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Technical Note

Metazoan Parasites of Mozambique tilapia (*Oreochromis mossambicus*) Native to Lake Urema, Mozambique

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Abstract: Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), an important aquaculture species investigated herein, is at risk and listed as vulnerable on the IUCN Red List. During a preliminary survey conducted in 2011, 30 Mozambique tilapia from Lake Urema in Gorongosa National Park in central Mozambique, were examined for metazoan parasites. Two groups of ectoparasites were recovered consisting of two species of copepods and a monogenean. Endoparasites retrieved were in the larval stage and included one trematode and one nematode species. This study provides new parasite records for *O. mossambicus* in Mozambique and forms a baseline for monitoring against the presence of ichthyoparasites associated with the introduction in neighbouring systems of non-native fish such as Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758), a fish of worldwide importance in capture fisheries and especially aquaculture.

Keywords: freshwater fish parasites; Gorongosa National Park; invasive; *Oreochromis niloticus*

Key Contribution: Tilapias are often kept in aquaculture and represent a major source of protein, especially in Africa. In a time of intense aquaculture and worldwide translocation of fishes, baseline data on the biodiversity and occurrence of fish parasites are of importance for aquaculture and conservation of native fauna. To date, only a few studies have reported fish parasites from Mozambique and this is the first record of parasites from Mozambique tilapia in Lake Urema.

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1. Introduction

Understanding parasite communities of aquatic systems can have important implications for conservation [1–3]. Here, we report a preliminary study of the metazoan parasites of the Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852) population occurring in Lake Urema in Gorongosa National Park (GNP). This work is important as this fish species is vulnerable in most of its native range, a troublesome invasive in some countries and an important aquaculture species [4]. Indeed, this cichlid has been widely anthropogenically introduced, leading to the co-introduction of its parasites e.g., in Australia [4] and Madagascar [5].

Since the mandate of GNP is to record, restore and conserve the indigenous fauna and flora therein, this study intended to investigate and compile a record of metazoan parasites associated with *O. mossambicus* from Lake Urema and to establish if the resident population of fish is at risk if alien introductions would occur. As fisheries and aquaculture will play an increasingly important role in providing food and nutrition to a growing world population in the future [6], knowledge regarding their parasites and the effect of parasites on their survival is of utmost importance. Indeed, this baseline information will also be valuable by improving the knowledge concerning parasites that can possibly be transferred between natural systems and aquaculture facilities and potentially save-guarding both these important entities.

2. Methods

2.1. Site Description

Lake Urema, a shallow reservoir lake with a mean depth of 1.64 m [7], is located within GNP in Sofala Province, central Mozambique [8,9] at 18°52′ S and 34°30′ E (Figure 1). The lake forms part of the Pungwe River catchment area of 31,150.5 km^2 with 4.7% of the catchment being situated in Zimbabwe and the remainder in Mozambique [10]. Water enters Lake Urema from rivers meandering across the Báruè Midlands, Cheringoma Plateau, and the Rift Valley. Due to the underlying geology, landscape geomorphology, and climatology, the water surface swells from 10 km^2 during the dry season to 200 km^2 in the rainy season with the overflow draining into the Pungwe River, which flows into the Indian Ocean some 80 km away [7,8].

