histological examination of female ovaries revealed the presence of previtellogenic and late-vitellogenic oocytes. This observation aligns with the typical ovarian development stages in teleost fishes (Tyler *et al.*, 1990), shedding light on the reproductive physiology of female kuhli loach. The identification of oocyte developmental stages is crucial for understanding the reproductive dynamics and timing of spawning events in this species. The presence of distinct stages of spermatogenesis in the testes and the classification of oocytes in the ovaries emphasize the reproductive maturity and potential breeding capabilities of the individuals under study.

The study highlights the presence of sexual dimorphism in pectoral fin rays among kuhli loaches, suggesting potential roles in reproductive behavior. The significant differences in pectoral fin rays (PeFR) between male and female kuhli loach individuals are indicative of a potential sexually dimorphic feature. There are 7–8 weak rays and one hard ray (P.I.7–P.I.8) in the male pectoral fin, while in females consist of 8–10 weak rays and no hard rays (P.8–P.10). Such distinctions in fin ray counts have been reported in various fish species and are often linked to reproductive strategies, locomotion, or environmental adaptations (Endruweit, 2016). In contrast, no significant differences were detected in pelvic fin rays (PFR), dorsal fin rays (DFR), anal fin rays (AFR), and various body stripes between male and female kuhli loaches. This lack of dimorphism in these traits suggests that they may not be strongly influenced by sexual selection or reproductive strategies in this species.

The presence of consistent barbels (BrB) and body stripes number, without significant differences between genders, indicates potential similarities in general body morphology and coloration. These features may serve purposes

unrelated to sexual dimorphism, such as environmental adaptation, predator avoidance, or prey capture (Ajuria Ibarra *et al.*, 2019; Johnston *et al.*, 2017; Fikri *et al.*, 2022; Urban *et al.*, 2022). The analysis of yellow stripes (YS) and brown stripes (BS) number on both sides of the body did not reveal significant differences between males and females. These stripes may be involved in camouflage, communication, or species recognition, and their similarity between genders suggests a shared adaptive function.

The present study delves into the morphometric characters of male and female kuhli loach, shedding light on potential sexual dimorphism within the species. The results reveal intriguing variations in certain anatomical features, stimulating discussions on their ecological and reproductive implications.

The lack of significant differences in total length (TL), fork length (FL), and standard length (SL) between male and female kuhli loaches suggests overall size similarity between the sexes. The discovery is consistent with prior research on various loach species, revealing limited differences in body length between genders, suggesting a likelihood of shared ecological roles and resource utilization (Baras *et al.*, 2012; Endruweit, 2016; Prakoso *et al.*, 2017; Sharma *et al.*, 2014).

Notably, head depth (HD) exhibited a significant difference between the genders, indicating potential divergence in head morphology. Head dimensions are often associated with feeding adaptations and ecological niche partitioning (Kerfoot and Schaefer, 2006; Hidayat *et al.*, 2024). The observed difference in head depth may imply variations in feeding strategies or microhabitat utilization between male and female kuhli loaches. Males may have adaptations for feeding on harder prey or utilizing specific substrates, while females might be better suited for softer prey or different habitats. These variations reduce competition by allowing each sex to exploit different ecological niches, enhancing resource partitioning and coexistence.

Fin dimensions play a crucial role in fish locomotion, stability, and courtship displays. Our

results demonstrate significant differences in dorsal fin height (DFH), dorsal fin base length (DBL), anal fin height (AFH), pectoral fin height (PFH), and ventral fin height (VFH) between male and female individuals. These distinctions in fin morphology suggest potential roles in reproductive behaviors, with adaptations for courtship rituals and displays (Passos *et al.*, 2015).

The distances from the anal fin to the caudal fin end (DACE) and caudal fin base (DACB) exhibited significant differences between males and females. These measurements are indicative of caudal peduncle length and may influence swimming performance and agility. Sexual dimorphism in caudal peduncle dimensions could be linked to differences in swimming behaviors, predator avoidance, or reproductive strategies (Taugbøl *et al.*, 2020; Sabrina *et al.*, 2023).

The observed sexual dimorphism in body depth (BD) within male and female kuhli loaches prompts speculation about potential reproductive functions associated with this morphological difference. Body depth, defined as the vertical distance between the dorsal and ventral surfaces of the fish, plays a crucial role in buoyancy, hydrodynamics, and, in the context of reproductive biology, may indeed be linked to the accommodation of eggs during spawning (Manoharan *et al.*, 2019; Sharma *et al.*, 2014; Tahir *et al.*, 2024).

Understanding sexual dimorphism in the kuhli loach has practical implications for conservation efforts. Knowledge of sex-specific traits and reproductive anatomy can aid in the development of targeted conservation strategies, especially in the face of potential threats to specific populations. Furthermore, this information can guide the management of captive breeding programs, contributing to the long-term sustainability of the species.

CONCLUSION

This study illuminates the sexual dimorphism, reproductive anatomy, and morphometric characteristics of kuhli loach. Morphological distinctions indicate potential

adaptations for reproductive functions, while internal examinations reveal distinct stages of spermatogenesis in males and ovarian development in females. Meristic and morphometric analyses identify significant differences in pectoral fin rays, suggesting potential roles in reproductive behavior, while other traits remain similar between genders. These findings contribute to a comprehensive understanding of kuhli loach ecology and reproductive biology, with implications for conservation strategies and captive breeding programs aimed at ensuring the long-term viability of the species.

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AUTHORS' CONTRIBUTIONS

DSB: Conceptualization, Project administration, Resources, Validation, Writing – original draft. HK: Conceptualization, Formal Analysis, Resources, Software, Visualization, Writing – review and editing. MR, S, TM, ASM: Data curation, Formal Analysis, Resources. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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