Ecología Austral 33:632-640 Agosto 2023
Asociación Argentina de Ecología

Diversity, distribution, and ecology of the genus *Hyalella* Smith, 1874 (Crustacea: Amphipoda: Hyalellidae) in the Puna and the Altos Andes ecoregions of Argentina

Guillermo E. Hankel[1,✉]; A. Verónica Isa Miranda[2] & Carolina Nieto[1,3]

[1] *Instituto de Biodiversidad Neotropical, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Tucumán.* [2] *Instituto de Invertebrados, Fundación Miguel Lillo.* [3] *Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán.*

Abstract. We studied the diversity, distribution and ecology of *Hyalella* (a genus endemic to America that lives over a wide range of geographical altitudes) in 44 peatlands of the Puna and the Altos Andes ecoregions, in the NW of Argentina, mostly placed in the provinces of Salta and Catamarca. Our aim is to deepen our understanding of the distribution and ecology of these freshwater crustaceans in both ecoregions. Field collection was made between 2013 and 2017, and ecogeographical variables were also recorded. The crustaceans were identified by dissections under a microscope. We estimated biodiversity indices and made Whittaker curves to compare diversity between both ecoregions. We made correlations between *Hyalella* abundance and 11 ecogeographic variables. A principal components analysis was made to study relationships among each peatland and eco-geographical variables. A total of 6548 individuals, distributed among 4 species and 11 morphospecies, were collected. Diversity in the Puna was higher than in the Altos Andes, but was low in each individual peatland. *Hyalella fatimae* was the most abundant species in both ecoregions. The PCA revealed that altitude, rainfall, temperature, electrical conductivity and total dissolved solids were key variables that characterized the peatlands. The abundance of *Hyalella* was negatively correlated with altitude and annual mean rainfall, and positively correlated with the mean minimum temperature of the coldest month. *Hyalella* proved to be an important component of macroinvertebrates assemblages in these environments, capable of generating a species complex and endemism, as reported for other regions of South America. The peatlands are important wetlands in the arid region of the Puna and the Altos Andes, and deserve more attention in the present context of being threatened by climatic change and the advance of economic activities.

[Keywords: peatlands, Andean wetlands, aquatic macroinvertebrates, biodiversity]

Resumen. **Diversidad, distribución y ecología del género *Hyalella* Smith, 1874 (Crustacea: Amphipoda: Hyalellidae) en las ecorregiones Puna y Altos Andes de la Argentina.** Estudiamos la diversidad, la distribución y la ecología de *Hyalella* (género endémico de América que vive en un amplio rango altitudinal y ambiental) en 44 vegas de las ecorregiones Puna y Altos Andes (NO de la Argentina), mayormente en las provincias de Salta y Catamarca. Nuestro objetivo fue comprender mejor la distribución de estos crustáceos dulceacuícolas en ambas ecorregiones. Las colectas de campo se realizaron entre 2013 y 2017, y también se registraron variables ecogeográficas. Los crustáceos fueron identificados mediante disecciones bajo microscopio. Calculamos índices de diversidad y realizamos curvas de Whittaker para comparar la diversidad entre ambas ecorregiones. Hicimos correlaciones entre 11 variables ecogeográficas y la abundancia de *Hyalella*. Se realizó un análisis de componentes principales para estudiar las relaciones entre las vegas y las variables ecogeográficas. Se colectaron 6548 individuos en total, distribuidos entre 4 especies y 11 morfoespecies. La diversidad en la Puna fue mayor que en los Altos Andes, pero fue baja en cada una de las vegas individualmente. *Hyalella fatimae* fue la especie más abundante en ambas ecorregiones. El PCA mostró que la altitud, las precipitaciones, la temperatura, la conductividad eléctrica y los sólidos totales disueltos fueron variables importantes para caracterizar las vegas. La abundancia de *Hyalella* correlacionó negativamente con la altitud y las precipitaciones medias anuales, y positivamente con la temperatura mínima del mes más frío. *Hyalella* demostró ser un componente importante de los ensambles de macroinvertebrados en estos ambientes, capaz de generar complejos de especies y endemismos, como se reportó para otras regiones de Sudamérica. Las vegas son humedales clave en las regiones áridas de la Puna y los Altos Andes, y merecen más atención en el contexto actual, estando amenazadas por el cambio climático y el avance de las actividades económicas.

