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Nyctiphanes couchii as intermediate host for the acanthocephalan *Bolbosoma balaenae* in temperate waters of the NE Atlantic

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ABSTRACT: Cystacanths of the acanthocephalan *Bolbosoma balaenae* (Gmelin, 1790) were found encapsulated in the cephalothorax of the euphausiid *Nyctiphanes couchii* (Bell, 1853) from temperate waters in the NE Atlantic Ocean. Euphausiids were caught in locations outside the Ría de Vigo in Galicia, NW Spain, and prevalence of infection was up to 0.1%. The parasite was identified by morphological characters. Cystacanths were 8.09 ± 2.25 mm total length (mean ± SD) and had proboscises that consisted of 22 to 24 longitudinal rows of hooks, each of which had 8 or 9 hooks per row including 2 or 3 rootless ones in the proboscis base and 1 field of small hooks in the prebulbar part. Phylogenetic analyses of 18S rDNA and cytocrome *c* oxidase subunit I revealed a close relationship with other taxa of the family Polymorphidae (Meyer, 1931). The results extend northwards ot the known distribution of *B. balaenae*. Taxonomic affiliation of parasites and trophic ecology in the sampling area suggest that *N. couchii* is the intermediate host for *B. balaenae*, and we suggest that the whales *Balaenoptera physalus* (Linnaeus, 1758) and *B. acutorostrata* (Lacepède, 1804) are its definitive hosts. This life cycle is probably completed with or without paratenic hosts.

KEY WORDS: Acanthocephala · Cystacanths · *Bolbosoma balaenae* · Zooplankton · *Nyctiphanes couchii* · NE Atlantic

INTRODUCTION

The most frequent parasites in marine zooplankton appear to be protists hosted by copepods (Skovgaard & Saiz 2006). However, there are other groups of parasites, mostly platyhelminths, nematodes and acanthocephalans, which are also common and play a major part in both host life cycles and ecosystem food webs (Marcogliese 1995, 2002, 2004). Among zooplankton communities, euphausiids play an important role as intermediate hosts in the pelagic realm (Marcogliese 1995). They are able to attain massive biomasses and can form vast and dense swarms occupying one of the lowest trophic levels. Additionally they can be used by different types of parasites to reach their definitive host (Mauchline 1980, 1984, Marcogliese 2002).

Nyctiphanes couchii (Bell, 1853) is the most abundant euphausiid in European Atlantic waters and occurs very close to the European continental shelf. Locations where they are most abundant are near the Spanish coast, the Celtic Sea, the coasts of Ireland, Scotland and northeastern England and the entrance to the Skagerrak (Lindley 1977). In the NE Atlantic Ocean it is one of the main prey items for different cephalopods and fish species, which in turn become

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part of the diet of other vertebrates that serve as definitive hosts for a number of parasites. In addition, euphausiids are also a sizeable ingredient of the diet of some marine mammals (Raga et al. 2009). Despite the fact that parasites have great ecological importance, their recruitment (especially for acanthocephalans) in the lower levels of the food web and the role that they play is poorly understood.

Adult polymorphid acanthocephalans are intestinal parasites of marine mammals, fish-eating birds and waterfowl. *Bolbosoma* (Porta, 1908) and *Corynosoma* (Lühe, 1904) are two of the main genera of intestinal parasites that infect marine mammals (Aznar et al. 2006). The life cycle of *Bolbosoma* species is thought to involve pelagic marine zooplankton, especially pelagic euphausiids and copepods, as an intermediate host (Hoberg et al. 1993) and different species of fish as paratenic (transport) hosts (Raga et al. 2009). The juvenile forms of acanthocephalans are cystacanths, which are morphologically similar to the mature worms but differ from them in the size of the trunk and the degree of development of the sexual organs (Zdzitowiecki 1991, Hoberg et al. 1993). Moreover, these juvenile forms are widely considered to be the infective stage for definitive hosts. These cystacanths appear contracted with an introverted proboscis and neck inside cysts of the intermediate and paratenic hosts. The genus *Bolbosoma*, established for Acanthocephala from whales, contains 15 species (Amin 1985) and has a worldwide distribution (Measures 1992). Despite this, no previous data on the presence of *B. balaenae* cystacanths in euphausiids from the NE Atlantic Ocean are available. With the exception of Shimazu (1975) and Tsimbalyuk (1980), *Bolbosoma* has not been reported in euphausiids. Shimazu (1975) described the larvae of *B. caenoforme* (Heitz, 1920) from *Thyssanoessa longipes* (Brandt, 1851) and *T. raschi* (Sars, 1864) in the North Pacific Ocean. On the other hand, Tsimbalyuk (1980) found *Bolbosoma* sp. infecting *Thyssanoessa* sp. Recently Gómez-Gutiérrez et al. (2010) reported 3 Polymorphidae larval stages of *Nyctiphanes simplex* (Hansen, 1911) (probably *Bolbosoma* or *Corynosoma*) off the northwestern coast of Mexico.

Considering the background of the identification of parasites in the mesozooplankton communities from the NE Atlantic Ocean, the aims of this paper are to (1) undertake the taxonomic and molecular diagnosis of cystacanths of *Bolbosoma balaenae* found in communities of *Nyctiphanes couchii*, thereby ascertaining the role of krill in the life cycle of acanthocephalan parasites from northwest of the Iberian Peninsula, (2) provide data on the occurrence of cystacanths of *B. balaenae* in the *N. couchii* population and (3) explore the potential life cycle strategies for these cystacanths to reach their definitive host.

