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Identification and Morphological Evaluation of Protozoa in Hikong Alu and Hikong Bente, Lake Sebu

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ABSTRACT

This study aimed to identify and describe the protozoa found in Hikong Alu and Hikong Bente of Lake Sebu, South Cotabato. Protozoa are single-celled organisms that play vital roles in aquatic ecosystems, serving as decomposers, nutrient cycles, and indicators of water quality. The study used a descriptive-exploratory design, with water samples collected from three stations per site. Samples were prepared as wet mounts, stained with methylene blue or crystal violet, and observed under 4x and 10x magnifications. Results showed a total of 12 protozoa in Hikong Alu and 6 in Hikong Bente, including *Amoeba*, *Paramecium*, *Mastigophora*, and *Heterotrophic flagellates*. Protozoan presence varied across stations due to differences in water flow and nutrient levels. The study concludes that environmental factors influence protozoan diversity and abundance, providing baseline data for future biodiversity and conservation research. It is recommended that future researchers use higher magnifications (40x–100x), conduct seasonal sampling, analyze water parameters, check the pH level of the water in both falls, and ensure microscopy is performed within 24 hours of sampling to ensure optimal protozoa observation.

Keywords: *Protozoa, Morphology, Lake Sebu, Hikong Alu, Hikong Bente*

INTRODUCTION

Protozoa are single-celled eukaryotic microorganisms that play essential roles in aquatic ecosystems. They contribute to nutrient cycling, serve as food for higher organisms, and act as indicators of water quality (Weiss, 2023). Protozoa are usually between 5 and 1000 µm in size. Since all free-living protozoa require water to thrive, they are essentially aquatic and grow in brackish, freshwater (including soil), and marine environments. The group exhibits significant morphological and physiological diversity (Warren & Esteban, 2019). According to Anderson (2020), protozoa play critical roles in ecosystems: some serve as primary producers, others act as decomposers, and many function as links in food chains. By consuming bacteria, algae, and organic matter, protozoa release nutrients back into the environment, thereby supporting ecosystem productivity.

Internationally, protozoa have been studied extensively due to their sensitivity to environmental changes. According to Yuan et al. (2024), their study demonstrated that eutrophication and pesticide exposure significantly alter protozoan communities, reducing species richness when multiple stressors are present. These findings confirm that protozoa respond quickly to ecological disturbances, making them reliable bioindicators. In the Philippines, protozoan research has also highlighted their ecological and health significance. According to Milanez et al. (2020), their study successfully isolated *Acanthamoeba* species from freshwater sources, pointing to their dual role in ecology and potential risks to public health. Furthermore, the examined protozoan abundance in Lake Lanao had revealed strong associations between protozoan distribution and water parameters, reinforcing their value as indicators of lake trophic status (Camar Ameril et al., 2018). A study documented the diversity of free-living amoebae in freshwater ecosystems, emphasizing their ecological importance and potential as indicators for environmental monitoring (Milanez et al., 2022).

Lake Sebu (Lanaw Sbù), located in South Cotabato, Mindanao, Philippines, is a culturally rich and ecologically significant freshwater system. Surrounded by mist-covered mountains and fertile forests, it has been recognized as a biodiversity hotspot and a vital resource for surrounding communities (Lakbaypinas, 2025). Hikong Bente, one of the seven waterfalls in the area, provides a dynamic aquatic habitat likely to harbor diverse protozoan communities. Lake Sebu faces potential threats from human activities, which could impact protozoan biodiversity and overall ecosystem health. Changes in water quality might alter protozoan community structures, affecting their ecological functions. Studying protozoa in Hikong Bente can offer valuable insights into the lake's ecological status and the role of protozoa in its food web and nutrient dynamics. Given protozoa's capacity to serve as indicators of water quality, examining these microorganisms can contribute to understanding and potentially managing environmental impacts on Lake Sebu. Although numerous studies have been carried out across different geographic scales, baseline information on protozoa in Lake Sebu remains limited. Addressing this gap through morphological evaluation of protozoa in Hikong Bente can enrich understanding of freshwater biodiversity and support conservation and management strategies in the region.

Objectives of the Study

The study aims to identify the diversity of protozoans exists in Hikong Bente and Hikong Alu, Lake Sebu. Additionally, the morphological characteristics of protozoans will also be determined. To identify and conduct morphological evaluations of protozoa present in Hikong Alu and Hikong Bente, Lake Sebu. To quantify protozoans from the stream line of hikong alu and hikong bente, lake sebu.

1. To determine the morphological characteristics of the collected protozoans using microscopy.
2. To identify the collected protozoans up to the genus level only.

Literature Review

Protozoa

Protozoa are a varied group of single-celled eukaryotic organisms that significantly contribute to nutrient mineralization in soil, making these nutrients accessible to plants and other organisms in the ecosystem. They're often colloquially called "one-celled animals" because they exhibit animal-like traits such as movement and predation, and unlike plants and many algae, they lack a cell wall. These microorganisms can live freely or be parasitic, feeding on organic matter like other microorganisms, tissues, and debris. Protozoa reproduce either sexually or asexually, with asexual binary fission being common; sexual reproduction forms include isogamy and the more prevalent anisogamy. Beyond soil, protozoa are vital in aquatic food chains. In polluted waters, many protozoa species consume organic matter, serving as food for insect larvae and worms, which are then eaten by larger creatures like fish, lobsters, and crabs – ultimately entering the human food chain. In specific ecosystems like the Godavari River, notable protozoa include *Paramecium, Euglena, Coleps, and Bursaridium difficile,* highlighting their diversity in aquatic environments (Rai et al., 2023).

