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Characterizing ecoregions in Argentinian Patagonia using extant continental ostracods

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Abstract: In order to characterize Patagonian (Argentina) ecoregions using non-marine ostracods, their associations in 69 environments were assessed. Twenty eight taxa were recorded, including 12 endemic of the Neotropical region. Our results indicate that Patagonian ostracods are mainly influenced by electrical conductivity (EC), altitude, pH, and temperature; and shows a correlation with Argentinian ecoregions. Assemblage I is composed of sites located at high altitude in the Andean Patagonian forest ecoregion. Host waters have low temperature, EC and pH, and support as representative species *Cypris pubera, Eucypris virens, Bradleystrandesia fuscata, Tonacypris lutaria* and *Amphicypris nobilis*. Assemblage II, related to mid-altitude environments in the Patagonian Steppe ecoregion, thrived in waters with moderate to high EC, and alkaline pH values. Dominant species includes *Limnocythere rionegroensis, L. patagonica, E. virgata, Riocypris whatleyi, Riocypris sarsi, Newnhamia patagonica, Kapcypridopsis megapodus, Ilyocypris ramirezi* and *Penthesinelula incae*. Assemblage III inhabited environments within Monte and Espinal ecoregions, situated in the eastern part of the study area at low altitude, EC moderate and temperate waters, supporting *Heterocypris hyalinus, Amphicypris argentinensis, Sarscypridopsis aculeata, Cypridopsis vidua, Herpetocypris intermedia* and *Chlamidotheca incisa*. Our results indicates that Argentinian Patagonia hosts a diverse ostracod fauna and highlights their capacity as proxies in ecological and palaeoenvironmental studies.

Key words: Argentina, ecology, ecoregions, freshwater, Ostracoda, recent.

INTRODUCTION

Ostracods are bivalved microcrustaceans, which inhabit all types of aquatic environments (Mesquita-Joanes et al. 2012, Martínez-García et al. 2015). Their carapace, composed of low magnesium calcite, is commonly preserved in the sediments, giving these organisms a great potential as paleoenvironmental proxies (Holmes & Chivas 2002). Abiotic factors (salinity, water temperature, pH, water depth, substrate, oxygen content, water ionic composition, flow rate or permanence of a water body),

biotic factors (submerged vegetation, food availability) and biologic interactions (predation, competence and parasitism) control the ostracod distribution and abundance (Palacios-Fest et al. 1994, Schwalb 2003, Mezquita et al. 2005, Ruiz et al. 2013, Coviaga et al. 2018b). In this context, their sensitive ecological response to environmental changes favours the use of ostracods as bioindicators (Martínez-García et al. 2015).

Patagonia is a cold temperate region (approx. 700000 km^2) situated in southern South America, within the Neotropical region. From many points

of view, it represents a rather unique region. It is a closest temperate land to the Antarctic Peninsula, this is caused for the presence of the Antarctic Circumpolar Current which controls the climate of the Southern oceans (Rabassa 2008a). On the other hand, Patagonia is the only region which is permanently affected by the westerly wind belt influencing the composition and distribution of the regional biota (Villa Martínez & Moreno 2007). Additionally, it has one of the most marked and abrupt bioclimatic transitions in the world, where it is expected to detect more quickly and in a more marked way the impacts of global change over ecosystems (Kitzberger 2012).

In the last 20 years the knowledge of ostracods in Patagonia Argentina has been improved. Schwalb et al. (2002) and Cusminsky et al. (2005) analysed modern lacustrine ostracods and their oxygen and carbon isotopic signatures in a wide range of recent environments in the lakes Cari-Laufquen (42 °S) and Cardiel (49 °S) area. This first dataset characterized modern ostracod assemblages in different environmental types: springs, permanent and ephemeral ponds. Ramón Mercau et al. (2012) explored ostracod preferences with respect to the hydrochemistry of their host water. Afterwards, Ramón Mercau & Laprida (2016) assembled their extant ostracod dataset from inland waters in southern Patagonia to calibrate a new transfer function inferring electrical conductivity from sedimentary archives as a quantitative salinity proxy. Coviaga (2016) and Coviaga et al. (2018a) studied 40 sites along a W-E transect of 650 km length in northern Patagonia, compiling an ostracod dataset and assessing their distribution and abundance to abiotic environmental variables. Ramos et al. (2015, 2017) analysed the morphological variation of several lacustrine ostracod species using geometric morphometric techniques and explored their relation with

reproductive strategies and environmental factors. Despite these recent efforts studying non-marine ostracods in the Patagonian region, a large area with innumerable water bodies along the N-S gradient is still unknown. Detailed studies are necessary in the designated area to complement missing biodiversity data in these unique aquatic ecosystems.

Multidisciplinary studies in Argentina defined an ecoregion as a geographically defined territory dominated by certain geomorphological and climatic conditions, containing a distinct assemblage of natural communities and species (Burkart et al. 1999). These authors defined 18 ecoregions in Argentina based on geomorphology, climate, vegetation and faunal distribution (Figure 1). The latter category includes diverse species of birds, mammals, reptiles and amphibians; missing the chance to utilise the diversity of invertebrates, including ostracods (Burkart et al. 1999, Brown et al. 2006). It is important to highlight that out of 18 ecoregions, Patagonia holds only four: Andean - Patagonian forest, Patagonian steppe, Monte and Espinal.

The aims of this work are to: (1) enhance our understanding of the regional Patagonian ostracod fauna; (2) evaluate the relationship between ostracod species and physical and chemical parameters of host waters along W-E precipitation gradients (1200 to 160 mm/year, respectively); and (3) relate their distribution to the Patagonian ecoregions proposed by Burkart et al. (1999). The underlying hypotheses are: i) the distribution of Patagonian ostracods is associated to physical and/or chemical gradients of the host waters, and thus ii) the distribution of Patagonian ostracod assemblages can be associated with Argentinian ecoregions.
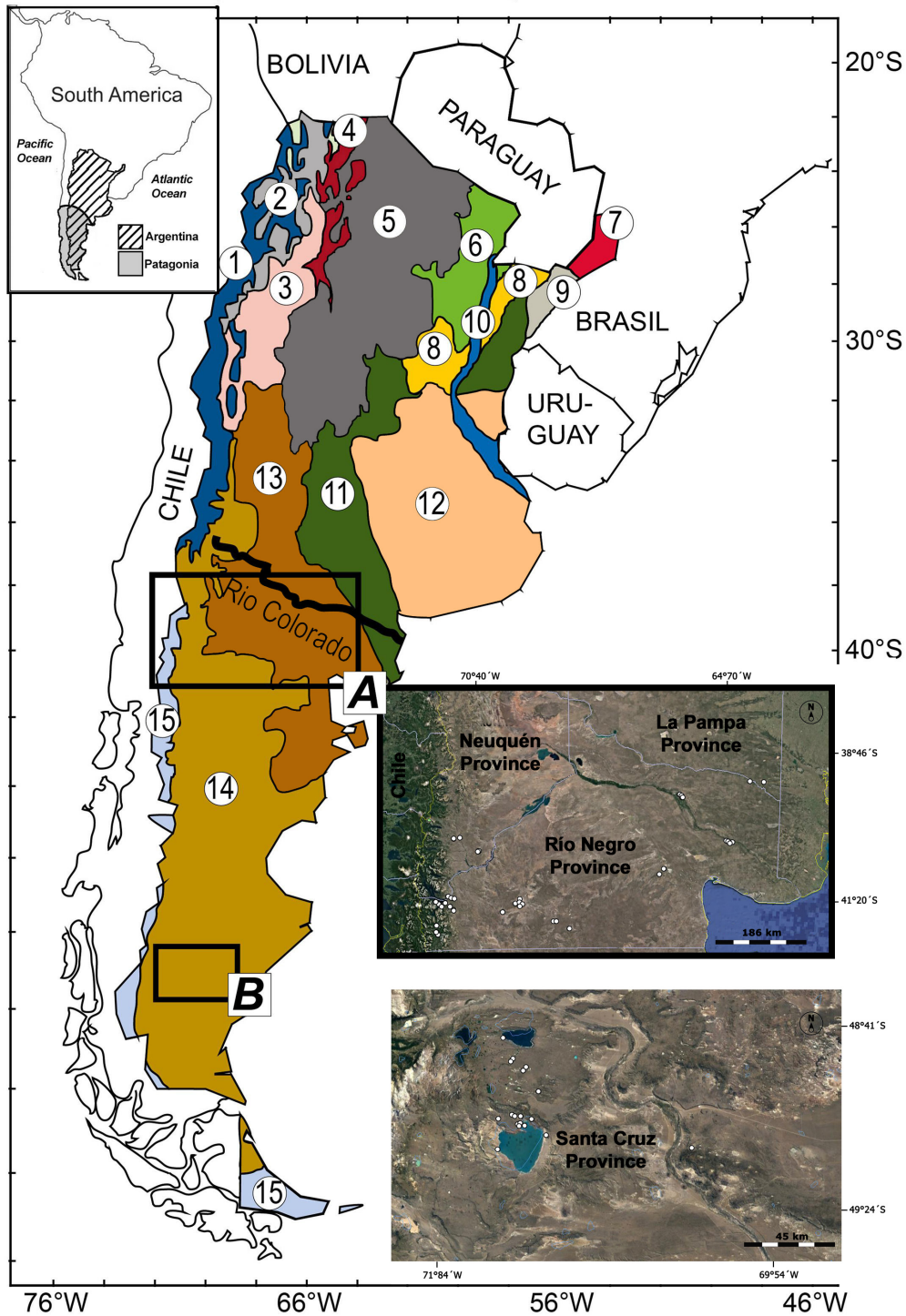
Figure 1. Study area and Argentinian ecoregions (after Burkart et al. 1999) (a) 1 – Altos Andes (dark blue), 2 – Puna (light gray), 3 – Monte de Sierras y Bolsones (light red), 4 – Selva de las Yungas (dark red), 5 – Chaco Seco (gray), 6 – Chaco Húmedo (green), 7 – Selva Paranense (red), 8 – Esteros del Iberá (yellow), 9 – Campos y Malezales (gray), 10 – Delta e Islas del Paraná (blue), 11 – Espinal (dark green), 12 – Pampa (light orange), 13 – Monte de Llanuras y Mesetas (dark orange), 14 – Estepa Patagónica (dark yellow), 15 – Bosques Patagónicos (light blue), redrawn from Burkart et al. (1999). Regional location, boxes indicate study areas A and B and location of the sampling sites (b) sampling sites in study areas A and B (see text for details).

