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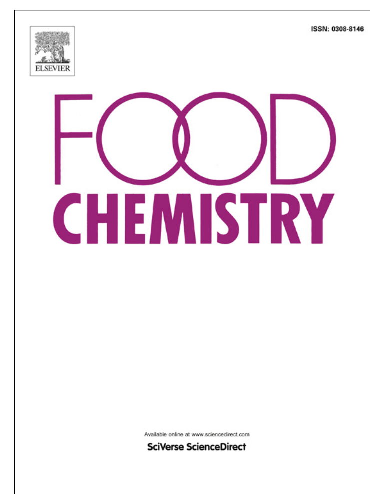

Developed of a method for the genetic identification of ling species (*Genypterus spp.)* in seafood products by FINS methodology

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1 **Developed of a method for the genetic identification of ling species (*Genypterus*
2 *spp.)* in seafood products by FINS methodology**

3

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5

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8

9 **Abstract**

10 In the present work a method of authentication of *Genypterus* and their substitute
11 species was developed, by means of Polymerase Chain Reaction (PCR) technique
12 followed by phylogenetic analysis (FINS, *Forensically Informative Nucleotide*
13 *Sequencing*). The methodology developed allows the identification of all the studied
14 species using the mitochondrial *cytochrome oxidase subunit I* gene (*COXI*) as molecular
15 marker.

16 Substitutions of the species belonging to *Genypterus* genera by other species with minor
17 value can take place, since in a lot of seafood products , is not possible the assignation
18 to a particular species based on morphological traits, because it are removed in the
19 transformation process.

20 In this work several methodological strategies were developed and all of them allow the
21 authentication of the studied species in any kind of products, from fresh or frozen fish,
22 to ready-cooked meal.

23 Therefore, the proposed methodology can be used as a routine method to avoid the
24 mislabelling in the marketing of *Genypterus* species. Also this methodological
25 approximation is suitable to assess the correct seafood traceability of the products
26 elaborated from the mentioned species.

27

28 **KEYWORDS**: *Genypterus*, Genetic Identification, FINS, *Cytochrome oxidase subunit I, COXI.*
29 Ling, Kingklip.

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33 **INTRODUCTION**
34
35 Ophidiiformes order, a group of marine fish, are characterised by having pelvic fins with
36 0-2 soft rays, located below the anterior part of the head, at the operculum, or in front of
37 it; they also have long dorsal and anal fins, extending to and usually joined to the caudal
38 fin; and fins with soft rays. This order include 5 families, one of them, Ophidiidae,
39 contains four subfamilies and 50 genera, the subfamily Ophidiinae are known as cusk-
40 eels are characterised for having pelvic fins supported by contains the genus
41 *Genypterus*. This genus include 6 species (Bisby, Roskov, Orrell, Nicolson,
42 Paglinawan, Bailly, et al., 2011): *Genypterus blacodes* that inhabit the Southwest
43 Atlantic, the Chilean, New Zealand and Australian coast; *G. maculatus and G. chilensis*
44 *that* occurs in the Chilean coast; *G. tigerinus* wich is distributed in Australian coast; *G.*
45 *capensis* that is found in South African coast; and *G. brasiliensis* which are distributed
46 in Atlantic coast of South America (Figure 2).
47 Ling are fish with a commercial importance, especially in the South hemisphere, they
48 are very appreciated and widely consumed (Heyden, Barendse, Seebregts, & Matthee,
49 2010). In other countries, like Spain, these fish are also appreciated and usually
50 purchased frozen. The total world catch of *G. blacodes* in 2007 was approximately
51 50.000 tons (FAO, FIGIS, Fisheries and Aquaculture Department). These data reflect
52 the importance of this taxonomic group as resource for human consumption.
53 Overexploitation of appreciated species has leaded in the past to loss of the resource, in
54 the case of some *Genypterus* species, like the *G. blacodes*, the Federal Fisheries Council
55 of Argentina (CFP) has established a total allowable catch (TAC) of 18000 tons for
56 2010, because landings has decreased steadily since 2006 from 20.000 t to 15.000 in
57 2009. However, there is a need for the control of fishing beyond the established TACs,
58 these practices widely known as illegal, unregulated and unreported fishing (IUU) are
59 the main problem for the correct management of exploited fish. Fish species
60 identification can be very difficult when fish are processed on board or onshore and is
61 sold in the markets without the main external morphological characters. There are
62 labelling regulations in most important commercial areas which establish which species
63 should be labelled under a particular commercial name. For instance, the European
64 Commission Regulation 104/2000 and 2065/2001 establish dispositions relative to the
65 information of consumers about fish products and the traceability control (EC, 2000,
66 2001).

