

1 **Molecular identification of whole squids and calamari at fairs and markets in**
2 **regions of Latin America**

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31

32 **Abstract**

33 In recent decades, the commercial importance of cephalopods has increased considerably,
34 being an important fishing resource around the world. However, during the preparation
35 for commercialization of those species, especially those sold in markets, they suffer the
36 process known as “calamari” which includes removing and separating the head, arm, skin
37 or even having the body structure cut into rings, which ends up making it difficult or often
38 prevents the identification of the species, which can lead to replacements. In this sense,
39 the present study aimed to use the large ribosomal region, *rrnL* (also known as 16S rDNA)
40 to genetically identify cephalopod species sold in markets and fairs in Latin America.
41 Whole and processed samples were collected from supermarkets and directly from local
42 fishers the approximate collection location. Each generated sequence was submitted to
43 the website *Genbank* for molecular comparison and included in the database for
44 subsequent genetic identification. Comparison of sequences using the *Blastn* revealed the
45 presence of eight species that are widely traded in the Latin American region. Our results
46 indicate labeling errors in samples from the State of Pará that contained the species
47 *Dosidicus gigas* (d’ Orbigny, 1823) found only in the Pacific Ocean and were generically
48 labeled as “National Lula”. No type of substitution was found among the samples that
49 were being sold at fairs and markets, only labeling errors. Thus, our results demonstrate
50 the effectiveness of the *rrnL* for identifying species and evaluating labeling errors.

51

52 **Keywords:** Cephalopods, Fishery Resources, Species identification, Labeling.

53 **Introduction**

54 In recent decades, the commercial importance of cephalopods has grown notably
55 in volume of trade (Caddy and Rodhouse 1998; Watson and Pauly 2001; Jereb and Roper,
56 2010). The number of cephalopod species that are targets of commercial fisheries has
57 grown in recent times (FAO 2022), and given this, they represent the third largest marine
58 group consumed by the human population (the largest two being finfish and crustaceans)
59 (Gleadall et al 2024). And when it comes to commercial value, members of the family
60 Ommastrephidae represent the largest volume of commercialization and global
61 importance (FAO 2016). Loliginids represent the second family of interest for global
62 captures due to their distribution limited to the continental shelf, in tropical and temperate
63 regions around the world, as well as the high quality of the species' meat (Jereb and Roper

64 2010; Sales et al. 2011), having a high protein value, a wide variety of essential amino
65 acids and extremely low fat levels, making regular consumption of the species part of
66 healthy and balanced diets (Anfaco-Cecopesca 2018; USDA 2018).

67 Currently, cephalopods represent about 7% of seafood production, their landings
68 have increased since 1961, reaching a record of captured and commercialized tons in 2020
69 (FAO, 2022). In terms of cephalopods catches and consumption, Asian, European,
70 African and North American countries have the ten largest fishing fleets in the world
71 (FAO 2020), with Spain, Italy and Japan being the largest consumers (FAO 2016; 2018).

72 In South America, *Dosidicus gigas* D'orbigny 1835 has been widely explored,
73 being exported by Peru to more than 50 countries, where several attempts are being made
74 to diversify new products made with the species among them, canned, pre-cooked, roasted
75 and others (Vieites et al. 2019). This high demand for export means that the
76 commercialization of entire species is greatly reduced or made difficult, with the need for
77 processing often resulting in a longer shelf life of derived products (Anfaco-Copesca
78 2018; USDA 2018).

79 However, processing most often removes or damages diagnostic features that are
80 important for correct species identification through traditional taxonomic characteristics
81 (Di Pinto et al. 2013). In the case of cephalopods, there is the removal of the intestines,
82 skin, arms, tentacles, fins and head, where the species are often cut into rings or tubes
83 (Chapela et al 2003; Johnson 2007), thus removing all morphological characteristics
84 which are used to identify species as sex, or sexual maturity, a process that make all
85 species be commercialized as calamari (Roper and Mangold 1998; Chapela et al. 2003).

86 These modifications make taxonomic identification difficult or even impossible,
87 making it more prone to economic fraud where highly valued species, such as those from
88 the family Loliginidae, are replaced by species of lower commercial value, such as those
89 from the Ommastrephidae, which becomes a concern for the international commercial
90 market (Johnson 2007; Chapela et al. 2003), or often, unintentional substitution, where
91 due to lack of systematic knowledge, suppliers themselves are unable to correctly identify
92 the species, assigning them umbrella terms that shelter many different species (Cawthorn
93 et al. 2013; Kroetz et al. 2020; Sharrad et al. 2023).

94 Molecular methods have been widely used, firstly, to identify new species or
95 populations of different species, thus providing a complement to pure systematic studies,
96 with their applicability in the identification of species already consolidated in several
97 different taxonomic groups (Virgilio et al. 2010; Hollingsworth et al. 2011, Gales et al.

98 2022; Rodrigues-Filho et al. 2023), especially DNA barcode methods (Hebert et al. 2003).
99 This technique is based on analyzing the variability of a short nucleotide sequence to
100 assess differences between species (Hebert et al. 2003). The cytochrome oxidase subunit
101 I (*coxI*) gene was initially proposed by Hebert et al. (2003), this region of the mitogenome
102 includes both primary and conserved sites, as well as a suitable sequence of variation that
103 allows differentiation between species (Hellberg et al. 2011).

