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Prevalence and molecular characterization of Myxobolus parasites from freshwater and saltwater fishes in Mwanza and Dar-es-Salaam, Tanzania

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Abstract

Background: Myxobolus species are Myxosporean parasites affecting various fish, causing diseases that weaken populations and result in substantial economic losses in aquaculture and fisheries. Despite extensive studies in Asia, Europe, and the Americas, there is limited research on Myxobolus in Africa, particularly in Tanzania, where fisheries and aquaculture are vital for fish populations, ecosystem health, and economic growth.

Methods: This study investigated the prevalence and genetic characteristics of Myxobolus parasites in 384 fish samples from Dar es Salaam (Indian Ocean) and Mwanza (Lake Victoria). Samples were examined for cysts and spores using microscopy, followed by molecular characterization through PCR amplification of the 18S rDNA gene, Sanger sequencing, phylogenetic analysis, and genetic distance evaluation.

Results: The overall prevalence of Myxobolus was 12%, with a significantly higher prevalence in Mwanza (21.88%) compared to Dar es Salaam (2.08%). Statistical analysis revealed significant associations between prevalence, fish species, and locality. Phylogenetic analysis identified two genetic lineages within a monophyletic group, clustering with Myxobolus species from Israel, Egypt, and Ghana, suggesting potential novel species. Genetic distance analysis indicated greater variation in saltwater samples compared to freshwater.

Conclusion: These findings highlight the higher prevalence of Myxobolus in freshwater and emphasize the need for targeted management strategies, continued surveillance, and research to safeguard fish populations and sustain aquaculture.

Keywords: Myxobolus, Prevalence, PCR, 18s rDNA gene, Phylogenetic analysis, Genetic distance, Aquaculture, Tanzania

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Background

Saltwater and freshwater fishes are integral in food security, where they serve as a primary protein source for millions of people worldwide. Tanzania has abundant water resources from freshwater (lakes, rivers, and dams) to Marine water (Territorial Sea, coastal line, and Exclusive Economic Zone) which is a potential source of fisheries and aquaculture production. Fish provide essential nutrients and contribute to the overall nutritional needs of the population, while also supporting the livelihoods of thousands involved in fishing, processing, and distribution [1,2]. Additionally, fish populations offer valuable ecosystem services, including water filtration, nutrient cycling, and coastal protection, which support industries like tourism, recreation, and coastal development, highlighting their multifaceted economic importance [3,4]. However, a wide range of pathogens affect fish production in different water sources, including bacteria, viruses, fungi, and parasites. Myxobolus is a genus of parasitic organisms belonging to phylum Cnidaria, that infect various host species including fishes, amphibians, birds, elasmobranchs, reptiles, as well as mammals as intermediate hosts and annelids or bryozoans as final hosts. Myxobolus parasites in fishes have been reported around the world [5,6,7] where they are commonly found in freshwater with some other species found in marine (brackish water), and terrestrial habitats [8,9]. Myxobolus has more than 978 Myxosporean species where M. celebralis, M. pseudodispar, M. cyprini, M. squamalis and M. artus are some of the most commonly reported species [10].

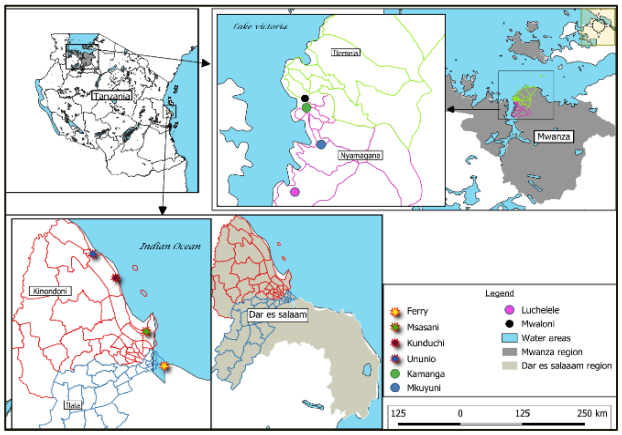
Some of other species recently reported and characterized include M. grassi, M. bejeranoi, and M. dajiangensis [11,12,13,14]. In Africa, Myxobolus species have been reported in Cameroon [15,16], Benin [17], Egypt [18,19], Kenya, Uganda, and Ethiopia [20] revealing the ecological significance of these parasites in fishes and fish industries. Myxobolus parasites produce cysts that inflict significant pathogenic effects on various organs of fish including gills, heart, liver, kidneys, spleen, gallbladder, gonads, intestine, and urethra, subsequently causing various diseases in fishes. Myxosporeans have been linked to severe diseases such as whirling disease [21], Proliferative kidney disease [22], and Gill disease, caused by several Myxobolus species [23] affecting both aquaculture and wild fish populations [24]. Additionally, Myxobolus parasites can cause skin and muscle lesions, leading to localized inflammation, tissue damage, and reduced swimming performance [25,26,27]. The presence of Myxobolus parasites in fish populations can lead to reductions in population size due to increased mortality rates, and impaired reproduction, ultimately affecting the sustainability of fisheries, aquaculture, and the economy [28,29]. From a public health perspective, Myxobolus parasites have been reported in patients with gastrointestinal symptoms [30], in immunosuppressed patients [31], and also in human feces through ingestion of infected fishes [32]. Myxobolus parasites have been well documented in other continents including Asia, Europe, and American countries but comparably fewer studies have been documented in African countries. However, there is scarce to no information on documented and published studies of Myxobolus parasites in Tanzania despite their importance in fisheries and aquaculture sectors. Molecular characterization techniques are employed to identify and differentiate Myxobolus species. The utilization of molecular methods provides accurate distinction between different Myxobolus species and strains. Furthermore, such genetic data may provide insights into transmission dynamics, host specificity, and potential host-switching events. Moreover, updated and additional data are needed to better understand Myxobolus divergence, interactions with fish hosts' environment, and implications for fisheries management and aquaculture. Addressing these gaps will aid in developing effective disease control strategies and ensuring sustainable fish populations and food security. This study will provide the baseline information on molecular characteristics and assess the prevalence of Myxobolus parasites to reveal the extent of infection among fish populations and elucidate the distribution patterns of these parasites in aquatic ecosystems in saltwater and freshwater fishes from Dar es Salaam and Mwanza, Tanzania. The results are of utility in disease surveillance, taxonomic classification, and the development of targeted control measures to effectively manage Myxobolus infections and promote the health and conservation of saltwater and freshwater fish populations in the studied regions of Tanzania.

