Molecular phylogeny and divergence time estimates in pennatulaceans (Cnidaria: Octocorallia: Pennatulacea)

Francisco J. García-Cárdenas [1], Mónica Núñez-Flores [1,2], Pablo J. López-González [1]

[1] Biodiversidad y Ecología Acuática. Departamento de Zoología, Facultad de Biología, Universidad de Sevilla, Reina Mercedes 6, 41012 Sevilla, Spain.
(FJG-C) (Corresponding author) E-mail: frangarca@us.es. ORCID iD: https://orcid.org/0000-0002-1503-9552
(MN-F) E-mail: nuez.monica@gmail.com. ORCID iD: https://orcid.org/0000-0002-9692-1909
(PJL-G) E-mail: pjlopez@us.es. ORCID iD: https://orcid.org/0000-0002-7348-6270
[2] Programa de Doctorado en Sistemática y Biodiversidad, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Chile.

Summary: Pennatulaceans are an important component of benthic marine communities usually related to soft bottoms. Despite their important ecological role, as yet little is known about their origin and divergence time. The first attempts to establish phylogenetic relationships among genera date from the early 20th century, when only morphological characters were available. In the last decade, phylogenetic analyses based on mitochondrial DNA sequences from a selected number of species have proposed a different hypothetical ancestor for this group, but their intergeneric relationships remain obscure. The present study is based on a combination of mitochondrial and nuclear markers (*mtMutS*, *Cox1* and *28S* rDNA), adding new molecular information about the phylogenetic relationships among the pennatulacean genera, including 38 new sequences belonging to 13 different species. Some of the phylogenetic relationships inferred in the present study question the current classification of sea pens based on morphology (at different taxonomic levels), clearly indicating that the two main groups Sessiliflorae and Subselliflorae, some of their main families (e.g. Pennatulidae, Umbellulidae, Virgulariidae) and some genera (e.g. *Umbellula*, *Veretillum*) are non-monophyletic. In addition, the veretillids, traditionally considered the most primitive pennatulaceans, are not shown as the earliest-diverging taxon. Moreover, an analysis of divergence time performed here suggested that the origin of the pennatulaceans dates from the Lower Cretaceous (Berriasian, ~144 Ma), in agreement with their sparsely known fossil record, while the initial divergence of most extant genera occurred in the Oligocene and Miocene times.

Keywords: phylogeny; *mtMutS*; *Cox1*; *28S*; Pennatulacea; sea pen; divergence time.

Filogenia molecular y estimación del tiempo de divergencia en pennatuláceos (Cnidaria: Octocorallia: Pennatulacea)

Resumen: Los pennatuláceos son un componente importante de las comunidades bentónicas marinas generalmente relacionados con fondos blandos. A pesar de su importante papel ecológico, la información sobre su origen y tiempo de divergencia es aún escasa. Los primeros intentos de establecer relaciones filogenéticas entre géneros datan de principios del siglo XX, cuando sólo estaban disponibles caracteres morfológicos. En la última década, los análisis filogenéticos basados en secuencias de ADN mitocondrial procedentes de un limitado número de especies han propuesto un ancestro hipotético diferente para este grupo, pero sus relaciones intergenéricas permanecen oscuras. La presente investigación está basada en una combinación de marcadores mitocondriales y nuclear (*mtMutS*, *Cox1* y ADNr *28S*), aportando nueva información molecular sobre las relaciones filogenéticas entre los géneros de pennatuláceos, incluyendo 38 nuevas secuencias pertenecientes a 13 especies. Algunas de las relaciones filogenéticas inferidas en el presente estudio cuestionan la actual clasificación de las plumas de mar basada en la morfología (a diferentes niveles taxonómicos), indicando claramente que los dos grupos principales Sessiliflorae y Subselliflorae, algunas de sus principales familias (por ejemplo Pennatulidae, Umbellulidae, Virgulariidae) y algunos géneros (por ejemplo *Umbellula*, *Veretillum*) son no-monofiléticos. Asimismo, los veretílidos, tradicionalmente considerados los pennatuláceos más primitivos, no se muestran como el taxón divergente más antiguo. Además, un análisis del tiempo de divergencia realizado en este trabajo sugirió que el origen de los pennatuláceos data del Cretácico Inferior (Berriasiano, ~ 144 Ma), de acuerdo con su escasamente conocido registro fósil, mientras que la divergencia inicial de la mayoría de los géneros existentes ocurrió en tiempos del Oligoceno y Mioceno.

Palabras clave: filogenia; *mtMutS*; *Cox1*; *28S*; Pennatulacea; plumas de mar; tiempos de divergencia.

INTRODUCTION

Anthozoan cnidarians are considered to be one of the most important marine bio-constructors, and they often dominate on rocky substrata (Roberts et al. 2006, Mortensen et al. 2008). These structurally complex communities provide refuge and food for both larval and adult stages of a rich associated fauna by establishing numerous symbiotic relationships as well as trophic interactions (Sammarco and Coll 1992, Roberts et al. 2010, Baillon et al. 2012). Soft bottoms account for about 95% of ocean depths (Cognetti et al. 2001), but they are an unstable substrate for the settlement of most of the anthozoan species and genera dominating in rocky areas (Chia and Crawford 1973). However, several anthozoans show adaptations (e.g. hooked basal parts in the antipatharian genus *Schizopathes*; tree-root bases in some species of the soft coral genus *Anthomastus* and the isidid *Isidella*; or elongated bodies in the order Ceriantharia) to life in these habitats (see Jungersen 1927, Tiffon 1987, Opresko 2002, among others). Among the octocorals, pennatulaceans seem to be the most specialized group, with significant morphological adaptations, such as the presence of a muscular peduncle serving as an anchor system on soft sediments (Herklots 1858, Tixier-Durivault 1965, Williams 2011), although a few rock-inhabiting sea pen species have been discovered, modifying the basal portion of the peduncle as a holdfast for attachment to rocky substrata (Williams and Alderslade 2011).

The order Pennatulacea contains more than 200 species considered valid, distributed in 37 genera and 14 families (Williams 2011, 2015, García-Cárdenas et al. 2019). They are present in all oceans, with a bathymetric distribution ranging from intertidal zones to a depth of about 6100 m (Williams 2011). Some sea pen species form extensive meadows modifying the habitat and increasing the local diversity because of the rich fauna associated with them (Hughes 1998, Baillon et al. 2014, Clippele et al. 2015). This important ecological role has been recognized by including pennatulaceans and their associated megafauna in the OSPAR list of threatened and/or declining species and habitats (Jones et al. 2000, Curd 2010).

Pennatulaceans are colonial organisms with a muscular peduncle anchoring the colony to soft substrata, and a polypary (or rachis) where zooids are found (Herklots 1858, Kükenthal 1912). Both the colonial structure and common tissues are generated from the initial polyp, called the oozooid (Williams et al. 2012). The rest of the polyps arise by lateral budding of its body wall, and at least two kinds are distinguished: the autozooids (with a crown of eight collector tentacles, responsible for feeding and reproductive functions) and the siphonozooids (without a crown of collector tentacles, sometimes having just one with varying degrees of development) that are responsible for water exchange to and from the colonial interior (Tixier-Durivault 1965, Williams et al. 2012). Two other types, mesozooids and acrozooids, can be present (the former in *Pennatula* and *Ptilella* and the latter in *Pteroeides*) (see Williams et al. 2012, García-Cárdenas et al. 2019).

The proposed classification for octocorals by Hickson (1930), based on colonial forms and sclerite morphological diversity, was subsequently modified by Bayer (1981), who divided them into three main groups. Among them, pennatulaceans (O. Pennatulacea) are clearly distinguished from other octocorals [stolonate, soft corals and gorgonians (O. Alcyonacea) and blue corals (O. Helioporacea)] by the above-mentioned colonial structure (Williams 1995, Daly et al. 2007, Pérez et al. 2016). However, the first attempts to establish the possible phylogenetic relationships among the pennatulacean genera are attributed to Kölliker (1870), who used the morphological similarities and structural complexity of colonies to propose a common origin for the group (Kölliker 1880, Kükenthal and Broch 1911). Kükenthal (1915) proposed a classification in which the families were divided into two suborders: Sessiliflorae (polyps are directly located on rachis) and Subselliflorae (polyps are grouped forming high ridges or lateral leaves). Other proposals developed in the following decades (Hickson 1937, Bayer 1956, 1981) continued to delve into details such as autozooid and siphonozooid distribution, as well as the shape and ornamentation of the sclerites (Williams 1995, Fabricius and Alderslade 2001, López-González and Williams 2002). The current classification of pennatulaceans is exclusively based on the aforementioned morphological set of characters (Williams 1997). More recently, molecular analyses have revealed possible homoplasies (e.g. the arrangement of autozooids in polyp leaves) and the consideration of non-monophyletic groupings within Pennatulacea (Dolan et al. 2013, Kushida and Reimer 2018, García-Cárdenas et al. 2019).

Hickson (1916), in agreement with Kükenthal (1912) and Niedermeyer (1913), considered the veretillids (Veretillidae) to be the most primitive pennatulaceans, the order probably being derived from an alcyoniid ancestor with radial colony symmetry (related to the soft coral genus *Anthomastus*). These thoughts on the basal group of the pennatulaceans were considered feasible until the end of the 20th century (Williams 1994, 1997). The incorporation of molecular analysis to phylogenetic reconstruction in octocorals [based on mitochondrial markers *msh1* (henceforth *mtMutS*) and *ND2*] has strongly supported gorgonians of the family Ellisellidae (one of the five families traditionally included in the Suborder Calcaxonia; see Grasshoff 1999) as the sister group of the pennatulaceans (McFadden et al. 2006). Additional molecular markers, such as *Cox1* and the nuclear *28S* rDNA, corroborated the close relationship between ellisellids and pennatulaceans (McFadden et al. 2010). Consequently, Williams (2019) proposed two alternative scenarios for the placement of pennatulaceans: 1) pennatulaceans must be included in the Calcaxonia along with the five previously recognized gorgonian families (Ellisellidae, Ifalukellidae, Primnoidae, Chrysogorgiidae and Isididae; see Grasshoff 1999); and 2) ellisellids must be removed from the Calcaxonia and included together with pennatulaceans in a clade named Actinaxonia (*sensu* Williams 2019). However, these hypotheses need to be tested using a more comprehensive molecular phylogeny of octocorals.

Although there were discrepancies regarding the sister group of pennatulaceans, both the morphological and molecular methodological approaches recognized the monophyletic origin of pennatulaceans which, according to the oldest undisputed fossil record of pennatulaceans, might have occurred in the Late Cretaceous (Reich and Kutscher 2011).

McFadden et al. (2014) complemented their initial mitochondrial barcode for octocorals (*Cox-1+igr1+msh1*, McFadden et al. 2011) with a nuclear segment (*28S* rDNA), constituting this multilocus sequence (*mtMutS+Cox1+28S* rDNA), which has been considered a more accurate barcode for identifying species of octocorals and is useful in the identification of intra- and intergeneric relationships of a selected group of pennatulaceans (García-Cárdenas et al. 2019).

Most recent phylogenetic studies in pennatulaceans based on mitochondrial genes (*mtMutS* and *ND2*) have proposed the existence of four main clades (Dolan et al. 2013, Kushida and Reimer 2018). However, given the low evolution rate of the mitochondrial genome in octocorals, and its uniparental inheritance, phylogenetic hypotheses relying solely on mtDNA could be biased, while the integration of both mitochondrial and nuclear markers (such as *28S*) must be preferable (Bilewitch and Degnan 2011, McFadden et al. 2014, Núñez-Flores et al. 2020).

