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1 Article Category: Notes and comments

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3 **Tardigrades in the city: a review of diversity patterns in response to**
4 **urbanization**

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19 Running title: Urban tardigrades

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25 **ABSTRACT**

26 In different taxonomical groups, the number of species found in urban
27 environments tends to decline compared to adjacent non-urban environments. It is
28 unclear whether tardigrades also conform to this pattern of diversity decline in
29 cities. Tardigrades are microscopic invertebrates which have been understudied,
30 despite the fact that they are cosmopolitan and found in all types of habitats. Due
31 to their capability to withstand extreme conditions, tardigrades should be able to
32 successfully thrive in urban environments. Here, all available information about
33 tardigrade diversity in cities was compiled. It was quantitatively determined that
34 tardigrade diversity declines in urban areas compared to adjacent rural areas.
35 Geographically closer cities are also likely to harbor a more similar set of
36 tardigrade species. In comparison to other groups like mammals and birds, there
37 are no tardigrade species consistently found in most studied cities. In fact, most
38 urban tardigrades have only been found in one single city. Ultimately, the species
39 of tardigrades found in a given city will normally depend on the set of species
40 already living in the adjacent native environments. One question that deserves
41 further investigation is why only a subset of such native species is able to colonize
42 the new environmental niches available in cities.

43

44 **Keywords:** Tardigrada, tardigrades, diversity, urbanization, urban ecology

45

46

47 **INTRODUCTION**

48 In most groups of organisms, urbanization leads to a decline in diversity, although
49 the few successful urban species can be very abundant (Grimm *et al.* 2008). In
50 contrast, some other groups may experience a heightened diversity in urban
51 environments, as it can be the case with bees (Fortel *et al.* 2014). The diversity
52 pattern for tardigrades in urban environments remains unclear. Tardigrades are
53 microscopic invertebrates, which are cosmopolitan and present in all types of
54 ecosystems, including urban environments (Nelson 2002). Terrestrial tardigrades
55 can potentially be found in any sample of moss or lichen, and they are known for
56 surviving under extreme conditions (e.g. extremely low and high temperatures,
57 lack of oxygen, lack of water, exposure to radiation levels that would kill most
58 other organisms, and extreme high pressure) (Schill 2019). Consequently,
59 tardigrades should a priori be unaffected by urban stressors, and thus species
60 richness should be similar in urban areas and in neighboring rural areas. However,
61 in the studies in which tardigrade diversity has been investigated in both rural and
62 urban sites, the pattern seems to be for the number of species to be lower in urban
63 sites (de Peluffo *et al.* 2006; Johansson *et al.* 2011; Meyer *et al.* 2013; Rocha *et al.*
64 2016).

65
66 A possible decline in tardigrade diversity in cities could involve pollution as a main
67 explanatory factor (Roberts & Zimmer 1990; Steiner 1994). For example, in Zürich,
68 the number of tardigrade species decreased with increasing levels of air SO₂
69 (Steiner 1994). The negative effect of pollution on tardigrade diversity seems
70 further supported by studies reporting fewer tardigrade species in polluted sites
71 (Hohl *et al.* 2001; Vargha *et al.* 2002) or in response to experimental exposure to
72 pollutants (Steiner 1995). The lower pH and lower humidity normally found in
73 cities have also been used to explain lower tardigrade diversity in urban
74 environments (Meininger *et al.* 1985), although at least in one city pH levels could
75 not explain differences in tardigrade diversity between rural and urban areas
76 (Johansson *et al.* 2011).

77

78 Tardigrades remain a very understudied group, and this is particularly the case in
79 the context of urban ecology (Rocha *et al.* 2016). However, tardigrades can be a

80 very powerful model to investigate the challenges and opportunities encountered
81 by urban colonisers. Tardigrades are found in cities worldwide, they are easy to
82 sample in large numbers in short periods of time, and they can easily be
83 transported across countries. Consequently, tardigrades can be used to understand
84 worldwide patterns of colonisation and adaptation to urban environments. It is
85 thus important to have a preliminary understanding of the effect of urbanization
86 on tardigrade diversity as a first step to guide future studies.

