

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Occurrence of ascaridoid nematodes in *Illex coindetii*, a commercially relevant cephalopod species from the Ligurian Sea (Northwest Mediterranean Sea)

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1737057> since 2020-06-17T22:26:58Z

Published version:

DOI:10.1016/j.foodcont.2020.107311

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

1 **Occurrence of *ascaridoid* nematodes in *Illex coindetii*, a commercially relevant cephalopod**
2 **species from the Ligurian Sea (Northwest Mediterranean Sea)**

3
4 Vasco Menconi¹, Paolo Pastorino^{1,2,*}, Erika Astrid Virginie Burioli^{1,3}, Marzia Righetti¹, Tommaso
5 Scanzio¹, Livio Favaro^{1,4}, Maria Cristina Bona¹, Elena Pavoletti⁵, Alessandro Dondo¹, Marino
6 Prearo¹

7
8 ¹ Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, via Bologna 148,
9 10154 Torino, Italy

10 ² Dipartimento di Scienze della Vita, Università degli Studi di Trieste, via Giorgieri 10, 34127
11 Trieste, Italy

12 ³ LABÉO Frank Duncombe, 1 route de Rosel – Saint Contest – 14053 Caen, France;

13 ⁴ Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, via
14 Accademia Albertina 13, 10123 Torino, Italy

15 ⁵ Servizio Veterinario ASL Vercelli, Area Igiene della produzione, trasformazione,
16 commercializzazione, conservazione e trasporto degli alimenti di origine animale e loro derivati,
17 Largo Giusti 13, 13100 Vercelli, Italy.

18
19 *corresponding author: Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta,
20 via Bologna 148, 10154 Torino, Italy. E-mail: paolo.pastorino@izsto.it (P.P.); telephone: +39
21 0112686295

22
23 **Keywords:** *Anisakis pegreffii*; *Anisakidae*; food safety; *Hysterothylacium* sp.; food-borne parasite

24
25 **Abstract**

26 In this study we investigated the occurrence of larval *ascaridoid nematodes* in *Illex coindetii*, a
27 commercially relevant cephalopod, via a cross-sectional prevalence study (January 2015 to
28 February 2018) in two fishing areas (Savona and Piombino) in the Ligurian Sea (northwest
29 Mediterranean Sea). A total of 745 specimens of *I. coindetii* were caught by local fisherman
30 through bottom trawling. *Parasitological examination revealed ascaridoid nematodes (L3) larvae*
31 *(n=9): Hysterothylacium sp. (n=6) and Anisakis spp. (n=3). All larvae morphologically referred to*
32 *the genus Anisakis were identified by PCR-RFLP as A. pegreffii. The prevalence of*
33 *Hysterothylacium sp. infestation was 0.88% for the Savona and 0.56% for the Piombino sampling*
34 *site; the prevalence of A. pegreffii was 0.18% for Savona and 1.11% for Piombino and did not differ*

35 between the two sampling sites (chi-square test; $p=0.0848$). Better knowledge of the distribution of
36 **ascaridoid nematodes, especially of *Anisakis* species** is essential for defining their epidemiological
37 role and the potential risk for consumer health.

38

39 **1. Introduction**

40 Anisakid nematodes have a complex life cycle that progresses through four larval stages (L1–L4)
41 and involves a broad spectrum of hosts (crustaceans, cephalopods, fish, marine mammals, and sea
42 birds). The heteroxenous life cycle involves marine mammals as definitive hosts, crustaceans as
43 first intermediate hosts, and fish, squid and other invertebrates as intermediate or paratenic hosts
44 (Kjøie 1993; **Mattiucci et al., 2018**). Infection is transmitted through the marine food web (Klimpel
45 and Palm 2011).

46 Humans become accidental hosts after consuming raw or semi-cooked fish or cephalopods infected
47 with third-stage larvae (Audicana and Kennedy, 2008). The consumption of improperly processed
48 or uncooked seafood (i.e., sushi and sashimi, salted or smoked herring, Nordic gravlax, Lomi Lomi,
49 **salmon, American cebiche and marinated anchovies**) is a key factor in human infection (Audicana
50 et al., 2002). The most common symptoms of human anisakiasis are abdominal pain, nausea,
51 emesis, and mild fever (Arizono et al., 2012). In most clinical cases, the larvae have penetrated the
52 stomach mucosa; larvae have rarely been found in the small intestine and even more rarely in the
53 colonic mucosa (Arizono et al., 2012). **Several episodes of allergic reactions have been described as
54 a possible consequence of *Anisakis* spp. infection, even with the ingestion of well-cooked fishery
55 products (Daschner et al., 2000; Mattiucci et al., 2013).**

56 Public health authorities and the seafood industry are aware that fish-borne zoonotic nematode
57 infection can affect both consumer health and the economy of fish production. In response to these
58 issues, the European Food Safety Authority (EFSA) published a Scientific Opinion on risk
59 assessment of parasites in fishery products (EFSA, 2010). Nonetheless, the zoonotic implications
60 and the distribution and epidemiology of anisakid nematodes in the Mediterranean Sea remain
61 poorly understood. Furthermore, the bulk of published data refer to the occurrence of *Anisakis* spp.
62 larvae in teleost species. Few studies have described the occurrence of **ascaridoid** larvae in
63 cephalopods from the Mediterranean Sea (Gestal et al., 1999; Farjallah et al., 2008; Angelucci et al.,
64 2011; Petrić et al., 2011; Serracca et al., 2013; Picó-Durán et al., 2016; **Goffredo et al., 2019**).

