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Chronicles of *Kyphosus* in the Mediterranean Sea: new records and complete mitogenomes support the scenario of one expanding fish species

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The Mediterranean Sea is a biodiversity hotspot, being home to a vast array of marine species. Furthermore, seawater warming is facilitating the arrival and spread of new thermophilic species, posing a severe threat to biodiversity. Among the species currently extending their range and increasing in abundance in Mediterranean waters, sea chubs (genus *Kyphosus*) are one of the most enigmatic. One challenge arises from the high phenotypic similarity between the two congeneric species documented in the basin: *Kyphosus vaigiensis* and *Kyphosus sectatrix*. Their resemblance has often led to identification challenges, resulting in incorrect or omitted species-level classifications. Therefore, despite the growing presence of these fish in the Mediterranean, it remains unclear whether only one or both species are experiencing a demographic increase and range extension. To date, there have been 26 reports of *Kyphosus* individuals in the Mediterranean Sea, documented in 24 separate papers. Here, we reviewed the history of the genus in the basin and provided 13 new records of these fish from multiple localities along Mediterranean coasts. In addition, we sequenced the entire mitogenomes of two specimens, assessed their phylogenetic relationships with published *Kyphosus* mitochondrial DNAs from around the world, and conducted detailed morphological and meristic analyses on one of them, allowing us to provide accurate species-level identifications. Our results indicate that *K. vaigiensis* is the species currently expanding its range in the Mediterranean Sea, while *K. sectatrix* is still very rare and only sporadically reported. Notably, our mitogenome data indicate that Mediterranean *K. vaigiensis* individuals most likely came from Atlantic waters, while there is no evidence to support an entrance through the Red Sea or any other anthropogenic vector. Finally, the potential ecological and fishing impacts associated with the proliferation of these fish in the region are discussed.

KEYWORDS

Kyphosus vaigiensis, Mediterranean Sea, mitochondrial genome, morphological analyses, taxonomy, thermophilic species

1 Introduction

The distribution and population dynamics of many marine species are constantly varying due to climate change and anthropogenic activities. This shifting process is particularly evident in the Mediterranean Sea, where the emerging scenario is of critical concern (Chatzimentor et al., 2023). In fact, albeit the basin hosts a rich and unique biodiversity, its communities are undergoing rapid changes due to many factors, with the increase and spread of thermophilic and non-indigenous species being among the major ones (Bianchi et al., 2019; Tiralongo et al., 2020).

Among the species currently expanding in Mediterranean waters, sea chubs (Kyphosidae) are one of the most intriguing taxonomic groups. In fact, due to the high phenotypic similarities between some *Kyphosus* species, their identification is often problematic, and involves the assessment of several characters, including counting of soft rays in the anal and dorsal fins, scale rows along the body, gill rakers, as well as the evaluation of colour patterns, relative body proportions, and relative size and placement of fins (Knudsen and Clements, 2013). Despite numerous taxonomic studies and reviews attempting to establish a clear and unique classification for the genus (Sakai and Nakabo, 1995, 2004, 2006; Knudsen and Clements, 2013, 2016), conflicting results persist, leaving the taxonomy of the genus very elusive.

From an ecological perspective, sea chubs are strictly herbivorous fishes, feeding primarily on macroalgae, on both temperate and tropical reef areas. In some areas such as Western Australia, they represent a large proportion of coastal fishes, considerably impacting algal biomass (Knudsen and Clements, 2016). Sea chubs are also considered highly valued as food, being generally caught with handline, gillnet and spear (Sakai, 2003). However, the fishing statistics for species of the Kyphosidae family are generally included in broader fisheries reports rather than being reported separately.

In the Mediterranean Sea, two species belonging to the genus *Kyphosus* have been reported so far: *Kyphosus sectatrix* (Linnaeus, 1758) and *Kyphosus vaigiensis* (Quoy & Gaimard, 1825). In European Atlantic waters both species are present, but *K. vaigiensis* seems to be the most common, probably due to its greater mobility (Bañón and de Carlos, 2022). These two taxa have often been reported under

different names, later recognised as junior synonyms or misspelled names (Knudsen and Clements, 2013, 2016); for example, *K. incisor* for *K. vaigiensis* (Orsi Relini et al., 2010; Azzurro et al., 2013; Bilecenoglu et al., 2013), or *K. sectator* for *K. sectatrix* (Šoljan, 1963; Merella et al., 1998; Hemida et al., 2004; Francour and Mouine, 2008). Besides, specimens have often been misidentified; for example, original observations of *K. sectatrix* have later been attributed to *K. vaigiensis* (Ligas et al., 2011 and Elbaraasi et al., 2013; Knudsen and Clements, 2013 and Goren et al., 2016).

Table 1 lists the 26 *Kyphosus* spp. records reported in Mediterranean waters so far, with details on the date of observation, locality, number of specimens, size, depth, and fishing gear. Despite the wealth of literature, most articles report sightings of a single individual, and all are referred to a single locality. Overall, the large majority of sea chub observations are attributed to *K. vaigiensis*. One study also proposed the presence of a third species (Knudsen and Clements, 2013): the authors indicated that the (alleged) *K. sectatrix* specimen from Palermo (Italy) reported by Doderlein (1883) could be *Kyphosus bigibbus* Lacepède, 1801. The number of scales on the lateral line of this specimen (80) slightly falls outside the variability of both *K. bigibbus* and *K. sectatrix*, while the other morphometric and morphological characteristics are coherent with both (Doderlein, 1883; Knudsen and Clements, 2013). However, the same study refers to *K. bigibbus* as "not known from the Mediterranean", leaving doubts about the status of this specimen. Nevertheless, a later study on the same specimen, preserved at the Museum of Zoology, University of Palermo, refers to it as *K. sectatrix* (Lo Brutto, 2017). To date, no concrete evidence has been provided about the presence of a third *Kyphosus* species in the Mediterranean Sea.

In addition to morphometric and morphological studies, molecular analyses of mitochondrial DNA (mtDNA) have been also employed to study *Kyphosus* species. All of these studies have assessed mtDNA sequence variation at the level of a single gene or a few genes: cytochrome oxidase subunit 1 (COI), cytochrome b, and ND2 genes, as well 12S and 16S RNA genes (Yagishita et al., 2002; Kiparissis et al., 2012; Mannino et al., 2015; Vella et al., 2016). These genes have often been used as useful barcoding regions to identify fish species, particularly the COI gene (Ardura et al., 2013; Mohanty et al., 2015; Bingpeng et al., 2018). However, while this multi-gene

TABLE 1   Previous records of *Kyphosus* species in the Mediterranean Sea, and associated relevant data (updated up to February 2024).

