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Resurrection of the sea pen genus *Ptilella* Gray, 1870 and description of *Ptilella grayi* n. sp. from the NE Atlantic (Octocorallia: Pennatulacea)

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Summary: The order Pennatulacea covers a group of specialized and morphologically distinct octocorals found in all oceans from intertidal areas to more than 6000 m in depth. Sea pens constitute an important structural component in marine soft-bottom communities by increasing the complexity of these environments. Despite being both morphologically distinctive and ecologically important, the taxonomy and systematics of sea pens is still poorly understood. Recent molecular studies have shown the existence of convergent morphological features, making the current familial distribution of genera unstable. The genus *Pennatula* Linnaeus, 1758 was one of the first described octocoral genera. It is the type genus of its family, Pennatulidae. Colonies of this genus have a characteristic morphology. Recent sampling efforts in the northeastern Atlantic have provided a number of colonies initially attributable to the genus *Pennatula*. Both morphological and molecular (*mtMutS, Cox1* and *28S* genes) study of this material supports the polyphyletic nature of this genus and the need to resurrect the genus *Ptilella* Gray, 1870 to accommodate these and other species. A new species, *Ptilella grayi* n. sp., is described and illustrated. The species *Pennatula bayeri* is proposed to be a junior synonym of *Pennatula bellissima* (here also considered in the genus *Ptilella*).

Keywords: NE Atlantic; Pennatulacea; *Ptilella grayi*; sea pen; new species.

Resurrección del género de pluma de mar Ptilella Gray, 1870, y descripción de *Ptilella grayi* n. sp. del Atlántico NE (Octocorallia: Pennatulacea)

Resumen: El orden Pennatulacea abarca a un grupo de octocorales especializados y morfológicamente diferenciados, distribuidos por todos los océanos, desde las zonas intermareales hasta más de 6000 metros de profundidad. Las plumas de mar constituyen un importante componente estructural en las comunidades marinas de fondo blando, incrementando la complejidad de estos ambientes. A pesar de ser característicos morfológicamente, e importantes ecológicamente, la taxonomía y la sistemática de las plumas de mar es aún pobremente conocida. Recientes estudios moleculares han mostrado la existencia de características morfológicas convergentes, haciendo inestable la actual distribución familiar de los géneros. El género *Pennatula* Linnaeus, 1758, fue uno de los primeros géneros de octocorales descritos. Es el género tipo de su familia, Pennatulidae. Las colonias de este género tienen una morfología característica. Los recientes esfuerzos de muestreo en el noreste Atlántico han proporcionado un número de colonias inicialmente atribuibles al género *Pennatula*. Tanto el estudio morfológico como molecular (genes *mtMutS*, *Cox1* y *28S*) de este material respaldan la naturaleza polifilética de este género, y la necesidad de resucitar al género *Ptilella* Gray, 1870 para acomodar a éstas y otras especies. Una nueva especie, *Ptilella grayi* n. sp., es descrita e ilustrada. La especie *Pennatula bayeri* es propuesta como sinónimo reciente de *Pennatula bellissima* (aquí también considerada en el género *Ptilella*).

Palabras clave: noreste Atlántico; Pennatulacea; *Ptilella grayi*; pluma de mar; nueva especie.

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INTRODUCTION

The anthozoan fauna from deep-sea zones is still poorly known, despite recent international efforts having improved our general knowledge of the diversity and ecology of deep-sea benthic communities. Sea pens are a group of specialized and morphologically distinct octocorals found in all oceans, from intertidal areas to more than 6000 m in depth (Bayer 1956, Williams 2011). The order Pennatulacea includes more than 200 species in 35 genera and 14 families (López-González et al. 2001, López-González and Williams 2002, Williams 2015). Sea pens constitute an important structural component in marine soft-bottoms communities, increasing the complexity of these environments in a role similar to that of other coral groups such as gorgonians and scleractinians, which are typically found on a more rocky substrata (Sale 1977, Done 1999). The ecological importance of the recently discovered rock-pens (Williams and Alderslade 2011) is yet to be evaluated, although they could potentially also contribute similarly to scleractinians and gorgonians by providing yet another environmental niche in rocky seabeds. Moreover, it has been demonstrated that sea pens are often used as a refuge or nurseries for demersal fauna (Sammarco and Coll 1992, Baillon et al. 2012). The ecological importance of sea pen beds is internationally recognized, with these being included in red lists of marine environments threatened by anthropogenic activities (see OSPAR Commission 2010).

Among sea pens, the genus *Pennatula* Linnaeus, 1758 was one of the first described octocoral genera. It is the type genus of its own family, Pennatulidae. Colonies assigned to this genus exhibit a high consistency of morphological characters, being typically pinnate (feather-like) in shape, with well-developed polyp leaves, with polymorphic zooids. Autozooids arranged along the ventral edge of the polyp leaves, as well as siphonozooids and sometimes mesozooids at the base of the polyp leaves or on the rachis, and with the sclerites along the colony mainly as three-flanged needles (see Kükenthal 1915, Williams 1995a).

Recent molecular studies in octocorals have identified the order Pennatulacea as a monophyletic group (McFadden et al. 2006, Dolan et al. 2013, Kushida and Reimer 2018). However, the monophyly of the various supra-familial and familial pennatulacean groupings that have been proposed historically has recently been questioned by these same molecular studies (McFadden et al. 2006, Dolan et al. 2013, Kushida and Reimer 2018).

Although Dolan et al. (2013: 615) does not reject the possible monophyly of some genera such as *Kophobelemnon*, *Pennatula* is clearly identified as polyphyletic, suggesting the need for a redefinition of this genus. This will provide a solution to the current problem that morphological criteria used to identify members of the genus *Pennatula* have been found to only poorly resolve their phylogenetic relationships when compared with analysis based on DNA sequence data (see Dolan et al. 2013: 614-615). More recently (Kushida and Reimer 2018), the genera *Umbellula*, *Pennatula* and *Kophobelemnon* were also shown as polyphyletic groups. When a polyphyletic or paraphyletic group is detected from molecular evidence, morphological characters are re-examined to identify those characters concordant with the molecular clades, and to solve potential homoplastic situations according to nomenclatural rules (Lowther et al. 2004, Fleck et al. 2008). The delimitation of monophyletic groupings and the establishment of their relationships is a common problem in evolutionary biology research (e.g. Wheeler and Nixon 1990, Crisp and Chandler 1996, Brummitt 2002, among many others).

The current conception of the cosmopolitan genus *Pennatula* includes at least 14 valid species (Williams 2011). However, some of these species have descriptions based on single and frequently poorly preserved specimens, or on specimens lacking those characteristics currently in use for reliable diagnosis (Kölliker 1880, Kükenthal 1915, Hickson 1916).

In recent years the benthic component of a range of surveys carried out in the northeastern Atlantic by Marine Scotland Science (MSS) has provided an interesting collection of pennatulaceans, many of which have formed the basis for this study. The material examined was collected over the period 2007-2016 during various demersal trawl surveys carried out by MSS on board the research vessel MRV *Scotia*.

In this study, certain colonies initially attributed to two chromatic forms of the northeastern Atlantic species *Pennatula grandis* Ehrenberg, 1834 were collected. However, detailed morphological and molecular study of these specimens highlighted differences, suggesting further consideration of the current genus *Pennatula* as a polyphyletic grouping. The genus placement of Ehrenberg's species and the need for the resurrection of a forgotten sea pen genus is discussed, and a new species is proposed.

METHODS

Sampling

The material studied was collected using a large demersal trawl (supplemented occasionally with a small ground gear net that was deployed directly underneath the main net deployed) on banks and terraces to the west of the Outer Hebrides, Scotland, during the Rockall Haddock Survey (2010), the OFFCON Rockall Survey (2011), the Rockall Anglerfish Survey (2010, 2013 and 2014) and the Deepwater Time Series (2008, 2009 and 2011), and to the North East of Scotland during the North Sea Anglerfish Survey (2012). Further occurrences from similar surveys over the period 2005-2016 are used to improve the known distribution (Fig. 1; Suplementary material Table S1). Overall, these surveys covered a depth range of 42 to 2145 m.

All material analysed from the *Scotia* cruises was collected using a demersal fish trawl with both the codend and the full body of the net being thoroughly examined for specimens after each deployment. Pennatulaceans of all types, including the new specimens studied here, were rarely recorded in the codend but commonly encountered meshed in the wings or belly

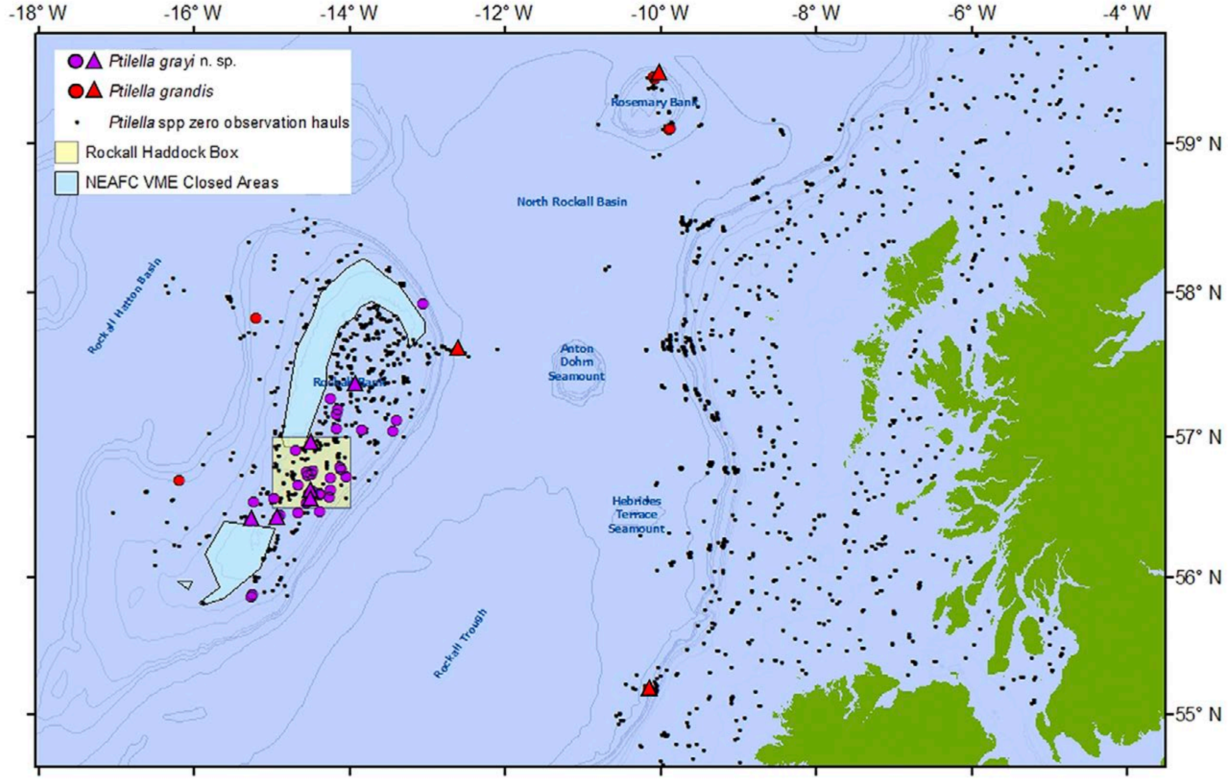

Fig. 1. – Known distribution of *Ptilella grayi* n. sp. (violet symbols) and *Ptilella grandis* (red symbols) in the study area and hauls with no occurrences (black dots). Symbols represent approximate midpoints of hauls. Circles indicate sampling stations where the species was recorded only, while triangles indicate sampling stations where the species was both recorded and examined over the course of this study. Map © Crown copyright.

of the trawl. Observations from a total of 1541 trawl deployments were used to provide distributional data on the genus. Temperature data close to the seabed were recorded using either a vertical deployment of the Seabird 19+ CTD profiler or a Star-Oddi DST logger mounted on the headline of the net. Both gave temperatures of the water within 3 to 5 m of the seabed. The relevant information on materials and sampling stations of these and other comparative material used in this paper is compiled in Table 1.

Pennatulacean colonies were sorted, labelled and fixed in buffered formalin (5% in seawater). After the fixation period, colonies were preserved in 70% ethanol. A certain number of colonies were directly fixed in 100% ethanol for further molecular studies.

For comparative purposes, the morphology of additional *Pennatula* species was also examined. These colonies were collected during various benthic surveys and over different geographical areas: Antarctica (BIOROSS), the northeastern Atlantic-Arctic (BIOICE), the northeastern Atlantic (*Scotia* cruises, INDEMARES Chica), the southeastern Atlantic (BENGUELA VIII), and the Mediterranean (INDEMARES Alborán). A subsample of these were sequenced (Table 2).

