

# Microbial diversity and trophic components of two high altitude wetlands of the Chilean Altiplano

## Diversidad microbiana y componentes tróficos de dos humedales de altura del altiplano chileno

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### ABSTRACT

This study examines the limnology and ecology of two high altitude wetlands, Lirima (19°51'24 S; 68°55'02 W; 4000 m asl) and Caya (20°37'21 S; 68°58'28 W; 3700 m asl), located in the Chilean Altiplano. Both wetlands are formed by the evaporitic remnant basins of paleolakes which occupied an extensive area of what today is known as the Altiplano. These systems have a negative hydrological balance, receiving their water from groundwater, snow melt and limited seasonal rains. An ongoing negative water balance and the sediment characteristics in the region have accelerated the salinization process in these systems, as shown by their present physicochemical characteristics. Nutrient values were typical of mesotrophic to eutrophic systems. The ionic content classifies Lirima as a sodium sulfated wetland and Caya as a calcium chloride one. Conductivity values ranged between 778 µS/cm at Lirima to 2100 µS/cm at Caya, and were reflected in the differences in biodiversity found in these systems. The Lirima wetland supports a population of the endemic fish *Orestias* aff. *agassii* found in several Evolutionary Significant Units (ESU) across the region. Microbial diversity in the water column was characterized by the presence of 5 bacterial phyla and related genera (e.g. *Psychrobacter*, *Bacillus*, *Erythobacter*, *Halomonas*). We present information on several key ecosystem components including macrophytes, plankton, benthos, fish and birds. This descriptive paper highlights the unusual limnological and biological characteristics of high altitude wetlands and highlights the importance of describing their biological communities across levels of organisation (e.g. microbial through to higher vertebrates) as well as their functional role, interactions and sensitivity to changes in water availability.

**KEYWORDS:** Altiplano, biodiversity, endemism, evolutionary significant units, salinity.

### RESUMEN

Este estudio examina la limnología y ecología de dos lagos de altura, los humedales de Lirima (19°51'24 S; 68°55'02 W; 4000 m asl) y Caya (20°37'21 S; 68°58'28 W; 3700 m asl) que están ubicados en el Altiplano Chileno, representando cuencas evaporíticas remanentes de antiguos paleolagos los cuales ocuparon una amplia zona en lo que hoy conocemos como Altiplano. Estos sistemas tienen un balance hídrico negativo, recibiendo su agua desde fuentes freáticas, derretimiento de nieves y escasas lluvias estacionales. Las características del suelo junto con la demanda de agua en la región han acelerado el proceso de desalinización en estos sistemas siendo reflejado en las características químicas actuales. Los valores de nutrientes fueron típicos de sistemas mesotróficos a eutróficos. El contenido iónico clasifica Lirima como un humedal sodio sulfatado y el humedal de Caya como un humedal de cloruro de calcio. Los valores de conductividad fluctuaron entre 778 µS/cm en Lirima a 2100 µS/cm en Caya, reflejándose en las diferencias de biodiversidad encontrada en estos sistemas. El humedal de Lirima conserva el pez endémico *Orestias* que representa Unidades Evolutivas Significativas (ESU) en la región. La diversidad microbiana en muestras de agua estuvo caracterizada por la presencia de 5 filo bacterianos y géneros relacionados (e.g. *Psychrobacter*, *Bacillus*, *Erythobacter*, *Halomonas*). Los componentes tróficos estudiados

incluyeron macrófitas, plancton, bentos, peces, anfibios y aves. Este artículo descriptivo destaca las inusuales características limnológicas y biológicas de los humedales de altura poniendo atención a la importancia de describir comunidades en distintos niveles de organización biológica (desde tapetes microbianos hasta vertebrados superiores), pero también sus funciones, interacciones y sensibilidad a cambios en la disponibilidad de agua.

**PALABRAS CLAVE:** Altiplano, biodiversidad, endemismo, unidades evolutivas significativas, salinidad.

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## INTRODUCTION

The Chilean Altiplano, with an average altitude of over 3000 meters above sea level, includes a large and varied number of freshwater ecosystems within its boundaries from its northern (18°S, Lake Chungará) to southern limits (27°S, Lake Negro Francisco). To date, 50 different closed hydrographic basins have been identified, which together present significant variation in physicochemical (e.g. ionic concentrations) and geomorphologic characteristics (Risacher *et al.* 2003a, 2003b; Moreno *et al.* 2009). These systems are internationally important but are largely undescribed, limiting our understanding of them and the ability of managers and scientists to protect them and the goods and services they provide from human impacts.

The evaporitic basins of the Chilean Altiplano originate from paleolakes that occupied the extensive area of what today is known as the Altiplano and the Atacama Desert (Chong 1988; Marquet . 1998). Today, these systems have a negative hydrological balance, with water loss by evaporation outweighing hydrological inputs via groundwater, snow melt and limited seasonal rains (Aceituno 1997; Salazar 1997; Keller & Soto 1998).

Studies of the taxa inhabiting aquatic ecosystems in the Altiplano have raised important questions with regard to their evolutionary history, biogeographic relationships and evolutionary adaptations to the significant abiotic stresses that vary at daily, seasonal and interannual scales (e.g. temperature, UV radiation, water availability). In shallow lentic systems in the Altiplano, known locally as *bofedales*, this has resulted in the repeated presence of genetically distinct and locally adapted Evolutionary Significant Units (ESU) in different taxa, for instance the fish *Orestias* aff. *agassii* Valenciennes (Vila *et al.* 2010).

