



Labelling assessment and molecular authentication of squid-based products from two major European cities

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ABSTRACT

This study provides the first assessment of labelling compliance and species authenticity of squid-based products sold in two major EU cities, Brussels and Milan. A total of 198 products were collected from supermarkets, fishmongers and the food service sector. They were analysed through label evaluation and molecular authentication using DNA barcoding (mono-species samples) and DNA metabarcoding (multi-species samples). Mislabelling was detected in 13.3% of products in Brussels and 30.2% in Milan. Substitution patterns frequently involved the replacement of high-value Mediterranean or Northeast Atlantic species with lower-priced imported taxa. When mislabelling rates were recalculated by restricting the analysis to products allowing species-level verification, comparable mislabelling frequencies were observed between the two cities (27.3% for Brussels and 26.5% for Milan). This indicates that differences between cities are largely driven by national seafood nomenclature systems, with the widespread use of umbrella commercial designations in Belgium hindering species-level verification. Statistical analyses indicated no significant association between mislabelling frequency and product category or retailer type. Overall, these results highlight transparency gaps in the squid markets analysed and support the need for harmonised labelling requirements, including mandatory species information for processed products and the food service sector.

1. Introduction

Ensuring accurate labelling of seafood products is a central objective of food control policy, underpinning consumer protection, market transparency, and traceability along the supply chain. Mislabelling (or misdescription), defined as the use of explicitly false claims or distorted information on labels or packaging (Winkler et al., 2023), is considered one of the most concerning forms of food fraud in the EU market (Giusti et al., 2023a; Kroetz et al., 2020; Reilly, 2018). A recent overview by the European Commission on food fraud notifications within the Alert and Cooperation Network (ACN) included mislabelling as a key issue affecting the fishery sector, alongside microbiological contamination and the presence of heavy metals (European Commission, 2024). Seafood mislabelling, which sometimes involves the intentional substitution of high-value species with less expensive ones for economic gain,

can also concern the falsification of geographical origin, with possible impacts on fisheries sustainability (Cusa et al., 2025). These labelling discrepancies not only compromise consumer trust but also hinder traceability and regulatory enforcement (Naaum et al., 2016). Furthermore, mislabelling may result from the trade of products derived from illegal, unreported, and unregulated (IUU) fishing, with negative consequences for the conservation and management of declining populations (Helyar et al., 2014). Beyond economic and ecological concerns, mislabelling also poses potential risks to human health, particularly when species at higher risk of containing toxins, allergenic species, or products containing elevated levels of heavy metals are incorrectly identified and enter the consumer market (Di Maggio et al., 2025; Giusti et al., 2026; Naaum et al., 2016; Ryburn et al., 2022).

In the European Union (EU), labelling requirements for seafood are established by Regulation (EU) No 1379/2013, hereinafter referred to as

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the Common Market Organisation (CMO) Regulation for fishery and aquaculture products (CMO Regulation, 2013). This Regulation mandates that consumers be provided with the product's commercial designation and scientific name, its geographical origin (catch area for wild-caught products or country of production for aquaculture products), the production method (e.g. caught or farmed), and, in the case of wild-caught products, the fishing gear used. Each Member State must establish, publish, and regularly update a list of the commercial designations authorised in its territory for the sale of fishery and aquaculture products. The CMO Regulation applies to seafood products such as fresh and frozen unprocessed or filleted items, as well as salted, dried, and smoked seafood. By contrast, it does not extend to processed products such as canned, prepared, preserved, or semi-preserved items, which only fall under the broader Regulation (EU) No 1169/2011 on food information to consumers. Food business operators are nonetheless encouraged to provide consumers with the voluntary information foreseen by the CMO Regulation even for products that do not fall within its scope, in order to enhance market transparency (D'Amico et al., 2016). Similarly, neither the CMO Regulation nor any other EU-wide law obliges restaurants or other catering establishments to display detailed information about seafood products, unlike the mandatory labelling required for products sold by retailers.

Cephalopod-based products (Class Cephalopoda), including octopus, squid, and cuttlefish, are especially prone to mislabelling. This reflects a combination of factors, including their high species diversity, morphological plasticity, the lack of widely standardised identification protocols, and their frequent commercialisation in non-whole or processed forms (Gleadall et al., 2024; Nguyen et al., 2025).

In Northern Europe, value-added items in which raw cephalopods are enhanced through additional processing such as breeding or seasoning are common. In Southern Europe, retail counters are dominated by fresh cephalopod products, which are often obtained by defrosting frozen imports (CBI, 2022). In addition to single-species products, the EU seafood market increasingly includes multi-species products, particularly in the form of processed or composite preparations (e.g. surimi-based products, seafood balls, rolls, stuffed products, or mixed seafood preparations). These products contain mixtures of different seafood species and, in some cases, non-seafood ingredients such as fish from other taxonomic groups or terrestrial animal products used as fillers or flavouring components.

Distribution occurs mainly through supermarkets, wholesalers, and food service operators, including restaurants and canteens (CBI, 2022).

To date, few studies have specifically investigated mislabelling in cephalopod products sold in the EU market. However, those studies available have reported variable – but often substantial – mislabelling rates. For instance, Fernández et al. (2020) documented frequent substitution of high-value European squid species with imported ones in Spain and Italy, with a mislabelling rate of 31% in processed products sold in supermarkets. In Greece, Giagkazoglou et al. (2024) found an overall mislabelling rate of 40.4%, for octopus, squid, and cuttlefish products sold by fishmongers, open markets, supermarkets, and restaurants. More recently, a study conducted in France revealed that 35% of cephalopod dishes were mislabelled, containing jumbo flying squid instead of octopus (Günther et al., 2024). Despite few cephalopod-specific studies, mislabelling rates for cephalopod products have nonetheless been reported in broader seafood authentication surveys in EU countries (Grbin et al., 2025; Guardone et al., 2017; Pardo et al., 2018; Pardo & Jiménez, 2020; Tinacci et al., 2023). Collectively, these studies relied on genetic tools to verify the authenticity of cephalopod products.

DNA-based methods are currently the most widely applied approaches for assessing the species composition of seafood products, and their use is also promoted at the regulatory level (CMO Regulation, 2013). Among these, DNA barcoding, which relies on sequencing short, standardised gene regions, has been employed extensively for the authentication of fish and seafood mono-species samples (i.e. products

which only contain a single individual from a single species) (Fernandes et al., 2021; Giusti et al., 2023a; Mitchell & Hellberg, 2016; Nehal et al., 2021). Nevertheless, DNA barcoding shows important limitations when applied to multispecies samples, which can contain many different species, as conventional Sanger sequencing typically recovers only the dominant sequence, thereby overlooking minor constituents. To address this issue, Next Generation Sequencing (NGS) technologies, high-throughput platforms capable of generating millions of reads simultaneously, have become the most suitable tools for authenticating complex food matrices (Giusti et al., 2024). When DNA barcoding markers are analysed through NGS platforms, the approach is referred to as metabarcoding or amplicon sequencing (Fernandes et al., 2021; Taberlet et al., 2012). This combined approach enables the simultaneous identification of multiple species within a single sample, providing a more comprehensive overview of species composition and representing a powerful tool in seafood authenticity testing, particularly for detecting hidden or undeclared ingredients in processed products (Lorusso et al., 2024).

