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# Identification and prevalence of *Anisakis pegreffii* and *A. pegreffii* × *A. Simplex* (s.s.) hybrid genotype larvae in Atlantic horse Mackerel (*Trachurus trachurus*) from some North African Mediterranean coasts

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ABSTRACT

Atlantic horse mackerel is one of the most popular fish for North African consumers. Mackerel constitutes a common host for the *Anisakis* species of infective larvae (L3). *A. pegreffii* and *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype were identified using PCR-RFLP and entire ITS-DNA sequencing protocol. This research investigated the prevalence and mean intensity of *A. pegreffii* and its hybrid form in randomly collected mackerel samples throughout spring to summer seasons from the Libyan western coast. Briefly, 55 out of 240 (22.9%) fish samples were confirmed to be infected and the prevalence of *A. pegreffii* reached 22.08% with mean intensity of infection 29.13 ± 2.43 parasite/fish, while the prevalence of *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype was 0.8% with mean intensity of infection 22 ± 0.85 parasite/fish. The prevalence of infection noticeably elevated during the summer season to reach 30.8%. Additionally, female fish samples were heavily infected as the mean intensity of infection was 34.75 ± 1.27 parasite/fish. Furthermore, results indicated that the highest intensity of infection was recorded in older and female mackerel. To sum up, the current study has provided substantial information about the impacts, diversity and epidemiology of *A. pegreffii* and its hybrid form in North African waters.

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## Introduction

Anisakids (Nematoda: Anisakidae) are potential human seafood borne pathogens/ allergens, that pose a serious impact on the health and economy of the fisheries' industry. Fishermen and handlers are also at risk of contracting anisakiasis as well as developing occupational asthma caused by the inhalation of antigens from *A. simplex* (Daschner et al., 2000; Purello-D'Ambrosio et al., 2000; Serracca et al., 2014).

Adult *Anisakis* species reside in marine mammals, and the female worm lays eggs in their feces, which then become embryonated in sea water to develop first-stage larvae (L1). The larvae molt turn into second-stage larvae (L2) and then hatch to become free-swimming. The hatched L2 will then be ingested by crustaceans to grow into third-stage larvae (L3) which are infective to fish and squid. Third stage larvae (L3) migrate from fish intestines to their tissues where they grow to reach 3 cm in length. If ingested by humans through eating raw or undercooked infected marine fish, L3 will molt twice and develop into adult worms that penetrate the gastric and intestinal mucosa, causing the symptoms of anisakiasis (Kagei, 1968; Klimpel et al., 2004; Nagasawa, 1990).

Anisakiasis is a serious human disease caused by accidental ingestion of L3upon consuming raw or lightly preserved infected fish (Baptista-Fernandes et al., 2017). The disease triggers allergic host defenses as the ingestion of infested seafood can lead to an acute gastrointestinal disease associated with abdominal pain,

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nausea and diarrhea. Massive eosinophilic infiltration and granulomas in the gastrointestinal tract can occur if the larvae were not removed. Re-infection causes severe anaphylactic reactions and extra-gastrointestinal or ectopic forms with symptoms varying from gastrointestinal pathologies to deadly anaphylactic shock (Daschner and Pascual, 2005; Nieuwenhuizen, 2016; Nieuwenhuizen and Lopata, 2014).

The genus *Anisakis* contains nine species and mainly two species of the *A. simplex* complex: *A. simplex* sensu-stricto (s.s.) and *A. pegreffii* have been associated with infections in humans (Audicana and Kennedy, 2008; Mattiucci and Nascetti, 2008). *A. pegreffii* is similar to *A. simplex* (s.s.) in its pathogenic potential to trigger human anisakiasis when engulfed with contaminated food(Jeon and Kim, 2015). The first human anisakiasis with *A. simplex* was reported in the 1960s, and during the 1990s it was well established that even the consumption of dead larvae in seafood can cause severe hypersensitivity reactions. This may also occur upon exposure to allergens from dead worms through airborne or skin contact routes. These facts make *Anisakis* spp dangerous both dead and alive (Audicana et al., 2002; Audicana and Kennedy, 2008).

