Vol. 128: 249–258, 2018
https://doi.org/10.3354/dao03226

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published June 4

Taxonomic status and epidemiology of the mesoparasitic copepod *Pennella balaenoptera* in cetaceans from the western Mediterranean

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ABSTRACT: *Pennella balaenoptera* is a mesoparasitic copepod that has been reported in at least 17 cetacean species. Subtle morphological differences in the first antennae of adult females have been used to discriminate this species from *P. filosa*, a species infecting fishes. Other morphological traits are unreliable because of their high plasticity, and no molecular data are available to confirm the taxonomic status of *P. balaenoptera* as an independent species. We found no consistent morphological differences of the first antennae between *P. balaenoptera* and *P. filosa* collected from cetaceans and fish in the western Mediterranean. Molecular data on the mitochondrial cytochrome oxidase subunit I failed to show reciprocal monophyly for the 2 species, and nucleotide divergence between them was low (mean ± SD [range]: 4.1 ± 0.006% [0.5–8.9]). Thus, *P. balaenoptera* and *P. filosa* are considered conspecific. We also obtained data on infection parameters of *P. balaenoptera* based on 450 individuals of 6 cetacean species stranded on the Spanish Mediterranean coast between 1980 and 2017. Prevalence was significantly lowest in the most coastal species, the bottlenose dolphin *Tursiops truncatus* (3.6%) and highest in the most oceanic species, Cuvier's beaked whale *Ziphius cavirostris* (100%). This suggests that the life cycle of *P. balaenoptera* is primarily oceanic. Interestingly, *P. filosa* also occurs in the oceanic realm infecting large fishes. This ecological similarity further supports the hypothesis that *P. balaenoptera* and *P. filosa* are conspecific.

KEY WORDS: *Pennella balaenoptera* · *Pennella filosa* · mtCOI · Integrative taxonomy · Cetaceans · Oceanic fish · Host specificity

INTRODUCTION

Pennella Oken, 1816 is a genus of mesoparasitic copepods infecting cephalopods, fish and marine mammals with ca. 9 valid species described to date (Hogans 2017b). Although specific details of the complete life cycle are unknown for any species, it includes, as in other members of the Pennellidae, a brief planktonic phase with 2 naupliar stages, and a copepodid stage that infects a first host (presumably a cephalopod) and subsequently develops into a chalimus and an adult stage. After mating on the first host, the inseminated female seeks a second host (a fish or marine mammal) to which it attaches and then produces eggs that are released to the environment (Arroyo et al. 2002, Boxshall & Halsey 2004). To attach, inseminated females penetrate into the hosts' musculature or blubber so that the cephalothorax, holdfast horns and neck remain deeply embedded in the host's tissue, whereas the trunk, genital complex

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and the brush-like abdominal process protrude through the host's body surface (Boxshall 1986, Hogans 1987b, 2017b).

Adult females of *Pennella* spp. exhibit high host specificity, with each species infecting 1 or 2 host species, except in 2 cases. First, *P. filosa* (Linnaeus, 1758) occurs in a wide range of large pelagic marine fishes including scombrids of the genus *Thunnus* South, 1845, the ocean sunfish *Mola mola* (Linnaeus, 1758) and the swordfish *Xiphias gladius* Linnaeus, 1758 (Hogans 1987a). Second, *P. balaenoptera* Koren and Danielssen, 1877 has been reported in at least 17 species of whales, dolphins, porpoises and, exceptionally, pinnipeds (see Table S1 in the Supplement at www.int-res.com/articles/suppl/d128p249_supp.pdf) and is the only species of *Pennella* infecting marine mammals.

The taxonomic discrimination between *P. balaenoptera* and *P. filosa* has been based on subtle morphometric differences observed in adult females (Hogans 2017b). In particular, a qualitative assessment indicated that the second segment of the 3-segmented first antenna is relatively longer, and all segments are slimmer, in *P. filosa* than in *P. balaenoptera* (Hogans 1987b). Other morphological traits lack diagnostic value because they are subject to enormous morphological plasticity depending on the site of attachment (Abaunza et al. 2001, Hogans 2017b). The shape of the cephalothorax and the length of the holdfast horns and neck can vary between specimens collected from the same host species, or even from the same host individual, due to the need of lesser or greater grip while penetrating the muscle or the blubber of hosts (Hogans 1987b, 2017b). The observation that each species infects distantly related host groups could support the decision of separating them as valid taxa (Hogans 1987b). However, morphological variability, such as that observed in *P. balaenoptera* and *P. filosa*, has led to taxonomic uncertainties, and molecular analyses thus offer an opportunity to shed light on the discussion of what defines a legitimate species (Hogans 2017b).