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67 *Genypterus* species are valued for the high quality of their meat, but there are some
68 hygienic-sanitary aspects linked to the fish quality, as for instance the strictness of
69 hygienic-sanitary conditions of transformation plants, which are worse in third world
70 countries than in developed ones.
71 The main way of marketing of these species is whole specimens, tails, slices, fillets or
72 minced meat, especially in countries of the Northern hemisphere, this fact difficulties its
73 correct identification because usually they are devoid of morphological characters.
74 However, as mentioned before the existence of legal constraints to exploitation of some
75 of these species could lead to a deliberate or unintentional substitution of some of these
76 species. This fact can be overcome by the application of non-morphological analytical
77 methods, DNA-based methods developed for the genetic identification of species
78 belonging to different taxonomic groups, as for instance cephalopods (Santaclara,
79 Espiñeira, & Vieites, 2007), hakes (Sanchez, Quinteiro, Rey-Mendez, Perez-Martin, &
80 Sotelo, 2009), bivalves (Espiñeira, González-Lavín, Vieites, & Santaclara, 2009a), or
81 anchovies (Santaclara, Cabado, & Vieites, 2006).
82 There are not published studies dealing with the problem of species identification of
83 species of the genus *Genypterus*, only Larrain *et al*. describes the sarcoplasmic
84 electrophoretic patterns for *G. blacodes*, but since the study did not consider the patterns
85 of other *Genypterus* species is not suitable for the identification within the genus
86 (Larraín, Abugoch, Quitral, Vinagre, & Segovia, 2002). Since the exploitation of this
87 group of species is very important to some countries there have been some works
88 dealing with the assessment of the population structure of *G. blacodes* by the use of
89 microsatelli loci (Ward & Reilly, 2001).
90 In the present work one method for the genetic identification of species belonging to the
91 genus *Genypterus* and related species was designed. It is based on the PCR
92 amplification and phylogenetic analysis, using the *COXI* gene as molecular marker. The
93 importance of the present work lies in the fact that up to now there is not any work
94 about the genetic identification of the six species of the genus *Genypterus*. Moreover,
95 *Brotula multibarbata* and *Kataetyx rubrirostris* were included as out-group because
96 these species could be used as substitute of *Genypterus* in some elaborated products.
97 The methods such as the herein proposed are necessary to improve the quality of fish
98 products, since they will allow to guarantee their correct traceability and authenticity.

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99 **MATERIALS AND METHODS**

100 **Sample collection, storage and DNA extraction**

101 Samples of different *Genypterus* species were collected from several locations around
102 the world. Each sample contained a variable number of individuals comprised among 2
103 and 5 (Table 1). When it was possible, the individuals were identified attending to
104 morphological characters (Nielsen, Cohen, Markle, & Robins, 1999). In other cases, the
105 samples were authenticated in origin by collaborators.

106 DNA was extracted from 30 mg of muscle in fresh and frozen samples, according to the
107 method described by Blanco *et al*. (Blanco, Pérez Martín, & Sotelo, 2008). The purity
108 and concentration of the extracted DNA was measured using a UV-vis
109 spectrophotometer (*Nanodrop 2000, Thermo*). Samples and DNA extractions were
110 appropriately labelled and stored at -80ºC for subsequent studies.

111

112 **PCR amplification and DNA sequencing**

113 The primers used in this work were designed from the following sequences obtained in
114 the *National Center for Biotechnology Information* (NCBI) database: EF609355 (*G.*
115 *tigerinus*); EU074432, EU074431 (*G. brasiliensis*); EU074430, EU074429, EU074428,
116 EU074427, EU074426, EF609354 (*G. blacodes*). These sequences were aligned with
117 BioEdit version 7.0.0 (Hall, 1999), allowing the location of conserved regions for
118 primer design.

119 In all cases PCR reactions were carried out in a total volume of 50 µL with the
120 following composition: 100-300 ng of template DNA were added to PCR mix
121 consisting of 0.8 mM of dNTP mix (*GenScript*), 5 µL of 10X buffer, 2 mM of $MgCl_2$,
122 0.75 unit of *Green Taq DNA Polymerase* (*GenScript*), 0.8 µM of each primer (*Sigma*
123 *Genosys*) and molecular biology grade water (5 Prime) needed to adjust the final
124 volume.

