Food Control

Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat

--Manuscript Draft--

Manuscript Number:	FOODCONT-D-20-02792R1
Article Type:	Research Paper
Keywords:	Species identification, exotic meat, reptiles, EU legislation, illegal trade
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Abstract:	A molecular approach (DNA barcoding and phylogenetic analysis) using mitochondrial COI and 16SrRNA genes was used to identify species in crocodile dried food products (meat and feet) and skin sold on the Chinese market and generically labelled as “鳄鱼” (crocodiles). All the 80 collected samples (100%) were identified at species level and five of them were also identified at sub-species level using the COI gene. Limits of the DNA barcoding approach related to the presence of sequences from misidentified specimens on official genetic databases (Genbank and BOLD) were encountered. The only DNA barcoding method was successfully applied for the species identification of 47 (58.7%) samples (42 using the COI and 5 using the 16SrRNA) while the support of the phylogenetic analysis was considered in 7 (8.7%) samples (performed using the 16SrRNA gene). For the remaining 26 samples (43.3%) the species identification was only achieved by phylogenetic analysis using the COI gene. Three species were overall detected: Crocodiles siamensis (n= 44; 55%), C. porosus (n= 29; 36.2%) and Caiman crocodilus (n= 7; 8.7%) with the sub-species C. crocodylus crocodylus in 5 out of the 7 cases. Although the traceability system of these products in China presented evident shortcomings, outcomes from this study appeared comforting since all the three species are among the most reared for meat production and can plausibly feed the market requests. Interestingly, only one of these species is included among those considered by the new EU legislation on reptile meat. Therefore, although Chinese crocodilian-based products are still not allowed to be imported in the EU market, a future law up-dating could not be excluded considering the relevance of the Chinese exports for the EU . Outcomes from this study, other than allowing to monitor products through the whole food value chain, contribute to enrich the scientific pool of data from which EU food imports legislation draw upon.

Dear Editor,

please find enclosed the manuscript entitled "*Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat*" to be considered for publication in Food Control.

The consumption of crocodiles (order Crocodylia) has worldwide grown over the years and, to date, crocodilian meat is appreciated in many Asian, African, Australian and American countries as healthy alternative to conventional livestock. Despite the development of farming system in which crocodiles are reared both for skin and meat production, illegal trades of crocodile-based products, that in the past wiped out many populations worldwide, are still present. Differently from other countries, the EU legislation framework specifically targeting reptile meat, including crocodilian, has been set up only in very recent times.

At present, literature dealing with the molecular identification of crocodilian species in products sold on the market are few. In particular, to the best of our knowledge, no surveys on the Chinese market were performed. Although Chinese crocodilian-based products are still not allowed to be imported in the EU market, a future law up-dating could not be excluded considering the relevance of the Chinese exports for the EU. Thus, considering that DNA-based methods can assist the monitoring of crocodile trade especially when processed products, difficult to identify, are involved, a molecular approach based on both DNA barcoding and phylogenetic analysis used COI and 16SrRNA as genetic marker was applied for identifying species in 60 crocodile dried food products (40 meat and20 feet) and 20 skin products sold on the Chinese market generically labelled as "鳄鱼" (crocodiles in English). All the samples (100%) were unequivocally allocated to species level and five of them were also identified at sub-species level using the *COI* gene. Limits of the DNA barcoding approach related to the presence of sequences from misidentified specimens on official genetic databases were encountered. Three species were overall detected: *C. siamensis* (n= 44; 55%), *C. porosus* (n= 29; 36.2%) and *Caiman crocodilus* (n= 7; 8.7%) with the sub-species *C. crocodylus crocodylus* in 5 out of the 7 cases. The dried food products (meet and feet) (n=60) were mostly identified as *C. siamensis* (n=34; 56.7%), followed by *C. porosus* (n=21; 35%) and *C. crocodylus* (with the relative sub-species) (n=5; 8.3%). Skin products (n=20) were only identified as *C. porosus* (n=12; 60%) and *C. siamensis* (n=8; 40%). Although the traceability system of these products in China presented evident shortcomings,

outcomes from this study appeared comforting since all the three species are among the most reared for meat production and can plausibly feed the market requests.

Therefore, A proper market monitoring on a continuous basis is strongly recommended and, in this respect, the technique used in this study could represent a valid control tool to face the criminality operating within illegal channels, to ensure a valid traceability system and to guarantee a proper consumers' information. Also, outcomes from this study may contribute to enrich the scientific pool of data from which EU food imports legislation draw upon.

The manuscript has not been published elsewhere nor is it being considered for publication elsewhere. All authors have approved this manuscript, agree to the order in which their names are listed, declare that no conflict of interests exists and disclose any commercial affiliation.

Best Regards
Jing Wen

Dear Editor, we are sending you back the revised version of the manuscript/paper entitled "Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat". We are pleased for the comments received by the reviewers and we thank them for the tips they gave us to ameliorate the paper.

Reviewers' comments:

Reviewer #1: The manuscript entitled as "Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat" is a report to detect the crocodile species in the crocodile products using DNA barcoding method. I think the topic of the manuscript is interesting and important. Therefore, I recommend the publication after following minor correction:

Abstract: The abstract is clearly and concisely written

Introduction:

Lines 46-47: All abbreviations (e.g. PUFA, SAF etc.) should be in full form in first time use. **Done**

Line 135: the sequences of used primers to be included.

The primers sequences were added.

Materials and Methods:

The sequences of the used primers should be included, only references are not suitable. A brief description of amplification and sequencing conditions should be added.

This section has been improved as requested.

Results:

The authors claimed that their extracted DNA quality and quantity are good compared to others, however, they did not include their results.

The results were briefly reported in this section (line 154-156).

Discussion:

The authors did not include any sub-section in the discussion section of the obtained results. Authors should discuss their results in discussion section. Authors should also discuss the possible reason why the COI gene-based barcode unable to amplify or identify the samples (S6, S32, S41, S14, S36, S50 etc.) which were tested by 16S rRNA gene-based barcode.

Overall, the COI gene was not amplified in 12 out of the 80 analysed samples (15%). Based on our experience, the possibility that a molecular target could be not amplified in a small percentage of samples despite the good quality of the extracted total DNA is nothing out of the ordinary. In fact, the presence of PCR inhibitors (especially in the case of commercial samples) or a reduced annealing performance of the PCR primers may subsist. For this reason, we preliminary set up the analytical protocol by selecting

an alternative molecular target in case of amplification failures. However, sentence has been added in the discussion section to better point out this aspect (see line 245-246).

Fig.: Label the Fig. 2 & provide the Fig. 3.

Figure 2 caption was already provided in the manuscript. However, it has been revised.

Figure 3 is now provided, and its caption has been revised.

Reviewer #2: Manuscript ID: FOODCONT-D-20-02792

Journal: Food Control Title: Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat. Authors: Xia Zhang, Andrea Armani, Alice Giusti, Jing Wen, Sigang Fan, Xiaoguo Ying

The manuscript is focused on the monitoring of crocodile dried food products and skin sold on the market in two Chinese provinces. Samples were chosen proportionally according to the product type, however, no one was properly labeled. A molecular approach of DNA barcoding and phylogenetic analysis were used for species identification. As the crocodilian-based meat products are not allowed at European market, aim of this study was also to contribute to enrich the scientific pool of data from which EU food imports legislation draw upon. It is a pilot study, as the standard method identifying crocodile-based products has not yet been selected. The manuscript lay out, form and content well suits the Food control journal and the objectives and reasons for the research are well explained. "Introduction" contains all the necessary information well-ordered in logical sequence. I appreciate the extensive separate paragraph „Discussion", which covers all aspects that could affect the reproducibility of the results. The objective of the paper could be of potential interest, however, several corrections should be accomplished, so minor revision is recommended.

Comments to the authors

1/ Please, explain, why all samples weren´t analysed using the same method/s of identification and some of them were analysed by DNA barcoding, others by phylogenetic analysis and others by both of the methods. To achieve the European legislation allowing the import of the crocodile meat, one standard detection method would be needed. By Your opinion which one would be appropriate?

This aspect was already discussed in section 4.2. We found that the only DNA barcoding approach did not allow to achieve the species level in all the samples. However, this limit is attributable to the presence of wrongly deposited sequences rather than the method itself. To answer the last question, the DNA barcoding method may be elected as standard method for species identification at EU level but, as reported in this study, a preliminary analysis aimed at evaluating the genetic database reliability and eventually discarding wrongly deposited sequences is needed.

2/ Do You think that the proper labelling of the crocodilian-based products imported from China to Europe will be achieved in the future?

Considering the relevance of the Chinese exports for the EU this eventuality is advocated. However, considering the current shortcomings involving the Chinese

labelling system (for seafood, as well as for many other foods), we think this scenario will be not achieved in the short term.

Lines 46, 47, 69: Please, write the abbreviations „PUFA", "SFA" and „IUCN" in full text, or write the full text in the brackets after the abbreviations.

Done

Lines 67, 91 and 216: Reduce, please, the number of references and choose only the most valid ones.

Done

Lines 114-118: Write in details the procedures of DNA extraction, amplification and sequencing. The citation of methods used is not sufficient, as the full text of the manuscript Zeng et al. (2019) is not freely accessible.

This section has been improved as requested.

Figure 2: The caption of this figure is missing.

Figure 2 caption was already provided in the manuscript. However, it has been revised.

Molecular authentication of crocodile dried food products (meat and feet) and skin sold on the Chinese market: implication for the European market in the light of the new legislation on reptile meat

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Abstract

A molecular approach (DNA barcoding and phylogenetic analysis) using mitochondrial *COI* and *16SrRNA* genes was used to identify species in crocodile dried food products (meat and feet) and skin sold on the Chinese market and generically labelled as "鳄鱼" (crocodiles). All the 80 collected samples (100%) were identified at species level and five of them were also identified at sub-species level using the *COI* gene. Limits of the DNA barcoding approach related to the presence of sequences from misidentified specimens on official genetic databases (Genbank and BOLD) were encountered. The only DNA barcoding method was successfully applied for the species identification of 47 (58.7%) samples (42 using the *COI* and 5 using the *16SrRNA*) while the support of the phylogenetic analysis was considered in 7 (8.7%) samples (performed using the *16SrRNA* gene). For the remaining 26 samples (43.3%) the species identification was only achieved by phylogenetic analysis using the *COI* gene. Three species were overall detected: *Crocodiles siamensis* (n= 44; 55%), *C. porosus* (n= 29; 36.2%) and *Caiman crocodilus* (n= 7; 8.7%) with the sub-species *C. crocodylus crocodylus* in 5 out of the 7 cases. Although the traceability system of these products in China presented evident shortcomings, outcomes from this study appeared comforting since all the three species are among the most reared for meat production and can plausibly feed the market requests. Interestingly, only one of these species is included among those considered by the new EU legislation on reptile meat. Therefore, although Chinese crocodilian-based products are still not allowed to be imported in the EU market, a future law up-dating could not be excluded considering the relevance of the Chinese exports for the EU. Outcomes from this study, other than allowing to monitor products through the whole food value chain, contribute to enrich the scientific pool of data from which EU food imports legislation draw upon.

