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Untangling the cephalopod market: Authentication of seafood products in Greece with DNA-barcoding

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ABSTRACT

Cephalopod products are very popular in Greece and are considered a delicacy. However, heavy processing and marketing practices interfere with visual inspection and impede morphological identification, allowing species substitution. Mislabeling of seafood products remains a worldwide issue despite existing labeling regulations at local and European level. For the detection of fraudulent products, a variety of identification methods have been developed, however DNA barcoding remains the most favored. This study aims to investigate the cephalopod species sold in the country's seafood market and assess their mislabeling rates. Two mitochondrial genes, the cytochrome *c* oxidase subunit I (COI) and the 16S ribosomal RNA (16S), were selected for the analyses. A total of 156 samples were collected from fishmongers, markets, and restaurants across four cities. Identification was successful in 93.58% of the samples and 59 discrepancies (40.41%) between the label and the identified species were recorded. However, in some cases, substitution might have been unintentional, caused by negligence, lack of detailed information on the label, and the overall low awareness of the legislation by retailers. High mislabeling rates were estimated, especially when compared against the average global substitution rate in seafood products but align with those reported by studies on cephalopod products. This study is the first to investigate mislabeling rates in cephalopod commodities in Greece. With the aim of a transparent seafood trade, our results highlight the need for monitoring of all seafood products available in the country's market.

1. Introduction

Over the last 50 years, human population has increased and along with it, the world's seafood demand (Azad et al., 2016; FAO, 2023). The term "seafood" includes all aquatic animals (fish, mollusks, crustaceans, and echinoderms) that are edible and are available on the market (Stamatis et al., 2015). The global seafood consumption has increased with an average rate of 3% annual (FAO, 2022). The consumption of aquatic animal foods reached from 9 kg (in 1964) to approximately 22 kg per person per year in 2016 (FAO, 2016), followed by a slight decline to 20.2 kg (FAO, 2022). Seafood consumption in the European Union reached 14.6 million tons in 2018 and is expected to rise to 16.1 million tons by 2030 (EU, 2018). Cephalopod exploitation has been following a similar trend over the last decades, with continuous growth over the last 20 years and an average annual global production exceeding 3.5 million tons (FAO, 2022).

Cephalopods are marine organisms with short life cycle, that belong to the molluscan class of Cephalopoda; they have high nutritional value and constitute an important source of human nutrition (Doubleday et al., 2016). In the global food trade, fishery products are included in the most internationally traded commodities and cephalopods account for 4% of the total traded volume (FAO, 2016; Pardo et al., 2018). Globally, they are usually classified and traded under three macro categories: octopus, squids, and cuttlefishes (Arkhipkin et al., 2015; Wen et al., 2015). The families of Loliginidae and Ommastrephidae (squids) are the most commercially important groups of cephalopods, with an annual production of 1.27 million tons in 2017, that is approximately 33.7% of the overall cephalopod production (Shi et al., 2020).

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Approximately 75% of all seafood consumed in Europe is imported from other regions, with the main importers and consumers being Italy and Spain (FAO, 2016; Pardo & Jimenez, 2020). Due to the globalization of the seafood trade and the overall decline of fish stocks globally, the need for strict labeling regulations and traceability systems have become essential (Minoudi et al., 2020; Tamm et al., 2016). Nonetheless, the introduction of foreign species in local markets renders the control of such products even more difficult (Barbuto et al., 2010).

For these reasons, the EU has introduced a variety of rules and regulations over the last 20 years. For example, the 178/2002/EC regulation was introduced in 2002, establishing the European Food Safety Authority, to prevent fraudulent and deceptive practices along with any food adulteration. This regulation applies to all production, processing and distribution stages of food and feed, and establishes procedures for issues affecting their safety. Regulation 1169/2011 confirmed and specified the labeling requirements of foodstuff traded in the EU (Stamatis et al., 2015). In 2017, the 2017/625/EC established rules for official controls, to ensure the correct application and enforcement of the existing legislation, in relation to human health, animal health, plant health and welfare. Specifically for seafood, the EU established Regulation 1379/2013, regarding mandatory labeling information for fishery and aquaculture products. All seafood products should therefore be traded with the common and scientific names of each product, the production method, the area of origin, and the fishing gear used. Additionally, every country-member is required to publish a list of all aquatic species traded in its markets, along with their common and scientific names (D'Amico et al., 2016). In Greece, this list was first published in 2015 (Official Government Gazette 475/Issue B'/27-3-2015, No. 1750/32219) and was renewed in 2021 (Official Government Gazette 343/Issue B'/31-1-2021). It includes aquatic animals traded in the country, accompanied by their scientific names and a current Greek common name (http://www.alieia.minagric.gr/node/42). Restaurants in Greece, however, are not required to state scientific names on their menus, therefore labeling regulations in this part of the food chain become nugatory (Minoudi et al., 2020). Similarly, the existing labeling regulations do not mandate for canned seafood products to include any species scientific names.

Food fraud occurs when food products are traded in a false or misleading way and is characterized by its intentional nature in the form of economic gain (Moore et al., 2012). The most common categories of food fraud are removal, addition and replacement of authentic substances by non-authentic ones, without the consumer's knowledge, and usually for economic gain (De Vries, 2009). For example, mislabeling falls under the replacement category and occurs when one breed or species is substituted and traded under the name of another (Rasmussen & Morrissey, 2008; Stamatis et al., 2015). It is recognized as a persistent global problem of the seafood industry, with worldwide reports for a variety of commercial species (Hanner, Becker, Ivanova, & Steinke, 2011; Galal-Khallaf et al., 2014; Minoudi et al., 2020; Pardo & Jimenez, 2020; Giagkazoglou et al., 2022). Numerous negative consequences have already been associated with mislabeling of seafood, false reporting of fishing areas, fishing gears, and the overall production. Those consequences range from economic fraud to health complications in humans, as well as over exploitation of fish stocks and destruction of marine habitats (Pardo et al., 2018; Zhang et al., 2023). The health concerns raised by the trade of cephalopods are not limited to allergens and toxic metal bioaccumulation. Algal toxins can be introduced in the human food chain through contaminated cephalopods (Lopes et al., 2013), while some species can be venomous to humans (e.g., blue-ringed octopus, *Hapalochlaena lunulata*) (Wu et al., 2014). Therefore, correct labeling of traded cephalopods along with the area of origin, are of utmost importance.