[Palabras clave: vegas, humedales andinos, macroinvertebrados acuáticos, biodiversidad]

Editor asociado: Alejandro Loydi

Recibido: 16 de Febrero de 2023
Aceptado: 19 de Mayo de 2023

✉ guillehankel@gmail.com

Introduction

Hyalella Smith, 1874 is a genus of freshwater crustaceans that is endemic to America (Baldinger 2004), and it is the only genus in the family Hyalellidae that occurs throughout the Nearctic and Neotropical biogeographic regions (Bueno et al. 2014; Rangel et al. 2022). The species in this genus can be found in various types of freshwater environments, such as lakes, ponds, streams and subterranean waters. They can also be found associated with aquatic vegetation or burrowed in the bottom (da Silva Castiglioni and Bond Buckup 2008), over a wide range of geographical altitudes, from sea level to more than 4000 m a. s. l. (Peralta and Isa Miranda 2019).

There are 99 species described in the genus (Isa Miranda and Peralta 2022; Limberger et al. 2022; Marrón-Becerra and Hermoso-Salazar 2022; Rangel et al. 2022); 72 are from South America and 13 from Argentina. Until now, most of these studies have been based on morphology characters. However, in recent years, several analyses including molecular evidence revealed some cryptic species or species complexes.

In Argentina, the Central Andean Highlands includes two ecoregions, namely the Altos Andes (with the steepest slopes) and the Puna (an upraised flatland). The Altos Andes encompass the high peaks of the Andean Mountains ranges, above 4000-4200 m a. s. l. The Puna is a high-altitude plateau with elevations above 3000 m a. s. l., making it the highest plateau in the world after Tibet in terms of both altitude and size. Together, both ecoregions have a wide distribution with more than 14.3 million hectares. It is characterized by an arid climatic condition with an annual precipitation range of 100 to 400 mm (Cabrera 1976).

In the last years, different field trips made possible an extensive collection of the peatlands in the Puna, mainly in the provinces of Catamarca and Salta. The *Hyalella* genus is dominant in the region (Nieto et al. 2017), although its differentiation between species remains unknown. In this study, we report the presence of several morphospecies of the genus *Hyalella* in the study region. We also provide information on the diversity and abundance of *Hyalella* in each peatland and ecoregion and examine the relationship between these characteristics and the abiotic conditions of the water and landscape. Furthermore, we compare the diversity of *Hyalella* between the two ecoregions in the study area. Our aim was to deepen our understanding of the distribution and ecology of these freshwater crustaceans in these ecoregions.

Materials and Methods

The study area covers 14210000 hectares in the Altos Andes of NW Argentina (Izquierdo et al. 2015) (Figure 1), within the Central Andean Puna and the Altos Andes ecoregions (Olson et al. 2001), including part of the provinces of Jujuy, Salta, Catamarca, La Rioja and San Juan. The area extends from an elevation of 3200 to ~6900 m a. s. l., at the top of the highest mountains. The total area of peatlands in the study area is estimated at 110873 hectares (0.78% of the total study area) (Izquierdo et al. 2015), between 3005 and 5141 m a. s. l., with an average elevation of 4056 m a. s. l. (Izquierdo et al. 2015). The climate is very dry, with a NE-SW aridity gradient. The mean annual precipitation ranges from less than 100 mm in the SW to 400 mm in the NE of the region. Average annual temperature ranges between 9 °C and -4 °C (Cabrera 1976).