The material from the Scotia cruises referred to here is deposited in the National Museum of Scotland (NMS), in the Natural History Museum in London (NHM), in the Museu de Zoologia de Barcelona (MZB) and in the collection of the research group Biodiversidad y Ecología Acuática of the University of Seville (BECA).

External morphology and SEM study

Sclerites of different parts of the colonies were prepared for SEM study employing the standard methodology described by several authors (e.g. Bayer and Stefani 1988), and permanent mounts were made for examination using light microscopy. Sclerite dimensions and illustrations are based on the holotype or indicated lot. Colony and sclerite terminology follows Bayer et al. (1983).

DNA extraction, PCR amplification and sequencing

Total genomic DNA was extracted from ethanol (EtOH)-preserved specimens using the EZNA DNA kit (OmegaBiotech), following the manufacturer's instructions. Two mitochondrial regions, *mtMutS* (=*msh1*) and *Cox1*, plus a nuclear region, *28S* ribosomal DNA, were sequenced. These three markers are concatenated as this has been considered an octocoral barcode (McFadden et al. 2014). The start of the *mtMutS* region was amplified using the primers ND42599F and MUT3458R (France and Hoover 2002, Sánchez et al. 2003). The *Cox1* region was amplified using the primers COII8068F and COIOCTR (France and Hoover 2002, McFadden et al. 2004). The *28S* nuclear ribosomal gene (*28S* rDNA) was amplified using the primers 28S-Far and 28S-Rar (McFadden and van Ofwegen 2013). Each PCR used 0.5 U of DNA Stream Polymerase (BIORON), 0.2 mM

Table 1. – Sampling data associated with the Atlantic material of *Ptilella* compared in this paper. Abbreviations: NMS, National Museum of Scotland; MZB, Museu de Zoologia de Barcelona; NHM, Natural History Museum in London; BECA, Biodiversidad y Ecología Acuática of the University of Seville; n.a., not available; i.c., incomplete colony.

Species	Status	Registration code	Cruise	Cruise no. (haul)	Area and coordinates Start - Stop	Dept range (m)	Date	Nº colonies (total length; mm)
Ptilella grayi n. sp.	Holotype	NMS.Z.2019.2.1	Rockall Haddock Survey 2011	1011S (S11426)	Rockall Bank, NE Atlantic 56°32.63’N 14°30.92’W 56°33.97’N 14°28.78’W	198-202	30 Aug 2011	1 (474)
Ptilella grayi n. sp	Paratype	NMS.Z.2019.2.2	Rockall Anglerfish Survey 2010	0410S (S10150)	Rockall Bank, NE Atlantic 56°25.22’N 15°14.67’W 56°22.91’N 15°18.75’W	246-261	6 Apr 2010	1 (254)
Ptilella grayi n. sp	Paratype	NMS.Z.2019.2.3	Rockall Haddock Survey 2011	1011S (S11426)	Rockall Bank, NE Atlantic 56°32.63’N 14°30.92’W 56°33.97’N 14°28.78’W	198-202	30 Aug 2011	1 (471)
Ptilella grayi n. sp	Paratype	MZB 2018-0761	Rockall Haddock Survey 2011	1011S (S11426)	Rockall Bank, NE Atlantic 56°32.63’N 14°30.92’W 56°33.97’N 14°28.78’W	198-202	30 Aug 2011	1 (391)
Ptilella grayi n. sp		NMS.Z.2019.2.4	OFFCON 2012	0712S (S12287)	Rockall Bank, NE Atlantic 57°22.33’N 13°54.87’W 57°21.36’N 13°57.73’W	146-152	24 Jul 2012	1 (305)
Ptilella grayi n. sp		NMS.Z.2019.2.5	Rockall Anglerfish Survey 2013	1413S (S13371)	Rockall Bank, NE Atlantic 56°36.59’N 14°26.65’W 56°35.81’N 14°32.89’W	197-199	24 Oct 2013	1 (450)
Ptilella grayi n. sp		MZB 2018-0762	Rockall Anglerfish Survey 2013	1413S (S13372)	Rockall Bank, NE Atlantic 56°55.42’N 14°31.33’W 56°58.65’N 14°28.61’W	179-188	24 Oct 2013	1 (450)
Ptilella grayi n. sp		BECA OPEN-335	Rockall Anglerfish Survey 2013	1413S (S13371)	Rockall Bank, NE Atlantic 56°36.59’N 14°26.65’W 56°35.81’N 14°32.89’W	197-199	24 Oct 2013	1 (575)
Ptilella grayi n. sp		NHMUK 2019.1	Rockall Anglerfish Survey 2013	1413S (S13371)	Rockall Bank, NE Atlantic 56°36.59’N 14°26.65’W 56°35.81’N 14°32.89’W	197-199	24 Oct 2013	1 (570)
Ptilella grayi n. sp		MZB 2018-0763	Rockall Anglerfish Survey 2013	1413S (S13371)	Rockall Bank, NE Atlantic 56°36.59’N 14°26.65’W 56°35.81’N 14°32.89’W	197-199	24 Oct 2013	1 (545)
Ptilella grayi n. sp		BECA OPEN-338	Rockall Anglerfish Survey 2013	1413S (S13371)	Rockall Bank, NE Atlantic 56°36.59’N 14°26.65’W 56°35.81’N 14°32.89’W	197-199	24 Oct 2013	1 (572)
Ptilella grayi n. sp		BECA OPEN-339	Rockall Anglerfish Survey 2014	0414S (S14141)	Rockall Bank, NE Atlantic 56°25.40’N 14°57.37’W 56°26.56’N 14°54.75’W	206-215	14 Apr 2014	1 i.c. (115)
Ptilella grayi n. sp		BECA OPEN-340	Rockall Anglerfish Survey 2014	0414S (S14141)	Rockall Bank, NE Atlantic 56°25.40’N 14°57.37’W 56°26.56’N 14°54.75’W	206-215	14 Apr 2014	1 i.c. (90)
Ptilella grandis (Ehrenberg, 1834)		NMS.Z.2019.2.6	Deepwater Time Series 2008	1108S (S08385)	Hebridean Slope, NE Atlantic 55°13.79’N 10°08.87’W 55°08.49’N 10°10.66’W	1018-1078	14 Sept 2008	1 (256)
Ptilella grandis (Ehrenberg, 1834)		MZB 2018-0759	OFFCON 2011	0711S (S11264)	NE Rockall, NE Atlantic 57°36.37‘N 12°36.66‘W 57°35.94‘N 12°36.98‘W	1601-1605	30 Jun 2011	1 (182)
Ptilella grandis (Ehrenberg, 1834)		NHMUK 2019. 2	North Sea Anglerfish Survey 2012	0412S (S12220)	S Norwegian Sea, NE Atlantic 61°55.05’N 02°28.94’E 61°52.66’N 02°34.22’E	390-396	29 Apr 2012	1 (510)
Ptilella grandis (Ehrenberg, 1834)		BECA OPEN-143	BIOICE	516 (3507)	South Iceland, NE Atlantic 61°54.95’N, 19°17.02’W	1595	3 Sept 2002	1 (114)
Ptilella inflata (Kükenthal, 1910)		NMS.Z.2019.2.7	Benguela VIII	67	Namibia, SE Atlantic 23°28.03’S 13°05.08’E	418	23 Jul 1985	1 (203)
Ptilella inflata (Kükenthal, 1910)		NHMUK 2019. 3	Benguela VIII	67	Namibia, SE Atlantic 23°28.03’S 13°05.08’E	418	23 Jul 1985	1 (207)
Ptilella inflata (Kükenthal, 1910)		MZB 2018-0760:	Benguela VIII	67	Namibia, SE Atlantic 23°28.03’S 13°05.08’E	418	23 Jul 1985	1 (227)
Ptilella bellissima (Fowler, 1888)	Holotype	BMNH (1888.6.28.1)	n.a.	n.a.	Bahamas Islands, NW Atlantic	n.a.	14 Feb 1888	1 (178)

of dNTPs, 0.3 μM of each primer and approximately 30 ng of genomic DNA, and was brought to a final volume of 25 μL with H_2O. The *mtMutS* PCR was carried out using the following cycle profile: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. The *Cox1* PCR used the same cycle profile with 58ºC as the annealing temperature and 40 s for extension duration on each of the 35 cycles. The

Table 2. – Pennatulaceans included in the molecular phylogenetic analyses. Species in bold are those sequenced for this study. Note that all GenBank sequences are presented here with the names as they appear in GenBank and their original publications.

Family / Species	Catalog numbers (or additional information)	Geographic area	*mtMutS*(=*msh1*)	*Cox1*	*28S*
Pennatulidae					
Ptilella grandis	BECA OPEN-143	South Iceland, NE Atlantic	**MK603844**	**MK603860**	**MK603854**
Ptilella grandis	NMS.Z.2019.2.6	Hebridean Slope, NE Atlantic	**MK603843**	**MK882496**	**MK882494**
***Ptilella grayi* n. sp.** (Holotype)	NMS.Z.2019.2.1	Rockall Bank, NE Atlantic	**MK603847**	**MK882497**	**MK882495**
***Ptilella grayi* n. sp.** (Paratype)	NMS.Z.2019.2.2	Rockall Bank, NE Atlantic	**MK603846**	**MK603856**	**MK603853**
Pennatula rubra	BECA OPEN-139	Alborán, Mediterranean Sea	**MK603845**	**MK603857**	**MK603852**
Pennatula phosphorea	BECA OPEN-453	Sea of the Hebrides, NE Atlantic	**MK603848**	**MK603858**	**MK882492**
Pennatula phosphorea	BECA OPEN-454	Gulf of Cádiz, NE Atlantic	**MK603850**	**MK603861**	**MK882491**
***Pennatula* sp.**	BECA OPEN-152	Ross Sea, Antarctica	**MK603849**	**MK603859**	**MK882493**
*Pennatula phosphorea**	MBARI K2-06-070110-01	USA, Eastern Pacific	KX904975[5]	KX904957[5]	
*Pennatula phosphorea**	NWFSC 34212-045	USA, Eastern Pacific	JN866531[2]	KF874190[2]	
Ptilosarcus gurneyi	NWFSC 34212-082	USA, Eastern Pacific	JN866536[2]	KF874195[2]	
Ptilosarcus gurneyi	NWFSC 34213-020	USA, Eastern Pacific	JN866540[2]	KF874201[2]	
Virgularidae					
Acanthoptilum gracile	NWFSC 34210-026	USA, Eastern Pacific	JN866525[2]	KF874184[2]	
Acanthoptilum gracile	NWFSC 34212-029	USA, Eastern Pacific	JN866529[2]	KF874188[2]	
Echinoptilidae					
Actinoptilum molle	RMNH Coel.40822	n.d.	GQ342491[3]	GQ342414[3]	JX203738[4]
Protoptilidae					
Distichoptilum gracile	NTM C14561	AUS, Tasman Sea,	DQ302866[1]	GQ342454[3]	JX203739[4]
Stachyptilidae					
Gilibelemnon octodentatum	BECA OPEN-452	Seymour Island, Antarctica	**MK603841**	**MK603855**	**MK603851**
Renillidae					
Renilla muelleri	SCF-FLA	USA, Gulf of Mexico	DQ297432[1]	NC_018378[1]	
Renilla sp.	UF4000	Off California coast, Pacific	GQ342526[3]	GQ342455[3]	
Outgroup (Ellisellidae)					
Viminella sp.	RMNH Coel.40032	n.d.	JX203794[4]	JX203852[4]	JX203703[4]
Viminella sp.	RMNH Coel.40813	n.d.	GQ342493[3]	GQ342416[3]	JX203705[4]

Abbreviations: NMS, National Museum Scotland; NHM, Natural History Museum (London, UK); MZB, Museu de Zoologia de Barcelona (Spain); BECA, Biodiversidad y Ecología Acuatica (Seville, Spain); MBARI, Monterey Bay Aquarium Research Institute (California, U.S.A.); NWFSC, Northwest Fisheries Science Center (Seattle, U.S.A.); NIWA, National Institute of Water and Atmospheric Research (Auckland, New Zealand); NOCS, National Oceanography Centre (Southampton, UK), RMNH, Rijksmuseum van Natuurlijke Historie (Leiden, Netherlands); NTM, Museum and Art Gallery of the Northern Territory (Darwin city, Australia); SCF, Collection of S.C. France (U.S.A.); UF, Florida Natural History Museum (Florida; U.S.A.). n.d.: no data.*: Both specimens are from the North Eastern Pacific. However, despite these specimens being originally identified as *Pennatula phosphorea*, according to the phylogenetic hypothesis presented in this paper (see Fig. 5) we consider these identifications as doubtful. We suggest these findings as indicative of a morphospecies and thus deserving of further research.
References: (1) McFadden et al. 2006; (2) Elz et al. unpublished; (3) Brockman and McFadden 2012; (4) McFadden and van Ofwegen 2012; (5) Everett et al. 2016.

