

Nyctiphanes couchii as intermediate host for *Rhadinorhynchus* sp. (Acanthocephala, Echinorhynchidae) from NW Iberian Peninsula waters

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ABSTRACT: In the mesozooplanktonic community of the coastal upwelling system of the Ría de Vigo (NW Spain), the euphausiid *Nyctiphanes couchii* has been identified for the first time in temperate waters of the NE Atlantic as the intermediate host for cystacanths of *Rhadinorhynchus* sp. Parasites were identified using morphological characters described in 20 cystacanths. The hooks of the proboscis were arranged in 14 rows of 26 hooks each, while the hooks of the basal circle were only slightly erected and were longer than remaining spines. A maximum-likelihood estimation (ML) tree inferred from the 18S rRNA data set of Palaeacantocephala revealed that our specimens belong to a highly supported clade with *Rhadinorhynchus* sp., *Pararhadinorhynchus* sp. and *Transvena annulospinosa*. Nonetheless, our morphological and phylogenetic analyses suggested that the status of *Rhadinorhynchus pristis* should be re-examined. The prevalences of parasites were 0.0019% and 0.0001% for frontal and coastal summer communities, and 0.0068% and 0.0008% for coastal and oceanic autumn communities, respectively. The presence of these cystacanths in different mesozooplankton communities throughout the study suggests that the recruitment of parasites may be affected by the oceanography.

KEY WORDS: Cystacanth · Euphausiid · *Nyctiphanes couchii* · *Rhadinorhynchus* sp. · Zooplankton · Acanthocephalan · NE Atlantic

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INTRODUCTION

Acanthocephalans, or thorny-headed worms, are in adult stages obligate endoparasites of the intestine of vertebrates (Nickol 1985, Marcogliese 1995, Garey et al. 1996, Nickol et al. 2002, Taraschewski 2005). The cystacanths of this genus are the infective stage. Morphologically, they are similar to the mature worms, but differ in the size of the trunk and the degree of development of the sexual organs (Hoberg et al. 1993, Zdzitowiecki 1991).

Among zooplankton communities, euphausiids play an important role as intermediate hosts in the

pelagic realm (Marcogliese 1995). They are able to attain massive biomasses that form vast and dense swarms occupying one of the lowest trophic levels. Moreover, they can be used by different parasites to reach their definitive host (Mauchline 1980, 1984, Marcogliese 2002). *Nyctiphanes couchii* (Bell, 1853) is the main euphausiid in the European continental shelf and one of its areas of higher concentrations is situated near the Spanish coast (Roura et al. 2013). This species is also one of the main prey items of different fish species, which in turn are involved in the diet of potential definitive hosts (Pascual et al. 1996, Marcogliese 2002). Some reports (e.g. Sars 1885, Shi-

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mazu 1975, Lindley 1977, Tsimbalyuk 1980, Gómez-Gutiérrez et al. 2010, Gregori et al. 2012) recognised some species of cystacanths infecting the thoracic organs of adults of several species of krill.

Moreover, it is well-known that within the Palaeacanthocephala, a few members of *Rhadinorhynchus* (Lühe, 1911) have experienced considerable speciation in aquatic environments (Taraschewski 2005), where they are able to infect important commercial fishes such as Scombridae (Rego et al. 1985, Rego 1987), Xiphidae (Hogans et al. 1983), Belonidae, Carangidae, and Bramidae in the Atlantic Ocean (www.nhm.ac.uk/research-curation/research/projects/host-parasites/database/index.jsp).

As far as we know, no previous data on the presence of *Rhadinorhynchus* sp. (Rudolphi, 1802) in euphausiids from the NE Atlantic are available. With the exception of Rodrigues et al. (1975) and Rego et al. (1985), *Rhadinorhynchus* has not been reported in this area. In addition, these authors found adults of this genus infecting scombrid fishes.

Despite parasites having a great ecological and economic significance in NE Atlantic waters, their recruitment to the zooplankton level is poorly understood. Therefore, the aims of this study were to (1) report the role of euphausiids in the life cycle of *Rhadinorhynchus* sp. in NW Iberian Peninsula waters; (2) provide data about parasite morphology, genetic and demographic infection values; and (3) discuss the controversy with the genetic identification of *R. pritsits* (Rudolphi, 1802).

MATERIALS AND METHODS

Biological sampling

The zooplankton samples were caught in the Ria de Vigo in Galician waters, NW Iberian Peninsula, onboard the RV 'Mytilus' (Fig. 1). Ten surveys were undertaken in the summer (2, 4, 9 and 11 July) and autumn (26 September, and 1, 3, 9, 10 and 14 October) of 2008. Samples were collected by double oblique towing, using a 750 mm diameter bongo net equipped with 375 μm mesh. At a ship's 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 s^{-1} . The Bongo was

equipped with a current meter, which enabled calculation of the volume of water filtered during the haul, thus permitting an estimation of zooplankton abundance (no. m^{-3}). The sample was filtered using a 500 μm sieve and fixed on board with 100% ethanol. Samples were later transferred to 70% ethanol in the laboratory and stored at -20°C .