The association of *P. balaenoptera* with cetaceans is intriguing because it is among the few species of parasitic copepods infecting mammals (see Boxshall 2005). Interestingly, *P. balaenoptera* has been proposed as an indicator of compromised health in cetacean populations (Vecchione & Aznar 2014). This circumstance begs a deeper understanding of the ecological relationship between *P. balaenoptera* and its marine mammal hosts. Unfortunately, epidemiological information is very scarce: we are aware of only 2 studies reporting infection parameters of *P. balaenoptera*. Aznar et al. (2005) examined striped dolphins *Stenella coeruleoalba* (Meyen 1833) in the western Mediterranean (global host sample size = 144) and found prevalence values ranging from 12.9 to 40.3%, with median intensities from 2 to 15 ind. per dolphin. More recently, Ólafsdóttir & Shinn (2013) reported a prevalence of 10.3% and mean intensity of just 1.6 individuals in 188 common minke whales, *Balaenoptera acutorostrata* Lacépède, 1804, from Icelandic waters. What factors drive contact and recruitment of *P. balaenoptera* into different cetacean species is an open question.

Over the last decades, we have had the opportunity to collect data and samples of *P. balaenoptera* from several cetacean species stranded along the central Mediterranean coast of Spain. We also have had access to samples of *P. filosa* from swordfish and ocean sunfish in the same area. This situation has given us the opportunity to (1) re-examine the taxonomic status of *P. balaenoptera* based on morphological and molecular data and (2) provide and compare epidemiological data on this species in 6 cetacean species from this geographical region. Together, the results can significantly improve our understanding of the identity and host–parasite relationships between *P. balaenoptera* and its cetacean hosts.

MATERIALS AND METHODS

Data collection

Specimens of *Pennella balaenoptera* were obtained from dead cetaceans stranded along the central Mediterranean coast of Spain (40° 31′ N, 0° 30′ E and 37° 50′ N, 0° 45′ W) between 1980 and 2017 (Table 1). Access to carcasses was authorized by the Wildlife Service of the Valencian Regional Government. Specimens of *P. filosa* were obtained from a swordfish that stranded in the same area on 25 July 2016, and from ocean sunfishes accidentally caught in a traditional tuna fishery at La Azohía in May 2005 (Cartagena, Spain; 37° 33′ N, 1° 15′ W) (Table 1).

Taxonomic study

For morphological analysis, 6 specimens of *P. balaenoptera* were collected from 3 striped dolphins, 6 specimens of *P. filosa* from 2 ocean sunfishes and 2 specimens of *P. filosa* from 1 swordfish (Table 1). All parasites used in the study were post metamorphic

Table 1. Collection data of adult females of *Pennella balaenoptera* and *P. filosa* from the Spanish Mediterranean used for morphometrics and molecular identification of species. 'N' is the number of individual hosts examined for *Pennella* spp. GenBank accession numbers of newly generated sequences are given in brackets

Pennella species	Host	N	Number of specimens	
			Morphology	Molecular identification
P. balaenoptera	Striped dolphin *Stenella coeruleoalba*	326	6	2 [MG701288] [MG701293]
	Common dolphin *Delphinus delphis*	20		3 [MG701287] [MG701290] [MG701291]
	Long-finned pilot whale *Globicephala melas*	16		1 [MG701292]
	Risso's dolphin *Grampus griseus*	28		1 [MG701289]
	Bottlenose dolphin *Tursiops truncatus*	55		
	Cuvier's beaked whale *Ziphius cavirostris*	5		
P. filosa	Swordfish *Xiphias gladius*	1	2	3 [MG701282] [MG701285] [MG701286]
	Ocean sunfish *Mola mola*	5	6	2 [MG701283] [MG701284]

females; they were excised from hosts along with the surrounding tissue, which was later carefully removed from the cephalothorax and neck of each specimen. 'Clean' specimens were conserved in 70% ethanol for later examination.

The first antennae of each specimen were removed from the basis. Due to their tiny size, the right and left first antenna of 1 specimen of *P. balaenoptera*, and 1 of *P. filosa*, respectively, were broken in the process. Each intact antenna was placed in saline on a cover glass and drawn with the aid of a light microscope connected to a drawing tube (200×). Maximum width and length of each segment was measured on drawings. Setal armature was described from 2 individuals of *P. filosa* and 2 of *P. balaenoptera*.

The morphometric comparison between *P. balaenoptera* and *P. filosa* was based on measurements of the left antenna; in the single specimen of *P. filosa* in which the left antenna was broken, the right antenna was used instead. Linear mixed models were employed to test whether the length:width ratio of each segment was larger in *P. filosa*; 'species' was considered a fixed factor, and 'specimen' as a random factor. A *t*-test was also used to determine whether the relative length of the second segment was longer in *P. filosa*. Finally, a MANOVA was used to explore further potential morphometric differences of the first antenna between *P. balaenoptera* and *P filosa*, using all morphometric distances as dependent variables and 'species' as a fixed factor.

