*Zoological Studies*

# Native Fauna of Tardigrades from Two Natural Areas of the Argentina Republic

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Understanding and preserving biodiversity in natural habitats is crucial due to their rapid degradation and destruction. The meiofauna of natural areas is less well known than the macroscopic life. Tardigrades are common in limno-terrestrial meiofauna and can indicate environmental conditions. In this study, we expand our understanding of the taxonomy and ecology of tardigrade communities in two natural reserves in Argentina by examining the environmental factors that can affect them and the species that could be used as indicators. In 2018, sampling occurred in the Parque Luro Provincial Reserve (province of La Pampa) and the Poligono A Municipal Ecological Reserve (province of Salta). Samples were taken from epiphytic communities that grow on the bark of trees. Various environmental and microhabitat factors were taken into account. In the province of La Pampa, there were 1326 specimens of five species, and in the province of Salta, there were 212 specimens of nine species. The tardigrade communities in Salta exhibited statistically higher diversity than those in La Pampa. Temperature and moisture affected the tardigrade community of Parque Luro Provincial Reserve, while the microhabitat thickness affected those of Poligono A Municipal Ecological Reserve. The species turnover shaped the community of Salta, while nesting shaped the community of La Pampa.

**Key words:** Biodiversity, Ecology, South America, Geography, La Pampa, Salta

## BACKGROUND

The majority of knowledge regarding the biodiversity of natural areas has centred on macroscopic life, but their meiofauna are relatively unknown and are likely to be as diverse as or more diverse than their macrobiota (Bartels and Nelson 2007). Studies on invertebrates (Ims et al. 2007; O'Gorman et al. 2012; Gauthier et al. 2013) can demonstrate the effects of changes in biodiversity on various ecosystems. Despite this, little attention has been paid to the fundamental role that limno-terrestrial invertebrates play in natural ecosystems (Summerhayes and Elton 1923; Zmudczyńska-Skarbek et al. 2017; Koltz et al. 2018; Gillespie and Vincent 2019), understanding their role and the changes that their communities may experience due to global warming (Van der Putten et al. 2010; Gillespie and Vincent 2019).

Tardigrades, micro-metazoa measuring 100 to 1200 μm and one of the few animal phyla with

extensive cryptobiotic capabilities, are a component of the limno-terrestrial meiofauna (Kinchin 1994; Møbjerg et al. 2018). As a result of their evolutionary history, tolerance for unfavourable conditions, small size, and likely long-distance dispersal by the wind (Jørgensen et al. 2007; Incagnone et al. 2015; Nelson et al. 2015; Rivas et al. 2019; Roszkowska et al. 2021), tardigrades inhabit a wide variety of environments, including those with extreme conditions, such as deep waters, glaciers, and mountain peaks (Zawierucha et al. 2015; Nelson et al. 2018). Under optimal conditions, they can attain extremely high densities up to hundreds of individuals per 1 g of dry moss sample (Zawierucha et al. 2016). Some authors point to the ability of tardigrades to serve as environmental indicators, capable of responding to altitudinal changes (Nelson 1975; Dastych 1988; Guil et al. 2009; Kaczmarek et al. 2011), environmental disturbances (González-Reyes et al. 2020), and indicating substrate fertility (Porazińska et al. 2002; Zawierucha et al. 2015 2016), among other factors. Several studies (Guil et al. 2009; Nelson et al. 2018; González-Reyes et al. 2020; Ostertag et al. 2022) have reported that environmental disturbances (natural and anthropic) alter the abundance, diversity and/or complexity of tardigrade communities.

The Federal System of Protected Areas (SiFAP) of Argentina consists of 533 natural areas, of which 57 are of national jurisdiction under the Administration of National Parks and 476 are administered by the provinces with some type of provincial, municipal, university, private, or mixed management (https://www.argentina.gob.ar/ambiente/areas-protegidas). Rocha et al. (2016) for the Yungas ecoregion conducted the only research on tardigrade assemblages in natural areas of Argentina. Consequently, the purpose of this study is to contribute to the taxonomic and ecological understanding of tardigrade communities in two Argentine natural reserves, including areas of Selva Montana in the Yungas ecoregion (Salta) and the Espinal ecoregion (La Pampa). This study aims to determine the community structure of tardigrades in two natural areas from distinct ecoregions by evaluating environmental factors that may have an impact on them and searching for indicator species.

## MATERIALS AND METHODS

### Study Area

The tardigrades were collected from lichen and moss communities on tree trunks in two natural areas of Argentina, one of which was the Parque Luro Provincial Reserve (ecoregion of the Espinal) and the Poligono A Municipal Ecological Reserve (ecoregion of the Yungas). The Parque Luro Provincial Reserve (36°54'49"S, 64°15'41"W) is located in the centre-east of the province of La Pampa at 175 m a.s.l (Fig. 1). It encompasses 7,608 hectares and is the largest natural reserve of calden (*Neltuma caldenia*) in the world, containing dunes, lagoons, and flora and fauna of the Pampean mountain. This reserve represents the Espinal ecoregion, which is a wide Chaco-Pampean plain that is flat to gently undulating and has a temperate, dry climate with a pronounced water deficit and an annual precipitation average of 676 mm. In La Pampa, this ecoregion is characterised by a psamophyllous pasture of *Stipa* spp. and *Poa* spp. with islets of calden forests as well as *Geoffroea decorticans*, *Jodina rhodifolia*, *Neltuma caldenia*, *Neltuma flexuosa* and a shrub layer of *Aloysia gratissima*, *Condalia microphylla* and *Lycium chilense* (Albera 2002; Subsecretaría de Ecología 2004; Duval et al. 2017).