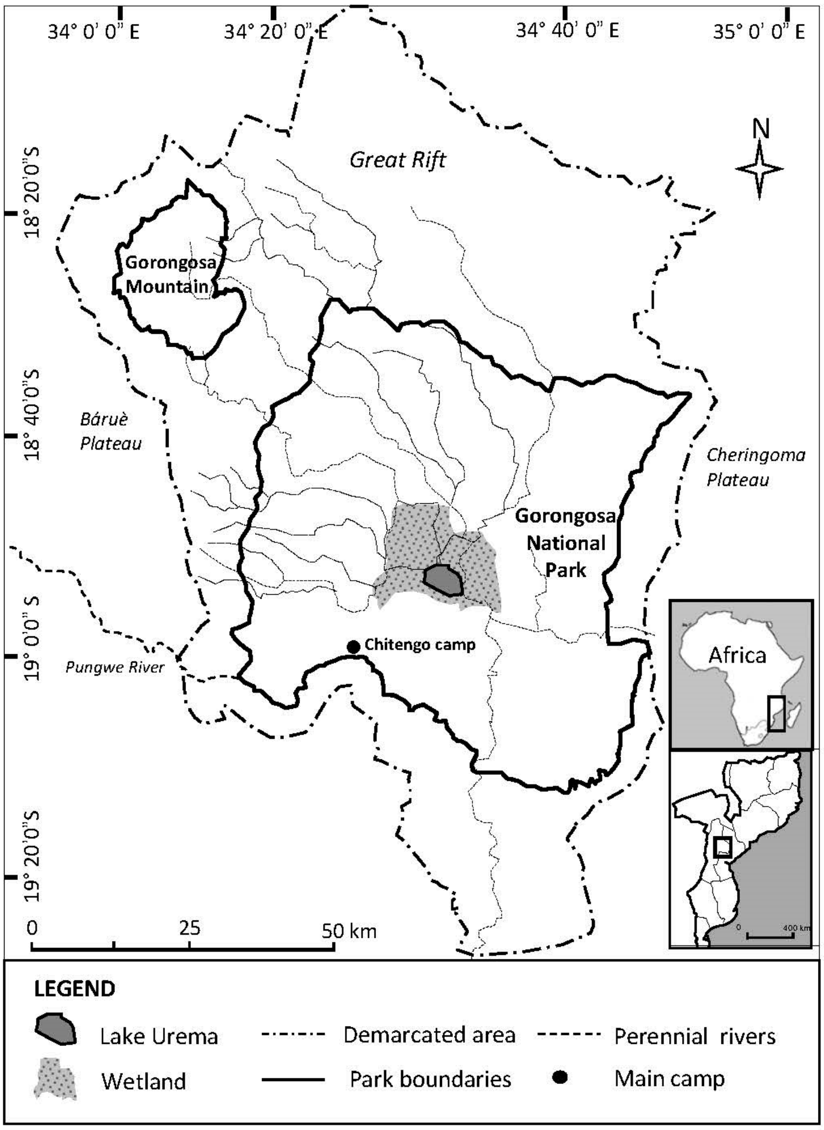


Figure 1. The location of Lake Urema in the Gorongosa National Park (GNP), Sofala Province, Mozambique. Inserts illustrate the locality of Mozambique within Africa and the locality of Sofala Province within Mozambique.

2.2. Sampling of Fish and Parasite Collection

Fish were captured using a seine net. This study only intended to collect metazoan parasites. Once removed from the net, specimens were visually examined for the presence of mobile ectoparasites. These were removed using a fine brush or a pair of forceps and placed into glass vials filled with lake water for further processing at a field laboratory. Mucus smears, taken by scraping the length of the body and fins with a microscope slide, were examined using a stereomicroscope (Model: Leica EZ4, Leica Microsystems, Wetzlar, Germany). The total length of fish was measured (mm) and recorded. Fish were euthanised and the gills, eyes, and internal organs removed and placed into separate Petri dishes containing distilled water and examined for parasites using a stereomicroscope. The body cavity, body fat, and muscle tissue were thoroughly scrutinised for encysted parasites.

Monogeneans were mounted onto slides in either glycerine ammonium picrate (GAP) or glycerine jelly. All parasitic crustaceans were preserved in 70% ethanol. Endoparasites were fixed and preserved according to methods outlined in Madanire-Moyo [11]. Monogeneans were identified following standard procedures by Douëllou [12] and Pariselle and Euzet [13], digeneans according to Gibson et al. [14] and Chibwana and Nkwengulila [15], nematodes as prescribed by Anderson [16], and copepods as by Oldewage and van As [17] and Robinson and Avenant-Oldewage [18]. Due to time constraints, the organs of specimens not processed in the field were preserved in ethanol to be examined later.

Parasite prevalence (%) with confidence limits, based on the Clopper and Pearson intervals, and mean abundance (MA) and mean intensity (MI) with bootstrap confidence limits (BC_a), for each parasite species, were calculated using Quantitative Parasitology 3.0 (QP 3.0: [19]) following Rózsa et al. [20]. Bootstrapping (BC_a) for MA and MI was set at 2000 replications.

