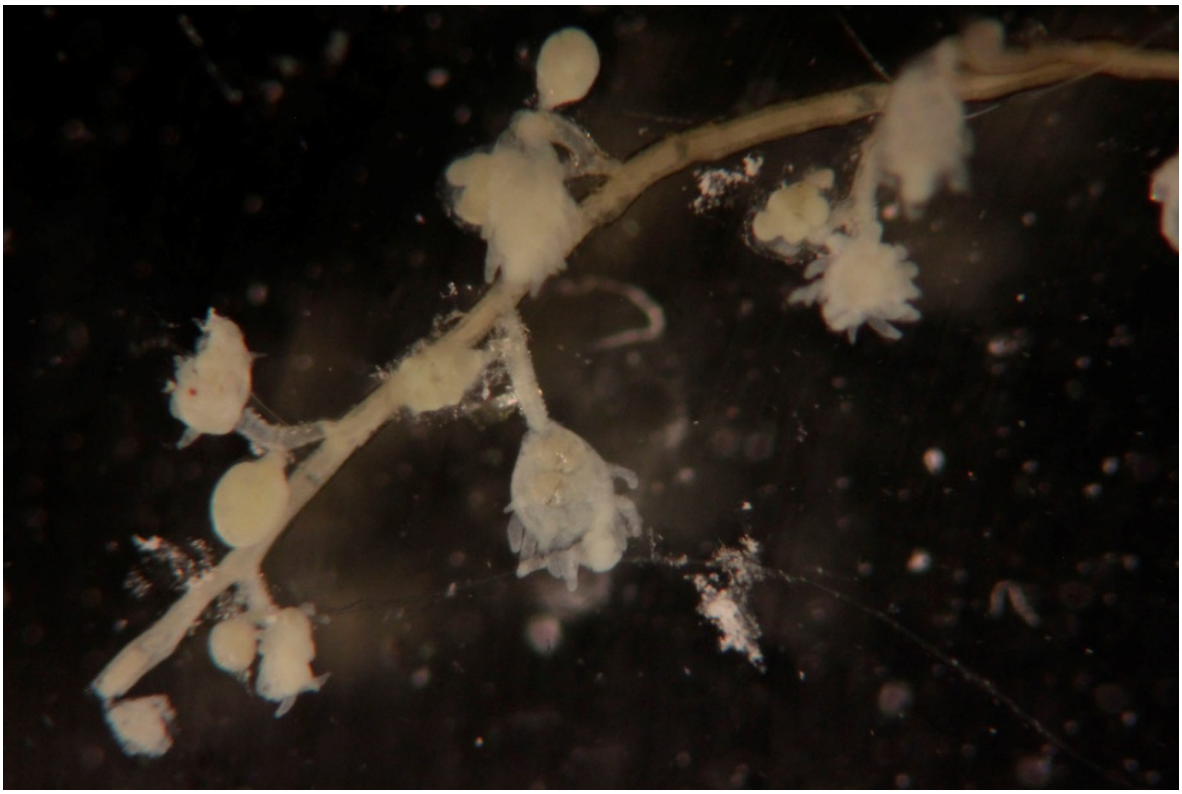
RECLAMATION

Managing Water in the West

Range expansion of the invasive hydroid, *Cordylophora caspia* (Pallas, 1771), in Colorado River reservoirs

Research and Development Office
Science and Technology Program
(Final Report) ST-2017-1626-01



U.S. Department of the Interior
Bureau of Reclamation
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Mission Statements

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The Department of the Interior protects and manages the Nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its trust responsibilities or special commitments to American Indians, Alaska Natives, and affiliated island communities.

