Exploring the trends of adaptation and evolution of sclerites with regards to habitat depth in sea pens

Yuka Kushida [Corresp., 1, 2, 3, 4], Yukimitsu Imahara [2, 5, 6], Wee Hin Boo [7], Iria Fernandez-Silva [8], Jane Fromont [9], Oliver Gomez [9], Nerida Wilson [9, 10], Taeko Kimura [11], Shinji Tsuchida [12], Yoshihiro Fujiwara [12], Takuo Higashiji [13], Hiroaki Nakano [14], Hisanori Kohtsuka [15], Akira Iguchi [2, 16], James Davis Reimer [4, 17]

1 Faculty of Geo-Environmental Science, Rissho University, Kumagaya, Saitama, Japan

2 Geological Survey of Japan, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

3 International Center for Island Studies Amami Station, Kagoshima University, Amami, Kagoshima, Japan

4 Molecular Invertebrate Systematics and Ecology Laboratory, Graduate School of Engineering and Science, University of the Ryukyus, Nishihara, Okinawa, Japan

5 Kuroshio Biological Research Foundation, Otsuchi, Kochi, Japan

6 Octocoral Research Laboratory, Wakayama, Wakayama, Japan

7 Institut Perubahan Iklim, Universiti Kebangsaan Malaysia, Selangor, Darul Ehsan, Malaysia

8 Department of Biochemistry, Genetics and Immunology, Campus Universitario, University of Vigo, Vigo, Spain

9 Collections & Research, Western Australian Museum, Welshpool, Western Australia, Australia

10 School of Biological Sciences, University of Western Australia, Perth, Western Australia, Australia

11 Department of Life Sciences, Graduate School of Bioresources, Mie University, Tsu, Mie, Japan

12 Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa, Japan

13 Okinawa Churaumi Aquarium, Okinawa Churashima Foundation, Motobu, Okinawa, Japan

14 Shimoda Marine Research Center, University of Tsukuba, Shimoda, Shizuoka, Japan

15 Misaki Marine Biological Station, Graduate School of Science, University of Tokyo, Miura, Kanagawa, Japan

16 Research Laboratory on Environmentally-Conscious Developments and Technologies [E-Code], National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki, Japan

17 Tropical Biosphere Research Center, University of the Ryukyus, Nishihara, Okinawa, Japan

Corresponding Author: Yuka Kushida
Email address: ykushida@ris.ac.jp

Octocorals possess sclerites, small elements comprised of calcium carbonate ($CaCO_3$) that are important diagnostic characters in octocoral taxonomy. Among octocorals, sea pens comprise a unique order (Pennatulacea) that live in a wide range of depths. Habitat depth is considered to be important in the diversification of octocoral species, but a lack of information on sea pens has limited studies on their adaptation and evolution across depth. Here, we aimed to reveal trends of adaptation and evolution of sclerite shapes in sea pens with regards to habitat depth via phylogenetic analyses and ancestral reconstruction analyses. Colony form of sea pens is suggested to have undergone convergent evolution and the loss of axis has occurred independently across the evolution of sea pens. Divergences of sea pen taxa and of sclerite forms are suggested to depend on habitat depths. In addition, their sclerite forms may be related to evolutionary history of

the sclerite and the surrounding chemical environment as well as water temperature. Three-flanged sclerites may possess the tolerance towards the environment of the deep sea, while plate sclerites are suggested to be adapted towards shallower waters, and have evolved independently multiple times. The common ancestor form of sea pens was predicted to be deep-sea and similar to family Pseudumbellulidae in form, possessing sclerites intermediate in form to those of alcyonaceans and modern sea pens such as spindles, rods with spines, and three-flanged sclerites with serrated edges sclerites, as well as having an axis and bilateral traits.

Exploring the trends of adaptation and evolution of sclerites with regards to habitat depth in sea pens

Yuka Kushida*[1,2,3,4], Yukimitsu Imahara[2,5,6], Wee Hin Boo[7], Iria Fernandez-Silva[8], Jane Fromont[9], Oliver Gomez[9], Nerida Wilson[9,10], Taeko Kimura[11], Shinji Tsuchida[12], Yoshihiro Fujiwara[12], Takuo Higashiji[13], Hiroaki Nakano[14], Hisanori Kohtsuka[15], Akira Iguchi[2,16], James Davis Reimer[4,17]

[1] Faculty of Geo-Environmental Science, Rissho University, 1700 Magechi, Kumagaya, Saitama 360-0194, Japan
[2] Geological Survey of Japan, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8567, Japan
[3] International Center for Island Studies Amami Station, Kagoshima University, 15-1 Naze-Minatomachi, Amami, Kagoshima 894-0026, Japan
[4] Molecular Invertebrate Systematics and Ecology Laboratory, Graduate School of Engineering and Science, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0123, Japan
[5] Octocoral Research Laboratory, 300-11 Kire, Wakayama 640-0351, Japan
[6] Kuroshio Biological Research Foundation, 560 Nishidomari, Otsuchi, Kochi 788-0333, Japan
[7] Institut Perubahan Iklim, Universiti Kebangsaan Malaysia, 43600 Bangi UKM, Selangor Darul Ehsan, Malaysia
[8] Department of Biochemistry, Genetics and Immunology, Campus Universitario, University of Vigo, 36310 Vigo, Spain
[9] Collections & Research, Western Australian Museum, 49, Kew St, Welshpool, Western Australia 6106, Australia
[10] School of Biological Sciences, University of Western Australia, Crawley, Perth, Western Australia 6009, Australia
[11] Department of Life Sciences, Graduate School of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507, Japan
[12] Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima, Yokosuka, Kanagawa 237-0061, Japan
[13] Okinawa Churaumi Aquarium, Okinawa Churashima Foundation, 424 Ishikawa Motobu, Okinawa 905-0206, Japan
[14] Shimoda Marine Research Center, University of Tsukuba, 5-10-1, Shimoda, Shizuoka 415-0025, Japan
[15] Misaki Marine Biological Station, Graduate School of Science, The University of Tokyo, 1024 Koajiro, Misaki, Miura, Kanagawa 238-0225, Japan
[16] Research Laboratory on Environmentally-Conscious Developments and Technologies [E-Code], National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8567, Japan

40   [17] Tropical Biosphere Research Center, University of the Ryukyus, 1 Senbaru, Nishihara,
41   Okinawa 903-0123, Japan
42
43   Corresponding Author:
44   Yuka Kushida[1,2,3,4]
45   1700 Magechi, Kumagaya, Saitama 360-0194, Japan
46   Email address: ykushida@ris.ac.jp
47

Abstract

49   Octocorals possess sclerites, small elements comprised of calcium carbonate ($CaCO_3$) that are
50   important diagnostic characters in octocoral taxonomy. Among octocorals, sea pens comprise a
51   unique order (Pennatulacea) that live in a wide range of depths. Habitat depth is considered to be
52   important in the diversification of octocoral species, but a lack of information on sea pens has
53   limited studies on their adaptation and evolution across depth. Here, we aimed to reveal trends of
54   adaptation and evolution of sclerite shapes in sea pens with regards to habitat depth via
55   phylogenetic analyses and ancestral reconstruction analyses. Colony form of sea pens is
56   suggested to have undergone convergent evolution and the loss of axis has occurred
57   independently across the evolution of sea pens. Divergences of sea pen taxa and of sclerite forms
58   are suggested to depend on habitat depths. In addition, their sclerite forms may be related to
59   evolutionary history of the sclerite and the surrounding chemical environment as well as water
60   temperature. Three-flanged sclerites may possess the tolerance towards the environment of the
61   deep sea, while plate sclerites are suggested to be adapted towards shallower waters, and have
62   evolved independently multiple times. The common ancestor form of sea pens was predicted to
63   be deep-sea and similar to family Pseudumbellulidae in form, possessing sclerites intermediate in
64   form to those of alcyonaceans and modern sea pens such as spindles, rods with spines, and three-
65   flanged sclerites with serrated edges sclerites, as well as having an axis and bilateral traits.
66

Introduction

68   The order Pennatulacea, within the subclass Octocorallia (Cnidaria: Anthozoa), includes the sea
69   pens and sea pansies. Sea pens are unique octocorals as they have adapted to a life on marine soft
70   bottoms, and it is estimated there are about 200 species in the world's oceans (Fabricius and
71   Alderslade, 2001; Williams, 2011). The primary polyp, called an oozooid, is the central part of
72   the sea pen colony and includes the rachis and peduncle, and is a characteristic trait. The
73   peduncle enables sessile life by acting as an anchor via embedding in soft sediments. The rachis
74   hosts two to four different types of polyps, including autozooids, which have functions related to
75   feeding and reproduction, and siphonozooids for exhaling water from canals, and sometimes
76   other types of zooids (mesozooids, acrozooids) (Williams, 2011; Williams et al., 2012). Sea pens
77   have utility in understanding evolution in class Anthozoa as they have unique ecological traits
78   such as unique morphology and differentiation of zooids, active mobile behavior and
79   bioluminescence, all likely stemming from benthic life on sandy bottoms. However, sea pens

80   have been sparsely studied, and basic information on their taxonomy, diversity and phylogeny
81   remains limited (e.g. Kushida and Reimer, 2019).
82   Colony forms and internal morphology, such as types of sclerites and axes, are both
83   important traits for the identification of sea pens. Sea pen colony forms are diverse, and include
84   various shapes such as feather-like, club-like, whip-like, and capitate colonies (*Renilla* species),
85   as well species with a cluster of polyps at the top of the colony (*Umbellula* species) (Imahara et
86   al., 2014). Sclerites are small skeletal elements composed of a kind of calcium carbonate
87   ($CaCO_3$), polycrystalline calcite, which aggregates in the soft tissues of most octocorals
88   (Fabricius and Alderslade, 2001). The shapes of sea pen sclerites are simpler than those in other
89   octocorals, and include needle, rod, oval, and plate forms. Some sea pens also have sclerites with
90   three longitudinal flanges or warts (Bayer, 1983; Williams, 1995). Sclerites help support colonies
91   as well as act in preventing feeding by predators (Lewis and von Wallis, 1991; West, 1998).
92   Across evolutionary time, Metazoa have evolved biominerals such as sclerites and spicules at
93   least twenty times, and improving our knowledge of octocoral sclerites can provide relevant
94   information to help understand biomineralization in other taxa (Knoll, 2003). Furthermore,
95   information on biomineralization in octocorals is lacking, and thus the accumulation of
96   knowledge is important (Conci et al., 2021). Finally, most sea pen taxa except *Renilla*,
97   *Echinoptilum*, *Actinoptilum*, and some *Veretillum* spp. have a more or less long central axis, or
98   alternately a short axis consisting of calcium carbonate (Williams, 1995, 2011).
99   The traditional classification system of sea pens was primarily established from the end
100 of the 19th Century to the early 20th Century (e.g. Kölliker, 1869, 1870, 1872, 1875, 1880;
101 Kükenthal and Broch, 1911; Kükenthal, 1915; Hickson, 1916). Kükenthal (1915) developed and
102 established two suborders, Sessiliflorae and Subselliflorae, based on external morphology.
103 However, the validity of these two suborders has been in doubt due to results of cladistic
104 (Williams, 1995) and molecular phylogenetic studies, which instead have either shown four large
105 clades within Pennatulacea (Dolan et al., 2013; Kushida and Reimer, 2019; García-Cárdenas et
106 al., 2020 (maximum likelihood analyses)), or two clades and an unstable grouping of other taxa
107 (García-Cárdenas et al., 2020; Bayesian analyses). In addition, colony forms of sea pens have
108 been suggested to be the result of convergent evolution (Dolan et al., 2013; Kushida and Reimer,
109 2019), making the current taxonomy of Pennatulacea confusing due to inconsistencies between
110 diagnostic morphology and molecular data (Dolan et al., 2013; Kushida and Reimer, 2019;
111 García-Cárdenas et al., 2020). Additionally, in the case of the axis, most species with no axis are
112 located within a single phylogenetic clade (=clade 2 *sensu* Dolan et al. (2013) and Kushida and
113 Reimer (2019); Clade II *sensu* García-Cárdenas et al. (2020)). This clade 2 includes *Renilla* sp.,
114 *Echinoptilum* sp. and *Actinoptilum molle* (Kükenthal, 1910). However, some species of
115 *Veretillum* also lack an axis (Williams, 1995), but are located in another phylogenetic clade
116 (=clade 1 *sensu* Kushida and Reimer, 2019; Clade I *sensu* García-Cárdenas et al. (2020)). Thus,
117 it is highly possible sea pens have lost their axes more than once across their evolution.
118   Among the taxonomic characters of sea pens, sclerites are an important component, but
119 the evolution of sea pen sclerites has not been studied well. In the case of Caribbean gorgonian

