Papéis Avulsos de Zoologia
Museu de Zoologia da Universidade de São Paulo

ARTICLE

# *Brachionus rotundiformis* Tschugunoff, 1921 from the *Brachionus plicatilis* species complex (Rotifera: Monogononta): A new record from Galápagos Archipelago, Ecuador

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**Abstract.** The presence of the rotifer species *Brachionus rotundiformis* from the *B. plicatilis* species complex in Lake Arcturo, a saline lake in the Genovesa Island of the Galápagos Islands, is here reported. This is the first record of the species for the rotifer fauna of Ecuador as well as of the species complex to the Galápagos Islands. This finding is consistent with the idea of high dispersion capacity, and of cosmopolitan distribution of this species complex. Because Genovesa Island is uninhabited, passive transport by wind currents and zoochory by migrant birds seem to emerge as the most plausible factors in this process of colonization. Integrative studies on the morphological variations, genetic, molecular, and ecological aspects are still required to further understand the process of dispersion and the ecology of this member of the *B. plicatilis* species complex in this remote and isolated locality, and the exact taxonomical position of the island's population to the other members of the complex.

**Key-Words.** Brachionidae; Geographical distribution; Neotropics; Rotifers; South America.

## INTRODUCTION

*Brachionus plicatilis* Müller, 1786 is one of the best known monogonont rotifers. This taxon has been extensively studied, owing to its successful use in aquaculture (Lubzens, 1987; Lubzens *et al.,* 2001) and ecotoxicology (Kostopoulou *et al.,* 2012; Rico-Martínez *et al.,* 2017; Won *et al.,* 2017) and presently constitutes a model organism in ecological and evolutionary studies (Declerck & Papakostas, 2017; Serra & Fontaneto, 2017).

Traditionally, *B. plicatilis* has been considered a cosmopolitan morphologically variable taxon associated to saline waterbodies (Ahlstrom, 1940; Koste, 1978). However, detailed analyses on their morphology (Fu *et al.,* 1991a), allozyme patterns (Fu *et al.,* 1991b), temperature-driven fecundity patterns (Hirayama & Rumengan, 1993), karyotype differences (Rumengan *et al.,* 1991) as well as the outcome of assortative mating experiments (Fu *et al.,* 1993; Gómez & Serra, 1995; Rico-Martínez & Snell, 1995) contributed in the recognition that *B. plicatilis* is not a single species, but a species complex.

Initially, the complex was considered to consist of two different morphologically distinguishable morphotypes, namely 'S' (small) and 'L' (large), based on the average size difference of individu-

**Pap. Avulsos Zool., 2019; v.59: e20195921**
http://doi.org/10.11606/1807-0205/2019.59.21
http://www.revistas.usp.br/paz
http://www.scielo.br/paz
Edited by: Marcelo Veronesi Fukuda
Received: 12/10/2018
Accepted: 03/04/2019
Published: 23/04/2019

ISSN On-Line: 1807-0205
ISSN Printed: 0031-1049
ISNI: 0000-0004-0384-1825

als of comparable developmental sex and age. Segers (1995) formally recognized the two as belonging to different species-level taxa, and consequently re-established the names *Brachionus rotundiformis* Tschugunoff, 1921 and *B. plicatilis sensu stricto* (s.s.) for the S and L morphotypes, respectively.

Subsequently, more size-forms were recognized (Yúfera, 2001) and three clades that corresponded to three size groups were suggested, namely the Large (L), Small medium (SM) and Small small (SS) clades (Ciros-Pérez *et al.,* 2001; Mills *et al.,* 2017). The advent of low-cost DNA sequencing methodologies, and critically, the application of molecular-based species delimitation techniques (*e.g.,* Fontaneto *et al.,* 2015), allowed the recognition of more species at the *B. plicatilis* species complex. Currently, besides *B. plicatilis* s.s. and *B. rotundiformis,* the described species are *Brachionus ibericus* Ciros-Pérez, Gómez & Serra, 2001 and *Brachionus koreanus,* Hwang, Dahms, Park & Lee, 2013 as SM morphotypes, and *Brachionus manjavacas* Fontaneto, Giordani, Melone & Serra, 2007 and *Brachionus asplanchnoidis* Charin, 1947 as L-morphotype (Ciros-Pérez *et al.,* 2001; Fontaneto *et al.,* 2007; Hwang *et al.,* 2013; Michaloudi *et al.,* 2017; Mills *et al.,* 2017).

