

# Food Control

## Molecular authentication of mushroom products: first survey on the Italian market --Manuscript Draft--

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Abstract:	<p>For the first time, mushroom products sold on the Italian market were authenticated using DNA barcoding. The analysis was performed on 71 market products (MPs) of different types (canned, dried, frozen, ready-to-cook-dishes) collected in supermarket chains. The ITS complete region was amplified and sequenced. The obtained sequences were then submitted to a BLAST analysis against both an internal dataset developed in a previous study and GenBank for species identification. Issues during amplification and sequencing were especially highlighted for canned products, plausibly due to the processing. The internal dataset was able to support the identification at species level. However, the need to improve it with sequences of commercially relevant species it is essential to increase its identification capability. Cases of suspected mislabeling were observed, with <i>Agaricus bisporus</i> and <i>Bjerkandera adusta</i> respectively found in two products labeled as <i>Boletus edulis</i> and its group. The presence of <i>B. adusta</i>, a not edible plant pathogen, also highlighted a scarce attention in the application of good hygiene and manufacturing practices. Since only 1 or 2 sequence/s were produced from each MP, we cannot exclude that mislabeling rate (2.8%) may be under-estimated. Specific guidelines on sampling strategy should be therefore fixed. Also, the use of metabarcoding, able to identify all the species present in a sample, should be considered.</p>
Suggested Reviewers:	<p>Bryn T.M. Dentinger b.dentinger@kew.org He is author of the following paper: What's for dinner? Undescribed species of porcini in a commercial packet</p> <p>Nicholas H. Oberlies nicholas_oberlies@uncg.edu He is author of the following paper: DNA barcoding for identification of consumer-relevant mushrooms: A partial solution for product certification?</p> <p>Paloma Morán paloma@uvigo.es She is author of the following paper: It's what's inside that counts: DNA-barcoding of porcini (<i>Boletus</i> sp., Basidiomycota) commercial products reveals product mislabelling</p>

Dear Editor,

please find enclosed the manuscript entitled “**Molecular authentication of mushroom products: first survey on the Italian market**” to be considered for publication in Food Control.

The significant shift of consumers' food habits to more sustainable diets, often involving vegetarian meals, has largely contributed to the global increase of mushrooms consumption. Although mushrooms are commonly consumed in the EU, there are no regulations exclusively targeting these products. In Italy, the mushroom scientific name must be declared on the label of products offered for sale on the market, except for prepared food products (sauces, creams, risottos, pasta), which bear the wording "with mushrooms", for which there is no obligation of further specifications. Therefore, a proper identification of mushroom species in the context of official control activities is pivotal.

In this study, 71 mushroom products (canned, dried, frozen, ready-to-cook-dishes) sold on the Italian market were authenticated using DNA barcoding for the first time. The ITS region was amplified and sequenced and the obtained sequences were submitted to a BLAST analysis against both an internal dataset developed in a previous study and GenBank for species identification.

The internal dataset was able to support the identification at species level. However, the need to improve it with sequences of commercially relevant species it is essential to increase its identification capability. Cases of suspected mislabeling were observed, with *Agaricus bisporus* and *Bjerkandera adusta* respectively found in two products labeled as *Boletus edulis* and its group. The presence of *B. adusta*, a not edible plant pathogen, also highlighted a scarce attention in the application of good hygiene and manufacturing practices. Since only 1 or 2 sequence/s were produced from each MP, we cannot exclude that mislabeling rate (2.8%) may be under-estimated.

This study provided useful data on mushroom national market, that were used to improve the internal dataset with ITS sequences of commercially relevant species. Given the high number of mushroom specimens possibly contained in a commercial product, and the possibility to found more than one species in a single product, the use of more advanced molecular tools, such as metabarcoding, may be preferable in the future. In fact, only mono-species products can be successfully authenticated using DNA barcoding. Thus, in line with the objectives of the research project funded by the National Ministry of Health described in Giusti et al. (2020), the already multi-species mushroom products will be authenticated by means of metabarcoding in a future study.

Regards,

Alice Giusti

**Dear Editor, we are sending you back the revised version of the manuscript entitled “Molecular authentication of mushroom products: first survey on the Italian market”. The manuscript has been improved according to the suggestions provided by Reviewers.**

Reviewer #1: - Review each of the references that I use. - Highlight the importance of the authenticity of the mushrooms and what effects would be caused by not carrying out this process during marketing if it could be considered a risk to the consumer

**Dear Reviewer #1, issues related to the commercialization/consumption of toxic species are already considered in the manuscript (see line 89-96). However, as you suggested, a sentence related to the importance of the authentication for preventing the ingestion of toxic species has been added in the conclusion (see line 356-358).**

Reviewer #2: Manuscript titled "Molecular authentication of mushroom products: first survey on the Italian market " (FOODCONT-D-23-00280) by Giusti et al. aims a study of the authentication of different types of mushroom products collected on the Italian market using DNA barcoding. In this study, authors made a comparative study for species authentication among the obtained sequences of the Internal Transcribed Spacer DNA region against both an internal dataset developed in a previous study and a BLAST analysis (GenBank). Suspected cases of mislabeling were observed.

Study is original and fairly well structured. Approach, methodologies and analysis used in this work are satisfactory. Results are well presented in a comprehensive way and sufficiently discussed. Its content in general justifies the length. Language is clear and understandable.

In my opinion, this manuscript deserves to be published in Food Control Journal because it provides comprehensive analytical results and it offers new insight in the relationship between legislation and control methods of the labeling of mushroom commercial products. In addition, I believe this work is a good reference for other researchers.