120   octocorals, Sánchez et al. (2003) mapped morphological traits such as sclerites and axis
121   mineralization onto the molecular phylogenetic relationships of gorgonians. Their study found
122   the type of surface sclerite present to be very variable, and suggested the diversity of sclerites
123   may be due to their functional morphology, as it is known that sclerite forms can vary in
124   response to the environment (West et al., 1993). Quattrini et al. (2020) examined anthozoan
125   skeleton evolution through deep time with regards to ocean conditions with a robust
126   phylogenetic tree. This study noted octocorals have obtained and lost their skeletal forms
127   multiple times across their evolution, and that the skeletal composition of octocorals appears to
128   be able to adapt more readily to changing ocean chemistry when compared to the skeletons of
129   scleractinian hard corals. In addition, these important species may be affected by the modern
130   climate change situation. For example, it is known that higher pCO_2 from ocean acidification
131   reduces biomineralization in the red coral *Corallium rubrum* (Cerrano et al., 2013; Rastelli et al.
132   2020), and thus climate change may threaten the rich diversity of octocorals.
133   However, there have only been a few studies on the evolution of sea pens using
134   phylogenetic and morphological information due to extremely limited available genetic
135   information on sea pens (e.g. Williams, 2011; Dolan et al., 2013; Kushida and Reimer, 2019;
136   García-Cárdenas et al., 2020). Kushida and Reimer (2019) mapped the presence of three-flanged
137   sclerites onto their molecular phylogeny, and found almost all species in clade 1, which includes
138   many shallow water species, lacked three-flanged sclerites, while all taxa in clades 2, 3, and 4
139   had three-flanged sclerites with the exception of the shallow water species of *Scytalium* (clade 2)
140   and deep-sea *Umbellula* sp. 2 (clade 4, *sensu* Dolan et al., 2013; Kushida and Reimer, 2019). In
141   addition, in Kushida and Reimer's (2020) formal description of a *Calibelemnon* species, which
142   focused on sea pens within clade 1, they hypothesized that the common ancestor of a molecular
143   *Anthoptilum – Umbellula – Calibelemnon* clade may have lost their rachis sclerites when
144   ancestral species moved from shallow to deeper waters. Thus, it seems that sclerite evolution
145   may be related to environmental factors such as habitat depth. However, our evolutionary
146   knowledge of sea pens based on direct observations is still fragmentary at best.
147   Sea pens as a group inhabit a wide range of habitats, living embedded in sandy and
148   muddy bottoms from the intertidal zone to the deep sea down to 6260 m (*Umbellula*) and from
149   tropical to polar regions (Williams, 2011; 2021). Although reports of some sea pen taxa are very
150   limited (e.g. genera *Echinoptilum*, *Calibelemnon*), it is known that 71% of sea pen families (ten
151   of 14 families) and about half of described sea pen genera have a global distribution (Williams,
152   2011). Additionally, instead of regional distributions, it is known that sea pen distributions are
153   apparently based on bathymetric ranges. Accordingly, Williams (2011) divided sea pens into
154   three major groups based on their bathymetric ranges: shallow-water species (0-400 m), mid-
155   depth species (400-2000 m), and deep-water species (2000-6260 m). The variability of sea pens
156   and their transition to the deep sea was treated in Pasternak (1989). Similarly, recent work on
157   gorgonians has shown bathymetric gradients act as drivers of diversification (Sánchez et al.,
158   2021).

159   Here, we focused on the sclerites of sea pens, aiming to explore the trends of sclerite
160 adaptation and evolution in relation to the habitat depth of sea pens. We observed sclerites from
161 various species, analyzed their molecular phylogenetic relationships, and overlapped sclerite
162 traits onto a phylogenetic tree to resolve the following questions: 1) Are there any relationships
163 between phylogenetic topology and sclerite forms? and 2) Are there any relationships between
164 habitat depth and sclerite forms?
165
166 ## Materials & Methods
167
168 **Specimen sampling**
169 All specimens utilized in this study and their GenBank information
170 (https://www.ncbi.nlm.nih.gov/genbank) are listed in Supplemental table S1. In this study, we
171 utilized novel specimens as well as specimens from Kushida and Reimer (2019) in our
172 morphological and molecular examinations. Novel specimens were collected from Japanese
173 waters (Supplemental table S1) via a variety of sampling methods. Mesophotic and deep-sea
174 specimens were collected during the 18th JAMBIO (Japanese Association for Marine Biology)
175 Coastal Organism Joint Survey off Shimoda, Japan in December 2018, T/RV *Seisui-maru* (Mie
176 University) cruise No. 1903 off Kii Peninsula, Japan in April 2019, R/V *Kaimei* cruise KM19-
177 05C (JAMSTEC; Japan Agency for Marine-Earth Science and Technology) off Iwate, Japan in
178 August 2019, and ROV cruises conducted by Okinawa Churaumi Aquarium off Okinawa, Japan
179 in 2018 to 2019. Additional specimens were collected by reef walking (*Cavernularia* sp.;
180 NSMT-Co1761) and scuba diving (< 40 m), as well as by use of dredges, beam trawl nets, an
181 ROV, and a bottom sampler for deeper waters specimens. Furthermore, two preserved specimens
182 from the Western Australian Museum were utilized (Reg. No: WAM Z44543), collected during
183 the Antarctic Circumnavigation Expedition 2016-2017. Field surveys requiring permits were
184 conducted under Ministry of Agriculture, Forestry and Fisheries (農林水産省指令３０水管第
185 ２７０９号).
186 It should be noted that although we utilized a relatively comprehensive set of taxa in this study,
187 we were not able to acquire specimens of all genera of sea pens, and there are some taxa missing
188 in this study. In this research, habitat depths were categorized based on Williams (2011) and
189 Kushida and Reimer (2020) as "categories" Shallow (0–50 m), Mesophotic (50–200 m),
190 Shallower Deep (200–400 m), Medium Deep (400–2000 m), and Deep (2000–6260 m).
191 Furthermore, we also set broader "groups" than our initial depth category definitions; a shallower
192 group consisting of the shallow + mesophotic + shallower deep categories (shallower than 400
193 m; shallow-water species *sensu* Williams 2011), and a deeper group consisting of the medium
194 deep + deep categories (deeper than 400 m; mid-depth species + deep-water species *sensu*
195 Williams 2011) for analyses as described below. We divided the depth groups at 400 m based on
196 the distribution patterns of sea pens as described by Williams (2011).
197
198 **Morphological observations**

199 The external morphology of specimens was observed with a stereoscopic microscope (Leica S8
200 APO, Leica Ltd., Tokyo). In this study, surface sclerites such as in the rachis, tentacles, and
201 polyp leaves (if present) were focused on because these parts are considered to be strongly
202 affected by the surrounding environment (Sánchez et al., 2003). For sclerite observations, tissues
203 surrounding sclerites were dissolved in sodium hypochlorite (household bleach). After this, the
204 sclerites were rinsed at least three times with water, with a final rinse of ethanol. After aligning
205 isolated sclerites on SEM stubs under a stereomicroscope, sclerites were coated with Pd/Au for
206 observation by a JEOL JSM-6060LV SEM (JEOL, Tokyo) operated at high vacuum at an
207 acceleration voltage of 15 kV.

208

209 **Molecular phylogenetic analyses and trait analyses**
210 The protocols of DNA extraction and amplification in this study were similar to those in Kushida
211 and Reimer (2019). We utilized sequences from the mitochondrial mutS-like protein DNA
212 mismatch repair gene (mtMutS) and the NADH dehydrogenase subunit 2 gene (ND2) as well as
213 sequences of the cytochrome c oxidase I (COI) region following McFadden et al. (2014). To
214 construct robust and comprehensive phylogenetic trees, we additionally utilized published
215 sequences in GenBank from Dolan et al. (2013), Kushida and Reimer (2019), Kushida and
216 Reimer (2020), and García-Cárdenas et al. (2019). These sequences were aligned and
217 concatenated using Geneious v.8.1.3 (Kearse et al., 2012). The total length of the resulting
218 alignment was 1812 bp (COI: 651 bp, mtMutS: 696 bp, ND2: 465 bp) and included 74
219 sequences. Newly acquired sequence information was deposited in NCBI GenBank (National
220 Center for Biotechnology Information, America) (http://www.ncbi.nlm.nih.gov/genbank)
221 (Supplemental table S1). As in Kushida and Reimer (2019), sequences from a species of
222 Ellisellidae, sister taxa to sea pens, were utilized as outgroup.
223   To construct phylogenetic trees, maximum likelihood (ML) and Bayesian (B) analyses
224 were conducted under the general time-reversible model with discrete gamma distribution and
225 invariant sites (GTR+G+I) as suggested by model selection in MEGA X (Kumar et al., 2018).
226 Rapid boot strapping and search for best-scoring ML tree were chosen. Bootstrap replicates were
227 performed 1000 times in Geneious v.8.1.3 (Kearse et al., 2012). For constructing the Bayesian
228 tree, analyses were conducted in Mr. Bayes v. 3.2.2 (Huelsenbeck and Ronquist, 2001; Ronquist
229 et al., 2012) under the the Hasegawa-Kishino-Yano model with gamma distribution (HKY+G)
230 model. Markov chain Monte Carlo (MCMC) chains were run for 10,000,000 generations and
231 sampling frequency was set each 200 generations. The first 480,000 generations were discarded
232 as burn-in (average standard deviation of frequency: 0.007987). The average standard deviation
233 of frequency was 0.001552 in the final generation.
234   The ecological traits of these depth groups and sclerite shapes (no sclerites, plates,
235 smooth rods and needles, three-flanged sclerites, spindles) were mapped onto the ML
236 phylogenetic tree to estimate the ancestral state of sea pen habitat depth. The analyses were
237 performed in Mesquite v. 3.6.1 with maximum likelihood (ML) method with a Mk1 likelihood
238 model (Maddison and Maddison 2019).

239   The morphological information for specimens with sequences in GenBank was based on
240   Williams (1995), López-González and Williams (2002), Dolan (2008), and García-Cárdenas et
241   al. (2019).
242
243   **Statistical analyses**
244   Statistical analyses were conducted in R Console (4.1.0) with RStudio (1.4.1106) User Interface
245   (R Core Team, 2021; RStudio Team, 2021) to test the relationships among three categories:
246   phylogenetic clades (n=4; clade 1, 2, 3, 4), depth groups (n=2; shallower group, deeper group),
247   and sclerites (n=5; no sclerites, plates, smooth rods and needles, three-flanged sclerites,
248   spindles). Three contingency tables were constructed based on the pairwise comparison of these
249   three categories (clade, depth, sclerite shape). A Pearson's chi-squared test was conducted on
250   each table, and standardized residual post-hoc tests with Bonferroni's correction were conducted
251   to detect significant relationships between the categories.
252
253   # Results
254
255   **Phylogenetic analyses**
256   The ML and B phylogenetic trees of the concatenated mitochondrial COI, mtMutS, and ND2
257   sequences (Figure 1) showed a topology divided into four large, well-supported clades; clade 1
258   (ML bootstrap values/B posterior probabilities = 99/1.00), clade 2 (ML/B = 97/1.00), clade 3
259   (ML/B = 96/1.00), and clade 4 (ML/B = 86/1.00).
260   Clade 1 included a *Virgularia* – Virgulariidae spp. – *Pteroeides* subclade (ML/B =
261   62/0.83), a *Sclerobelemnon* – Veretillidae subclade (ML/B = 100/1.00), and a *Anthoptilum* –
262   *Umbellula* – *Calibelemnon* subclade (ML/B = 79/0.99). Within the *Virgularia* – Virgulariidae sp.
263   – *Pteroeides* subclade, we observed a small *Virgularia* – Virgulariidae spp. (ML/B = 100/1.00)
264   and *Pteroeides* (ML/B = 99/1.00) clades. Within the *Sclerobelemnon* – Veretillidae subclade
265   (ML/B = 100/1.00), *Veretillum*, *Cavernulina* and *Cavernularia* species were nested with
266   *Sclerobelemnon* species. Within the *Anthoptilum* – *Umbellula* – *Calibelemnon* subclade, we
267   observed an *Anthoptilum* subclade (ML/B = 85/1.00), and an *Umbellula* – *Calibelemnon*
268   subclade (ML/B = 80/1.00) with *Umbellula* (ML/B = 76/1.00) and *Calibelemnon* (ML/B =
269   100/1.00) clades.
270   Clade 2 included a sole representative of *Stylatula elongata* Verrill, 1864, as well as a
271   *Protoptilum* subclade (ML/B = 98/1.00), and a large subclade (ML/B = 93/1.00) that included a
272   *Stachyptilum* – *Pennatula* subclade (ML/B = 100/1.00), a *Renilla* – *Pennatula* – *Echinoptilum* –
273   *Gilibelemnon* subclade (ML/B = 71/0.86), a *Ptilella* clade (ML/B = 100/1.00), a *Scytalium* clade
274   (ML/B = 100/1.00), a *Distichoptilum* clade (ML/B = 99/1.00), and a single *Distichoptilum*
275   representative. Within the *Renilla* – *Pennatula* – *Echinoptilum* – *Gilibelemnon* subclade, there
276   was a *Renilla* – *Pennatula* – *Echinoptilum* clade (ML/B = 97/1.00), with *Renilla* and *Pennatula*
277   as sister taxa (ML / B = 91/0.99).

