

# Helminth parasites of the dwarf sperm whale *Kogia sima* (Cetacea: Kogiidae) from the Mediterranean Sea, with implications on host ecology

M. Santoro<sup>1,\*</sup>, F. Di Nocera<sup>1</sup>, D. Iaccarino<sup>1</sup>, P. Cipriani<sup>2,3</sup>, I. Guadano Procesi<sup>4</sup>,  
F. Maffucci<sup>5</sup>, S. Hochscheid<sup>5</sup>, C. Blanco<sup>6</sup>, A. Cerrone<sup>1</sup>, G. Galiero<sup>1</sup>, G. Nascetti<sup>4</sup>,  
S. Mattiucci<sup>2</sup>

<sup>1</sup>Istituto Zooprofilattico Sperimentale del Mezzogiorno, 80055 Portici, Italy

<sup>2</sup>Department of Public Health and Infectious Diseases, Section of Parasitology, Sapienza University of Rome, 00185 Rome, Italy

<sup>3</sup>Institute of Marine Research (IMR), Norder, 5817 Bergen, Norway

<sup>4</sup>Department of Ecological and Biological Sciences, Tuscia University, 01100 Viterbo, Italy

<sup>5</sup>Marine Turtle Research Center, Stazione Zoologica Anton Dohrn, 80055 Portici, Italy

<sup>6</sup>Unitat de Zoologia Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València, 46071 Valencia, Spain

**ABSTRACT:** Limited data exist on the occurrence of the dwarf sperm whale *Kogia sima* in the Mediterranean Sea and its parasite fauna. Here, the occurrence of the anisakid species *Anisakis physeteris* and *A. pegreffii* in the stomach chambers of an adult female dwarf sperm whale, stranded in southern Italy, is reported. In addition, the occurrence of *Phyllobothrium delphini* larvae infecting the blubber of the caudal peduncle region was recorded. *A. physeteris* and *A. pegreffii* represent the 2 parasite species of the genus, mostly distributed in the Mediterranean Sea in fish and squids. The finding of *A. pegreffii* and *A. physeteris* in the dwarf sperm whale represents a new record in this host species for the Mediterranean Sea. The study of gastrointestinal content also revealed a massive presence of cephalopod beaks identified as belonging to pelagic squids including the umbrella squid *Histioteuthis bonnellii*, the reverse jewel squid *H. reversa*, the long-armed squid *Chiroteuthis veranii*, and the comb-finned squid *Ctenopteryx sicula*. The feeding habits of the dwarf sperm whale, as well as the occurrence of these squid residuals in the cetacean host, suggest that these squid species play a major role in maintaining the life cycle of anisakid parasite species and *P. delphini*.

**KEY WORDS:** *Kogia sima* · Mediterranean Sea · *Anisakis physeteris* · *Anisakis pegreffii* · *Phyllobothrium delphini* · Squid beaks · Host ecology

Resale or republication not permitted without written consent of the publisher

## INTRODUCTION

The genus *Kogia* comprises 2 cetacean species, the pygmy sperm whale *Kogia breviceps* de Blainville, 1838 and the dwarf sperm whale *Kogia sima* Owen, 1866, which were not recognized as separate species until the mid 1960s (Taylor et al. 2012). The dwarf sperm whale appears to be distributed widely in off-

shore waters of tropical and warm temperate zones and inhabits shelf-edge and slope waters, where it primarily feeds on deep-water cephalopods (Taylor et al. 2012). In the Mediterranean Sea, the only records of kogiid species in the past are limited to 2 stranded individuals of the dwarf sperm whale, both from Italian waters (Baccetti et al. 1991, Bortolotto et al. 2003). The first refers to a decomposed carcass

\*Corresponding author: mario.santoro@izsmportici.it

found at the Foce Chiarone located between the Latium and Tuscany regions (Tyrrhenian Sea) (Baccetti et al. 1991). The second was a male found on the western coast of Sicily in Eraclea Minoa (Agrigento) (Bortolotto et al. 2003). In both cases, there is no evidence that parasites and/or gastrointestinal content were studied, nor was a genetic analysis of the host performed. Here for the first time, records on the parasites, gastrointestinal food contents, and the genetic identification of a dwarf sperm whale stranded in the Mediterranean Sea in 2017 are presented.

## MATERIALS AND METHODS

### Sampling

An adult female of *Kogia* sp., weighing 116 kg and measuring 195 cm in total length, was found stranded on the beach of Trentova (Agropoli) (40° 20' 56" N, 14° 58' 29" E) in the Salerno province of southern Italy on 4 February 2017. The whale was in a decomposition grade code 3 and showed good nutritional status. During necropsy, blubber, heart, blood vessels, trachea, lungs, urinary bladder, liver, gallbladder, kidneys, pancreas, uterus, oesophagus, stomach chamber, and intestine were examined for helminths. Organs and tissues were opened and surfaces examined visually, then washed through a 100 µm mesh screen. The remaining washed material from each organ was examined carefully under a dissecting microscope, and any helminths were collected and rinsed in saline solution. The adult, L4 larval and pre-adult forms of *Anisakis* spp. were identified to the genus level on the basis of morphological characters (Mattiucci et al. 2014, 2018). Anisakid nematodes were counted and stored at –80°C for molecular identification. Cestode larvae were preserved in 70% alcohol before identification under the light microscope following Agustí et al. (2005). Gastrointestinal content was collected in order to examine and identify any remaining prey items. Cephalopod beaks were recovered from stomach and intestine and identified following the methods used by Clarke (1986) and Xavier & Cherel (2009).