Mislabeling could also be the result of illegal, unreported, and unregulated fishing (IUU), with negative impact on conservation and management efforts of declining populations (Agnew et al., 2009; Sadovy de Mitcheson et al., 2018). On that regard, however, cephalopod populations appear to be increasing in the last 60 years, despite their growing exploitation (Doubleday et al., 2016). The reasons behind this seemingly bizarre trend could be related to the decrease of natural predators, along with the changes in environmental ocean conditions (Doubleday et al., 2016). However, their annual global catches appear to substantially fluctuate, reaching a peak in 2014 (4.9 Mt), and decreasing to 3.7 Mt by 2020 (FAO, 2022). The world's annual cephalopod catch mainly consists of just three species (*Todarodes pacificus, Dosidicus gigas*, and *Illex argentinus*), all transboundary and highly migratory stocks, rendering stock assessment extremely difficult (Gleadall et al., 2024). Additionally, the European Union does not routinely assess cephalopod stocks. In Greece, all the 49 species (Official Government Gazette 343/Issue B'/31-1-2021, No. 1750/32219) that are legally traded are evaluated by the International Union for Conservation of Nature (IUCN) as Least Concern (LC) or Data Deficient (DD).

In the market, seafood products are usually processed and transformed before they reach consumers, to increase their palatability and reduce their perishability (Barbuto et al., 2010). With extensive processing, morphological characteristics are usually partially and/or completely removed, and many products have similar taste and appearance, thus morphological identification can be proven difficult. Such practices increase the risk of food fraud and species substitution (Barbuto et al., 2010; Pazartzi et al., 2019). Particularly, cephalopods are marketed in many forms, whole or in pieces (rings, tentacles, and wings), fresh, frozen or in cans. For fresh whole specimens, morphological identification can be achieved via visual inspection and the use of morphological keys (Yalla & Mohanraju, 2019). However, even for those cases, identification can be proven troublesome, as it requires a high level of expertise (Cheng et al., 2023; Martínez et al., 2002).

For this reason, several analytical methods have been developed, with DNA barcoding being the most widely used (Böhme et al., 2019). This technique is based on the amplification and sequencing of a standard, short genetic region, and the comparison of the resulted sequences against the ones deposited in online databases (Minoudi et al., 2020). DNA barcoding has been used globally for the detection of food fraud and species identification, for a variety of seafood products (Griffiths et al., 2013; Chin et al., 2016; Pardo & Jimenez, 2020). In Greece, a few scientific studies have been published, revealing moderate to high mislabeling rates (Garcia-Vazquez et al., 2011; Giagkazoglou et al., 2022; Minoudi et al., 2020; Pardo et al., 2018; Pazartzi et al., 2019; Stamatis et al., 2015; Triantafyllidis et al., 2010). However, no scientific study investigated mislabeling rates of invertebrates in the Greek seafood market up-to-date.

Therefore, this study aims to investigate the cephalopod trade in Greece and estimate the mislabeling rates in the market. The partial sequencing of two mitochondrial genes, the cytochrome oxidase subunit I (COI) and the 16S ribosomal RNA (16S) were used to identify species sold under the umbrella terms of "chtapodi" (octopus), "kalamari" (squid), "thrapsalo" (squid), and "soupia" (cuttlefish) (Table 1). Additionally, we investigated the patterns of species utilization between

Table 1
Labels recorded in Greek markets by this study, along with genus distinction per category.

Labels recorded in Greek markets	Species
Chtapodi, Chtapodi Indo-Irinikou, Chtapodi Indikou, Chtapodi Mexikou, Chtapodi Elliniko, Plokami Chtapodi, Plokami Chilis, Kapnisto Chtapodi	Octopus of the genus *Octopus*
Moschios, Moschochtapodo	Octopus of the genus *Eledone*
Kalamari, Kalamarakia, Kalamari Gnisio Xondro, Kalamari Indias, Kalamari Californias	Squids of the genus *Doryteuthis, Loligo, Uroteuthis*
Thrapsalo, Thrapsalo (Apopsigmeno), Thrapsalo Irinikou, Plokami Thrapsalo	Squids of the genus *Stenoteuthis, Notododarus, Illex, Dosidicus*
Soupia, Soupia Indias	Cuttlefish of the genus *Sepia*
Seafood mix, Seafood salad	Combination of cephalopods

different market (fresh, frozen, canned, and cooked) and retailer types (open markets, fishmongers, supermarkets, and restaurants). Finally, we examined whether mislabeling was affected by the sampling location and the commercial value of the specimens.

2. Materials and methods

2.1. Sample collection and storage

A total of 156 samples of cephalopods (octopuses, cuttlefishes, and squids) were collected between September 2021 and March 2023. Different marketed seafood products were targeted (fresh, frozen, canned, and cooked) to provide a wide range of representation in our analyses. Commercialized seafood products were collected under a variety of labels, directly purchased at random from fishmongers, open markets (i.e., street retailers), supermarkets, and restaurants. Samples were purchased in four cities of Northern Greece (Kavala: n = 23, Komotene: n = 24, Volos: n = 24, Thessaloniki: n = 85; Fig. 1, Table 2). Frozen and canned samples were only collected from the largest city, Thessaloniki, as it was speculated that seafood distributers usually supply the same brands in markets around Greece, with minimal differences between locations. A minimum of 10 samples were collected for the fresh and cooked product categories, from each location respectively. Samples were transferred to the lab, and the interior part of each individual sample was cut into smaller pieces and stored separately at −20 °C, until further analysis. Cooked and canned samples were rinsed with sterile water prior to analysis. Each sample was given a code, and marketing information such as label (common and scientific name), sampling location, date and pricing were recorded. The category (fresh, frozen, canned, and cooked) of the samples was also recorded, along with their respective market type (open markets-OM, supermarkets-SM, fish mongers-FM, and restaurants- RE).