Methods

Study area

This study was conducted in Mwanza and Dar es Salaam. In Dar es Salaam, sampling was carried out in two districts, Kinondoni and Ilala, at sites included Kivukoni, Msasani, Ununio, and Kunduchi along the Indian Ocean. In Mwanza, sampling sites were located in Nyamagana and Ilemela districts, at Mkuyuni, Mwaloni, Kamanga, and Luchelele along Lake Victoria, as illustrated in Figure 1.

Figure 1: A map of Mwanza and Dar es Salaam regions depicting selected study



sites. The map was created using QGIS software version 3.36.1 and shape files from DIVA-GIS (retrieved on 10 May 2024).

Dar es Salaam, situated at 6°48′S, 39°17′E, covers 1,393 km² and experiences a hot, humid tropical climate. It is bordered to the east by the Indian Ocean and is surrounded by the Pwani Region. Major activities in Dar es Salaam include fishing and trade. In contrast, Mwanza is located at 2°31′S, 32°55′E, spanning 25,233 km², and lies between 1,200 and 1,400 meters above sea level. The region experiences a tropical savanna climate, characterized by warm temperatures throughout the year and distinct wet and dry seasons. Mwanza is bordered to the north by Lake Victoria and Kagera Region, and to the east, south, and west by Mara, Simiyu, Shinyanga, and Geita Regions respectively. The main economic activities in Mwanza are fishing and agriculture.

Study design and selection of study area,

A cross-sectional study design was employed to establish the prevalence and molecular characteristics of Myxobolus parasites in Mwanza along Lake Victoria (freshwater body) and Dar es Salaam along the Indian Ocean (saltwater body). Mwanza was purposively selected due to its role as a major mainland freshwater fish export depot, its significance in supplying fish for domestic purposes, the presence of common fish species that host the targeted parasite, and the existence of prior studies on this parasite in Lake Victoria [20], from other countries. Dar es Salaam was chosen as a counterpart location due to its proximity to the Indian Ocean, a saltwater fishing hotspot, and the presence of previous studies on the parasite from other country, which support the consideration of parasite transmission in this area [33]. Systematic sampling was employed to select aquaculture and fishery sites within each region, beginning with a random starting point at fish landing sites or markets then from the starting point, additional sites were chosen at regular intervals to ensure representative sampling. Study sites were chosen for their varying fishing intensities, pollution levels, and ecological conditions, allowing the study to assess the combined effects of human activity and environmental factors on fish health and parasite prevalence. Within each selected market or landing site, fish were systematically chosen from the fish sellers and fishermen to capture variability. Sample collection occurred in the morning and evening to introduce variability, minimize sampling bias, and account for diurnal fluctuations. This

approach allowed for the collection of a diverse range of fish from all selected sites from March to April of 2024.

Sample size

Cochran prevalence formula for estimation of sample size, N=Z2PQ/E2, where N= sample size, Z=1.96 (the number of standard deviations from the mean) corresponding to the desired confidence level, P=50% prevalence, Q= 1-P, E=5% precision error was employed in the study [34]. A total of 384 fish samples were included in the study, where 192 fish samples were obtained from each study location (Mwanza and Dar es Salaam) after calculations.

