Tropical Biomedicine 42(4): 361-369 (2025)
https://doi.org/10.47665/tb.42.4.001

RESEARCH ARTICLE

The Rinconada Lake System (RLS) as a Potential Reservoir of Protozoan Parasites in Camarines Sur, Philippines

Paet, J.M.Q.[1,3*], Rojas, S.M.[2], Torres, J.F.[3]

[1]Research Development and Management Division, Bicol University, Legazpi City, 4500, Philippines
[2]Advanced Molecular Technologies Laboratory, Research Institute for Tropical Medicine, Muntinlupa City, Metro Manila, 1781, Philippines
[3]Biology Department, College of Science, Bicol University, Legazpi City, 4500, Philippines
*Corresponding Author: jmqpaet@bicol-u.edu.ph

ARTICLE HISTORY

Received: 14 March 2025
Revised: 14 September 2025
Accepted: 1 October 2025
Published: 31 December 2025

ABSTRACT

Protozoan and helminthic parasitism remain significant health and economic burdens, particularly in developing countries such as the Philippines. This study evaluated the Rinconada Lake System (RLS) in Camarines Sur, comprising Lakes Buhi, Bato, and Baao, as a potential reservoir for medically important parasites. Microscopic examination of surface water samples from 20 stations revealed no helminthic ova, which may have settled in sediments, but detected potential coccidian oocysts of *Cryptosporidium* spp. (55%, 11/20) and *Cyclospora* sp. (40%, 8/20). Culture-based detection of free-living amoeba was at 60% (36/60), showing organisms resembling *Acanthamoeba* spp. (35% or 21/60) significantly linked to Lake Baao and Lake Bato ($P < 0.01$) likely reflecting greater anthropogenic influence. The metabarcoding confirmed the presence of protozoan parasites in the lakes, such as *Echinamoeba* sp. and *Vermamoeba vermiformis*, while no helminthic parasites were detected using current methods. Additional microbial eukaryotes of medical relevance, such as fungi and algae, were also identified. Retrospective analysis of the available health records from surrounding communities showed that *Entamoeba histolytica* was the most documented case for 4 years (47.8% or 75/157), followed by various helminthic infections (49.7% or 78/157), predominantly caused by *Ascaris lumbriocoides*. There was no significant association with the patient's residence or sex, except for significantly higher cases in those aged 0-10 years. In conclusion, findings suggest that the RLS may serve as an environmental reservoir for opportunistic protozoan parasites, while its role in helminthic transmission requires further investigation. Based on current evidence, emphasis on surveillance and mitigation strategies in communities surrounding the RLS, especially for protozoan parasites, is highly recommended.

Keywords: Amoeba; *Ascaris*; coccidian oocysts; metabarcoding.

INTRODUCTION

Neglected tropical diseases (NTDs) are a significant public health concern that imposes a considerable disease burden in the Asia-Pacific region, including the Philippines (Hotez & Ehrenberg, 2010; Leonardo *et al.*, 2020). These chronic and debilitating conditions that persist among vulnerable populations include intestinal helminthiases, foodborne trematode infections, trachoma infections, lymphatic filariasis, schistosomiases, and arboviral infections, among others (Hotez & Ehrenberg, 2010). Most NTDs are associated with parasitic, vector-borne, foodborne, and zoonotic agents, and many are transmitted through environmental contamination *via* the fecal-oral routes, often through ingestion of or contact with contaminated food and water sources (Archer *et al.*, 2020). Despite the attention to public health control strategies, climate change exacerbates the impact of NTDs by affecting the sensitivity of the life cycle and transmission dynamics of infectious agents exposed to environmental factors such as air, water, and soil (Booth, 2018). Such can increase the vulnerability of marginalized groups, considering their dependence on shared community resources that may serve as potential reservoirs for infectious agents.

The Rinconada Lake System (RLS), which includes Lake Buhi, Lake Bato, and Lake Baao, is among the vital freshwater resources in the Bicol Region, particularly in the province of Camarines Sur. These lakes are recognized as an important ecosystem providing essential ecological services that sustain the livelihoods of residents and serve as a habitat for a wide variety of flora and fauna. Lake Buhi plays a critical role in fisheries, irrigation, domestic water supply, and navigation, whereas Lake Bato and Lake Baao are primarily used for aquaculture and irrigation to nearby agricultural lands (Regis & Dela Cruz, 2006). Resource utilization by the locals and other societal sectors has already negatively impacted these lakes, and ongoing anthropogenic activities could further exacerbate the deterioration of this ecosystem and increase the risk of biological contamination. Parasitic contamination, in particular, may contribute to the NTD burden by exposing the vulnerable local population to waterborne pathogens.

Published by Malaysian Society of Parasitology and Tropical Medicine.

Water-related parasitic contamination, especially from protozoan parasites and helminths, has already been documented in various water bodies, including lakes, rivers, and ponds within the Philippines and the broader Southeast Asian regions (Onichandran *et al.*, 2014; Noda *et al.*, 2009). Helminth ova, particularly of *Ascaris, Schistosoma*, and hookworms, along with protozoan oocysts were documented in recreational or environmental water systems in Malaysia and Thailand (Kumar *et al.*, 2014). In the local setting, *Acanthamoeba* sp. has been detected in Lake Buhi *via* molecular identification, highlighting the presence of potentially pathogenic protozoa in the RLS waters (Hagosojos *et al.*, 2020). While the majority of documented diarrheal waterborne outbreaks linked to parasitic protozoa occur outside Asia, ongoing studies have already detected similar pathogens in Asian waters. As of 2017, only 1% of the global outbreaks linked to waterborne parasitic protozoa were recorded in Asia, highlighting the scarcity of data and the need for further studies to assess waterborne NTD risks in the region (Lim & Nissapatorn, 2017).

To address the knowledge gap regarding the status of the Philippine freshwater systems, this study aimed to detect potential helminthic or protozoan parasitological contaminations of the RLS. Likewise, this study sought to determine the possible role of the RLS in the ongoing health burden of parasitic NTDs in the province of Camarines Sur using secondary data.

