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NOTE

Range Expansion of the Exotic Zooplankter *Cercopagis pengoi* (Ostroumov) into Western Lake Erie and Muskegon Lake

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ABSTRACT. *Previously reported from Lakes Ontario and Michigan, the nonindigenous zooplankter* Cercopagis pengoi *was found for the first time in western Lake Erie, the Detroit River, and Muskegon Lake, Michigan, during summer 2001. A native of the Ponto-Caspian region,* C. pengoi *is currently expanding its range in North America. Analysis of mitochondrial gene ND5 sequences confirmed that the Lake Erie haplotype is identical to that reported previously from Lakes Ontario and Michigan and the Finger Lakes, New York. These findings support the hypothesis that* C. pengoi's *range expansion in the Great Lakes likely resulted from inter-lake transfer of ballast water, rather than from additional introductions from European locations. Pleasure-craft traffic operating between Lake Michigan and Muskegon Lake is likely responsible for this inland transfer of* Cercopagis, *a trend that likely will increase due to human activities.*

INDEX WORDS: Cercopagis pengoi, *genetics, size structure, Lake Erie, nonindigenous species, cladocera, zooplankton.*

INTRODUCTION

Nonindigenous species (NIS) invaded the Great Lakes at an alarming rate during the 20th century, largely as a consequence of ballast water discharge by transoceanic ships (Ricciardi 2001). Currently, the Great Lakes are home to at least 162 nonindigenous species of fish, invertebrates, pathogens, plants, and algae (Mills *et al.* 1994, Ricciardi 2001). Approximately 70% of NIS discovered in the Great Lakes since 1985 are native to the Ponto-Caspian region of southeastern Europe, a region encompassing the Black, Azov, and Caspian seas (Ricciardi and MacIsaac 2000). Many of these invasions appear to be the result of the establishment of invasion corridors that either directly or indirectly link the Great Lakes with Ponto-Caspian waterbodies (MacIsaac *et al.* 2001). In addition, it is possible that some Ponto-Caspian invaders, including zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. bugensis*), may have facilitated subsequent invaders, a process termed "invasional meltdown" (Simberloff and Von Holle 1999, Ricciardi 2001). Several NIS introduced to North America from the Ponto-Caspian region either rapidly expanded their range after establishing in the lower Great Lakes (*D. polymorpha*) or are in the early stages of this process (*Echinogammarus ischnus, D. bugensis*; Nalepa *et al.* 2001).

One of the newest Ponto-Caspian invaders to the Great Lakes is the "fishhook" waterflea *Cercopagis pengoi* (MacIsaac *et al.* 1999). This species was initially discovered in Lake Ontario in 1998, though by the following year had spread to upper and lower

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Lake Michigan and to the Finger Lakes region of New York (Ojaveer *et al.* 2001, Makarewicz *et al.* 2001, Charlebois *et al.* 2001). It is potentially dispersed alive in ballast or other waters, or via movement of its resting eggs either naturally (waterfowl or fish) or by human-mediated vectors (contaminated fishing line, ballast water, etc.). Confirmed in this study is further range expansion of *C. pengoi* in the Great Lakes and an adjacent inland waterbody, and an examination of the genetic identity of these individuals to determine whether additional introductions from Eurasia have occurred.

METHODS

Vertical (64 µm mesh) and horizontal (253 µm mesh) plankton samples were collected using 0.5 m diameter plankton nets during summer 2001. Daytime horizontal tows were conducted for a period of 10 minutes, at idle speed (1m/sec) and ~2 m depth, on 17 August 2001. Daytime vertical tows were made of the whole water column on ten sampling dates between 8 August and 6 September 2001 at eight sampling sites in western Lake Erie. Additional daytime vertical hauls of the entire water column or horizontal tows (5 minutes at ~2 m depth) were collected at six locations between the upper Detroit River and lower Lake Huron on 30 August 2001 using the 253 µm mesh plankton net. Additional vertical hauls and horizontal tows were made on 8 September 2001 using the 253 µm mesh plankton net in the western basin of Muskegon Lake. This inland waterbody is connected to Lake Michigan where *C. pengoi* has been reported previously. Samples were preserved using either 4% sugared formalin or, for samples to be used in genetic analyses, 95% ethanol. Zooplankton was enumerated using dissecting microscopes at 6× to 40× magnifications (Leica and Wild). Body length measurements of *C. pengoi* were made from the top of the head to the base of the caudal process after Grigorovich *et al.* (2000) using an image analysis system (Vision Gauge).