Samples for histological examination of all organs and tissues examined for parasites were fixed in 10% neutral phosphate-buffered formalin and processed by routine methods into paraffin blocks, which were cut into 3 µm thick sections and stained with hematoxylin and eosin. Because dolphin morbillivirus infection (DMV) represents a common cause of ceta-

cean stranding along the Mediterranean coasts (Centelleghes et al. 2017), a brain sample was examined for DMV by RT-PCR restriction fragment length polymorphism (RFLP) according to the methodology used in Verna et al. (2017).

### Host identification

The cetacean was identified to species using the morphometric keys provided by Barros & Duffield (2003) and McAlpine (2009). DNA sequence analysis on a tissue sample of the cetacean was used to confirm the morphological identification. Total genomic DNA was extracted from approximately 25 mg of muscle with a NucleoSpin® Tissue kit (Machery-Nagel, Düren, Germany) following the manufacturer's recommendations. A ~700 bp fragment was amplified from the cytochrome *b* gene (*cytb*), one of the most commonly used markers for DNA-based species identification in cetaceans (Viricel & Rosel 2012), using primer pairs L14724 and H15387 (Palumbi et al. 2002). PCR reactions were carried out in 50 µl volumes using the following conditions: initial denaturation at 95°C for 5 min followed by 34 cycles of 95°C for 60 s, 49°C for 60 s, and 72°C for 60 s, followed by a final extension of 72°C for 7 min. A negative control (template-free PCR reactions) was used to test for contamination. PCR products were checked by agarose gel electrophoresis and purified using a High Pure PCR Product Purification Kit (Roche Diagnostic).

Sequence reactions were obtained with the BigDye Terminator Cycle Sequencing technology (Applied Biosystems), purified in automation using the Agencourt CleanSEQ Dye terminator removal kit (Agencourt Bioscience Corporation), and a robotic station Biomek FX (Beckman Coulter). Products were analyzed on an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, ThermoFisher Scientific). Forward and reverse chromatograms were analysed and assembled using the software package SeqManII (DNASTAR). The resulting sequence was used to perform a BLAST® search (Altschul et al. 1990) in the GenBank database ([www.ncbi.nlm.nih.gov/blast/](http://www.ncbi.nlm.nih.gov/blast/)).

### Genetic/molecular identification of *Anisakis* spp.

A total of 108 *Anisakis* spp. specimens were identified to the species level by a multi-marker genotyping approach. The length of each frozen nema-

tode was first measured; cephalic and caudal ends were preserved in 70% alcohol, while a portion of tissue of the nematode was used to perform genetic/molecular identification. The last included both nuclear and mitochondrial markers: i.e. 3 diagnostic allozyme loci (Mattiucci et al. 2009), DNA sequences analysis of mitochondrial (mtDNA) cytochrome C oxidase subunit II [*cox2*], 629 bp (Mattiucci et al. 2014) and nuclear (elongation factor EF1  $\alpha$ -1 of nDNA, 409 bp) genes (Mattiucci et al. 2016). The EF1  $\alpha$ -1 of nDNA was analysed as a further nuclear marker to detect the possible occurrence of hybrid genotypes between *A. pegreffii* and *A. simplex* (s.s.), as indicated in Mattiucci et al. (2016). The diagnostic allozyme loci (*Adk-2*, *Pep C-1*, and *Pep C-2*) were analysed, according to established procedures (see Mattiucci et al. 2001), on 108 *Anisakis* spp. specimens.

The total DNA was extracted using the Quick-gDNA MiniPrep (column format) by Zymo Research from 2 mg of homogenized tissue from each nematode following the manufacturer's protocol (Levsen et al. 2018). The mitochondrial *cox2* gene was amplified using the primers 211F (5'-TTT TCT AGT TAT ATA GAT TGR TTY AT-3') and 210R (5'-CAC CAA CTC TTA AAA TTA TC-3') (Mattiucci et al. 2014). PCR was carried out according to the previously described procedures (Mattiucci et al. 2014). The sequences obtained at the mtDNA *cox2* locus for the sequenced nematodes were compared with those in GenBank: *A. simplex* (s.s.) (DQ116426), *A. pegreffii* (DQ116428), *A. berlandi* (KC809999), *A. typica* (DQ116427), *A. ziphidarum* (DQ116430), *A. nascettii* (FJ685642), *A. physeteris* (DQ116432), *A. brevispiculata* (DQ116433), and *A. paggiae* (DQ116434).

