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An additional record of *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) (Osteichthyes, Kyphosidae) from Sicily clarifies the confused situation of the Mediterranean kyphosids

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Abstract

The lowfin chub, *Kyphosus vaigiensis*, is reported for the first time off Favignana Island, Sicily, central Mediterranean Sea. The specimen was identified on the basis of morphometric and meristic characters as well as mitochondrial DNA sequences (COI and 16S-rDNA). Two, perhaps three, *Kyphosus* species—*K. bigibbus*, *K. sectatrix* and *K. vaigiensis*—have been occasionally recorded in the Mediterranean. These species occur both in the Atlantic and Indo-Pacific regions but it is likely they entered the Mediterranean through the Strait of Gibraltar. However, it is unclear whether they have established reproductive native populations in the Mediterranean.

Key words: Mediterranean Sea, Egadi Islands, Sicily, *Kyphosus vaigiensis*, new record, COI, 16S

Introduction

The taxonomy of the genus *Kyphosus* (Lacepède, 1801) "has been confused to the point where many authors did not attempt species level identification" (Knudsen & Clements, 2013a: 5). Two recent revisions (Knudsen & Clements, 2013a; Sakai & Nakabo, 2014) addressed this uncertainty; yet remain at odds concerning the identity and number of species that have been recorded in the Atlantic Ocean and Mediterranean Sea (Table 1).

The two revisions differ in the number of *Kyphosus* species occurring in the areas adjacent to the Mediterranean Sea. Knudsen & Clements (2013a) identified four widespread species (*Kyphosus bigibbus, K. vaigiensis, K. cinerascens, K. sectatrix*) occurring both in the Atlantic and Indo-Pacific areas, whereas Sakai & Nakabo (2014), identified three species (*K. atlanticus, K. bosquii, K. incisor*) in Atlantic region and six species (*K. vaigiensis, K. bigibbus, K. cinerascens, K. sydneyanus, K. pacificus, K. hawaiiensis*) in the Indo-Pacific region. However, the two revisions mainly differ on the acceptance of some valid species (Table 1). Consequently, understanding which species occur in the Mediterranean and by which route they entered is a challenge.

According to Knudsen & Clements (2013a), the species already recorded in the Mediterranean are *K. vaigiensis* and *K. sectatrix.* Knudsen & Clements (2013a) used digital radiographic photography to examine osteological characters, and statistically analysed morphometric and meristic characters, in addition to molecular phylogenetic analysis. They consider that the lowfin chub, *K. vaigiensis* (Quoy & Gaimard, 1825), is widely distributed, with records in the Atlantic, Pacific and Indian Oceans (including the Red Sea) and treated *K. incisor* (Cuvier *in* Cuvier & Valenciennes, 1831) as a junior synonym. Similarly, they consider *K. sectatrix* (Linnaeus, 1758) to be "much more widespread than previously thought" (Knudsen & Clements, 2013a: 58) and present in the Mediterranean. Sakai & Nakabo (2014), in their revision of the Atlantic and Eastern Pacific *Kyphosus* species,

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consider *K. bosquii* (Lacepède, 1802), the only species they recognize as occurring in the Mediterranean Sea, to be the valid name for *K. sectatrix*.

Following the revision by Knudsen & Clements (2013a), the first recorded specimen of *K. vaigiensis* in the Mediterranean was captured off Almuñécar, Granada, Spain, in 1998 (identified as *K. incisor*, Azzurro *et al.,* 2013). Two specimens were caught off Camogli, Ligurian Sea, Italy, in 2009 (identified as *K. incisor*, Orsi-Relini *et al.,* 2010). Another specimen captured in the Ligurian Sea (identified as *K. saltatrix,* an invalid name for *K. sectatrix*, by Ligas *et al.,* 2011) was recognized as *K. vaigiensis* following molecular analysis and re-examination of its morphological characters (Knudsen & Clements, 2013a). In 2013, yet another specimen of *K. vaigiensis* was captured off Almuñécar (identified as *K. incisor*, Peña-Rivas & Azzurro, 2013).

