

**Table S1.** Species, sample size (*n*), and GenBank accession number for DNA sequences used for *in silico* Yaqui Catfish assay development. eDNAAssay = environmental DNA classification tool.

Species name	Common name	<i>n</i>	GenBank accession	Mismatches			
				eDNAAssay AP	Forward primer	Reverse primer	Probe
<u>Target species</u>							
<i>Ictalurus pricei</i>	Yaqui Catfish	10	KJ496298-9; EF491723-8; EF590205-6	0.815–0.874	1	0	0
<u>Non-target species</u>							
<i>Ictalurus furcatus</i>	Blue Catfish	3	EF491729; KM576102; AF484159	0.429	3	2	2
<i>Ictalurus lupus</i>	Headwater Catfish	2	AY458885; AY327267	0.328	2	2	2
<i>Ictalurus punctatus</i>	Channel Catfish	8	EU490914; AB069646; AF482987; AY184253; JN015529; AF477829; AY184254; AY458886	0.328	3	3	3
<i>Ameiurus melas</i>	Black Bullhead	3	KT804702; AY184273; AY184263	0.362	4	7	3
<i>Noturus miurus</i>	Brindled Madtom	2	KM363024; KM363026	0.282	7	4	4
<i>Prietella phreatophila</i>	Mexican Blindcat	1	AY458884	0.167	4	4	4
<i>Pylodictis olivaris</i>	Flathead Catfish	3	AY458887; AF484161; DQ790748;	0.267	3	4	3
<i>Gila purpurea</i>	Yaqui Chub	1	JX443021.1	0.246	6	6	9
<i>Cyprinella formosa</i>	Beautiful Shiner	1	EU082519.1	0.226	3	7	10
<i>Catostomus bernardini</i>	Yaqui Sucker	1	EU668881.1	0.225	5	6	9
<i>Poeciliopsis sonoriensis</i>	Yaqui Topminnow	1	DQ138944.1	0.305	4	2	7
<i>Oncorhynchus mykiss</i>	Rainbow Trout	1	KU872710.1	0.207	5	3	7
<i>Oncorhynchus chrysogaster</i>	Mexican Golden Trout	1	JX960801.1	0.207	5	3	7
<i>Cyprinus carpio</i>	Common Carp	1	DQ868875.1	0.237	5	6	5
<i>Carassius auratus</i>	Goldfish	1	AB368694.1	0.237	4	5	4

Species name	Common name	<i>n</i>	GenBank accession	Mismatches			
				eDNAAssay AP	Forward primer	Reverse primer	Probe
<i>Campostoma ornatum</i>	Mexican Stoneroller	1	JF343098.1	0.211	5	5	9
<i>Gila pulchra</i>	Conchos Chub	1	KF514253.1	0.246	6	7	9
<i>Gila robusta</i>	Roundtail Chub	1	FJ913816.1	0.249	5	7	8
<i>Agoisa chrysogaster</i>	Longfin Dace	1	KY398931.1	0.213	5	8	7
<i>Codoma ornata</i>	Ornate Shiner	1	AY281061.1	0.243	4	8	9
<i>Pimephales promelas</i>	Fathead Minnow	1	GQ184522.1	0.244	5	9	8
<i>Carpionodes carpio</i>	River Carpsucker	1	AF454867.1	0.237	7	6	7
<i>Catostomus wigginsi</i>	Opata Sucker	1	EU668781.1	0.213	3	6	8
<i>Catostomus plebeius</i>	Rio Grande Sucker	1	KU697935.1	0.231	5	7	9
<i>Poeciliopsis prolifica</i>	Blackstripe Livebearer	1	AF412147.1	0.273	4	2	8
<i>Poeciliopsis monacha</i>	Headwater Livebearer	1	AF047346.1	0.37	3	2	6
<i>Poeciliopsis occidentalis</i>	Gila Topminnow	1	AF412144.1	0.305	4	2	7
<i>Micropterus salmoides</i>	Largemouth Bass	1	KX588092.1	0.242	3	5	8
<i>Lepomis cyanellus</i>	Green Sunfish	1	JF742828.1	0.196	5	4	6
<i>Lepomis microlophus</i>	Redear Sunfish	1	JF742834.1	0.183	4	4	8
<i>Lepomis megalotis</i>	Longear Sunfish	1	AY828977.1	0.296	5	2	9
<i>Gambusia krumholzi</i>	Spotfin Gambusia	1	JX679663.1	0.289	6	1	8
<i>Gambusia affinis</i>	Mosquitofish	1	KP059011.1	0.239	6	7	7
<i>Homo sapiens</i>	Human	1	JN034136.1	0.21	4	6	7

**Table S2.** Environmental DNA assays for detecting Yaqui Catfish and Channel Catfish using quantitative polymerase chain reaction (qPCR). T<sub>m</sub> = primer melting temperature.

