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# Population genetic analysis of *Anisakis simplex s.l.* and *Anisakis pegreffii* (Nematoda, Anisakidae) from parapatric areas and their contact zone

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#### ABSTRACT

Genetic markers (ribosomal DNA and mitochondrial DNA) were used for molecular dissection of the *Anisakis simplex sensu lato* (*s.l*). *complex* populations. Host fish were caught off Moroccan coasts, where only *Anisakis pegreffii* is present, the sympatric area comprising Spanish coasts, and the Little Sole Bank fishing area from Nordeast Atlantic Ocean where the only present species is *A. simplex sensu stricto*(*s.s.*). Sequence variations in the amplification products were then assessed indirectly by digestion with restriction endonucleases or directly by sequencing for 623 L3 larvae. The sequences were used to infer the relationships between the two species under study using various methodological approaches. We reveal the high genetic diversity of *Anisakis simplex s.s.* and *A. pegreffii* in both mitochondrial and nuclear genes. We detected 10 and 2 fixed differences between *A. simplex s.s.* and *A. pegreffii* in the Cox2 and ITS1, respectively. We found a proportion of putative hybrids below 20% with similar figures on the Atlantic and Mediterranean coasts. Moroccan hybrids were more similar to *A. pegreffii* reflecting backcrosses between these mixed genotypes and his ancestor *A. pegreffii*. We discuss the possible interpretation of these putative hybrids.

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#### 1. Introduction

In the past, most of the controversy regarding the systematics of anisakids was due to the exclusive use of morphological characteristics of L3 larvae to distinguish between species, especially in cases where the existence of sibling species was suspected. Applying molecular techniques has helped to resolve this problem. One of these complex sibling species is *Anisakis simplex sensu lato* (*s.l.*), which is made up of 3 species, *Anisakis simplex sensu stricto* (*s.s.*), *Anisakis pegreffii* and *Anisakis berlandi*, distinguishable via isoenzymes and molecular markers (D'Amelio et al., 2000; Kijewska et al., 2000; Nadler and Hudspeth, 2000; Valentini et al., 2006; Mattiucci and Nascetti, 2008; Cavallero et al., 2011; Mattiucci et al., 2013). However, this differentiation is not so clear in areas where *A. simplex s.s.* and *A. pegreffii* are sympatric, as it occurs along the coasts of the Iberian Peninsula and Japan, where it is possible to find specimens with combined allozyme or genetic patterns between these two species (Abollo et al., 2003; Martín-Sánchez et al., 2005; Hermida et al., 2012; Cipriani et al., 2015). The detection of these putative hybrids has been the cause of some controversy in terms of its interpretation mainly due to the large recovery of larval forms and to the rare observation of adult hybrids. Recently, adult *A. pegreffii*, *A. simplex s.s.* and hybrid individuals of the two species have been

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recovered in sympatry in the same host specimen (*Stenella coeruleoalba*) found stranded on the Sicilian coast by Cavallero et al. (2011). Until now adult hybrids were described solely in *Balenoptera acutorostrata* from the Japanese waters (Umehara et al., 2006). Differential biomedical importance traits as pathogenic and allergenic potential have been demonstrated between both sibling species (Romero et al., 2013; Arcos et al., 2014) giving more value to the taxonomic differentiation of these species. This highlights the need for us to focus on apparent hybridization phenomena.

The genetic structure of populations of a species is revealed to us by the distribution of the genetic variation between specimens of that species over different spatial scales and is conditioned by the study method chosen. Natural hybridization between species has been recorded in many groups of organisms. These phenomena may have profound impact on the dynamics of populations and species. Our objective, achieved through the study of parasites taken from different hosts and various geographical origins where these species are sympatric and parapatric, was to contribute to the existing knowledge on the distribution of the genetic variation of these two *Anisakis* species, *A. simplex s.s* and *A. pegreffii*, and their putative hybrids.



Fig. 1. Map of the area showing the geographical distribution of the different capture locations and the relative proportions of A. simplex s.s., A. pegreffii and hybrids.

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