Recently, a *Kyphosus* specimen (Figure 1) was caught off Favignana Island, western Sicily, Italy (central Mediterranean Sea). Here, we integrate morphological and meristic characters with analysis of mitochondrial DNA segments to identify this specimen. Its stomach contents were also examined to provide qualitative and quantitative information on its diet.

TABLE 1. Some taxonomic incongruences between the revisions by Knudsen & Clements (2013a) and Sakai & Nakabo (2014) on the *Kyphosus* species this paper is interested in.

According to Knudsen & Clements (2013a):		
Valid name	Synonyms	Species range
K. bigibbus	*K. (syn. Pimelepterus) bosquii*	Atlantic–Mediterranean–Indo-Pacific
K. cinerascens		Atlantic–Mediterranean–Indo-Pacific
K. sectatrix	*K. pacificus*	Atlantic–Mediterranean–Indo-Pacific
K. vaigiensis	*K. incisor*	Atlantic–Mediterranean–Indo-Pacific
According to Sakai & Nakabo (2014):		
Valid name		Species range
K. atlanticus	(formerly as *K. sectatrix*)	Atlantic
K. bosquii	(formerly as *K. sectatrix*)	Atlantic–Mediterranean
K. incisor		Atlantic
K. bigibbus		Indo-Pacific
K. cinerascens		Indo-Pacific
K. pacificus		Indo-Pacific
K. vaigiensis		Indo-Pacific

Material and methods

Morphology and stomach content analysis. A single specimen was caught on 17 September 2013 off Favignana Island (37°55'34"N, 12°19'16"E, Marine Protected Area "Egadi Islands", western Sicily), in shallow waters, approximately 3 m depth, on a hard bottom. It was caught by fishing line at night, likely attracted by the fisherman's light.

The individual was photographed and identified on the basis of meristic and morphometric characters reported by Knudsen & Clements (2013a). Measurements were taken with a dial caliper and converted to percent of standard length (SL). The stomach was removed and its contents examined under light microscope (Zeiss, Stemi SV 11 Apo), and weighed to the nearest 0.01 g.

Muscle tissue was excised and preserved in absolute alcohol for subsequent DNA analysis. The specimen was frozen and deposited, together with muscle tissue and archival organic DNA, in the Zoological Museum of the University of Palermo (MZPA, accession number PL-391; Figure 1).

Extraction of DNA and PCR. Total genomic DNA extraction was carried out using the *Genomic DNA Extraction Kit Tissue* (*RBCBioscience,* Taiwan). Two target mitochondrial markers were amplified using universal primers. A 680 base pair (bp) region of the cytochrome *c* oxidase subunit I (COI) gene was amplified using primers

Fish-R1 and Fish-F1 (Ward *et al.*, 2005), and a 561-bp region of the 16S ribosomal RNA gene was amplified using primers 16Sar and 16Sbr (Palumbi *et al.,* 1991). All PCRs were performed in 25 µL volumes containing 1 × Incomplete NH4 Reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.5 U DFS-*Taq* DNA polymerase (*Bioron GmbH,* Germany), 1 µM of each primer, 80–100 ng of template DNA.

