

Integrative taxonomy supports the presence of two species of *Kyphosus* (Perciformes: Kyphosidae) in Atlantic European waters

Rafael Bañón^{1,2}, David Barros-García^{3,4}, Alejandro de Carlos⁵

¹ Instituto de Investigaciones Marinas, CSIC, c/ Eduardo Cabello 6, 36208 Vigo, Pontevedra, Spain.

² Grupo de Estudos do Medio Mariño (GEMM), Puerto deportivo s/n 15960 Ribeira, A Coruña, Spain. (RB) (Corresponding autor) E-mail: anoplogaster@yahoo.es. ORCID-iD: <http://orcid.org/0000-0001-6038-9335>

³ Centro de Apoyo Científico y Tecnológico a la Investigación, Universidad de Vigo, c/ Fonte das Abelleiras s/n, 36310 Vigo, Spain.

⁴ Programa de Doctorado en Metodología y Aplicaciones en Ciencias de la Vida, Facultad de Biología, Universidad de Vigo, c/ Fonte das Abelleiras s/n, 36310 Vigo, Spain.

(DB-G) E-mail: davbarros@uvigo.es. ORCID-iD: <http://orcid.org/0000-0002-5283-2605>

⁵ Departamento de Bioquímica, Genética e Inmunología, Facultad de Biología, Universidad de Vigo, c/ Fonte das Abelleiras s/n, 36310 Vigo, Spain.

(AC) E-mail: adcarlos@uvigo.es. ORCID-iD: <http://orcid.org/0000-0003-0138-4918>

Summary: The taxonomic identification of one *Kyphosus sectatrix* and two *Kyphosus vaigiensis* specimens caught in the European Atlantic waters of Galicia, northwestern Spain, was carried out by means of integrative taxonomy, combining the examination of morphological characters and DNA barcodes. Taxonomical assignment based on morphological characters of these specimens was tested by comparing their COI sequences with available data of *Kyphosus* deposited in public repositories. The resulting neighbour-joining tree defined four clades corresponding to Barcode Index Number (BIN) and indicated that some nucleotide sequences from *Kyphosus*, previously deposited, probably originate from misidentified specimens, as would be expected from cryptic and sympatric species. The specimens of *Kyphosus vaigiensis* represent a new record for the waters of Galicia and the northernmost record in the eastern Atlantic. This kind of herbivorous tropical fishes could play an important role in the tropho-dynamic context of this temperate coastal ecosystem.

Keywords: *Kyphosus vaigiensis*; *Kyphosus sectatrix*; molecular systematic; northern limit; Galician waters; ichthyogeography.

Identificación de dos especies de *Kyphosus* (Perciformes: Kyphosidae) en aguas del Atlántico europeo mediante taxonomía integrativa

Resumen: Un ejemplar de *Kyphosus sectatrix* y dos de *Kyphosus vaigiensis* fueron capturados en aguas del Atlántico europeo, en Galicia, al noroeste de España. La identificación se llevó a cabo mediante taxonomía integradora, combinando el examen de caracteres morfológicos y códigos de barras de ADN. La asignación taxonómica basada en los caracteres morfológicos de estos especímenes fue testada comparando sus secuencias de COI con los datos de *Kyphosus* depositados en los repositorios públicos. El árbol filogenético resultante definió cuatro clados correspondientes al número de índice de códigos de barras (BIN), e indicó que algunas secuencias de nucleótidos de *Kyphosus*, previamente depositadas, provienen probablemente de especímenes identificados erróneamente, como cabría esperar de especies crípticas y simpátricas. Los especímenes de *Kyphosus vaigiensis* representan un registro nuevo para las aguas de Galicia y el más septentrional en el Atlántico oriental. La proliferación de peces tropicales herbívoros podría desempeñar un papel importante en el contexto trofo-dinámico del ecosistema costero templado.

Palabras clave: *Kyphosus vaigiensis*; *Kyphosus sectatrix*; identificación molecular; límite norte; aguas de Galicia; ictiogeografía.

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INTRODUCTION

The number of species in the perciform family Kyphosidae is controversial, mainly depending on the number of genera included. According to Nelson (2016), this family contains 53 species divided into 14 genera, and Froese and Pauly (2017) recognize the same number of genera but 54 species. On the other hand, Knudsen and Clements (2013) reduce this number to 12 species in 2 genera. The sea chubs *Kyphosus* species typically inhabits shallow waters over sandy, rocky or grassy bottoms around coral reefs, mainly in the Atlantic, Indian and Pacific Oceans, and juveniles are commonly found among floating algae or below flotsam (Tortonese 1986).

Only two species of *Kyphosus* have been reported so far in the northeastern Atlantic and the Mediterranean: the Bermuda sea chub *Kyphosus sectatrix* (L., 1758) and the yellow sea chub *Kyphosus incisor* (Cuvier, 1831) (Tortonese 1986). Over the last few decades kyphosid catch records for the Atlantic and Mediterranean have experienced an increased number of observations. For the Mediterranean Sea, new records of *K. sectatrix* (Ligas et al. 2011, Kiparissis et al. 2012) and *K. incisor* (Azzurro et al. 2013) have been reported successively. For the European Atlantic waters, specimens of *K. sectatrix* have been reported sporadically in the Macaronesian islands, south of Portugal, northwest of Spain and in the Bay of Biscay (Bañón et al. 2010, Canas et al. 2005, Quérou et al. 2009).

