

Characterization of limnoterrestrial Tardigrades (Tardigrada) in some locations in Caracas, Venezuela

Caracterización de los Tardígrados (Tardigrada) limnoterrestres en algunas localidades de Caracas, Venezuela

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Abstract

The objective of this research was to characterize and identify the tardigrade genera present in limnoterrestrial communities in four locations in the Gran Caracas (Sebucán, San Bernardino, Facultad de Ciencias UCV, and Guatire) and to evaluate their distribution and diversity in urban environments. Samples of mosses and lichens were collected from different substrates, processed by controlled hydration, and filtered with graduated sieves to concentrate the specimens. Taxonomic identification was performed under an optical microscope (100–320x) using morphological criteria of claws, cuticle, and bucco-pharyngeal apparatus, following the systematic arrangement of (Pilato and Binda, 2010) and the updated species list (Degma et al., 2021). A total of 670 individuals were recorded, of which 240 were identified to genus level, grouped into the classes Eutardigrada and Heterotardigrada, with 15 genera belonging to seven families. Sebucán and the Faculty of Sciences had the highest richness (14 genera each), while San Bernardino had the lowest (7 genera), showing a pattern of diversity associated with local microclimatic conditions. The beta diversity index ($\beta = 1.2$) indicated low turnover between communities, and the Jaccard index reflected high similarity between Sebucán and the Faculty of Sciences ($\approx 87\%$). Twelve new genera were recorded for Venezuela, doubling the known national diversity and consolidating the country as one of the most diverse in Tardigrades of the Neotropics. The results support the urban ecology hypothesis: urbanization reduces taxonomic richness and promotes communities dominated by resistant genera such as Minibiotus and Minilentus.

Keywords: Tardigrada; Eutardigrada; Heterotardigrada; cryptobiosis; limnoterrestrial organisms.

Resumen

El objetivo de esta investigación fue caracterizar e identificar los géneros de tardígrados presentes en comunidades limnoterrestres de cuatro localidades (Sebucán, San Bernardino, Facultad de Ciencias UCV y Guatire) de la Gran Caracas, Venezuela, y evaluar su distribución y diversidad en ambientes urbanos. Las muestras de musgos y líquenes fueron recolectadas de distintos sustratos, procesadas mediante hidratación controlada y filtradas con tamices graduados para concentrar los ejemplares. La identificación taxonómica se realizó bajo microscopio óptico (100–320x) utilizando criterios morfológicos de garras, cutícula y aparato bucofaríngeo, siguiendo el arreglo sistemático (Pilato y Binda, 2010), así como la lista actualizada de especies (Degma et al., 2021). Se registraron 670 individuos, de los cuales 240 fueron identificados hasta el nivel de género, agrupados en las clases Eutardigrada y Heterotardigrada, con 15 géneros pertenecientes a siete familias. Los sitios de Sebucán y la Facultad de Ciencias presentaron la mayor riqueza (14 géneros cada uno), mientras que San Bernardino mostró la menor (7 géneros), evidenciando un patrón de diversidad asociado a las condiciones microclimáticas locales. El índice de diversidad beta ($\beta = 1,2$) indicó bajo recambio entre comunidades, y el índice de Jaccard reflejó alta similitud entre Sebucán y la Facultad de Ciencias ($\approx 87\%$). Se registraron 12 nuevos géneros para Venezuela, duplicando la riqueza nacional conocida y consolidando al país como uno de los más diversos en Tardígrados del Neotrópico. Los resultados apoyan la hipótesis de la ecología urbana: la urbanización reduce la riqueza taxonómica y promueve comunidades dominadas por géneros resistentes como Minibiotus y Minilentus.

Palabras clave: Tardigrada; Eutardigrada; Heterotardigrada; criptobiosis; organismos limnoterrestres.

1. Introduction

Tardigrades (from the Latin *tardigradus*, meaning “slow-moving”), a term coined by Spallanzani in 1776 (Harmunt., 2018) and commonly known as water bears, are microscopic (50–1,200 µm) bilaterally symmetrical, protostome, and schizocoelous animals. Despite their small size, they possess a highly complex morphology and anatomy (León-Espinosa., 2018). A unique characteristic of this group is their ability to enter cryptobiosis, a state of dormancy that allows them to colonize a wide variety of microhabitats through the passive dispersal of cryptobionts, eggs, and active adults, withstanding extreme conditions such as high or sub-zero temperatures, desiccation, hypoxia, osmotic stress, and even vacuum and space radiation. For this reason, tardigrades are considered extremophile organisms (Nelson., 2018; Muñoz and Capote., 2019).