Anisakids parasitize a wide range of commercial marine fishes such as Sea Hake (*Merluccius merluccius*); Anchovy (*Engraulis encrasicholus*); Tuna (*Sardasarda*); Sardine (*Sardina pilchardus*), Atlantic horse mackerel (*Trachurus trachurus*) and blue jack mackerel (*T. picturatus*), and are globally reported in the North-eastern Atlantic and Northern waters of Europe, Portugal, Mediterranean Sea, Adriatic Sea and the Pacific, Atlantic waters of North America and Namdae River, South Korea (Buselic et al., 2017; Costa et al., 2016, 2003; Mattiucci et al., 2013; Mladineo et al., 2017; Setyobudi et al., 2010).

Several reports demonstrated that Mediterranean Sea has a prevailing distribution of *A. pegreffii* and a minimal presence of *A. simplex* (s.s.) in hybrid form with *A. pegreffii* in Atlantic horse mackerel (Costa et al., 2016; Mattiucci et al., 2008; Vincenzo et al., 2015). Significant positive correlations were found between Atlantic horse mackerel's host length and *A. simplex* occurrence, as well as its abundance in the Atlantic coast of Morocco (Shawket et al., 2017).

There were limited studies related to *Anisakis* spp larvae infection in North African Libyan waters (Abusdel, 2016; Farjallah et al., 2008b; Kassem and Bowashi, 2015). Therefore, the objectives of the current research are to accurately identify the potentially infective larvae *of Anisakis* spp. and explore their prevalence in Atlantic horse mackerel collected from North African Libyan waters.

## Materials and methods

### *Fish sample collection, processing and morphological identification of anisakid nematodes*

Atlantic horse mackerel (*T. trachurus*) samples were captured during spring and summer seasons of the year 2014. A total number of 240 fish were collected equally on a monthly basis by the Lampara fishing method from the area that extended from Tajura (east of Tripoli city) to Janzur (west of Tripoli city) at a rate of 20 fish/each fishing time. After collection, samples were transported to the Poultry and Fish Diseases Laboratory (PFDL), Faculty of Veterinary Medicine, University of Tripoli under refrigeration (4 °C) and were examined within 24 h.

Each fish sample was flushed with saline, and then the total length (TL) and body weight were determined. Approximate age of Atlantic horse mackerel samples was estimated by measuring TL and examining the whole otoliths with a light microscope, and then approximate fish age was estimated by identifying and counting annuli (Waldron and Kerstan, 2001). Furthermore, host samples were examined for the parasites as soon as morphometric measures were done. Briefly, peritoneal cavity and digestive tract of fish samples were examined after making an incision along the ventral line from the anus to the mouth opening. Fish meat (muscles) was removed from the spine and immersed in warm water for several minutes to facilitate the migration of the parasite outside the fish (Eissa, 2016; Stoskopf, 1993).

The collected *Anisakis* larvae were washed in saline solution for thirty minutes, then the larvae were relaxed in distilled water for ten minutes and killed in 80 °C hot glycerin alcohol (1 part glycerin: 3 parts 95% ethanol) according to the method adopted from (Oguz and Oktener, 2007). The retrieved larvae were cleared in lactophenol and examined under stereo-microscope.

Larvae were morphologically identified using the standard morphological criteria described by Quiazon et al. (2009) and Soewarlan et al. (2014). Briefly, the measured morphological parameters were the body width, esophagus length, ventriculus length/width and mucron length. The morphometric assessment keys were conducted as previously described (Cannon, 1977; Mattiucci et al., 2014; Shamsi et al., 2009a, b). Preliminary identified anisakid nematodes were preserved in 70% ethanol for further molecular identification.

