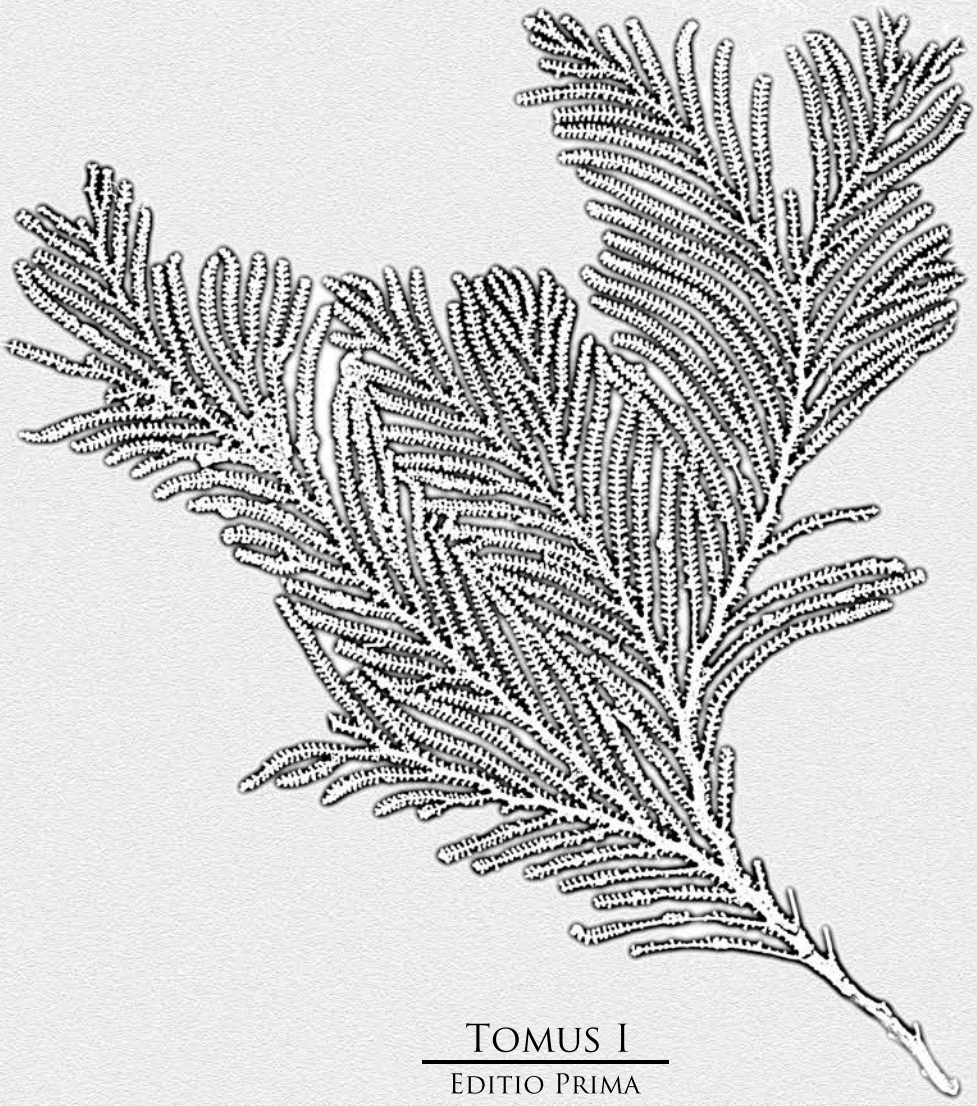
REBECA ZAPATA GUARDIOLA
FACULTAD DE BIOLOGÍA
DEPARTAMENTO DE ZOOLOGÍA

PhD THESIS

DIVERSITY AND EVOLUTION OF ANTARCTIC GORGONIANS (OCTOCORALLIA, PRIMNOIDAE), DISTRIBUTION, GROWTH AND REPRODUCTIVE PATTERNS

DIVERSIDAD Y EVOLUCIÓN DE GORGONIAS ANTÁRTICAS (OCTOCORALLIA, PRIMNOIDAE), DISTRIBUCIÓN, CRECIMIENTO Y ASPECTOS REPRODUCTIVOS



Tomus I

Editio Prima

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D. Pablo J. López González, Profesor Titular del Departamento de Zoología de la Universidad de Sevilla, y D. Josep Maria Gili i Sardà, Profesor de Investigación del Departamento de Biología Marina y Oceanografía de l'Institut de Ciències del Mar (CSIC) de Barcelona

CERTIFICAN:

Que Dña. Rebeca Zapata Guardiola, licenciada en Biología Marina por la Universidad de La Laguna, ha realizado bajo su dirección y supervisión el trabajo titulado "Diversity and Evolution of Antarctic gorgonians (Octocorallia, Primnoidae), Distribution, Growth and Reproductive Patterns", considerando que reúne todas las condiciones necesarias para ser presentado y defendido como Tesis Doctoral.

Sevilla, 20 de Diciembre de 2013.

Fdo: Pablo J. López González

Fdo: Josep Maria Gili i Sardà

El trabajo que se presenta en esta memoria ha sido realizado gracias a la concesión de una ayuda predoctoral de Formación de Personal Investigador con referencia BES-2007-17202, asociada al proyecto de investigación POL2006-06399, “CAMBIO CLIMATICO EN LA ANTARTIDA: UNA APROXIMACION DESDE EL ACOPLAMIENTO PELAGO-BENTICO A LOS EXTREMOS DEL MAR DEL WEDDELL (CLIMANT)”, concedida por el Ministerio de Educación y Ciencia en junio de 2007. La estancia en el National Museum of Natural History, Smithsonian Institution, fue posible gracias a la concesión de una ayuda complementaria para estancias breves en el extranjero durante el año 2009 del Ministerio de Educación y Ciencia. La estancia en el Institut de Ciències del Mar de Barcelona, Consejo Superior de Investigaciones Científicas (ICM-CSIC), así como la estancia en el Natural History Museum, London (NHM) fueron posibles gracias a la concesión de Ayudas Complementarias para realizar estancias breves durante los años 2010 y 2011 del Ministerio de Ciencia e Innovación.

El coneixement és verament fascinant perquè sempre queda terra nova per descobrir. Tota activitat humana en tant que humana comença amb el coneixement, i sense ell l'home i la dona no faria res del que fa.

Jordi Llimona, 1996

Cualquier mujer tiene dentro de sí, el coraje suficiente para llegar hasta el fin del mundo.

Josefina Castellví, 1996

Be very, very careful what you put into that head, because you will never, ever get it out.

Cardinal Wolsey (1471-1530)

El éxito es un 99% de fracaso.

Soichiro Honda

A la família,

Als amics,

Als companys,

A tots aquells que m'he trobat pel camí i m'han donat suport

I en especial a l'Adrià...

"Gràcies per seguir aquí després de tant de temps"

Agradecimientos

Nunca se me han dado demasiado bien las palabras y menos aún expresar mis sentimientos a los demás...y si no preguntarle a cualquiera...mi sentido del tacto es nulo. De hecho era conocida como la "*borderline*" término que sorprendentemente es utilizado en el campo de la psiquiatría para referirse al Trastorno Límite de la Personalidad, que se caracteriza principalmente por la desregulación emocional, el pensamiento extremadamente polarizado y las relaciones interpersonales caóticas...creo que no hacen falta más explicaciones...aunque sinceramente, no sé cómo he podido llegar hasta aquí!

A lo que iba...que esta ocasión bien requiere un esfuerzo por mi parte para transmitir aquello que siento, y agradecer todo cuanto tengo, todo cuanto he conseguido y todo cuanto he necesitado durante todos estos años, porque toda la gente que ha estado a mi lado merece ese reconocimiento por mi parte que aunque mal que me pese sí, soy egoísta y mucho pero siempre soy y seré muy agradecida.

Que padres hubieran imaginado lo que les esperaba cuando su hija de 11 años volviendo un día del colegio les dijo toda entusiasmada "Mamá quiero ser bióloga!". Desde aquel mismo momento fue una especie de obsesión, aunque sin saber exactamente lo que significaba el término Biología y menos aún lo de ser Bióloga, el camino a seguir ya estaba escrito y nada ni nadie podría cambiarlo.

¿Qué padres hubieran imaginado por todo lo que tendrían que aguantar? peleas, discusiones, lágrimas, incertidumbre pero también por las celebraciones y las risas, por todas y cada una de las despedidas y reencuentros, por aquellas horas esperando en el aeropuerto y por tenerme siempre en un trocito de su corazón y no olvidar que formo parte de sus vidas, por apoyarme durante más de 15 años en mi sueño...por todo esto y mucho más...GRACIAS. Y no me olvido de mis hermanos ni de l'avi! Us estimo a tots molt!! I no sabéis cuanto me duele no haber estado a vuestro lado mientras crecíais porque yo iba por media España intentando lograr mi sueño, siento haberme perdido cumpleaños, risas, confesiones de hermanas y peleas (bueno de hecho esto último no! Creo que quedé escarmentada con la última que tuvimos eh Esa?!), en fin que teniu una germana, una neta i una filla per sempre! No ho oblideu mai!

Adrià i que haig de dir de la teva família són tots un encant, m'han obert amb els braços oberts, agraeixo de tot cor la seva preocupació i ànims durant tot aquest temps, en especial a la teva mare Inge i als teus avis. Ich werde euch immer in meinem Herzen tragen. Danke schön!!

Y que hay de mis "nuevas familias"….si si…y es que en el transcurso de todo este tiempo he ganado mucho! En Estados Unidos me adoptaron como una hija más y debo agradecerles desde todo mi corazón a la familia Henry-Gallardo el calor que me dieron estando lejos de todo, de mi país, de mi idioma y de mi gente…algo impagable. I miss you all so much!!! Thanks for everything!! Y en Sevilla…quien me lo iba a decir…adoro a esos peques!! Antonio (el payaso por excelencia) y Marina (la presumida)…como me he reído yo con ellos!! Y no me olvido de la Cuca, eh!! Gracias Mamen y Luis por hacer que me sienta una vez más en un hogar de verdad.

En fin, que he estado *casi* como en casa! Hasta mi prima Cristina estaba en Sevilla! Qué cosas! Las dos primas de regreso a los orígenes…la yaya no se lo hubiera imaginado, eh? Qué fuerte! Pero me alegro muchísimo que acabaras aquí, porque como he dicho soy egoísta y que hayamos coincidido en esta época de nuestras vidas…ha sido un aire fresco para mi, de desconexión de todo lo relacionado con la Uni y mis "gorgoñas" como dice Nando. Valoro muchísimo tu amistad y espero no perderla…muchas gracias prima, a ti y a tus chicos sevillanos por cuidarme taaaaaanto y dejarme ser la *Tata Guay*!

Ahora es el turno de mis niñas…si es que…con quien me iba a reír yo si no es con ellas y de ellas?! Jajaja, que hubiera echo yo sin esos largos y gratificantes jacuzzis?? Y sin esas miradas y risas cómplices? De esas escapadas cafeterianas a desahogarnos de nuestros males indígenas…ay ay ay! Y aunque se metieran conmigo por motivos varios…ahora que pienso…se metían conmigo por cualquier cosa y a todas horas!! Que si… el coche es de gasolina? Sí, la mitad del depósito….uff! estoy llena!...dos tortelini?!...dientes de hojalata…catalanucha…Betis 3-Barça 1…pero creo… que aún las quiero!! Qué hubiera hecho sin Iri y sin Badi??!! Hmmm……

Tantas horas en el lab es lo que tiene….pasan a ser tu familia, amigos y compañeros. Me alegra haber compartido tantas horas en el 8 con el resto del grupo, Espe, Mercedes, Cesar…todos me han aportado algo durante estos años, me han enseñado a ver las cosas desde distintos puntos de vista, me han aconsejado, me han sermoneado, me han regañado, me han animado, me han apoyado y hasta me han intentado enseñar a bailar Sevillanas (aunque sin éxito)… sin ellos no hubiera sido lo mismo! Chicos…no me echéis de menos!!

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I que dir dels "nois del JM" (bé així és com us dic quan parlo sobre vosaltres…jeje), tot i que no he passat els moment super xungus de la tesi amb vosaltres (sort per vosaltres!) us haig de donar un 10! Me sentit acollida com a una més tot i ser una estranya que venia de terres llunyanes…que m'alegro haver-vos conegut a tots incloent-hi les noies (of course!), gràcies per ensenyar-me a fer el cafè (tot i que dubto que el que faig jo us agradi…jejeje) i que em poséssiu al dia de tots els cotis (fer safareig m'encanta!!!) SOU ELS MILLORS! I Bego? Que haig de dir de tu, eh?! Encara que no t'ho creguis m'has ensenyat molt! I m'has aportat moltíssim! M'encantes com a persona i espero conservar aquesta amistat plena de sinceritat, ets una

gran persona i et mereixes el millor per això sempre podràs comptar amb mi!! Forever and Ever!!!!!!!! (un pèl cursi potser?)

Y …no os agobies….que ya termino…mi último y mayor agradecimiento se lo merece alguien muy especial para mi, a estas alturas ya debes saber quién eres...y sino…malament, malament!! Me has apoyado desde el inicio, desde que mandé la solicitud ya sabias que me iba a ir a más de 1000 km de distancia…y aun con una relación recién empezada me animaste y me apoyaste en todo momento (seria que querías librarte de mi para hacer la tuya, eh???!! Bueno, mejor no pensar en eso…pá por si!!), no tengo palabras (quizás sí otras cosas…jajaja!!!) para agradecerte todo lo que has tenido que soportar más que nadie…te has comportado como un santo, la piedra angular que me ha ayudado a aguantar y sobrellevar todos estos años…sin ti y tu amor (porque en el fondo sé que estas enamorado de mi…aunque tu lo niegues!) no seria quien soy, tu calma y tu capacidad de observación y positividad me han ayudado a mejorar como persona. Sempre seré el teu BitXo. Adrià t'estimo.

Y ahora…….? Ahora QUÉ?!!!!!!!!!! No me quiero desanimar así que a ser positivos que ahora empieza una nueva etapa y como dice una canción de Aqua: Imagination, life is your creation!

GRACIAS a todos por apoyarme hasta el final.

R.

Resumen

El continente Antártico así como sus aguas circundantes se encuentran bajo protección legal desde 1959, fecha en la que se firmó el Tratado Antártico y gracias al cual hoy en día la Antártida es considerada como uno de los lugares del planeta donde la belleza natural y la pureza de los ambientes que alberga todavía se preservan. Debido a esta peculiaridad la Antártida resulta un escenario ideal dónde la fauna marina se ha diversificado y adaptado a unas condiciones peculiares, convirtiéndola en un laboratorio natural para estudiar procesos ecológicos y evolutivos en la formación de nuevas especies, cuya dinámica evolutiva puede utilizarse como referencia para establecer estándares que permitan explicar los cambios que se han producido a nivel mundial durante las últimas décadas así como extrapolar su futura dinámica.

En esta Tesis Doctoral se abordan cuestiones de carácter biológico sobre la fauna bentónica, una de las comunidades más ricas en especies del planeta. Gracias al material recolectado durante casi dos décadas en áreas principalmente del mar de Weddell y el mar de Ross, se ha podido estudiar la diversidad de gorgonias antárticas (Cnidaria: Anthozoa: Octocorallia: Primnoidae) en las regiones Antártica y Subantártica, sus patrones de distribución geográfica y batimétrica, así como sus relaciones filogenéticas además de aportar nueva información sobre los patrones reproductivos y de crecimiento .

El estudio taxonómico ha permitido, hasta el momento, la identificación y descripción de 4 géneros, 2 subgéneros y doce especies nuevas para la ciencia. Además la asignación de tres nuevas combinaciones, resultado de revisiones taxonómicas, hace que la clasificación de la familia Primnoidae sea algo más comprensible, aunque todavía es necesario encontrar un consenso unificado sobre los caracteres a utilizar en la clasificación de géneros y especies. La fauna de primnoidos en el océano Austral se ha estimado en 69 especies con un alto grado de endemismo (81%), lo que podría sugerir una baja tasa de dispersión a través del frente polar. Además la región Antártica es más diversa y más endémica que la Subantártica, en concreto la zonas correspondientes a la península Antártica, que además es la más afín con la zona correspondiente al cono sur de América del sur, lo que sugeriría la presencia de un punto caliente de biodiversidad donde gracias a la conexión entre ambas zonas hubieran existido procesos evolutivos que llevaron a un mayor nivel de especiación en la región Antártica. Los estudios batimétricos podrían sugerir que la dispersión de las especies estudiadas se hubiera producido primeramente alrededor de la región Antártica con una posterior radiación desde las zonas de la plataforma hacia el fondo lo que hubiera facilitado la dispersión a la región Subantártica. Los estudios de filogenia basados en la familia Primnoidae parecerían apoyar un origen de la fauna en el océano Austral que posteriormente habría irradiado hacia los océanos colindantes.

Para los estudios de los patrones tanto reproductivos como de crecimiento, se ha estudiado la especie *Thouarella variabilis,* donde a pesar de tener una baja tasa de fecundidad (1 larva por pólipo), los resultados podrían sugerir 2 eventos reproductivos al año, aunque datos durante la época invernal así como de la evolución de la larva hasta la formación del primer pólipo son

altamente necesarios para poder caracterizar con exactitud el ciclo reproductivo. El estudio de los anillos de crecimiento mediante distintas técnicas ha revelado bajas tasas de crecimiento y una elevada longevidad para los primnoidos cuando son comparados con especies de zonas templadas. Además se ha observado la presencia de unos anillos internos que a falta de validar la anualidad o no de los mismos, podrían datar gorgonias antárticas con más de 180 años o bien correlacionarse con variaciones intraanuales debido a fluctuaciones ambientales.

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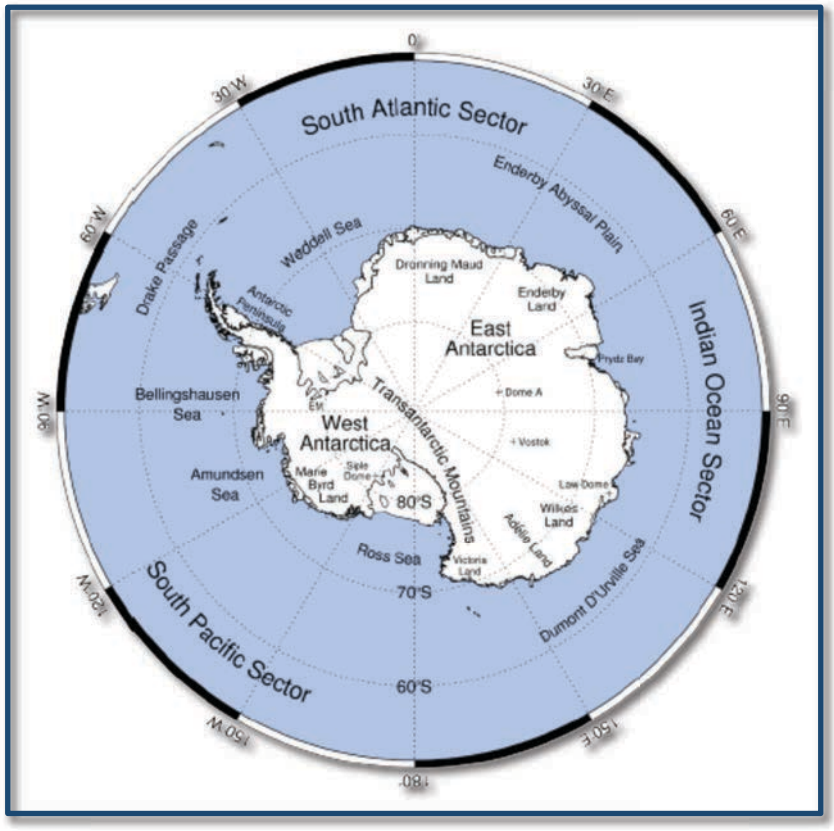
Introducción

1.1 Descripción del área de estudio

1.1.1 El océano Austral

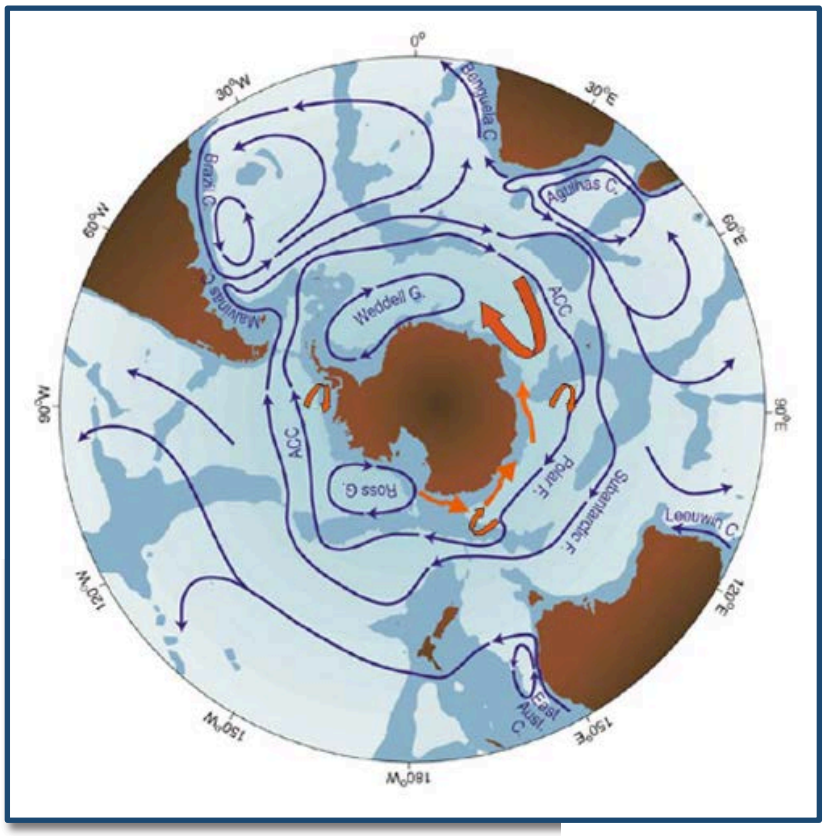
Desde el punto de vista geopolítico y definido por el Tratado Antártico (1 de Diciembre de 1959), el océano Austral se define como cualquier masa de agua ubicada al sur de los 60º de latitud sur (Map 1.1). Sin embargo, esta definición no tiene en cuenta las necesidades específicas antárticas desde el punto de vista oceanográfico, topográfico o biológico además de no incluir las regiones Subantárticas.



Map 1.1.- Mapa de la Antártida mostrando algunas localidades incluidas dentro de los 60ºS (Turner *et al.* 2009).

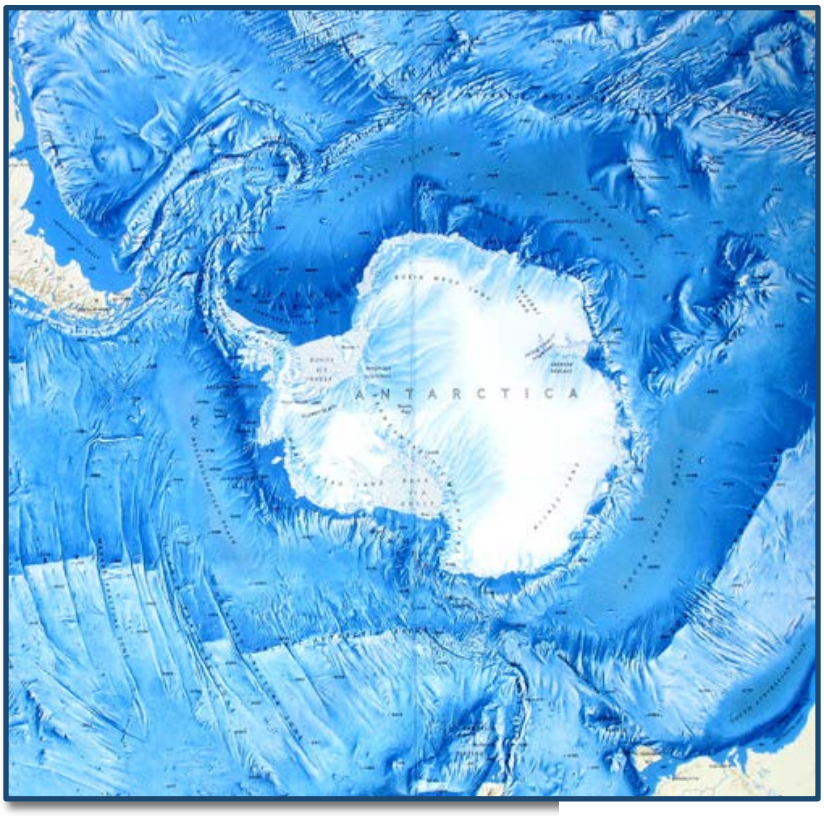
Desde el punto de vista oceanográfico, el océano Austral está limitado al norte por la mayoría de los frentes y corrientes, a lo que se le llama la *Convergencia Antártica*, y al sur por el continente antártico (Map 1.2). En la frontera norte se forma la *Corriente Circumpolar Antártica* (ACC, por sus siglas en inglés), que limita al norte con el *Frente Subantártico* (SF, por sus siglas en inglés) y al sur por el *Frente Polar* (PF).

La ACC forma una barrera permeable con poco intercambio de calor entre las cálidas aguas del norte y las aguas frías del océano Austral. Por lo tanto, existe un gradiente de temperatura, salinidad y densidad muy fuerte en ambos lados de la ACC.



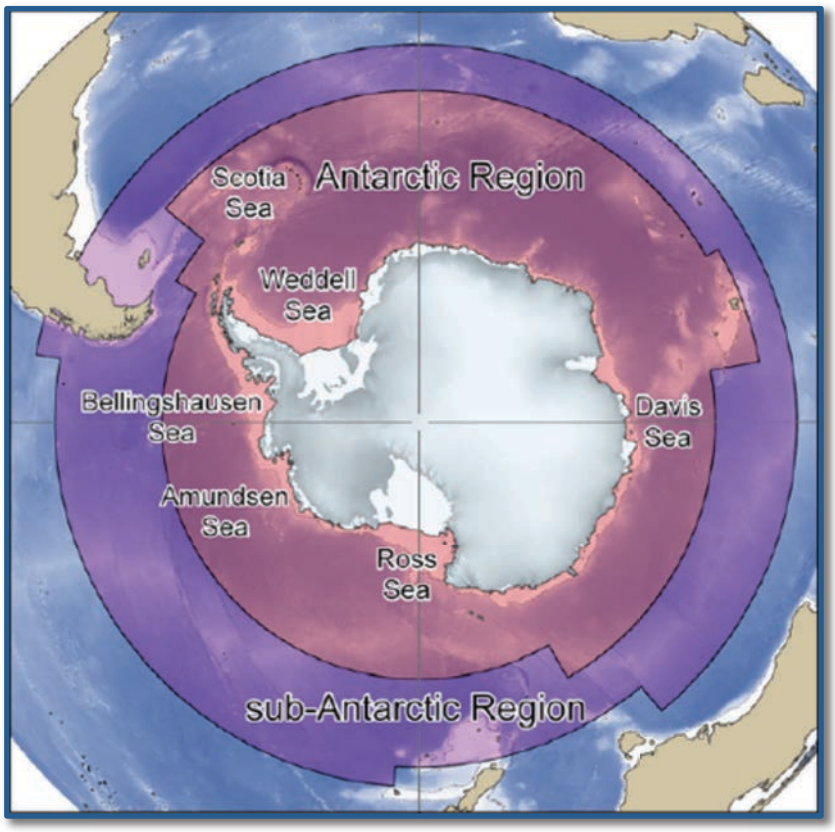
Map 1.2.- Mapa esquemático de las principales corrientes al sur de los 20º. **F**, frente; **C**, corriente, **G**, giro (Rintoul *et al.* 2001).

Desde el punto de vista topográfico, el océano Austral consiste en la plataforma continental Antártica, el talud asociado a las llanuras abisales circundantes a las dorsales oceánicas Atlántico-Indica, Indica sudoriental y Pacífico-Antártica, además de las islas Antárticas del Arco de Scotia y la meseta de Kerguelen (Map 1.3). El área total comprende cerca de 34.8 millones de kilómetros cuadrados, de los cuales hasta 21 pueden estar cubiertos por hielo durante el invierno y unos 7 durante el verano austral (Aronson *et al.* 2007). La plataforma continental en la Antártida alcanza una profundad promedio de 500 m (en el resto de océanos suele ser de 100-200 m), aunque se encuentran zonas que llegan a alcanzar los 1000 m o más de profundidad, debido supuestamente al peso de la masa de hielo sobre el continente (Clarke & Johnston 2003). Por esta razón, de manera convencional, se ha señalado la isobata de 1000 m para designar el borde de la plataforma continental Antártica e inicio del talud (Clarke & Johnston 2003), mientras que la de 3000 m marcaría el fin del talud y el inicio de las llanuras abisales. Además, existen zonas que pueden llegar a alcanzar más de 7200 m de profundidad como ocurre en la fosa de las islas Sandwich del Sur.



Map 1.3.- Mapa topográfico del fondo del océano Austral (www.mappery.com)

Por último, cuando se hace referencia a la biogeografía de las especies en el océano Austral y la biorregionalización del océano se destacan principalmente dos regiones con diferentes características biológicas: la región Antártica y la región Subantártica (Hedgpeth 1969, Griffiths *et al.* 2009). La región Antártica se extiende desde el continente antártico hasta el PF, incluyendo las islas del Arco de Scotia, Georgia del Sur, isla Bouvet y la meseta de Kerguelen (Map 1.4). La región Subantártica limita al sur con el PF, y al norte con el frente subtropical, excediendo el norte del SF, incluyendo de esta forma el sur de la Patagonia, las islas Malvinas y las aguas al sur de Tasmania y Nueva Zelanda (Map 1.4).

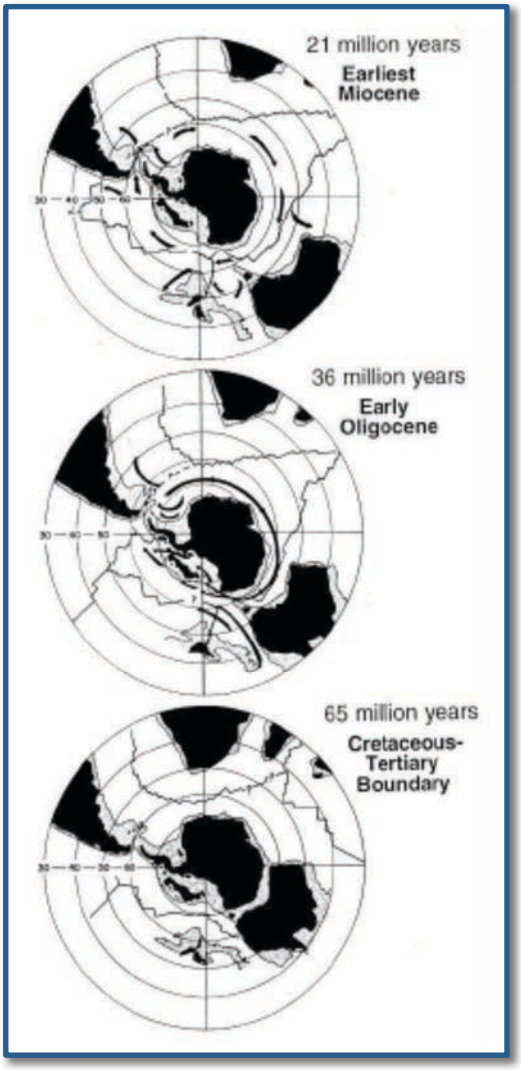


Map 1.4.- Mapa donde se muestran las dos regiones del océano Austral, la región Antártica y la región Subantártica (Griffiths 2010).

1.1.2 El ecosistema antártico

El aislamiento de la Antártida y del océano Austral del resto de los océanos del mundo ocurrió en varias etapas durante millones de años. Los inicios de la fragmentación del supercontinente Gondwana datan de hace 165 millones de años (Ma), pero el primer paso importante en el aislamiento de la Antártida fue la separación del bloque [Antártida - Australia] y del bloque [India - Madagascar] hace unos 100 Ma, seguido de la separación del bloque [Antártida - Australia] y Nueva Zelanda que se produjo entre 95 y 75 Ma. Finalmente, la apertura de la vía marítima de Tasmania ocurrió hace 35 Ma (Map 1.5), y separó finalmente la Antártida de Australia (Brandt *et al.* 2007, Rogers 2007).

El total aislamiento de la Antártida se considera efectivo sólo a partir de la apertura del pasaje de Drake, que separa la península Antártica del extremo sur de la Patagonia y Tierra del Fuego. La fecha de este evento es muy discutida, entre 40 y 15Ma. Algunos afirman que la apertura completa del pasaje de Drake ya era efectiva antes del establecimiento de la vía marítima de Tasmania (Brandt *et al.* 2007). Otros autores sustentan que la formación del pasaje fue un proceso que duró entre los 29 y 15 Ma (Map 1.6), aunque se cree que un flujo de proto-ACC ya estaba presente desde la apertura de la región de Tasmania, coincidiendo con el inicio de la glaciación Antártica del periodo terciario (Katz *et al.* 2011).

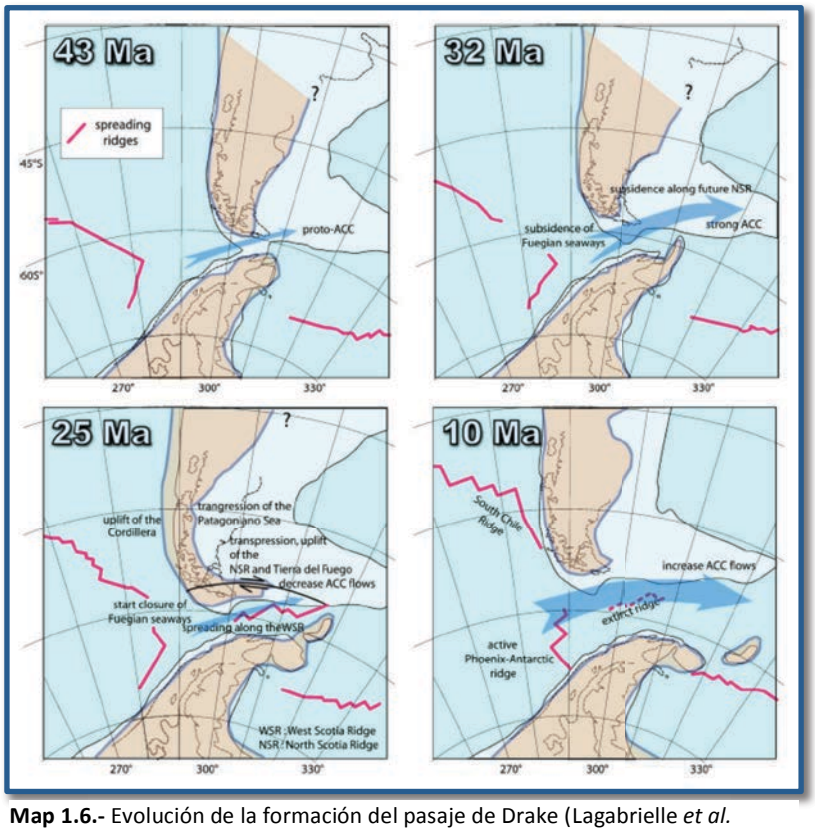


Map 1.5.- Mapas mostrando la progresiva separación de la Antártida y otros continentes del hemisferio sur. Se muestran las aperturas oceánicas que llevaran a la formación de la ACC (Turner *et al.* 2009).

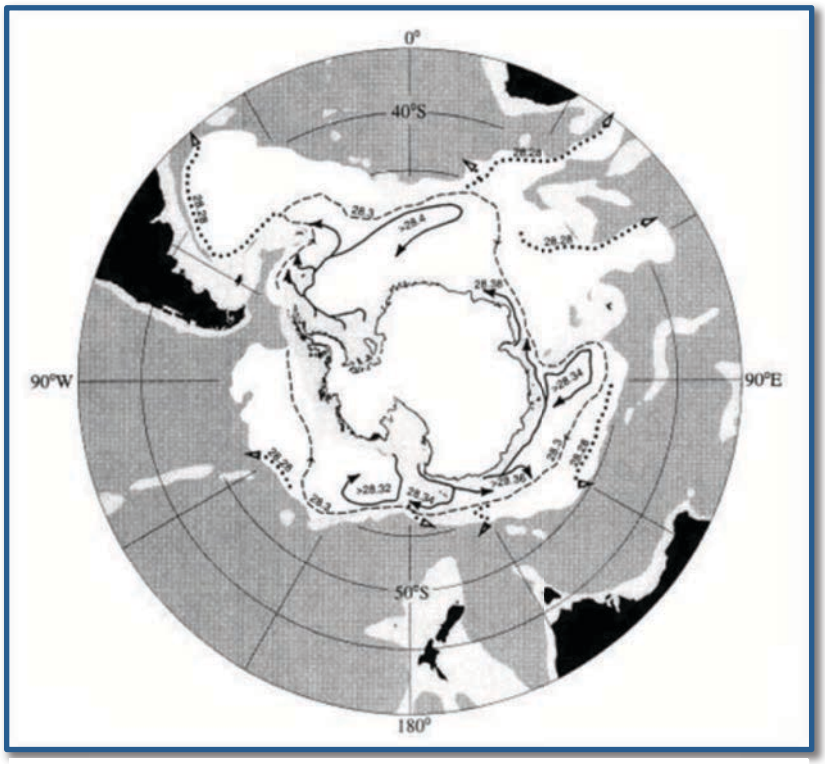
Sin embargo, la Antártida no está compuesta por un único bloque continental. Se cree que anterior a la glaciación Antártica, durante el cenozoico temprano (~60 Ma), existió una comunicación marítima entre los proto-mares de Ross y Weddell a través del pasaje situado entre los bloques oriental y occidental de la Antártida. Este pasaje trans-Antártico se mantendría abierto hasta el Oligoceno, e incluso hasta el Mioceno medio (20 Ma). Desde entonces, al menos dos eventos de reapertura de este pasaje han sido constatados mediante el registro geológico: uno durante el calentamiento del Plioceno (4 Ma) y el otro durante los últimos 1,1 Ma (Barnes & Hillenbrand 2010).

En la configuración actual de continentes y océanos, el océano Austral desempeña un papel clave en la hidrodinámica global, contribuyendo de forma significativa al ingreso de agua fría y rica en oxígeno en la circulación termohalina global. La ACC es la única corriente que da completamente la vuelta al mundo. Con una longitud de unos 20.000 km, la ACC atraviesa los océanos Atlántico, Índico y Pacífico, actuando además como una vía de intercambio de temperatura y salinidad de un océano a otro (Turner *et al.* 2009). La corriente circumpolar Antártica es la corriente de superficie dominante en esta región y está influenciada principalmente por los vientos Antárticos que soplan de oeste a este.

El hundimiento del agua que se extiende alrededor de la Antártida más allá de la plataforma continental y del talud para formar el *agua antártica de fondo* (AABW, por sus siglas en inglés), oxigena la mayor parte de los fondos oceánicos a nivel global (Orsi *et al.* 1999), y proporciona un ambiente frío bastante uniforme que permite la supervivencia de los organismos bentónicos. El océano Austral tiene tres áreas principales de formación del AABW: el mar de Weddell, el mar de Dumont d'Urville y el mar de Ross (Map 1.7). Estudios realizados sobre pulpos de profundidad (Strugnell *et al.* 2008) proponen que durante la formación de la circulación termohalina global (gracias a la exportación del AABW) se originó la radiación evolutiva de la fauna profunda desde la Antártida hacia el resto de los océanos.



Map 1.6.- Evolución de la formación del pasaje de Drake (Lagabrielle *et al.* 2009).



Map 1.7.- Presentación esquemática de las áreas de formación del Agua Antártica de Fondo (AABW) (Orsi *et al.* 1999).

1.1.3 Características ambientales y biológicas del océano Austral

Al igual que en la mayoría de los océanos, la productividad primaria en el océano Austral es principalmente una función dependiente de la luminosidad. Pero en estas latitudes, la productividad primaria es muy baja durante el invierno austral y máxima en primavera, alcanzando valores de hasta 0,1 mg Chl/l (Turner *et al.* 2009). Esto es debido a la marcada variación estacional de la luz solar, aunque la temperatura del agua se mantiene bastante constante a lo largo del año.

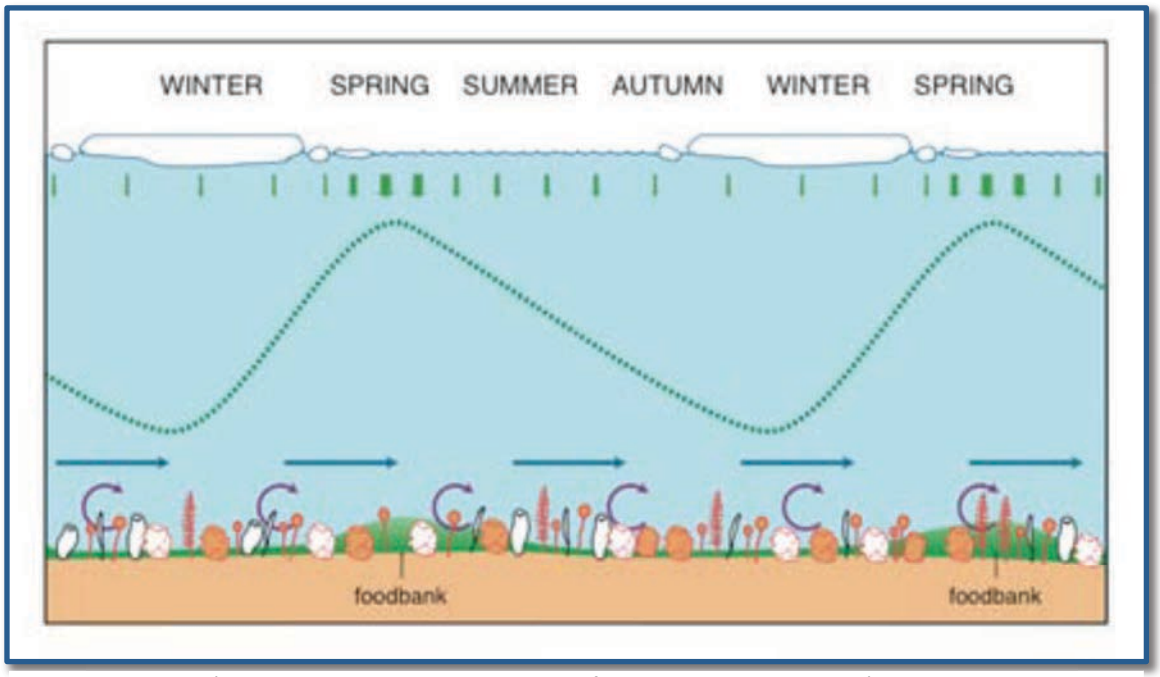


Figure 1.1.- Vista sinóptica de los procesos estacionales del flujo vertical de materia orgánica que se inicia en primavera (línea verde discontinua), las flechas indican los procesos de transporte lateral y resuspensión (Turner *et al.* 2009).

A pesar de esta dinámica en la columna de agua, la biomasa de organismos bentónicos es muy importante en la plataforma continental antártica durante todo el año. A principios de la primavera, el fitoplancton procedente del deshielo no es consumido inmediatamente por el zooplancton en aquellas zonas donde las corrientes son más débiles. Este fitoplancton sedimenta en el fondo de la plataforma continental y se acumula en los llamados "bancos de alimentos" o "alfombras verdes", creando así una fuente potencial de alimento para los organismos bentónicos (Gutt *et al.* 1998, Turner *et al.* 2009). Las corrientes actúan en la resuspensión permanente de la materia orgánica de alta calidad nutricional, creando una fuente de alimento para los organismos bentónicos suspensívoros (Smith *et al.* 2006). Estos suspensívoros antárticos son además capaces de alimentarse de partículas de pequeño tamaño, en contraste con los suspensívoros de otras latitudes que únicamente se alimentan de zooplancton (Orejas *et al.* 2003).

Gracias a la resuspensión debida a las corrientes de marea y a la calidad nutricional de los sedimentos, existen unas condiciones tróficas bentónicas prácticamente constantes a lo largo del año. Estos sucesos proporcionan la base para un nuevo modelo que ayuda a explicar la elevada biomasa de las comunidades bentónicas alrededor de la Antártida incluso cuando la

entrada de alimentos a la zona eufótica es escasa debido a que la superficie del océano se encuentra cubierta por hielo durante los meses de invierno. Durante el invierno austral, los procesos de alimentación que ocurren en el fondo marino, pueden ser la clave para comprender la alta productividad del sistema al principio de la primavera y de la elevada biomasa del ecosistema bentónico (Gili *et al.* 2006).

De esta forma, la materia orgánica estaría disponible todo el año para el bentos antártico de la plataforma continental (Fig. 1.1). El flujo de la materia orgánica generada principalmente al inicio de la primavera se observa que es mayor durante la primavera y posteriormente disminuye a lo largo del verano y otoño para ser mínima en invierno, cuando la mayor parte del océano Austral está cubierto de hielo. La materia orgánica no consumida en primavera, se acumulará en los "bancos de alimentos" y será resuspendida durante todo el año por las corrientes de marea (Turner *et al.* 2009).

1.1.4 Biodiversidad antártica: Origen de la fauna Antártica

Con la apertura del pasaje de Drake se completó la separación de la Antártida y América del Sur. Este hecho permitió la formación de la ACC y el establecimiento del PF, actuando de manera eficaz como barreras (o filtros) para la migración de la fauna en ambas direcciones.

A diferencia de otros grandes ecosistemas marinos, las aguas de la plataforma continental alrededor de la Antártida se asemejan a una cuenca cerrada, aislada de otras áreas de la plataforma del hemisferio sur por la distancia, los patrones actuales de corrientes y las temperaturas del agua. El océano Austral se caracteriza por su relativa constancia, que no estabilidad, de sus condiciones físicas (Arntz *et al.* 1994). Encontramos temperaturas bajas pero estables, las fluctuaciones de salinidad son bajas y el aporte terrígeno es escaso. Sin embargo, ciertas condiciones como el régimen de luz, la cobertura de hielo marino, el efecto de los icebergs sobre los fondos, las plataformas de hielo y la variación de las corrientes y de los patrones de circulación, fluctúan intensamente. Y, aunque el ecosistema del bentos antártico (al igual que el de los mares profundos) comparado con otros ecosistemas bentónicos marinos tiene condiciones físicas constantes destacables, está expuesto a más variabilidad física y perturbaciones de lo que se había pensado (Arntz *et al.* 1994). Estas condiciones de aislamiento se han desarrollado durante los últimos 40 millones de años, en los que la fauna marina se ha adaptado a un hábitat nuevo, y donde sus áreas de distribución se han visto muy limitadas. Las tasas de endemismo llegan a alcanzar el 97% en algunos grupos marinos. Sin embargo, a pesar de los avances que se están consiguiendo gracias a una mejor toma de muestras junto a nuevos enfoques en filogenia molecular y filogeografía, el origen de la fauna actual del océano Austral sigue siendo un tema controvertido.

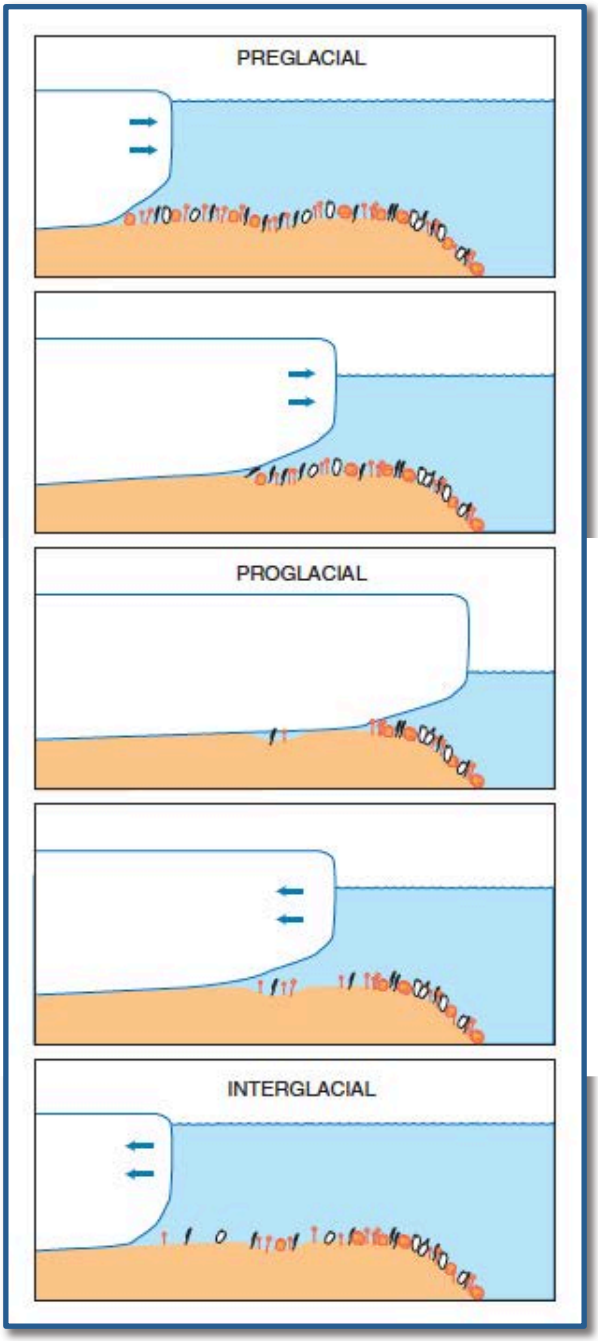


Figure 2.2.- Diagrama del desplazamiento de la capa de hielo durante los periodos glaciales e interglaciares y sus efectos sobre los organismos de la plataforma continental (Gili *et al.* 2006).

Actualmente existen dos teorías que tratan de explicar el origen de la fauna antártica, la sumersión y la emergencia evolutiva polar (Aronson *et al*. 2007). En la primera, algunos taxones de aguas poco profundas se habrían desplazado a zonas más profundas mientras que en la segunda serian los taxones de aguas profundas y abisales los que habrían colonizado la plataforma continental Antártica (Brandt e*t al*. 2007). Ambas teorías no son mutuamente excluyentes ya que desde la creación del océano Austral, la dispersión y diversificación de la fauna antártica ha evolucionado en un sentido (sumersión) u otro (emersión) dependiendo del grado de aislamiento que ha tenido el océano Antártico.

Estos patrones están claramente ligados a la historia glacial de la Antártida. Brey *et al.* (1996) demostraron que muchos taxones de plataforma en la Antártida tienen rangos batimétricos más extensos que los taxones comparables de las plataformas continentales de otros lugares. Esto sugiere que el movimiento de entrada y salida de aguas más profundas, impulsado por los ciclos glaciales, puede representar una historia general evolutiva para la fauna. Durante la glaciación, la capa de hielo de la plataforma continental se vio aumentada, erosionando el fondo, y en algunos casos erradicando los ecosistemas bentónicos. La fauna antártica ha experimentado una sucesión de periodos glaciares e interglaciares, donde varios autores se han preguntado cómo esta fauna ha persistido en estos ambientes. En este escenario, habría existido una fauna asociada a la plataforma continental, y

otra asociada a los taludes (Gili *et al.* 2006).

El avance de la plataforma de hielo durante la glaciación llevaría a la extinción de la fauna de plataforma, y durante el período interglaciar (deshielo) las plataformas ya libres de hielo (y parcial o totalmente desprovistos de vida) serian recolonizadas por la fauna asociada a los taludes (Fig. 1.2). La distribución euribática de estas especies sería una respuesta adaptativa a los ciclos de glaciaciones a los que estuvieron sometidos.

En resumen, los intercambios faunísticos entre la plataforma y las aguas profundas de la Antártida se debieron en gran medida a la naturaleza generalmente profunda de las plataformas, y por la ausencia de un fuerte gradiente térmico entre las zonas profundas y poco profundas en el océano Austral.

1.1.5 Características del bentos antártico

A finales del siglo XIX se llevó a cabo la *Challenger Expedition* (1872-1876), la primera expedición con fines científicos que exploraría el fondo oceánico y traería nuevos conocimientos sobre la oceanografía descriptiva y la distribución de organismos en el bentos marino. En 1882 se celebró el Primer Año Polar Internacional con la participación de más de una docena de países, gracias al cual se promovió la investigación polar y durante los años posteriores grandes expediciones se llevaron a cabo como la *Deutschen Tiefsee Expedition* (1898-1899), la *Deutschen Südpolar Expedition* (1901-1903), la *Swedish Antarctic Expedition* (1901-1903), la *Discovery Expedition* (1901-1904), la *Seconde Expeditione Antarctique Française* (1908-1910), o la *Australasian Antarctic Expedition* (1911-1914) que contribuyeron de manera excepcional al conocimiento de la historia natural Antártica.

Las condiciones polares a la que los organismos que ahí se encuentran están sometidos hacen que muestren una alta adaptación fisiológica a esas condiciones particulares, lo que les puede llevar a utilizar determinadas estrategias reproductivas (Clarke 1992) que les serian más favorables o bien a la ausencia de determinados grupos como los decápodos, que no habrían sido capaces de adaptarse (Aronson & Blake 2001). En la actualidad, la Antártida alberga una biodiversidad excepcionalmente rica, abundante y altamente endémica (Kükenthal 1924, Arntz *et al.* 1994, Clarke & Johnston 2003, Brandt *et al.* 2007), especialmente en zonas de la plataforma continental y las plataformas en torno a las islas Subantárticas, consideradas como uno de los "puntos calientes" de biodiversidad a nivel mundial.

Existen tres masas de tierra que se extienden en el océano austral hacia el continente antártico, y cuyas plataformas continentales asociadas representan un posible camino de colonización o dispersión para los organismos hacia y/o desde el continente antártico. Desde América del Sur, el arco de Scotia, se curva mediante sus islas hasta la extensión más al norte de la Antártida, la península Antártica. Nueva Zelanda y Australia proporcionan un camino más fragmentado a través del Archipiélago indo-malayo. Aunque Sudáfrica se encuentra a una gran distancia de la Antártida, proporciona un contacto de aguas superficiales hacia el norte con el océano Índico en su vertiente oriental y con el Atlántico en el occidental (Dell 1972). Sin embargo, todos los grupos zoológicos no están representados por igual y muchas de las especies del océano Austral están aún lejos de ser descubiertas y formalmente descritas.

Una de las características en la fauna bentónica del Océano Austral, es la ausencia de grandes depredadores (tiburones, crustáceos decápodos de los grupos Brachyura y Anomura) o por su baja diversidad (peces teleósteos, rayas) en la alta región antártica (Aronson & Blake 2001, Thatje *et al.* 2005a, Aronson *et al.* 2007). Estos depredadores han desaparecido o visto reducida su diversidad en la zona de la plataforma continental antártica como consecuencia de los procesos de glaciación al no tener la capacidad fisiológica para soportar las bajas temperaturas presentes. La ausencia de estos grandes depredadores originó el incremento en la abundancia de otros grupos, como estrellas de mar y crinoideos en ciertas áreas de la plataforma continental antártica (Aronson & Blake 2001). El bentos, que representa el 88% de las especies animales conocidas en el océano Austral, se compone principalmente de invertebrados bentónicos, donde dominan los suspensívoros (Arntz *et al.* 1994, Gutt *et al.* 2004, Gili *et al.* 2006). Los principales grupos de suspensívoros presentes son las esponjas, tunicados, briozoos, cnidarios y equinodermos (Fig. 1.3). Muchos de estos organismos actúan además como soporte de organismos epibiontes (Gutt 2000). La depredación en la Antártida se debe principalmente al resultado de depredadores poco móviles, como serian las anémonas, estrellas de mar, gasterópodos, isópodos, nemertinos y picnogónidos (Aronson & Blake 2001).

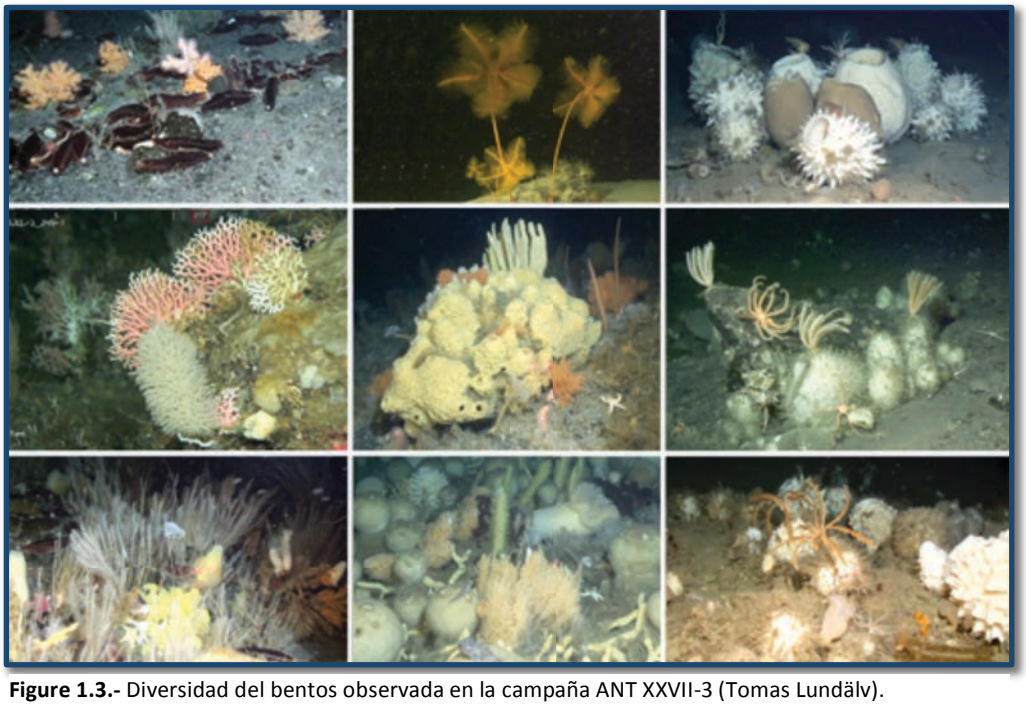


Figure 1.3.- Diversidad del bentos observada en la campaña ANT XXVII-3 (Tomas Lundälv).

Sin embargo, en los últimos años, se ha citado la aparición de grandes depredadores en la Antártida especialmente de decápodos anomuros. Éstos han sido localizados en los alrededores de la isla Bouvet, en las laderas de la plataforma continental de la península Antártica y en el mar de Bellingshausen (Thatje *et al.* 2005b, 2008). Estos decápodos llegan desde las aguas subantárticas a través de las llanuras abisales circundantes, y a través de las islas a lo largo del Arco de Scotia (Thatje *et al.* 2005b). Algunos ejemplares de una especie subantártica de centolla (crustáceo braquiuro) fueron identificados a finales de 1980 cerca de la isla Rey Jorge (islas Shetland del Sur), probablemente transportada en el agua de lastre de

algún buque (Barnes *et al.* 2006). El éxito de estas invasiones se ha supuesto que se debe al calentamiento de las aguas en la Antártida occidental (Barnes *et al.* 2006, Aronson *et al.* 2007, Thatje *et al.* 2008). Si estos fenómenos persisten, el bentos antártico de la plataforma continental podría sufrir las consecuencias de estos grandes depredadores, y en primera instancia aquellos grupos con escasos depredadores como estrellas de mar y crinoideos.