We collected macroinvertebrates in 49 peatlands, during five austral summer seasons (December to March) from 2013 to 2017 (Supplementary Materials 1-Table S1). These macroinvertebrates samples were obtained using a D-frame net (mesh size 300 μm). The number of sampling units per peatland ranged from two to ten depending on the size and heterogeneity of each peatland. All samples were taken from 10:00 to 17:00 h, with a time window of a standardized effort of 30 minutes. All collected materials were placed in vials of 15 mL with 96% ethyl alcohol. In the laboratory, each sample was identified and quantified with the aid of a stereomicroscope. Between two and four males from each site for identification at the species level. Samples were dissected under a stereomicroscope using Brunson as an immersion solution. Dissected structures were observed under the microscope. Identification at the species level was based on previous taxonomic work on *Hyalella* species (Grosso and Peralta 1999; González and Watling 2001, 2003; Peralta and Isa Miranda 2019; González et al. 2020; Isa Miranda and Peralta 2022). The examined material was deposited at the IBN (Instituto de Biodiversidad Neotropical, CONICET-UNT), Tucumán, Argentina.

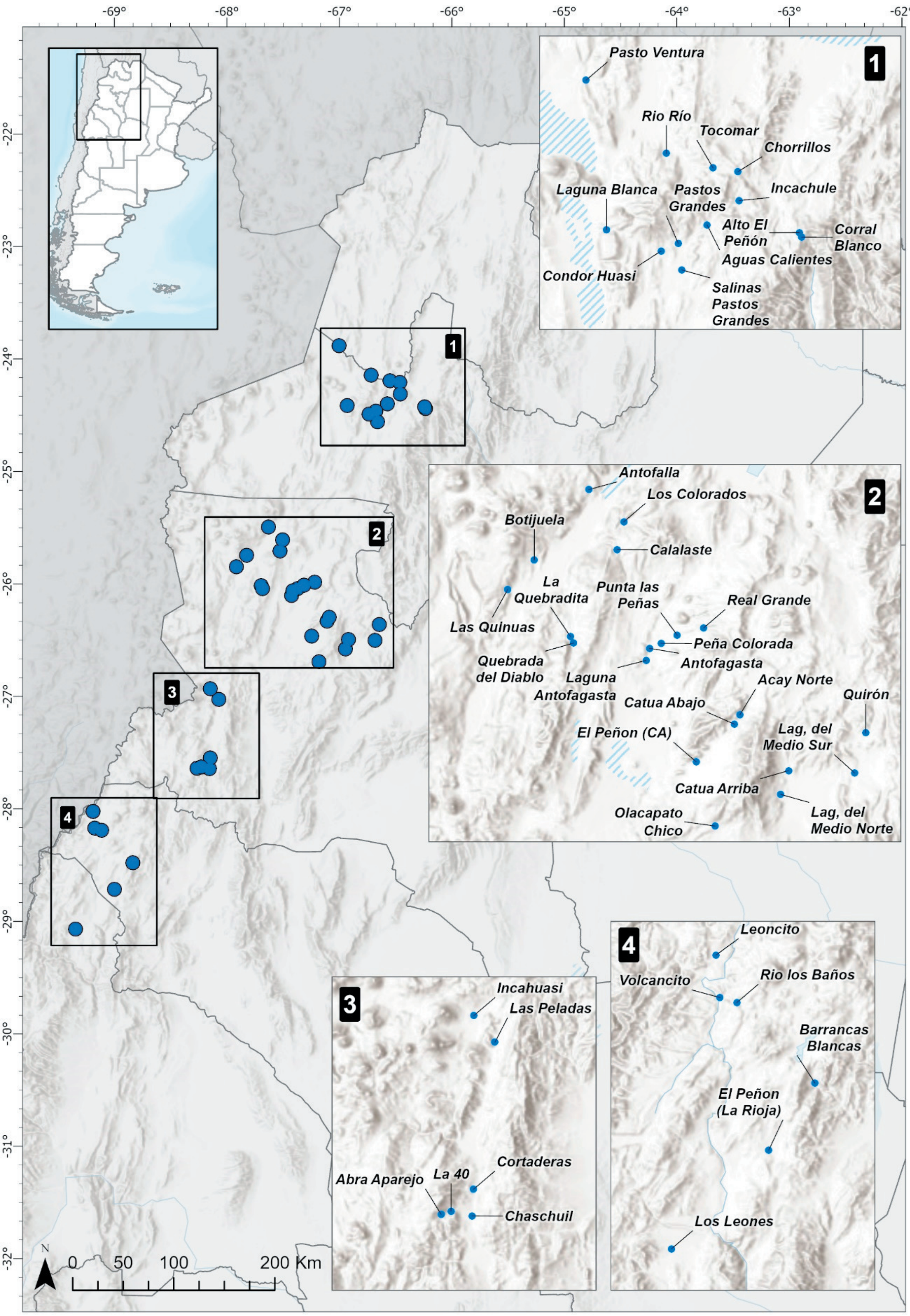

Figure 1. Map of the geographical location of the studied peatlands.

Figura 1. Mapa que muestra la ubicación geográfica de las vegas estudiadas.

At each sampling point we also recorded the altitude and different physical and chemical characteristics of water (Supplementary Materials 1-Table S1), measured with Horiba Multiparameter Water Quality Meters U-52. Variables related to the water column were the water temperature (TWAT, °C), pH, oxidation-reduction potential (ORP, mV), conductivity (COND, μS/cm), turbidity (TURB, NTU) and dissolved oxygen (%DO). Other variables such as NDVI, peatland area (AREA, m^2), minimum water temperature (TMIN, °C) and air temperature mean (TAIR, °C) were taken from Izquierdo et al. (2020).

Whittaker curves were made to describe the relative importance of *Hyalella* in the assemblages of the Puna and the Altos Andes. We made a single curve to illustrate the relative importance of *Hyalella* in each peatland. Furthermore, the individuals found in the peatlands of the Puna and the Altos Andes were added by ecoregion to elaborate the comparative curves.

