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## Description of a New Epibiotic Relationship (Suctorian - Copepoda) in NE Atlantic waters: from Morphological to Phylogenetic Analyses

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### ABSTRACT

*Paraeuchaeta hebes* is one of the most important carnivorous copepods in the coastal upwelling system off Galician waters (Ría de Vigo, NE Atlantic). A suctorian epibiont of the genus *Pelagacineta* was found attached to the surface of these copepods. The abundance and distribution on the copepod surface was analyzed, taking into account the sex of the crustacean, revealing some preference for females and also a different attachment point in both sexes. The morphological and molecular study allowed us to identify a new species of this Suctorian epibiont as *Pelagacineta hebensis*. A maximum-likelihood estimation (ML) tree inferred from the 18S rRNA gene revealed that this species belongs to the Phyllopharingea, showing a highly supported sister relationship with *Paracineta limbata*.

### INTRODUCTION

Epibiotic associations are common in marine crustaceans. This facultative association which involves two organisms (the epibiont and the basibiont) is known as

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3 epibiosis (Wahl 1989). The term epibiont comprises organisms that, during the sessile  
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5 phase of their life cycle, are fixed to the surface of a living substratum, while the  
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7 basibiont carries and constitutes a support for the epibiont (Threlkeld *et al.* 1993). An  
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9 important number of ciliates have been described as epibionts in many crustacean  
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11 groups like amphipods, branchiopods, copepods, ostracods, mysids, euphausiids or  
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13 decapods (Fernandez-Leborans *et al.* 2002; Fernandez-Leborans *et al.* 1997; Fernandez-  
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15 Leborans and Tato-Porto 2000a, b; Fernandez-Leborans and Tato-Porto 2002). Some of  
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17 these crustaceans may constitute an important part of the zooplankton (Roura *et al.*  
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19 2013), which act as substrata for the epibionts and also as intermediate or final hosts of  
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21 different parasite species (Chatton 1920; Fernandez-Leborans *et al.* 2002; Fernandez-  
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23 Leborans and Tato-Porto 2002; Gómez *et al.* 2009; Gregori *et al.* 2012, 2013; Ho and  
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25 Perkins 1985; Skovgaard *et al.* 2012; Skovgaard *et al.* 2005; Skovgaard *et al.* 2007;  
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27 Skovgaard and Saiz 2006). Among ciliate species, suctorians have been described as  
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29 epibionts of copepods (Fernandez-Leborans and Tato-Porto 2000a). These stalked  
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31 ciliates do not penetrate the tegument of the copepod. However, the effects produced to  
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33 the host are widely studied (Fernandez-Leborans 2010).

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39 Copepods are by far, the most abundant organisms on earth, as well as a key link of  
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41 marine food webs. As previously mentioned, the copepod surface seems to be a suitable  
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43 habitat for many genera of Suctorian epibionts: *Acineta*, *Branchyosoma*, *Conchacineta*,  
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45 *Cucumophrya*, *Choanophrya*, *Dentacineta*, *Dentacinetides*, *Ephelota*, *Lecanophrya*,  
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47 *Lecanophryella*, *Loricodendron*, *Ophryodendron*, *Paracineta*, *Pelagacineta*,  
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49 *Praethecacineta*, *Pseudocorynophrya*, *Rhabdophrya*, *Rhyncheta*, *Thecacineta*,  
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51 *Tokophrya*, *Trematosoma* and *Trichophrya* have been described on Fernandez-Leborans  
52  
53 and Tato-Porto (2000a). Although Fernandez-Leborans and Tato-Porto (2000a)  
54  
55 extensively reviewed the species of copepod acting as basibionts, *Paraeuchaeta hebes*  
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3 Giesbrecht, 1888 was not mentioned in their work. This copepod is one of the most  
4 important carnivorous found in the mesozooplanktonic communities of the Galician  
5 coastal upwelling system (Roura *et al.* 2013). Several specimens of *P. hebes* were found  
6 with an unknown ciliate colonizing their bodies.  
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12 Accordingly, the aim of this work was to carry a morphological study to identify  
13 the epibiont, accompanied with a detailed study of their location on the body of *P. hebes*  
14 to study if the epibiont display any preference for certain parts of the copepod  
15 Moreover, a molecular analysis was carried to confirm the phylogenetic position of the  
16 epibiont and supply additional molecular information for future studies on this  
17 assemblage.  
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## 26 MATERIALS AND METHODS

### 27 *Biological sampling*

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30 The zooplankton samples were caught in the Ría de Vigo (NW Iberian  
31 Peninsula) on board of the RV *Mytilus* (Fig.1). Ten surveys were undertaken at night, in  
32 the summer and autumn of 2008. Samples were collected by double oblique towing,  
33 using a 750 mm diameter bongo net equipped with 375  $\mu\text{m}$  mesh. At a ship's speed of 2  
34 knots, the bongo net was first lowered and stabilized near the bottom for a period of 15  
35 min, then hauled to the surface at  $0.5 \text{ ms}^{-1}$ . The sample was fixed on board with 100%  
36 ethanol. Samples were later transferred to 70% ethanol in the laboratory and stored at -  
37  $20^{\circ}\text{C}$ . Six species of the most abundant copepods were analysed for epibionts within the  
38 samples collected in summer: *Acartia clausii* Giesbrecht, 1889, *Temora longicornis*  
39 Müller O.F. 1785, *Calanus helgolandicus* Claus, 1863, *Calanoides carinatus* Krøyer,  
40 1849, *Centropages chierchiae* Giesbrecht, 1889 and *Paraeuchaeta hebes*.  
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### *Collection and processing of epibionts*