A pesar del aumento en las campañas oceanográficas desde finales del siglo XX, la diversidad del bentos del océano Austral está aún lejos de ser plenamente conocida (Brandt *et al.* 2007, Rogers 2007, De Broyer *et al.* 2011). Algunas regiones antárticas de la plataforma continental han sido poco exploradas, por ejemplo, entre el mar de Weddell y la bahía de Prydz o entre los mares de Davis y Dumont d'Urville. Además, el conocimiento que tenemos sobre la fauna que habita las cuencas profundas que rodean las áreas de la plataforma continental antártica es aún muy pobre. Sólo las campañas ANDEEP (Antarctic benthic deep-sea biodiversity) se han dedicado a estos ambientes en el sector atlántico del océano Austral (Brandt *et al.* 2007).

Desde finales del siglo XX, muchos investigadores interesados en la diversidad del bentos del océano Austral se sorprendieron al descubrir que algunos grupos tenían una diversidad oculta, revelada gracias a estudios moleculares. Algunos ejemplos con complejos de especies crípticas son los moluscos (Linse *et al.* 2007, Allcock *et al.* 2011), los artrópodos (Havermans *et al.* 2011), los equinodermos (Ward *et al.* 2008), los anélidos (Schüller 2011) y los nemertinos (Thornhill *et al.* 2008). El uso de herramientas moleculares en la estandarización de las comparaciones "barcoding" y la filogeografía muestran ser valiosas fuentes de información que a menudo revelan esta diversidad oculta. Sin embargo, otros estudios han puesto de manifiesto una cierta homogeneidad genética molecular dentro del bentos antártico, revelando una gran capacidad de dispersión e intercambio genético entre poblaciones alejadas (Raupach *et al.* 2010, Arango *et al.* 2011).

1.2 La protección en el océano Austral

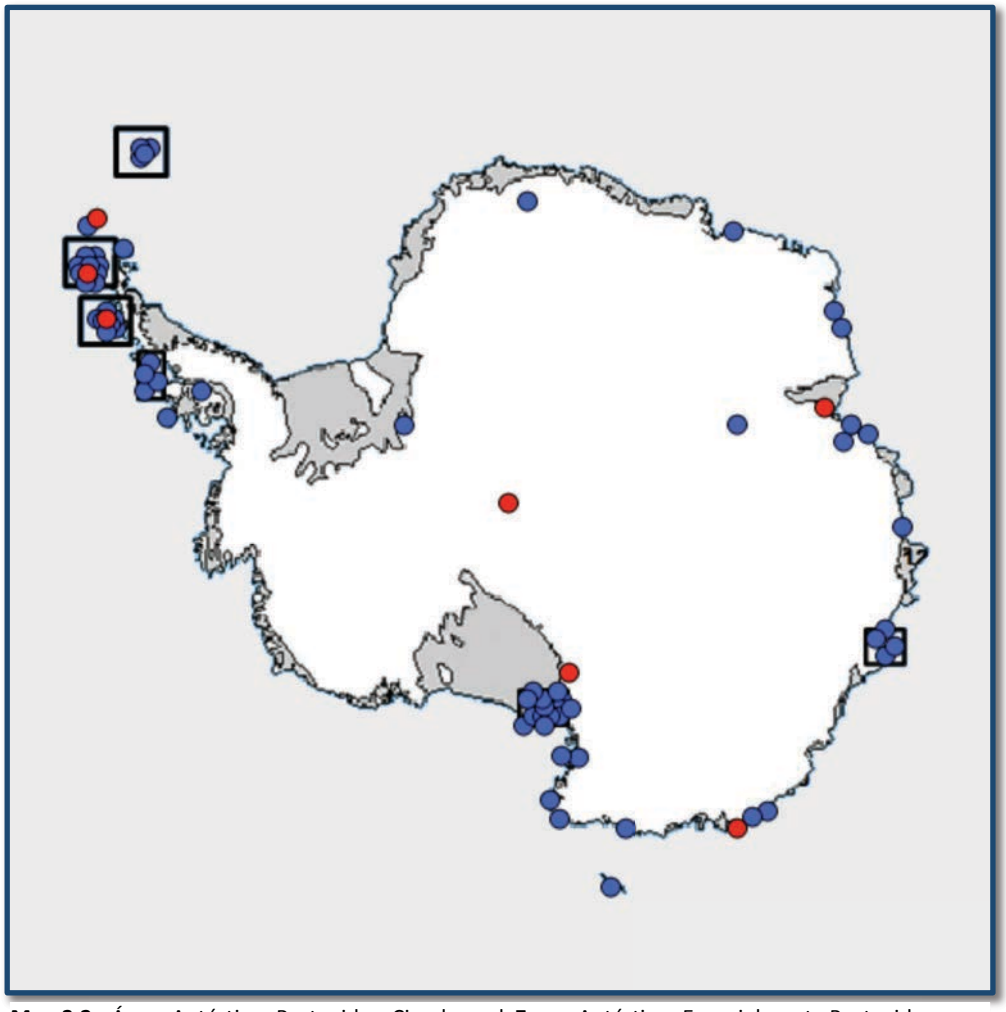
La protección del continente Antártico y del océano Austral tiene el apoyo internacional definitivo gracias a la firma del Tratado Antártico del 1 de diciembre de 1959. Su principal propósito es asegurar en interés de la humanidad que la Antártida debe continuar siendo utilizada exclusivamente para fines pacíficos y no convertirse en escenario u objeto de disputas internacionales.

Posteriormente en las Reuniones Consultivas se adoptaron varias recomendaciones al tratado, entre ellas el Protocolo al Tratado Antártico sobre la Protección del Medio Ambiente (Madrid, 3 de Octubre de 1991). En este protocolo se retira el nombramiento de la Antártida como Área de Especial Conservación para proteger el territorio Antártico y sus ecosistemas dependientes y asociados para pasar a designar la Antártida como una reserva natural, dedicada a la paz y la ciencia.

1.2.1 El Sistema del Tratado Antártico y las Áreas Protegidas

La finalidad del Sistema del Tratado Antártico (STA) así como el del Protocolo sobre la Protección del Medio Ambiente es asegurar el uso de la Antártida para fines pacíficos. Para

lograr tales fines se establece que cualquier zona (incluyendo las marinas) es susceptible de ser designada como una Zona Antártica Especialmente Protegida (ASPA, de sus siglas en inglés) o como una Zona Antártica Especialmente Administrada (ASMA, de sus siglas en ingles).



Map 3.2.- Áreas Antárticas Protegidas. Circulo azul, Zonas Antárticas Especialmente Protegidas (ASPAs); circulo rojo, Zonas Antárticas Especialmente Administradas (ASMAs).

Zonas Antárticas Especialmente Protegidas

Se denomina así a cualquier zona terrestre o marina que posea unos valores científicos, estéticos, históricos o naturales sobresalientes, cualquier combinación de estos valores, o las investigaciones científicas en curso o previstas.

Actualmente existen 71 ASPAs designadas, principalmente distribuidas alrededor de la península Antártica, las islas Shetland del Sur i del mar de Ross (círculos azules en el Map 3.2).

Los criterios utilizados para identificar y designar estas zonas están basados en un marco ambiental y geográfico sistemático:

- Zonas libres de toda interferencia humana, que puedan servir en futuras comparaciones.
- Ejemplos representativos de los principales ecosistemas.

- Áreas con agregados de especies importantes o poco habituales.
- Localidades tipo o el único hábitat conocido de cualquier especie.
- Zonas de especial interés para las investigaciones científicas en curso o previstas.
- Zonas de excepcional valor estético o natural.
- Cualquier otra zona donde convenga proteger los valores anteriormente mencionados.

Zonas Antárticas Especialmente Administradas

Se denomina así a cualquier zona terrestre o marina donde se lleven a cabo actividades en curso o en un futuro para evitar posibles conflictos, mejorar la cooperación y reducir al mínimo los impactos ambientales.

Actualmente existen 7 ASMAs designadas, principalmente distribuidas alrededor de la península Antártica, el Polo Sur, el mar de Ross y la Antártida oriental (círculos rojos en el Map 3.2), donde se localizan la mayoría de las bases Antárticas nacionales.

1.2.2 Gestión de las Áreas Protegidas

Para poder evaluar y seleccionar nuevas áreas protegidas se deben tener en cuenta los atributos y componentes más importantes que queremos proteger e identificarlos de manera clara; además de identificar el uso que queremos darle a esas áreas (Table 3.1).

Categorías de Protección	Categorías de Uso
ecosistemas	investigación científica
hábitats	conservación
colección de especies	económica
especies (tipos)	turística
paisaje/estéticas	
intrínseco	

Table 3.1.- Categorías de protección y uso para la identificación nuevas áreas protegidas.

Una vez han sido identificados los valores a proteger es importante llevar a cabo un estudio de impacto ambiental donde se incluyan análisis de los posibles riesgos medioambientales, así como la calidad y viabilidad a la hora de evaluar la idoneidad de que una área sea designada como una Zona Antártica Especialmente Protegida o Administrada (Table 3.2).

Calidad	Riesgo Medioambiental	Viabilidad
representatividad	actividades e impacto humanos	límites
tipicismo	procesos naturales	conflictos
diversidad	variabilidad y viabilidad naturales	tamaño
importancia ecológica	amenazas no antárticas	herramientas de gestión
grado de interferencia	urgencia por protección	plazo/duración
educación potencial	incertidumbre científica	facilidad de acceso
ciencia y seguimiento		logística

Table 3.2.- Criterios de evaluación para designar áreas protegidas.

1.3 Descripción de los organismos en este estudio

1.3.1 Los Octocorales

Pertenecen al filo cnidarios, son organismos relativamente simples con simetría radial que suele enmascarar otras simetrías, poseen dos capas de células embrionarias (diblásticos), el ectodermo y el endodermo a partir de las cuales se desarrollaran las estructuras del individuo adulto; la matriz extracelular que queda entre los dos epitelios de la pared del cuerpo se denomina mesoglea (Fig. 1.4). Al conjunto de mesoglea y epitelios de las zonas comunes de la

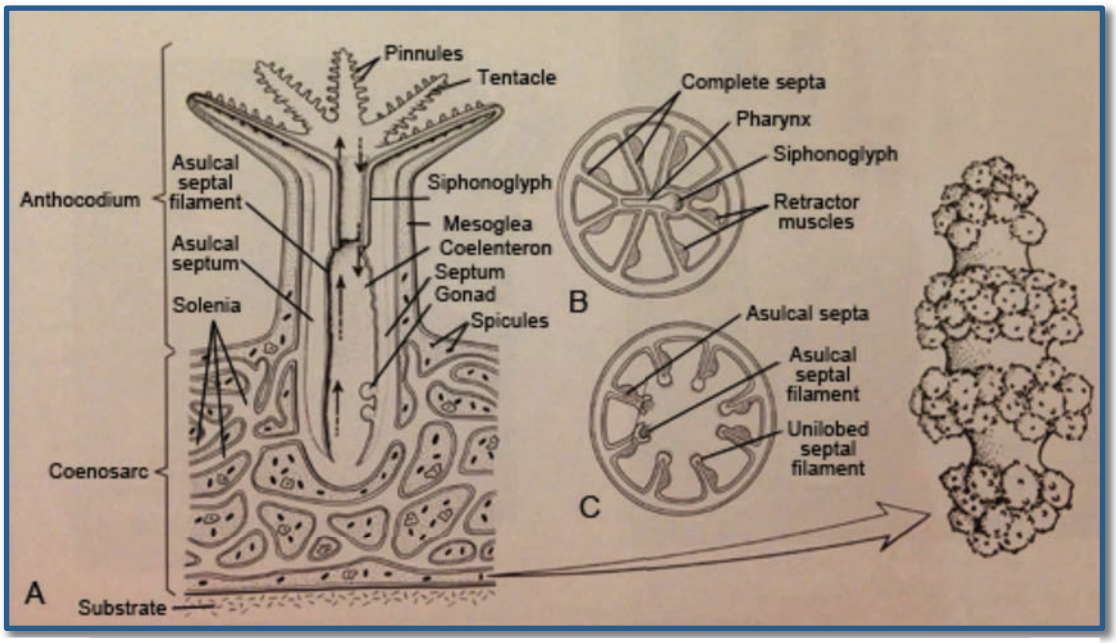


Figure 1.4.- Anatomía del pólipo de un octocoral. **A**, corte longitudinal de un pólipo, la flecha señala una espícula típica de la mesoglea; **B**, corte transversal a la altura de la faringe; **C**, corte transversal proximal sin alcanzar la faringe (Ruppert *et al*. 2004).

colonia se denomina cenénquima. En estos organismos la digestión se realiza en la cavidad gastrovascular, la cual se encuentra dividida por tabiques carnosos en disposición radial, llamados mesenterios. Los octocorales son animales exclusivamente marinos y sésiles, y la gran mayoría son coloniales. Las formas de crecimiento de las colonias son variadas, encontramos desde colonias masivas, encostrantes, filiformes, ramificadas con distintos

patrones (dicotómica, irregular, pennada, liradas, etc.) a las más complejas en estructura presentes en los pennatulaceos (Fig. 1.5) pero los pólipos mantienen una estructura octámera similar.

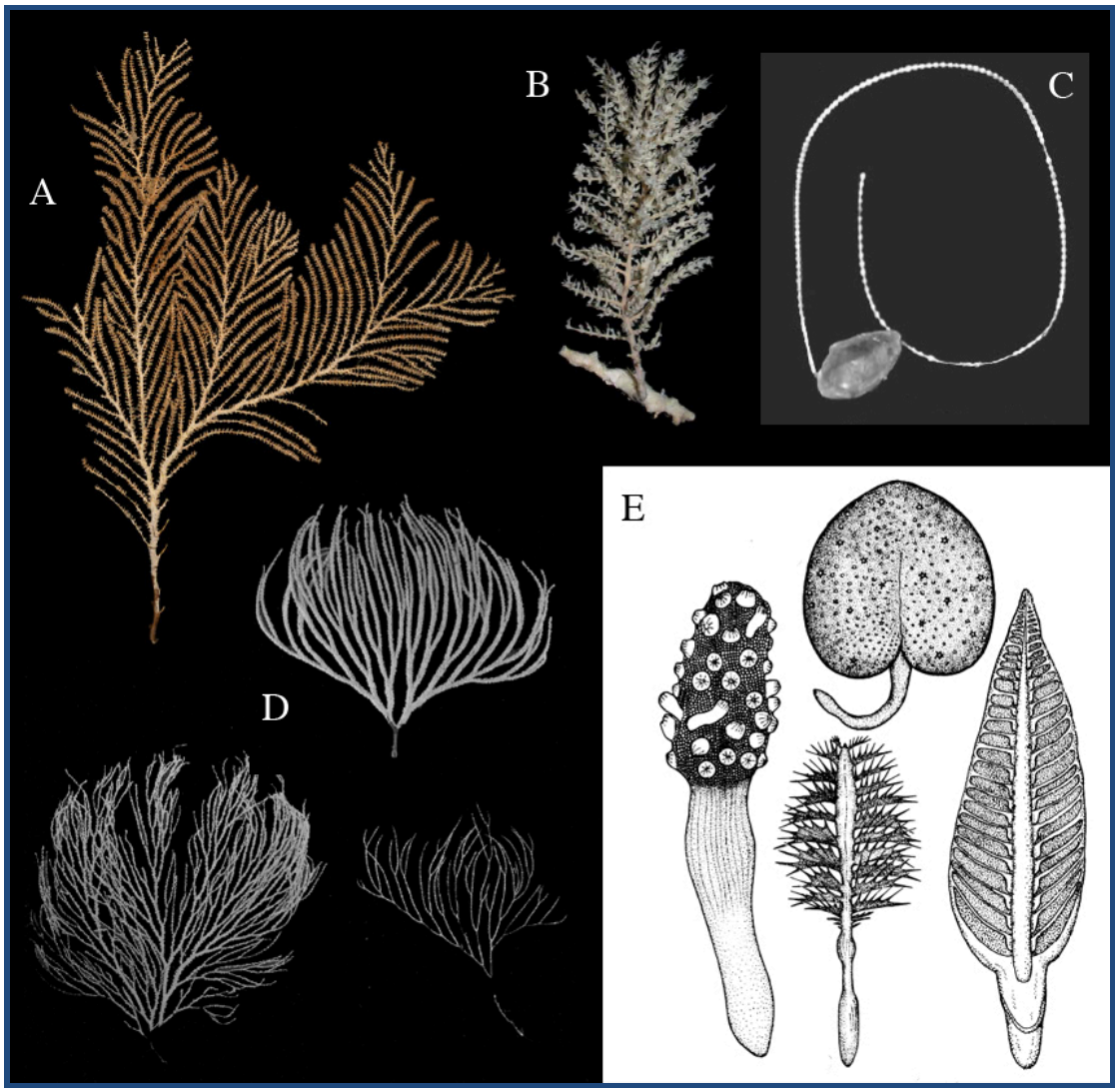


Figure 1.5.- Variabilidad de formas coloniales. **A**, *Plumarella castellviae* (foto: Zapata-Guardiola *et al*. 2013) ; **B**, *Tauroprimnoa austasensis* (foto: Zapata-Guardiola & López-González 2010b); **C**, *Primnoella delicatissima* (foto: Cairns 2006); **D**, *Fannyella kuekenthali* (foto: Bayer 1998); **E**, pennatulaceos (foto: Williams 1997).

En octocorales se han llegado a describir hasta 5 tipos de pólipos según su función (Williams *et al.* 2012). Sin embargo lo más común es encontrar colonias con pólipos monomórficos que suelen ser fértiles (autozooides). Este modelo básico de pólipo, que es el más común, consiste en una columna tubular de 0.3 mm (*Thouarella minuta*) a 21 mm de diámetro (*Umbellula monocephalus*), dónde en su extremo oral encontramos el disco oral rodeado por ocho tentáculos pinnados. Existe una gran variabilidad tanto en el número de pínnulas como en su tamaño (Fig. 1.6), llegando incluso a estar ausentes (género *Acrossota*). La boca continua en una faringe tubular comprimida lateralmente que desciende desde el disco oral y se abre en la cavidad gastrovascular. En uno de los extremos laterales (considerado ventral) de la faringe se encuentra un surco ciliado llamado sifonoglifo (Fig. 1.7).

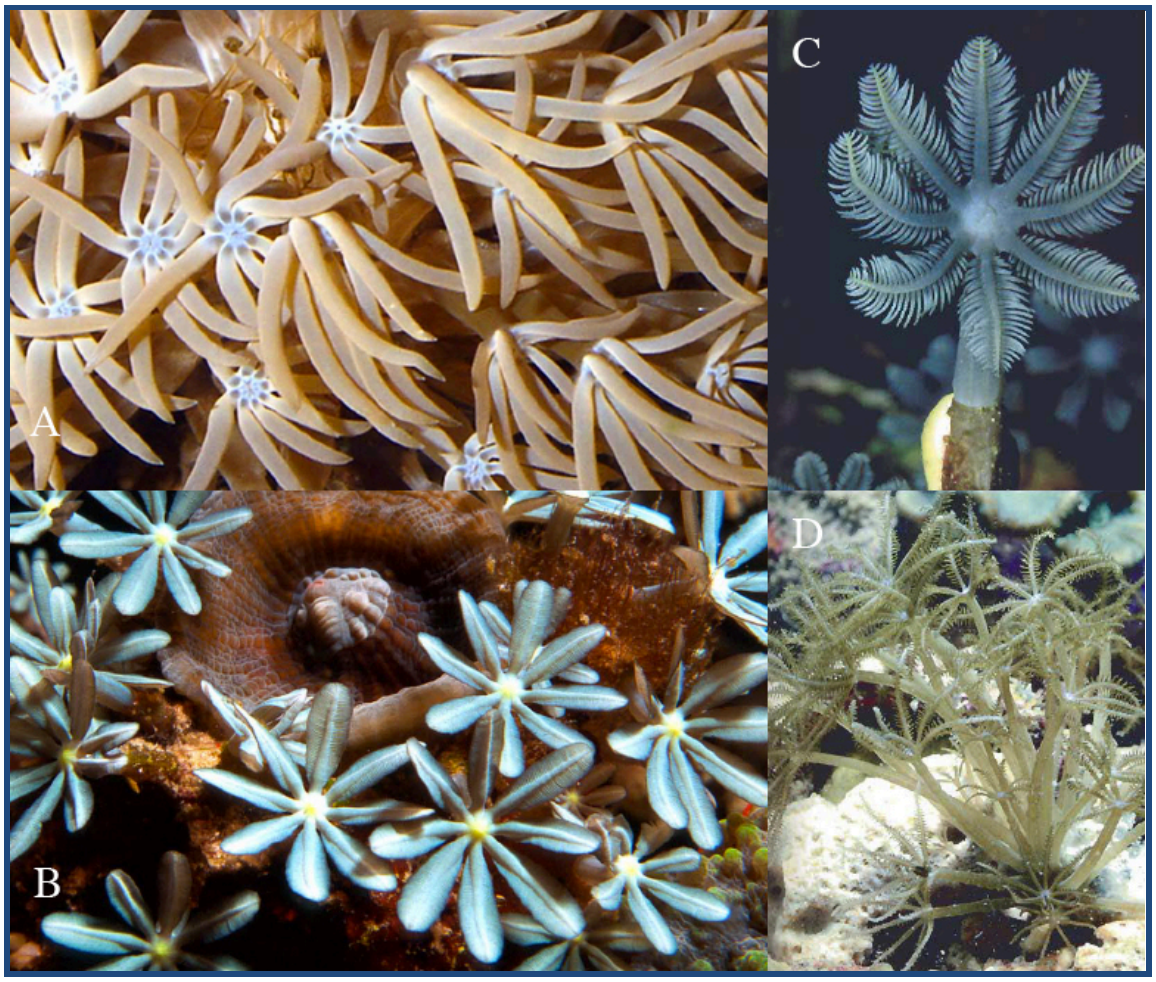


Figure 1.6.- Variabilidad de tentáculos y sus pínnulas. **A**, *Acrossota*; **B**, *Knopia octocontacanalis*; **C**, *Clavularia*; **D**, *Sansibia*. **Fotos**: **A** y **B** Alderslade & McFadden 2007, **C** y **D** Fabricius & Alderslade 2001.

Durante el desarrollo embrionario la faringe (estomodeo) se forma de la invaginación del ectodermo en el lado del blastoporo. La cavidad gastrovascular se encuentra dividida por ocho mesenterios completos pareados unilobulares, estos mesenterios son pliegues de la gastrodermis que flanquea una capa de mesoglea.

Los dos septos que se encuentran opuestos al sifonoglifo se llaman septos asulcales. Las células reproductivas se forman en todos los mesenterios excepto en los asulcales, que son estériles. La mayoría de los octocorales son gonocóricos aunque el hermafroditismo también se ha observado en corales blandos (Achituv & Benayahu 1990). Las células germinales se originan en la gastrodermis y son recubiertas por una capa de mesoglea recubierta a la vez por una de gastrodermis (Gutiérrez-Rodríguez & Lasker 2004). Posteriormente las células germinales primordiales forman agregados que mediante un pedicelo se anclan a los mesenterios en la zona basal de los pólipos (Achituv & Benayahu 1990). A medida que los productos sexuales crecen en tamaño la cavidad del pólipo se amplía.

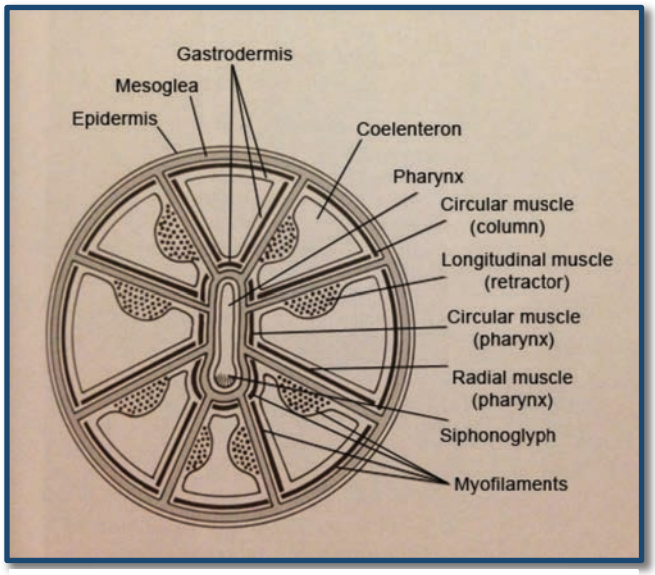


Figure 1.6.- Detalle de un corte transversal de un pólipo a la altura de la faringe (Ruppert *et al.* 2004).

Tras la fecundación, que puede ser interna (Benayahu & Loya 1983) o externa en la columna de agua (Dahan & Benayahu 1997) o en la superficie de la colonia hembra (Gutiérrez-Rodríguez & Lasker 2004) y la posibilidad de un cierto cuidado parental que puede darse en el interior (Brito *et al.* 1997) o en la superficie de la colonia hembra (Benayahu & Loya 1983), la larva que se origina es una larva plánula.

Las cavidades gastrovasculares de los pólipos se encuentran comunicadas entre si mediante un sistema de tubos gastrodérmicos llamados solenia. La superficie de la colonia se encuentra cubierta por la epidermis (originada por el ectodermo), mientras que el epitelio que recubre la cavidad gastrovascular es la gastrodermis (originada por el endodermo).

Los elementos esqueléticos inorgánicos se segregan en la mesoglea del cenénquima y pólipos, y están formados por escleritos de carbonato cálcico (Fig. 1.8), sueltos o fusionados. Además, pueden existir elementos estructurales axiales formados por una proteína córnea (gorgonina) que puede formar ejes con distintos patrones de ramificación, estar en diferente grado de impregnación por carbonato cálcico, o alternarse por segmentos con estos materiales inorgánicos. En ocasiones, también se observan cutículas córneas, especialmente en las zonas basales de los corales blandos. En algunos octocorales (*p.ej. Leptogorgia*, *Pseudopterogorgia*), la parte proximal de los pólipos puede penetrar en el interior del cenénquima, denominándose antostele a la región basal donde se recoge el antocodio (porción distal del pólipo que se introduce en el antostele cuando éste se retrae, Fig. 1.9).

1.3.2 Gorgonias Antárticas: los primnoidos

El ecosistema bentónico antártico es uno de los más ricos en número de especies (Clarke & Crame 1992), siendo además comparable con aquellos ecosistemas de zonas tropicales en valores de biomasa. Las comunidades de organismos suspensívoros, entre los que se incluyen los octocorales, dominan los fondos oceánicos antárticos de plataforma (Gili *et al.* 1997). Los octocorales además, son uno de los grupos mejor representados tanto en abundancia como en diversidad en estas comunidades (Starmans *et al.* 1999), siendo las gorgonias los más característicos. Estabilizan el substrato proporcionando un hábitat óptimo para el asentamiento de otras especies (Arntz *et al.* 1994), y al contrario que en otras latitudes, donde entre seis y diez familias son fácilmente identificables, los cambios ambientales sufridos en el

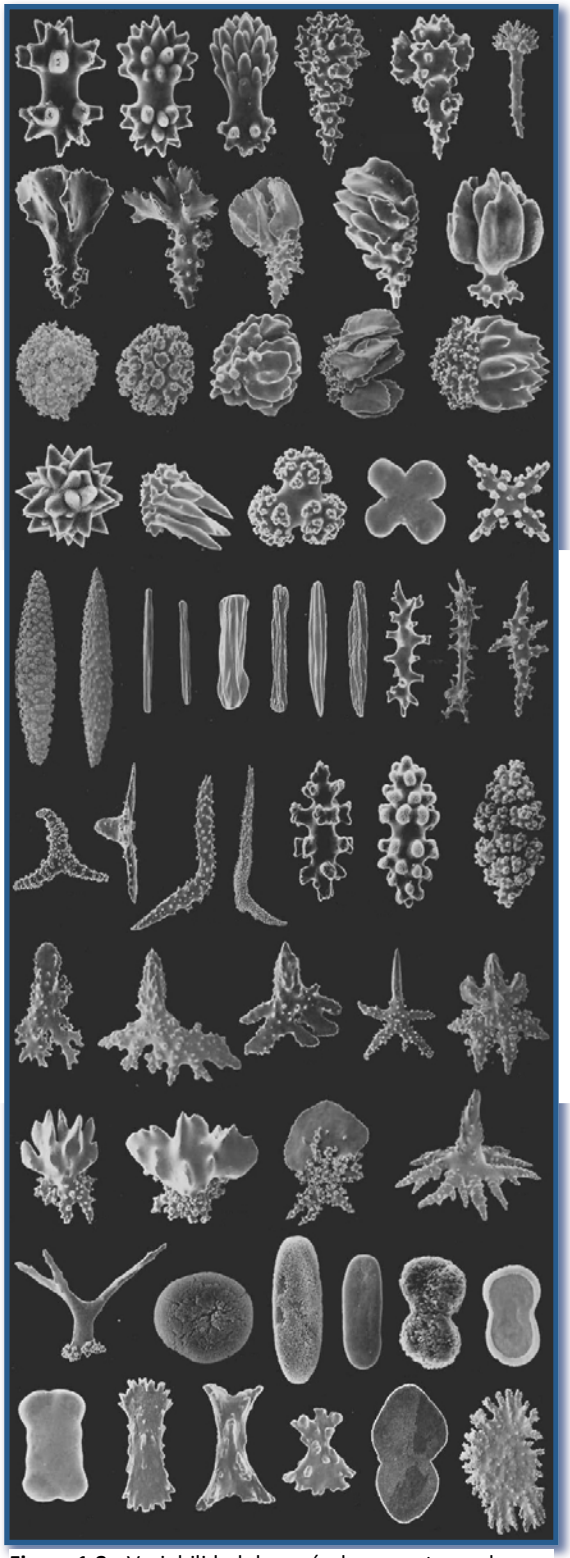


Figure 1.8.- Variabilidad de espículas en octocorales (modificado de Bayer *et al.* 1983).

continente antártico y las consecuentes implicaciones sobre la biota, redujeron principalmente a dos el número de familias presentes en aguas antárticas, Primnoidae e Isididae.

Recientemente se ha encontrado una nueva especie de la familia Chrysogorgidae (Cairns 2002), perteneciente al igual que las anteriormente mencionadas al suborden Calcaxonia. Ambas familias, especialmente Primnoidae, diversificaron ocupando los nichos donde habitualmente se encontraban otras familias como Acanthogorgiidae, Plexauridae, o Subergorgiidae. Aunque López-González (2006) describió recientemente un nuevo género de la familia Plexauridae en el Mar de Escocia. Aunque primnoidos e isididos dominan actualmente estos ambientes antárticos (Bayer 1996a; López-González & Gili 2002), es la familia Primnoidae quien acumula el mayor número de especies y géneros endémicos. El grado de endemismo a nivel genérico ronda más del 50%, la mitad de los géneros a nivel mundial son endémicos de aguas antárticas, y ocurre lo mismo a nivel de especies.

La primera referencia que se encuentra sobre un primnoido data de 1763, cuando el naturalista noruego Johan Ernst Gunnerus describió un ejemplar como *Gorgonia resedaeformis* (actualmente *Primnoa resedaeformis*). Aproximadamente cien años después, expediciones de gran magnitud y transcendencia como la *Challenger Expedition* (1872-76), *Thetis Expedition* (1898), *Valdivia Expedition* (1898-99) o la *Siboga Expedition* (1899-1900), entre muchas otras, aportaron una visión del sistema natural marino hasta entonces desconocida.

Desde entonces, diversos autores han contribuido sustancialmente al conocimiento de primnoidos en los océanos de todo el mundo (p.ej. Wright & Studer 1889; Versluys 1906; Gravier 1914; Kükenthal 1924; Bayer & Stefani 1988; Cairns & Bayer 2009). Sin embargo, las aportaciones más recientes sobre la biodiversidad de este grupo de gorgonias se ha concentrado en las regiones de mares y océanos de aguas templadas y tropicales (Cairns & Bayer 2002, 2003, 2004, 2005; Cairns 2006, 2007, 2010). En la región que ocupa el presente proyecto, la Antártida, son pocas las contribuciones taxonómicas hacia la familia Primnoidae (Bayer 1996b; López-González *et al.* 2002). Gracias a la mejora tecnológica para describir e identificar especies ahora el nivel de exactitud es mayor y debido a esto varios géneros algo confusos han sido revisados recientemente (*Mirostenella* en Zapata-Guardiola *et al.* 2013; *Thouarella* en Taylor *et al.* 2013) y la asignación de las especies está siendo modificado (Cairns & Bayer 2009; presente trabajo). La revisión y la consiguiente reasignación, en algún género ya existente (*Thouarella*) o en uno nuevo (*Faxiella* y *Primnocapsa*), de las especies previamente incluidas en el género *Amphilaphis*, hace más comprensible la actual clasificación de la familia Primnoidae.

Aunque necesitamos contribuciones más completas y exhaustivas para llegar a un consenso con los caracteres usados para agrupar especies y géneros. Actualmente podemos encontrar caracteres taxonómicos (p.ej. el patrón de ramificación de la colonia o la disposición de los pólipos en las ramas) usados para distinguir géneros (p.ej. entre *Plumarella* y *Mirostenella*), que también son usados para distinguir especies del mismo género (p.ej. en el género *Thouarella*). Estudios morfológicos y moleculares sobre las especies de primnoidos podrían ayudar a identificar caracteres morfológicos genéricos y específicos, así como el número de posibles homoplasias, su evolución a través de la familia y el establecimiento de relaciones entre los diferentes taxones.

Durante los últimos años la sistemática molecular ha despertado un gran interés en la comunidad científica. Aplicar las técnicas de secuenciación genómica para obtener las huellas digitales (barcode) de cualquier organismo complementa nuestro conocimiento sobre él y nos puede llegar a replantear la sistemática tradicional basada en caracteres morfológicos. Los primeros estudios en octocorales han analizado comparativamente las secuencias de distintos genes mitocondriales en busca de aquellos más idóneos para cada grupo taxonómico (France & Hoover 2001, 2002; Sánchez *et al.* 2003; McFadden *et al.* 2004, 2006). Mientras que en la familia Isididae se ha observado que el gen *mtMutS* da buenos resultados (France 2007), en la familia Gorgoniidae el gen *ITS2* parece soportar y corroborar observaciones basadas en caracteres morfológicos (Aguilar & Sánchez 2007) aunque para especies del Mar Mediterráneo no ha aportado información filogenética útil (Calderón *et al.* 2006, Gori *et al.* 2012). En primnoidos, por el momento, no se han realizado estudios moleculares para explorar las relaciones filogenéticas internas, aunque un número de secuencias se encuentran disponibles en GenBank.

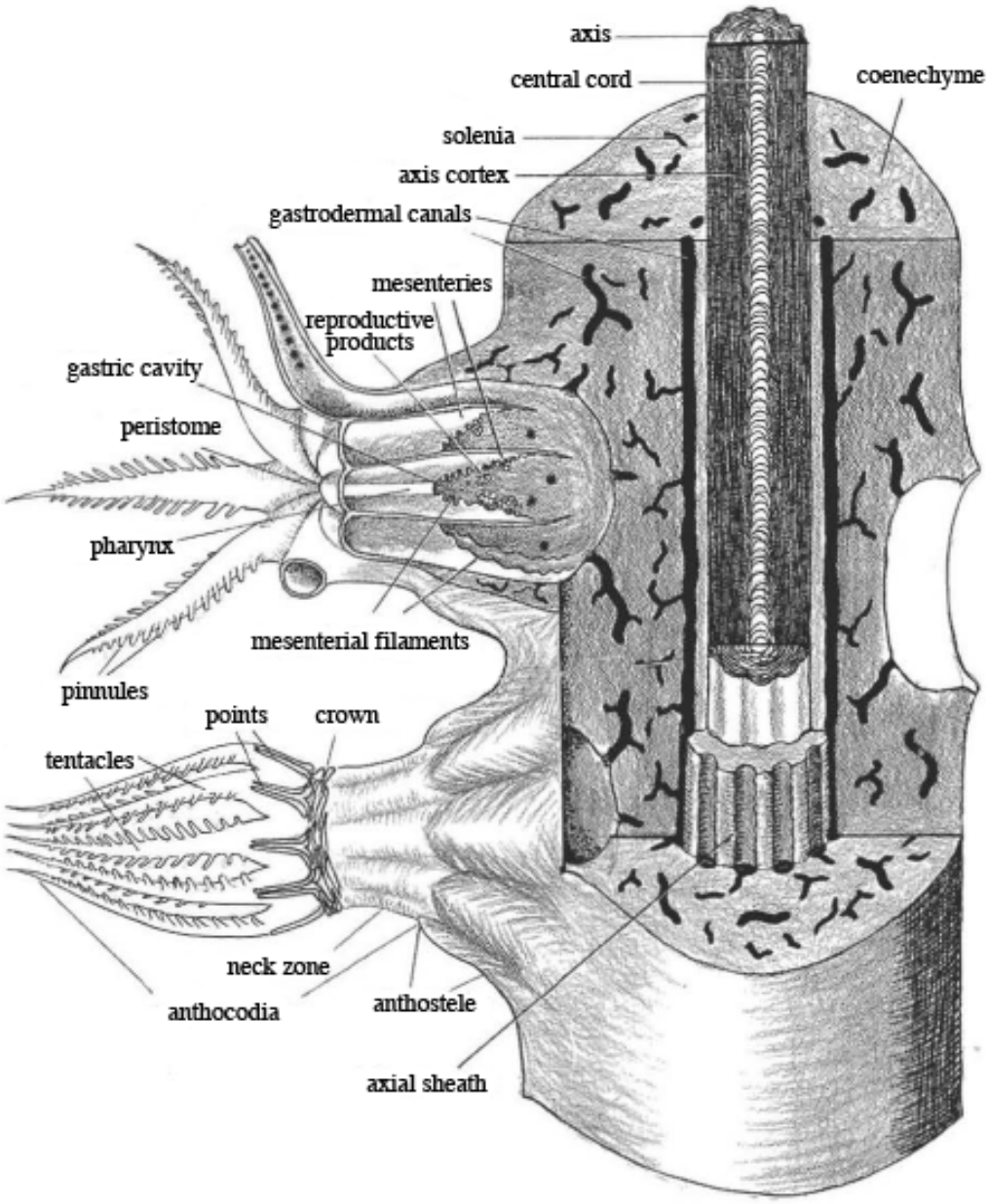


Figure 1.9.- Diagrama general de la anatomía de una gorgonia donde se observa la estructura interna del pólipo y de sus elementos esqueléticos (Bayer *et al.* 1983).

Igualmente sorprende observar el reducido número de estudios dedicados al conocimiento de la biología de estas especies de gorgonias, siendo como son, el principal grupo de octocorales presentes en los fondos antárticos. Los estudios señalan a los primnoidos como especies gonocóricas con un modo de reproducción sexual, mediante la incubación y desarrollo de la larva en el interior de los pólipos en las colonias hembra, hasta el momento de su liberación al medio (Brito 1997; Orejas *et al.* 2007). Se ha sugerido que la morfología de la colonia (en abanico o en hisopo) está relacionada con la captura del alimento, y que por lo tanto puede estar jugando un papel importante en cuanto a la estrategia reproductiva elegida (Orejas *et al.* 2007). La liberación de una larva por pólipo y por estación ha sido propuesta para aquellas

especies que presentan ovocitos en dos estadios de desarrollo distintos simultáneamente. Sin embargo, la explicación de una liberación continua durante todo el año también ha sido propuesta (Brito 1997). Aunque algunos estudios han tratado de caracterizar el proceso de gametogénesis en gorgonias antárticas (Brito 1997; Orejas *et al.* 2002, 2007), información sobre el proceso de fertilización, planulación, asentamiento larvario y/o reclutamiento siguen siendo una incógnita. Todos ellos son importantes para dar explicación a las distribuciones espaciales y los patrones en los que se estructuran las comunidades.

Como ocurre en otros grupos de invertebrados marinos antárticos, es esperable que las gorgonias también muestren una distribución en parche. Brito (1997) sugiere que la larva lecitotrófica de estos organismos (no pelágica y sin capacidad para alimentarse) se asienta rápidamente después de su liberación al medio, resultando en una baja dispersión. Sin embargo, se ha visto que algunas especies de primnoidos muestran una distribución circumantártica (Zapata-Guardiola & López-González, datos no publicados) sugiriendo la posibilidad de que otros factores estén influyendo en la distribución de estos parches (Gutt 2000).

1.3.3 Importancia ecológica

Desde un punto de vista trófico los octocorales pertenecen a los denominados suspensívoros pasivos que dependen de la disponibilidad de alimento suspendido en la columna de agua para cubrir la demanda de energía (Jørgensen 1990). La dieta de las gorgonias presenta un amplio espectro pudiendo alimentarse de las fracciones más finas del seston como ciliados y dinoflagelados (2 µm - 50 µm) hasta de zooplancton (~3 mm) (Orejas *et al.* 2003). Esta plasticidad trófica les puede suponer una ventaja que atenúe las fluctuaciones estacionales que sufre la comunidad planctónica de la que se alimentan, y cambiar su dieta en función del tipo de presa disponible o de las condiciones medioambientales (Fabricius *et al.* 1995). Esta capacidad demuestra la gran eficiencia de estos organismos a subsistir con el alimento más abundante que haya disponible en el medio así como de obtener la energía necesaria de manera más efectiva con una dieta mixta (Coma *et al.* 1998). Sin embargo las corrientes juegan un papel decisivo en la captura, eficiencia y calidad de las presas. Las gorgonias se alimentan pues de las partículas que caen al fondo (flujo vertical) y gracias a la dinámica del flujo de agua y de la resuspensión estas partículas pueden estar más tiempo en suspensión (Wildish & Kristmanson 1997). Existen además procesos de advección lateral (flujo horizontal) que permiten el transporte de las partículas a zonas alejadas del origen de hundimiento de las partículas (Dunbar *et al.* 1989). A gran escala se ha observado como la distribución y riqueza de la fauna está ligada a la disponibilidad de alimento debido a estos procesos laterales de transporte (Rosenberg 1995). La renovación del agua cerca del fondo es muy importante y gracias a los procesos hidrodinámicos de resuspensión y de advección se facilita la captura de alimento por las gorgonias durante prácticamente todo el año explicando las densas poblaciones que encontramos en la Antártida (Fahrbach *et al.* 1992). Estos flujos permiten que las colonias puedan invertir menos energía en alimentación y utilizarla en otros procesos como en crecimiento y reproducción (Young & Braithwaite 1980).

La estabilidad de la población dependerá pues de unas condiciones hidrodinámicas constantes y de una producción planctónica (Fréchette & Lefaivre 1990). Sin embargo en la Antártida existe una gran fluctuación en la cobertura de hielo (limita la luz) lo que influye en la producción primaria y por lo tanto en la cantidad y calidad de las partículas que precipitan al fondo (Arntz *et al.* 1994). Durante el invierno austral donde la producción primaria se ve severamente mermada, los procesos hidrodinámicos de resuspensión y transporte lateral juegan un papel relevante pues son la principal vía por la cual las gorgonias pueden obtener el alimento (Grebmeier & Barry 1991). En estos períodos, donde la cantidad de alimento es escaso en el medio, el metabolismo de las colonias se vuelve más lento para disminuir el gasto de energía (Lasker 1981). Organismos con metabolismos bajos de aguas frías presentan unas tasas de crecimiento altamente eficientes con unos costes de mantenimiento reducidos (Clarke 1991, 1998). Mientras que en regiones templadas los organismos invierten de manera estacional en la reproducción (Coma *et al.* 1998), en ambientes polares encontramos un balance entre el metabolismo basal y la longevidad del organismo lo que resulta en un esfuerzo reproductivo constante a lo largo de su vida (Dayton 1989).

Una alimentación continuada de la materia orgánica que se deposita en el fondo procedente de organismos de la columna de agua gracias a los procesos de resuspensión, advección lateral, transporte a grandes distancias y aprovechamiento del hidrodinamismo junto con la capacidad que tienen las gorgonias para alimentarse de un amplio rango de alimento disponible compensan las bajas temperaturas y las condiciones oligotróficas gracias a una mayor eficiencia metabólica y reducción de costes. Las gorgonias pues juegan un papel importante en los procesos de transferencia de energía desde los sistemas pelágicos a los sistemas bentónicos (Arntz *et al.* 1999).

En las comunidades bentónicas tanto de mares templados como polares las gorgonias son los cnidarios más destacados, ya que suelen ser uno de los organismos más comunes que además juegan un papel muy importante en muchos ecosistemas por pertenecer a los llamados "ecosystem engineers" (Brazeau & Lasker 1989, Orejas *et al.* 2002). Entre sus características encontramos que son capaces de estructurar y estabilizar el ecosistema donde se encuentran (Mitchell *et al.* 1993) proveyendo de una compleja estructura tridimensional, gracias a la cual proporcionan distintos hábitats para distintos organismos asociados. La complejidad de estas estructuras tridimensionales viene determinada en gran medida por la velocidad de las corrientes y de la disponibilidad de alimento en la columna de agua, así pues las gorgonias y demás suspensívoros pasivos suelen encontrarse en zonas de corrientes, favoreciendo así la captura de alimento y la resuspensión del sedimento en los hábitats generados (Kim & Lasker 1997), e incrementando la abundancia y la diversidad de la fauna asociada (Jones *et al.* 1994, Mortensen *et al.* 1995).

Las gorgonias son uno de los cnidarios más longevos, se han aportado datos que estiman la edad de *Primnoa resedaeformis* en 320 años (Risk *et al.* 2002), tienen bajas tasas de crecimiento (0.15 cm/año) y de reproducción, y están caracterizadas por adoptar estrategias del tipo K (Grigg 1989). Normalmente gonocóricas, las gorgonias suelen incubar sus larvas internamente dentro de los pólipos o en el exterior mediante una producción y posterior liberación de los gametos. La dieta y la reproducción de varias especies de gorgonias han sido

descritas, sin embargo la biología de la mayoría de ellas sigue siendo poco conocida aunque datos como la estructura poblacional, el crecimiento, la ecología trófica y su ciclo reproductor son alguna de las claves para entender las influencias medioambientales y antropogénicas de cualquier comunidad (Dayton 2003).

1.4 Objetivos

La infinidad de formas de vida marina que albergan las aguas antárticas ha atraído el interés de numerosos investigadores, principalmente en el campo de la taxonomía y de la sistemática (Brandt *et al.* 2007). Lamentablemente, se estima que solamente se conoce el 25% de la biodiversidad existente en las zonas de plataforma de la Antártida (Gutt *et al.* 2004). Durante las últimas décadas el número de campañas oceanográficas ha incrementado notablemente y consecuentemente un gran número de muestras han sido recolectadas. La oportunidad de poder estudiar todo ese material contribuirá enormemente al conocimiento sobre la diversidad y el funcionamiento de las comunidades Antárticas.

El presente proyecto propone contribuir al estudio la diversidad de gorgonias (Cnidaria: Anthozoa) en la región Antártica y Subantártica e identificar posibles hipótesis evolutivas sobre su diversificación y distribución, con especial interés sobre su papel en el origen de la actual fauna bentónica antártica. Finalmente se propone realizar un estudio para completar los conocimientos biológicos sobre las estrategias reproductivas en gorgonias de aguas polares, así como de crecimiento.

1.4.1 Objetivos Generales

Los principales objetivos de la presente memoria son los siguientes:

- Realizar una revisión taxonómica de primnoidos recolectados en expediciones antárticas recientes.
- Obtener una visión general de la distribución del principal grupo de gorgonias antárticas, la familia Primnoidae en el océano Austral.
- Proponer áreas de protección en la Antártida.
- Comprender las relaciones filogenéticas entre las gorgonias antárticas.
- Obtener una primera aproximación en los diferentes análisis de datación en gorgonias antárticas.
- Completar el conocimiento en los patrones de reproducción en gorgonias antárticas.

1.4.2 Objetivos Específicos

Para abordar estos objetivos generales, el estudio se centra en las especies antárticas pertenecientes a la familia Primnoidae, la familia dominante en número de géneros, especies y abundancia en el océano Austral. Se proponen los siguientes objetivos específicos:

- Identificar las especies de primnoidos recolectadas en 11 expediciones a la Antártida con especial énfasis en aquellas incluidas en el género *Thouarella*.
- Descripción formal de aquellas formas de primnoidos nuevas para la Ciencia.

- Redescribir especies ya conocidas de primnoidos con el fin de obtener descripciones más precisas e imágenes de mayor resolución.
- Comparar la fauna de primnoidos en relación a las principales áreas biogeográficas para el océano austral.
- Comparar batimétricamente la fauna de primnoidos entre las regiones Antártica y Subantártica.
- Identificar las potenciales Áreas Especialmente Protegidas Antárticas para la conservación de primnoidos y sus comunidades.
- Analizar filogenéticamente la familia Primnoidae mediante caracteres genéricos morfológicos.
- Analizar filogenéticamente el género *Thouarella* a través de la morfología de sus escleritos.
- Correlacionar los resultados con la distribución biogeográfica observada.
- Discutir el posible origen del género y su dispersión, con especial énfasis en las especies australes.
- Analizar filogenéticamente las especies del género *Thouarella* mediante análisis moleculares en el entorno de otros géneros de primnoidos.
- Comparar los resultados de las filogenias morfológicas y moleculares.
- Aproximación a los procesos de datación.
- Correlacionar la estimación de la edad a través de las imágenes obtenidas al microscopio electrónico de barrido.
- Correlacionar las estimaciones de edad con los análisis de ^{210}Pb.
- Diseñar un protocolo efectivo para estimar la edad y la tasa de crecimiento en gorgonias antárticas.
- Identificar patrones en la gametogénesis.
- Identificar los estadios de desarrollo de los productos sexuales.
- Estimar la reducción en tamaño de los productos sexuales tras los protocolos de estudio que implican la deshidratación del material.
- Estimar el volumen ocupado por los productos sexuales.

CHAPTER 2

Taxonomy of Primnoidae

2.1 Introduction

The first described primnoid species was *Gorgonia resedaeformis* Gunnerus, 1763 from the coast of Norway. Since 1812 this species is known as *Primnoa resedaeformis*, when Lamoroux described the earliest primnoid genus. But it wasn't until 1857 when Milne Edwards coined the term *Primnoacées* (interpreted to be as the first available name for the family Primnoidae), a category between family and genus, to include two genera (*Primnoa* and *Muricea*). One year later Gray (1858) referred to the family as Primnoadae, and finally was corrected by Verrill (1868) with the current name of Primnoidae.

At the end of the 19th century and the beginning of the 20th century, there was an important effort on polar science motivated by the First International Polar Year in 1882, where more than a dozen countries where involved. During those years great expeditions were carried out like the *Deutschen Tiefsee Expedition* (1898-1899), the *Deutschen Südpolar Expedition* (1901-1903), the *Swedish Antarctic Expedition* (1901-1903), the *Discovery Expedition* (1901-1904), the *Seconde Expeditione Antarctique Française* (1908-1910), the *Australasian Antarctic Expedition* (1911-1914) among others which extensively contribute to the knowledge of the marine natural history.

But the interest for marine organisms was not only focused on Polar Regions, during those decades the exploration of the sea was on the top interests and priorities. Expeditions in the vicinity of Australia (*Thetis Expedition*, 1898), Japan (*Sixten Bock's Expedition*, 1914), Indonesia (*Siboga Expedition*, 1899), Hawaii (*Albatross Expedition*, 1902) among others where also important to increase the knowledge of Primnoidae species.

Numerous species where described as new for science, however despite of the great effort of the authors many of these descriptions are poor and vague to distinguish the species using the original descriptions, making necessary to consult the type specimens which, unfortunately, are in some cases in very bad conditions or lost.

Consequently numerous reports, including new species where published and the taxonomy of Primnoidae was not very stable at the beginning. Authors like Gray (1870) included 13 genera into the family, many of which are now considered to be in other families, and created new families for species that are now included into the Primnoidae family. Verrill (1883) took out some species from Primnoidae family. Studer (1887) divided the genera in four subfamilies without any explanation; afterwards Wright & Studer (1889) included the diagnoses for those 4 subfamilies. Some authors followed this subfamilial arrangement until recent times, except for Versluys (1906) and Kinoshita (1908). Versluys provided a complete analysis of the family and proposed a preliminary evolutionary tree on the diversification of the genera. Authors like Kinoshita, Nutting or Kükenthal were very prolific octocoral researchers, the later giving the first complete and extensive revision of Primnoidae species (Kükenthal 1924). Although other researchers contributed to the knowledge of primnoids (Thomson & Ritchie 1906, Molander

1929, Aurivillus 1931, Deichman 1936, Madsen 1944) was Frederick M. Bayer who since 1950 started to revise the genera included in this family, and becoming the major expert on the field in the modern times with more than 20 publications. His successor at the Smithsonian Institution, Stephen Cairns, has continued his labour and he is becoming one of the current specialists on Primnoidae family, focused on the western Atlantic and Hawaiian species.

The Antarctic waters certainly have one of the highest abundance and diversity of cold-water corals in the world (López-González & Gili 2002). Accordingly, during last years new species of Antarctic primnoids have been described (Zapata-Guardiola & López-González 2010a, 2010b). However, the taxonomy of the Antarctic octocorals has not been revised since 1924.

2. 2 Objectives

It is the purpose of this chapter to taxonomically revise the Antarctic primnoid species found in the recently Antarctic expeditions, with special emphasis in bottlebrush colonial morphology related species currently included in, at least, three primnoid genera, *Thouarella*, *Plumarella*, and *Fannyella*. The present work implies that a higher generic and subgeneric diversity is present in this gorgonian family. The following specific objectives have been addressed:

- Identify primnoid species collected in 11 Antarctic expeditions with special interest of those forms initially attributable to the genus *Thouarella*.
- Update descriptions and illustrations of those old known primnoid species, identifying those undescribed ones.
- Formal description and illustration of those undescribed detected species.
- Elaborate updated artificial key of primnoid genera and species for those treated genera.
- Critical discussions about the utility of the characters currently used in the taxonomy of this gorgonian group.

2.3 Material and Methods

2.3.1 Material origin

For the purpose of this chapter we studied materials collected from eleven recent Antarctic research expeditions, however to correctly identify the specimens and re-describe some primnoid species, type specimens held in European, American and Australian museums have also been analysed.

2.3.1.1 Research Expeditions

The materials studied were collected in eleven Antarctic research expeditions between 1996 and 2008 on board the research vessels R/V *Polarstern*, R/V *Tangaroa* and R/V *Italica*. The expeditions mainly include six Antarctic research projects EASIZ, ANDEEP, LAMPOS, BENDEX, CLIMANT, and BioRoss. The project CLIMANT was included in the International Polar Year (2007-2008). Three of these expeditions, ANT XV-3, ANT XVII-3 and ANT XXI-2 were developed under the framework of the EASIZ programme (Ecology of the Antarctic Sea Ice Zone) of SCAR

(Scientific Committee on Antarctic Research). The aim of EASIZ was to improve the understanding of the structure and dynamics of the Antarctic coastal and shelf marine ecosystem, emphasizing in biodiversity, biogeography, ecology, the impact of disturbances and the bentho-pelagic coupling in the Antarctic ecosystem. The international project ANDEEP (ANtarctic benthic DEEP sea biodiversity) focused on the distributional patterns of the benthic fauna at different scales from local to global to observe the colonisation and exchange processes of the deep-sea fauna or the importance of the Antarctic region as a possible source for deep-sea benthic taxa in other oceans. While LAMPOS (Latin American Polarstern Study), grounds on the study of the biogeographic and evolutionary links between the Magellan Region and the Antarctic continent, focussing on the benthic fauna. The aim of BENDEX programme (Benthos Disturbance Experiment) was the continuity of the EASIZ programme, quantify the dynamics of the shelf ice and the movement of icebergs and reproduce artificially these perturbations to follow recolonization and succession processes in order to deduce the rules concerning the resilience of benthic marine ecosystems and pioneer species to help conservation management. BioRoss (Biodiversity of the Ross Sea) was aimed at improving our understanding and knowledge of the biodiversity of the Ross Sea region. The survey provided also scientific information to support New Zealand's efforts to establish a protected area around, and including, the Balleny Islands archipelago. Another benefit was valuable baseline biodiversity information for the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR). Finally, the IPY project CLIMANT (CLIMate change in ANTarctica) focused on a pelagic-benthic coupling approach to the extremes of the Weddell Sea to observe the recolonization after the collapse of Larsen platforms. The general data about the research expeditions are shown in the following table:

Expedition	Acronym	Start date	End date	Season	Area
ANT XIII-3	EASIZ I	26 Jan 1996	15 Mar 1996	Summer	eWS
ANT XIII-4		17 Mar 1996	20 May 1996	Autumn	WS-KGI-DP
ANT XV-3	EASIZ II	13 Jan 1998	26 Mar 1998	Summer	eWS-PA
ANT XVII-3	EASIZ III	18 Mar 2000	11 May 2000	Autumn	eWS-PA
ANT XIX-3	ANDEEP I	23 Jan 2002	26 Feb 2002	Summer	DP-eSI-EI-deep eWS
ANT XIX-5	LAMPOS	01 Apr 2002	01 May 2002	Autumn	SA-PA
ANT XXI-2	BENDEX	16 Nov 2003	15 Jan 2004	Spring	eWS
TAN 0402	NZ BioRoss	26 Jan 2004	05 Mar 2004	Summer	Ross Sea
ITALICA XIX	VLT-2004	01 Feb 2004	05 Mar 2004	Summer	Victoria Land
ANT XXIII-8	CLIMANT	23 Nov 2006	30 Jan 2007	Spring	WS-KGI-DP
ANT XXIV-2	ANDEEP-SYSTCO	28 Nov 2007	04 Feb 2008	Spring	eWS

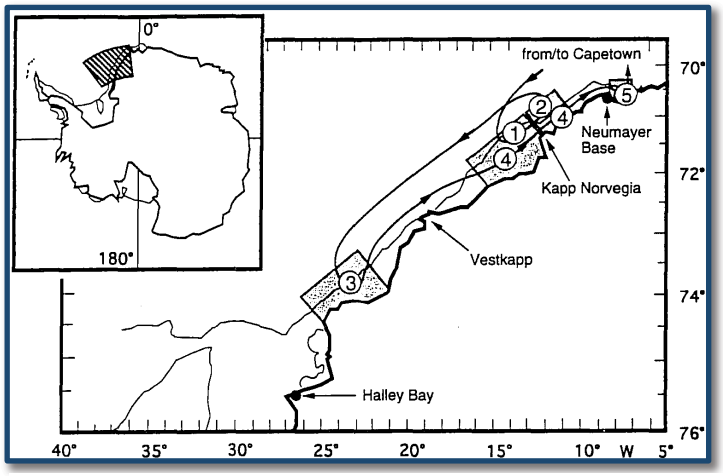
Table 2.1.- DP: Drake Passage; **eSI**: east Sandwich Islands; **EI**: Elephant Islands; **eWS**: east Weddell Sea; **SA**: Scotia Arc; **PA**: Peninsula Antarctica.