### *Molecular identification of A. pegreffii and A. pegreffii × A. simplex (s.s.) hybrid genotype members using PCR-RFLP and rDNA sequencing protocol*

Identification of retrieved anisakid nematode larvae at the species level was done by Restriction Fragment Length Polymorphism Analysis of PCR-Amplified Fragments (PCR-RFLP)using the standard protocol (http://www.iss.it/binary/crlp/cont/MI_04_website_EN.pdf) approved by the European Union Reference Laboratory for Parasites –*Instituto Superiore di Sanità* (ISS) at the department of infectious, parasitic and immuno-mediated diseases, unit of gastro-enteric and tissue parasitic diseases (Rome, Italy). Briefly, the procedures combined the standard PCR for amplification of Internal Transcribed Spacer (locus ITS "ITS1, 5.8S and ITS2" of ribosomal DNA) using the oligonucleotides primers (**NC5** 5'-GTAGGTGAACCTGCGGAAGGATCATT-3' and **NC2** 5'-TTAGTTTCTTTTCCTCCGCT-3') with RFLP protocol using *Hin*fI and/or *Hha*I restriction enzymes (D'Amelio et al., 2000) to allow the unambiguous authentication of all epidemiologically relevant anisakidae species. Accordingly, the restriction of digestion by the ITS fragments with the *Hin*fI enzyme allows the distinguishing of *A. pegreffi*, *A. shupakovi*, *A. ziphidarium*, *A. typical*, *A. physeteris* from *A. simplex* (s.s.), *A. simplex* C and *Pseudoterranova*. Also, using ITS sequence restriction digestion with *Hha*I enzyme, *A. simplex,* s.s can be distinguished from *A. simplex* C (La Rosa et al., 2006).

For molecular authentication of the hybrid genotype, the ITS PCR amplicons were excised from the gel, and the DNA was extracted from the gel using GF-1 AmbiClean kit (Vivantis, Malaysia) and sequenced using cycle sequencing PCR reaction with BigDye® Terminator v3.1 Kit (AB-Applied Bioscience), then sequenced in four-capillary ABI PRISM® 3100-Avant Genetic Analyzer. The chromatogram files were displayed and manually edited using ChromasPro version 2.1.6 software (Technelysium, Australia). Subsequently, BLAST search on NCBI (http://www.ncbi.nlm.nih.gov/pubmed) and Clustal W multiple sequence alignment was applied for the examined consensus sequences to compare with the previously published sequences.

### *Assessment of A. pegreffii and A. pegreffii × A. simplex (s.s.) hybrid larvae prevalence and mean intensity of infection*

Prevalence of *Anisakis* spp. L3 infection in Atlantic horse mackerel fish samples was calculated by dividing the number of infected

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fishes by the total number of examined fish and was expressed as a percentage. Mean intensity of infection is the average intensity of *A. pegreffii* or *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype members among the infected Atlantic horse mackerel fish samples (i.e. the total number of parasites found in a sample divided by the number of fish hosts infected with that parasite) and expressed as parasite/fish (Bush et al., 1997).

### *Statistical analysis*

One-way Analysis of Variance (ANOVA) was implemented using SPSS version 16 software to test the significant differences among the means' intensity of *Anisakis* spp. infection in relation to the variation in seasons, gender, age and different organs of infected fish samples. The statistical significance level was set at $p < 0.05$ for all tests. Tukey's procedure was used as a *post hoc* test.

## Results

### *Morphological identification of anisakid L3*

Morphological examination revealed that larvae were contained in white to creamy-colored cyst encompassing the larvae which are coiled in a tight spiral. When larvae were freed from the cyst, the anterior end of each of them had three relatively small, inconspicuous lips with an obvious center offset, forward projecting tooth (antero-ventral projecting boring tooth). The glandular region and excretory pore opening follow the relatively long esophagus on ventral side at the anterior end, between elementary sub ventral lips (Fig. 1).

The morphometric measures of anisakid nematode larvae are shown in Table 1. Two forms of the worm, including active wriggling and static coiling, were found. *Anisakis* species identification depended on the presence of a rounded tail possessing a mucron and a long ventriculus with an oblique ventricular-intestinal junction (Fig. 1). Cuticle was transversely striated, wrinkled irregularly close the tail. Esophagus is characterized by anterior muscular and posterior glandular nature. It was obviously visible in living larvae.

