

# Species diversity and morphometrics of tardigrades in a medium-sized city in the Neotropical Region: Santa Rosa (La Pampa, Argentina)

J. R. Peluffo, A. M. Rocha & M. C. Moly de Peluffo

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## Abstract

*Species diversity and morphometrics of tardigrades in a medium-size city in the Neotropical Region: Santa Rosa (La Pampa, Argentina).*— Tardigrade diversity was studied in a medium-sized city in the Neotropical Region: Santa Rosa (La Pampa, Argentina). Samples were collected between February 1999 and January 2000 from lichens and mosses growing on sidewalk trees of the urban and periurban area. Five species of tardigrades were found, i.e., *Echiniscus rufoviridis* du Bois-Reymond Marcus, 1944, *Macrobiotus areolatus* Murray, 1907, *Ramazzottius oberhaeuseri* (Doyère, 1840), *Milnesium* cf. *tardigradum* and a non-described species of *Macrobiotus*. Only one species, *M.* cf. *tardigradum*, was found in areas with high levels of vehicle traffic. Results are compared with those from cities in the Nearctic and Palearctic regions. Measurements and *pt* index values (percentage ratios between the length of the structure considered and the buccal tube length) are provided for *M. areolatus*, *R. oberhaeuseri* and *M.* cf. *tardigradum*. Amongst the characters considered, the *pt* index for the stylet support insertion shows the least intraspecific variation. This character is also independent from body length and buccal-tube length.

Key words: Tardigrades, Neotropical fauna, Urban environment, Biotic homogenization, Morphometric analysis, Medium-size cities.

## Resumen

*Diversidad y morfometría de tardígrados de una ciudad mediana de la región Neotropical: Santa Rosa (La Pampa, Argentina).*— Se estudió la diversidad de tardígrados en una ciudad mediana de la Región Neotropical: Santa Rosa (La Pampa, Argentina). Las muestras se recolectaron entre febrero de 1999 y enero de 2000 de líquenes y musgos que crecían sobre árboles de vereda de áreas urbanas y periurbanas. Se encontraron cinco especies de tardígrados: *Echiniscus rufoviridis* du Bois-Reymond Marcus, 1944, *Macrobiotus areolatus* Murray, 1907, *Ramazzottius oberhaeuseri* (Doyère, 1840), *Milnesium* cf. *tardigradum* y una especie no descrita de *Macrobiotus*. *M.* cf. *tardigradum* fue la única especie encontrada en áreas con alto tránsito vehicular. Los resultados se comparan con los de ciudades de las Regiones Neártica y Paleártica. Se brindan medidas y valores del índice *pt* (relación porcentual entre la longitud de la estructura considerada y la longitud del tubo bucal) para *M. areolatus*, *R. oberhaeuseri* y *M.* cf. *tardigradum*. Entre los caracteres considerados, el índice *pt* correspondiente a la inserción del soporte de los estiletes es el que muestra la menor variabilidad intraespecífica. Además, este carácter es independiente del largo total del cuerpo y del largo del tubo bucal.

Palabras clave: Tardígrados, Fauna neotropical, Ambiente urbano, Homogenización biótica, Análisis morfométrico, Ciudades medianas.

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Julio R. Peluffo, Alejandra M. Rocha & María C. Moly de Peluffo, Fac. de Ciencias Exactas y Naturales, Univ. Nacional de La Pampa, Uruguay 151, 6300 Santa Rosa, La Pampa, Argentina.

Corresponding author: J. R. Peluffo. E-mail: [molpel@speedy.com.ar](mailto:molpel@speedy.com.ar)

## Introduction

The phylum Tardigrada includes meiofaunal organisms that live in all environments. These include terrestrial environments where they can be found to colonise in the soil, in leaf litter and turf, and in lichen and moss communities growing on rocks, trees or other suitable substrates.