More than 700 stations where sampled during these eleven Antarctic expeditions, and more than 1500 lots of primnoids were found. Although not all the collected material could be analysed, up to date more than 400 specimens have been studied.

2.3.1.2 Sampling area

ANT XIII-3

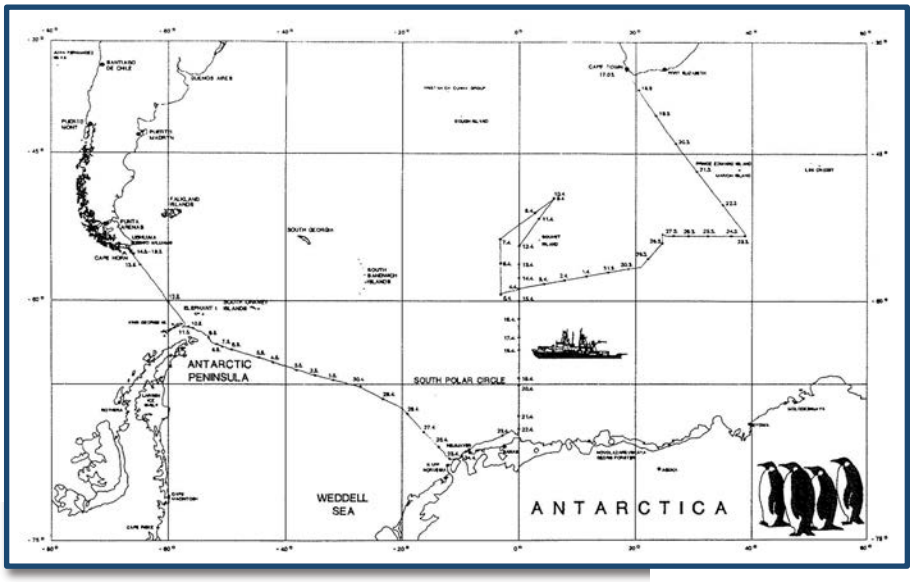
EASIZ-I cruise mainly focused on Eastern Weddell Sea. The first principal area of study was off Cape Norvegia (1 in map 2.1) to undertake benthic sampling according to bottom topography features, which include an inner shelf depression, perpendicular to the coastline. North to Cape Norvegia we found the iceberg "cemetery" (2 in map 2.1) where, parallel to benthic work, fishing was carried out. Fishing was done in the area south of Vest Cape (3 in map 2.1) complemented by benthic sampling. Finally a short transect perpendicular to the coast of Cape Norvegia and a transect along the coastline (4 in map 2.1) was carried out, and during the logistic work at Neumayer Base (5 in map 2.1) the last sampling was done.



Map 2.1.- Map of investigation area during ANTXIII-3. Numbers indicate planned sequence of working areas.

ANT XIII-4

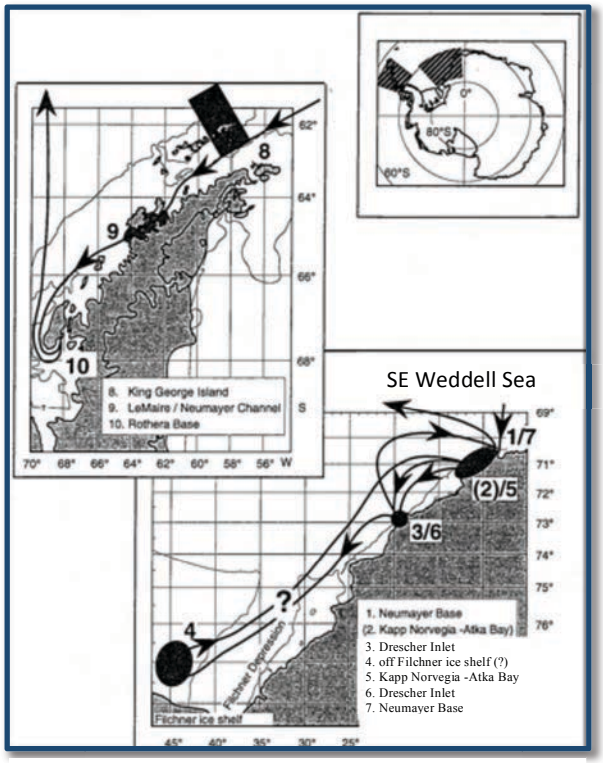
During ANT XIII-4, the benthic programme was mainly performed in the second part of the cruise, at the Drake Passage area. The main purpose was to investigate the ecological relationship between the marine fauna of the Antarctic Peninsula and the southernmost part of South America. Stations were carried out on transects between 2500 and 100 m where all trawled gears and cores were deployed successfully.



Map 2.2.- Cruise track of ANT XIII-4.

ANT XV-3

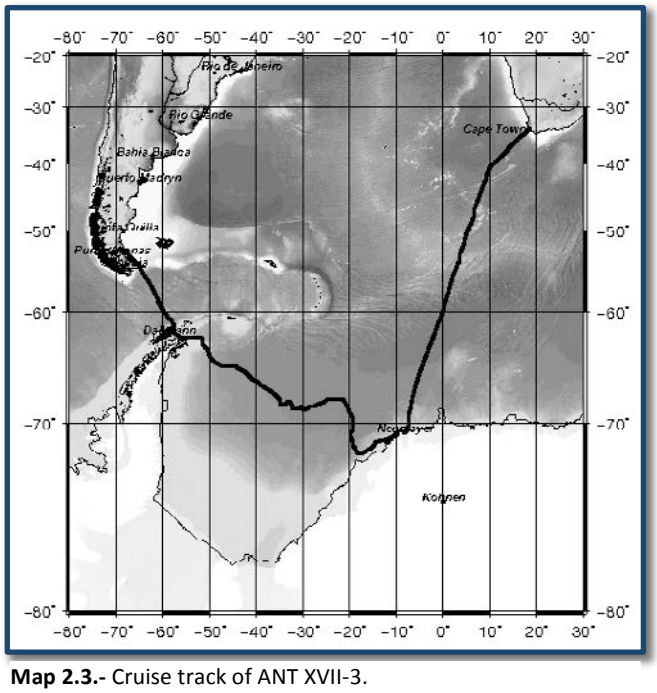
EASIZ cruise II continued the same approach as EASIZ I (ANT XIII-3), concentrating on few localities (box concept) and a limited number of joint projects rather than on large-scale sampling. The first part of the cruise was carried out at Cape Norvegia and Atka Bay area, while the second part was focused on Peninsula Antarctica, from King George Island to Rothera Base at Adelaide Island.



Map 2.3.- Map of investigation during ANT XV-3. Numbers indicate planned sequence of working areas.

ANT XVII-3

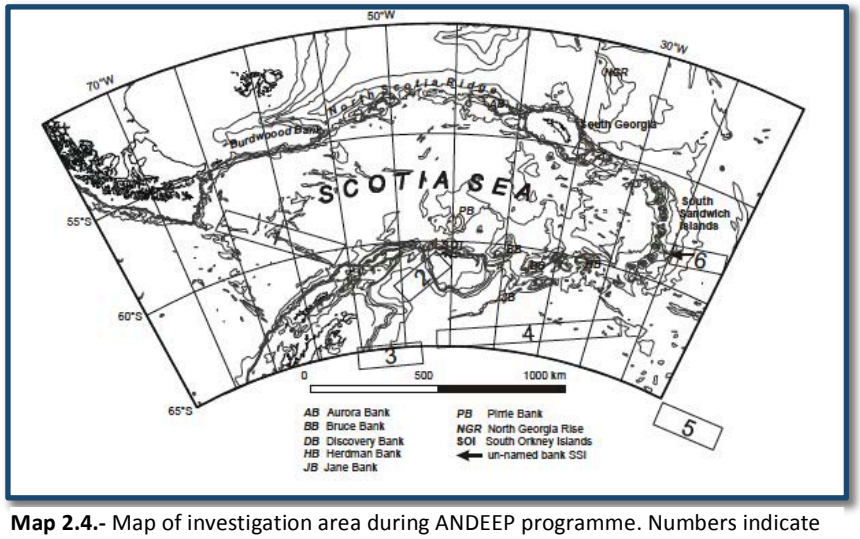
As during the two preceding cruises EASIZ I and EASIZ II, the third cruise of SCAR's international EASIZ programme was focused on limited areas to be studied. Exactly as in EASIZ cruise II, the first part of the cruise was carried out at Cape Norvegia and Atka Bay area, while the second part was focused on Peninsula Antarctica.



Map 2.3.- Cruise track of ANT XVII-3.

ANT XIX-3

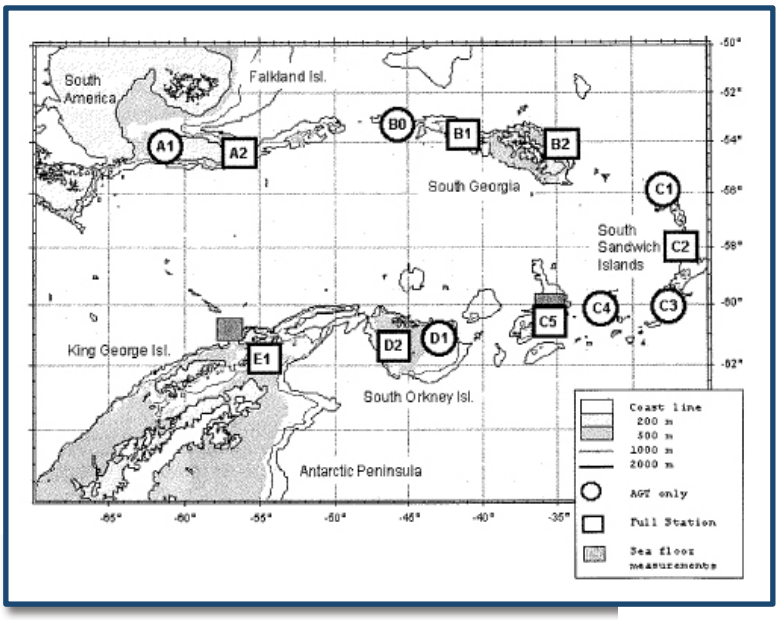
ANDEEP-I cruise belongs to a series of three expeditions focusing on the Antarctic benthic deep-sea biodiversity to understand its role in ecosystem functioning, and requirements for its conservation. Sampling efforts were carried out in the he target area 1 (map 2.5) in the Drake Passage on the Shackelton Fracture Zone. In addition, other stations off Elephant Islands, King George and Livingstone Islands were also sampled.



Map 2.4.- Map of investigation area during ANDEEP programme. Numbers indicate target areas.

ANT XIX-5

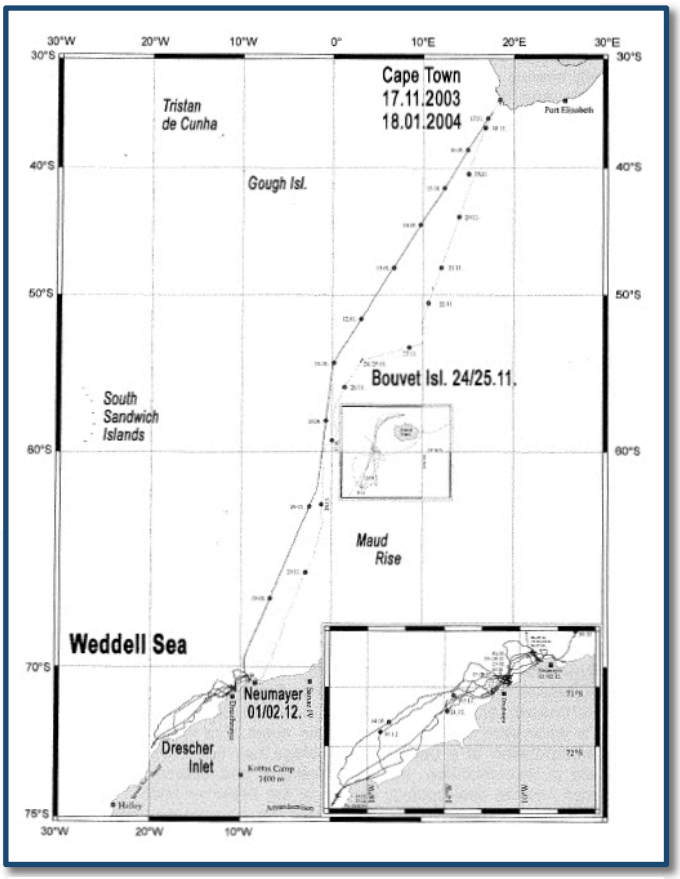
The LAMPOS cruise was planned to study the biogeographic and evolutionary links between the Magellan Region and the Antarctic continent. For this purpose, work focussed on the benthic fauna, following the Scotia Arc and concentrating biological sampling between 200 and 600 m depth. LAMPOS is also complementary to ANDEEP cruises, which were intended to investigate recolonization via the deep sea.



Map 2.6.- Map of investigation area during ANT XIX-5. Numbers indicate planned sequence of working stations.

ANT XXI-2

During ANT XXI-2 (BENDEX) cruise the effort was mainly carried out at eastern Weddell Sea, between Atka Bay and Drescher Inlet, with a focus on the iceberg rest place areas between Atka and Cape Norvegia, appropriate to study dynamics of ice shelf edge and iceberg movements. Studies about biodiversity and biogeography were also carried out around Bouvet Island.



Map 2.7.- Cruise track of ANT XXI-2.

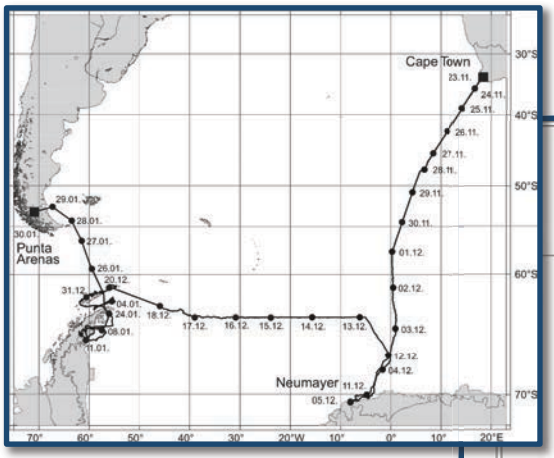
TAN 0402 and ITALICA XIX

The New Zealand Biodiversity Strategy Programme, in cooperation with the Italian Antarctic Research Programme carried out BioRoss to improve the understanding and knowledge of the biodiversity of the Ross Sea region. Coastal benthic studies were conducted along the northwest Ross Sea (Cape Adare and Victoria Land) (Italica XIX) and close inshore at the Balleny Islands (TAN 0402).

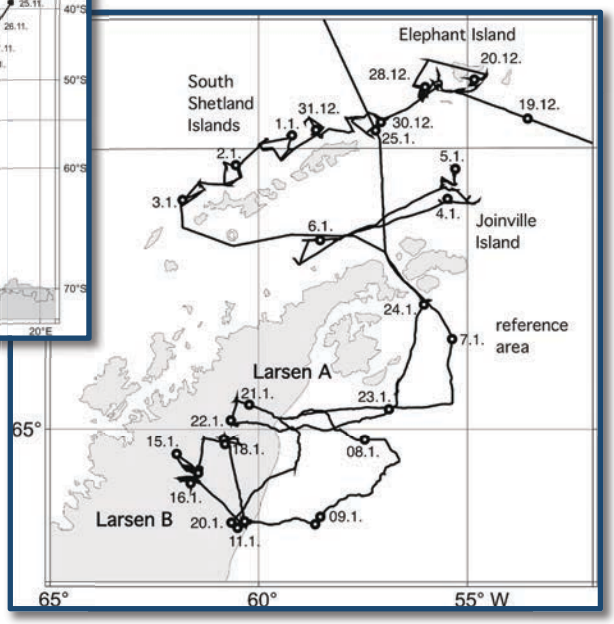
Map 2.8.- Map of investigation area during Bio-Ross joint programme.

ANT XXIII-8

The sampling sites in ANT XXIII-8 cruise were mainly on the Peninsula Antarctica's Islands (South Shetland Islands and Elephant Island) and the recently opened area beneath the former Larsen A and B ice shelves to produce a comprehensive bentho-pelagic coupling interpretation of a poorly known ecosystem.

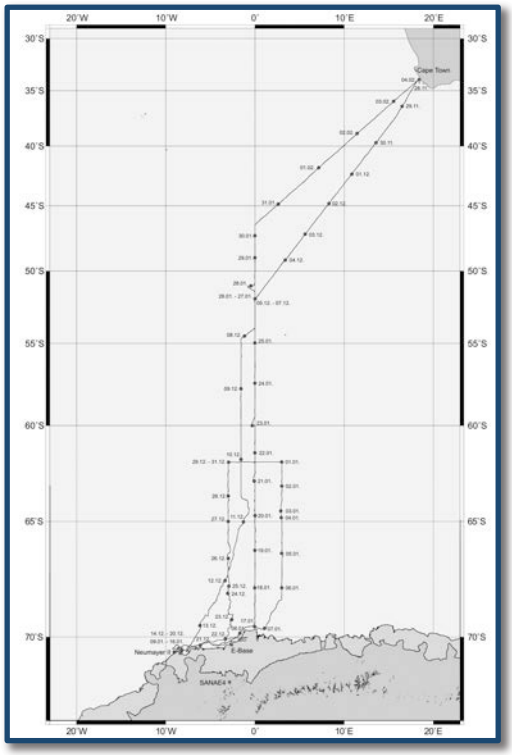


Map 2.9.- Cruise track of ANT XXIII-8.



Map 2.10.- Cruise track detail in the Peninsula Antarctica and Shouth Shetland Islands.

ANT XXIV-2



The cruise performed research in the Lazarev sea and had also sampling around Neumayer station with the purpose to better understand the upper ocean physical and biological processes influenced by sea ice and their linkage through the water column to the deep-sea abyss and its biogeochemistry and impact on biodiversity.

Map 2.11.- Cruise track of ANT XXIV-2.

2.3.1.3 Sampling methods

The materials were collected by several sampling equipment. However, Agassiz and Bottom trawl were the two more used gears to collect the specimens.

- Agassiz Trawl (AGT): demersal trawl consisting of a heavy rectangular iron frame with a wide-meshed net used to catch the benthic fauna (mesh size approx. 1 cm). The Agassiz trawl is constructed such that it does not matter which side is up or down when landing on the seafloor.
- Bottom Trawls (BT): consists of a fishing net ending in a codend, which retains the catch (mesh size approx. 1 cm). Normally the net has two lateral wings extending forward from the opening. The mouth of the trawl is framed by headline and groundrope. It is designed and rigged to catch species living on or near the bottom. Bottom contact with the gear is needed for successful operations.
- Epibenthic Sledge (EBS): developed specifically to capture medium sized peracarids, the EBS is equipped with two nets to catch these organisms, a fine-meshed "epinet" (300 µm) directly above ground and a slightly coarser-meshed "supranet" (500 µm) on top. Each net ends in a cup, which collects the captured organisms.

Figure 2.1.- Different gears used to collect studied material. **A-C**, Agassiz trawl: **A**, front view; **B,** on deck; **C**, catching. **D,** Bottom trawl. **E**, TV grab. **F-G**, Van Veen grab: **F**, closed; **G**, opened. **H-I**, Rauschert dredge: **H**, lateral view with upper rubber mat unfolded; **I**, lateral view with rubber mat folding over to protect the nets. **J-K**, Epibenthic sledge: **J**, setting up; **K**, lateral view. **L-M**, Giant box corer: **L**, catching; **M**, lateral view.

- Giant Box Corer (GKG): used to remove the sediment from the seafloor, especially large organisms (up to about 10 cm) living in the sediment. The 50x50x70 cm box penetrates deeply into the sediment after the gear landed on the seafloor.
- Rauschert Dredge (RD): small and simple gear, composed of a symmetrical metal frame that can land on the bottom of the sea on either of its sides and be pulled at the end of a cable of one hundred meters. Behind this frame, two nets (an inner one of 0.5 mm mesh and an outer one of strong 10 mm mesh to protect the first one) are attached to collect the animals that live on the surface of the sediment.
- Van Veen grab (GVVL): is a bottom sediment lightweight grab sampler designed to take large samples in soft bottoms by its clam shell-type scoop setup.

After sampling, all mixed material (crustaceans, octocorals, sponges, …) was deposited on deck and quickly sorted into the main taxonomical groups. Octocorals were kept into sea water until they were sorted in the wet laboratory inside the research vessel. After the preliminary sorting, labelling and taking photographs of the fresh specimens, octocoral colonies were fixed in buffered formalin 10% or ethanol 70%. Once the specimens got to the university they were transferred to ethanol 70%.

2.3.1.4 Museums

During the preliminary identification of the specimens there were several misinterpretations due to the lack of standardized descriptions with lack of taxonomical information. Because of that, several museum type materials were consulted in order to revise those doubtful primnoid specimens. In many cases type specimens were very fragile and only a small portion of the original colony could be consulted, in the worst cases the colony lacked almost all its polyps and none could be analysed. The study of those type specimens let us to re-describe some primnoid species, and acquire an accurate identification of our specimens.

Figure 2.2.- One of the collection cabinets at the Natural History Museum in London with primnoid specimens. Jars with red labels are type specimens.

2.3.2 Taxonomic methodology

2.3.2.1 Morphological Study

First of all when examining a primnoid like specimen, we have to focus on the external anatomy. In the present study we mainly followed the morphological and anatomical terms applied to octocorallia given by Bayer *et al.* (1983).

The characteristics of the colony and the polyps are basically the two main features to take into account to identify a primnoid specimen. The main characters are:

- General colonial branching pattern (flagelliform, dichotomous, bottlebrush…).
- Presence/absence and general arrangement of the polyps on the main stem, branches and branchlets (irregular, spiral, pairs, whorls… number of polyps per cm, etc.).
- Size and shape of the polyps are useful characters (taking into account its associated variability), although total size of the colony is a continuous character that should be considered with caution.
- General arrangement of scales on polyps (number of rows and number of sclerites per row) as well as general morphology of these polyp scales (polyp body, marginals, operculars, and tentacular positions) and coenenchyme (arranged in one or two layers).
- Detailed SEM observations of the ornamentation of scales at the different positions.
- Colour information of living specimens is still scarcely recorded. Colour information of preserved axis is usually noted in descriptions.

The lab material used in order to examine these features were:

- Digital caliper: to measure the diameter of the main stem, density of polyps per centimetre of branchlet, size of branchlets.
- Plastic tape measure: to measure the colony size.
- Stereo microscope MOTIC SMZ-140 series.
- Microscope MOTIC.
- Scanning Electron Microscope Philips XL30.

In primnoids, one of the most important taxonomic characters are the number of sclerites per longitudinal row on polyps, their size and shape, as well the coenenchymal sclerites. The best methodology to study and describe the sclerites is thanks to the Scanning Electron Microscope (SEM). However, depending if we need observation of the arrangement of the sclerites in polyps and coenenchyme or detailed observation of the ornamentation of these sclerites, the previous preparation of the sclerites will be different.

Figure 2.3.- Sclerites cleaning process under a light microscope.

- Sclerites attached to polyp body wall: carefully remove some polyps or whorls of polyps from the branchlet. Keep them in a glass cup filled with water. Prepare three more glass cups, fill the first one with sodium hypochlorite solution (bleach), the second one with hydrogen peroxide and the third one with water. Under a stereo microscope transfer one polyp or a fragment of the branchlet with attached polyps each time to the bleach cup for a few seconds (3-8"), then transfer to the hydrogen peroxide cup and, when the effervescence is ended, transfer it again to the bleach cup and again to the hydrogen peroxide cup until the epidermic tissue has been removed and sclerites are clearly visible and still attached to the polyp body wall. Keep them in the final water cup to clean up the remnant chemicals.

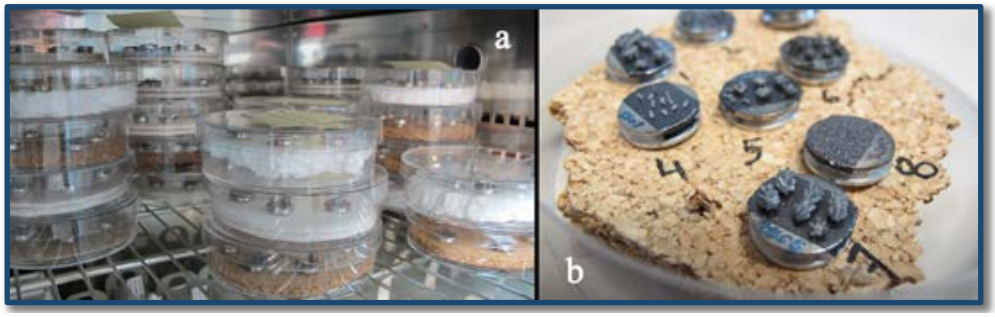


Figure 2.4.- a, collection of SEM stubs inside the heater; **b**, detail of coated SEM stubs.

- Free sclerite observations from polyp or coenenchyme: carefully remove a couple of polyps from the branchlet and immerse them in sodium hypochlorite solution until all organic parts are dissolved and sclerites are free at the bottom of the cup. Add 2-3 drops of hydrogen peroxide and let the effervescence stop, add more drops to remove the adherent tissue remnants if necessary. Clean up by water-washing several times to replace the chemicals from the sclerites (Fig. 2.3).

In both cases the next step is to dehydrate the sclerites, however the procedure is different for each kind of sample.

- Sclerites attached to polyp body wall: polyps with sclerites attached to its body wall are dehydrated in an ethylic alcoholic series from 70 to 100%, and subsequently critical-point-dried with hexamethyldisilazane (3-5 minutes).
- Free sclerites or coenenchymal sclerites: using a glass Pasteur pipet to avoid losing sclerites by adherence to the wall pipet in plastic pipets, we transfer the sclerites from the water cup to a microscope slide with the minimum amount of water to decrease the time of drying. Let the sclerites dry under a light focus.

Once the samples are dehydrated they are mounted directly onto aluminium Philips XL-30 SEM stubs (Fig. 2.4) that had been previously coated with a double-sided conductive carbon adhesive tape. Once polyps and sclerites are mounted on the stubs then are coated with gold-palladium target in a Edwards Scancoat six SEM sputter coater to mainly reduce beam damage, sample charging and to increase thermal conduction. Stubs are finally ready to be observed with a Philips XL30 SEM (Fig. 2.5).

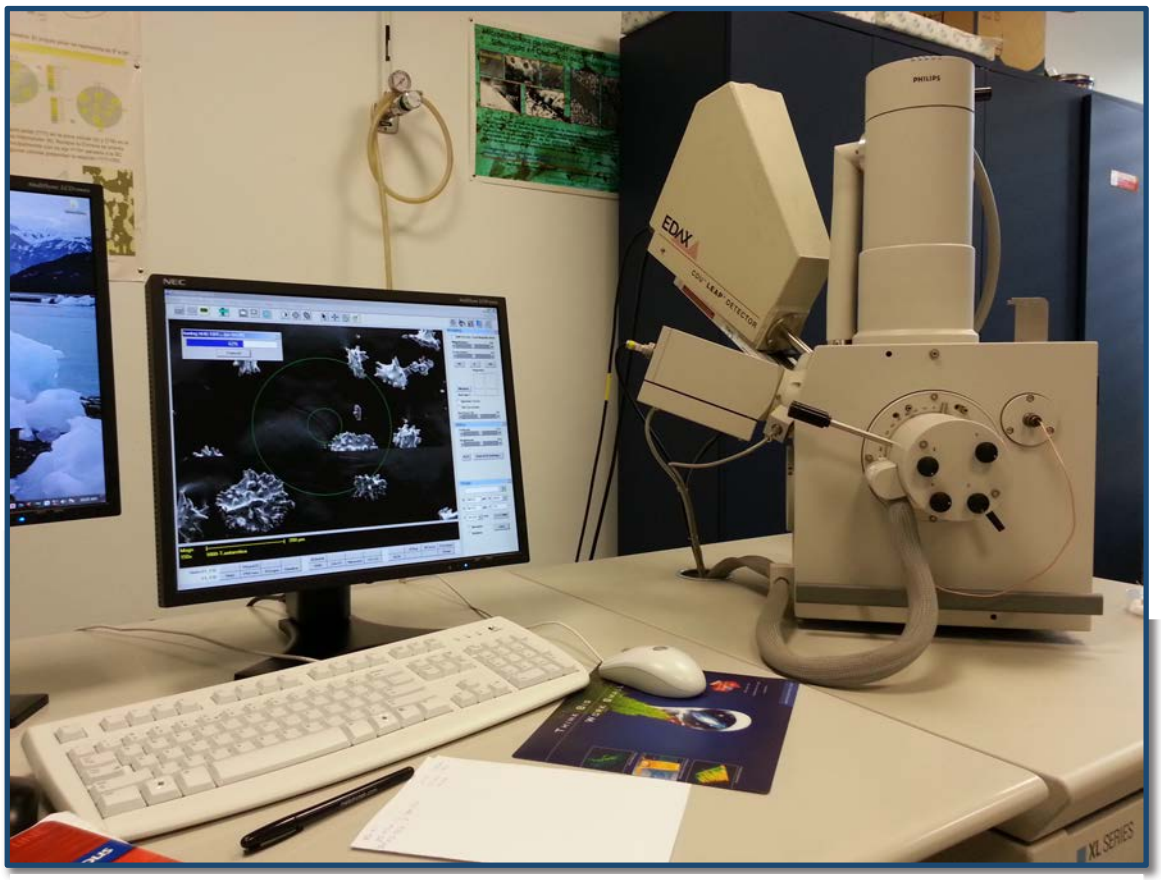


Figure 2.5.- Scanning Electron Microscope at University of Seville, Philips XL30-II.

2.3.2.2 Specimen description

To identify the specimens we mainly follow the most recent proposal of classification given by Cairns and Bayer (2009). Some slightly modifications have been included, new genera and species are introduced, and one of primnoid genus is no longer considerate valid and thus the species included have been reorganized.

To describe the specimens we use a defined structure to make easily the future comparison of the primnoid species. A species description includes a first part talking about the colony characteristics (size, colour, branching pattern…), a second one describing polyps (size, arrangement…), and a last part referring to the characteristics (size, shape, number…) of each type of sclerites (operculars, marginals and body and coenenchymal scales).

2.3.2.3 Storage

During the examination, the material was stored in the collection of the research group "Biodiversidad y Ecología de Invertebrados Marinos" of the University of Seville, Spain. Once the specimen was identified, some material remained in the Octocoral Reference Collection of the research group (BEIM-CRO), while the majority of the material was deposited at the following museums:

Australian Museum, Sydney, Australia (AM)
National Institute of Water & Atmospheric Research, Wellington, New Zealand (NIWA)
National Museum of Natural History, Smithsonian Institution, Washington D.C., USA (USNM)
Natural History Museum, London, United Kingdom (NHMUK)
Natural History Museum, University of Oslo, Norway (NHM)
Museu de Zoologia de Barcelona, Barcelona, Spain (MZB)
Museo Nazionalle dell'Antartide, Genova, Italy (MNA)
Museum für Naturkunde, Berlin, Germany (ZMB)
Muséum National d'Histoire Naturelle, Paris, France (MNHN)
Museum of Comparative Zoology, Harvard University, Cambridge, USA (MCZ)
Museum of Natural History, Wroclaw University, Poland (MNHW)
P.P. Shirshov Institute of Oceanology, Russian Academy of Sciences, Russia (IORAS)
The Bavarian State Collection of Zoology, Munich, Germany (ZSM)
Zoological Museum of Amsterdam, Amsterdam, the Netherlands (ZMA)
Zoologisches Institut uns Zoologisches Museum, Hamburg, Germany (ZMH)

Figure 2.7.- Octocoral Reference Collection of the research group (BEIM-CRO) at University of Seville.

2.4 Results

Twenty-six primnoid species have been studied in the descriptive taxonomic part of this memory. These species have been reunited in eight genera and six subgenera. Most of the species show bottlebrush colony growth, but species with planar dichotomous and moderate sympodial patterns are also included in the present study. Among all these taxa, eleven species, four genera, and two subgenera have been described as new to Science during the elaboration of this work in the framework of this PhD Thesis. The remaining species were poorly described or confused in the literature, and its taxonomic morphologic revision permit a better placement among the primnoid genera currently considered valid.

Some of the studied species, belonging to different museum collections, do not have their distribution in the SO waters. However in order to place correctly the antarctic and subantarctic materials in the systematics of the group, it has been necessary to re-describe those taxa, some of them becoming additional species new to Science.

2.4.1 Systematics of studied species

Kingdom Animalia

Phylum Cnidaria

Class Anthozoa

Subclass Octocorallia

Order Alcyonacea Lamoroux, 1812

Suborder Calcaxonia Grasshoff, 1999

Family Primnoidae Milne Edwards, 1857

Genus *Digitogorgia* Zapata-Guardiola and López-González, 2010

***Digitogorgia brochi* Zapata-Guardiola and López-González, 2010**

***Digitogorgia kuekenthali* Zapata-Guardiola and López-González, 2010**

Genus *Fannyella* Gray, 1872

Subgenus *Scyphogorgia* Cairns and Bayer, 2009

***Fannyella* (*Scyphogorgia*) *abies* (Broch, 1965)**

Genus *Mirostenella* Bayer, 1988

***Mirostenella articulata* Bayer, 1988**

Genus *Plumarella* Gray, 1870

Subgenus *Dicholaphis* (Kinoshita, 1907)

***Plumarella* (*Dicholaphis*) *bayeri* (Zapata-Guardiola and López-González, 2010)**

***Plumarella* (*Dicholaphis*) *diadema* (Cairns, 2006)**

***Plumarella* (*Dicholaphis*) *undulata* (Zapata-Guardiola and López-González, 2010)**

Subgenus *Faxiella* Zapata-Guardiola and López-González, 2012

***Plumarella* (*Faxiella*) *abietina* (Studer, 1894)**

***Plumarella* (*Faxiella*) *delicatula* (Thomson and Rennet, 1931)**

Subgenus *Verticillata* Zapata-Guardiola *et al.*, 2013

***Plumarella* (*Verticillata*) *castellviae* Zapata-Guardiola *et al.*, 2013**

Genus *Primnocapsa* Zapata-Guardiola and López-González, 2012

***Primnocapsa plumacea* (Thomson and Mackinnon, 1911)**

Genus *Scopaegorgia* Zapata-Guardiola and López-González, 2010

***Scopaegorgia liouvillei* (Gravier, 1913)**

Genus *Tauroprimnoa* Zapata-Guardiola and López-González, 2010

***Tauroprimnoa austasensis* Zapata-Guardiola and López-González, 2010**

Genus *Thouarella* Gray, 1870

Subgenus *Euthouarella* Kükenthal, 1915

***Thouarella* (*Euthouarella*) *laxa* Versluys, 1906**

***Thouarella* (*Euthouarella*) *vitjaz* Zapata-Guardiola and López-González, 2012**

Subgenus *Epithouarella* Kükenthal, 1915

***Thouarella* (*Epithouarella*) *dispersa* Kükenthal, 1912**

***Thouarella* (*Epithouarella*) *grandiflora* Kükenthal, 1912**

***Thouarella* (*Epithouarella*) *regularis* (Wright and Studer, 1889)**

***Thouarella* (*Epithouarella*) *viridis* Zapata-Guardiola and López-González, 2010**

Subgenus *Thouarella* Gray, 1870

***Thouarella* (*Thouarella*) *andeep* Zapata-Guardiola and López-González, 2010**

***Thouarella* (*Thouarella*) *antarctica* (Valenciennes, 1846)**

***Thouarella* (*Thouarella*) *brucei* Thomson and Ritchie, 1906**

***Thouarella* (*Thouarella*) *minuta* Zapata-Guardiola and López-González, 2010**

***Thouarella* (*Thouarella*) *pendulina* (Roule, 1908)**

***Thouarella* (*Thouarella*) *variabilis* Wright and Studer, 1889**

Genus *Tokoprymno* Bayer, 1996

***Tokoprymno anatis* Zapata-Guardiola and López-González, 2010**

2.4.2 Primnoid species description

Diagnosis

Calcaxonia with a cross section of axis revealing a pattern of undulating concentric layers of calcified material embedded in gorgonin, resulting from a longitudinal pattern of radiation. Polyps non-retractile, and body wall with calcareous scales, usually imbricated and distinguished by a cruciform extinction pattern under polarized light because of radial crystal orientation.

Type genus

Primnoa Lamoroux, 1812

Distribution

Worldwide, predominantly at slope depths, ranging from 8 to 5850 m depth.

Artificial key to the genera of the family Primnoidae

1. Polyps united in groups forming polyp leaves placed along axis as in some pennatulaceans ……..……..”**Subfamily *Ainigmaptilinae”*: *Ainigmaptilon***
2. Polyps individually distinct or united basally, but not united in groups forming polyp leaves ……..”**Subfamily *Primnoinae”***
   a. Polyps adnate to coenenchyme except for oral region.
      i. Colonies dichotomous, large, and robust, terminal branches long and flexible; polyps large, arranged in close set of whorls, abaxial side covered by two rows of narrow, sickle-blade-shaped sclerites; distalmost polyp scales not differentiated as operculum ……..***Armadillogorgia***
      ii. Colonies closely pinnate, slender and plumose, side branches short and stiff; polyps small, not in whorls, biserial, or in close spirals, directed obliquely upward, abaxial side with only one longitudinal row of scales, adaxial side extremely short and adnate to coenenchyme, lacking scales below marginal; operculum well developed, tall, conical, the triangular opercular scales fitting closely together……...***Pseudoplumarella***
   b. Polyps not adnate to coenenchyme, perhaps appressed, inclined or perpendicular.
      i. Polyps having sclerites in the form of thick plates, not imbricating but closely fitted as in mosaic, not aligned in regular rows……..……***Microprimnoa***
      ii. Polyps having sclerites in the form of scales, thin or thick, clearly imbricating and aligned in regular rows, at least on immature polyps; adaxial body wall rows may be missing or vestigial.
         1) Polyps having sclerites aligned in five to eight or more complete, well-developed rows on all sides of polyp or randomly, at least on immature polyps, resulting in adaxial side of polyp being completely covered with scales.
            a) Sclerites of mature polyps in multiple (more than eight) rows, the longitudinal alignment of eight regular rows present only in immature polyps; distalmost scales not differentiated as well-organized operculum.

i) Colonies robust, dichotomous; polyps large, curved inward toward axis, polyps arranged in whorls; numerous distal body scales with strong apical keel…………………………………………………………………………………………..***Aglaoprimnoa***

ii) Colonies small, delicate, pinnate; polyps small, not curved inward; polyps arranged biserially (not in whorls); distal body scales lack keel but close over retracted tentacles and mouth…………………………………………………..….***Primnoeides***

b) Sclerites of polyps in five to eight longitudinal rows; distalmost scales differentiated as operculum.

i) Marginal scales of polyps form a circumoperculum that folds over bases of opercular scales.

(1) Outer surface of abaxial and lateral body scales, including marginal and submarginals, with a well-defined, transverse, serrate, spinose, or granular ridge extending across the greatest width of the sclerite, separating the exposed distal part from the proximal part covered by the distal margin of the next lower scale. The transverse ridge is continuous with lateral and distal margins and forms a shallow concavity (the ascus scale) on upper surface of sclerite.

(a) Exposed outer surface of body wall scales sculptured with a serrate or spinose transverse ridge; inner surface of opercular scales ridged.

(i) Colonies flagelliform; marginal scales without apical spine ………..***Onogorgia***

(ii) Colonies branched; marginal and sometimes submarginal scales with a strong, smooth apical spine.

1.- Colonies bottlebrush shaped, with numerous simple twigs arising from all sides of main stems……………….....***Fannyella*** **(*Scyphogorgia*)**

2.- Colonies dichotomously to quasy pinnately branched ………………………..……………………………..…..…..***Fannyella*** **(*Cyathogorgia*)**

(b) Exposed outer surface of body wall scales sculptured with low, smooth projections and distinguished from the covered portion by a transverse row of granules or tubercles along a more or less thickened boundary between exposed and concealed part of scale; inner surface of opercular scales with a strong apical keel most prominent on abaxial and outer laterals…………………………………………………………….…………..***Metafannyella***

(2) No distinct boundary separating exposed distal part of body scales from proximal part covered by scale below.

(a) Colonies flagelliform (unbranched), sometimes unattached…..***Convexella***

(b) Colonies abundantly branched.

(i) Calyces with eight rows of equal-sized body wall scales.

1.- Branching dichotomous…………………………………………….…..***Pyrogorgia***

2.- Branching bottlebrush……………………………………………..….***Digitogorgia***

(ii) Calyces with six to eight rows of body wall scales, the two adaxial rows having smaller and fewer number scales especially near calyx base, these rows being covered by broadened adjacent inner laterals.

1.- Seven marginal scales..... ***Scopaegorgia***

2.- Eight marginal scales.....***Thouarella***

a.- Marginal scales lacking a spine or with a short distal spine .....***Thouarella* (*Epithouarella*)**

b.- Marginals with well-developed distal spine.

i.- Polyps arranged in pairs or whorls.

1/ Colonies pinnate or bottlebrush....***Thouarella* (*Euthouarella*)**

2/ Colonies dichotomous branched..***Thouarella* (*Diplocalyptra*)**

ii.- Polyps isolated.....***Thouarella* (*Thouarella*)**

ii) Marginal scales of polyp do not fold over bases of opercular scales.

(1) Colonies unbranched or very sparsely branched.

(a) Polyps with eight marginal scales; polyps fused basally.....***Callozostron***

(b) Polyps with four marginal scales; polyp bases not fused .....***Candidella*** (in part)

(2) Colonies abundantly branched.

(a) Eight marginal scales.

(i) Marginal scales offset from opercular scales; inner face of operculars keeled.

1.- Opercular scales with a locking device, each vertex of the proximal inner surface of operculars has an irregular mound articulating with the corresponding mound of adjacent ones; polyps isolated arranged in spirals.....***Primnocapsa***

2.- No locking device on opercular scales; polyps arranged in whorls, pairs or isolated; marginal scales with a tubular extension (a flute).....***Parastenella***

(ii) Marginal scales aligned with opercular scales.

1.- Calcified axis interrupted by organic nodes at points of bifurcation .....***Mirostenella***

2.- Calcified axis not interrupted by organic nodes at points of bifurcation.

a.- Inner surface of opercular scales keeled; brood polyps common .....***Tokoprymno***

b.- Inner surface of opercular scales not keeled; brood polyps rare.

i.- Distal edges of body wall scales pectinate; inner faces of sclerites smooth (not tuberculate).....***Acanthoprimnoa***

ii.- Distal edges of body wall scales spinose or serrate; inner face of sclerites tuberculate.....***Plumarella***

1/ Polyps arranged isolated.

a/ Polyps arranged all side branches with no order ……..……..***Plumarella* (*Dicholaphis*)**

b/ Polyps arranged alternating..…… ***Plumarella* (*Plumarella*)**

2/ Polyps arranged in pairs or whorls.

a/ Polyps arranged in pairs……..***Plumarella* (*Faxiella*)**

b/ Polyps arranged in whorls..……***Plumarella* (*Verticillata*)**

(b) Four to five marginal scales.

(i) Four marginal scales.

1.- Only one abaxial row of large body scales; colonies bottlebrush in shape.……..…***Tauroprimnoa***

2.- Body scales not arranged in longitudinal rows; colonies uniplanar dichotomous……..***Candidella*** (in part)

(ii) Five marginal scales.

1.- Only one abaxial row of large body scales; colonies bottlebrush in shape.……..…***Dasystenella***

2.- Two rows of abaxial body scales; colonies closely pinnate ……..…***Pterostenella***

2) Polyps having sclerites aligned in complete, well-developed rows only on abaxial and sometimes outer-lateral sides, the adaxial side having a few, small or no sclerites below the adaxial marginal scales.

a) Abaxial side of polyps protected by large scales; distalmost eight scales form a well-differentiated operculum.

i) Polyps crowded irregularly around stems, not in regular whorls ……..……..…***Primnoa***

ii) Polyps arranged in pairs or whorls around stems.

(1) Abaxial body wall scales arranged in a single longitudinal row of large scales ……..……..***Perissogorgia***

(2) Abaxial body wall scales arranged in two or more longitudinal rows.

(a) Body wall scales in two rows.……..…"**Subfamily *Calyptrophorinae***"

(i) Two pairs of large abaxial body wall scales.

1.- Both pairs of abaxial scales extend around body as a solid ring, the members inseparably fused along abaxial and adaxial symphysis ……..……..***Calyptrophora***

2.- Abaxial scales extend around body but are not solidly fused along abaxial and adaxial symphysis.

a.- One pair of infrabasal scales.……..…***Paracalyptrophora***

b.- Two or more pairs of infrabasal scales.……..…***Arthrogorgia***

(ii) Three to five pairs of abaxial body wall scales.

1.- Three or four pairs of abaxial scales enclose body; no inner- or outer-lateral scales.

a.- Six marginal scales; calyces arranged unilinearly….***Australogorgia***

b.- Four marginal scales (including two small adaxial scales); calyces arranged in downward pointing whorls……..***Narella***

2.- Five pairs of abaxial body wall scales; one pair of both inner- and outer-laterals present……...***Paranarella***

(b) Body scales in four, six, or eight longitudinal rows.

(i) Circumopercular scales (marginals) fold over bases of opercular scales.

1.- Colonies unbranched (flagelliform) or weakly branched with long whip-like branches; body sclerites are thin, smooth, imbricating scales……..***Primnoella***

2.- Colonies dichotomously branched (lyriform), end branches long; body sclerites are thick ascus scales……..***Fannyella* (*Fannyella*)**

(ii) Circumopercular scales (marginals) do not fold over bases of opercular scales.

1.- Colonies unbranched; polyps stand perpendicular to branch ……..***Arntzia***

2.- Colonies branched (pinnate, dichotomous); polyps appressed to branch, inclined upward.

a.- Body scales sculptured with ridges, crests, or smooth granules; coenenchymal scales nearly smooth or sculptured by radial or anastomosing ridges; colonies mostly pinnate, plumose, rarely dichotomous……..***Callogorgia***

b.- Body scales sculptured with finely serrate or tuberculate crests closely radiating from depositional centre; coenenchymal scales sculptured with crowded tubercles; colonies mostly dichotomous, sometimes openly pinnate or quasi-dichotomous ……..***Fanellia***

b) Abaxial side of polyps covered by numerous small warty plates not aligned in regular longitudinal rows except in small, immature individuals; distalmost sclerites not differentiated as opercular scales……..***Ophidiogorgia***

Genus *Digitogorgia* Zapata-Guardiola and López-González, 2010

Digitogorgia Zapata-Guardiola and López-González, 2010b:317.—Zapata-Guardiola and López-González, 2010d:56.

Diagnosis

Primnoidae with bottlebrush colonies and simple branchlets. Polyps elongated, cylindrical, markedly curved upwards to the branch, and arranged in whorls. Accessory operculars eight in number, stick shaped, close to eight operculars. Marginal scales without thorn or keel, eight in number, folding over operculars. Opercular and marginal scales, with pointed digitations distally, attenuated in body scales. Body scales in eight complete longitudinal rows. Coenenchyme with two layers: outer layer of oval shaped scales, inner layer with irregular tuberculate sclerites.

Geographical and bathymetrical distribution

At present, *Digitogorgia* has been reported from Subantarctic regions like Burdwood Bank, and Isla Nueva; between 111 and 2500 m in depth.

Etymology

The generic name combines *digito*- with reference to the distally pointed digitations especially present in opercular, and marginal scales, and *-gorgia*, a common sufix in gorgonian generic names, gender being feminine.

Type species

Digitogorgia kuekenthali Zapata-Guardiola and López-González, 2010.

***Key to the species of the genus* Digitogorgia**

1. Up to 9 scales in the longitudinal abaxial row; accessory opercular scales tear-pointed shape; opercular scales elongated and multi digitated……***D. kuekenthali***
2. 12 scales or above in the longitudinal abaxial row; accessory opercular scales leaf shape; opercular scales less elongated and digitated……..***D. brochi***

Digitogorgia brochi Zapata-Guardiola and López-González, 2010b

(Figures 2.8–2.12)

Thouarella (*Euthouarella*) *brucei*, Broch, 1965:27–28, pl. 4, fig. 11–13.
Digitogorgia brochi Zapata-Guardiola and López-González, 2010d:57.

Examined material

Holotype: NHM B969, "Brategg" Expedition, "Tromso-Tral 2", 54°43'S, 60°14'W, Burdwood Bank, Subantarctica, 111.5 m depth, 09 March 1948.

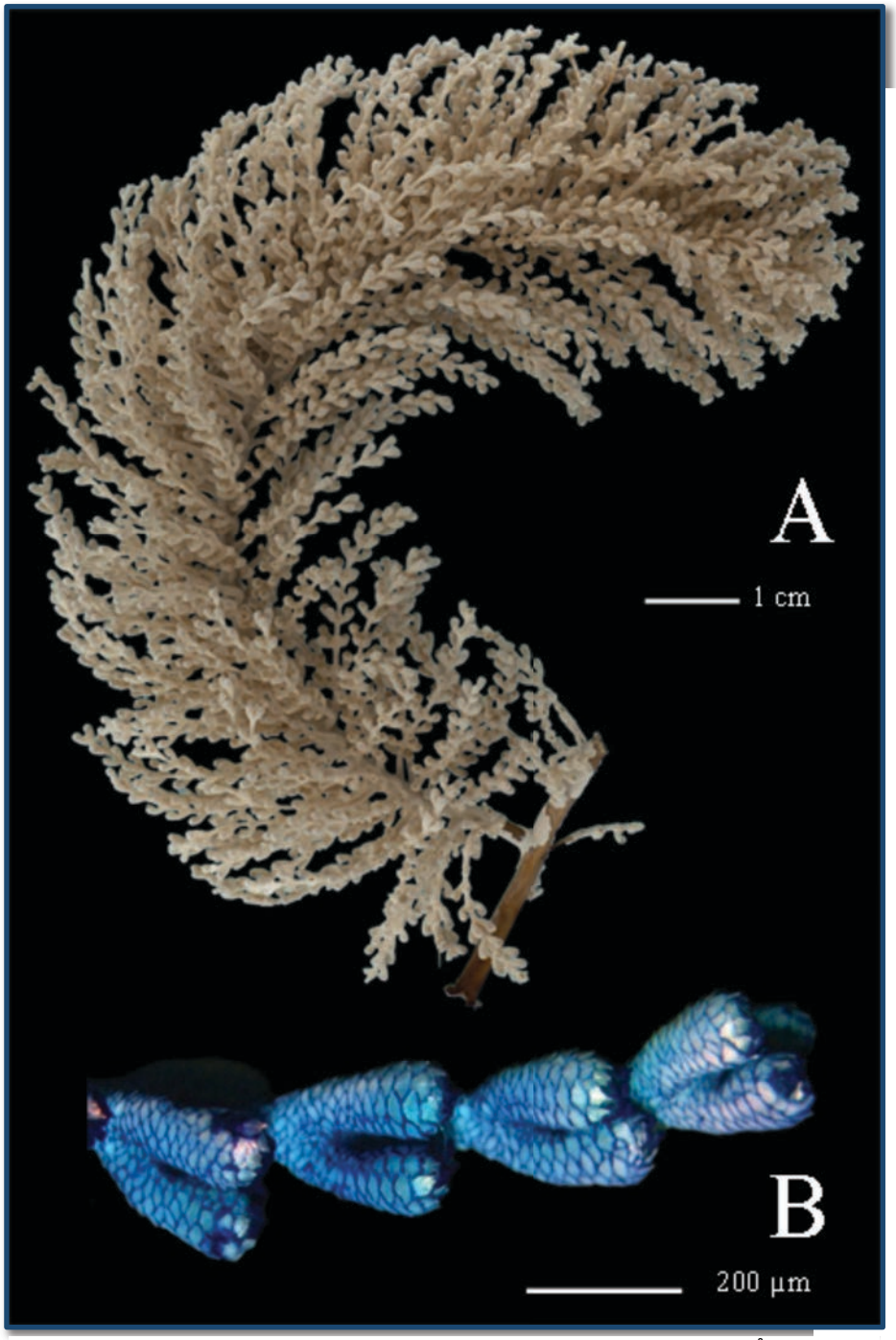


Figure 2.8.- *Digitogorgia brochi*, holotype (NHM B969): **a**, whole colony, photo: Åse Wilhelmsen NHM, Oslo; **b**, detail of a branchlet.

Description of the holotype

Fragment of a colony (Fig. 2.8a), probably a main side branch, of 16 cm in total height and about 4.5 cm in width, with simple branchlets (Fig. 2.8b) up to 3cm in length arising all around the branch forming a bottlebrush shape. Axis brown. Main side branch basal axis diameter 2.3 mm. Main stem basal axis diameter 2.9 mm. Polyps (Fig. 2.9) on branchlets in whorls of 3, 4–5 whorls per cm, directed upwards. Polyps relatively elongate, slightly clavate, about 1.6–2.2 mm in height and 0.6–0.8 mm in diameter.

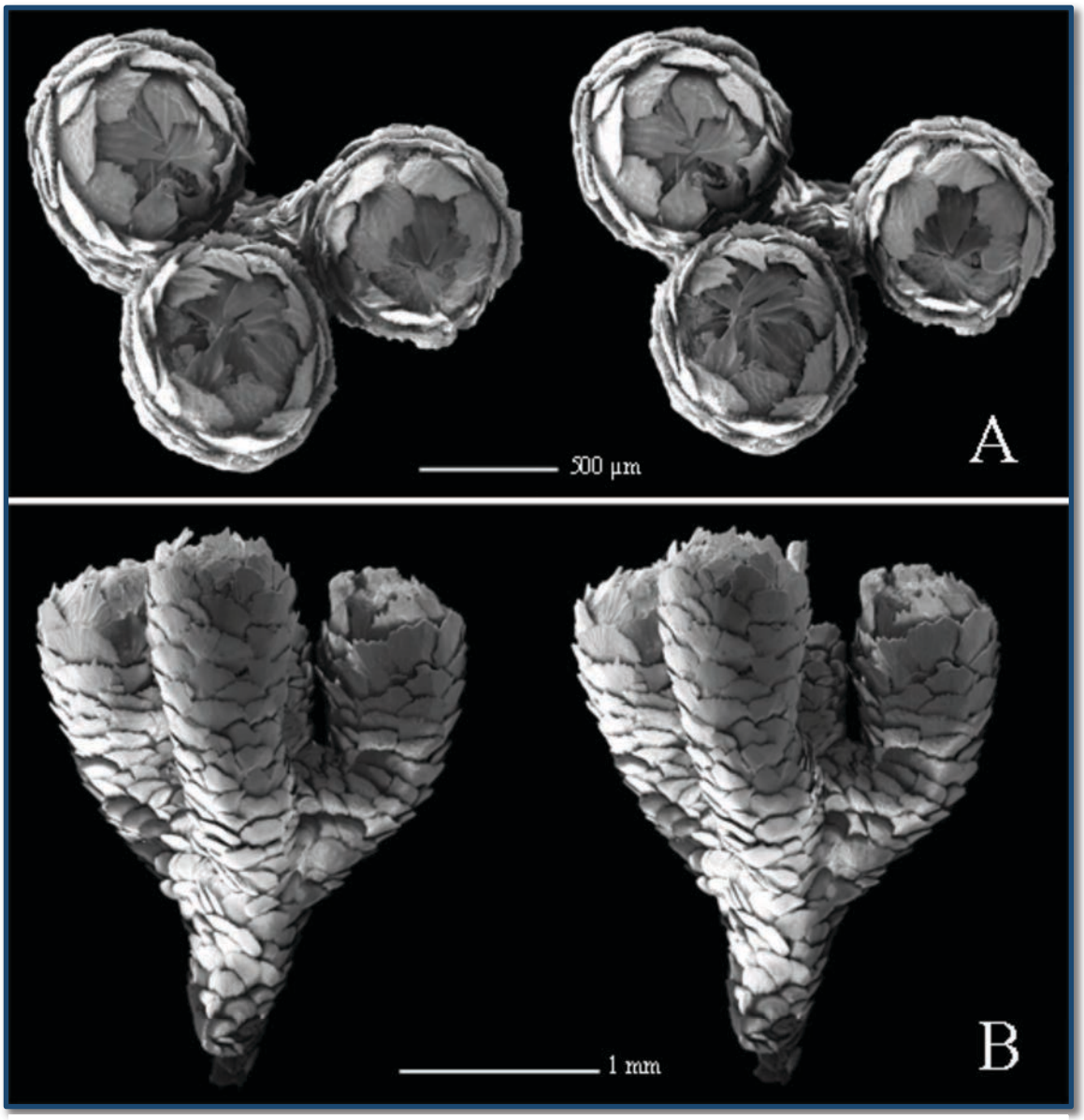


Figure 2.9.- *Digitogorgia brochi*, holotype (NHM B969): **a**, whorl of polyps, oral view, stereo pair; **b**, whorl of polyps, stereo pair.

