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Uncovering the Intraspecific Variation of *Lernaea* Parasites and Their Organ-Specific Distribution in *Tor* Fish (*Tor tambroides*)

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ABSTRACT

This study aimed to examine the species diversity of *Lernaea*-infesting *Tor* fish and determine the infestation patterns based on the organs preferred by the parasites. A total of 740 *Lernaea* specimens were collected and morphologically identified, revealing five species of *Lernaea* infesting the *Tor tambroides* fish, namely *L. cyprinacea*, *L. vastatrix*, *L. oryziphila*, *L. papuensis*, and one species remaining unidentified. *Lernaea cyprinacea* showed as the most dominant species found. Violin plot analyses revealed varying infestation patterns across different fish species and host organs, with the mouth and fins emerging as the most frequently targeted sites. Although the Kruskal-Wallis test did not indicate a statistically significant difference in infestation levels among organs, the observed trends suggest adaptive strategies employed by *Lernaea* and point to their potential ecological implications. Furthermore, the detection of an unidentified *Lernaea* species highlights the need for further taxonomic and molecular investigations to uncover hidden diversity within this parasitic genus.

INTRODUCTION

Tor tambroides, known as Tambra fish, is one of Indonesia's native fish species, also popularly referred to as the Goldfish or Java Salmon, making it a target for many ornamental fish enthusiasts. Additionally, *Tor* species hold significant economic value and nutritional potential due to their richness in protein, essential minerals, and balanced amino acids, making them a valuable food source for diverse populations **(Sharma *et al.*, 2023)**. *Tor tambroides* are increasingly being integrated into aquaculture systems, which is crucial for meeting the growing global demand for fish products **(Indra *et al.*, 2024)**, including in Indonesia, where *Tor* fish farming continues to be developed **(Rumondang *et al.*, 2023; Indra *et al.*, 2024)**.

However, there are several challenges and opportunities associated with *Tor* fish farming, including parasitic outbreaks. It is undeniable that parasites in fish remain a threat to the sustainability of aquaculture operations. Among the various parasitic organisms that infect fish, copepod parasites of the genus *Lernaea* are a significant concern, particularly in freshwater

species. The presence of a hook organ on the anterior of *Lernaea* enables these ectoparasites to severely impact the health of fish populations and contribute to substantial economic losses in fish farming. An infestation of *Lernaea* can be fatal, as evidenced by a reported case of 100% mortality in a single fish heavily infected with more than 30 adult *Lernaea cyprinacea* individuals **(Balagtas *et al.*, 2024)**.

Various reports of *Lernaea* infestations have been documented by the **East Java Provincial Fish and Environmental Health Laboratory (2024)**, where lernaeosis was found in *Tor soro* cultured in the Pasuruan district, East Java province, with 100% prevalence. Another report on *Lernaea* infestation **(Shatrie *et al.*, 2011)** found six species of *Lernaea* infesting Irian arowana (*Scleropages jardinii*) in Merauke, Jakarta, Bogor, and Depok: *Lernaea cyprinacea* found in the eyes, scales, fins, and gill filaments; *L. devastatrix* on the ventral antero; *L. lophiara* on the medial and gills; *L. oryzophila* on the ventral and fins; *L. papuensis* on the fins; and *Lernaea* sp., a suspected new species, on the ventro-posterior and fins.

Despite the presence of *Lernaea* infesting several fish species, their diversity and the ecological factors influencing host preferences remain largely unexplored. *Lernaea* exhibit complex host interactions influenced by both host species and morphological variability. Understanding specific parasite-host interactions is crucial for determining effective parasite control and prevention strategies. Furthermore, the predilection of *Lernaea* species for specific fish hosts and their distribution patterns needs to be further investigated to develop practical solutions for managing their impact on fish populations.