In the present contribution, a phylogenetic reconstruction on the internal relationships among pennatulacean taxa (suborders, families and genera) is carried out. A wide taxonomic coverage (providing 38 new pennatulacean sequences, 13 *mtMutS*, 13 *Cox1,* and 12 *28S*), based on the previously proposed concatenated barcode for octocorals (*mtMutS, Cox1,* and *28S*) is used for that purpose. Phylogenetic relationships proposed in previous molecular studies are discussed, as well as the monophyletic or non-monophyletic nature of sea pen families and genera. Also included here for the first time is a divergence time estimation for pennatulaceans, which provides insights into the origination time of the different lineages comprising sea pens.

METHODS

Sampling

The materials examined herein were collected during various surveys over different geographical areas and sampling programmes: the northeastern Arctic-Atlantic (BIOICE programme), the northeastern Atlantic (*Scotia* cruises, INDEMARES Chica), the Mediterranean Sea (INDEMARES Alborán, INDEMARES Cap de Creus), the southeastern Atlantic (BENGUELA VIII) and the Antarctic Peninsula and the eastern Weddell Sea (*Polarstern* cruises ANT XVII/3, ANT XIX/5, and ANT XXIII/8) (see Table 1).

During the different expeditions, the specimens were sorted and labelled on board. The colonies (or a tissue sample from each one) were directly fixed in 100% ethanol for further molecular studies. The remaining part of the colonies was fixed in hexamethylenetetramine-buffered 4% formalin-seawater or 70%

ethanol. After the fixation period, all colonies were preserved in 70% ethanol. The sequenced voucher specimens are deposited in the Museu de Zoologia de Barcelona (MZB), in the Muséum National d'Histoire Naturelle (MNHN) in Paris and in the collection of the Biodiversidad y Ecología Acuática research group of the University of Seville (BECA).

Molecular analyses

DNA extraction and PCR profiles.

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* and *Cox1*) and a nuclear region (*28S* rDNA) were sequenced. 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 CO-IOCTR (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 of dNTPs, 0.3 μM of each primer and approximately 30 ng of genomic DNA, and it 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 *28S* PCR used the same cycle as the *Cox1* profile, but with 50ºC as the annealing temperature. The PCR products were purified using the NucleoSpin® Extract II DNA Purification Kit, following the manufacturer's instructions. The purified products were electrophoresed on an ABI PRISM® 3730xl genetic analyser, and sequence traces were edited using Sequencher™ v4.0.

Phylogenetic analyses

The new sequences were compared with homologous sequences obtained from GenBank (Table 1). Any sequences from GenBank showing doubtful identity were discarded for our analyses, especially some attributed to the genus *Anthoptilum* (already detected by Kushida and Reimer 2018). The placement of this genus was considered according to a recent study revealing that *Anthoptilum* species (*Anthoptilum grandiflorum* MK91965 and *Anthoptilum* sp. 1 MK919656) have the same gene order as the bamboo corals Isididae sp. (EF622534) and *Acanella eburnea* (EF672731) (Hogan et al. 2019). According to previous molecular hypotheses (McFadden et al. 2006, 2010, McFadden and van Ofwegen 2012), a set of ellisellid gorgonians was selected as an outgroup for the phylogenetic reconstruction (Brockman and

Table 1. – Pennatulaceans included in molecular phylogenetic analyses in Figure 1. Species in bold are those sequenced for this study. Note that all GenBank sequences are presented here along with the names as they appear in GenBank and their original publications (including numbers or letters).

Taxa	Catalogue nos. (or additional information)	Geographic area	mtMutS	Cox1	28S
Veretillidae					
Cavernularia pusilla	BECA OPEN-465	NW Mediterranean Sea	MT968957[1]	MT952706[1]	MT951908[1]
Veretillum cynomorium	BECA OPEN-462	NW Mediterranean Sea	MT968958[1]	MT952707[1]	MT951909[1]
Anthoptilidae					
Anthoptilum grandiflorum	NMS.Z.2019.25.16	Greenland	MK919655[12]	MK919655[12]	
Anthoptilum sp. 1	NMS.Z.2019.25.1	Whittard Canyon	MK919656[12]	MK919656[12]	
Funiculinidae					
Funiculina quadrangularis	NWFSC 34210-032	USA, eastern Pacific	JN866526[2]	KF874185[2]	
Funiculina sp. 1	BECA OPEN-466	N Atlantic	MT968959[1]	MT952708[1]	MT951910[1]
Funiculina sp. 2	BECA OPEN-132	NE Atlantic	MT968960[1]	MT952709[1]	MT951911[1]
Stachyptilidae					
Gilibelemnon octodentatum	BECA OPEN-452	Seymour Island, Antarctica	MK603841[10]	MK603855[10]	MK603851[10]
Halipteridae					
Halipteris sp.	BECA OPEN-167	NE Atlantic	MT968961[1]	MT952710[1]	MT951912[1]
Halipteris californica	NWFSC 34213-022	USA, eastern Pacific	JN866542[2]	KF874203[2]	
Halipteris finmarchica	NTM-C014596	W Pacific (Tasman Sea, AUS)	DQ302868[5]	GQ342425[3]	JX203741[7]
Halipteris willemoesi	NWFSC 34212-052	USA, eastern Pacific	JN866533[2]	KF874192[2]	
Kophobelemnidae					
Kophobelemnon sp. 1	BECA OPEN-141	NE Atlantic	MT968962[1]	MT952711[1]	MT951913[1]
Kophobelemnon sp. 2	BECA OPEN-151	SE Atlantic	MT968963[1]	MT952712[1]	MT951914[1]
Kophobelemnon sp. 3	NMS.Z.2019.25.5	Whittard Canyon	MK919661[12]	MK919661[12]	
Kophobelemnon sp. 4	NMS.Z.2019.25.6	Whittard Canyon	MK919662[12]	MK919662[12]	
Kophobelemnon macrospinum	NTM-C014985	W Pacific (Tasman Sea, AUS)	DQ302865[5]	GQ342429[3]	JX203742[7]
Pennatulidae					
Gyrophyllum hirondellei	MNHM OCT.A.579	N Atlantic	MT968964[1]	MT952713[1]	MT951915[1]
Gyrophyllum sp.	NTM-C014392=NOR89/53	W Pacific (Tasman Sea, AUS)	DQ302869[5]	JX203865[7]	JX203740[7]
Ptilella grayi	NMS.Z.2019.2.2	Rockall Bank, NE Atlantic	MK603843[10]	MK603856[10]	MK603853[10]
Ptilella grandis	BECA OPEN-143	South Iceland, NE Atlantic	MK603844[10]	MK603860[10]	MK603854[10]
Pennatula aculeata	NMS.Z.2019.25.7	Whittard Canyon	MK919663[12]	MK919663[12]	
Pennatula rubra	BECA OPEN-139	Alborán, Mediterranean Sea	MK603845[10]	MK603857[10]	MK603852[10]
Pennatula phosphorea	BECA OPEN-453	Sea of Hebrides, NE Atlantic	MK603848[10]	MK603858[10]	MK882492[10]
Pennatula sp.	BECA OPEN-152	Ross Sea, Antarctica	MK603849[10]	MK603859[10]	MK882493[10]
Pteroeides griseum	BECA OPEN-140	NW Mediterranean Sea	MT968965[1]	MT952714[1]	MT951916[1]
Ptilosarcus gurneyi	NWFSC 34212-082	USA, eastern Pacific	JN866536[2]	KF874195[2]	
Umbellulidae					
Umbellula huxleyi	BECA OPEN-161	NE Atlantic	MT968966[1]	MT952715[1]	MT951917[1]
Umbellula sp. A	BECA OPEN-464	Antarctica	MT968967[1]	MT952716[1]	
Umbellula sp. B	BECA OPEN-463	Antarctica	MT968968[1]	MT952717[1]	MT951918[1]
Umbellula sp. 1	NMS.Z.2019.25.12	Whittard Canyon	MK919669[12]	MK919669[12]	
Umbellula sp. 2	NMS.Z.2019.25.13	Porcupine Bank, Ireland	MK919670[12]	MK919670[12]	
Umbellula sp. 3	NMS.Z.2019.25.14	Whittard Canyon	MK919671[12]	MK919672[12]	
Virgulariidae					
Virgularia mirabilis	BECA OPEN-310	Mediterranean Sea	MT968969[1]	MT952718[1]	MT951919[1]
Virgularia schultzei	RMNH Coel. 40823	Western Cape Province, S. Africa	GQ342527[3]	GQ342459[3]	JX203743[3]
Acanthoptilum gracile	NWFSC 34213-027	USA, eastern Pacific	JN866544[2]	KF874205[2]	
Renillidae					
Renilla sp.	CSM-2010-UF4000	E Pacific, Gulf of Panama	GQ342526[3]	GQ342455[3]	
Renilla muelleri	SCF-FLA	E Pacific	DQ297432[5]		
Renilla muelleri	n. d.	n. d.		NC_018378[8]	
Protoptilidae					
Protoptilum carpenteri	NMS.Z.2019.25.10	Whittard Canyon	MK919667[12]	MK919667[12]	
Distichoptilum gracile	NTM-C014561	W Pacific (Tasman Sea, AUS)	DQ302866[5]	GQ342454[3]	JX203739[7]
Echinoptilidae					
Actinoptilum molle	RMNH Coel. 40822	Eastern Cape Province, S. Africa	GQ342491[3]	GQ342414[3]	JX203738[7]
Ellisellidae					
Nicella sp.	AMQ-2013	NW Atlantic	KC788269[9]	KC788231[9]	
Nicella sp.	AMQ-2016	NW Atlantic			KX890219[9]
Ctenocella schmitti	JAS-238	NW Atlantic	JN227995[4]	FJ268628[11]	
Verrucella sp.	NTM-C014982	Tasman Sea, AUS	DQ302864[5]		
Verrucella sp.	RMNH Coel. 40030	West Papua, Indonesia		JX203851[7]	JX203704[7]
Viminella sp. 1	RMNH Coel. 40032	West Papua, Indonesia	JX203794[7]	JX203852[7]	JX203703[7]

Abbreviations: NMS, National Museum Scotland Smithsonian (Scotland, UK); MNHM, Muséum National d'Histoire Naturelle (Paris, France); BECA, Biodiversidad y Ecología Acuática (Seville, Spain); MBARI, Monterey Bay Aquarium Research Institute (California, USA); NWFSC, Northwest Fisheries Science Center (Seattle, USA); NTM, Museum and Art Gallery of the Northern Territory (Darwin city, Australia); RMNH, Rijksmuseum van Natuurlijke Historie (Leiden, Netherlands); SCF, Collection of S.C. France (USA); JAS, Collection of J. A. Sánchez; AMQ, Collection of A. M. Quattrini; n.d., no data.
References: (1) This study; (2) Elz et al. unpublished; (3) Brockman and McFadden 2012; (4) Pante et al. 2012; (5) McFadden et al. 2006; (6) Everett et al. 2016; (7) McFadden and van Ofwegen 2012; (8) Kayal et al. 2013; (9) Quattrini et al. 2013; (10) García-Cárdenas et al. 2019; (11) France and Pante unpublished; (12) Hogan et al. 2019.

McFadden 2012, Everett et al. 2016, Kushida and Reimer 2018).

Individual genes were tested for substitution saturation using the DAMBE software (Xia et al. 2003, Xia and Lemey 2009). *MtMutS*, *Cox1* and *28S* showed low levels of substitution saturation at the third position.

The set of new sequences obtained in this study (*mtMutS*, *Cox1*, *28S*) and those from GenBank were aligned using the MUSCLE alignment method implemented in MEGA 6 (Tamura et al. 2013). The concatenated dataset involved 45 nucleotide *mtMutS*, 45 *Cox1* and 27 *28S* sequences. The alignment was 704 bp for *mtMutS* (63% conserved positions), 775 bp for *Cox1* (80% conserved), 790 bp for *28S* (54% conserved) and 2323 bp for the concatenated *mtMutS+Cox1+28S* dataset. After alignment, the best nucleotide substitution model was selected using Modeltest implemented in MEGA 6, according to the Akaike information criterion and hierarchical likelihood ratio test values. The phylogenetic reconstruction was obtained by applying the maximum likelihood and Bayesian inference methods. The maximum likelihood method was carried out in MEGA 6 using the nearest neighbour interchange heuristic method and 1000 bootstrap replications. The selected nucleotide substitution model was T92+G for the concatenated *mtMutS+Cox1+28S*. 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. For comparative purposes and discussion, the clade designations I-IV used in previous phylogenetic studies (Dolan et al. 2013, Kushida and Reimer 2018) were used here, although some of these might not be supported by our study.