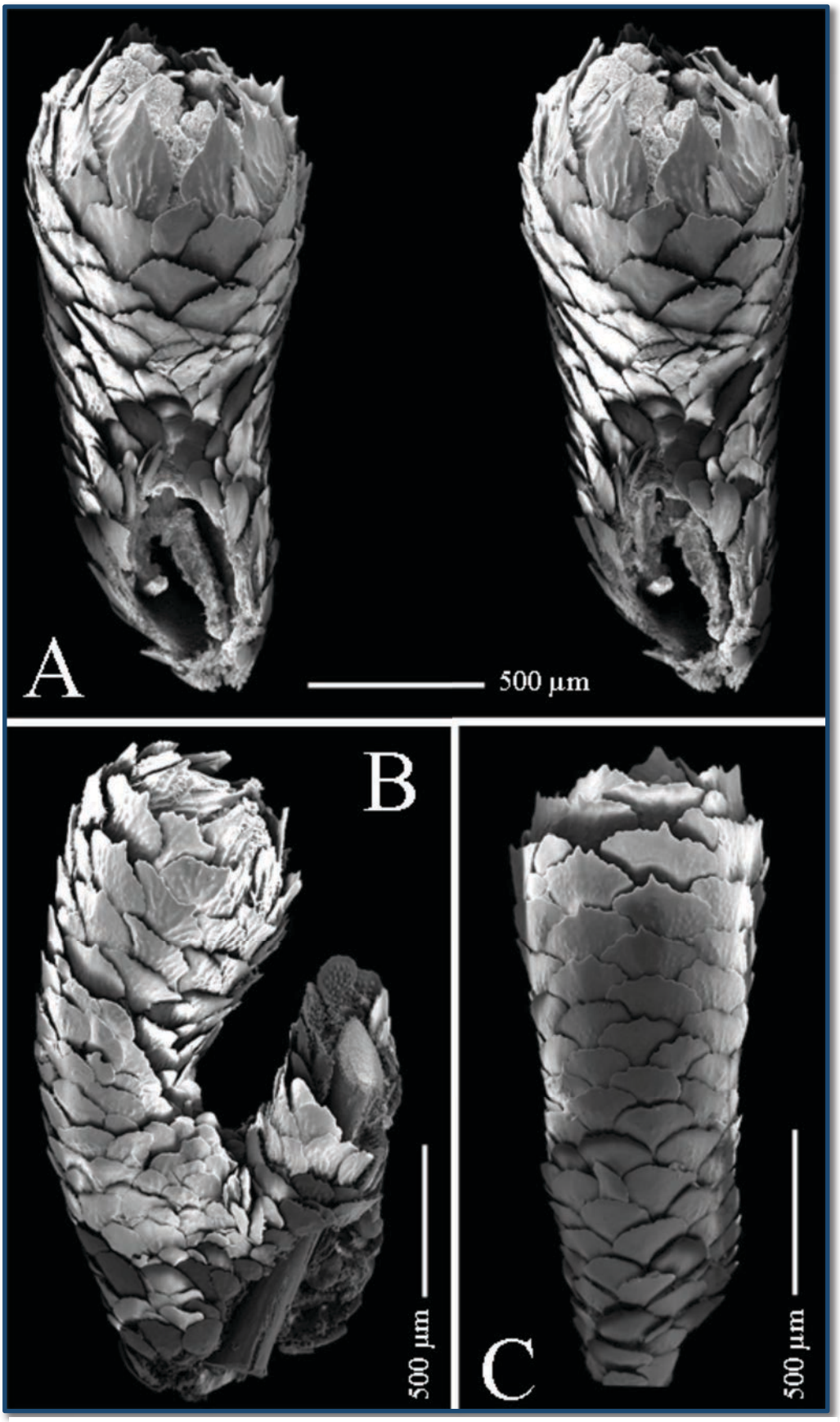


Figure 2.10.- *Digitogorgia brochi*, holotype (NHM B969): **a**, polyps, adaxial view, stereo pair; **b**, polyp, lateral view; **c**, polyp, abaxial view.

Polyp body with eight complete longitudinal rows of scales, those in adaxial rows slightly smaller. About 6–7 scales in each adaxial row (Fig. 2.10a) and 12–13 scales on each abaxial row (Figs. 2.9b, 2.10c). Adaxial rows slightly disorganized basally, but without any reduction in number of rows (Fig. 2.9). Accessory operculars (Fig. 2.11a) eight in number, 0.25–0.44 mm in height and 0.08–0.14 mm in width, more or less triangular and pointed. Proximal inner surface tuberculate, covering about 20–50% of their length, distal inner surface smooth. Outer surface quite granular with several warts on the most proximal portion. Basal margin with digitate processes. Opercular scales (Fig. 2.11b) eight in number, 0.30–0.45 mm in height and 0.16–0.24 mm in width, more or less triangular or oval shaped. Proximal inner surface tuberculate, covering around half of their length, distal surface smooth without keel or thorn. Proximal outer surface granular with several warts on the most proximal part, distal portion quite smooth. Basal margin often with long digitate processes, free margin entire laterally but digitate apically. Marginal scales (Fig. 2.11c) eight in number, 0.33–0.43 mm in height and 0.23–0.46 mm in width, broad, oval shaped. Proximal inner surface tuberculate, covering up to 80% of the length, distal inner surface smooth, without keel or thorn. Outer surface granular, most proximal part with several warts. Basal margin with long digitate processes, free margin entire laterally but digitate apically. Body scales (Fig. 2.12a) more-or-less oval to fan shape, 0.17–0.37 mm in height and 0.26–0.45 mm in width. Inner surface almost completely tuberculate, outer surface granular with several warts on the most proximal part. Free margin as in marginal scales. Coenenchymal sclerites (Fig. 2.12b) in two layers: outer layer with round, oval-shaped sclerites, 0.11–0.24 mm in maximum length, inner surface tuberculate, outer surface granular or smooth, free margin entire or scalloped; inner layer with irregular tuberculate sclerites, 0.06–0.08 mm in maximum length.

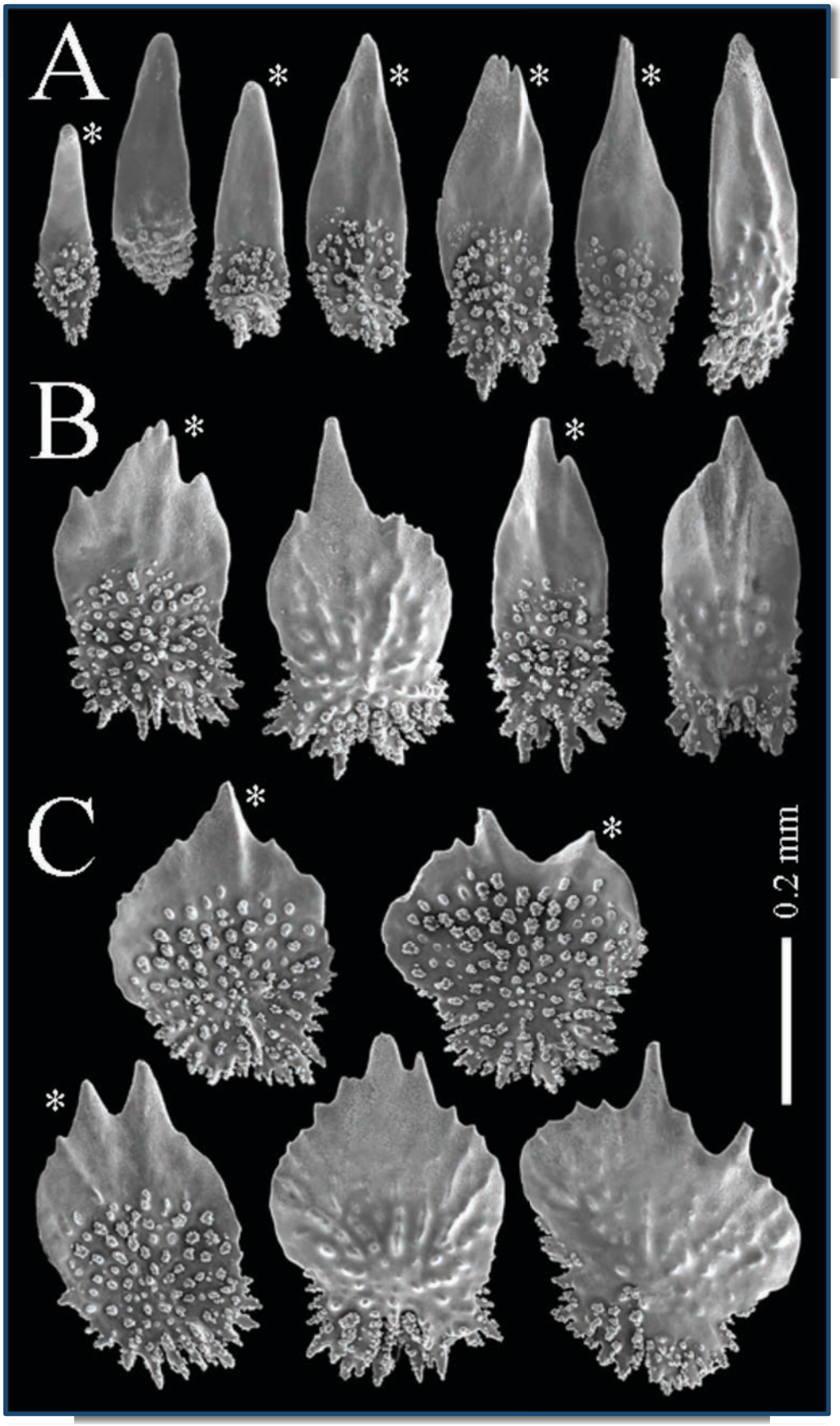


Figure 2.11.- *Digitogorgia brochi*, holotype (NHM B969): **a**, accessory opercular scales; **b**, opercular scales; **c**, marginal scales. ∗ inner surface view.

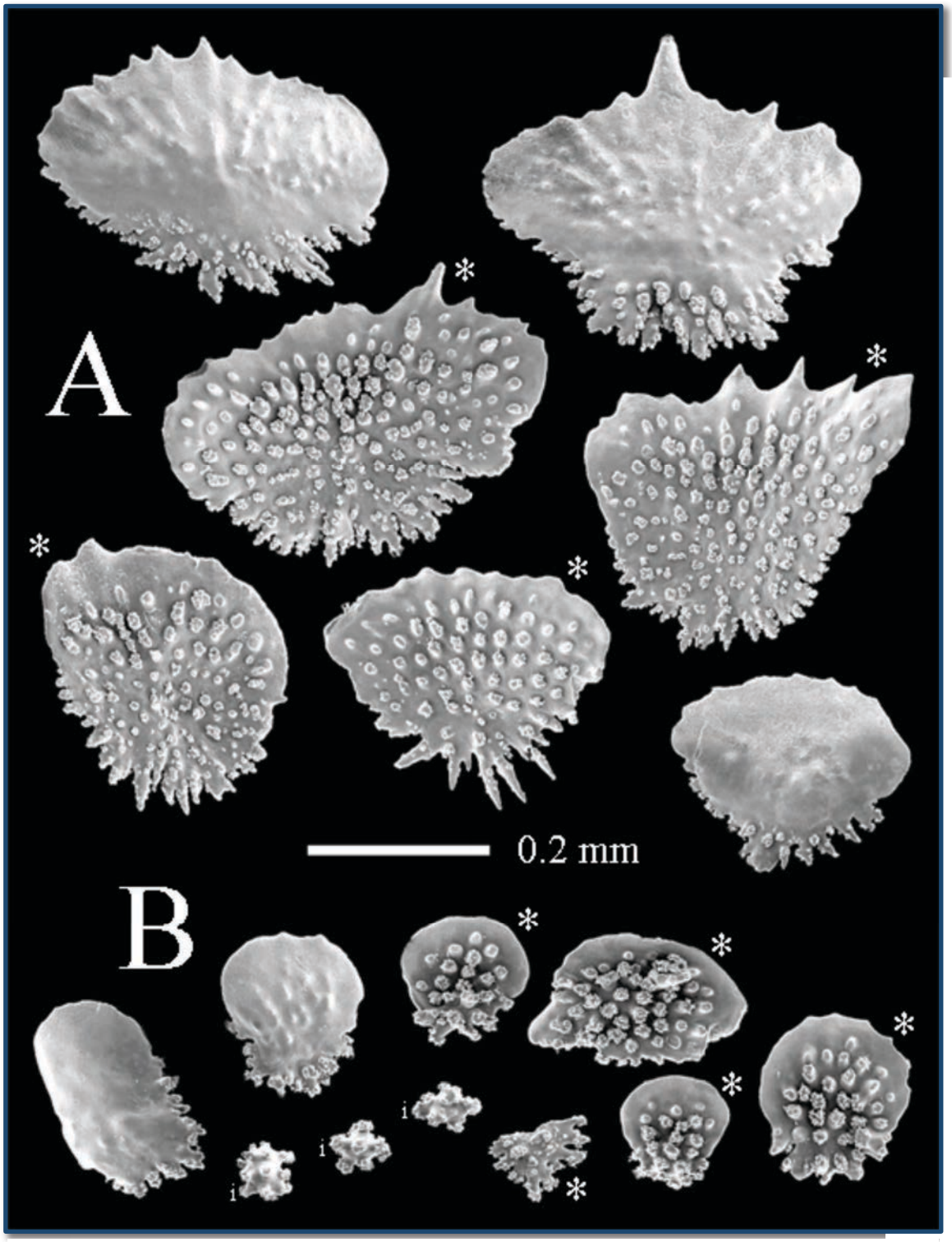
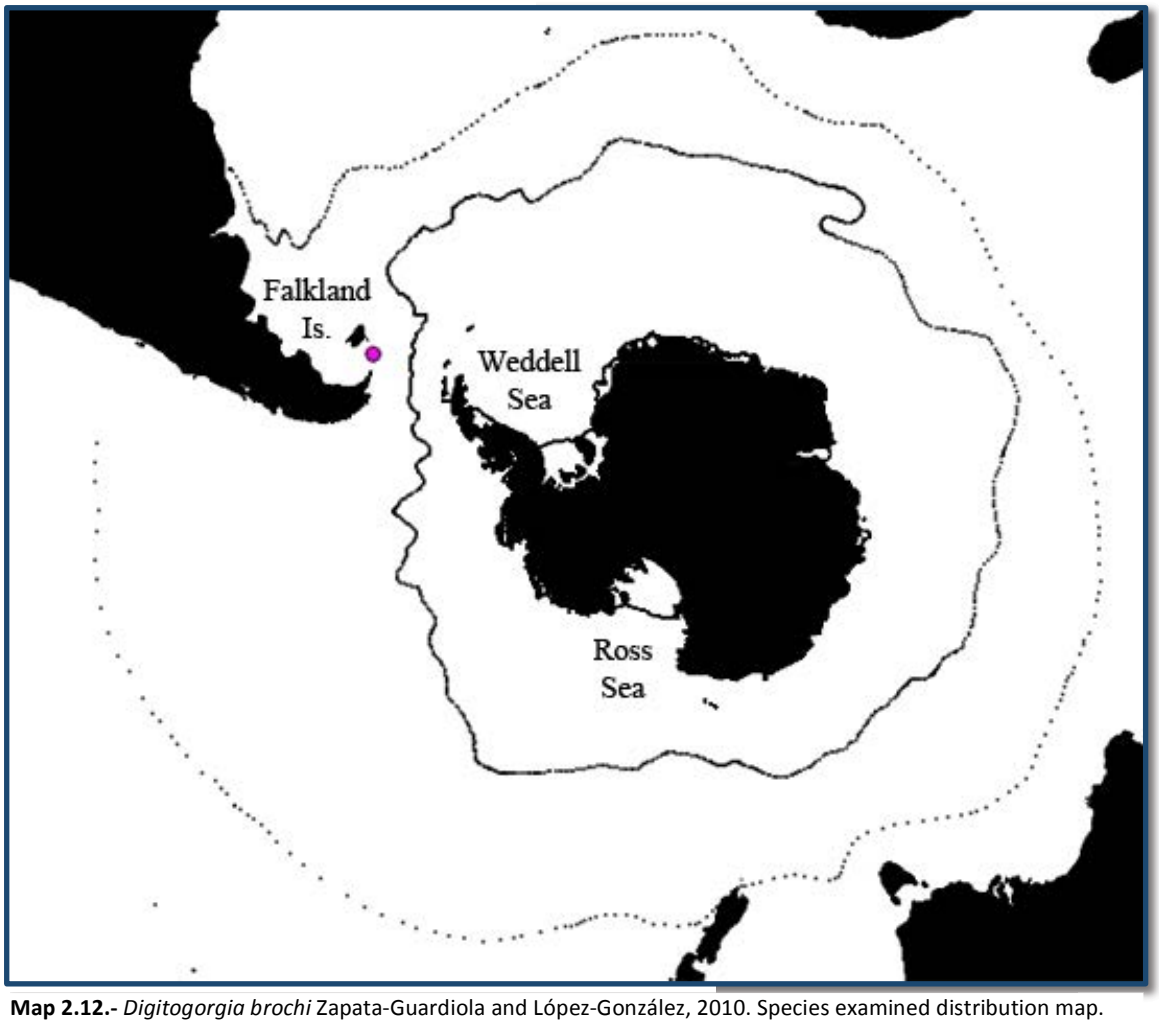


Figure 2.12.- *Digitogorgia brochi*, holotype (NHM B969): **a**, body scales; **b**, coenenchymal scales. **i**, scales from inner layer. ∗ inner surface view.

Geographical and bathymetrical distribution

At present, *Digitogorgia brochi* has only been reported from Burdwood Bank, Subantarctica (Map 2.12), at a depth of 111.5 m.



Map 2.12.- *Digitogorgia brochi* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Pink circle, holotype**.**

Etymology

This species is dedicated to Hjalmar Broch, in recognition of his contribution to our knowledge of octocorals.

Digitogorgia kuekenthali Zapata-Guardiola and López-González, 2010b

(Figures 2.13-2.18)

Digitogorgia kuekenthali Zapata-Guardiola and López-González, 2010b:317.—Zapata-Guardiola and López-González, 2010d:63.

Examined material

Holotype: ZIZMH (C11740), ANT XIX/5, stn PS61/150-01, 54°30.22'S, 56°08.20'W, Burdwood Bank, Subantarctica, 286.3–291.6 m depth, 6 April 2002.

Paratypes: USNM (1128575), ANT XIII/4, stn PS40/114-01, 55°33.40'S, 65°54.60'W, south east of Isla Nueva, Subantarctica, 2,468 m depth, 18 May 1996, two fragments of colony; BEIM (CRO-0030), ANT XIII/4, stn PS40/114-01, 55°33.40'S, 65°54.60'W, south east of Isla Nueva, Subantarctica, 2468 m depth, 18 May 1996, two fragments of colony.

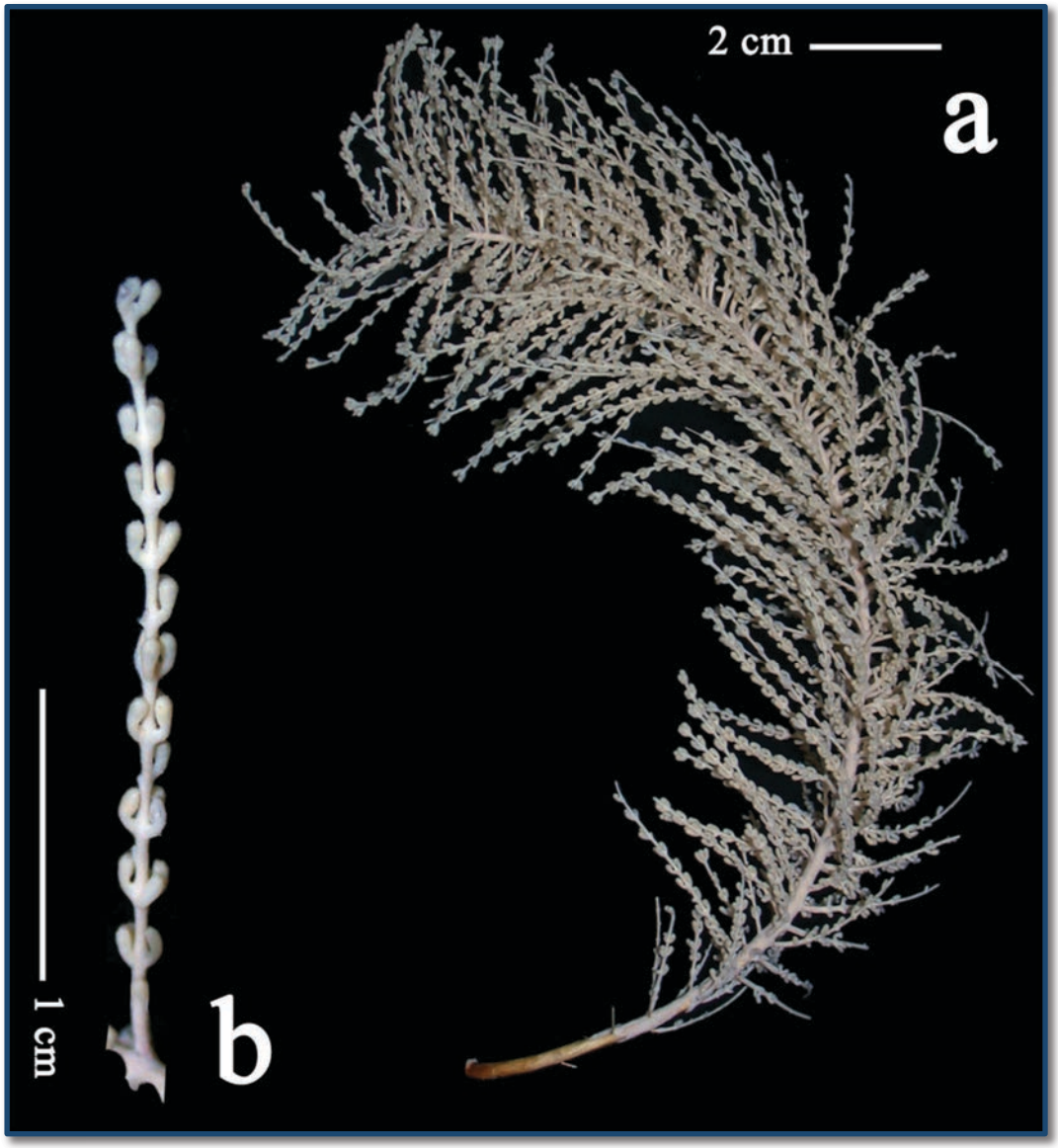


Figure 2.13.- *Digitogorgia kuekenthali*, holotype (ZIZMH C11740): **a**, whole colony; **b**, detail of a branchlet.

Description of the holotype

Bottlebrush colony (Fig. 2.13a) 21 cm in total height and 6.4 cm in width, with simple branchlets (Fig. 2.13b) arising all around the main stem, up to 4.4 cm in length. Axis light brown to grey in colour, without holdfast. Basal axis diameter of 1.3 mm Polyps (Fig. 2.14a) on branchlets placed in whorls, occasionally in pairs, 3–4 polyps per whorl and 3–4 whorls per cm and directed upwards. Polyps not present on main stem.

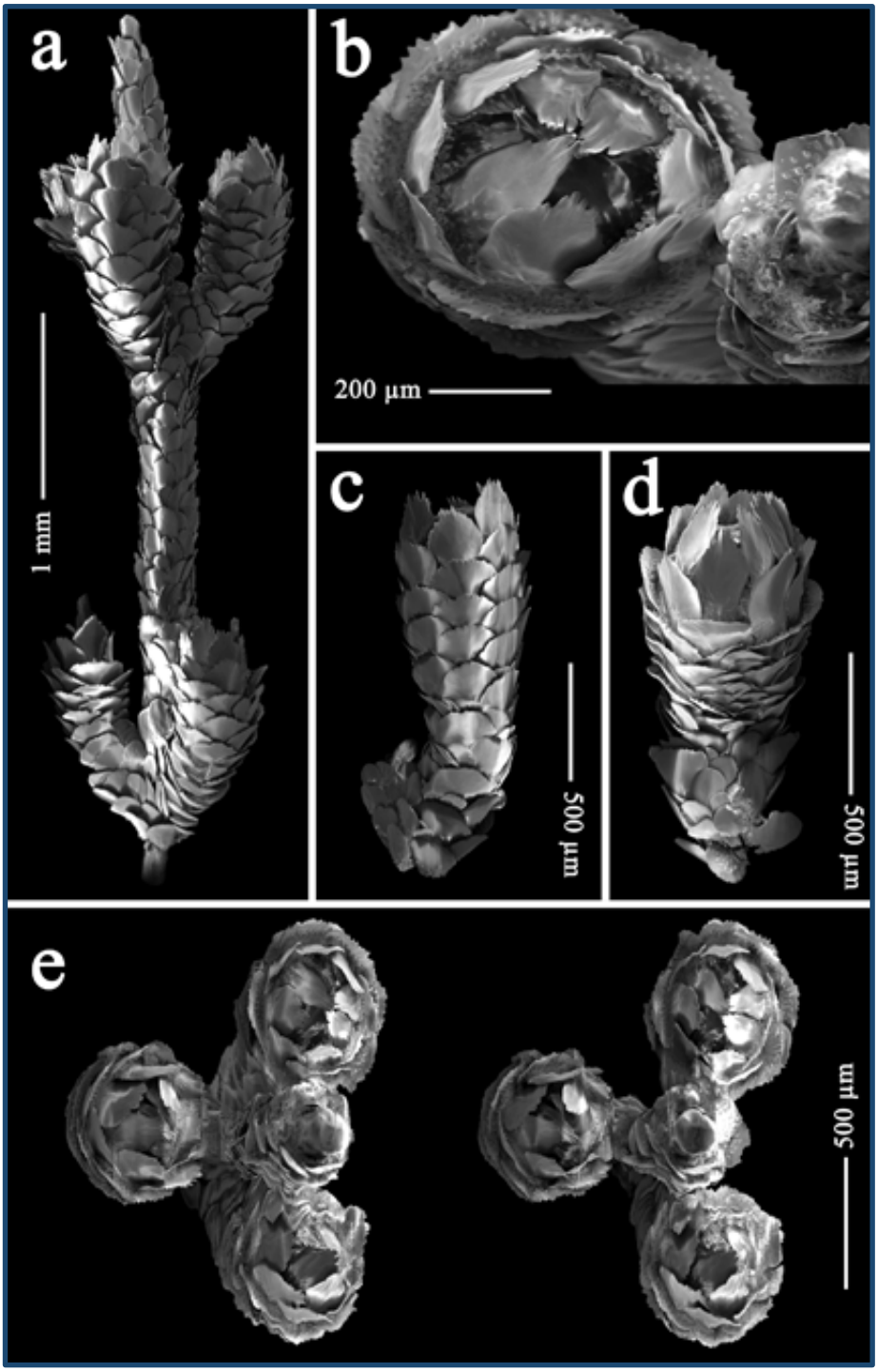


Figure 2.14.- *Digitogorgia kuekenthali*, holotype (ZIZMH C11740): **a**, detail of branchlet; **b**, polyp on oral view; **c**, polyp on lateral view; **d**, polyp on adaxial view; **e**, whorl on oral view, stereo pair.

Polyps relatively elongated, cylindrical, about 1.24–1.86 mm in height and 0.47–0.63 mm in diameter; with a conical operculum. Polyp body with eight complete longitudinal rows of scales, those in adaxial rows smaller. The two adaxial rows are slightly disorganized, but without any reduction in number of rows, even down to the base (Figs. 2.14d, 2.15a) There are 8–9 scales on each abaxial row (Figs. 2.14c and 2.15c). Accessory operculars (Fig. 2.16a) eight in number, 0.13-0.42 x 0.05-0.1 mm, stick or spoon shaped. Proximal inner surface tuberculate, covering about 40–60% of their length. Distal inner surface of scale smooth. Outer surface quite granular. Basal and distal margin with digitate processes. Accessory opercular scales situated under opercular scales. Opercular scales (Fig. 2.16b) eight in number, 0.31-0.48 x 0.10-0.16 mm, with similar shape as accessory operculars. Proximal inner surface tuberculate, covering around half of their length; distal inner surface quite smooth, outer distal surface granular. Basal margin with tuberculate processes, free margin smooth laterally but digitate apically. Marginal scales (Fig. 2.16c) eight in number, 0.34-0.43 x 0.13-0.29 mm, oval shaped. Proximal inner surface tuberculate, covering up to 80%. Distal inner surface smooth, without keel. Proximal outer surface granular, distal outer surface smooth. Basal margin with tuberculate processes, free margin smooth laterally but toothed apically. Body scales (Fig. 2.17a) round, 0.24–0.35 mm in maximum length. Inner surface almost completely tuberculate, outer surface slightly granular. Free margin smooth or partly toothed, basal margin with tuberculate processes. Coenenchymal scales (Fig. 2.17b, 2.18) round, oval shaped, 0.13–0.27 mm in maximum length. Inner surface tuberculate, outer surface smooth. Basal margin warty with digitate processes, free margin smooth.

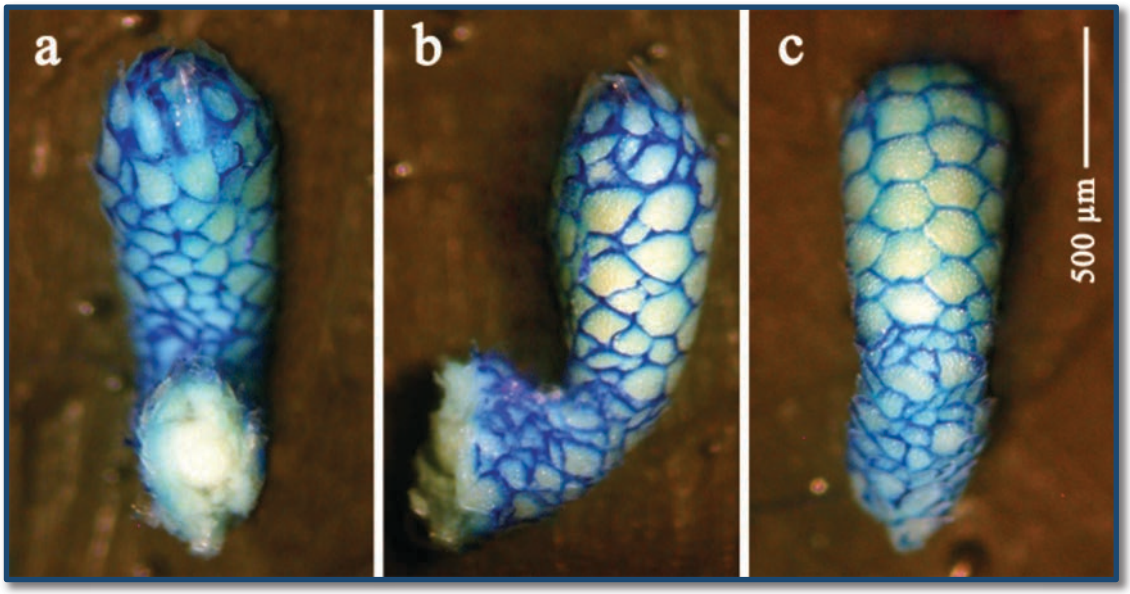
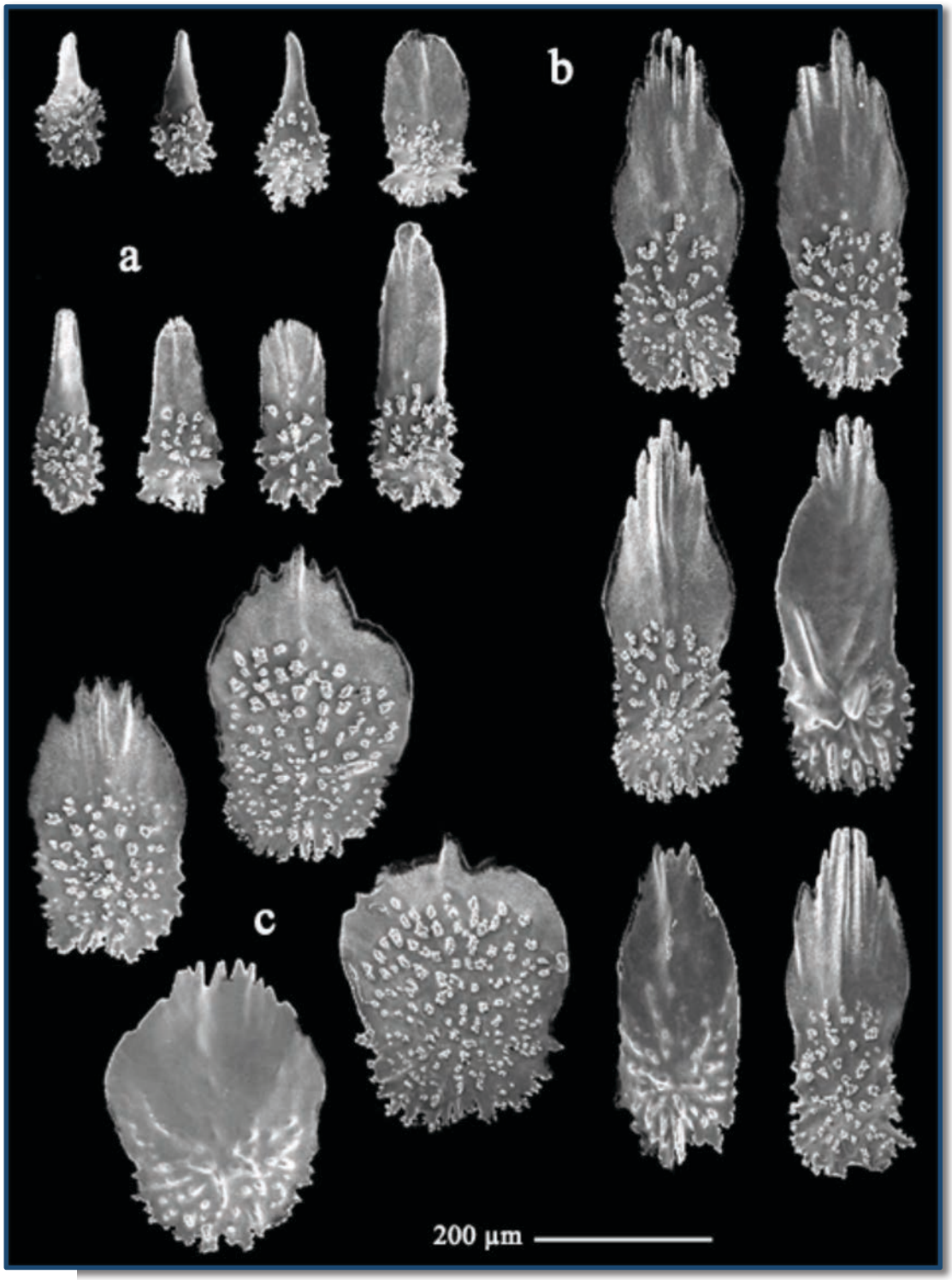


Figure 2.15.- *Digitogorgia kuekenthali*, holotype (ZIZMH C11740): **a**, polyp on adaxial view; **b**, polyp on lateral view; **c**, polyp on abaxial view.

Variations from holotype

The fragments of the additional material have a general colonial structure quite similar to that of the holotype. One of the fragments shows a lateral branch of 11.4 cm, which has similar simple branchlets as the main stem. Polyps reach up to about 2 mm and are arranged in

whorls of two, three or four, and up to five whorls per centimetre. Distribution and form of the sclerites from polyps and coenenchyme are similar to that of the holotype.



Fi gure 2.16.- *Digitogorgia kuekenthali*, holotype (ZIZMH C11740): **a**, accessory opercular scales; **b**, opercular scales; **c**, marginal scales.

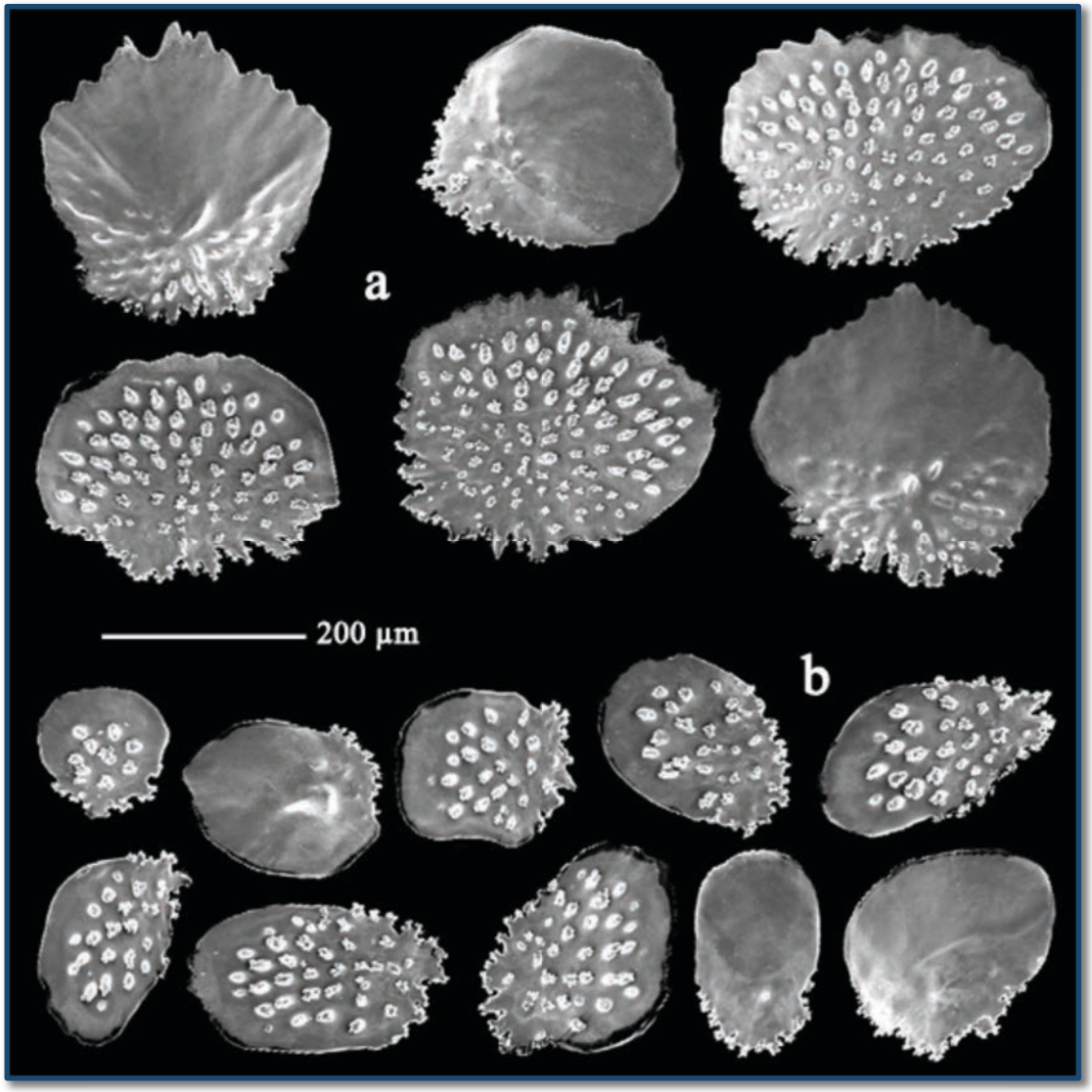


Figure 2.17.- *Digitogorgia kuekenthali*, holotype (ZIZMH C11740): **a**, body scales; **b**, coenenchymal scales.

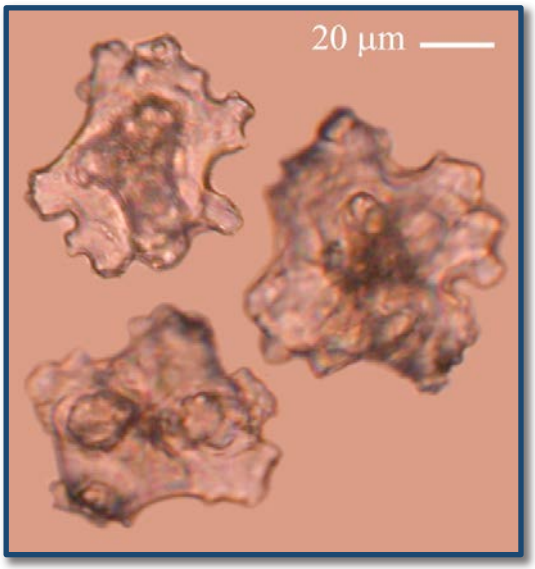
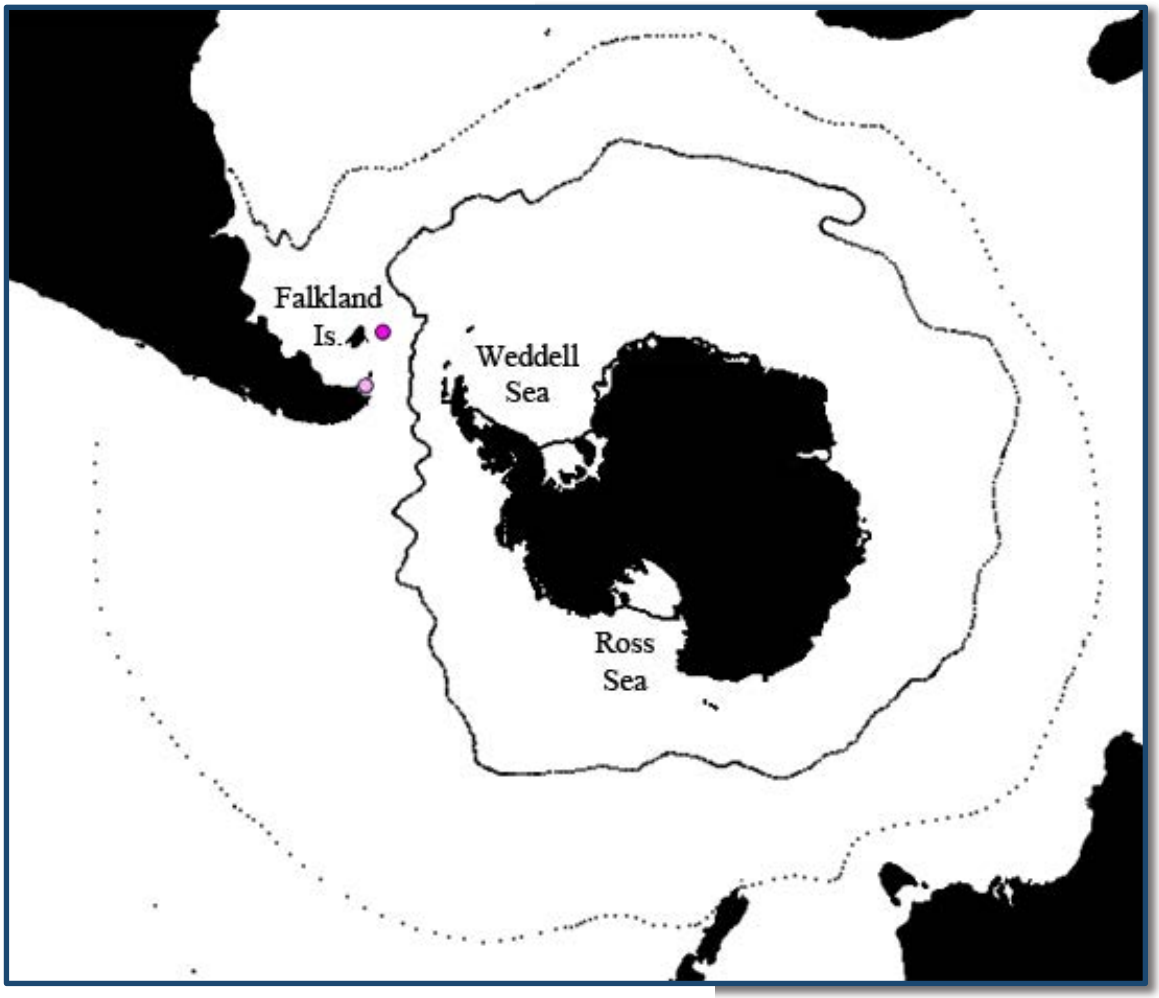


Figure 2.18.- *Digitogorgia kuekenthali*, holotype (ZMH C11740). Coenenchymal scales from inner layer, optical microscope view.

Geographical and bathymetrical distribution

At present, *Digitogorgia kuekenthali* is only known from Burdwood Bank, and from south east of Isla Nueva, Subantarctica (Map 2.13), between 286.3 and 2468 m in depth.



Map 2.13.- *Digitogorgia kuekenthali* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Dark pink, holotype; light pink, paratype.

Etymology

The specific name *kuekenthali* is chosen in honour of Prof. W. Kükenthal, in recognition of his valuable contributions to the knowledge of Octocorallia taxonomy.

Genus *Fannyella* Gray, 1872

Fannyella Gray, 1872:744.—Bayer, 1990:774; 1998:164-165 [in part: *F. rossii*, *F. kuekenthali*].—Zapata-Guardiola and López-González, 2010e.
Ascolepis Thomson and Rennet, 1931:20-23.—Bayer, 1981:936 [in part: not fig. 66, key to genus].—Bayer and Stefani, 1989:454 [key to genus].

Diagnosis (modified from Bayer 1998, modifications in bold)

Primnoinae with uniplanar and dichotomously branching, often lyrate, **also bottlebrush colonies**. Polyps upward, appressed to stem, straight or adaxially incurved, and arranged in whorls or in spirals. Opercular scales eight in number. Marginal scales also eight in number, forming a circumoperculum that can fold over the operculars. Marginals and body scales distinctively shaped as ascus scales, aligned in eight longitudinal rows. Adaxial scales often reduced in size and number.

Geographical and bathymetrical distribution

At present, *Fannyella*, has a circumantarctic distribution, from Peninsula Antarctica and Weddell Sea to the Ross Sea, between 40 and 900 m in depth.

Etymology

The generic name is dedicated after a lady who was friend of Mrs. Hooker, who took great interest in the results of Captain Ross's Antarctic voyage.

Type species

Fannyella rossii Gray, 1872

***Key to the species of the genus* Fannyella**

1. Colonies bottlebrush with simple branchlets…… **Subgenus *Scyphogorgia***: ***F. abies***
2. Colonies uniplanar, multiflabellate with a dichotomously branching.
   a. Polyps covered by 6 longitudinal rows of body wall scales, adaxial row usually absent resulting in a naked face, marginal scales not spinose or keeled……..**Subgenus *Fannyella***
      i. Polyps arranged in whorls……..***F. rossii***
      ii. Polyps isolated in spirals……***F. kuekenthali***
   b. Polyps covered by 8 longitudinal rows of body wall scales, spinose marginal scales keeled……..**Subgenus *Cyathogorgia***: ***F. spinosa***

Subgenus *Scyphogorgia* Cairns and Bayer, 2009

Diagnosis (from Cairns and Bayer 2009)

Bottlebrush colonies, with a discoidal base usually attached to a hard substrate. Polyps arranged in whorls; calyces appressed, facing upward not fused basally. Small operculum present, distal inner surface of opercular multiridged, without keel. Opercular aligned with marginal scales. Eight marginal ascus scales, with transverse ridge separating smooth distal portion from tuberculate proximal portion, fold over the operculum, forming a circumoperculum; distal margin spinose, inner surface with a longitudinal keel. Polyps protected by eight imbricate longitudinal rows of body wall ascus scales; two abaxial rows with a variable number of scales in each row, usually more than five. The abaxial submarginals also spinose; those more proximal with spines progressively less prominent. Perimeter of cup-like structure of ascus scales finely serrated. Adaxial row quite short but present, resulting in complete coverage of the polyp. Coenenchymal scales in two layers: outer layer composed of plates with a smooth outer surface, inner layer composed of tuberculate spindles that compose the walls of longitudinal stem canals.

Geographical and bathymetrical distribution

To the South Orkney Islands (Coronation Island), South Shetland Islands (Elephant Island), the Antarctic Peninsula and Cape Adare and Possession Islands from the eastern Ross Sea, between 94 and 550 m in depth.

Etymology

The subgeneric name combines the Greek word *skyphos*- meaning cup, in allusion to the cup-shaped body wall ascus scales, and -*gorgia*, a common suffix in gorgonian generic names, gender being feminine.

Type species

Fannyella abies (Broch, 1965).

Fannyella (*Scyphogorgia*) *abies* (Broch, 1965)

(Figures 2.19-2.23)

Thouarella (*Euthouarella*) *abies* Broch, 1965:29–30; pl. 5, figs 14–16, pl. 7, fig. 22.
Thouarella abies —Bayer 1998:171, 173 (in text).
Fannyella (*Scyphogorgia*) *liouvillei* —Cairns and Bayer 2009:28, 36; figs 8i–p (in part: *Thouarella abies*).
Fannyella (*Scyphogorgia*) *abies* —Zapata-Guardiola and López-González, 2010e:2004.

Examined material

Holotype: NHM B970, "Brategg" Expedition, "Tromso-Tral 2", 6 nautical miles north of Coronation Island, South Orkneys, Antarctica, 274.2–310.76 m depth, 30 November 1959, one colony.

Additional material: CRO-0047 and CRO-0050, ANT XVII-3, stn 158-01, 63°04.70'S, 57°31.60'W, South Shetland Islands, Antarctica, 94–95 m depth, 26 April 2000, one colony each; CRO-0045, TAN0402, stn 14, 71°43.88'S, 171°45.00'E, Cape Adare, Antarctica, 451 m depth, 5 February 2004, one colony; CRO-0046, TAN0402, stn 20, 71°44.44'S, 171°38.64'E, Cape Adare, Antarctica, 400–415 m depth, 5 February 2004, one colony fragment; NIWA 60493, TAN0402, stn 91, 72°16.61'S, 171°26.94'E, Cape Adare, Antarctica, 409–414 m depth, 14 February 2004, five fragments of colonies and one colony; MNA 2463 and MNHN-1K.2009–810, VLT ITALICA (XIX), stn A-1, 71°15.5'S, 170°41.9'E, Cape Adare, Antarctica, 515 m depth, 15 February 2004, one colony and three fragments respectively; MNA 2464, VLT ITALICA (XIX), stn H-OUT-2 (ter), 72°17.1'S, 170°29.9'E, Possession Islands, 369–388 m depth, 17 February 2004, two fragments of colonies; MNA 2465, VLT ITALICA (XIX), stn H-OUT-3 (bis), 72°17.4'S, 170°26.4'E, Possession Islands, 205–258 m depth, 17 February 2004, two fragment of colonies and three colonies fragmented; NIWA 60494, TAN0402, stn 105, 71°15.45'S, 170°38.08'E, Cape Adare, Antarctica, 462–470 m depth, 18 February 2004, five fragments of colonies and five colonies; NIWA 60495, TAN0402, stn 186, 71°30.72'S, 171°25.51'E, Cape Adare, Antarctica, 389–390 m depth, 27 February 2004, two colonies; NIWA 60496, TAN0402, stn 188, 71°32.85'S, 171°6.67'E, Cape Adare, Antarctica, 280–286 m depth, 27 February 2004, one colony; CRO-0049, ANT XXIII-8, stn 608-01, 61°11.34'S, 54°43.17'W, Elephant Island, Antarctica, 293 m depth, 20 December 2006, one colony; CRO-0051, ANT XXIII-8, stn 687-01, 62°35.19'S, 54°45.99'W, south Elephant Island, Antarctica, 263 m depth, 4 January 2007, one colony; CRO-0048, ANT XXIII-8, stn 693-01, 62°25.84'S, 55°35.07'W, south Elephant Island, Antarctica, 243 m depth, 5 January 2007, one broken colony.

Description of the holotype

Colony (Fig. 2.19a) 36 cm total height and 6 cm width, bottlebrush with simple branchlets (Fig. 2.19b) up to 4 cm long, all around. Axis dark brownish, broken in its proximal portion, without holdfast. Polyps (Figs. 2.19b, 2.20a) straight and directly upward to branchlets, placed in whorls, three to five polyps per whorl (Fig. 2.20b) and seven or eight whorls per centimetre. Polyps not present on main stem. Polyps (Fig. 2.20) cylindrical, about 1.00–1.67 mm height and up to 0.49 mm diameter. Polyp body with six longitudinal rows of scales, six or seven scales on each longitudinal abaxial row (Fig. 2.20c) overlapping one another, and adaxial row partially naked with two or three scales (Fig. 2.20d).

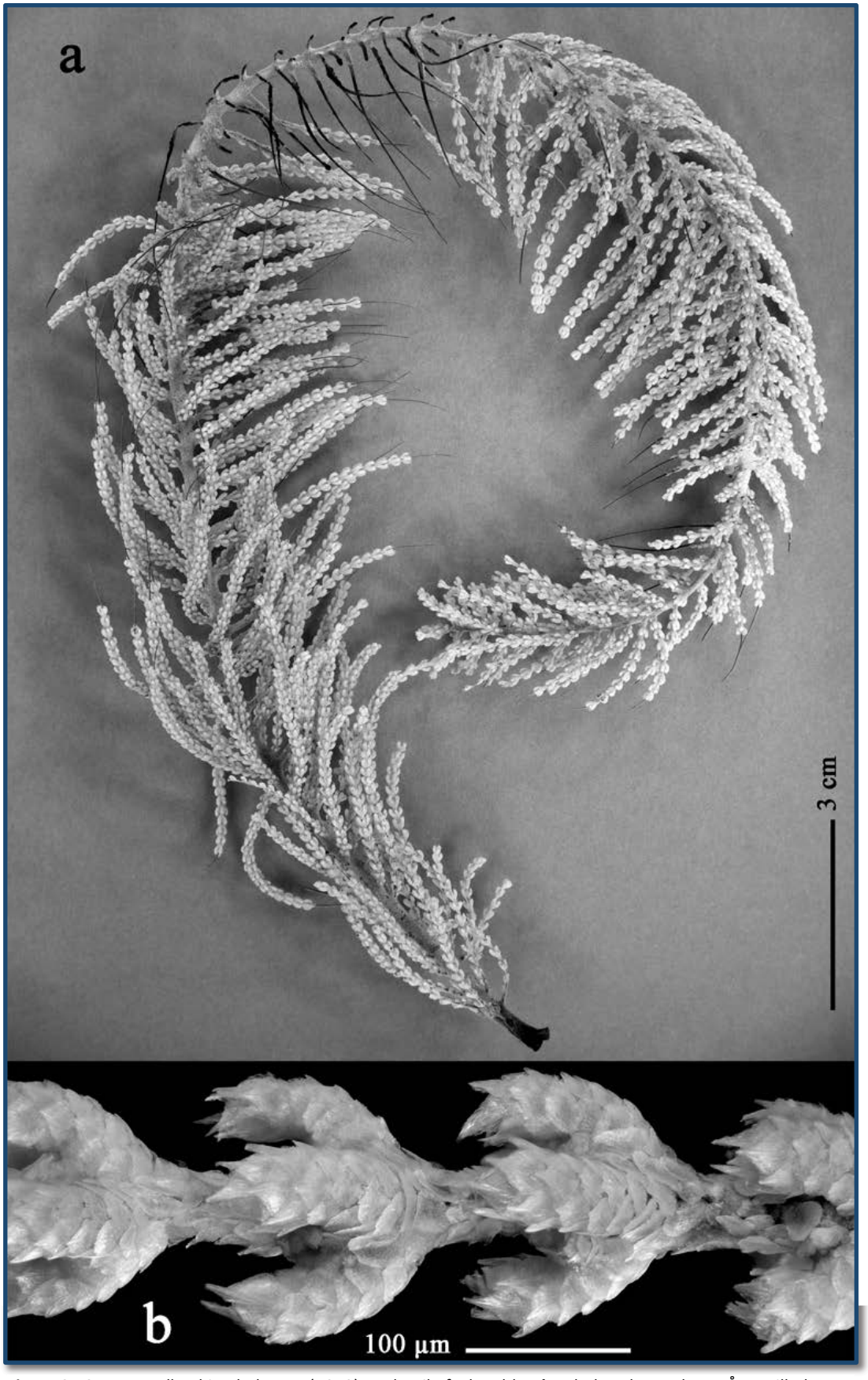


Figure 2.19.- *Fannyella abies*, holotype (B970): **a**, detail of a brachlet; **b**, whole colony. Photo: Åse Wilhelmsen NHM, Oslo.

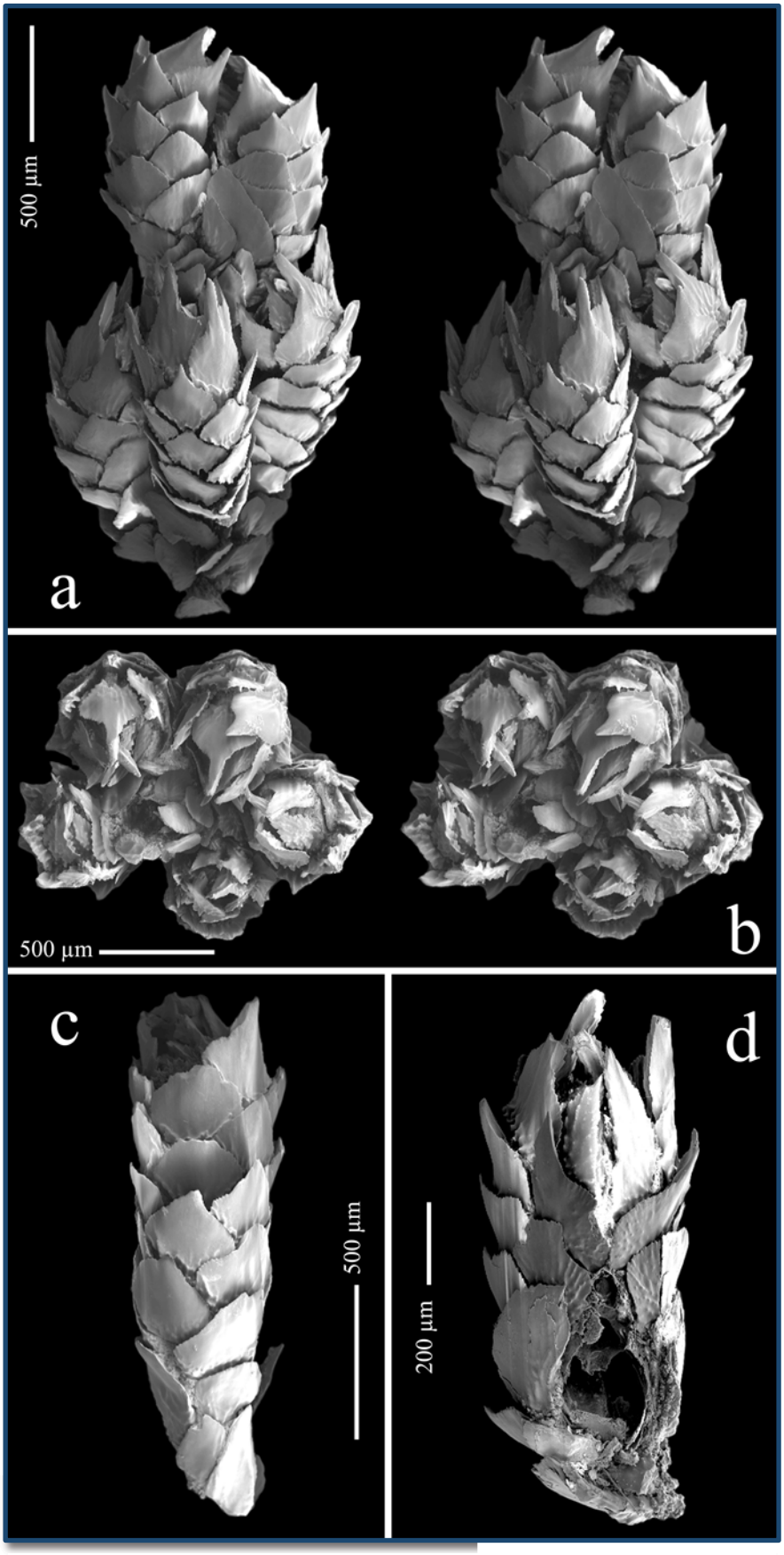


Figure 7.20.- *Fannyella abies*, holotype (B970): **a**, whorl of polyps, stereo pair; **b**, whorl on oral view, stereo pair; **c**, polyp on outer-lateral view; **d**, polyp on adaxial view.

