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RESEARCH NOTE

Stowaways in the catch: Identification of *Xenobrama microlepis* in the haul fishery for *Brama australis*

Polizones en la captura: Identificación de *Xenobrama microlepis* en un lance de pesca de *Brama australis*

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Abstract.- Species that occur in low abundances and that are morphologically similar to the target fish are often undetectable in fisheries hauls; however, they add to the catch statistics of the target species. Using mitochondrial DNA and phylogenetic approaches we identified 6 individuals of the species *Xenobrama microlepis* in a haul targeting the southern Ray's bream (*Brama australis*) taken by artisanal fishermen close to Chilean coast. The presence of *X. microlepis* increases the regional marine biodiversity of fishes in Chilean waters, and fisheries managers should pay attention to the hidden biodiversity in the fishery statistics.

Key words: Molecular taxonomy, hidden biodiversity, barcode, COI

INTRODUCTION

Species that occur in low abundances and that are morphologically similar to the main target species are often undetectable in fisheries hauls, and thus they increase the fishery statistics of the target species (*i.e.*, Ardura *et al*. 2013, Wang *et al*. 2017). However, by using molecular tools it is possible to resolve this issue by identifying these species and quantifying the marine biodiversity they represent in a specific area (*e.g.*, Wang *et al*. 2017). This approach has made significant contributions to the characterization of biodiversity, revealing new species and resolving the status of morphologically similar species (*e.g.*, Zemlak *et al*. 2009, Smith *et al*. 2011).

In the Chilean marine ecosystem, a total of 1182 species of fish have been recorded (Pequeño 1989), however, the number of fish species that remain to be described from the oceanic zone remains unknown (CONAMA 2008). In this context, the family Bramidae is an oceanic fish group with 22 described species (Froese & Pauly 2005), that are considered rare (with the exception of some Brama's species) (Paulin 1981). This rarity is well-supported by several first records in last decade (*e.g.*, Gutierrez *et al*. 2005, De La Cruz & Cota 2008, Carvalho-Filho *et al.* 2009), mainly through by-catch in fisheries activities. Recently, in the southern Ray's bream (*Brama australis* Valenciennes, 1837) fishery blackish individuals were observed that were similar to *B. australis* caught in the same haul. This suggested that the Bramidae maybe more diverse than previously thought. In this context, the use of molecular data and phylogenetic approaches has had a big influence on contemporary taxonomy (Harley 2009), and may be useful in determining the taxonomic status of these blackish individuals.

Hebert *et al*. (2003) proposed the use of the Cytochrome Oxidase I fragment (hereafter COI) of mitochondrial DNA (mtDNA) as a highly informative sequence useful for the identification of different taxonomic groups, and this fragment has been used for several marine taxa (*e.g.*, fishes Ward *et al*. 2005, crustaceans Costa *et al*. 2007, and polychaetes Canales-Aguirre *et al*. 2011). Finally, although the use of only one gene has been controversial (see Dasmahapatra & Mallet 2006, Valentini *et al*. 2009) its usefulness in marine taxa, such as fishes, has been highly valued to date (*e.g.*, Ward *et al*. 2005, Zemlak *et al*. 2009, Karahan *et al*. 2017).

In this study, the molecular identification of *Xenobrama microlepis* Yatsu & Nakamura (1989), collected from one haul of the southern Ray's bream artisanal fishery in the south of the Chilean Exclusive Economic Zone was described. The taxonomic determination was made using a COI fragment sequence from mitochondrial DNA, and phylogenetic approaches.

Materials and methods

To evaluate of the taxonomic rank assignment, i) mtDNA attributes, and ii) theoretical knowledge of nested phylogenetic hierarchy were used. Differences in mtDNA composition among the species are provided by the mutations that accumulate slowly and constantly in species over time (Harley 2009); previous studies have supported interspecific genetic differences in marine fishes using COI (Ward *et al*. 2005). Lastly, the nested phylogenetic hierarchy, resulting in similar clades (*i.e.*, reciprocal monophyly), provides support for assigning the individuals sampled to specific species.

A total of 25 individuals were collected from a single haul by artisanal fishermen in Calbuco, Chile (-43.41°S, -75.16°W) and taken at a depth of 40 m. Each organism from one haul was visually inspected, and a number of blackish but morphologically similar individuals were identified (Fig. 1). Muscular tissue samples were obtained from each individual and stored in 96% ethanol for molecular analysis.