87

88 Here I compiled all available information to date on tardigrade diversity in cities to
89 quantitatively answer two main questions: (i) whether there is a consistent decline
90 in the number of tardigrade species in urban sites compared to non-urban sites
91 across cities; and (ii) whether the similarities between cities in their urban
92 tardigrade communities can be explained by the geographical distance separating
93 those cities.

94

95 **METHODS**

96 I made a comprehensive search in Web of Science on 2 April 2017, compiling
97 results from several searches using the terms “tardigrad*” or “water bear” plus
98 “urban” or “city”. After a preliminary filtering, I considered a total of 73
99 publications. From these 73 publications, only those that reported the number of
100 tardigrade species within a city were considered in the analyses (most of those 73
101 publications did not include any urban samples). A few publications in which
102 tardigrades were not identified at the species level (e.g. Pérez-Pech *et al.* 2016)
103 were also excluded. This selective process resulted in 10 relevant publications
104 (Table 1). From these 10 publications, the following information was extracted: the
105 total number of species in urban sites (using information only present in figures
106 when necessary), the total number of species in rural sites (when available), and
107 the number of samples analyzed in each habitat type.

108

109 In all studies, urban sampling took place across the whole city, including highly
110 urban sites. Although samples mostly consisted of mosses and lichens, there were
111 considerable differences among studies: in General Pico, Santa Rosa, Cincinnati,
112 and Salta, moss and lichen samples were collected from trees; in Belfast, samples

113 consisted of lichen exclusively on lime trees; in Lake Charles, samples included
114 mosses, lichens, plants, and leaf litter; in Zürich, samples were exclusively mosses
115 on walls; in Fresno and Tokyo, lichen and moss samples were collected from
116 several substrates, including trees, rocks, concrete and soil; in Nice, lichen and
117 moss samples were complemented with samples from artificial substrates like
118 pavement. Extraction of tardigrades from samples in most studies involved
119 rehydration with water and collection of tardigrades from the suspension. Only
120 two studies used extraction methods involving a funnel and movement of
121 tardigrades along a gradient (Meininger *et al.* 1985; Steiner 1994).

122

123 All statistical tests were implemented in R (R Core Team 2014). Values are
124 reported as mean \pm SD. Significance level (α) was set at 0.05. Differences in
125 tardigrade richness between urban and rural sites were determined using paired *t*-
126 tests, considering either the total number of species in each type of habitat or the
127 number of species divided by the number of samples analysed. A Mantel test was
128 used to calculate the relationship between a matrix of similarities between cities
129 based on the occurrence of tardigrade species and a matrix of geographical
130 distances between cities. Diversity similarities were calculated as Jaccard distances
131 between cities using the binary data in Table 2. Lower distance values indicated
132 cities with similar tardigrade communities (e.g. General Pico and Santa Rosa, in
133 Argentina). The geographical distances between each two cities were obtained
134 from www.distancecalculator.net. The function `mantel` (package `vegan`) was used
135 to run the Mantel tests, selecting 9999 permutations.

136

137 **RESULTS**

138 The mean number of tardigrade species found in cities was 6.52 ± 2.5 (range = 2-
139 10 species), when considering all available studies (Table 1). When comparing
140 rural and urban areas from studies in which both habitat types were sampled,
141 tardigrade diversity was significantly lower in urban areas (7.2 ± 1.81 species)
142 than in rural areas (13.03 ± 4.83 species; paired *t*-test: $t_5 = -4.57$, $p = 0.006$; Fig. 1).
143 Species richness was also lower in urban sites than in rural sites after controlling
144 for the different sampling effort in both habitats (paired *t*-test: $t_5 = -3.89$, $p = 0.01$).
145 The decline in tardigrade diversity in urban sites compared to rural sites (where

146 0% decline would indicate the same number of species in rural and urban sites,
147 and 50% decline would indicate that the total number of species in urban sites was
148 half than that in rural sites) ranged from 32.6% to 52.94%. That is, in all cities
149 investigated to date there is a substantial decline in tardigrade diversity compared
150 to rural sites (Table 1).