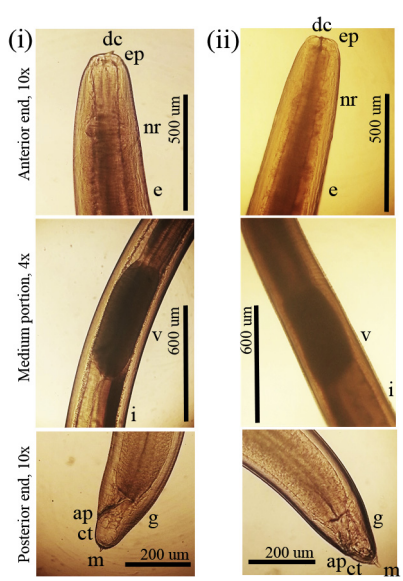


**Fig. 1.** Micrographs of *A. pegreffii* L3 (i) and *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype L3 (ii). Cuticular tooth (dc), excretory pore (ep), nerve ring (nr) esophagus (e), ventriculus (v), intestine (i), glands (g), analpore (ap), conical tail (ct), mucron (m).

**Table 1**
Morphological criteria of *A. pegreffii* and *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype L3 retrieved from Atlantic horse mackerel samples. Total number of retrieved L3 = 1588.

| Morphological characters | *A. pegreffii* (mm) | *A. pegreffii* × *A. simplex (s.s.)* (mm) |
|---|---|---|
| Total body length | 18 (11–25) | 9.00–17.5 |
| Maximum bodywidth | 0.49 (0.38–0.60) | 0.25–0.56 |
| Distance of nerve ring to anterior end | 0.23 (0.20–0.26) | 0.19–0.23 |
| Length of esophagus | 1.48 (1.04–1.93) | 0.56–1.75 |
| Length of ventriculus | 0.64 (0.50–0.79) | 0.20–0.40 |
| Width of ventriculus | 0.18 (0.12–0.25) | 0.12–0.20 |
| Length of tail | 0.07 (0.04–0.10) | 0.04–0.09 |
| Length of mucron | 0.02 (0.01–0.03) | 0.01–0.03 |

A rectangle ventriculus with slanted esophago-intestinal junction was also reported.

### *Molecular identification of A. pegreffii and A. pegreffii ×A. simplex (s.s.) hybrid genotype L3*

Molecular identification of the retrieved larvae species was done using PCR-RFLP at the European Union ISS reference laboratory. Hence, *A. pegreffii* and its hybrid form were identified from the Atlantic horse mackerel samples and their banding pattern of ITS amplicons that had been digested with *Hha*I and *Hin*FI. The restriction enzymes were found to be typical to those previously reported at the banding pattern of *A. pegreffii* and *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype (Costa et al., 2016; D'Amelio et al., 2000; Umehara et al., 2006). Accordingly, all individual samples of *Anisakis* spp. L3 showed two visible bands of the same banding pattern (532–419 bps) when ITS PCR product was digested with *Hha*I restriction enzyme (Fig. 2a). While *Hin*fI digested ITS PCR