125 PCR were carried out in a GeneAmp®PCR System 9700 (*Applied Biosystems*), in the
126 following conditions: a preheating step at 95º C for 3 min, 35 cycles of amplification
127 (95 ºC for 30 s, 52 ºC for 1 min, 72 ºC for 1 min 30 s) and a final extension step of 72
128 ºC for 5 min.

129 PCR amplicons were visualized on 2% agarose gels (*Pronadisa*) in 0,5X TBE buffer
130 (*Sigma*) with RedSafe™ 1X (*iNtRON Biotechnology*) using the Mini-Sub®Cell GT
131 system (*Bio-Rad*). DNA fragments were visualized using the Gel Documentation

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132 System *Gel Doc XR System* and the software *Quantity One®* *v 4.5.2* (*Bio-Rad*). The
133 *O´GeneRuler™ 100 bp DNA Ladder* (*Fermentas*) was used to estimate the size of the
134 amplicons.
135 The PCR products were purified using the *Nucleospin Extract II kit* (*Macherey-Nagel*)
136 according to the manufacturer's instructions. The concentration and purity were
137 estimated by means of a *NanoDrop 2000 Spectrophotometer* (*Thermo Scientific*).
138 Subsequently were sequenced on an *ABI Prism 310 Genetic Analyzer* (*Applied*
139 *Biosystems*) using the primers of the PCR amplification and the *BigDye Terminator*
140 *Cycle Sequencing Ready Reaction Kit v 1.1* (*Applied Biosystems*) following the
141 supplier's recommendations.
142 The electropherograms obtained were analyzed using the *DNA Sequencing Analysis*
143 *Software v 3.4* (*Applied Biosystems*) and manually corrected with *Chromas v 1.45 (Mc*
144 *Carthy, 1996)*. The nucleotidic sequences obtained were aligned with *BioEdit v 7.0*
145 software (Hall, 1999) and used for estimating intraspecific and interspecific distances
146 with *DnaSP v 4.0 (Rozas, Sanchez-DelBarrio, Messeguer, & Rozas, 2003).*
147
148 **Development of FINS methodology using *COXI* gene**
149 The sequences herein obtained and those ones downloaded from *GenBank* database
150 were used to carry out the phylogenetic analysis. The following DNA sequences
151 belonging to *Genypterus* species were downloaded from the NCBI database:
152 HM007745, HM007744, HM007743, HM007742, HM007741, HM007740,
153 HM007739, HM007738, HM007737, HM007736, HM007735 (*G. capensis*); EF609355
154 (*G. tigerinus*); EU074430, EU074429, EU074428, EU074427, EU074426 (*G.*
155 *blacodes*); EU074432, EU074431 (*G. brasiliensis*).
156 The phylogenetic analyses were carried out with *Mega 3.0* (Kumar, Tamura, & Nei,
157 2004) using the Tamura-Nei model to calculate the genetic distances between
158 sequences. The inference of the phylogenetic tree was carried out with the Neighbor-
159 Joining method (Saitou & Nei, 1987). The species *Brotula multibarbata* and *Cataetyx*
160 *rubrirostris* were used as outgroup (Accession numbers FJ237650 and GU440264
161 respectively). The reliability of the groups was evaluated by means of bootstrap test
162 with 2000 replications.
163
164
165

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166 **Methodological Validation**

167 Individuals of the different species were authenticated on the basis of their
168 morphological traits. These reference individuals were used to manufacture different
169 products. The most extreme treatment applied to the samples was the sterilization in a
170 steel retort at 115ºC for 50 min, with 1.2 bars of overpressure.

171 Products were analysed with the methods developed in the present work. Results of the
172 species assignment on the basis of morphology and genetic tests were compared.

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173 **RESULTS AND DISCUSSION**

174 **DNA extraction, amplification by PCR and sequencing**

175 The *COXI* gene has been widely used as molecular markers in the genetic identification
176 of a great number of species belonging to different taxa (Espiñeira, González-Lavín,
177 Vieites, & Santaclara, 2008; Ward, Zemlak, Innes, Last, & Hebert, 2005). The selection
178 of mitochondrial molecular markers versus nuclear ones relies on its numerous
179 advantages. Among them, mitochondrial molecular markers, generally, evolve much
180 faster than nuclear markers, and besides there are several copies of mt DNA inside a cell
181 (Mackie, Pryde, González-Sotelo, Medina, Perez-Martín, Quinteiro, et al., 1999) and
182 therefore the abundance of stand is higher allowing more effective PCR amplifications
183 in comparison to nuclear DNA.