**Dear Reviewer #2, thank you very much for the appreciation on the proposed study. All the suggestions provided have been taken into consideration during the revision process.**

Minor comments

L177-178 It seems an incomplete sentence. Please revise

**The sentence has been amended**

L151 "The 71 mushroom products...." Were they from different brands? Please, specify. Moreover, if you have information such as: "prepared on...." or "canned on....", "preparation date..", in my opinion they could be useful information to add in order to indicate if there is a temporality of the species used in the different products.

**These information have been added in the text of the manuscript (see line 153-155).**

L220-237 although this is not a purely biochemical journal, I would recommend reporting the range of the length of the obtained PCR fragments, to indicate the differences between species and the real difficulties of obtaining intact DNA from canned or differently prepared products.

**The range of the expected amplicons has been added in material and methods section (line 178). All the obtained sequences were in this length range, with no particular differences among product type.**

L306 ...and it is....Please, add the letter "i"

**Done**

# **Molecular authentication of mushroom products: first survey on the Italian market**

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## Abstract

For the first time, mushroom products sold on the Italian market were authenticated using DNA barcoding. The analysis was performed on 71 market products (MPs) of different types (canned, dried, frozen, ready-to-cook-dishes) collected in supermarket chains. The ITS complete region was amplified and sequenced. The obtained sequences were then submitted to a BLAST analysis against both an internal dataset developed in a previous study and GenBank for species identification. Issues during amplification and sequencing were especially highlighted for canned products, plausibly due to the processing. The internal dataset was able to support the identification at species level. However, the need to improve it with sequences of commercially relevant species it is essential to increase its identification capability. Cases of suspected mislabeling were observed, with *Agaricus bisporus* and *Bjerkandera adusta* respectively found in two products labeled as *Boletus edulis* and its group. The presence of *B. adusta*, a not edible plant pathogen, also highlighted a scarce attention in the application of good hygiene and manufacturing practices. Since only 1 or 2 sequence/s were produced from each MP, we cannot exclude that mislabeling rate (2.8%) may be under-estimated. Specific guidelines on sampling strategy should be therefore fixed. Also, the use of metabarcoding, able to identify all the species present in a sample, should be considered.

**Keywords:** ITS region, DNA barcoding, mislabeling, frauds, *Boletus* sp., *Agaricus* sp.

## 1. Introduction

Mushrooms are the spore-producing structures of certain fungi typically consisting of a stalk and a cap (Jensen-Vargas & Marizzi, 2018). According to a recent review, 3283 mushroom species have been confirmed as edible or conditionally edible (Li et al., 2021). The significant shift of consumers' food habits to more sustainable diets, often involving vegetarian meals, has largely contributed to the global increase of mushrooms consumption; in fact, they can be considered a valid alternative to animal-based protein given their excellent nutritional properties (Feeney, Miller, & Roupas, 2014; Giusti et al., 2020). According to the most recent data, the global mushroom market size, that was valued at USD 50.3 billion in 2021, is expected to expand at a compound annual growth rate (CAGR) of 9.7% from 2022 to 2030 (Market Analysis Report, 2022). Fresh mushrooms dominated the market (revenue share 89.5% in 2021), but processed forms, namely dried, frozen and canned is expected to witness a revenue-based CAGR of 10.2% in the same period (Market Analysis Report, 2022). Mushrooms available on the market are either cultivated or harvested in the wild. The most cultivated mushrooms in the world are the common mushroom/button mushroom (*Agaricus bisporus*), the shiitake mushroom (*Lentinula edodes*), the oyster mushroom (*Pleurotus* spp.), the jelly ear or Judas's ear (*Auricularia auricula-judae*), the straw mushroom (*Volvariella volvacea*), and the enoki mushroom or velvet shank (*Flammulina velutipes*) while the most famous wild mushrooms are porcini (*Boletus* spp.), chanterelle (*Cantharellus* spp.), trumpet chanterelle (*Craterellus* spp.), horn of plenty or black trumpet (*Craterellus cornucopioides*), morel (*Morchella* spp.), and the fairy ring mushroom (*Marasmius oreades*) (Dimopoulou, Kolonas, Mourtakos, Androutsos, & Gortzi, 2022; Royse, Baars, & Tan, 2017). Porcini are among the most sought-after edible mushroom species, and also the most harvested species worldwide (Rivas-Ferreiro, Otero, & Morán., 2023).

The Asia Pacific is the leading region in the mushroom market, and China is the largest producer accounting for about 75% of the total global output. China is the largest exporter of porcini, mostly destined to the European market (Detinger & Suz, 2014). The European Union (EU) is one of the

83 key mushroom consumers, and the market, relying majorly on imports, is especially represented by  
84 the button, the shiitake and the oyster mushrooms. In Italy, where mushroom farming is still unable  
85 to satisfy the high national request, cultivated mushrooms are highly imported, besides China, from  
86 the Eastern Europe (Giusti et al., 2021). However, there is a long recreational tradition of collecting  
87 wild mushrooms, mainly porcini, Chantarelle (*C. cibarius*), and the nationally called “Ovulo”  
88 (*Amanita caesarea*).

89 As well known, the consumption of some toxic mushroom species can provoke toxin-induced  
90 disease that can affect gastrointestinal, neurological, renal, and hepatic systems and, in the worst  
91 cases lead to death (Govorushko, Rezaee, Dumanov, & Tsatsakis, 2019; White et al., 2019).  
92 However, some edible species can become toxic if improperly collected, transported, stored, and  
93 cooked (Nieminem & Mustonen, 2020; White et al., 2019). Given the risks related to the trade in  
94 non-edible species, since 1981 the Codex Alimentarius has published a specific standard for edible  
95 fresh or processed mushrooms marketing, quality, hygiene, and labelling (Codex Alimentarius,  
96 1981).