Zooplankton estimation

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

Collection and processing of cystacanths

All zooplankton components of the samples were examined for acanthocephalans using a stereomicroscope (20 \times). Parasites were removed from the host using dissection material under the stereomicroscope. Cystacanths were identified by examining the body and proboscis according to Petrochenko (1956, 1958), Cable & Linderoth (1963), Yamaguti (1963), Zdzitowiecki (1989), and Arai (1989). The number

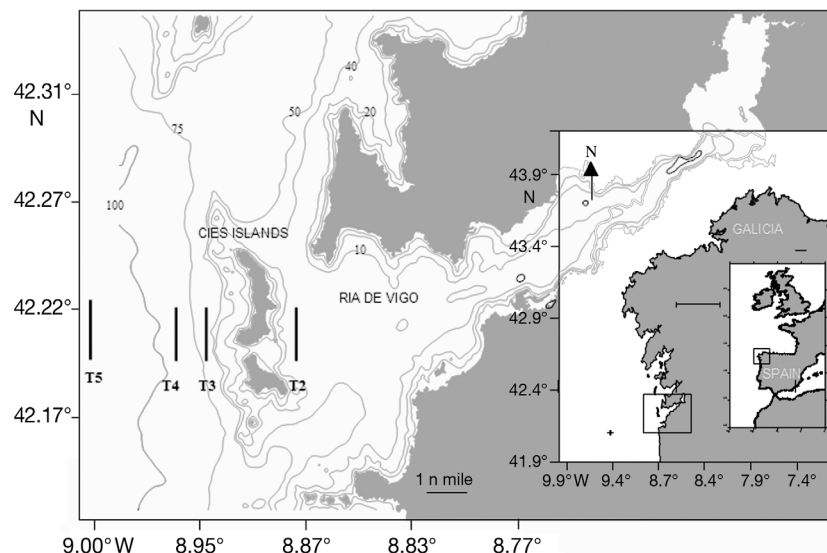


Fig. 1. Sampling area off the Ría de Vigo in Galician waters, NE Atlantic. T2–5: transects 2–5

and distribution patterns of the proboscis armature and variations in the spination of the anterior part of body are considered the most important determining features (Miller & Dunagan 1985). Morphological study was carried out to determine the cystacanth species. As the cystacanths normally presented the proboscis, neck, and part of the anterior trunk introverted, we dissected them to evert these structures. The body was cleared using the protocol described by Gregori et al. (2012) because this method does not damage DNA. The caudal part of the body was used for DNA extraction.

Scanning electron microscopy preparations in a Philips XL 30 were used to clarify the morphological examination. Infection parameters were estimated following Bush et al. (1997) and Rózsa et al. (2000). Sterne's exact 95 % confidence interval (CI) was calculated for prevalence (Reiczigel 2003).

Genomic DNA extraction and PCR amplification

Genomic DNA was isolated using the Qiagen DNeasy™ Tissue Kit according to the manufacturer's instructions. DNA quality and quantity was checked in a NanoDrop® ND-1000 spectrophotometer and in 1 % agarose gel. The primers 18SU467F (5'-ATC CAA GGA AGG 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 subunit (18S) ribosomal RNA gene. PCRs were performed in a total volume of 25 µl containing 1 µl of genomic DNA (150–200 ng), PCR buffer at 1× concentration, 1.5 mM MgCl₂, 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 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 PCRs.

DNA sequencing and phylogenetic analysis

Positive PCR products were cleaned for sequencing using ExoSAP-IT® (USB Corporation). Sequences were subjected to BLASTn analyses against available sequences from GenBank, through web servers of the National Center for Biotechnology Information (USA). All 18S rRNA sequences present in GenBank of the Class Palaeacanthocephala were

Table 1. Species and GenBank accession numbers of taxa used for 18S rDNA analyses

Taxon	GenBank accession no.
<i>Southwellina hispida</i>	JX014228, EU267809
<i>Arhythmorhynchus brevis</i>	AF064812
<i>Pseudocorynosoma constrictum</i>	EU267800
<i>Ibirhynchus dimorpha</i>	CQ981436
<i>Hexaglandula corynosoma</i>	EU267808
<i>Pseudocorynosoma anatarium</i>	EU267801
<i>Polymorphus minutus</i>	EU267806
<i>Profilicollis botulus</i>	EU267805
<i>Polymorphus</i> sp.	AF064815
<i>Polymorphus altmani</i>	AF001838
<i>Andracantha gravida</i>	EU267802
<i>Corynosoma enhydri</i>	AF001837
<i>Corynosoma magdaleni</i>	EU267803
<i>Corynosoma strumosum</i>	EU267804
<i>Bolbosoma vasculosum</i>	JX014225
<i>Bolbosoma balaenae</i>	JQ040306
<i>Plagiorhynchus cylindraceus</i>	AF001839
<i>Centrorhynchus conspectus</i>	U41399
<i>Centrorhynchus</i> sp.	AY830155
<i>Centrorhynchus microcephalus</i>	AF064813
<i>Serrasentis sagittifer</i>	JX014227
<i>Gorgorhynchoides bullocki</i>	AY830154
<i>Transvena annulospinosa</i>	AY830153
<i>Pararhadinorhynchus</i> sp.	HM545903
<i>Rhadinorhynchus pristis</i>	JQ061133
<i>Rhadinorhynchus</i> sp.	AY062433
<i>Acanthocephalus dirus</i>	AY830151
<i>Acanthocephalus lucii</i>	AY830152
<i>Filisoma bucerium</i>	AF064814
<i>Filisomaa rizalinum</i>	JX014229
<i>Echinorhynchida</i> sp.	EU732662
<i>Acanthocephaloides propinquus</i>	AY830149
<i>Echinorhynchus gadi</i>	AY218123, U88335, JX014222
<i>Rhadinorhynchus pristis</i>	JX014226
<i>Rhadinorhynchus lintoni</i>	JX14224
<i>Pomphorhynchus laevis</i>	JX014223, AY218124, AY423346
<i>Pomphorhynchus tereticollis</i>	AY423347
<i>Dentitruncus truttae</i>	JX460865
<i>Illiosentis</i> sp.	AY830158
<i>Pseudoleptorhynchoides lamothei</i>	EU090950
<i>Koronacantha pectinaria</i>	AF092433, AY830157
<i>Leptorhynchoides thecatus</i>	AF001840
<i>Pomphorhynchus bulbocoli</i>	AF001841
Outgroup: <i>Rotaria rotatoria</i>	AY218121
<i>Plationus patulus</i>	DQ297712

downloaded for phylogenetic analyses (n = 49). Additionally, 2 rotiferan sequences were downloaded as an outgroup, due to their close relationship with the acanthocephalans (García-Varela et al. 2000). Table 1 shows the species used for phylogenetic analyses and their accession numbers. These 18S rRNA sequences were aligned using MAFFT v5.7 (Katoh et al. 2002) with default settings. GBlocks