Species	Assay component	Sequence (5'-3')	T <sub>m</sub> (°C)	Optimal concentration (nM)
Yaqui Catfish	Forward primer	GCCTCACATCAAGCCCGAG	60.3	300
	Reverse primer	GTTTAGAAGTGTGGAGGAGGGGA	59.5	900
	Probe	6FAM-CACTACTACTCTCTATCTTAG-MGBNFQ	68	250
Channel Catfish	Forward primer	TTAGCCCGCGGAATACAAATC	59.9	900
	Reverse primer	GGACCAATTAACAGTGCGGTG	60.2	900
	Probe	6FAM-TGGTTCCTCTCTAATCTA-MGBNFQ	69	250

**Table S3.** Species, sample size (*n*), and GenBank accession number for DNA sequences used for *in silico* Channel Catfish assay development. eDNAAssay = environmental DNA classification tool.

Species name	Common name	<i>n</i>	GenBank accession	eDNAAssay AP	Mismatches		
					Forward primer	Reverse primer	Probe
			<u>Target species</u>				
<i>Ictalurus punctatus</i>	Channel Catfish	9	AF482987; MF621716- MF621718; MF621720- MF621722; OL477732-33	0.805	0	0	0
			<u>Non-target species</u>				
<i>Ictalurus pricei</i>	Yaqui Catfish	2	KJ496298-9	0.143	3	3	2
<i>Ictalurus furcatus</i>	Blue Catfish	3	MG570460-1; KM576102	0.102	3	4	4
<i>Ameiurus melas</i>	Black Bullhead	1	KT804702	0.055	0	3	0
<i>Ameiurus natalis</i>	Yellow Bullhead	2	MF621735; MG570406	0.074	4	7	4
<i>Ameiurus catus</i>	White Bullhead	3	MG570464-5; MH324423	0.076	3	7	5
<i>Ameiurus nebulosus</i>	Brown Bullhead	2	MG621733-4	0.051	4	6	5
<i>Noturus flavus</i>	Stonecat	1	AF528020.2	0.177	5	3	2
<i>Noturus exilis</i>	Slender Madtom	3	AF527996-8	0.08	5	4	5
<i>Noturus albater</i>	Ozark Madtom	2	AF528025-6	0.083	4	4	4
<i>Noturus taylori</i>	Caddo Madtom	1	KP013089	0.163	4	3	4
<i>Pyloodictis olivaris</i>	Flathead Catfish	3	MF621728-30	0.175	4	7	4
<i>Catostomus bernardini</i>	Yaqui Sucker	1	FJ751807	0.05	7	7	4
<i>Poeciliopsis sonoriensis</i>	Yaqui Topminnow	1	MK860197	0.053	5	7	10
<i>Oncorhynchus mykiss</i>	Rainbow Trout	1	AB889396	0.03	4	8	7
<i>Cyprinus carpio</i>	Common Carp	1	KJ511883	0.083	7	6	4
<i>Carassius auratus</i>	Goldfish	1	AB368639	0.056	5	6	5
<i>Gila robusta</i>	Roundtail Chub	1	DQ536424	0.059	8	7	5
<i>Pimephales promelas</i>	Fathead Minnow	1	KT278765	0.026	9	7	5
<i>Carpionodes carpio</i>	River Carpsucker	1	AP006763	0.083	8	6	8
<i>Poeciliopsis monacha</i>	Headwater Livebearer	1	KX229692	0.052	5	9	9
<i>Poeciliopsis occidentalis</i>	Gila Topminnow	1	MK860198	0.042	6	7	10
<i>Micropterus salmoides</i>	Largemouth Bass	1	AP014537	0.066	4	7	8
<i>Lepomis cyanellus</i>	Green Sunfish	1	KC427094	0.027	5	7	4
<i>Gambusia affinis</i>	Mosquitofish	1	AP004422	0.053	7	6	7

**Table S4.** Species used for *in vitro* testing of the Yaqui Catfish assay. Origin refers to the U.S. state where each sample was collected.