The present study aimed to assess the labelling compliance and authenticity of squid-based products collected from different points of sale in two major European cities, Brussels (Belgium) and Milan (Italy). Squid-based products were selected given their relative popularity and their supply chains, which involve species from different ocean basins. The sampling encompassed products obtained from supermarkets, fishmongers, restaurants, and canteens and included fresh, frozen, and processed products (i.e. prepared and preserved). The compliance of the product labelling was assessed in the light of the CMO Regulation. Mono-species samples were then subjected to DNA barcoding, and multi-species samples to DNA metabarcoding, to assess their authenticity and evaluate potential cases of mislabelling.

2. Material and methods

2.1. Sampling

The collection period spanned February to May 2025. Due to the non-probabilistic nature of the sampling, which was driven by product availability at the time of purchase, no formal calculation of sample size was undertaken. A total of 198 squid-based products were purchased in Brussels (n = 98) and Milan (n = 100). Of these products, 94 (47.5%) were collected from supermarkets, 66 (33.3%) from the food service sector (restaurants), and 38 (19.2%) from fishmongers, including fishmongers in weekly street markets, distributed across the two cities. Based on their market presentation and the apparent degree of transformation at the point of sale, these products were classified as processed (n = 138; 70.2%), fresh (n = 46; 22.7%), or frozen (n = 14; 7.1%) (details of the samples are reported in Supplementary Material 1). This categorisation does not necessarily correspond to the Combined Nomenclature (CN) classification under the EU customs code, which implements the Harmonised System for tariff and trade purposes (CN Regulation, 2024). CN classification depends on the precise processing steps and composition of each product and therefore requires a case-by-case assessment. The analytical categories used in this study (“fresh”, “frozen”, “processed”) reflect only how products are presented at retail. Additionally, 150 samples (75.8%) were mono-species and 48 (24.2%) were multi-species. The mono-species samples included those containing a single squid species or more than one species (squid and/or other) in which the squid component was clearly visible. Multi-species samples consisted of highly processed mixtures where such a distinction was not possible (e.g., seafood balls, rolls, or surimi). First, the declared squid species, reported as the scientific name and/or commercial designation, was recorded based on the information provided on product labels, in-store displays, or restaurant menus. Then, from each product, an aliquot of tissue (at least 100 mg) was collected, placed into Eppendorf tubes containing 70% ethanol, and subsequently submitted to the FishLab, Department of Veterinary Sciences, University of Pisa, for

analysis. For frozen products, samples were thawed prior to tissue collection, while fresh and processed products were sampled directly. In multi-species samples, two subsamples were taken from different portions of the product and combined into a single composite sample before preservation in ethanol.

2.2. Label analysis

The compliance of labels with the CMO Regulation was only evaluated for fresh ($n = 46$) and frozen ($n = 14$) squid-based products, as processed products do not fall within the scope of the Regulation. Specifically, the labels or, in the case of non-prepacked products, the information displayed at the point of purchase, were checked for the presence of the scientific name and associated commercial designations and origin. This label analysis was also performed, on the same products, by Cusa & Vulperhorst (2025). However, in the present study, the label compliance was also evaluated by checking the compliance of commercial designations against the official lists of commercial designations accepted in Italy and Belgium (European Commission, n.d.). As regards processed products ($n = 138$), which include prepared and preserved products purchased in supermarkets and restaurants, the voluntary declaration of commercial designation and/or the official scientific name was also verified.

2.3. Molecular authentication: total DNA extraction and evaluation

For the molecular authentication, mono-species samples ($n = 150$) were analysed using DNA barcoding, whereas multi-species samples ($n = 48$) were analysed using metabarcoding. Total DNA was extracted from all the mono-species samples ($n = 150$) using the protocol described in Armani et al. (2012), starting from 50 mg of tissue. For the multi-species samples, total DNA was extracted using the NucleoSpin Food kit (Macherey-Nagel), following the manufacturer's instructions and starting from 50 mg of tissue. Procedural blanks, containing no tissue, were included as negative controls in the analysis for metabarcoding. Total DNA concentration was assessed using a NanoDrop One Microvolume UVVis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) DNA purity was evaluated using the A260/A280 and A260/A230 absorbance ratios, which were interpreted as indices of acceptable purity. Extracts were considered suitable for downstream analyses when the A260/A280 ratio and A260/A230 fell within 1.80–2.00/2.20 following established criteria (Boesenberg Smith et al., 2012; Nakayama et al., 2016).

Spectrophotometric measurements ensured the suitability of each DNA extract for subsequent amplification and sequencing. Statistical analyses pertaining to DNA yield and purity are outlined in Section 2.7.

2.4. DNA barcoding analysis of mono-species squid-based samples

2.4.1. DNA amplification and sequencing

A ~655 bp fragment of the Cytochrome Oxidase I (*COI*) gene was selected as the primary DNA barcoding target and amplified using the universal primer pair LCO1490 (5'-GGTCAACAAATCATAAAGA-TATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') designed by Folmer et al. (1994). When *COI* amplification proved unsuccessful, an alternative target was utilised, namely a 190–220 bp region of the 16S rRNA gene (*16S*), which was amplified using the primer pair 16S f_var (5'-AGTTACCATAGGGATAACAGCGTAATTTG-3') and 16S r_var (5'-GACGAGAAGACCCTAATGAGCTTT-3') designed by Chapela et al. (2002). Details of the PCR protocols and cycling programs are presented in Supplementary Material 2. PCR products (5 μ l) were checked on 2% agarose gels (GellyPhorLE, Euroclone, Milan, Italy) stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Hayward, CA, USA). Fragment size was verified under a UV transilluminator by comparison with the SharpMass™ 50-DNA molecular marker (Euroclone, Milan, Italy). PCR products were sent to an external sequencing provider

for analysis.

2.4.2. Sequence editing and taxonomic assignment

The resulting sequences were processed in BioEdit v.7.7 and aligned using the ClustalW algorithm embedded in the alignment editor (Hall, 1999). Taxonomic assignment of the resulting barcodes was conducted by querying GenBank reference sequences via the Megablast algorithm implemented in the Basic Local Alignment Search Tool (BLAST) interface (Altschul et al., 1990). For *COI* sequences, potential species identification was assigned to matches with 100% query coverage and $\geq 98\%$ identity (Hebert et al., 2003). In the case of *16S* sequences, species-level identification was assigned to matches with 100% query coverage and 99–100% identity (Tinacci et al., 2018).