MATERIALS AND METHODS

Collection and processing of larvae

The zooplankton sample was collected off the coast of Ría de Vigo in Galician waters, NW Iberian Peninsula, in 1 tow of length 1 nautical mile (from 42.21° N, 09° W to 42.23° N, 09° W) (Fig. 1) with the RV 'Mytilus' in July 2008. The sample was collected by double oblique towing, using a 750 mm diameter bongo net with 375 µm mesh size. At a ship speed of 2 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15 min, then hauled to the surface at 0.5 m^{-1}. The bongo net was equipped with a current meter, which allowed the volume of water filtered during the haul to be calculated, thus permitting zooplankton abundance (as

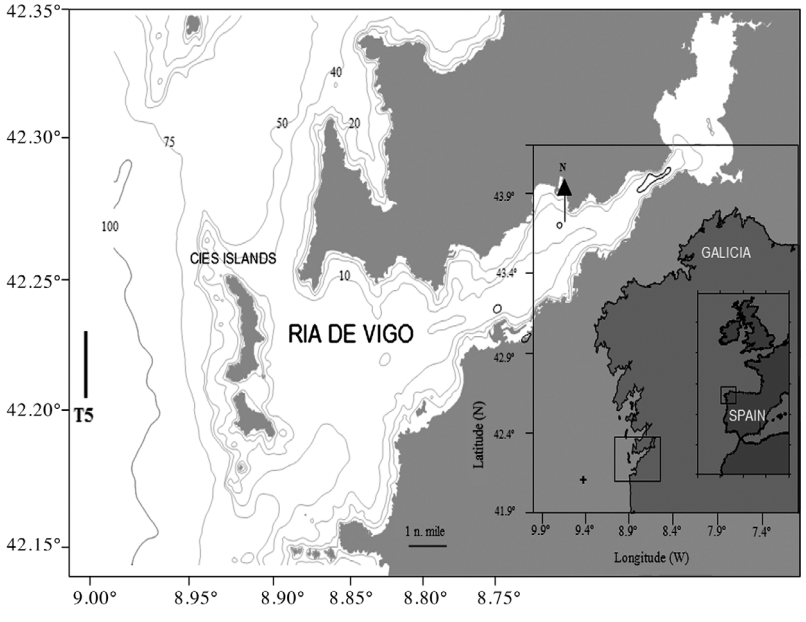

Fig. 1. Sampling transect (T5) off the Ría de Vigo in Galician waters, NE Atlantic

no. organisms m^{-3}) to be estimated. The sample was filtered with a 500 µm mesh sieve and fixed onboard with 100% ethanol. In the laboratory samples were transferred to 70% ethanol and stored at −20°C.

The abundance of the different zooplankton taxa was calculated after counting a subsample obtained from a Folsom splitter (Omori & Ikeda 1984). Organisms were identified to the lowest possible taxonomic level. Species diversity was calculated by using the Shannon-Wiener and Species Evenness Indices (Omori & Ikeda 1984, Guisande et al. 2006). The number of euphausiids was estimated by the method of calculating precise replica (Andrew & Mapstone 1987).

All the zooplankton components of the sample were examined for acanthocephalans with a stereomicroscope (20×). When cystacanths were found, they were extracted from the host by dissection. Cystacanths were analyzed by using morphological methods. The morphological characters used for identification followed Delyamure (1955), Petrochenko (1956, 1958), Yamaguti (1963) and Zdzitowiecki (1991). The most important diagnostic characters for generic and specific assignation of species within Polymorphidae are the proboscis armature and the pattern of trunk spination, particularly of the anterior trunk (Zdzitowiecki 1991, Aznar et al. 2006 and references therein). Since the proboscis, the neck and part of the anterior trunk were introverted in all specimens, we cut the trunk posterior to the proboscis receptacle and dissected it to evert the proboscis and the anterior trunk. This body part was dissected and cleared with lactophenol to improve the visibility of structures. The rest of the body was cleared before DNA extraction. We cleared them by using different concentrations of glycerine and 70% ethanol in order to observe the degree of sexual development. The sample was initially washed in a clearing solution that consisted of 1 part pure glycerine and 3 parts 70% ethanol for 5 min. For the second wash we used equal volumes of glycerine and 70% ethanol for 5 min. For the third wash we used 3 parts glycerine and 1 part 70% ethanol for 10 min, and the final wash contained 100% glycerine. This method allowed us to extract DNA from the body because the organisms were not destroyed. The following measurements were recorded: body length and width, foretrunk width (the typical bulb of *Bolbosoma*, see Amin & Margolis 1998) and length of receptacle and proboscis. When possible, the number of rows of hooks and number of hooks per row were counted, and hooks were measured.

Prevalence and intensity were estimated as described by Bush et al. (1997). The CI for prevalence was calculated by the method described by Agresti & Coull (1998) using an R macro developed by S. Dorai-Raj (http://rss.acs.unt.edu/Rdoc/library/binom/html/binom.confint.html, accessed 13 October 2011). Comparisons of prevalences were performed by using Fisher's exact tests (Rózsa et al. 2000).

