



Three new cryptic species of the freshwater zooplankton genus *Holopedium* (Crustacea: Branchiopoda: Ctenopoda), revealed by genetic methods

CHAD L. ROWE¹, SARAH J. ADAMOWICZ² & PAUL D. N. HEBERT^{3,4}

Biodiversity Institute of Ontario, Department of Integrative Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada.

²*Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, N2L 3G1, Canada.*

E-mail: ¹clrowe@gmail.com; ²sadamowi@scimail.uwaterloo.ca; ³phebert@uoguelph.ca;

⁴*Corresponding author*

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Abstract

Molecular approaches have greatly advanced our understanding of species diversity and biogeography in the cladoceran crustaceans. Here, we provide the first large-scale examination of taxonomic diversity in the genus *Holopedium* Zaddach, 1855, by characterizing patterns of allozyme, mtDNA, and morphological variation from a total of 193 sites from three continents, including collections from near the type localities for the two generally recognized species, *Holopedium gibberum* Zaddach, 1855, and *Holopedium amazonicum* Stingelin, 1904. Allozyme data were only available for North American samples but revealed the presence of four species. Divergence patterns in the mitochondrial cytochrome *c* oxidase subunit I (COI) gene supported those species, as well as a fifth taxon endemic to South America. The five putative species are separated by substantial sequence (8.7–24.5%) and allozyme (0.36–1.54 Nei's distance) divergences, while intraspecific genetic diversity was generally limited in comparison. Although two of these species exhibited little morphological differentiation from their closest relatives, and diagnostic traits were not found among the characters considered, a population-level approach revealed significant morphological differences among all pairs of taxa. We therefore

present both an allozyme key and a morphological/geographic key to all species, as well as new or augmented descriptions for all five species. *H. gibberum* s.s. is distributed in Europe and across arctic North America, while its cryptic sister species, *H. glacialis* n. sp., is widely distributed across temperate North America. *H. amazonicum* s.s. is apparently restricted to the Amazon basin, *H. atlanticum* n. sp. occurs in lakes along the eastern margin of North America, while *H. acidophilum* n. sp. occurs sporadically across North America along a narrow band of middle latitudes. Due to high morphological variability within species, as well as the detection of cryptic diversity, we suggest that genetic analyses should be performed on populations from other geographic regions and should always accompany the recognition of new species of *Holopedium*.

Key words: allozymes, Cladocera, COI, Crustacea, cryptic species, freshwaters, mitochondrial DNA, morphology, species description, taxonomy

Introduction

The past twenty years have seen a paradigm shift in taxonomic perceptions for the cladoceran crustaceans and many other freshwater invertebrate groups. The traditional view of low species diversity and cosmopolitan distributions was derived from the observations of early workers who noted that many freshwater invertebrates exhibited little morphological variation over vast geographic distances (Lyll 1832; Darwin 1859), as well as great dispersal ability (Darwin 1882). Mayr (1963) described this biogeographic pattern as arising from the homogenizing effects of gene flow, and, indeed, the resting eggs of these organisms do possess several characteristics that would appear to make them ideal passive agents of dispersal (Fryer 1996). Capable of being transported by wind and surviving passage through avian digestive systems (reviewed in De Meester *et al.* 2002), resting eggs also often possess sticky spines or protuberances that facilitate attachment to waterfowl, and they are produced in the greatest numbers when waterfowl migration is at its peak (Fryer 1996). Propensity for dispersal is also supported by the rapid colonization of northern habitats following deglaciation for many cladocerans.

However, despite this capacity for dispersal, detailed morphological and genetic investigations have revealed high levels of taxonomic diversity and endemism (e.g. Frey 1982, 1985, 1987; DeMelo & Hebert 1994; Taylor *et al.* 1996, 1998; Colbourne *et al.* 1998; Petrusek *et al.* 2004). Moreover, genetic information has challenged the view that gene flow is sufficient to maintain genetic cohesion among cladoceran populations on a continental — let alone a global — scale (Boileau *et al.* 1992; Hebert & Taylor 1997). Founder effects, combined with rapid population increase and local adaptation, may be important factors that restrict gene flow in the face of dispersal of propagules (Boileau *et al.* 1992; De Meester *et al.* 2002). Thus, local genetic differentiation and continental or regional endemism, as opposed to cosmopolitanism, have become established features of our understanding of cladoceran diversity.

Despite the high biotic and abiotic variability among aquatic habitats, such genetic divergence is often not associated with morphological change in zooplankton species. Detailed phylogenetic frameworks have allowed researchers to address key questions regarding how molecular and morphological evolution proceeds in these lineages (e.g. King & Hanner 1998). For example, is morphological similarity among species due to convergence, cosmopolitanism, introgression, or shared ancestry (Taylor *et al.* 1996)? Habitat-linked convergence and introgression have played important roles in morphological evolution in the cladocerans (Colbourne *et al.* 1997; Schwenk *et al.* 2000) and these processes have tended to be associated with cases of more rapid morphological evolution. However, the overwhelming answer in most cases has been that shared ancestry, combined with a slow pace of morphological evolution, is the culprit for past cases of diversity underestimation. Thus, combined genetic and morphological approaches have proven both necessary and fruitful for assessing species diversity and for investigating the evolutionary history of the Cladocera.

The genus *Holopedium* Zaddach, 1855 is an example of a cladoceran taxon still regarded as being both broadly distributed and species poor. Its representatives are widely distributed in softwater lakes throughout