Zoosyst. Evol. 100 (1) 2024, 49–59 | DOI 10.3897/zse.100.113707

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für Natur MUSEUM FÜR NATURKUNDE BERLIN

First occurrence of the genus *Pleurobranchaea* Leue, 1813 (Pleurobranchida, Nudipleura, Heterobranchia) in British waters, with the description of a new species

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https://zoobank.org/579ED8AF-40E7-4FE4-A8AF-ED87567D5DCC

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Academic editor: M. Glaubrecht ♦ **Received** 5 October 2023 ♦ **Accepted** 28 November 2023 ♦ **Published** 26 January 2024

Abstract

In the north-eastern Atlantic and Mediterranean Sea, the pleurobranchid genus *Pleurobranchaea* Leue, 1813 is represented by two species, *Pleurobranchaea meckeli* (Blainville, 1825) and *Pleurobranchaea morosa* (Bergh, 1892). The former is a well-known species distributed from northern Spain to Senegal and the Mediterranean Sea, while the second is a poorly-described species. In this contribution, species delimitation analyses (ABGD and COI/16S *p*-distances) identified a third undescribed *Pleurobranchaea* species from samples collected in south-western UK waters and the Gulf of Cadiz (SW Spain). This new species, *Pleurobranchaea britannica* **sp. nov.**, is also supported by several morphological synapomorphies. The British specimens constitute the first occurrence of the genus *Pleurobranchaea* in UK waters.

Key Words

Atlantic Ocean, Gulf of Cadiz, Mediterranean Sea, molluscan diversity, *Pleurobranchaea britannica*, Pleurobranchaeidae, southwest UK, systematics

Introduction

The family Pleurobranchaeidae Pilsbry, 1896 was established for heterobranchs, characterised by an oval body, broad oral veil, rolled rhinophores and variable colours. Members of this family have a bipinnate gill in the middle of the right side, which may or may not be covered by the mantle (García-Gomez and Cervera 2011). The latter character led to their inclusion together with the Umbraculida (Order Notaspidea). However, phylogenetic analyses, based on morphological and molecular data (Martynov and Schrödl 2009; Göbbeller and Klussmann-Kolb 2010), demonstrated that the Pleurobranchaeidae should be considered as a separate group. Under an alternative classification scheme, Wägele and Willan (2000) introduced the Nudipleura to unite Pleurobranchoidea and Nudibranchia. Some characteristics the Nudipleura have in common are: the loss of the shell, the presence of papillae on the notum, a hermaphroditic reproductive system with simultaneous maturation of the gametes and obligate cross-fertilisation involving copulation.

Members of the Pleurobranchaeidae are active hunters of invertebrates and typically inhabit sedimentary substrates. The family is ubiquitous and they can be

found from the intertidal zone to the circalittoral. The family is considered monophyletic and comprises three genera: *Pleurobranchaea* Leue, 1813, *Euselenops* Pilsbry, 1896 and *Pleurobranchella* Thiele, 1925. The genus *Pleurobranchaea* constitutes 15 valid species (Alvim et al. 2014; MolluscaBase 2023a) that inhabit temperate or tropical waters across a wide geographical range (Muniain et al. 2007; Alvim et al. 2014). The type species of the genus, *Pleurobranchaea meckeli* (Blainville, 1825), is the only well-known species from European waters (Bergh 1897; Vayssière 1901; Marcus and Gosliner 1984). This species has been recorded in many localities from the Mediterranean Sea, as well as in several localities around the Atlantic Iberian coasts, Madeira, Canary Islands, Azores (Cervera et al. 2004) and Cape Verde Archipelago (Vayssière 1901). Marcus and Gosliner (1984) described two additional species of *Pleurobranchaea*, based on preserved material collected from Turkey, Israel (*P. notmec*) and Algiers (*P. vayssierei*). However, Cervera and Garcia-Gomez (1988), following Willan (pers. comm.), considered both names to be junior synonyms of *P. meckeli*. Bergh (1892) described *P. morosa* from a single specimen collected from the Pico-Faial Channel (Azores) at 130 m depth. No further records are attributed to this second species, since Marcus and Gosliner (1984) did not include *P. morosa* in their review of the Pleurobranchaeidae due to its insufficient description.

The northernmost record of *Pleurobranchaea* in the eastern Atlantic had been from northern Spain (Cervera et al. 2004), but recent bottom trawls conducted along the English Channel in 2018 and 2019, as well as in the Gulf of Cadiz (SW Iberian Peninsula) in 2020 collected specimens of an unknown pleurobranchid with an external appearance slightly different from *Pleurobranchaea meckeli.* During the surveys, the collected specimens were initially considered as a different morphotype of *P. meckeli*, but a detailed examination of the external and internal anatomy revealed marked differences from all other species of the genus. Subsequently, a phylogenetic analysis, based on two mitochondrial (cytochrome-oxidase subunit I and 16SrRNA) and one nuclear (Histone 3) marker, in conjunction with species delimitation analyses, supported the status of this morphotype as a new and previously undescribed species, which is formally described in the present paper.

Materials and methods

Taxon sampling and molecular data

Fourteen specimens of an undescribed species of *Pleurobranchaea* were collected during two different campaigns in southern England (Fig. 1A) and one in the Gulf of Cadiz (Fig. 1B). The surveys in the Western Channel and Celtic Sea occurred during the 2018 and 2019 Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Quarter One South West EcoSystem (Q1SWECOS) surveys. This survey series used two commercially rigged 4 m beam trawls with 80 mm cod-end mesh, with one of the trawls fitted with a 40 mm liner to facilitate the collection of epibenthic species. The bathymetric range covered by the trawls was approximately 20–200 m. The survey conducted by the Instituto Español de Oceanografía (IEO-CSIC) in the Gulf of Cadiz, offshore between Faro and Cadiz was collected by beam trawl of 190 cm and the effective height was 60 cm above the bottom with a duration of 15 min.

The material collected in these campaigns was deposited either in the Natural History Museum of London (NHM) or in the National Museum of Natural Sciences of Madrid (MNCN) (see Table 1). Twenty-four specimens of three different species (11 *Pleurobranchaea meckeli*, 4 *P. maculata* and 14 of the new species) were sequenced in this study to obtain partial sequences of two mitochondrial (cytochrome-oxidase subunit I or COI and 16SrRNA or 16S) and one nuclear (Histone 3 or H3) markers.