Water availability is key to the function and maintenance of life in these high altitude freshwater ecosystems. This extends from the varied primary producer communities that include contributions from fringing macrophytes, microalgae and microbial mats (Mitsh & Gosselink 2000; Dorador *et al.* 2008; Riveros *et al.* 2012) that are often exposed to desiccation, through to the higher taxa that

consume and transfer energy and nutrients as part of the foodwebs such as fish (Vila *et al.* 2013).

A series of recent studies have highlighted that high altitude wetlands have unusual microbial communities (Bacteria and Archaea) that include the presence of previously undescribed lineages of Bacteria (Dorador *et al.* 2013). These taxa participate actively in different biogeochemical cycles (Dorador *et al.* 2008a) and exhibit marked temporal and spatial variation according to gradients of salinity, temperature and solar radiation (Dorador *et al.* 2008b; 2010). To date, there has been little consideration of the trophic role of microbial taxa in these ecosystems.

Across the Altiplano, the majority of aquatic ecosystems remain undescribed, limiting our ability to understand their ecological importance and sensitivity to human impacts. Here, we provide a first description of microbial diversity and key trophic components of two undescribed and contrasting wetlands (Lirima and Caya), located at high altitude in the Tarapaca Region of northern Chile. These ecosystems, like other wetlands located across the Chilean Altiplano, are subject to elevated risk due to high and increasing water demand and ongoing climate change (Valdés-Pineda *et al.* 2014): this work therefore contributes to the basic knowledge of threatened aquatic environments in northern Chile.

## MATERIALS AND METHODS

### DESCRIPTION OF STUDY SITES

We examined two high-altitude wetlands: Lirima (19°51'24 S; 68°55'02 W; 4000 m asl) and Caya (20°37'21 S; 68°58'28 W; 3700 m). Both systems are shallow remnants of earlier deeper lakes from the Tarapacá Ravine area. The Tarapacá Ravine rises in the Andean highlands where annual inputs of water are not greater than 150 mm/year (Aceituno 1997, Risacher *et al.* 2003). The climatic regime of the Tarapacá Ravine is pluvial, with higher freshets during summer due to summer rains in the Altiplano. The Tarapacá Ravine system receives waters from the Coscaya River and then forms the Caya wetland. Further downstream, the system drains the Lirima plains where it forms the Lirima wetland. This river

presents higher freshets during winter and summer due to winter and summer rains (June–July and January–March) (MOP-DGA, CADE-IDEPE 2004) (Figs. 1 and 2).

Aquatic ecosystems in the region are commonly used as sources for water supply for industrial processes such as mining and agriculture. The Caya wetland currently has 11 applications to extract water for the mining industry and 3 existing abstraction points which are likely affecting both water levels of the wetland and its function. In contrast, Lirima wetland has no existing applications for water extraction request to date (Morales, 2011).

In order to characterise the different systems, we used a Geographical Information System (GIS) approach using a number of different GIS (ENVI 4.4, IDRISI ANDES 15.01, ARC GIS 9.3 & ARC View 3.3). Satellite images were obtained from the Earth Science Data Interface (ESDI) of the Global Land Cover Facility, University of Maryland, USA, with two views from 1999 and 2003 from the Landsat 7 satellite, sensor ETM+ with 30 meter spatial resolution. During November 2009 we made a series of measurements of physicochemical and biological characteristics in the Caya and Lirima wetlands. Chemical and biological variables were obtained from replicated water samples. Due to the difficulties involved in accessing wetlands in this region, and the stresses involved with reduced water availability, these observations were made during a period with minimum water flow (Maximum water depth in Caya = 0.3 m; Lirima = 0.8 m) with the aim of characterizing the system under extreme conditions, allowing the identification of the key abiotic factors that drive ecosystem function under such conditions.

#### PHYSICAL AND CHEMICAL VARIABLES

Water temperature was measured ( $\pm 0.1^\circ\text{C}$ ) with digital Hanna thermometer; conductivity and total dissolved solids (TDS) were measured with a VWR portable conductivity meter, and pH with a portable WTW pH meter ( $\pm 0.1$  pH unit). Salinity (NaCl) was measured ( $\pm 0.1$ ) with a portable VWR salinometer. Dissolved oxygen concentrations were estimated using the Winkler method (APHA, 2001), and percentage saturation was estimated using values of dissolved oxygen, altitude and temperature. Total phosphorous (TP) and total nitrogen (TN) were measured according to Mühlhauser *et al.* (1986). Sulfates ( $\text{SO}_4$ ) were measured with the turbidity method of Göltnerman *et al.* (1978). Bicarbonates ( $\text{HCO}_3$ ), carbonates ( $\text{CO}_3$ ) and the cations Na, K, Ca and Mg were measured by atomic absorption (APHA 2005).