278   Clade 3 included a *Gyrophyllum* clade (ML/B = 100/1.00), and a large subclade (ML/B =
279   99/1.00). This large subclade included a *Funiculina* subclade (ML/B = 100/1.00), and a nested
280   clade (ML/B = 55/0.67) formed of a *Kophobelemnon* subclade (ML/B = 100/1.00) and a
281   *Kophobelemnon* – unidentified specimen subclade (ML/B = 77/0.93) including a
282   *Kophobelemnon* subclade (ML/B = 100/1.00).
283   Clade 4 included an Pseudumbellulidae clade (ML/B = 88/1.00) and sequences from
284   single representatives of *Balticina finmarchica* (Sars, 1851) and *Scleroptilum grandiflorum*
285   Kölliker, 1880
286   Shallow water specimens were included in clades 1 and 2 and a single specimen of
287   shallow water *Funiculina quadrangularis* (Pallas, 1766) was in clade 3. Almost all sea pens from
288   mesophotic depths were included in clade 2, except Pennatulacea sp. in clade 3. Shallower deep
289   specimens were located in clades 1, 2 and 3. Medium deep and deep water specimens were
290   located in all clades. Each clade included specimens of various colony shapes (Figures 2-4).
291   The results of the ML ancestral reconstruction (Figure 5) suggested the last common
292   ancestor of all sea pens was likely from deeper than 400 m (proportional likelihood = 0.95). The
293   habitat depths of each common ancestor of clade 1, clade 2, clade 3 and clade 4 were also deeper
294   than 400 m (proportional likelihoods: clade 1 = 0.82, clade 2 = 0.90, clade 3 = 0.99, clade 4 =
295   0.99). Furthermore, the sclerite shape of the common ancestor of all sea pens was likely the
296   three-flanged sclerite (proportional likelihood = 0.99). The common ancestor of clade 1 had lost
297   sclerites (proportional likelihood = 0.94), and subsequently several taxa re-obtained sclerites
298   such as plates, smooth rods or needles, while only the deep-sea species *Umbellula thomsoni*
299   (Kölliker, 1874) re-obtained three-flanged sclerites. In addition, the shapes of sclerites of the
300   common ancestors of other clades were also likely three-flanged structures (proportional
301   likelihoods: clade 2 = 0.99, clade 3 = 0.99, clade 4 = 0.99). Subsequently, *Scytalium* species
302   evolved plate sclerites in shallow water, while Pennatulacea sp. (WAM Z44543) and
303   *Solumbellula monocephalus* Pasternak, 1964 developed spindles. (Figure 5).
304
305   **Sclerite observations**
306   The results of sclerite observations from representative specimens are listed in Supplemental
307   table S2. Regarding trends of sclerite forms of each clade, species in clade 1 exclusively had
308   plates, smooth rods or smooth needle sclerites, or no sclerites at all (Fig. S1), while species in
309   clade 2 had three-flanged rods or needles as well as plates for shallow water species of *Scytalium*
310   (Fig. S2). Species in clade 3 also had three-flanged rods or needles or spindles with warts (Fig.
311   S3). Due to the limited number of specimens in clade 4, we could only examine a single
312   specimen of *Pseudumbellula* sp. (NSMT-Co1772). This *Pseudumbellula* specimen had several
313   different types of sclerites, including rods with spines, spindle, three-flanged rods in tentacles,
314   and three-flanged rods and three-flanged spindles with serrated edges in the rachis (Figure.6). In
315   addition, broken three-flanged sclerites were observed in multiple clades in this study. These
316   damaged sclerites had broken flanged portions with an undamaged central part (Figure 7).
317

318   **Statistical analyses**
319   Depth preferences among clades (Supplemental table S3) showed species from shallower depths
320   were higher in number in clades 1 (n=15) and 2 (n=13), and rarely from clades 3 (n=3), with
321   none present in clade 4 (n=0). On the other hand, clades 1 to 3 had equal numbers of species
322   (n=9) at deeper depths, and clade 4 included only deeper species (n=5). There was a significant
323   difference among the depth groups with clades (X^2=10.21, p = 0.009). However, the
324   standardized residual (|2.73|) did not detect any significant relationship between each depth
325   group with clade.
326   Comparisons between clades and sclerites (Supplemental table S4) showed the majority
327   of species from clade 1 (n=13) did not possess sclerites, and the other clade 1 species contained
328   exclusively plates (n=6), or smooth rods or needles (n=4). On the other hand, clades 2, 3 and 4
329   had mostly three-flanged sclerites. There was a significant difference among sclerite shapes in
330   the rachis by clade (X^2=58.93, p = 0.0004). The standardized residual (|3.02|) test showed the
331   consistency of no sclerites and three flanged sclerites found in clade 1 and of three-flanged
332   sclerites in clade 2.
333   Furthermore, plates and smooth rods or needles were found only in the shallower group
334   (Supplemental table S5). Three-flanged sclerites, spindles and species with no sclerites were
335   observed in both depth groups. There was a significant difference between the two depth groups
336   with regards to sclerite types present (X^2=15.45, p = 0.0009). In addition, plates were
337   significantly more likely to be present in the shallower group and significantly less likely to be in
338   the deeper group based on standardized residual test with Bonferroni's correction (|2.80|). Of
339   note, when we conducted supplemental statistical analyses between clades and sclerite forms by
340   uniting plates and smooth rods or needles as "simple surface sclerites", significant relationships
341   between clades 1 and 2 – none, clade1 – simple surface sclerites, and clades 1 and 2 – three-
342   flanged sclerites were detected (|2.95|).
343
344   # Discussion
345
346   **Unexpected diversity of sea pens**
347   During this study, we examined several remarkable specimens. Among these was a specimen of
348   Virgulariidae sp. (NSMT-Co1780) that was collected off Ofunato, Iwate, Japan, at a depth of 400
349   m (Figure 2c). This specimen had thick polyp leaves and thin rod sclerites that were intermediate
350   between plates and rods in their tentacles (Fig. S1). At first, this specimen was thought to be a
351   species of *Scytalium*, which have thin polyp leaves and plate-like thin rod sclerites, however, this
352   specimen's phylogenetic position was closer to *Virgularia* species rather than to *Scytalium*
353   species. Therefore, based on its phylogenetic position, it is possible that this species belongs to
354   an undescribed genus.
355   Another Virgulariidae sp. specimen (NSMT-Co1779), collected from Tanabe Bay,
356   Wakayama, Japan, at depths between 609 and 612 m (Figure 2b), was similar to *Virgularia* and
357   *Calibelemnon* species as it did not have sclerites in its rachis and polyps. At first observation this

358   specimen appeared to belong to *Calibelemnon* (Kimura et al., 2019) as the polyps were paired
359   and there were no terminal polyps. However, this specimen was phylogenetically located as a
360   sister taxon to Virgulariidae sp. (NSMT-Co 1780), and was not close to *Calibelemnon*
361   specimens. Thus, it is possible that this specimen represents another undescribed genus within
362   Virgulariidae.
363   Finally, two specimens of an unknown taxa of Pennatulacea sp. (WAM Z44543, Z43174)
364   from the Western Australia Museum collection (Figure 4e), which were collected from sub-
365   Antarctic Candlemas Island at a depth of 185 m, were unusual, as their colony forms were
366   intermediate between *Kophobelemnon* and *Umbellula* species. However, these specimens did not
367   have axes, and had spindle-like sclerites. Based on these two specimens' phylogenetic position
368   within clade 3, it is possible that these specimens belong to an undescribed family, or alternately
369   that there is a need to reconsider the taxonomic definition of family Kophobelemnidae.
370   In the past two decades, novel sea pen genera have been reported from the Antarctica
371   region and from the deep sea (e.g. López-González and Williams, 2002, López-González et al.,
372   2022). These previous findings and our current results suggest that the diversity of sea pens
373   likely exceeds our current understanding, and that further surveys both of shallow waters
374   (Kushida and Reimer, 2019) and the deep sea are needed. Further specimen collections from a
375   wide range of locations should help to resolve knowledge gaps currently hampering sea pen
376   research.
377
378   **Phylogenetic relationships among taxa**
379   The general topology of our tree supported the results of Dolan et al. (2013), Kushida and
380   Reimer (2019), and the ML tree in García-Cárdenas et al. (2020). García-Cárdenas et al. (2020),
381   who recovered different results based on their ML and Bayesian analyses, mentioned the
382   possibility of bias from using only mtDNA sequences, and their 28S rDNA sequences appear
383   useful in making the support of clades 1 and 2 stronger, and in demonstrating the unstable status
384   of clades 3 and 4 in their Bayesian tree. However, the utility of the combination of mtMutS and
385   ND2 for genus- or family-level analyses has been supported by previous studies (e.g. McFadden
386   et al., 2006; Quattrini et al., 2020). As well, the unstable lower support values of clades 3 and 4
387   in the Bayesian tree in García-Cárdenas et al. (2020) could be related to the limited taxon
388   sampling in their study. Alternately, the lower support values of clades 3 and 4 may be caused by
389   past patterns of diversification and extinction (i.e. hard polytomy) rather than data limitations
390   (i.e. soft polytomy). Further verification via more robust next generation analyses as in Quattrini
391   et al. (2020) and McFadden et al. (2021) may help to resolve this issue.
392   So far, at least seven families of sea pens (Virgulariidae, Pennatulidae, Scleroptilidae,
393   Kophobelemnidae, Protoptilidae, Stachyptilidae, Umbellulidae) are known as polyphyletic
394   based on the molecular results of precious studies (e.g. Dolan et al. (2013) and Kushida and
395   Reimer (2019)); and the results of both of these past studies were supported by the current study.
396   The original family Umbellulidae was recently separated to two families, Umbellulidae and
397   Pseudumbellulidae (López-González et al. 2022), and the current study is the first report of

398 Pseudumbellulidae species from the northwestern Pacific Ocean (Japan), with specimens from
399 the shallowest depths reported thus far; 694–752 m (*Pseudumbellula* sp. NSMT-Co1772). The
400 genus *Kophobelemnon* may also be rendered paraphyletic if unknown Pennatulacea sp.
401 specimens (WAM Z44543, WAM Z43174) are not assigned to an expanded-concept
402 *Kophobelemnon*. The genus *Pennatula* was also recovered as polyphyletic, with species located
403 in the *Stachyptilum – Pennatula* subclade (ML/B = 100/1) and in the *Renilla – Pennatula –*
404 *Echinoptilum – Gilibelemnon* subclade (ML/B = 71/0.86) within clade 2. The genera *Ptilella* and
405 *Alloptilella* were recently resurrected and established as separate from *Pennatula* by García-
406 Cárdenas et al. (2019) and Li et al. (2021), respectively, based on the polyphyly of genus
407 *Pennatula*. As polyp leaves have appeared more than once due to convergent evolution in
408 Subselliflorae, it is not surprising that additional taxa in subclass Subselliflorae, such as
409 *Pennatula*, have turned out to be polyphyletic.
410
411 **Adaptation and evolution of sea pens across habitat depths**
412   The community structure of octocorals and sea pens is known to be strongly related to
413 bathymetry (Braga-Henriques et al., 2013; Williams, 1992; Williams, 2011). Furthermore,
414 octocorals are suggested to be distributed by their habitat depths to avoid overlap between
415 closely related taxa (Pante et al., 2012). Although habitat depth seems to play an important role
416 in the divergence and evolution of octocorals, the divergence and evolution patterns of sea pens
417 along depth have not been well studied to date (Dolan et al., 2013; Kushida and Reimer, 2019;
418 Hogan et al., 2019; García-Cárdenas et al., 2019; 2020).
419   In this study, we overlaid our observations of sclerites in the rachis from representative
420 sea pen specimens onto the phylogenetic topologies within Pennatulacea, and then observed
421 trends of adaptation and evolution of sea pens in each clade, considering colony forms, axis, and
422 sclerites. Our results reconfirmed that colony forms of sea pens have undergone convergent
423 evolution as previously suggested by Dolan et al. (2013) and Kushida and Reimer (2019). As
424 polyp leaves have evolved independently multiple times in the Subselliflorae, we hypothesize
425 that these structures allow effective feeding in the water column above soft sediments.
426 Additionally, the loss of axis has independently evolved in *Renilla* species (in clade 2),
427 *Echinoptilum* (in clade 2), and Pennatulacea sp. (in clade 3) (Figure 1).
428   Our results demonstrate that there are trends in habitat depths for sea pens in different
429 clades, and thus that the divergence of sea pens may depend at least partially on habitat depth
430 (Figure 5). Shallow water species (0–50 m) were exclusively located in clades 1 and 2 in
431 addition to a sole species of *F. quadrangularis* (Pallas, 1766) within clade 3, while deep-sea
432 species were present in all clades. As well, most mesophotic sea pens were included in clade 2.
433 Although standardized residual analyses did not detect significant relationships between each
434 depth group with clade, this may be due to the presence of only a single *Anthoptilum –*
435 *Umbellula – Calibelemnon* subclade, which includes most all deep-sea taxa, in clade 1. Thus,
436 undersampling of some habitats may still be affecting analyses. Furthermore, almost all members
437 of clade 3 and 4 were from deep sea.