MATERIALS AND METHOD

Study area and fish collection

Tor tambroides weighing between 100–150 grams and measuring 22–30cm in length were collected from aquaculture ponds in Pasuruan, East Java, Indonesia, located at -7.75685196298582° south latitude and 112.93500627578787° east longitude (Fig. 1). This site was selected due to reported cases of *Lernaea* infestation in farmed *Tor tambroides*. Infested fish were individually transferred to clean tanks, anesthetized, oxygenated, and reared for observation. *Lernaea* parasites were found on various body regions, including the lateral, dorsal, anteroventral, gills, mouth, eyes, anal region, dorsoventral, caudal, and pectoral fins. The parasites were carefully removed using tweezers, preserved in 70% ethanol, and documented based on quantity and predilection site. Samples were then sent to the Laboratory of Anatomy, Faculty of Fisheries and Marine, Universitas Airlangga, for further identification.

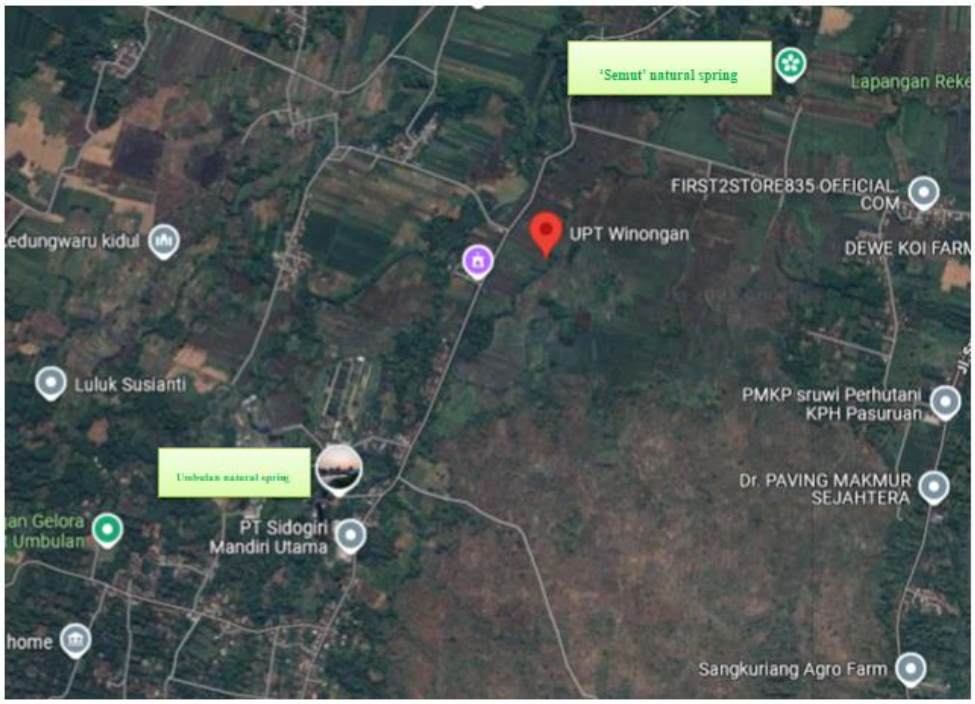

Fig. 1. Sampling site

Lernaea observation

Lernaea specimens were collected from various regions of the fish body, including the body surface, anteroventral region, mouth, eyes, and gills. Morphological examinations were conducted using a compound microscope (OPTIKA B-192, Italy) and a stereo microscope (Nikon SMZ460, Japan). Illustrations of *Lernaea* specimens were produced using a lucida binocular microscope (Nikon E-E-200 with camera set, Japan). Morphometric measurements were performed using Optika ProView software, and species identification was conducted based on the taxonomic keys provided by **Harding (1950)**, **Boxshall (1981)**, **Robinson and Oldewage (1996)**, **Ho and Kim (1997)**, and **Boxshall *et al.* (1997)**.

Statistical analysis

The distribution pattern of each *Lernaea* species across different fish organs was visualized using violin plots generated in R Studio **(R Core Team, 2020)**. An ANOVA test was applied to assess whether there were significant differences in infestation levels among the different organs. The hypotheses tested were:
H_0: There is no significant difference in the mean infestation among fish organs.
H_1: There is a significant difference in the mean infestation among fish organs.