Species (formerly reported)	Reference (see also)	YRec	Locality	Nspec	Size: TL-W	Depth (in m)	Method	Evidence
K. sectatrix (*K. sector*)	Šoljan (1963) (Dulčić and Ahnelt, 2006)	1846	Near Trieste (Italy)	1	195 mm	/	F	Mor
K. sectatrix (*Pimelepterus boscii* var. *sicula*)	Doderlein (1883) (Lo Brutto, 2017)	1883	Arenella (Palermo, Italy)	1	300 mm	/	F	Mor
K. sectatrix (*Pimelepterus bosci*)	Ariola (1904)	1903	Genoa (Italy)	1	225 mm	SW	F	Mor
K. sectatrix (*K. sector*)	Merella et al. (1998)	1996	Mallorca (Spain)	1	216 mm (172.3 g)	600	PS	Mor

(Continued)

TABLE 1 Continued

Species (formerly reported)	Reference (see also)	YRec	Locality	Nspec	Size: TL-W	Depth (in m)	Method	Evidence
K. vaigiensis (*K. incisor*)	Azzurro et al. (2013)	1998	Almuñécar (Granada, Spain)	1	310 mm (452 g)	/	TN	Mor
K. sectatrix (*K. sectator*)	Hemida et al. (2004)	2003	Annaba (Algeria)	6+	485 mm (675 g*)	10-20	GN, T	Mor
K. sectatrix	Hattour (2006)	2005	Salakta (Tunisia)	1	415 mm (1700 g)	6	S	Mor
K. sectatrix (*K. sectator*)	Francour and Mouine (2008)	2006	Saint-Tropez (France)	1	489 mm (1535.4 g)	8	S	Mor
K. vaigiensis (*K. incisor*)	Orsi Relini et al. (2010)	2009	Camogli (Italy)	2	480 mm (1800 g)	SW	M	Mor
K. vaigiensis (*K. sectatrix*)	Ligas et al. (2011) (Knudsen and Clements, 2013)	2009	Meloria Banks (Livorno, Italy)	1	495 mm (1650 g)	5	S	Mor
Kyphosus sp. (*K. saltatrix*)	Lelong (2012)	2010	Galite Islands (Tunisia)	4	200-250mm	3-4	P	Pho
K. vaigiensis (*K. saltatrix*)	Elbaraasi et al. (2013) (Goren et al., 2016)	2010	Benghazi (Libya)	1	475 mm (1630 g)	7	S	Mor
K. sectatrix (*K. saltatrix*)	Kiparissis et al. (2012) (Knudsen and Clements, 2013)	2011	Strofades Islands (Greece)	1	265 mm (352.2 g)	16	S	Mor + Mol
K. vaigiensis (*K. incisor*)	Bilecenoglu et al. (2013)	2013	Almuñécar (Granada, Spain)	1	460 mm (1640 g)	15	TN	Mor
K. vaigiensis	Mannino et al. (2015)	2013	Favignana Island (Italy)	1	475 mm	3	FL	Mor + Mol
K. vaigiensis	Groud et al. (2021)	2013	Annaba (Algeria)	1	415 mm (1270 g)	/	TN	Mor
K. vaigiensis	Vella et al. (2016)	2015	Delimara (Malta)	1	157 mm	2	FR	Mor + Mol
K. vaigiensis	Goren et al. (2016)	2016	Ashdod (Israel)	1	420 mm	/	S	Mor + Mol
Kyphosus sp. (*K. sectatrix*)	Ragkousis et al., 2023	2016	Galite Islands (Tunisia)	1	/	15-20	/	Vis
K. vaigiensis	Gerovasileiou et al. (2017)	2016	Limassol (Cyprus)	1	272 mm (403 g)	33	TN	Mor
K. vaigiensis	Ragkousis et al., 2023	2017	Mugiarro (Malta)	1	/	12	F	Mor + Mol
Kyphosus sp.	Al Mabruk et al. (2021)	2017	El Montazah beach (Alexandria, Egypt)	1	/	9	S	Pho
K. vaigiensis	Kiyağa et al. (2019)	2018	İskenderun Bay (Turkey)	1	531.1 mm (2270 g)	4	FR	Mor + Mol
K. vaigiensis (*Kyphosus* sp.)	Kousteni et al. (2022)	2021	Villefranche-sur-Mer (France)	1	485 mm (2045 g)	3-4	S	Mor
K. vaigiensis	Ragkousis et al., 2023	2021	Carini (Italy)	1	/	7	S	Pho
K. vaigiensis	Fitori et al. (2022)	2021	Tolmeitha (Libya)	2	/	20	TN	Mor

Data include: species (in brackets, the identification originally given by the authors); reference (in brackets, other works that further discussed the observation changing the species-level ID); YRec, year in which the specimen(s) was/were recorded; NSpec, number of observed specimens (in brackets, the number of measured specimens); size in Total Length (TL) or Weight (W), for records VI and IX, size is provided for a single specimen; depth of observation/capture (in meters or SW, shallow waters); method of observation (F, unspecified fishing, PS, purse seine, TN, trammel net, GN, gill nets, T, trawling, S, spearfishing, M, mugginara, a small net trap fixed to the rocks, P, photography, FL, fishing line, FR, fishing rod); evidence (Pho, photographic/visual, Mol, molecular, Mor, morphological, Vis, visual). Records not supported by sound morphological or molecular evidence were regarded as *Kyphosus* sp.

approach allows comparisons across multiple mtDNA regions, it also makes it difficult to compare published data from specimens of diverse global populations.

In this work, we present additional records of *Kyphosus* specimens from numerous localities along the Mediterranean coasts, with related relevant data on the species, date, locality, size, depth, and technique of observation. Moreover, to facilitate comprehensive mtDNA comparisons with previously published data, we completely sequenced the mitogenome of two of the *Kyphosus* specimens. This multidisciplinary approach allowed us to perform a species-level identification of reported individuals, to document a large-scale increase of the species *Kyphosus vaigiensis* in Mediterranean Sea, to define that Mediterranean *K. vaigiensis* specimens are of Atlantic origin and to compare the Mediterranean emerging scenario with the one earlier proposed for Galician (Atlantic) waters (Bañón and de Carlos, 2022).

2 Materials and methods

2.1 Data collection

Our new records were collected during the AlienFish campaign (specimens I-XIII, Table 2). The AlienFish project was launched in 2012, aiming to monitor the distribution of rare, thermophilic, and non-indigenous fish species along the Mediterranean coasts. The initiative relies on contributions from citizens, who often use social networks to provide interesting and otherwise unavailable information (Tiralongo et al., 2020). As the project is based in Italy, most of the new records here reported were collected through Italian Facebook groups, including both marine biology and fishing groups. In particular, we used these groups to search for photos and videos of *Kyphosus* species, and hence contacted the authors to ask information about the observation, and the permission to use the published photographic material. With this approach data about the date, locality, number of specimens, size, depth and fishing technique were gathered (Table 2).

One specimen (specimen XII) was physically collected and examined by the authors. It was weighed to the nearest 1 g and subjected to an accurate morphological and meristic analyses. Sex determination was performed through macroscopic analysis of the mature gonads present in specimen XII. Stomach content was also inspected. Part of another fish (specimen X) was kept frozen by the fisherman and later collected and analysed by the authors; morphological measurements were collected as well for this fish. Molecular analyses, including DNA extraction and whole mitogenome sequencing, were conducted for both specimens X and XII.