Genomic DNA extraction and PCR amplification

Genomic DNA was isolated by using Qiagen DNeasy™ Tissue Kit according to manufacturer's instructions. DNA quality and quantity was checked in a spectrophotometer (ND-1000, Nanodrop Technologies) and in 1% agarose gel. The 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) were used to amplify approximately 700 bp of the cytochrome oxidase *c* subunit I (COI), and the primers 18SU467F (5-ATC CAA GGA AGG CAG CAG GC-3) and 18SL1310R (5-CTC CAC CAA CTA AGA ACG GC-3) (Suzuki et al. 2008) were employed to amplify approximately 900 bp of the small 109 subunit (18S) ribosomal RNA gene.

PCR reactions were performed in a total volume of 25 µl containing 1 µl of genomic DNA (150 to 200 ng), PCR buffer at 1× concentration, 1.5 mM $MgCl_2$, 0.2 mM nucleotides (Roche Applied Science), 0.3 µM primers and 0.625 U *Taq* DNA polymerase (Roche Applied Science). The cycling protocol for the COI gene was 2 min at 94°C, 35 cycles with 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, followed by 7 min at 72°C. The cycling protocol for the 18S rRNA gene was 2 min at 94°C, 35 cycles with 30 s at 94°C, 1 min at 55°C and 2 min at 72°C, followed by 7 min at 72°C. All PCRs were carried out in a TGradient thermocycler (Biometra) and a negative control (no DNA) was included for each set of PCR reactions. PCR products were separated on a 2% agarose (in 1× Tris-acetic EDTA buffer) gel, stained with ethidium bromide and scanned in a GelDoc XR documentation system (Bio-Rad Laboratories).

DNA sequencing and phylogenetic analysis

The positive PCR products were cleaned for sequencing by using ExoSAP-IT© (USB) as supplied by the manufacturer. Sequencing was performed by Secugen (Madrid, Spain). The chromatograms were analyzed with ChromasPro v. 1.41 (Technelysium). To complete the partial sequence of the 18S rDNA

gene, the specific primer Bolbo-F1 (5-CTA TCG CCA ACG CTT TAT CT-3) was designed by using the program Primer-3 (Rozen & Skaletsky 2000). Sequences were subject to basic local alignment search tool (BLAST) analyses with BLASTn against available sequences from GenBank, through web servers of the National Center for Biotechnology Information (USA). For phylogenetic analyses the sequences obtained were aligned with other acanthocephalan sequences available on GenBank. Taxa used for 18S rDNA and COI analyses are listed in Table 1; the rotifer *Rotaria rotatoria* was used as the outgroup. Alignment was accomplished by using the Clustal W algorithm in MEGA v. 3.1 software (Kumar et al. 2004) with settings at defaults: gap opening/gap extension penalties = 15/6.66 for both pairwise and multiple alignments, and with transitions weighted at 0.5. Maximum parsimony analysis was conducted by using the close neighbour interchange (CNI) heuristic option with initial trees by random addition of 1000 replicates, a search level of 1 and bootstrap values calculated over 100 replicates. Minimum evolution analysis was performed by using the Nucleotide Maximum Composite Likelihood model, the CNI heuristic option with a search level of 2, and bootstrap values were calculated over 1000 replicates.

Nucleotide sequence data reported in this paper are available in the GenBank under the Accession No. JQ040303–6.

Table 1. Species and GenBank accession number of taxa used for (a) 18S rDNA and (b) COI analyses

Taxon and authority	GenBank no.
(a) 18S rDNA analysis	
Acanthocephaloides propinquus (Dujardin, 1845)	AY830149
Andracantha gravida (Alegret, 1941)	EU267802
Corynosoma magdaleni (Montreuil, 1958)	EU267803
Corynosoma strumosum (Rudolphi, 1802)	EU267804
Echinorhynchus gadi (Zoega, 1776)	AY218123
Gorgorhynchoides bullocki (Cable & Mafarachisi, 1970)	AY830154
Hexaglandula corynosoma (Travassos, 1915)	EU267808
Ibirhynchus dimorpha (Schmidt, 1973)	GQ981436
Pararhadinorhynchus sp.	HM545903
Polymorphus minutus (Goeze, 1782)	EU267806
Profilicollis botulus (Van Cleave, 1916)	EU267805
Pseudocorynosoma constrictum (Van Cleave, 1918)	EU267800
Rhadinorhynchus sp.	AY062433
Southwellina hispida (Van Cleave, 1925)	EU267807
Transvena annulospinosa (Pichelin & Cribb, 2001)	AY830153
Outgroup: *Rotaria rotatoria* (Pallas, 1766)	AY218121
(b) COI analysis	
Andracantha gravida	EU267822
Arhythmorhynchus frassoni (Molin, 1858)	EU189484
Corynosoma strumosum	EF467871
Echinorhynchus gadi	AY218095
Hexaglandula corynosoma	EU189488
Ibirhynchus dimorpha	GQ981438
Polymorphus brevis (Van Cleave, 1916) Travassos, 1926	EF467861
Profilicollis botulus	EF467862
Pseudocorynosoma anatarium (Van Cleave, 1945)	EU267821
Rhadinorhynchus sp.	DQ089712
Southwellina hispida	FJ824189
Transvena annulospinosa	DQ089711
Outgroup: *Rotaria rotatoria*	EU499879

Table 2. Zooplankton groups represented in the plankton sample obtained off the coast of Ría de Vigo, Galicia, Spain. n = number of individuals; *A* = Abundance (n m^{-3}); % = percent composition of selected zooplankton categories. Total volume filtered in the tow was 410.37 m^3