For molecular analysis, we obtained a small piece (~2 mm^3) of tissue from the trunk of 1 specimen of *P. balaenoptera* from 1 Risso's dolphin, 1 from 1 long-finned pilot whale, 3 from 2 common dolphins, 2 from 2 striped dolphins, 3 from 1 swordfish and 2 from 1 ocean sunfish (Table 1). Total genomic DNA from each specimen was extracted using the Isolate II Genomic DNA Kit (Bioline), following the manufacturer's recommendations. Prior to DNA extraction, ethanol from each sample was replaced with 500 µl of TE buffer (0.001 M TrisHCl, pH 7.5, 0.001 M EDTA, pH 8). Partial mitochondrial cytochrome oxidase subunit I (mtCOI) was amplified with primers LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994), and with jgLCO1490 (5'-TNT CNA CNA AYC AYA ARG AYA TTG G-3') and jgHCO2198 (5'-TAN ACY TCN GGR TGN CCR AAR AAY CA-3') (Geller et al. 2013). Thermocycling profiles for gene amplification were as follows: initial denaturation at 94°C for 5 min, 38 cycles of 94°C for 45 s, 48°C for 45 s, 72°C for 80 s, and a final extension at 72°C for 7 min when using the LCO1490/HCO2198 set of primers (Raupach et al. 2015), and initial denaturation at 94°C for 2 min, 30 cycles of 94°C for 1 min, 48°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 7 min when using the jgLCO1490/jgHC2198 set of primers (Geller et al. 2013). Amplicons were purified with a NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel) and sequenced in both directions on an Applied Biosystems ABI 3730 XL automated sequencer by Macrogen Europe (Amsterdam). Contigs were assembled in BioEdit 7.0.5.3, and sequence identity was verified using the Basic Local Alignment Search Tool (BLAST).

Newly generated sequences from *P. balaenoptera* and *P. filosa* were aligned using the online version of Mafft (https://mafft.cbrc.jp/alignment/software/) with all other sequences of the Pennellidae available from GenBank and *Caligus rogercresseyi* Boxshall and Bravo, 2000 (Caligidae, Siphonostomatoida) as the outgroup. The Hasegawa, Kishino and Yano model with gamma distribution and invariant sites (HKY+G+I) was selected as the best model that fit the alignment according to Akaike's Information Criterion (AIC) applied in JModelTest 2.1.4 (Darriba et al. 2012). Phylogenetic trees were constructed through Bayesian inference using MrBayes 3.1 (Huelsenbeck & Ronquist 2001) and through maximum likelihood (ML) using MEGA 6 (Tamura et al. 2013) under the selected model of evolution. Posterior probabilities (PPs) were obtained after 4 Markov chain Monte Carlo chains ran for 10 000 000 generations, with 1 topology saved after 100 generations. Consensus trees were constructed using a 'burn-in' of 69 for estimating 'sump' and 'sumt' after the average standard deviation of split frequencies was <0.001. The ML bootstrap values were estimated after 1000 replicates, and the heuristic tree searching strategy was Subtree-Pruning-Regrafting. Clades were considered highly supported when PPs were >90% and ML bootstrap values were >80%. From the aligned dataset, uncorrected pairwise p distance matrices were computed with the number of base differences per site using MEGA 6.

Epidemiological analysis

Data of *P. balaenoptera* were collected from 450 individuals of 6 species of cetaceans stranded between 1980 and 2017 (see Table 4). All carcasses showed a light to moderate state of decomposition sensu Geraci & Lounsbury (2005). Total body length and blubber thickness of each host were measured. The 95% CI for prevalence (frequency of hosts infected with *P. balaenoptera*) per cetacean species was calculated with Sterne's exact method (Reiczigel 2003), and for mean intensity (no. of *P. balaenoptera* per infected host) with the bias-corrected and accelerated bootstrap method using 10 000 replications (Rózsa et al. 2000, Reiczigel et al. 2013). Pairwise differences in the prevalence of *P. balaenoptera* between cetacean species were tested with Fisher tests, and differences in mean abundances with a Kruskal-Wallis test with post hoc comparisons (Conover 1999). In multiple comparisons, probability values were corrected by the sequential Bonferroni procedure (Rice 1989). The package SPSS v. 22.0 was used to perform all statistical analyses.

RESULTS

Morphological and molecular comparison of *Pennella balaenoptera* and *P. filosa*

In toto female specimens of *P. balaenoptera* and *P. filosa* are shown in Fig. 1. Regardless of species, there were no significant differences between the morphometry of the right and left first antennae (MANOVA, Wilk's lambda = 0.776, $F_{6,17}$ = 0.818, p =

