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Multi-spatial contamination of environmental aquatic matrices with *Cryptosporidium*: a climate, health, and regulatory framework for the Philippines

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Abstract

Background: *Cryptosporidium* is a waterborne global pathogen causing diarrhea primarily in infants and immuno-compromised individuals. The Philippines is a tropical country susceptible to the influences of climate change and water crises. To date, the country has no existing epidemiologic data, regulation, or strategy for monitoring *Cryptosporidium* in freshwater systems. We, therefore, endeavored to provide evidence on the multi-spatial contamination of *Cryptosporidium* in environmental aquatic matrices using low-cost, user-friendly, and sustainable strategies and submit implications on the presence of *Cryptosporidium* in freshwater systems in a climate, health, and regulatory framework.

Results: Here, we present the microscopic detection of *Cryptosporidium* oocysts in low-volume (50 mL) environmental samples of surface water (SW), sediments (BW), and substrate-associated biofilm (SAB) and in 1 L bulk SW investigated by PCR. The multi-spatial distribution of *Cryptosporidium* oocysts in the low-volume (50 ml) aquatic matrices based on microscopy was highest at 69% (20/29) in SW and lowest at 50% (13/26) in BW. Immunofluorescence technique provided the highest microscopic positivity rate with 59% (17/29), 38% (10/26), and 50% (10/20) detection in SW, BW, and SAB, respectively. The detection and identification of *Cryptosporidium* in 1 L bulk SW by PCR and sequence analysis was recorded in total at 21% (6/29) in sampling sites where the differential identification of *C. parvum, C. hominis,* and *Cryptosporidium* spp. was 7% (2/29), 10% (3/29), and 3% (1/29), respectively.

Conclusions: We report the microscopical and first molecular epidemiologic data of *Cryptosporidium* from the most significant environmental freshwater systems in the Philippines. The presence of the two main human and animal pathogenic species *C. parvum* and *C. hominis* from the largest lakes and major water reservoirs in the country calls for sustainable solutions in safeguarding the quality of freshwater resources in a climate, health, and regulatory approach.

Keywords: *Cryptosporidium*, Biofilms, Climate, Sediments, Lakes, Freshwater, Philippines

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Background

Cryptosporidiosis is the second leading cause of infantile mortality next to rotavirus infection [1] and was listed in the 'Neglected Disease Initiative' of the World Health Organization [2]. *Cryptosporidium* has a low infectious dose (1–10 oocysts) and causes morbidity in both the immunocompromised and immunocompetent populations [3, 4].

Masangkay *et al. Environ Sci Eur*   (2020) 32:121

Page 2 of 11

Cryptosporidium ranks first in parasitic protozoans causing waterborne infections in developed and developing countries [5, 6]. The common practice in water quality assessment is the testing of surface water samples used for drinking water preparation. Water sediments were explored but in the form of sludge from wastewater treatment facilities [7, 8] and rarely in environmental sampling [9]. Investigators involved in such studies have, therefore, recommended the simultaneous testing of disturbed water samples as a by-product of zoonotic and anthroponotic activities [10].

Biofilms may be formed at solid–liquid (substrate-associated) as well as water–air (floating) interface, and may harbor pathogenic microorganisms [11]. Floating biofilms, for example, are mainly composed of Gram-negative bacteria, which produce a polysaccharide matrix to hold the microcosm together [12], and, through time, potentially trap and accumulate waterborne pathogens [13–15].

Hundreds of efforts have been described in addressing selected components of the problems encountered in the monitoring of *Cryptosporidium* in water with the objectives to define the quantity, viability, and human infectivity of oocysts [16]. This provides a quantitative understanding of the public health risks concerning water supply and water treatment strategies, which can provide foundations for water reservoir management, treatment designs, operation, supply, monitoring, and regulation [17].

The provision of low-cost, user-friendly, and sustainable recovery methods for *Cryptosporidium* in high (50–100 L) and standard-volume (5–10 L) water samples like flotation and flocculation have been previously described [16, 18–23]. However, *Cryptosporidium* identification in water samples still proves to be challenging and requires expertise in identification through a plethora of technical methodologies [24].

According to the WHO Drinking water key facts of 2018, contaminated drinking water is the cause of diarrhoeal deaths estimated to be at 502,000 per year. 844 million people lack access to drinking water service, and 423 million utilize well and spring water with 159 million still dependent on untreated surface water from lakes, ponds, rivers, and streams. Daunting challenges to the access of clean drinking water are climate change, population growth, demographic changes, and urbanization, thereby leading to a forecast of having the world's population living in water-stressed areas by 2025 [25]. The first mention of climate change in any medical literature was more than 30 years ago by Professor Alexander Leaf in a 1989 article titled 'Potential health effects of global climatic and environmental changes' [26]. Fast forward to the present, a clear understanding of climate

change direction, magnitude, and tempo is still elusive or unknown to the general population and is barely studied within the framework of human health. The Southeast Asian region and its populace are highly susceptible to the health effects of climate change and water crises [27]. To date, research that delves into the relationship of non-communicable and communicable diseases with the changing climate and how it influences the availability of and access to clean water is still a rarity [28].

