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Eurytemora carolleeae in the Laurentian Great Lakes revealed by phylogenetic and morphological analysis

Adrian A. Vasquez [a,*], Patrick L. Hudson [b], Masanori Fujimoto [a], Kevin Keeler [b], Patricia M. Armenio [b], Jeffrey L. Ram [a]

[a] Department of Physiology, Wayne State University School of Medicine, 5374 Scott Hall, 540 E. Canfield St., Detroit, MI 48201, USA
[b] US Geological Survey, Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, USA

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ABSTRACT

In the Laurentian Great Lakes, specimens of *Eurytemora* have been reported as *Eurytemora affinis* since its invasion in the late 1950s. During an intensive collection of aquatic invertebrates for morphological and molecular identification in Western Lake Erie in 2012-2013, several specimens of *Eurytemora* were collected. Analysis of these specimens identified them as the recently described species *Eurytemora carolleeae* Alekseev and Souissi 2011. This result led us to assess *E. carolleeae's* identifying features, geographic distribution and historical presence in the Laurentian Great Lakes in view of its recent description in 2011. Cytochrome oxidase I (COI) DNA sequences of *Eurytemora* specimens were identified as closer (2 - 4% different) to recently described *E. carolleeae* than to most *E. affinis* sequences (14% different). *Eurytemora* from other areas of the Great Lakes and from North American rivers as far west as South Dakota (Missouri River) and east to Delaware (Christina River) also keyed to *E. carolleeae*. Morphological analysis of archival specimens from 1962 and from all the Great Lakes was identified as *E. carolleeae*. Additionally, *Eurytemora* drawings in previous publications were reassessed to determine if the species was *E. carolleeae* and are reported here. Additional morphological characters that may distinguish North American *E. carolleeae* from other taxa are also described. We conclude that *E. carolleeae* is the correct name for the species of *Eurytemora* that has inhabited the Great Lakes since its invasion, as established by both morphological and COI sequence comparisons to reference keys and sequence databases in present and archival specimens.

Introduction

In the Laurentian Great Lakes, introductions of non-native copepods have occurred over several decades (Engel, 1962; Horvath et al., 2001; Hudson and Bowen, 2002). However, some publications that list copepods have either mischaracterized their native distribution (Drake and Lodge, 2007a, 2007b; Mills et al., 1993) and/or the taxonomy of the species (Reid and Hudson, 2008). The introduction of the estuarine copepod *Eurytemora* to the Great Lakes was noticed quickly because it is easily distinguished from native calanoid copepods by its long caudal ramus, long pointed metasomal wings, and relatively shorter antennae. *Eurytemora* was likely introduced to the Great Lakes due to the construction and opening of the St. Lawrence Seaway since introductions of many non-native freshwater tolerant marine taxa coincided with the opening of the Seaway or followed shortly thereafter (Mills et al., 1993). *Eurytemora* sp. was first recorded in Lake Ontario at the genus level in 1958 (Anderson and Clayton, 1959) and thereafter reported as *Eurytemora affinis* Poppe 1880 in all the Great Lakes (Mills et al., 1993). However, the recent description (Alekseev and Souissi, 2011) of *Eurytemora carolleeae* Alekseev and Souissi, 2011 raised questions about the appropriate identification of *Eurytemora* populations in the Great Lakes which the present study seeks to answer.

Significant work has been completed in studying the life history, mechanisms of invasion and biogeography of *Eurytemora* taxa in North America (Cabrol et al., 2015; Dodson et al., 2010; Favier and Winkler, 2014; Lee, 1999; Lee and Frost, 2002; Posavi et al., 2014; Winkler et al., 2008). *Eurytemora*, typically identified as *E. affinis*, has been known to play an important role as a dominant grazer in marine, estuarine, and freshwater systems and is considered to be a cosmopolitan species due to its broad biogeographic range encompassing subtropical to subarctic areas (Lee, 2000; Suarez-Morales et al., 2008). Historically, this coastal-estuarine copepod was considered to be a marine species (Croskery, 1978). Nevertheless, surveys within freshwater systems in North America and Mexico have identified *Eurytemora* clades far from the marine coastline (Lee and Frost, 2002; Suarez-Morales et al., 2008). Evolutionary and physiological osmoregulatory adaptations may have enabled *Eurytemora* taxa to invade freshwater environments from its typical saline habitats (Johnson et al., 2014; Lee, 1999; Posavi et al., 2014).