Two taxonomic views on *Kyphosus* with different perspectives have recently been published. Knudsen and Clements (2013) re-examine this genus within a world revision of the family based on both morphological and molecular characteristics, recognizing 12 valid species. According to these authors, four species are present in the Atlantic Ocean and the Mediterranean Sea: the brown chub *Kyphosus bigibbus* Lacepède, 1801, the brassy chub *Kyphosus vaigiensis* (Quoy and Gaimard, 1825), the blue sea chub *Kyphosus cinerascens* (Forsskål, 1775) and *K. sectatrix*. These authors consider *K. incisor* a junior synonym of *K. vaigiensis*.

A different taxonomic view of *Kyphosus* in the Atlantic and eastern Pacific Oceans, and using only morphologic characteristics, was provided by Sakai and Nakabo (2014), who only recognize 11 species, and share only 6 species with the revision presented by Knudsen and Clements (2013), stating that only three of them are present in the Atlantic Ocean and the Mediterranean Sea: *Kyphosus atlanticus* Sakai and Nakabo, 2014, *Kyphosus bosquii* (Lacepède, 1802) and *K. incisor*. According to Sakai and Nakabo (2014), *K. sectatrix* in the Atlantic Ocean is found to represent two species, *K. bosquii* and a new species, *K. atlanticus*.

Many of the morphological discrepancies between the two mentioned revisions have been recently reported by Gilbert (2015), who considers *K. atlanticus* a junior synonym of *K. sectatrix*, following Knudsen and Clements (2013).

The practice of an integrative taxonomy approach (Dayrat 2005) that gathers information from different sources is advisable when identifications based on mor-

phology alone are inadequate for distinguishing between species. DNA barcoding has been considered an efficient aid to traditional taxonomy and is designed to facilitate fast and accurate species identification from a short, standardized DNA sequence (Hebert et al. 2003). In its strictest sense, DNA barcoding addresses only a limited aspect of the taxonomic process, by matching DNA sequences to “known” species, the latter being delimited with traditional (e.g. morphological) methodologies. In this context, the role of barcodes is to provide a methodology for assigning unidentified specimens to already characterized species (Hebert et al. 2003). This is of great help to the end users of taxonomy, and is helping to make more rapid progress in species identification and delimitation of species groups (Ratnasingham and Hebert 2007). Where species are simply unknown or no attempts have been made to delimit them, the barcode approach as originally intended is inadequate in its applicability and should be applied with precaution. It is generally assumed for the majority of vertebrate species that DNA markers such as mtDNA-COI can be used to distinguish between species, and therefore the barcoding approach is based on the assumption that the variation within species of vertebrates is smaller than between species (Ratnasingham and Hebert 2007). As a consequence, DNA barcoding has the potential to aid taxonomic studies and help to clarify cases of potential synonymy and delimitation of cryptic species (Grant et al. 2011). Limitations of using mtDNA to infer species boundaries include: retention of ancestral polymorphism, male-biased gene flow, selection on any mtDNA nucleotide, introgression following hybridization, and paralogy resulting from transfer of mtDNA gene copies to the nucleus (Moritz and Cicero 2004). Despite their benefits and pitfalls, mtDNA-COI barcode sequences and their ever increasing taxonomic coverage have been considered an unprecedented resource for taxonomy and systematics studies and also function as a diagnostic tool.

DNA barcoding is recognized as an important new tool that can be usefully applied to help resolve taxonomic issues in fishes (Ward et al. 2009), based on the development of a reference library of barcode sequences from vouchered specimens. The analysis of validated DNA barcodes for cluster recognition provides an efficient approach for recognizing putative species (operational taxonomic units, OTU). The Barcode Index Number (BIN) system is a persistent registry for animal OTUs recognized through sequence variation in the mtDNA-COI barcode region (Ratnasingham and Hebert 2013).

Molecular data including DNA sequences of *Kyphosus* species are scarce in the scientific literature. Markers like mitochondrial cytochrome b, 12S and 16S rDNAs have been used to identify *K. sectatrix* in Greek waters (Kiparissis et al. 2012). Currently 45 records of mtDNA-COI from *Kyphosus* are available from the BOLD reference database (April 2016). Of these, 42 are assigned to species level and represent 5 species. Some barcodes from *K. vaigiensis* were obtained during the barcoding identification of marine fishes from Japan (Zhang and Hanner 2011) and in the identification of *K. vaigiensis* from the Mediterranean Sea (Mannino et al. 2015). Se-

Table 1. – List of specimens employed in this investigation.