184 In this work, we evaluate the *COXI* gene to identify all the species contained in the
185 genus *Genypterus*. The first step of the proposed methodology is the DNA extraction.
186 The method evaluated for this purpose worked correctly, both in fresh and in processed
187 products. The quality and quantity of DNA obtained (evaluated by means of optical
188 measurements) were optimal to successfully amplify the DNA by PCR in all cases. The
189 spectrophotometric values obtained at 260 nm were higher than100 ng/μL, and the
190 260/280 ratio was between 1.8 and 2.0. These values and ratios show a low amount of
191 proteins and RNA in the DNA extracts.

192 A fragment of the *COXI* gene was amplified by PCR using the GenCoi forward and
193 reverse primers herein designed (GenCoi-F 5´-GAG CCG GAA TAG TAG GAA CAG
194 CCT T-3´ and GenCoi-R 5´-GGG TCT CCT CCT CCC GCA GGG TC-3´). This
195 fragment was called *Fragment COXI-610*. Also an internal primer (GenInt-R) was
196 designed in order to amplify a shorter fragment (*Fragment COXI-207),* by using it
197 together with GenCoi-F primer (Figure 1). The sequence of this primer is the following:
198 5´- ATG CTA TGT CGG GGG CG -3´.

199

200 DNA amplification with the primers sets herein designed (GenCoi-F/GenCoi-R and
201 GenCoi-F/GenInt-R (internal fragment)) generated a 610 and 207 bp fragment
202 respectively in all of the samples included in the present study (Table 1).

203

204 The genetic distances among the obtained *COXI* gene sequences and those available in
205 the Gene Bank, reveal that the average intraspecific distance was of 0.002, and the
206 interspecific one was one order of magnitude higher than the intraspecific ones (the

207 mean value for these was 0.032). These distances were calculated using the *Tamura-Nei*
208 model of nucelotidic substitution. The species *G. blacodes* and *G. tigerinus* contribute
209 to this distance, since in these species were detected 3 and 2 haplotypes respectively,
210 while the other species a unique haplotype was detected.
211

212 **Development of the genetic method: PCR product amplification**

213 A methodological strategy was developed for the authentication of *Genypterus* species
214 in processed products, based on the amplification of the *Fragment COXI-207*. DNA
215 extracted from fish which is submitted to transformation process, especially those
216 involving a severe thermal treatment, it is not suitable for amplification with *Fragment*
217 *COXI-610,* because the thermal treatment generates DNA fragmentation. Santaclara *et*
218 *al.* amplified a maximum fragment size of 208 bp to ensure amplification from canned
219 products DNA (Santaclara, Espiñeira, & Vieites, 2007). Pardo *et al.* under certain
220 conditions, amplified fragments higher than 200 bp from canned products (Pardo &
221 Pérez-Villareal, 2004). For this reason, we establish the use of the *Fragment COXI-610*
222 in fresh, frozen, and even moderate thermal treated fish (temperature without pressure);
223 and the use of the *Fragment COXI-207* for products elaborated with thermal treatment
224 and pressure.
225

226 **Phylogenetic analysis: FINS methodology**

227 The FINS technique was evaluated because it has been extensively used in the past for
228 the genetic identification of fish and shellfish as for instance sharks (Blanco, Pérez
229 Martín, & Sotelo, 2008), scombroids (Espiñeira, González-Lavín, Vieites, & Santaclara,
230 2009b) or anchovies (Santaclara, Cabado, & Vieites, 2006). The main advantage of this
231 technique is that uses the information of all nucleotidic positions of the amplified DNA,
232 in front of the RFLP (*Restriction Fragment Length Polymorphism*), Real-Time PCR, or
233 other techniques which only assess a low number of nucleotidic positions (the targets of
234 the used enzymes in the case of RFLP, and the positions of probe and primers in the
235 case of Real-Time PCR).
236 FINS allows the genetic identification of species by means of phylogenetic analyses
237 using a DNA sequence database. Thus, reference sequences belonging to
238 morphologically identified individuals and the sequence of an unknown individual are
239 compared. From this analysis a distance matrix is constructed which is used for building
240 a graphic representation of the matrix, the phylogenetic tree, which allows the genetic