Prior to the ANOVA test, data normality was assessed using the Shapiro-Wilk test. Due to limited data, infestation in the operculum and eyes was excluded from the ANOVA analysis.

RESULTS AND DISCUSSION

We identified five morphological variations of *Lernaea* infesting *Tor tambroides*. These five forms were distributed across several anatomical regions, including the operculum, fins,

mouth, anteroventral area, gills, and eyes (Table 1). A total of 740 *Tor tambroides* individuals were found to be infested, with *Lernaea cyprinacea* being the most frequently encountered species, while *Lernaea oryzophila* was the least prevalent.

Commonly referred to as the "anchor worm," *L. cyprinacea* is a parasitic copepod known to infect a wide range of freshwater fish species globally. Its infestation patterns exhibit considerable variability in terms of host specificity, anatomical attachment sites, prevalence, and infection intensity. This parasite has been documented in several fish families, including cichlids (*Oreochromis mossambicus*, *O. placidus*, *Tilapia rendalli*), cyprinids (*Labeo altivelis*, *Barbus paludinosus*), clariids (*Clarias gariepinus*), centrarchids (*Micropterus salmoides*), and characids (*Hydrocynus vittatus*) **(Dalu *et al.*, 2012; Rasmi *et al.*, 2020; Smit *et al.*, 2023)**.

Infestation typically occurs in the ventral and caudal regions of the host, with the head being a less common site. Frequently targeted anatomical sites include the fins, gills, skin, and ocular region. The morphological variation among *Lernaea* species infesting *Tor tambroides* is notably reflected in the structure of the holdfast organ (Fig. 2). Although the holdfast remains a key diagnostic feature for species identification, molecular analysis conducted by **Hua *et al.* (2019)** revealed that two morphologically distinct *Lernaea* species based on holdfast structure were, in fact, conspecific.

Moreover, the anatomical attachment site on the host can influence holdfast morphology, underscoring its importance in species differentiation **(Robinson & Avenant-Oldewage, 1996; Hue *et al.*, 2019)**.

Table 1. Distribution of *Lernaea* species in the body organs of *Tor tambroides*

Infested organs	Number of Species				
	Lernaea cyprinacea	*Lernaea vastatrix*	*Lernaea oryziphila*	*Lemaea papuensis*	Unidentified
Operculum	76	-	-	-	-
Fins	13	9	9	28	37
Mouth	247	138	-	-	-
Anteroventral	51	13	-	-	39
Gills	5	-	-	47	26
Eyes	2	-	-	-	-
Total Parasites	394	160	9	75	102

Lernaea cyprinacea was the dominant species most frequently observed infecting *Tor tambroides*, with the mouth being the most heavily infested organ (247 parasites), followed by the operculum (76 parasites). This suggests a high level of adaptability and tissue compatibility in L. cyprinacea. In contrast, *L. vastatrix* was recorded on fewer anatomical sites, with a total of 160 individuals, the majority (138 parasites) concentrated in the mouth region, indicating a narrower ecological niche.

L. oryzophila exhibited a highly localized distribution, found exclusively in the fins (9 individuals), while *L. papuensis* demonstrated a clear preference for the gills (47 parasites) and fins (28 parasites), suggesting organ-specific specialization.

Additionally, unidentified *Lernaea* specimens were collected from multiple sites, predominantly in the fins (37 individuals), anteroventral region (39), and gills (26). These findings point to the potential presence of undescribed taxa or significant morphological variation within the genus. The unidentified specimens could not be classified due to ambiguous holdfast morphology, which hindered accurate identification. A similar issue was reported by **Hua *et al.* (2019)**, who encountered difficulty in distinguishing species with indistinct or overlapping holdfast features.