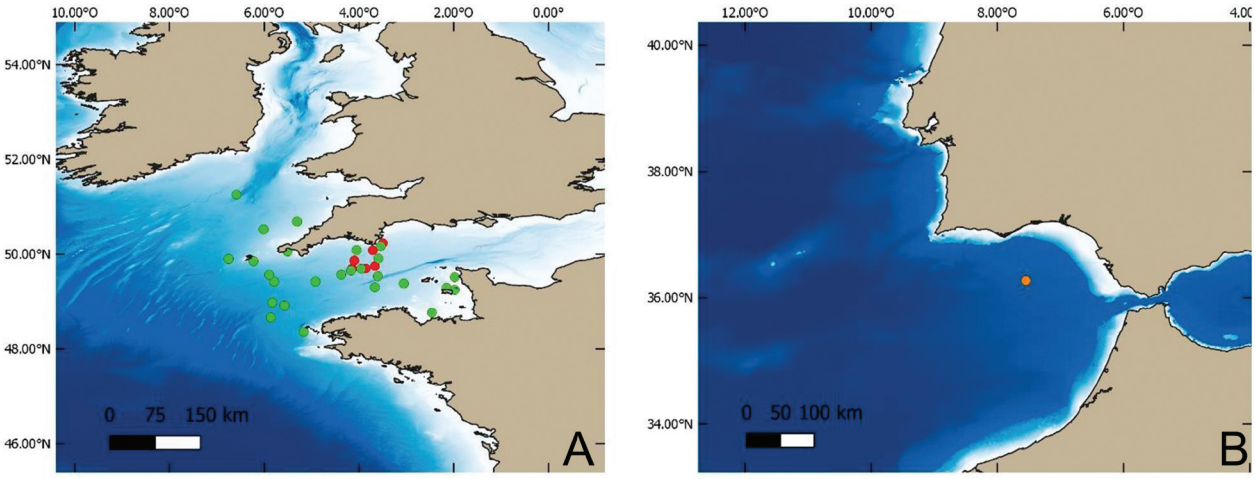

Figure 1. Sampling stations where *Pleurobranchaea britannica* sp. nov. material was collected. **A.** The map on the left shows the south of England: the red dots refer to the 2018 campaign and the green dots to the 2019 campaign; **B.** The map on the right shows part of Spain and the orange dot is where samples were collected in 2019.

Additionally, sequences of these genes from ten closely-related pleurobranchid taxa were added from GenBank, including two additional species of *Pleurobranchaea* (*P. californica* and *P. inconspicua*) and *Bathydoris aioca* (Er. Marcus and Ev. Marcus, 1962), *Bathydoris clavigera* (Thiele, 1912) and *Berthella plumula* (Montagu, 1803) as outgroups (Table 1).

Morphological analysis

The samples were stored in 95%–100% ethanol to allow DNA extraction. Two specimens were dissected by dorsal incision. The internal morphology of the reproductive system was examined and drawn using a Leica Wild M8 dissection microscope. The buccal bulb was removed and placed in a 10% sodium hydroxide (NaOH) solution for three days until the radulae and jaws were cleaned of the surrounding tissue. The radula and jaws were then rinsed in demineralised water and rinsed at least twice with 96% ethanol before drying. Clean radulae and jaws were mounted on SEM stubs with dissection pins and coated with gold for examination with a Nova NanoSEM scanning electron microscope (SEM) at the Central Services of Scientific and Technological Research (SC-ICYT) unit of the University of Cadiz.

DNA extraction and amplification

Genomic DNA was extracted from foot tissue of specimens using the DNeasy Blood & Tissue Kits of Qiagen (Qiagen, Inc., Valencia, Ca., USA) and stored in extraction buffer at –20 °C prior to amplification. Partial sequences of H3 were amplified by polymerase chain reaction (PCR) using the universal primers H3F and H3R (Colgan et al. 2000), while universal and specific primers or their combination were used for the COI and 16S (Table 2).

Table 1. List of species used for this study including sample locality, voucher numbers and GenBank accession numbers.

Species	Locality	Voucher numbers	GenBank accession numbers		
			COI	16S	H3
Berthella plumula (Montagu, 1803)	Ballyhenry Is., Northern Ireland, UK	CASIZ 193034	MK542770	MK542742	MK542803
Prodoris clavigera Thiele, 1912	South Shetland I., Elephant I., Antartica	CASIZ 167553	JX274106	JX274067	KP940463
Bathydoris aioca Er. Marcus & Ev. Marcus, 1962	California	CPIC 01053	KP153283	KP153249	KP153316
Pleurobranchaea maculata Quoy & Gaimard, 1832	Auckland, New Zealand	–	JN675223	–	–
Pleurobranchaea maculata	Auckland, New Zealand	–	JN675222	–	–
Pleurobranchaea maculata	Auckland, New Zealand	–	JN675221	–	–
Pleurobranchaea maculata	Auckland, New Zealand	–	JN675220	–	–
Pleurobranchaea californica MacFarland, 1966	California	–	–	FJ917440	–
Pleurobranchaea inconspicua Bergh, 1897	Patagonia, Argentine, Atlantic Ocean	MNCN 15.05/94846	–	OR723442	OR715121
Pleurobranchaea meckeli (Blainville, 1825)	Blanes, Spain, Mediterranean Sea	–	AY345026	–	–
Pleurobranchaea meckeli	Blanes, Spain, Mediterranean Sea	–	–	–	EF133470
Pleurobranchae meckeli 005	Castellaneta Marina, Italy, Mediterranean Sea	MNCN 15.05/94841	–	OR723434	OR715119
Pleurobranchaea meckeli 006	Castellaneta Marina, Italy, Mediterranean Sea	MNCN 15.05/94842	OR687335	OR723435	OR715118
Pleurobranchaea meckeli 008	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/94843	OR687340	OR723441	OR715116
Pleurobranchaea meckeli 010	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/94844	OR687336	OR723436	OR715117
Pleurobranchaea meckeli 016	Laayoun, Morocco, Atlantic Ocean	MNCN 15.05/94845	OR687341	OR723437	–
Pleurobranchaea meckeli 21.1	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/94837	OR687337	OR723438	OR715120
Pleurobranchaea meckeli 21.2	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/94838	OR687339	OR723440	OR715115
Pleurobranchaea meckeli 21.3	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/94839	OR687338	OR723439	OR715114
Pleurobranchaea meckeli 22.1	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/94840	OR807509	–	–
Pleurobranchaea britannica sp. nov. 17.1	Southwest of England	NHMUK 20230086	OR687348	OR723452	OR715123
Pleurobranchaea britannica sp. nov. 17.2	Southwest of England	NHMUK 20230087	OR687349	OR723446	OR715122
Pleurobranchaea britannica sp. nov. 17.3	Southwest of England	NHMUK 20230085	OR687351	OR723453	OR715124
Pleurobranchaea britannica sp. nov. 17.4	Southwest of England	NHMUK 20230088/1	OR687347	OR723444	OR715125
Pleurobranchaea britannica sp. nov. 18.1	Southwest of England	MNCN 15.05/200180	–	OR723447	–
Pleurobranchaea britannica sp. nov. 18.2	Southwest of England	NHMUK 20230089	–	–	OR715126
Pleurobranchaea britannica sp. nov. 18.3	Southwest of England	NHMUK 20230090	–	–	OR715127
Pleurobranchaea britannica sp. nov. 18.4	Southwest of England	NHMUK 20230091	–	–	OR715128
Pleurobranchaea britannica sp. nov. 20.1	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/200181	OR687345	OR723448	OR715132
Pleurobranchaea britannica sp. nov. 20.2	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/200182	OR687343	OR723449	OR715133
Pleurobranchaea britannica sp. nov. 20.3	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/200183	OR687346	OR723450	OR715129
Pleurobranchaea britannica sp. nov. 20.4	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/200184	OR687342	OR723451	OR715130
Pleurobranchaea britannica sp. nov. 20.5	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/200185	OR687344	OR723445	OR715134
Pleurobranchaea britannica sp. nov. 20.6	Gulf of Cadiz, Spain, Atlantic Ocean	MNCN 15.05/200186	OR687350	OR723443	OR715131