The total genomic DNA was isolated using a CTAB 2X method (Murray & Thompson 1980), and quantified in an Eppendorf biophotometer®, and finally the template DNA was diluted to 20 ng μl^{-1} for further PCR amplifications. The COI fragment (~655 bp) was amplified using the primers, PCR conditions, and thermal regime described by Ward *et al*. (2005). The PCR products were visualized in 1.5% agarose gel stained with ethidium bromide. The samples were sequenced at the Macrogen® (Korea) in an automated DNA sequencer (Model 3730xl; Applied Biosystems). Low quality ends sequences were trimmed per individual, and then aligned using the SEQUENCHER v4.8 (GeneCodes Corp.) software.

To determine the closest relatives of each individual, preliminary similarity searches against sequences available in the GenBank database were made using the Basic Local Alignment Search Tool (BLAST, Altschul *et al*. 1990), and an e-value equal to 1 in order to be more stringent. The e-value is the number of BLAST hits (alignment) that are expected to be found by chance, thus, low e-values can be inferred as homology. The COI fragment from all individuals surveyed in this study showed a high degree of similarity percentage ≥ 99% with species of the family Bramidae, therefore these high-similarity sequences of each species from GenBank were selected for further phylogenetic analyses (Table 1). The sequence selection criteria were: (1) that these sequences had a similar length (size in bp) to those obtained in the present study, permitting the optimization of the number of homologous characters used; and (2) included the greatest number of possible representative members of

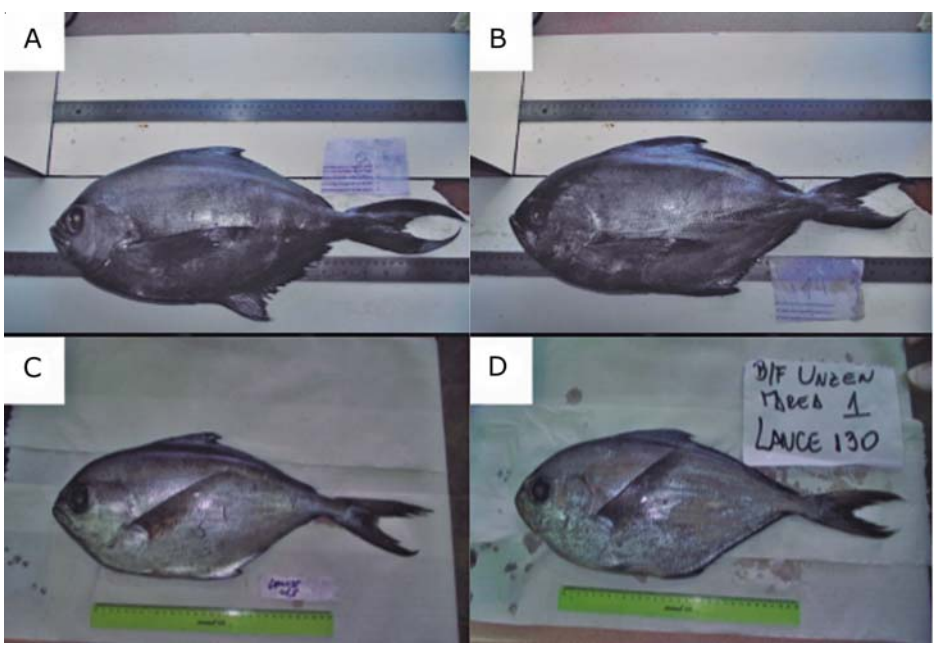

Figure 1. Two specimens of *Xenobrama microlepis* (A and B), and 2 specimens *Brama australis* (C and D) caught by artisanal fishermen on the Chilean coast / Dos especímenes de *Xenobrama microlepis* (A y B), y 2 especímenes de *Brama australis* (C y D) capturados por pescadores artesanales en las costas chilenas

Table 1. The COI sequences of the family Bramidae obtained from GenBank. In bold haplotypes obtained from this study / Secuencias del fragmento COI de la familia Bramidae obtenidas desde GenBank. En negrita los haplotipos obtenidos en este estudio