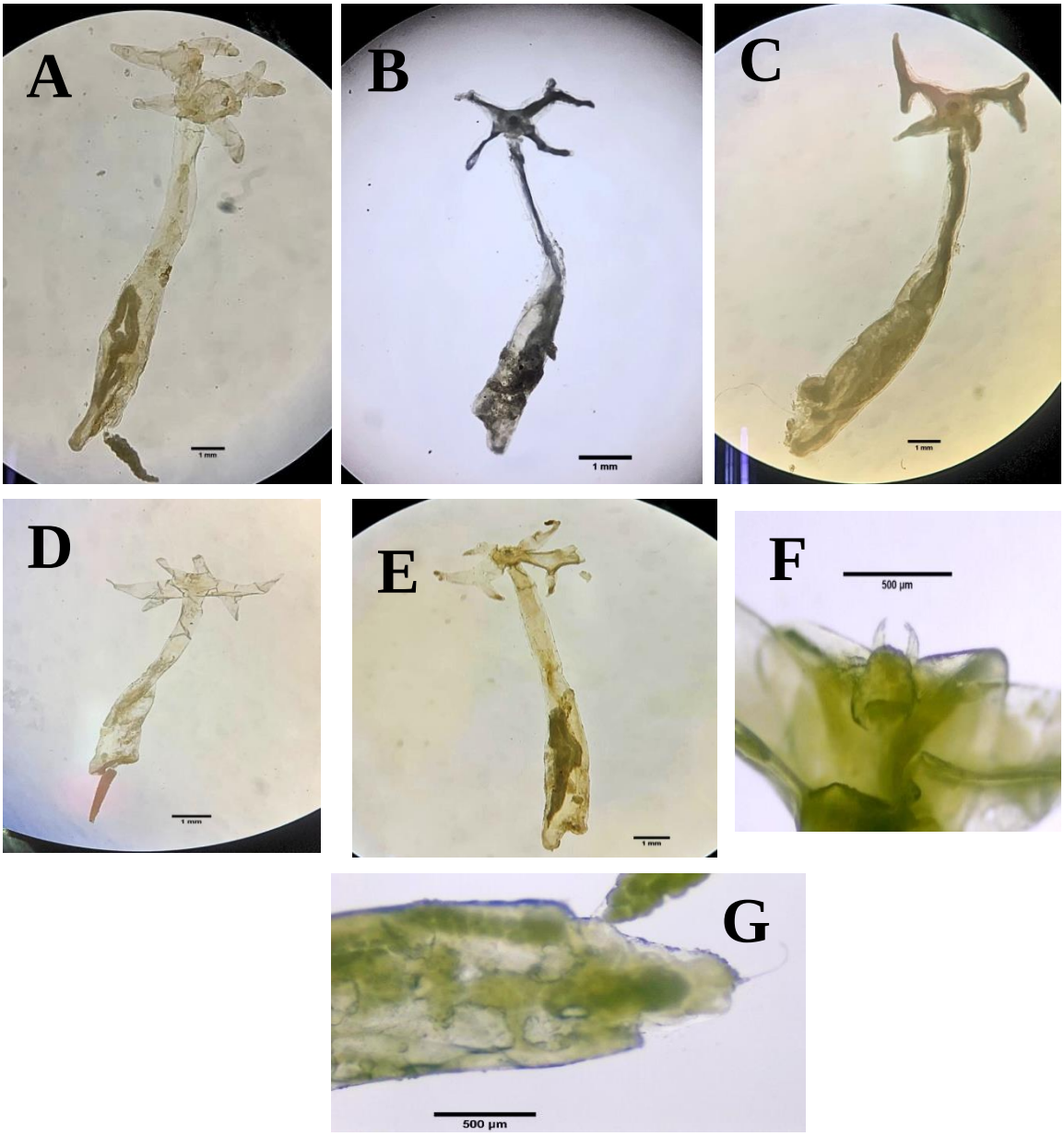

Fig. 2. Morphology of *the Lernaea-Infected Tor tambroides*. A. *Lernaea papuensis*, B: *Lernaea oryzophila*, C: Lernaea *devastratix*, *D: Lernaea cyprinacea*, E*: Lernaea* unidentified, F*:* Anterior part, G: Posterior part *(*Ramus with setae on uropod. A-E: 40x magnification, 1 mm scalebar. F-G: magnification 100x, scalebar 500μm

Lernaea species exhibit a preference for specific attachment sites on their hosts, commonly targeting the fins, gills, and skin. The ventral and caudal regions are particularly favored, whereas the head is generally the least preferred site. *Lernaea cyprinacea*, a copepod parasite of freshwater fish, is known to infest a wide variety of hosts across several families—*Cyprinidae*, *Amiidae*, *Catostomidae*, *Salmonidae*, *Sciaenidae*, and *Umbridae*—as well as tadpoles of *Rana* spp. **(Barros *et al.*, 2021)**.