Table 2. List of primers used for this study.

Gene	Primer ID	Sequence (5'to-3')	Annealing Temperature (°C)	Source
COI	LCO1490 (F)	GGTCAACAAATCATAAAGATATTGG	50	Folmer et al. (1994)
	HCO2198 (R)	TAAACTTCAGGGTGACCAAAAATCA	50	Folmer et al. (1994)
	Pluero26F (F)	GAGTTGGGGACTTCAGGAGC	46	This study
	Pluro526R (R)	AATAGCCCCCGCCAATACTG	46	This study
	jgLCO1490 (F)	TITCIACIAAYCAYAARGAYATTGG	50	This study
	jgHCO2198 (R)	TAIACYTCIGGRTGICCRAARAAYCA	50	This study
16S	Sar-L (F)	CGCCTGTTTATCAAAAACAT	52	Palumbi (1996)
	Sbr-H (R)	CCGGTCTGAACTCAGATCACGT	52	Palumbi (1996)
	F52 (F)	ATAGCCGCGGTACTTTGACC	55	This study
	R384 (R)	AGTCCAACATCGAGGTCACA	55	This study
H3	H3F (F)	ATGGCTCGTACCAAGCAGAGVGC	58	Colgan et al. (1998)
	H3R (R)	ATATCCTTRGGCATRATRGTGAC	58	Colgan et al. (1998)

Reactions took place in a total volume of 25 µl including 2 µl of template DNA, 1 µl of both forward and reverse primers (10 µM), 2.5 µl of dNTP (2 mM), a gene-dependent amount of magnesium chloride (25 mM), 0.25 µl of Qiagen DNA polymerase (5 u/µl), 5 µl of "Q-solution" (5×) and 2.5 µl of Qiagen buffer (10×; Qiagen Taq PCR Core Kit cat. no. 201225). Magnesium chloride ($MgCl_2$) amounts were 4.5 µl for COI and 16S and 3 µl for H3. The COI fragment was amplified with initial denaturation step for 1 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, an annealing step for 30 s at 52 °C (universal primer) or 50–46 °C (specific primer) and 30 s at 72 °C. A final extension step for 3 min at 72 °C was added to ensure extension. For 16S, the thermal cycle profile began with denaturation for 1 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 16 s at 52 °C (universal primer) or 16 s at 55 °C (specific primer) and 30 s at 72 °C, with a final extension step for 3 min at 72 °C. Finally, the H3 amplification was performed with an initial denaturation for 2 min at 95 °C, followed by 40 cycles of 30 s at 94 °C, an annealing step for 30 s at 58 °C and 1 min at 72 °C, with a final extension step at 72 °C for 7 min. A negative control (no template) was included in each reaction. PCR products were visualised by electrophoresis on a 2% agarose gel and those viable were purified and amplified in both directions by Macrogen Inc. All new sequences obtained were deposited in GenBank.

Nucleotide sequence alignment and phylogenetic reconstruction

All DNA chromatograms were assembled and edited using Geneious version 10.0.9 (http://www.geneious.com, Kearse et al. 2012). The sequences were aligned with MAFFT v.7.402 server (Katon and Standley 2013) using the L-INS-i iterative refinement algorithm via the CIPRES Portal Science Gateway (Miller et al. 2010). The alignments were further optimised by eye using AliView (Larsson 2014) and trimmed to 328 bp (H3), 658 bp (COI) and 450 bp (16S). These partitions were subsequently concatenated with FASconCAT-G v.1.0, resulting in 1436 bp alignment. Pairwise uncorrected *p*-distances between each available taxon were conducted for the COI and 16S gene using PAUP v.4.0 (Swofford 2002).

The best model of evolution was determined with jModelTest 2.1.10 (Darriba et al. 2012). The model identified with the Akaike Information Criterion (AIC) (Akaike 1998) was the Tamura-Nei model (TrN+G) for H3 and the general time-reversible model (GTR+G) for COI and 16S. Phylogenetic analyses were conducted under two optimal criteria: Maximum Likelihood (ML) and Bayesian Inference (BI). The ML phylogenetic trees were inferred using RAxML v.8.2.12 (Stamatakis 2014) under the GTRGAMMA model. Node support was assessed with rapid bootstrap analysis with 1000 replicates. Analysis stopped the search for bootstrap after 100 replicates with the autoMRE-based bootstopping criterion. Values ≥ 70% were interpreted as significant nodal support (Hillis and Bull 1993).