REGIONAL SETTING AND STUDY AREA

Patagonia is the southern part of South America extending southward of Río Colorado, between 36 °S to 55 °S (Rabassa 2008b) (Figure 1). The region is affected by the Austral borders of semi-permanent anticyclones of the Pacific and Atlantic oceans (Compagnucci 2011). Since the Late Miocene, when the Andean Cordillera reached its highest elevations, the Westerlies flow coming from the Pacific has been partially blocked by this mountain chain. As a result, rainfall occurs mainly on the western (Chilean) sector of the cordillera whereas the amount of precipitation diminishes towards the east, causing humid conditions in Andean Patagonia and a semi-arid climate in the extra Andean region of Argentina (Compagnucci 2011).

Several ecoregions have been recognized in Patagonia based on its climate, geomorphology, soil types and vegetation. From west to east they have been named as: Andean Patagonian forest, Patagonian steppe, Monte, and Espinal ecoregions (Burkart et al. 1999). The Patagonian forest is characterized by the Andean mountains range that provides a typical glacial to fluvial geomorphology to the area. The climate is wet (800 to 3500 mm/year precipitation) and temperate to cool (5.4 to 9.0 °C average mean temperature) (Burkart et al. 1999). The soils are rich in organic matter and the vegetation is characterized by *Nothofagus* forests. To the east, the Patagonian steppe ecoregion includes numerous landscape steps from the Andean piedmont to the Atlantic Ocean coast (Paruelo et al. 1998). The climate is temperate to cool with low precipitation, especially in the eastern sector (200 mm/year); the mean annual temperature range from 10 to 14 °C in the north to 5 to 8 °C to the south of the region (Burkart et al. 1999, Matteucci 2012). The soils are poorly developed, characterized by low organic matter

contents and scrubby vegetation (Paruelo et al. 2005). The Monte, encompassing steppes and plains, is characterized by temperate (mean annual temperature range from 10 to 14 °C) and dry climate, with precipitation ranging between 100 to 200 mm/year (Matteucci 2012). The soils are poorly developed, and the vegetation is mainly represented by scrub steppe, such as "jarilla" (*Larrea* spp.). Eventually, the Espinal ecoregion is represented by the Calden district (*Prosopis caldenia* forests; Menéndez & La Roca 2006) and is characterized by drained plains poorly developed soils and gently slopes, with a warm to semiarid climate (precipitation from 300 to 600 mm/year, temperature between 15 to 16 °C) (Menéndez & La Roca 2006). Westerly air masses originating from the Pacific anticyclone dominate over most of Patagonia (Paruelo et al. 1998). However, the Espinal ecoregion is also influenced by northeast winds generated by the Subtropical Atlantic anticyclone (Menéndez & La Roca 2006).

The study area presented in this contribution is an extension of the study area that began in 1998, with the Patagonian Lake Drilling Project (PATO) (Schwalb et al. 2002). It includes two Patagonian sectors: Sector A, the northern area, delimited for 39° to 41.6° S and 64° to 71.5° W approximately (Río Negro province, western part of Neuquén province and southwest of La Pampa province) and, Sector B, the southern area, placed at 48° S - 71° W (western of Santa Cruz province) (Figure 1).

Sector A is placed in three geological Provinces, from the west to the east are: the North Segment of the Cordillera Patagónica Septentrional, the Precordillera Patagónica and the Somún Cura Massif. In all of them, rocks of a metamorphic basement of medium to high degree and Paleozoic plutonites covered by Mesozoic marine and continental Jurassic and Eocretacic deposits outcrop. These rocks were

intruded by the Patagonian Batholith (Ramos 1999). Sector B is placed at the northern end of the Southern Patagonian Plateau, between the Deseado Massif to the north and the Southern Patagonian Cordillera to the west. In the region outcrop only the marine and continental deposits of the Upper Cretaceous and the Cenozoic age, as well as extensive basaltic flows of Middle to Upper Miocene age. To the east, peneplans covered with "rodados patagónicos" of Pliocene-Pleistocene age are developed (Ramos 1999). Since the Miocene Patagonia suffered significant climatic variations with the recurrence of multiple cold-warm climatic cycles (Rabassa 2008a). Both sectors show Pleistocene sequence of lacustrine sediments, whereas the Holocene also encompasses alluvial, colluvial and aeolian deposits (Whatley & Cusminsky 1999). Geomorphological evidences indicates that the last glaciations not affected the eastern area of sector A (Lago Cari Laufquen) neither sector B (Lago Cardiel) (Gilli et al. 2005, Ariztegui et al. 2008).

MATERIALS AND METHODS

Field and laboratory work

Sixty-nine permanent, ephemeral, ponds and spring aquatic environments of Patagonia were sampled during the Austral spring and summer of the years 1998, 2001, 2011, 2012, 2013 (Schwalb et al. 2002, Ramos et al. 2017, Coviaga et al. 2018a) (Table SI - Supplementary Material). At each site ostracods were recovered from surface sediment and/or from the water-sediment interface. Mud samples were taken from seeps and ponds by scraping over the sediment surface with a plastic bag. In lakes Cardiel, Cari-Laufquen Grande and Cari-Laufquen Chica, samples were taken along a transect ranging from 5 to 35 m water depth using an Ekman grab sampler from a rubber boat. Sediment from ponds and small

lakes was sampled from shore with a bolapipe dredge (Benson & Kaesler 1965) (Schwalb et al. 2002). The water-sediment interface samples were recovered using a hand net (D frame 200 μm mesh aperture) along a 1- 6m long transect, depending on the environment. Samples were fixed with ethanol (70%). Simultaneously, pH, water temperature (T, °C), and electrical conductivity (EC, mScm^{-1}) were measure *in situ* (Schwalb et al. 2002, Coviaga et al. 2018a). Additionally, water samples were taken for ionic analysis.

In the laboratory, the concentration of major cations (K$^+$, Na$^+$, Ca^{2+} and Mg^{2+}) was measured using atomic absorbance spectrometry (Perkin Elmer Analyst 100).

All adult ostracod individuals were sorted under a stereomicroscope. Representative specimens were measured, and dissected under stereoscopic microscopes (Olympus SZ30 and SZ61 and Nikon SMZ-645). Taxonomic identification was done based on carapaces, valves and appendices following Van Morkhoven (1963), Martens (1990), Cusminsky & Whatley (1996), Meisch (2000), Cusminsky et al. (2005), Karanovic (2012) and Coviaga et al. (2018b). The zoogeographical distribution of each taxon was determined according to Martens & Behen (1994), Martens et al. (2008, 2013) and Coviaga et al. (2018a).

