Prevalence and molecular identification of Anisakidae larvae un *Scomber scombrus* and *Merluccius merluccius* from the Canary markets.

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INTRODUCTION

Anisakidosis is an emerging zoonosis caused by the ingestion of the infective stage of Anisakidae nematodes in raw or undercooked seafood and a variety of fish. This explains why more than 90% of anisakidosis cases are reported in Japan, compared to most of the rest of the European countries. However, the number of cases from other countries has been increasing recently, as diagnostic methods improve and culinary habits change. The main causative agents belonging to *Anisakis* genus are *Anisakis simplex* sensu stricto (s.s) and *A. pegreffii*, sibling species of the *A. simplex* (s,l,) complex (1). Accidental consumption of these ascarid larvae can cause gastrointestinal and/or allergic symptomatology (2). Reliable identification of *A. simplex* (s.s.) and *A. pegreffii*, can be achieved with molecular analysis (3). The identification of Anisakidae species and geographical regions that are of high risk may help to prevent anisakidosis cases. Fish consumption in the Canary Islands is high since many of the typical dishes of gastronomy are based on the use of fish or marine products, mackarel (*Scmber scombrus*) and european heak (*Merluccius merluccius*), are two popular fishes for the Canary Islands (Spain) consumers. Therefore, the present study aimed to analyze the health status of these two species, in order to prevent possible cases of anisakidosis.

MATERIALS AND METHODS

A total of 80 fresh fish samples, 60 for *S. scombrus* and 20 for *M. merluccius*, were obtained from different markets on the island of Tenerife, all the individuals came from the North-Eastern Atlantic Sea, corresponding to the FAO (Food and Agriculture Organization of the United Nations) fishing areas 27 and 34, respectively. Some of the morphological characters of taxonomic interest were analyzed, such as the absence or presence of a tooth at the apical end, the mucron at the caudal end, the shape of the esophagus among others .The specific identification of *Anisakis* larvae was carried out using the molecular technique PCR-RFLP (restriction fragment length polymorphism). The entire ITS region was amplified using NC5 and NC2 primers and sequenced. The sequences of ITS were aligned with published sequences in GenBank database (NCBI) using Clustal method. Phylogenetic analyses was carried out using MEGA X.

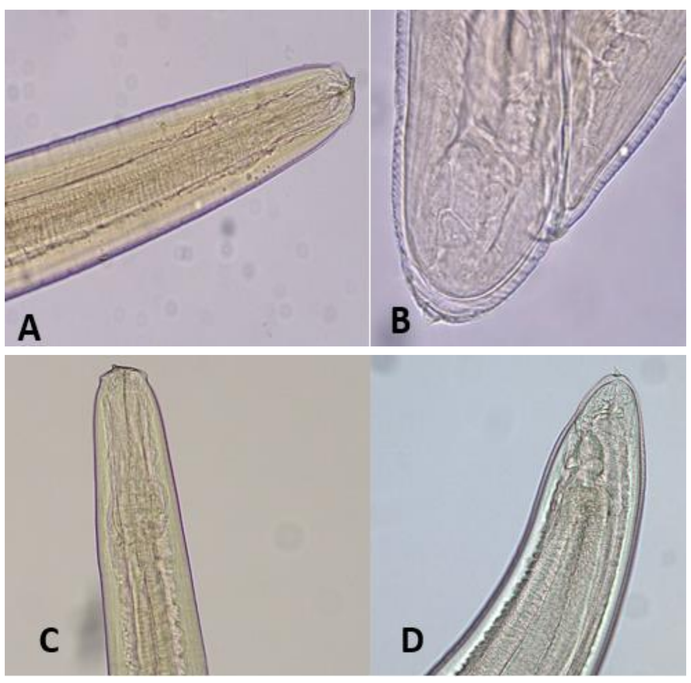

Figure 1. *Anisakis pegreffii* and *Anisakis simplex*. A. Anterior region of *A. pegreffii*; B. Caudal region of *A. pegreffii*; C. Anterior region of *A. simplex*; D. Caudal region of *A. simplex*. Images acquisition was performed with a Leica DM750 microscope.

RESULTS AND DISCUSSION

The prevalence obtained for Anisakidae larvae was 46.6% for *S. scombrus* and 40% for *M. merluccius.* Morphologically, all larvae have been identified as *Anisakis* Type 1 (**Figure 1**). The species identified were *A. simplex* s.s. (23.75%) and *A. pegreffii* (10%) (**Figure 2**). *Anisakis simplex* s.s was found in *S. scombrus* and *M. merluccius*, whilst *A. pegreffi* was found only in *M. merluccius.* The present study reveals a high prevalence of anisakids and the presence of two of the main responsible species of anisakidosis worldwide, in two fish species commonly consumed in the Canary Islands. The specific identification of the causative agent of anisakiasis is important since the degree of pathogenicity depends on the species, assuming to *A. simplex* (s.s.) a greater risk for the consumers, since it presents a higher pathogenicity (4). These results provide a precedent for future studies on the health situation of other species of consumption interest in the Canary Archipelago to avoid food safety problems.

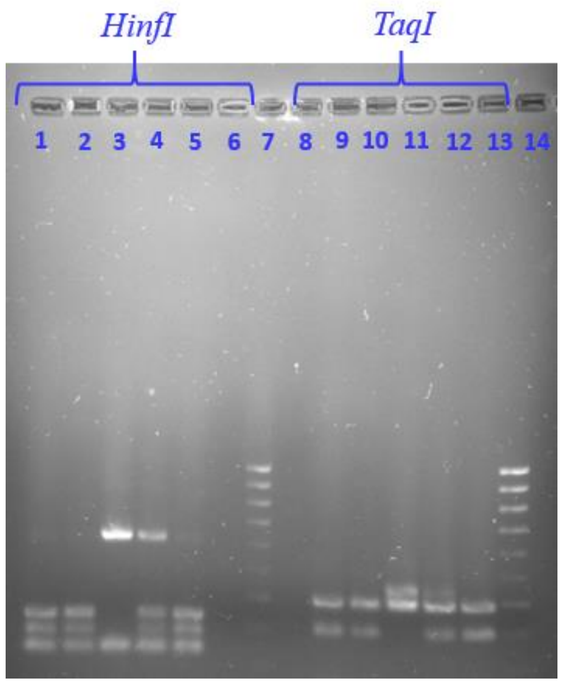

Figure 2. PCR-RFLP profiles of anisakids ITS amplicon, digested with *HinfI* and *TaqI* restriction enzymes. Results obtained for the enzyme *HinfI*: 1,2,4,5: *Anisakis pegreffii*; 3: *Anisakis simplex*; 6: negative control. Results obtained for the enzyme *TaqI*: 9,10,12,13: *Anisakis pegreffii*; well 11: *Anisakis simplex*; well 8: negative control

FUNDING

Funded by “Red Atlántica para la vigilancia Sanitaria de los productos Pesqueros y Acuícolas – RASPA (MAC2/1.1a/305)” project (Universidad de La Laguna; co-financed by funds FEDER to 85%). KGL and EIR granted Beca M-ULL (2019); EBG granted Beca FPI Gobierno de Canarias: Consejería de Economía, Industria y Comercio y Conocimiento, and co-financed by Fondo Social Europeo (85%) (TESIS 2021010056).

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