Taxon and authority	n	*A*	%
Amphipoda			
Gammaridea (Latreille, 1802)	85	0.21	0.03
Brachyura zoeae	2462	6.00	0.78
Copepoda			
Acartia clausii (Giesbrecht, 1889)	7421	18.08	2.34
Calanoides carinatus (Kroyer, 1849)	72 258	176.08	22.78
Calanus helgolandicus (Claus, 1863)	35 155	85.67	11.08
Centropages chierchiae (Giesbrecht, 1889)	3966	9.67	1.25
Paraeuchaeta hebes (Giesbrecht, 1888)	8637	21.05	2.72
Euphausia			
Nyctiphanes couchii adults (Bell, 1853)	69 954	170.47	22.05
N. couchii calyptopis	34 432	88.78	11.49
N. couchii furcilia	49 387	120.35	15.57
Fish larvae	511	1.25	0.16
Mysidacea	1918	4.68	0.61
Cephalopoda			
Sepiola atlantica (Orbigny, 1839)	7	0.02	0.0022
Ophiuroidea larvae	511	1.25	0.16
Paguridae megalopa	479	1.17	0.15
Polychaeta larvae	5	0.01	0.0013
Siphonophora	1535	3.74	0.48
Stomatopoda			
Meiosquilla desmaresti (Risso, 1816)	479	1.17	0.15
Thaliacea	21 175	51.60	6.68
Total	317 174	772.94	100
Shannon-Wiener index (*H*)	1.555		
Species Evenness index	0.149		

RESULTS

The composition, total abundance and density of taxa collected in the zooplankton samples, as well as the Shannon-Wiener Index and Species Evenness Index values for the entire assemblage, are shown in Table 2. The most abundant taxon was *Nyctiphanes couchii* (Euphausiacea) which contributed ~49.09% to the total zooplankton community. Adults of this species made up ~22.05% of the total sample, whereas furcilia and calyptopis larvae contributed ~15.57 and ~11.49%, respectively. Copepods contributed ~41.68% of the sample, in which *Calanoides carinatus* were the most abundant species of this group (~22.78%), followed by *Calanus helgolandicus* at 11.08%. Thaliacea was also relatively abundant and contributed ~6.68%. The remaining zooplanktonic taxa contributed just ~2.52% (Table 2).

A total of 70 uncoloured cystacanths putatively identified as belonging to the family Polymorphidae were removed from the thorax of adult individuals of *Nyctiphanes couchii* (Fig. 2A). All cystacanths had the neck and proboscis invaginated within the foretrunk and the proboscis receptacle, respectively. After dissection, diagnostic details for a specific assignation were revealed, and all specimens were identified as *Bolbosoma balaenae*. No evidence of genital development was observed in any specimen. A brief morphological description follows.

The cystacanth body was cylindrical (length: 8.09 ± 2.58 mm [mean ± SD], n = 60; width at middle length: 0.41 ± 0.08 mm, n = 47), and had a funnel-shaped bulb (width: 0.68 ± 0.13 mm, n = 59) at the foretrunk (Fig. 2B), with a wide apex and narrow base (Fig. 2C). A somatic armature was present, and the single field of trunk spines was restricted to the prebulbar part or the foretrunk and composed of 4 to 6 irregular circles of small spines adjacent to the neck (no. of specimens observed = 38). The bulb was unarmed. The proboscis was cylindrical (length: 0.69