Bayesian Inference analyses were conducted using MrBayes v.3.2.7 (Ronquist et al. 2012). Two runs were performed in parallel with four independent MCMC chains (one heated, three cold) and default priors. The analyses were run for 5,000,000 generations, saving a tree every 1000 generations and discarding the first 1250 trees of each analysis as "burn-in." Nodal support was estimated as posterior probabilities (PP), with values ≥ 90% taken as significant (Huelsenbeck and Rannala 2004).

Species delimitation analyses

Species delimitation analyses involved two methods: (a) the Automatic Barcode Gap Discovery (ABGD; Puillandre et al. 2012), through a simple distance matrix, based on the COI and 16S genes (generated in MEGA v.7.0.18) as input file under default intra- and interspecific priors (pmin = 0.001; pmax = 0.10) in 10 steps and with a relative gap width of 1.5; (b) a pairwise genetic distance matrix, based on the COI sequences generated in PAUP v.4.0 (Swofford 2002).

Results

Systematics

Superorder Nudipleura Wägele & Willan, 2000
Order Pleurobranchida Gray, 1827
Superfamily Pleurobranchoidea Gray, 1827
Family Pleurobranchaeidae Pilsbry, 1896
Genus *Pleurobranchaea* Leue, 1813

***Pleurobranchaea britannica* sp. nov.**
https://zoobank.org/81238088-87DA-4163-81F8-06AEB3ADDCAA

Material examined. ***Holotype:*** NHMUK 20230085, 18 mm preserved length, (49°54'5.306"N, 6°45'7.056"W), southern England, 103 m depth, Apr 2019. ***Paratypes:*** NHMUK 20230087, 19 mm preserved length, (49°35'59.389"N, 4°39'48.485"W) southwest England, 91.98 m depth, Mar 2018; NHMUK 20230086, 18 mm preserved length, (49°42'13.429"N, 4°6'28.514"W) southwest England, 81.12 m depth, Mar 2018; NHMUK 20230091, 22 mm preserved length, (50°5'10.929"N, 3°41'34.436"W) southwest England, 68.94 m depth, Mar 2018; MNCN 15.05/200180, 24 mm preserved length, (50°2'41.978"N, 4°3'33.805"W) southwest England, 75.69 m depth, Mar 2018, dissected specimen; NHMUK 20230090, 18 mm preserved length, (49°54'5.306"N, 6°45'7.056"W), southwest England, 103 m depth, Apr 2019; NHMUK 20230089, 19 mm preserved length, (49°54'5.306"N, 6°45'7.056"W), southwest England, 103 m depth, Apr 2019; NHMUK 20230088/1, 20 mm preserved length, (49°54'5.306"N, 6°45'7.056"W), southwest England, 103 m depth, Apr 2019, dissected specimen; MNCN 15.05/200181, 7 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Feb 2020; MNCN 15.05/200182, 8 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Feb 2020; MNCN 15.05/200183, 11 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Feb 2020; MNCN 15.05/200184, 10 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Feb 2020; MNCN 15.05/200185, 11 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Feb 2020; MNCN 15.05/200186, 9 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Feb 2020. ***Additional material*:** MNCN 15.05/94837, 41 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Mar 2020; MNCN 15.05/94838, 43 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Mar 2020; MNCN 15.05/94839, 48 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Mar 2020; MNCN 15.05/94840, 42 mm preserved length, (36°16'19.56"N, 7°32'52.8"W) Gulf of Cadiz, 555 m depth, Mar 2020.

Diagnosis. Body oval, large, translucent with a minute cream/ochre pigmentation. Some specimens with opaque white specks irregularly spread all over mantle, oral veil, gill and posterior region of the foot not covered by the mantle. Rhinophores with dark spots on the front and white ones on the back. Gill bipinnate, with 15–18 pairs of pinnules and smooth rachis. Caudal spur absent. Outermost radular teeth bicuspid. Seminal receptacle short; bursa copulatrix at the end of the vagina and directly fused to it.

Description. ***External morphology*** (Fig. 2). Body oval and large, with a rough mantle forming irregular polygons delimited by shallow grooves (Fig. 2A, B). Base colour translucent with a minute cream to ochre pigmentation, which may not always be present. Opaque white specks might appear irregularly spread all over mantle, oral veil, gill and posterior region of the foot not covered by the mantle. Speckles density variable. Viscera partially visible through mantle in lighter individuals. Posterior part of foot round with no caudal spur (Fig. 2A). Moreover, no pedal gland was observed. Sole patterned. Anterior part of rhinophores brown and posterior covered with close white dots (Fig. 2A). Oral veil with trapezoid shape and fused with mantle where rhinophores are inserted. Veil front edge not smooth, but slightly irregular. Some specimens with series of white specks at veil corners. Gill located on the right side of the body, clearly visible and not covered by the mantle. Gill bipinnate, with 15–18 pairs of pinnules and smooth rachis. Gill with same base colour, white grains almost always present and variable in density, being visible on rachis and pinnules. Genital openings in front of gill and nephropore, clearly visible since it is covered by a circular fleshy papilla, which may have white dots. Anus opens above the 6th and 7th pinnule of the gill.

Internal anatomy (Figs 3, 4) Radula almost rectangular with no rachidian teeth. Radular formulae are: 35 × (53–50).0.(53–50) (NHMUK 20230088/2); 33 × (54–56).0.(54–56) (MNCN15.05/200180) (Fig. 3A). All teeth with two long and blade-shaped cusps. The outermost cusp is larger, while the one facing the centre of the radula is smaller and sometimes covered by the next tooth. Innermost teeth slightly more elongated and with finer tips, while outermost teeth with rounder tip (Fig. 3B). Jaws elongated. Anterior part of jaw elements hexagonal, hand-shaped, with 4 to 9 denticles along anterior edge. The jaw elements have a depression in the middle (Fig. 3C, D).