Sample collection and transportation

Fish were collected from their landing sites and/or fish markets in Mwanza and Dar es Salaam for one month (March – April 2024). Six fish species were sampled, Mackerel (Rastrelliger kanagurta), Spangled emperor (Lethrinus nebulosus), and Bigeye trevally (Caranx sexfasciatus) from Dar es Salaam and the Nile perch (Lates niloticus), Nile tilapia (Oreochromis niloticus) and Lake Victoria squeaker (Synodontis victoriae) from Mwanza. All the collected fish samples were bought from the fish markets and fishermen, and identified based on a protocol similar to that employed by [51] and then stored in a cool box containing ice packs for preservation. The samples were then transported to the parasitology laboratory at Sokoine University of Agriculture in Morogoro for parasite examination.

Identification of features of collected fish samples

The identification of fish sex was done in the parasitology laboratory, based on observation of morphological features particularly the genital papilla located posterior to the fish's anus. Slight distinctions in the genital papilla were utilized to sort out the fish sexes where the female genital papilla was identified with the presence of an oviduct in addition to the urinary pore unlike that of the males. The weight of the fish was measured by using an electronic weighing machine model (SF-400) while the fish length was measured by using a standard measuring ruler. The location of sample collection, fish sex, weight, and length were recorded.

Parasites (Myxobolus cysts/spores) detection, fish dissection and storage

Collected fish were observed to identify the presence of the parasites (cysts) in the skin, scales, eyes, operculum, and fins with the aid of a stereomicroscope (OPTA-TECH, Warsaw, Poland) with a 4x objective lens. This was followed by fish dissection by using a sterilized surgical blade to allow examination of cysts infesting the fish's internal organs including gills, intestines, liver, heart, kidney, spleen, and muscles. Samples (dissected organs) were placed onto Petri dishes and examined for the presence of cysts using a stereo microscope (OPTA-TECH, Warsaw, Poland) with a 4x objective lens. Detected and extracted tissue cysts were placed on the microscopic slides and crushed by using sterilized syringe needles to release spores, followed by the addition of one drop of distilled water to form a wet smear which was then covered with coverslips. The smear was then observed under the light microscope (OPTA-TECH, Warsaw, Poland) with an objective lens of 100x and oil immersion to identify and measure the spores belonging to the Myxobolus parasites based on guidelines set by [36]. Measurements were taken by using a calibrated eyepiece micrometer. The samples that tested positive upon microscopy were carefully preserved in falcon tubes containing 70% ethanol to await further molecular analysis.

Extraction of Myxobolus DNA

Genomic DNA was extracted using the Quick-DNA TM Universal kit (ZYMO Research, USA) following the manufacturer's instructions. Each tissue sample in the Eppendorf tube received 95 µl of nuclease-free water, 95 µl of solid tissue buffer, and 10 µl of proteinase K, and was then incubated at 55°C for 3 hours to ensure solubility. After incubation, the sample was centrifuged at 12,000 x g for one minute, and the supernatant was carefully transferred to a clean tube. Subsequently, 400 µl of genomic binding buffer was added to each 200 µl volume of transferred supernatant. This mixture was then transferred to a Zymo-spin TM IIC-XL column placed in a collection tube and centrifuged at 12,000 x g for 1 minute. The DNA purification process included three wash steps with various wash buffers per the protocol instructions. This involved using 400 µl of DNA pre-wash buffer once, followed by two washes with 700 µl and 200 µl of g-DNA wash buffer, respectively. The purified DNA extracted was then eluted using 50 µl of DNA elution buffer. Before storage, the concentration and quality of extracted DNA were measured using a NanoDrop TM spectrophotometer. Finally, the extracted DNA was stored at -20°C for future use.

PCR amplification, gel electrophoresis, and amplicon sequencing

PCR amplification was conducted targeting the 18S rDNA gene using forward (MC5F: 5'-CCT GAG AAA CGG CTA CCA CAT CCA-3') and reverse (MC3R:5'-GAT TAG CCT GAC AGA TCA CTC CAC GA-3') primers [37]. The Master mix prepared for the 25 µL PCR reaction per tube comprised an enzyme premix of 12.5 µl contained DNA polymerase enzyme, dNTPs, buffer and Mg+, and other components including template DNA 5 µl, nuclease-free water 4 µl, Bovine Serum Albumin (BSA) 2.5 µl, and the primers 1 µl. Amplification conditions were as follows; an initial denaturation step at 95˚C for 5 minutes, followed by 35 cycles of denaturation at 95˚C for 60 seconds, annealing temperature at 60˚C for 60 seconds, and an extension at 72˚C for 90 seconds. A final extension step at 72˚C for 5 minutes to terminate the amplification end process. The amplicons were separated by 1.5% agarose gel stained with ethidium bromide at 120 Volts for 45 minutes. Visualization and documentation were done by a gel documentation system (Vilber, Germany). A volume of 20 µL from each positive PCR product was sent to Macrogen Company (Europe, Netherlands), where the 18S rDNA gene sequences were generated via dideoxynucleotide cycle sequencing (Sanger sequencing). The Applied Biosystem's 96 Capillary 3730xl DNA Analyzer (Thermo Fisher Scientific, Carlsbad, CA, USA) was used to sequence each sample in both forward and reverse directions preceded by purification of the PCR products.