65 Cephalopods are important predators in the marine trophic chain (Boyle and Pierce 1994; Clarke,
66 1996). Furthermore, the global landings of cephalopods have increased with growing consumer
67 appreciation. Broadtail shortfin (*Illex coindetii*) is a benthic ommastrephid squid commonly fished
68 in the Mediterranean Sea by bottom trawl (Lefkaditou et al., 2008; Picó-Durán et al., 2016). It is

69 widely distributed throughout the Mediterranean, the eastern Atlantic (from south of Great Britain
70 to Namibia), and the western Atlantic (from the Caribbean to the Gulf of Mexico and the Straits of
71 Florida) (Sanchez et al., 1998). *I. coindetii* has been recorded up to 1100 m depth, but commercial
72 catches are normally obtained at an average depth of 60-400 m (Guerra 1992; Jereb and Roper,
73 2010). Records of cephalopods caught at depths greater than 500 m are few and come mainly from
74 the western Mediterranean (Quetglas et al., 2000). **With this study we wanted to investigate the
75 prevalence and the anatomical location of ascaridoid nematodes infestation, as well as the
76 geographical distribution of ascaridoid nematodes in *I. coindetii* from the Ligurian Sea.** Our
77 findings may inform the creation of risk maps of cephalopod consumption.

78

79 **2. Material and Methods**

80 2.1 Study area and squid sampling

81 Between January 2015 and February 2018 (3-year monitoring period), a cross-sectional prevalence
82 study was carried out on **ascaridoid nematodes** recovered from broadtail shortfin (*Illex coindetii*). A
83 total of 745 specimens of *I. coindetii* were caught by local fisherman through bottom trawling in
84 two fishing areas: Savona (n=565) and Piombino (n=180) in the Ligurian Sea (Fig. 1).

85 Squid samples were put in cold boxes at 4°C and transferred to the Fish Diseases Laboratory of the
86 Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta for analysis. For each
87 specimen, species identification and measurement of mantle length (cm) were performed.
88 Anatomopathological and parasitological examination focused on the occurrence of larval
89 **ascaridoid nematodes**. The internal organs were placed in Petri dishes with physiological saline
90 solution and left for 30 min under a heat source to allow mobilization of the anisakid larvae
91 (Serracca et al., 2013). The visceral surfaces were visually inspected and observed under a
92 stereomicroscope (Zeiss Stemis V8, Zeiss, Oberkochen, Germany) to detect the presence of **larval**
93 **ascaridoid nematodes**. The muscle tissue was sectioned in thick slices (< 5 mm) and analysed by
94 transillumination (UVP White Light Transilluminators, TW-43, Analytik Jena AG, Jena, Germany).
95 The muscle tissue and visceral organs were digested separately using an enzymatic digestion
96 method based on the Codex Alimentarius Commission (Codex Alimentarius Commission, 2004)
97 and EU Regulation (EC) No. 2075/2005 (European Commission, 2005). The larvae were rinsed in
98 deionized water and counted. Morphological identification was performed by light microscopy
99 (Olympus BX40, Olympus, Tokyo, Japan). The anterior and posterior parts from each nematode
100 were cleared using Amman's lactophenol to allow morphological identification. Morphological
101 classification was performed as described in Berland (1961). A small piece of the mid-part of the
102 larvae morphologically identified as *Anisakis* spp. underwent molecular analysis by polymerase

103 chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the protocol
104 reported by Serracca et al. (2013).

105

106 2.2 Statistical analysis

107 Normality of data was checked using the Shapiro-Wilk test. The non-parametric Mann-Whitney U
108 test was used to check for differences in mantle length (the biometric parameter used to measure
109 squid size) of *I. coindetii* samples from the two sampling sites (Arkhipkin, 1996). The prevalence of
110 nematode infestation was calculated for the two sampling sites (Bush et al., 1997) and the
111 differences between them were tested using the chi-square test. The 95% confidence intervals (95%
112 CI) were also presented. The criterion for significance was set at $p < 0.05$. Statistical analyses were
113 performed using GraphPad Prism version 8.0.2.

114

115 3. Results

116 The range in mantle length was 3-15 cm (8.65 ± 2.11 cm mean \pm standard deviation [\pm SD]) for the
117 squid from the Savona sampling site and 7-20 cm (10.67 ± 2.69 cm) for the squid from the Piombino
118 site. A significant difference in mantle length between the two sites was noted (Mann-Whitney U
119 test; $p=0.0001$).