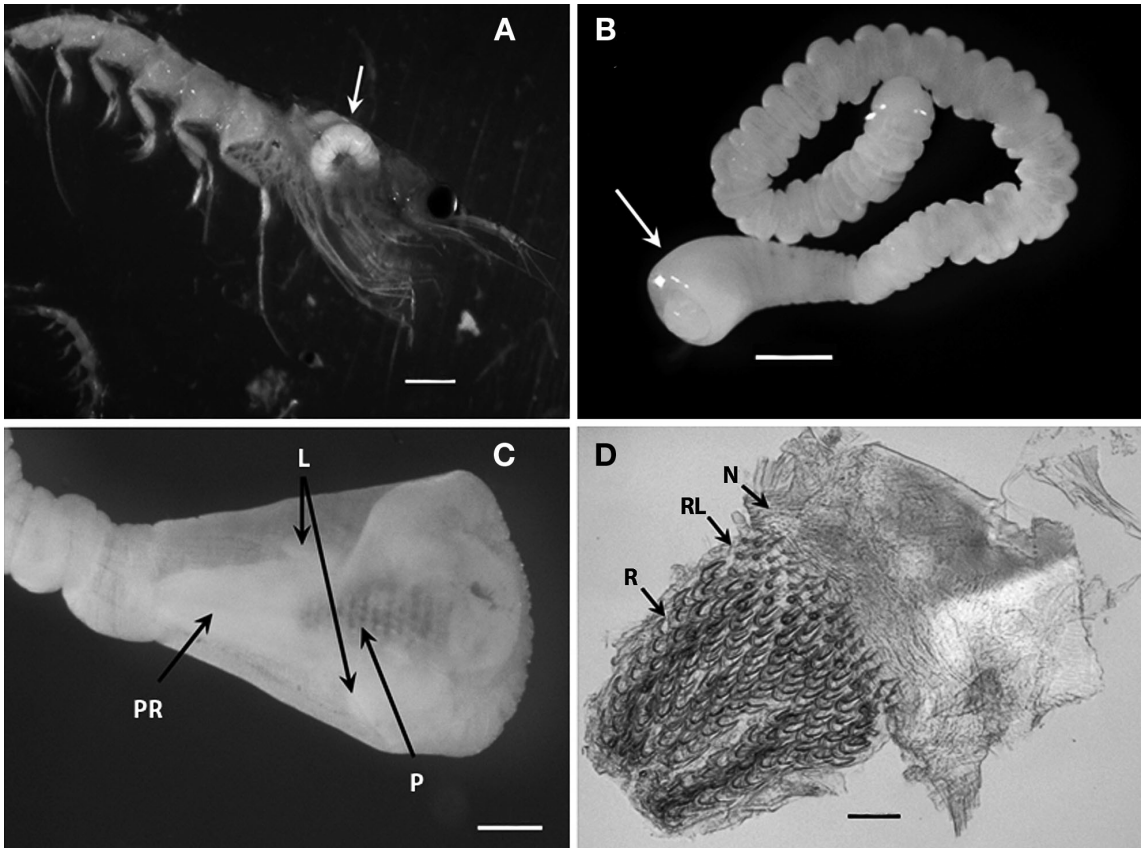

Fig. 2. *Bolbosoma balaenae*. (A) Cystacanth (arrow) inside the cephalothorax of *Nyctiphanes couchii*. Scale bar = 1 mm. (B) General view of the cystacanth. Arrow: the bulb characteristic of *Bolbosoma*. Scale bar = 500 µm. (C) Detail of the anterior part of the body showing the funnel-like-shaped bulb that contains the proboscis (P), its receptacle (PR) and lemniscis (L). Scale bar = 200 µm. (D) General view of the proboscis after dissection of the cystacanth showing the neck (N), rootless hooks (RL) and hooks with roots (R). Scale bar = 100 µm

± 0.48 mm, n = 45; width: 0.15 ± 0.08 mm, n = 41) and had 22 to 24 rows of hooks with 8 to 9 hooks per row, 6 with roots and 2 to 3 rootless (30 specimens observed) (Fig. 2D). Hook (H) lengths (in µm) were as follows: H1 (39.9 ± 7.8, n = 29); H2 (44.3 ± 8.1, n = 29); H3 (55.2 ± 11.4, n = 27); H4 (59.2 ± 7.3, n = 26); H5 (61.1 ± 6.1, n = 25); H6 (63.8 ± 6.0, n = 25); H7 (67.7 ± 9.2, n = 26); H8 (68.5 ± 5.9, n = 25); H9 (66.2 ± 0.2, n = 2). Voucher specimens were deposited at the Museo do Mar de Galicia, Vigo, Spain, with the accession number MDMG8012011.

The mean prevalence (95% CI) of *Bolbosoma balaenae* in *Nyctiphanes couchii* was 0.045% (0.035 to 0.057%). Considering only adults of *N. couchii*, the prevalence was 0.100% (0.079 to 0.127%). All host individuals harboured single specimens of *B. balaenae*. A comparison of prevalence between *N. couchii* and arthropod taxa in the zooplankton sample with at least 1000 individuals is shown in Table 3. Only 2 copepod species had a significantly lower prevalence than that of the total sample of *N. couchii*, but all copepod species had significantly lower prevalence than that of *N. couchii* when only adults of the latter species were considered (Table 3).

Table 3. Results of Fisher's exact tests that compare the prevalence of the acanthocephalan *Bolbosoma balaenae* in the total sample and the sample of adults only of the euphausiid *Nyctiphanes couchii* with that in other arthropod taxa collected in samples of zooplankton caught off Ría de Vigo, Galicia, Spain. Only taxa with ≥1000 individuals are included. Brachyura zoeae were excluded because their small size precluded infections with *B. balaenae* (see also Table 2). n = number of individuals

Taxon	n	Prevalence (%) (95% CI)	p-value (*N. couchii*) Total	Adult
Euphausiacea				
N. couchii	155 773	0.04 (0.03–0.06)		
Copepoda				
Acartia clausii	7421	0 (0–0.04)	0.078	0.002
Calanoides carinatus	72 258	0 (0–0.00)		
Calanus helgolandicus	35 155	0 (0–0.00)	<0.001	<0.001
Centropages chierchiae	3996	0 (0–0.08)	<0.001	<0.001
Paraeuchaeta hebes	8637	0 (0–0.03)	0.053	<0.001
Paraeuchaeta sp.	4797	0 (0–0.06)	0.277	0.023
Mysidacea sp.	1918	0 (0–0.16)	1.000	0.268

