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Can consumers avoid mislabelling? Genetic species identification provides recommendations for shrimp/prawn products

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Abstract

BACKGROUND: Crustaceans of the superfamily Penaeoidea (e.g., shrimps and prawns) are among the most commercially available aquatic products worldwide. However, there are few studies regarding not only the presence but also the characteristics of mislabelling in these food products. Such information would be helpful for consumers in order to avoid the typical problems associated with mislabelling (e.g., health and economic issues). For this reason, this work considers Penaeoidea mislabelling by comparing different products (frozen, fresh, boiled), and sources (hypermarkets, supermarkets and fishmongers) from Spain (Europe).

RESULTS: A total of 94 samples from 55 different products were collected, representing 19 different species from 13 genera. Mitochondrial DNA (COI gene) was amplified, revealing mislabelling in almost 30% of supermarket products and almost exclusively found in frozen samples (95% of the total) regardless of its price. In addition, products from the Pacific Ocean seem to be particularly susceptible to mislabelling.

CONCLUSIONS: All in all, recommendations for the consumer in order to avoid mislabelling of prawns include purchasing them fresh from fishmongers; aquaculture products must not be avoided. This study represents, to our knowledge, the first attempt to provide recommendations to consumers based on DNA analyses in order to avoid mislabelling in food products. Further research is therefore required to provide such recommendations in different food products, particularly those that are processed, packaged and/or frozen.

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Keywords: COI; customer recommendations; DNA barcoding; food; mislabelling; prawn; shrimp

INTRODUCTION

Historically, crustaceans of the superfamily Penaeoidea (such as shrimps and prawns) have been among the most commercially important aquatic products worldwide. Currently, according to the United Nations Food and Agriculture Organization (FAO), the majority of their catches and production are destined for markets in the high-income regions of Europe, North America and Japan.¹ Shrimp consumption in the European Union (EU) is 1.47 kg per capita per year (49% wild and 51% farmed), with warm water shrimp and Argentine red shrimp being the most commonly consumed species.² The supply of this product in the EU is to a large extent dependent on imports, mainly from Ecuador, India, Vietnam, Thailand, Indonesia, Argentina, and Greenland.^{2,3}

Taking these factors into account, the labelling rules needed to trace seafood and protect consumers' rights and health are essential. In this regard, seafood labelling is regulated in the EU, and the law stipulates that, among other things, seafood products must be labelled with the full scientific name of the species together with the common name (Regulation EU No. 1379/2013). However, despite efforts to regulate and ensure the traceability of seafood

products, recent studies have revealed cases of mislabelling, showing that the seafood chain is particularly vulnerable to fraud, mainly due to species substitution and mislabelling.⁴ Guardone *et al.*⁵ conducted a study at the Border Inspection Post of Livorno-Pisa (Italy) on the non-compliance of labelling of fishery products imported from non-European countries. They found that 22.5% of the products analysed were incorrectly labelled with the highest mislabelling rate observed for cephalopods (43.8%), followed by crustaceans (17.0%) and fish (14.0%).

Seafood fraud (mislabelling being one of the main incidents involved on it⁶) can occur for a variety of reasons, from unintentional inaccurate identification or misunderstanding of

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regulations to deliberate deception to increase profits, or even hiding illegally caught species by falsely pretending they are cultured. Therefore, it is not only important for consumers from an economic and human health perspective,⁷ but also for species conservation/overfishing.⁸

For the identification of mislabelling of seafood products, a fundamental first step is to correctly identify the species. This can be difficult using morphology-based approaches, especially in the case of Penaeoidea superfamily, as morphological differences between some species can be subtle, particularly in closely related species that differ only by minor genital differences. In comparison, DNA-based techniques such as the use of DNA barcode markers (recently reviewed in Fernandes *et al.*⁹ and Antil *et al.*¹⁰), which can be used for a wide variety of food matrices, have proven to be very useful for correct species identification applied to seafood authentication.¹¹ Among the molecular markers used, DNA sequencing of the cytochrome c oxidase I (COI) mitochondrial gene (DNA barcoding) has provided numerous successful examples demonstrating its reliability for the identification of economic aquatic species including penaeid species.^{12–14} One of the advantages of using DNA barcoding over morphological characterization is the identification of species after processing of fish and shellfish.¹⁵ On the negative side, a major drawback is the dependence on the availability of complete and robust databases, such as GeneBank or Bold System, and the availability of species-specific reference sequences in these databases.⁹ Different surveys based on the DNA barcoding approach have been conducted in Spanish markets^{16,17} and in restaurants¹⁸ to detect mislabelling and fraudulent species substitutions in fish products. However, barcoding studies that focus on the detection of mislabelling of commercial shrimp products are still limited, with previous work in commercial prawns/shrimps showing between 22% and 66% of mislabelling in different markets.^{19–24}