120 Parasitological examination revealed **ascaridoid nematodes** larvae (n=9) (Table 1):
121 *Hysterothylacium* sp. (n=6, 5 of which from the Savona and 1 from the Piombino site) and *Anisakis*
122 spp. (n=3, 1 of which from the Savona and 2 from the Piombino site). We found third larval stage
123 (L3) of *Anisakis* larvae type I (*sensu* Berland, 1961) and third larval stage (L3) of *Hysterothylacium*
124 sp. All larvae morphologically referred to the genus *Anisakis* were identified as *A. pegreffii* by
125 PCR-RFLP.

126 None of the **ascaridoid nematodes** had penetrated the musculature but were found encapsulated on
127 the surface of the peritoneal membrane lining the mantle cavity. The prevalence of *A. pegreffii*
128 infestation was 0.18% (95% confidence interval [CI] 0 - 0.52) for the Savona site and 1.11% (95%
129 CI 0 - 2.64) for the Piombino site; the prevalence of *Hysterothylacium* sp. was 0.88% (95% CI 0 -
130 2.64) for the Savona site and 0.56% (95% CI 0.01 - 3.06) for the Piombino site. There was no
131 difference in the prevalence of *A. pegreffii* between the two sites ($\chi^2=2.97$; $p=0.0848$).

132

133 4. Discussion

134 With this study we investigated the occurrence of *Anisakis pegreffii* and *Hysterothylacium* spp. in *I.*
135 *coindetii* from the Ligurian Sea. Few studies **to date** have described the occurrence of **ascaridoid**

136 **nematodes** in *I. coindetii* from the Mediterranean Sea. This knowledge gap poses a potential health
137 risk for squid consumption.

138 Published data show a higher prevalence of **ascaridoid nematodes** infestation in *I. coindetii* than in
139 our samples: Gestal et al. (1999) found a prevalence of 4.8 % for *A. simplex* and of 28 % for
140 *Hysterothylacium* spp. (n=42; Tyrrhenian Sea); Angelucci et al. (2011) reported a prevalence of 50
141 % for *Anisakis* spp. but only in 4 samples from the Sardinian Sea; Picó-Durán et al. (2016) reported
142 a prevalence of 11.4 % for *A. pegreffii*, 16.6 % for *A. physeteris*, 0.8 % for *A. simplex* x *A. pegreffii*
143 hybrid, and 4.1 % for *Hysterothylacium* spp. in samples from Spanish Mediterranean areas (n=123).
144 All specimens of *I. coindetii* (n=137) from Apulia (Italy) analysed by Goffredo et al. (2019) tested
145 negative for anisakid nematode larvae. Finally, Serracca et al. (2013) found only one larva of
146 *Hysterothylacium* spp. (prevalence 1.6 %; n=60) in samples from the Ligurian Sea.

147 **Cipriani et al. (2019) also investigated the occurrence of ascaridoids nematodes in squids but**
148 **belonged to the species *Illex argentinus* (n=70) caught off the Falkland Islands (South Atlantic**
149 **Ocean). Prevalence values of *Anisakis pegreffii* (15.7 %) and *Hysterothylacium aduncum* (2.9 %)**
150 **were higher in comparison to our results, probably due to the different environmental conditions of**
151 **ocean waters that affect the biological cycle of ascaridoids nematodes.**

152 In our sample, the average mantle length (8.65 cm for the Savona and 10.67 cm for the Piombino
153 sampling site) **was smaller than** in the study samples mentioned above, in which the mean mantle
154 length reached up to 36.5 cm (Gestal et al., 1999; Petrić et al., 2011; Serracca et al., 2013; Picó-
155 Durán et al., 2016). The higher prevalence the previous studies reported may stem from the fact that
156 older *I. coindetii* host more nematodes than young ones since squids change their food habits as
157 they grow. *I. coindetii* change diet during the life cycle: juveniles generally prey on crustaceans
158 (Euphausiacea, Penaeidae and Phrosinidae) before switching to fish and cephalopods as adults
159 (Boyle and Rodhouse, 2005; Rosas-Luis et al., 2014). Broadtail shortfin most likely acquires
160 anisakid larvae through the predation of infected euphausiids or from small teleosts (Pascual et al.,
161 1996; Abollo et al., 2001). Based on the results of this study and previous findings, it may be
162 assumed that the consumption of smaller *I. coindetii* carries a lower health risk for nematode
163 zoonoses.

164 This study is the first to reveal *A. pegreffii* identified at the species level by PCR-RFLP in *I.*
165 *coindetii* from the Ligurian Sea. This finding is consistent with published data documenting it as the
166 most widespread species in the Mediterranean Sea (Mattiucci and Nascetti, 2006). Furthermore, our
167 observation of visceral infestation of **ascaridoid nematodes** larvae is shared by several previous
168 studies (Smith, 1984; Gestal et al., 1999; Petrić et al., 2011) but not by Picó-Durán et al. (2016)
169 who found 10 larvae of *Hysterothylacium* sp. in the muscle tissue.