In contrast, the Poligono A Municipal Ecological Reserve (24°43'39.63"S, 65°30'11.71"W) is situated in the province of Salta between 700 and 1500 m a.s.l. and encompasses a total area of 66.5 hectares (Fig. 1). This reserve protects the Yungas environment, which is a subtropical humid forest with a distinct dry season (April to October) and sporadic snowfall in the winter (Brown et al. 2001). Along the reserve's altitude gradient, there are two levels of vegetation of the Yungas with different physical and floral characteristics: a) Selva pedemontana, between 400 and 900 m a.s.l. in the piedmont and low-altitude mountain ranges, with an average annual rainfall of 820 mm, represented by *Calycophyllum multiflorum*, *Phyllostylon rhamnoides*, and several species of timber value, such as *Anadenanthera colubrina* var. *cebil*, *Cedrela augustifolia*, *Handroanthus impetiginosus*, *Myroxylon peruiferum* and *Pterogtne nitens*, among others (Brown and Malizia 2004); and b) Selva montana, on the slopes of the mountains, between 900 and 1600 m a.s.l., with an average annual rainfall of 1800 mm (Bianchi and Yañez 1992; Arias and Bianchi 1996), represented by *Blepharocalix salicifolius*, *Cinnamomum porphyrium*, *Eugenia uniflora*, *Ficus maroma*, *Inga edulis*, *I. saltensis*, *Nectandra pichurim*, *Ocotea puberula* and *Tipuana tipu* among others (Rodríguez and Silva 2012).

In 2018, tardigrades were collected from two different sites in each reserve; for Yungas, each site corresponded to an altitudinal level. Three transects separated by more than 300 m were established at each sampling site, for a total of six transects per reserve. In each transect, four trees separated by more than 100 m were chosen and one was used as backup material (*voucher*) for future genetic analysis and/or SEM (Fig. 2). Using an 11 mm diameter punch, samples of lichen

and moss were collected from each tree at approximately chest height (1.3 m). The sample sizes were selected following Rocha et al. (2016) and González-Reyes et al. (2020). The total number of samples taken was 48, of which 12 correspond to *voucher*, and were kept at room temperature in paper envelopes. Of that total, 36 were subsequently hydrated for 42 hours, and adults, molts, and eggs were separated using micropipettes. Individuals were placed in an oven at 60°C to induce asphyxiation before being fixed in 10% formaldehyde that had been neutralised. For microscopic examination, the material was affixed to polyvinyl lactophenol. A Leica DM500 binocular microscope was utilised for the identification of the tardigrades and their eggs. The collected material was classified at the lowest taxonomic level possible (species).

## Microhabitat environmental variables

On the basis of the research of Moly de Peluffo et al. (2006), Peluffo et al. (2007), Guil et al. (2009), González-Reyes et al. (2020), and Ostertag et al. (2022), four microhabitat environmental variables (moisture, temperature, pH, and thickness) that can affect tardigrade communities were considered. Temperature and moisture microhabitat were measured using a HANNA thermo-hygrometer with an external dome; pH was determined using the method proposed by Ostertag et al. (2022); and microhabitat thickness was determined according to the recommendations of González-Reyes et al. (2020) and Ostertag et al. (2022).

## Data analysis

## Alpha diversity: the structure of communities in each reserve

First, the species diversity and total abundance per reserve area (Salta-La Pampa) were recorded. The positivity rate of the samples was used as a metric to
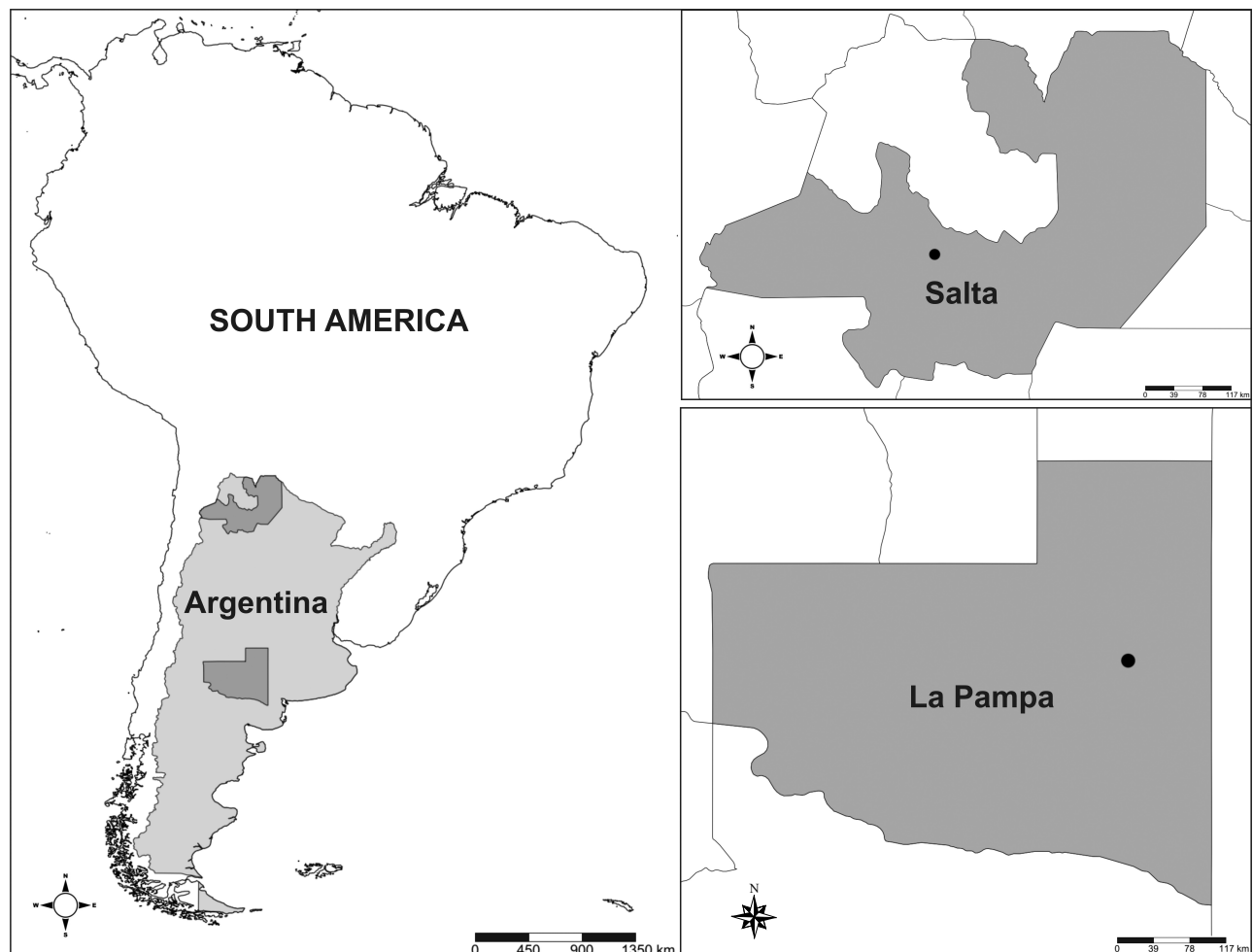