Data analysis

Cluster analysis using the Unweighted Pair Group Mean Averages (UPGMA) with Jaccard's similarity index was applied to display clustering relationships among sites based on binary data of species' occurrence (presence/absence). Species with only one occurrence were eliminated from the analysis to increase their power. Past 3.10 software (Hammer et al. 2001) was used for cluster analysis.

Canonical Correspondence Analysis (CCA) was performed to analyze the relationship between ostracod species and environmental variables (Ter Braak 1995). A unimodal method was used because only the response to the presence /absence of ostracod species (i.e. binary) was considered (Lepš & Šmilauer 2003). Environmental variables were standardized. Additionally, the following parameters were log transformed to conform to normality; using log(x+1) for altitude and conductivity, log(x+10) for Na^+, K^+ and Ca^{2+}, and log(x+100) for Mg^{2+}; temperature and pH remained untransformed. For CCA analysis, rare species were down-weighted, and environmental variables were added by manual forward selection using Monte Carlo permutation test (999 unrestricted permutations) to evaluate their statistical significance. Variables were included if they significantly ($p<0.05$) contributed to the remaining variance, with emphasis on independent variables with highest ecological relevance in case of similar contributions (Van der Meeren et al. 2010). The strength of the relationship between environmental variables and data was assessed using a series of partial and constrained CCAs with one environmental variable entered at a time. CCA was carried out with CANOCO V5.1 (Ter Braak & Šmilauer 2012).

Combined cluster analysis, and CCA results in a Geographical Information System (GIS) allowed us identifying spatial variation of ostracod assemblages.

RESULTS

Species Occurrence

A total of 28 ostracod species were identified. *Ilyocypris ramirezi* was the most frequent species, recorded in 27 sites; followed by *Heterocypris incongruens*, *Riocypris whatleyi* and *Eucypris virens* that were present in 14-16 sites. *Limnocythere cusminskyae, Darwinula stevensoni, Eucypris labyrinthica* and *Heterocypris salina* were present at only one site each (Table II).

About 18% of the species have a cosmopolitan distribution and inhabit more than three zoogeographical regions; 21% of taxa are present in three zoogeographical regions (Paleartic (PA), Neartic (NA) and Neotropical (NT); 11% belong to two regions (NT, PA) and 50% are endemic (present only in the NT region). Table III shows the environmental range where the 12 Neotropical species were recorded (see Table II for details). *Ilyocypris ramirezi* displays the widest environmental range of temperature, pH, EC, and Mg^{2+} concentration; *Riocypris whatleyi* and *Eucypris virgata* show the largest range for K^+ and Na^+ concentrations while *R. whatleyi* is tolerant to Ca^{2+} variations. On the other hand, *Eucypris cecryphalium* has the narrowest range for temperature, pH, EC Mg^{2+} and Na^+ concentrations, and *Penthesinelula incae* and *Amphicypris argentinensis* exhibit the smallest range for K^+ and Ca^{2+} concentrations, respectively.

Species distribution and environmental physicochemical characterization

The dendrogram resulting from cluster analysis (Figure 2) allowed distinguishing between eight clusters at 0.15 units of similarity. Clusters 1 and 2 are composed by only one site each, C1 and LJ, respectively, Cluster 3, defined for 17 water bodies, these clusters are located at the western sector of the study area (Figure 3a). Cluster 4 groups' three sites, and Cluster 5 seven, both clusters are located on the eastern sector of the study area (Figure 3a). Cluster 6 is composed of 13 sites, and Cluster 7 encompasses 27, sites of these groups are placed in sectors A and B of the study area (Figures 3a and b). Cluster 8 is defined by three sites located at 48° S at the eastern sector (Figure 3b). The main environmental parameters are summarized in Table IV.

Table II. List of identified ostracods and their occurrence in the study area (codes of sampling sites as in Table SI). Abbreviations: NT = Neotropical; AT = Afrotropical; AU = Australasian; NA = Nearctic; PA = Palaearctic; OL = Oriental; PAC = Pacific Oceanic Island; Sex. = sexual; Sex.-Parth. = sexual and parthenogenetic; Parth. = parthenogenetic. An asterisk denotes the species with only one occurrence that were excluded from the statistical analysis.

Species	Code	Zoogeographic Region	Reproduction type	N° of sites
Superfamily Cytheroidea Baird, 1850				
Family Limnocytheridae Klie, 1938				
Limnocythere cusminskyae Ramón Mercau et al., 2014 *	*Lc*	NT	Sex.	1
Limnocythere patagonica Cusminsky & Whatley, 1996	*Lp*	NT	Sex.-Parth.	3
Limnocythere rionegroensis Cusminsky & Whatley, 1996	*Lr*	NT	Sex.-Parth	10
Superfamily Darwinuloidea Brady & Norman, 1889				
Family Darwinulidae Brady & Norman, 1889				
Darwinula stevensoni (Brady & Robertson, 1870) *	*Dv*	NT, AT, AU, NA, OL, PA	Parth.	1
Penthesinelula incae (Delachaux, 1928)	*Pi*	NT	Parth.	5
Superfamily Cypridoidea Baird, 1845				
Family Cyprididae Baird, 1845				
Amphicypris argentinensis Fontana & Ballent, 2005	*Aa*	NT	Sex.	2
Amphicypris nobilis Sars, 1901	*An*	NT, NA, PA	Sex.	3
Bradleystrandesia fuscata (Jurine, 1820)	*Bf*	NT, NA, PA	Parth.	4
Chlamydotheca incisa (Claus, 1892)	*Ci*	NT, PA	Parth.	2
Cypridopsis vidua (O.F. Müller, 1776)	*Cv*	NT, AT, NA, PA, PAC	Parth.	6
Cypris pubera O.F. Müller, 1776	*Cp*	NT, NA, PA	Parth.	2
Eucypris cecryphalium Cusminsky et al., 2005	*Ec*	NT	Parth.	2
Eucypris labyrinthica Cusminsky & Whatley, 1996 *	*El*	NT	Parth.	1
Eucypris virens (Jurine, 1820)	Ev	NT, AT, AU, NA, PA	Parth.	15
Eucypris virgata Cusminsky & Whatley, 1996	*Evg*	NT	Parth.	9
Herpetocypris intermedia Kaufmann, 1900	*Hi*	NT, PA	Parth.	2
Heterocypris hyalinus Klie, 1930	*Hh*	NT	Sex.	7
Heterocypris incongruens (Ramdohr, 1808)	*Hig*	NT, AT, AU, NA, OL, PA, PAC	Parth.	16
Heterocypris salina (Brady, 1868) *	*Hs*	NT, NA, PA	Parth.	1
Kapcypridopsis megapodus Cusminsky et al., 2005	*Km*	NT	Parth.	7
Potamocypris smaragdina (Vávra, 1891)	*Ps*	NT, NA, PA	Parth.	6
Potamocypris unicaudata Schäfer, 1943	*Pu*	NT, NA, PA	Parth.	8
Riocypris sarsi (Daday, 1902)	*Rs*	NT	Parth.	3
Riocypris whatleyi Coviaga et al., 2018b	Rw	NT	Sex.	14
Sarscypridopsis aculeata (Costa, 1847)	*Sa*	NT, AT, AU, NA, PA	Parth.	5
Tonnacypris lutaria (Koch, 1838)	*Tl*	NT, PA	Parth.	9
Family Ilyocyprididae Kaufmann, 1900				
Ilyocypris ramirezi Cusminsky & Whatley, 1996	*Ir*	NT	Parth.	26
Family Notodromadidae Kaufmann, 1900				
Newnhamia patagonica (Vávra, 1898)	*Np*	NT	Parth.	5

The sites with the highest diversities are placed in cluster 7 (Ñe=8, Lag6=6). Also, in clusters 2, 3, 5 and 6 species diversity (5) is highest (LJ, R15, PAG and CA, respectively). In cluster 8, the site with maximum diversity is EN = 4, and in cluster 4 is BPChChB = 3. Cluster 1 (C1) presents a diversity =1 (Figure 2; for abbreviation details see Table SI).

The surveyed sites are located from 5 m to 1161 m above sea level (a.s.l.); cluster 7 shows the widest altitudinal range (5 to 1159 m a.s.l.), while cluster 5 displays the narrowest (66 to 134 m a.s.l.). Surface water temperature of sites range between 6 °C to 34 °C, although cluster 7 presents the widest temperature range (7.5-34.1 °C). All cluster sites show a high temperature variation coefficient. Most of the water bodies are slightly acid to moderately basic (pH range = 6.0-10.8). Cluster 7 presents the greatest pH variation and cluster 8 the lowest. 85% of the sites have limnetic to oligohaline waters (EC range = 75-3,106 mS cm^{-1}); and solely ten sites, belonging to clusters 6 and 7, are meso- to euhaline (Venice Symposium 1958) (EC range = 11,390-51,694/cm). Clusters 5, 6 and 7 exhibit the widest ranges of K$^+$, Ca^{2+} Mg^{2+} and Na$^+$ ionic concentrations, while clusters 4, 6, 3 and 8 experience the lowest, respectively (Table IV).