Free-living amoebas (FLAs), protozoa found in diverse aquatic habitats, have been isolated from freshwater fishes in Lake Taal, Philippines, highlighting their presence in tropical freshwater ecosystems. Molecular identification and phylogenetic analysis have characterized FLAs like *Vermamoeba vermiformis,* which was isolated from the gut of *Oreochromis niloticus* (tilapia), contributing insights into their genetic relationships and environmental distributions. Such findings expand understanding of protozoan diversity in freshwater fish hosts and prompt considerations of their ecological roles and potential implications for host health, underscoring the utility of molecular tools in exploring these microorganisms in Lake Taal and similar aquatic environments (Milanez et al., 2017).

Protozoa are single-celled eukaryotic organisms capable of living as parasites or free-living entities, interacting with diverse environments and hosts ranging from bacteria to humans. They represent a significant source of parasitic diseases, claiming over a million lives annually worldwide due to complications from infections. Notable pathogenic protozoa include *Trypanosomatidae* (like *Trypanosoma brucei* causing sleeping sickness, *T. cruzi* causing Chagas disease, and *Leishmania species* causing leishmaniasis) and *Apicomplexa* (involved in malaria and toxoplasmosis). These parasites have complex life cycles involving multiple hosts and cellular interactions, with many infecting host cells. Despite high infection and mortality rates, particularly in low-income developing regions, current therapies are limited, underscoring the urgent need for effective drugs and public health initiatives. The *Trypanosomatidae* family, part of the Kinetoplastida order, features flagellated protists with a distinctive kinetoplast. Pathogenic *trypanosomatids* like *T. brucei* (sleeping sickness in Africa), *T. cruzi* (Chagas disease in Latin America), and *Leishmania* species (leishmaniasis globally in tropical/subtropical areas) are classified by the WHO as neglected diseases affecting impoverished populations lacking efficient treatments. Research indicates the importance of understanding protozoa like ciliated protozoa in freshwater ecosystems, correlating with pathogens like *Giardia duodenalis* and *Cryptosporidium spp*., highlighting contamination risks in water sources supplying millions, as seen in studies from Brazil's Atibaia River. Such findings stress continuous monitoring needs for water quality and underscore protozoa's role in environmental and public health contexts (Bonatti et al., 2023).

According to Weerakoon et al. (2018), in tropical regions, particularly within impoverished communities, co-parasitism is a common phenomenon that contributes significantly to disease burden. While intestinal helminth infections like *Schistosoma japonicum* have been widely documented, the prevalence and impact of diseases caused by intestinal protozoa (IP) remain underexplored in the Philippines. This study provides an in-depth examination of polyparasitism in a rural community in Northern Samar, specifically focusing on co-infections involving intestinal protozoa and *Schistosoma japonicum*, shedding light on the interplay of these parasitic infections in this setting.

The study explored the connection between protozoan species and environmental factors in the Nile River, emphasizing their possible role as indicators of water quality. Protozoans were collected monthly from six locations along a gradient of environmental conditions. The majority of identified protozoan species were ciliates, with *Paradileptus elephantinus* being the most prevalent and omnivorous species. The findings indicated that aluminium, fluoride, and turbidity had a detrimental impact on both abundance and biomass, while dissolved oxygen and potassium had a positive effect on biomass. The prevalent species *Centropyxis aculeata* were linked to variables associated with runoff, whereas bacterivorous ciliates such as *Colpidium colpoda, Glaucoma scintillans, and Vorticella convallaria* were connected to heterotrophic bacteria, phytoplankton biomass, and total organic carbon. This study underscores the potential of protozoans as bioindicators for assessing water quality in the Nile River (El-Tohamy et al., 2024).

Freshwater sponges in Lake Buhi actively filter large volumes of water to acquire nutrients, and in doing so, they can accumulate contaminants like the parasitic protozoans *Cryptosporidium* and *Giardia*, which are major pollutants in aquatic ecosystems. Researchers examined five different aquatic components from Lake Buhi: surface water, water sediments, substrate-associated biofilms, and five sponge samples, looking for *Cryptosporidium* and *Giardia (oo)cysts*. They used modified Kinyoun's acid-fast stain to detect

Cryptosporidium and immunofluorescence staining to detect both *Cryptosporidium* and *Giardia*. Scanning Electron Microscopy helped visualize the sponges' structures and simulate contamination with *Cryptosporidium parvum oocysts*. Results showed that water sediments and substrate-associated biofilms had 20% positivity for *Cryptosporidium oocysts*. Overall, 60% of the aquatic samples tested positive for *Cryptosporidium*. Notably, all five freshwater sponges (identified as *Radiospongilla cfr. philippinensis*) tested positive for *Cryptosporidium* (100%), and one sponge tested positive for *Giardia* (20%). Experiments spiking sponges with *Cryptosporidium parvum oocysts* showed these parasites can fit into and get trapped in the sponge's water channels. The presence of *Cryptosporidium* and *Giardia (oo)cysts* in these sponges indicates contamination with infectious stages of these parasites. This study suggests freshwater sponges like *Radiospongilla cfr. philippinensis* could serve as ecological indicators – acting as reservoirs and potential biomonitors for waterborne parasitic protozoans in aquatic ecosystems, offering a promising perspective on using them to track contamination contamination (Masangkay et al., 2022).

Finding pathogens, bacteria, and protozoa in various treated urban wastewaters and connecting biological pollution to wastewater treatment plant operations are the goals of this study project. The makeup of bacteria and parasites is taken into consideration while examining the potential for water reuse. *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Clostridium perfringens (spore), Salmonella species, Legionella species, helminth eggs, Giardia, and Cryptosporidium species* are among the bacteria and protozoa that were examined, and *amoebae* that live freely (FLA). The primary distinction between the chosen municipal wastewater treatment plants (MWTPs), which are situated in Navarra, Spain, is that some of them employ natural lagoons for tertiary treatment. According to the results of the bacteriological identification, the pollution was primarily of fecal origin. In certain MWTPs, the use of natural lagoons as a tertiary treatment had a significant disinfecting effect. Furthermore, whereas FLA was found in every instance, disease parasites like *Giardia and Cryptosporidium* were not found in the samples under investigation (Mosteo et al., 2013).

