UNIVERSITY OF SOUTHAMPTON

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Phylogenetics, Systematics and Biogeography of Deep-Sea Pennatulacea (Anthozoa: Octocorallia)

Evidence from molecules and morphology

Submitted by

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I hereby declare that no part of this thesis has been submitted for a degree to the University of Southampton, or any other University, at any time previously. The material included is the work of the author, except where expressly stated.

30 September 2008, Emily Dolan

University of Southampton

Abstract

Faculty of Engineering, Science and Mathematics
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PHYLOGENETICS, SYSTEMATICS AND BIOGEOGRAPHY OF DEEP-SEA PENNATULACEA (ANTHOZOA: OCTOCORALLIA): EVIDENCE FROM MOLECULES AND MORPHOLOGY

by Emily Dolan

Despite its extreme environmental conditions, the deep sea harbours a unique and species-rich fauna of mostly unknown age and phylogeny. Pennatulids (Anthozoa: Octocorallia) are a group whose taxonomy and phylogenetic relationships remain poorly known and little studied, in spite of their abundance and ecological importance in soft-bottom communities. Phylogenetic analysis of a combination of partial *ND2* and *msh1* sequences produced well-supported phylogenetic relationships for representative deep-sea (and shallow-water) pennatulids at familial, generic and specific taxonomic levels. Generally, molecular data were congruent with current classification and previous phylogenetic reconstructions of the O. Pennatulacea based on morphology. Discrepancies were evident concerning the finer details for some families and genera: this can be attributable to the high frequency of homoplasy in pennatulids where reversals in evolution have led to taxa that possess apomorphic character states that are analogous with plesiomorphic traits. Genetic analysis gave strong support that highly-derived taxa occur in both shallow and deep water and that many may have differentiated and dispersed from the deep sea to the shallows. The Renillidae, which is considered one of the most primitive shallow-water families, evolved recently from deep-water ancestors. Conversely, the bathyal Anthoptilidae was the most primitive of families, and although more evidence is required, pennatulids as a group may have originated in deep water.

The systematics of the exclusively deep-sea genus *Umbellula*, which contains forty-two species, remains unclear despite the repeated attempts of revision. Incorporating new morphological and distributional data from the examination of recently collected material, together with type specimens, genetic analysis, and a critical study of the literature, fifteen *Umbellula* species are here considered valid, including three new to science. Eight species lack sclerites in the autozooids, *U. magniflora*, *U. encrinus*, *U. antarctica*, *U. carpenteri* and *Umbellula* sp.1 n. sp. (quadrangular axes), and *U. huxleyi* and *U. pellucida* (round axes); and seven possess autozooid sclerites, *U. thomsoni* and *U. hemigymna* (quadrangular axes), and *U. monocephalus*, *U. aciculifera*, *U. durissima*, *Umbellula* sp.2 n. sp. and *Umbellula* sp.3 n. sp. (round axes).

Biogeographic data and genetic evidence supported the hypothesis that species of *Umbellula* differentiated in the Indo-Pacific. Many radiated southwards to the Antarctic and later north into the Atlantic, E Pacific, Indian and Arctic oceans, occupying bathyal and abyssal depths. Other, older species that evolved via a separate evolutionary pathway, may have originated in the Indo-Pacific, and dispersed to the Subantarctic (*U.* sp.2 n. sp.) or Indian and Atlantic oceans (*U. monocephalus*). Further, morphological examination of *Umbellula* showed it adapted to the oligotrophic conditions of the deep sea by reducing the number but increasing the size of the autozooids, and in doing so, enlarged the food-catchment area; abyssal species have done so even more extremely.

Acknowledgements

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It has been a difficult journey at times, and I couldn't have done it without those close to me. So, thank you to my Mum and Stu; you were a tower of support and were with me every step of the way. Thanks to my brother, Simon, for all your encouragement, and for putting a roof over my head when I worked in London! Many thanks to my Dad and Linda, Melanie and Andrew, you always believed in me. My friends, Clara and Lev, Fifi, and Nelia; you all kept my spirits high through good times and bad. Een grote "dank u wel", Marijke and Ivan, for your care and support.

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“From the time of Pliny humans believed that there was no life in the deep. It took the historic expedition in the ship [HMS] *Challenger* between 1872 and 1876 to prove Pliny wrong; its deep-sea dredges and trawls brought up living things from all depths that could be reached. Yet even in the twentieth century, scientists continued to imagine that life at great depth was insubstantial, or somehow inconsequential. The eternal dark, the almost inconceivable pressure, and the extreme cold that exist below one thousand meters were, they thought, so forbidding as to have all but extinguished life. The reverse is in fact true...[Below 200 meters] lies the largest habitat on earth...

...Perhaps one-day man will be more like aqua man, and roam the ocean depths with the fish creatures alike...”

(Flannery, 2007)

Preface

Situated beyond the continental shelves is the deep-sea floor, which comprises 50 % of the surface of the Earth. This vast environment therefore, could be considered the most typical on the planet, and its inhabitants as typical life forms (Gage and Tyler, 1991). However, because of its remoteness and difficulties in observing and sampling fauna, very little is known about the biology of the deep sea. Indeed, it was once believed that the realms beyond 600 m depth were entirely devoid of life. Through pioneering oceanographic voyages in the nineteenth and twentieth centuries, together with modern research techniques and expeditions, the deep sea is now believed to be the most biologically diverse ecosystem on Earth (Grassle and Maciolek, 1992). Some of the most striking revelations in the recent history of deep-sea biology were the discovery of luxuriant animal communities at deep-sea hydrothermal vents and cold seeps (Corliss and Ballard, 1977), and the extensive distribution of deep-sea coral reef ecosystems (Veron, 1995). The history of deep-sea research is well documented (Le Danois, 1948; Menzies et al., 1973; Mills, 1983; Gage and Tyler, 1991; Van Dover, 2000), and thus this topic shall not be reiterated here.

Commonly known as sea pens, pennatulids are colonial anthozoans belonging to the suborder Octocorallia. Although they are eurybathic (intertidal to >6000 m), pennatulids most often inhabit bathyal and abyssal depths or cold waters (Kükenthal, 1915; Rice et al., 1992; Keller and Pasternak, 2001), where they sometimes form dense aggregations (Langton et al., 1990). Indeed, pennatulids are often abundant megafaunal filter feeders in the deep sea (Tyler, 2003), and perhaps form the most diverse cnidarian group here. Pennatulids show a high proportion of cosmopolites, and occupy extensive regions of the seafloor from the tropics to polar regions. One factor driving this is their ability to exploit soft or unstable substrata, giving them a huge advantage over other octocorals, which require hard substrata for their attachment. Sediments prevail on the shelf, slope, bases of seamounts, and abyssal plains, and thus, by their very nature, it is clear why pennatulids contribute significantly to deep-sea ecosystems.

In addition to exerting a major influence on benthic community structure, pennatulids are also likely to be among taxa that are especially vulnerable to trawling impact of commercial fishing. A study on benthic invertebrate by-catch from a deep-water trawl fishery working in the Chatham Rise area, New Zealand, found that pennatulids comprised 12.2 % of taxa recorded in tows from the flat areas of the seamount. However, this figure may be a gross underestimation of the number of pennatulids that are actually affected by trawling: their ability to anchor themselves in the sediment means pennatulids often avoid capture, but many species are inflexible and therefore it is highly likely that trawling could damage, or even break, the axis of such colonies. Populations of these fragile, long-lived animals may nearly be exterminated by a single passage of a trawl, and perhaps are unable to recover quickly. Trawling other deep-water biodiversity hotspots, such as canyons (Morais et al., 2007), may also have serious repercussions on pennatulid populations.

Despite their ecological importance and vulnerability, pennatulids are a poorly known and little studied group (Williams, 1995b), and only a fraction of research has been conducted on those that inhabit the deep sea. These studies are restricted to the ecology and reproduction of *Pennatula aculeata* (Langton et al., 1990; Eckelbarger et al., 1998); investigations on aspects of ecology and distribution of the genera *Kophobelemnon* and *Umbellula* in the NE Atlantic (Rice et al., 1992; Tyler et al., 1995); and distribution of pennatulid species in southern Africa (Williams, 1990; Williams, 1992a) and Brazil (Castro and de Medeiros, 2001). Williams (1992b; 1995c; 1997b) initiated modern systematic and biogeographic analyses, mainly focusing on shallow-water species, which provide a firm foundation for research into their deep-water counterparts. No studies have been conducted on the evolutionary history of deep-sea pennatulids, and our understanding of species diversity and distribution of important deep-sea genera, such as *Umbellula*, is meagre.

Accurate classification systems are crucial in the field of deep-sea biology, not only because they provide the means to identify species, but also because they provide a framework around which fauna can be studied; systematic biology, which uses evolutionary relationships to understand biogeography and adaptation, is linked inextricably with conservation (Dimmick et al., 1999). In this context, this thesis presents the first phylogenetic and systematic study of deep-sea pennatulids, and a reassessment

of the classification of the genus *Umbellula* together with a biogeographical and morphological approach to its origins and adaptations, respectively.

Chapter One gives an outline of the general background that the remainder of this thesis will draw upon. Starting with phylum Cnidaria and working towards order Pennatulacea, it provides an overview to the morphology, classification, and systematics of pennatulids; and identifies gaps in our current understanding. The finer aspects of pennatulid systematics and classification, and details on phylogeny, and biogeography are introduced at the beginning of chapters two, three and four, together with further details on research aims. Chapter Two examines the systematic and phylogenetic relationships among pennatulid families inferred from molecular data, with special emphasis on the evolutionary history of deep-sea pennatulids, and a reassessment of aspects of their classification. Chapter Three presents a taxonomic revision of the exclusively deep-sea genus *Umbellula*, and includes three species new to science. This research incorporates new morphological and distributional data from the examination of recently collected material, together with type specimens, and additional geographical data from the literature, plus a study of the phylogenetic relationships among *Umbellula* species with reference to morphological traits. In Chapter Four, the biogeography of *Umbellula* is evaluated. Further, an analysis of morphological variability of species of *Umbellula* with depth is addressed in the final section of this chapter. Chapter Five presents the conclusions of this study by summarising the outcomes of the research and pointing the direction of future work arising from this project.

Chapter One

General Background

1.1 Systematics of Octocorallia (Cnidaria: Anthozoa) and General Morphological Structure

1.1.1 Phylum Cnidaria

Cnidarians are among the most primitive eumetazoans, and their divergence from other animals must have occurred in the Precambrian. The phylum Cnidaria contains fauna found exclusively in aquatic, mostly marine, environments, and includes the familiar hydras, jellyfish, sea anemones, sea pens, and hard and soft corals. The unifying characteristic of the cnidarians is the possession of nematocytes, specialised stinging cells that carry structures called nematocysts (Ruppert and Barnes, 1994). Symmetry in cnidarians is radial, and in combination with the vibrant colours displayed in many species, these animals are often incredibly striking (Fig 1.1).

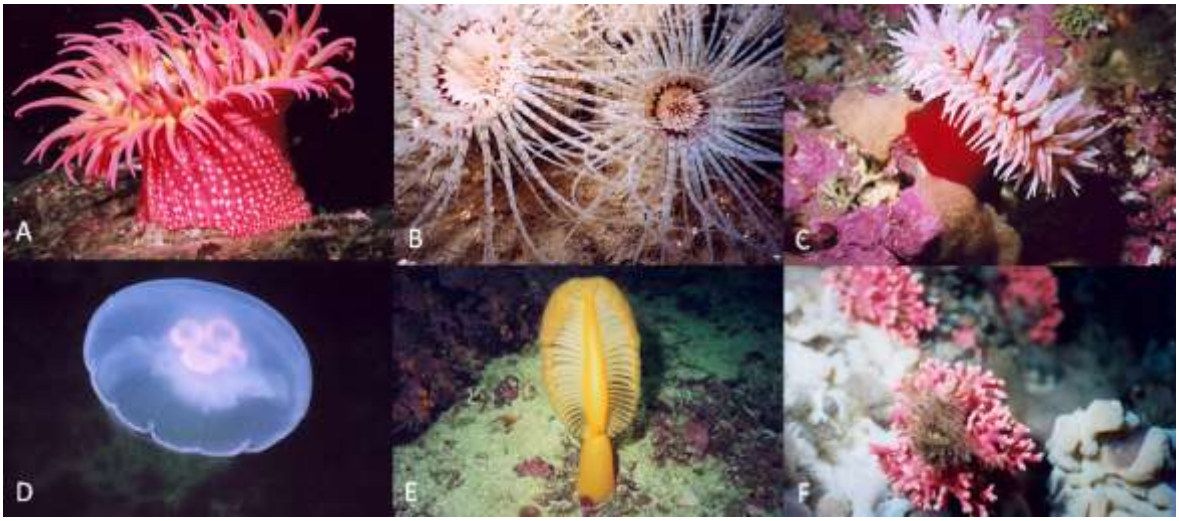

Figure 1.1 Cnidarian exemplars: A White spotted anemone, *Urticina lofotensis* (Hexacorallia: Actinaria); B Tube dwelling anemones, *Pachycerianthus fimbriatus* (Hexacorallia: Ceriantharia); C Fish eating anemone, *Urticina piscivora* (Hexacorallia: Actinaria); D Moon jelly, *Aurelia labiata* (Scyphozoa: Semaeostomeae); E Orange sea pen, *Ptilosarcus guerneyi* (Octocorallia: Pennatulacea); F Pink hydrocoral, *Stylaster* sp. (Hydrozoa: Anthoathecatae) (Photographs by Janna Nichols © 2007).

Cnidarians possess a gut cavity lined by endoderm, termed the gastrovascular cavity, or coelenteron, which functions not only for digestion, but also in circulation. The gastrovascular cavity is aligned with the long-axis of the animal and has only one opening, which functions as both the mouth and the anus. A circle of tentacles, representing evaginations of the body wall, surrounds the mouth to aid the capture and ingestion of food. The body wall consists of two types of tissue, the outer epidermis bearing sensory cells and nematocysts, and the inner gastrodermis responsible for digestion and reproduction. Between these two layers lies the mesoglea, which may be a thin basal laminar, or a thick acellular or cellular connective tissue (Ruppert and Barnes, 1994).

Six classes of Cnidaria are recognised: Anthozoa, Cubozoa, Hydrozoa, Scyphozoa, Stauromedusae, and the extinct Conulata. However, in a revision of the phylum, Petersen (1979) raised Anthozoa from its previous status as a Class to a subphylum[1], and in doing so, a new subphylum was also proposed, Medusozoa, to include the remaining cnidarian classes. With only a few exceptions, Medusozoa have two forms in their life history: a free-swimming medusa that reproduces sexually; and a sessile polyp that generally propagates by asexual reproduction. Anthozoans are exclusively marine; its members are either colonial or solitary, and in contrast to the Medusozoa, the free-swimming stage is completely absent.

1.1.2 Class Anthozoa

Two anatomically related structures characterise anthozoans, the actinopharynx and the mesenteries (Fig. 1.2), which are unique among cnidarians polyps (Fautin and Mariscal, 1991). The actinopharynx is a tubular gullet extending from the mouth some distance to the coelenteron. A densely ciliated groove located on the internal surface of the actinopharynx, the siphonoglyph, is responsible for driving water into the coelenteron. Within the coelenteron, a canal system transports the water to the rest of the colony, functioning for both respiratory purposes and inflating the polyp by hydrostatic pressure. In pennatulids, the single siphonoglyph is exceptionally well developed in the siphonozooids (see Section 1.2.1.2).

[1] Concerning systematic classification, many texts do not recognise Anthozoa as a subphylum; thus, for reasons of wide usage, Anthozoa is herein considered a Class.

The mesenteries are longitudinal sheets of tissue that partition the coelenteron into a series of chambers, extending radially from the body wall; some reach all the way to the actinopharynx (complete mesenteries). Below the actinopharynx the inner edge of the mesenteries are free, forming convoluted mesenteric filaments provided with cilia, gland cells, and nematocysts. Longitudinal retractor muscles of the mesenteries allow the polyp to retract in many species (Manuel, 1988; Fautin and Mariscal, 1991).

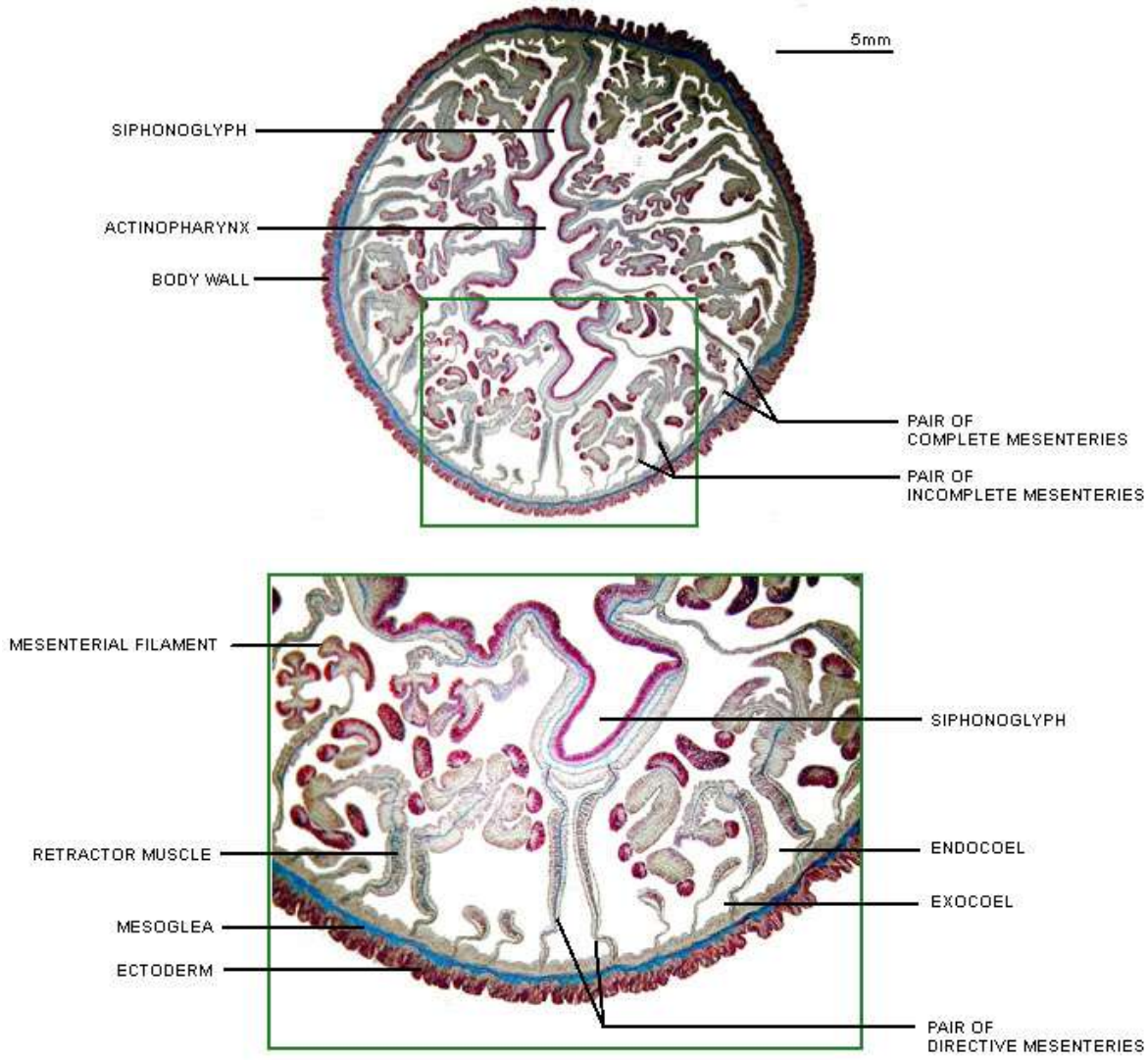

Figure 1.2 Cross-section of an anthozoan, the sea anemone *Megalactic* sp., at the level of the actinopharynx (http://www.tolweb.org/Anthozoa, © Adorian Ardelean).

Molecular sequence data (France et al., 1996; Berntson et al., 1999) support the division of Anthozoa into two Subclasses, Octocorallia and Hexacorallia, which are externally distinguished by the number and form of the polyp tentacles (Hyman, 1940). Octocorals are always colonial, usually composed of small polyps that are uniform and relatively simple in structure. Each polyp has eight pinnately branched tentacles that surround the

mouth, and eight radially arranged mesenteries dividing the coelenteron. In contrast, the polyps of hexacorals are usually larger, and have a higher degree of structural complexity, than those of octocorals; and tentacles are never pinnate. Despite being named Hexacorallia, almost no members have six tentacles. The number of tentacles and mesenteries a single polyp possesses can vary between groups from six to numerous, although never eight, an exclusive trait of Octocorallia (Manuel, 1988).

The divisions within Anthozoa have a complex history, in terms of their classification. Hyman (1940) partitioned the Class into Alcyonaria (Octocorallia) and Zoantharia (Hexacorallia), based largely on polyp symmetry, and tentacle form and number as outlined above. Wells and Hill (1956) recognised a third Subclass, Ceriantipatharia (also listed in Dunn, 1982), based on similarity of the ceriantharian larval stage to the antipatharian polyp, and various other morphological affinities between the two groups. Yet, differences in gross morphology (Hand, 1966), nematocysts (Hand, 1966; Schmidt, 1974), and evidence from DNA (France et al., 1996; Song and Won, 1997; Berntson et al., 1999) do not support the monophyly of the Ceriantharia and Antipatharia. Indeed, four divisions were proposed by Hand (1966) (Antipatharia, Ceriantharia, Zoantharia, and Alcyonaria) based on morphology, and while the most complete molecular data available (Berntson et al., 1999) do not support the monophyly of the Ceriantharia and Antipatharia, placement of the Ceriantharia remains uncertain. The Ceriantharia may merit subclass status, but more data are necessary to determine the phylogenetic position of cerianthids (Berntson et al., 1999). Following the suggestions of Hyman (1940), Antipatharia and Ceriantharia are widely considered as orders of the Subclass Zoantharia (Hexacorallia).

1.1.3 Subclass Octocorallia

1.1.3.1 Classification and General Morphology of Octocorallia

Forming a well-defined morphologic group, octocorals share several uniting characteristics: tentacle number and structure, the number and structure of the mesenteries, and all are colonial. Within the octocorals, three morphologically distinct orders are defined, Alcyonacea (soft corals and sea fans), Helioporacea (blue corals), and

Pennatulacea (sea pens). Although there is some genetic evidence to suggest otherwise (Berntson et al., 2001, see Section 2.1, Chapter Two), these orders clearly delineate as three separate, natural groups (McFadden et al., 2006). The age of octocorals, and time of evolutionary separation between the three orders is unknown, since the skeletal components (sclerites, Fig. 1.3) of pennatulids and alcyoniids are small and quickly wash away after the colony dies (Fabricius and Alderslade, 2001), and thus fossil records are poor (Bayer, 1956).

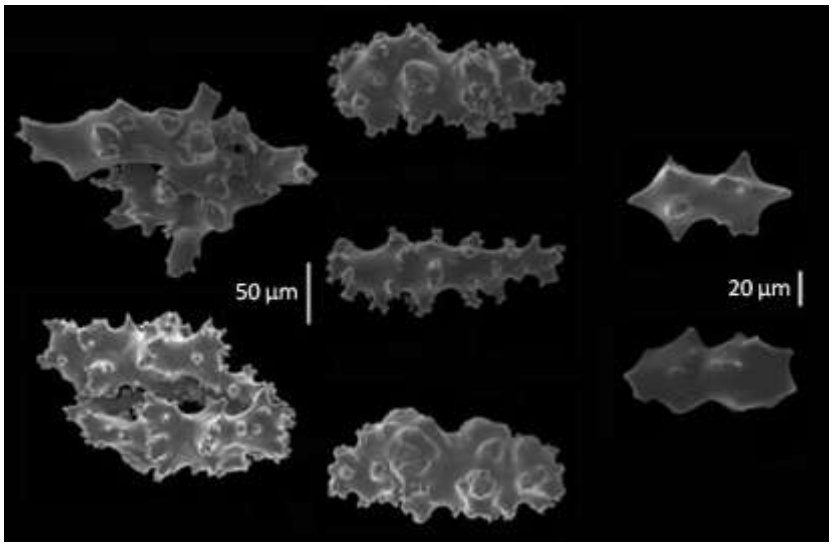

Figure 1.3 Sclerites of the soft coral *Telesto fruticulosa* (Adapted from SERTC http://www.dnr.sc.gov/marine/sertc/octocoral%20guide/Telesto_fruticulosa.htm).

The blue coral, *Heliopora coerulea*, is one of the most isolated of living animals; it is the only known species of its genus, and the only member of the family Helioporidae. Helioporid is the only octocoral that forms a massive aragonite skeleton, like hard corals, or fire corals, and thus is more readily preserved: this living relic has fossil relatives known from more than 100 million years ago (Gregory, 1899; Fabricius and Alderslade, 2001).

Alcyoniids and pennatulids do not produce calcium carbonate skeletons; instead, they contain minute, spiny skeletal elements called sclerites. Aside from their taxonomic utility in species identification, sclerites provide these corals with some degree of support and give their flesh a spiky, grainy texture that may also function to deter predators (Fig. 1.3). Pennatulids distinguish themselves from alcyoniids by their large, central, primary polyp, which is supported internally by a calcareous axis. Also unique to the pennatulids is the peduncle, a muscular 'foot' that digs into sand or mud, anchoring the colony in the soft substratum (see Section 1.2.1 for more detail on pennatulid morphology).

In contrast to pennatulids, alcyoniids require a hard substratum on which to attach. The system of classifying alcyoniids has a complex history (Bayer, 1981). The past has seen families separated into seven orders: Protoalcyonaria, Stolonifera, Telestacea, Gastraxonia, Xeniacea, Alcyonaria, and Gorgonacea. However, over the years, as previously described species were examined in more detail, and as new species were discovered, it became increasingly clear that intermediate forms between these groups prevented the definition of any clear boundaries. Bayer (1981) recognised that species form a complete series from simple soft corals to complex gorgonians (sea fans), and thus adopted the single Order, Alcyonacea. Within the alcyoniids, two groups of gorgonians, the Holoxonia and the Calcaxonia, differ in axis construction where no intermediate forms have yet been found (Fabricius and Alderslade, 2001).

1.1.3.2 Taxonomy and Systematics of Octocorallia

Octocorals are considered a group with a complex taxonomy, and its classification has posed countless problems in the past and present day (for example Kölliker, 1880; Danielssen and Koren, 1884; Jungersen, 1904; Thomson and Ritchie, 1906; Kükenthal and Broch, 1911; Broch, 1913; Thomson, 1915; Hickson, 1916; Kükenthal, 1919; Madsen, 1944; Bayer, 1956; Broch, 1958; Tixier-Durivault, 1964; Tixier-Durivault and D'Hondt, 1974; Bayer, 1981; Grasshoff, 1981; Williams, 1995b; Williams and Alderslade, 1999). One factor responsible for the difficulty in classifying the group is the high level of morphological variability among species; and the paucity of taxonomic characters together with poor, often conflicting species descriptions, have led to the misclassification of many taxa. Thus, taxonomists have unjustifiably split or grouped families, genera or species.

Prior to the late twentieth century, Kükenthal and Broch (1911), Kükenthal (1915), and Hickson (1916) represented the major monographic works on pennatulid systematics, and Kükenthal (1919) and Aurivillius (1931) on alcyoniids. However, modern systematics on octocorals has stressed the need for a review of the taxonomy of the group at family and genus levels (Bayer, 1956; Williams, 1990; Berntson et al., 1999; Berntson et al., 2001; Sánchez, 2001; Lopez-Gonzalez and Williams, 2002; Sánchez et al., 2003a; McFadden et

al., 2006). A series of workshops[2], focusing on octocoral taxonomy, aimed at addressing the problems incurred in the classification of the group. The major outcomes of the first of these (1981) was to standardise the use of taxonomic terms from historic literature, often written in German and French, to the English language, thus to facilitate proper, precise usage among octocoral scientists. The terms were complied to create the 'Illustrated Trilingual Glossary of Morphological and Anatomical Terms Applied to Octocorallia' (Bayer et al., 1983). This was later modified after discussions in a second workshop (2002), in order to alter terms inaccurately defined, inadequately coined or obsolete, and to add terms taken from new publications. A third octocoral workshop (2003) emphasised the need to develop collaborative efforts between taxonomists and molecular systematists, and work towards understanding and applying molecular phylogenetics to octocoral classification.

To date, molecular studies of octocorals have addressed a variety of questions at different systematic levels, generally starting with well-established taxa. They range from population studies carried out at species and genus levels (McFadden, 1999; Song and Lee, 2000; McFadden and Hutchinson, 2004) to broader surveys of family-level systematics and phylogeny on selected sections of octocorals (Berntson et al., 2001; France and Hoover, 2001; France and Hoover, 2002; Sánchez et al., 2003a; b). Only phylogenetic and taxonomic studies based on morphological characters have been made pertaining to pennatulids (Williams, 1989; 1992b; 1995a; c; d; 1997a; b; Lopez-Gonzalez et al., 2000; 2001; Pérez and Ocampo, 2001; Lopez-Gonzalez and Williams, 2002).

Until recent years, mitochondrial (*16s* gene) and nuclear (*18s* gene) molecular markers developed for octocoral studies were only useful for resolving genus- and family-level relationships among octocorals, and could not resolve deeper (Subordinal or ordinal) or shallower (intrageneric) relationships because of high degrees of gene conservation (France et al., 1996; Berntson et al., 2001). However, the development of markers for the mitochondrial protein-coding genes, *msh1*, and NADH-dehydrogenase subunits 2 (*ND2*) and 6 (*ND6*) (France and Hoover, 2001; McFadden et al., 2004) have unveiled better-resolved phylogenies within the octocorals (Sánchez et al., 2003b; McFadden et al., 2006). Moreover, this breakthrough in octocoral research will allow scientists, both systematists

[2] http://www.calacademy.org/research/izg/orc_home.html

and taxonomists alike, to resolve questions concerning classification and evolutionary history of families, genera, and possibly species within the group.

1.2 Order Pennatulacea (Anthozoa: Octocorallia)

1.2.1 Biology of Pennatulacea

The gross structure of pennatulids was relatively well documented following the pioneering oceanographic voyages in the nineteenth and early twentieth centuries (Kölliker, 1870; Lindahl, 1874; Kölliker, 1880; Danielssen and Koren, 1884; Marshall, 1887; Jungersen, 1904; Kükenthal, 1915; Hickson, 1916; 1937). Modern histological techniques have provided further information on the internal anatomy and gametogenic biology of the deep-water species *Kophobelemnon stelliferum* (Rice et al., 1992), *Umbellula* sp. (Tyler et al., 1995), and *Pennatula aculeata* (Eckelbarger et al., 1998); and the shallower-water species *Ptilosarcus guerneyi* (Chia and Crawford, 1973), *Virgularia juncea* (Soong, 2005), and *Pennatula phosphorea* (Edwards and Moore, 2008).

1.2.1.1 Gross Structure

The pennatulids are the most advanced of octocorals in terms of their colonial complexity, functional specialisation of polyps, and colonial integration (Hickson, 1909; Bayer, 1956; 1973; Brusca and Brusca, 2003), and indeed, are one of the most spectacular forms of sessile megabenthos found in the marine environment (Fig.s 1.4; 1.5). Uniquely, mature colonies develop from a single large, elongated primary polyp, the oozooid, which extends the length of the colony forming a central axis. Also exclusive to the pennatulids is the character of a muscular peduncle, located at the most proximal portion of the oozooid. The peduncle may be expanded or deflated by peristaltic contractions, and functions to anchor the colony into soft substrata such as sand, mud, or abyssal ooze. The distal region of the primary polyp, the rachis, gives rise to dimorphic secondary polyps by lateral budding of its body wall: the autozooids are typical feeding polyps, whilst the siphonozooids serve as intakes for water, which circulates within the colony and helps to keep it upright. A central axial rod of calcium carbonate provides further

support to the colony, and often, calcareous sclerites are present within the mesoglea for the same purpose.

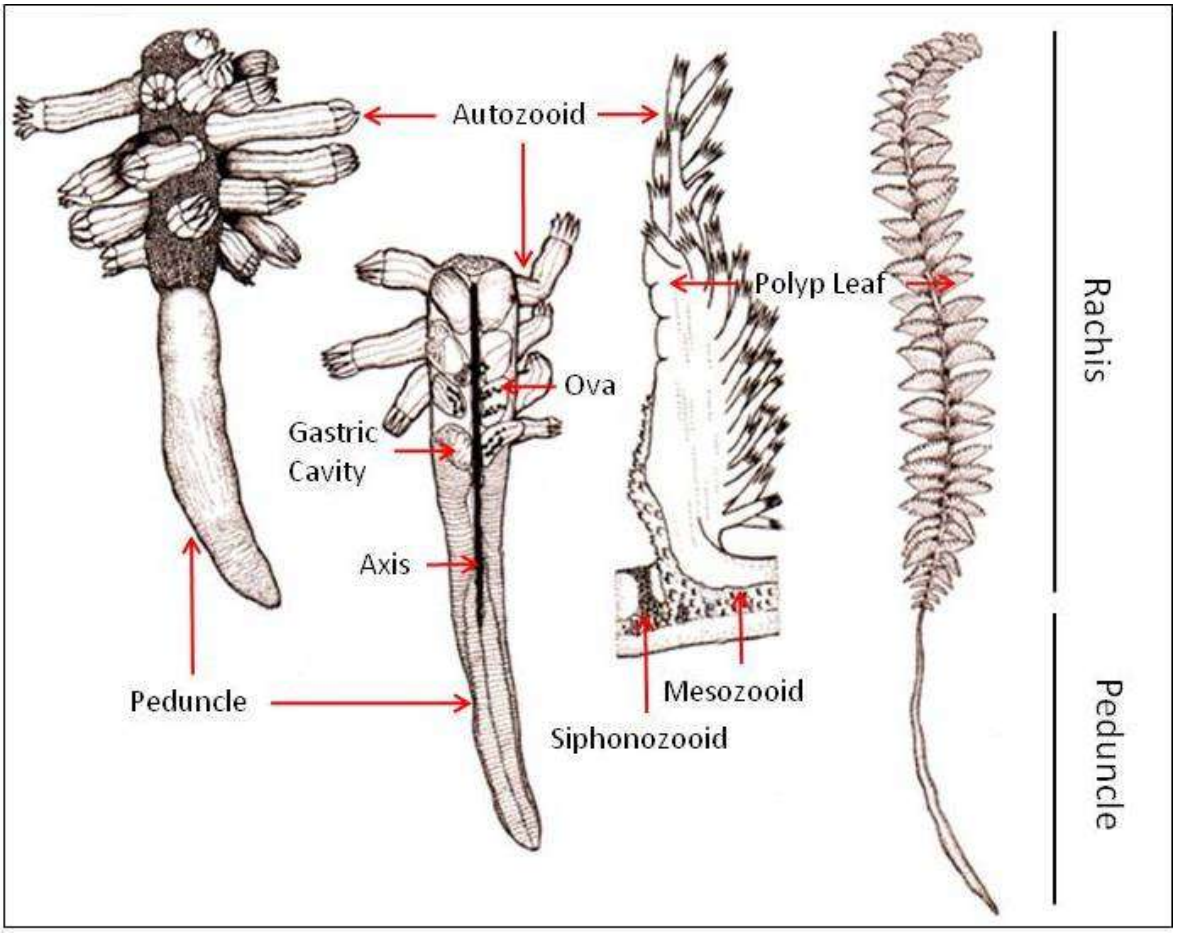

Figure 1.4 Pennatulid gross morphology (adapted from Williams http://research.calacademy.org/research/izg/seapenmorphology.htm).

Although all pennatulids comprise of the basic structure described above, colony forms are quite variable owing to evolution and adaptation to their particular environment (Fig. 1.5). The unusual sea pansy, *Renilla*, is morphologically adapted to live in turbulent benthic areas by having a horizontally expanded rachis, which offers less resistance to water flow than the vertically elongated rachis of other pennatulids (Kastendiek, 1976). The peduncle can be up to five centimetres in diameter and can be distended further, better anchoring the colony in the sand flats it typically inhabits. A cluster of modified siphonozooids forms an outlet valve that releases water to deflate the colony: if the colony is on a sand bar at low tide, it will deflate and in doing so, *Renilla* is actually able to crawl about on its leaf-like primary polyp in order to find refuge in deeper water. Other pennatulid colony forms include those that have fused autozooids creating 'leaves', or raised ridges, and those that have autozooids clustered at the distal end of the rachis,

pompon-like. This last morphologic form, comprising of species of the genus *Umbellula*, are highly adapted to the trophic conditions of the deep sea, and their large, clustered autozooids are directed upwards enabling them to capture the sparse, flocculated food-particles that reach the seabed. Likewise, bathyal and abyssal species of *Kophobelemnon* have adapted to low nutrient conditions by possessing autozooids that are large, relative to colony size.

Figure 1.5 Pennatulid colony form exemplars. A The sea pansy *Renilla* sp. with its horizontally expanded rachis; B *Pennatula phosphorea* has autozooids arranged on 'leaves'; C The autozooids of *Umbellula* sp. arranged at the distal end of the colony.

1.2.1.2 The Polyps

Some features of anthozoan polyps, such as the actinopharynx, mesenteries and siphonoglyph, were outlined in Section 1.1.2. Here, further details of octocoral polyp morphology are provided, with specific reference to pennatulids.

Species of octocorals with one polyp type are termed monomorphic, and are restricted to the Order Alcyonacea. However, some alcyoniids and all pennatulids are polymorphic, possessing a second, smaller type of polyp, the siphonozooid. Siphonozooids usually lack tentacles, or have rudimentary alternatives, and function in colony irrigation. A third polyp type, the mesozooid, only present in a few pennatulid species, is an intermediate structure between autozooids and siphonozooids.

Both autozooids and siphonozooids are essentially composed of a cylindrical or tubular structure termed the column, which terminates at its distal end in a transverse oral disc. As with other anthozoans, the coelenteron is partitioned into a series of chambers by radially arranged mesenteries; in octocorals however, there are always eight of these.

Their lines of attachment to the column and disc, which are often externally visible, are called mesenteric insertions. Mesenteries are complex in structure since they bear the organs of digestion, reproduction and various muscles. The base of the polyps is embedded in a common tissue mass, the coenchyme.

At the free end of the autozooids, a slit-like mouth is centrally located which is surrounded by eight, hollow marginal tentacles (Fig 1.6). These tentacles have finger-like extensions along each side, called pinnules (Fig 1.6), which give them a feathery appearance and greatly enhance both the inner and outer surface areas of the autozooid. Pinnate tentacles are mobile and contractile, and densely covered with sensory cells enabling the autozooid to detect and grab impacting food particles. In shallow-water species, the tentacles are often filled with symbiotic zooxanthellae, which provide further means of attaining nutrition. Nematocysts, located on the epidermis of the autozooid and tentacles, aid capture of some small zooplankton.

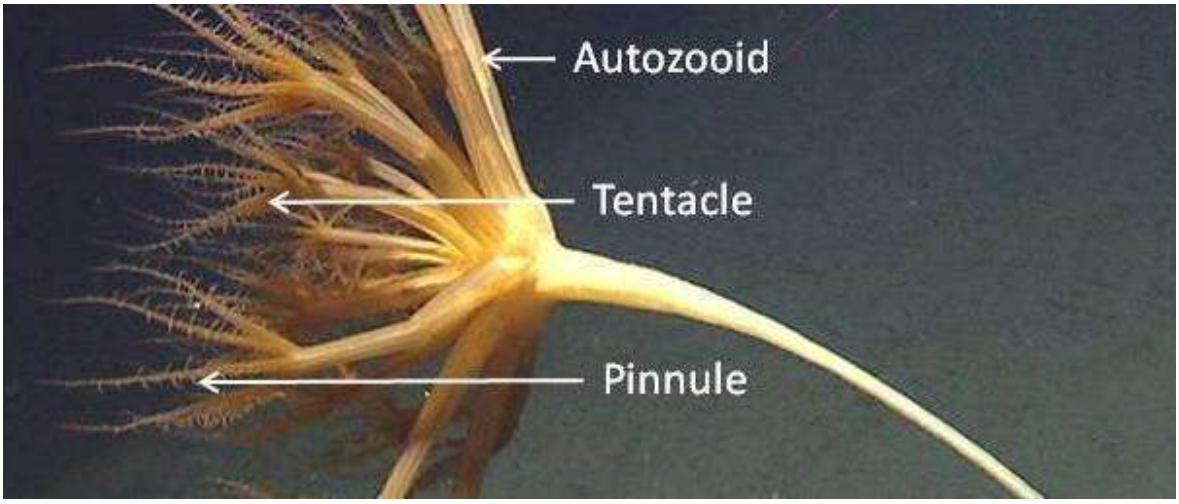

Figure 1.6 Photograph illustrating position of autozooid tentacles and pinnules.

As previously mentioned, the siphonozooids usually lack tentacles, or have rudimentary alternatives. However, some species of pennatulids have been observed to have a single long, fine tentacle (Danielssen and Koren, 1884); pinnule-type structures have also been observed associated with siphonozooid tentacles, and in contrast to autozooid tentacles, pinnules form a row along one side. Siphonozooids function to irrigate the colony, and thus the associated siphonoglyph, a structure responsible for driving water into the coelenteron, is exceptionally well developed in these polyps; this is particularly the case for pennatulids.

1.2.2 Taxonomy and Systematics of Pennatulacea

Very few authors have attempted to deal with the subject of taxonomy and systematics of pennatulids, the majority of which are in need of revision (Kölliker, 1870; Koch, 1878; Kölliker, 1880; Marshall, 1887; Kükenthal and Broch, 1911; Kükenthal, 1915; Hickson, 1916). Through sporadic sampling and a lack of knowledge concerning variability, early work created a substantial amount of confusion in pennatulid classification whereby families, genera or species were unjustifiably split or grouped. Extensive collecting in many different geographical localities and detailed comparison of material was necessary to assess the degree of variation in many taxa due to genetic, geographical, or ecological differences.

Williams (1992b; 1995a; c; 1997b) initiated modern-day phylogenetic studies of the group (further details are discussed in Chapter Two); and made detailed regional accounts that included some taxonomic information on virtually the entire group (Williams, 1990), plus taxonomic descriptions of shallow-water (Williams, 1989; 1995d) and deep-sea species (Williams, 1995a). Further, Williams (1995b) compiled a synopsis of all living genera, including keys to families and genera, and a much needed reassessment of pennatulid classification. Other modern taxonomic studies on pennatulids comprise Zamponi and Perez (1995), Lopez-Gonzalez et al. (2000; 2001), and Lopez-Gonzalez and Williams (2002).

The work of Williams and others summarised above have provided important advances in pennatulid research, and significantly improved our understanding concerning the classification of many families, genera and species. Thirty-four genera in fifteen families of living pennatulids are currently recognised. These are listed in Table 1.1, which also outlines comparative morphological characters among genera. Moreover, it is now understood that less than half the 436 nominal species are valid (Williams, 1995b). Nevertheless, several previously unknown species have recently been described (Williams, 1995a; d; Zamponi and Perez, 1995; Lopez-Gonzalez et al., 2000; 2001; Lopez-Gonzalez and Williams, 2002), and it is very likely that further additions will be made in the future. Accordingly, it is thought that the existing pennatulid fauna of the world comprises around 200 species (Williams, 1990).

Table 1.1 Comparative characters for the genera of Order Pennatulacea (adapted from Williams, 1995b to include newly described genera).

Family	Genus	Axis	Symmetry	Polyp Leaves	Three-Flanged Sclerites	Permanent Calyces	Depth (m)
Anthoptilidae	*Anthoptilum*	X	BL				155–3150
Chunellidae	*Calibelemnon*	X	BL				100–1275
	Chunella	X	BL				818–1200
	Amphiacme		BL				818–1200
Echinoptilidae	*Actinoptilum*		R		X	S	12–333
	Echinoptilum		R or BL		X	S	50–628
Funiculinidae	*Funiculina*	X	BL		X	S	60–2600
Halipteridae	*Halipteris*	X	B	RR	X	S	36–1950
Kophobelemnidae	*Kophobelemnon*	X	BL		X		36–4400
	Malacobelemnon	X	BL				42–60
	Sclerobelemnon	X	BL				10–472
Pennatulidae	*Crassophyllum*	X	BL	X		F	30–650
	Pennatula	X	BL	X	X	F	520–1266
	Ptilosarcus	X	BL	X	X	S	18–2825
	Sarcoptilus	X	BL	X		S or F	9–320
Pteroeididae	*Pteroeides*	X	BL	X	X	S	0–68
	Gyrophyllum	X	BL	X		S?	0–145
Protoptilidae	*Protoptilum*	X	BL		X	S	250–4000
	Distichoptilum	X	BL		X	S	650–4300
Renillidae	*Renilla*		BL		X		0–70
Scleroptilidae	*Scleroptilum*	X	BL		X		510–4200
Stachyptilidae	*Stachyptilum*	X	BL		X	S	36–950
	Gilibelemnon	X	BL			S	110-378
Umbellulidae	*Umbellula*	X	BL or R		*		210–>6100
Veretillidae	*Amphibelemnon*		R		X	S	91-227
	Cavernularia	*	R				3–320
	Cavernulina	X	R				30–62
	Lituaria	X	R				3–150
	Veretillum	*	R				6–220
Virgulariidae	*Acanthoptilum*	X	BL	X	X	S	3–529
	Scytaliopsis	X	BL	X		F	up to 460
	Scytalium	X	BL	X		S	18–180
	Stylatula	X	BL	X	X	S or F	0–1020
	Virgularia	X	BL	X		F	0–1100

X = present; * present or absent; BL = bilateral; R= radial; S = sclerites present; F = fleshy; RR= raised ridges, not distinct polyp leaves.

Chapter Two

Phylogeny and Systematics of Deep-Sea Pennatulacea (Anthozoa: Octocorallia)

A molecular analysis based on mitochondrial protein-coding sequences

2.1 Introduction

Octocorals (Cnidaria: Anthozoa) are ecologically diverse and important members of a wide variety of marine communities, from the warm shallow-water tropics to the cold depths of the deep sea where they are often abundant megafaunal filter feeders (Tyler, 2003). Indeed, there are approximately twice as many species of deep-sea octocorals in the Gulf of Alaska (Etnoyer and Morgan, 2005) as there are shallow-water scleractinian corals (~50 spp.) in the Caribbean (Veron, 1995). Forming a well-defined morphologic group, octocorals share several uniting characteristics: nematocyst complement, tentacle number and structure, and the number and structure of the mesenteries (divisions within the gastrovascular cavity).

In contrast to other major groups of cnidarians for which there is a long and rich history of phylogenetic study (for example Veron et al., 1996; Collins et al., 2006), our knowledge of historical relationships within the octocorals is poor and under-studied (Bayer, 1981). Endeavours to improve our understanding have been impeded by a scarcity of useful taxonomic characters, a high frequency of homoplasy (parallelisms, convergences, and reversals), and unusually high degrees of intraspecific variability (Williams, 1992b). Systematic work in the past has focused mainly on alpha-taxonomy (Kölliker, 1880; Hickson, 1916; Bayer, 1955; Williams, 1992b; 1997b) yet the difficulty in polarising taxonomic characters for phylogenetic reconstructions has been exacerbated by the near absence of octocorals in the fossil record (Bayer, 1956).

The advent of molecular approaches has considerably improved our understanding of the evolutionary relationships among anthozoans: phylogenetic analysis based on partial sequences of the *16S* rDNA (France et al., 1996), partial and complete sequences of the *18S* rDNA (Song and Won, 1997) a combination of both genes (Bridge et al., 1995; Brugler and France, 2007), or the entire mitochondrial genome (Medina et al., 2006; Brugler and France, 2007), have suggested that Octocorallia is the sister taxon to all other anthozoan orders. Likewise, molecular evidence has verified Octocorallia as a group of unquestionable monophyly (Berntson et al., 1999; Berntson et al., 2001; McFadden et al., 2006).

To understand further sub-ordinal relationships within the octocorals, mitochondrial molecular markers were developed for the octocoral-specific gene, *msh1* (France and Hoover, 2001; McFadden et al., 2004). Unlike nuclear DNA, which is inherited from both parents and in which genes are rearranged in the process of recombination, there is usually no change in mitochondrial DNA from female parent to offspring. Although mitochondrial DNA also recombines, it does so with copies of itself within the same mitochondrion. Because of this and because the mutation rate of the mitochondrial genome exceeds that of the nuclear genome by a factor of ~10 (Brown et al., 1979), mitochondrial DNA is a powerful tool for tracking ancestry for high-resolution phylogenetic analysis. Yet the cnidarian mitochondrial genome is believed to evolve at rates up to twenty times slower than in other animal groups (Romano and Palumbi, 1997), and thus has been uninformative for phylogenetic reconstructions at low taxonomic levels in cnidarians. Analyses of *16S* and *12S* ribosomal DNA and the protein-coding genes *cytochrome oxidase I* (*COI*), *cytB,* and *ATPase-6* have revealed levels of sequence divergence that are typically less than 1% among congeneric species and less than 6% among confamilial genera (Best and Thomas, 1993; France et al., 1996; Romano and Palumbi, 1997; Medina et al., 1999; van Oppen et al., 1999; Fukami et al., 2000; France and Hoover, 2002). Likewise, non-coding regions have shown similar levels of conservation (Ma´rquez et al., 2002) and accordingly, phylogenetic resolution has been mainly limited to the level of orders (France et al., 1996), families (Romano and Palumbi, 1996), or occasionally genera (Fukami et al., 2000).

Nevertheless, it is now recognised that all octocorals exhibit the mitochondrial protein-coding gene, *msh1*, a homologue of the bacterial DNA mismatch repair gene, *mutS* that is

not known to occur in any other cnidarians or metazoans (Pont-Kingdon et al., 1995; Culligan et al., 2000). The *msh1* gene is believed to evolve two times faster than either *ND3* or *ND4L* (France and Hoover, 2001), making it potentially informative for family- and genus-level phylogenetic analyses. Mitochondrial sequence data using a combination of *ND2* and *msh1* genes has unveiled better resolved phylogenies within the octocorals (Sánchez et al., 2003b; McFadden et al., 2006), some findings of which are incongruent with nuclear sequence data (see below).

Within the Octocorallia, the order Pennatulacea (sea pens) can be readily distinguished based on morphology (Bayer, 1956; 1973). The pennatulids are the most advanced of octocorals in terms of their colonial complexity, functional specialisation of polyps, and colonial integration (Hickson, 1909; Bayer, 1956; 1973) and perhaps form the most diverse cnidarian group in the deep sea. Uniquely, mature colonies develop from a single large primary polyp that produces secondary polyps by lateral budding of its body wall. Also exclusive to the pennatulids, is the character of a muscular peduncle, which anchors the colony by peristaltic contractions into soft substrata such as sand, mud, or abyssal ooze. Yet based on nuclear *18S* rDNA sequences the origins of this morphologically well-defined group were not resolved (Berntson et al., 2001). Unexpectedly, O. Pennatulacea was found to be polyphyletic because of the inclusion of the pennatulid *Umbellula* sp. in a clade with the alcyoniids, *Anthomastus* and *Corallium*. This result, however, was not supported by mitochondrial data (*ND2* and *msh1*), which recovered the pennatulids as a monophyletic order (McFadden et al., 2006).

Kükenthal and Broch (1911) and Kükenthal (1915) developed a higher classification scheme of two suborders (and six sections) within the pennatulids: the Sessiliflorae for the taxa with polyps emanating directly from the rachis and the Subselliflorae for the taxa with polyps located on polyp leaves or raised ridges. Williams (1995b) discusses that although the Subselliflorae form a holophyletic clade, the Sessiliflorae should be considered paraphyletic since the group does not contain all descendants from a common ancestor, suggesting this classification scheme is of nominal value only.

Very few authors have attempted to deal with the subject of phylogeny and the origins of the pennatulids, the majority of which are in need of revioson (Kölliker, 1870; Koch, 1878; Kölliker, 1880; Marshall, 1887; Kükenthal and Broch, 1911; Kükenthal, 1915; Hickson,

1916). Williams (1992b) initiated modern phylogenetic study of the group based on a cladistical analysis of morphological characters for nine of the fifteen pennatulid families. Intra-generic cladistical analysis based on morphology is limited to the deep-sea species *Gyrophyllum sibogae*, *Pennatula inflata*, *Ptilosarcus undulatus*, *Sarcoptilus grandis*, *Crassophyllum cristatum*, and *Pteroeides spinosum* (Williams, 1995a) and inter-generic analysis of the shallow-water sea pansy, *Renilla* (Pérez and Ocampo, 2001). The first to address phylogeny of the pennatulids was Kölliker (1870; 1880), who considered deep-sea taxa (principally *Umbellula* and *Protoptilum*) to be primitive offshoots of the pennatulacean prototype: "These simpler forms are probably also the oldest, and may be regarded as the last remnants of an extinct primary creation". Also, Kölliker (1870) considered shallow-water Veretillidae as highly specialised forms derived from kophobelemnoid ancestors. Koch (1878) disputed this, postulating that veretillids are transitional forms between the alcyoniids and pennatulids. Marshall (1887) suggested that high diversity in deep-sea pennatulids and the derived nature of *Umbellula* makes them highly specialised and less primitive than their shallower-water counterparts (referring to *Funiculina*). Similarly, Kükenthal and Broch (1911) considered *Umbellula* to be highly derived, and veretillids more primitive; *Protoptilum* and *Funiculina* were considered members of closely-related families. Williams (1992b; 1995a) supported these findings, adding that *Funiculina* is more derived than the veretillids but less derived than *Umbellula* and *Pteroeides*.

There is still much speculation with regards to the origins of pennatulids. Many believe that the Ediacaran and Burgess Shale frond-like fauna are fossilised pennatulacean-like octocorals (Bergström, 1991). However, 'similarities' i.e. the lateral branches of the frond-like fossils and the polyp leaves of many pennatulids appear to be non-homologous and not even functionally convergent (Williams, 1997b). Instead, Williams (1997b) proposed that pennatulids evolved from a soft coral ancestor similar to the alcyoniid genus *Anthomastus*. While molecular evidence founded on both mitochondrial and nuclear sequences (Berntson et al., 1999; Berntson et al., 2001; McFadden et al., 2006) suggests that *Anthomastus* may be more closely related to the pennatulids than other soft corals, these data do not support a sister relationship. Instead, there is strong evidence to support the calcaxonian sea fan family Ellisellidae as the sister group to the order Pennatulacea (McFadden et al., 2006), a relationship Bayer (1955) proposed on the basis of observed similarities in the axial structure of the two groups. As such, it is now

believed that the calcaxonian skeletal axis and the axis of the pennatulids are of single-evolutionary origin, having been derived from that of a calcaxonian ancestor (McFadden et al., 2006).

It is now understood that pennatulids exhibit morphological character changes as evolutionary events within different lineages such as development of bilateral symmetry and lateral processes such as polyp leaves or ridges, concentration and localisation of feeding polyps, and the reduction in the number and size of sclerites (Williams, 1992b). Furthermore, distributional and phylogenetic data based on morphology support the hypothesis that pennatulids first differentiated in tropical shallow-water and subsequently dispersed to and diversified in temperate and polar regions, and to all ocean depths, as well as the shallow-water tropics (Williams, 1997b). Williams (1997b) stated that "Primitive, mostly tropical shallow-water taxa are represented by *Cavernularia* and *Veretillum*, while variously derived deeper-water taxa of widespread distribution include *Funiculina*, *Chunella*, *Umbellula*, *Pennatula*, *Gyrophyllum*, *Distichoptilum*, and *Kophobelemnon*. *Pteroeides* is an example of a derived taxon represented mostly in tropical shallow-water".

Aims and Objectives

To date, octocoral systematics and phylogenetic research has tended to focus on the higher taxonomic groups, and very little work has been conducted at the familial level, particularly regarding the pennatulids. Until recently, the published history on the systematics and evolution pertaining to the pennatulids spans the period 1870-1916. Modern phylogenetic studies of deep-sea pennatulids were based on morphology and distribution (Williams, 1992b; 1997b): to date, there are no phylogenetic or systematic studies based on molecular data. The recent collections of pennatulids for molecular analysis, representing a suite of taxa of wide geographic and bathymetric scope, have enabled a reassessment of the systematics and phylogenetic relationships among 10 of the 15 pennatulid families. This study offers the first genetic analysis of O. Pennatulacea and addresses the following questions:

1. Is the current classification scheme of O. Pennatulacea supported by molecular systematics?

2. Is there molecular evidence to support the higher classification scheme of Kükenthal and Broch (1911) and Kükenthal (1915)?
3. What do molecular analyses tell us about the evolutionary history of pennatulids?
4. Are the two mitochondrial protein-coding genes *ND2* and *msh1* useful for addressing phylogenetic questions within O. Pennatulacea?

2.2 Materials and Methods

2.2.1 Specimens

A total of 132 frozen and ethanol-preserved pennatulid specimens were used in this study, collected during a variety of research cruises and sources. Samples included representatives from all oceans (Atlantic, Arctic, Indian, Pacific and Southern), ranging in depth from 12 m to 4229 m (Fig. 2.1; Table 2.1). Individuals were identified to genus level and to species level whenever possible.