2.2. DNA extraction, amplification, and sequencing

Genomic DNA was extracted following different extraction methods. Initially, DNA was extracted from fresh, frozen, and cooked samples according to a modified CTAB protocol, using 70 mg of tissue (Hillis et al., 1996). Where this failed, DNA was obtained from 30 mg of tissue using the QIAamp DNA mini kit (Qiagen, Hilden Germany). For the canned samples, DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden Germany), according to the manufacturer's

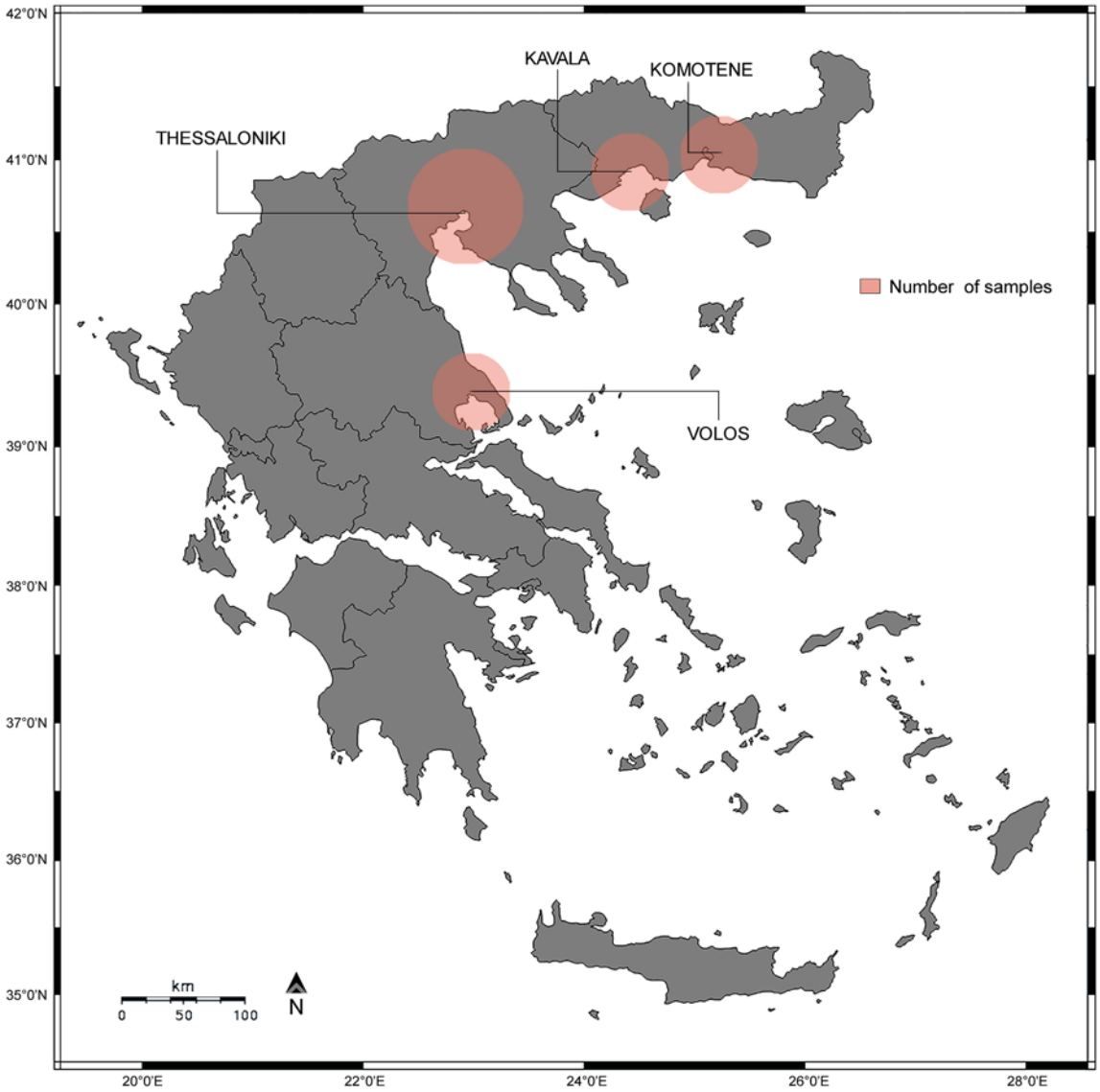

Fig. 1. Approximate sampling locations of cephalopod collected between September 2021 and March 2023. Number of collected samples: Komotene(24), Kavala (23), Thessaloniki(85), Volos(24).

Table 2
Number of samples, species and mislabeling rates per sampling location.

City	Number of samples	Number of species	Mislabeled	Fresh	Frozen	Cooked	Canned
Kavala (KA)	23	9	8	11	0	12	0
Kometene (KO)	24	9	9	9	0	14	0
Thessaloniki (TH)	85	18	31	19	29	10	27
Volos (VO)	24	11	11	11	0	13	0

instructions. The quality and quantity of the extracted DNA was determined on a Quawell DNA/Protein Analyzer 3000 by absorbance measurements and calculation of A260/A280 and of A260/230 ratios, according to manufacturer's indications. DNA was also visualized on a 1% agarose gel, stained with Midori Green DNA dye (Nippon Genetics Europe GmbH, Germany). DNA extracted using the CTAB based protocol, was purified using the microClean purification kit (Gel company, USA). DNA extracted from canned samples, was purified using the DNA Clean & Concentrator kit (ZYMO Research, USA). DNA purifications were performed to remove PCR inhibitors from DNA extracts. Purified DNA was stored at −20°C until further analysis.

Two mitochondrial (mtDNA) genes (COI and 16S rRNA) were selected, with the COI gene serving as the main marker for the analysis. Both genes have been successfully and repeatedly used in cephalopod DNA barcoding studies (Galal-Khallaf et al., 2021; Shi et al., 2020; Velasco et al., 2021; Wen et al., 2017). Approximately, a 700 bp segment of the COI gene was amplified using the universal primer pair LCO1490/HC02198 (Folmer et al., 1994) (Table 3). Two different PCR mixes were used for the amplification. Initially, the Optima PCR HotStart Ready Mix with dye (FastGene) and where this failed, the KAPA2G Robust HotStart Ready Mix (KAPA Biosystems, South Africa). All PCR reactions were conducted in 25 μL volume. The reaction mixtures for the FastGene PCR mix contained 1.5 μL of template DNA, 1.25 μM of each primer (10 μM), 12.5 μL PCR mix and completed with PCR-grade water up to 25 μL. PCR cycling conditions were set with an initial denaturation at 95 °C for 3 min, followed by 40 cycles at 95 °C for 1 min, 50 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min. The reaction mixtures for the KAPA PCR mix contained 1 μL of template DNA, 1.25 μM of each primer (10 μM), 12.5 μL PCR mix and 9 μL PCR-grade water. PCR cycling conditions were set following manufacturer's recommendations, with an initial denaturation at 95 °C for 2 min, followed by 35 cycles at 95 °C for 15 s, 50 °C and 55 °C for 15 s, 72 °C for 15 s, and a final extension at 72 °C for 5 min. All PCR runs included negative controls.