**Fig. 1.** Map of South America showing the two study areas.

compare the distribution of tardigrades in each sampled area (Positivity Rate = (Number of positive samples / Total samples) * 100). Using PAST programme 3.08 (Hammer et al. 2001) and the bootstrapping method (Krebs 1999), we determined if there were statistically significant differences in species richness and abundance values between reserves. For this, we used the abundance and richness values of each sample (18 per reserve) (Table S1). In addition, we utilised a rarefaction-extrapolation analysis based on individuals at the same sample coverage level, with 95% confidence intervals (CIs) and 100 permutations using the iNEXT programme (Hsieh et al. 2013) to compare species richness among reserves and sites within the reserve. This method guarantees that samples are compared with equal completeness, regardless of sample size, allowing for more robust inferences regarding the species richness pattern of the community (Chao and Jost 2012). "True diversity" based on species richness and the effective number of species in each community was also compared. Consequently, three measures of true diversity were considered: $^0D$, the value of which is equivalent to species richness ($^0D = S$) and is insensitive to relative species abundance; $^1D$ (Shannon exponential); and $^2D$ (the inverse of the Simpson index), in which species are regarded as the value of diversity proportionally weighted to community abundance (Hill 1973; Jost 2006 2007; Tuomisto 2010a b 2011; Moreno et al. 2011). In addition, the SPADE programme (Chao and Shen 2010) estimated the true diversity expected (Jost 2006 2007) by utilising the ACE (abundance-based coverage estimator) ($^0D$) estimator for highly heterogeneous communities: CVrare > 0.8 (Chao and Shen 2010), MLEbc (bias-corrected Shannon
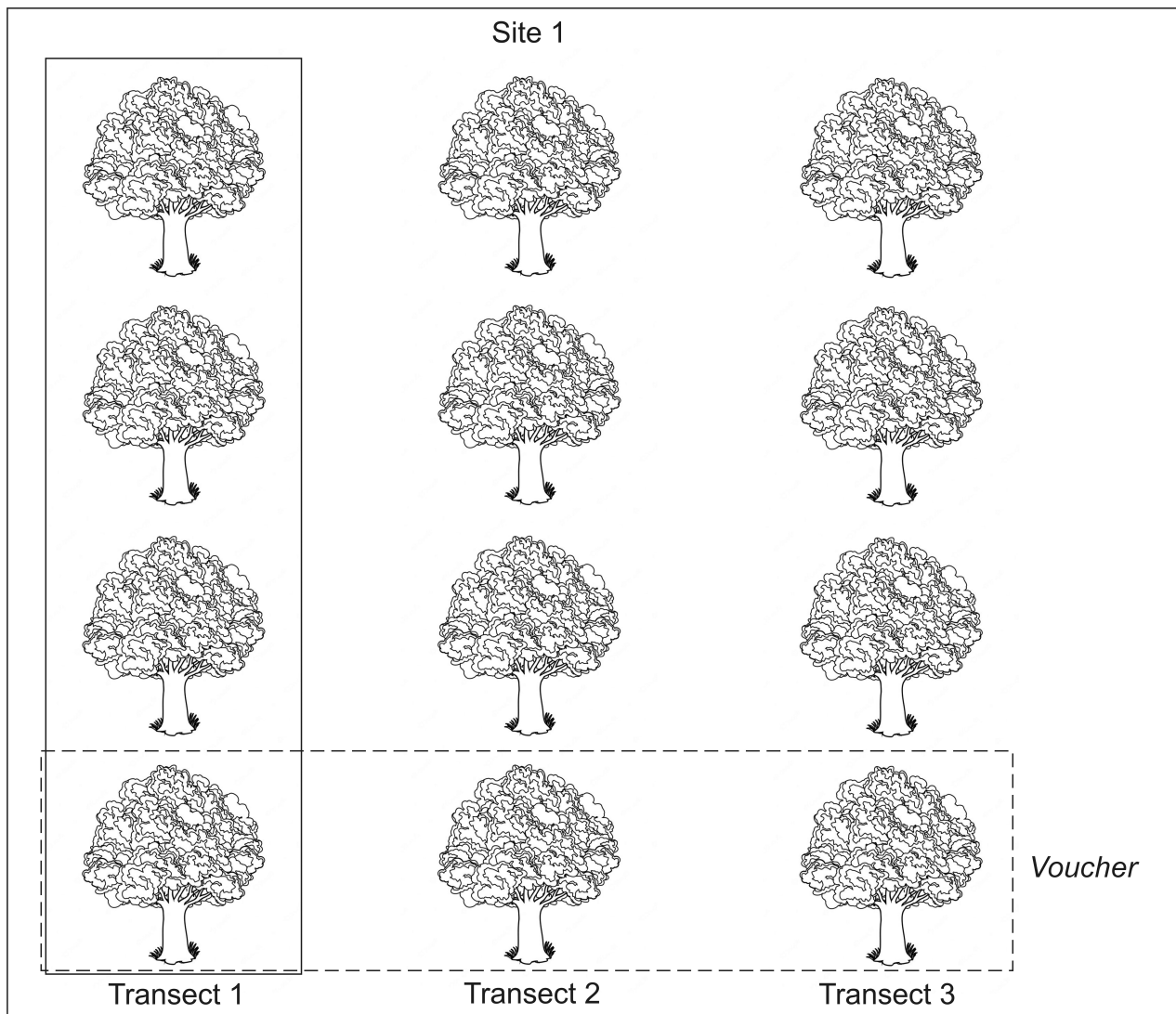


