Screening of cyanotoxin producing genes in Ecuadorian freshwater systems

Triagem de genes produtores de cianotoxinas em sistemas de água doce do Equador

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Abstract: Aim: This study pursued the detection of *cyr* and *mcy* genes to assess the presence of cylindrospemopsin (CN) and microcystin (MC) potential producers in Ecuadorian water bodies. **Methods:** Environmental DNA (eDNA) was extracted from eight water bodies and one wastewater treatment plant (WWTP) from Ecuador. A nested PCR was designed to amplify *mcy*B, *cyr*E, and *cyr*J genes in these environmental samples. PCR products were sequenced and blasted against GenBank database. **Results:** Potential CN producers were found in seven water bodies and the WWTP. *cyr*E amplification revealed three variants belonging to *Raphidopsis* and *Aphanizmenon* species and one for *cyr*J with around 90% identity with *Raphidiopsis and Oscillatoria* species. Four water bodies presented the same variant for *mcy*B similar to *Microcystis panniformis* with 99% of identity. **Conclusions:** This study contributes new data on the presence of toxic cyanobacteria strains and provides new molecular tools to assess cyanotoxin hazards in Ecuadorian water bodies.

Keywords: cylindrospermopsin; microcystin; nested PCR; Andes; Amazon.

Resumo: Objetivo: Este estudo buscou a detecção de genes *cyr* e *mcy* para avaliar a presença de potenciais produtores de cilindrospemopsina (CN) e microcistina (MC) em corpos d'água equatorianos. **Métodos:** O DNA ambiental (eDNA) foi extraído de oito corpos d'água e uma estação de tratamento de águas residuais (ETAR) do Equador. Uma "nested" PCR foi projetada para amplificar os genes *mcy*B, *cyr*E e *cyr*J nessas amostras ambientais. Os produtos de PCR foram sequenciados e pesquisados no banco de dados GenBank. **Resultados:** Potenciais produtores de CN foram encontrados em sete corpos d'água e na ETAR. A amplificação de *cyr*E revelou três variantes pertencentes à espécies dos gêneros *Raphidopsis* e *Aphanizomenon* e uma para *cyr*J com cerca de 90% de identidade com espécies dos gêneros *Raphidiopsis* e *Oscillatoria*. Quatro corpos d'água apresentaram a mesma variante para *mcy*B semelhante a *Microcystis panniformis* com 99% de identidade. **Conclusões:** Este estudo contribui com novos dados sobre a presença de cianobactérias tóxicas e fornece novas ferramentas moleculares para avaliar os riscos de cianotoxinas em corpos d'água equatorianos.

Palavras-chave: cilindrospermopsina; microcistina; nested PCR; Andes; Amazônia.

Graphical Abstract
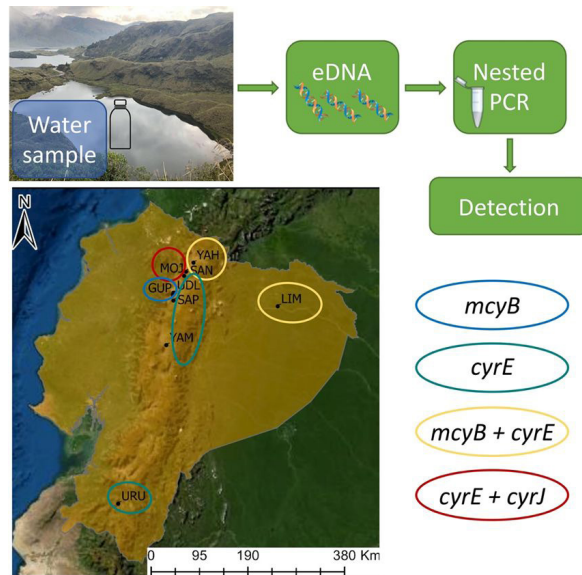
1. Introduction

The proliferation of harmful algae or harmful algal blooms (HABs) can severely impact water quality and present risks to public health (Gobler, 2020). Concerns about inland HABs are commonly related to cyanobacteria, which potentially produce undesirable secondary metabolites, including cyanotoxins (CNs). Exposure to cyanotoxins can lead to adverse health outcomes and directly affect biodiversity and ecosystem services (Percival & Williams, 2013). Other negative effects include degradation of water quality for drinking and recreational use (Abeysiriwardena et al., 2018; Carmichael & Boyer, 2016; Metcalf et al., 2018).