As commented earlier, there are few works describing not only the presence but also the characteristics of mislabelling in shrimps and prawns to date^{22–25}; however, there are no specific recommendations for consumers that have been done based on them. For this reason, we have collected and barcoded a high number of shrimp/prawn samples from different establishments (hypermarkets, supermarkets and fishmongers) to assess the degree of mislabelling in Spanish food services. In addition to mislabelling detection, the aim of the study was to examine potential links between such mislabelling and certain products, in order to be able to provide useful recommendations to consumers so that they can avoid such practices, which unfortunately is not a common information presented in mislabelling bibliography.

MATERIAL AND METHODS

Sampling

A total of 55 commercial products, including fresh, frozen, and boiled (Fig. 1), were obtained over a 1-year period (October 2021 to June 2022) from different supermarkets (ten) and fishmongers (three) in the Madrid area (Madrid centre and surroundings) and Toledo (Table 1), Spain. Samples were randomly taken from whatever was available at the time of sampling. The origin of some of these commercial products ($n = 6$) was aquaculture. Two different individuals (samples) were taken from each product. Fresh samples were immediately frozen and stored at -20°C until laboratory analysis. Table 1 lists all samples recording to declared commercial and scientific name, capture zone (FAO), type of product and price. Labels showed 19 products at species

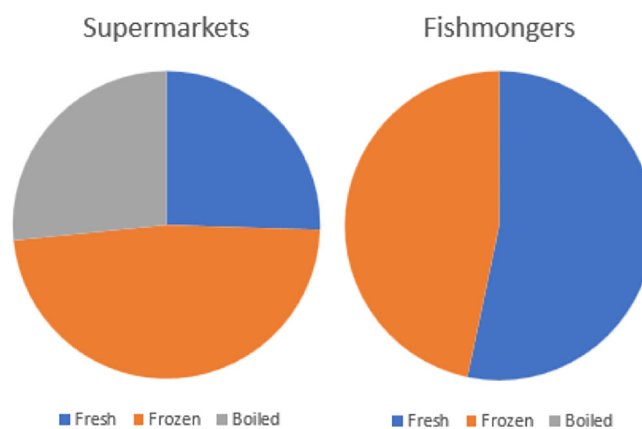


Figure 1. Proportions of fresh, frozen, or boiled samples obtained from supermarkets (left) or fishmongers (right).

level and four products at genus level (Table 1), according to the package labels.

DNA extraction, PCR amplification and DNA sequencing

A small piece of tissue (< 0.1 mg) from the tail was used for genomic DNA isolation. Genomic DNA was extracted using NZY Plant/Fungi Isolation kit (Nzytech, Lisboa, Portugal) and eluted in $50\ \mu\text{L}$. A primer pair (LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO 2198: 5'-TAACTTCAGGGTGACCAAAAAATCA-3')²⁶ amplifying a fragment of almost 700 bp of the COI was employed for DNA amplifications. Polymerase chain reaction (PCR) conditions included a total volume of $20\ \mu\text{L}$, containing $2\ \mu\text{L}$ of DNA, $10\ \mu\text{L}$ of NZYtaq II 2x master mix (Nzytech), $0.8\ \mu\text{L}$ of each primer ($5\ \mu\text{mol/L}$) and 0.01% BSA (bovine serum albumin). PCR programme consisted of an initial denaturation step for 3 min at 95°C followed by 35 cycles of denaturation for 90 s at 94°C with an annealing for 25 s at 45°C and an extension for 30 s and a final extension step for 1 min at 72°C . PCR products were analysed on agarose gels (1.5%) including negative controls, and sequenced on an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA) at the UCM Genomic Unit.

Data and statistical analyses

Sequences were edited with MEGA 11 software²⁷ and compared against the GenBank database at the National Centre for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). The identification obtained by barcoding was compared with the species information collected at establishments/labels. To assign the sample identification, three values of percentage sequence identity, greater than 98%, 99% and 100% of the reference specimen to the query sequence were considered. Samples were classified as mislabelled if the species declared for the product did not match the species identified by barcoding.