No.	BOLD Process ID	Species name	Country	GenBank Acc. No.
1	ABFJ021-06	<i>Kyphosus vaiigiensis</i>	Japan	JF952771
2	ABFJ232-07	<i>Kyphosus vaiigiensis</i>	Japan	JF952770
3	BAHA219-08	<i>Kyphosus sectatrix</i>	Bahamas	JQ839801
4	BIM473-16	<i>Kyphosus vaiigiensis</i>	Israel	—
5	BZLWA537-06	<i>Kyphosus</i> sp.	Belize	JQ840121
6	BZLWC068-06	<i>Kyphosus</i> sp.	Belize	JQ840890
7	BZLWE009-08	<i>Kyphosus incisor</i>	Belize	JQ841613
8	CFSAN073-11	<i>Kyphosus incisor</i>	United States	KF461190
9	DSFSF676-09	<i>Kyphosus bigibbus</i>	South Africa	GU804959
10	DSFSG575-11	<i>Kyphosus sectatrix</i>	South Africa	KF489619
11	DSFSG612-11	<i>Kyphosus sectatrix</i>	South Africa	KF489620
12	DSLGA090-10	<i>Kyphosus bigibbus</i>	South Africa	—
13	DSLGA091-10	<i>Kyphosus bigibbus</i>	South Africa	—
14	DSLGA1112-11	<i>Kyphosus bigibbus</i>	South Africa	—
15	DSLGA1120-11	<i>Kyphosus vaiigiensis</i>	South Africa	—
16	DSLGA1121-11	<i>Kyphosus vaiigiensis</i>	South Africa	—
17	DSLGA397-10	<i>Kyphosus bigibbus</i>	South Africa	—
18	DSLGA398-10	<i>Kyphosus vaiigiensis</i>	South Africa	—
19	DSLGA684-10	<i>Kyphosus bigibbus</i>	South Africa	—
20	DSLAR424-08	<i>Kyphosus bigibbus</i>	South Africa	—
21	DSLAR425-08	<i>Kyphosus bigibbus</i>	South Africa	—
22	DSLAR426-08	<i>Kyphosus bigibbus</i>	South Africa	—
23	DSLAR427-08	<i>Kyphosus bigibbus</i>	South Africa	—
24	DSLAR428-08	<i>Kyphosus bigibbus</i>	South Africa	—
25	FOAJ442-09	Perciformes	Indonesia	GU674403
26	GBGCA11901-15	<i>Kyphosus vaiigiensis</i>	Spain	KP116934
27	GBGCA11902-15	<i>Kyphosus vaiigiensis</i>	Spain	KP116935
28	GBGCA11903-15	<i>Kyphosus vaiigiensis</i>	Italy	KR013046
29	LIDMA357-10	<i>Kyphosus bigibbus</i>	Belize	HQ987871
30	MBFA125-07	<i>Kyphosus vaiigiensis</i>	French Polynesia	JQ431874
31	MFSP091-09	<i>Kyphosus incisor</i>	Brazil	JX124794
32	MFSP2072-11	<i>Kyphosus incisor</i>	Brazil	JQ365389
33	MFSP413-10	<i>Kyphosus sectatrix</i>	Brazil	JQ365390
34	MFSP536-10	<i>Kyphosus sectatrix</i>	Brazil	JQ365391
35	MLIII601-08	<i>Kyphosus vaiigiensis</i>	Belize	GU224526
36	SBF352-11	<i>Kyphosus cinerascens</i>	Madagascar	JQ350079
37	TOBA235-09	<i>Kyphosus sectatrix</i>	Trinidad and Tobago	JQ842912
38	TOBA410-09	<i>Kyphosus</i> sp.	Trinidad and Tobago	JQ842911
39	TZMSB180-04	<i>Kyphosus vaiigiensis</i>	South Africa	JF493713
40	TZMSC478-05	<i>Kyphosus bigibbus</i>	South Africa	JF493712
41	TZMSC479-05	<i>Kyphosus vaiigiensis</i>	South Africa	JF493714
42	TZSAL621-13	<i>Kyphosus bigibbus</i>	South Africa	—
43	TZSAL622-13	<i>Kyphosus bigibbus</i>	South Africa	—
44	—	<i>Kyphosus cinerascens</i>	Philippines	KF009602
45	—	<i>Kyphosus cinerascens</i>	Japan	NC013138
46	—	<i>Kyphosus sectatrix</i>	Spain	KT780867

quences of mtDNA-COI from *K. cinerascens* and *K. vaiigiensis* have been obtained in relation studies aimed at resolving cryptic diversity in Indo-Pacific coral-reef fishes (Hubert et al. 2012). Barcodes of *K. sectatrix* and *K. incisor* have been used to identify marine fishes of Sao Paulo State in Brazil (Ribeiro et al. 2012).

The aim here is to identify specimens of *Kyphosus* caught in European Atlantic Galician waters by combining morphological identifications in relation to their mtDNA-COI sequences. Since OTUs often tend to show high concordance with vertebrate species delimitations, this approach can be used to support species identifications based on morphological characters (Ratnasingham and Hebert 2013).

MATERIAL AND METHODS

Sample collection and specimen assignation

Three specimens belonging to the genus *Kyphosus* were recorded on the Galician coasts (NW Spain) in 2013 and 2014 by recreational spear fishermen and professional artisanal fishers. Specimens were first

preserved frozen and later thawed and measured to the nearest mm, and meristic characters were determined according to Knudsen and Clements (2013). Morphology-based identifications, taxonomical nomenclature and classification were carried out following Knudsen and Clements (2013). Afterwards, the fish were fixed in 10% formalin, transferred to 70% ethanol and finally deposited in the fish collection of the Museum Luis Iglesias de Ciencias Naturais (MHNUSC) in Santiago de Compostela (Galicia, Spain).