CNs include anatoxins, cylindrospermopsins, L-beta-N-methylamino-L-alanine, microcystins, nodularins, and saxitoxins (Meriluoto et al., 2017). Among them, microcystins (MCs) and CNs have emerged in the past few decades as freshwater cyanobacterial toxins of increasing concern (Adamski et al., 2020; Corbel et al., 2014; Massey & Yang, 2020). In 1996, the death of 52 dialysis patients due to liver failure was traced back to water contaminated with cyanobacteria and the presence of MCs in a Brazilian hospital (Carmichael, 2001). These tragic incidents in Brazil triggered interest in assessing the toxicity of MCs and monitoring environmental concentrations. MCs may be typically classified as hepatotoxin, however, their effects extend beyond the liver. For instance, chronic exposure to MC also has harmful effects on the renal system including necrosis, hemorrhages,

and infiltration of leukocytes (Milutinović et al., 2002). In the gastrointestinal system, colorectal/rectal carcinomas in humans may be worsened by microcystin exposure (Zhou et al., 2002). CN was also responsible for a significant human-poisoning incident reported on Palm Island, Australia, in 1979 (Antunes et al., 2015). CN was characterized as hepatotoxic in the early 1990s (Ohtani et al., 1992), but later has also been identified as potentially genotoxic, dermatotoxic, developmentally toxic, and carcinogenic (de la Cruz et al., 2013), mostly based on findings in mammals. Several studies using human hepatic cells or rodent models have identified pathological and metabolic changes in the liver by CN exposure (Huguet et al., 2019; Seawright et al., 1999). In addition, CN has been shown to cause oxidative stress, increase DNA strand breaks, and decrease natural cell apoptosis in mammalian hepatocytes and blood lymphocytes (Hercog et al., 2017; Štraser et al., 2013)

Cylindrospermopsis raciborskii, renamed *Raphidiopsis raciborskii* in 2018 (Aguilera et al., 2018), was identified as a producer of CN by Hawkins, et al.(1985). Since then, it has been shown that this molecule is also produced by cyanobacteria belonging to other genera such as *Anabaena, Aphanizomenon, Dolichospermum, Lyngbya, Raphidiopsis,* and *Umezakia* (Kokociński et al., 2017; Stüken et al., 2006). MC and/or CN has been reported to be present in water bodies in Germany (Mantzouki et al., 2018; Wiedner et al., 2008), the United States (Howard et al., 2017; Loftin et al.,

2016), Brazil (Bittencourt-Oliveira et al., 2011; Lorenzi et al., 2018; Walter et al., 2018), Spain (Cirés et al., 2014), and Ecuador (Van Colen et al., 2017), among others.

However, cyanobacterial toxins, including MC and CN, are not routinely monitored in all parts of the world due to the unavailability of expensive analytical equipment, training capacity, and difficulty in culturing and preparing samples for analysis (Abeysiriwardena et al., 2018). Even though several standardized protocols in cyanotoxin analysis have been developed specifically for liquid chromatography-mass spectrometry (LC-MS/MS) (Haddad et al., 2019; Meriluoto et al., 2017), this method is expensive, leading to cyanotoxin monitoring efforts being concentrated in rich and developing countries (Haddad et al., 2019). An easier and more economical way to assess the presence of cyanotoxins, especially for countries with limited facilities such as Ecuador, is the detection of toxin synthesis-related genes by PCR using environmental DNA (eDNA) extracted from water samples as a template. In fact, eDNA is one of the best options for biomonitoring and screening the presence of potentially toxic species (Seymour, 2019). The description of microcystin synthesis genes (*mcy*A-J) (Christiansen et al., 2003; Nishizawa et al., 2000) and the gene cluster responsible for cylindrospermopsin biosynthesis (*cyr*A-O) (Mihali et al., 2008) enable the design of toxic strain-specific primers to test the presence of MC- and CN-producing cyanobacteria by using PCR techniques (Lei et al., 2019; Zhang et al., 2014).

