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Short Communication

Identification of three *Ictalurus* species in Mexico using Cytochrome Oxidase I gene sequencing

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ABSTRACT. Channel catfish, *Ictalurus punctatus*, is one of the most important fish on Mexican aquaculture and despite its productive potential, there is very few information regarding the distribution of *Ictalurus* species in Mexican territory. Sequencing of Cytochrome Oxidase I gene has proved to be very useful for the identification of fish specimens and sub-products. In the present, we report the molecular identification of 93 feral, commercial and domestic catfish individuals. All domestic fish were positively identified as channel catfish. Commercial samples were identified as *Ictalurus punctatus*, *Ictalurus lupus*, and *Ictalurus furcatus*, providing evidence that catfish represents a generic label for separate fish species. We also report the presence of feral *I. punctatus* on eight novel locations within Mexican territory, *I. lupus* on three and *I. furcatus* in one. Thus, we propose that molecular identification by DNA barcoding represents a powerful tool in the study of catfish populations and sub-products.

Keywords: *Ictalurus*, channel catfish, DNA, barcoding, genetic, fish traceability.

Mexico contributes with nearly 10% of the world fish diversity (CONABIO, 2014). 543 out of 3000 species are subject to commercial exploitation in the country (SAGARPA, 2013). However, it has been proposed that 86% of the species on Earth and 91% in the ocean still need to be described (Mora *et al.*, 2011). Channel catfish *Ictalurus punctatus* is one of the Mexican freshwater fishes with the highest presence in American aquaculture. For its exploitation on aquaculture and fisheries, channel catfish have been introduced in 20 states of the Mexican territory (Lara-Rivera *et al.*, 2015). Reservoirs are common receptacles for fry and adult releases in order to promote local subsistence fishing. Since there are no reports of neither prior nor subsequent studies of these releases, it is uncertain if wild and domestic populations have mixed at some point. On the other hand, fishermen are not familiar with the taxonomical distinctive characteristics of the different species of the genus *Ictalurus*, which leads to misidentification of fish from the net and all the way to the market. In order to identify a specimen, there is need of two things: first, a specialized taxonomist, and second, specimens in an ideal state of preservation. DNA barcoding has proven to be a powerful tool for rapid, non-morphological and accurate species identification and discovery (Ward *et al.*, 2005). Sequencing of a 648 bp region from the Cytochrome Oxidase 1 (COI) region allows comparison and identification of species based on similarities among related groups (Clare *et al.*, 2007). Among the most useful applications of DNA barcoding are identification of fish products and sub-products (such as fillets, nuggets and conserves), mislabeled products (Jacquet & Pauly, 2008; Wong & Hanner, 2008; Galal-Khallaf *et al.*, 2014) and recognition of endangered or potentially risky species (Galimberti *et al.*, 2013; Quinto *et al.*, 2016). There is very few information regarding channel catfish distribution on Mexican continental waters and nowadays, *I. punctatus* is considered to be an invasive species in Mexico (González *et al.*, 2014), since the production began from fish imported from the USA instead of Mexican

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native populations. Therefore, the aim of the present work was the molecular identification of Mexican catfish using a DNA barcoding approach.