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Range expansion of the invasive hydroid, *Cordylophora caspia* (Pallas, 1771), in Colorado River reservoirs

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Executive Summary

Cordylophora caspia (Pallas, 1771) colonies have significant biofouling potential at hydropower facilities, which can increase maintenance and cause system failure. In 2015, *C. caspia* colonies were observed at dams along the Lower Colorado River system in Lake Powell UT, Lake Mead NV/AZ, Lake Mohave AZ/NV, and Lake Havasu AZ/CA. The hydroid was serendipitously found on settlement plates deployed for invasive dreissenid mussel monitoring. Species identification was confirmed by taxonomy and molecular analysis, and phylogenetic reconstruction was performed to identify the relationship of sequenced COI fragments relative to those from *C. caspia* specimens collected at other geographic locations.

This research was published as a Rapid Communication in BioInvasions Records (2016) Volume 5, Issue 3: 133-137. Please see the full article in Appendix A.

Contents

Main Report

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Appendix A – BioInvasions Records, Rapid Communication

Range expansion of the invasive hydroid, *Cordylophora caspia* (Pallas, 1771), in Colorado River reservoirs

Cordylophora caspia in Colorado River reservoirs

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Abstract
Cordylophora caspia (Pallas, 1771) colonies have significant biofouling potential at hydropower facilities, which can increase maintenance and cause system failure. In 2015, *C. caspia* colonies were observed at dams along the Lower Colorado River system in Lake Powell UT, Lake Mead NV/AZ, Lake Mohave AZ/NV, and Lake Havasu AZ/CA. The hydroid was serendipitously found on settlement plates deployed for invasive dreissenid mussel monitoring. Species identification was confirmed by taxonomy and molecular analysis, and phylogenetic reconstruction was performed to identify the relationship of sequenced COI fragments relative to those from *C. caspia* specimens collected at other geographic locations.

Key words
colonial hydroid, *Cordylophora*, biofouling, benthic predator, biofilm

Introduction
Cordylophora caspia (Pallas, 1771) is a colonial hydroid originating from the Black and Caspian Seas. This hydroid occurs in brackish and freshwater environments globally (Arndt 1989, Folino-Rorem 2015, Smith 2001). *C. caspia* has a global distribution due to transport in ballast water, increased boat travel and water connectivity, and the ability to acclimate and proliferate in varying salinities (Roos 1979, Folino-Rorem 2015, and Bij de Vaate et al. 2002) ranging from 0-

30 ppt (Arndt 1989). *C. caspia* colonies grow on hard surfaces and consist of polyps specialized for feeding (hydranths) and for reproduction (gonophores). Mature ova are retained in the female gonophores, and male gonophores release sperm that penetrate the female gonophore and fertilize the eggs. The embryos develop in the gonophore and are released as free-swimming, ciliated planulae which disperse from the parent colony before attaching to substrates and forming new colonies. *C. caspia* does not have a medusoid stage (Pennak 1991). Colony growth and dispersal also occurs via asexual budding, via body fragments (hydrorhiza and hydrocauli), and via temperature and drought resistant menonts (Roos 1979).

C. caspia is carnivorous, and is considered a benthic predator that feeds on small crustaceans, worms, insect larvae, watermites and other zooplankton and benthic invertebrates (Bij de Vaate et al. 2002, Smith et al. 2002). Folino (2000) suggests that *C. caspia* may contribute to a restructuring of benthic and pelagic freshwater communities. Colonies also have serious negative economic impacts associated with biofouling (Folino-Rorem 2015), as they prefer solid substrates for settlement. *C. caspia* has been found colonizing and clogging intake tunnels, filters, condenser tube sheets, power plant pipes, and drinking water treatment plants in Europe and the United States (Lipsey and Chimney 1978, Jenner and Janssen-Mommen 1993, Moreteau and Khalanski 1994, Folino-Rorem and Indelicato 2005).

Since its introduction into the United States, *C. caspia* has been documented in at least 17 states (USGS and the World Register of Marine Species). Although Peck et al. identified this hydroid in Lake Mead, NV in 1987; this location is not commonly recognized in many range distribution maps and lists for the species. This paper describes the discovery of *C. caspia* in additional reservoirs along the Colorado River and genetically confirms identification.