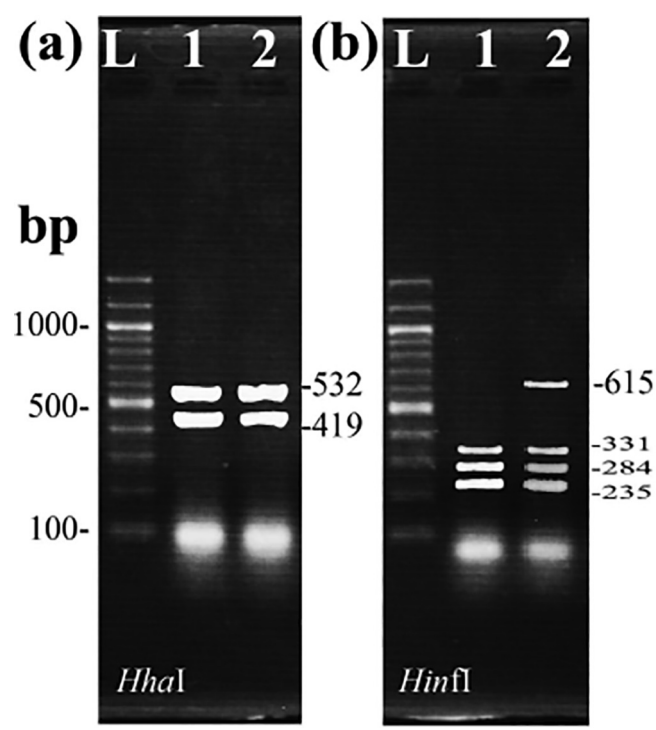


**Fig. 2.** Representation of PCR-RFLP profiles of anisakid ITS amplicon digested with *Hha*I (a) and *Hin*fI (b) restriction enzymes. (L: Ladder, 1- *A. pegreffii* and 2-*A. pegreffii* × *A. simplex* (s.s.) hybrid genotype isolated from Atlantic horse mackerel samples.

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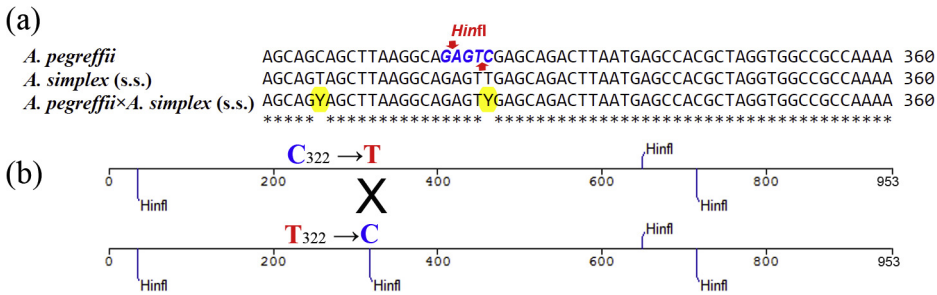


**Fig. 3.** (a) Clustal W multiple sequence alignment of ITS loci of *A. pegreffii*, *A. simplex* (s.s.) and *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype showing hybridization at C/T bases at 306 and 322 positions. (b) *Hinf* I restriction enzyme map of *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype.

product of both *A. pegreffii* and *A. pegreffii* × *A. simplex* (s.s.); which they had shared in three visible bands with banding pattern (331, 284 and 235 bps). *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype differentiation was inferred from displaying a forth visible bandat 615 bps (Fig. 2b).

Sequence analysis of amplified entire ITS loci of presumptively identified hybrid larvae revealed that they consist of 953 bp and all of the sequences were 99% identical to *A. pegreffii* and *A. pegreffii* × *A. simplex* (s.s.) isolate 444 from Italian waters (accession number: JF412028). In addition, Clustal multiple sequence alignment of *A. pegreffii* × *A. simplex* (s.s.) ITS entire region revealed that the hybridization had occurred at heterozygotes Y(C/T) bases at 306 and 322 positions which were C in *A. pegreffii* and T in *A. simplex* (s.s.) (Fig. 3a). *Hin*fI restriction enzyme map of *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype showed the effect of replacing C322 by T on the sequence of *Hinf*I site, which resulted in the characteristic PCR-RFLP profile banding pattern (Fig. 3b).

### *Prevalence and mean intensity of infection of A. pegreffii and A. pegreffii × A. simplex (s.s.) hybrid genotype L3*

Out of 240 examined Atlantic horse mackerel fish samples, only 55 (22.91%) were infected with a total number of 1588 *Anisakis* spp. L3.1544 (97.22%) L3 were identical to *A. pegreffii* and 44 (2.77%) L3 were identical to *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype (Table 2). *A. pegreffii* is more prevalent in 22. 08% of the examined fish samples than *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype, which had been found in only 0.8% of the examined fish samples with mean intensity of infection reaching 29.13 ± 2.43 and 22 ± 0.85 parasite/fish, respectively (Table 2).