These communities and their tardigrades have been well studied in non-urban environments (e.g. Nelson, 1975; Ito, 1995; Bertolani & Rebecchi, 1996; Jönsson, 2003; Michalczyk & Kaczmarek, 2003). Conversely, urban tardigrades are less known. The works of Séméria (1981, 1982) in France, Meininger et al. (1985) in the USA, Utsugi (1986) in Japan and Steiner (1994a, 1994b, 1994c) in Switzerland are among the few that have been published world-wide. In Argentina, the tardigrade fauna is known mainly from papers of the 1980's (Claps & Rossi, 1981, 1984, 1988; Maucci, 1988; Rossi & Claps, 1980, 1989), but none of them deals with urban tardigrades. During the past few years some work has been carried out on city-dwelling tardigrades (Peluffo et al., 2002; Rocha et al., 2002; Moly de Peluffo et al., 2006).

Increasingly rapid urbanization, with the peculiar conditions it imposes upon organisms living in urban areas, has become a major concern in conservation biology (Shochat et al., 2006). A consequence of this process is the global decrease in diversity and the increased biota similarity among cities. McKinney & Lockwood (1999) defined biotic homogenization as "the replacement of local biota with non-indigenous species". His concept was later extended by Olden & Rooney (2006) to describe "the broader, overarching ecological process by which formerly disparate biotas lose biological distinctiveness at any level of organization, including in their genetic, taxonomic and functional characteristics".

The effects of urbanization differ according to the class of organism involved. Urban tardigrades elicit a number of questions on this issue, such as, a) what is the diversity of tardigrades in a city? b) is it different from that in the surrounding rural areas? c) can an internal gradient or intersite differences be detected within a city? d) do different cities host different faunas? e) if so, how different are these faunas? f) do populations of the same species living in different cities show morphometric variations? and g) are such differences due to phenotypic plasticity or genetic differences?

Exact identification of the species considered is required to answer these questions. The taxonomic status of several taxa therefore needs to be clarified before any further work can be undertaken.

Many wide-ranging species that are considered cosmopolitan, such as *Milnesium tardigradum* Doyère, 1840, may in fact be species complexes (Nelson, 2002). Besides, as many original descriptions are based on a small number of specimens,

the range of intraspecific, ontogenetic, and sexual variation remains unknown (Kinchin, 1994).

Such systematic problems increase when the South American tardigrade fauna is considered, as knowledge of this group is still very limited (Pilato et al., 2003).

The aim of this paper was to provide data on tardigrade species found in a medium-sized city in central Argentina, i.e., Santa Rosa (La Pampa), and compare them to those found in other cities, providing data for a preliminary "biogeography of urban tardigrades". We also performed a morphometric analysis in an attempt to contribute elements towards a better characterization of the urban tardigrades inhabiting the Neotropical Region.

## Material and methods

Between February 1999 and January 2000 samples were collected from mosses and lichens growing on different tree species in the city of Santa Rosa. Trees in public areas mainly include specimens of *Robinia pseudoacacia* and several species of *Fraxinus*. Also, and mainly in non-paved areas, there are specimens of the autochthonous *Prosopis caldenia*. Samples were treated following the usual methodology (e.g., Ramazzotti & Maucci, 1983). Specimens were mounted in Faure's medium or polyvinyl-lactophenol.

Tardigrades were measured with an eyepiece micrometer. Measurements were taken following Pilato (1981), Binda & Pilato (1990), Bertolani & Rebecchi (1993) and Kinchin (1996). Percentage ratios between the length of the structure considered and the buccal tube length ( $\rho$ ) were calculated according to Pilato (1981).