The EF1  $\alpha$ -1 nuclear gene was amplified using the primers EF-F (5'-TCC TCA AGC GTT GTT ATC TGT T-3') and EF-R (5'-AGT TTT GCC ACT AGC GGT TCC-3') (Mattiucci et al. 2016). The PCR procedures followed those reported in Mattiucci et al. (2016). In particular, because the primers at that locus are to date only available for species of the *A. simplex* (s.l.) complex, the sequencing of the EF1  $\alpha$ -1 gene of the nDNA was carried out on only those 18 specimens of *A. pegreffii* previously identified based on the allozyme diagnostic loci and mtDNA *cox2* locus (see 'Results'). The sequences obtained at the EF1  $\alpha$ -1 locus were then compared at the diagnostic positions (i.e. 186 and 286) with those previously analysed and deposited in GenBank (Mattiucci et al. 2016).

A Bayesian inference (BI) tree, inferred from the mtDNA *cox2* gene sequences obtained, was built in relation to the *cox2* gene sequences previously se-

quenced at the same locus from other *Anisakis* spp. The analysis was performed using MrBayes 3.1 (Huelsenbeck & Ronquist 2005) with the TRN+I+G substitution model as implemented in jModelTest 2.1 (Darriba et al. 2012). The parameters for the selected model were I = 0.487 and G = 0.783, chosen with Akaike's information criterion (AIC) (Posada & Buckley 2004). For the Bayesian analysis, 4 incrementally heated Markov Chains (using default heating values) were run for 1 000 000 generations, sampling the Markov Chains at intervals of 100 generations. The *burninfrac* was fixed at 0.25. Posterior probabilities were estimated and used to assess support for each branch in the inferred phylogeny, where p = 95% is indicative of significant support (Reeder 1995); *Toxocara canis* and *Ascaris suum* were used as outgroups.

## RESULTS

### Sampling

A total of 1348 anisakid specimens were collected from the stomach chambers (Fig. 1). The adult anisakid specimens were first assigned morphologically to the genus *Anisakis*; some nematodes were L4 or pre-adult stages, with a mean ( $\pm$  SD) length of 19.5  $\pm$  5.6 (range: 10.0–32.0) mm. A total of 43 cestode larvae (merocercoid) found in the blubber of the caudal peduncle region were morphologically identified as *Phyllobothrium delphini*.

More than 100 cephalopod beaks were recovered from the whale's stomach (Fig. 1) and intestine, but just 15 of them were sufficiently well preserved for species identification. Beaks were identified as belonging to 4 species of pelagic squid including the umbrella squid *Histioteuthis bonnellii* (N = 4), the reverse jewel squid *H. reversa* (N = 9), the long-armed squid *Chiroteuthis veranii* (N = 1), and the comb-finned squid *Ctenopteryx sicula* (N = 1). Mediterranean seagrass *Posidonia oceanica* was also found in the stomach chambers (Fig. 1). No significant gross or microscopic pathological findings were seen in the carcass or sectioned tissues. No obvious specific cause of death was noticed. Results of the RT-PCR RFLP analysis of the brain for detection of DMV infection were negative.

### Host identification

The provisional identification of the stranded cetacean as a dwarf sperm whale using morphologi-

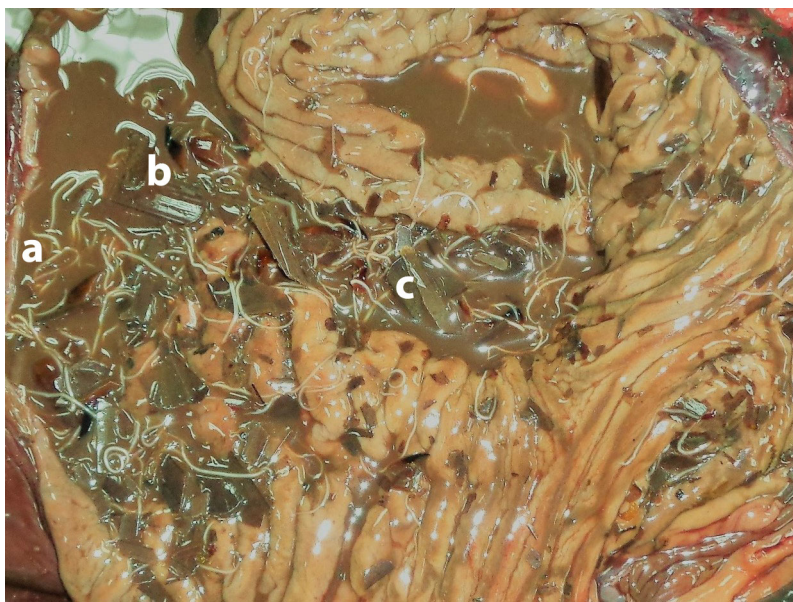


Fig. 1. Stomach of the dwarf sperm whale *Kogia sima* showing numerous *Anisakis* spp. individuals (a) and squid beaks (b). Remains of Mediterranean sea grass *Posidonia oceanica* (c) are also visible

cal diagnostic features was confirmed by the DNA sequencing approach. BLAST analysis of the *cytb* sequence obtained (accession number: MG252607) provided the highest match of 100 % over 596 bp to *Kogia sima* voucher SEFSC (accession number: EU517708).

