First report of infestation by a parasitic copepod (Pennella balaenopterae) in a harbour porpoise (Phocoena phocoena) from the Aegean Sea: a case report

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ABSTRACT: An adult, female harbour porpoise (*Phocoena phocoena relicta*) was found stranded on the southern Aegean Sea coast of Turkey. Thirteen holes made by copepods were observed on the lateral sides of the porpoise. The copepods were identified as *Pennella balaenopterae*, based on the morphological characteristics and measurement. Tissue samples were collected from embedded parts of parasites, histopathologically examined and panniculitis findings were observed. Although this parasite copepod had been reported on several marine mammals, this is the first report in the harbour porpoise, and in the Aegean Sea.

Keywords: copepod; Pennella balaenopterae; harbour porpoise; ectoparasite; southern Aegean Sea

Parasitic diseases are a significant health problem in marine mammals. In the marine environment, generally, ectoparasites cling to the surface of marine mammals in some way when transmitting to their next stage host and cause skin damage (Geraci and Aubin 1987). Six pennellid species were reported in Balaenopteridae and Delphinidae (Aznar et al. 2001). Although other species of the genus are found embedded in the muscles of numerous marine hosts, Hogans (1987) reported that *Pennella balaenopterae* (Koren and Danielssen 1877) is the only species in this genus that parasitises marine mammals (Abaunza et al. 2001). According to Raga et al. (2009), *Pennella* species are probably transmitted to pelagic cetaceans from oceanic fish.

The life cycles of copepods are complex. Only the adult female and the first naupliar stage have been described with certainty (Hogans 1987; Arroyo et al. 2002). After the nauplii stage, copepodids become adult and mate by using fish, molluscs or cephalopods as an intermediate host.

To produce the offspring, free-swimming inseminated females need to attach to a cetacean as a definitive host for feeding on blood and body fluids (Dailey 2001; Aznar et al. 2005; Raga et al. 2009). Turner (1905) observed that males do not attach to their hosts. This parasite has been reported on striped dolphins (*Stenella coeruleoalba*) in the western Mediterranean (Aznar et al. 2005), and on fin whales (*Balaenoptera physalus*), Cuvier's beaked whales (*Ziphius cavirostris*), Risso's dolphins (*Grampus griseus*) and bottlenose dolphins (*Tursiops truncatus*) in the Adriatic Sea (Brzica 2004). The first report from the Turkish eastern Mediterranean coast concerned a fin whale (Cicek et al. 2007).

In the Mediterranean basin, the harbour porpoise is found predominantly in the Black Sea. There have been, however, several records of sightings/strandings in the Turkish Straits System and also, to a lesser degree, some records in the Aegean Sea, almost always restricted to the northern part (Frantzis 2009; Tonay and Dede 2013).

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In this paper, the parasitic copepod *P. balaeno-pterae* is reported for the first time on a phocoenid species, the harbour porpoise (*Phocoena phocoena relicta*), in the Aegean Sea.

Case description

On 10 January 2013, an adult female (body length 141.5 cm) emaciated harbour porpoise (Phocoena phocoena relicta) was found stranded dead on the coast of the village of Torba on Bodrum Peninsula, Turkey (37° 4.904'N, 27° 27.714'E). The porpoise was in bad nutritive condition, its blubber was thin, and the longissimus dorsi (LD) muscle and neck were visibly concave. Its right lateral side was damaged and internal organs were almost decomposed (Decomposition Condition Code 4 according to Rowles et al. (2001)). This individual was the first stranding record of the harbour porpoise in the southern Aegean Sea coast of Turkey (Tonay and Dede 2013). At the gross necropsy, the cepholathorax and neck parts of individuals of P. balaenopterae were found embedded in the blubber and muscle. There were 13 holes made by ectoparasites on the body (Figure 1). Haemorrhage was observed around the holes (Figure 2). Concordantly, ectoparasites were settled around the vena jugularis, abdominal wall vein plexus and on the spinal cord of the peduncle (Figures 1 and 2). Ectoparasites were carefully collected using forceps and fixed in 70% ethanol and 5% glycerine. Fourteen parasite pieces were collected, nine pieces belonging to cephalothorax and five pieces of thoracic and abdominal regions. Embedded parts, i.e. cephalothorax, neck and ovisacs were yellowish; trunk and abdomen were darker (Figures 3 and 4).