**Fig. 2.** Sampling design scheme: sampling site showing each transect and *voucher*.

diversity estimator) ($^1$D) and MVUE (minimum variance unbiased estimator) ($^2$D) (Moreno et al. 2011). The completeness of the inventory of each site was determined as the ratio between the expected richness (ACE) and the observed species richness. Whittaker abundance rank curves were utilised to compare the community structure of tardigrades in the studied reserves. These graphs illustrate the differences in equitability and species richness (Magurran 2004) and are the most suitable method for illustrating the impact of environmental factors (Jeanneret et al. 2003).

## Analysis of beta diversity and its components

Using PC-ORD 7.04 (McCune and Mefford 2016), we conducted a Detrended Correspondence Analysis (DCA) followed by a Canonical Correlation Analysis (CCA) to determine the degree of association/ similarity of the sets of species from the studied sites and their relationship with the microhabitat variables chosen. Due to the limited number of specimens captured by each sample, a primary matrix with abundance data per transect was utilised as well as a secondary matrix in which sample-level variables were considered (Tables S1, S2). The variables were previously subjected to a Principal Component Analysis (PCA) using the programmes PC-Ord ver. 7.04 (McCune and Mefford 2016) and PAST ver.3.0 (Hammer et al. 2001) in order to evaluate and overcome the problems of autocorrelation between them by employing the correlation matrix. As this is the recommended transformation for environmental variables (McCune and Grace 2002), they were then relativized by their standard deviation (mean = 0, variance = 1). The Multi-Response Permutations Procedure (MRPP) analysis validated the clusters generated by the ordering.

Then, in each reserve, the beta diversity was partitioned into its two components using Sorensen's dissimilarity ($\beta_{SOR}$) to determine its changes, where: $\beta_{SIM}$ represents the dissimilarity between communities due to species turnover, and $\beta_{SNE}$ represents the nesting of assemblages (Baselga 2010). This analysis was performed with the software R (R Core Team 2022), using the package BETAPART (Baselga et al. 2013) (Appendix 1). As an ecological tool, nesting analysis has been used to describe patterns of species presence and the underlying causes of these alterations. In this system, the species composition of small assemblages in nested systems represents a subsample of the species composition of larger assemblages (Ulrich et al. 2009).

## Species indicator

The Indicator Value (IndVal) proposed by Dufrêne and Legendre (1997) was used to identify indicator species or species assemblages in each reserve. This analysis is based on the degree of reserve exclusivity (specificity) and reserve fidelity (frequency of occurrence within the same site). Species with a high indicator value are referred to as "indicator species," while those with intermediate values (50–70%) are referred to as "detector species" (McGeoch et al. 2002). The statistical significance of the indicator was evaluated using the Monte Carlo test and 4999 permutations. The test was conducted using the PC-ORD 7.04 software (McCune and Mefford 2016).

## RESULTS

The Parque Luro Provincial Reserve (PL) has a 94% positivity rate for tardigrades per sample, with 1326 specimens from five registered species; the Municipal Ecological Reserve Polygon A (PA) has a 33% positivity rate, with 212 specimens from nine reported species. Six of the identified species are new to science and will be published soon: two from the genus *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar and Wolf, 2009, two from the genus *Echiniscus* C.A.S. Schultze, 1840, one from the genus *Minibiotus* R.O. Schuster, 1980 (in Schuster et al. 1980) and one from the genus *Ramazzottius* Binda and Pilato, 1986.

*Pseudechiniscus saltensis* Rocha, Doma, González-Reyes and Lisi, 2020, *Minibiotus* sp. nov., *Echiniscus* sp. nov. 1, *Echiniscus* sp. nov. 2, *Doryphoribius cephalogibbosus* Rocha, Doma, González-Reyes and Lisi, 2020, and *Paramacrobiotus* sp. nov. 2 were unique to PA, while *Barbaria* cf. *rufoviridis* (du Bois-Reymond Marcus, 1944) and *Macrobiotus kristenseni* Guidetti, Peluffo, Rocha, Cesari and Moly de Peluffo, 2013 were unique to PL. In both communities, Echiniscidae Thulin, 1928 accounted for more than 67% of the tardigrades identified (PL = 68% and PA = 70.5%) (Table 1). The PA selva montana sites (PAM) had the greatest species richness (S = 7), whereas the PL sites (PL1, PL2) had the lowest (S = 5). In contrast, the abundance of PL2 (N = 1145) was greater than that of the Yungas selva pedemontana sites (PAP) (N = 38). Differences in abundance values between native communities were statistically significant ($p < 0.001$), while differences in species richness were not ($p = 0.586$) (Table 1).

The interpolation-extrapolation analysis revealed that by interpolating the smaller number of individuals (N = 212) and extrapolating twice (N = 424), the PA communities were statistically significantly more diverse than those of PL, with their confidence intervals not overlapping (Fig. 3a). Likewise, among sites pairs,

the PAM community was more diverse than the PAP community, even though they appeared to be similar. In contrast, PL1 and PL2 were differentiated, with PL1 exhibiting greater diversity. Sites diversity exhibited a gradient from the PAP and PAM communities to the PL1 and PL2 communities (Fig. 3b).