Opercular scales (Fig. 2.21a) eight in number, 0.17–0.38 by 0.09–0.2 mm, triangular with acute tip or small thorn, two adaxial scales smaller. Proximal inner surface with large tubercles, covering at least half of their longitude; distal surface ridged, with a weak keel. Outer surface strongly granulated forming ridges. Basal margin with digitate processes, free margin serrated. Marginal scales (Fig. 2.21b) eight in number, 0.35–0.50 by 0.19–0.29 mm, triangular with acute tip or small thorn. Proximal inner surface with large tubercles. Distal inner surface granulated forming ridges longitudinally near tip. Outer surface with transverse crest dividing the scale into two distinct parts: distal smooth and proximal tuberculate. Basal margin with digitate processes, free margin serrated.

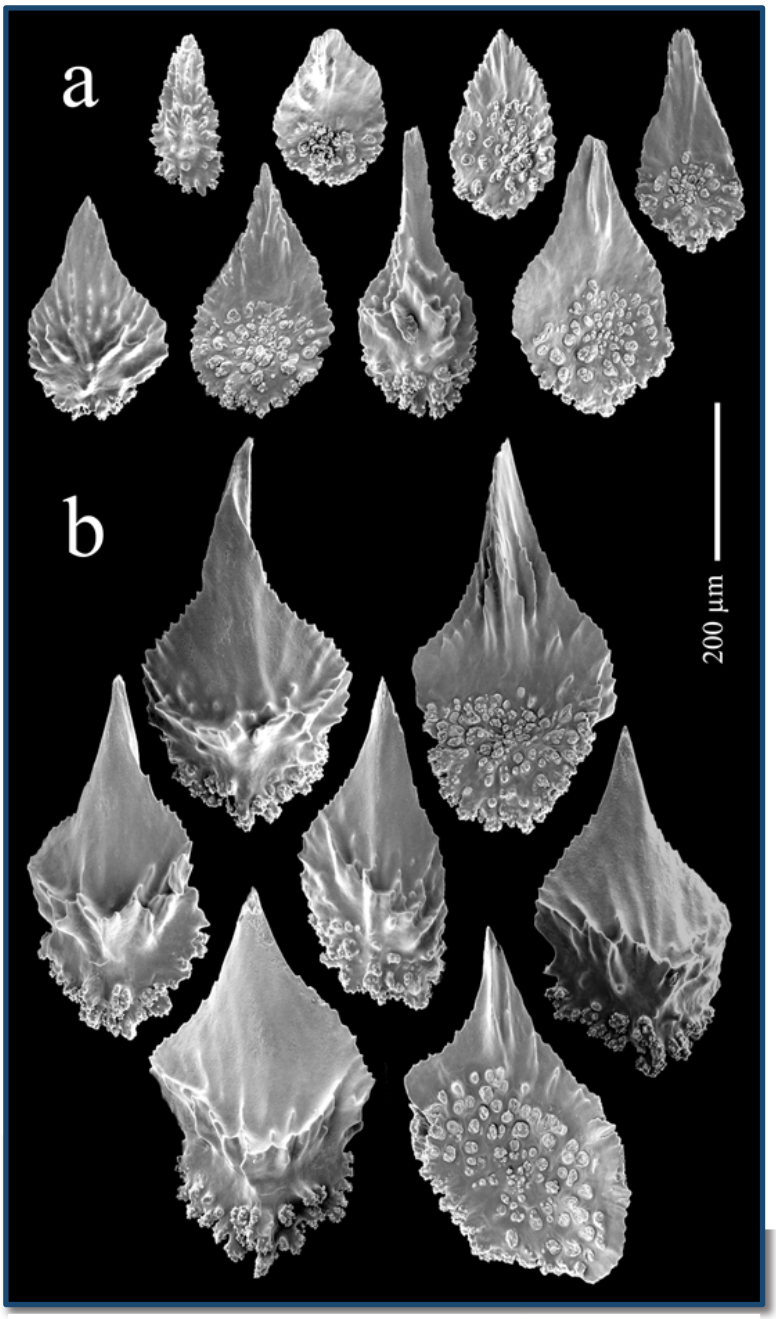


Figure 2.21.- *Fannyella abies*, holotype (B970): **a**, opercular scales; **b**, marginal scales.

Body scales (Fig. 2.22a) fan-shaped with pointed tips, 0.27–0.38 by 0.26–0.38 mm. Proximal inner surface almost completely covered by large tubercles, distal inner surface ridged. Outer surface and free margins as in marginal scales. Coenenchymal scales (Fig. 2.22b) round, oval-shaped, 0.20–0.48 mm maximum length. Inner surface tuberculate, large warts, outer surface smooth. Free margin smooth or finely serrated.

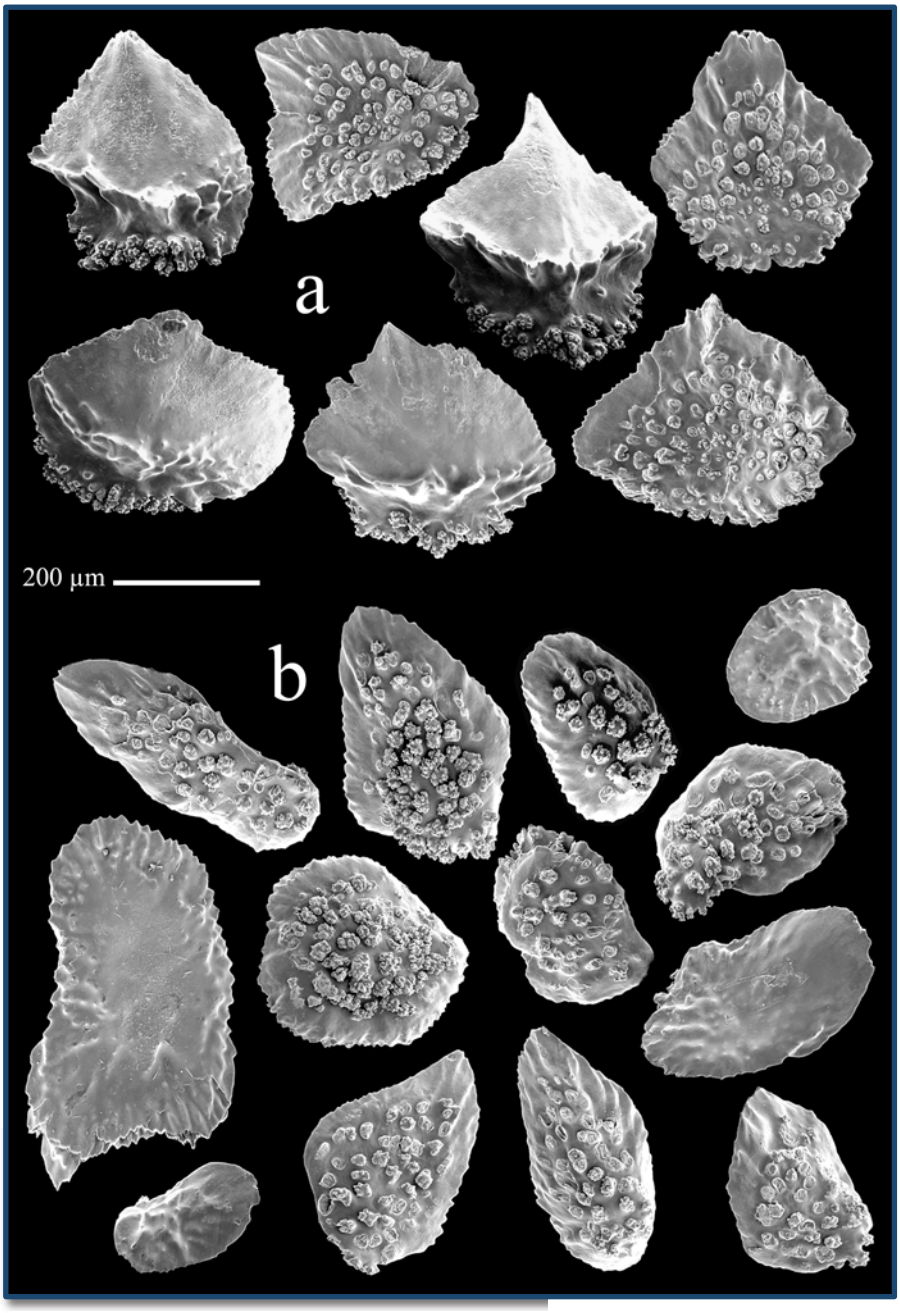


Figure 2.22.- *Fannyella abies,* holotype (B970): **a**, body scales; **b**, coenenchymal scales.

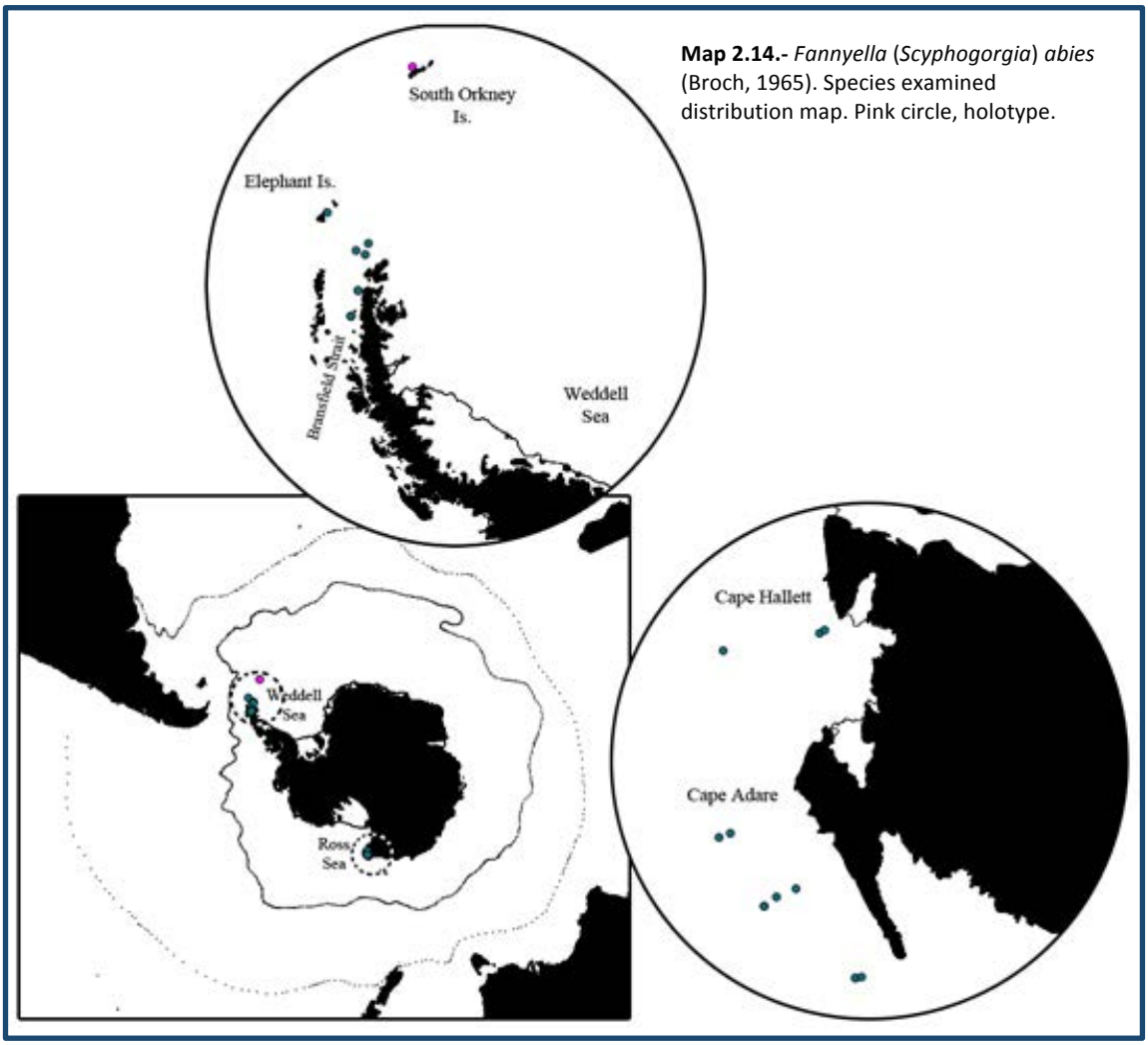
Figure 2.23.- Bottlebrush colony shape variability in *Fannyella abies*: **a**, CRO-0049, preserved material; **b**, MNA 2463, living material; **c**, CRO-0048, living material.

Variations from holotype

The general colonial structure of the studied specimens is quite similar to that of the holotype. Single bottlebrush colonies up to 36 cm height and 7 cm width, some colonies divided basally giving up to seven main side branches (Fig. 2.23) of up to 45 cm height and 6.8 cm width each, giving a total colony height of 48.5 cm and a total width of 18 cm. Branchlets simple, up to 76 mm. Branchlets quite crowded, more than 12 branchlets per centimetre, sometimes giving the false impression of being ramified at their base. Polyps arranged in three to eleven whorls, usually four to six. Whorls from four to nine per centimetre, usually six or seven. Single polyps also present on main stem. Calyces from 0.85 to 2.2 mm height and from 0.44 to 0.66 mm width, inclined towards the branchlet, composed of five to eight scales in each longitudinal abaxial row. Tentacular scales, eight in number, range from 0.24 to 0.63 mm height and from 0.07 to 0.25 mm width. Marginal scales in groups of eight range from 0.43 to 0.85 mm height and from 0.22 to 0.5 mm width. Body wall scales range from 0.24 to 0.45 mm height and from 0.23 to 0.60 mm width; coenenchymal scales with a maximum length of 0.09–0.50 mm. Sclerites form and distribution are as in the holotype. Living colonies (Fig. 2.23b,c) orange in colour, while preserved colonies (Fig. 2.23a) have a dark brown stem and creamy white coenenchyme and polyps.

Geographical and bathymetrical distribution

At present, *Fannyella abies* (Broch, 1965) is known from the South Orkney Islands (Coronation Island), South Shetland Islands (Elephant Island), the Antarctic Peninsula and Cape Adare and Possession Islands from the eastern Ross Sea (Map 2.14) between 94 and 550 m in depth. Cairns and Bayer (2009) reported additional material and locations of this species as *Fannyella* (*Scyphogorgia*) *liouvillei* (at least the lots USNM 58156, USNM 82981, and USNM 82980).



Map 2.14.- *Fannyella* (*Scyphogorgia*) *abies* (Broch, 1965). Species examined distribution map. Pink circle, holotype.

Etymology

The specific name *abies* seems to be presumably chosen because of the resemblance of the colony to the European silver fir *Abies alba*.

Genus *Mirostenella* Bayer, 1988

Mirostenella Bayer, 1988:251-252.—Bayer and Stefani, 1989:454 [key to genus].—Cairns and Bayer, 2009:38-39 [in part: not *M. delicatula*].—Zapata-Guardiola, López-González and Gili, 2013:231.

Diagnosis (modifications from Bayer (1988) in bold)

Primnoidae with a planar colony shape that is dichotomously branched. **Straight** polyps arranged in pairs or whorls of 3**–6**, **almost perpendicular or inclined upward towards branchlets**, with 8 complete rows of body scales. **Thorny** marginal scales **with smooth outer surface** aligned with opercular scales, which **do not fold over them**. Calcified axis interrupted by organic nodes at points of bifurcation.

Remarks

In the original diagnosis of genus *Mirostenella,* Bayer (1988) used the distinct calcified axis with organic nodes as a taxonomic character at generic level. However, after having examined the available material of the type species and the new species, we consider this a character at the specific level.

Geographical and bathymetrical distribution

At present, *Mirostenella*, has been reported from South Georgia Island, Antarctica, between 200 and 700 m in depth.

Etymology

From Latin *mirus*, extraordinary, wonderful, from *miror-*, to be astonished at, and *-stenella*, name applied to a genus of primnoid gorgonians by J.E. Gray, in allusion to the similar arrangement of the polyps.

Type species

Mirostenella articulata Bayer, 1988

Mirostenella articulata Bayer, 1988

(Figures 2.24-2.27)

Mirostenella articulata Bayer, 1988:251-256.—Cairns and Bayer, 2009:28 (in list), 38-39, fig. 10 r-a'.—Zapata-Guardiola, López-González and Gili, 2013:231-234.

Examined material

Holotype: USNM 79959, USAP, Eltanin 22, stn 1536, 54°28.98'S, 39°22.02'W, South Georgia Island, off West Tip of Island, Scotia Sea, Subantarctica, 659-686 m depth, 8 February 1966, one colony.

Paratype: USNM 79960, with the same sampling data as the holotype, two colonies.

Additional material: BEIM (CRO-58), *Polarstern* cruise ANT XIX-5, stn PS61-164-01, 53°23.8'S, 42°42.03'W, Shag Rocks, west South Georgia, Antarctica, 312-322 m depth, 9 April 2002, two colonies, one of them with holdfast; MZB (2012-0484), *Polarstern* cruise ANT XIX-5, stn PS61-167-01, 53°23.68'S, 42°42.23'W, Shag Rocks, west South Georgia, Antarctica, 306-342.7 m depth, 9 April 2002, one fragment of colony.

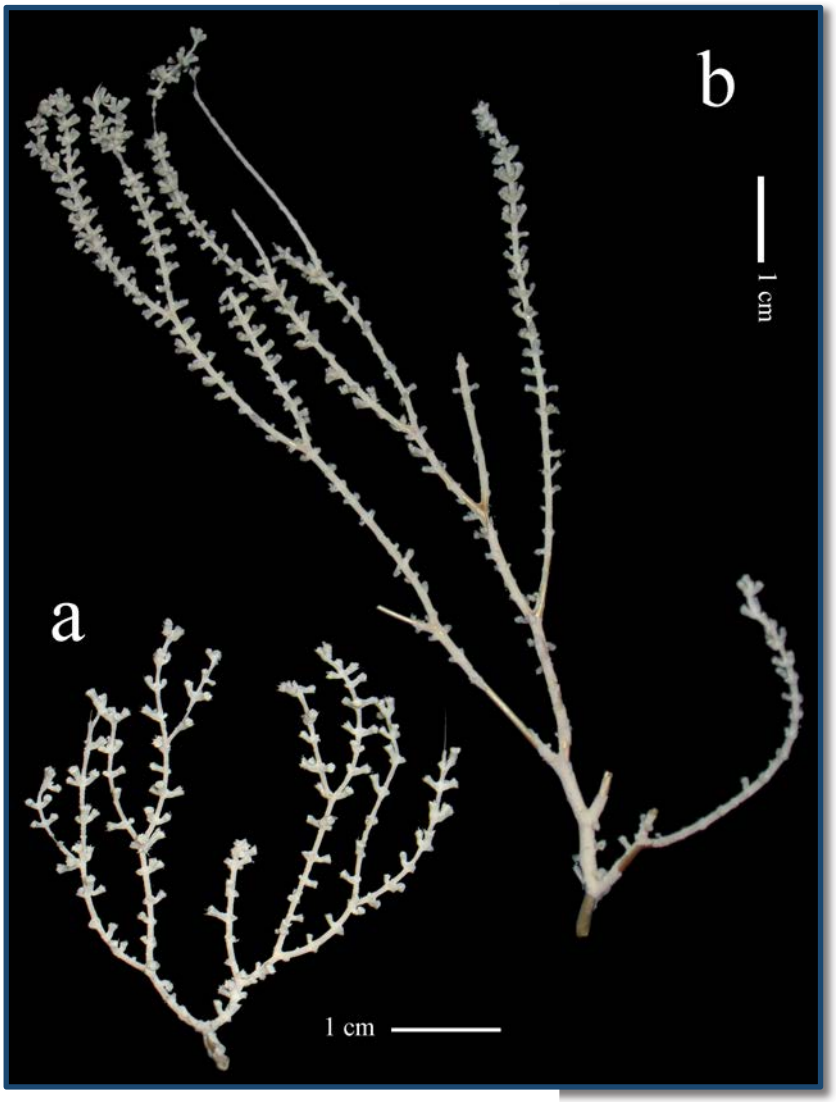


Figure 2.24.- *Mirostenella articulata*, BEIM(CRO-58): **a**, **b**, two colonies.

Description of the additional material examined

Smaller colony (Fig. 2.24a) of 4.5 cm in total height and about 4 cm in width, with holdfast. Larger colony fragmented (Fig. 2.24b) of 10.5 x 8 cm. Fragment of colony of 16 x 3 cm. Plane colonies dichotomously branched, internodes about 5-33 mm in length, terminal branchlets up to 55 mm. Axis ochre in colour, dark brown, more distinct in distalmost nodes. Basal axis diameter up to 1.2 mm and 0.9 cm long before the first bifurcation in the small colony. Polyps straight, almost perpendicular or inclined upward branchlets, arranged in whorls (Fig. 2.25), 3-6 polyps per whorl and 4-6 whorls per cm.

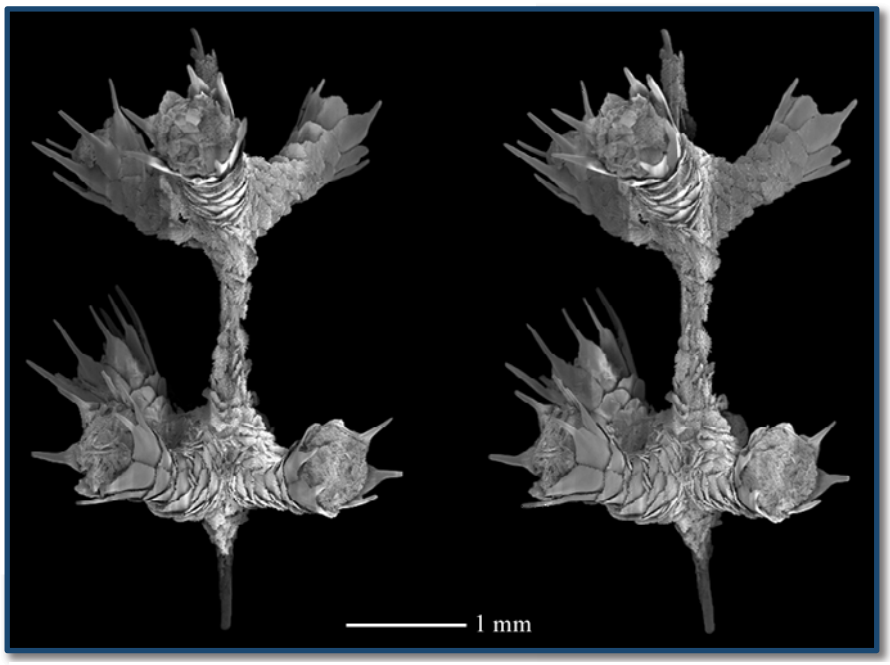


Figure 2.25.- *Mirostenella articulata*, BEIM (CRO-58). Detail of a branchlet, stereo pair.

Polyps (Figs. 2.25, 2.26) funnel shape; about 1.0–2.0 mm in height and 0.48–0.7 mm in diameter. Polyp body with 8 longitudinal rows of scales, 5–6 scales in each abaxial row and 4 scales in each adaxial row; proximal scales disorganized and overlapping one another. Eight opercular scales (Fig. 2.27a) generally isosceles triangle-shaped, 0.43–0.58 x 0.21–0.28 mm, although some sclerites are strongly reduced in width on the distal two thirds, 0.09–0.13 mm in width distally. Inner surface tuberculated, crowded at least on the proximal half of scale, forming short longitudinal ridges distally, but keels are absent. Outer surface granulated. Basal margin tuberculated, free margin finely serrated. Eight marginal scales, 0.64–1.09 x 0.23–0.32 mm in size (Fig. 2.27b), basal part oval shaped supporting a long projecting thorn that is round in cross-section, almost smooth, and occupies more than one-third of the total sclerite length. Inner surface of basal part of scale completely tuberculated, without a keel; outer surface almost completely smooth, with only a few tubercles on most proximal border. Basal and free margin with minute irregular processes. Body scales (Fig. 2.27c) oval-fan shape, 0.17–0.39 x 0.23–0.40 mm. Inner surface tuberculated; outer surface granulated, proximal border with tubercles. Margin with irregular processes. Coenenchymal sclerites (Fig. 2.27d) round, oval, rectangular-shaped, 0.14–0.45 mm in maximum length; inner surface tuberculated, outer surface granulated, free margin with irregular processes.

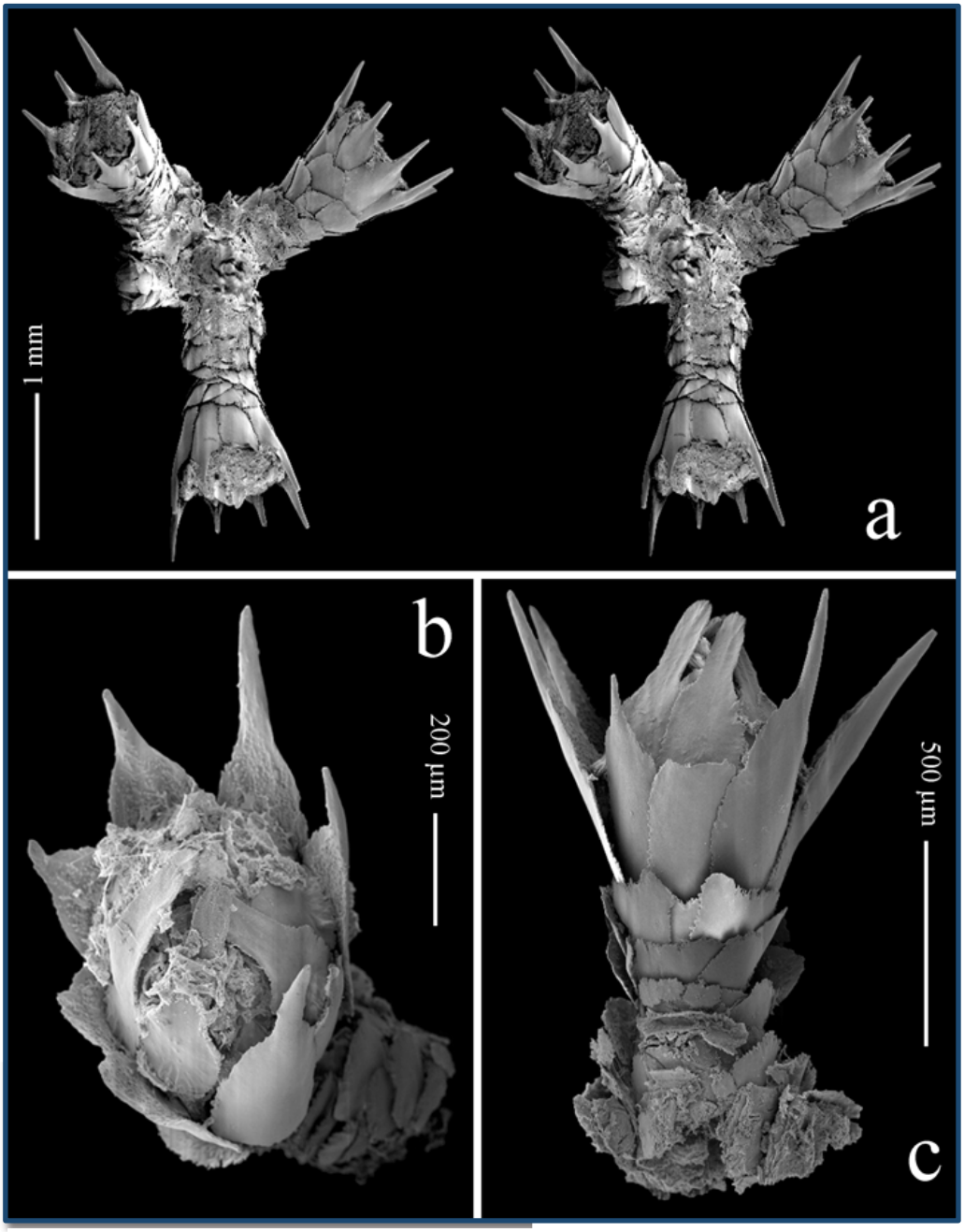


Figure 2.26.- *Mirostenella articulata*, BEIM (CRO-58): **a**, polyp whorl, adaxial view, stereo pair; **b**, polyp, oral view; **c**, polyp, adaxial view.

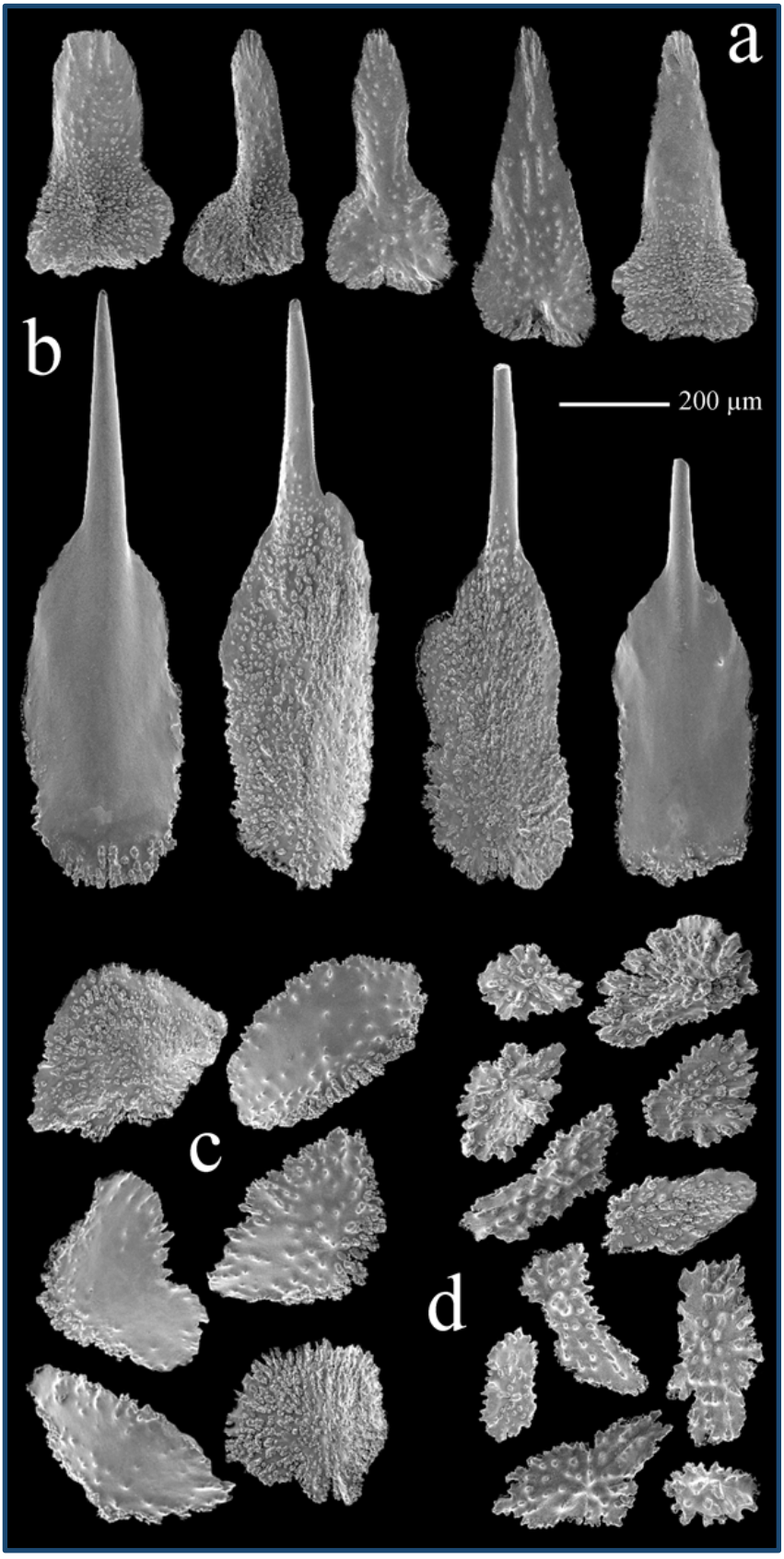
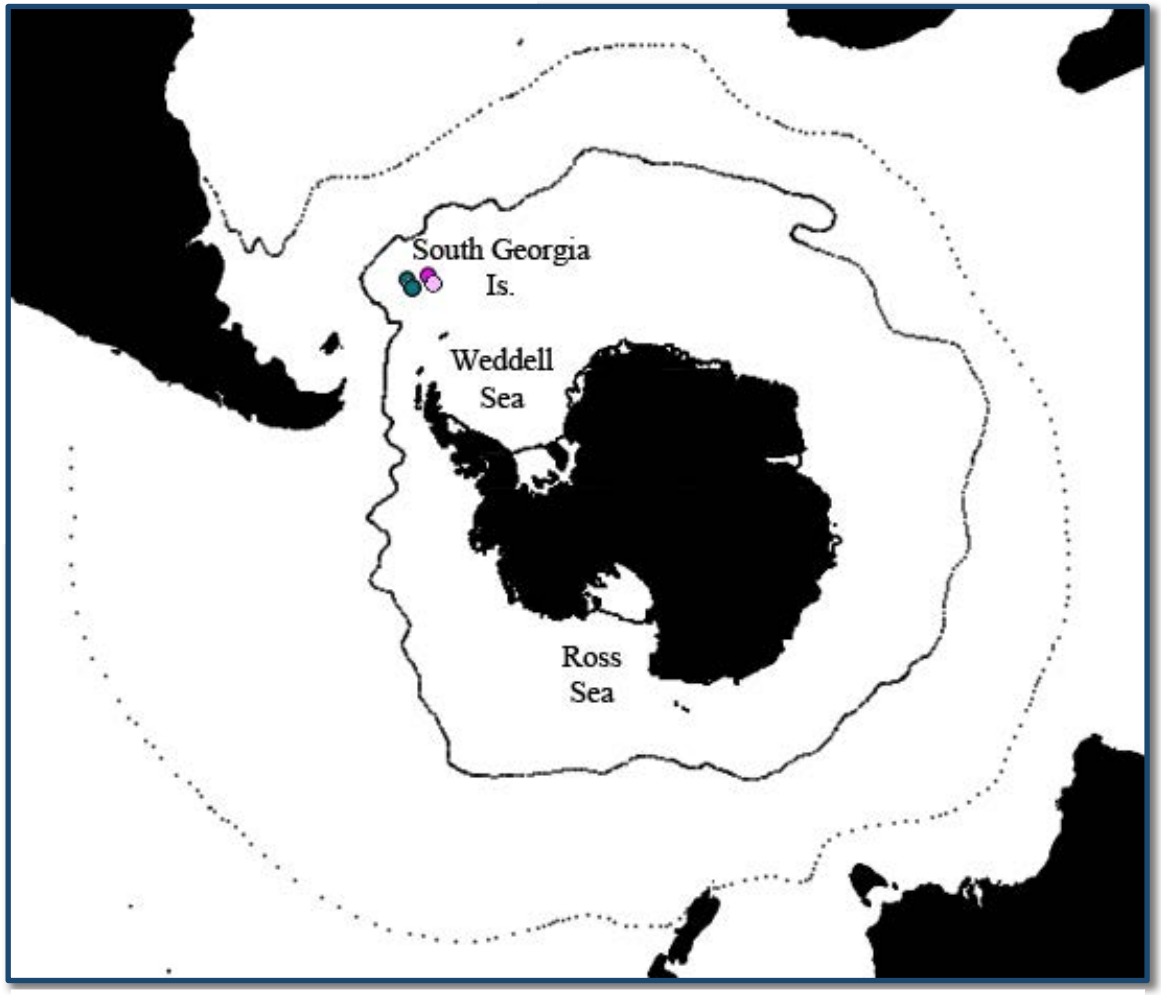


Figure 2.27.- *Mirostenella articulata*, BEIM (CRO-58): **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal scales.

Geographical and bathymetrical distribution

At present, *Mirostenella articulata* Bayer, 1988, is only known from South Georgia Island, Antarctica (Map 2.15), between 201-210 m in depth, 312-342.7 m in depth, and 659-686 m in depth.



Map 2.15.- *Mirostenella articulata* Bayer, 1988. Species examined distribution map. Dark pink, holotype; light pink, paratype.

Etymology

The specific name derives from the Latin word *articulare* meaning "divide into distinct parts" presumably referred to the divisions of the calcified axis produced by the organic nodes at the bifurcation points.

Genus *Plumarella* Gray, 1870

Cricogorgia Milne Edwards, 1857:6, pl. B2, fig. 6 [nomen nudum].
Dicholaphis Kinoshita, 1907:230-231; 1908b:24-27.—Cairns and Bayer, 2009:26.
Primnodendron Nutting, 1912:70.
Plumarella Gray, 1870:36.—Studer, 1887:51.—Wright and Studer, 1889:xlix, 73-74.—Versluys, 1906:13-14.—Kinoshita, 1908a:6-8.—Kükenthal, 1915:144-145 [key to genus and species]; 1919:340-343 [key to genus and species]; 1924:255 [key to genus and species].—Deichmann, 1936:155 [key to genus].—Bayer, 1956:F220; 1961:293 [illustrated key to genus].—Cairns and Bayer, 2004c:448-449 [key to western Atlantic species].—Cairns and Bayer, 2009:39 [revision].—Cairns, 2010:422.—Cairns, 2011:7.—Zapata-Guardiola and López-González, 2012:16.—Zapata-Guardiola, López-González and Gili, 2013:232-233.

Diagnosis (Zapata-Guardiola and López-González 2013)

Primnoidae colonies usually uniplanar, alternately pinnately branched or dichotomously branched and may also be bottlebrush in shape. Polyps may occur in an alternate or opposite biserial, or in whorls on branchlets and also crowded on all sides of branchlets in no order. Polyps covered by 8 complete rows of body scales, adaxial rows sometimes reduced in number and size. Marginal scales arranged in one circlet of scales, which do not fold over the opercular scales, may have ridges, spines and other ornamentations in their inner surface but never will be keeled.

Geographical and bathymetrical distribution

At present, *Plumarella* has been reported from western Pacific, Patagonia, northwest Atlantic, Subantarctica, between 10 and 2743 m in depth.

Etymology

The generic name combines *pluma*- a Latin word meaning “feather” and *–ella*, a Latin suffix meaning “little, small”, in reference to its colony shape.

Type species

Gorgonia penna Lamarck, 1815.

***Key to species of the genus* Plumarella**

1. Polyps singly placed around stem and branchlets.
   a. Polyps placed on alternating sides of branchlets.……**Subgenus *Plumarella***
      i. Marginal scales not pointed.
         1) Body wall scales without keel.
            a) up to 6 scales in the longitudinal abaxial row.
               i) 2 scales in the longitudinal adaxial row.……..***P. lata***
               ii) 3-4 scales in the longitudinal adaxial row
                  (1) Outer side of body wall scales smooth.……***P. delicatissima***
                  (2) Outer side of body wall scales granulated.
                     (a) Polyps close fitting.……***P. flabellata***

(b) Polyps spread……***P. alba***

iii) 5 scales in the longitudinal adaxial row.

(1) Branching alternate pinnate; colonies often large (up to 33 cm).

(a) Body wall scales smooth.

(i) Closely-pinnate branching; opercular scales elongate and granular; 10-12 polyps/cm……...***P. pellucida***

(ii) Loosely-pinnate branching; opercular scales shorter and smooth; 14-21 polyps/cm……..***P. laxiramosa***

(b) Body wall scales granular.

(i) Distal edges of marginal scales of some polyps in a colony slightly angled (but not spinose)……..***P. pourtalesii var. obtusa***

(ii) Distal edges of marginal scales straight to slightly rounded.

1.- 11-13 polyps/cm; distance between polyps on one side of branch 1.0-1.2 mm……..***P. pourtalesii var. typical***

2.- 14-16 polyps/cm; distance between polyps 0.5-0.8 mm ……...***P. pourtalesii var. robusta***

(2) Branching dichotomous; colonies fairly small (less than 11 cm) ……..***P. dichotoma***

b) 6 scales or above in the longitudinal abaxial row.

i) 6-8 scales in the longitudinal abaxial row……..***P. circumoperculum***

ii) 10 scales in the longitudinal abaxial row……..***P. gracilis***

2) Body wall scales with keel……..……***P. dofleini***

ii. Marginal scales pointed.

1) Marginal scales without an apical spine.

a) 5-6 scales in the longitudinal abaxial row……***P. acuminata***

b) 10 scales in the longitudinal abaxial row……...***P. penna***

2) Marginal scales with an apical spine.

a) Body wall scales with a pectinate lateral and distal edges……..……..***P. recta***

b) Body wall scales without a pectinate lateral and distal edges.

i) 4-6 scales in the longitudinal abaxial row.

(1) 2-3 scales in the longitudinal adaxial row.

(a) Polyps small, up to 0.7 mm height……..……***P. longispina***

(b) Polyps large, over 1 mm height……..…***P. adhaerans***

(2) 4 scales in the longitudinal adaxial row……..……..***P. aculeata***

(3) 5 scales in the longitudinal adaxial row……..……..***P. spicata***

ii) 6-7 scales in the longitudinal abaxial row.

(1) 8-10 polyps/cm……..***P. spinosa***

(2) 14-22 polyps/cm……***P. aurea***

iii) 8 scales in the longitudinal abaxial row.……***P. rigida***

b. Polyps placed on all sides of branchlets……**Subgenus *Dicholaphis***

i. Colonies dichotomously branching.

1) Marginal scales with a log thorn.……***P. bayeri***

2) Marginal scales without a thorn.……***P. delicata***

ii. Colonies pinnately branched or shaped as a bottlebrush.

1) Colonies bottlebrush in branching.

a) Marginal scales with a long thorn.

i) Accessory opercular scales present……***P. diadema***

ii) Accessory opercular scales absent……***P. undulata***

b) Marginal scales without a thorn.

i) Polyps large (3.0–3.6 mm), 9–15 polyps/cm; tentacular rodlets present; coenenchymal membrane often present between bases of branchlets ……***P. nuttingi***

ii) Polyps small (1.0–1.4 mm), 30–35 polyps/cm; tentacular rods absent; no coenenchymal membrane between branchlets……***P. superba***

2) Colonies pinnately branched.

a) Marginal scales (exclusive of adaxials) bear elongate spines consisting of over 60% of length of scale; marginal spine spiny or ridged on all surfaces.

i) Colonies consists of a single branch from which branchlets originate in a pinnate manner; marginal spines finely ridged; tentacular rodlets present; species occurs deeper than 800 m……***P. robusta***

ii) Colonies consists of several major branches from which branchlets originate in a pinnate manner; marginal spines coarsely spiny; tentacular rodlets absent; species occurs at depths shallower than 700 m……***P. echinata***

b) Marginal scales (exclusive of adaxials) have a pointed or straight distal edge but never produced into an elongate spine; inner surface of marginal spines smooth.

i) Marginal scales rectangular, with a straight distal edge; opercular scales quite elongate, with a L:W over 2.5; species occurs deeper than 2500 m ……***P. profunda***

ii) Marginal scales triangular, with a pointed distal edge; opercular scales triangular but with a L:W less than 2.5; species occurs at depths shallower than 500 m.

(1) Polyps small (0.9–1.2 mm); body wall scales thick and coarsely serrate; tentacular rodlets absent……***P. aleutiana***

(2) Polyps larger (2.0–2.4 mm); body wall scales thin, concave above, and finely

serrate; tentacular rodlets present……***P. hapala***

2. Polyps placed in pairs or whorls around stem and branchlets.
   a. Polyps placed in pairs……**Subgenus *Faxiella***
      i. Large polyps (2.5-3.5 mm); 2 polyps/cm…….. ***P. abietina***
      ii. Small polyps (1-1.8 mm); 4 polyps/cm……..***P. delicatula***
   b. Polyps placed in whorls……..**Subgenus *Verticillata: P. castellviae***

Subgenus *Dicholaphis* (Kinoshita, 1907)

Diagnosis

Plumarella in which polyps occur on all sides of branchlets.

Geographical and bathymetrical distribution

Aleutian Islands and off Japan, 40-2514 m.

Etymology

The subgeneric name combines the Latin prefix dicho- meaning two or divided in two, in allusion to the dichotomic ramification of the type species, and –laphis meaning leaf.

Type species

Dicholaphis delicata Kinoshita, 1907.

Plumarella (*Dicholaphis*) *bayeri (*Zapata-Guardiola and López-González, 2010e)

(Figures 2.28-2.30)

Thouarella (*Thouarella*) *bayeri* Zapata-Guardiola and López-González, 2010e:133.
Plumarella (*Plumarella*) *bayeri*, Cairns, 2011:8 (listed).

Examined material

Holotype: ZIZMH C11738, ANTXIX/5, stn PS61/164-01, 53°23.80'S, 42°42.03'W, west South Georgia, Antarctica, 312.5 to 321.6 m depth, 9 April 2002.

Paratypes: ZIZMH C11739, with the same sampling data as the holotype, two colonies and two fragments. USNM 1123419 , with the same sampling data as the holotype, one colony and two fragments.

Additional material: US 6441, ANTXIX/5, stn PS61/160-01, 53°23.75'S, 44°45.12'W, Shag Rocks, Antarctica, 434 m depth, 9 April 2002, one colony; BEIM CRO-021, ANTXIX/5, stn PS61/164-01, 53°23.80'S, 42°42.03'W, west South Georgia, Antarctica, 312.5 to 321.6 m depth, 9 April 2002, two colonies (one of them without holdfast) and two fragments; BEIM CRO-022 and US 1431, ANTXIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, west South Georgia, Antarctica, 306.0 to 342.7 m depth, 9 April 2002, two fragments.

Description of the holotype

Colony uniplanar (Fig. 2.28a), 9.6 cm in height and 4 cm in width, dichotomously branched, internodes about 6-27 mm in length, unbranched terminal twigs up to 26 mm.

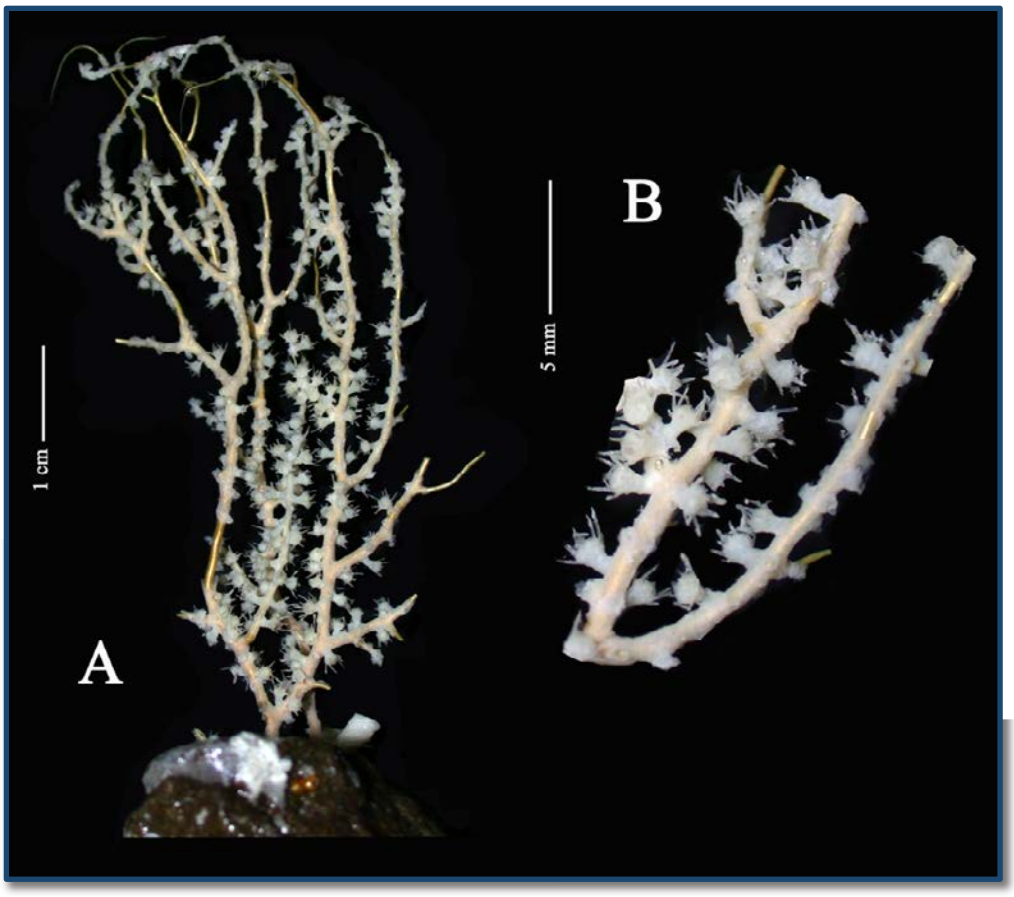


Figure 2.28.- *Plumarella bayeri*, holotype ZIZMH C11738: **a**, whole colony; **b**, detail of a branchlet.

Axis bronze in colour, stiff and firmly attached to hard substrate by a white, calcareous, discoidal holdfast, basal axis diameter of 1.3 mm and 21 mm height until the first division. Polyps perpendicular to stem (Fig. 2.28b), present on main stem and branches in a roughly alternating arrangement, occasionally opposite (i.e. paired), with little tendency toward pairs or whorls, 9-12 polyps per cm.

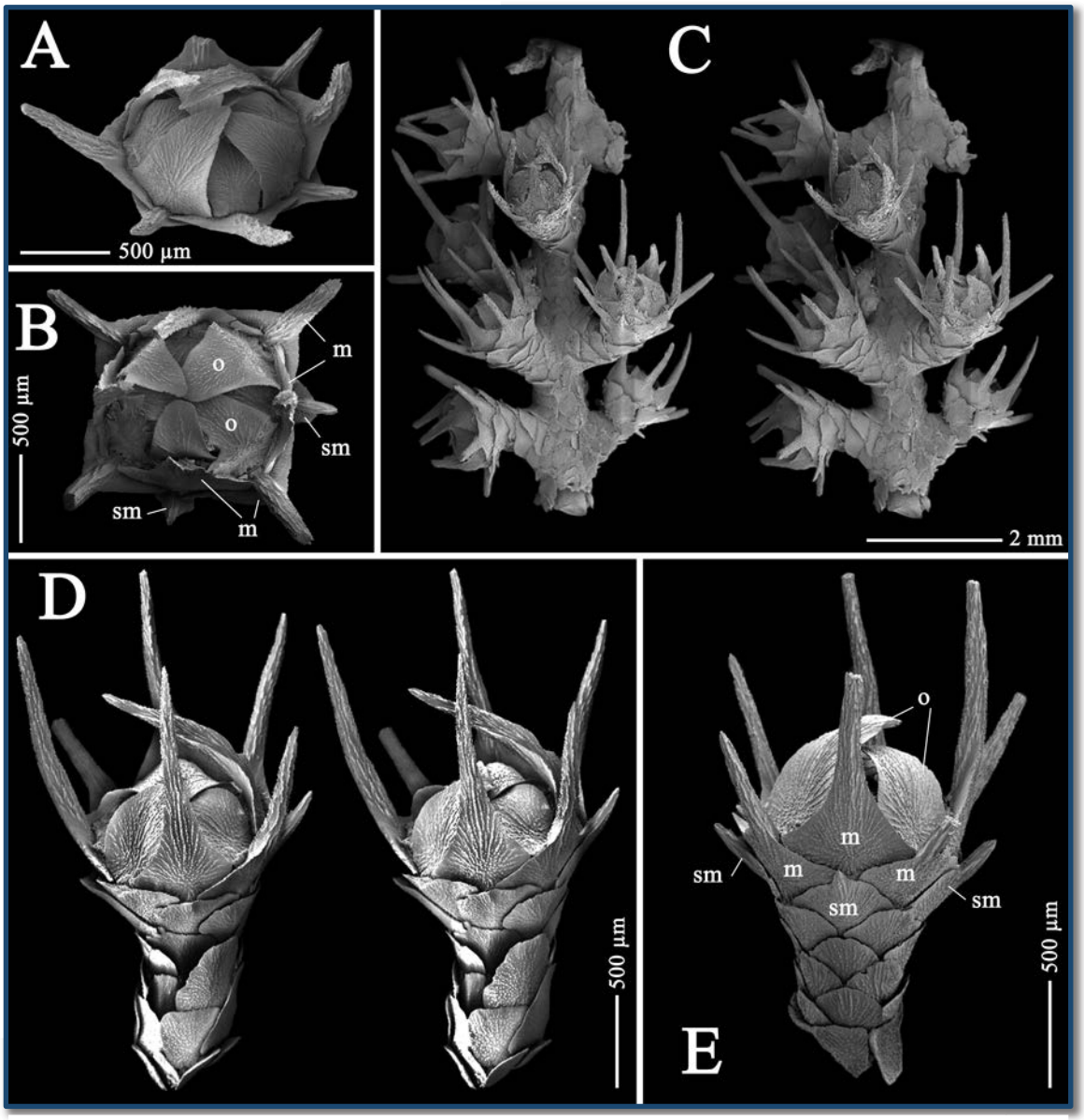


Figure 2.29.- *Plumarella bayeri*, holotype ZIZMH C11738: **a** and **b**, polyps in oral view; **c**, detail of a branchlet, stereo pair; **d**, polyps on latero-adaxial view, stereo pair; **e**, polyps in lateral view. Abbreviations: **o**, opercular scales; **m**, marginal scales; **sm**, submarginal scales.

Polyps (Fig. 2.29) relatively short, cylindrical to club-shaped distally, about 1.6-2.4 mm in height and 0.47-0.91 mm in diameter without including sclerite spines, which may extend considerably beyond the operculum. Polyp body with seven longitudinal rows of scales, 4-5 transversal rows of scales on each longitudinal abaxial row overlapping one another. Second accessory opercular scales in variable number, from absent to four (rarely seven), stick-shaped. Where present, on the inner surface of first accessory opercular scales very close together.

First accessory opercular scales in numbers of eight, arranged in two alternate cycles of four scales: inner cycle (Fig. 2.30a) with small, 0.27-0.41 x 0.09-0.14 mm, narrow, stick-shaped tentacular scales; outer cycle (Fig. 2.30b) with bigger, 0.22-0.42 x 0.14-0.22 mm, broad, arrowhead-like scales. Proximal inner surfaces tuberculate, covering about one third in length, distal inner surface smooth, without keel. Outer surface quite smooth. Basal margin with digitate processes, free margin finely serrated. Opercular scales, 0.62-1.06 x 0.41-0.54 mm, arranged in two alternate cycles of four scales: inner cycle (Fig. 2.30c) with blunt tips and squarish base; outer cycle (Fig. 2.30d) with isosceles-shaped scales. Inner surface like that in accessory opercular scales. Outer surface with radial granules from nucleus on proximal portion. Free margin finely serrated.

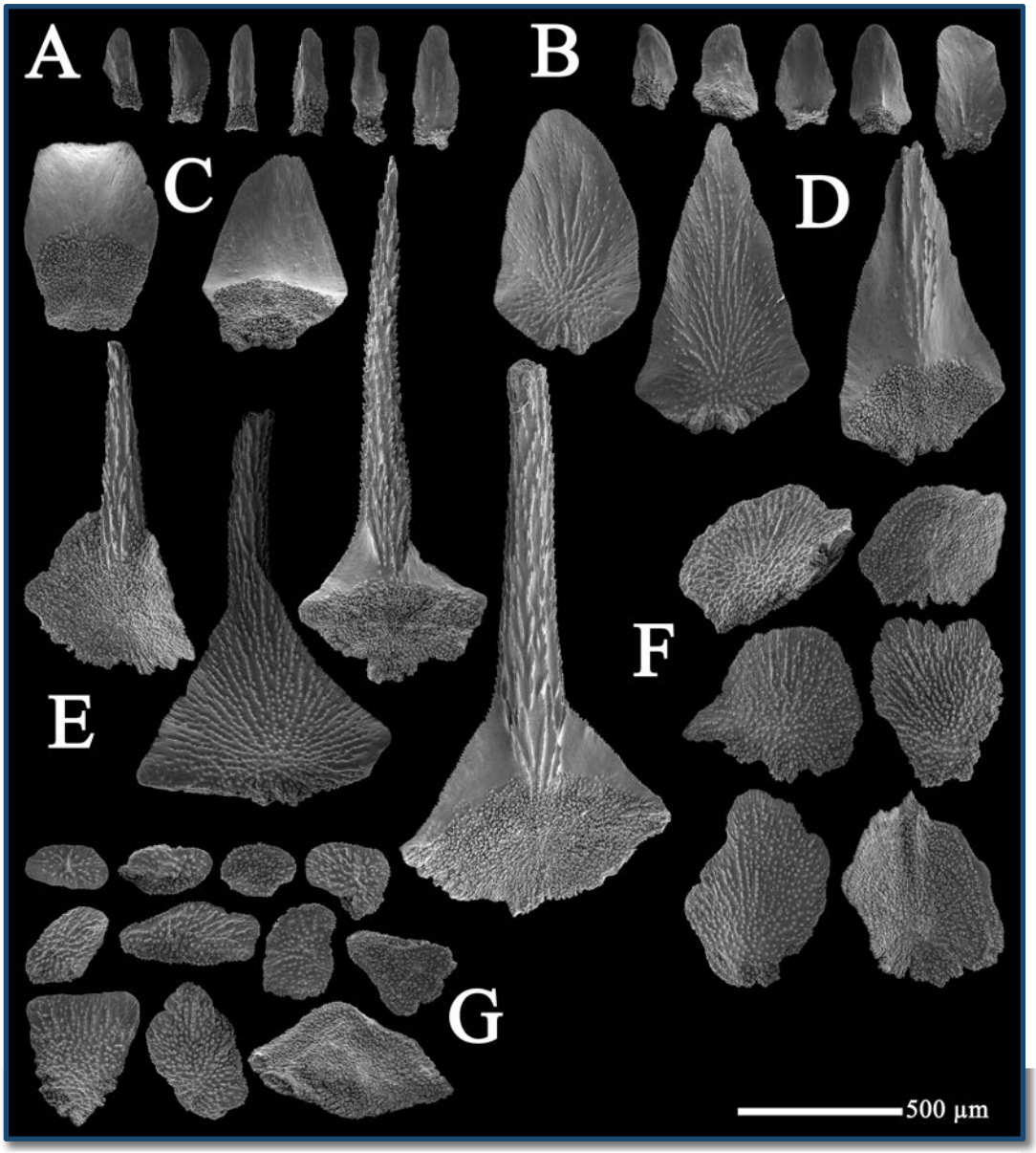


Figure 2.30.- *Plumarella bayeri*, holotype ZIZMH C11738: First accessory opercular scales from inner **a** and outer **b** alternate cycle. Opercular scales from inner **c** and outer **d** alternate cycle. Marginal scales **e**. Body scales **f**. Coenechymal scales **g**.