Keywords

Species identification, exotic meat, reptiles, EU legislation, illegal trade

1. Introduction

The consumption of meat from species belonging to the Order Crocodylia (crocodiles, alligators, caimans and gavials) has a millennia long tradition in rural areas and in marginalized regions (Eaton et al., 2010; Sandalj, Treydte, & Ziegler, 2016). Outside these regions, it has long provoked disagreement in most of consumers (Cawthorn & Hoffman, 2016; Huang, Tsai, Liu, Syue, & Su, 2018). However, people increasingly showed curiosity to try new "adventurous foods" (Hoffman, Crafford, Muller, & Schutte, 2003; Hoffman & Cawthorn, 2013; Ahmad Nizar, Ali, Hossain, Sultana, & Ahamad, 2018) because eating this type of unconventional meat is the "par excellence" exotic experience (Cawthorn & Hoffman, 2016; Ahmad Nizar et al., 2018; Huang et al., 2018). Edible parts of crocodiles are recognized as medicinal products rather than as food in parts of Asia and Africa. In Chinacrocodile meat is thought to promote longevity, strengthen the body, replenish Qi, relieve asthma, and treat a myriad of other ailments (Deng, Chan, Deng, & Li, 2011; Williams Williams, Moshoeu, & Alexander, 2016). However, in addition to its use as medical product, crocodilian meat is appreciated for its organoleptic features such as its flavour lying between chicken, fish and veal and for its balanced content of nutrients even better respect to that of conventional livestock (Hoffman & Cawthorn, 2013; Canto et al., 2015; Černíková et al., 2015). The meat is in fact high in protein and has a good ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acid (SFA) (Hoffman, 2008). Moreover, together with other exotic meat, it can contribute to global food security by providing high quality animal proteins (Cawthorn & Hoffman, 2016). The development of breeding programs in North, Central and South America, Africa (e.g. Zimbabwe,

South Africa, Zambia), Asia (e. g. Thailand) and Australia, where national codes of practice for crocodilian farming have been developed (Manolis & Webb, 2016), has contributed to the increasing request of crocodile meat in many areas of the world (EFSA, 2007). Moreover, in many commercial breeding programs a certain number of hatchlings must be returned to the wild allowing the restocking (Fitzsimmons et al., 2002). Farming, other than reducing the hunting pressure for skin collection, have helped to consolidate the exotic meat niche in the international market (Saadoun, Cabrera, Terevinto, & del Puerto, 2014). Currently, crocodilian meat is widely consumed in Africa, Australia, Asia, US, and South America (Cawthorn & Hoffman, 2016).

As regards Europe, the citizens' practice in consuming crocodilian meat is still very limited, and the available data on to market consumption of this product are mainly aggregated with those related to the entire reptile category. Overall, imports from third countries of fresh, chilled or frozen meat and edible offal of reptiles, including crocodilian meat, have shown an upward trend over the last 10 years with an increase of over 50% in the quantity imported during the period 2007-2017 and an average yearly import in the European Union (EU) of nearly 100 tons (Eurostat's reference database for detailed statistics on international trade in goods, 2017). In Europe, it is especially imported into Belgium, Denmark, Germany, Spain, and UK (EFSA, 2007).

The EU legislation framework targeting reptile meat, including crocodilian, has been set up only in very recent times (Table 1SM). This new legislative framework clearly suggests that this foodstuff is expected to acquire a certain share of the EU market in the coming years. Accordingly, reptile meat may only be imported into the EU from certain third countries, and it must be produced in plants approved to export

and properly listed (Commission Implementing Regulation EU 2019/626). Currently Chinese plants are not included in the list, but future implementations of the countries list could occur.

However, it should be considered that the illegal hunting and trades of crocodiles, crocodile-based products (especially skin) and crocodiles meat have continued to date also endangering their survival in natural habitats (~~Meganathan Dubey, & Haque, 2009;~~ Eaton et al., 2010; ~~Jogayya, Meganathan, Dubey, & Haque, 2013; Meganathan, Dubey, Jogayya, & Haque, 2013; Ahmad Nizar et al., 2018~~). Currently, six crocodilian species are on the International Union for Conservation of Nature (IUCN) Red List as "Critically Endangered" and many other are reported as "Endangered" or "Vulnerable" (https://www.iucnredlist.org/). All living crocodiles were included in Appendix I (most species) or Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora (CITES) (https://www.cites.org/). The state of Amazonas is considered the largest producer of illegal alligator meat in the world, with the main markets being Brazil and Colombia (Marioni, Botero-Arias, & Fonseca-Junior, 2013; Carreira & Sabbag, 2015); crocodile carcasses were recorded as illegally imported from Africa to Europe (Chaber, Allebone- Webb, Lignereux, Cunningham, & Rowcliffe, 2010); a huge volume of illegal crocodile materials was confiscated in Kuala Lumpur (Malaysia) (Ahmad Nizar et al., 2018).

The increasing consumers demand and the economic weight of some Third countries, especially China, within the EU market, might lead to a further regulatory review for the imports of crocodilian products, especially considering the traceability lacks that have been reported for Chinese food products (D'Amico et al., 2014; Armani et al., 2015a). Factually, illegal Chinese alligator meat has already appeared in the markets (Yan et al., 2005). In addition to wildlife concern, crocodile is an issue in halal foods since they are prohibited for Muslims (Cawthorn &

Hoffman, 2016). Finally, health issues related to its allergenic properties (Ballardini et al., 2017) and the presence of biological hazards, especially *Salmonella* spp. and *Trichinella* spp. as well as chemical should not be underestimated (Huchzermeyer, 1997; Pozio, Owen, Marucci, & La Rosa, 2004; EFSA, 2007; Magnino et al., 2009; Schneider et al., 2012; FDA, 2020).

Based on all these premises, it is evident that proper checks at the point of entry should be performed, in order to guarantee the freely distribution of legal and safe products throughout the internal market, ensuring the traceability as well as the consumer and animal protection in accordance to the basic principles of the EU food law. In this respect, the monitoring of crocodile-based products trade can be assisted by molecular tools based on DNA analysis, especially when processed products, difficult to identify, are involved (Eaton et al., 2010). DNA based methods have been widely applied to authenticate food products with the aim to ensure their quality and safety, especially in the seafood sector, where fraudulent practices involving cases of species substitution are worldwide reported (Pardo, Jiménez, & Pérez-Villarreal, 2016). While in the past the DNA based approach in the context of meat traceability was limited (Galimberti et al., 2013), the situation has changed since many studies aimed at meat identification have been produced in the past years (Cottenet et al., 2016; Hellberg, Hernandez, & Hernandez, 2017; Hossain et al., 2019; Cottonet, Blancpain, Chuah, & Cavin, 2020).

At present studies aimed at detecting and/or identifying crocodile species in meat and skin products sold on the market are few (Yan et al., 2005; Unajak et al., 2011; Kitpipit, Sittichan, & Thanakiatkrai, 2014; Ahmad Nizar et al., 2018; Ahmad Nizar et al., 2019). Moreover, to the best of our knowledge, no surveys on the Chinese market were performed, since the unique dated study dealing with Chinese market exclusively proposed a molecular method to detect the illegal presence of the endangered Chinese alligators (*Alligator sinensis*) in commercial meat and skin

96 (Yan et al., 2005). Considering the above-mentioned relevance of Chinese foodstuff's exports for the EU, a preliminary evaluation of the
97 national status respect to the market of crocodile-based products should be performed. For this reason, in this study a molecular approach (DNA
98 barcoding and phylogenetic analysis) based on double genetic marker analysis (*COI* and *16SrRNA*) was applied for identifying species in
99 crocodile dried food products (meat and feet) sold on Chinese market generically labelled as "鳄鱼" (crocodiles in English). In addition, also
100 skin samples were collected and analysed due to their use as medical products by the traditional medicine. By providing data on the mainly
101 exploited crocodile species, this study may both facilitate the transparency in the market chain of crocodiles and contribute to enrich the
102 scientific pool of data on which EU legislation draw upon.

103 **2. Materials and Methods**

104 ***2.1. Sampling***

105 The sampling criterion was established with the aim to have a picture as close to reality as possible of the crocodile-based products currently
106 commercialized on the Chinese market. Crocodile skin, although not representing a food items, were also analyzed for this purpose. Therefore,
107 sampling was conducted to include a proportional number of products per type, according to the market availability. A total of 80 samples were
108 collected: 60 dried food products (40 meat and 20 feet) and and 20 skin samples. Sixty-four and 16 products were purchased from seafood shops
109 in Guangzhou and Zhanjiang province (China), respectively (Table 1). All the samples were sold without reference to any species on the label. A
110 selection of the samples collected in this study are shown in Fig. 1. The products' price was also considered.