170 The role of *I. coindetii* in the *Anisakis* spp. life cycle may be limited by their shorter lifespan
171 compared to fish (Packard, 1972). Nevertheless, they make up an important part of the diet of
172 odontocete cetaceans (definitive hosts) (Smith, 1984). The Ligurian Sea, also designated as the
173 Pelagos Sanctuary for Cetaceans, is a protected area where cetaceans are more abundant than in the
174 rest of the western Mediterranean Sea (Di Sciara et al., 1993; Azzellino et al., 2008; Serracca et al.,
175 2013). Anisakidae transmission pathways are strictly habitat-dependent and involve a wide
176 spectrum of invertebrates and intermediate or paratenic fish hosts (Klimpel and Rückert 2005;
177 McClelland 2005; Palm and Klimpel, 2007). Despite predisposing factors present in both sampling
178 sites (Pelagos Sanctuary for Cetaceans), the survey data show a very low prevalence of *Anisakis* and
179 *Histerothylacium* species.

180 Further investigations on cephalopods, as well as parasite hosts, may improve our knowledge of the
181 food web structure and better define their role in the **ascaridoid nematodes** life cycle.

182

183 **5. Conclusions**

184 Cephalopods are intermediate or paratenic hosts in the biological cycles and transmission of several
185 parasites (Petrić et al., 2011). This study provides new information on the distribution of **ascaridoid**
186 **nematodes** larvae in broadtail shortfin from the Ligurian Sea. Improving knowledge of zoonotic
187 food-borne parasites is a milestone in health risk assessment of fish consumption (Menconi et al.,
188 2020). The survey data suggest that the risk of fish-borne zoonotic nematode infection after the
189 consumption of broadtail shortfin from the Ligurian Sea is very low. Prevalence rates, low
190 consumption of raw and/or undercooked cephalopods, and the tendency of the larvae to encapsulate
191 in the visceral compartment rather than in the muscle tissue of the host bear this out. Further studies
192 are needed for detailed risk assessment of **ascaridoid nematodes** larvae in other commercially
193 relevant cephalopod species to define consumer health risk.

194

195 **Acknowledgments**

196 This research was partially funded by the Italian Ministry of Health (**project IZS PLV 12/18 RC**).

197

198 **Declaration of competing interest**

199 None.

200

201 **References**

202

203 Abollo, E., Gestal, C., & Pascual, S. (2001). *Anisakis* infestation in marine fish and cephalopods
204 from Galician waters: an updated perspective. *Parasitology Research*, 87(6), 492-499.
205 <https://doi.org/10.1007/s004360100389>.

206 Angelucci, G., Meloni, M., Merella, P., Sardu, F., Madeddu, S., Marrosu, R., Petza, F., & Salati, F.
207 (2011). Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods
208 sampled from waters off Sardinia. *Journal of Food Protection*, 74(10), 1769-1775.
209 <https://doi.org/10.4315/0362-028X.JFP-10-482>.

210 Arkhipkin, A. (1996). Geographical variation in growth and maturation of the squid *Illex coindetii*
211 (Oegopsida, Ommastrephidae) off the north-west African coast. *Journal of the Marine Biological*
212 *Association of the United Kingdom*, 76(4), 1091-1106. [https://doi.org/10.1017/](https://doi.org/10.1017/S0025315400040984)
213 [S0025315400040984](https://doi.org/10.1017/S0025315400040984).

214 Arizono, N., Yamada, M., Tegoshi, T., & Yoshikawa, M. (2012). *Anisakis simplex* sensu stricto and
215 *Anisakis pegreffii*: biological characteristics and pathogenetic potential in human anisakiasis.
216 *Foodborne pathogens and disease*, 9(6), 517-521. <https://doi.org/10.1089/fpd.2011.1076>.

217 Audicana, M. T., Ansotegui, I. J., de Corres, L. F., & Kennedy, M. W. (2002). *Anisakis simplex*:
218 dangerous dead and alive? *Trends in Parasitology*, 18(1), 20-25. [https://doi.org/10.1016/S1471-](https://doi.org/10.1016/S1471-4922(01)02152-3)
219 [4922\(01\)02152-3](https://doi.org/10.1016/S1471-4922(01)02152-3).

220 Audicana, M. T., & Kennedy, M. W. (2008). *Anisakis simplex*: from obscure infectious worm to
221 inducer of immune hypersensitivity. *Clinical Microbiology Reviews*, 21(2), 360-379.
222 <https://doi.org/10.1128/CMR.00012-07>

223 Azzellino, A., Gaspari, S., Airoidi, S., & Nani, B. (2008). Habitat use and preferences of cetaceans
224 along the continental slope and the adjacent pelagic waters in the western Ligurian Sea. *Deep Sea*
225 *Research Part I: Oceanographic Research Papers*, 55(3), 296-323. [https://doi.org/10.1016/](https://doi.org/10.1016/j.dsr.2007.11.006)
226 [j.dsr.2007.11.006](https://doi.org/10.1016/j.dsr.2007.11.006).

227 Berland, B. (1961). Nematodes from some Norwegian marine fishes. *Sarsia*, 2(1), 1-50.
228 <https://doi.org/10.1080/00364827.1961.10410245>.

229 Boyle, P., & Rodhouse, P. (2005). Cephalopods: ecology and fisheries. Blackwell Publishing,
230 London, pp. 464.

231 Boyle, P. R., & Pierce, G. J. (1994). Fishery biology of northeast Atlantic squid: an overview.
232 *Fisheries Research*, 21(1-2), 1-15. [https://doi.org/10.1016/0165-7836\(94\)90093-0](https://doi.org/10.1016/0165-7836(94)90093-0).