A total of 93 samples were used for the analysis (Table 1); 17 samples representing the most important aquaculture farms in Mexico, 70 samples were obtained from free-living catfish from 5 reservoirs, 5 rivers, and 1 lake. Additionally, 5 commercial samples, labeled as “catfish” were used for a comparative analysis. Samples consisted of anal and adipose fins. DNA was extracted using the commercial kit GenElute Mammalian Genomic DNA Miniprep (SIGMA Aldrich®) following the directions provided by the manufacturer. DNA integrity was verified on 1.5% agarose gels. DNA concentration was calculated using a Nano Drop 2000 C spectrophotometer (Thermo Scientific v 1.1). In order to amplify 651 bp fragment from the 5’ end of mitochondrial COI gene, PCR reactions were conducted using primer cocktails C_FishF1t1 and C_FishR1t1 as described by Ivanova *et al.* (2007) and Wong *et al.* (2011). The amplification reactions were performed in a total volume of 10 µL and included 0.5X Buffer (Promega ®), 0.2 mM of each deoxynucleotide triphosphate (dNTPs), 2.5 mM $MgCl_2$, 0.2 pmol of each primer, 100 ng of genomic DNA, and 1.2 U µL^{-1} Taq DNA polymerase (Promega®). The reactions were conducted using an MJ Research 3130 Thermal Cycler under the following conditions: an initial denaturation at 95°C for 2 min; 35 cycles of 95°C for 30 s, 52°C for 40 s and 72°C for 1 min; and concluded with a final elongation step of 72°C for 10 min followed by a hold at 4°C. Amplified PCR products were subsequently cleaned by the Exo-SAP method (Dugan *et al.*, 2002); 1 µL of PCR product and 0.5 µL EXO SAP-IT (Affymetrix) were incubated for 15 min at 37°C and 80°C for 15 min. Thereafter, 1 µL of each purified PCR product was labeled using the BigDye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems Inc., CA, USA); 4.5 µL of sterile miliQ water, 2.0 µL of BigDye Sequencing Buffer (400 mM Tris-HCl pH 9.0 and 10 mM $MgCl_2$), 2.0 µL of Ready Reaction RR-100 and 0.2 pmol Forward Primer F2_t1 for a total reaction mixture of 10 µL. A sequencing reaction program was run as follows: an initial denaturation at 96°C for 1 min; 25 cycles of 96°C for 10 s, 50°C for 05 s and 60°C for 4 min, followed by a hold at 4°C. Then, 5 µL were taken from the resulting sequencing reaction and added with 22.5 µL and 5.0 µL SAM™ and BigDye^{R} XTerminator™ (Applied Biosystems Inc., CA, USA) solutions respectively, incubated with agitation of 1000 rpm for 30 min at 24°C, then centrifuged at 10000 for 10 min and supernatant transferred to a 96 well plate and sequenced on an ABI 31306l Genetic Analyzer (Applied Biosystems Inc., CA, USA). Sequences were edited using Chromas Lite v 2.1.1 (Technelysium, Pty Ltd.). Voucher sequences from GenBank, reference sequences from BOLD (Altschul *et al.*, 1990) databases and consensus sequences of each species generated from this study were compared and aligned using the CLUSTALW software on MegAlign Pro (DNASTAR Inc., Madison, WI). Sample identification based on the sequence similarity approach was carried out using two databases; BOLD and GenBank. The highest percent pairwise identity of the consensus sequence from each species blasted (BLASTN) against NCBI were compared to the percent specimen similarity scores of the consensus sequence from each species within the BOLD-IDS (BOLD Identification System) (Ratnasingham & Hebert, 2007). As commonly applied in DNA barcoding, sequence divergences were estimated by the Kimura 2-parameter substitution model and a phylogenetic tree was constructed with MegAling using an improved version of the Neighbor-Joining algorithm of Satou and Nei (Gascuel, 1997). The robustness of the maximum parsimony tree was assessed by performing bootstrapping analysis with 1000 replicates, and gaps removed by complete deletion (Felsenstein, 1985).

Table 1 shows the comprehensive barcoding identification results based on GenBank or BOLD databases. Both databases revealed identity in the range of 96-100% for consensus sequences of three species (*Ictalurus punctatus, I. furcatus,* and *I. lupus*). GenBank-based identification for all species yielded an alignment E-value of 0.0.BOLD-IDS results were in agreement with GenBank results in the identification of these species, yielding 100% identity. However, some entrees on FISH-BOLD were marked as "private" and therefore, even when there could be a more reliable match for the target sequence, it cannot be identified at this moment. All domestic samples used for aquaculture were positively identified as *I. punctatus* (Table 1). Concerning free-living individuals, most samples were also identified as channel catfish, with the exception of two individuals from Pilón and Corona Rivers in Tamaulipas, identified as *I. lupus*. With respect to commercial fish, only two samples matched *I. punctatus* COI sequence: a sample from Panuco River, a natural border between the Mexican States Tamaulipas and Veracruz, was identified as channel catfish, same as a fish purchased from a random fish market located on the center of the State of Tamaulipas. At that same location, another specimen was identified as *I. lupus*. A fish collected at Chapala Lake, in the State of Jalisco was also identified as *I. lupus*. Another related species, *I. furcatus* was found at Champayán Lagoon, located southern state in Tamaulipas.

