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NOTE

Pennella balaenoptera actively select injured cetacean skin as attachment sites, making them potentially useful forensic tags

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ABSTRACT: Cetaceans harbor multiple epibionts on their external surface, and these attach to particular microhabitats. Understanding what drives the selection of attachment sites is relevant for refining the use of epibionts as indicators of their hosts. We report on about 100 females of the mesoparasitic copepod *Pennella balaenoptera* attached to a dead Cuvier's beaked whale *Ziphius cavirostris* stranded in Tunisia (western Mediterranean); the first report of *P. balaenoptera* in this country. The copepods were exclusively attached to numerous incisive, likely anthropogenic, wounds found on the host's skin. This finding suggests that newly recruited females may actively seek skin areas where physical penetration is facilitated; a factor that may help explain patterns of microhabitat selection by *Pennella* spp., and perhaps other pennellids, on their hosts. The estimated age of parasitization by *P. balaenoptera* (supported by age estimations of the co-occurring epibiotic barnacle *Conchoderma virgatum*) also suggests that the cetacean host likely survived these injuries, at least initially, and the presumed cause of death was starvation due to entanglement in a fishing net.

KEY WORDS: Cetacean health · Parasite attachment · Skin injury · Epibiont · Forensics · Indicator

1. INTRODUCTION

The skin of cetaceans is a microhabitat for multiple epibiotic animals (Ten et al. 2022). Although some epizoites can also use inanimate objects as substrata (e.g. floating debris; Frick & Pfaller 2013), most species have developed obligate associations with cetaceans and are potentially useful tools to obtain biological information on their hosts (see Ten et al. 2022 and references therein). Most epizoites have specific attachment sites on their hosts; thus, understanding the factors driving microhabitat selection is relevant to take full advantage of their potential as indicators. For instance, the spatial distribution of epibiotic barnacles mirrors specific hydrodynamic patterns in cetaceans (e.g. Briggs & Morejohn 1972, Carrillo et al. 2015, Moreno-Colom et al. 2020).

Pennella balaenoptera Koren & Danielssen, 1877 — recently suggested to be conspecific with *P. filosa* (Linnaeus, 1758) (see Fraija-Fernández et al. 2018, Suyama et al. 2021) — is a mesoparasitic copepod that has been reported on at least 24 cetacean species (Ten et al. 2022). The definitive hosts (i.e. cetaceans and large teleosts) are only parasitized by inseminated females, which seek them to grow and produce eggs (Arroyo et al. 2002, Boxshall & Halsey 2004). The cephalothorax penetrates the skin and remains deeply embedded in the blubber, allowing the parasite to feed on blood and body fluids while its trunk, genital complex, and abdominal plumes hang out of

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the skin (Abaunza et al. 2001, Hogans 2017). The presence of large numbers of *P. balaenoptera* has been considered as an indicator of poor host health (Mackintosh & Wheeler 1929, Aznar et al. 2005, Vecchione & Aznar 2014). However, it is unclear how this increased recruitment is related to the patterns of attachment, and questions remain regarding whether females of *P. balaenoptera* select specific settlement sites and whether there are atypical areas of colonization when hosts are sick. Elucidating these questions could shed light on the factors driving microhabitat selection and how they could refine the use of this species as a health indicator. In this study, we provide field evidence that females of *P. balaenoptera* may actively seek skin areas where physical penetration is facilitated, e.g. by injuries. We also illustrate the use of *P. balaenoptera* as a forensic tag that helps reconstruct the circumstances surrounding the host's death.

2. MATERIALS AND METHODS

On 22 December 2022, a fresh carcass (code 2 sensu Geraci & Lounsbury 2005) of a 5.6 m long female Cuvier's beaked whale *Ziphius cavirostris* Cuvier, 1823 was found stranded at Mgasseb Beach in Nefza, Tunisia (western Mediterranean Sea, 37° 03′ 52.9′′ N, 8° 57′ 21.8′′ E). The flukes of the animal were entangled in a large piece of fishing net that had also trapped a wooden pallet (Fig. 1A). The dissection was performed by a team from the National Institute of Sea Sciences and Technologies (INSTM), which operates the national stranding network established in 2004. The animal was emaciated, and the stomach and intestines were devoid of food remains or any other objects. Incised, non-bleeding wounds of varying extent and depth (ca. 5–15 mm) were observed at the anterior region, i.e. from the snout to the blowhole (Fig. 1). Most incisions had a thickened ridge and followed a vertical arrangement, being spaced ca. 2–6 cm apart; 3 of them were large and showed a less defined arrangement; and there was a group of 5 horizontal incisions right behind the commissure (Fig. 1B,C).