97 Although mushrooms are commonly consumed in the EU, there are no EU regulations  
98 exclusively targeting these products. The general hygienic rules relating to the processing,  
99 conservation, packaging, and sale, as well as official control of fungi, are therefore regulated by the  
100 EU rules on food (legislation is described in Giusti et al., 2022a). About consumer information,  
101 mushrooms and mushroom based products fall within the scope of Regulation (EU) No. 1169/2011  
102 which governs food labeling. In Italy, the Presidential Decree of 14 July 1995 n. 376 (PD  
103 376/1995), imposes that the mushroom scientific name must be declared on the label of mushroom-  
104 based products offered for sale on the market, except for prepared food products (sauces, creams,  
105 risottos, pasta), which bear the wording "with mushrooms", for which there is no obligation of  
106 further specifications (PD 376/1995).

107 Therefore, a proper identification of mushroom species in the context of official control activities  
108 is pivotal and, in this respect, the aforesaid PD 376/1995, also set up the Mycological Inspectorates,



109 composed of a team of expert mycologists. Their activities consist mainly in the morphological  
110 identification of mushroom species to prevent poisoning phenomena (Giusti et al., 2022a).  
111 Mushroom identification is usually based on the observation of phenotypic characters, macroscopic  
112 structures of the fruit body, or microscopic structure of the spores. However, this identification is  
113 challenging, especially in processed forms that are often sold sliced and/or dried (Jensen-Vargas et  
114 al., 2018; Giusti et al., 2021). Therefore, official laboratories (e. g. Experimental Zooprophyllactic  
115 Institutes) should use alternative and more effective identification techniques. Among the various  
116 available, DNA analysis is the method of choice.

117 Since 2003, DNA barcoding proposed by Hebert, Ratnasingham, & De Waard (2003) has been  
118 claimed to be the most efficient method to identify living beings and it has also become a key player  
119 in food authentication (Galimberti, Sandionigi, Bruno, Bellati, & Casiraghi, 2015; Galimberti et al.,  
120 2019) especially used for fish and seafood products (Fernandes, Amaral., & Mafra, 2021). Also,  
121 with the advent of the Next Generation Sequencing (NGS) Technologies, metabarcoding (i. e. DNA  
122 Barcoding combined with NGS) represent an attractive method in the field of food authentication,  
123 although still poorly applied (Haynes, Jimenez, Pardo, & Helyar, 2019). For mushroom species  
124 identification, the Internal Transcribed Spacer (ITS) region (Gardes & Bruns, 1993), has been  
125 chosen as the universal DNA barcode (Schoch et al., 2012). To date, most studies using DNA  
126 barcoding for species identification in mushrooms have taxonomic purposes, while few studies  
127 were performed to the authenticate mushrooms or mushroom commercial products from the market  
128 (Table 1). The more extensive available survey was performed in China (Zhang et al., 2021) while  
129 the other studies were conducted in USA (Jensen-Vargas & Marizzi, 2018; Raja, Baker, Little, &  
130 Oberlies, 2017), UK (Detinger & Suz, 2014) and Spain (Rivas-Ferreiro et al., 2023). The study by  
131 Cutler, Bradshaw, & Dentinger (2021) was the only applying metabarcoding to mushroom products  
132 sold in the USA. Except for the study by Zhang et al. (2021), the number of samples analyzed in the  
133 other above-mentioned studies was rather low (Table 1). Moreover, not all of them evaluated  
134 mislabeling in the analyzed products (Table 1). The National Experimental Zooprophyllactic

Institute of Lazio and Tuscany together with the regional Mycological Inspectorate, the Tuscany Mycological Groups Association (AGMT) and the Department of Veterinary Sciences of the University of Pisa recently implemented a research project funded by the Ministry of Health aimed at developing an analytical method to optimize the official control activity of nationally marketed mushroom products (Giusti et al., 2020). The project already provided an ITS-based genetic dataset to support the identification of the wild and cultivated mushroom species in the Italian territory (Giusti et al. 2021). Also, the compliance to European and National safety and labelling requirements of wild and cultivated mushrooms and mushroom-based products sold on the Italian market was assessed (Giusti et al., 2022a). In this study, the efficiency in identifying mushroom species contained in commercial products sold in Italy using DNA barcoding of the ITS region together with the dataset built by Giusti et al. 2021 was evaluated. The internal dataset efficiency especially represents a basic starting point for the future development of a metabarcoding procedure targeting fungal products, that is the main objective of an ongoing project also founded by the Ministry of Health (Current Research Grant IZS 07/21 RC, 2021).

## 2. Material and Methods

### 2.1. Sampling

The 71 mushroom products (MPs) (29 canned, 22 dried, 11 frozen, 9 ready to cook dishes such as pasta/risotto/pizza/polenta with mushrooms) included in this study (Table 2) represented a sub-population of a more extensive sampling including 98 MPs. The samples, belonging to 27 different brands, were purchased during 2019 and analyzed before the minimum durability (from September 2020 to July 2023). They were collected in different supermarket chains in Tuscany (Italy) in a context of a survey aimed at checking the presence of label mandatory information required by the EU and national legislation (Giusti et al., 2022a). In particular, the MPs used in this study were uniquely represented by those declaring the presence of a single species on the label (mono-species MPs). The MPs were considered as monospecies also when the term “*Boletus edulis* and its group” was reported, that according to the Italian legislation refers to the trade name porcini in dried

161 mushrooms (PD 376/1995). For each MPs, three tissue samples (each likely referable to a distinct  
162 fungal specimen) were randomly selected, for a total of 213 tissue samples. All the samples were  
163 stocked, in accordance with the storage instructions on the MPs label, at room temperature or -20°C  
164 until molecular analysis.