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241 identification of species because samples belonging to the same species are grouped into
242 the same clade (shortest genetic distance). The bootstrap method can be used to obtain
243 the statistical support of the different groups obtained in the phylogenetic tree. It has
244 been calculated that bootstrap values higher or equal to 70% usually correspond to a
245 probabilities higher or equal to 95% which means that the topology is close to the real
246 (Hillis & Bull, 1993), giving a quantitative measurement of the certainty of the
247 assignment of a sample to a particular species.
248 The phylogenetic tree constructed from 561 bp sequences (*Fragment COXI-610* without
249 primers) shows that all the sequences belonging to individuals of the same species are
250 grouped in the same cluster (Figure 3). All clusters are strongly supported, with
251 bootstrap values higher than 86, allowing the reliable assignation of each individual to a
252 particular species. Also, bootstrap values higher than 80 were obtained in the
253 phylogenetic reconstruction carried out with the 165 bp fragment (*Fragment COXI-207*
254 without primers) (Figure 4), showing that both fragments allow the reliable assignment
255 of an unknown sample to its species.
256 Therefore, both proposed strategies allow the amplification of a long enough DNA
257 fragment suitable for the successful discrimination of all commercially important
258 *Genypterus* and analogous species, even in transformed products where the DNA can be
259 highly degraded.
260
261 **Methodological Validation**
262 The results of the genetic analyses of the samples manufactured in the pilot plant of the
263 *Marine Research Institute* were in accordance with the expected based on the
264 morphology characterization. Therefore, the developed method showed a specificity of
265 100%. Also it was checked that the canning process or any other kind of processing,
266 such as smoking, salting, or canning do not affect to the correct operation of the
267 developed methods.
268
269 In conclusion, the technique described in this paper is a useful tool to authenticate the
270 *Genypterus* species in all kind of products, from fresh fish to processed products. It can
271 be useful to verify the correct labelling of fishing products elaborated with these
272 species. Also, this methodology could be useful to check importations and exportations
273 of these species and therefore to trace these products or even determine their
274 geographical origin, thus permitting the control of the allocated total allowable catch

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275 (TACs) for these species, contributing to avoid Illegal, Unregulated and Unreported
276 (IUU) fishing practices.
277 Moreover the analysis can be carried out in less than two working days, and it allows a
278 high throughput screening of all kind of samples. Also, different methodological
279 strategies are proposed, which permit the analysis of fresh, frozen or processed products
280 with total reliability.

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287 **LITERATURE CITED**

288 Bisby, F. A., Roskov, Y. R., Orrell, T. M., Nicolson, D., Paglinawan, L. E., Bailly, N.,
289 Kirk, P. M., Bourgoin, T., Baillargeon, G., & Ouvrard, D. (2011). Species 2000
290 & ITIS Catalogue of Life: 2011 Annual Checklist. Digital resource at
291 www.catalogueoflife.org/annual-checklist/2011/. Species 2000: Reading, UK. .
292 Blanco, M., Pérez Martín, R. I., & Sotelo, C. G. (2008). Identification of Shark Species
293 in Seafood Products by Forensically Informative Nucleotide Sequencing (FINS).
294 *Journal of Agricultural and Food Chemistry, 56* 9868-9874.
295 EC. (2000). European Commission, Council Regulation No 104/2000 of 17 December
296 1999 on the Common Organisation of the Markets in Fishery and Aquaculture
297 Products.
298 EC. (2001). Commission Directive 2001/101/EC of 26 November 2001 amending
299 Directive 2000/13/EC of the European Parliament and of the Council on the
300 approximation of the laws of the Member States relating to the labelling,
301 presentation and advertising of foodstuffs.
302 Espiñeira, M., González-Lavín, N., Vieites, J. M., & Santaclara, F. J. (2008).
303 Development of a method for the genetic identification of flatfish species on the
304 basis of mitochondrial DNA sequences. *Journal of Agricultural and Food*
305 *Chemistry, 56*(19), 8954-8961.
306 Espiñeira, M., González-Lavín, N., Vieites, J. M., & Santaclara, F. J. (2009a).
307 Development of a Method for the Genetic Identification of Commercial Bivalve
308 Species Based on Mitochondrial 18S rRNA Sequences. *Journal of Agricultural*
309 *and Food Chemistry, 57*(2), 495-502.
310 Espiñeira, M., González-Lavín, N., Vieites, J. M., & Santaclara, F. J. (2009b).
311 Development of a method for the identification of scombroid and common
312 substitute species in seafood products by FINS. *Food Chemistry, 117*(4), 698-
313 704.
314 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and
315 analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.*(41), 95-98.
316 Heyden, S., Barendse, J., Seebregts, A., & Matthee, C. A. (2010). Misleading the
317 masses: detection of mislabelled and substituted frozen fish products in South
318 Africa. *ICES Journal of Marine Science, 67*(1), 176-185.
319 Hillis, D. M., & Bull, J. J. (1993). An Empirical-Test of Bootstrapping as a Method for
320 Assessing Confidence in Phylogenetic Analysis. *Systematic Biology, 42*(2), 182-
321 192.
322 Kumar, S., Tamura, K., & Nei, M. (2004). MEGA3: Integrated software for molecular
323 evolutionary genetics analysis and sequence alignment. *Briefings in*
324 *Bioinformatics, 5*(2), 150-163.
325 Larraín, M. A., Abugoch, L., Quitral, V., Vinagre, J., & Segovia, C. (2002). Capillary
326 zone electrophoresis as a method for identification of golden kinglip
327 (*Genypterus blacodes*) species during frozen storage. *Food Chemistry, 76*, 377-
328 384.
329 Mackie, I. M., Pryde, S. E., González-Sotelo, C., Medina, I., Perez-Martín, R.,
330 Quinteiro, J., Rey-Mendez, M., & Rehbein, H. (1999). Challenges in the
331 identification of species of canned fish. *Trends in Food Science & Technology,*
332 *10*(1), 9-14.
333 Mc Carthy, C. (1996). Chromas version 1.45. *School of Health science, Griffifth*
334 *University, Gold Coast Campus, Queensland, Australia.*
335 Nielsen, J. G., Cohen, D. M., Markle, D. F., & Robins, C. R. (1999). *FAO species*
336 *catalogue. Ophidiiform fishes of the world (Order Ophidiiformes). An annotated*