Rondello Bonatti et al. (2023) carried out a comprehensive molecular characterization of waterborne protozoa in surface water and sediments across Brazil, with particular emphasis on ciliated protozoa. Their taxonomic survey provided insights into the diversity and distribution of these organisms, while also exploring their correlation with *Giardia duodenalis* and *Cryptosporidium* spp., two protozoan parasites of major concern for human and animal health. By employing molecular tools, the researchers were able to identify a wide range of ciliate taxa and demonstrate how their presence and abundance could be linked to the occurrence of pathogenic protozoans in aquatic environments. This study not only contributes to the understanding of protozoan ecology in Brazilian freshwater systems but also underscores the importance of monitoring these organisms as indicators of water quality and potential health risks.

Strazdaitė-Žielienė et al. (2022) investigated the presence of *Sarcocystis* parasites in environmental water samples, addressing a major research gap since most studies on these protozoans have focused on animal tissues. The researchers tested different molecular approaches to detect *Sarcocystis*, including variations in sample volume, sporocyst isolation techniques, and conventional PCR methods. They further developed species-specific primers to improve detection accuracy. Among the evaluated approaches, nested PCR targeting the cox1 gene with species-specific primers proved to be the most reliable, successfully detecting *Sarcocystis* DNA in 97.4% of the analyzed samples (111 out of 114). This study represents the first PCR-based identification of multiple *Sarcocystis* species in environmental water, including *S. bovifelis, S. cruzi, S. hirsuta, S. arieticanis, S. tenella, S. capracanis, S. bertrami,* and *S. miescheriana*. As a pilot study, their findings provide significant groundwork for developing standardized and effective methods to monitor *Sarcocystis* in aquatic environments, highlighting the importance of water as a potential route of protozoan transmission.

Morphological Evaluation of Protozoa

Protozoa are eukaryotic, single-celled microorganisms that can have a variety of shapes, including elongated, spherical, or oval forms, and some that change shape over the course of their life cycle. Larger ones can be seen without magnification, and their diameters range from 1 μm to 2 mm. Similar to animal cells, protozoa can move during specific stages of life, have no cell walls, and consume food particles. Some *phytoflagellate* protozoa, on the other hand, resemble plants and obtain their energy from photosynthesis. The cell structures of protozoa are similar to those of animals. Certain protozoa can be seen moving quickly in water under a microscope by swimming with flagella or cilia, which are short, hair-like appendages (Pelczar et al., 2025).

Protozoa are unicellular eukaryotic organisms that exist as parasites or free-living entities, interacting with diverse environments and hosts. They cause significant diseases like malaria (*Plasmodium* spp.), sleeping sickness (*Trypanosoma brucei*), Chagas disease (*T. cruzi*), leishmaniasis (*Leishmania* spp.), and toxoplasmosis (*Toxoplasma*), leading to over a million deaths annually worldwide. *Trypanosomatidae,* part of the order *Kinetoplastida*, are flagellated protists with a distinctive kinetoplast; they have complex life cycles involving various hosts and cells. These pathogens are classified as neglected diseases by the WHO, affecting vulnerable populations in developing regions with limited treatment options, underscoring the urgent need for effective therapies and public health initiatives to improve outcomes. Research on protozoa like soil testate *amoebae* also highlights morphological and biometric variations crucial for species identification, as seen in studies characterizing species like those in the *Nebela* group (Duque et al., 2013).

According to Ma et al. (2022), tiny sand-loving (*psammophilic*) ciliates along Qingdao's coast in China found several marine species called *karyorelicteans*. They focused on four *Remanella species*, including two new ones: *Remanella elongata* and *Remanella aposinica*. The others were *R. rugosa* (studied in detail for the first time here) and *R. unicorpusculata*. *R. elongata* stands out with complex granules on its surface, fewer big nuclei, and a longer body. *R. aposinica* differs with specific cilia patterns (14-17 right rows; 24-37 dikinetids inside mouth area). Researchers suggest *R. rugosa var. unicorpusculata* should be called a full species, *Remanella unicorpusculata*, based on new data. They looked at genetic info (SSU rDNA) for all four and found *Remanella* species form a branch related to another genus, *Loxodes*, with decent genetic support.

The research of Galal (2022) conducted in Lake Manzala tracked the lake's physico-chemical parameters alongside the prevalence and biodiversity of zooplankton, with a focus on protozoan organisms. Protozoa emerged as the most common zooplankton group, with ciliates being predominant, followed by flagellates and then sarcodines. Organisms were concentrated using cold centrifugation at 7°C and examined via a Carl-Zeiss Jena inverted microscope. Findings indicated Lake Manzala's pollution levels are notably higher than those in the Nile branches, likely attributable to unchecked discharges of pollutants from multiple provinces, oil contaminants from the Mediterranean, and sewage. Comparing current data to past records suggests some advancements have been made, though further urbanization and ecological management are seen as necessary for progress on both ecological and economic fronts.

Rhizopods (including naked and testate amoebae) and *actinopods* (like *heliozoa*) are often overlooked in studies of lake plankton, largely due to methodological and taxonomic challenges. However, research suggests these *sarcodines* – comprising *amoebae* and *heliozoans* – can be significant components of freshwater plankton communities, irrespective of the lake's trophic status or geographical location. While generally not as numerous as *heterotrophic nanoflagellates, planktonic sarcodines* can be as abundant or even more so than ciliates, highlighting their potential ecological importance. Separate studies focusing on ciliated protists in Lake Weishan Wetland, China, identified four epibiotic peritrichous ciliates, including two known species *(Epistylis cambari and Epistylis lwoffi*) and two new species (*Parapiosoma typicum and Orborhabdostyla gracilis*), leading to the proposal of a new genus, *Parapiosoma*. Phylogenetic analyses based on genetic sequence data indicate the *Epistylididae* family encompasses morphospecies with distinct evolutionary lineages, suggesting *Parapiosoma* might represent a novel taxon at the family level (Wu et al., 2023).