## Study area

Santa Rosa (36° 39' S, 64° 17' W) is located in the central-eastern part of the province of La Pampa (fig. 1), at 177 m a.s.l. According to the bioclimatic classification of Argentina (IRAM 11.603, 1996), it corresponds to zone IIIa, defined as warm-temperate. Within this zone, it is placed in the southernmost sector, near the boundary with zone IVc (cold-temperate). The average maximum temperatures in summer are in the 30° range and the average minimum temperatures in winter are around 0°C. The daily thermal ranges are over 15°C. Annual average rainfall is approximately 640 mm. The stable population is around 100,000. Santa Rosa is a regional commercial and administrative centre and has over 24 industrial enterprises spread over an area of 134 hectares. Its characteristics are those of an intermediate or medium-sized city: according to Llop Torné & Bellet Sanfeliu (1999), an intermediate city is defined by its population — 20,000 to 200,000 — and the role it plays in the surrounding regions for which it serves as a reference centre.

## Results

Tardigrades were found in 127 of the 157 samples studied. Active individuals and free laid eggs were found as well as exuvia with and without eggs. This allowed specific identification of tardigrades. They belonged to five species: *Echiniscus rufoviridis* du Bois–Reymond Marcus, 1944, *Macrobiotus areolatus* Murray, 1907, *Ramazzottius oberhaeuseri* (Doyère, 1840), *Milnesium cf. tardigradum* and *Macrobiotus* sp. The last species, which was present in 18% of the samples, is new to science and will be fully described in a forthcoming paper.

The greatest specific richness per sample was 4 and was recorded in a bush placed in the central square.

Class: Heterotardigrada Marcus, 1927

Order: Echiniscoidea Marcus, 1927

Family: Echiniscidae Thulin, 1928

Genus: *Echiniscus* Schultze, 1840

### *Echiniscus rufoviridis* du Bois–Reymond Marcus, 1944

Observations: present in 32% of the samples. This is a Neotropical species based on specimens collected in Brazil in 1944 and recently rediscovered and mentioned for several localities in Argentina by Peluffo et al. (2002).

Class: Eutardigrada Marcus, 1927

Order: Parachela Schuster, Nelson, Grigarick and Christenberry, 1980

Family: Macrobiotidae Thulin, 1928

Genus: *Macrobiotus* Schultze, 1834

### *Macrobiotus areolatus* Murray, 1907

Observations: cosmopolitan species. In Santa Rosa it was found in a non-systematic sampling of a paved area with medium to heavy vehicle traffic. The eggs are typical for the species. Table 1 and figures 2A and 3A illustrate morphometric data and results of statistical analysis. The *pt* index with the smallest coefficient of variation corresponds to the insertion level of stylet supports on the buccal tube followed by the row length of placoids and the width of the buccal tube. On the other hand, the *pt* index values of the insertion level of the stylet support show the highest independence with respect to the body length ( $y = -0.0009x + 77.585$ ;  $r^2 = 0.0004$ ) and with respect to the buccal tube length ( $y = -0.0284x + 78.564$ ;  $r^2 = 0.0075$ ).

Family: Hysibiidae Pilato, 1969

Genus: *Ramazzottius* Binda and Pilato, 1986

### *Ramazzottius oberhaeuseri* (Doyère, 1840)

Observations: cosmopolitan species, present in 18% of the samples. Egg morphology coincides perfectly with that described for this species. It was absent from samples collected in areas with



Fig. 1. Map of South America, Argentina and La Pampa indicating the location of Santa Rosa.

Fig. 1. Mapa de América del Sur, Argentina y La Pampa indicando la ubicación de Santa Rosa.

heavy vehicle traffic. Table 2 and figures 2B and 3B illustrate morphometric data and results of statistical analysis. The *pt* index with the smallest coefficient of variation corresponds to the insertion level of stylet supports on the buccal tube. The values of *pt* index for this character show the greatest independence respect to body length ( $y = 0.0005x + 56.184$ ;  $r^2 = 0.0002$ ) and respect to buccal tube length ( $y = 0.0189x + 55.768$ ;  $r^2 = 0.0014$ ).