Family	Species name	Common name	Sample size	Origin	
Ictaluridae	<i>Ictalurus pricei</i>	Yaqui Catfish	11	Arizona	
	<i>Ictalurus punctatus</i>	Channel Catfish	5	Montana	
	<i>Noturus flavus</i>	Stonecat	1	Montana	
	<i>Amieurus natalis</i>	Yellow Bullhead	2	Montana	
	<i>Amieurus melas</i>	Black Bullhead	2	Colorado	
Cyprinidae	<i>Cyprinus carpio</i>	Common Carp	1	Wyoming	
	<i>Agosia chrysogaster</i>	Longfin Dace	1	New Mexico	
	<i>Pimephales promelas</i>	Fathead Minnow	1	New Mexico	
	<i>Cyprinella lutrensis</i>	Red Shiner	1	New Mexico	
	<i>Semotilus atromaculatus</i>	Creek Chub	1	New Mexico	
	<i>Gila pandora</i>	Rio Grande Chub	1	Colorado	
	<i>Rhynchichthys osculus</i>	Speckled Dace	1	Arizona	
	<i>Tiaroga cobitis</i>	Loach Minnow	1	Arizona	
	<i>Gila nigrescens</i>	Chihuahua Chub	1	New Mexico	
	<i>Gila robusta</i>	Roundtail Chub	2	Colorado, Arizona	
	<i>Meda fulgida</i>	Spikedace	1	Arizona/New Mexico	
	Catostomidae	<i>Pantosteus clarkii</i>	Desert Sucker	1	New Mexico
		<i>Catostomus commersonii</i>	White Sucker	1	New Mexico
<i>Xyrauchen texanus</i>		Razorback Sucker	1	New Mexico/Arizona	
Centrarchidae	<i>Pomoxis nigromaculatus</i>	Black Crappie	1	Montana	
	<i>Micropterus dolomieu</i>	Smallmouth Bass	1	Oregon	
	<i>Micropterus salmoides</i>	Largemouth Bass	1	Montana	
	<i>Pomoxis annularis</i>	White Crappie	1	Montana	
	<i>Lepomis cyanellus</i>	Green Sunfish	1	Washington	
Cottidae	<i>Cottus bairdii</i>	Mottled Sculpin	1	Utah	
Percidae	<i>Sander vitreus</i>	Walleye	1	Washington	
	<i>Perca flavescens</i>	Yellow Perch	1	Washington	
Salmonidae	<i>Salvelinus fontinalis</i>	Brook Trout	1	Idaho	
	<i>Oncorhynchus apache</i>	Apache Trout	1	Arizona	
	<i>Oncorhynchus gilae</i>	Gila Trout	1	New Mexico	
	<i>Oncorhynchus clarkii bouvieri</i>	Yellowstone Cutthroat Trout	1	Nevada	
	<i>Oncorhynchus mykiss gairdnerii</i>	Redband Trout	1	California	

**Table S5.** Species used for *in vitro* testing of the Channel Catfish assay. Origin refers to the U.S. state where each sample was collected.

Family	Species name	Common name	Sample size	Origin
Ictaluridae	<i>Ictalurus punctatus</i>	Channel Catfish	1 6	MontanaOklahoma
	<i>Ictalurus pricei</i>	Yaqui Catfish	3	Arizona
	<i>Ictalurus lupus</i>	Headwater Catfish	6	Texas
	<i>Ictalurus furcatus</i>	Blue Catfish	3	Oklahoma
	<i>Ameiurus natalis</i>	Yellow Bullhead	2 1	MontanaNew Mexico
	<i>Ameiurus melas</i>	Black Bullhead	2	Colorado
	<i>Pylodictis olivaris</i>	Flathead Catfish	3	Oklahoma
	<i>Noturus flavus</i>	Stonecat	1	Montana
Catostomidae	<i>Catostomus insignis</i>	Sonora Sucker	1	New Mexico
	<i>Pantosteus clarkii</i>	Desert Sucker	1	New Mexico
	<i>Catostomus plebeius</i>	Rio Grande Sucker	1	New Mexico
	<i>Catostomus commersonii</i>	White Sucker	1	New Mexico
	<i>Xyrauchen texanus</i>	Razorback Sucker	1	Arizona
Centrarchidae	<i>Pomoxis annularis</i>	White Crappie	1	Montana
	<i>Lepomis cyanellus</i>	Green Sunfish	1	New Mexico
	<i>Micropterus salmoides</i>	Largemouth Bass	1	Montana
Cyprinidae	<i>Semotilus atromaculatus</i>	Creek Chub	1	New Mexico
	<i>Cyprinus carpio</i>	Common Carp	1	Wyoming
	<i>Gila nigrescens</i>	Chihuahua Chub	1	New Mexico
	<i>Cyprinella lutrensis</i>	Red Shiner	1	New Mexico
	<i>Rhinichthys osculus</i>	Speckled Dace	1	New Mexico
	<i>Agosia chrysogaster</i>	Longfin Dace	1	New Mexico
	<i>Gila pandora</i>	Rio Grande Chub	1	Texas
	<i>Ptychocheilus lucius</i>	Colorado Pikeminnow	1	Utah
Umbridae	<i>Umbra limi</i>	Central Mudminnow	1	Montana