We employed two diversity indexes: the Shannon-Wiener (W) and the Simpson (1-D). These indices were independently estimated for the *Hyalella* community —including all peatlands— and for the Puna and the Altos Andes. Indexes were calculated with the vegan package (Oksanen et al. 2019). We performed a principal component analysis (PCA) on the set of 12 physic-chemical and ecogeographic variables measured in the peatlands (Supplementary Materials 1-Table S1). The variables were centered and scaled with the R package scales (Wickham and Seidel 2020), and PCA was done with the R package ade4 (Dray and Dufour 2007). This step allowed us to frame the study of community structure in general and *Hyalella*, particularly within a synthetic representation of physical-chemical variability. Also, we perform correlations (Pearson or Spearman, as appropriate) to elucidate relationships between *Hyalella* abundances and the abiotic variables. All analyses were made with the R statistical software (R core team 2020).

Results

Hyalella diversity

We collected macroinvertebrates in 49 peatlands; 44 of them reported *Hyalella* (Supplementary Materials 2-Table S2). *Hyalella* species were a very important component in the macroinvertebrates' community of the peatlands. Of the 22872 macroinvertebrates collected, 28% were *Hyalella*, and in 38% of the peatlands, more than 50% of the density were *Hyalella* individuals (Figure 2). The range of relative abundance of *Hyalella* in the different peatlands oscillated between 2.4 and 91%. A total of 6548 individuals were identified, belonging to 4 species and 11 morphospecies (Supplementary Materials 2-Table S2). *H. fatimae* and *H. puna* were the most representative (50% of the total).

The diversity indexes obtained for the complete assemblage were 2.029 (Shannon) and 0.81 for Simpson. The abundance of *Hyalella* was negatively correlated with ALT (P<0.05, Pearson correlation, r=-0.37) and RAIN (P<0.01, Spearman correlation, r_s=-0.41), positively correlated with TMIN (P<0.05, Spearman correlation, rs=0.34) and TURB (P<0.06, Spearman correlation, r_s=0.29), the latter being marginally significant.