We tested whether there was a statistical correlation between the average price (euro per kilogram, €/kg) of the products and the percentage of mislabelling by using Mann–Whitney test with PAST 4.0 software.²⁸

RESULTS AND DISCUSSION

In total, 94 samples from 55 products (meaning 1.71 samples per products) yielded successful COI PCR amplification and were therefore useful for the study of mislabelling (Table 1). Within

Table 1. Shrimp and prawn commercial products obtained in the Madrid area (Madrid centre and surroundings) and Toledo are shown in the table as products and sample numbers, scientific name of the label (product label), fishing area (FAO or aquaculture), type of product (fresh, frozen and boiled), price (euro per kilogram), shop [supermarkets (S) and fishmonger (F)]. The species identified (COI species) by BLAST (<https://www.ncbi.nlm.nih.gov/BLAST>), and the percentage of identification (% Ident) between the amplified and the most similar sequence existing in GeneBank. The presence of mislabelling was annotated with Yes or No in column Mislab.

| Product | Sample | Label | Area | Product | Price | Shop | COI species | % Ident | Mislab |
|---------|--------|-----------------------------------|--------------------------------|---------|-------|------|-----------------------------------|---------|--------|
| St01 | Sa01 | <i>Parapenaepsis stylifera</i> | Indian Ocean (FAO n° 51) | Frozen | 12.5 | S | <i>Mierspenaeopsis hardwickii</i> | 99.50 | Yes |
| St02 | Sa02 | <i>Palaemon serratus</i> | Atlantic Ocean (FAO n° 27) | Fresh | 69.8 | F | <i>Palaemon serratus</i> | 98.38 | No |
| St02 | Sa03 | <i>Palaemon serratus</i> | Atlantic Ocean (FAO n° 27) | Fresh | 69.8 | F | <i>Palaemon serratus</i> | 100 | No |
| St03 | Sa04 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n° 34) | Frozen | 21.9 | F | <i>Parapenaeus longirostris</i> | 99.68 | No |
| St03 | Sa05 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n° 34) | Frozen | 21.9 | F | <i>Parapenaeus longirostris</i> | 99.84 | No |
| St04 | Sa06 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n° 34) | Fresh | 27.9 | F | <i>Parapenaeus longirostris</i> | 99.35 | No |
| St04 | Sa07 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n° 34) | Fresh | 27.9 | F | <i>Parapenaeus longirostris</i> | 99.66 | No |
| St05 | Sa08 | <i>Parapenaeus longirostris</i> | Mediterranean Sea (FAO n°37.1) | Fresh | 59.9 | F | <i>Parapenaeus longirostris</i> | 99.83 | No |
| St05 | Sa09 | <i>Parapenaeus longirostris</i> | Mediterranean Sea (FAO n°37.1) | Fresh | 59.9 | F | <i>Parapenaeus longirostris</i> | 100 | No |
| St06 | Sa10 | <i>Litopenaeus vannamei</i> | Aquaculture | Fresh | 15.9 | F | <i>Pennaeus vannamei</i> | 100 | No |
| St06 | Sa11 | <i>Litopenaeus vannamei</i> | Aquaculture | Fresh | 15.9 | F | <i>Pennaeus vannamei</i> | 100 | No |
| St07 | Sa12 | <i>Pleoticus muelleri</i> | Atlantic Ocean (FAO n°4) | Frozen | 22.95 | S | <i>Pleoticus muelleri</i> | 98.89 | No |
| St07 | Sa13 | <i>Pleoticus muelleri</i> | Atlantic Ocean (FAO n°4) | Frozen | 22.95 | S | <i>Pleoticus muelleri</i> | 99 | No |
| St08 | Sa14 | <i>Pandalus borealis</i> | Atlantic Ocean (northeast) | Frozen | 15.5 | S | <i>Pandalus borealis</i> | 99.67 | No |
| St08 | Sa15 | <i>Pandalus borealis</i> | Atlantic Ocean (northeast) | Frozen | 15.5 | S | <i>Pandalus borealis</i> | 100 | No |
| St09 | Sa16 | <i>Plesiopenaeus edwardsianus</i> | Pacific Ocean | Frozen | 27.5 | S | <i>Palaemon varians</i> | 99.84 | Yes |
| St10 | Sa17 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n° 34) | Fresh | 33.17 | S | <i>Parapenaeus longirostris</i> | 100 | No |
| St10 | Sa18 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n° 34) | Fresh | 33.17 | S | <i>Parapenaeus longirostris</i> | 99.68 | No |
| St11 | Sa19 | <i>Pleoticus muelleri</i> | Atlantic Ocean (southwest) | Fresh | 12.99 | S | <i>Pleoticus muelleri</i> | 100 | No |
| St11 | Sa20 | <i>Pleoticus muelleri</i> | Atlantic Ocean (southwest) | Fresh | 12.99 | S | <i>Pleoticus muelleri</i> | 100 | No |
| St12 | Sa21 | <i>Penaeus vannamei</i> | Honduras/Nicaragua | Fresh | 8.95 | S | <i>Penaeus vannamei</i> | 99.69 | No |
| St12 | Sa22 | <i>Penaeus vannamei</i> | Honduras/Nicaragua | Fresh | 8.95 | S | <i>Penaeus vannamei</i> | 98.37 | No |
| St13 | Sa23 | <i>Parapenaeus longirostris</i> | Mediterranean Sea | Fresh | 29.95 | S | <i>Parapenaeus longirostris</i> | 100 | No |
| St13 | Sa24 | <i>Parapenaeus longirostris</i> | Mediterranean Sea | Fresh | 29.95 | S | <i>Parapenaeus longirostris</i> | 98.89 | No |
| St14 | Sa25 | <i>Parapenaepsis stylifera</i> | Indian Ocean | Frozen | 12.67 | S | <i>Mierspenaeopsis hardwickii</i> | 98.30 | Yes |
| St15 | Sa26 | <i>Parapenaepsis spp</i> | Indian Ocean | Frozen | 16.54 | S | <i>Metapenaeus brevicornis</i> | 99.83 | Yes |
| St15 | Sa27 | <i>Parapenaepsis spp</i> | Indian Ocean | Frozen | 16.54 | S | <i>Metapenaeus brevicornis</i> | 99.32 | Yes |
| St16 | Sa28 | <i>Solenocera melantho</i> | Pacific Ocean | Frozen | - | S | <i>Solenocera melantho</i> | 99.36 | No |
| St17 | Sa29 | <i>Pleoticus muelleri</i> | Atlantic Ocean (southwest) | Frozen | 19.95 | F | <i>Pleoticus muelleri</i> | 99.05 | No |