The Piper diagram of cations shows that most water sampling sites are sodium-potassium-enriched (Figure S4 - Supplementary Material). Only one site from cluster 7 (Lag 6) has magnesium as the dominant cation (magnesium water type) and five sites from cluster 3 (TE1, TE2, TE3, Ef and Ñl2) do not have a dominant cation type (calcium, magnesium, sodium or potassium 75%-30% Na$^+$+K$^+$). Sites of clusters 3, 5 and 7 have the broadest compositional variation, while the sites of clusters 4 and 8 have the narrowest variation in the sodium-potassium type water composition. The presence of the ions in the water could come from the weathering of the igneous and the metamorphic rocks that outcrop in the areas.

Species, environmental variables and sites relations

CCA results performed to analyze the relationship between ostracod species and environmental variables show that the first two axes (Figure 5) account for 70% (CCA1: 38.2%, CCA2: 31.8%) of the cumulative percentage variance of the species-environment relation. The species-environment correlations are 0.842 for axis 1 and 0.836 for axis 2. Monte Carlo permutation test shows that all the canonical axes are significant ($p < 0.001$). Forward selection reveals that five variables explain a significant amount of variance in the ostracod assemblages (Tables Va, b). According to the CCA, the most significant abiotic predictors of assemblages' composition were EC, altitude and pH, followed by Mg^{2+} concentration and water temperature. CCA1 is highly and negatively correlated with EC, pH and Mg^{2+} concentration, while CCA2 is highly correlated with (positively) temperature and (negatively) altitude.

In the CCA triplot (Figure 5) three assemblages are recognized. Assemblage I includes *E. virens*, *B. fuscata*, *A. nobilis*, *T. lutaria* and *C. pubera* and is associated to environments located at moderate to high altitude with cold waters of low conductivity, pH and Mg^{2+} concentration. This group is present in sites of clusters 1 and 3, located at the western part of the study area A (39-41°S /71°W) (Figure 3a, Table IV). Assemblage II is formed by species associated to a gradient from moderate to high values for EC, pH and Mg^{2+} concentration, ranging from the lowest to the highest they are: *H. incongruens*, *P. incae*, *E. cecryphalium*, *P. unicaudata*, *N. patagonica*, *I. ramirezi*, *L. patagonica*, *P. smaragdina*, *K. megapodus*, *R. sarsi*, *E. virgata*, *R. whatleyi* and *L. rionegroensis*. This Assemblage II was present in sites of clusters 2, 6, 7 and 8, located at

Table III. Tolerance range of endemic species for the examined parameters, number of occurrences in parenthesis. Abbreviations: Alt. = altitude, T = temperature, EC = electrical conductivity. For species abbreviation see Table II.

	Alt. (m s.n.m.)	T (°C)	pH	EC (µS/cm)	K⁺ (mg/l)	Ca⁺² (mg/l)	Mg⁺² (mg/l)	Na⁺ (mg/l)
Lp (4)	276 – 909	6.6 – 17	8.5 – 9.6	381 – 1234	0.1 – 29.0	0.2 – 28.0	3.6 – 68.0	0.5 – 281.0
Lr (10)	276 – 810	6.6 – 30.0	8.8 – 9.6	1234 – 51694	1.1 – 199.0	7.1 – 58.5	3.6 – 324.2	199.1 – 8713.2
Pi (5)	304 – 956	7.9 – 20.2	7.8 – 9.0	424 – 1169	1.1 – 1.9	6.3 – 30.2	3.6 – 15.3	40.6 – 188.1
Aa (2)	116 – 123	20.3 – 20.8	9.9 – 9.9	1738 – 2200	9.5 – 14.9	14.0 – 14.0	39.0 – 42.0	287.0 – 387.0
Ec (2)	381 – 895	11.0 – 12.0	9.1 – 9.1	466 – 580	4.3 – 11.8	19.9 – 50.9	19.3 – 24.6	20.8 – 33.7
Evg (9)	276 – 900	7.5 – 17.0	8.5 – 9.6	247 – 51278	0.1 – 265.3	0.2 – 58.5	3.6 – 134.1	0.5 – 8713.2
Hh (7)	59 – 126	17.3 – 28.7	7.6 – 9.9	215 – 7090	1.2 – 46.0	14.0 – 55.4	2.6 – 71.4	43.0 – 1522.0
Km (7)	276 – 422	7.9 – 16.0	7.8 – 10.8	883 – 6153	0.7 – 9.3	8.5 – 13.9	1.0 – 22.0	18.6 – 1046.3
Rs (3)	525 – 909	12.0 – 13.5	8.8 – 9.5	466 – 9945	3.3 – 29.0	8.7 – 20.7	8.2 – 68.0	33.7 – 1740.7
Rw (14)	276 – 930	6.6 – 27.0	8.1 – 9.6	381 – 51278	0.1 – 265.3	0.2 – 61.0	1.8 – 134.1	0.5 – 8713.2
Ir (27)	59 – 956	7.5 – 34.1	7.6 – 10.8	215 – 51694	0.1 – 127.8	0.2 – 51.0	0.0 – 324.2	0.5 – 8688.0
Np (5)	116 – 900	8.0 – 20.3	7.8 – 9.9	247 – 1738	1.3 – 11.8	13.9 – 50.9	3.7 – 42.0	18.4 - 287

T = temperature, EC = electrical conductivity. For species abbreviation see Table II.

moderate altitude from 40-41° S as well as at 48° S, and up to 70-71° W of the study areas A and B (Figures 3a, b, Table IV). The Assemblage III, consisting of *C. vidua, H. intermedia, C. incisa, H. hyalinus, S. aculeata* and *A. argentinensis*, shows preferences for water bodies located at low altitudes and characterized by temperate waters with moderate EC, Mg²⁺ concentration and pH. This group corresponds to sites of cluster 4, 5 and some sites of cluster 7, located in the eastern part of the study area A (39-41°S /65°W) (Figure 3a, Table IV).