TABLE 2   New records of *Kyphosus* specimens in the Mediterranean Sea reported in this study, with associated relevant data.

N	Species	Date	Location	Coordinates in WGS 84		Nspec	Size: TL-W	Depth (in m)	Method	Evidence
				N	E					
I	*K. vaigiensis*	8 Nov 2003	Maratea (Italy)	39.991	15.702	7 (2)	1980 g, 1750 g	10-12	S	Pho
II	*Kyphosus* sp.	27 Oct 2010	Calvi (France)	42.580	8.724	1	~210 mm	4	P	Pho
III	*K. vaigiensis*	5 Sep 2020	Tirrenia (Italy)	43.640	10.285	1	~1500 g	~3	S	Pho
IV	*K. vaigiensis*	27 Sep 2020	Marina di Andora (Italy)	43.950	8.151	1	~2000 g	2	S	Pho
V	*K. vaigiensis*	10 Jul 2021	Marinella (Italy)	37.580	12.841	1	2531 g	5.5	S	Pho
VI	*K. vaigiensis*	12 Aug 2021	Penisola Magnisi (Italy)	37.153	15.241	1	~2000 g	9	S	Pho
VII	*K. vaigiensis*	18 Oct 2021	Giardini-Naxos (Italy)	37.819	15.273	1	~1000 g	4.5	S	Pho
VIII	*K. vaigiensis*	15 Jun 2022	San Lorenzo (Italy)	36.767	15.117	1	2075 g	17.5	S	Pho
IX	*K. vaigiensis*	3 Jul 2022	Pantelleria (Italy)	36.791	12.054	2 (1)	~2000 g	8	S	Pho
X	*K. vaigiensis*	13 Sep 2022	Genova (Italy)	44.398	8.904	1	520 mm	7	S	Mol
XI	*K. vaigiensis*	14 Nov 2022	Scilla (Italy)	38.250	15.689	2 (1)	3000 g	7	S	Pho
XII	*K. vaigiensis*	6 Dec 2022	Roccella Ionica (Italy)	38.304	16.354	1	558 mm, 2620 g	7	GN	Mor + Mol
XIII	*K. vaigiensis*	21 Dec 2022	Brindisi (Italy)	40.663	17.972	1	460 mm, 1800 g	3	S	Pho

NSpec, number of observed specimens (in brackets; the number of captured specimens); size in Total Length (TL) or Weight (W); depth of observation/capture (in meters); method of observation (S, spearfishing; P, photography; GN, gill nets); evidence (Pho, photographic; Mol, molecular; Mor, morphological). Specimen X has recently been reported by Azzola et al. (2024), without molecular and morphological descriptions.

2.2 DNA extraction

Small portions of the fish were stored in absolute alcohol. Using clean tweezers, the tissue was picked, washed with Milli-Q® water, and transferred to a 1.5 mL tube. Samples were then pestled and DNA was extracted using phenol-chloroform as in Lombardo et al. (2022).

2.3 Long-range PCR amplification of the entire mitogenome

We designed two primer pairs on the basis of *Kyphosus* mtDNA sequences available in GenBank to amplify two partially overlapping amplicons covering the entire mitogenome. Primer: (i) forward: 5'-TTGTTCACTGATTCCCGCTGT-3', reverse: 5'-ATTGTGTGAGGGGTCGGAATG-3'; (ii) forward: 5'-CACCTG CCAACCCACTAGTAAC-3', reverse: 5'-TGTAGGCGTCTG GGTAGTCTG-3'. PCR reactions were performed in a volume of 25 μL, containing 12.5 μL of GoTaq® Long PCR Master Mix (Promega, USA), 0.75 μL of each primer (final concentration of 0.3 μM), 1 μL of template DNA (107 ng/μL for specimen X, 64 ng/μL for specimen XII) and 10 μL of nuclease-free water. DNA amplification started with 2 min of initial denaturation (94°C) followed by 30 cycles of: 30 sec at 94°C, 30 sec at 57°C, 9 min at 65°C; at the end, a final extension cycle of 10 min at 72°C was performed. 3 uL of the amplification products were checked by 1% agarose gel electrophoresis and ethidium bromide staining.

Amplified mtDNA amplicons were purified using QIAquick spin columns (Qiagen, Redwood City, CA, USA) and then quantified by the Quantus™ fluorometer (Promega) using the QuantiFluor® ONE dsDNA system. About 50 nanograms for each of the two amplicons were combined for library preparation.

2.4 Library preparation and sequencing

The two complete mitogenome sequences were obtained by Next Generation Sequencing on a MiSeq Illumina platform. Nextera™ DNA Flex Library Prep was used to prepare the sequencing libraries, following the steps described in the manufacturer's protocol: tagmentation of input DNA, amplification of tagmented DNA with the addition of premixed dual-indexed adapters (Nextera™ DNA CD Indexes), and PCR clean-up. Libraries were then quantified by the Qubit 4 Fluorometer (Thermo Fisher). We then run the pooled normalized library on the 4150 TapeStation System (Agilent) and diluted to 4nM with RSB resuspension buffer. Five microliters of pooled libraries were denatured with 5 μl of NaOH (0.2N), diluted to the loading concentration of 6 pM (600 μl final volume) using HT1 hybridization buffer and sequenced on a MiSeq system (Illumina) using paired-end sequencing with a MiSeq Reagent Kit v. 2 (2×250 cycles).

2.5 Analysis of mitogenome sequence data

The raw MiSeq sequencer data were output in BCL format, demultiplexed, and converted to FASTQ format with the Illumina® bash package, bcl2fastq2 Conversion Software v2.20. FASTQ files were analysed with Geneious v. 8.1.9 (https://www.geneious.com) and initially aligned to a published mitogenome of *K. vaigiensis* (GenBank accession number OP035071). This alignment allowed us to export the consensus of sample number X (Table 2), which was then employed as the reference sequence in this study. Variant calling was performed by setting the threshold for heteroplasmies at 30% of reads and considering only mutations in the range 30%–70% as heteroplasmic in phylogenetic analyses. The two mitogenomes were completely sequenced with an average depth of 9481X and 11166X for samples X and XII, respectively. Finally, mitogenome sequences were exported in the standard FASTA format and deposited in GenBank (accession numbers: PP178641 for specimen X, PP178642 for specimen XII).

2.6 Phylogenies of COI and complete mtDNA coding-region

In order to assess mtDNA affinities and ancestral sources of the two sequenced fish, previously published COI sequences (614 bp from nucleotide position (np) 5564 to np 6177) belonging to the species *K. vaigiensis* were downloaded from GenBank and added to our database to build a phylogenetic tree including worldwide specimens. Sequences labeled as *K. incisor* were included as well due to their synonymy with *K. vaigiensis* (Knudsen and Clements, 2013), also verified in this article. Besides, we also included, when available, one COI sequence for other *Kyphosus* species to assess the relationships of *K. vaigiensis* with its congeneric counterparts. The choice of the COI gene reflects the wealth of available sequences in GenBank.