151

152 There was a positive association between diversity similarities and geographical
153 distances between cities, i.e. geographically closer cities tended to have more
154 species in common (Mantel test: $r = 0.53$, $p = 0.003$).

155

156 **DISCUSSION**

157 Species richness of tardigrades was lower in urban sites than in adjoining rural
158 sites. This was the case for all available studies making a direct comparison
159 between urban and rural sites. However, it must be noted that the number of
160 available studies is very low, especially since the considered cities are distributed
161 worldwide. The low sample sizes prevented considering the effect of confounding
162 variables like sampling effort, types of substrates sampled, extraction
163 methodologies, and ecological differences between cities. Despite the low
164 statistical power of this study, the overall result is consistent and offers interesting
165 research venues for future studies.

166

167 There are several factors that have been used to explain the decline in tardigrade
168 diversity in urban areas, including increased pollution, lower humidity and lower
169 pH in cities (Hohl *et al.* 2001; Meininger *et al.* 1985; Vargha *et al.* 2002). It is still
170 unclear, however, which ones of these factors may determine the set of species
171 that can be found in any given city. It must be noted that each species may be
172 affected differently by one or more of these factors. In particular, pollution seems
173 an obvious candidate to explain declines in diversity, despite the fact that
174 tardigrades have remarkable abilities to sustain all sorts of environmental
175 stressors. For example, in Zürich, the number of tardigrade species decreased with
176 increasing levels of air SO₂ (Steiner 1994) and the abundances of two tardigrades
177 were significantly correlated with air pollution (Steiner 1994). In contrast, in

178 Lithuania the same set of tardigrade species were found in lichens beside
179 highways and in unpolluted sites (Šatkauskienė 2012).

180

181 It is apparent that not all tardigrade species are able to colonize cities to the same
182 extent. What remains to be explained is why in different cities we find a different
183 community of tardigrades (Johansson *et al.* 2011). Only a few tardigrade species
184 were found in several cities (although not in all of them), and most urban
185 tardigrades have so far been identified in only one city. It would not seem that any
186 eusynanthropic (completely adapted to the urban environment) tardigrade exists
187 (Luniak 2004), i.e. a tardigrade equivalent to the Norway rat or the feral pigeon.
188 The set of species that can be found in a given city may thus be explained by an
189 impoverishment of the higher diversity that exists in the rural matrix where the
190 city is sited. Only in one study, conducted in Fresno, CA, USA, were most of the
191 urban species not found in the adjoining rural areas (Johansson *et al.* 2011). In all
192 other cases, most of the urban species were also found in the surrounding rural
193 areas (Meininger *et al.* 1985; Meyer *et al.* 2013; Rocha *et al.* 2016; Séméria 1981,
194 2002). If the tardigrade species found in a city depend on the species already
195 established in the territory around the city, we would expect that closer cities will
196 have more urban species in common than distant cities. Indeed, a statistically
197 significant relationship was found between the similarity in the species found in
198 any two given cities and the geographical distance between them.

199

200 It is possible that the low levels of tardigrade diversity reported for urban
201 environments reflects insufficient sampling effort. Some tardigrade species found
202 in rural areas may also be present in urban areas but in such low numbers that the
203 relatively low sampling effort performed in some previous studies were not able to
204 detect such species in urban sites. Furthermore, some tardigrade species living in
205 urban areas may do so in different habitats than moss and lichen, which are the
206 habitats that are normally sampled in tardigrade studies (Séméria 2002). Any
207 tardigrade species thriving in alternative urban habitats but not in natural habitats
208 within a city may have thus gone undetected in previous studies. For example, in
209 the Mexican city of Chetumal some tardigrade species that were not found in moss
210 samples were however present in road sediment, including the recently

211 discovered species *Doryphoribius chetumalensis*, so far only found in this type of
212 habitat (Pérez-Pech *et al.* 2016, 2017a).