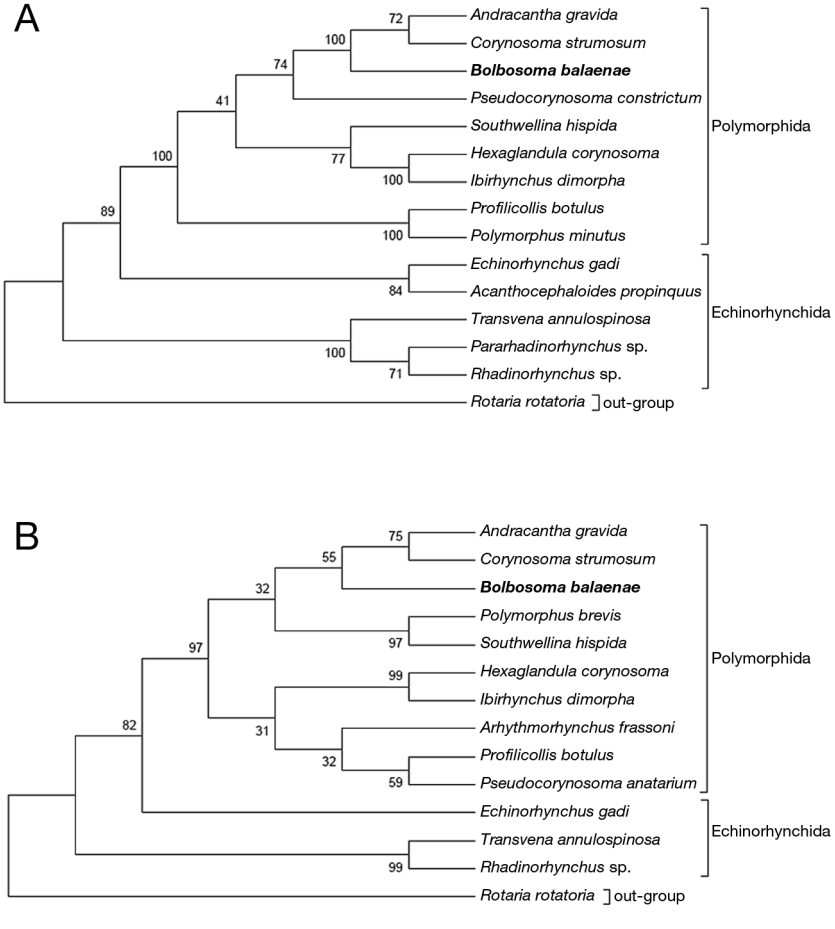

Fig. 3. *Bolbosoma balaenae.* Phylogenetic relationship among acanthocephalan sequences inferred by the minimum evolution algorithm within MEGA 4.1. (A) Analysis of 18S rDNA sequences. (B) Analysis of COI sequences. Numbers indicate the bootstrap confidence values of 10 000 replicates

Molecular characterization

The amplified and sequenced 18S rDNA and COI regions of the acanthocephalans were 885 and 512 bp in length, respectively. The G+C contents of the sequenced genes were 48.5% for 18S rDNA and 36.4% for COI. Submission to the BLAST server showed that the most similar sequences in GenBank were the 18S rDNA of *Corynosoma strumosum*, *C. magdaleni*, *C. enhydri* (Morozov, 1940) and *Andracantha gravida* with identity values of 99%, and the COI of *C. strumosum* with an identity value of 81%. Alignment of sequences showed that 490 sites (59%) were conserved, 333 (40.1%) were

variable, 193 (23.2%) were parsimony informative and (16.7%) were singleton for 18S rDNA. Of the COI sites, 150 (30.8%) were conserved, (67.9%) were variable, 219 (45.0%) were parsimony informative and 30 (17.4%) were singleton. Phylogenetic analyses using the minimum evolution (ME) and minimum parsimony (MP) methods yielded a similar tree topology, placing the species within the Polymorphidae. Phylogenetic analyses of the 18S rDNA revealed a close relationship to the sequences of *C. strumosum* and *A. gravida*, with bootstrap values of 100% for ME analysis (not shown) and 96% for MP analysis (Fig. 3). For COI phylogenetic analyses, a similar dendrogram was obtained but it was supported with low bootstrap values. The trees also showed that the Polymorphidae form a monophyletic group, which is supported by high bootstrap values (>95%).

DISCUSSION

Within the Polymorphidae, the main diagnostic characters to identify taxa are the number and arrangement of proboscis hooks and the patterns of trunk armature (Schmidt 1973, Amin 1982, Aznar et al. 2006). For identification purposes, one advantage of these characters is that they are already visible at the cystacanth stage and remain unmodified during the adult development (Van Cleave 1952). This is the reason why some *Bolbosoma* species currently considered as valid have been described from cystacanths alone, e.g. *B. caenoforme* and *B. heteracanthis* (see Petrochenko 1958). Amin (1985) and Golvan (1994) listed 14 valid species within the genus *Bolbosoma*. Twelve species are common to both classification schemes, i.e. *B. balaenae*, *B. bobrovoi* (Krotov & Delyamure, 1952), *B. brevicolle* (Malm, 1867), *B. caenoforme* (Heitz, 1920), *B. capitatum* (Linstow, 1880), *B. hamiltoni* (Baylis, 1929), *B. heteracanthis* (Heitz, 1917), *B. nipponicum* (Yamaguti, 1939), *B. physeteris* (Gubanov, 1952), *B. scomberomori* (Wang, 1980), *B. tuberculata* (Skrjabin, 1970) and *B. vasculosum* (Rudolphi, 1819). However, Amin (1985) considered *B. thunni* (Harada, 1935) as a valid species whereas Golvan (1994) considered that it could be synonymous with *B. vasculosum*, as did more recent authors (Costa et al. 2000). Amin (1985) also considered 2 subspecies within *B. turbinella* (Diesing, 1851), i.e. *B. turbinella turbinella* (Diesing, 1851) and *B. turbinella australis* (Skrjabin, 1972). Golvan (1994), however, regarded these subspecies as separate species. Later, Amin & Margolis (1998) synonymized *B. capitatum* and *B. physeteris*.