Samples were collected during the following research cruises: a suite of frozen and ethanol-preserved material obtained from the Benthic CROZET cruise (D300) aboard the RRS *Discovery* (National Oceanography Centre, Southampton); ethanol-preserved specimens collected aboard the *Western Flyer* with the ROV *Tiburon* (Monterey Bay Research Institute) off Monterey; several ethanol-preserved specimens acquired by Edward McCormack (Marine Institute, Galway) from the NE Atlantic; five ethanol-preserved specimens from Marguerite Bay, Antarctica, collected aboard RRS *James Clark Ross* during JCR166 with the ROV *Isis* (National Oceanography Centre, Southampton); three ethanol-preserved specimens obtained from the NE Atlantic during HERMES cruises aboard RRS *James Cook* (JC10 and JC11) with the ROV *Isis* (National Oceanography Centre, Southampton); an array of specimens preserved in ethanol collected during the Oceans 2020 voyages, courtesy of National Institute of Water and Atmospheric Research (New Zealand); and a further two specimens were obtained from the Indian Ocean off Sumatra by Paul Tyler (National Oceanography Centre, Southampton) on board *The Performer*.

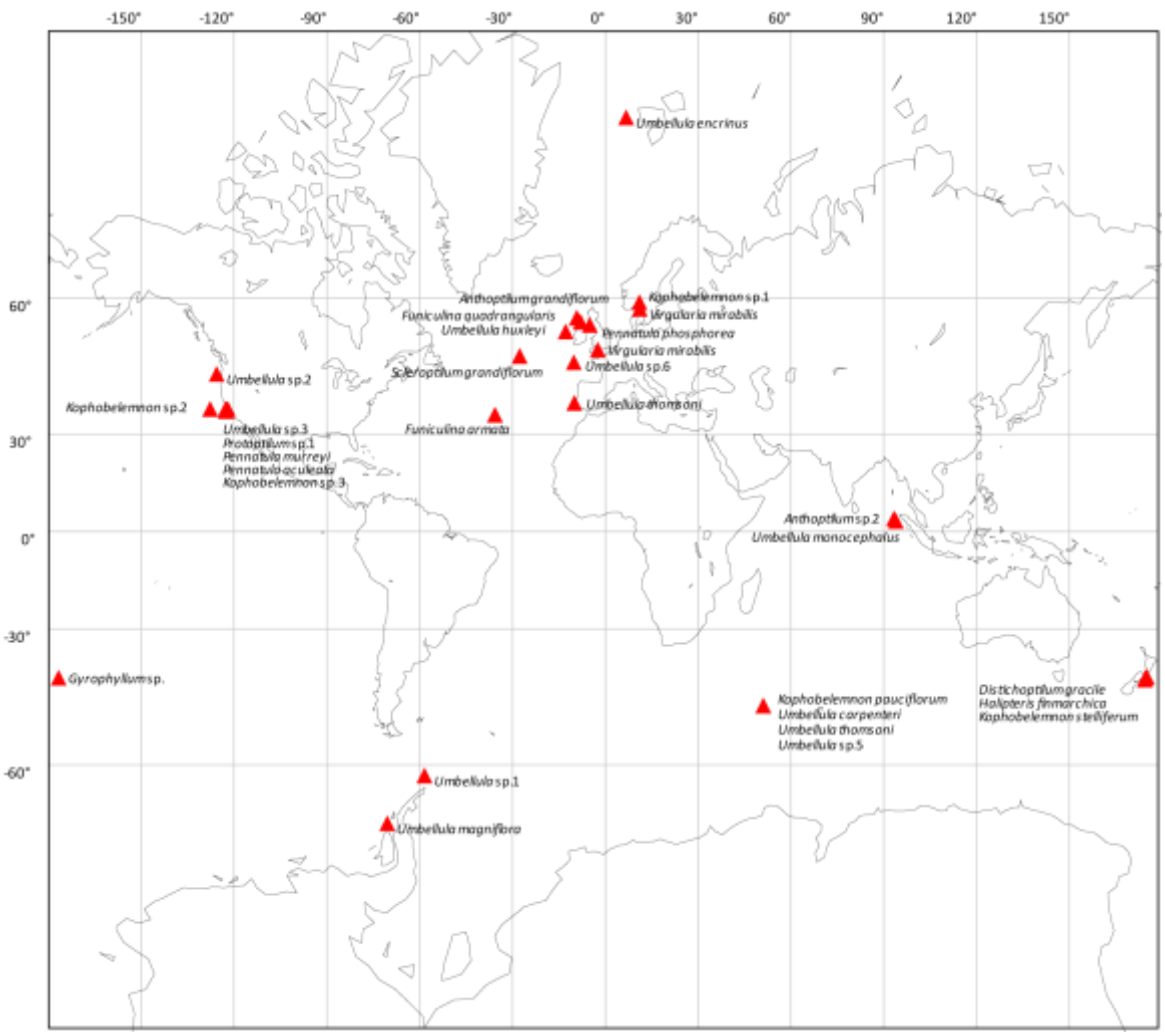

Figure 2.1 Location of all specimens attained for molecular analysis (map source: PanMap, Diepenbroek et al., 2000).

Additional material was obtained from a variety of sources: samples collected by means of SCUBA diving from Portland Harbour (Dorset, UK) and preserved in ethanol; ethanol-preserved tissue of an Arctic specimen acquired by Peter Lamont of the Scottish Association of Marine Science; two specimens donated by Hans G. Hansson, Tjärnö Marine Biological Laboratory, University of Gothenburg, taken from the Koster Channel, Sweden; eleven ethanol-preserved specimens from the Southern Ocean courtesy of Rhian Waller (Woods Hole Oceanographic Institution); specimens and ethanol-preserved material obtained from the collections housed at the California Academy of Sciences, courtesy of Gary Williams; and further material was obtained through the Millport Marine Station, Scotland.

Table 2.1 Molecular specimens: Dates of collection, location, depth (m).

Taxon	Date	Latitude	Longitude	Depth	Location/Station
Family Anthoptilidae					
Anthoptilum grandiflorum	2006	56.6636	-9.2018	1053	NE Atlantic
Anthoptilum sp.1	02/2006	?	?	?	S Atlantic, Subantarctic
Anthoptilum sp.2	05/2005	03.4009	94.0091	1714	Sumatra, Indian Ocean
Family Funiculinidae					
Funiculina armata	06/2007	35.2985	-35.6451	350	NE Atlantic
Funiculina quadrangularis	2006	55.9300	-07.9900	173	NE Atlantic
Family Halipteridae					
Halipteris finmarchica	04/2007	-43.1712	174.4670	555	New Zealand, W Pacific
Family Kophobelemnidae					
Kophobelemnon pauciflorum	12/2005	-48.9368	51.0650	4189	Crozet, S Atlantic
Kophobelemnon stelliferum	04/2007	-42.5425	175.1415	1812	New Zealand, Pacific
Kophobelemnon sp.1	05/2007	59.3333	11.0167	70	Koster Channel, Sweden
Kophobelemnon sp.2	11/2006	36.7792	-125.7560	2456	Monterey, S of canyon
Kophobelemnon sp.2	11/2006	36.7792	-127.7560	2456	Monterey, S of canyon
Kophobelemnon sp.3	11/2006	36.2580	-122.6800	3208	Monterey, S of canyon
Family Pennatulidae					
Pennatula aculeata	11/2006	36.7792	-122.7560	2456	Monterey, S of canyon
Pennatula phosphorea	03/2006	55.3667	-05.0167	55	Millport, NE Atlantic
Pennatula murrayi	12/2005	-48.9368	51.0650	4189	Crozet, S Atlantic
Pennatula murrayi	11/2006	36.2580	-122.6800	3208	Monterey, S of canyon
Family Protoptilidae					
Distichoptilum gracile	04/2007	-42.6452	177.8693	1211	New Zealand, Pacific
Distichoptilum gracile	11/2006	36.7792	-126.7560	2456	Monterey, S of canyon
Distichoptilum gracile	11/2006	36.7792	-128.7560	2456	Monterey, S of canyon
Protoptilum sp.	11/2006	36.2580	-122.6800	3208	Monterey, S of canyon
Family Pteroeididae					
Gyrophyllum sp.	2006	53.8968	-10.0315	1580	NE Atlantic
Gyrophyllum sp.	04/2007	-42.7048	-178.3403	997	New Zealand, Pacific
Family Scleroptilidae					
Scleroptilum grandiflorum	07/2007	49.2447	-27.7102	2190	Mid-Atlantic Ridge
Family Umbellulidae					
Umbellula carpenteri	12/2005	-48.9368	51.0650	4189	Crozet , S Atlantic
Umbellula carpenteri	12/2005	-49.0191	51.0753	4189	Crozet, S Atlantic
Umbellula encrinus	07/2001	78.9680	06.7150	1400	Arctic Ocean
Umbellula huxleyi	2006	54.1325	-12.8150	1512	NE Atlantic
Umbellula magniflora	01/2007	-68.1968	-70.5110	840	Marguerite Bay, Antarctica
Umbellula monocephalus	2005	04.1602	93.3179	4229	Indian ocean
Umbellula thomsoni	12/2005	-48.9368	51.0650	4189	Crozet, S Atlantic
Umbellula thomsoni	06/2007	38.3755	-09.9782	3476	Cascais Canyon, Atlantic
Umbellula sp.1	07/2007	47.9268	-10.2092	4040	Whittard Canyon, Atlantic
Umbellula sp.2	12/2005	-48.9368	51.0650	4189	Crozet, S Atlantic
Umbellula sp.3	?	-61.6717	-58.4667	390.0	King George Is, Antarctica
Umbellula sp.4	?	45.3033	-125.6750	2633	Oregon, Cascadia Plain
Umbellula sp.5	?	36.7667	-122.0333	650	Monterey Canyon, Pacific
Umbellula sp.6	1992	Aquacultured			Monterey Canyon, Pacific
Family Virgulariidae					
Virgularia mirabilis	06/2007	58.2453	11.0925	36.5	Sweden, Atlantic
Virgularia mirabilis	03/2006	50.5896	-2.4274	12	Portland, Dorset, UK

2.2.2 DNA Extraction

From 103 of 132 pennatulid specimens, total genomic DNA was extracted from 15-25 mg of polyp tissue using Qiagen DNeasy extraction kits according the manufacturer's instructions: DNA from 53 of these was used for the final analysis in this study. Eluted DNA samples were run on 1% agarose gels to check for contamination and quality. Pennatulid tissue tended to yield high concentrations of DNA as detected on the NanoDrop ND-1000 spectrophotometer (Labtech International), and often had to be significantly diluted to obtain optimum concentrations of 2 ng μl^{-1}. As such, it was not necessary to add cetyltrimethylammonium bromide (CTAB) to the extraction buffer, a reagent that has proved to be effective at removing polysaccharides that are abundant in coral tissues (Berntson et al., 1999) and often interfere with DNA extraction.

2.2.3 Primers, Amplification and Sequencing

Six different genes were examined for their suitability for sequence analysis of pennatulids: part of the mitochondrial enzyme-complex gene, succinate dehydrogenase (*SDH*); the non-coding region of the mitochondrial genome between *COI* and *COII* (*COI-COII* intergenic spacer); the large subunit of the mitochondrial ribosomal DNA gene (*16S rDNA*); the small subunit of the nuclear ribosomal DNA gene (*18S rDNA*); and two mitochondrial protein-coding genes, NADH-dehydrogenase subunit 2 (*ND2*) and *msh1*, a homologue of the bacterial DNA mismatch repair gene, *mutS*.

Pipette tips with filter barriers were used throughout PCR preparation to guard against contamination of the reactions. Negative controls (without DNA template) were included during the PCRs.

2.2.3.1 Succinate Dehydrogenase and COI-COII Intergenic Spacer

Using the web-based software, Primer3 (Rozen and Skaletsky, 2000), a pair of primers was designed to amplify a portion of the mitochondrial enzyme-complex gene, succinate dehydrogenase (*SDH*):

SDHPeF 5'-ATGTCGTGAAGGCATTTGTG-3'

SDHPeR 5'-CAATTTCTATATTACTTATCTGGTT-3'

The following primers were used to amplify the *COI -COII* intergenic spacer (Smith et al., 2004):

COII7816 5'-GACCAATACCATTGATG-3'

COI8492 5'-CAATCATTACTGGCATTA-3'

S. France (unpublished).

Each 50 µl PCR reaction contained: 5µl of 10X PCR buffer, 2 µl of 3 mM $MgCl_2$, 2 µl of 0.2 mM dNTP, 5 µl of "Q-solution", 0.5 µl of *Taq* Polymerase (all reagents from Qiagen), 2 µl of each 10 pmol primer and 5 µl of 2 ng DNA template. Amplification was then carried out over 35 cycles of 1 minute at 96°C, 1 minute at 48°C, 1.5 minute at 72°C, followed by a 5 minute extension step at 72°C. PCR products were separated by electrophoresis on 1% agarose gels in a TBE buffer, stained with ethidium bromide, and viewed under ultraviolet light to check for the quality of amplification.

2.2.3.2 *16S* rDNA

Universal primers were successfully used for PCR amplification of partial sequences of the *16S rDNA*-encoding gene for the shallow-water pennatulid *Pennatula phosphorea* and the deep-sea pennatulid *Umbellula carpenteri*:

16Sar (5'-CGCCTGTTTATCAAAAACAT-3'),

16Sbr (5'-CCGGTTTGAACTCAGATCATG-3')

(Palumbi et al., 1991).

The PCR solution contained the following in 50 µl volumes: 5µl of 10X PCR buffer, 2 µl of 3 mM $MgCl_2$, 2 µl of 0.2 mM dNTP, 5 µl of "Q-solution", 0.5 µl of *Taq* Polymerase (all reagents from Qiagen), 2 µl of each 37.5 pmol primer and 5 µl of 2 ng DNA template. The thermal cycle parameters of the PCR reaction were the same as those outlined above for succinate dehydrogenase and the non-coding region. PCR Products were visualised on 1% agarose gels, then purified using QIAquick PCR purification kits (Qiagen). Cycle sequencing reactions were performed using BigDye cycle sequencing kits (PE Applied Biosystems) according to manufacturer's instructions. The sequencing reaction products were purified using Qiagen DyeEx v.2 spin kits, and dried with a vacuum centrifuge, re-suspended in 10 µl formamide, heated for 3 minutes at 96°C and cooled for 3 minutes on ice prior to sequencing. Sequences were detected on an ABI 3100 automated sequencer.

Modifications to the PCR protocol were made by implementing annealing temperatures of 45°C, 47°C and 55°C for the consistent amplifications of *16S rDNA* for all pennatulid specimens. Also for this purpose, endeavours to amplify a smaller region of the *16S rDNA* encoding gene were performed using a pair of internal primers and applying the same protocol:

LP16SF 5'-TTGACCGGTATGAATGGTGT-3

LP16SR 5'-TCCCCAGGGTAACTTTTATC-3

(Le Goff-Vitry et al., 2004).

2.2.3.3 *18S* rDNA

Initially, modified versions of the universal eukaryotic primers A and B (Medlin et al., 1988) with polylinkers removed were used to amplify *18S rDNA*:

Uni A 5'-AACCTGGTTGATCCTGCCAGT-3'

Uni B 5'-TGATCCTTCTGCAGGTTCACCTAC-3'

(Berntson et al., 1999).

The PCR solutions contained the following in 50 µl volumes: 5µl of 10X PCR buffer, 2 µl of 3 mM $MgCl_2$, 2 µl of 0.2 mM dNTP, 5 µl of "Q-solution", 0.5 µl of *Taq* Polymerase (all reagents from Qiagen), 2 µl of each 10 pmol primer and 5 µl of 2 ng DNA template. Amplification was then carried out over 35 cycles of 45 seconds at 94°C, 45 seconds at 55°C, 90 seconds at 72°C, followed by a 5 minute extension step at 72°C. The product was visualised on 1% agarose gel for each sample. PCR products were purified using QIAquick PCR purification kits (Qiagen). Cycle sequencing reactions were performed, using BigDye cycle sequencing kits (PE Applied Biosystems) according to manufacturer's instructions. The sequencing reaction products were purified using Qiagen DyeEx spin kits and sequences were detected on an ABI 3100 automated sequencer.

PCR products could not be successfully sequenced probably as a result of the long length of the fragment (~1800 base pairs, bp), and accordingly the following three pairs of internal primers were used, selected from a combination of universal primers and a set of octocoral-specific primers designed by Berntson et al. (1999):

Uni A 5'-AACCTGGTTGATCCTGCCAGT-3'	(1) ~1-536 bp
536R 5'-WATTACCGCGGCKGCTG-3'	(1)
514F 5'-GTGCCAGCMGCCGCGG-3'	(2) ~514-1200 bp

1200R 5'-GGGCATCACAGACCTG-3' (2)

1055F 5'-GGTGGTGCATGGCCG-3' (3) ~1055-1800 bp

Uni B 5'-TGATCCTTCTGCAGGTTCACCTAC-3' (3)

(Berntson et al., 1999).

Primer pairs (1) and (3) amplified consistently, whereas primer pair (2) always failed to amplify. The following primers were created by reversing 536R 5'-WATTACCGCGGCKGCTG-3' and 1055F 5'-GGTGGTGCATGGCCG-3' to make forward and reverse primers respectively and PCRs were carried out as before:

536F 5'-CAGCMGCCGCGGTAATW-3' (2) ~356-1055 bp

1055R 5'-CGGCCATGCACCACC-5' (2)

2.2.3.4 *ND2* and *msh1*

The following primers were used to amplify NADH-dehydrogenase subunit 2 (*ND2*):

16647F 5'-ACACAGCTCGGTTTCTATCTACCA-3'

ND21418R 5'-ACATCGGGAGCCCACATA-3'

(McFadden et al., 2004).

For amplification of *msh1*, the following primer pair was used:

ND42599F 5'-GCCATTATGGTTAACTATTAC-3'[1]

Mut-3458R 5'-TSGAGCAAAAGCCACTCC-3'[2]

(France and Hoover, 2002)[1]; (Sánchez et al., 2003b)[2].

The PCR solutions contained (in 50 µl volumes): 5µl of 10X PCR buffer, 2 µl of 3 mM $MgCl_2$, 2 µl of 0.2 mM dNTP, 5 µl of "Q-solution", 0.5 µl of *Taq* Polymerase (all reagents from Qiagen), 2 µl of each 10 pmol primer and 5 µl of 2 ng DNA template. Amplification was then carried out over 35 cycles of 90 seconds at 94°C, 90 seconds at 58°C, 60 seconds at 72°C, followed by a 5 minute extension step at 72°C. PCR products were visualised on 1% agarose gels. For specimens that yielded no visible PCR product, a second PCR reaction was performed using 1 µl of PCR product diluted 1/20 with ultrapure water, from which 5 µl of diluted template was used, and amplified over 40 cycles.

For purification, all products were run out on 1% agarose gels, and the amplified product was excised with sterile scalpels, visualised under ultraviolet light. DNA was purified by means of QIAquick Gel Extraction kits (Qiagen) according the manufacturer's instructions. Clean PCR products were sent to Macrogen Ltd, Korea, for sequencing.

2.2.4 Sequence Analysis of ND2 *and* msh1

Both strands of corresponding sequences were aligned in the sequence alignment program BioEdit using ClustalW (Thompson et al., 1994) with default alignment parameters, and then corrected by eye to produce a consensus sequence. A Blast search was performed in GenBank (Benson et al., 2006, http://www.ncbi.nlm.nih.gov/) and the matching homologous pennatulid sequences (an additional 5 sequences for *ND2* and 4 for *msh1*, Table 2.2) were retained for subsequent alignment to complement the analysis of 25 (*ND2*) and 29 (*msh1*) distinct genotypes in this study. Two members of the closely-related calcaxonid family (Ellisellidae), *Ctenocella barbadensis* and *Verrucella* sp., were chosen as the outgroup (also from GenBank, McFadden et al., 2006).

These sequences were analysed together in two data sets for *ND2* and *msh1* respectively, and a third data set of *ND2* and *msh1* combined. For each data set, sequences were aligned in MEGA4 (Tamura et al., 2007) with ClustalW (Thompson et al., 1994) using the default alignment settings, and trimmed to the shortest sequence.

Table 2.2 List of taxa for which GenBank sequences were used in the phylogenetic analyses, and its corresponding gene, accession number and author.

Taxon	Location	Gene	Accession #	Author
Family Anthoptilidae				
Anthoptilum murrayi	Tasman Sea, AUS	*ND2*	DQ302938	McFadden et al. (2006)
Family Kophobelemnidae				
Sclerobelemnon theseus	Columbia	*ND2*	DQ311678	McFadden et al. (2006)
Sclerobelemnon theseus	Columbia	*msh1*	DQ311679	McFadden et al. (2006)
Kophobelemnon macrospinosum	Tasman Sea, AUS	*ND2*	DQ302937	McFadden et al. (2006)
Kophobelemnon macrospinosum	Tasman Sea, AUS	*msh1*	DQ302865	McFadden et al. (2006)
Family Pennatulidae				
Pennatula sp	Tasman Sea, AUS	*ND2*	DQ302943	McFadden et al. (2006)
Pennatula sp	Tasman Sea, AUS	*msh1*	DQ302870	McFadden et al. (2006)
Family Pteroeididae				
Pteroeides sp	Tasman Sea, AUS	*ND2*	DQ302944	McFadden et al. (2006)
Pteroeides sp	Tasman Sea, AUS	*msh1*	DQ302871	McFadden et al. (2006)
Family Renillidae				
Renilla muelleri	GOM, Florida, USA	*ND2*	DQ297451	McFadden et al. (2006)
Renilla muelleri	GOM, Florida, USA	*msh1*	DQ297432	McFadden et al. (2006)
Outgroup: Family Ellisellidae				
Ctenocella barbadensis	Unknown	*ND2*	AY534736	McFadden et al. (2006)
Ctenocella barbadensis	Unknown	*msh1*	AY533651	McFadden et al. (2006)
Verrucella sp	Tasman Sea, AUS	*ND2*	DQ302936	McFadden et al. (2006)
Verrucella sp	Tasman Sea, AUS	*msh1*	DQ302864	McFadden et al. (2006)

The program Modeltest3.7 (Posada and Crandall, 1998) was used to determine the optimal probabilistic model of sequence evolution by using the Akaike Information Criterion for each alignment. Phylogenies were constructed using PAUP* Portable version 4.0b10 for Windows (Swofford, 1993) for maximum-likelihood, maximum parsimony and neighbour-joining analyses. Maximum likelihood analyses were run using a heuristic search with TBR branch-swapping, for 100 bootstrap replicates with the following model parameters chosen by Modeltest3.7: TVM+G for *msh1*; k81uf+I+G for *ND2*; and GTR+G+I for the combined analysis. For maximum parsimony, a heuristic search with TBR branch-swapping was used, for 1000 bootstraps with a maximum of 1000 trees saved per replicate. Neighbour-joining (distance method) was conducted for 1000 bootstrap replicates. A Bayesian analysis was performed using the program MrBayes Version 3 (Huelsenbeck and Ronquist, 2001), setting the likelihood model according to Modeltest3.7 estimations, for 10,000,000 generations (burnin=10,000). Trees were displayed in TreeView version 1.6.6 (Page, 1996).

2.3 Results

2.3.1 PCR Optimisations and Primers

During the early stages of research, methods for sequencing octocorals to resolve relationships at the family- and genera-level were still in their infancy. While there were only a few molecular sequences publicly available for the order Alcyonacea, sequence data obtained from pennatulids were restricted to one or two unknown species. Consequently, a preliminary study to identify useful primers for the consistent amplification of a variety of regions of mitochondrial and nuclear DNA was undertaken (summarised in Table 2.3).

Forward and reverse primers, SDHPeF and SDHPeR respectively, were designed to amplify a portion of the mitochondrial enzyme-complex gene, succinate dehydrogenase. Unfortunately, these primers were not suitable and failed to anneal to the succinate dehydrogenase fragment.

Table 2.3 List of primers, target gene fragment, primer sequence and primer reference.

Primer	Gene	Sequence (5'-3')	Reference
SDHPeF	*SDH*	ATGTCGTGAAGGCATTTGTG	E. Dolan/A. Rogers (unpublished)
SDHPeR	*SDH*	CAATTTCTATATTACTTATCTGGTT	E. Dolan/A. Rogers (unpublished)
COII7816	*NCR*	GACCAATACCATTGATG	S. France (unpublished)
COI8492	*NCR*	CAATCATTACTGGCATTA	S. France (unpublished)
16Sar	*16S*	CGCCTGTTTATCAAAAACAT	Palumbi et al., 1991
16Sbr	*16S*	CCGGTTTGAACTCAGATCATG	Palumbi et al., 1991
LP16SF	*16S*	TTGACCGGTATGAATGGTGT	Le Goff-Vitry et al., 2004
LP16SR	*16S*	TCCCCAGGGTAACTTTTATC	Le Goff-Vitry et al., 2004
Uni A	*18S*	AACCTGGTTGATCCTGCCAGT	Berntson et al., 1999
Uni B	*18S*	TGATCCTTCTGCAGGTTCACCTAC	Berntson et al., 1999
536R	*18S*	WATTACCGCGGCKGCTG	Berntson et al., 1999
514F	*18S*	GTGCCAGCMGCCGCGG	Berntson et al., 1999
1200R	*18S*	GGGCATCACAGACCTG	Berntson et al., 1999
1055F	*18S*	GGTGGTGCATGGCCG	Berntson et al., 1999
536F	*18S*	CAGCMGCCGCGGTAATW	E. Dolan/A. Rogers (unpublished)
1055R	*18S*	CGGCCATGCACCACC	E. Dolan/A. Rogers (unpublished)
16647F	*ND2*	ACACAGCTCGGTTTCTATCTACCA	McFadden et al., 2004
ND21418R	*ND2*	ACATCGGGAGCCCACATA	McFadden et al., 2004
ND42599F	*msh1*	GCCATTATGGTTAACTATTAC	France and Hoover, 2002
Mut-3458R	*msh1*	TSGAGCAAAAGCCACTCC	Sánchez et al., 2003b

Primers, COII7816 and COI8492 (S. France, unpublished), for the *COI-COII* intergenic spacer of the mitochondrial genome, failed to amplify pennatulid DNA under a variety of annealing temperatures and template concentrations. Although the *COI-COII* intergenic spacer is known to exhibit greater variability than some other mitochondrial genes (NADH dehydrogenase subunits *ND2*, *ND3* and *ND6*), and may contain useful species-specific markers, its short length (<122 base pairs in many octocorals) limits its phylogenetic utility (McFadden et al., 2004). For this reason, it was decided not to persevere with optimising protocols to amplify this non-coding gene.

Universal primers, 16Sar and 16Sbr (Palumbi et al., 1991), proved unsuccessful for the consistent amplification of the mitochondrial *16S* rDNA region for all pennatulid specimens, even following modifications to the PCR protocol. An endeavour to amplify a smaller fragment of the *16S* rDNA gene was performed using a pair of internal primers, LP16SF and LP16SR (Le Goff-Vitry et al., 2004), but as with the universal primers, it was not possible to amplify consistently the *16S* rDNA region for all pennatulids. The *16S* rDNA gene is thought to exhibit especially low levels of divergence in octocorals (France et al., 1996; France and Hoover, 2002) and with this in mind it seemed unwise to persist

with optimising protocols for sequencing this gene for phylogenetic analysis of pennatulids. For this reason, the use of *16S* for this study was abandoned.

Universal primers, Uni A and Uni B (Berntson et al., 1999), successfully amplified a long fragment of the nuclear rDNA gene, *18S*. However, PCR products could not be sequenced, most likely because of the great length of the gene (*18S* is approximately 1800 base pairs long in anthozoans). To overcome this, the gene was amplified in three sections using the primers of Berntson et al. (1999) (Uni A and 536R; 1055F and Uni B) and modified versions of these, 536F and 1055R (E. Dolan and A. Rogers, unpublished).

Yet *18S* is too invariant to resolve relationships among families and genera in octocorals (Berntson et al., 2001), and so is often not useful for lower lever phylogenetic analysis. However, while this search for useful primers was being conducted, McFadden et al. (2006) were simultaneously developing protocols to sequence octocorals. Primers for the two mitochondrial protein-coding genes, *ND2* (NADH-dehydrogenase subunit 2) and *msh1* (a mutS homologue), were designed for this purpose. These primers (16647F and ND21418R, and ND42599F and mut-3458R, respectively) amplified consistently for all pennatulid genomic DNA. Thus, exacerbated by time and financial constraints to amplify and sequence three-fold with internal primers, work on *18S* was abandoned for this study to pursue analysis of the potentially more useful genes, *ND2* and *msh1* (McFadden et al., 2006).

2.3.2 Sequences

For the two mitochondrial protein-coding genes, *ND2* and *msh1*, 41 and 39 samples respectively (a total of 47 individuals) were of high enough quality for analysis following amplifications and sequencing. Amplifications were often impeded by DNA deterioration in many older specimens and those stored in <90% EtOH: such samples produced poor quality reads that were not used in the final analysis.

Table 2.4 Taxa for which partial sequences of *ND2* and *msh1* were obtained, the number of individuals sequenced and length of gene fragment.

Taxa	*ND2* Seq.d	Bp	*Msh1* Seq.d	Bp
Family Anthoptilidae				
Anthoptilum grandiflorum	1	-	1	-
Anthoptilum sp.1	2	717	1	772
Anthoptilum sp.2	1	717	1	-
Family Funiculinidae				
Funiculina armata	1	687	1	758
Funiculina quadrangularis	1	-	1	758
Family Halipteridae				
Halipteris finmarchica	1	681	1	748
Family Kophobelemnidae				
Kophobelemnon pauciflorum	2	687	3	758
Kophobelemnon stelliferum	1	687	1	758
Kophobelemnon sp.1	1	687	2	758
Kophobelemnon sp.2	1	687	1	758
Kophobelemnon sp.3	3	687	3	-
Family Pennatulidae				
*Pennatula aculeata**	1	687	2	758
Pennatula murrayi	2	687	1	776
*Pennatula phosphorea**	2	687	2	767
Family Protoptilidae				
Distichoptilum gracile	2	687	1	757
Protoptilum sp.1	3	687	1	757
Family Pteroeididae				
Gyrophyllum sp.1	2	687	2	749
Family Scleroptilidae				
Scleroptilum grandiflorum	1	688	1	748
Family Umbellulidae				
Umbellula carpenteri	3	688	4	739
Umbellula encrinus	1	685	1	739
Umbellula huxleyi	3	688	3	733
Umbellula magniflora	1	685	3	739
Umbellula monocephalus	1	689	1	758
*Umbellula thomsoni***	1	703	1	739
*Umbellula thomsoni***	1	703	1	745
Umbellula sp.1	1	689	1	752
Umbellula sp.2	1	684	1	752
Umbellula sp.3	1	-	1	-
Umbellula sp.4	1	-	1	-
Umbellula sp.5	1	-	1	-
Umbellula sp.6	4	-	1	-
Family Virgulariidae				
Virgularia mirabilis	2	712	2	749
Total	50	41	48	39

Seq.d, number of sequences obtained for each taxon; Bp, indicates sequence length, expressed in number of nucleotides (base pairs); - indicates where sequencing was unsuccessful after amplification; *, sequences showed no variation between these two species for *ND2*; **, sequences differed between members of this species (*msh1* only).

A total of 30 sequences of both *msh1* and *ND2* mitochondrial protein-coding genes combined, corresponding to 24 distinct genotypes of 11 pennatulid genera and 9 families were determined for this study (Table 2.4). A further 11 sequences for *ND2* and 9 for *msh1* were obtained (also Table 2.4). Wherever possible, at least two representatives of each species were sequenced. In nearly all cases, sequences were identical between individuals of the same species, however where two sequences differed, both sequences were included in the phylogenetic analysis (viz. *U. thomsoni*). Five pennatulid sequences taken from GenBank were incorporated in the analysis of both genes, bringing the total number of genera in this study to 14, representing 10 of the 15 pennatulid families. An additional *ND2* sequence (*Anthoptilum murrayi*) was also obtained from GenBank.

The *ND2* gene fragment was found to be less variable than *msh1*: *ND2* sequences of *P. aculeata* and *P. phosphorea* were invariant between individuals, whereas the corresponding *msh1* sequences revealed differences in haplotype (marked by * in Table 2.4). This was also the case for *U. thomsoni*: *msh1* showed interspecific variation, whereas *ND2* sequences were invariant among this species (marked by ** in Table 2.4). This implies that *msh1* is less conserved than *ND2* in pennatulids and provides further evidence that the *msh1* gene evolves faster than other mitochondrial genes (France and Hoover, 2001).

2.3.3 Alignments

The new *ND2* fragments ranged from 684 to 717 nucleotides in length (Table 2.4). The alignment of all *ND2* sequences revealed 3 insertions/deletions (indels). Differences in length were mainly attributable to a large indel near the 3' end of the fragment, with noteworthy insertions of 8 amino acids (24 base pairs) in *Anthoptilum* spp. and *Virgularia mirabilis* sequences. This variable region was removed in the final analysis, however, when aligned sequences were trimmed to match those shorter ones from GenBank. *Umbellula* spp. displayed a deletion of one amino acid (leucine) near the 3' end, representing those species without sclerites in the polyp/rachis tissue. *Halipteris finmarchica* possessed a unique deletion of 7 bp.

The new *msh1* fragments ranged from 733 to 776 nucleotides in length (Table 2.4). The alignment of all *msh1* sequences revealed a higher number of indels than *ND2*. A highly variable region near the 3' end of the fragment consisted of a unique insertion for *Anthoptilum* sp.1 followed by a number of insertions/deletions for all taxa. As found for *ND2*, *Umbellula* presented indels corresponding to those species with and without sclerites in the polyp/rachis tissue.

Despite these and other indels, nucleotide sequences of both genes maintained the correct reading frame, and therefore gaps were not removed from the alignments for phylogenetic analysis. Phylogenetic trees with the gaps removed produced similar topologies, but the basal nodes remained unresolved polytomies. The nucleotide alignment of the two genes combined was 1578 bp in length and included 719 bp of *ND2* and 859 bp of *msh1* (gaps treated as fifth characters). The final nucleotide alignment with the shorter GenBank sequences included, however, was 1196 bp in length (465 bp and 731 bp for *ND2* and *msh1*, respectively). Of these 1196 nucleotides, 865 characters were invariant, and 197 of 331 variable sites were parsimony-informative (gaps treated as 'missing').

2.3.4 Outgroup

Two members of the sea fan family (Ellisellidae), *Ctenocella barbadensis* and *Verrucella* sp. (GenBank), were used to root the trees for analysis containing all sequences herein analysed. This outgroup was chosen based on strong evidence to suggest the ellisellids are the sister taxon to the pennatulids (McFadden et al., 2006), and follows the suggestion that outgroups should be monophyletic with the ingroup in a wider phylogenetic context (Smith, 1994).

2.3.5 Trees

For the combined dataset of *ND2* and *msh1* partial sequences, Bayesian (Fig. 2.2), maximum parsimony (Fig. 2.3), maximum-likelihood (App. Fig. A1), and neighbour-joining (App. Fig. A2) analyses all recovered very similar topologies.

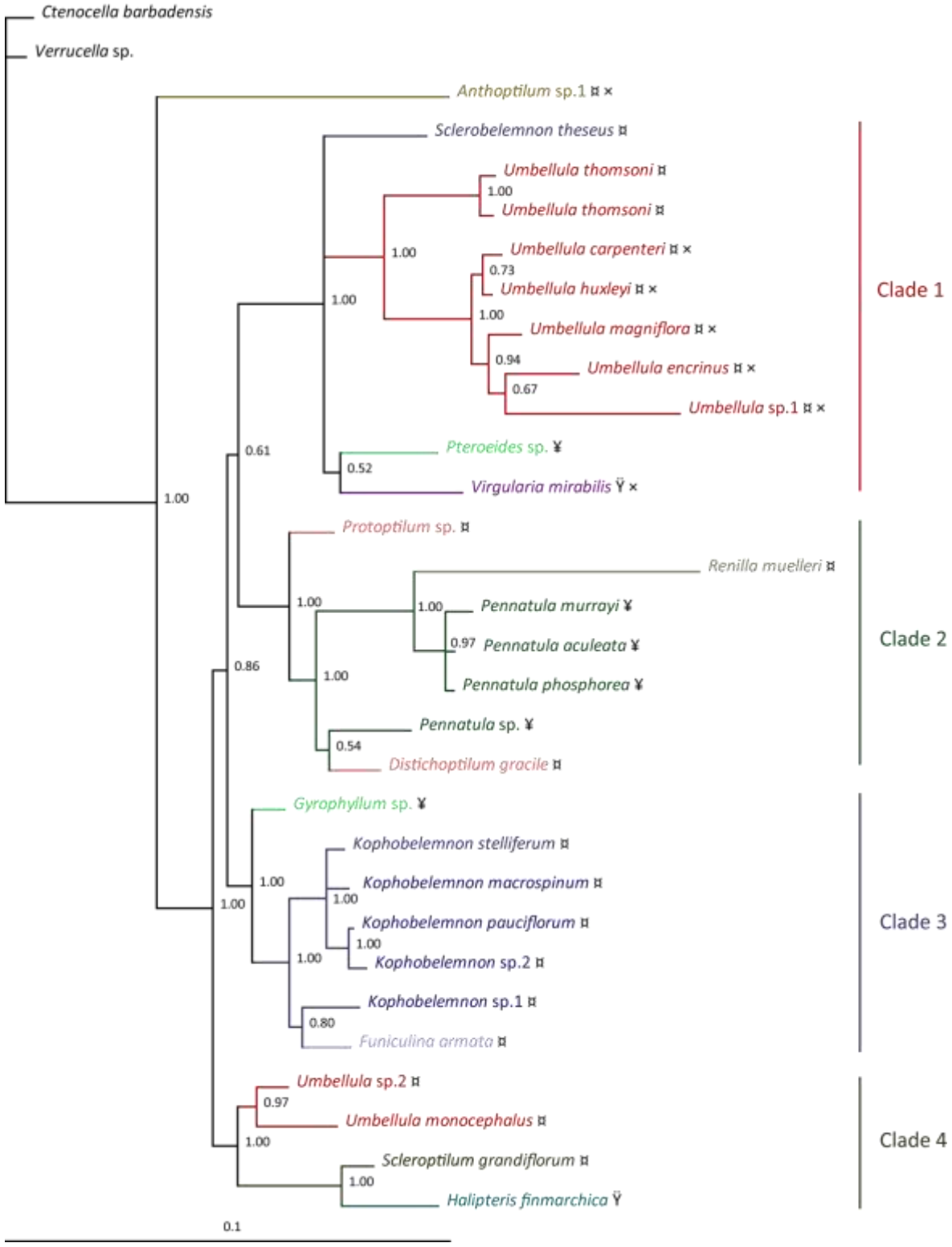

Figure 2.2 Phylogenetic relationships among 10 families in O. Pennatulacea for the combined analysis of *ND2* and *msh1*. Bayesian likelihood tree, 50% majority-rule consensus of 35,622 trees (10^7 generations; burnin=10,000); values at nodes are posterior probabilities; scale bar is the expected changes per site. Colours represent families; ¤ Sessiliflorae; ¥ Subselliflorae (polyp leaves); Ÿ Subselliflorae (polyp ridges); × Sclerites absent from polyps and rachis.

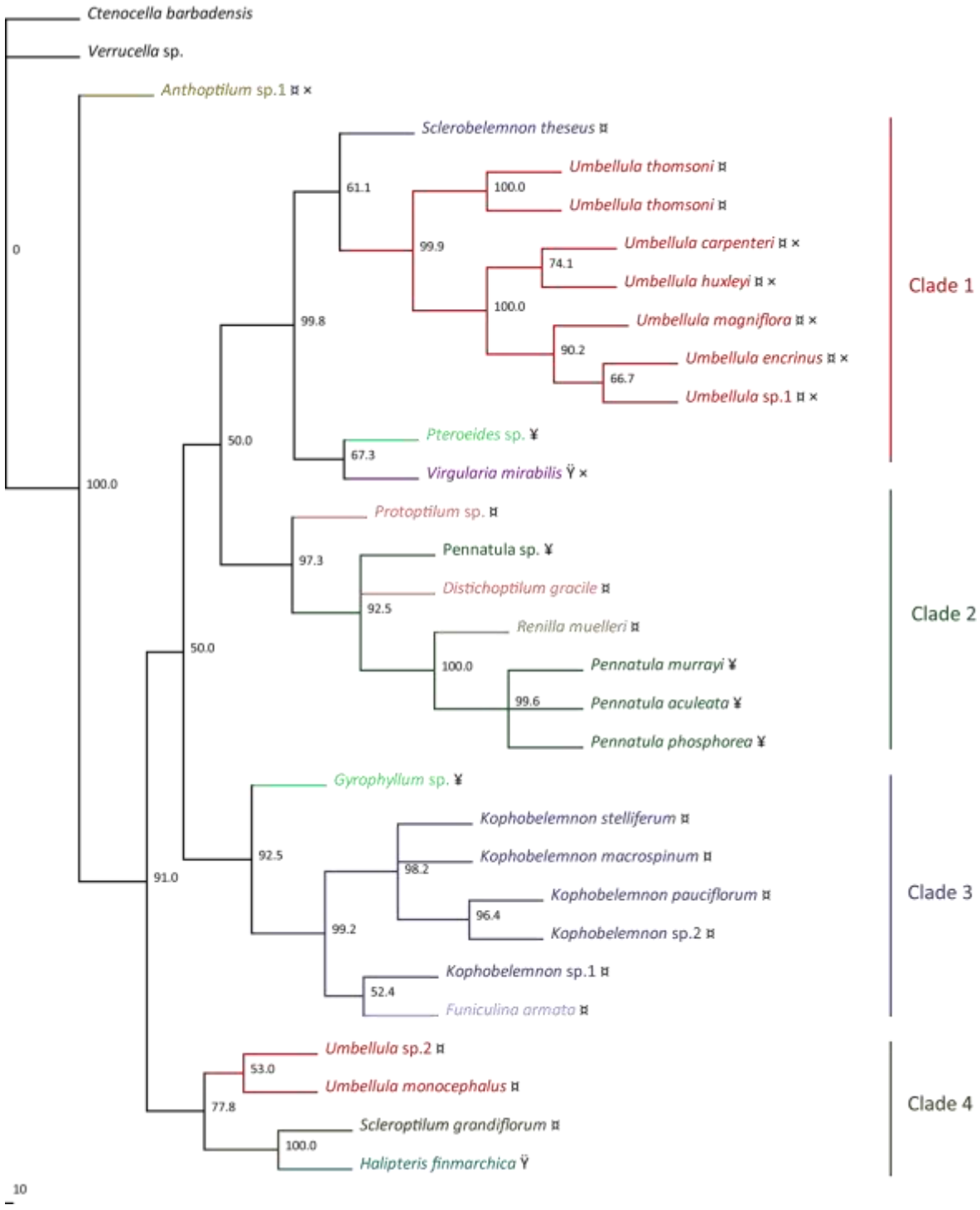

Figure 2.3 Phylogenetic relationships among 10 families in O. Pennatulacea for the combined analysis of *ND2* and *msh1*. Maximum parsimony tree, 50% majority-rule consensus (length = 579, consistency index = 0.66, retention index = 0.76; and homoplasy index = 0.34); values at nodes are percentages from 1000 bootstrap replicates with maxtrees = 100; scale bar indicates number of nucleotide changes. Colours represent families; ¤ Sessiliflorae; ¥ Subselliflorae (polyp leaves); Ÿ Subselliflorae (polyp ridges); × Sclerites absent from polyps and rachis.

Phylogenetic analysis of each gene separately produced trees similar in topology but only weakly supported or less resolved relative to the combined trees (App. Fig. A3; A4): *ND2* proved to be the less informative of the two genes. Single-gene analyses however, allowed the examination of the phylogenetic positions of some taxa for which it was possible to sequence only one of the two genes. As such, the results presented herein are the combined data for Bayesian and maximum parsimony analyses, unless otherwise stated.

The majority of the nodes that were well supported (>86%) by the Bayesian posterior probabilities (Fig. 2.2) also had strong support (>77%) from maximum parsimony bootstrap values (Fig. 2.3).

Four distinct and well-supported clades of pennatulids were recovered in all analyses. The largest clade included *Sclerobelemnon theseus* (F. Kophobelemnidae), most members of the monogeneric family, Umbellulidae, as well as *Pteroeides* sp. (F. Pteroeididae) and *Virgularia mirabilis* (F. Virgulariidae). A second clade included all members of F. Pennatulidae (represented here by *Pennatula* spp.), F. Protoptilidae (*Protoptilum* sp. and *Distichoptilum gracile*), and F. Renillidae (*Renilla muelleri*). The third clade was represented by *Gyrophyllum* sp. (F. Pteroeididae), all *Kophobelemnon* spp. (F. Kophobelemnidae), and the monogeneric F. Funiculinidae (*Funiculina armata* and *Funiculina quadrangularis*, the latter of which only *msh1* sequences were obtained, App. Fig. A3). The fourth clade included two further members of Umbellulidae, plus F. Scleroptilidae and F. Halipteridae (*Scleroptilum grandiflorum* and *Halipteris* sp., respectively). To simplify, these clades are referred to as Clades 1 to 4.

Anthoptilum sp.1 (F. Anthoptilidae) separated from the above-mentioned clades and occupied a basal position to all other pennatulids analysed. The exclusion could be expected as the aligned *msh1* fragment displayed a large and unique insertion (as mentioned in Section 2.3.3). On the basis of analyses of *ND2* alone (App. Fig. A4) two additional species, viz. *A. murrayi* (GenBank) and *Anthoptilum* sp.2, clustered together, but their relationship with *Anthoptilum* sp.1 was unresolved, like many of the taxa in these trees based on *ND2* only. However, analysis of *ND2* data, before sequences were further trimmed for the inclusion of the (shorter) GenBank sequences, produced trees in which *Anthoptilum* sp.2 occupied a basal position (trees not presented here). Thus, the

monogeneric F. Anthoptilidae can be considered one of the most primitive of pennatulid families.

Further pertaining to *Anthoptilum* sp.1, the consensus tree built by Bayesian inference produced a long branch for this taxon. However, the problem of 'long-branch attraction' is reduced in larger phylogenies such as those presented in this study. Moreover, the overall topology of the tree is identical to that created through maximum-parsimony (as well as maximum likelihood and neighbour-joining analyses, App. Fig.s A1; A2), in which a shorter branch was resolved for *Anthoptilum* sp.1. As such, conclusions regarding the speed of evolution in *Anthoptilum* cannot be made with any certainty without testing for uniform rate of evolution among taxa.

The results provide no evidence for the division of O. Pennatulacea into the suborders Subselliflorae (taxa with polyps located on polyp leaves or raised ridges) and Sessiliflorae (taxa with polyps emanating directly from the rachis) as proposed by Kükenthal and Broch (1911) and Kükenthal (1915) (specific aim #2), thus these divisions can be considered of nominal value only.

Clade 1

This clade was dominated by all but two members of the monogeneric family, Umbellulidae (*Umbellula* spp.), which was further split into two groups: those with sclerites in the polyp/rachis tissue, and those without. The position of *Umbellula* spp. in the trees was well supported and strongly suggests that this group of exclusively deep-sea taxa is of most recent descent: those without sclerites being the most derived.

Pteroeides (F. Pteroeididae) and *Virgularia* (F. Virgulariidae) form the sister taxa to *Umbellula*. Although the relationship between these two genera was only moderately supported, they consistently grouped together within this clade in all analyses.

The relationship of *Sclerobelemnon* to the other pennatulids was poorly resolved, though all trees inferred that *Sclerobelemnon* is not of the family Kophobelemnidae, as currently classified.

Clade 2

Pennatula spp. divided into two separate groups within this clade: this provides strong evidence for synapomorphic traits within this morphologically-distinct genus. Furthermore, the topology of this clade implies that *Protoptilum* is the least derived taxon of the group: *Distichoptilum*, *Pennatula* and the shallow-water sea pansy, *Renilla*, having more recently evolved from a common, *Protoptilum*-like ancestor.

The consensus tree built by Bayesian inference produced a long branch for *Renilla muelleri*. Liken to that of *Anthoptilum* sp.1, a shorter branch was resolved for *Renilla* in those trees created through maximum-parsimony, as well as maximum likelihood and neighbour-joining analyses (App. Fig.s A1; A2), and thus 'long-branch attraction' is not considered a problem (see above).

Clade 3

Gyrophyllum sp. is the most primitive taxon of this clade: the trees suggest that *Kophobelemnon* and *Funiculina* descended from a *Gyrophyllum*-like ancestor. Furthermore, all *Kophobelemnon* spp. and the monogeneric F. Funiculinidae, represented here by *Funiculina armata*, form a well supported group within this clade in all analyses. This is further supported on the basis of analyses of *msh1* alone, where the additional species, *Funiculina quadrangularis* (App. Fig. A3) formed a close relationship with *Funiculina armata* and *Kophobelemnon* sp.1. This strongly suggests that *Kophobelemnon* and *Funiculina* belong to the same family and that Kophobelemnidae (Gray, 1860) is the senior synonym of Funiculinidae (Gray, 1870).

The separation of *Gyrophyllum* and *Pteroeides* (Pteroeididae) in all analyses provides strong evidence to suggest these two genera are not members of the same family.

Clade 4

The inclusion of two members of *Umbellula* in this clade strongly suggests that F. Umbellulidae, and therefore the genus *Umbellula*, is polyphyletic. The position these species occupy in the trees infers that these members of *Umbellula* are primitive in

relation to the majority of the pennatulids analysed, and differentiated earlier than the many other *Umbellula* species. The separation is surprising considering *Umbellula* spp. are morphologically distinct from all other pennatulids: species of this genus have exceptionally large and localised polyps, situated at the most distal portion of the rachis. Furthermore, these traits are considered highly specialised and adapted.

The topology of all trees suggests a close relationship between *Halipteris finmarchica* Sars 1851 (F. Halipteridae, Williams, 1995b) and *Scleroptilum grandiflorum* Kölliker 1880 (F. Scleroptilidae, Jungersen, 1904), which clearly form a separate, well-supported group in all analyses. This provides strong evidence to suggest that these morphologically similar taxa should be reclassified under the junior family synonym, Scleroptilidae.

1.4 Discussion

The recent collections of pennatulids for molecular analysis, representing a suite of taxa of wide geographic and bathymetric scope, have enabled a reassessment of the systematics and phylogenetic relationships of the 10 of the 15 families on a genetic level.

Phylogenetic analysis of partial sequences from the NADH-dehydrogenase subunit 2 (*ND2*) and the mutS homologue (*msh1*) combined produced well-supported phylogenetic relationships for representative deep-sea (and shallow-water) pennatulids at familial, generic and specific taxonomic levels. Bayesian, maximum parsimony, maximum-likelihood, and neighbour-joining analyses all recovered very similar topologies for a combined dataset, and *ND2* and *msh1* genes analysed separately. However, *ND2* was found to be more conserved than *msh1*, suggesting that the latter evolves faster and is the more phylogenetically informative of the two genes.

1.4.1 Phylogeny

Williams (1992b) postulated that the veretillid genera of the shallow-water tropics possess the most plesiomorphic characters of all extant pennatulids: radially arranged and evenly distributed polyps that emanate directly from a short rachis, which are fully

retractile; and smooth sclerites. O. Pennatulacea diversified in these tropical seas from a veretillid-like ancestor, subsequently differentiating and dispersing to great depth and away from the tropics into temperate and polar regions. Highly derived taxa also occupy shallow-water tropical seas and are thus sympatric with the more primitive forms.

While the data presented in this study do not include any members of Veretillidae and are mainly focused on deep sea pennatulids, historical patterns surmised by Williams (1992b) are still evident. It is clear that highly-derived taxa exist both in shallow- and deep-water. The genus *Umbellula*, which are an exclusively deep sea and cosmopolitan taxon, represents one of the more diverse groups, many species of which are perhaps the most advanced, thus supporting the hypothesis that various taxa radiated and diversified in the deep sea, some of which are of (most) recent descent. Williams (1992b) suggested that of the shallow-water forms, *Virgularia* and *Pteroeides* are highly modified based on the development of polyp leaves/ridges. Molecular data are congruent with this, trees also inferring that *Renilla* may be added to the list. Moreover, molecular data have revealed that *Renilla* evolved from a *Protoptilum*-like ancestor (as too did *Distichoptilum* and *Pennatula*). Thus, while pennatulids may have initially diversified and radiated from the shallows (Williams, 1992b), many may have subsequently differentiated and dispersed from the deep sea into shallow water.

Contrary to Williams (1992b; 1995a), molecular data suggest that many *Umbellula* species have evolved more recently than *Pteroeides*, *Virgularia* and *Pennatula*. While Williams (1995a) also considered *Umbellula* to be highly derived, he hypothesised that the shallower water family, Pteroeididae, is the most evolutionary advanced group based on both the presence of well-developed polyp leaves and the restriction of the siphonozooids to the polyp leaves: all other pennatulids have siphonozooids present on the rachis. Yet it is now understood that the more derived *Umbellula* share a common ancestor with both *Pteroeides* and *Virgularia*, demonstrating on a genetic level that concentration and localisation of sessile feeding polyps, and loss of sclerites in the rachis/polyps, are more recently evolved morphological adaptations than the characters of polyp leaves (with fully retractable polyps) and siphonozooid zonation. Renillidae (the sea pansy) was considered primitive, and a close relation to Veretillidae and Echinoptilidae, having more characteristics in common with Echinoptilidae than any other pennatulid family (Pérez and Ocampo, 2001). Contrary to this, molecular data presented

here tell us that *Renilla*, with its foliate rachis is, in fact, highly derived and is closely related to Pennatulidae.

Of the deeper water taxa, *Sclerobelemnon* was considered the most primitive based on its short rachis (rachis elongation being a derived character state) and the presence of multiserial polyps: biserial, whorled, clustered and those on leaves/ridges being highly derived traits (Williams, 1992b; 1997b). Yet genetic analysis has revealed that *Sclerobelemnon* may not be quite as primitive as previously thought. Although its position in the tree was not supported with high bootstrap values, it can be inferred that *Sclerobelemnon* is more derived than, for example, *Gyrophyllum* (F. Pteroeididae) with its fleshy, polyp leaves. The bathyal family, Anthoptilidae, on the other hand, occupied a basal position in the trees, thus suggesting that this monogeneric family comprises of some of the most primitive of deep water pennatulids: such colonies are elongated, with non-retractile polyps directly emanating from the rachis. Curiously, members of Anthoptilidae do not possess sclerites in their rachis/polyps which suggests that extinct taxa may have also lacked this trait and that the presence of sclerites in many families is in fact a derived state (as too is the subsequent loss of sclerites in *Umbellula*, see below).

Analysis of mitochondrial genes clearly demonstrates the high frequency of homoplasy in pennatulids. The most obvious example is in *Umbellula* (monogeneric F. Umbellulidae), a group that is morphologically distinct from any other pennatulid genera/family with its polyps localised in a cluster at the most distal portion of the colony. *Umbellula* underwent convergent evolution from two different lineages, indicating on a genetic level that localisation of feeding polyps is a synapomorphic trait: Umbellulidae comprises of some of the most primitive and most recently evolved taxa. The adaptation of sclerite loss, as seen in many *Umbellula* species, can be considered apomorphic within this genus, as these species evolved from a recent, common ancestor within a single lineage. Anthoptilidae and *Virgularia* also lack rachis/polyp sclerites, thus this character can be considered synapomorphic in relation to other pennatulid families/genera. Yet, as mentioned above, members of Anthoptilidae are the most primitive of pennatulids herein analysed, thus the loss of sclerites can be considered a reversal to a more primitive state in both *Virgularia* and many *Umbellula* species. The derived nature of polyp leaves/ridges expressed in many genera (*Pteroeides*, *Pennatula*, *Virgularia*, *Gyrophyllum* and *Halipteris*) can be considered synapomorphic as these taxa follow several different lineages. Within

Pennatula, there too are synapomorphic traits, manifested as tubular autozooids with spiculiferous calyces and terminal teeth that emanate from lateral leaves: these features could be considered a function of parallel evolution since *Pennatula* spp. divided into two groups in the trees. Furthermore, the trees suggest that *Kophobelemnon* and *Funiculina*, taxa that both have polyps emanating directly from the rachis, derived from a *Gyrophyllum*-like ancestor. Such reversals infer that the character of polyps emanating directly from the rachis is not always a symplesiomorphic trait i.e. a shared primitive state, but is also a synapomorphy (shared derived state) in some taxa.

Although more evidence is required, it could be that O. Pennatulacea originated and diversified in the deep sea, and subsequently invaded shallow waters: the deep-sea family, Anthoptilidae, occupied a basal position on the trees, suggesting that this may be the case. Moreover, deep-sea and shallow-water taxa group together in two clades (Clades 1 and 2), and considering the positions the shallow taxa occupy within the trees, the invasion of the shallows from the deep may have occurred on at least two occasions. A recent phylogenetic study on stylasterid corals found similar results. Data suggested that this important group of tropical shallow-water fauna may have evolved from deep-water ancestors; and invaded the shallow-water tropics three times, with one additional invasion of the shallow-water temperate zone (Lindner et al., 2008).

1.4.2 Systematics and Classification

The high frequency of homoplasies (parallelisms, convergences and reversals) outlined above have led to misleading (morphological) evidence of relationships between genera and, consequently, many pennatulids have been misclassified (Kölliker, 1880; Kükenthal and Broch, 1911; Kükenthal, 1915; Hickson, 1916; Williams, 1995a). This problem has been exacerbated by the sheer paucity of morphological characters of rigorous taxonomic value.

Kükenthal and Broch (1911) and Kükenthal (1915) developed a higher classification scheme for O. Pennatulacea, consisting of two suborders and six sections (equivalent to Superfamily rank). The suborder Subselliflorae encompassed those pennatulids with a feather-like appearance where polyps are positioned on leaves or raised ridges. Those

belonging to the much larger suborder, Sessiliflorae, lack leaves/ridges and instead polyps emanate directly from the rachis. The sections are based on growth form, whether radiate, foliate, biserial, verticillate, rush-shaped and feather-shaped. However, the more recent identification and comparison of characters of many pennatulids has shown that this higher classification scheme, together with the work of Kölliker (1869; 1880) and Studer (1901) regarding the subordinal, familial and subfamilial levels, is problematic and largely inadequate (Williams, 1992b; 1995b; 1997b).