213

214 Which native species are able to colonize a city may depend on the particular
215 nature of that city, including the set of pollutants being produced and accumulated
216 and the environmental conditions in its geographical area (e.g. yearlong extreme
217 temperatures and rain patterns). That is, not all cities may provide conditions that
218 are optimal for the same tardigrade species. In Zürich, for example, Steiner (1994,
219 1995) described *Macrobotus persimilis* as being able to endure high levels of
220 pollution, whereas he considered *Macrobotus hufelandi* to be adversely affected by
221 air pollution (Steiner 1994). However, *M. persimilis* has not been found in any
222 other city, whereas *M. hufelandi* is present in most cities investigated so far.

223

224 Meyer *et al.* (2013) pointed out that most urban species described at the time were
225 eutardigrades. Terrestrial tardigrades are divided into the two Classes
226 Eutardigrada and Heterotardigrada (Bertolani *et al.* 2014). The data compiled in
227 this study confirm that all urban tardigrades, except those in the genus *Echiniscus*,
228 belong to the Class Eutardigrada. Similar results have been found in studies
229 characterizing tardigrades at the genus level (Pérez-Pech *et al.* 2017b). The
230 Eutardigrada is the largest Class of tardigrades, but this fact alone cannot explain
231 the much higher success of eutardigrades in urban environments.

232

233 Tardigrade abundance in a given sample is normally similar in rural and urban
234 sites (Meyer *et al.* 2013). In fact, in some cities tardigrade abundance can be higher
235 in urban sites than in rural sites (Rocha *et al.* 2016). Therefore, urban
236 environments may not be particularly inhospitable to those tardigrade species that
237 are able to colonize and get established in cities. However, it is unclear whether the
238 success of urban tardigrades relies on morphological or physiological adaptations
239 to the urban environment; or whether the transition from rural areas to urban
240 areas does not require the involvement of any genetic adaptation or phenotypic
241 plasticity in those species that successfully colonize cities. More research is
242 definitely needed on the establishment of tardigrade species in cities worldwide.
243 As some of the differences between previous studies may have arisen due to

244 variation in sampling effort, collection and extraction methodology, equipment
245 used, or taxonomical expertise, I suggest that the optimal approach to understand
246 how different types and intensities of urbanization affect tardigrade diversity and
247 abundance may require the same research group to survey in different cities and
248 across urban gradients while using the same sampling methodology.
249 Unfortunately, across-city replication is still rare in urban ecology studies (Bonier
250 2012).

251

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255

256 **REFERENCES**

- 257 Bertolani, R., Guidetti, R., Marchioro, T., Altiero, T., Rebecchi, L., Cesari, M. (2014)
258 Phylogeny of Eutardigrada: New molecular data and their morphological
259 support lead to the identification of new evolutionary lineages. *Molecular*
260 *Phylogenetics and Evolution* 76, 110-126. doi: 10.1016/j.ympev.2014.03.006
- 261 Bonier, F. (2012) Hormones in the city: endocrine ecology of urban birds.
262 *Hormones and Behavior* 61, 763-772. doi: 10.1016/j.yhbeh.2012.03.016
- 263 de Peluffo, M. C. M., Peluffo, J. R., Rocha, A. M., Doma, I. L. (2006) Tardigrade
264 distribution in a medium-sized city of central Argentina. *Hydrobiologia* 558,
265 141-150. doi: 10.1007/s10750-005-1413-9
- 266 Fortel, L., Henry, M., Guilbaud, L., Guirao, A. L., Kuhlmann, M., Mouret, H., Rollin, O.,
267 Vaissière, B. E. (2014) Decreasing abundance, increasing diversity and
268 changing structure of the wild bee community (Hymenoptera: Anthophila)
269 along an urbanization gradient. *Plos One* 9, e104679. doi:
270 10.1371/journal.pone.0104679
- 271 Grimm, N. B., Faeth, S. H., Golubiewski, N. E., Redman, C. L., Wu, J., Bai, X., Briggs, J.
272 M. (2008) Global change and the ecology of cities. *Science* 319, 756-760. doi:
273 10.1126/science.1150195
- 274 Hohl, A. M., Miller, W. R., Nelson, D. R. (2001) The distribution of tardigrades
275 upwind and downwind of a Missouri coal-burning power plant. *Zoologischer*
276 *Anzeiger* 240, 395-401. doi: 10.1078/0044-5231-00047

277 Johansson, C., Calloway, S., Miller, W. R., Linder, E. T. (2011) Are urban and rural
278 tardigrade (Tardigrada) communities distinct and determined by pH: A case
279 study from Fresno County, California. *Pan-Pacific Entomologist* 87, 86-97.