Since *mcy*A, *mcy*B, and *mcy*C genes are essential for MC production, any of them are commonly used for detecting potential producers (Kurmayer et al., 2004; Massey & Yang, 2020; Mikalsen et al., 2003; Thomas & Dittmann, 2005; Via-Ordorika et al., 2004). However, the case of CN production by the *cyr* cluster is more controversial than MC. It has been reported in the genus *Aphanizomenon* that CN-producing and non-CN-producing strains possess *cyr*A, *cyr*B, and *cyr*C genes encoding polyketide synthases (PKS) and peptide synthetases (PS) putatively involved in CN synthesis (PKS) (Rasmussen et al., 2008; Schembri et al., 2001; Shalev-Alon et al., 2002). Thus, the detection of these genes may not guarantee that these strains produce CN. A study by Mihali et al. (2008) revealed that *cyr*J, a sulfotransferase-encoding gene, was present only in CN-producing strains,

supporting the involvement of this gene in the biosynthesis of CN.

The aim of our work was to perform a molecular screening of MC and CN producers in some Ecuadorian water bodies considering all the information above. The sequence of the amplified genes revealed the presence of *cyr* genes related to *Raphidiopsis* and *Aphanizomenon* and the presence of *mcy* genes related to *Microcystis*. Therefore, this study confirms the existence of potential CN and MC producers in Ecuador, reveals a new variant of the *cyr*J gene, and provides new tools to assess cyanotoxin hazards in Ecuadorian water bodies.

2. Materials and Methods

2.1. Study area

Eight water bodies and one wastewater treatment plant (WWTP) were chosen for this research (Figure 1; Table 1), with details as follows: i) Yahuarcocha Lagoon is located in Imbabura province. It is a eutrophic lagoon, since it is a recipient of wastewater discharges, fertilizers, and cattle grazing (Echeverría-Almeida & Athens, 2016). ii) San Pablo is a β-mesotrophic (moderate pollution) lake also located in Imbabura. Its poor conditions are due to the presence of chemical residues used in plant fertilization and livestock care (Rodríguez-Ayala et al., 2018). iii) Limoncocha Lagoon is in the Limoncocha Biological Reserve in the southwestern part of Sucumbios province. This eutrophic water body receives domestic wastewater
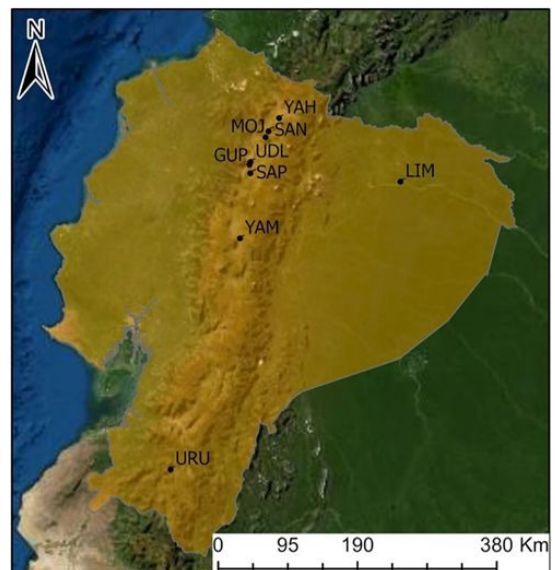

Figure 1. Map of Ecuador showing the sampling sites. Name codes of sampling sites are listed in Table 1.

Table 1. List of the sampling sites along with the name code, type of water body, longitude, latitude, and altitude. In meters above sea level (m asl), trophic status and temperature (Te).

Code	Water body	Longitude	Latitude	Altitude (m asl)	Trophic Status	Te (ºC)
GUA	Guápulo Artificial Pond	-78.472955	-0.197881	2597	β-mesotrophic	13
LIM	Limoncocha Lagoon	-76.619404	-0.406792	244	eutrophic	28
MOJ	Mojanda Lagoon	-78.273007	0.140353	3742	β-mesotrophic	11
SAN	San Pablo Lake	-78.237102	0.215909	2664	β-mesotrophic	21
SAP	San Pedro River	-78.460100	0.296226	2446	α-mesotrophic	18
UDL	UDLA WWTP	-7.458998	-0.162997	2837	-	-
URU	Uruzhapa Artificial Lake	-7.438013	-3.948732	1816	β-mesotrophic	18
YAH	Yahuarcocha Lake	-78.108984	0.376199	2191	β-mesotrophic	21
YAM	Yambo Lake	-78.58589	-1.104991	2579	eutrophic	21