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337 *and illustrated catalogue of pearlfishes, cusk-eels, brotulas and other*
338 *ophidiiform fishes known to date.* (Vol. 18).
339 Pardo, M. A., & Pérez-Villareal, B. (2004). Identification of commercial canned tuna
340 species by restriction site analysis of mitochondrial DNA products obtained by
341 nested primer PCR. *Food Chemistry, 86*(1), 143-150.
342 Rozas, J., Sanchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA
343 polymorphism analyses by the coalescent and other methods. *Bioinformatics,*
344 *19*(18), 2496-2497.
345 Saitou, N., & Nei, M. (1987). The Neighbor-Joining Method - a New Method for
346 Reconstructing Phylogenetic Trees. *Molecular Biology and Evolution, 4*(4), 406-
347 425.
348 Sanchez, A., Quinteiro, J., Rey-Mendez, M., Perez-Martin, R. I., & Sotelo, C. G.
349 (2009). Identification of European hake species (*Merluccius merluccius*) using
350 real-time PCR. *Journal of Agricultural and Food Chemistry, 57*(9), 3397-3403.
351 Santaclara, F. J., Cabado, A. G., & Vieites, J. M. (2006). Development of a method for
352 genetic identification of four species of anchovies: *E. encrasicolus*, *E. anchoita*,
353 *E. ringens* and *E. japonicus*. *European Food Research and Technology, 223*(5),
354 609-614.
355 Santaclara, F. J., Espineira, M., Cabado, G., Aldasoro, A., Gonzalez-Lavín, N., &
356 Vieites, J. M. (2006). Development of a method for the genetic identification of
357 mussel species belonging to *Mytilus*, *Perna*, *Aulacomya*, and other genera.
358 *Journal of Agricultural and Food Chemistry, 54*(22), 8461-8470.
359 Santaclara, F. J., Espiñeira, M., & Vieites, J. M. (2007). Genetic identification of Squids
360 (Families *Ommastrephidae* and *Loliginidae*) by PCR-RFLP and FINS
361 Methodologies. *Journal of Agricultural and Food Chemistry, 55*, 9913-9920.
362 Ward, R. D., & Reilly, A. (2001). Development of microsatellite loci for population
363 studies of the pink ling, *Genypterus blacodes* (Teleostei: Ophidiidae). *Molecular*
364 *Ecology Notes, 1*, 175-175.
365 Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA
366 barcoding Australia´s fish species. *Philosophical Transactions of the Royal*
367 *Society, Biological Sciences, 360*, 1847-1857.