Table 1. Continued

| Product | Sample | Label | Area | Product | Price | Shop | COI species | % Ident | Mislab |
|---------|--------|---------------------------------|---------------------------------|---------|-------|------|-------------------------------------|---------|--------|
| St17 | Sa30 | <i>Pleoticus muelleri</i> | Atlantic Ocean (southwest) | Frozen | 19.95 | F | <i>Pleoticus muelleri</i> | 99.37 | No |
| St18 | Sa31 | <i>Trachypenaeus spp.</i> | Pacific Ocean (FAO n°61) | Frozen | 17.25 | S | <i>Metapenaeopsis barbata</i> | 99.87 | Yes |
| St18 | Sa32 | <i>Trachypenaeus spp.</i> | Pacific Ocean (FAO n°61) | Frozen | 17.25 | S | <i>Pleoticus muelleri</i> | 99.08 | Yes |
| St19 | Sa33 | <i>Palaemonetes varians</i> | Atlantic Ocean (northeast) | Boiled | 8.99 | S | <i>Palaemon varians</i> | 99.84 | No |
| St19 | Sa34 | <i>Palaemonetes varians</i> | Atlantic Ocean (northeast) | Boiled | 8.99 | S | <i>Palaemon varians</i> | 99.84 | No |
| St20 | Sa35 | <i>Nephrops norvegicus</i> | Atlantic Ocean (east) | Fresh | 11.89 | S | <i>Penaeus sp.</i> | 99.47 | Yes |
| St21 | Sa36 | <i>Litopenaeus vannamei</i> | Venezuela | Fresh | 7.95 | S | <i>Litopenaeus vannamei</i> | 99.84 | No |
| St21 | Sa37 | <i>Litopenaeus vannamei</i> | Venezuela | Fresh | 7.95 | S | <i>Litopenaeus vannamei</i> | 99.84 | No |
| St22 | Sa38 | <i>Litopenaeus vannamei</i> | Ecuador | Fresh | 9.95 | S | <i>Litopenaeus vannamei</i> | 99.01 | No |
| St22 | Sa39 | <i>Litopenaeus vannamei</i> | Ecuador | Fresh | 9.95 | S | <i>Litopenaeus vannamei</i> | 99.18 | No |
| St23 | Sa40 | <i>Parapenaeopsis stylifera</i> | Indian Ocean (FAO n°51, 57) | Frozen | 12.67 | S | <i>Parapenaeopsis hardwickii</i> | 100 | Yes |
| St24 | Sa41 | <i>Solenocera melantho</i> | FAO n°61 | Frozen | 15.68 | S | <i>Solenocera crassicornis</i> | 100 | Yes |
| St24 | Sa42 | <i>Solenocera melantho</i> | FAO n°61 | Frozen | 15.68 | S | <i>Parapenaeus fissuroides</i> | 99.40 | Yes |
| St25 | Sa43 | <i>Pandalus borealis</i> | Atlantic Ocean (north) | Frozen | 14.67 | S | <i>Pandalus borealis</i> | 99.84 | No |
| St25 | Sa44 | <i>Pandalus borealis</i> | Atlantic Ocean (north) | Frozen | 14.67 | S | <i>Pandalus borealis</i> | 100 | No |
| St26 | Sa45 | <i>Pleoticus muelleri</i> | Atlantic Ocean (FAO n°41) | Frozen | 20.84 | S | <i>Pleoticus muelleri</i> | 98.50 | No |
| St27 | Sa46 | <i>Penaeus vannamei</i> | Aquaculture (Ecuador) | Frozen | 8.69 | S | <i>Penaeus vannamei</i> | 100 | No |