and agricultural leachate (Carrillo et al., 2021). iv) San Pedro River is part of the upper basin of Guayllabamba in Pichincha province and is increasingly influenced by anthropogenic activities with direct sewage discharges. The sample point is located downstream in the Sangolquí district and is α-mesotrophic (strong pollution) (Ríos-Touma et al., 2022). v) Yambo is a lagoon located in Cotopaxi province and is considered eutrophic since it is a direct recipient of waste from nearby private complexes and the devastation of nearby vegetation (Orquera & Cabrera, 2020). vi) Uruzhapa is a β-mesotrophic artificial-lake situated in Uruzhapa, San Pedro de la Bendita, in Loja province. Its water is used exclusively for irrigating local crops. vii) Guápulo: β-mesotrophic artificial pond located in Guápulo Park in Quito. viii) Mojanda Lagoon is β-mesotrophic due to the constant extension of agricultural activities into the paramo ecosystem above 3,000 m asl, threatening the quality of water and causing lower amounts of precipitation, which in turn creates more concentrated levels of contaminations in the outflow (Schutz, 2014). ix) The WWTP is situated in "Universidad de las Américas" and its purpose is to treat the sewage generated from the facilities. Water treatment is mainly based on the use of trickling filters and sedimentation tanks.

2.2. Environmental sampling

A total of 8 planktonic samples (one from each water body except WWTP) and 8 biofilm samples (one from WWTP and each water body except Limoncocha) were collected from May to June 2019. Planktonic samples comprised 2L subsurface water (0.5m deep) from each location whereas biofilm samples were scrubbed off the upper surface

of three to five submerged stones, 10 to 20 cm in diameter, using a toothbrush (Kobayasi & Mayama, 1982). Samples were kept cold until their transfer to the laboratory. Access to freshwater ecosystems was conferred by the Environment Ministry of Ecuador (MAE) via the permission codes MAE-DNB.CM-2018-0093.

2.3. DNA extraction

Extraction was performed using commercially available kits following the manufacturer's instructions. For planktonic samples, 2L of collected water was filtered in a 0.45μm Whatman® mixed cellulose ester membrane. The filter was scrubbed and the DNA from the resultant material was extracted with PureLink™ Microbiome DNA Purification Kit. Using the same kit, biofilm samples were extracted without the previous processing for water samples. DNA concentration and purity of the samples were evaluated using a NanoDrop spectrophotometer (ThermoFisher Scientific). DNA was stored at –20 °C for further analyses.

2.4. Nested-PCR analyses

To check the success of the DNA extraction and the absence of inhibition in the PCR reaction, the amplification of the region 16S rDNA gene was carried out as a positive control using the primers CYA106F / CYA781R (Nübel et al., 1997) and PCR conditions, as outlined by Ballesteros et al. (2021). eDNA samples with a positive amplification for the 16S were considered suitable for further analysis.

A nested PCR assay was developed to enhance the specificity and signal amplification of the *mcy*B, *cyr*E, and *cyr*J genes in environmental samples (Fan et al., 2009). Two sets of primers (outer and

inner) were designed for the *mcy*B and *cyr*E genes with the Primer-BLAST tool, developed at the NCBI web page (Ye et al. 2012). The sequences from seven species belonging to the *Microcystis* genus were downloaded (January 2019) to design mcyB primers (*M. aeruginosa* CP020771, *M. viridis* AP019314.11, *M. panniformis* CP011339.1, *M. ichthyoblabe* KJ818158.1, *M. botrys* KJ818139.1, *M. smithii* KJ818124.1, *M. novacekii* KJ818122.1) and four sequences for cyrE and cyrJ primers (*Raphidiopsis curvata* KJ139745.1, *Cylindrospermopsis raciborskii* KJ139743.1, *Aphanizomenon* sp GQ385961.1, *Oscillatoria* sp FJ418586.4). Because of the small size of the amplification product, one set of primers was designed for the *cyr*J gene including one reverse and two forward primers and the reverse primer was combined with a forward primer from a previous study (Mankiewicz-Boczek et al., 2012) to perform the second PCR. The primers used are listed in Table 2, along with the sequence and position from the first nucleotide of the start codon.