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368 **FIGURES**
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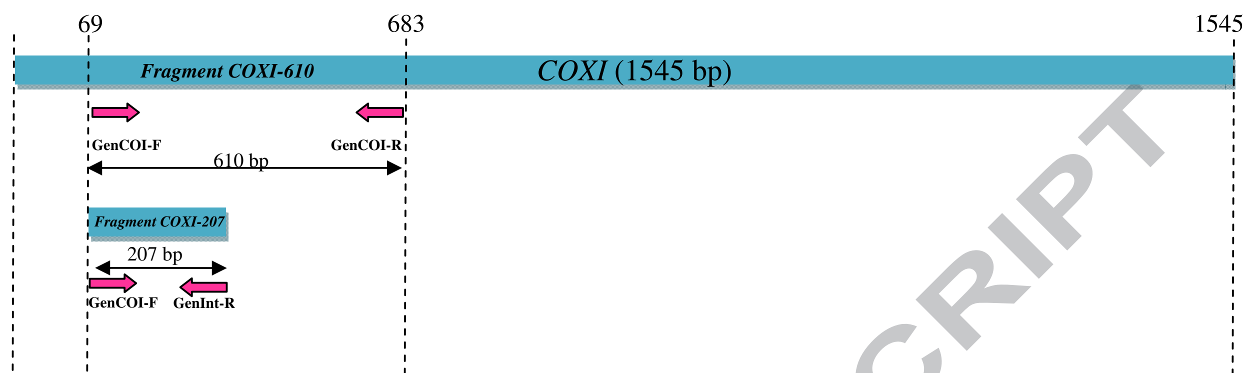

378 **Figure 1**. Location and size of the DNA fragments (*COXI*) amplified in this work and
379 the position of the primer sets used

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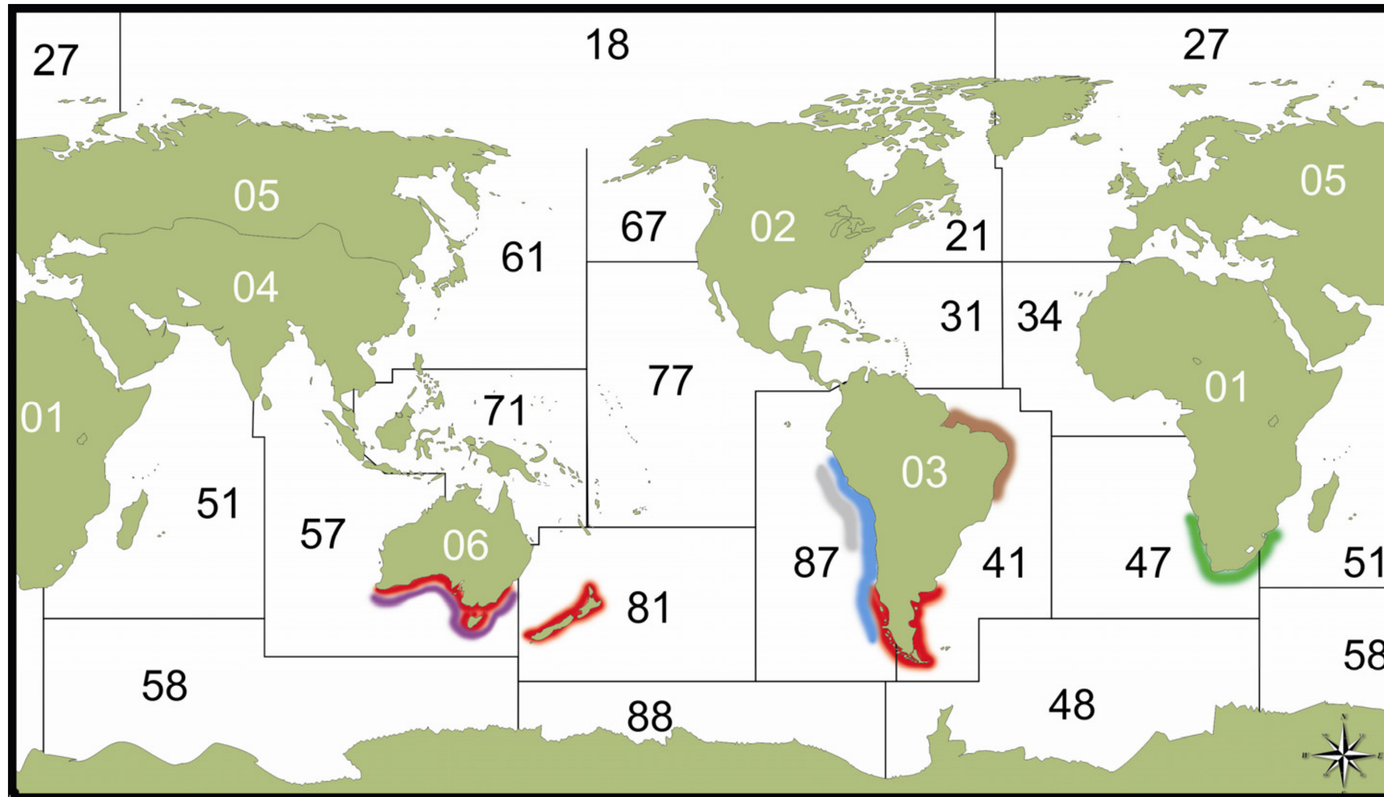
380 **FIGURE 2**