Order: Apochela Schuster, Nelson, Grigarick and Christenberry, 1980

Family: Milnesiidae Ramazzotti, 1962

Genus: *Milnesium* Doyère, 1840

### *Milnesium cf. tardigradum*

Observations: *Milnesium tardigradum* Doyère, 1840 is a cosmopolitan species. *Milnesium cf. tardigradum* is present in 89% of the samples. In areas with heavy vehicle traffic it was the only species recorded. Table 3 and figures 2C and 3C illustrate morphometric data and results of statistical analysis. The *pt* index with the smallest coefficient of variation corresponds to the insertion level of stylet supports on the buccal tube followed by those corresponding to claw length of the fourth pair of legs. The values of *pt* index that show the greatest independence from the body length are those of the main branch length of the claws of the fourth pair of legs ( $y = 0.0047x + 55.298$ ;  $r^2 = 0.0083$ ). They are

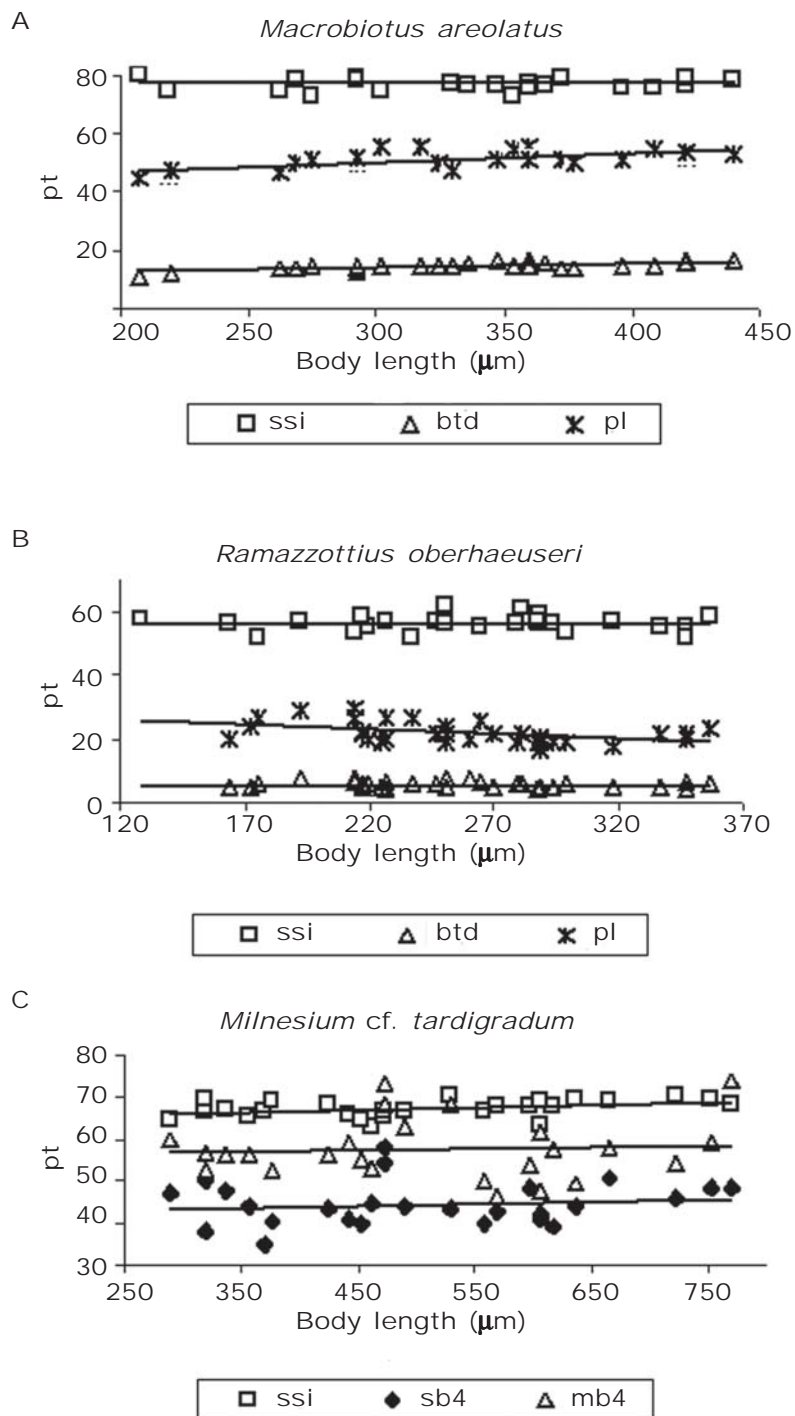


Fig. 2. The *pt* index values plotted against body length. The tendency lines are shown: ssi. Insertion level of stylet supports on the buccal tube; btd. Diameter of the buccal tube; pl. Macroplacoid row length; sb4. Basal claws + secondary branch length of the fourth pair of legs; mb4. Main branch length of claws of the fourth pair of legs.

Fig. 2. Valores del índice *pt* obtenidos en función de la longitud total del cuerpo. Se muestran las líneas de tendencia: ssi. Nivel de la inserción del soporte de los estiletes sobre el tubo bucal; btd. Diámetro del tubo bucal; pl. Longitud de la hilera de macroplacoides; sb4. Longitud de la uña basal + longitud de la rama secundaria del cuarto par de patas; mb4. Longitud de la rama principal de la uña del cuarto par de patas.