Seasonal changes, gender and approximate age of host fish were found to affect the susceptibility of Atlantic horse mackerel to *A. pegreffii* L3 infection (Table 3). The prevalence of infection in the spring season was 15%, this value was exaggerated in the summer season to reach 30.8% (Fig. 4). Furthermore, the prevalence of infection in female (20.6%) was higher than male fish samples (25.6%). In addition, female samples significantly suffered a higher intensity of infection34.75 ± 1.27 ($p < 0.05$) parasite/fish than male samples. Furthermore, both prevalence and intensity of infection were directly proportional to the age of the host, especially when Atlantic horse mackerel was getting as old as 6 years; they were significantly contaminated with higher intensity of *A. pegreffii* than younger ones (Fig. 4). Distribution of *Anisakis* spp. L3 in meat (muscle) and edible organs of infected fish is presented in Table 4. The data revealed that gonads were the most heavily infected organs with *A. pegreffii* L3; with significantly higher infection intensity in the ovaries than that in the testicles (Fig. 5). These data suggest that the consumption of male, young horse mackerel fish in the spring season can reduce the human risk of anisakiasis.

**Table 2**
Prevalence and mean intensity of infection of *A. pegreffii* and *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype L3 in Atlantic horse mackerel samples. Total samples number = 240.

| | Total | *A. pegreffii* | *A. pegreffii* × *A. simplex (s.s.)* |
|---|---|---|---|
| Number of infected fish | 55 | 53 | 2 |
| Number of retrieved L3 | 1588 | 1544 | 44 |
| Prevalence% | 22.9 | 22.08 | 0.8 |
| Mean intensity of infection* (parasite/fish) | 28.87 ± 2.42 | 29.13 ± 2.43 | 22 ± 0.85 |

*Mean ± Standard Error (SE)

## Discussion

PCR-RFLP and sequencing of ribosomal ITS locus allowed the authentication and characterization of *Anisakis* spp., as well as the finding of putative hybrids or recombinant genotypes such as those between *A. pegreffii* and *A. simplex* (s.s.). This recombination was first described in Iberian Peninsula waters as a hybrid zone for the two sibling species in an area of sympatry; the recombinant individuals may be a product of inter-specific hybridization (Abollo et al., 2003).

Subsequently, these hybrid forms were also reported in Japanese waters and the Mediterranean Sea (Cavallero et al., 2012; Chaligiannis et al., 2012; Farjallah et al., 2008b; Meloni et al., 2011; Umehara et al., 2006). A more recent survey was conducted on the occurrence of *A. simplex* (s.s.) and *A. pegreffii* hybrid forms on

**Table 3**
Distribution of infective *A. pegreffii* L3 in Atlantic horse mackerel individuals according season, gender and age.

| Variance | | Total number of fish samples | Number of infected fish | Number of retrieved L3 |
|---|---|---|---|---|
| Seasonal | Spring | 120 | 18 | 533 |
| | Summer | 120 | 37 | 1055 |
| Gender | Male | 131 | 27 | 615 |
| | Female | 109 | 28 | 973 |
| Age (years) | 3 | 84 | 4 | 22 |
| | 4 | 95 | 17 | 407 |
| | 5 | 15 | 8 | 194 |
| | 6 | 46 | 26 | 965 |

