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***Brachionus leydigii* (Monogononta: Ploima) reported from the western basin of Lake Erie**

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Abstract

Several species of non-indigenous planktonic invertebrates have historically been introduced to the Laurentian Great Lakes. Previous introductions of non-indigenous planktonic invertebrates to the Great Lakes have been crustacean zooplankton, specifically Cladocera and Copepoda. This report documents the first known occurrence of *Brachionus leydigii* var. *tridentatus* (Zernov, 1901) in Lake Erie and possibly the first detection of a non-indigenous rotifer species in the Laurentian Great Lakes. The specimen was collected from a U.S. EPA monitoring station in the western basin of Lake Erie on April 4, 2016.

Keywords

Zooplankton; Great Lakes; New species record; Biological monitoring

Introduction

Brachionidae belongs to the Rotifera order Ploima and after Nottomatidae (with 23 genera) and Dicranophoridae (12 genera) is the third family with most numerous (eight) genera, and the fifth most specious family with 105 known species. Among the 8 genera of Brachionidae the genus *Brachionus* is the most specious with 37 known species. *Brachionus* is characterized by strong polymorphism resulting with many morphs in each species. *Brachionus leydigii* (Cohen, 1862) belongs to the sixth most polymorphic species of the genus and to the seventh largest species exceeding the length of 300 µm. *Brachionus leydigii* has four subspecies (*leydigii*, *quadratus*, *tridentatus*, *rotundus*) out of them *B. leydigii* var. *tridentatus* (Zernov, 1901) is known from the most diverse aquatic habitats including small and large reservoirs, large rivers, ponds, canals, ditches, polders, estuaries and in the littoral of lakes (Bł dzki, 1989; De Ridder and Segers, 1997). The morphology of the Lake Erie *B.*

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leydigii specimen warrants assignment to the subspecies *B. leydigii* var. *tridentatus*. Reports regarding the introduction of non-indigenous rotifers in the western hemisphere are sparse (De Paggi, 2002; Bezerra-Neto et al., 2004; Nicholls, 2016). We hereby report the first known occurrence of *B. leydigii* from Lake Erie and possibly, the first documented detection of a non-indigenous rotifer species in the Laurentian Great Lakes.

Methods

Sample collection and analysis

Zooplankton samples were collected across all five Laurentian Great Lakes aboard the U.S. Environmental Protection Agency's (U.S. EPA) *R/V Lake Guardian* as part of the U.S. EPA Great Lakes National Program Office's (GLNPO) long-term biological monitoring program. Samples were collected with 0.5 m diameter 63 μm and 153 μm mesh nets towed vertically through the water column following a standard operating procedure (EPA SOP, 2013) for field sampling. In the shallow western basin of Lake Erie, samples using both meshes were collected from 2 m above the lake bottom to the surface. Samples were preserved in a 4% buffered sugar formalin solution treated with rose bengal dye.

Rotifers are typically counted from the tow taken with the 63 μm mesh net as the larger 153 μm mesh net does not capture sufficient numbers of these small taxa. The single *B. leydigii* specimen was an exception, being found in the 153 μm mesh net sample during crustacean zooplankton enumeration. A Folsom plankton splitter was used to divide the sample in half and repeated until 200–400 individuals per sub-sample were reached. These two smallest subsamples were analyzed first. Second, two additional larger subsamples representing 2 and 4 times the split factor of the smallest subsample were analyzed for sub-dominant and rare taxa following a standard operating procedure (EPA SOP, 2016) for crustacean zooplankton sample analysis. After detecting *B. leydigi* in a subsample, the corresponding 63 μm mesh net sample from the same date and location was processed (methods below) for additional *B. leydigii* specimens without success.

In addition to the crustacean enumeration described above, the 63 μm mesh net sample was analyzed for microzooplankton following a different method. These organisms were counted from separate 1 mL aliquots withdrawn with a Hensen-Stempel pipette from the appropriate, thoroughly homogenized split. The goal is to enumerate 200–400 rotifers and copepod nauplii (dreissenid veliger are enumerated as well but are not included toward the 200–400 total) in an original count as well as a duplicate count. These are referred to as A and B counts. The 1 mL aliquot is placed in a Sedgwick-Rafter cell and covered with a glass coverslip. All microzooplankton are identified and enumerated under a compound microscope at 100 \times magnification following a standard operating procedure (EPA SOP, 2016) for microzooplankton sample analysis. Microphotographs were taken with an ACCU-SCOPE Excelis HD camera attached to an OLYMPUS CX41 compound microscope, extended depth of field images were created using CaptaVision PC Imaging Software. Specimen measurements were taken using an OLYMPUS CX41 compound microscope with a drawing tube and a GTCO CalComp DrawingBoard VI.