DNA extraction, polymerized chain reaction (PCR) amplification and nucleotide sequencing

Total DNA was purified from 25 mg of muscle tissue taken from each specimen using the spin-column protocol of the Tissue DNA Extraction Kit (Omega-Biotek). The standard 5' barcoding region of the COI gene (ca. 650 bp) was amplified by PCR using the primers set C_FishF1t1-C_FishR1t1 (Ivanova et al. 2007). Polymerase chain reaction was carried out using Phire Green Hot Start II DNA Polymerase (Thermo Scientific, Waltham, MA, USA); mixtures contained a

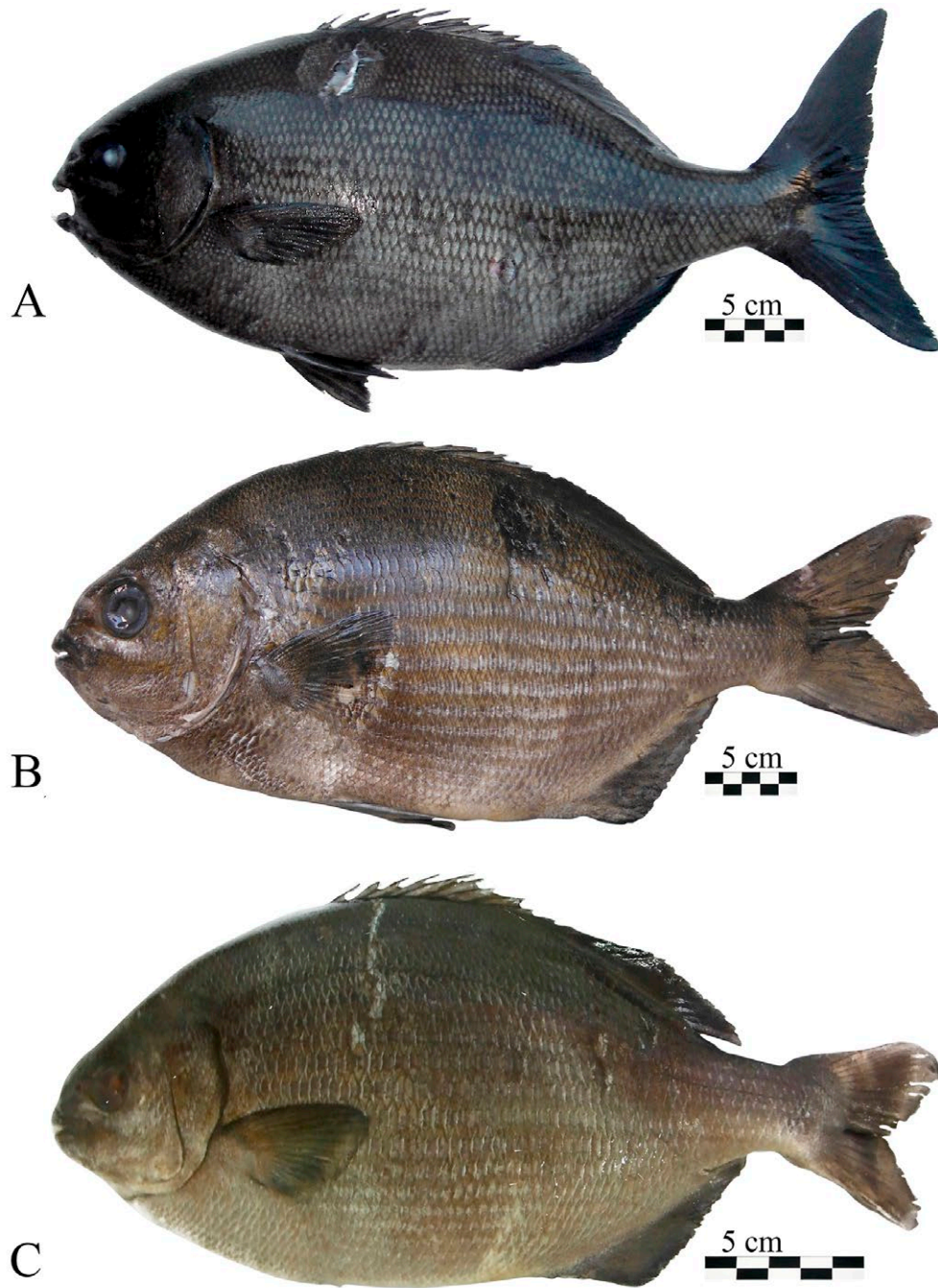


Fig. 1. – *Kyphosus* specimens caught in Galician waters: *K. sectatrix*, MHNUSC 25018-1, 450 mm total length (A); *K. vaigiensis*, MHNUSC 25017-1, 482 mm total length (B); *K. vaigiensis*, MHNUSC 25017-2, 280 mm total length (C).

final volume of 50 μ L and included 19 reaction buffer, 200 μ M of each dNTP, 0.1 μ M of each primer and 1 μ L of polymerase enzyme; between 50 and 100 ng of template DNA was added. PCR reaction products were visualized on 1.2% agarose gels (Seakem LE Agarose) stained with ethidium bromide and, due to the specificity of the results, purified directly with ExoSAP-IT (USB) following the manufacturer's instructions. DNA sequencing reactions were carried out in the forward and reverse senses using the M13F (-21) and M13R (-27) primers and the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The resulting products were resolved on an ABI 3130 Genetic Ana-

lyser (Applied Biosystems, Foster City, CA, USA) and the consensus sequences were obtained after removing the primer-bind regions and assembling the direct and reverse traces with SeqScape v2.5 (Applied Biosystems, Foster City, CA, USA).