Basibionts (the six species of copepods above mentioned) were separately counted by sex and examined for epibionts using a stereomicroscope (20 x). When epibionts were detected, they were isolated and treated using the silver carbonate technique, according to the procedure described by Fernandez-Leborans and Castro de Zaldumbide (1986), and also with methyl green and neutral red. The distribution and number of epibionts on the anatomical parts of the basibionts was further analyzed. Sizes of epibionts were determined using an ocular micrometer. Light microscope images and morphometry of the epibionts were obtained using Image Analysis (KS300 Zeiss). Scanning electron microscopy (SEM) preparations in a Philips XL 30 were used to enhance the morphological examination. Voucher specimens were deposited at the Natural History Museum of London, UK, with the accession numbers NHM 2013.4.2.2, NHM 2013.4.2.3, NHM 2013.4.2.4, and NHM 2013.4.2.5.

### *Genomic DNA extraction and PCR amplification*

Genomic DNA was isolated using Qiagen DNeasy™ Tissue Kit according to manufacturer's instructions. DNA quality and quantity was checked in a spectrophotometer Nanodrop® ND-1000 (Nanodrop technologies, Inc) and in 1% agarose gel. 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 a little fraction (521-788 bp) of the small subunit (18S) ribosomal RNA gene. PCR reactions were performed in a total volume of 25 µl containing 1 µl of genomic DNA (50-100 ng), 2.5µl 10x PCR buffer, 0.2 µl MgCl<sub>2</sub>, 0.5µl nucleotides (Roche Applied Science), 0.75µl 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, 30 s at 55 °C and 2 min at 72 °C, followed by 7 min at 72 °C.

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3 All PCRs were carried out in a TGradient thermocycler (Biometra) and a negative  
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5 control (distilled water) was included for each set of PCR reactions.  
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#### 8 *DNA sequencing and phylogenetic analysis*

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10 Positive PCR products were cleaned for sequencing using ExoSAP-IT<sup>®</sup> (USB  
11 corporation). Sequences were subject to BLASTn analyses against available sequences  
12 from GenBank through web servers of the National Center for Biotechnology  
13 Information (USA). All 18S rRNA sequences present in GenBank of the Class  
14 Phyllopharyngea were downloaded for phylogenetic analyses (n=17). Additionally, two  
15 sequences belonging to Nassophorea and Kariolelictea were used as outgroup, due to its  
16 close relation with the Phyllopharyngea. Table 1 shows the species used for  
17 phylogenetic analyses and their accession numbers. These 18S rRNA sequences were  
18 first aligned using Clustal W implemented in Bioedit 7.0 (Hall 1999). GBlocks  
19 (Castresana 2000) were then used to identify and remove highly divergent regions and  
20 poorly aligned positions. Afterwards, a substitution model was selected under the  
21 Akaike information criterion (Akaike 1974) as implemented in jModeltest (Posada  
22 2008). The GTR+I+G (Tavaré 1986) model was chosen to infer the evolutionary history  
23 by using the Maximum Likelihood (ML) method. The analysis involved 26 nucleotide  
24 sequences with a total of 364 conserved sites in the final dataset. Bootstrap probabilities  
25 with 1000 replications were calculated to assess reliability on each node of the ML tree.  
26 Evolutionary analyses were conducted in MEGA5 (Tamura *et al.* 2011).  
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## 48 **RESULTS**

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50 The suctorians observed on *Paraeuchaeta hebes* (Fig. 2A, B) were identified as  
51 loricate ciliates. Their lorica was thecostyle type (prolongation of the stalk) and was  
52 surrounded, as much, a half lower body of the ciliate (Fig. 2C). The funnel-shaped  
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3 lorica was 84.60-108.00  $\mu\text{m}$  long (Fig. 2C), with a maximum width of 88.36-118.70  
4  
5  $\mu\text{m}$ . Some young specimens presented a reduced lorica like a hat-shaped structure in  
6  
7 contact with the rear end of the ciliated body. The lorica extended through the posterior  
8  
9 part of the body in a narrow stalk (85.60-233.00  $\mu\text{m}$ ), which finished on an oval basal  
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11 disk (Fig. 2D). Longitudinal striations were clearly observed covering the stalk surface  
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13 (Fig. 2E). The body of the suctorian was ovoid (Fig. 2F) with a length of 60.16-97.60  
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15  $\mu\text{m}$  and 50.76-70.83  $\mu\text{m}$  in width (Table 2). Numerous tentacles sticking out through the  
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17 different parts of the surface of the body thus they were not in contact with the lorica  
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19 (Fig. 2G). There were 54-142 similar capitate tentacles that were highly contractile (Fig.  
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21 2H). The macronucleus (Ma) was located centrally in the body and it was oval,  
22  
23 sometimes transversely elongated (31.20-40.36  $\mu\text{m}$  long, 23.20-32.84  $\mu\text{m}$  width). Near  
24  
25 the Ma was a small and dense spherical micronucleus (Fig 2I). Some specimens showed  
26  
27 buds in their body (Fig. 3A). The budding is endogemmic, with a unique bud  
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29 (monogemmic) or with more than one (polygemmic) (Fig. 3B, 3C). These buds will  
30  
31 develop into asymmetric and elongated swimmers with a long between 17.40-20.80  $\mu\text{m}$   
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33 and a width between 7.20-8.80  $\mu\text{m}$  (Fig. 3D).  
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#### 39 *Location on the basibiont*