Total DNA was extracted from *C. pengoi* using standard proteinase K methods (Makarewicz *et al.* 2001, Cristescu *et al.* 2001). A primer pair for *C. pengoi* was developed based on published mitochondrial NADH dehydrogenase subunit 5 (mtND5) sequences (Cristescu *et al.* 2001). A 666-base pair (bp) fragment of the mtND5 gene was amplified using the primers CerPND5 (5′—TT TAT ATC GGG ATT AGT GGC—3′; and 5′—TCC CCA TGA GAT ACT GTA AGC—3′). The balance of the PCR was double-distilled water, manufacture-supplied PCR buffer, $MgCl_2$, dNTPs, and Platinum *Taq* DNA polymerase (GibcoBRL). Reactions were run at an annealing temperature of 50°C (60 seconds) for 40 cycles. A 72°C extension cycle (90 seconds) and a 94°C denaturing cycle (60 seconds) were also used in a PTC-225 Peltier Thermal Cycler (MJ Research). The PCR product was purified and sequenced. Sequences obtained were aligned with published sequences stored in GenBank using OMIGA (1.1).

RESULTS AND DISCUSSION

Cercopagis pengoi was collected at three sites in the western basin of Lake Erie—two sites in open water and one near the mouth of the Detroit River—as well as at one site in the Detroit River (Fig. 1). Population density was very low, averaging 1 ind./m^3 at two of the sampling sites (Sites C and D, Fig. 1). However, *C. pengoi* dominated collections in the lower Detroit River and in western Lake Erie near the river inflow (Sites A and B, Fig. 1) where the species accounted for ~26% of macro-

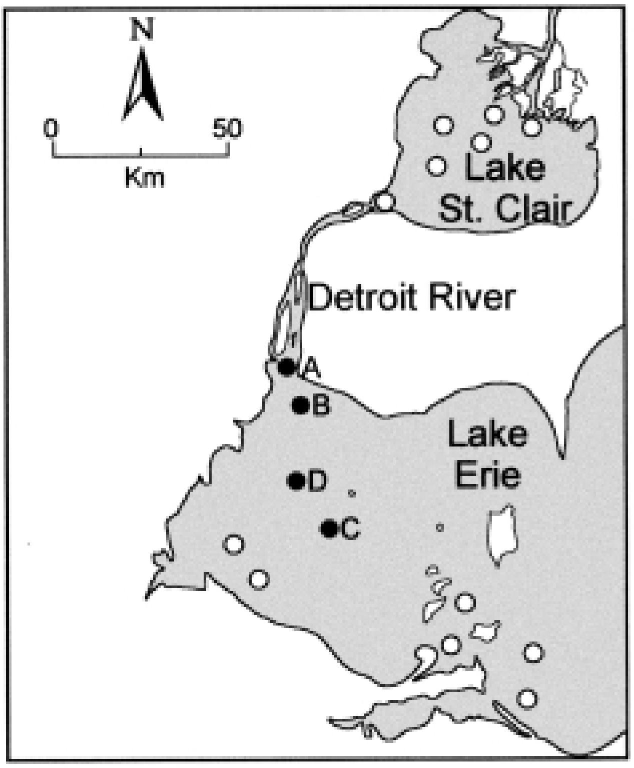

FIG. 1. Sampling sites in the western basin of Lake Erie and the Detroit River where **Cercopagis** ***was collected (filled circles). Sampling sites where*** **C. pengoi** ***was not found also are shown (open circles).***

TABLE 1. Mean (± SD) body lengths (mm) of female* Cercopagis pengoi *collected at four sites in western Lake Erie and the Detroit River, exclusive of the caudal appendage. Number of individuals in each class is provided.