## 165 **2.2. Molecular analysis**

166 *2.2.1. Total DNA extraction and evaluation.* The total DNA extraction protocol described by  
167 Giusti et al. (2021) was applied to all the 213 tissue samples. The total DNA concentration and  
168 purity were evaluated with Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies,  
169 Wilmington, DE, US) by two subsequent measurements of the absorbance value at 260 nm and  
170 calculation of A260/A280 and of A260/A230 ratio according to manufacturer's indications. Ratios  
171 A260/A280 and A260/A230 around 2.0 were considered as satisfactory values of DNA purity. Each  
172 DNA sample was stored at -20 °C until further analysis.

173 *2.2.2. ITS region amplification, sequencing and sequences editing.* The complete ITS region was  
174 amplified from the 213 DNA samples using the PCR protocol described in Giusti et al. (2021),  
175 using the fungal specific primers ITS-1F/ITS-4 (Gardes & Bruns, 1993; White, Bruns, Lee, &  
176 Taylor, 1990), the most used for mushroom products authentication in literature (Table 1). Positive  
177 reaction was assessed by electrophoresis on a 2% agarose gel. The presence of amplicons of the  
178 expected length [ranging between 600 and 800 bp \(Giusti et al., 2021\)](#) and the PCR products  
179 concentration was assessed by comparison with the standard marker SharpMass™ 50-DNA ladder  
180 (Euroclone Spa, Milano, Italy). Then, PCR products presenting the expected amplicon, with at least  
181 a minimum sufficient concentration (5ng/μl) [were stored to be purified and sequenced.](#) In the event  
182 of successful amplification of all 3 extracted DNA samples from each MP, the 2 PCR products with  
183 the highest concentration were sent to sequencing, and the residual PCR product was stored at -  
184 20°C for further analysis in case of sequencing failure or non-compliances. In case that 2 or 1 PCR  
185 product/s were obtained from each MP, all of them were sent to sequencing. The sequencing was  
186 carried out at the Experimental Zooprophyllactic Institute of Lazio and Tuscany (Italy).

187 Chromatograms of forward and reverse sequences of each sample were checked, searching for  
188 putative reading errors, and these were corrected using BioEdit version 7 (Hall, 1999).

189 *2.2.3 BLAST analysis and species identification: databased comparison.* All the obtained ITS  
190 sequences were submitted to a BLAST analysis against the mushroom ITS gene dataset built by  
191 Giusti et al. (2021) in Geneious Prime version 2022.0.1 (Kearse et al., 2012). Since some species  
192 declared on the MPs labels were not included in this dataset, also a BLASTN analysis was  
193 performed against GenBank (<https://blast.ncbi.nlm.nih.gov/>) and the results were compared. For the  
194 BLAST analysis performed in Geneious Prime version 2022.0.1 (Kearse et al., 2012), a weighted  
195 score for the hit provided by the software and comprising the e-value, the pairwise identity and the  
196 coverage was considered. For the BLASTN analysis performed in GenBank, the percent identity  
197 was considered. In both cases, a value  $\geq 97$ –100% was selected as the threshold for species  
198 identification (Giusti et al., 2021; Raja et al. 2017).

199 *2.2.4 Assessment of label information.* Outcomes from section 2.2.3 were compared to data  
200 obtained from MPs labelling analysis previously conducted in Giusti et al. (2022a). In particular,  
201 the mislabelling rate in relation to the scientific name was calculated. Cases of non-compliance  
202 between the species declared in MPs label and species identified by molecular analysis were  
203 discussed.

## 204 **3. Results & Discussion**

### 205 ***3.1. Sampling***

206 The 71 mushroom-based products (MPs) (29 canned, 22 dried, 11 frozen, 9 ready to cook dishes  
207 such as pasta/risotto/pizza/polenta with mushrooms) (Table 2) collected in a previous study (Giusti  
208 et al., 2022a) were here analyzed to test the ability of DNA barcoding targeting ITS gene and the  
209 internal database built in Giusti et al. (2021) in authenticate mushroom products and support official  
210 controls.

211 Compared with most of the available studies aimed at authenticating commercial mushroom  
212 (Table 1), the sample number is rather higher; thus, this study represents one of the first survey  
213 targeting a commodity as yet under-investigated worldwide.

214 Overall, the majority of collected MPs (n=36; 50.7%) declared the presence of “*B. edulis* and its  
215 group”, followed by *A. bisporus* (n=33; 46.5%); the remained 2 MPs were labeled as  
216 *Kuehneromyces mutabilis* and *Pholiota nameko*, respectively (Table 2). The MPs labeled as “*B.*  
217 *edulis* and its group” were mostly dried (22 out of 36; 61.1%), but also frozen (8 MPs; 22.2%),  
218 canned (3 MPs; 8.3%) and ready to cook dishes (3 MPs; 8.3%), proving that such definition is also  
219 used in mushroom products, other than dried, despite no legislative requirements. The MPs labeled  
220 as *A. bisporus* were instead mostly canned (24 out of 33; 72.7%), as well as both the MPs labeled as  
221 *K. mutabilis* and *P. nameko* (Table 2). This first analysis allowed to gain more knowledge on the  
222 present status of the mushroom national market.