The Republic of the Philippines, at present, has limited literature on *Cryptosporidium*, and no molecular identification in water supplies exist [29, 30]. Therefore, we aimed to establish inceptive data at a nationwide scale on the microscopic and molecular prevalence of *Cryptosporidium* from the largest lakes and major water reservoirs in the Philippines, using low-cost, user-friendly, and sustainable methods, while discussing our findings through the lenses of climate, health, and regulation to unravel its implications.
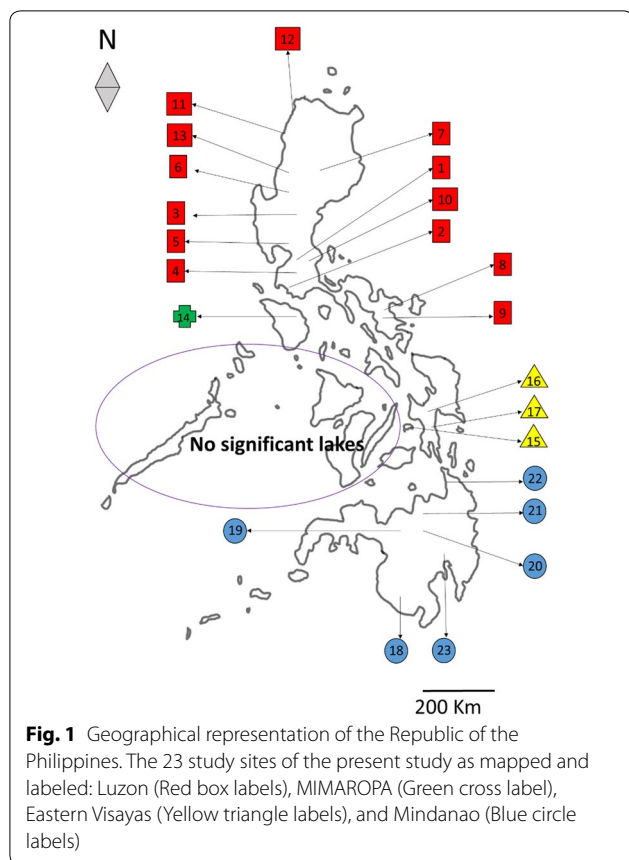
Materials and methods
Study sites
The study sites covered the three major Islands of the Philippines namely: Luzon ($n = 13$), Visayas ($n = 3$), Mindanao ($n = 6$), and a study site in the MIMAROPA (Mindoro, Marinduque, Romblon, and Palawan) region ($n = 1$). Characteristics of the study sites such as surface area, elevation in meters above sea level (MASL), coordinates, water temperature, average precipitation level, and date and time of collection are listed in Additional file 1. The 23 study sites were mainly composed of the largest lakes and major freshwater reservoirs in the Philippines (Fig. 1). These study sites were further divided into sampling sites ($n = 29$) composed of sampling points ($n = 72$) (Additional files 2 and 3) and were chosen based on (1) significance to the Philippines and its inhabitants, (2) road access, (3) presence of human settlements, (4) anthropogenic activities as well as tourism and recreational activities, and (5) presence of aquaculture.

Sample collection, processing, and microscopy panel
Surface water samples (SW) were collected in 50 mL sterile polyethylene containers from no more than 30 cm below the water surface. Sediments with bottom water (BW) were collected from a water depth of 1 m along the shoreline that was composed of one-part sediments and four-part bottom water. Substrate-associated biofilms (SAB) were harvested from an area with a depth of 1 m along the shoreline where aquatic plants and rocks were present. Short segments of small aquatic plants were collected and washed with sterile distilled water to remove non-adherent cells and cut to small portions to fit inside

Masangkay *et al. Environ Sci Eur*   (2020) 32:121

Page 3 of 11

Fig. 1 Geographical representation of the Republic of the Philippines. The 23 study sites of the present study as mapped and labeled: Luzon (Red box labels), MIMAROPA (Green cross label), Eastern Visayas (Yellow triangle labels), and Mindanao (Blue circle labels)

the 50 mL sterile polyethylene containers and suspended in 50 mL sterile distilled water. In the absence of aquatic plants, adherent biofilms (approximately 2 g wet weight) were scraped from rocks no more than 30 cm below the water surface. Samples were transported to the laboratory and processed within 48 h after collection. Each sample was vortexed for 1 min to dislodge adherent cells from any larger organic substances and debris to evenly distribute the solids throughout each sample matrix and left to stand for 5 min to settle heavier solids. The SW, BW, and SAB sample suspensions were each manually filtered through a 1.2 μm pore size Glass microfiber filter (Whatman™) fitted inside 50 mL plastic syringes. Glass microfiber filters were recovered and placed on sterile disposable polyethylene plates, where the filtered sediments were scraped using a sterile inoculating loop and 5 mL sterile distilled water as eluent. The 5 mL eluates were transferred to sterile test tubes and centrifuged at 1500 g for 15 min where 3 mL of the supernatants were discarded and the remaining 2 mL and pellet were mixed to form a suspension and stored in microcentrifuge tubes for smearing within 24 h. From each pellet suspension, a 1 cm-diameter smear (25 μL) was prepared, fixed with absolute methanol, and stained. The traditional