First, PCR was performed in reactions of 20 µL, with Phusion High-Fidelity polymerase (Thermo FisherTM) containing 1x Master Mix with HF buffer, 0.25 µM of each outer primer (N1Fw/ N1Rv), and 25 – 100 ng of extracted eDNA. The first PCR was performed for 35 cycles as follows: initial denaturation step at 98 °C for 2 min, subsequent denaturation step at 98 °C for 30 s, annealing step at 53 °C (*mcy*B and *cyr*E) and 58 °C (*cyr*J) for 20 s, extension step at 72 °C for 30 s, and final extension for 2 min at 72 °C. One microliter of the amplified product was used as a DNA template for the second PCR. The second PCR was run as follows: 98 °C for 30 s, then 25 cycles of 98 °C for 15 s, 58 °C (*mcy*B and *cyr*E) and 55ºC (*cyr*J)

for 15 s and 72 °C for 20 s, and finally 72 °C for 1 min. Every PCR assay included a negative control containing all reagents without the DNA sample. PCR products of the second PCR were analyzed on a 1.5% w/v agarose gel stained with ethidium bromide (0.5 ng L^{-1}) and visualized using the GelDoc XR system (Bio-Rad).

2.5. Sequencing and sequence analysis

Amplicons of the expected size were purified using AccuPrep PCR/Gel Purification Kit (Bioneer) and sequenced at the sequencing service of Universidad de Las Américas (Quito, Ecuador). Sequences were edited with MEGA X software (Kumar et al., 2018) and submitted to a search in the GeneBank database at the NCBI website using the Basic Local Alignment Tool (BLASTn) (McGinnis and Madden, 2004).

The sequences obtained were registered in the NCBI nucleotide database under the following accession numbers: *mcy*B "MZ615179"; *cyr*E1 "MZ615175"; *cyr*E2 "MZ615176"; *cyr*E3 "MZ615177"; *cyr*J "MZ615178".

3. Result and Discussion

We assessed eight water bodies from urban and rural areas in Ecuador and one WWTP situated in "Universidad de las Americas" facilities to detect *mcy*B and *cyr*J/E genes. Although most studies report planktonic cyanobacterium hazards, previous works demonstrated that in both planktonic and biofilm samples, there might be the potential for cyanotoxin production (Belykh et al., 2016; Lajeunesse et al., 2012; Mohamed et al., 2006; Smith et al., 2011; Zupančič et al., 2021). Thus, we took samples from plankton and biofilms from

Table 2. Primers designed to amplify *mcy*B, *cyr*E, and *cyr*J by nested PCR. Primers named as N1 were used for the first PCR and N2 for the second round, except for CyrJN1Rv, which was used for PCR1 and 2. The symbol * indicates that the primer was retrieved from Mankiewicz-Boczek, et al. (2012). Primer positions are referred to as the ATG from *Microcystis viridis* (*mcy*) and *Raphidiopsis raciborskii* (*cyr*).

Target gene	Primer name	Sequence (5'→ 3')	Position
mcyB	*Mcy*N1Fw	AGAATCCGCAGGGAATAGCG	282
	*Mcy*N1Rv	CTTGGGCATTAGCAAGGGGA	1208
	*Mcy*N2Fw	AAAAGCCGGGGGAGCTTATG	460
	*Mcy*N2Rv	ACCGCTACTTCTGTTGGTCC	1137
cyrE	*Cyr*EN1Fw	ATGGGGCGATGGTTTCAGTT	597
	*Cyr*EN1Rv	CCATTTGCATACGACTCGGC	1479
	*Cyr*EN2Fw	GCGATTCAATCTGTCAGCCG	644
	*Cyr*EN2Rv	GCTTCATGCACCTGTCCAGT	1328
cyrJ	*Cyr*JN1Fw	CCACGCAGTTTTGGTACAGTG	33
	*Cyr*JN1Rv	CCCCTACAGCCTGACAAAGC	560
	*Cyr*JN2Fw*	TTCTCTCCTTTCCCTATCTCTTTATC	103

every water body except Limoncocha due to the lack of solid submerged substrates. The WWTP sample was taken from the trickling filter biofilm. Four primers were used to perform a nested PCR to amplify *mcy*B and *cyr*E, and just three for *cyr*J due to the small size of this gene. This method is well-known for its high sensitivity and specificity when facing low concentrations of target DNA, as is the case when using eDNA as a template (Fan et al., 2009). In some samples, the second PCR generated multiple bands, as previously reported in similar works (Mankiewicz-Boczek et al., 2012; Stefanova et al., 2020). In these cases, we consider as positive results the appearance of a band of the expected size which was extracted from the agarose gel and sequenced.