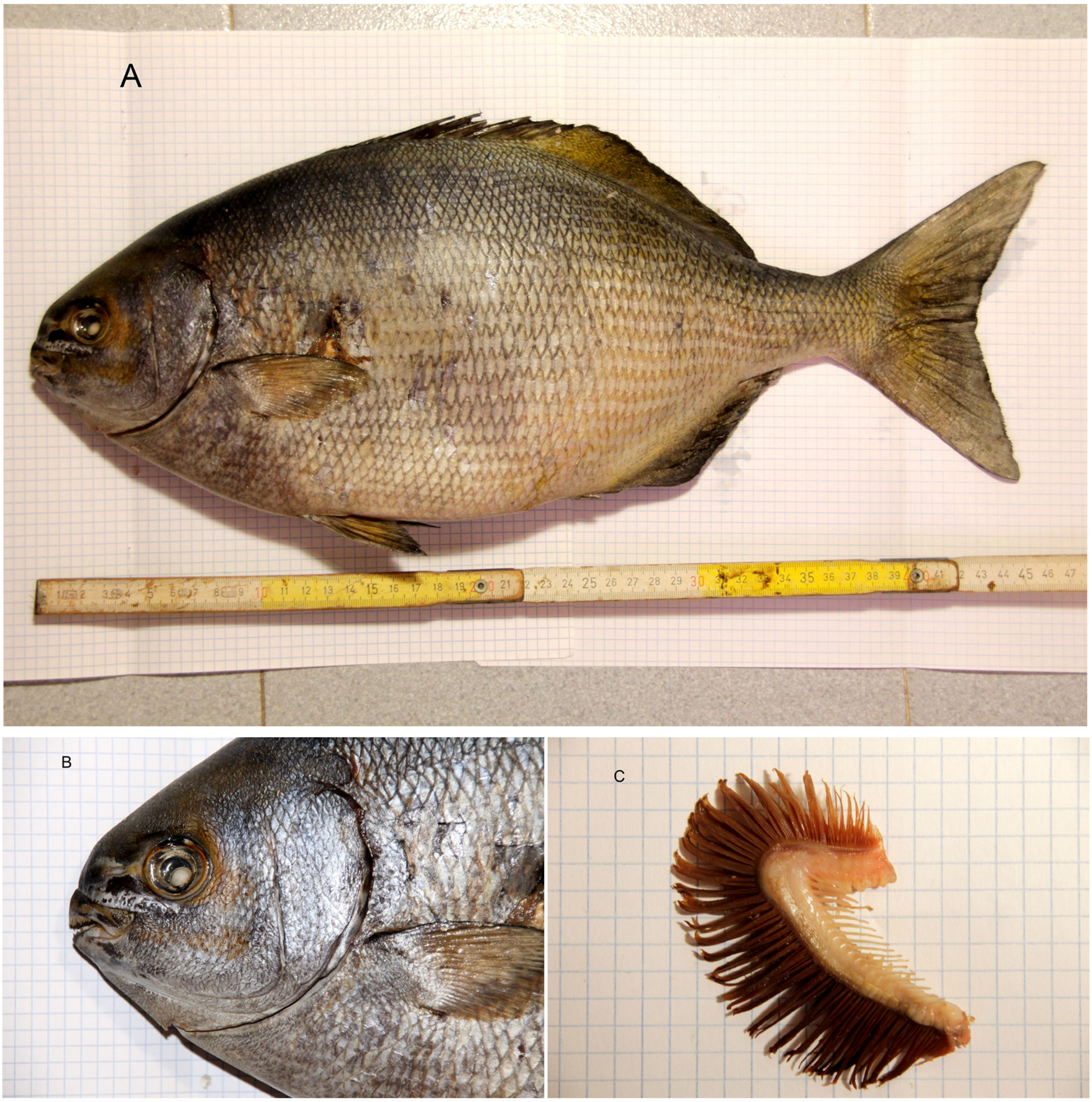

FIGURE 1. *Kyphosus vaigiensis* (Quoy & Gaimard, 1825) recorded off Favignana Island (37°55'34"N, 12°19'16"E; Egadi Islands Marine Protected Area, Italy). **A**: lateral view; **B**: head; **C**: first gill arch.