Regardless of these slight differences between classification schemes, *B. balaenae* is the only species of *Bolbosoma* that has a single field of trunk spines restricted to the prebulbar foretrunk; in the remaining species, spines cover the bulb to a variable extent (Meyer 1933, Van Cleave 1953, Petrochenko 1958, Zdzitowiecki 1991, Measures 1992, Amin & Margolis 1998, Costa et al. 2000, and references therein). The number of circles of prebulbar spines seems to vary among specimens of *B. balaenae*; Meyer (1933) mentioned 6 circles, whereas Zdzitowiecki (1991) mentioned up to 10 circles, and Van Cleave (1953) reported specimens even without spines, but he made it clear that this was a natural condition. Accordingly, the diagnostic character of somatic armature would allow a ready assignment of the cystacanths collected in this study as *B. balaenae*; the number of circles of spines observed in our sample may be considered within the range of natural variability of this species. In addition, our identification is confirmed based on the patterning of proboscis hooks. The proboscis of cystacanths collected in this study had 22 to 24 rows of hooks with 8 to 9 hooks per row, 6 with roots and 2 to 3 rootless. As far as we know, this combination of characters is shared only with previous descriptions of *B. balaenae.* Only 3 species, *B. australis*, *B. brevicolle* and *B. nipponicum*, have a number of rows of hooks that overlaps with that of *B. balaenae*; however, the number of hooks per row in these species is lower (range, 5 to 7) (see Meyer 1933, Van Cleave 1953, Petrochenko 1958, Zdzitowiecki 1991, Measures 1992, Amin & Margolis 1998, Costa et al. 2000, and references therein).

Phylogenetic analyses shows that this species is well embedded in the family Polymorphidae and is included as a sister taxa to *Corynosoma strumosum* and *Andracantha gravida*. The phylogenetic trees herein obtained are in accordance with previous molecular and morphological phylogenetic hypotheses for acanthocephalans, which support the monophyly of this group (García-Varela et al. 2000, 2002, 2011). The absence of DNA sequences deposited in the GenBank for *Bolbosoma balaenae* only allows placing the species within the phylogeny of the group. However, the sequences deposited are useful as reference material for future comparisons with sequences of adults of *B. balaenae*. Interestingly, *Corynosoma*, *Andracantha* and *Bolbosoma* are the only genera within the Polymorphidae in which the majority or all species occur in marine hosts: *Corynosoma* mainly in pinnipeds, *Andracantha* in cormorants and *Bolbosoma* in cetaceans (see Hoberg et

al. 1993, Aznar et al. 2006). The position of a member of *Bolbosoma* in the phylogenetic tree of the Polymorphidae opens new avenues to interpret the history of associations between these acanthocephalans and marine mammals. In particular, it is possible that the ancestor that was the origin of these forms came from a single colonization event in the marine realm. The phylogenetic trees obtained here are in accordance with the results described previously by García-Varela et al. (2000, 2002, 2011).

Apart from 3 sporadic records in oceanic odontocetes, i.e. the northern bottlenose whale *Hyperoodon ampullatus* (Lacépède, 1804) (see Delyamure 1955), spinner dolphins *Stenella longirostris* (Gray, 1828) and spotted dolphins *S. attenuata* (Gray, 1846) (see Dailey & Perrin 1973), *Bolbosoma balaenae* has frequently been reported in at least 7 mysticetes species worldwide, including the bowhead whale *Balaena mysticetus* (Linnaeus, 1758), sei whale *Balaenoptera borealis* (Lesson, 1828), common minke whale *B. acutorostrata* (Lacépéde, 1804), fin whale *B. physalus* (Linneus, 1758), blue whale *B. musculus* (Linneaus, 1758), humpback whale *Megaptera novaeangliae* (Borowski, 1781) and grey whale *Eschrichtius robustus* (Lilljeborg, 1861) (Zdzitowiecki 1991, Dailey et al. 2000, and references therein). In the Atlantic Ocean *B. balaenae* has been found in bowhead, common minke, sei, blue and fin whales. Fin whales occupy principally a wide area between the 40° and 55° N latitudes and migrate southward during winter months (Raga et al. 1986). It is a common species in coastal waters where upwelling events occur (Jefferson et al. 1993, Aguilar 2009). It is considered a generalist and euryphagous. In the northern hemisphere its diet is mainly composed of krill *Meganyctiphanes norvegica* (Sars, 1856) and *Thysanoessa inermis* (Kryer, 1846), other zooplanktonic crustaceans, fishes and small squids (Perrin et al. 2009), especially schooling fish such as capelin, herring, cod, sardine and mackerel (Nemoto 1959, Klumov 1963 and Mitchell 1975, all as cited in Measures 1992). Consequently this whale probably acquires *B. balaenae* from infected fish that probably serve as paratenic hosts. Reports of juvenile *Bolbosoma* sp. in fish implicate members of Scombridae, Scorpaenidae, Carangidae, Trichiuridae, Gempylidae, Salmonidae, Berycidae, Lophotidae, Gadidae and Belonidae (Measures 1992, www.nhm.ac.uk/research-curation/research/projects/host-parasites/index.html).

The known geographic distribution of the euphausiid *Nyctiphanes couchii* includes Galician waters in NW Spain, where it is relatively abundant (Lindley 1977). As a component of the mesozooplankton, the krill is part of the diet of decapods, cephalopods (Pascual et al. 1996), fish and marine mammals (Mauchline 1980). This places in one of the lowest trophic levels, allowing the transmission of the cystacanths towards a definitive host throughout predator–prey interactions (Marcogliese 1995).