Data analysis

Collected data were coded and then entered in Microsoft Excel 2017 spreadsheets. The prevalence of Myxobolus parasites from freshwater and saltwater fish was determined by computing the

proportions of fish that were infected by Myxobolus parasites within the populations. Prevalence of Myxobolus parasite infestation was hence calculated using the following formula; P = No /NT × 100 %, Where P: Prevalence, No: number of positive samples, and NT: number of total samples tested. The results were then expressed as a percentage. The SPSS software version 26, created by IBM Corporation, Armonk, NY, USA in 2017, on the other hand, was employed where the Chi-square test was used to assess the statistical significance between the prevalence of parasites and various study variables (locality, sex, length, weight, and species of fishes). Findings were considered statistically significant when the p-value was less than 0.05.

Molecular analysis

Generated sequences were checked for quality, trimmed, edited, to remove low-quality and ambiguous bases, correcting sequencing errors, then assembled into consensus sequences using Bio Edit version 7.2.5. The consensus sequences of 18S rDNA in Myxobolus were then searched for similarity in the National Center for Biotechnology Information (NCBI) GenBank using the nucleotide Basic Local Alignment Search Tool (BLASTn), the sequences were considered are same species if the percentage similarities were 98% to 100%. The consensus sequences and sequences retrieved from NCBI GenBank were aligned using the ClustalW statistical algorithm built in Evolutionary Genetics Analysis (MEGA) version 11. The phylogenetic tree was constructed by using the Neighbor-Joining method [38] and 1000 bootstrap replications [39], by using MEGA software version 11 to evaluate the relatedness of Myxobolus spp isolated from this study with those from other published studies [40]. The evolutionary distances were computed using the Kimura 2-parameter method [41]. Genetic pairwise distance analysis for sequences was computed using the Tamura 3-parameter model in MEGA 11 software [42,40].

Results

Fish species morphometrics and demographics

Three hundred and eighty-four fishes of six fish species were collected from Dar es Salaam and Mwanza in selected fish markets and or land sites. The species were Lates niloticus, Oreochromis niloticus, Synodontis victoriae, Rastrelliger kanagurta, Lethrinus nebulosus, and Caranx sexfasciatus. The fish species collected had a weight ranging from 14–288.5 g, and a length ranging from 10.1–26.5 cm. The greatest average length, mean length, mean weight, as well as weight range, was observed on fish belonging to the Lates niloticus species. Out of the 384 fishes collected, 42.97% were female (n=165) and 57.03% were male (n=219) as shown in Table 1.

Table 1. Morphometric and demographic characteristics of fish samples (n=384).

Variables	***Rastrelliger kanagurta***	***Lethrinus nebulosus***	***Caranx sexfasciatus***	***Lates niloticus***	***Oreochromis niloticus***	***Synodontis victoriae***
Total No. of sample	64	64	64	64	64	64
Samples Sex	Male:40	Male:37	Male:29	Male:42	Male:36	Male:35
	Female:24	Female:27	Female:35	Female:22	Female:28	Female:29
Location	Ferry:16	Ferry:16	Ferry:16	Mwaloni:16	Mwaloni:16	Mwaloni:16
	Ununio:16	Ununio:16	Ununio:16	Mkuyuni:16	Mkuyuni:16	Mkuyuni:16
	Msasani:16	Msasani:16	Msasani:16	Luchelele:16	Luchelele:16	Luchelele:16
	Kunduchi:16	Kunduchi:16	Kunduchi:16	Kamanga:16	Kamanga:16	Kamanga:16
Average length(cm)(SD)	18.186(2.26)	18.241(2.99)	19.16(2.8)	23.32(1.86)	20.47(2.59)	22(1.72)
Length Size range(cm)	14.5-25.1	10.1-24.5	13.7-25.2	19-30	14.3-26.5	18-26
Mean weight(g)(SD)	62.69(27.49)	24.91(5.209)	96.34(41.66)	147.311(42.8)	140.19(54.38)	94.81(19.19)
Weight range(g)	16-139	14-53	32-224	80-281	66-288.5	48-126

Morphological and morphometric identification of Myxobolus cysts and spores

Cysts were white, round to ellipsoidal, and measured up to 6 mm in diameter. Myxospores were identified based on their distinctive morphology: an elongated, oval body with two equal-sized polar capsules (Figure 2). A total of 46 fish (12%) were found to be infected with Myxobolus cysts from Mwanza and Dar es Salaam. The myxospores body measured (14.2-17 µm) in length, and (7.5–10.2 µm) in width, and the two polar capsules were equal in size and elongated, measuring (5.1–7 µm) in length and (1.2–2.2 µm) in width.