The actual number of species in the complex worldwide has been established to be at least fifteen (Mills *et al.,* 2017; Serra & Fontaneto, 2017). The recent overview by Mills *et al.* (2017) has confirmed the cosmopolitan distribution of each species thus refuting the suggestion that individual species may have narrower geographic distributions, but also noted gaps in the known distribution of the complex.

Studies on rotifer fauna of continental Ecuador are scarce and relatively recent (Koste & Bötterg, 1989, 1992). After these seminal papers, only a small number of studies detailing on rotifers from inland water bodies of Ecuador has been published (De Cruz, 1998; Torres & Palacios, 2007; Quimi, 2014; Valencia, 2015). Similarly, the rotifer fauna of the Galapagos Islands has been very little studied. Only three references are available, and none mentioned the *B. plicatilis* species complex (De Smet, 1989; Segers, 1991; López *et al.,* 2018).

In this paper, the presence of the rotifer *B. rotundiformis* from the *B. plicatilis* species complex is reported in Lake Arcturo, a saline crater lake located on Genovesa Island, the Galápagos Archipelago, Ecuador. This is the first record of this taxon for Ecuador and the first record of the species complex to Galápagos Islands.

## MATERIAL AND METHODS

### Sampled locality

Analyzed material was collected at Lake Arcturo (WGS84 coordinates: 00°19'40"N, 89°57'30"W) which sits at sea level on the center of Isla Genovesa, an uninhabited, 17 km$^2$ island located in the northeastern Galápagos Archipelago (Fig. 1). The lake is circular with 500 m in diameter, 27.5 m deep at its maximum depth, and is surrounded by steep, 60 m crater walls (Fig. 2). A dense red mangrove (*Rhizophora mangle* Linnaeus, 1753) thicket