Orden	Family	Genus	Species	Accession number
Perciformes	Bramidae	*Brama*	*australis*	**KT455472, KT455473, KT455475, KT455480, KT455485, KT455486**
			brama	AB639848, AB639849, AB639857, AB639858, EF609300, HQ611083, HQ611084, HQ611085, HQ611086, JF492978, JF492979, JF492980, JF492981, JF492982
			dussumieri	KJ020208, KJ020209
			japonica	EU400169, FJ164426, GU440256, JF952690, JN242501, JN242502, JN242503, JN242504, JN242505
			orcini	KF4899508
		Eumegistus	*illustris*	AP012497, NC_022485
		Taractes	*asper*	EU400170, GU440550, AP012498, NC_022486
			rubescens	JF952875
		Taractichthys	*longipinnis*	AB639845, EF609476
			steindachneri	EF609477
		Xenobrama	*microlepis*	EF609495, **KX497160, KX497161**
		Gempylus	*serpens*	AP012502

each morphological species of the family Bramidae, in order to optimize the species allocation and strengthen the phylogenetic analysis results. Finally, the sequenced individuals were compared with a database of 43 selected sequences of 11 Bramidae species (50% of the species available in GenBank) and one outgroup (*Gempylus serpens*) (Table 1) that was chosen for its close relationship to the Bramidae (Miya *et al*. 2013). All different haplotypes were deposited in GenBank (Table 1). Seven sequences exhibited a noisy sequencing signal and were therefore discarded prior to further phylogenetic analysis.

The species identification from the haul was based on a phylogenetic reconstruction using 2 methods: the first based on genetic distance, and the second based on Bayesian theorem. For the distance method, a Neighbor Joining (NJ) tree (Saitou & Nei 1987) was built using the Kimura 2-parameter model (K2P, Kimura 1980). This method has been frequently used in studies focused on the identification of species using COI (*e.g.*, Ward *et al*. 2005, Costa *et al*. 2007, Canales-Aguirre *et al*. 2011). The analysis involved 55 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 569 positions in the final dataset. The NJ tree was obtained using the software MEGA7 (Kumar *et al*. 2016), and node support was obtained by 10,000 bootstrap. For the Bayesian approach, a phylogenetic tree was built using the mixture model described by Pagel & Meade (2004, 2005), and the general time-reversible (GTR) evolutionary sequence model (Rodríguez *et al*. 1990). This model was used because the GTR model usually fits real data better than the simpler models (Sumner *et al*. 2012). This permitted the fitting of more than one model of sequence evolution, for which there were 2 GTR models. The Bayesian majority rule tree

was obtained using the software BAYESPHYLOGENIES1.1 (Pagel & Meade 2004). The setting parameters used for the NJ tree followed Ward *et al*. (2005), and for the Bayesian approach followed Hernández *et al*. (2013).

Results and discussion

Based on BLAST, 6 individuals that matched *Xenobrama microlepis* (GenBank access number: EF609495) were recorded, exhibiting a similarity of 99%, and an e-value with a statistical significance of zero, which suggests evidence for homology, and therefore that they correspond to *X. microlepis* species. All other samples sequenced were assigned to *B. australis*, as was expected. The most divergent pairwise species comparison was between *Brama dussumieri* and *Taractichthys steindachneri* (*i.e.*, K2P distance= 0.192 substitutions per site). The mean divergence between *B. australis* and *X. microlepis* was 0.164 substitutions per site. The phylogenetic tree built using the NJ and the Bayesian approach indicated that all 6 individuals that were matched with *X. microlepis* in BLAST, were assigned to a clade with high support (*i.e.*, bootstrap = 100% and posterior probability = 1) (Fig. 2). The same method assigned the rest of the individuals to the *B. australis* clade (Fig. 2). Both clades, *B. australis* and *X. microlepis*,