438

The common ancestor of sea pens

440   *Pseudumbellula* sp. (NSMT-Co1772) had rods with spines, spindles, and three-flanged
441 rods in tentacles, and three-flanged rods and three-flanged spindles with serrated edges in the
442 rachis. The sclerite shapes of this specimen, particularly rods with spines and spindles in their
443 tentacles and the serrated edge structures in the rachis, resemble the sclerites of alcyonacean
444 species (e.g. see family Ellisellidae section in Fabricius and Alderslade, 2001). Dolan et al.
445 (2013) considered that deep-sea taxa may be the origin of sea pens. The current study
446 hypothesizes that the ancestral sea pen in the deep sea had an axis, bilateral traits, three-flanged
447 sclerites, and no calyces (see also Kushida and Reimer 2019). These characters agree well with
448 the morphology of species of Umbellulidae and/or Pseudumbellulidae. From the findings of the
449 current study, it is possible that the common ancestor of sea pens was similar to
450 Pseudumbellulidae, which is a deep-sea taxon, having sclerites intermediate in shape between
451 alcyonaceans' spindles and sea pens' three-flanged sclerites, as well as an axis, and bilateral
452 traits.
453   Interestingly, some keratoisidid sclerites resembled those of *Pseudumbellula* (both rods
454 with spines and a sclerite structure with serrated edges) (Watling and France, 2011; Dueñas et al.
455 2014). Even though Kerastoisididae is not a sister taxa of Pennatulacea, it seems these sclerite
456 shapes have evolved independently in Octocorallia (Saucier et al. 2021).

457

Evolution of sea-pen sclerites across habitat depths

459   We observed relationships of sea pen sclerite forms with clades and habitat depths, and
460 thus consider that the divergence of sclerite forms of sea pens may also depend on habitat depth
461 (Supplemental table S4, Figure 5). Sclerites shapes in the rachis are significantly diverse,
462 especially in clade 1. Species in clade 1 have smooth rods, needles, plates, or no sclerites in
463 shallower species, while the deeper species we examined lacked sclerites except for *Umbellula*
464 *thomsoni* (Kölliker, 1874), which had three-flanged sclerites. Almost all species in clades 2, 3,
465 and 4 exclusively had three-flanged sclerites. From the results of sclerites observation and
466 molecular data, we propose a possible scenario of sclerite evolution; 1) the common deep-sea
467 ancestor of sea pens included intermediate sclerites between alcyonacean spindles and sea pen
468 three-flanged sclerites (as in Figure 6), 2) sea pen sclerites became simplified by losing serrated
469 edges and three-flanged sclerites became dominant, and 3) various sclerite forms (plates, smooth
470 rods and needles, spindles and no sclerites) appeared (Figure 5). Furthermore, we confirmed the
471 characteristic evolutionary pattern of the appearance of plates and smooth rods and needles when
472 sea pens advance to shallow waters. Our study also supports the hypothesis of Quattrini et al.
473 (2020), who suggested that octocoral skeletal structures can easily adapt to their surrounding
474 environment. From the results of Quattrini et al. (2020) and this study, sea pen species that have
475 specific types of sclerites may have adapted to their surrounding environments.
476   In the current study, three-flanged sclerites were predicted to have been present in the
477 deep-sea common ancestor of sea pens, and were confirmed in species from various depths in all

478   clades. The reason why sea pens produce three-flanged sclerites remains unknown. We believe
479   there may be a relationship with at least these factors: 1) evolutionary background, and 2) water
480   temperatures. Similarly, it has previously been theorized that the surrounding environment may
481   affect the primary crystallization process that forms sclerites (Weiner and Addadi, 2011).
482   Firstly, producing three-flanged sclerites may be associated with a species evolutionary
483   background. The presence of this type of sclerite is suggested to be a relatively conservative trait
484   that originated in the deep sea (Figure 5). Thus, perhaps many sea pens produce three-flanged
485   sclerites simply as a preserved character from their evolutionary past.
486   Another possible factor may be the surrounding chemical traits of the marine
487   environment (Quattrini et al. 2021). For example, water temperatures may also be associated
488   with the process of sclerite production. From research on scleractinians, it is known that
489   biomineralization is affected by water temperatures as well as Mg/Ca ratios. When the Mg/Ca
490   ratio is low, growth rates of scleractinian skeletons are faster at high water temperatures and
491   slower at low temperatures (Higuchi et al. 2017). In the deep sea, it is known that the amounts of
492   water temperature changes are different at different depths. In particular, a thermocline were the
493   water temperature changes widely generally exists at depths from hundreds of meters to 1000 m.
494   In this study, sea pen species below 400 m that have sclerites were shown to exclusively have
495   three-flanged sclerites. Thus, sclerite shape may possibly be affected by some kind of
496   environmental restriction, and it may be that simple and robust sclerites such as smooth rods or
497   needles are comparatively difficult to produce in low temperature environments. To verify this
498   hypothesis, further molecular ecological experiments are needed.
499   In addition, almost all specimens from below 400 m in this study had three-flanged
500   sclerites, if sclerites were present in the rachis (Figure 5). Additionally, in clade 2, *Pennatula* sp.
501   (NSMT-Co1774) from mesophotic depths (77–102 m) and *Renilla* sp. from shallow water (5 m)
502   also had three-flanged sclerites. Interestingly, the three-flanged structures of these two specimens
503   were not as distinct as those of species living in deeper waters. However, in this research, we
504   could not examine specimens of sea pen genera with three-flanged sclerites that inhabit <400 m
505   in depth, such as *Actinoptilum*, *Ptilosarcus*, *Grasshoffia*, and *Acanthoptilum*. Increasing the
506   number of examined taxa will help improve our understanding of these sclerites and their
507   evolution and importance.
508   The structural forms of biominerals that result from their producing process are known
509   to help in damage tolerance in other marine invertebrates (Yang et al. 2022). In sea pens, we
510   hypothesize that three-flanged sclerites are possibly adaptations to deep-sea conditions. Our
511   observations of broken three-flanged sclerites support this hypothesis, as in such damaged
512   sclerites the main central parts were still intact and only the flanged parts were broken (Figure 7).
513   Even if sclerites are chemically vulnerable, and despite strong physical pressure, three-flanged
514   sclerites' function in structural colony support can be maintained with the loss of the flanged part
515   of a sclerite. For similar reasons, serrated edge ornamentation may also be an adapted trait to
516   protect chemically vulnerable sclerites. We observed damaged sclerites in deep-sea
517   *Pseudumbellula* specimens that had undamaged main central parts and only damaged serrated

518 edge ornamentations (Figure 7). The flanges and serrated-edges may be the sclerite parts that are
519 initially broken while protecting the main central parts and the overall sclerite function. Thus, sea
520 pens with three-flanged sclerites may be able to better withstand deep-sea environmental
521 pressures without making costly huge robust sclerites.
522   The acquisition of three-flanged sclerites may also affect sea pens' ecological traits.
523 Kushida et al. (2020a) reported on mole-like burrowing behavior and bending of *Echinoptilum*
524 sp. emerging from within sand, and these behaviors may exert physical pressure on sea pen
525 colonies. *Echinoptilum* sp. may be enabled to perform such behaviors due to the presence of
526 three-flanged sclerites (Kushida et al. 2020a). Even if there are strong physical pressures via
527 moving within the sand, the main central part of three-flanged sclerites remains.
528   In the cases of other marine animals, sponges (Porifera) and echinoderms
529 (Echinodermata) also have calcareous small elements of $CaCO_3$ (spicules) embedded within their
530 tissues. The shapes of spicules in these marine invertebrates are different from those found in sea
531 pen sclerites. However, it is known that concentrical ultrastructural layers in spicules can be
532 found across taxa (e.g. calcareous sponges and sea urchins; Sethmann and Wörheide, 2008). In
533 addition, these structures make spicules strong against fracture stress as the structure can
534 distribute physical stress and deflect propagating fractures (Sethmann and Wörheide, 2008).
535 Although our current study did not include the observation of the ultrastructure of sclerites in sea
536 pens, and thus our examinations are from an external point of view, we believe it to be possible
537 that three-flanged structures in sea pens have tolerance towards physical pressures for similar
538 reasons as observed in sponges and echinoderms.
539   In contrast, plate sclerites are suggested to be a trait adapted towards shallow waters (<
540 400 m), as plates were commonly observed in shallow water species in significantly higher
541 numbers (Supplemental table S5). As well, simple surface sclerites (plates + smooth rods or
542 needles) were also shown to be significantly more common in clade 1, which includes many
543 shallow water species, and were also seen in shallow-water *Scytalium* species in clade 2 (plates).
544 Thin plate sclerites without ornamentation may cost less energy to produce compared to three-
545 flanged sclerites (Kushida and Reimer, 2020). On the other hand, the supersaturated situation in
546 shallow water may be related to the production of robust smooth rods and needles in shallow
547 water sea pens.
548   Furthermore, sea pen species with no sclerites in the rachis are thus far only known from
549 clade 1 (Kushida and Reimer, 2020). In the taxa utilized in the molecular phylogenetic analyses,
550 the condition of having no sclerites in the rachis has been reported from *Virgularia* and
551 *Calibelemnon* species, and from almost all *Umbellula* species except *Umbellula thomsoni* (Dolan
552 (2008) and *Anthoptilum* species in clade 1 (Williams, 1995; Kushida and Reimer, 2020). Thus,
553 no sclerites is suggested to be a derived trait of sea pens. This is consistent with the suggestion of
554 Quattrini et al. (2021) that loss of sclerites is most likely a derived condition. However, some
555 genera such as *Porcupinella*, *Chunella* and *Amphiacme* in family Chunellidae that inhabit depths
556 between 1000 and 6000 m do not have sclerites (Williams, 1990; 2021). However, these taxa
557 have not yet been phylogenetically examined, and this information is needed to resolve not only

558 the evolution of sclerite loss, but also to help resolve taxonomic problems between Chunellidae
559 and Scleroptilidae (Kushida and Reimer, 2020; Williams 2021). A lack of sclerites in the rachis
560 has been observed in species living across a wide range of depths (shallow, medium deep and
561 deep). It is also known that several other octocoral taxa do not have sclerites (Fabricius and
562 Alderslade, 2001; Lau and Reimer, 2019). Similarly, *Virgularia* aff. *gustaviana*, which also has
563 no sclerites, is an ecosystem engineer forming sea pen fields with remarkably high densities
564 (Kushida et al., 2020b). By not producing sclerites and saving energy, faster growth rates may
565 increase colony numbers in sandy or muddy bottoms, although there may be higher predation
566 risks due to the lack of sclerites. We expect that sclerite types can affect a species' ecological
567 traits. Furthermore, research into sea pen species that lack sclerites may provide additional
568 information in resolving key questions of biomineralization.
569   The lack of sclerites in polyp tentacles has been observed across a wide range of sea pen
570 taxa. Thus, the presence or absence of tentacle sclerites may not be related to sea pens'
571 phylogenetic relationships or to habitat depths. However, the shapes of sclerites in tentacles are
572 considered to be related to habitat depth, as they are basically similar to the structures of sclerites
573 in the rachis.
574   Finally, sclerites embedded in sea pens are relatively more simple and smoother than
575 those observed in many other octocoral species. Among octocorals, alcyonaceans spend their life
576 attached to substrates and do not move from their habitat, and prevent predation from other
577 marine animals with their sclerites. However, many sea pens can actively move, including
578 withdrawal behavior and mole-like burrowing (in *Echinoptilum*), and thus apparently at least
579 some sea pens can escape from predators via such movements (Imahara and Ogawa, 2006;
580 Ambroso et al., 2013; Chimienti et al., 2018; Kushida and Reimer, 2020a). Therefore, sea pens
581 may not need the complex and uneven protective sclerites seen in alcyonacean species due to
582 their ability to move.
583