DISCUSSION

Taxonomy and biogeography

We found 28 species in 69 samples (Figure 2); several previously identified species such as *Eucypris fontana* and *E. cecryphalium* (Cusminsky & Whatley 1996, Cusminsky et al. 2005) have required taxonomic revision. The reasons behind this revision is beyond the scope of this article, but the readers may find more details in Coviaga et al. (2018b) and Pérez et al. (2019).
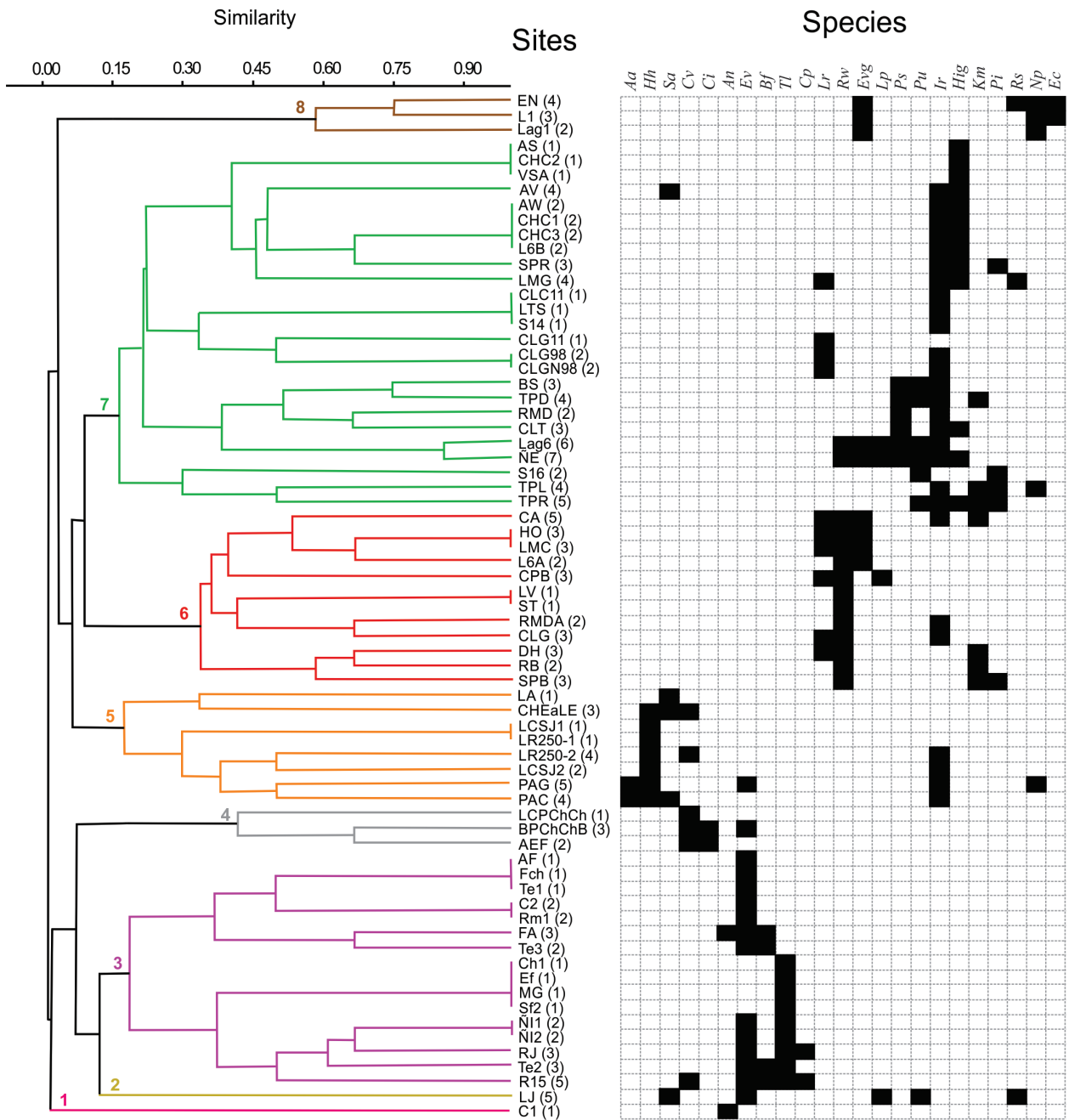
Figure 2. Dendrogram of sites and their species. Dendrogram showing eight clusters (1 – 8) separated by 0.15 units of similarity, obtained using the Unweighted Pair Group Method (UPGMA) and Jaccard Similarity Index. Coffenetic Correlation Coefficient (CCC) = 0.8307. Abbreviation of species names are shown in Table II. Numbers between parentheses indicate number of species at each site.
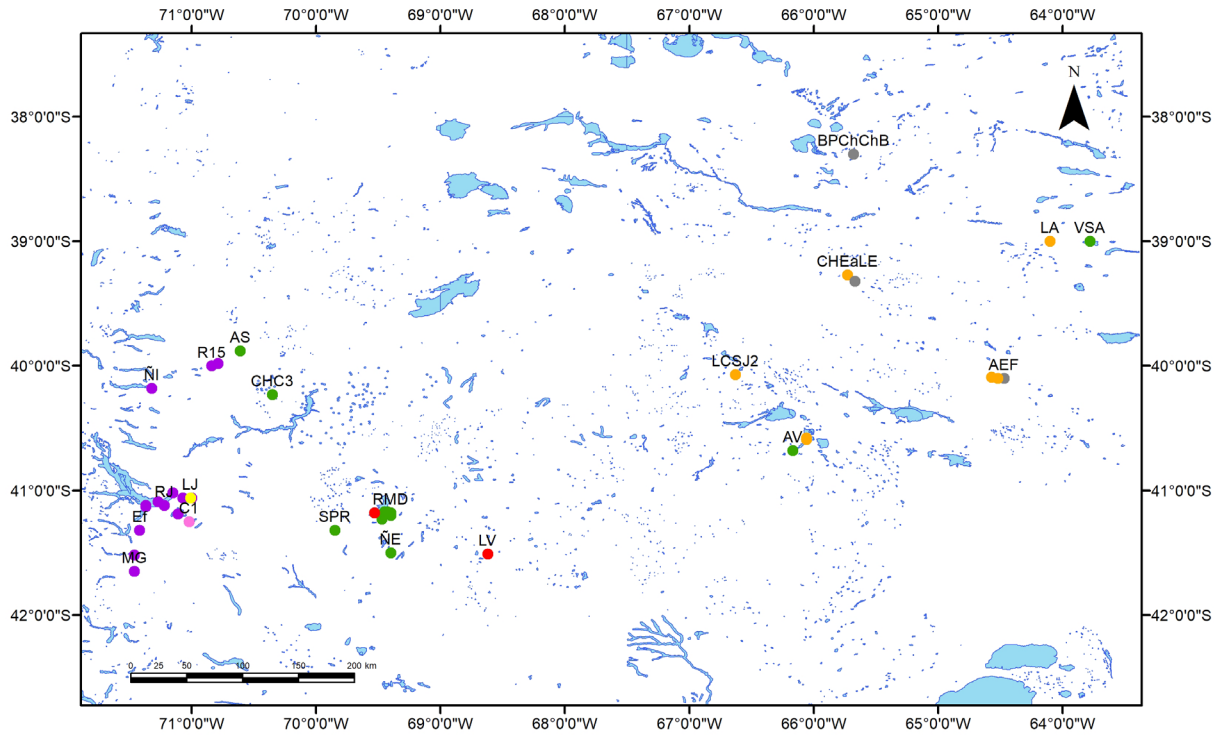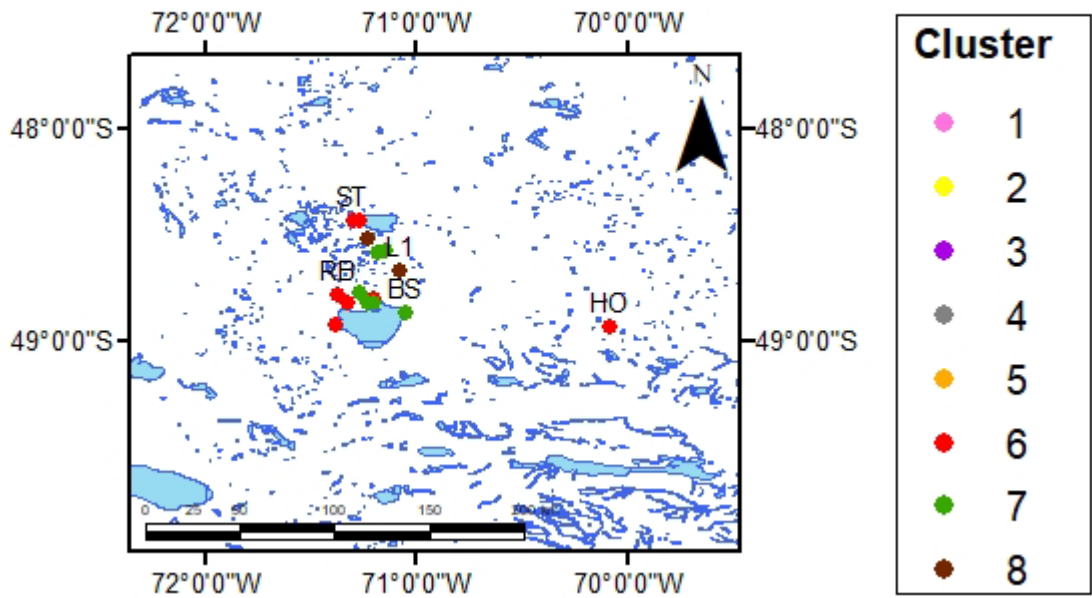
Figure 3. Map of sites grouped after cluster Analysis. **a** sites are located at 41 °S, in the study area A; whereas **b** sites are situated at 48 °S in the study area B.

Table IV. Range of examined parameters in the different habitats of clusters, median values in parenthesis. Abbreviations: T = temperature, EC = electrical conductivity, Alt. = altitude.