#### Genetic/molecular identification of *Anisakis* spp.

Of the 108 *Anisakis* spp. specimens, 18 (16.6 %) (all L4 or pre-adult stages) were genetically identified as *A. pegreffii* according to the alleles observed at the diagnostic loci, i.e. *Adk-2*<sup>100</sup>, *Pep C-1*<sup>100</sup>, and *Pep C-2*<sup>100</sup>; while, based on the genotypes observed at the same loci, i.e. *Adk-2*<sup>97</sup>, *Pep C-1*<sup>110</sup>, and *Pep C-2*<sup>108</sup>, 90 specimens (83.3 %) (all adult stages) were identified as belonging to the species *A. physeteris*.

Additionally, the mtDNA *cox2* sequences of those 90 specimens identified as *A. physeteris* by allozyme loci matched, 100 or 99 % those sequences available in GenBank for that parasite species, at the same locus. Analogously, the 18 mtDNA *cox2* sequences of those individuals identified by allozyme loci as *A. pegreffii* were a 100 % match of *A. pegreffii* sequences, previously deposited in GenBank. Eight sequences of mtDNA *cox2* were deposited in GenBank with the accession numbers: MG076944, MG076945, MG076946, MG076947 for *A. pegreffii*;

and MG076948, MG076949, MG076950, MG076951 for *A. physeteris*.

Finally, the 18 *Anisakis* spp. specimens identified as *A. pegreffii* by allozyme diagnostic loci and mtDNA *cox2* were also sequenced at a further nuclear locus, i.e. the partial sequence of the EF1  $\alpha$ -1 gene that confirmed the homozygote genotypes of *A. pegreffii*. The 4 EF1  $\alpha$ -1 of nDNA gene sequences we obtained from *A. pegreffii* were deposited in GenBank (accession numbers: MG076940, MG076941, MG076942, MG076943).

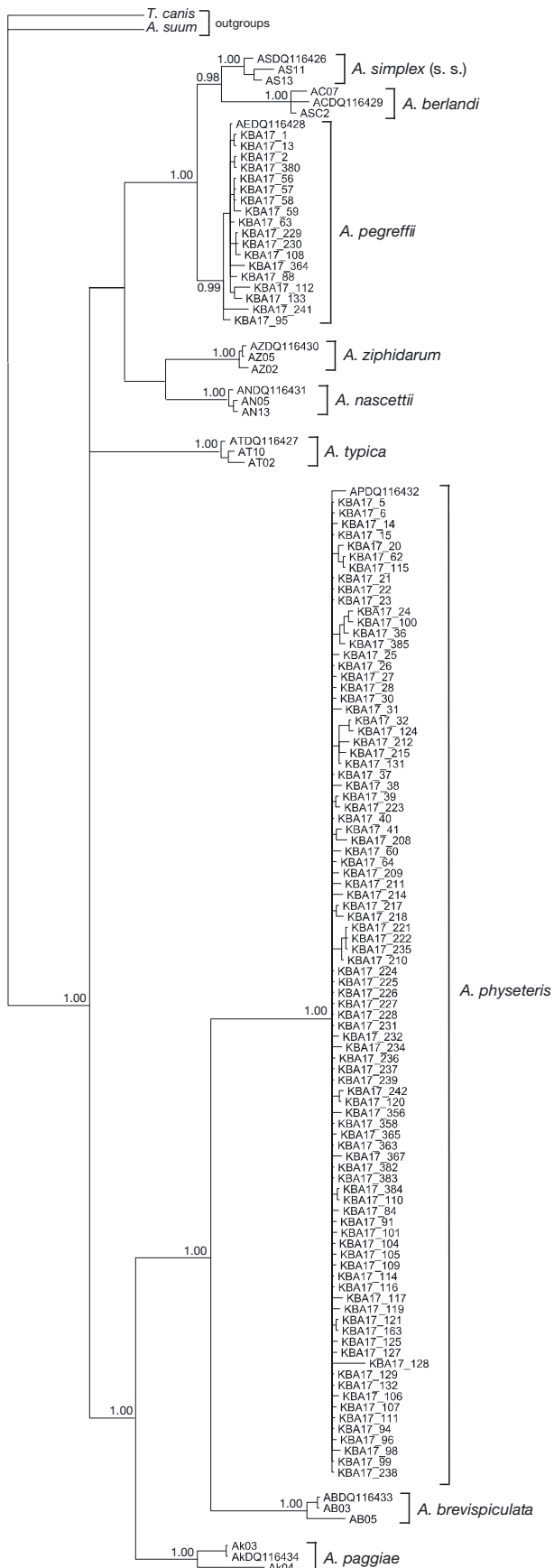
Phylogenetic analysis inferred from BI (Fig. 2) showed that the 90 *A. physeteris* sequences and the 18 sequences of *A. pegreffii* obtained here clustered in 2 distinct and well supported phylogenetic clades, also including the previously deposited sequences of these species (Fig. 2).