111 ***2.2. DNA extraction, amplification and sequencing***

Total DNA extraction was performed using the TIANamp Marine Animals DNA Kit (TIANGEN, China) according to the manufacturer's instructions. The qualities and quantities of the DNA from each sample were determined with a U-1800 spectrophotometer (Hitachi, Japan). The primer pair LCO1490 (5'- GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'- TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994) was used for the amplification of an expected 658 bp region of the cytochrome oxidase subunit I gene (*COI*). An alternative primer pair 16SFI (5'-AAAGCATTCTGCCTACACCTGAAA-3') and 16SRI (5'-TTGTGTTGGCTGCTTTAAGGCCTA-3') (Jogayya, Meganathan, Dubey, & Haque, 2013) was used for the amplification of an expected ~600bp region of the 16S ribosomal RNA gene (*16SrRNA*) in case of failure of the *COI* amplification. PCR amplification were performed using 100 ng of template DNA and 50 µL master mix containing 2 µL each primer (10 µmol/L), 5 µL of 10×Ex Taq buffer (20 mmol/L Mg2+ plus), 4 µL dNTP mixture (2.5 mmol/L each, TaKaRa, Japan), and 0.25 µL Ex Taq DNA polymerase (2 U/µL) (TaKaRa, Japan). PCR amplifications were carried out in a C1000 touch thermal cycler (Bio-Rad, USA). For the COI gene the amplification conditions were a denaturing step at 94 °C for 3 min, 30 cycles of 42 s at 94 °C for denaturation, 30 s at 48 °C for annealing and 50 s at 72 °C for extension, and a final extension at 72 °C for 10 min. Amplification conditions for the 16S rRNA gene were 94 °C for 5 min of initial denaturation followed by 30 cycles of: denaturation at 95°C for 30 s; annealing at 63°C for 30 s, extension at 72°C for 30 s and amplification ended with a 7 min final extension step followed by a 4°C hold. The PCR products were analyzed by 1.2% agarose gel (11.5×6 cm) electrophoresis at 160 V for 30 min. The lengths of the fragments were determined by comparison with the DL2000 DNA ladder (TaKaRa, Japan). PCR products were purified with the AxyPrep™ DNA Gel Extraction Kit (Axygen, USA) and sequenced in both directions with the Applied Biosystems 3730 Automatic Sequencer.

2.3. Sequence editing and comparison with databases

The sequences were analyzed with the Chromas lite v2.23 software and aligned using Editseq software (DNASTAR Lasergene Version 7.1.0) and Jellyfish v1.4 software. The final sequences were queried by Basic Local Analysis Search Tool (BLAST) selecting the program Highly similar sequences (megablast) and, in the case of *COI*, also by the BOLD Identification System (ID's) selecting the program Species Level Barcode Records (Ratnasingham & Hebert, 2007) against the reference sequences available on GenBank (http://www.ncbi.nlm.nih.gov) and Barcode of Life Data system (BOLD) (http://www.boldsystems.org/), respectively. A match with a sequence similarity of at least 98% was used to designate potential species identification for the *COI* (Hebert, Cywinska, Ball, & deWaard, 2003). As regards the *16SrRNA*, a specific identification was attributed only for identity values of 99-100% (Armani et al., 2015b), due to the lower interspecific variability of this gene.

2.4 Phylogenetic analysis and species identification

A phylogenetic analysis was performed on both the *COI* and the *16SrRNA* genes. To do this, all the species from Alligatoridae, Crocodylidae and Gavialidae families were searched on the Reptile Database (www.reptile-database.org). Subsequently, for all the retrieved species all the available *COI* and *16SrRNA* sequences were searched on GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and, in the case of the *COI* gene, also from BOLD (Table 2SM). For each gene, all the retrieved sequences were first aligned with Geneious R7 software (Kearse et al., 2012) and those that did not match with the *COI* and *16SrRNA* fragment analysed in this study (section 2.2) were discarded. Thus, two distinct genetic datasets were constructed, one for *COI* and one for *16SrRNA*, containing the retained sequences (Table 2SM) and the sequences obtained from this study. Both the datasets were used to construct a Neighbor-Joining (NJ) phylograms using the Kimura 2-parameter model (Kimura, 1980)

with 1000 bootstrap re-samplings in MEGA-X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Where available, five representative sequences for each species were used.

3. Results

3.1 Samples collection: market price and labelling

The crocodile products collected in this study were characterized by relatively high prizes. Meat and feet products were sold at variable price of 320, 380, 440, 560 RMB (~79, 108, 138US$)/kg and even 720 RMB (~177 US$)/kg, respectively. The prices variability was only related to the different shops in which the products were sold. In addition, no correlation exists between price, information reported on the label and species identified by the molecular analysis (see section 3.3). In fact, all the products collected in this survey only reported on the label the generic Chinese name "鳄鱼" (crocodiles in English) referring to the taxonomical group (order Crocodylia). The Chinese terms 鳄鱼肉, 鳄鱼皮, 鳄鱼爪 were used on the labels of crocodile meat, skin and feet, respectively.

3.2 DNA extraction, amplification, sequencing, and sequence editing

Although a certain degree of DNA degradation, low average DNA concentrations and low purity was reported for crocodile processed products (Eaton et al., 2010; Ahmad Nizar et al., 2018), our results revealed a good DNA quality and concentration, as ~~for all the collected samples~~the spectrophotometric analysis confirmed medium high yield and quality (A260/A280 and A260/A230 ratio >2.0) for all the collected samples (data not shown). Therefore, as expected, all the 80 DNA samples produced at least one amplicon suitable for sequencing and one

159 readable sequence. In particular, the *COI* gene was successfully amplified from 68 samples (35 meat, 16 feet, and 17 skin samples) while the
160 *16SrRNA* gene from 12 samples (5 meat, 4 feet and 3 skin samples). All the *COI* sequences were 658 bp in length, corresponding to 100% of the
161 expected amplicons. Also, all the *16SrRNA* sequences reached 100% of the expected amplicon length (ranging from 544 to 546 bp due to the
162 presence of species-specific insertions and deletions). For both the genes, the sequences were conventionally grouped together if identical. In
163 particular, five sequences group were obtained from *COI* and five from *16SrRNA.* Samples from each group were detailed in Table 1.

164 ***3.3 Sequences comparison with genetic databases and phylogenetic analysis***

165 *3.3.1 COI gene.* As reported in Table 1, the sequences belonging to the group 1 (40 samples) showed a 100% identity value with three
166 sequences (EU621816, EF581859 and MH999467) from *C. siamensis* (Crocodylidae) using both BLAST and BOLD IDs systems. Both the
167 sequences belonging to the group 2 (15 samples) and the group 3 (7 samples) showed 100% and 99.85% identity value with sequences deposited
168 as *C. siamensis*; additionally, identity values ranging from 98.92% to 99.85% (group 2) and 98.77% 100% (group 3) with sequences deposited as
169 *C. porosus* were observed. According to Srikulnath, Thongpan, Suputtitada, & Apisitwanich (2012), which has proved that the complete mtC
170 DQ353946 sequence (matching with group 2 and 3) might be an intraspecific variation of *C. porosus* instead of *C. siamensis*, it was highly
171 probable that sequences from group 2 and group 3 were *C. porosus.* However, given the high identity value with the sequence NC_008795
172 which was reported as *C. siamensis,* it was not possible unequivocally to allocate the sequences to a species only the basis of the sequence
173 comparison. The sequences belonging to the group 4 (4 samples) and the group 5 (2 samples) were identified as *C. crocodylus* (Alligatoridae)
174 using both BLAST and BOLD IDs systems with identity values of 98-99.43% and 98.93-99.24%, respectively. In particular, the group 5 was

allocated to the sub-species *C. crocodylus crocodylus* (Table 2), while the group 4 was not possible identified at the sub-species level since the BOLD analysis also showed identity values higher than 98% with *C. crocodylus chiapasius* (Table 1). To further investigate the results obtained by the sequences comparison a phylogenetic analysis was conducted. To do this a preliminary selection of the sequences were performed and 21 species were considered for the *COI* and *16SrRNA* sequences retrieving. For *C. crocodylus,* also the 4 valid sub-species were included (Table 2SM) on the basis of the outcomes of the sequences' comparison. All the BOLD available sequences were mined from Genbank, so they were considered once with the Genbank accession number (Table 2SM). Of the 410 initially retrieved sequences for *COI*, 364 were retained in the genetic dataset (Table 2SM). The NJ phylograms essentially confirms the results obtained for the groups 1, 4 and 5 by the sequences comparison. Sequences from group 1 clustered with sequences from *C. siamensis* that showed a 100% identity during databases comparison, including the sequence EF581859, which was proved to belong to the real *C. siamensis* (Srikulnath et al., 2012), and separately from the cluster containing the sequence DQ353946 which according to the same study was actually *C. porosus.* Sequences from group 4 were clearly identified as *C. crocodyus crocodylus* as clustering with sequences from group 5 within the cluster containing *C. crocodylus crocodylus* sequences (JN311638-40) and separately from the *C. crocodylus chiapasius* cluster (Fig. 2). As regards the groups 2 and 3, the cluster containing the mtC sequence from properly identified *C. siamensis* (EF581859) and in which sequences from group 1 were located, was separated from both the cluster containing *C. porosus* sequences (HM490354-56 and AJ810453) and the cluster containing sequences from group 2 and group 3, which actually appeared to include intraspecific variation of *C. porosus.* In addition to the mtC sequence DQ353946 which was already proved to be *C. porosus* instead of *C. siamensis* (Srikulnath et al., 2012), also the mtC NC_008795 seems to belong to *C. porosus* in this study. Therefore,

191 samples from group 2 and group 3 probably belonged to *C. porosus*. The analysis of the deposited sequences showed that the sequences
192 MH999467, EF581859 and EU621816 were true *C. siamensis,* while DQ353946, NC 008795 and DQ273698 may be intra-specific variation of
193 *C. porosus* (Fig. 2). Additionally, the sequences deposited as private on BOLD and reported as *C. crocodylus chiapasius* (Table 1) probably
194 belonged to misidentified specimens.

195 *3.3.2 16SrRNA gene.* The BLAST analysis conducted on the sequences belonging to the group 1 (3 samples) showed a 100% identity value
196 with *C. siamensis* (Table 1). The sequences belonging to the group 2 (3 samples), the group 3 (1 sample) and the group 4 (3 samples) were
197 instead identified as *C. porosus* since the unique *C. siamensis* sequence matching with them was the mtC DQ353946 (intraspecific variation of
198 *C. porosus*) (see section 3.3.1) (Table 1). Finally, sequences from group 5 (2 samples) showed a 100% identity value with *C. crocodylus*. For the
199 phylogenetic analysis, of the 98 initially retrieved sequences for *COI*, 43 were retained in the genetic dataset (Table 2SM). The NJ phylogram
200 constructed with *16SrRNA* sequences (Fig. 3) confirmed the outcomes of the BLAST analysis and especially proved that sequences belonging to
201 the group 2, 3 and 4 were *C. porosus* (mtC DQ353946 and mtC NC_008795 were both proved to be *C. porosus* instead of *C. siamensis* in
202 previous sections).