In order to observe the effect of the mitochondrial or nuclear marker used, additional phylogenies were obtained based on the individual markers and the concatenated *mtMutS+Cox1*. The conditions of phylogenetic reconstruction were similar to those described above. The selected nucleotide substitution models used were T92+G+I for *mtMutS*, *Cox1* and *mtMutS+Cox1*; and K2+G for *28S*. Resulting trees were included in supplementary material (Figs S2-S5). When substitution saturation was detected, two additional Bayesian inferences were carried out in the same conditions, using (A) the 1st, 2nd and 3rd codon positions of mt-genes; and (B) only the 1st and 2nd positions (excluding possible saturated positions at the 3rd codon). The resulting trees were practically identical, only with slight differences in certain node support values (for example from 0.96 PP to 0.98 PP). This indicates that the little saturation detected with DAMBE does not influence the genus relationships showed here.

Analysis of time divergence

The analysis of time divergence was undertaken within a Bayesian framework in BEAST 2.5.0 (Bouckaert et al. 2014), using the 45 taxa from which at least two of the three loci were available. BEAST allows topologies to be considered "fixed" or estimated to accommodate for phylogenetic uncertainty (Bouckaert el al. 2014, Drummond and Rambaut 2007). Here, we chose the second approach because the posterior node probabilities of Bayesian phylogeny were relatively low in few cases. Several works have emphasized the importance of the rigorous selection of an appropriate clock model (e.g. Duchêne et al. 2014), and to this end four models were compared: i) relaxed with an exponential local type distribution; ii) relaxed with a log-normal type distribution; iii) random local; and iv) strict. For each model, we estimated their marginal likelihoods using the nested sampling approach implemented in the NS package of BEAST 2 (Maturana et al. 2018) and used the Bayes factor to obtain the best-supported model. In each of these models, the three partitions were treated as linked, and we used a pure-birth (Yule) tree prior (Bouckaert el al. 2014, Drummond and Rambaut 2007, Drummond and Bouckaert 2015). The Yule model is a simple model of speciation that is generally more appropriate for considering sequences from different species (Drummond and Bouckaert 2015). The absolute estimates of divergence times were calculated after one-fossil calibrations (see Fossil calibration section below). For the best-supported model of molecular clock, two independent runs of 100 million generations, sampling every 10000 generations, were performed. Runs were considered complete with effective sample sizes greater than 200 for all parameters (Rambaut et al. 2018). LogCombiner (Rambaut and Drummond 2010) was used to combine the log files from the independent BEAST runs. TreeAnnotator (Rambaut and Drummond 2010) was used to summarize resulting tree samples into a single consensus tree using the maximum clade credibility, mean height options and discarding 10% of trees as burn-in. The maximum clade credibility summarized the 95% highest posterior density (HPD) limits of the node age.

Fossil calibration. The oldest undisputed pennatulacean fossil comes from several Late Cretaceous beds in Europe and North America (Reich and Kutscher 2011). These forms were assigned to the genera *'Graphularia'* (4 spp.) and *Glyptosceptron* (1 sp.; see details in Reich and Kutscher 2011), which are from the lower to upper Maastrichtian (66-72.1 Ma) of the Netherlands, Germany, Belgium, USA and Ukraine. The other well-recognized fossil record of pennatulaceans is from the Early to Middle Eocene (41.2-47.8 Ma) of Trinidad (Pointe-a-Pierre Formation) based on the recognition of *Virgularia presbytes* (Bayer, 1955), a genus with extant representatives. The age of the most recent common ancestor of the genus *Virgularia* (ca. 41.2 Ma) was set as an offset, while the mean and standard deviation of the log-normal distribution was set as M=2 and S=1. We avoid using the age of the oldest undisputed pennatulacean fossil in our time calibration analyses (Reich and Kutscher 2011) because it would certainly restrict the early origin of this poorly fossiliferous clade. In the following paragraphs we use the standard abbreviation 'Ma' (= million years ago) for the age of a specific moment in the geological past (Aubry et al. 2009).

RESULTS

Phylogenetic analysis

General overview

Our phylogenetic analysis based on the concatenated *mtMutS+Cox1+28S* data sets showed the distribution of a set of sea pen genera into two related and well-supported clades [Clade I and Clade II, bootstrap (bst) >75%, posterior probability (PP) >0.90], and the rest of genera into two groupings whose relationships varied slightly depending on the phylogenetic method used (Fig. 1). The Bayesian inference method grouped the genera *Gyrophyllum*, *Kophobelemnon* and *Halipteris*, as well as a sequence attributed to a species of *Umbellula* (*Umbellula* sp. 2 MK919670) with strong support (0.98 PP), and not including the genus *Funiculina,* which formed a separate well-supported clade. The maximum likelihood method grouped the genera *Gyrophyllum*, *Funiculina*, and *Kophobelemnon* with strong support (96% bst) into what we have named Clade III, as a sister group of Clade I-Clade II, while *Umbellula* sp. 2 was reunited with the genus *Halipteris*, constituting a relatively poorly supported Clade IV (60% bst) (see Fig. 1, bottom box).

The distribution of genera obtained was not in agreement with the old commonly used suborders (Sessiliflorae and Subselliflorae). The genera with polyp leaves (previously under Subselliflorae) were located in different clades (*Pteroeides* and *Virgularia* within Clade I but *Ptilella*, *Acanthoptilum, Ptilosarcus* and *Pennatula* within Clade II), while species without polyp leaves (previously under Sessiliflorae) were found throughout all the groupings. As a result of this, the traditional suborders Sessiliflorae and Subselliflorae can no longer be recognized here as monophyletic groupings.

Colonies with radial symmetry (characterized by their cylindrical-clavate colonial shape and zooids without permanent calyces distributed evenly over the entire surface of rachis), represented here by the genera *Cavernularia*, *Veretillum* and *Actinoptilum*, were observed within Clades I and II (Fig. 1), while colonies with bilateral symmetry were widely distributed throughout the tree. These examples of morphological distribution illustrate the lack of concordance between molecular and traditional morphological groupings.

Status of traditional families

Of the 14 families currently in use in Pennatulacea, 12 were included in our phylogenetic analysis (Fig. 1, families are represented by symbols in the tree). Those families represented here by a single genus (such as Anthoptilidae, Funiculinidae, Stachyptilidae, Halipteridae, Kophobelemnidae, Renillidae and Echinoptilidae), most of them with more than a single species, showed a common origin, except for Umbellulidae (see below). Those families with two or more genera (such as Protoptilidae, Pennatulidae and Virgulariidae) were not recovered as monophyletic groups, except

for Veretillidae, whose genera *Cavernularia* and *Veretillum* (including its type species *V. cynomorium*), were reunited within Clade I with strong support (bst =100%, PP=1).

The protoptilid genera *Protoptilum* and *Distichoptilum,* although located within the same Clade II, were not shown close to one another, the latter being the sister group of *Ptilella* (bst =49%, PP=1). The pennatulid genera were distributed within different clades: *Pteroeides* within Clade I with moderate support (bst =43%, PP=0.95); *Pennatula* and *Ptilosarcus* within Clade II with strong support (bst =92%, PP=0.98); and *Gyrophyllum* as the sister group of *Kophobelemnon* (PP=0.98). *Ptilella* (included tentatively in Pennatulidae) was placed within Clade II with strong support (bst =99%, PP=1) but not close to the other pennatulids *Pennatula* and *Ptilosarcus*. The two virgulariid genera (*Virgularia* and *Acanthoptilum*) were placed within two different clades: *Virgularia* within Clade I with moderate support (bst =43%, PP=0.95), and *Acanthoptilum* within Clade II with strong support (bst =99%, PP=1). The family Echinoptilidae represented here by the genus *Actinoptilum* was placed within Clade II, as the sister group of a clade including *Renilla*, *Acanthoptilum, Ptilosarcus* and *Pennatula,* with strong support (bst =99%, PP=1). The family Renillidae represented by the genus *Renilla* was located within Clade II with strong support (bst =100%, PP=1), as the sister group of the virgulariid genus *Acanthoptilum*. The family Umbellulidae represented by the genus *Umbellula* was placed within Clade I with strong support (bst =100%, PP=1), as the sister group of *Anthoptilum* spp. However, a sequence identified as *Umbellula* sp. 2 was related to *Kophobelemnon, Gyrophyllum* and *Halipteris* (PP=0.98). In the maximum likelihood hypothesis (see Fig. 1, bottom box), this sequence was only close to *Halipteris* spp. with moderate support (bst= 60%) (see Discussion). The family Funiculinidae, represented by the genus *Funiculina* with strong support (bst =100%, PP=1), had an unsupported location outside these previously described groupings, although in the maximum likelihood tree these sequences constituted the sister group of *Kophobelemnon*, with low support (bst=34%). Our results indicated that the placement of *Funiculina* spp. is mainly due to the addition of the nuclear segment *28S* and not only due to the method used (Table S1). Phylogenies (maximum likelihood and Bayesian inference) based on *mtMutS* (Fig. S2), *Cox1* (Fig. S3) and the combined *mtMutS+Cox1* (Fig. S4) supported the inclusion of *Funiculina* within Clade III or IV, in contrast to the phylogeny based on *28S* (Fig. S5) showing an unsupported placement outside the mentioned groupings (see Table S1 and Discussion). Finally, the family Anthoptilidae represented by the genus *Anthoptilum* was within Clade I with moderate support (bst =79%, PP=0.95) with *Umbellula* spp. as the sister group.

Clade classification in Pennatulacea

Our results show that supra-generic groupings distributed in clades resulting from molecular analyses are

Fig. 1. – Phylogenetic relationships in the order Pennatulacea based on Bayesian inference (BI) for combined regions *mtMutS+Cox1+28S*. A partial phylogeny based on ML is shown in the bottom box (see Results for additional comments), highlighted by asterisk are those groupings that change according to BI method (see Discussion). Posterior probability and bootstrap supporting values are indicated.

inconsistent with the traditional grouping of families based on morphological features, as there are genera with different colonial forms gathered in the same

clade. Clade I with strong support (bts=98%, PP=1) is here formed by a mixture of bilateral, radial, elongated and clavate colony growth (Fig. 1).

Clade II (bts=99%, PP=1) gathered species belonging to six families of bilateral symmetry, except for *Actinoptilum* (radial symmetry). This clade includes genera with long-flagelliform colonies (e.g. *Protoptilum*, *Distichoptilum*), pansy-shaped (e.g. *Renilla*), with polyp leaves of moderate development (e.g. *Acanthoptilum*) and with well-developed polyp leaves (e.g. *Ptilella*). Among these genera, *Protoptilum, Distichoptilum* and *Ptilella* seem to be the earliest-diverging taxa, the *Pennatula* grouping being the most derived one.

The final grouping was made up of species belonging to four families covering different colony morphologies, including colonies with and without polyp leaves (e.g. *Gyrophyllum* and *Kophobelemnon*, respectively), or elongated and long-flagelliform forms (e.g. *Halipteris*). Finally, *Funiculina* sequences constituted a separate grouping, without a clear relationship to previously mentioned clades.