#### DISCUSSION

This is the first parasitological study of a dwarf sperm whale stranded in the Mediterranean Sea. To date, 9 species belonging to the genus *Anisakis* have been identified worldwide. These species possess distinct gene pools and are reproductively isolated, as demonstrated by means of nuclear markers (allozyme data) (see Mattiucci et al. 2018). The existence of 9 species as distinct phylogenetic units has been demonstrated by various phylogenetic analyses, as inferred from both nuclear and mitochondrial genes (Mattiucci & Nascetti 2008, Cavallero et al. 2011, Mattiucci et al. 2014, 2018). The nuclear and mitochondrial genetic/molecular markers used in the present study allowed the identification of the 2 species *A. physeteris* and *A. pegreffii* from the dwarf sperm whale.

Previous reports on the detection of *Anisakis* spp. in the dwarf sperm whale are scattered throughout its host range. By means of genetic/molecular tools, the dwarf sperm whale has been indeed recognized, outside the Mediterranean basin, as the main host of *A. brevispiculata* and *A. paggiae* (Mattiucci et al. 2005, 2018, Mattiucci & Nascetti 2008); while, occasionally, adult specimens of *A. typica*, *A. physeteris*, *A. ziphidarum* (Cavallero et al. 2011, Klimpel & Palm 2011, Quiazon et al. 2013, Kuhn et al. 2016, Di Azevedo et





al. 2017), and L4 of *A. berlandi* (Shamsi et al. 2012) were also identified. For instance, *A. physeteris* in the dwarf sperm whale has been reported from the Atlantic coast of the USA (Cavallero et al. 2011). Thus, the finding of *A. pegreffii* and *A. physeteris* in this cetacean is a new host record for the Mediterranean Sea.

Knowledge concerning cetacean distribution and ecology in the Mediterranean Sea is limited. Therefore, any information on a cetacean species, including its parasite fauna, may help elucidate its ecology. Identification of anisakid species from a given host provides useful insights into the geographical distribution, definitive host preference and life cycles of genetically identified species of the genus *Anisakis* (Mattiucci & Nascetti 2008, Mattiucci et al. 2009, 2014). Indeed, the life cycles of *Anisakis* spp. involve crustaceans, fish, and squid as intermediate/paratenic hosts and marine mammals as definitive hosts (Mattiucci & Nascetti 2008, Klimpel & Palm 2011). Sea turtles and birds are also reported as accidental hosts, being infected when ingesting fish hosts harbouring larval stages of *Anisakis* spp. (Santoro et al. 2010a,b, Shamsi et al. 2017). A host–parasite association likely resulting from co-evolutionary processes between the parasite *A. physeteris* and its main host, the sperm whale *Physeter macrocephalus*, has been previously suggested (Mattiucci & Nascetti 2008). Also, a host–parasite association between the sperm whales of the family Kogiidae (i.e. *Kogia breviceps* and *K. sima*) and the other 2 species of the *A. physeteris* (s.l.) complex (i.e. *A. brevispiculata* and *A. paggiae*) has been postulated (Mattiucci & Nascetti 2008). In the Mediterranean Sea, several hundred *A. physeteris* have been observed in a few stranded sperm whales *P. macrocephalus* (Mazzariol et al. 2011, Mattiucci et al. 2018), whose gastric contents also included several hundreds of cephalopod beaks belonging to the same squid species identified in the present study. Additionally, *A. physeteris*, like other larval type II *Anisakis* species of the ‘*physeteris* clade’, may use squids rather than fishes as primary intermediate/paratenic hosts (Mattiucci & Nascetti 2008, Mattiucci et al. 2018). Angelucci et al. (2011) found a mixed infection of *A. pegreffii* and *Anisakis* type II larvae (presumably *A. physeteris*) in the flying squid *Todarodes sagittatus* in the waters off Sardinia,

Fig. 2. Bayesian inference tree based on mtDNA *cox2* gene sequences of *A. physeteris* and *A. pegreffii* collected from dwarf sperm whale, shown in relation to other known *Anisakis* spp. sequences

Mediterranean Sea. *Anisakis* type II larvae, in mixed infection with type I larvae, were also found in the Humboldt squid *Dosidicus gigas* off the Chilean coast (Pardo-Gandarillas et al. 2009) and in some Argentine shortfin squid *Illex argentinus* samples from Falkland waters (P. Cipriani unpubl. data). Therefore, the finding of adult *A. physeteris* in a dwarf sperm whale suggests that this nematode species is adapted to host species belonging to the Physeteroidea clade. As the ecology of the dwarf sperm whale in the Mediterranean Sea is similar to that of the sperm whale, infection with *A. physeteris* may have been acquired by preying upon those squid species (Spitz et al. 2011) that occur in the deeper water layers of the southern Tyrrhenian Sea, as well as in other basin waters of the Mediterranean Sea (Bello 2008, Romeo et al. 2012). Further, in 2 sperm whales stranded in successive events during 2011 and 2013 along the Adriatic coast (Mazzariol et al. 2011, S. Mattiucci & P. Cipriani unpubl. data), several hundred *A. physeteris* was found and, interestingly, also a very high number of beaks belonging to the same squid species as those identified in the present study (S. Mattiucci & P. Cipriani unpubl. data). The presence of 4 pelagic squid species (umbrella, reverse jewel, long-armed, and comb-finned squids) identified on the basis of their beak morphology, together with a massive presence of *Anisakis* spp. specimens, suggests that these cephalopod species play a role in transmitting the nematodes to the dwarf sperm whale. To date, the recognized intermediate/paratenic squid hosts included only the lesser flying squid *Todaropsis eblanae*, the Angolan flying squid *Todarodes angolensis*, the flying squid, and the southern shortfin squid *Illex coindetii* for *A. pegreffii*; and the umbrella squid, the Humboldt squid, the southern shortfin squid, the flying squid, and the angel squid *Ancistroteuthis lichtensteinii* for *A. physeteris* (Mattiucci et al. 2018).