Diversity in the Puna and the Altos Andes

Most of the peatlands present only one or two species or morphospecies of *Hyalella*. The maximum diversity occurred at the Antofagasta site, where there were two species and two morphospecies. The most common species in the Puna and the Altos Andes ecoregions was *H. fatimae* (Figure 3; Supplementary Materials 2-Table S2). *Hyalella fossamancinii* was also very important, being the third most abundant taxon in the Puna, and the second in the Altos Andes (Figure 3). There are no exclusive species in either ecoregion. In the Altos Andes, we found fewer species/morphospecies of *Hyalella* (6 species/morphospecies), in contrast to the Puna, where all 15 species/morphospecies were present. The three most abundant taxa (*H. fatimae*, *H. fossamancinii* and *H. sp3*) added together become 70% of the *Hyalella* abundance. In the Puna ecoregion, in addition to *H. fatimae* and *H. fossamancinii*, *H. puna* was significant, representing the second most abundant taxon. Among these three taxa, it represents ~70% of the *Hyalella* abundance. Other taxa with lower importance were *H. kochi*, *H. sp6*, and *H. sp4*, which, added to the previous taxa, accumulate almost 90% of abundance.

The Simpson index indicated a similar dominance in both ecoregions (Altos Andes=0.79, Puna=0.8), although the Shannon index pointed to the Puna as the most diverse ecoregion (Altos Andes=1.61, Puna=1.96).

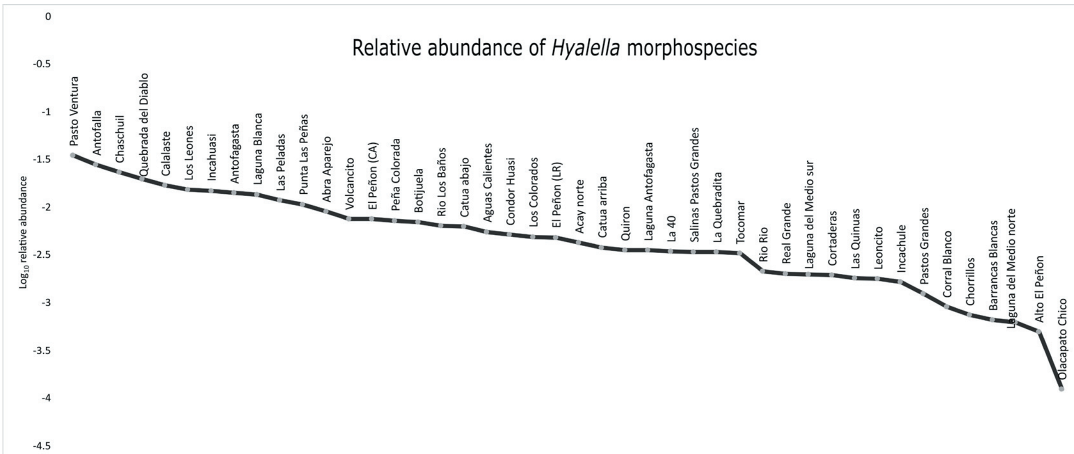

Figure 2. Whittaker curve of *Hyalella* relative importance in the macroinvertebrates assemblage in each studied peatland.

Figura 2. Curva de Whittaker mostrando la importancia relativa de *Hyalella* en el ensamble de macroinvertebrados en cada una de las vegas estudiadas.