280 Luniak, M. (2004) Synurbization - adaptation of animal wildlife to urban
281 development. In: W. W. Shaw, L. K. Harris, L. W. VandDruff (eds) *International*
282 *Urban Wildlife Symposium*, pp. 50-55

283 Meininger, C. A., Uetz, G. W., Snider, J. A. (1985) Variation in epiphytic
284 microcommunities (tardigrade-lichen-bryophyte assemblages) of the
285 Cincinnati, Ohio area. *Urban Ecology* 9, 45-61. doi: 10.1016/0304-
286 4009(85)90016-6

287 Meyer, H. A., Hinton, J. G., Samletzka, C. A. (2013) Water bears in the Anthropocene:
288 a comparison of urban and woodland tardigrade (Phylum Tardigrada)
289 communities in Southwestern Louisiana, USA. *Journal of Limnology* 72, 123-
290 127. doi: 10.4081/jlimnol.2013.s1.e15

291 Nelson, D. R. (2002) Current status of the Tardigrada: Evolution and ecology.
292 *Integrative and Comparative Biology* 42, 652-659. doi: 10.1093/icb/42.3.652

293 Peluffo, J. R., Rocha, A. M., de Peluffo, M. C. M. (2007) Species diversity and
294 morphometrics of tardigrades in a medium-sized city in the Neotropical
295 region: Santa Rosa (La Pampa, Argentina). *Animal Biodiversity and*
296 *Conservation* 30, 43-51.

297 Pérez-Pech, W. A., Anguas-Escalante, A., Cutz-Pool, L. Q., Guidetti, R. (2017a)
298 *Doryphoribius chetumalensis* sp. nov. (Eutardigrada: Isohypsibiidae) a new
299 tardigrade species discovered in an unusual habitat of urban areas of Mexico.
300 *Zootaxa* 4344, 345-356. doi: 10.11646/zootaxa.4344.2.9

301 Pérez-Pech, W. A., Cutz-Pool, L. Q., Guidetti, R., Blanco-Piñón, A. (2016) Primer
302 registro genérico de tardígrados, habitantes del área urbana de Chetumal
303 Quintana Roo, México. *Entomología Mexicana* 3, 912-918.

304 Pérez-Pech, W. A., Guidetti, R., Anguas-Escalante, A., Cutz-Pool, L. Q., Blanco-Piñón,
305 A. (2017b) Primer registro genérico de tardígrados para Pachuca Hidalgo,
306 México y áreas circundantes. *Entomología Mexicana* 4, 681-687.

307 R Core Team (2014). R: A language and environment for statistical computing. R
308 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0,
309 URL <http://www.R-project.org>

310 Roberts, D., Zimmer, D. (1990) Microfaunal communities associated with epiphytic
311 lichens in Belfast. *The Lichenologist* 22, 163-171. doi:
312 10.1017/s0024282990000111

313 Rocha, A. M., González-Reyes, A., Corronca, J., Rodríguez-Artigas, S., Doma, I., Repp,
314 E. Y., Acosta, X. (2016) Tardigrade diversity: an evaluation of natural and
315 disturbed environments of the province of Salta (Argentina). *Zoological*
316 *Journal of the Linnean Society* 178, 755-764. doi: 10.1111/zoj.12476

317 Šatkauskienė, I. (2012) Microfauna of lichen (*Xanthoria parietina*) in Lithuania:
318 diversity patterns in polluted and non-polluted sites. *Baltic Forestry* 18, 255-
319 262.