(Castresana 2000) were then used to identify and remove highly divergent regions and poorly aligned positions. Afterwards, a substitution model was selected under Akaike's information criterion (Akaike 1974) as implemented in jModeltest (Posada 2008). The GTR+I+G (Tavaré 1986) model was chosen to infer the evolutionary history using the maximum likelihood (ML) method. The analysis involved 51 nucleotide sequences with a total of 582 conserved sites in the final data set. Bootstrap probabilities with 1000 replications were calculated to assess reliability on each node of the ML tree. Evolutionary analyses were conducted in MEGA5 (Tamura et al. 2011).

RESULTS

A total of 20 cystacanths infecting *Nyctiphanes couchii* were found in different samples. Their prevalences (95% CI) are presented in Table 2. Estimations of accompanying zooplankton taxa and abundance, Shannon-Weaver index and species evenness where larvae were found are recorded in Table 3. Complete information about mesozooplankton composition in each community is available in Roura et al. (2013). Cystacanths were removed from the thoracic organs (Fig. 2A) of *N. couchii* adults. The body of the cystacanths was cylindrical and in most samples their neck and proboscis were invaginated. A detailed description is given for male and female specimens pooled because sex could not always be determined. Morphometric measurements are given as means \pm SD [range], with sample size in parentheses.

Description

Trunk long, uniformly cylindrical, 10.5 ± 3.9 mm [5.3–19.5 mm] long ($n = 18$) \times 0.55 ± 0.37 mm [0.33–1.87 mm] wide ($n = 15$), spinose anteriorly. Trunk spines in 2 fields separated by unarmed zone (Fig. 2B,C). Anterior trunk spines adjacent to neck, arranged in 2 or 3 circles, the 3rd ventrally not complete, posterior field restricted to ventral area (Fig. 2B), with ca. 10–14 rows of spines, reaching $23 \pm 3.5\%$ [15–28%] of trunk length ($n = 15$). Trunk spines 82 ± 20 μ m [53–118 μ m] long ($n = 163$ from 12 specimens), slightly longer in the posterior region, embedded in cuticular sheath. Neck cylindrical, 0.22 ± 0.10 mm [0.13–0.39 mm] long \times 0.18 ± 0.06 mm [0.12–0.23 mm] wide ($n = 5$). Proboscis slender, cylindrical, 2.26 ± 0.43 mm [1.55–3.32 mm] long \times 0.20 ± 0.06 mm [0.11–0.33 mm] wide ($n = 16$) (Fig. 2D). Hooks arranged in 14 (rarely 13) rows of 26 hooks (rarely 23–25) each ($n = 16$). Hooks of basal circle only slightly erected and longer than remaining spines (Fig. 2D). Range of hook (H) length (in μ m) at base as follows ($n = 10$ specimens except in H24–26, for which $n = 6$): H1: 93–131; H2: 62–99; H3: 79–98; H4: 67–110; H5: 74–110; H6: 69–111; H7: 78–120; H8: 87–114; H9: 88–124; H10: 94–128; H11: 90–123; H12: 91–122; H13: 94–118; H14: 86–110; H15: 77–111; H16: 85–123; H17: 96–122; H18: 87–113; H19: 92–116; H20: 85–114; H21: 77–122; H22: 85–111; H23: 68–108; H24: 85–99; H25: 68–98; H26: 66–88. Proboscis receptacle considerably longer than proboscis, 3.8 ± 0.5 mm [2.9–4.7 mm] long ($n = 16$) \times 0.18 ± 0.06 [0.22–0.28] mm wide ($n = 4$). Lemnisci not extending past receptacle, generally hidden behind

Table 2. *Nyctiphanes couchii* population divided into 3 different stages in each community during 2008, where cystacanths were found from Ría de Vigo, Galicia, NW Atlantic, Spain, in different samplings: summer frontal (SF), summer coastal (SC), autumn ocean (AO) and autumn coastal (AC). T5B: transect 5 at bottom; T2B: transect 2 at bottom; T3B: transect 3 at bottom. N: number of individuals estimated; Ab: abundance (no. m^{-3}); Population % [CI]: prevalence in the population [95% confidence interval]; Adults % [CI]: prevalence in adults [95% confidence interval]

Community	SF		SC		AO				AC			
	T5B, 2 Jul N	Ab	T2B, 2 Jul N	Ab	T5B, 26 Sep N	Ab	T5B, 1 Oct N	Ab	T5B, 9 Oct N	Ab	T3B, 10 Oct N	Ab
Calyptopis	36433	88.78	1423426	2097.96	2496	11.30	4060	25.52	475	2.13	165120	436.46
Furcilia	49388	120.35	37415	55.15	4512	20.44	480	3.02	175	0.78	92160	243.61
Adults	69955	170.47	328	0.48	3600	16.31	2620	16.47	2175	9.76	1920	5.07
Population	155775	380.60	1461169	2154.59	10608	48.05	7160	45.01	2825	13.67	259200	685.14
No. infected	3		1		12		1		1		2	
Population % [CI]	0.0019 [0.0004–0.002]		0.0001 [0.0000–0.00013]		0.068 [0.0372–0.0445]				0.0008 [0.0001–0.0008]			
Adults % [CI]	0.0043 [0.0125–0.0009]		0.3047 [0.0000–0.0169]		0.0014 [0.0912–0.2797]				0.1042 [0.0126–0.3758]			