2.3. Water Parameters

The survey was undertaken prior to the onset of the rainy season. Water temperature, dissolved oxygen, pH, conductivity, and salinity were taken near the surface and bottom of the water column using a handheld YSI multiparameter meter (Model: YSI 556 MPS, Yellow Springs, OH, USA).

2.4. Ethical Considerations

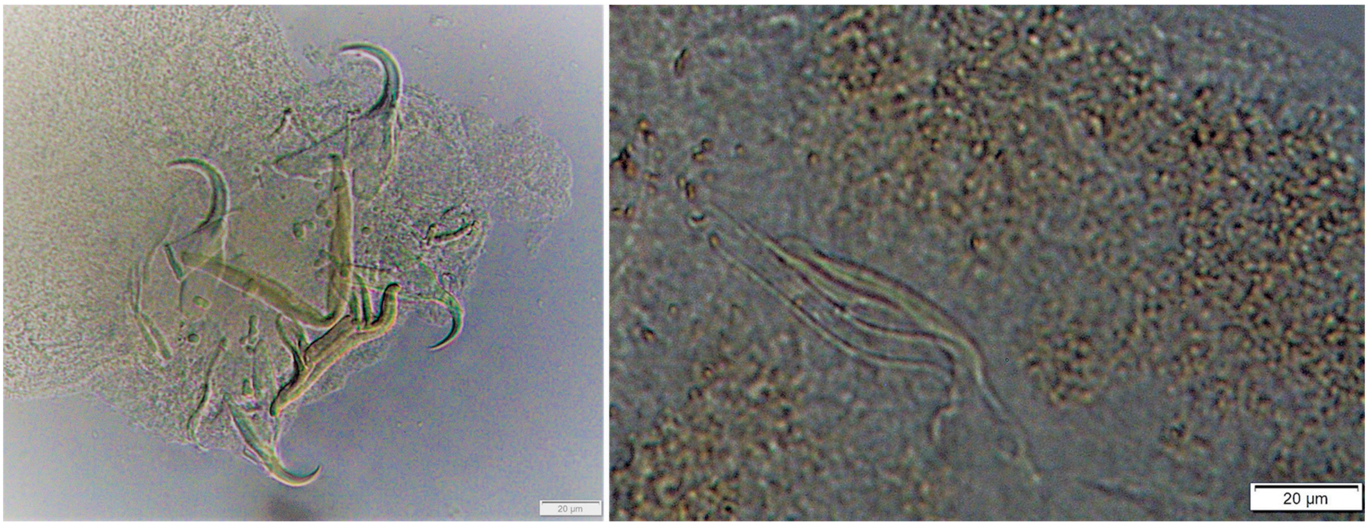
The protocol was approved following the GNP mandate and guidelines. Fish were euthanised by severing the spinal cord whilst covering the eyes with a damp cloth. Handling and treatment of animals were in accordance with the guidelines of the South African Council on Animal Care [21].

3. Results

Thirty individuals of *O. mossambicus*, having a mean (±standard deviation) total length of 287.6 (±20.8) mm, were captured, from which five parasite taxa were recorded.