Results

A single female specimen of *B. leydigii* var. *tridentatus* was detected in a preserved plankton sample collected from the U.S. EPA monitoring station ER92 (41.951°N/82.68701°W) on April 4, 2016 at 01:36 AM Eastern Standard Time. Net tows at station ER92 were collected at a depth of 11 m to the water's surface. Twenty open water monitoring stations were sampled across Lake Erie and only one *B. leydigii* specimen was detected at a single station (Fig. 1). No additional *B. leydigii* specimens were detected at EPA monitoring stations in Lake Erie in the spring or summer of 2016. And no additional specimens of *B. leydigii* were collected from ER92 on April 5, 2017. Environmental conditions at the time of sample collection were as follows: water column was iso-thermal, water temperature was 5.3 °C, and chlorophyll-a concentration was 3.6 µg/L.

Based on the condition of the specimen's internal anatomy it is our judgement that this animal was alive at the time of collection. Lorica length of the specimen as measured from the anterior margin of the dorsal plate (not including the anterior spines) to the posterior margin of the dorsal plate (not including the posterior spines) was 222 µm. Lorica width of the specimen as measured at the widest point of the lorica was 215 µm. The lengths of the anterior spines as measured from the base to the distal ends were as follows; anteriomedian spines measured 37 µm (left) and 43 µm (right), anteriointermediate spines measured 24 µm (left) and 25 µm (right), anteriolateral spines measured 17 µm (left) and 22 µm (right). The length of the medial posterior spine as measured from the base to the distal end was 14 µm. The foot while only partly extended measured 86 µm long and the toes measured 14 µm (left) and 19 µm (right) long.

Morphological description

The morphology of *B. leydigii* is fairly unique and recognizable within the Great Lakes *Brachionus* assemblage. In comparison to native Great Lakes *Brachionus* species, *B. leydigii* var. *tridentatus* has a somewhat square lorica (Fig. 2a), comprised of 3 plates: dorsal, ventral, and basal. The lorica surface is finely textured with a distinctive but relatively subtle polygonal pattern which may also help distinguish it from the nominal form (Fig. 2b). The anteriodorsal margin is characterized by 6 spines (Fig. 3a) with the anteriomedian spines longest and curved somewhat outward. The anterioventral margin is characterized by a relatively shallow medially placed U-shaped sinus (Fig. 3b). The posterior margin is characterized by small posteriolaterally placed spines (Fig. 4a). The ventral foot opening is relatively large and club-shaped (Fig. 4b). Three broad based posterior spines are placed directly below the foot opening, the medial spine being the shortest (Fig. 5). The foot is annulated and fairly long if fully extended and terminates in two toes (Fig. 5).

Discussion

The Lake Erie specimen was initially identified as *B. leydigii* (Cohen, 1862) based on Ahlstrom (1940). Subsequently, the specimen was determined to be the infrasubspecific variant *B. leydigii* var. *tridentatus* based on Kutikova (1970) and Koste (1978). The nomenclature *B. var. tridentatus* (Zernov, 1901) was assigned to the specimen in question in reference to the Rotifer World Catalog website (Jersabek and Leitner, 2013) and Segers et al.