Table 3. Mesozooplankton taxa collected in each community during 2008, where cystacanths were found from Ría de Vigo, Galicia, NW Atlantic, Spain, in different samplings: summer frontal (SF), summer coastal (SC), autumn ocean (AO) and autumn coastal (AC). T5B: transect number 5 at bottom; T2B: transect 2 at bottom; T3B: transect 3 at bottom. N: number of individuals estimated; Ab: abundance (no. m⁻³). Volumes filtered per transect were 410.37, 678.48, 220.78, 159.07, 222.92, and 3783.2 m³, respectively. -: taxon not found

Community	SF		SC		AO				AC			
	T5B, 2 Jul N	Ab	T2B, 2 Jul N	Ab	T5B, 26 Sep N	Ab	T5B, 1 Oct N	Ab	T5B, 9 Oct N	Ab	T3B, 10 Oct N	Ab
MEROPLANKTON												
Cephalopoda												
Loliginidae	-	-	8	0.01	2	0.01	-	-	1	0.004	4	0.01
<i>Octopus vulgaris</i>	-	-	1	0.001	1	0.005	3	0.01	2	0.01	13	0.03
Sepioidae	7	0.08	1	0.001	-	-	-	-	-	-	3	0.01
Cirripedia												
Cirripedia larvae	-	-	4267	6.29	-	-	-	-	-	-	170880	451.69
Echinodermata												
Echinoidea larvae	-	-	1422	2.10	-	-	-	-	-	-	3840	10.15
Ophiuroidea larvae	512	1.28	28444	41.92	-	-	-	-	-	-	353280	933.82
Fish												
Fish eggs	-	-	-	-	-	-	-	-	-	-	1920	5.07
Fish larvae	512	1.25	1046	1.54	29	0.13	2	0.01	6	0.03	1969	5.20
Gastropoda												
Gastropoda larvae	-	-	12800	18.87	-	-	-	-	25	0.11	24960	65.98
Isopoda												
Aegidae	-	-	50	0.07	-	-	-	-	-	-	24	0.06
Decapoda												
Alpheidae zoeae	-	-	1422	2.10	-	-	-	-	25	0.11	-	-
Brachyura juvenile	-	-	7	0.01	2	0.01	1	0.01	-	-	-	-
Brachyura megalopa	-	-	-	-	-	-	-	-	-	-	1920	5.07
Brachyura zoeae	2463	6.00	45511	67.08	-	-	40	0.25	25	0.11	24960	65.98
Crangonidae zoeae	-	-	1422	2.10	-	-	-	-	-	-	-	-
Paguridae megalopa	480	1.17	-	-	-	-	-	-	-	-	-	-
Paguridae zoeae	-	-	4267	6.29	-	-	20	0.13	-	-	9600	25.38
Palaemonidae zoeae	-	-	1422	2.10	-	-	-	-	-	-	-	-
<i>Pisidia longicornis</i> megalopa	-	-	-	-	-	-	-	-	-	-	1920	5.07
<i>Pisidia longicornis</i> zoeae	-	-	15644	23.06	-	-	-	-	-	-	17280	45.68
<i>Porcellana platycheles</i> zoeae	-	-	5689	8.38	-	-	-	-	-	-	15360	40.60
Processidae zoeae	-	-	533	0.92	-	-	-	-	-	-	600	11.41
<i>Jaxea nocturna</i>	-	-	1422	2.10	-	-	-	-	-	-	-	-
Amphipoda												
Caprellidea	-	-	6	0.009	-	-	4	0.02	1	0.004	-	-
Gammaridea	85	0.201	65	0.10	6	0.03	2	0.01	24	0.11	176	0.46
Stomatopoda												
<i>Meiosquilla desmaresti</i>	480	1.17	-	-	-	-	-	-	-	-	-	-
Polychaeta												
Polychaeta larvae	5	0.01	49	0.07	35	0.16	6	0.04	8	0.04	21	0.06
HOLOPLANKTON												
Appendicularia												
Appendicularia	-	-	7111	10.48	-	-	-	-	-	-	71040	187.78
Amphipoda												
Hyperidea	-	-	1	0.001	5	0.02	9	0.06	17	0.08	13	0.03
Chaetognatha												
Chaetognatha	-	-	11942	17.60	1861	8.43	1220	7.67	775	3.48	96000	253.76
Cnidaria												
Cnidaria	-	-	-	-	-	-	-	-	-	-	5760	15.22
Cladocera												
<i>Evadne nordmanni</i>	-	-	14222	20.96	-	-	-	-	-	-	36480	96.43
<i>Podon intermedius</i>	-	-	36978	54.50	-	-	-	-	-	-	55680	147.18
Hydrozoa												
Siphonophora	1536	3.74	5317	7.84	74	0.33	-	-	300	1.35	1920	5.07

Table 3 (continued)