28S PCR used the same cycle as the *Cox1* profile, but with 50ºC as the annealing temperature. PCR products were purified using the NucleoSpin® Extract II DNA Purification Kit, following the manufacturer's instructions. Purified products were electrophoresed on an ABI PRISM® 3730xl genetic analyser, and sequence traces were edited using Sequencher™ v4.0.

According to the molecular phylogeny of Dolan et al. (2013) and Kushida and Reimer (2018), *Pennatula* species were included in Clade II. As no clear basal relationships have been demonstrated among the three pennatulacean clades, for the present study sequences of two ellisellids from GenBank were selected as outgroups. The set of new sequences obtained in this study and those from GenBank (see Table 2) were aligned using MUSCLE and then implemented in MEGA5 (Tamura et al. 2011). After alignment, the best nucleotide substitution model was selected using Modeltest implemented in MEGA 5 according to Akaike Information Criterion and hierarchical likelihood ratio test (hLRT) values. The phylogenetic reconstruction was obtained by applying maximum likelihood and Bayesian inference methods. The maximum likelihood method was carried out in MEGA 5 using the nearest neighbour interchange heuristic method and 1000 bootstrap replications. The selected nucleotide substitution model was T92+G+I for the concatenated *mtMutS*+*Cox1*+*28S* data set. The Bayesian inference was carried out with MrBayes v3.1.2 (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003), using the substitution model GTR+G (lset nst=6 rates=gamma) and 10^7 generations, and discarding 25% of the initial trees.

RESULTS

Subclass OCTOCORALLIA Haeckel, 1866
Order Pennatulacea Verrill, 1865
Family Pennatulidae Ehrenberg, 1834
Genus *Ptilella* Gray, 1870

Ptilella Gray 1870: 21; Koren and Danielssen 1874: 422, 1877: 82.
Pennatula, Kölliker 1872: 136 (in part); Kölliker 1880: 4 (in part); Grieg 1892: 10 (in part); Jungersen 1904: 11 (in part); Kükenthal and Broch 1910: 348 (in part); Broch 1913: 28 (in part); Kükenthal 1915: 81 (in part); Hickson 1916: 181 (in part), 1937: 123; Williams 1995a: 125 (in part); López-González et al. 2001: 70 (in part); Altuna 2015: 2 (in part).
Pennatula (*Ptilella*), Verrill 1883: 3, 1885: 532 (in part).

Diagnosis (modified from Gray 1870: 21). Colonies pinnate. Rachis bilaterally symmetrical throughout. Rachis with a central dorsal track naked of zooids. Rachis-peduncle limit with a distinct thickening or swelling, the thickening sometimes forming an edged ring at the thickest point. Axis circular in section, present throughout the entire colony. Polyp leaves large, conspicuous and fan-shaped. Autozooids in groups of 3-4 (occasionally 2) polyps on ventral edge of polyp leaves, groups of polyps in oblique lines. Anthocodiae retractile into spiculiferous, tubular and eight-toothed calyces. Siphonozooids numerous, on the axillae of polyp leaves. Mesozooids on rachis (on both sides of longitudinal naked dorsal track) and on the proximal 2/3 part of dorsal edge of polyp leaves. Dorsal edge of polyp leaves without zooids distally. Sclerites three-flanged and rods. Sclerites at the upper and lower parts of rachis-peduncle limit distinctly differentiated (three-flanged spindles in upper portion, but smaller rods in lower portion).

Type species. *Pennatula borealis* Sars, 1846 (=*Pennatula grandis* Ehrenberg, 1834), by monotypy.

Remarks. Four Atlantic species are here attributed to this genus: *Ptilella grandis* (Ehrenberg, 1834) comb. nov. (N Atlantic), *Ptilella bellissima* (Fowler, 1888), (NE Atlantic), *Ptilella inflata* (Kükenthal, 1910) n. comb. (W Indian and SE Atlantic), and *Ptilella grayi* n. sp. (NE Atlantic). Furthermore, the morphological descriptions of another four Pacific species, *P. naresi* Kölliker (1880: 2), *P. pearceyi* Kölliker (1880: 4), *P. murrayi* Kölliker (1880: 5), and *P. moseleyi* Kölliker (1880: 6), suggest that these could also belong to the genus *Ptilella*. They are here tentatively included in *Ptilella*, although revised and updated morphological and molecular information is necessary to establish this conclusively (see also Discussion). The molecular analysis carried out in this paper suggests the need for a re-evaluation of the previously considered morphological variability within the genus *Pennatula*. Our study reveals that an historical attempt to segregate some of these characters had already been undertaken by Gray (1870) in describing the genus *Ptilella*. As this is in concordance with our molecular data, *Ptilella* is resurrected here.

Ptilella grayi n. sp.

(Figs 1-3)

http://zoobank.org/9844EDA5-AAEC-4884-A054-6CBDE378BE15

Holotype: NMS.Z.2019.2.1, Rockall Haddock Survey cruise 1011S (haul S11426), 56°32.63N 14°30.92W to 56°33.97N 14°28.78W, northeastern Atlantic, 198-202 m depth, 30 Aug 2011.

Paratypes: NMS.Z.2019.2.2, Rockall Anglerfish Survey 2010 cruise 0410S (haul S10150), 56°25.22N 15°14.67W to 56°22.91N 15°18.75W, northeastern Atlantic, 246-261 m depth, 6 Apr 2010. NMS.Z.2019.2.3, Rockall Haddock Survey cruise 1011S (haul S11426), 56°25.22N 15°14.67W to 56°22.91N 15°18.75W, northeastern Atlantic, 198-202 m depth, 30 Aug 2011. MZB 2018-0761, Rockall Haddock Survey cruise 1011S (haul S11426), 56°25.22N 15°14.67W to 56°22.91N 15°18.75W, northeastern Atlantic, 198-202 m depth, 30 Aug 2011.

See Table 1 for additional materials.

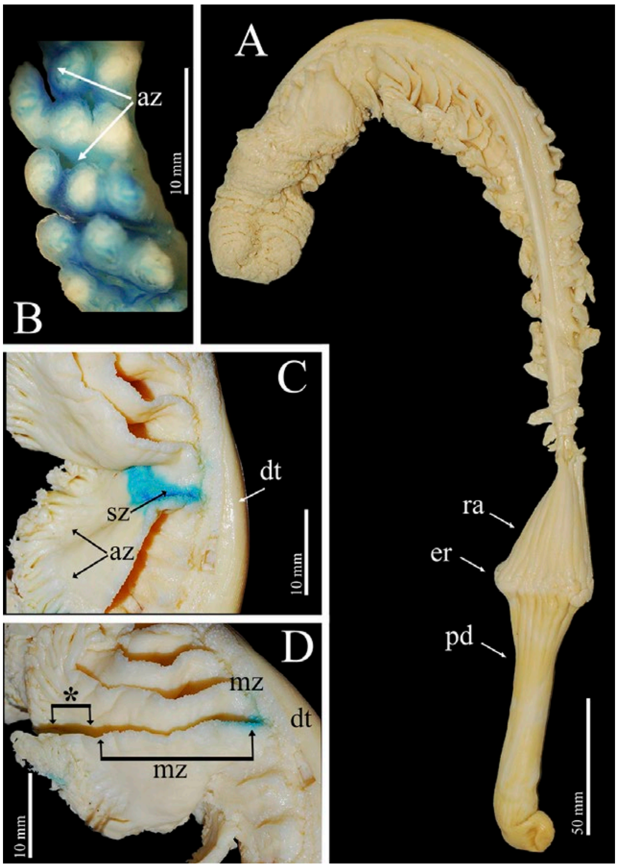

Fig. 2. – *Ptilella grayi* n. sp. Holotype (NMS.Z.2019.2.1). A, whole colony, showing the edged ring (er) betwen peduncle (pd) and rachis (re); B, detail of autozooids (az) in oblique rows, sectioned basally; C, dorsal view of polyp leaves showing the location of mesozooids (mz), siphonozooids (sz) and the distal part devoid of polyps (asterisk); D, area between two polyp leaves from latero-dorsal view showing the location of siphonozooids (sz), autozooids (az) on the ventral edge of the polyp-leaf, and the naked dorsal track (dt). Note that some parts in B to D are stained with methylene blue for increasing contrast.

Description of the holotype. Colony elongate, pinnate, and erect (Fig. 2A), 457 mm in length in preserved state. Axis present throughout colony, rounded in cross section, 5 mm in maximum diameter. Rachis bilaterally symmetrical, 354 mm in length (77% of overall length) and 42 mm in width, with a distinctive naked dorsal and ventral track visible along the rachis. Rachis-peduncle limit with a prominent edged ring (Fig. 2A: er). Peduncle 103 mm in length (23% of overall length) and 36 mm in width at the widest point (the thickening). Rachis with 94 polyp leaves, inserted obliquely and extending ventrally upward, leaves gradually increasing in size along the rachis until the mid-zone, then decreasing in size towards the distal part. Autozooids numerous (approximately 50-60 per polyp-leaf), well developed (up to 5 mm in length, up to 2 mm in width), arranged in groups of 3-4 (occasionally 2), forming oblique rows along the polyp-leaf edge (Fig. 2B: az). Anthocodiae retractile into permanent spiculiferous calyces. Calyces densely spiculated and distinctly eight-toothed. Siphonozooids minute, about 0.35-0.50 mm in diameter, numerous, on the axillae of polyp leaves (Fig. 2C: sz). Mesozooids well developed, 0.50-0.75 mm in diameter, numerous, situated on the latero-dorsal surface of rachis and at the dorsal edge of polyp leaves (Fig. 2D: mz). Dorsal edge of polyp leaf with distinctive zone free of zooids distally (Fig. 2D:*).

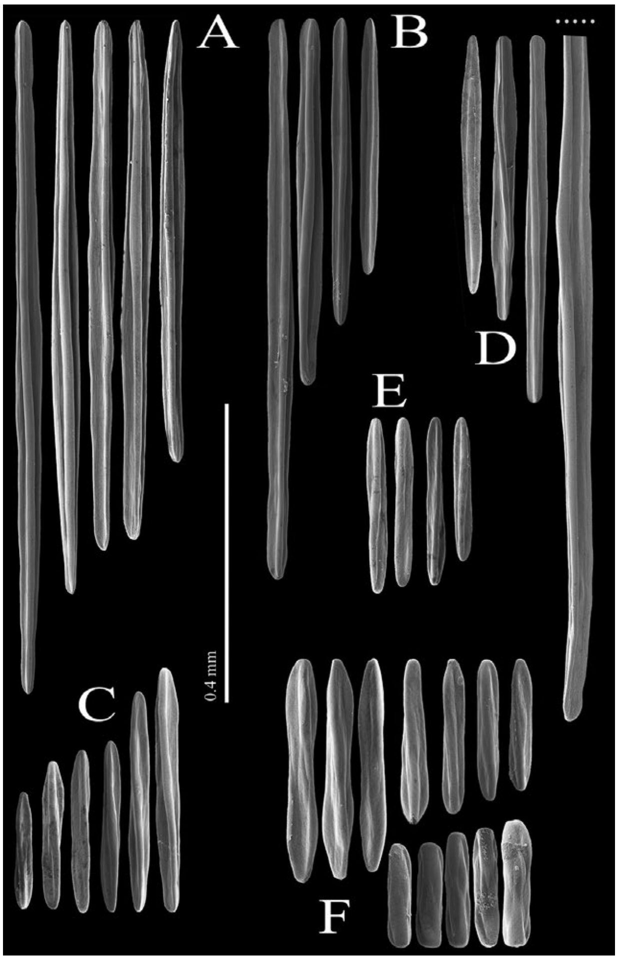

Fig. 3. – *Ptilella grayi* n. sp. Holotype (NMS.Z.2019.2.1). SEM photographs of sclerites. A, calyces; B, mesozooids; C, siphonozooids; D, polyp leaves; E, dorsal track; F, rachis-peduncle limit, above edged ring (upper row) and below edged ring (lower row).

Sclerites in calyces three-flanged rods, up to 1.20 mm in length (Fig. 3A). Sclerites absent in walls and tentacles of autozooids. Sclerites of mesozooids three-flanged rods, up to 0.80 mm in length (Fig. 3B). Sclerites of siphonozooids short, three-flanged rods, up to 0.45 mm in length (Fig. 3C). Sclerites of polyp-leaf surfaces three-flanged rods, up to 1.45 mm in length (Fig. 3D). Sclerites in dorsal track scarce, short three-flanged rods, up to 0.40 mm in length (Fig. 3E). Sclerites above edged ring in conspicuous three-flanged rods, up to 0.30 mm in length (Fig. 3F, upper row). Sclerites below edged ring short rods, up to 0.18 mm in length (Fig. 3F, lower row).