* Corresponding author.
E-mail address: adrian.amelio.vasquez@gmail.com (A.A. Vasquez).

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E. affinis has a geographic range that spans the northern hemisphere and habitat types that range from hypersaline salt marshes to fresh water suggesting a cryptic species complex (Dodson et al., 2010). Sequences of the mitochondrial cytochrome oxidase I (COI) gene have been shown to be very useful for distinguishing calanoid and harpacticoid copepods including cryptic and sibling species in biogeographic studies (Gutierrez-Aguirre et al., 2014; Laakmann et al., 2013; Miracle et al., 2013; Peterson et al., 2013). Previous genetic analyses of the COI gene in *Eurytemora* populations described specimens from the Great Lakes as belonging to an Atlantic clade of *E. affinis* (Lee and Frost, 2002; Winkler et al., 2008). Phylogenetic analysis of North American *Eurytemora* collected from several marine and freshwater sites, including specimens from Lake Michigan and the Detroit River, revealed several distinct clades but did not distinguish any differences in the morphological characters of the specimens associated with the different clades using keys available at that time (Lee and Frost, 2002). Recently, Alekseev and Souissi (2011) identified *E. carolleeae* as a previously undescribed sibling species to *E. affinis* native to the North American Atlantic coast, with distinct characters to enable its morphological identification. *E. carolleeae* was also reported to be a potentially new invasive copepod in the Baltic Sea and European Atlantic coast estuaries first based on COI sequence data and then through taxonomic identification (Alekseev et al., 2009; Sukhikh et al., 2013). *E. carolleeae* observations in North America were from the Chesapeake Bay and the St. Lawrence estuary with the possibility of distributions in the inland waters of the Great Lakes to Mexico (Alekseev and Souissi, 2011). COI sequence data was used to corroborate the morphological identification of the *E. carolleeae* invasion of the Baltic Sea and European Atlantic coast estuaries (Sukhikh et al., 2013). These recent analyses indicated more than one species of *Eurytemora* contributed to the Great Lakes invasion, which led us to re-examine the classification of *Eurytemora* specimens collected in the Great Lakes.

In order to determine which *Eurytemora* species or clade had invaded the Great Lakes, this present study used morphological and COI molecular barcoding methods to identify *Eurytemora* taxa. Morphological analysis was carried out for archival specimens from the Great Lakes dating back to 1962, and we reviewed drawings and photographs in past literature. Additionally, this paper describes our analysis of samples collected in 2012–2014 from the Great Lakes and from rivers as far west as South Dakota and east to Delaware to determine the possible distribution and morphological variation associated with this species complex.

Methods

Sampling

Specimens of *Eurytemora* came from various locations in the Great Lakes including western Lake Erie, Detroit River, Lake St. Clair, Lake Huron, Lake Michigan (including from Muskegon Lake, an estuarine lake of Lake Michigan (Weinke et al., 2014)), and two river systems, Christina River in Delaware and Missouri River (Lewis and Clark Lake) in South Dakota (Fig. 1). Plankton samples from western Lake Erie were collected using a hand-thrown Wisconsin plankton tow net with a 80 μm mesh (Wildco, USA) during the summer of 2012 and 2013 at sites in and near Toledo Harbor, Ohio USA. Fourteen sites were repeatedly sampled over the 2012 and 2013 summer months beginning in May and ending in August (see Electronic Supplementary Material (ESM) Appendix S1). Samples were split and preserved in 80% ethanol for molecular analysis and in Lugol's solution for morphological analysis. The sample in Lugol's solution was shipped to EcoAnalysts (Moscow, ID) for taxonomic analysis. Sampling during 2014 was limited to spot locations using either a Wisconsin net near shore or in shoreline aquatic vegetation using a bucket and multiple grabs, filtered with an 80 μm sieve and stored in 91% isopropyl alcohol.