233 Bush, A. O., Lafferty, K. D., Lotz, M., Shostak, A.W. (1997). Parasitology meets ecology. *Journal*
234 *of Parasitology*, 83(4), 575-583.

235 Clarke, M. R. (1996). The role of cephalopods in the world's oceans: an introduction. *Philosophical*
236 *Transactions of the Royal Society of London. Series B: Biological Sciences*, 351, 977–1112.
237 <https://doi.org/10.1098/rstb.1996.0088>.

238 Codex Alimentarius Commission (2004). Standard for salted Atlantic herring and salted sprat.
239 *Codex Stan*, 244-2004, 1–8.

240 Daschner, A., Alonso-Gómez, A., Cabañas, R., Suarez-de-Parga, J. M., & López-Serrano, M. C.
241 (2000). Gastroallergic anisakiasis: borderline between food allergy and parasitic disease - Clinical
242 and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*.
243 *Journal of Allergy and Clinical Immunology*, 105(1), 176-181. [https://doi.org/10.1016/S0091-](https://doi.org/10.1016/S0091-6749(00)90194-5)
244 [6749\(00\)90194-5](https://doi.org/10.1016/S0091-6749(00)90194-5).

245 Di Sciara, G. N., Venturino, M. C., Zanardelli, M., Bearzi, G., Borsani, F. J., & Cavalloni, B.
246 (1993). Cetaceans in the central Mediterranean Sea: distribution and sighting frequencies. *Italian*
247 *Journal of Zoology*, 60(1), 131-138. <https://doi.org/10.1080/11250009309355800>.

248 EFSA (2010). Panel on Biological Hazards (BIOHAZ). Scientific opinion on risk assessment of
249 parasites in fishery products. *EFSA Journal*, 8(4), 1543. <https://doi.org/10.2903/j.efsa.2010.1543>.

250 European Commission (2005). Commission Regulation (EC) No. 2075/2005 of 5 December 2005
251 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the*
252 *European Union*, 338, 75-76.

253 Farjallah, S., Slimane, B. B., Busi, M., Paggi, L., Amor, N., Blel Said, K., & D'Amelio, S. (2008).
254 Occurrence and molecular identification of *Anisakis* spp. from the North African coasts of
255 Mediterranean Sea. *Parasitology Research*, 102(3), 371. [https://doi.org/10.1007/s00436-007-0771-](https://doi.org/10.1007/s00436-007-0771-9)
256 [9](https://doi.org/10.1007/s00436-007-0771-9).

257 Gestal, C., Belcari, P., Abollo, E., & Pascual, S. (1999). Parasites of cephalopods in the northern
258 Tyrrhenian Sea (western Mediterranean): new host records and host specificity. *Scientia Marina*,
259 63(1), 39-43. <https://doi.org/10.3989/scimar.1999.63n139>.

260 Goffredo, E., Azzarito, L., Di Taranto, P., Mancini, M.E., Normanno, G., Didonna, A., Faleo, S.,
261 Occhiochiuso, G., D'Attoli, L., Pedarra, C., Pinto, P., Cammilleri, G., Graci, S., Sciortino, S., &
262 Costa, A. (2019). Prevalence of anisakid parasites in fish collected from Apulia region (Italy) and
263 quantification of nematode larvae in flesh. *International Journal of Food Microbiology*, 292, 159-
264 170. <https://doi.org/10.1016/j.ijfoodmicro.2018.12.025>.

265 Guerra, A. (1992). Mollusca, Cephalopoda. In: Ramos, M. A. (ed.), “Fauna Ibérica”. Museo
266 Nacional de Ciencias Naturales, CSIC Madrid: 327.

267 Jereb, P., & Roper, C. F. (2010). Cephalopods of the world. An annotated and illustrated catalogue
268 of cephalopod species known to date. *FAO Species Catalogue for Fishery Purposes*, 4(2), 262.

269 Klimpel, S., & Palm, H. W. (2011). Anisakid nematode (Ascaridoidea) life cycles and distribution:
270 increasing zoonotic potential in the time of climate change? In: Progress in parasitology, Springer,
271 Berlin, Heidelberg, 201-222.

272 Klimpel, S., & Rückert, S. (2005). Life cycle strategy of *Hysterothylacium aduncum* to become the
273 most abundant anisakid fish nematode in the North Sea. *Parasitology Research*, 97(2), 141-149.
274 <https://doi.org/10.1007/s00436-005-1407-6>.

275 Køie, M. (1993). Aspects of the life cycle and morphology of *Hysterothylacium aduncum*
276 (Rudolphi, 1802) (Nematoda, Ascaridoidea, Anisakidae). *Canadian Journal of Zoology*, 71(7),
277 1289-1296. <https://doi.org/10.1139/z93-178>.