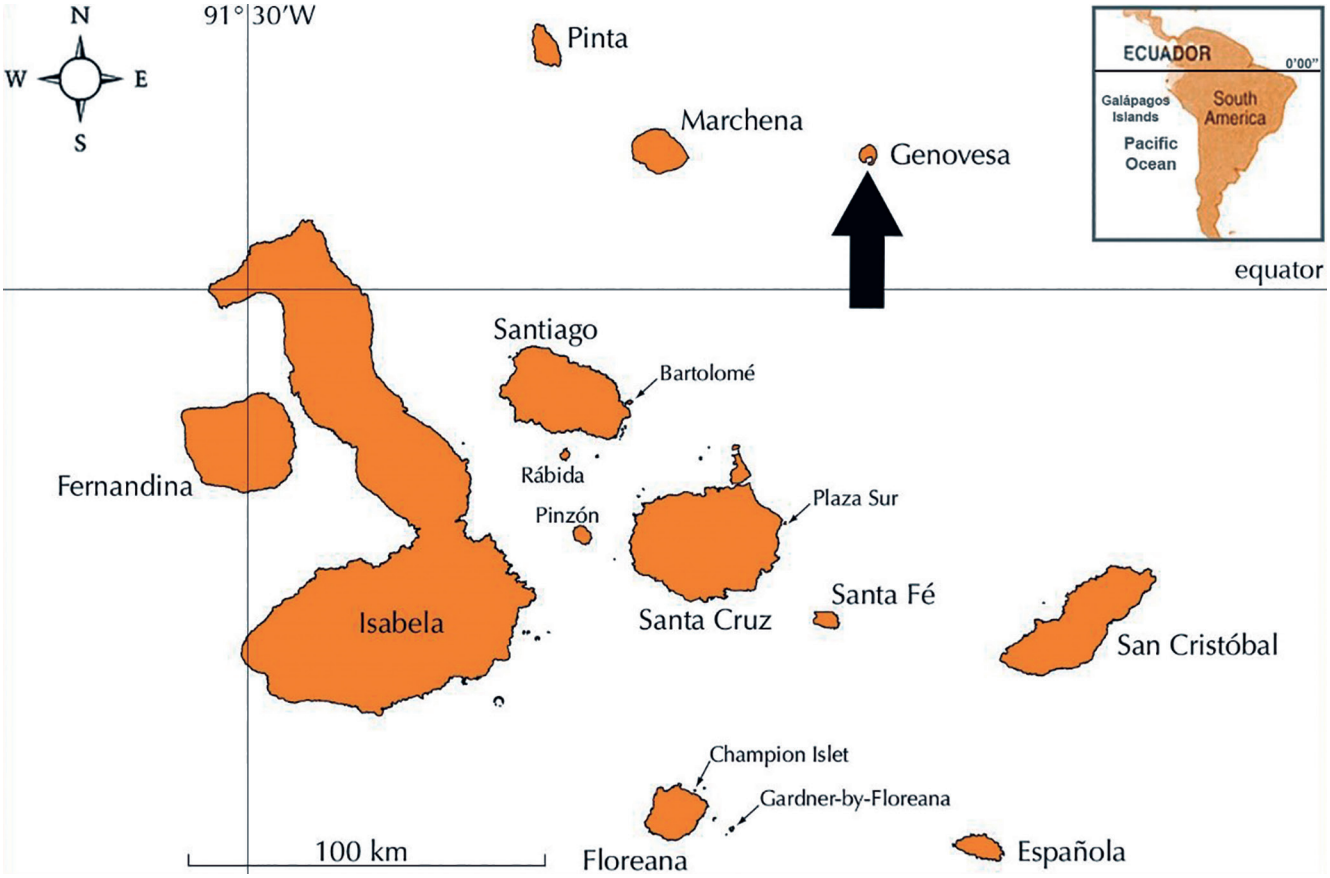


**Figure 1.** Map of the Galápagos Archipelago showing the location of the Genovesa Island and Lake Arcturo.

**Figure 2.** Photo of Lake Arcturo.

covers the lakeshore. The northeastern shore of the lake has a shallow-water beach area, whereas the southern part of the basin has much steeper bathymetry. Although the lake is connected to the ocean through many fissures in the island basalt a distinct seawater inlet is not obvious. Stable isotope values of water, as well as major cations and anions, indicate that the lake is hypersaline, with salinity roughly 1.5 times that of seawater (Conroy *et al.,* 2014). The lake is frequented by large numbers of seabirds and is an outstanding example of guanotrophy. Primary production in the epilimnion is high, and the hypolimnion is usually anoxic. The lake is probably stratified during the entire year (Walker & Likens, 1975; Bryhn, 2009). There appears to be no fish in the lake. The invertebrate fauna is dominated by corixid bugs and tanaid shrimps (Howmiller, 1969). More information on this lake is given in Conroy *et al.* (2014, 2015).

### Samples and examination of the material

Samples for the present study were collected on September 9th, 2004, using a standard conical plankton net (mesh size 63 µm). They were fixed with 4% formalin solution and the individuals of the *B. plicatilis* complex were analyzed under a Leitz Laborlux S optical microscope. Photographs for each of the 20 individuals were taken with an adjusted camera Canon Power shot A650 IS, and morphometric measurements were obtained using ImageJ (Abramoff *et al.,* 2004). A total of 18 lorica dimensions were measured based on Fu *et al.* (1991a), Ciros-Pérez *et al.* (2001), Proios *et al.* (2014), and Michaloudi *et al.* (2017). Examined material is deposited in the rotifers collection of Dr. Evangelia Michaloudi in Department of Zoology, Aristotle University of Thessaloniki, Thessaloniki, Greece. Other voucher specimens were deposited in the collection of aquatic invertebrates of Dr. Miriam Steinitz-Kannan in Department of Biological Sciences, Northern Kentucky University, Highland Heights, Kentucky, U.S.A, and in the rotifer collection of Dr. Carlos López in Escuela de Acuicultura y Pesquería, Universidad Técnica de Manabí, Bahía de Caráquez, Ecuador.