The phylogenetic status of tardigrades has been the subject of extensive debate. They are currently placed within the superphylum Ecdysozoa, which groups organisms that shed their cuticle in a process called ecdysis (Aguinaldo et al., 1997). It is believed that tardigrades may have originated in a marine environment before colonizing limnic and terrestrial habitats (Edgecombe., 2010). Historically, they were linked to arthropods or nematodes due to morphological similarities such as the triradial pharynx or anhydrobiosis (Kinchin., 1994). However, modern molecular analyses confirm their inclusion in Ecdysozoa alongside nematodes (Borner et al., 2014).

Within this context, various phylogenetic hypotheses have been proposed. The Tactopoda hypothesis (Budd., 2001) posits Arthropoda and Tardigrada as sister groups, based on structural similarities such as the cuticle, the ganglionated ventral nerve cord, and the presence of a trilobate brain (Telford et al., 2008; Persson et al., 2014). In contrast, the Lobopodia hypothesis (Nielsen., 2012) places them as a sister group to a clade comprising Arthropoda and Onychophora, supported by recent molecular analyses (Campbell et al., 2011). Although there is no definitive consensus, both hypotheses acknowledge the evolutionary affinity of tardigrades with panarthropods.

Various experiments have been conducted to assess the survival of tardigrades. *Hypsibius exemplaris* possesses genes capable of detecting ionizing radiation, maintaining DNA integrity, and constantly triggering the repair of genetic material (Allicahuaman-Huayua., 2024).

Currently, the phylum Tardigrada consists of the classes Heterotardigrada, Mesotardigrada, and Eutardigrada. The class Mesotardigrada is monotypic and is based on the species *Thermozodium esakii*, discovered in hot springs in Japan; this species is now considered doubtful due to the loss of specimens of that genus following a seismic event (León-

Espinosa., 2018). The Heterotardigrada include marine, coastal, and semi-terrestrial species grouped into the orders Arthrotardigrada and Echiniscoidea. The former has been described as a paraphyletic group based on molecular data (Jørgensen et al., 2010; Guil and Giribet., 2012), while the latter is considered monophyletic (Nichols et al., 2006). For their part, the Eutardigrada, predominant in limnoterrestrial environments, comprise the orders Apochela and Parachela (Schuster et al., 1980), and their monophyly has been confirmed by genetic analyses. Recent molecular studies also suggest that Eutardigrada and Heterotardigrada are sister groups within the phylum (Nelson and Rebecchi., 2010).

Morphologically, tardigrades have elongated bodies and bilateral symmetry, with four pairs of lobopodial legs ending in claws (Figure 1). The body is divided into five “segments”: one cephalic, three trunk segments, and one caudal segment, with sizes ranging from 50 to 1200 µm, exceptionally reaching 2 mm (Nelson and Adkins., 2001). The mouth may be frontal or subventral, and the legs are located in a lateroventral position. Heterotardigrada possess sensory appendages (cirri, papillae, and dorsal or lateral spines), whereas Eutardigrada lack them, except for some lateral papillae in the order Apochela (León-Espinosa., 2018).



Figure 1. Morphological diversity of tardigrades (Møbjerg et al., 2018). a) Eutardigrade in active state. b) Eutardigrade in cryptobiosis. c) Heterotardigrade in active state. d) Heterotardigrade in cryptobiosis.

The taxonomy of the group is based on the morphology of sclerotized structures (Nelson and Rebecchi., 2010):

The claws (Figure 2) are of great diagnostic value. In Heterotardigrada, four symmetrical claws may be smooth or have a ventral spur (Møbjerg et al., 2018); in Eutardigrada, the claws form diplo claws joined in pairs (León-Espinosa., 2018).