Sequences were aligned with Sequencher v. 4.9 (Sequencher® version 4.9 DNA sequence analysis software, Gene Codes Corporation, Ann Arbor, MI USA), and a maximum parsimony tree was built manually, indicating nucleotide positions that differed among mtDNAs, with respect to the newly established *K. vaigiensis* reference sequence (specimen X). The same dataset of COI sequences used to build the MP tree was employed for the construction of a Median-Joining network with the software Network 10.2.0.0 (www.fluxusengineering.com).

We also constructed a maximum parsimony (MP) tree with the whole coding-region of five *K. vaigiensis* mitogenomes - the two that we sequenced and three previously published (GenBank accession numbers OP057015, OP035071 and OP035143). Their control-region sequences (876 base pairs from np 15668 to np 16543) were excluded from this tree, similarly to previous mitogenome studies of other animal species (Battaglia et al., 2022; Lombardo et al., 2022) due to the particularly high mutation rate of mtDNA control regions, which often limits their phylogenetic informativeness. The *Kyphosus cinerascens* (Forsskål, 1775) complete mitogenome (GenBank accession number NC_013138) was employed as outgroup.

3 Results

3.1 Collected records

Thirteen new records were collected (I-XIII, Table 2), involving a total of 21 specimens, captured or spotted from 2003 to 2022.

Interestingly, despite the AlienFish campaign is still ongoing, no *Kyphosus* record was collected during 2023. As most of the exploited Facebook groups were Italian, the majority of records came from Italy (12 records). At the same time, we also report one record from a French locality (specimen II), dating back to 2010. Figure 1 shows the geographical source of each new and previous record on a Mediterranean scale, while specific information about each new observation is provided in Table 2.

3.2 Morphological traits and measurements

The clear horizontal golden rows observable in almost all our specimens (examples in Figure 2) indicate their belonging to the species *K. vaigiensis* (Knudsen and Clements, 2013).

Morphological and meristic traits measured for specimens X and XII (Table 3) also corroborate the classification as *K. vaigiensis* (Figure 3). The fish showed a head profile that was gently convex in front of the eye, 14 dorsal rays, 12 anal rays, 9 and 23 gill rakers on the upper and lower limbs of the first gill arch, respectively; it had an eviscerated weight of 2400 g (vs. 2620 g of full total weight). Furthermore, the analysis of the stomach content revealed the presence of 80 g of algal material. Macroscopic analysis of gonads indicated that it was a mature male.

3.3 Molecular and phylogenetic analyses

The MP tree of COI sequences reported in Figure 4 and the network in Supplementary Figure 1 confirm that *K. vaigiensis* mtDNAs share nine distinctive COI transitions and form a well defined species-specific branch. However, it should be also noted that two mtDNAs from Japan (n. 44, JF952771) and Myanmar

(n. 45, MH235651) cluster in the *K. cinerascens* branch despite their postulated classification as *K. vaigiensis*. As shown in the tree, *K. cinerascens* is the closest congeneric species to *K. vaigiensis*, with sequence divergence values for different mtDNA regions in the range 2.5% - 3.0% (Table 4), which are close to the postulated interspecies COI thresholds for fish species (Ward et al., 2009). In contrast, *K. sectatrix*, the other Mediterranean species, is most closely related to other species (*K. hawaiiensis* and *K. bigibbus*).

The COI sequences of our specimens X and XII are members of the *K. vaigiensis* branch, thus fully supporting morphological and meristic evidence. The COI sequence from specimen XII (reported as n. 33 in Figure 4) is identical to two *K. vaigiensis* sequences (n. 31-32) from Galician waters (Bañón and de Carlos, 2022) and belongs to a branch, defined by the transition at np 5919, which also includes mtDNAs (n. 29-30) from Brazil, thus indicating a likely Atlantic ancestral source for specimen XII (Figure 4). In contrast, the sequence of the specimen X (n. 1 in Figure 4) showed 100% identity with 25 previously published sequences, including not only the Mediterranean specimen (n. 22) reported by Mannino et al. (2015) in Sicily and specimens from European (n. 15-20) and American Atlantic waters (n. 8-9, 23-26), but also specimens from the Red Sea (n. 4) and many other extremely distant locations (South Africa, South East Asia, Polynesia, Hawaii) (Supplementary Table S1).

The phylogenetic relationships of *Kyphosus* mtDNAs were also assessed at a much higher level of molecular and phylogenetic resolution by comparing whole mtDNA coding regions (15667 bp) (Figure 5). The MP tree reported in Figure 5 is much more informative than the one in Figure 4, albeit including only five mtDNAs (n. 1-2, 24, 27 and 33). Each of the five mitogenomes bears a distinct haplotype, clustering into two well-defined haplogroups that we termed Kv1 and Kv2. The latter includes two Hawaiian mitogenomes (n. 2 and 27), while haplogroup Kv1 encompasses our
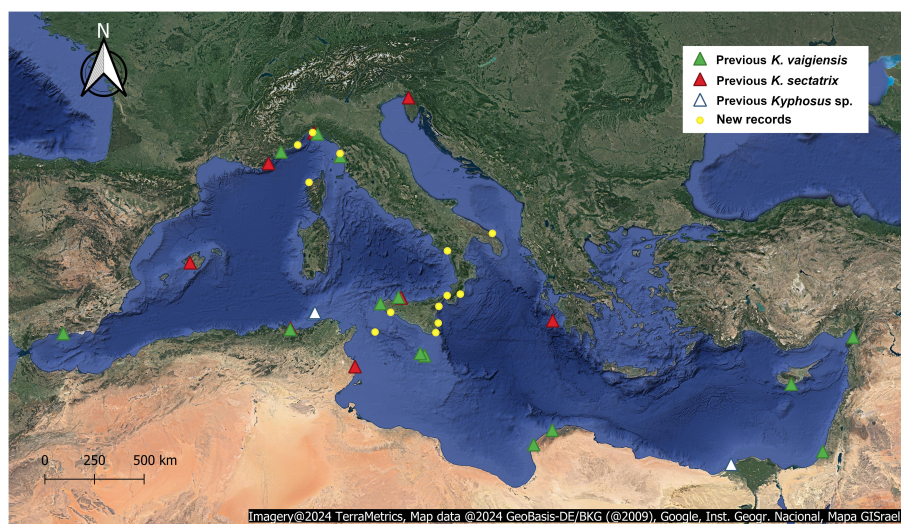

FIGURE 1
Previous records of *K. vaigiensis* (green triangle) and *K. sectatrix* (red triangle), and new *Kyphosus* records hereby reported (yellow dot). We considered as "*Kyphosus* sp." the records reported by Lelong (2012) and Al Mabruk et al. (2021) (white triangle), since the provided photographic material is not adequate to allow species-level identifications. Map in Spherical Mercator (EPSG:3857).

