

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

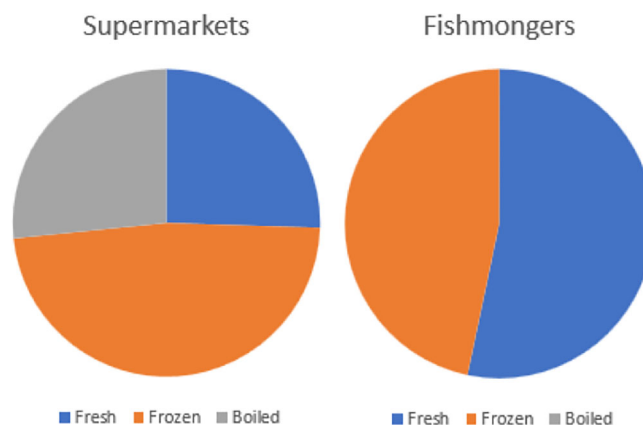


Figure 1. Proportions of fresh, frozen, or boiled samples obtained from supermarkets (left) or fishmongers (right).

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>Pennaeus vannamei</i>	99.84	No
St30	Sa53	<i>Penaeus vannamei</i>	Aquaculture (Nicaragua/Ecuador)	Boiled	9.6	S	<i>Pennaeus 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. Mislabelling 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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