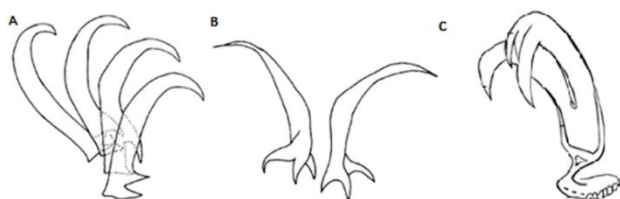


Figure 2. Typical morphology of claws in (a) a specimen of the Echiniscidae family. (b) a specimen of the Carphanidae family. (c) A typical eutardigrade claw. (Nelson y col., 2020).

The cuticle (Figure 3) is chitinous and permeable, consisting of an epicuticle, an intracuticle, and a procuticle. Heterotardigrada exhibit dorsal plates and ornamentation, whereas Eutardigrada have smooth or porous cuticles and are referred to as “naked tardigrades” (León-Espinosa., 2018).

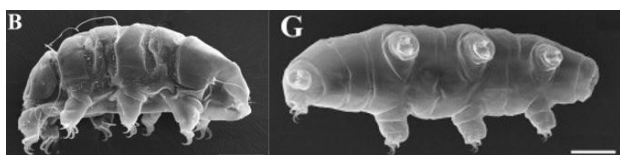


Figure 3. Electron micrographs of tardigrades: (B) *Echiniscus* sp. showing cuticular plates, spines, and filaments (Heterotardigrade); (G) *Paramacrobiotus* (Eutardigrade).

The buccopharyngeal apparatus (Figure 4) has a complex feeding system consisting of a buccal tube, stylet, and muscular pharynx, with morphological variations that reflect different feeding habits: carnivorous, herbivorous, or microbivorous (Pilato and Binda, 2010; León-Espinosa., 2018).

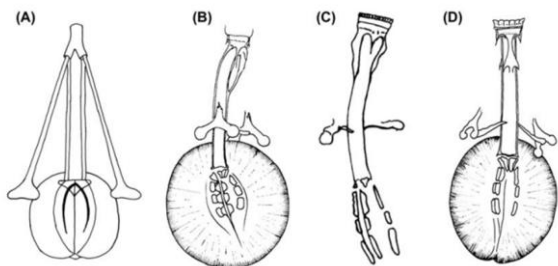


Figure 4. Diagram of the buccopharyngeal apparatus. (León-Espinosa, 2018).

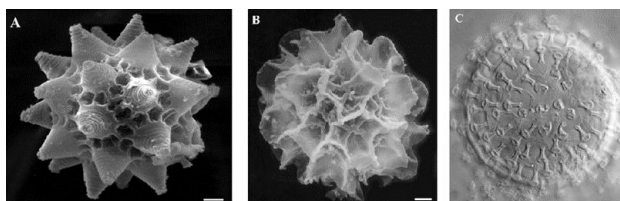


Figure 5. Electron micrographs of tardigrade eggs: (B) *Echiniscus* sp. showing cuticular plates, spines, and filaments (Heterotardigrade); (G) *Paramacrobiotus* (Eutardigrade).

Eggs (Figure 5) are structures used for taxonomic identification, particularly in Eutardigrada, featuring specific

ornamentations (pores, reticulations, processes) and ranging in size from 50 to 235 μm (Bertolani and Rebecchi., 1993).

In terms of their internal anatomy, tardigrades lack circulatory and respiratory systems; their body cavity is filled with fluid and bounded by the integument (Møbjerg., 2018). The digestive system is divided into anterior, middle, and posterior intestines, with a cloaca in Eutardigrada and a pre-anal gonopore in Heterotardigrada. The nervous system is of the “ladder” type, with four pairs of ventral ganglia connected longitudinally (Schultze et al., 2014). They possess a single dorsal gonad suspended by ligaments (Bertolani., 2001) and exhibit both gonochoric and hermaphroditic reproduction. Fertilization can be external, within the exuvia, or internal via copulation (Jørgensen et al., 1999). In the face of extreme environmental conditions, tardigrades exhibit adaptive mechanisms that include cryptobiosis, osmoregulation, diapause, and encystment (Guidetti and Møbjerg., 2018). Cryptobiosis is a reversible ametabolic state induced by environmental stress and comprises several types:

Anhydrobiosis, characterized by the almost complete loss of water and the formation of a “barrel-shaped” structure with suspended metabolism, from which the organism can recover upon rehydration (Guidetti and Møbjerg., 2018). Cryobiosis, which allows organisms to survive at temperatures below $-196\text{ }^{\circ}\text{C}$ thanks to proteins that inhibit the formation of ice crystals (Nelson and Rebecchi., 2010). Osmobiosis, a response to high osmotic pressures in marine and limnoterrestrial euryhaline species (Nelson et al., 2010). Anoxibiosis, induced by the absence of oxygen; the organism becomes immobile and rigid, able to survive from hours to several days (Nelson and Rebecchi., 2010). Encystment, the formation of oval cysts with a thickened cuticle and reduced metabolism, capable of surviving for months without food (Nelson and Rebecchi., 2010).