Conclusions
585   This study increases our knowledge on the phylogeny, adaptation, evolutionary history,
586 and diversity of sea pens. Our results suggest that sea pen diversification is related to habitat
587 depth (Figure 8), and that sclerite shape may be related to adaptation towards the surrounding
588 seawater chemical environment, and specifically to water temperatures. The common ancestor of
589 sea pens is estimated to have had three-flanged structures and lived in the deep sea. As sea pens
590 diversified into shallower water, they may have changed the characters of their sclerites;
591 obtaining plates, smooth rods or needles, or even losing sclerites. In addition, sea pen colony
592 forms have experienced convergent evolution in soft sediment environments, and the axis of
593 some sea pen species has been independently lost. Furthermore, we hypothesize that the shapes
594 of sclerites in the rachis are associated to the ecological traits of each sea pen species, such as
595 movement or the ability to form sea pen fields. Recently, the ecological importance of sea pens
596 has been recognized. For example, sea pen fields have been designated as Vulnerable Marine
597 Ecosystems (VMEs) by the Northwest Atlantic Fisheries Organization NAFO and as

598 "Threatened and/or Declining Habitat" by the Oslo and Paris Conventions (OSPAR) (e.g.
599 Kushida et al., 2020b). As the biomineralization of octocorals is an important factor in create
600 their biodiversity, understanding the mechanisms of sea pens' ecological traits, including their
601 roles as ecosystem engineers via more basic and ecological studies are urgently needed.
602   Changing ocean conditions across time likely prompted changing habitat depths in
603 various sea pen species, and these depth shifts may be related to the evolution of sclerites in sea
604 pens. To resolve this hypothesis, time divergence estimation is an effective method to trace the
605 evolution of sea pens. It is known that fossil records of sea pens are sparse and almost
606 completely consist of axes (Reich et al., 2011). Furthermore, the first confirmed fossil record of a
607 sea pen is from the Cretaceous (70–83 mya; Anderson, 1979; Reich et al., 2011). Interestingly, a
608 divergence time calculation of sea pens studied based on mtMutS, COI, and 28SrDNA sequences
609 by García-Cárdenas et al. (2020) estimated the origin of sea pens to be in the lower Cretaceous
610 (~144 Ma). However, this estimate is far more recent than robust estimates by Quattrini et al.
611 (2020) and McFadden et al. (2021), who suggested 300 Ma ~ 400 Ma with time-calibrated
612 phylogenetic trees from hundreds of ultra-conserved elements and exon loci. Combining the
613 morphological data of sea pens with genome-wide phylogenetic analyses is a logical next step in
614 understanding the evolution of sea pen forms.
615

Acknowledgements

617 The authors thank all researchers and the crew of the T/RV *Seisui-maru* (Mie University) cruise
618 1903, all researchers and crew of the R/V *Kaimei*, and operators of the KM-ROV during the
619 KM19-05C cruise, staff of Okinawa Churaumi Aquarium (Okinawa Churashima Foundation),
620 Honmei Kishimoto, captain of the *Daini-Kuroshio-Maru*, and the Motobu Fisheries Cooperative,
621 all staff of Shimoda Marine Research Center (University of Tsukuba), Amakusa Marine
622 Biological Laboratory (Kyushu University), Dr. Hiroki Kise (National Institute of Advanced
623 Industrial Science and Technology) and Dr. Naoto Jimi (Nagoya University) for specimen
624 collection, all technical staff in Vigo University (Spain) for sequencing, Dr. Euichi Hirose
625 (University of the Ryukyus) for access to SEM. We also thank Dr. Claudio Vasapollo for editing
626 this manuscript, and the reviews of Dr. Les Watling and two anonymous reviewers that greatly
627 improved this manuscript.

628

References

630 **Ambroso S, Dominguez-Carrió C, Grinyó J, López-González PJ, Gili JM, Purroy A,**
631   **Requena S, Madurell T. 2013.** In situ observations on withdrawal behaviour of the sea
632   pen *Virgularia mirabilis*. *Marine Biodiversity* 43: 257–258.
633   https://doi.org/10.1007/s12526-013-0172-5.
634 **Bayer FM, Grasshoff M, Verseveldt J. 1983.** *Illustrated trilingual glossary of morphological*
635   *and anatomical terms applied to Octocorallia.* Leiden: E. J. Brill.

636 **Braga-Henriques A, Porteiro FM, Ribeiro PA, Matos VD, Sampaio Í, Ocaña O, Santos RS.**
637   **2013.** Diversity, distribution and spatial structure of the cold-water coral fauna of the
638   Azores (NE Atlantic). *Biogeosciences* 10: 4009–4036

639 **Chimienti G, Angeletti L, Mastrototaro F. 2018.** Withdrawal behaviour of the red sea pen
640   *Pennatula rubra* (Cnidaria: Pennatulacea). *The European Zoological Journal* 85: 64–
641   70. https://doi.org/10.1080/24750263.2018.1438530.

642 **Conci N, Vargas S, Woerheide G. 2021.** The biology and evolution of calcite and aragonite
643   mineralization in Octocorallia. *Frontiers in Ecology and Evolution* 9: 623774.
644   https://doi.org/10.3389/fevo.2021.623774

645 **Dolan E. 2008.** Phylogenetics, systematics and biogeography of deep-sea Pennatulacea
646   (Anthozoa: Octocorallia): evidence from molecules and morphology. Doctoral
647   dissertation, University of Southampton.

648 **Dolan E, Tyler PA, Yesson C, Rogers AD. 2013.** Phylogeny and systematics of deep-sea sea
649   pens (Anthozoa: Octocorallia: Pennatulacea). *Molecular Phylogenetics and Evolution*
650   69: 610–618. https://doi.org/10.1016/j.ympev.2013.07.018.

651 **Dueñas, LF, Alderslade P, Sánchez JA. 2014.** Molecular systematics of the deep-sea bamboo
652   corals (Octocorallia: Isididae: Keratoisidinae) from New Zealand with descriptions of
653   two new species of *Keratoisis*. *Molecular Phylogenetics and Evolution*, 74: 15–28.
654   https://doi.org/10.1016/j.ympev.2014.01.031

655 **Farrell JW, Prell WL. 1991.** Pacific $CaCO_3$ preservation and $\delta^{18}O$ since 4 Ma: paleoceanic and
656   paleoclimatic implications. *Paleoceanography* 6: 485–498.
657   https://doi.org/10.1029/91PA00877

658 **Fabricius KK, Alderslade PP. 2001.** *Soft corals and sea fans: a comprehensive guide to the*
659   *tropical shallow water genera of the central-west Pacific, the Indian Ocean and the Red*
660   *Sea.* Townsville: Australian Institute of Marine Science (AIMS),.

661 **García-Cárdenas FJ, Drewery J, López-González PJ. 2019.** Resurrection of the sea pen genus
662   *Ptilella* Gray, 1870 and description of *Ptilella grayi* n. sp. from the NE Atlantic
663   (Octocorallia: Pennatulacea). *scientia marina* 83: 261–276.
664   https://doi.org/10.3989/scimar.04845.26A.

665 **García-Cárdenas FJ, Núñez-Flores M, López-González PJ. 2020.** Molecular phylogeny and
666   divergence time estimates in pennatulaceans (Cnidaria: Octocorallia: Pennatulacea).
667   *scientia marina* 84: 317–330. https://doi.org/10.3989/scimar.05067.28A.

668 **Greene SE, Ridgwell A, Kirtland Turner S, Schmidt DN, Pälike H, Thomas E, Greene L K,**
669   **Hoogakker BAA. 2019.** Early Cenozoic decoupling of climate and carbonate
670   compensation depth trends. *Paleoceanography and Paleoclimatology* 34: 930–945.
671   https://doi.org/10.1029/2019PA003601

672 **Hickson SJ. 1916.** The Pennatulacea of the Siboga Expedition: with a general survey of the
673   order. *Siboga Expedition Monographs.* 14: i=x+1–265, pls. 1=10, 1 chart.

674 **Higuchi T, Shirai K, Mezaki T, Yuyama I. 2017.** Temperature dependence of aragonite and
675   calcite skeleton formation by a scleractinian coral in low mMg/Ca seawater. *Geology*
676   45: 1087–1090. https://doi.org/10.1130/G39516.1
677 **Hogan RI, Hopkins K, Wheeler AJ, Allcock AL, Yesson C. 2019.** Novel diversity in
678   mitochondrial genomes of deep-sea Pennatulacea (Cnidaria: Anthozoa: Octocorallia).
679   30: 764–777. https://doi.org/10.1080/24701394.2019.1634699.
680 **Huelsenbeck JP, Ronquist F. 2001.** MRBAYES: Bayesian inference of phylogenetic trees.
681   *Bioinformatics* 17: 754–755.
682   http://146.6.100.192/users/CH391L/Handouts/MRBAYES.pdf
683 **Imahara Y. Ogawa K. 2006.** Rediscovery of *Virgularia juncea* (Octocorallia, Pennatulacea)
684   from a tidal marsh in Okinawa, with a short note on its peculiar behavior. *Proceedings*
685   *of the 10th International Coral Reef Symposium*. 1: 9–13.
686 **Imahara Y, Iwase F, Namikawa H. 2014.** *The Octocorals of Sagami Bay.* Kanagawa: Tokai
687   University Press.
688 **Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A,**
689   **Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A. 2012.**
690   Geneious Basic: an integrated and extendable desktop software platform for the
691   organization and analysis of sequence data. *Bioinformatics* 28: 1647–1649.
692   https://doi.org/10.1093/bioinformatics/bts199.
693 **Kimura T, Kimura S, Kakui K, Hookabe N, Kuramochi T, Fujita T, Ogawa A, Kobayashi**
694   **I, Jimi N, Okanishi M, Yamaguchi H, Hirose M, Yoshikawa A, Fukuchi J,**
695   **Shimomura M, Kashio S, Uyeno D, Fujiwara K, Naruse T, Kushida Y, Kise H,**
696   **Maekawa Y, Nakamura T, Okumura J, Tanaka K. 2019.** Benthic deep-sea fauna in
697   south of the Kii Strait and the Sea of Kumano, Japan. Second report. *Annals of Field*
698   *Research and Technology Mie University* 17: 1–29.
699 **Knoll AH. 2003.** Biomineralization and evolutionary history. *Reviews in Mineralogy and*
700   *Geochemistry* 54: 329–356.
701 **Kölliker RA von. 1869.** Anatomisch-systematische Beschreibung der Alcyonarian. Erste
702   Abtheilung: Die Pennatuliden. Abhandlungen von der Senckenbergischen naturf
703   orschenden. *Gesellschaft* 7: 109-256, pls. 1-10.
704 **Kölliker RA von. 1870.** Anatomisch-systematische Beschreibung der Alcyonarian. *Erste*
705   *Abtheilung: Die Pennatuliden. 7. Gattung: Halisceptrum, Herklots. Abhandlungen von*
706   *der Senckenbergischen naturf orschenden. Gesellschaft* 7: 487-602, pls. 1-17.
707 **Kölliker RA von. 1872.** Anatomisch-systematische Beschreibung der alcyonarian. *Erste*
708   *Abtheilung: Die Pennatuliden. Abhandlungen der Senckenbergischen Naturforschenden*
709   *Gesellschaft*, 8: 85-275, pos. 1-24.
710 **Kölliker RA von. 1875.** Die Pennatulide *Umbellula* und zwei neue Typen der Alcyonarien.
711   *Festschrift zur Feier des fünfundzawinzigjährigen Bestehens der physicalisch-*
712   *medicinsichen Gesellschaft in Würzburg* 1-23pp, pls. 1, 2.

713 **Kölliker RA von. 1880.** Report on the Pennatulida, dredged by H. M. S. *Challenger* during the
714   years 1873-1876. *Report on the Scientific Results of the Exploring Voyage of H.M.S.*
715   *Challenger* 1873–76, Zoology, 1, 1-41, pls. 1-11.

716 **Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018.** MEGA X: Molecular Evolutionary
717   Genetics Analysis across computing platforms. Molecular Biology and Evolution. 35:
718   1547–1549. https://doi.org/10.1093/molbev/msy096.

719 **Kushida Y, Reimer JD. 2019.** Molecular phylogeny and diversity of sea pens (Cnidaria:
720   Octocorallia: Pennatulacea) with a focus on shallow water species of the northwestern
721   Pacific Ocean. *Molecular Phylogenetics and Evolution*131: 233–244.
722   https://doi.org/10.1016/j.ympev.2018.10.032.

723 **Kushida Y, Reimer JD. 2020.** Description of the sea pen *Calibelemnon hinoenma* sp. nov. from
724   shallow waters in southern Japan. *Marine Biodiversity* 50: 1–10.
725   https://doi.org/10.1007/s12526-020-01132-1.

726 **Kushida Y, Higashiji T, Reimer JD. 2020a**. First observation of mole-like burrowing behavior
727   observed in a sea pen. *Marine Biodiversity.* 50: 29. https://doi.org/10.1007/s12526-020-
728   01054-y.

729 **Kushida Y, Kise H, McFadden CS, Reimer JD. 2020b.** A sea pen field in shallow water in the
730   Amakusa Islands, southern Japan. *Plankton and Benthos Research* 15: 259–268.
731   https://doi.org/10.3800/pbr.15.259.

732 **Kükenthal W. 1915.** Pennatularia. Das Tierreich. 43: 1–132. Verlag von R. Friedländer und
733   Sohn, Berlin.

