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BRIEF COMMUNICATION

First genetic record of the non-native muzzled blenny *Omobranchus punctatus* (Teleostei: Blenniidae) in the Atlantic Coast of Central and South America

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Abstract

In this study we sequenced two mitochondrial (COI and 16S rRNA) and one nuclear (18S rRNA) gene fragment of an introduced muzzled blenny (*Omobranchus punctatus*) specimen collected from the Orinoco Delta (Gulf of Paria estuary) in Venezuela. This is the first genetic data generated for this species' introduced range in Central and South America, suggesting an introduction from the Indian Ocean.

KEYWORDS
ballast water, biofouling, Caribbean, DNA barcoding, exotic fish, introduction pattern

The muzzled blenny *Omobranchus punctatus* (Valenciennes, 1836) is a coastal, brackish fish that can be naturally found near river mouths or mangroves, in tidal pools and inhabiting the interstices of rock substrates (Ismail & Clayton, 1990; Springer & Gomon, 1975). It is considered native to the Indo-Pacific region, stretching from the Persian Gulf to South-East Asia, Japan, northern Australia and the Fiji Islands (Chargulaf *et al.*, 2011; Springer & Gomon, 1975).

Springer and Gomon (1975) documented the first record of *O. punctatus* outside its natural range in 1930, on the West coast of Trinidad (Gulf of Paria), based on Fowler's (1931) taxonomic work. Since then, the species has been recorded from several localities of the Atlantic Coast of Central and South America (Figure 1), including Panamá, Colombia, Venezuela, and other localities in Trinidad and Brazil (Cervigón, 1966; Contente *et al.*, 2015; Costa *et al.*, 2011; Garzón-Ferreira, 1989; Gerhardinger *et al.*, 2006; Lasso *et al.*, 2004; Lasso-Alcalá *et al.*, 2011; Loebmann *et al.*, 2010; Springer & Gomon, 1975; Vilar *et al.*, 2011; Williams, 2002). Additionally, it has been found in the eastern coast of Africa (Fricke *et al.*, 2018; Lasso-Alcalá *et al.*, 2011; Maugé, 1967; Springer & Gomon, 1975), in the Suez Canal (Bath, 1980), the Mediterranean Sea (Golani, 2004; Psomadakis