Methods

Cordylophora caspia was collected serendipitously on settlement plates that were deployed to monitor invasive quagga mussel (*Dreissena rostriformis bugensis*, Andrusov, 1897) settlement at dams along the Lower Colorado River system. Black PVC plates (14.7 cm x 14.7 cm) were installed at Glen Canyon Dam, Lake Powell UT (36.9375 N, 111.4837 W), Hoover Dam, Lake Mead NV/AZ (36.0163 N, 114.7372 W), Davis Dam, Lake Mohave AZ/NV (35.1967 N, 114.5683 W), and Parker Dam, Lake Havasu AZ/CA (34.2966 N, 114.1394 W) in 2014 (Figure 1). Plates were positioned at depths of approximately 10, 20, and 30 m below the surface in the forebay at each dam, and were analyzed for quagga mussel settlement monthly.

C. caspia colonies were visually (Figure 2) and microscopically (Figure 3) observed on plates. Taxonomic identification of specimens was confirmed by John Beaver and Thomas Renicker at BSA Environmental Services, INC. DNA barcoding for genetic identification of tissue samples was performed by the Reclamation Detection Laboratory for Exotic Species (RDLES) using established protocols (Keele et al. 2014).

DNA was isolated using the DNEasy Blood & Tissue Extraction Kit (Qiagen Inc., Valencia, CA USA), and a 710 bp fragment of the mitochondrial cytochrome oxidase 1 (COI) gene was PCR amplified using the primers LCO1490: 5′-GGTCAACAAATCATAAAGATATTGG-3′ and HCO2198: 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ (Folmer et al. 1994). PCR amplification was performed with AmpliTaq Gold DNA Polymerase (ThermoFisher Scientific,

Grand Island, NY, USA), with an initial denaturation of 10 minutes at 95°C, followed by 40 rounds of amplification with 1 minute at 95°C, 1 minute at 40°C, and 1.5 minutes at 72°C, and a final extension of 10 minutes at 72°C. Resulting PCR products were sent to a commercial laboratory for DNA sequencing. The forward and reverse DNA sequences from each amplified fragment were aligned to each other and to on-line databases (DNA Bold and NCBI Blast) to identify the source organism (Appendix 1).

Phylogenetic reconstruction was performed to identify the relationship of sequenced COI fragments relative to those previous published (Folino-Rorem et al. 2009, Wollschlager et al. 2013) for *C. caspia* specimens collected at other geographic locations. COI sequences collected in the study were aligned to sequences retrieved from GenBank (Appendix 2) using MUSCLE (Edgar 2004). Phylogenetic reconstruction was performed using MrBayes version 3.2.1 (Ronquist et al. 2012). The analysis was run for 5,000,000 generations, with 4 runs of 2 chains each. The analysis was run with a GTR+I+G likelihood model of sequence evolution, chosen based on analysis of the dataset with jModelTest (Posada 2008). The first 1,250,000 generations of the run were discarded as burn-in prior to calculation of a consensus tree and posterior probabilities.

Results and Discussion

C. caspia was taxonomically and genetically confirmed at sites along the Colorado River system in UT, NV, AZ, and CA. Colonies were first noticed on settlement plates at Lake Mead in December 2014, and once identified were regularly found at all other locations on plates at 10, 20, and 30 m. *C. caspia* was previously identified as *Cordylophora lacustris* in Lake Mead in a report by Peck et al. (1987) who commonly found this hydroid coating buoy anchor ropes, submerged tree branches, and un-sedimented rock surfaces at about 15 meters or below.