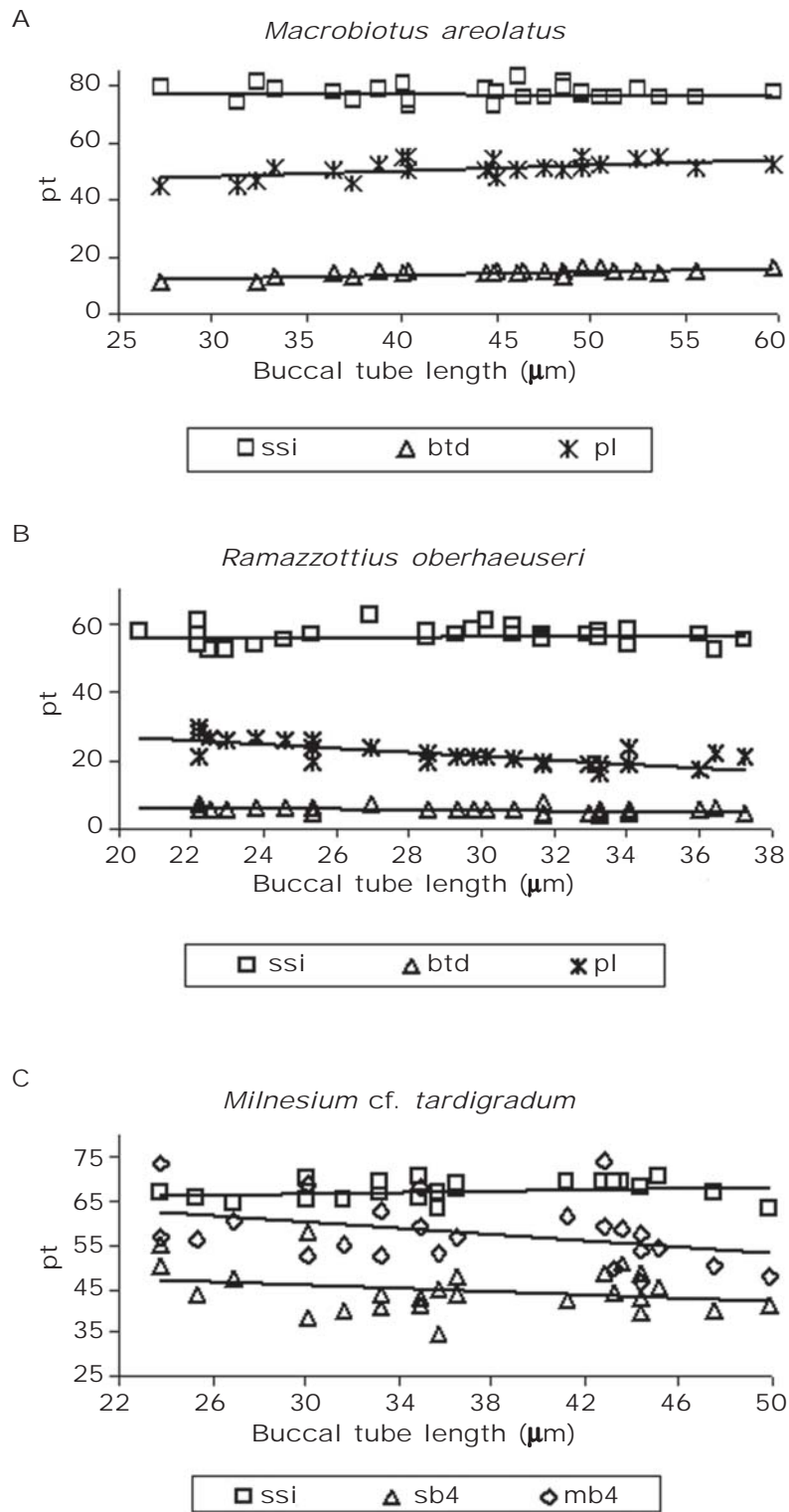


Fig. 3. The *pt* index values plotted against buccal tube length. The tendency lines are shown. (For abbreviations see fig. 2.)

Fig. 3. Valores del índice *pt* obtenidos en función de la longitud del tubo bucal. Se muestran las líneas de tendencia. (Para las abreviaturas, ver la fig. 2.)

Table 1. Summary of morphometric data (in  $\mu\text{m}$ , except *pt*) for *Macrobiotus areolatus*: bl. Body length; btl. Buccal tube length; btd. Diameter of the buccal tube; ssi. Insertion level of stylet supports on the buccal tube; pl. Macroplacoid row length; 1° pl. First macroplacoid length; 2° pl. Second macroplacoid length; 3° pl. Third macroplacoid length; C. Character; Min. Minimum; Max. Maximum; SD. Standard deviation; CV. Coefficient of variation.