2.5. DNA metabarcoding analysis of multi-species squid-based samples

2.5.1. Library preparation and sequencing

The primer pair 16S f_var and 16S r_var designed by Chapela et al. (2002) (Section 2.4.1) was added to Illumina adapter sequences 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG- [forward primer] and 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG- [reverse primer] and used to amplify the *16S* target region from all the DNA samples obtained from the 48 multi-species samples. The amplification was performed in duplicate, for a total of 96 samples. Procedural blanks were also amplified. Details of the PCR protocol and cycling program are provided in Supplementary Material 2. The amplification of fragments of the expected length was assessed as described in Section 2.4.1. DNA libraries were prepared according to the Illumina® 16S Metagenomic Sequencing Library Preparation protocol (Illumina, 2013). For library purification steps, the NucleoMag® NGS Clean-up and Size Select kit (Macherey-Nagel GmbH & Co. KG, Germany) was used. The libraries were pooled, and the pool was denatured and diluted until 8 pM before MiSeq loading. The pool was loaded using MiSeq Reagent Kit v2 (300-cycles), and the run included a 20% PhiX Control Kit v3 (Illumina) to serve as an internal control.

2.5.2. Bioinformatic analysis and taxonomic assignment

Folders containing fastq files with raw reads obtained after Illumina sequencing were processed to generate amplicon sequence variants (ASVs) using the DADA2 R package (Callahan et al., 2016). Representative sequences for each ASV were taxonomically assigned using BLAST (Altschul et al., 1990) against the GenBank nucleotide database (Benson et al., 2013), and species-level identification was performed as described in Section 2.4.2. Taxonomic assignment data were aggregated by summing read counts from technical duplicates. For each sample, taxa representing less than 1% of the total sequences were considered to be either potential contaminants introduced during product manufacturing or possible cross-contamination during analysis and were therefore excluded (1% filtering threshold) for the final authentication of products.

2.6. Comparison of molecular results with label information

The results of molecular authentication were compared with the information provided on the labels of the products (Section 2.2.) to identify potential mismatches. This comparison was carried out according to the following criteria: (i) for products where the scientific name of the species was reported (either mandatorily or voluntarily), it was directly compared with the species identified through molecular analysis; (ii) when only the commercial designation was reported, and this was included in the official lists of commercial designations accepted in Italy or Belgium (depending on the sampling location), the molecularly identified species was compared with the species (or list of species) associated with that designation.

2.7. Statistical analyses

For DNA barcoding data, the Shapiro-Wilk and Levene's tests were used to assess data normality and homogeneity of variances, respectively. As the assumptions for parametric tests were not met, the non-parametric Kruskal–Wallis test was applied to compare DNA yield and purity among fresh, frozen, and processed products. For DNA metabarcoding data, diversity indices including Shannon (H') and Simpson (1–D) were calculated from aggregated read counts using the *vegan* R package (version 2.7-1). To explore potential associations between product category or retail channel and the frequency of mismatches, chi-square tests (χ^2) or Fisher's exact test were performed according to the category sample sizes. The analysis considered the presence/absence of mismatching as a categorical variable and compared its distribution across collection sites (i.e. fishmongers, supermarkets, and the food service sector), product categories (i.e. fresh, frozen, and processed). In all cases, a p-value <0.05 was considered statistically significant. Statistical analyses were performed using R software (version 4.4.0).

3. Results and discussion

3.1. Sampling

In Brussels, a total of 98 products were sampled from 59 retail locations (12 supermarkets, 16 fishmongers, and 31 restaurants). Most of the products originated from supermarkets (n = 43; 43.9%) and the food service sector (n = 34; 34.7%), followed by fishmongers (n = 21; 21.4%). Most supermarket samples were processed products (n = 34; 79.1% of supermarket samples), with a smaller share of frozen (n = 5; 11.6% of supermarket samples) and fresh (n = 4; 9.3% of supermarket samples) products. All of the products from the food service sector were processed (i.e. cooked and/or battered) (n = 34; 100%), while those from fishmongers were almost entirely fresh (n = 20; 95.2%) (Fig. 1). In Milan, a total of 100 products were obtained from 56 retail locations (14 supermarkets, 13 fishmongers, and 29 restaurants). These products were mainly purchased from supermarkets (n = 51; 51.0%) and the food service sector (n = 32; 32.0%), followed by fishmongers (n = 17; 17.0%). Supermarket products consisted predominantly of processed products (n = 38; 74.51% of supermarket samples), along with frozen (n = 8; 15.7% of supermarket samples) and fresh (n = 5; 9.8% of supermarket samples) products. Similar to Brussels samples, all of the food service sector products were processed (n = 32; 100%). Products from fishmongers were exclusively fresh (n = 17; 100%) (Fig. 1).

In both Brussels and Milan, the average number of samples collected

per site varied by retailer type. Multiple samples were often obtained from an individual supermarket (approximately 3–4 products per site on average), reflecting the wider assortment and availability of squid-based products in large retail chains. This pattern also results from the fact that a single supermarket can commercialise multiple brands, allowing the collection of several distinct products within the same outlet. In contrast, only one sample per site was generally obtained from restaurants, and one or two samples per site from fishmongers, as each establishment was considered a single “brand,” being directly responsible for the labelling of their products and likely exhibiting consistent labelling behaviour across all items sold.

Also, in both cities, supermarkets were dominated by processed products, potentially reflecting the reliance of large-scale retail on seafood items with longer shelf-lives and standardised formats, and the preference of supermarket consumers for pre-prepared or pre-cooked products. As expected, the food service sector exclusively offered processed products in the form of prepared dishes. By contrast, fishmongers were almost entirely associated with fresh products, including fresh squid rings, confirming their traditional role in seafood distribution. It is essential to underscore that the results offer an exploratory snapshot of the products available for purchase during the defined study period, rather than a comprehensive assessment of the squid retail market in Brussels and Milan. Nonetheless, the findings align with broader trends reported in a recent Eurobarometer survey on EU consumer habits and purchasing preferences for fishery and aquaculture products (European Commission, 2025). Specifically, the 2025 Eurobarometer survey indicates that in Belgium, frozen and processed products are preferred over fresh alternatives, largely due to convenience and price sensitivity. Belgian consumers prioritise cost and shelf-life, with a notable decline in weekly consumption of fresh products compared to previous years. In contrast, Italian consumers maintain a cultural and culinary preference for fresh products which are typically purchased from local fishmongers or open-air markets, with emphasis on visual freshness, origin, and harvest date. However, frozen products appear increasingly popular due to their convenience and longer shelf life, particularly among younger consumers and urban households, and processed products also hold a significant share of the Italian market (IMARC Group, 2024, pp. 2025–2033).