MATERIALS AND METHODS

Sampling Stations and Collection of Water Samples

An ocular inspection was conducted to establish a total of 20 main sampling stations, with eight in Lake Buhi, seven in Lake Bato, and five in Lake Baao (Figure 1). Lake Buhi is a volcanic freshwater lake of approximately 17 km^2 that supports aquaculture, irrigation, and domestic use, and is most notable as the habitat of the endemic sinarapan (*Mistichthys luzonensis*). Lake Bato, at about 28 km^2, is among the ten largest lakes in the Philippines, sustaining local fisheries and wildlife while serving as an important component of the Bicol River drainage system. In contrast, Lake Baao is the smallest (~2 km^2) and shallowest in the Rinconada Lake System, shrinking considerably during the dry months and uniquely functioning as a direct drainage source for adjacent rice farms. Three replicate substations were set up in each lake within a 1–5-meter range of the main sampling stations and were described as (1) near any major/minor tributary, (2) near the residential area or commercial establishments, (3) sites near aquaculture pens or agricultural

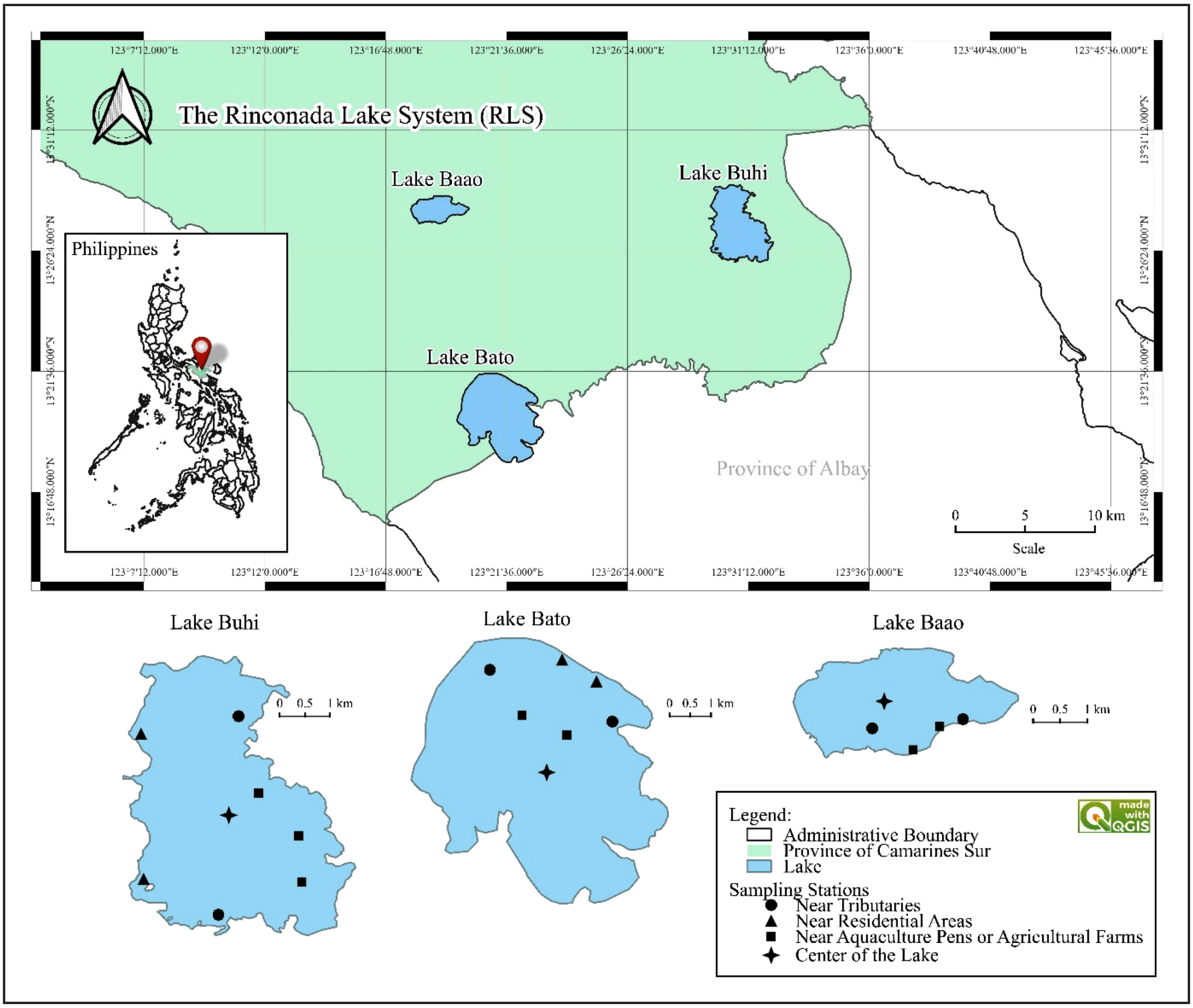

Figure 1. Map of the study location. Pinned in red on the Philippine map (inset) is the Province of Camarines Sur, where the Rinconada Lake System (RLS) is located. The RLS includes Lake Buhi, Lake Bato, and Lake Baao, with a total of 20 sampling stations established for the collection of water samples for helminthic and protozoan parasite detection.

farms, and (4) the perceived center of the lake as per the guide. GPS coordinates were recorded using Garmin eTrex® 22x. Surface water grab samples were obtained by filling a 10 L PVC collapsible water container, which was used for parasite microscopy- and culture-based detection methods. Meanwhile, 5 L grab water samples per main station were also collected for parasitome analysis using eDNA metabarcoding. This cross-sectional study was conducted during the dry months of February to April 2022.

Parasite Detection Using Direct Microscopic Methods

Approximately 1 L of the grab water sample per replicate substation was filtered through a 0.2 µm nitrocellulose membrane filter. The filtered particulates were carefully scraped off using distilled water with 0.1% Tween 20 and Lugol's iodine solution. The suspension was transferred to a clean microscope slide and then viewed under high-power magnification to detect the presence of parasitic organisms. Similarly, using the cold method, acid-fast staining was done in 1 L filtered samples pooled from three replicate substations. The scraped samples were air-dried in clean glass slides and then fixed with methanol for 30 seconds. Fixed samples were stained with Kinyoun's carbol fuchsin for 1 minute, destained with acid alcohol for 2 minutes, and counterstained with alkaline methylene blue for 2 minutes, with rinsing using distilled water after each step. The slides were left to dry, and parasites were observed under oil immersion objectives. Identification of parasites was done using the World Health Organization's guide (WHO, 2019) and the morphological description from the Centers for Disease Control and Prevention website, DPDx (CDC, 2019a).

Culture-based Method for the Detection of Free-living Amoeba

Before collecting samples, media for the non-axenic culture of free-living amoeba were prepared. *Escherichia coli* was first grown overnight at 37°C in nutrient broth (NB). Also, a minimal medium was prepared using phosphate buffered solution (pH = 7.0) with 1.5% agar (PBSA), supplemented with 50 µg/ml fluconazole during the first growing procedure. In the final non-axenic culture, heat-killed 250 µl of overnight NB bacterial culture was spread-plated onto the PBSA, cooled to approximately 15-20°C during storage, and pre-warmed to room temperature prior to use. After sampling, 1 L of the grab water samples from each replicate substation were filtered in a 0.22 µm nitrocellulose membrane and then plated in the seeded PBSA. The set-up was incubated at 37°C for 7 days. Microscopy was conducted to detect the presence of free-living amoeba in the samples, utilizing Lugol's iodine stain for better resolution of the images when needed.