Williams (1995b) suggested that Sessiliflorae should be considered a paraphyletic taxon, since it is based on the symplesiomorphy of polyps arising directly from the rachis and thus does not contain all descendants from a common ancestor. Whilst molecular data support the division of Sessiliflorae, the group should in fact be considered polyphyletic since it does not contain the most recent common ancestor of all its members; and the character of polyps emanating directly from the rachis is an apomorphic state in some families (viz. Kophobelemnidae and Funiculinidae). Williams (1995b) on the other hand, considered the Subselliflorae as a holophyletic clade based on synapomorphy of polyp leaves, adding that *Renilla* represents an autapomorphic clade and thus forms a natural group. Molecular analysis, however, clearly separated the Subselliflorae, thus the classification into two suborders has nominal value only (as too are the sections, Williams, 1995b).

Historically, there has been much discussion concerning how to classify the members of the families Pennatulidae and Pteroeididae. Kölliker (1869) originally unified the subfamilies 'Pennatulinae' and 'Pteroeidinae' into one family and then subsequently elevated the status of the subfamilies to separate families (Kölliker, 1880). Studer (1901) placed the new genus *Gyrophyllum* in the family Pteroeididae, but Kükenthal and Broch (Kükenthal and Broch, 1911) disputed this stating the presence of three-flanged sclerites are characteristic of the family Pennatulidae; Kükenthal (1915) made distinction between Pennatulidae and Pteroeididae based on sclerite morphology. More recently, Williams (1995a) recognised that *Gyrophyllum* represents a morphological intermediate between the two families and suggested that Pennatulidae and Pteroeididae represent a single holophyletic taxon. On this basis, it was proposed that only one family be recognised, the Pennatulidae, comprising the six genera *Gyrophyllum*, *Pennatula*, *Ptilosarcus*, *Sarcoptilus*, *Crassophyllum*, and *Pteroeides*. Genetically, however, these do not form a natural group,

and instead *Gyrophyllum* and *Pteroeides* (Pteroeididae), and *Pennatula* (Pennatulidae) separated into three different clades. Further work incorporating more species representatives is required to make any firm conclusions on the reclassification of these three genera, but it seems that *Gyrophyllum* may form a family in its own right, 'Gyrophyllidae'. There is now some evidence to suggest a close relationship between *Pteroeides* and *Virgularia*, and as such, these genera could be considered members of the same family.

Molecular data have revealed other inconsistencies with our current understanding of pennatulid systematics. In the past, *Protoptilum* and *Funiculina* were considered members of closely related families (Kükenthal and Broch, 1911; Williams, 1997b), but molecular evidence strongly suggests that *Protoptilum* is a primitive taxon that shares a common ancestry with the more recently evolved *Pennatula*. Moreover, *Funiculina* and *Kophobelemnon* are closely related and could therefore be considered members of the same family (junior synonym, Kophobelemnidae, Gray, 1860). *Sclerobelemnon*, which is currently classified under the family Kophobelemnidae because of its morphological affinities with *Kophobelemnon*, is not closely related to the other members the family. Conversely, *Halipteris finmarchica* (F. Halipteridae) and *Scleroptilum grandiflorum* (F. Scleroptilidae) are very closely related, which suggests that these morphologically similar taxa should be reclassified under the junior family synonym, Scleroptilidae (Jungersen, 1904).

1.4.3 Conclusions

This study is the first of its kind and provides important information on the evolutionary relationships among O. Pennatulacea. Variation in *ND2* and *msh1* mitochondrial protein-coding genes is adequate to resolve phylogenetic relationships among pennatulid families; *msh1* is a more rapidly evolving gene, and thus useful in differentiating between all genera, and many (if not all) pennatulid species, and in combination with *ND2*, these genes resolve all subordinal taxonomic levels.

Molecular data are congruent, on the whole, with current classification and previous phylogenetic reconstructions of O. Pennatulacea based on morphological characters

(Williams, 1992b; 1995b; c; 1997b). Discrepancies are evident concerning the finer details for some families and genera.

Genetic analysis gave strong support that highly-derived taxa occur in both shallow- and deep-water, together with more primitive pennatulid species. Furthermore, these new data suggest that many taxa may have differentiated and dispersed from the deep sea to shallow water: for example Renillidae, which has been considered one of the most primitive shallow-water families, is highly derived. Thus, the results reveal that shallow-waters are not only a source of pennatulid diversity (Williams, 1992b), but have accumulated species and lineages from the deep sea. Although more evidence is required, it could be that O. Pennatulacea originated and diversified in the deep sea, and subsequently invaded shallow waters, on at least two occasions.

This study supports the findings of many previous authors that the following characters are apomorphic: sessile polyps; complete loss of sclerites in the feeding polyps and rachis; and clustering of polyps or the presence of polyp leaves and raised ridges. However, reversals in evolution have led to taxa that possess derived character states that are analogous with plesiomorphic (primitive) traits, thus making phylogenetic reconstructions based on morphology problematic.

The high frequency of homoplasy in pennatulids has led to many misinterpretations, in terms of the systematics of the group: the traditional classification system still holds true (if only for nominal value), but it is clear that without a (more) comprehensive dataset, any inferences made regarding systematics are limited. Despite this, it can be concluded that many families (and genera) of pennatulids do not represent monophyletic groups. The suborders Sessiliflorae and Subselliflorae are polyphyletic and thus are of nominal value only. This too is the case for members of the families Pennatulidae, Pteroeididae, and Kophobelemnidae whose classification is in need of revision. Halipteridae is possibly synonymous with Scleroptilidae, and Funiculinidae with Kophobelemnidae.

Chapter Three

A Systematic Account of the Genus *Umbellula* (Pennatulacea: Umbellulidae)

A revision using morphological, molecular and distributional data with descriptions of three new species

3.1 Introduction

Pennatulid systematics is an equivocal and disputative area; our knowledge concerning this order is far from adequate and a great deal of further research is required to attain the objective of worldwide synthesis of the classification of this diverse group. Within the order Pennatulacea, the family Umbellulidae contains the single genus *Umbellula*, the species of which represent the most enigmatic and unusual of pennatulids. Colonies of Umbellulidae possess a long, slender stem: the autozooids of which are uniquely clustered at the extreme upper end of this, rather than distributed down the length of the colony as in all other families. Mature colonies can possess forty or more autozooids, or as little as one; autozooid leaves and calyces are absent, and thus anthocodiae are non-retractile; siphonozooids are present on the rachis at the base of the autozooids, below the terminal autozooid-cluster and on the stem; sclerites are only present in some species, and totally absent in others (with the exception of minute oval bodies often present in the peduncle); and the conspicuous axis is present throughout the colony, being round or quadrangular in cross-section.

In 1752 the Jutland voyager Adrians, Captain of the whaling vessel *Brittania*, captured two peculiar looking specimens from 432 m depth off the coast of Greenland. This was the very first discovery of the extraordinary *Umbellula*: “Each of the two plants was broken into three pieces, which accident, however, did not hinder me from laying it before me according to its complete form and size” (from Gray, 1860). The specimens were dried, and the larger handed to the Englishman John Ellis (1753), by whom it was described in his work on Corallines. The smaller of the two was passed to the German, Christlob

Mylius and described in 'An Account of a new Zoophyte' (1754), and referring to Ellis' findings wrote: "He saw a Number of Animals where I had seen a Flower; he saw so many Polypules, as I had seen Pieces of the Flower; he took that for a Supporter of the Polypules, what I had taken for a Stalk; and called Eggs, what I had called Seeds. I was increasing the Vegetable Kingdom, by adding a new Subject, and he was enlarging the Number of Animals".

Ellis (1753; 1755) found the animal to be a species of "cluster-polype", or "*Hydra marina arctica*". Following comparison with the '*Encrinos*' or '*Lilium lapidem*' of the palaeontologists, he gave good justification for its standing as a separate genus. Linnaeus (1767) classified it as "*Vorticella encrinus*", though it was finally named "*Ombellula*" by Cuvier (1798). The misnomer "*Umbellula*" was originally scribed by Gray (1870) while cataloguing the collection of the British Museum (London) yet subsequent authors continued with this spelling. For reasons of long term and widespread usage, this study employs the incorrect spelling, *Umbellula*. Unfortunately, both original specimens have disappeared and imperfect descriptions are all that remain.

More than a century passed before this remarkable genus was rediscovered. In 1871, a further two specimens of *Umbellula* were sampled during the Swedish Expedition to Greenland and Newfoundland. Supposedly differing from the first (now known as *Umbellula encrinus* Lindahl, 1874), Lindahl (1874) described two new species, viz. *U. miniacea* and *U. pallida*, which were later synonymised by Kölliker (1875) under the name *U. lindahli*. Kölliker (1880) undertook the description of the pennatulids collected by the Challenger Expedition in 1873-1876. He described eight new species of *Umbellula*: *U. durissima*, *U. güntheri*, *U. thomsoni*, *U. leptocaulis*, *U. huxleyi*, *U. carpenteri*, and *U. magniflora*. Danielssen and Koren (1884) presented a detailed taxonomic account based on twelve specimens of *U. encrinus* in various stages of development, obtained from the Norwegian North Atlantic Expedition in 1876-1878. Other significant contributions to taxonomic descriptions of *Umbellula* include Jungersen (1904), Danish Ingolf-Expedition, 1895-1896; Kükenthal and Broch (1911), *Valdivia* Expedition, 1898-1899; Kükenthal (1915); Hickson (1916), *Siboga* Expedition, 1899-1900; Broch (1957), Swedish Deep-Sea Expedition, 1947-1948; Broch (1958), *Discovery* Expedition, 1927-1937; and Pasternak (1962; 1964; 1975; 1993).

The systematics of the genus *Umbellula*, which contains forty-two species, is still unclear despite the repeated attempts of revision (Kükenthal, 1915; Hickson, 1916; Broch, 1958; Pasternak, 1962). A substantial amount of confusion has arisen in the literature since findings of this genus were rare and so sporadic that each individual discovery was often described as a new species. Moreover, new species descriptions from isolated specimens led to a general lack of knowledge concerning variability, thus sufficient valuable diagnostic features for the species were not recognised. The paucity of morphological characteristics of true taxonomic value in distinguishing between *Umbellula* species has further complicated the situation: often characters that are correlated with dimensions of colony anatomy or number of autozooids have been used inappropriately. This method of distinguishing between species can be ambiguous and unreliable since these 'characters' may be altered depending on the degree of contraction or differences in ontogenetic stages. Furthermore, the majority of the species were not sufficiently described, diagnoses were short, and accompanying figures were of poor quality. Such factors led to a proliferation of putative species in the literature, which is the most regrettable example of unjustified splitting into species in the whole group of pennatulids.

Realising the necessity of a revision, Kükenthal (1915) accounted thirty-five species, fifteen of which he regarded as ambiguous in an attempt to reduce the number of nominal species. Hickson (1916) recognised that *Umbellula* spp. fall into two main groups, viz. those with sclerites, and those without. However, he was of the opinion that the shape of the axis, whether quadrangular or round in cross-section, was only of subordinate value taxonomically and believed all *Umbellula* spp. lacking sclerites to be genetically the same, thus synonymising many species including *U. pellucida* (quadrangular axis) with *U. huxleyi* (round axis) (Hickson, 1937). The problem of species misnomers and axis shape was exacerbated by incorrect descriptions, where the shape of specimens' stems or peduncles with their cover of soft tissue had been described instead of the internal axis (Marshall, 1887), or where axes described as 'square/quadrangular with rounded edges' (essentially 'round') were taken to be the same form as the quadrangular axes possessing four longitudinal grooves (Hickson, 1916).

The work of Broch (1958) remains the most comprehensive revision of *Umbellula*, in which he defined species based on non-variable features such as presence/absence of

sclerites and their form, and the shape of the axis in cross-section. He bravely synonymised many taxa, recognising seven species of *Umbellula*: *U. durissima*, *U. thomsoni*, *U. huxleyi*, *U. spicata*, *U. pellucida*, *U. lindahli*, and the type species of the genus, *U. encrinus*, although later he regarded this as an ecological variant of *U. lindahli* (Broch, 1961). Pasternak (1962) undertook an extensive review of Antarctic and sub-Antarctic *Umbellula*, disagreeing with Broch's (1958) synonymy of *U. antarctica* with *U. lindahli*, arguing that they are morphologically distinct species. Pasternak later went on to describe two new species, *U. monocephalus* (Pasternak, 1964) and *U. hemigymna* (Pasternak, 1975). Presently, at least nine species are considered valid (Williams, 1995b): *U. encrinus*, *U. lindahli*, *U. pellucida*, *U. huxleyi*, *U. spicata* without sclerites; and *U. durissima*, *U. monocephalus*, *U. thomsoni*, *U. hemigymna* with sclerites.

Aims and Objectives

It is recognised that extensive collecting in many different geographical localities and detailed comparison of material is necessary to assess the degree of variation in many taxa due to genetic, geographic, or ecological differences (Williams, 1990). With a vast collection of *Umbellula* spp. from the NE Atlantic and numerous additional specimens from all world oceans, together with type specimens, genetic data, and a critical study of the literature pertaining to *Umbellula*, the present work aims at revising the systematics of this baffling genus. A dichotomous key (plus a glossary of pennatulid terms) and a detailed synopses of fifteen species of *Umbellula* incorporating emended diagnoses, is presented, and includes three species new to science. Additionally, distributional and bathymetric information is examined for nearly all nominal species to illustrate patterns in occurrence, once assigned the true species name.

The present work is not only aimed for specialists in the discipline of octocoral systematics, but also offers a guide for other biologists in the identification of material from benthic surveys and other studies.

3.2 Materials and Methods

3.2.1 Specimens

From a variety of research cruises over the period of June 1974 to July 2007, 257 individuals of *Umbellula* were studied (Table 3.1; Fig. 3.4, Section 3.3.6). Most specimens formed part of the extensive Discovery Collections (National Oceanography Centre, Southampton, UK) obtained from the NE Atlantic: these were fixed at sea in formalin (borax-buffered 4 % formaldehyde in seawater) and transferred 70 % propan-2-ol. Further material, preserved in 96 % ethanol for genetic analysis, was collected during the following research cruises and sources: the Benthic CROZET cruise (D300) aboard the RRS *Discovery* (National Oceanography Centre, Southampton); several specimens acquired by Edward McCormack (Marine Institute, Galway) from the NE Atlantic; five specimens from Marguerite Bay, Antarctica, collected during *James Clarke Ross* cruise 166 with the ROV *Isis* (National Oceanography Centre, Southampton); specimens obtained from the NE Atlantic during HERMES cruises aboard RRS *James Cook* (JC10 and JC11) with the ROV *Isis* (National Oceanography Centre, Southampton); and a further two specimens were obtained from the Indian Ocean on board *The Performer* by P. Tyler (National Oceanography Centre, Southampton). All material is housed at the National Oceanography Centre, Southampton (UK). In addition, for genetic analysis a piece of tissue from a colony of *U. encrinus* was acquired courtesy of the Scottish Association for Marine Science, Oban, UK.

Specimens were examined by means of a stereo microscope and their dimensions recorded; where there were many representatives of a particular species only 10 to 20 specimens were measured, depending on variability.

Table 3.1 List of *Umbellula* spp. used in this study, number of specimens (#), date of collection, and location.

Species	#	Date	Depth (m)	Latitude	Longitude	Ocean: Location
U. aciculifera	1	21/04/1978	1533	50.0733	-11.9883	NE Atlantic: Goban Spur
U. aciculifera	1	07/06/1979	1789.5	49.5017	-13.3317	NE Atlantic: Goban Spur
U. aciculifera	1	13/10/1979	1600	49.5500	-12.5667	NE Atlantic: Goban Spur
U. aciculifera	1	21/08/1984	1357.5	51.7060	-13.0960	NE Atlantic: Porcupine Seabight
U. aciculifera	1	25/09/2000	1691	51.1482	-12.0653	NE Atlantic: Porcupine Seabight
U. carpenteri	2	06/09/1989	4860	48.8417	-16.3833	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	07/04/1997	4843	48.8685	-16.4443	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	3	17/07/1997	4845	48.8683	-16.4250	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	23/07/1997	4848.5	48.8660	-16.4102	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	06/07/1997	4842	48.8660	-16.4102	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	18/03/1998	4840	48.8263	-16.4620	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	30/04/1998	4836	48.8940	-16.7100	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	05/10/1998	4825.5	48.9822	-16.7537	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	04/10/2002	4842.5	48.9567	-16.2950	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	05/10/2002	4842	48.8967	-16.1800	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	06/06/1979	4510	49.7317	-15.0767	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	22/06/1985	4652.5	49.5045	-14.8170	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	06/05/1988	4850	48.8033	-16.5033	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	21/05/1991	4840.5	48.8017	-16.5333	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	22/05/1991	4842.5	48.8483	-16.5017	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	25/05/1991	4846	48.8617	-16.5567	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	22/10/1997	4841.5	48.8183	-16.6400	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	3	26/04/1999	4834	48.7017	-16.8583	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	28/04/1999	4836.5	48.7483	-16.6750	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	30/04/1999	4839	48.7817	-16.6933	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	02/05/1999	4844	48.4400	-15.6617	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	4	09/09/1989	4860	48.7967	-16.5833	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	12/09/1996	4837.5	48.7957	-16.2613	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	04/04/1997	4845.5	48.9367	-16.3795	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	27/07/1997	4848.5	48.8567	-16.7233	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	11/03/1998	4824.5	48.8385	-16.6217	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	25/05/1991	4846	48.8617	-16.5567	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	2	22/10/1997	4841.5	48.8183	-16.6400	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	1	05/10/1998	4825.5	48.9822	-16.7537	NE Atlantic: Porcupine Abyssal Plain
U. carpenteri	5	27/12/2005	4189.5	-48.9368	51.0650	S Indian: Crozet
U. carpenteri	3	29/12/2005	4189.0	-49.0192	51.0753	S Indian: Crozet
U. durissima	1	18/09/2000	3987	50.1987	-14.6560	NE Atlantic: Porcupine Abyssal Plain
U. encrinus	1	01/07/2001	1400	78.9680	6.7150	Arctic
U. hemigymna	1	27/09/1981	3810	50.0150	-14.1133	NE Atlantic: Porcupine Abyssal Plain
U. huxleyi	1	01/10/2000	1909	50.8980	-11.9740	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	27/04/2001	1200	49.8317	-11.7350	NE Atlantic: Irish continental slope/rise
U. huxleyi	10	30/10/1978	1750	56.7667	-9.8000	NE Atlantic: Irish continental slope/rise
U. huxleyi	15	01/06/1979	972.5	51.9067	-12.8983	NE Atlantic: Irish continental slope/rise
U. huxleyi	6	02/07/1979	1872.5	51.1133	-13.2783	NE Atlantic: Irish continental slope/rise
U. huxleyi	3	08/07/1979	980	51.4417	-13.4017	NE Atlantic: Irish continental slope/rise
U. huxleyi	3	16/10/1979	1057.5	49.3867	-12.0167	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	16/10/1979	1260	49.3917	-12.3583	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	20/10/1979	942.5	51.7417	-13.2467	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	05/08/1980	1312	51.6017	-13.0700	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	07/06/1980	1257.5	51.2767	-13.3883	NE Atlantic: Irish continental slope/rise

Table 3.1 continued…

Species	#	Date	Depth (m)	Latitude	Longitude	Ocean: Location
U. huxleyi	5	08/11/1980	1027.5	51.3633	-13.4567	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	08/11/1980	787.5	51.6963	-13.4423	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	21/05/1981	940	51.7833	-13.2183	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	16/09/1981	1975	51.0900	-12.9300	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	18/11/1982	485	51.8367	-13.0850	NE Atlantic: Irish continental slope/rise
U. huxleyi	4	21/02/1982	1975	49.8450	-12.3817	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	26/03/1982	750	51.8883	-13.3200	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	06/05/1983	1100	51.4950	-13.2033	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	29/09/1983	1016	49.5450	-11.8850	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	20/08/1984	525	52.0817	-13.4783	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	07/11/1984	1240	51.6917	-13.9400	NE Atlantic: Irish continental slope/rise
U. huxleyi	4	31/05/1991	885.5	51.7700	-13.2550	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	24/04/1978	1396	49.3717	-12.8183	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	20/10/1979	942.5	51.7417	-13.2467	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	05/08/1980	1312	51.6017	-13.0700	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	07/06/1980	1257.5	51.2767	-13.3883	NE Atlantic: Irish continental slope/rise
U. huxleyi	5	08/11/1980	1027.5	51.3633	-13.4567	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	08/11/1980	787.5	51.6963	-13.4423	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	21/05/1981	940	51.7833	-13.2183	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	16/09/1981	1975	51.0900	-12.9300	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	18/11/1982	485	51.8367	-13.0850	NE Atlantic: Irish continental slope/rise
U. huxleyi	4	21/02/1982	1975	49.8450	-12.3817	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	26/03/1982	750	51.8883	-13.3200	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	06/05/1983	1100	51.4950	-13.2033	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	29/09/1983	1016	49.5450	-11.8850	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	20/08/1984	525	52.0817	-13.4783	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	07/11/1984	1240	51.6917	-13.9400	NE Atlantic: Irish continental slope/rise
U. huxleyi	4	31/05/1991	885.5	51.7700	-13.2550	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	24/04/1978	1396	49.3717	-12.8183	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	22/09/1983	1005	56.6000	-9.2833	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	01/06/2006	998.5	53.8967	-13.0582	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	21/09/1983	1265	56.7667	-9.2500	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	19/05/1983	2195	57.2833	-10.2667	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	01/06/2006	997.5	54.0382	-13.0582	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	01/06/2006	1458.5	56.7338	-9.3502	NE Atlantic: Irish continental slope/rise
U. huxleyi	7	01/06/2006	1496	54.1325	-13.8160	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	01/06/2006	734.5	55.2718	-10.0652	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	09/11/1980	2645	50.4367	-13.3467	NE Atlantic: Irish continental slope/rise
U. huxleyi	1	27/09/1983	2487.5	49.9167	-12.9683	NE Atlantic: Irish continental slope/rise
U. huxleyi	2	17/04/1985	977.5	56.7167	-9.1833	NE Atlantic: Irish continental slope/rise
U. magniflora	5	22/01/2007	840	-68.1968	-70.5110	Southern: Marguerite Bay, Antarctica
U. monocephalus	1	05/09/1989	4846	48.7883	-16.4883	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	12/09/1989	4865	48.8667	-16.4017	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	4	04/09/1996	4839.5	48.8822	-16.7158	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	12/09/1996	4839.5	48.8388	-16.5498	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	12/10/1996	4837.5	48.7957	-16.2613	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	04/04/1997	4845.5	48.9367	-16.3795	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	05/04/1997	4847	48.8800	-16.3580	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	4	07/04/1997	4843	48.8693	-16.5887	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	10/07/1997	4843.5	48.8750	-16.6552	NE Atlantic: Porcupine Abyssal Plain

Table 3.1 continued…

Species	#	Date	Depth (m)	Latitude	Longitude	Ocean: Location
U. monocephalus	1	11/07/1997	4843.5	48.7338	-16.5470	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	17/07/1997	4845	48.8685	-16.4443	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	06/07/1997	4842	48.8660	-16.4102	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	4	10/03/1998	4843	48.8450	-16.4723	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	11/03/1998	4824.5	48.8385	-16.6217	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	18/03/1998	4840	48.8263	-16.4620	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	22/03/1998	4833.5	48.9298	-16.4923	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	3	23/03/1998	4833	48.9062	-15.6655	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	30/04/1998	4836	48.8940	-16.7100	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	05/10/1998	4825.5	48.9822	-16.7537	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	05/10/2002	4842	48.8967	-16.1800	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	20/07/1982	3485	50.0217	-13.9667	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	05/12/1986	4841.5	48.8667	-15.9500	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	10/12/1986	4870	48.2583	-16.2900	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	22/05/1991	4842.5	48.8483	-16.5017	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	24/05/1991	4846	48.8783	-16.6417	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	07/04/1994	4835	48.8583	-16.6867	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	14/04/1994	4844.5	48.8950	-16.6133	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	15/04/1994	4845.5	48.9250	-17.0017	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	19/04/1994	4844.5	48.9100	-16.7900	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	2	20/10/1997	4841.5	48.7817	-16.8283	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	3	22/10/1997	4841.5	48.8183	-16.6400	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	29/04/1999	4837	48.7900	-16.8150	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	02/05/1999	4844	48.4400	-15.6617	NE Atlantic: Porcupine Abyssal Plain
U. monocephalus	1	01/05/2004	4229	4.1602	93.3179	NE Indian: W of Indonesia
U. pellucida	3	03/06/1974	550	43.4500	-124.8267	NE Pacific: Oregon
U. thomsoni	3	12/11/1977	3753	50.0533	-13.8433	NE Atlantic: Porcupine Abyssal Plain
U. thomsoni	2	20/07/1982	3485	50.0217	-13.9667	NE Atlantic: Porcupine Abyssal Plain
U. thomsoni	1	28/08/2001	4298.5	49.6505	-14.3212	NE Atlantic: Porcupine Abyssal Plain
U. thomsoni	2	27/12/2005	4189.5	-48.9368	51.0650	S Indian: Crozet
U. thomsoni	1	29/06/2007	3476	38.3755	-9.9782	NE Atlantic: Cascais canyon
U. sp.1 n. sp.	1	03/07/2007	4040	47.9268	-10.2092	NE Atlantic: Whittard Canyon
U. sp.2 n. sp.	1	27/12/2005	4189.5	-48.9368	51.0650	S Indian: Crozet
U. sp.3 n. sp.	1	08/11/1977	4073.5	49.8367	-14.1217	NE Atlantic: Porcupine Abyssal Plain

3.2.2 Type Specimens and Literature

Many of the type specimens are housed at the Natural History Museum (London, UK): these were studied and photographed for reference. Furthermore, much of the literature pertaining to *Umbellula* is very old, dating back to 1753: such references are archived in the Natural History Museum. Since these reports and monographs are very delicate and should not be exposed to bright light, they could not be photocopied. To overcome this, each page was photographed with a digital camera (without flash) and the references compiled as PDF documents. Some of the literature is written in German and Russian: the former was translated by J. Ingels (Department of Marine Biology, Ghent University,

Belgium) and the latter by D.M. Miljutin and M. Miljutina (Laboratory of Coastal Researches, Russian Federal Research Institute of Fisheries and Oceanography, Moscow, Russia).

3.2.3 *Sclerite Analysis*

3.2.3.1 Light Microscope

Temporary microscope slides were prepared in order to examine the sclerites of various specimens to aid species identification. Small pieces of tissue (2-3 mm^2) were removed from the pinnules, tentacles, polyp wall, rachis, stem and peduncle of each specimen using a scalpel and/or scissors and placed on microscope slides. One or two drops of 100 % sodium hypochlorite were placed on the tissue using a teat-pipette and the tissue was left to dissolve for a few seconds before applying cover slips. Sclerites were immediately observed under the compound microscope before they dissolved. Slides were rinsed under running water so they could be reused.

3.2.3.2 Scanning Electron Microscope

Of the seven *Umbellula* spp. that possess sclerites in their tissue, five species were more or less in a reasonable enough condition to extract sclerites to attain SEM images (Table 3.2).

Table 3.2 *Umbellula* spp. (and fixative/preserve) from which sclerites were extracted for SEM analysis

Species	Fixative/Preserve
Umbellula aciculifera	Formalin
Umbellula hemigymna	Formalin
Umbellula monocephalus	Ethanol
Umbellula sp.2 n. sp.	Ethanol
Umbellula sp.3 n. sp.	Formalin

To isolate sclerites from the mesoglea, small pieces of tissue were dissolved in buffered sodium hypochlorite solution. When insufficiently buffered, hypochlorite will corrode the calcareous sclerites resulting in modification of their shape, dimensions and fine details, and even completely dissolving them. The ability of sclerites to resist dissolution can vary

according to the manner in which the specimen has been preserved; formalin-fixed specimens are likely to possess damaged sclerites which are readily dissolved in hypochlorite.

Borax is traditionally used as a buffer. A saturated solution was made by adding borax to Mille-Q until it could no longer dissolve and so settled out. From this stock solution, a working solution was made up of three parts borax solution, seven parts Mille-Q. The buffer was diluted in this way to prevent crystals forming that stick to the sclerites.

Small pieces of tissue (2-4 mm^2) were removed from the pinnules, tentacles, polyp wall, rachis, stem and peduncle of each specimen using a scalpel and/or scissors and placed in embryo dishes containing borax buffer. To this, 2-10 µl of sodium hypochlorite was added, the volume depending on tissue thickness, preservation and sclerite size. Tissue was left to dissolve slowly: it took up to a week for some of the larger sclerites to become dislodged from the tissue. Tissue was frequently checked under the stereo microscope, and any intact sclerites that had fallen from the dissolving tissue were removed by means of micropipette and kept in buffer. Larger sclerites deeply buried into the tissue took longer to isolate, and consequently the buffer-hypochlorite solution became saturated with dissolved material. In such cases, the tissue was gently removed and the process continued in fresh buffer.

Isolated sclerites were washed in clean buffer followed by two rinses in 100% ethanol. Intact sclerites were separated and grouped together within the embryo dish using a mounted eyelash tool; this ensured as many sclerites as possible were sucked up in 5-10 µl of ethanol. These were then carefully pipetted onto the SEM adhesive stub and arranged using the mounted eyelash tool, whilst wet. When the alcohol evaporated, the sclerites became firmly attached to the stub. Stubs were subsequently gold coated and visualised with a Leo 1450 VP (variable pressure) Scanning electron microscope.

Temporary light microscope slides were made throughout to ensure sclerites, particularly the smaller types, were intact, as seen under higher magnification of the compound microscope. To make the slides, sclerites were pipetted onto a microscope slide and covered with a cover slip.

3.2.4 Axis Analysis

The main colony supporting structure, the axis, is characterised by its shape in cross-section, and is an important trait specific to pennatulid species. In this study, the terms "round" and "quadrangular" were used to define the shape of the axis for *Umbellula* species (Fig. 1). The stems of *Umbellula* colonies were cut in cross-section and the tissue stripped to reveal the shape of the axis beneath.

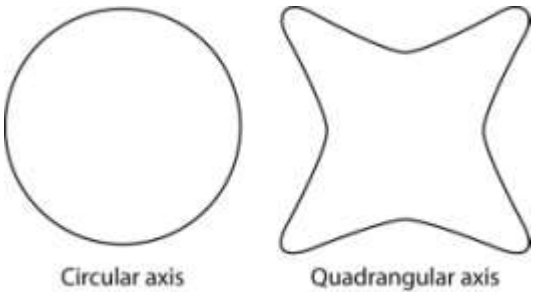

Figure 3.1 The two forms of axes (in cross-section) characteristic of *Umbellula* species.

3.2.5 Molecular Analysis

The DNA of eight species of *Umbellula* were analysed to produce a phylogenetic tree to infer systematic relationships between species in relation to their morphology. For methods, please refer to Chapter Two.

3.2.6 Geographic and Bathymetric Distribution

Geographic and bathymetric data were obtained from the literature for all species of *Umbellula*. Maps were created for the literature data and that of the newly collected material (Table 3.1) using the computer software, PanMap (Diepenbroek et al., 2000).

3.3 Results and Discussion

3.3.1 Glossary of Morphological and Anatomical Terms Applied to Pennatulacea

Adapted from Bayer et al. (1983)

Anthocodia	That portion of the polyp (autozooid) that protrudes into the water i.e. the distal part of an autozooid that bears the mouth and the tentacles and encloses the pharynx and gastric cavity. Often termed the polyp body
Anthocodiae	Plural of anthocodia
Anthostele	The proximal portion of the polyp (autozooid) that is embedded in the rachis
Asulcal side	The side of the polyp opposite the siphonoglyph
Autozooid	Polyp with eight well-developed tentacles and mesenteries; the only kind of polyp in a monomorphic species, the larger polyp in dimorphic species. Often just termed polyp
Autozooid-cluster	The portion of the rachis from which the autozooids bud in *Umbellula* spp.
Axis	The inner supporting structure of Pennatulacea (and Gorgonacea); it may be calcareous or horny
Coenenchyme	The colonial tissue between the polyps, consisting of mesoglea usually containing sclerites and penetrated by the network of solenia (small canals lined with gastrodermis) and the larger gastrodermal canals
Colony	A group of interconnected, genetically identical, elementary functional units, the polyps
Dimorphism	The presence of two kinds of polyps: autozooids and siphonozooids
Dorsal side	In pennatulid colonies, that side of the colony derived from the asulcal side of the primary autozooid, often where the axis can be seen beneath the rachis
Dorsal track/midline	The more or less naked strip extending along the rachis between the autozooids along the dorsal side
Gastric cavity	Interior space of an autozooid
Mesenterial filaments	The thickened convoluted edges of the mesenteries
Mesenteries	Thin, radial, non-calcareous partitions joining the pharynx to the body wall and dividing the gastrovascular cavity of the polyp
Mesoglea	The jelly-like substance separating the two epithelial layers and containing more or less numerous cells, including scleroblasts and cell strings
Needle	Pertaining to sclerite form: long, thin, smooth monaxial sclerite
Nutrient canals	In pennatulids, the four main canals formed by the gastric cavity of the primary autozooid, one dorsal, two lateral, and one ventral, extending the length of the colony and interconnected by smaller canals, the solenia
Oozoid	The persistent and modified primary autozooid of pennatulids
Peduncle	The lower part of the pennatulid colony used as an anchor, lacking polyps (autozooids or siphonozooids)
Pharynx	The tubular passageway between the mouth and the gastric cavity

Pinnules	The lateral processes of a tentacle
Plate	Pertaining to sclerite form: flat sclerite of diverse outline, often oval; normally found in the peduncle
Polyp	Any individual of the octocorallian colony regardless of anatomical structure, but usually equivalent to autozooid
Polyp body	Refer to Anthocodia
Primary autozooid	The first autozooid of a colony (founder polyp) formed by metamorphosis of the planula larva, becomes the axial oozooid (autozooid) in pennatulids
Rachis	The autozooid-bearing (polypiferous) portion of pennatulid colonies
Rachis-swelling	The enlarged portion of the rachis below the autozooid-cluster
Rod	Pertaining to sclerite form: straight or curved monaxial sclerite, blunt at both ends
Sclerite	A calcareous element, irrespective of form, in the mesoglea
Secondary autozooid	Those polyps that develop after the primary autozooid
Siphonoglyph	(=Sulcus) The strongly ciliated groove extending down one side of the pharynx
Siphonozooid	A polyp with strongly developed siphonoglyph and reduced tentacles or none, commonly reduced mesenterial filaments; usually smaller than the autozooids
Spindle	Pertaining to sclerite form: straight or curved monaxial sclerite, pointed at both ends
Stem	The long, slender region of the colony below the rachis and above the peduncle
Sulcal side	The side of the polyp nearest the siphonoglyph
Three-flanged	Pertaining to sclerite form: rod, needle or spindle with three longitudinal flanges
Tubercles	Pertaining to sclerite topology: sclerites ornamented with perturbations/warts
Ventral side	In pennatulid colonies, that side of the colony derived from the sulcal side of the primary autozooid, often the opposite side to where the axis can be seen beneath the rachis

3.3.2 Key to the Fifteen Species of Umbellula

1 Colonies without sclerites in the autozooids and rachis……2
- Colonies with sclerites in the autozooids and rachis……9

2 Colonies with quadrangular axes (no sclerites)……3
- Colonies with round axes (no sclerites)……8

3 Colonies small (<100 mm); bilateral symmetry; 3-7 autozooids; axis often protrudes above the rachis spine-like……*U. carpenteri*
- Colonies tall (500 to >2000 mm) or smaller (<350 mm); radial symmetry; >7 autozooids; axis does not protrude above the axis spine-like ……4

4 Colonies tall; few autozooids (8-12) arranged in a single concentric circle (1-3 autozooids sometimes inside this circle)……5

- Colonies either tall (500 to >2000 mm) with numerous autozooids (30-45); or smaller (<350) with numerous autozooids (25-30); autozooids arranged in >>1 concentric or irregular whorls……..6

5 Siphonozooids present between the anthocodiae and on the most distal region of the rachis in the field between the anthocodiae; each siphonozooid possess an obviously branched tentacle; large mucous cells in the ectoderm.....*U. magniflora*
- Siphonozooids absent between anthocodiae and distal field encircled by the anthocodiae; siphonozooids possess a single tentacle that is not obviously branched; no large mucous cells in the ectoderm.....*Umbellula* sp.1 n. sp.

6 Colonies small (<350 mm) and slender; siphonozooids absent between the anthocodiae.....*U. pellucida*
- Colonies >350 mm and slender or stout; siphonozooids present between anthocodiae 7

7 Colonies tall (1000-2000 mm) and slender; autozooids in concentric whorls……*U. encrinus*
- Colonies stout and shorter (~500 mm); autozooids arranged in irregular whorls……...*U. antarctica*

8 Rachis short; numerous, crowded autozooids (~45 in colonies 500-600 mm tall) arranged in a tight cluster at the most distal part of the rachis-swelling; anthocodiae not especially long and slender……..*U. huxleyi*
- Autozooid-bearing portion of the rachis especially long; autozooids less numerous (~25 in mature colonies) spaced along the tassel-like rachis; anthocodiae especially long and slender .....*U. spicata*

9 Colonies with quadrangular axes (with sclerites)……..10
- Colonies with round axes (with sclerites)……11

10 Sclerites numerous; anthocodiae not especially long and slender; tentacles thick and robust……..*U.thomsoni*
- Sclerites not numerous but sparsely distributed throughout, only aggregating in parts of the asulcal side of the anthocodiae (proximally); anthocodiae very long and slender; tentacles fine, long and slender……...*U. hemigymna*

11 Mature colonies with one very large primary autozooid, no secondary autozooids; monaxial sclerites throughout……..*U. monocephalus*
- Colonies with more than one autozooid (primary plus secondary autozooids); sclerites of two types, monaxial and three-flanged……..12

12 Colonies with bilateral symmetry; monaxial sclerites 1.5-1.6 mm only occurring in the tentacles; anthocodiae often a distinctive milky-blue.....*U. aciculifera*
- Colonies with radial symmetry; or bilateral symmetry with large, encrusting monaxial sclerites >2 mm in tentacles and anthocodiae; anthocodiae never disinctive milky-blue in colour.....13

13 Colonies display bilateral symmetry; rachis dorso-ventrally flattened; large wart-like siphonozooids form a rhomboid-shaped plate on the dorsal rachis……..*U. durissima*
- Colonies display radial symmetry; rachis not dorso-ventrally flattened; siphonozooids small and do not form a rhomboid-shaped plate on the dorsal rachis.....14

14 Rachis conical below autozooid-cluster; large monaxial sclerites restricted to the anthocodiae and tentacles, do not occur in the rachis……*Umbellula* sp.2 n. sp.
- Rachis spherical; large monaxial sclerites in the tentacles and occasional ones in the rachis, do not occur in the anthocodiae……*Umbellula* sp.3 n. sp.

3.3.3 Taxonomic Descriptions

In this section all twelve valid *Umbellula* spp. are revised, and a further three new species are described. This section includes synonymised species[1], type material, material examined, key taxonomic descriptors, emended diagnosis, differential diagnosis and remarks, and discussion, wherever applicable. Many descriptions incorporate information on intraspecific variability and ontogeneity. Species are classified in two groups: Group A, those species without sclerites in the rachis/autozooids; and Group B, those species possessing sclerites in the rachis/autozooids.

Group A: *Umbellula* spp. without sclerites

3.3.3.1 *Umbellula magniflora* Kölliker 1880; *Umbellula encrinus* Linnaeus 1758; *Umbellula antarctica* Kükenthal and Broch, 1911

Umbellula magniflora, *U. encrinus,* and *U. antarctica* are morphologically very similar species, and are characterised by a complex classification history. Therefore, these three species are integrated together in this section. Furthermore, an emended diagnosis is not included for *U. encrinus* or *U. antarctica*: the present author only superficially studied a remarkably large specimen of *U. encrinus* during a visit to the Scottish Association for Marine Science, Oban, UK (Plate 2), from which a piece of tissue was kept for genetics; and a single specimen of *U. antarctica* at the Natural History Museum, London, UK.

Umbellula magniflora Kölliker 1880

Umbellula magniflora	Kölliker 1880
Umbellula rigida	Kükenthal and Broch 1911
Umbellula carpenteri	Kükenthal 1915
Umbellula carpenteri pars	Broch 1957 (specimens 7 and 8)
Umbellula lindahli pars	Broch 1958 (specimens B, D and E)
Umbellula magniflora	Pasternak 1962
Umbellula magniflora	Pasternak 1970
Umbellula magniflora	Pasternak 1975
Umbellula lindahli	Williams 1990
Umbellula magniflora	Pasternak 1993

[1] Species name followed by '?' indicates the present author is unsure of whether the species is synonymous; 'pars' indicates that not all specimens are synonymous

Type Material

Location unknown.

Material Examined

Marguerite Bay, Antarctica, Southern Ocean (68.1968° S; 70.5110° W), 840 m, collected by means of the ROV, *Isis*, on board the RRS *James Clarke Ross*, 22/01/2007: 5 specimens preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Axis quadrangular with four longitudinal grooves
- No sclerites in autozooids/rachis
- Colonies tall and slender (~1000 to 2500 mm)
- Approximately 8 to 12 autozooids arranged in a single concentric circle; 1 to 3 often positioned within this in mature specimens (colonies > 800 mm)
- Autozooid-bearing portion of the rachis short and chalice-shaped
- Siphonozooids dense, possessing a long branched-tentacle, often giving the colony a 'hairy' appearance
- Siphonozooids form elevated petal-shaped zones on the chalice-shaped portion of the rachis and extend upwards between each autozooid
- Endowed with large mucous cells in the ectoderm of the tentacles and autozooids

Emended Diagnosis Plate 1; Table 3.3; Fig. 3.1

The following description is based on five fine exemplars of *U*. *magniflora* from the Southern Ocean. All measurements were made immediately onboard the RRS *James Clarke Ross* upon collection and prior to preservation, and as such closely represent those of the living specimens (Table 3.2). The careful method of acquisition by the manipulator arm of the ROV, *Isis*, has allowed for the first time, the study of totally unscathed representatives of this species. Further to this, live video footage and photographs were obtained *in situ* (Plate 1, Fig. A(v)).

The colonies are considered mature: two possessing 10, two possessing 9, and one possessing 8 autozooids, and range in total height from 980 mm to 440 mm. Autozooids are arranged in a single rosette at the distal portion of the rachis. The polypiferous

portion of the rachis is short, and pendulous when out of its natural habitat. The autozooids are closely packed so that there are no gaps between neighbours: the primary autozooid can be easily distinguished from the others since it is set in slightly within the ring. Below the autozooid cluster, the rachis is chalice-shaped becoming elongated and conical proximally, gently tapering into the stem.

Each anthocodia is slim, large and cylindrical. They have the characteristic longitudinal striations corresponding to the internal mesenteries and have acquired lateral creases, presumably resulting from contraction subsequent to capture since these features were not observed in *in-situ* images of the same specimens (Plate 1, Fig. A(v)).

The autozooid tentacles are approximately 1 to 1.5 times as long as the anthocodiae and thin but comparatively robust. Pinnules are spread along the tentacles with small gaps between, and are very long. They appear to differ in length but do not alternate in size down tentacle, pairs directly opposed each other and the smaller pinnules apparently aligned further from the tentacle edge than the longer ones. The tissue composing the tentacles has an undulating surface probably caused by the presence of mucus cells: upon collection, the specimens were placed in a water-filled tray in which bubbles of polysaccharides were observed escaping from the tentacles. Mucous appears to be characteristic of these cold-water forms, and Danielssen and Koren (1884) found specimens of *U. encrinus* enveloped with mucous.

Siphonozooids are densely packed over the rachis-cluster (including the distal zone in the centre of the concentric circle of polyps, and the dorsal midline), the lower rachis, the upper stem and the lower stem above the peduncle: where the sarcosoma thins on the middle portion of the stem there is a tendency for fewer siphonozooids. Characteristically, from each siphonozooid a long, branched single tentacle emanates; these branches restricted to one side of the tentacle. Siphonozooid tentacles give the colony a 'hairy' appearance and are approximately two to three times longer on the rachis where they measure 2 to 3 mm (lesser degree of retraction?). This feature was also observed by Broch (1958) when describing *U. lindahli* (=*U. magniflora*) from the Discovery collections (1927-1937). In one specimen, many of the siphonozooid tentacles of the rachis are retracted thus eliminating its shaggy look. Siphonozooids of the stem are flat and are not obvious, marked only by the presence of the extended tentacle (tentacles

of the stem/peduncle interface are up to 1.1 mm long). On the rachis however, siphonozooids are manifested as domes covering the surface tissue and form somewhat elevated petal-shaped zones which extend upwards between each autozooid. This last feature is very distinctive in this species.

The axis is thin and relatively inflexible, quadrangular in section with typical longitudinal grooves and pronounced keels with rounded edges. Where the axis enters the rachis of the autozooid cluster, it either is positioned dorsally as marked by the presence of a ridge/spine or enters centrally: in both cases, the axis cannot be directly observed through the sarcosoma of the rachis.

The peduncle is an elongated swelling, quadrangular in section, the upper limit marked by the absence of siphonozooid tentacles. No sclerites can be found here.

The largest specimen is fecund and large oocytes/sperm bundles (up to 0.8 mm) are present in the rachis, as seen where an autozooid has been removed for molecular analysis. Otherwise, the gametes cannot be seen bulging in the anthocodiae or through the sarcosoma. There is no level of transparency in the sarcosoma of the autozooids/rachis. The sarcosoma is very thin on the stem from below the lower rachis to the peduncle.

Table 3.3 Dimensions (mm) of *U. magniflora* colonies from Marguerite Bay, Antarctica, 840 m; L, length; W, width.

U. magniflora	**1**										**2**								**3**									
Colony L	980.0										643.0								590.0									
Axis W	1.4-2.2										1.1-2.8								1.9-2.1									
Stem W	1.4-2.2										1.1-2.8								1.95-2.2									
Peduncle L	98.0										71.0								74.0									
Peduncle W	5.0										3.6								4.5									
Rachis swelling L	15.0										11.0								13.0									
Total rachis L	22.0										68.0								120.0									
Rachis W	13.5										8.3								10.0									
N autozooids	10.0										9.0								10.0									
Autozooid L	28.0	27.	32	28	32	29	30	28	32	29	25.0	20	17	21	20	23	22	28	29	22.0	22	22	23	17	22	21	22	23	25
Autozooid W	5.0										4.0									4.5									
Tentacle L	37.0	33	44	44	42	49	35	19	40	47	32.0	36	16	24	23	23	23	24	27	33.0	33	29	29	21	25	34	33	30	30
Tentacle W	1.1										1.3									1.3									
Pinnule L (long)	3.9										2.7-3.0									2.7									
Pinnule L (short)	0.5										0.3-0.4									0.8-0.6									
Pinnule W (long)	0.3										0.3									0.3									
Pinnule W (short)	0.2										0.15									0.2									
Siphonozooids (large)	0.5										0.4-0.55									0.3									
Siphonozooids (small)	0.25										0.2-0.4									0.1-0.2									

U. magniflora	**4**									**5**							
Colony L	450.0									440.0							
Axis W	0.5-1.2									0.8-0.8							
Stem W	0.9-1.6									0.9-2.0							
Peduncle L	59.0									51.0							
Peduncle W	5.5									3.6							
Rachis swelling L	11.0									8.0							
Total rachis L	27.0									16.0							
Rachis W	7.5									7.5							
N autozooids	9.0									8.0							
Autozooid L	16.0	17	17	10	13	16	14	18	20	10.0	12	9	12	10	12	15	15
Autozooid W	3.0									3.0							
Tentacle L	20.0	23	23	12	20	22	24	17	20	15.0	14	16	14	15	16	15	16
Tentacle W	1.4									0.6							
Pinnule L (long)	2.2									1.1							
Pinnule L (short)	0.6									0.5							
Pinnule W (long)	0.2									-							
Pinnule W (short)	0.1									-							
Siphonozooids (large)	0.3									-							
Siphonozooids (small)	0.1									-							

Umbellula encrinus Linnaeus 1758

Clusterpolype	Ellis 1753
Zoophytum grønlandicum	Mylius 1753
Hydra marina arctica	Ellis 1755
Isis encrinus (*U. encrinus encrinus*)	Linnaeus 1758
Pennatula encrinus	Pallas 1766
Pennatula encrinus	Ellis and Zolander 1766
Vorticella encrinus	Linnaeus 1767
Vorticella encrinus	Esper 1791
Ombellula	Cuvier 1798
Umbellularia groenlandica	Lamarck 1801
Umbellularia encrinus	Blainville 1830
Umbellularia encrinus	Ehrenberg 1832
Umbellularia encrinus	Blainville 1834
Umbellularia groenlandica	Dana 1847
Umbellularia encrinus	Milne-Edwards 1857
Umbellularia groenlandica	Milne-Edwards 1857
Umbellularia groenlandica	Herklots 1857
Umbellularia groenlandica	Richiardi 1869
Umbellularia groenlandica	Gray 1870
Umbellularia groenlandica	Kölliker 1872
Umbellula encrinus	Lindahl 1874
Umbellula miniacea	Lindahl 1874
Umbellula pallida	Lindahl 1874
Umbellula Lindahlii	Kölliker 1874
Umbellula encrinus	Marenzeller 1878
Umbellula encrinus	Danielssen and Koren 1884
Umbellula bairdii?	Verril 1885
Umbellula encrinus ambigua	Fischer 1889
Umbellula lindahli	Jungersen 1904
Umbellula encrinus	Jungersen 1904
Umbellula encrinus encrinus	Kükenthal 1915
Umbellula encrinus ambigua	Kükenthal 1915
Umbellula lindahli	Kükenthal 1915
Umbellula carpenteri pars	Broch 1957

Type Material

Location unknown

Material Examined

Arctic Ocean (78.9680 °N; 06.7150 ° E), 1400 m, 07/01; 1 specimen, colony fixed in formalin (borax-buffered 4 % formaldehyde in seawater), a portion of which was removed (peduncle and one anthocodia) prior to fixing and preserved in 96 % ethanol preserved.

Key Taxonomic Descriptors

- Axis quadrangular with four longitudinal grooves
- No sclerites, only small oval bodies in the peduncle
- Tall, slender colonies (>2000 mm)

- Mature colonies with numerous (> 40), crowded autozooids arranged in concentric whorls; radial symmetry
- Autozooid-bearing portion of the rachis short and chalice-shaped
- Siphonozooids numerous and possess a single branched-tentacle; present between anthocodiae
- Large mucous cells in the ectoderm of the tentacles and autozooids

Umbellula antarctica Kükenthal and Broch, 1911

Umbellula encrinus var. *antarctica*	Kükenthal 1902
Umbellula antarctica	Kükenthal and Broch, 1911
Umbellula antarctica	Kükenthal 1915
Umbellula lindahli pars	Broch 1958
Umbellula lindahli	Pasternak 1962
Umbellula lindahli	Pasternak 1993

Type Material

Location unknown

Material Examined

Natural History Museum, London. '*Umbellula lindahli*' (Broch, 1958), Discovery Stn 371, Southern Ocean, S Sandwich Islands, 99-161 m.

Key Taxonomic Descriptors

- Axis quadrangular with four longitudinal grooves
- No sclerites in the rachis /autozooids
- Short and stout, mature colonies rarely reaching 830 mm, normally ~500 mm
- Autozooids very numerous (up to 40), arranged in irregular whorls; radial symmetry
- Autozooid-bearing portion of the rachis short and chalice-shaped
- Siphonozooids numerous on the rachis and between the anthocodiae
- Axis exceptionally thick (~3-5 mm)
- Thick peduncle, ~18 mm in colonies 450 mm tall

Differential Diagnosis and Remarks: *Umbellula magniflora, Umbellula encrinus* and *Umbellula antarctica*

Umbellula encrinus inhabits the Arctic and N Atlantic oceans, whereas *U. magniflora* and *U. antarctica* are generally restricted to the high latitudes of the southern hemisphere. Molecular analysis of *U. encrinus* and *U. magniflora* suggests they are genetically different (Fig. 3.3, Section 3.3.5), and thus should be regarded as two separate species. Their morphological differences, however, are much more subjective: this too is the case for *U. antarctica* for which molecular data are wanting.

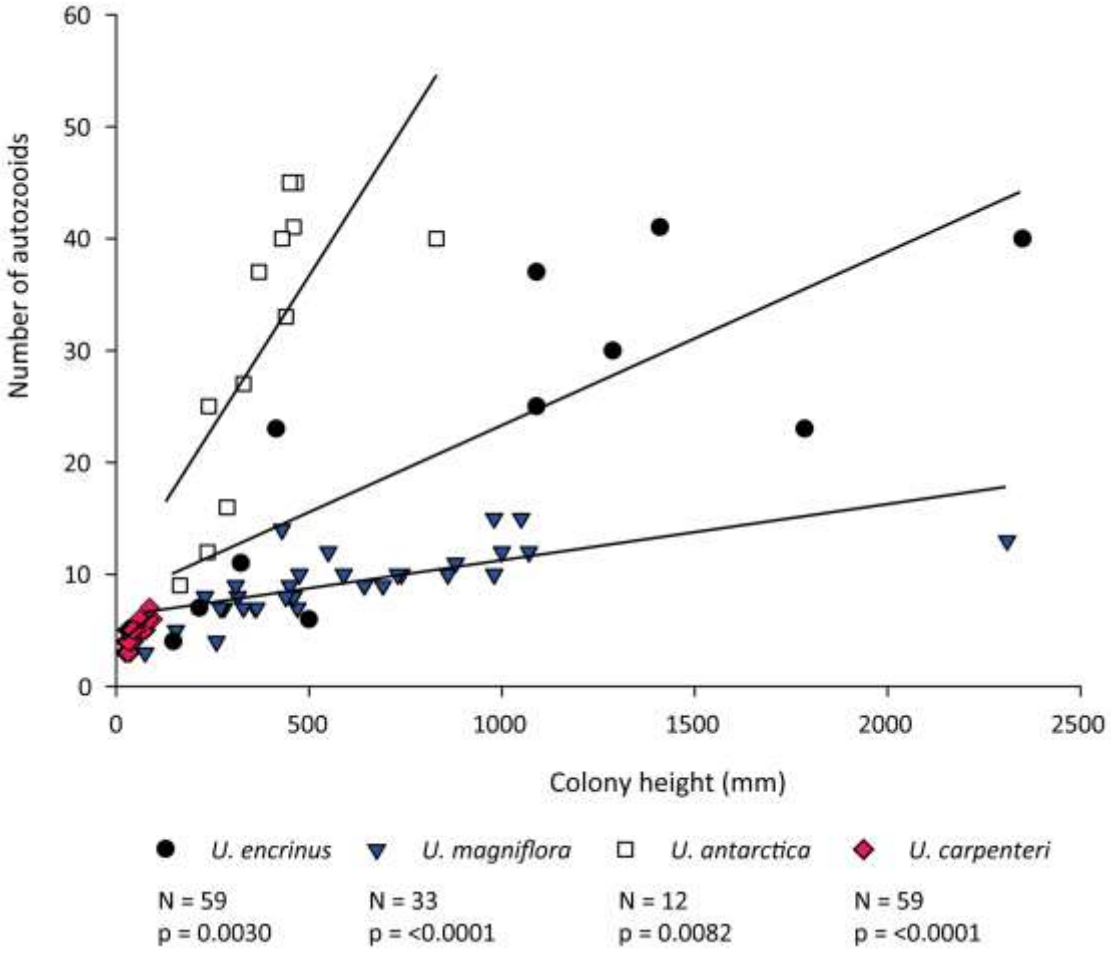

Figure 3.1 Relationship between colony height and number of autozooids for *U. encrinus*, *U. magniflora* and *U. antarctica* (and *U. carpenteri* for comparison, see Section 3.3.3.2, and discussion below). Regression lines highlight the differences between the four species: N, number of specimens; p, p-value for analysis of variance. Data taken from the literature (Lindahl, 1874; Kölliker, 1880; Jungersen, 1904; Kükenthal and Broch, 1911; Broch, 1957; 1958; Pasternak, 1962; Williams, 1990; Pasternak, 1993) and new material (*U. magniflora* and *U. carpenteri* only, see Tables 3.3 and 3.4).

Umbellula encrinus is very similar in general appearance to *U. magniflora*: both grow very tall in height (colonies exceeding 2000 mm); have quadrangular axes with longitudinal grooves; the clusters display radial symmetry with autozooids positioned in whorl(s) at

the most distal part of the short chalice-shaped rachis; both possess numerous siphonozooids each endowed with a long branched tentacle; and are enveloped with mucous. However, *U. magniflora* has considerably less autozooids than the *U. encrinus*: a colony of *U. magniflora* 2310 mm tall was recorded to have only 13 autozooids (Pasternak, 1962), whereas *U. encrinus* 2350 mm in height had 40 autozooids (Jungersen, 1904): this is highlighted in Fig. 3.1.

Of the two southern species, *U. antarctica* too has considerably more autozooids than *U. magniflora* (Fig. 3.1). Such colonies have autozooids arranged in very irregular whorls (in *U. magniflora* they form a single concentric circle, with one or two in the centre of this in mature specimens), and are much stronger, more robust forms, being thicker and stouter in the stem and peduncle than either *U. encrinus* or *U. magniflora*. The colonies of these two species are tall and slender, whereas *U. antarctica* seldom reaches heights above 500 mm (the largest recorded was 830 mm).