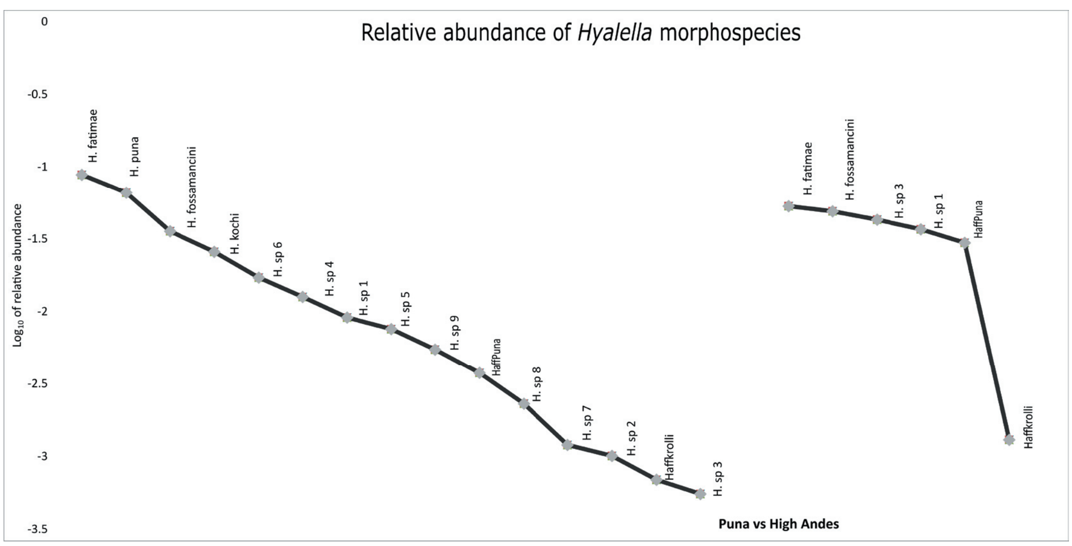

Figure 3. Whittaker curves to contrast the most abundant *Hyalella* species in each ecoregion.

Figura 3. Curvas de Whittaker comparando las especies de mayor abundancia en cada una de las ecorregiones.

Peatland ecogeographic variables

The ALT of the sampled peatlands oscillated between 3200 and 4700 m, with a mean of 3900 m. Most of the peatlands had an alkaline pH (mean=8.11, median=7.97), although an acidic pH was also recorded (minimum=4.66) (Supplementary Materials 1-Table S1). The TWAT fluctuated between 6 °C to 22 °C, with a mean and median of 14 °C. COND ranged between hyperfresh, 46 uS/cm (Nieto et al. 2017), to hypersaline (11400 uS/cm), but the median was 360 uS/cm. Five of the peatlands were hyper fresh, 33 were fresh, and the rest was saline. RAIN had a mean and median near to 100 mm, and a range of 112 mm. T MIN coincided in mean and median about -6 °C. Usually, %DO was under saturation (mean and median=83%). TURB generally was above 0, with a mean of 21.9 NTU, a median of NTU and a maximum of 203.9 NTU.

In the PCA, the first (axis 1, eigenvalue 3.56) and the second (axis 2, eigenvalue 2.6) principal components explained 32.4% and 23.6% of the total variability, respectively. Axis 1 was influenced by ALT and RAIN (positive correlation of 0.94 and 0.72, respectively)