Time-tree analysis

The estimated marginal likelihood for the time-tree analysis under different clock models indicated that the relaxed log-normal is the best supported one (Table S2). The result of this model is represented in Figure 2 (and Fig. S1). The divergence dates inferred by the Bayesian relaxed clock analyses indicated Lower Cretaceous age (Berriasian age; inferred mean age of 144.3 Ma; HPD 95%: 65.7–249.1 Ma) for the origin of Pennatulacea. In addition, the four main lineages within Pennatulacea were originated soon after, with Clade I and II diverged during the Hauterivian age (Lower Cretaceous; inferred mean age of 132.4 Ma; 95% HPD: 64.1–229.9 Ma). The groupings obtained in the BEAST analysis were similar to those from MrBayes, even considering the uncertain affinities of the genus *Funiculina* (Figs 2, S1). The last genus appears to begin its diversification in the middle Miocene (inferred mean age of 15.6 Ma; 95% HPD: 2.2–36.4 Ma). The most recent common ancestor of the genera *Halipteris*, *Kophobelemnon* and *Gyrophyllum* is from the Coniacian age (Upper Cretaceous; inferred mean age of 89.3 Ma; 95% HPD: 35.2–161 Ma). The beginning of the diversification of most genera with more than one sequence, excepting *Virgularia*, *Gyrophyllum* and *Ptilella*, occurred during Oligocene and Miocene times (ranging from 33.9 to 5.3 Ma). Finally, veretillids (including *Cavernularia* and *Veretillum*), appear to have diversified only after the late Eocene (inferred mean age of 36.9 Ma; 95% HPD: 10.3-70 Ma).
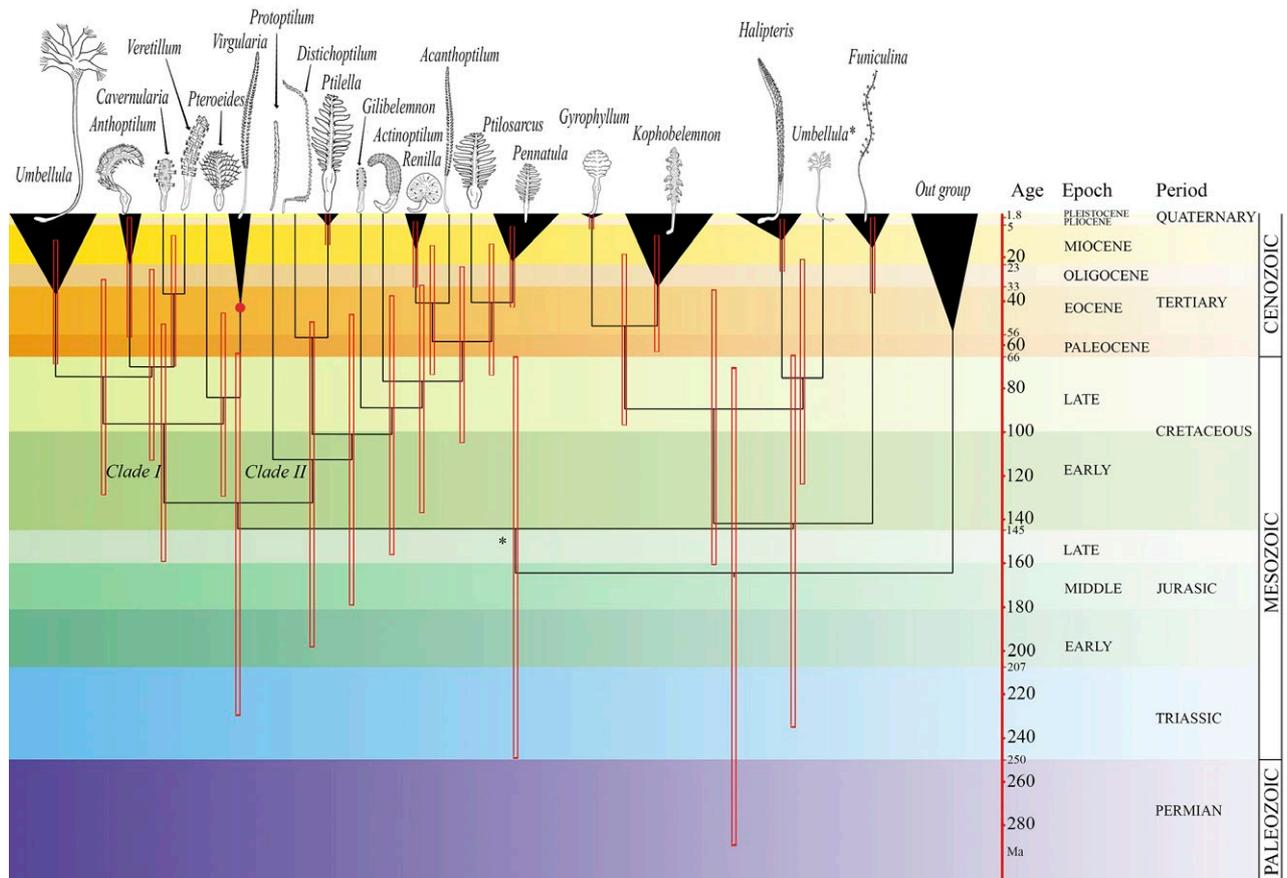

Fig. 2. – Calibrated tree from Time Divergence Analysis (based on *mtMutS+Cox1+28S*) showing the divergence among genera, and the diverse pennatulacean colonial morphologies. Red dot indicates fossil calibration point. The confidence intervals of the divergence age estimates are indicated at each node.

DISCUSSION

Monophyly of basal divergences in pennatulaceans

Hickson (1916:131) had already highlighted the difficulty of carrying out systematic studies on Pennatulacea based on features such as colonial symmetry because of the wide range of variation of those characters overlapping among genera and species. As has happened before with other octocoral groups, there is an evident lack of agreement between the current systematics of pennatulaceans based on morphological characters (see Kükenthal 1915, Hickson 1930, Williams 1997, among others) and phylogenetic hypotheses generated from molecular data (McFadden et al. 2006, Dolan et al. 2013, Kushida and Reimer 2018, among others). The presence of homoplastic characters (e.g. convergence and reversals) is a serious concern in the formulation of a reliable evolutionary hypothesis of pennatulaceans based only on morphology (Landing et al. 2015, Pérez et al. 2016).

However, several synapomorphies observed in sea pens have traditionally been used to differentiate pennatulaceans from other octocorals and support them as a monophyletic group (Hickson 1930, 1937, Williams 1994). In the last decades, despite the inclusion of unsuitable markers or doubtful sequences that questioned this common origin (see results of Berntson et al. 1999, 2001), most of the molecular studies carried out have corroborated the monophyletic origin of pennatulaceans with strong support (McFadden et al. 2006, Everett et al. 2016, Kushida and Reimer 2018).

The use of the two traditional suborders, Sessiliflorae and Subselliflorae (Kükenthal 1915), until the early 1990s (e.g. Hickson 1916, Tixier-Durivault 1965, Williams 1990) has been avoided in recent decades since Williams (1995: 136) questioned the validity of these groupings. In monographic works the different families were simply listed from the most structurally simple colonies (radial forms such as veretillids) to the most structurally complex ones (bilaterals bearing well-developed polyp leaves such as pennatulids), the latter often being considered from a morphological point of view as the most derived forms (Williams 1997, López-González and Williams 2002). In the present study, using a molecular barcode for octocorals and a wider taxa sampling range, this traditional classification into suborders has been clearly shown as a non-monophyletic one, in agreement with previous molecular studies (McFadden et al. 2006, Dolan et al. 2013, Kushida and Reimer 2018). Therefore, although Sessiliflorae and Subselliflorae are still mentioned from a practical point of view (e.g. Yesson et al. 2012, Hogan et al. 2019), it is evident that they are non-monophyletic groupings, the internal morphological evolution of each clade still being difficult to understand.

Current status of traditional families

Morphological traits have been used to categorize the extant pennatulaceans into 14 families, 37 genera and approximately 200 considered valid species (Williams 2011, 2015, García-Cárdenas et al. 2019) according to the most important contributions concerning the order Pennatulacea (e.g. Kükenthal and Broch 1910, Kükenthal 1915, Hickson 1916, among many others). However, recent molecular studies have failed to recover the monophyly of some of these families, such as Umbellulidae, Pennatulidae, Virgulariidae, Protoptilidae, Scleroptilidae, Stachyptilidae and Kophobelemnidae (Dolan et al. 2013, Kushida and Reimer 2018). The present study, including seven genera not previously sequenced and additional mitochondrial sequences and molecular markers, confirmed the non-monophyletic nature of some of these families (such as Protoptilidae, Virgulariidae or Pennatulidae).

The family Veretillidae, which includes most of the structurally simplest and radial colonial forms observed in pennatulaceans, have been considered since the early 19th century as transitional forms between soft corals and pennatulaceans (Koch 1878). The idea that Veretillidae and Echinoptilidae were the earliest-diverging taxa in phylogenies based on morphology spread during the early (Kükenthal and Broch 1910, Niedermeyer 1913, Hickson 1916) and late (Williams 1994, 1997) 20th century and the transition to the 21st century (López-González and Williams 2002), before molecular approaches postulated a different origin and basal relationships of pennatulaceans from those of the rest of the octocoral taxa (McFadden et al. 2006, Dolan et al. 2013). In contrast to that traditional idea, and following later molecular postulates, our phylogeny does not support the hypothesis that Veretillidae and Echinoptilidae are located at a basal position, exemplifying the ancestral sea pen morphology.

Recent phylogenies based on the mitochondrial markers *ND2* and *mtMutS* including sequences of the veretillid genera *Veretillum* and *Cavernulina* showed them to be closely related to sequences of the genus *Sclerobelemnon* (Kophobelemnidae) and placed all of them within Clade I with moderate support (Kushida and Reimer 2018: 9). Our research includes new additional veretillid sequences belonging to *Cavernularia* (*C. pusilla*) and *Veretillum* (including the type species *V. cynomorium* Pallas, 1766). In our phylogeny these veretillids (*Cavernularia* and *Veretillum*) are gathered in a clade with strong support (bst= 100%, PP= 1) within Clade I (Fig. 1). An additional exploratory maximum likelihood analysis based on *mtMutS* including all available *mtMutS* sequences from GenBank (Fig. S2) revealed that sequences attributable to the Pacific species *Veretillum* sp. 1 (MK133435) and *Veretillum* sp. 2 (MK133539, MK133545, MK133526) from Kushida and Reimer (2018) (although falling into the same Clade I) were not close to our sequence of the type species *V. cynomorium*, but rather close to *Cavernulina* and *Sclerobelemnon* sequences, in a different grouping. Thus, the inclusion of Pacific and Mediterranean (type species) sequences in a single genus would result in a paraphyletic taxon. This fact suggests the need for a detailed morphological study of Pacific colonies, as they could belong to a different genus, despite their possible similar morphological appearance. In short, with current information, the family Veretillidae

seems to be a monophyletic grouping within Clade I (Fig. 1). The gathering of further molecular data on the remaining veretillid genera (such as *Lituaria* or *Amphibelemnon*) would be desirable in order to better delineate the monophyletic nature of the current list of genera included in the family Veretillidae.

The family Echinoptilidae (including *Actinoptilum* and *Echinoptilum*) was traditionally considered the other earliest-diverging taxon in the phylogeny of pennatulaceans, mainly because of its radially symmetrical rachis (or at least in its distal portion) (Kükenthal and Broch 1911, Niedermeyer 1913, Williams 1992). Kushida and Reimer (2018) considered radial symmetry only for those species included in Veretillidae (*Veretillum*, *Cavernulina*) within Clade I, the rest of taxa in Clade I and all taxa within the Clades II, III and IV being bilaterals (Kushida and Reimer 2018:5). Nevertheless, both echinoptilid genera exhibit radial symmetry in colonies to different degrees (Williams 1995). The phylogeny proposed by these authors showed the genus *Echinoptilum* as a derived taxon with strong support within Clade II, with *Renilla-Pennatula* as the sister group. In our study, the sequence attributable to the other echinoptilid genus, *Actinoptilum molle*, was also located as a derived taxon within Clade II as the sister group of a [(*Renilla-Acanthoptilum*)-(*Ptilosarcus-Pennatula*)] clade.