However, characters such as length, thickness and number of autozooids are frustratingly subjective without numerous specimens for comparison. *Umbellula antarctica* may well be an environmental variant of *U. encrinus* or *U. magniflora*, but it is believed here that this is not the case and the differences between these three are apparent in the figure (3.1) and plates of *U. magniflora* and *U. encrinus* (Plates 1 and 2, respectively).

Discussion: *Umbellula magniflora, Umbellula encrinus* and *Umbellula antarctica*

The published history pertaining to *U. encrinus* and the morphologically similar species highlighted above is very complex: authors have often unjustifiably spilt the group, or conversely made distinctive species synonymous. Poor descriptions (often contradictory) and sporadic collections of material have exacerbated these problems. Furthermore, it is not unusual that the same author revises their opinion of the systematics of these *Umbellula* forms (*U. magniflora*, *U. antarctica*, *U. encrinus* plus *U. carpenteri*) several times, and extremely so within a short period of time (cf. Broch, 1957; 1958; 1961).

Umbellula encrinus was the first species of the genus to be discovered in 1753 at 79˚ N in the Arctic waters off Greenland. The two colonies substantially exceeded one metre in

height: Ellis (1753) described the larger (1785 mm) under the designation 'Clusterpolype', and Mylius (1753; 1755) the smaller specimen (1287 mm) under the designation 'Zoophytum gønlandicum'. More than a century elapsed before it was rediscovered: in 1871 two specimens were recovered from Omenak Fjord and Baffin's Bay (Greenland), regarded by Lindahl (1874) as two new species, namely *U. pallida* and *U. miniacea* respectively. Kölliker (1875) considered Lindahl's specimens synonymous, naming them *U. Lindahlii* (='*U. lindahli*') after their founder. Yet Danielssen and Koren (1884) believed *U. pallida* and *U. miniacea* to be synonymous with *U. encrinus* (as well as the southern *U. magniflora*, Kölliker 1880). Through very detailed accounts based on 12 specimens of differing developmental stages they concluded that *U. pallida* and *U. miniacea* are "partially developed (younger) specimens of the old, venerable *Umbellula encrinus*". Drawing from the descriptions, dimensions and figures of these authors it is unquestionable that this is correct. Hence, it is the belief of the present author that *U. lindahli* and its synonyms, *U. pallida* and *U. miniacea* (Kölliker, 1875) are junior synonyms of the older Linnaean name, *U. encrinus*.

Jungersen (1904) wrongly discarded the synonymy of *U. lindahli* with *U. encrinus* and described two new specimens of '*U. lindahli*', the first of which was a juvenile *U. monocephalus*, an undescribed species until the 1960s (Pasternak, 1964); and of the second he stated: "This specimen agrees very well in appearance with Lindahl's two specimens from Baffins Bay and Omenak Fjord, and especially with the one he has called *U. pallida*…it [is] certain that our specimen belongs to exactly the same species as that of Lindahl, and on the other hand also very probable that it is a species different from that of *U. encrinus*". The differences he highlighted between the two were that *U. encrinus* is "more robust" and "shorter stalked" than *U. lindahli*. Considering *U. lindahli* is based on young specimens of *U. encrinus* (as explained by Danielssen and Koren, 1884), it would therefore be more fragile and slender, and thus proportionally longer stalked. Jungersen (1904) goes on to describe five specimens of *U. encrinus*, focusing on the mature specimens. Likewise, Kükenthal (1915) assigned less mature specimens to *U. lindahli* i.e. those with thin stems and less numerous autozooids in a narrow hanging cluster; and more mature specimens with long stems and less slender rachis to *U. encrinus*.

Twenty-four years prior to this, Kölliker (1880) described no fewer than seven new species of *Umbellula* from the *Challenger* Expedition (1873-1876) including *U. magniflora*

from the Southern Ocean: "The only specimen of this *Umbellula* brought home by the *Challenger* is in a very bad state of preservation; nevertheless it is of great interest, as it is the only known *Umbellula* which resembles the *Umbellula* of Ellis and Mylius so much that it seems to be the same species, or at least come very near to it". Kölliker was describing what is herein also considered *U. magniflora*: molecular analysis of *U. encrinus* from the Arctic and *U. magniflora* from the Antarctic has provided evidence to suggest that Kölliker (1880) was justified in raising these two species (Fig. 3.3, Section 3.3.5).

Kükenthal (1902) who studied the first Antarctic specimens of *Umbellula* considered them subspecies of *U. encrinus* , namely *U. encrinus* var. *antarctica* which later was raised to a separate species, *U. antarctica* (Kükenthal and Broch, 1911; Kükenthal, 1915); and Hickson (1916) assigned three specimens to this name. Based on their accounts (and observations of a specimen in the Natural History Museum) it is clear that they are not describing *U. magniflora* (colonies only 465 mm tall possessed approximately 45 autozooids) and is a different species. However, *U. antarctica* later became incorrectly synonymised with *U. lindahli* by Broch (1958).

Broch (1957) regarded *U. carpenteri* as a separate species in a detailed account of its development, but later made it synonymous with *U. magniflora* and *U. antarctica* (Broch, 1958), considering them a developmental series of *U. lindahli*: the "carpenteri-magniflora-antarctica line". *Umbellula carpenteri* herein maintains its species status (see Section 3.3.3.2). However, like the majority of species ascribed to this genus, the diagnosis for *U. carpenteri* is contradictory: Kölliker (1880) was the first to describe *U. carpenteri*, but he based his descriptions on two (or more?) species, thus making its characters open to interpretation. Broch (1958) based his diagnosis of *U. carpenteri* on Kölliker's specimens D and E (=*U. magniflora*) and so his interpretations seem reasonably justifiable at first. Yet when one studies Broch's account of *U. lindahli*, he is without doubt describing two different species: there certainly is not a developmental pattern correlating number of autozooids and colony height. This is demonstrated in Fig. 3.1, which uses data taken from Broch's (1958) descriptions of *U. lindahli* but dividing them into, in Broch's words, "Typical *magniflora*" and "Typical *antarctica*".

Pertaining to *U. encrinus* and *U. lindahli*, Broch (1958) states the following: "…their size differs so greatly that it is generally easy to distinguish between specimens of the two

'species', and at present it is hard to define the limits exactly so *encrinus* is considered as a separate species", seemingly referring to younger (*U. lindahli*) and more mature (*U. encrinus*) specimens. In his key, Broch (1958) states for *U. lindahli* that autozooids are large and numerous but not especially crowded, whereas *U. encrinus* is more robust and has larger autozooids than *U. lindahli*. Three years later Broch (1961), in his revision, suggested that *U. lindahli* is synonymous with *U. encrinus*, and claimed that the Arctic variants do not distinguish themselves as ecologically determined, morphological forms. In doing so, he wrongfully grouped *U. carpenteri*, *U. magniflora*, and *U. antarctica* with *U. encrinus*.

However, molecular evidence provides us with information that Broch (and others) did not have, and strongly suggests that *U. magniflora* and *U. encrinus*, although closely related, are genetically different. The genetic identity of *U. antarctica* remains enigmatic for the time being. As such, the present study considers those with numerous autozooids from the Arctic and north Atlantic oceans as *U. encrinus*; and those from the Antarctic and high latitudes of the southern hemisphere, *U. antarctica* and *U. magniflora*, the latter of which possesses fewer autozooids. However, their full geographical distribution is yet to be determined.

In conclusion, the present author considers *U. magniflora*, *U. antarctica*, and *U. encrinus* as valid species, and *U. lindahli* (*U. pallida* and *U. miniacea*) synonymous with *U. encrinus*. Further investigation by means of genetics is fundamental to improve our understanding of the distribution of these species and to support or discard their classifications. It is of the opinion of the author that many new species will be revealed through molecular barcoding.

3.3.3.2 *Umbellula carpenteri* Kölliker 1880

Umbellula carpenteri pars	Kölliker 1880 (specimen A only)
Umbellula carpenteri pars	Broch 1957 (specimens 1-5 only)

Type Material

Natural History Museum, London. Paralectotype specimen, Kölliker's Specimen 'A' designated on pg 23 (Kölliker, 1880), from Challenger Stn 156, Southern Ocean, SW of Australia (-62.4300° S; 95.7300° E) 3612 m, or Challenger Stn 157, S Indian Ocean (-53.9200° S; 108.9200° E) - specimen was combined with other species where origins of individuals were not specified; Plate X fig. 39b (Kölliker, 1880).

Material Examined

Type material (see above).

Porcupine Abyssal Plain, NE Atlantic Ocean (48.8894° N; 16.3969° W), 4510-4860 m, collected by means of trawl (OTSB) and epibenthic sledge over the period of 06/06/1979 to 05/10/2002: 45 specimens fixed in formalin (borax-buffered 4 % formaldehyde in seawater) and stored in formalin/70 % propan-2-ol.

Crozet Islands, S Indian Ocean (48.9368° S; 51.0650° E), 4182-4195 m, collected by means of OTSB 27/12/2005: 9 specimens, preserved in 96 % ethanol.

Crozet Islands, S Indian Ocean (49.0192° S; 51.0753° E), 4187-4191 m, collected by means of OTSB 29/12/2005: 9 specimens, preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Quadrangular axis: uppermost end often extending above rachis as a short, slender spine
- No sclerites in autozooids/rachis
- Colonies very small (<100 mm in height)
- Distinctly bilateral symmetry with 3-7 autozooids

Emended Diagnosis Plates 3 and 4; Table 3.4; Fig. 3.1

These specimens (63 in total) are consistently very small, colonies never exceeding 100 mm in height. The number of autozooids each possess is few, ranging from 3 in the smallest colonies to 6 /7 in the tallest. There is a high degree of bilateralism so that the

first two secondary autozooids bud from the rachis laterally, positioned opposite each other and a little asymmetrically below the primary autozooid. Subsequent autozooids bud ventro-laterally at the distal portion the rachis. The primary autozooid can be easily identified: it either is positioned at a right angle to the axis on the ventral field, or extends above the rachis in line with the axis (particularly in larger specimens). The autozooids are placed somewhat apart and there is a rather conspicuous distance between the primary autozooid and the secondary autozooids. The dorso-ventrally flattened rachis is inferior and can hardly be distinguished from the stem. In fecund colonies, however, the rachis becomes swollen where the oocytes are stored in the mesenteries of the anthostele, as seen through the sarcosoma. The rachis has a tendency to bend towards the ventral field, or spiral in highly contracted specimens.

Anthocodiae are not cylindrically uniform in preserved colonies and relative to colony size, fairly large (anthocodiae of the primary autozooid up to 9.2 mm in length and of the secondary autozooids, 8.7 mm). The sarcosoma of the anthocodiae is thick so that the internal anatomy is not discernable in formalin-fixed specimens. For those preserved in alcohol, the anthocodiae are opaque and the eight mesentery septa can be seen within. Longitudinal striations corresponding to the mesenteries are present in all specimens and are more pronounced in those preserved in alcohol.

Each autozooid consistently presents short, robust tentacles; the mouths are oval and undulated where the tentacles join. The short, fragile pinnules are spaced along the tentacle with gaps in-between having a tendency for longer and shorter pinnules to alternate. Sclerites are absent from the autozooids and rachis.

Siphonozooids are small (~0.2 to 0.5 mm diameter) occurring on the rachis dorsally, ventrally and laterally, between the anthocodiae, and extending down the rachis away from the cluster and down the stem. A very narrow bare strip along the mid-dorsal line appears free of siphonozooids. Siphonozooids occur as raised warts covering the distal rachis observed to possess a single short tentacle, and are approximately three times larger here. Siphonozooids become smaller, flatter and less dense away from the cluster. There is a tendency for three siphonozooids to be positioned either side of the stem: these are not in defined rows.

Table 3.4 Dimensions (mm) of a representative sample of *U. carpenteri* from the Porcupine area, NE Atlantic, 4510-4860 m; and Crozet, S Indian Ocean, 4182-4195 m.

#	Colony L	Na	La_1	La_2	La_3	La_4	La_5	La_6	La_7	Wa_1	Wa_2	Wa_3	Wa_4	Wa_5	Wa_6	Wa_7	Lt	Wt	Lped	Wped
NE Atlantic																				
1	24.8	3	2.1	-	2.2					1.9	-	1.8					2.7	0.6	2.9	0.7
2	26.6	3	3.7	1.6	1.3					2.0	1.6	1.1					3.0	0.6	3.0	0.8
3	32.5	3	-	3.0	3.9					-	2.6	2.3					6.3	0.6	2.9	0.8
4	44.0	4	-	6.0	6.8	6.0				-	2.8	2.5	2.0				5.1	0.5	5.0	1.1
5	24.0	4	-	1.2	4.1	1.6				-	1.3	1.5	0.8				4.5	0.6	5.0	1.0
6	34.0	5	3.5	3.1	1.5	1.6				2.5	3.1	1.2	1.3				3.5	0.5	2.7	0.6
7	40.0	5	-	3.0	6.0	3.4	3.6			-	2.9	2.5	2.0	2.6			4.6	0.7	6.3	1.5
8	38.0	5	6.1	4.0	-	3.8	4.9			2.1	3.8	-	2.0	3.1			4.8	0.6	5.0	1.1
9	40.0	5	11.0	6.5	10.0	7.0	6.8			4.4	3.0	2.5	2.1	2.0			7.0	1.6	5.0	1.5
10	46.0	5	6.3	5.0	7.0	-	5.0			3.8	2.4	3.5	-	2.0			6.4	1.2	6.1	1.1
11	35.0	5	6.1	3.7	5.5	3.5	4.4			2.5	2.4	2.5	2.2	2.2			6.1	0.8	7.0	1.1
12	35.6	5	7.3	3.8	6.0	2.3	4.1			2.1	2.7	2.7	1.5	2.1			5.0	0.7	7.5	1.4
13	40.0	5	7.9	3.1	6.3	4.9	3.4			2.3	2.4	2.7	2.1	1.4			6.9	0.9	7.6	1.2
14	41.1	5	7.5	3.4	8.2	4.1	4.8			2.2	2.8	2.3	2.2	2.3			6.8	1.1	7.1	1.3
15	62.4	5	7.9	3.4	8.2	4.2	5.6			3.1	3.4	2.3	2.3	3.8			6.1	1.4	8.0	2.0
S Indian																				
16	70.0	5	4.5	5.0	7.6	7.4	2.1			3.0	2.2	2.0	3.5	1.1			11.0	1.2	8.0	2.8
17	77.0	5	5.6	7.9	8.4	9.4	7.0			3.3	2.8	2.1	2.2	2.4			15.0	1.2	9.0	2.0
18	72.0	5	7.0	4.8	8.0	7.1	6.8			3.9	3.7	2.6	3.0	3.0			9.0	1.3	7.0	1.5
19	88.0	6	6.4	7.7	11.7	12.0	7.1	7.2		4.7	3.9	4.3	5.2	2.4	2.0		16.0	1.1	10.0	2.2
20	95.0	6	8.0	7.2	8.6	10.3	9.1	2.7		3.0	2.7	3.0	2.4	3.8	1.9		11.0	1.3	11.0	2.4
21	84.0	6	5.0	4.1	8.4	8.2	7.6	5.5		3.9	3.5	2.7	2.2	2.7	2.0		11.0	1.4	11.0	1.6
22	60.0	6	6.0	7.0	8.6	10.2	5.1	4.7		2.8	2.5	2.5	2.6	2.9	2.9		11.0	1.4	9.0	1.6
23	86.0	7	6.8	5.8	5.7	5.5	8.8	4.4	5.4	2.9	2.3	2.4	2.7	2.7	2.0	2.1	11.0	1.3	8.0	2.0

Colony L, total length colony; Na, number of autozooids; La_1-7, length of autozooid; Wa_1-7, width of autozooid; Lt, length of tentacle; Wt, width of tentacle; Lped, length of peduncle; Wped, width of peduncle

The stem is covered by a relatively thick layer of sarcosoma. In alcohol preserved specimens, the tissue of the stem and proximal rachis has become stretched, laterally flattening the stem. In a few colonies, the axis is twisted and curved probably resulting from contraction, sometimes leading to spiralled colonies.

The thin axis (~0.2 mm inside the rachis, 0.5 to 0.7 mm below) is moderately flexible and quadrangular in section, possessing four longitudinal keels with rounded edges. The axis can be seen to pass up through the rachis, and often is not embedded in the wall of the primary autozooid but juts out above the rachis as a short spine, where it thins to about the width of a hair. This characteristic is fairly typical for this species and has not been observed in any other *Umbellula*, but is often a trait in abyssal species of the genus *Kophobelemnon*. For those colonies where the axis does not jut above the rachis, the axis is embedded in the wall of the primary anthocodia. In both cases, the axis is fine and appears rounded in section where it terminates. The peduncle is a small, slightly elongated swelling at the tip of the stem and possesses sporadic, minute corpuscle-type sclerites.

Differential Diagnosis and Remarks: *Umbellula carpenteri*

We know from molecular analysis of DNA sequences taken from the S Indian Ocean/Subantarctic samples that *U. carpenteri* is genetically different from the southern *U. magniflora* and northern *U. encrinus* (Fig. 3.3, Section 3.3.5). DNA sequences were not obtained for *U. carpenteri* from the NE Atlantic, but based on morphology there is no reason to believe that these do not belong to the same species. Colonies of *U. carpenteri* have previously been found in the equatorial Atlantic (Broch, 1957) giving further reason to believe that *U. carpenteri* exists in both northern and southern high latitudes. Thus, *U. carpenteri* should be considered a cosmopolitan abyssal species, unless future genetic analysis proves otherwise.

However, the small size of the colonies prompts one to think that these are young specimens of perhaps another species (such as *U. antarctica*?). Since 63 individuals were studied by the present author, all taken from different cruises, different years and different times of the year, it seems highly unlikely that this species attains heights

significantly larger than those presented herein. One could argue that the larger, more mature forms, escaped capture being too firmly anchored in the sediment, but the fact that there are many large specimens in the Discovery Collections (National Oceanography Centre), the majority of which collected by trawl and epibenthic sledge, and often at the same time as these specimens, contradicts this argument. Furthermore, colonies of *Kophobelemnon pauciflorum*, an abyssal species of wide geographical distribution (pers. ob.) and common to both areas from which *U. carpenteri* were obtained for this study, are also of restricted height (~100 mm). Thus, *U. carpenteri* is characterised by its bilateral symmetry, short height, and few autozooids. Figure 3.1 (Section 3.3.3.1) illustrates the differences in relation to colony height and number of autozooids between the other species that share morphological affinities with *U. carpenteri* (*U. magniflora*, *U. encrinus* and *U. antarctica*).

Discussion: *Umbellula carpenteri*

Kölliker (1880) first described *U. carpenteri* collected during the voyages of HMS *Challenger* (St 156 and St 157) and assigned five specimens under this name, specimens A to E. Unfortunately, Kölliker did not specify exactly which station each colony originated, and all of them were amalgamated into a single jar: specimen C is missing, but the remainder are in the Natural History Museum, London. All are figured in his paper, and with these and his measurements, it was possible to work out which descriptions refer to which specimens. Of these five, only one is considered to be *U. carpenteri* (specimen A) in this study.

In his diagnosis of *U. carpenteri*, Kölliker (1880) gave intermediate or contradicting characters, mentioning "…a very interesting gradation from bilateral to an apparently irregular arrangement of the polyps", the latter arrangement referring to specimens D and E, mature *U. magniflora*, and specimen B, a young colony of *U. magniflora*. Consequently, subsequent authors have incorrectly assigned specimens under the name *U. carpenteri* based on this confusing description (e.g. Broch, 1957). Note that Kölliker (1880) most likely based much of his description on specimen E, the largest of the colonies.

Broch (1957) described the development of the autozooid cluster of 16 specimens assigned to *U. carpenteri* obtained during the Swedish Deep-Sea Expedition, 1947-1948. However, only specimens 1 to 5 from 5275 m in the equatorial Atlantic (01.05° N, 18.67° W) were indeed *U. carpenteri* as described above. These were the five smallest colonies (31 to 43 mm in height), possessing only a few autozooids (3 to 5), and displaying bilateral symmetry. Also from the same haul, two medium sized specimens were obtained, 180 and 288 mm in height, and each possessing 5 and 6 highly-contracted autozooids, respectively. Yet going by his description of the autozooid arrangement, these latter specimens in fact belong to *U. magniflora*. Of the largest colonies (>1000 mm), Broch writes "two large specimens from the same haul possibly give us the definitive, normal arrangement of the polyps in outgrown specimens….In both specimens, the comparatively large autozooids are placed in two wreaths. The outer circle consists of 9 autozooids…whereas the inner circle has only 6 autozooids". However, these large specimens are both from a haul located approximately 40 degrees north of the 'younger specimens', and are likely to belong to *U. encrinus*.

A year later, Broch (1958) recognised that Kölliker erroneously grouped more than one species, and thus made *U. carpenteri* synonymous with *U. lindahli*, along with *U. magniflora* and *U. antarctica* (see discussion in Section 3.3.3.1).

In conclusion, it is of the opinion of the present author that *U. carpenteri*, based on its genetic and morphologic uniqueness, should maintain its species status and the above description should be used for its diagnosis, based on Kölliker's (1880) specimen A.

3.3.3.3 *Umbellula* sp.1 n. sp.

Material Examined

Whittard Canyon, NE Atlantic Ocean (47.9268° N; 10.2092° W), 4040 m, collected by means of ROV, *Isis*, on board the RRS *James Cook*, 03/07/2007: 1 specimen, colony fixed in formalin (borax-buffered 4 % formaldehyde in seawater), a portion of which was removed (peduncle and one anthocodia) prior to fixing and preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Axis quadrangular with four longitudinal grooves
- No sclerites in rachis/autozooids
- Few large cylindrical autozooids arranged in a concentric circle; radial symmetry
- Siphonozooids numerous and large; do not occur between the anthocodiae or on the distal rachis field encircled by the anthocodiae
- No large mucous cells in the ectoderm
- Long, slender stem

Taxonomic Description Plate 5; Table 3.5

This single specimen does not fit any previous species descriptions of *Umbellula*. Although sharing similar traits with to *U. encrinus* and *U. magniflora*, it is genetically different (see Differential Diagnosis and Remarks below) and for this reason, it is herein regarded as a new species. The colony is broken in the lower stem; nonetheless, it is complete and well preserved and provides a good representation of this *Umbellula* form. However, it is not possible to provide information on intraspecific variation or stages of development, and as such, the following description can only provide an indication of the characters specific to this species.

The length of the colony is 368 mm from the base of the peduncle to the tip of the rachis. Seven fully developed autozooids (including one removed for molecular analysis) are arranged in a single concentric circle at the most distal end of the rachis swelling only marginally spaced apart: the smallest (and youngest) of which is positioned proximally on the ventral field. The rachis here is a bulbous calyx, while the lower (proximal) portion narrows into a cylindrical thickening which gradually tapers into the stem.

The large anthocodiae are straight, cylindrical, and rather chunky and long. They each possess prominent longitudinal ribs corresponding to the internal mesenteries, but do not have the transversal wrinkles often associated with contraction. The sarcosoma of the anthocodiae is translucent in this formalin-fixed specimen, through which the internal anatomy can be seen. It is interesting that the sarcosoma of the autozooid preserved separately in ethanol for molecular analysis is not translucent and has a different colouration (white-grey as opposed to pale brown).

Unfortunately, many of the tentacles are missing, being either retracted or cropped in some cases, thus making reliable measurements of them, and their associated pinnules, impossible. However, in the case of one of the autozooids, half the tentacles still remain semi intact: these are very thin and delicate, and probably quite long but measurements of this structure are not reliable in such a specimen. The fine pinnules are spaced down the length of the tentacles and were not observed to alternate in length.

Siphonozooids are dense conical-shaped studs on the rachis, forming tongue-like tapering zones towards the anthocodiae. However, siphonozooids were not observed on the spaces between the anthocodiae or on the most distal field of the rachis in the area where the autozooids encircle. Siphonozooids are present on the dorsal midline, and densely cover the rachis below the swelling and down to the stem. Occasionally, a single tentacle can be seen projecting from the siphonozooids of the upper rachis. Siphonozooids were not found on the stem.

The stem is long and very thin, the sarcosoma of which is also thin but becoming fleshier towards the lower swelling of the rather subtle and elongated peduncle. The axis is quadrangular with deep grooves and keels with rounded edges.

Table 3.5 Dimensions (mm) of a single colony of *Umbellula* sp.1 n. sp. from the Whittard Canyon, NE Atlantic, 4040 m; L, length; W, width.

***Umbellula* sp.1 n. sp.**							
Colony L	368.0						
Axis W	1.4						
Stem W	1.4						
Peduncle L	89.0?						
Peduncle W	3.1						
Rachis swelling L	73.0						
Rachis W	12.0						
N autozooids	7.0						
Autozooid L	26.0	36.0	37.0	32.0	21.5	28.0	30.0
Autozooid W	5.3	6.3	6.1	6.1	3.0	6.1	6.0
Tentacle L	9.0?						
Tentacle W	0.4						
Pinnule L	-						
Pinnule W	-						
Siphonozooids	0.35						

Neither the tentacles, autozooids, rachis or the stem possess sclerites in their mesoglea. The peduncle however, contains numerous broad oval sclerites.

The specimen is gravid, with oocytes bursting out of the rachis where one of the anthocodia has been removed, but appear not to distort the shape of the remaining anthocodiae. Interestingly, an oocyte was observed within one of the tentacles when observed under the microscope. This phenomenon was also seen in specimens of other species during this present study.

Differential Diagnosis and Remarks: *Umbellula* sp.1 n. sp.

This new species is morphologically very similar to the northern dwelling *U. encrinus*, and the southern *U. magniflora* and *U. antarctica*. Characters such as axis shape, presence/absence of sclerites, and symmetry are not sufficient to distinguish these species since they all have the same character states in common: to differentiate, less convincing characteristics need to be employed. In the section on *U. magniflora*, *U. encrinus* and *U. antarctica* comparisons were made based on the number of autozooids in relation to colony height: yet with only one exemplar, and therefore no indication of autozooid development, the average number of autozooids of mature colonies, and its height limitations, it is not possible to make such comparisons.

However, it can be said that autozooids are much fewer in *Umbellula* sp.1 n. sp. compared to colonies of *U. encrinus*, and *U. antarctica* in particular, for specimens of similar height. The anthocodiae of *Umbellula* sp.1 n. sp. are larger than those of the other species of this form of similar height (*U. magniflora*, *U. encrinus* and *U. antarctica*), thus distinguishing it from the rest based on these ambiguous characters. A further difference is the distribution of the siphonozooids: siphonozooids are not present on the rachis in the field encircled by the anthocodiae and also do not occur between the anthocodiae themselves, whereas this is not a trait of the other three species. This new species does not possess large mucous cells in the ectodermal layer, which is characteristic of *U. magniflora* and *U. encrinus*.

There is molecular evidence to suggest that *Umbellula* sp.1 n sp. is genetically different from either *U. encrinus* or *U. magniflora* (DNA sequence data for *U. antarctica* is wanting), and that it is most closely related to *U. encrinus* (Fig. 3.3, Section 3.3.5). This is not surprising considering they show a morphological resemblance and both are only known

to inhabit the higher latitudes of the northern hemisphere. It is possible that *Umbellula* sp.1 n. sp. with its small number of large autozooids has adapted to the abyssal depths of the N Atlantic, whereas *U. encrinus* with its numerous smaller autozooids inhabits the shallower cold-waters of the Arctic and N Atlantic Oceans. Without further information on their geographical distribution and depth range, however, this can only be speculated.

3.3.3.4 *Umbellula pellucida* Kükenthal 1902

Umbellula pellucida	Kükenthal 1902
Umbellula pellucida	Kükenthal and Broch 1911
Umbellula pellucida	Kükenthal 1915
Umbellula pellucida	Hickson 1916
Umbellula huxleyi	Hickson 1937
Umbellula pellucida	Broch 1958
Umbellula pellucida	Pasternak 1964

Type Material

Location unknown.

Material Examined

Off Oregon, NE Pacific Ocean (43.4500° N; 124.8267° W), 550 m, collected by means of OTSB, 03/06/1974: 3 specimens, preserved in 75 % ethanol.

Key Taxonomic Descriptors

- Axis quadrangular with four longitudinal grooves
- No sclerites, only small oval bodies in the peduncle
- Colonies slender, small: <350 mm in height, only exceptionally exceeding 300 mm
- Autozooids numerous (25-30), the anthocodiae of which are small (~30 mm in length; 4-5 mm width) and crowded; no apparent symmetry, indistinct bilateral symmetry in immature colonies (?)
- Tentacles approximately equal in length to the anthocodiae
- Rachis manifests as a distinct conical swelling on top of which sit the anthocodiae
- Siphonozooids absent from the interspaces between anthocodiae

Differential Diagnosis and Remarks: *Umbellula pellucida*

Having only very briefly studied the above-mentioned material during a visit to the California Academy of Science, San Francisco, an emended diagnosis has not been included in this account. However, it is of the opinion of the present author that *U. pellucida* is indeed a true species, thus the following differential diagnosis is mainly based on information from the literature (Kükenthal, 1915; Hickson, 1916; 1937; Broch, 1958).

There are six species of *Umbellula* herein considered true taxon that do not possess sclerites in their rachis and autozooids, each having axes quadrangular in section with four longitudinal grooves: *U. magniflora*, *U. encrinus*, *U. carpenteri*, *Umbellula* sp.1 n. sp. and *U. pellucida*.

Colonies of *U. pellucida* occur in waters generally <1600 m depth: they are one of the smallest *Umbellula* species, only exceptionally exceeding 300 mm in height, and possess numerous (25 to 30), small autozooids. One other species of *Umbellula* is known to be of restricted height, namely *U. carpenteri*: mature colonies of this species are <100 mm tall. However, not only are these generally smaller, they differ in the following ways: autozooids few (~7); symmetry bilateral; the rachis is an inferior enlargement of the upper stem; tentacles consistently much shorter than anthocodiae; and furthermore, they are only known to inhabit abyssal depths. The one recorded specimen of *Umbellula* sp.1 n. sp., as accounted in this study, was also found at a depth >4000 m, and is morphologically distinct by its few, very large anthocodiae. Large colonies of *U. magniflora* also have very few autozooids: a colony of this species 2310 mm tall was recorded to have just 13 (Pasternak, 1962). Colonies of *U. antarctica*, on the other hand, possess numerous autozooids, and are generally not very tall (~500 mm). However, they are remarkably thick and stout in comparison to the slender colonies of *U. pellucida*: specimens ~440 mm have peduncles ~18 mm thick and axes 3-5 mm in diameter. The slender colonies of *U. encrinus* are rather tall, often exceeding heights 2000 mm, and siphonozooids occur between the anthocodiae: this latter feature is also a trait of *U. magniflora*, *U. carpenteri* and *U. antarctica*, but they seem consistently absent from *U. pellucida*.

In terms of geographical distribution, *U. pellucida* is comparatively common in the Indian Ocean, inhabiting relatively shallow depths, rarely exceeding 1600 m; this is the first account of this *U. pellucida* from the Pacific Ocean.

Discussion: *Umbellula pellucida*

There are very few records of *U. pellucida* in the literature: Kükenthal (1902) made the first description of this species, and later Kükenthal and Broch (1911) and Kükenthal (1915) based on the same material, Hickson (1916), and more recently Broch (1958) and Pasternak (1964). Hickson (1937) described *U. pellucida* under the misnomer *U. huxleyi*. All previous recorded specimens were collected from the Indian Ocean, but now *U. pellucida* is known to inhabit the Pacific Ocean suggesting a cosmopolitan distribution. Broch (1958) gives a detailed review of this species.

3.3.3.5 *Umbellula huxleyi* Kölliker 1880

Umbellula huxleyi pars	Kölliker 1880 (specimen D only)
Umbellula huxleyi	Kükenthal and Broch 1911
Umbellula gracilis	Broch 1913
Umbellula gracilis	Kükenthal 1915
Umbellula huxleyi	Kükenthal 1915
Umbellula Weberi	Hickson 1916
Umbellula huxleyi	Broch 1958

Type Material

Natural History Museum, London. Lectotype specimen, Challenger Stn 235, NW Pacific, S of Tokyo, Japan (35.1800° N; 135.6500° E) 1033 m, reg. no. 1881. 2.11.25; Plate IX Fig. 37 (Kölliker, 1880).

Material Examined

Type material (see above).

Porcupine Seabight and along continental slope/rise (NW Ireland), NE Atlantic Ocean (49.3170° N to 57.2833° N; 9.1833° W to 13.9400° W), 483-2645 m, collected by means of trawl (OTSB) and epibenthic sledge over the period of 24/04/1978 to 01/06/2006: 108 specimens, fixed in formalin (borax-buffered 4 % formaldehyde in seawater), stored in formalin/70 % propan-2-ol; 11 specimens preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Axis round
- No sclerites, only small oval bodies in the peduncle
- Autozooids numerous, arranged in indistinct circles manifested as a concentrated pompon at the most distal end of the rachis; radial symmetry
- Autozooid-bearing portion of rachis short, gradually narrowing below
- Anthocodiae not large in proportion to colony size; tentacles of equal length to anthocodiae

Emended Diagnosis Plate 6 and 7; Table 3.6

Colonies are tall and slender, reaching heights > 675 mm. Autozooids are irregularly positioned in indistinct whorls at the most distal portion of the rachis, thus creating a kind of 'pompon', with no apparent pattern although having a radial arrangement. Younger specimens take on an indistinctly bilateral form seen from the dorsal aspect only. The autozooid-bearing portion of the rachis is short and the numerous autozooids (up to ~45 in the largest specimens) are tightly crowded here: those of the outer whorls are more spaced. Below, the rachis gradually decreases in breadth until it blends with the stem. In some specimens, this portion has become contorted and twisted.

The anthocodiae are slender and relatively small, those of the outer whorls tending to be smaller than those deep within the cluster. However, this is not exclusive and often young autozooids are located in the internal whorls, and thus there is no apparent pattern of development. The tentacles are approximately of equal length to the anthocodiae and are thin; each possessing long, fine pinnules situated almost opposing each other along their length: these do not alternate in size and are positioned with spaces between them.

Siphonozooids are numerous and cover the entire rachis, including the dorsal midline. They occur between the anthocodiae and normally form tapering tongues below the autozooid-cluster: in younger specimens, these may be absent. Siphonozooids are 2-3 times larger between anthocodiae, and are dome-shaped. Many posses a single tentacle; these tentacles are much more obvious in the larger siphonozooids situated between the autozooids (~1.5 times the height of the siphonozooid). The tentacles of the smaller

siphonozooids appear as rudimentary points. The larger siphonozooids may have longer tentacles since the anthocodiae offer protection from damage and/or cropping, or it may well be that the tentacles have retracted in those siphonozooids lower down the rachis. Siphonozooids of the stem are sparse in the main, but more numerous in some; the occasional threadlike tentacle can be seen to emanate in well-preserved specimens.

The sarcosoma of the stem is consistently thick in comparison to other species. The axis is round in cross-section and very flexible, particularly below the rachis where it thins. The peduncle manifests as a strong thickening of the proximal stem, and is often contracted to take on the form of the internal nutrient canals and thus is quadrangular in cross-section. Small broad sclerites are present in the peduncle.

The colour of the colonies varies from brownish-pink to cream-grey.

Table 3.6 Dimensions (mm) of a representative sample of *U. huxleyi* from the Porcupine Abyssal Plain, NE Atlantic, 483-2645 m; L, length; W, width.

U. huxleyi	1	2	3	4	5
Colony L	288.0	363.0	487.0	558.0	676.0
Axis W	1.3	1.1	1.1	1.9	2.5
Stem W	1.8	1.7	1.9	3.6	3.4
Peduncle L	51.0	6.6	65.0	85.0	100.0
Peduncle W	4.0	86.0	4.2	7.0	10.4
Rachis swelling L	16.6	39.0	40.0	68.0	83.0
Rachis W	7.0	11.0	12.6	10.0	30.0
N autozooids	18	30	21.0	42	~45
Autozooid L (average)	13.3	15.0	18.2	21.4	26.5
Autozooid W (average)	3.0	3.5	3.9	4.5	4.5
Tentacle L	14.6	11.5	17.2	23.5	27.3
Tentacle W	0.4-0.5	0.5	0.6	0.8-1.1	0.7
Pinnule L	1.0	1.0	0.9	0.9	0.9-1.1
Pinnule W	0.30	0.09	0.08	0.20	0.30
Siphonozooids (distal rachis)	0.5-0.8	0.6	0.8	0.5	0.5-0.6
Siphonozooids (proximal rachis)	0.2	0.3	0.4	0.3	0.3

Differential Diagnosis and Remarks: *Umbellula huxleyi*

Two species lacking sclerites and with round axes belong to *Umbellula*, namely *U. huxleyi* and *U. spicata*. *Umbellula huxleyi* is characterised by its concentrated pompon-like autozooid cluster and numerous, small anthocodiae arranged in indistinct whorls; *U. spicata*, on the other hand, is characterised by the long, spaced tassel of very slender, long anthocodiae.

Discussion: *Umbellula huxleyi*

Umbellula huxleyi was described by Kölliker (1880) from the *Challenger* Expedition based on four specimens (A-D), writing "Axis indistinctly quadrangular" in his account. Broch's (1958) revision of *Umbellula* outlines the problems regarding this diagnosis, recognising that Kölliker based his descriptions on two species: specimens A and B having quadrangular axes were considered to be '*U. lindahli*' and specimen D, on which most of Kölliker's description was based and having a round axis throughout, *U. huxleyi* (specimen C is missing from the collection at the Natural History Museum, London). Having studied these specimens, the present author is in agreement with Broch (1958), with the exception of his diagnosis of specimens A and B, which are probably *U. pellucida* (or undescribed species?).

Broch (1913) gave a detailed description of a specimen from the NE Atlantic ascribing it to *U. gracilis*, but in his revision, Broch (1958) made *U. gracilis* synonymous with *U. huxleyi*: by these accounts, this seems justifiable and thus *U. gracilis* is regarded a synonym of *U. huxleyi*.

Hickson (1916) described a new species, *U. weberi* (originally named *U. Weberi*, Hickson 1916), from the material collected during the Siboga Expedition and taken from a similar area as Kölliker's *U. huxleyi* (N Pacific), writing "*U. Weberi* appears to be most closely related to *U. encrinus* of the North Atlantic Ocean, but it is a more slender form with more numerous autozooids…and there are no deep grooves in the axis". For this specimen he summarises "*Umbellula* of slender habit, without spicules, with about 30 small autozooids in a specimen 485 mm in length". From these descriptions and associated figures, it is believed that Hickson (1916) was describing *U. huxleyi* and therefore *U. weberi* is a junior synonym of this species. Hickson's (1937) *U. huxleyi* is probably *U. pellucida*, based on the "quadrangular axis marked by four longitudinal grooves", as first surmised by Broch (1958).

3.3.3.6 *Umbellula spicata* Kükenthal 1902

Umbellula spicata	Kükenthal 1902
Umbellula valdiviae	Kükenthal 1902
Umbellula spicata	Kükenthal and Broch, 1911
Umbellula valdiviae	Kükenthal and Broch, 1911
Umbellula spicata	Broch 1958

Type Material

Location unknown.

Material Examined

None.

Key Taxonomic Descriptors

- Axis round
- No sclerites in the autozooids/rachis
- Autozooid-bearing portion of rachis long, autozooids spaced along this
- Anthocodiae very long, slender; numerous (~25)
- Irregular arrangement of autozooids
- Colony slender

Differential Diagnosis and Remarks: *Umbellula spicata*

There are no exemplars of this species available for this study, thus an emended diagnosis could not be made. Nonetheless, having studied the literature and associated figures (Kükenthal, 1902; Kükenthal and Broch, 1911; Broch, 1958), it is the belief of the present author that *U. spicata* is a valid species.

As mentioned in the previous section, there are two species of *Umbellula* that lack sclerites and have axes round in cross-section, namely *U. huxleyi* and *U. spicata*. *Umbellula huxleyi* is characterised by its concentrated pompon-like autozooid cluster and numerous, small anthocodiae arranged in indistinct whorls: *U. spicata*, on the other hand, possess a lengthy tassel of very slender, long anthocodiae with comparatively large interspaces between them.

Only known from the Indian Ocean, at depths of ~470 to 1280 m

Discussion: *Umbellula spicata*

In his review of *U. spicata*, Broch (1958) made *U. valdiviae* a junior synonym of this species, considering the character differences Kükenthal and Broch (1911) attributed to separate the two insufficient to justify a division. These characters are as follows: *U. valdiviae* has a weak, more slender stem (this is not perceivable in the figures); the rachis is not so contracted in *U. valdiviae* as in *U. spicata*; and the tentacle pinnules alternate in size, whereas they increase successively in length towards the end of the tentacle in *U. spicata*. Broch (1958) stated that these features are a result of contraction and the present author is inclined to agree: the descriptions and excellent figures presented by Kükenthal and Broch (1911) do not bring to light character differences of taxonomic value and thus *U. valdiviae* and *U. spicata* are considered synonymous.

Group B: *Umbellula* spp. with sclerites

3.3.3.7 *Umbellula thomsoni* Kölliker 1874

Umbellula thomsoni	Kölliker 1874
Umbellula thomsoni	Kölliker 1875
Umbellula thomsoni	Kölliker 1880
Umbellula güntheri	Kölliker 1880
Umbellula leptocaulis	Kölliker 1880
Umbellula simplex	Kölliker 1880
Umbellula Köllikeri?	Kükenthal and Broch 1911
Umbellula güntheri	Broch 1913
Umbellula güntheri	Kükenthal 1915
Umbellula leptocaulis	Kükenthal 1915
Umbellula Köllikeri?	Kükenthal 1915
Umbellula güntheri	Broch 1957
Umbellula thomsoni	Broch 1958
Umbellula thomsoni	Pasternak 1962
Umbellula thomsoni	Pasternak 1970
Umbellula thomsoni	Grasshoff 1972
Umbellula thomsoni	Pasternak 1975
Umbellula thomsoni	Pasternak 1993

Type Material

Natural History Museum, London. Lectotype specimen, Kölliker's Specimen 'A' designated on pg 243 (Kölliker, 1874); reg. no. 1881. 2.11.23. Type specimen *U. güntheri* Köll 1880 from Challenger Stn 106, Atlantic, just N of Equator (1.7000 ° N; -25.2300° W) 3383 m; Plate IX fig. 35 (Kölliker, 1880).

Material Examined

Type material (see above).

Porcupine Abyssal Plain, Nr Goban Spur, NE Atlantic Ocean (49.6505° N to 50.0533° N; 13.8433° W to 14.3212° W), 3485-4298 m, collected by means of trawl over the period of 12/11/1977 to 28/08/2001: 5 specimens, fixed in formalin (borax-buffered 4 % formaldehyde in seawater), stored in formalin/70 % propan-2-ol.

Crozet, S Indian Ocean (48.9368° S; 51.0650° E), 4182-4195 m, collected by means of OTSB, 27/12/2005: 2 specimens, preserved in 96 % ethanol.

Cascais Canyon, NE Atlantic Ocean (38.3755° N; 9.9782° W), 3476 m, collected by means of ROV, *Isis*, on board the RRS *James Cook*, 29/06/2007: 1 specimen, preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Quadrangular axis with four longitudinal grooves
- Sclerites in every part of the mesoglea; none very large (< 0.86 mm) and all three-flanged
- Palpable bilateral symmetry displayed by the autozooids
- Tentacles broad and short (≤ length of anthocodia)
- Siphonozooids often tall; dense on rachis, form narrow tongues between the anthocodia
- Autozooids of preserved specimens possess transversal and longitudinal wrinkles

Emended Diagnosis Plate 8 and 9; Table 3.7

Specimens perfectly match the diagnosis of Kölliker (1880) and the more detailed descriptions of Broch (1957; Broch, 1958). Colonies are distinctly bilateral, autozooids positioned pair-wise on the lateral surfaces of the rachis. The rachis itself is dorso-ventrally flattened, the breadth of which varies depending on degree of contraction and level of fecundity in gravid colonies, but is generally an inferior elongated keel-like swelling of the stem. The primary autozooid is located at the most distal portion of the rachis (at its tip), from which it emanates in a continuous line with the longitudinal axis of the colony, sometimes set in slightly towards the ventral field or sometimes marginally above this. The first secondary autozooids bud from the rachis approximately at 45˚ to the longitudinal axis so that the distal ends of the anthocodia are directed ahead. In more

mature colonies, subsequent autozooids bud ventro-sublaterally on the surface of the proximal rachis and are directed ahead and slightly upwards. Autozooids, although not tightly packed, are positioned so that there is very little space between them.

The anthocodiae of all specimens have undergone, to different degrees, remarkable levels of contraction manifested as transversal wrinkles: longitudinal striations are also apparent corresponding to the internal mesenterial septa. Tentacles are approximately equal to or slightly shorter than the anthocodiae and are broad and strong. Pinnules are short and approximately equal in size, closely positioned along the tentacle.

Siphonozooids are very numerous and dense on the rachis. Dorsally, they form narrow tongues up the rachis swelling from its base, continuing on the tight interspaces between the autozooids. Siphonozooids are absent from the dorsal midline of the rachis but cover the entire surface of the ventral field. Generally, they are conical bodies, 0.4 to 0.6 mm in height and breadth at their bases, and are largest amongst the anthocodia at the most distal portion of the rachis, presumably, where they have been guarded against abrasion.

Sclerites are small ranging from 0.20 to 0.86 mm in length and 0.02 to 0.09 mm in breadth. They are three-flanged needles with dentate edges throughout the rachis, autozooids and tentacles. Sclerites of the anthocodiae are orientated with a tendency to be parallel to the longitudinal axes of the autozooids. Those surrounding the siphonozooids frequently form a kind of calyx ending with several points. Sclerites of the stem tend to be smaller and broader (0.13 to 0.2 mm by 0.025 to 0.051 mm), and are more tuberculated than those of the rachis and autozooids.

The sarcosoma of the stem is thin. The quadrangular axis within possesses longitudinal keels with rounded edges, and is remarkably inflexible and brittle so that the majority of specimens have broken away from their peduncle upon collection. The axis can be seen to enter the rachis dorsally where it forms a ridge along the dorsal midline before terminating in the primary autozooid. This feature often leaves the rachis keeled, with the autozooids angled in towards the ventral field.

Peduncle is an inferior thickening of the lower stem. Colonies are straw-coloured throughout.

Table 3.7 Dimensions (mm) of *U. thomsoni* colonies from the Porcupine Abyssal Plain, NE Atlantic, 3485-4298 m (specimens 1-3, 5-7); Cascais Canyon, NE Atlantic, 3476 m (specimen 4); Crozet, S Indian Ocean, 4182-4195 (specimen 8). L, length; W, width.

U. thomsoni	**1**					**2**						**3**								
Colony L	-					-						-								
Axis W	0.3					-						-								
Stem W	0.8					-						-								
Peduncle L	-					-						-								
Peduncle W	-					-						-								
Rachis swelling L	9.0					-						4.1								
Rachis W	4.0					6.0						7.0								
N autozooids	5.0					6.0						9.0								
Autozooids L	11.0	12.5	8.6	7.3	8.0	5.0	9.5	8.6	8.0	-	10.0	11.6	12.0	14.0	15.0	13.0	10.0	15.0	13.5	13.0
Autozooids W	4.4	4.5	3.5	4.4	4.0	5.0	5.0	4.2	4.5	-	5.6	4.0	4.0	5.3	6.0	4.6	4.2	5.0	5.5	5.5
Tentacle L	-	9.0	9.0	7.3	9.0	-	9.0	8.6	7.4	-	10.8	7.0	9.5	10.3	12.3	9.4	-	10.1	9.4	10.0
Tentacle W	-	0.8	0.7	1.0	1.0	-	1.2	1.3	1.1	-	1.5									
Pinnule L	-																			
Pinnule W	-																			
Siphonozooids	-																			

U. thomsoni	**4**					**5**						**6**								
Colony L	388.0					-						-								
Axis W	1.1					0.6						0.9								
Stem W	1.3					1.2						1.5								
Peduncle L	16.0					-						-								
Peduncle W	1.6					-						-								
Rachis swelling L	16.3					22.0						22.0								
Rachis W	6.0					5.0						8.0								
N autozooids	5.0					6.0						9.0								
Autozooids L	12.3	16.0	17.9	21.0	19.0	8.5	9.0	8.0	7.5	8.5	7.5	8.0	8.0	13.0	10.0	12.5	9.5	11.0	11.0	11.5
Autozooids W	4.1	5.7	7.0	7.0	6.9	4.3	4.7	4.1	4.5	4.9	4.5	3.0	4.3	4.0	4.5	4.5	4.5	4.9	3.9	4.0
Tentacle L	12.2	12.6	17.5	17.4	14.9	-						-	9.0	12.0	12.0	14.3	-	12.0	10.0	11.0
Tentacle W	1.4	1.4	1.8	1.6	2.1	-						-	1.4	1.2	1.1	1.2	-	1.2	1.2	1.2
Pinnule L	2.4					-						3.0								
Pinnule W	0.4					-						0.2								
Siphonozooids	0.2-0.3					0.5						0.6								

Table 3.7 continued… Dimensions (mm) of *U. thomsoni* colonies from the Porcupine Abyssal Plain, NE Atlantic, 3485-4298 m (specimens 1-3, 5-7); Cascais Canyon, NE Atlantic, 3476 m (specimen 4); Crozet, S Indian Ocean, 4182-4195 (specimen 8). L, length; W, width

U. thomsoni	**7**										**8**										
Colony L	660.0										-										
Axis W	2.7										1.6										
Stem W	2.9										1.7										
Peduncle L	19.0										-										
Peduncle W	3.0										-										
Rachis swelling L	30.0										35.0										
Rachis W	11.0										15.0										
N autozooids	10.0										11.0										
Autozooids L	5.0	17.0	17.0	17.0	18.0	21.0	24.0	20.0	20.0	18.0	22.0	18.0	21.0	21.0	20.0	20.6	11.0	11.3	21.0	18.0	19.3
Autozooids W	6.0	6.1	5.6	4.8	4.5	5.0	5.8	5.6	5.5	5.6	5.3	4.8	5.4	5.0	5.8	5.1	4.6	4.0	5.0	4.9	4.8
Tentacle L	-	10.0	13.2	11.6	11.0	12.4	13.0	11.3	18.0	11.0	13.0	11.0	13.5	13.0	15.0	15.0	12.0	12.3	11.0	14.1	15.0
Tentacle W	-	1.8	1.9	1.5	1.4	1.5	1.9	1.4	2.0	1.3	1.6	1.6	1.5	1.6	1.8	1.7	1.1	1.3	1.5	1.5	1.5
Pinnule L	1.0										1.8										
Pinnule W	0.2										0.3										
Siphonozooids	0.3										1.4										

Differential Diagnosis and Remarks: *Umbellula thomsoni*

Umbellula thomsoni and *U. hemigymna* are the only known species to possess both quadrangular axes and sclerites in their autozooids and rachis. However, features such as the distribution of the sclerites, and the form of the tentacles and associated pinnules are the main features that distinguish them from each other. For a more detailed account of these differences, refer to Section 3.3.3.8 *Umbellula hemigymna*.

Colonies of *U. thomsoni* from the NE Atlantic and S Indian Oceans are genetically very similar (differences probably attributable to intraspecific variability), and thus are regarded as two populations of the same species (Fig. 3.3, Section 3.3.5). This provides strong molecular evidence to suggest that *U. thomsoni* is cosmopolitan. Previous authors have reported *U. thomsoni* from the Atlantic (Kölliker, 1880; Broch, 1913; 1957; Grasshoff, 1972; Pasternak, 1993) and Indian Oceans (Pasternak, 1964), as well as the Pacific (Pasternak, 1970) and Southern Oceans (Pasternak, 1993). Thus, *U. thomsoni* is believed to inhabit four of our five world oceans, populating abyssal depths in both the northern and southern hemispheres.

Discussion: *Umbellula thomsoni*

Umbellula thomsoni was first described by Kölliker (1874/75), and later re-described in 1880 along with a further four *Umbellula* species possessing sclerites in their rachis' and autozooids (Kölliker, 1880). Of these, three were regarded synonymous with *U. thomsoni* (Broch, 1957; 1958), namely *U. güntheri*, *U. leptocaulis* and *U. simplex*.

Following a revision of the descriptions and figures given by Kölliker (1880) and Broch (1913; 1957; 1958), along with the lectotype specimen (*U. güntheri*) at the Natural History Museum and those new specimens available for this study, the same conclusion as Broch (1958) has been reached: the differences Kölliker attributes do not make for a sufficient *fundementum divisionis*, and are thus regarded a function of development and/or intraspecific variability and contraction upon preservation.

A further species, *U. köllikeri* (originally named *U. Köllikeri*, Kükenthal, 1902), was described that fits the description of *U. thomsoni* perfectly, with the exception that sclerites are apparently absent from the autozooids and rachis. Kükenthal and Broch (1911) also described this species based on the same specimen and their Fig. shows a colony with remarkable likeness to *U. thomsoni*. It is also plausible that *U. köllikeri* is the senior synonym of *U. hemigymna* (Section 3.3.3.8), a colony known to possess very few, sparsely distributed sclerites. Nonetheless, with hesitation, *U. köllikeri* has herein been made synonymous with *U. thomsoni* based on overall morphology, but this grouping should be regarded with some caution.

Thomson (1915) described *U. aciculifera*, which was made synonymous with *U. thomsoni* by Broch (1958): subsequent authors (Pasternak, 1962; 1964; 1970; 1975; Williams, 1990; Pasternak, 1993) have up held this synonymy; however, *U. aciculifera* is regarded by the author as a valid and distinctive species (see Section 3.3.3.10).

3.3.3.8 *Umbellula hemigymna* Pasternak 1975

Umbellula hemigymna Pasternak 1975

Type Material

Holotype, 1 exemplar from Stn 1207, Caribbean Sea, Grenada Basin (13.3100° N; 62.9900° W), 3000 m; P.P. Shirsov's Institute of Oceanology, Moscow.

Material Examined

Porcupine Abyssal Plain at the base of Porcupine Seabight, NE Atlantic Ocean (50.0150° N; 14.1133° W), 3800-3820 m, collected by means of OTSB, 27/09/1981: 1 specimen, fixed and stored in formalin (borax-buffered 4 % formaldehyde in seawater).

Key Taxon Descriptors

- Axis quadrangular with four longitudinal grooves
- Sclerites present in the stem, rachis, autozooids, tentacles and pinnules
- Sclerites all small (<0.6 mm in length) and three-flanged
- Sclerites not numerous and distributed sparsely, aggregating only in parts of the colony

- Autozooids long and narrow
- Rachis displays bilateral symmetry
- Tentacles long and thin
- Siphonozooids large and numerous on the rachis

Emended Diagnosis Plate 10 and Plate 17; Table 3.8

One individual of *U*. *hemigymna* fitting the detailed description of Pasternak (1975) is described. Since the discovery of this species, no other records have been published, and because the description is only publicly available in Russian, the remarks herein will also consider the diagnostic characters discussed by the original author, as well as providing further information on phenotypic variability. Thus, to avoid confusion the new specimen will be referred to as the Discovery specimen (from the Discovery Collections, National Oceanography Centre, Southampton).

The Discovery specimen is stored in formalin and is quite damaged, the stem being broken in four places, and the lower stem and peduncle missing. The four exemplars detailed by Pasternak (1975) were also broken along the fragile axis, although complete. These specimens possessed 4 to 7 autozooids and therefore were presumably younger than the Discovery specimen, which has 14 autozooids in total (11 fully grown, one younger, one semi-rudimentary with short tentacles, and one rudimentary form, lacking tentacles). The primary autozooid is situated at the tip of the rachis, set in slightly towards the ventral side. The secondary autozooids from the dorsal aspect are located pair-wise on the lateral sides of the rachis; younger and smaller autozooids are located ventrally, on the proximal portion of the rachis. The radial symmetry of the cluster is distorted by the shape of the flattened rachis, thus the cluster displays bilateral symmetry liken to *U*. *thomsoni* and *U*. *aciculifera*.

Pasternak (1975) describes the autozooids as comparatively tall and slender, yet those of the Discovery specimen are up to 4.5 times longer than these, but having approximately the same width (autozooids reminiscent of *Umbellula spicata*). However, this may be a function of age: since the Discovery specimen is more mature, the autozooids are larger. A second difference is the rachis shape, described as short and wedge-shaped by Pasternak: the rachis of the Discovery specimen is an elongated swelling, ovoid in shape.

Again, this could be attributed to differences in maturity, intraspecific variability, or perhaps translational error, and do not furnish sufficient *fundementum divisionis*.

The bodies of the autozooids are almost cylindrical, with a slight narrowing in the middle region. The walls possess 8 longitudinal striations corresponding to the mesenterial septa, whereas transversal rugosity is absent (with the exception of occasional creases). The long, fine tentacles are approximately equal in length to the autozooid bodies, and are laterally flattened. Pinnules are short but slender, being of equal size, and spaced along the tentacles so there are gaps between them, this distance increasing towards the distal end of the tentacle. The tentacles of the younger polyps are relatively more robust and shorter.

Siphonozooids are very numerous. They occupy the whole central field at the ventral face of the rachis where they form narrow lateral tongues that taper between the bases of the autozooids. Dorsally, siphonozooids are everywhere except the midline of the rachis where the axis can be seen beneath the surface, and the small areas at the bases of the autozooids. The siphonozooids are truncated cones 0.15 to 0.3 mm in diameter and up to 0.6 mm in height (the contracted ones 0.2 mm high). Pasternak (1975) notes that the 8 radial striations are usually discernable at their surface, and that the mouth opening is visible in some cases. Below the rachis swelling, siphonozooids become few and far between. It is difficult to say without making histological sections if the siphonozooids continue down the length of the stem, but this region of the colony appears to be wanting (Pasternak made no mention of siphonozooids below the rachis).