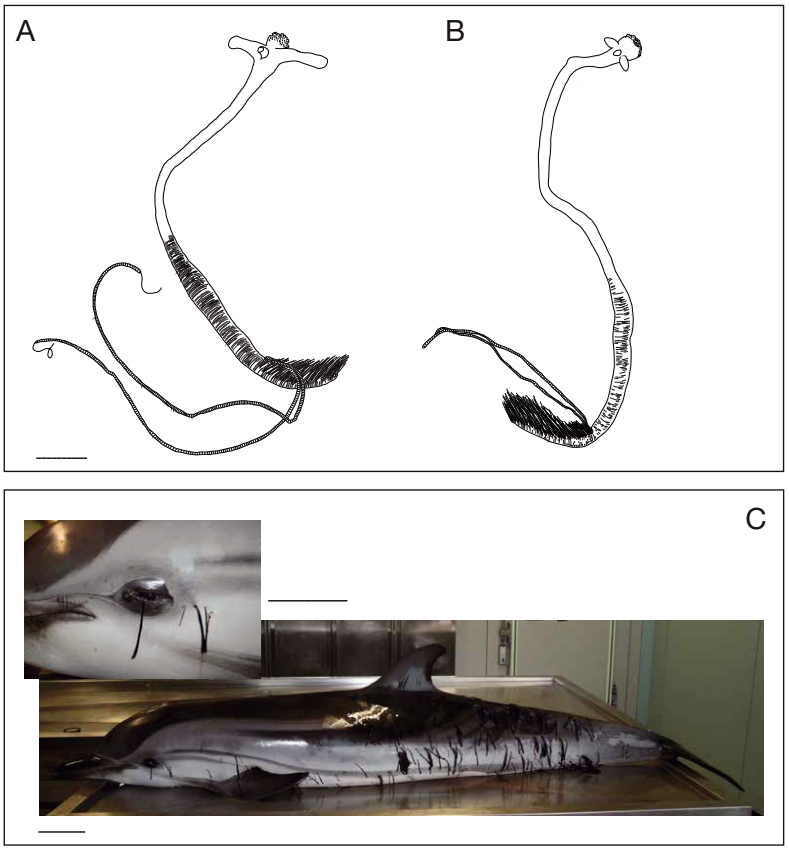

Fig. 1. Habitus profile of 2 female specimens of (A) *Pennella balaenoptera* and (B) *P. filosa*. (C) Lateral view of a heavily parasitized striped dolphin *Stenella coeruleoalba* from the Western Mediterranean. Scale bars = (A,B) 1 mm, (C) 10 cm

Table 2. Morphometric measurements of the 3 segments of the first left antenna of *Pennella balaenoptera* collected from striped dolphins *Stenella coeruleoalba* and *P. filosa* from swordfish *Xiphias gladius* and ocean sunfish *Mola mola* from the central Mediterranean coast of Spain. Measurements are expressed in mm ± SD (range). L: length; W: width

	Pennella balaenoptera (n = 5)		*Pennella filosa* (n = 8)	
	Length	Width	Length	Width
1st segment	1.25 ± 0.29 (1.00–1.74)	0.84 ± 0.22 (0.65–1.22)	1.13 ± 0.21 (0.91–1.48)	0.78 ± 0.10 (0.65–0.91)
2nd segment	1.31 ± 0.39 (0.74–1.83)	0.54 ± 0.17 (0.35–0.78)	1.05 ± 0.22 (0.74–1.44)	0.55 ± 0.07 (0.44–0.65)
3rd segment	1.00 ± 0.18 (0.87–1.30)	0.43 ± 0.04 (0.39–0.48)	0.97 ± 0.14 (0.78–1.22)	0.47 ± 0.11 (0.26–0.61)
Ratio L:W 1st segment	1.51 ± 0.24 (1.21–1.86)		1.48 ± 0.31 (1.09–1.88)	
Ratio L:W 2nd segment	0.43 ± 0.13 (0.24–0.58)		0.55 ± 0.16 (0.39–0.88)	
Ratio L:W 3rd segment	2.33 ± 0.57 (1.91–3.33)		2.17 ± 0.53 (1.50–3.17)	
Relative length of 2nd segment	0.36 ± 0.08 (0.22–0.44)		0.33 ± 0.05 (0.27–0.42)	

0.571). Morphometric values for the first left antenna of *P. balaenoptera* and *P. filosa* are given in Table 2. Contrary to our expectations, average values of the ratio of segment length: width in the 3 antennal segments were slightly smaller in *P. filosa* than in *P. balaenoptera* (Table 2), but the difference was not significant ($F_{1,35}$ = 0.04, 1-tailed p = 0.818). Likewise, the relative length of the second antennal segment was slightly shorter in *P. filosa*, but the difference with *P. balaenoptera* was not significant (*t*-test, *t* = −1.192, df = 11, 1-tailed p = 0.742). The MANOVA did not reveal any other difference in the morphometry of the first antenna of both species (Wilk's lambda = 0.552, $F_{6,6}$ = 1.233, p = 0.403).