320 Schill, R. O. ed. (2019) *Water bears: the biology of tardigrades*. Springer

321 Séméria, Y. (1981) Recherches sur la faune urbaine et sub-urbaine des Tardigrades
322 muscicoles et lichénicoles. I. Nice-ville. *Bulletin mensuel de la Société*
323 *linnéenne de Lyon* 50, 231-237.

324 Séméria, Y. (1982) Recherches sur la faune urbaine et suburbaine des Tardigrades
325 muscicoles et lichénicoles. II. L'espace sub-urbain : les hauteurs orientales de
326 Nice-Ville. *Bulletin mensuel de la Société linnéenne de Lyon* 51, 315-328.

327 Séméria, Y. (2002) Les Tardigrades des surfaces passantes de la ville de Nice.
328 Recherches sur la faune des Tardigrades urbains et sub-urbains de la ville de
329 Nice (Alpes-Maritimes France). *Bulletin mensuel de la Société linnéenne de*
330 *Lyon* 71, 244-250.

331 Steiner, W. A. (1994) The influence of air pollution on moss-dwelling animals: 2.
332 Aquatic fauna with emphasis on Nematoda and Tardigrada. *Revue Suisse de*
333 *Zoologie* 101, 699-724.

334 Steiner, W. A. (1995) The influence of air pollution on moss-dwelling animals: 5.
335 Fumigation experiments with SO₂ and exposure experiments. *Revue Suisse*
336 *de Zoologie* 102, 13-40.

337 Utsugi, K. (1985) Urban tardigrades in Tokyo. *Zoological Science* 2, 1006.

338 Vargha, B., Ötvös, E., Tuba, Z. (2002) Investigations on ecological effects of heavy
339 metal pollution in Hungary by moss-dwelling water bears (Tardigrada), as
340 bioindicators. *Annals of Agricultural and Environmental Medicine* 9, 141-146.

341
342

343 FIGURE LEGENDS

344

345 **Fig. 1** Tardigrade biodiversity in relation to urbanization. Each line connects the

346 number of species in rural and urban sites in the same city.

347

348 **Table 1.** Available data on tardigrade richness in urban environments. Studies are
 349 ordered by population size in ascending order.

City	Popul ation	# sampl es (% sampl es contai ning tardig rades)	# Urban specie s	# Rural species	% Urban decline in richness	References
General Pico, Argentina	52,000	56 (98.2 %)	5			(de Peluffo <i>et al.</i> 2006)
Lake Charles, LA, USA	72,000	40 (68%	8	17	52.94	(Meyer <i>et al.</i> 2013)
Santa Rosa, Argentina	100,00 0	157 (80.9 %)	5			(Peluffo <i>et al.</i> 2007)
Cincinnati, OH, USA	385,40 9	5 ¹	5	8	37.5	(Meininger <i>et al.</i> 1985)
Nice, France	400,00 0	88 (61.4 %)	8	16	50	(Séméria 1981, 1982, 2002)
Belfast, UK	487,41 7		2			(Roberts & Zimmer 1990)
Fresno, CA, USA	502,00 0	73 (38.3 %)	10	19	47.37	(Johansson <i>et al.</i> 2011)
Salta, Argentina	535,30 3	144	6	9	33.33	(Rocha <i>et al.</i> 2016)

Zürich, Switzerland	836,28	80	6.2	9.2	32.61	(Steiner
	4					1994)
Tokyo, Japan	30,303	191 ²	10			(Utsugi
	,794					1985)

350 Human population size offers a crude proxy for city size. I used the population
351 sizes stated in the publications; otherwise I found the population size for the time
352 the study was conducted in worldpopulationreview.com. ¹ 5 sites across the city
353 but unclear how many samples per site. ² 191 'locales' across the 23 wards of
354 Tokyo, but unclear how many samples per site.

355

356

Table 2. Tardigrade species found in cities.