Multiple specimens of *Pennella balaenoptera* were found attached to many of the incisions (see Section 3). Due to the difficulty associated with conducting fieldwork because of the partial immersion of the whale in the water, an attempt was made to collect as many specimens as possible, mostly the protruding part of them. The ventral side of the beaked whale could not be examined in detail due to the animal's large size (Fig. 1). Morphometric measurements of the parasites were obtained using the software ImageJ (version 1.53t; NIH) based on *in situ* photographs or those of collected specimens. The length of the protruding part of the bodies (i.e. abdomen + trunk + sometimes part of the neck) of 65 specimens could be measured to the nearest mm from *in situ* photographs.

A conservative estimate of the time since the attachment of *P. balaenoptera* was inferred from data in the available literature. This was corroborated by estimating the time since attachment (a proxy for age) of *Conchoderma virgatum* Spengler, 1789; an epibiotic barnacle that was attached to some of the larger *P. balaenoptera* (see Section 3). Barnacle age estimates were based on the von Bertalanffy growth equations in Dalley & Crisp (1981) and Eckert & Eckert (1987) that use capitulum length, which was measured for all 12 individuals.

3. RESULTS AND DISCUSSION

A total of 130 pennellid parasites (all dead at the time of sampling) were attached to several incisions but none were found in the surrounding areas or other body parts (Fig. 1). The parasite's abdomen (mean ± SD length: 15.5 ± 3.1 mm, n = 49) exhibited dark pigmentation and abdominal plumes expanding outwards; the trunk (mean length: 22.7 ± 5.1 mm, n = 15) had a striated contour and was also dark-pigmented. Some of the largest specimens (mean abdomen length: 17.3 ± 3.1 mm, n = 8) presented egg strings. The neck (mean length: 34.3 ± 9.2 mm, n = 14) was comparatively thin and exhibited a pale pink color. The cephalic region could be examined in 3 young specimens (total length: 54.1 ± 7.1 mm, abdomen length: 8.1 ± 0.5 mm, n = 3); it was globose and covered with papillae, and had 2 lateral holdfast horns and 4 pairs of swimming legs. Specimens were identified morphologically as *Pennella balaenoptera* based on Abaunza et al. (2001). Although there are numerous records of *P. balaenoptera* in the Mediterranean Sea, this is the first report in Tunisia (see Ten et al. 2022).

Narrow incisions or wound edges seemed to be the most common attachment sites for *P. balaenoptera*, and individuals attached to the same incisions sometimes varied greatly in size (Fig. 2), indicating that they attached in several colonization events. Overall, we found a wide range of body sizes, with the length of the protruding section ranging from 9 to 77 mm and a mean (±SD) of 37.8 ± 16.5 mm. Inseminated pennellid females propel their bodies into the tissues of their hosts by vigorous growth, but how the breakdown of