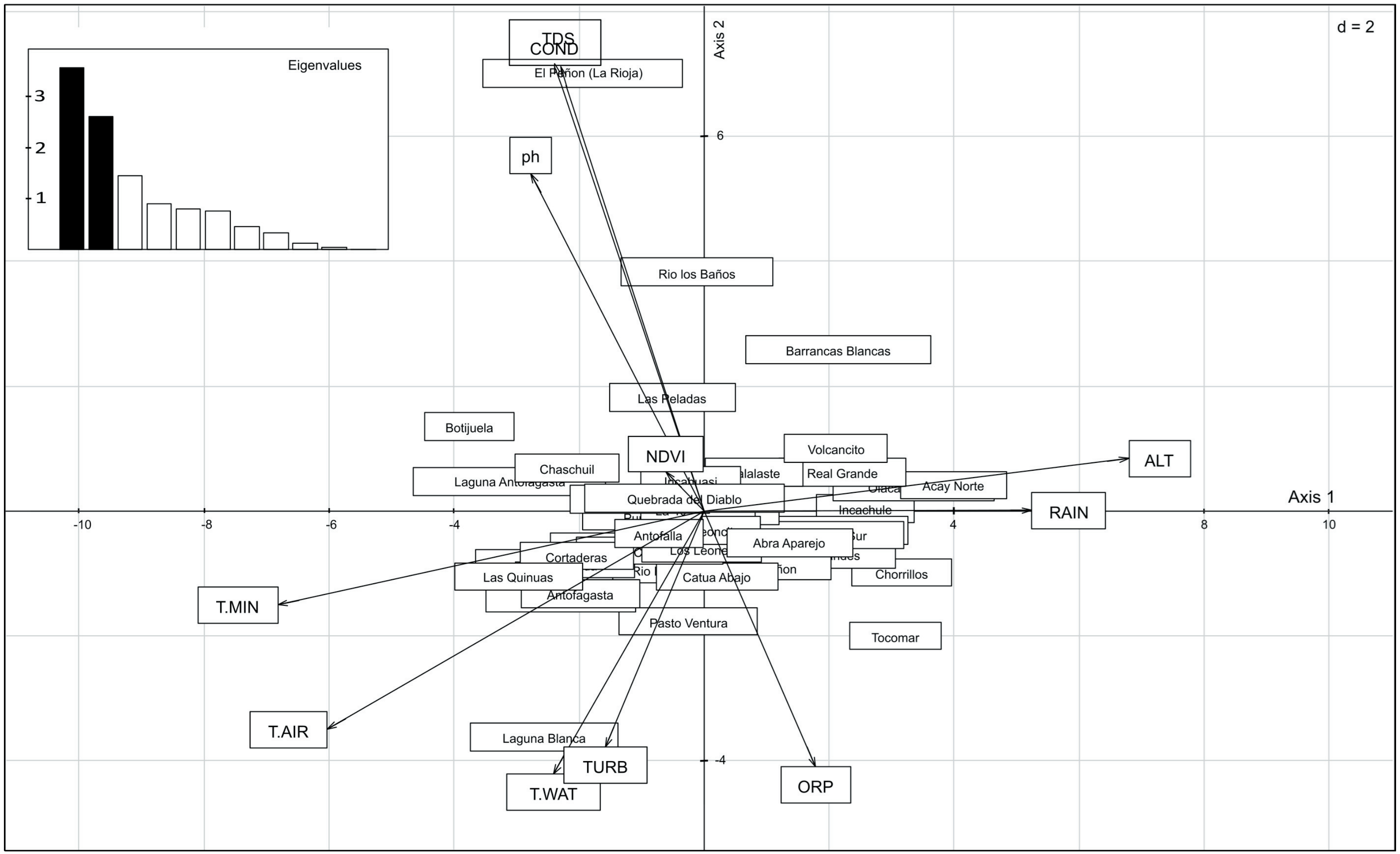

Figure 4. PCA plot showing the relationships between the eco-geographical variables recorded and the peatlands. The inset figure shows the eigenvalue of each principal component.

Figura 4. Gráfico del PCA que muestra el ordenamiento de las vegas estudiadas según las variables eco-geográficas registradas. La figura inserta en la esquina superior muestra el eigenvalor de cada componente principal.

and TMIN and TAIR (negative correlation of -0.94 and -0.83, respectively), while axis 2 was majorly influenced by COND and TDS (positive correlation of 0.80 for both variables) (Figure 4). Most of the sites clustered closely near the center. The El Peñón La Rioja site was segregated toward up because it presented the highest COND and TDS. Laguna Blanca was discriminated toward the down due to its high TURB, and Río Los Baos and Barrancas Blancas were slightly apart toward up because they recorded the lowest TAIR.

Discussion

This study evaluated the *Hyalella* diversity of the genus in peatlands of the Puna and the Altos Andes ecoregions of Argentina. In this way, we can 1) recognize 4 species and 11 morphospecies distributed in 44 peatlands; 2) identify species/morphospecies in each ecoregion (Puna-Altos Andes); 3) correlate ecogeographical variables with the abundance of the genus, and 4) group the peatlands according to the physicochemical variables.

Although 4 species and 11 morphospecies in this region are presumably high, this genus has a similar background. The Lago Titicaca reported 13 endemic species (González and Watling 2003) and 12 BINs (Barcode Index Number System) (Ratnasinghan and Herbert 2013) reported by Andamowicz et al. (2018). Even these last authors suggested that this lake could serve as a 'cradle of diversification' to *Hyalella* (Andamowicz et al. 2018). In Uruguay, Waller et al. (2022) revealed a complex of species —the 'curvispina complex'— and reported greater diversity than originally expected.