(2015) which both noted *Brachionus leydigii* var. *tridentatus* as a “junior subjective synonym” of *Brachionus leydigii* (Cohn, 1862). The genus *Brachionus* is large, specious and well known for morphological variability; in the Laurentian Great Lakes 12 species have previously been reported (Grothe and Grothe, 1977; Stemberger, 1979; Jersabek et al., 2003). The Great Lakes native *B. variabilis* could potentially be mistaken for *B. leydigii* based on the 6 anterior spines, somewhat similar shaped lorica, and posterior protuberance placed dorsally to the foot opening. But *B. variabilis* is characterized by a flattened or rounded posterior protuberance rather than pointed or triangular broad based posterior spines (Fig. 5) as present in *B. leydigii*. In open water areas of the Great Lakes, *Brachionus* species are most abundant during summer months in the western basin of Lake Erie (Barbiero and Warren, 2011). Members of the genus are primarily littoral and often associated with eutrophic environments (Stemberger, 1979). Only a single female specimen of *B. leydigii* var. *tridentatus* was detected from Lake Erie in 2016 and no evidence of reproduction such as carried eggs were observed associated with the specimen. If the species is newly introduced it is likely that planktonic densities are still very low and may elude detection with most subsampling enumeration methods. For these reasons the establishment status of this species in Lake Erie cannot be determined and is therefore unknown. However, it should be stated that the ability of rotifers to reproduce parthenogenetically can allow for rapid colonization of new habitats (Wallace et al., 2006).

B. leydigii is widely distributed in the eastern hemisphere including reports from Europe (Sládek, 1983), Asia (Jersabek and Bolortsetseg, 2010), and Australia (Koste and Shiel, 1987). *B. leydigii* has even been reported from some saltwater environments including the Baltic Sea, the North Sea (Fontaneto et al., 2006) and saline lakes (Viayah and Špoljar, 2012). Kutikova (1970) reported the infrasubspecific variant *B. leydigii* var. *tridentatus* from large lakes including Lake Baikal. De Ridder and Segers (1997) suggested *B. leydigii* var. *tridentatus* is only known to the Palearctic and is widely distributed in this zoogeographic region; across much of Europe into the Caspian region.

The native distribution of *B. leydigii* and its subspecies are poorly understood in the western hemisphere, but some reports are likely credible. Kofoid (1908) reported *B. leydigii* from the Illinois River as occurring from May until August. However, with no sketches associated with the Kofoid (1908) report, the species cannot be verified with certainty. Ahlstrom (1934) reported *B. leydigii* (Cohen, 1862) from Florida within a list of rotifers surveyed in the state but did not include descriptions or sketches of the specimens collected. Ahlstrom (1940) in reference to *B. leydigii* remarked “I have seen material from England, Sweden, India, China, and? Ohio” but made no mentions of his 1934 reference to the species presence in Florida. Ahlstrom (1940) included sketches and measurements of all *B. leydigii* specimens mentioned except for the specimens reported from Ohio, making it impossible to verify the Ohio note. Dumont (1983) did report *B. leydigii* (Cohen, 1862) as present in North America but based on Ahlstrom’s uncertain 1940 Ohio report. Reports of *B. leydigii* in North America are sparse and date primarily to the early twentieth century (Kofoid, 1908; Ahlstrom, 1934; Ahlstrom, 1940). *B. leydigii* has not been previously reported from South American waters (Dumont, 1983). Ahlstrom’s, 1934 inclusion of *B. leydigii* (Cohen, 1862) on a list of rotifers surveyed in the state of Florida suggests *B. leydigii* may be native to North America. Additionally, *B. leydigii* has been reported from the River Antigua in Vera-

cruz, Mexico further supporting the presence of this species in the Western Hemisphere (Nandini et al., 2017).

Ballast associated collections of *B. leydigii* have been reported from the Great Lakes in recent years. A single specimen of *B. leydigii* (Cohen, 1862) was collected in ballast water from the upper wing ballast tanks of a trans-oceanic vessel in Hamilton Harbor, Lake Ontario (Bailey et al., 2005b). Additionally, specimens of *B. leydigii* were successfully hatched from the residual ballast sediment of four trans-oceanic vessels entering the Great Lakes (Bailey et al., 2005a). The detection of *B. leydigii* var. *tridentatus* from plankton in the western basin of Lake Erie may possibly be the result of a ballast related introduction. If so, this and other recent detections of non-indigenous species (Connolly et al., 2017) in Great Lakes may indicate that the Great Lakes are still vulnerable to species introductions. Microscopic taxa in particular, may be easily transported by various vectors and present a risk for introductions which may go unnoticed without regular biological monitoring efforts.