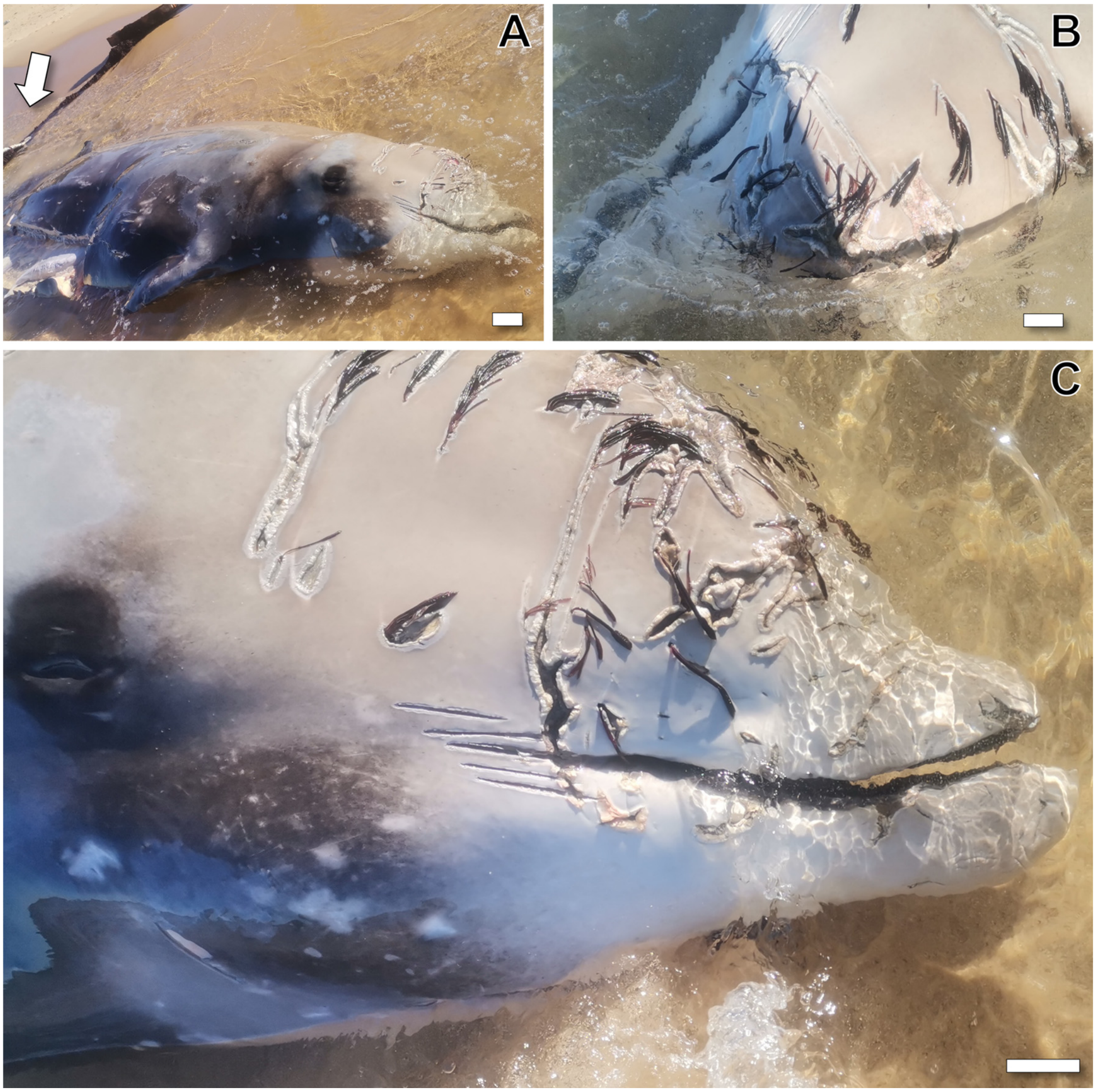


Fig. 1. A dead Cuvier's beaked whale *Ziphius cavirostris* stranded on Mgasseb Beach (Tunisia, western Mediterranean). (A) The tail fluke was entangled in a fishing net (white arrow) that had also trapped a wooden pallet. Note that the abdomen was dissected to collect the viscera. (B,C) The rostrum showed numerous incisions harboring high loads of the copepod *Pennella balaenoptera*. Scale bars = (A) 10 cm, (B,C) 5 cm

these tissues is conducted is not fully understood (Kabata 1979). Interestingly, the presented evidence suggests that females of *P. balaenoptera* selected spots where penetration was easier (i.e. incised wounds vs. intact skin), provided that the surrounding dermis is tight enough to hold an attached individual in place, hence their preference for narrow incisions or wound edges versus open wounds. The presence of female clusters in some injuries (Fig. 2) provides evidence that penetrating cetacean skin is difficult. Given that these aggregations cannot be related to mating (as for other epibiotic taxa; e.g. Moreno-Colom et al. 2020), they are likely associated with the benefits of using the pathways previously generated by other females, or by some sort of conjoint penetration (see also Fig. 3 in Vecchione & Aznar 2014).