381



382–405

406 **Figure 2.** Distribution map of the *Genypterus* species included in the present study. **Blue**: *G. chilensis*; **Gray**: *G. maculatus*; **Red**: *G. blacodes*;
407 **Green**: *G. capensis*; **Violet**: *G. tigerinus*; and **Brown**: *G. brasiliensis*.

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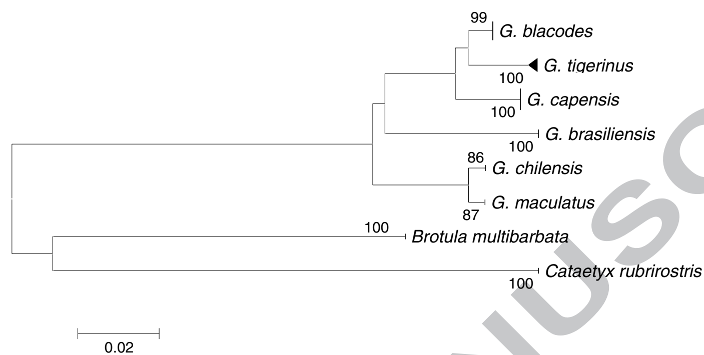
408 **Figure 3**

409

410



411

412 **Figure 3.** Neighbor-Joining tree of genetic relationships among studied species, carried

413 out from the alignment of 561 bp sequences.

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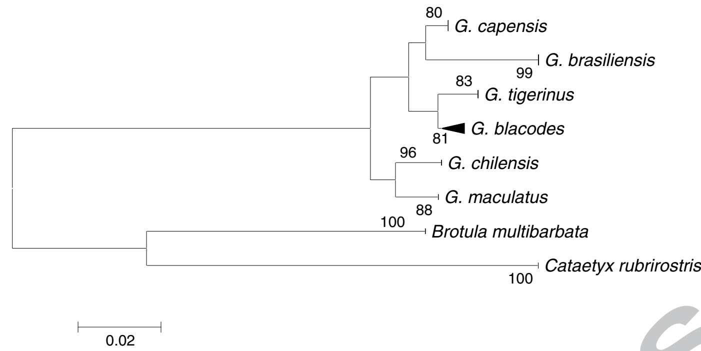
414 **Figure 4**



415

416 **Figure 4.** Neighbor-Joining tree of genetic relationships among studied species, carried
417 out from the alignment of 165 bp sequences.

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420 **TABLES**
421
422 **Table 1.** Samples included in this work and location.
423

Scientific name	Common name	Samples	FAO Area
Genypterus blacodes	Pink cusk-eel	8	87, 41
Genypterus brasiliensis	Pink ling	2	41
Genypterus capensis	Kingklip	2	47
Genypterus chilensis	Red cusk-eel	2	87
Genypterus maculatus	Black cusk-eel	2	87
Genypterus tigerinus	Rock ling	2	57
Brotula multibarbata	Goatbeard brotula	2	71, 61, 57, 51
Cataetyx rubrirostris	Rubynose brotula	2	77, 67

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433 **HIGHLIGHTS**
434 1
435 This paper describes a method for the genetic identification of *Genypterus* species
436
437 2
438 The method is based on FINS methodology (DNA sequencing and phylogenetic
439 analyses)
440
441 3
442 The method comprises the all the *Genypterus* species and allows identify them.
443
444 4
445 The proposed method was validated and can be applied to commercial products
446
447 5
448 This tool is suitable for studies of traceability and labelling of all kind of products
449

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