3.2. Label analysis

Overall, 30.6% of products collected in Brussels (30/98) fell within the scope of CMO Regulation, as they were fresh or frozen. When focusing on available species and geographical catch location

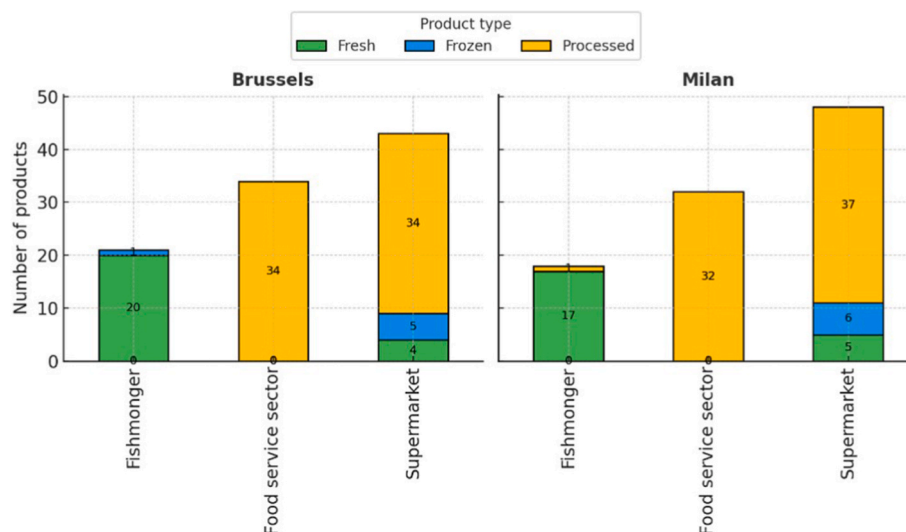


Fig. 1. Distribution of product types (fresh, frozen, processed) according to retailer type in Brussels (left, n = 98) and Milan (right, n = 100).

information, only six out of these 30 products (20.0%) were compliant with the CMO Regulation, while the remaining 24 products (80.0%) did not comply with respect to one or more required details. Specifically, 21 products lacked the mandatory scientific name. In addition, six products used commercial designations that are not included in the Belgian official list of commercial designations (three of these also lacked the scientific name), represented by: (i) “*encornet géant*” (instead associated with *Dosidicus gigas* in the French official list; three products); (ii) “*chipirón*” (associated with five species of *Loligo* spp. in the Spanish official list; one product); (iii) “*encornet argentin*” (similar to “*encornet rouge argentin*” associated with *Illex argentinus* in the French official list; one product); and (iv) “*giant squid*” (in English; one product). The use of commercial designations not included on the Belgian official list but present on the French or Spanish lists may suggest that part of the product was imported or processed elsewhere in the EU, where such terms are legally recognised. This highlights how the commercial name used on the label can indirectly provide insights into the supply chain and processing origin of the product, reflecting the transnational dimension of seafood trade. Moreover, the Belgian official list of commercial designations only includes the umbrella terms *calmar/encornet*, associated with the family-level scientific names Ommastrephidae and Loliginidae. While such general terms ensure a certain degree of flexibility, they also reduce the granularity of labelling and hinder species-specific traceability, especially when compared to other Member States that provide more detailed nomenclature. This situation may complicate consumer information and create inconsistencies in the implementation of CMO Regulation across the EU. Factually, the use of umbrella terms has been identified as a relevant factor that contributes to and potentially enhances the misleading of consumers (Cawthorn et al., 2018; Zhang et al., 2024a).

As regards the products collected in Milan, 30 of 100 (30.0%) fell within the scope of the CMO Regulation, and 13 out of those 30 products (43.3%) were compliant with this regulation. Among the 17 (56.7%) non-compliant product labels, 12 lacked the mandatory scientific name, four did not report any commercial designation (despite providing the scientific name), and one reported a commercial designation not included in the Italian official list of commercial designations (“*Loligo calamari*”). It is worth noting that the Italian official list of commercial designations differs from the Belgian list in that it is much more detailed and does not rely on umbrella terms. As highlighted by Tinacci et al. (2019), one of the main strengths of the Italian list of seafood trade names is its high level of detail, which reduces ambiguity by minimising the use of generic categories. This structural difference with the Belgian list may explain the opposite pattern observed: in Milan, where the official list requires more detailed information, non-compliance was often related to the use of vague or overly generic designations. In contrast, in Brussels, where the list is less detailed and allows umbrella terms, non-compliance was more frequently due to missing information.

As regards the analysis of voluntary information, among the 68 products collected in Brussels that fell outside the scope of CMO Regulation, 16 (23.5%) voluntarily reported the scientific name of the squid species in the ingredient list. However, one of these products was found to be non-compliant, as the commercial designation did not correspond to the declared scientific name. In Milan, 15 out of 70 products (21.4%) that were outside the scope of the CMO Regulation voluntarily reported the scientific name. Among them, six products (40.0%) were non-compliant, providing commercial designations that did not correspond to the declared scientific names. Although more than a decade has passed since the European Commission first recommended the voluntary provision of scientific names for processed seafood products, this information was only reported for roughly 20% of products sampled in both cities and that fell outside the scope of the CMO Regulation. This limited uptake suggests that voluntary guidance alone has not been sufficient to ensure widespread implementation. In light of the persistent mislabelling observed, these findings support the need to extend mandatory labelling requirements to all types of seafood, including

processed products, thereby improving transparency and consumer protection. This need is further underscored by the growing number of advocacy initiatives and institutional campaigns calling for enhanced traceability, clearer origin declarations, and stricter enforcement of labelling standards (Oceana, 2025). These efforts reflect a broader policy momentum within the EU aimed at addressing fraud vulnerabilities and restoring consumer trust in fishery and aquaculture products (European Commission, 2020; European Parliament, 2023).

3.3. Total DNA extraction and evaluation

A suitable total DNA yield was obtained from all of the 198 squid-based products analysed. For the subset of products analysed with DNA barcoding ($n = 150$), the average DNA concentration was 1222.2 ng/ μ l (range: 19–4250 ng/ μ l). Spectrophotometric evaluation confirmed medium-to-high quality (average A260/A280 and A260/A230 ratios of 2.04 and 1.94, respectively). The spectrophotometric assessment of DNA extracts from fresh ($n = 46$), frozen ($n = 14$), and processed ($n = 90$) products used for DNA barcoding did not reveal significant differences in DNA yield or purity ratios among the three categories. Mean DNA concentrations were comparable across groups, with 1138.8 ± 700 ng/ μ l for fresh products, 1437.1 ± 680 ng/ μ l for frozen products, and 1230.4 ± 924 ng/ μ l for processed products. DNA concentrations, 260/280 and 260/230 ratio absorbance ratio were not normally distributed ($p < 0.01$; Shapiro-Wilk Test) and Levene's test revealed no homogeneity of variances ($p < 0.05$) among the three categories of products. Product categories showed no statistically significant differences in DNA concentration ($p = 0.372$, $X^2 = 1.98$, $df = 2$; Kruskal–Wallis test). Similarly, the 260/280 absorbance ratio was highly consistent across categories ($2.03\text{--}2.04 \pm 0.031\text{--}0.076$) with no statistically significant differences ($p = 0.78$, $X^2 = 0.51$, $df = 2$; Kruskal–Wallis test), indicating an overall absence of protein contamination. The 260/230 ratio, instead, showed a trend towards lower values in processed samples (1.88 ± 0.432) compared to fresh (2.01 ± 0.338) and frozen (2.09 ± 0.282), although this difference did not reach statistical significance ($p = 0.094$, $X^2 = 4.73$, $df = 2$; Kruskal–Wallis test). This suggests that, despite comparable yields and 260/280 ratios, DNA extracted from processed products may be more prone to co-extraction of residual contaminants such as salts or polysaccharides, reflecting the higher level of matrix complexity and industrial treatments these samples undergo. In the case of products analysed with metabarcoding ($n = 48$) a lower average DNA concentration was observed (370.5 ng/ μ l; range 11–2091 ng/ μ l), with average ratios A260/A280 and A260/A230 of 2.01 and 2.03, respectively (Supplementary Material 3). The lower DNA concentration observed in this case is most likely attributable to the use of a commercial extraction kit. Indeed, DNA isolation with commercial kits employing silica-based adsorption columns have been observed to give lower yields of DNA (Kopecká et al., 2014).