Marginal scales, seven (eight) in number (Fig. 2.30e), basal part of scale equilateral triangle-shaped projecting a long thorn, round in section, 1.1-1.83 x 0.52-0.83 mm in length (including thorn), thorn more than three-quarters of total sclerite length, with numerous longitudinal ridges on all sides. Outer surface with granules radially arranged from nucleus on proximal portion. Free margin serrated, proximal margin with digitate processes. Remaining body scales (Fig. 2.30f) with tendency to circular or oval shape, 0.38-0.63 mm in maximum length. Inner surface completely tuberculate, outer surface with granules radiating from nucleus on proximal portion. Free margin finely serrated, basal margin with digitate processes. Coenenchymal scales (Fig. 2.30g) more diverse in shape, ranging from round to irregular elongated polygons, 0.18-0.45 mm in maximum length. Surface with similar ornamentation to body scales. Margin quite smooth, some serrated, or with digitate processes.

Variations from holotype

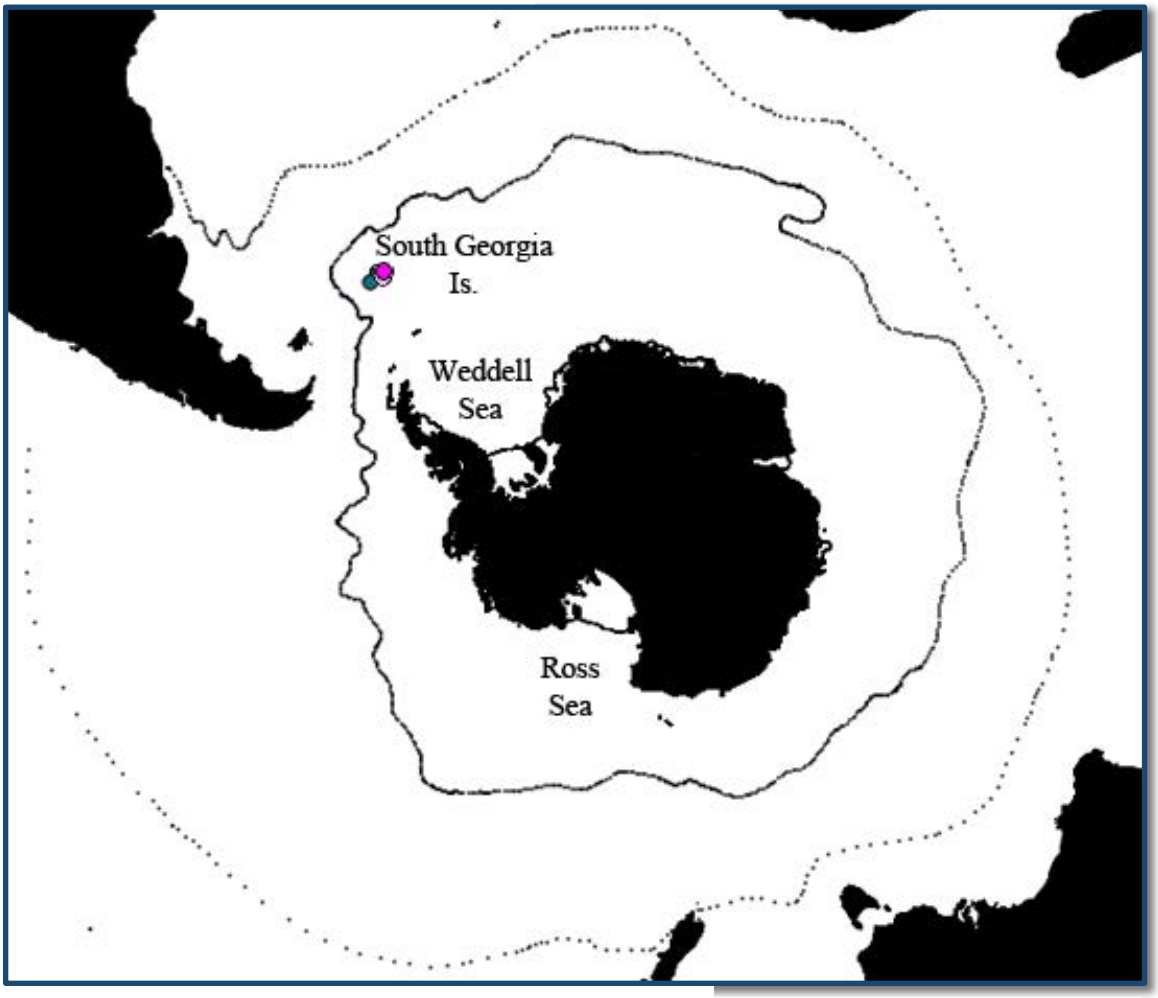
The general colonial structure of the paratypes and additional examined material is quite similar to that of the holotype. Colonies reach up to 8 cm in height and 5 cm in width. The ramification pattern is somewhat elongated or broad. The main stem (before the first dichotomic division) is 6-13 mm in length. The polyp form and distribution are as in the holotype. Second accessory opercular scales can vary from absent to four in number as in the holotype. The outer cycle of opercular scales in some colonies or fragments of paratypes are less pointed and isosceles-shaped

Geographical and bathymetrical distribution

At present, *Plumarella bayeri* is known only from west of South Georgia Island, Antarctica (Map 2.16), between 306 and 342.7 m in depth.

Etymology

The specific name *bayeri* is chosen in honour of Prof. F.M. Bayer, in recognition of his valuable contributions to the knowledge of Octocorallia taxonomy.



Map 2.16.- *Plumarella* (*Dicholaphis*) *bayeri* (Zapata-Guardiola and López-González, 2010). Species examined distribution map. Dark pink, holotype, light pink, paratype.

Plumarella (*Dicholaphis*) *diadema* (Cairns, 2006)

(Figures 2.31-2.34)

Thouarella (*Thouarella*) *diadema* Cairns, 2006:181.
Thouarella (*Thouarella*) *sardana* Zapata-Guardiola and López-González, 2010e:136.
Plumarella (*Plumarella*) *diadema*, Cairns, 2011:8 (listed).

Holotype: USNM 1078187, SEM stub B219-222, C1177-1179, Calypso 1776, 24°54.4'S, 44°26'W, off São Sebastião, Brazil, 1000 m depth, 1 colony fragment.

Examined material

Holotype: ZIZMH C11740, ANTXIX/5, stn PS61/164-01, 53°23.80'S, 42°42.03'W, west of South Georgia, Antarctica, 312.5 to 321.6 m depth, 9 April 2002.

Paratypes: ZIZMH C11741, with the same sampling data as the holotype, three colonies and seven fragments. USNM 1123420,with the same sampling data as the holotype, two colonies.

Additional material: US 1685, ANTXIX/5, stn PS61/150-01, 54°29.64'S, 56°8.13'W, east Burdwood Bank, Subantarctica, 286-291 m depth, 6 April 2002; US 6429, ANTXIX/5, stn PS61/153-01, 54°30.04'S, 56°8.33'W, east Burdwood Bank, Subantarctica, 277-296 m depth, 6 April 2002, three colonies and three fragments; BEIM CRO-023 and US 6442 ANTXIX/5, stn PS61/160-01, 53°23.75'S, 44°45.12'W, west of South Georgia, Antarctica, 434 m depth, 9 April 2002, one colony each; US 6428, ANTXIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, north west South Georgia, Antarctica, 306-343 m depth, 9 April 2002, three colonies and four fragments; BEIM CRO-024, ANTXIX/5, stn PS61/174-01, 54°24.47'S, 35°36.81'W north of South Georgia, Antarctica, 278 m depth, 11 April 2002, one fragment.

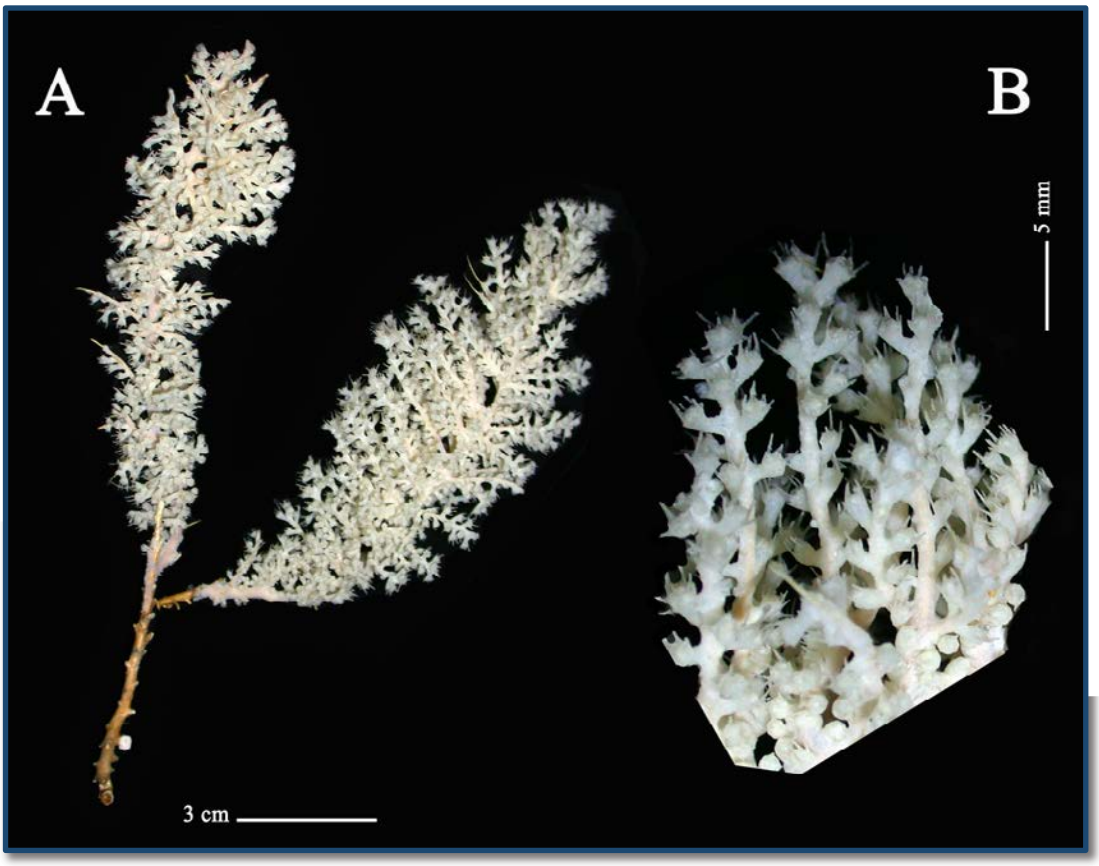


Figure 2.31.- *Plumarella diadema,* holotype ZIZMH C11740: **a**, whole colony; **b**, detail of a branchlet.

Description of the holotype

Colony bottlebrush (Fig. 2.31a), 15.3 cm in length, with two main branches from the main stem, probably part of a larger colony. One of the branches 12.2 cm in height and 4.9 cm in width, and the other one 10.4 cm in height and 3 cm in width; stiff branchlets (Fig. 2.31b) simple or bifurcate on their bases, about 1-2.5 cm in length. Axis bronze in colour, stiff. Basal axis diameter of 2.2 mm and 35 mm height until the first main branches. Polyps almost perpendicular to stem (Figs. 2.31b, 2.32c), present on main stem and branches, arranged in a spiral (Fig. 2.32c), occasionally opposite on proximal part of branches, with no tendency toward pairs or whorls. 9-10 polyps per cm. Polyps (Fig. 2.32) relatively short, cylindrical to club-shaped distally; about 2.1-3 mm in height and 0.74-0.93 mm in diameter without including submarginal thorns, which may extend considerably beyond the operculum. Polyp body with 7 longitudinal rows of scales, 5 transverse rows of scales on each longitudinal abaxial row overlapping one another.

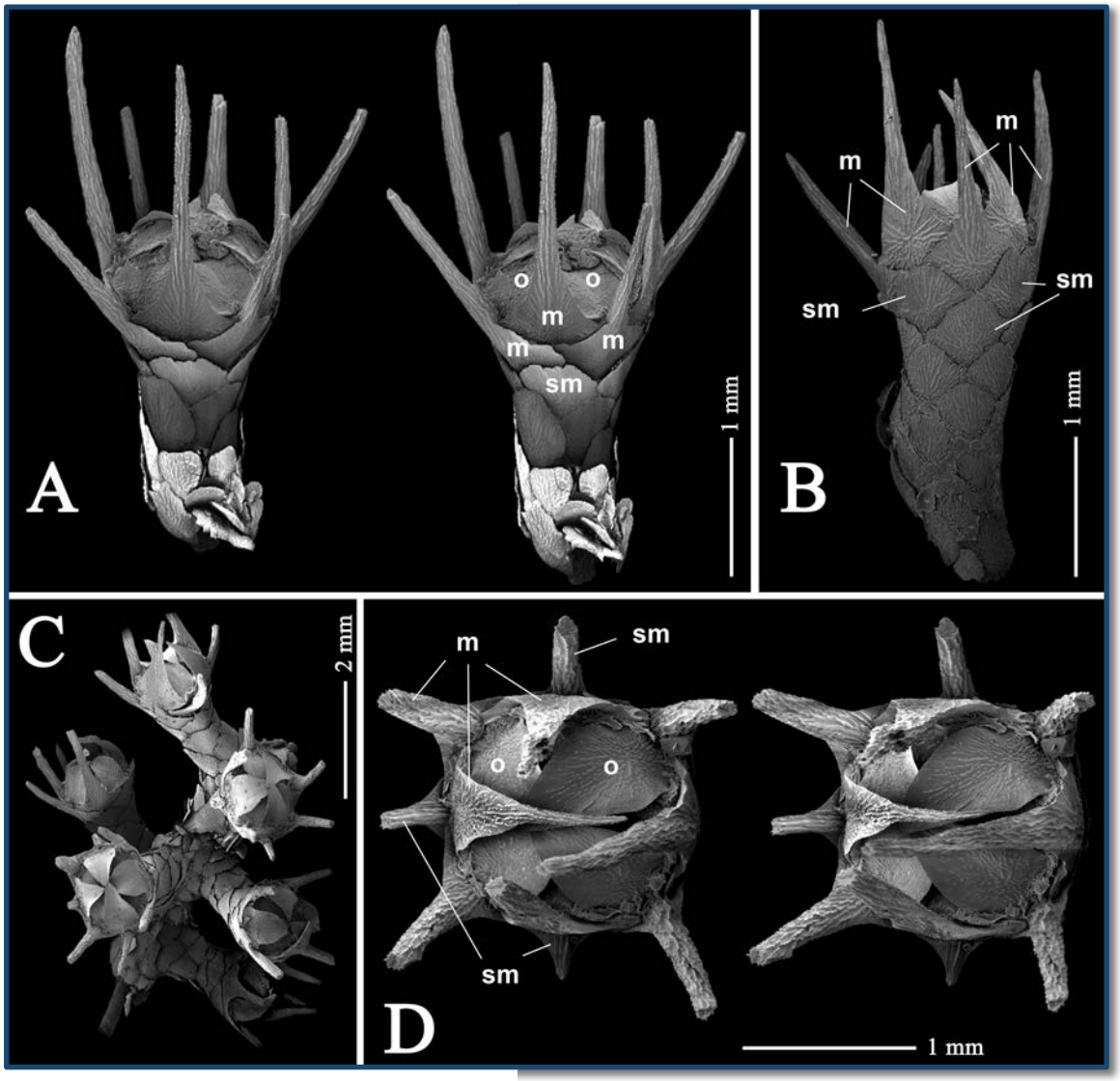


Figure 2.32.- *Plumarella diadema,* holotype ZIZMH C11740: **a**, polyps in adaxial view, stereo pair; **b**, polyps in abaxial view; **c**, detail of branchlet; **d**, polyps in oral view, stereo pair. Abbreviations: **o**, opercular scales; **m**, marginal scales; **sm**, submarginal scales.

Four to six accessory opercular scales (Fig. 2.33a), each small, higher than broad, and 0.41-0.61 x 0.16-0.23 mm. Proximal half of inner surface tuberculate, smooth distally, without keel. Outer surface quite smooth. Basal margin with digitate processes, free margin finely serrated.

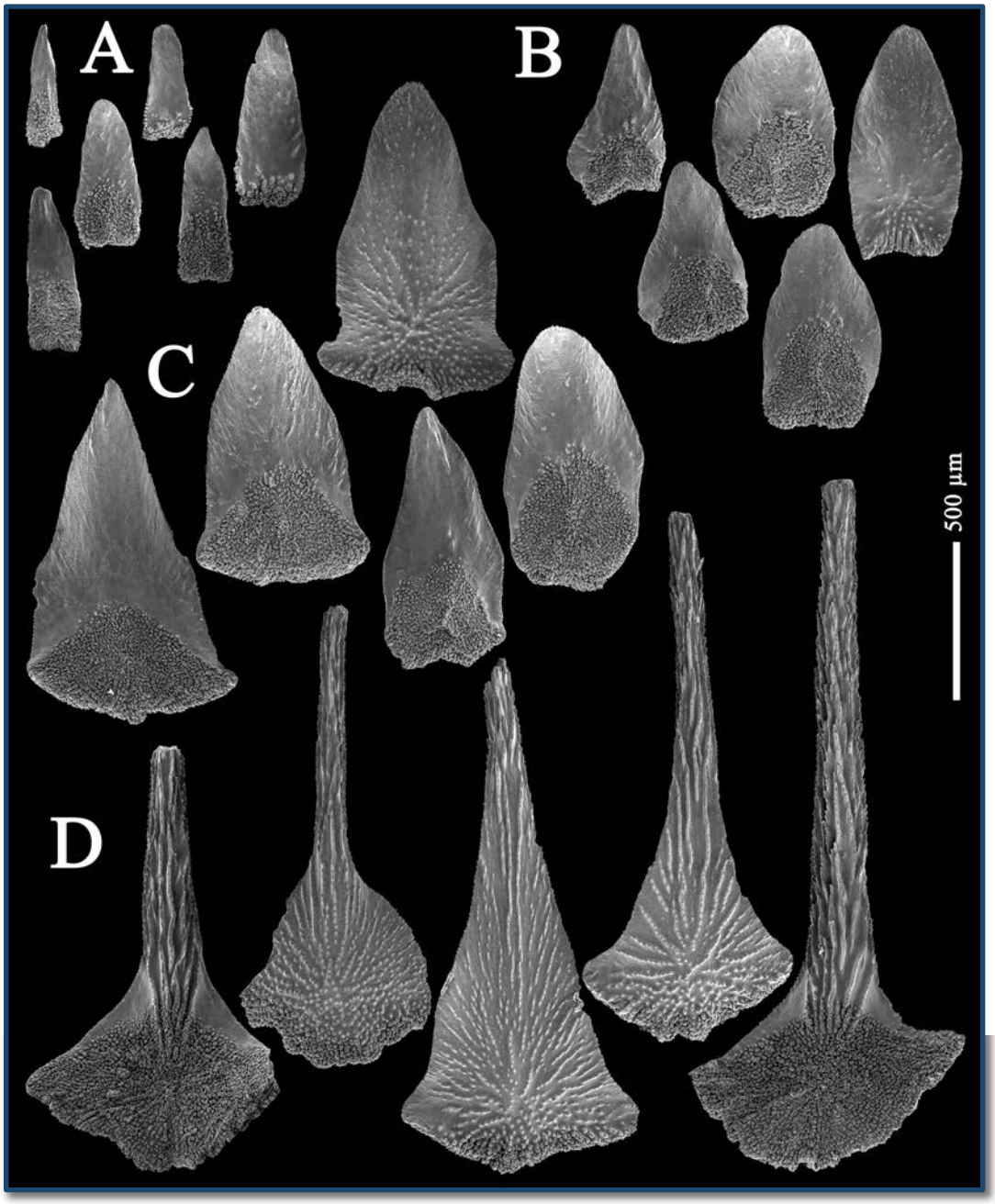


Figure 2.33.- *Plumarella diadema,* holotype ZIZMH C11740: **a**, accessory opercular scales; opercular scales from inner **b** and outer **c** alternate cycle; **d**, marginal scales.

Opercular scales, 0.63-1.2 x 0.33-0.69 mm, arranged in two alternate cycles of four scales: inner cycle (Fig. 2.33b) with oval tips and square base; outer cycle (Fig. 2.33c) with isosceles-shaped scales. Inner surface as in accessory opercular scales; outer surface with radial granules from nucleus on proximal portion. Free margin finely serrated. Marginal scales (Fig. 2.33d) seven to eight in number, basal part of scale equilateral triangle–shaped projecting a high and

nearly cylindrical thorn, 1.5-2.5 x 0.63-0.91 mm (including thorn), thorn more than three-quarters of total sclerite length. Thorn with numerous longitudinal ridges on all sides. Inner surface tuberculate covering part of thorn base, with distal smooth areas. Outer surface with radial granules from nucleus on proximal portion. Free margin finely serrated, proximal margin with digitate processes. Body wall scales (Fig. 2.34a) fan-shaped, with tendency to square or oval shape, 0.58-1.17 mm in maximum length. Inner surface completely tuberculate, outer surface covered with radial, often pointed granules from nucleus on proximal portion. Free margin finely serrated, basal margin with digitate processes. Coenenchymal scales (Fig. 2.34b) more diverse-shaped from circular to irregular elongated polygons, 0.22-0.8 mm in maximum length. Surface with similar characteristics to body scales. Margin from quite smooth to with digitate processes.

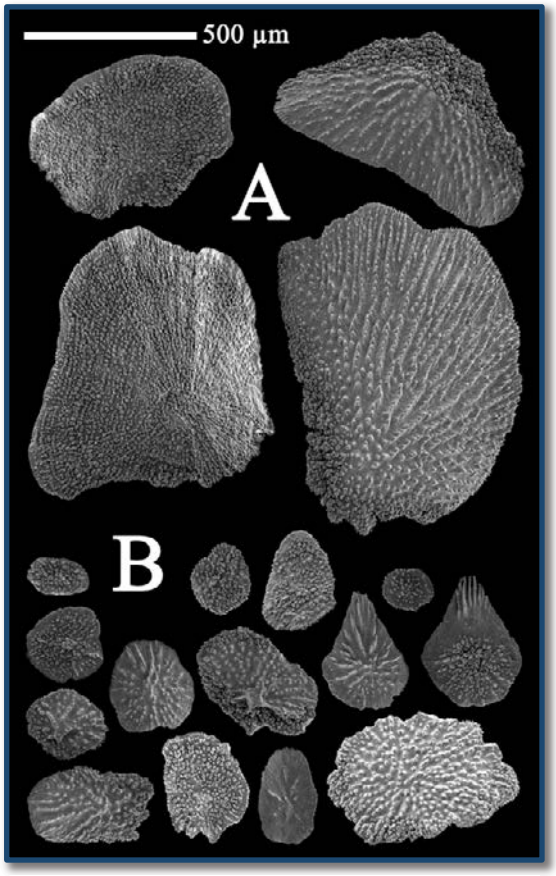


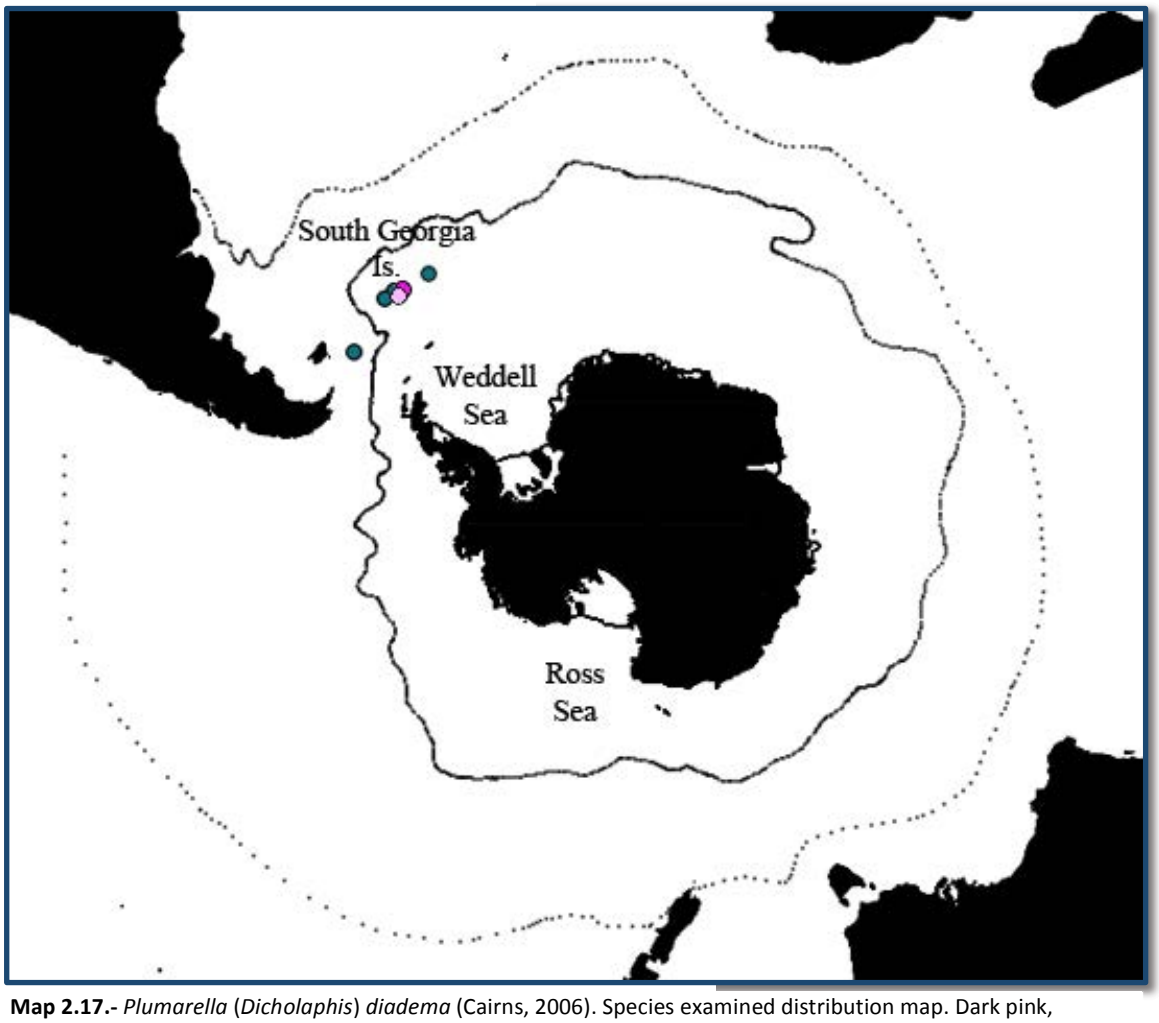
Figure 2.34.- *Plumarella diadema*, holotype ZIZMH C11740: **a**, body scales; **b**, coenenchymal scales.

Variations from holotype

The general colonial structure of the paratypes and additional examined material is quite similar to that of the holotype. The colonies have the base attached to a hard substrate by a white, calcareous, discoidal holdfast. The basal portion of the colony, first 3-11 mm, is devoid of branchlets. The colonies only show one main stem and a variable number of branchlets, the size of the colonial fragments varying from 4 to 14.5 cm in height and from 2.5 to 5 cm in width. The branchlets of some fragments are bent towards one side. The polyps are slightly shorter than in the holotype, 1.2-1.5 mm. The accessory opercular scales can vary from 3 to 6 (usually more than 4) in number. The distribution and the form of the sclerites from polyps and coenenchyme are as in the holotype.

Geographical and bathymetrical distribution

At present, *Plumarella diadema* is known from South Georgia Island area, Antarctica (Map 2.17), between 278 and 434 m in depth, and from the type locality off São Sebastião, Brazil at 1000 m in depth.



Map 2.17.- *Plumarella* (*Dicholaphis*) *diadema* (Cairns, 2006). Species examined distribution map. Dark pink, holotype, light pink, paratype.

Etymology

The species name is diadema (Greek for crown), treated as a noun in apposition, is an allusion to the tall spines of the crown-like marginal sclerites.

Plumarella (*Dicholaphis*) *undulata* (Zapata-Guardiola and López-González, 2010e)

(Figures 2.35-2.37)

Thouarella (*Thouarella*) *undulata* Zapata-Guardiola and López-González, 2010e:139.
Plumarella (*Plumarella*) *undulata,* Cairns, 2011:8 (listed).

Examined material

Holotype: ZIZMH C11742, ANTXIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, west of South Georgia, Antarctica, 306.0 to 342.7 m depth, 9 April 2002.

Paratypes: ZIZMH C11743, with the same sampling data as the holotype, two colonies and 12 fragments. USNM 1123421, with the same sampling data as the holotype, two colonies (one of them without holdfast).

Additional material: BEIM CRO-025, ANTXIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, west of South Georgia, Antarctica, 306.0 to 342.7 m depth, 9 April 2002,one colony. BEIM CRO-026, ANTXIX/5, stn PS61/160-01, 53°23.75'S, 44°45.12'W west of South Georgia, Antarctica, 434 m depth, 9 April 2002, one fragment. BEIM CRO-027, ANTXIX/5, stn PS61/164-01, 53°23.80'S, 42°42.03'W, west of South Georgia, Antarctica, 312.5 to 321.6 m depth, 9 April 2002, three colonies and 13 fragments.

Description of the holotype

Colony bottlebrush (Fig. 2.35a), 10.1 cm in height and 3.3 cm in width without holdfast, probably part of a larger colony; branchlets stiff, bifurcate on their base, about 1.2-2.7 cm in length. Axis bronze in colour, stiff, basal axis diameter of 1.12 mm.

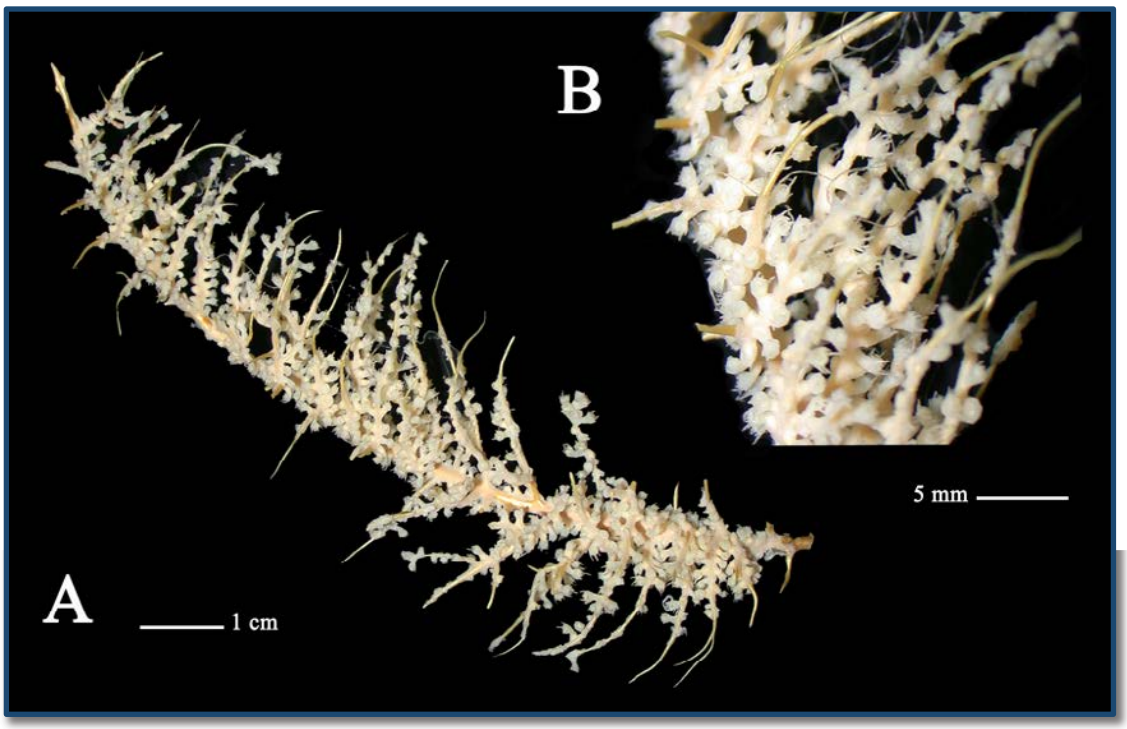


Figure 2.35.- *Plumarella undulata*, holotype ZIZMH C11742: **a**, whole colony; **b**, detail of a branchlet.

Polyps almost perpendicular to stem (Fig. 2.35b), singly placed, present on main stem and branchlets, occasionally opposite on proximal part of branches, but without tendency toward pairs or whorls, 10-11 polyps per cm. Polyps (Fig. 2.36) relatively short, cylindrical to club-shaped distally; about 1.7-2.4 mm in height and 0.51-0.65 mm in diameter without including thorns from marginal scales, which may extend considerably beyond the operculum. Polyp body with seven longitudinal rows of scales, five scales on each longitudinal abaxial row overlapping one another.

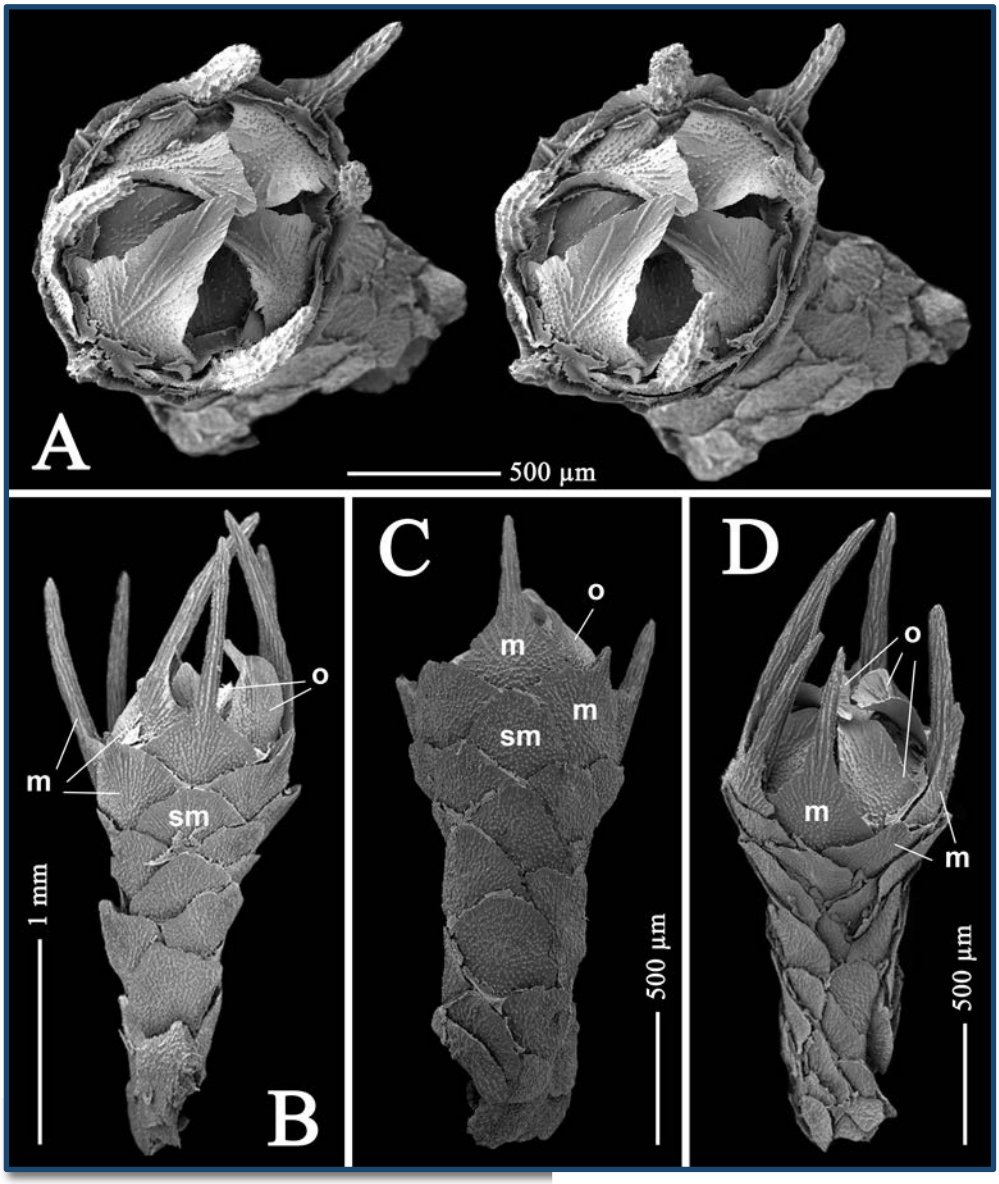


Figure 2.36.- *Plumarella undulata*, holotype ZIZMH C11742: **a**, polyps in oral view, stereo pair; **b**, polyp in latero-adaxial view; **c**, polyp in abaxial view; **d**, polyp in adaxial view. Abbreviations: **o**, opercular scales; **m**, marginal scales; **sm**, submarginal scales.

Opercular scales in numbers of eight, arranged in two alternate cycles of four scales: inner cycle (Fig. 2.37a) small, 0.24-0.50 x 0.11-0.33 mm, with oval tips; outer cycle (Fig. 2.37b) larger, 0.51-0.69 x 0.36-0.53 mm, bell-shape scales with convex inner distal surface. Proximal half of inner surface tuberculate, distal inner surface smooth, without keels. Outer surface with

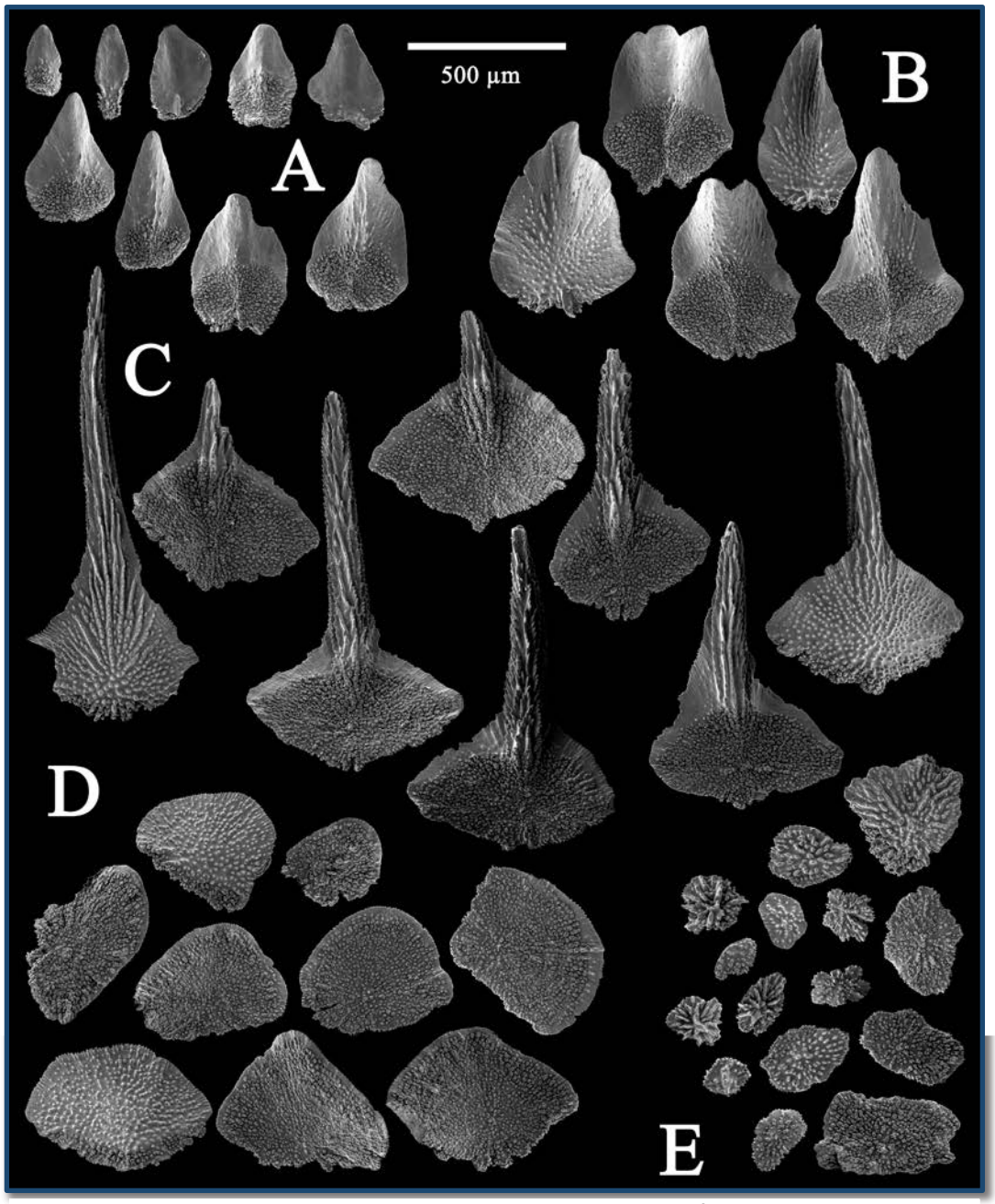


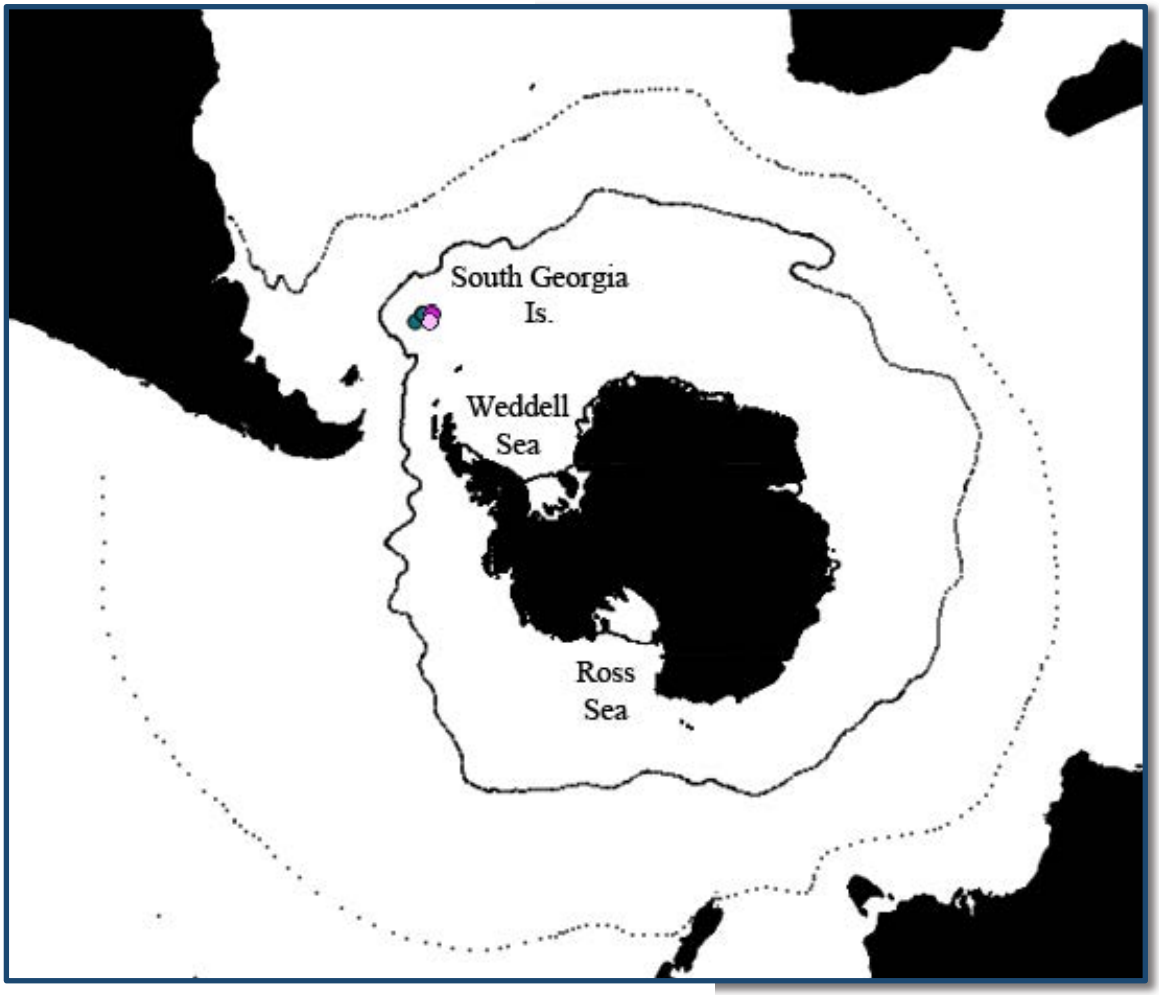
Figure 2.37.- *Plumarella undulata*, holotype ZIZMH C11742: Opercular scales from inner **a** and outer **b** alternate cycle; **c**, marginal scales; **d**, body scales; **e**, coenenchymal scales.

smooth granules radiating from nucleus. Basal margin with digitate processes, free margin quite straight. Scales distinctly undulate medially, more pronounced in the outer cycle. Marginal scales (Fig. 2.37c), eight in number, arranged in two alternate cycles of four scales. Basal part of scale obtuse triangle-shaped projecting a high thorn nearly round in section, 0.69-1.47 x 0.53-0.70 mm (including thorn), thorn 64-80% of total sclerite length. Thorn with numerous longitudinal ridges on all sides. Inner surface tuberculate, covering part of thorn base, with distal smooth areas. Outer surface with smooth granules covering all scales. Free margin finely serrated, proximal margin with digitate processes. Body scales (Fig. 2.37d), fan-shaped, with tendency to oval shape, 0.34-0.61 mm in diameter. Inner surface completely tuberculate, outer surface covered with smooth granules. Free margin finely serrated, basal

margin with digitate processes. Coenenchymal scales (Fig. 2.37e) more diverse shaped from circular to irregular elongated polygons, 0.12-0.46 mm in diameter. Surface with similar characteristics to body scales; some of them with radial ridges of granules distinctly elevated. Margin irregular due to presence of tubercles.

Geographical and bathymetrical distribution

At present, *Plumarella undulata* is known only from west of South Georgia Island, Antarctica (Map 2.18), between 306 and 434 m in depth.



Map 2.18.- *Plumarella* (*Dicholaphis*) *undulata* (Zapata-Guardiola and López-González, 2010). Species examined distribution map. Dark pink, holotype, light pink, paratype.

Etymology

The species name is the feminine form of the Latin adjective *undulatus*, meaning undulate, in reference to the undulation of the tentacular scales.

Subgenus *Faxiella* Zapata-Guardiola and López-González, 2012

Diagnosis

Plumarella with a planar colony shape and pinnate branching. Cylindrical-shaped, elongate polyps placed in pairs and curved upward toward the stem and branchlets. Opercular and marginal scales with longitudinal distal ridges. Adaxials smaller and slightly disorganized but always present.

Geographical and bathymetrical distribution

West coast of Central America to the Galapagos and off Macquarie Island, Subantarctica, between 2743 and 3181 m in depth.

Etymology

The subgeneric name comes from *fax*, the Latin word for torch, in reference to the shape of the polyps. Gender: feminine.

Type species

Amphilaphis abietina Studer, 1894.

Plumarella (*Faxiella) abietina* (Studer, 1894)

(Figures 2.38-2.40)

Amphilaphis abietina Studer, 1894:65.—Menneking, 1905:255-260; pl.8, fig. 7-8; pl. 9, fig. 17-20.—Versluys, 1906:22.—Cairns and Bayer, 2009:28.
Thouarella (*Amphilaphis*) *abietina*, Kükenthal, 1915:149.—Kükenthal, 1919:410-411.—Kükenthal, 1924:290.
Plumarella (*Faxiella*) *abietina*, Zapata-Guardiola and López-González, 2012:16.
Plumarella abietina, Cairns, 2011:8 (listed).
not *Thouarella abietina*, Pasternak, 1981:49-50.

Examined material

Type: MCZ 4802, Albatross Expedition, stn Alb-3399, 1°7'N, 81°4'W, west coast of central America to the Galapagos, 3181 m depth, 1891.

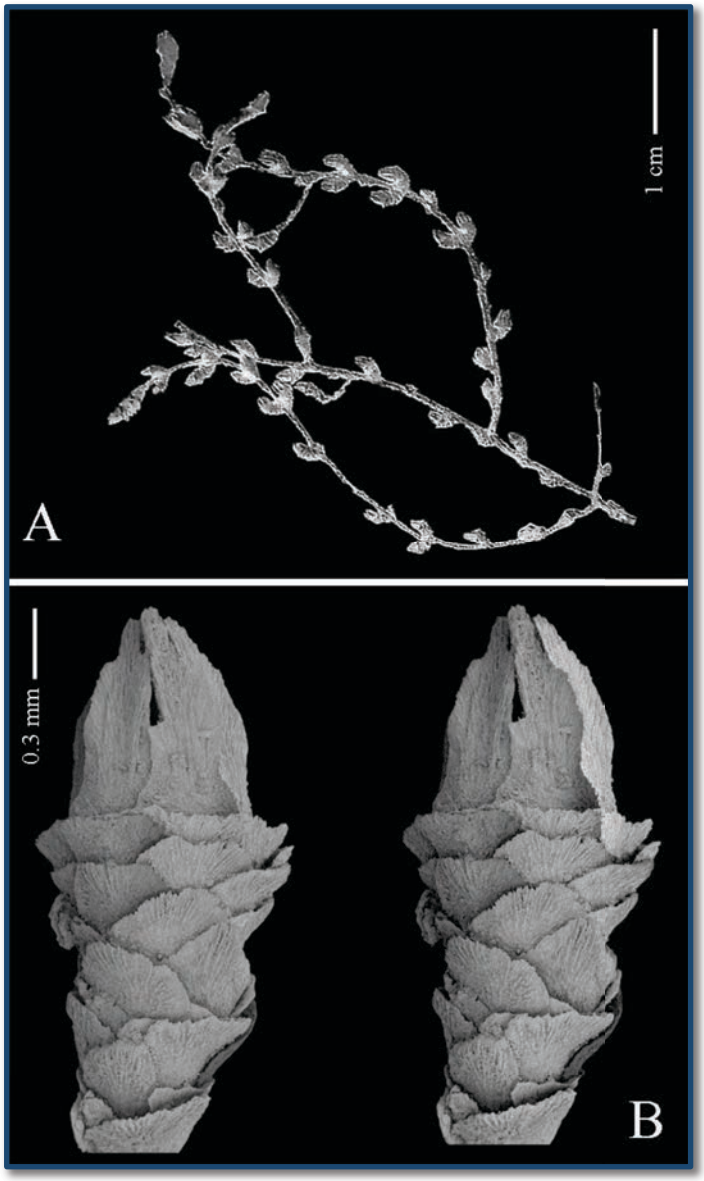


Figure 2.38.- *Plumarella* (*Faxiella) abietina* (Studer, 1894), holotype (MCZ 4802); **a**, fragment of colony from Menneking, 1905: pl. 8, Fig. 7; **b**, polyp on abaxial view, stereo pair. [Photo: Stephen D. Cairns, NMNH, Washington].

Description of the holotype

Fragment of colony ramified in one plane (Fig. 2.38a) 6 cm in high and 3 cm wide; ramified up to the third order, with slender branchlets up to 5 cm long. Axis without holdfast, with a basal diameter of 1 mm. The polyps are curved upward to the stem and branchlets, placed in pairs and sparsely arranged with 2-3 mm between them; 2 pairs of polyps per cm. They are relatively elongate (Figs. 2.38b, 2.39a), cylindrical, up to 2.6 mm tall and 1.0-1.2 mm in diameter, with a high conical, well-developed operculum.

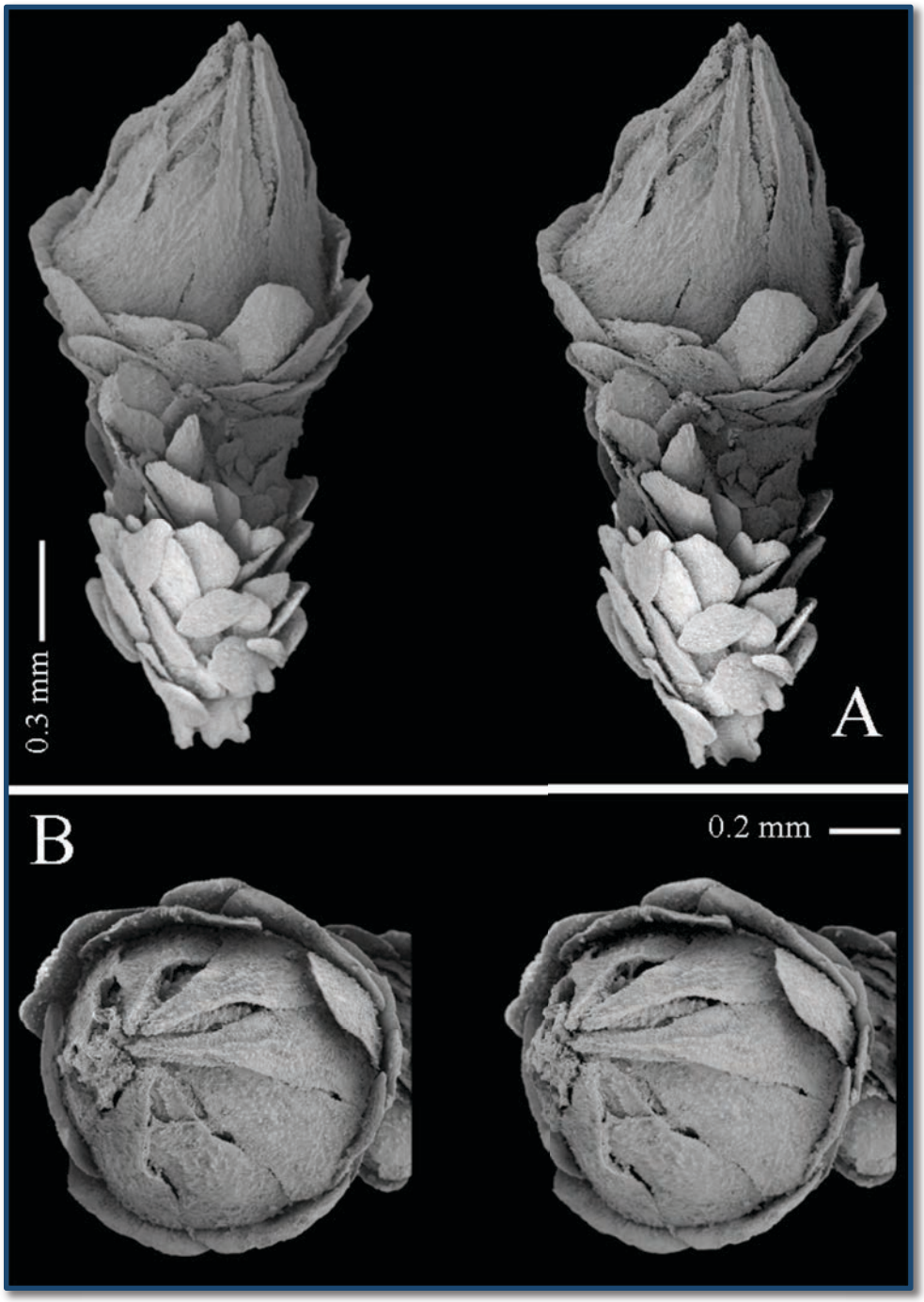


Figure 2.39.- *Plumarella* (*Faxiella) abietina* (Studer, 1894), holotype (MCZ 4802): **a**, polyp on adaxial view, stereo pair; **b**, polyp on oral view, stereo pair. [Photo: Stephen D. Cairns, NMNH, Washington].

Polyp body with 8 rows of scales, 6 in each abaxial row (Fig. 2.38b) and 5-6 smaller and slightly disorganized scales in each adaxial row (Fig. 2.39a). Eight opercular scales (Figs. 2.39b, 2.40a), 0.74-1.22 x 0.24-0.46 mm, shaped like elongated isosceles triangles. Proximal inner surface tuberculate covering up to half of the length; distal part with numerous longitudinal ridges. Outer surface granular and forming ridges distally. Free margin finely serrated. Marginal and body scales (Fig. 2.40b) roughly square to oval-shaped, 0.31-0.64 mm maximum length. Inner surface almost completely tuberculate, outer surface granular, with distal longitudinal ridges. Free margin finely serrated. Scales decreasing in size from abaxial to adaxial side. Eight marginal scales aligned with opercular scales without folding over them; slightly disorganized on the adaxial side.