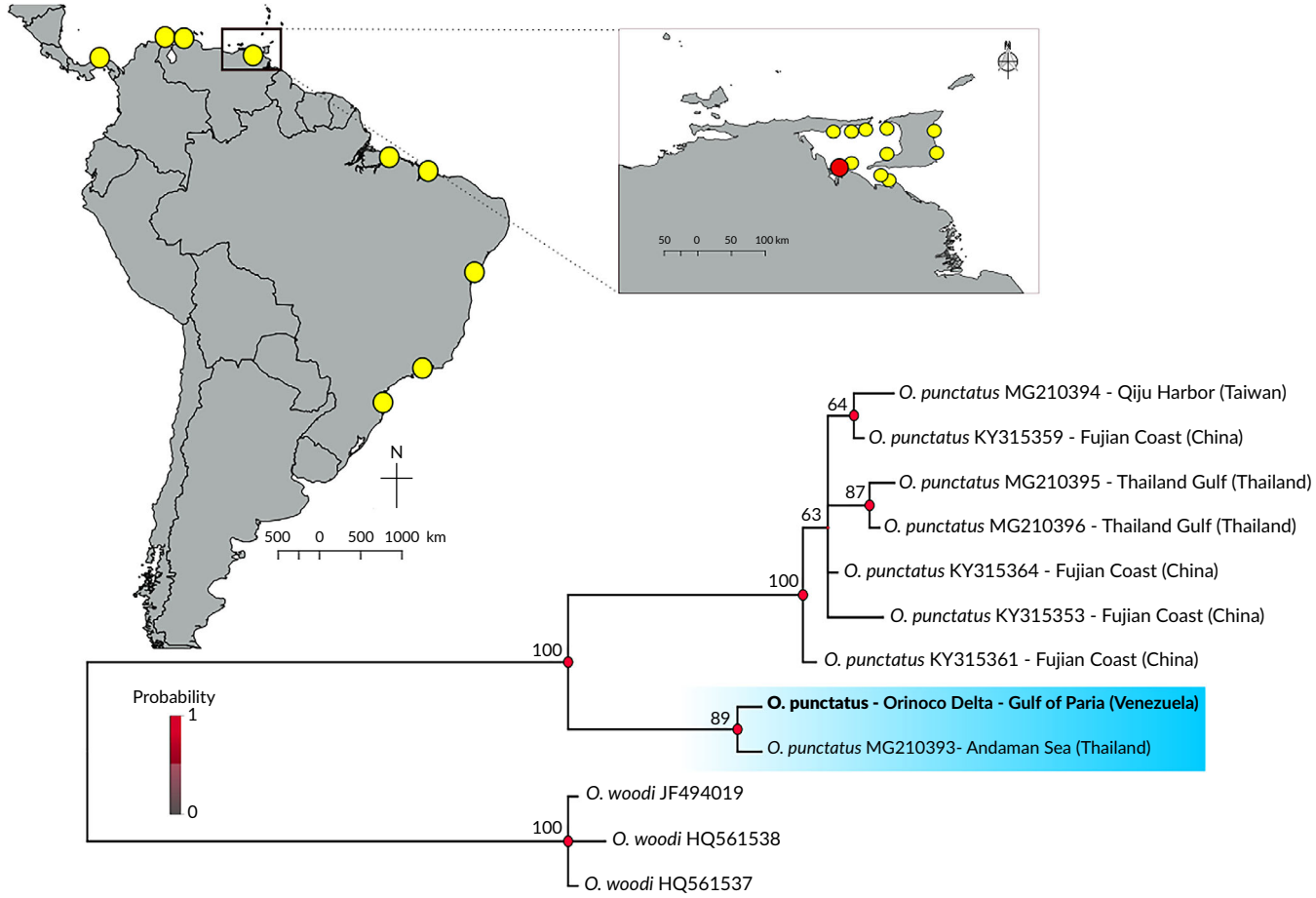

FIGURE 1 Bayesian consensus tree based on the *Omobranchus punctatus* sequenced individual (in bold black) and GenBank samples. The tree was rooted with *O. woodi*. Bayesian posterior probabilities are represented by colour and node size and values by nodes correspond to bootstrap support given by the maximum likelihood analyses. The red circle in the map represents the locality where this specimen was collected. Yellow circles correspond to localities where the species was previously recorded (see Lasso-Alcalá *et al.*, 2011 for full detailed distribution)

et al., 2012) and in the Red Sea (Bogorodsky & Randall, 2018; Golani & Bogorodsky, 2010; Golani & Fricke, 2018).

In its introduced range, *O. punctatus* has been frequently found associated with fouling communities of artificial structures such as boats and aquaculture fish cages, but also inhabiting natural substrates (under rocks in tidal pools), most of them located near major ports and seaways (Contente *et al.*, 2015; Gerhardinger *et al.*, 2006; Golani, 2004; Lasso *et al.*, 2004; Lasso-Alcalá *et al.*, 2011; Loebmann *et al.*, 2010; Soares *et al.*, 2011).

Therefore, biofouling and ballast water have been identified as the most likely vectors responsible for the introduction and dispersion of this species (Contente *et al.*, 2015; Gerhardinger *et al.*, 2006; Golani, 2004; Lasso-Alcalá *et al.*, 2011; Springer & Gomon, 1975). In this sense, the ever-increasing magnitude and efficiency of global maritime trade together with the high tolerance of *O. punctatus* to both a wide range of salinity levels and adverse environmental conditions (Contente *et al.*, 2015; Gerhardinger *et al.*, 2006; Lasso-Alcalá *et al.*, 2004, 2008, 2011; Lasso-Alcalá & Lasso, 2011), suggest possible future introductions of this species to new regions (Briggs, 2012; Wonham *et al.*, 2000).