staining employed were Modified Kinyoun's (MK) and Modified Safranin Methylene Blue (SMB), while fluorescence techniques utilized were Auramine (Aura) and Direct Antibody Fluorescence Technique (IFT) (Waterborne Inc. USA). Each smear was examined under 200 oil immersion fields (OIF) and 200 high power fields (HPF) for traditional and fluorescence microscopy, respectively [31, 32]. Microscopic *Cryptosporidium* oocyst identification was compared with *C. parvum* positive controls (Waterborne Inc., USA) based on measurements and morphologic characteristics referenced from the CDC [33]. Theoretical approximates of oocyst counts per 50 mL sample matrix were extracted from the range of oocysts visualized in 200 microscopic fields per 1 cm-diameter (25 μL) smear area [32].

Molecular analysis

Molecular detection through Polymerase Chain Reaction (PCR) was performed on 1 L bulk SW samples from each sampling site ($n = 29$) to provide sufficient genetic material for DNA extraction. Each 1 L bulk SW sample was vacuum-filtered through a 47 mm diameter, 1.2 μm pore size glass microfiber filter (Whatman™) using a simple Buchner funnel, and electric-operated diaphragm pump (Fisher Scientific Pte Ltd). The filtered material was harvested from the glass microfiber filter while still wet by eluting with sterile distilled water and gently scraped using a sterile scalpel blade. The eluted material was placed in sterile 10 mL test tubes and centrifuged at 1500 g for 15 min [34] after which the supernatant was discarded and the pellet was stored in 2 mL PCR tubes. DNA extraction was performed using the QIAamp® PowerFecal® Pro DNA Kit (Qiagen, Germany) with one cycle of freeze–thaw (FT) pretreatment performed on 500 μL pellet suspension by overnight freezing in −7 °C and boiling in 100 °C digital dry bath (BIO-RAD) for 10 min. PCR was performed following previously published protocols [35] with modifications of using a non-nested protocol with primer set Cry18S-S2 (5′-GGTGAC TCATAACTTTACGG-3) and Cry18S-As2 (5′-ACGCTA TTGGAGCTGGAATTAC-3′). Amplicons were visualized by gel electrophoresis on a 1.5% agarose gel stained with ethidium bromide applied with 180 volts electricity for 15 min using PowerPac™ Basic (BIO-RAD). Amplicons from PCR-positive samples were sent to a commercial sequencing company (Macrogen South Korea). Sequences were aligned using Clustal W of Bioedit with a careful visual inspection of gaps and ambiguous sequences and were deposited in GenBank. Phylogenetic and molecular evolutionary analysis was conducted using Mega version 7 which was based on the best tree model with bootstrap set at 1000 replicates [36].

Masangkay *et al. Environ Sci Eur* (2020) 32:121

Page 4 of 11

Results and discussion

Microscopic detection of *Cryptosporidium* oocysts

A total of 179 environmental aquatic matrices were collected, which comprised of SW ($n = 72$), BW ($n = 68$), and SAB ($n = 39$) (Additional file: 2 and 3). Among the microscopy tests employed, IFT consistently provided the highest positivity with 59% (17/29), 38% (10/26), and 50% (10/20) detection in SW, BW, and SAB, respectively (Table 1). MK, SMB, and Aura also proved useful in terms of being cost-effective, but delivered lower detection rates. *Cryptosporidium* oocysts from environmental aquatic matrices were morphologically consistent with published descriptions and images of *Cryptosporidium* oocysts from the CDC and demonstrated consistent morphologic features when compared to *C. parvum* positive control (Fig. 2a to d). Under 1000× magnification of light microscopy (MK/SMB), *Cryptosporidium* oocysts from sample matrices were round, 4–6 μm in diameter, and stained bright red against a blue background (Fig. 2e and f). IFT confirmation of *Cryptosporidium* oocysts under 400× magnification visualized round oocysts measuring 4–6 μm in diameter that fluoresced bright-apple-green against a black background (Fig. 2h). In the present study, microscopic examination of 1 cm-diameter smear area (25 μL) under 200 microscopic fields using light microscopy (MK/SMB; 1000X magnification) and fluorescence microscopy (Aura/IFT; 400X magnification) mostly reported a range of 0–1 *Cryptosporidium* oocysts in 25 μL pellet suspension (Additional file 3). This number of oocysts translates theoretically to oocyst counts of 0–2000 per 50 mL. In the current study, the microscopic confirmation of *Cryptosporidium* oocysts with IFT [34] agrees with the 55% detection in raw and finished waters in North-eastern Spain [37], with variations in results attributed to water qualities unique to each sampling site and types of aquatic matrices tested [38]. The microscopic detection and the theoretical approximate of 0–2000 oocysts per 50 mL or 0–40,000 oocysts per 1 L bulk SW sample suggest high contamination compared to lower estimates reported in Thailand [39], Australia [40], and Iran [41]. The higher oocyst count in the current study can be attributed to several factors. Methodologically, the direct filtration, elution, and pelleting of low-volume (50 mL) aquatic matrices supposedly minimized the potential loss of oocysts if processed using multiple sequential steps [16, 34], while the standardized smear area of 1 cm diameter (25 μL), systematic, and exhaustive microscopy (200 fields per 1 cm-diameter smear area) aided in increased detection (Additional file 3: Table S3), where the results provided a clear picture of the distribution of *Cryptosporidium* spp. oocysts in environmental