Community	SF		SC		AO				AC			
	T5B, 2 Jul N	Ab	T2B, 2 Jul N	Ab	T5B, 26 Sep N	Ab	T5B, 1 Oct N	Ab	T5B, 9 Oct N	Ab	T3B, 10 Oct N	Ab
Euphausiacea												
<i>Nyctiphanes couchii</i> calyptopis	36433	88.78	1423426	2097.96	2496	11.30	4060	25.52	475	2.13	165120	436.46
<i>N. couchii</i> furcilia	49388	120.35	37415	55.15	4512	20.44	480	3.02	175	0.78	92160	243.61
<i>N. couchii</i> adult	69955	170.47	328	0.48	3600	16.31	2620	16.47	2175	9.76	1920	5.08
Copepoda												
Calanoidea												
<i>Acartia clausi</i>	7421	18.08	93867	138.35	1263	5.721	260	1.63	3350	15.03	99840	263.91
<i>Candacia armata</i>	–	–	1422	2.10	–	–	–	–	–	–	–	–
<i>Calanoides carinatus</i>	72259	176.08	2844	4.19	1600	7.25	20	0.13	575	2.58	7680	20.30
<i>Calanus helgolandicus</i>	35156	85.67	1422	2.10	1853	8.39	100	0.63	475	2.13	–	–
<i>Centropages chierchiae</i>	3966	9.67	14222	20.96	–	–	20	0.13	75	0.34	1920	5.07
<i>Centropages typicus</i>	–	–	–	–	–	–	–	–	–	–	1920	5.07
<i>Clausocalanus</i> spp.	–	–	–	–	–	–	100	0.63	100	0.45	3840	10.15
<i>Diaixis pygmaea</i>	–	–	1422	2.10	–	–	–	–	25	0.11	–	–
<i>Isias clavipes</i>	–	–	1422	2.10	–	–	–	–	–	–	1920	5.07
<i>Mesocalanus tenuicornis</i>	–	–	–	–	253	1.14	–	–	–	–	–	–
<i>Metridia lucens</i>	–	–	1422	2.10	1432	6.48	–	–	100	0.45	–	–
<i>Paracalanus parvus</i>	–	–	–	–	253	1.14	–	–	575	2.58	1920	5.07
<i>Paraeuchaeta hebes</i>	8637	21.05	–	–	7326	33.18	220	1.38	850	3.81	1920	5.07
<i>Paraeuchaeta</i> sp.	4797	11.69	–	–	20463	92.68	1700	10.69	2225	9.98	15360	40.60
<i>Pseudocalanus elongatus</i>	–	–	–	–	–	–	20	0.13	25	0.11	–	–
<i>Subeucalanus crassus</i>	–	–	–	–	–	–	20	0.13	–	–	–	–
<i>Temora longicornis</i>	–	–	28444	41.92	–	–	–	–	–	–	9600	25.38
Cyclopoidea												
<i>Oithona plumifera</i>	–	–	–	–	–	–	–	–	250	1.12	5760	15.22
Mysidacea	1919	4.68	–	–	528	2.39	360	2.26	475	2.13	1920	5.07
Thaliacea												
Salpida	21175	51.60	4267	6.29	3874	17.54	1780	11.19	1900	8.52	13440	35.53
Polychaeta												
<i>Tomopteris</i> spp.	–	–	–	–	–	–	–	–	25	0.11	–	–
Total	317185	773.93	1814417	2674.37	51466	233.11	13067	82.15	15084	67.66	1321863	3503.90
Shannon's Index (H')	1.55	0.507	2.12	1.49	2.31	2.37						
Evenness index	0.54	0.139	0.71	0.48	0.69	0.64						

it but apparently reaching half of proboscis receptacle ($n = 2$). Terminal gonopore. A voucher specimen was deposited at the Natural History Museum of London, UK, with the accession number NHMUK 2013.4.2.1

Amplified sequences of 18S rRNA ranged from 814 to 819 bp. These sequences are available on GenBank under the accession numbers JQ061133–JQ061136. BLAST search showed close homology (98%) with the 18S rRNA of *Rhadinorhynchus* sp. and *Pararhadinorhynchus* sp. (Johnston & Edmonds, 1947). The ML tree inferred from the 18S rRNA data set of Palaeacantocephala revealed that our specimens belong to a highly supported clade (bootstrap values of 100), with *Rhadinorhynchus* sp., *Pararhadinorhynchus* sp. and *Transvena annulospinosa* (Pichelin & Cribb 2001) (Fig. 3). Unexpectedly, sequences of *Rhadinorhyn-*

chus pristis and *R. lintoni* (Cable & Linderoth 1963), recently described by Verweyen et al. (2011), are nested in a highly supported group with *Pomporhynchus* (Monticelli, 1905) species, displaying homologies of 99% with those species. Comparing our data against the sequences of Verweyen et al. (2011) revealed homology of only 84%. The ML tree showed the monophyly of Polymorphida and the paraphyly of Echinorhynchida, the 2 orders found within Palaeacantocephala. In fact, Rhadinorhynchidae was the most polyphyletic family among the Echinorhynchida.

DISCUSSION

Cystacanth found in *Nyctiphanes couchii* can be undoubtedly assigned to the genus *Rhadinorhynchus*