Variations. Colonies grow to a considerable size, as evidenced by living specimens being encountered up to 581 mm length and up to 312 g in weight (weighed wet with excess water blotted off).

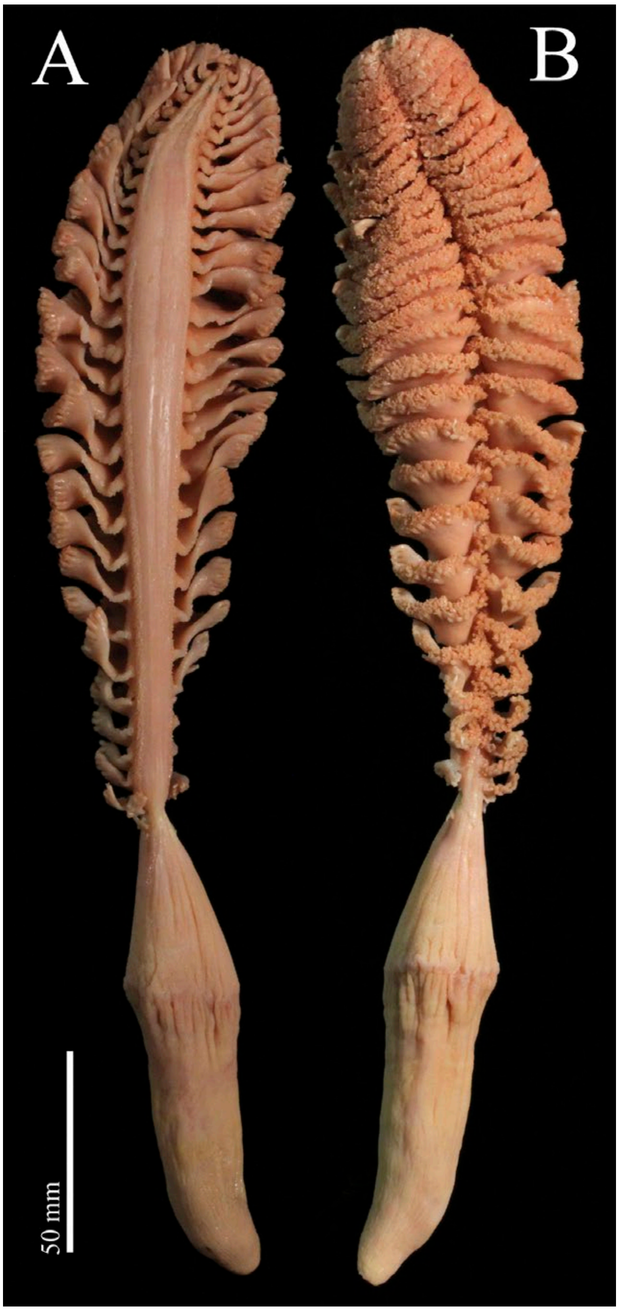

Fig. 4. – *Ptilella grayi* n. sp. (NMS.Z.2019.2.4). Newly collected specimen, showing a more relaxed rachis-peduncle limit, but still with a distinct edged ring. A, dorsal view; B, ventral view. Both images © Crown copyright.

The general colonial structure of the paratype colonies and additional material examined is similar to that of the holotype (see Figs S1 and S2). All preserved colonies examined ranged between 254 and 575 mm in length. Rachis bilaterally symmetrical with 64-98 polyp leaves. Autozooids numerous, arranged in oblique groups of 2-4 along the ventral edge of polyp leaves, 4.0-6.5 mm in length and 1.5-2.0 mm in width. Siphonozooids 0.35-0.50 mm in diameter, on axillar proximal areas of polyp leaves. Mesozooids 0.45-0.75 mm in diameter, numerous, on two lateral bands flanking a naked dorsal track. Axis rounded in cross section, up to 5.4 mm in max diameter. Sclerites as in the holotype: those of calyces up to 1.40 mm in length (Fig. S3A); those of the mesozooids up to 0.80 mm in length (Fig. S3B); those of the siphonozooids up to 0.50 mm in length (Fig. S3C); those of the polyp-leaf surfaces up to 1.40 mm in length (Fig. S3D); those of the dorsal track scarce, up to 0.40 mm in length (Fig. S3E); those close to the rachis-peduncle limit up to 0.35 mm in length above edged ring (Fig. S3F, upper row), and up to 0.17 mm in length below edged ring (Fig. S3F, lower row).

Living colonies range in colour from light yellow/orange (Fig. 4) through to a delicate salmon pink, the latter having been observed in some small specimens. Preserved examined colonies are a light fleshy to dirty white colour in ethanol. Sclerites are colourless.

Etymology. The name *grayi* is chosen in honour of Dr. John Edward Gray (1800-1875), in recognition of his contributions to the knowledge of sea pens and of his being the first to recognize and describe *Ptilella* as a different genus to *Pennatula*.

Distribution and associated fauna. At present, *Ptilella grayi* n. sp. is only known from the upper Rockall Bank area (west of the Outer Hebrides in Scotland) from a depth range of 145-389 m (Fig. 1).

The bottom temperature at these localities had a range of 8.40°C to 9.73°C over the survey period of April to October during the years 2005-2016.

The species is spread over the upper, shallow gradient area of Rockall Bank, but it is broadly centred within the Rockall Haddock Box (RHB) and spreading somewhat towards both the NE and SW. The RHB encloses the entirety of ICES statistical rectangle 42D5 and has been subject to restrictions on mobile commercial fishing since 2001 by agreement between the North East Atlantic Fisheries Commission (NEAFC) and the European Union in order to reduce mortality of stocks of juvenile haddock. To the south and west of the observed distribution of *Pt. grayi* n. sp. lie two further areas that have been closed by the NEAFC to all forms of commercial fishing: the North-West Rockall Bank, which has been closed since 2007, and the South-West Rockall Area (Empress of Britain Bank), which has been closed since 2013. These closed areas are in place to protect vulnerable marine ecosystems (VME) from anthropogenic degradation, the VME in these cases being aggregations of *Lophelia pertusa*. Whether the distribution of *Pt. grayi* n. sp. extends into these areas is, however, presently unknown. Despite an extensive sampling effort, the species has not been recorded in the vicinity of Rockall islet itself. Similarly, *Pt. grayi* n. sp. has not been recorded at all to date on the upper shelf to the east of the Rockall Trough, despite intensive sampling there over a lengthy timeframe (2010-2017 for the upper shelf and 2005-2016 for the shelf slope) and a wide depth range (42-2147 m).

The area of *Pt. grayi* n. sp. distribution, along with the other parts of the upper Rockall Bank, is a productive area for demersal gadoids, particularly haddock, but also prominent are blue whiting (*Micromesistius poutassou*), poor cod (*Trisopterus minutus*), Norway haddock (*Sebastes viviparus),* bluemouth (*Helicolenus dactylopterus*), lesser argentine (*Argentina sphyraena*) and silvery pout (*Gadiculus argenteus thori*). In addition, megrim (*Lepidorhombus whiffiagonis*), grey gurnard (*Eutrigla gurnardus*), anglerfish (*Lophius* ssp.) and blue skate (*Dipturus flossada*) are common (all data available at the public website http://www.ices.dk/marine-data/data-portals/Pages/DATRAS.aspx).

Invertebrates frequently recorded in the same hauls as *Pt. grayi* n. sp. include another sea pen commonly encountered on top of Rockall Bank, *Funiculina quadrangularis*, and the associated brittlestar *Asteronyx lovenii*. Others include the scleractinian *Caryophyllia smithii*, the holothurian *Parastichopus tremulus*, the crustacean *Pagurus forbesii*, the asteroids *Hippasteria phrygiana*, *Stichastrella rosea*, *Astropecten irregularis* and *Luidia cilaris*, the echinoids *Gracilechinus* spp., and various actinarian spp.

In contrast, *Pt. grandis* was observed on the Donegal continental slope, Rockall Plateau and Rosemary Seamounts over a depth range of 682-1605 m, with the associated fish and invertebrate assemblages varying greatly with depth. This study also records this species in the southern Norwegian Sea at a depth of 380-404 m, and to the South Iceland at a depth of 1595 m.

Phylogenetic analyses

In our *mtMutS*+*Cox1*+*28S* hypothesis (Fig. 5), the sequences obtained for *Ptilella grayi* n. sp. and *Pt. grandis* were reunited in a distinct and well-supported clade (Bst >90%, PP >0.9), far from those related to the available concatenated sequences of *Pennatula* species, which are also in a well-supported clade (Bst >90%, PP >0.9). In this phylogenetic hypothesis, species of different genera such as *Actinoptilum, Acanthoptilum, Renilla, Ptilosarcus* and *Gilibelemnon* are placed between *Ptilella* and *Pennatula* sequences with *Ptilella* diverging earlier than *Pennatula.*

Our results demonstrate that *Ptilella* and its closest morphological genus *Pennatula* are different by 19 substitutions in *mtMutS*, 27 in *Cox1* and 110 in *28S*. Moreover, 13 nucleotide mutations in *mtMutS* and 20 in *Cox1* result in silent mutations, while a number of nucleotide mutations (6 in *mtMutS* and 7 in *Cox1*) imply a change of Aa (see Table S2).

An incomplete *mtMutS* sequence of *Pt. inflata* from Namibia was obtained during the molecular works carried out for this paper. Despite several amplifications, it was not possible to obtain a clean sequence that could be used in this study in order to characterize *Pt. inflata*. However, at least 17 of the 19 substitutions differentiating *Ptilella* from *Pennatula* in *mtMutS* were in agreement with its placement in *Ptilella*.

DISCUSSION

Historical remarks on *Ptilella* and its type species

Pennatula grandis was succinctly described by Ehrenberg (1834: 290) based on an old specimen in the Museum of Berlin. Later on, Sars (1846: 17) described and illustrated *Pennatula borealis* based on material collected in Norway. The latter nomenclature and species were widely accepted thereafter by most of Sars's contemporary octocoral researchers, such as Koren and Danielssen (1856), Milne Edwards and Haime (1857), Herklots (1858), Gray (1860), Richiardi (1869) and Kölliker (1869-1872).

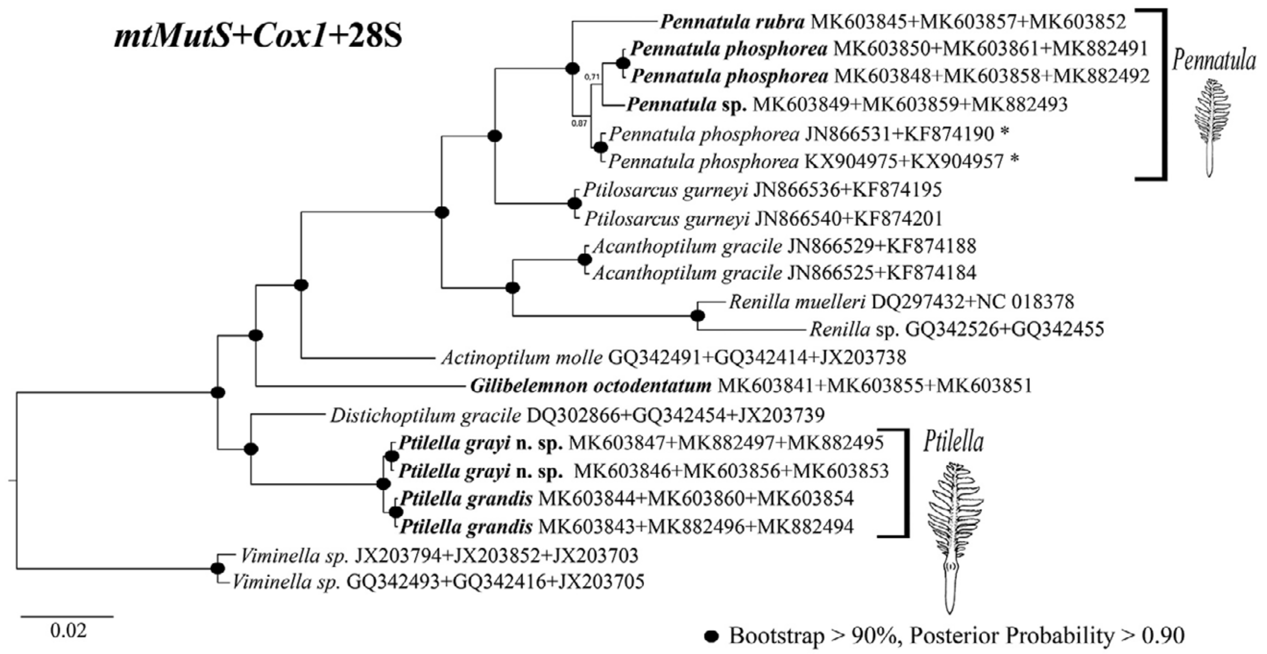

Fig. 5. – Bayesian analysis showing the phylogenetic relationships of *Ptilella*, *Pennatula* and other genera and species of sea pens (Clade II of Dolan et al. 2013, and Kushida and Reimer 2018) based on the concatenated mitochondrial sequences *mtMutS*+*Cox1*+*28S*. Sequences provided by this study are in bold. See Table 2 for a complete list of species and GenBank accession numbers. The asterisks indicate a pair of sequenced samples from the northeastern Pacific originally identified as *Pennatula phosphorea*. According to the results of the present analysis, which includes specimens of *Pennatula phosphorea* from the northeastern Atlantic [the original distribution area indicated by Linnaeus (1758: 818) and Ellis 1764: 420], the identity of the Pacific specimens is considered here as doubtful and deserving of additional morphological and molecular study.