#### BIOLOGICAL VARIABLES

Bacterial abundance was estimated from water samples using DAPI staining (Porter & Feig, 1980). Samples

were fixed in the field with 2% formaldehyde, filtered in polycarbonate filters with  $0.22 \mu\text{m}$  pore size and posteriorly analyzed by epifluorescence microscopy (Olympus BX51). Culturable bacterial diversity was determined using mineral media using acetate and DMSO as energy sources (Atlas 1995). The samples used to determine the diversity of culturable bacteria were obtained from water samples where field temperatures fluctuated between  $21\text{--}25^\circ\text{C}$  collected from the Lirima wetland. Isolates were identified through their 16S rRNA sequences, which were obtained by PCR and direct sequencing (Dorador *et al.* 2009). Their closest relatives were determined by BLAST search (<http://www.ncbi.nlm.nih.gov/blast>) and the classifier tool in RDP II (<http://www.cme.msu.edu/rdp>). Chlorophyll *a* concentration was measured according to Montecino & Cabrera (1982). Phytoplankton samples were obtained with a net  $40 \mu\text{m}$  size and fixed with Lugol. In the laboratory, phytoplankton were identified and counted following the Utermöhl method using an inverted microscope (Olympus CK2) according to Villafaña & Reid (1995), in 10 ml and 25 ml sedimentation cells during 24 hrs and 48 hrs respectively. Rivera *et al.* (1982), Parra & Bicudo (1995) and Diaz & Maidana (2005) were used for taxonomic identification of phytoplankton to the genus level. Macrophytes were collected and identified according to Ramírez and San Martín (2006), with Point Quadrat methodology, with a sampling each two meters at ten points using an inter-point distance of 10 cm.

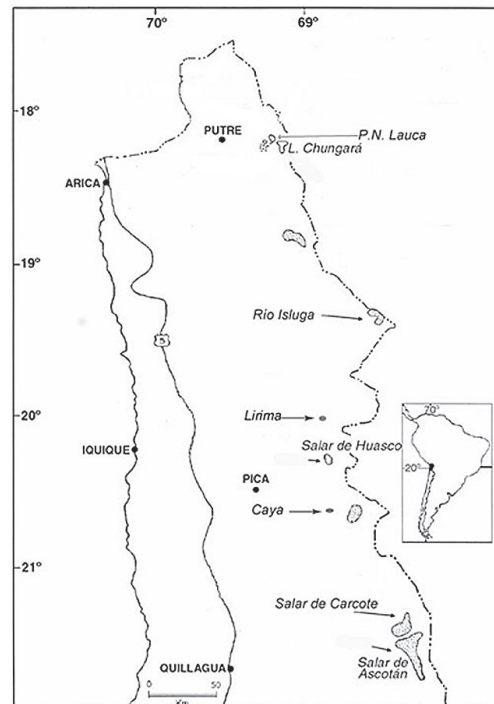


FIGURE 1. Map of the Altiplanic region, northern Chile. Study sites.

FIGURA 1. Mapa de la Región Altiplánica, norte de Chile. Sitios de Estudio.