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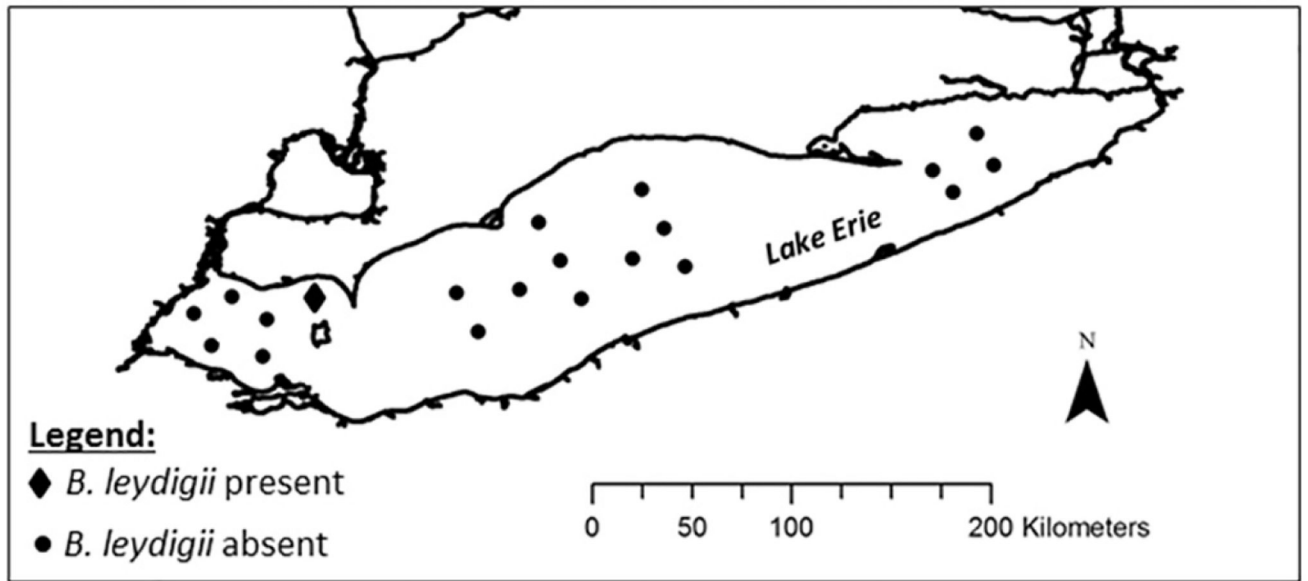


Fig. 1.
U.S. EPA GLNPO long-term monitoring stations in Lake Erie.