| St27 | Sa47 | <i>Penaeus vannamei</i> | Aquaculture (Ecuador) | Frozen | 8.69 | S | <i>Penaeus vannamei</i> | 98.77 | No |
| St28 | Sa48 | <i>Pandalus borealis</i> | Atlantic Ocean (north) | Frozen | 14.6 | S | <i>Pandalus borealis</i> | 99.83 | No |
| St28 | Sa49 | <i>Pandalus borealis</i> | Atlantic Ocean (north) | Frozen | 14.6 | S | <i>Pandalus borealis</i> | 99.84 | No |
| St29 | Sa50 | <i>Penaeus vannamei</i> | Aquaculture (Ecuador) | Fresh | 12 | S | <i>Penaeus vannamei</i> | 100 | No |
| St29 | Sa51 | <i>Penaeus vannamei</i> | Aquaculture (Ecuador) | Fresh | 12 | S | <i>Penaeus vannamei</i> | 100 | No |
| St30 | Sa52 | <i>Penaeus vannamei</i> | Aquaculture (Nicaragua/Ecuador) | Boiled | 9.6 | S | <i>Penaeus vannamei</i> | 99.84 | No |
| St30 | Sa53 | <i>Penaeus vannamei</i> | Aquaculture (Nicaragua/Ecuador) | Boiled | 9.6 | S | <i>Penaeus vannamei</i> | 99.68 | No |
| St31 | Sa54 | <i>Penaeus spp</i> | Aquaculture (Ecuador) | Boiled | 12 | S | <i>Penaeus vannamei</i> | 99.50 | No |
| St32 | Sa55 | <i>Penaeus longirostris</i> | Atlantic Ocean | Fresh | 9 | S | <i>Penaeus longirostris</i> | 100 | No |
| St32 | Sa56 | <i>Penaeus longirostris</i> | Atlantic Ocean | Fresh | 9 | S | <i>Penaeus longirostris</i> | 99.33 | No |
| St33 | Sa57 | <i>Pleoticus muelleri</i> | Atlantic Ocean (FAO n°41) | Fresh | 10.50 | S | <i>Pleoticus muelleri</i> | 99.08 | No |
| St34 | Sa58 | <i>Penaeus brevirostris</i> | Atlantic Ocean (FAO n°77) | Fresh | 9.99 | S | <i>Farfantepenaeus brevirostris</i> | 100 | No |
| St34 | Sa59 | <i>Penaeus brevirostris</i> | Atlantic Ocean (FAO n°77) | Fresh | 9.99 | S | <i>Farfantepenaeus brevirostris</i> | 98.97 | No |
| St35 | Sa60 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n°35) | Fresh | 13.95 | S | <i>Parapenaeus longirostris</i> | 100 | No |
| St35 | Sa61 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (FAO n°35) | Fresh | 13.95 | S | <i>Parapenaeus longirostris</i> | 100 | No |
| St36 | Sa62 | <i>Palaemon serratus</i> | Celtic Sea | Boiled | 49.95 | S | <i>Palaemon serratus</i> | 98.58 | No |
| St36 | Sa63 | <i>Palaemon serratus</i> | Celtic Sea | Boiled | 49.95 | S | <i>Palaemon serratus</i> | 99.20 | No |
| St37 | Sa64 | <i>Aristeus alcocki</i> | Indian Ocean (west) | Frozen | 29.4 | S | <i>Aristeus alcocki</i> | 100 | No |
| St38 | Sa65 | <i>Solenocera melantho</i> | China | Frozen | 12.5 | S | <i>Solenocera melantho</i> | 99.72 | No |
| St38 | Sa66 | <i>Solenocera melantho</i> | China | Frozen | 12.5 | S | <i>Pleoticus robustus</i> | 99.81 | Yes |