Prevalence of Myxobolus parasites in Mwanza and Dar es Salaam

The prevalence of the parasite in the study sites was established based on the presence of Myxobolus cysts/spores' infestation in fish. Three species of fish which are Lates niloticus, Oreochromis niloticus, and Caranx sexfasciatus were infested with Myxobolus parasites. The overall prevalence of Myxobolus parasites in fishes was 12% (46/384), being greater in Mwanza with 21.88%, (42/192) than in Dar es Salaam with 2.08%, (4/192). In selected study sites, the infestation was observed only in Mkuyuni, Kamanga, Mwaloni, Luchelele, and Ferry with 31.3% (15/48), 20.8% (10/48), 20.8% (10/48), 14.6% (7/48), 8.3% (4/48) prevalences respectively. Only fish gills were observed to be infested with multiple infestations of Myxobolus cysts. The results of this study show that host sex, length, and weight categories had no significant effect on the prevalence of Myxobolus parasites, with p-values 0.115, 0.066, and 0.105 respectively as shown in Table 2.2. It was found that fish species, and locality, are significant factors for Myxobolus parasites both having a p-value of 0.001(Table 2).

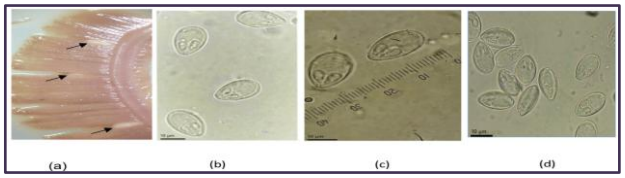


Figure 2. Cysts (a) of Myxobolus parasites under a stereo microscope (OPTA-TECH, Warsaw, Poland) at 40x total magnification power and spores (b, c, and d) of Myxobolus parasites under a light microscope (OPTA-TECH, Warsaw, Poland) with 1000x total magnification power (Source: author 'photos taken in SUA's Parasitology laboratory in examined fishes).

Table 2. The Chi-square (χ^2) association between the prevalence of Myxobolus parasites and variable parameters (n=384)

variable	Categories (N=individual collected)	Infested(n)	Prevalence (n/N*100)	Odds ratio	CI[a] (95%, Lower limit-upper limit)	χ^2	p-value
Fish species	*Oreochromis niloticus*(N=64)	20	31.25%	1.23	0.00-0.46	81.107	0.001
	Lates niloticus(N=64)	22	34.38%				
	Caranx sexfasciatus (N=64)	4	6.25%				
	Rastrelliger kanagurta, (N=64)	0	0				
	Lethrinus nebulosus (N=64)	0	0				
	Synodontis victoriae(N=64)	0	0				
Sex	Female (N=165)	15	9.09%	0.565	0.04-0.19	2.484	0.115
	male(N=219)	31	14.154%				
Location	Mwanza (N=192)	42	21.88%	13.424	0.00-0.28	35.663	0.001
	Dar es salaam (N=192)	4	2.08%				
Length (13.7-30cm)	Below Q1(N=94)	6	5.8%	1.304	0.01-0.25	7.201	0.066
	Q1-Q2(N=97)	13	13.30%				
	Q2-Q3(N=90)	9	11%				
	Above Q3(N=103)	18	17.81%				
Weight(g)	Light(N=227) Medium(N=127) Heavy(N=30)	21 19 6	9.3% 15% 20%	0.579	0.05-0.35	4.503	0.105

*Prevalence is calculated as the number of infected fishes divided by the total number of fishes sampled; **Q1, Q2, and Q3 refer to the first, second, and third quartiles of the length distribution, respectively.

Polymerase Chain Reaction (PCR) amplification of Myxobolus DNA

DNA from the 18S rDNA gene of the Myxobolus parasite was isolated from 30 positive samples (cysts and spores). These samples were selected as representative and subsequently used for nucleic acid extraction. Visualization revealed successful amplification of the 18S rDNA gene from all analyzed samples, resulting in clear 1050 bp bands on the gel (Figure 2).

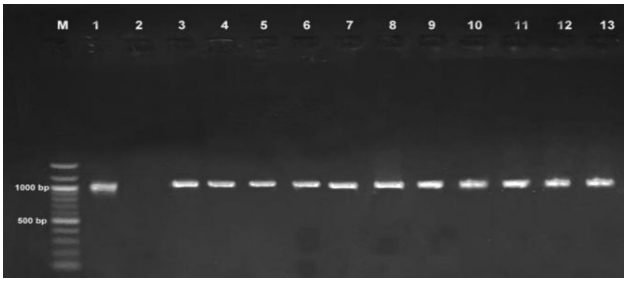


Figure 3. Gel electrophoresis of amplified partial coding region of the 18S rDNA gene of Myxobolus spp. M is a DNA ladder while lane number 1 and 2 are positive and negative controls respectively, Lanes 3-11 are test samples of freshwater fishes, and lanes 12-13 are test samples of saltwater fishes.