In the Indonesian context, the presence of *L. cyprinacea* is believed to have been introduced during the 17th–18th centuries through international trade involving Koi fish (*Cyprinus carpio*), a member of the *Cyprinidae* family. It is likely that the ectoparasite was introduced either through infested fish or via parasite eggs attached to the fish body, which subsequently hatched in the local aquatic environment. Given that *Tor soro* (*Cypriniformes: Cyprinidae*) and Koi fish belong to the same family and share similar physiological and habitat characteristics, *L. cyprinacea* has a strong potential to infest *Tor* species as well.

In the current study, *L. cyprinacea* was found to be the dominant species infesting *Tor tambroides*, consistent with findings reported by **Abbas *et al.* (2014)**. Another species, *L. devastatrix*, has previously been reported in Indonesia, infesting Irian Arowana (*Scleropages jardinii*) (*Osteoglossiformes: Osteoglossidae*) in Merauke and several other regions, including Jakarta, Depok, and Bogor **(Shatrie *et al.*, 2011)**. According to **Cepriadi *et al.* (2010)**, the ornamental fish trade has extended the distribution of *S. jardinii* from Papua to Surabaya, East Java. This trade network facilitates the potential spread of *Lernaea* infestations into new geographic regions **(Adriany *et al.*, 2020)**.

The discovery of *L. vastatrix* in *Tor* populations may also be attributed to its adaptability to fast-flowing aquatic environments, making *Tor soro*, a species native to swift waters, a likely host. However, limited recent studies have reported infestations of *L. oryzophila* and *L. papuensis*, indicating either their restricted distribution or underreporting in Indonesian waters.

Distribution pattern of *Lernaea* in *Tor tambroides*

The distribution patterns of *Lernaea* species in cultured *Tor tambroides* populations were visualized using violin plots (Fig. 3). The plots reveal distinct differences in infestation patterns among the *Lernaea* species. *L. cyprinacea* and *L. vastatrix* exhibit wide and asymmetrical distributions, suggesting these species are more evenly dispersed and prevalent in pond-cultured fish. In contrast, species such as *L. papuensis* and unidentified *Lernaea* forms show a narrower distribution range, reflecting lower prevalence and possibly more organ-specific or host-specific infestation behavior.

These differences highlight the variability in infestation dynamics even among *Lernaea* species infecting the same host. Such variation underscores the importance of species-level identification and site-specific monitoring to understand parasite-host interactions and inform effective management strategies in aquaculture systems.