PAP was 1.67, 1.42 and 1.05 times more diverse than the PL2, PAM and PL1 communities, respectively considering the 1D values. The completeness of the inventory was good, exceeding 90% (Table 2).

The assemblages of species of the communities of the different ecoregions was different (Fig. 4). Both were strongly dominated by a single species, *B.* cf. *rufoviridis* in PL and *P. saltensis* in PA. *Milnesium pelufforum* Rocha, González-Reyes, Ostertag and Lisi, 2022, *Ramazzottius* sp. nov. and *M. kristenseni* were important in the La Pampa assemblages; while

*Minibiotus* sp. nov., *Paramacrobiotus* sp. nov. 1, *Echiniscus* sp. nov. 1, *D. cephalogibbosus* and *Echiniscus* sp. nov. 2 were prevalent in Salta. A species of the genus *Paramacrobiotus* (*Paramacrobiotus* sp. nov. 1) was not very abundant in PL, but displayed intermediate abundance in Salta (Fig. 4).

## Beta diversity: the relationship between species assemblages and microhabitat environmental variables

The DCA revealed that the first two axes accounted for 50.5% of the total variance observed (35.3% and 15.5%, respectively) and confirmed the gradient organisation of tardigrade assemblages in the studied reserves. The gradient length along axis 1 was 4.2 ($p = 0.01$) with an eigenvalue of 0.88, and

**Table 1.** Species richness and abundance of tardigrades recorded in the Parque Luro Provincial Reserve, the Poligono A Municipal Reserve, and between the sites analysed in each reserve

| Family | Species | PL | | PA | |
|---|---|---|---|---|---|
| | | PL1 | PL2 | PAM | PAP |
| Doryphoribiidae | *Doryphoribius cephalogibbosus* Rocha, Doma, González-Reyes, Lisi, 2020 | 0 | 0 | 3 | 3 |
| Total family | | **0** | | **6** | |
| Echiniscidae | *Barbaria* cf. *rufoviridis* (du Bois-Reymond Marcus, 1944) | 88 | 820 | 0 | 0 |
| | *Echiniscus* C.A.S. Schultze, 1840 | | | | |
| | *Echiniscus* sp. nov. 1 | 0 | 0 | 0 | 8 |
| | *Echiniscus* sp. nov. 2 | 0 | 0 | 6 | 0 |
| | *Pseudechiniscus saltensis* Rocha, Doma, González-Reyes, Lisi, 2020 | 0 | 0 | 117 | 19 |
| Total family | | **908** | | **150** | |
| Macrobiotidae | *Macrobiotus kristenseni* Guidetti, Peluffo, Rocha, Cesari, Moly de Peluffo, 2013 | 28 | 64 | 0 | 0 |
| | *Minibiotus* R.O. Schuster, 1980 | | | | |
| | *Minibiotus* sp. nov. | 0 | 0 | 34 | 2 |
| | *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar, Wolf, 2009 | | | | |
| | *Paramacrobiotus* sp. nov. 1 | 6 | 1 | 10 | 5 |
| | *Paramacrobiotus* sp. nov. 2 | 0 | 0 | 0 | 1 |
| Total family | | **99** | | **52** | |
| Milnesiidae | *Milnesium pelufforum* Rocha, González-Reyes, Ostertag, Lisi, 2022 | 23 | 187 | 1 | 0 |
| Total family | | **210** | | **1** | |
| Ramazzottiidae | *Ramazzottius* sp. nov. (Doyère, 1840) | 36 | 73 | 3 | 0 |
| Total family | | **109** | | **3** | |
| Total abundance | | **1326** | | **212** | |

PL = Parque Luro Provincial Reserve; PL1 = Parque Luro Provincial Reserve site 1; PL2 = Parque Luro Provincial Reserve site 2; PA = Poligono A Municipal Reserve; PAM = selva montana of Poligono A Municipal Reserve; PAP = selva pedemontana of Poligono A Municipal Reserve.
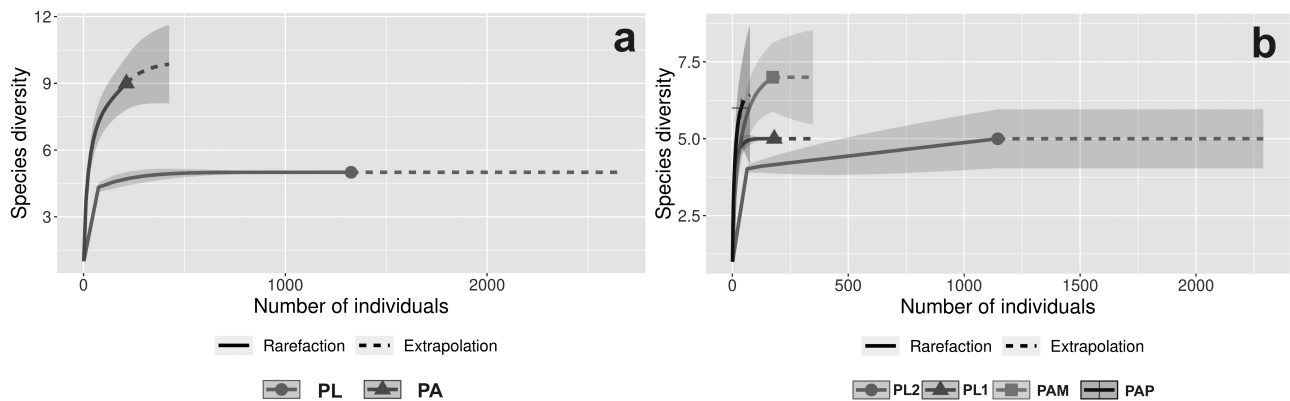
**Fig. 3.** Rarefaction curves based on individuals comparing: a- natural sites of Parque Luro Provincial Reserve (PL) and Poligono A Municipal Reserve (PA); and b- between Parque Luro Provincial Reserve 1 (PL1) and 2 (PL2), selva montana of Poligono A Municipal Reserve (PAM) and selva pedemontana of Poligono A Municipal Reserve (PAP).
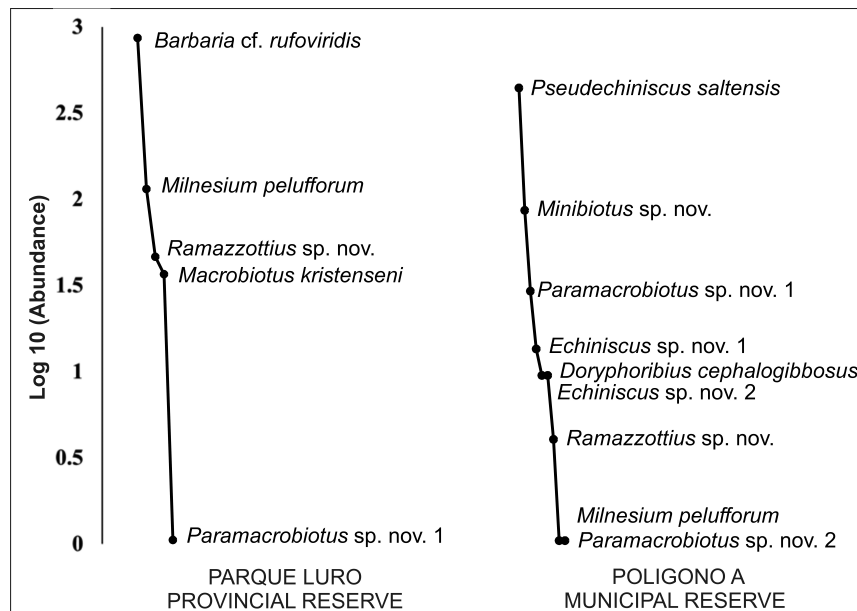