3.4. DNA barcoding analysis of mono-species squid-based samples

3.4.1. DNA amplification and sequencing

Overall, 149 out of 150 DNA samples were successfully amplified. In details, 126 samples (84.6%) were amplified using the primer pair by Folmer et al. (1994). The remaining 23 DNA samples (15.4%) were amplified using the primer pair by Chapela et al. (2002). All 149 PCR products were successfully sequenced.

3.4.2. Taxonomic assignment

Detailed results of the taxonomic assignment of sequences are provided in Supplementary Material 4. Species-level identification was reached in 139 out of 149 products (93.3%). In the remaining ten products (6.7%), the identity scores obtained only supported assignment at the genus level. The most prevalent species found were *Dosidicus gigas* ($n = 33$; 22.1%), *Doryteuthis gahi* ($n = 32$; 21.5%), *Uroteuthis duvaucelii* ($n = 21$; 14.1%), *Illex argentinus* ($n = 19$; 12.8%), and *Loligo vulgaris* ($n =$

15; 10.1%) (Fig. 2). The species with the lowest prevalence were *Decorisepia madokai*, *Loligo reynaudii*, *Loliolus japonica*, *Octopus vulgaris*, and *Sepia officinalis*, detected in one product each (0.7%) (Fig. 2). The genus-level identification concerned five products identified as *Illex* sp. and six as *Mytilus* sp. In the latter case, the identification of a genus belonging to the class Bivalvia could be due to sampling errors, arising from the difficulty of recognising specific squid tissue in prepared and mixed samples containing more than one species. For *Illex* sp., the molecular target used (COI) did not allow discrimination between *I. coindetii* and *I. illecebrosus* (Supplementary Material 4); therefore, authentication was retained at the genus level.

3.5. DNA metabarcoding analysis of multi-species squid-based products

3.5.1. Library preparation and sequencing

All 96 samples were successfully amplified using the selected primer pair. No amplification was observed in the procedural blanks, which were therefore excluded from subsequent analytical steps. The libraries presented an average size of 377 bp (range 365–390 bp), which resulted from the target length (190–220 bp) added to illumina adapter sequences and indexes.

3.5.2. Bioinformatic analysis and taxonomic assignment

Results of the bioinformatic analysis are reported in Supplementary Material (Supplementary Material 5). An average of 3159.73 sequences (DS±19303.42) was initially obtained from the samples (input). After the bioinformatic filtration based on sequences quality, an average of 24684.55 sequences (DS±17551.95) was retrieved, with an average retention percentage of 76.1%. A low number of sequences was obtained only for one duplicate sample (87-B; input: 137; retained: 90). However, the other duplicate provided a good number of retrieved sequences (input: 16293; retained: 9718) to be used to properly authenticate that product. After the taxonomic assignment, an average percentage of 88.32 sequences was properly assigned to a species using the established identity value and query coverage. In four samples (59B-a; 59B-b; 78B-a; 78B-b), no sequences with significant similarity to the reference database were found, so that these samples were not assigned to any species. In the other six samples, the percentage of assigned sequences was low (112B-a; 112B-b; 15M-a; 15M-b) or medium-low (13M-a; 13M-b) (Supplementary Material 5). Results of taxonomic assignments (92 squid-based samples, excluding 59B-a; 59B-b; 78B-a; 78B-b) are detailed in Supplementary Material 6a (results of each technical duplicate) and 6b (results of data aggregated). The analysis of diversity indices calculated on the aggregated metabarcoding data revealed an overall low

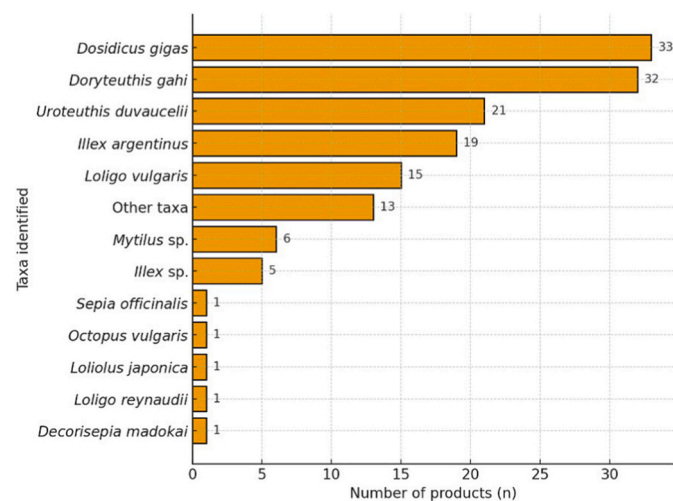


Fig. 2. Taxonomic assignment of mono-species samples analysed using DNA barcoding.

taxonomic heterogeneity across the samples. The Shannon index accounts for both species richness and evenness, increasing with higher numbers of taxa and a more even distribution of sequences among them, whereas the Simpson index reflects the probability that two randomly selected sequences belong to different taxa. Values of both indices close to zero indicate low diversity and strong dominance of a single taxon within the sample (Magurran, 2021). Shannon index values were close to zero for most samples, indicating the predominance of a single species within the molecular communities. Similarly, Simpson index values were very low, confirming high dominance and very limited evenness among detected taxa (Supplementary Material 7). A slight diversification was only observed in a subset of samples, although even in these cases Shannon and Simpson values remained low, reflecting a taxonomic composition still largely dominated by one or few taxa. By applying 1% data filtering, which was fixed according to the literature (Giusti et al., 2024), it was observed that the taxonomic composition of products was largely dominated by *D. gigas*, which was consistently the most abundant species across samples, often exceeding 80% and in several cases approaching 100% of the sequences. In addition, other cephalopod species, such as *Sthenoteuthis oualaniensis*, *Ommastrephes bartramii*/*O. brevimanus*, *Uroteuthis chinensis*, and *O. vulgaris* were also detected at high relative abundances in specific samples (Supplementary Material 6). These results highlight the strong dominance of a few cephalopod species in the analysed products. Comparison with the DNA barcoding of mono-species samples (Fig. 2) highlighted an overlap with respect to the dominant presence of *D. gigas*. Nevertheless, some species found using DNA barcoding, such as *D. gahi* and *I. argentinus*, were not detected at relevant levels by metabarcoding. This discrepancy may be explained by the different typologies of products analysed. DNA barcoding was in fact applied also to fresh and frozen mono-species samples; in this respect, *D. gahi* (Patagonian squid), a species of major commercial importance for South American fisheries (particularly in the Falkland Islands and Argentina) (Arkhipkin et al., 2015) is mainly exported as whole frozen products or as “tube and tentacles” presentations (CBI, 2022), and it is not commonly used in multi-ingredient or highly processed seafood products marketed in the EU. Similarly, *I. argentinus* (Argentine shortfin squid), the most heavily caught squid species in South America, is mostly commercialised as frozen blocks or whole products, and only rarely used in mixed or processed preparations (CBI, 2022).