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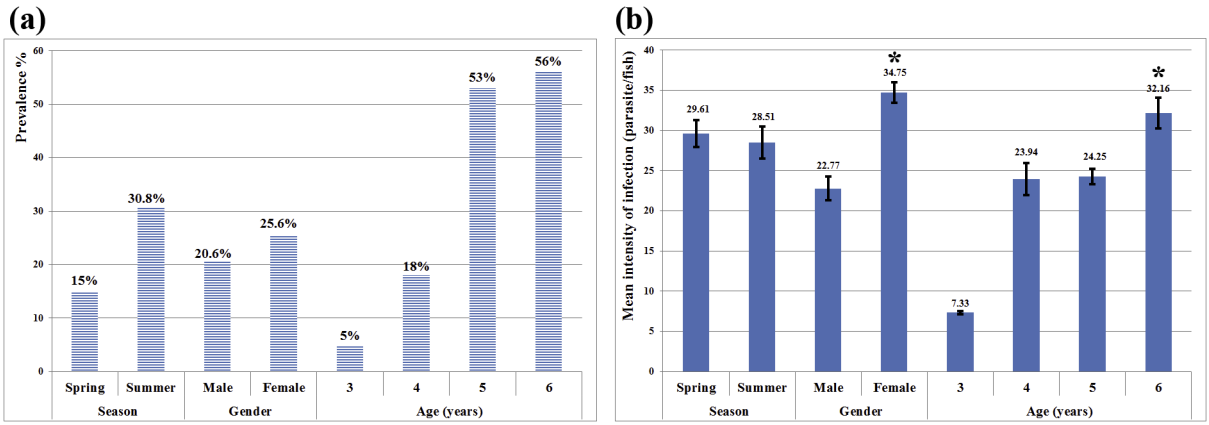


**Fig. 4.** Prevalence (a) and mean intensity of infection (b) of *A. pegreffii* L3 in Atlantic horse mackerel samples in relation to seasonal changes, gender and approximate age of fish. Mean ± SE, asterisks*indicate statistical significance ($p < 0.05$).

**Table 4**
Distribution of infective *Anisakis* spp L3 among various tissues of infected Atlantic horse mackerel samples.

| Number of infected fish | | | Number of retrieved L3 | |
|---|---|---|---|---|
| Tissue type | *A. pegreffii* | *A. pegreffii* × *A. simplex* | *A. pegreffii* | *A. pegreffii* × *A. simplex* |
| Meat (muscle) | 24 | 2 | 99 | 1 |
| Stomach | 35 | 2 | 278 | 21 |
| Intestine | 40 | 2 | 253 | 12 |
| Pyloric caeca | 38 | 2 | 215 | 4 |
| Abdominal cavity | 9 | 2 | 17 | 2 |
| Ovaries | 25 | 0 | 414 | 0 |
| Testes | 20 | 2 | 268 | 4 |

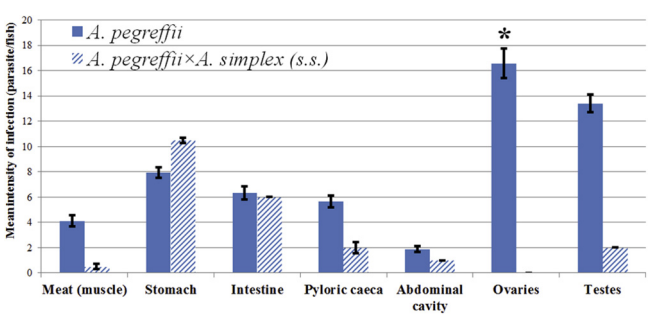


**Fig. 5.** Comparing means intensity of infections with *A. pegreffii* and/or *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype in Atlantic horse mackerel samples in relation to their distribution in different host tissues. Mean ± SE, asterisks*indicate statistical significance ($p < 0.05$).

fish samples caught off the coasts of Sicily (Southern Italy), hence 17% of the examined larvae was found as *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype (Costa et al., 2016). In the current study, 97% of the examined larvae were identified as *A. pegreffii* and 3% was identified as *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype.

Throughout the past decade, nematodes of zoonotic importance as *Anisakis* spp were reported in marine fishes throughout the Mediterranean coasts of North African countries including Morocco, Tunisia and finally Libya (Eissa et al., 2015; Farjallah et al., 2008b; Fernandez-Jover et al., 2010). In the current study, morphometric assessments indicated that anisakid nematode larvae retrieved from Atlantic horse mackerel, have majorly coincided with the standard morphological criteria of *A. pegreffii* described elsewhere (Eissa et al., 2015; Quiazon et al., 2009).