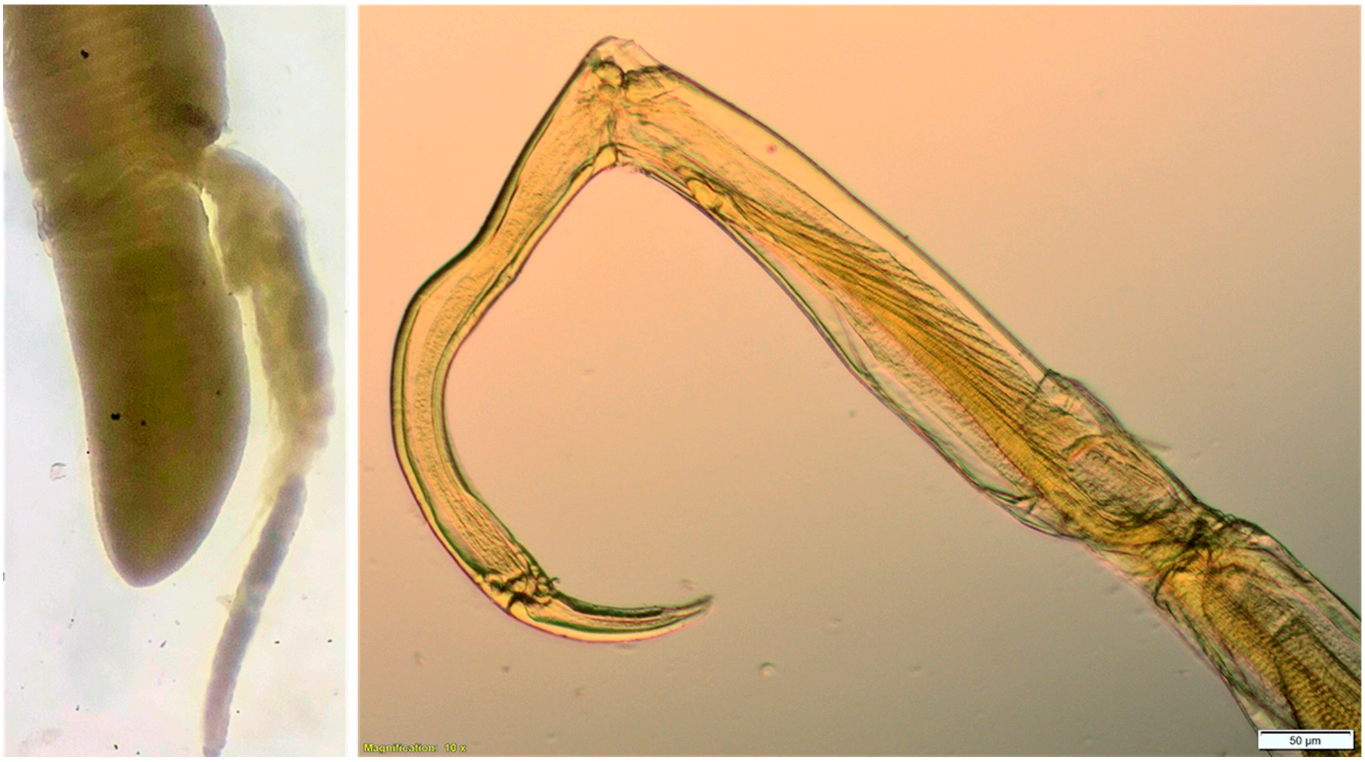
3.1. Parasites Retrieved

Ectoparasites recovered included copepods and monogeneans. The monogenean *Cichlidogyrus halli* (Price & Kirk, 1967) (Figure 2), was recovered from the gills with a non-specific distribution. Copepods recorded were *Ergasilus mirabilis* Oldewage & van As, 1987 (Figure 3) recovered from the gills and *Lernaea cyprinacea* Linnaeus, 1758 (Figure 4), from the skin. Specimens of *E. mirabilis* collected were mainly attached close to the gill arch near the base of the filaments, with a small number occurring in close proximity to the distal end of the filaments. For most, tissue damage was noted in the attachment area. *Lernaea cyprinacea* occurred mainly on the ventral and lateral regions with visible tissue damage leading to secondary infections. Two groups of larval endoparasites were recorded, i.e., metacercariae of the trematode *Euclinostomum* Travassos, 1928 (Figure 5A) embedded in muscle tissue and the third stage larvae of the nematode *Contracaecum* Railliet & Henry, 1912 (Figure 5B) from the body cavity in the proximity of the liver.