GenBank blastn alignment of COI sequences from hydroids sampled from Lake Powell, Lake Mead, and Lake Mohave showed highest similarity to *C. caspia* sequences for all specimens. All sampled sequences showed 99% identity to a reference *C. caspia* COI sequence (GenBank accession number: KC489509.1). Phylogenetic analysis of COI sequences isolated from *C. caspia* in the Colorado River placed all the specimens sampled within the previously identified clade 1A (Figure 4; Folino-Rorem et al. 2009, Wollschlager et al. 2013). This clade includes samples collected from a wide variety of geographic locations, including Lake Michigan, Lake Erie, Virgina, Panama, and the Illinois River and tributaries. Although the limited geographic sampling and resolution within the tree do not allow a definitive identification of the source population for *C. caspia* in the Colorado River, the presence of specimens from the Upper Mississippi River and Great Lakes watersheds in the 1A clade suggests possible origins for the invasion. It is also intriguing that all other specimens in this clade were collected from exclusively freshwater habitats, like that of the Colorado River. This is in contrast to the other *C. caspia* clades (1B, 2A, and 2B), which contain representatives collected from waters with a range of salinities. The possibility that "*C. caspia*" is a complex of genetically distinct species or subspecies with different ecological tolerances has been suggested previously (see Folino 2000), and bears further investigation.

During microscopic examination of *C. caspia*, feeding polyps were regularly observed to contain dreissenid mussel larvae (Figures 5). Dense quagga mussel populations are found in the Lower

Colorado River (Mahon 2011), and it is possible that *C. caspia* is benefiting from the high numbers of mussel veligers available as prey, facilitating range expansion and increased population densities. *C. caspia* has previously been reported to co-occur with invasive populations of dreissenid quagga and zebra mussels, feeding on mussel larva and using mussel shells as a substrate (Olenin and Leppäkoski 1999, Folino-Rorem et al. 2006). The hydroid's filamentous structure may enhance or facilitate recruitment of dreissenid mussels by providing additional surface area for settlement (Dean and Hurd 1980, Moreteau and Khalanski 1994), although it is possible that the two species compete for suitable substrate for colonization (Walton 1996, Folino-Rorem 2015). Given that the native range of *C. caspia* overlaps with those of zebra and quagga mussels, it may be that hydroid colonies have translocated with, and have benefitted from, the range expansion of invasive dreissenid mussels.

C. caspia growth rates have been found to increase non-linearly with temperature, suggesting that a small increase in temperature can result in a large increase in growth rate (Meek et al. 2012). Positive growth rates occur at temperatures above 14°C and the peak growth rate occurs above 19°C (Meek et al. 2012). Temperatures at lower Colorado River Reservoirs are commonly above 20°C during the summer months, suggesting that *C. caspia* growth rate has the potential to be maximized. The presence of *C. caspia* in the Colorado River system may be of concern to local facility managers as there are several hydropower facilities located along the Colorado River, and *C. caspia* has the potential to cause significant biofouling issues at these sites.

Acknowledgements
The authors of this paper would like to thank Reclamation's Research and Development Program for funding the research that resulted in this finding. Also thank you to Denise Hosler (team lead of the Reclamation Detection Laboratory for Exotic Species) and Mark Nelson (retired Reclamation biologist) for their help with this research.