278 Lefkaditou, E., Politou, C. Y., Palialexis, A., Dokos, J., Cosmopoulos, P., & Valavanis, V. D.
279 (2008). Influences of environmental variability on the population structure and distribution patterns
280 of the short-fin squid *Illex coindetii* (Cephalopoda: Ommastrephidae) in the Eastern Ionian Sea.
281 *Hydrobiologia*, 612, 71-90. <https://doi.org/10.1007/s10750-008-9490-1>.

282 **Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., & Nascetti, G. (2018). Molecular epidemiology
283 of *Anisakis* and anisakiasis: an ecological and evolutionary road map. In D. Rollinson, & J. R.
284 Stothard (Eds.), *Advances in Parasitology* (pp. 93-263) Academic Press.
285 <https://doi.org/10.1016/bs.apar.2017.12.001>**

286 **Mattiucci, S., Fazii, P., Paoletti, M., De Rosa, A., Salomone Megna, A., Glielmo, A., Bruschi, F.,
287 De Angelis, M., Costa, A., Meucci, C., Sorrentini, L., Calvaruso, V., & Nascetti G. (2013)
288 *Anisakiasis* and gastroallergic reactions associated with *Anisakis pegreffii* infection, Italy .
289 *Emerging Infectious Disease*, 19(3): 496-499. <https://doi.org/10.3201/eid1903.121017>**

290 Mattiucci, S., & Nascetti, G. (2006) Molecular systematics, phylogeny and ecology of anisakid
291 nematodes of the genus *Anisakis* Dujardin, 1845: an update. *Parasite*, 13(2): 99-113.
292 <https://doi.org/10.1051/parasite/2006132099>

293 McClelland, G. (2005) Nematoda (roundworms). In: Rohde K (ed) "Marine parasitology". CABI
294 Publishing, Wallingford, 104-115.

295 Menconi, V., Manfrin, C., Pastorino, P., Mugetti, D., Cortinovis, L., Pizzul, E., Pallavicini, A., &
296 Prearo, M. (2020). First Report of *Clinostomum complanatum* (Trematoda: Digenea) in European
297 Perch (*Perca fluviatilis*) from an Italian Subalpine Lake: A Risk for Public Health? *International
298 journal of environmental research and public health*, 17, 1389.
299 <https://doi.org/10.3390/ijerph17041389>

300 Packard, A. (1972). Cephalopods and fish: the limits of convergence. *Biological Reviews*, 47(2),
301 241-307. <https://doi.org/10.1111/j.1469-185X.1972.tb00975.x>.

302 Palm, H. W., & Klimpel, S. (2007). Evolution of parasitic life in the ocean. *Trends in Parasitology*,
303 23(1), 10-12. <https://doi.org/10.1016/j.pt.2006.11.001>

304 Pascual, S., Gonzalez, A., Arias, C., & Guerra, A. (1996). Biotic relationships of *Illex coindetii* and
305 *Todaropsis eblanae* (Cephalopoda, Ommastrephidae) in the northeast Atlantic: evidence from
306 parasites. *Sarsia*, 81(3), 265-274. <https://doi.org/10.1080/00364827.1996.10413624>.

307 Petrić, M., Mladineo, I., & Šifner, S. K. (2011). Insight into the short-finned squid *Illex coindetii*
308 (Cephalopoda: Ommastrephidae) feeding ecology: is there a link between helminth parasites and
309 food composition? *Journal of Parasitology*, 97(1), 55-62. <https://doi.org/10.1645/GE-2562.1>.

310 Picó-Durán, G., Pulleiro-Potel, L., Abollo, E., Pascual, S., & Muñoz, P. (2016). Molecular
311 identification of *Anisakis* and *Hysterothylacium* larvae in commercial cephalopods from the Spanish
312 Mediterranean coast. *Veterinary Parasitology*, 220, 47-53. <https://doi.org/10.1016/j.vetpar.2016.02.020>.

314 Quetglas, A., Carbonell, A., & Sánchez, P. (2000). Demersal continental shelf and upper slope
315 cephalopod assemblages from the Balearic Sea (north-western Mediterranean). Biological aspects
316 of some deep-sea species. *Estuarine, Coastal and Shelf Science*, 50(6), 739-749.
317 <https://doi.org/10.1006/ecss.1999.0603>.

318 Rosas-Luis, R., Villanueva, R., & Sánchez, P. (2014). Trophic habits of the Ommastrephid squid
319 *Illex coindetii* and *Todarodes sagittatus* in the northwestern Mediterranean Sea. *Fisheries Research*,
320 152, 21-28. <https://doi.org/10.1016/j.fishres.2013.10.009>.

321 Sanchez, P., Gonzalez F., Jereb P., Laptikhovskiy V. V., Mangold K., Nigmatullin C., Ragonese S.
322 (1998). *Illex coindetii*. In: "Squid recruitment dynamics: The commercial *Illex* species", P. G.
323 Rodhouse, E. G. Dawe, and R. K. O'Dor (eds.). FAO Fisheries Technical Paper 376, FAO, Rome,
324 Italy, 59-76.