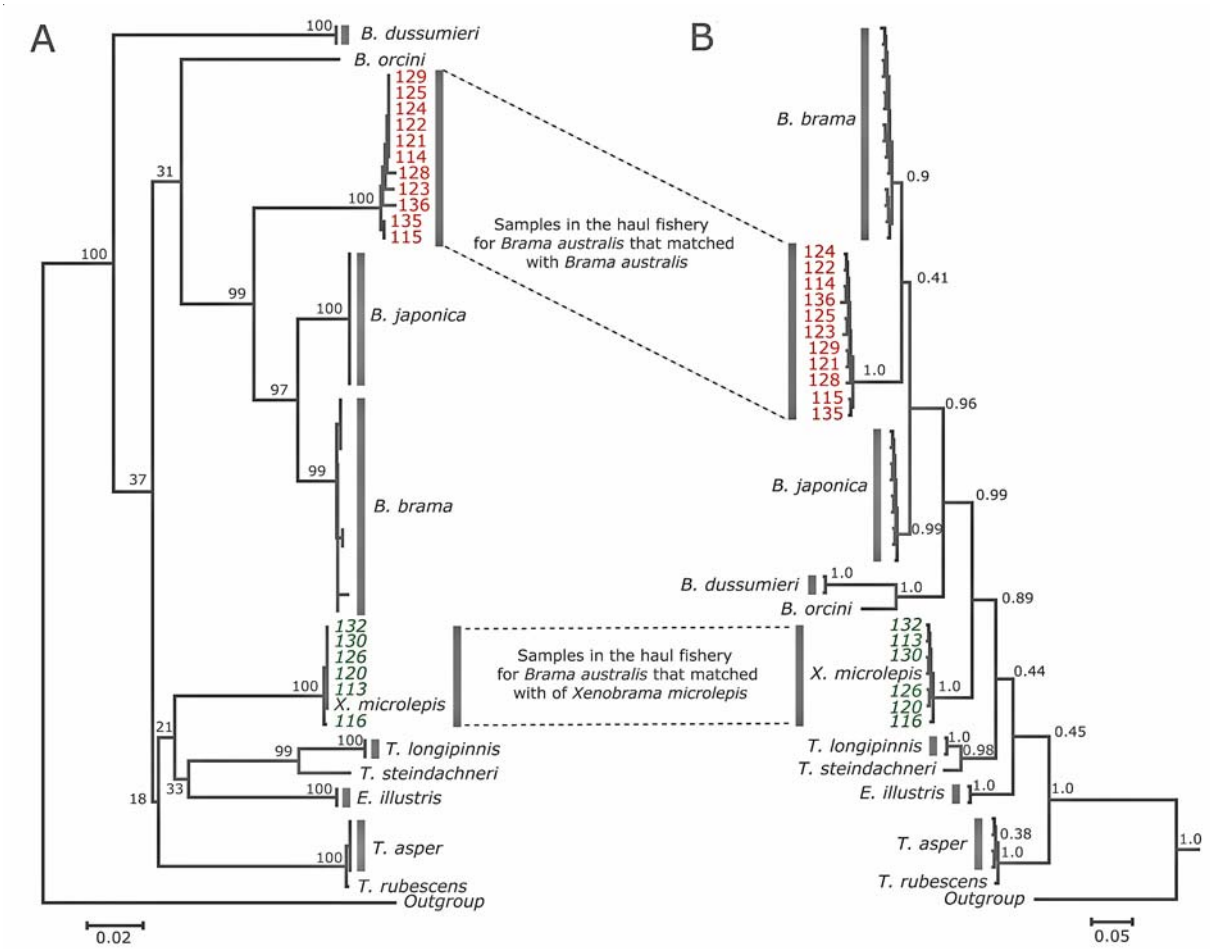

Figure 2. A) The neighbor-joining tree based on Kimura 2-parameter distance. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. B) The majority rule tree of the 900 phylogenetic trees by means of the Bayesian approach. The values above the nodes correspond to the bootstrap support (A), and posterior probability (B) / A) Árbol del vecino más cercano basado en la distancia de Kimura 2-parámetros. El árbol esta dibujado a escala, con los largos de rama en las mismas unidades que las distancias evolutivas usadas para inferir el árbol filogenético. B) Árbol de la regla de la mayoría de los 900 árboles filogenéticos mediante aproximación Bayesiana. Los valores sobre los nodos corresponden al soporte de bootstrap (A) y la probabilidad *a posteriori* (B)

were identified as monophyletic, supporting their taxonomic status. In addition, this was reinforced given that clades are distantly related within the tree of the family built in this study. The results from this genetic survey show high values of divergence and posterior probability that supported the morphology-based taxonomic delimitation of the Bramidae species, confirming the usefulness of the COI gene fragment as a molecular marker for fast and accurate taxonomic determination in marine fishes (Ward *et al*. 2005, Zemlak *et al*. 2009).