The family Protoptilidae, including the genera *Protoptilum* and *Distichoptilum*, is here recognized as a non-monophyletic taxon, in agreement with Kushida and Reimer (2018). The family Renillidae, constituted by the genus *Renilla*, was not close to Veretillidae (as was suggested from morphology, see Williams 1997, Pérez and Ocampo 2001), supporting the results of Dolan et al. (2013) and Kushida and Reimer (2018). In our phylogeny, *Renilla* is the sister group of *Acanthoptilum* (Virgulariidae), and both are a sister group to *Ptilosarcus-Pennatula* (Pennatulidae). The family Anthoptilidae, represented here by *Anthoptilum* sp. 1 MK919656 and *A. grandiflorum* MK919655, was placed within Clade I as the sister group of *Umbellula*, and not occupying an ancestral position as was suggested by Dolan et al. (2013, Fig. 1). As commented above, the two best-represented families of pennatulaceans Pennatulidae and Virgulariidae (Williams 1995) are here recognized as non-monophyletic groupings, in agreement with Dolan et al. (2013) and Kushida and Reimer (2018).

The family Umbellulidae is shown here as a non-monophyletic grouping, as observed in previous works (Dolan et al. 2013, Kushida and Reimer 2018) (Figs 1, 2, S2). As the type species of *Umbellula*, *U. encrinus* (Linnaeus, 1758), falls into Clade I, that set of sequences has to be considered as the genus *Umbellula*. Although the genus *Umbellula* was involved in old hypotheses postulating the deep-sea origin of pennatulaceans (Kölliker 1880), other authors (Marshall 1883) recognized *Umbellula* as a highly modified form. In the 20th century, the Umbellulidae was considered as a highly derived family among the authors who supported the shallow-water origin of pennatulaceans (Kükenthal and Broch 1911, Williams 1997, among others).

Our research supports a later divergence of *Umbellula* within Clade I (Fig. 2).

The inclusion in our study of the nuclear marker 28S was useful in order to reinforce the support of Clades I and II. However, it also highlighted the unstable location of the genera *Funiculina* and *Halipteris* (see Figs 1, S2-S5, and Table S1) depending on the phylogenetic approach used. Clade III and Clade IV identified by Kushida and Reimer (2018) were recovered in our study (with high or relatively low support, bst 96% and 60%, respectively) only when the maximum likelihood method was used, while Bayesian inference indicated a different phylogenetic hypothesis. Therefore, the inclusion of the 28S locus allows us, on the one hand, to reinforce the support of Clades I and II, but also provides evidence of the instability in clades proposed by previous studies, probably requiring greater taxonomic and molecular sampling.

In general, our results, based on a combination of mitochondrial and nuclear segments (*mtMutS+Cox1+28S*), indicate the monophyletic origin of most of sea pen genera with strong support (1 PP). This is one of the few common points in which morphology and molecular studies agree.

Time divergence estimation in pennatulaceans

Very little is known about the time frame of pennatulacean evolution, and our study is the first attempt to build a time-calibrated phylogeny based on Bayesian relaxed molecular clock analysis. The Lower Cretaceous (Berriasian, ~144 Ma) estimation for the divergence of the Pennatulacea from their sister group Ellisellidae is slightly older than those recently inferred (estimated age of its most recent common ancestor as 82-125 Ma; see Bilewitch 2014). However, it is highly consistent with previous studies regarding estimations of the divergence time of Calcaxonia (with which pennatulaceans have been related; 120-300 Ma; Park et al. 2012), as well as with the age of the oldest undisputed pennatulacean fossils so far recovered (Reich and Kutscher 2011).

The Cretaceous is well-known as a greenhouse period caused largely by increased CO_2 from elevated global volcanic activity (e.g. Takashima et al. 2006, Prokoph et al. 2008), but it also is the last stage in the Gondwana break-up, and displays high rates of seafloor spreading, high sea levels (as much as 260 m above the present) and high ocean temperatures (surface waters >35°C and deep-ocean water >20°C), as well as evidence for changes in global ocean circulation (Haq et al. 1987, Pearson et al. 2001, Friedrich et al. 2012). The global climatic conditions during the Berriasian, when the origin of pennatulaceans likely occurs, were generally arid and the sea level was rather low (Föllmi 2012). The Berriasian also coincides with an extinction phase (Tithonian/Berriasian), which, however, apparently affected continental life more strongly than marine life (Föllmi 2012). In addition, during the Lower Cretaceous several oceanic anoxic events occurred, representing time intervals of usually relatively short duration (<1 Ma) in which intermediate

and bottom-water masses became depleted in oxygen (Föllmi 2012).

Our results therefore reinforce the notion that the Cretaceous was a pivotal time in octocoral evolution, because several clades appear to begin their diversification in this epoch (e.g. primnoids; Taylor et al. 2013). The main clades recovered here appear to have diverged quickly (Hauterivian-Berriasian) after their initial pennatulacean origins (Fig. 2), and this finding indicates that the early phase of their diversification took place in a greenhouse period and especially in periods of dramatic environmental changes (Prokoph et al. 2008, Föllmi 2012). Consequently, changes in sea temperature and oceanic anoxic events would have played a role in forcing pennatulacean diversification, and probably also vertical displacement (submersion and emersion of lineages) throughout the complete colonizable bathymetry, as it has been demonstrated that sea pens are one of the zoological groups present over the widest bathymetric range (0-6100 m depth) (Williams 2011). Our results also provide further support for a late origination of veretillids (late Eocene), contrasting with the early notion based on morphological features previously discussed.

Finally, it is evident that the single available fossil calibrations used on the present work have an impact on the quality of the resulting chronogram. We thus stress that this chronogram must be regarded as the first attempt to produce a time-calibrated sea pen tree. Only after achieving a wider sampling of molecular information will we be able to refine or even test the interpretations offered in the present work.

ACKNOWLEDGEMENTS

REFERENCES

Aubry M.P., Van Couvering J.A., Christie-Blick N., et al. 2009. Terminology of geological time: Establishment of a community standard. Stratigraphy 6: 100-105.

Baillon S., Hamel J.F., Wareham V.E., et al. 2012. Deep cold-water corals as nurseries for fish larvae. Front. Ecol. Environ. 10: 351-356.
https://doi.org/10.1890/120022

Baillon S., Hamel J.F., Mercier A. 2014. Diversity, distribution and nature of faunal associations with deep-sea pennatulacean corals in the Northwest Atlantic. PloS ONE 9: e111519.
https://doi.org/10.1371/journal.pone.0111519

Bayer F.M. 1956. Octocorallia. In: Moore R.C. (eds), Treatise on invertebrate paleontology. Part F. Coelenterata. Geol. Soc. America Univ. Kansas Press. New York and Lawrence Kansas, pp. 166-231.

Bayer F.M. 1981. Key to the genera of Octocorallia exclusive of Pennatulacea (Coelenterata: Anthozoa), with diagnoses of new taxa. Proc. Biol. Soc. Wash. 94: 902-947.

Berntson E.A., France S.C., Mullineaux L.S. 1999. Phylogenetic relationships within the class Anthozoa (phylum Cnidaria) based on nuclear 18S rDNA sequences. Mol. Phylogenet. Evol. 13: 417-433.
https://doi.org/10.1006/mpev.1999.0649

Berntson E.A., Bayer F.M., McArthur A.G., et al. 2001. Phylogenetic relationships within the Octocorallia (Cnidaria: Anthozoa) based on nuclear 18S rRNA sequences. Mar. Biol. 138: 235-246.
https://doi.org/10.1007/s002270000457

Bilewitch J.P. 2014. The roles of morphological diversification, depth range expansions and a novel gene in the evolution of the Octocorallia. Unpublished Phd thesis, The University of Queensland, Australia.

Bilewitch J.P., Degnan S.M. 2011. A unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual self-contained function. BMC Evol. Biol. 11: 1-14.
https://doi.org/10.1186/1471-2148-11-228

Bouckaert R., Heled J., Kühnert D., et al. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. PloS Comput. Biol. 10: e1003537.
https://doi.org/10.1371/journal.pcbi.1003537

Brockman S.A., McFadden C.S. 2012. The mitochondrial genome of *Paraminabea aldersladei* (Cnidaria: Anthozoa: Octocorallia) supports intramolecular recombination as the primary mechanism of gene rearrangement in octocoral mitochondrial genomes. Genome Biol. Evol. 4: 882-894.
https://doi.org/10.1093/gbe/evs074

Chia F.S., Crawford B.J. 1973. Some observations on gametogenesis, larval development and substratum selection of the sea pen *Ptilosarcus guerneyi*. Mar. Biol. 23: 73-82.
https://doi.org/10.1007/BF00394113

Clippele L.H., Buhl-Mortensen P., Buhl-Mortensen L. 2015. Fauna associated with cold water gorgonians and sea pens. Cont. Shelf. Res. 105: 67-78.
https://doi.org/10.1016/j.csr.2015.06.007

Cognetti G., Sarà M., Magazzù G. 2001. Biología marina. Ed. Ariel, Barcelona. 619 pp.

Curd A. 2010. Background Document for sea pen and burrowing megafauna communities., Biodiversity Series. OSPAR Commission, Ospar Convention for the Protection of the Marine Environment of the Northeast Atlantic, 26 pp.

Daly M., Brugler M.R., Cartwright P. 2007. The phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 1668: 127-182.
https://doi.org/10.11646/zootaxa.1668.1.11

Dolan E., Tyler P.A., Yesson C., et al. 2013. Phylogeny and systematics of deep-sea sea pens (Anthozoa: Octocorallia: Pennatula-

cea). Mol. Phylogenet. Evol. 69: 610-618.
https://doi.org/10.1016/j.ympev.2013.07.018

Drummond A.J., Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7: 214.
https://doi.org/10.1186/1471-2148-7-214

Drummond A.J., Bouckaert R.R. 2015. Bayesian evolutionary analysis with BEAST. Cambridge University Press.
https://doi.org/10.1017/CBO9781139095112

Duchêne S., Lanfear R., Ho S.Y. 2014. The impact of calibration and clock-model choice on molecular estimates of divergence times. Mol. Phylogenet. Evol. 78: 277-289.
https://doi.org/10.1016/j.ympev.2014.05.032

Everett M.V., Park L.K., Berntson E.A., et al. 2016. Large-scale genotyping-by-sequencing indicates high levels of gene flow in the deep-sea octocoral *Swiftia simplex* (Nutting 1909) on the west coast of the United States. PloS ONE 11: e0165279.
https://doi.org/10.1371/journal.pone.0165279

Fabricius K., Alderslade P. 2001. Soft corals and sea fans. Australian Institute of Marine Science. Australia. 264 pp.

Föllmi K. B. 2012. Early Cretaceous life, climate and anoxia. Cretaceous Res. 35: 230-257.
https://doi.org/10.1016/j.cretres.2011.12.005

France S.C., Hoover L.L. 2002. DNA sequences of the mitochondrial COI gene have low levels of divergence among deep-sea octocorals (Cnidaria: Anthozoa). Hydrobiologia 471: 149-155.
https://doi.org/10.1023/A:1016517724749

Friedrich O., Norris R.D., Erbacher J. 2012. Evolution of middle to Late Cretaceous oceans-a 55 my record of Earth's temperature and carbon cycle. Geology 40: 107-110.
https://doi.org/10.1130/G32701.1

García-Cárdenas F.J., Drewery J., López-González P.J. 2019. Resurrection of the sea pen genus *Ptilella* Gray, 1870 and description of *Ptilella grayi* n. sp. from the NE Atlantic (Octocorallia, Pennatulacea). Sci. Mar. 83: 261-276.
https://doi.org/10.3989/scimar.04845.26A

Grasshoff M. 1999. The shallow water gorgonians of New Caledonia and adjacent islands (Coelenterata: Octocorallia). Senchenb. Biol. 78: 1-245.