When amplification and/or sequencing with the COI failed, a 600 bp fragment of the 16S rRNA gene was amplified, using the universal primer pair 16Sar/16Sbr (Palumbi, 1996) (Table 3). PCR was conducted in 25 μL and the reaction mixtures included the same reagents and volumes described before. The reactions performed using the FastGene PCR mix and were conducted with an initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 1 min, 53 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR using the KAPA mix were conducted according to the manufacturer's recommendations and annealing temperature at 50 °C. All PCR runs included negative controls.

Both mitochondrial fragments were separated on 1.5% agarose gel electrophoresis, stained with Midori Green DNA dye. A FastGene 100 bp DNA Ladder (Nippon Genetics Europe GmbH, Germany) was applied to assess the quality and quantity of the fragments. PCR products were purified and sequenced at the International Hellenic University (Department of Agriculture, Greece), using an ABI 3500 Genetic Analyzer (Applied Biosystems, USA).

2.3. Data analysis

All resulted sequences were manually checked and edited using BioEdit 7.2.6 (Hall, 1994). Each sequence was primarily compared against the ones available on GenBank and analyzed using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To ensure a high level of accuracy, the COI sequences were also compared against the Animal Identification System in BOLD, specifically in the Species Level Barcode Records Database. Species were identified with the top match, and the identity threshold was set at 98% for both databases. Only sequences with high homology (≥98%) were included in the analysis. The sequences were not submitted to any international online database, since the results were not corroborated with morphological identifications by an expert (where it was possible e.g. fresh and frozen samples). All generated sequences are available in Supplementary Table 3. For samples that species level identification was not possible through database comparisons alone, phylogenetic trees were constructed. The results of the molecular identification and the phylogenetic analysis were compared against the information stated on the label (common and scientific name) of each product. In case of a discrepancy between the two, the sample was declared mislabeled.

2.4. Phylogeny

Phylogenetic analyses were carried out using MEGA 11.0 (Tamura et al., 2021). Sequences were aligned using the ClustalW algorithm incorporated in MEGA (Thompson et al., 1994). Pairwise genetic distances were calculated using the Kimura-2-Parameter (K2P) model (Kimura, 1980) and both trees (Neighbour Joining and Maximum Likelihood) were constructed with 1000 bootstrap replicates (Saitou & Nei, 1987). Reference sequences for the COI gene were downloaded from GenBank (Supplementary Table 1).

2.5. Statistical analyses

Statistical analyses were carried out using R 3.5.1 (https://cran.r-project.org/) and PAST-6 (Hammer et al., 2001). A non-parametric analysis of similarity (ANOSIM) was performed, with each sample/data representing one point, using the Bray-Curtis distance measure. The effect of sampling location and label on patterns of cephalopods on sale was also examined. A SIMPER analysis was performed to estimate the contribution of each species to differences between categories. For this part of the analysis, the recorded labels were grouped together according to their genus and their legal designation (Table 1). Finally, a two-way PERMANOVA analysis was performed to evaluate the effects of mislabeling to price, in relation to location and the retailers.

Table 3
Sequencing primers used for cephalopod species identifications in this study.

Target Gene	Primer Name	Primer sequence (5′-3′)	Tm	Amplicon Length (bp)	Reference
16S rRNA	16SH	5′-CCGGTCTGAACTCAATCACG-3′	55.7	600	Palumbi (1996)
	16SL	5′-CGCCTGTTTAACAAAAACAT-3′	49.6		
COI	LCO1490 {F}	5′-GGTCAACAAATCATAAAGATATTGG-3′	56.4	700	Folmer (1994)
	HCO2198 {R}	5′-TAAACTTCAGGGTGACCAAAAAATCA-3′	58.5		

3. Results

3.1. DNA extraction, amplification, and sequencing evaluation

DNA extractions were successful for all collected samples, however the quantity and quality of the DNA varied between different categories. On average, DNA extractions on the frozen and fresh categories resulted in higher quality and quantity of DNA, with longer DNA fragments, followed by the cooked samples. Differences in both quantity and quality of DNA were observed in the canned products, where the DNA had low concentrations with short fragments. Despite several attempts, ten samples failed amplification or sequencing in both targeted genes. Specifically, PCR amplifications were successful for all samples of the fresh, frozen, and cooked categories, yet failed for seven canned samples. Sequencing failed for one fresh and two cooked samples as well. Overall, 146 sequences were obtained for the COI gene and 27 sequences for the 16S rRNA gene. The length of the sequences ranged between 448 and 655 bp (average length 630 bp) for the COI gene and between 480 and 547 bp (average length 500 bp) for the 16S rRNA gene (Supplementary Table 3).

3.2. Species identification

Out of the 156 collected samples, a total of 146 (93.58%) were successfully identified (Supplementary Table 2). In most cases, barcode searches in BLAST and BOLD databases produced singular top matches with ≥98% confidence on species assignment (Supplementary Table 2). For six specimens of the Sepiidae family, database comparisons resulted in more than one top match with the COI gene and were identified through the 16S rRNA marker (Supplementary Table 2). For 11 samples of the *Illex* (9) and *Uroteuthis* (2) genera, species level identification was not possible, whereas for 17 samples belonging to the *Nototodarus* genus, species level identification was only possible after phylogenetic analysis.

Totally, species identification was successful for 135 samples (92.46%). We identified 19 species across ten genera (*Doryteuthis, Dosidicus, Eledone, Illex, Loligo, Nototodarus, Octopus, Sepia, Stenoteuthis, Uroteuthis*) and five families (Eledonidae, Loiginidae, Octopodidae, Ommastrephidae, Sepiidae). Most of the identified samples belonged to the common octopus (*O. vulgaris*, n = 27, 18.49%), followed by the New Zealand arrow squid (*N. sloanii*, n = 17, 11.64%), and the Indian squid (*Uroteuthis duvaucelii*, n = 17, 11.64%) (Fig. 2). Five species (*Doryteuthis gahi, Sepia aculeata, Sepia vecchioni, Stenoteuthis oualaniensis*, and *Uroteuthis chinensis*) were the least common, with only one individual identified (Fig. 2, Supplementary Table 2). Eleven samples (7.5%) were identified at the genus level (Supplementary Table 2), as they resulted to equivalent top matches to multiple species and the phylogenetic analysis did not provide species level identification. Nine of those belong to the *Illex* genus and two belong to the *Uroteuthis* genus.