Several individuals of the facultative epibiotic barnacle *Conchoderma virgatum*, also dead at the time of sampling, were found attached to the trunk of 10 individuals of *P. balaenoptera*; 2 of them harbored 2 barnacles, and 8 harbored just one. Based on measurements taken from photographs (Fig. 3B), the length of the trunk + abdomen of *P. balaenoptera* harboring barnacles ranged from 37 to 52 mm (mean ± SD: 42.5 ± 5.5 mm, n = 10), and the capitulum length of

Fig. 2. Detail of the skin lacerations on the frontal body of a dead Cuvier's beaked whale *Ziphius cavirostris* stranded on Mgasseb Beach (Tunisia, western Mediterranean). The wounds harbored high loads of the copepod *Pennella balaenoptera*, including individuals of a broad size range. Scale bar = 2 cm

C. virgatum ranged from 3.5 to 11.5 mm (mean ± SD: 7.1 ± 2.4 mm, n = 12).

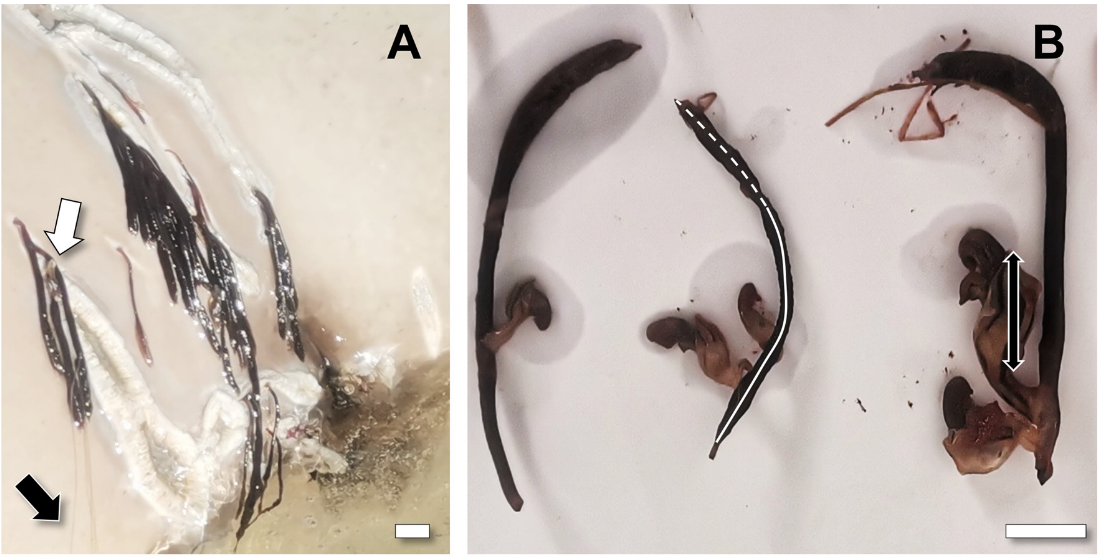
The finding of ovigerous females (Fig. 3A) suggests that the first recruitment of *P. balaenoptera* occurred long before the whale died. Although no precise accounts exist on the life span of *Pennella* spp., experimental infections of another species within the family Pennellidae, i.e. *Lernaeocera branchialis* (Linnaeus, 1767), are informative. Khan (1988) indicated that inseminated females of this species may need 12–14 wk to develop the egg strings, for a life span of 9–10 mo; other life span estimates for *L. branchialis* range from 8 wk to over 1 yr (van Damme & Hamerlynck 1992, see also Brooker et al. 2007). The specimens of *P. balaenoptera* from this study were collected from a warm-blooded host at a lower latitude, and both factors could have speeded up the parasite's growth. However, it is also clear that such a large species of *Pennella* is likely to experience a comparatively slower development and greater longevity (Hogans 2017). Therefore, it seems reasonable to assume that the first recruitment of these parasites to the Cuvier's beaked whale occurred at least 1 mo prior to the host's death, and most likely earlier.