Table 1 Summary of microscopic results of low-volume (50 mL) aquatic matrices

Sampling site ($n = 29$)	Microscopy and sample matrix		
	SW	BW	SAB
Luzon			
S1 Laguna de bay	1,3,4	1,4	–
S2 Taal Lake	3,4	–	3
S3 Pantabangan Lake	3,4	4	–
S4.1 Sampaloc Lake	3,4	–	3
S4.2 Bunot Lake	–	–	3,4
S4.3 Palakpakin Lake	–	–	nc
S4.4 Mojicap Lake	–	3,4	–
S4.5 Yambo Lake	–	–	–
S4.6 Pandin Lake	–	–	nc
S4.7 Kalibato Lake	3,4	–	–
S5 Ipo water reservoir	4	–	nc
S6 Magat water reservoir	1,3,4	1,2,3,4	nc
S7 San Roque water reservoir	1,3	1,2,3,4	nc
S8 Buhi Lake	–	4	1,4
S9 Bato Lake	–	–	–
S10 Cavinti/Lumot Lake	–	Nc	nc
S11 West Pudoc fish ponds	4	4	4
S12 Paoay Lake	4	1	nc
S13 Ambuklao water reservoir	3,4	Nc	1,3,4
Mimaropa			
S14 Naujan Lake	4	4	1,3,4
Visayas			
S15 Sakanaw Lake	4	–	–
S16 Danao Lake	1	1,3	–
S17 Bito Lake	–	1,2,3	1,3,4
Mindanao			
S18 Sebu Lake	1,4	1,4	4
S19 Pulangi water reservoir	4	–	nc
S20 Bukidnon farm irrigation	4	nc	nc
S21 Lanao Lake	1,3,4	–	2,3,4
S22 Mainit Lake	1,3,4	4	4
S23 Tagunay River	3	–	4
Matrix prevalence	69% (20/29)	50% (13/26)	60% (12/20)
Rates of detection			
1	24% (7/29)	27% (7/26)	20% (4/20)
2	0% (0/29)	12% (3/26)	5% (1/20)
3	38% (11/29)	19% (5/26)	35% (7/20)
4	59% (17/29)	38% (10/26)	50% (10/20)

SW, surface water; BW, sediments with bottom water; SAB, substrate-associated biofilms; 1 Modified Kinyoun (MK); 2 Safranin Methylene Blue (SMB); 3 Auramine (Aura); 4 Immunofluorescence (IFT); nc, no sample collected;– negative

Details of sampling areas such as date and time of collection, location, coordinates, temperature, rain, surface area, and elevation can be found in Additional file 1

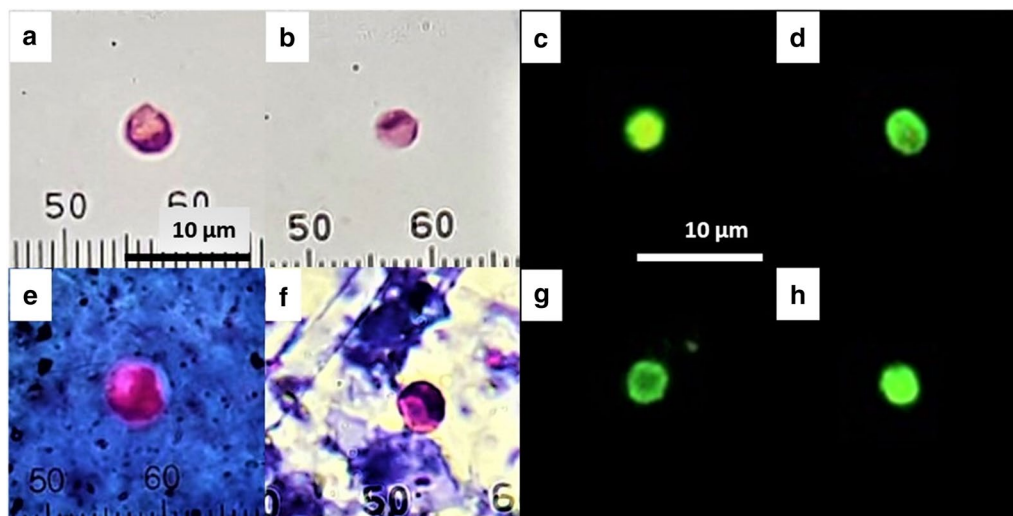A detailed breakdown of microscopy panel results per sampling point can be found in Additional files 2 and 3