Species identification for the four marketing categories (fresh, frozen, canned, and cooked) varied (Fig. 3). It was successful for all collected frozen samples (100%). Fresh and cooked samples followed with 88% and 83.67% success in species level identification, respectively, with the canned samples having the lowest success rates (74.07%). Genus level identification was possible for 12.24% of the cooked and 10% of the fresh products (Fig. 3). The highest level of unidentified samples was recorded in the canned products (25.92%, Supplementary Table 2) due to unsuccessful amplification of both targeted genes. For canned products, mini barcode amplifications were attempted using the primer pair described by Armani et al. (2015), without success.

3.3. Phylogeny

Two phylogenetic trees were generated to assist with the species level identification of the *Nototodarus* sp. specimens. The first neighbour-joining tree showed that all *Nototodarus sloanii* sequences but one were grouped with our samples (Supplementary Fig. 1). Most *Nototodarus gouldi* sequences were grouped in a single cluster. Finally, the *Loligo forbesii* and the *Octopus vulgaris* sequences were used as outgroups and formed two separate clusters. Two sequences of *N. gouldi* and *N. sloanii* (HM888020, HM888021) did not group with the rest of the specimens (Supplementary Fig. 1). The second neighbour-joining tree did not clarify species level identification of the *Illex* sp. specimens and was therefore excluded from the analysis.

3.4. Identification of mislabeling incidents

Only 48 of the 156 collected samples were sold with all labeling information (common name, scientific name, and fishing location). For 66 samples, no information regarding the location was included and for 107 samples no scientific name was included on the label. Only one sample had no labeling information recorded in the packaging. Cooked and canned samples were evaluated using the generic name (chtapodi, kalamari, thrapsalo, and soupia, Supplementary Figs. 2a, 2b, 2c, 2d;

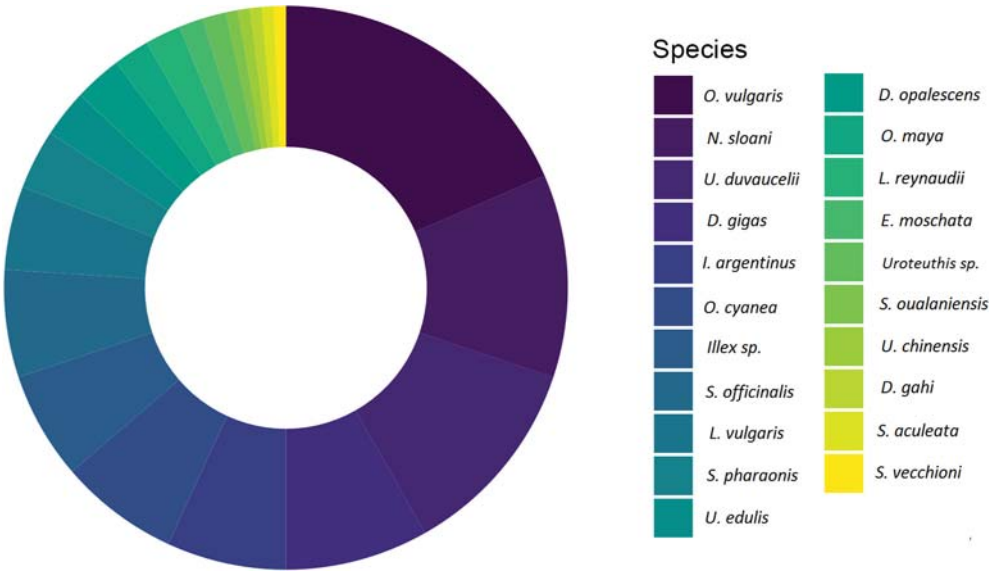

Fig. 2. Composition of the identified species in this study.