Sclerites occur in the mesoglea of the stem, rachis, autozooids, tentacles and pinnules; however, they are rare and hardly discernible and as such can go unnoticed when the colony is briefly examined. Unfortunately, the sclerites of the Discovery specimen have been damaged by formalin making them fragile and are readily dissolved in sodium hypochlorite. This has meant that the finer details of the edges have been lost, and the SEM images are poor (Plate 17).

Sclerites are small, three-flanged rods of similar size. Those of the rachis are the most numerous, generally distributed irregularly between the siphonozooids but not present within the siphonozooids themselves and instead encircling them, a feature not noted by

Pasternak (1975). In the zones where siphonozooids are absent, at the bases of the autozooids, sclerites form sparse aggregations consisting of 20 to 30 at most. The sclerites of the autozooids are orientated with the long axis parallel to the long axis of the polyp bodies (again a feature not noted by Pasternak, 1975) and are dispersed at a great distance from each other. These are restricted to the proximal portions of the autozooids and mainly only in the limits of 3 to 4 inter-mesenterial bands of the asulcal side, 10 to 40 in each. Rachis and autozooid sclerites are quite equal, 0.250 to 0.575 in length and 0.035 to 0.060 mm in width.

Sclerites of the tentacles are usually orientated along its axis, as too are those of the pinnules. The latter are most densely aggregated in the proximal parts of the pinnules but do not form a solid armature characteristic for other *Umbellula* species. Sclerites of the tentacles are ~0.3 mm in length and 0.036 to 0.047 mm wide, and those of the pinnules are smaller being 0.185 to 0.292 in length and 0.015 to 0.022 mm wide.

The sclerites of the stem are few in number and orientated parallel to the axis. They are broader and flatter in section, 0.207 to 0.251 mm long, 0.051 to 0.074 mm wide in the Discovery specimen. Conversely, the stem sclerites of Pasternak's (1975) specimens were narrower reaching only 0.025 mm in width. This aside, the sclerites of the Discovery specimen are a perfect match to those of Pasternak's (1975) descriptions and measurements.

The axis is quadrangular in section, strongly keeled with rounded edges, and has a high degree of flexibility. Where the axis of the Discovery specimen has been broken, the four keels have come apart from each other, and much of the axis has been flattened so that the keels have paired and splayed laterally. The stem sarcosoma is thin and compressed, taking on the quadrangular shape of the axis inside.

The colour of the rachis and autozooids of Pasternak's (1975) alcohol-fixed specimens are bluish-grey and the pinnules are brown. The formalin-fixed and preserved Discovery specimen is pallid and straw coloured throughout.

In fecund specimens, mesenteries packed with oocytes do not distort the shape of the autozooids thus maintaining their slender appearance.

Table 3.8 Dimensions (mm) of *U. hemigymna* colonies from Porcupine Abyssal Plain, NE Atlantic, 3800-3820 m (1); Caribbean Sea, 2655-3000 m (2-5), Pasternak 1975; L, length; W, width.

U. hemigymna	1														***2***	3	4	5
Colony L	-														504.0	570.0	572.0	249.0
Axis W	1.3																	
Stem W	1.5														0.8	1.0	0.8	0.7
Peduncle L	-														14.0	22.0	15.0	5.0
Peduncle W	-														2.5	2.5	1.5	1.0
Rachis swelling L	48.0														19.0			
Rachis W	16.0														7.0	7.0	6.0	3.5
N autozooids	14.0														6.0	7.0	4.0	7.0
Autozooid L	33.0	37.0	29.0	7.0	32.0	55.0	30.0	28.6	32.0	26.0	36.0	26.0	5.0	2.6	11.0	12.0	10.0	11.0
Autozooid W	4.5	4.4	5.0	3.7	3.8	3.4	3.0	4.5	4.3	4.5	3.7	4.5	3.7	1.5	4.5	5.0	3.0	3.0
Tentacle L	19.0	43.0	27.0	5.0	19.0	30.0	-	34.0	23.0	20.0	19.0	19.0	1.0	-	12.0	13.0	12.0	18.0
Tentacle W	0.7-1.0																	
Pinnule L	0.6																	
Pinnule W	0.1																	
Siphonozooids W	0.15-0.3														0.15-0.3			
Siphonozooids H	0.6														0.6			

Differential Diagnosis and Remarks: *Umbellula hemigymna*

U. *hemigymna* has affinities with *U*. *thomsoni*: both possess small three-flanged sclerites in their mesoglea, and their axes are quadrangular and strongly keeled. However, these two species differ from each other by the number of sclerites and the character of their distribution. The sclerites of *U*. *thomsoni* are numerous throughout forming a solid armature in the sarcosoma. Conversely, the sclerites of *U*. *hemigymna* are not numerous in the least, distributed sparsely and aggregating only in several parts of the colony. Further to this, the autozooids of *U*. *hemigymna* are longer and narrower than those of *U. thomsoni*; the tentacles of *U*. *hemigymna* are much finer than the thick, robust tentacles of *U*. *thomsoni*; and the pinnules are spaced along the tentacles in *U*. *hemigymna*, not closely packed like in *U. thomsoni*. Pasternak (1975) made mention that these two species also differ by the construction of the siphonozooids: those of *U*. *hemigymna* are taller and more easily discernible. This character, however, should be treated with some caution since the specimens of *U. thomsoni* described herein, particularly the more mature colonies, have rather tall and highly visible siphonozooids implying that Pasternak's comparison was based on the young specimens of *U. thomsoni* described in the same paper. In truth, perhaps all the morphological differences that distinguish the two are of an ambiguous nature, but there is no denying that the specimens of *U. thomsoni* available for this study are quite different in general appearance, thus with hesitation these are regarded as two species.

It must be noted that Williams (1995b) in his synopses of living genera of pennatulids mistakenly assigned *U. hemigymna* to those *Umbellula* species without sclerites.

The species name, *U. hemigymna*, indicates its main feature: the small number of sclerites and their presence on the abcaulinal (asulcal) side of the autozooids only. Hemi- derived from the Greek *hēmi*, meaning half, and -gymna from the Greek *gymnos* meaning naked or bare.

3.3.3.9 *Umbellula monocephalus* Pasternak 1964

Umbellula lindahli	Jungersen 1904
Umbellula durissima	Broch 1957
Umbellula durissima	Broch 1958
Umbellula monocephalus	Pasternak 1964
Umbellula thieli	Grasshoff 1972

Type Material

Holotype, 1 exemplar from N Indian Ocean (-01.9200° S; 83.0800° E), 4911 m; P.P. Shirsov's Institute of Oceanology, Moscow.

Material Examined

Porcupine Abyssal Plain, NE Atlantic Ocean (48.2583° N to 50.0217° N; 13.9667° W to 17.0017° W), 3485-4870 m, collected by means of trawl (OTSB) and epibenthic sledge over the period of 20/07/1982 to 05/10/2002: 54 specimens, fixed in formalin (borax-buffered 4 % formaldehyde in seawater), stored in 70 % propan-2-ol.

W of Sumatra, Indonesia, NE Indian Ocean (04.1602° N; 93.3179° E), 4229 m, collected by means of ROV, 01/05/2004: 1 specimen, preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Round axis
- Sclerites numerous, all monaxial, round in section and of two size classes: larger type >3mm in length; smaller type ~0.5 mm
- One large autozooid (no secondary autozooids)

Emended Diagnosis Plate 11 and 18; Table 3.9

Colonies of this very characteristic species reach heights >1000 mm. Uniquely, *U. monocephalus* possess only one exceptionally large autozooid, positioned at the most distal portion of the rachis occupying a ventral position. The rachis is laterally flattened and curves towards the ventral field with the axis running along the dorsal edge like a spine: distally, the axis bends inwards forming a hook within the proximal portion of the anthocodia. In strongly contracted specimens, the rachis spirals in on itself. The beginning of the distal portion of the rachis is marked by the presence of siphonozooids: it is elongated and gradually tapers to become continuous with the stem. Often the rachis is swollen with gametes that reside in the space below the pharynx.

The great autozooid is laterally flattened, and is complemented with extraordinarily large tentacles that are approximately twice the length of the anthocodia: these are dorso-ventrally flattened and very broad, furnished with proportionally short, robust pinnules that are spaced along the tentacles with gaps in-between.

The siphonozooids are numerous, small (~0.3 mm diameter) and flat, occupying the stem and rachis. They form tapering narrow fields up the distal portion of the rachis in the region where the anthostele resides underneath the sarcosoma: these siphonozooid tongues correspond with the internal mesenteries of the single autozooid.

Sclerites are numerous in every part of the mesoglea, all round in cross-section, and consisting of two size classes. Large sclerites up to 3 mm in length encrust the aboral aspect of the pinnules, their axes running parallel with its long axis, and adjoin the strong band of similar large sclerites that are located on the axis of the tentacle. Orally, sclerites are absent from the tentacles and pinnules. Small sclerites (~0.5 mm) encrust the tissue of the anthocodia orientated with its long axis, and upon the rachis, small sclerites form bands between the siphonozooid tongues; sclerites of the stem are far less dense than in other parts of the colony.

Table 3.9 Dimensions (mm) of a representative sample of *U. monocephalus* from NE Indian Ocean, 4229 m (1); and the Porcupine Abyssal Plain, NE Atlantic, 3485-4870 m (2, 3); L, length; W, width.

U. monocephalus	1			2			3		
Colony L	>747.0			-			-		
Axis W	1.4-3			2.5-5.5			-		
Stem W	-			3.9-6.0			-		
Peduncle L	-			-			-		
Peduncle W	-			-			-		
Rachis L	77.0			183.0			-		
Rachis(upper) W	12.0			20.0			6.5		
Rachis (lower) W	7.0			10.0			4.0		
Autozooid L	10.0			91.0			58.0		
Autozooid W	12.0			28.0			11.0		
Tentacle L	22.0	28.5	35.0	77.0	50.0	56.0	41.0	48.0	45.0
Tentacle W	5.8	5.0	5.4	12.0	12.0	12.0	7.0	7.9	8.0
Pinnule L	5.0-5.5			8.0-10.5	10.5	8.0	4.6-7.5		
Pinnule W	1.0-1.4			1.6-2.0	1.6	1.6	1.3-1.4		
Siphonozooids	0.3			0.3			0.3		

The axis is round in cross-section, and not flexible in the least: in large specimens, it can exceed 40 mm in width. The peduncle is an elongated thickening of the proximal stem.

Differential Diagnosis and Remarks: *Umbellula monocephalus*

This taxon is unmistakeable with its single, great autozooid. However, in the past small colonies have been erroneously considered juvenile forms of other species (see below). Thus, *U. monocephalus* can further be distinguished by the presence of sclerites that, uniquely, are all round in cross-section (monaxial): there are no three-flanged types.

Discussion: *Umbellula monocephalus*

Umbellula monocephalus was not recognised as a species until Pasternak described it in the 1960s (Pasternak, 1964). However, specimens of this unique taxon were collected much earlier than this: Jungersen (1904) described two exemplars of *U. lindahli*, the first of which was *U. monocephalus*. This is apparent from his figures (Plate III, Fig.s 37-39), which depict a juvenile colony with a single, large autozooid, and this together with the rachis present morphological features that exactly correspond with those outlined above. However, his description is misleading, and referring to the specimen's only 'secondary autozooid', writes "This rudiment [of a polyp] projects only quite slightly as a low truncate cone with a cleft-like oral aperture: the eight septa with their filaments are distinctly begun, but no trace of arms is seen. The length of this rudiment is 0.32 mm, the breadth 0.154 mm". There is no doubt that he was referring to a siphonozooid. Further, he adds, "The calcareous axis shines distinctly though the thin sarcosoma, and shows the characteristic quadrangular form, with rather deeply concave surfaces and ridge-like projecting edges". This was clearly a mistake, and perhaps the sarcosoma of the stem had contracted in such a way that it misled Jungersen in his diagnosis.

Broch (1957) made a similar misdiagnosis, and classifies a juvenile *U. monocephalus* under the misnomer *U. durissima*, based on the presence of sclerites and round axis. In a subsequent paper, Broch (1958) uses the characters of this specimen to validate *U. durissima* in a review of the genus, *Umbellula* (see discussion in Section 3.3.3.11). Grasshoff (1972) described what he considered a new species, *U. thieli* (=*U. monocephalus*), overlooking the fact that Pasternak (1964) had described it already.

3.3.3.10 *Umbellula aciculifera* J. Stuart Thomson 1915

Umbellula durissima?	J. Stuart Thomson and Ritchie 1906
Umbellula aciculifera	J. Stuart Thomson 1915
Umbellula thomsoni	Williams 1990

Type Material

Natural History Museum, London. Syntype specimen, S Atlantic, Bouvetinsel (-33.5200 ° S; 16.6500 ° E), 2231 m.

Material Examined

Type material (see above).

Porcupine Seabight and Goban Spur, NE Atlantic Ocean (49.5017° N to 51.7060° N; 11.9883° W to 13.0960° W), 1357.5-1789.5 m, collected by means of OTSB over the period of 21/04/1978 to 25/09/2000: 5 specimens, fixed in formalin (borax-buffered 4 % formaldehyde in seawater) and stored in formalin/70 % propan-2-ol.

Key Diagnostic Descriptors

- Round axis
- Small sclerites: round, monaxial (0.5-1.6 mm in length); three-flanged (<0.3 mm in length)
- Large, cylindrical autozooids, distinct bilateral symmetry
- Tentacles very short and robust
- Siphonozooids small, extend to the bases of the anthocodiae
- Anthocodiae distinctively milky-blue in colour; autozooid mouths, dark brown

Emended Diagnosis Plates 12, 13, 14 and 17; Table 3.10

The five specimens of this distinctive species agree well with the description given by Thomson (1915). Since *U. aciculifera* is currently considered synonymous with *U. thomsoni* (e.g. Broch, 1958; Pasternak, 1962; Williams, 1990), the following description provides details on individual specimens to account for intraspecific variability and ontogeneity.

The most mature specimen of *U. aciculifera* herein described has 12 autozooids in total (9 fully grown, 3 developing). The anthocodiae extend laterally either side of the rachis,

demonstrating a high level of bilateral symmetry and in doing so, the autozooid-bearing portion of the rachis is longer than that of the younger specimens. The primary autozooid of all specimens extends in line with the longitudinal axis of the colonies, flush with the dorsal side of the rachis. The oldest secondary autozooids are located pair-wise on the lateral sides of the rachis. Younger autozooids bud ventro-sublaterally from the most distal and ventral portion of the rachis, with those younger still, budding below these. Thus, the dorsal field completely lacks autozooids.

Of the most mature specimen, the 9 fully grown anthocodiae are large and robust, and near cylindrical in form becoming slightly wider towards the mouths. The young autozooids (3 in total) are truncated cones, flattened at their apex where rudimentary tentacles emanate. The sarcosoma of the anthocodiae is thick and rubbery so that the characteristic wrinkles normally resulting from contraction, nor the ribs corresponding to the mesenterial septa, are present. Instead, their ectoderm is remarkably smooth and non-transparent.

The tentacles of the fully grown autozooids are exceptionally short and robust, tapering towards the end, away from the mouth. Pinnules too are short and thick, and are closely positioned along the tentacles becoming shorter distally.

The rachis is covered with numerous small siphonozooids (<0.3 mm diameter) which appear as pits as opposed to conical studs, probably resulting from damage of long-term storage in formalin. Siphonozooids occur on the narrow interspaces between the anthocodiae and up to their bases, and the slender portion below the autozooid cluster, but are absent from the dorsal midline. On the stem, siphonozooids are few and far between and form a line either side of the stem continuing down to the slight swelling of the peduncle.

Numerous minute sclerites can be seen in the sarcosoma of the anthocodiae, tentacles and pinnules; these are less numerous in the rachis. Sclerites of the aboral surface of the tentacles are round spindles, 0.5 to 1.6 mm in length and up to 0.16 mm at their widest point. Those of distal portion of the rachis, anthocodiae, and the proximal parts of the tentacles are three-flanged rods and spindles (<0.3 mm in length) that have dentate edges. Sclerites of the stem are smaller (0.075 mm by 0.027 mm) and also three-flanged

and beset with tubercles, twisted screw-like along the longitudinal axis. Those of the peduncle are broad and flat, the surface of which is rough having low, rounded protuberances; these sclerites are 0.069 to 0.091 mm in length, 0.026 to 0.031 mm wide. Sclerites have a tendency to concentrate in the holes left from the degraded siphonozooids but these are likely to be the ones that survived dissolution subsequent to fixation.

The tissue covering the axis is thick and tough, so much so that it can easily be pushed back to reveal the axis beneath. The axis is thick and relatively inflexible, and round in section (tending towards square in its upper most part? William, 1990), and never quadrangular with longitudinal grooves. The peduncle manifests as an inferior, elongated swelling of the lower stem region.

The colour of the upper rachis and proximal portions of the autozooids are strikingly milky-blue fading basally to white. Tentacles are white, whilst the stem and peduncle are straw-coloured. The oral sides of the tentacles and autozooid mouths are chocolate brown, but under close inspection, this was found to be 'dirt' which can be scrapped off the tentacles with forceps: the mouths remain brown. Oocytes can be seen in the anthosteles where slices of tissue have been removed for sclerite analysis.

The second specimen has seven autozooids arranged as above, with younger anthocodiae positioned on the proximal portion of the ventral surface of the rachis. Bilateral symmetry is manifested, but this presumably younger specimen has fewer autozooids that limit the length of the autozooid-bearing portion of the rachis. The round axis becomes contorted in the rachis swelling indicating a great degree of contraction in this specimen and here it becomes much finer (1 mm in diameter).

As described above, the anthocodiae are a distinctive milky-blue, the sarcosoma thick and smooth, and numerous minute sclerites are present in the surface tissue. In this formalin-stored specimen, siphonozooids are small pits in the surface of the rachis with sclerites often more dense in these pits and no sign of a tentacle. Siphonozooids cover the dorsal rachis, and a siphonozooid-free patch can be seen where the axis passes close to the surface at the dorsal midline. However, above this area, siphonozooids appear again and oddly, a tongue of siphonozooids extends up the anthocodia of the primary autozooid.

Although autozooids are tightly packed at the distal portion of the rachis-swelling, siphonozooids are present in the narrow spaces between. Oocytes within the anthosteles can be seen where the ectoderm of the rachis has been cut away.

The last three specimens are presumably younger than the above two specimens, only possessing 6 autozooids per colony. The degree of bilateralism has further diminished, and the autozooids of the least developed specimen are almost concentrically located at the extreme distal end of the rachis. This young specimen is stored in formalin, and as before, siphonozooids are minute pock-holes in the upper rachis swelling which extend to the very base of the anthocodiae, and cover the entire rachis (with the exception of a small, narrow area along the dorsal midline). At the lower (proximal) portion of the rachis the siphonozooids become far less obvious and it is impossible to say with any certainty whether they continue down the stem.

The other two younger specimens of *U. aciculifera* are stored in alcohol and thus possess more typical, yet small, siphonozooids: they are not dents but instead are flat being flushed with the rachis surface. These specimens are harder due to dehydration from the alcohol, and are highly contracted.

Umbellula aciculifera were often found to possess epizoic zoanthids attached to the stem/lower rachis (Plate 12, Fig E; Plate 13, Fig. A); this phenomenon has also been observed in this species by previous authors (Thomson, 1923). Interestingly, the present author has not observed such epizoons on any other *Umbellula* spp. making its occurrence even more curious. One possible explanation could be the nature of the sarcosoma: the comparatively thick tissue may be ideal for the organisms to attach. Alternatively, the physical parameters of the surrounding environment where *U. aciculifera* dwell may be perfect for the zoanthids to thrive.

Table 3.10 Dimensions (mm) of *U. aciculifera* colonies from Porcupine area, NE Atlantic, 1357.5-1979.5 m; L, length; W, width.

U. aciculifera	**1**							**2**						**3**										
Colony L	>470.0							470.0						428.0										
Axis W	1.9							1.4						1.9										
Stem W	2.0							22.0						2.0										
Peduncle L	-							10.0						93.0										
Peduncle W	-							2.8						4.2										
Rachis swelling L	3.5							8.6						41.0										
Rachis (Lower) W	12.0							10.0						19.0										
Rachis (Upper) W								10.0																
N autozooids	7.0							6.0						12.0										
Autozooid L	23.0	26.0	22.0	5.0	26.0	20.0	30.0	18.0	18.0	11.0	25.0	20.0	2.0	25.0	19.0	23.0	24.0	30.0	30.0	31.0	34.0	29.0	5.0	1.5	0.6
Autozooid W	10.0	11.0	8.5	4.0	9.0	7.0	10.0	9.6	11.0	4.0	9.0	10.0	2.6	8.0	9.0	7.0	8.0	10.0	8.0	10.0	10.0	8.0	4.0	2.6	2.0
Tentacle L	8.0	15.0	7.0	2.0	8.0	7.0	8.0	10.0	7.0	2.0	10.0	13.0	R	8.0	7.0	7.5	9.0	9.1	8.4	7.0	10.0	7.5	R	R	R
Tentacle W	4.0	4.5	3.0	1.0	3.0	3.8	4.0	2.6	3.0	1.0	2.3	4.0	R	2.0	2.3	2.0	3.1	2.1	2.2	2.0	3.0	2.8	R	R	R
Pinnule L	1.0							2.0						0.9										
Pinnule W	0.3							0.3						0.4										
Siphonozooids	0.3							0.2						0.3										

U. aciculifera	**4**						**5**					
Colony L	420.0						410.0					
Axis W	1.2						1.5					
Stem W	2.0						2.0					
Peduncle L	7.2						8.8					
Peduncle W	2.3						5.0					
Rachis swelling L	5.4						7.3					
Rachis (Lower) W	2.5						4.0					
Rachis (Upper) W	14.0						8.0					
N autozooids	6.0						6.0					
Autozooid L	12.5	20	13	18	8	15.8	18.0	22	21	18	21	19
Autozooid W	10.0	9.5	6.5	9.5	6.4	9.0	8.0	9.0	10.0	11.0	9.0	8.0
Tentacle L	7.0	6.5	4.0	7.0	7.0	6.0	5.0	4.2	8.0	5.0	6.0	4.0
Tentacle W	4.0	3.5	1.5	3.0	2.0	3.0	3.5	2.5	3.1	2.1	1.0	1.5
Pinnule L	1.4						1.4					
Pinnule W	0.3						0.2					
Siphonozooids	0.1						0.1					

Differential Diagnosis and Remarks: *Umbellula aciculifera*

Until now *U. aciculifera* was regarded synonymous with *U. thomsoni*. However, it differs in the following ways: the axis is round and does not possess the sharply quadrangular axis with the characteristic longitudinal grooves of *U. thomsoni*; the sclerites are of two types, round and three-flanged, whereas *U. thomsoni* only has three-flanged sclerites; siphonozooids do not form tapering tongues on the rachis like *U. thomsoni*, but instead wrap round the base of the anthocodiae; the tentacles are far shorter and thicker than those of *U. thomsoni*; and lastly, the colour of *U. aciculifera* is milky-blue, and although colour is herein regarded as an ambiguous trait, there is no denying that this is characteristic and unique to this species.

Other species, however, share more affinities with *U. aciculifera*, namely *U. monocephalus*, *U. durissima* and two new species, *Umbellula* sp.2 and *Umbellula* sp.3, which all possess the two sclerite types and have rounded axes. However, they all have numerous large sclerites (>1.225 mm of which most are >2 mm) in their tentacles and/or anthocodiae; the largest sclerites of *U. aciculifera* are between 0.5 mm and 1.6 mm, only occurring in the tentacles.

Discussion: *Umbellula aciculifera*

Umbellula aciculifera was first described by Thomson (1915), but was possibly found a little earlier by the same author (Thomson and Ritchie, 1906) assigned to the name *U. durissima*. Much later, *U. aciculifera* was made synonymous with *U. thomsoni* by Broch (1958) who had clearly never laid eyes upon an example of this distinctive and beautiful species. With this said, the description given by Thomson (1915) was incomplete and a little confusing. Of the stalk/axis, Thomson writes “The stalk is obviously quadrangular externally in its upper part, but lower down it tends to become rounded, though even at this part if a rough section is made the axis is seen to be quadrangular, with however, a tendency to a circular or oval form”. It is not clear whether he is referring to the axis or the stem, but either way, there is no mention of longitudinal grooves characteristic of a ‘true quadrangular axis’. Williams (1990) finds the axis of *U. thomsoni* (=*U. aciculifera*) to be rounded quadrangular, but never sharply quadrangular (i.e. square with rounded

corners at its upper and round below this, going by his figure), and makes mention that the shape of the axis in cross-section is an invalid differentiating character in *U. thomsoni*. It is clear, however, the reason for this is because *U. aciculifera* and *U. thomsoni* are different species. Further to this, Thomson (1915) failed to notice the round sclerites and only refers to the three-flanged types: *U. thomsoni* is known to possess only three-flanged sclerites, thus providing Broch (1958) a further reason to make *U. aciculifera* synonymous with *U. thomsoni*.

A specimen of *U. aciculifera* as identified by Thomson (1923) is housed at the Natural History Museum, London (see Plate 12, Fig. B), together with the lectotype specimen of *U. güntheri* (=*U. thomsoni*). Study of these specimens, as well as those available for this study, has confirmed that they are, without doubt, two different species and thus the name *U. aciculifera* should be reinstated.

3.3.3.11 *Umbellula durissima* Kölliker 1880

Umbellula durissima	Kölliker 1880
Umbellula dura	Thomson and Henderson 1906
Umbellula dura	Kükenthal 1915
Umbellula durissima	Kükenthal 1915
Umbellula durissima	Pasternak 1964
Umbellula durissima	Pasternak 1975

Type Material

Natural History Museum, London. Holotype specimen from Challenger Stn 234, NW Pacific, S of Tokyo, Japan (34.1200° N; 138° E), 1033 m, reg. no. 1881. 2.11.21; Plate VIII Fig.s 32 and 33 (Kölliker, 1880).

Material Examined

Type material (see above).

Porcupine Abyssal Plain at the base of the Porcupine Seabight, NE Atlantic Ocean (50.1987° N; 14.6560° W), 3972-4002 m, collected by means of OTSB, 18/09/2000: 1 specimen, fixed and stored in formalin (borax-buffered 4 % formaldehyde in seawater).

Key Taxonomic Descriptors

- Axis round, highly flexible
- Sclerites exceptionally numerous in every part of ectoderm, encrusting the tissue: large, monaxial needles; and small, three-flanged rods
- Principle sclerites of tentacles/anthocodiae > 2 mm in length; smaller three-flanged sclerites, <0.5 mm, occur throughout colony
- Siphonozooids large and dense on the rachis, raised high above the surface of the rachis and form a rhomboid-shaped plate here
- Rachis dorso-ventrally flattened

Emended Diagnosis Plate 15; Table 3.11

The following description is based only on one specimen, but is an excellent exemplar of *U*. *durissima* as first described by Kölliker (1880). It is, however, incomplete, fragile and brittle: the peduncle and lower stalk wanting, and storage in formalin has led to deterioration with time so that many of the sclerites have fallen from the tissue.

The autozooids, of which there are only three, are large relative to the proportions of the colony. The primary autozooid is positioned at the distal end of the rachis, and the two secondary autozooids extend laterally on either side of this, thus exhibiting bilateral symmetry. Contraction of the specimen together with the highly sclerite-encrusted tissue has distorted the shape of the anthocodiae so their original form cannot be recognised. The rachis from which they emanate is dorso-ventrally flattened and corresponds to the "flat rhomboid expansion of the stalk" of the original description (Kölliker, 1880). Autozooid tentacles are approximately the same length as the anthocodiae and are thick and robust. The pinnules are short and thick, positioned along the tentacles without gaps between each one.

Siphonozooids are large wart-like studs surrounded by sclerites, and are easily visible and dense on the rachis. Few siphonozooids occur between the anthocodiae, yet the tongue-like zonation often formed by siphonozooids on the dorsal aspect of the rachis is not present. Instead, they form a rhomboid-shaped plate over the rachis (Plate 15; and also see Kölliker 1880, Plate VIII, Fig. 33). Siphonozooids are present below the cluster on the

lower rachis/upper stem, and although continue down the stem, they occur in low densities here.

Sclerites are numerous in every part of the coenenchyme. There are of two types: large (> 2 mm), monaxial needles, slightly granulated in texture, and round in cross-section with swollen ends; and smaller (~ 0.3 mm to 0.5 mm) three-flanged rods with dentate edges. The larger type form a band, two sclerites wide, down the aboral side of the tentacles, also occurring on the anthocodiae, although with no apparent pattern. As mentioned, the specimen has deteriorated somewhat, so it is probable that this specimen once possessed the "eight lines [of sclerites] on the body wall of the polyp, directly continuous with those on the stems of the tentacles" as described by Kölliker (1880). The large sclerites are restricted to the autozooids and tentacles. The smaller sclerites, however, are present in all areas (pinnules, tentacles, autozooids, rachis and stem), and are so numerous that there is very little (if any) tissue between the sclerites themselves.

The stem is very thin and highly encrusted with sclerites of the smaller type. The axis is round in section, feebly calcified and highly flexible.

The autozooids are grey-blue in colour underneath the numerous white sclerites. The tentacles are brown on the oral side, as too are the mouths of the autozooids.

Table 3.11 Dimensions (mm) of *U. durissima* colonies from the Porcupine Abyssal Plain, NE Atlantic, 3972-4002 m; L, length; W, width.

U. durissima			
Colony L	-		
Axis W	0.7		
Stem W	0.6-0.8		
Peduncle L	-		
Peduncle W	-		
Rachis L	20.0		
Rachis W	4.9		
N autozooids	3.0		
Autozooid L	10.0	8.0	8.0
Autozooid W	3.5	3.0	3.0
Tentacle L	4.5		
Tentacle W	1.1		
Pinnule L	0.7		
Pinnule W	0.4		
Siphonozooids	0.2-0.3		

Differential Diagnosis and Discussion: *Umbellula durissima*

Kölliker (1880) characterised *U. durissima* very well when making the first description of this species collected during the *Challenger* Expedition (1873-1876). Hickson (1916) and much later, Grasshoff (1972), described the same two specimens from the *Siboga* Expedition, and designated them under the name *U. durissima* based on the sclerites and the shape on the axis. However, they failed to notice that these specimens differed in the following ways: *U. durissima* has distinct bilateral symmetry, while their specimens had radial symmetry with autozooids located in a 'typical umbellulate shape'; they described a 'calyx-shaped' rachis, whereas the rachis of *U. durissima* is dorso-ventrally flattened; and they did not consider the fact that the siphonozooids of *U. durissima* are large wart-like studs that sit tall on the rachis, whilst the siphonozooids of their specimens were much smaller and flatter (observation from the Fig., Grasshoff 1972). The specimens they described are herein considered a new species, *Umbellula* sp.2 n. sp. (see Section 3.3.3.12).

Broch (1957) assigned a specimen to *U. durissima*, but he was clearly describing *U. monocephalus* with its "great, single autozooid" and large sclerites, all of which round in section. In a following paper, Broch (1958) revised the genus *Umbellula*, in which he validated *U. durissima* giving the morphological characteristics of *U. monocephalus*. However, Broch (1957; 1958) made *U. dura* synonymous with *U. durissima*, and it is in the opinion of the present author that these should remain synonymous based on the revision of *U. dura* given by Kükenthal (1915).

3.3.3.12 *Umbellula* sp.2 n. sp.

Umbellula durissima Hickson 1916
Umbellula durissima Grasshoff 1972

Material Examined

Crozet Islands, S Indian Ocean (48.9368° S; 51.07650° E), 4182-4195 m, collected by means of OTSB 27/12/2005: 1 specimen, preserved in 96 % ethanol.

Key Taxonomic Descriptors

- Axis round, thin
- Sclerites in every part of the mesoglea: large monaxial sclerites in the tentacles and autozooids; small three-flanged dentate needles throughout
- Rachis conical below cluster, distinct
- Few autozooids located in a concentric circle; radial symmetry

Taxonomic Description Plates 16 and 18; Table 12

The description herein is founded on a single individual and therefore it is plausible that the characters given are not fully adequate, and does not provide information on intraspecific variability. Nevertheless, its characters do not fit earlier diagnoses and as such is regarded a new species. This alcohol-preserved specimen is in good condition and is complete.

The colony is 456 mm in height and possesses four fully mature autozooids. These are well spaced and positioned in a concentric circle at the most distal end of the rachis. The radial symmetry is strongly pronounced: the primary autozooid is not set in from the autozooid-ring as found in other radially-symmetrical species (e.g. *U. magniflora*) and is only discernable from the secondary autozooids by the presence of the axis visible beneath the sarcosoma. The rachis is a well-defined conical swelling and the autozooids are directed distally away from this. Below, the rachis abruptly narrows where the stem begins.

Autozooids are short and thick, and almost cylindrical: the contracted walls leave the autozooids with an elevated girdle at their most distal ends and together with the tentacles form ‘crowns’ in this preserved specimen. Transversal wrinkles are minimal and

the longitudinal striations often associated with the internal mesenteries are absent. The tentacles are of equal length to the anthocodiae, and are thick and robust. Short, thick pinnules are closely positioned next to one another along the length of the each tentacle.

Small siphonozooids densely blanket the rachis swelling and just below the swelling where the rachis abruptly narrows into the stem. They are numerous between the autozooids occurring up to their bases and do not form tapering tongued-zones. The dorsal midline is devoid of siphonozooids. Siphonozooids were also not observed on the stem, but that is not to say they are not present here and may well have been worn away.

Sclerites are present in all parts of the mesoglea and are of two types: large monaxial rods with rounded blunt-ends, granulated and without tubercles (>1.225 mm length); and small, three-flanged sclerites that are twisted screw-like around the longitudinal axis with dentate edges and rounded ends (<0.504 mm length).

Table 3.12 Dimensions (mm) of *Umbellula* sp.2 n. sp. colony from the Porcupine Abyssal Plain, NE Atlantic, 4182-4195 m; L, length; W, width.

***Umbellula* sp.2 n. sp.**				
Colony L	456.0			
Axis W	1.3			
Stem W	1.4			
Peduncle L	10.0			
Peduncle W	2.0			
Rachis swelling L	31.0			
Rachis W	12.0			
N autozooids	4.0			
Autozooid L	15.0	15.5	15.0	12.0
Autozooid W	6.6	5.0	6.1	5.4
Tentacle L	15.0	14.0	16.0	13.0
Tentacle W	1.6	1.6	1.3	1.3
Pinnule L	1.6			
Pinnule W	0.5			
Siphonozooids	0.5			

Sclerites of the pinnules are monomorphic, consisting of small three-flanged dentate needles, 0.277 to 0.442 in length, 0.027 to 0.045 mm width. The sclerites of the tentacles consist of the larger and smaller types: the smaller being quite equal in size and form with those of the pinnules, distributed evenly throughout the tissue; and the larger, monaxial sclerites of two size classes (1.225 mm in length by 0.156 mm width; 2.716 mm length by 0.222 mm width) that form rows along the aboral axes of the tentacles. Those of the autozooids also consist of the two sclerite forms, the smaller being approximately twice

the size as those of the pinnules and tentacles (0.608 to 0.818 by 0.068 to 0.083) and having no particular orientation. Rachis sclerites mainly occur between the siphonozooids rather than in the siphonozooids themselves, and are all small and three-flanged consisting of two varieties: straight three-flanged dentate needles similar to the pinnules and tentacles in form and size; and three-flanged spindles that narrow in the middle and possess larger tubercles. Sclerites of the stem are three-flanged dentate needles (0.121 to 0.361 mm in length and 0.045 to 0.058 mm width). Those of the peduncle are flattened spindles, and are either longer and narrower (~0.300 mm by ~0.060 mm), or broader and shorter (~0.180 by ~0.095 mm) relative to each other.

The round axis is flexible and very slender, narrowing towards the rachis where the sarcosoma is very thin. It enters the conical rachis centrally, above which it forms a sinusoidal spine buried in the sarcosoma of the primary autozooid.

The peduncle is slender and manifests as an inferior elongated thickening of the proximal stem.

This alcohol-preserved specimen is straw-coloured throughout.

Differential Diagnosis and Remarks: *Umbellula* sp.2 n. sp.

Four other species of *Umbellula* possessing sclerites in the mesoglea of the rachis and autozooids, and each with a round axis are described herein: *U. monocephalus*, *U. aciculifera*, *U. durissima* and a new species, *Umbellula* sp.3 n. sp. (see Section 3.3.4.12): for a differential diagnosis, please refer to Section 3.3.4.12.

Discussion: *Umbellula* sp.2 n. sp.

Two exemplars of this new species were possibly collected from the W Pacific during the *Siboga* Expedition (1899-1900). Hickson (1916) described these specimens under the misnomer *U. durissima* on the basis of the sclerite forms: up until this time the only other species possessing sclerites (all having the three-flanged type only) assigned to *Umbellula*

were *U. thomsoni*, *U. güntheri*, *U. leptocaulis* and *U. simplex*, the latter three being synonymous with the first. Much later, Grasshoff (1972) redescribed Hickson's specimens also under the name *U. durissima* and included figures of the colonies themselves. These descriptions and images are a perfect match with *Umbellula* sp.2 n. sp.

3.3.3.13 *Umbellula* sp.3 n. sp.

Material Examined

Porcupine Abyssal Plain, base of Porcupine Seabight, NE Atlantic Ocean (49.8367° N; 14.1217° W), 4043-4104 m, collected by means of OTSB trawl, 08/11/1977: 1 specimen, fixed and stored in formalin (borax-buffered 4 % formaldehyde in seawater), Discovery Collections, National Oceanography Centre, Southampton.

Key Taxonomic Descriptors

- Axis round
- Sclerites numerous but not encrusting: large monaxial needles in the tentacles and rachis; small three-flanged rods throughout
- Distinctly spherical rachis
- Few autozooids located in a concentric circle; radial symmetry
- Siphonozooids small and flat, very numerous

Taxonomic Description Plates 15 and 17; Table 3.13

The following description is based on one specimen only and it is therefore plausible that the characters given are not fully sufficient. Furthermore, it is incomplete, having the lower stalk and peduncle missing, as well as the distal portion of one autozooid. Despite this, it clearly does not match the descriptions of previous authors and as such is herein regarded as a new species.

The short rachis of this specimen is different from any other species of *Umbellula*. Distally, it forms an almost perfect sphere looking from any aspect, below which it abruptly narrows and merges with the stem. The four autozooids are spaced on the

extreme distal region of this sphere, forming a concentric ring at the top of the rachis and in doing so displaying radial symmetry.

The anthocodiae are cylindrical and moderately large, superficially creased corresponding to the eight mesenteries within. Their sarcosoma is opaque in this formalin-preserved specimen, through which the internal anatomy can be seen. The majority of one of the anthocodia is missing, but going by the width of the remaining stump, it is probable that it is of similar magnitude to the other three: there are no signs of developing autozooids. Autozooid tentacles are fairly short, being only slightly longer than the anthocodiae, and are thick and strong. Pinnules are closely aligned along the tentacles. They are short and thick, and all of similar size.

Siphonozooids are diminutive but still discernable with the naked eye, giving a granulated appearance to the surface of the rachis. Here, they are very numerous and densely cover the rachis and the areas between the anthocodiae with very little space between them. Surrounding the anthocodiae at their bases, the distinctive tongue-shape pattern often seen on the rachis in many *Umbellula* species is absent. They are wanting on the ridge formed by the axis along the dorsal midline, but this may have resulted from abrasion. The siphonozooids become fewer as they continue down below the rachis swelling and onto the stem. Here, they are flatter, and difficult to see under high magnification. No tentacle was observed emanating from any of the siphonozooids, but that is not to say that they are absent altogether and may be retracted or have worn off.

The sclerites of this specimen take on two forms: large (2.73 to 3.06 mm in length) cylindrical needles, granulated in texture but without protuberances; and smaller (0.21 to 0.54 mm in length) three-flanged rods with dentate edges. The largest sclerites are more or less restricted to the aboral side of the tentacles forming a line down their axis: the occasional large sclerite can be seen embedded in the rachis, but the autozooids themselves are completely devoid of this type. The small sclerites of the autozooids range in size from 0.29 mm to 0.52 mm in length, and those smaller sclerites of the rachis are of two size classes: 0.36 to 0.40 mm and 0.21 to 0.24 mm in length, the latter type often taking on a slightly curved shape. In the main, sclerites surround the siphonozooids, but do not encrust them as seen in *U. durissima*; this is also the case for

the stem. Overall, sclerite densities are lower in this specimen comparatively, although still very numerous.

Table 3.13 Dimensions (mm) of *Umbellula* sp.3 n. sp. colony from the Porcupine Abyssal Plain, NE Atlantic, 4043-4104 m; L, length; W, width.

***Umbellula* sp.3 n. sp.**				
Colony L	-			
Axis W	0.8			
Stem W	0.7			
Peduncle L	-			
Peduncle W	-			
Rachis swelling L	14.0			
Rachis W	11.0			
N autozooids	4.0			
Autozooid L	12.0	10.0	-	12.0
Autozooid W	4.1	4.0	4.0	4.0
Tentacle L	13.0	11.0	-	12.0
Tentacle W	1.2	0.8	-	1.0
Pinnule L	0.4			
Pinnule W	0.2			
Siphonozooids	0.2			

The sarcosoma of the stem is moderately thick, and surrounds a round and remarkably flexible axis. This can be seen to pass into the spherical rachis, where it terminates half way to the distal end.

Autozooids of this formalin-preserved specimen are white in colour, through which the blue-grey colouration of the mesenteries/pharynx can be seen within. The rest of the colony is white, including the tentacles and pinnules. The opaque nature of the sarcosoma of the anthocodiae allows one to see oocytes aligned beneath, and also within the anthostele inside the rachis.

Differential Diagnosis and Remarks: *Umbellula* sp.3 n. sp.

There are five *Umbellula* species possessing sclerites in their rachis and autozooids, each also having axes that are round in section: *U. monocephalus*, *U. aciculifera*, *U. durissima*, *Umbellula* sp.2 n. sp. and *Umbellula* sp.3 n. sp. Colonies of *U. monocephalus* have only one very large autozooid on its rachis, and its sclerites are all needles, round in section; three-flanged sclerites are absent. The sclerites of *U. aciculifera* are three-flanged and

monaxial rods/spindles, all of which are small (<1.6 mm). Furthermore, *U. aciculifera* colonies have distinct bilateral symmetry, and are instantly recognisable by their milky-blue colouration. Colonies of *U*. *durissima* possess both large, round sclerites and the small, three-flanged types but differ in the following ways: they have high densities of encrusting sclerites, display bilateral symmetry, are dorso-ventrally flattened with a rhomboid-shaped rachis and possess large siphonozooids that are raised high above the surface of the ectoderm.

The new species, *Umbellula* sp.3 n. sp., perhaps most closely resembles *Umbellula* sp.2 n. sp.: both possess the two sclerite types, few autozooids and radial symmetry. However, these two species differ from each other by the distribution of the sclerites and the shape of the rachis. The large sclerites of *Umbellula* sp.3 n. sp. occur in the tentacles and rachis and not in the anthocodiae themselves, whereas *Umbellula* sp.2 n. sp. has large sclerites in the anthocodiae but not in the rachis. *Umbellula Umbellula* sp.3 n. sp. has a spherical rachis, while the rachis of *Umbellula* sp.2. sp. is conical.

We know from molecular analysis (Fig. 3.3, Section 3.3.5) that the genus *Umbellula* is polyphyletic and that *Umbellula* sp.2 n. sp. is genetically most closely related to *U. monocephalus*, which together form a separate clade from the other sequenced *Umbellula* species presented. Unfortunately, sequences were not obtained for the other three above-mentioned species that share morphological affinities with *Umbellula* sp.2 n. sp. and *U. monocephalus* (*U. durissima*, *U. aciculifera* and *Umbellula* sp.3 n. sp.) and thus their systematic relationships are yet to be determined. Since there is high degree of homoplasy in this genus, cladistic analysis based on morphology is by no means reliable to determine this, and consequently further molecular analysis is fundamental to improve our understanding of systematic and phylogenetic relationships of these similar morphological forms.

3.3.4 A Note on Useful Morphological Characters for Umbellula *Classification*

After extensive study of specimens and the literature pertaining to *Umbellula*, it became increasingly apparent that very few morphological characteristics are of taxonomic value when distinguishing between *Umbellula* species: of these only sclerites and axial shape can be regarded wholly unambiguous. The presence of sclerites, and indeed their shape and size, is by far the strongest feature in assigning species the correct name. Of course, this poses problems in identifying those *Umbellula* without sclerites or among species with similar sclerites. In such specimens, the shape of the axis in cross-section is significant. However, the taxonomic importance of axial shape is not universally agreed: Hickson (1937) believed all *Umbellula* spp. lacking sclerites to be genetically the same, grouping many species, including *U. pellucida*, with *U. huxleyi*. However, these are certainly distinct species, having quadrangular axes and round axes, respectively. The problem of species misnomers and axis shape has been exacerbated by incorrect descriptions, where the shape of specimens' stems or stalks with its cover of soft tissue has been described instead of the internal axis (Marshall, 1887), or where axes described as square/quadrangular with rounded edges (essentially 'round') were taken to be the same form as the quadrangular axes possessing four longitudinal grooves (Hickson, 1916; Williams, 1990). Further pertaining to axes, authors have referred to degree of flexibility as characteristic for different species, correlating it with axis shape and extent of calcification: round axes generally being rather poorly or almost uncalcified, whereas quadrangular axes are as a whole heavily encrusted with lime (Broch, 1958). However, large specimens of *U. monocephalus* have tremendously thick, round axes, which are totally inflexible, and thus the degree of flexibility is often correlated with colony maturity/axis dimensions and not axis shape.

Colony symmetry, that is the arrangement of autozooids upon the rachis whether bilateral or radial, is of great importance. One could argue that symmetry is a function of development: Broch (1958) upon discussing the "carpenteri-magniflora-antarctica line", made these three species synonymous with '*U. lindahli*', on the basis that they were different developmental stages of the same species, younger forms exhibiting bilateral symmetry (=*U. carpenteri*). This has not been found to be the case here, and instead symmetry was consistent among all species.

Often characters that are correlated with dimensions of colony anatomy or number of autozooids have been used in the past and have also been employed in this study. This method of distinguishing between species can be ambiguous and unreliable, as these 'characters' may be altered depending on factors such as varying degrees of contraction of the preserved material, ecological variability, and ontogeneity. However, the use of such characters are essential in distinguishing between species of similar form and when used with some caution, have been found to be reliable especially when used in combination with other characters. For example, the length of the autozooid-bearing portion of the rachis in *U. spicata* is far longer than that of *U. huxleyi*: this is the main character that distinguishes these two as separate species. Of course, older colonies of *U. spicata* will have more autozooids and therefore, a longer rachis, whereas colonies of *U. huxleyi* have tight 'pompon' autozooid-clusters positioned on a short rachis, thus making these two quite distinctive (in their mature form). Evidently, distinguishing between younger forms becomes tricky on this basis, and then one has to look at the positioning of the autozooids, and perhaps the relative lengths of the anthocodiae. A second example is that the number of autozooids in *U. encrinus* distinguishes it from *U. magniflora*: a colony of *U. encrinus* 2000 mm tall would perhaps possess forty autozooids, whereas a colony of *U. magniflora* of the same height would have only twelve.

The length of the tentacles in proportion to the length of the anthocodiae, whether shorter than, equal to or greater than its length, has been described many times previously. It is of the opinion of the present author that although tentacles can vary from specimen to specimen of a particular species (resulting from contraction, retraction, cropping), tentacle length appears to be a conservative feature in *Umbellula* spp.: the very short, stumpy tentacles consistent in *U. aciculifera* are remarkably different from the long, fine tentacles of *U. magniflora*, for example.

Another character considered important is siphonozooid distribution: although these can be easily damaged through abrasion, their occurrence between the anthocodiae and beyond the autozooid-cluster is characteristic in some species and not in others, as too is the pattern they form on the dorsal side of the autozooid-bearing portion of the rachis. Their shape/size can be of importance, for example *U. durissima* has large wart-like siphonozooids, whereas in *U. aciculifera* they are very flat and not easily discernable. The possession of a single tentacle is not considered characteristic: although many species

were found without these, it is of the opinion of the present author that most, if not all, siphonozooids possess a tentacle, which can be overlooked or absent in damaged specimens.

Colour has often been used in species descriptions, though its taxonomic value is of little significance. Specimens' colouration, and indeed transparency can vary tremendously depending on the nature of fixation/preservation: colonies fixed in formalin compared with pieces of the same specimen but fixed in ethanol were found to be quite different from each other. However, *U. aciculifera* is certainly distinctive with its milky-blue colouration, but it must be noted that this is not necessarily a taxonomic trait of this species.

The thickness of sarcosoma is also a dubious quality, and can vary according to state of preservation. With that said, there appears to be some conservation within particular species: *U. huxleyi* and *U. aciculifera* tend to be much fleshier of the stem than *U. thomsoni*, for example.

3.3.5 Phylogenetic Analysis to Infer Systematic Relationships within Umbellula

The Phylogenetic tree presented below (Fig. 3.3) is based on DNA sequence data (*msh1* and *ND2* genes combined) attained from Chapter Two for eight species of *Umbellula* and uses the closely related taxa inferred from the original tree (Fig.s 2.2; 2.3, Chapter Two) as the outgroups.

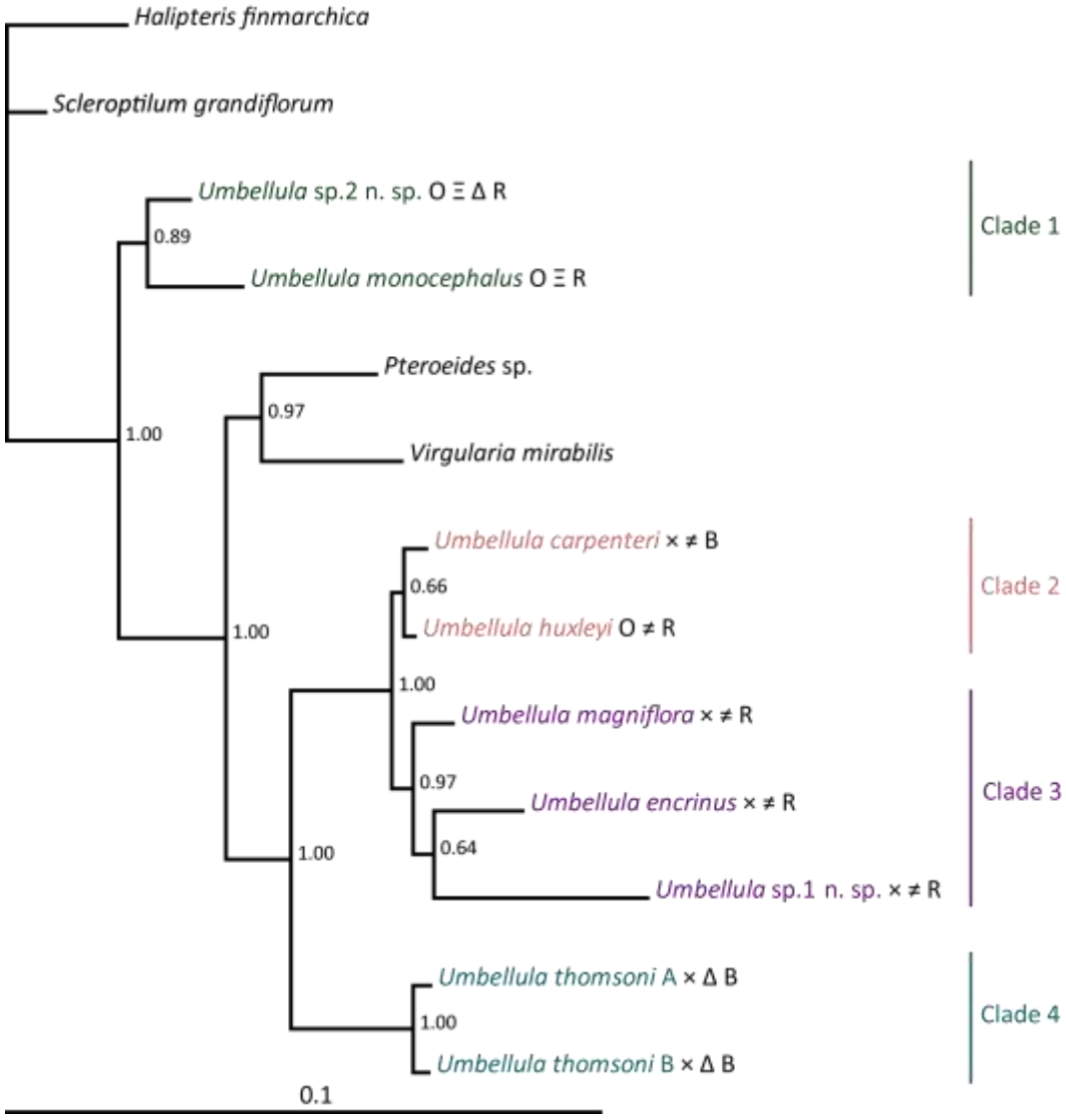

Figure 3.3 Phylogenetic relationships among 8 species of *Umbellula*. Bayesian likelihood tree, 50% majority-rule consensus of 105 trees (10^6 generations; burnin=1000); values at nodes are posterior probabilities; scale bar indicates number of nucleotide substitutions per site. The following species were used as outgroups: *Virgularia mirabilis*, *Pteroeides* sp., *Scleroptilum grandiflorum* and *Halipteris finmarchica*; *U. thomsoni* A and B are from the Indian and Atlantic oceans respectively. Colours represent the four major clades referred to in the text. Symbols indicate the following characters: × Quadrangular axis; O Round axis; Ξ Round sclerites; Δ Three-flanged sclerites; ≠ Sclerites absent; B Bilateral symmetry; R Radial symmetry.

The tree illustrates differences in *ND2* and *msh1* partial sequences between species considered synonymous (e.g. Broch, 1958): the positions of *U. carpenteri*, *U. magniflora* and *U. encrinus* on the tree, and indeed the differences in branch lengths, strongly suggests that these species are genetically distinct species.

The tree further suggests that *Umbellula* is a polyphyletic genus: that is to say, *Umbellula* underwent convergent evolution from at least two different lineages as demonstrated by this non-exclusive set of representatives. Of the two main *Umbellula* clades, the largest is split into three additional clades: Clades 1, 3 and 4 are well-supported with high bootstrap values (≥0.89), whereas Clade 2 is not as well-supported (bootstrap value of 0.66), but its taxa consistently group together in all trees constructed for all analyses (see App. Fig.s A1; A2).

Clade 1 consists of two species, *viz. U. monocephalus* and *Umbellula* sp.2 n. sp., which represent those *Umbellula* spp. with axes that are round in cross-section together with the possession of sclerites in their autozooids and rachis: sclerites of *U. monocephalus* are exclusively monaxial, whereas *Umbellula* sp.2 n. sp. has monaxial and three-flanged sclerites. Evidently, these closely related species are the least derived of those *Umbellula* spp. analysed.

The taxa of Clade 2 and *U. magniflora* (Clade 3) evolved from a common ancestor. Clades 2 and 3 represent those species that do not possess sclerites in their autozooids/rachis and the tree suggests that loss of sclerites in many *Umbellula* spp. is a derived trait: it could be speculated that a 'skeleton' is not so important in the deep sea where currents are weak, or possibly sclerite-loss may be related to ocean chemistry. Unexpectedly, Clade 2 is by represented *U. carpenteri* and *U. huxleyi*, though this relationship is not highly supported (bootstrap value 0.66). *Umbellula carpenteri* has a quadrangular axis with four longitudinal grooves of the same form as those species that comprise Clade 3 (and Clade 4), and colonies possess very few autozooids that are bilaterally arranged, traits shared with *U. thomsoni* which also has a quadrangular axis but possesses sclerites (Clade 4). Conversely, *U. huxleyi* has an axis round in cross-section, and possesses numerous autozooids arranged in whorls (radial symmetry). Thus, *U. huxleyi* is morphologically most similar to those species of Clade 3, with the exception of axis shape.

Clade 3 is comprised of *U. magniflora*, *U. encrinus* and *Umbellula* sp.1 n. sp., the former of which shares its most recent ancestor with the latter two more recently evolved species. The grouping of these taxa is not surprising: these species lack sclerites, all possess quadrangular axes, and have autozooids arranged in concentric circles. Yet, *U. encrinus* is characterised by having a far greater number of autozooids than either *U. magniflora* or *Umbellula* sp.1 n. sp. (its autozooid-cluster very similar to that of *U. huxleyi*), and so the relationship of *Umbellula* sp.1 n. sp. with *U. encrinus*, albeit not highly supported (bootstrap value of 0.67), is surprising on a morphological basis: one would expect *U. magniflora* and *Umbellula* sp.1 n. sp. (few autozooids) to be most closely related to each other. In terms of distribution, however, *U. encrinus* and *Umbellula* sp.1 n. sp. both occupy the high latitudes of the northern hemisphere (see section 3.3.6), whereas *U. magniflora* is most common to the high latitudes of the southern hemisphere, and thus one could hypothesise that the northern dwelling forms radiated from southern ancestors common to *U. magniflora*.

The taxa of Clades 2 and 3 share a common (but not most recent) ancestor with Clade 4. Clade 4 is composed of two specimens of just one species, *U. thomsoni*. The tree indicates that representatives of this taxon from the Indian (*U. thomsoni* A) and Atlantic (*U. thomsoni* B) oceans are genetically very similar, and the small differences in sequences are probably not sufficient to separate them into two species (NB a genetic study of these populations is required to confirm this using a more reliable species-specific marker). *Umbellula thomsoni* is characterised by the possession of small, three-flanged sclerites (no monaxial forms), and a quadrangular axis with four longitudinal grooves, and few autozooids that are bilaterally arranged.