Fig. 2 *C. parvum* positive control and *Cryptosporidium* spp. oocysts from environmental aquatic matrices. **a–d** positive control; **a** *C. parvum* (MK); **b** *C. parvum* (SMB); **c** *C. parvum* (Aura); **d** *C. parvum* (IFT); **e** S1 SW (MK); **f** S6 BW (SMB); **g** S13 SAB (Aura); **h** S19 SW (IFT); **a, b, e**, and **f** 1000X magnification; **c, d, g**, and **h** 400× magnification; scale bars = 10 μm

freshwater systems all over the country. Matrix wise, the analysis of raw/untreated water potentially offers higher oocysts contamination compared to treated water [42]. However, the actual density of *Cryptosporidium* oocysts in some of the investigated SW samples was suspected to be below the theoretical estimates and *Cryptosporidium* DNA was successfully amplified only in six out of the 29 bulk SW samples (Table 2). The theoretical estimates require further verification also through the performance of recovery efficiency in the future. Theoretical estimates should not be taken as absolute counts, since the oocysts

are unevenly dispersed in water or they may be associated with substrates [9, 31, 32, 43]. In addition, different concentration methods offer varying degrees of recovery efficiencies [37, 44]. It should be noted that the low-volume (50 mL) sampling strategy models the theoretical amount of water that could be accidentally ingested or inhaled by an individual during anthropogenic activities in water systems. This defines functional data on the minimum volume of environmental water that can potentially harbor and transmit *Cryptosporidium* oocysts to humans and animals [32].

Table 2 Molecular detection of *Cryptosporidium* spp. in 1 L bulk surface water samples

Site	Molecular results	GenBank Accession no.	*Cryptosporidium* prevalence		
			Island	Sampling site	Study site
Luzon			15% (2/13)	21% (6/29)	26% (6/23)
S4.5 Yambo Lake	*Cryptosporidium parvum*	MT221250			
S13 Ambuklao water reservoir	*Cryptosporidium hominis*	MT221550			
Visayas			33% (1/3)		
S17 Bito Lake	*Cryptosporidium* spp.	MT234345			
Mindanao			50% (3/6)		
S19 Pulangi water reservoir	*Cryptosporidium parvum*	(MT219974)			
S21 Lanao Lake	*Cryptosporidium hominis*	(MT221440)			
S23 Tagunay River	*Cryptosporidium hominis*	(MT221442)			
			Cryptosporidium spp. sampling site prevalence		
			C. parvum		7% (2/29)
			C. hominis		10% (3/29)
			Cryptosporidium spp.		3% (1/29)

Masangkay *et al. Environ Sci Eur*   (2020) 32:121

Page 6 of 11

Molecular identification of *Cryptosporidium* species

Molecular analysis of 1 L bulk SW samples confirmed six sampling sites were contaminated with *C. parvum, C. hominis,* and other *Cryptosporidium* spp. (Table 2 and Fig. 3). Luzon sampling sites had a recorded *Cryptosporidium* prevalence of 15% (2/13) with *C. parvum* in Yambo Lake and *C. hominis* in Ambuklao water reservoir. Visayas sampling sites presented with 33% (1/3) prevalence, with Bito Lake isolate registered under *Cryptosporidium* species. Mindanao sampling sites recorded a 50% (3/6) prevalence with *C. parvum* in Pulangi water reservoir, *C. hominis* in Lanao Lake, and another *C. hominis* isolate in Tagunay River. On a nationwide scale, the molecular prevalence of *Cryptosporidium* was in total at 21% (6/29) in sampling sites where the differential identification of *C. parvum, C. hominis,* and *Cryptosporidium* spp. was at 7% (2/29), 10% (3/29), and 3% (1/29), respectively (Table 1). The sequences of the environmental aquatic isolates were deposited in GenBank under the accession numbers MT221250, MT221550, MT234345, MT219974, MT221440, and MT221442 with reference sequences listed in Table 3. The molecular prevalence of *Cryptosporidium* from our Philippine study sites (26%) agrees with reports in water supplies in Turkey (27%) [21]. In addition, the differential prevalence of *C. parvum* in the present study (7%) is also similar to the molecular confirmation of *C. parvum* in a multi-use catchment

in South Australia [40]. The amplification of the 18S rRNA locus using PCR offered lower cost as compared to RT-PCR or qPCR assays with the non-nested protocol responsible for reducing 5–6 h of run-time per batch of 12–15 samples. Furthermore, the diagnostic sensitivity (96.9%) and specificity (98.4%) of 18S rRNA PCR for *Cryptosporidium* is high, with an analytical sensitivity of 1–10 oocysts per reaction [45]. The applied non-nested protocol did not seem to affect the PCR sensitivity as it enabled the detection of *C. parvum* (GenBank accession number MT221250) in S4.5 Yambo Lake even if it was negative for all four microscopy methods in all three 50 mL counterparts (Tables 1 and 2). Microscopy positive but PCR-negative samples may be attributed to the density and characteristics of suspended solids (Additional file 4) and abundant PCR inhibitors because of high-industrially polluted environmental study sites [10, 21, 37, 43]. A low ratio of PCR-positive results was also reported in Finland [46], France [47], Hungary [48], and Spain [49] with a case of PCR-negative results in all 20 study sites in North-eastern Spain despite IFT detection of *Cryptosporidium* [37].