203 ***3.4 Final species identification of the collected samples***

204 All the collected samples (100%) were finally identified at species level and five of them were also identified at sub-species level using the
205 *COI* gene (Table 2). The only DNA barcoding approach successfully applied for the species identification of 47 (58.7%) samples (42 using the
206 *COI* and 5 using the *16SrRNA*) while the support of the phylogenetic analysis was considered in 7 (8.7%) samples (performed using the

207 *16SrRNA* gene). For the remained 26 samples (32.5%) the species identification was only achieved by the means of the phylogenetic analysis
208 (performed using the *COI* gene). *C. siamensis*, *C. porosus* (Crocodylidae) and *C. crocodylus* (Alligatoridae) were identified in 55% (n=44),
209 36.2% (n=29) and 8.7% (n=7) of the samples, respectively. Five out of the 7 *C. crocodylus* were also identified at sub-species level as *C.*
210 *crocodylus crocodylus.* The dried food products (meet and feet) (n=60) were mostly identified as *C. siamensis* (n=34; 56.7%), followed by *C.*
211 *porosus* (n=21; 35%) and *C. crocodylus* (with the relative sub-species) (n=5; 8.3%). Skin products (n=20) were only identified as *C. porosus*
212 (n=12; 60%) and *C. siamensis* (n=8; 40%).

213 **4. Discussion**

214 ***4.1 Samples collection: price and labelling analysis***

215 The prices of the products analysed in this study seemed to agree with previous data referring to 2004 and could be related to the fact that, in
216 China, crocodile meat is considered a delicacy and luxury food item (Xubing & Rui, 2004). This is also demonstrated by the fact that in the two
217 of the most prestigious restaurants in the Bangkok area specialise in crocodile meat dishes many of the customers are Chinese citizens
218 (https://www.fatg.com.au/meat-game/meat-products/crocodile). As regards the products' labelling, it is easily understandable that the utilization
219 of the generic umbrella term "crocodile" is not appropriate to accurately described the species belonging to the aforesaid taxa. This evidence still
220 highlights the lack of a proper labelling and traceability system for the Chinese food chain (Armani et al., 2012; Xiong et al., 2016; Wen et al.,
221 2017; Zeng et al., 2019). Indeed, these shortcomings were also highlighted in the labelling system of Chinese seafood products imported to EU

(D'Amico et al., 2014) so that, in a context of potential entry in the Community market, the labelling system should be properly revised and harmonised with EU requirements (see section 4.4).

4.2 Selection of the molecular markers and limits of the DNA barcoding approach

Mitochondrial DNA (mtDNA) is a good target for phylogenetic reconstruction at several taxonomic levels (Avise, 2000; Rastogi et al., 2007; Panday, Jha, Thapa, Pokharel, & Aryal, 2014) and complete mtDNAs have been so far published for crocodilian species (Janke & Arnason, 1997; ~~Janke, Erpenbeck, Nielsson, & Arnason, 2001~~; ~~Wu et al., 2003~~; Yan, Feng, Li, & Wu, 2010; ~~Man, Yishu, Peng, & Xiaobing, 2011~~; Srikulnath et al., 2012). Opposed to crocodile food products, literature dealing with the molecular identification of crocodilian species from tissue or blood samples is wide (Fitzsimmons et al., 2002; ~~Li, Wu, Ji, Yan, & Amato, 2007~~; Meganathan, Dubey, & Haque, 2009; Eaton et al., 2010; ~~Man et al., 2011~~; Srikulnath et al., 2012; Jogayya et al., 2013; ~~Bloor, Ibánez, & Viloria‐Lagares, 2015~~; ~~Shirley, Villanova, Vliet, & Austin, 2015~~). In this taxon, the effectiveness of the mitochondrial cytochrome b gene (*cytb*) as species-specific marker has been established for a long time (Yan et al., 2005; Meganathan et al., 2009; Unajak et al., 2011; Srikulnath et al., 2012). Short fragments from this gene were proposed for the analysis of commercial meat products (Yan et al., 2005; Unajak et al., 2011; Ahmad Nizar et al., 2019). Other mitochondrial genes have been proved as equally efficient for this purpose; Srikulnath et al. (2012), which constructed and compared distinct phylograms using the complete *cytb*, and the *COI*, proved that the two molecular markers produced similar tree topologies. The standard *COI* barcoding region for identifying the members of animal kingdom proposed by Hebert et al. (2003) was successfully used for detecting crocodile species in bushmeat (Eaton et al., 2010) and for distinguishing threatened species in India (Meganathan, Dubey, Jogayya, & Haque, 2013). Meganathan et al. (2013)

affirmed that the use of *COI* as molecular marker for crocodiles had been restricted due to lack of reference sequences respect to *cytb*. However, the higher taxonomic coverage of genetic databases observed in the last years, and the growing confidence of the scientific community in the Barcode of Life Data (BOLD) system (http://www.boldsystems.org/) may act as incentive for selecting *COI* as elective marker.

Among mitochondrial genes, also the *16SrRNA* was proved as effective for forensic identification of crocodile species; in particular, Jogayya et al. (2013) projected two primer pairs for the amplification of two partial *16SrRNA* sequences of six crocodile species which should be later combined to obtain a larger region (~1290 bp) showing a sufficient inter-species variability. Recently, Ahmad Nizar et al. (2018) affirmed that an analytical approach based on the use of two molecular markers provides better security because if one is broken down, the alternative target can complement the missing target. Factually, the double-gene approach in crocodile species identification had been also previously applied by other authors (Unajak et al., 2011; Bloor, Ibánez, & Viloria- Lagares, 2015~~Bloor et al., 2015~~; Shirley, Villanova, Vliet, & Austin, 2015~~Shirley et al., 2015~~). For all the above-mentioned reasons, we chose to select two distinct molecular marker such as *COI* and *16SrRNA* for identifying crocodile species. The *16SrRNA* was selected as alternative marker given its well-known higher conservation degree respect to other mitochondrial genes (Hebert et al., 2003), and this aspect could increase the possibility to amplify DNA from a broader range of species especially in case of *COI* gene amplification failure. In fact, also in this study we were not able to amplify the *COI* from all the samples.

In this study, the only DNA barcoding approach did not allow to unequivocally identify all the samples at species level (section 3.4). Based on the observed results, these shortcomings were not to be attributed to the method itself, given the fact that both the selected markers showed an inter-species variability that allowed to discriminate species within this taxon. However, the presence in official databases of sequences from

misidentified specimens could make less reliable, or even distort, the analysis outcomes. The taxonomic inconsistencies of official databases were already highlighted by other studies dealing with DNA barcoding (Vella, Vella, Karakulak, & Oray, 2017; Giusti et al., 2019). Therefore, the preliminary sequences analysis and the construction of reliable internal datasets represent fundamental steps before approaching this method.

4.3 Crocodilian species identification: conservation status and farming

Overall, data arising from this study showed the similar market situation that was described fifteen years ago. Crocodilian meat available on Chinese market belonged to farmed crocodiles and particularly the Saltwater crocodile (*C. porosus*) and the Siamese crocodile (*C. siamensis*) (Yan et al., 2005). The Saltwater crocodile is considered as least concern according to the IUCN Red List (iucnredlist.org) and it is one of the most widely distributed of all crocodilians, ranging from southern India and Sri Lanka, throughout southeast Asia, east through the Philippines to Micronesia, and down through Indonesia, Papua New Guinea and the Solomon Islands to northern Australia (Webb, Manolis, & Brien, 2010). This species represents the focus of crocodilian farming in Australia and it is also farmed in Papua New Guinea and in a number of Asian countries (Cawthorn & Hoffman, 2016).

The Siamese crocodile, native to the most of Southeast Asia regions, is already extirpated in the wild or nearly extinct from 99% of its original range except Cambodia due to threat from human disturbance, habitat occupation and illegal capture. It was even believed that the species was almost or completely extinct in the wild in 1992 (Simpson & Bezuijen, 2010). Therefore, this species is critically endangered in wild while it is reared extensively in Thailand and Cambodia (Cawthorn & Hoffman, 2016). Since both the above-mentioned species found in this

study are currently widely farmed, it is highly plausible that illegal trade channels were not involved and that the analysed products belonged to reared specimens.

The Saltwater and the Siamese crocodiles are furthermore widely raised together (Fitzsimmons et al., 2002; Srikulnath et al., 2012). Hybrids between these two species naturally occur as they remain fertile in captivity and, in many farms, they have been intentionally hybridized (Fitzsimmons et al., 2002; EFSA, 2007; Srikulnath et al., 2012). The Saltwater crocodile/Siamese crocodile hybrids are significantly reared in Africa (Zimbabwe, South Africa) (EFSA, 2007) and Asia (Thailand, Bangladesh) (Srikulnath et al., 2012; Hossain, Jaman, Ahmed, Rahman, & Uddin, 2013).

The spectacled caiman, a species that is mainly found in Central and South America, From Oaxaca, Mexico, to Central and South America to Paraguay River and Argentina (Saadoun et al., 2014), was instead for the first time found in crocodile meat products sold on the Chinese market. Even though it is still mainly bred for obtaining skins, the production of meat from *Caiman spp.* is considered for long time an alternative to increase the farmer's income (Cossu, Gonzáles, Wawrzkiewicz, Moreno, & Vieites, 2007). In this respect, the spectacled caiman is highly reared in Colombia and Brazil, while other caiman species such as the yacare caiman (*C. yacare*) and the broad-snouted caiman (*C. latirostris*) are more exploited in Bolivia and Argentina, respectively (Cawthorn & Hoffman, 2016). In 2015, farms in South America produced meat and skin of yacare caimans with an estimated value of approximately $900,000 USD per year (Carreira & Sabbag, 2015). Moreover, C. *crocodilus* is also highly reared for meat production in Taiwan, because its meat is particularly appreciated as pale and tender with a characteristic mild taste (Huang et al., 2018) and for this reason it is highly plausible that, also in this case, farmed specimens were involved in the meat products

analysed in this study. It is equally plausible that, for all the three found species, we were dealing with products imported from other Asian countries (e. g. Thailand) or from Australia, due to the fact that the attempting to develop crocodile farming in China has been unsuccessful (Guo et al., 2018). Thailand is in fact the leader in crocodile farming since 1990s, especially exporting skins to Europe and meat to Asian countries; Australia, whose crocodile industry is increasingly growing, seriously produced for the Asian market since 2011, when a crocodile-meat-export agreement with the Chinese government was signed (https://www.fatg.com.au/meat-game/meat-products/crocodile). However, our hypothesis could not be undoubtedly assumed given the inadequacy of the traceability system that trace back products to their origin. Furthermore, we do not have to forget that caiman meat still represents the largest illegal crocodile trade in the world (Marioni et al., 2013) and the eventual presence of illegal products on the national market should not be excluded. In this context, the implementation of a legislation specifically addressed to products identification through the whole food value chain is highly advocated.