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43 Overall, 39,030 copepods divided into 3,152 *C. helgolandicus*, 14,930 *C.*  
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45 *carinatus*, 1,240 *C. chiercheae*, 10,785 *A. clausii*, 2,680 *P. hebes* and 6,242 *T.*  
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47 *longicornis* were examined for protozoans. The suctorian ciliates were exclusively  
48  
49 found attached to the surface of *P. hebes*. A total of 114 males carried about 643  
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51 epibionts whereas 228 females bore about 1,461 (Table 3). Ciliates were encountered on  
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53 the buccal appendages in a very low percentage. The preferred sites of attachment  
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55 differed among sexes. The percentage of attachment of the epibionts in males, in  
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3 decreasing order of importance was: leg 5 (L<sub>5</sub>), leg 4 (L<sub>4</sub>), urosome (U), metasome (M),  
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5 leg 3 (L<sub>3</sub>), caudal ramus (CR), cephalosome (C) and genital segment (G). In females  
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7 was: G, U, CR, M, C, L<sub>4</sub>, L<sub>3</sub>, leg 2 (L<sub>2</sub>) (Fig. 4).  
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11 *Taxonomic position.*  
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13  
14 Phylum Ciliophora Doflein, 1901  
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16  
17 subphylum Intramacronucleata Lynn, 1996  
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19  
20 class Phyllopharyngea De Puytorac et al., 1974  
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22  
23 subclass Suctorian Claparède & Lachmann, 1858  
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25  
26 order Endogenida Collin, 1912  
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28  
29 family Tokophryidae Jankowski in Small & Lynn, 1985  
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31  
32 genus *Pelagacineta* Jankowski, 1978  
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35 *Pelagacineta hebensis* sp. n.  
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38 *Diagnosis of Pelagacineta hebensis* sp. n.  
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41 *Pelagacineta hebensis* has an ovoid body, often wider than long, with a length of  
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43 84.60-108.00 µm, and a width of 88.36-118.70 µm. A funnel-shaped lorica, thecostyle  
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45 type, surrounds at least half of the lower body of the ciliate. The lorica is extended  
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47 through the posterior part of the body in a narrow stalk, which is finished on an oval  
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49 basal disk. The surface of this stalk is covered with longitudinal striations. The tentacles  
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51 are capitate and highly contractile. They all (54-142) start from different points of the  
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53 body surface that is not in contact with the lorica. Macronucleus is oval and centrally  
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55 located in the body however, sometimes, it is transversely elongated (31.20-40.36 µm  
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3 long). Near to the macronucleus is placed a small, dense and spherical micronucleus.  
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5 Endogenous budding in the apical area occurs in a unique bud (monogemmic) or more  
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7 than one (polygemmic). The buds will develop into asymmetric and elongated swimmers  
8  
9 with a mean length of 17.40-20.80  $\mu\text{m}$ . The host is *Paraeuchaeta hebes* (Copepoda). *P.*  
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11 *hebensis* may be mainly found on the female host on genital segment, urosome, caudal  
12  
13 ramus and metasome. On the male host they may be mainly found on the leg 5, leg 4,  
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15 urosome and metasome. Its geographical distribution is on the continental shelf at Ría  
16  
17 de Vigo (N.E. Atlantic waters, Galician coast, Spain).  
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### 20 21 22 *Phylogenetic analysis*

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25 Amplified sequences of 18S rRNA ranged from 521 to 788 bp. These sequences  
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27 are available on GenBank under the accession numbers XXXXXXXXXXXXX. BLAST  
28  
29 search showed close homology (95%) with the 18S rRNA of *Paracineta limbata*  
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31 (Maupas, 1881) Collin, 1912 and distant homology (86%) with *Acineta flava* Kellicott,  
32  
33 1885. The 18S rRNA genealogy showed that the class Phyllopharyngea contains a  
34  
35 monophyletic subclass, Suctoria (Gao *et al.* 2012; Gong *et al.* 2008; Gong *et al.* 2009;  
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37 Li and Song 2006; Pan *et al.* 2012). The families Discophryidae, Prodiscophryidae and  
38  
39 Heliophryidae, were clustered in the order Evaginogenida with a strong bootstrap  
40  
41 support (99%). Nevertheless, the proximity between *Discophrya collini* Root, 1914 and  
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43 *Prodiscophrya* sp. Kormos, 1935 (supported by a strong bootstrap, 99%) suggested that  
44  
45 they are more probably, the same species (Fig. 5). Ephelotidae was grouped with a  
46  
47 strong bootstrap support (96%) within Exogenida. Moreover, Endogenida included two  
48  
49 families (Tokophryidae and Acinetidae) with a moderate bootstrap support (66% ML).  
50  
51 Contrary to expectations, the ML tree inferred from the 18S rRNA data set of  
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53 Phyllopharyngea revealed that our specimens (*Pelagacineta hebensis*) belong to a  
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3 highly supported clade (bootstrap values of 100), with *Paracineta limbata* (Fig. 5)  
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5 within Tokophryidae (Endogenida). Moreover, the position of *Acineta flava* remained  
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7 unresolved.  
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## 10 DISCUSSION