FIGURE 2
Seven of our reported *Kyphosus* specimens: **(A)** record I; **(B)** record VII; **(C)** record VIII; **(D)** record IX; **(E)** record X; **(F)** record XIII. The golden horizontal rows that are typical of *K. vaigiensis* are evident in the photos.

two rather divergent (22 transitions/transversions) Mediterranean mitogenomes (n. 1 and 33) together with mitogenome n. 24, which is from the Atlantic (Belize) (Figure 5), thus further supporting the Atlantic Ocean as ancestral source for the Mediterranean *K. vaigiensis* population.

4 Discussion

The recent expansion of sea chubs in Mediterranean waters has posed several challenges in accurately identifyingspecies due to the high morphological similarity between *K. vaigiensis* and *K. sectatrix* (Knudsen and Clements, 2013), and has prevented authors from giving species-specific identifications (Kousteni et al., 2022). At the same time, several specimens have been misidentified or reported under a synonymous name (see Table 1). Our data, based on a combined approach encompassing citizen science, morphological and molecular methods, appear to fully address this issue and might represent a valuable approach for other studies concerning similar and difficult-to-identify species.

As for our morphometric and morphological data, an important character, very useful for visual identification from photos, is the presence of yellow/golden horizontal rows along the sides of *K. vaigiensis* (Knudsen and Clements, 2013), well visible in fresh and sometimes in preserved specimens and not present in adults of the congeneric *K. sectatrix*. Specimen II (Table 2) has been photographed underwater and the apparent absence of the

horizontal golden rows casts doubt on its species-level identification: were these rows missing or simply not visible in that photo due to the particular exposure, angle, and brightness? Similarly, for specimen VI, we only have a frame of a spearfishing video, where the golden rows are not appreciable. In our opinion, this is likely due to the lack of definition of the image, and to a general difficulty in observing the rows in underwater photos. Indeed, this specimen was spotted in Sicily in 2021, and all the other specimens spotted in the same region and period correspond to *K. vaigiensis*.

Therefore, there is no concrete morphometric/morphological evidence to support the presence of *K. sectatrix* among our records, and it is highly likely that all specimens here reported correspond to *K. vaigiensis*.

The recent record in the bay of Villefranche-sur-Mer, reported as "*Kyphosus* sp." (Kousteni et al., 2022), is probably referred to *K. vaigiensis* as well. In fact, morphometric and meristic counts match the variability of the species observed by other authors (Knudsen and Clements, 2013; Mannino et al., 2015; present study). Besides, the clear yellow/golden rows present in the original figure (Kousteni et al., 2022) further support this hypothesis (Knudsen and Clements, 2013).

As for our mtDNA data, the two sequences from Japan (n. 44, JF952771) and Myanmar (n. 45, MH235651) labeled as *K. vaigiensis*, but clustering within the *K. cinerascens* clade in the COI-based phylogeny (Figure 4), could result from an event of hybridization between the two species, as suggested by other

TABLE 3 Morphometric and meristic data collected on specimen X and XII caught at Genoa (Italy, Ligurian Sea) in September 2022 and at Roccella Ionica (Italy, Ionian Sea) in December 2022.

Specimen	Morphometry	mm	%TL	Meristics/ Weights	counts
X	TL	524	100	/	/
	FL	471	89.89	/	/
	SL	423	80.73	/	/
	HL	102	19.47	/	/
	Body Depth	190	36.26	/	/
	CP Depth	52	9.92	/	/
	EY	20	3.82	W (g)	~2000
	PAL	229	43.70	/	/
XII	TL	558	100	DS	XI
	FL	502	89.96	DR	14
	SL	451	80.82	AS	III
	HL	104	18.64	AR	12
	Body Depth	202	36.20	PR	21
	CP Depth	55	9.86	GillRakUpper1stArch	9
	EY	22	3.94	GillRakLower1stArch	23
	PAL	244	43.73	GillRakTotal1stArch	30
	6th DSL	55	9.86	IncisorTeethUJ	32
	4thDRL	34	6.09	IncisorTeethLJ	34
	2nd ARL	55	9.86		
	PectFL	79	14.16	W (g)	2620
	PelvFL	75	13.44	EvW (g)	2400

TL, total length; FL, fork length; SL, standard length; HL, head length; CP Depth, depth of the caudal peduncle; EY, eye diameter; PAL, preanal-length; DSL, dorsal spine length; DRL, dorsal ray length; ARL, anal ray length; PectFL, pectoral fin length; PelvFL, pelvic fin length; DS, dorsal spines; DR, dorsal rays; AS, anal spines; AR, anal rays; PR, pectoral rays; GillRakUpper1stArch, number of gill rakers in the upper limb of the branchial arch of the first gill; GillRakLower1stArch, number of gill rakers in the lower limb of the branchial arch of the first gill; GillRakTotal1stArch, number of gill rakers in the whole branchial arch of the first gill; IncisorTeethUJ, incisiviform teeth on the upper jaw; IncisorTeethLJ, incisiviform teeth in the lower jaw; W, total body weight; EvW, eviscerated body weight.

authors (Bañón et al., 2017); K. vaigiensis and K. cinerascens are indeed genetically very close to each other (Table 4). However, to the best of our knowledge, no study has ever evaluated the presence of hybridization between the two species. The COI-based tree shows a general lack of geographic clustering for K. vaigiensis populations. Indeed 26 out of the 42 mtDNA sequences (~62%) in the K. vaigiensis branch, including the Mediterranean specimen X, are represented by the same COI haplotype, despite the geographical sources of the specimens encompass all oceans (Atlantic, Pacific and Indian Ocean). One exception is represented by a branch (defined by the transition at np 5919) that includes five mtDNAs, four from Atlantic sources, plus one from the Mediterranean (our specimen XII), thus suggesting a Gibraltar entry route for the latter. A general lack of genetic structure of K. vaigiensis mtDNA sequences from all over the world has been also reported in previous studies (Vella et al., 2016). This might be interpreted as supportive of the notion that K. vaigiensis covers very long distance migrations (Sakihara et al., 2015). However, it most likely indicates that, as previously shown for many other animal species, the assessment of only short

segments of mtDNA does not permit the identification of structure at the population level and the understanding of a species' origin and spread (Lombardo et al., 2022).

The latter scenario is supported by our entire coding-region tree, despite the fact that it encompasses only five mitogenome sequences. MtDNAs n. 1 and 33 differ for only one mutation in the COI phylogeny (Figure 4), but are 22 mutations apart in the coding-region phylogeny (Figure 5). Moreover, the two Mediterranean mitogenomes cluster together in the same haplogroup (Kv1) with a Belize specimen, while the two from Hawaii form a distinct sister haplogroup (Kv2), further suggesting an Atlantic origin of Mediterranean sea chubs. An Atlantic source fits also with the timing of the recent expansion of K. vaigiensis in Galician waters (Bañón and de Carlos, 2022), which appears to be simultaneous to the expansion of the species in the Mediterranean Sea.