Tabla 1. Resumen de datos morfométricos (en  $\mu\text{m}$ , excepto para *pt*) de *Macrobiotus areolatus*: bl. Longitud total del cuerpo; btl. Longitud del tubo bucal; btd. Diámetro del tubo bucal; ssi. Nivel de la inserción del soporte de los estiletes sobre el tubo bucal; pl. Longitud de la hilera de macroplacoides; 1° pl. Longitud del primer macroplacoides; 2° pl. Longitud del segundo macroplacoides; 3° pl. Longitud del tercer macroplacoides; C. Carácter; Min. Mínimo; Max. Máximo; SD. Desviación estándar; CV. Coeficiente de variación.

C	Mean	Min.	Max.	SD	<i>pt</i> mean	<i>pt</i> min.	<i>pt</i> max.	<i>pt</i> SD	<i>pt</i> CV	n
bl	329.24	207.40	439.20	64.27						26
btl	44.31	27.30	59.66	8.06						26
btd	6.55	3.03	9.61	1.54	14.44	11.11	16.33	1.31	9.07	25
ssi	34.23	21.74	46.52	6.26	77.31	72.50	83.22	2.64	3.41	26
pl	22.49	12.14	31.35	4.97	50.88	44.44	55.47	3.14	6.71	24
1° pl	6.71	3.40	9.80	1.71	15.12	12.37	18.23	1.61	10.62	25
2° pl	5.18	2.02	8.43	1.57	11.57	7.41	15.09	1.73	14.95	25
3° pl	7.54	3.70	10.11	1.95	16.97	11.44	20.85	2.16	12.73	25