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14 The suctorian ciliate observed on the copepod *Paraeuchaeta hebes* belongs to  
15  
16 the genus *Pelagacineta*. Like their congeners, they are marine loricate ciliates with a  
17  
18 thecostyle lorica. This ovoid and transversely rounded ciliate did not present  
19  
20 actinophores and possessed a unique group of capitate and contractile tentacles that in  
21  
22 other species of this genus may appear forming two groups. The stalk expands  
23  
24 anteriorly to form the lorica. Macronucleus (Ma) elongated and often branched.  
25  
26 Reproduction by multiple endogenous budding. Swimmers ovoid, partially ciliated with  
27  
28 several longitudinal kineties. Attached to copepods or marine algae (Curds 1987).  
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33 The ciliates found could belong to the genus *Paracineta* or *Pelagacineta*.  
34  
35 Notwithstanding, the specimens here studied differed from *Paracineta* in the  
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37 arrangement of tentacles, which normally are placed in the apical end of the body's  
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39 suctorian of this species. However, when the lorica is too small tentacles are able to  
40  
41 radiate from the other areas. Conversely, the tentacles of our specimens are placed  
42  
43 anywhere on the surface of the body which is not covered by the lorica. The  
44  
45 distinguishing feature of the *Paracineta* is the exogenous budding, while the most  
46  
47 marked feature in our specimens is the endogenous budding, mono or polygemmic with  
48  
49 asymmetric buds. The mode of asexual reproduction has been largely used to group  
50  
51 these ciliates into the subclass Suctoria (Lynn 2008). Our specimens are different from  
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53 *Paracineta gaetani* Sewell, 1951 in the length of the stalk, the Ma shape and the asexual  
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55 reproduction. *P. gaetani* is characterized by a rigid stalk, which is shorter than the  
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3 lorica, their Ma is spherical and their budding is exogenous. A stalk three times longer  
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5 than the lorica, transversally ovoid and rounded macronucleus and endogenous budding  
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7 marks the specimens here studied.  
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11 Among *Pelagacineta*, four species have been described: *Pelagacineta*  
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13 *campanula* Schröder, 1907, *P. interrupta* Jankowski, 1978, *P. dibdalteria* Parona, 1881  
14  
15 and *P. euchaetae* Sewell, 1951. Differences among them are summarized in Table 4.  
16  
17 From a fore said comparison table 4 it is noted that our specimens slightly resemble *P.*  
18  
19 *campanula*, which have a dorso-ventrally compressed and discoidal body in contrast  
20  
21 with a not compressed and ovoid body in the samples studied. While the basal disk of  
22  
23 the stalk is striated in *P. campanula*, in our specimens is longitudinally striated. An  
24  
25 elongated and very branched Ma is typical in *P. campanula* whereas, our individuals  
26  
27 present an oval Ma. An outermost circle of tentacles, that are patently shorter, surrounds  
28  
29 the tentacles in *P. campanula*. This last feature is absent in our studied samples. Taking  
30  
31 into account the morphological differences between the suctorians analysed here and  
32  
33 other similar species, we have concluded that the observed suctorian belong to a new  
34  
35 species, which we have named *Pelagacineta hebensis* in reference to the copepod where  
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37 they were found. Consequently, these data constitute both the registration of a new  
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39 basibiont and geographical distribution for the genus *Pelagacineta*.  
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#### 45 *Phylogenetic analysis*

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49 In previous studies (Gong *et al.* 2008; Gong *et al.* 2009; Li and Song 2006; Pan  
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51 *et al.* 2012), the Subclass Suctorina was strongly confirmed as a monophyletic clade  
52  
53 containing three major Orders so far represented by 18S rRNA phylogeny. This is  
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55 consistent with the traditional taxonomy based on the shared morphological characters  
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57 (Lynn 2008). The 18S rDNA have been broadly used as a taxonomic tool to clarify the  
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3 taxonomy of Phyllopharyngea at the species level (Gong *et al.* 2008; Gong *et al.* 2009;  
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5 Li and Song 2006; Pan *et al.* 2012). Our genealogy showed that the Subclass Suctoria  
6  
7 contained three monophyletic orders Evaginogenida, Exogenida and Endogenida which  
8  
9 are strongly supported by some monographic works (Dovgal 1996, 2002; Lynn 2008).  
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11 Despite the fact that *Dyscophrya collini* and *Prodiscophrya* sp. have been included in  
12  
13 the Order Evaginogenida, very close to *Heliophrya erhardi* Dragesco et al., 1955, they  
14  
15 showed identical sequences (Pan *et al.* 2012). Hence we considered that these two  
16  
17 species that belong to different families should be revised. According to the  
18  
19 phylogenetic tree here obtained, *Acineta flava* clade has been unsolved. This species  
20  
21 could be belonging to whatever three genus that appeared next to it (*Tokophrya*, *Acineta*  
22  
23 or *Pelagacineta*). Nevertheless, in an attempt to resolve the position of *A. flava*, we  
24  
25 removed *Tokophrya infusionum* (Stein, 1859) Buetschli, 1889 and *T. lemnarum* Stein,  
26  
27 1859 from the phylogenetic analysis. Surprisingly, *A. flava* was grouped with *T.*  
28  
29 *quadripartita* Claparede and Lachmann, 1859 (bootstrap of 45%) and clearly appeared  
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31 separated from the other *Acineta* analyzed (73%). This finding leads us to suspect that  
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33 *A. flava* should also be reviewed. Regarding Exogenida, which is represented by  
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35 *Ephelota* species, showed monophyly within the subclass Suctoria (Li and Song 2006),  
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37 whereas Tokophryidae (Exogenida) demonstrated paraphyly. According with Lynn  
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39 (2008) seven genera have been included in the Tokophryidae family where we can  
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41 found *Pelagacineta* and *Tokophrya*. Our phylogenetic tree showed that *Pelagacineta*  
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43 genus could be included in Acinetidae as Fernandez-Leborans and Tato-Porto (2000a)  
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45 showed in their review. Thereby, Tokophryidae and Acinetidae will be monophyletic  
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47 clades into Suctoria. Unexpectedly, our results also showed that *Paracineta limbata*  
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49 belongs to the Endogenida Order as a sister taxon of *Pelagacineta hebensis*. This close  
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51 proximity between this two species could be explained if *P. limbata* was erected to the  
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3 *Pelagacineta* genus. Due to the asexual reproduction is an important feature, which  
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5 group species of the Suctoria, *P. limbata* should be clustered with *Ephelota* species  
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7 because this species showed exogenous budding. Moreover, we strongly suggested that  
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9 *P. limbata* sequences should be revised.  
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12 The present study provides new phylogenetic information about Suctorians,  
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14 taking into account that only 16 sequences of this species-rich group have been  
15  
16 sequenced and represented on 18S rDNA phylogenetic trees. To determine details of  
17  
18 their relationships in these highly specialized organisms, more data are definitely  
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20 needed.  
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#### 23 24 25 *Specificity, distribution on the host and ecology* 26