Sequence alignment and molecular analysis

Forty-three mtDNA-COI sequences assigned to *Kyphosus* of at least 600 bp were retrieved from BOLD and GenBank, including the sequence FOAJ442-09 from Zhang and Hanner (2011). The final dataset, including the three Galician specimens, comprised 46

Table 2. – Morphometric and meristic data of the *K. sectatrix* specimens caught in Galician waters.

	<i>K. sectatrix</i> MHNUSC 25018-1	Knudsen and Clements (2013)
L _T (mm)	450	31-538
L _S (mm)	354	26-448
As % of L _S :		
Head length	25.7	21.0-34.2
Snout length	7.3	5.0-9.0
Postorbital length	11.3	10.6-16.7
Eye diameter	5.1	4.7-11.9
Upper jaw length	7.3	2.6-9.4
Interorbital length	10.2	8.6-13.9
Predorsal length	34.5	31.1-41.2
Preanal length	61.0	55.3-67.3
Prepectoral length	22.6	20.1-31.1
Preventral length	35.6	29.6-40.3
Base dorsal length	48.6	40.3-62.5
Anal base length	22.3	16.2-23.4
Pectoral length	18.9	13.3-24.1
Ventral length	14.4	11.8-19.0
Caudal peduncle length	20.9	12.1-23.8
Caudal peduncle depth	9.9	8.8-14.3
Body depth	42.7	38.7-54.7
Body width	15.8	11.3-20.7
2° anal fin ray length	9.3	8.5-15.2
4° dorsal fin ray length	7.3	4.8-11.9
Meristic features:		
Dorsal fin rays	XI+12	X-XI+11-12
Anal fin rays	III+11	III+10-12
Pectoral fin rays	18	17-20
Ventral fin rays	I+5	I+5
Gill rakers	7+17	5-8+14-18
Scales in lateral line	73	63-76
Pored scales in lateral line	58	51-61
Scale rows above lateral line	12	10-14

sequences with a total length of 651 nucleotides. The nucleotide and their deduced amino acid alignments

Table 3. – Morphometric and meristic data of *K. vaigiensis* specimens caught in Galician waters.

	<i>K. vaigiensis</i> MHNUSC 25017-1	<i>K. vaigiensis</i> MHNUSC 25017-2	Knudsen and Clements (2013)
L _T (mm)	482	280	51-479
L _S (mm)	390	220	41-444
As % of L _S :			
Head length	24.4	24.1	21.5-33.1
Snout length	7.9	6.8	4.4-9.5
Postorbital length	11.8	11.4	11.1-15.2
Eye diameter	5.4	5.9	4.9-11.8
Upper jaw length	7.7	7.3	4.9-11.8
Interorbital length	10.5	11.4	9.6-14.2
Predorsal length	39.7	32.3	30.3-41.9
Preanal length	59.7	56.8	54-63.4
Prepectoral length	23.8	26.8	22.4-33.5
Preventral length	34.6	32.7	31.5-41.6
Base dorsal length	50.3	48.2	41.9-56.1
Anal base length	26.2	23.2	15.1-26.2
Pectoral length	16.7	17.7	14.5-20.8
Ventral length	15.1	17.3	14.1-19.7
Caudal peduncle length	20.5	19.1	15.4-21.5
Caudal peduncle depth	10.0	10.9	8.6-15.1
Body depth	41.8	43.2	35.3-48.9
Body width	16.7	13.2	10.4-23.4
L 2° anal fin ray	10.0	11.4	6.9-14
L 4° dorsal fin ray	6.9	9.1	4.2-12.5
Meristic features:			
Dorsal fin rays	XI+14	XI+14	X-XI+13-15
Anal fin rays	III+13	III+13	III+12-14
Pectoral fin rays	20	19	17-20
Ventral fin rays	I+5	I+5	I+5
Gill rakers	10+22	10+22	5-10+16-23
Scales in lateral line	78	78	63-80
Pored scales in lateral line	60	59	52-63
Scale rows above lateral line	13	11	9-15

were built with the MUSCLE algorithm (Edgar 2004) with pairwise deletion. The specimens employed in the analysis are listed in Table 1.

The molecular analysis was conducted using the neighbour-joining (NJ) (Saitou and Nei 1987) method in MEGA 6.0 (Tamura et al. 2013). The nucleotide substitution model employed was p-distance and the confidence limits were tested though a bootstrap procedure (Felsenstein 1985) with 2000 replicates. The resulting tree was edited using TreeGraph (Stöver and Müller 2010).