Our results are in consistence with other studies that had retrieved *A. pegreffii* from horse mackerel in Mediterranean waters (Abattouy et al., 2014; Abattouy et al., 2011; Costa et al., 2016). Moreover, the prevalence of *A. pegreffii* infection recorded here is similar to that reported in other studies that have utilized other species such as European hake (*Merluccius merluccius*) (Farjallah et al., 2008a) and the black scorpionfish (*Scopaena porcus*) (Eissa et al., 2015). On the other hand, *A. simplex* from horse mackerel was reported off the Atlantic coast of Morocco with prevalence that reached 35.28% and a mean intensity of infection that was up to 18.75 parasite/fish (Shawket et al., 2017).

In this study, the seasonal highest prevalence of infection reported in the summer was in agreement with that published by Abattouy et al. (2014) who declared that the prevalence of infection by *A. pegreffii* in horse mackerel was highest during the autumn and summer. In addition, the high infection rates during the summer season, compared to those of the spring season, consistently agreed with those obtained by Eissa (2002). It appears that water temperature has a great impact on enhancing the life cycle with consequent increase in the prevalence during summer compared to the spring season.

The relation between prevalence of infection and sex in fish species was also obvious in the current findings as females showed greater prevalence of *Anisakis* spp than males in examined Atlantic horse mackerel. Similar results were reported in hake, *Merluccius gayi* from Chile (Carvajal and Cattan, 1985). George-Nascimento et al. (1983) concluded that sexually mature females had significantly higher prevalence of infections with *Anisakis* spp than males in the Chilean jack mackerel. The immunosuppressive nature of stress hormones (corticosterols) that are known to be much higher in mature spawning females than males may explain this prevalent increase in females(Kubokawa et al., 2001). Females are also exposed to hormonal stress during the maturity and spawning periods that render them more liable to diseases than males (Eissa et al., 2015).

The results also indicated high prevalence of *Anisakis* spp in older fish than younger ones with maximum infection percentage

in the 6 years old category. This higher infection prevalence could be attributed to the prolonged durations of exposure and predation along the long lifetime of such a category. It has been postulated that the number of ascaridoid larvae that accumulate in marine fish is proportional with age, predatory behavior and increased feeding rates (Abollo et al., 2001; Manfredi et al., 2000; Smith, 1984). Furthermore, the results confirmed that infected organs in examined fishes included the stomach, intestine, pyloric ceca, abdominal cavity and gonads, with the latter being the most infected organ. This result was consistent with that of Tantanasi et al. (2012) who reported that most of L3 stage of *Anisakis* spp. retrieved from Atlantic horse mackerel was found in the gonads rather than on other organs. While Setyobudi et al. (2011) reported that nematodes were mostly found in the muscles surrounding the visceral organs (98.00%), with the mean intensity of 68.26 ± 47.83 (larvae/infected host) and only a few anisakid larvae were found in pyloric ceca, liver and other organs.

The voracious eating behavior of Atlantic horse mackerel may explain its great chance of engulfing lots of the marine crustaceans infected with L3 of *Anisakis* spp which invade fish intestinal wall to peritoneal cavity then move with the blood circulation, with special preference to hematogenous organs (Setyobudi et al., 2011). Food safety considerations in relation to *A. pegreffii* require avoidance of consumption of raw or thermally unprocessed or salted fish types which have high prevalence of infection (Serracca et al., 2014). Thus, as a prophylactic measure, it is advised to avoid old age female Atlantic horse mackerel especially in the summer season and encourage the consumption of young male fish.

## Conclusion

The current study has provided the African fish diseases/food safety library with substantial records on the geographical distribution, prevalence and intensity of *A. pegreffii* and its hybrid form in Atlantic horse mackerel from North African waters. Also, it emphasized the importance of recording *A. pegreffii* × *A. simplex* (s.s.) hybrid genotype mandates for future research on ecology, distribution and impacts of anisakid hybrid genotypes in marine fisheries through the Mediterranean Sea.

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