**Fig. 4.** Whittaker curves showing the rank-abundance relationship of the species registered in the native sites of the province of La Pampa (Parque Luro Provincial Reserve) and Salta (Poligono A Municipal Reserve).

**Table 2.** Observed and expected values of true diversity ($^0D$, $^1D$, $^2D$), coefficients of variation and percentage of inventory completeness

| Site | Observed Diversity | | | Estimated Diversity | | | |
|---|---|---|---|---|---|---|---|
| | $^0D$ (species richness) | $^1D$ (Shannon exponential) | $^2D$ (Inverse of Simpson Index) | $^0D$ (ACE) | $^1D$ (MLEbc) | $^2D$ (MVUE) | % of inventory completion |
| PAM | 7 | 2.82 | 2.02 | 7.60 | 2.87 ± 0.77 | 2.03 ± 0.42 | 92.10 |
| PAP | 6 | 4.02 | 3.11 | 6.60 | 4.33 ± 0.75 | 3.30 ± 0.23 | 90.90 |
| PL1 | 5 | 3.80 | 3.15 | 5.00 | 3.84 ± 0.19 | 3.19 ± 0.16 | 100.00 |
| PL2 | 5 | 2.41 | 1.83 | 5.00 | 2.40 ± 0.07 | 1.83 ± 1.16 | 100.00 |

PAM = selva montana of the Poligono A Municipal Reserve; PAP = selva pedemontana of the Poligono A Municipal Reserve; PL1 = Parque Luro Provincial Reserve site 1; PL2 = Parque Luro Provincial Reserve site 2; ACE: abundance-based coverage estimator; MLEbc: bias-corrected Shannon diversity estimator; MVUE: minimum variance unbiased estimator.

0.28 along axis 2. The CCA analysis subsequently explained 35% of the total variance along the first axis ($p = 0.001$) and 26% along the second axis (Fig. 5). The first eigenvalue was 0.88 while the second was 0.28. After sorting, the MRPP analysis revealed statistically significant differences between all sites compared to A = 0.216 ($p = 0.004$); however, there were no statistically significant differences between sites in PL and PA (A = -0.014; $p = 0.519$). Moisture (r = 0.805) and temperature (r = 0.543) were negatively correlated along the first axis, whereas microhabitat thickness (r = 0.461) was positively correlated and pH (r = 0.089)

did not show any correlation (Fig. 5).