*A. pegreffii* is the dominant species of its genus in the Mediterranean Sea, being widespread in several pelagic and demersal fish and rarely found in squid species (Mattiucci & Nascetti 2008, Anastasio et al. 2016, Cipriani et al. 2018a,b). In Atlantic waters, the northern limit of its geographical range is the Spanish–Portuguese border (Mattiucci & Nascetti 2008, Klimpel & Palm 2011, Kuhn et al. 2016). Infection by *A. pegreffii* may have been acquired by the dwarf sperm whale through consumption of squid species or fish species such as the Atlantic horse mackerel *Trachurus trachurus* or the European hake *Merluccius merluccius*, which are infected by this parasite in the Mediterranean Sea (Spitz et al. 2011, Mladineo &

Poljak 2014, Blažeković et al. 2015, Cipriani et al. 2018b, Levsen et al. 2018).

The presence of *A. pegreffii* in the stomach chambers of the dwarf sperm whale in our study, and the absence of *A. simplex* (s.s.), whose geographical range is mostly in Atlantic waters, suggests that the dwarf sperm whale had recently been feeding in the Mediterranean Sea. However, the possibility that some of the food remains found in the stomach came from species consumed in Atlantic waters cannot be completely excluded. All the identified specimens of *A. pegreffii* were at L4 or pre-adult stage with a mean length of 19.5 (10.0–32.0) mm, much smaller than the 41.6 (33.0–55.0) mm described for the adult stage of *A. pegreffii* (Mattiucci et al. 2014, 2018). This finding could be explained as a recent infection of the cetacean before it stranded. Alternatively, kogiids may be an unsuitable host for the full development of *A. pegreffii* into the adult reproductive stage, as adult *A. pegreffii* have only been found in oceanic dolphins and baleen whales (Mattiucci et al. 2018), likely a result of host–parasite co-adaptation and co-evolutionary processes (Mattiucci & Nascetti 2008, Mattiucci et al. 2018).

Furthermore, taking into account that cephalopod species are considered as the second intermediate host of *Phyllobothrium delphini*, our data suggest that the recovered squid species from the dwarf sperm whale could also play a role in the life cycle and transmission of this parasite in the Mediterranean Sea. *P. delphini* merocercoids are common in off-shore cetaceans feeding on fish and/or cephalopods (Aznar et al. 2007). Although the life cycle of *P. delphini* has not yet been elucidated, marine mammals may act as intermediate host for this parasite, and large predatory and/or scavenger pelagic sharks are the most likely definitive host (Agustí et al. 2005, Aznar et al. 2007). Since tetraphyllideans are usually found at the plerocercoid stage in fish, cephalopods, and other marine invertebrates, and since dwarf sperm whales feed intensively on cephalopods, it is plausible that the *P. delphini* infection was acquired by the consumption of squid prey.

In conclusion, the species of anisakids found in this study, together with previous stranding events of dwarf sperm whales in the Mediterranean Sea, suggest the existence of a dwarf sperm whale population in this basin. Also, we conclude that *A. physeteris* maintains its life cycle in the Mediterranean Sea by using both physeteroid species as hosts, i.e. the sperm whale (Mazzariol et al. 2011, Mattiucci et al. 2018, S. Mattiucci & P. Cipriani unpubl. data) and the dwarf sperm whale (present study). Further, the mas-

sive presence of *A. physeteris* in this dwarf whale, similar to that previously observed in stranded sperm whales (Mazzariol et al. 2011, Mattiucci et al. 2018, S. Mattiucci & P. Cipriani unpubl. data), coupled with the massive accumulation of squid beaks in the stomach of these cetacean hosts, suggests that the life cycle of the parasite is well established and maintained in the Mediterranean Sea. Thus, the occurrence of *A. physeteris* could be used as an ecological indicator to monitor the stability of trophic webs, which include the above-mentioned physeteriids and squid species, in the Mediterranean Sea ecosystem (Mattiucci & Nascetti 2008).

**Acknowledgements.** We thank Raimondo Pannone of the Molecular Biology and Sequencing Service of the Stazione Zoologica Anton Dohrn, Naples (Italy), for his assistance in the genetic analysis of the dwarf sperm whale sample.