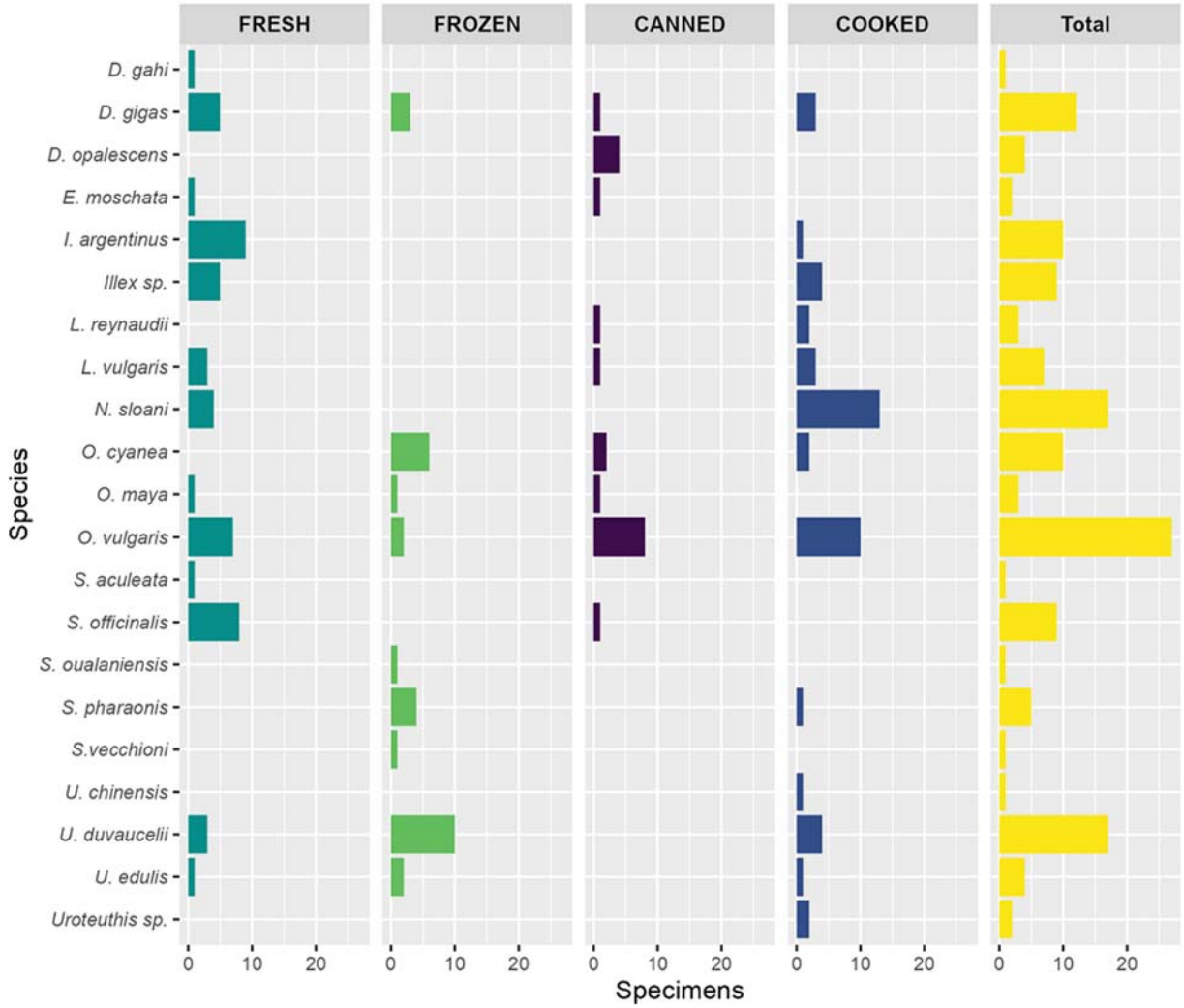

Fig. 3. Composition of the identified species in this study per marketing categories (fresh, frozen, canned, and cooked).

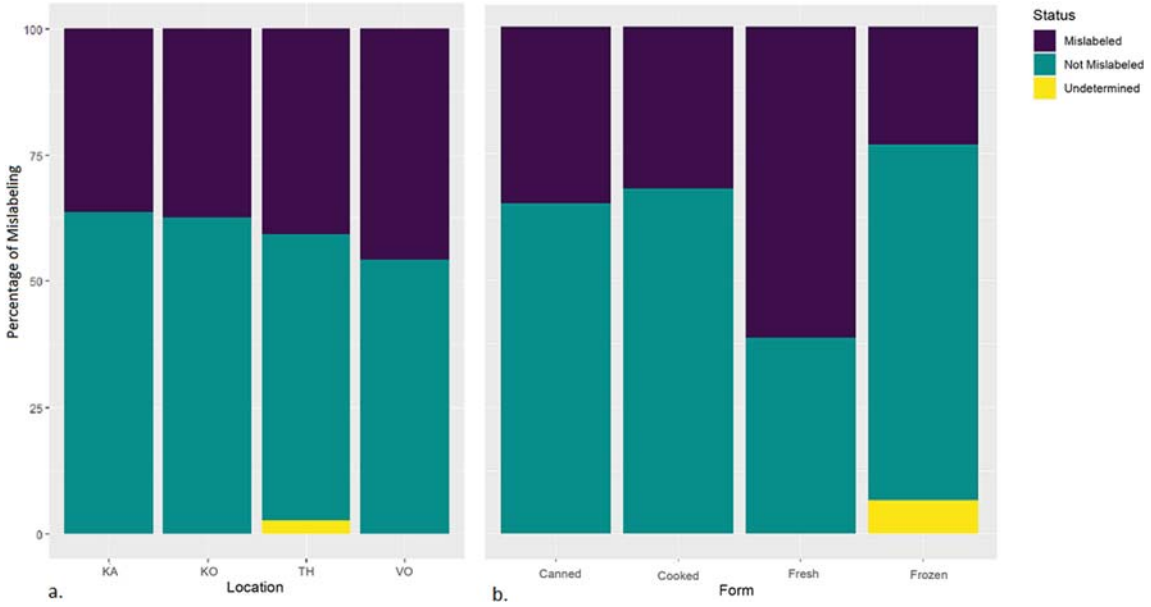

Fig. 4. Schematic representation of the mislabeling rates identified in this study, per a) sampling locations and b) marketing categories (fresh, frozen, canned, and cooked).

Table 1) recorded in the menu and the can label, as species-specific legal designations are not required. Overall, the generic name provided was deemed sufficient to evaluate their labeling status. Moreover, 59 (40.41%) cases of discrepancy between the market labels and DNA identification results were detected amongst the 146 identified samples (Table 3). For two samples, labeling evaluation was not possible. In those cases, no specific information (scientific/common name) was included in the packaging, and therefore recorded as undetermined (Fig. 4, Supplementary Table 2).

When comparing across the four marketing categories, fresh products recorded the highest mislabeling rates, with 66.66% (Fig. 4b), followed by canned (35%) and cooked products (31.91%). The lowest rates were recorded in the frozen category with 23.33% of the identified samples (Fig. 4b). Additionally, the highest mislabeling rates were recorded in Volos (45.83%) and the lowest in Kavala with 36.36% (Fig. 4a).

Overall, 59 discrepancies were recorded between the products' label (common and scientific name) and the DNA barcoding results. The vast majority (37) of the mislabeled products belonged in the broad category of squids (Supplementary Table 2). In Greece, squids are separated in two generic categories, based on their common names (kalamari and thrapsalo, Table 1). Fourteen cases of substitution were recorded between "kalamari" and "thrapsalo" products. According to the Official Government Gazette 343/Issue B'/31-1-2021, the label "kalamari" belongs to *Loligo vulgaris*. However, we identified seven cases where the label "kalamari" was used to describe different species i.e. *U. duvaucelii*, *U. edulis, L. reynaudii*, and *D. gahi*. Similarly, the label "thrapsalo" belongs to the species *Ommastrephes bartramii, Todarodes sagittatus* and *Todaropsis eblanae*. Our study recorded eleven cases, where the label "thrapsalo" was used to describe products that belonged to *I. argentinus, S. oualaniensis, D. gigas* and *N. sloani*. Finally, five cases of substitution were discovered where *D. gigas* (thrapsalo Irinikou) was labeled as *O. vulgaris* or *O. mimus*.