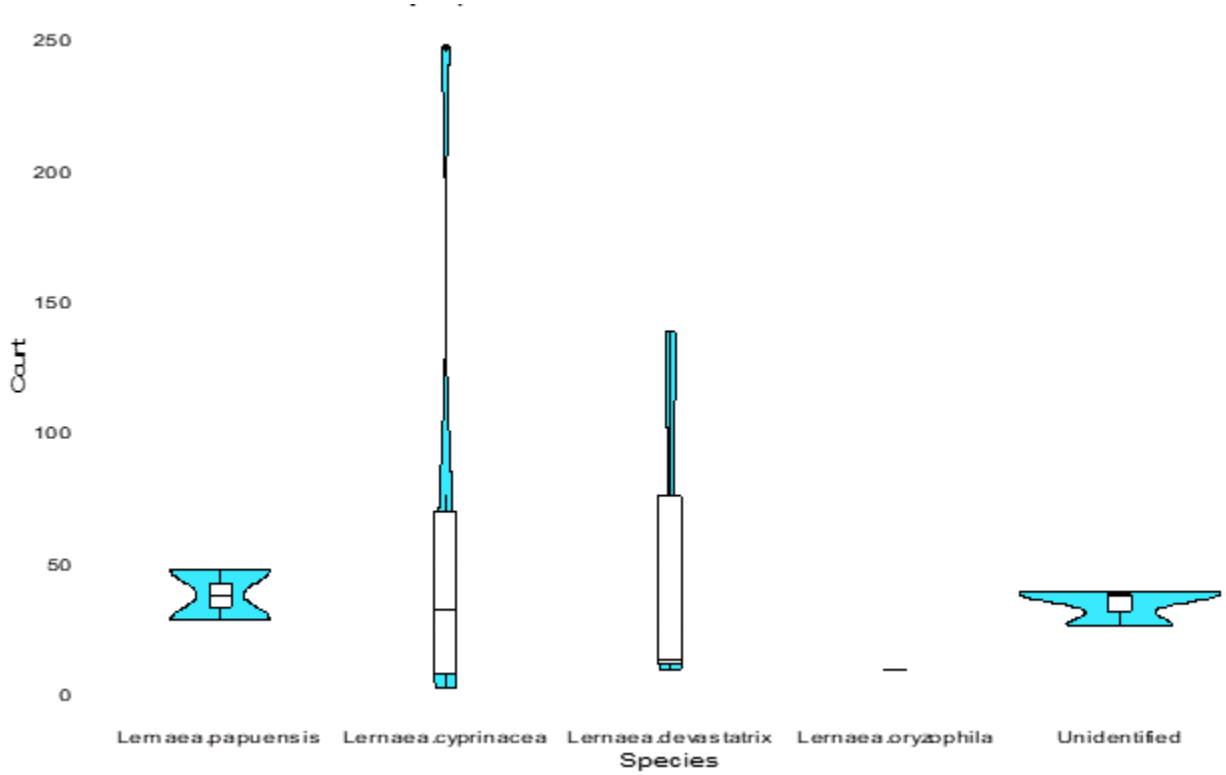

Fig. 3. Distribution pattern of *Lernaea* based on the species distribution in *Tor tambroides*

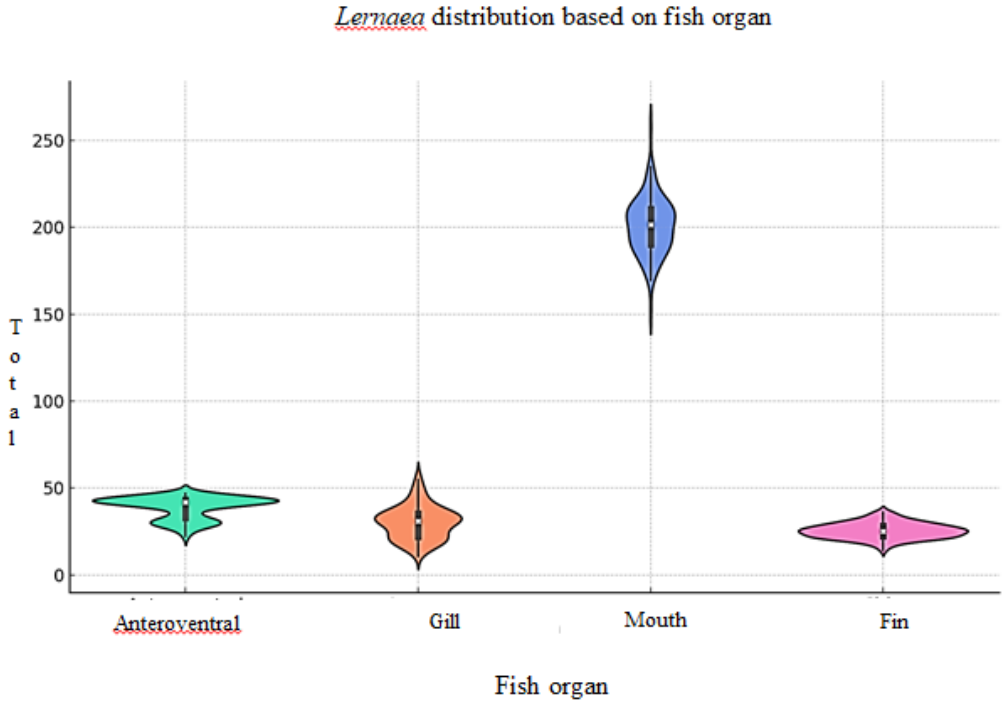

Fig. 4. Distribution of *Lernaea* based on organs in *Tor tambroides*

The violin plot analysis revealed distinct infestation patterns of *Lernaea* across different anatomical sites of *Tor tambroides*. The anteroventral organ exhibited an asymmetrical violin shape with a narrow lower section, indicating that most infestation values clustered within the 40–50 range, with a median of approximately 43. The upper concentration suggests that *Lernaea* infestation is notably dominant in this region.

In contrast, the gill organ displayed a symmetrical violin plot with a wide spread, reflecting high variability in infestation values. The median hovered around 30, while the shape's central widening suggested an even distribution of parasites, without pronounced skew or outliers.

The mouth organ demonstrated a markedly different distribution. Its violin plot appeared narrow at the extremes but highly dense at the center, indicating a sharp peak of infestation values. The median, close to 200, signaled a much higher infestation rate compared to other organs. This dense central concentration confirms the consistent dominance of *Lernaea* in the mouth, identifying it as the most heavily infested organ in this study. While previous research has often reported high infestation rates on fins and body surfaces **(Afriandini & Suwartiningsih, 2021; Nur *et al.*, 2022)**, the present findings highlight the mouth as the primary site of infestation.

The fin organs showed a moderate distribution that closely resembled a normal curve. With a median of around 25 and a symmetrical, stable violin shape, the data suggest balanced infestation levels across this site compared to others. Overall, the violin plot analysis reinforced that *Lernaea* infestations vary significantly across host organs, each showing a distinct distribution profile.

Based on the Kruskal-Wallis test, a chi-square value of 9.5324 with 5 degrees of freedom yielded a *p*-value of 0.08962. As this exceeds the 5% significance level, the result indicates no statistically significant difference in *Lernaea* infestation levels between the various organs.

Among the infected body parts, the fin was the only organ where all *Lernaea* species recorded in this study were found. However, the highest number of individual parasites was documented in the mouth (385 individuals). The gill lamella and anteroventral region each hosted three species—*L. cyprinacea*, *L. devastatrix*, and an unidentified *Lernaea*. The mouth region was infested by *L. cyprinacea* and *L. devastatrix*, whereas the operculum and eyes were each infested exclusively by *L. cyprinacea*.

Though *Lernaea* can attach to nearly all external fish surfaces, they are frequently found in the gills, mouth, and nasal cavities **(Nur *et al.*, 2022)**. The infestation intensity across organs varied: ventral (103 individuals), fins (96), gills (78), operculum (76), and eyes (2). According to **Abbas *et al.* (2014)** and **Muchlisin *et al.* (2014)**, ectoparasites prefer organ sites that offer ease of access, minimal hydrodynamic disturbance (which may dislodge parasites), and sufficient space and nutrients for growth and reproduction.

The results confirm that the mouth and fins are the most frequently infested organs, likely due to their accessibility and potentially thinner epithelial layers, providing optimal conditions for parasite attachment. These findings underscore the diverse infestation strategies and organ preferences exhibited by different *Lernaea* species, with important implications for targeted parasite management in aquaculture settings. Furthermore, the presence of unidentified *Lernaea* species in multiple anatomical sites suggests the possibility of undescribed or misidentified taxa, reinforcing the need for continued morphological and molecular investigation.

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CONCLUSION

These findings highlight the diversity of *Lernaea* species parasitizing *Tor tambroides*, with *Lernaea cyprinacea* identified as the predominant species infecting this host. The study also underscores the need for a more targeted approach to managing *Lernaea* infestations in aquaculture, particularly through enhanced surveillance of high-risk organs and the identification of potentially new or cryptic species. A deeper understanding of organ-specific parasitism and species-level variation is essential for advancing fish health management strategies and ensuring the long-term sustainability of *Tor* fish farming systems.

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