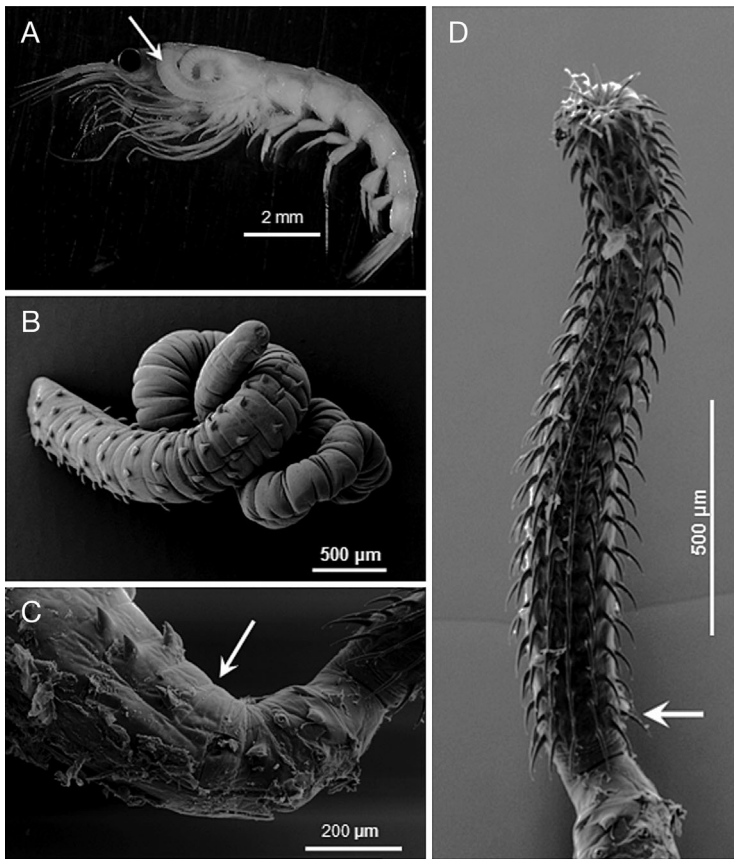


Fig. 2. Cystacanth of *Rhadinorhynchus* sp. from *Nyctiphanes couchii*. (A) Single infection with one cystacanth of *Rhadinorhynchus* sp. in the cephalothorax cavity (arrow). (B) The spines of the anterior part of the body, general view. (C) Two fields of spines separated by a space without spines (arrow). (D) Cystacanth's proboscis. Basal circle perpendicular hooks (arrow)

based on the patterns of trunk armature and proboscis morphology (see Petrochenko 1956, Golvan 1969, Amin et al. 2011). *Pararhadinorhynchus* and *Transvena*, which belong to the same clade as *Rhadinorhynchus* (Fig. 3), can be readily distinguished using their taxonomy because a key character for species of the genus *Pararhadinorhynchus* is the absence of trunk spines. The morphotype of species of *Transvena* is distinguished from other acanthocephalans because their trunk possesses a single ring of posteriorly pointing spines, at or near the junction between the neck and trunk (Pichelin & Cribb 2001). In contrast, all our examined specimens possess extended fields of spines on the trunk.

According to the most recent key to species of *Rhadinorhynchus* (Amin et al. 2011), our specimens belong to a group of 20 species that combine 2 character states that are apparently stable: (1) trunk spines in 2 fields clearly separated by an unarmed

zone; and (2) dorsal spines absent in the posterior field. Among these, only a single species, namely *Rhadinorhynchus saltatrix* (Troncy & Vassiliades 1973), exhibits the combination of a proboscis armature with 14 rows of hooks and a field of posterior trunk spines with >10 spine rows (Amin et al. 2011). The description of *R. saltatrix* is well detailed but based only on a few specimens, 5 males and 8 females (Troncy & Vassiliades 1973). Our specimens agree with the description made by Troncy & Vassiliades (1973) except that the number of hooks per row that they report is 24 in *R. saltatrix* while 26 hooks per row are more frequently found in our specimens. However, it is important to note that our specimens closely resemble *R. pristis* and *R. selkirki* (Van Cleave, 1921), except that the number of spine rows in the posterior field of the trunk is smaller (≤ 10) in these species (see Amin et al. 2011).

From the above results a key question is the range of variability of this spiny field, and the factors that may influence this trait. For instance, there is evidence of clear sexual differences of this trait in many species (see e.g. Petrochenko 1958, Cable & Linderroth 1963, Troncy & Vassiliades 1973). In contrast, the status of *Rhadinorhynchus pristis* is currently rather confused. Amin et al. (2011) list 7 species that, in their opinion, were erroneously identified as *R. pristis*, but they consider *R.*

selkirki as a valid species. In contrast, Chandler (1934) and Petrochenko (1956) consider *R. selkirki* as synonym of *R. pristis*. To compound the problem, other available descriptions of *R. pristis* (e.g. Rego 1987, Arai 1989, Bunkley-Williams & Williams 1996) were not included in the revision by Amin et al. (2011) and do not fulfil the diagnostic traits of this species sensu these authors.

According to the above morphological discussion, we should tentatively identify our specimens as *Rhadinorhynchus saltatrix*, pending a critical re-examination of *R. pristis* and related species.

Once the morphological identification was confirmed, genetic homology using 18S rRNA sequences allowed us to assign the cystacanths to the genus *Rhadinorhynchus* (Fig. 3). The 18S (SSU sequences) have been broadly used in different research as a taxonomic tool to clarify the taxonomy of acanthocephalans at the species level (Near et al. 1998, Gar-