Water quality assessment strategies and multi-spatial distribution of *Cryptosporidium*

The detection of *Cryptosporidium* oocysts requires adjusting sampling and processing details to fit specific
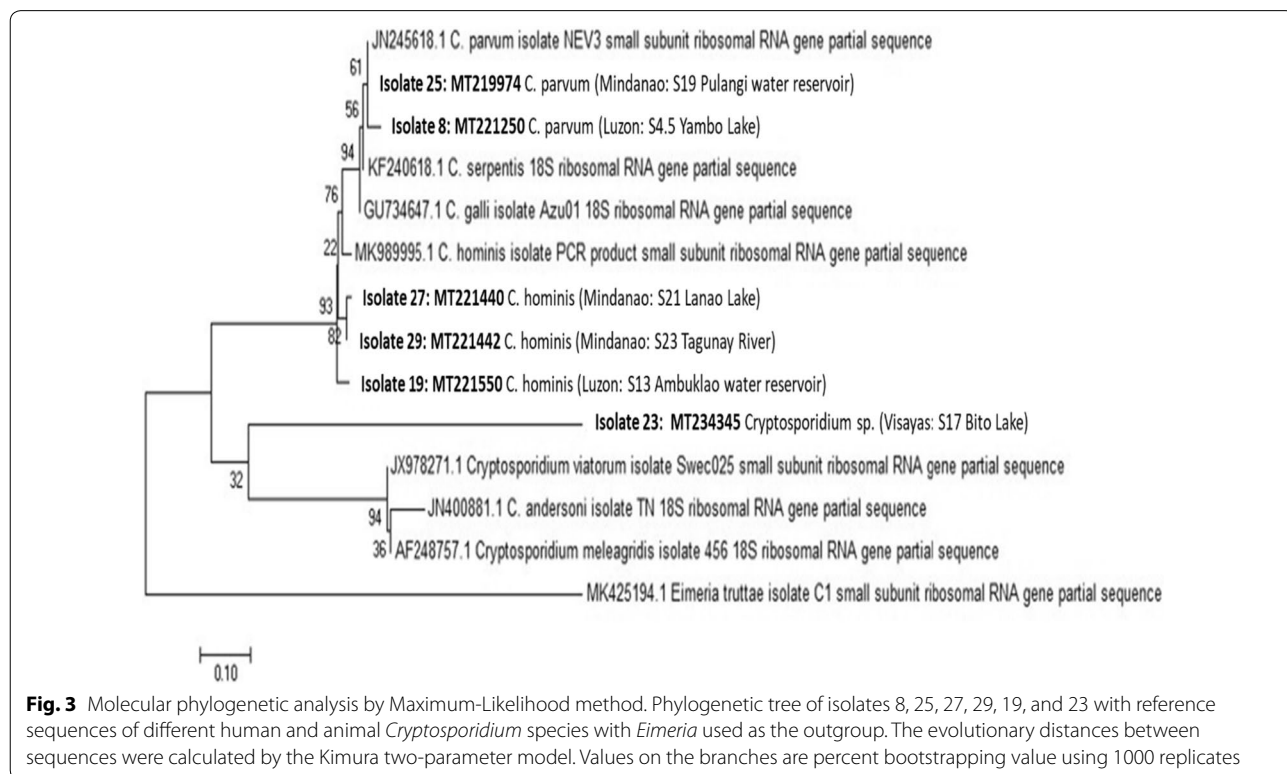

Fig. 3 Molecular phylogenetic analysis by Maximum-Likelihood method. Phylogenetic tree of isolates 8, 25, 27, 29, 19, and 23 with reference sequences of different human and animal *Cryptosporidium* species with *Eimeria* used as the outgroup. The evolutionary distances between sequences were calculated by the Kimura two-parameter model. Values on the branches are percent bootstrapping value using 1000 replicates

Masangkay *et al. Environ Sci Eur*   (2020) 32:121

Page 7 of 11

Table 3 Reference sequences used in the present study

Reference sequences	Source/Country	GenBank accession no.
Human pathogens representative		
C. parvum isolate NEV3 ssrRNA gene partial sequence	Diarrheic calf feces/Turkey	JN245618.1
C. hominis isolate PCR product ssrRNA gene partial sequence	Freshwater sponge/Philippines	MK989995.1
C. viatorum isolate Swec025 ssrRNA gene partial sequence	Humans/Kenya	JX978271.1
C. meleagridis isolate 456 18S rRNA gene partial sequence	Human feces/[a]	AF248757
Mammalian representative		
C. andersoni isolate TN 18S ribosomal RNA gene partial sequence	Calf/India	JN400881.1
Avian representative		
C. galli isolate Azu01 18S rRNA gene partial sequence	*Cyanocompsa brissonii* (ultramarine grosbeak), feces/Brazil	GU734647.1
Reptilian representative		
C. serpentis 18S rRNA gene partial sequence	*Pantherophis guttatus* (corn snake), feces/Brazil	KF240618.1
Outgroup		
Eimeria truttae isolate C1 small subunit rRNA gene partial sequence	*Salmo trutta* (Brown trout), pyloric caeca and intestinal content/Spain	MK425194.1