For general examination a magnifying glass was used. Size measurements of parasites were made



Figure 1. Holes made by *Pennella balaenopterae* (marked with 1–13) on the lateral sides of the harbour porpoise stranded on the southern Aegean coast of Turkey

using a tape measure and digital calipers. Photos were taken under the binocular stereo microscope. Identification was made and morphometric characteristic of the parasites were examined according to Hogans (1987) and Abaunza et al. (2001). Total body length (mean \pm S.D.) was 117.4 \pm 32.4 mm (n = 6); cephalothorax was sub-cylindrical with a flattened anterior end. Anterior ends were commonly covered with papillae. Two large papillae were present near the margin of the anterior end of the dorsal surface (Figure 4). The lengths of the lateral horns were 13.85 mm and 13.05 mm. Lateral horns were cylindrical and unbranched. The neck and trunk were cylindrical. Between the abdomen and trunk there were oviduct orifices. The abdomen was cylindrical and the ventral surface was covered by plumes of different lengths. The mean length of the abdomen plumes was 4.8 ± 1.6 mm (n = 13). The mean lengths of other regions were as follows: abdomen, 19.8 ± 2.4 mm (n = 3); trunk, $30.9 \pm 5.5 \text{ mm } (n = 4); \text{ neck, } 66 \pm 16.8 \text{ mm } (n = 4);$ abdomen and trunk, 51.47 ± 8.9 mm (n=3); and also an ovisac was measured as 71.65 mm.

The tissue samples collected from embedded parts of parasites were fixed with 10% of neutral buffered formalin. Samples were processed for routine paraffin embedding using a Leica TP1020 Tissue Processor (dehydration in several grades of alcohol, clearing with xylene and embedding in paraffin). Five µm-thick sections were cut using a rotary microtome and stained with haematoxylin and eosin (H&E). The tissue sections were examined and photographed using an optical microscope with a camera attachment (Olympus BX50).



Figure 2. Embedded *Pennella balaenopterae* on the right side of the peduncle



Figure 3. One of the *Pennella bal-aenopterae* individuals (neck, trunk, abdomen and ovisacs) found on the harbour porpoise



Figure 4. Cephalothorax of one of the *Pennella balaenopterae* specimens

Histopathological examination of tissue samples obtained from skin and blubber revealed a number of parasite sections in blubber (Figure 5) surrounded by necrotic tissue, bacteria and an outer delineated layer of connective tissue infiltrated by neutrophils and mononuclear cells (Figures 6A and 6B). While some sections of skin were normal (Figure 6C), other sections were compatible with panniculitis, i.e. degenerated and necrotised lipocytes, mineralisation seen as dark blue colour, and increased connective tissue among lipocytes (Figure 6D).

DISCUSSION

According to Raga et al. (2009), female individuals of *Pennella* mainly burrow, generally on the back and belly of marine mammals to anchor their heads. In cetaceans vein vessels are more superficial in the back and abdominal parts for thermoregulation (Ponganis 2002). Parasites probably prefer these