Milne Edwards and Haime (1857: 211) pointed out that Ehrenberg's description of *Pennatula grandis* accorded well with that of Sars's *Pennatula borealis*, using this last nomenclature. Richiardi (1869: 31) also considered *Pennatula grandis* a synonym of *Pennatula borealis*.

Gray (1870: 21) erected the genus *Ptilella* based on *Pennatula borealis* to include a number of distinctive morphological features including "*pinnules membranaceous, broad, rounded, fringed with three close parallel series of short polype-cells on the edge*".

Kölliker (1872: 136) had the opportunity to examine the original material of *Pennatula grandis* deposited in the Museum of Berlin. He provided a diagnosis of the species as *P. borealis* and synonymized Ehrenberg's species without being aware of the description of the genus *Ptilella*. However, this act was contrary to the principle of priority. Kölliker (1872) also described an additional specimen of 350 mm in length deposited in the collection of the Museum of Copenhagen.

Koren and Danielssen (1874: 422) summarized the discussion on the identity of *P. grandis* and *P. borealis* in favour of the former nomenclature by priority of Ehrenberg's contribution. These authors also recognized the differences of *P. borealis* (as the type species of *Ptilella*) from all other *Pennatula* species. Their report is the first to use the nomenclature *Ptilella grandis* (Ehrenberg). This name was also adopted in a further contribution by the same authors (Koren and Danielssen 1877: 82).

Kölliker (1880: 4), however, did not recognize the genus *Ptilella*, considering that several rows of autozooids on the ventral border of the polyp leaves and the row of mesozooids on the dorsal edge of the polyp leaves could be included within the variability of the genus *Pennatula*. Verrill (1883, 1885) considered *Ptilella* a subgenus of *Pennatula*, as *Pennatula* (*Ptilella*) *borealis* Sars. Subsequent contributions by Grieg (1892: 10), Storm (1901), Jungersen (1904). Kükenthal and Broch (1910) and Broch (1913) do not consider *Ptilella* at all but simply mention the species as *Pennatula grandis*.

Despite this, Balss (1910: 54) started to form groups among the species in *Pennatula* that he considered valid, while Kükenthal and Broch (1911: 350) considered within the genus *Pennatula* a "*Grandis-Gruppe*" that brought together *P. grandis*, *P. naresi* and *P. inflata* by the presence of polyp leaves placed in oblique lines and polyps present along at least part of the dorsal margin of the polyp leaves. These authors described a poorly preserved specimen from the North Atlantic as *Pennatula* aff. *inflata*, thus clearly establishing the close relationship of *P. inflata* with *P. grandis* and *P. bellissima*. The intrageneric group "Grandis" (without taxonomic category) was not considered in subsequent important contributions (e.g. Kükenthal 1915, Hickson 1916: 181). However, Hickson (1937: 123) again mentioned a "Grandis-Group" when reporting *P. inflata* from the Maldive Archipelago, considering in that moment the possible synonym of *P. borealis* and *P. bellissima* as *P. grandis,* and even *P. inflata* as a synonym of the latter.

More recently, *Ptilella* was either considered a synonym of *Pennatula* (Williams 1995a: 126) or disappeared from the scarce specialized literature that was published (e.g. Altuna 2015). Thus, *Pennatula grandis* is reported in general contributions on North Atlantic benthic communities (e.g. Edinger et al. 2007, Hamoutene et al. 2008, Murillo et al. 2011, Baker et al. 2012, Baillon et al. 2012).

In the present contribution, the genus *Ptilella* is considered different to *Pennatula* because of the following set of characters:

1) the lack of zooids along the dorsal track; 2) the autozooids on the margins of polyp leaves being arranged in an oblique group of 3-4 (occasionally 2) polyps, giving the impression of several (3-4) series along the polyp-leaf border; 3) a distinctive thickening at the boundary between rachis and peduncle, often giving the appearance of an edged ring while exhibiting different sclerite morphology in the upper and lower part of this thickening; 4) the insertion of polyp leaves on the rachis being distinctly oblique and extending ventrally upward; and 5) DNA sequences (*mMutS*, *Cox1* and *28S*) being divergent from those of other pennatulacean genera (see phylogenetic analyses).

In accordance with the differences described, it follows that the diagnosis of *Pennatula* needs to be amended to include characters that separate it clearly from *Ptilella.*

Diagnosis of *Pennatula* (modified from Williams 1995a: 126)

Colonies pinnate. Symmetry of rachis bilateral throughout. *Rachis-peduncle limit just under the lower polyp leaves, as a narrowing, without a distinct thickening or swelling with a prominent edged ring at the rachis-peduncle limit*. Axis circular in section, present throughout length of colony. Polyp leaves present, usually large and conspicuous, deltoid, sickle-shaped or fan-shaped. Autozooids in *one single row along the ventral edge* of polyp leaves. Anthocodiae retractile into permanent spiculiferous calyces. Calyces tubular, with eight terminal teeth. Siphonozooids on rachis, and between polyp leaves. Mesozooids on rachis, or on basal dorsal margin of polyp leaves. Sclerites three-flanged needles on calyces, mesozooids, siphonozooids, inconspicuous three-flanged rods on surface of peduncle, and small ovals in interior of peduncle (mostly >0.1 mm long).

Although a revision of the species in the genus is needed, according to Williams (1995a) the following list of species can be tentatively considered valid in the genus *Pennatula*: *P. aculeata* Danielssen, 1860 (North Atlantic); *P. delicata* Tixier-Durivault, 1966 (Madagascar); *P. fimbriata* Herklots, 1858 (Japan, Philippines); *P. indica* Thomson and Henderson, 1906 (Indian Ocean); *P. phosphorea* Linneaus, 1758 (northeastern Atlantic and Mediterranean, Cosmopolitan ?); *P. rubra* (Ellis, 1761) (Mediterranean Sea); and *P. prolifera* Jungersen, 1904 (North Atlantic).

As we state above, our phylogenetic hypothesis based on mitochondrial and nuclear markers places the type species of *Ptilella,* (*Pennatula grandis*), far from those sequences from the type species of the genus *Pennatula*, (*P. phosphorea)*, or at least, taking into account that the genus *Pennatula* is in need of revision from the sequenced species and specimens sharing the morphological characters defined for *Pennatula phosphorea* in this paper and the most relevant references (e.g. Kükenthal 1915, Kükenthal and Broch 1911) (see Figs 5 and 6).

Comparison of *Ptilella* species

In this paper, four Atlantic species are considered to be in the genus *Ptilella* (*Pt. grandis*, *Pt. grayi* n. sp. *Pt. inflata*, and *Pt. bellissima*) on the basis of a number of morphological features. Corroborative molecular information is currently available for two of them (*Pt. grandis*, *Pt. grayi)*, and partially so for *Pt. inflata* (see above). Molecular grade tissue is, however, lacking from the old single specimen of *Pt. bellissima* due to the preservation method used.

In the late 19th century, Professor Albert R. von Kölliker described four Pacific sea pen species and ascribed them to the genus *Pennatula*: *P. naresi* Kölliker (1880: 2), *P. pearceyi* Kölliker (1880: 4), *P. murrayi* Kölliker (1880: 5), and *P. moseleyi* Kölliker (1880: 6). The original descriptions of these species agree with some of the characters considered here for *Ptilella*: 1) the obliquely inserted polyp leaves on the rachis; 2) at least two series of autozooids on the ventral edge of the polyp leaves; 3) the dorsal track free of zooids; and 4) mesozooids on the proximal dorsal part of the polyp leaves. While taking into account of the current state of knowledge of these species, their morphological features thus support their tentative inclusion in *Ptilella* here. However, it is important that their position be reassessed following an improved morphological and molecular understanding of these species in the future.

Ptilella grandis and *Pt. grayi* n. sp. are clearly differentiated from *Pt. bellissima* and *Pt. inflata* by their more rigid and spiculose colonies (especially visible on the upper and lower polyp leaf surfaces), a higher overall number of polyp leaves and a higher number of autozooids per polyp leaf. Moreover, the two former species have a much stronger separation between rachis and peduncle with an edged ring (see Figs 2A, S1A, and S2E for *Pt. grayi* n. sp.; Figs 7A and 8C for *Pt. grandis*). This structure is much more marked in *Ptilella grandis* and *Pt. grayi* n. sp. than in *Pt. bellissima* (see Figs 7C and 8I) and *Pt. inflata* (see Figs 7B and 8F), where the upper and lower surfaces are more continuous in structure.

Ptilella bellissima was described by Fowler (1888) based on a single specimen from the Bahamas (northwestern Atlantic) as *Pennatula bellissima*, now deposited in the Natural History Museum in London (BMNH 1880.6.28.1). More than a century later, Castro and Medeiros (2001: 154) described a new species of a large *Pennatula* (up to 350 mm in total length) from the Brazilian coast as *Pennatula bayeri*. After the comparison of both original descriptions and the holotype of *Pt. bellissima*, we believe *P. bayeri* to be a junior synonym of *Pt. bellissima*. As mentioned by Fowler (1888), Castro and Medeiros (2001), and as illustrated in the present paper, *Pt. bellissima* dif-