Sequencing and Nucleotide Sequence Analysis of the 18S rDNA Gene

Each of the sequences (PQ387947- PQ387954) generated via automated dideoxynucleotide cycle sequencing was subjected to a BLASTn search to reveal homology through percentage identity, query cover, and E-value comparison. A list of the most homologous sequences for each sample subjected to BLASTn was identified. Close homologous sequences from Egypt (Myxobolus sp. 2 HS-2015 strain Oreo-8) and Israel (Myxobolus bejeranoi n.sp.) emerged as the candidate homologs for comparative analysis based on the closest query coverage, similarity and e value. The results are summarized in Table 2.3.

Phylogenetic Inference of Evolutionary Relationships

The phylogenetic analysis of 23 nucleotide sequences, including eight candidate sequences from this study with accession numbers (PQ387950, PQ387948, PQ387949, PQ387947, PQ387951, PQ387952, PQ387953, PQ387954) and 15 reference sequences obtained from NCBI's online repository, provided significant insights into the evolutionary relationships among these groups of sequences. The phylogenetic inference revealed that the candidate sequences formed two main genetic lineages within a single monophyletic group. Furthermore, these candidate sequences clustered within specific clades of the Myxobolus genus with very close to Myxobolus bejeranoi from Israel, and Myxobolus species from Egypt and Ghana. Furthermore, given that all species analyzed belong to the genus Myxobolus, the generated phylogenetic tree suggests a complex evolutionary history within this genus, characterized by multiple distinct clades representing divergent evolutionary lineages (Figure 2.4). Kudoa mirabilis and Kudoa thyrsites sequences were used as out-group sequences.

Table 3. Comparison of 18S rDNA Gene Sequences via BLASTn (n=384)

Accession number of Query sequence	Sampling Locality	Host Species	Sequences producing significant alignments with query sequence in NCBI	% Similarity	E-Value	Query cover
PQ387947	Dar es Salaam (Ferry)	*Caranx sexfasciatus*	MF401455.1	89.6%	0.0	84%
PQ387948	Mwanza (Luchelele)	*Lates niloticus*	KP990670.1	90.7%	0.0	84%
PQ387949	Mwanza (Kamanga)	*Oreochromis niloticus*	MF401455.1	90.6%	0.0	79%
PQ387950	Mwanza (Mwaloni)	*Oreochromis niloticus*	MF401455.1	90.6%	0.0	83%
PQ387951	Mwanza (Mwaloni)	*Oreochromis niloticus*	MF401455.1	90.5%	0.0	83%
PQ387952	Mwanza (Mkuyuni)	*Lates niloticus*	KP990670.1	90.8%	0.0	81%
PQ387953	Mwanza (Kamanga)	*Oreochromis niloticus*	MF401455.1	90.4%	0.0	82%
PQ387954	Mwanza (Mkuyuni)	*Lates niloticus*	KP990670.1	90.3%	0.0	79%