RESULTS

Morphological aspects

Kyphosus sectatrix (L., 1758) (Fig. 1A)

Material examined. MHNUSC 25018-1, 450 mm L_T, total length, 16 August 2013, Malpica, 43.325°N 8.810°W, 2-3 m depth.

Description. Body oval and moderately compressed; head short, 4.9 times in TL; mouth small and terminal; snout slightly greater than eye diameter; pelvic fin short, 1.8 times in head length; caudal fin not deeply emarginated. Colour dusky grey in body and fins, lighter ventrally, without yellow lines patents. The main morphometric and meristic characteristics are presented in Table 2.

Habitat and distribution. Adults are found on rocky reefs or reef flats down to 25 m depth. Juveniles are

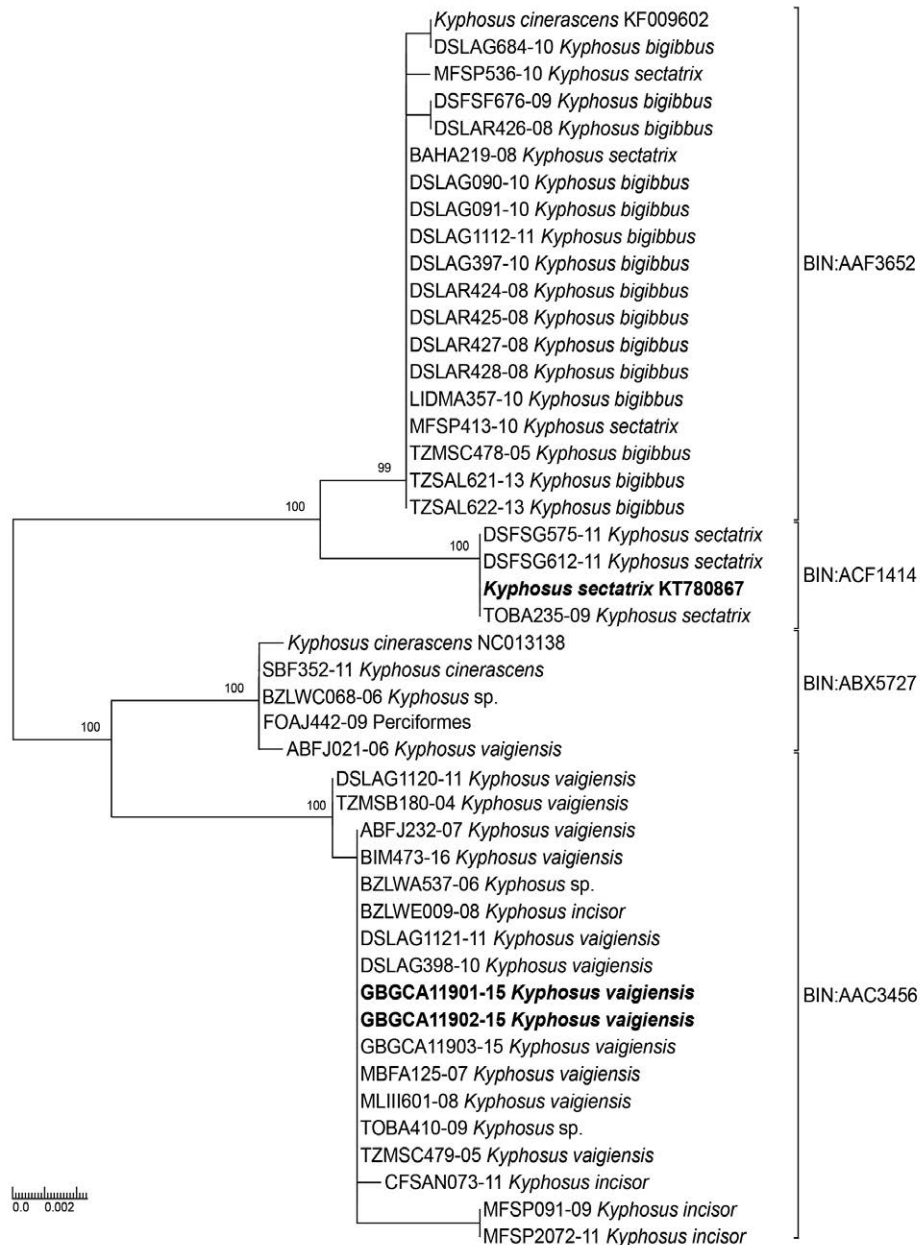


Fig. 2. – Neighbour-joining tree of *Kyphosus* COI sequences. Numbers by the nodes indicate bootstrap supports. Sequences from European Atlantic waters of Galicia specimens are in bold letters. BIN: Barcode Index Number.

pelagic, associated with flotsam, and can be encountered in the open ocean. Widely distributed in the west Pacific, to at least the Revillagigedo Islands, the east Pacific, the Indian Ocean, the Red Sea, the Atlantic and the Mediterranean (Knudsen and Clements 2013).