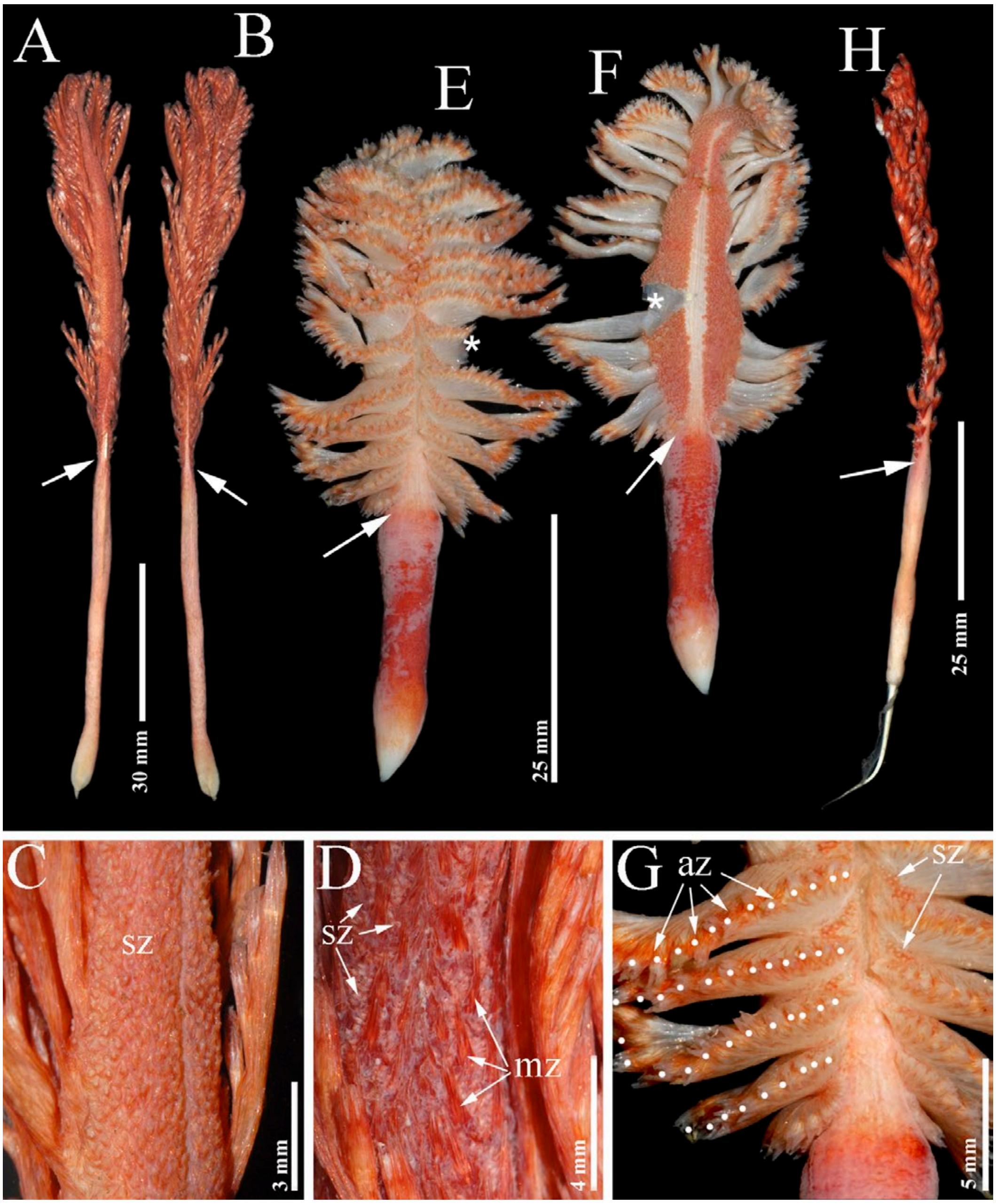

Fig. 6. – Species of the genus *Pennatula* used in this paper for the comparative morphological and/or molecular study. A-C, *Pennatula phosphorea* (BECA OPEN-139); A, dorsal view; B, ventral view; C, detail of the distal part of the rachis in dorsal view showing the presence of siphozooids (sz) only covering the dorsal area. Notice the narrow dorsal track. D, *Pennatula aculeata* (BECA OPEN- 206), detail of distal part of the rachis in dorsal view showing the presence of minute siphozooids (sz) among the large mesozooids (mz) covering the dorsal area. E-G, *Pennatula rubra* (BECA OPEN-453); E, ventral view; F, dorsal view; G, detail of the rachis in ventral view showing the nearly perpendicular orientation of the polyp leaves at their insertion points. Note position of autozooids (indicated by white dots), not to be confused with the siphonozooids (sz), which are present on the rachis limits between two consecutive polyp leaves. H, *Pennatula* sp. (BECA OPEN-152); G, ventro-lateral view, the polyp leaves are shown oriented distally due to the fixation process. Notice limit between rachis and peduncle (arrowed) in all sequenced colonies. Asterisks indicate damage incurred during the study as part of the examination of sclerome or the tissue sampling process.

fers from *Pt. inflata* by its slender triangular and less fleshy polyp leaves, and well-spaced (sometimes difficult to follow) groups of 2-3 autozooids along the ventral edge of the polyp leaves. Castro and Medeiros (2001: 157) discussed the possibility of synonymy between *Pennatula bayeri* (=*Pt. bellissima*) and *Pt. inflata*, but we consider them as two clearly morphologically distinct taxa. It is important that future molecular studies (based on appropriately fixed material) should further explore the internal relationships of *Ptilella* species. Particular attention should be paid to further specimens of *Pt. bellissima*, in order to correctly evaluate the variability in the distribution of mesozooids around the insertion point of the polyp leaves and the autozooids.

The most immediately evident feature differentiating *Ptilella grandis* and *Pt. grayi* n. sp. is the colour: dark-red to red-brown both alive and pre-

Fig. 7. – Species of the genus *Ptilella* used in this paper for the comparative study; A, *Ptilella grandis* (NMS.Z.2019.2.6); B, *Ptilella inflata* (NMS.Z.2019.2.7); C, *Ptilella bellissima* (BMNH 1888.6.28.1) holotype.

served in the former but light fleshy to dirty white in the latter in the preserved state and light yellow to light pink in living specimens. In the examined material the maximum observed lengths of sclerites in *Pt. grandis* and *Pt. grayi* n. sp. tends to be overall slightly larger in the former species (calyces 1.70 vs. 1.40 mm; mesozooids 1.0 vs. 0.80 mm; siphonozooids 0.80 vs. 0.50 mm; polyp leaves 1.11 vs. 1.40 mm; dorsal track 0.50 vs. 0.40; rachis-peduncle limit 0.50 vs. 0.35 above edged ring and 0.16 vs. 0.17 mm below edged ring).

The molecular comparison of *Pt. grayi* n. sp. vs *Pt. grandis* revealed the following substitutions: at the positions 249 (A/C) and 672 (C/A) in *mtMutS*; at the positions 372 (A/G) and 624 (C/A) in *Cox1*; and at the positions 166 (C/A), 210 (A/G) and 519 (G/C) in *28S* (see Table S3). In *mtMutS*, both substitutions imply the change of Aa (Met by Ile, and Phe by Leu, respectively), although in both cases all Aa are nonpolar. In *Cox1*, both are silent substitutions. Although the genetic distance obtained in *mtMutS* did not exceed 0.30%, the more conserved mitochondrial marker *Cox1* also showed differences between these species, which are additionally supported by the differences in the nuclear *28S*.

According to our current knowledge, *Pt. grayi* n. sp. seems to have an overlapping but somewhat more geographically restricted and shallower bathymetric distribution than *Pt. grandis* (145-389 m and 90-2700 m in depth, respectively). Moreover, the new species is only known, for the moment, from the NE Atlantic, though *Pt. grandis* has been reported from both sides of the North Atlantic Ocean. The densities of *Pt. grayi* n. sp. on Rockall Bank are presently unknown and cannot be inferred from swept area estimates using numbers caught per haul, as it is likely that demersal trawl is an inefficient method of capturing large sea pens that are anchored in the sediment. Moreover, the exact design of trawl used varied with the survey that the specimens were obtained fr.0om.

On the polyphyletic family Pennatulidae and the familial relationships in Clade II

The traditional family Pennatulidae comprises six genera: *Gyrophyllum*, *Pennatula*, *Ptilosarcus*, *Sarcoptilus*, *Crassophyllum* and *Pteroeides* (Williams 1995a, 1995b). Dolan et al. (2013) showed a consensus phylogenetic tree suggesting a non-monophyletic Pennatulidae. Although the monophyly of Pennatulidae was not

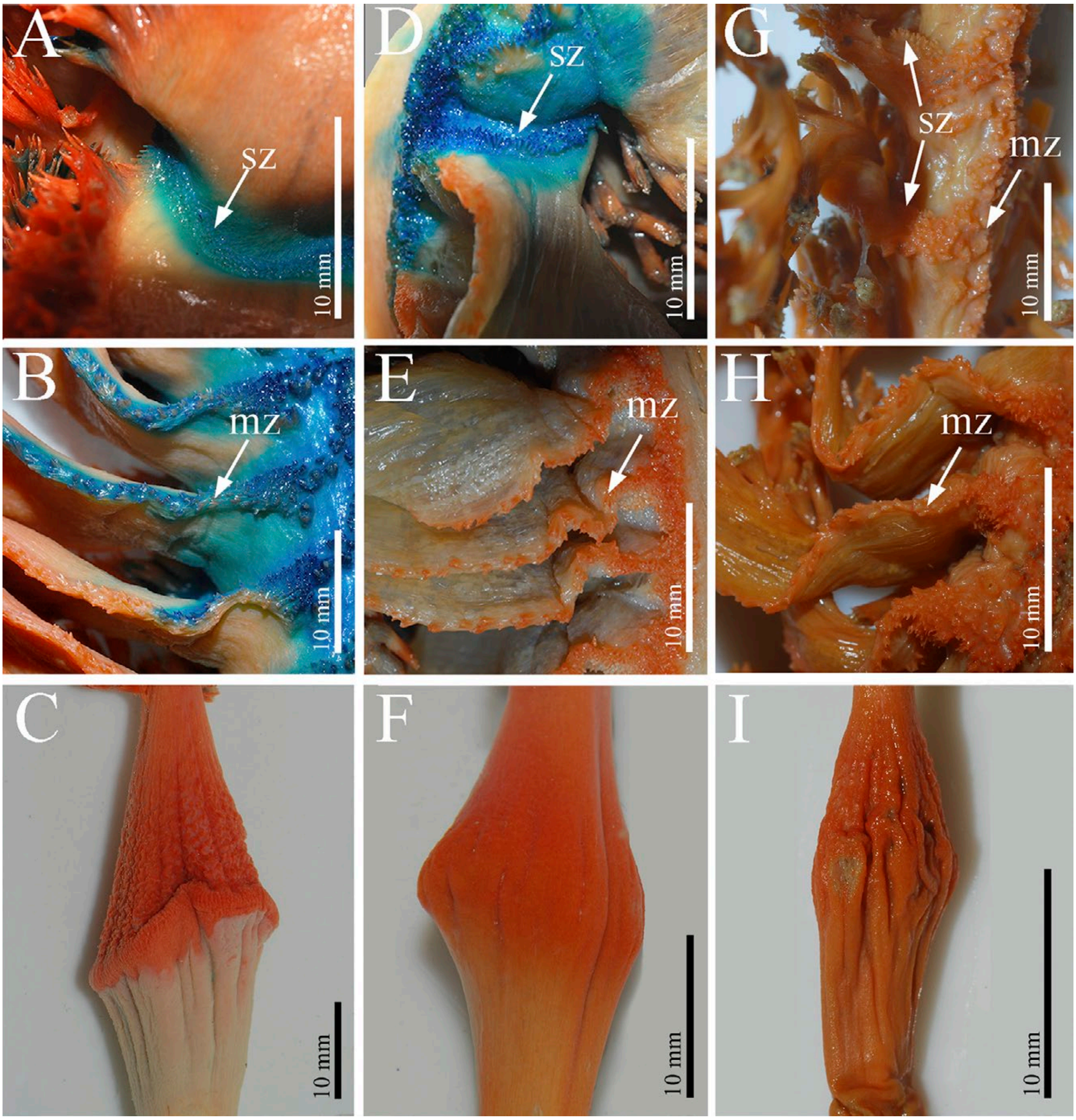

Fig. 8. – Details of different parts of colonies of *Ptilella* species used for comparison in this study. Vertical rows of images: A-C, *Ptilella grandis;* D-F, *Ptilella inflata*; G-I, *Ptilella b ellissima*. Horizontal rows of images: A, D and G, dorso-lateral view showing arrangement of siphonozooids (sz) in pads at base of polyp leaves; B, E and H, dorsal view of polyp leaves showing mesozooids (mz) on their dorsal edges; C, F and I, thickening at the limit between rachis and peduncle.

formally rejected by an SH test, in that study this family formed a non-natural group, placing the genera *Pteroeides*, *Pennatula*, and *Gyrophyllum* into three different clades (Clades I, II and III, respectively). Clade II based on two mitochondrial markers (*mtMutS*+*ND2*) included the genera *Pennatula*, *Renilla, Distichoptillum* and *Protoptilum*, the two former genera being sister groups. Later, results offered by Kushida and Reimer (2018) based on those same mitochondrial (*mtMutS*+*ND2*) again confirmed the polyphyletic nature of the current Pennatulidae, showing the same distribution of the genera *Pteroeides*, *Pennatula* and *Gyrophyllum*. In that study, Clade II again included the genus *Pennatula* as the sister group of *Renilla*, and both related to genera of different families: *Echinoptilum* (Echinoptilidae), *Stachyptilum* (Stachyptilidae), *Distichoptilum* and *Protoptilum* (both Protoptilidae), and *Scytalium* and *Stylatula* (both Virgulariidae). In our present phylogenetic analyses for Clade II we present a more complete molecular coverage, including two mitochondrial (*mtMutS*, *Cox1*) markers and a nuclear marker (*28S*), showing a well-supported genus *Pennatula* with *Ptilosarcus* as the sister group, with *Renilla* being the sister group of *Acanthoptillum* (see Fig. 5). *Pennatula* species were well separated from the resurrected genus *Ptilella*, which diverges early. Future sea pen studies utilizing a more complete set of taxonomic units (families, genera and species) and molecular markers may completely change our previous understanding of the distribution of genera among all the possible familial units in this specialized group of octocorals. We recognise the current incomplete and unstable state of taxonomic knowledge of the Pennatulidae and our placement of Ptilella within this family is to be regarded as tentative. We consider this as pending a thoroughly comprehensive review that will also achieve the segregation of diagnostic morphological characters across those well supported familial units.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available through the online version of this article and at the following link: http://scimar.icm.csic.es/scimar/supplm/sm04845esm.pdf

Table S1. – Data associated with all hauls where *Ptilella grayi* n. sp. and *Ptilella grandis* were observed, including the number of colonies recorded at each.

Table S2. – Nucleotide substitution implying amino acid changes in the mitochondrial loci *mtMutS* and *Cox1*, between the sequences of *Ptilella* and *Pennatula* used in the molecular analyses, once homologous sites of a datamatrix including *Ptilella* and *Pennatula* sequences are aligned (see Table 2 and Fig. 5).

Table S3. – Nucleotide and amino acid differences in the mitochondrial loci *mtMutS* and *Cox1*, and nucleotide differences in the nuclear *28S* between *Ptilella grayi* n. sp. and *Pt. grandis*. Based on a datamatrix including only *Ptiella* and *Pennatula* species (see Table 2 and Fig. 5).