Cycling conditions for PCR amplifications consisted of an initial 95°C denaturation step for 5 minutes followed by 35 cycles of 60 sec at 95°C, 60 sec at 48°C, and 60 sec at 72°C, with a final extension at 72°C for 8 min and a final cooling at 4°C. The resulting amplified DNA fragments were purified with the *QIAquick PCR Purification Kit* (*Qiagen*), and were sequenced in forward direction, with an Applied Biosystems (ABI) 3730*xl* DNA analyzer and the sequences were deposited in GenBank with the Accession numbers: KR013046 and KR013047. The mitochondrial sequences of the specimen were compared with sequences of congeneric species deposited in the Barcode of Life Data Systems (BOLD, http://www.barcodinglife.org) (Ratnasingham & Hebert, 2007) and GenBank (http://www.ncbi.nlm.nih.gov) databases (Table 2).

TABLE 2. Details of the COI and 16S reference sequences of *Kyphosus* species downloaded and showed in the NJ tree.

Accession Number (A.N.) and Specimen Voucher	Sampling site	Reference
COI		
Kyphosus vaigiensis		
JQ431874 (voucher MBIO207.4)	South Pacific Ocean (Moorea, French Polynesia)	Hubert *et al.* (2012)
JF952770 (voucher ISZ9)	North Pacific Ocean (Yokohama, Japan)	Zhang & Hanner (2011)
JF493714 (voucher Smith 189.3-2)	South Indian Ocean (KwaZulu-Natal, South Africa)	sequence unpublished (1)
GU224526 (voucher MFL1494)	North Atlantic Ocean (Gulf of Mexico, Belize)	Valdez-Moreno *et al.* (2010)
Kyphosus incisor [*syn. vaigiensis* sensu Knudsen & Clements (2013a)]		
JQ841613	North Atlantic Ocean (Gulf of Mexico, Belize)	Weigt *et al.* (2012)
KF461190	North Atlantic Ocean, (Gulf of Mexico, USA)	Deeds *et al.* (2014)
JX124794	South Atlantic Ocean (Brazil)	sequence unpublished (2)
JQ365389	South Atlantic Ocean (Brazil)	Ribeiro *et al.* (2012)
Kyphosus sectatrix		
JQ365390	South Atlantic Ocean (Brazil)	Ribeiro *et al.* (2012)
JQ839801	North Atlantic Ocean (Gulf of Mexico, Belize)	Weigt *et al.* (2012)
Kyphosus cinerascens		
AP011061	North Pacific Ocean (Japan)	Yagishita *et al.* (2009)
Kyphosus bigibbus		
KF489620	South Indian Ocean (Walters Shoal, South Africa)	sequence unpublished (3)
16S		
Kyphosus vaigiensis		
AY530871	South Pacific Ocean (Great Barrier Reef, Australia)	Bellwood *et al.* (2004)
KC136382	**Mediterranean Sea**, eastern Ligurian Sea (Italy)	Knudsen & Clements (2013a)(4)
Kyphosus cinerascens		
AP011061	North Pacific Ocean (Japan)	Yagishita *et al.* (2009)
Kyphosus sectatrix		
JN007799	**Mediterranean Sea**, Ionian Sea (Greece)	Kiparissis *et al.* (2012)
Kyphosus bigibbus		
KC136508	South Pacific Ocean (Western Australia)	Knudsen & Clements (2013b)

(1) uploaded by Steinke, D., Zemlak, T.S., Connell, A.D., Heemstra, P.C. & Hebert, P.D.N.—University of Guelph, Canada.
(2) uploaded by Ribeiro, A.O. & Oliveira, C.—Universidade Estadual Paulista, Brazil.
(3) uploaded by Steinke, D., Connell, A.D. & Zemlak, T.S.—University of Guelph, Canada.
(4) specimen recorded by Ligas *et al.* (2011) and erroneously identified as *K. sectatrix* (Knudsen & Clements, 2013a).

Sequence alignment and successive analyses were implemented with MEGA 6 software (Tamura *et al.,* 2013). Measuring of genetic differentiation was based on Kimura-two-Parameter (K2P) model (Kimura, 1980). Unrooted Neighbor-Joining (NJ; Saitou & Nei, 1987) trees were built and the nodes were supported by a high proportion (> 90%) of replicates in the bootstrap analysis (Felsenstein, 1985).