### 223 **3.2. Molecular analysis**

224 **3.2.1. Total DNA extraction and evaluation.** The concentration of the total DNA extracted from  
225 all the 213 tissue samples ranged from 9.4 ng/μl to 3817.9 ng/μl (mean 901.4 ng/μl), with quality  
226 ratios A260/A280 1.7-2.3 (mean 2.1) and A260/230 0.3-2.5 (mean 1.8). For each product type, both  
227 medium-high (260/280nm > 2.0 and 260/230 nm >1.7) and low quality (260/280 <1.8 and the  
228 260/230 nm <1.4) total DNA samples were obtained. Literature reports that several processing  
229 treatments (freezing, acidification, lyophilization, pasteurization, sterilization) are known as  
230 potentially leading to DNA degradation and fragmentation, with an appreciable decrease in its  
231 qualitative-quantitative yield (Gryson, 2010; Lo & Shaw, 2018). In this study, no appreciable  
232 differences emerged from the analysis of the spectrophotometric results between the different  
233 product types (canned, dried, frozen, ready-to-cook dishes) in terms of apparent total DNA  
234 extraction efficiency (Table 3) and the observed cases of poor spectrophotometric quality might be  
235 plausibly related to: 1) the presence organic compounds residues not effectively removed by the  
236 used extraction protocol (e. g. in case of canned in oil products), 2) high content of free nucleic

237 acids resulting from the products acidification and the application of high temperatures (e. g.  
238 products lyophilized and sterilized after canning); 3) removal of DNA from the sample during the  
239 thawing phase due to cellular breakdown and subsequent shrinkage of dissolved molecules in water  
240 (Gryson, 2010).

241 3.2.2. *ITS region amplification, sequencing and sequences editing.* The ITS region was  
242 successfully amplified (presence of specific amplification band with concentration >5ng/μl) from  
243 162 out of 213 DNA samples (76%). As reported in Table 4, the lowest amplification rate was  
244 observed in canned products (54 amplified samples out of 87, 62.1%). Of the other 51 DNA  
245 samples, 23 (10.8%) were not amplified (absence of amplification band) – especially involving  
246 ready-to-cook dishes (25.9% of sample of this type) and canned (13.8%) (Table 4) - and 28 (13.1%)  
247 were considered as not usable for sequencing since one or more non-specific amplification bands  
248 were visualized by electrophoresis or the PCR product showed a concentration below the limit set  
249 for sequencing (<5ng/μl), of which 75% was represented by canned (24.1% of sample of this type)  
250 (Table 4).

251 Despite the cases of not amplified DNA or not usable PCR products, at least 2 DNA samples  
252 were successfully amplified from 88.7% of the MPs (63 out of 71 MPs). Specifically, successful  
253 amplification was obtained 1) from all the 3 DNA samples extracted from each MP in 28 cases  
254 (39.4% of the total MPs), 2) from 2 DNA samples in 35 cases (49.3%) and 3) only 1 DNA sample  
255 in the remaining 8 cases (11.3%) (Table 5).

256 Based on the established sequencing criteria (section 2.2.2), 134 PCR products were initially  
257 sequenced, and one additional PCR product was sent to sequencing following the non-compliance  
258 observed in the BLAST analysis (section 3.2.3) (Table 5). Overall, 130 usable sequences were  
259 obtained, since sequencing failures were registered for 5 PCR products from canned MPs. Thus,  
260 canned MPs were found to be the most problematic type in both amplification and sequencing  
261 phases. In this respect, it was observed that technological food processes applied to the MPs might  
262 affect the DNA amplifiability: first, they can cause DNA fragmentation that make difficult to

263 generate ITS barcodes, as the primer binding sites may be degraded (Chung, Drábek, Opel, Butler,  
264 & McCord, 2004); also, secondary metabolites could inhibit PCR by decreasing the Taq-  
265 polymerase's activity, (Jensen-Vargas & Marizzi, 2018). The fact that canned products were  
266 especially involved may be plausibly due to oxidation and hydrolysis phenomena linked to the  
267 acidification of the product and treatment with high temperatures, associated with the presence of  
268 PCR inhibitors such as polyphenols (Gryson, 2010; Lo & Shaw, 2018). Raja et al. (2017)  
269 highlighted similar issues in commercial fungal powdered and edible mushrooms from grocery  
270 stores, ascribing the impossibility to obtain PCR products to the manufacturing process and, in  
271 particular, to the effects of exposing mushrooms to very high temperatures, with consequent  
272 degradation and fragmentation of DNA. The same authors described double amplification bands for  
273 some DNA samples belonging to the same product batch (Raja et al., 2017).

274 *3.2.3. BLAST analysis and species identification: databased comparison.* Respect to the species  
275 declared on the MPs labels, the mushroom ITS gene dataset built by Giusti et al. (2021) and used as  
276 reference for the BLAST analysis in this study did not contain sequences from *A. bisporus* and *P.*  
277 *nameko*, since it was built with ITS sequences from wild local species. In this respect, we supposed  
278 that also cultivated or wild species from other countries that might have been found in the analyzed  
279 MPs were not included in this dataset. In the light of improve the internal dataset with ITS  
280 sequences of worldwide commercially relevant mushroom species and consequently enforce the  
281 official control activity of nationally-marketed mushroom products, the analysis performed in this  
282 study was pivotal. In fact, the sequences obtained from this study were finally included in the  
283 internal dataset, and a further production from other reference specimens was planned within the  
284 Current Research Grant IZS 07/21 RC (2021). In this study, given the current absence of such  
285 comprehensive internal dataset, a genetic database having a higher taxonomic coverage such as  
286 GenBank was also used to assist and support the analysis. We did not use the established Barcode  
287 of Life Data System (BOLD) identification system because there were too few ITS records on

288 BOLD to return a successful match, as found in our previous study (Giusti et al. 2021) and also  
289 highlight by Jensen-Vargas & Marizzi (2018).