This is the first time that *Bolbosoma balaenae* has been found in the euphausiid *Nyctiphanes couchii*, which probably acts as an intermediate host. Hoberg et al. (1993) and Gómez-Gutiérrez et al. (2010) suggested that euphausiids and copepods could be the intermediate hosts of the genus *Bolbosoma* and Acanthocephala in general. Nevertheless, only 2 studies have reported the genus *Bolbosoma* in euphausiids (Shimazu 1975, Tsimbalyuk 1980). In our samples we found *Bolbosoma* only in adults of *N. couchii*, despite the fact that we studied a great number of copepod species and other zooplankton components. This reinforces the idea proposed by Nickol et al. (2002) in which different genera of Polymorphidae are specialized in a specific fraction of zooplankton. Consequently, it is possible that *B. balaenae* is specific for this species of krill (*N. couchii*) in the NE Atlantic Ocean.

We did not find *Bolbosoma balaenae* larvae in the Euphausiacea larvae (calyptopis and furcilias, which were 0.8 to 2.20 mm and 2.2 to 5.5 mm in total length, respectively), nor in the copepod species or other taxa from our sample; this fact could be due to the large size of these cystacanths (8.09 mm). We speculate that only adults of *Nyctiphanes couchii* could harbour them in their body cavity (12 to 17 mm length). The larger copepods in our sample were *Calanus helgolandicus*, *Calanoides carinatus* and *Paraeuchaeta hebes*, and none exceeded a total length of 3 mm. The smaller ones were *Acartia clausii*, *Centropages chierchiae* and *Temora longicornis* (Müller O.F., 1785), whose size is always smaller than 2 mm. Despite finding Mysidacea with a similar size to euphausiids in our sample, we did not find cystacanths inside. Probably this is due to the low numbers in our sample or because they occupy a different niche and have a different behaviour. Consequently, it seems that the smaller crustaceans cannot harbour cystacanths nor act as intermediate host for these acanthocephalans.

Additionally, none of the examined *Nyctiphanes couchii* showed more than one cystacanth per individual. Therefore, it seems that krill are able to harbour only one cystacanth. Nevertheless it is desirable to carry out experimental infections to validate the null hypothesis of host size as a limiting factor for infection intensity or determine whether the dilution of the pelagic realm could be that factor. It is true that

multiple infections can reduce survival rates and perhaps we did not find more than one cystacanth in a host because these hosts died. Also, we only found cystacanths in the cephalothorax of krill, as did Shimazu (1975), Sars (1885), Lindley (1977) and Gómez-Gutiérrez et al. (2010), who also found Polymorphidae acanthocephalans in different species of krill. On the other hand, different Polymorphidae cystacanths have been reported in crabs with frequencies of more than one individual per crab. Nickol et al. (2002) detected infections of *Arhythmorhynchus* (Lühe, 1911) and *Hexaglandula* (Petrochenko, 1950) in fiddler crabs. In that case they found a crab that harboured a maximum of 3 cystacanths while the rest of the crabs examined harboured only 1 per individual. Balboa et al. (2009) detected the same larval stage harboured by several Brachyura (Linnaeus, 1758) and Anomura (Macleay, 1838), which showed differences in the intensity of infection. Experimental infections are required to determine whether size is the limiting factor for infection intensity.

The low prevalence (0.1%) observed in adults of *Nyctiphanes couchii* is usually considered a feature of an invertebrate intermediate host, especially in zooplankton communities (Marcogliese 1995). This is mainly because of the effect of dilution in the pelagic realm where finding a suitable intermediary host may be difficult. In spite of this low prevalence, most predators ingest large quantities of crustaceans and thus acquire prevalent and heavy infections (Marcogliese 1995). The information on larval acanthocephalans in euphausiid communities is very scanty and most of this information comes from studies on lake and river ecosystems where euphausiids, being marine organisms, are not found. Moreover the low prevalence is common for intermediate crustacean hosts (Uznanski & Nickol 1980, Ashley & Nickol 1989). Despite different ecosystem and transmission pathways, the prevalence (0.1%) agrees with those found for different acanthocephalan genera and platyhelminthes compiled in zooplanktonic communities (Marcogliese 1995).

Although there is information on other acanthocephalan species infecting zooplanktonic groups, no data on their prevalence is available (Hubschman 1983 and Wilson & Hubschman 1985 as cited in Marcogliese 1995, Dezfuli 1996, Bush et al. 2001, Kakizaki et al. 2003).

Owing to the high abundance of *Balaenoptera physalus* off the west coast of the Iberian Peninsula, we suggest that this whale could be a definitive host of *Bolbosoma balaenae* in this geographical region, which supplies and supports an infective population in the euphausiids. We also consider that the minke whale and other mammals could be additional suitable definitive hosts of this parasite in the NE Atlantic Ocean. However, we cannot rule out the possibility that the life cycle of *B. balaenae* could also involve pelagic fishes and cephalopods as paratenic hosts, which in turn are an important commercial resource in the area studied (http://conselleriamar.xunta.es/web/pesca/datos-producion).

Owing to the high importance of the euphausiids within the marine pelagic trophic webs, and taking into account that they are a key component, directly and/or indirectly, in the dietary habits of many cephalopods, fishes and marine mammals, we suggest that it would be interesting to undertake further studies focused on the possible interactions between the recruitment of *Bolbosoma balaenae* to *Nyctiphanes couchii* driven by the upwelling events that occur in this area of the NE Atlantic Ocean.

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