These mechanisms, together with the ancestral cryptobiosis present in all lineages (Hygum et al., 2016), explain the extraordinary resistance of tardigrades to desiccation, extreme temperatures, hypoxia, and radiation, establishing the phylum as one of the most resilient animal groups on the planet.

The overall objective of this study is to characterize and identify, down to the lowest possible taxonomic level, the tardigrades present in limnoterrestrial samples collected at various locations in the Capital Region and the state of Miranda. To this end, the previously described taxonomic, morphological, and ecological aspects will be integrated, applying a detailed analysis of sclerotized structures such as claws, cuticle, buccopharyngeal apparatus, and eggs, which are diagnostic characteristics of the species. The research will not only seek to characterize local diversity but will also provide valuable data on the distribution of these microorganisms in specific

habitats in Venezuela, laying the groundwork for the characterization and understanding of the biogeographic and ecological patterns of this phylum in certain locations within the Gran Caracas.

2 Materials and Methods

The study area was the Capital Region of Venezuela, which consists of the Capital District and the states of Miranda and La Guaira. The Capital District, in turn, comprises the Libertador municipality and four municipalities in the state of Miranda: Baruta, Chacao, El Hatillo, and Sucre. The terrain is mountainous, with the Caracas Valley (the largest) bounded to the north by the Cordillera de la Costa (Waraira Repano National Park or El Ávila) and to the south by the Cordillera del Interior, the former being the higher of the two (maximum elevation: Pico Naiguatá at 2,765 m above sea level). The climate is highland tropical, with an average annual temperature of 21.6 °C and annual precipitation ranging from 900 to 1,300 mm in the Caracas Valley. In higher areas, above 1,500 m a.s.l., rainfall may be higher (>2,000 mm) due to orographic rainfall that concentrates clouds at these altitudes (INAMEH, 2013). Cloud forests form on the high peaks; at lower thermal levels, between 800 and 1,500 m a.s.l., the predominant natural vegetation is semideciduous forest and xerophytic forest on the northern slope facing the coast (Steyermark and Huber, 1978).

The locations where the sampling was conducted were the San Bernardino neighborhood in the Libertador Municipality at 10°30'49"N -66°53'55"W, the Sebucán neighborhood in the Municipality of Sucre 10°30'05"N -66°50'11"W, and the UCV Faculty of Sciences in Las Tres Gracias 10°29'13"N -66°53'41"W, and the Villa Hermosa Residential Complex located in the city of Guatire (10°28'48"N -66°33'19"W).

Sampling sites in terrestrial habitats were selected based on the visual presence of conspicuous moss and lichen communities. At these sites, all substrates were sampled (sampling units: living and dead trees, rocks, and man-made structures). Samples of moss and/or lichen mats were collected by scraping the substrate surface with a spatula. In other locations where the substrate was continuous (such as a wall), samples were collected systematically across the surface.

The collected samples were immediately placed in pre-labeled paper bags, allowing the plant material to dry slowly and naturally. In this way, the tardigrades had sufficient time to enter an anhydrobiotic state.

The collection and processing of the tardigrades were carried out in several stages.

The Degma (2010) method was used to concentrate the

sample:

A small fragment of the sample (approximately 5 x 5 cm) was taken and submerged in filtered water to prevent any possible contamination. The sample was then refrigerated for 24 hours to allow the tardigrades sufficient time to emerge from their anhydrobiotic state. The hydrated sample was then placed in a Petri dish, where entomological forceps were used to mechanically break up the plant material, with the aim of hydrating all specimens, eggs, and exuviae. The broken-up sample was transferred to a beaker, where more water was added, and each fragment was vigorously stirred with glass rods to dislodge as many organisms as possible from the plant material.