734 **Kükenthal W, Broch H. 1911.** Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition
735   auf dem Dampfer "Valdivia" 1898–1899. 13, Lieferung. 2, 113–576.

736 **Lau YW, Reimer JD. 2019.** Zooxanthellate, sclerite-free, and pseudopinnuled octocoral
737   *Hadaka nudidomus* gen. nov. et sp. nov. (Anthozoa, Octocorallia) from mesophotic
738   reefs of the southern Ryukyus Islands. *Diversity* 11: 176.
739   https://doi.org/10.3390/d11100176.

740 **Lewis JC, von Wallis E. 1991.** The function of surface sclerites in gorgonians (Coelenterata,
741   Octocorallia). Biology Bulletin 183: 275–288. https://doi.org/10.2307/1542099.

742 **López-González PJ, Williams GC. 2002.** A new genus and species of sea pen (Octocorallia:
743   Pennatulacea: Stachyptilidae) from the Antarctic Peninsula. Invertebrate Systematics
744   16: 919–929. https://doi.org/10.1071/IT01027.

745 **López-González, PJ, Drewery J, Collins A. 2022.** When distant relatives look too alike: a new
746   family, two new genera and a new species of deep-sea *Umbellula*-like sea pens
747   (Anthozoa, Octocorallia, Pennatulacea). *Invertebrate Systematics*, 36: 199–225.

748 **Maddison WP, Maddison DR. 2019.** Mesquite: a modular system for evolutionary analysis.
749   Version 3.61. https://www.mesquiteproject.org

750 **McFadden CS, France SC, Sánchez JA, Aderslade P. 2006.** A molecular phylogenetic
751   analysis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-

752   coding sequences. *Molecular Phylogenetics and Evolution* 41: 513–527.
753   https://doi.org/10.1016/j.ympev.2006.06.010.
754 **McFadden CS, Brown AS, Brayton C, Hunt CB, van Ofwegen LP. 2014.** Application of
755   DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies
756   agree with morphological estimates of species richness in Palau. *Coral. Reefs* 33: 275–
757   286. https://doi.org/10.1007/s00338-013-1123-0.
758 **McFadden CS, Quattrini AM, Brugler MR, Cowman PF, Dueñas LF, Kitahara MV, Paz-**
759   **García DA, Reimer JD, Rodríguez E. 2021.** Phylogenomics, Origin, and
760   Diversification of Anthozoans (Phylum Cnidaria). *Systematic Biology* 70: 635–647.
761   https://doi.org/10.1093/sysbio/syaa103.
762 **Pante E, France SC, Couloux A, Cruaud C, McFadden CS, Samadi S, Watling L. 2012.**
763   Deep-sea origin and in-situ diversification of chrysogorgiid octocorals. *PLoS ONE* 7:
764   e38357. https://doi.org/10.1371/journal.pone.0038357.
765 **Pasternak FA. 1989.** On the variability of sea-pens (Octocorallia: Pennatulacea) connected with
766   the transition to the deep-water existence. Trudy Instituta Okeanologii, Akademii Nauk
767   SSSR 124: 165–173. [in Russian with English Summary]
768 **Pytkowicz RM. 1970.** On the carbonate compensation depth in the Pacific Ocean. *Geochimica*
769   *et Cosmochimica Acta* 34: 836–839.
770 **Quattrini AM, Rodríguez E, Faircloth BC, Cowman PF, Brugler MR, Farfan GA, Hellberg**
771   **ME, Kitahara MV, Morrison CL, Paz-García DA, Reimer JD, McFadden CS.**
772   **2020.** Palaeoclimate ocean conditions shaped the evolution of corals and their skeletons
773   through deep time. *Nature Ecology & Evolution* 4: 1531–1538.
774   https://doi.org/10.1038/s41559-020-01291-1.
775 **Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L,**
776   **Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: Efficient Bayesian phylogenetic
777   inference and model choice across a large model space. *Systematic Biology* 61: 539–
778   542. https://doi.org/10.1093/sysbio/sys029.
779 **R Core Team. 2021.** R: A Language and Environment for Statistical Computing. R Foundation
780   for Statistical Computing. Vienna, Austria. https://www.R-project.org (accessed 1 June
781   2021)
782 **RStudio Team. 2021.** RStudio: Integrated Development Environment for R; RStudio, PBC:
783   Boston, MA, USA.
784 **Saucier EH, France SC, Watling L. 2021.** Toward a revision of the bamboo corals: Part 3,
785   deconstructing the Family Isididae. *Zootaxa*, 5047: 247–272.
786   https://doi.org/10.11646/zootaxa.5047.3.2
787 **Sánchez JA, McFadden CS, France SC, Lasker HR. 2003.** Molecular phylogenetic analyses
788   of shallow-water Caribbean octocorals. *Marine Biology* 142: 975–987.
789   https://doi.org/10.1007/s00227-003-1018-7.

790   **Sánchez JA, González-Zapata FL, Prada C, Dueñas LF. 2021.** Mesophotic gorgonian corals
791   evolved multiple times and faster than deep and shallow lineages. *Diversity* 13: 650.
792   https://doi.org/10.3390/d13120650
793   **Sethmann I, Wörheide G. 2008.** Structure and composition of calcareous sponge spicules: a
794   review and comparison to structurally related biominerals. *Micron* 39: 209–228.
795   https://doi.org/10.1016/j.micron.2007.01.006.
796   **Watling L, France SC. 2011.** A new genus and species of bamboo coral (Octocorallia: Isididae:
797   Keratoisidinae) from the New England seamounts. *Bulletin of the Peabody Museum of*
798   *Natural History* 52: 209–220. https://doi.org/10.3374/014.052.0202
799   **Weiner S, Addadi L. 2011.** Crystallization pathways in biomineralization. *The Annual Review*
800   *of Materials Research* 41: 21–40. https://doi.org/10.1146/annurev-matsci-062910-
801   095803
802   **West JM, Harvell CD, Walls AM. 1993.** Morphological plasticity in a gorgonian coral
803   (*Briareum asbestinum*) over a depth cline. *Marine Ecology-Progress Series* 94: 61–69.
804   **West JM. 1998.** Plasticity in the sclerites of a gorgonian coral: tests of water motion, light level,
805   and damage cues. *Biology Bulletin* 192: 279–289. https://doi.org/10.2307/1542721.
806   **Williams GC. 1990.** The Pennatulacea of southern Africa (Coelenterata, Anthozoa). *Annals of*
807   *the South African Museum* 99(4): 31–119.
808   **Williams GC. 1992.** Biogeography of the octocorallian coelenterate fauna of southern Africa.
809   *Biological Journal of the Linnean Society* 46: 51–401. https://doi.org/10.1111/j.1095-
810   8312.1992.tb00869.x.
811   **Williams GC. 1995.** Living genera of sea pens (Coelenterata: Octocorallia: Pennatulacea):
812   illustrated key and synopses. *Zoological Journal of the Linnean Society* 113: 93–140.
813   https://doi.org/10.1111/j.1096-3642.1995.tb00929.x.
814   **Williams GC. 2011.** The global diversity of sea pens (Cnidaria: Octocorallia: Pennatulacea).
815   *PLoS ONE* 6, e22747. https://doi.org/10.1371/journal.pone.0022747.
816   **Williams GC. 2021.** The deep-sea pennatulacean genus *Porcupinella* – with the description of a
817   new species from Tasmania (Anthozoa, Octocorallia, Chunellidae). *ZooKeys* 1019: 1–
818   14. https://doi.org/10.3897/zookeys. 1019.61789.
819   **Williams GC, Hoeksema BW, van Ofwegen LP. 2012.** A fifth morphological polyp in
820   pennatulacean octocorals, with a review of polyp polymorphism in the genera
821   *Pennatula* and *Pteroeides* (Anthozoa: Pennatulidae). Zoological Studies 51, 1006–1017.
822   **Yang T, Chen H, Jia Z, Deng Z, Chen L, Peterman EM, Weaver JC, Li L. 2022.** A damage-
823   tolerant, dual-scale, single-crystalline microlattice in the knobby starfish, *Protoreaster*
824   *nodosus*. *Science* 375: 647–652. https://doi.org/10.1126/science.abj9472.
825

Figure 1

COI+mtMutS+ND2 phylogenetic tree constructed by ML method.

The numbers on each branch indicate the ML/B = bootstrap values/posterior probabilities. The black circles on each node indicate the ML/B = 100/1.00. Each color indicates each family; light blue: Virgulariidae, red: Pennatulidae, green: Kophobelemnidae, yellow: Veretillidae, black: Anthoptilidae, dark navy: Umbellulidae, orange: Scleroptilidae, blue green: Protoptilidae, yellow green: Stachyptilidae, pink: Renillidae, light purple: Echinoptilidae, brown: Funiculinidae, Dark purple: Pseudumbellulidae, light blue green: Halipteridae, blue: Pennatulacea sp. The bars at the right side show clades. The small boxes on branches indicate events estimated by maximum parsimony methods; yellow: loss of axis, red: polyp leaves or raised ridges present. Scale indicates substitutions per site.

CLADE

71/1
Virgularia sp. YK86
Virgularia cf. *gustaviana* YK210
Virgularia gustaviana YK220
Virgulariidae sp. YK317
Polyp leaves present
62/0.83
68/0.77
Virgulariidae sp. YK323
99/1
Pteroeides sp. YK245
Pteroeides caledonicum YK216
Pteroeides caledonicum YK215
99/1
Sclerobelemnon theseus
Sclerobelemnon sp. YK283
94/0.99
Cavernulina sp. YK102
Cavernulina sp. YK100
Veretillum sp. YK96
Sclerobelemnon sp. YK282
72/0.82
62/0.85
Cavernularia sp. YK300
Veretillum sp. YK248
85/1
Anthoptilum sp. 1
Anthoptilum grandiflorum
52/0.80
79/0.99
76/1
Umbellula thomsoni
Umbellula huxleyi
Umbellula carpenteri
91/1
Umbellula magniflora
80/1
98/0.99
Umbellula sp.1
58/0.77
Umbellula encrinus
Calibelemnon hinoenma YK340
Calibelemnon hinoenma YK341

Clade 1

Polyp leaves present
Stylatula elongata
91/1
Protoptilum carpenteri YK316
98/1
Protoptilum carpenteri
Protoptilum sp.
97/1
Stachyptilum dofleini YK53
Polyp leaves present
Pennatula cf. *fimbriata* YK292
Loss of axis
Renilla muelleri
Renilla sp. YK166
57/-
91/0.99
Renilla reniformis YK167
80/1
96/1
Pennatula phosphorea
Pennatula aculeata
Polyp leaves present
Pennatula murrayi
97/1
Pennatula sp. YK88
Pennatula sp. YK89
98/1
Loss of axis
Echinoptilum sp. YK22
93/1
71/0.86
Echinoptilum sp. YKOCA325
Echinoptilum sp. YK281
Polyp leaves present
Gilibelemnon octodentatum
Ptilella grandis
Ptilella grayi
Polyp leaves present
Scytalium martensii YK250
Scytalium sp. YK271
99/1
Distichoptilum gracile
Distichoptilum gracile
Distichoptilum sp. YK321

Clade 2

Polyp leaves present
Gyrophyllum sp.
Gyrophyllum sp.
96/1
Funiculina quadrangularis
Funiculina armata
Loss of axis
Pennatulacea sp. WAM78
Pennatulacea sp.WAM23
99/1
Kophobelemnon macrospinum
Kophobelemnon pauciflorum
64/0.95
Kophobelemnon sp. 3
77/0.93
Kophobelemnon sp. YK263
87/1
Kophobelemnon sp. 1
55/0.67
98/1
Kophobelemnon sp. YK298
62/0.93
Kophobelemnon sp. YK319
Kophobelemnon sp. YK318
90/0.96
Kophobelemnon sp. 4
88/1
Pseudumbellula sp. YK320
Pseudumbellula sp. 2
86/1
Solumbellula monocephalus
Raised ridges present
Halipteris finmarchica
Scleroptilum grandiflorum

Clade 3

Clade 4

Viminella sp. YK104
98/1
Junceella sp. YK136
Ellisella sp. YK122

Outgroup

0.02

Figure 2

Specimens in clade 1.