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28 Despite the large number of copepods examined, *Pelagacineta hebensis* was  
29  
30 only found on *Paraeuchaeta hebes*, a crustacean for which it seems to show a clear  
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32 preference. In the report of Fernandez-Leborans and Tato-Porto (2000b) *P. euchaetae*  
33  
34 was detected on *Calanus helgolandicus*, which was one of the dominant species in our  
35  
36 samples, however in our coastal region *C. helgolandicus* was free of this epibionts. The  
37  
38 rest of the copepods here studied were the dominant species in the samples collected  
39  
40 (Roura *et al.* 2013) however, only *P. hebes* carried this epibiont demonstrating the  
41  
42 specificity above mentioned.  
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46  
47 Gender preferences have also been detected since an important number of  
48  
49 epibionts, were most frequently attached to females. This phenomenon has widely been  
50  
51 accepted as a feature among basibiont females (Carman and Dobbs 1997; Fernandez-  
52  
53 Leborans 2010; Walkusz and Rolbiecki 2007; Xu and Burns 1991). Moreover,  
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55 protozoan epibionts are able to show preferences on certain parts of the crustacean  
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3 basibionts e.g. *Ophryodendron* sp. Claparède and Lachmann, 1859 on the caudal ramus  
4  
5 of *Lichomolgus singularipes* Humes and Ho, 1968 (Humes and Ho 1968). Walkusz and  
6  
7 Rolbiecki (2007) found some individuals of *Paracineta* sp. attached exclusively on the  
8  
9 prosome of *Metridia longa* Lubbock, 1854 and *Paraeuchaeta norvegica* Boeck, 1872.  
10  
11 Furthermore, Fernandez-Leborans *et al.* (2005) described that *Ephelota* spp. Wright,  
12  
13 1858 were distributed over the cephalothorax, genital segment, abdomen and caudal  
14  
15 branches of the copepod. Among females of *Paraeuchaeta hebes*, suctorians were most  
16  
17 frequently attached to the genital segment, urosome, caudal ramus and metasome. By  
18  
19 contrast in males the attachment was Leg 5, Leg 4, urosome and metasome. This  
20  
21 different distribution could be related with the reproduction behaviour where genital  
22  
23 segment of females are related with Leg 5 of males. Likely when males deposit their  
24  
25 sperm sac on females genital pore, they became infested with the epibiont. In this sense  
26  
27 different behaviour during mating could establish differences found between sexes. The  
28  
29 location of *Pelagacineta hebensis* concentrated on the posterior part of the basibiont  
30  
31 body coincided with that reported by Evans *et al.* (1979); Fernandez-Leborans and  
32  
33 Tato-Porto (2000a); Sherman and Schaner (1965); Walkusz and Rolbiecki (2007). The  
34  
35 posterior locations on the copepod basibiont could protect the epibionts from the water  
36  
37 friction (when copepod swimming). Moreover, swimming appendages could provide  
38  
39 epibionts with food, faecal particles facilitating the capture of food by the suctorian,  
40  
41 thereby increasing the density of ciliates in these regions (Fernandez-Leborans 2010;  
42  
43 Fernandez-Leborans and Tato-Porto 2000b).

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51 Despite the fact that apparently, there was not a detrimental effect of the  
52  
53 presence of *P. hebensis* on the basibionts since they do not penetrate into the copepod's  
54  
55 tegument, some indirect negative effects can occur on them. Suctorians can affect  
56  
57 negatively the basibiont survival (with heavy colonization), increasing the predation risk  
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3 of the basibiont (by modifying chemical signals acting on mobility, decreasing sensory  
4 activity, increasing energy costs, substantial shift of interactions among species). Their  
5 effects on biological functions are also wide, because of nutrient competition, inhibition  
6 of moulting, increased of weight and friction with water, trans-epidermal impeded  
7 exchanges, etc.(Fernandez-Leborans 2010; Wahl *et al.* 2012). In this way Weissman *et*  
8 *al.* (1993) recorded slower sinking rates in *Acartia hudsonica* Pinhey, 1926, when  
9 infested with solitary peritrich, *Rhabdostyla* sp. Kent, 1881, suggesting that the epibiont  
10 may increase burden drag forces, thereby impeding locomotion and increasing energy  
11 expense by the host.  
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24 In summary, our investigation has provided evidence that the new epibiont  
25 species *Pelagacineta hebensis* sp. n is found in adult individuals of the copepod  
26 *Paraeuchaeta hebes* from NE Atlantic waters. It is the first time that this copepod is  
27 observed as a basibiont for suctorian species. The new epibiont is described both with  
28 morphological and molecular techniques, contributing to enlarge the DNA sequences  
29 available for the class Phyllopharyngea. Finally, our results show that the suctorian *P.*  
30 *hebensis* has a marked preference for sexual appendages and the posterior part of the  
31 body of *P. hebes* females. Next studies should test if the oceanographic conditions  
32 affect the colonization of *P. hebensis* on *P. hebes*, as well as to determine if the sexual  
33 fitness of the copepod is affected by the presence of the suctorian epibiont.  
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*Acknowledgements*