There are 22 species in the family Bramidae, of which 2 are economically important (*i.e., Brama brama* and *B. australis*) and 3 have been recorded in the Chilean Exclusive Economic Zone (Pequeño1989). This family is mainly distributed in epipelagic warm and temperate waters (Nelson 2006) and has been considered uncommon, excluding the *Brama* genus (Paulin 1981). First records in several countries support this unusual situation (*e.g.*, *B. caribbea*, Gutiérrez *et al*. (2005); *Pteraclis aesticola*, Agüero & Gómez (2008); *Eumegistus brevorti* and *Taractes rubescens*, Carvalho-Filho *et al*. (2009)). In the Bramidae family, *X. microlepis* –bronze bream– is widely distributed in the South Pacific Ocean (Yatsu & Nakamura 1989), and it is a monotypic genus that was described in 1989 and which exhibits morphological characteristics that differentiate it from other Bramidae. It has been rarely identified in the by-catch of the Ray's bream fishery (*B. brama*) in New Zealand waters, and never in Chilean waters, where the southern Ray's bream (*B. australis*) fishery occurs. Although *X. microlepis* is included in the latest reviewed and annotated checklist of fishes from Chile (Pequeño 1989), the record is based on the information published by Yatsu & Nakamura (1989) and not on fresh specimens collected from the Chilean Exclusive Economic Zone, until the present study. The diversity of Bramidae species suggests that new research into its ecology, biogeography and evolutionary biology should be conducted. Thus, the contributions of new molecular information (*e.g.*, COI) increases the likelihood of filling this gap, and in the near future identifying their phylogenetic relationships and divergence time, which are currently unknown and should be investigated further.

Fisheries landing statistics for *B. australis* started 20 years ago, and were collected from Chilean artisanal fishermen, as until 2017 there is no regulation of this species, and so little attention is being paid to its by-catch. This is because there have been no by-catch studies and *X. microlepis* has not been previously recorded. The possibility of this observation of *X. microlepis* in the by-catch is a unique event without further studies of the by-catch associated with *B. australis*. However, given that the first observation of *X. microlepis* in waters close to the Chilean Exclusive Economic Zone occurred 28 years ago (Yatsu & Nakamura 1989), it seems odd that it has not been observed since. This suggests that its presence in the by-catch has been persistently overlooked, but no evidence exists to support this. A likely explanation may be related to a shift in distributional range of the species as a result of climate change, as has been suggested for many marine fishes (Perry *et al*. 2005, Booth *et al*. 2011). Nevertheless, this is only a hypothesis and should be tested in future studies.

The Chilean fishery authority-National Fishery and Aquaculture Service (SERNAPESCA in Spanish)- must be aware of their presence to avoid increasing the catch records of *B. australis*. Erroneous species identification can lead to unsustainable fisheries when fish landings records are not accurate (Crego *et al*. 2012, Ardura *et al*. 2013). For example, *Trichiurus* spp., from Taiwan, are being managed as one species given the lack knowledge concerning species composition. However, Wang *et al*. (2016) using genetic data revealed there were 3 species in the catch, but with differences in their composition. They highlighted that different life histories and uneven proportions among *Trichiurus* spp. could give clues to their differential susceptibilities to fishing. In New Zealand fisheries, 3 closely related species of Bramidae are caught (*i.e*., *B. brama*, *B. australis*, and *X. microlepis*), but although their presence in hauls is recognized, all these species are recorded as *Brama* spp., because there is no easy way to identify them (Ministry for Primary Industries, 2015). Thus, we also cannot know whether *X. microlepis* will start to be caught more frequently, but if so in the future it should be included in the list of exploited Chilean marine resources, even if the landing is scarce. Thus, further research should begin in order to establish a baseline for this species.

Finally, based on present results conclusions are: a) COI can differentiate easily between *Brama* spp. and *X. microlepis*; b) the Chilean government through its Subsecretary of Fishery and Aquaculture (SUBPESCA) should be paying attention to *X. microlepis* or other species in the landing of *B. australis*, and finally c) the presence of *X. microlepis* increases the regional marine biodiversity of fishes in Chilean waters.

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