The Automatic Barcode Gap Discovery (ABGD) analysis (Puillandre *et al.*, 2012) was used to detect a barcode gap in the COI dataset. The COI sequences were uploaded to the ABGD web interface and were run with the default settings. K2P distance was tested to infer from the data a model-based confidence limit for maximum intraspecific and interspecific divergence (Puillandre *et al.*, 2012).

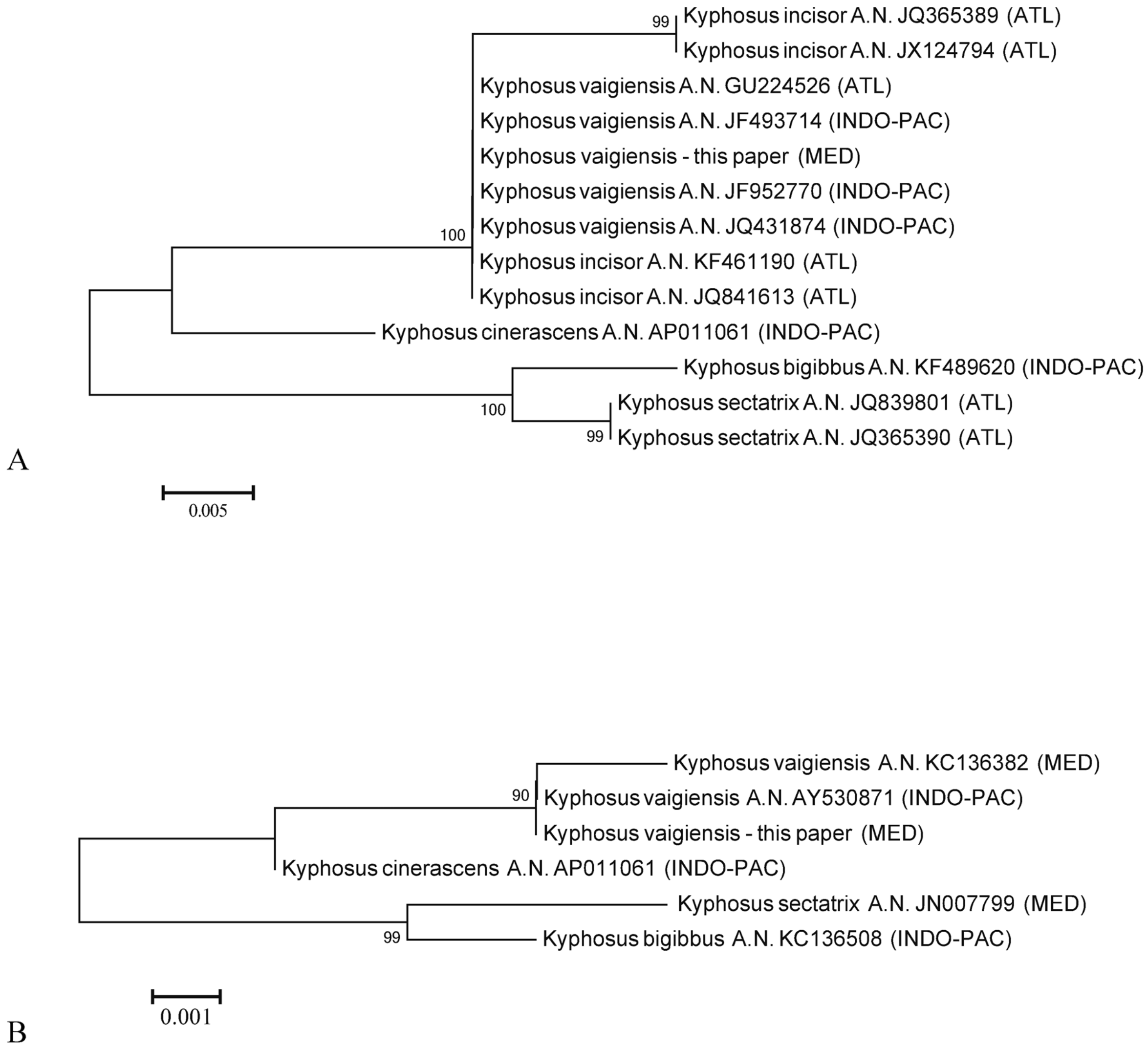

FIGURE 2. NJ tree constructed on K2P model including reference data from GenBank with the present data from: (A) 627-bp COI sequences and (B) 524-bp 16S sequences. Captures from the Mediterranean area (MED), the Atlantic area (ATL), and the Indo-Pacific area (INDO-PAC). (The names of species are reported as uploaded from the GenBank except for A.N. KC136382).

Results

Morphology and stomach content analysis. The specimen presents the following main meristic and morphological characters: elongate and oval shaped body, scale rows horizontally along body and alternately golden and bluish, dorsal and anal fin very low, dorsal fin with XI spines and 13 soft rays, anal fin with III spines and 11 soft rays, pectoral fin with 17–20 rays, caudal fin moderately emarginated, teeth incisor-shaped, scales on interorbital region, external side of first gill arch with large number of gill rakers on lower limb (22) and upper limb (8), lateral line with 55 pored scales, longitudinal row with 60 scale rows. All examined characteristics are reported in Table 3.

The stomach contents (84 g) consisted mainly of seaweeds (78 g): mostly *Ulva* spp. (68 g), with the remainder composed of brown algae such as *Dictyopteris polypodioides* (A.P. De Candolle) J.V. Lamouroux, *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *Halopteris* spp. and *Padina pavonica* (Linnaeus) Thivy. Terrestrial vegetables (tomatoes, lettuce) (2 g) and the remains of small fishes (4 g) used as bait or found intermixed with algae were also present.

TABLE 3. Proportional measurements (in % SL) and counts of the *Kyphosus vaigiensis* specimen caught off Favignana Island. SL: Standard length.

Proportional measurements (in % SL)		**Counts**	
Total length (mm)	475	Dorsal fin spines	XI
Fork length (mm)	445	Dorsal fin soft rays	13
Standard length (mm)	380	Anal fin spines	III
Head length	26.3	Anal fin soft rays	11
Body width	15.8	Pectoral fin rays	20
Body depth	44.7	Pelvic fin rays	I, 5
Caudal peduncle length	10.5	Caudal fin rays	17
Caudal peduncle depth	9.2	Gill rakers upper limb on first gill arch	8
Snout length	5.3	Gill rakers lower limb on first gill arch	22
Eye diameter	3.9	Gill rakers, total on first gill arch	30
Interorbital width	9.2	Pored scales in lateral line	55
Upper jaw length	5.3	Scale rows above lateral line	11
Preanal length	65.8	Scale rows below lateral line	21
Dorsal fin base length	55.3	Scale rows in longitudinal row	60
Soft dorsal fin base length	26.3	Incisor-like teeth on upper jaw	25
Spinous dorsal fin base length	23.7	Incisor-like teeth on lower jaw	24
6th dorsal spine length	10.5	Cheek scales	16
4th dorsal ray length	7.9		
2nd anal ray length	10.0		
Pectoral fin length	18.4		
Anal fin base length	26.3		
Caudal fin length	23.7		
Pelvic fin length	14.5		

Sequences comparison with co-generic species. The COI sequence of the Sicilian specimen matched (100% similarity) the reference sequences of *K. vaigiensis* and two sequences identified as *K. incisor* (A.N. KF461190 and JQ841613), building a *vaigiensis-incisor* clade, within which a deeper lineage of *K. incisor* was also scored (Figure 2A). These last two specimens of *K. incisor* (A.N. JQ365389 and JX124794), captured in the southern Atlantic Ocean (Brazil), showed genetic differences that deserve further investigations.

For all the COI barcodes, K2P distances ranged from 0.000 to 0.062 (Figure 2A).

Although the number of reference sequences for the 16S marker were limited, some sequences of specimens captured in Mediterranean were available, as labeled with "MED" in the NJ tree (Figure 2B). For the whole 16S dataset, K2P distances ranged from 0.000 to 0.017, where the highest value corresponded to the interspecific variation between *K. vaigiensis* and *K. sectatrix*. The 16S pattern was congruent with the one for the COI gene, supporting the existence of a *K. vaigiensis* clade. The COI and 16S sequences of the Sicilian specimen matched sequences of Atlantic specimens of *K. incisor,* and Atlanto-Indo-Pacific specimens of *K. vaigiensis*, as a result of K2P distance values (intra *vaigiensis-incisor* clade distance: 0.000–0.011 for COI; 0.0–0.004 for 16S).

The 16S sequence of the examined Sicilian specimen also supported the placement of the *K. sectatrix* specimen recorded by Ligas *et al.* (2011) (A.N. KC136382) into *K. vaigiensis* (Knudsen & Clements, 2013a) (Figure 2B). By contrast, the 16S barcode of the *K. sectatrix* specimen collected in the Mediterranean Ionian Sea (Kiparissis *et al.,* 2012) maintained its own genetic identity, suggesting that this specimen was correctly identified. In fact, *K. sectatrix* and *K. bigibbus* (Lacepède, 1801) fall out as distinct sister species (Figure 2A).

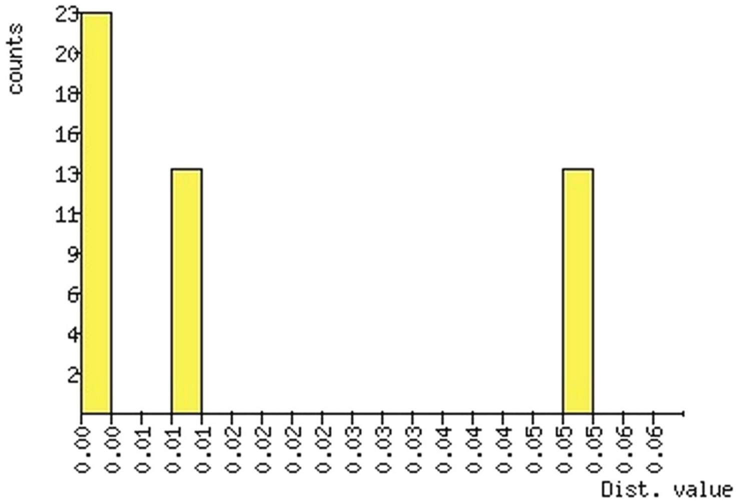

FIGURE 3. Distribution of COI pairwise K2P distance values, performed by ABGD, showing the "barcode gap" between the putative maximum co-specific divergence (0.01) and the minimum congeneric divergence (0.05).

Discussion

Being aware of the controversial taxonomic status of *Kyphosus* species and the historical problems in delimiting the different species and identifying them, we carefully examined the specimen collected off Sicily. Its morphometric and meristic data matched those of *K. vaigiensis* reported in Knudsen & Clements (2013a). The mitochondrial DNA sequences matched those of *K. vaigiensis* as well: its COI and 16S sequences were placed within a monospecific clade together with haplotypes from Indo-Pacific *K. vaigiensis* and Atlantic *K. incisor*, the latter consequently having been synonymized with *K. vaigiensis* by Knudsen & Clements (2013a).

Its full stomach indicates that the Sicilian specimen was in good condition and agrees with its known herbivorous habits (Clements & Choat, 1997).

Overall, the data presented here resolve the taxonomy of the Sicilian specimen, and highlights some problems with Sakai & Nakabo's taxonomy (2014).

The specimen collected in the Ionian Sea (*K. saltatrix*, in Kiparissis *et al.,* 2012) "matches *K. sectatrix* in both morphological and molecular… characters" (Knudsen & Clements, 2013a: 60). These authors consider the specimens recorded by Tortonese (1954), Merella *et al.* (1998), Hemida *et al.* (2004) and Francour & Mouine (2008) as *K. sectatrix,* yet caution: "Whether other previous reports of *K. sectatrix* from the Mediterranean are valid is difficult to say" (Knudsen & Clements, 2013a: 60). The NJ trees clearly support the genetic identity of this Ionian specimen as *K. sectatrix*, and indicate a pattern consistent with the present-day existence in the Mediterranean basin of two *Kyphosus* species, namely *K. sectatrix* and *K. vaigiensis*. Such conclusion does not solve the discrepancies between the two mentioned revisions dealing with the "*sectatrix*-case". In fact, the *sectatrix* name is treated by Knudsen & Clements (2013a) as a widespread Atlantic–Mediterranean–Indo-Pacific species, while Sakai & Nakabo (2014) split it into two endemic Atlanto–Mediterranean species, and the only specimens analyzed here comes from Atlanto–Mediterranean region.

However, the *vaigiensis-incisor* clade supports the existence of a wide ranging species, over the Atlantic–Mediterranean–Indo-Pacific region, which matches the revision by Knudsen & Clements (2013a). The ABGD analysis was applied to the COI dataset to prove the efficiency of the DNA barcoding in delimiting the Mediterranean species. According to the ABGD analysis the *vaigiensis-incisor* clade seems to be well delimited. Nonetheless, it is increasingly recognised that accurate species delineation requires a multilocus approach taking into account two or more independent markers from the same individuals, which necessarily involves sequencing nuclear genes (see Knudsen & Clements, 2013a).

The presence of *Kyphosus* species in the Mediterranean Sea is not a recent event. An older specimen, collected in the Gulf of Palermo, Sicily, and described as *Pimelepterus boscii sicula* by Doderlein (1883), was regarded as junior synonym of *K. bigibbus* (Lacepède, 1801) by Knudsen and Clements (2013a), but considered as *incertae sedis* by Sakai and Nakabo (2014).

Doderlein's description reports 80 scales in longitudinal row (Doderlein, 1883), exceeding the number of scales of all known species of *Kyphosus*. Fortunately, the stuffed specimen is preserved in the Zoological Museum of the University of Palermo (MZPA, accession number P-247; Figure 4), and its re-examination confirmed that it was an error, indeed we counted 56 scales in the longitudinal row. According to Knudsen and Clements (2013a), this character together with dorsal fin spines (XI), dorsal fin soft rays (12), anal fin spines (III), anal fin soft rays (11) and the length of the second anal fin ray (8.6% SL) lead to identify it as *K. sectatrix*.

The specimen has also been erroneously classified in the collection of the Museum, and further molecular analyses are advisable.

It is noteworthy to point out that the past *Kyphosus* record captured in 1883 (Doderlein, 1883) proves that such genus is capable of dispersal covering long distances and the ability to survive in and colonize a different geographical range. Golani *et al.* (http://www.ciesm.org/atlas/Kyphosusincisor.php, viewed January 11, 2015) considered that *K. vaigiensis* (reported as *K. incisor*) entered the Mediterranean as "ship associated introductions via Gibraltar". Zenetos *et al.* (2012) considered the records (again reported as *K. incisor*) as stemming from natural range expansion rather than a human mediated introduction. The two species—*K. sectatrix* and *K. vaigiensis*—recorded in the Mediterranean also occur in the Atlantic and Indo-Pacific (including the Red Sea) (Knudsen & Clements, 2013a). Whether these species have established populations in the Mediterranean, or represent rare, or occasional, ephemeral entries through the Suez Canal or the Strait of Gibraltar, remains unknown, though the records in the Alboran Sea and the western Mediterranean suggest the latter is the likelier event.

FIGURE 4. specimen stored at the Zoological Museum of the University of Palermo (MZPA), accession number P-247 (see the text for the details).

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