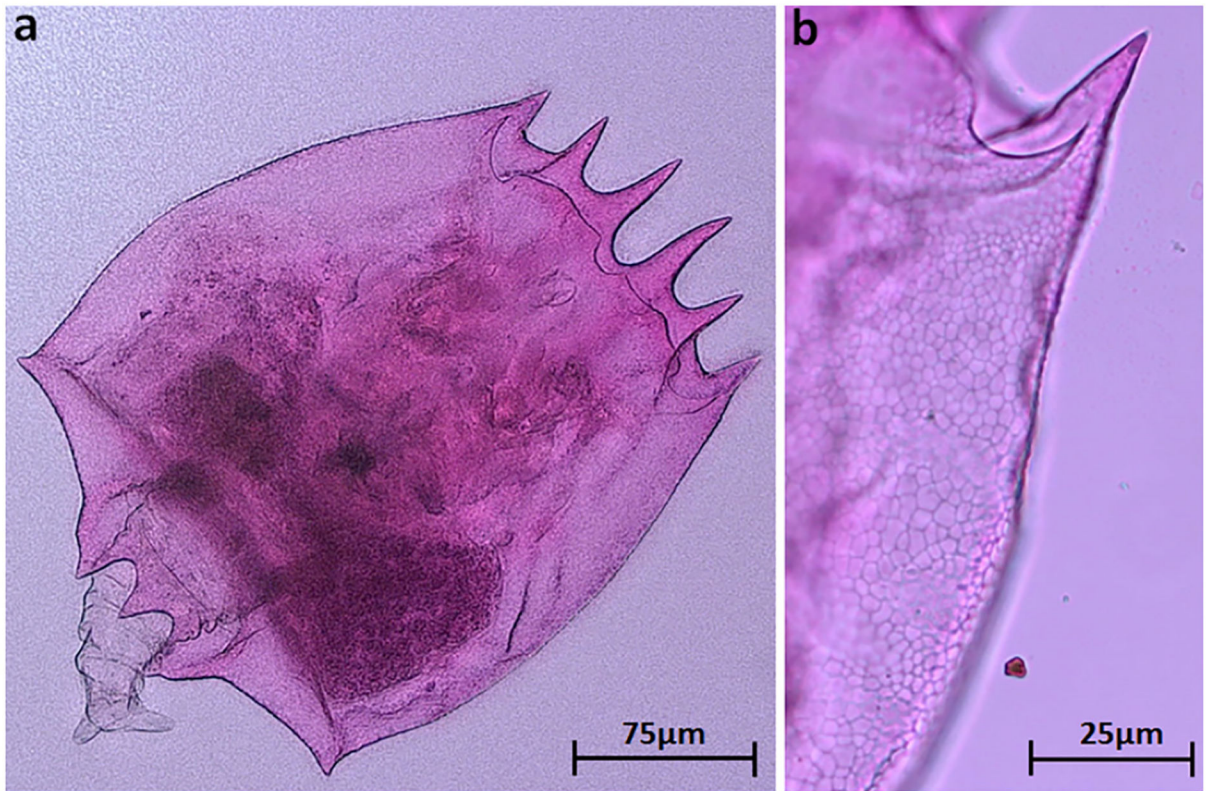


Fig. 2.
(a) Extended depth of field image of Lake Erie *B. leydigii* var. *tridentatus* specimen. (b) Polygonal pattern on lorica surface of *B. leydigii* var. *tridentatus*.

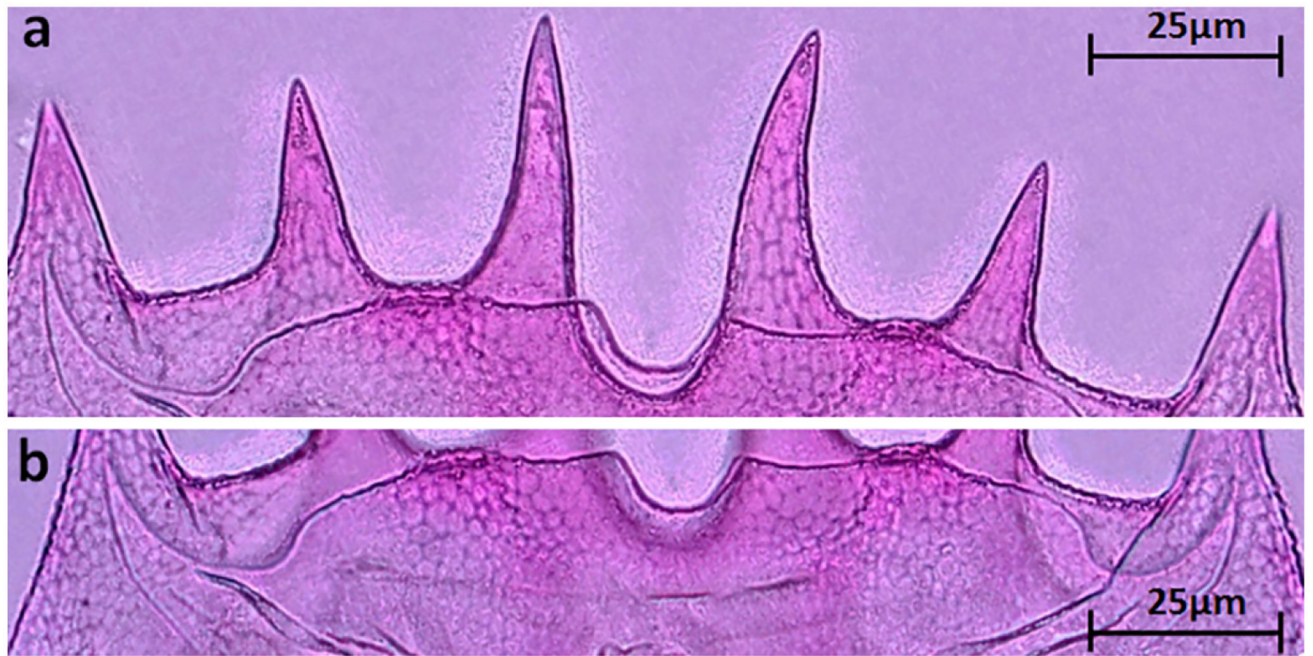


Fig. 3.
(a) Anterior spines on dorsal plate of *B. leydigii* var. *tridentatus*. (b) Anterior margin of ventral plate of *B. leydigii* var. *tridentatus*.