Kyphosus vaigiensis (Quoy and Gaimard, 1825)
(Fig. 1B, C)

Material examined. MHNUSC 25017-1, 482 mm L_T , 21 January 2014, between Cape Finisterre and Punta Cabanas; 42.887°N 9.264°W, 4-6 m depth; MHNUSC 25017-2, 280 mm TL, 27 April 2014, “Pedras Negras”, O Grove (Ría de Arousa), 42.887°N 9.264°W, 8 m depth, in a rock crevice.

Description. Body oval and moderately compressed; head short, 5.1 and 5.3 times in TL; mouth

small and terminal; snout slightly greater than eye diameter; pelvic fin a little shorter than pectoral fin, 1.6 and 1.4 times in head length; caudal fin not deeply emarginated. Colour dusky grey in body and fins, lighter ventrally; golden yellow horizontal scale rows along body from operculum to caudal fin and head with two gold stripes, one below eye and the other behind eye. The main morphometric and meristic characteristics are presented in Table 3.

Habitat and distribution. Adults are usually found close to the shore and the coastline, while juveniles are associated with flotsam and can be encountered in the open ocean close to the surface. Widely distributed in the Pacific, Indian and Atlantic Oceans, and also in the Mediterranean Sea (Knudsen and Clements 2013).

Table 4. – Genetic distances (% of p-distances) among COI sequences of kyphosid BIN (range values are shown in brackets).

	Within BIN	BIN: AAC3456	Between BIN BIN: ABX5727	BIN: ACF1414
BIN: AAC3456	0.2 (0-0.8)			
BIN: ABX5727	0.2 (0-0.3)	2.6 (2.3-3.2)		
BIN: ACF1414	0	5.1 (4.9-5.8)	4.5 (4.5-4.6)	
BIN: AAF3652	0.1 (0-0.3)	4.7 (4.5-5.5)	4.1 (4.0-4.3)	1.6 (1.5-1.7)

Molecular analysis

Three COI DNA sequences were obtained from specimens of *Kyphosus* caught in Galician waters. An alignment of 651 nucleotide positions was built that, when translated, gave in all cases an amino acid sequence of 217 residues. When nucleotide positions were compared in all 46 sequences, a total of 598 conserved and 53 variable positions were found, from which 49 were parsimony-informative.

A graphic representation of divergences among specimens was created in the form of a consensus tree (Fig. 2) that grouped the sequences according to BIN and showed in its general topology all the *Kyphosus* sequences divided into two highly supported clades. One clade included all the sequences previously assigned to *K. sectatrix* and *K. bigibbus*, which clustered in two different, well-supported groups. The first group consisted of 19 sequences, 15 of them identified as *K. bigibbus*, three as *K. sectatrix* and one as *K. cinerascens*. The average genetic distance among them was 0.01% (Table 4), constituting BIN: AAF3652. The second group comprised four voucher specimens that yield a single haplotype identified as *K. sectatrix*, including KT780867 obtained in this study, corresponding to BIN: ACF1414. The genetic distance between these two BINs varied between 1.50% and 1.70% (Table 4).

In the second clade, the first node delimited two well-supported groups. The first contained five sequences, two of them previously associated with *K. cinerascens*, one as *Kyphosus* sp., and the last one labelled “Perciformes”, with an average genetic distance among them of 0.20% (Table 4), constituting BIN: ABX5727. The second group comprised a total number of 18 sequences that together constitute BIN: AAC3456, from which 12 were identified as *K. vaigiensis*, including the two Galician specimens (GBGCA11901-15 and GBGCA11902-15). In addition, four sequences were named as *K. incisor* and two as *Kyphosus* sp. The average genetic distance among them was 0.20% (Table 4).

The barcoding results thereby support the identification of the specimens made by morphological examination.

DISCUSSION

Morphologically, the examined *K. sectatrix* and *K. vaigiensis* specimens could be differentiated mainly by dorsal and anal soft ray counts (14 dorsal fin and 13 anal fin soft rays in *K. vaigiensis* compared with 12 dorsal fin and 11 anal fin soft rays in *K. sectatrix*) and the number of gill rakers (32 in *K. vaigiensis* and 24 in *K. sectatrix*), which agree with previous taxonomical reports (Knudsen and Clements 2013, Mannino et al. 2015).

In the NJ tree constructed from COI DNA sequences from *Kyphosus*, each of the three Galician specimens was placed within well-defined clades together with individuals from other geographical areas. The Galician sequences of *K. vaigiensis* grouped with sequences previously obtained from specimens of *K. incisor* and *K. vaigiensis* of Atlantic, Mediterranean and Indo-Pacific areas, including BIN: ACC3456, thereby supportive of considering *K. incisor* a synonym of *K. vaigiensis* (Knudsen and Clements 2013, Mannino et al. 2015). The *K. sectatrix* sequence obtained formed a robust clade with another three sequences from South Africa and the western Atlantic assigned to the same species under the designation BIN: ACF1414, producing all together a single haplotype.