Cluster	Alt. (m s.n.m.)	T (°C)	pH	EC (µS/cm)	K⁺ (mg/l)	Ca⁺² (mg/l)	Mg⁺² (mg/l)	Na⁺ (mg/l)
1	1161 (1161)	22.7 (22.7)	6 (6)	75 (75)	6.2 (6.2)	0.3 (0.3)	2.6 (2.6)	4.5 (4.5)
2	909 (906)	13.5 (13.5)	9.5 (9.5)	1220 (1220)	29.0 (29.0)	20.7 (20.7)	68 (68)	281 (281)
3	657 – 1045 (826)	8.1 - 21.7 (13.1)	6 - 8.1 (7.3)	24 – 289 (100)	0.1 – 6.4 (0.9)	0.6 – 24.9 (11.9)	0.3 – 9.4 (4.6)	2.4 – 13.4 (7.1)
4	66 – 134 (121)	18.5 – 27 (24.4)	7.5 – 8.8 (8.1)	215 – 2040 (348)	1.2 – 6.2 (1.4)	17.6 – 101.0 (21.6)	3.0 – 48.0 (4.3)	16 - 271 (36)
5	59 – 126 (69)	17.3 – 28.7 (20.7)	7.6 – 9.9 (8.6)	215 – 10950 (1762)	1.2 – 50 (12.2)	14.0 – 629.0 (48.1)	2.6 – 168.6 (40.5)	43 – 1633 (268.0)
6	276 – 930 (598)	6.6 – 27 (15.5)	8.1 - 9.6 (9.1)	883 – 51278 (4215)	0.7 – 265 (10.5)	7.1 – 61.0 (12.5)	1.81 – 134.1 (30.8)	18.6 – 8713 (674)
7	5 – 1159 (525)	7.5 – 34.1 (17)	7.8 – 10.8 (8.6)	257 – 51694 (960)	0.1 – 128 (3.2)	0.2 – 305 (23.3)	0 – 324.2 (12)	0.5 – 8688 (158)
8	820 – 900 (895)	8 – 12 (11)	9.1 – 9.2 (9.1)	247 – 580 (466)	4.0 – 11.8 (4.3)	19.9 – 50.9 (21.3)	3.8 – 24.6 (19.3)	18.4 – 33.7 (20.8)

Table Va. Summary statistics for CCA analysis.

	Axis 1	Axis 2	Axis 3	Axis 4	Total Inertia
Eigenvalues	0.5843	0.4874	0.2342	0.1446	7.183
Pseudo-canonical correlation	0.8425	0.8359	0.7157	0.5780	
Cumulative percentage variance					
of response data	8.13	14.92	18.18	20.19	
of fitted response data	38.20	70.06	85.38	94.83	
Sum of all eigenvalues					7.183
Sum of all canonical eigenvalues					1.529

Table Vb. Main results of Canonical Correspondence Analyses (CCA) for ostracods presence/absence data based on the 69 sites of Patagonia. a) Sumary statistics for the first four axes of CCA. b) Ranking environmental variables in importance by their simple (left) and conditional (right) effects obtained by forward selection. *p* =significance level of the effect, as obtained with a Monte Carlo permutation test under the null model with 999 random permutations.

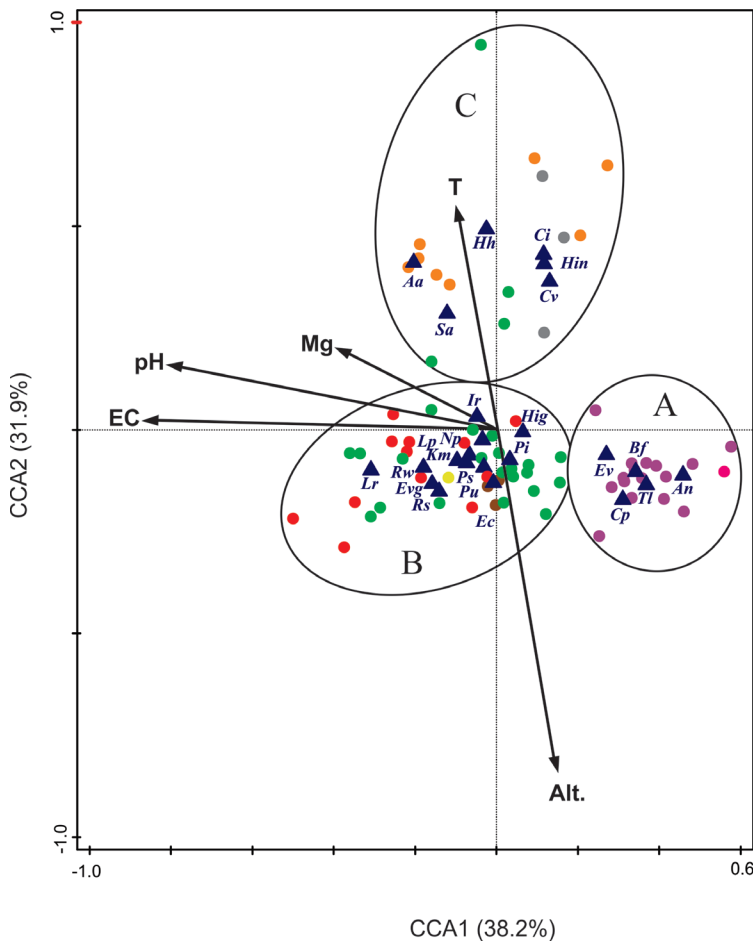| Name	Simple term effects			Name	Conditional term effects		
	Explains %	pseudo-F	*p*		Explains %	pseudo-F	*p*
EC	7.0	5.0	0.002	EC	7.0	4.2	0.002
pH	6.7	4.8	0.002	Alt.	5.6	4.2	0.002
Alt.	5.6	4.0	0.002	pH	3.1	2.4	0.004
Na^+	5.5	3.9	0.002	Mg^{+2}	2.9	2.3	0.012
T	3.8	2.6	0.002	T	2.7	2.1	0.004
Mg^{+2}	3.4	2.4	0.006	Ca^{+2}	1.3	1.0	0.42
K^+	3.2	2.2	0.002	Na^+	1.3	1.0	0.394
Ca^{+2}	2.2	1.5	0.112	K^+	2.0	1.6	0.056

Figure 5. Canonical Correspondence Analysis triplot shows the relationships between 23 species (triangles), selected physicochemical environmental variables (arrows; temperature = T, pH, electrical conductivity = EC, altitude = Alt., Mg=Mg concentration), and sites (points). Colour site points: pink cluster 1, yellow cluster 2, violet cluster 3, gray cluster 4, orange cluster 5, red cluster 6, green cluster 7, and brown cluster 8. I: assemblage of Andean - Patagonian forest ecoregion; II: assemblage of Patagonian Steppe ecoregion; III: assemblage of Monte and Espinal ecoregions. Abbreviation of species names are listed in Table II.

Fifty percent of the species in our study are endemic to the Neotropical zoogeographical region. Of 333 non-marine ostracod species described from the Neotropics, 250 (75%) are endemic (Martens et al. 2008). It is noteworthy that, the number of species is considerably less than what is presently known from Paleartic and Afrotropical regions (Meisch 2000). This pattern is probably skewed by an incomplete exploration for many areas in the Neotropics; which likely leads to an underestimation of specific richness (Martens et al. 2008, Yavuzatmaca et al. 2015). Additionally, the exclusive record of some species, such as *B. fuscata, P. unicaudata, H. salina, A. nobilis,* in recent sediments suggest that many species registered in Patagonia are recent colonizer. At the end of the 19th century,

immigrants arrived at Patagonia from different European countries and built many small cities (e.g. San Carlos de Bariloche, Gaiman, etc.), probably bringing resilient eggs attached to their imported farm equipment (Koenders et al. 2012).

Species- environments and ecoregions relationship

The combination of cluster analysis and CCA results allows us to recognize three types of ostracod assemblages (I, II and III) based on environmental features. These assemblages are correlated with the ecoregions recognized in our study area (Figures 6a and 6b, Table VI) (Burkart et al. 1999).