Identification of channel catfish species in Mexico has several connotations. First of all, it can be inferred

Table 1. Sample ID, collection site, location types and BLASTN results showing species identity, and accession number. *Blast performed only on FishBol Database and therefore, no further information of alignments is available.

Sample ID	Collection site	Location type	Species	Identity (%)	Max. score	Coverage (%)	E-value	Accession number
1150	Purificación River, Tamaulipas	River	*I. punctatus*	100	1203	95	0	JF292380.1
1151	Purificación River, Tamaulipas		*I. punctatus*	91	811	51	0	HQ024943.1
1152	Purificación River, Tamaulipas		*I. punctatus*	99	1173	52	0	JF292354.1
1153	Purificación River, Tamaulipas		*I. punctatus*	93	79	12	8e – 11	KF558290.1
1154	Purificación River, Tamaulipas		*I. punctatus*	88	374	31	2e – 99	EU524678.1
1155	Purificación River, Tamaulipas		*I. punctatus*	99	1181	94	0	AF482987.1
1157	Purificación River, Tamaulipas		*I. punctatus*	100	1203	92	0	JF292392.1
COX17	Purificación River, Tamaulipas		*I. punctatus*	99	1151	90	0	JF292353.1
COX18	Purificación River, Tamaulipas		*I. punctatus*	98	1053	86	0	JF292353.1
COX19	Purificación River, Tamaulipas		*I. punctatus*	99	1122	91	0	JF292353.1
COX20	Purificación River, Tamaulipas		*I. punctatus*	99	736	89	0	JF292353.1
1173	Pilón River, Tamaulipas		*I. punctatus*	99	1197	92	0	JF292353.1
1174	Pilón River, Tamaulipas		*I. punctatus*	100	1203	81	0	JF292380.1
1176	Pilón River, Tamaulipas		*I. punctatus*	99	1219	87	0	AF482987.1
1177	Pilón River, Tamaulipas		*I. punctatus*	99	1205	87	0	AF482987.1
1178	Pilón River, Tamaulipas		*Ictalurus lupus*	98	1140	92	0	JN026911.1
1179	Pilón River, Tamaulipas		*I. punctatus*	99	1214	64	0	AF482987.1
COX14	Pilón River, Tamaulipas		*I. punctatus*	99	1072	87	0	JF292353.1
COX15	Pilón River, Tamaulipas		*I. punctatus*	99	1107	88	0	JF292353.1
COX16	Pilón River, Tamaulipas		*I. punctatus*	99	1077	85	0	JF292353.1
1231	Corona River, Tamaulipas		*Ictalurus lupus*	98	1094	96	0	JN026910.1
1231	Corona River, Tamaulipas		*Ictalurus lupus*	98	1138	93	0	JN026911.1
1203	Corona River, Tamaulipas		*I. punctatus*	100	1203	95	0	JF292380.1