#### LITERATURE CITED

- Agustí C, Aznar FJ, Olson PD, Littlewood DT, Kostadinova A, Raga JA (2005) Morphological and molecular characterization of tetraphyllidean merocercoids (Platyhelminthes: Cestoda) of striped dolphins (*Stenella coeruleoalba*) from the Western Mediterranean. *Parasitology* 130:461–474
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215:403–410
- Anastasio A, Smaldone G, Cacace D, Marrone R and others (2016) Inactivation of *Anisakis pegreffii* larvae in anchovies (*Engraulis encrasicolus*) by salting and quality assessment of finished product. *Food Control* 64:115–119
- Angelucci G, Meloni M, Merella P, Sardù F and others (2011) Prevalence of *Anisakis* spp. and *Hysterothylacium* spp. larvae in teleosts and cephalopods sampled from waters off Sardinia. *J Food Prot* 74:1769–1775
- Aznar FJ, Agustí C, Littlewood DT, Raga JA, Olson PD (2007) Insight into the role of cetaceans in the life cycle of the tetraphyllideans (Platyhelminthes: Cestoda). *Int J Parasitol* 37:243–255
- Baccetti N, Cancelli F, Renieri T (1991) First record of *Kogia simus* (Cetacea, Physeteridae) from the Mediterranean Sea. *Mammalia* 55:152–154
- Barros NB, Duffield DA (2003) Unravelling the mysteries of pygmy and dwarf sperm whales. *Strandings Newsletter of the Southeast US Marine Mammal Stranding Network*. NOAA Tech Memo NMFS-SEFSC-521p
- Bello G (2008) Cephalopoda. *Biol Mar Mediterr* 15:318–322
- Blažeković K, Pleić IL, Đuras M, Gomerčić T, Mladineo I (2015) Three *Anisakis* spp. isolated from toothed whales stranded along the eastern Adriatic Sea coast. *Int J Parasitol* 45:17–31
- Bortolotto A, Papini L, Gili C, Tumino G, Mazzariol S, Pavan G, Cozzi B (2003) First record of a dwarf sperm whale, *Kogia sima* (Owen, 1866) stranded alive along the coasts of Italy. *Proc 31st Symp Eur Assoc Aquat Mamm, Tene-rife*. Centro Studi Cetacei, Pescara
- Cavallero S, Nadler SA, Paggi L, Barros NB, D'Amelio S (2011) Molecular characterization and phylogeny of anisakid nematodes from cetaceans from south eastern Atlantic coasts of USA, Gulf of Mexico, and Caribbean Sea. *Parasitol Res* 108:781–792
- Centelleghé C, Beffagna G, Palmisano G, Franzo G and others (2017) Dolphin Morbillivirus in a Cuvier's beaked whale (*Ziphius cavirostris*), Italy. *Front Microbiol* 8:111
- Cipriani P, Sbaraglia G, Palomba M, Giulietti L and others (2018a) *Anisakis pegreffii* (Nematoda: Anisakidae) in European anchovy *Engraulis encrasicolus* from the Mediterranean Sea: fishing ground as a predictor of parasite distribution. *Fish Res* 202:59–68
- Cipriani P, Sbaraglia G, Paoletti M, Giulietti L and others (2018b) The Mediterranean European hake, *Merluccius merluccius*: detecting drivers influencing the *Anisakis* spp. larvae distribution. *Fish Res* 202:79–89
- Clarke MR (1986) A handbook for the identification of cephalopod beaks. Clarendon Press, Oxford
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModel-Test 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772
- Di Azevedo MI, Carvalho VL, Iniguez AM (2017) Integrative taxonomy of anisakid nematodes in stranded cetaceans from Brazilian waters: an update on parasite's hosts and geographical records. *Parasitol Res* 116:3105–3116
- Huelsenbeck JP, Ronquist F (2005) Bayesian analysis of molecular evolution using MrBayes. In: Nielsen R (ed) *Statistical methods in molecular evolution*. Springer, New York, NY, p 183–226
- Klimpel S, Palm HW (2011) Anisakid nematode (Ascaridoidea) life cycles and distribution: increasing zoonotic potential in the time of climate change? In: Mehlhorn H (ed) *Progress in parasitology, Parasitology Research Monographs* 2. Springer, Berlin, p 201–222
- Kuhn T, Cunze S, Kochmann J, Klimpel S (2016) Environmental variables and definitive host distribution: a habitat suitability modelling for endohelminth parasites in the marine realm. *Sci Rep* 6:30246
- Levsen A, González AF, Mattiucci S, Cipriani P and others (2018) A survey of zoonotic nematodes of commercial key fish species from major European fishing grounds—introducing the FP7 PARASITE exposure assessment study. *Fish Res* 202:4–21
- Mattiucci S, Nascetti G (2008) Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. *Adv Parasitol* 66:47–148
- Mattiucci S, Paggi L, Nascetti G, Abollo E and others (2001) Genetic divergence and reproductive isolation between *Anisakis brevispiculata* and *Anisakis physeteris* (Nematoda: Anisakidae). *Int J Parasitol* 31:9–14
- Mattiucci S, Nascetti G, Dailey M, Webb SC, Barros N, Cianchi R, Bullini L (2005) Evidence for a new species of *Anisakis* Dujardin, 1845: morphological description and genetic relationships between congeners (Nemto-da: Anisakidae). *Syst Parasitol* 61:157–171
- Mattiucci S, Paoletti M, Webb SC (2009) *Anisakis nascettii* n. sp. (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. *Syst Parasitol* 74:199–217
- Mattiucci S, Cipriani P, Webb SC, Paoletti M and others (2014) Genetic and morphological approaches distinguish the three sibling species of the *Anisakis simplex* species complex, with a species designation as *Anisakis berlandi* n. sp. for *A. simplex* sp. C (Nematoda: Anisakidae). *J Parasitol* 100:199–214