Parasitome in the RLS Using Next-Generation Sequencing

Environmental DNA samples were collected from the grab water samples, as previously described. Upon arrival at the laboratory, samples were immediately filtered using a 0.22 µm nitrocellulose membrane. Sites and replicates for each sampling station were pooled as one sample and used for DNA extraction (Promega Wizard® Genomic DNA Purification Kit) and PCR amplification, library preparation, and sequencing. For protozoan detection, amplification was conducted using the EUKAF (5'-GCCGCGGTAATTCCAGCTC-3') and EUKAR (5'-CYTTCGYYCTTGATTRA-3') primer pairs (Moreno *et al.*, 2018), while for helminths and other eukaryotes, the 563F (5'-GCCAGCAVCYGCGGTAAY-3') and 1132R (5'-CCGTCAATTHCTTYAART-3') primer pairs were used (Hugerth *et al.*, 2014; Kounosu *et al.*, 2019). Amplification conditions included an initial denaturation at 95°C for 3 minutes, followed by 25 cycles of denaturation (95°C for 30 seconds), annealing (40°C for 30 seconds), extension (72°C for 15 seconds), and a final extension at 72°C for 5 minutes. Amplification products were used for the amplicon DNA library preparation following the 16S Metabarcode Sequencing Library Preparation protocol using the Herculase II Fusion DNA Polymerase Nextera XT Index V2 Kit. Paired-end reads were generated using the Illumina Sequencing platform with a read length of around 301 bp. A total of 11 metabarcodes for bioinformatics was used and analyzed through the Nextflow Ampliseq pipeline (version 2.7.1) with embedded quality control (primer trimming, sequence evaluation, and quality filtering), generation of the amplicon sequence variants (ASV) using DADA2, taxonomic classification using PR2 databases, with other output reports such as a bar plot, QIIME2 diversity, and quality control reports (Straub *et al.*, 2020).

Retrospective Analysis of Parasitic NTDs in the RLS Communities

Various health facilities in the municipalities of Buhi, Bato, and Baao were visited to gather data on patients infected with parasites. Seven out of the nine facilities visited provided disaggregated data on parasite-infected patients from 2019-2022. The only information extracted from the records was the patient's name, year of testing, age, sex, and address of the parasite-infected patient. The data was manually encoded in a spreadsheet, and the frequency of infection was noted. Only patients residing in any of the municipalities (Buhi, Baao, or Bato) were included in the final data consolidation. Statistical analysis was done using IBM SPSS v.26 software for the Chi-square association test. In addition, an informal, structured interview was conducted with representatives of the facility as key informants, focusing mainly on the methods used in parasite detection and their experience with parasite control and mitigation.

RESULTS

Microscopy Showed No Helminths, but Protozoan Parasites were Detected

A total of 60 wet mounts with Lugol's iodine and 20 acid-fast stained slides were prepared for the preliminary inspection of human parasites in the surface water samples. After thorough inspection, no parasitic ova matching the descriptors in the identification key were observed. Most of the observations were clearly artifacts, appearing mostly as unicellular algae, phytoplankton, zooplankton, or their remains.

Acid-fast staining was done to specifically identify oocysts of the coccidian species stained red with their characteristic size range: *Cryptosporidium* spp. (4.2 µm to 5.4 µm), *Cystoisospora* spp. (25 µm to 30 µm), and *Cyclospora* spp. (7.5 µm to 10 µm). In the 20 sampling sites established, 95% (19/20) were positive for at least one coccidian oocyst, while all lakes contained at least one kind of coccidian parasite (Figure 2a-d). The most commonly detected was putative *Cryptosporidium* spp. (Figure 2a-b) at 55% (11/20), followed by *Cyclospora* spp. (Figure 2c-d) at 40% (8/20). No observations conforming to *Cystoisospora* spp. morphology were observed on any of the sites. In this study, *Cryptosporidium* positivity in Lake Buhi, Lake Bato, and Lake Baao was at 75% (6/8), 42.9% (3/7), and 60% (3/5), respectively. Meanwhile, *Cyclospora* positivity was at 25% (2/8), 57.1% (4/7), and 40% (2/5), respectively. No significant association was found between lakes and percent positivity ($P = 0.45$).

Culture-based detection of free-living amoeba (FLA) also yielded positive results for growth. Trophozoites of amoebas were observed in wet mounts after 4 days of incubation of filtered water samples. The observed morphologies of the trophozoites with long and broad pseudopodia were thought to resemble those of *Balamuthia* (Figure 2e) in contrast to the thin spine-like acanthopodia of *Acanthamoeba* spp. Other possible FLA were also noted, showing typical amoeboid bodies (Figure 2f) with various morphology of amoebic encystation observed upon prolonged incubation. Smooth to wrinkled circular cysts were also observed, which is hard to assign taxonomically since most amoebae cysts are circular in shape (Figure 2g).

Overall, 60% (36/60) of the plates tested positive for FLA, while the observed stellate cysts (Figure 2h), which are presumptively identified as *Acanthamoeba* spp. were observed at 35% (21/60). Culturable FLAs were found in all lakes with the following prevalence:

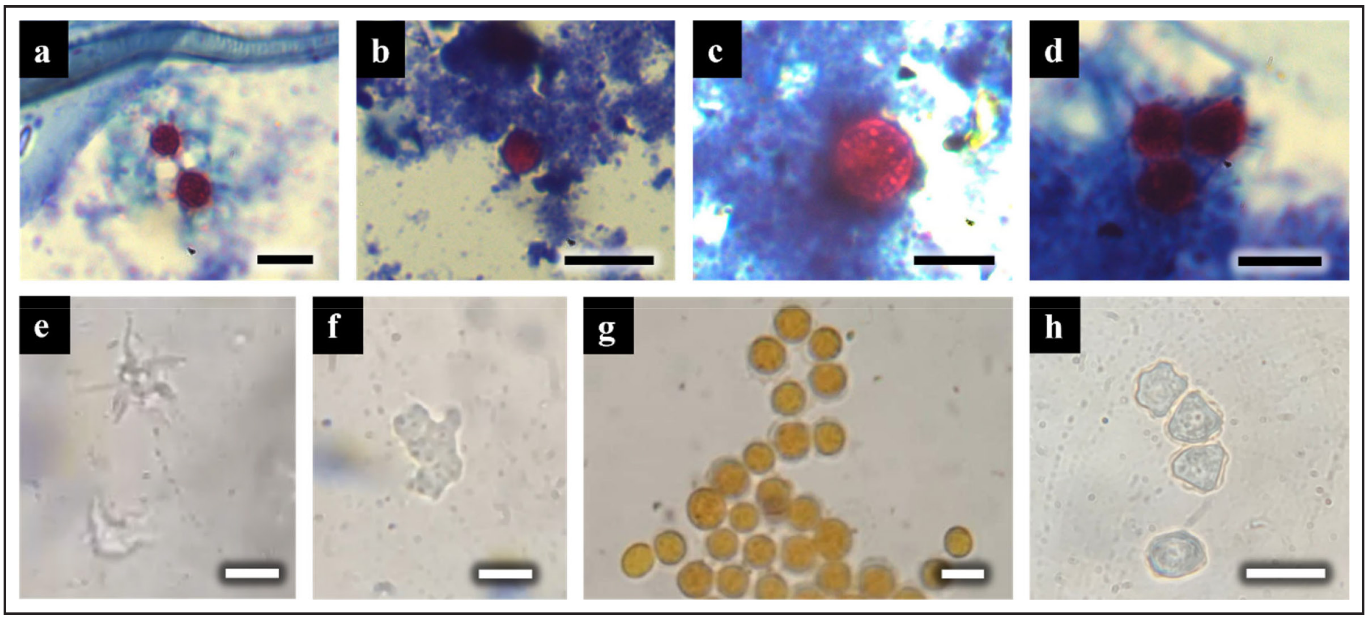

Figure 2. Photomicrographs of prepared slides from filtered RLS surface water (a-d) and unstained and stained wet mounts from FLA-positive culture (e-h). Acid-fast stained slides viewed under OIO of putative (a-b) *Cryptosporidium* spp. and (c-d) *Cyclospora* spp. based on size and morphology. Trophozoite forms of representative amoeba slides showing (e) broad finger-like projections of the pseudopodia and (f) typical amoeboid form at 7-10 days post-inoculation. FLA encystation at 14 days post inoculation showed cysts with (g) a rough exocyst and Lugol's iodine-stained endocyst, as well as (h) an endocyst of varying polygonal shapes (unstained). The scale bar in black and white is set at 10 and 20 µm, respectively.

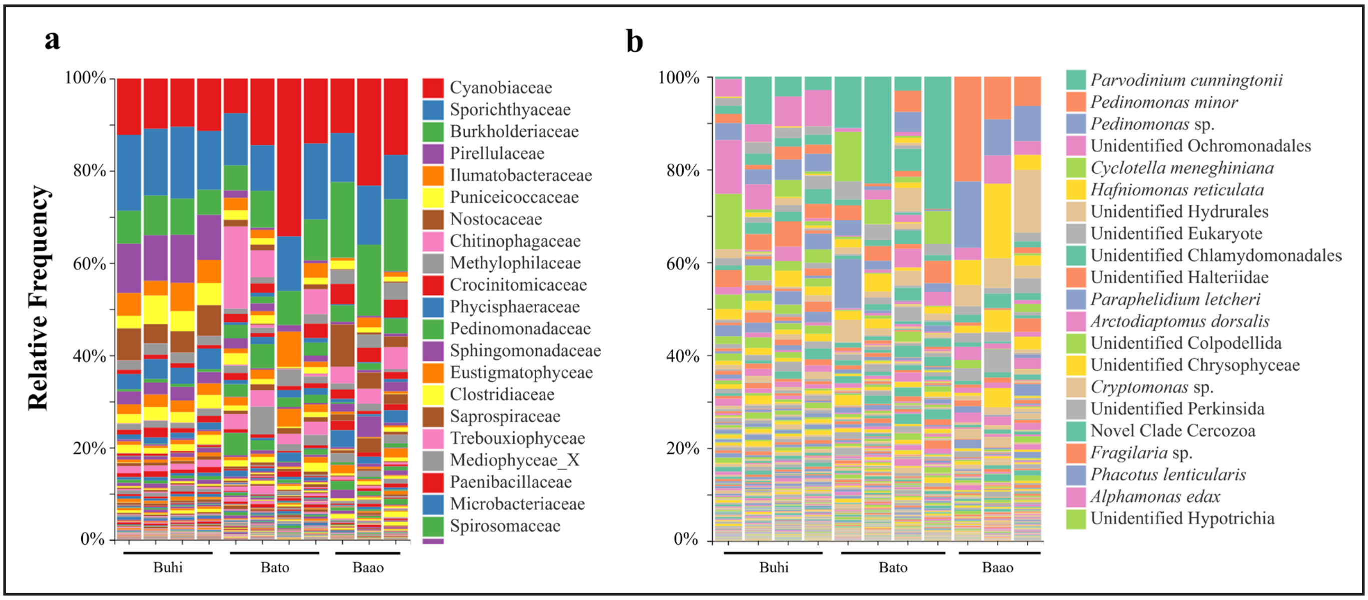

Figure 3. Relative abundance of different domains and families detected at the RLS. Organism profile at the family level detected using the (a) 563F/1132R and (b) EukaF/EukaR primer pairs. Representative families shown have the most abundant identified sequences.

Lake Buhi at 50% (14/24), Lake Bato at 47.6% (10/21), and Lake Baao at 93.3% (14/15). A significantly higher number of FLA-positive plates were associated with Lake Baao than expected cases ($P = 0.01$). Moreover, the highest proportion of plates positive for putative *Acanthamoeba* spp. was observed in Lake Baao at 80% (12/15), followed by Lake Bato at 23.8% (5/21) and Lake Buhi at 16.7% (4/24). A significant association between high positive results in *Acanthamoeba* spp. ($P < 0.001$) with both Lake Baao and Lake Bato were observed more than the expected values.

Potentially Pathogenic Organisms Detected in RLS Using Metabarcoding

Parasite metabarcode profiling using the universal primers of Hugherth *et al.* (2014) to target helminthic and other eukaryotic organisms did not yield satisfactory results, comprising mostly of untargeted bacterial amplified products (Figure 3a). This limited the taxonomic resolution at the species level because the reference database was not optimized for the non-specific amplicons. Kounosu *et al.* (2019) supported the findings of this study, where their evaluation of the 563F/1132R primer pair showed bias toward the Domain Bacteria given a complex sample source. Hence, future parasite profiling efforts may consider other suggested primers designed that are more specific to parasitic helminths and protozoans.

Meanwhile, the EUKAF/EUKAR primers successfully amplified eukaryote eDNA (Figure 3b). Metabarcoding was able to confirm the presence of several Amoebozoa species, including *Angulamoeba* sp., *Vermamoeba vermiformis*, *Chaos* sp., *Echinamoeba* sp., *Copromyxa microcystidis*, and *Vannella miroides.* These amoeba species accounted for less than 1% of the total sequence reads, indicating a relatively lower abundance compared to other eukaryotic microbes. While parasitic organisms were not well-represented during the sequencing (as in the case of the presumptive coccidian oocysts and FLAs detected from other methods), several other medically important microorganisms including fungi and toxin-producing algae were observed in the RLS (Table 1).

Epidemiology of Parasitic Infections in the Municipalities of Buhi, Bato, and Baao

A general survey of the various health facilities in communities associated with the lakes showed that no strong parasite detection efforts are being undertaken. Key informants cited the heavy reliance on the government's national deworming programs to control parasite infections. Microscopy-based detection methods are still the only method used in all facilities visited using only the usual wet mount technique coupled with iodine staining, as in most stool examinations. Currently, there is no epidemiological survey being conducted to determine the prevalence of infections in any of these communities. Detection primarily occurs by chance when the patient undergoes fecalysis, either upon the physician's advice or as needed during employment. Furthermore, routine fecalysis of patients visiting hospitals was not observed in the facilities surveyed unless requested by the patient or the attending physicians. Lastly, no proper database management was observed in these communities, and record-keeping was mainly through manual entry to logbooks. Information regarding patients who tested positive for parasite infection was incomplete in most cases and often omitted from records since the record keepers are usually familiar with the patients' details.