Assemblage I (Andean - Patagonian forest ecoregion)

This assemblage is composed by sites of clusters 1 and 3, located at medium to high altitude and characterized by waters of low values in temperature, EC, pH and ion concentrations (Figure 5). Andean-Patagonian forest ecoregion is the only region that presents several sites with waters not dominated by high sodium and potassium concentrations Specifically, the Ef, TE1, ÑI2, TE2 and TE3 sites exhibit no dominant type waters (Figures S4), which are located in the western area (near the forest/steppe ecotone) from Northern Patagonia (Figure 1). In the Andean-Patagonian forest ecoregion precipitations rise towards to the west, at the base of the Andes Mountains (Paruelo et al. 1998, Premoli et al. 2006) and possibly control the observed decreasing sodium trend. This assemblage is characterized by *C. pubera, E. virens, B. fuscata, T. lutaria* and *A. nobilis*. This association was previously reported by Coviaga et al. (2018b) as typical fauna of Northern Patagonia environments with cold and low conductivity waters. High precipitation rates in the Andean forest ecoregion lead to low salinity lacustrine environments, and therefore supporting this ostracod association (Coviaga et al. 2015, 2018a). *Eucypris virens*, *B. fuscata* and *T. lutaria* are present in lakes with no dominant type waters ($Na^+ + K^+ - Mg^{2+} - Ca^{2+}$). *Eucypris virens* appears in most of the sites of this sector. This is a cosmopolitan species with a broad ecological tolerance and morphological plasticity (Meisch 2000, Pieri et al. 2006, Martins et al. 2009), although this species prefers environments of low temperature and salinity in Patagonia Patagonia (Ramón Mercau & Laprida 2016, Coviaga et al. 2018a). Additionally, this taxon has been previously found in an environment very rich in organic matter which appears to favour its occurrence (Martínez-García et al. 2015). This

is in accordance with our findings and dominant characteristics of the soils of the Andean - Patagonian forest ecoregion (Burkart et al. 1999). *Eucypris virens* frequently exists with *T. lutaria*, suggesting similar ecological requirements (Yilmaz & Külköylüoğlu 2006, Coviaga et al. 2018a). Additionally, *T. lutaria* appears as a single species in sites located in the westernmost sector, suggesting that probably this species tolerates and prefers lower temperature than *E. virens*. In agreement with this hypothesis, *T. lutaria* was also recognized in Holsteinian Interglacial sediments in Northern Europe (Griffiths et al. 1998); and presented a lower environmental tolerance index (ETI, Curry 1999) for the temperature (2.6 for *T. lutaria* versus 3.9 for *E. virens*) in Patagonian environments (Coviaga et al. 2018a). *Bradleystrandesia fuscata* and *A. nobilis* are species widely distributed, with records in the Neotropical, Neartic and Paleartic regions. Both taxa prefer cold waters of low to moderate conductivity (Meisch 2000, Schwalb et al. 2002, Cusminsky et al. 2005, Coviaga et al. 2018a), also in accordance with the characteristics of the Andean - Patagonian forest environments.

Type I was the only assemblage without endemic species. This is probably due to all the environments sampled in this ecoregion are located near disturbed human settlements. In this context, cosmopolitan taxa can play an important role structuring these types of habitats due to their ability to tolerate wide ranges of environmental variables. In accordance to this, comparative studies in Turkey showed that cosmopolitan species outnumber non-cosmopolitan species in disturbed sites, which can be inferred also to poor water quality (Külköylüoglu 2004). These results again highlight that an increase in sampling efforts in pristine aquatic environments within the Andean
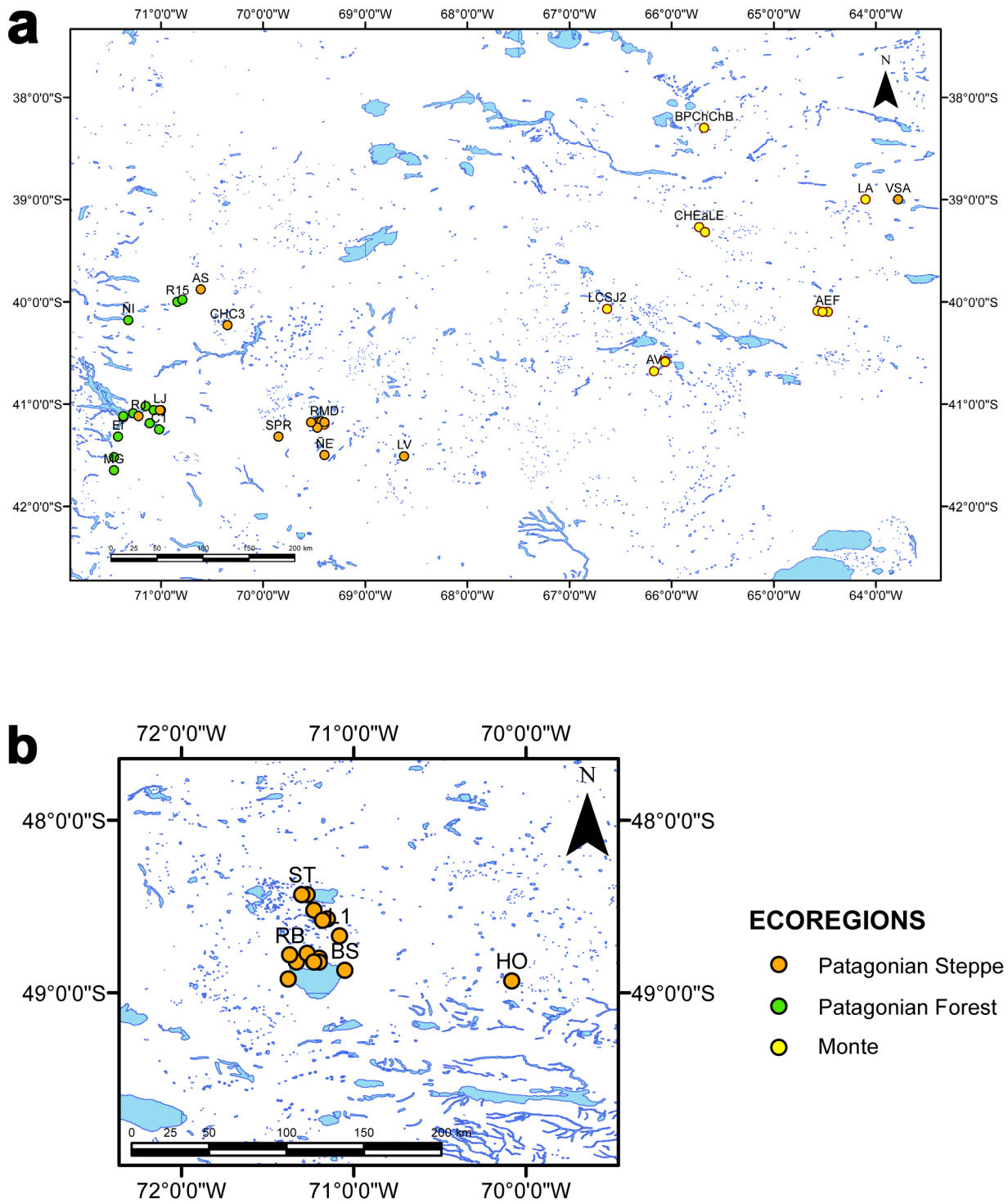
Figure 6. Sampled sites categorized by Patagonian ecoregions, **a** sites located at 41°S and **b** sites situated at 48 °S.

- Patagonian forest ecoregion is necessary to characterize its native ostracod fauna.

Assemblage II (Patagonian Steppe ecoregion)

The sites conforming to this assemblage belong to clusters 2, 6, 7 and 8, and are located at moderate altitude and characterized by waters of moderate to high EC, and alkaline pH values (Figure 5). This assemblage presents the highest diversity, with the largest number of endemic species such as *L. rionegroensis, L. patagonica, E. virgata, R. whatleyi, R. sarsi, N. patagonica, K. megapodus, E. cecryphalium, I. ramirezi* and *P. incae*. Both *I. ramirezi* and *E. virgata* inhabit a wide range of environments such us springs, permanent, semi-permanent and ephemeral waters showing high tolerance to variable salinity and ionic concentrations (Schwalb et al. 2002, Cusminsky et al. 2005, 2011, Ramón

Mercau et al. 2012, Ramón Mercau & Laprida 2016, Coviaga et al. 2018a). *Ilyocypris ramirezi* shows a eurytopic behaviour, being present at most sites and exhibiting a broad tolerance range to water temperature, pH, EC and Mg^{2+} concentration; allowing it to be associated with the majority of the other species present in this sector. However, its presence is closely related to flowing waters of low to moderate conductivity, confirming its use as indicator of lotic and lentic environments fed by streams and springs (Schwalb et al. 2002, Cusminsky et al. 2005, Coviaga et al. 2018a). *Eucypris cecryphalium* and *P. incae* present narrow tolerance range to EC and Mg^{2+} concentration. The former taxon was found in permanent waters, with low to medium conductivity (Cusminsky et al. 2005). On the other hand, *P. incae* (named *Darwinula* sp. in Schwalb et al. 2002 and Cusminsky et al. 2005)

Table VI. Range of examined parameters in the different ecoregions, median values in parenthesis. Abbreviations: T = temperature, EC = electrical conductivity, Alt. = altitude.