	Sampling sites with *Cercopagis* shown in Figure 1			
Developmental stage	Site A	Site B	Site C	Site D
instar I female (reproductive status undeterminable)	0.77 ± 0.10 (n = 8)	0.85 ± 0.06 (n = 13)	0.92 ± 0.17 (n = 5)	
instar I parthenogenetic female	0.85 ± 0.02 (n = 3)	0.78 ± 0.15 (n = 4)		
instar II parthenogenetic female	1.04± 0.12 (n = 14)	1.06 ± 0.14 (n = 12)		
instar III parthenogenetic female	1.33 ± 0.20 (n = 60)	1.41 ± 0.18 (n = 73)	1.18 (n = 1)	1.13 (n = 1)
sexual female	1.35 (n = 1)	1.48 ± 0.28 (n = 3)		

zooplankton (> 200 µm) abundance and had an approximate density of 226 ind./m^3. Body lengths of individuals were consistent among sites (Table 1). No significant differences were detected with respect to female body length among sites (ANOVA, $F_{1,8}$ = 0.067, p = 0.803), although body size varied by instar stage (Table 1). This finding was independent of sample location (Table 1). Also, two males were collected at Site B; one instar I (0.7 mm) and one instar III (1.2 mm). Combining both sexes and all instar stages, mean body length ± SD was 1.27 mm ± 0.28 (n = 107) for Site B, and 1.22 mm ± 0.26 (n = 86) for Site A. As expected, body size of *C. pengoi* varied by instar stage, a finding consistent with previous studies on the population structure of this species (Grigorovich *et al.* 2000). Furthermore, individuals from Lake Erie and the Detroit River were similar in body size to those reported in Lake Ontario (Makarewicz *et al.* 2001). The presence of sexual females and males suggests that resting eggs, and potentially a "seed bank" for population development in future years, were likely produced in western Lake Erie. Additional sampling in 2001 in Lake Superior (12 sites, I. Duggan, pers. comm.), and Lakes St. Clair and Huron (this study) failed to detect the presence of *Cercopagis*. However, based on range expansion patterns noted for *Bythotrephes*, *Cercopagis* is predicted to establish in these lakes in the near future.

Individuals collected from the western basin of Lake Erie and sequenced for the mitochondrial ND5 gene were the same haplotype as those reported from Lake Ontario, Lake Michigan, and the Finger Lakes, NY (Cristescu *et al.* 2001). The presence of the same haplotype throughout the Great Lakes region suggests the basin was colonized only once, following which the species has been dispersed within the basin. Lake Erie receives very little ballast discharge, either of saline water from "Ballast On Board" (BOB) vessels or those that enter the Great Lakes in "No Ballast On Board" (NOBOB) status with only residual water (Colautti *et al.* 2003). It is possible that the Lake Erie population originated from the Baltic Sea, where the same haplotype is fixed; however based on the proximity of invaded sources upstream and downstream in the Great Lakes, and on patterns in shipping traffic (Colautti *et al.* 2003), this is unlikely.

Cercopagis pengoi also was detected in Muskegon Lake, adjacent to Lake Michigan. The examined sample contained both parthenogenetic females (stages I [n = 110], II [n = 16], and III [n = 36]) and males (stages I [n = 38] and II [n = 2]). Population density was low, approximately 1 ind/m^3. This is the first report of *Cercopagis* to an inland waterbody outside of the Finger Lakes region of New York State and indicates that this species is expected to continue to invade inland lakes.

Although natural mechanisms (waterfowl plumage) could potentially disperse *C. pengoi*, human-mediated activities are far more likely to account for the observed spread of the species throughout the Great Lakes basin. Initially introduced to Lake Ontario *via* ballast water from a transoceanic vessel (MacIsaac *et al.* 1999), spread to Lake Michigan was likely caused by movement of ballast water by the domestic "laker" fleet, or, less likely, by transoceanic vessels that entered the Great Lakes in "NOBOB" status (Colautti *et al.* 2003). Colonization of the lower Detroit River and western Lake Erie could have resulted from advective movement of individuals from northern Lake Michigan, or by discharge of ballast water by an upbound ship travelling from Lake Ontario. Sampling conducted in late summer 2001 in the upper reaches of the Detroit River and in Lake St. Clair failed to detect the presence of *Cercopagis* (T. Therriault and H. MacIsaac, unpubl. data), as did sampling in northern Lake Huron (T. Therriault and R. Colautti, unpubl. data), the central and eastern basins of Lake Erie (D. Kane, unpubl. data), and throughout Lake Superior (I. Duggan, unpubl. data). Therefore, downstream drift of individuals from an undetected upstream population is unlikely to account for *Cercopagis* populations in the lower Detroit River or western Lake Erie. Given its current distribution and numerous modes of possible transfer, it is highly likely that this species will spread throughout the Great Lakes basin and into more inland lakes in a manner similar to that of the confamiliar *Bythotrephes*.

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