### Partition of Beta Diversity

The partitioning of β diversity into its two components, species turnover ($\beta_{SIM}$) and nestedness ($\beta_{SNE}$) revealed that their proportions differ between the PL and PA sites. These differences in species assemblage composition between sites were primarily due to species turnover ($\beta_{SIM} = 71.1\%$) within the PA, while nestedness ($\beta_{SNE} = 100\%$) was observed in PL assemblies. In turn, there was both turnover (59.9%) and nestedness (40.1%)
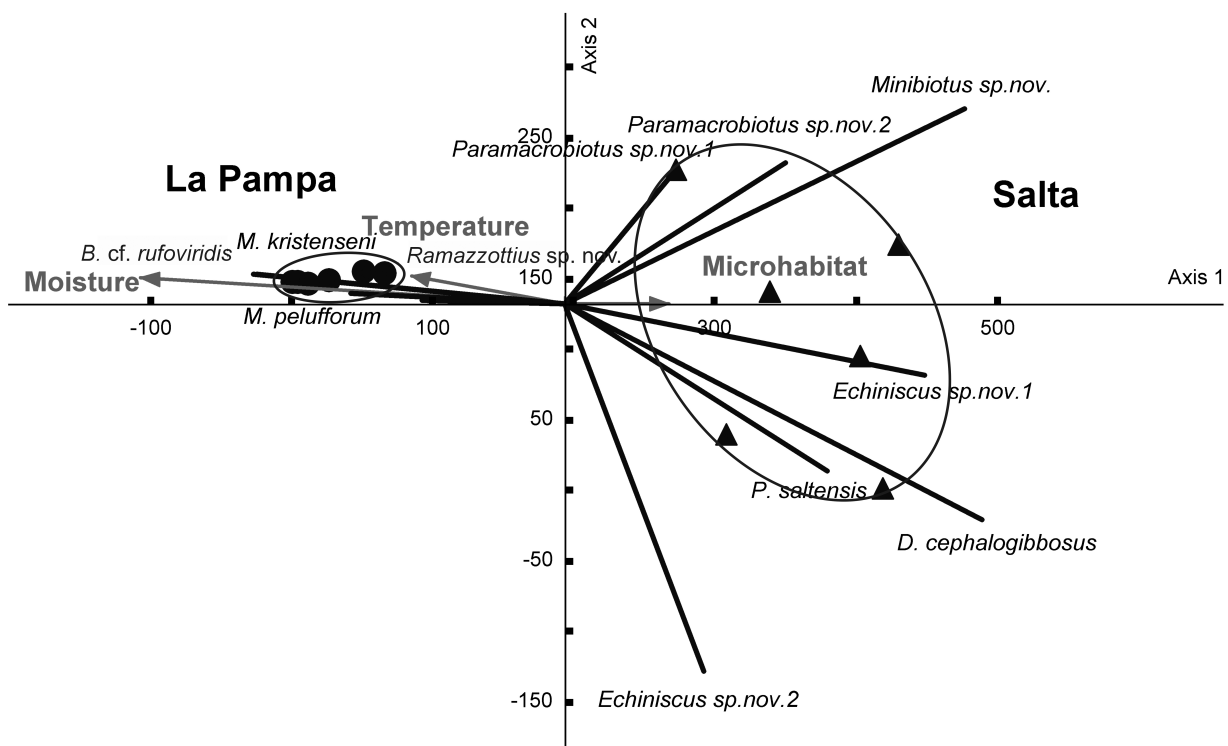


**Fig. 5.** Ordering by CCA analysis of the 12 sampling transects in the natural sites of the province of La Pampa (six circles) and Salta (six triangles), showing a general pattern of relationship with microhabitat environmental variables.

**Table 3.** Values of beta diversity in the Parque Luro Provincial Reserve and in the Poligono A Municipal Reserve and between the sites analysed

| Site | $\beta_{SIM}$ | % | $\beta_{SNE}$ | % | $\beta_{SOR}$ |
|---|---|---|---|---|---|
| PL | 0.00 | - | 0.17 | 100.00 | 0.17 |
| PA | 0.50 | 71.10 | 0.20 | 28.80 | 0.70 |
| PL vs PA | 0.40 | 69.90 | 0.17 | 30.00 | 0.57 |
| PAM | 0.40 | 59.90 | 0.27 | 40.10 | 0.67 |
| PAP | 0.33 | 59.90 | 0.22 | 40.10 | 0.56 |
| PAM vs PAP | 0.33 | 86.60 | 0.05 | 13.30 | 0.38 |

PL= Parque Luro Provincial Reserve; PA= Poligono A Municipal Reserve; PAM= selva montana of Poligono A Municipal Reserve; PAP= selva pedemontana of Poligono A Municipal Reserve; $\beta_{SIM}$ = dissimilitude due to species turnover between communities; $\beta_{SNE}$ = nesting of the assemblages; $\beta_{SOR}$ = beta diversity.

in the PAM and PAP assemblages (Table 3).

## Indicator species of each reserve

IndVal analysis revealed four tardigrade species for La Pampa (*B.* cf. *rufoviridis*, *M. kristenseni*, *Ramazzottius* sp. nov., *M. pelufforum* and one for Salta communities (*P. saltensis*) (Table 4).

## DISCUSSION

Studies on tardigrade diversity patterns in Argentina have focused primarily on urban areas (Moly de Peluffo et al. 2006; Peluffo et al. 2007; Rocha et al. 2016, 2020; González-Reyes et al. 2020; Ostertag et al. 2022). In contrast, there are few studies for the native areas of Argentina (Rocha et al. 2016), and only for the province of Salta; this is the first contribution to protected native areas of La Pampa. Similar to the findings of Zawierucha et al. (2015), we found a notably high percentage of positive samples in La Pampa province (94%), but a much lower percentage in the Yungas (33%). This leads us to believe that the native environments of the Espinal of La Pampa still exhibit greater uniformity in their environmental conditions on a local scale, thereby increasing the likelihood of locating tardigrades. In contrast, in the Yungas, the elevational gradient, changes in the heterogeneity of the habitat, and the pronounced climatic seasonality would favor a patchy spatial distribution of tardigrade communities (Zawierucha et al. 2015).

Herbivorous species dominate the community structure of the analysed native habitats (*Doryphoribius cephalogibbosus*, *Echiniscus* sp. nov. 1, *Echiniscus* sp. nov. 2, *Macrobiotus kristenseni*, *Minibiotus* sp. nov., *Pseudechiniscus saltensis*, *Ramazzottius* sp. nov. and *Barbaria* cf. *rufoviridis*). Some authors suggest that the possible decline of predatory species may be attributable to competition with other invertebrate predators (Vecchi et al. 2022). The trophic structure of the native communities in Argentina was distinct from that of the urban communities (González-Reyes et al. 2020; Ostertag et al. 2022).