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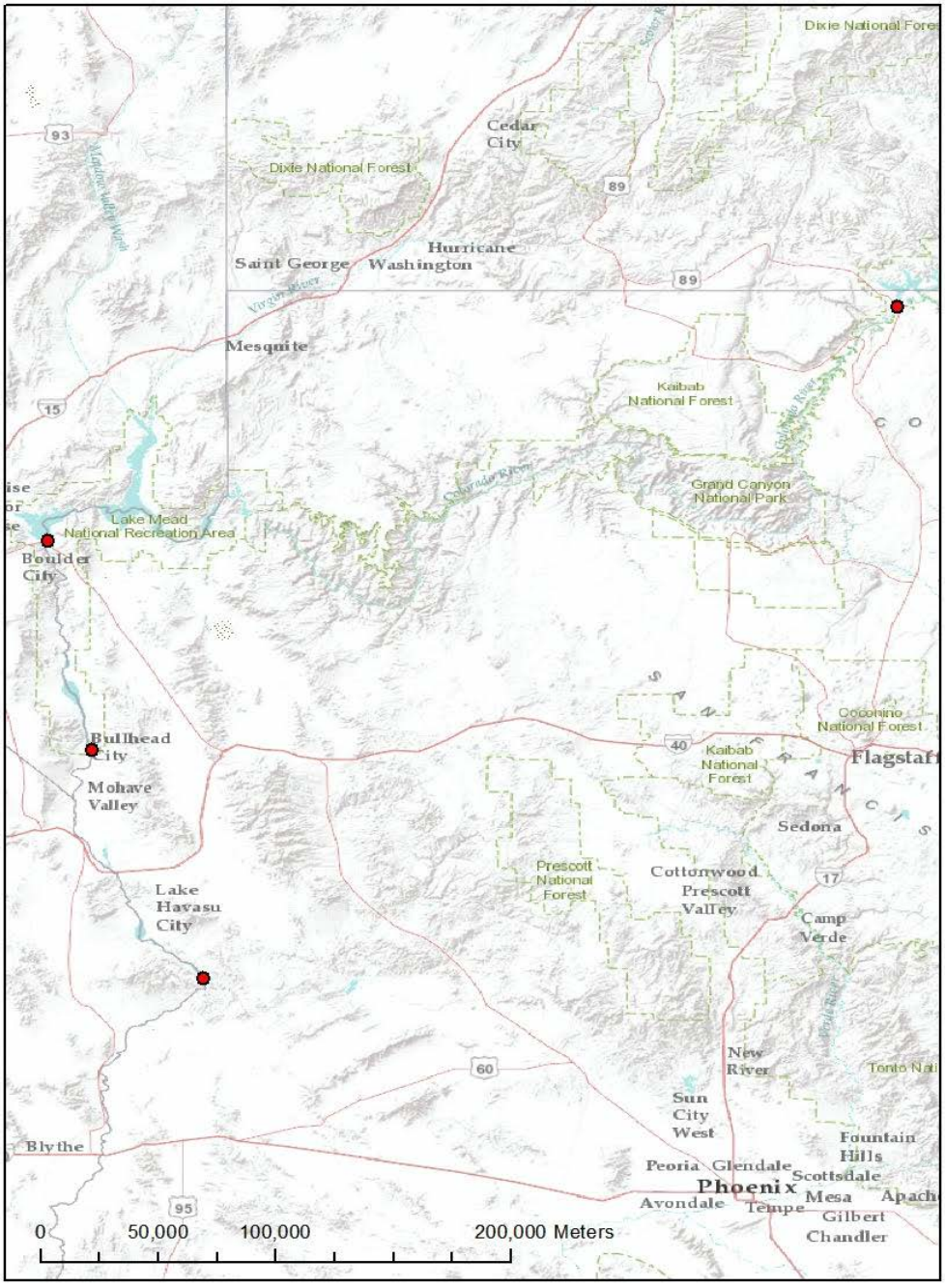

Figure A 1. Dots indicate locations along the Colorado River where quagga mussel settlement plates were deployed and where *C. caspia* colonies were collected.

Figure A 2. *C. caspia* colony growth on a settlement plate at Lake Mead, NV in the forebay near the Hoover Dam.