Overall, our combined morphological and molecular results indicate that K. vaigiensis is the sea chub species currently expanding in Mediterranean waters, while K. sectatrix, albeit present, is to date very rare. This is also partially explained by the
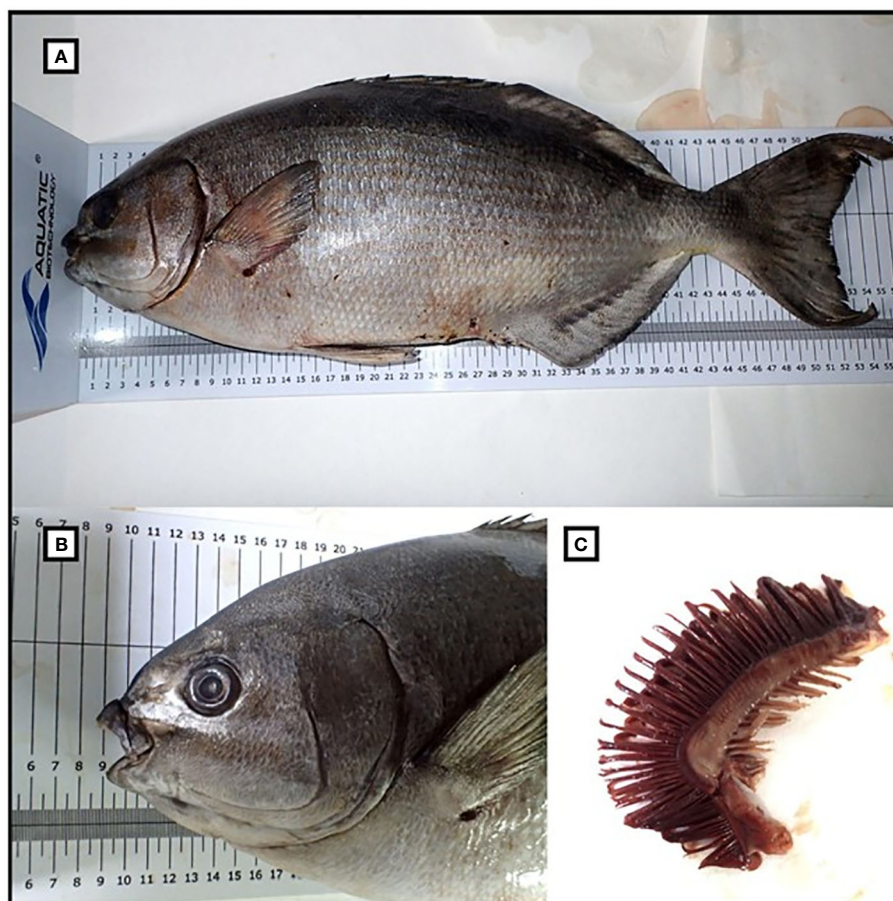
FIGURE 3
(A) *Kyphosus vaigiensis* specimen XII caught at Roccella Ionica (Italy, Ionian Sea) on December 2022; **(B)** particular of the head; **(C)** first gill arch with gill filaments (left side on the photo) and gill rakers (right side).

different mobility of the two species. Indeed, while *K. vaigiensis* is capable of undertaking extensive migrations along coastal waters, *K. sectatrix* exhibits a markedly more limited range of movements (Eristhee and Oxenford, 2001; Sakihara et al., 2015; Bañón and de Carlos, 2022). Furthermore, in agreement with other authors that evidenced the difficulty to evaluate whether old records of *K. sectatrix* in the basin are valid or not (Knudsen and Clements, 2013), we hypothesise that other records of *K. vaigiensis* might be present among them.

According to available literature (Knudsen and Clements, 2016; Knudsen et al., 2019), the closest species to *K. vaigiensis* are in the order *Kyphosus ocyurus* (Jordan and Gilbert, 1882), *Kyphosus elegans* (Peters, 1869), and *K. cinerascens*. The limited divergence between *K. vaigiensis* and *K. cinerascens* (Figure 4; Table 4) shows that further mitochondrial analyses on *K. ocyurus* and *K. elegans* are also needed. Their complete mitogenome sequences would help to confirm or dismiss the distinction as separated species.

Unfortunately, it was not possible to physically obtain all the specimens here reported and thus provide their molecular and meristic data. This limitation is intrinsic to all citizen science-based projects. However, citizen science is probably the only available approach for documenting a large-scale expansion of similar fish species, and

combined with meristic and molecular data of the obtained specimens, allows to reach sound results (Castejón-Silvo et al., 2023).

Interestingly, the great majority of our observations (11 out of 13 in Table 2) are from spearfishermen. As previously reported in other studies (Tiralongo et al., 2020 and references therein), this underlines the importance of recreational fishing, and in particular spearfishing, in monitoring the expansion of certain species, which could travel unnoticed by professional fishermen, divers and other sea-user categories. Furthermore, being these species mainly herbivorous, sport techniques that use hooks and baits or artificial baits are probably not very efficient in catching these fish, which are instead much more easily captured with spearguns, due to their rather considerable size.