The occurrence of sequences assigned to other animal species was also observed. In several samples, sequences assigned to pork (*Sus scrofa*) were detected at non-negligible proportions (Supplementary Material 6). Notably, the proportion of pork DNA exceeded that of cephalopods in seven products, where *S. scrofa* accounted for the majority of sequences (ranging from ~66% to >98%), while cephalopod sequences were either minor or nearly absent. Beyond pork DNA, a subset of samples also contained sequences from other fish species such as *Gadus chalcogrammus*, *Dicentrarchus labrax*, and *Merluccius paradoxus* (Supplementary Material 4). A wide range of fish species was observed, in particular in certain processed products such as squid surimi, balls, and stuffed squid ravioli. In these types of processed products, it is not uncommon to detect the presence of ingredients other than squid, such as fish or pork (Giusti et al., 2025; Ho et al., 2020; Zhang et al., 2024b; Zhang et al., 2025). Factually, products with higher Shannon and Simpson values corresponded to cases where either pork or multiple fish species were detected in addition to the main cephalopod component.

It should be noted that only the occurrence of species could be determined, rather than their exact proportion in a mixed sample, as metabarcoding read proportions do not always reflect the exact share of ingredients in a sample due to PCR amplification biases and primer affinity differences (Giusti et al., 2023b; Hu et al., 2025). Moreover, the application of a 1% filtering threshold, although consistent with current literature (Giusti et al., 2024), may have excluded rare ingredients present at trace levels.

3.6. Comparison of molecular results with label information

For the products collected in Brussels, molecular results were compared with the information on the labels of 90 out of the 98 products. The remaining eight products were excluded for the following reasons: (i) four products were molecularly identified as non-target seafood (*Mytilus* spp.) (Supplementary Material 4); (ii) two products (seafood pasta sauces) did not provide additional information regarding the scientific name or commercial designation of the cephalopods

contained in the preparation; and (iii) two products (analysed using metabarcoding) yielded an insufficient number of reliable sequences for molecular authentication. In Brussels, mismatches were observed in 12 out of the 90 products included in the analysis (13.3%). Most of these involved fresh products purchased at fishmongers, where many products declared as *Loligo* spp. (“chipirons”) were identified as *D. gahi* or *D. gigas* (Table 1). The substitution of *Loligo* spp., which are typically harvested in the Mediterranean or Northeast Atlantic and regarded as high-value products, with *D. gahi*, a Patagonian squid imported in large volumes

Table 1

Mislabeled squid-based products showing a mismatch between the declared species (commercial designation and/or scientific name) and the species identified using molecular analysis (DNA barcoding and metabarcoding).

Product	Sampling city	Sampling site	Product category	squid ingredient reported on the label	Name of the species according to lists of commercial designations accepted in Italy or Belgium	Species identified molecularly
4B	Brussels	Fishmonger	Fresh	<i>Dosidicus gigas</i>	-	<i>Doryteuthis gahi</i>
5B	Brussels	Fishmonger	Fresh	Chipirons	<i>Loligo</i> spp.	<i>Doryteuthis gahi</i>
23B	Brussels	Fishmonger	Fresh	Chipirons (<i>Loligo</i> spp.)	<i>Loligo</i> spp.	<i>Dosidicus gigas</i>
25B	Brussels	Fishmonger	Fresh	Chipirons (<i>Loligo</i> spp.)	<i>Loligo</i> spp.	<i>Doryteuthis gahi</i>
27B	Brussels	Fishmonger	Fresh	Chipirons (<i>Loligo</i> spp.)	<i>Loligo</i> spp.	<i>Doryteuthis gahi</i>
50B (1)	Brussels	Supermarket	Processed	<i>Uroteuthis duvaucelii</i> or <i>Illex argentinus</i>	-	<i>Acanthosepion pharaonis</i>
52B	Brussels	Supermarket	Fresh	<i>Architeuthis</i> sp.	-	<i>Dosidicus gigas</i>
74B (2)	Brussels	Supermarket	Frozen	<i>Dosidicus gigas</i> or <i>Loligo</i> spp.	-	<i>Illex argentinus</i>
76B	Brussels	Supermarket	Fresh	<i>Dosidicus gigas</i>	-	<i>Octopus vulgaris</i>
118B	Brussels	Fishmonger	Fresh	Chipirons	<i>Loligo</i> spp.	<i>Doryteuthis gahi</i>
115B	Brussels	Supermarket	Processed	<i>Illex argentinus</i>	-	<i>Acanthosepion pharaonis</i>
125B	Brussels	Supermarket	Processed	Calamar	all species in Loliginidae and Ommastrephidae	<i>Acanthosepion pharaonis</i>
2M	Milan	Supermarket	Fresh	Totano	<i>Illex coindetii</i> , <i>Ommastrephes bartramii</i> , <i>Todarodes sagittatus</i> , <i>Todaropsis eblanae</i>	<i>Uroteuthis duvaucelii</i>
4M	Milan	Food service sector	Processed	<i>Dosidicus gigas</i>	-	<i>Doryteuthis gahi</i>
11M	Milan	Supermarket	Processed	Totano	<i>Illex coindetii</i> , <i>Ommastrephes bartramii</i> , <i>Todarodes sagittatus</i> , <i>Todaropsis eblanae</i>	<i>Uroteuthis singhalensis</i>
13M	Milan	Supermarket	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	Several fish species; <i>Sepia recurvirostra</i> , <i>Dosidicus gigas</i>
13M (2)	Milan	Supermarket	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	Several fish species; <i>Sepia recurvirostra</i> , <i>Dosidicus gigas</i>
13M (3)	Milan	Supermarket	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	Several fish species; <i>Sepia recurvirostra</i> , <i>Dosidicus gigas</i>
16M	Milan	Fishmonger	Fresh	<i>Loligo</i> spp.	-	<i>Doryteuthis gahi</i>
22M	Milan	Supermarket	Fresh	<i>Illex coindetii</i>	-	<i>Illex argentinus</i>
26M	Milan	Supermarket	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Dosidicus gigas</i>
28M	Milan	Supermarket	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Dosidicus gigas</i>
29M	Milan	Supermarket	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Sus scrofa</i> , <i>Dosidicus gigas</i>
32M	Milan	Supermarket	Fresh	<i>Uroteuthis edulis</i>	-	<i>Dosidicus gigas</i>
33M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Illex argentinus</i>
34M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Illex argentinus</i>
35M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Dosidicus gigas</i>
35M (2)	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Dosidicus gigas</i>
36M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Sus scrofa</i> , <i>Dosidicus gigas</i>
37M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Illex argentinus</i>
38M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Illex argentinus</i>
39M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Sus scrofa</i> , <i>Dosidicus gigas</i>
42M	Milan	Supermarket	Processed	Totano atlantico	<i>Illex argentinus</i> , <i>Illex illecebrosus</i>	<i>Uroteuthis duvaucelii</i>
47M (2)	Milan	Supermarket	Processed	<i>Uroteuthis chinensis</i>	-	<i>Uroteuthis duvaucelii</i>
50M	Milan	Supermarket	Frozen	<i>Uroteuthis chinensis</i>	-	<i>Uroteuthis duvaucelii</i>
55M	Milan	Supermarket	Processed	<i>Illex coindetii</i>	-	<i>Illex argentinus</i>
55M (2)	Milan	Supermarket	Processed	<i>Illex coindetii</i>	-	<i>Decorsepia madokai</i>
86M	Milan	Supermarket	Frozen	Calamaro del Pacifico	<i>Doryteuthis opalescens</i> , <i>Heterololigo bleekeri</i> , <i>Loliolus beka</i> , <i>Loliolus japonica</i> , <i>Uroteuthis chinensis</i> , <i>Uroteuthis sibogae</i>	<i>Uroteuthis duvaucelii</i>
87M	Milan	Supermarket	Frozen	<i>Uroteuthis chinensis</i>	-	<i>Uroteuthis duvaucelii</i>
89M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Dosidicus gigas</i>
91M	Milan	Food service sector	Processed	Calamaro	<i>Loligo vulgaris</i> , <i>Loligo forbesii</i>	<i>Dosidicus gigas</i>