Microscopy of Protozoa

In recent years, new methods such as scanning transmission electron microscopy (STEM) have made studying biological samples faster and more effective. Unlike traditional transmission electron microscopy (TEM), STEM can analyze thicker slices (up to 1 µm) and, when combined with cryo-electron tomography, enables 3D imaging of samples in their natural wet state. This technique, called cryo-scanning transmission electron tomography (cryo-STET), was first introduced in 2014 and has opened new insights into the structure and ultrastructure of protozoans. Protozoa, important waterborne pathogens, have received less attention compared to bacteria in drinking water studies. Research on a large subtropical city's drinking water system revealed protozoa were widespread in tap water, with amoebae being dominant. The bacteria associated with protozoa included potential pathogens, especially those hosted by amoebae. Current disinfection methods were ineffective against protozoa and their bacteria, while ultrafiltration membranes surprisingly supported amoebae and their linked bacterial growth. These findings highlight that viable protozoa and their associated bacteria in drinking water may pose risks to public health (Mai et al., 2023).

Kahraman et al. (2024) introduced an advanced approach for protozoa detection using the YOLOv4 deep learning algorithm applied to microscopic imaging. Their study targeted freshwater protozoa such as *Paramecium* and *Stylonychia pustulata*, creating a dataset from various water sources to train and validate the model. The YOLOv4-based system achieved remarkable performance, with an overall accuracy of 97%, an f1-score of 0.95, precision of 0.92, sensitivity of 0.98, and a mean average precision (mAP) of 0.9752. These results demonstrated its superiority over conventional identification methods in terms of both speed and precision. Beyond the algorithm, the researchers also developed a user-friendly desktop application for practical use, enabling real-time protozoa recognition. This innovation holds great promise for environmental monitoring, parasitology, water quality assessment, and ecosystem preservation, offering rapid and reliable analysis of protozoa in diverse aquatic contexts.

According to Villaruel and Camacho (2023), the Tadlac Lake that is formerly used for aquaculture, experienced severe water quality degradation culminating in a massive fish kill in 1999. Aquaculture was subsequently banned to allow for natural rehabilitation. This study assessed the trophic status of the lake through zooplankton community analysis and physico-chemical parameters from October 2017 to March 2018. A total of 25 zooplankton species were recorded, dominated by *Rotifera* (46.19%), followed by *Copepoda* (36.70%) and *Cladocera* (17.11%). Eutrophic indicator species such as *Brachionus forficula, Keratella tropica, and Brachionus havanaensis* were the most abundant taxa. Statistical analyses showed significant temporal variation in zooplankton density, with environmental parameters such as dissolved oxygen, conductivity, pH, biological oxygen demand, and temperature strongly influencing community structure. Findings confirmed that Tadlac Lake remains eutrophic with moderate organic pollution, highlighting the need for further management strategies to control nutrient enrichment and improve water quality.

The detection of protozoan pathogens depends on either the direct observation of the specific causal agent in clinical samples or the identification of particular immune responses in the host. While a significant portion of protozoan diagnostics continues to use microscopy, molecular methods, especially polymerase chain reaction (PCR)-based techniques, have become increasingly important in recent years. One major benefit of molecular methods is their capability to identify organisms at a level below the genus, something that light microscopy often cannot achieve. Serological tests are highly beneficial for diagnosing tissue parasites and most extra-intestinal infections, although they are less effective in cases of acute infections with short incubation periods and in patients with weakened immune systems. Rapid card tests, which can detect parasite antigens and host antibodies, are available for various key protozoan parasites and are particularly useful in field settings (Walochnik & Aspöck, 2012).

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METHODS

Research Design

This research is a descriptive and exploratory study that focuses on analyzing and identifying the morphological characteristics of protozoa. The study was conducted at Hikong Alu and Hikong Bente in Lake Sebu, where researchers collected protozoan samples from the waterfall streamlines. It aims to describe the physical appearance and unique features of protozoa found in these areas, examining how they live in freshwater environments and how they are morphologically evaluated. The research design combines both qualitative and quantitative approaches to provide a comprehensive understanding of protozoan morphology. Classical and modern techniques were utilized to ensure accuracy and reliability of the findings.

Research Locale

This study was conducted at Hikong Alu and Hikong Bente, the first and second waterfall of the Seven Falls located in Lake Sebu, South Cotabato, Philippines. Lake Sebu is a first-class municipality in the province of South Cotabato, situated in the SOCCSKSARGEN Region of Mindanao. Hikong Alu, also called the "Passage Falls" and Hikong Bente, also called the “immeasurable falls,” are two of the seven waterfalls of Lake Sebu and major tourist attractions. Surrounded by rich vegetation, they provide a freshwater habitat suitable for diverse aquatic organisms, including protozoa. The site was selected for its ecological importance, accessibility, and relevance to freshwater biodiversity and conservation.