Subsequently, each sample was placed on top of a series of sieves graded from largest to smallest mesh size (0.6 to 0.3 mm). After shaking the set of sieves, the wash water was collected in another beaker. The container was then rinsed again with more distilled water. Next, the material remaining in the sieves was rinsed with plenty of distilled water, concentrating the tardigrades in the finest sieve.

Tardigrades, their eggs, and exuviae were collected from the concentrate as described below:

The contents of the finest sieve were placed in a Petri dish using a pipette filled with distilled water, leaving only a thin film of sediment. Subsequently, using a dropper, the specimens were transferred to a Boerner-style perforated plate with 10 wells or compartments (each 1.5 cm in diameter), to which a small amount of distilled water was added. The area surrounding the specimens was cleared using entomological pins. In this way, the tardigrades could be observed under a Wild-Heerbrugg M5A stereoscopic microscope (20-40x). The tardigrades found in each well were extracted using a pair of entomological needles and placed in Eppendorf tubes, to which a few drops of glacial acetic acid had previously been added. This was done to organize, straighten, and fix the organisms within a few minutes.

The specimens were processed using the systematic arrangement (Pilato and Binda, 2010) cross-referenced with the "Current Checklist of Tardigrada Species" (Degma et al., 2021). Individuals were identified to the taxonomic rank of the genus using a Leica DMIL inverted optical microscope (100-320x). The diagrams and descriptions available in the most recently published literature (Degma et al., 2021) were used.

With regard to community parameters, the genera present at the different sites were first recorded in a presence/absence table. The total number of genera (richness) per site was also determined.

Alpha (average local richness), beta (replacement), and

gamma (total or global richness) diversities were calculated (Ricklefs and Miller., 2000). Beta diversity (β) was obtained from the ratio of gamma diversity (γ) to alpha diversity (α):

$$\gamma = \alpha \times \beta \Rightarrow \beta = \frac{\gamma}{\alpha}$$

The Jaccard index (I_j) was used to measure the similarity between the communities at two sampling sites, regardless of the taxonomic composition of both sites (Real and Vargas., 1996). The equation is expressed as follows:

$$I_j = \frac{c}{a + b - c}$$

Where:

- a: the number of species present at site A.
- b: the number of species present at site B.
- c: the number of species present at both sites, A and B.

This index ranges from 0 to 1, representing environments that are completely dissimilar and completely similar to one another, respectively. The organisms were counted and reported in absolute and relative frequencies (p_i). The latter were expressed as percentages obtained using the following equation:

$$\%p_i = \frac{n_i}{N} \times 100$$

Where n_i is the total number of individuals in a genus and N is the total number of individuals across all genera. Relative abundances were plotted as stacked bar charts. These data were plotted as bar charts.

3 Results and Discussion

The study of tardigrades at four locations (the Sebuacán residence, the San Bernardino residence, the UCV Faculty of Sciences, and the Villa Hermosa residential complex in Guatire) resulted in the collection of 670 individuals in total, constituting a significant quantitative database for a study of mesofauna in urban environments. The distribution of specimens was notably uneven across the areas, suggesting the existence of contrasting microenvironmental conditions. Sebuacán stood out with the highest concentration, contributing 270 individuals (40.3% of the total sample), followed by the Faculty of Sciences, which contributed 170 individuals (25.4%). In contrast, Guatire and San Bernardino showed lower counts, with 100 (14.9%) and 130 (19.4%) individuals, respectively.

This uneven distribution of specimens suggests the existence of contrasting microenvironmental conditions among the studied areas. The high abundance recorded in Sebuacán and the Faculty of Sciences reflects environments with more stable and favorable microclimates, while the lower counts in San Bernardino and Guatire could be associated with conditions that are more restrictive for the development of these communities.

Of the total sample, 240 individuals (35.8% of the total) could be identified to the genus level. Taxonomic identification allowed the specimens to be classified into the Classes Eutardigrada and Heterotardigrada.

The Class Eutardigrada was represented by the Orders Apochela (Family Milniisidae) and Parachela (Families Calohypsibidae, Eohypsibidae, Doryphoribiidae, Macrobiotidae, and Murrayidae), while the Class Heterotardigrada was represented solely by the Order Echiniscoidea (Family Echiniscidae).