1202	Corona River, Tamaulipas		*I. punctatus*	100	11.03	92	0	JF292353.1
1205	Corona River, Tamaulipas		*I. punctatus*	99	1182	91	0	JF292386.1
1206	Corona River, Tamaulipas		*I. punctatus*	99	1142	93	0	JF292353.1
1207	Corona River, Tamaulipas		*I. punctatus*	99	1098	91	0	JF292354.1
1209	Corona River, Tamaulipas		*I. punctatus*	99	1035	92	0	JF292353.1
1210	Corona River, Tamaulipas		*I. punctatus*	92	981	92	0	JF292360.1
1533	Bravo River, Tamaulipas		*I. punctatus*	99	1219	96	0	AF482987.1
1532	Bravo River, Tamaulipas		*I. punctatus*	99	987	77	0	JF292353.1
1580	Bravo River, Tamaulipas		*I. punctatus*	99	1240	61	0	AF482987.1
1530	Bravo River, Tamaulipas		*I. punctatus*	99	1219	96	0	AF482987.1
1505	Bravo River, Tamaulipas		*I. punctatus*	99	1219	96	0	AF482987.1
1529	Bravo River, Tamaulipas		*I. punctatus*	99	1214	96	0	AF482987.1
1535	Bravo River, Tamaulipas		*I. punctatus*	99	1208	96	0	AF482987.1
1509	Bravo River, Tamaulipas		*I. punctatus*	99	1214	96	0	AF482987.1
PAN13	Pánuco River, Veracruz and Tamaulipas borderline		*I. punctatus*	87				*BCFB131-06
1096	Gustavo Díaz Ordaz Dam, Sinaloa	Reservoir	*I. punctatus*	89	795	82	0	JF292392.1
1104	Gustavo Díaz Ordaz Dam, Sinaloa		*I. punctatus*	89	852	57	0	AF482987.1
1109	Gustavo Díaz Ordaz Dam, Sinaloa		*I. punctatus*	100	1131	57	0	JF292353.1
1132	Gustavo Díaz Ordaz Dam, Sinaloa		*I. punctatus*	94	1020	58	0	JF292387.1
1137	Gustavo Díaz Ordaz Dam, Sinaloa		*I. punctatus*	86	610	48	1e -170	JF292388.1
1139	Gustavo Díaz Ordaz Dam, Sinaloa		*I. punctatus*	100	1208	65	0	HQ024943.1
1092	Gustavo Díaz Ordaz Dam, Sinaloa		*I. punctatus*	99	1177	97	0	JF292392.1
1233	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	98	828	98	0	HQ024943.1
1234	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	99	929	90	0	JF292362.1
1235	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	99	1216	96	0	AF482987.1
1236	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	99	1099	87	0	JF292353.1
1298	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	94	922	61	0	JF292392.1
1299	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	100	1203	92	0	JF292392.1
1240	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	99	1171	91	0	JF292392.1