Mcy primers were designed to amplify the *mcy*B gene from the *Microcystis* genus, since these species are abundant, have numerous bloom occurrences, and commonly produce MC (Massey & Yang, 2020). The nested PCR was positive for samples from Guápulo (biofilm), Limoncocha (plankton), San Pablo (plankton and biofilm), and Yahuarcocha (plankton and biofilm) with the amplification of a fragment of the *mcy*B gene of the expected size (687 bp) (Table 3). Despite the existence of multiple alleles described for *Microcystis* sp. (Massey & Yang, 2020), we detected the same gene variant for the four water bodies. The BLASTn showed only one mismatched nucleotide with *mcy*B gene from *Microcystis panniformis* that has been previously reported as an MC producer in the neotropical region (Bittencourt-Oliveira et al., 2011).

The primers for *cyr*J were designed to amplify genes from the main genera described as putative CN producers, such as *Oscillatoria*, *Cylindrospermopsis, Raphidiopsis curvata,* and *Aphanizomenon* (Adamski et al., 2020). The only positive sample for this gene was eDNA isolated from Mojanda biofilm (Table 3). The closest matches, with an identity of around 90%, were *Raphidiopsis raciborskii*, *R. curvata*, and *Oscillatoria* sp., with the latter taxa being already described as a benthic cyanobacterium genus (Gaget et al., 2017).

*Cyr*E was amplified in Limoncocha (plankton), Uruzhapa (plankton), Yambo (plankton), Yahuarcocha (biofilm), San Pablo (biofilm), and Mojanda (biofilm) (Table 3). The sequence obtained from Limoncocha, Yambo, WWTP, Yahuarcocha and Mojanda locations was named *cyr*E1, while the one from Uruzhapa and San Pedro River was given the name *cyr*E2, and the sequence retrieved from San Pablo Lake became *cyr*E3. *cyr*E1 was most similar (99.4% identity) to *cyr*E3. The variation in nucleotide sequence leads to a single amino acid change from phenylalanine (*cyr*E1) to leucine (*cyr*E3). *Cyr*E2 nucleotide sequence had 98.2% identity to *cyr*E3 (four amino acid changes) and 97.2% to *cyr*E1 (five different amino acids) (Figure 2). The *cyr*E1 and *cyr*E3 sequences revealed a 100% identity with the same gene from species belonging to the *Raphidiopsis* genus, specifically *Raphidiopsis raciborskii* and *Raphidiopsis curvata*, respectively. Nevertheless, *cyr*E2 differed from cyrE1 and cyrE3. The closest match to the *cyr*E2 sequence at the GenBank was 98% identity to *cyr*E related to *Aphanizomenon* sp., which has been described as a benthic cyanobacterium genus (Gaget et al., 2017). In South America, most isolated strains from *R. raciborskii* are reported to produce saxitoxins (Hoff-Risseti et al., 2013), although CN producers have also been found in Brazil and Venezuela (Antunes et al., 2015; Mowe et al., 2015), and Ecuador (Ballesteros et al., 2021; Van Colen et al., 2017; Venegas et al., 2018). Even though *R. racibosrkii* has been reported in Yahuarcocha and San Pablo in association with the presence of CN (Van Colen et al., 2017), *cyr*J was not amplified from samples in these

Table 3. List of the sampling points showing the variants of the amplification and sequencing of the assessed genes in this study: mcyB1 from *Microcystis panniformis*; cyr E1 from *Raphidiopsis raciborskii*; cyrE2 from *Aphanizomenon* sp; cyrE3 from *Raphidiopsis curvata* n.d.: not detected. (b)= biofilm samples. Name codes of sampling sites are listed in Table 1 . (p)=plankton samples.

