

SPECIES IDENTIFICATION IN PETFOODS BY USING BLAST ANALYSIS OF A FRAGMENT OF THE MITOCHONDRIAL 16S RIBOSOMAL RNA GENE (16SRRNA).

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The aim of this work was to verify the label information of pet food for cats, reporting the commercial denomination of Bianchetto among the ingredients, by sequencing and BLAST analysis of a short fragment of the mitochondrial 16SrRNA gene

Fifteen samples of petfood for cats were collected from the retail market. Three fish per samples were analyzed. After DNA extraction, performed according to Armani et al. (2011) (1), the DNA degradation pattern was assessed by electrophoretic analysis. On the basis of the 108 sequences available in GenBank belonging to the order Clupeiformes and Osmeriformes, four different primers were designed and used in combination with those reported in Armani et al. (2012) (2), for the amplification of short fragments of the mitochondrial 16SrRNA gene with a length ranging from 77 to 246 pb. Such fragments were preventively tested by BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=blastSearch&SHOW_DEFAULTS=on&LINK_LOC=blasthome) to assess their discriminatory power at inter and intra specific level. After PCR amplification, the samples associated to the expected amplicon were sequenced and the 45 sequences obtained were analyzed using the program Clustal W in Bioedit version 7.0.9.0 (3) and identified by BLAST analysis.

The BLAST analysis returned an identity values of 100% with different species of the genus *Encrasicholina*. In particular, the most part of the samples were identified at the species level as *E. heteroloba*, and *E. punctifer*.

The obtained results confirms that the molecular marker selected in this study can be used for the identification of species belonging to the Clupeiformes order, allowing a discrimination even among close species. All the 15 market pet food samples were mislabeled. In fact, while the species identification performed by molecular analysis clearly showed the presence of the juvenile form of *Encrasicholina* sp, also known as tropical anchovies, all the labels reported the commercial denomination of Bianchetto, which, in Italy, is allowed only for the juvenile form of *Sardina pilchardus* (4).

1) Armani, A., Castigliero, L., Tinacci, L., Gianfaldoni, D., & Guidi, A. (2011). Molecular characterization of icefish, (Salangidae family), using direct sequencing of mitochondrial cytochrome b gene. *Food Control*, 22, 888-895. 2) Armani, A., Castigliero, L., Tinacci, L., Gandini G., Gianfaldoni, D., & Guidi, A. (2012d). A rapid PCR-RFLP method for the identification of *Lophius* species. *European Food Research and Technologies*, 235, 253-263. 3) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp* 41:95-98. 4) 12) Decreto Ministeriale 14 gennaio 2005. Denominazione in lingua italiana delle specie ittiche di interesse commerciale, ai sensi del Regolamento (CE) n. 2065/2001 della Commissione del 22 ottobre 2001. G.U n. 33 del 10 febbraio 2005