Discussion of *Umbellula* Systematics: Molecules vs. Morphology

The paucity of morphological characters of taxonomic value in pennatulids makes their classification and systematics difficult, and the genus *Umbellula* is no exception. Williams (1995a) performed a cladistical analysis based on morphology to resolve some problematic aspects of the literature pertaining to the systematics of the genus *Gyrophyllum* in relation to other genera that share common traits: *Pennatula*, *Ptilosarcus*, *Sarcoptilus*, *Crassophyllum* and *Pteroeides*. Yet the phylogenetic trees presented in

Chapter Two (Fig.s 2.2; 2.3) reveals quite a different genetic lineage to Williams': representative species of *Gyrophyllum*, *Pennatula* and *Pteroeides* were placed in three separate clades.

Molecular data presented here provide evidence that presence/absence of sclerites, and indeed axial shape, are the principal morphological traits of systematic value in *Umbellula* spp.: those species with sclerites formed two exclusive clades, one consisting of taxa with round axes (Clade 1), and another with a taxon possessing a quadrangular axis (Clade 4); while Clades 2 and 3, composed of taxa without sclerites, are closely related. However, if presence/absence of sclerites is important in *Umbellula* systematics, one might expect different relationships between those species with sclerites. *Umbellula monocephalus* is closely related to *Umbellula* sp.2 n. sp. (Clade 1), but possesses only monaxial sclerites, whereas *Umbellula* sp.2 n. sp. has both monaxial and three-flanged sclerites. The sclerites of *U. thomsoni* (Clade 4) are exclusively three-flanged and thus one could speculate that *Umbellula* sp.2 n. sp. is intermediate between *U. monocephalus* and *U. thomsoni* based on these characters, but this is not the case. However, this is explained by the fact that *Umbellula* is polyphyletic: the taxa of Clade 1 follow a different lineage to Clades 2, 3 and 4 and thus the taxa of Clade 1 would have to be excluded if one wanted to perform cladistic analysis based on morphological traits similar to Williams' (1995a) study.

As mentioned, the tree suggests that the character of axis shape is of importance in *Umbellula* systematics with most clades consisting of taxa with exclusively round or quadrangular axes. Yet species of each axis type are found within the same clade (Clade 2). This too is the case for colony symmetry (bilateral versus radial) and whether mature colonies possess numerous, crowded autozooids or very few, well-spaced autozooids. This also rings true of other characters not discussed in this section such as length of tentacles, siphonozooid distribution and rachis shape. Thus, such traits are not as important as the character of sclerites and axis shape, but cannot be disregarded for separating species.

We understand from Chapter Two that O. Pennatulacea underwent a high frequency of homoplasy: this is evident in the *Umbellula* tree of the present chapter representing eight species, which shows this genus underwent convergent evolution from two different lineages. As accounted in the previous section (Section 3.3.3), there are 15 known

species of *Umbellula*: with DNA sequence data for a greater number of species, further lineages may come to light. This information, combined with the very few characters to distinguish between species of *Umbellula*, perhaps renders systematic (and phylogenetic) relationships based on morphology futile for many (if not all) *Umbellula* species and possibly many other families/genera of pennatulids.

3.3.6 *Comments on Global Occurrence of* Umbellula *Species*

Figure 3.4 shows the distribution of the recently collected *Umbellula* spp. used in this study. Figure 3.5 and Table 3.14 show the distribution of *Umbellula* spp. from the literature, and incorporates the original species name given by the author and the name it has herein been made synonymous with: these nicely illustrate patterns in species distribution, once assigned the true species name, and correlate well with the maps of new material.

The genus *Umbellula* is cosmopolitan with representatives occurring in all oceans at depths of 210 to 6275 m.

Umbellula aciculifera occurs in the Porcupine Seabight/Goban Spur of the NE Atlantic and off South Africa in the E Atlantic (914.5 to 2231 m). A possible representative was also found in the Scotia Sea of the Southern Ocean at 3186 m.

Umbellula antarctica is a southern dwelling species, usually found inhabiting the Southern Ocean, but is also known to occur in the S Atlantic and S Pacific Oceans, with one occurrence as far north as Japan. This species has a large bathymetric range, occupying depths as shallow as 310 m (off Bouvet Islands, Southern Ocean) to 6275 m (Southern Ocean).

Umbellula carpenteri inhabits abyssal depths (3566 to 5275) in the Atlantic, S Indian, S Pacific and Southern Oceans.

Umbellula durissima in known to inhabit the Pacific at a depth 1033 m, but has now been found much deeper on the Porcupine Abyssal Plain, NE Atlantic (3987 m).

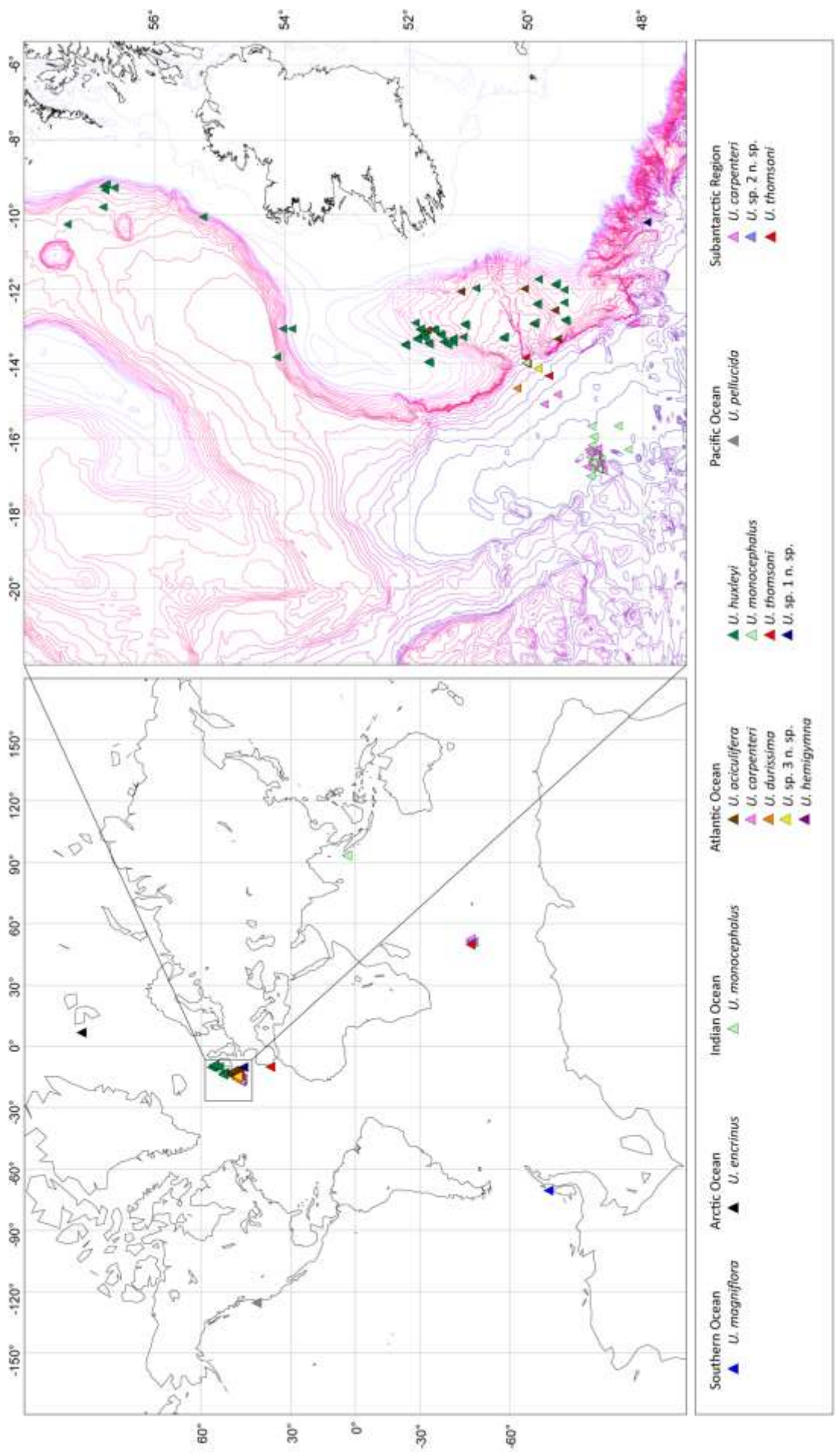

Figure 3.3 Distribution of *Umbellula* species collected for this study.

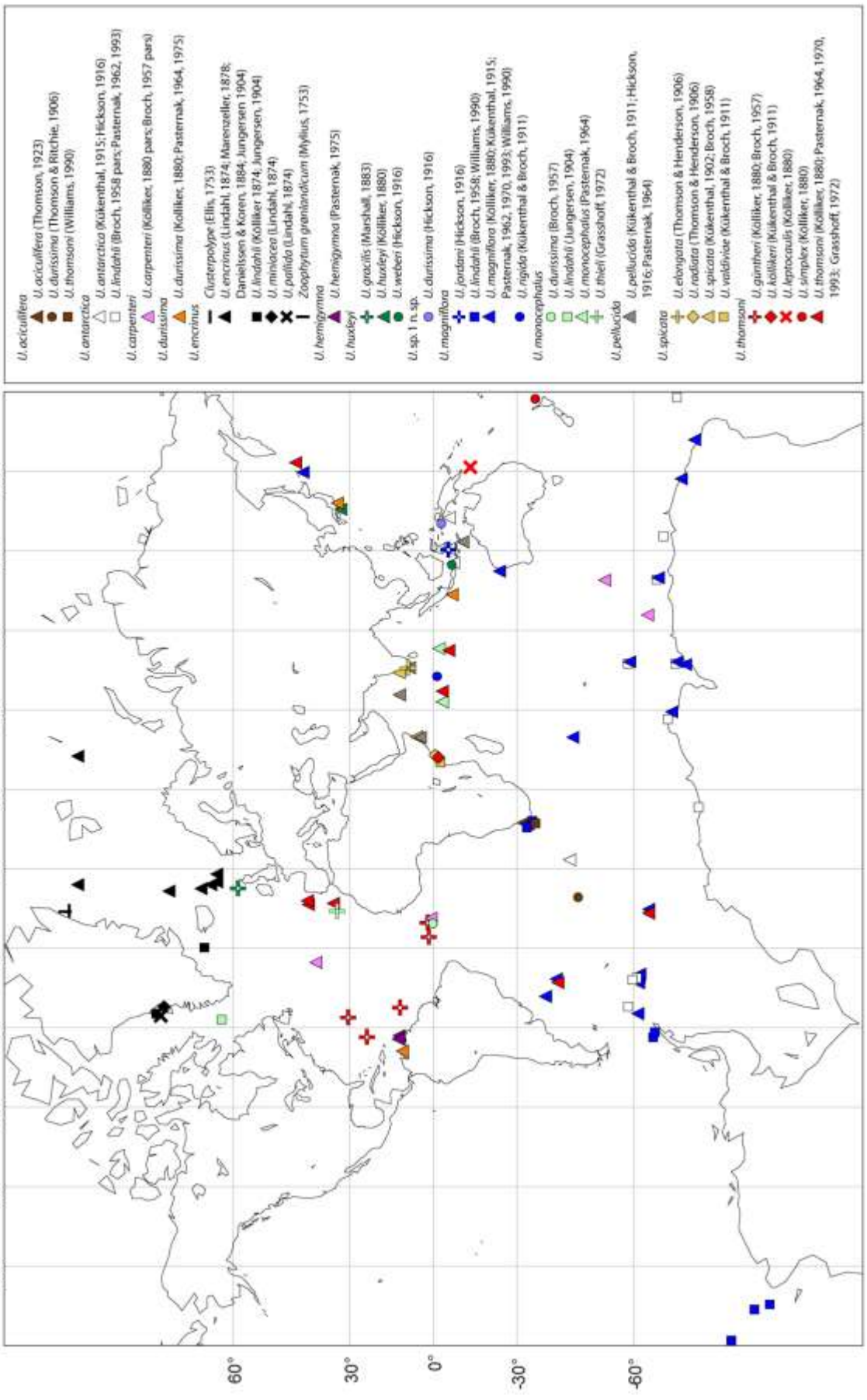

Figure 3.4 Distribution of *Umbellula* species using data from a critical study of the literature. Each true species (triangles) and its associated synonyms (various symbols) have been designated a colour.

Umbellula encrinus is a northern dwelling species, occurring at the high latitudes of the N Atlantic and Arctic Oceans; those of the Atlantic tending to live at deeper depths (740 to 4356.5 m) than those of the Arctic where the bottom water is cold (210 to 1829 m).

Umbellula hemigymna was first described from the Caribbean Sea (NW Atlantic) and is now known from the NE Atlantic. It is a deep-dwelling species, occurring at 2655 to 3810 m depth.

Umbellula huxleyi occurs in the N Atlantic and N Indian Oceans as well as the Indo-Pacific. It occurs at shallower depths of 220 to 1872.5 m but has been found up to 2487.5 m in the NE Atlantic.

Umbellula magniflora is mainly documented from the southern hemisphere, mostly common to the Southern Ocean but also occurring in the SW Atlantic and S Indian Oceans, with one occurrence in the N Pacific. This species has a large depth range: 280 to 6275 m in the Southern Ocean; 5185 to 5225 in the Atlantic; 1600 m in the S Indian Ocean.

Umbellula monocephalus is known from the N and equatorial Atlantic and Indian Oceans occupying abyssal depths of 3956 m to 5275 m.

Umbellula pellucida is common to the Indian Ocean but is now known to occur in the NE Pacific. It is a shallower dwelling species, occupying depths rarely exceeding 1600 m.

Umbellula spicata is only known to occur in the Indian Ocean. Here, it occupies shallower depths of 659 to 1188.5 m.

Umbellula thomsoni has a widespread geographical distribution occurring in the E and W Atlantic, N and S Pacific, N and S Indian, and Southern Oceans. It is an abyssal species inhabiting depths of 3383 to 6162 m. *Umbellula köllikeri* has been made synonymous with *U. thomsoni*, although this is considered dubious (see Discussion, Section 3.7). This taxon was found in the NW Indian Ocean at 1668 m.

Umbellula sp.1 n. sp. is only known to occur in the NE Atlantic (Whittard Canyon) at a depth of 4040 m.

Umbellula sp.2 n. sp. is an abyssal species inhabiting the S Indian Ocean (Subantarctic) at 4189.5 m. However, it was possibly found in the shallower waters of the W Pacific at 567 m (Hickson, 1916).

Umbellula sp.3 n. sp. has only been found on the Porcupine Abyssal Plain, NE Atlantic to date. Here it occupies abyssal depths (4073.5 m).

Table 3.14 List of nominal *Umbellula* species in the literature (Nominal species name), its true species name as identified by the present author (Species), author of description and original naming (Author), depth (m), latitude and longitude (Lat and Long respectively), and information on sampling stations etc. (Notes).

Nominal species name	Species	Author	Depth	Lat	Long	Ocean/Sea: Location	Notes
Clusterpolype	*U. encrinus*	Ellis 1753	432	79.00	-16.50	Arctic: Greenland	*Brittania*
Crinillum siedenburgii	*U. encrinus*	Lindahl 1874	4938	-6.67	126.78	Banda Sea (W Pacific): Indonesia	
Hydra marina arctica	*U. encrinus*	Ellis 1755	432	79.00	-16.50	Arctic: Greenland	*Brittania*
Isis encrinus	*U. encrinus*	Kükenthal 1915				Arctic	*Valdivia* Expedition (1852-1914)
U. aciculifera	*U. aciculifera*	Thomson 1915	914.5			E Atlantic: Cape Point	Green mud
U. aciculifera	*U. aciculifera*	Thomson 1923	2231	-33.52	16.65	E Atlantic	St 524
U. antarctica	*U. antarctica*	Kükenthal 1915	450	-45.43	3.40	S Atlantic: Bouvetinsel	*Valdivia* Expedition (1852-1914)
U. antarctica	*U. antarctica*	Hickson 1916	1060	-7.28	115.47	Pacific: Bali Sea	*Siboga* Expedition St 17
U. antarctica	*U. antarctica*	Hickson 1916	1158	-5.67	120.75	Pacific: nr Saleyer	*Siboga* Expedition St 211
U. antarctica	*U. antarctica*	Hickson 1916	310	-5.67	132.43	Pacific: Kei Is.	*Siboga* Expedition St 254
U. antarctica	*U. antarctica*	Kükenthal & Broch 1911	457			Southern: Boutvet Islands	German Deep-Sea Expedition St 131
U. bairdii	*U. encrinus?*	Verrill 1885	4356.5			NE Atlantic	
U. carpenteri	*U. carpenteri pars*	Kölliker 1880	3612	-62.43	95.73	Southern: SW of Australia	Challenger St 156; diatomaceous ooze
U. carpenteri	*U. carpenteri pars*	Kölliker 1880	3566	-53.92	108.92	S Indian: SW of Australia	Challenger St 157; diatomaceous ooze
U. carpenteri	*U. magniflora*	Kükenthal 1915				Southern: Antarctica	*Valdivia* Expedition (1852-1914)
U. carpenteri	*U. carpenteri pars*	Broch 1957	5275	1.05	-18.67	Equatorial Atlantic	Swedish Deep-Sea Expedition St 342
U. carpenteri	*U. magniflora pars*	Broch 1957	5275	1.05	-18.67	Equatorial Atlantic	Swedish Deep-Sea Expedition St 342
U. carpenteri	*U. encrinus*	Broch 1957	4570	40.55	-35.40	N Atlantic	Swedish Deep-Sea Expedition St 387
U. durissima	*U. durissima*	Kölliker 1880	1033	34.12	138.00	NW Pacific: S of Tokyo, Japan	Challenger St 234; 2.3˚C
U. durissima	*U. aciculifera*	Thomson &Ritchie 1906	3186	-48.10	-10.08	Scotia Sea: Antarctica	Voyage of S.Y. Scotia; pebbles, diatom ooze
U. durissima	*Umbellula* sp.2 n.sp	Hickson 1916	567	-3.45	131.00	W Pacific: Ceram, Indonesia	*Siboga* Expedition
U. durissima	*U. monocephalus*	Broch 1957	5275	1.05	-18.67	Equatorial Atlantic	Swedish Deep-Sea Expedition St 342
U. durissima	*U. durissima*	Pasternak 1964	4544	-6.82	103.43	E Indian	
U. durissima	*U. durissima*	Pasternak 1975	1085	11.77	-68.88	Caribbean Sea: Curacao Is	
U. elongata	*U. spicata*	Thomson & Henderson 1906	659	9.49	75.63	N Indian	
U. encrinus	*U. encrinus*	Lindahl 1874	740	79.72	-52.05	N Atlantic: Baffin's Bay, Greenland	
U. encrinus	*U. encrinus*	Marenzeller 1878	210	79.00	42.48	Arctic: E of Franz-Joseph Land	Austro-Hungarian Expedition; specimen lost at sea
U. encrinus	*U. encrinus*	Danielssen & Koren 1884	914	63.00	-2.00	NE Atlantic: Norway/Faroes	The Norwegian North-Atlantic Expedition St 18
U. encrinus	*U. encrinus*	Danielssen & Koren 1884	914	63.00	-5.00	NE Atlantic: Norway/Faroes	The Norwegian North-Atlantic Expedition St 31
U. encrinus	*U. encrinus*	Danielssen & Koren 1884	914	64.00	-6.00	NE Atlantic: Norway/Faroes	The Norwegian North-Atlantic Expedition St 87
U. encrinus	*U. encrinus*	Danielssen & Koren 1884	1829	79.00	-6.00	Arctic: NW of Spitzbergen	The Norwegian North-Atlantic Expedition St 362
U. encrinus	*U. encrinus*	Jungersen 1904	1394	65.57	-7.52	Arctic: Faroes	Ingolf Expedition St 105
U. encrinus	*U. encrinus*	Jungersen 1904	678	70.08	-8.43	Arctic: S of Jan Mayen	Ingolf Expedition St 116
U. encrinus ambigua	*U. encrinus*	Kükenthal 1915				Arctic	*Valdivia* Expedition (1852-1914)

Table 3.14 continued…

Nominal species name	Species	Author	Depth	Lat	Long	Ocean/Sea: Location	Notes
U. encrinus encrinus	*U. encrinus*	Kükenthal 1915				Arctic	*Valdivia* Expedition (1852-1914)
U. gracilis	*U. huxleyi*	Marshall 1883	1015	59.01	-7.22	NE Atlantic: W of Rona	Warm area
U. gracilis	*U. huxleyi*	Broch 1913	1365			NE Atlantic: E of Canaries	*Michael Sars* Deep-Sea Expedition St 41
U. güntheri	*U. thomsoni*	Kölliker 1880	3383	1.78	-25.23	Atlantic: Just N of equator	Challenger St 106; 1.8˚C
U. güntheri	*U. thomsoni*	Broch 1913	5500			E Atlantic: W of Canaries	*Michael Sars* Deep-Sea Expedition St 47 & 48
U. güntheri	*U. thomsoni*	Broch 1957	5275	1.05	-18.67	Equatorial Atlantic	Swedish Deep-Sea Expedition St 342
U. güntheri	*U. thomsoni*	Broch 1957	5038.5	12.37	-52.00	W Atlantic	Swedish Deep-Sea Expedition St 363
U. güntheri	*U. thomsoni*	Broch 1957	5855	24.20	-63.38	NE Atlantic: nr Caribbean	Swedish Deep-Sea Expedition St 371
U. güntheri	*U. thomsoni*	Broch 1957	5470	30.32	-55.93	NW Atlantic: Sargasso Sea	Swedish Deep-Sea Expedition St 376
U. hemigynma	*U. hemigymna*	Pasternak 1975	3000	13.31	-62.99	Caribbean Sea: Grenada Basin	
U. hemigynma	*U. hemigymna*	Pasternak 1975	2655	12.93	-62.98	Caribbean Sea: Curacao Is.	
U. huxleyi	*U. huxleyi*	Kölliker 1880	1033	35.18	135.65	N Pacific: S of Tokyo, Japan	Challenger St 235; 3.3˚C
U. huxleyi	*U. huxleyi*	Kükenthal & Broch 1911	296			N Indian	German Deep-Sea Expedition St 208
U. huxleyi	*U. pellucida*	Hickson 1937	220			Gulf of Aden: off Aden	John Murray Expedition St 194
U. huxleyi	*U. pellucida*	Hickson 1937	2001			Arabian Sea: S Arabia	John Murray Expedition St 185
U. huxleyi	*U. pellucida*	Hickson 1937	797			W Indian: Maldives Archipelago	John Murray Expedition St 143
U. huxleyi	*U. huxleyi*	Broch 1958	600			E Atlantic: BIs.sagos Is., N Africa	Longhurst's specimens housed at NHM
U. jordani	*U. magniflora*	Hickson 1916	115	-5.67	120.75	Indo-Pacific: S Sulawesi	*Siboga* Expedition St 211
U. köllikeri	*U. thomsoni?*	Kükenthal & Broch 1911	1668	-1.80	41.98	NW Indian	German Deep-Sea Expedition St 250
U. leptocaulis	*U. thomsoni*	Kölliker 1880	4462	-13.83	151.82	S Pacific: SE of New Guinea	Challenger St 181; red clay
U. lindahli	*U. encrinus*	Kölliker 1874 (1875, 1885)	223	71.45	-53.97	NW Atlantic: Umanak Fjord	U. miniacea Lindahl 1874
U. lindahli	*U. monocephalus*	Jungersen 1904	2624	61.83	-56.35	NW Atlantic: Davis Straits	Ingolf Expedition St 36
U. lindahli	*U. encrinus*	Jungersen 1904	1039	64.75	-29.10	NE Atlantic: Denmark Straits	Ingolf Expedition St 90
U. lindahli	*U. encrinus*	Kükenthal 1915				Arctic: off Greenland	*Valdivia* Expedition (1852-1914)
U. lindahli	*U. antarctica*	Broch 1958	245			Southern: Palmer archipelago	Discovery St 180; @150 m 0.00˚C
U. lindahli	*U. magniflora*	Broch 1958	335	-63.65	-62.98	Southern: Palmer archipelago	Discovery St 181; 0.40˚C
U. lindahli	*U. magniflora*	Broch 1958	278	-63.65	-61.03	Southern: Palmer archipelago	Discovery St 182; 0.12˚C
U. lindahli	*U. magniflora*	Broch 1958	114.5			Southern: S Sandwich Is.	Discovery St 366
U. lindahli	*U. magniflora*	Broch 1958	125.5			Southern: S Sandwich Is.	Discovery St 371; @150 m 0.44˚C
U. lindahli	*U. magniflora*	Broch 1958	645	-77.59	-163.83	Ross Sea: Antarctica	Discovery St 1644; @585 m ~1.85˚C
U. lindahli	*U. magniflora*	Broch 1958	475	-76.28	-165.70	Ross Sea: Antarctica	Discovery St 1645; @450 m ~1.85˚C
U. lindahli	*U. magniflora*	Broch 1958	567	-74.06	-177.41	Ross Sea: Antarctica	Discovery St 1652; @541 m ~1.90˚C
U. lindahli	*U. antarctica*	Broch 1958	550	-59.18	-51.33	Southern: S Shetland Is.	Discovery St 1948
U. lindahli	*U. antarctica*	Broch 1958	114.5			Southern: S Sandwich Is.	Discovery St 366
U. lindahli	*U. antarctica*	Broch 1958	125.5			Southern: S Sandwich Is.	Discovery St 371; @150 m 0.44˚C
U. lindahli	*U. antarctica*	Pasternak 1962	1320	-59.10	78.10	Southern: Antarctic/Sub-Antarctic	Soviet Antarctic Expedition St 176; diatom ooze

Table 3.14 continued…

Nominal species name	Species	Author	Depth	Lat	Long	Ocean/Sea: Location	Notes
U. lindahli	*U. antarctica*	Pasternak 1962	280	-67.15	78.05	Southern: Olaf Prydz Bay	Soviet Antarctic Expedition St 185; muddy gravel
U. lindahli	*U. antarctica*	Pasternak 1962	277.5	-65.98	57.13	Southern: N of Enderby Land	Soviet Antarctic Expedition St 204; muddy sand
U. lindahli	*U. antarctica*	Pasternak 1962	200	-70.32	23.85	Southern: N of Princess Ragnhild	Soviet Antarctic Expedition St 232; mud and pebbles
U. lindahli	*U. antarctica*	Pasternak 1962	685	-64.22	109.82	Southern: N of Sabrina Coast	Soviet Antarctic Expedition St V; mud, boulders
U. lindahli	*U. antarctica*	Pasternak 1962	400	-65.30	126.17	Southern: N of Banzare Coast	Soviet Antarctic Expedition St 331; grey mud
U. lindahli	*U. antarctica*	Pasternak 1962	700	-67.35	179.88	Southern: Scott Is.	Soviet Antarctic Expedition St 377; mud, boulders
U. lindahli	*U. magniflora*	Williams 1990	2926.5	-34.62	17.05	E Atlantic: Cape of Good Hope	
U. lindahli	*U. magniflora*	Williams 1990	2826	-33.60	16.25	E Atlantic: W of Dassen Is.	
U. lindahli	*U. magniflora*	Williams 1990	490	-35.37	18.76	E Atlantic: S of False Bay	
U. lindahli	*U. antarctica*	Pasternak 1993	6275	-60.84	-41.18	Southern	
U. lindahli	*U. antarctica*	Pasternak 1993	5847.5	-60.87	-40.98	Southern	
U. lindahli	*U. antarctica*	Pasternak 1993	3700	-60.01	-41.24	Southern	
U. magniflora	*U. magniflora*	Kölliker 1880	2926	-46.27	49.62	Southern: E of Kerguelen Is.	Challenger St 147; globigerina ooze; 0.8˚C
U. magniflora	*U. magniflora*	Kükenthal 1915	1600	-23.95	112.28	Indian	
U. magniflora	*U. magniflora*	Kükenthal 1915	1600			S Indian: E of Kerguelen	*Valdivia* Expedition (1852-1914)
U. magniflora	*U. magniflora*	Pasternak 1962	1320	-59.10	78.10	Southern: Antarctic/Sub-Antarctic	Soviet Antarctic Expedition St 176; diatom ooze
U. magniflora	*U. magniflora*	Pasternak 1962	280	-67.15	78.05	Southern: Olaf Prydz Bay	Soviet Antarctic Expedition St 185; muddy gravel
U. magniflora	*U. magniflora*	Pasternak 1962	630	-68.35	77.08	Southern: Olaf Prydz Bay	Soviet Antarctic Expedition St 187; mud with pebbles
U. magniflora	*U. magniflora*	Pasternak 1962	1270	-66.35	59.27	Southern: N of Enderby Land	Soviet Antarctic Expedition St 202; diatom ooze
U. magniflora	*U. magniflora*	Pasternak 1962	910	-64.22	109.82	Southern: N of Sabrina Coast	Soviet Antarctic Expedition St V; mud, boulders
U. magniflora	*U. magniflora*	Pasternak 1962	910	-67.75	147.17	Southern: N of King George V Land	Soviet Antarctic Expedition St 335; clay with pebbles
U. magniflora	*U. magniflora*	Pasternak 1962	675	-69.57	161.92	Southern: N of Oates Land	Soviet Antarctic Expedition St 336; mud, boulders
U. magniflora	*U. magniflora*	Pasternak 1970	6112.5	44.12	149.57	NE Pacific	Vitayazj' St 5633
U. magniflora	*U. magniflora*	Pasternak 1993	5115	-62.61	-15.30	Southern: Antarctica	St 4104
U. magniflora	*U. magniflora*	Pasternak 1993	6275	-60.84	-41.18	Southern: Antarctica	St 4086
U. magniflora	*U. magniflora*	Pasternak 1993	5266.5	-60.77	-41.06	Southern: Antarctica	St 4089
U. magniflora	*U. magniflora*	Pasternak 1993	5847.5	-60.87	-40.98	Southern: Antarctica	St 4090
U. magniflora	*U. magniflora*	Pasternak 1993	4320	-60.71	-41.05	Southern: Antarctica	St 4094
U. magniflora	*U. magniflora*	Pasternak 1993	3080	-60.68	-54.68	Southern: Antarctica	St 4097
U. magniflora	*U. magniflora*	Pasternak 1993	5185	-41.76	-41.59	SW Atlantic	St 4107
U. magniflora	*U. magniflora*	Pasternak 1993	5225	-38.66	-48.17	SW Atlantic	St 4109
U. miniacea	*U. encrinus*	Lindahl 1874	732	70.72	-52.05	NW Atlantic: Baffins Bay	Grey ooze
U. monocephalus	*U. monocephalus*	Pasternak 1964	4630	-3.17	63.00	N Indian	RV *Vitiaz*
U. monocephalus	*U. monocephalus*	Pasternak 1964	4911	-1.92	83.08	N Indian	RV *Vitiaz*
U. pallida	*U. encrinus*	Lindahl 1874	223	71.45	-53.97	NW Atlantic: Umenak Fjord	Light grey clay
U. pellucida	*U. pellucida*	Kükenthal & Broch 1911	628	6.40	49.53	NW Indian: Coast of Somalia	German Deep-Sea Expedition St 265
U. pellucida	*U. pellucida*	Kükenthal 1915	628			Indian: Somaliland	*Valdivia* Expedition (1852-1914)

Table 3.14 continued…

Nominal species name	Species	Author	Depth	Lat	Long	Ocean/Sea: Location	Notes
U. pellucida	*U. pellucida*	Hickson 1916	918	-10.80	123.38	Indian: Nr Rotti	*Siboga* Expedition St 300
U. pellucida	*U. pellucida*	Pasternak 1964	265	9.18	75.88	Indian	
U. pellucida	*U. pellucida*	Pasternak 1964	481	12.83	65.65	Indian	
U. radiata	*U. spicata*	Thomson & Henderson 1906	1188.5	13.00	76.00	N Indian: Aden-Bombay	J.E. Purton; unrecorded specimen housed at NHM
U. radiata	*U. spicata*	Thomson & Henderson 1906	897	-0.45	42.79	Indian: Andaman Is.	
U. rigida	*U. magniflora*	Kükenthal & Broch 1911	2919	-1.95	73.32	N Indian	German Deep-Sea Expedition St 220
U. simplex	*U. thomsoni*	Kölliker 1880	3749	-36.17	178.00	N Pacific	Challenger St 246; 1.3˚C
U. spicata	*U. spicata*	Kükenthal 1902	741	6.40	49.73	NW Indian: Coast of Somalia	Type specimen
U. spicata	*U. spicata*	Kükenthal & Broch 1911	741			NW Indian: Coast of Somalia	German Deep-Sea Expedition St 266
U. spicata	*U. spicata*	Broch 1958	1189	13.00	76.00	N Indian: Aden - Bombay	Unrecorded specimen at NHM; JE Purton
U. thieli	*U. monocephalus*	Grasshoff 1972	3956	33.77	-15.55	NE Atlantic	RV *Meteor*
U. thomsoni	*U. thomsoni*	Kölliker 1880	3886	35.33	-13.07	NE Atlantic: Portugal/Madeira	Challenger St 7; 2.0˚C
U. thomsoni	*U. thomsoni*	Pasternak 1964	4115	-5.60	82.37	Indian	RV *Vitiaz*
U. thomsoni	*U. thomsoni*	Pasternak 1964	3490	-3.17	67.00	Indian	RV *Vitiaz*
U. thomsoni	*U. thomsoni*	Pasternak 1970	6162.5	46.10	153.30	NW Pacific: E of Japan	Vitayazj' St 5609
U. thomsoni	*U. thomsoni*	Pasternak 1993	5115	-62.61	-15.47	Southern: Antarctica	St 4104
U. thomsoni	*U. thomsoni*	Pasternak 1993	5185	-41.76	-41.59	SW Atlantic	St 4107
U. thomsoni	*U. thomsoni*	Grasshoff 1972	5318	42.64	-13.52	NE Atlantic	St 24
U. thomsoni	*U. thomsoni*	Grasshoff 1972	5315	42.90	-13.42	NE Atlantic	St 25
U. thomsoni	*U. aciculifera*	Williams 1990	1650	-34.67	17.75	E Atlantic: SW of Cape Point	Holotype *U. aciculifera* Thomson 1915
U. valdiviae	*U. spicata*	Kükenthal & Broch 1911	748	-3.12	40.76	NW Indian: Coast of Somalia	German Deep-Sea Expedition St 249
U. valdiviae	*U. spicata*	Kükenthal & Broch 1911		-27.07	42.79	NW Indian	German Deep-Sea Expedition St 253
U. weberi	*U. huxleyi*	Hickson 1916	1018	-7.47	115.40	Indo-Pacific: Malay Archipelago	*Siboga* Expedition St 18
Zoophytum grønlandicum	*U. encrinus*	Mylius 1753	432	79.00	-16.50	Arctic: Greenland	*Brittania*

3.4 Summary

Umbellula Species

- There are fifteen species of *Umbellula* considered valid including three newly described: those without sclerites are *U. magniflora*, *U. encrinus*, *U. antarctica*, *U. carpenteri* and *Umbellula* sp.1 n. sp. (quadrangular axes), and *U. huxleyi* and *U. pellucida* (round axes); those with sclerites are *U. thomsoni* and *U. hemigymna* (quadrangular axes), and *U. monocephalus*, *U. aciculifera*, *U. durissima*, *Umbellula* sp.2 n. sp. and *Umbellula* sp.3 n. sp. (round axes).
- *U. lindahli* (Kölliker, 1875) is synonymous with *U. encrinus* (Linnaeus, 1767) as the former species was based on young colonies of the latter.
- *U. aciculifera* (currently synonymous with *U. thomsoni*), and *U. antarctica*, *U. carpenteri* and *U. magniflora* (currently synonymous with *'U. lindahli'*) are reinstated as species based on morphological and molecular data.

Characters and Systematics

- Species of the genus *Umbellula* are distinguishable by the presence/absence of sclerites and the form of these sclerites; and shape of the axis in cross-section (round/square with rounded corners and quadrangular with four longitudinal grooves).
- Other characters to consider are colony symmetry (bilateral vs. radial); distribution of siphonozooids; distribution of autozooids along the rachis; number of autozooids in mature specimens; and relative tentacle length.
- The genus *Umbellula* is polyphyletic: this is evident in the *Umbellula* tree representing eight species, which shows this genus underwent convergent evolution from two different lineages.

Geographic and Bathymetric Occurrence

- The cold waters of the Arctic and subarctic (N Atlantic) are dominated by *U. encrinus*, whereas *U. antarctica* and *U. magniflora* are generally restricted to the Southern Ocean and subantarctic waters. *Umbellula hemigymna* is only known to

occur in the N Atlantic, while most other species have a wider distribution, with the exception of *U. spicata* and *U. pellucida* which are most common in the Indian Ocean, with only one record of the latter from the Pacific.

- Those species considered predominately bathyal are *U. aciculifera*, *U. huxleyi*, *U. pellucida* and *U. spicata*; those predominately abyssal are *U. carpenteri*, *Umbellula* sp.3 n. sp., *U. hemigymna*, *U. monocephalus*, *U. thomsoni* and *Umbellula* sp.1 n. sp.; and those eurybathic species are *U. antarctica*, *U. durissima*, *U. encrinus*, *U. magniflora*, and possibly *Umbellula* sp.2 n. sp..

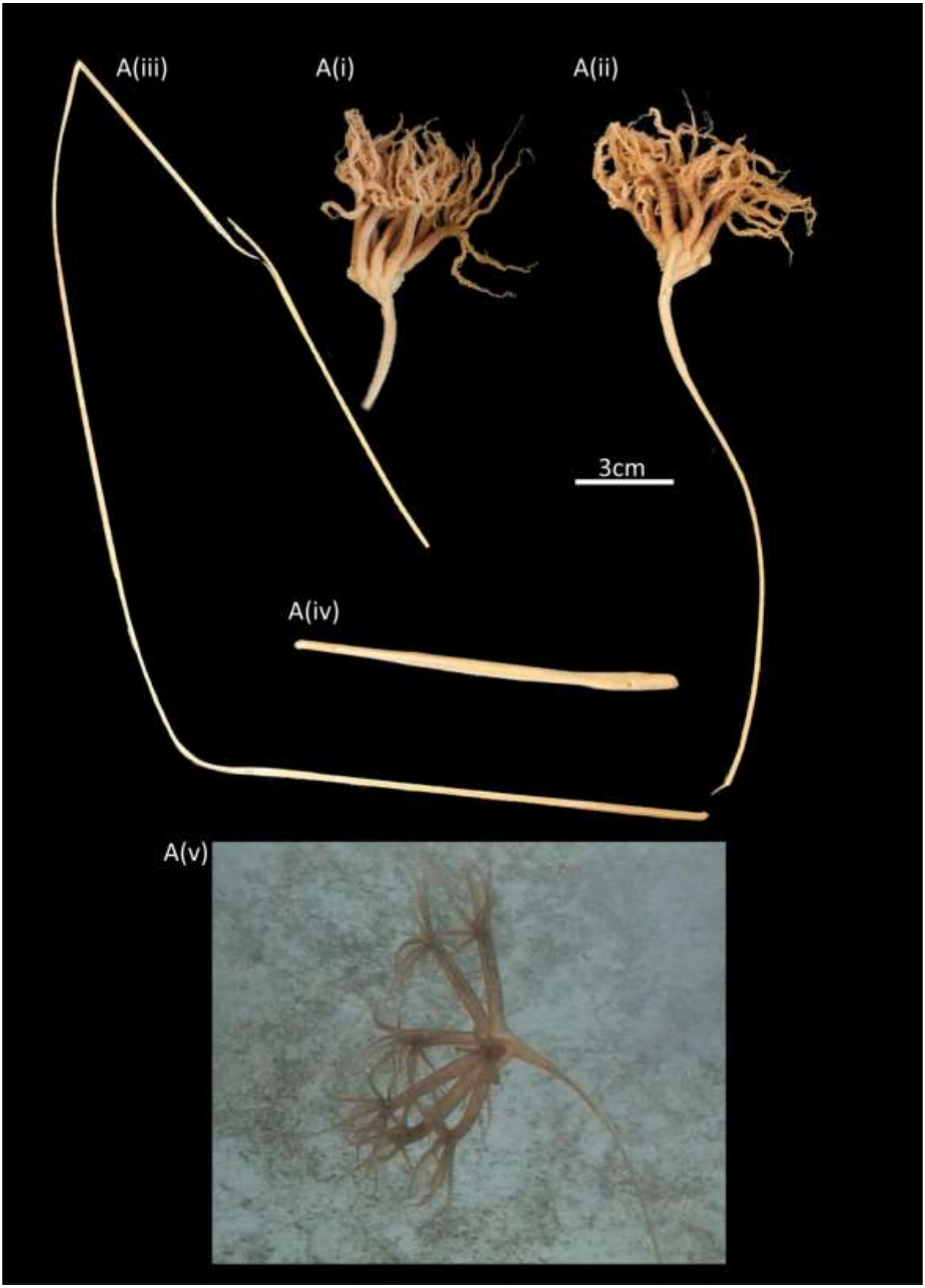

Plate 1 *Umbellula magniflora*. Marguerite Bay, Antarctica, 840 m: A(i) Ventral; A(ii) Dorsal; A(iii)-(iv) Stem and peduncle; A(v) *In situ* image.

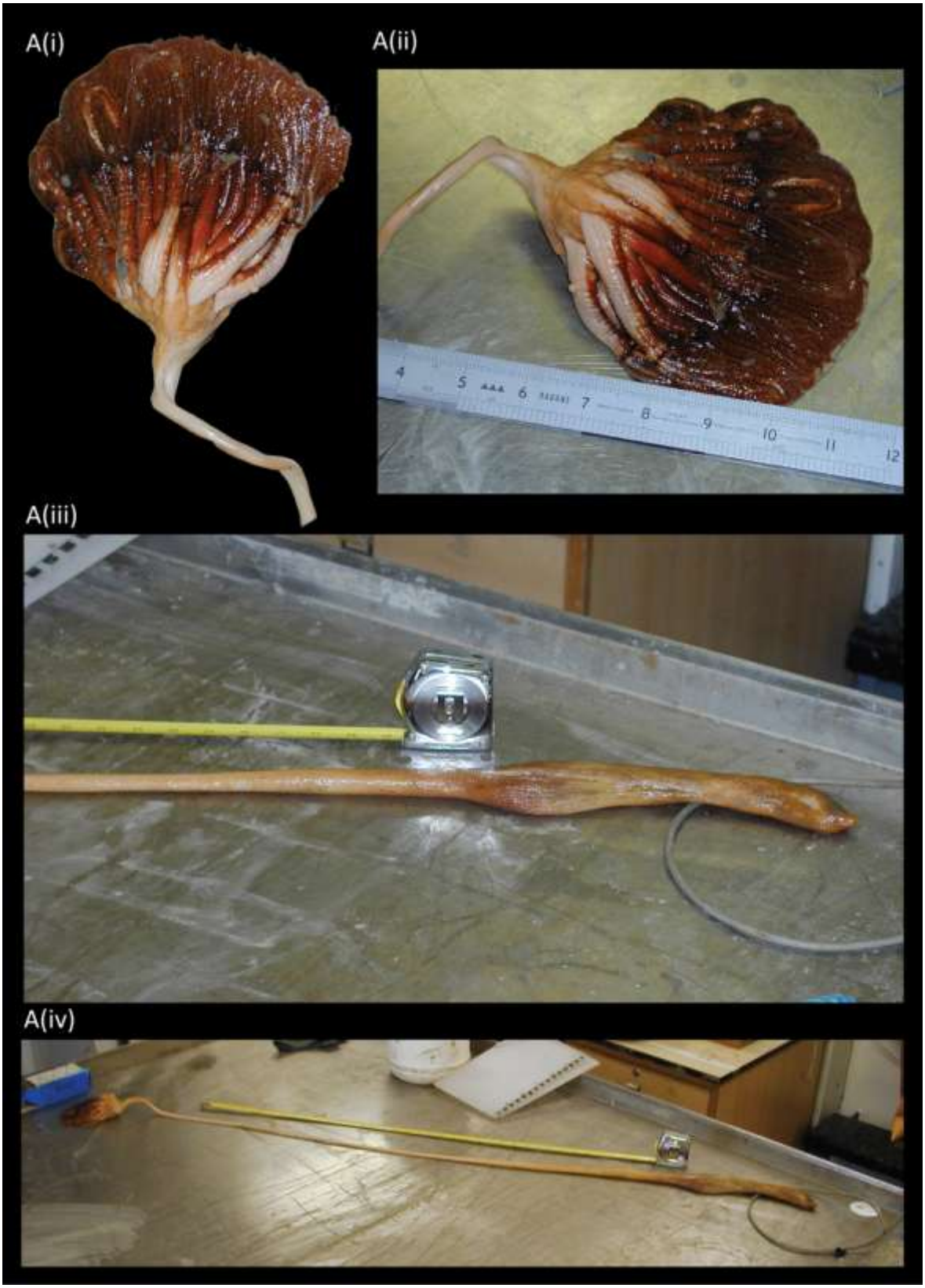

Plate 2 *Umbellula encrinus*. Arctic, 1400 m: A(i)-(ii) Autozooid cluster; A(iii) Peduncle; A(iv) Entire colony (tape measure is 1 m).

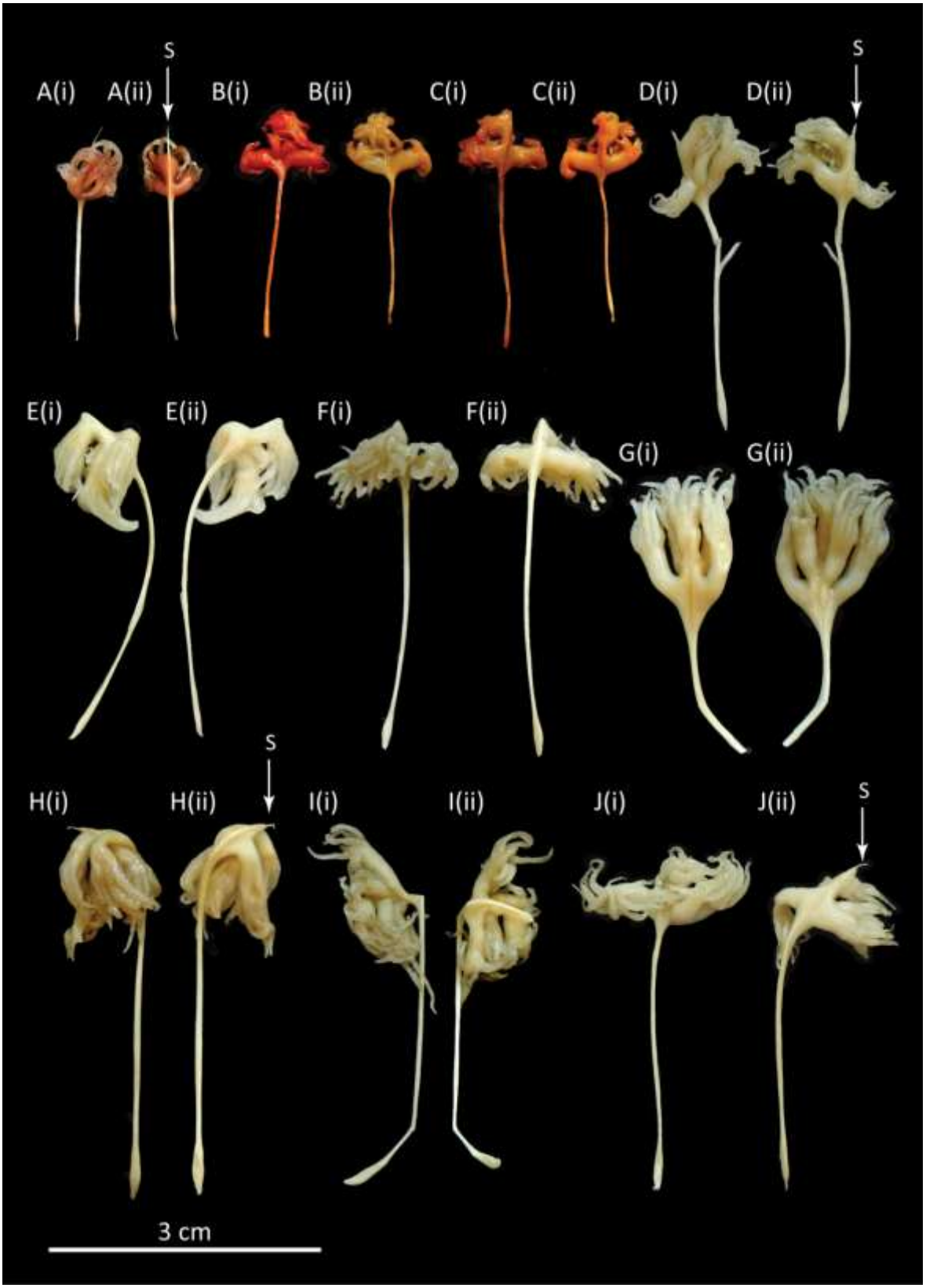

Plate 3 *Umbellula carpenteri*. A-J, Porcupine Abyssal Plain, NE Atlantic, 4510-4860 m: A(i)-J(i) Ventral view of colonies; A(ii)-J(ii) Dorsal view of colonies. S, Spine created by axis extending above the rachis.

Plate 4 *Umbellula carpenteri*. K-O, Crozet, S Indian, 4187-4191 m. Oo, Oocytes within the mesenteries; S, Spine created by axis extending above the rachis.

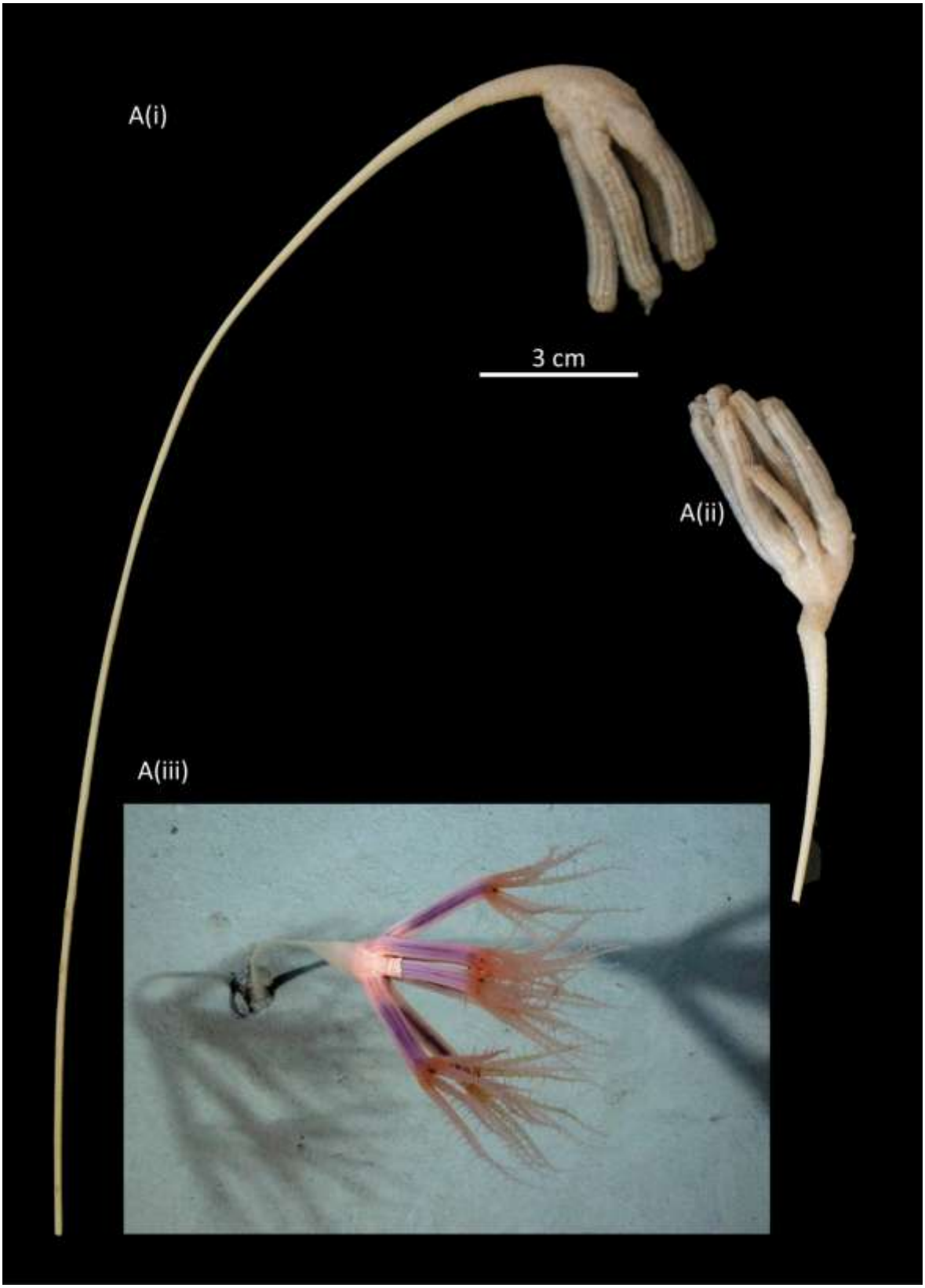

Plate 5 *Umbellula* sp.1 n. sp. Whittard Canyon, NE Atlantic, 4040 m: A(i) Ventral; A(ii) Dorsal; A(iii) *In situ* image.

Plate 6 *Umbellula huxleyi*. Irish continental slope/rise, NE Atlantic, 2010 m: A(i) Ventral; A(ii) Dorsal.

Plate 7 *Umbellula huxleyi*. Irish continental slope/rise, NE Atlantic, 1496 m: B(i) Ventral; B(ii) Dorsal.

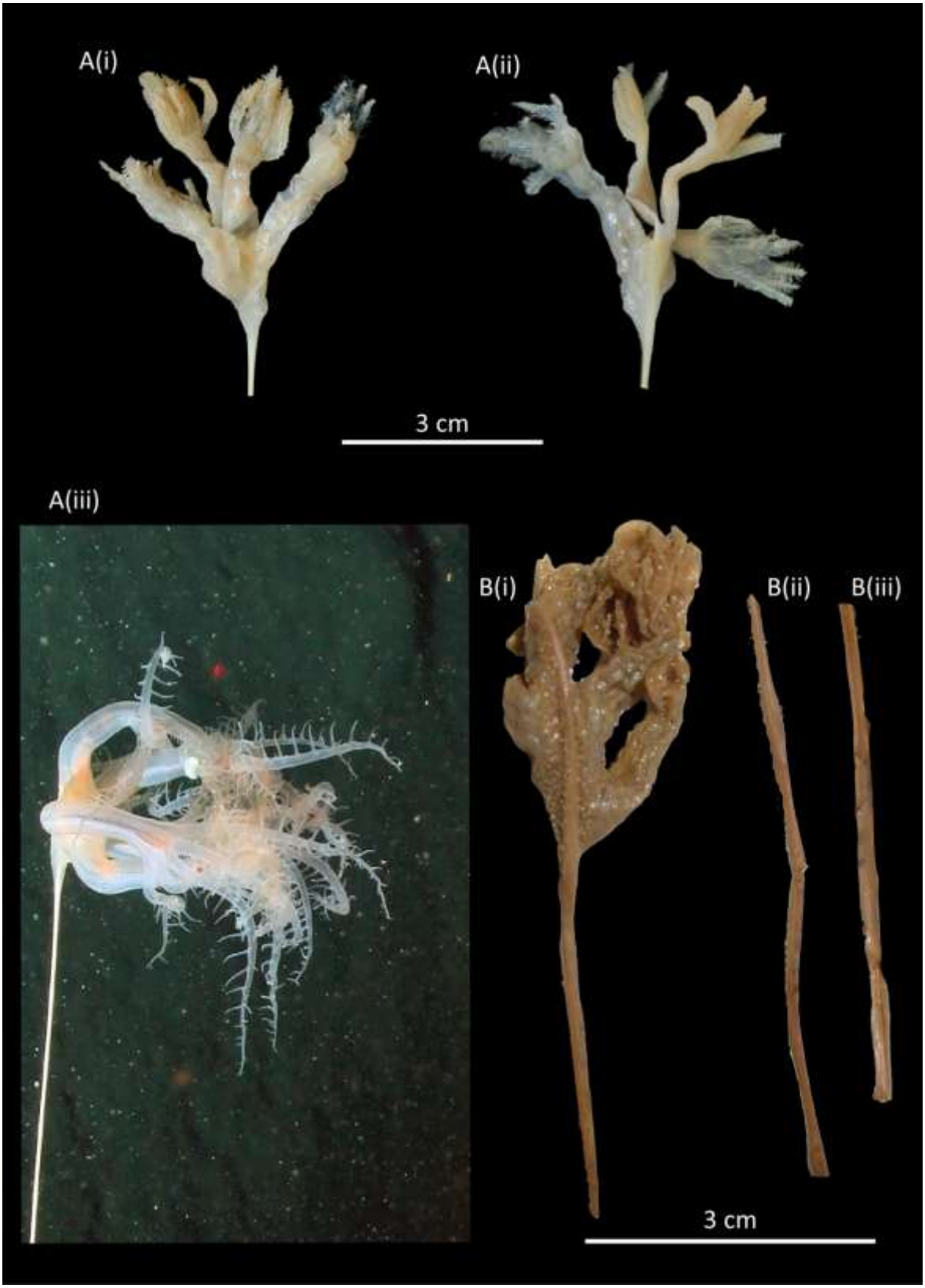

Plate 8 *Umbellula thomsoni*. A, Cascais Canyon, NE Atlantic, 3476 m; B, Equatorial Atlantic, 3383 m, *U. güntheri* (=*U. thomsoni*) type specimen (Natural History Museum specimen, Kölliker 1880): A(i) Ventral; A(ii) Dorsal; A(iii) *In situ* image; B(i) Dorsal view of upper colony; B(ii)-(iii) Stem pieces.