a: *Virgularia* cf. *gustaviana* (NSMT-Co 1776), 9 m in depth, b: Virgulariidae sp. (NSMT-Co 1779) 609-612 m, c: Virgulariidae sp. (NSMT-Co 1780) 400 m, d: *Pteroeides caledonicum* (NSMT-Co1773), 10 m, e: *Sclerobelemnon* sp. (NSMT-Co 1765), 207 m, f: *Cavernulina* sp. (NSMT-Co1763), 20 m, g: *Veretillum* sp. (NSMT-Co1762), 17 m, h: *Calibelemnon hinoenma* (NSMT-Co 1709), 32 m. Scale bars = 1 cm. a: living specimen b-h: preserved specimens in 99.5% ethanol.
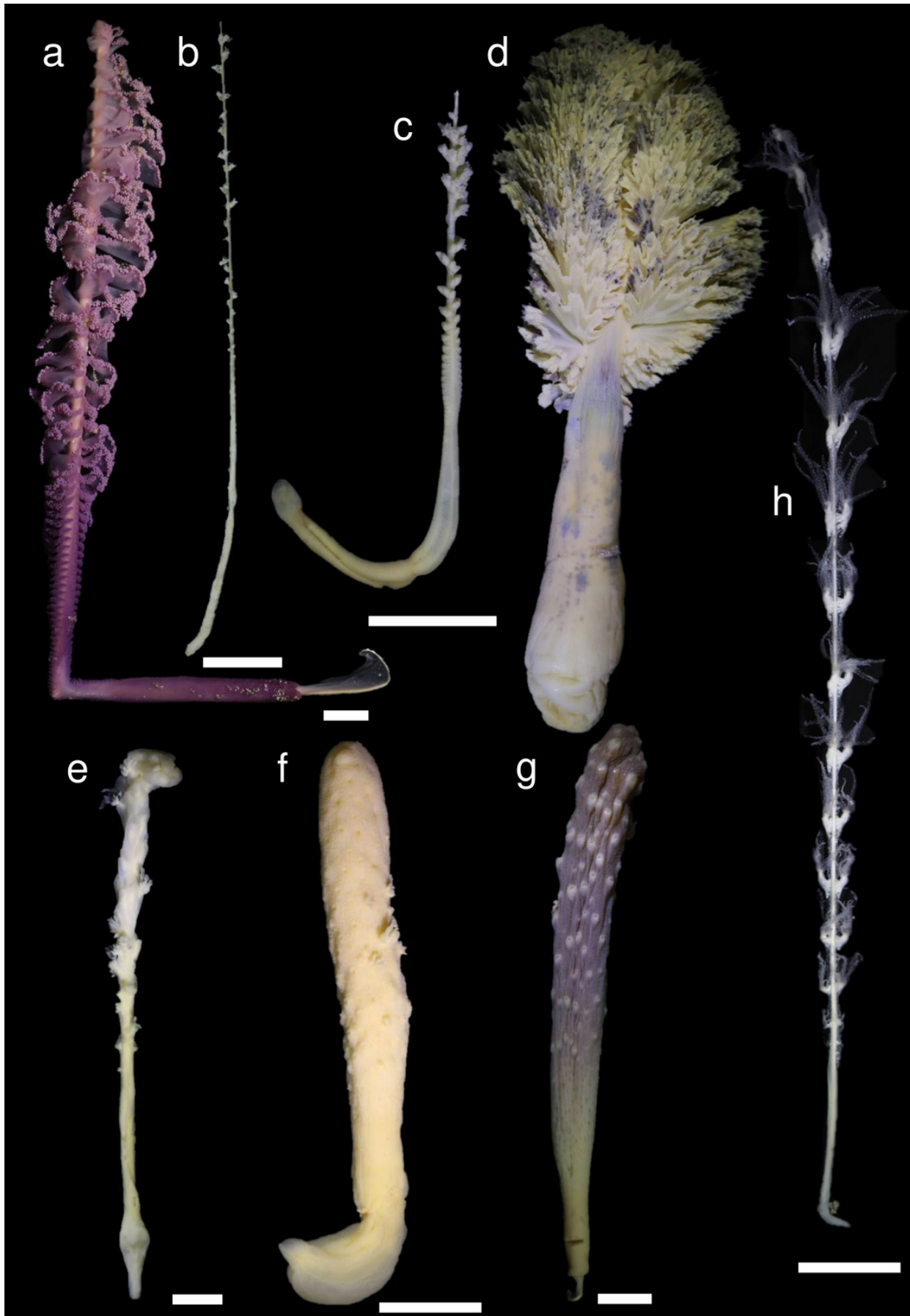
Figure 3

Specimens in clade 2.

a: *Protoptilum carpenteri* (NSMT-Co1771), 609-612 m in depth, b: *Stachyptilum dofleini* (NSMT-Co1769), 95-187 m, c: *Echinoptilum* sp. (NSMT-Co1764), 118 m, d: *Distichoptilum* sp. (NSMT-Co1770), 694-752 m, e: *Renilla reniformis* (NSMT-Co1760), 5 m, f: *Pennatula* sp. (NSMT-Co1774), 77-102 m, g: *Pennatula* cf. *fimbriata* (YK292), 190 m, h: *Scytalium* sp. (NSMT-Co1778), 22 m. Scale bars = 1 cm. All preserved specimens in 99.5% ethanol.
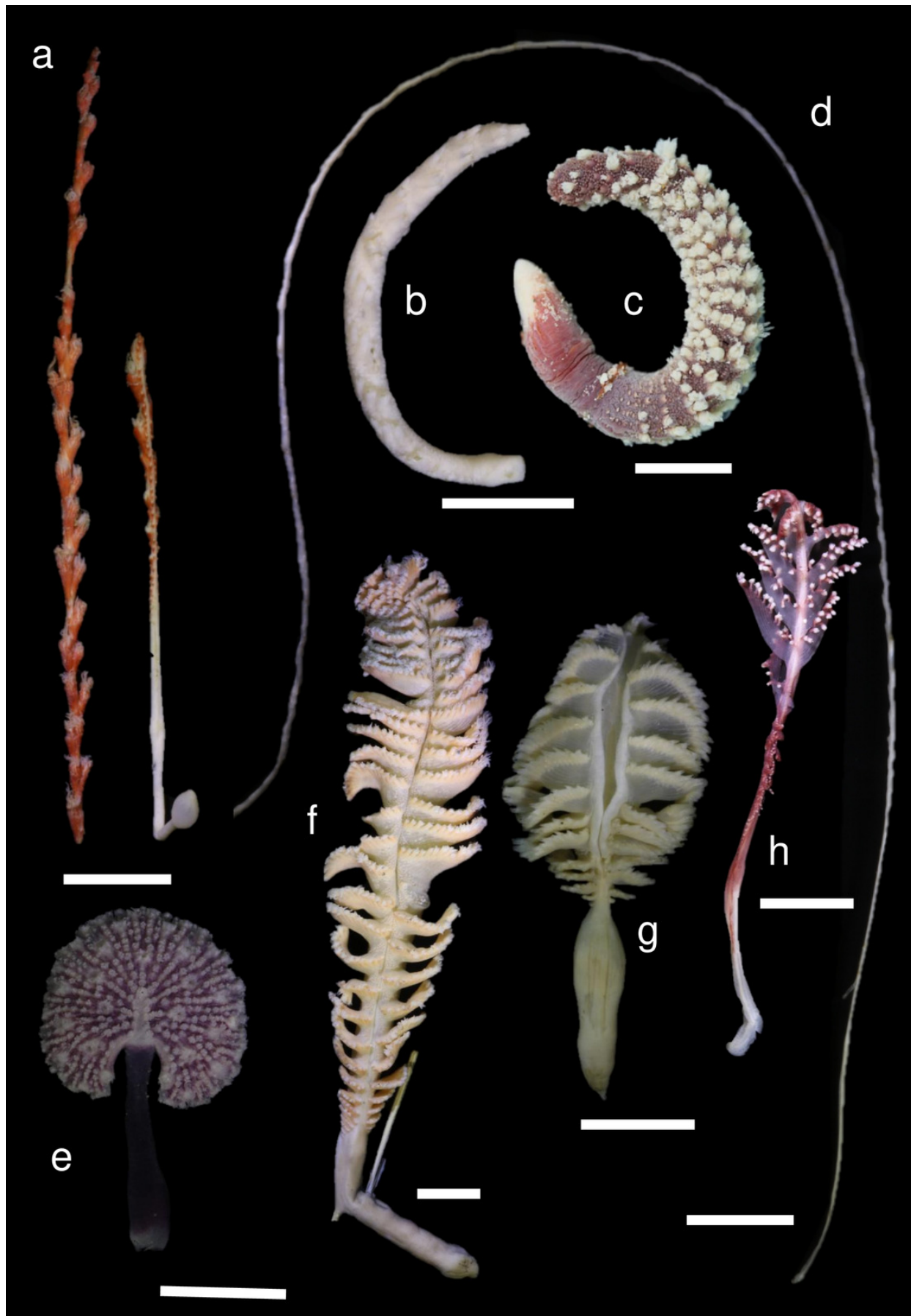
Figure 4

Specimens in a-e: clades 3 and f: clade 4.

a: *Kophobelemnon* sp. (NSMT-Co 1768), 694-752 m in depth, b: *Kophobelemnon* sp. (NSMT-Co 1767), 694-752 m, c: *Kophobelemnon* sp. (NSMT-Co1766), 400-450 m, d: *Kophobelemnon* sp. (YK263), 729-793 m, e: Pennatulacea sp. (WAMZ44543), 185 m, f: *Pseudumbellula* sp. (NSMT-Co1772), 694-752 m. Scale bars = 1 cm. All preserved specimens in 99.5% ethanol.
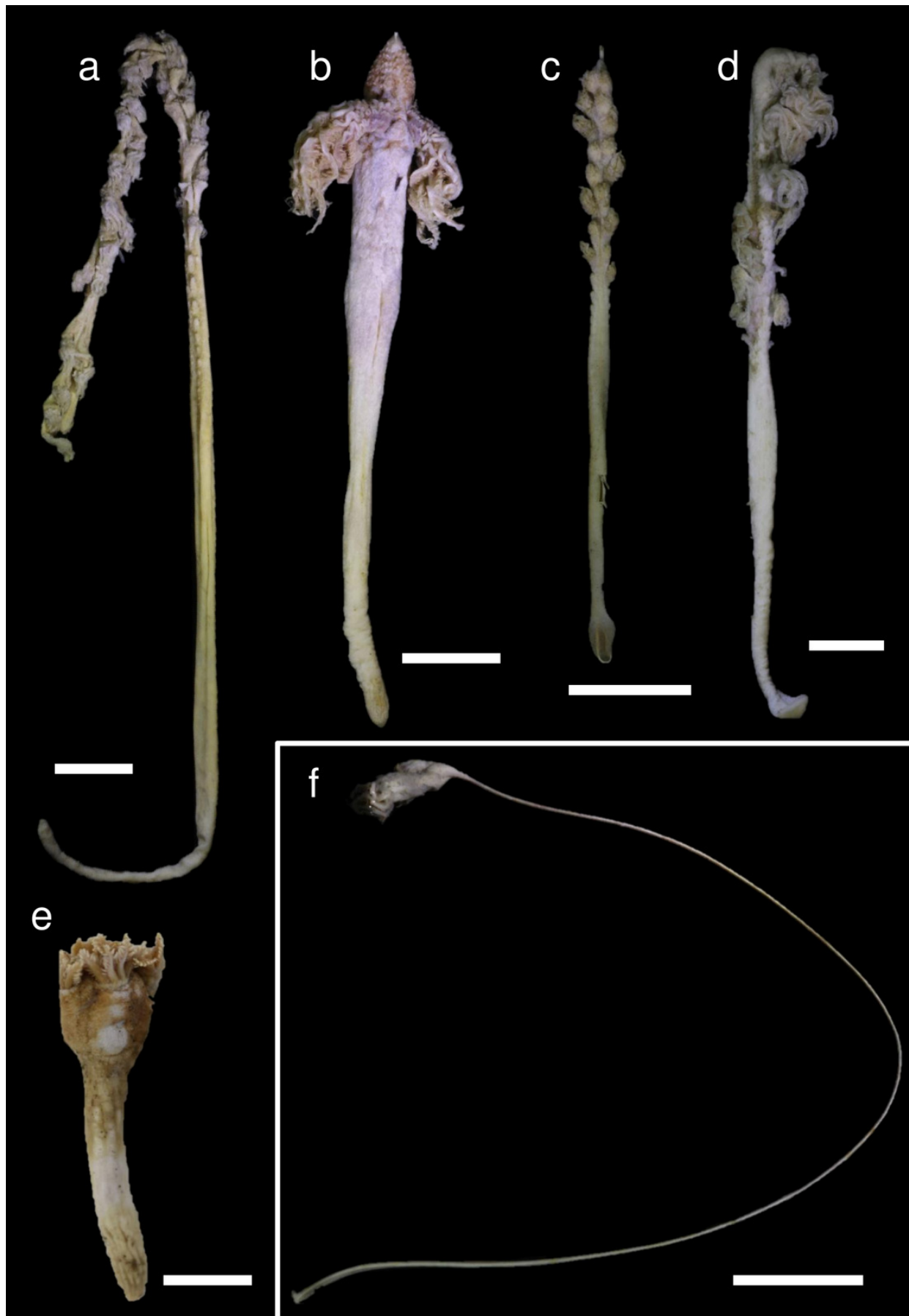
Figure 5

Ancestral state reconstruction for habitat depth by ML method.