Haq B.U., Hardenbol J., Vail P.R. 1987. Chronology of fluctuating sea levels since the Triassic. Science 235: 1156-1167.
https://doi.org/10.1126/science.235.4793.1156

Herklots J.A. 1858. Notices pour servir à l'étude des polypiers nageurs ou pennatulidés. Bijdragen tot de Dierkunde 7: 1-31.
https://doi.org/10.1163/26660644-00701001

Hickson S.J. 1916. The Pennatulacea of the Siboga Expedition, with a general survey of the order. Siboga-Expeditie Monographs 14, Livr. 77: 265 pp.

Hickson S.J. 1930. On the Classification of the Alcyonaria. Proc. Zool. Soc. Lond. 100: 229-252.
https://doi.org/10.1111/j.1096-3642.1930.tb00975.x

Hickson S.J. 1937. The Pennatulacea. Scientific Rep. John Murray Expedition, 1933-v1934 4: 109-130.

Hogan R.I., Hopkins K., Wheeler A.J., et al. 2019. Novel diversity in mitochondrial genomes of deep-sea Pennatulacea (Cnidaria: Anthozoa: Octocorallia). Mitochondr. DNA Part A. 30: 764-777.
https://doi.org/10.1080/24701394.2019.1634699

Hughes D.J. 1998. Sea pens and burrowing megafauna (volume III). An overview of dynamics and sensitivity characteristics for conservation management of marine SACs. Scottish Association for Marine Science (UK Marine SACs Project). 105 pp.

Huelsenbeck J.P., Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754-755.
https://doi.org/10.1093/bioinformatics/17.8.754

Jones L.A., Hiscock K., Connor D.W. 2000. Marine habitat reviews, a summary of ecological requirements and sensitivity characteristics for the conservation and management of Marine SAC's. Peterborough: Joint Nature Conservation Committee (UK Marine SAC's Project Report). 178 pp.

Jungersen H.F.E. 1927. *Anthomastus*. The Danish Ingolf-Expedition. Vol. V. Bianco Luno. Copenhagen.

Kayal E., Roure B., Philippe H., et al. 2013. Cnidarian phylogenetic relationships as revealed by mitogenomics. BMC Evol. Biol. 13: 5.
https://doi.org/10.1186/1471-2148-13-5

Koch G. von. 1878. Notiz über die Zooide von Pennatula. Zool. Anz. 1: 103-104.

Kölliker R.A. 1870. Anatomisch-Systematische Beschreibung der Alcyonararien. I. Die Pennatuliden. Abh. Senckenb. Naturforsch. Ges. 7: 487-602.

Kölliker R.A. 1880. Report on the Pennatulida dredged by HMS Challenger during the years 1873-1876. Report of the Scientific Results of the Voyage of H. M. S. Challenger during the years 1873-76. Zoology 1(2): 1-41.

Kükenthal W. 1912. Der Stammbaum der Seefedern. Verhandlungen der international Zoologischen Kongress Jena 8: 563-570.

Kükenthal W. 1915. Pennatularia. Das Tierreich. 43: 1-132. Verlag von R. Friedländer und Sohn, Berlin.

Kükenthal W., Broch H. 1910. System und Stammesgeschichte der Seefedern. Zool. Anz. 36: 222-230.

Kükenthal W., Broch H. 1911. Pennatulacea. Wissenschaftliche Ergebnisse der deutschen Tiefsee-Expedition "Valdivia" 13: 113-576.

Kushida Y., Reimer J.D. 2018. Molecular phylogeny and diversity of sea pens (Cnidaria: Octocorallia: Pennatulacea) with a focus on shallow water species of the northwestern Pacific Ocean. Mol. Phylogenet. Evol. 131: 233-244.
https://doi.org/10.1016/j.ympev.2018.10.032

Landing E., Antcliffe J.B., Brasier M.D., et al. 2015. Distinguishing Earth's oldest known bryozoan (Pywackia, late Cambrian) from pennatulacean octocorals (Mesozoic-Recent). J. Paleontol. 89: 292-317.
https://doi.org/10.1017/jpa.2014.26

López-González P.J., Williams G.C. 2002. A new genus and species of sea pen (Octocorallia: Pennatulacea: Stachyptilidae) from the Antarctic Peninsula. Invertebr. Syst. 16: 919-929.
https://doi.org/10.1071/IT01027

Marshall A.M. 1883. Report on the Pennatulida dredged by H.M.S. Triton. Trans. R. Soc. Edinb. 32: 119-152.
https://doi.org/10.1017/S0080456800026697

Maturana R.P., Brewer B.J., Klaere S., et al. 2018. Model selection and parameter inference in phylogenetics using nested sampling. Syst. Biol. 68: 219-233.
https://doi.org/10.1093/sysbio/syy050

McFadden C.S., van Ofwegen L.P. 2012. Stoloniferous octocorals (Anthozoa, Octocorallia) from South Africa, with descriptions of a new family of Alcyonacea, a new genus of Clavulariidae, and a new species of Cornularia (Cornulariidae). Invertebr. Syst. 26: 331-356.
https://doi.org/10.1071/IS12035

McFadden C.S., van Ofwegen L.P. 2013. Molecular phylogenetic evidence supports a new family of octocorals and a new genus of Alcyoniidae (Octocorallia, Alcyonacea). Zookeys 346: 59-83.
https://doi.org/10.3897/zookeys.346.6270

McFadden C.S., Tullis I.D., Hutchinson M.B., et al. 2004. Variation in coding (NADH dehydrogenase subunits 2, 3, and 6) and noncoding intergenic spacer regions of the mitochondrial genome in Octocorallia (Cnidaria: Anthozoa). Mar. Biotechnol. 6: 516-526.
https://doi.org/10.1007/s10126-002-0102-1

McFadden C.S., France S.C., Sánchez J.A., et al. 2006. A molecular phylogenetic analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences. Mol. Phylogenet. Evol. 41: 513-527.
https://doi.org/10.1016/j.ympev.2006.06.010

McFadden C.S., Sánchez J.A., France S.C. 2010. Molecular phylogenetic insights into the evolution of Octocorallia: a review. Integr. Comp. Biol. 50: 389-410.
https://doi.org/10.1093/icb/icq056

McFadden C.S., Benayahu Y., Pante E., et. al. 2011. Limitations of mitochondrial gene barcoding in Octocorallia. Mol. Ecol. Resour. 11: 19-31.
https://doi.org/10.1111/j.1755-0998.2010.02875.x

McFadden C.S., Brown A.S., Brayton C., et al. 2014. Application of DNA barcoding in biodiversity studies of shallow- water octocorals: molecular proxies agree with morphological estimates of species richness in Palau. Coral Reefs 33: 275-286.
https://doi.org/10.1007/s00338-013-1123-0

Mortensen P.B., Buhl-Mortensen L., Gebruk A.V., et al. 2008. Occurrence of deep-water corals on the Mid-Atlantic Ridge based on MAR-ECO data. Deep-Sea Res. Part II 55: 142-152.
https://doi.org/10.1016/j.dsr2.2007.09.018

Niedermeyer A. 1913. Über einige histologische Befunde an *Veretillum cynomorium*. Zool. Anz. 43: 263-270.

Núñez-Flores M., Gomez-Uchida D., López-González P.J. 2020. Molecular and morphological data reveal three new species of *Thouarella* (Anthozoa: Octocorallia: Primnoidae) from the Southern Ocean. Mar. Biodivers. 50: 30.
https://doi.org/10.1007/s12526-020-01053-z

Opresko D.M. 2002. Revision of the Antipatharia (Cnidaria: Anthozoa). Part II. Schizopathidae. Zool. Opresko Meded. Leiden 76: 411-442.

Pante E., France S.C., Couloux A., et al. 2012. Deep-sea origin and in-situ diversification of chrysogorgiid octocorals. PloS ONE 7: e38357.
https://doi.org/10.1371/journal.pone.0038357

Park E., Hwang D.S., Lee J.S., et al. 2012. Estimation of divergence times in cnidarian evolution based on mitochondrial protein-coding genes and the fossil record. Mol. Phylogenet. Evol. 62: 329-345.
https://doi.org/10.1016/j.ympev.2011.10.008

Pearson P.N., Ditchfield P.W., Singano J., et al. 2001. Warm tropical sea surface temperatures in the Late Cretaceous and Eocene epochs. Nature 413: 481-487.
https://doi.org/10.1038/35097000

Pérez C.D., Ocampo F.C. 2001. Cladistic analysis of the pennatulacean genus *Renilla* Lamarck, 1816 (Coelenterata, Octocorallia). J. Nat. Hist. 35: 169-173.
https://doi.org/10.1080/00222930150215305

Pérez C.D., de Moura Neves B., Cordeiro R.T., et al. 2016. Diversity and distribution of Octocorallia. In: Goffredo S., Dubinsky Z. (eds). The Cnidaria, Past, Present and Future. Springer, Cham, Switzerland, pp. 109-123.
https://doi.org/10.1007/978-3-319-31305-4_8

Prokoph A., Shields G.A., Veizer J. 2008. Compilation and time-series analysis of a marine carbonate $\delta^{18}O$, $\delta^{13}C$, $^{87}Sr/^{86}Sr$ and $\delta^{34}S$ database through Earth history. Earth-Science Reviews 87: 113-133.
https://doi.org/10.1016/j.earscirev.2007.12.003

Quattrini A.M., Georgian S.E., Byrnes L., et al. 2013. Niche divergence by deep-sea octocorals in the genus *Callogorgia* across the continental slope of the Gulf of Mexico. Mol. Ecol. 22: 4123-4140.
https://doi.org/10.1111/mec.12370

Rambaut A., Drummond A.J. 2010. TreeAnnotator version 1.6.1. University of Edinburgh, Edinburgh, UK.
http://beast.bio.ed.ac.uk

Rambaut A., Drummond A.J., Xie D., et al. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67: 901-904.
https://doi.org/10.1093/sysbio/syy032

Reich M., Kutscher M. 2011. Sea pens (Octocorallia: Pennatulacea) from the Late Cretaceous of northern Germany. J. Paleontol. 85: 1042-1051.
https://doi.org/10.1666/10-109.1

Roberts J.M., Wheeler A.J., Freiwald A. 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. Science 312: 543-547.
https://doi.org/10.1126/science.1119861

Roberts J.M., Wheeler A.J., Freiwald A., et al. 2010. The biology and geology of deep-sea coral habitats. Oceanography 23: 226-227.
https://doi.org/10.5670/oceanog.2010.105

Ronquist F., Huelsenbeck J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.
https://doi.org/10.1093/bioinformatics/btg180

Sammarco P.W., Coll J.C. 1992. Chemical adaptations in the Octocorallia: evolutionary considerations. Mar. Ecol. Prog. Ser. 88: 93-104.
https://doi.org/10.3354/meps088093

Sánchez J.A., McFadden C.S., France S.C., et al. 2003. Molecular phylogenetic analyses of shallow-water Caribbean octocorals. Mar. Biol. 142: 975-987.
https://doi.org/10.1007/s00227-003-1018-7

Takashima R., Nishi H., Huber B.T., et al. 2006. Greenhouse world and the Mesozoic ocean. Oceanography 19: 64-74.
https://doi.org/10.5670/oceanog.2006.07

Tamura K., Stecher G., Peterson D. et al. 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol. Biol. Evol. 30: 2725-2729.
https://doi.org/10.1093/molbev/mst197

Taylor P.D., Berning B., Wilson M.A. 2013. Reinterpretation of the Cambrian 'Bryozoan' Pywackia as an Octocoral. J. Paleontol. 87: 984-990.
https://doi.org/10.1666/13-029

Tiffon Y. 1987. Ordre des Cérianthaires. In: Grassé P (ed) Traité deZoologie: Anatomie, Systématique, Biologie - Cnidaires/Anthozoaires - Tome III. Masson, Paris, pp. 210-256.