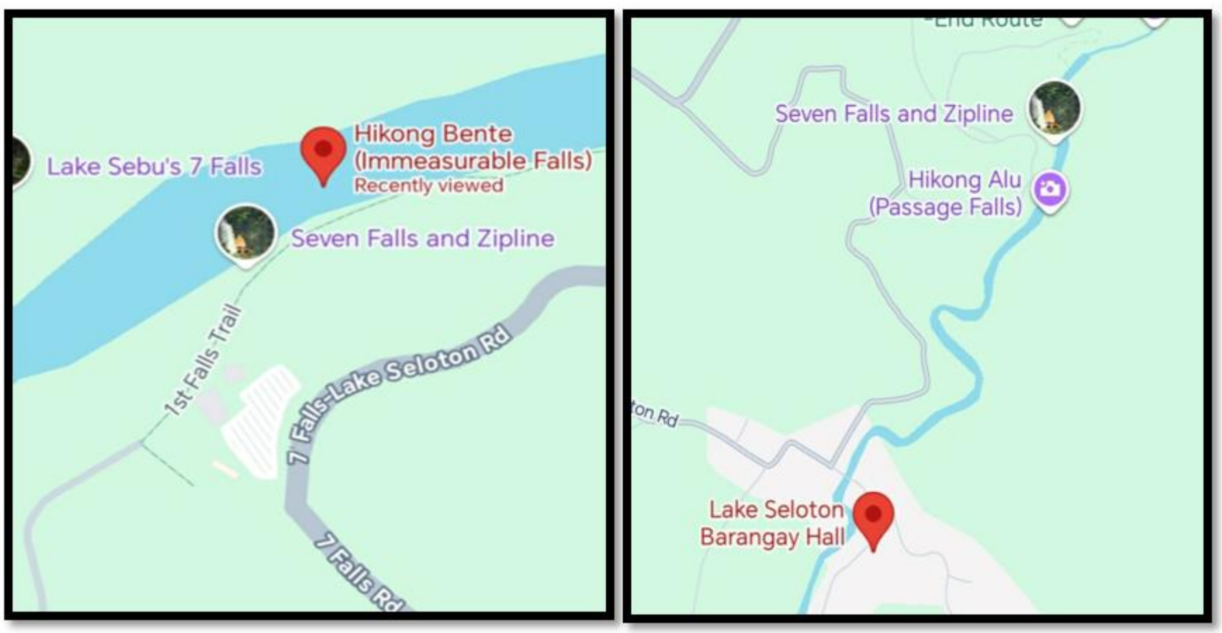

Figure 1. *Map of the Municipality of Lake Sebu, South Cotabato, showing Barangay Seloton and the location of Hikong Bente (Immeasurable Falls) and Hikong Alu (Passage Falls).*

Sampling Technique

A purposive sampling method was employed to collect protozoan samples from different points along the waterfall streams of Hikong Bente and Hikong Alu, Lake Sebu. This method was chosen to specifically target areas where protozoa are most likely to be present and exhibit high diversity. Sampling focused on shallow zones with organic matter, midstream flow, and downstream areas potentially influenced by human activity. Each waterfall site was divided into three sampling stations, and three samples were collected per station to ensure data accuracy and representativeness. Sterile 60 mL specimen bottles were used for collecting samples by submerging them approximately 10–20 cm below the water surface, facing upstream to minimize contamination. A 3 mL syringe served as an alternative to a transfer pipette for transferring the water samples into the specimen bottles, and it was rinsed with distilled water after each use to prevent cross-contamination. The distance between each station was measured using a measuring tape, maintaining a 15-meter interval between points. All collected specimen bottles were placed in sealed plastic bags, properly labeled, stored in coolers, and immediately transported to the laboratory for further analysis and morphological examination of the protozoa.

Sample Preparation

In the laboratory, drops of the collected water samples were taken using a dropper and placed onto glass slides, then covered with cover slips to prepare wet mounts for observation. To enhance the visibility of cellular structures and facilitate identification of protozoan species, some samples were treated with simple stains, specifically methylene blue and crystal violet, which were also applied using a dropper. The prepared slides were then examined under a microscope to study the morphology and distinguish different protozoan species.

Microscopy

Protozoan samples were examined using a compound light microscope under 4x and 10x objectives, as these magnifications provided sufficient visibility for observation. Wet mounts were first examined under low power (4x) to locate organisms, followed by 10x for a more detailed study of morphology, movement patterns, and internal structures. Selected samples were treated with methylene blue and crystal violet to enhance visibility of cellular structures and facilitate species identification. When available, phase-contrast microscopy was used to observe live specimens. Observed protozoa were photographed using a smartphone camera for documentation and subsequently used for classification and comparison with standard taxonomic references.

RESULTS AND DISCUSSION

Quantity Of Protozoa

Table 1. *Protozoan count in each station and sample oh Hikong Alu.*

FALLS	STATION	SAMPLE	NO. OF PROTOZOANS OBSERVED	Total no. of protozoan
HIKONG ALU	1		4	7
		2	2	
		3	1	
	2	1	0	0
		2	0	
		3	0	
	3	1	0	5
		2	4	
		3	1	

The total number of protozoa identified in Hikong Alu was 12. Among the three stations sampled, Station 1 showed the highest protozoan presence, with 7 organisms observed. The findings from Hikong Alu showed a total of twelve protozoans across all three sampling stations, revealing noticeable differences in microhabitat conditions within the waterfall area. Station 1 contained the highest number of protozoa, with seven individuals recorded. This suggests that Station 1 may offer more favorable environmental conditions, such as calmer water flow, greater organic matter, and higher microbial food availability—factors known to support protozoan survival and reproduction. The Centers for Disease Control and Prevention (CDC, 2024) notes that protozoa thrive in moist environments that have adequate nutrients and limited disturbance. Station 3 recorded five protozoans, indicating that certain areas within the station also provide suitable microhabitats. In contrast, no protozoans were identified in Station 2. This absence may be associated with strong water turbulence or limited organic material, conditions that hinder the establishment of protozoan communities. These results align with findings from the U.S. Geological Survey showing that high water velocity can negatively affect microbial settlement and distribution (USGS, 2023).