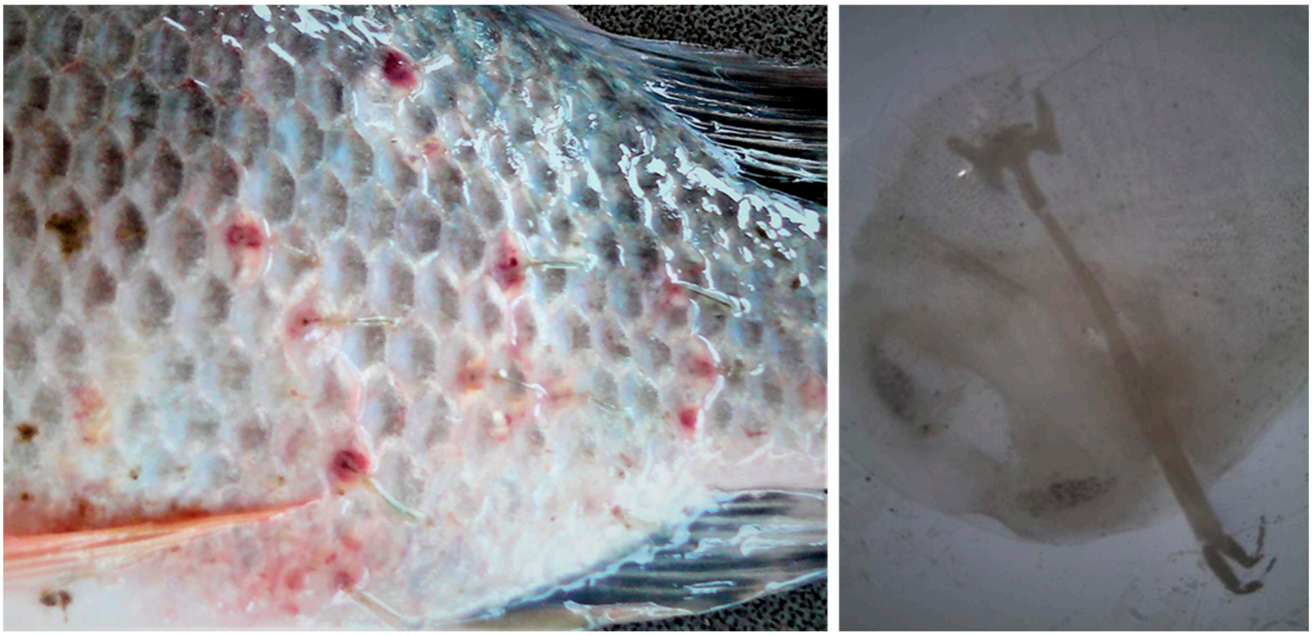
(**A**) (**B**)

Figure 2. *Cichlidogyrus halli*; (**A**) = haptoral parts; (**B**) = male copulatory organ (MCO).



(**A**) (**B**)

Figure 3. *Ergasilus mirabilis*; (**A**) = attached to the gill filament; (**B**) = second antenna (smooth with single terminal tip).



(**A**) (**B**)

Figure 4. *Lernaea cyprinacea*; (**A**) = Embedded in the skin (note signs of inflammation); (**B**) = removed from the fish and still attached to the ventral side of the scale.

(**A**) (**B**)

Figure 5. Larval endoparasites; (**A**) = *Euclinostomum* sp.; (**B**) = *Contracaecum* sp.

The parasite community was dominated by ectoparasites whereby all hosts were infected with *E. mirabilis* and *C. halli* and 90.0% by *L. cyprinacea* (Table 1). The prevalence, MA, and MI of ectoparasites were notably higher than those of endoparasites (Table 1). Although not all the fish examined were infected with *L. cyprinacea*, this parasite had the highest recorded MA and MI, ranging from 2–24 parasites per infected host (Table 1). Fish with no *L. cyprinacea* attached displayed lesions from previous infestations by this parasite, indicating that all fish inspected were infected at some point.

Table 1. Prevalence (P), mean abundance (MA), mean intensity (MI), and intensity range (IR) of parasites from 30 *Oreochromis mossambicus* sampled from Lake Urema during a survey undertaken in October 2011. The upper and lower confidence levels (CL) for P, MA, and MI are given in parentheses.

Parasite Group	Parasite Species	Infected Site	P (%)	95% CL for P (%)	MA	95% CL for MA	MI	95% CL for MI	IR
Monogenea	*Cichlidogyrus halli*	Gills	100.0	(88.4–100.0)	5.9	(4.6–7.1)	5.9	(4.7–7.1)	2–15
Trematoda	*Euclinostomum* sp. *	Muscle	16.7	(3.8–30.7)	0.2	(0.0–0.3)	1.0	(NC)	1
Nematoda	*Contracaecum* sp. *	Body cavity	60.0	(40.6–77.3)	0.9	(0.6–1.2)	1.5	(1.2–1.8)	1–3
Copepoda	*Ergasilus mirabilis*	Gills	100.0	(88.4–100.0)	8.0	(6.0–10.7)	8.0	(6.0–10.4)	2–28
	Lernaea cyprinacea	Skin	90.0	(69.3–96.2)	10.4	(7.3–12.5)	11.6	(8.7–14.0)	2–24

* larval forms. (NC) = Not calculated due to intensity being constant.