We are indebted to the captain, crew and technicians of R/V “Mytilus” (IIM, CSIC Vigo), for their assistance in collecting the zooplankton samples. We are grateful to Mariana Cueto, Félix Álvarez, Alexandra Castro and Juan Hernández for assisting us with technical analysis, sample classifying and parasites-epibionts separation. We would like to thank Juan Abella who helped us improving the text. This study was supported by the projects CAIBEX (CTM-2007- 66408-CO2-01), LARECO (CTM-2011-25929). Maria Gregori and A.R. were granted by Pre-Doc JAE (CSIC) co-financed with Fondo Social Europeo (ESF) funds. Part of the equipment used was supported with FEDER Funds.

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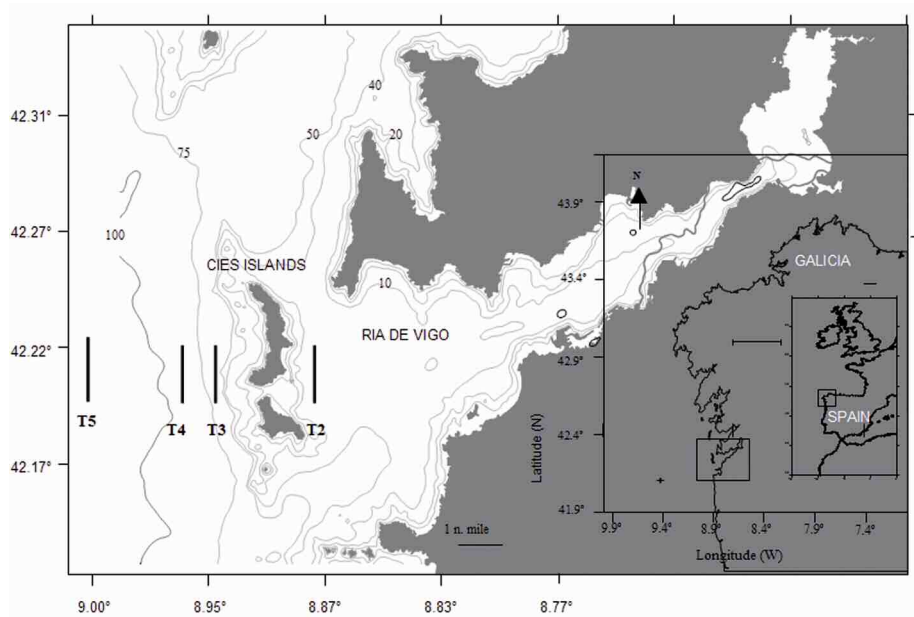
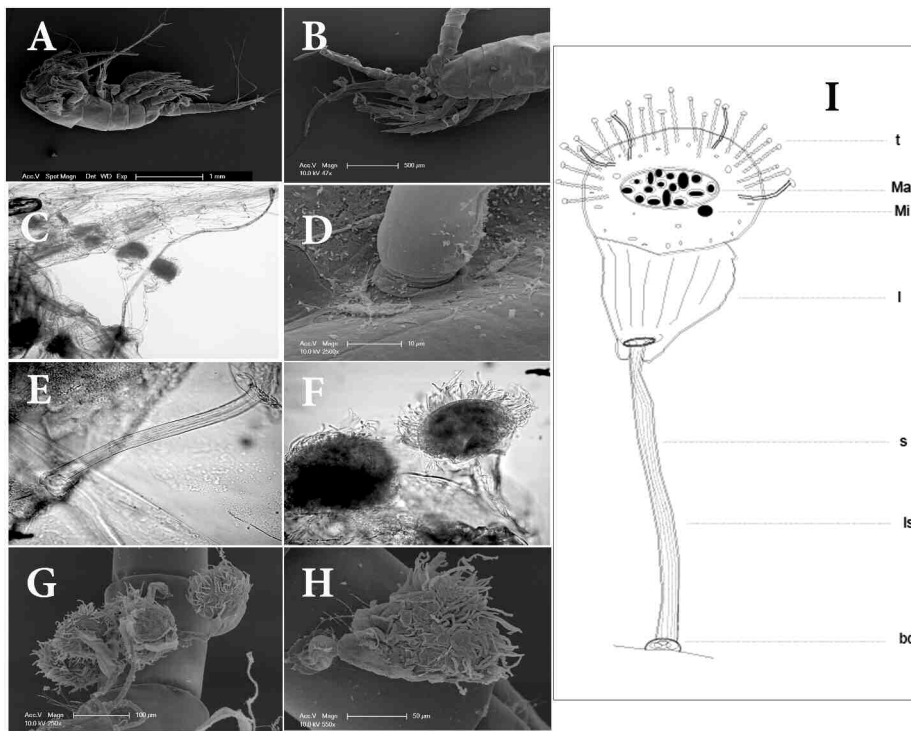
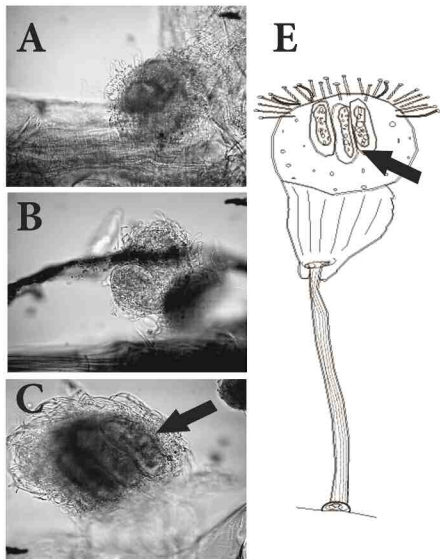