Reproductive system (Fig. 4) begins with the hermaphroditic duct which first widens into the ampulla and thereafter narrows and divides into two parts: one entering the prostate gland and the other continuing to oviduct. The prostate gland is composed of small and pyramidal-shaped papillae. Exiting from prostate, vas deferens entering the penial sac, anchored by a retractor muscle to the inner body wall. Inside the sac, penis relatively straight, with a couple of twists, but not coiled and apparently not cuticularised. The oviduct

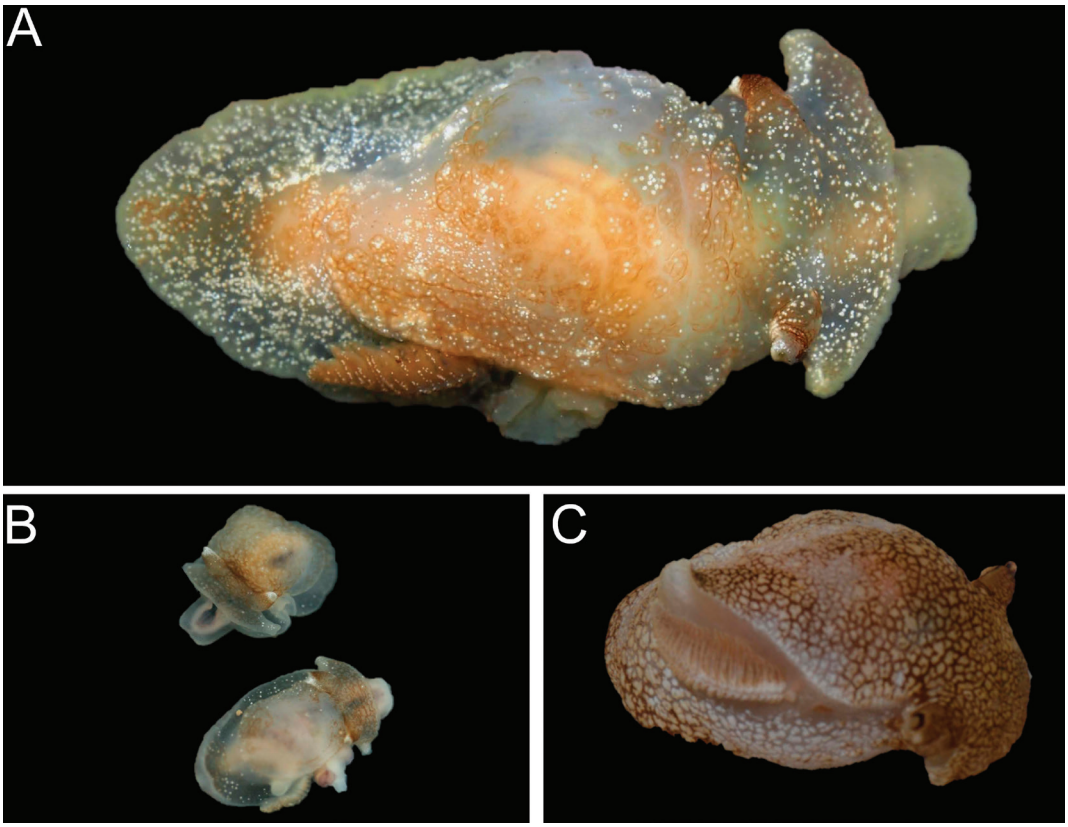

Figure 2. A. Living specimens of *Pleurobranchaea britannica* sp. nov. collected on Survey CEND 0518, southwest England. Photo by Ross Bullimore (NHMUK 20230085); **B.** Two young individuals of *P. britannica* sp. nov. from the Gulf of Cadiz, Spain (MNCN 15.05/200181; MNCN 15.05/200182); **C.** Specimen of *P. meckeli* from Morocco, Mediterranean Sea (MNCN 15.05/94845).

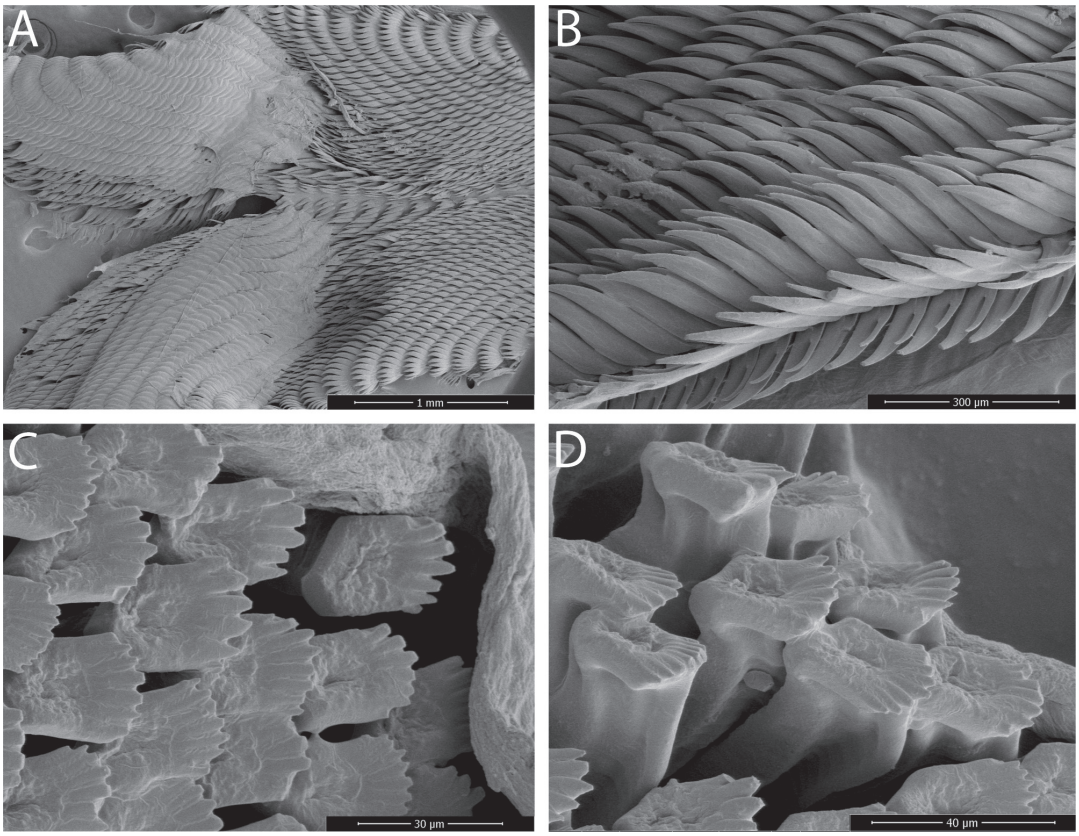

Figure 3. *Pleurobranchaea britannica* sp. nov. Scanning electron micrographs of radula and jaw. **A.** Complete radula (MNCN15.05/200180); **B.** Lateral teeth of radula (MNCN15.05/200180); **C.** View from above of the anterior part of the jaw (MNCN15.05/200180); **D.** Lateral view of the anterior part of the jaw (MNCN15.05/200180).