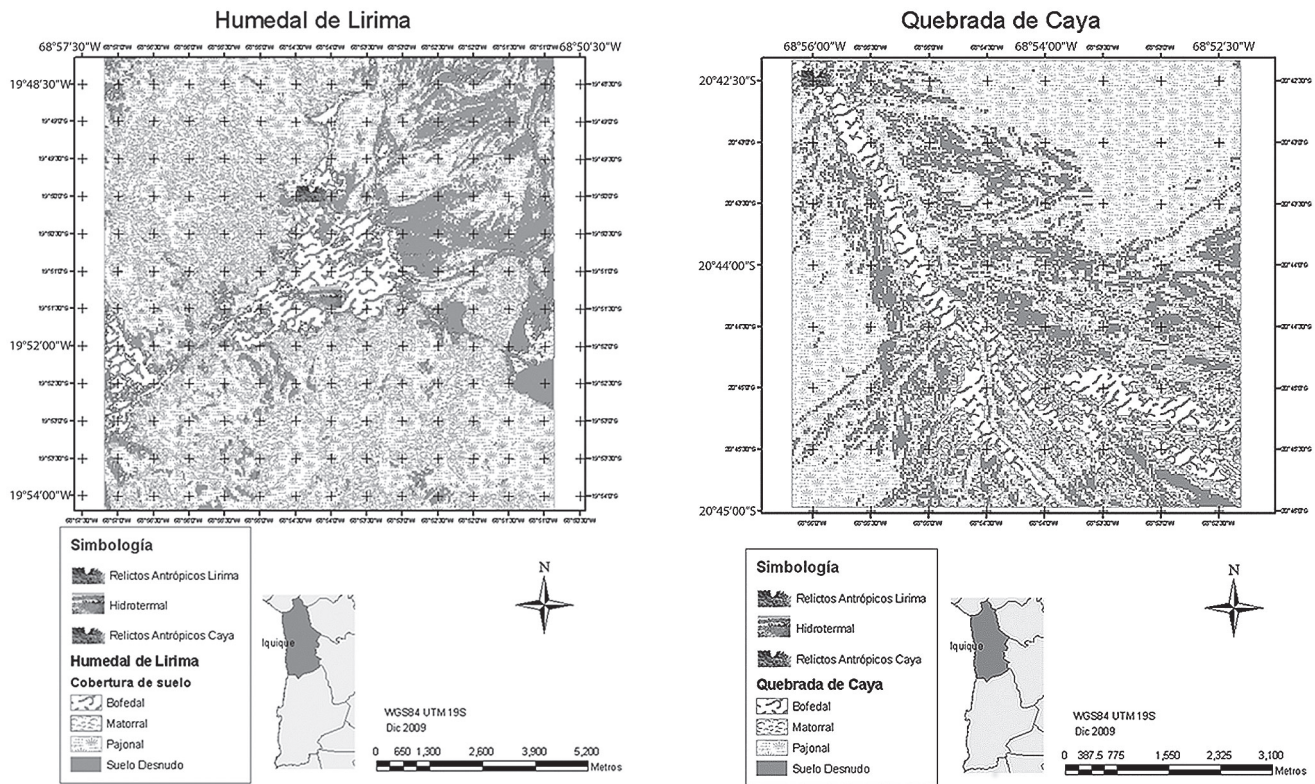


FIGURE 2. Classification of land cover in Lirima and Caya based on images of Landsat ETM+, from 1999.

FIGURA 2. Clasificación de la cobertura de suelo en Lirima y Caya basados en imágenes Landsat ETM+, de 1999.

Zooplankton samples were obtained by filtering 10 L of water of the water column with a zooplankton net 120  $\mu\text{m}$  size and fixed with 10% buffered formaldehyde. In the laboratory, they were counted in a Bogorov cell (Wetzel & Likens 1991). For taxonomic identification the taxonomic keys of Araya & Zuñiga (1985) were used. Benthic fauna samples were obtained with a Surber sampler and identified according to Fernández & Domínguez (2001) and Figueroa *et al.* (2003). Fish and amphibians were captured with electrofishing equipment and identified in the laboratory according to Vila *et al.* (2006). The avian communities using the wetlands were assessed over one hour in each system at midday, counted and identified in the field.

## RESULTS

Landcover in the catchment of the study wetlands is heterogeneous and characterized by seven distinct cover types corresponding to: i) Xerophytic grassland, ii) Bofedal (low vegetation forming “cushions”) iii) Stream (superficial river bed), iv) Bush (50 to 150 cm shrub formation), v) Naked

soil, vi) Anthropological relicts and vii) Hydrothermal waters, (Ahumada & Faundez, 2009) (Figs. 1 and 2).

TABLE 1 details the physicochemical characteristics recorded from the Lirima and Caya wetlands. Water temperature was within the typical range of Altiplano systems, with high values during the day due to the diurnal heating of the shallow high Andean systems ( $>20^\circ\text{C}$ ). Conductivity analysis showed values of 778  $\mu\text{S}/\text{cm}$  at Lirima, characteristic of freshwater systems (Wetzel 2001). Conductivity was higher in the Caya wetland, reaching 2100  $\mu\text{S}/\text{cm}$ . Values of pH were basic, fluctuating between 8.3 and 8.7 due to the salt concentration of these systems.

Oxygen values were high for high altitude systems, especially in Caya, and varied between 10 and 14 mg/L at Lirima and Caya respectively. In the Lirima wetland, the oxygen saturation percentage reached 100% and at Caya it increased to 130%. TP values were especially high in Lirima (169.9  $\mu\text{g}/\text{L}$ ) which corresponds to high productivity or eutrophic systems. Caya presented lower values of TP (67.8  $\mu\text{g}/\text{L}^{-1}$ ), in the range of mesotrophic systems (Ryding

& Rast 1992). Values of TN were relatively high and correspond to systems from meso- to eutrophic in Lirima and Caya, reaching 1631.6 and 2421.6  $\mu\text{g/L}$ , respectively (Ryding & Rast 1992; Wetzel 2001). Chloride values were high in Caya (450 mg/L) and lower in Lirima with 67 mg/L. Sulfate values were 82.5 mg/L and 172 mg/L at Lirima and Caya, respectively. Bicarbonate values were low and fluctuated between 26.5 mg/L and 79.3 mg/L for Lirima and Caya, respectively.  $\text{CO}_3^{-2}$  values were 15 mg/L at Lirima and under the detection limit at Caya. According to the cation concentrations, Lirima represents a system with predominance of sodium ( $\text{Na} < \text{Ca} < \text{K} < \text{Mg}$ ). Caya, by contrast, has dominance of calcium ( $\text{Ca} < \text{Na} < \text{Mg} < \text{K}$ ). Figure 3 summarizes the anionic and cationic values for both systems in a Maucha diagram (Wetzel 2001), which highlights the proportionality of ion concentrations in both wetlands.

TABLE 1. Physical and chemical variables of the Caya and Lirima systems (average of replicated samples).

TABLA 1. Variables físicas y químicas de los sistemas de Caya y Lirima (promedio de réplicas de muestras).