Table 2. *Protozoan count in each station and sample oh Hikong Bente.*

FALLS	STATION	SAMPLE	NO. OF PROTOZOANS OBSERVED	Total no. of protozoan
HIKONG BENTE	1	1	3	5
		2	2	
		3	0	
	2	1	0	0
		2	0	
		3	0	
	3	1	1	1
		2	0	
		3	0	

The total number of protozoa observed in Hikong Bente was 6. The protozoan distribution in Hikong Bente displayed a similar pattern to Hikong Alu. Station 1 contained the highest count with five protozoans, suggesting that favorable environmental conditions may be consistently present in specific areas of the waterfall. According to the National Institutes of Health (NIH, 2021), protozoans often flourish in nutrient-rich habitats with abundant decaying organic matter. Station 3 showed only one protozoan, reflecting a more limited suitability for sustaining protozoan life. Meanwhile, Station 2 contained no protozoans across all samples, indicating that ecological conditions there may be unfavorable. This pattern supports ecological research demonstrating that protozoan populations decline sharply in unstable or nutrient-poor freshwater conditions (ScienceDirect, 2020). The parallel between both waterfalls highlights how microhabitat stability strongly influences protozoan abundance.

Morphological Evaluation Of Protozoa

Table 3. Morphological Evaluation of the collected Protozoa in Hikong Alu.

Station	Sample	Shape	Internal organelles	Locomotion structure	Surface structure
1	1	Round shape	Has nucleus	Has flagella	Has a clear cell membrane
	2	Round and oval shape	Has vacuole	No locomotion	Has shell and hair like a Parasitic protozoan
	3	Oval shape	Has nucleus	Has broken flagellum	Clear cell membrane
2	1	N/A	N/A	N/A	N/A
	2	N/A	N/A	N/A	N/A
	3	N/A	N/A	N/A	N/A

3	1	N/A	N/A	N/A	N/A
	2	Brick shape	Cytoplasmic dots	Has broken flagellum	Has a thick cell membrane
	3	Oval shape	Has nucleus	Has cilia	Has a thick cell membrane

Morphological observations from Hikong Alu revealed a variety of protozoan forms that reflect differing adaptive strategies. Station 1 showed organisms with round to oval bodies, some containing visible nuclei, vacuoles, and flagella—structures essential for movement and nutrient processing. The University of Minnesota (2023) emphasizes that vacuoles are vital for regulating water balance and digestion in protozoans. One sample displayed features resembling a parasitic protozoan, indicated by its shell-like covering and hair-like projections. This may suggest the presence of a cyst-forming stage or a species adapted to fluctuating environmental conditions commonly found near waterfalls, where changes in water flow can trigger protective structural responses. Station 2 yielded no protozoa, suggesting unsuitable environmental conditions for cellular structures to sustain themselves, possibly due to strong currents or low nutrient availability. In Station 3, more structurally complex organisms were observed, including a brick-shaped organism with cytoplasmic dots and a damaged flagellum, as well as a ciliated organism resembling Paramecium. Britannica (2024) notes that Paramecium species possess thick membranes and coordinated cilia, enabling efficient movement in calm freshwater habitats. The morphological diversity observed in Hikong Alu reflects differences in microhabitat conditions across stations and highlights how even subtle variations in substrate stability, organic matter, and water turbulence can significantly influence the structural development and survival of protozoan populations.

Table 4. *Morphological Evaluation of the collected Protozoa in Hikong Bente.*

Station	Sample	Shape	Internal organelles	Locomotion structure	Surface structure
1	1	Irregular shape	Has nucleus and granular iinside	Pseudopodia	Has clear cell membrane
	2	Irregular shape	Vacuolated	Pseudopodia	Has clear cell membrane
	3	N/A	N/A	N/A	N/A
2	1	N/A	N/A	N/A	N/A
	2	N/A	N/A	N/A	N/A
	3	N/A	N/A	N/A	N/A
3	1	Pentagon shape / oval shape	Nucleus is missing	Has broken flagellum	Has thick cell membrane
	2	N/A	N/A	N/A	N/A
	3	N/A	N/A	N/A	N/A

Hikong Bente exhibited fewer morphological variations compared to Hikong Alu, indicating lower protozoan diversity. In Station 1, organisms with irregular shapes, granular interiors, and pseudopodia were observed, consistent with amoeboid protozoans. Amoebas commonly inhabit moist, nutrient-rich environments where they actively move and capture food through pseudopodia (Cleveland Clinic, 2024). Many samples contained only algae, suggesting limited conditions for protozoan growth and possibly a greater competition for space or nutrients among microorganisms. In Station 3, a pentagon-shaped organism with a thick membrane and broken flagellum was identified, possibly reflecting environmental stress or physical disturbance. Research from the University of California (2020) shows that microorganisms exposed to fluctuating environmental conditions often exhibit structural damage. The frequent absence of protozoa across multiple samples in Hikong Bente indicates that the site may have harsher or more variable

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ecological conditions, such as inconsistent water flow, reduced organic content, or greater physical pressure from moving sediments. These factors collectively suggest that only highly resilient or stress-tolerant protozoan forms are able to survive in this environment.

Identification of Protozoans (Genus Level)

Table 5. *Identification of Protozoa in Hikong Alu.*

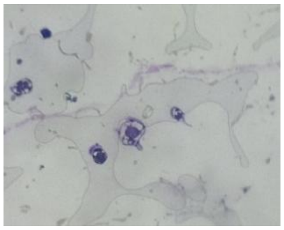
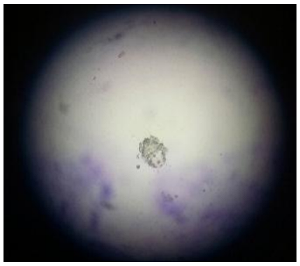
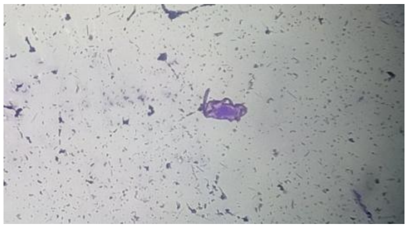
Station	Sample	Observation	Possible Classification (Genus/Group)
1	1	Round, bubble-like organism	Amoeba 
	2	Parasite-like presence	Parasitic protozoan 
	3	Oval shape, nucleus, broken flagellum	Heterotrophic flagellate 