followed by the *pt* index values of the secondary branch length of the same claws ( $y = 0.0056x + 41.823$ ;  $r^2 = 0.0224$ ) and the insertion level of the stylet supports ( $y = 0.0058x + 64.447$ ;  $r^2 = 0.1539$ ). As to the buccal tube length, the *pt* index values that show the greatest independence are those of the secondary branch length of claw IV ( $y = -0.167x + 50.825$ ;  $r^2 = 0.0592$ ), followed by the *pt* index values of the insertion level of the stylet supports ( $y = 0.0737x + 64.644$ ;  $r^2 = 0.0739$ ).

## Discussion

Two of the species found in Santa Rosa —*Milnesium* cf. *tardigradum* and *Ramazzottius oberhaeuseri*— were also recorded by Moly de Peluffo et al. (2006) in General Pico; *M. tardigradum* and *R. oberhaeuseri* were recorded in Europe by Séméria (1981) in Nice and by Steiner (1994b) in Zurich.

*M. areolatus* is a species that has not been recorded within the cities except in the Neotropical

Table 2. Summary of morphometric data (in  $\mu\text{m}$ , except *pt*) for *Ramazzottius oberhaeuseri*. (For abbreviations see table 1.)

Tabla 2. Resumen de datos morfométricos (en  $\mu\text{m}$ , excepto para *pt*) de *Ramazzottius oberhaeuseri*. (Para las abreviaturas, ver tabla 1.)

C	Mean	Min.	Max.	SD	<i>pt</i> mean	<i>pt</i> min.	<i>pt</i> max.	<i>pt</i> SD	<i>pt</i> CV	n
bl	252.74	128.22	356.30	54.89						39
btl	29.37	20.61	37.26	4.63						36
btd	1.61	1.27	2.38	0.28	5.50	3.81	7.50	0.89	16.2	35
ssi	16.55	11.65	20.61	2.83	56.14	51.72	60.71	2.29	4.1	29
pl	6.33	4.76	7.93	0.63	21.72	16.67	30.00	3.22	14.8	35

Table 3. Summary of morphometric data (in  $\mu\text{m}$ , except *pt*) for *Milnesium cf. tardigradum*: mb4. Main branch length of claws of the fourth pair of legs; sb4. Basal claws + secondary branch length of the fourth pair of legs. (For other abbreviations see table 1.)

Tabla 3. Resumen de datos morfométricos (en  $\mu\text{m}$ , excepto para *pt*) de *Milnesium cf. tardigradum*: mb4. Longitud de la rama principal de la uña del cuarto par de patas; sb4. Longitud de la uña basal + la rama secundaria del cuarto par de patas. (Para las otras abreviaturas, ver tabla 1.)

C	Mean	Min.	Max.	SD	<i>pt</i> mean	<i>pt</i> min.	<i>pt</i> max.	<i>pt</i> SD	<i>pt</i> CV	n
bl	508.11	288.9	770.37	139.61						26
btl	37.00	23.78	49.94	7.55						26
btd	13.72	7.13	25.37	4.24	36.99	23.68	59.26	7.96	21.5	26
ssi	24.62	15.90	31.90	5.48	67.37	63.11	70.53	2.05	3.0	26
mb4	21.17	13.48	31.71	4.23	57.72	46.43	74.07	7.24	12.5	25
sb4	16.43	11.1	22.20	3.48	44.65	34.67	57.89	5.18	11.6	26

Region. In Argentina, it was recorded in urban samples of General Pico (Moly de Peluffo et al., 2006) and in non-systematic surveys in other cities.

As pointed out by Séméria (1982) for some species found in Nice, *Echiniscus rufoviridis* and *Macrobiotus sp.*, even if not cosmopolitan show such tolerance to urban conditions that it allows them to appear in cities. *Echiniscus rufoviridis* has been recorded in several cities ranging from its original locality in Brazil to central Argentina (Peluffo et al. 2002). However, non-systematic surveys in other Neotropical cities have rendered negative results.