widens slightly forming a bilobed seminal receptacle, narrowing before entering laterally into the muscular vagina. Copulatory bursa spherical, placed at the distal end of vagina. Copulatory bursa not very muscular, its wall being delicate and thinner than the vagina's. Female gland and vagina join laterally, very close to the female orifice. There are two different genital openings: the opening closest to the nephropore is the female one, the further one is male.

Etymology. The species name in Latin refers to the British waters where this species was initially found.

Distribution. The species has been found in a number of locations in the southwest of UK waters and the Gulf of Cadiz, see Fig. 1, but we hypothesise that it could probably be distributed throughout the Atlantic coast of Spain, Portugal and France up to the southwest approaches to the English Channel.

Type locality and habitat. South-western England (see Fig. 1A). Collected from a range of depths (70–110 m) and a range of substrates that include areas of mosaic rock and mixed sediments and areas of muddier sediments.

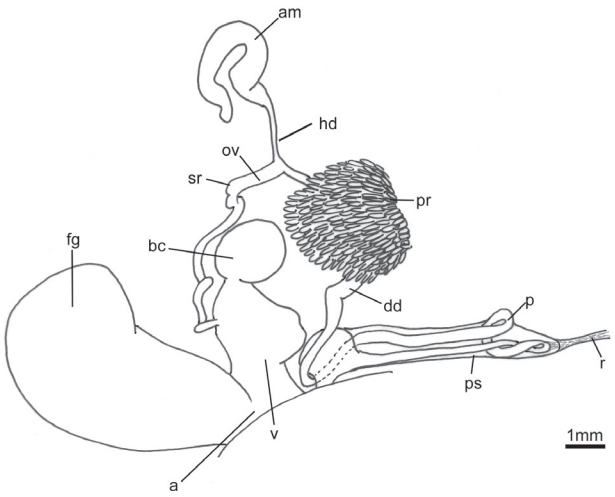

Figure 4. *Pleurobranchaea britannica* sp. nov. Reproductive system (NHMUK 20230088/5). Abbreviations: a – atrium; am – ampulla; bc – bursa copulatrix; dd – deferent duct; fg – female gland; hd – hermaphroditic duct; ov – oviduct; p – penis; pr – prostate; r – retractor muscle; ps – penis sac; sr – seminal receptacle; v – vagina.

Phylogenetic analysis

We obtained 17 sequences for COI, 20 for 16S and 21 for H3 genes. The combined data set (COI+H3+16S) provided better resolution than the COI, 16S and H3 separately. Fig. 5 shows the phylogenetic hypothesis based on the combined dataset (COI+H3+16S) constructed by Bayesian Inference (BI). The topology of the Maximum Likelihood (ML) tree was very similar to that obtained by BI (former not shown). All the sequences of the new species clustered in a single, well-supported clade (PP = 1; BS = 88), included in a broader well-supported clade (PP =1; BS = 80) together with *Pleurobranchaea inconspicua*, *P. maculata* and *P. californica*. All the specimens of *Pleurobranchaea meckeli* clustered in a different and maximum supported clade (PP =1; BS = 100) including the remaining species of *Pleurobranchaea* we analysed.

Uncorrected *p*-distances (%) between *P. britannica* sp. nov. and the species *P. maculata* and *P. meckeli* ranged from 12.7% to 18.3%, respectively (Table 3).

The ABGD species delimitation analysis recovered three putative species, based on the COI gene with both Jukes-Cantor (JC69) and Kimura (K80) parameters, while for the 16S alignment retrieved four putative species. These differences are due to the fact that sequences of *P. inconspicua* and *P. californica* were not available for COI, whereas for 16S, there were no data for *P. maculata*.

Table 3. Maximum and minimum COI gene pairwise uncorrected *p*-distances (%).

Species	*P. meckeli*	*P. maculata*
P. meckeli		
P. maculata	16.2–17.5	
P. britannica sp. nov.	16.3–18.3	12.7–13.9

Discussion

Our analyses support, from both the molecular and morphological approaches, the existence of *Pleurobranchaea britannica* sp. nov. as a distinct species. The geographical range of this species and that of *P. meckeli* partially overlap, but they can be distinguished by both external and internal characters. Externally, the new species lacks a caudal spur and has a transparent cream-coloured base with variation in the density of white spots and the mottled or "net" pattern which can be darker or lighter. *Pleurobranchaea meckeli* exhibits a conspicuous caudal spur and has a brown net pattern all over the body (Fig. 2A). Furthermore, the colour pattern of the rhinophores of *P. britannica* is characteristic with dark spots on the front and white spots on the back, respectively. Other differences between *P. meckeli* and *P. britannica* are summarised in the Appendix 1. This Appendix also includes the main differences between the known species from the Atlantic Ocean and Mediterranean Sea. For example, we also observed differences in the radula formula and the outermost teeth which are bicuspid in *P. britannica*, while they are unicuspid in *P. meckeli*. In the reproductive system, we can find several clear differences: the seminal receptacle of *P. meckeli* is larger than in *P. britannica* and, therefore, easier to observe. The bursa copulatrix in *P. meckeli* is not directly connected to the vagina, but with a small tube that connects the two structures. In the new species, the bursa copulatrix is located at the end of the vagina, directly connected with it. Another difference can be observed in the structure of the penis since, in *P. meckeli*, it performs various loops within the penal sac, while in *P. britannica*, it does not.