Despite the several *K. vaigiensis* records here presented, which have never similarly been reported in Mediterranean waters, in our opinion, even this *Kyphosus* species still remains quite rare in the basin. In fact, the 13 collected records are the result of a collection campaign of the AlienFish project that involves hundreds of professional and recreational fishermen. Nevertheless, it is likely that the ongoing seawater warming will favour the expansion of *K. vaigiensis* in the Mediterranean Sea in the next future, similarly to what is already happening for other fish species of Atlantic origin,
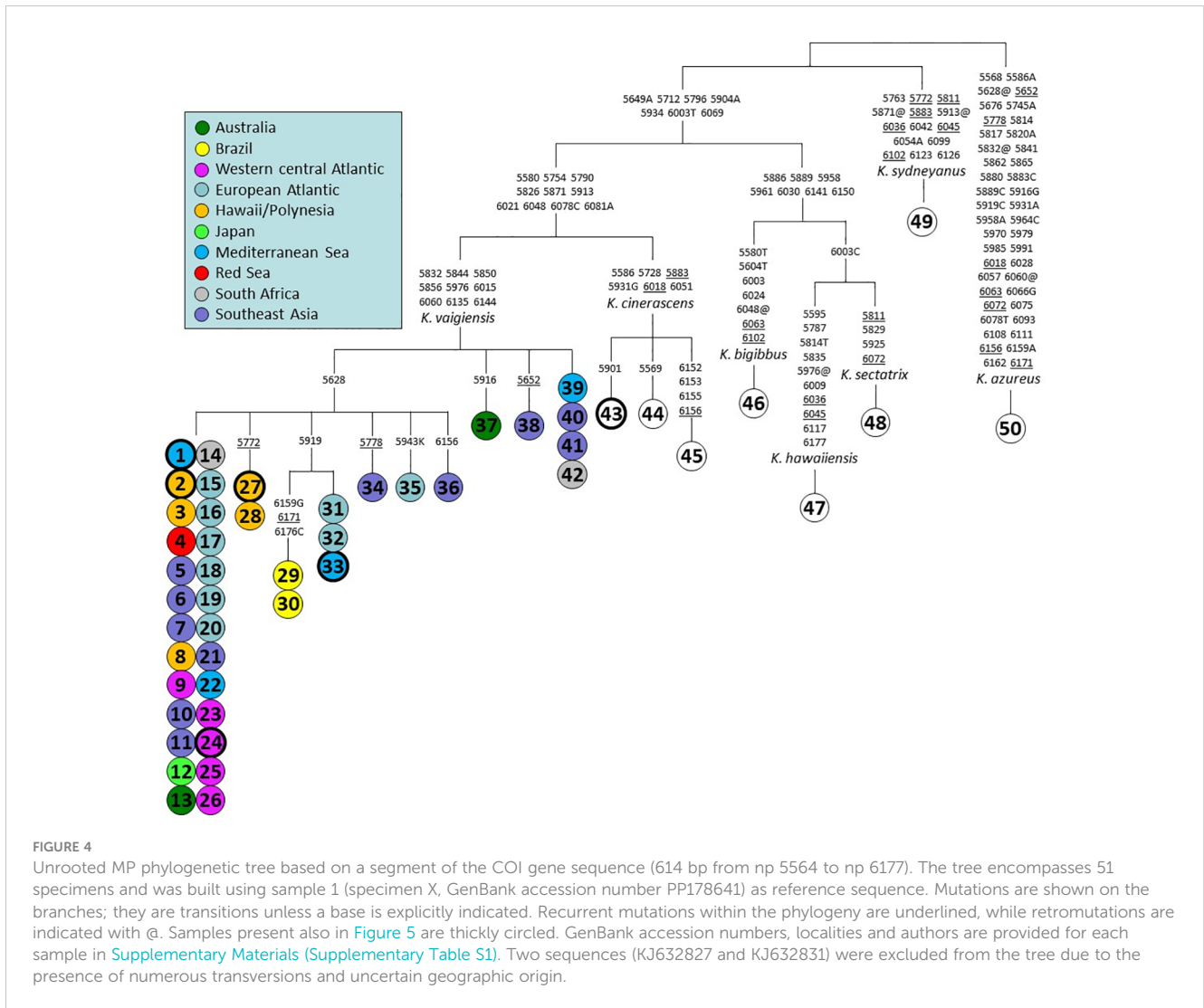
FIGURE 4
Unrooted MP phylogenetic tree based on a segment of the COI gene sequence (614 bp from np 5564 to np 6177). The tree encompasses 51 specimens and was built using sample 1 (specimen X, GenBank accession number PP178641) as reference sequence. Mutations are shown on the branches; they are transitions unless a base is explicitly indicated. Recurrent mutations within the phylogeny are underlined, while retromutations are indicated with @. Samples present also in Figure 5 are thickly circled. GenBank accession numbers, localities and authors are provided for each sample in Supplementary Materials (Supplementary Table S1). Two sequences (KJ632827 and KJ632831) were excluded from the tree due to the presence of numerous transversions and uncertain geographic origin.
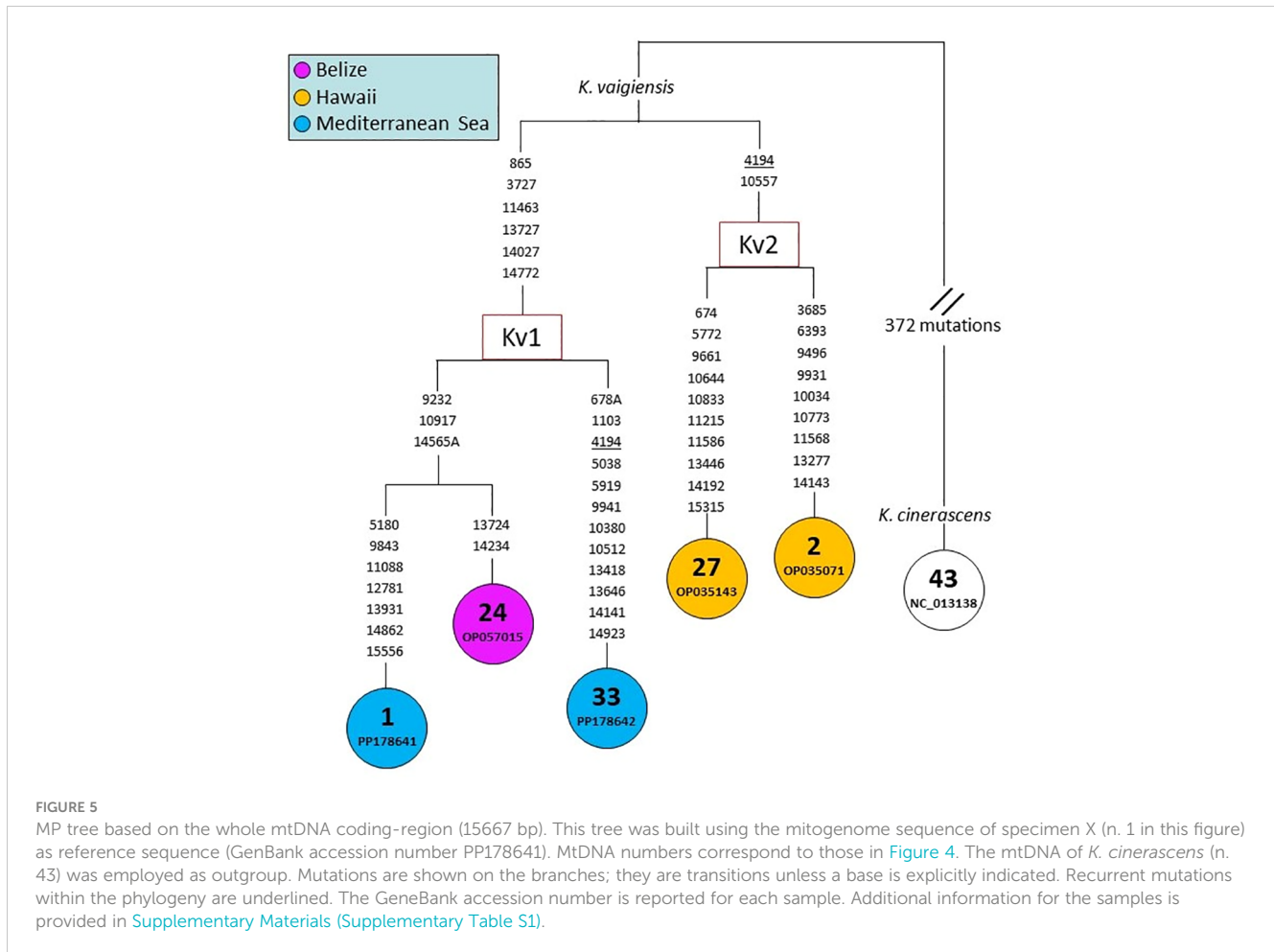
namely *Parapristipoma octolineatum* (Valenciennes, 1833) (Tiralongo et al., 2023).

