

Supplemental File 1: Species and associated gene sequences used to design an eDNA assay for detection of Longnose Darter (*Percina nasuta*) in Missouri, USA.

Caudal fin clips were collected from Gilt Darter (*Percina evides*), Common Logperch (*P. caprodes*), and Longnose Darter (*P. nasuta*) specimens in the St. Francis River in Missouri, USA and from a Slenderhead Darter (*P. phoxocephala*) specimen in the North Fork of the Salt River in Missouri, USA. Tissues were extracted with DNeasy Blood and Tissue Kit (Qiagen), and we amplified a portion of the *cytochrome b*, mitochondrial (*mt-cyb*) gene with primers GLU and THR (Near et al. 2000). PCR reactions consisted of 50 µL total volume with 0.2 U AmplitaqGold (Applied Biosystems), 5 µL 10X Gold Buffer, 0.08 µL dNTPs, 30 µg BSA, 0.5 µM of each primer, 2.5 mM MgCl<sub>2</sub>, and 3 µL genomic DNA. Reactions were subject to an initial denature of 95°C for 10 min followed by 40 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 2 min. After cycles, a final extension of 72°C for 10 min completed the reaction. We sequenced with terminal primers and internal primers (cytb-intF; 5'CCGGTTCTTCGCCTTCCATTTCTCT and cytb-intR: 5'GTCTTTATAGGAGAAGTAGGGGTGG) designed with an alignment of *Percina* spp. including *P. nasuta*, *P. phoxocephala* and *P. brucethompsoni*. Cycle sequencing and electrophoretic analysis was performed by the University of Missouri DNA Core facility on an ABI 3730xL (Applied Biosystems). We deposited the resulting sequences into GenBank (Table S1; MZ254679, MZ254680, MZ254683, MZ254684, MZ254685, MZ254686).

Supplemental Table 1. Reference *mt-cyb* sequences used to design a qPCR assay for longnose darter (*Percina nasuta*) eDNA in southeast Missouri, USA.

Species	Common Name	GenBank Accession Numbers
<i>Percina brucethompsoni</i>	Ouachita Darter	KM209992, KM209993 KT880217, EF587839, KC211182, EU379093, EU046670, DQ493484, AF386550, DQ493483, DQ493485, DQ493490,
<i>Percina caprodes</i>	Common Logperch	EF587840, MZ254679 AY374284, AF375947, AF375938, AF375942, AF375945, AF375941, AF375939, AF375946, AF375943, AF375955, AF375954, AF375952, AF375951, AF375948, AF375949,
<i>Percina evides</i>	Gilt Darter	MZ254680
<i>Percina maculata</i>	Blackside Darter	AF045353, AF386557
<i>Percina nasuta</i>	Longnose Darter	HQ128264, KM209988, AF386561, KM209987, KM209986, AF386562, KM209989, KM209990, MZ254685, MZ254686 KM210000, KM209996, AY374289, AF386563, KM210003, KM210011, KM209994, KM210013, KM210030, KM210028, KM210010, KM209998, KM210029, KM210014, KM210017, KM209995, KM209997, KM210021, KM210022, KM210004, KM210001, KM209999, KM210025, KM210005, KM210002, KM210009, KM210006, KM210007, KM210026, KM210027, KM210008, MZ254683, MZ254684
<i>Percina phoxocephala</i>	Slenderhead Darter	
<i>Percina sciera</i>	Dusky Darter	HQ128270, EF613205, EF613206, AF386573, EF613204

#### Reference

Near, T.J.; Porterfield, J.C.; Page, L.M. Evolution of cytochrome b and the molecular systematics of Ammocrypta (Percidae: Etheostomatinae). *Copeia* **2000**, *2000*, 701-711.