290 All the initially obtained 129 ITS sequences were assigned to a species using both the databases,  
291 with all the 71 MPs identified at specie level. *Agaricus bisporus* was actually identified in the 33  
292 MPs declaring its presence in the label. The 2 MPs labeled as *K. mutabilis* and *P. nameko* were  
293 likewise identified with these species. Out of the 36 MPs labeled as “*B. edulis* and its group”, 16  
294 (44.4%) were identified with *B. reticulatus*, 8 (22.2%) with *B. aereus*, 5 (13.9%) with *B. edulis*, 4  
295 (11.1%) with *B. aereus* and *B. edulis*, and 1 (2.8%) with *B. aereus* and *B. reticulatus*; in the  
296 remained 2 MPs, *B. reticulatus* was found as mixed with species belonging to other genera, namely  
297 *A. bisporus* and *Bjerkandera adusta* (smoky polypore) (Table 2). Since the presence of this latter  
298 species appeared particularly unconventional, we decided to produce an additional sequence from  
299 the residual PCR product stored at -20°C to confirm the result (passing from 129 to 130 usable  
300 sequences). This was identified as *B. adusta* likewise. Specifically, the label of this product  
301 clearly specified that it was handcrafted *B. adusta*, commonly known as the smoky polypore or  
302 smoky bracket, is a not edible mushroom acting as plant pathogen that causes white rot in live trees,  
303 but most commonly appears on dead wood (Ostry, O'Brien, & Anderson, 2011). Since 2 out of 3  
304 sequences produced from this product were identified as *B. adusta*, a scarce attention in the  
305 application of Good Hygiene Practice (GHP) and good manufacturing Practice (GMP) by the  
306 company production can be hypothesized. In fact, even supposing that other wild species can be  
307 gathered together with *Boletus* sp., the accidental presence of *B. adusta* would be understandable in  
308 arboriferous mushrooms (e. g. *Agrocybe* spp.) respect to porcini. In the same samples also contained  
309 *A. bisporus* and it is not possible to establish if the presence of *A. bisporus* was unintentional (e. g.  
310 co-presence of *Boletus* spp. and *A. bisporus* in the processing plant) or intentional, especially  
311 considering that the price of *Boletus* spp. is higher than *A. bisporus*. According to the national  
312 legislation (PD 376/1995), the trade name “*B. edulis* and its group” can refer, besides *B. edulis*, to  
313 other three *Boletus* spp., namely *B. aereus*, *B. reticulatus* and *B. pinicola* (current name *B.*



314 *pinophilus* according to [www.speciesfungorum.org](http://www.speciesfungorum.org)). Thus, all the MPs identified with these *Boletus*  
 315 spp. (*B. edulis*, *B. aereus* and *B. reticulatus*) or their mix can be considered as properly labeled.  
 316 Thus, based on our findings, only two MPs contained species different from those reported on the  
 317 label (Table 2) and can be considered as mislabeled (mislabeling rate 2.8%). However, we consider  
 318 appropriate to specify that, since only 1 or 2 sequence/s were produced from each MP, we cannot  
 319 exclude that mislabeling cases involving the presence of non-declared species might also affect  
 320 other MPs. In this respect, the necessity to establish a standard sampling strategy for the analysis of  
 321 a significative number of samples should be remarked in the field of food authentication, as we  
 322 already highlighted (Giusti, Malloggi, Tinacci, Nucera, & Armani, 2022b). Although data for these  
 323 products are scarce in literature, cases of mislabeling were reported: Rivas-Ferreiro et al. (2023)  
 324 found *B. edulis* in only three out of the nine products labelled as containing this species; in its place  
 325 other *Boletus* spp. (mainly belong to the relative group) and/or poorer quality Boletaceae (*Imleria*  
 326 *badia*, *Sutorius* spp., *Tylopilus* spp.) were found, and even the fungal parasite *Hypomyces*  
 327 *chrysospermus* (Rivas-Ferreiro et al., 2023). Jensen-Vargas & Marizzi (2018) found that four out of  
 328 ten analyzed samples were labeled correctly at both the species and genus level, while the remained  
 329 samples were labeled at the correct genus level, but they were unable to resolve them down to the  
 330 species level, as there were minor discrepancies between the used databases. In this respect, the  
 331 availability of a comprehensive and validated reference set of DNA sequence data from target  
 332 species is crucial to provide accurate and verifiable molecular identification in mushrooms (Giusti  
 333 et al., 2021; Hibbett et al., 2016; Nilsson et al., 2014; Schoch et al., 2014). Cuttler et al. (2021)  
 334 found that that only five out of the sixteen products ~~analysed~~[analyzed](#) by means of metabarcoding  
 335 had an ingredient label that accurately described the species composition of the contents, and most  
 336 of the products that claimed to include porcini consisted instead of cultivated species (button  
 337 mushrooms, oyster mushrooms, shiitake) and wild-collected *Suillus* spp. Metabarcoding, being able  
 338 to possibly identify all plants, animal, fungal and microalgae ingredients in food commodities  
 339 (Haynes et al., 2019), actually represents a captivating method for the authentication of this type of

340 products on a large scale. On the one hand, all the mushroom species present in a product could be  
341 detected, given the capability to simultaneously sequence all the DNA molecules, including those  
342 present in trace amounts. On the other hand, the overall sample number may be increased a lot  
343 thank to the possibility to pooled them in a unique sequencing reaction.