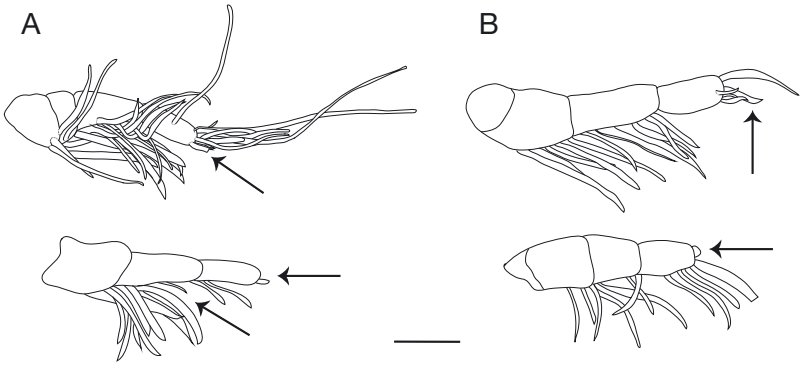

Fig. 2. First antennae of 2 specimens of (A) *Pennella balaenoptera* and (B) *P. filosa*. Areas in which setae were apparently lost or broken are shown with an arrow. Scale bar = 0.1 mm

The first antennae of 2 specimens of *P. filosa* and 2 of *P. balaenoptera* are shown in Fig. 2. The number of setae was highly variable, even between individuals of the same species. A detailed examination revealed that some setae had been lost or broken (Fig. 2B). Using a complete antenna of 1 specimen of *P. balaenoptera* from a striped dolphin, we found 10 setae in the apical region, 12 setae in the second segment and 7 in the third segment (Fig. 2A).

A total of 7 new sequences of the mtCOI were obtained for *P. balaenoptera* and 5 new sequences for *P. filosa*, which are now available on GenBank (see Table 1 for accession numbers). Complete alignment, including new sequences of *Pennella* spp. and all other available sequences of the Pennellidae, was 449 bp long. In the phylogenetic tree obtained, all sequences of *Pennella* spp. were clustered together in a highly supported clade (PP and ML = 100%; Fig. 3). Thus, specimens of *P. filosa* and *P. balaenoptera* did not constitute reciprocally monophyletic groups. Mean ± SD nucleotide divergence between sequences of *P. balaenoptera* and *P. filosa* was 4.1 ± 0.006% (range 0.5–8.9%; Table 3). The highest nucleotide divergence occurred between sequences of *P. filosa* from the ocean sunfish (3.9–8.9%) and sequences of *P. balaenoptera* from the striped dolphins (3.4–8.9%) compared to all other sequences studied.

Infection parameters of *P. balaenoptera*

Infection parameters of *P. balaenoptera* in the 6 cetacean species studied are shown in Table 4. Prevalence of *P. balaenoptera* in cetaceans ranged from 3.6% in the bottlenose dolphin and 100% in the Cuvier's beaked whale. Pairwise Fisher tests (with p-values corrected by the sequential Bonferroni correction) indicated the following pattern of statistically significant differences in prevalence of *P. balaenoptera* ($p < 0.05$) in bottlenose dolphin < other cetaceans < Cuvier's beaked whale.

The mean number of *P. balaenoptera* per cetacean host was generally low, ranging from 2.0 in the bottlenose dolphin to 23.1 individuals in striped dolphins (Table 4). The maximum number was found in a