**Table 1.** Summary statistics (minimum, maximum, mean and standard error, in µm) for the selection of morphometric traits measured for the *B. rotundiformis* individuals from Lake Arcturo, Genovesa Island. Lorica measurements following Fu *et al.* (1991a); Ciros-Pérez *et al.* (2001); Proios *et al.* (2014); Michaloudi *et al.* (2017).

| * Lorica measurements | Min | Max | Mean | St. error |
|---|---|---|---|---|
| a | 130.83 | 199.81 | 164.29 | 17.08 |
| c | 107.44 | 152.52 | 123.92 | 14.63 |
| b | 64.08 | 103.11 | 84.70 | 10.77 |
| m | 39.90 | 73.96 | 57.01 | 8.52 |
| e | 18.89 | 30.89 | 23.65 | 4.03 |
| h | 6.73 | 20.64 | 14.43 | 3.79 |
| l | 8.97 | 19.56 | 14.29 | 3.65 |
| g | 5.31 | 12.42 | 10.03 | 2.19 |
| k | 17.02 | 24.68 | 20.58 | 2.08 |
| j | 20.38 | 29.31 | 24.44 | 2.97 |
| o | 18.60 | 27.67 | 23.36 | 2.92 |
| n | 8.71 | 26.12 | 14.52 | 4.68 |
| f | 14.61 | 28.15 | 21.88 | 3.59 |
| d | 0.00 | 26.90 | 15.33 | 7.43 |
| i | 60.92 | 100.46 | 84.16 | 11.13 |
| p | 10.59 | 30.43 | 22.51 | 6.03 |
| q | 15.25 | 25.37 | 20.14 | 3.07 |
| r | 12.375 | 23.64 | 15.94 | 2.73 |

## RESULTS AND DISCUSSION

Based on the morphometrical analysis as well as the morphology of the examined individuals (Fig. 3, Table 1), it is evident that the taxon found corresponds to the SS-type morphological group of the species complex. More specifically our specimens are characterized by the small lorica length (131-200 µm) that falls within the range described by Ciros-Pérez *et al.* (2001) for *B. rotundiformis,* as well as the pattern of the anterodorsal spines with the second spine being shorter (g: 5-12 µm) compared to the other two (h: 7-21 µm, j: 20-29 µm) (Table 1). The anteroventral side also corresponds well with the *B. rotundiformis* morphology (Fig. 3). This feature has recently been proven to hold strong diagnostic features in *Brachionus* species (Michaloudi *et al.,* 2017, 2018). Despite the clear morphological resemblance of our specimens with *B. rotundiformis* we should mention that Mills *et al.* (2017) phylogenetically recognized two clades in the SS group, namely clade SS1 corresponding to *B. rotundiformis,* and clade SS2 whose morphological identity is yet unknown.

The *B. plicatilis* species complex is considered cosmopolitan (Serra & Fontaneto, 2017) and is found in brackish and estuarine habitats of continental Ecuador (De Cruz, 1998; Torres & Palacios, 2007) and other regions of the Neotropics (Koste & José de Paggi, 1982; José de Paggi & Koste, 1995; Vásquez *et al.,* 1998; Garraffoni & Lourenço, 2012; Ferrando & Claps, 2016). Particularly in the Neotropical zone, the species *B. rotundiformis* has been reported only from Mexico (Sarma *et al.,* 2000), Brazil (Garraffoni & Lourenço, 2012) and Argentina (Ferrando & Claps, 2016). Of the two clades proposed by Mills *et al.* (2017) phylogenetically recognized in the SS group, the