Species	Class	Order	Family	GP	LC	SR	C	B	Z	N	F	S	T	Total
<i>Milnesium reticulatum</i>	Eutardigrada	Apochela	Milnesiidae		X									1
<i>Milnesium tardigradum</i>	Eutardigrada	Apochela	Milnesiidae	X		X		X		X	X		X	6
<i>Astatumen bartosi</i>	Eutardigrada	Parachaela	Hypsibiidae				X							1
<i>Diphascon oculatum</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Diphascon scoticum</i>	Eutardigrada	Parachaela	Hypsibiidae				X							1
<i>Eremobiotus alicatai</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Hypsibius canadensis</i>	Eutardigrada	Parachaela	Hypsibiidae										X	1
<i>Hypsibius convergens</i>	Eutardigrada	Parachaela	Hypsibiidae						X					1
<i>Hypsibius dujardini</i>	Eutardigrada	Parachaela	Hypsibiidae		X					X				2
<i>Hypsibius pallidus</i>	Eutardigrada	Parachaela	Hypsibiidae							X				1
<i>Isohypsibius granulifer</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Isohypsibius marcellinoi</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Isohypsibius prosostomus</i>	Eutardigrada	Parachaela	Hypsibiidae						X					1
<i>Isohypsibius silvicola</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Isohypsibius sismicus</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Ramazzottius anomalus</i>	Eutardigrada	Parachaela	Hypsibiidae								X			1
<i>Ramazzottius oberhaeuseri</i>	Eutardigrada	Parachaela	Hypsibiidae	X		X				X	X		X	5
<i>Macrobiotus echinogenitus</i>	Eutardigrada	Parachaela	Macrobiotidae		X									1

<i>Macrobiotus harmsworthi</i>	Eutardigrada	Parachaela	Macrobiotidae	X					X	2	
<i>Macrobiotus hibiscus</i>	Eutardigrada	Parachaela	Macrobiotidae				X			1	
<i>Macrobiotus hufelandi</i>	Eutardigrada	Parachaela	Macrobiotidae			X	X	X	X	X	6
<i>Macrobiotus persimilis</i>	Eutardigrada	Parachaela	Macrobiotidae					X		1	
<i>Macrobiotus recens</i>	Eutardigrada	Parachaela	Macrobiotidae						X	1	
<i>Minibiotus acadianus</i>	Eutardigrada	Parachaela	Macrobiotidae	X						1	
<i>Minibiotus hufelandioides</i>	Eutardigrada	Parachaela	Macrobiotidae						X	1	
<i>Minibiotus intermedius</i>	Eutardigrada	Parachaela	Macrobiotidae			X			X	3	
<i>Paramacrobiotus areolatus</i>	Eutardigrada	Parachaela	Macrobiotidae	X	X	X		X	X	5	
<i>Paramacrobiotus richtersi</i>	Eutardigrada	Parachaela	Macrobiotidae	X				X		2	
<i>Echiniscus arctomis</i>	Heterotardigrada	Echiniscoidea	Echiniscidae						X	1	
<i>Echiniscus japonicus</i>	Heterotardigrada	Echiniscoidea	Echiniscidae						X	1	
<i>Echiniscus rufoviridis</i>	Heterotardigrada	Echiniscoidea	Echiniscidae	X		X			X	3	
<i>Echiniscus testudo</i>	Heterotardigrada	Echiniscoidea	Echiniscidae					X		1	

Cities are ordered by population size, being smallest in General Pico and largest in Tokyo. Species are listed by class, then by order, then by family, and then in alphabetical order. Only identified species are listed, and thus the number of species listed under a city may be lower than the number indicated in Table 1. GP: General Pico, Argentina (de Peluffo *et al.* 2006); LC: Lake Charles, LA, USA (Meyer *et al.* 2013); SR: Santa Rosa, Argentina (Peluffo *et al.* 2007); C: Cincinnati, OH, USA (Meininger *et al.* 1985); B: Belfast, UK (Roberts & Zimmer 1990); Z: Zürich, Switzerland (Steiner 1994); N: Nice, France (Séméria 1981, 2002); F: Fresno, CA, USA (Johansson *et al.* 2011); S: Salta, Argentina (Rocha *et al.* 2016); T: Tokyo, Japan (Utsugi 1985).