Sample code	*mcy*B sequence	*cyr*E sequence	*cyr*J sequence
GUP	*mcyB1(b)*	n.d.	n.d.
LIM	*mcyB1(p)*	*cyrE1(p)*	n.d.
MOJ	n.d.	*cyrE1(b)*	*cyr*J1*(b)*
SAN	*mcyB1(p,b)*	*cyrE2(b)*	n.d.
SAP	n.d.	*cyrE3 (p)*	n.d.
UDL	n.d.	*cyrE1(p)*	n.d.
URU	n.d.	*cyrE2(p)*	n.d.
YAH	*mcyB1(p,b)*	*cyrE1(b)*	n.d.
YAM	n.d.	*cyrE1(p)*	n.d.

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         *         20         *         40         *         60
cyrE2 : HAFHSPLMEPILGQFRRVANTITYRPPQINLVSNVTGARVYKEIAIPDYWVRHLQETVRF :  60
cyrE3 : HAFHSPLMEPILGQFRRVANTITYRPPQINFVSNVTGGQVYKEIAIPDYWVRHLQETVRF :  60
cyrE1 : HAFHSPLMEPILGQFRRVANTITYRPPQINFVSNVTGGQVYKEIAIPDYWVRHLQETVRF :  60

         *         80         *         100        *
cyrE2 : ADGVKVLHEQNVNFMLEIGPKPTLLGMVELQSSENPSSMPMMMPSLRENRSD : 112
cyrE3 : ADGVKVLHEQNVNFMLEIGPKPTLLGMVELQSSENPSMPMMMPSLRQNRSD  : 112
cyrE1 : ADGVKVLHEQNVNFMLEIGPKPTLLGMVELQSSENPSMPMMMPSLRQNRSD  : 112
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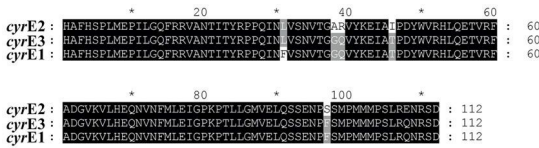
Figure 2. Alignment of cyrE sequences translated into amino acids.

water bodies. The sequence of this gene amplified from Mojanda showed high variation with those available at the GenBank, which suggests that the varieties of *cyr*J present in these water bodies might not be amplified by the primers used in this work.

Except for Mojanda Lagoon, all water bodies assessed in this study might present cyanobacterial blooms due to their trophic status and temperature. The average water temperature of Yahuarcocha and San Pablo Lakes is 21 °C and is comparable to that of lowland subtropical lakes, despite their locations at 2,190 and 2,700 m asl, respectively (Kurmayer et al., 2004). Furthermore, the lack of a cold season allows cyanobacterial blooms to persist for more than one year and allows tropical fish species to survive in the lakes (Van Colen et al., 2017). The same principles could apply to the eutrophicated lagoon of Yambo, also positive for *cyr*E from *R. raciborskii*. This water body is located at 2,500 m asl with an average water temperature of 18.5 ºC. Limoncocha Lagoon also fulfills the environmental conditions for this species to bloom (Mowe et al., 2015; Padisák, 1997; Padisák & Reynolds, 1998; Whitton & Potts, 2002), as it is highly eutrophicated due to natural processes and sewage from nearby Limoncocha village and has a temperature ranging from 28 °C to 32.23 °C (Gómez Durañona, 2005). The presence of *cyr*E from *R. raciborskii* sample in this lagoon was consistent with the report of this cyanobacterium by Venegas et al. (2018). Mojanda Lagoon, where also *cyr*E1 sequence was detected, is located at 3,710 m asl with an average temperature of approximately 13.5 ºC, this lagoon is mesotrophic due to anthropic activities such as livestock. Thus, it is the least likely to develop a HAB (Schutz, 2014). Therefore, the lack of positives in planktonic samples in Mojanda Lagoon might be due to a lower cyanobacterial density in planktonic samples than benthic ones.

The characterization of the cyanotoxin genes present in Ecuadorian water bodies is the first step towards developing tools to detect the production of cyanotoxins through qPCR techniques, as described by Lei et al. (2019). Furthermore, this country is an outstanding set to study the effects of global warming as

there is an almost constant temperature gradient along the slope from the Andes range down to the amazon region throughout the year (Cuesta et al., 2019). Therefore, the current study might be a humble first step towards future screenings that might anticipate the consequences of global warming in cyanotoxin production in the Andean region of Ecuador.

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