Knowing the product origin could also allow to select products coming from more sustainable productions such as ranching or harvesting instead of farming. Ranching is in fact a rearing that depends on the presence of a sustainable wild population in which collections of eggs or hatchlings are regulated by quotas established by wildlife authorities. This system contributes to the replenishment of the wild population by the restocking of a certain percentage of the animals reared in captivity (Magnino et al., 2009; Marioni et al., 2013).

4.4 Crocodilian species identification: implication for the EU market in the light of the current legislation

With the enactment of the Regulation (EU) 2017/625, where the scope of the previous EU official controls regulations was expanded to the entire agri-food chain, a relevant focus was addressed to lay down requirements for the entry into the EU of consignments of animals and goods

301 from third countries in order to ensure their compliance with EU in the area of food and food safety. With this mind, the imports data and market
302 share of certain unconventional products of animal origin (such as reptiles, including crocodiles) were also considered (Table 1SM). In the
303 Commission Delegated Regulation (EU) 625/2019 the reptile meat was defined as the edible parts, either unprocessed or processed, derived
304 from authorised farmed reptiles. Three crocodile (*Crocodylus johnstoni*, *C. niloticus*, *C. porosus*) and one alligator (*Alligator mississippiensis*)
305 species were included in the category, together with other reptilian taxa (Commission Delegated Regulation EU 625/2019). However, the future
306 inclusion of other crocodilian species cannot be excluded on condition that they are authorised in accordance with Regulation (EU) 2015/2283
307 on novel food and listed in the relative Commission Implementing Regulation (EU) 2017/2470. Therefore, although crocodilian products from
308 China are still not included, the current relevance of Chinese exports for EU market might lead to might lead to a further regulatory review for
309 the imports of crocodilian products, also in term of marketed species. Among the species found in this study only *C. porosus* is currently
310 reported in the approved list of reptiles and allowed to be imported (Commission Delegated Regulation EU 625/2019); however, considering the
311 current commercial relevance of *C. siamensis* in South-east Asia, it is not excluded that also this species will be authorised as novel food
312 (Regulation EU 2015/2283) once imports from China are considered. Therefore, data from this study could produce useful data to on crocodile
313 specie that could be considered for importation in future, in analogy with what regularly happens for seafood. The official lists of seafood must
314 be in fact updated on the basis of trade inputs and in response to the expansion of the variety of species, present, in transit or permanently
315 introduced on the national market (Tinacci, Giusti, Guardone, Luisi, & Armani, 2019). It should be however pointed out that Chinese
316 government should first improve its traceability system to comply with EU legislation (Regulation EU 1169/2011) since the outcomes from this

study highlighted substantial shortcomings (see section 4.1). In addition, also EU legislation on biological and chemical risks management should be complied.

Conclusions

This study represents the first aimed at investigating crocodile food products (meat and feet) and skin on the Chinese market. Overall, the average prices of the collected products confirmed that, while in rural areas wild bushmeat represents an important protein and additional income source, in urban areas it often represents a luxury food. Consequently, this unconventional item may be particularly subjected to fraudulent practices that can affect not only the consumers' right of making a conscious choice but can also hamper the fair trade facilitating the illegal commerce. The outcomes of this study appeared comforting respect to the data regarding the illegal hunting practices which have over the years strongly wipe out many crocodilian populations worldwide, since all the species found in the analysed products are reported among the most reared for meat production. However, a proper market monitoring on a continuous basis is strongly recommended especially in the presence of a too vague labelling system such as those applied to the products analysed in this study. Finally, this study highlighted the marketing of products belonging to a species not even considered in the EU list of reptiles, showing that the molecular approach not only allow to support products traceability but also provide useful data that can be used for the continuous improvement of the EU legislations.

Acknowledgments and Funding

This work was supported by the National Natural Science Foundation of China (No. 31872571), the Foundation of Department of Education of Guangdong Province (Nos. 2019KZDXM018, 2020ZDZX1042), the Technology Program of Shaoguan (200811114531607), the Scientific Research Project of Zhejiang Province (No. 2019C02075), the Fund of Zhejiang Ocean University (Nos. 11135090218, 11105090618) and the Foundation of Shaoguan University (Nos. XJ2020000601, 433-99000614).

Conflict of interest

Authors declare no conflict of interest

Figures caption

Fig. 1. Dried crocodile food products analyzed in this study. a-c = dried skin; d-f = dried feet; g-j = dried meat. Bar = 2 cm.

Fig. 2. NJ phylogram created in MEGA-X (Kumar et al., 2018) with *COI* sequences. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site.

Fig. 3. NJ phylogram created in MEGA-X (Kumar et al., 2018) with *16SrRNA* sequences. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site.

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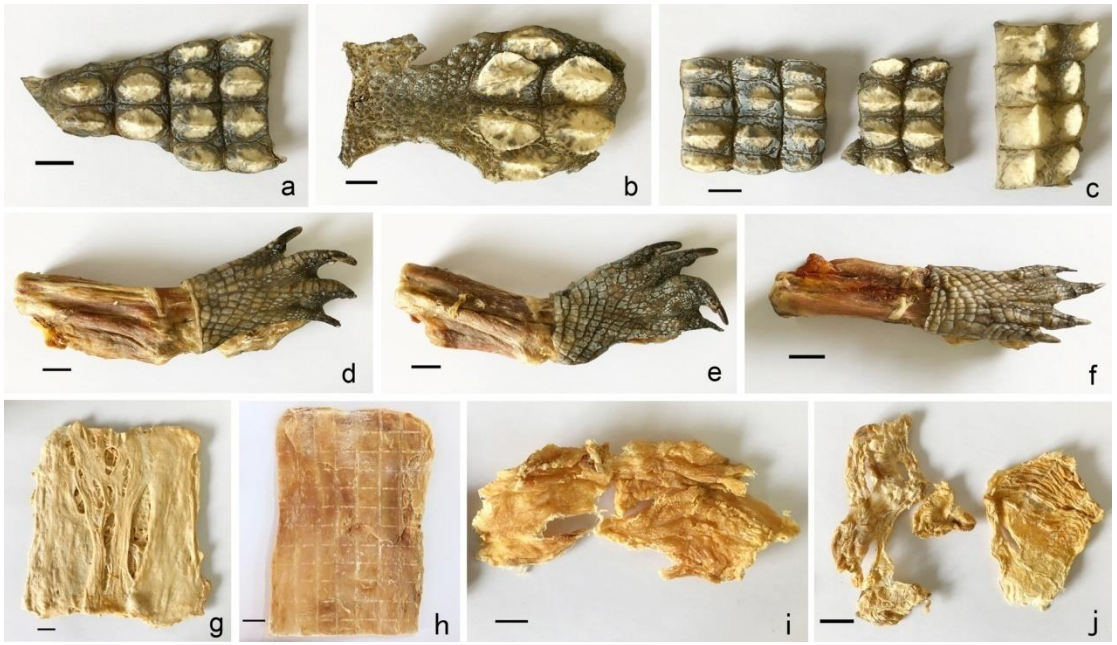

Figure 1. Dried crocodile food products analyzed in this study. a-c = dried skin; d-f = dried feet; g-j = dried meat. Bar = 2 cm.

560 **Table 1**. *COI* and *16SrRNA* DNA barcoding results; samples from which identical sequences were obtained were grouped together. DQ353946*
561 (mitochondrion complete) might be the intraspecific variation of *C. porosus* according to Srikulnath et al. (2012).

Molecular target	Group	samples	ID values (Genbank)	ID values (BOLD)
mtCOI	1	S1-S5, S7-10, S15, S18, S21-S24, S31, S33-S35, S42-43, S57-75	100% *Crocodylus siamensis* (MH999467; EF581859; EU621816)	100% *Crocodylus siamensis* (MH999467; EF581859; EU621816)
	2	S11-13, S30, S37-40, S44-47, S76-78	100% *C. siamensis* (DQ353946*)	100% *C. siamensis* (NC_008795; DQ353946*)
			98.94-99.85% *Crocodylus porosus* (DQ273698; NC_008143; HM490344; AJ810453; EU621815)	98.92-99.85% *Crocodylus porosus* (DQ273698; EU621815; HM490344-63; NC_008143; AJ810453)
	3	S19-20, S25-28, S49	98.78-100% *Crocodylus porosus* (DQ273698; NC_008143; HM490344; AJ810453; EU621815)	98.77-100% *Crocodylus porosus* (DQ273698; NC_008143; HM490344-63; AJ810453; EU621815)
			99.85% *Crocodylus siamensis* (DQ353946*)	99.85% *Crocodylus siamensis* (DQ353946*; NC_008795)
	4	S51-52, S55-56	99.09-99.22% *Caiman crocodylus crocodylus*	98.98-99.43% *Caiman crocodylus crocodylus* (private

			(JN311638; JN311640)	sequences)
				98-98.12% *Caiman crocodylus chiapasius* (private sequences) X
	5	S79-80	98.03-99.06% *Caiman crocodylus crocodylus* (JN311638; JN311640)	98.81-99.24% *Caiman crocodylus crocodylus* (private sequences)
16S rRNA	1	S6, S32, S41	100% *Crocodylus siamensis* (EF581859)	-
	2	S14, S36, S50	99.63% *Crocodylus siamensis* (DQ353946*)	-
			99.63% *Crocodylus porosus* (DQ273698)	
	3	S16	100% *Crocodylus siamensis* (DQ353946*)	-
			99.27-100% *Crocodylus porosus* (DQ273698; NC_008143; AJ810453)	
	4	S17, S29, S48	99.82% *Crocodylus siamensis* (DQ353946*)	-