In the city of Santa Rosa there are none of the species with high tolerance to urban pollution that have been mentioned for northern hemisphere cities, i.e., *Macrobiotus hufelandi* Schultze, 1833 (Séméria, 1981, 1982), *Diphascion (Adropion) scoticum* Murray, 1905 (Meininger et al., 1985) and *Macrobiotus persimilis* Binda & Pilato, 1972 (Steiner, 1994b). In Santa Rosa the species that shows the greatest tolerance to urban pollution due to vehicle transit is *M. cf. tardigradum*. This coincides with data from General Pico (Moly de Peluffo et al., 2006).

The low diversity of tardigrades in Santa Rosa and the maximum specific richness for any one site are common to those of other cities studied (Moly de Peluffo et al., 2006). Santa Rosa and General Pico share their five species of tardigrades, while Nice and Zurich share four. One of them, i.e., *R. oberhaeuseri*, appears in all four cities.

Upon observation of tardigrade distribution in Santa Rosa, the statement—for other animals—by Jokimäki & Kaisanlahti-Jokimäki (2003), applies, i.e., "Urbanization cannot be seen as a process that monotonically increases the similarity of bird communities". Thus, the maximum richness of four species of tardigrades occurs in the central area of the city, i.e., the central square of c. 1 ha. This suggests that the negative effect of intense vehicle traffic on

species richness observed in the epiphytic communities on sidewalk trees, decreases rapidly with increased distance from the street. It may be possible that the strong seasonal winds characteristic of this region have a dispersant effect on the potential atmospheric pollutants produced by vehicles. This and other particular cases seem to confirm the need to enrich the traditional gradient analysis with consideration of other factors, including socio-economic variables (Kinzig et al., 2005).

Similarities among tardigrade taxocenoses in cities suggest that these animals are also undergoing a process of biotic homogenization linked to urbanization, although in a different measure according to the level of organization and spatial scale considered. It appears that homogenization reaches a taxonomic level at a regional scale. At a global scale there appears to be a functional homogenization that produces the following similarities: a) reduced specific richness, b) different tolerant species in cities of different biogeographic regions, and c) tolerant species common to different cities in a same biogeographic region. The last case requires study if these species show intercity population similarities regarding density, relative abundance and morphometric characteristics.

In the three species recorded in Santa Rosa for which morphometric data was analyzed, *M. areolatus*, *R. oberhaeuseri* and *M. cf. tardigradum*, the *pt* index with the lowest variation coefficient was clearly that corresponding to the insertion level of stylet supports on the buccal tube (3–4.1%). This confirms the low variability mentioned for this character by Pilato (1981). The same was recorded by Bertolani & Rebecchi (1993) for species of the *Macrobiotus hufelandi* group. In his study in tardigrades from the Caucasus, Biserov (1997) did not provide the variation coefficient of *pt* indices. However, calculation of such coefficients based on

the data provided in his tables shows that the *pt* index of the insertion level of stylet supports on the buccal tube is also that which varies the least, except for one species of *Ramazzottius*.

As recorded by Pilato (1981) for *Isohypsibius elegans*, in the three species analyzed in this study, the values of *pt* index for the insertion level of stylet supports on the buccal tube were independent from the body length and the length of buccal tube.

Morphometric analysis of the data presented herein provides a foundation for a better characterization of the tardigrade species living in cities of the Neotropical Region and adds to our knowledge of the morphometric differences amongst their populations.

Future studies should allow the degree of similarity among the urban tardigrade taxocenoses to be measured at different organization levels and different spatial scales. If morphometric differences were detected among populations of species common to different cities, molecular studies should be useful to elucidate whether the differences are due to phenotypic plasticity or genetic differences. Such investigation should also contribute towards the uncovering of distribution patterns of urban tardigrades and the underlying mechanisms that produce them, as well as towards the testing of any such hypotheses.

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