SITE	CAYA	LIRIMA
Depth (m)	1	1
Total nitrogen ( $\mu\text{g/L}$ )	2421.6	1631.9
Total Phosphorus ( $\mu\text{g/L}$ )	67.8	169.9
$\text{Cl}^-$ (mg/L)	450	67
$\text{HCO}_3^-$ (mg/L)	79.3	26.5
$\text{CO}_3^{-2}$ (mg/L)	0.0	15.0
$\text{Na}^+$ (mg/L)	188.3	91.1
$\text{K}^+$ (mg/L)	18.6	16.3
$\text{Ca}^{+2}$ (mg/L)	261.1	60.9
$\text{Mg}^{+2}$ (mg/L)	40.1	9.2
Chl <i>a</i> (mg/L)	5.4	2.9
Dissolved oxygen (mg/L)	12.5	8.9
$\text{SO}_4^{-2}$ (mg/L)	172.1	82.5
Temperature ( $^{\circ}\text{C}$ )	24.1	22.1
Conductivity ( $\mu\text{S/cm}$ )	2100	778
pH	8.3	8.7

Bacteria present in Lirima water samples were analyzed by DAPI, estimating an average concentration of  $3 \times 10^4$  cell/ml. Microbial cells were mainly grouped in a filamentous matrix. In the hydrothermal waters of Lirima wetland, the presence of highly pigmented microbial mats was common. In total we analyzed 30 16S rRNA gene sequences from 61 bacterial isolates. The isolates were members of Gammaproteobacteria (43%), Alphaproteobacteria (13%), Firmicutes (37%), Actinobacteria (3.3%) and Bacteroidetes (3.3%). Most of the isolates exhibited high sequence identity (>98%) with the following genera: *Psychrobacter*, *Halomonas*, *Aeromonas*, *Pseudomonas*, *Paracoccus*, *Brevundimonas*, *Rhizobium*, *Exiguobacterium*, *Bacillus*, *Planococcus*, *Dietzia* and *Chryseobacterium* (Fig. 4). Most of these taxa use different organic carbon sources and also exhibit adaptations to thrive under extreme environmental conditions such as low temperature (*Psychrobacter*, *Chryseobacterium*), high salt concentration (*Halomonas*) and desiccation (*Bacillus*) (Table 2).

Chlorophyll *a* values were in direct accordance with the amount of dissolved nutrients in these wetlands, especially TN in Caya. These values fluctuated between 5.4  $\mu\text{g/L}$  in Lirima and 2.9  $\mu\text{g/L}$  in Caya, which corresponds to mesotrophic systems (Wetzel 2001). As in the majority of the Altiplano wetlands, microalgae were mostly represented by yellow silica algae or diatoms (Diaz & Maidana 2005). At Caya there was a higher abundance of cyanobacteria in correspondence with higher values of total nitrogen. Their abundance reached a total of 135.6 and 108.9 units/L in Caya and Lirima, respectively (Table 3). The macrophyte communities at both locations were limited: at Lirima macrophytes were dominated by “water pine“, *Myriophyllum* sp., followed by *Lilaeopsis* sp.; at Caya only *Lilaeopsis*, *Patosia* and *Ranunculus* were recorded (Table 3). Zooplankton abundance was low and dominated by Cladocera in both wetlands. Ostracoda followed in abundance, (Table 4). Benthic fauna in both wetlands were mostly composed of the crustacean genus *Hyalella*, which is generally very abundant in shallow waters associated with macrophytes (Table 5).

The bird community of the two systems included 15 species, including the Andean seagull *Larus serranus* (Tschudi) classified as a rare species, and the vulnerable species Andean goose *Chloephaga melanoptera* (Eyton), (Table 6). Fish were only recorded Lirima where karachi *Orestias* aff *agassii* Valenciennes were present; this genus is endemic to the Altiplano area and all of its species are classified as endangered, due to their low population number and restricted distribution according to the current Environmental Ministry species classification rules (<http://www.mma.gob.cl/clasificacionespecies/>) (Table 6).

TABLE 2. Phylogenetic affiliation of bacterial isolates obtained from water samples of Lirima wetland based on 16S rRNA gene sequence identity with GenBank and Ribosomal Database Project (RDP).

TABLA 2. Afilación filogenética de aislados bacterianos obtenidos desde muestras de agua del humedal de Lirima basados en la identidad de secuencia del gen ribosomal ARN 16S con las bases de datos GenBank y Ribosomal Database Project (RDP).

Bacterial strain	First hit in BlastN (accession number) (% coverage/ % sequence identity)	Phylogenetic affiliation (RDP) (Phyla/ Subphyla, Genus)
9-11	<i>Psychrobacter</i> sp. BSw20963B (GU166134) (99/96)	Gammaproteobacteria, <i>Psychrobacter</i>
L8-11	<i>Psychrobacter marincola</i> strain J82 (JX976309) (100/100)	Gammaproteobacteria, <i>Psychrobacter</i>
L52-32	<i>Halomonas</i> sp. SB135_6 (EU308331) (100/99)	Gammaproteobacteria, <i>Halomonas</i>
L5-11	<i>Aeromonas hydrophila</i> pc104A (CP007576) (95/99)	Gammaproteobacteria, <i>Aeromonas</i>
L33-41	Clone 661206 (DQ404928) (100/99)	Gammaproteobacteria, <i>Pseudomonas</i>
L66-21	<i>Erythrobacter</i> sp. JL660 (EF512713) (100/99)	Alphaproteobacteria, <i>Erythrobacter</i>
L37-41	<i>Paracoccus marcusii</i> strain zzx35 (KJ009431) (100/99)	Alphaproteobacteria, <i>Paracoccus</i>
L51-21	Clone CT1C2BB10 (JQ427853) (100/99)	Alphaproteobacteria, <i>Brevundimonas</i>
L48-41	<i>Rhizobium</i> sp. AB3 (KC870058) (100/99)	Alphaproteobacteria, <i>Rhizobium</i>
L11-11	<i>Exiguobacterium</i> sp. Pb-WC11087 (JX913841) (100/99)	Firmicutes, <i>Exiguobacterium</i>
L2-11	<i>Bacillus</i> sp. 58B112Y11 (KC815825) (100/99)	Firmicutes, <i>Bacillus</i>
L47-MH	Clone QNSW58 (FJ384526) (100/99)	Firmicutes, <i>Bacillus</i>
L39-42	Clone ncd2231c10c1 (JF193171) (100/100)	Firmicutes, <i>Sinobaca</i>
L46-12	Clone QNSW58 (FJ384526) (100/99)	Firmicutes, <i>Bacillus</i>
L6-11	Clone QNSW58 (FJ384526) (100/99)	Firmicutes, <i>Bacillus</i>
L21-22	<i>Dietzia psychralcaliphila</i> strain DSM 44820 (FJ468331) (99/99)	Actinobacteria, <i>Dietzia</i>
L62-21	<i>Chryseobacterium</i> sp. L2 (KF358274) (100/99)	Bacteroidetes, <i>Chryseobacterium</i>