Table 1. Continued

| Product | Sample | Label | Area | Product | Price | Shop | COI species | % Ident | Mislab |
|---------|--------|-----------------------------------|-----------------------------|---------|-------|------|-----------------------------------|---------|--------|
| St39 | Sa67 | <i>Penaeus vannamei</i> | South America | Frozen | 15.3 | S | <i>Penaeus vannamei</i> | 100 | No |
| St39 | Sa68 | <i>Penaeus vannamei</i> | South America | Frozen | 15.3 | S | <i>Pleoticus muelleri</i> | 99.84 | Yes |
| St40 | Sa69 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (central) | Frozen | 13.95 | S | <i>Parapenaeus longirostris</i> | 99.84 | No |
| St40 | Sa70 | <i>Parapenaeus longirostris</i> | Atlantic Ocean (central) | Frozen | 13.95 | S | <i>Parapenaeus longirostris</i> | 99.84 | No |
| St41 | Sa71 | <i>Parapenaeus sp.</i> | Atlantic Ocean (central) | Frozen | 29.95 | S | <i>Parapenaeus longirostris</i> | 99.84 | No |
| St42 | Sa72 | <i>Penaeus notialis</i> | Mediterranean Sea | Frozen | 19.98 | S | <i>Fantapenaeus aztecus</i> | 100 | Yes |
| St43 | Sa73 | <i>Penaeus notialis</i> | Mediterranean Sea | Frozen | 20.98 | S | <i>Fantapenaeus aztecus</i> | 100 | Yes |
| St44 | Sa74 | <i>Penaeus vannamei</i> | Ecuador | Frozen | 8.98 | S | <i>Litopenaeus vannamei</i> | 100 | No |
| St45 | Sa75 | <i>Melicertus latisulcatus</i> | Indian Ocean (west) | Frozen | 23 | S | <i>Melicertus latisulcatus</i> | 99.20 | No |
| St45 | Sa76 | <i>Melicertus latisulcatus</i> | Indian Ocean (west) | Frozen | 23 | S | <i>Melicertus latisulcatus</i> | 100 | No |
| St46 | Sa77 | <i>Penaeus monodon</i> | Madagascar | Boiled | 29.95 | S | <i>Penaeus monodon</i> | 99.69 | No |
| St46 | Sa78 | <i>Penaeus monodon</i> | Madagascar | Boiled | 29.95 | S | <i>Penaeus monodon</i> | 99.49 | No |
| St47 | Sa79 | <i>Penaeus latisulcatus</i> | Indian Ocean (west) | Boiled | 26.95 | S | <i>Melicertus latisulcatus</i> | 98.78 | No |
| St48 | Sa80 | <i>Penaeus kerathurus</i> | Ionian Sea | Fresh | 54.95 | S | <i>Penaeus kerathurus</i> | 99.79 | No |
| St48 | Sa81 | <i>Penaeus kerathurus</i> | Ionian Sea | Fresh | 54.95 | S | <i>Penaeus kerathurus</i> | 99.79 | No |
| St49 | Sa82 | <i>Parapenaeopsis stylifera</i> | Indian Ocean (FAO n°51, 57) | Frozen | 11.99 | S | <i>Parapenaeopsis hardwickii</i> | 99.53 | Yes |
| St49 | Sa83 | <i>Parapenaeopsis stylifera</i> | Indian Ocean (FAO n°51, 57) | Frozen | 11.99 | S | <i>Parapenaeopsis hardwickii</i> | 99.69 | Yes |
| St50 | Sa84 | <i>Aristeus alcocki</i> | Indian Ocean (FAO n°51, 57) | Frozen | 17.99 | S | <i>Penaeus indicus</i> | 98.75 | Yes |
| St50 | Sa85 | <i>Aristeus alcocki</i> | Indian Ocean (FAO n°51, 57) | Frozen | 17.99 | S | <i>Mierspenaeopsis hardwickii</i> | 99.20 | Yes |
| St51 | Sa86 | <i>Plesiopenaeus edwardsianus</i> | — | Frozen | 24.99 | S | <i>Litopenaeus vannamei</i> | 99 | Yes |
| St51 | Sa87 | <i>Plesiopenaeus edwardsianus</i> | — | Frozen | 24.99 | S | <i>Aristeomorpha foliacea</i> | 99.66 | Yes |
| St52 | Sa88 | <i>Penaeus vannamei</i> | South America, aquaculture | Frozen | 9.99 | S | <i>Penaeus vannamei</i> | 99.53 | No |
| St52 | Sa89 | <i>Penaeus vannamei</i> | South America, aquaculture | Frozen | 9.99 | S | <i>Penaeus vannamei</i> | 99.07 | No |
| St53 | Sa90 | <i>Penaeus vannamei</i> | South America, aquaculture | Frozen | 15.99 | S | <i>Penaeus vannamei</i> | 99.54 | No |
| St53 | Sa91 | <i>Penaeus vannamei</i> | South America, aquaculture | Frozen | 15.99 | S | <i>Penaeus vannamei</i> | 99.53 | No |
| St54 | Sa92 | <i>Parapenaeus longirostris</i> | Senegal | Frozen | 30 | F | <i>Parapenaeus longirostris</i> | 100 | No |
| St55 | Sa93 | <i>Penaeus vannamei</i> | Ecuador | Frozen | 12 | F | <i>Penaeus vannamei</i> | 99.69 | No |
| St55 | Sa94 | <i>Penaeus vannamei</i> | Ecuador | Frozen | 12 | F | <i>Penaeus vannamei</i> | 99.53 | No |