Approximately 152 disaggregated records were retrieved from various facilities across the three municipalities, with Baao having the highest data kept (75.5%), followed by Bato (21.9%) and Buhi (2.6%) from 2019-2022 (Table 2). Most of the cases recorded were the protozoan parasite *Entamoeba histolytica* (47.8%), which causes gastrointestinal infection that manifest as diarrhea cases. Furthermore, various helminthic infections were prevalent such as *Ascaris lumbriocoides* (41.4%), *Trichuris trichiura* (4.5%), and hookworms (3.8%). Other protozoan infections, which are believed to be *Iodamoeba* sp., *Balantidium* sp., and *Blastocystis* sp., accounted for the remaining 2.5%. Most of the data was gathered in 2020, with a significant decrease in detections to about three-fold in 2021. Also, no reported cases of FLAs causing encephalitis or keratitis infections were reported in all of the municipalities. Hospital records of patients residing either near or far from the lakes showed no significant association with any of the parasites detected ($P > 0.10$). Similarly, no significant association was observed in parasite infection records from the different municipalities related to the RLS ($P > 0.12$) as well as between the sexes ($P > 0.31$). Meanwhile, significantly higher cases of helminthic and protozoan infections ($P < 0.001$) were liked to patients aged 0-10 years.

DISCUSSION

Roundworms, whipworms, and hookworms are among the major helminthic parasites collectively referred to as soil-transmitted helminths (STHs), which is consistent with their non-detection in the waters of RLS. While STHs are typically associated with soils, they have also been reported in fresh vegetable produce, largely attributed to manure contamination from humans and animals in farming areas (Ordoñez *et al*., 2018; Vizon *et al*., 2019). The absence of parasitic ova in the water, even in sites near agricultural farms or residential zones, may therefore reflect minimal or undetectable fecal waste inputs into the RLS. However, helminth eggs are known to interact strongly with sediments in aquatic systems, where they readily settle and become incorporated into cohesive particles, thereby reducing their detectability in the overlying water column (Sengupta *et al*., 2012). This is further supported by findings that higher egg counts are often recovered from sediments located adjacent to contamination sources (Amoah *et al*., 2020). Taken

Table 1. Presence of other potential medically important microorganisms in the RLS

Detected Microorganisms	Buhi				Bato				Baao		
	NT	NR	NA	C	NR	NT	NA	C	NT	NA	C
Malassezia furfur	-	-	-	-	-	-	+	-	-	-	-
Malassezia restricta	-	-	-	-	-	-	+	-	-	-	-
Malassezia globose	-	-	-	-	-	+	-	-	-	-	-
Rhinosporidium seeberi	-	-	-	-	+	-	-	-	-	-	-
Nitzschia palea	-	-	-	-	+	+	+	+	+	+	+
Nitzschia amphibia	+	+	-	+	-	-	-	-	-	-	-
Gonyostomum semen	-	-	-	-	+	-	+	-	-	-	-
Cyclotella meneghiniana	+	+	+	+	+	+	+	+	+	-	-
Cyclotella scaldensis	-	-	-	-	-	+	+	+	-	-	-
Cryptomonas marssonii	+	-	-	-	+	+	+	+	+	+	-
Cryptomonas curvata	-	-	+	-	+	+	+	+	-	+	-
Cryptomonas pyrenoidifera	+	-	-	-	+	+	+	+	+	+	-
Cryptomonas paramecium	-	-	-	-	-	-	-	-	+	+	-
Cryptomonas obovate	-	-	-	-	-	+	+	+	-	-	-
Chlamydomonas noctigama	-	+	-	-	-	+	-	-	-	-	-
Chlamydomonas reinhardtii	-	+	-	-	+	+	+	+	+	+	+
Chlamydomonas applanata	-	-	-	-	-	-	-	-	+	+	-
Chlamydomonas fasciata	-	-	-	-	+	-	+	-	+	-	-
Chlamydomonas zebra	-	-	-	-	-	+	-	+	-	-	-

Legend: NT is Near Tributary, NR is Near Residential, NA is Near Aquaculture/Agricultural Farms, and C is Center of the lake.

Table 2. Chi-square test of independence between parasite infection data (2019-2022) and demographic profile

Profile	***Entamoeba***		χ^2 (p-value)	***Ascaris***		χ^2 (p-value)	***Trichuris***		χ^2 (p-value)	***Hookworm***		χ^2 (p-value)
	Positive	Negative		Positive	Negative		Positive	Negative		Positive	Negative	
Lake-Associated Communities												
Near	22	18	0.0252 (0.874)	15	25	1.28 (0.258)	2	38	0.157 (0.692)	1	39	2.75 (0.097)
Far	17	15		8	24		1	31		4	28	
Sex												
Male	37	43	0.646 (0.422)	36	44	0.345 (0.557)	5	75	1.04 (0.308)	3	77	0.017 (0.895)
Female	38	34		29	43		2	70		3	69	
Municipality												
Buhi	4	0	4.22 (0.121)	0	4	3.43 (0.180)	0	4	2.36 (0.307)	0	4	0.286 (0.867)
Bato	16	17		16	17		0	33		1	32	
Baao	55	60		49	66		7	108		5	110	
Age (years)												
0 to 10	42	61	10.7 (0.014)	55	48	16.9 (<0.001)	6	97	2.69 (0.442)	3	100	5.49 (0.139)
11 to 25	9	6		1	14		0	15		2	13	
26 to 60	9	2		2	9		1	10		1	10	
60 and above	15	8		7	16		0	23		0	23	

together, these observations indicate that STH eggs introduced into aquatic environments tend to accumulate in nearshore or benthic sediments, underscoring the importance of including sediment and bentho-pelagic sampling in future investigations.