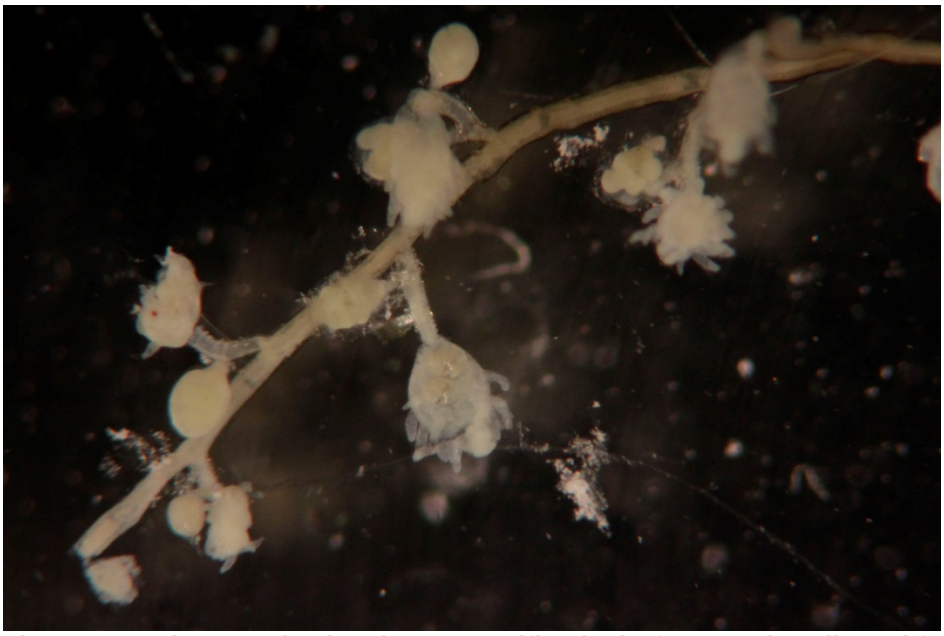

Figure A 3. Microscopic view (30 X magnification) of *C. caspia* collected from Lake Mohave, NV/AZ