TABLE 3. Abundance of microalgae in Lirima and Caya (Units per L) (average of replicated samples). Number of individuals/L of zooplankton in the Lirima and Caya systems (\* absent) (average of replicated samples).

TABLA 3. Abundancia de microalgas en Lirima y Caya (unidades por litro) (promedio de muestras replicadas). Número de individuo/Litro en los sistemas de Lirima y Caya (\*ausente) (promedio de muestras replicadas).

MICROALGAE	LIRIMA units/L	CAYA units/L	ZOOPLANKTON	LIRIMA ind/L	CAYA ind/L
Diatoms			Copepoda		
<i>Cocconeis</i>	14.7	25.6	Ciclopoidea		
<i>Navicula</i>	65.4	71.6	<i>Eucyclops serrulatus</i>	0.1	*
<i>Synedra</i>	18.2	24.0	Harpacticoidea	*	0.4
<i>Cimatopleura</i>	2.1	1.6	Cladocera		
<i>Nitzschia</i>	2.0	2.0	<i>Alonella excisa</i>	*	0.3
<i>Fragilaria</i>	2.4	3.6	<i>Chydorus sphaericus</i>	0.4	*
<i>Surirella</i>	1.2	1.6	Rotifera		
<i>Gomphonema</i>	2.0	2.0	<i>Keratella cochlearis</i>	0.1	*
Cyanobacteria			Rotifer spp 1	0.1	*
<i>Anabaena</i>	0.9	2.8	Ostracoda	0.2	0.8
Desmids	*	0.8			
Total	108.9	135.6			



FIGURE 3. B: Maucha diagrams characterising the anionic and cationic content of the wetlands of Caya and Lirima.

FIGURA 3. B: Diagramas de Maucha con el contenido aniónico y catiónico de los sistemas de Caya y Lirima.

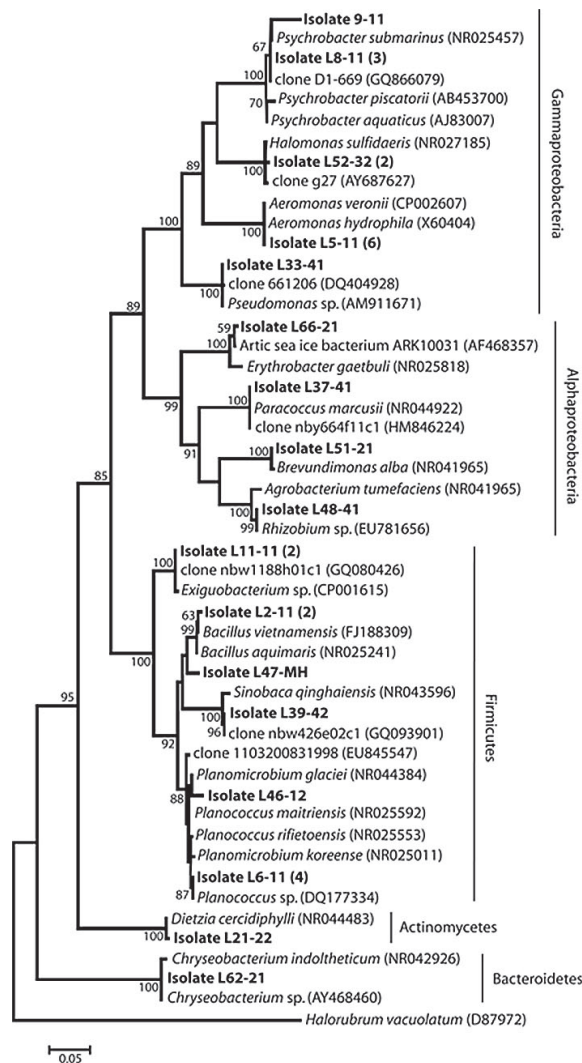


FIGURE 4. Phylogenetic tree inferred from partial 16S rRNA gene sequences (~900 bp) from isolates bacterial phylotypes of Lirima wetland. The tree was constructed using maximum likelihood. Phylotypes are highlighted in bold and the number of isolates per phylotypes are shown in brackets. The evolutionary distance was inferred using general time reversible method. Bootstrap values over 50% are shown. Scale bar indicate 0.5 site substitutions. *Halorubrum vacuolatum* was used as outgroup.