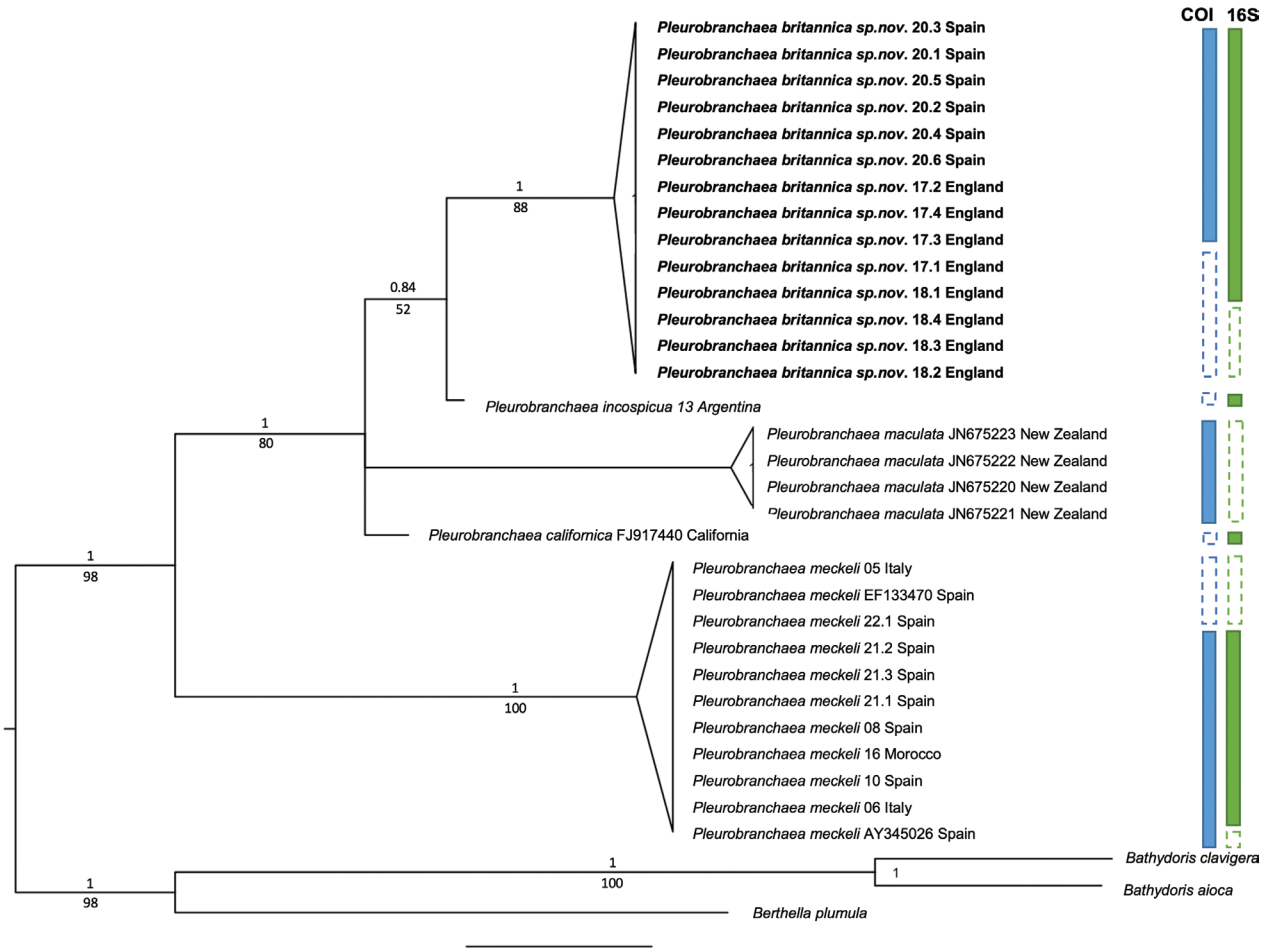

Figure 5. Phylogenetic hypothesis of *Pleurobranchaea* systematics, based on concatenated dataset (COI+16S+H3) inferred by Bayesian analysis. Significant support values are given as BI posterior probabilities (below branch) and ML bootstrap percentages (above branch). Rectangles are automatic barcode gap discovery for the COI and 16S dataset. White rectangles indicate the lack of those sequences in the alignment.

Pleurobranchaea britannica sp. nov. does not correspond to the poor descriptions of other animals currently accepted as synonyms of *P. meckeli* (see MolluscaBase (2023b)). For example, *Pleurobranchaea dellechiaii* Véany, 1846 has a minimal external description, but the mantle has red dots even after death, which are not present in the new species. *Pleurobranchaea notmec* Marcus, Ev. and Gosliner 1984 has a caudal spur, absent in *P. britannica* and the penis is much longer (as in *P. meckeli*) compared to the new species. *Pleurobranchaea vayssierei* Marcus, Ev. and Gosliner 1984 also has a caudal spur and an elongated penis wrapped in the penal sac, but the vagina is long and quite narrow unlike *P. britannica* which has a wide vagina which connects the bursa copulatrix to the outside. Those last two names (*P. notmac* and *P. vayssierei*) were proposed from preserved material without any information provided of the living individuals' colouration.

All these morphological and anatomical differences which separate *P. britannica* sp. nov. as a standalone species are supported by our genetic data. In fact, individuals identified as *P. britannica* form a single well-supported taxon (PP = 1; BS = 88), which is also separate from *P. meckeli*, the geographically closest species. To date, the only European (excluding the Azores) species of the genus *Pleurobranchaea meckeli*, has not been recorded further north than Iberian coasts. Therefore, *P. britannica* represents the first record of the genus *Pleurobranchaea* in British waters and the second valid species from European seas.

Acknowledgements

We thank the survey scientists and crew of "RV Cefas Endeavour" their help for the collection of the specimens of the new species from the English Channel. Likewise, Carlos Farias (Spanish Institute of Oceanography, IEO) donated the material of *Pleurobranchaea britannica* n. sp. and *P. meckeli* from the Gulf of Cadiz collected during several IEO oceanographic campaigns ARSA. This study has been partially supported by the project 'Desentrañando la diversidad criptica en las regiones Lusitánica y Mediterránea: Heterobranquios marinos (Mollusca), Sílidos (Annelida) y Caprélidos (Arthropoda, Pancrustacea) como casos de estudio' funded by the University of Cadiz (PR2018-039) to J. L. Cervera.

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Appendix 1

Table A1. Summary of diagnostic features of species of *Pleurobranchaea*.