- ✦ Mattiucci S, Acerra V, Paoletti M, Cipriani P and others (2016) No more time to stay 'single' in the detection of *Anisakis pegreffii*, *A. simplex* (s. s.) and hybridization events between them: a multi-marker nuclear genotyping approach. *Parasitology* 143:998–1011
- ✦ Mattiucci S, Cipriani P, Levsen A, Paoletti M, Nascetti G (2018) Molecular epidemiology of *Anisakis* and Anisakiasis: an ecological and evolutionary road map. *Adv Parasitol* 99:93–263
- ✦ Mazzariol S, Di Guardo G, Petrella A, Marsili L and others (2011) Sometimes sperm whales (*Physeter macrocephalus*) cannot find their way back to the high seas: a multidisciplinary study on a mass stranding. *PLOS ONE* 6:e19417
- McAlpine DF (2009) Pygmy and dwarf sperm whales. In: Perrin WF, Würsig B, Thewissen JGM (eds) *Encyclopedia of marine mammals*. Academic Press, San Diego, CA, p 936–938
- ✦ Mladineo I, Poljak V (2014) Ecology and genetic structure of zoonotic *Anisakis* spp. from Adriatic commercial fish species. *Appl Environ Microbiol* 80:1281–1290
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G (2002) The simple fool's guide to PCR. University of Hawaii, Honolulu, HI
- ✦ Pardo-Gandarillas MC, Lohrmann KB, Valdivia AL, Ibáñez CM (2009) First record of parasites of *Dosidicus gigas* (d'Orbigny 1835) (Cephalopoda: Ommastrephidae) from the Humboldt current system off Chile. *Rev Biol Mar Oceanogr* 44:397–408
- ✦ Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Syst Biol* 53:793–808
- ✦ Quiazon KM, Santos MD, Yoshinaga T (2013) *Anisakis* species (Nematoda: Anisakidae) of dwarf sperm whale *Kogia sima* (Owen, 1866) stranded off the Pacific coast of southern Philippine archipelago. *Vet Parasitol* 197: 221–230
- ✦ Reeder TW (1995) Phylogenetic relationships among phrynosomatid lizards as inferred from mitochondrial ribosomal DNA sequences: substitutional bias and information content of transitions relative to transversions. *Mol Phylogenet Evol* 4:203–222
- ✦ Romeo T, Battaglia P, Pedà C, Perzia P, Consoli P, Esposito V, Andaloro F (2012) Pelagic cephalopods of the central Mediterranean Sea determined by the analysis of the stomach content of large fish predators. *Helgol Mar Res* 66:295–306
- ✦ Santoro M, Badillo FJ, Mattiucci S, Nascetti G and others (2010a) Helminth communities of loggerhead turtles (*Caretta caretta*) from Central and Western Mediterranean Sea: the importance of host's ontogeny. *Parasitol Int* 59:367–375
- ✦ Santoro M, Mattiucci S, Paoletti M, Liotta A, Degli Uberti B, Galiero G, Nascetti G (2010b) Molecular identification and pathology of *Anisakis pegreffii* (Nematoda: Anisakidae) infection in the Mediterranean loggerhead sea turtle (*Caretta caretta*). *Vet Parasitol* 174:65–71
- ✦ Shamsi S, Gasser R, Beveridge I (2012) Genetic characterisation and taxonomy of species of *Anisakis* (Nematoda: Anisakidae) parasitic in Australian marine mammals. *Invertebr Syst* 26:204–212
- ✦ Shamsi S, Briand MJ, Justine JL (2017) Occurrence of *Anisakis* (Nematoda: Anisakidae) larvae in unusual hosts in Southern hemisphere. *Parasitol Int* 66:837–840
- ✦ Spitz J, Cherel Y, Bertin S, Kiszka J, Dewez A, Ridoux V (2011) Prey preferences among the community of deep-diving odontocetes from the Bay of Biscay, Northeast Atlantic. *Deep-Sea Res I* 58:273–282
- Taylor BL, Baird R, Barlow J, Dawson SM and others (2012) *Kogia sima*. The IUCN Red List of Threatened Species 2012: e.T11048A17695273
- ✦ Verna F, Giorda F, Miceli I, Rizzo G and others (2017) Detection of morbillivirus infection by RT-PCR RFLP analysis in cetaceans and carnivores. *J Virol Methods* 247:22–27
- ✦ Viricel A, Rosel PE (2012) Evaluating the utility of *cox1* for cetacean species identification. *Mar Mamm Sci* 28:37–62
- Xavier JC, Cherel Y (2009) Cephalopod beak guide for the Southern Ocean. British Antarctic Survey, Cambridge

Editorial responsibility: Stephen Raverty,  
Abbotsford, British Columbia, Canada

Submitted: December 7, 2017; Accepted: May 30, 2018  
Proofs received from author(s): August 2, 2018