The native status of *K. vaigiensis* in the Mediterranean requires special attention. In fact, the species has often incorrectly been considered "alien" (Groud et al., 2021; Azzola et al., 2024, and references therein), probably based on the assumption that the first records in the basin are referred to the last three decades (Table 1). However, following widely accepted rules regarding terminology in the science of biological invasions (Pyšek et al., 2004; Crees and

TABLE 4 Sequence divergence between *K. vaigiensis* (specimen X) and *K. cinerascens* (GenBank accession number NC_013138).

MtDNA region	Number of transitions/transversions	%
Whole coding-region (15667 bp)	387	2.47
Protein-coding genes (11407 bp)	347	3.04
Entire COI gene (1551 bp)	39	2.51
COI fragment (614 bp, from np 5564 to np 6177)	17	2.77

Turvey, 2015; Essl et al., 2018), naturally expanding species must be considered part of the native biota, and not alien taxa. Some authors proposed that *K. vaigiensis* could be able to follow vessels and that this could facilitate its introduction in the basin (Azzurro et al., 2013; Lo Brutto, 2017), and mentioned Nelson (1994) as support for such a possibility. However, neither this version, nor the latest version of Nelson's book "Fishes of the World" report this particular behaviour for the species (Nelsonet al., 2016). In all cases, the spread and increasing abundance of *K. vaigiensis* in the Mediterranean has been substantially witnessed only in the last decade (Table 1, Table 2), and thus vessel-following cannot explain alone the expansion of this fish in the basin. Other authors even suggested that the species might have been introduced by ballast waters (Groud et al., 2021). In our opinion, this is rather unlikely, and unnecessary to explain the current expansion of the fish. Indeed, previous research suggested a thermophilic nature of *K. vaigiensis*: for example, in Maltese waters, the species was caught in an area with increased sea temperature due to thermal effluent discharges from a power station (Vella et al., 2016). The high number of individuals captured in southern Italian regions between 2021 and 2022 now suggests the presence of a stable population of the species

FIGURE 5

MP tree based on the whole mtDNA coding-region (15667 bp). This tree was built using the mitogenome sequence of specimen X (n. 1 in this figure) as reference sequence (GenBank accession number PP178641). MtDNA numbers correspond to those in Figure 4. The mtDNA of *K. cinerascens* (n. 43) was employed as outgroup. Mutations are shown on the branches; they are transitions unless a base is explicitly indicated. Recurrent mutations within the phylogeny are underlined. The GeneBank accession number is reported for each sample. Additional information for the samples is provided in Supplementary Materials (Supplementary Table S1).

in the southern (warmer) Mediterranean sectors, indicating a natural expanding phenomenon and subsequent establishment of this species in the area. This is perfectly in agreement with the ability of a species able to migrate 251 km in 33 days (Sakihara et al., 2015). Hence, current data suggest the natural range expansion from the western Atlantic to Mediterranean waters of this thermophilic species.

Since all the available data support the notion that *K. vaigiensis* populations represent a natural expansion from the Atlantic Ocean through the Strait of Gibraltar, the species must not be considered "alien" nor "exotic" for the Mediterranean, but just part of the native fauna of the basin (Essl et al., 2018). Alternatively, following Essl et al. (2019), the species can be called "neonative" (i.e. a taxon that expanded its range due to biophysical changes in the environment, but not as a result of human transport or dispersal corridors creation).

The possible ecological impacts of a substantial expansion of *K. vaigiensis* are difficult to predict. *Kyphosus* species are typically herbivorous (Clements and Choat, 1997), and their diet significantly contributes to the structure and dynamics of marine benthic communities, as their grazing can influence the composition and abundance of algae and associated species. Additionally, the feeding behaviour of *Kyphosus* species can affect the marine ecosystems indirectly, influencing habitat availability

and resources for other species. However, given the currently limited abundance of the fish in the Mediterranean, its impacts are far from being comparable to the ones caused by other expanding species such as the herbivorous rabbitfish species (*Siganus* spp.) (Escalas et al., 2022; Vella et al., 2023).

In a broader context, the increasing proliferation of thermophilic fish species in the Mediterranean Sea raises concerns about potential ecological and economical effects on ecosystem and fishing activities (Coco et al., 2022; Nota et al., 2023). As sea temperatures continue to rise, the Mediterranean ecosystem faces unpredicted shifts in species distribution and biodiversity. As these ecological dynamics evolve, it is imperative to prioritize initiatives aimed at understanding the drivers and consequences of species expansion in the basin.

To conclude, the recent proliferation of sea chubs in the Mediterranean Sea presents a challenge in species recognition, primarily due to the morphological similarity between *K. vaigiensis* and *K. sectatrix*. This has led to misidentification and the reporting of specimens under synonymous names. Here we proposed a multidisciplinary approach, incorporating both citizen science, morphological and molecular methods, which can be applied also to the study of other expanding species (Pires et al., 2023; Michail et al., 2024). This approach provided valuable insights into the ongoing expansion of *K. vaigiensis*, indicating this species

as the sea chub currently expanding its range in the Mediterranean Sea. The results obtained by other authors in Galician waters, together with the golden rows clearly visible on the majority of our reported specimens and our morphological and molecular evidence, altogether support the scenario that the species currently expanding in the Mediterranean is *K. vaigiensis*, while to date, there is no concrete reason to hypothesize a similar expansion pattern for *K. sectatrix*. Genetic analyses suggest that the origin of these *K. vaigiensis* specimens has to be considered Atlantic, and albeit a human introduction of the species or the entry/exit through the Suez Canal cannot be excluded, there is no evidence in this regard for the moment. In the current scenario of global change and water warming of the Mediterranean Sea, the increase of thermophilic species puts the attention on ecological and economic risks associated with their presence.

Moreover, several biological and ecological aspects of *K. vaigiensis* remain still largely unknown, hence the urgent need to orient further research in aspects such as the life span and larval planktonic phases of the species, especially in response to changing ocean temperatures. The adaptation of invading species to the new habitats usually involves many species-specific ecological and genetic processes (Nota et al., 2024). Therefore, understanding these and others life history traits of this thermophilic species is crucial to know and predict its distribution and possible ecological impacts.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because the two sequenced fish were collected already dead from the fishermen.

Author contributions

AN: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. FT: Writing – review & editing, Writing – original draft, Resources, Project administration, Formal analysis, Data curation, Conceptualization. AS: Writing – review & editing, Validation, Methodology, Investigation. AT: Writing – review & editing, Writing – original draft, Validation, Resources, Funding acquisition. AO: Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Formal analysis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1411111/full#supplementary-material

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