The putative age of the collected individuals of *C. virgatum* argues in favor of this hypothesis. Based on von Bertalanffy growth equations (Dalley & Crisp 1981, Eckert & Eckert 1987), the largest barnacles in our sample were predicted to be ca. 2–3 wk old (Fig. 3C). This approach was previously attempted to estimate the entanglement duration of a shark (Wegner & Cartamil 2012). In our case, it is unclear whether individuals of *C. virgatum* require a minimum size of *P. balaenoptera* to attach, although all barnacles were found on relatively large females. In any event, it should take several days for newly recruited females of *P. balaenoptera* to metamorphose (e.g. it takes 5 d in *L. branchialis*) and grow enough to protrude and enable barnacle attachment. Thus, 3–4 wk seems to be the most conservative estimate of the time required for the whole settlement process of *P. balaenoptera*.

According to the above evidence, the Cuvier's beaked whale may have not died as a result of the incisive wounds; the large size variability of females of *P. balaenoptera* suggests a protracted recruitment of individuals after the wounds were produced. The origin of these injuries is unknown. Deeply incised wounds exhibiting a regular arrangement, such as we observed, can be caused by propeller blades (Byard et al. 2012). However, the co-occurrence of horizontal, narrowly spaced incisions, as well as open wounds spanning into different directions, cannot allow us to rule out additional or alternative agents (see, e.g. Moore et al. 2013). For instance, stab wounds might have been produced by fishers, in an attempt to release the whale when it became entangled. In any event, the entanglement of the whale's tail in a piece of fishing net with a pallet could have led to an unbearable drag and the eventual death of the animal by starvation.

4. CONCLUSIONS

This report demonstrates the potential of *Pennella balaenoptera* to draw inferences not only on the hosts' health but also to be used as a forensic tag. The latter application could be used to refine the investigation of the scarring process and the relationship of wounds with the host's death by adding a supplementary timescale. Also, the present findings suggest further venues to understand how the females of *P. balaenoptera* select specific spots on the cetacean body. We hypothesize that, within nutrient-rich body regions (i.e. well-irrigated areas), *P. balaenoptera*



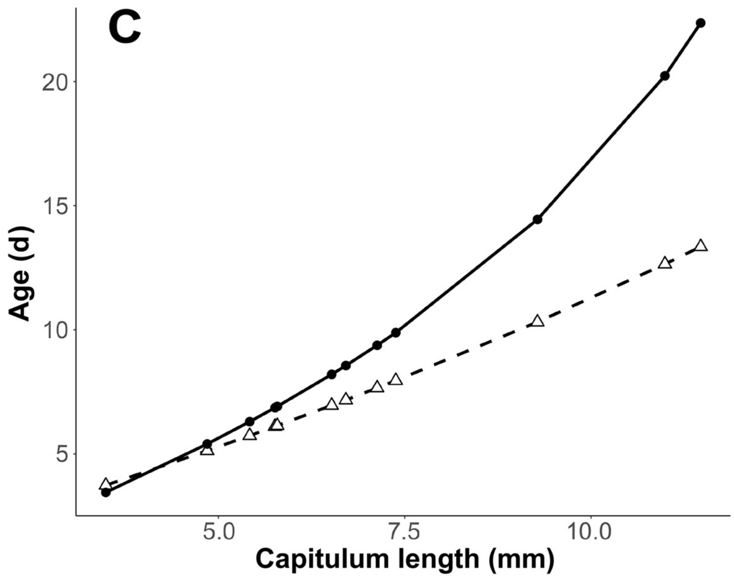


Fig. 3. Epibiotic species on a Cuvier's beaked whale *Ziphius cavirostris* stranded on Mgasseb Beach (Tunisia, western Mediterranean). (A) Some specimens of *Pennella balaenoptera* presented egg strings (black arrow) and 1–2 barnacles of the species *Conchoderma virgatum* were found on some large *P. balaenoptera* (white arrow). (B) Morphometric measurements of the epibionts: abdomen and trunk length of *P. balaenoptera* (dotted and solid white line, respectively) and capitulum length of *C. virgatum* (double-headed black arrow). Scale bars = 1 cm. (C) Age estimation of *C. virgatum* based on the von Bertalanffy growth equations in Eckert & Eckert (1987), solid line and dots; and Dalley & Crisp (1981), dashed line and triangles

would take advantage of using spots with (micro)-injuries and/or where the dermis is thinner. This, along with immunological impairment, could help explain why emaciated hosts are more easily infested (see Vecchione & Aznar 2014). These hypotheses are testable and deserve further attention in the future.

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