Supplementary File 1

DNA Extractions

DNA was extracted from fin tissue and individual metacercariae in 400 µl of 5% Chelex containing 0.1 mg/ml proteinase K, incubating at 60°C for 2-12 hours, and heating to 100°C for 8 minutes.

Fish Host COI Amplification

PCR reactions (50 μL) contained 2.0 μL of DNA extraction, 200 μM each dNTP, 2.0 mM MgCl2, 0.5 μM each primer, 1x Taq buffer and 1.25 units GoTaq DNA polymerase (Promega). PCR amplification consisted of 2 min at 94°C, followed by 35 cycles of 40 sec at 94°C, 40 sec at 52°C (*Lepomis*) or 45°C (*Pomoxis* and *Micropterus*) and 1 min at 72°C, and a final extension for 8 min at 72°C. All host PCR products were purified using QIAquick gel extraction kits (Qiagen).

*Posthodiplostomum* COI and ITS1 Amplification

COI PCR reactions were as described for fish hosts and amplification consisted of 2 min at 94°C, followed by 35 cycles of 30 sec at 94°C, 30 sec at 42°C and 1 min at 72°C and a final extension for 8 min at 72°C. PCR conditions for the ITS1 region were as described for COI, except for a 50°C annealing temperature, using 40 cycles. COI and ITS1. PCR products were purified and sequenced as described for fish hosts.

Bayesian Analyses

COI and ITS1 analyses sampled across GTR model space during the MCMC analysis by setting nst=mixed and rates=gamma. MCMC searches utilized three heated chains and were run for 5,000,000 (COI) and 1,000,000 (ITS1) generations with sampling every 500 (COI) and 100 (ITS1) generations.

Maximum Likelihood Analyses

Maximum Likelihood analyses incorporated the HKY+G (gamma shape parameter = 0.214) and K2P models of sequence evolution for COI and ITS1, respectively, as selected by the Bayesian information criterion (BIC) of MEGA7.

GenBank and Outgroup Information for Phylogenetic Analyses

Phylogenetic analyses included *Posthodiplostomum* sp. 3 (GenBank Accession #s HM064800.1 & HM064951.1 for COI and ITS1, respectively), *Posthodiplostomum* sp. 8 (GenBank Accession #s HM064876.1 & HM064962.1 for COI and ITS1, respectively), and *Posthodiplostomum* sp. 2 (GenBank Accession # HM064797.1 was used for COI, but sequences of this species were not available for ITS1). Sequences from *Diplostomum* sp. 15 (GenBank Accession # KR271125.1) and *Diplostomum* sp. 6 (GenBank Accession # KX037901.1) were used as outgroups for COI analyses and sequences from *Diplostomum baeri* (GenBank Accession # AY123042.1) and *Diplostomum huronense* (GenBank Accession # AY123044.1) were used as outgroups for ITS1 analyses.