Fig. S1. – *Ptilella grayi* n. sp. Paratype (NMS.Z.2019.2.3). A, whole colony; B, dorsal view of polyp leaves and naked dorsal track showing the location of mesozooids; C, detail of autozooids on the ventral edge of the polyp leaves; D, detail of oblique rows of autozooids, sectioned basally, stained with methylene blue to increase contrast.

Fig. S2. – *Ptilella grayi* n. sp. Paratype (NMS.Z.2019.2.3). A, polyp-leaf sectioned from the base, lateral view; B, detail of dorsal area of a polyp-leaf showing autozooids (az) and mesozooids (mz), and distal area free of zooids (*); C, detail of dorsal basal part of polyp leaves showing siphonozooids (sz) and mesozooids (mz); D, detail of arrangement of siphonozooids (sz) in pad at the base of a polyp leaf; E, distinctive thickening at the rachis-peduncle limit.

Fig. S3. – *Ptilella grayi* n. sp. Paratype (NMS.Z.2019.2.3). SEM photographs of sclerites. A, calyces; B, mesozooids; C, siphonozooids; D, polyp leaves; E, dorsal track; F, rachis-peduncle limit, above edged ring (upper row) and below edged ring (lower row).

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Resurrection of the sea pen genus *Ptilella* Gray, 1870 and description of *Ptilella grayi* n. sp. from the NE Atlantic (Octocorallia: Pennatulacea)

Francisco J. García-Cárdenas, Jim Drewery, Pablo J. López-González

Supplementary material

Table S1. – Data associated with all hauls where *Ptilella grayi* n. sp. and *Ptilella grandis* were observed including the number of colonies recorded at each.

Species	Cruise	Cruise no (haul)	Area	Coordinates Start - Stop	Depth range (m)	Date	No. colonies
Ptilella grayi n. sp.	Rockall Haddock Survey 2005	1205S (S05357)	Rockall Bank, NE Atlantic	56º35.49N 14º31.57W-56º36.52N 14º28.72W	197-198	06-Sep-05	3
Ptilella grayi n. sp.	Rockall Haddock Survey 2007	1207S (S07371)	Rockall Bank, NE Atlantic	57º09.39N 14º12.16W-57º09.59N 14º08.94W	166-170	13-Sep-07	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2007	1207S (S07386)	Rockall Bank, NE Atlantic	56º32.78N 14º32.25W-56º31.32N 14º34.00W	198-203	12-Sep-07	2
Ptilella grayi n. sp.	Rockall Haddock Survey 2008	1108S (S08358)	Rockall Bank, NE Atlantic	56º28.03N 14º41.91W-56º27.82N 14º38.40W	199-202	06-Sep-08	5
Ptilella grayi n. sp.	Rockall Haddock Survey 2008	1108S (S08360)	Rockall Bank, NE Atlantic	56º35.43N 14º32.20W-56º36.37N 14º29.54W	197-199	06-Sep-08	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2009	0409S (S09168)	Rockall Bank, NE Atlantic	56º43.54N 14º34.52W-56º46.84N 14º33.03W	187-195	5-May-09	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2009	0409S (S09169)	Rockall Bank, NE Atlantic	56º37.05N 14º25.39W-56º36.63N 14º31.29W	191-200	5-May-09	3
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2009	0409S (S09170)	Rockall Bank, NE Atlantic	57º17.82N 14º14.45W-57º14.44N 14º14.93W	170-178	6-May-09	3
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2009	0409S (S09172)	Rockall Bank, NE Atlantic	57º22.21N 13º52.59W-57º21.17N 13º58.32W	145-153	6-May-09	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2009	1209S (S09339)	Rockall Bank, NE Atlantic	57º03.65N 14º08.69W-57º02.74N 14º11.61W	167-191	07-Sep-09	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2009	1209S (S09347)	Rockall Bank, NE Atlantic	56º36.11N 14º29.97W-56º35.20N 14º32.73W	194-201	09-Sep-09	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2009	1209S (S09349)	Rockall Bank, NE Atlantic	56º43.69N 14º30.18W-56º44.51N 14º29.62W	192-198	09-Sep-09	2
Ptilella grayi n. sp.	Rockall Haddock Survey 2009	1209S (S09350)	Rockall Bank, NE Atlantic	56º47.90N 14º05.97W-56º46.39N 14º08.07W	193-198	10-Sep-09	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2010	0410S (S10150)	Rockall Bank, NE Atlantic	56º25.22N 15º14.67W-56º22.91N 15º18.75W	238-262	06-Apr-10	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2011	0911S (S11426)	Rockall Bank, NE Atlantic	56º32.63N 14º30.92W-56º33.97N 14º28.78W	198-202	30-Aug-11	12
Ptilella grayi n. sp.	Rockall Haddock Survey 2011	0911S (S11427)	Rockall Bank, NE Atlantic	56º35.18N 14º24.27W-56º36.48N 14º22.06W	204-209	30-Aug-11	2
Ptilella grayi n. sp.	Rockall Haddock Survey 2011	0911S (S11430)	Rockall Bank, NE Atlantic	56º42.54N 14º03.67W-56º43.71N 14º01.29W	251-255	30-Aug-11	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2011	0911S (S11432)	Rockall Bank, NE Atlantic	57º02.48N 13º52.08W-57º03.75N 13º49.62W	182-186	30-Aug-11	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2012	0412S (S12141)	Rockall Bank, NE Atlantic	55º51.10N 15º19.53W-55º52.24N 15º13.35W	365-375	14-Apr-12	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2012	0412S (S12145)	Rockall Bank, NE Atlantic	56º32.34N 14º55.91W-56º35.17N 15º00.02W	195-225	15-Apr-12	3
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2012	0412S (S12146)	Rockall Bank, NE Atlantic	56º53.19N 14º44.00W-56º55.71N 14º39.29W	179-192	15-Apr-12	1
Ptilella grayi n. sp.	OFFCON 2012	0712S (S12287)	Rockall Bank, NE Atlantic	57º22.33N 13º54.87W-57º21.36N 13º57.73W	146-152	15-Apr-12	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2013	1113S (S13304)	Rockall Bank, NE Atlantic	56º43.65N 14º14.44W-56º41.83N 14º14.62W	196-203	14-Sep-13	2
Ptilella grayi n. sp.	Rockall Haddock Survey 2013	1113S (S13305)	Rockall Bank, NE Atlantic	56º36.15N 14º22.46W-56º34.79N 14º24.46W	206-209	14-Sep-13	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2013	1113S (S13306)	Rockall Bank, NE Atlantic	56º36.05N 14º30.02W-56º35.75N 14º33.40W	196-199	14-Sep-13	6
Ptilella grayi n. sp.	Rockall Haddock Survey 2013	1113S (S13307)	Rockall Bank, NE Atlantic	56º40.01N 14º39.58W-56º39.38N 14º40.49W	195-198	14-Sep-13	2
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2013	1413S (S13371)	Rockall Bank, NE Atlantic	56º36.59N 14º26.65W-56º35.81N 14º32.89W	197-199	24-Oct-13	82
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2013	1413S (S13372)	Rockall Bank, NE Atlantic	56º55.42N 14º31.33W-56º58.65N 14º28.61W	179-188	24-Oct-13	2
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2013	1413S (S13373)	Rockall Bank, NE Atlantic	57º10.17N 14º11.27W-57º12.81N 14º06.85W	165-171	24-Oct-13	4
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2014	0414S (S14141)	Rockall Bank, NE Atlantic	56º25.40N 14º57.37W-56º26.56N 14º54.75W	206-212	14-Apr-14	2
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2014	0414S (S14144)	Rockall Bank, NE Atlantic	56º41.94N 14º32.66W-56º45.50N 14º32.42W	193-199	15-Apr-14	2
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2015	0415S (S15142)	Rockall Bank, NE Atlantic	56º36.04N 14º14.27W-56º32.91N 14º16.67W	260-299	11-Apr-15	1

Species	Cruise	Cruise no (haul)	Area	Coordinates Start - Stop	Depth range (m)	Date	No. colonies
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2015	0415S (S15143)	Rockall Bank, NE Atlantic	56º36.27N 14º26.94W-56º35.43N 14º32.96W	194-205	12-Apr-15	17
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2015	0415S (S15144)	Rockall Bank, NE Atlantic	56º29.97N 14º21.34W-56º27.17N 14º24.64W	281-287	12-Apr-15	3
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2015	0415S (S15157)	Rockall Bank, NE Atlantic	56º25.35N 14º56.29W-56º27.76N 14º51.81W	204-214	14-Apr-15	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2015	0415S (S15159)	Rockall Bank, NE Atlantic	56º34.02N 15º13.28W-56º30.69N 15º15.78W	266-289	14-Apr-15	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2015	1115S (S15351)	Rockall Bank, NE Atlantic	56º35.93N 14º31.35W-56º36.48N 14º28.27W	196-198	31-Aug-15	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2015	1115S (S15352)	Rockall Bank, NE Atlantic	56º36.95N 14º16.25W-56º38.10N 14º13.72W	226-231	31-Aug-15	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2015	1115S (S15356)	Rockall Bank, NE Atlantic	57º02.91N 13º25.79W-57º01.49N 13º27.53W	270-278	01-Sep-15	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2016	0416S (S16137)	Rockall Bank, NE Atlantic	57º36.48N 12º57.47W-57º34.60N 12º57.59W	280-290	09-Apr-16	1
Ptilella grayi n. sp.	Rockall Anglerfish Survey 2016	0416S (S16164)	Rockall Bank, NE Atlantic	56º15.82N 16º14.36W-56º12.51N 16º15.49W	358-389	13-Apr-16	2
Ptilella grayi n. sp.	Rockall Haddock Survey 2016	1216S (S16365)	Rockall Bank, NE Atlantic	56º33.53N 14º29.33W-56º32.22N 14º31.75W	199-201	05-Sep-16	3
Ptilella grayi n. sp.	Rockall Haddock Survey 2016	1216S (S16366)	Rockall Bank, NE Atlantic	56º46.45N 14º27.75W-56º44.81N 14º29.19W	186-193	05-Sep-16	3
Ptilella grayi n. sp.	Rockall Haddock Survey 2016	1216S (S16368)	Rockall Bank, NE Atlantic	56º47.22N 14º05.67W-56º45.63N 14º07.23W	193-200	05-Sep-16	1
Ptilella grayi n. sp.	Rockall Haddock Survey 2016	1216S (S16372)	Rockall Bank, NE Atlantic	57º07.74N 13º23.21W-57º06.31N 13º25.09W	215-223	06-Sep-16	1
Ptilella grandis	Deepwater Time Series 2008	1108S (S08385)	Donegal Slope, NE Atlantic	55º13.79N 10º08.87W-55º08.49N 10º10.66W	1018-1045	14-Sep-08	1
Ptilella grandis	OFFCON 2011	0711S (S11264)	Rockall Basin, NE Atlantic	57º36.37N 12º36.66W-57º35.94N 12º36.98W	1601-1605	30-Jun-11	1
Ptilella grandis	North Sea Anglerfish Survey 2016	0412S (S12220)	S Norwegian Sea, NE Atlantic	61º55.05N 02º28.94E-61º52.66N 02º34.22E	380-404	29-Apr-12	1
Ptilella grandis	MoreDeep 2014	1314S (S14379)	Rosemary Seamount, NE Atlantic	59º26.55N 10º09.34W-59º26.60N 10º02.14W	859-889	15-Sep-14	1
Ptilella grandis	MoreDeep 2014	1314S (S14382)	Rosemary Seamount, NE Atlantic	59º05.37N 09º54.16W-59º06.40N 09º51.58W	1052-1075	16-Sep-14	1
Ptilella grandis	Rockall Anglerfish Survey 2016	0416S (S16151)	West Rockall Bank, NE Atlantic	56º57.12N 14º15.98W-56º56.60N 14º21.78W	723-761	11-Apr-16	1
Ptilella grandis	Rockall Anglerfish Survey 2016	0416S (S16155)	West Rockall Bank, NE Atlantic	56º36.27N 14º26.94W-56º35.43N 14º32.96W	682-727	11-Apr-16	1
Ptilella grandis	MoreDeep 2016	1316S (S16379)	Rosemary Seamount, NE Atlantic	59º06.16N 09º52.17W-59º05.16N 09º54.75W	1061-1100	19-Sep-16	2

Table S2. – Nucleotide substitution implying amino acid changes in the mitochondrial loci *mtMutS* and *Cox1*, between the sequences of *Ptilella* and *Pennatula* used in the molecular analyses, once homologous sites of a datamatrix including *Ptilella* and *Pennatula* sequences are aligned (see Table 2 and Fig. 5).