325 Serracca, L., Cencetti, E., Battistini, R., Rossini, I., Prearo, M., Pavoletti, E., Fioravanti, M. L.,
326 Righetti, M., Di Donfrancesco, B., & Ercolini, C. (2013). Survey on the presence of *Anisakis* and
327 *Hysterothylacium* larvae in fishes and squids caught in Ligurian Sea. *Veterinary Parasitology*,
328 196(3-4), 547-551. <https://doi.org/10.1016/j.vetpar.2013.02.024>.

329 Smith, J. W. (1984). Larval ascaridoid nematodes in myopsid and oegopsid cephalopods from
330 around Scotland and in the northern North Sea. *Journal of the Marine Biological Association of the*
331 *United Kingdom*, 64(3), 563-572. <https://doi.org/10.1017/S0025315400030253>.

332

333

334

335

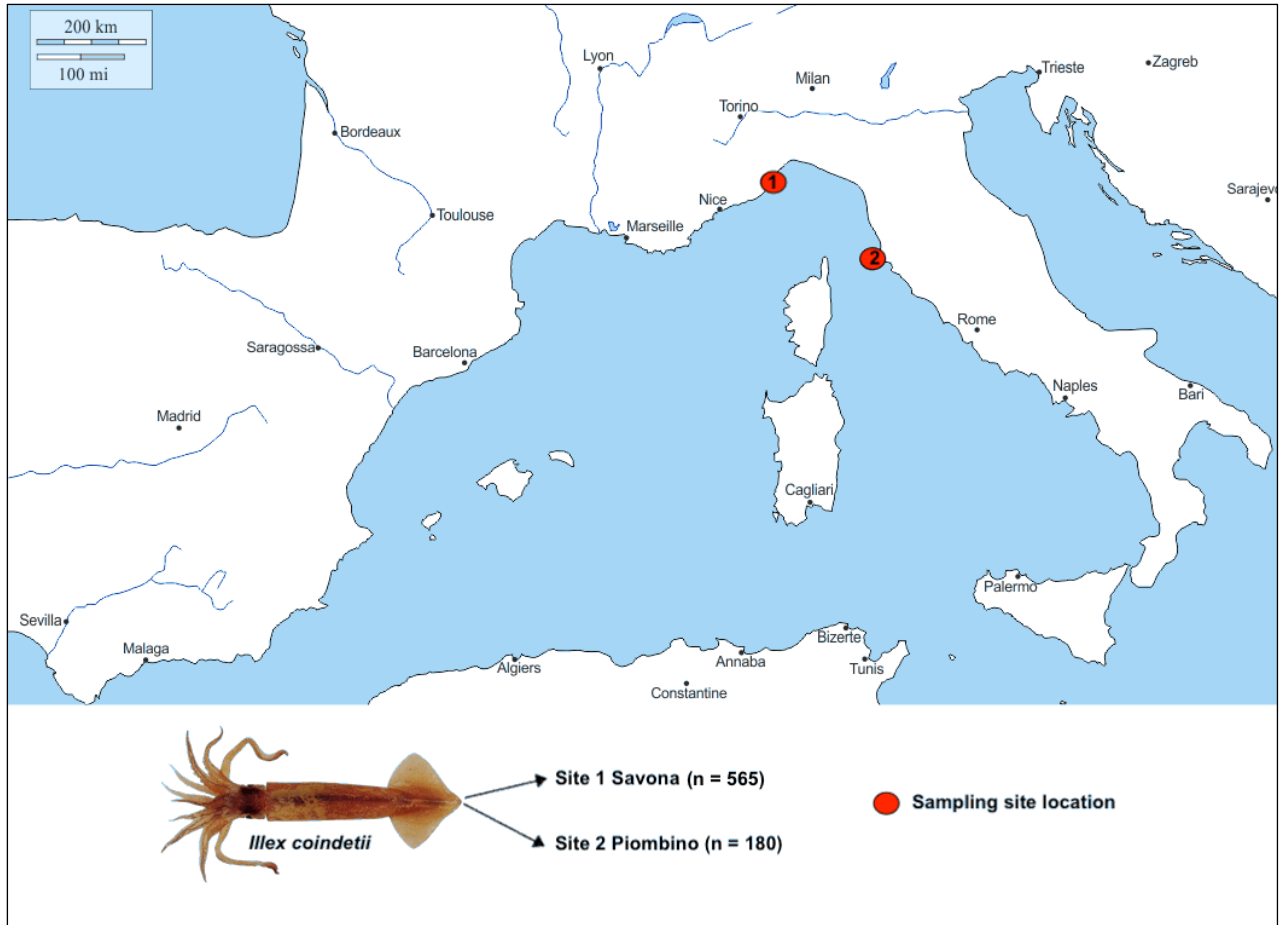
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369

Table 1. Mantel length (cm) and number (N) of *Illex coindetii* caught from the two sites with the number and mean intensity of infestation by *Hysterotylacium* sp. and *Anisakis pegreffii*.

Sampling site	<i>Illex coindetii</i>	Mantel length	<i>Hysterotylacium</i> sp.		<i>Anisakis pegreffii</i>	
	N	cm (mean \pm SD)	N	mean intensity	N	mean intensity
Savona	565	8.65 \pm 2.11	5	1	1	1
Piombino	180	10.67 \pm 2.69	1	1	2	1

370

371 **Figure 1.** Western Mediterranean Sea and location of sampling sites (in red). Site 1 (Savona):
372 44°17'25.58"N 8°39'6.23"E; site 2 (Piombino): 42°56'34.164"N 10°25'37.713"E.