			99.45-99.82% *Crocodylus porosus* (DQ273698; NC_008143; AJ810453)	
	5	S53-54	100% *Caiman crocodylus* (AJ404872)	-

577 **Table 2. Final species identification of the collected samples.** The used molecular marker and the analytical method were reported.

sample type	sample code	n	gene	analytical tool	Identified species
meat	S41	1	*16SrRNA*	DNA barcoding	*Crocodylus siamensis*
	S42-S43	2	*COI*	DNA barcoding	*Crocodylus siamensis*
	S44-S47	4	*COI*	phylogenetic analysis	*Crocodylus porosus*
	S48	1	*16SrRNA*	DNA barcoding + phylogenetic analysis	*Crocodylus porosus*
	S49	1	*COI*	phylogenetic analysis	*Crocodylus porosus*
	S50	1	*16SrRNA*	DNA barcoding + phylogenetic analysis	*Crocodylus porosus*
	S51-S52	2	*COI*	phylogenetic analysis	*Caiman crocodylus crocodylus*
	S53-S54	2	*16SrRNA*	DNA barcoding	*Caiman crocodylus*
	S55-S56	2	*COI*	phylogenetic analysis	*Caiman crocodylus crocodylus*
	S57-S75	19	*COI*	DNA barcoding	*Crocodylus siamensis*
	S76-S78	3	*COI*	phylogenetic analysis	*Crocodylus porosus*
	S79-S80	2	*COI*	DNA barcoding	*Caiman crocodylus crocodylus*
feet	S1-S5	5	*COI*	DNA barcoding	*Crocodylus siamensis*
	S6	1	*16SrRNA*	DNA barcoding	*Crocodylus siamensis*
	S7-S10	4	*COI*	DNA barcoding	*Crocodylus siamensis*
	S11-S13	3	*COI*	phylogenetic analysis	*Crocodylus porosus*
	S14	1	*16SrRNA*	DNA barcoding + phylogenetic analysis	*Crocodylus porosus*
	S15	1	*COI*	DNA barcoding	*Crocodylus siamensis*
	S16-S17	2	*16SrRNA*	DNA barcoding + phylogenetic analysis	*Crocodylus porosus*
	S18	1	*COI*	DNA barcoding	*Crocodylus siamensis*
	S19-S20	2	*COI*	phylogenetic analysis	*Crocodylus porosus*
skin	S21-S24	4	*COI*	DNA barcoding	*Crocodylus siamensis*
	S25-S28	4	*COI*	phylogenetic analysis	*Crocodylus porosus*
	S29	1	*16SrRNA*	DNA barcoding + phylogenetic analysis	*Crocodylus porosus*
	S30	1	*COI*	phylogenetic analysis	*Crocodylus porosus*
	S31	1	*COI*	DNA barcoding	*Crocodylus siamensis*
	S32	1	*16SrRNA*	DNA barcoding	*Crocodylus siamensis*
	S33-S35	3	*COI*	DNA barcoding	*Crocodylus siamensis*
	S36	1	*16SrRNA*	DNA barcoding + phylogenetic analysis	*Crocodylus porosus*
	S37-S40	4	*COI*	phylogenetic analysis	*Crocodylus porosus*

578

1. Crocodile species in Chinese food (meat and feet) and skin were molecularly verified.
2. 100% of the samples were identified although limits of DNA barcoding were encountered.
3. *Crocodylus siamensis* (55%), *C. porosus* (36.2%) and *Caiman crocodilus* (8.7%) were found.
4. Only one species is currently allowed to be imported into EU among those detected
5. EU food imports legislation may draw upon from data obtained from this study.

*Conflict of Interest Form

Declarations of interest: none

CRediT author statement

Xia Zhang: **Investigation, Data curation, writing Original Draft**

Andrea Armani: **Writing - Review & Editing, Supervison**

Alice Giusti: **Writing - Review & Editing**

Jing Wen: **Conceptualization; Writing - Review & Editing, Funding aquisiton**

Sigang Fan: **Conceptualization; Writing - Review & Editing**

Xiaoguo Ying: **Conceptualization; Writing - Review & Editing**

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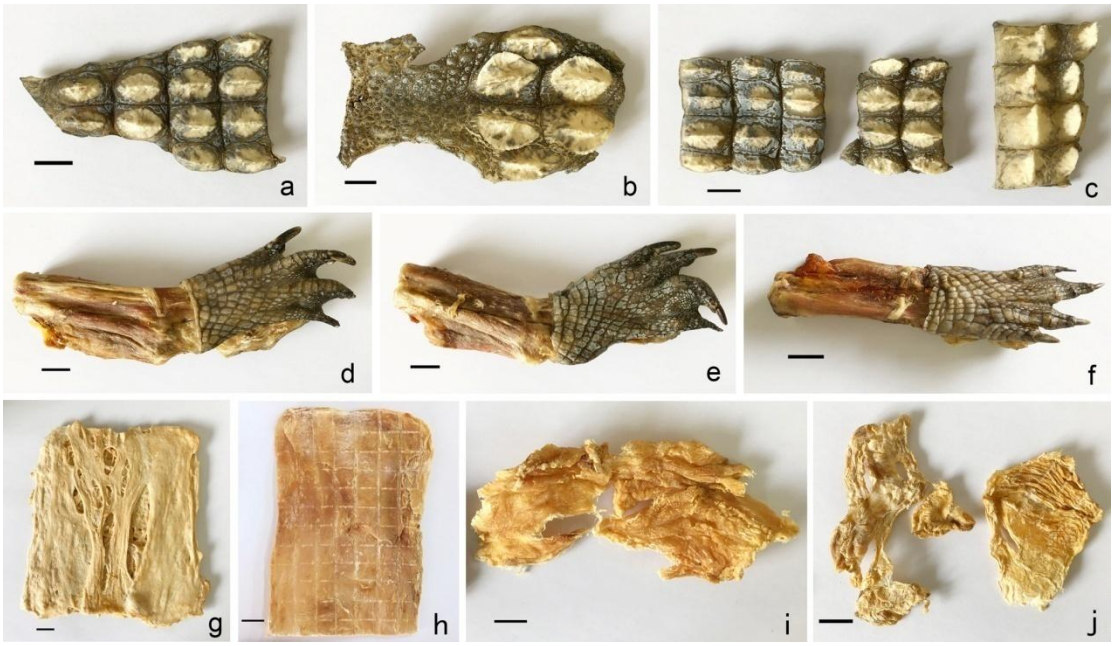
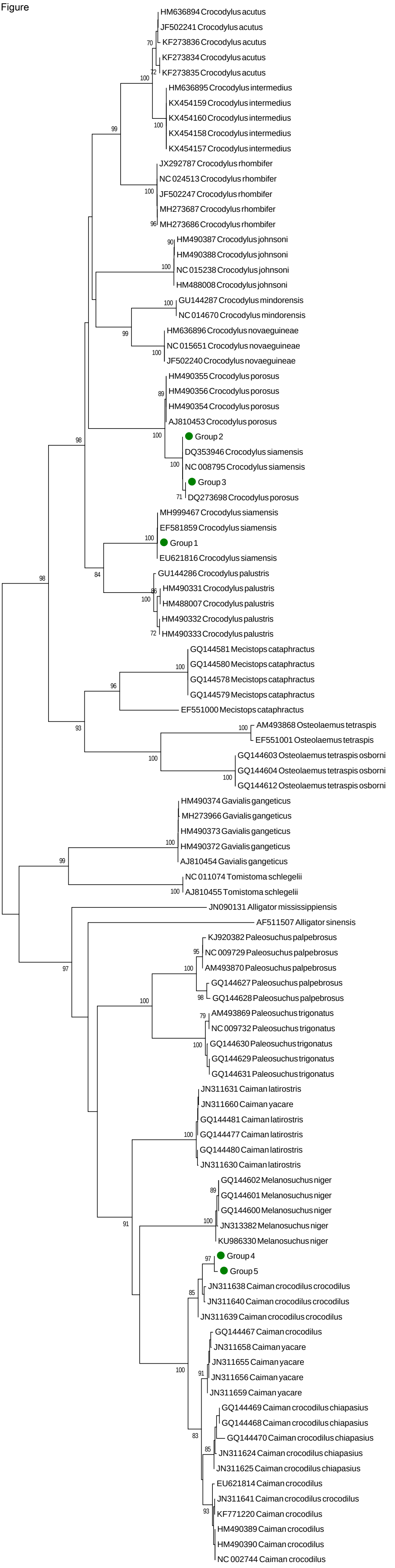
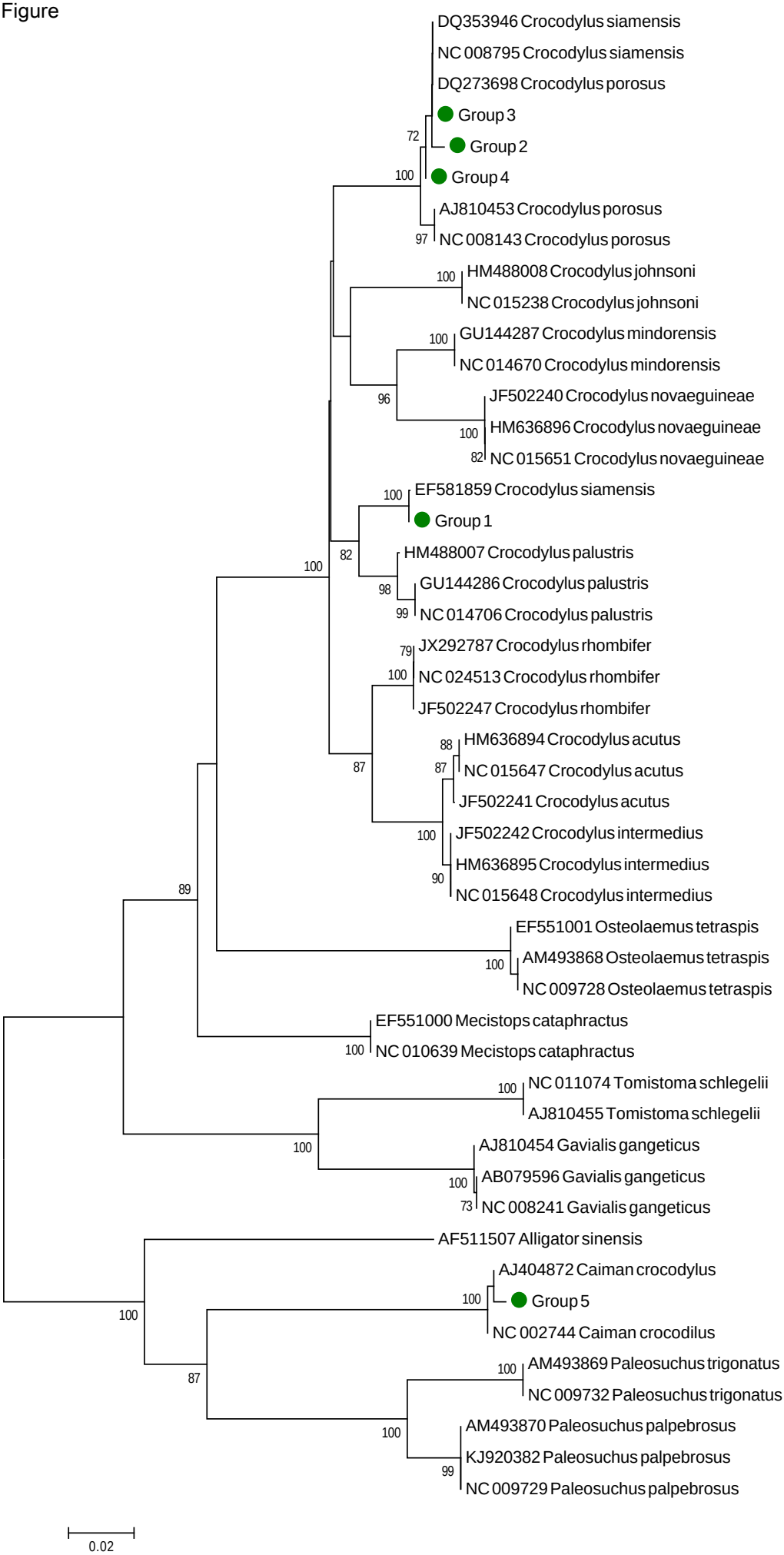


Fig. 1. Dried crocodile food products analyzed in this study. a-c = dried skin; d-f = dried feet; g-j = dried meat. Bar = 2 cm.

Figure


HM636894 Crocodylus acutus
JF502241 Crocodylus acutus
KF273836 Crocodylus acutus
KF273834 Crocodylus acutus
KF273835 Crocodylus acutus
HM636895 Crocodylus intermedius
KX454159 Crocodylus intermedius
KX454160 Crocodylus intermedius
KX454158 Crocodylus intermedius
KX454157 Crocodylus intermedius
JX292787 Crocodylus rhombifer
NC 024513 Crocodylus rhombifer
JF502247 Crocodylus rhombifer
MH273687 Crocodylus rhombifer
MH273686 Crocodylus rhombifer
HM490387 Crocodylus johnsoni
HM490388 Crocodylus johnsoni
NC 015238 Crocodylus johnsoni
HM488008 Crocodylus johnsoni
GU144287 Crocodylus mindorensis
NC 014670 Crocodylus mindorensis
HM636896 Crocodylus novaeguineae
NC 015651 Crocodylus novaeguineae
JF502240 Crocodylus novaeguineae
HM490355 Crocodylus porosus
HM490356 Crocodylus porosus
HM490354 Crocodylus porosus
AJ810453 Crocodylus porosus
Group 2
DQ353946 Crocodylus siamensis
NC 008795 Crocodylus siamensis
Group 3
DQ273698 Crocodylus porosus
MH999467 Crocodylus siamensis
EF581859 Crocodylus siamensis
Group 1
EU621816 Crocodylus siamensis
GU144286 Crocodylus palustris
HM490331 Crocodylus palustris
HM488007 Crocodylus palustris
HM490332 Crocodylus palustris
HM490333 Crocodylus palustris
GQ144581 Mecistops cataphractus
GQ144580 Mecistops cataphractus
GQ144578 Mecistops cataphractus
GQ144579 Mecistops cataphractus
EF551000 Mecistops cataphractus
AM493868 Osteolaemus tetraspis
EF551001 Osteolaemus tetraspis
GQ144603 Osteolaemus tetraspis osborni
GQ144604 Osteolaemus tetraspis osborni
GQ144612 Osteolaemus tetraspis osborni
HM490374 Gavialis gangeticus
MH273966 Gavialis gangeticus
HM490373 Gavialis gangeticus
HM490372 Gavialis gangeticus
AJ810454 Gavialis gangeticus
NC 011074 Tomistoma schlegelii
AJ810455 Tomistoma schlegelii
JN090131 Alligator mississippiensis
AF511507 Alligator sinensis
KJ920382 Paleosuchus palpebrosus
NC 009729 Paleosuchus palpebrosus
AM493870 Paleosuchus palpebrosus
GQ144627 Paleosuchus palpebrosus
GQ144628 Paleosuchus palpebrosus
AM493869 Paleosuchus trigonatus
NC 009732 Paleosuchus trigonatus
GQ144630 Paleosuchus trigonatus
GQ144629 Paleosuchus trigonatus
GQ144631 Paleosuchus trigonatus
JN311631 Caiman latirostris
JN311660 Caiman yacare
GQ144481 Caiman latirostris
GQ144477 Caiman latirostris
GQ144480 Caiman latirostris
JN311630 Caiman latirostris
GQ144602 Melanosuchus niger
GQ144601 Melanosuchus niger
GQ144600 Melanosuchus niger
JN313382 Melanosuchus niger
KU986330 Melanosuchus niger
Group 4
Group 5
JN311638 Caiman crocodilus crocodilus
JN311640 Caiman crocodilus crocodilus
JN311639 Caiman crocodilus crocodilus
GQ144467 Caiman crocodilus
JN311658 Caiman yacare
JN311655 Caiman yacare
JN311656 Caiman yacare
JN311659 Caiman yacare
GQ144469 Caiman crocodilus chiapasius
GQ144468 Caiman crocodilus chiapasius
GQ144470 Caiman crocodilus chiapasius
JN311624 Caiman crocodilus chiapasius
JN311625 Caiman crocodilus chiapasius
EU621814 Caiman crocodilus
JN311641 Caiman crocodilus crocodilus
KF771220 Caiman crocodilus
HM490389 Caiman crocodilus
HM490390 Caiman crocodilus
NC 002744 Caiman crocodilus
0.02

Figure


DQ353946 Crocodylus siamensis
NC 008795 Crocodylus siamensis
DQ273698 Crocodylus porosus
Group 3
Group 2
Group 4
AJ810453 Crocodylus porosus
NC 008143 Crocodylus porosus
HM488008 Crocodylus johnsoni
NC 015238 Crocodylus johnsoni
GU144287 Crocodylus mindorensis
NC 014670 Crocodylus mindorensis
JF502240 Crocodylus novaeguineae
HM636896 Crocodylus novaeguineae
NC 015651 Crocodylus novaeguineae
EF581859 Crocodylus siamensis
Group 1
HM488007 Crocodylus palustris
GU144286 Crocodylus palustris
NC 014706 Crocodylus palustris
JX292787 Crocodylus rhombifer
NC 024513 Crocodylus rhombifer
JF502247 Crocodylus rhombifer
HM636894 Crocodylus acutus
NC 015647 Crocodylus acutus
JF502241 Crocodylus acutus
JF502242 Crocodylus intermedius
HM636895 Crocodylus intermedius
NC 015648 Crocodylus intermedius
EF551001 Osteolaemus tetraspis
AM493868 Osteolaemus tetraspis
NC 009728 Osteolaemus tetraspis
EF551000 Mecistops cataphractus
NC 010639 Mecistops cataphractus
NC 011074 Tomistoma schlegelii
AJ810455 Tomistoma schlegelii
AJ810454 Gavialis gangeticus
AB079596 Gavialis gangeticus
NC 008241 Gavialis gangeticus
AF511507 Alligator sinensis
AJ404872 Caiman crocodylus
Group 5
NC 002744 Caiman crocodilus
AM493869 Paleosuchus trigonatus
NC 009732 Paleosuchus trigonatus
AM493870 Paleosuchus palpebrosus
KJ920382 Paleosuchus palpebrosus
NC 009729 Paleosuchus palpebrosus
72
100
97
100
100
96
100
82
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82
100
98
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79
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88
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100
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73
100
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100
100
99
0.02