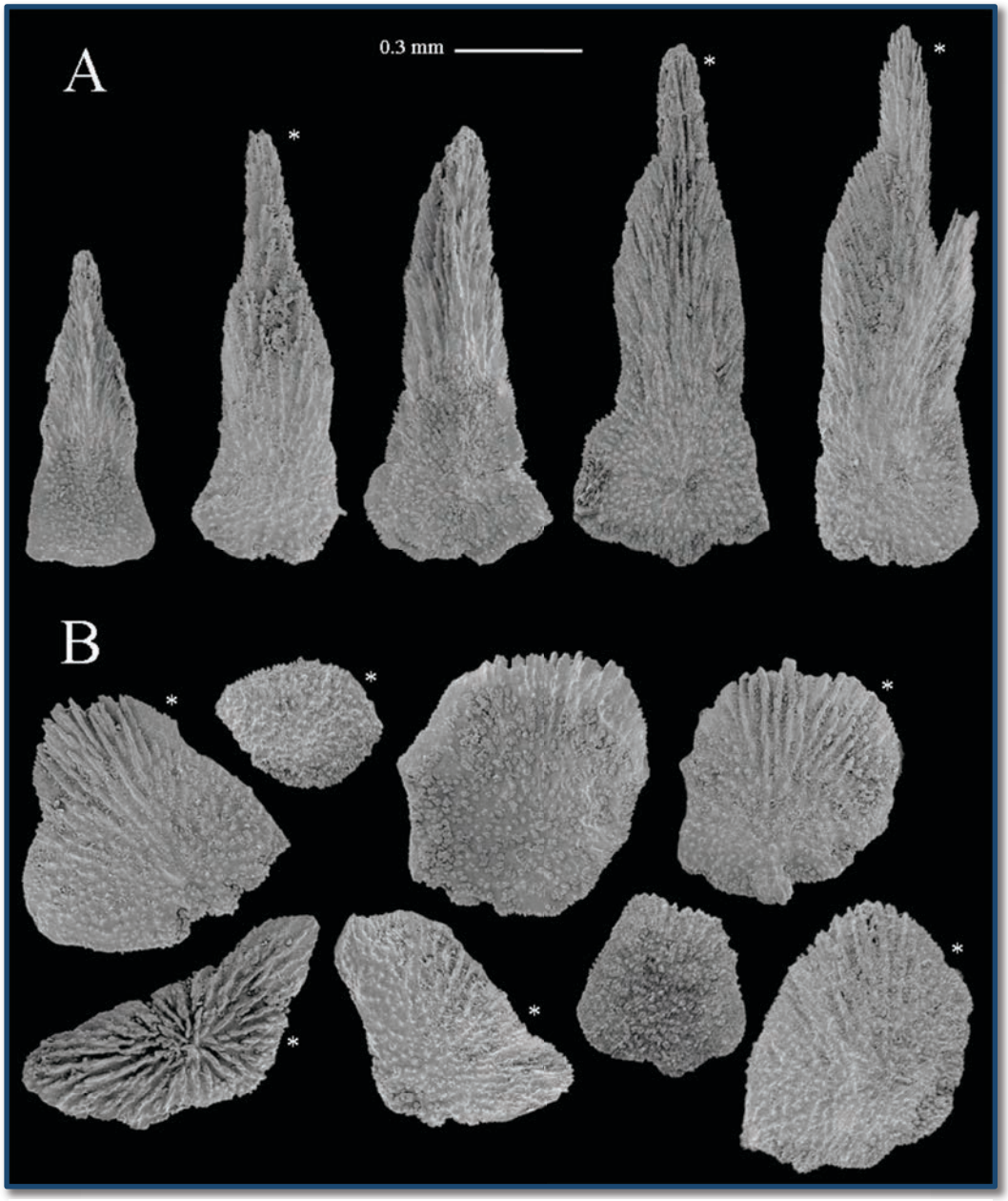
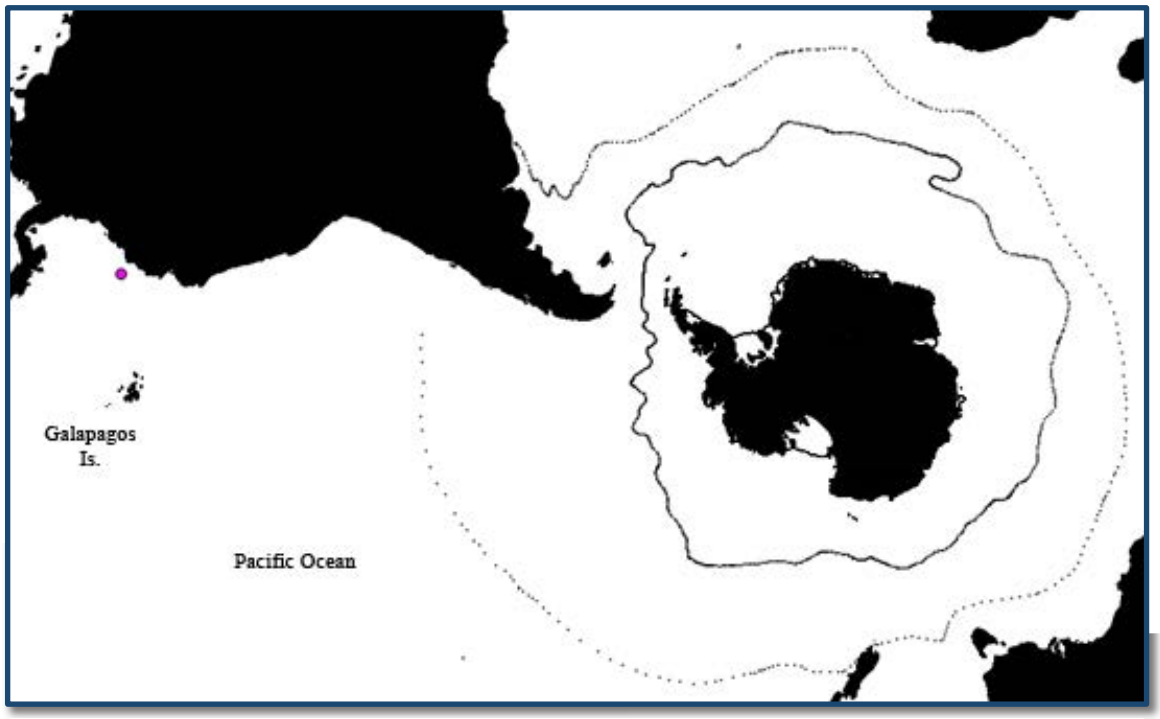


Figure 2.40.- *Plumarella* (*Faxiella) abietina* (Studer, 1894) n. comb., holotype (MCZ 4802): **a**, opercular scales; **b**, marginal and body scales. [*outer surface view; photo: Stephen D. Cairns, NMNH, Washington].

Geographical and bathymetrical distribution

Plumarella (*Faxiella*) *abietina* is only known from the west coast of Central America to the Galapagos (Map 2.19), at a depth of 3181 m.



Map 2.19.- *Plumarella* (*Faxiella*) *abietina* (Studer, 1894). Species examined distribution map. Dark pink, holotype, light pink, paratype.

Etymology

The specific epithet *abietina* is derived from the Latin for a fir tree (*Abies* genus).

Plumarella (*Faxiella*) *delicatula* (Thomson and Rennet, 1931)

(Figures 2.41-2.42)

Dicholaphis delicatula Thomson and Rennet, 1931:30, pl. 9, fig. 8-9, pl. 12, fig. 4.
Mirostenella delicatula, Cairns and Bayer, 2009:28 (in list), 38-39.
Plumarella (*Faxiella*) *delicatula,* Zapata-Guardiola, López-González and Gili, 2013:233.

Examined material

Holotype: AM (G13266), Australasian Antarctic Expedition 1914, 54°36'S, 158°48'E, off Macquarie Island, Subantarctica, 2743 m depth.

Remarks

The current state of conservation of this type material is very poor for the comparative taxonomic study here proposed. The colony is partially decalcified, and the few polyps and sclerites present are often fractured. Without enough material to work on, no SEM analysis could be carried out for polyps and branchlets; only one or two polyps were dissolved and analysed by SEM to observe the sclerite shape and ornamentation.

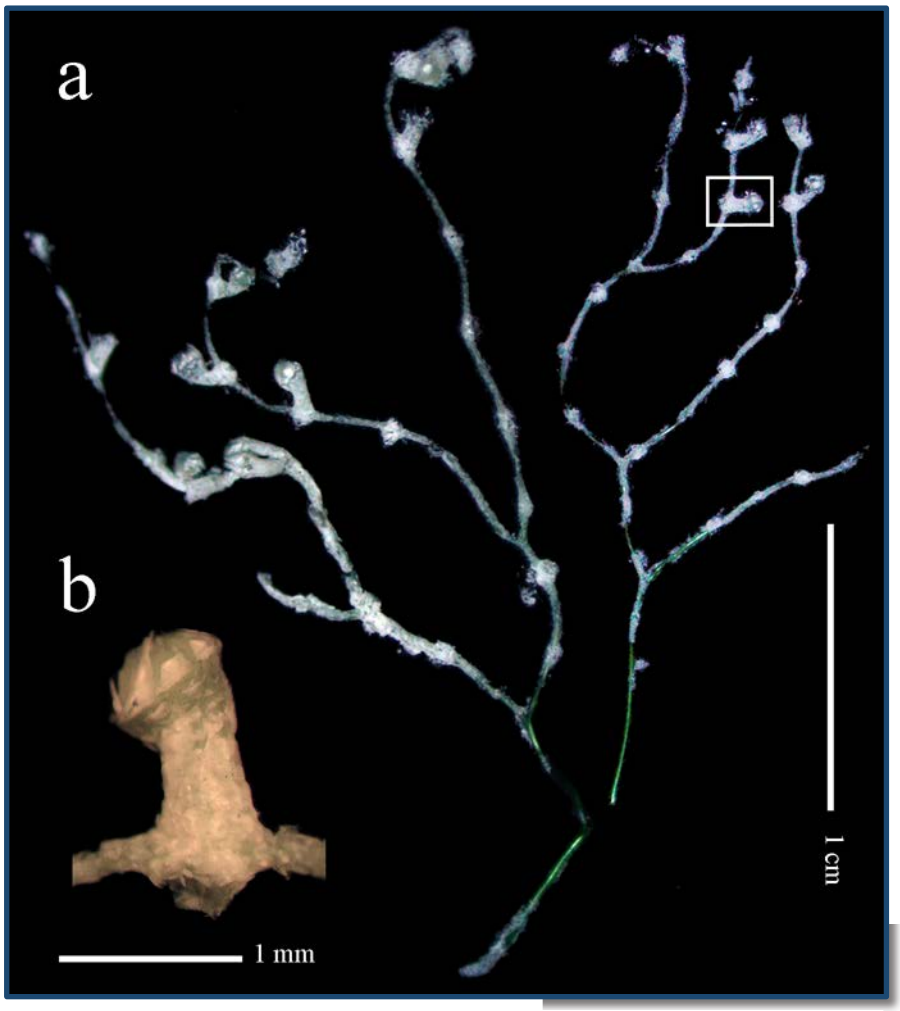


Figure 2.41.- *Plumarella* (*Faxiella*) *delicatula*, holotype (AM G13266): **a**, colony in two fragments; **b**, detail of a polyp indicated in a, lateral view.

Description of the holotype

Two delicate uniplanar fragments (Fig. 2.41a), 2.6 cm and 3.3 cm tall and about 1 cm and 1.5 cm wide respectively, dichotomously branched, internodes about 4-7 mm in length, terminal branchlets up to 18 mm. Polyps cylindrical, about 1.1-1.8 mm long and 0.37-0.69 mm in diameter (Fig. 2,41b), curved upward towards the branchlets and placed in pairs, 4 pairs per cm (Fig. 2.41a). Outer surface of polyp scales smooth (Fig. 2.42), inner surface tuberculated. Free margin smooth. Marginal scales about 0.52 x 0.3 mm, body scales (Fig. 2.42bs) about 0.16-0.28 mm in maximum diameter and coenenchymal scales (Fig. 2.42cs) about 0.09 mm in maximum diameter.

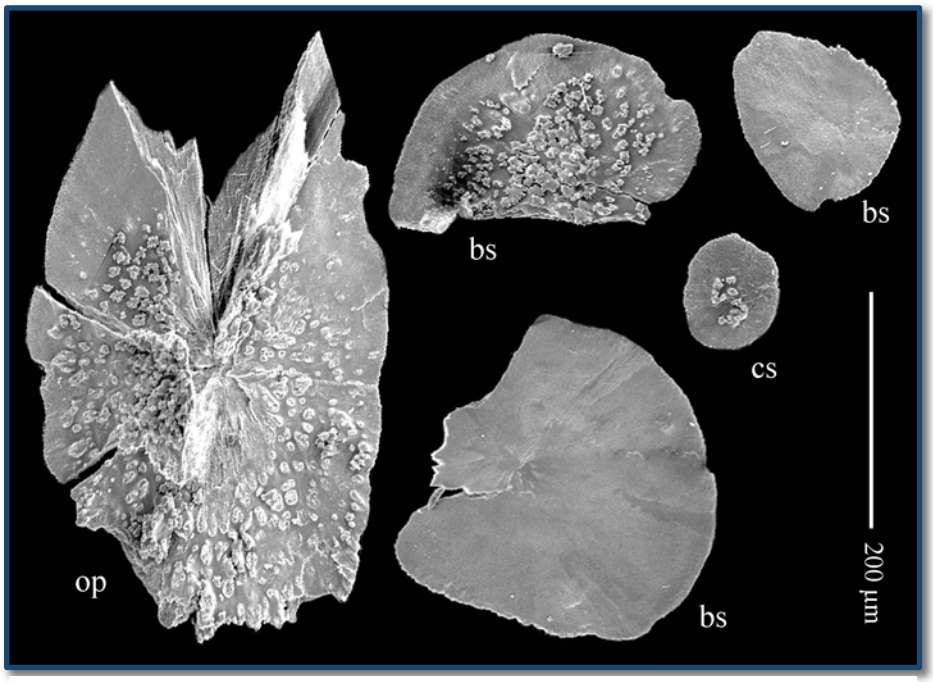


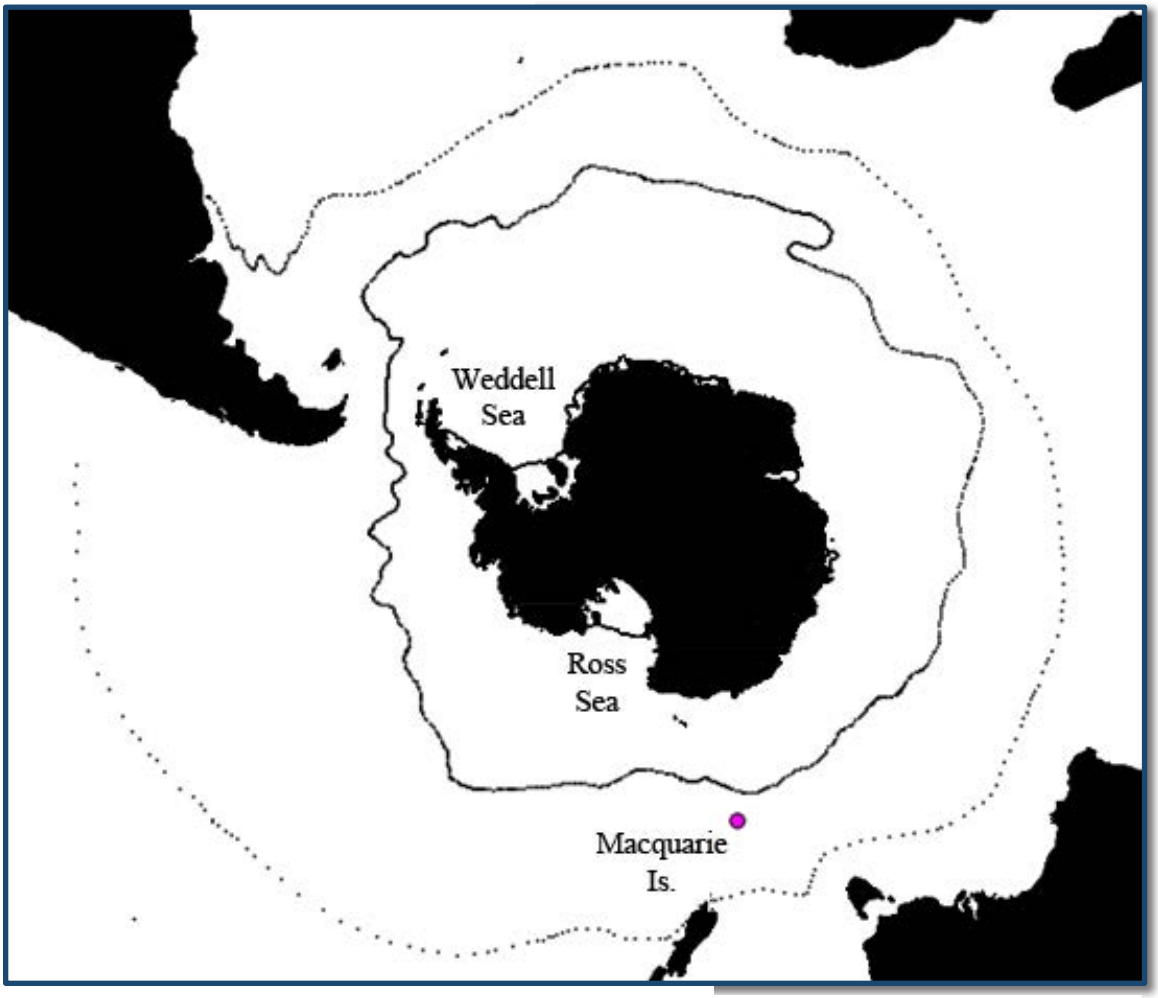
Figure 2.42.- *Plumarella* (*Faxiella*) *delicatula*, holotype (AM G13266): Scales. Note the fragmented state of the material examined. Abbreviations: **op**, opercular scale; **bs**, body scales; **cs**, coenenchymal scale.

Geographical and bathymetrical distribution

At present, *Plumarella* (*Faxiella*) *delicatula* is known from off Macquarie Island (Map 2.20), Subantarctica, at 2743 m in depth.

Etymology

The specific name *delicatula* was presumably chosen by the general delicacy of the colony.



Map 2.20.- *Plumarella* (*Faxiella*) *delicatula* (Thomson and Rennet, 1931). Species examined distribution map. Pink circle, holotype.

Subgenus *Verticillata* Zapata-Guardiola, López-González and Gili, 2012

Diagnosis

Plumarella in which funnel-shaped polyps occur straight and almost perpendicular to stem, placed in whorls along branchlets.

Geographical and bathymetrical distribution

Subantarctic waters, from Tierra del Fuego and the Falkland Islands to Burdwood Bank in the Scotia Sea, between 120 and 2044 m in depth.

Etymology

From the modern Latin adjective verticillatus that means “disposed in or forming whorls” which comes from the Latin noun verticillus “whorl”, in reference to the arrangement of polyps in whorls on branchlets. Gender: feminine.

Type species

Plumarella (*Verticillata*) *castellviae* Zapata-Guardiola, López-González and Gili, 2012.

Plumarella (*Verticillata*) *castellviae* Zapata-Guardiola, López-González and Gili, 2013

(Figures 2.43-2.46)

Plumarella (*Verticillata*) *castellviae* Zapata-Guardiola, López-González and Gili, 2013:236.

Examined material

Holotype: USNM 1099626, *LMG* 06-05, stn 2, 53°48‘S, 64°54‘W, off Isla Grande, Tierra del Fuego, Subantarctica, 120 m depth, 15 May 2006, one dry colony.

Paratypes: USNM 1128562, with the same sampling data as the holotype, nine fragmented colonies; NHMUK 2011.41, Discovery Expeditions, RSS *William Scoresby*, stn WS 877, 52°35.4‘S, 61°3.6‘W, south west of Falkland Islands, Subantarctica, 350 m depth, 4 April 1932, two fragments; USNM 100787 (plus SEM stubs 1609, 1610), USNM 100795 and USNM 1019337, USAP, *Eltanin* 6, stn 339, 53°07.9‘S, 59°24‘W, west of Beauchene Island, Falkland Islands, Subantarctica, 512-586 m depth, 3 December 1962, one, three and 20 fragmented colonies respectively; USNM 58167 (plus SEM stubs 285, 297, 298) and USNM 88822 (plus SEM stub 2236), USAP, *Eltanin* 9, stn 740, 56°06‘S, 66°18‘W, east of Cape Horn, Drake Passage, Tierra del Fuego, Subantarctica, 384-494 m depth, 18 September 1963, ten and five colonies respectively; USNM 77392 (plus SEM stubs 1568, 1569), USAP, *Eltanin* 22, stn 1592, 54°45‘S, 55°37‘W, Burdwood Bank, Scotia Sea, Subantarctica, 1647-2044 m depth, 14 March 1966, ten fragmented colonies; USNM 100789 (plus SEM stubs 1591, 1592), USAP, *Hero* 715, stn 885,

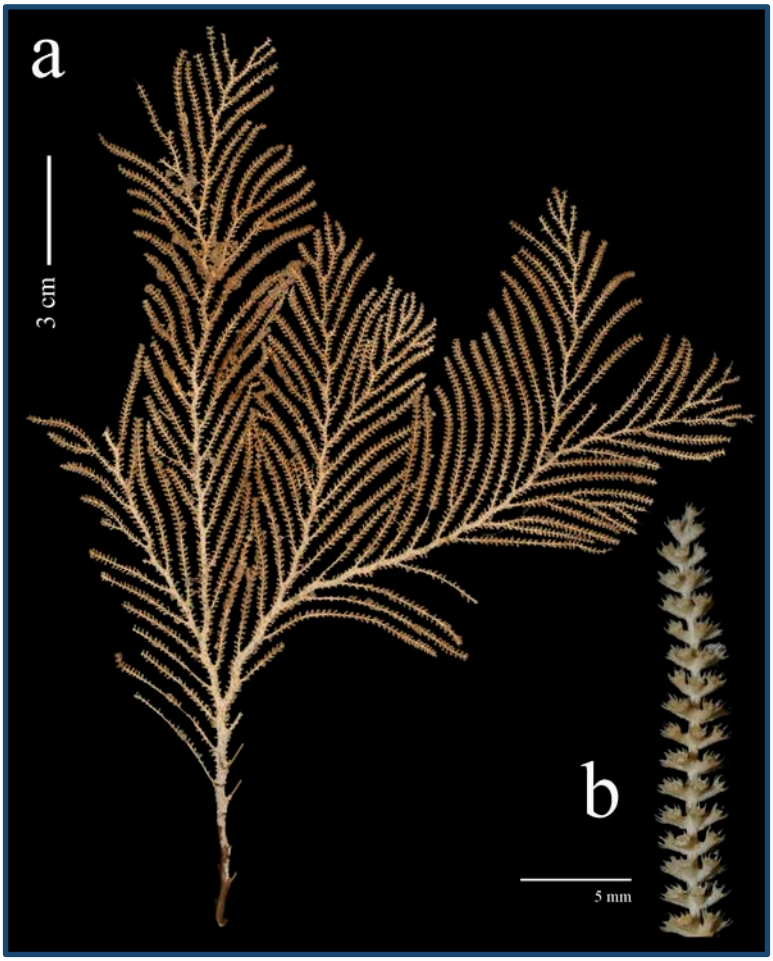


Figure 2.43.- *Plumarella* (*Verticillata*) *castellviae*, holotype (USNM 1099626): **a**, whole colony; **b**, detail of a branchlet.

54°55‘S, 64°9.7‘W, Staten Island, Tierra del Fuego, Subantarctica, 493-511 m depth, 30 October 1971 one colony; BEIM (CRO-57), *Polarstern* cruise ANT XIII/4, stn 40/111, 55°28.8‘S, 66°04.5‘W, southeast of Isla Nueva, Tierra del Fuego, Subantarctica, 1147 m depth, 17 May 1996, one fragment; MZB (2012-0485), *Polarstern* cruise ANT XIII/4, stn 40/115, 55°27.4‘S, 66°05.7‘W, southeast of Isla Nueva, Tierra del Fuego, Subantarctica, 580 m depth, 18 May 1996, numerous fragments; MZB (2012-0486), *Polarstern* cruise ANT XIX/5, stn PS61/150-1, 54°30.22‘S, 56°8.20‘W, south east of Falkland Islands, Antarctica, 286.3-290.3 m depth, 6 April 2002, two fragmented colonies; MZB (2012-0487), *Polarstern* cruise ANT XIX/5, stn PS61/153-1, 54°31.22‘S, 56°8.93‘W, south east of Falkland Islands, Antarctica, 277.2-295.8 m depth, 6 April 2002, one colony.

Description of the holotype

Uniplanar colony (Fig. 2.43a) 25 cm tall and about 20 cm wide, moderate sympodial branching with branchlets up to 6 cm in length, simple or branched up to the sixth order. Axis brown, broken in its proximal portion, without holdfast. Axial nodes without reduction of calcification. Basal axis diameter about 3 mm. Polyps funnel-shaped, straight, about 0.9-1.4 mm in height and 0.37-0.50 mm in diameter, almost perpendicular to branchlet and placed in whorls (Fig. 2.43b); 5-7 polyps per whorl (Fig. 2.44a) and 6-8 whorls per cm (Fig. 2.43b).

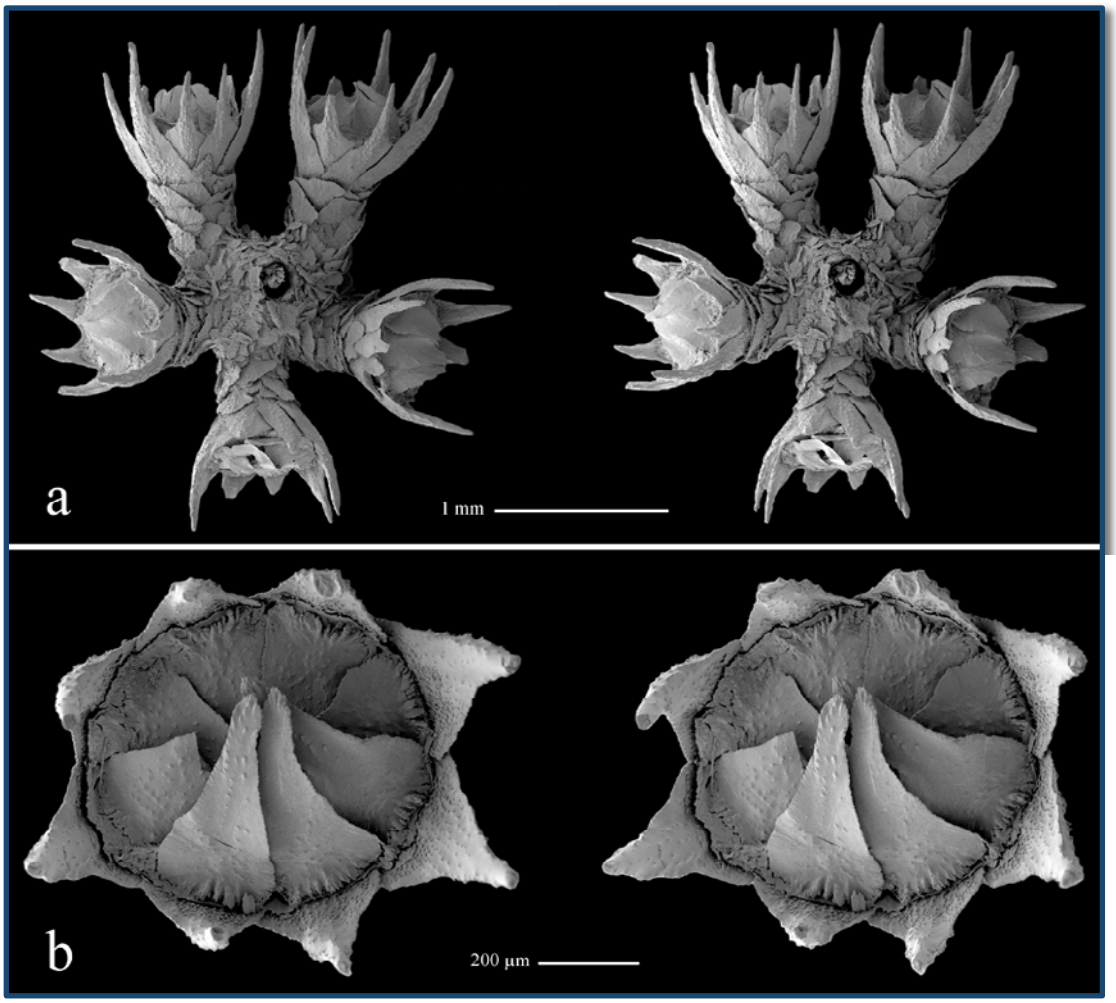


Figure 2.44.- *Plumarella* (*Verticillata*) *castellviae*, holotype (USNM 1099626): **a**, polyp whorl, adaxial view, stereo pair; **b**, polyp, oral view, stereo pair.

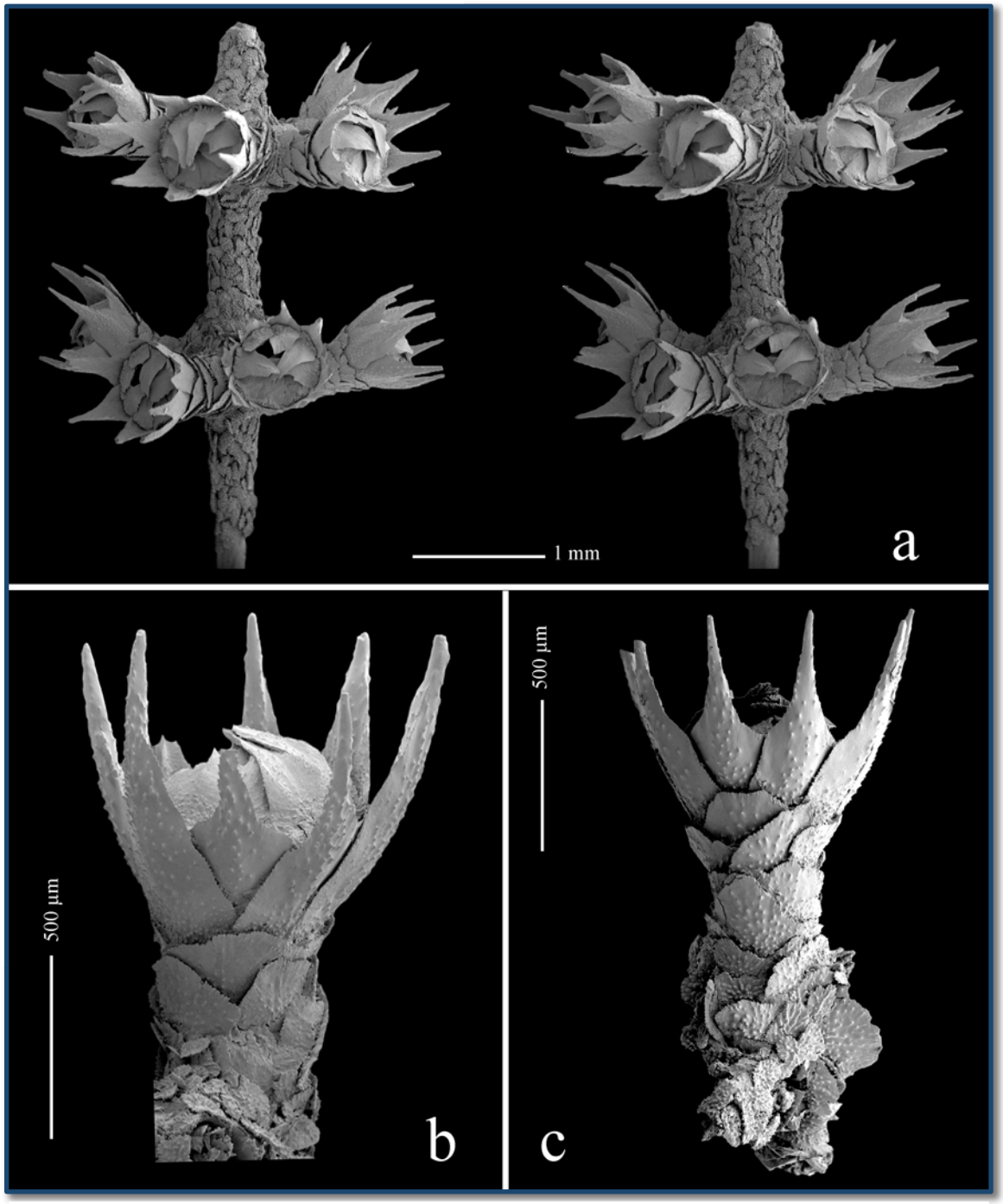


Figure 2.45.- *Plumarella* (*Verticillata*) *castellviae*, holotype (USNM 1099626): **a**, polyp whorls, stereo pair**; b**, polyp, adaxial view; **c,** polyp, abaxial view.

Polyp body with 8 longitudinal rows of scales overlapping one another, 3 scales in each adaxial row (Fig. 2.45b), 5-6 scales in each abaxial row but disorganized proximally (Fig. 2.45c). Operculum low with 8 triangle-shaped scales (Fig. 2.44b, 2.46a) 0.40-0.47 x 0.19-0.26 mm in size. Proximal half of inner surface tuberculated, distal half smooth, without keel. Outer surface radially granulated. Basal margin tuberculated, free margin finely serrated almost smooth. Eight marginal scales (Fig. 2.46b), 0.67-0.99 x 0.26-0.32 mm, spoon-shaped with distal thorn. Inner surface tuberculated until thorn base; pointed granules on thorn surface. Outer surface with pointed granules arranged longitudinally, proximal part tuberculated. Free margin

finely serrated, basal margin tuberculated. Body scales (Fig. 2.46c) oval-fan shaped, 0.18-0.37 x 0.21-0.37 mm. Inner surface almost completely tuberculated. Proximal outer surface tuberculated, distal outer surface granulated, basal outer margin tuberculated, free margin finely serrated. Coenenchymal scales (Fig. 2.46d) irregular oval shaped, 0.06-0.24 mm in maximum length; inner surface tuberculated, outer surface granulated.

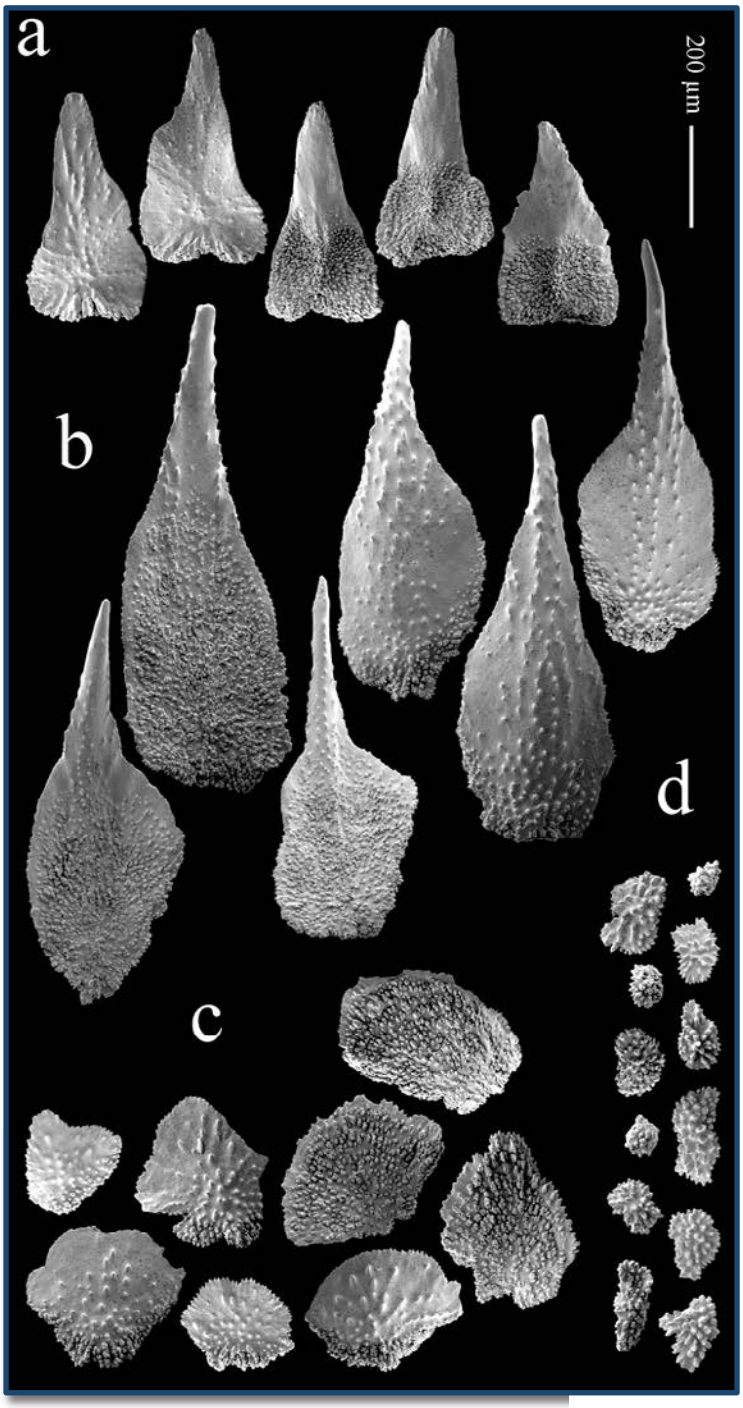


Figure 2.46.- *Plumarella* (*Verticillata*) *castellviae*, holotype (USNM 1099626): **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal scales**.**

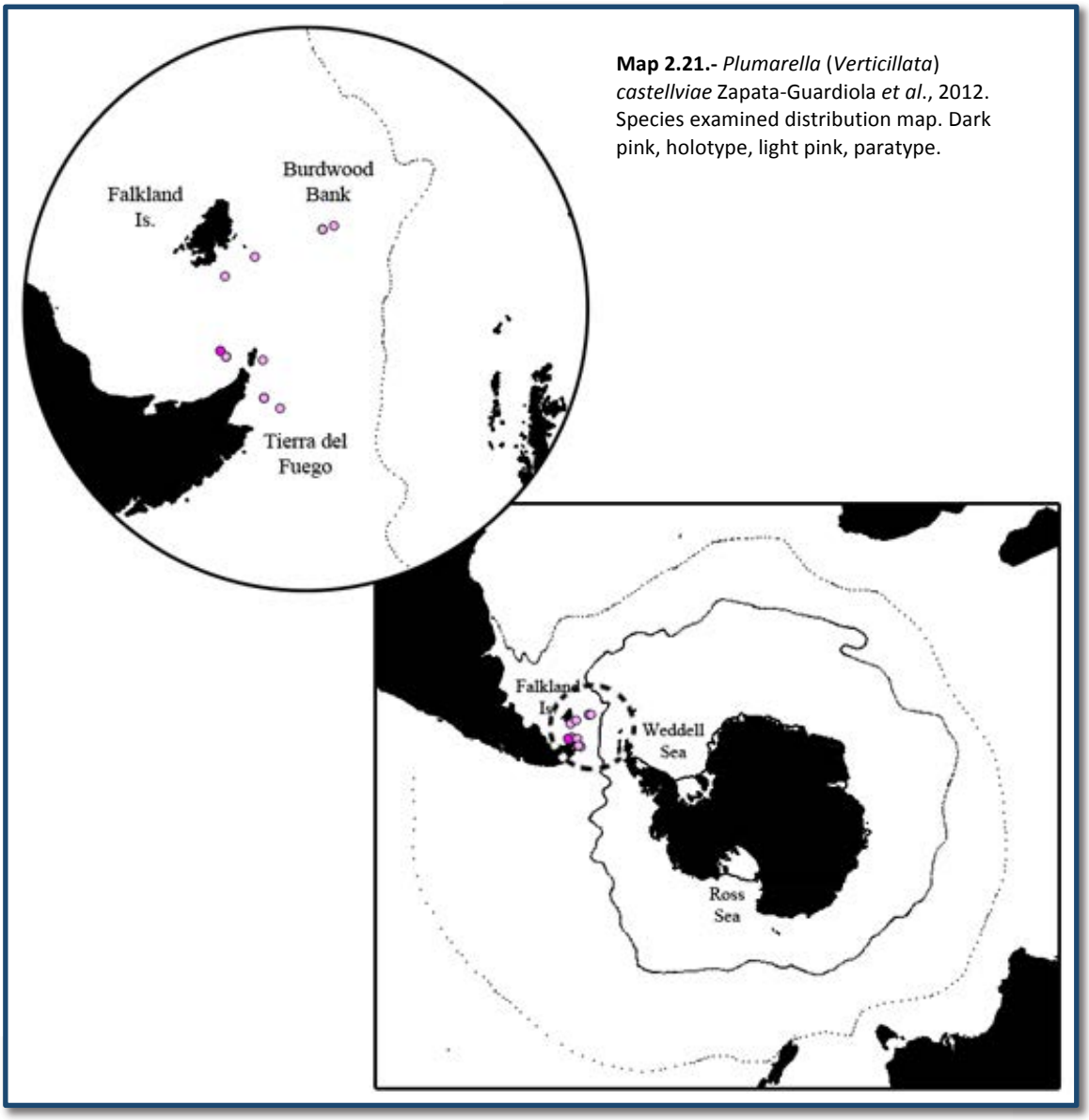
Variations from holotype

The colony shape varies from uniplanar to a bushy appearance. Their size range is between 11-25 cm tall and 6-20 cm wide. Polyps are arranged in whorls from 4 to 7, and their density

ranges from 5 to 8 whorls per centimetre. The funnel form of the polyps is similar to those of the holotype, from 0.8 to 1.6 mm long and from 0.3 to 0.5 mm in diameter. The distribution and form of the sclerites from polyps and coenenchyme are as in the holotype.

Geographical and bathymetrical distribution

At present, *Plumarella castellviae*, is known from Subantarctic waters (Map 2.21), from Tierra del Fuego and the Falkland Islands to Burdwood Bank in the Scotia Sea, between 120 and 2044 m in depth.



Map 2.21.- *Plumarella* (*Verticillata*) *castellviae* Zapata-Guardiola *et al*., 2012. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Etymology

The species is named after Dr. Josefina Castellví (Institute of Marine Sciences, Spain) in recognition of her major contribution to the understanding of Antarctic ecosystems and, such as the first manager of the Spanish Antarctic Program, for being a key person for the Spanish Antarctic research. Gender: feminine.

Genus *Primnocapsa* Zapata-Guardiola and López-González, 2012

Primnocapsa Zapata-Guardiola and López-González, 2012:19.

Diagnosis

Primnoidae with a planar colony shape and dichotomously branched. Short polyps club-shaped, slightly curved upward to stem and branchlets, singly placed and arranged in spirals. Eight opercular scales with the inner surface keeled and with 2 irregular basal mounds. Eight marginal scales with strong radial ridges on the distal inner surface, offset from the opercular scales. Polyps completely covered by 8 longitudinal rows of body scales reduced in number and size from distal to basal part. Body scales reduced adaxially but always present. Coenenchyme with a single layer of round shaped scales with a central bulk on the inner surface.

Geographical and bathymetrical distribution

At present, *Primnocapsa* is only known from the type species off Barrenjoey, Australia, between 54.6 and 73.12 m depth.

Etymology

The generic name combines *primno*– a common prefix in reference to the gorgonian family, and –*capsa*, a Latin word meaning box, in reference to the perfect closed operculum like the capsule fruits of some herbaceous plant groups. Gender: feminine.

Type species

Amphilaphis plumacea Thomson and Mackinnon, 1911.

Primnocapsa plumacea (Thomson and Mackinnon, 1911)

(Figures 2.47-2.49)

Amphilaphis plumacea Thomson and Mackinnon, 1911:680-681; pl. 65, fig. 3; pl. 68, fig. 3; pl.74. –Cairns and Bayer, 2009:28 (listed).
Thouarella (*Amphilaphis*) *plumacea*, Kükenthal, 1915:149.—Kükenthal, 1919:414.—Kükenthal, 1924:291-292; text fig. 163.
Primnocpasa plumacea, Zapata-Guardiola and López-González, 2012:20.

Examined material

Holotype: AM G12123, Thetis Expedition, "11 miles E. by N. of Barrenjoey", 54.6-73.12 m depth.

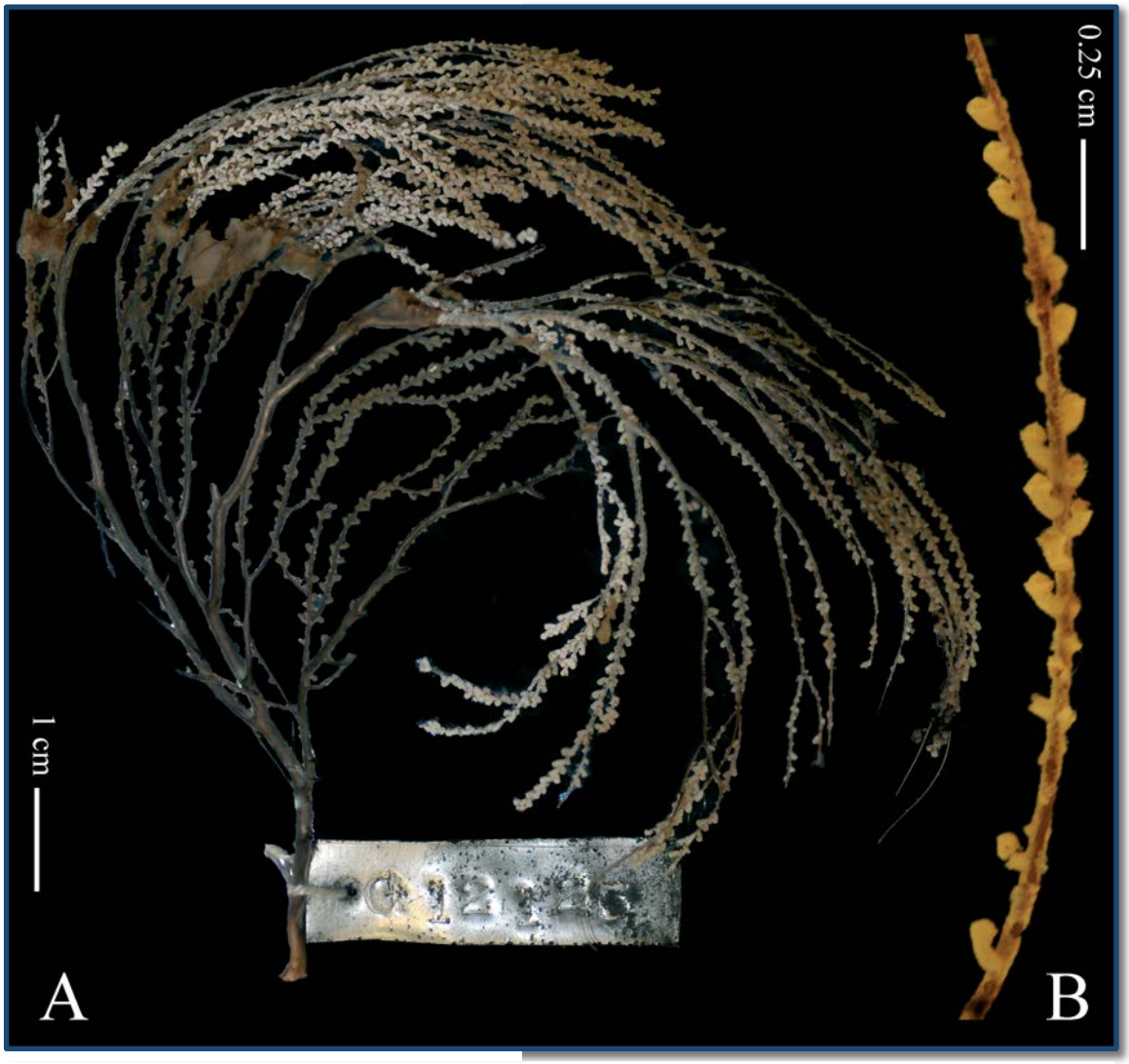


Figure 2.47.- *Primnocapsa plumacea* (Thomson and Mackinnon, 1911), holotype (AM G12123): **a**, whole colony; **b**, detail of branchlet.

Description of the holotype

Planar colony, dichotomously branched (Fig. 2.47a), 14 cm high and 5 cm wide, internodes about 7-21 mm long and terminal branchlets up to 29 mm. Axis dark brown, stiff, holdfast present. Basal axis diameter 2 mm. Polyps slightly curved upward to stem and branchlets (Fig. 2.47b), singly placed, and arranged in spirals, 11-15 polyps per cm. They are relatively short (Fig. 2.48), club-shaped, up to 1.2 mm tall and 0.36-0.64 mm in diameter, with a conical operculum. Polyp body with 8 rows of scales that are reduced in size and number adaxially. Each abaxial row has 8-12 scales (Fig. 2.48a) and each adaxial row has 2-3 scales (Fig. 2.48c). Marginal scales offset from opercular scales (Fig. 2.48b).

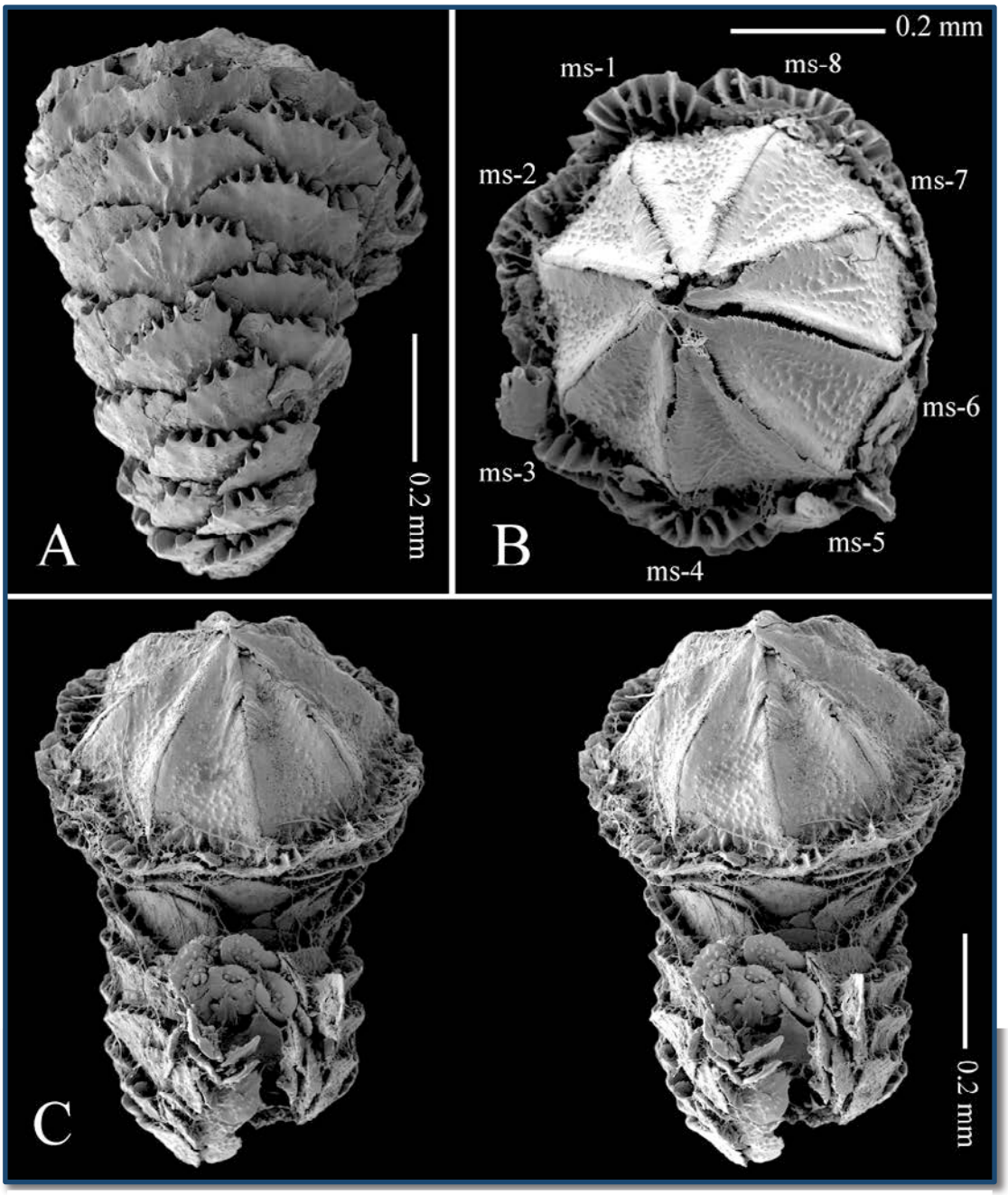


Figure 2.48.- *Primnocapsa plumacea* (Thomson and Mackinnon, 1911), holotype (AM G12123): **a**, polyp on abaxial view; **b**, polyp on oral view; **c**, polyp on adaxial view, stereo pair. [**ms**= marginal scales].

Eight opercular scales (Figs. 2.48b, 2.49a), 0.31-0.48 x 0.22-0.28 mm, isosceles triangle-shaped with an acute apex. Each vertex of the proximal inner surface has an irregular mound articulating with the corresponding mound of adjacent operculars, distal inner surface with a strong prominent keel. Inner surface of free lateral borders and the distal keel with granules forming ridges; remaining inner surface tuberculate. Outer surface of the lateral free borders grooved or smooth, remaining outer surface granular. Free margin finely serrated.

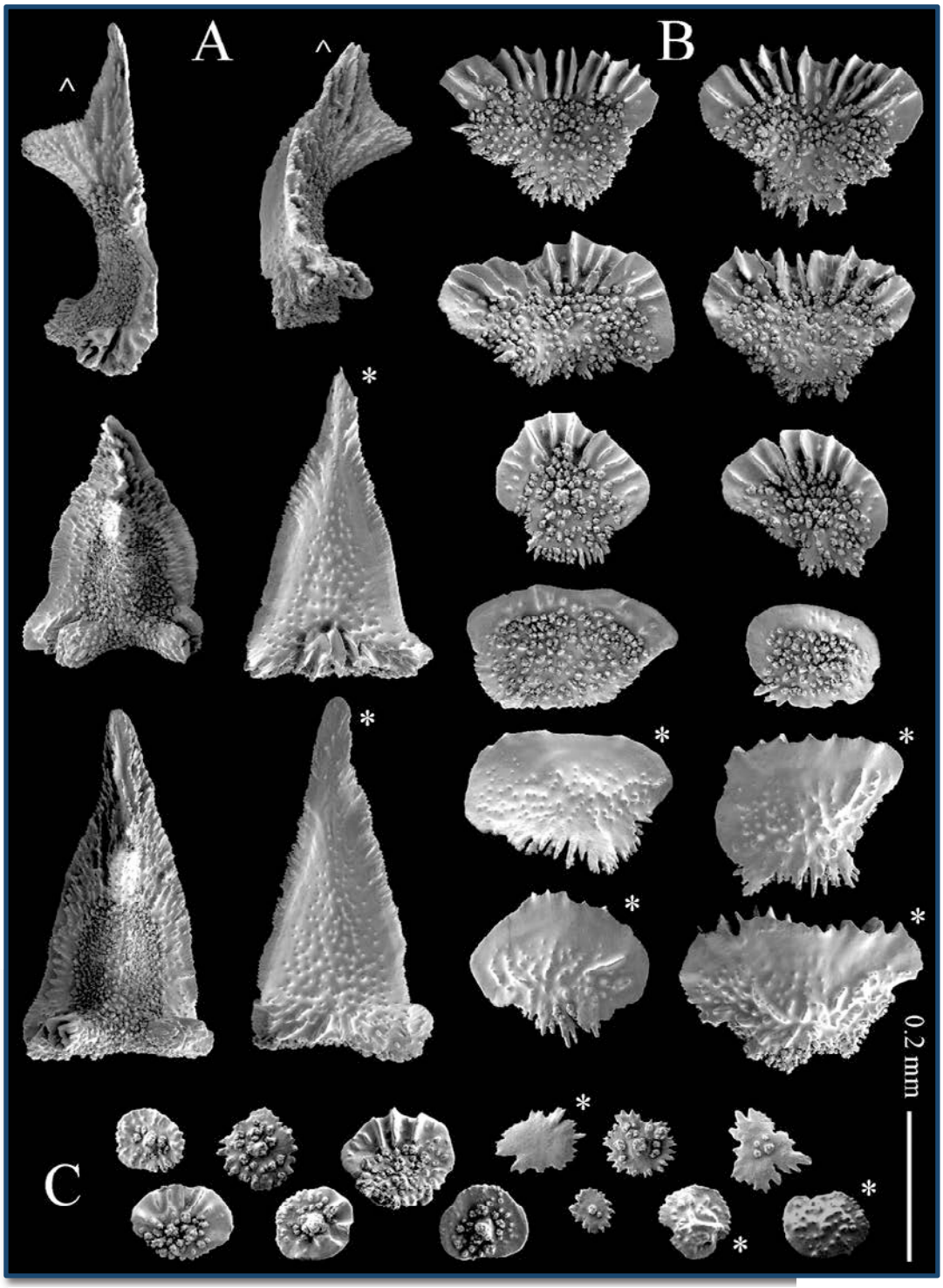
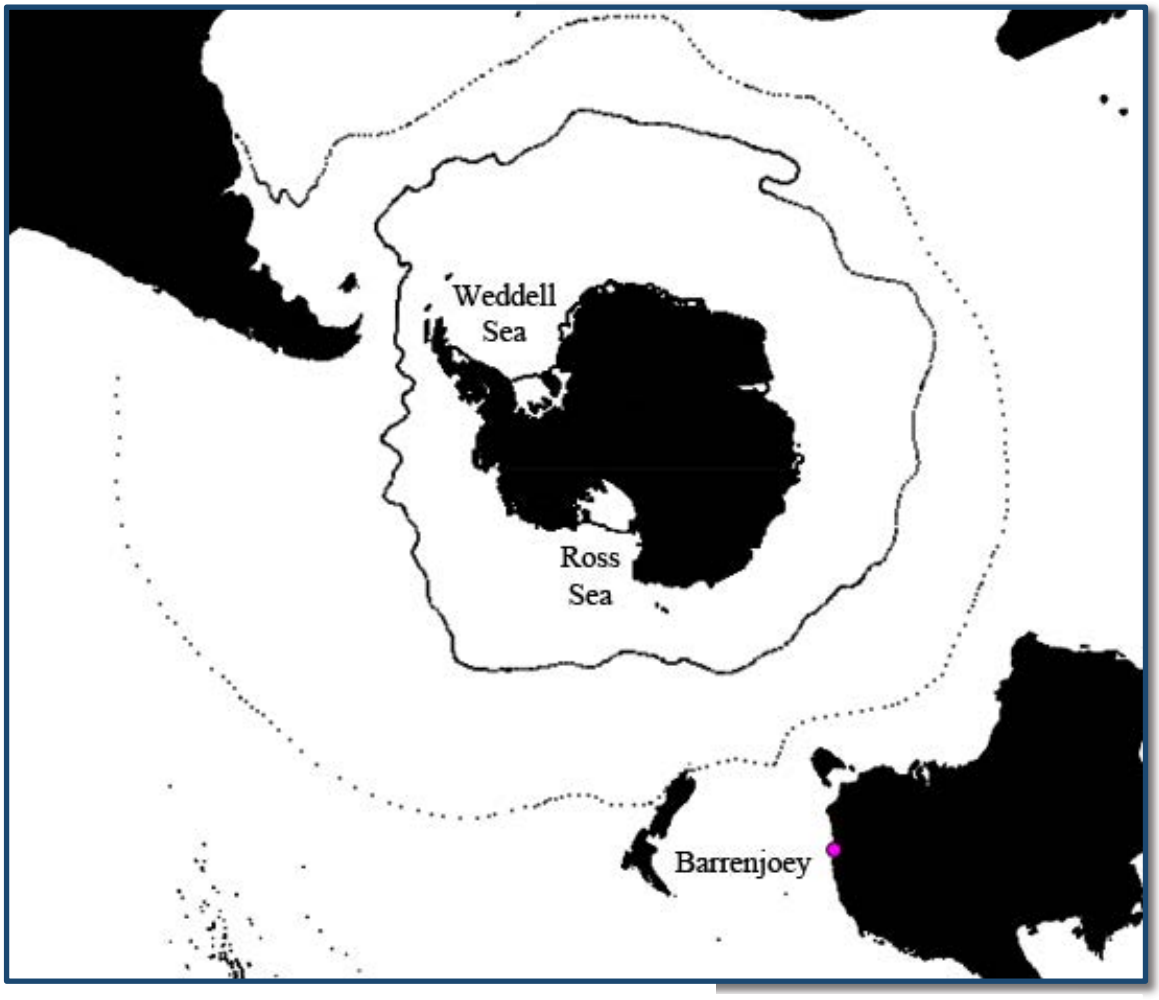


Figure 2.49.- *Primnocapsa plumacea* (Thomson and Mackinnon, 1911), holotype (AM G12123): **a**, opercular scales; **b**, marginal and body scales; **c**, coenenchymal sclerites. [* outer surface view; ^ lateral view].

Abaxial marginals and body scales (Fig. 2.49b) fan-shaped, 0.11-0.25 x 0.14-0.33 mm with the proximal inner surface tuberculate covering at least 75% of the scale and strong, distal, radial ridges; adaxials without ridges. Outer surface granular. Free margin finely serrated, basal margin with digitate processes. Scales decrease in number and size from abaxial to adaxial side and from distal to basal portion. The 8 marginal scales offset from opercular scales. Coenenchymal sclerites (Fig. 2.49c) round in shape, 0.06-0.15 mm in maximum diameter. Inner surface tuberculate with a central bulk, edge quite smooth or ridged, outer surface from quite smooth to granular. Margin quite smooth, or with digitate processes.