[a] Not declared

water qualities. The summary of microscopy results per sampling site revealed that the spatial distribution of *Cryptosporidium* oocysts in aquatic matrices was highest in SW at 69% (20/29) and lowest in BW at 50% (13/26) (Table 1). *Cryptosporidium* oocysts exist among other naturally occurring particles in various water samples including sediments, sewage water, surface, and groundwater, even in chlorinated swimming pools and hot springs [9, 24, 50–52]. However, investigations delving into interactions with biofilms are limited (14, 57). Seasonal changes influencing biofilm profile and retention of *Cryptosporidium* oocysts have been described [53, 54]; water depth influences biofilm thickness and other biologic masses like freshwater sponges may facilitate protection against UV radiation, although largely known as requiring an animal cell for its complex biological progression [31, 32, 55, 56], *Cryptosporidium* excystation and developmental stages have been demonstrated in in-vitro *Pseudomonas aeruginosa* biofilms [14] and even in in-vitro axenic cultures [57]. It is, therefore, of great interest, to further expand *Cryptosporidium* investigations in biofilms in aquatic and fluid systems.

Low-volume (50 mL) water sampling for the detection of *Cryptosporidium* was reported in two previous studies that demonstrated its utility in PCR analysis in a river sample in China [43] and the first MK/PCR detection of *C. hominis* in a freshwater sponge [31]. Low-volume sampling provided the advantages of ease of collection, transport, multiple matrix sampling, large sampling site coverage, reproducibility, adjusted density of suspended solids, and significantly lower test cost, and enables large-scale surveys, as was the case for the present study. Multiple matrix sampling proved its benefits in increasing the detection of *Cryptosporidium* oocysts and decreased occasions of declaring false-negative microscopy results in sampling sites where positivity in SW agreed with either BW and/or SAB results. More importantly, on some occasions, negative results in SW proved positive in BW and/or SAB (Table 1).

Water contamination with *Cryptosporidium* in a climate, health, and regulatory framework

The Philippine archipelago is located in Southeast Asia, a region of the globe that is highly susceptible to the influences of climate change and water crises [26]. Research on the health effects of climate change is estimated to be only half of that in other areas impacted by the same like agriculture and forestry. To date, poor research output on the effects of climate change to health somewhat infers that most health scientists are still unaware of the present and impending mortality and morbidity this iceberg of a problem truly poses [28]. According to the Institute of Health Metrics and Evaluation (IHME), Global Burden of Disease study in 2017, contaminated water sources were the leading risk factor for child deaths due to diarrhea [58]. Globally, climate models predict precipitation to become more intensive over most land areas, where North America, Europe, and Asia may experience the greatest increase in heavy downpours [59]. A study on the availability of freshwater that can be used for drinking and growing food has shown that two of three people on earth live in areas, where water consumption is twice as great as availability [60]. The El Niño phenomenon has and continually affects the growth of crops and the

Masangkay *et al. Environ Sci Eur*   (2020) 32:121

Page 8 of 11

availability of clean drinking water in the Philippines [61, 62]. This represents an unknown situation on the compounded effects of altered patterns in human consumption, levels of nutritional intake and dehydration, as well as the development of non-transmissible and waterborne diseases in relation to climate change.

As early as 2001, heavier precipitation events have influenced the increasing reports of outbreaks of waterborne diseases including hepatitis A, cholera, *E. coli*, leptospirosis, and cryptosporidiosis due to increasing population, urban landscapes, and overflow of sewer systems [63]. Similarly, the absence of sewer systems promotes waterborne disease outbreaks and so does the lack of rain and scarcity of freshwater supply [64]. In the Philippines, even its metropolis is not well equipped with sewer systems connected to water treatment facilities; rather, open sewer systems are the norm which contributes to the continuous contamination and deterioration of environmental freshwater systems by way of pollution with domestic and industrial wastes [65]. Ironically, these polluted freshwater resources are, at present, the primary sites utilized for aquaculture, irrigation of agricultural lands, human, and veterinary activities which may play a significant role in the cycling of waterborne diseases to humans, animals, and the environment [32].

To date, limited efforts have been exerted in *Cryptosporidium* research in unraveling its prevalence in freshwater resources in the Philippines; making concepts relative to *Cryptosporidium* neglected or relatively unknown among local water quality analysts and healthcare practitioners. This lack of information is a gap in understanding *Cryptosporidium* and may be responsible for non-diagnosis and/or misdiagnosis in healthcare, which can be evidenced by the last report of *Cryptosporidium* diagnosis in cancer patients in the Philippines 15 years ago [66]. Non-inclusion in local quality standards for water potability leads to an accumulated underreported incidence at a nationwide scale.