Tixier-Durivault A. 1965. Quelques octocoralliaires australiens. Bull. Mus. Natl. Hist. Nat. 4: 705-716.

Williams G.C. 1990. The Pennatulacea of southern Africa (Coelenterata, Anthozoa). Ann. S. Afr. Mus. 99: 1-120.

Williams G.C. 1992. Biogeography of the octocorallian coelenterate fauna of southern Africa. Biol. J. Linn. Soc. 46: 351-401.
https://doi.org/10.1111/j.1095-8312.1992.tb00869.x

Williams G.C.1994. Biotic diversity, biogeography and phylogeny of pennatulacean octocorals associated with coral reefs in the Indo-Pacific. Proc. 7th Int. Coral Reef Symp. 1994: 739-745.

Williams G.C. 1995. Living genera of sea pens (Coelenterata: Octocorallia: Pennatulacea): illustrated key and synopses. Zool. J. Linn. Soc. 113: 93-140.
https://doi.org/10.1006/zjls.1995.0004

Williams G.C. 1997. Preliminary assessment of the phylogeny of Pennatulacea (Anthozoa: Octocorallia), with a reevaluation of Ediacaran frond-like fossils, and a synopsis of the history of evolutionary thought regarding the sea pens. Proc. 6th Int. Conf. Coel. Biol. 1997: 497-509.

Williams G.C. 2011. The Global Diversity of Sea Pens (Cnidaria: Octocorallia: Pennatulacea). PloS ONE 6: e22747.
https://doi.org/10.1371/journal.pone.0022747

Williams G.C. 2015. A new genus and species of pennatulacean octocoral from equatorial West Africa (Cnidaria, Anthozoa, Virgulariidae). Zookeys 546: 39-50.
https://doi.org/10.3897/zookeys.546.6344

Williams G.C. 2019. A new genus and species of enigmatic gorgonian coral from the Ryukyu Archipelago, northwestern Pacific, with a discussion of calcaxonian systematics (Cnidaria, Anthozoa, Octocorallia). Zootaxa 4701: 417-433.
https://doi.org/10.11646/zootaxa.4701.5.2

Williams G.C., Alderslade P. 2011. Three new species of pennatulacean octocorals with the ability to attach to rocky substrata (Cnidaria: Anthozoa: Pennatulacea). Zootaxa 3001: 33-48.
https://doi.org/10.11646/zootaxa.3001.1.2

Williams G.C., Hoeksema B.W., van Ofwegen L.P. 2012. A fifth morphological polyp in pennatulacean octocorals, with a review of polyp polymorphism in the genera *Pennatula* and *Pteroeides* (Anthozoa: Pennatulidae). Zool. Stud. 51: 1006-1017.

Xia X., Lemey P. 2009. Assessing substitution saturation with DAMBE. In: Lemey P., Salemi M., et al. (eds) The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny. Cambridge University Press, Cambridge, pp. 615-630.
https://doi.org/10.1017/CBO9780511819049.022

Xia X., Xie Z., Salemi M., et al. 2003. An index of substitution saturation and its application. Mol. Phylog. Evol. 26: 1-7.
https://doi.org/10.1016/S1055-7903(02)00326-3

Yesson C., Taylor M.L., Tittensor D.P., et al. 2012. Global habitat suitability of cold-water octocorals. J. Biogeogr. 39: 1278-1292.
https://doi.org/10.1111/j.1365-2699.2011.02681.x

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/sm05067esm.pdf

Fig. S1. – Calibrated tree from time divergence analysis. Phylogenetic relationships were based on Bayesian inference methods for combined regions *mtMutS+Cox1+28S*. Bars indicate the 95% highest posterior density with the inferred mean age. Red dot indicates fossil calibration point.

Fig. S2. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the marker *mtMutS*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table S3 for species and GenBank accession numbers used in this tree.

Fig. S3. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the marker *Cox1*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table 1 for species and GenBank accession numbers used in this tree.

Fig. S4. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the concatenated mitochondrial markers *mtMutS+Cox1*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table 1 for species and GenBank accession numbers used in this tree.

Fig. S5. – Phylogenetic relationships in the order Pennatulacea

based on the maximum likelihood method for the nuclear marker *28S*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table 1 for species and GenBank accession numbers used in this tree.

Table S1. – Different composition of Clade III according to the markers and phylogenetic methods used. G, *Gyrophyllum*; K, *Kophobelemnon*; F, *Funiculina*; H, *Halipteris*; ML, maximum likelihood method; BI, Bayesian inference.

Table S2. – Results from the molecular clock model comparisons.

Table S3. – GenBank accession number of *mtMutS* sequences used in Figure S2.

Molecular phylogeny and divergence time estimates in pennatulaceans (Cnidaria: Octocorallia: Pennatulacea)

Francisco J. García-Cárdenas, Mónica Núñez-Flores, Pablo J. López-González
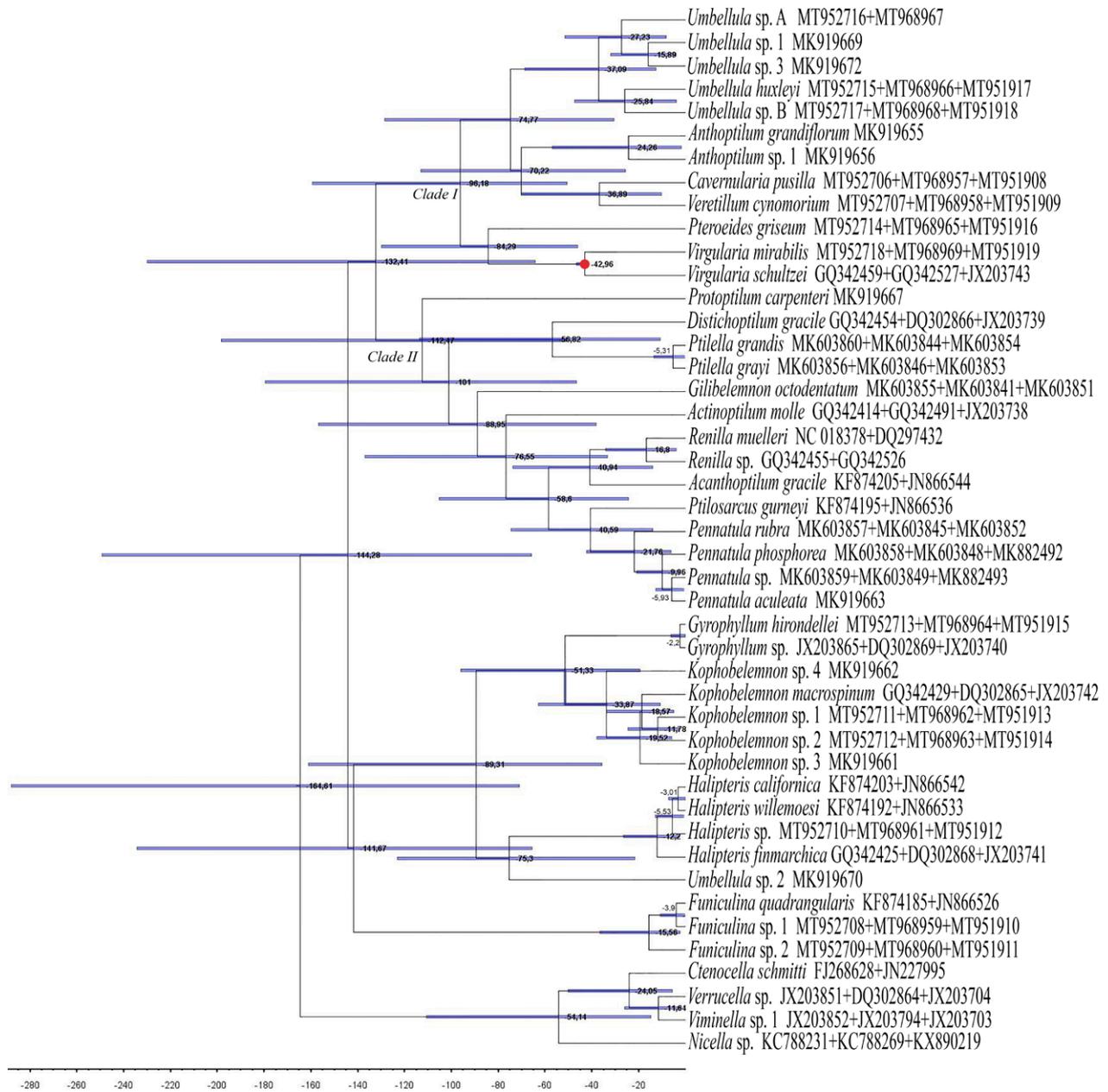
Supplementary material

Fig. S1. – Calibrated tree from time divergence analysis. Phylogenetic relationships were based on Bayesian inference methods for combined regions *mtMutS+Cox1+28S*. Bars indicate the 95% highest posterior density with the inferred mean age. Red dot indicates fossil calibration point.

mtMutS

Umbellula spp.
1/98
Calibelemnon sp. MK133473
1/99 *Umbellula* spp.
Cavernularia pusilla MT968957
Veretillum cynomorium MT968958
1/78 *Sclerobelemnon theseus* DQ311679
1/86 //──*Sclerobelemnon* sp. MK133376
1/85 *Veretillum* sp. 1 MK133435
Veretillum sp. 2 MK133526
1/93 *Cavernulina* sp. 1 MK133372
Cavernulina sp. 2 MK133439
Anthoptilum sp.1 MK919656

Clade I
1/97
Pteroeides spp.
1/99

Virgularia spp.
1/99

Stylatula elongata JX023275
0.99/84
Protoptilum spp.
1/99 *Scytalium* spp.
0.99/96 0.99/86
Pennatula sp. DQ302870
Clade II
1/99 *Ptilella* spp.
1/99 *Distichoptilum* spp.
Gilibelemnon octodentatum MK603841
Actinoptilum molle GQ342491
Stachyptilum dofleini MK133401
//──*Echinoptilum macintoshi* MK133436
Ptilosarcus gurneyi JN866521
1/99 *Renilla* spp.
0.95/76 0.99/97
Acanthoptilum gracile JN866544
0.97/81

Pennatula spp.

1/99 *Gyrophyllum* spp.