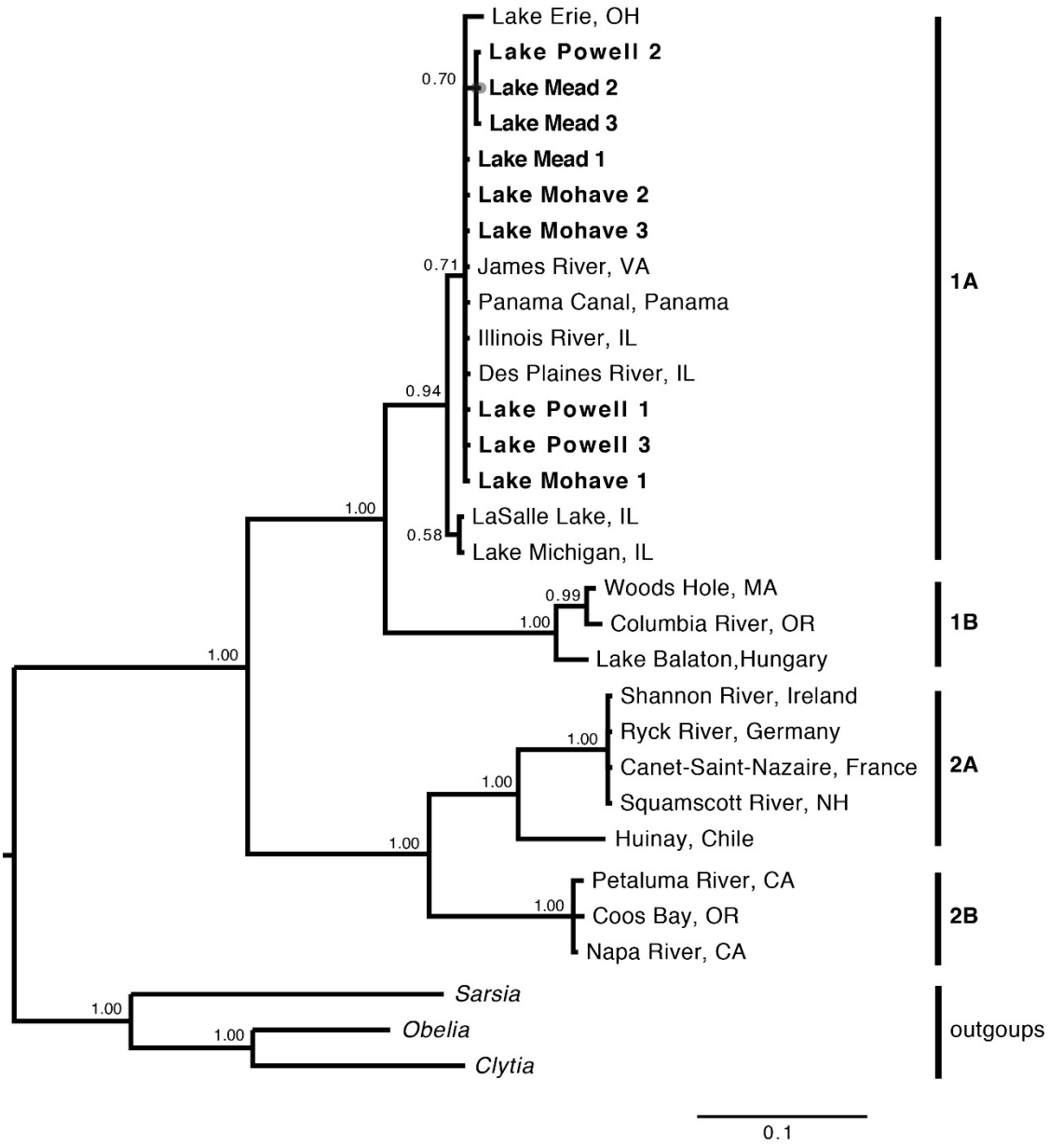

Figure A 4. Phylogenetic analysis in of *C. caspia* COI sequences. Phylogram from Bayesian likelihood analysis with 4 independent runs of 5,000,000 generations each. Posterior probabilities are shown for nodes with values greater than 0.50. Scale bar below the tree represents 0.1 nucleotide substitutions per site. Clade names are presented to the right of the tree.

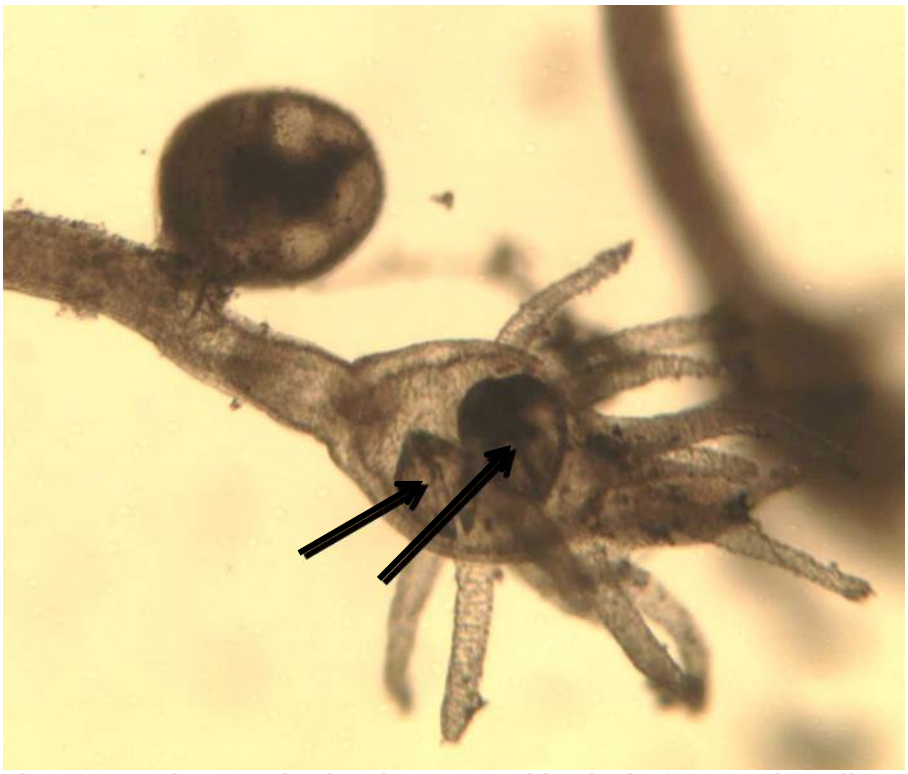

Figure A 5. Microscopic view (105X magnification) of *C. caspia,* collected from Lake Havasu AZ/CA with two quagga mussel veligers (arrows) inside a feeding polyp

Appendix B – Sample and Sequencing Information

Table B 1. Geographic and gene information for *C. caspia* samples analyzed for this study.