Molecular data, and especially DNA barcoding, have been recognized as a powerful and simple tool for identifying and monitoring nonindigenous species (Comtet *et al.*, 2015; Darling & Blum, 2007; Hebert *et al.*, 2003; Rius *et al.*, 2015), including fishes (Almeida *et al.*, 2018; Barman *et al.*, 2018; Collins *et al.*, 2013). In this sense, molecular data has not only been used for early detection of such species (Almeida *et al.*, 2018; Comtet *et al.*, 2015) but also to provide crucial insights into their patterns of introduction (Comtet *et al.*, 2015; Rius *et al.*, 2015). Although there is some molecular data available on *O. punctatus* inside its native region, to date there are no sequences available for the species in its introduced range. In the present study, one individual collected from a rocky beach at Pedernales (Orinoco Delta – Gulf of Paria, Venezuela) (Figure 1) was barcoded by sequencing two mitochondrial [cytochrome c oxidase subunit I (COI) and 16 ribosomal RNA (16S rRNA)] and one nuclear [18S ribosomal RNA (18S rRNA)] gene fragment. The individual was collected as a part of a faunal survey with ethical guidelines as described in Lasso *et al.* (2004). Specifically, this fish specimen is deposited in the fish collection of the Museo de Historia Natural La Salle (MHNLS 17220), in Caracas, Venezuela; and it was collected as a part of the Project

TABLE 1 Primers and PCR conditions used for amplification of *Omobranchus punctatus* individual from Orinoco Delta – Gulf of Paria (Venezuela)

Primer	Sequence (5'-3')	Source	PCR conditions
COI			
VF2_t1	TGTAAAACGACGGCCAGTCAACCAA CCACAAAGACATTGGCAC	Ivanova *et al.*, (2007)	94°C (1'); [x5] 94°C (30''), 50°C (40''), 72°C (1'); [x35] 94°C (30''), 54°C (40''), 72°C (1'); 72°C (10')
FishF2_t1	TGTAAAACGACGGCCAGTCGACTA ATCATAAAGATATCGGCAC		
FishR2_t1	CAGGAAACAGCTATGACACTTCAGGG TGACCGAAGAATCAGAA		
FR1d_t1	CAGGAAACAGCTATGACACCTCAGGG TGTCCGAARAAYCARAA		
16S rRNA			
16Sar-L	CGCCTGTTTATCAAAAACAT	Palumbi *et al.*, (1991)	94°C (4'); [x40] 94°C (45''), 52°C (50''), 72°C (1'); 72°C (10')
16Sbr-H	CCGGTCTGAACTCAGATCACGT		
18S rRNA			
SSU 3F	CACCGCCCGTCGCTACTACCG	Jarman *et al.*, (2013)	94°C (4'); [x40] 94°C (20''), 58°C (1'), 72°C (2'); 72°C (10')
SSU 3R	GGTTCACCTACGGAAACCTTGTTACG		

Biological Assessment and Socio Economical Aspects of the Aquatic Ecosystems of the Gulf of Paria and Orinoco Delta, Venezuela, under the Program AquaRAP (2002–2004). The care and use of experimental animals complied with Venezuelan animal welfare laws, guidelines and policies as approved by the Instituto Nacional de Pesca y Acuicultura (2002). The specimen was collected with a hand net and, after tissue collection, it was fixed in a 10% formaldehyde solution. No surgical procedures were performed, and no procedures that cause lasting harm to the fish were carried out (see page 216 in Lasso *et al.*, 2004 for additional sampling information).

Whole-genomic DNA was extracted from a small amount of muscle tissue using the PureLink Genomic DNA Mini Kit (Invitrogen, Paisley, UK), according to the manufacturer's protocol. Primers for amplification and PCR conditions are listed in Table 1. Mitochondrial COI gene was amplified using a M13-tailed cocktail of universal fish primers (Ivanova *et al.* 2007). PCR product purification and sequencing were outsourced to a commercial company (GENEWIZ, Leipzig, Germany).

The obtained sequences were checked and edited using Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA), and thereafter submitted to GenBank [accession numbers MN907119 (COI), MN901493 (16S) and MN901496 (18S)]. Species-level identification was performed using GenBank's BLASTn search (Altschul *et al.*, 1990). Because 16S and 18S rRNA sequences are not available in GenBank for this species, only the mitochondrial COI sequence was used for phylogenetic analyses. Such analyses were performed using all other available COI sequences from *O. punctatus* in GenBank (all of them from its native range; accession numbers MG210393-MG210396, KY315353, KY315359, KY315361 and KY315364). In addition, three sequences of the closely related species *O. woodi* (Gilchrist & Thompson, 1908) (accession numbers HQ561537, HQ561538 and JF494019) were selected as the outgroup.