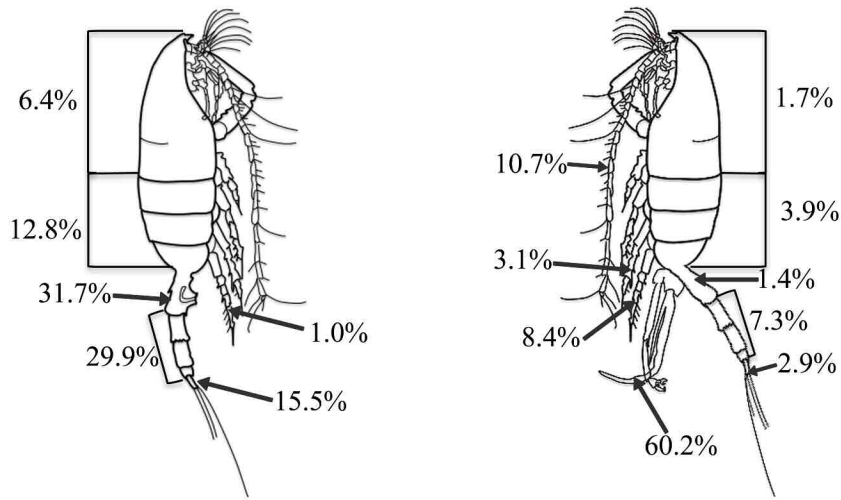
Fig. 1 Sampling area showing the transects where mesozooplankton samples were collected.



**Fig. 2** Light and SEM micrograph of (A) *Pelagacineteta hebensis* attached to the female of *Paraeuchaeta hebes*. (B) *P. hebensis* attached to *P. hebes* male. (C) Two specimens attached to the surface of the genital segment of the female basibiont. Lorica thecostyle type. (D) Oval basal disk on the attachment point surrounded with epibiont bacteria. (E) Longitudinal striations on the stalk. (F) Ovoid suctorian body. (G) Numerous tentacles sticking out through the different parts of the surface of the suctorian body thus they were not in contact with the lorica. (H) Similar capitate tentacles that were highly contractile. (I) Schematic *P.hebensis* where is shown tentacles (t), macronucleus (Ma), micronucleus (Mi), lorica (l), stalk (s), striated stalk (ls) and basald disk (bd).

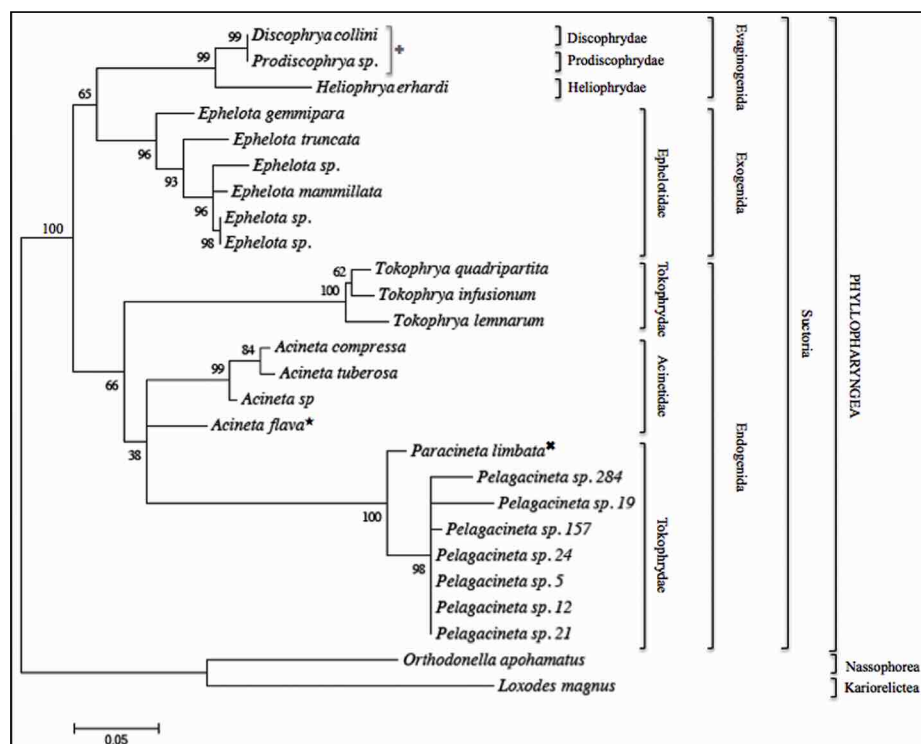


**Fig. 3** *Pelagacinetta hebensis* light micrograph of a specimen. (A) Buds were shown in the body. (B) Monogemmic budding (C) Polygemmic budding. (D) Schematic individual with asymmetric developed buds that give rise to swarmers (arrow).



**Fig. 4.** Percentages of distribution of the epibionts (*Pelagacineta hebensis*) in *Paraeuchaeta hebes* females (left) and males (right), among sex. From right side: *Paraeuchaeta hebes* female. From left side: *P. hebes* male.