Ecoregion	Alt. (m s.n.m.)	T (°C)	pH	EC (μS/cm)	K+ (mg/l)	Ca+2 (mg/l)	Mg+2 (mg/l)	Na+ (mg/l)
Andean-Patagonian forest	657 – 943 (816)	8.1 - 19.8 (11.9)	7.2 - 8.1 (7.4)	24 – 289 (97)	0.3 - 5.5 (0.9)	3.3 - 21.7 (13.7)	0.3 - 7.3 (4.6)	2.4 - 13.4 (6.6)
Patagonian steppe	116 – 1161 (806)	6.6 - 34.1 (16.0)	6.0 - 9.9 (8.8)	52.8 - 51693.6 (1079)	0.1 - 265.3 (4.2)	0.2 - 78.1 (15.7)	0.0 - 324.2 (13.2)	0.5 - 8713,2 (151.3)
Monte	59 – 134 (63.5)	8.7 - 28.7 (24)	7.5 - 10.8 (8.3)	215.0 - 7090.0 (534)	1.2 - 46.0 (2.2)	11.7 - 100.5 (48.1)	1.0 - 71.4 (11.3)	16.0 - 1522.0 (93.6)
Espinal	5 – 77 (41)	20.6 - 22.4 (21.5)	7.9 - 8.3 (8.1)	10950 – 11390 (11170)	49.8 - 57.4 (53.6)	305.2 - 629.4 (467.3)	141.2 - 168.6 (154.9)	1633 – 1800 (1716.5)

was recorded in springs with low conductivity at moderate to high latitudes in southern Patagonia (48°S). Ramón Mercau & Laprida (2016) found *P. incae* in temporary to permanent environments and considered it as limnetic and stenohaline species. The others species characterising Assemblage II exhibit a moderate to high tolerance range to water temperature, EC, pH and Mg^{2+} concentrations. *Limnocythere patagonica* is characteristic of permanent lakes and ponds at 41° and 48° S latitude, associated with temperate to cold waters of moderate conductivity (Coviaga et al. 2018a). In agreement, Ramón Mercau et al. (2012) and Ramón Mercau & Laprida (2016) characterized this species as stenohaline taxa, with preference for limnetic to oligohaline waters. *Riocypris whatleyi* and *R. sarsi* have been recorded in ephemeral and permanent environments. The former reaches its maximum abundance in permanent lakes, preferring moderately saline conditions (Coviaga et al. 2018a). However, this species is able to inhabit waters with a wide range of solute composition and salinity (see Table III) (Cusminsky et al. 2005, Ramón Mercau et al. 2012, Ramón Mercau & Laprida 2016, Coviaga et al. 2018b). On the other hand, *R. sarsi* has lower EC tolerance range than *R. whatleyi* (see Table III), reaching its maximum abundance in temporary environments and could be considered as a stenohaline limnetic taxon with preferences for water conductivity around 1,000 μS cm^{-1} (Coviaga et al. 2018b). *Limnocythere rionegroensis*, found in environments at both 41°S and 48°S, presents a wide EC tolerance range (see Table III), and is considered as a mesohaline to polyhaline taxon (Cusminsky et al. 2005, Ramón Mercau et al. 2012, Ramón Mercau & Laprida 2016, Ramos et al. 2017). *Newnhamia patagonica, K. megapodus, H. incongruens, P. unicaudata* and *P. smaragdina* are also present in the Patagonian Steppe ecoregion, but were recorded in waters

of moderate values of EC, ionic concentration and pH (Figure 5, Table III). The majority of these species was found in all types of environments, associated with waters of low to medium conductivity (Schwalb et al. 2002, Cusminsky et al. 2005, 2011, Ramón Mercau et al. 2012, Ramón Mercau & Laprida 2016, Coviaga et al. 2018a). This suggests their preferences for limnetic to oligohaline environments (Ramón Mercau & Laprida 2016, Coviaga et al. 2018a).

The Patagonian steppe ecoregion is considered 50% arid, 5% hyperarid and only 9% as sub-humid ecoregion based on the potential evapotranspiration/mean annual precipitation ratio (Paruelo et al. 1998, 2005). Thus, the species recorded in this sector frequently inhabits environments with high evaporation/precipitation ratios, characteristic of this ecoregion.

Assemblage III (Monte and Espinal ecoregions)

The sites conforming this assemblage are situated at low altitude in the eastern side of the study area (clusters 4, 5 and 7). This sector is represented by temperate waters (Figure 5) occupied by both endemic species, *H. hyalinus* and *A. argentinensis*, and cosmopolitan taxa *S. aculeata, C. vidua, H. intermedia* and *C. incisa*. All of them prefer temperate waters, above 20 °C, with moderate to high conductivity (Coviaga et al. 2018a). *Heterocypris hyalinus* and *A. argentinensis* inhabit low altitudes (59 to 126 m a.s.l.; Table III). The former has broad ecological tolerance ranges (Table III) and is widespread in the Monte ecoregion, associated with stagnant, temperate, and moderate conductivity waters (Coviaga et al. 2018a). *Amphicypris argentinensis* shows narrow values of altitude, T, pH, EC and Mg^{2+} concentration (Table III). We found this species in oligohaline waters, whereas previously it was recorded in the Buenos Aires province (Pampa

ecoregion), showing a preference for temperate waters of high conductivity (Fontana & Ballent 2005). *Cypridopsis vidua* is a cosmopolitan and swimming species, which inhabits a wide range of aquatic, principally vegetated, habitats (Meisch 2000, Meisch et al. 2019) with relatively wide physical and chemical parameters (Meisch 2000, Külköylüoğlu et al. 2007, Külköylüoğlu & Sarı 2012, Martínez-García et al. 2015). *Sarscypridopsis aculeata* displays a broad tolerance to physical and chemical parameters. However, this cosmopolitan taxon prefers temperate and slightly saline waters bodies, and is rare in pure freshwater environments (Meisch 2000, Mischke et al. 2003, Coviaga et al. 2018a).

Our results did not allow differentiating a characteristic ostracod fauna between the Monte and Espinal ecoregions, although both ecoregions present different environmental features, i.e. the Monte ecoregion has a higher mean annual precipitation and lower mean annual temperature than the Espinal ecoregion (Burkart et al. 1999). This is likely a consequence of the small number of environments sampled in the Espinal ecoregion (n=2) and the proximity of these sites to the Monte ecoregion. Additionally, *L. cusminskyae* and *H. salina* were sampled only in Espinal sites. Until now, these species were only mentioned in Buenos Aires province (Ramón Mercau et al. 2014) and Buenos Aires and Mendoza provinces (Bertels & Martínez 1990, D'Ambrosio 2014) respectively, suggesting that it is necessary to obtain more information to characterize the ostracoda fauna in this ecoregion.

CONCLUSIONS

Our research present the largest data set of extant non-marine ostracods in Patagonia, Argentina, analysing 69 aquatic environments including permanent, and ephemeral lakes, ponds and springs. According to our CCA results the ostracods responds to EC, altitude, pH, and temperature, showing a significant correlation to the ecoregions recognized by Burkart et al. (1999) in this part of South America: Andean-Patagonian forest, Patagonian steppe, Monte and Espinal. However, our results did not allow to differentiate between the ostracoda fauna of Monte and Espinal ecoregions, suggesting that it is still necessary to obtain additional information to characterize the ostracod ecological requirements in these ecoregions. The Andean - Patagonian forest was the only ecoregion without endemic species, probably due to human disturbance. Therefore, an increase in sampling efforts in the Andean - Patagonian forest ecoregion is necessary, should emphasize on pristine environments, to characterize the native ostracod fauna.

According to the distribution of ostracod assemblages and their relationship with the EC, altitude, pH, and water temperature, three ostracod associations have been recognized within the following Patagonian ecoregions: I) Andean-Patagonian forest ecoregion represented by *C. pubera, E. virens, B. fuscata T. lutaria* and *A. nobilis*, and characterized by environments of moderate altitudes, high precipitation and moderate temperature, with waters of low temperature, EC, pH and ionic concentrations; II) Patagonian steppe ecoregion with *L. rionegroensis, L. patagonica, E. virgata, R. whatleyi, R. sarsi, N. patagonica, K. megapodus, P. smaragdina, P. unicaudata, E. cecryphalium, P. incae* and *H. incongruens* as characteristic species and distinguished by waters with moderate to high EC, and alkaline pH values in environments of moderate temperature, low precipitation and median altitude and; III) Monte ecoregion represented by *S. aculeata, C. vidua, H. hyalinus, H. intermedia, C. incisa* and *A.*

argentinensis situated at low altitude sites, with temperate and moderate conductivity waters.

Our results compile the knowledge about extant non-marine ostracod assemblages in Patagonia and highlight their capacity as proxies to conduct ecological and hydroclimatic studies especially in the context of community response to global change. However, we propose that the biodiversity in aquatic ecosystems of Patagonia are still under characterized in comparison to analogous ecoregions of the world.

Acknowledgments

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Table SI.
Figure S4.

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