Table 5.1. *Data Gathered in station one from Hikong Alu, Lake sebu.*

The protozoans in Hikong Alu Station 1 demonstrated diverse characteristics indicating the presence of multiple taxonomic groups. In Table 5.1 of Hikong Alu, Station 1 Sample 1, the researchers observed a round, bubble-like organism, which may indicate the presence of simple protozoan forms. The round, bubble-like organism observed in Sample 1 resembles an amoeba, which thrives in freshwater environments rich in nutrients (Mayo Clinic, 2023). Sample 2 exhibited parasite-like features, suggesting the possibility of organic contamination or nutrient accumulation that supports parasitic organisms. Sample 3 contained an oval organism with a visible nucleus and a damaged flagellum, indicating a heterotrophic flagellate that may have experienced physical stress from water movement. Flagellates, according to the University of Michigan Museum of Zoology (2023), play a major role in consuming bacteria and maintaining nutrient cycling in aquatic ecosystems. Overall, Station 1 exhibited a diverse range of microscopic organisms, suggesting that this area provides suitable environmental conditions such as organic content and moderate water flow.

Station	Sample	Observation	Possible Classification (Genus/Group)
2	1	No protozoa observed, mostly algae and other waste from the water.	none
	2	No protozoa observed, mostly algae and other waste from the water.	none
	3	No protozoa observed, mostly algae and other waste from the water.	none

Table 5.2. *Data Gathered in station two from Hikong Alu, Lake sebu.*

In Table 5.2 of Hikong Alu, Station 2 Samples 1, 2, and 3 revealed no observable protozoa under microscopic examination. No protozoa were observed in any of the samples collected from Hikong Alu Station 2. The presence of algae and debris without protozoans suggests that this area may lack the necessary stable conditions for protozoan survival. The World Health Organization (WHO, 2023) notes that protozoa are highly sensitive to environmental disturbances and require stable, nutrient-rich microhabitats. The complete absence of protozoa in Station 2 indicates that it may be the most ecologically affected area within Hikong Alu.

Station	Sample	Observation	Possible Classification (Genus/Group)
3	1	No protozoa observed, mostly algae and other waste from the water.	none
	2	Brick shape, cytoplasmic dots, damaged flagellum	Mastigophora 
	3	Shape like *Paramecium*	Paramecium 

Table 5.3. *Data Gathered in station three from Hikong Alu, Lake sebu.*

In Table 5.3 of Hikong Alu, Station 3 Sample 1 showed no protozoan presence. However, Sample 2 displayed a brick-shaped organism with visible dot-like structures and a broken flagellum, which suggests

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that it may belong to the phylum Mastigophora, a group characterized by flagella used for locomotion. Research shows that flagellated protozoa commonly occupy freshwater environments where they feed on microbial resources (ScienceDirect, 2022). Another sample resembled a Paramecium, which typically inhabits calm freshwater ecosystems with moderate levels of organic matter (Britannica, 2024). The varied observations suggest that although Station 3 may not be consistently favorable, it supports protozoan presence in specific microzones. This pattern indicates that protozoan distribution within Station 3 may be influenced by subtle environmental variations such as localized nutrient concentrations, microhabitat shelter, or slight differences in water flow. The absence of protozoa in Sample 1 could be attributed to harsher microconditions—possibly stronger currents, limited organic matter, or recent disturbances—while Samples 2 and 3 reveal that certain pockets still provide suitable conditions for survival. The presence of Mastigophora-like organisms and Paramecium suggests that Station 3 hosts a mix of protozoan niches, each supporting species with different ecological requirements. Overall, these findings highlight the complexity of microhabitats within a single station and emphasize the importance of examining multiple samples to accurately assess protozoan diversity and distribution.

Table 6. *Identification of Protozoa in Hikong Bente.*

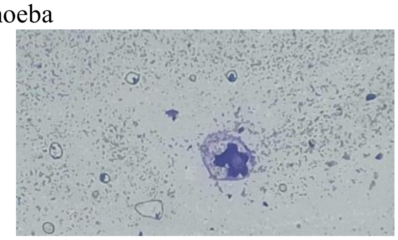
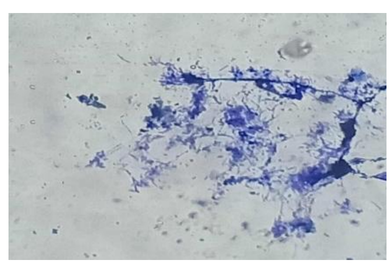
Station	Sample	Observation	Possible Classification (Genus/Group)
1	1	Irregular shape, pseudopods	Amoeba 
	2	Irregular shape, pseudopods	Amoeba 
	3	Presence of algae and slime molds	Myxomycetes 

Table 6.1. *Data Gathered in station one from Hikong Bente, Lake sebu.*

Station 1 of Hikong Bente displayed the highest protozoan diversity in the area. In Table 6.1 of Hikong Bente, Station 1 Samples 1 and 2 revealed the presence of amoebas, characterized by their irregular shape and pseudopod movement. The detection of amoebas suggests a nutrient-rich and moist environment favorable to their survival. The Centers for Disease Control and Prevention (2024), highlights that amoebas are commonly found in freshwater environments with abundant organic matter. Additionally, the presence of myxomycete-like organisms alongside algae suggests a highly moist and nutrient-enriched microhabitat. According to the Missouri Botanical Garden (2023), slime molds such as myxomycetes typically grow in areas containing decaying vegetation and moisture. This richness in microbial life indicates that Station 1 provides the most suitable conditions among all Hikong Bente stations.