Similarly, nine discrepancies were recorded in the broad category of *Octopus* (Supplementary Table 2). In five cases, *O. vulgaris* (chtapodi) products were marketed under squid labels (i.e., kalamari and thrapsalo) while for the rest, a different species of octopus (i.e. *O. cyanea, O. maya* and *E. moschata*) was sold under the label of *O. vulgaris*.

3.5. Species comparison among cities and retailer types

The highest species diversity was observed in the largest city, Thessaloniki (number of species = 18), followed by Volos (number of species = 11), whereas in Kavala and Komotene nine species were recorded (Table 3). Species utilization in comparison with the labels was significant (ANOSIM, $R = 0.183$, $p < 0.001$, Supplementary Table 4). Additionally, all labels differentiated from the others, except for the "Thrapsalo'' vs "Kalamari'' that were significantly similar (ANOSIM, $R = 0.082$ $p < 0.001$, Supplementary Table 4) driven mainly by *N. sloani* and *U. duvaucelii* (SIMPER, Supplementary Table 4). Similarly, the labels "Soupia'' and "Seafood mix'' were significantly similar (ANOSIM, $R = 0.127$, $p = 0.330$, Supplementary Table 4). However, the Seafood mix category contains a small number of samples, and therefore should not be considered. The comparison of species utilization between different locations was significant (ANOSIM, $R = 0.020$, $p = 0.024$). All locations were similar with the exception of Komotene vs Thessaloniki (ANOSIM, $R = 0.036$, $p = 0.004$, Supplementary Table 4) and Komotene vs Kavala (ANOSIM, $R = 0.045$, $p = 0.031$, Supplementary Table 4). Additionally, mislabeling rates among different retailers was significant (ANOSIM, $R = 0.037$, $p < 0.001$, Supplementary Table 4). Pairwise comparisons revealed similarities between all retailers, except for "OM'' (Open Market) vs "SM'' (Super Market) and "OM'' vs "FM'' (Fish Mongers) (Supplementary Table 4).

The two-way PERMANOVA analysis indicated no significant differences in prices of products sold among the different locations (two-way PERMANOVA, $F = 0.860$, $P = 0.117$), in relation to mislabeling rates (two-way PERMANOVA, $F = 1.396$, $P = 0.065$). The interaction between the factors suggests no effect to the prices (two-way PERMANOVA, $F = -16.836$, $P = 0.975$). Significant differences were found in prices of products sold by different retailers (two-way PERMANOVA, $F = 2.159$, $P = 0.036$), but differences between the price and mislabeling rates are not significant (two-way PERMANOVA, $F = 2.146$, $P = 0.062$). The nonsignificant interaction (two-way PERMANOVA, $F = -16.09$, $P = 0.998$) between the two factors suggests no effect to the prices as well (Supplementary Table 4).

4. Discussion

4.1. Sampling

This investigation represents the first large scale assessment of cephalopod mislabeling in Greece. Previous seafood studies were mainly focused on fish products (Garcia-Vazquez et al., 2011; Giagkazoglou et al., 2022; Giovos et al., 2020; Minoudi et al., 2020; Pardo et al., 2018; Pazartzi et al., 2019; Stamatis et al., 2015; Triantafyllidis et al., 2010). For example, Pardo et al. (2018) included 11 seafood samples from Greece and Cyprus, however the number of cephalopods was not specified. Here, various steps were followed to ensure that this study is representative of the country's market and to warrant the independence of the analyses. Firstly, sampling was carried out in different time periods (between September 2021 and March 2023), and different sampling locations in northern Greece were selected to avoid the collection of the same products. Additionally, with the objective of covering the highest range of products possible, different market presentations were targeted, such as fresh, frozen, canned, and cooked (obtained from restaurants).

4.2. DNA barcoding and database misnomers

DNA barcodes were successfully generated and subsequently identified 135 out of 146 samples at the species level, supporting the efficiency of the two selected mitochondrial genes (COI and 16S rRNA). The use of the 16S rRNA gene was necessary for the species-level identification of the samples belonging to the Sepiidae family, where the database analysis of the COI gene provided more than one equivalent top matches. Species level identification can be proven difficult among cephalopod products (Gleadall et al., 2024; Guardone et al., 2017). A growing number of researchers has acknowledged the misidentification issues that online databases, such as Bold and GenBank, face (Cheng et al., 2023). Errors, misnomers, and overall inconsistencies are common in both GenBank and BOLD databases (Cheng et al., 2023; Wannell et al., 2020). These errors are not an isolated issue, as discrepancies have been recorded for a variety of families (Cheng et al., 2023; Pazartzi et al., 2019). There is a great need for improvement of the existing databases, to increase taxonomic resolution and accuracy. Updating of the lodged sequences would aid in more reliable species identification (Wannell et al., 2020). The human factor has a major role in these cases, as errors can occur due to data or sample confusion, contamination, or morphological misidentification of specimens (Cheng et al., 2023). For intact specimens, morphological identification should be performed prior to DNA barcoding, and serve as corroborating evidence. However, this option is not always available when external characteristics are removed. In those cases, barcoding results cannot be validated and therefore their inclusion in online databases and international repositories should be discouraged.

4.3. Reconsideration of seafood trade official lists

On average, along the past decade, Greece imports approximately 11.860 tons of cephalopod products per year, from countries all over the world (European Market Observatory for Fisheries and Aquaculture Products-EUMOFA) (https://eumofa.eu/Data). All products are

imported without species distinction and under the ''cephalopod'' umbrella term (https://eumofa.eu/Data). Our results revealed the sale of 19 species sold under a variety of commercial names. This number corresponds to 38.77% of the 49 species with legal designations in the country (Official Government Gazette 343/Issue B'/31-1-2021, No. 1750/32219). Indeed, Mediterranean countries overall include more species in their lists than other countries of Europe (Gleadall et al., 2024). However, among the 135 samples identified at a species level in this study, we discovered one incident where one species (*S. vecchioni*) was not included in the Official Government Gazette.

Similar cases, perhaps in different locations/cities other than the ones targeted in this study, is strongly suspected. Such issues have been recorded in lists published by other countries of the EU, as well (e.g., Bulgaria) (Tinacci et al., 2022). Furthermore, unaccepted scientific names such as *Octopus dollfusi* (currently identified as *Amphioctopus aegina*) still exist (Gleadall et al., 2024). The continuous monitoring of the cephalopod trade in Greece and the overall modernization of the Official Government Gazette are strongly advised, focusing on the inclusion of new species and the update of the existing common names. With the goal of ensuring a fair and transparent market, the reconsideration of these lists and their modernization should follow the approach of one species-one name, as it is advocated by Tinacci et al. (2019).