The current study provides the first molecular epidemiologic data of *Cryptosporidium* from the largest lakes and major water reservoirs in the country, where four major water reservoirs were confirmed to be contaminated with human pathogenic species of *Cryptosporidium* namely: Ambuklao (*C. hominis*), Pulangi (*C. parvum*), Lanao lake (*C. hominis*), and Tagunay river (*C. hominis*). Two lakes that are major sources of livelihood and aquaculture were also found to be contaminated with *C. parvum* (Yambo Lake) and *Cryptosporidium* spp. (Bito Lake). The immunocompromised population is at high risk of contracting *Cryptosporidium* infection [67]. *Cryptosporidium* has been reported from children undergoing chemotherapy [68], immunodeficiency, and organ transplant patients in Iran [69]. Cryptosporidiosis in pediatric liver transplants

recipients has been reported to cause significant complications [70]. Malaysia reported *C. parvum* and *C. hominis* genotypes from HIV patients [71]. Poland reported a higher frequency of *Cryptosporidium* infections from colorectal cancer patients [72]. A Southeast Asian systematic review and meta-analysis revealed a significant relationship between cryptosporidiosis and the risk of chronic diarrhea in people with HIV [67]. Although case reports from the immunocompetent population are rare, it should be speculated whether underlying conditions may contribute to susceptibility to *Cryptosporidium* infection or trigger the progression of the same as a case in Poland reported *C. meleagridis* from an immunocompetent patient with adenocarcinoma [73]. Furthermore, a 2019 survey in the Czech Republic reported a widespread and chronic low-level exposure from 301 blood donors [74]. The molecular confirmation of *Cryptosporidium* in freshwater systems in the Philippines suggests unknown effects contributed by chronic low-level exposures, the potential for human infection, or even worse, outbreak scenarios following the ingestion of contaminated water due to the presence of human pathogenic species *C. parvum* and *C. hominis* [75–77]. In Israel, a 14 year study consisting of 522 *Cryptosporidium* cases submitted to its government the inclusion of *Cryptosporidium* among the mandatory notifiable diseases for timely detection of outbreaks [78].

To date, there is no policy in the Philippines on the detection and monitoring of *Cryptosporidium* and other relevant waterborne protozoan pathogens (WBPP) in waters for human use. The results of the present study warrant the inclusion of *Cryptosporidium* and other WBPP in water quality assessment of freshwater resources and drinking water. The formulation of initiatives for the simultaneous testing of SW, BW, and SAB will provide important data in the multi-spatial distribution of *Cryptosporidium* oocysts within aquatic systems, along with increased chances of detection [32].

The data conceived from the present study are the first national report on the microscopic and molecular prevalence of *Cryptosporidium* in environmental aquatic matrices. This can stimulate further research, the development of both private and governmental initiatives, and sustainable solutions in monitoring and safeguarding the quality of environmental freshwater and drinking water resources. A climate and health approach is necessary for the comprehension, prevention, mitigation, and management of *Cryptosporidium* contamination and infection.

Conclusions

Microscopy results demonstrated an abundance of *Cryptosporidium* oocysts in surface water, sediments, and substrate-associated biofilms from environmental

Masangkay *et al. Environ Sci Eur*   (2020) 32:121

Page 9 of 11

freshwater systems. The results of the present study provided the first national molecular epidemiologic data on the distribution of *Cryptosporidium parvum* and *C. hominis* in the Republic of the Philippines. Employing low-volume (50 mL and 1 L), multiple matrices, and multiple sampling area strategies is a low-cost, user-friendly, sustainable, and reproducible method of detecting *Cryptosporidium* from environmental aquatic matrices, and can be performed even in resource-limited settings. A standardized and systematic microscopic examination for the optimal detection of *Cryptosporidium* oocysts from low-volume environmental aquatic matrices complemented with 18S rRNA PCR and sequencing seems to be a successful strategy. The climate and health framework calls for the formulation of a national policy in the inclusion of *Cryptosporidium* and other waterborne protozoan pathogens in water quality assessment and monitoring. It would be interesting to have samples from different seasons in the year. This initiative is indispensable in preventing and mitigating unforeseen effects of chronic low-level exposures, cases of infections, and potential outbreak scenarios.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12302-020-00410-w.

 Additional file 1: Consolidated details of Study sites, sampling sites, and sampling points in the Philippines.

Additional file 2: *Cryptosporidium* spp. low-volume microscopy panel and bulk water PCR results.

Additional file 3: Raw data on *Cryptosporidium* spp. oocyst counts in 200 microscopic fields per 1 cm diameter (25μL) smear.

Additional file 4: Filtered sediments of 1 L bulk water samples from sampling sites. Filtration of low-volume (1 L) bulk water with 1.2 μm pore size glass microfiber filter is efficient in recovering dispersed sediments from surface water samples and can be used for high turbidity aquatic matrices by simply changing the filter once full. Different sampling sites show different density and physical characteristics of suspended solids.

Abbreviations

MIMAROPA: Mindoro, Marinduque, Romblon, and Palawan; MASL: Meters above sea level; SW: Surface water; BW: Sediments; SAB: Substrate-associated biofilms; MK: Modified Kinyoun's staining; SMB: Modified Safranin Methylene Blue staining; Aura: Auramine staining; IFT: Immunofluorescence test; OIF: Oil immersion field; HPF: High power field; CDC: Center for Disease Control and Prevention; PCR: Polymerase chain reaction; FT: Freeze thaw; ssrRNA: Small subunit ribosomal Ribonucleic acid; UV: Ultraviolet; IHME: Institute of Health Metrics and Evaluation; HIV: Human immunodeficiency virus; WBPP: Waterborne protozoan pathogens.

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Masangkay *et al. Environ Sci Eur* (2020) 32:121

Page 10 of 11

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Page 11 of 11

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