The NJ tree obtained showed a number of probably wrongly assigned individuals, in agreement with the complexity described for *Kyphosus* (Knudsen and Clements 2013). As an example, Zhang and Hanner (2011) obtained two barcodes of *K. vaigiensis* during the identification of marine fishes from Japan. They remarked that the K2P genetic distance between the specimens ABFJ021-06 (identified as *K. vaigiensis*) and the BOLD specimen FOAJ442-09 (identified as *K. cinerascens*) was 0.2%, which is in stark contrast with the 2.7% intraspecific genetic value found in their *K. vaigiensis* group. However, their specimen ABFJ021-06 (*K. vaigiensis*) grouped with *K. cinerascens* individuals in BIN: ABX5727, while ABFJ232-07 (*K. vaigiensis*) grouped with *K. vaigiensis*/*K. incisor* individuals (BIN: AAC3456). The short genetic distances between ABFJ021-06 and FOAJ442-09 can be explained by misidentification of the former, or by a hybridization phenomenon. Therefore, the high intraspecific distance reported for *K. vaigiensis* (2.7%) could be due to misidentification or a case involving cryptic species.

Mannino et al. (2015) published an NJ tree of *Kyphosus* COI sequences constructed with a K2P model using data from GenBank to form a 627 bp alignment, which included sequence DSFFSG612-15, assigned to *K. bigibbus*. In the present work, the latter grouped with three *K. sectatrix* specimens (BIN: ACF1414), sharing the same haplotype. In fact, this sequence is now re-assigned as *K. sectatrix* in the BOLD database. Two *K. sectatrix* sequences (BAHA219-08 and MFSP413-10) mentioned in the study by Mannino et al. (2015) shared the same haplotype found in the present study with another 12 sequences identified as *K. bigibbus* (BIN: AAF3652).

The barcoding technique has been successfully integrated with the traditional morphological analysis in the systematic study of fishes in the context of an integrative taxonomy (Dayrat 2005). Application of a combination of both morphological and molecular

barcoding identification of species is recommendable in all taxonomic studies of fishes, and especially for problematic groups like *Kyphosus*, with a rather uniform morphology and only subtle variations among species. The result of barcoding allowed the species assignment of these north Atlantic specimens and supported the hypothesized assignment based on their morphological identification, from an integrative taxonomy point of view.

Both *Kyphosus* described here are warm-water species found northwards of their usual distribution ranges, with *K. vaigiensis* being the northernmost confirmed occurrence in the eastern Atlantic. The presence of *Kyphosus* species in the Mediterranean and the European Atlantic has been related to the warming of waters (Bañón 2004, Canas et al. 2005). A rise of 0.24°C per decade has been observed in the Galician sea waters since 1974 (Gómez-Gesteira et al. 2011), with a decrease in the extension and intensity of the upwelling seasons, responsible for the presence of colder coastal surface waters in summer, and an increase in the extension and intensity of the downwelling seasons, which favour the poleward current (Álvarez-Salgado et al. 2008).

Relationships between water temperature and *Kyphosus* abundance and distribution have also been found at other latitudes, supporting the presence and abundance of these species as indicators of global warming at temperate latitudes. In western Japan, where this genus is more common, it has been observed that the number of caught specimens decreases as the water temperature decreases (Yamaguchi et al. 2010). In southeastern Australia, the silver drummer *Kyphosus sydneyanus* (Günther, 1886) has expanded its distribution range and abundance in response to climate change (Last et al. 2011).

As occurred with other warm-water affinity fishes found in Galician waters, *K. vaigiensis* was previously recorded in the Mediterranean Sea, where it was reported under the synonym, *K. incisor* (Azzurro et al. 2013), a misidentification of *K. sectatrix* (Ligas et al. 2011) finally clarified by Knudsen and Clements (2013), or with its current name (Mannino et al. 2015). This seems to confirm a general and gradual northward displacement of these species in the eastern Atlantic, using the Gibraltar Strait as an escape valve in this migration to the north. This is a general trend also noted in other tropical and subtropical species such as *Pisodonophis semicinctus* (Richardson, 1848) and the lesser amberjack *Seriola fasciata* (Bloch, 1793) (Bañón et al. 2010), which have also been recently found in Galician waters. *K. vaigiensis* is one of the most widely distributed species of sea chubs, being present across the Pacific, Indian and Atlantic oceans, and also the Mediterranean Sea (Knudsen and Clements 2013), which is in agreement with the present results.

An environmental tropicalization could increase the *Kyphosus* population in Galician waters, which would adversely affect the seaweed abundance (Vergés et al. 2016). The family Kyphosidae sensu Knudsen and Clements (2013) is a strictly herbivorous family, with morphological and physiological traits suited for con-

sumption of algae, such as the ability to perform microbial fermentation in their guts. Herbivorous fishes have a significant effect on macroalgal vegetation, not only in tropical but also in warm temperate waters, and *Kyphosus* species are very important for understanding the feeding damage inflicted on seaweed beds by herbivorous fishes (Yamaguchi et al. 2010).

The poleward-flowing boundary currents are creating ocean warming hotspots around the globe, enabling the range expansion of tropical species and increasing their grazing rates in temperate areas (Vergés et al. 2014). For example, Franco et al. (2015) found 45 times more herbivorous fishes in a “warm” than in a “cool” region in the neighbouring Portuguese coast. Further research effort is needed in order to reveal the ecological consequences of *Kyphosus* species as “natural invaders” of new temperate habitats.

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