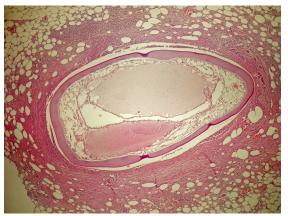


Figure 5. Parasite section, H&E, 100×

parts of the body due to the ease of reaching body fluids. In our case parasites were found on these parts of the body. In the report of Hogans (1987), the parasites were young individuals based on abdomen plume lengths. Dorsal horns were rarely seen in the cephalotorax of *P. balaenopterae* in this same study (Hogans 1987). Abaunza et al. (2001) reported that the dorsal horn is needed for a better grip on the whale due to the thickness of blubber. Presumably because of the ages of the parasites and the thin blubber of the host (1.4 cm in Dorsomedian line), horns were small compared to those in Hogans (1987). When fragmented subsamples of the parasites were put together, lateral horns were observed in cephalothorax, but there were no dorsal horns, which is in good agreement with previous studies (Turner 1905; Hogans 1987; Abaunza et al. 2001). Cornaglia et al. (2000) described Pennella sp. in the subcutaneous adipose tissue of a striped dolphin, on the skin of a Risso's dolphin and in a bottlenose dolphin. They described histopathological changes as moderate inflammatory reaction with lymphohistiocytic elements, eosinophils and microhaemorrhages infiltrating the dermis around the parasitic formations in the striped dolphin and infiltration of lymphocytes and eosinophils, with micro haemorrhages in the Risso's dolphin. Dailey et al. (2002) reported P. balaenopterae in a northern elephant seal (Mirounga angustirostris) which caused severe inflammation surrounding degenerated portions of cuticles. They presumed that the parasite's inability to penetrate the keratinised skin of pinnipeds in contrast to the skin of cetaceans caused this serious inflammatory reaction. In our case, histopathological findings resembled

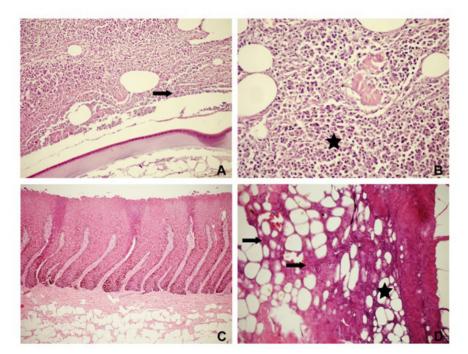


Figure 6. Microscopic views of embedded parts. (A) Parasite section surrounded by necrotic tissue and bacteria (arrow), H&E, 200×. (B) Neutrophil and mononuclear cell infiltration (star) in subdermis, H&E, 400×. (C) Intact cutis, H&E, 100×. (D) Degenerated and necrotized lipocytes, mineralisation (star) and increased connective tissue (arrows) H&E, 200×

the above reports. Copepod cuticles were not degenerated and less severe typical lesions were seen around the parasites' lateral horns as described by Dailey (2002). Neutrophil and mononuclear cell infiltration and capillary distension seen especially around the embedded parts of the parasites also match the observations of Cornaglia et al. (2000).

P. balaenopterae females have been reported in various marine mammals but this is the first record in the harbour porpoise and in any phocoenid species in general. *Pennella balaenopterae* is the largest known copepod in the world but the life history of *Pennella* species is not well understood. Salinity, temperature, oxygen content and current system influence parasitic copepods on fish (Jones 1998). It has been described that epizoic barnacles and *Pennella* fell off their host during the migration to colder waters (Olafsdottir and Shinn 2013). If *Pennella* fall off during the migration to colder waters, scars should be observed. Although *P. balaenopterae* is widely distributed in the world's oceans, it has not yet been reported from the Black Sea.

From genetic analyses, the harbour porpoise in our study has been found to share common haplotypes with harbour porpoises in Black Sea waters as well as in the Turkish Straits System (Tonay et al. 2014). In the Aegean Sea, changes in diet and the surrounding environment of harbour porpoises may result in deteriorating health condition, making it more susceptible to parasitic infestation. On the other hand, harbour porpoises can be suitable

hosts for *P. balaenopterae* but they do not come across this parasite in the Black Sea. Further studies should be performed on *P. balaenopterae* to elucidate their poorly understood life cycle and ecology and also regarding the presence of harbour porpoises in the Aegean Sea. For further analyses samples have been deposited at the parasite collection of Marine Biology Department, Faculty of Fisheries, Istanbul University.

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