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Microecology and Virulence of Some Pathogens in Gill Biotope of the Catfish Host, *Clarias gariepinus* (Burchell, 1822): Does Organism Body Size Matter?

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ABSTRACT

This study investigated the diverse metazoan pathogens inhabiting the gill biotope of the African sharptooth catfish, *Clarias gariepinus*. The pathogens identified include the viviparous gyrodactylid monogeneans *Macrogyrodactylus clarii* and *Gyrodactylus rysavyi*, the copepod *Ergasillus sieboldi*, and oviparous monogeneans from the genus *Quadriacanthus*. Among these, *E. sieboldi* was the largest, measuring 2500µm (2200-2700) in length, followed by *M. clarii* at 1950µm (1900-2000) and *G. rysavyi* at 1500µm (1350-1600). In contrast, *Quadriacanthus* was the smallest, measuring 500µm (430-560), while encysted metacercaria were 200µm (180-220) long. Notable variability in spatial distribution and population sizes of these pathogens suggests that factors beyond space and food supply may influence their occurrence. The study revealed significant species-specific differences in morphological features, feeding behaviors, attachment organ design, and reproductive strategies. Monogeneans primarily feed on gill epithelium, have simple life cycles, and utilize haptoral attachments, while *E. sieboldi* consumes blood from gill capillaries, has a complex twelve-stage life cycle, and employs specialized segmented antennae. Spatially, *Quadriacanthus* prefers the distal areas, while *E. sieboldi* and *M. clarii* inhabit proximal regions, with *Gyrodactylus* favoring the borders of the gill filaments. Two primary factors influencing this distribution are the specific demands of each parasite species and their interactions. Additionally, unlike *Quadriacanthus* and *Macrogyrodactylus*, *Gyrodactylus* and *E. sieboldi* exhibit skillful swimming behaviors, though all monogeneans exhibit leech-like movement. Lastly, the impact of encapsulated digenean metacercarial cysts on the gill's resident organisms remains to be explored.

INTRODUCTION

Typically, fish gills are composed of arches, filaments, lamellae and rakers. The gill is one of the most vital fish organs due to its physiological role (ammonia nitrogen excretion, filter-feeding, respiration and osmoregulation). The rakers offer the fish protection against debris. Gill filaments are feathery arrangements that are held by the curved gill arch. Gill lamellae increase surface respiratory area to optimize gas exchange process between water and blood. The gill lamellae extend from the gill filaments. Gas exchange primarily occurs at the gill lamellae, where the respiratory area is particularly only one cell layer thick **(Wilson & Laurent, 2002)**. The gill acts as a barrier (microbial, chemical, physical, and immunological). In addition to the above mentioned advantages, the gill apparatus of the catfish, *Clarias gariepinus* is supplemented with an accessory breathing tool, namely respiratory tree or dendritic organ **(Karlina & Luthfi, 2018, Mashaly *et al.*, 2023)**. Fish gills preferred habitat are monogeneans, digeneans, crustaceans, etc.

Many problems would be faced by the gill residents in fish; these gill parasites frequently possess unique modifications that enable them to adhere to the gill tissues and to consume the biological fluids or nutrients derived from the fish. Gill parasites can have specific attachment features like hooks, suckers, or sticky glands as part of their adaptations. Additionally, they may have undergone changes to evade host immunity. Gill dwellers of *Clarias gariepinus* should experience two varying environments: microenvironment (i.e. host-mediated immune responses) and macroenvironment (i.e. abiotic and biotic elements of host-parasite system) **(EL Sayed *et al*., 2023)**.

The present study aimed to throw light on some ecological and behavioral adaptations (maneuvers) of the common gill parasites on *Clarias gariepinus*, namely the viviparous gyrodactylid monogeneans *Macrogyrodactylus clarii* Gussev, 1961 and *Gyrodactylus rysavyi* Ergens 1973, the copepod crustacean *Ergasillus sieboldi* von Nordmann, 1832, and the oviparous monogenean species of the genus *Quadriacanthus* Paperna, 1961 (Dactylogyridae). A great deal of attention was paid to the microhabitat deterioration of each parasites and contribution of the body size and viability of parasites to their population size with potential histopathological changes on gills induced on by these parasites' movement.

MATERIALS AND METHODS

A total of 552 specimens of the African sharptooth catfish, *Clarias gariepinus* Burchell, 1822 were caught from the Damietta Branch of the River Nile. Agricultural Drain and a minor freshwater stream were surveyed for gill parasites between September 2021 and August 2022. Gills were dissected and individual gill arches were examined for metazoan parasites with the aid of a high-power stereomicroscope. Observations were made of the microhabitat preference for each parasite species. A fraction of formalin-fixed gills were processed for histopathological investigation according to standard methods for hematoxylin and eosin staining. Under stereomicroscope, monogenean worms were dislodged from the gill filaments and gill lamellae and differentiated owing to comparison of the body measurements, construction of the copulatory structures, dimensions of the hamuli, hooks, and connective bars of the haptor. To dislodge monogeneans off their attachment loci on the gills, a sharp needle was introduced gently underneath the clinging haptor, and then the parasite was pushed gently and transported to the dish. The ecoparasitological indices prevalence, mean intensity and abundance of the helminth parasites were calculated according to **Bush *et al*. (1997)**. Prevalence is the number of host individuals infected/infested by one or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species; abundance is the total number of individuals of a particular parasite species divided by the total number of hosts of that species examined (infected and non-infected); and mean intensity is the total number of worms of a particular parasites species divided by the number of hosts infected with that parasite. Movement patterns of each parasite species were clarified under stereomicroscope along with observations made by handlens magnifying device.

RESULTS

Metazoan parasite species inhabiting the gill biotope of the African sharptooth catfish, *Clarias gariepinus* Burchell, 1822 include the viviparous gyrodactylid monogeneans *Macrogyrodactylus clarii* Gussev, 1961 and *Gyrodactylus rysavyi* Ergens 1973, the copepod crustacean *Ergasillus sieboldi* von Nordmann, 1832, and the oviparous monogeneans of the genus *Quadriacanthus* Paperna, 1961 (Dactylogyridae). *Ergasillus sieboldi* recorded the largest body size (Length: 2500µm, Breadth: 400µm), followed by *Macrogyrodactylus clarii* (Length: 1950µm, Breadth: 300µm) and *Gyrodactylus rysavyi*, while *Quadriacanthus* recorded the smallest body size (Length: 500µm, Breadth: 120µm). Moreover, the encysted digenean metacercaria impregnated into the cartilage supporting the gill filaments measured (Length: 200µm, Breadth: 50µm). There was a marked variability in the microhabitat preferences and abundance of the studied parasite species.

The infestation parameters (abundance, prevalence and mean intensity) of *Ergasillus* were 0.03, 2 and 1.33%; *Macrogyrodactylus*: 0.44, 15.88 and 2.03%; *Gyrodactylus*: 0.74, 15.49% and 2.70; *Quadriacanthus*: 1.73, 38.94 and 2.99%; metacercariae: 2.20, 34.75 and 6.54%, respectively.

Ergasillus sieboldi sucks blood from capillaries in the underlying gill tissues and causes robust tissue fragmentation and rupture of the blood capillaries and high compression of underlying gill tissues (Figs. 1-4). The monogenean species feed upon the gill epithelium, and rarely *Macrogyrodactylus* worms feed upon epithelial cells and scanty amount of blood corpuscles and mucous cells blended in the epithelial tissue (Figs. 2-9), *Quadriacanthus* feeds upon epithelial cells (Fig. 10) and is implanted deeply into underlying epithelium. Digenean cyst relies on deep insertion (encapsulation) inside the gill tissues, particularly the cartilage supporting delicate filaments.

Fig. (11) shows a non-pregnant, well-fed, *Macrogyrodactylus* worm with stripped or banded intestinal limbs (red double arrowhead). A cluster of embryonic cells could be recognized inside the uterus (green asterisk). Fig. (12) illustrates a pregnant, malnourished, *Macrogyrodactylus* worm with almost empty intestinal limbs (red double arrowhead) which are lifted to the lateral border of the body proper of parent worm.

Three *Macrogyrodactylus* generations could be recognized one inside the other (H1, H2 and H3). Observations on alive members of all studied monogenean microorganisms indicated that they feed regularly upon epithelial gill tissues. However, the amount of food engulfed by individual *Macrogyrodactylus* worms was much more than those consumed by *Gyrodactylus* and *Quadriacanthus* ones.

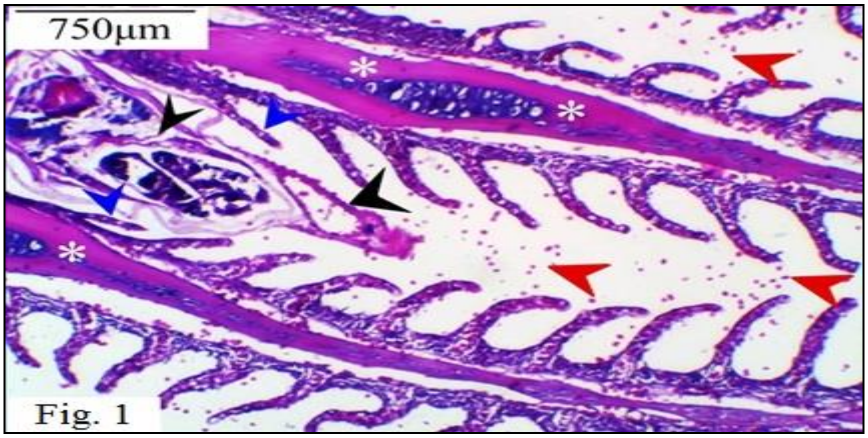

Fig. 1. An individual of *Ergasillus* (black arrowhead) located between two adjacent gill filaments of *C. gariepinus* with an ample amount of blood corpuscles (red arrowhead) leaking from a blood vessel ruptured by the sharp tearing tool (mouth parts) or punctured by the piercing, needle-like tool (second antennae). Note the pressure exerted on the gill lamellae (blue arrowhead) in the vicinity of the parasite. Cartilage supporting gill filament (asterisk) seems normal

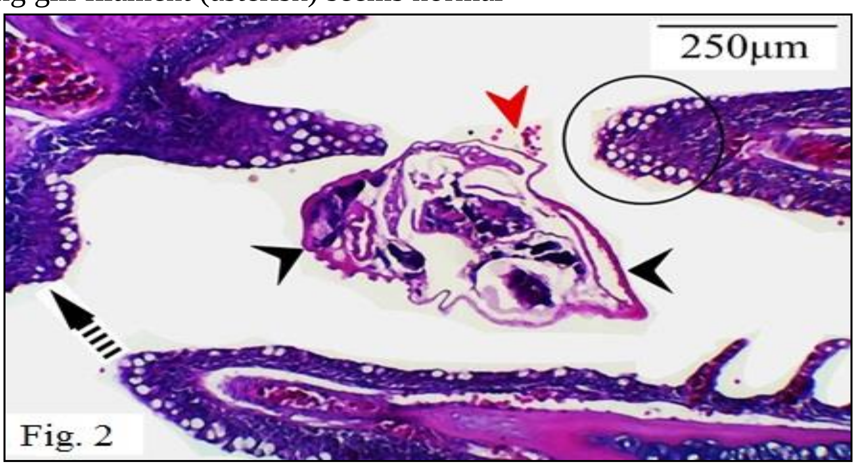

Fig. 2. Adhesive attitude and histopathological features generated by *Ergasillus*. Note a fragment of blood corpuscles (red arrowhead) migrating towards the anterior head region of *Ergasillus* (black arrowhead). Note also the marked compression of the gill tissues facing the head region and that in the adjacent filament (black intermittent arrow) of the parasite. Atrophy of epithelial layers of the filament and distortion of the form of the filament. Excessive mucous cells develop at the interface between the host tissue and resident parasite (black-outlined circle)

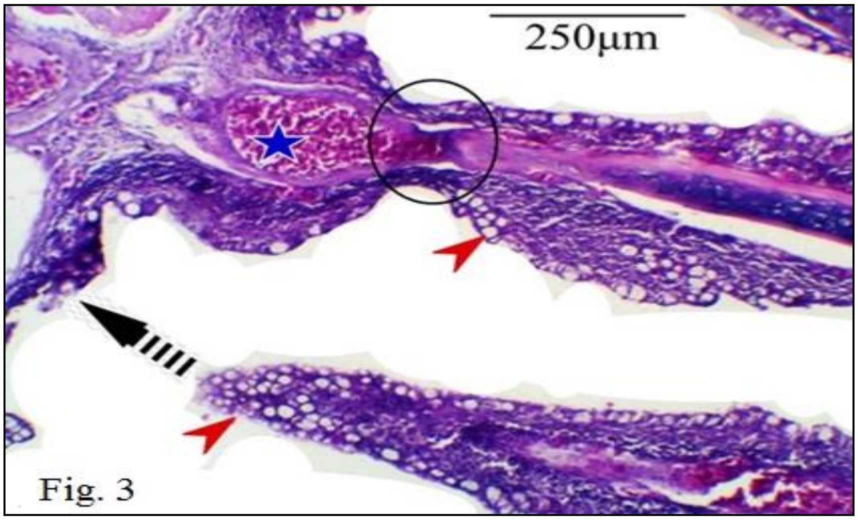

Fig. 3. Gill tissues facing head region of *Ergasillus*, Deep robust compression of the filament epithelium, cessation of blood of the branchial vessel, atrophy of epithelial layers of the filament and distortion of the form of the filament. On the other hand, regarding the adjacent gill filament, the gill tissues facing the sclerite(s) of the second antenna, the compressed tissues are lowered below this scope. Proliferation of mucous cells could be recognized adjacent to this compressed epithelium

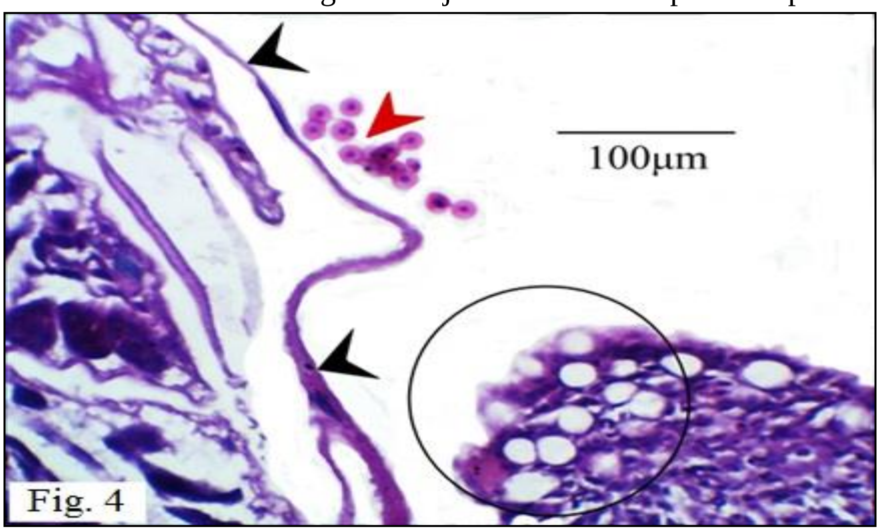

Fig. 4. Histological features of the gill filament tissues facing the anterior head region of *Ergasillus* (P). Note that blood corpuscles (red arrowhead) are the primary food item of this copepod. Note also the desquamation of the affected epithelial layers of the filament and distortion of the form of the filament (black outlined circle)

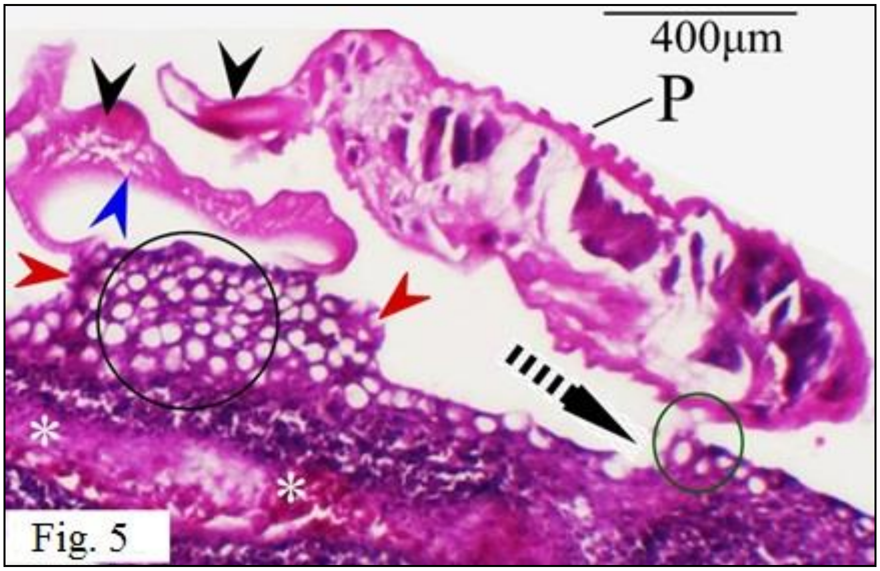

Fig. 5. *Macrogyrodactylus* worm (P) exerting a haptoral (blue arrowhead) suction force over a patch of the gill epithelium that proliferated intensively into mucous cells (black-outlined circle). Note that the haptor (black arrowhead) has no role in attachment. A feeding pit (intermittent black arrow) and inflammation (red arrowhead) exist under the anterior region of body proper of the parasite. Mucous cells are engulfed (green-outlined circle) along with epithelial cells. The blood flow in interrupted (asterisk)

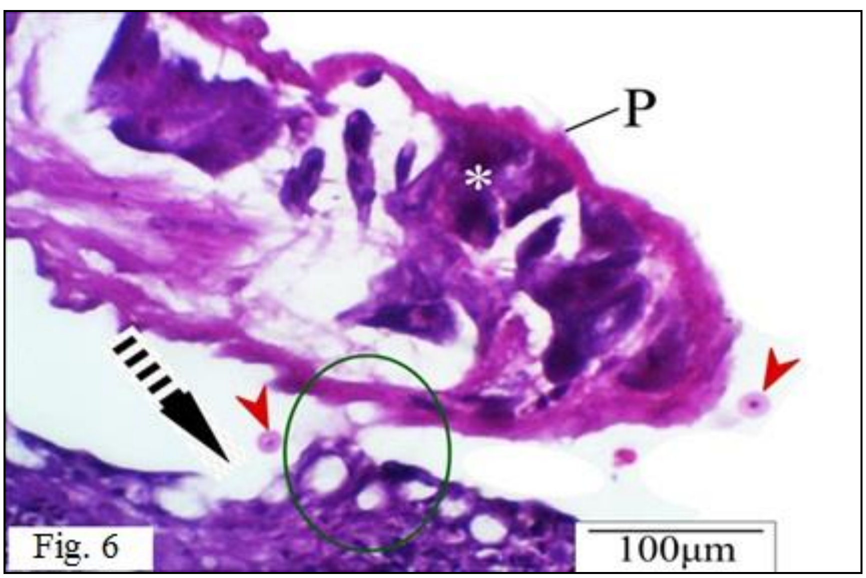

Fig. 6. Close view of the attachment site of *M. clarii*. Asterisk, pharyngeal glands of the parasite; black arrowhead, tegumental folds; blue arrowhead, part of the wall of the haptor; red arrowhead, a red blood cell moving into the mouth of the parasite; yellow-outlined circle, mucous cells underneath the haptor

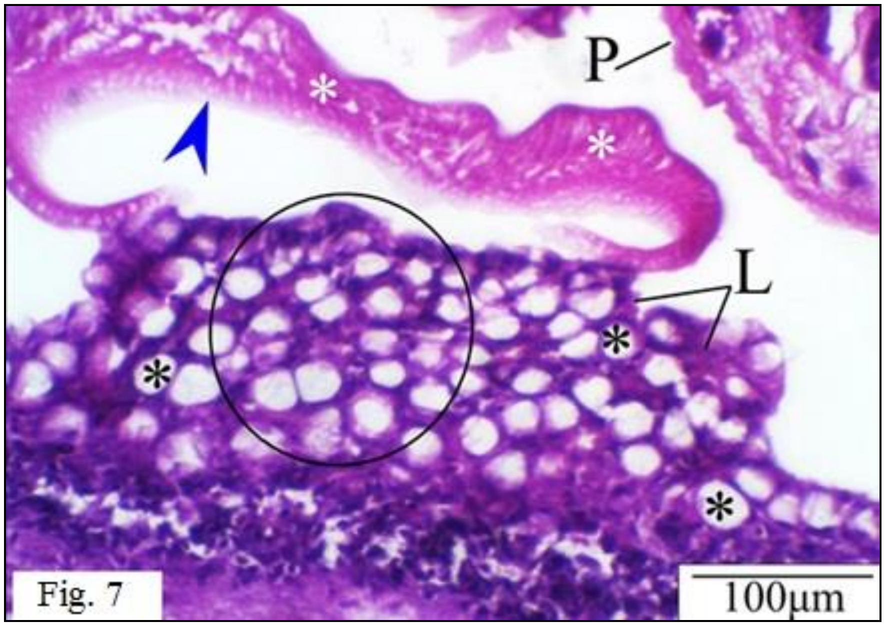

Fig. 7. Close view of the haptoral (blue arrowhead) attachment site of *M. clarii* (P). Black asterisk, mucous cells; white asterisk, haptoral muscles; L, lymphocyte. Black-outlined circle, intensive mucous biomass

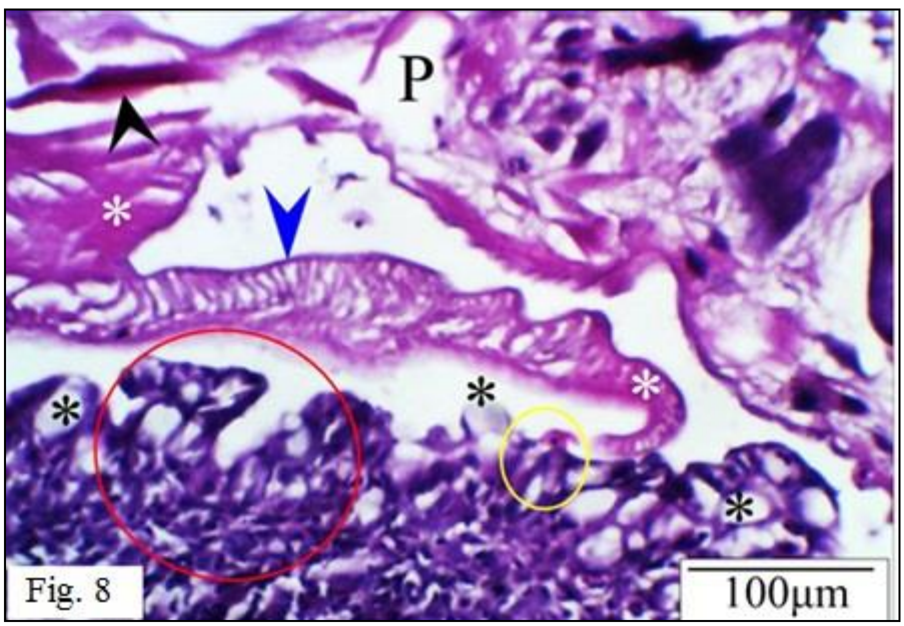

Fig. 8. Close view of the attachment site of *M. clarii*. Asterisk, pharyngeal glands of the parasite; black arrowhead, hamulus; blue arrowhead, part of the wall of the haptor; red-outlined circle, degenerated gill epithelium; yellow-outlined circle, marginal hooklet

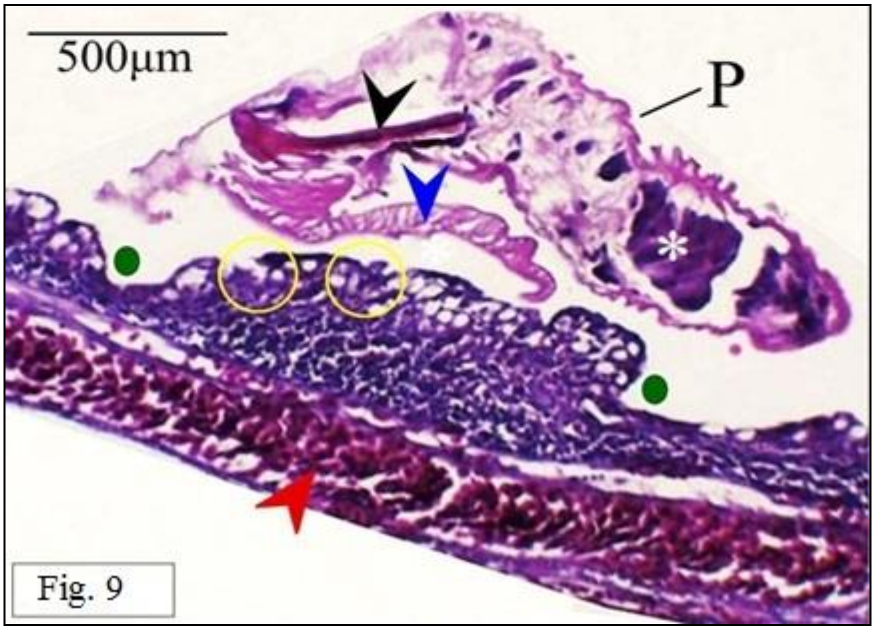

Fig. 9. *Macrogyrodactylus* worm (P) exerting a haptoral (blue arrowhead) suction over gill epithelium that underwent degeneration (yellow-outlined circle). Note that haptor (black arrowhead) is located away from attachment spot. Two invaginations could be noticed at the border of the sucked epithelial patch (green solid circle). Blood flow is normal (red arrowhead)

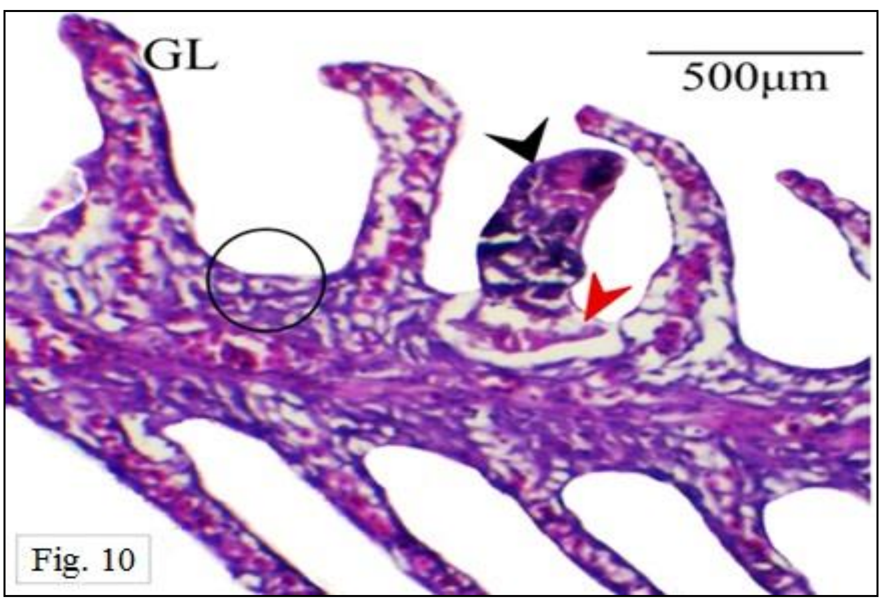

Fig. 10. *Quadriacanthus* (black arrowhead) firmly implanted into gill epithelium utilizing haptoral elements (red arrowhead). Compare this feature with normal epithelium (black-outlined circle)

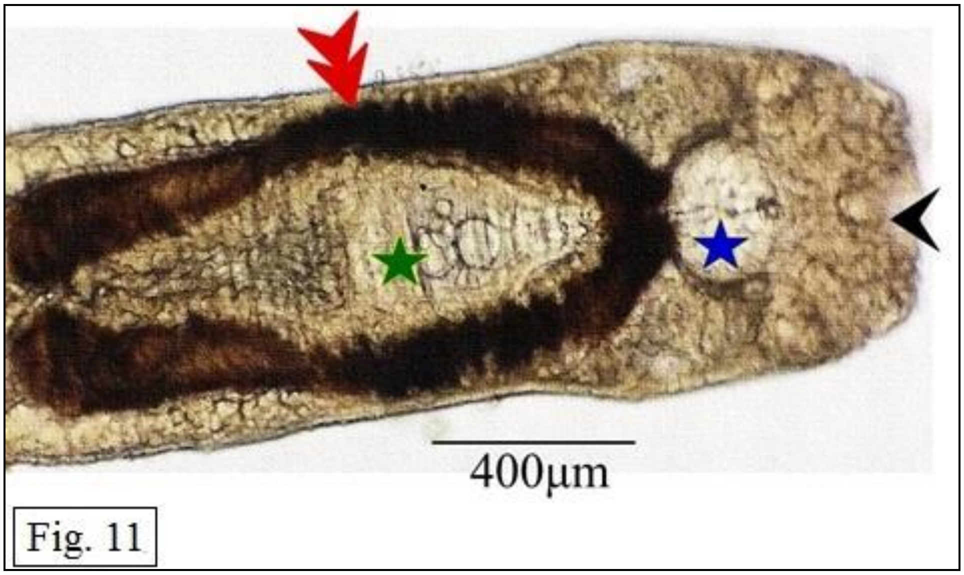

Fig. 11. Non-pregnant, well-fed, *Macrogyrodactylus* worm with stripped or banded intestinal limbs (red double arrowhead) and incubate a cluster of embryonic cells (green asterisk). Blue Asterisk, Pharynx; Black arrowhead, anterior head region

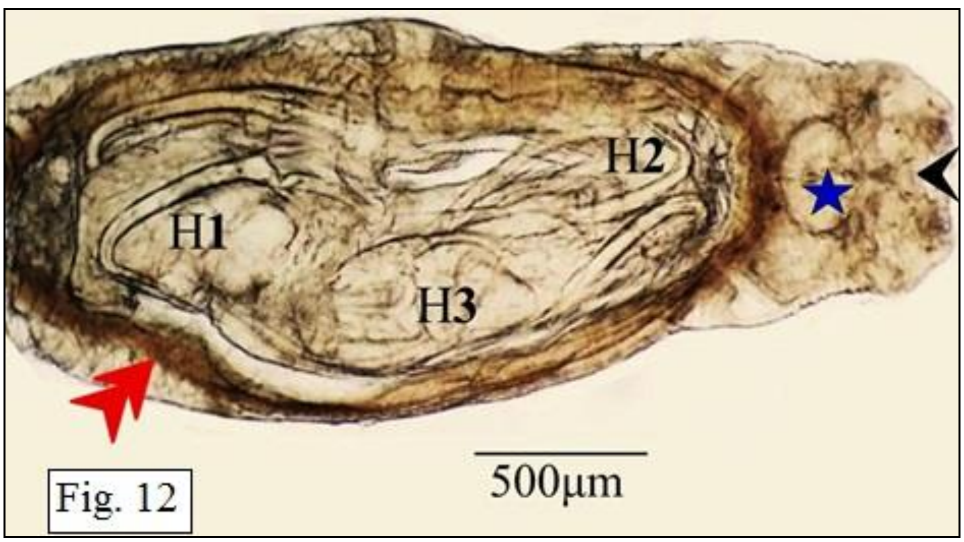

Fig. 12. Pregnant, malnourished, *Macrogyrodactylus* worm with almost empty intestinal limbs (red double arrowhead) and shifted to the lateral margin of the body proper of parent worm. Blue Asterisk, Pharynx; Black arrowhead, anterior head region; H1, first generation; H2, second generation; H3, third generation

Concerning spatial distribution of these parasites on the gill biotope, frequent observations on immediately fixed host individuals in 10% formaldehyde revealed that *Quadriacanthus* worms occupied the distal area, while *Ergasilus sieboldi* preferred proximal area of the gill filaments. *Macrogyrodactylus clarii* preferred proximal area of the gill filaments, with dominant existence across interhemibranchial septum. *Gyrodactylus* species favors the afferent and efferent margins of the gill filaments.

Unlike *Quadriacanthus* and *Macrogyrodactylus*, *Gyrodactylus* and *Ergasillus* are skillful swimmers. Leech-like movement is a common behavior in all investigated monogeneans. *Gyrodactylus* was observed to propel its body across the water column by displaying momentary successive looping and unlooping movements in coordination with upside down leech-like movements when it reaches the superficial layer of water. Gyrodactylid monogeneans exhibited frequent translocation over microsettlement sites, while *Ergasillus* was rarely, but wisely translocated.

DISCUSSION

Gyrodactylid monogeneans such as *Gyrodactylus* and *Macrogyrodacntylus* are skin and gill browsing parasites appearing initially to navigate and terminally to land on a definite microhabitat which provides in need of nourishment and safe haven **(El-Naggar *et al*., 2001; Robio–Godoy *et al*., 2010)**. Gyrodactylids exhibit diverse behavioral traits appropriate to the behavioral and ecological characteristics of the fish host **(Bakke *et al*., 2007; Olstad *et al*., 2007)**; these behavioral traits comprise the feeding habits, hydrodynamic forces generated by gill ventilation, acrobat near–surface displays, ability to pervade terrestrial habitat for a brief time and distribution and orientation across the water column **(El–Naggar, 2012)**.

The Nile catfish *Clarias gariepinus* remains suspended in the muddy substrate and can walk out of the water at night in search of land–based food items to satisfy their hunger or swim across noticeably shallow stream segments **(Bishai & Khalil, 1997; Dadebo, 2000; Booth *et al*., 2019)**. This behavioral aspect may interfere with the existence of these tiny microorganisms in two ways: it probably undermine the feeding activity, communication regime, transmission attempts and proliferation of monogenean populations. **El–Naggar (2012)** suggested that embedding of the catfish host in the mud at the riverbed probably facilitates the dislodgement of the fragile-fixed microorganisms exposed to the volatile circumstances such as the friction posed on the microhabitats (clinging sites) and macrohabitats (fish and water) they experience. **Mashaly *et al.* (2019)** reported that habitat degradation may have an impact on monogeneans' microhabitat specialization and site selection. Furthermore, the existence of the catfish away from the water body likely brought the skin and its biota at the mercy of drought conditions, particularly in the superficial layers of the skin epidermis upon which monogenean worms graze.

Oviparous monogeneans belonging to genus *Quadriacanthus* attained the smallest body dimensions, while the copepod crustacean *Ergasillus sieboldi* recorded the largest body size, followed by *M. clarii* and *Gyrodactylus rasavyi*. According to **Bromagen**

(2022), body size reveals features of the biography, ecology, and evolution of the living organism. This plays a critical role in success or failure during biological interactions (e.g. competition, reproduction, microhabitat selection, etc.). Usually, there is a negative association between body size and population density in nature **(Bromagen, 2022)**. This assumption is in line with our data; the abundance of adult (feeding and reproducing) stage of the oviparous *Quadriacanthus* species was higher than those of the cohabitants (*Macrogyrodactylus*, *Gyrodactylus* and *Ergasillus*). On the other hand, the abundance of encysted metacercaria topped other gill residents. Yet the gill biotope is regarded as a food- and space-rich resource, other drivers may play a pivotal role in the survival of these tiny parasites.

Abundance and body size typically have a negative correlation across all species. Definite resource constraints among parasite species are probably not as severe as those faced by free-living organisms. The body size of the parasite linked negatively with infection intensity and positively with prevalence among fish helminth endoparasites **(Poulin, 1999)**. Conversely, there was a positive correlation found between the body size of copepod ectoparasites and their frequency and intensity in fish. The reason behind these divergent patterns could be the stronger correlation between endoparasite body size and intensity-dependent regulation in comparison to ectoparasites **(Poulin, 1999)**.

Harrison’s Rule and Poulin’s Increasing Variance Hypothesis are the two main ideas explaining the relationship between the size of the parasite and that of its host. Harrison's Rule states that the size of a parasite's body and its host’s body are positively correlated. This was first predicated on Launcelot Harrison’s findings that there is a positive correlation between the body sizes of bird lice and those of their hosts **(Leung, 2022)**. Since then, it has been discovered that Harrison's Rule also applies to a large variety of other parasites, with some noteworthy exceptions reported in specific types of bird lice, parasitic copepods of fish, and monogeneans. It appears that the causal processes underlying this trend vary depending on the parasite-host system **(Leung, 2022)**.

There were parasite species-specific variances in the morphology, mode of feeding, buildup of attachment tools and reproductive potential. For example, *E. sieboldi* sucks blood sips from accessible capillaries, has more complex life cycle involving 12 phases and utilizes segmented, claw II antennae. Monogeneans grazes on the gill epithelium, have direct and simple life cycle and attach to underlying epithelium by haptor, while digenean cysts rely on deep insertion (encapsulation) into the gill tissues, particularly the cartilage supporting the delicate filaments. **Baillie *et al*. (2019)** noted distinct morphological changes between anterior and posterior dactyli in different species sharing the same attachment mechanism, as well as between outwardly and internally attaching cymothoids. For anterior dactyli, allometric effects are strong, but not for posterior dactyli. Species that attach to the mouth have more variation in their shapes than species that attach to the gills. There is no proof that the shape of the dactylus and the parasite mode are associated in a way that is distinctive to a particular clade. These authors concluded that attachment morphology seems to be mostly driven by parasite mode. This probably reflects a number of aspects of the ecology of parasites, such as eating and the requirements for attachment in various microhabitats. Though the gill biotope provides an ample amount of food (blood, epithelium, mucus) and an infinite nesting places that is assumed to create an appropriate circumstances for survival and prosperity of the gill inhabitants, there was a marked variability in the spatial distribution and population size of these cohabitants. This indicates that other factors, rather than space and food supply, may play a pivotal role in parasite occurrence.

With respect to microhabitat selection, *Quadriacanthus* worms occupied the distal area, while *E. sieboldi* preferred proximal area of gill filaments. *M. clarii* preferred proximal area of gill filaments, with dominant existence across the interhemibranchial septum in the present study. Unlike *Quadriacanthus* and *Macrogyrodactylus*, *Gyrodactylus* and *Ergasillus* are skillful swimmers **(El-Naggar *et al*., 2001, 2004)**. Leech-like movement is a common behavior in all investigated monogeneans. Gyrodactylid monogeneans exhibited frequent translocation over microsettlement sites, while *Ergasillius* was rarely translocated. Frequent migration over the microhabitat patches likely expose movable organisms to many threats, for example sweeping by the hydrodynamic forces, predation by aquatic cleaners, displacement by antagonistic interactions. **Rohde (2013)** suggested two types of parasite communities, namely species-rich, interactive and species-poor, non-interactive communities. A non-interactive community has an unsaturated niche space and parasitic species that do not interact, whereas an interactive community is shaped by interspecific interactions and a saturated niche space.

Gill metazoan parasite community of *C. gariepinus* comprises moderate number of species (three of the genus *Quadriacanthus*, one of the genus *Gyrodactylus*, one of the genus *Macrogyrodactylus*, one of the genus *Ergasillus*, in addition to metacercariae encapsulated and confined to a specific loci inside the cartilage). Generally, all parasite species scored low abundance levels, except for metacercarial cysts that intensively invaded and impregnated into gills. It is assumed that this community is structured by intraspecific interactions. The ability of encapsulated metacercarial cysts to disturb existence of gill inhabitants remains questionable.

Ergasillus sieboldi induces strong tissue fragmentation, rupture of the blood capillaries, and significant compression of the underlying gill tissues by sucking blood from capillaries in the gill tissues beneath. The gill epithelium is the source of food for monogenean species, while *Macrogyrodactylus* worms mostly feed on epithelial cells and a small number of blood corpuscles and mucous cells mixed into the epithelial tissue. *Quadriacanthus* worms also feed on epithelial cells and are deeply implanted into the underlying epithelium. Digenean cysts are reliant on encapsulation (deep insertion) within the gill tissues, namely in the cartilage that supports the fragile filaments.

The pathological effects of ectoparasites were described by **Vankara *et al*. (2022)** as follows: fusion of secondary lamella; hyperplasia of the gill filaments and the epithelial cells; propagation of bronchial tips; thinning of the central axis; deshaping, shortening, and fusion of secondary gill lamellae epithelium; severe degenerative and necrotic changes in gill filaments and secondary lamellae; curling of secondary lamellae and proliferation of mucous cells. *Wallago attu* gills suffer significant damage from ectoparasites, which reduces the fish's ability to breathe through its gills. Early pathology investigations can determine the degree of parasite damage, allowing for the implementation of different diagnostic programs and best management techniques in aquaculture to increase productivity.

Mohammadi *et al*. (2012) obtained similar pathological results while studying parasitic infestation of skin and gill on Oscar (*Astronotus ocellatus*) and discus (*Symphysodon discus*), and **Dias *et al*. (2021)** discovered similar results when studying *Colossoma macropomum* (Serrasalmidae). The amount of parasites on the gills directly correlates with the severity of respiratory injury. Significant alterations in osmoregulation or respiratory failure, a reduction in body weight and condition factor, and eventual mortality are all possible outcomes of more severe gill damage. Cell injury and hemorrhages at the site of adhesion were caused by ectoparasites such monogeneans and copepods, which attach to the principal lamellae with their legs and anchors. A high number of cell nuclei within the gut of gill parasites is evident, where the parasite feeds on the oozed blood that has leaked from the hemorrhagic site. Other parasites secrete exogenous enzymes to break down the host fish's gill filaments **(Noga, 2010; Padua *et al*., 2015)**. Although the hooks of monogenean parasites and copepods mechanically harm gill epithelial cells, scraping and sucking activities generated by the parasites created the most histopathological damage **(Derwa, 1995; Endrawes, 2001; Hanna, 2001)**.

CONCLUSION

In summary, this research sheds light on the intricate community of metazoan pathogens residing in the gill biotope of the African Sharptooth catfish, *Clarias gariepinus*. The study identifies several key pathogens, including the viviparous monogeneans *Macrogyrodactylus clarii* and *Gyrodactylus rysavyi,* the copepod *Ergasillus sieboldi*, and the oviparous monogeneans of the genus *Quadriacanthus*. Each of these organisms exhibits unique morphological traits and feeding behaviors that reflect their adaptations to the gill environment.

Our data show that these parasites vary significantly in body size and spatial distribution, indicating that factors other than resource availability may influence their existence and abundance. The distinct preferences for specific gill regions highlight the ecological dynamics at play, with *Quadriacanthus* favoring distal areas, while *E. sieboldi* and *M. clarii* are more commonly found in proximal regions.

Furthermore, the study underscores the necessity of understanding how these parasites interact with their host, particularly in terms of potential histopathological effects on gill tissues. The potential of these infections to cause tissue damage can have major consequences for the host fish's health, reducing respiratory efficiency and overall well-being.

Finally, this study not only improves our understanding of the biodiversity inside *C. gariepinus's* gill biotope, but it also emphasizes the importance of future research into the ecological roles of these infections and their consequences for fish health and aquatic ecosystems.

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