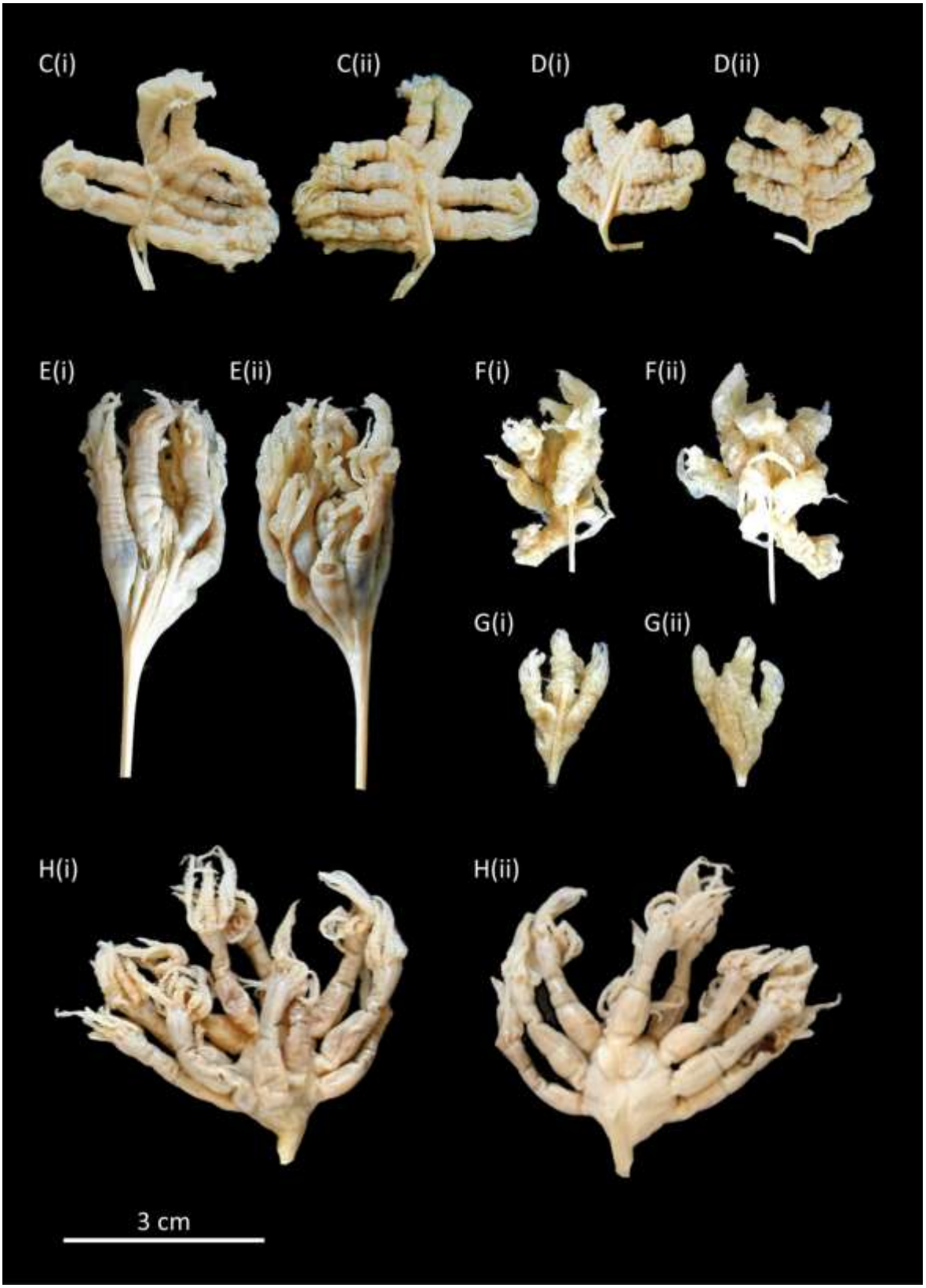

Plate 9 *Umbellula thomsoni*. A-G, Porcupine Abyssal Plain, NE Atlantic, 3485-4298 m; H, Crozet, S Indian, 4182-4195 m: A(i)-H(i) Ventral; A(ii)-H(ii) Dorsal.

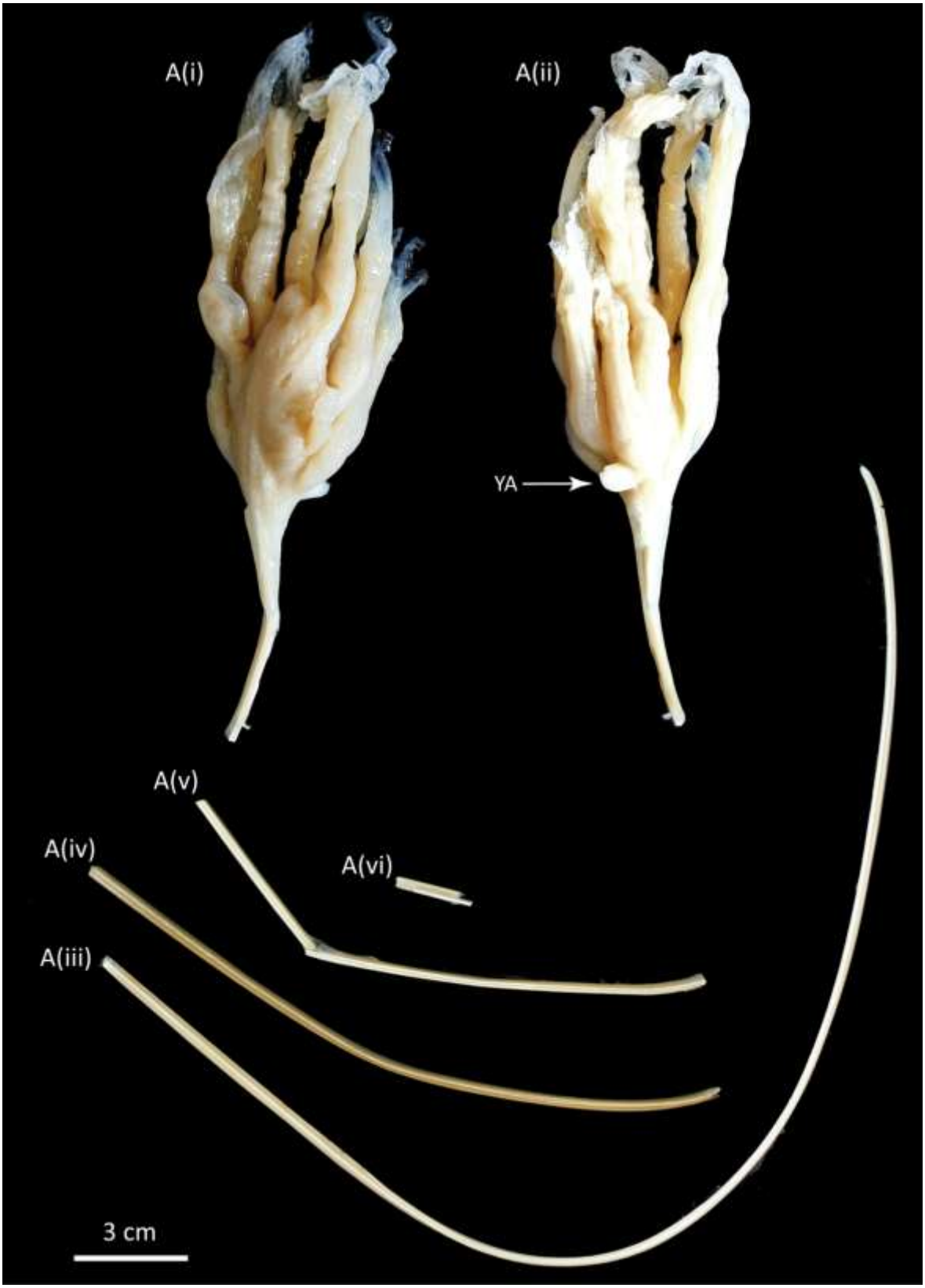

Plate 10 *Umbellula hemigymna*. Porcupine Abyssal Plain, NE Atlantic, 3810 m: A(i) Ventral; A(ii) Dorsal; A(iii)-(v) Stem pieces. YA, Young autozooid.

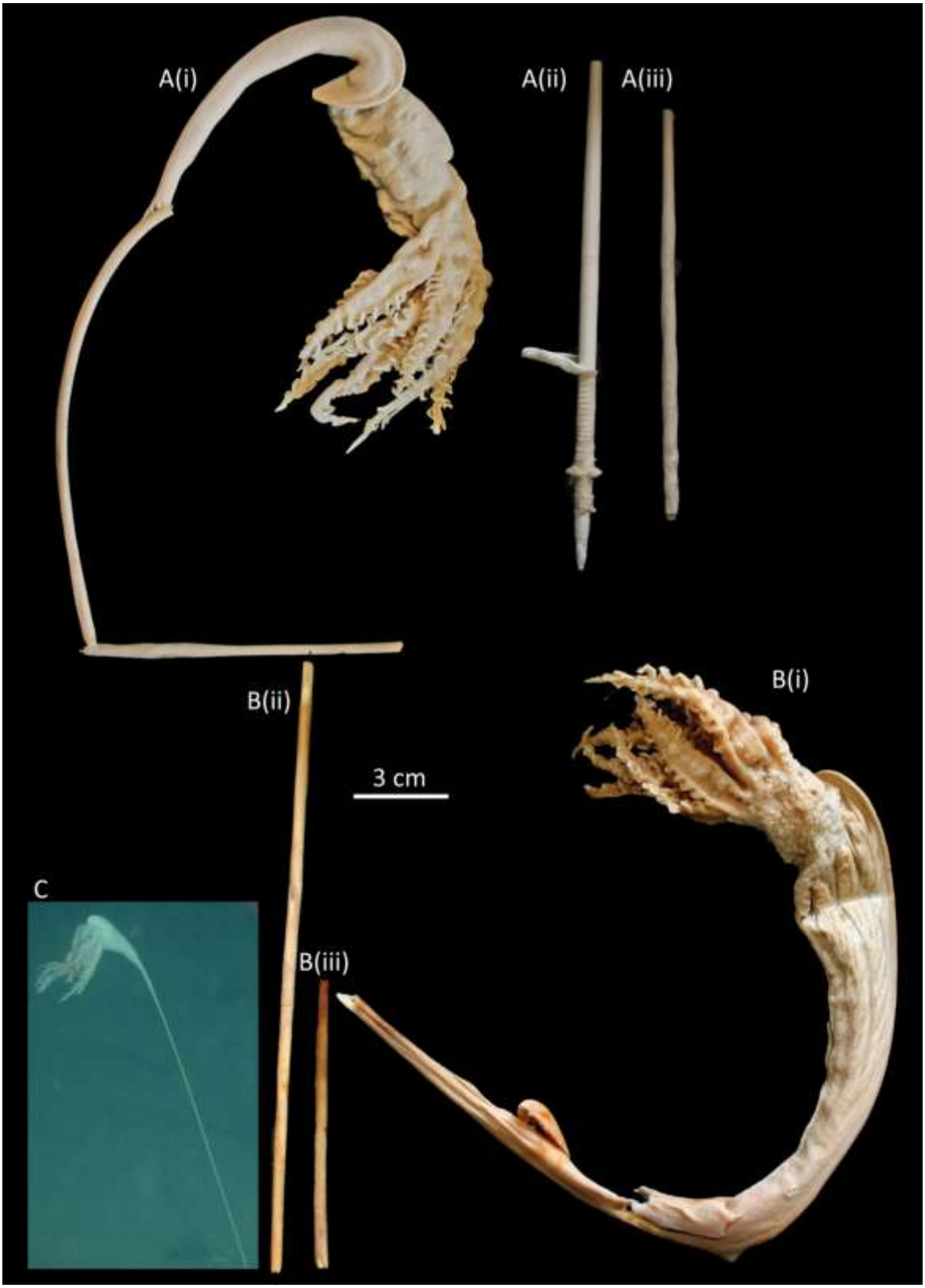

Plate 11 *Umbellula monocephalus*. A-B, Porcupine Abyssal Plain, NE Atlantic, 3485-4870 m; C, W of Sumatra, NE Indian, 4229 m: A(i) Upper colony; A(ii)-(iii) Stem pieces; B(i) Upper colony; B(ii)-(iii) Stem pieces; C *In situ* image.

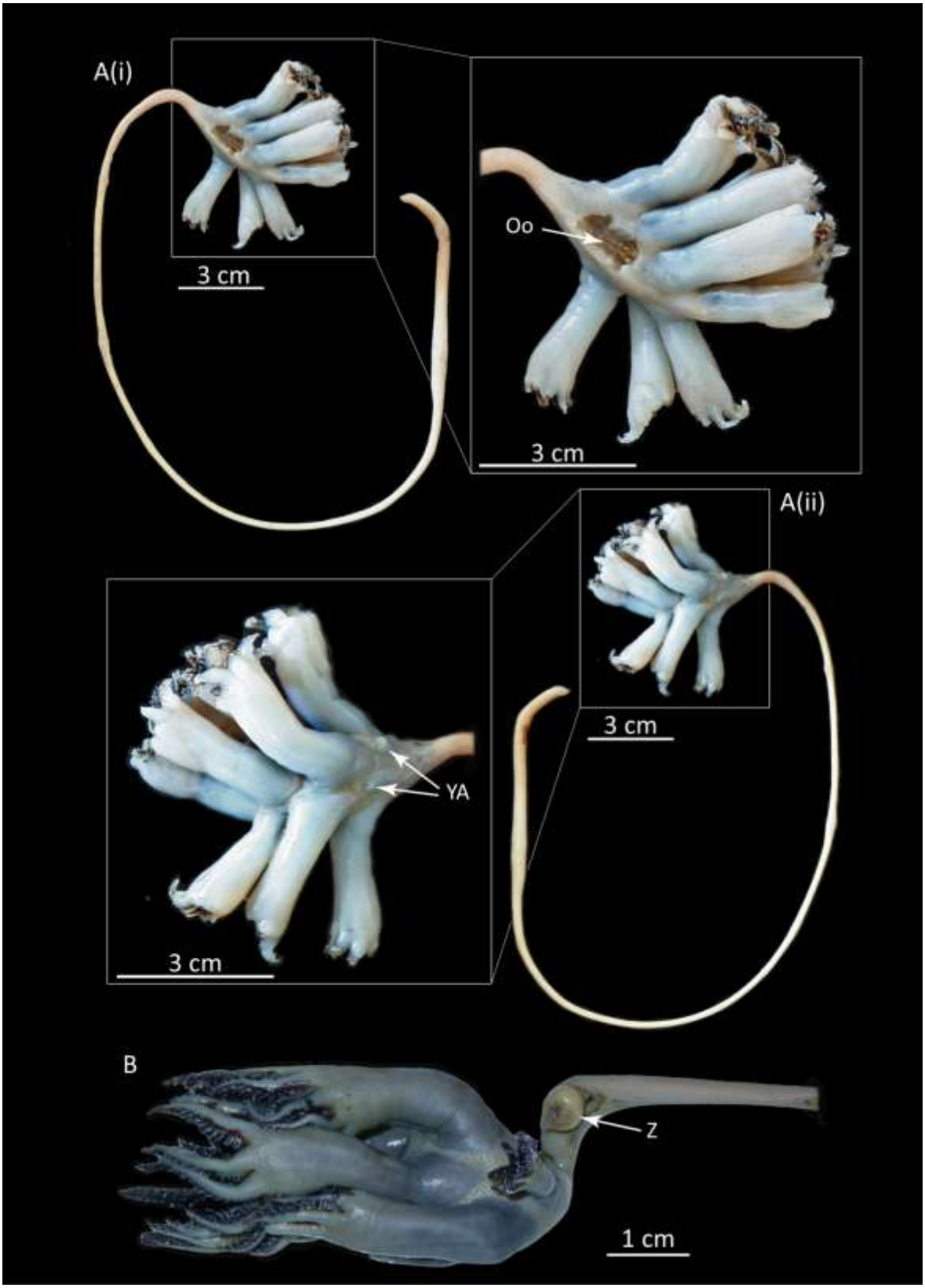

Plate 12 *Umbellula aciculifera*. A, Porcupine Seabight/Goban Spur, NE Atlantic, 1357 m; B, E Atlantic, 2231 m (Natural History Museum specimen, Thomson, 1923): A(i) Dorsal; A(ii) Ventral; B Dorsal view of upper colony. Oo, Oocytes within the mesenteries; YA, Young autozooid; Z, Zoanthid.

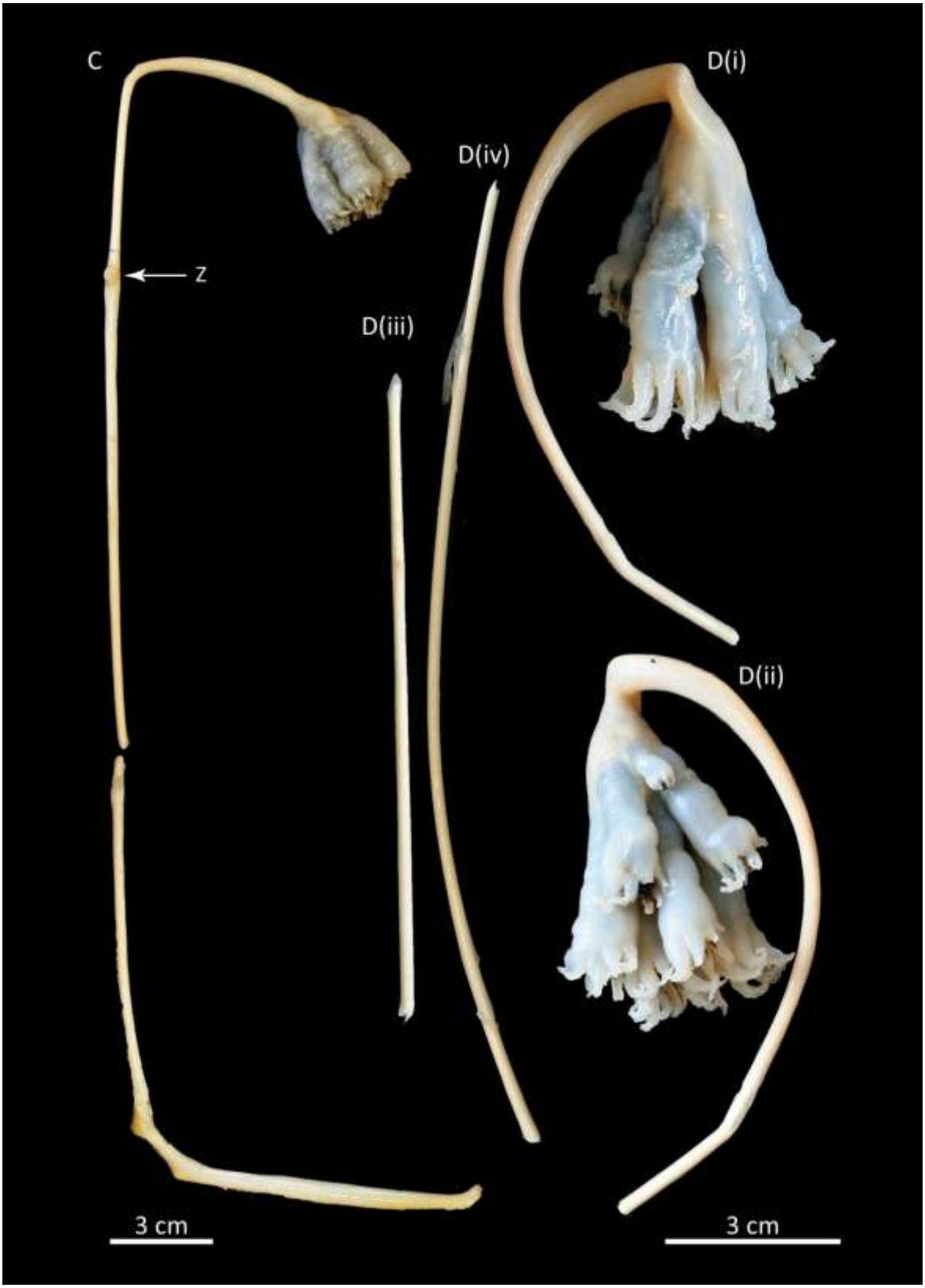

Plate 13 *Umbellula aciculifera*. C-D, Porcupine Seabight/Goban Spur, NE Atlantic, 1533-1789.5 m; C Lateral; D(i) Dorso-lateral; D(ii) Ventral; D(iii)-(iv) Stem pieces. Z, Zoanthid.

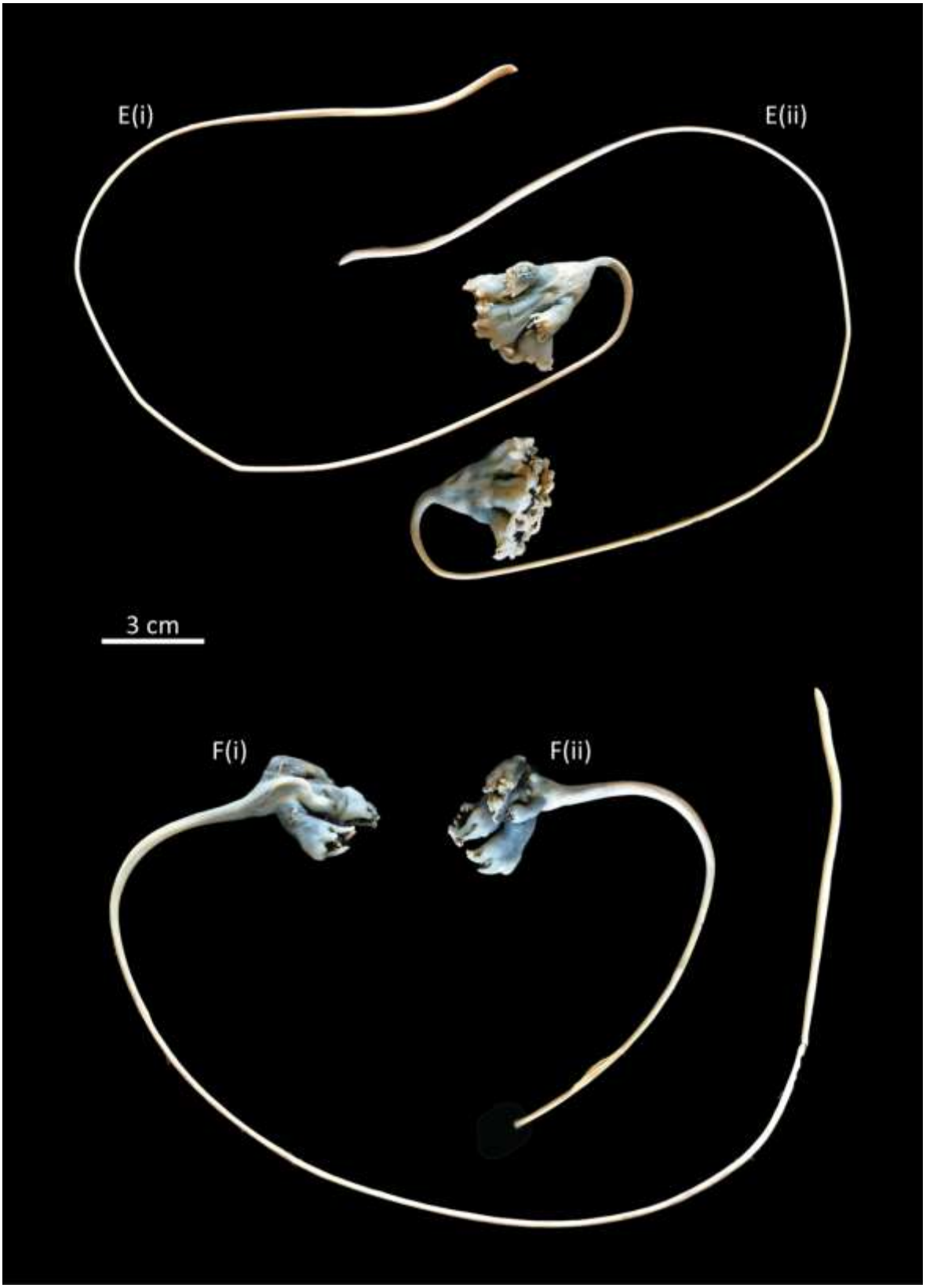

Plate 14 *Umbellula aciculifera*. E-F, Porcupine Seabight/Goban Spur, NE Atlantic, 1600-1691 m; E(i), F(ii) Ventral; E(ii), F(i) Dorsal.

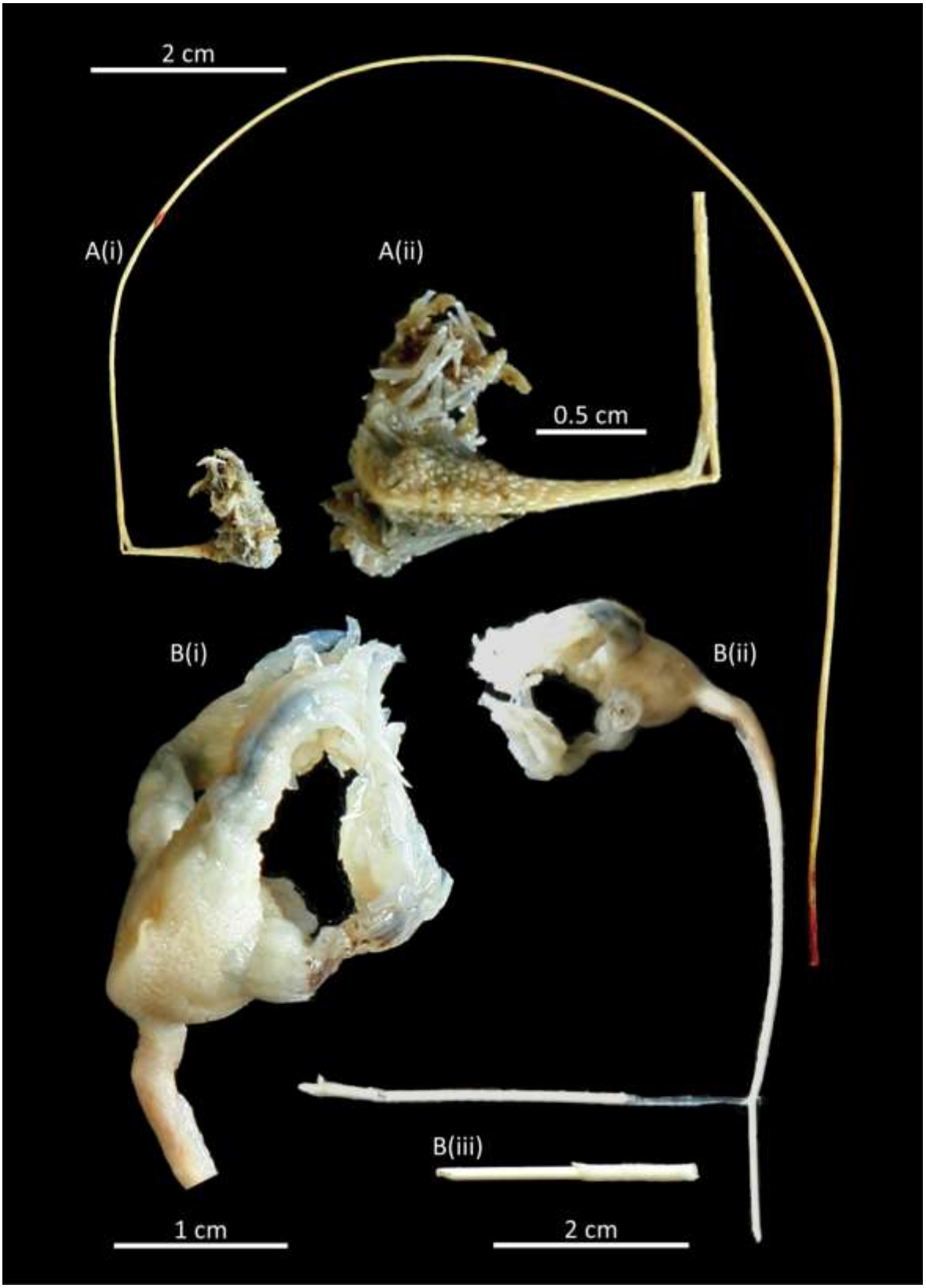

Plate 15 *Umbellula durissima*, *Umbellula* sp.3 n. sp. A, *U. durissima*: A(i) Ventral; A(ii) Dorsal. B, *Umbellula* sp.3 n. sp.: B(i) Dorsal; B(ii) Ventral; B(iii) Stem piece.

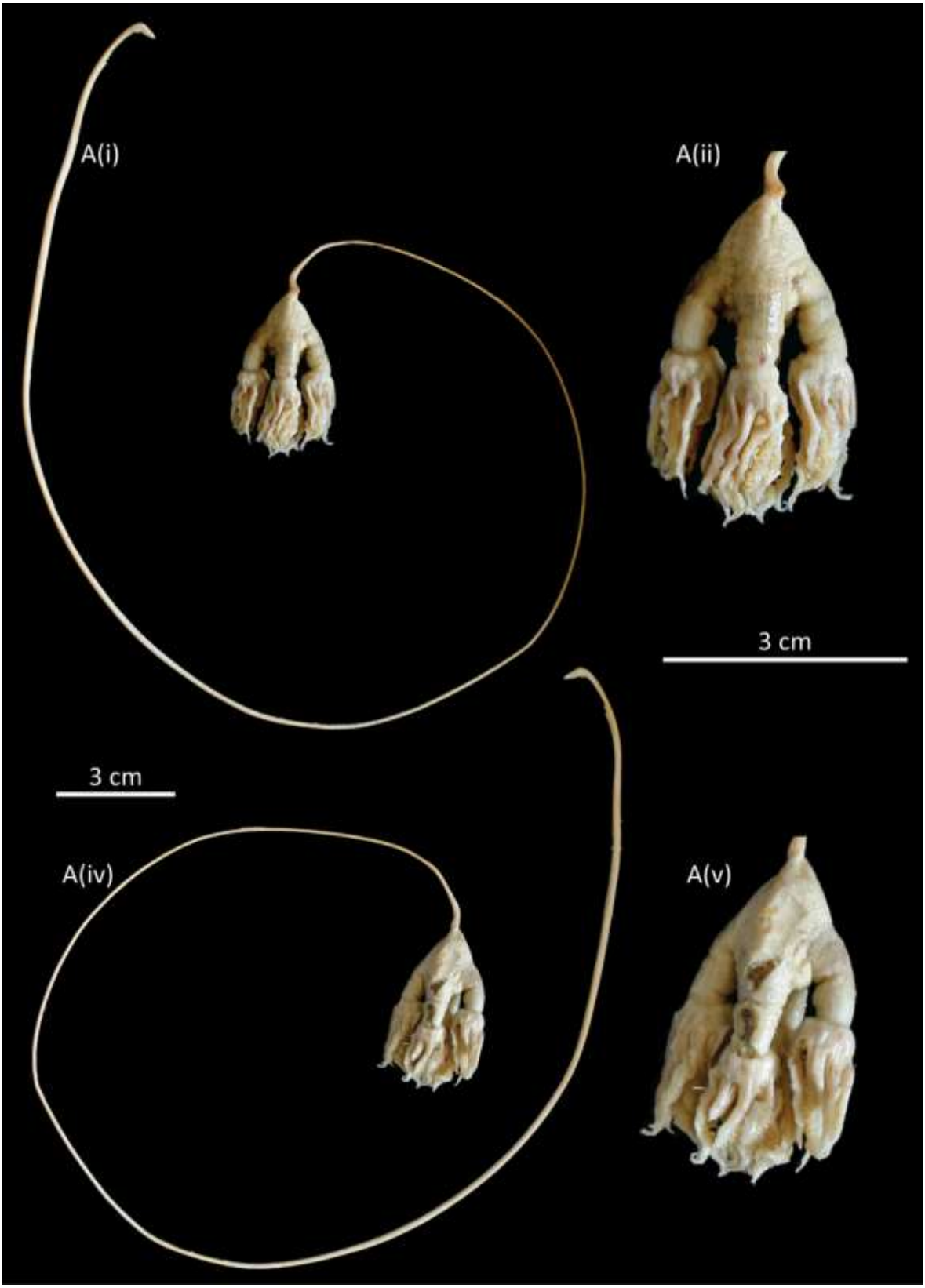

Plate 16 *Umbellula* sp.2 n. sp. Crozet, S Indian, 4189.5 m: A(i)-A(ii) Ventral; B(i)-(ii) Dorsal.

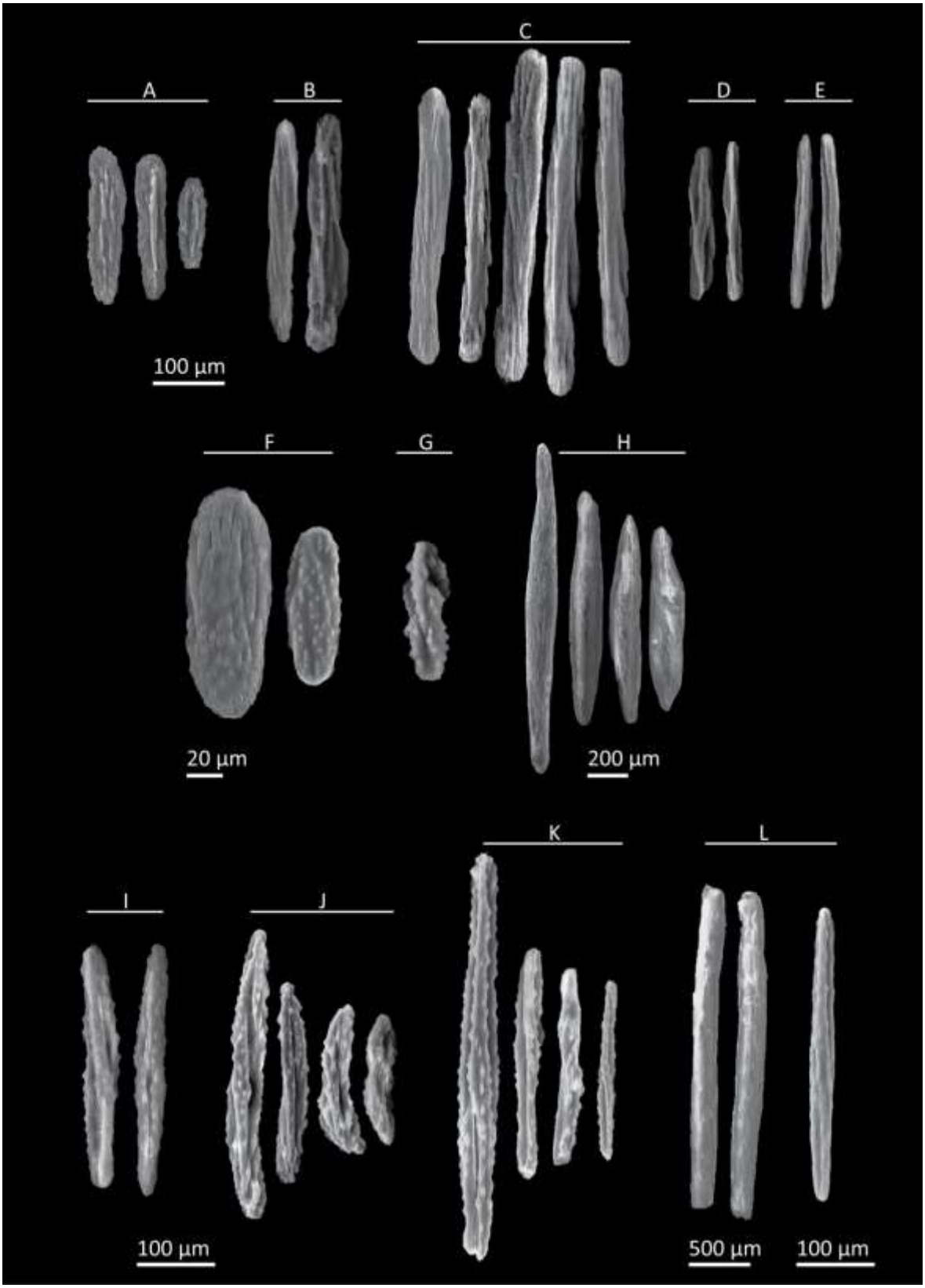

Plate 17 Sclerites. A-E *U. hemigymna*: A, Stem; B, Rachis; C, Autozooid; D, Tentacle; E, Pinnule. F-H *U. aciculifera*: F, Peduncle; G, Stem; H, Autozooid /rachis. I-L *Umbellula* sp.3 n. sp.: I, Stem; J, Rachis; K, Autozooid; L, Tentacle/pinnule.

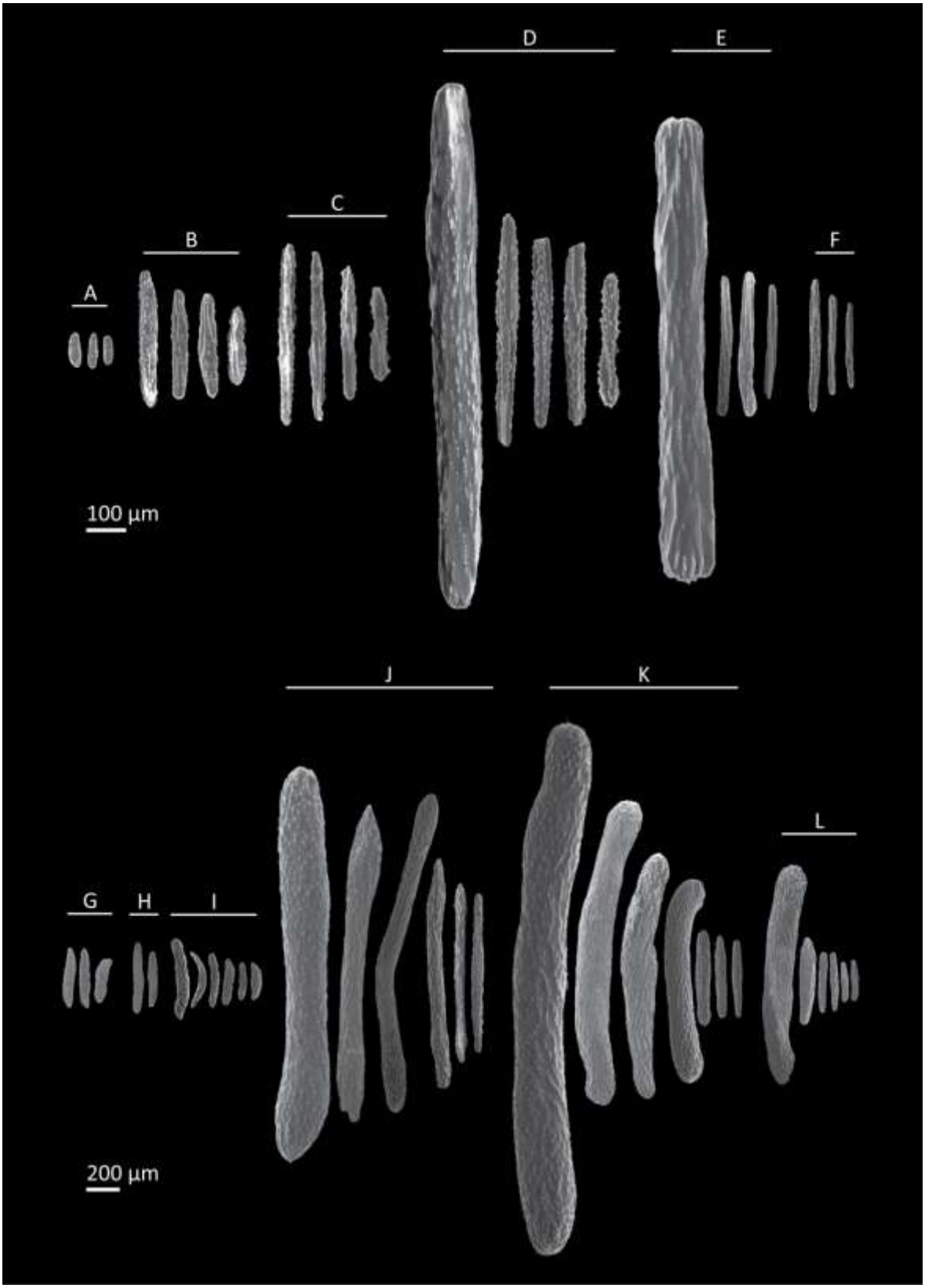

Plate 18 Sclerites. A-E *Umbellula* sp.2 n. sp.: A, Peduncle; B, Stem; C, Rachis; D, Autozooids; E, Tentacle; F, Pinnule. G-L *U. monocephalus*: G, peduncle; H, Stem; I, Rachis; J, Autozooid; K, Tentacle; L, Pinnule.

Chapter Four

Biogeography of the Deep-Sea Genus *Umbellula* and its Morphological Variability with Depth

4.1 Introduction

The discovery of high species richness in the deep sea (Grassle and Maciolek, 1992) has led us to question the origin and evolution of marine biodiversity, and with this, came the study of the biogeography of such fauna. The first conception of the deep sea was that it was a single biogeographic province, sharing species with cosmopolitan distributions; such views were derived from the humdrum of abyssal fauna collected during the worldwide voyages of HMS *Challenger,* 1870-72. Whilst this opinion was upheld by Bruun (1957), others discerned differences within the World Ocean, dividing it into multiple biogeographic regions and provinces (Ekman, 1935; 1953; Vinogradova, 1959; Madsen, 1961; Vinogradova, 1979; 1997).

Geographical patterns in the distribution of species (or higher taxa) and the causes for those patterns are not well understood for deep-sea fauna. This situation arises, in part, because of the vastness of the habitat, lack of sampling and sampling limitations, and problems in identifying species (Tyler, 2003). Thus, biogeographic classification systems are far less developed than in terrestrial, coastal and continental shelf areas. It is known that abyssal species tend to show restricted geographic distributions to one ocean or region as the abyssal plains are divided by the continents and mid-ocean ridges (Vinogradova, 1997); yet, some taxa, for example the holothurian, *Onierophanta mirabilis*, are almost completely cosmopolitan (Hansen, 1975). Latitudinal variability also has an influence on the distribution of species suggesting that historical factors, surface productivity patterns and other parameters correlated with latitude influence species diversity (Rex et al., 1993; Keller and Pasternak, 2001).

Octocorals occur throughout the world's oceans, over all latitudes and from the littoral to the greatest depths of the ocean floor; yet it is in the shallow-water tropics that they reach maximal species diversity (Williams, 1992b; Fabricius and Alderslade, 2001). Similarly, bathyal octocorals display highest biodiversity at low latitudes (Keller and Pasternak, 2001). Pennatulids are eurybathic (intertidal to >6000 m), most often inhabiting bathyal and abyssal depths or cold waters (Kükenthal, 1915; Rice et al., 1992; Keller and Pasternak, 2001), but like other octocorals, pennatulids show maximum species diversity in the shallow-water tropics (Williams, 1992b). In contrast to other octocorals, however, the highest species diversity of bathyal pennatulids is reached at the high latitudes of the northern hemisphere (Keller and Pasternak, 2001). It is speculated that the age of deep-water corals, and the history of modern ocean formation, are the main factors determining their latitudinal distribution.

Whilst the biogeography and origins of scleractinian corals have been well documented (Veron, 1995 and the references therein; Bellwood and Hughes, 2001), such studies on octocorals are inadequate. This is because the paucity of octocorals in the fossil record (Bayer, 1956) makes extrapolating historical biogeographic patterns problematic. Thus, very few biogeographic studies have been conducted on octocorals beyond regional scale (Williams, 1992a; b) or studies are restricted to certain genera of particular regions (Lopez-Gonzalez et al., 2001; Cairns and Bayer, 2002; Lopez-Gonzalez and Williams, 2002; Cairns and Bayer, 2003; 2004a; b; 2008).

Williams (1992a) established that 20 % of pennatulid species found in South African waters are cosmopolitan, and of those species considered endemic to southern Africa, all occur shallower than 333 m. The high number of pennatulid cosmopolites or species with wide-ranging distributions is driven by their ability to exploit soft or unstable substrata, and thus pennatulids occupy extensive regions of the seabed. Consequently, there is a low degree of endemism in pennatulids when compared to other octocorals; no alcyoniid cosmopolites are presently recorded. A biogeographic and phylogenetic assessment of the shallow water Indo-Pacific pennatulid fauna suggested that Veretillidae and the Echinoptilidae were the least derived of the extant pennatulids: these are at present concentrated in the relatively shallow waters of the Indo-Pacific, while a great variety of more derived forms are present worldwide and show extensive bathymetric ranges (Williams, 1992b; 1997b). Hence, Williams (1992b; 1997b) postulated that pennatulids

may have differentiated in the shallow waters of the Indo-Pacific and subsequently diversified and dispersed to all depths of the temperate and polar regions, as well as the tropics.

In order for the pennatulids to occupy new niches, such as those encountered with depth, and to exploit these efficiently, adaptive changes were paramount. Food availability declines with depth and distance from the coast, and as a consequence of these gradients the deep-sea benthic fauna change rapidly with depth down the continental margin and into the abyss (Gage and Tyler, 1991). The genus *Umbellula* is cosmopolitan and eurybathic, and species within *Umbellula* represent some of the oldest and youngest pennatulid species (Chapter 2).

This chapter aims to evaluate the biogeography and possible origins of the deep-sea genus *Umbellula* (family Umbellulidae), and to test whether species originally differentiated in the Indo-Pacific. Having revised *Umbellula* (Chapter 3) it is now possible to get a fuller understanding of the biogeographical distributions of the species. Prior to this, species were incorrectly synonymised or split, making comparisons between oceanic regions of the world erroneous (Hickson, 1916; Broch, 1957; 1958; Pasternak, 1962). An understanding of the historical relationships of *Umbellula* spp. and their relative ages, inferred from genetic analyses, gives further insight into the origins of this genus. Furthermore, observations concerning the morphological variability in *Umbellula* spp. are presented to infer adaptations to the deep sea: *Umbellula* is a cosmopolitan and eurybathic genus and thus is apposite for such a study.

4.2 Materials and Methods

4.2.1 Sources of Data

Data were accumulated from the examination of preserved specimens (refer to Section 3.2.1 and Table 3.1, Chapter 3) and critically analysing the literature (important sources included Lindahl, 1874; Kölliker, 1875; 1880; Danielssen and Koren, 1884; Jungersen, 1904; Kükenthal and Broch, 1911; Kükenthal, 1915; Hickson, 1916; 1937; Broch, 1957; 1958; Pasternak, 1962; Pasternak, 1970; Pasternak, 1973; 1975; Williams, 1990;

Pasternak, 1993). Also, refer to Section 3.2.2 and Table 3.14, Chapter 3, pertaining to the literature data.

4.2.2 Biogeography

Umbellula species were mapped using the computer software, PanMap (Diepenbroek et al., 2000) and biogeographic zones were overlaid in Adobe Illustrator. The zoogeographic zonation scheme proposed by Vinogradova (1979) was adapted for the recognition of biogeographic regions and provinces of the World Ocean[1]. This scheme was originally designed for the abyssal ocean floor and was composed of 3 regions, 6 sub-regions and 8 provinces:

Pacific-North-Indian deep-sea region
- Pacific sub-region
  - North-Pacific abyssal province
  - West-Pacific abyssal province
  - East-Pacific abyssal province
- North-Indian Ocean sub-region

Atlantic deep-sea region
- Arctic sub-region
- Atlantic sub-region
  - North-Atlantic abyssal province
  - West-Atlantic abyssal province
  - East-Atlantic abyssal province

Antarctic deep-sea region
- Antarctic-Atlantic sub-region
- Antarctic-Indian-Pacific sub-region
  - Indian Ocean abyssal province
  - Pacific abyssal province

For the purpose of this study, however, the limits of the zones were extended to include bathyal areas adjacent to the continental shelves. Furthermore, the Atlantic deep-sea region was divided to separate the Atlantic from the Arctic, and the Pacific-North-Indian deep-sea region was divided to separate the Indo-Pacific from the East Pacific. This scheme was appropriate since it regards *Umbellula* species occurrences, and is similar to that proposed by Ekman (1935) who divided the Arctic from the Atlantic; and considers the conclusions of Madsen (1954; 1961) who showed deep-sea echinoderms to be more isolated from the eastern Pacific, whereas those from the Indian and Atlantic oceans were

[1] A new scheme is now proposed (Watling, in press), but was not publicly available at the time of writing.

most closely related. Thus, the biogeographic categories employed are as follows, which recognises 5 regions and 9 provinces:

Indo-Pacific region
- North-Pacific province
- West-Pacific province
- North-Indian province

East Pacific region
Atlantic region
- North-Atlantic province
- West-Atlantic province
- East-Atlantic province

Arctic region
Antarctic region
- Antarctic-Atlantic province
- Antarctic-Indian province
- Antarctic-Pacific province

Species were assigned a faunistic category based on their occurrence in the Indo-Pacific region and their distribution within the other biogeographic regions (see Table 4.1 for a full list of categories and explanations).

4.2.3 Bathymetric Variation in Body Morphology in Species of Umbellula

To analyse bathymetric distributions of species, depth data were obtained from the literature and benthic samples (Section 4.2.1), and plotted in SigmaPlot 10.0. The method of acquiring samples by means of trawl and sledge was considered 'quantitative' for the analysis of abundance in asteroids (Howell et al., 2002) and bivalves (Olabarria, 2005). However, the lower portion of pennatulid colonies (the muscular peduncle) is buried in the sediment and acts as an anchor; this, together with the flexible nature of the stem in *Umbellula* colonies, often enables them to avoid capture. Accordingly, samples only give an indication of presence (and to some extent, absence) but do not provide information on relative or absolute abundances. Since samples were collected by means of trawl and epibenthic sledge, quantitative abundance data could not be obtained, and thus treatment of the data was limited. Accordingly, a review on morphological variability with depth was conducted. For this, the maximum number of autozooids was evaluated for each *Umbellula* species from the specimens, and similar data were obtained from the literature (Section 4.2.1), and analysed together with the bathymetric data.

4.3 Results

4.3.1 Biogeography

The biogeographic data are presented in Table 4.1 and Fig. 4.1.

Table 4.1 Biogeographic categories and faunistic categories of *Umbellula* spp.

Species	Biogeographic Category IP_{NP}	IP_{WP}	IP_{NI}	EP	Atl_N	Atl_W	Atl_E	Arc	An_A	An_I	An_P	Faunistic Category
U. aciculifera					√				√			NP
U. antarctica		√	√						√	√	√	AS
U. durissima	√	√	√		√	√			√			C
U. carpenteri					√		√			√		NP
U. encrinus					√			√				NP
U. hemigymna					√!	√!						NP
U. huxleyi	√	√	√		√		√					S
U. magniflora	√	√	√	?					√	√	√	AS
U. monocephalus			√		√		√					S
U. pellucida			√	√								EL
U. spicata			√									En
U. thomsoni	√	√	√		√	√	√		√	√		C
Umbellula sp.1 n. sp.					√!							U
Umbellula sp.2 n. sp.			√							√		EL
Umbellula sp.3 n. sp.					√!							U
Total per province	4	5	9	1*	10	3	4	1	5	5	2	
Total per region	9			1*	10			1	7			

Biogeographic Category

Indo-Pacific region	IP_{NP}	North-Pacific province
	IP_{WP}	West-Pacific province
	IP_{NI}	North-Indian province
East-Pacific region	EP	East-Pacific province
Atlantic region	Atl_N	North-Atlantic province
	Atl_W	West-Atlantic province
	Atl_E	East-Atlantic province
Arctic region	Arc	Arctic region
Antarctic region	An_A	Antarctic-Atlantic province
	An_I	Antarctic-Indian province
	An_P	Antarctic-Pacific province

√	Present
!	Full distribution unknown
?	Presence suspected
*	No. of species expected to be greater

Faunistic Category

En	Endemic to the Indo-Pacific
EL	Extralimital: primarily Indo-Pacific distribution but also extending into other areas
AS	Antarctic and Subantarctic: Present in the Indo-Pacific, and distributed in the Antarctic and/or Subantarctic
C	Cosmopolitan distribution: cosmopolitan taxa are present in both temperate and tropical areas of the Atlantic, Indian, Pacific and southern oceans
S	Scattered distribution: scattered taxa are found in various regions of the World Ocean but are not widespread enough to be considered cosmopolitan
NP	Not present in the Indo-Pacific
U	Not present in the Indo-Pacific but extent of distribution unknown

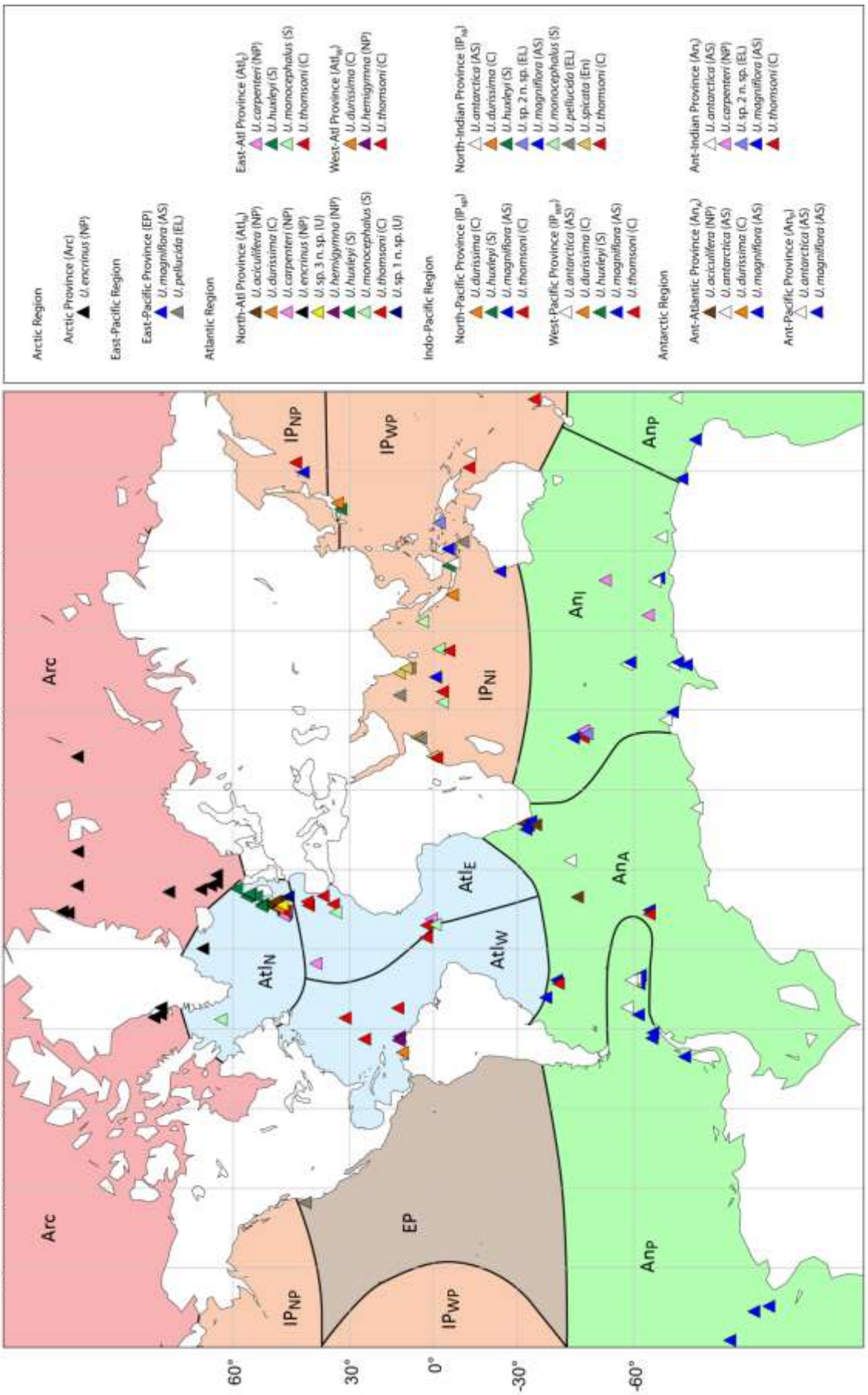

Figure 4.1 Biogeographical map of *Umbellula* species. Provinces adapted from Vinogradova (1979).

The genus *Umbellula* is cosmopolitan, distributed throughout all biogeographic regions of the World Ocean. The most species of *Umbellula* are in the Atlantic, which harbours 10 of the 15 known species of *Umbellula* (66.7 % of total species); within this region, the North-Atlantic province possesses all 10 of these species, whereas the West-Atlantic province and East-Atlantic province display lower diversities, with 3 and 4 species respectively. Only one species, the cosmopolitan *U. thomsoni*, is common to all the provinces within the Atlantic region.

The Indo-Pacific harbours 9 species in total (60.0 %). However, the provinces within this region are far more balanced in terms of diversity than those of the Atlantic: the North-Pacific province has 4 species, the West-Pacific has 5 and the East-Pacific has 9; and of these, 4 are common to all provinces of the Indo-Pacific, *U. durissima*, *U. huxleyi*, *U. magniflora* and *U. thomsoni*. The first and the last two species have a cosmopolitan distribution, *U. huxleyi* is scattered, whereas *U. magniflora* is widely distributed throughout the Antarctic and Subantarctic.

The Antarctic region has 7 species of *Umbellula* (46.7 % of total species), 5 of which occur in the Antarctic-Atlantic province, 5 in the Antarctic-Indian province and 2 from the Antarctic-Pacific province. Of these, 2 *Umbellula* species are common to all provinces within the Antarctic region, namely *U. antarctica* and *U. magniflora*, the distribution of which is mainly restricted to the southern hemisphere. Although evidence is still not available, the distribution of *U. antarctica* and *U. magniflora* may be influenced by the existence of circumpolar deep water.