Taxonomy

For identifying specimens of *Eurytemora* we used several characters to separate *E. carolleeae* from its congener *E. affinis*, including a large outside dent on the mandible and setal segmentation on the caudal rami and swimming legs, which we documented in some of our specimens. However, for routine separation we chose to use the wing-like outgrowths on the genital double-somite (Fig. 2a) and a small spine near the distal seta insertion point in P5 (Fig. 2b) in females, and the naked caudal rami (Fig. 2c) and cylindrical shape (length/width (L/W) ratio > 1.3) of the second segment of the exopod (also known as the basipod) on the left P5 (Fig. 2d) in the male to identify specimens of *E. carolleeae* as described in Alekseev and Souissi (2011). These characters were either easily seen under a dissecting microscope or when the P5 was placed under a coverslip on a slide and viewed under a compound microscope at higher magnification. In addition, Great Lakes specimens from the US Geological Survey Great Lake Science Center collections of alcohol preserved plankton samples and specimens archived on microscope slides were examined (Table 1) using the same characteristics. A similar analysis was applied, when possible, to drawings and photographs in descriptions of *Eurytemora* in previous publications from studies in the Holarctic region (see list in Table 2).

To further characterize *E. carolleeae* morphologically, the presence/absence and placement of setae on the fifth leg of female and male specimens were analyzed. To supplement this, drawings of the female and male fifth legs of *Eurytemora* in references listed in Table 2 were reviewed for setae presence and placement. Dr. Eduardo Suarez-Morales from El Colegio de la Frontera Sur (ECOSUR), Chetumal, Mexico assisted us by further confirmation of his observations of setae placement and contributed additional morphometrics of the female and male fifth legs of his specimens reported in Suarez-Morales et al. (2008) and evaluated morphological differences. *Eurytemora* specimens analyzed by Dr. Suarez-Morales are deposited in the collection of Zooplankton of ECOSUR under Colina Lake ECO-CHZ-03662, and Balmorhea Lake ECO-CHZ-03440, 03441. Comparisons to the specimens in this study were used to investigate a basis for possible diagnostic characters to further separate the *E. affinis* complex.

DNA extraction

Individual ethanol-preserved specimens were lysed in ATL lysis buffer (cat. no. 19076, Qiagen, Hilden, Germany), with Proteinase K (cat. no. 19133, Qiagen, Hilden, Germany), followed by DNA purification with the DNAeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) and Qiagen Spin Columns according to standard protocols (https://www.qiagen.com/us/resources/resourcedetail?id=6b09dfb8-6319-464d-996c-79e8c7045a50&lang=en). Elution with Low TE (Invitrogen, Carlsbad, CA) used a small volume (28 μl) since the resultant purified DNA is from a single microscopic organism.

Polymerase chain reaction (PCR)

Purified DNA was amplified by PCR using COI forward primer HCO2198 and reverse primer LCO1490 (Folmer et al., 1994) prepared as stock solutions of 10 pmol/μl. DNA was added to PCR reactions at a quantity of 1.5 μl per 25 μl reaction. PCR master mix contained 0.5 μl of each forward and reverse primer stock solutions, 12.5 μl of SSO Advanced Universal SYBR Green Supermix (BioRad, Irvine, CA), and 10 μl sterile water. Reactions were run on an iCyclerQ Realtime thermocycler (BioRad, Irvine, CA), initiated by heating to 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, 51 °C for 30 s, 72 °C for 1 min, and then a final extension of 72 °C for 7 min followed by a hold at 15 °C until further processing within 3 h. PCR products were visualized on 1% agarose gels with SYBR Safe DNA Gel Stain (Invitrogen, Grand Island, NY), and images were documented with a DarkReader