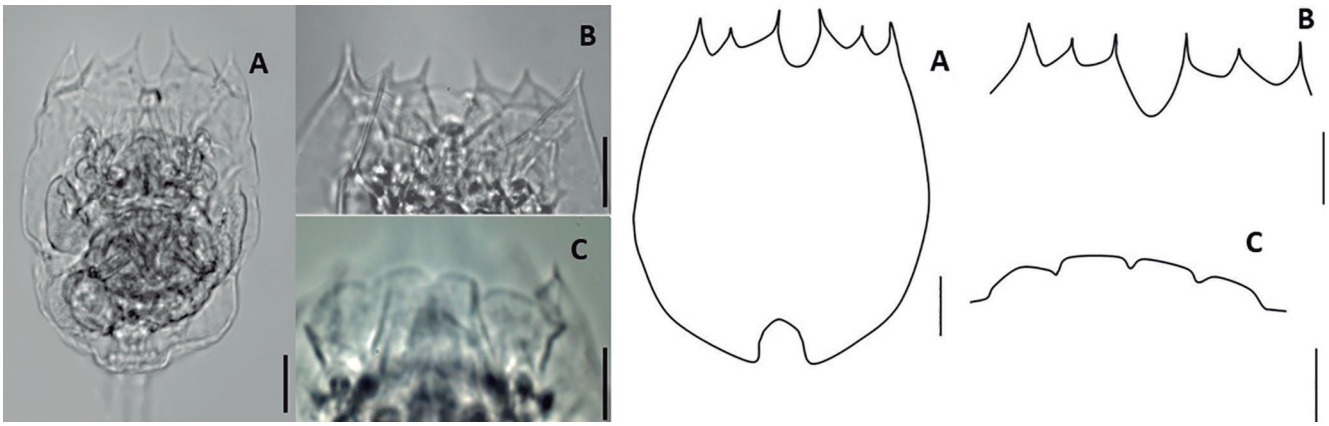


**Figure 3.** *Brachionus rotundiformis* microphotographs and drawings. (A) Dorsal view (B) anterodorsal margin; (C) anteroventral margin (Scale bars: 25 µm).

clade *B. rotundiformis* (SS1) is a widespread species found in all seven continents, while clade SS2 has only been recorded in two continents.

The presence of *B. rotundiformis* in Lake Arcturo in Genovesa Island is consistent with the great dispersal ability in this species complex (Serra & Fontaneto, 2017). Among the mechanisms of dispersion of rotifers indicated by Segers & De Smet (2008), passive transport by wind currents and zoochory by migrant birds emerge as most plausible factors in the process of colonization. In the Galápagos Archipelago, about 27 species of migrant birds are found (Windenfeld, 2006) and particularly Lake Arcturo is inhabited by large seabird populations (Howmiller, 1969). Since Genovesa Island is uninhabited, the effects of human activities are reduced to visiting tourists. However, the number of human visitors to the islands has increased exponentially in the last 30 years and may be associated with the recent colonization of *Keratella cochlearis* in San Cristóbal Island (López *et al.*, 2018). Still in the case of Genovesa, we hypothesize that human activities are less important in the introduction of rotifers compared to the other inhabited islands. More studies are required to understand the process of dispersion of this complex species in Galápagos islands. Due to the remoteness and isolation of the studied locality, this record of *B. rotundiformis* is particularly important in relation to the processes of dispersion and speciation of the species complex.

## ACKNOWLEDGEMENTS

Suggestions and criticisms of referees improved a first version of the manuscript. Thanks to staff of the Centro del Agua y Desarrollo Sustentable (CADS) of ESPOL for support in laboratory. Plankton collections on the Galápagos, including Lake Arcturo were funded by The US National Science Foundation, NOAA and National Geographic grants to MSK and her colleagues. We thank the Charles Darwin Research Station and the Galápagos National Park Foundation for providing logistic support including permits to study Lake Arcturo (Proyecto Nº PC 08-04). Special thanks for María Belén López for the help with the map.

**Authors' contribution:** MSK collection of samples; CL identified the material; EM, GW, HS confirmed identification, took the digital images, made drawings and made measurements; MSK, CL, EM, GW, SP, DF and HS wrote the text.

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Published with the financial support of the Committee of
"Programa de Apoio às Publicações Científicas Periódicas da USP" (SIBi-USP)