4.4. Mislabeling incidents

During the last decade, seafood trade and mislabeling rates are investigated in the Greek markets (see references in 4.1). The first large scale investigation reported relatively low rates (12.9%) of mislabeling (Minoudi et al., 2020). A similar trend (13.5%) was reported in rays and skates (Giagkazoglou et al., 2022). Conversely, higher rates (56%) were discovered on elasmobranch meat products (Pazartzi et al., 2019). The reported mislabeling rates, however, remain higher than the average global substitution rate (8%) (Luque & Donlan, 2019) and the European rate (6%) (EU, 2015). Various factors are responsible for the variance in mislabeling rates among the different studies in Greece (Minoudi et al., 2020) and they are mostly affected by the legislation and the marketed species. Therefore, whether mislabeling is considered common in the country's seafood market is not yet clear, and the investigation is still ongoing (Giagkazoglou et al., 2022; Pazartzi et al., 2019).

In the south of Europe, and Greece in particular, cephalopods are highly appreciated and considered a delicacy by consumers. However, available research on this seafood product remains scarce (Pardo & Jimenez, 2020). A higher percentage of mislabeled cephalopod commodities (43.8%) was recorded in Italy, when compared against other seafood categories such as fish (14%) and crustaceans (17%) (Guardone et al., 2017). The overall mislabeling rates in Spain, Iceland, Finland, and Germany were approximately 50%, while mislabeling rates for cephalopods reached 60% (Pardo et al., 2018). DNA barcoding analysis of seafood products sold in Spain recorded similar rates of mislabeling (58%) (Pardo & Jimenez, 2020). A more recent investigation of the Italian market recorded high levels of mislabeling but significantly lower (33%) than the rates reported by Pardo et al. (2018; 2020) (Maggioni et al., 2020). Finally, a recent study concluded that 42% of the *L. vulgaris* products, 26.1% of the *S. officinalis* products and 7.3% of the *O. vulgaris* products were mislabeled in Italy (Giusti et al., 2023). Our results on cephalopod products revealed similar mislabeling rates (40, 41%), a rate similar to the aforementioned studies, but well above the average global and European rates.

In the current study, 59 mislabeling incidents detected by molecular tools were discovered, with at least 21 caused by the lack of detailed information on the label. For example, samples identified as *Illex argentinus*, were labeled as "thrapsalo" instead of "thrapsalo Argentinis". A small number of substitution cases (seven) of a low value product (thrapsalo) with a high value product (kalamari/chtapodi) were identified amongst our frozen and canned samples. Incidents as such could be explained by negligence or mishandling during processing and/or packaging, however, they could be attributed to intentional fraudulent practices. Fourteen cases of substitution were recorded between "kalamari" and "thrapsalo" products, suggesting a possible economic motive behind the mislabeling and therefore, intentional fraud is strongly suspected. Almost all mislabeling cases recorded in restaurants fall under this category, as restaurants in Greece are not obligated to provide the legal designations, rather than the generic category that the product belongs to (Minoudi et al., 2020).

Similarly, products identified as *D. gigas* were marketed as "chtapodi", the legal designation of *O. vulgaris*; this is a clear example of intentional fraudulent substitution. For some cases, the label only mentioned the generic word "tentacle" and after a verbal inquiry the retailer identified the product as octopus, while for others, the common name "chtapodi" was included in the label. In the Greek market, *D. gigas* products are usually heavily processed, and are often in pieces, in frozen packages or in cans. When sold fresh, tentacles are marketed with their suction cups removed. The removal of external morphological characteristics along with the low consumer awareness renders such substitution possible. Similar incidents have been reported by previous studies, where *D. gigas* and *Eledone cirrhosa* were considered the main substitute species of *O. vulgaris* (Espiñeira & Vieites, 2012). Surprisingly, no significant increase in price was recorded between the fraudulent and the correctly labeled *D. gigas* products. This may simply reflect the need of the retailer to sell the product, rather than the increased economic gain.

4.5. Differences among location, retailers, and label types

Our analysis identified significant differences among labels, although significant similarities were identified between ''Kalamari'' and ''Thrapsalo'', particularly as most of the mislabeled samples fall in these two categories. The results are not a surprise as both categories describe different species of squids, with the first one having a higher market value in Greece. Similarities in the use of umbrella labeling terms by various retailers could be attributed to the supply chain of cephalopods (imported and non). Most of the identified species belong to species with Atlantic, Pacific, and Indian geographical distributions. Our results identified significant differences in prices for products sold by different retailers, in relation to the labeling practices. Those results were expected, as traditionally in Greece, open markets are offering lower prices for the same products to consumers than supermarkets and fishmongers. The non-significant interaction between price and mislabeling rates across the different retailers, suggests that economic gain and mislabeling may not have a causative relationship.

5. Conclusions

Our results clearly demonstrate how vulnerable consumers are to fraudulent practices of the seafood market. Efforts for the implementation of the existing legal framework are an important first step, and regular official controls could pressure retailers into more transparent labeling practices. In light of the increasing seafood consumption and the great amounts of imported goods, reliable, effective and meaningful traceability is paramount. Despite the existence of an extensive list of species-specific designations for cephalopods, improvements can still be made. The curation of the published lists by each country member is needed, while the homogenization, when possible, of trade names used among the different EU countries could be an important step towards a more transparent seafood trade. Specifically in Greece, restaurants are not obliged to declare any species-specific legal designations on their menus; therefore, this part of the food chain could be more susceptible to substitution. Including restaurants under the current framework could be beneficial. Additionally, public awareness efforts could prove invaluable, enhance consumer awareness of the legal framework around legal designations and establish the importance of a transparent food market. Future controls should include more seafood categories that until now have never been evaluated.

CRediT authorship contribution statement

Zoi Giagkazoglou: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dimitrios Loukovitis:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis. **Chrysoula Gubili:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis. **Dimitrios Chatziplis:** Writing – review & editing, Writing – original draft, Resources, Investigation, Formal analysis. **Avraam Symeonidis:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Anastasia Imsiridou:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All information is provided in the supplementary material

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2024.110523.

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