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**Fig. 5** Maximum likelihood tree based on 18S rRNA gen showing the phylogenetic relationship of *Pelagacineta hebensis* sp. n. among Suctoria. ML (the number showed in the tree) bootstrap support values over 30% are given. Sequences inferred by the minimum evolution algorithm within MEGA 5. Analysis of 18S rDNA sequences after 1000 bootstraps.

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

<i>Paracineta limbata</i> (Maupas, 1881) Collin, 1912	FJ865207
<i>Acineta flava</i> Kellicott, 1885	HM140400
<i>Acineta tuberosa</i> Ehrenberg, 1833	FJ865206
<i>Acineta compressa</i> Claparède and Lachmann, 1859	FJ865205
<i>Acineta</i> sp. Ehrenberg, 1833	AY332717
<i>Ephelota mammillata</i> Dons, 1918	EU600181
<i>Ephelota gemmipara</i> Hertwig, 1876	EU600180
<i>Ephelota truncata</i> Fraipont, 1878	EU600182
<i>Ephelota</i> sp. Kent, 1882	DQ834370
<i>Ephelota</i> sp.	AY331804
<i>Ephelota</i> sp.	AF326357
<i>Tokophrya quadripartita</i> Claparède and Lachmann, 1859	AY102174
<i>Tokophrya lemnarum</i> Stein, 1859	AY332717
<i>Tokophrya infusionum</i> (Stein, 1859) Bütschli, 1889	JQ723984
<i>Discophrya collini</i> Root, 1914	L26446
<i>Prodiscophrya</i> sp. Kormos, 1935	AY331802
<i>Heliophrya erhardi</i> Saedeleer & Tellier, 1930	AY007445
<i>Loxodes magnus</i> Stokes, 1887	L31519
<i>Orthodonella apohamatus</i> Lin et al., 2004	DQ232761

**Table 2.** Biometry of *Pelagacineta hebensis*. Measurements in  $\mu\text{m}$ . Ma = macronucleus; SD = standard deviation; SE = standard error. N=30.

	<b>Mean</b>	<b>SD</b>	<b>SE</b>	<b>Min - Max</b>
<b>Body length</b>	72.09	12.86	4.54	60.16 - 97.60
<b>Body width</b>	59.94	6.72	2.87	50.76-70.83
<b>Number of tentacles</b>	82.87	27.66	9.78	54.00 - 142.00
<b>Tentacles length</b>	32.31	6.33	2.24	21.80 - 43.70
<b>Lorica length</b>	91.70	6.91	2.44	84.60 - 108.00
<b>Lorica max. width</b>	99.13	9.58	3.38	88.36 - 118.70
<b>Stalk length</b>	173.82	50.56	17.87	85.60 - 233.00
<b>Stalk width</b>	14.36	1.43	0.50	12.50 - 16.92
<b>Length of basal disc</b>	20.75	0.20	0.07	20.50 - 221.05
<b>Ma Length</b>	34.94	2.59	0.91	31.20 - 40.36
<b>Ma width</b>	26.38	2.98	1.05	23.20 - 32.84
<b>Bud length</b>	19.02	1.14	0.40	17.40 - 20.80
<b>Bud width</b>	7.85	0.57	0.20	7.20 - 8.80
<b>N. buds</b>	2.25	0.88	0.31	1.30 - 3.00



**Table 3** Number of *Paraeuchaeta hebes* examined for epibionts. NInf = number of non infected copepods. Inf = number of infected copepods. N°Epib = number of epibionts found on copepod surface. MInt±SE = Intensity average ± standard error. Max = maximum number of epibionts found on copepods surface. Min = minimum number of epibionts found on copepods surface.

<b>P. hebes</b>	<b>NInf</b>	<b>Inf</b>	<b>N°Epib</b>	<b>MInt±SE</b>	<b>Max</b>	<b>Min</b>
<b>Males</b>	733	114	643	5,64±0.52	30	1
<b>Females</b>	1605	228	1461	6,41±0.35	31	1
<b>Total</b>	<b>2338</b>	<b>342</b>	<b>2104</b>	<b>5,40±0.29</b>	<b>31</b>	<b>1</b>

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**Table 4.** Comparison between the different species of Pelagacineta. N = number

	Species of <i>Pelagacineta</i>				Present paper
	<i>P. interrupta</i>	<i>P. campanula</i>	<i>P. dibdalteria</i>	<i>P. euchaetae</i>	
<b>Body length</b>	100-140	100-150	50-60	50-90	60-97
<b>Body shape</b>	Dorso-ventrally compressed and discoidal				Not compressed and ovoid
<b>N. groups of tentacles</b>	2	1	-	2	1
<b>N. tentacles</b>	10-40	-	2	-	54-142
	(each group)				
<b>Tentacle length</b>	-	-	-	36	39-79
<b>Stalk length</b>	2-3 times	1-3 times	≤	<	1-3 times
	lorica length	lorica length	lorica length	lorica length	lorica length
					(84-233 long)
<b>Stalk width</b>	20-30	-	-	-	12-16
<b>Ma shape</b>	Variable	Elongated	Sausage-shaped	Variable	Oval
	(horseshoe, C, X, ramified)	and highly		(elongate, curved)	elongated
<b>N. buds</b>	1-4	Multiple	-	56 long	1-3
<b>Lorica</b>	Funnel-like	Cup-shaped (130-140 long)	Cup-shaped	257 long	Funnel-like (84-89 long)
<b>Habitat</b>	<i>Euchaeta</i>	Marine algae		<i>Euchaeta</i>	<i>Paraeuchaeta</i>
	<i>Metridia</i> (antarctic)			(antarctic)	<i>hebes</i>
					N.E. Atlantic
					Ría de Vigo