#### 344 4. Conclusion

345 This is the first Italian study aimed at authenticating commercial mushroom products using DNA  
346 barcoding. Suspected cases of mislabeling were observed, although we think that specific and  
347 exhaustive guidelines on sampling strategy should be fixed (also by legislation) for investigating  
348 mislabeling in commercial products, as for other typologies of control on food. This type of study  
349 provided useful data on mushroom national market, that have been used to improve the internal  
350 dataset with ITS sequences of commercially relevant species. Given the high number of mushroom  
351 specimens possibly contained in a commercial product, and the possibility to found more than one  
352 species in a single product, the use of more advanced molecular tools, such as metabarcoding, may  
353 be preferable in the future. In fact, only mono-species products can be successfully authenticated  
354 using DNA barcoding. Thus, [in](#) line with the objectives of the research project funded by the  
355 National Ministry of Health described in Giusti et al. (2020), the already [collected](#) multi-species  
356 mushroom products will be authenticated by means of metabarcoding in a future study. [This will](#)  
357 [also allow to better safeguard consumer's health in case of fraudulent substitution involving toxic](#)  
358 [species.](#)

359

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362

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364

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## Figures



**Figure 1.** Different types of mushroom market products (MPs) analyzed in this study. The MPs brand was removed. A: canned; B: dried; C: frozen; D: Ready-to-cook dishes.



491 **Tables**

492

Reference	Type of product	Country	Method/gene	Sample size (n)	Primer pair	Mislabeling evaluation
Detinger & Suz (2014)	Dried porcini ( <i>Boletus</i> spp.)	UK	DNA barcoding (complete ITS)	15 pieces from 1 packet	ITS1F <sup>(a)</sup> /ITS4 <sup>(b)</sup>	no
Raja et al. (2017)	Powdered and edible mushroom (not specified)	USA	DNA barcoding (complete ITS)	33	ITS5 <sup>(c)</sup> /ITS4 <sup>(b)</sup>	no
Jensen-Vargas & Marizzi (2018)	Dried and fresh	USA	DNA barcoding (complete ITS)	10 pieces from 2 packets	ITS1F <sup>(a)</sup> /ITS4 <sup>(b)</sup>	yes
Cutler et al. (2021)	dried, powdered, soups, pasta sauces, flavor enhancers	USA	Metabarcoding (ITS-2)	16	5.8SFun/ITS4Fun	yes
Zhang et al. (2021)	Not specified	China	DNA barcoding (complete ITS)	3585	ITS5 <sup>(c)</sup> /ITS4 <sup>(b)</sup>	no
Rivas-Ferreiro et al. (2023)	Dried porcini ( <i>Boletus</i> spp.)	Spain	DNA barcoding (complete ITS)	9	ITS1F <sup>(a)</sup> /ITS4 <sup>(b)</sup>	yes

493 **Table 1.** Available literature on commercial mushroom product's molecular authentication. Main data on sampling and analytical methods are reported. The studies are listed  
494 chronologically. (a) Gardes & Bruns (1993); (b) White et al., 1990; (c) Gardes, White, Fortin, Bruns, & Taylor (1991); (d) Taylor et al. (2016)

MP	Type	Label info	Sequences (n)	ID (internal dataset)	ID (GenBank)	Final ID
1	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
2	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
3	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
4	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
5	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
6	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
7	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
8	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
9	canned	<i>B. edulis</i> and its group	1	<i>B. edulis</i>	<i>B. edulis</i>	<i>B. edulis</i>
10	canned	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
11	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
12	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
13	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
14	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> * <sup>[a]</sup>	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
15	canned	<i>P. nameko</i>	2	* <sup>[c]</sup>	<i>P. nameko</i>	<i>P. nameko</i> <sup>[b]</sup>
16	canned	<i>B. edulis</i> and its	3	* <sup>[c]</sup> (2 seq)	<i>B. adusta</i> (2 seq)	<i>B. adusta</i> <sup>[b]</sup> , <i>B. reticulatus</i>

		<b>group</b>		<b><i>B. reticulatus</i> (1 seq)</b>	<b><i>B. reticulatus</i> (1 seq)</b>	
17	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
18	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
19	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
20	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
21	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
22	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
23	canned	<i>A. bisporus</i>	1	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
24	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
25	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
26	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
27	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
28	canned	<i>K. mutabilis</i>	2	<i>K. mutabilis</i>	<i>K. mutabilis</i>	<i>K. mutabilis</i>
29	canned	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
30	dried	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
31	dried	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
32	dried	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
33	dried	<i>B. edulis</i> and its group	1	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
34	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
35	dried	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
36	dried	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
37	dried	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
38	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
39	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
40	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
41	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
42	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
43	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i>	<i>B. edulis</i>	<i>B. edulis</i>
44	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i> (1 seq) <i>B. aereus</i> (1 seq)	<i>B. reticulatus</i> (1 seq) <i>B. aereus</i> (1 seq)	<i>B. reticulatus</i> ; <i>B. aereus</i>
45	dried	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
46	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i>	<i>B. edulis</i>	<i>B. edulis</i>
47	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i> (1 seq); <i>B. aereus</i> (1 seq)	<i>B. edulis</i> (1 seq) <i>B. aereus</i> (1 seq)	<i>B. edulis</i> ; <i>B. aereus</i>