Species	Radula	Inner and outermost tooth	Mantle and foot	Caudal Spur	Pedal gland	Jaw (denticles on jaw elements)	Rinophores	Veil	Gill	Seminal receptacle	Vagina and bursa copulatrix	Penis	Genital opening	Distribution	References
P. agassizii (Bergh, 1897)	32 x 98.0.98	Bicuspid, but 17 outermost unicuspid	–	Present	–	4–8	–	–	32 pinnules	A single bulge	–	Muscular without cuticle	–	Western Atlantic: Florida, Great Bahama Bank, Gulf of Mexico	Marcus and Gosliner (1984); Alvim et al. (2014)
P. bubala (Maecus and Gosliner, 1984)	35 x 100.0.100	Bicuspid, 20–30 outmost teeth unicuspid	Mantle extends over the foot on both sides; pattern of dark brown pigment and irregular whitish blotches	Absent	Present	4–14 rarely more than 10	–	A row of small tubercles	26 pinnules, smooth rachis	–	Long glandular vagina and bilobed bursa copulatrix	The stylet is similar to that of *P. tarda*	Protruding, with flap on hind border	From Atlantic coast of Cape Península to Inhaca, Mozambique	Marcus & Gosliner (1984); Alvim et al. (2014)
***P. britannica* sp. nov.**	**36 × 59–58.0.59–58**	**Bicuspid, outmost tiny secondary cusp**	**Mantle not covering foot; translucent or cream base colour, irregular white spots**	**Absent**	**Absent**	**4–9**	**Rolled and pointed, are separate; the anterior part of the tip is brown and the posterior is covered with white dots**	**Trapezoid shape and is fused with the mantle, irregular front edge**	**15–18** pinnules, **bipinnate, smooth rachis**	**Slightly bilobed**	**At the distal end of muscular vagina is the spherical copulatory bursa**	**Not cuticularised penis is relatively straight, with a couple of twists**	**Covered by a circular fleshy papilla**	**Atlantic coast of Spain, Portugal and France up to the English Channel**	
P. gela (Er. Marcus and Ev. Marcus, 1966)	42 × 57.0.57	Bicuspid, sometimes	Black foot sole with light	–	Present	8–14	–	–	18–26 pinnules	Bilobed or trilobed	Small, rounded bursa copulatrix	Well–developed crest along cuticular translucent stylet, coiling five to six times	With dorsal flap	West Africa	Marcus and Gosliner (1984); Alvim et al. (2014)
P. inconspicua (Bergh, 1897)	30 X 64.0.64	Bicuspid, sometime s tiny secondary cusp or unicuspid	Mantle is reduced, not covering foot; translucent white, with reticulate pattern of brown lines and white dots	Present	Present	5–11	Smooth, translucent brown with some whitish stains, It is fused with the mantle	Broad with singular row of sensory papillae along the anterior edge	20–26 pinnules, unipennate rachis	Bilobed	Large rounded bursa copulatrix and muscular vagina	Cuticular stylet, translucent white, coiling 10–11 times; Penis large, cylindrical, sometimes projecting	Surrounded by fold with triangular papilla	Northern Brazil, Western Atlantic, Mediterranean, West Africa	Marcus and Gosliner (1984); Muniain, Ardila, Cervera (2006); Alvim et al. (2014); WoRMS (2023)

Species	Radula	Inner and outermost tooth	Mantle and foot	Caudal Spur	Pedal gland	Jaw (denticles on jaw elements)	Rinophores	Veil	Gill	Seminal receptacle	Vagina and bursa copulatrix	Penis	Genital opening	Distribution	References
P. meckeli (Leue, 1813)	46 × 71.0.71	Bicuspid, 1–5 outermost unicuspid	Mantle is smaller than the foot; brown or dark grey crosslinked variable, background colour is cream, but sometimes there are white areas	Present	Present	–	As long as the tentacles, blunt, cylindrical	Irregular front edge and with pointed ends; row of papillae, the sides of the veil produced into a pointed cephalic tentacle with split sides	23–25 pinnules, bipinnate, alternate knobs on rachis	Globular	Vagina elongated and connected at the end with a round bursa copulatrix	Elastic cuticular stylet with a high crest, stylet coiled 6–10 times	Surrounded by thick fold	Mediterranean, Atlantic, including Azores, Cape Verde Islands	Marcus and Gosliner (1984); García-Gómez et al. (2011); Alvim et al. (2014)
P. morosa (Bergh, 1892)	37 × 70–68.0.68– 70	–	–	–	Present	5–7	–	–	15 pinnules	–	–	–	–	Azores	Bergh (1892)
P. obesa (Verrell, 1882)	31–34×75–90.0.75–90	Bicuspid, the outermost is unicuspid	Mantle is smooth, swollen extending far out over the foot	Present	Present	Variable number of denticles	Smooth	A single row of papillae	26–35 pinnules, smooth rachis	Lobate and glandular	Vagina and bursa copulatrix are large	Cuticular stylet present	–	North-western Atlantic	Bergh, (1892); Marcus and Gosliner (1984); Alvim et al. (2014)
P. spiroporphyra (Alvim, Simon & Pimenta, 2014)	30–32 × 40–44.0.40–44	Bicuspid with smaller tiny cusp	Mantle margin reduced, not covering foot, mantle with tiny flap at end of gill	Present	Present	1–5	Rolled, separated	Broad, thin, connected to head region; deep notch in apical third or quarter of oral tentacles; several large rounded papillae forming one row	17–23 pinnules, unipinnate, tuberculated rachis	Many enlargements	–	Penis large, cylindrical; cuticular stylet with 12–14 coils	Surrounded by thick fold, with triangular papillae	Rio de Janeiro	Alvim et al .(2014); WoRMS (2023)
P. tarda (Verrill, 1880)	70.0.70	Bicuspid, six outermost unicuspid	Smooth mantle and about the same size as foot	Sometime there is a short spur	Present	5–7	Typical for the genus	Tubercles	20–30 pinnules, Tuberculated	Ciliated and serial	Vagina is short and wide	Cuticular stylet present	With or without flap	Western and south-eastern Atlantic: from Martha's Vineyard to south of Cuba; from Angola to Agulhas Bank	Vayssiere (1901); Marcus and Gosliner (1984); Alvim et al. (2014)