All COI sequences were aligned using MUSCLE (Edgar, 2004) as implemented in MEGA X (Kumar *et al.*, 2018), and trimmed to a length of 602 bp for the final alignment. Phylogenetic analysis were conducted using Bayesian inference (BI) in MrBayes v3.2.6 (Ronquist *et al.*, 2012) and maximum likelihood (ML) in Garli v2.0.1 (Zwickl, 2006). Data partitioning by codon was applied to minimize saturation effects of codon positions and to account for different rates of evolution of each one. Partition Finder v2.1.1 (Lanfear *et al.*, 2016) was used to estimate the best-fit model of sequence evolution for the dataset, according to the Akaike Information Criterion (Akaike, 1974). The following models were applied to COI partitions: SYM (1st position), F81 (2nd position) and HKY (3rd position). For the BI analysis, two independent runs (each with four chains for 2×10^7 generations) were performed. Trees and parameters were sampled every 1000 generations, with the heating parameter set to 0.25. Stationarity was reached when the average standard deviation of the split frequencies was lower than 0.01. A majority-rule consensus tree was estimated combining results from both analyses, after discarding 25% of the total samples as burn-in. ML analysis was performed using 10 independent searches and 1000 bootstrap replicates. The convergence between tree topologies was confirmed by examining log likelihood values across searches. The SumTrees command from the package DendroPy (Sukumaran & Holder, 2010) was used to summarize non-parametric bootstrap support values for the best tree after generating a majority-rule consensus tree. The consensus tree inferred for each phylogenetic approach was visualized and rooted using FigTree v1.4.3 (Rambaut, 2017).

The highest similarity from the BLAST search (99.17%) matched a sequence of *O. punctatus* from Trang Province off the Thailand coast in the Andaman Sea (accession number MG210393, Gibbs *et al.*, 2018). Accordingly, phylogenetic reconstructions supported the initial species-level assignment (Figure 1), with sequences from *O. punctatus*

retrieved as monophyletic and grouped into two well-supported clades: one containing *O. punctatus* sequenced from Taiwan, China and the Gulf of Thailand, and the other containing the sequence from Trang Province and the one generated in the present study (Figure 1). Genetic divergence between both clades was high (minimum 3.5% and a minimum of 25 substitutions). However, genetic divergence between our sample and that from Trang Province (Andaman Sea – Thailand, MG210393) was only 0.3% (two substitutions). Although molecular data are still scarce, the geographic distribution of these two clades suggests a phylogeographic break separating populations located east of the Thai-Malay Peninsula, from those located to the West of the peninsula (Gibbs *et al.*, 2018). Indeed, a major biogeographic break for several marine taxa, including fishes, is roughly located in that region (the Sundra Shelf Barrier, SSB) (reviewed in Rocha *et al.*, 2007), and could be the reason for the observed phylogeographic division.

Based on the available genetic data, we suggest that source populations of *O. punctatus* in the Orinoco Delta–Gulf of Paria (Venezuela) could originate west from the SSB. This corroborates the morphological affinity found between the Caribbean populations and Western Thailand (Springer & Gomon, 1975). According to these authors, *O. punctatus* could have been introduced into Trinidad by accidental transfer on slave boats from the Bay of Bengal (Madras or, most probably, Calcutta region) (Springer & Gomon, 1975). Later, Lasso-Alcalá *et al.* (2008) confirmed morphological similarities between Venezuelan specimens and those from Springer and Gomon (1975). Therefore this species could have spread from Trinidad, through ballast water or biofouling, to Venezuela, Colombia and the Atlantic entrance of the Panama Canal (Golani, 2004; Lasso-Alcalá *et al.*, 2011; Springer & Gomon, 1975).

However, further molecular studies including both native (especially India) and other non-native populations from the Atlantic Coast of Central and South America are needed to shed light on the possible introduction routes of *O. punctatus*. In addition, further studies focusing on species biology, population dynamics and behaviour are important to understand if this species represents a risk for native populations, providing useful information for the effective management of this introduced species.

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AUTHOR CONTRIBUTIONS

O.L-A. and M.J.J. conceived and designed the analysis. O.L-A. collected the *Omobranchus* sample. M.P.C. carried out all the molecular work and performed all molecular analyses under the supervision of R.X. M.P.C. took the lead in writing the manuscript with input from all authors. Finally, all authors discussed the results and contributed to the final manuscript.

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