3.2. Water Parameters

The mean (±standard deviation) recorded for water temperature, dissolved oxygen, conductivity, and salinity was 27.45 (±0.16) °C, 3.21 (±0.04) mg/L, 0.10 (±0.00) mS·cm^{-1} and 0.03 (±0.00) ppt, respectively. The pH measured was 6.50 throughout the water column of ~1 m.

4. Discussion

4.1. Lake Condition and Parasite Burden

All water measurements were consistent with pre-flood conditions [7]. Factors from the external environment such as water temperature and water quality may affect the parasite itself or the intermediate hosts that are needed to complete the life cycle. The environmental conditions determine the general character of the parasite fauna as a whole and the absence, presence, and abundance of parasites depends thus on the availability of a suitable environment for them. The water temperature was high which is normally favorable for invertebrate intermediate hosts as well as for most ectoparasite groups such as monogeneans [22,23] and copepods [24]. The effects of seasonal changes in water quality and levels, in combination with fish size, fish gender, and the region within the lake e.g., inlet vs. outlet, on parasite burden of *O. mossambicus* and other fish species require investigation.

4.2. Known Parasite Records and Distribution

It is important to gain knowledge of the parasite composition of fish species in their native environment, to attain baseline information for when invasions may occur in the future. As this lake is within a protected area, knowledge gained here will add to the continual need to conserve and protect the biodiversity in Lake Urema. A study by Firmat et al. [25] is the only record of parasites from *O. mossambicus* in Mozambique, however totally isolated from Lake Urema and approximately 700 km apart. During the above-mentioned study they recorded five species of monogenean gill parasites from *O. mossambicus*. *Gyrodactylus* spp. are reported from the skin of this fish from South Africa [26,27] and invasive *O. mossambicus* in the Philippines [28]. Besides gill monogeneans, three species of endoparasitic monogeneans of *Enterogyrus* [11,28] were recorded for *O. mossambicus* in South Africa. More monogenean species were thus expected; however, only *C. halli* was recorded in this study. A potential loss of monogenean species has been proposed to result from the host population going through a genetic bottleneck event [29]. This could have been the case if Lake Urema was originally colonised by only a small number of Mozambique tilapias. Another explanation can be a temporal variation in infection due to an immune response by fish [30]. *Cichlidoyrus halli* has been reported from *O. mossambicus* in Mozambique [25], South Africa [28,31,32], cultured *O. mossambicus* in Japan [33], as well as various cichlid hosts, including introduced populations belonging to various tilapia species, from other African countries [5,13,34,35]. With all fish in this study infected with *C. halli*, the prevalence was notably higher than reported in the aforementioned studies, besides the study by

Firmat et al. [25] where a 100% prevalence was recorded at Linlangalinwe in the Changane River (lower Limpopo Drainage System, Mozambique). The MI levels for *C. halli* reported here were comparable to the findings by Madanire-Moyo et al. [31] and Maneepitaksanti and Nagasawa [33] for the same parasite and host species.

The larval stages of endoparasites collected in this study could not be identified to the species level since the reproductive organs, used to characterise the worms, were not fully developed. The occurrence of larval stages of *Euclinostomum* spp. and *Contracaecum* spp. has been widely reported in cichlids from southern Africa by Khalil and Polling [34], Scholz et al. [35] and Tavakol et al. [36]. The MI for *Contracaecum* sp. recorded here corresponds well with findings by Tavakol et al. [36] for *O. mossambicus* sampled from a number of impoundments in South Africa. The prevalence of *Contracaecum* sp. in this study was however notably higher than that reported by Tavakol et al. [36] but similar to that reported by Sara et al. [30] for the same fish species. *Contracaecum* sp., with a prevalence of 97.5%, was retrieved from *C. gariepinus* in Lake Urema by Dumbo [37]. *Cyprinus carpio* Linnaeus, 1758 was indicated by Boane et al. [38] to have a prevalence of 22.0% and 17.4% of *Contracaecum* sp. infestation from Lagoon Chuáli and the Limpopo River, Mozambique, respectively. The current study is the first to record *Contracaecum* sp. from *O. mossambicus* in Mozambique. The prevalence and MI of *Euclinostomum* sp. recorded reflect well with results by Britz et al. [39] and Olivier et al. [28] for the same species of fish in South Africa. This observation is the first record of this parasite from Mozambique.