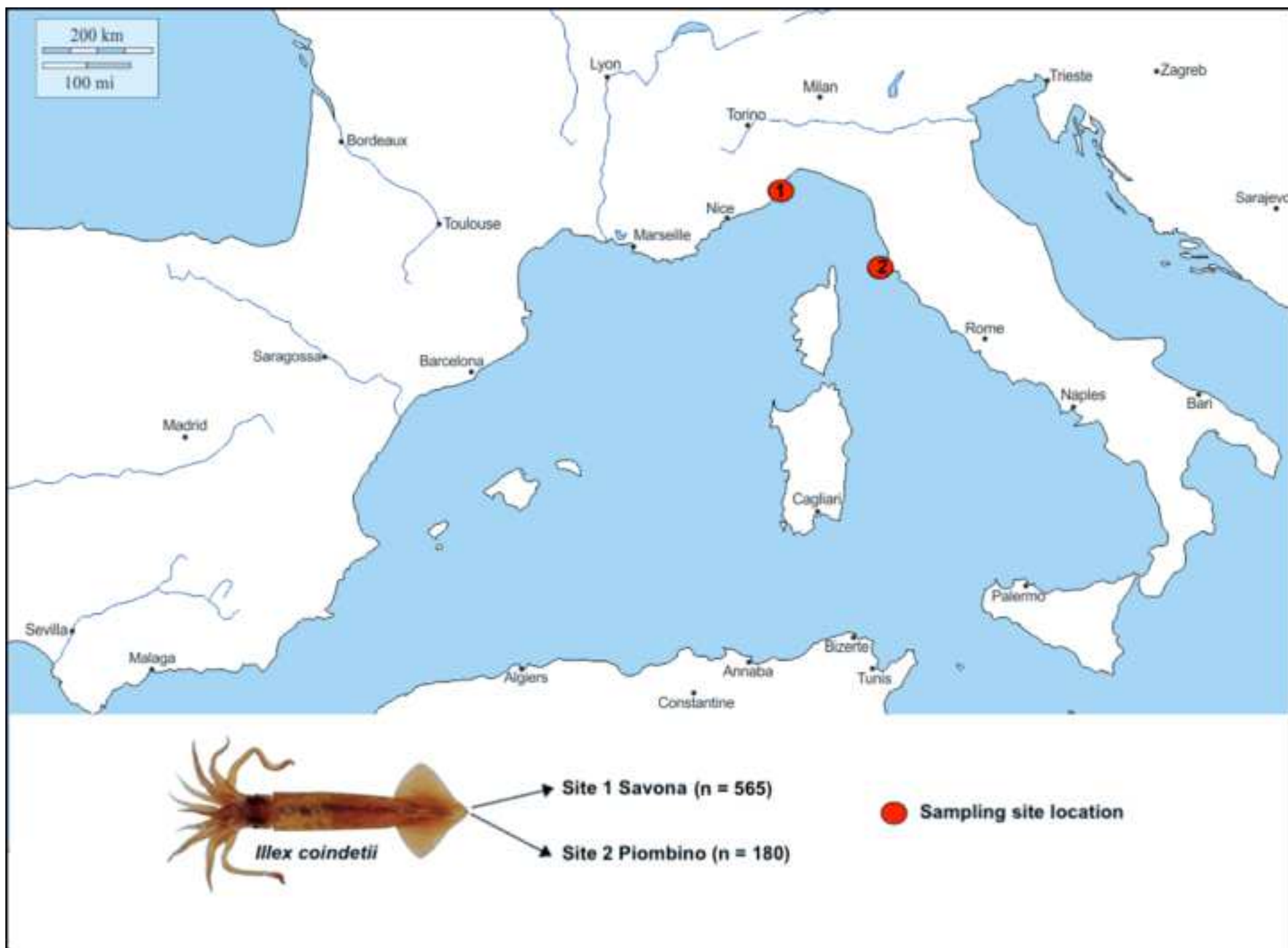


373

374

375

Figure 1



Credit Author Statement

Conceptualization: **Vasco Menconi, Paolo Pastorino, Maria Cristina Bona, and Marino Prearo**;
Data curation: **Vasco Menconi, Paolo Pastorino and Maria Cristina Bona**; Investigation: **Vasco Menconi, Paolo Pastorino, Erika Astrid Virginie Burioli, Marzia Righetti, Tommaso Scanzio, Livio Favaro, Maria Cristina Bona, Elena Pavoletti, Alessandro Dondo and Marino Prearo**;
Methodology: **Vasco Menconi, Paolo Pastorino and Marino Prearo**; Supervision: **Marino Prearo**;
Writing – original draft: **Vasco Menconi and Paolo Pastorino**; Writing – review & editing: **Vasco Menconi, Paolo Pastorino and Marino Prearo**. All authors have read and agreed to the published version of the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: