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Larvae of *Sulcascaris sulcata* (Nematoda: Anisakidae), a parasite of sea turtles, infect the edible purple dye murex *Bolinus brandaris* in the Tyrrhenian Sea

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ABSTRACT

Anisakid nematodes are among the most common helminth parasites of the marine realm, particularly important for their implications with human infections and/or allergies. Members of the Anisakidae use aquatic mammals, birds and fish as definitive hosts, and crustaceans, fish and molluscs as intermediate/paratenic hosts. *Sulcascaris sulcata,* the only species in the monotypic genus *Sulcascaris*, represents the exception being a parasite of sea turtles as adult. The recent findings of larvae of *S. sulcata* in scallops (*Pecten jacobeus* and *Aequipecten opercularis*) and Mediterranean mussels *Mytilus galloprovincialis* intended for human consumption from the Mediterranean Sea caused concern regarding the sanitary control of edible molluscs and consumer safety. Herein, we investigated the larval anisakids collected from the purple dye murex, *Bolinus brandaris,* harvested for human consumption from the Central Mediterranean Sea (Tyrrhenian Sea). Morphological study and sequence analysis of the internal transcribed spacer regions of the ribosomal DNA and the mitochondrial cytochrome *c* oxidase subunit 2 gene locus revealed the occurrence of fourth stage larvae of *S. sulcata* in 16% of the purple dye murex examined. The present study adds the purple dye murex to the list of the known intermediate hosts of this parasite in the Mediterranean Sea and the northern coast of Campania region as site where individuals of loggerhead turtle and purple dye murex may become infected. This is the first study reporting an anisakid nematode in edible gastropods. Epidemiological features of infection in the purple dye murex and implications for gastropod safety and risk for consumers are discussed.

1. Introduction

The purple dye murex, *Bolinus brandaris* (Linnaeus, 1758), is a common gastropod species, present throughout the whole Mediterranean Sea, with its geographical distribution spreading also in the Atlantic waters, restricted to the coasts of Portugal and Morocco (Sabelli et al., 1990; Poppe & Goto, 1991; Houart, 2001). *Bolinus brandaris* is among the most valuable edible gastropod species with high commercial value (reaching prices around 20 to 25 € per kg for first sale) in several countries of the Mediterranean basin, where it is used to prepare traditional and fine seafood dishes for human consumption (Martín et al., 1995; Vasconcelos et al., 2008, 2012). In particular, the purple dye murex is regularly harvested for marketing in France, Italy, Spain, Greece and Portugal, and occasionally in Tunisia and Turkey (Martín et al., 1995; Vasconcelos et al., 2008, 2012).

Parasitic infections and related allergic reactions associated to the consumption of seafood are a serious public health problem worldwide with about 56 million of human cases reported (World Health Organization, 2012; Nieuwenhuizen & Lopata, 2013; Bao et al., 2017, 2019; Mattiucci et al., 2018). Human fishery product-borne parasitic diseases may be caused by ingestion of viable parasites, or as allergic (hypersensitivity) reactions against parasite antigens present in seafood. The most important helminth parasites in fishery products implicated with human infections and/or allergies are some anisakid nematodes within the genera *Anisakis* and *Pseudoterranova* (Arizono et al., 2011; Nieuwenhuizen & Lopata, 2013; Bao et al., 2017, 2019; Mattiucci et al., 2018). Members of the Anisakidae family, which also include the genera *Contracaecum*, *Phocascaris*, *Pulchrascaris*, and *Terranova,* typically use aquatic mammals, birds and fish as definitive hosts, and crustaceans (krill), fish and molluscs as intermediate/paratenic hosts (Mattiucci

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et al., 2018; Ángeles-Hernández et al., 2020). *Sulcascaris sulcata,* the only species in the monotypic genus *Sulcascaris*, represents an exception since it is a parasite of sea turtles, with molluscs (bivalves and gastropods) acting as intermediate hosts (Sprent, 1977; Lichtenfels et al., 1978, 1980; Berry & Cannon, 1981; Marcer et al., 2020; Santoro et al., 2019, 2020).

The recent molecular identification of larvae of *S. sulcata* in scallops (*Pecten jacobeus* and *Aequipecten opercularis*) from the Adriatic Sea (Marcer et al., 2020; Pretto et al., 2020) and Mediterranean mussels *Mytilus galloprovincialis* from the Tyrrhenian Sea (Santoro et al., 2020) intended for human consumption raised concern regarding both public health and fishery economic losses for the potential restrictions of mollusc marketing by national and international sanitary regulations (Marcer et al., 2020; Pretto et al., 2020; Santoro et al., 2020). An experimental study performed on fish, chicken, and cat suggested that *S. sulcata* can mature to the adult stage and reproduce only in sea turtles representing their final host; however, since *S. sulcata* is a species related to zoonotic anisakid parasites, the potential public health concerns should be monitored and evaluated (Berry & Cannon, 1981).

Records of larval forms of nematodes in edible marine gastropods are rare and limited to the finding of *Echinocephalus pseudouncinatus* (a parasite of elasmobranchs as adult) encysted in the foot of the pink abalone, *Haliotis corrugata* and green abalone *Haliotis fulgens* in southern California (see Lauckner, 1980). However, during a parasitological survey of free-ranging molluscs from the coast of Campania region of southern Italy, viable larvae of nematodes were found in the tissues of the purple dye murex harvested for human consumption. Herein, using an integrative taxonomic approach, we identified those larvae as belonging to *S. sulcata* revealing another intermediate host in the life cycle of this parasite, and reporting for the first time the occurrence of an anisakid nematode in an edible gastropod from the Mediterranean basin.

2. Materials and methods

2.1. General data and parasitological study

During May 2021, a total of 56 specimens of purple dye murex were harvested by local fisherman from the coast of Baia Domizia (Caserta municipality) at 5–10 m depth on sand bottom (Fig. 1). Within 4 h after the sampling, the specimens of purple dye murex were transferred, in a cooler with ice packs, to the laboratory, where they were identified to the species level according to their morphological characters (Sabelli et al., 1990) and kept for 48 h at +4 °C until parasitological analyses were performed. Subsequently, they were measured (shell length: SL) to the nearest 0.1 cm using an electronic calliper. After removing the shell by cracking it in a bench vice, soft parts of the organism were isolated and the sex was determined before the parasitological inspection by examination of the sexual organs (Vasconcelos et al., 2012). Then, each gastropod specimen was cut along the dorsal mid-line of the mantle to expose and inspect mantle organs (ctenidium, osphradium, hypobranchial gland, pallial gonoduct, and hindgut), and to access the cephalopodium that was dorsally cut as well. Internal organs (proboscis, salivary glands, oesophagus, gland of Leiblein, and kidney) were removed from the cephalopodial cavity and visceral spire, placed individually in plastic Petri dishes, opened, and inspected for the presence of metazoan parasites under a dissecting microscope. The foot of each specimen was dissected in small pieces (0.2 cm × 0.2 cm approximately) and examined under the dissecting microscope. Parasites found embedded in the tissues were extracted using scissors and tweezers. Larval nematodes were measured (mean total body length and width ±

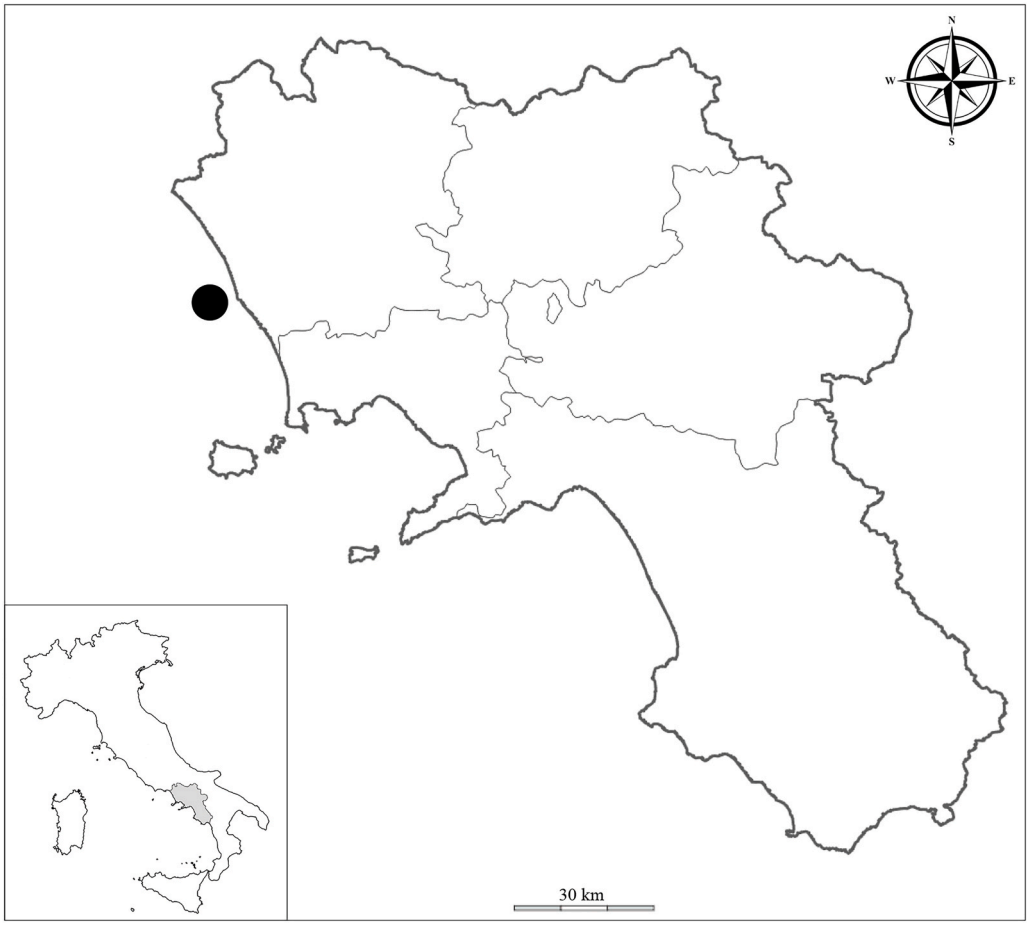

Fig. 1. Sampling location (black dot) of individuals of purple dye murex studied for anisakid nematodes.

standard deviation followed by range in parenthesis), studied and photographed using a dissecting microscope and a compound microscope both equipped with ZEN 3.1 imaging system (Zeiss). All the detected nematodes were subsequently counted, washed in physiological saline solution, and preserved in 70% ethanol or frozen at −20 °C. Extremities of each larva were clarified in Amman's lactophenol and morphologically identified using a compound microscope according to available identification keys (Lichtenfels et al., 1978; Berry & Cannon, 1981), while a portion of the middle body tract was used for molecular analysis.

For scanning electronic microscopy (SEM), both extremities of three larvae were fixed overnight in 2.5% glutaraldehyde, then transferred to 40% ethanol (10 min), rinsed in 0.1 M cacodylate buffer, postfixed in 1% OsO4 for 2 h, dehydrated in ethanol series, and critical point dried and sputter-coated with platinum. Observations were made using an EVO HD15 Zeiss scanning electron microscope operating at 5.0 kV.

Finally, prevalence of infection was defined as the number of purple dye murex infected with 1 or more larvae of *S. sulcata*; abundance was measured as the number of individuals of *S. sulcata* in a single purple dye murex regardless of whether or not the host is infected (Bush et al., 1997).

2.2. Molecular analysis of larvae of S. sulcata

In order to confirm the identity of larvae, morphologically attributed to *S. sulcata*, the total genomic DNA from ~2 mg of 11 larvae was extracted using Quick-gDNA Miniprep Kit (ZYMO RESEARCH) following the standard manufacturer-recommended protocol.

The ITS region of rDNA, including first internal transcribed spacer (ITS-1), the 5.8S gene, the second transcribed spacer (ITS-2), and ~70 nucleotides of the 28S gene, was amplified using the primers NC5 (forward; 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′) and NC2 (reverse; 5′-TTAGTTTCTTTTCCTCCGCT-3′) (Zhu et al., 1998). PCRs were carried out in a 15 μL volume containing 0.3 μL of each primer 10 mM, 2.5 μL of $MgCl_2$ 25 mM (Promega), 1.5 μL of 5 × buffer (Promega), 0.3 μL of DMSO 0.3 mM, 0.3 μL of dNTPs 10 mM (Promega), 0.15 μL (5 U/μL) of Go-*Taq* Polymerase (Promega) and 2 μL of total DNA. PCR temperature conditions were the following: 94 °C for 5 min (initial denaturation), followed by 30 cycles at 94 °C for 30 s (denaturation), 55 °C for 30 s (annealing), 72 °C for 30 s (extension) and followed by post-amplification at 72 °C for 5 min. Additionally, the mtDNA *cox*2 locus was sequenced using the primers 211F (5′-TTTTCTAGTTATATA GATTGRTTYAT-3′) and 210R (5′-CACCAACTCTTAAAATTATC-3′) (Nadler et al., 2000; Valentini et al., 2006). PCRs were carried out in a 25 μL volume containing 0.5 μL of each primer 10 mM, 3 μL of $MgCl_2$ 25 mM (Promega), 5 μL of 5 × buffer (Promega), 0.5 μL of DMSO 0.3 mM, 0.5 μL of dNTPs 10 mM (Promega), 0.3 μL (5 U/μL) of Go-*Taq* Polymerase (Promega) and 3 μL of total DNA. The amplification protocol was performed using the following conditions: 94 °C for 3 min (initial denaturation), followed by 35 cycles at 94 °C for 30 s (denaturation), at 46 °C for 1 min (annealing), at 72 °C for 90 s (extension), and a final extension at 72 °C for 10 min.

The successful PCR products were purified, and Sanger sequenced through an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems), using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies).

The obtained sequences were analysed, edited, and compared with those available in GenBank using BLASTn (Morgulis et al., 2008). The present sequences and those downloaded from GenBank were aligned with MEGAX (Kumar et al., 2018). The alignments were pruned to 661 bp and 501 bp for the ITS region and *cox*2 gene locus, respectively. Nucleotide-sequence divergences were calculated using Kimura's two-parameter (K2P) model (Kimura, 1980) with 1000 bootstrap re-samplings by MegaX (Kumar et al., 2018). The K2P model provides the best metric when genetic distances are low (Nei & Kumar, 2000). This analysis involved 58 ITS sequences (from 22 larvae and 25 adults from GenBank database and 11 larvae from this study) and 50 *cox*2 sequences (from 20 larvae and 19 adults from GenBank and 11 larvae from this study).

Representative sequences of *S. sulcata* here obtained were deposited in GenBank with the accession numbers MZ647950-54 (ITS region of the rDNA) and MZ671783-87 (mtDNA *cox*2).

3. Results

3.1. General data and parasitological study

Out of the 56 gastropods, 22 were males (ranging in SL from 57.3 to 76.7) and 34 females (ranging in SL from 49.4 to 74.9 cm). Overall 15 nematode larvae were collected from 9 (2 males: 64.8–65.7 cm in SL; and 7 females ranging in SL from 56.9 to 74.9 cm) of the purple dye murex (Fig. 2). All larvae were whitish in colour, and on the basis of morphological characters (i.e., squarish lips, constriction at the base of the lips, interlabia, teeth and caecum appearance, excretory pore position, conical tail with a mucronate tip and longitudinal ridges on the cuticle) were all morphologically assigned to the genus *Sulcascaris* showing morphological features of fourth-stage larvae (Figs. 3 and 4). The following measurements were achieved on 11 larvae. They were: 25.1 ± 9624.37 mm in body length (range: 15.5–51.5 mm) and 0.5 ± 57.66 mm in body width (range: 0.4–0.6 mm). Prevalence and abundance of infection were 16% and 0.37, respectively. The maximum and minimum number of larvae per gastropod were 6 and 1, respectively. Most (n = 7) of the infected gastropods harboured 1 larva; the remaining had 2 and 6 larvae, respectively. Regarding the tissue distribution, out of the 15 larvae, 6 (40%) were encysted on the external surface of the anterior oesophagus at the level of the proboscis base (Fig. 2A), 3 (20%) were found in the mantle cavity (Fig. 2B), and 2 each (13.3%) were found in the cephalopodial cavity, foot (Fig. 2C), and in the gland of Leiblein.

3.2. Molecular analysis of larvae of S. sulcata

The sequences (850 bp) of the ITS region of the rDNA obtained from the larvae showed 100% identity with the sequences of *S. sulcata* previously obtained from Tyrrhenian Sea and available in GenBank (accession number MN699442). The mtDNA *cox*2 sequences (580 bp), matched at 99–100% with those sequences of *S. sulcata* obtained from the same geographical area and deposited in GenBank (accession number MN991206). The distance values (±standard deviation) between *S. sulcata* larvae here examined and *S. sulcata* adults from GenBank database resulted to be K2P = 0.0006 ± 0.000 at the ITS region and K2P = 0.003 ± 0.001 at the *cox*2 gene locus, respectively. Very similar distance values were found between the larvae here investigated and those available in GenBank (K2P = 0.0009 ± 0.000 at the ITS region and K2P = 0.003 ± 0.001 at the *cox*2 gene locus).

4. Discussion

Sulcascaris sulcata is a parasite with a worldwide distribution that infects sea turtle species [i.e, loggerhead turtle (*Caretta caretta*), green turtle (*Chelonia mydas*), and Kemp's ridley turtle (*Lepidochelys kempii*)] from Mediterranean, Caribbean, Southern and Western Atlantic, and Western Pacific (Sprent, 1977; Lichtenfels et al., 1978; Berry & Cannon, 1981; Werneck et al., 2008; Santoro et al., 2010, 2019). The loggerhead turtle, which feeds mostly on bivalves and gastropods, represents its main definitive host (Berry & Cannon, 1981; Santoro et al., 2019). According to the available literature, the occurrence of *S. sulcata* in the Mediterranean population of loggerhead turtles is restricted to its eastern basin and the Tyrrhenian Sea (Sey, 1977; Santoro et al., 2010, 2019; Gračan et al., 2012; Gentile et al., 2021) with prevalence of infection ranging from 16.5% to 33.3% depending by geographical area and timing of sampling (Gračan et al., 2012; Santoro et al., 2010, 2019;

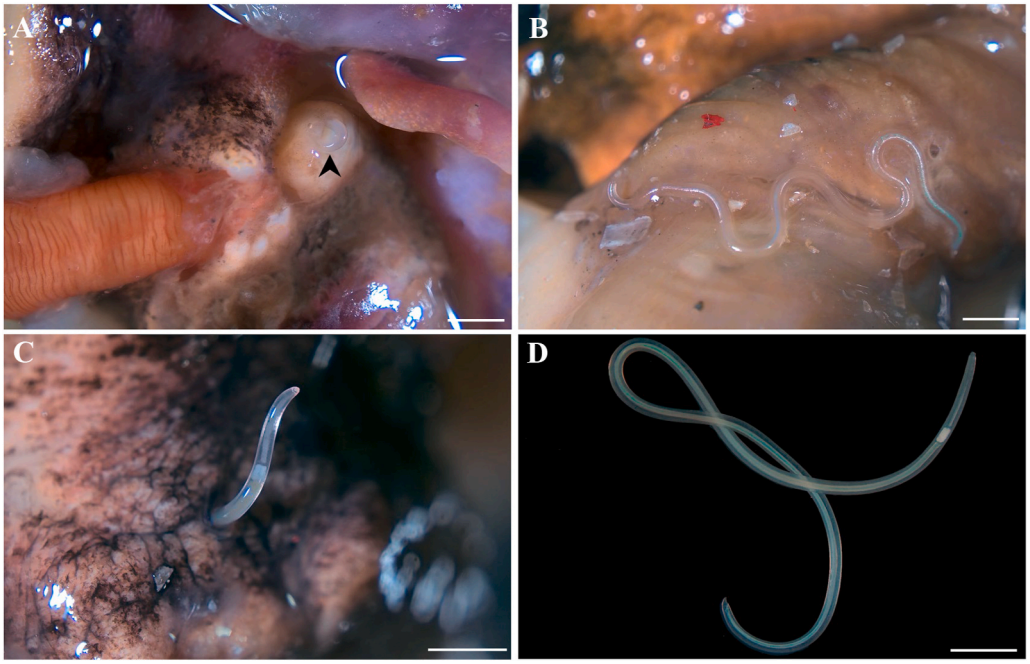

Fig. 2. Purple dye murex infected with larvae of *Sulcascaris sulcata*. A larva (arrow) encysted on the external surface of the anterior oesophagus at level of the proboscis base (A). A larva free in the mantle cavity (B). A larva in the wall of the foot (C). A whole larva (D). Scale bars = 2000 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

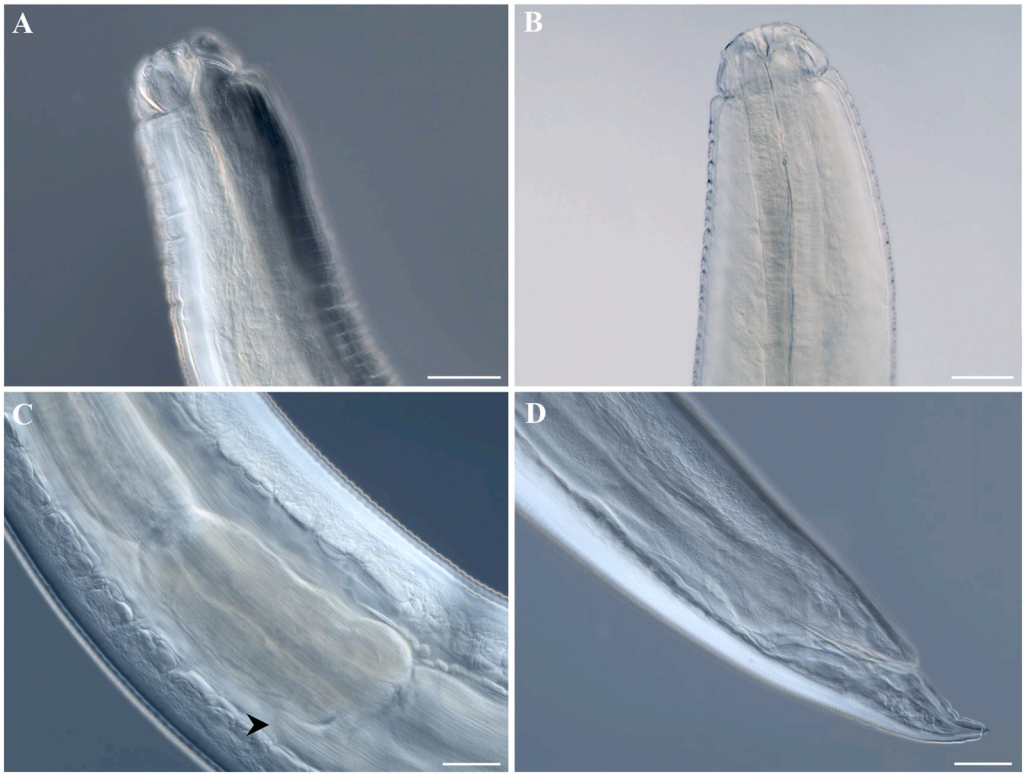

Fig. 3. Microscopic features of larvae of *Sulcascaris sulcata* found in the purple dye murex. Anterior extremity (A, B), ventriculus with caecum (arrow) (C) and posterior extremity (D). Scale bars = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

Gentile et al., 2021). A recent study found that loggerhead turtles from the Adriatic Sea show a higher infection level than those from Tyrrhenian Sea (Santoro et al., 2019). It has been suggested that the higher value of infection is related to the particular environmental conditions of this latter basin, hosting greatest abundance of molluscan intermediate hosts (Gračan et al., 2012; Santoro et al., 2019).

Regarding the life cycle of *S. sulcata*, it is known that sea turtles become infected by ingesting molluscs infected with fourth-stage larvae.

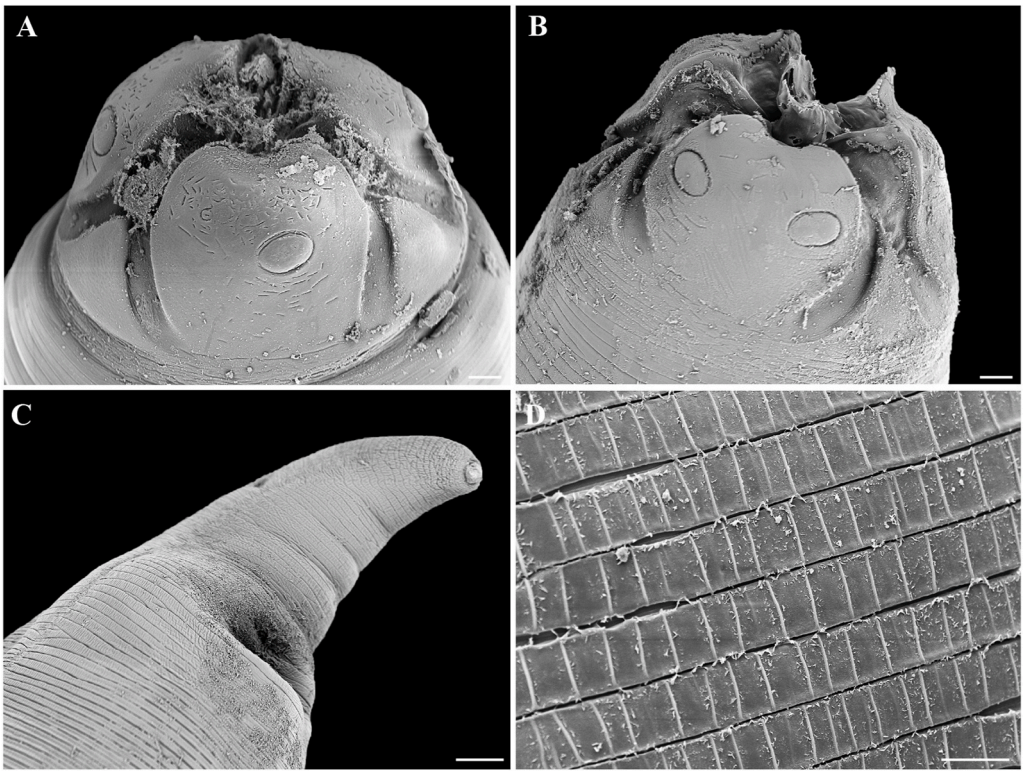

Fig. 4. Scanning electronic microscopy of larvae of *Sulcascaris sulcata* found in the purple dye murex. Anterior (A, B) and posterior (C) extremities and cuticle (D). Scale bars, A, B, D = 10 μm; C = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article).

Parasites mature and live in the stomach of the host, shedding eggs that disperse in the marine environment with faeces. Eggs hatch in 5 day at 25 °C, releasing third-stage larvae which penetrate the molluscs (bivalves and gastropods) where they develop to fourth-stage larva within 3–4-month post infection (Berry & Cannon, 1981). Successful experimental infections of molluscs occurred by driving the third stage larvae into the inhalant siphon of bivalves and into gastropods. Up to about 55 days after infection, larvae were recovered from the adductor muscle, digestive gland, and gonads of the bivalves *Melina ephippium* and *Pinctada* spp., and from the gonads and digestive gland of the gastropod *Polinices sordidus*. No successful experimental infection occurred when molluscs were exposed to *S. sulcata* eggs at various developmental stages (Berry & Cannon, 1981).

Regarding the dynamic of infection in the wild, since the third-stage larvae after being laid settle on the bottom (almost immobile) and live only a few days (3–4 days at 25 °C; Berry & Cannon, 1981), the infection of the intermediate hosts is strongly associated with local definitive host abundance, being the locality where the specimens of purple dye murex were harvested an important feeding area for large loggerhead turtles, as confirmed by satellite telemetry studies (Luschi et al., 2018; Chimienti et al., 2020). The purple dye murex is a generalist carnivore, able to prey on bivalves and other gastropods, and a carrion feeder that may be attracted by the fecal material of infected sea turtles near which the larvae are settled. Additionally, it has been observed that in marine gastropods coprophagy may be an alternative feeding behaviour progressively adopted at high individual densities because it avoids competition for habitual resources and provides an alternative food source (Lopez & Levinton, 1978; López-Figueroa & Niell, 1988). Moreover, the occurrence of the purple dye murex aggregations around a single food item is common (Martín et al., 1995; Vasconcelos et al., 2008, 2012) and this feeding behaviour may favour the infection of several gastropod individuals with the larvae hatched from the large number of nematode eggs released by a single faecal pellet.

According to the known life history of the parasite, it is plausible that the specimens of purple dye murex became infected by sucking the third stage larvae through their inhalant siphon, taking advantage of the respiratory current directed towards the mantle. The site of localization of larvae in the gastropods here investigated suggests this route of infection since most larvae were found in the mantle or in the cephalopodial organs, which are in close proximity with the mantle. Furthermore, this infection route has been demonstrated in infection experiments in bivalves (Berry & Cannon, 1981).

Regarding the prevalence of infection in the intermediate hosts so far retrieved in the Mediterranean, fourth-stage larvae have been found in 35 out 54 (64.81%) *P. jacobaeus* and 1 out 10 (10%) *A. opercularis* from the Adriatic Sea (Marcer et al., 2020; Pretto et al., 2020), and in 5 of 363 (1.4%) *M. galloprovincialis* from the Tyrrhenian Sea (Santoro et al., 2020). Prevalence of infection is unknown for gastropods naturally infected with larvae of *S. sulcata* from Florida and Australian waters (namely *Busycon canaliculatum*, *Fasciolaria lilium hunteria* and *Pleuroploca gigantea* [Neogastropoda] and *Cipraea* tigris and *Lunatia heros* [Littorinimorpha]) (see Lichtenfels et al., 1980; Santoro et al., 2020), however studies from the same geographical areas reported a variable prevalence in bivalves (i.e., Mactridae, Pectinidae, Pinnidae, and Spondylidae) ranging from 4 to 78% depending by geographical site and timing of sampling (see Lichtenfels et al., 1980). The observed prevalence of infection with infective stages of *S. sulcata* strongly suggests the purple dye murex as intermediate host of this parasite for the loggerhead turtle in the Tyrrhenian Sea and the northern coast of Campania region, a key site where individuals of purple dye murex and loggerhead turtle may become infected. Interestingly, it has been observed that thick shells of gastropods as the purple dye murex as well other species that may serve as intermediate host of *S. sulcata*, able to protect the soft body of the mollusc from most predators, can be only crushed by the robust jaws of larger loggerhead turtle individuals (Bustard, 1972). Indeed, studies on feeding ecology of loggerhead turtles in the Central Mediterranean reported Gastropoda as the second most important taxon retrieved from their stomach (Casale et al., 2008), with the purple dye

murex resulting among the preferred gastropod prey of larger turtles (Casale et al., 2018). This is in agreement with the observation that the infection with *S. sulcata* in the Central Mediterranean occurs in larger loggerhead turtles (Santoro et al., 2010, 2019), which are able to crush the robust shell of the purple dye murex.

The consumption of purple dye murex in Mediterranean countries, as a common edible gastropod, strongly suggests that the infection by an anisakid nematode, here reported for the first time, should be monitored. According to the European Commission's Food and Veterinary Office (European Commission, 2015), the sanitary controls on live gastropods harvested from non-classified production areas from most European countries are absent or inappropriate, while the method for the assessment of the viability of marketing gastropods are based exclusively on the external visual inspection, and the toxicological and microbiological parameters used for bivalves (European Commission, 2004). In the light of the above, it is evident that those methods make it impossible to visualize the larvae in the tissues of the gastropod under the shell.

In the markets, the purple dye murex is commercialized as live and consumed mainly as cooked, however in few regions of southern Italy this gastropod is consumed as marinated raw seafood. The presence of anisakid larvae compromises the quality and safety of fishery products, representing a cause of concern for consumers, official control authorities and seafood businesses (D'Amico et al., 2014; Bao et al., 2017, 2019). Although the results of experimental infections on homoeothermic species (cat and chicken) minimised the possibility of infection in humans (Berry & Cannon, 1981), it cannot be excluded that, as for other anisakid species (namely *Anisakis simplex* (s.s.) and *A. pegreffii*), thermostable proteins (allergens), responsible for IgE-hypersensitization in the immune response of the accidental human hosts, may pose a risk for sensitized consumers after consumption of seafood infected with dead larvae, even when it has been properly cooked or previously frozen (Nieuwenhuizen & Lopata, 2013; Bao et al., 2017; Caballero & Moneo, 2004; Mattiucci et al., 2017; Palomba et al., 2019).

In absence of specific parasite sanitary inspections on marine gastropods, corrective measures should be applied to avoid that snails infected with viable anisakid larvae arrive to the consumer. The scope of such measures would include both the reduction of potential risk of zoonosis and the control of the perception of the problem by the consmers. In fact, although the larvae can lose their pathogenicity through freezing and/or cooking, the products may be unsuitable for sale because of the repellent aesthetical impact they could have on the consumer, that could discourage any further consumption of that seafood product (D'Amico et al., 2014; Bao et al., 2017, 2019). A possible suggestion to seafood industry could be the marketing of the gastropods without shells permitting to visualize the edible tissues; in addition, the retailers could perform a deferred evisceration at the time of retail as suggested for fishes infected with anisakid larvae (D'Amico et al., 2014). Moreover, based on the general food law concept that all food must be safe and free from harmful substances or potential zoonotic pathogens, the harvest of edible gastropods from the sites known as feeding areas of sea turtles should be at least avoided.

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Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Mario Santoro: Conceptualization, Methodology, Validation, Supervision, Formal analysis, Investigation, Writing – original draft. **Marialetizia Palomba:** Methodology. **Maria Vittoria Modica:** Methodology, Writing – review & editing.

References

Ángeles-Hernández, J. C., Gómez-de Anda, F. R., Reyes-Rodríguez, N. E., Vega-Sánchez, V., García-Reyna, P. B., Campos-Montiel, R. G., Calderón-Apodaca, N. L., Salgado-Miranda, C., & Zepeda-Velázquez, A. P. (2020). Genera and species of the Anisakidae family and their geographical distribution. *Animals, 10*. https://doi.org/10.3390/ani10122374. Article 2374.

Arizono, N., Miura, T., Yamada, M., Tegoshi, T., & Onishi, K. (2011). Human infection with *Pseudoterranova azarasi* roundworm. *Emerging Infectious Diseases, 17*, 555–556. https://doi.org/10.3201/eid1703.101350

Bao, M., Pierce, G., Pascual, S., González-Muñoz, M., Mattiucci, S., Mladineo, I., Cipriani, P., Bušelić, I., & Strachan, N. J. C. (2017). Assessing the risk of an emerging zoonosis of worldwide concern: Anisakiasis. *Scientific Reports, 7*. https://doi.org/10.1038/srep43699. Article 43699.

Bao, M., Pierce, G. J., Strachan, N. J. C., Pascual, S., González-Muñoz, M., & Levsen, A. (2019). Human health, legislative and socioeconomic issues caused by the fish-borne zoonotic parasite *Anisakis*: Challenges in risk assessment. *Trends in Food Science & Technology, 86*, 298–310. https://doi.org/10.1016/j.tifs.2019.02.013

Berry, G. N., & Cannon, L. R. G. (1981). The life history of *Sulcascaris sulcata* (Nematoda: Ascaridoidea), a parasite of marine molluscs and turtles. *International Journal for Parasitology, 11*, 43–54.