Station	Sample	Observation	Possible Classification (Genus/Group)
2	1	No protozoa observed, mostly algae and other waste from the water	none
	2	No protozoa observed, mostly algae and other waste from the water	none
	3	No protozoa observed, mostly algae and other waste from the water	none

Table 6.2. *Data Gathered in station two from Hikong Bente, Lake sebu.*

In Table 6.2 of Hikong Bente, Station 2 Samples 1 to 3 showed no protozoa under microscopic observation. This absence may be due to unfavorable environmental conditions such as increased water movement or low nutrient levels. The lack of organisms could also indicate minimal organic debris or environmental factors that disrupt microscopic habitation. Similar to Hikong Alu Station 2, no protozoa were identified in any sample from Hikong Bente Station 2. The dominance of algae and absence of protozoans demonstrate that the habitat conditions in this station may be too unstable to support protozoan communities. The University of Toronto (2022) emphasizes that protozoa require stable environmental conditions to maintain normal feeding and movement behaviors. These findings indicate that Station 2 is one of the most ecologically limiting sites in both waterfalls.

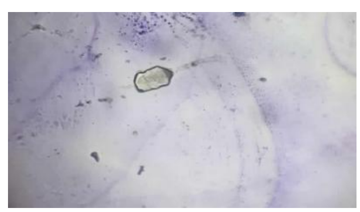
Station	Sample	Observation	Possible Classification (Genus/Group)
3	1	Protozoan with broken flagella, thick capsid, pentagon shape; nucleus absent	Paramecium 
	2	No protozoa observed, mostly algae and other waste from the water	none
	3	No protozoa observed, mostly algae and other waste from the water	none

Table 6.3. *Data Gathered in station three from Hikong Bente, Lake sebu.*

In Table 6.3 of Hikong Bente, Station 3 Sample 1 presented a protozoan with a thick capsid, broken flagellum, and a pentagon-like shape, though the nucleus was not visible. These characteristics suggest a possible dormant or stressed state, potentially due to environmental pressure. Such characteristics indicate environmental stress, nutrient deficiency, or physical disturbance. Britannica (2024) explains that Paramecium species are highly sensitive to changes in water quality and typically rely on stable freshwater

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conditions. Samples 2 and 3 exhibited no protozoa, indicating that protozoan presence within this station is limited to very specific microsites. The lack of protozoans in the other samples suggests that Station 3 provides highly limited support for protozoan life. Overall, Station 3 shows minimal protozoan activity, suggesting selective survival due to environmental constraints.

Summary of Findings

This study observed the protozoa specimens collected from the Hikong Alu and Hikong Bente, Lake Sebu, this study reveals varying levels of protozoa's diversity and abundance across the different stations. Station 1 of Hikong Alu, a round, bubble-like organism observed in Sample 1 suggests the presence of simple protozoan forms that thrive in stable water conditions. Sample 2 contained parasitic organisms, possibly indicating organic contamination or nutrient-rich material that supports parasitic growth. Meanwhile, Sample 3 showed an oval-shaped organism with a visible nucleus and a damaged flagellum, which may belong to a flagellated heterotrophic protozoan group. Station 2 of Hikong Alu, no protozoa were observed in Samples 1, 2, and 3. Likely due to factors such as high-water turbulence, low organic content, or unfavorable environmental conditions. This indicates lower biological activity at Station 2 compared to other sampling areas. Station 3 of Hikong Alu, sample 1 showed no protozoa, while Sample 2 revealed a possible *Mastigophoran* organism, and Sample 3 indicated the presence of a *Paramecium*. These findings suggest that certain microzones in Station 3 can support protozoan life despite generally low diversity.

Station 1 of Hikong Bente, *amoebas* were observed in Samples 1 and 2, indicating a nutrient-rich and moist environment. Sample 3 showed abundant *algae* and possible *myxomycetes*, suggesting high organic content and moisture. Station 2 of Hikong Bente - no protozoa were observed in Samples 1 to 3, likely due to unfavorable conditions such as strong water movement, low nutrients, or minimal organic matter, indicating limited microscopic life in the area. Station 3 of Hikong Bente - Sample 1 showed a protozoan with features suggesting a dormant or stressed state, while Samples 2 and 3 had none. This indicates minimal protozoan activity and suggests that only specific microsites within the station can support survival under environmental stress.

The findings suggest that protozoa's diversity and abundance are influenced by environmental factors such as, water turbulence, organic contents and also moisture levels. The presence of these protozoa shows a suitable habitat, and their absence is maybe because of environmental stress or even limitations. Overall, these findings can be used as a basis for further research in Lake Sebu.

CONCLUSION

This study concludes that various protozoa are present in the waters of Hikong Alu and Hikong Bente, Lake Sebu, with noticeable differences in protozoan distribution among stations. Hikong Alu displayed a limited presence of oval, bubble-like, and possible *Paramecium* and *Mastigophora*-type organisms, while Hikong Bente showed more *amoebas*, *algae*, and *myxomycetes* in selected samples. Several stations in both areas showed no protozoan activity, suggesting environmental conditions that may not support protozoan growth. These findings highlight the ecological importance of protozoa as indicators of water quality and contributors to nutrient cycling. However, limitations such as sample degradation and morphological similarities may affect identification accuracy. Overall, this study provides useful baseline information on protozoan diversity in these freshwater sites.

Recommendations

For future researchers, it is recommended to collect multiple water samples from each station to ensure proper replication and increase the accuracy of results. Make sure that the sterile syringe is thoroughly rinsed with distilled water to minimize contamination. Follow consistent sampling procedures, including gentle mixing and staining with iodine solution and methylene blue to enhance visibility and facilitate protozoan identification. Store all samples in coolers, label them correctly, and examine them immediately using a compound light microscope (10x, 40x, and 100x oil immersion). It is also advised that microscopy be conducted within 24 hours after sampling to preserve protozoa and ensure reliable identification. Adjust the lighting as needed to observe protozoan morphology clearly, and ensure that all necessary materials and chemicals are available in the laboratory. It is also recommended to analyze key water parameters such as pH, temperature, and turbidity to better assess water quality, identify possible contamination sources, and evaluate habitat suitability for aquatic life.

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