A total of 15 genera were recorded, distributed across seven families (Figure 6 and Table 1), demonstrating the community's heterogeneity. The order Apochela was represented by the family Milniisidae (the only family in this order); Parachela included five families (Calohypsibidae, Eohypsibidae, Doryphoribiidae, Macrobiotidae, and Murrayidae), grouping the majority of freshwater tardigrades; while Echiniscoidea was represented exclusively by the family Echiniscidae. These results reflect a taxonomically diverse community, with a predominance of lineages typical of limnoterrestrial environments.

In terms of genus richness by site, it was notably higher at the Faculty of Sciences and Sebuacán, with 14 genera each, indicating high taxonomic diversity in environments with more stable microclimates. In contrast, San Bernardino had the lowest richness, with only 7 genera approximately half that of the richest sites suggesting that a few genera account for a very high proportion of the community.

Six genera were found to be ubiquitous (present at all four sites): *Calcarobiotus*, *Minibiotus*, *Minilentus*, and *Xerobiotus* (all from the family Macrobiotidae), as well as *Macroversum* and *Murrayon* (both from the family Murrayidae). The ubiquity of these genera suggests a high degree of ecological plasticity and resistance to the environmental variations typical of urban ecosystems. Likewise, the predominance of the family Macrobiotidae underscores its importance in the colonization of habitats dominated by urban mosses and lichens.

The pattern of relative abundance by location revealed clear differences in community structure. At the Faculty of Sciences, *Minilentus* (31%) predominated, followed by *Minibiotus* (22%), indicating a relatively balanced

community but with marked dominance. In San Bernardino, *Minibiotus* (28%), *Murrayon* (22%), and *Xerobiotus* (22%) accounted for a large portion of the abundance, reflecting a less diverse and more dominated community. In Sebuacán, *Minibiotus* (25%) was the most abundant genus, followed by *Calcarobiotus* and *Xerobiotus* (13% each), while in Guatire, *Minibiotus* (17%) was also dominant, followed by *Minilentus* and *Murrayon* (both at 11%). These patterns demonstrate how community structure responds to specific local conditions.

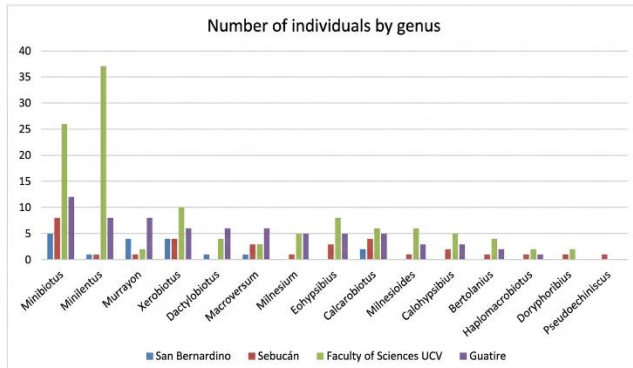


Figure 6. Graphical comparison of the number of individuals by gender found in the four locations sampled.

Table 1. Characterization of collected individuals. Taxa identified at sampling points. (X = presence).

Class	Order	Family	Genus	San Bernardino	Sebuacán	UCV Faculty of Science	Guatire
Eutardigrada	Apocheila	Milnesiidae	<i>Milnesioides</i>		X	X	X
			<i>Milnesium</i>		X	X	X
		Calophypallidae	<i>Calophypallus</i>		X	X	X
			<i>Berolanius</i>		X	X	X
		Eolypsiidae	<i>Eolypsius</i>		X	X	X
	Parscheila	Doryphoribidae	<i>Doryphoribius</i>		X	X	
			<i>Calcarobiotus</i>	X	X	X	X
		Macrobiosidae	<i>Minibiotus</i>	X	X	X	X
			<i>Minilentus</i>	X	X	X	X
			<i>Xerobiotus</i>	X	X	X	X
			<i>Dactylobiotus</i>	X		X	X
		Murrayidae	<i>Macroverum</i>	X	X	X	X
			<i>Murrayon</i>	X	X	X	X
	Heterotardigrada	Echiniscoides	<i>Pseudoechiniscus</i>		X		

The diversity analysis indicated an average alpha diversity (α) of 12 genera per site and a gamma diversity (γ) of 15 genera in total (see Table 2). The beta (β) index, calculated as the ratio γ/α , was 1.2. According to Whittaker's (1960) conceptualization, species diversity in a series of community samples across a given range of environments results from the combination of alpha and beta diversities, where the latter represents the magnitude of change in community composition along an environmental gradient.