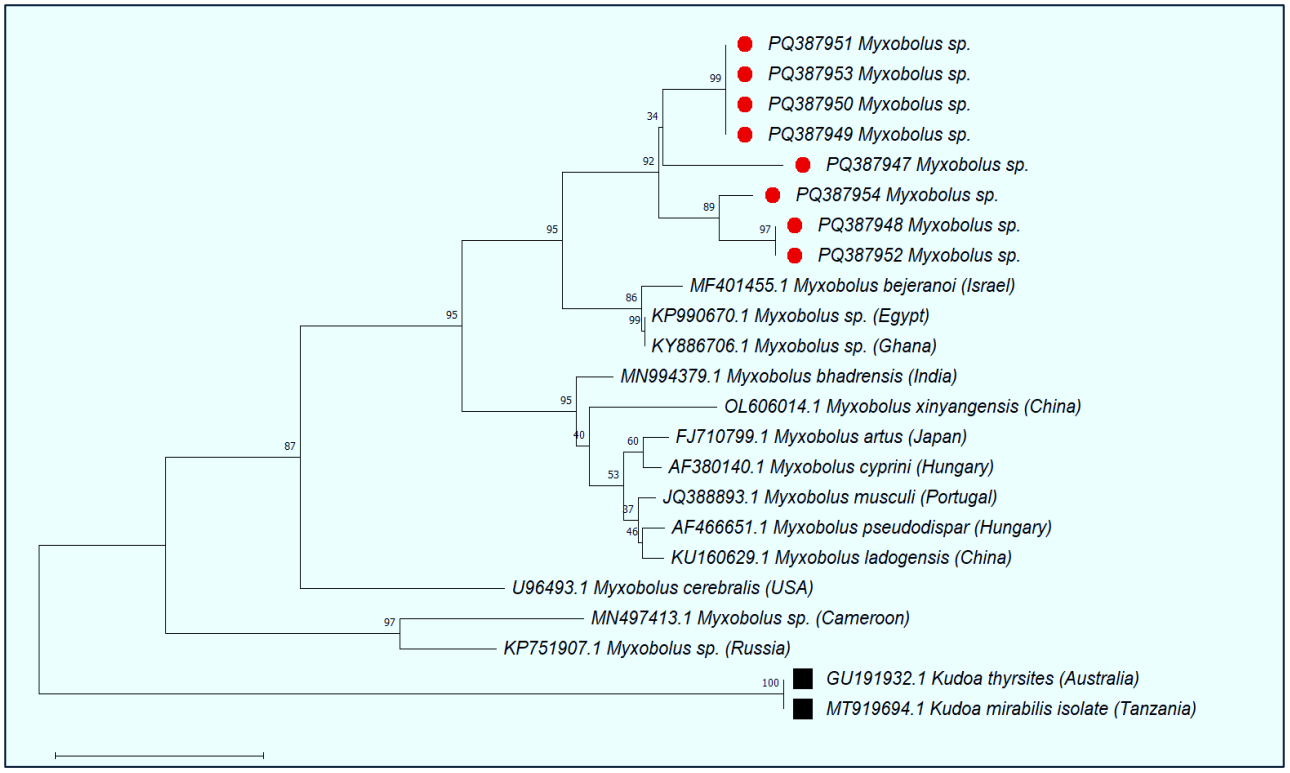


Figure 4. Phylogenetic tree of Myxobolus species nucleotide sequences. The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There was a total of 187 positions in the final dataset. Candidate study sequences are denoted by red dots, and outgroup sequences are represented by black squares while the rest are those retrieved from NCBI.

Genetic divergence

The genetic distance between the candidate sequences was uneven, with the high distance observed on Dar es Salaam sequence (PQ387947) compared to the rest of the candidate sequences. Furthermore, lowest genetic distances were noted between sequence PQ387949, PQ387950, PQ387951, and PQ387953 and also, they observed with the same genetic distance (0.001) between them. Low distance observed between sequence PQ387948, PQ387952 (0.009) and they have >2% genetic distance with sequence PQ387954 (Table 4).

Table 4. Genetic divergence between Myxobolus sp. Sequences (N=384).

	PQ387947	PQ387948	PQ387949	PQ387950	PQ387951	PQ387952	PQ387953	PQ387954
PQ387947								
PQ387948	0.071							
PQ387949	0.041	0.061						
PQ387950	0.042	0.061	0.001					
PQ387951	0.042	0.058	0.001	0.001				
PQ387952	0.067	0.009	0.060	0.061	0.061			
PQ387953	0.043	0.060	0.003	0.001	0.001	0.062		
PQ387954	0.066	0.025	0.064	0.067	0.067	0.022	0.069	

*Triangular Matrix indicates pairwise genetic distance per estimate

Discussion

The study established the prevalence and molecular characteristics of Myxobolus parasites in freshwater and saltwater fish from Mwanza and Dar es Salaam, Tanzania. The overall prevalence of Myxobolus parasites was 12%, with a higher occurrence in fish from freshwater ecosystems. These findings align with the identification of Myxobolus species, such as Myxobolus awadhii [43], which underscores the diversity and specificity of these parasites in freshwater fish, particularly in gill infections [44,45]. Also, several studies have reported a high prevalence of Myxobolus parasites in freshwater fishes in Russian Far East, particularly Sakhalin Island and the Maritime Province and a few species found in saltwater fishes across the world's oceans, including the North Atlantic, South Atlantic, North Pacific, South Pacific, and Indian Ocean [46,33]. This notable difference in parasite prevalence between the two study regions is likely attributable to their respective geographical and chemical variations, suggesting that environmental factors or variations in local ecological conditions may play a critical role in influencing the prevalence of Myxobolus parasites [47]. The data indicate that Myxobolus parasites exhibit species-specific patterns, with Lates niloticus being mostly susceptible to infection. This finding aligns with observations from Cameroon, where similarly high prevalence rates were reported in this species [8,48,49]. This finding suggests a potential species-specific susceptibility to Myxobolus infections, which are often

more susceptible due to their life cycles and habitat preferences, which could have significant implications for fisheries targeting these species. Furthermore, the higher prevalence of these parasites in Lates niloticus can have a significant impact on its health, growth rates, and market value [50]. The high prevalence of Myxobolus parasites in Lates niloticus underscores the need for robust parasite monitoring and management strategies in aquaculture and fisheries operations within this region. Statistical analysis further supports initial findings on regional variation and species-specific prevalence of the candidate parasite ($p < 0.05$). Inferential statistical finding is in line with that provided by [51] who reported significant spatial and temporal variations in prevalence across host species and locations. Highlight that, both species-specific and regional are significant factors for the presence of Myxobolus parasites. In contrast, other candidate variables did not show statistically significant effects, which align with [52], indicating that factors such as fish size and sex may not significantly influence the prevalence of Myxobolus parasites. These findings underscore the necessity for targeted health management strategies in both aquaculture and fisheries, with a particular focus on species-specific and regional factors. Understanding of regional disparities in Myxobolus prevalence can inform sustainable management practices and conservation strategies. Despite robust amplification and extensive database searching, sequence similarity was insufficient for definitive species-level identification as reported by [6,12]. This suggests that the amplified sequences may represent novel strains, closely related species, or potentially even new species within the Myxobolus genus. Hence, a phylogenetic analysis was conducted to refine species identification and further explore the evolutionary relationships, give the lower similarity percentages observed in the BLASTn results. Phylogenetic analysis revealed that the eight candidate sequences formed two distinct lineages within a monophyletic Myxobolus clade, indicating shared ancestry. Notably, Tanzanian Myxobolus sequences clustered with those from Israel, Egypt, and Ghana, suggesting a common evolutionary history and potential long-distance dispersal mechanisms. The clustering of these geographically distant species in the phylogeny suggests potential long-distance dispersal mechanisms, such as host migration or human activities [53]. This finding underscore significant biogeographical patterns and raises concerns about the potential dissemination of parasitic diseases among fish populations in Africa and the Middle East. The rapid spillover of an African parasite with a complex life cycle into the Middle East has been observed, potentially impacting aquaculture and wild fish populations [54]. The saltwater fish sample from Dar es Salaam exhibited the longest branch length, indicating a higher degree of genetic divergence compared to the freshwater fish samples from Mwanza. [55] demonstrated, distinct ecotypes can emerge due to ecological divergence, leading to localized genetic differences and potentially even taxonomic distinctions. This suggests that the saltwater population has experienced older divergence events or a faster evolutionary rate. The geographical isolation of Dar es Salaam and the unique ecological pressures of the saltwater environment likely contributed to this divergence. Genetic distance analysis revealed a significantly higher genetic distance in the saltwater sample, suggesting a different species from the freshwater samples due to distinct evolutionary lineages.

In contrast, the low genetic distances among freshwater sequences suggesting the presence of two different species in freshwater since the genetic distance is the same (0.001) among the four sequences (PQ387949, PQ387950, PQ387951, and PQ387953). Another two sequences PQ387948, PQ387952 had slight genetic differences (0.009), suggesting that they belong to the same species. However, the sequence PQ387954 is considered different strain since the genetic distance is greater than 2%. This Aligns with the isolation-by-distance model, where geographic distance limits gene flow and promotes genetic divergence [56,57]. Indicates close evolutionary relationships and limited genetic divergence, likely forming two distinct specie due to recent common ancestry or similar selective pressures. These findings suggest that the saltwater adaptation of the parasite may have influenced host-parasite interactions, potentially contributing to the observed genetic divergence.

Furthermore, it could affect the parasite's host range and pathogenicity, with implications for disease transmission in marine fish populations. Contrary to the assumption of homogeneity in marine populations, evidence suggests significant genetic structuring and local adaptation, often driven by parasites [58,59]. These results emphasize the importance of environmental factors in shaping evolutionary pathways and highlight the need for further research on the mechanisms driving parasite evolution in diverse aquatic ecosystems.

Conclusion

This study provides the first baseline data on Myxobolus parasites in Tanzanian freshwater and saltwater fish, marking the first documented occurrence of these parasites in the country. Notably, this research identifies new Myxobolus species, including from a saltwater host, expanding the known diversity of this genus in Tanzania. The presence of Myxobolus in various fish species underscores the need for further investigations into their potential impact on fish health and fisheries. Long-term monitoring, particularly focusing on seasonal variations in infection rates, is recommended to better understand the dynamics of these parasites. Additionally, research on Myxobolus infections in annelid hosts, which serve as definitive hosts, is necessary, as no such studies currently exist in Tanzania. A comprehensive epidemiological assessment of these parasites will contribute to a deeper understanding of their distribution and ecological significance within Tanzanian aquatic ecosystems.

Abbreviation

BLASTn: Nucleotide Basic Local Alignment Search Tool; MEGA: Molecular Evolutionary Genetics Analysis; MNUAT: Mwalimu Nyerere University of Agriculture and Technology; NCBI: National Center for Biotechnology Information; PCR: Polymerase Chain Reaction; SUA: Sokoine University of Agriculture

Declaration

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Availability of data and materials
Data will be available by emailing julianamkwama@gmail.com

Authors' contributions
Conceptualization, J.L.M and E.M.M; sample collection, J.L.M; laboratory analyses, J.L.M; formal analysis, J.L.M, and A.A.C; writing—original draft preparation, J.L.M; writing—review, and editing, G.G.B, A.A.C, and E.M.M. All authors have read, and agreed to this published version of the manuscript.

Ethics approval and consent to participate
We conducted the research following the declaration of Helsinki. Ethical clearance for this study was approved by the directorate of research, consultancy, and technology transfer (DPRTC) of the Sokoine University of Agriculture, Morogoro Tanzania (Ref. no: DPRTC/R/186/23) issued on 21 June 2024. All protocols and procedures for sample collection, and sample processing in this study complied with the direction of the Ethics Committee, Sokoine University of Agriculture.

Consent for publication
Not applicable

Competing interest
The authors declare that they have no competing interests.

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