Fifteen species of *Ergasilus* have been recorded throughout Africa [35,40–42], however, no published records of *Ergasilus* spp. from *O. mossambicus* exist, making this study a new host record for this parasite genus. Other *Ergasilus* spp. have, however, been recorded from *Oreochromis* spp. as well as other cichlids. *Ergasilus mirabilis* with a prevalence of 7.3% was reported for *C. gariepinus* from Lake Urema by Dumbo [37] and a MI far greater than for *O. mossambicus* recorded here. A range of host fishes throughout southern Africa, including some squeakers [41] and parrotfish [43], is reported to harbour this copepod.

Lernaea cyprinacea had an aggregated distribution on the body of the fish. This parasite, commonly known as anchor worm, is an alien species to Africa and an opportunistic host generalist in that it infests various families of fish and amphibians [44–46]. It is a cosmopolitan parasite that is widely distributed due to the translocation of edible and ornamental cyprinids [46–49]. In southern Africa, *L. cyprinacea* has been reported from *O. mossambicus* in Lake Victoria [18] and a number of water bodies in Zimbabwe [50,51] and South Africa [18,52–54]. Although this is the first record of this parasite from *O. mossambicus* in Mozambique, it has been reported for *C. carpio* in this country by Boane et al. [38]. Another lernaeid, *Lernaea barnimiana* (Hartmann, 1865) is also known to occur in the southern African region and has been reported from *O. mossambicus* [35]. High infestation levels of copepods are expected in Lake Urema given that this group is known to thrive in shallow waters at high temperatures [50,55–57]. The prevalence and MI of anchor worms reported from South Africa are lower [18] whereas those in Zimbabwe [50] (associated with shallow and warm water) are higher than in the current study. As with studies by Dezfuli et al. [58], Lester and Hayward [59], and Vinobaba [60], tissue damage that can lead to secondary infections, was observed at the attachment sites for both copepod species reported here. Infection by anchor worms has been associated with poor general health [54,61,62] and thus poses a great threat to native fish in its non-native range as well as to aquaculture practices.

All parasite species recorded here may be transferred to and become established within aquaculture facilities and have a negative impact on aquaculture fish stocks and therefore the economic viability of this sector [6,63]. Parasitic infestation has been associated with a reduction in host weight, growth, fecundity, and in severe cases mortality [5,62,64,65]. Knowledge regarding parasitic threats and potential stressors for fish may reduce economic strain on a volatile sector making it more sustainable in the future.

4.3. Zoonotic Importance

The fish-borne zoonotic trematodes and nematodes reported here may pose a health risk as they can be transmitted to humans consuming raw or undercooked fish [66–70]. Dried salted fish is a common product in rural areas and is being offered by local traders [71]. This is of special concern as fishermen from nearby Muaredzi, some 30 km from the lake, have access to catch and supply the village inhabitants with fresh fish. There is also small-scale extensive aquaculture production taking place within Sofala Province where farmers are stocking from natural systems, posing a risk of parasite transfer. Therefore, appropriate measures of control should be established and implemented. Park authorities and educators working at surrounding rural schools should attempt to make the general public and fish farmers aware of the zoonotic nature of some fish parasites and the risks they may pose to human health.

Author Contributions: W.J.S.: collected samples; conceptualisation; analysed the data; writing—original draft preparation. W.J.L.-P. and N.A.G.M.: writing—review and editing; funding acquisition; resources. M.P.M.V.: writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: At the time when the study was undertaken neither the University of Limpopo (UL) nor the Gorongosa National Park administration had an ethics committee. The researchers are currently working under UL ethics approval (AREC/01/2020:IR) issued on 14 February 2020. The Animal Research Ethics Committee (AREC) is registered with the National Health Research Ethics Council, Registration Number: AREC-290914-017.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to being intellectual property.

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Conflicts of Interest: The authors declare no conflict of interest.

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