FIGURA 4. Árbol filogenético inferido de secuencias parciales del gen 16S del rRNA (~900 pares de bases) de filotipos bacterianos aislados del humedal de Lirima. El árbol fue construido usando Máxima Verosimilitud. Filotipos son resaltados en negrita y el número de aislados por filotipo son mostrados en paréntesis. La distancia evolutiva fue inferida usando el método general de tiempo reversible. Los valores de Bootstraps mostrados son sobre el 50%. La barra de escala indica 0.5 sustituciones por sitio. *Halorubrum vacuolatum* fue utilizado como grupo externo.

TABLE 4. Percentage coverage of aquatic macrophytes.

TABLA 4. Vegetación de macrófitas acuáticas.

Plant genera	LIRIMA	CAYA
	Plant cover (%)	Plant cover (%)
<i>Myriophyllum</i>	80	
<i>Lilaeopsis</i>	30.7	46.0
<i>Eleocharis</i>	6.7	-
<i>Oxychloe</i>	5.3	-
<i>Patosia</i>	-	49.3
<i>Ranunculus</i>	-	39.3

TABLE 5. Benthic macroinvertebrates recorded from the Lirima and Caya systems (individuals/m<sup>2</sup>).

TABLA 5. Fauna bentónica de los sistemas de Lirima y Caya (Individuos/m<sup>2</sup>).

CLASS	ORDER	FAMILY	GENUS	LIRIMA	CAYA
Arachnoidea	Acari	Hydrozetidae	<i>Hydrozetes</i>	-	391
		Hydrobatidae	<i>Atractidella</i>	-	19
		-	Undetermined species	-	2
Crustacea	Amphipoda	Hyalellidae	<i>Hyalella</i>	83	158
Hirudinea	-	-	-	-	11
Insecta	Coleoptera	Elmidae	<i>Austrelmis</i>	-	7
	Diptera	Chironomidae	<i>Chironomus</i>	-	52
	Hemiptera	Corixidae	-	-	1
	Odonata	Coenagrionidae	-	-	4
Total individuals				83	645

TABLE 6. Presence of aquatic birds and others associated with the Lirima and Caya systems. (×: presence).

TABLA 6. Presencia de aves asociadas a los sistemas de Lirima y Caya (×: presencia)

COMMON NAME	SCIENTIFIC NAME	LIRIMA	CAYA	CONSERVATION STATE
Red-backed sierra finch	<i>Phrygilus dorsalis</i> (Cabanis)	×	×	
Blue-and-white swallow	<i>Pygochelidon cyanoleuca</i> (Lafresnaye y D'Orbigny)	×	×	
Andean gull	<i>Larus serranus</i> (Tschudi)	×		R
Flamingo	<i>Not identified</i>	×		
Chilean teal	<i>Anas flavirostris oxyptera</i> (Meyen)	×		
Lesser rhea	<i>Pterocnemia pennata tarapacensis</i> Chubb	×	×	
Mountain caracara	<i>Phalcoboenus megalopterus</i> (Meyen)	×	×	
Andean Condor	<i>Vultur gryphus</i> (Linnaeus)	×		V
Puna tinamou	<i>Tinamotis pentlandii</i> (Vigors)	×		V
Andean goose				
<i>Chloephaga melanoptera</i> (Eyton)	×			V
	×			
Puna ibis	<i>Plegadis ridgwayi</i> (Allen)	×		V
Crested duck	<i>Lophonetta specularioides alticola</i> (Ménégaux)	×	×	
Greenish Bellow-finch	<i>Sicalis olivascens</i> (Tschudi)		×	
Rufous-naped ground-tyrant	<i>Muscisaxicola rufivertex pallidiceps</i> (Hellmayr)		×	
White-winged cinclodes	<i>Cinclodes atacamensis</i> (Philippi)		×	
Black siskin	<i>Carduelis atrata</i> (Lafresnaye y D'Orbigny)		×	

V= Vulnerable; R= Rare. V= Vulnerable; R= Rara.