The insignificant association between lakes and percent positivity indicates that all lakes may potentially serve as reservoirs for putative *Cryptosporidium* and *Cyclospora* infection. Coccidian protozoan parasites like *Cyclospora cayetanensis* and *Cryptosporidium* spp. are the causative agents of food and waterborne intestinal parasitic outbreaks, cyclosporiasis, and cryptosporidiosis, respectively (CDC, 2019b, 2019c). Humans can acquire both diarrheal pathogens by consuming food and water contaminated with the parasite, but the latter can also infect a wide range of vertebrate animals. Residents and visitors to developing countries are more likely to become infected due to poor sanitation, but both cases occur worldwide (Almeria *et al.*, 2019; Caravedo & White, 2022). Both can also be detected in stool sample examination using acid-fast staining; however, conventional microscopy methods, which have lower sensitivity, are still widely used including the testing facilities in Camarines Sur. In the Philippines, there is a lack of epidemiological data and mitigation strategies for cryptosporidiosis and cyclosporiasis, with most research focusing on potential sources of contamination. Oocysts of *Cryptosporidium* and *Cyclospora* spp. were previously detected in a major watershed in Metro Manila with a general positivity of 72% (Masangkay, 2020). In other related works, *Cryptosporidium* spp. was found in several freshwater systems in the Philippines with a prevalence of 15%, 33%, and 50% in Luzon, Visayas, and Mindanao samples, respectively (Masangkay *et al.*, 2020). Most *Cryptosporidium* spp. identified through 18S ribosomal DNA in the same study were *C. parvum* and *C. hominis*, known to cause acute gastroenteritis. The prevalence of *Cyclospora cayetanensis* in diarrheic patients from Iloilo was associated with their main source of water, which is a deep well (Buerano *et al.*, 2008). Other protozoan parasites were observed from the same study of almost 3,500 patients with diarrhea, such as *Giardia lamblia*, *Cryptosporidium*, *Cyclospora* spp., and *Isospora* spp., with varying rates of prevalence at 0.03-2.0%. Tracking of fecal contamination sources in Laguna Lake also showed tributaries to be positive for *C. parvum*, *C. muris*, *C. hominis*, and *C. galli*, later traced to either human or agricultural sources (de la Peña *et al*., 2021). Likewise, the characteristics of the lakes i.e., being closely linked to anthropogenic activities such as aquaculture, farming, and nearby human settlements, most likely contributed to the presence of these protozoan parasites.

Although the culture-based methods were able to detect and characterize FLA, isolated FLAs require further taxonomic verification *via* molecular methods to ascertain their identity and potential pathogenicity (Samba-Louaka *et al.*, 2019). Given the current use of Lake Bato and Lake Baao for aquaculture and farming, there is an increased risk of potential FLA infections among users who are in close contact with these environments. Most of the FLAs detected using the EUKAF/EUKAR primer pair have no parasitic infection records except for *Echinamoeba* sp., which may harbor pathogenic bacteria (Wannasan *et al.*, 2013), and *V. vermiformis*, which can cause keratitis and similarly harbor pathogenic bacterial endosymbionts and was also previously isolated from Lake Taal water and fish gut (Milanez *et al.*, 2017). Both *Echinamoeba* sp. and *V. vermiformis* were detected in Lake Buhi, while no helminths and other human parasites were found in any of the lakes using a DNA-based method. Additionally, previously detected *Acanthamoeba* spp. in Lake Buhi which were known to persist even without preservation were also not detected (Masangkay *et al.*, 2024). This highlights the current limitations of parasitome metabarcoding with the primers used and may also reflect a reduced parasitic load during the sampling period (i.e., dry season) leading to less sensitive detection.

On the other hand, different *Malassezia* species, including *M. furfur*, *M. restricta*, and *M. globosa*, detected in Lake Bato are known causative yeast agents of pityriasis versicolor, seborrheic dermatitis and dandruff (Gaitanis *et al.*, 2013). An emerging aquatic protistan parasite, *Rhinosporidium seeberi*, also detected in Lake Bato, has previously been described to cause a human oral or nasal mucosal infection called rhinosporidiosis (Fredricks *et al.*, 2000). *Nitzschia* species, i.e., *N. palea* and *N. amphibia* from all lakes are related to the marine diatom *N. pungens*, known to produce domoic acid-causing amnesic shellfish poisoning (Bates *et al.*, 1989). *Gonyostomum semen* in Lake Bato is known to produce mucilaginous threads that cause skin irritation in humans and is also related to the marine raphidophytes that form harmful algal blooms (HAB) (Hongve *et al.*, 1988). Other algae detected are also noted to contribute to HAB, including *Cyclotella* species (*C. meneghiniana* and *C. scaldensis*), *Cryptomonas* species (*C. marssonii*, *C. curvata*, *C. pyrenoidifera*, *C. paramecium*, and *C. obovata*), and *Chlamydomonas* species (*C. noctigama*, *C. reinhardtii*, *C. applanata*, *C. fasciata*, and *C. zebra*), all found in the RLS.

As expected, parasite diagnostics in low-resource communities rely on actual observational cases when the infection becomes severe, such as during the expulsion of worms. Unfortunately, the microscopy-based techniques facilities near RLS used for parasite detection have very limited detection capabilities if not coupled with concentration protocols or specific staining methods (WHO, 2019). The decrease in the infection data in 2021 was mainly due to the COVID-19 pandemic, which limited the movement of individuals seeking medical consultations. Based on previous health records, it is believed that sources of parasite infection may not be directly linked to the lakes since none of them were detected in the preliminary assessment.

With the limited tests and diagnostic procedures in the communities surrounding the RLS, current parasite prevalence or incidence may be poorly documented. For helminthic parasite detection, where microscopy-based detection is reliable, it is hypothesized that the RLS may not be directly related to its infection cycle. However, *E. histolytica* cases may appear unusually high and might not be the only causative agent for the reported diarrheagenic cases in the RLS communities. It is difficult to ascertain the identity of *E. histolytica* using microscopic methods alone, even with trained eyes, as it looks morphologically similar to *Entamoeba dispar* and other non-pathogenic cysts of amoeba (Rivera *et al*., 2004). In fact, several trained personnel and medical technologists can over-diagnose *E. histolytica* cases by 4-5% (Salazar *et al.*, 1990). Therefore, better detection methods are necessary to provide more accurate epidemiological information, such as DNA-based methods, as previously demonstrated in *E. histolytica* (Rivera *et al.*, 1996, 2020; Rivera & Ong, 2013). Likewise, other potential parasites may be causing these diseases, as in the case of the coccidian parasites detected in the lakes' water samples, which are also known to cause diarrhea in humans. Regular stool examinations cannot specifically detect coccidian oocysts in samples without proper staining or specifically designed markers such as those used in enzyme-linked immunosorbent assays or ELISA tests (Rivera *et al*., 2004). Moreover, the high positivity in patients aged 0-10 years indicates the continued vulnerability of children to parasite infections not only on helminths but also for protozoan parasites.

CONCLUSION

This study assessed the Rinconada Lake System (RLS) as a potential reservoir for parasitic infections. Findings indicate the potential of opportunistic protozoan infections in the RLS which are likely influenced by anthropogenic activities, while helminthic parasites were notably absent in surface waters, suggesting that future investigations should focus on sediments as potential reservoirs.

The detection of other medically relevant microorganisms, including fungi and algae, through metabarcoding will also be valuable in enhancing health awareness. More importantly, the heavy reliance on mass drug administration programs that specifically target helminths, without parallel routine monitoring, highlights a critical gap in proactive health management, as protozoan infections remain prevalent and continue to significantly affect vulnerable groups such as children. The study highlights the need for a more comprehensive approach in parasitic infection control programs of the communities, extending beyond a sole focus on helminths. Enhancing epidemiological surveillance for prevalent parasites observed in the communities can be done by improving poor data management. The findings emphasize the need for tailored and sustainable health interventions that address the specific parasitic landscape of the studied areas, especially in terms of controlling waterborne protozoans.