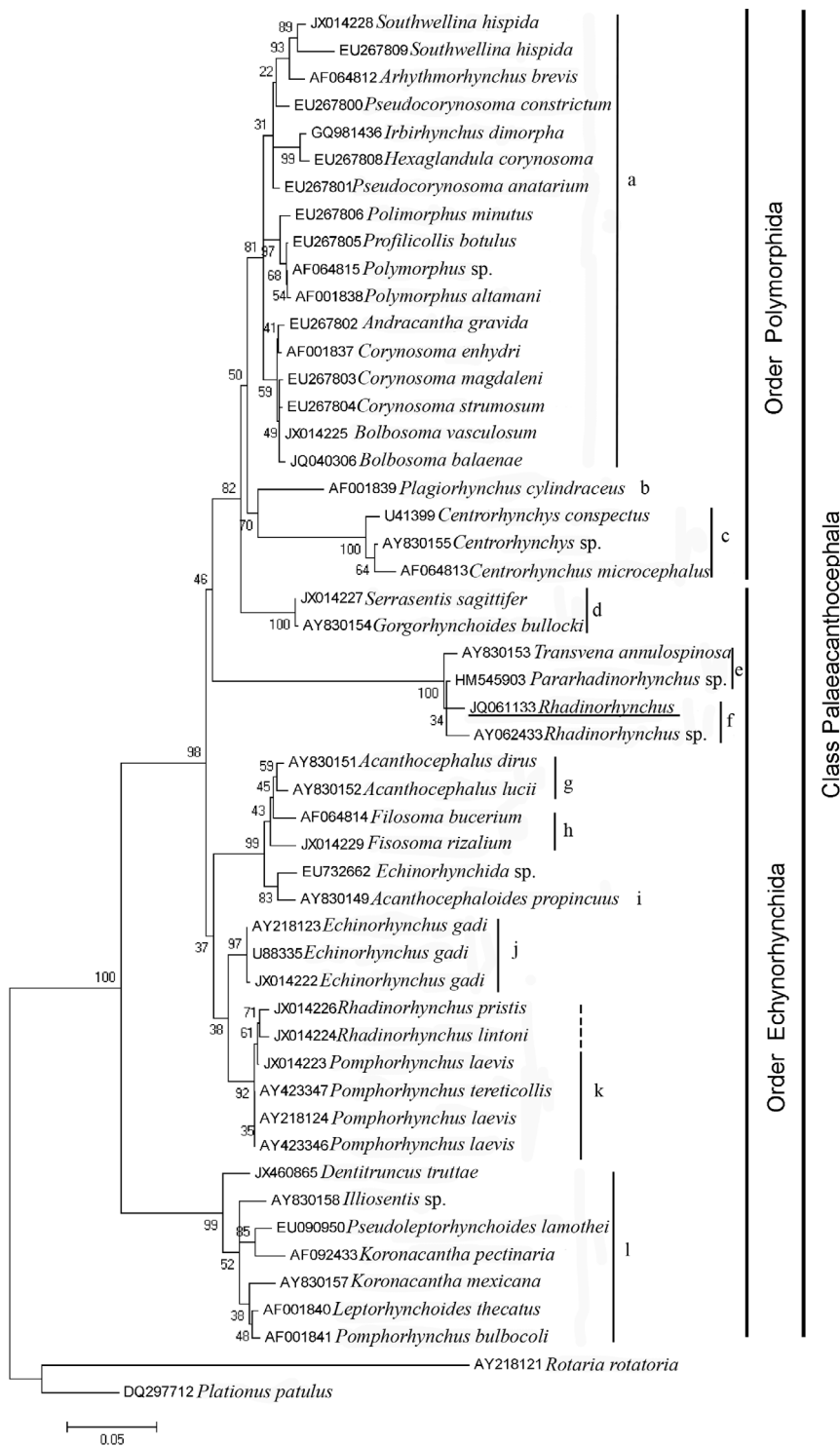


Fig. 3. Maximum likelihood consensus tree after 1000 bootstraps showing the phylogenetic relationships of the Palaeacanthocephala using 18S rRNA sequences and Rotifera as the outgroup. Abbreviations: a, Polymorphidae; b, Plagiorhynchidae; c, Centrorhynchidae; d, Rhadinorhynchidae; e, Transvenidae; f, Rhadinorhynchidae; g, Echinorhynchidae; h, Cavisomidae; i, Arhythmacanthidae; j, Echinorhynchidae; k, Pomphorhynchidae; l, Illiosentidae. Underlined species correspond with cystacanths found infecting *Nyctiphanes couchii*

cía-Varela et al. 2000, 2002, Herlyn et al. 2003, Near 2002, García-Varela & Nadler 2005, 2006, García-Varela & González-Oliver 2008, Gregori et al. 2012). At the genetic level, our identification disagrees with the results obtained by Verweyen et al. (2011), whose sequences correspond with *R. pristis* and *R. lintoni*. Phylogenetic analyses showed almost the same topology, but the position of *Rhadinorhynchus* species is markedly different. Our sequences appeared in a well-supported clade with members of the family Transvenidae, *Transvena annulospinosa* and *Rhadinorhynchus* sp. as reported by García-Varela & Nadler (2005) and García-Varela & González-Oliver (2008). However, *R. pristis* and *R. lintoni* identified by Verweyen et al. (2011) appeared within the *Pomporhynchus* group. This contradiction is due to the absence of *Pararhadinorhynchus* sp. and *Rhadinorhynchus* sp. sequences in the Bayesian analysis carried out by Verweyen et al. (2011). The omission of these 2 sequences placed their *R. pristis* and *R. lintoni* far from *Transvena annulospinosa*, which was a clade strongly supported with a bootstrap confidence of 100% (García-Varela & Nadler 2005, García-Varela & González-Oliver 2008). Our results suggest that genetic identification of *Rhadinorhynchus* by Verweyen et al. (2011) should be revised. Another explanation would be that *Rhadinorhynchus* is a polyphyletic genus, since Rhadinorhynchidae is a polyphyletic family as shown by morphological, molecular and cladistic studies (Herlyn et al. 2001, Monks 2001, García-Varela et al. 2002, García-Varela & Nadler 2005, García-Varela & González-Oliver 2008). It is clear that the paraphyly of Rhadinorhynchidae requires re-examination and reclassification and even the creation of new fami-

lies. As suggested by Pichelin & Cribb (2001) and García-Varela & Nadler (2005), the group formed by *Serrasentis sagittifer* (Linton, 1889) and *Gorgorhynchoides bullocki* (Cable & Mafarachisi, 1970), plus *Golvanorhynchus* (Noronha, Fabio & Pinto, 1978), which form a sister group of Polymorphida, should be removed from the Rhadynorhynchidae. Our results support the transfer of *Leptorhynchoides* (Kostylev, 1924) and *Pseudoleptorhynchoides* (Salgado-Maldonado, 1976) (both Rhadynorhynchidae) to the family Illiosentidae, as suggested by García-Varela & González-Oliver (2008). This way the Illiosentidae would be a monophyletic clade. In our work the order Polymorphida is monophyletic, in contrast with the works of García-Varela & Nadler (2005), García-Varela & González-Oliver (2008) and Verweyen et al. (2011). This difference may result from the larger data set analysed in this work, 49 Palaeacanthocephalan sequences, versus the 19, 20 and 36 used by García-Varela & Nadler (2005), García-Varela & González-Oliver (2008) and Verweyen et al. (2011), respectively. Apart from *R. pristis* and *R. lintoni*, our phylogenetic analysis highlights the possible misidentification of *Pomporhynchus bulbocoli* (Linkins in Van Cleave, 1919) as a member of the monophyletic clade Illiosentidae and *Echinorhynchida* sp. (Cobbold, 1879) (Fig. 3).