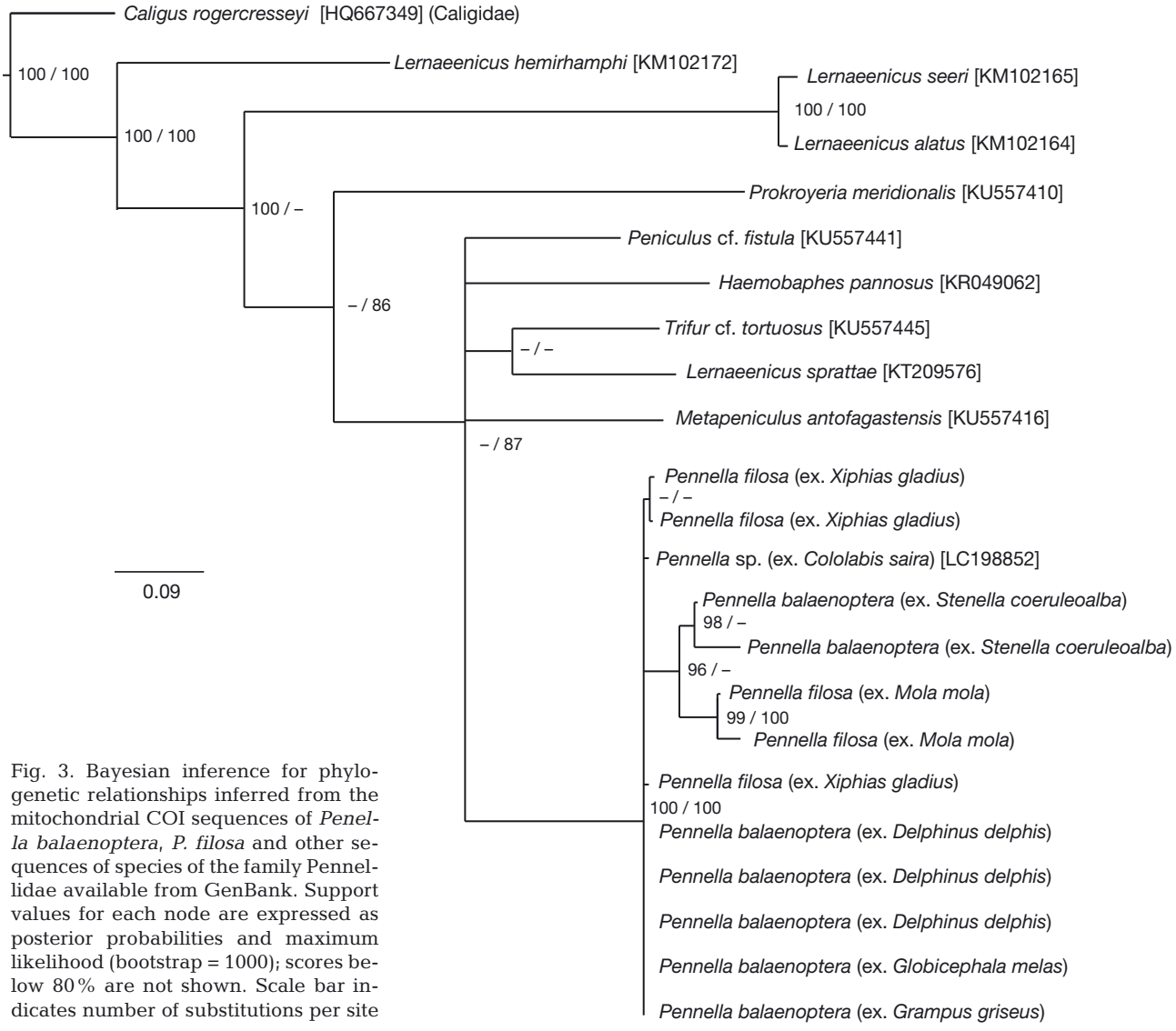

Fig. 3. Bayesian inference for phylogenetic relationships inferred from the mitochondrial COI sequences of *Pennella balaenoptera*, *P. filosa* and other sequences of species of the family Pennellidae available from GenBank. Support values for each node are expressed as posterior probabilities and maximum likelihood (bootstrap = 1000); scores below 80% are not shown. Scale bar indicates number of substitutions per site

striped dolphin (344 individuals). Mean abundance of *P. balaenoptera* differed significantly between cetacean species (Kruskal-Wallis test, χ^2 = 31.61, df = 5, $p < 0.001$). The post hoc comparison indicated that the abundance was significantly lower in bottlenose dolphins than in all other species ($p < 0.013$ in all comparisons). Abundance was also significantly higher in Cuvier's beaked whales than in all other species ($p < 0.019$ in all comparisons).

DISCUSSION

Taxonomic status of *Pennella balaenoptera*

Among copepods of the Siphonostomatoida (e.g. Morales-Serna et al. 2014, González et al. 2016), and specifically among members of the Pennellidae (e.g. Castro-Romero et al. 2016, Hogans 2017b, 2018), morphological polymorphism often precludes a clear delimitation of species. Indeed, Hogans (1987b) used subtle differences in the structure of the first antenna to differentiate *P. balaenoptera* and *P. filosa*, i.e. a slimmer appendage and longer second segment in *P. filosa*. However, Hogans (1987b) did not provide measurements of the segments of the first antenna for either species. In the present study, these putative differences could not be substantiated based on examinations and measurements using light microscopy. It should be noted that the power of statistical tests was low because of small sample size, but the fact that the pattern of morphometric differences was just the opposite to the one that should be expected would cast clear doubt that these putative diagnostic traits are reliable.

Table 3. Matrix of genetic distances between each pair of sequences of *Pennella balaenoptera* and *P. filosa* used in this study. Lower left half shows the number of base pair differences per site between each pair of sequences (expressed as percentages), and upper right half shows their standard error estimates. Pf: *Pennella filosa*; Pg: *Pennella balaenoptera*; Cs: *Cololabis saira*; Mm: *Mola mola*; Xg: *Xiphias gladius*; Dd: *Delphinus delphis*; Gm: *Globicephala melas*; Gg: *Grampus griseus*; Sc: *Stenella coeruleoalba*