Bush, A. O., Lafferty, K. D., Lotz, J. M., & Shostak, W. (1997). Parasitology meets ecology on its own terms: Margolis et al. Revisited. *The Journal of Parasitology, 83*, 575–583. https://doi.org/10.2307/3284227

Bustard, H. R. (1972). *Sea Turtles. Their natural history and conservation*. London: Collins.

Caballero, M. L., & Moneo, I. (2004). Several allergens from *Anisakis simplex* are highly resistant to heat and pepsin treatments. *Parasitology Research, 93*, 248–251. https://doi.org/10.1007/s00436-004-1099-3

Casale, P., Abbate, G., Freggi, D., Conte, N., Oliverio, M., & Argano, R. (2008). Foraging ecology of loggerhead sea turtles *Caretta caretta* in the central Mediterranean Sea: Evidence for a relaxed life history model. *Marine Ecology Progress Series, 372*, 265–276.

Casale, P., Broderick, A. C., Camiñas, J. A., Cardona, L., Carreras, C., Demetropoulos, A., Fuller, W. J., Godley, B. J., Hochscheid, S., Kaska, Y., Lazar, B., Margaritoulis, D., Panagopoulou, A., Rees, A. F., Tomás, J., & Türkozan, O. (2018). Mediterranean Sea turtles: Current knowledge and priorities for conservation and research. *Endangered Species Research, 36*, 229–267. https://doi.org/10.3354/esr00901

Chimienti, M., Blasi, M. F., & Hochscheid, S. (2020). Movement patterns of large juvenile loggerhead turtles in the Mediterranean Sea: Ontogenetic space use in a small ocean basin. *Ecology and Evolution, 10*, 6978–6992. https://doi.org/10.1002/ece3.6370

D'amico, P., Malandra, R., Costanzo, F., Castigliego, L., Guidi, A., Gianfaldoni, D., & Armani, A. (2014). Evolution of the *Anisakis* risk management in the European and Italian context. *Food Research International, 64*, 348–362. https://doi.org/10.1016/j.foodres.2014.06.038

European Commission. (2004). Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for the hygiene of foodstuffs, 853/2004/CE. *Official Journal, L 139/55, 30/04/2004*. Retrieved from http://data.europa.eu/eli/reg/2004/853/2021-01-01. (Accessed 16 June 2021).

European Commission. (2015). Overview report bivalve molluscs. In *DG health and food safety*. Publications Office of the European Union. https://doi.org/10.2772/64443.

Gentile, A., Amato, T., Gustinelli, A., Fioravanti, M. L., Gambino, D., Randazzo, V., Caracappa, G., Vicari, D., & Arculeo, M. (2021). Helminth infection of the loggerhead sea turtle *Caretta caretta* along the coasts of sicily and the north- west Adriatic Sea. *Animals, 11*. https://doi.org/10.3390/ani11051408. Article 1408.

Gračan, R., Bursic, M., Mladineo, I., Kučinić, M., Lazar, B., & Lacković, G. (2012). Gastrointestinal helminth community of loggerhead sea turtle *Caretta Caretta* in the Adriatic Sea. *Diseases of Aquatic Organisms, 99*, 227–236. https://doi.org/10.3354/dao02490

Houart, R. (2001). *A review of the recent mediterranean and North-eastern Atlantic species of muricidae*. Rome: Evolver.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution, 16*, 111–120.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). Mega X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*, 1547–1549. https://doi.org/10.1093/molbev/msy096

Lauckner, G. (1980). Diseases of Mollusca: Gastropoda. In O. Kinne (Ed.), *Diseases of marine animals* (pp. 311–400). John Wiley & Sons.

Lichtenfels, J. R., Bier, J. W., & Madden, P. A. (1978). Larval anisakid (*Sulcascaris*) nematodes from Atlantic molluscs with marine turtles as definitive hosts. *Transactions of the American Microscopical Society, 97*, 199–207.

Lichtenfels, J. R., Sawyer, T. K., & Miller, G. C. (1980). New hosts for larval *Sulcascaris* sp. (Nematoda, Anisakidae) and prevalence in the calico scallop (*Argopecten gibbus*). *Transactions of the American Microscopical Society, 99*, 448–451. https://doi.org/10.2307/3225656

López-Figueroa, F., & Niell, F. X. (1988). Feeding behaviour of *Hydrobia ulvae* (Pennant) in microcosms. *Journal of Experimental Marine Biology and Ecology, 114*, 153–167.

Lopez, G. R., & Levinton, J. S. (1978). The availability of microorganisms attached to sediment particles as food for *Hydrobia ventrosa* Montagu (Gastropoda, Prosobranchia). *Oecologia, 32*, 263–375.

Luschi, P., Mencacci, R., Cerritelli, G., Papetti, L., & Hochscheid, S. (2017). Large-scale movements in the oceanic environment identify important foraging areas for

loggerheads in central Mediterranean Sea. *Marine Biology, 165*. https://doi.org/10.1007/s00227-017-3255-1. Article 4.

Marcer, F., Tosi, F., Franzo, G., Vetri, A., Ravagnan, S., Santoro, M., & Marchiori, E. (2020). Updates on ecology and life cycle of *Sulcascaris sulcata* (Nematoda: Anisakidae) in mediterranean grounds: Molecular identification of larvae infecting edible scallops. *Frontiers in Veterinary Science, 7*. https://doi.org/10.3389/fvets.2020.00064. Article 64.

Martín, P., Sánchez, P., & Ramón, M. (1995). Population structure and exploitation of *Bolinus brandaris* (Mollusca: Gastropoda) off the Catalan coast (northwestern Mediterranean). *Fisheries Research, 23*, 319–331.

Mattiucci, S., Cipriani, P., Levsen, A., Paoletti, M., & Nascetti, G. (2018). Molecular epidemiology of *Anisakis* and anisakiasis: An ecological and evolutionary road map. *Advances in Parasitology, 99*, 93–263. https://doi.org/10.1016/bs.apar.2017.12.001

Mattiucci, S., Colantoni, A., Crisafi, B., Mori-Ubaldini, F., Caponi, L., Fazii, P., Nascetti, G., & Bruschi, F. (2017). IgE sensitization to *Anisakis pegreffii* in Italy: Comparison of two methods for the diagnosis of allergic anisakiasis. *Parasite Immunology, 39*(7). https://doi.org/10.1111/pim.12440. Article e12440.