Conflict of Interest

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS

Special thanks to Bicol University for funding the parasite in the RLS NTDs project (RD-2022-B-2-008), and to the local government units of Baao, Bato, and Buhi, as well as the various health and medical testing facilities, for their support and cooperation.

REFERENCES

Amoah, I.D., Kumari, S., Reddy, P., Stenström, T.A. & Bux, F. (2020). Impact of informal settlements and wastewater treatment plants on helminth egg contamination of urban rivers and risks associated with exposure. *Environmental Monitoring and Assessment* **192**: 713. https://doi.org/10.1007/s10661-020-08660-0

Almeria, S., Cinar, H.N. & Dubey, J.P. (2019). *Cyclospora cayetanensis* and cyclosporiasis: An Update. *Microorganisms* **7**: 317. https://doi.org/10.3390/microorganisms7090317

Archer, J., O'Halloran, L., Al-Shehri, H., Summers, S., Bhattacharyya, T., Kabaterine, N.B., Atuhaire, A., Adriko, M., Arianaitwe, M., Stewart, M. *et al*. (2020). Intestinal schistosomiasis and giardiasis co-infection in Sub-Saharan Africa: Can a one health approach improve control of each waterborne parasite simultaneously? *Tropical Medicine and Infectious Disease* **5**: 137. https://doi.org/10.3390/tropicalmed5030137

Bates, S.S., Bird, C.J., Freitas, A.S.W. de, Foxall, R., Gilgan, M., Hanic, L.A., Johnson, G.R., McCulloch, A.W., Odense, P., Pocklington, R. *et al*. (1989). Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from Eastern Prince Edward Island, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **46**(7): 1203-1215. https://doi.org/10.1139/f89-156

Booth, M. (2018). Climate change and the neglected tropical diseases. *Advances in Parasitology* **100**: 39-126. https://doi.org/10.1016/bs.apar.2018.02.001

Buerano, C., Lago, C., Matias, R., de Guzman, B., Izumiyama, S., Yagita, K. & Natividad, F. (2008). Identification of *Cyclospora* and *Isospora* from diarrheic patients in the Philippines. *Philippine Journal of Science* **137**: 11-15.

Caravedo, M.A. & White, A.C. (2022). Treatment of cryptosporidiosis: nitazoxanide yes, but we can do better. *Expert Review of Anti-Infective Therapy* **21**: 167-173. https://doi.org/10.1080/14787210.2023.2160704

CDC. (Centers for Disease Control and Prevention). (2019a). *CDC - DPDx - Parasites A-Z Index*. Centers for Disease Control and Prevention. https://www.cdc.gov/dpdx/az.html. Accessed 03 February 2025.

CDC. (Centers for Disease Control and Prevention). (2019b). *CDC - DPDx - Cryptosporidiosis*. CDC. https://www.cdc.gov/dpdx/cryptosporidiosis/index.html. Accessed 03 February 2025.

CDC. (Centers for Disease Control and Prevention). (2019c). *CDC - DPDx - Cyclosporiasis*. https://www.cdc.gov/dpdx/cyclosporiasis/index.html. Accessed 03 February 2025.

dela Peña, L.B.R.O., Labrador, K.L., Nacario, M.A.G., Bolo, N.R. & Rivera, W.L. (2021). Microbial source tracking of fecal contamination in Laguna Lake, Philippines using the library-dependent method, rep-PCR. *Journal of Water and Health* **19**: 762-774. https://doi.org/10.2166/wh.2021.119

Fredricks, D.N., Jolley, J.A., Lepp, P.W., Kosek, J.C. & Relman, D.A. (2000). *Rhinosporidium seeberi*: A Human Pathogen from a Novel Group of Aquatic Protistan Parasites. *Emerging Infectious Diseases* **6**(3): 273-282. https://doi.org/10.3201/eid0603.000307

Gaitanis, G., Velegraki, A., Mayser, P. & Bassukas, I.D. (2013). Skin diseases associated with Malassezia yeasts: Facts and controversies. *Clinics in Dermatology* **31**: 455-463. https://doi.org/10.1016/j.clindermatol.2013.01.012

Hagosojos, B., Masangkay, F.R., Fernandez, J.B., Lazaro, J.A., Medroso, D.E., Olaguera, B., Pastores, C.M., Santos, J. & De, G. (2020). Molecular identification of *Acanthamoeba* sp. in Lake Buhi, Philippines. *Annals of Parasitology* **66**: 111-114.

Hongve, D., Løvstad, Ø. & Bjørndalen, K. (1988). *Gonyostomum semen* – a new nuisance to bathers in Norwegian lakes. *SIL Proceedings 1922-2010* **23**: 430-434. https://doi.org/10.1080/03680770.1987.11897957

Hotez, P.J. & Ehrenberg, J.P. (2010). Escalating the global fight against neglected tropical diseases through interventions in the Asia Pacific Region. In: Important Helminth Infections in Southeast Asia: Diversity and Potential for Control and Elimination, Part A, Zhou, X.-N., Bergquist, R., Olveda, R. & Utzinger, J. (editors). pp. 31-53.

Hugerth, L.W., Muller, E.E.L., Hu, Y.O.O., Lebrun, L.A.M., Roume, H., Lundin, D., Wilmes, P. & Andersson, A.F. (2014). Systematic design of 18S rRNA gene primers for determining eukaryotic diversity in microbial consortia. *PLoS ONE* **9**: e95567. https://doi.org/10.1371/journal.pone.0095567

Kounosu, A., Murase, K., Yoshida, A., Maruyama, H. & Kikuchi, T. (2019). Improved 18S and 28S rDNA primer sets for NGS-based parasite detection. *Scientific Reports* **9**: 15789. https://doi.org/10.1038/s41598-019-52422-z

Kumar, T., Onichandran, S., Lim, Y.A.L., Sawangjaroen, N., Ithoi, I., Andiappan, H., Salibay, C.C., Dungca, J.Z., Chye, T.T., Sulaiman, W.Y.W. *et al*. (2014). Comparative Study on Waterborne Parasites between Malaysia and Thailand: A New Insight. *The American Journal of Tropical Medicine and Hygiene* **90**: 682-689. https://doi.org/10.4269/ajtmh.13-0266

Leonardo, L., Hernandez, L., Magturo, T.C., Palasi, W., Rubite, J.M., de Cadiz, A., Moendeg, K., Fornillos, R.J., Tabios, I.K., Mistica, M. *et al*. (2020). Current status of neglected tropical diseases (NTDs) in the Philippines. *Acta Tropica* **203**: 105284. https://doi.org/10.1016/j.actatropica.2019.105284

Lim, Y.A.L. & Nissapatorn, V. (2017). Transmission of waterborne parasites in the Association of Southeast Asian Nations (ASEAN): Overview and direction forward. *Food and Waterborne Parasitology* **8-9**: 75-83. https://doi.org/10.1016/j.fawpar.2017.08.001

Masangkay, F.R. (2020). Increased detection of *Cryptosporidium* and *Cyclospora* spp. oocysts in a major Philippine watershed following rainfall events. *Asian Journal of Biological and Life Sciences* **8**(3): 111-116. https://doi.org/10.5530/ajbls.2019.8.18

Masangkay, F.R., Milanez, G.D., Somsak, V., Kotepui, M., Tangpong, J. & Karanis, P. (2020). Multi-spatial contamination of environmental aquatic matrices with *Cryptosporidium*: a climate, health, and regulatory framework for the Philippines. *Environmental Sciences Europe* **32**: 121. https://doi.org/10.1186/s12302-020-00410-w

Masangkay, F.R., Rona, F.C. IV, Poricallan, A.J.G., Padua, R.L.M., Reyes, M.S.S., Ricacho, J.K.M., Samar, M.J.E., Santos, M.C.I., Tangpong, J., Kotepui, M. *et al*. (2024). First report of biological contaminants in household water in the Philippines and long-term viability of *Acanthamoeba* species in one-year-old household water sediment. *Journal of Water and Health* **22**(11): 2194-2205. https://doi.org/10.2166/wh.2024.391

Milanez, G.D., Masangkay, F.R., Thomas, R.C., Ordona, M.O.G.O., Bernales, G.Q., Corpuz, V.C.M., Fortes, H.S.V., Garcia, C.M.S., Nicolas, L.C. & Nissapatorn, V. (2017). Molecular identification of *Vermamoeba vermiformis* from freshwater fish in lake Taal, Philippines. *Experimental Parasitology* **183**: 201-206. https://doi.org/10.1016/j.exppara.2017.09.009

Moreno, Y., Moreno-Mesonero, L., Amorós, I., Pérez, R., Morillo, J.A. & Alonso, J.L. (2018). Multiple identification of most important waterborne protozoa in surface water used for irrigation purposes by 18S rRNA amplicon-based metagenomics. *International Journal of Hygiene and Environmental Health* **221**: 102-111. https://doi.org/10.1016/j.ijheh.2017.10.008

Noda, S., Hoa, N.T.V., Uga, S., Thuan, L.K., Aoki, Y. & Fujimaki, Y. (2009). Parasite egg contamination of water and air in a suburban area of Hanoi, Vietnam. *Tropical Medicine and Health* **37**: 55-61. https://doi.org/10.2149/tmh.2009-04

Onichandran, S., Kumar, T., Salibay, C.C., Dungca, J.Z., Tabo, H.A., Tabo, N., Tan, T.-C., Lim, Y.A., Sawangjaroen, N., Phiriyasamith, S. *et al*. (2014). Waterborne parasites: a current status from the Philippines. *Parasites & Vectors* **7**: 244. https://doi.org/10.1186/1756-3305-7-244

Ordoñez, K.N., Lim, Y.A.-L., Goh, X.T. & Paller, V.G.V. (2018). Parasite contamination of freshly harvested vegetables from selected organic and conventional farms in the Philippines. *Pertanika Journal of Tropical Agricultural Science* **41**: 1741-1756.

Regis, E. & Dela Cruz, F. (2019). Environmental State of Lakes Bato and Baao, Camarines Sur. Institute for Environmental Conservation and Research, Ateneo de Naga University. https://www.adnu.edu.ph/inecar/research-projects/completed/environmental-state-of-lakes-bato-and-baao-camarines-sur/. Accessed 03 February 2025.

Rivera, P.T., Rivera, W.L. & Solon, J.A.A. (2004). Protozoan infections. In: Philippine Textbook of Medical Parasitology, Belizario, V.Y. Jr. & De Leon, W.U. (editors) 2nd edition. Manila, Philippines: University of the Philippines Manila, pp. 23-67.

Rivera, W.L., Aquino, I.M.C., De Cadiz, A.E., Villacorte, E.A., Tongol-Rivera, P.N. & Kanbara, H. (2020). Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* among residents of an urban slum area in Manila, Philippines as detected by the polymerase chain reaction. *Annals of Parasitology* **66**: 547-553.

Rivera, W.L. & Ong, V.A. (2013). Development of loop-mediated isothermal amplification for rapid detection of *Entamoeba histolytica*. *Asian Pacific Journal of Tropical Medicine* **6**: 457-461. https://doi.org/10.1016/s1995-7645(13)60074-7

Rivera, W.L., Tachibana, H., Silva-Tahat, M.R.A., Uemura, H. & Kanbara, H. (1996). Differentiation of *Entamoeba histolytica* and *E. dispar* DNA from cysts present in stool specimens by polymerase chain reaction: its field application in the Philippines. *Parasitology Research* **82**: 585-589. https://doi.org/10.1007/s004360050169

Salazar, N.P., Pasay, C.J., Avenido, A.O., Macapasir, S.R., Lena, M.J., Maguinsay V.M., Angus, P.R. & Bandalan, J.M. (1990). Detection of *Entamoeba histolytica* in routine stool examination. *Philippine Journal of Microbiology and Infectious Disease* **19**: 57-60.

Samba-Louaka, A., Delafont, V., Rodier, M.-H., Cateau, E. & Héchard, Y. (2019). Free-living amoebae and squatters in the wild: ecological and molecular features. *FEMS Microbiology Reviews* **43**: 415-434. https://doi.org/10.1093/femsre/fuz011

Sengupta, M.E., Andersen, T.J., Dalsgaard, A., Olsen, A. & Thamsborg, S.M. (2012). Resuspension and settling of helminth eggs in water: Interactions with cohesive sediments. *Water Research* **46**: 3903-3912. https://doi.org/10.1016/j.watres.2012.04.033

Straub, D., Blackwell, N., Langarica-Fuentes, A., Peltzer, A., Nahnsen, S. & Kleindienst, S. (2020). Interpretations of environmental microbial community studies are biased by the selected 16S rRNA (Gene) amplicon sequencing pipeline. *Frontiers in Microbiology* **11**: 1-18. https://doi.org/10.3389/fmicb.2020.550420

Vizon, K.C.C., Battad, Z.G. & Castillo, D.S.C. (2019). Contamination of food-borne parasites from green-leafy vegetables sold in public markets of San Jose City, Nueva Ecija, Philippines. *Journal of Parasitic Diseases* **43**: 651-657. https://doi.org/10.1007/s12639-019-01144-0

Wannasan, A., Uparanukraw, P., Songsangchun, A. & Morakote, N. (2013). Potentially pathogenic free-living amoebae in some flood-affected areas during 2011 Chiang Mai flood. *Revista Do Instituto de Medicina Tropical de Sτo Paulo* **55**: 411-416. https://doi.org/10.1590/s0036-46652013000600007

WHO. (World Health Organization). (2019). Bench Aids for the Diagnosis of Intestinal Parasites, 2nd edition. Geneve: World Health Organization. https://www.who.int/publications/i/item/9789241515344. Accessed 03 February 2025.