Loci	Nucleotide positions	Nucleotide triplet *Ptilella* / *Pennatula*	Aa position	Aa substitution *Ptilella / Pennatula*
mtMutS	34	ATG / GTA(GTG)[1]	12	Met (M) / Val (V)
	74	CCG / CAG	25	Pro (P) / Glm (Q)
	164	GCT / GTT	55	Ala (A) / Val (V)
	197	GGA / GAA	66	Gly (G) / Glu (E)
	199	GTA / ATA	67	Val (V) / Ile (I
	533	GCC / GTC	181	Ala (A) / Val (V)
Cox1	226	TTG / ATG	76	Leu (L) / Met (M)
	271	TCA / GCA	91	Ser (S) / Ala (A)
	371	GCG(GCA)[2] / GTA	124	Ala (A) / Val (V)
	388	ATT / CTT	130	Ile (I) / Leu (L)
	439	AGT / CTA(TTA)	147	Met (M) / Leu (L)
	568	GTA / ATA	190	Val (V) / Ile (I)
	574	GTT / ATT	192	Val (V) / Ile (I)

[1] and [2], the third base of this triplet is a variable position within that genus (interspecific variability). The substitutions implying intergeneric differences occur in the first base of the triplet in 1, but in the second base in 2.

Table S3. – Nucleotide and amino acid differences in the mitochondrial loci *mtMutS* and *Cox1*, and nucleotide differences in the nuclear *28S* between *Ptilella grayi* n. sp. and *Pt. grandis*. Based on a datamatrix including only *Ptiella* and *Pennatula* species (see Table 2 and Fig. 5).

Loci	Nucleotide (position) *Pt. grayi* / *Pt. grandis*	Amino acid (position) *Pt. grayi* / *Pt. grandis*
mtMutS	(249) A / C	(83) Met / Ile
	(672) C / A	(225) Phe / Leu
Cox1	(372) A / G	(124) Ala / Ala
	(624) C / A	(208) Ile / Ile
28S	(166) C / A	
	(210) A / G	
	(519) G / C	

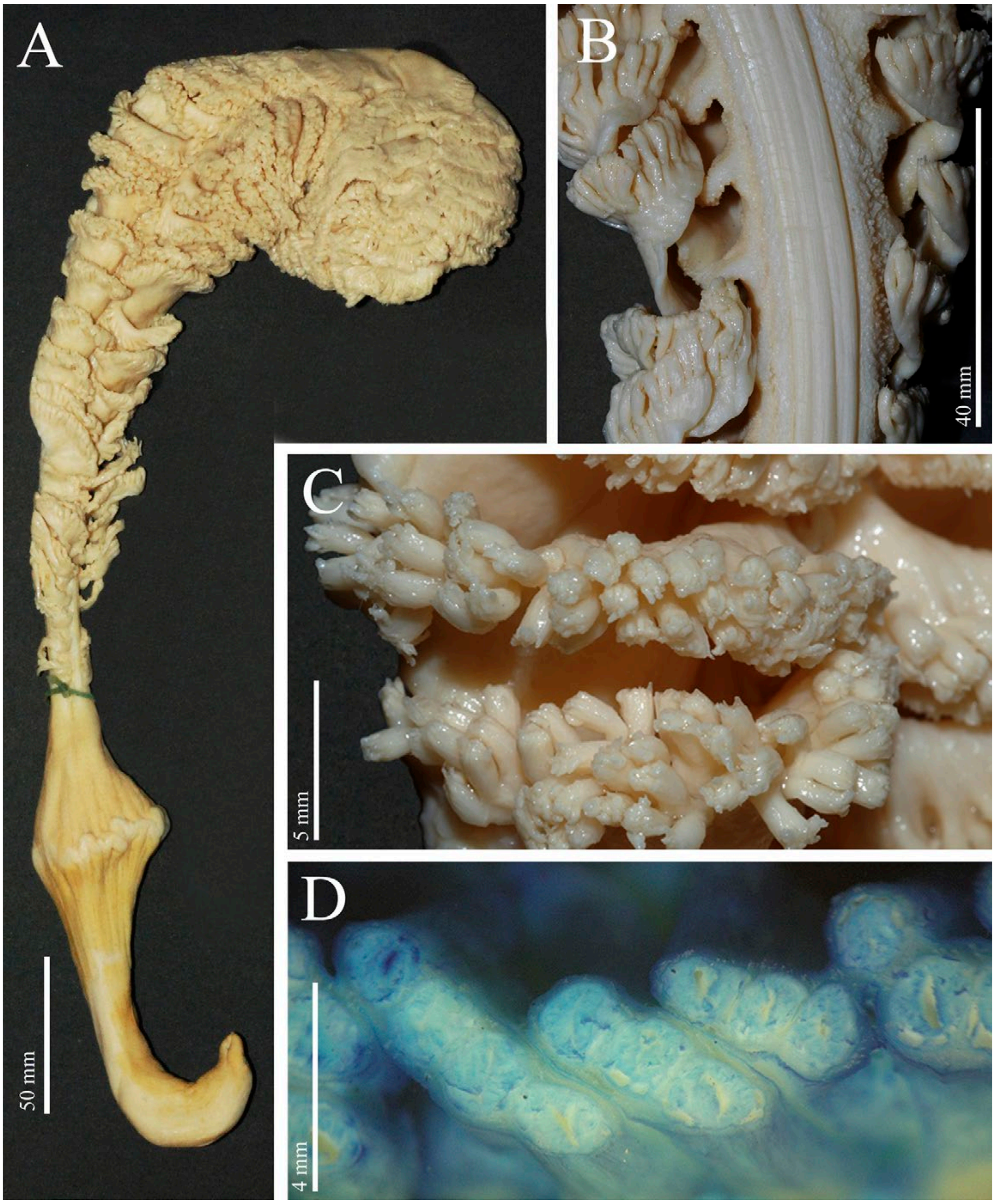

Fig. S1. – *Ptilella grayi* n. sp. Paratype (NMS.Z.2019.2.3). A, whole colony; B, dorsal view of polyp leaves and naked dorsal track showing the location of mesozooids; C, detail of autozooids on the ventral edge of the polyp leaves; D, detail of oblique rows of autozooids, sectioned basally, stained with methylene blue to increase contrast.

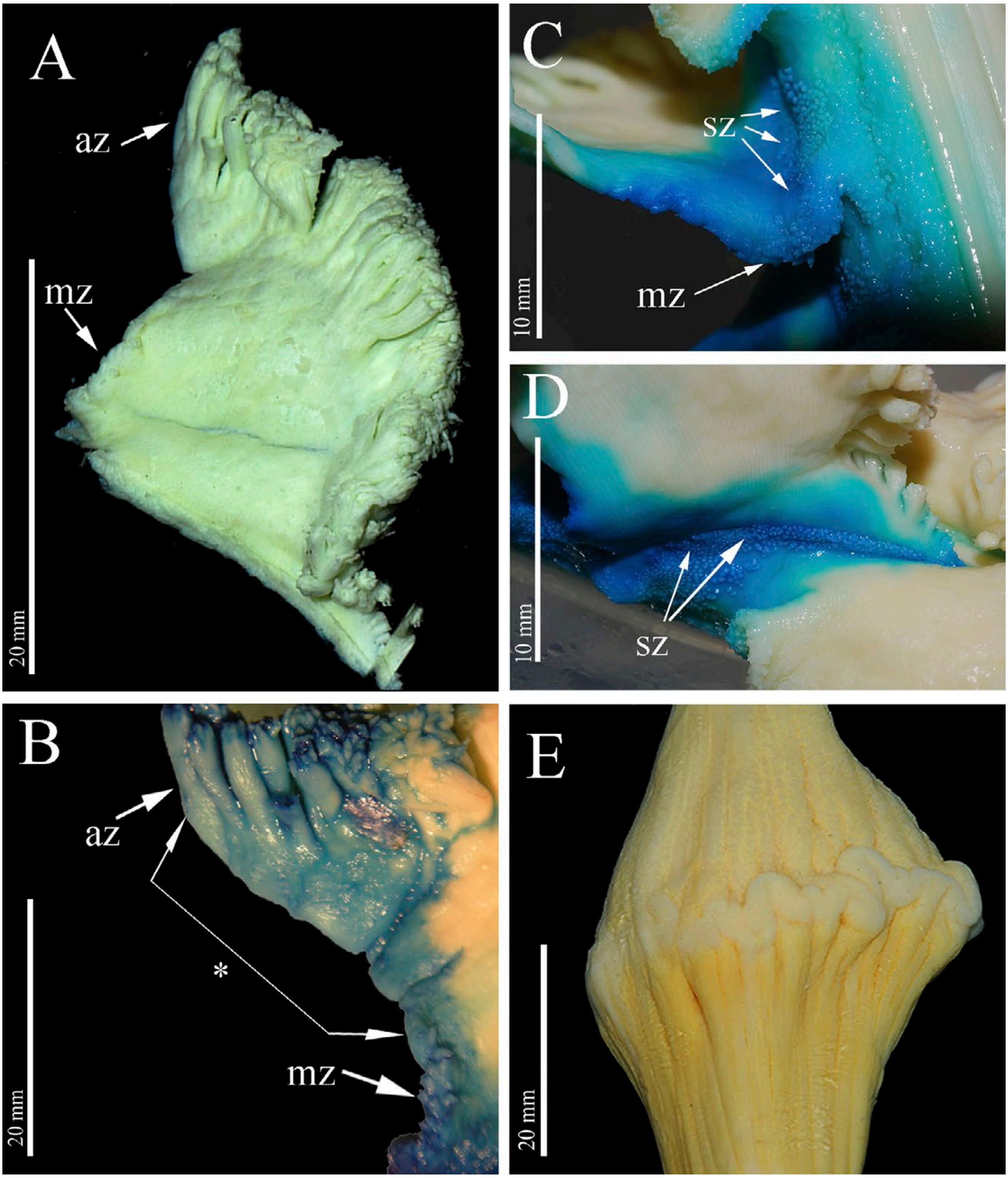

Fig. S2. – *Ptilella grayi* n. sp. Paratype (NMS.Z.2019.2.3). A, polyp-leaf sectioned from the base, lateral view; B, detail of dorsal area of a polyp-leaf showing autozooids (az) and mesozooids (mz), and distal area free of zooids (*); C, detail of dorsal basal part of polyp leaves showing siphonozooids (sz) and mesozooids (mz); D, detail of arrangement of siphonozooids (sz) in pad at the base of a polyp leaf; E, distinctive thickening at the rachis-peduncle limit and edged ring. B, C and D stained with methylene blue to increase contrast.

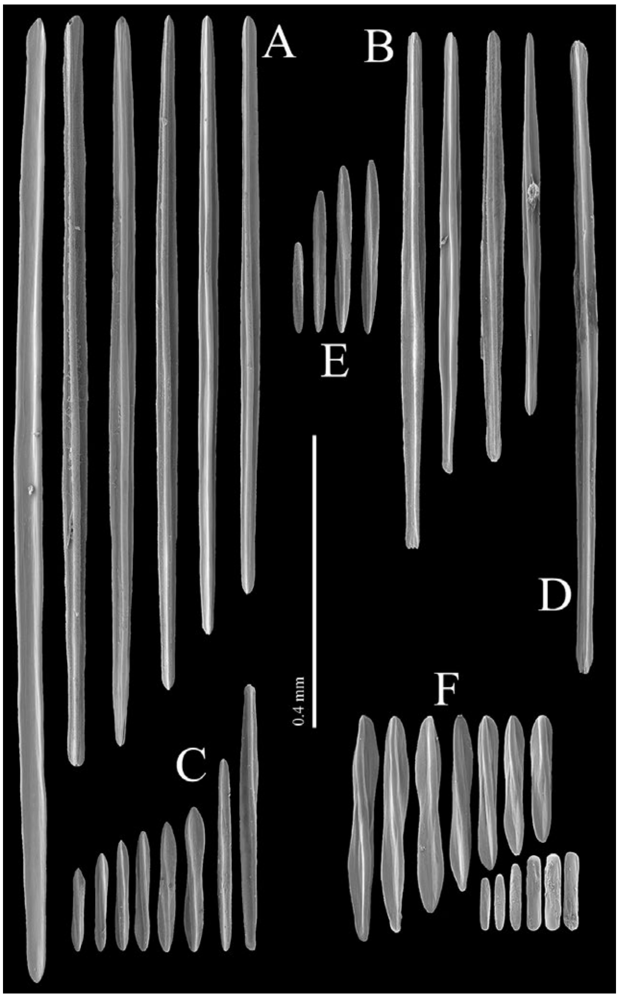

Fig. S3. – *Ptilella grayi* n. sp. Paratype (NMS.Z.2019.2.3). SEM photographs of sclerites. A, calyces; B, mesozooids; C, siphonozooids; D, polyp leaves; E, dorsal track; F, rachis-peduncle limit, above edged ring (upper row) and below edged ring (lower row).