from South America, points to the replacement of local, higher-value taxa with cheaper and more abundant imported species. Similarly, *D. gigas*, which is widely available in global markets, appeared as a substitute in one case. Another notable case included a product labelled as *Architeuthis* sp. but identified as *D. gigas*. Indeed, *Architeuthis* spp. (giant squids) are deep-sea cephalopods that typically inhabit mesopelagic and bathypelagic zones well beyond continental shelf depths, and are therefore known almost exclusively from strandings, deep-ocean trawl bycatch, or predator stomach contents rather than from directed commercial fisheries (Guerra et al., 2011; Wada et al., 2020). To the best of our knowledge, no reports document *Architeuthis* being actively harvested or processed in the seafood industry. Therefore, the declaration of *Architeuthis* sp. on the product's label should be treated as a red flag for mislabelling, most likely as a mistake rather than voluntary action. In this case, the most likely explanation is the confusion and misuse of common names. Indeed, in many contexts, the term “giant squid” is incorrectly applied to *D. gigas* (jumbo flying squid). For instance, in the Italian official list of seafood trade names, the commercial designation of *D. gigas* is “*Totano gigante del Pacifico*”, while in the French official list it is “*encornet géant*” (European Commission, n.d.).

In Milan, label information was compared with molecular results for 96 of the 100 products collected. Similarly to Brussels, four squid-based products were excluded for the following reasons: (i) two products were molecularly identified as non-target seafood (*Mytilus* spp.) (Supplementary Material 4); (ii) one product was labelled as seafood salad without additional information on the cephalopod species included in the preparation; and (iii) one product (analysed using metabarcoding) yielded an insufficient number of reliable sequences for molecular authentication. In Milan, mismatches were detected in 29 of the 96 analysed squid-based products (30.2%), with a higher frequency compared to Brussels. In Milan, mismatching cases were also more widespread and heterogeneous than in Brussels, spanning fishmongers, supermarkets, and the food service sector. Similarly to the findings in Brussels, several squid-based products collected in Milan also revealed the substitution of locally recognised Mediterranean species with imported oceanic species. Fresh supermarket products labelled as *I. coindetii* were instead identified as *I. argentinus*, a Southwest Atlantic species heavily exploited in Argentine fisheries and widely exported to Europe (Fernández et al., 2020). Moreover, several processed products generically marketed as “Calamaro” – a designation that in the Italian Official List of Seafood Trade Names is associated with *Loligo vulgaris* and *L. forbesii* – were found to contain either *D. gigas* or *I. argentinus*. Both taxa are large-bodied ommastrephids characterised by high availability and relatively low prices in international markets (Arkhipkin et al., 2015; Giagkazoglou et al., 2024), and their recurrent presence in mislabelled products reflects an economically motivated substitution pattern. This evidence mirrors observations from other European studies, which reported that high-value Mediterranean or Northeast Atlantic species are often replaced by imported squid from South America or the Indo-Pacific (Fernández et al., 2020; Giagkazoglou et al., 2024; Grbin et al., 2025; Tinacci et al., 2023). Such practices not only mislead consumers but also undermine the transparency of supply chains, highlighting how global trade dynamics can directly influence the authenticity of squid products available on local European markets. Some products that were declared as *Uroteuthis chinensis* were identified as *Uroteuthis duvaucelii* (Table 1). This substitution most likely reflects the taxonomic complexity and morphological similarity within the genus *Uroteuthis*. Both species are Indo-Pacific squid that share overlapping morphological traits, especially once processed (e.g., frozen, cleaned, or cut into rings), making them extremely difficult to distinguish by visual inspection alone (Jereb & Roper, 2005). In practice, these species are often harvested together and may enter the same supply chains, where misidentification can occur unintentionally during processing or labelling. However, intentional substitution cannot be ruled out: *Uroteuthis duvaucelii* is generally considered a more abundant and widely distributed resource, potentially more accessible in

large-scale fisheries than *Uroteuthis chinensis*, which is locally valued in Asian markets (Arkhipkin et al., 2015).

The detection of DNA from non-cephalopod animal species, especially pork, in some multi-species products warrants careful interpretation. Under EU legislation, the presence of additional animal-derived ingredients in processed seafood products is permitted, provided that such components are correctly declared in the list of ingredients in accordance with Regulation (EU) No 1169/2011. In the present study, however, the label assessment was specifically focused on the information related to the declared squid species (scientific name and/or commercial designation), in line with the objectives of evaluating cephalopod traceability and compliance with the CMO Regulation. As a result, the conformity of labels with respect to the declaration of other animal ingredients was not systematically assessed. Therefore, the detection of non-cephalopod DNA cannot be interpreted as evidence of mislabelling in this study.

The frequency of mislabelling differed across product categories and retail types (Fig. 3). In Brussels, mislabelling occurred predominantly in fresh products (33.3%), while lower rates were observed in frozen (16.7%) and processed products (4.4%). In Milan, the highest rates were detected in frozen (37.5%) and processed products (31.4%), with fresh items showing a comparatively lower incidence (18.2%). When comparing retail types, mislabelling in Brussels was mainly associated with fishmongers (28.6%), followed by supermarkets (14.0%), whereas no mismatches were recorded in the food service sector due to the use of broad umbrella terms such as “calamar” or “calamari” in restaurants and canteens. In Milan, mislabelling was more frequent in the food service sector (34.4%) and supermarkets (33.3%) than in fishmongers (5.9%).