them, 23 different labels were found, 19 of them declaring species level and four only at genus level, which means around four samples per species or genus. The samples collected belong to 13 different genera in total (including the genus of the samples at species level and at genus level): *Aristeus*, *Litopenaeus*, *Melicertus*, *Palaemon*, *Palaemonetes*, *Pandalus*, *Parapenaeopsis*, *Parapenaeus*,

Penaeus, *Pleoticus*, *Pleioopenaeus*, *Solenocera*, and *Trachypenaeus*. The origin of such samples was very diverse, including different areas of the Atlantic, Indian, and Pacific Oceans, but also the Mediterranean Sea and aquaculture from South America (Table 1). All of them have available COI sequence in GenBank (<https://www.ncbi.nlm.nih.gov/genbank>, accessed 31 May 2023).

Most of the samples were bought frozen ($n = 52$; 55.3% of the total), but there were also 32 samples (34%) fresh and ten boiled (10.6%). Samples were bought in supermarkets ($n = 79$; 84% of the total samples) or fishmongers ($n = 15$; 15.9% of samples) with a variety of prices, ranging 8.69–30 €/kg in frozen samples, 7.95–69.8 €/kg in fresh samples, and 8.99–49.95 €/kg in boiled samples (global mean of 20.2 €/kg, standard deviation = 13.79). Fresh samples were the 25.5% in supermarkets and the 53.3% in fishmongers, with no boiled samples in this last kind of establishment.

Considering BLAST identifications with more than 98% of identity, DNA amplification and subsequent sequence comparison showed 21 samples from the total of 94 samples (22.34%) as mislabelled. Such samples, as indicated on the product label, belong to six different genera and nine different species. Almost all of the incorrectly labelled samples were frozen ($n = 20$; 95.45%), the price ranged from 12 to 27.5 €/kg, and all of them were bought in supermarkets (no mislabelled samples were detected in fishmongers), and none of them were produced in aquaculture.

When an identity rate greater than 99% was considered for the species level assignment, the results were very similar to those of 98% because only one sample showed a sequence similarity between 98% and 99% (more specifically, 98.75%). That means 20 samples mislabelled (24.09% from the 83 samples which had more than 99% similarity), which included the same six different genera and nine different species of those from 98% similarity. Almost all of them ($n = 19$, 95%) were also frozen, the price range was the same (12–27.5 €/kg), none of them come from aquaculture, and all were purchased from a supermarket.

Finally, only 26 samples from the 94 (therefore 27.65%) could be assigned to the species level with a 100% identity rate in BLAST analyses. Mislabeling was detected in four of them (15.38%). All these samples were frozen and bought in supermarkets, with no aquaculture samples.

Mislabelling has been here detected in prawns sampled in Spanish markets. The percentage of samples mislabelled using 99% of DNA sequence similarity was 24.09%, with no differences ($\chi^2 = 0.004$, $P = 0.95$) when using 98%, according to previous works comparing percentage of DNA similarity for species identification.²⁹ However, this percentage was lower (15.38%) when a 100% identity score was considered for assigning the molecular identification of the species. This occurs because only 26 samples showed 100% identity with sequences uploaded to the database, probably due to the low number of COI sequences (haplotypes) of these animals are present in GenBank. For these reasons, we discuss hereon the 99% similarity results, which reported, as stated earlier, 24.09% of mislabelling in prawns sampled in Spanish markets. Previous work in commercial prawns/shrimps had been found between 22% and 66% of mislabelling in different markets.^{19–24} This proportion of mislabelling is also similar to others previously found in fish served in restaurants,¹⁸ fish served in Metro Vancouver,³⁰ or seafood in caterings.¹³

Interestingly, mislabelling was almost exclusively found in frozen samples (95.45% of the total), and all of them were bought in supermarkets (no mislabelled samples were detected in fishmongers or coming from aquaculture). This agrees with previous studies showing mislabelling as occurring in seafood processing.¹⁶ In addition, mean price of the mislabelled samples 16.9 and 21.11 €/kg in the no-mislabelled samples, but no statistical differences in mislabelling were found due to price (Mann–Whitney $U = 692$, $P = 0.79$), so mislabelling seems to be common, independent of product price.

In relation to sample geographic origin, nearly half of the mislabelled samples came from the Indian Ocean, but only 5.5% of the corrected labelled samples came from this ocean. The majority of the corrected labelled samples (45.2%) came from the Atlantic Ocean. This could lead to identify the Indian Ocean as a potential area for prawn mislabelling. Previous research on seafood mislabelling proposed that it was more prevalent in Pacific species than in others.¹⁶ In agreement with this, the Pacific samples of this work ($n = 6$) were mislabelled in the majority ($n = 5$, 83.3%), confirming the Pacific area as a potential source of mislabelling also in prawns.

In addition to all this, several findings of this study point to deliberateness in the found mislabelling:

- (1) When analysing per products (samples included in the same product bag or pool), the presence of at least one mislabelled sample was detected in 16 products (29.09% of the total). In such mislabelled products, when two samples were successfully amplified by PCR ($n = 7$), mislabelling occurred in both samples in more than half of cases ($n = 5$, 71.43%). Interestingly, in most of these cases ($n = 4$, 80%; products St18, St24, St50 and St51), the two mislabelled species were different species.
- (2) The found mislabelling included in most cases (76.19%) of species of different genus of the labelled species, are usually different in their morphology. Mislabelling was found in fresh/boiled products only in one sample (Sa35, fresh), which is more easily identified by visualization than frozen products (these samples are usually individuals without head and exoskeleton).
- (3) There were two samples in which the natural distribution of the species of the label and the molecular-identified species were very different. In the sample Sa66, the species of the label (*Solenocera melanthero*) is distributed across Asia and Australia waters, and the molecular-identified species (*Pleoticus robustus*) is distributed across American waters. In the other case (sample Sa68) the species of the label (*Penaeus vannamei*) the eastern Pacific coast from northern Mexico to northern Peru, meanwhile the actual species (*Pleoticus muelleri*) inhabits the other side of the South America across the southwest Atlantic, from southeast Brazil to Argentine Patagonia.

CONCLUSION

All in all, mislabelling in prawn products in Spain is high (close to 30% of products in supermarkets), occurs independently of its price, and points to be deliberate. It has only been found in supermarkets, and not in fishmongers, and the vast majority in frozen products, with no aquaculture ($n = 13$) samples mislabelled. The Pacific area seems to be especially affected for this practice. As a positive outcome of this study, none of the detected species involved in the mislabelling are present in the IUCN Red List of Threatened Species (<https://www.iucnredlist.org>, accessed 10 July 2023).

Taking all these findings into consideration, recommendations for the consumer in order to avoid mislabelling in shrimp/prawns would include:

- (1) To buy fresh or boiled products, because the majority of the mislabelled samples were frozen.

- (2) To buy in fishmongers, because no mislabelling was found at these establishments.
- (3) Do not avoid aquaculture samples because no mislabelling was detected on them. However, be careful with other associated problems of aquaculture such as contaminants, antibiotics, or sustainability, among others.^{31–33}
- (4) Do not consider price (e.g., high price) to try to avoid mislabelling, because no different outcomes were found.
- (5) Buy samples from the Atlantic Ocean, and not from the Indian and Pacific Oceans, because mislabelling is very much common in these last two areas.

Following these recommendations, the probabilities of buying mislabelled prawn products are low, thus it could help consumers to avoid the health,³⁴ economic,³⁵ and conservation⁸ problems directly related to mislabelling. This last information is very important, because this is, to our knowledge, the first time that information revealed by DNA barcode analysis has been used to provide recommendations to help customers to avoid potential deceit in the seafood industry, highlighting its usefulness in helping people make informed decisions about the types of items and suppliers to choose in order to prevent food fraud and health hazards. Based on that, further research is necessary to make such recommendations in different food products, particularly those that are processed, packaged and/or frozen.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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