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*Pennella* sp. ex. Cs		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2	*P. filosa* ex. Mm	7.6		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
3	*P. filosa* ex. Mm	9.6	2.1		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
4	*P. filosa* ex. Xg	1.8	6.2	8.2		0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.01	0.01
5	*P. filosa* ex. Xg	2.1	6.4	8.0	1.6		0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
6	*P. filosa* ex. Xg	1.8	6.6	8.2	1.4	0.7		0.00	0.00	0.00	0.00	0.00	0.01	0.01
7	*P. balaenoptera* ex. Dd	1.4	6.2	8.2	0.5	1.1	0.9		0.00	0.00	0.00	0.00	0.01	0.01
8	*P. balaenoptera* ex. Dd	1.4	6.2	8.2	0.5	1.1	0.9	0.0		0.00	0.00	0.00	0.01	0.01
9	*P. balaenoptera* ex. Dd	1.4	6.2	8.2	0.5	1.1	0.9	0.0	0.0		0.00	0.00	0.01	0.01
10	*P. balaenoptera* ex. Gm	1.4	6.2	8.2	0.5	1.1	0.9	0.0	0.0	0.0		0.00	0.01	0.01
11	*P. balaenoptera* ex. Gg	1.4	6.2	8.2	0.5	1.1	0.9	0.0	0.0	0.0	0.0		0.01	0.01
12	*P. balaenoptera* ex. Sc	5.5	3.9	5.5	4.6	5.3	4.6	4.1	4.1	4.1	4.1	4.1		0.01
13	*P. balaenoptera* ex. Sc	6.9	7.3	8.9	6.4	7.1	6.4	5.9	5.9	5.9	5.9	5.9	3.4	

Table 4. Summary of infection parameters of *Pennella balaenoptera* in 6 cetacean species stranded on the central Mediterranean coast of Spain between 1980 and 2017

Cetacean	Sampled hosts	Infected hosts	Total no. of *P. balaenoptera*	Prevalence (%) (95% CI)	Intensity (95% CI)
Tursiops truncatus	55	2	2	3.6 (0.6–12.4)	2.0[a]
Delphinus delphis	20	5	24	25 (10.4–47.4)	6.0 (2.0–9.0)
Stenella coeruleoalba	326	59	1247	18 (14.2–22.7)	23.1 (11.1–48.2)
Grampus griseus	28	8	158	29 (14.2–48.2)	22.6 (3.9–50.7)
Globicephala melas	16	3	16	19 (5.3–43.6)	5.3 (2.0–7.7)
Ziphius cavirostris	5	5	36	100 (50–100)	9.0 (2.5–19.0)

[a]No confidence interval was obtained, as only 2 hosts were infected

We also attempted comparison of the setal armature of the first antenna in *P. filosa* and *P. balaenoptera*, but obtaining intact antennae was extremely difficult. Interestingly, the number of first-antennal setae was slightly different, and some setae were much longer in our specimen of *P. balaenoptera* than in those depicted by Hogans (1987b, 2017b). Thus, we agree with Hogans (2017a, 2018) that appendage structure has limited use in pennellids as a diagnostic trait at the species level. Hogans (1987b) was apparently aware of these problems, but he preferred to leave *P. filosa* and *P. balaenoptera* as separate species because each one also infects distantly related hosts. This ancillary criterion made sense to trace differences between morphologically plastic species before the advent of DNA profiling and molecular analysis. Questions concerning levels of host specificity as related to species distinctness can now be more accurately addressed.

Molecular information such as DNA barcoding has been a suitable methodology to detect species boundaries, particularly when morphological data are unreliable or conflicting. In particular, the mtCOI has been extensively used as a molecular marker for identifying closely related species (Hebert et al. 2003, Waugh 2007). Results from our molecular analysis strongly suggest that *P. balaenoptera* and *P. filosa* are conspecific. Firstly, we found no evidence of reciprocal monophyly (a key criterion for species delimitation, see Kizirian & Donnelly 2004, De Queiroz 2007) between specimens of *Pennella* collected from cetaceans and fish. On the contrary, specimens formed a polytomy, with a secondary cluster of sequences obtained from individuals from both cetaceans and fishes. Secondly, the genetic 'yardstick' for species delimitation using mtCOI across different animal taxa has been proposed to be ~8% of divergence (Hebert et al. 2003, Waugh 2007), and we

found just 4.1% of mean nucleotide divergence between mtCOI sequences of *P. balaenoptera* and *P. filosa*, and a maximum sequence divergence of 8.9% between *P. filosa* from the ocean sunfish and *P. balaenoptera* from the striped dolphin. The use of genetic 'yardsticks' has been criticized based on both theoretical and empirical grounds (e.g. Nadler 2002, Cognato 2006). In any event, for families of the Siphonostomatoida, including Pennellidae, sister species are separated by a higher genetic divergence than that observed in the present study, e.g. 16.5–22.3% for *Nesippus* spp. (Pandaridae) (Dippenaar et al. 2010), 6.3–27.5% for *Lepeophtheirus* spp. (Caligidae) (González et al. 2016) and 2.8–14.4% for *Trifur* spp. (Pennellidae) (Muñoz et al. 2015).

In summary, the results from our study indicate that *P. balaenoptera*, the more recently erected species, is a junior synonym of *P. filosa*. Analysis of molecular data collected from additional specimens parasitic on marine mammals and fishes from other geographical areas should be included to corroborate this synonymy.