Table 1SM. Current EU legislation dealing with reptile meat

EU Legislative reference	Article (point)	Legislative text
Commission Delegated Regulation (EU) 2019/625	2 (15)	*"Reptiles" means animals belonging to the species Alligator mississippiensis, Crocodylus johnstoni, Crocodylus niloticus, Crocodylus porosus, Timon Lepidus, Python reticulatus, Python molurus bivittatus or Pelodiscus sinensis.*
	2 (16)	*"Reptile meat" means the edible parts, either unprocessed or processed, derived from farmed reptiles, which are, when applicable, authorised in accordance with Regulation (EU) 2015/2283 and listed in Commission Implementing Regulation (EU) 2017/2470*
	3 (a)	***Animals and goods which are required to come from third countries or regions thereof included in the list referred to in Article 126(2)(a) of Regulation (EU) 2017/625.*** *Consignments of the following animals and goods intended for human consumption shall enter the Union only from a third country or region thereof included in the list for those animals and goods laid down in Articles 3 to 22 of Implementing Regulation (EU) 2019/626.* ***a)*** *products of animal origin, including reptile meat and dead whole insects, parts of insects or processed insects, for which Combined Nomenclature codes ('CN codes') have been laid down in Chapters 2 to 5, 15 and 16, and Harmonised System codes ('HS codes') under headings 1702, 1806, 2102, 2103, 2105, 2106, 2202, 2301, 2822, 2932, 3001, 3002, 3501, 3502, 3503, 3504, 3507, 3913, 4101, 4102, 4103, 4110 and 9602 of Part Two of Annex I to Regulation (EEC) No 2658/87, when these products are intended for human consumption.*
Commission Implementing Regulation (EU) 2019/626	19	***List of third countries authorised for the entry into the Union of reptile meat.*** *Consignments of reptile meat intended for human consumption shall only be authorised for the entry into the Union if they come from Switzerland, Botswana, Vietnam, South Africa or Zimbabwe.*
Commission Implementing Regulation (EU) 2019/627	73	***Ante-mortem and post-mortem inspection of reptiles.*** *Article 11 shall apply to the ante-mortem inspection of reptiles. Articles 12, 13 and 14 shall apply to the post-mortem inspection of reptiles. For the purpose of Article 13 (a)(i), a reptile will be considered as 0,5 livestock units.*
Commission Implementing Regulation (EU) 2019/628	24	***Model official certificate for the entry into the Union for placing on the market of reptile meat intended for human consumption.*** *To meet the certification requirements laid down in Articles 88, 89 and Article 126(2)(c) of Regulation (EU) 2017/625, the model official certificate set out in Part XII of Annex III to this Regulation shall be used for the entry into the Union for placing on the market of reptile meat intended for human consumption.*
	33	***Transitional provisions.*** *Consignments of products of animal origin accompanied by the relevant certificates issued accordance with Regulation (EC) No 2074/2005, Regulation (EU) No 211/2013 and Implementing Regulation (EU) 2016/759 may be accepted for the entry into the Union until 13 March 2020 provided that the certificate was signed before 14 December 2019. Until 13 March 2020, […] and consignments of reptile meat, insects and other products of animal origin referred to in Article 26 may enter the Union without certificate set out in Annex III of this Regulation.*
	Part XII	*MODEL OFFICIAL CERTIFICATE FOR THE ENTRY INTO THE UNION FOR PLACING ON THE MARKET OF REPTILE MEAT INTENDED FOR HUMAN CONSUMPTION*

References

Commission Delegated Regulation (EU) 2019/625 of 4 March 2019 supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council with regard to requirements for the entry into the Union of consignments of certain animals and goods intended for human consumption. OJ L 131, 17.5.2019, p. 18–30.

Commission Implementing Regulation (EU) 2019/626 of 5 March 2019 concerning lists of third countries or regions thereof authorised for the entry into the European Union of certain animals and goods intended for human consumption, amending Implementing Regulation (EU) 2016/759 as regards these lists. OJ L 131, 17.5.2019, p. 31–50.

Commission Implementing Regulation (EU) 2019/627 of 15 March 2019 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption in accordance with Regulation (EU) 2017/625 of the European Parliament and of the Council and amending Commission Regulation (EC) No 2074/2005 as regards official controls. OJ L 131, 17.5.2019, p. 51–100.

Commission Implementing Regulation (EU) 2019/628 of 8 April 2019 concerning model official certificates for certain animals and goods and amending Regulation (EC) No 2074/2005 and Implementing Regulation (EU) 2016/759 as regards these model certificates. OJ L 131, 17.5.2019, p. 101–194.

Table 2SM. *COI* and *16SrRNA* sequences (all available and retailed in this study) from Alligatoridae, Crocodylidae and Gavialidae species (www.reptile-database.org). *mitochondrion complete sequences

		COI sequences				16SrRNA sequences			
Family	Species (common designation)	all (AN)	all (n)	retailed (AN)	retailed (n)	all (AN)	all (n)	retailed (AN)	retailed (n)
Alligatoridae	*Alligator mississipiensis* (American alligator)	JN090131	1	JN090131	1	-	0	-	0
	Alligator sinensis (Chinese alligator)	AF511507*	1	AF511507*	1	AF511507*	1	AF511507*	1
	Caiman crocodilus (-)	EU621814 GQ144467 HM490389-90 KF771220 AJ404872* NC_002744*	7	EU621814 GQ144467 HM490389-90 KF771220 AJ404872* NC_002744*	7	AY233138 DQ916160 EU621804 AJ404872* NC_002744*	5	AJ404872* NC_002744*	2
	Caiman crocodilus crocodilus (Spectacled caiman)	EU260450-60 JN311638-41	15	JN311638-41	4	-	0	-	0
	Caiman crocodilus fuscus (Brown caiman)	EU260416-43 EU260447-49	31	-	0	-	0	-	0
	Caiman crocodilus chiapasius (-)	EU260444-46 GQ144468-71 JN311624-29	13	GQ144468-71 JN311624-29	10	-	0	-	0
	Caiman crocodilus apaporiensis (Rio Apaporis caiman)	-	0		0	-	0	-	0
	Caiman latirostris (Broad-snouted caiman)	GQ144477-82 JN311630-36 KX954053-89 MH161370	51	GQ144477-82 JN311630-36 KX954053-89 MH161370	51	AY239139 KX954015-51	38	-	0
	Caiman yacare (Jacaré caiman)	GQ144472-76 JN311651-62 KF771229	17	GQ144472-76 JN311651-62 KF771229	17	-	0	-	0
	Melanosuchus niger (Black caiman)	GQ144599-602 JN313382 KU986330 MH161368	7	GQ144599-602 JN313382 KU986330 MH161368	7	AY239140 KX954052	2	-	0
	Paleosuchus palpebrosus (Cuvier's dwarf caiman)	GQ144627-28 MH161366 AM493870* KJ920382* NC_009729*	6	GQ144627-28 MH161366 AM493870* KJ920382* NC_009729*	6	AY239141 AM493870* KJ920382* NC_009729*	4	AM493870* KJ920382* NC_009729*	3
	Paleosuchus trigonatus	EU260461	8	GQ144629-31	7	AY239142	3	AM483869*	2

	(Schneider's dwarf caiman)	GQ144629-31 JN313381 MH161365 AM483869* NC_009732*		JN313381 MH161365 AM483869* NC_009732*		AM483869* NC_009732*		NC_009732*	
Crocodylidae	*Crocodylus acutus* (American crocodile)	KF273834-41 KY994087-88 KY994090 KY994093-94 GQ144571 MH273685 JF502241* HM636894* NC_015647*	18	KF273834-41 KY994087-88 KY994090 KY994093-94 GQ144571 MH273685 JF502241* HM636894* NC_015647*	18	JF502241* HM636894* NC_015647*	3	JF502241* HM636894* NC_015647*	3
	Crocodylus cataphractus (Slender-snouted crocodile)	GQ144572-81 NC_010639* EF551000*	12	GQ144572-81 NC_010639* EF551000*	12	AY239147 NC_010639* EF551000*	3	NC_010639* EF551000*	2
	Crocodylus intermedius (Orinoco crocodile)	KX454157-60 JF502242* NC_015648* HM636895*	7	KX454157-60 JF502242* NC_015648* HM636895*	7	AY239146 JF502242* NC_015648* HM636895*	4	JF502242* NC_015648* HM636895*	3
	Crocodylus johnstoni (Australian freshwater crocodile)	HM490387-88 NC_015238* HM488008*	4	HM490387-88 NC_015238* HM488008*	4	NC_015238* HM488008*	2	NC_015238* HM488008*	2
	Crocodylus mindorensis (Philippine crocodile)	NC_014670* GU144287*	2	NC_014670* GU144287*	2	NC_014670* GU144287*	2	NC_014670* GU144287*	2
	Crocodylus novaeguineae New Guinea crocodile	JF502240* NC_015651* HM636896*	3	JF502240* NC_015651* HM636896*	3	JF502240* NC_015651* HM636896*	3	JF502240* NC_015651* HM636896*	3
	Crocodylus palustris (Mugger crocodile)	HM490323-43 GU144286* HM488007* NC_014706*	24	HM490323-43 GU144286* HM488007* NC_014706*	24	HM921186 GU144286* HM488007* NC_014706*	4	GU144286* HM488007* NC_014706*	3
	Crocodylus porosus (Estuarine crocodile)	EU621815 HM490344-63 AJ810453* DQ273698* NC_008143*	24	EU621815 HM490344-63 AJ810453* DQ273698* NC_008143*	24	AY770542 AJ810453* DQ273698* NC_008143*	4	AJ810453* DQ273698* NC_008143*	3
	Crocodylus rhombifer (Cuban crocodile)	MH273686-87 JF502247* JX292787*	5	MH273686-87 JF502247* JX292787*	5	AY239145 JF502247* JX292787*	4	JF502247* JX292787* NC_024513*	3

		NC_024513*		NC_024513*		NC_024513*			
	Crocodylus siamensis (Siamese crocodile)	EU62816 MH999467 EF581859* DQ353946* NC_008795*	5	EU62816 MH999467 EF581859* DQ353946* NC_008795*	5	EU621806 EF581859* DQ353946* NC_008795*	4	EF581859* DQ353946* NC_008795*	3
	Osteolaemus tetraspis (African dwarf crocodile)	EU159834-66 GQ144603-26 JN090128 JX627008-10 JX627012 JX627014-17 JX627019-23 JX627026 JX627028-33 JX627038 JX627040 JX627042 JX627044-45 JX627047 JX627050-51 JX627059-60 JX627063 JX627065-66 KM406125-26 KM406129-34 KM406136-44 KM406146-50 MH161367 AM493868* EF551001* NC_009728*	117	EU159834-66 GQ144603-26 JN090128 JX627008-10 JX627012 JX627014-17 JX627019-23 JX627026 JX627028-33 JX627038 JX627040 JX627042 JX627044-45 JX627047 JX627050-51 JX627059-60 JX627063 JX627065-66 KM406125-26 KM406129-34 KM406136-44 KM406146-50 MH161367 AM493868* EF551001* NC_009728*	117	AY239148 AM493868* EF551001* NC_009728*	4	AM493868* EF551001* NC_009728*	3
	Tomistoma schlegelii (False gharial)	JN090129 MH161364 AJ810455* NC_011074*	4	JN090129 MH161364 AJ810455* NC_011074*	4	AY239150 AJ810455* NC_011074*	3	AJ810455* NC_011074*	2
Gavialidae	*Gavialis gangeticus* (Indian gharial)	HM490364-86 MH161369 MH273966 AJ810454* AB079596*	28	HM490364-86 MH161369 MH273966 AJ810454* AB079596*	28	AY239149 GQ398138 AJ810454* AB079596* NC_008241*	5	AJ810454* AB079596* NC_008241*	3

		NC_008241*		NC_008241*					
Total			410		364		98		43