

SUPPLEMENTARY MATERIAL 3. Primers, reaction volumes, and cycling conditions for gene amplification and sequencing. The COI region was amplified and sequenced with primers FishF1 and FishR1 or FF2d and FR1d (Ivanova et al. 2007) and a custom-designed primer, the cytochrome b (cytb) region was amplified with primers L14724 and H15915 (Schmidt and Gold 1993) and sequenced with primers L1 and L3 (Kinziger and Wood 2003) and H15915, and rhodopsin and S7 were amplified and sequenced primarily with custom-designed primers, which were generally more effective than those from Sevilla et al. (2007) and Chow & Hazama (1998). Reaction volumes of 50 μ L contained 50–100 ng DNA, 1 \times reaction buffer (Life Technologies), 2.5 mM MgCl₂, 200 μ M each dNTP, 1 μ M each primer, 1 U Taq polymerase (Life Technologies). The PCR program was 94 °C/5 min, [94 °C/1 min, 55 °C/1 min, 72 °C/1 min 30 s \times 34 cycles], 72 °C/5 min. The quality and quantity of template DNA were determined by 1.6% agarose gel electrophoresis. The PCR products were cleaned using ExoSAP-IT™ PCR Product Cleanup Reagent (Life Technologies) and sequence data was generated at Eurofins Genomics (Louisville, KY) on an ABI3730XL sequencing machine.

Gene	Name	Direction	Sequence
COI	Fish F1	Forward	TCAACCAACCACAAGACATTGGCAC
	Fish R1	Reverse	TAGACTTCTGGGTGGCCAAAGAATCA
	Custom	Internal reverse	GGAAGAAGTCAAAGCTCATYTT
	FF2D	Forward	TTCTCCACCAACCACAARGAYATCGG
	FR1D	Reverse	CACCTCAGGGTGTGGAAGAACCAGAA
cytb	L14724	Forward	CCCACCCCTACTAAAAATC
	L1	Forward	CCCACCCCTACTAA
	L3	Forward	ACCCCTCCCCACATCAAAC
	H15915	Reverse	CAACGATCTCCGGTTTACAAGAC
	Rhodopsin	Forward	GTCTGCAAGCCCATCAGCAACTTCCG
Rhodopsin	Custom	Internal forward	GGATCCCTGGTTGCAGAAGA
	Rod-4R	Reverse	CTGCTTGTTTCATGCAGATGTAGAT
	Custom	Internal reverse	ACGCCATCATGGCTTGGCCCTTCA
	S7	Forward	TGGCCTCTTCCTTGGCCGTC
S7	Custom	Internal forward	CAGAGGTTAGTCGTCTACTTT
	S74R	Reverse	AACTCGTCTGGCTTTTCGCC
	Custom	Internal reverse	TGAACAGGCCGTTGTGT

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