The East-Pacific and the Arctic regions show the lowest species diversities (6.7 % of total species in each). In the East-Pacific, only *U. pellucida* has been recorded with any certainty. Nevertheless, other species are expected to occur here: '*U. magniflora*' has been observed in photographs taken from an ROV in the Monterey area, CA (L. Kuhnz, pers. comm.), and although it is not usually possible to identify correctly *Umbellula* spp. with this method, it is likely that more than one species occurs here. The Arctic region harbours only one known species, *U. encrinus*. This species is also present in the North-Atlantic province, but its distribution is limited, only occurring in the highest latitudes where cold Arctic bottom currents circulate.

Two species, *U. durissima* and *U. thomsoni*, are common to the main biogeographic regions (Indo-Pacific, Atlantic, and the Antarctic). However, there is a very low degree of endemism: of the 15 species of *Umbellula*, only one is endemic to Indo-Pacific region, namely *U. spicata*, from the North-Indian province. *Umbellula pellucida* is extralimital, commonly found in the North-Indian province but is also encountered in the East-Pacific region. *Umbellula* sp.2 n. sp. is also considered extralimital, but has only been recorded once from the Indo-Pacific and once from the Antarctic-Indian province. *Umbellula hemigymna* is endemic to the Atlantic (both the East-Atlantic and West-Atlantic provinces), but this relatively recently described species (Pasternak, 1975), may be present elsewhere. *Umbellula encrinus* can be considered endemic to the Arctic region, but it also occurs in the upper fringes of the North-Atlantic province.

4.3.2 Bathymetric Variation in Body Morphology in species of Umbellula

Figure 4.2 shows the depth ranges of *Umbellula* spp. and the maximum number of autozooids each species possesses. The figure illustrates the wide vertical distribution of species belonging to this eurybiotic genus, with depths ranging from 265 m (*U. pellucida*) to 6162 m (*U. thomsoni*). Aligned in order of median depth, there is a negative relationship between number of autozooids and depth; this is also illustrated in Fig. 4.3 which shows a significant correlation ($p=0.0002$) between these two variables.

Generally, the number of autozooids decreases the deeper the distribution of the species, and conversely, the relative size of the autozooids increases (not shown graphically). Species can be divided into two groups: those with numerous autozooids (25-40+) occupy the upper bathyal zone; *U. pellucida*, *U. spicata*, *U. encrinus* and *U. huxleyi* fall into this group; whereas those with few autozooids (1 to 15) inhabit the lower reaches of the bathyal zone, and abyssal depths. These species are *U. aciculifera*, *U. durissima*, *U. hemigymna*, *Umbellula* sp.1 n. sp., *Umbellula* sp.3 n. sp., *Umbellula* sp.2 n. sp., *U. thomsoni*, *U. carpenteri*, and *U. monocephalus*. *Umbellula antarctica* and *U. magniflora* have extensive eurybathic distributions and do not fit into either group; however, it is plausible that these may represent more species, and could be divided in the future.

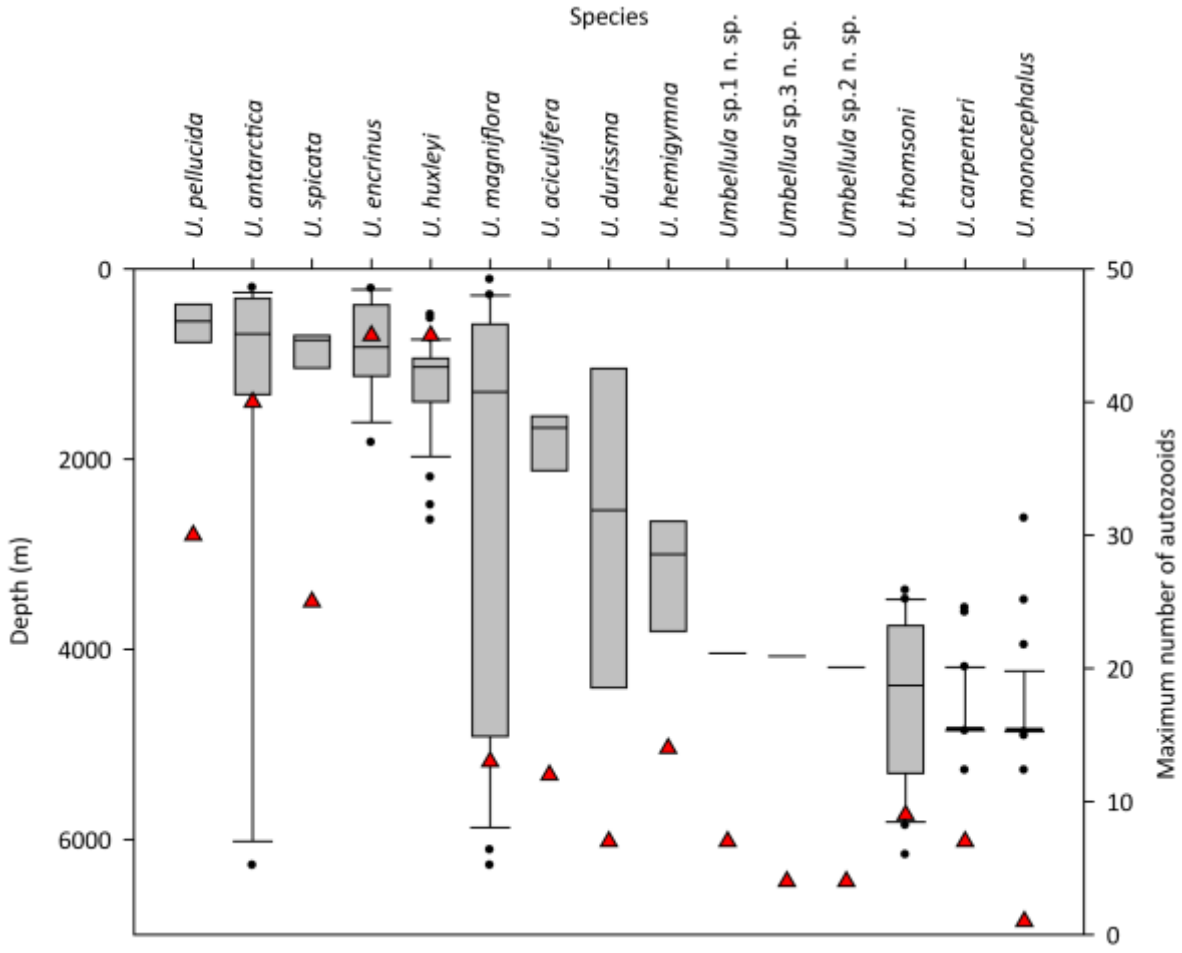

Figure 4.2 Box and whisker plot (right y-axis) showing bathymetric distribution of *Umbellula* spp.; horizontal bars inside the boxes are median depth values, and whiskers are standard error bars. Overlaid is a scatter plot (left y-axis) illustrating the maximum number of autozooids each species possesses (triangles).

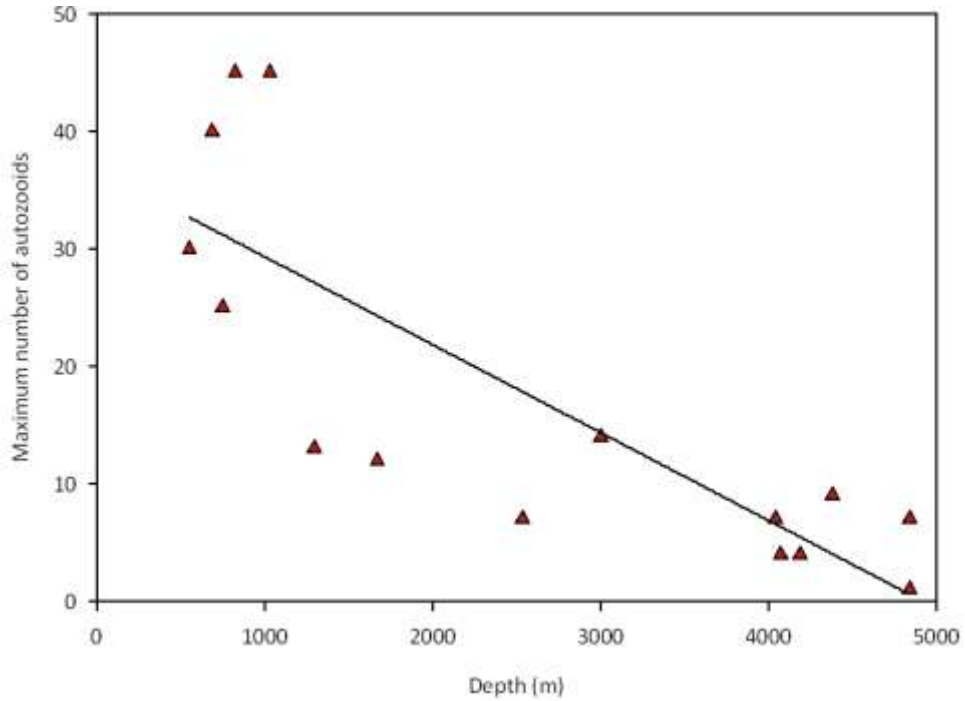

Figure 4.3 Relationship between depth and maximum number of autozooids for *Umbellula* spp. (p=0.0002). Each triangle represents a different species of *Umbellula*.

4.4 Discussion

4.4.1 Biogeography

Patterns of biodiversity and biogeography do not provide a clear indication of the geographic origins of the deep-sea genus *Umbellula*. High species diversity in the Indo-Pacific and Atlantic regions makes both these areas good candidates; and a low degree of endemism in all regions provides little information to distinguish between them. Sampling of the deep-waters of the Antarctic has been inadequate, and therefore it is not possible to make assumptions concerning species diversity for its respective provinces. Yet, historical data from molecular phylogenetic reconstructions (Chapter 2) suggest that those species that only occur in the Atlantic and Arctic are younger than species of *Umbellula* from the Indo-Pacific and fringing provinces in the Antarctic region. Biogeographic patterns and genetics, combined with the geological history of the modern ocean, suggest that that *Umbellula* originated from Indo-Pacific ancestors, subsequently differentiated and dispersed away from this area into the Antarctic, and later the Atlantic and Arctic, and E Pacific oceans. This occurred by two different evolutionary pathways. Such findings support those of Williams (1992b; 1997b), who postulated that the order Pennatulacea originally diversified in the Indo-Pacific, and the theories of Keller and Pasternak (2001) who suggested that bathyal corals (scleractinians, alcyoniids, and pennatulids) penetrated the higher latitudes of the southern hemisphere from the tropics before radiating to the northern latitudes (see Section 4.4.1.4 below).

4.4.1.1 Biodiversity of the genus *Umbellula* in the Indo-Pacific

The Indo-Pacific is perhaps the most diverse biogeographic region of the World Ocean (Williams, 1992b; Bellwood and Hughes, 2001). Many pennatulid species occupy sandy areas on, or adjacent to, the coral reefs, or are common in deeper waters of the sublittoral zones where soft sediments predominate. Pennatulids are also present (often in dense localised populations) in deeper portions of island arcs, or on continental shelves and slopes as well as abyssal plains (Williams, 1992b). The Indo-Pacific houses 60 % of known *Umbellula* species. Although our current knowledge of *Umbellula* distribution suggests that the Atlantic region has a marginally greater diversity (66.7 % of known

species), it is clear that we have a lot to learn about the number of species that belong to this genus and their distribution.

Two main factors explain why there are more species of *Umbellula* known from the Atlantic than the Indo-Pacific: sampling intensity and species identification. The N Atlantic, particularly the Irish continental margin, has been subject to rigorous sampling and two new species described from this area were discovered in this study alone (Sections 3.3.3.3 and 3.3.3.13, Chapter 3); but their full distribution is unknown. Although the Indo-Pacific has been extensively sampled in the past, the paucity of pennatulid taxonomists and difficulties in identifying species of *Umbellula* has meant that further new species have not been accounted for: a third new species, *Umbellula* sp.2 n. sp., was collected from the Subantarctic Indian Ocean (Section 3.3.3.12, Chapter 3), but was discovered earlier from the Indo-Pacific described under the misnomer, *U. durissima* (Hickson, 1916).

The biogeographic provinces of the Indo-Pacific are more balanced in terms of biodiversity than those of the Atlantic. This, coupled with the fact that the Indo-Pacific has endemic species, provides some evidence to suggest that *Umbellula* first diversified here. If we consider endemic and extralimital *Umbellula* spp. together, three species, *U. spicata,* and *U. pellucida* and *U.* sp.2 n. sp respectively, can be regarded as 'Indo-Pacific' taxa. *Umbellula hemigymna* is endemic to the Atlantic, but is poorly documented, thus the extent of its distribution is unknown. Likewise, *U.* sp.1 n. sp. and *U.* sp.3 n. sp. are only known from the Atlantic, but again, their distribution is unknown. *Umbellula encrinus* can be considered endemic to the Arctic, but biodiversity in these waters is low with only one species, a species that is the youngest of all those *Umbellula* spp. genetically analysed in Chapter 2, together with *Umbellula* sp.1 n. sp. from the Atlantic (see Section 4.1.1.3 below).

If it is the case that the Atlantic region is more diverse than the Indo-Pacific, this may have resulted from prolific and rapid speciation into new niches. Thus, *Umbellula* may have differentiated and diversified in the Indo-Pacific, but subsequently many more species differentiated in other regions, such as the Atlantic, where ecological differences among and within ocean basins (e.g. water masses and food fluxes) played a part in dispersion and speciation.

4.4.1.2 Distribution and Dispersal

The capacity of *Umbellula* to disperse great distances explains why species of this genus are rarely limited to a single oceanic basin. The distribution of higher taxa is unusually homogenous in the deep sea, but the ability of species to be widely spread depends on factors such as ecological differences among the basins, dispersal limitations and history (Gage and Tyler, 1991). Pennatulids are lecithotrophic, a mode of development where the free-swimming, non-feeding larva is pelagic (presumably near the bottom) for an unknown, but limited period of time. A study on the larvae of Antarctic echinoderms showed that those species with lecithotrophic development could survive for months to years by relying solely on the energy reserves present in the egg (Shilling and Manahan, 1994). Thus, a lecithotrophic mode of development allows species to disperse great distances without the need for external food sources. If we consider the Indo-Pacific species, *U. spicata* and *U. pellucida*, both inhabit 'shallow' deep-water: *U. spicata* occupies depths of 470 to 1280 m; and *U. pellucida* rarely exceeds 1600 m depth; and of those pennatulid species considered endemic to southern Africa, all were found from depths less than 333 m (Williams, 1992a). Perhaps upper bathyal pennatulids are adapted to more eutrophic environments than their abyssal relatives (see Section 4.4.2); or are unable to disperse great distances because of depth constraints; or maybe life history plays a role. A study on echinoderms suggests that the lecithotrophic larvae of deep-sea species of the Atlantic have longer larval periods when compared with shallow-water species, particularly those from warmer waters (Young et al., 1997).

Studies on the large lecithotrophic eggs of the bathyal echinoid *Phormosoma placenta* suggest lecithotrophic larvae are not demersal, but rather develop at, or near, the surface (Young and Cameron, 1987). It is conceivable that this is a limiting factor for many fauna invading the deep sea that are not situated in areas where surface/mid-water currents can carry the lecithotrophic larvae to great depth.

Umbellula encrinus appears to be restricted to the cold bottom waters of the Arctic and N Atlantic surrounding the southern coast of Greenland, and thus its distribution is perhaps limited by temperature constraints.

4.4.1.3 Historical Relationships within *Umbellula*: Genetic Evidence for Indo-Pacific Origins

If we refer back to Chapter 2, information pertaining to the relative ages of those *Umbellula* species analysed can be inferred from the phylogenetic trees (Fig.s 2.2 and 2.3). The phylogenetic analysis of *Umbellula* spp. and their closest relatives are presented here (Fig. 4.4) for convenience. The genus is polyphyletic, that is to say, species followed two different evolutionary paths. The species were divided as follows: *U. monocephalus* and *Umbellula* sp.2 n. sp. grouped together forming one clade; whereas *U. carpenteri*, *U. huxleyi*, *U. magniflora*, *U. encrinus*, and *Umbellula* sp.1 n. sp. formed a separate clade. For the purpose of this chapter, the first clade will be referred to as 'Group 1', and the latter, 'Group 2'.

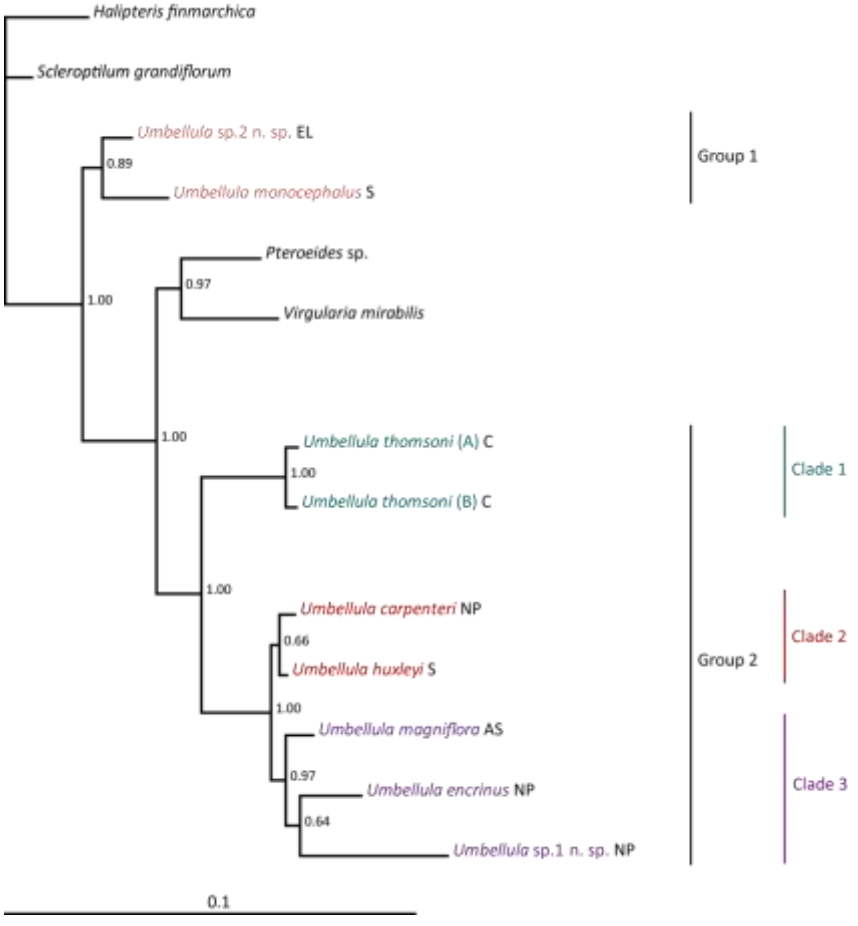

Figure 4.4 Phylogenetic relationships among 8 species of *Umbellula*. Bayesian likelihood tree, 50% majority-rule consensus of 105 trees (10^6 generations; burnin=1000); values at nodes are posterior probabilities; scale bar indicates number of nucleotide substitutions per site. The following species were used as outgroups: *Virgularia mirabilis*, *Pteroeides* sp., *Scleroptilum grandiflorum* and *Halipteris finmarchica*; *U. thomsoni* A and B are from the Antarctic-Indian and North-Atlantic provinces respectively; Faunistic categories are labelled in black adjacent to species name (for definitions, refer to Table 4.1).

Group 1 is the more primitive of the two groups, and contains *Umbellula* sp.2 n. sp., a species only known from the North-Indian and the Antarctic-Indian provinces; *U. monocephalus* has a scattered distribution but is common to the Indian and Atlantic regions. The closest relations to Group 1 were *Scleroptilum* and *Halipteris* (see Fig.s 2.1 and 2.2, Chapter 2, for resolved phylogenies), genera that, although they are widely distributed, are also common to the Indo-Pacific.

The younger species, Group 2, split into 3 clades: the cosmopolitan *U. thomsoni* formed one clade, separated from the others. *Umbellula carpenteri* and *U. huxleyi* formed a second clade: although both these species are present in the Atlantic, *U. huxleyi* is widely distributed in all the provinces of the Indo-Pacific, and *U. carpenteri* is distributed in the Antarctic-Indian province. A third clade consisted of *U. magniflora*, *Umbellula* sp.1 n. sp. and *U. encrinus*. Of these, *U. magniflora* was the most primitive, a species limited to the Indo-Pacific and the Antarctic; and crucially, the trees suggested that *U. encrinus* (Arctic species) and *Umbellula* sp.1 n. sp (N Atlantic species) descended from a *U. magniflora*-like ancestor. The closest relations to Group 2 were *Virgularia* and *Pteroeides*, the latter being regarded as an Indo-Pacific taxon (Williams, 1992b).

Therefore, it can be inferred that *Umbellula* sp.2 n. sp. and *U. monocephalus* are among the oldest species of *Umbellula*, derived from ancestors in the Indo-Pacific (the only region these two have in common). Along a separate evolutionary lineage, other species of *Umbellula* evolved and differentiated from taxa endemic to the Indo-Pacific; the youngest *Umbellula* species being endemic to the N Atlantic province and Arctic (*Umbellula* sp.1 n. sp. and *U. encrinus* respectively).

4.4.1.4 Radiation of *Umbellula* from the Indo-Pacific

It is hypothesised that *Umbellula* originally diversified in the Indo-Pacific, subsequently differentiated and dispersed away from this area into the Antarctic, and later the Atlantic and Arctic, and E Pacific oceans.

The near absence of octocorals in the fossil record makes extrapolating historical patterns of biogeography problematic. Keller and Pasternak (2001) considered the history of

modern ocean formation as the main factor determining the distribution of extant bathyal corals (scleractinians, alcyoniids, and pennatulids). In the Cenozoic era, Gondwana broke apart and the future Antarctica moved southward. Off the Antarctic coasts, dense, aerated, cold water (< 7 °C) masses began deepening at the end of the Miocene, which flowed down the continental slopes: these displaced the stagnant deep-waters created during the catastrophic warming event in the Lower Cretaceous that caused mass extinction of bathyal fauna. Presumably, those ancient warm-water fauna that were able to adapt to the new severe conditions of the Cretaceous, penetrated higher latitudes of the southern hemisphere following the pole-ward drift of the Gondwana southern plate: these were the pioneering species of the renewed deep waters. Among the octocorals, both primitive (Paragorgiidae, Kophobelemnidae) and advanced (Primnoididae, Isididae, Renillidae and Umbellulidae) families may have inhabited the margins of the Antarctic. Today, these octocoral families are the only inhabitants of Antarctica and the southern part of the South American shelf and slope. The northward influx of the Antarctic near-bottom waters continued, and by the Pleistocene (1.8 million years ago), the temperature had fallen to its modern value of 2 °C.

It was possibly during the Pleistocene that the genus *Umbellula* was able to advance northward, and inhabit the N Atlantic, E Pacific and Arctic basins; an exchange of bathyal species from the Pacific to the Atlantic via the Panama seaway could not have occurred since this closed ~ 3 million years ago (Lunt et al., 2008). The waters of the Antarctic shelf differ greatly from the lower bathyal and abyssal waters by high surface productivity and suspended detritus (Keller and Pasternak, 1996). Hence, *Umbellula* spp. managed to penetrate to the deep ocean, these species being highly adapted not only to cold water, but also to varying trophic environments. The occurrence of *U. encrinus* indicates that it must have penetrated into the Arctic region and into the cold bottom water of the Davis Strait and Baffin Bay from the Atlantic Ocean, and is possibly constrained by the Lomonosov Ridge, which prevents it crossing the Arctic Basin. However, sampling intensity in the eastern Arctic waters may account for the absence of *U. encrinus* in this area.

The older, abyssal species that composed ‘Group 1’ described above, were not constrained by bathyal water masses, and possibly dispersed from the Indo-Pacific prior

to the Pleistocene. This agrees with Keller and Pasternak (2001) who suggested that the bathyal coral fauna are probably younger (1.5 to 2 million years old) than the abyssal fauna based on the modern pattern of the bathyal coral distribution and history of modern ocean formation. Although the Panama gap could have provided a route for the exchange of abyssal species from the Pacific to the Atlantic, the absence of genetic data, and the near absence of biogeographic data for *Umbellula* species in the E Pacific prevents any further development of this theory.

4.4.2 Variability of Umbellula: *Morphological Adaptation to the Deep Sea*

The previous sections highlighted the low degree of endemism in the genus *Umbellula* and the extensive regions of benthic environments it inhabits, from the cold deep-waters of the N Atlantic to the warm shallows of the tropics. One factor driving this is the ability of pennatulids to exploit soft or unstable substrata. This gives them a huge advantage over other octocorals, which require a hard substratum for their attachment. Moreover, pennatulids can inhabit all soft sediment types, from fine to medium or relatively coarse sediments such as sand, or abyssal ooze deposits. Sediments prevail on the shelf, slope, bases of seamounts, and abyssal plains, and thus, by their very nature, it is clear why pennatulids were able to colonise the main oceanic vertical zones. In the case of *U. magniflora*, colonies have been observed anchored in the sediments that had settled on rocky crags of cliffs at ~800 m depth in the Antarctic (pers. ob.).

Pennatulids are passive suspension feeders that use their tentacles to separate particles from the passing currents. The tentacles are pinnate, mobile and contractile, and densely covered with sensory cells enabling the feeding polyp, or autozooid, to detect and grab impacting food particles. Pennatulids are highly dependent on currents for feeding, and size and amount of food particles in the water column; such factors are variable with depth. The results presented here show that species of *Umbellula* show morphological variability with depth that imply adaptations to increase feeding efficiency (Lasker et al., 1983). Generally, the number of autozooids decreases the deeper the distribution of the species; however, the relative size of the autozooids increases. Lasker et al. (1983) found the differences in feeding rates between two species of the shallow-water alcyoniid genus, *Plexaura*, were attributable to differences in autozooid size, and depth. *Plexaura*

nina has larger autozooids and was found to have greater feeding rates than *P. homomalla*, a species with smaller autozooids. Furthermore, feeding rates of both species tended to be lower at 29 m than at 17 m, but feeding rates of *P. homomalla* at 17 m were equal to those of *P. nina* at 29 m.

Pasternak (1989) first analysed the changes in pennatulid morphology that originated from the colonisation of the deep sea. He noted that species with fewer (but larger) autozooids had larger food catchment areas than those with numerous (but smaller) autozooids. The shallower-water *U. encrinus* with 60 autozooids had a total catching area of 117 cm^2, whereas the smallest individual of the abyssal *U. monocephalus* with a single, great autozooid possessed a catching area of 139 cm^2; the biggest colony of this species had a catching area of 507 cm^2. The morphology of the tentacles also contributed to the surface area of the feeding-polyps: in *U. monocephalus* these are flattened in the transversal plane; this too is the case for the abyssal *U. thomsoni*, among others. In upper bathyal species such as *U. encrinus* and *U. huxleyi*, the tentacles are cylindrical or flattened in the sagittal plane of the autozooids. There is a similar trend in the genus *Kophobelemnon*. For example, *K. stelliferum* inhabits upper bathyal zones and has a long, cylindrical rachis and numerous autozooids. Abyssal *K. pauciflorum* and *K. macrospinum* colonies are short and clavate, possessing one and two autozooids respectively, but the relative and absolute size of the feeding apparatus is greater than their shallower water representatives.

A further adaptation in different species of *Umbellula* is size and diversity of the supporting sclerites. The shallow-water group, with their small autozooids do not possess sclerites, only small oval bodies in the peduncle. However, *U. monocephalus*, *U. thomsoni* and *Umbellula* sp.2 n. sp., *Umbellula* sp.3 n. sp. and *U. durissima* possess a range of large and small sclerites, the largest of which are normally restricted to the autozooids and in particular, the tentacles and pinnules. *Umbellula aciculifera* is transient between the shallow species (without sclerites) and the abyssal species (with sclerites): this species has numerous small sclerites, and thus a thick, rubbery epidermis supports the large, but short, autozooids. *Umbellula carpenteri* is the only known abyssal species not to possess sclerites. However, the autozooids of *U. carpenteri*, although large relative to colony size, are small in absolute terms, and do not require a supporting skeleton.

In situ video footage of colony orientation and feeding of *Umbellula* sp. at 600 m off the Bahamas, showed that the autozooids were deflected downstream of the current; never did any autozooid obscure another by being directly upstream. Individual tentacles were held out rigidly in order to maximise tentacular area, and pinnules, positioned down two sides of the tentacles, were inserted alternately rather than being directly opposed. Further, pinnules were observed to incline towards the oral end of the autozooids to form v-shaped feeding surfaces in cross-section (Tyler et al., 1995). Similar behaviour was observed for *Umbellula* sp.1 n. sp. at 4040 m in the Whittard Canyon (see Plate 5, Chapter 3).

Thus, in the more oligotrophic regions at great depth, pennatulid species tend to have fewer yet larger autozooids, and their tentacular surface area is maximised, and large sclerites support these. This has an advantage in two ways: firstly, deep-water species are able to encounter food particles more frequently with a larger surface area; and secondly, the size of the autozooids allows capture of bigger food particles i.e. they are macrophagous. This latter advantage is important since particles reaching the deep-seabed have undergone flocculation and are therefore larger than organic matter in the shallows. Such a trend towards increasing size, associated with macrophagy, in the feeding polyp has already been discerned amongst other coelenterate groups and is most noticeable amongst species of oligotrophic basins where macrophagy, or carnivory, becomes, in energetic terms, a better adaptation than microphagous suspension feeding (Gage and Tyler, 1991). Mobility of potential prey and food particle size has a profound effect on feeding in pennatulids with much smaller autozooids. Kastendiek (1976) conducted laboratory-feeding experiments on the sea pansy, *Renilla kollikeri*, a subtidal species that inhabits regions of strong turbulence. Observations showed that *R. kollikeri* had difficulty capturing motile prey: in 500 encounters between autozooids and *Artemia* (0.4 to 0.7 mm in length), only three nauplii were caught and ingested. Similarly, when *R. kollikeri* were fed smaller (0.1 mm) copepods, none were caught in the 100 encountered. While *R. kollikeri* was an inefficient gatherer of motile animals larger than or as motile as a calanoid copepod 0.1 mm long, *R. kollikeri* was able to ingest non-motile prey such as bits of mussel (0.1 to 0.4 mm in length), and *Dunaliella* sp., single-celled, motile algae (9 to 11 μm in length).

4.5 Summary

Umbellula has a wide bathymetric range, thriving at depths from less than 300 m to greater than 6000 m; and is cosmopolitan with very few endemic species. This is possible because of morphological adaptations. *Umbellula* is able to exploit the soft sediments that prevail in the deep sea because of its muscular foot that anchors colonies, and thus does not require rocks for attachment; and its lecithotrophic mode of development allows species to disperse great distances. The shortage of food supply away from continental margins means that effectiveness of catching organic particles or perhaps swimming organisms is paramount. Thus, species of *Umbellula* have adapted by reducing the number but increasing the size of their autozooids, and in doing so, increased the food-catchment area; abyssal species have done so even more extremely.

The near absence of pennatulids in the fossil record makes extrapolating historical patterns of distribution and their evolutionary history problematic. Biogeographic data together with genetic evidence support the hypothesis that species of *Umbellula* originally differentiated in the Indo-Pacific. From here, they may have moved southwards to the Antarctic and later radiated north into the Atlantic, E Pacific, Indian and Arctic oceans, occupying bathyal and abyssal depths. This possibly occurred during the Plio-Pleistocene, 1.8 million years ago. The abyssal species, *U. monocephalus* and *U.* sp.2 n. sp., are among the oldest, and evolved via a separate evolutionary pathway. These too may have originated in the Indo-Pacific, and dispersed to the Subantarctic (*Umbellula* sp.2 n. sp.) or Indian and Atlantic oceans (*U. monocephalus*). However, the near absence of biogeographic data from the E Pacific (mainly because of the lack of reliable identifications), means that one cannot rule out the possibility of an exchange of species through the Panama seaway prior to the Pliocene.

Chapter Five

Summary and Conclusions

The discipline of systematics plays a central role in all branches of biology, and is linked inextricably with conservation. In today's technology-orientated research world, it is important to realise the continuing value of systematics, the basic tenet of which is to combine diverse types of data to produce classifications that reflect the natural history of living organisms (Monis, 1999; Dimmick et al., 1999). Accurate classification systems are crucial in the field of deep-sea biology, not only because they provide the means to identify species, but also because they provide a framework around which deep-sea fauna can be studied. Thus, systematic studies can be invaluable to improve our understanding of deep-sea ecosystems and play a vital role in the documentation of the Earth's biological diversity.

The construction of such a classification system for pennatulids is hampered by their morphology and biology; with small skeletal elements (sclerites), pennatulids are absent in the fossil record; a high degree of homoplasy is problematic for the classification of many families and genera; and only a handful of morphological traits are useful in distinguishing between many species. It was in this context that this project was developed: examining the systematics and phylogeny of deep-sea pennatulids; and providing a detailed synopsis and reclassification, together with studies of morphological adaptations and biogeography, of species of the deep-sea genus *Umbellula*. This was achieved through molecular and morphological analyses, distribution data, and a critical study of the literature.

The first step to a deeper understanding of pennatulids was through genetic analysis. Recent collections, representing a suite of taxa of wide geographic and bathymetric scope, enabled a reassessment of the systematic and phylogenetic relationships of 10 of the 15 pennatulid families (Chapter Two). Phylogenetic analysis of partial sequences from the NADH-dehydrogenase subunit 2 (*ND2*) and the mutS homologue (*msh1*) combined

produced well-supported phylogenetic relationships for representative deep-sea (and shallow-water) pennatulids at familial, generic and specific taxonomic levels. *ND2* was found to be more conserved than *msh1*, suggesting that the latter gene evolves faster and is the more informative of the two genes for phylogenetic analyses in pennatulids.

Genetic analysis gave strong support that highly-derived taxa occur in both shallow- and deep-water, together with more primitive pennatulid species, as suggested by Williams (1992b). Furthermore, many taxa may have differentiated and dispersed from the deep sea to shallow water: Renillidae, which is considered one of the most primitive shallow-water families, was found to be of more recent descent, derived from deep-water ancestors. Conversely, the bathyal family, Anthoptilidae, was the most primitive of pennatulids analysed, and although more evidence is needed, it could be that O. Pennatulacea originated and diversified in the deep sea, and subsequently invaded shallow waters.

Molecular analysis revealed a frequency of homoplasy among pennatulids, and suggested that many families (and genera) do not represent monophyletic groups. The following characters are apomorphic (derived): sessile autozooids; complete loss of sclerites in the autozooids and rachis; and clustering of autozooids or the presence of polyp leaves and raised ridges. However, reversals in evolution have led to taxa that possess derived character states that are analogous with plesiomorphic (primitive) traits, thus making phylogenetic reconstructions based on morphology problematic.

The suborders Sessiliflorae and Subselliflorae are polyphyletic and thus are of nominal value only. This is also the case for members of the families Kophobelemnidae, Pennatulidae, and Pteroeididae whose classification is in need of revision. Williams (1995a) suggested that the genera *Gyrophyllum* and *Pteroeides* (F. Pteroeididae) belong to F. Pennatulidae, and in unpublished work[1] (G. Williams and S. Carins, 2006) only 14 pennatulid families are recognised. This present study provided strong evidence that members of Pteroeididae divide into two groups and none belong to Pennatulidae. Thus, the new family name, “Gyrophyllidae”, should be established to include members of the

[1] http://research.calacademy.org/research/izg/OCTOCLASS.htm#penna

genus *Gyrophyllum*. Halipteridae is possibly synonymous with Scleroptilidae, and Funiculinidae with Kophobelemnidae.

Species of *Umbellula* have several uniting characteristics. Colonies possess a long, slender stem; the autozooids are large and clustered at their extreme upper end, rather than distributed down the colony length as in all other genera; and autozooid leaves and calyces are absent, and thus anthocodiae are non-retractile. These traits are considered highly specialised adaptations. However, molecular data revealed that this morphologically distinct genus is polyphyletic: species of *Umbellula* underwent convergent evolution from two different lineages; some of its members are primitive in relation to the majority of pennatulids analysed, whereas others evolved most recently.
The paucity of taxonomic characters together with poor, often conflicting species descriptions, and a lack of understanding concerning intraspecific variation have led to the misclassification of many *Umbellula* species. Previous authors have unjustifiably split or grouped species: there are forty-two described species assignable to this genus, of which, up until this study, nine were considered valid (Williams, 1995b). The difficulties in classifying *Umbellula* species were addressed in Chapter Three.

Umbellula is a genus with very few morphological characters of taxonomic value: presence/absence of sclerites in the autozooids and rachis, and the form/size of the sclerites when present are perhaps the only characters all previous authors agree to be of value; axis shape, whether round or quadrangular in cross-section is considered of secondary importance; colony symmetry, size and number of autozooids, length of tentacles proportional to the anthocodiae, form and distribution of siphonozooids, and colony stoutness/slenderness are traits of an ambiguous nature and were often considered to be functions of development, contraction, or state of preservation. In Chapter Three, it was demonstrated that a combination of these characters are fundamental in distinguishing between species of *Umbellula*; this was further backed by genetic analysis. Fifteen species of *Umbellula* were recognised, including three species new to science. Eight species had sclerites absent from the autozooids and rachis, viz. *U. magniflora*, *U. encrinus*, *U. antarctica*, *U. carpenteri* and *Umbellula* sp.1 n. sp. (quadrangular axes), and *U. huxleyi* and *U. pellucida* (round axes); and seven possessed sclerites, viz. *U. thomsoni* and *U. hemigymna* (quadrangular axes), and *U. monocephalus*, *U. aciculifera*, *U. durissima*, *Umbellula* sp.2 n. sp. and *Umbellula* sp.3 n. sp. (round axes).

A dichotomous key and a glossary of pennatulid terms were devised in Chapter Three, intended not only for specialists in the field of octocoral systematics but also as a guide for other biologists who share the common need to identify material from benthic surveys and other studies. Hopefully, this work will help pave the way to improving our knowledge of an important component of the deep-sea megabenthos, its biodiversity, and distribution.

Expanding on the work of chapters two and three, a biogeographical study of *Umbellula* was presented in Chapter Four. Distribution data together with genetic evidence supported the hypothesis that species of *Umbellula* originally differentiated in the Indo-Pacific. From here, they may have moved southwards to the Antarctic and later radiated north into the Atlantic, E Pacific, Indian and Arctic oceans, occupying bathyal and abyssal depths. The abyssal species, *U. monocephalus* and *Umbellula* sp.2 n. sp., are among the oldest, and evolved via a separate evolutionary pathway. These too may have originated in the Indo-Pacific, and dispersed to the Subantarctic (*Umbellula* sp.2 n. sp.) or Indian and Atlantic oceans (*U. monocephalus*). However, further biogeographic data are necessary, particularly from the E Pacific, to confirm whether radiation of older *Umbellula* species (pre Pliocene) occurred from the Antarctic to the rest of the World Ocean or whether species dispersed from the Pacific to the Atlantic via the Panama seaway.

The adaptive nature of *Umbellula* species to the deep sea was also demonstrated in Chapter Four. This specialised genus thrives over a large depth range, and the shortage of food supply away from the continental margins means that effectiveness of catching organic particles or perhaps swimming organisms is paramount. Species of *Umbellula* have adapted by reducing the number but increasing the size of their autozooids, and in doing so, increased the food-catchment area; abyssal species have done so even more extremely.

In summary, this project presents the first phylogenetic and systematic study of deep-sea pennatulids, and a reassessment of the classification of the genus *Umbellula* together with a biogeographical and morphological approach to its origins and adaptations, respectively. However, this has only scratched the surface: a great deal more work is required to advance significantly our understanding of the group, not only from a curiosity perspective, but also to conserve biodiversity in the deep sea.

In terms of future work, the following points seem worthwhile to pursue as part of further investigations:

- Studies incorporating a larger dataset, representing many more species are paramount to improve our understanding of pennatulid systematics. In an historical context, DNA sequences of representative Veretillidae and Echinoptilidae should be included in phylogenetic analyses to test whether these represent the most primitive of extant pennatulids, as suggested by Williams (1992), or to test if pennatulids, as a group, are of deep-water origin. Such findings will improve our understanding of biogeography and patterns of radiation.
- A study to identify if sister-species pairs exist from the Caribbean and E Pacific could lead to genetic analyses of these to examine rates of species divergence.
- Since there are very few characters useful in distinguishing many species it is very difficult to justify separations or grouping of species based on morphology: DNA barcoding of all known species would aid classification and systematics.
- Quantitative video surveys of pennatulid populations would provide distribution and abundance data. Patterns in these data could be correlated with environmental factors such as bottom temperatures and currents, and food fluxes, and may provide information on habitat specifications and possibly allow for habitat prediction.

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Appendix

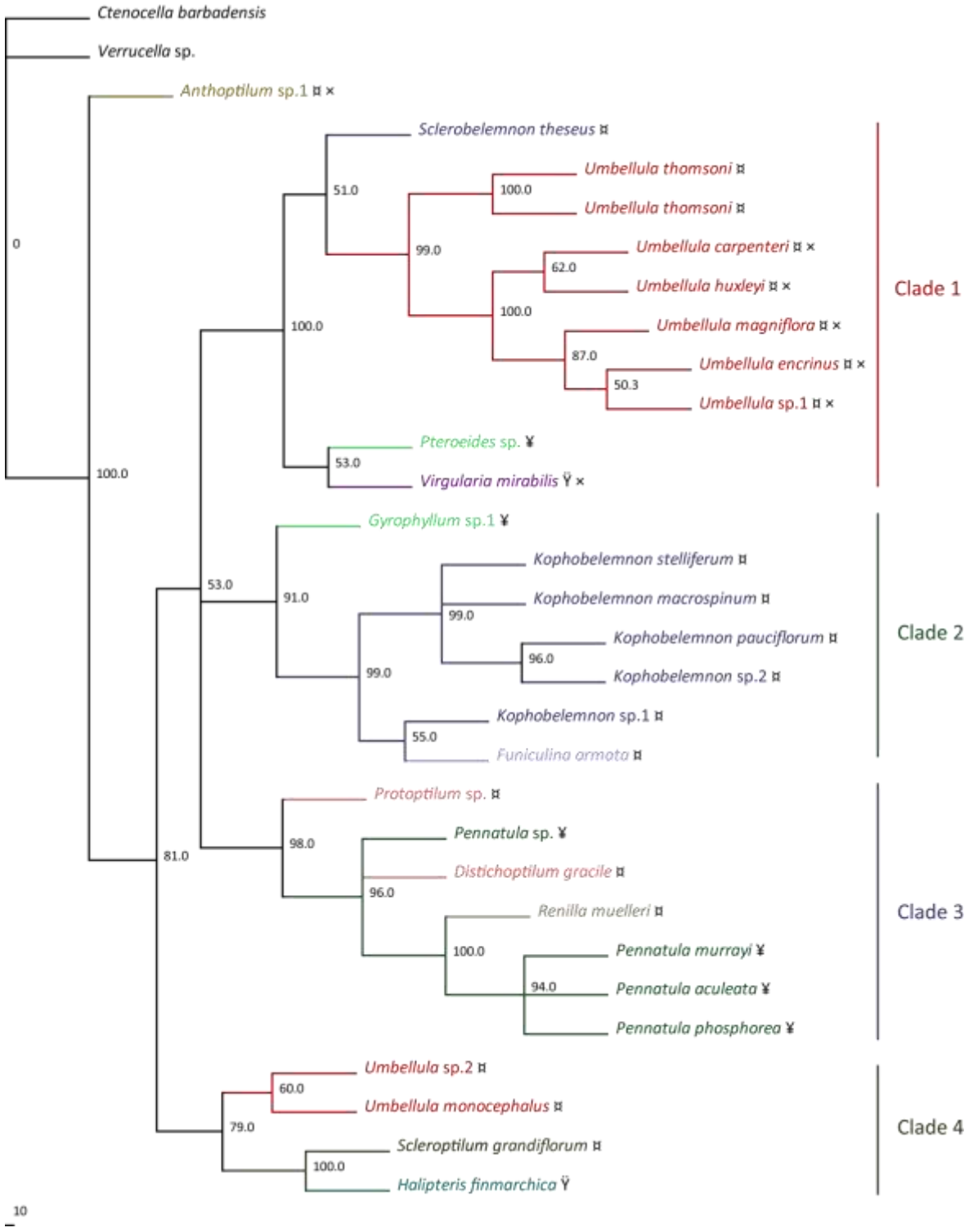

Figure A1 Phylogenetic relationships among 10 families in O. Pennatulacea for the combined analysis of *ND2* and *msh1*. Maximum likelihood tree, 50% majority-rule consensus, settings corresponded to the GTR+G+I model; values at nodes are percentages from 100 bootstrap replicates; scale bar is the number of nucleotide substitutions per site. Colours represent families; ¤ Sessiliflorae; ¥ Subselliflorae (polyp leaves); Ÿ Subselliflorae (polyp ridges); × Sclerites absent from polyps and rachis.

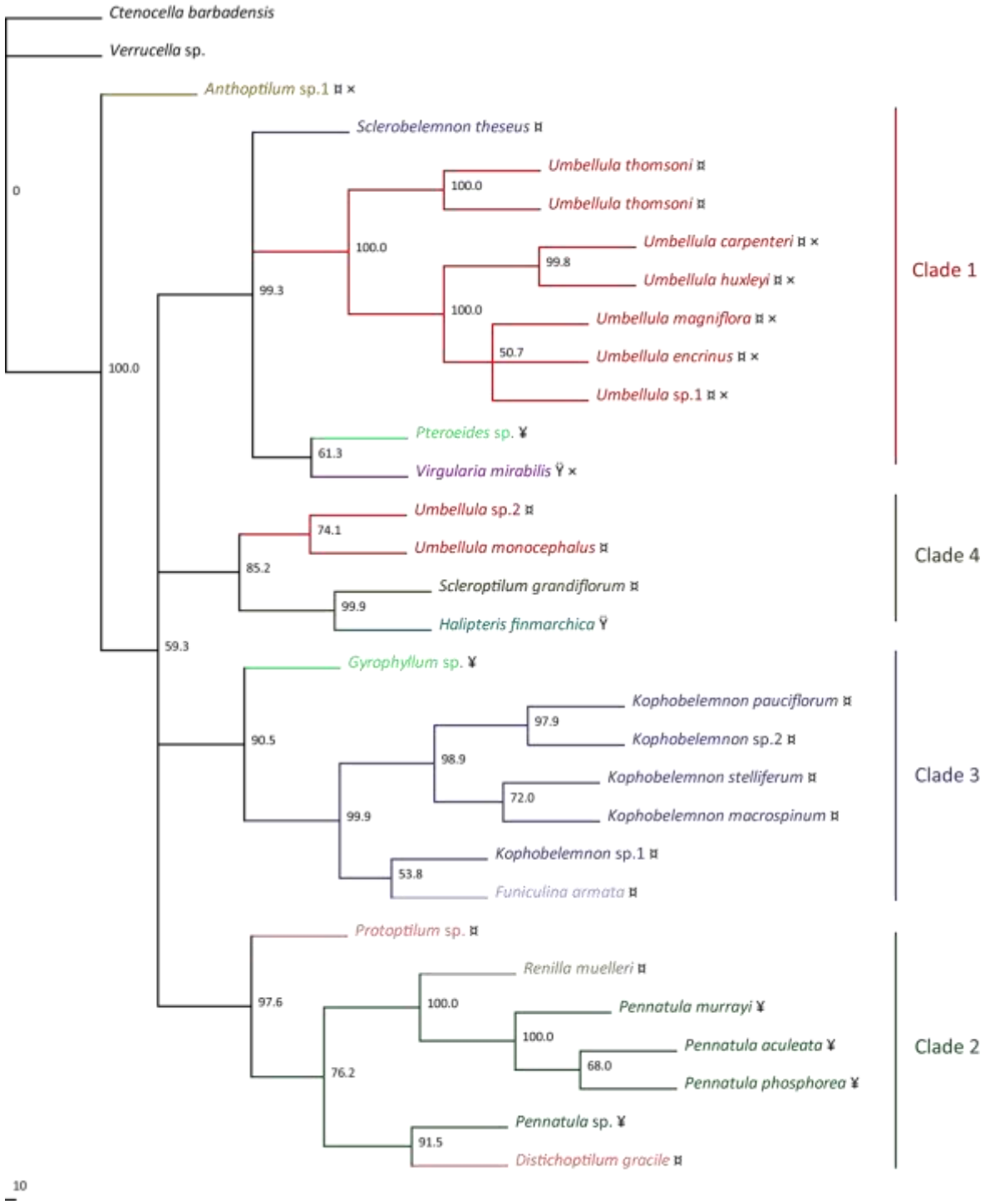

Figure A2 Phylogenetic relationships among 10 families in O. Pennatulacea for the combined analysis of *ND2* and *msh1*. Neighbour-joining tree; values at nodes are percentages from 1000 bootstrap replicates. Colours represent families; ¤ Sessiliflorae; ¥ Subsessiliflorae (polyp leaves); Ÿ Subsessiliflorae (polyp ridges); × Sclerites absent from polyps and rachis.

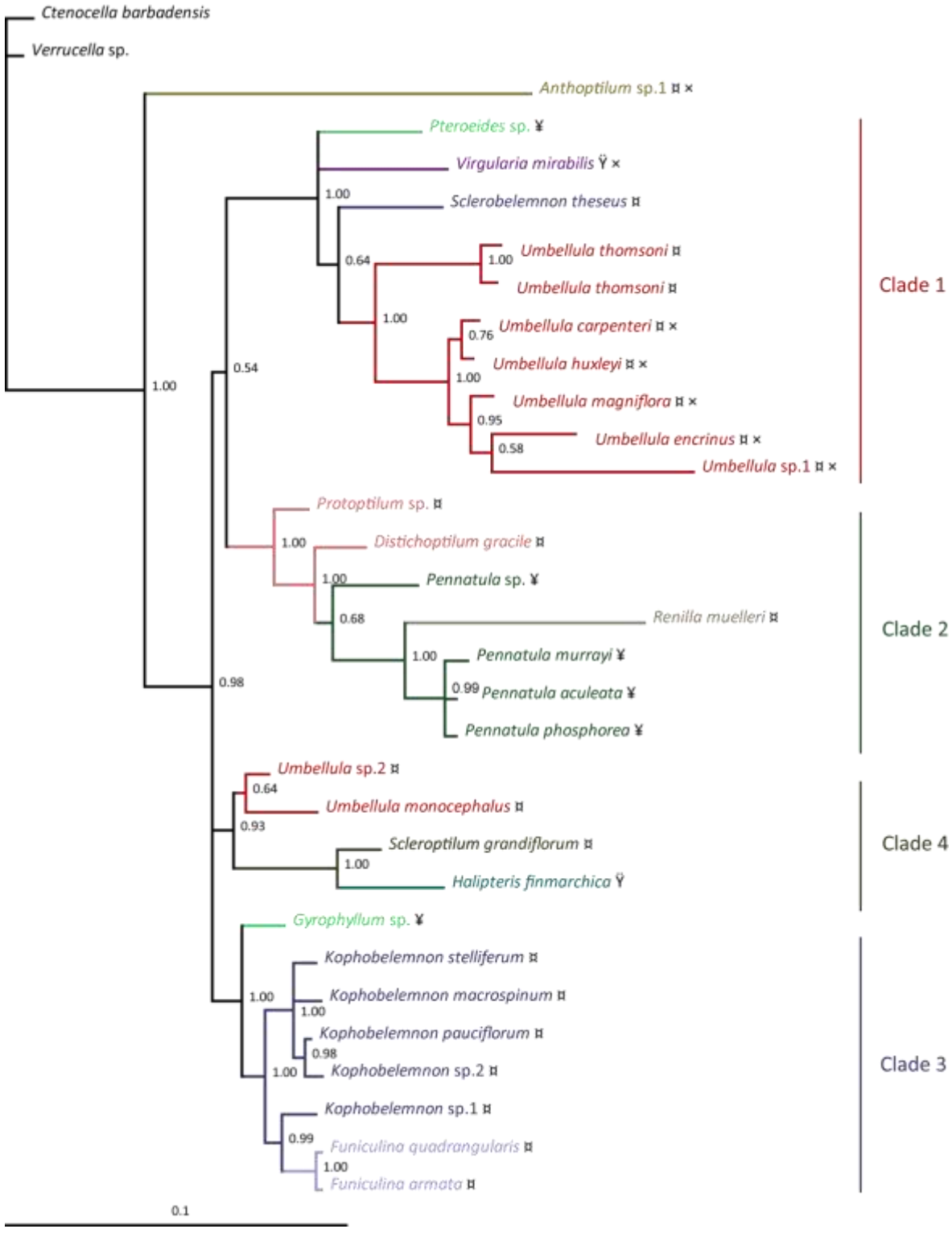

Figure A3 Phylogenetic relationships among 10 families in O. Pennatulacea for *msh1* only. Bayesian likelihood tree, 50% majority-rule consensus of 10,775 trees (10^6 generations; burnin=1000); values at nodes are posterior probabilities; scale bar is the expected changes per site. Colours represent families; ¤ Sessiliflorae; ¥ Subselliflorae (polyp leaves); Ÿ Subselliflorae (polyp ridges); × Sclerites absent from polyps and rachis.

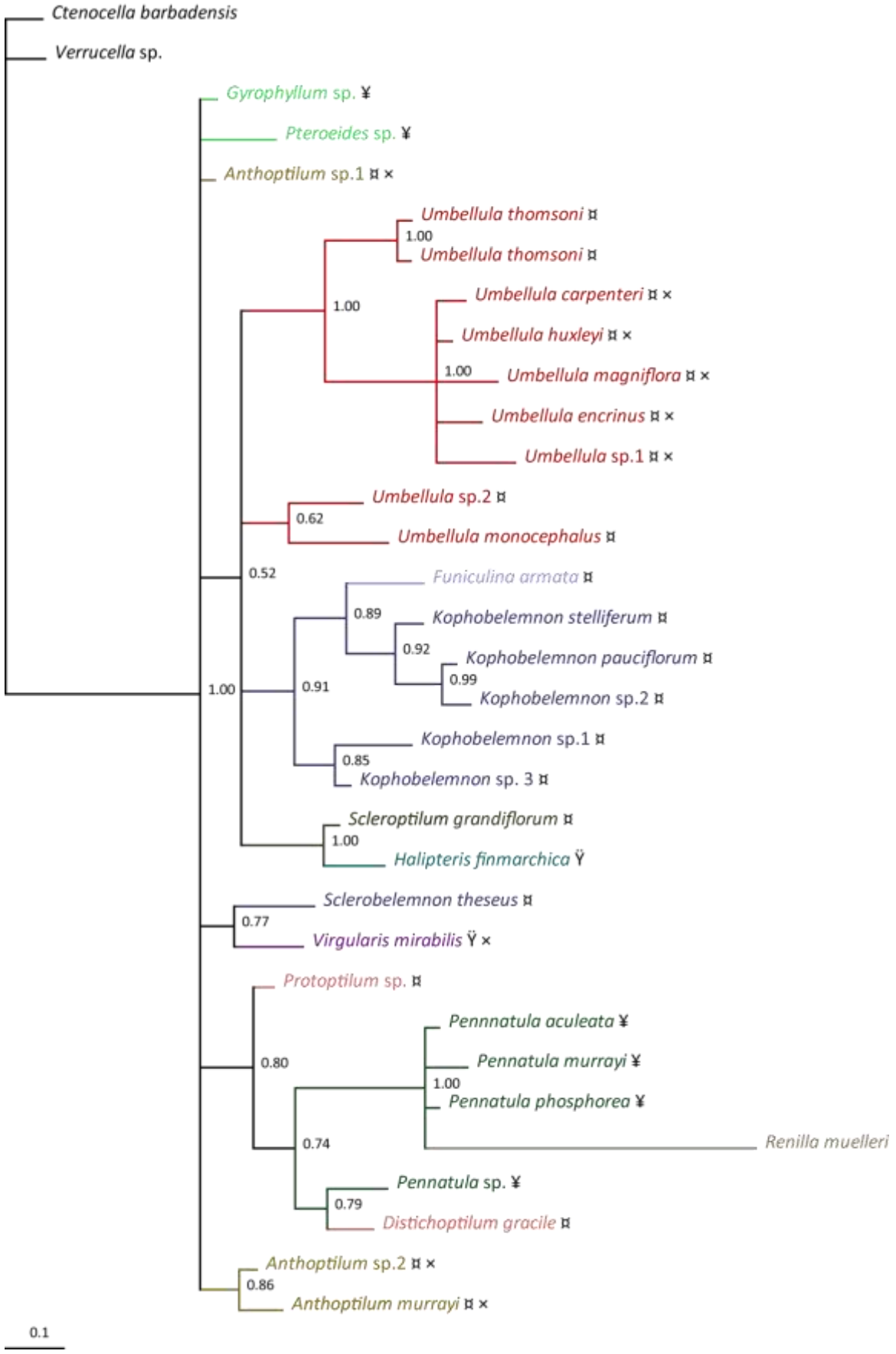

Figure A4 Phylogenetic relationships among 10 families in O. Pennatulacea for *ND2* only. Bayesian likelihood tree, 50% majority-rule consensus of 17,930 trees (10^6 generations; burnin=1000); values at nodes are posterior probabilities; scale bar is the expected changes per site. Colours represent families; ¤ Sessiliflorae; ¥ Subselliflorae (polyp leaves); Ÿ Subselliflorae (polyp ridges); × Sclerites absent from polyps and rachis.