Overall, the statistical analysis revealed that the mislabelling frequency was not significantly different among the categories of products (fresh, frozen, processed) ($p = 0.956$; Fisher's exact test). Accordingly, the chi-square tests did not reveal any significant association between mismatch frequency and retailer type ($\chi^2 = 1.59$, $p = 0.452$, $df = 2$). These results suggest that, within the dataset analysed, mislabelling events were not concentrated in a particular retail channel or product category but were instead distributed across different market segments. Although evidence from the broader seafood sector suggests that medium-to-highly processed products may be more prone to mislabelling than whole specimens, meta-analyses indicate that differences across product forms are not statistically significant (Giusti et al., 2023a; Luque & Donlan, 2019), which is in line with our results. Similarly, while several studies have reported higher rates of species substitution and misdescription in the food service sector (e.g., restaurants and canteens) compared to retail outlets (Pardo et al., 2018; Pardo & Jiménez, 2020; Warner et al., 2013), large-scale and cross-market analyses indicate that mislabelling can occur at comparable levels across different supply chain nodes, including wholesale, retail, and food service (Giusti et al., 2023a; Luque & Donlan, 2019). It should be noted however that the chances of catching instances of mislabelling are higher when the labels are more precise (e.g. supermarket products indicating species on label) compared to when they are vague, which is often the case for restaurants serving squid; the term “calamar” or “calamari” used by many restaurants, includes all squid species and prevents us from checking any kind of ‘species-level’ mislabelling.

When interpreting the markedly different mismatch rates observed in Brussels (13.3%) and Milan (30.2%), it is important to consider the role of national seafood trade nomenclatures. In Belgium, the official list of commercial designations relies on highly generic umbrella terms (e.g., “calamar”), which encompass all species within the families Loliginidae and Ommastrephidae. With such broad categories, it is difficult to unambiguously establish whether a mismatch has occurred. By contrast, the Italian official list of seafood names is considerably more specific and follows the fundamental principle of “one species – one name” (Oceana, 2015), thereby facilitating the detection of discrepancies whenever they occur. To further disentangle the effect of regulatory nomenclature from true supply chain misrepresentation, mislabelling rates were

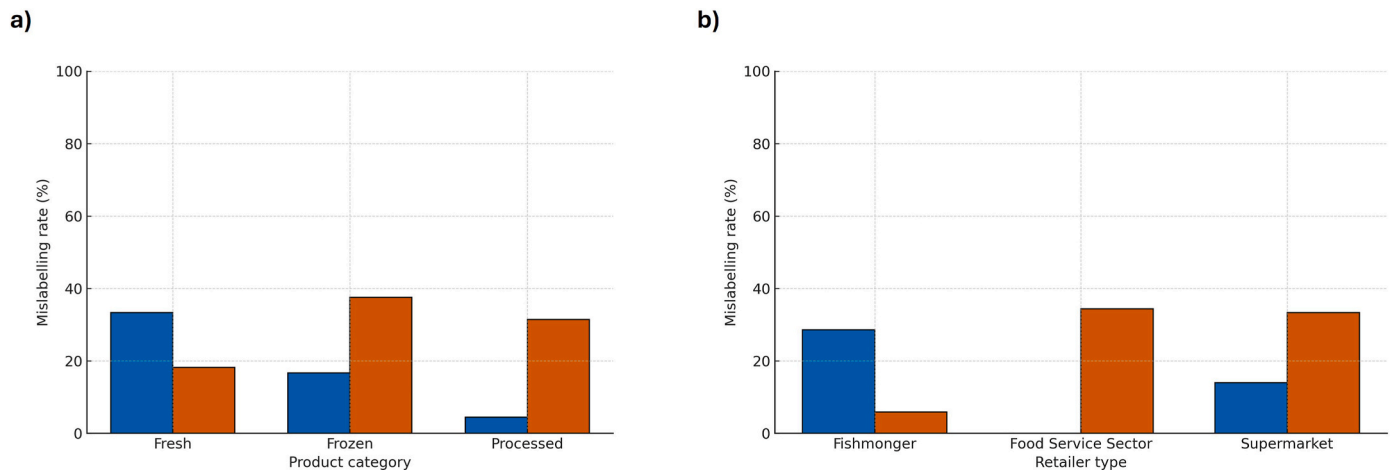


Fig. 3. Percentage of mislabelled squid-based products in Brussels (blue bars) and Milan (orange bars). (a) Mislabelling rates by product category (fresh, frozen, processed); (b) Mislabelling rates by retailer type (fishmonger, food service sector, supermarket). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

recalculated by restricting the analysis to products allowing species-level verification, namely those reporting a scientific name or a commercial designation linked to a defined list of species (22 products from Brussels and 34 from Milan). Under this conservative scenario, mislabelling was detected in 6 products in Brussels (27.3%) and 9 products in Milan (26.5%). This convergence confirms that the marked differences observed in the overall mislabelling rates are primarily driven by differences in national seafood nomenclature systems rather than by substantial variations in supply chain integrity.

Nonetheless, ensuring such specificity in national lists is crucial to guarantee product traceability, consumer protection, and market transparency.

A useful comparison can be drawn with the recent study by [Willette and Joaquin \(2025\)](#), who applied DNA barcoding and metabarcoding to squid and surimi products sold in grocery stores in Los Angeles under the U.S. regulatory framework. In contrast to the present study, they reported a 0% mislabelling rate for frozen squid products. However, this apparent full compliance largely reflects the permissiveness of the U.S. FDA Seafood List, which allows a broad range of cephalopod species to be marketed under the single acceptable market name “squid”. As noted by the authors, this approach results in technically correct but poorly informative labelling, masking substantial species diversity ([Willette & Joaquin, 2025](#)).

These results further confirm that differences in mislabelling rates across studies are strongly influenced by regulatory frameworks and nomenclature systems, rather than reflecting true differences in supply chain integrity.

4. Conclusions

This study provides the first comprehensive comparative assessment of squid-based products sold in two major EU cities, Brussels and Milan, combining label evaluation and molecular authentication through DNA barcoding and metabarcoding. Brussels and Milan represent two contrasting regulatory and market contexts, making them informative case studies. The results revealed that mislabelling is a relevant issue in both squid markets, with 13.3% of products in Brussels and 30.2% in Milan not matching the information declared on labels. Differences in national nomenclature systems for seafood trade names appear to strongly influence the apparent frequency of mislabelling, with the broader, family-level commercial designations permitted in Belgium reducing transparency compared to the more precise “one species–one name” approach adopted in Italy. Such structural inconsistency among Member States may hinder market transparency and complicate enforcement of

the CMO Regulation. Moreover, in markets where umbrella commercial designations are permitted, mislabelling cannot always be unambiguously detected, potentially leading to an underestimation of mismatch rates. The limited voluntary provision of scientific names on processed seafood products underscores the inadequacy of voluntary guidance to ensure full consumer information. Overall, the findings highlight the need for harmonised and stricter labelling requirements across the EU, extending the scope of the CMO Regulation to processed seafood products (including products sold by the food service sector) and promoting uniformity in national trade name lists.

CRediT authorship contribution statement

Alice Giusti: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lara Tinacci:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrea Armani:** Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Gabriele Spatola:** Writing – original draft, Methodology, Investigation. **Jiajie Hu:** Investigation. **Vanya Vulperhorst:** Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Allison L. Perry:** Writing – review & editing, Writing – original draft. **Marine Cusa:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors have no conflict of interests.

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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.2026.112069>.

Data availability

Data will be made available on request.

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