48	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i> (1 seq); <i>B. aereus</i> (1 seq)	<i>B. edulis</i> (1 seq) <i>B. aereus</i> (1 seq)	<i>B. edulis</i> ; <i>B. aereus</i>
49	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i>	<i>B. edulis</i>	<i>B. edulis</i>
50	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i> (1 seq); <i>B. aereus</i> (1 seq)	<i>B. edulis</i> (1 seq) <i>B. aereus</i> (1 seq)	<i>B. edulis</i> ; <i>B. aereus</i>
51	dried	<i>B. edulis</i> and its group	2	<i>B. edulis</i> (1 seq); <i>B. aereus</i> (1 seq)	<i>B. edulis</i> (1 seq) <i>B. aereus</i> (1 seq)	<i>B. edulis</i> ; <i>B. aereus</i>
52	frozen	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
<b>53</b>	<b>frozen</b>	<b><i>B. edulis</i> and its group</b>	<b>2</b>	<b><i>B. reticulatus</i> (1 seq) <i>A. bitorquis</i>*[a] (1 seq)</b>	<b><i>B. reticulatus</i> (1 seq) <i>A. bisporus</i> (1 seq)</b>	<b><i>B. reticulatus</i>; <i>A. bisporus</i><sup>[b]</sup></b>
54	frozen	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
55	frozen	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
56	frozen	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
57	frozen	<i>B. edulis</i> and its group	2	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
58	frozen	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
59	frozen	<i>B. edulis</i> and its group	2	<i>B. edulis</i>	<i>B. edulis</i>	<i>B. edulis</i>
60	frozen	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
61	frozen	<i>A. bisporus</i>	1	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
62	frozen	<i>B. edulis</i> and its group	1	<i>B. aereus</i>	<i>B. aereus</i>	<i>B. aereus</i>
63	Ready-to-cook dish	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
64	Ready-to-cook dish	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
65	Ready-to-cook dish	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>
66	Ready-to-cook dish	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
67	Ready-to-cook dish	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
68	Ready-to-cook dish	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
69	Ready-to-cook dish	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
70	Ready-to-cook dish	<i>B. edulis</i> and its group	2	<i>B. reticulatus</i>	<i>B. reticulatus</i>	<i>B. reticulatus</i>
71	Ready-to-cook dish	<i>A. bisporus</i>	2	<i>A. bitorquis</i> *[a]	<i>A. bisporus</i>	<i>A. bisporus</i> <sup>[b]</sup>

**Table 2.** Mono-species mushroom products (MPs) collected in Giusti et al. (2021) and analyzed in this study with final identification. Identification using the internal dataset built in Giusti et al. (2021) and GenBank are reported to compare the results. Mislabeled MPs are highlighted in bold. [a]: 97.1%-98.3% identity value. [b]: ID from GenBank was assessed as reliable since the internal dataset does not include this species; [c]: Low identity values (generally <90%) with other species.

Total DNA analysis	Canned	Dried	Frozen	Ready-to-cook dishes
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Concentration range (ng/μl)	13.0-3096.6	167.6-3817.9	9.4-2258.7	33.0-2162.0
Concentration mean (ng/μl)	447.1	1777.3	632.4	604.0
A260/A280 (range)	1.8-2.3	1.8-2.2	1.7-2.3	1.9-2.2
A260/A280 (mean)	2.1	2.1	2.1	2.1
A260/230 (range)	0.6-2.5	0.8-2.4	0.3-2.4	1.0-2.3
A260/230 (mean)	1.7	1.9	1.8	1.8

**Table 3.** Total DNA analysis reported as values of concentration and ratios A260/A280, A260/230 (range and mean) divided for product type.

Type (with total number)	Amplified (n; %)	Not amplified (n; %)	Not usable* (n; %)
Canned (n=87)	54 (62.1%)	12 (13.8%)	21 (24.1%)
Dried (n=66)	61 (92.4%)	2 (3%)	3 (4.5%)
Frozen (n=33)	28 (84.8%)	2 (6%)	3 (9%)
ready-to-cook (n=27)	19 (70.4%)	7 (25.9%)	1 (3.7%)
Total (n=213)	162 (76%)	23 (10.8%)	28 (13.1%)

**Table 4:** Amplification efficiency with amplified, not amplified and not usable samples; AR: Amplification rate; \*PCR product <5ng/μl or presence of non-specific amplification bands.

Successful amplification from each MP	MPs (n)	PCR products sent to sequencing
3	28	56 (28 x 2) (+1)*
2	35	70 (35 x 2)
1	8	8 (8 x 1)
Total		134 +1 = 135

**Table 5:** Successful amplification obtained from each MP and number of PCR products sent to sequencing according to the established sequencing criteria. \* the one additional PCR product was sent to sequencing after the non-compliances observed in the BLAST analysis.

## **Highlights**

1. 71 mushroom products sold in Italy were authenticated using DNA barcoding
2. The BLAST analysis was conducted using an internal dataset and GenBank
3. The internal dataset was able to support the identification at species level
4. The internal dataset should be improved with ITS sequences from commercial species
5. Mislabeling rate (2.8%) may be under-estimated and sampling strategy should be fixed

The authors have no conflict of interests

### **Author contributions**

Alice Giusti, Laura Gasperetti, Lara Tinacci, Andrea Armani: Conceptualization; Alice Giusti, Lara Tinacci: Data curation; Alice Giusti, Lara Tinacci, Enrica Ricci: Formal analysis; Laura Gasperetti: Funding acquisition; Alice Giusti, Lara Tinacci, Enrica Ricci, Roberto Narducci, Andrea Armani, Laura Gasperetti, Francesco Verdigi: Methodology; Laura Gasperetti, Andrea Armani: Project administration; Laura Gasperetti, Andrea Armani: Supervision; Alice Giusti, Lara Tinacci, Roberto Narducci: Roles/Writing - original draft; Alice Giusti, Laura Gasperetti, Andrea Armani: Writing - review & editing.

**A****B****C****D**