Due to its high diversity, this genus was also abundant and found very frequently in almost all peatlands (44 from 49). However, the high value of the Simpson index indicated that a high dominance occurs in many peatlands, where there were only one or two species of *Hyalella*. High abundance and distribution range were reported for some species of this genus, such as *H. curvispina*. It was reported in Andean Patagonian rivers (Miserendino 2001) and in headwater streams of Cordoba (Rodríguez et al. 2017), reflecting its importance in the aquatic macroinvertebrate assemblages.

H. fatimae and *H. fossamancinii* were the most common species collected in both the Puna and the Altos Andes ecoregions. It suggests that low atmospheric pressure and oxygen concentration related to the increase in altitude (Vinson and Hawkins 1998; Jacobsen 2003; Jacobsen et al. 2003) are not affecting these species. This is not consistent with previous research, in which we noted that aquatic communities were clearly affected by altitude, proposing this limit at 4200 m (Nieto et al. 2017) and indicating that diversity abruptly decreased above that limit. Anyway, in this study, we found that the Altos Andes presented less diversity than the Puna (6 *vs.* 15 species/morphospecies, respectively). Ecophysiological experiments are needed to understand the underlying mechanisms involved in this segregation. With the improvement of specific knowledge in *Hyalella*, we hope to enhance the comprehension of more tolerant species to limiting factors.

Besides altitude, *Hyalella* abundance was mainly affected by turbidity, minimum temperature and annual rain. Similar tendencies were found in assemblages of macroinvertebrates in Andean Patagonian rivers (Miserendino 2001), although she reported other variables affecting this group too, such as water velocity, conductivity and aquatic plants abundance among others. On the other hand, Waller et al. 2020 did not find a correlation between abundance *vs.* temperature and pH, suggesting high thermic plasticity for *H. curvispina*. The positive relationship between turbidity and *Hyalella* abundance could be related to the fact that certain *Hyalella* species are sediment biodisturbing, as reported by Bouvier et al. (2013). Therefore, a higher presence of *Hyalella* will imply a higher turbidity in the peatland.

A wide number of peatlands were studied in the Puna and the Altos Andes ecoregions in the NW of Argentina. Despite the extensive distance among some peatlands (e.g., the distance between Pasto Ventura and Los Leones is ~650 km), the PCA plot did not show a space ordering pattern and revealed high variability in the variables studied. Most of the sites were closely ordered near the center, reflecting few differences in the variables used. The main variables that structured the PCA were ALT, RAIN, TMIN, TAIR, COND, and TDS. In contrast, Coronel et al. (2004) analyzed the peatlands in the Tunari cordillera, in Bolivia, and found that those differed by a locality component (i.e., spatially) in their physical-chemical characteristics, not so in their morphometric or biotic features; although his PCA plot also showed a high variability pattern, the biological variables were the principal variables being structuring the PCA analysis in his study. The peatlands of both studies (this one and Coronel et al. 2004) had similar altitude and pH values, and the Bolivian peatlands presented lower values for electric conductivity.

The peatlands of the Puna and the Altos Andes ecoregions (both in Argentina and throughout South America) are essential ecosystems that regulate water flow and availability in these arid environments, function as carbon sinks and provide local cattle feed for human consumption (Oyague et al. 2015; Oyague and Cooper 2020). Moreover, these areas have exhibited a high degree of species diversity, as evidenced by the discovery of 4 species and 11 *Hyalella* morphospecies, surpassing the number of described species in Argentina.

The identification of 11 morphospecies in this study —putatively new to science— underscores the significance of continuing research on the biological diversity of peatlands. Traditional and molecular taxonomic methods are being employed to provide a comprehensive and precise description of these species. Further studies focusing on *Hyalella* and other invertebrates are expected to shed light on the evolutionary role of these environments as speciation factors in these organisms.

Acknowledgments. We want to thank Luciana Cristobal for elaborating on the map. AVIM thanks the Invertebrate Institute (Fundación Miguel Lillo) for providing institutional support for this research, and to Marcela Peralta (FML) for her constant advice. This work was funded by CONICET and Grants from PICT2012-1565, PICT 2016-2173, and PICT2019-2823 FONCYT and PIP 2021-652 CONICET to the research team.

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