Morgulis, A., Coulouris, G., Raytselis, Y., Madden, T. L., Agarwala, R., & Schäffer, A. A. (2008). Database indexing for production MegaBLAST searches. *Bioinformatics, 24*, 1757–1764. https://doi.org/10.1093/bioinformatics/btn322

Nadler, S. A., & Hudspeth, D. S. (2000). Phylogeny of the Ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: Hypotheses of structural and sequence evolution. *The Journal of Parasitology, 86*, 380–393. https://doi.org/10.1645/0022-3395

Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press.

Nieuwenhuizen, N. E., & Lopata, A. L. (2013). *Anisakis* – a food-borne parasite that triggers allergic host defences. *International Journal for Parasitology, 43*, 1047–1057. https://doi.org/10.1016/j.ijpara.2013.08.001

Palomba, M., Paoletti, M., Colantoni, A., Rughetti, A., Nascetti, G., & Mattiucci, S. (2019). Gene expression profiles of antigenic proteins of third stage larvae of the zoonotic nematode *Anisakis pegreffii* in response to temperature conditions. *Parasite, 26*. https://doi.org/10.1051/parasite/2019055. Article 52.

Poppe, G. T., & Goto, Y. (1991). *European seashells, polyplacophora, claudofoveata, Solenogastra, Gastropoda)*. Wiesbaden: Verlag Christa Hemmen.

Pretto, T., Vetri, A., Tosi, F., Ravagnan, S., Michelutti, A., Kapllan, S., Quaglio, F., & Arcangelli, G. (2020). Larval stages of *Sulcascaris sulcata* (Nematoda: Anisakidae) in scallops from the northern Adriatic Sea: Implications for seafood control and surveillance. *Italian Journal of Food Science, 32*(4). https://doi.org/10.14674/IJFS.1893. Article 769.

Sabelli, B., Giannuzzi-Savelli, R., & Bedulli, D. (1990). *Catalogo annotato dei molluschi marini del Mediterraneo*. Bologna: Edizioni Libreria Naturalistica Bolognese.

Santoro, M., Badillo, F. J., Mattiucci, S., Nascetti, G., Bentivegna, F., Insacco, G., Travaglini, A., Paoletti, M., Kinsella, J. M., Tomás, J., Raga, J. A., & Aznar, F. J. (2010). Helminth communities of loggerhead turtles (*Caretta Caretta*) from central and Western Mediterranean Sea: The importance of host's ontogeny. *Parasitology International, 59*, 367–375. https://doi.org/10.1016/j.parint.2010.04.009

Santoro, M., Marchiori, E., Iaccarino, D., Degli Uberti, B., Cassini, R., Di Nocera, F., Cerrone, A., Galiero, G., & Marcer, F. (2019). Epidemiology of *Sulcascaris sulcata* (Nematoda: Anisakidae) ulcerous gastritis in the Mediterranean loggerhead sea turtle (*Caretta caretta*). *Parasitology Research, 118*, 1457–1463. https://doi.org/10.1007/s00436-019-06283-0

Santoro, M., Marchiori, E., Palomba, M., Uberti, B. D., Marcer, F., & Mattiucci, S. (2020). The Mediterranean mussel (*Mytilus galloprovincialis*) as intermediate host for the anisakid *Sulcascaris sulcata* (Nematoda), a pathogen parasite of the Mediterranean loggerhead turtle (*Caretta caretta*). *Pathogens, 9*. https://doi.org/10.3390/pathogens9020118. Article 118.

Sey, O. (1977). Examination of helminth parasites of marine turtles caught along the Egyptian coast. *Acta Zoologica Academiae Scientiarum Hungaricae, 23*, 387–394.

Sprent, J. F. A. (1977). Ascaridoid nematodes of amphibians and reptiles: *Sulcascaris*. *Journal of Helminthology, 51*, 379–387.

Valentini, A., Mattiucci, S., Bondanelli, P., Webb, S. C., Mignucci-Giannone, A. A., Colom-Llavina, M. M., & Nascetti, G. (2006). Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *cox*2 sequences, and comparison with allozyme data. *The Journal of Parasitology, 92*, 156–166. https://doi.org/10.1645/GE-3504.1

Vasconcelos, P., Carvalho, S., Castro, M., & Gaspar, M. B. (2008). The artisanal fishery for muricid gastropods (banded murex and purple dye murex) in the Ria Formosa lagoon (Algarve coast, southern Portugal). *Scientia Marina, 72*, 287–298.

Vasconcelos, P., Moura, P., Barroso, C. M., & Gaspar, M. B. (2012). Reproductive cycle of *Bolinus brandaris* (Gastropoda: Muricidae) in the Ria Formosa lagoon (southern Portugal). *Aquatic Biology, 16*, 69–83. https://doi.org/10.3354/ab00434

Werneck, M. R., Martos Thomazini, C., Mori, E. Shigueru, Gonçalves, V. T., Gallo, B. M. G., & Da Silva, R. J. (2008). Gastrointestinal helminth parasites of loggerhead turtle *Caretta caretta* Linnaeus, 1758 (Testudines, Cheloniidae) in Brazil. *Pan-American Journal of Aquatic Sciences, 3*, 351–354. https://doi.org/10.1016/s0020-7519(98)00150-7

World Health Organization. (2012). Soil-transmitted Helminths. World Health Organization. Retrieved from http://www.who.int/intestinal_ worms/en/. (Accessed 1 April 2021).

Zhu, X., Gasser, R. B., Podolska, M., & Chilton, N. (1998). Characterisation of anisakid nematodes with zoonotic potential by nuclear ribosomal DNA sequences. *International Journal for Parasitology, 28*, 1911–1921. https://doi.org/10.1016/S0020-7519(98)00150-7