104 There have been many criticisms regarding the choice of the *coxI* gene as a
105 barcode region, such as the presence of pseudogenes (Rubinoff 2006; Rubinoff et al.
106 2006). For mollusk species this is a major problem since several studies have already
107 demonstrated the presence of alterations in the mitochondrial genome, including
108 reorganizations, gene duplications and deletions (Boore and Brown 1995; Boore 1999),
109 in addition to bi-parental inheritance in bivalve species (Hoeh et al. 1996). For cephalopod
110 species, the main limitations of the application of the *coxI* gene are found in the
111 Ommastrephidae family due to several species having two copies of this gene (Yokobori
112 et al. 2004), making its application as a DNA barcoding tool inappropriate for this group.
113 The large ribosomal region *rrnL* (also known as 16S rDNA) is a suitable alternative to be
114 used as a molecular region for species identification, due to its conserved nature and large
115 number of copies in mitochondria, having already been widely used in resolving
116 phylogenetic analysis in cephalopod species (Bonnaud et al. 1994; Warnke et al. 2004;
117 Sales et al. 2013; Sales et al. 2019; Costa et al. 2021).

118 Among the main advantages of using molecular methods in the identification of
119 fishery products is the fact that it is easy to amplify target regions, as well as the fact that
120 any portion of the body of a specimen or individuals from preparation processes such as
121 cooking, frying, canning can be used (Teletchea et al. 2005; Villanueva-Zaya et al. 2021;
122 Mottola et al. 2022). In this way, the applicability of molecular methods can help in the
123 correct identification of the species being commercialized, which can reveal not only
124 economic losses due to substitutions, as well as identify commercialization's of species
125 that may pose health risks (Von der Hayden et al. 2010) or the presence of endangered
126 species with prohibited capture, fishing and landing status, making it necessary to use
127 appropriate tools to confirm product labeling and avoid commercial fraud (Barbuto et al.
128 2010). Reliable molecular tools for barcoding DNA analysis have been developed to
129 protect consumers from food frauds and health hazards and to improve the monitoring of
130 endangered species due to overfishing and illegal commercial activities (Filonzi et al.
131 2023).

132 When complemented by molecular methods, market research can provide accurate
133 species identification, even for products that have been through the finning and
134 processing before sale (Palumbi 2007). DNA methods have been widely used for forensic
135 analysis of food products because they provide an efficient, informative, sensitive and
136 specific identification stimulus and because they can be applied to highly processed food
137 products (Hebert et al. 2003; Rasmussen and Morrissey 2008).

138 In Brazil, legislation on the cephalopod trade does not require all products to bear
139 the common name and scientific name of the species marketed on their labels, which can
140 lead to the intentional substitution of fish-based products, which can cause economic loss
141 to consumers. The European Union, in an attempt to seek solutions to reduce commercial
142 fraud, has instituted regulations (EC 104/200; EC 2065/2001) that require member
143 countries to make official lists available with scientific, common and commercial names
144 of fish species that are being marketed, as well as requiring that product labels also be
145 clearly informed (Brito et al. 2015).

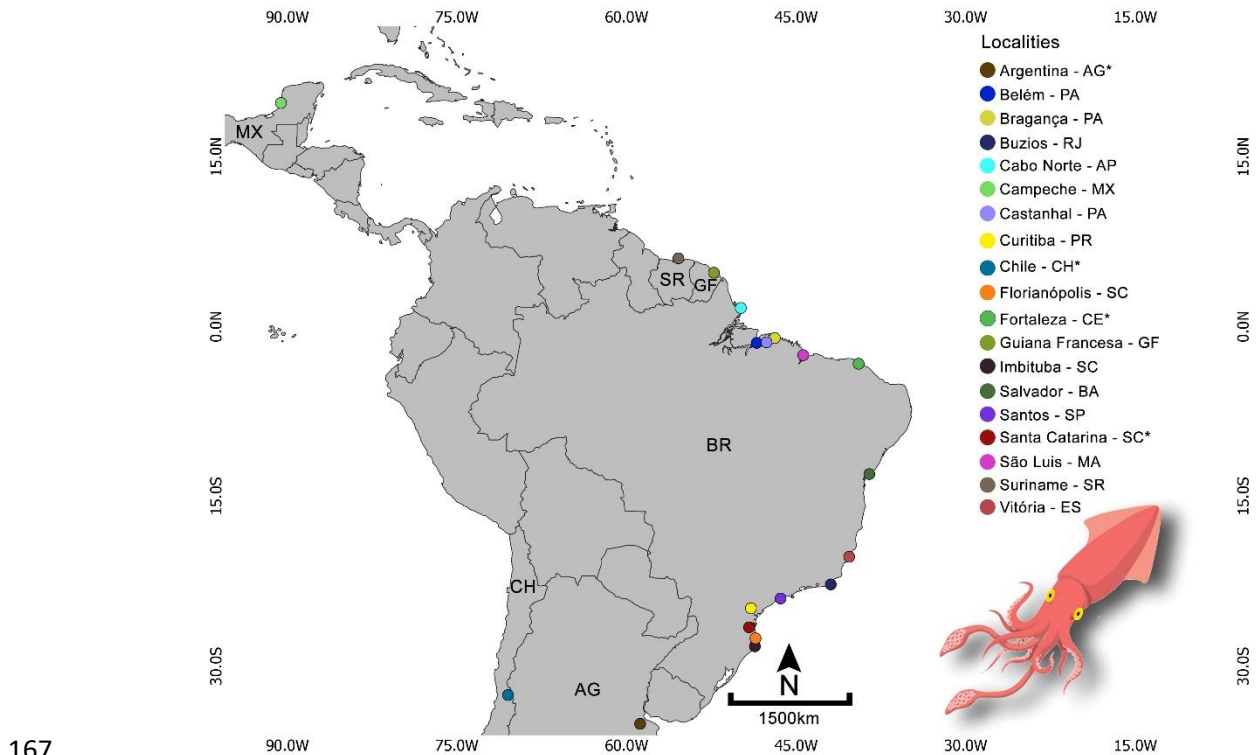
146 Taking into account all the problems exposed regarding the commercialization of
147 cephalopods, as well as the absence of official legislation labeling these products that are
148 sold in Brazil. The present study aimed to use the fragment of the *rrnL* to identify which
149 species of squid are being sold, both in the form of trays containing processed animals,
150 and specimens sold whole in markets and fish fairs in some Latin American countries
151 investigating whether there is evidence of intentional substitutions in the trade of
152 cephalopod species.

153 **Material & Methods**

154 **Sampling**

155 For the present study, both whole and processed squid were collected directly
156 from fishers or from supermarket chains in different regions of Brazil, Suriname, Mexico
157 and French Guiana. For some samples, the place of origin (fishing) and purchase was
158 different, with all information relating to these cases also duly recorded (samples
159 purchased in Fortaleza-Ceara state, Brazil, had identification of origin, coming from
160 Argentina and another package purchased in Belém-Para State, Brazil, also identified the
161 origin of the content as Chile, Figure 1). Subsequently, a small piece of muscle tissue was
162 removed from each sample stored in 70% ethanol and subsequently stored at -4°C until
163 DNA extraction. About samples purchased in a processed form, each ring contained in

164 the packaging was considered as a different sample. Each product tag with the assigned
165 generic identification name, as well as the name of the distributor and fishing company
166 were registered.



168 **Fig. 1:** Map showing all squid sampling places utilized in the present study. Colored circles correspond to
169 each localities. See Table 1 for further definitions for localities names marked with *. Abbreviations
170 represent the capitals name of each countries where: AG=Argentina; BR=Brazil; CH=Chile; GF=Guiana
171 Francesa; MX=Mexico; SR=Suriname. Map created with Ogis. Shapefile with projection WGS84. All
172 Editions were made on Photoshop Cc2023. Source: Shapefile IBGE.

173

174 DNA Extraction, PCR and Sequencing

175 Total DNA was extracted from muscle tissue using the CTAB DNA extraction
176 protocol (Doyle and Doyle 1987). The *rrnL* fragment was isolated and amplified by
177 polymerase chain reaction (PCR) using the primers: L1987 (5'-
178 GCCTCGCCTGTTTACCAAAAAC-3') and H2609 (5'-
179 CGGTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991). The reactions were carried
180 out containing a final volume of 15 μ l, with 2.4 μ l of dNTP (1.25mM), 1.5 μ l of buffer
181 solution (10X), 0.8 μ l of $MgCl_2$ (50 mM), 0.6 μ l of each primer, 0.12 μ l of Taq DNA

182 polymerase (5 U/ul), 1 µl of genomic DNA (200 ng/ul), and ultrapure water to complete
183 the final reaction volume.

184 Reactions were performed according to Sales et al. (2013), whose protocol
185 consists of: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation
186 at 94°C for 30 s, hybridization at 51°C for 1 min and extension at 72°C for 2 min, with
187 the final extension step of 72°C in a period of 7 min. The quality and size of the PCR
188 product were checked using 2% agarose gel electrophoresis. For sequencing of the
189 fragments obtained, the PCRs were previously purified using 65% isopropanol and 70%
190 ethanol, and the sequencing reactions were carried out with reagents from the BigDye
191 Terminator V3.1 Cycle Kit and then sequenced on the SeqStudio Genetic Analyzer
192 sequencer (Applied Biosystems).

193

194 Data analysis

195 The generated DNA sequences were aligned with the CRUSTAL W automatic
196 alignment tool (Thompson et al. 1997) implemented in the Bioedit v7.0.9.1 program (Hall
197 1999). Subsequently, the sequences underwent visual inspection for possible automatic
198 alignment errors, mainly in the hyper variable region of the *rrnL* region.

199 Two validation methods for molecular identification of the samples were used in
200 the present study. At first, the *Blastn* tool, available on the GenBank portal
201 (<https://www.ncbi.nlm.nih.gov/genbank/>) was used following a genetic similarity
202 criterion of 98% for the taxonomic signature specific to each sequence. Based on the
203 result of *Blastn*, sequences available from NCBI were aggregated for subsequent
204 phylogenetic inferences with the following species: *Doryteuthis pleii* Blainville, 1823;
205 *Doryteuthis pealeii* Lesueur, 1821; *Doryteuthis sanpaulensis* Brakonieccki, 1984;
206 *Lolliguncula brevis* Blainville, 1823; *Illex argentinus* Castellanos, 1960; *Dosidicus gigas*
207 d' Orbigny, 1835; *Nototodarus sloanii* Gray, 1849; *Uroteuthis duvaucelii* d' Orbigny,
208 1835. The number of sequences generated in this study with the access code, as well as
209 the sequences downloaded from GenBank are available in supplementary material 1.
210 Additionally, for species delimitation as a second identification method, the TIM+F+G4
211 evolutionary model was estimated by ModelFinder software (Kalyaanamoorthy et al.
212 2017), as the best model to our dataset. Subsequently, a Maximum Likelihood
213 phylogenetic tree was estimated with IQ-tree v2.2.0 program package (Minh et al. 2020)

214 using Ultrafast Bootstrap (Hoang et al. 2018) based on 1000 pseudoreplicates. The
215 software Figtree v1.4.4 (Rambaut 2010) was used to edit the phylogenetic tree. To
216 visualize the data, Barplot and PieDonut were generated in Software R (R Core Team
217 2022), using the “ggplot2” and “webr” packages, respectively.

218

219

220 **Results**

221 Molecular identification

222 A total of 552bp of the *rnl* mitochondrial gene fragment were generated from
223 181 squid samples. Comparison of sequences using the *Blastn* revealed the presence of
224 eight species that were widely traded throughout the Latin American region, namely: *D.*
225 *pleii*, *D. pealeii*, *D. sanpaulensis*, *L. brevis*, *I. argentinus*, *D. gigas*, *N. sloanii*, *N. gouldi*
226 and *U. duvalcelii* (Table 1).

227

228 All sequences evaluated in the present study, when compared to data from
229 Genbank, obtained an identification at species level with >98% similarity. Of the 181
230 samples identified, 18 samples showed 100% similarity with *D. pleii* (n=13), *D. pealeii*
231 (n=2) e *L. brevis* (n=3), from the following locations respectively: Bragança,
232 Florianópolis, Búzios, Campeche and Curitiba. The remaining of the identified samples
233 (47) had a minimum similarity value of 99.80% with *D. pleii* (n=9), *L. brevis* (n=28), *D.*
234 *sanpaulensis* (n=5) e *D. pealeii* (n=5), from the following locations: São Luís, Imbituba,
235 Santos, Cabo Norte (all four from Brazil), and Suriname.

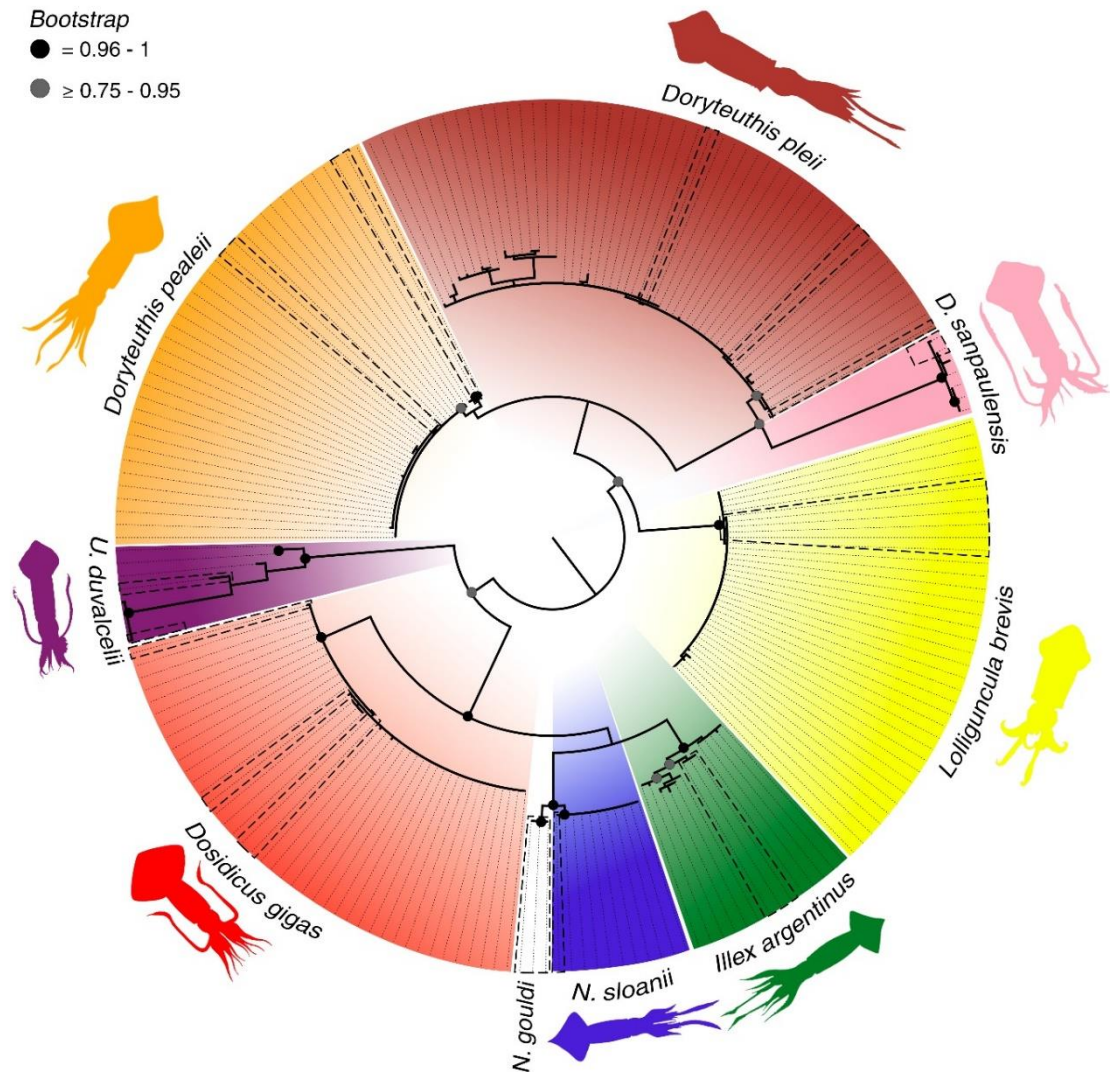
236 Furthermore, 11 samples showed a minimum similarity value of 99.79% with *I.*
237 *argentinus* (n=5) and *D. pleii* (n=6) coming from Santa Catarina, Argentina and
238 Suriname. 34 samples showed a similarity value of 99.59% with the species *D. pleii*
239 (n=8) and *D. pealeii* (n=26) from Salvador, Fortaleza, Vitória and Cabo Norte,
240 respectively. Among the samples from French Guiana and Belém, 11 samples showed a
241 minimum similarity value of 99.43% with the following species, *U. duvaucelii* (n=6), *D.*
242 *pleii* (n=4) and *D. gigas* (n=1). Finally, 36 samples obtained a similarity value of 99.41%
243 for *D. gigas*, from Chile (n=9) and Castanhal (n=27). Eight identified samples showed a
244 similarity of 99.39% with *D. pleii*, coming from Cabo Norte. Among the samples from

245 Florianopolis, six showed a minimum similarity value of 99.21% with *I. argentinus* while
246 10 remaining samples from French Guiana showed a value of 98.88% for *N. sloanii*. No
247 kind of species substitution (intentional or not) was found in the present study.

248 Twenty-nine sequences were added from GenBank, based on the degree of
249 similarity of previously identified sequences, 27 of which are related to species identified
250 with *Blastn* and three sequences of the species *N. gouldi*, resulting in a final alignment of
251 210 sequences. All sequences previously identified in *Blastn* were recovered in highly
252 supported clades, separating two genetically distinct families, Loliginidae and
253 Ommastrephidae, with five and three species identified, respectively. The results found
254 with the Maximum Likelihood tree were in agreement with the genetic similarity analysis
255 (NCBI-Blast), except in some cases referring to the structures within some species (Fig.
256 2).

257

258



259

260 **Fig 2.** Maximum Likelihood phylogenetic tree of the squid species identified in the
261 present study based on *rrnL* mitochondrial gene fragment (16S rDNA). Each species is
262 represented by its respective colored outline illustration: *D. pleii* – Brown; *D. pealeii* –
263 Orange; *D. sanpaulensis* – Pink; *L. brevis* – Yellow; *D. gigas* – Red; *U. duvalcelii* –
264 Purple; *N. sloanii* – Blue e *I. argentinus* – Green. Dashed shapes indicate the positioning
265 of DNA sequences obtained from GenBank that were associated to each respective
266 species clade. Image processing was done using the Adobe Photoshop Cc2023 software.

267

268 Within the family Loliginidae, three genera were recovered, *Loliginula*,
269 *Doryteuthis* and *Uroteuthis*, totalizing 123 individuals identified. The most abundant
270 species was *D. pleii* with 48 individuals forming a branch with 78% bootstrap support
271 associated with different regions (Table 1, Figure 2). *Dorytheuthis pealeii* corresponds to
272 the taxon with the second highest abundance among Loliginidae, totaling 33 identified

273 sequences forming two clades with support values of 96% and 78%, respectively.
274 *Lolliguncula brevis* (n=31) formed a group with the sequences downloaded from
275 Genbank according to the respective taxon with a support value of 97%. *Urotheutis*
276 *duvaucelii* (n= 6) was recovered with a high support value (99%), however there is
277 evidence of strong distinct grouping structures with sequences from GenBank.
278 *Dorytheuthis sanpaulensis* (n=5) formed a cluster with 100% support value with a
279 structure established between sequences from Santos-SP.

280 Species from three other genera were also identified, *Dosidicus*, *Illex* and
281 *Nototodarus* (all belonging to the family Ommastrephidae), with a total of 58 individuals.
282 The most abundant species for this family was *D. gigas* with 37 individuals forming a
283 branch with 99% support value. *Illex argentinus* (n=11) formed a clade with 98% support
284 with internal structuring being recovered with 87% support from sequences belonging to
285 samples from Florianopolis In the case of sequences of *N. sloanii* (n=10), 97% support
286 value separated them from *N. gouldi*, also demonstrating a support value of 99% that
287 highlights this difference between the sequences, since the grouping with *N. sloanii* got
288 the value of 97% together with the sequences from Genbank. In the previous analysis,
289 some sequences obtained high genetic similarity with *N. gouldi*, but it was possible to
290 observe the separation with a high bootstrap value among the congeners present in the
291 database.

292

293 Squid Processing level

294 Most of squid sampled (71%) were being sold in whole form, while the remaining
295 in processed form (29%) (Fig. 3a). The majority of individuals sold comprise Loliginidae,
296 especially individuals that were being sold (whole) in their entirety at fairs. The most
297 common species found was *D. pleii* (Fig. 3b) from the Southwestern Atlantic distributed
298 among the states of Amapá, Pará, Maranhão, Bahia, Rio de Janeiro, Espírito Santo and
299 Santa Catarina, all samples were being sold whole in supermarkets and fairs.

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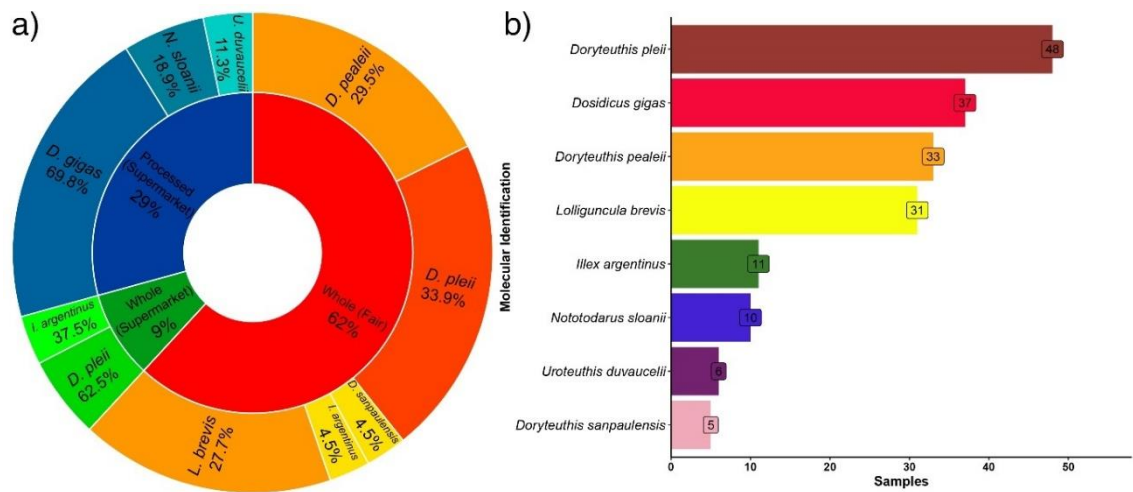
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310 **Fig 3.** Graphs demonstrating the quantified results of the present study. a) PieDonut
311 indicating the samples identified based on the type of processing and place of
312 commercialization of the squid. b) Barplot summarizing the abundance of
313 commercialized squid species.

314

315 The second most frequently found species was *D. gigas* purchased in the State of
316 Pará. In the case of this species, the individuals were being sold in a processed form (cut
317 into rings), containing generic information on the labels as “*Lula Nacional*” (National
318 Squid), “*Lula Brasileira*” (Brazilian Squid), or just “*Lula*” (Squid). The occurrence of
319 labeling errors was observed in 100% of the products, when these samples come from the
320 cities of Belém and Castanhal. Only one sample that was being sold in a supermarket in
321 Belem was being sold without any information on the product label, containing only on
322 the supermarket freezer label such as “Squid in rings”. The third most common species
323 was *D. pealeii* from Cabo Norte (Brazil) and Mexico. Another common species was *L.*
324 *brevis*, being present in fairs from two locations Cabo Norte and Santos. All samples
325 identified in the present was commercialized as whole being sold in fairs.

326

327 Among the less frequently traded species identified in this study were *I.*
328 *argentinus*, found both in fish markets in the city of Fortaleza but with Argentina as the
329 place of origin on the label, and supermarket chains in Santa Catarina as a whole. The
330 species *N. sloanii* was also identified less frequently, being sold in French Guiana in
processed form (salads) in supermarkets, followed by *U. duvaucelii* which was also sold

331 in French Guiana in the same way of *N. sloanii*, followed by *D. sanpaulensis* and for *D.*
332 *pealeii* from the Equatorial Northwest Atlantic, a genetically distinct lineage of *D. pealeii*
333 from North Atlantic (Sales et al. 2024).

334

335 **Discussion**

336 Incorrect labels end up deceiving OU jeopardizing consumers' conscious choices,
337 posing potential health risks (Tartre 2016), as well as generating financial losses for
338 consumers (von der Heyden et al. 2010). Additionally, from a conservation point of view,
339 they “mask” the commercialization of species with prohibited fishing status (Palmeira et
340 al. 2013; Zeng et al. 2019) or, when commercialized throughout the year, species that are
341 undergoing period of reproductive closure end up being captured, thus causing possible
342 future impacts on their recruitment and population stock (Stawitz et al. 2016).

343 Processing often removes or damages diagnostic characteristics that are important
344 for the identification of species through conventional taxonomy (Barbuto et al. 2010; Di
345 Pinto et al. 2013), which ends up contributing to the possible replacement of species of
346 greater value commercial for others of lower value. The results generated in the present
347 study indicate that a number of labeling errors occurred in products that had specimens
348 sold in 2015 in a processed form as squid rings sold with the generic name “Lula
349 Nacional” (National Squid), a name that suggests to the consumer that the product was
350 caught in Brazil. All samples derived from squid labeled this way were genetically
351 identified as *D. gigas*, a squid species found exclusively in the Pacific Ocean (Markaida
352 et al. 2005; Jereb and Roper 2010). A single sample that was being sold in processed form
353 at the supermarket in Belém, with identification of “*Lulas em anéis*” (Squid Rings) in
354 June 2022 which was also identified as *D. gigas*, was not in accordance with SDA
355 Ordinance No. 544, of March 14, 2022 (article 16 in paragraph 1), where it is emphasized
356 that “For products of the type *D. gigas*, it is mandatory to include scientific nomenclature,
357 such as the explanatory affix to the product's sales name”. This is the only species of
358 cephalopods for which there is an ordinance that regulates commercialization in all
359 Brazilian territory.

360 Nevertheless, the replacement of commercial species cannot always be
361 characterized as intentional commercial fraud. A portion of inappropriate labeling occurs
362 unintentionally, largely due to the morphological characters used to identify species that

363 can be easily mistaken, or simply when using vernacular names that are common to more
364 than one species. To a certain extent, mislabeling may be the result of difficulties in
365 identifying species, which occurs mainly in groups with few informative morphological
366 characters and with a long history of cryptic species, such as in the Loliginidae species
367 (Sales et al. 2013; Cheng et al. 2014; Costa et al. 2021, Sales et al. 2024a) and
368 Ommastrephidae (Fernández-Álvarez et al. 2020). Confusion can also arise due to
369 different species having the same vernacular name, or different common names in
370 different regions for the same species (Brito et al. 2015). However, usually the
371 substitutions of species, which always characterize commercial fraud, aims to obtaining
372 higher profits (Pauly et al. 2005; Worm et al. 2006).

373 Regarding the species purchased whole from markets in the Equatorial Northwest
374 Atlantic that were identified in the present study, some considerations must be made. The
375 squid fauna proposed for these regions is very similar to that reported for some regions
376 of the North Coast of Brazil (Jereb and Roper, 2010). The presence of *D. pleii*, *L. brevis*
377 and *D. pealeii* in these locations are in accordance with what has been proposed by recent
378 molecular reviews with specimens from the North Coast of Brazil (Sales et al. 2011; 2013;
379 2014; 2017; 2024a; Costa et al. 2021). Two species of *Doryteuthis* identified in the
380 present study are of high commercial importance in Brazil and in North America (Herke
381 and Foltz 2002; Jereb and Roper 2010; Postuma and Gasalla 2015), and, it is important
382 to clarify that the lineage of *D. pleii* commercialized in Brazil corresponds to the actual
383 species (Sales et al. 2017), while the lineage exploited in North America corresponds to
384 a not yet described species. The same specific delimitation is valid for *L. brevis* (Sales et
385 al. 2014) and, although this is not a commercially important species (Good et al. 2023),
386 on the North coast of Brazil it is often consumed by fishers (Sales et al 2024b). For *D.*
387 *pealeii*, the pattern is the opposite, with the lineage that occurs in Brazil being a new
388 undescribed species (Sales et al 2024a). According to our results, most of the samples
389 were in agreement with the fauna found on the Brazilian coast (Haimovici et al. 2009),
390 indicating that the species that were being sold at fairs come from artisanal fishing, which
391 contributes to the lack of supervision by the responsible governmental authorities, making
392 it difficult to verify frauds. The lack of evidence of substitution among the products that
393 were used in the present study denotes the difficulty in verifying the occurrence of
394 substitution in samples that are sold in Brazil, due to the lack of information on the label,
395 such as the name of the specific species name being sold for the consumer, thus making
396 it difficult to track the replacement of one species of squid by another of the same genus,

397 or family.

398 For the species in the present study, in relation to other species sold in markets in
399 Brazil and in Latin American countries utilized here, there was no case of commercial
400 fraud, but rather a labeling error, since the specimens were sold only as “Squid”. The
401 sequences that were identified as *U. duvaucelli* demonstrated evidence of strong different
402 clades with sequences originating from the GenBank. This result is in agreement with
403 other previous studies that already indicated the presence of multiple lineages within *U.*
404 *duvaucelli*.

405 *U. duvaucelii*, presents a relevant morphological and genetic polymorphism
406 within a wider geographic area (Sin et al. 2009), and is of commercial importance in the
407 Asian region, being mainly fished by artisanal fleets, but it already constitutes a high
408 volume of exports, specially from Thailand to other countries. This species represents
409 68% of the cephalopod species exported by India (Jereb and Roper 2010). The clade
410 formed in the Maximum Likelihood (ML) tree reinforces this finding, although, the
411 identification with *Blastn* suggests that all sequences generated in the present study
412 correspond to the taxon in question. Different morphotypes of *U. duvaucelii* are
413 recognized by commercial fishing. One of these are formed by a relatively large and a
414 smaller form from the Gulf of Aden and the Arabian Sea (Nesis 1987), and two other
415 forms are known, one more robust and one more slender from the Eastern Pacific
416 (Okutani 2005). In this sense, the diversity of the group may be underestimated,
417 significantly affecting the management and conservation of the species. The results of
418 molecular reviews carried out in recent years reinforce the presence of different species
419 within *U. duvaucelli* (Sales et al. 2013; Krishnan et al. 2022). One of the bottlenecks from
420 the point of view of fisheries management and resource exploitation arises precisely when
421 a given species targeted for exploitation has a cryptic species, because from the point of
422 view of management and commercial exploitation, it would not be a single species under
423 exploitation, but two or multiple at the same time, which can lead to a reduction in the
424 population stock of these lineages, as the reproductive period and breeding area are often
425 different between them (Awise 1996).

426 Finally, the sequences of *N. sloanii* were well separated in the phylogenetic tree,
427 although, for some sequences *Blastn* indicated a high similarity with *N. gouldi*. However,
428 in the same topology, *N. sloanii* and *N. gouldi* formed distinct clades with high support
429 value. One possible cause for this is the high level of similarity between species since
430 until recently *N. gouldi* was considered a subspecies of *N. sloanii*. According to the

431 information contained on the packaging label that these samples came from FAO fishing
432 area 81 (Southwest Pacific), which is consistent with the species' distribution area. It is
433 worth noting that the samples identified as *N. sloanii* came from French Guiana and were
434 being sold processed in supermarkets, containing all the information regarding the
435 labeling described by the European Union in the “EC 104/200” regulation; EC
436 2065/2001”. Data obtained from FAO indicate that global catches for this species
437 decreased in 2021, reaching a volume of 29,832 tons, data lower than the years 2020 and
438 2019, which had a volume of 41,928 and 43,795 tons, respectively.

439 As there is no comprehensive Brazilian legislation for cephalopods that requires
440 the common and scientific name of all species marketed labels, there is also no official
441 list containing the common, commercial, or scientific names of the species sold in the
442 country as well for Latin America Countries.

443 **Conclusion**

444 Based on the results of the present study, the samples identified as *D. gigas* that
445 were being sold in a processed form (rings) in supermarkets, were considered incorrect
446 labeling. For all other taxa identified in the present study, no occurrence of intentional
447 or unintentional replacement was found. Due to the fact that cephalopod species present
448 high morphological plasticity, the use of molecular tools becomes necessary for the
449 correct identification and labeling of species that are commercialized. With the high
450 levels of incorrect labeling recorded, the present study shows the importance of using
451 molecular methods that help in the identification of squid species that are
452 commercialized. The lack of specific Brazilian legislation on labeling for all Brazilian
453 fauna cephalopod (as such Latin America Countries) products may favor intentional and
454 unintentional substitutions, bringing problems such as economic fraud or species
455 substitutions. We are also relieved that our results continue to debunk the urban myth of
456 calamari substitutions with pig rectum (Grenoble, 2013).

457

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469

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471 **Conflicts of interest:** There are no conflicts of interest to declare.

472 **Ethics approval:** The research was developed under the ethical guidelines of
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474 **Consent to participate:** Authors declare consent to participate.

475 **Consent for publication:** Authors declare consent for publication if the manuscript is
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481

482 **Data Availability Statement:** The sequence data are in submission processes at the
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486

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740 **Table Legend**

741 **Table 1.** Squid species identified in the present study based on rrnL fragment (16S
742 rDNA). Data about sampling places, label identification, kind of processing, and genetic
743 similarity match is provided. Numbers above some of the sampling places indicate the
744 actual origin place from where the samples came: 1 – Santa Catarina State; 2 – Argentina;
745 3 – Chile. See Supplementary Material 1 for detailed table showing the *Blastn* results per
746 sample.

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748 **Supplementary Material**

749 Supplementary material 1: Metadata of the sequences used in the present study. In bold,
750 they indicate sequences downloaded from NCBI-Genbank.

751 Supplementary material 2: Maximum Likelihood (ML) phylogenetic tree with support
752 values.

753 Supplemental material 3: A) R Scrypt built to generate PieDonut. B) Datasheet used to
754 generate the graph.

755 Supplemental material4: A) R script built to generate the bar chart. B) Datasheet used to
756 generate the Barplot.

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