Continuation

Sample ID	Collection site	Location type	*Species*	Identity (%)	Max. score	Coverage (%)	E-value	Accession number
1241	Ramiro Caballero Dam, Tamaulipas		*I. punctatus*	99	1214	98	0	AF482987.1
1250	República Española Dam, Tamaulipas		*I. punctatus*	100	1221	93	0	JF292380.1
1251	República Española Dam, Tamaulipas		*I. punctatus*	100	1203	93	0	JF292392.1
1252	República Española Dam, Tamaulipas		*I. punctatus*	100	1214	96	0	AF482987.1
1253	República Española Dam, Tamaulipas		*I. punctatus*	100	1203	93	0	JF292392.1
1254	República Española Dam, Tamaulipas		*I. punctatus*	100	1225	96	0	AF482987.1
1255	República Española Dam, Tamaulipas		*I. punctatus*	100	1203	93	0	JF292392.1
1256	República Española Dam, Tamaulipas		*I. punctatus*	100	1203	93	0	JF292392.1
1257	República Española Dam, Tamaulipas		*I. punctatus*	100	1203	93	0	JF292392.1
1063	Vicente Guerrero Dam, Tamaulipas		*I. punctatus*	99	1162	94	0	JF292353.1
1064	Vicente Guerrero Dam, Tamaulipas		*I. punctatus*	99	1229	97	0	AF482987.1
1065	Vicente Guerrero Dam, Tamaulipas		*I. punctatus*	99	1225	96	0	AF482987.1
1450	La Boquilla Dam, Chihuahua		*I. punctatus*	100	1219	98	0	AF482987.1
1451	La Boquilla Dam, Chihuahua		*I. punctatus*	99	1201	96	0	AF482987.1
1452	La Boquilla Dam, Chihuahua		*I. punctatus*	99	1216	96	0	AF482987.1
1455	La Boquilla Dam, Chihuahua		*I. punctatus*	99	1197	96	0	AF482987.1
1457	La Boquilla Dam, Chihuahua		*I. punctatus*	100	1203	92	0	JF292392.1
1458	La Boquilla Dam, Chihuahua		*I. punctatus*	99	1227	96	0	AF482987.1
1461	La Boquilla Dam, Chihuahua		*I. punctatus*	99	1219	96	0	AF482987.1
1459	La Boquilla Dam, Chihuahua		*I. punctatus*	100	1203	93	0	JF292392.1
764	Sayula, Jalisco	Farm	*I. punctatus*	99	1147	89	0	JF292392.1
788	Sayula, Jalisco		*I. punctatus*	99	1227	97	0	AF482987.1
868	Briseñas, Michoacán		*I. punctatus*	100	1225	96	0	AF482987.1
874	Briseñas, Michoacán		*I. punctatus*	100	1225	97	0	AF482987.1
995	Villagrán, Zacatecas		*I. punctatus*	100	1221	97	0	AF482987.1
990	Villagrán, Zacatecas		*I. punctatus*	100	1225	96	0	AF482987.1
1413	San Fco. de Conchos, Chihuahua		*I. punctatus*	100	1227	96	0	AF482987.1
1422	San Fco. de Conchos, Chihuahua		*I. punctatus*	100	1203	96	0	AF482987.1
937	General Cepeda, Coahuila		*I. punctatus*	99	1221	96	0	AF482987.1
939	General Cepeda, Coahuila		*I. punctatus*	100	1203	93	0	JF292392.1
8	"La Doña", Tamaulipas		*I. punctatus*	94	647	59	0	KF558290.1
588	"Aquaque", Tamaulipas		*I. punctatus*	100	928	91	0	BCFB131-06
117	"Aquaque", Tamaulipas		*I. punctatus*	100				*ANGBF8222-12
75	"La Isla", Tamaulipas		*I. punctatus*	100	1227	96	0	AF482987.1
1049	Nuevo Padilla, Tamaulipas	Fish Market	*Ictalurus lupus*	98	1066	87	0	JN026911.1
1043	Nuevo Padilla, Tamaulipas	Fish Market	*I. punctatus*	98	1059	86	0	JF292353.1
COX6	Champayán, Tamaulipas	Fish Market (From Lagoon)	*Ictalurus furcatus*	94	985	88	0	JF292369.1
867	Chapala, Jalisco	Fish Market (From Lake)	*Ictalurus lupus*	98	1116	95	0	JN026911.1
			Ictalurus lupus	97	1064	92	0	JN026910.1
SL1	San Luis Missouri, USA	Reference from Farm	*I. punctatus*	99	813	90	0	JF292392.1
SL3	San Luis Missouri, USA		*I. punctatus*	100	1203	96	0	AF482987.1

that, in spite of the lack of attention that has been given to the species production, aquaculture is based on *I. punctatus* and not misleading species. An accurate and reliable identification of channel catfish provides a powerful tool for further market analysis of fish products and sub-products. It has been established that almost 25% of fish products in the United States are mislabeled (Wong & Hanner, 2008); most products are intentionally sold as more expensive species. Channel catfish, in particular, has faced great competition against bassa fish (*Pangasius* spp.) since it is produced at a much lower cost. However, the low-quality requirements of bassa fish make it a potential hazard to health and unfair competition for the channel catfish industry. In the present report, it was observed that when looking for "catfish", different species are available on fish markets. *I. lupus* and *I. furcatus* were presented as alternatives for channel catfish consumption but are likely dueto the local availability of fish or a lack of awareness from the consumers. The appropriate identification of species constitutes one of the main concerns regarding population management,

Table 2. *Ictalurus punctatus* previous and current distribution.