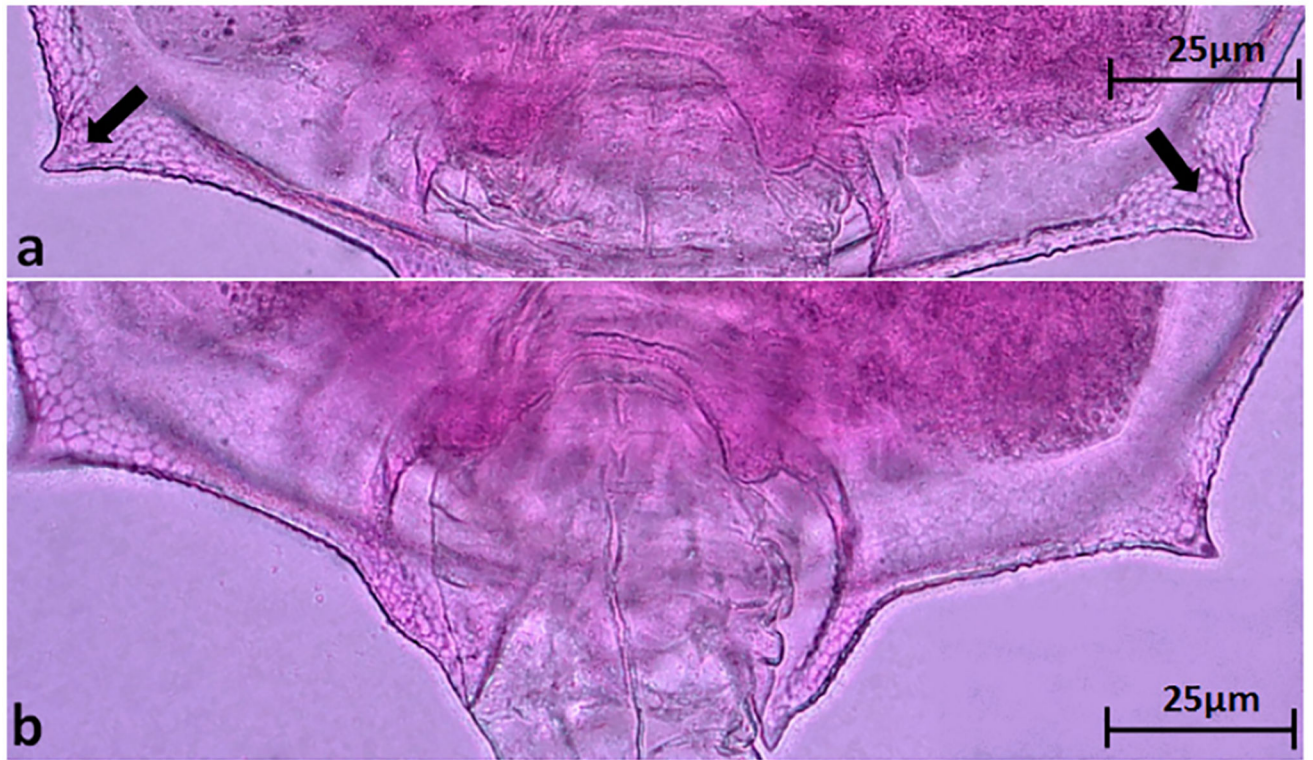


Fig. 4.

(a) Small posteriolateral spines on *B. leydigii* var. *tridentatus*. (b) Club-shaped foot opening on posterior margin of ventral plate of *B. leydigii* var. *tridentatus*.



Fig. 5. Posterior spines on posterior margin of dorsal plate of *B. leydigii* var. *tridentatus*; Partly extended foot and toes of *B. leydigii* var. *tridentatus*.