This is the first time that *Rhadynorhynchus* sp. has been found in the euphausiid *Nyctiphanes couchii*. Euphausiids are an essential and abundant nexus between the mesozooplankton and nekton, ingested by fishes, cephalopods (both acting as paratenic host) and birds (Deagle et al. 2007, Braley et al. 2010, Roura et al. 2012).

Reports of larval acanthocephalans acting as intermediate hosts in zooplankton are scarce. Among the Palaeacanthocephala some *Echinorhynchus corrugatus* have been found in *Euphausia krohnii* (Marcogliese 1995). *Bolbosoma caenoforme* has been found infecting *Thysanoessa longipes* and *T. raschii* (Shimazu 1975), whereas *Bolbosoma* sp. (Porta, 1908) has been detected in *Thysanoessa* sp. (Brandt, 1851) (Tsimbalyuk 1980). Lindley (1977) reported 3 larvae of Paleacanthocephala infecting the euphausiid *T. longicaudata*. Recently, 3 larval stages of Polymorphida (*Bolbosoma* or *Corynosoma*) were reported within *Nyctiphanes simplex* on the northwestern coast of Mexico (Gómez-Gutiérrez et al. 2010). Finally, cystacanths of *Bolbosoma balaenae* were found in *Nyctiphanes couchii* in the NE Atlantic (Gregori et al. 2012). Therefore, only acanthocephalans the orders Echinorhynchida and Polymorphida have been found in euphausiids. This fact may be

related to their final hosts, with Echinorhynchida infecting mainly teleost fishes and occasionally amphibians and reptiles, whereas Polymorphida infect mainly birds and marine mammals (Bush et al. 2001).

Rhadynorhynchus is a generalist at the definitive host level. However, at the zooplankton level, it demonstrated some specificity for krill. This specificity is reinforced because we did not find cystacanths of *Rhadynorhynchus* sp. in the larvae of *Nyctiphanes couchii* (calyptopis and furciliars with 0.8–2.20 and 2.2–5.5 mm in total length, respectively). We also did not find them in copepod species or other taxa, probably due to the large size of these cystacanths (~11.41 mm). We suspect that only adults of *N. couchii* could harbour them in their cephalothorax (12–17 mm length). Consequently, it seems that the smaller zooplankton organisms cannot harbour large cystacanths or act as intermediate hosts for these acanthocephalans. Moreover, the large size of the cystacanths we found probably acts to limit the intensity to 1, because none of the examined *N. couchii* showed more than one cystacanth per individual, as described in Gómez-Gutiérrez et al. (2010) and Gregori et al. (2012).

The prevalence of *Rhadynorhynchus* sp. within the euphausiid population was very low (Table 2). This is usually considered a feature of the zooplankton trophic level because of the dilution effect of the pelagic realm where it becomes difficult to find a suitable intermediary host (Marcogliese 2002). Nevertheless, since most predators ingest large quantities of krill, euphausiids become significant intermediate or paratenic hosts that originate high infection rates and intensities in the final parasite's hosts (Marcogliese 1995, 2002). Despite the fact that low prevalence is a feature at the zooplankton level, information about it is very scarce. Nevertheless, our prevalences are similar to those reported by Shimazu (1975) with infection rates about 0.219% in *Thysanoessa longipes* and 13.33% in *T. raschii*. Gómez-Gutiérrez et al. (2010) reported an average of prevalence about 3.1% in *Nyctiphanes simplex* and Gregori et al. (2012) reported a prevalence of 0.10% in *N. couchii*.

The ecological impact of cystacanths can be better understood if we consider the whole mesozooplanktonic community where the sample was taken. Roura et al. (2013) defined 6 characteristic mesozooplankton communities in the Ría de Vigo during the upwelling season following the bathymetric gradient, 3 in early summer and 3 in autumn, named as coastal, frontal and oceanic. These 6 communities

changed from summer to autumn due to a shift from relaxation-downwelled to upwelled conditions coupled with life-cycle changes in the zooplankton. We found cystacanths in the coastal (SC) and frontal (SF) summer communities as well as coastal (AC) and oceanic (AO) autumn communities (Table 3). A total of 2079 and 185 107 adults of *Nyctiphanes couchii* were counted in SC and SF, respectively, and therefore the number of potential infected adults would be 6 and 8 in these 2 communities. In autumn communities under upwelling conditions the number of adults of *N. couchii* was 3363 and 16 741 individuals in AC and AO. Accordingly, 4 and 24 would be the inferred number of infected adults in each community, respectively. These results suggest that the recruitment of parasites may be affected by the oceanography (Pascual et al. 2007).

In conclusion, we would like to emphasize that this is the first record of *Rhadinorhynchus* sp. in *Nyctiphanes couchii* in coastal waters of the NW Iberian Peninsula, with *N. couchii* probably acting through predator–prey interactions as an intermediate host. The results of our morphological and phylogenetic study, along with the available epidemiological information on *R. pristis* infection in Scombridae and Xiphidae fishes from the nearby Portugal coast, the Madeira Islands and the North Atlantic, suggest that the cystacanths herein described probably belong to this species (Rodrigues et al. 1975, Vassiliades 1982, Hogans et al. 1983, Rego 1987, Costa et al. 2004). However, we strongly recommend that a thorough review of the species, as well as the family Rhadinorhynchidae, should be carried out.

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