Monochrome circle color in the left tree indicates each depth group; white: the shallower group (0–400 m), black: the deeper group (400–6260 m) and each box's color each depth categories; yellow: shallow (0–50 m), red: mesophotic (50–200 m), green: shallower deep (200–400 m), blue: medium deep (400–2000 m), and black: deep (2000–6260 m). Circle colors in the right tree indicate each shape of sclerites in the rachis; gray: none, yellow: plates, red: smooth rods or needles, blue: three-flanged sclerites, yellow green: spindles. Each number in a circle indicates each clade.
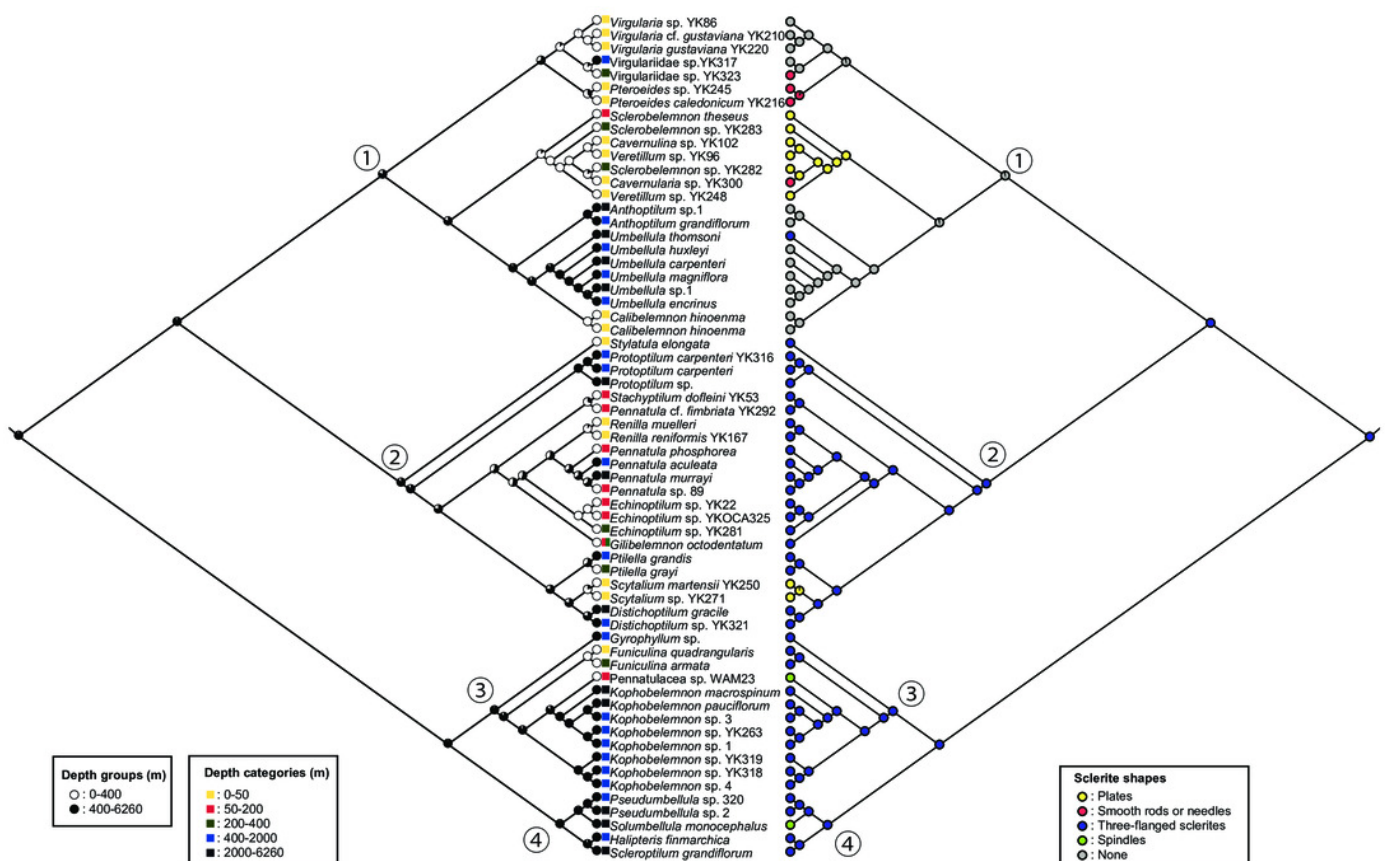
Figure 6

Sclerites in clade 4 (single specimen *Pseudumbellula* sp. (NSMT-Co1772)).

Abbreviations; Ten= tentacles, Ra= rachis. Scale bars = Ten: 200µm, Rachis = 100µm.
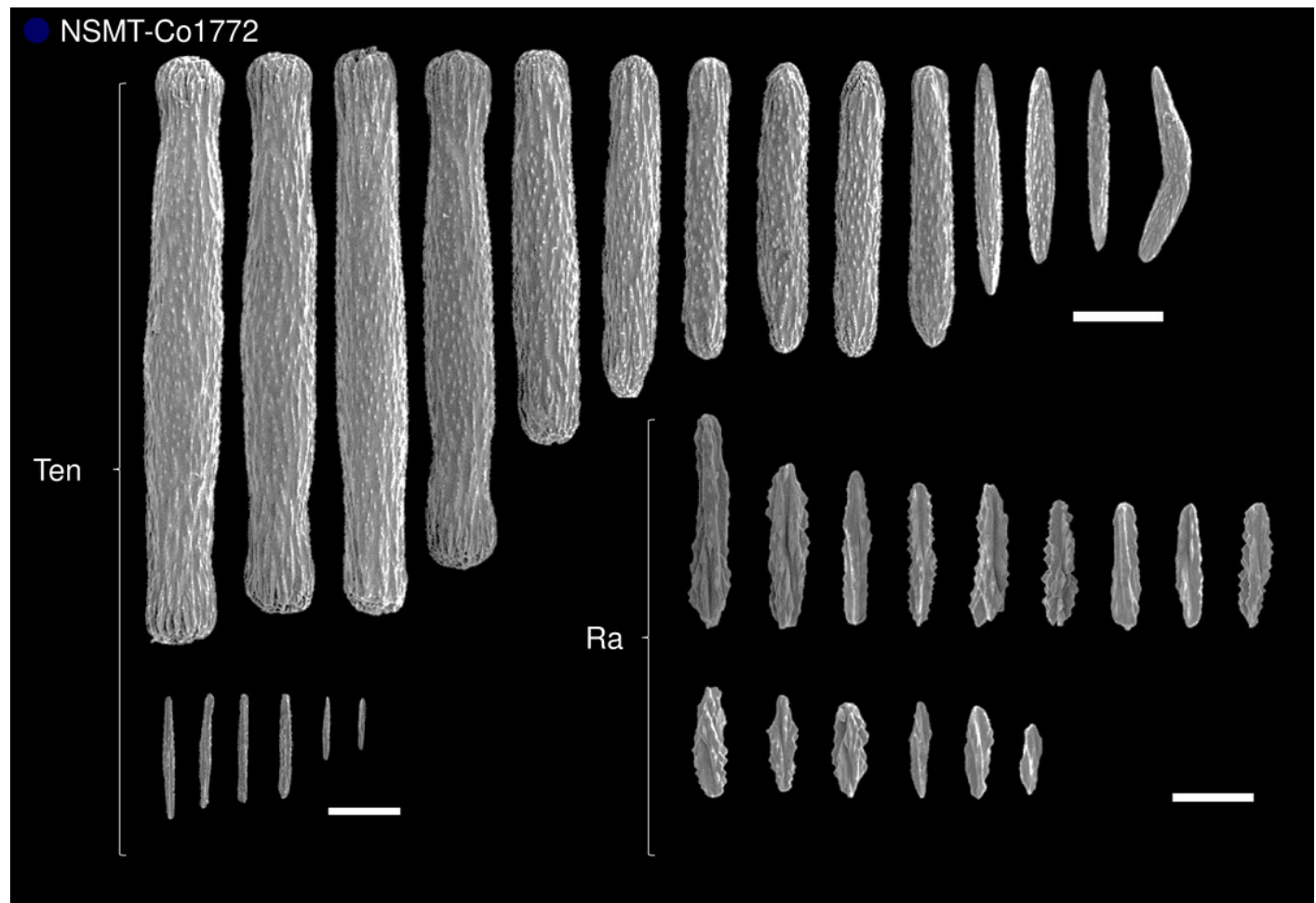
Figure 7

Broken three-flanged sclerites of different clades.

a: NSMT-Co1774 in clade 2, polyp leaves, b: NSMT-Co1772 in clade 4, rachis. Scale bar = 100 μm.
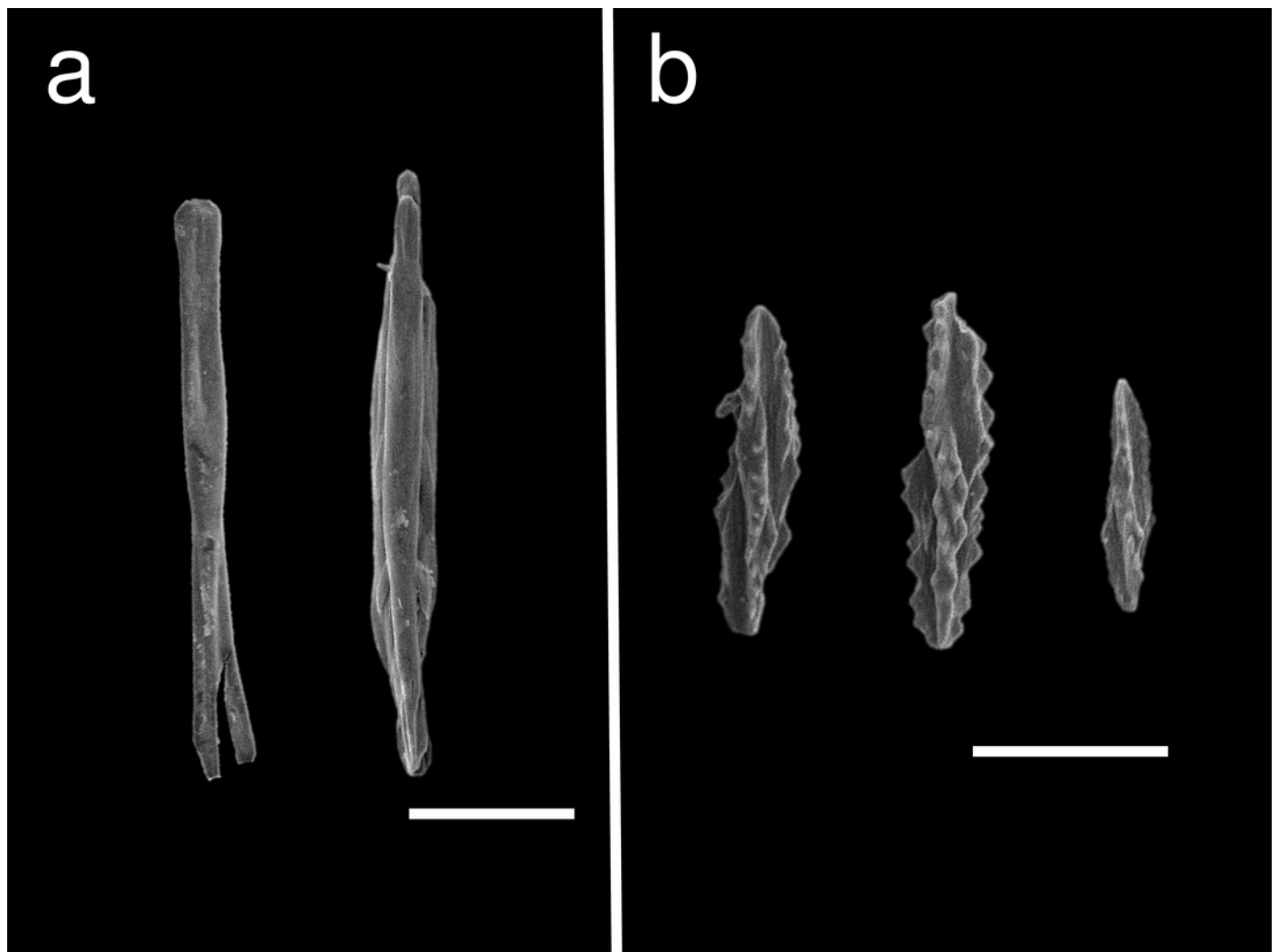
Figure 8

Summary of this study.

This study demonstrates that the diversification in order Pennatulacea is related to habitat depths and suggests sclerite shape may be affected by the surrounding environmental factors such as water temperature. Sclerites in each box indicate the representative sclerite shapes. Bar on the left indicates metalimnions of water temperature (℃).Bar on the right indicates depth (m).