Infection parameters of *P. balaenoptera* in cetaceans

Molecular data suggest that *P. balaenoptera* is a single species infecting several sympatric odontocetes in the western Mediterranean. However, infection levels differed significantly among host species, thus raising the question of whether it is a generalist parasite. Host specificity patterns are defined by 2 sequential filters, i.e. the contact filter, which excludes potential hosts that the parasite cannot reach, and the compatibility filter, which excludes contacted hosts in or on which the parasite cannot establish (Combes 2001) or, if the parasite can do so, some fitness components (e.g. size, fecundity) are significantly impaired (Mateu et al. 2011). Inseminated females of *P. balaenoptera* actively seek and select their final hosts and, therefore, host–parasite contacts would depend on the availability of host species where the life cycle develops. Interestingly, the prevalence and mean abundance of *P. balaenoptera* were minimal in the bottlenose dolphin and maximal in Cuvier's beaked whale, which represent 2 extremes of a bathymetric gradient of habitat use from coastal to oceanic Mediterranean waters (see Notarbartolo di Sciara & Birkun 2010). This could suggest that the life cycle of *P. balaenoptera* is primarily oceanic. Two further observations support this hypothesis. Firstly, of the 17 species of cetaceans in which *P. balaenoptera* has been reported to date, only 2 typically favour coastal habitats (Table S1). Secondly, *P. filosa* (now considered conspecific with *P. balaenoptera* according to our molecular evidence) largely occurs on oceanic fishes (Hogans 1987a, 2017b, Hernández-Trujillo et al. 2014).

Future studies should address whether there are additional factors influencing the likelihood of contact between *P. balaenoptera* and their cetacean hosts. For instance, positive relationships have been observed between host body size and abundance of ectoparasitic copepods (Poulin 1999). Interestingly, Cuvier's beaked whales are the largest species in our sample and could offer more contact surface for free-swimming females of *P. balaenoptera*. Likewise, there is the possibility that the compatibility filter also affects the establishment of *P. balaenoptera* depending on the cetacean species (or cetaceans vs. fishes, for that matter). For instance, the thickness of the blubber layer might influence the likelihood of successful attachment. Moreover, other specific fitness traits of females of *P. balaenoptera*, e.g. size or fecundity, could differ among cetacean species, or between cetaceans and fish. These issues will be addressed properly when a more comprehensive collection of data (including those obtained from *P. balaenoptera* from baleen whales) is available.

There are some examples of ectoparasitic copepods infecting distantly related hosts, but most of them represent instances of 'straggling' events (see Rivera-Parra et al. 2017). For instance, Ólafsdóttir & Shinn (2013) reported a typical ectoparasite of fish, i.e. *Caligus elongatus*, on some minke whales in Icelandic waters. In contrast, *P. filosa* (=*balaenoptera*) is widespread in both fish and cetaceans. The putative historical colonization of cetaceans by *P. filosa* represents an interesting case study because most host-switching events by metazoan parasites reported in cetaceans involve passive contact mechanisms such as trophic transmission (e.g. García-Varela et al. 2013, Fraija-Fernández et al. 2015). Other copepods infecting disparate host groups, like species of *Balaenophilus*, which infect baleen whales, marine turtles and manatees, use bodily contact of hosts for transmission (Badillo et al. 2007, Domènech et al. 2017). This pattern of host–parasite associations has been linked to a critical food resource for the parasite that is shared by all of its hosts, i.e. alpha-keratin (Badillo et al. 2007, Domènech et al. 2017). Thus, one may wonder what microhabitat conditions could be shared by fish and cetaceans for *P. filosa* (=*balaenoptera*) to actively infect both phylogenetically unrelated hosts.

Overall, morphological, molecular and epidemiological data presented in this study provide evidence for considering *P. balaenoptera* a junior synonym of *P. filosa*, which is the only mesoparasitic copepod that infects distantly related hosts such as fish and cetaceans. Molecular data from other geographical regions would contribute to a further understanding of the ecology, taxonomy and host–parasite relationships of this species.

Acknowledgements. We thank the Servicio de Vida Silvestre, Conselleria de Agricultura, Medio Ambiente, Cambio Climático y Desarrollo Rural (Generalitat Valenciana) and colleagues from the Marine Zoology Unit, for their assistance and support in obtaining data from stranded cetaceans. We are very grateful to Dr. W. E. Hogans and 2 anonymous reviewers who provided comments and suggestions that significantly improved the manuscript. This study was financially supported by projects CGL/2012/39545 and AGL2015/68405/R (Ministry of Economy and Competitiveness, Spain), PROMETEO II/2015/018 (Generalitat Valenciana), and by a Systematics Research Fund (Systematics Association and the Linnean Society of London) awarded to N.F.F., who holds a post-doctoral fellowship from Generalitat Valenciana APOSTD 2017/089.

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Editorial responsibility: Michael Moore, Woods Hole, Massachusetts, USA

Submitted: December 27, 2017; Accepted: March 20, 2018
Proofs received from author(s): May 10, 2018