In this study, the most dominant species belong to the class Heterotardigrada, specifically *B.* cf. *rufoviridis* in Parque Luro and *P. saltensis* in Poligono A, both of which have been identified as herbivores (Nelson et al. 2018) and appear to play a key role in the organisation of the remaining herbivorous species in the community. According to Vecchi et al. (2022), an increase in primary productivity could affect food production (*e.g.*, algae), thereby promoting the growth and reproduction of herbivorous species. *B.* cf. *rufoviridis* is a widespread species, documented in Argentina and Brazil (Kaczmarek et al. 2015), including urban, suburban, rural, and native Argentine environments (Peluffo et al. 2002 2007; Moly de Peluffo et al. 2006; Rocha et al. 2016 2023; González-Reyes et al. 2020; Ostertag et al. 2022). It has been documented in the urban communities of Salta, but not in the same habitats (González-Reyes et al. 2020), but not in the same habitats as *P. saltensis*. This leads us to believe that they may be potential competitors. *B.* cf. *rufoviridis* is a primary consumer and could be food for predatory species such as members of the genus *Milnesium* (Nelson et al. 2020). It could also play a functionally similar role to *R. oberhaeuseri*, a euryplastic species that is present in the city of Santa Rosa (Ostertag et al. 2022) due to its resistance to extreme urban conditions (Séméria 1981 1982; Meininger et al. 1985; Steiner 1994a b).

Other heterotardigrade species species belong to the genus *Echiniscus*. Due to their tolerance for dry conditions, species of this genus are known to be abundant in tree bark (Ito 1999). This may explain their increased representation in this study. Similarly, the presence of dorsal plates may reduce their capture ability and their palatability to predators. Their presence in PA can be explained by the fact that they are possibly

**Table 4.** Results of the IndVal analysis of indicator species for the two reserves, with their indicator values and *p* values, from the province of La Pampa (Parque Luro Provincial Reserve) and Salta (Poligono A Municipal Reserve), Argentina

| Reserve | Species | Indicator value | *p* |
|---|---|---|---|
| Parque Luro Provincial Reserve | *Macrobiotus kristenseni* | 100.00 | 0.0034** |
| | *Barbaria* cf. *rufoviridis* | 100.00 | 0.0034** |
| | *Milnesium pelufforum* | 99.50 | 0.0034** |
| | *Ramazzottius* sp. nov. | 97.30 | 0.0034** |
| Poligono A Municipal Reserve | *Pseudechiniscus saltensis* | 83.30 | 0.0138* |

** $p \leq 0.01$; * $p \leq 0.05$.

relic species, phylogenetically isolated, with restricted geographical distributions, and therefore qualify as stenothermic tardigrades (Gąsiorek 2022).

In general, eutardigrades are more diverse than heterotardigrades in cities (del Barco-Trillo 2019) and native areas (Guil et al. 2009; Rocha et al. 2016; Nelson et al. 2018), as evidenced by our findings in the analysed areas. The eutardigrades are primarily semi-terrestrial and limnic, whereas the heterotardigrades contain marine and semi-terrestrial species (Nelson et al. 2018). Among eutardigrades, *M. kristenseni* has only been reported twice by Guidetti et al. (2013) and by Ostertag et al. (2022) in the province of La Pampa, where it continues to be endemic. *Milnesium* Doyère 1840, however, is a common genus in native communities and diverse in urban ones (Moly de Peluffo et al. 2006; Peluffo et al. 2007; Vicente et al. 2013; Rocha et al. 2016 2022; Roszkowska et al. 2016a; González-Reyes et al. 2020; Ostertag et al. 2022). In this study, only *Milnesium pelufforum* was represented for native areas. This can be attributed to the preference of *M. pelufforum* for homogeneous, xeric, and more stable environments (temperature, moisture) in contrast to *Paramacrobiotus* sp. nov. 1, which prefers habitats with thicker microhabitats, such as the Yungas. Both genera exhibited antagonistic behaviours but played comparable trophic roles. Some species of *Paramacrobiotus* are known to feed on metazoans, as well as cyanobacteria, algae, and fungi; however, *Milnesium* typically feeds on whole prey, in addition to algae and fungal cells (Roszkowska et al. 2016b; Bryndová et al. 2020; Morek et al. 2020).

In the ecoregion of the Yungas, tardigrades are more diverse but less abundant than in the Espinal, as revealed by the diversity patterns observed here. This is due to a necessary temperature and humidity gradient for the development of La Pampa's native communities. On the contrary, it appears that the thickness of the microhabitat is crucial for the conservation of the diversity of Salta's tardigrade communities. The marked climatic seasonality in the Yungas could also result in a more heterogeneous environment. Different authors argue that heterogeneous environments promote communities with greater beta diversity (Anderson et al. 2006), thereby reducing nesting and species loss.

We were unable to demonstrate the effect of altitude on diversity in the Yungas in this study, and there is currently no consensus regarding the effect of altitude on tardigrade diversity (Zawierucha et al. 2015). It is known that species richness decreases with increasing altitude (Utsugi et al. 1997; Collins and Bateman 2001), and this may be the case given that the level of the Yungas at a lower altitude (between 400 and 900 m a.s.l.) had a greater abundance and richness of tardigrade species.

## CONCLUSIONS

Finally, we can conclude that the patterns of species diversity and community structures in the pristine areas of the two Argentine provinces differ from those previously observed in urban areas. If we consider the latitudinal differences observed in this study, the climate is not the only explanation for the assemblages of tardigrades; other factors, such as the evolutionary history of the species, the type of substrate, the altitude, and the specific characteristics of each habitat, could model these communities in a synergistic manner (Dastych 1987 1988; Beasley 1988; Schuster and Greven 2007 2013; Guil et al. 2009; Kaczmarek et al. 2011; Young et al. 2018). Taking into account current historical and ecological factors that have led or are leading both directly and indirectly to the assembly of tardigrade communities in natural areas, there is unquestionably much to investigate and elucidate to comprehend the structuring of tardigrade communities in natural areas. It is necessary to investigate the communities of tardigrades that inhabit natural environments.

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## Supplementary Materials

**Table S1.** Tardigrade abundance per species by sample. (download)

**Table S2.** Data of the variables collected by sample. (download)

**Appendix 1.** Script software R. (download)