1/98 *Kophobelemnon* spp.
1/91
Kophobelemnon sp.1 KF313837
1/95
Funiculina spp.
1/78

Umbellula spp.
Scleroptilum grandiflorum KF313847
1/99
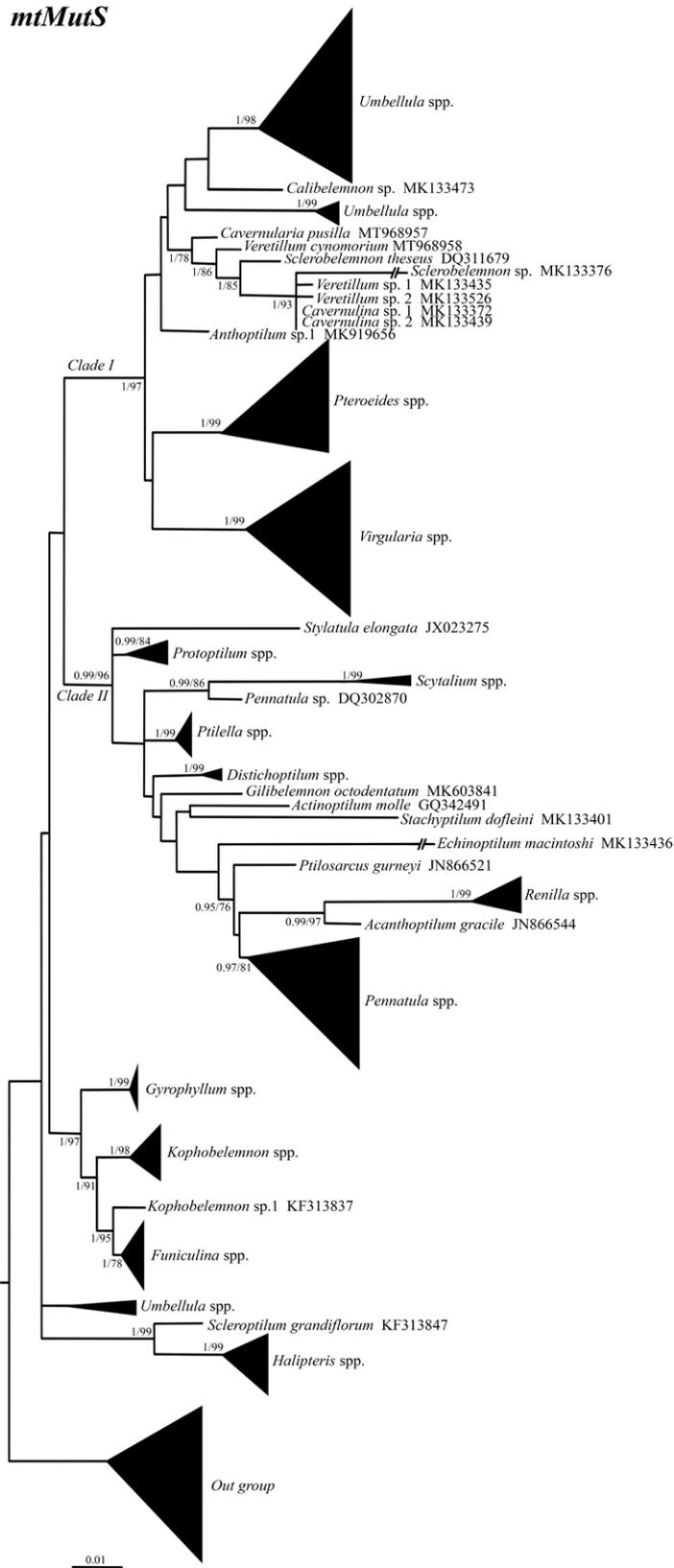1/99 *Halipteris* spp.

Out group

0.01

Fig. S2. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the marker *mtMutS*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table S3 for species and GenBank accession numbers used in this tree.

Fig. S3. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the marker *Cox1*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table 1 for species and GenBank accession numbers used in this tree.

Fig. S4. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the concatenated mitochondrial markers *mtMutS+Cox1*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table 1 for species and GenBank accession numbers used in this tree.

Fig. S5. – Phylogenetic relationships in the order Pennatulacea based on the maximum likelihood method for the nuclear marker *28S*. Posterior probability and bootstrap supporting values are indicated on the different nodes. See Table 1 for species and GenBank accession numbers used in this tree.
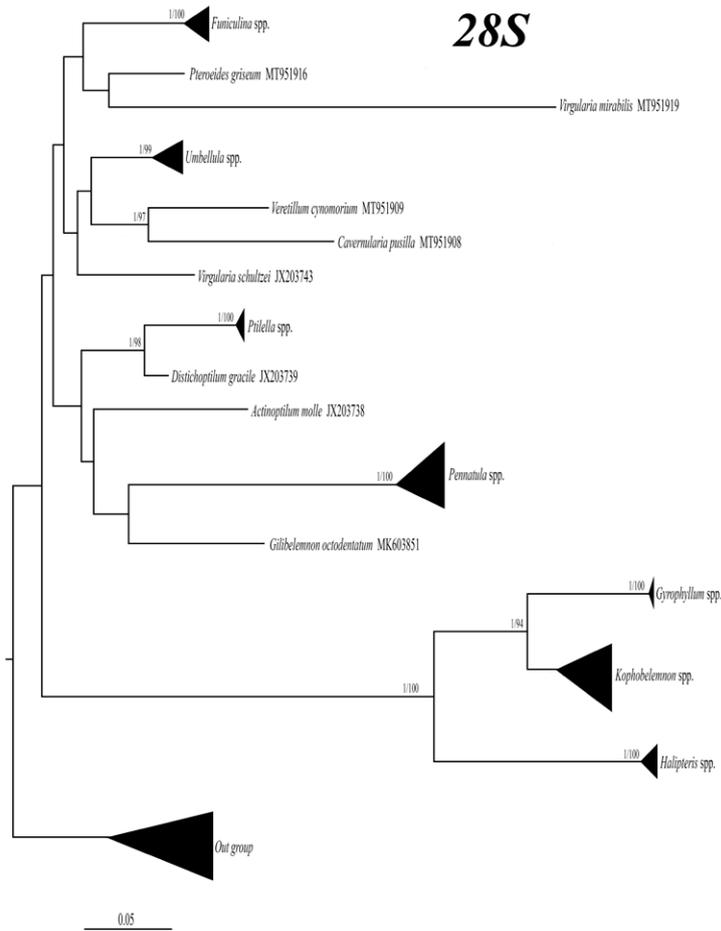
Table S1. – Different arrangement of genera in "Clade III" according to the markers and methods used. G, *Gyrophyllum*; K, *Kophobelemnon*; F, *Funiculina*; H, *Halipteris*; ML, maximum likelihood method; BI, Bayesian inference.

| Markers | CLADE III // out | | Source |
	ML	BI	
CoxI+mtMutS+28S	G-(K-F) // H	(G-K)-H // F	Present study (Fig. 1)
mtMutS	G-(K-F) // H	G-(K-F) // H	Present study (Fig. S2)
CoxI	G-K // F-H	G-K // F-H	Present study (Fig. S3)
CoxI+mtMutS	(G-K)-F // H	(G-K)-F // H	Present study (Fig. S4)
28S	(G-K)-H // F	(G-K)-H // F	Present study (Fig. S5)
mtMutS+ND2	G-(K-F) // H	G-(K-F) // H	Dolan et al. 2013, Kushida and Reimer 2018

Table S2. – Results from the molecular clock model comparisons.

Model	Marginal likelihood	Bayes factor
Relaxed log-normal	-13209.7	5.6
Relaxed exponential	-13215.3	209.2
Strict	-13424.5	1161.2
Random local	-14585.7	-

Table S3. – GenBank accession number of *mtMutS* sequences used in Figure S2.

Species	*mtMutS*	Species	*mtMutS*
Acanthoptilum gracile	JN866544	*Ptilella grayi*	MK603846
Actinoptilum molle	GQ342491	*Ptilosarcus gurneyi*	JN866521
Anthoptilum sp.1	MK919656	*Renilla muelleri*	DQ297432
Calibelemnon sp.	MK133473	*Renilla reniformis*	EU293803
Cavernularia pusilla	MT968957	*Renilla* sp.	GQ342526
Cavernulina sp.1	MK133372	*Renilla* sp.	MK133484
Cavernulina sp.2	MK133439	*Sclerobelemnon* sp.	MK133376
Distichoptilum gracile	KF313843	*Sclerobelemnon theseus*	DQ311679
Distichoptilum gracile	DQ302866	*Scleroptilum grandiflorum*	KF313847
Distichoptilum gracile	MK919657	*Scytalium martensi*	MK133361
Echinoptilum macintoshi	MK133436	*Scytalium* sp.1	MK133363
Funiculina armata	KF313833	*Stachyptilum dofleini*	MK133401
Funiculina quadrangularis	JN866526	*Stylatula elongata*	JX023275
Funiculina quadrangularis	JN866545	*Umbellula carpenteri*	KF313848
Funiculina quadrangularis	MK919658	*Umbellula encrinus*	KF313849
Funiculina sp.	JN227941	*Umbellula huxleyi*	KF313850
Funiculina sp.1	MT968959	*Umbellula huxleyi*	MK919668
Funiculina sp.2	MT968960	*Umbellula huxleyi*	MT968966
Gilibelemnon octodentatum	MK603841	*Umbellula magniflora*	KF313851
Gyrophyllum hirondellei	MT968964	*Umbellula* sp.	JN866562
Gyrophyllum hirondellei	KY039182	*Umbellula* sp.	JN866532
Gyrophyllum sp.	DQ302869	*Umbellula* sp.	JN227908
Gyrophyllum sp.	KF313845	*Umbellula* sp.	DQ302867
Gyrophyllum sp.	KF313846	*Umbellula* sp. 1	MK919669
Halipteris californica	JN866560	*Umbellula* sp. 3	MK919672
Halipteris californica	JN866542	*Umbellula* sp. A	MT968967
Halipteris finmarchica	DQ302868	*Umbellula* sp. B	MT968968
Halipteris finmarchica	KF313835	*Umbellula* sp.1	KF313855
Halipteris cf. *finmarchica*	MK919659	*Umbellula* sp.2	KF313856
Halipteris willemoesi	JN866533	*Umbellula* sp.2	MK919670
Halipteris sp.	MT968961	*Umbellula thomsoni*	KF313853
Kophobelemnon macrospinum	DQ302865	*Umbellula thomsoni*	KF313854
Kophobelemnon pauciflorum	KF313836	*Umbellula monocephalus*	KF313852
Kophobelemnon sp.1	KF313837	*Veretillum cynomorium*	MT968958
Kophobelemnon sp.1	MK919660	*Veretillum* sp.1	MK133435
Kophobelemnon sp.1	MT968962	*Veretillum* sp.2	MK133526
Kophobelemnon sp.2	KF313838	*Virgularia* cf. *gustaviana*	MK133518
Kophobelemnon sp.2	MT968963	*Virgularia* cf. *halisceptrum*	MK133359
Kophobelemnon sp.3	KF313839	*Virgularia* cf. *rumphi*	MK133423
Kophobelemnon sp.3	MK919661	*Virgularia mirabilis*	MT968969
Kophobelemnon sp.4	MK919662	*Virgularia mirabilis*	KF313857
Pennatula rubra	MK603845	*Virgularia mirabilis*	KF313858
Pennatula aculeata	MK919663	*Virgularia mirabilis*	MK919673
Pennatula aculeata	KF313840	*Virgularia schultzei*	GQ342527
Pennatula murrayi	KF313842	*Virgularia* sp.1	MK133378
Pennatula phosphorea	MK603848	*Virgularia* sp.3	MK133393
Pennatula phosphorea	JN866531	*Virgularia* sp.4	MK133400
Pennatula phosphorea	KF313841	*Virgularia* sp.5	MK133410
Pennatula phosphorea	KX904975	*Virgularia* sp.7	MK133424
Pennatula cf. *phosphorea*	MK133428	*Virgularia* sp.9	MK133449
Pennatula sp.	DQ302870	*Virgularia* sp.10	MK133462
Pennatula sp.	MK603849	OUTGROUP	
Protoptilum carpenteri	MK919667	*Ctenocella barbadensis*	AY533651
Protoptilum sp.	DQ297431	*Ctenocella schmitti*	JN227995
Protoptilum sp.	EU293804	*Ellisella* sp.	JN227994
Protoptilum sp.	KF313844	*Nicella americana*	KF803719
Pteroeides sp.	DQ302871	*Nicella carinata*	KF803720
Pteroeides griseum	MT968965	*Nicella obesa*	KF803727
Pteroeides caledonicum	MK133429	*Nicella toeplitzae*	KF803730
Pteroeides sp.1	MK133370	*Nicella* sp.	KC788269
Pteroeides sp.2	MK133387	*Nicella* sp.	KF803728
Pteroeides sp.2	MK133452	*Nicella* sp.	KC788269
Pteroeides sp.3	MK133371	*Nicella* sp.	KF803729
Pteroeides sp.4	MK133467	*Verrucella* sp.	DQ302864
Pteroeides sp.5	MK133521	*Viminella flagellum*	KF803745
Pteroeides sp.6	MK133527	*Viminella* sp.	GQ342493
Ptilella grandis	MK603844	*Viminella* sp.	JX203794