## DISCUSSION

The Lirima and Caya wetlands showed characteristic features of shallow systems known in the region as *bofedales* (Ramirez *et al.* 2002; Ahumada & Faundez 2009). Water levels were low in both systems reflecting the climatic characteristics of the region where rain is extremely limited (mean = 54.4 mm/year), and is exacerbated by high evaporation rates (Salazar 1997), which drive the chemical and physical characteristics of these shallow water systems. The high ionic concentrations recorded from the wetlands likely reflect the combined effects of climatic factors with high availability of ions from the underlying sediments. Sulfate values are relatively high for both systems, a common feature in Altiplano environments (Risacher *et al.* 2003b; Marquez *et al.* 2009). These systems are particularly sensitive to hydrological changes, which may affect nutrient availability and diminish dissolved oxygen content due to warming during the day, and increased salinity in both water and sediments. Reductions in water volume following extraction and evaporation results in increases in salinity and nutrient concentrations, due to the concentration of ions. Thus, in contrast to the wetlands typical of temperate regions, these systems do not make a stepwise shift from a clear water state to a turbid one (Scheffer & van Nes 2007); here, relatively minor hydrological changes may lead to a shift in ecosystem state, leading to a loss of species richness e.g. in higher taxa, reflecting the limited tolerance to salinity shown by the majority of freshwater invertebrates and vertebrates. It has been reported that spatial and temporal changes in the Huasco salar, located 40 km from these study sites, have resulted in a distinct microbial community (Dorador *et al.* 2008b; 2010) with likely consequences for higher trophic levels. Here, the culturable bacterial diversity obtained was restricted to the culture media used, nevertheless it was possible to describe at least five different bacterial phyla and 13 genera. The isolates obtained are functionally important, due to their role in recycling of organic matter using different sources of energy (e.g. *Psychrobacter*, *Pseudomonas*), anoxygenic photosynthesis (e.g. *Brevundimonas*) and nitrogen fixation (e.g. *Rhizobium*). Also the closest culturable relative to these taxa were previously isolated from different extreme environments exhibiting adaptations to a range of environmental stressors, as high salinity, cold temperatures and high solar radiation (Table 2). The diverse phylum found are in agreement with the main microbial groups found in other inland environments (e.g. Newton *et al.* 2011), but with the clear influence of high salt concentrations as the main factor modulating the aquatic culturable bacteria from the two wetlands. The prevalence of these taxa and their metabolic role could have important consequences for higher trophic levels, e.g. through provision of energy and the maintenance of suitable abiotic conditions.

Sodium chloride concentrations in the Caya wetland appears to be such to limit the presence of fish, as has been reported by Keller & Soto (1998) for the Ascotán salar. The presence and maintenance of suitable volumes of water as well as fringing vegetation is of primary importance in Atiplanic wetlands. Macrophytes are important due to their ability to retain nutrients and sediments, especially during the Altiplano rains, as well as the provision of substrate support and habitat for macroinvertebrates and fishes (Mitsch & Gosselink 2000; Gulati *et al.* 2005). The formation of the large salt pans in the Altiplano region was principally due to the climatic changes that have occurred in the region over geological time scales (Chong 1988). Nevertheless, during the last century, the salinization process has accelerated, largely due to increased water extraction. Here, water demand typically from agriculture and mining activities greatly outstrips water availability, resulting in decreased levels in many aquifers (Valdés-Pineda *et al.* 2014). The Altiplano is extremely sensitive to changes in the effective humidity (rains minus evaporation). Also, small changes in water reservoirs could produce important and amplified responses in the majority of the salt pans, by modifying morphological variables, geomorphologic processes, vegetation changes and other biogeochemical variations (Grosjean & Veit 2005). Solar radiation in the study area can be up to 20% greater than that recorded at sea level at the same latitude (Aceituno 1997) reaching values up to 1500 W/m<sup>2</sup> in the Altiplano (Piacentini *et al.* 2003). Solar radiation varies both annually and across daily cycles, producing abrupt changes in photosynthetic parameters and community structure (Hernandez *et al.* 2012). Phytoplanktonic photosynthesis inhibition has been studied in Lake Titicaca, showing a high degree of adaptation of the phytoplankton species to changes of the levels of radiation PAR, UVA and UVB (Villafaña *et al.* 1999) and likely operates in the wetlands studied here.

The two wetlands described here showed different physicochemical characteristics: Lirima can be characterized as a sodium sulfate wetland, and Caya as a calcium chloride wetland. Although both systems have relatively elevated nutrient concentrations, it appears that the difference in salinities between the two is associated with a reduction in biodiversity. This supports calls for conservation of minimum water levels in such sensitive ecosystems to maintain chemical quality (Risacher *et al.* 2003b) suitable for communities of microbes, plants and other taxa. Cartography shows that Caya is a shallow stream and Lirima is rather a pond with hydrothermal affluent; both have interesting relicts of former indigenous cultures (Villagrán & Castro 2003). Lirima sustains a low-density population of *Orestias* that has elevated biogeographical and evolutionary importance, since these fish are the only remnants of Atiplanic fish in this zone (Vila *et al.* 2010). The Lirima

population is an Evolutionary Significant Unit (ESU), due to its reproductive isolation and putative adaptation to local conditions and has conservational importance at both international and national scales (Waples 1991; Morales *et al.* 2011). The species of the genus *Orestias* are presently in danger of extinction across the entire Altiplano region (Vila *et al.* 2010). In this system we also observed the presence of the amphibian *Telmatobius*, also an important Altiplano genus (Velo 2006) (Data not shown). Twelve species of birds were observed at Lirima and ten in Caya, including the lesser rhea (*Pterocnemia pennata tarapacensis* Chubb) and flamingos, as an example of the importance of these wetlands as corridors for conservation of bird life in endangered and vulnerable conservation states (Salaberry & Tabilo 1990).

This descriptive paper highlights the unusual limnological and biological characteristics of high altitude wetlands in the Chilean Altiplano. Due to their international and national importance, and the ongoing and increasing demands for water in the region, there is a strong and pressing need for studies describing the physicochemical and biological characteristics of high altitude aquatic ecosystems across the Altiplano region. Such studies should look to not only describe communities across levels of biological organisation (e.g. microbial through to higher vertebrates) but also their function, interactions and sensitivity to changes in water availability.

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