Previously reported distribution	Present distribution (as found in this study)
• Balsas River (Mejía *et al.*, 2013). • Río Bravo Hydrological System. The "Río Bravo" Hydrological system includes Salado River, Álamo River, San Juan River, Bravo River, Anzaldúaz Dam, Retamal Dam and Madre Lagoon. • San Fernando Hydrological System "San Fernando" Hydrological System includes San Fernando, Potosí and Linares rivers. • Soto La Marina Hydrological System "Soto La Marina" Hydrological System is constituted by Soto La Marina, San Carlos, Pilón, Purificación, Corona, San Felipe, Santa Ana, San Marcos and Arroyo Grande rivers. • Guayalejo-Tamesí Hydrological System (Page & Burr, 1991; Pérez-Ponce & Choudhury, 2002). The "Guayalejo-Tamesí" Hydrological system includes Hieu, Nogales, San Vicente, Jaumave, Guayalejo, Sabinas, Frío, Las Flores, Mante, Tigre and Tamesí rivers.	Purificación River Pilón River Corona River Bravo River Pánuco River Vicente Guerrero Reservoir República Española Reservoir Gustavo Díaz Ordaz Reservoir La Boquilla Reservoir

such as biodiversity monitoring (Groves *et al*., 2002; Hajibabaei *et al*., 2007), recognition of mating and migratory behaviors (Sawyer *et al*., 2009), and population genetics by providing signals of the extent and nature of population divergences and facilitating comparative studies of population diversity (Taylor *et al*., 2003; Hebert *et al*., 2004). Taxonomic identification is usually dependent on the existence of a good quality specimen and the availability of a skilled professional. Molecular identification of wild specimens could be of utmost importance for the study of wild populations. Not only in the present it was possible to verify the reported distribution of *Ictalurus* species in Mexico such as the case of *I. furcatus* and *I. punctatus* on Bravo and Panuco rivers (García de Leon *et al.*, 2005), but we can also report the presence of free-living channel catfish, *Ictalurus punctatus*, on eight novel locations within Mexican territory: "Gustavo Díaz Ordaz", "República Española", "Ramiro Caballero", "Vicente Guerrero" and "La Boquilla" reservoirs and Purificación, Pilón and Corona rivers (Table 2).

On the other hand, *I. furcatus*, previously reported in Bravo and Yaqui River (Cuvier & Valenciennes, 1829) was now found on Champayán Lagoon, more than 400 km to the south. Moreover, *I. lupus*, previously reported in Balsas River and Ixtla River (Jordan & Snyder, 1900), was found at 3 novel locations: Pilón River, Corona River, and Chapala Lake. As it can be noted, *Ictalurus* species have expanded their original range. This can be attributed to the success of a growing industry. However, it should be noted that undocumented introductions, transportation, and releases could have a negative impact on recognized wild catfish populations. Channel catfish is considered as an invasive species on present days (González *et al.*, 2014) and any species with a little survival potential could be dangerous on a new ecosystem. Notwithstanding, the channel catfish should be rather seen as a species with a huge productive potential instead of a threat, while new tools emerge to greatly facilitate our understanding and ability to leverage its maximum potential.

The effectiveness of DNA barcoding was ascertained for channel catfish populations in Mexico. It was verified that aquaculture industry uses only the species *I. punctatus*. A wider distribution range was observed for three *Ictalurus* species. A total of 12 novel sites are here reported for *I. punctatus*, *I. furcatus* and *I. lupus*. When commercial samples were analyzed, it was found that *I. lupus* and *I. furcatus* were sold as generic "catfish", so it is proposed that molecular identification by DNA barcoding represents a powerful tool in the study of catfish populations and sub-products.

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