Table 2. Comparative Beta Diversity Index between Localities

Location	San Bernardino	Sebuacán	Faculty of Science	Guatire
San Bernardino	1	-	-	-
Sebuacán	1.43	1	-	-
Faculty of Science	1.33	1.07	1	-
Guatire	1.3	1.11	1.04	1

Table 3. Jaccard Similarity Index (JS)

Comparison	Similarity percentage (IJ)
Faculty of Science and Sebuacán	≈ 87%
Guatire and Sebuacán	≈ 69%
San Bernardino and other localities	≈ 47-54%

Guatire and Sebuacán showed intermediate similarity (≈69%), while San Bernardino exhibited the lowest values (47%–54%) compared to the other sites.

The high turnover observed in San Bernardino is due to its low species richness and the presence of unique genera or the absence of common genera, establishing it as an ecological outlier within the study. This pattern is relevant, as beta diversity is of great importance in ecology, biogeography, and conservation biology, serving as a key concept for understanding ecosystem dynamics, functioning, and management (Legendre et al., 2005).

The variations observed are consistent with the Urban Ecology Hypothesis. Authors such as Shochat et al. (2006) and Peluffo and Rocha (2007) argue that urbanization imposes pressures that lead to a decline in taxonomic richness due to vegetation fragmentation, alterations in the hydrological cycle, and the heat island effect. These factors tend to homogenize microhabitats, favoring resistant and generalist species, such as *Minibiotus* and *Minilentus*.

This hypothesis is supported by the microclimatic differences recorded among the study sites, which directly influence moisture availability and the environmental stability necessary for the development of limnoterrestrial communities. Sites with greater generic richness, such as Sebuacán and the Faculty of Sciences, exhibited more favorable conditions due to moisture retention associated with vegetation cover. In Sebuacán, the vegetation on the walls helped maintain adequate moisture levels, while at the Faculty of Sciences, the presence of gardens and evergreen trees contributed to a humid and relatively stable microclimate an essential condition for poikilohydric organisms such as mosses and the tardigrades that inhabit them.

In contrast, San Bernardino showed lower species richness, attributed to the presence of more restricted areas directly exposed to solar radiation, which increases dryness and water stress, creating a less favorable environment for the establishment and maintenance of moss communities and, consequently, their associated tardigrades.

Finally, this study documented 12 genera of tardigrades that had not previously been recorded in Venezuela. Adding these to the 6 previously reported genera (*Macrobiotus*, *Echiniscoides*, *Echiniscus*, and *Mopsechiniscus*), the country's total taxonomic record now stands at 18 genera. This finding ranks Venezuela as the fifth country

with the highest tardigrade biodiversity in the Neotropics, ahead of Argentina (15 genera), though still behind Mexico (30 genera) and Brazil (25 genera), highlighting the importance of urban studies in expanding biogeographic knowledge of the group.

4 Conclusions

The study revealed a diversity of limnoterrestrial tardigrades comprising 15 genera, mostly belonging to the class Eutardigrada, with a single representative of Heterotardigrada, and highlighted the addition of 12 new records for Venezuela. The analyzed communities showed an uneven distribution in terms of abundance, with Sebuacán being the site with the highest number of individuals, followed by the UCV Faculty of Sciences, both at intermediate elevations, while San Bernardino and Guatire Sebuacán had comparatively lower abundances.

Regarding community structure, the genera Minibiotus, Minilentus, and Xerobiotus dominated the limnoterrestrial tardigrade communities. San Bernardino stood out for having the greatest genus turnover and the highest dissimilarity compared to the other sites, suggesting a unique faunal composition. At the regional level, the results indicate that Venezuela has a lower tardigrade richness compared to other countries in the Neotropical region, highlighting the need to expand sampling and research efforts to better understand the actual diversity of the group in the country.

The expansion of the national record doubles the known diversity for Venezuela and demonstrates great potential for faunal discovery, especially in non-urban areas. At the continental level, the presence of *Milnesium* sp. in all countries confirms its status as a successful cosmopolitan genus

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