Species	Sample name	Sampling location	Latitude	Longitude	Depth	Collection date	% identity*	GenBank accession #
Cordylophora caspia	Lake Powell 1	Lake Powell, UT, USA	36.9375 N	111.4837 W	10m	1/14/2015	99%	KU695587
Cordylophora caspia	Lake Powell 2	Lake Powell, UT, USA	36.9375 N	111.4837 W	10m	1/14/2015	99%	KU695588
Cordylophora caspia	Lake Powell 3	Lake Powell, UT, USA	36.9375 N	111.4837 W	10m	1/14/2015	99%	KU695589
Cordylophora caspia	Lake Mead 1	Lake Mead, NV/AZ, USA	36.0163 N	114.7372 W	20m	10/22/2015	99%	KU695590
Cordylophora caspia	Lake Mead 2	Lake Mead, NV/AZ, USA	36.0163 N	114.7372 W	20m	12/15/2014	99%	KU695591
Cordylophora caspia	Lake Mead 3	Lake Mead, NV/AZ, USA	36.0163 N	114.7372 W	20m	12/15/2014	99%	KU695592
Cordylophora caspia	Lake Mohave 1	Lake Mohave, AZ/CA, USA	35.1967 N	114.5683 W	20m	7/14/2015	99%	KU695593
Cordylophora caspia	Lake Mohave 2	Lake Mohave, AZ/CA, USA	35.1967 N	114.5683 W	20m	7/14/2015	99%	KU695594
Cordylophora caspia	Lake Mohave 3	Lake Mohave, AZ/CA, USA	35.1967 N	114.5683 W	20m	7/14/2015	99%	KU695595

*Percent identity to *C. caspia* reference sequence (GenBank accession number: KC489509.1)

Table B 2. Accession numbers for *C. caspia* and outgroup COI sequences retrieved from GenBank for phylogenetic analysis.

Species	Sampling location	GenBank accession #
Cordylophora caspia	Lake Erie, OH, USA	KC489509.1
Cordylophora caspia	Huinay, Chile	EF540778.1
Cordylophora caspia	Canet-Saint-Nazaire, France	EF540783.1
Cordylophora caspia	Hungary	EF540787.1
Cordylophora caspia	Columbia River, OR, USA	EF540779.1
Cordylophora caspia	Coos Bay, OR, USA	EF540780.1
Cordylophora caspia	James River, VA, USA	EF540793.1
Cordylophora caspia	Illinois River, IL, USA	EF540785.1
Cordylophora caspia	Ryck River, Germany	EF540784.1
Cordylophora caspia	DesPlaines River, IL, USA	EF540781.1
Cordylophora caspia	Squamscott River, NH, USA	EF540782.1
Cordylophora caspia	LaSalle River, IL, USA	EF540789.1
Cordylophora caspia	Napa River, CA, USA	EF540790.1
Cordylophora caspia	Lake Michigan, IL, USA	EF540788.1
Cordylophora caspia	Panama Canal, Panama	EF540791.1
Cordylophora caspia	Shannon River, Ireland	EF540786.1
Cordylophora caspia	Petaluma River, CA, USA	EF540792.1
Cordylophora caspia	Woods Hole, MA, USA	EF540794.1
Clytia folleata	China	KF962082.1
Obelia sp.	China	KF962164.1
Sarsia tubulosa	China	JQ353758.1