Geographical and bathymetrical distribution

Primnocapsa plumacea is only known from the type locality off Barrenjoey, Australia (Map 2.22), between 54.6 and 73.12 m depth.



Map 2.22.- *Primnocapsa plumacea* (Thomson and Mackinnon, 1911). Species examined distribution map. Pink circle, holotype.

Etymology

The specific name refers to its delicate and graceful form, which has a certain resemblance to an uncurled ostrich plume.

Genus *Scopaegorgia* Zapata-Guardiola and López-González, 2010c

Scopaegorgia Zapata-Guardiola and López-González, 2010c:1997.

Diagnosis

Primnoidae with a bottlebrush colony shape with simple branchlets. Polyps cylindrical and straight to stem, arranged in whorls, not present on main stem. Opercular scales relatively large, pointed or with small thorn, keel present on the inner surface, eight in number. Marginal scales fan shaped with acute or rounded tip, without spine or keel, seven in number (two adaxial, two lateral and three abaxial). Body scales similar to marginal scales in shape and ornamentation. All adaxial sclerites smaller or reduced. Coenenchymal scales plate-like, with a deeper layer of tuberculate irregulars.

Geographical and bathymetrical distribution

At present, *Scopaegorgia*, has been reported from Bellingshausen Sea, eastern Weddell Sea, Elephant and South Shetland Islands and eastern Ross Sea, Antarctica, between 150 and 600 m in depth.

Etymology

The generic name combines *scopae*- meaning broom-shape, and -*gorgia*, a common suffix in gorgonian generic names. Gender: feminine.

Type species

Stenella liouvillei Gravier, 1913.

Scopaegorgia liouvillei (Gravier, 1913)

(Figures 2.50-2.54)

Stenella (*Dasystenella*) *liouvillei* Gravier, 1913:590; 1914:63–69, pl. 2, figs 9–11, pl. 4, fig. 20, text figs 77–85.
Scopaegorgia liouvillei, Zapata-Guardiola and López-González, 2010c:1998.

Examined material

Holotype: MNHN Oct.0000-0233, Seconde Expedition Antarctique Francaise (1908–1910), Pourquoi Pas?, dredge VIII, Marguerite Bay, Bellingshausen Sea, Antarctica, 176 m depth, 20 November 1909.

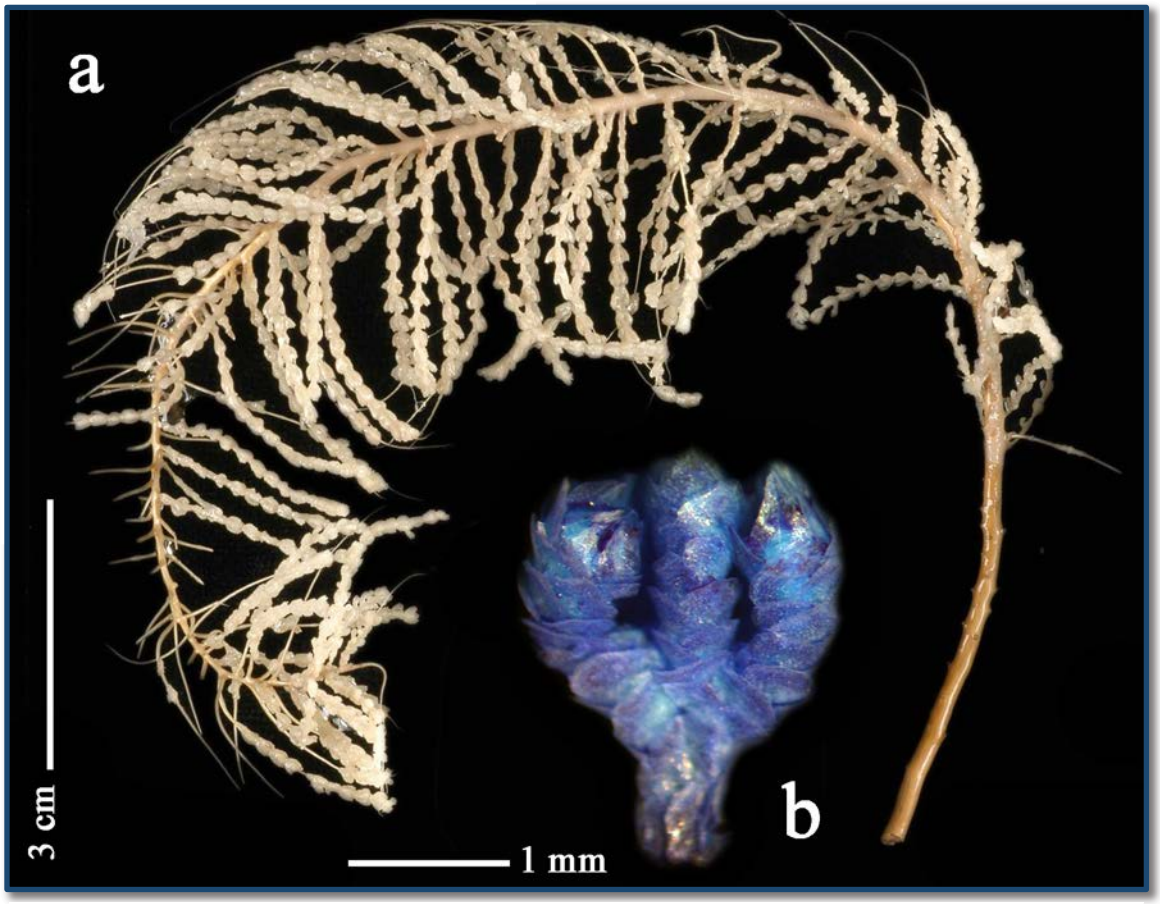


Figure 2.50.- *Scopaegorgia liouvillei*, holotype (MNHN Oct.0000–0233): **a**, whole colony; **b**, detail of a whorl. Photo: Aude Andouche, MNHN, Paris.

Additional material: CRO-0060, ANT XVII-3, stn 65-01, 71°17.60'S, 13°48'W, Cape Norvegia, Antarctica, 615 m depth, 31 March 2000, one colony; US 502, ANTXVII-3, stn 85-01, 71°12.19'S, 12°19'W, Cape Norvegia, Antarctica, 309-318 m depth, 2 April 2000, one colony; CRO-0061, ANT XVII-3, stn 109-01, 71°11.30'S, 12°18.50'W, Cape Norvegia, Antarctica, 311 m depth, 4 April 2000, one colony; ZIZMH 11746, ANT XIX/3, stn PS61/048-01, 61°09.82'S, 54°33.40'W, east of Elephant Island, Antarctica, 277.6–343.2 m depth, 30 January 2001, one broken colony; ZIZMH 11747, ANT XIX/5, stn PS61/052-01, 61°20.76'S, 55°13.80'W, south Rowett Island, Elephant Island, Antarctica, 264.0–270.0 m depth, 31 January 2001, four colonies; MNA 2460, ANT XIX/3, stn PS61/066-01, 60°53.13'S, 55°22.96'W, north of Seal Rocks, north-west of Elephant Island, Antarctica, 301.2–439.6 m depth, 4 February 2002, one colony; CRO-0055, ANT XIX/3, stn PS61/071-01, 60°58.83'S, 55°49.74'W, north-west of Elephant Island,

Antarctica, 160.0–187.2 m depth, 5 February 2002, one colony; MNA 2461, ANT XIX/3, stn PS61/111-01, 61°51.83'S, 59°15.71'W, north-west Bridgeman Islands, South Shetland Islands, Antarctica, 262.5–269.9 m depth, 16 February 2002, two colonies; US 1716, ANTXIX/3, PS61/121-01, 62°22.5'S, 61°26.58'W, north west of Livingston Island, South Shetland Islands, Antarctica, 297-363 m depth, 19 February 2002, two colonies; US 6438, ANT XIX/3, stn PS61/128-01, 62°41.37'S, 55°30.62'W, north Joinville Island, Peninsula Antarctica, Antarctica, 183-205 m depth, 21 February 2002, one colony; CRO-0059 and CRO-0064, ANT XIX/5, stn PS61/252-01, 61°23.45'S, 55°26.82'W, north of Gibbs Island, south-west of Elephant Island, Antarctica, 285.0–288.0 m depth, 25 April 2002, one colony each; CRO-0054, ANT XXI/2, stn PS65/276-1, 71°6.44'S, 11°27.76'W, Cape Norvegia, Antarctica, 277 m depth, 28 December 2003, one colony; MNA 2462, ANT XXI/2, stn PS65/292-01, 72°51.43'S, 19°38.62'W, West Cape, eastern Weddell Sea, Antarctica, 596.4–597.6 m depth, 31 December 2003, one colony;

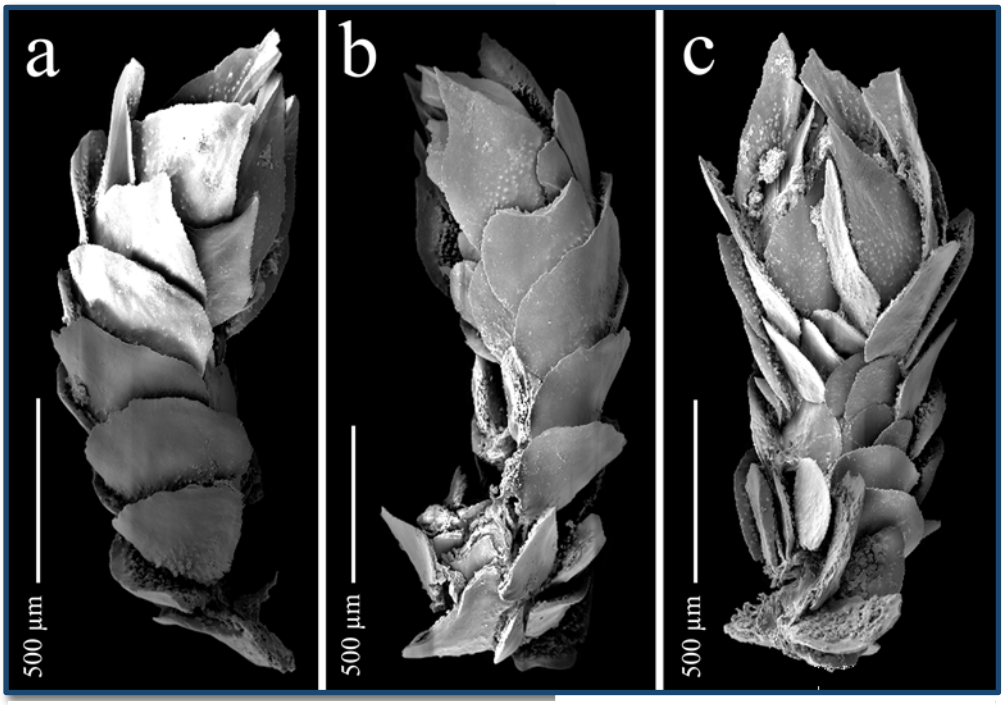


Figure 2.51.- *Scopaegorgia liouvillei*, holotype (MNHN Oct.0000–0233): **a,b**, polyps on lateral view; **c**, polyp on adaxial view.

CRO-0062, VLT ITALICA (XIX), stn H-OUT-4, 72°17.2'S, 170°23.9'E, Moubray Bay, Ross Sea, Antarctica, 204–208 m depth, 4 February 2004, one colony; CRO-0063, VLT ITALICA (XIX), stn H-OUT-2 (bis), 72°17.5'S, 170°29.4'E, Moubray Bay, Ross Sea, Antarctica, 339–353 m depth, 11 February 2004, one colony; US 6449, TAN0402, stn 59, 72°19.58'S, 170°27.48'E, Cape Hallet, Victoria Land, Antarctica, 231-236 m depth, 13 February 2004, two colonies; US 6338, TAN0402, stn 63, 72°19.3'S, 170°28.72'E, Cape Hallet, Victoria Land, Antarctica, 293-303 m depth, 13 February 2004, one colony; US 6362, VLT ITALICA (XIX), stn H-OUT-3 (bis), 72°17.6'S, 170°24.2'E, Cape Hallet, Victoria Land, Antarctica, 205-258 m depth, 17 February 2004, three colonies; CRO-0066 and US 6221, ANT XXIII/8, stn 605-01, 61°20.35'S, 55°29.16'W, north of Gibbs Island, southwest of Elephant Island, Antarctica, 151.3 m depth, 19 December 2006, two colonies, one broken colony and one colony; CRO-0053, ANT XXIII/8, stn 605-05, 61°20.27'S, 55°30.92'W, north of Gibbs Island, south-west of Elephant Island, Antarctica, 153.4 m depth, 20 December 2006, one colony; NHMB 1364, ANT XXIII/8, stn 608-01, 61°11.34'S, 54°43.17'W, east of Elephant Island, Antarctica, 293 m depth, 20 December 2006, two fragmented colonies; CRO-0065, ANT XXIII/8, stn 611-01, 60°58.90'S, 55°11.31'W, north of Elephant Island,

Antarctica, 215.1 m depth, 21 December 2006, one fragment; USNM 1139275, ANT XXIII/8, stn 654-06, 61°22.80'S, 56°03.84'W, north-west of Gibbs Island, south-west of Elephant Island, Antarctica, 342.3 m depth, 29 December 2006, one fragment; CRO-0052, ANT XXIII/8, stn 695-01, 63°00.55'S, 58°38.01'W, Bransfield Strait, Antarctica, 269.4 m depth, 6 January 2007, one colony; USNM 1139276, ANT XXIII/8, stn 696-01, 63°00.52'S, 58°49.68'W, Bransfield Strait, Antarctica, 360.5–361.2 m depth, 6 January 2007, one colony; US 6379, ANT XXIII/8, stn 697-01, 63°15.38'S, 59°3.94'W, Bransfield Strait, Antarctica, 329-408 m depth, 6 January 2007, one colony.

Description of the holotype

Colony (Fig. 2.50a) 23 cm total height and about 4 cm width, bottlebrush with simple branchlets, up to 3.4 cm long, all around. Axis ochre in colour, broken in its proximal portion, without holdfast. Basal axis diameter of 0.29 mm and 4.5 cm height up to the first branchlet.

Figure 2.52.- *Scopaegorgia liouvillei*, holotype (MNHN Oct.0000–0233). Whorl on abaxial view, stereo pair.

Polyps straight and directly upward to branchlet, placed in whorls (Fig. 2.50b), four or five polyps per whorl and four or five whorls per centimetre. Polyps not present on main stem. Polyps (Fig. 2.51) elongated, cylindrical, with a conical operculum; polyp about 1.6–2.3 mm in high and 0.51–0.67 mm in diameter. Polyp body with seven longitudinal rows of scales, six or seven scales on each longitudinal abaxial row (Fig. 2.52) overlapping one another. Eight opercular scales (Fig. 2.53a), large, 0.28–0.75 by 0.16–0.45 mm, shaped like an isosceles triangle with acute tip. Proximal inner surface tuberculated, covering at least half of their longitude; distal surface smooth with apical keel. Proximal outer surface granulated, distal quite smooth. Basal margin with digitate processes, free margin finely serrated. Two adaxial scales smaller, lancet-shaped with acute tip and with a small incipient keel on inner surface. Marginal scales (Fig. 2.53b) seven in number: two adaxials, two lateral and three abaxials;

0.45–0.64 by 0.34–0.45 mm, fan-shaped with acute tip, two adaxial marginal scales smallest 0.25–0.33 mm maximum length, round in shape. Proximal inner surface tuberculated, covering up to 80% of their longitude, in adaxials completely tuberculate, without spine. Distal inner surface smooth, inconspicuous or without keel. Outer surface smooth. Basal margin with digitate processes, free margin finely serrated. Body scales (Fig. 2.54a) oval-fan shape, 0.33–0.52 by 0.40–0.63 mm. Proximal inner surface almost completely tuberculate, distal inner surface smooth. Outer surface smooth. Free margin as in marginal scales. Coenenchymal scales (Fig. 2.54b) from outer layer round, oval-shaped, 0.13–0.48 mm maximum length; inner surface tuberculate, large warts, outer surface granulated or smooth, free margin finely serrated. Coenenchymal inner layer with tuberculate irregular sclerites.

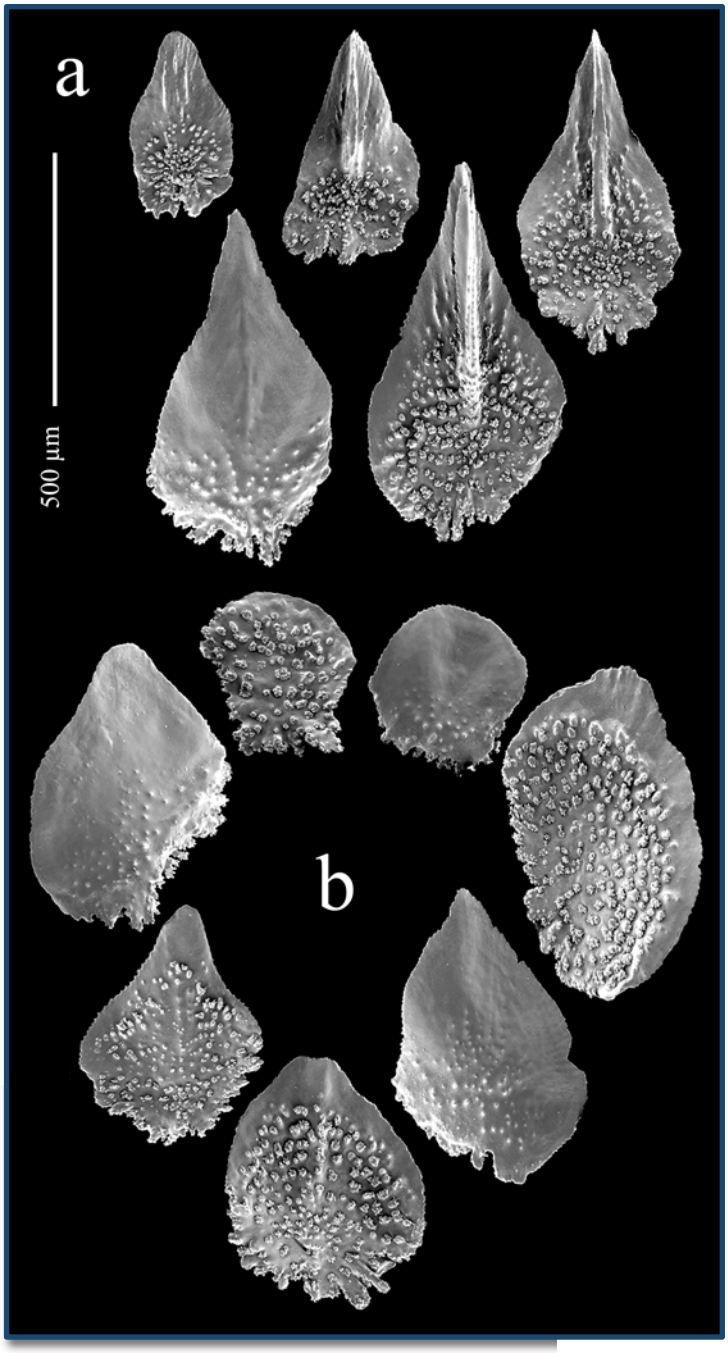


Figure 2.53.- *Scopaegorgia liouvillei*, holotype (MNHN Oct. 0000–0233): **a**, opercular scales; **b**, marginal scales.

Variations from holotype

The general colonial structure of the paratypes and additional material examined is quite similar to that of the holotype. They range in size between 13 and 47.5 cm height and 3.8 and 9.7 cm width. The simple branchlets, which can reach 5.3 cm long, arise from all around the main stem, sometimes so close that they seem to have their bases fused. Branchlets can be positioned more or less perpendicular to the stem, but generally they are inclined upwards. Polyps are arranged in whorls from four to eight, and their density ranges between four and seven whorls per centimetre. The form of polyps is similar to those in the holotype, from 1.4 to 2.3 mm height and from 0.49 to 0.72 mm diameter. Distribution and form of the sclerites from polyps and coenenchyme are as in the holotype.

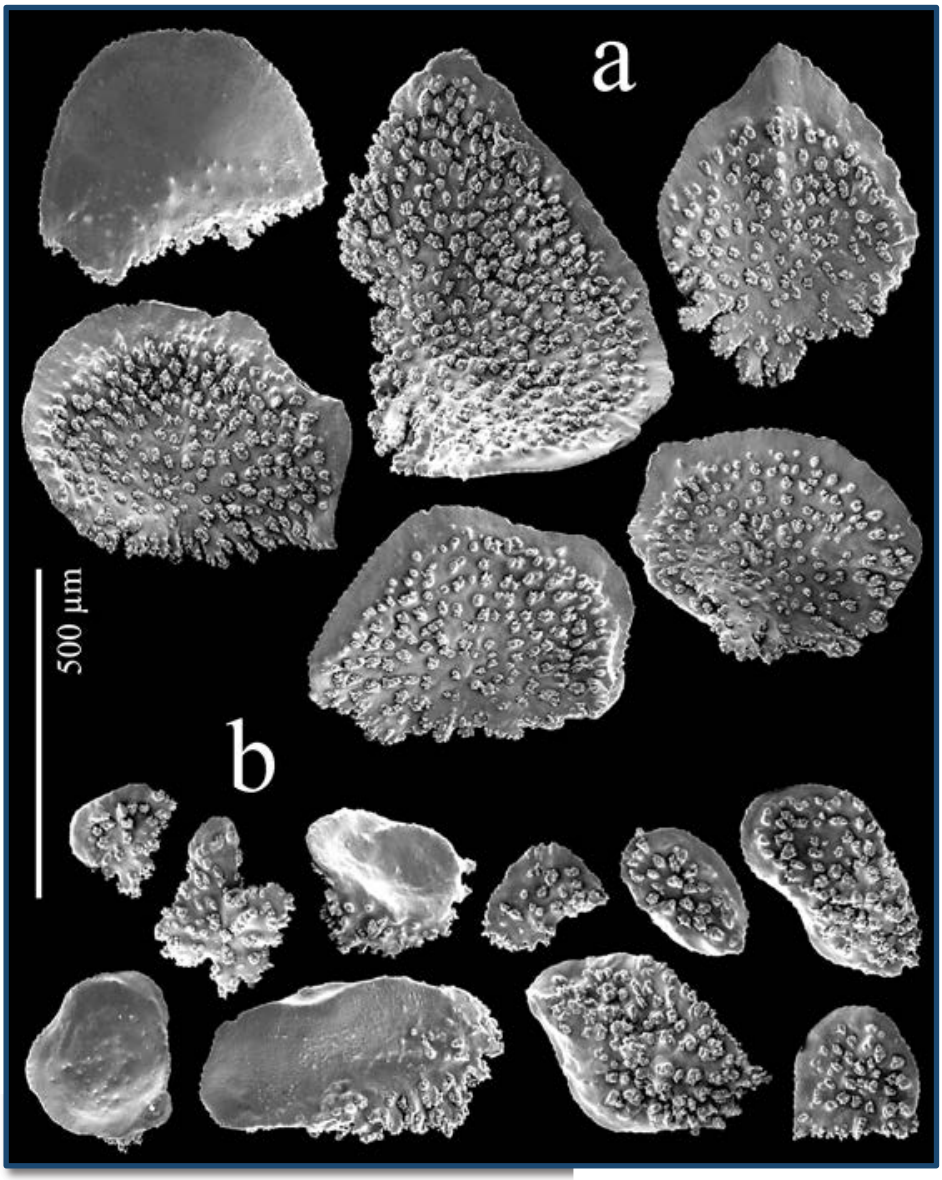
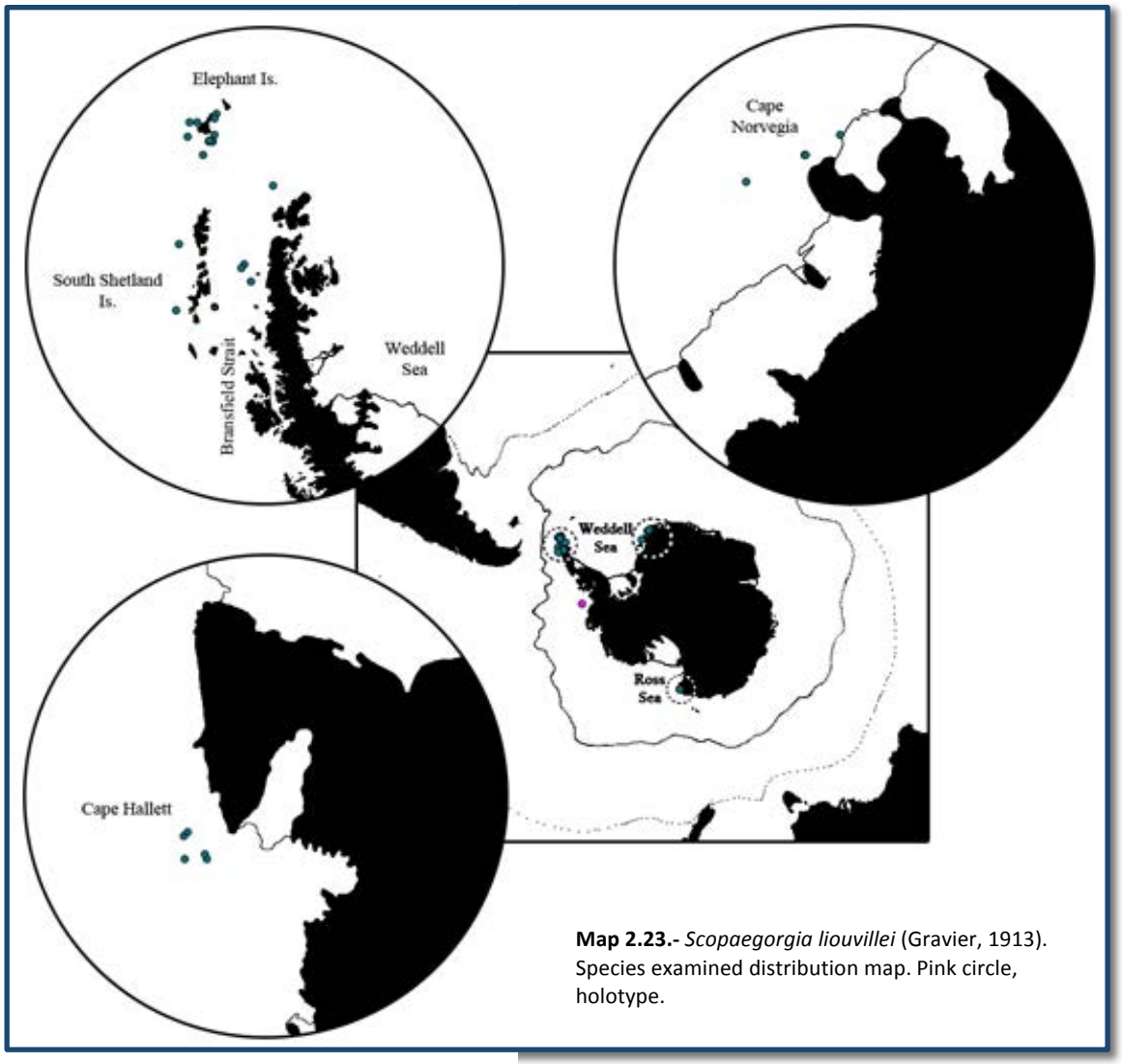


Figure 2.54.- *Scopaegorgia liouvillei*, holotype (MNHN Oct.0000–0233): **a**, body scales; **b**, coenenchymal scales.

Geographical and bathymetrical distribution

At present, *Scopaegorgia liouvillei* (Gravier, 1913), is known from Marguerite Bay, Bellingshausen Sea; Bransfield Strait, around Elephant and South Shetland Islands, eastern Weddell Sea (West Cape and Cape Norvegia) and Moubray Bay from the eastern Ross Sea, Antarctica (Map 2.23), between 151.3 and 597.6 m in depth.



Map 2.23.- *Scopaegorgia liouvillei* (Gravier, 1913). Species examined distribution map. Pink circle, holotype.

Etymology

This species is presumably dedicated to Joseph Liouville, a French mathematician.

Genus *Tauroprimnoa* Zapata-Guardiola and López-González, 2010b

Tauroprimnoa Zapata-Guardiola and López-González, 2010b:314

Diagnosis

Primnoidae with bottlebrush colonies and simple branchlets. Polyps cylindrical, more-or-less straight, angled slightly upwards and arranged in whorls. Opercular scales eight in number: four small alternating with four large; Marginal scales four in number not folding over the operculars, two large abaxials with strong, apical spine, and two smaller and squarish to round adaxials without a thorn. Polyps completely covered by five longitudinal rows of body scales, and only one abaxial row of large fan-shaped body scales. Remaining body scales small and rounded, reduced adaxially but always present. Coenenchyme with a single outer layer of oval-shaped scales.

Geographical and bathymetrical distribution

At present, *Tauroprimnoa* has only been reported from the type species locality in the eastern Weddell Sea, Antarctica, around 600 m in depth.

Etymology

The generic name combines the prefix *tauro*- with reference to the distinct two abaxial marginal scales—in the abaxial view, they resemble the horns of a bull, and *-primnoa*, a common suffix in reference to the gorgonian family, whose gender is feminine.

Type species

Tauroprimnoa austasensis Zapata-Guardiola and López-González, 2010.

Tauroprimnoa austasensis Zapata-Guardiola and López-González, 2010b

(Figures 2.55-2.59)

Tauroprimnoa austasensis Zapata-Guardiola and López-González, 2010b:314.

Examined material

Holotype: ZIZMH (C11738), ANT XXI/2, stn PS65/292-01, 72°51.43'S, 9°38.62'W, Austasen, eastern Weddell Sea, Antarctica, 596.4–597.6 m depth, 31 December 2003, one colony.

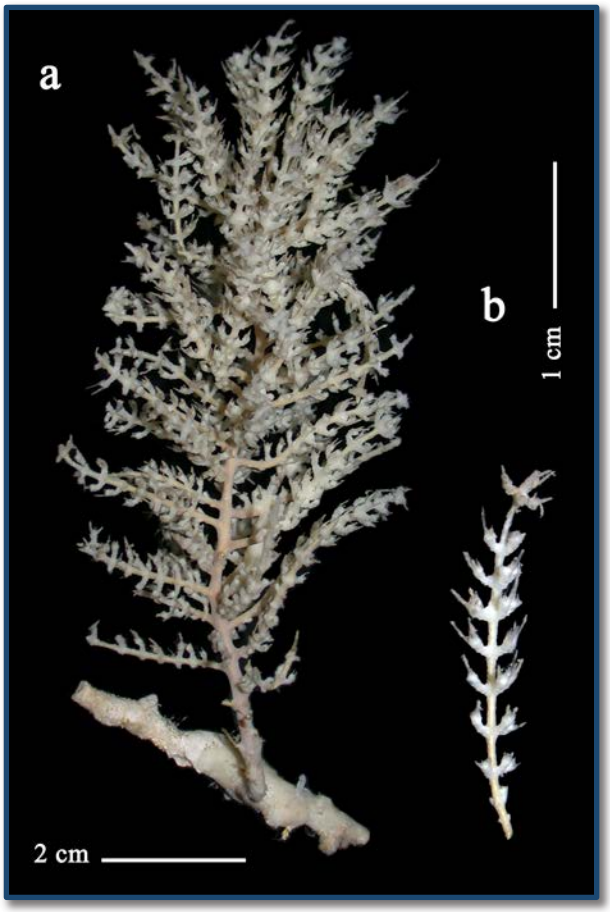


Figure 2.55.- *Tauroprimnoa austasensis*, holotype (ZIZMH-C11738): **a**, whole colony; **b**, detail of branchlet.

Paratypes: ZIZMH (C11739), USNM (1128573) and BEIM (CRO-0029) with the same sampling data as the holotype: four fragments, two broken colonies and one complete colony; four fragments, two colonies and one fragment of colony; and one complete colony, respectively.

Additional material: US 184, ANTXV-3, stn PS48/123, 73°35.8'S, 22°14.6'W, south VestKapp, Antarctica, 638 m depth, 07 February 1998, one colony; US 6413, ANTXV-3, stn PS48/167, 75°3.7'S, 27°20.7'W, Halley Bay, east Weddell Sea, Antarctica, 406 m depth, 12 February 1998.

Description of the holotype

Colony bottlebrush (Fig. 2.55a) of 10.2 cm in height and 4.5 cm in width, with simple, stiff branchlets up to 3 cm in length arising from the main stem. Axis ochre in colour, stiff and firmly attached to a hard substrate by a white, calcareous holdfast: basal axis diameter of 1.4 and 19 mm height until the first division. Polyps (Fig. 2.56) on branchlets, placed in whorls (Fig. 2.57), 3–5 polyps per whorl and 3–4 whorls per cm, straight and directed upwards. Polyps not present on main stem. Polyps cylindrical, about 1.2–2.4 mm in height and 0.49–0.64 mm in diameter, without including marginal thorns, and with a conical operculum. Opercular scales (Fig. 2.58a) eight in number, three types of sclerites are differentiated: (1) two adaxial

operculars (Fig. 2.58a—ad) more or less triangular and pointed, 0.59-0.71 x 0.15-0.27 mm; (2) two outer lateral operculars (Fig. 2.58a—ol) smaller with distal part more acuminate, 0.41-0.53 x 0.13-0.2 mm; (3) two abaxial and two inner lateral operculars (Fig. 2.58a—ab and il, respectively) larger, triangular, less slender than previous scales and with a wider proximal part, 0.65-0.94 x 0.29-0.49 mm.

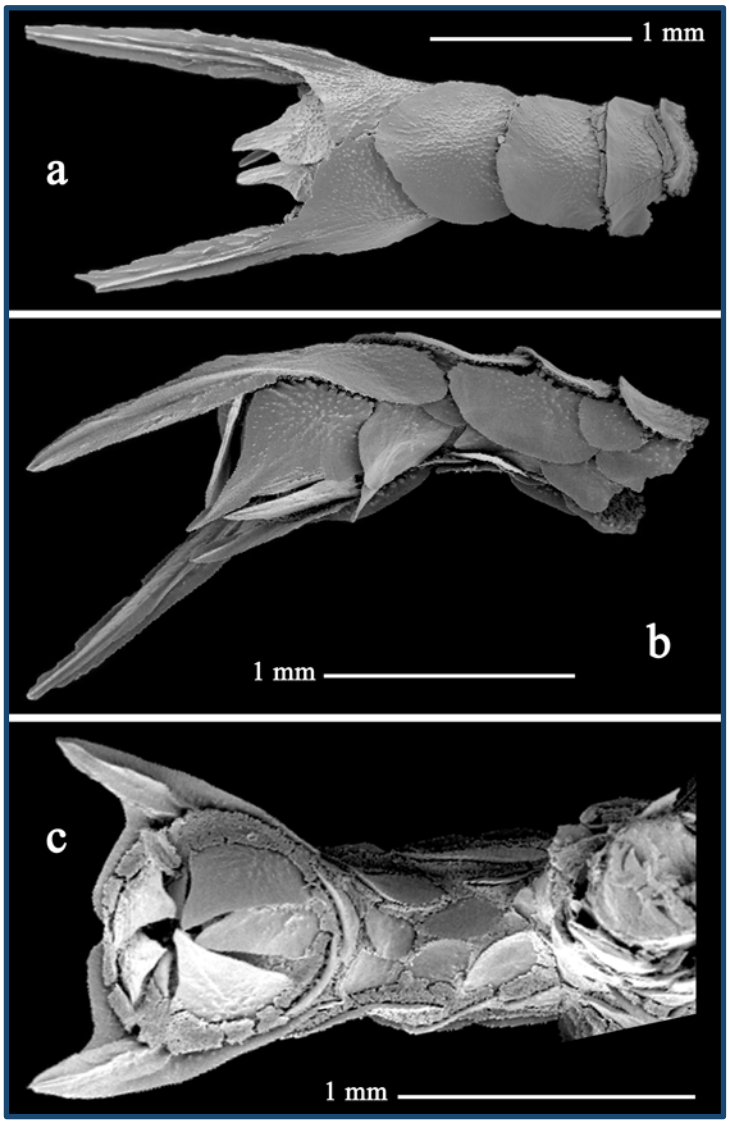


Figure 2.56.- *Tauroprimnoa austasensis*, holotype (ZIZMH-C11738): **a**, polyp on abaxial view; **b**, polyp on inner-lateral view; **c**, polyp on adaxial view.

Proximal inner surface with tubercles not densely represented, covering around a half of their length, medial distal keel poorly developed, mainly present in larger scales. Outer surface granulate. Basal margin with digitate processes, free margin finely serrated. Marginal scales (Figs. 2.58b,c) four in number. Adaxials and abaxials clearly differentiated. Two outer lateral (Fig. 2.58b) large, triangle-shaped projecting a strong thorn 1.80-2.34 x 0.70-0.76 mm (including thorn); thorn up to three-quarters of total sclerite length, with numerous longitudinal ridges on inner and lateral sides, outer thorn side flattened basally, inner surface of sclerites almost completely tuberculate; outer surface granular. Two adaxial (Fig. 2.58c)

smaller, squarish to more or less rounded, 0.37-0.49 x 0.27-0.47 mm in maximum length, inner surface almost completely tuberculate, sparsely distributed, outer surface quite smooth with some granules. Marginals with basal margin bearing digitate processes, and free margin finely serrated. Body scales in 5 longitudinal rows, 3 scales on the single longitudinal abaxial row of which the most distal is not a marginal (Fig. 2.56a), 2–3 scales on the inner lateral and adaxial rows (Figs. 2.56b,c). Body scales oval to round in shape, without thorns (Fig. 2.59a,b). Abaxial scales (Fig. 2.59a) wide strongly convex and large, with tendency to fan shape, 0.44-0.69 x 0.67-0.76 mm. Inner lateral and adaxial scales smaller, 0.25-0.4 x 0.25-0.73 mm (Fig. 2.59b). Body scales with inner surface almost completely tuberculate, outer surface from quite smooth to granular, being more or less spinose on abaxials. Free margin finely serrated, basal margin with tuberculate processes. Coenenchymal scales (Fig. 2.59c) round, oval shaped, 0.20–0.46 mm in maximum length. Surface and margin with similar characteristics to body scales.

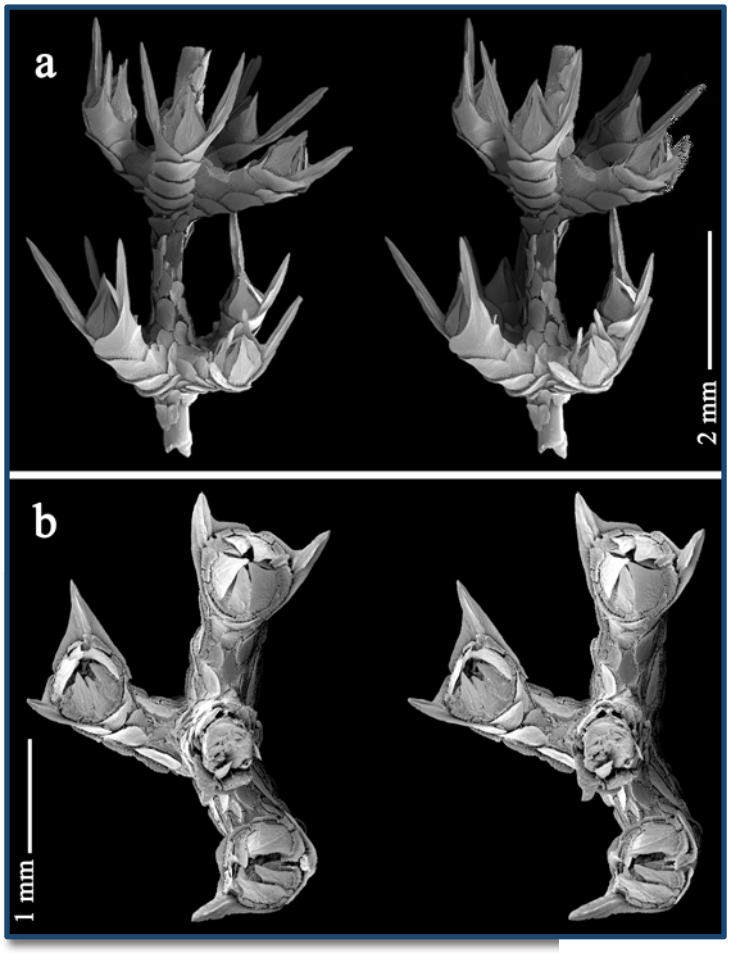


Figure 2.57.- *Tauroprimnoa austasensis*, holotype (ZIZMH-C11738): **a**, detail of branchlet, stereo pair; **b**, whorl on oral view, stereo pair.

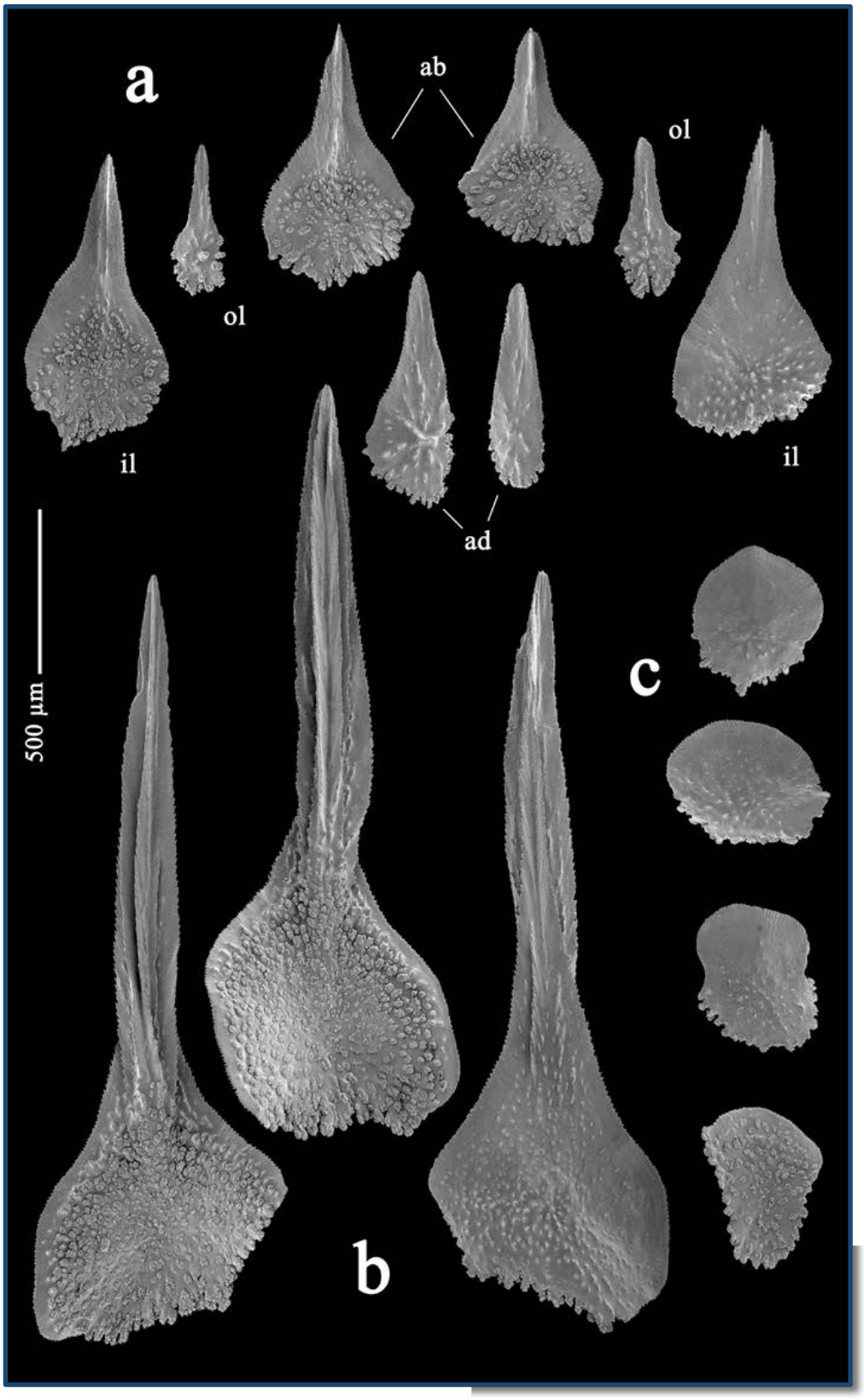


Figure 2.58.- *Tauroprimnoa austasensis*, holotype (ZIZMH-C11738): **a**, opercular scales; **b**, abaxial marginal scales; **c**, adaxial marginal scales. (**ab**) abaxial scales, (**ad**) adaxial scales, (**ol**) outer lateral scales, (**il**) inner lateral scales.

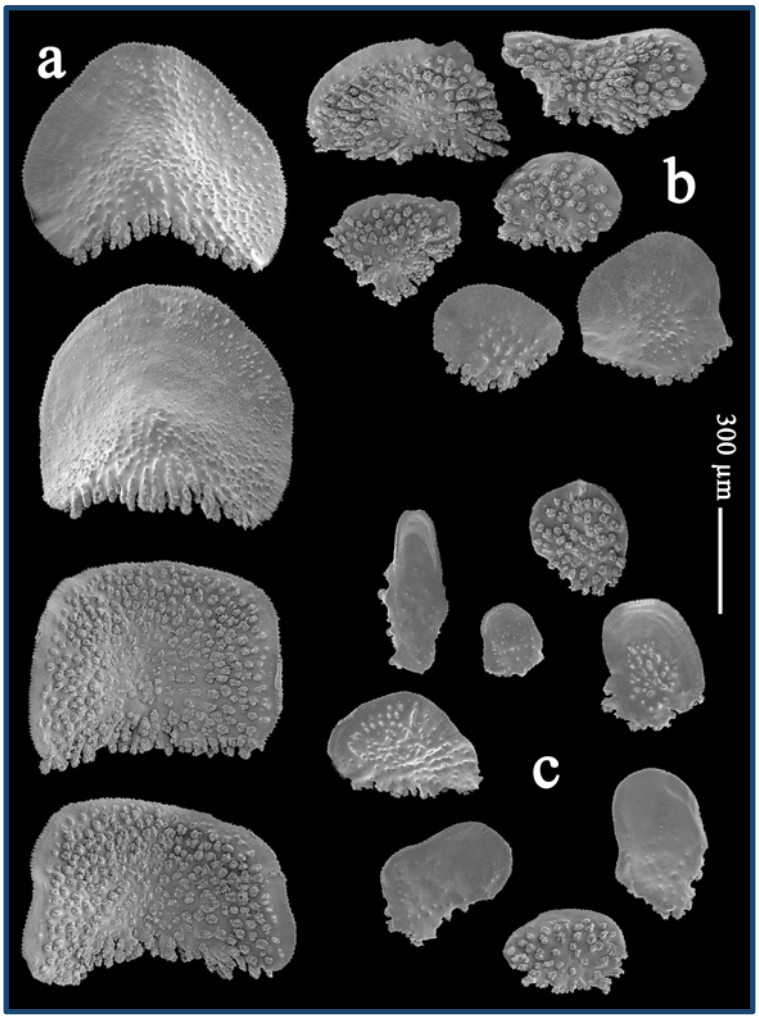


Figure 2.59.- *Tauroprimnoa austasensis*, holotype (ZIZMH-C11738): **a**, abaxial body scales; **b**, lateral and adaxial body scales; **c**, coenenchymal scales.

Variations from holotype

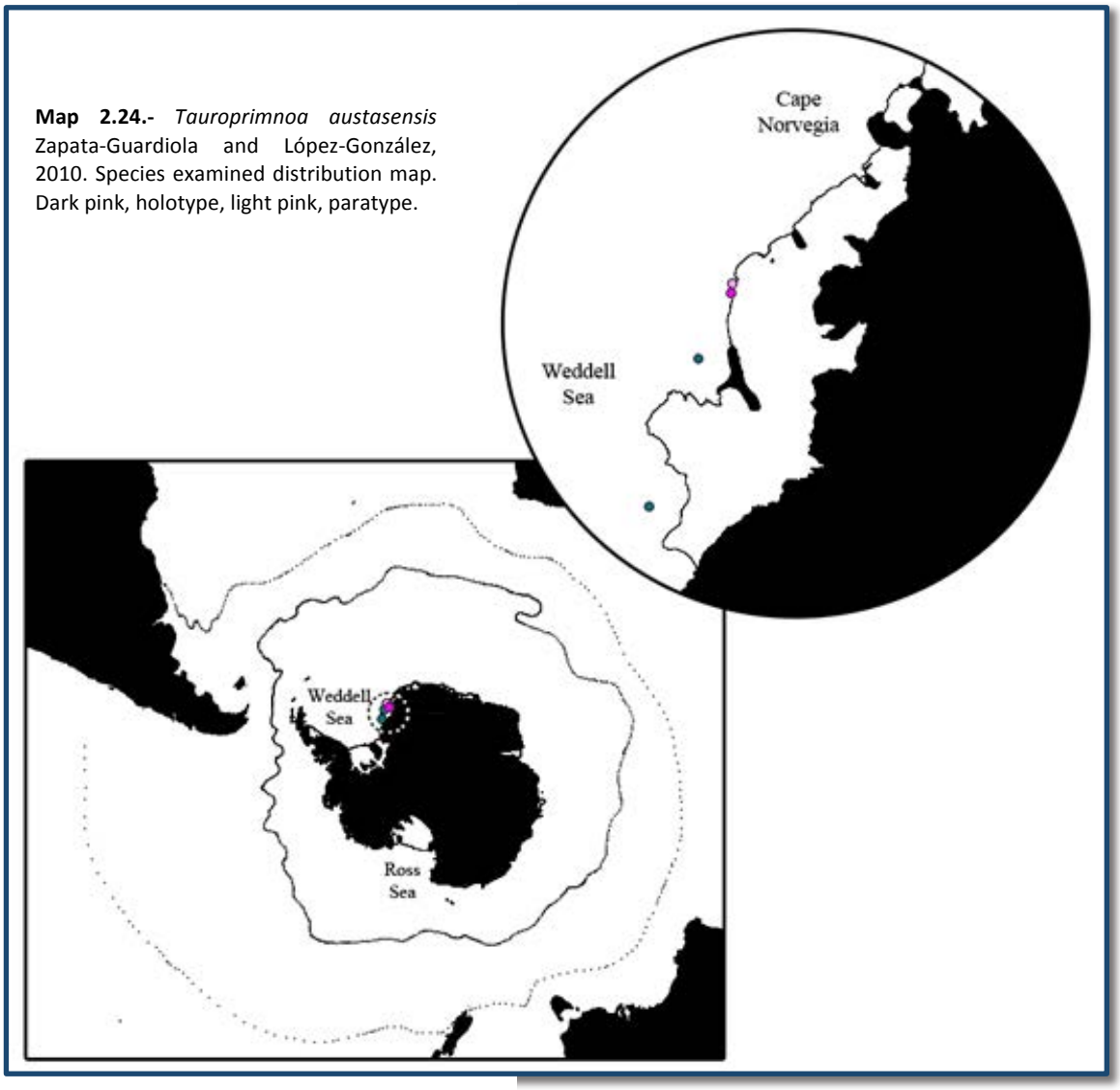
The paratypes and additional material are bottlebrush colonies and have a general structure similar to that of the holotype. The branchlets are simple, which arise from all around the main stem, up to 4 cm long, some of them bent to one side giving a false impression that their arrangement is in one plane. Furthermore, branchlets can be perpendicular or directed upwards, causing the colonies to show a range of width between 4.4 and 6.2 cm. The maximum height of the colonies examined is 11.5 cm. The polyps are present only on the branchlets and are arranged in whorls of three to seven. The number of whorls per centimetre can vary from 3 to 6. The shape of the polyps, their distribution and the shape of the sclerites are similar to those in the holotype.

Geographical and bathymetrical distribution

At present, *Tauroprimnoa austasensis* is only known from Austasen, eastern Weddell Sea, Antarctica (Map 2.24), around 597 m in depth.

Etymology

The specific name *austasensis* refers to the geographic area where the new species was found.



Map 2.24.- *Tauroprimnoa austasensis* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Genus *Thouarella* Gray, 1870

Primnoa Valenciennes, 1846:pl.12, figs. 2, 2a (no text, only images).—Milne-Edwards, 1857:140.—Gray, 1857:286; 1859:483.—Kölliker, 1865:135
Thouarella Gray, 1870:45; 1972:746.—Studer, 1887:50; 1888:649.—Wright and Studer, 1889:59–61 (in part), pl.11, 14.—Versluys, 1906:22–24.—Thomson and Henderson, 1906:38–41 (comparison table).—Kükenthal, 1907:202–208; 1908:10–11; 1912:292; 1919:405.—Bayer, 1956:F220; 1961:294 (key to genus); 1981:936 (key to genus).—Broch, 1965:24.—Stibane, 1987: 17–26, pl.1(4), 2(4).—Williams, 1992:277.—Cairns, 2006:175.—Zapata-Guardiola and López-González, 2010a:171.—Zapata-Guardiola and López-González, 2012:358.
Rhopalonella Roule, 1908:2-3, pl.1, figs. 5–8.
Thouarella (*Diplocalyptra*) Kinoshita, 1908:454. 457 (key to subgenus), pl.17, fig. 2; 1908:517–519; 1908d:52 (key to subgenus).—Cairns and Bayer, 2009:34-35.
Thouarella (*Euthouarella*) Kükenthal, 1915:149–150 (key to subgenus and species); 1919:414–415 (key to subgenus and species); 1924:292 (key to species).—Bayer, 1956:F220.—Bayer and Stefani, 1989:455 (key to subgenus).—Cairns, 2006:176, 187–188.—Cairns and Bayer, 2009:34.
Thouarella (*Parathouarella*) Kükenthal, 1915:150 (key to species); 1919:425–426 (key to species); 1924:296–297 (key to subgenus and species).
Thouarella (*Epithouarella*) Kükenthal, 1915:150-151 (key to subgenus and species); 1919:435 (key to subgenus and species); 1924:299 (key to subgenus and species).—Bayer, 1956:F220.—Cairns and Bayer, 2009:35.
Thouarella (*Thouarella*).—Bayer, 1956:F220.—Bayer and Stefani, 1989:455 (key to subgenus).—Cairns, 2006:176.—Cairns and Bayer, 2009:33-34.

Diagnosis

Primnoidae commonly with a bottlebrush colony shape and simple or ramified branchlets with also fan-shaped colonies. Polyps cylindrical to club shaped, upward or almost perpendicular to stem, straight or adaxially incurved and arranged single or in whorls. Opercular scales usually keeled, eight in number and arranged in two alternate cycles. Marginal scales also eight in number, alternating in two cycles, with a keel or multi keel folding over operculars in an indented way. Adaxial body scales often reduced.

Geographical and bathymetrical distribution

From the Atlantic and Pacific oceans to the Southern ocean, at depths ranging from 60 to 6400 m.

Etymology

The generic name is presumably dedicated to the Admiral Abel Aubert Du Petit-Thouars who found the specimen in Falkland Islands during the Voyage of the Vénus.

Type species

Primnoa antarctica Valenciennes, 1846.

Key to the species of the genus **Thouarella**

1. Polyps arranged in pairs or whorls.
   a. Colonies pinnate or bottlebrush……**Subgenus *Euthouarella***
      i. Colonies in one plane.
         1) Up to 6 pairs of polyps per centimetre.
            a) Polyps in pairs.
               i) 4 pairs of polyps per centimetre, 6-7 abaxial scales……***T. laxa***
               ii) 5 pairs of polyps per centimetre, 4-5 abaxial scales……***T. moseleyi***
            b) Polyps in pairs or whorls of 3……***T. flabellata***
         2) 6 pairs of polyps or more per centimetre.
            a) 6 pairs of polyps per centimetre ……..***T. tydemani***
            b) More than 6 pairs of polyps per centimetre.
               i) Polyps placed in pairs.
                  (1) Polyps less than 1 mm in height……***T. vitjaz***
                  (2) Polyps over 1 mm in height……..***T. tenuisquamis***
               ii) Polyps placed in whorls of three……..***T. carinata***
      ii. Colonies bottlebrush.
         1) Ridges on the inner surface of opercular and body wall scales……..***T. grashoffi***
         2) Absence of ridges on the inner surface of opercular and body wall scales ……..***T. hilgendorfi***
   b. Colonies dichotomously branched……..**Subgenus *Diplocalyptra***
      i. Polyps stand perpendicular to stem and branchlets……..***T. coronata***
      ii. Polyps stand inclined upward to stem and branchlets.
         1) 5 scales in the longitudinal abaxial row……...***T. parva***
         2) 6 scales or more in the longitudinal abaxial row……..***T. biserialis***
2. Polyps isolated arranged.
   a. Marginal scales lacking or with a short distal spine……**Subgenus *Epithouarella***
      i. Up to 8 scales in the longitudinal abaxial row.
         1) Funnel-shaped polyps.
            a) 5 scales in the longitudinal abaxial row……***T. dispersa***
            b) 6 scales or more in the longitudinal abaxial row……..***T. affinis***
         2) Club-shaped polyps.
            a) 8 polyps per centimetre……..***T. grandiflora***
            b) 14 polyps or more per centimetre……..***T. viridis***
      ii. 8 scales or more in the longitudinal abaxial row.

1) Up to 20 polyps per centimetre.

a) Polyps about 0.9-1.8 mm in height.....***T. regularis***

b) Polyps more than 2 mm in height.....***T. crenelata***

2) 21 polyps or more per centimetre.....***T. chilensis***

b. Marginal scales with a well-developed distal spine.....**Subgenus *Thouarella***

i. Up to 5 scales in the longitudinal abaxial row.

1) Polyps less than 2 mm in height.

a) Polyps less than 1 mm.....***T. minuta***

b) Polyps larger than 1 mm.

i) Polyps densely distributed, more than 25 polyps per centimetre.....***T. pendulina***

ii) Polyps sparsely distributed, less than 25 polyps per centimetre.

(1) Marginal scales with a long spine.....***T. variabilis***

(2) Marginal scales with a discreet spine.....***T. hicksoni***

2) Polyps larger than 2 mm in height.

a) Colony pinnate, uniplanar.....***T. bipinnata***

b) Colony bottlebrush.

i) Marginal scales with a distal thorn.....***T. andeep***

ii) Marginal scales without a distal thorn.

(1) Marginal scales with heavily ridged inner edge.....***T. striata***

(2) Inner edge of marginal scales relatively smooth.....***T. brucei***

ii. 6 scales or more in the longitudinal abaxial row.

1) Polyps less than 2 mm in height.

a) Polyps densely distributed, more than 25 polyps per centimetre.....***T. trilineata***

b) Polyps sparsely distributed, less than 25 polyps per centimetre.....***T. clavata***

2) Polyps larger than 2 mm in height.

a) Polyps densely distributed, more than 25 polyps per centimetre.

i) Marginal scales with heavily ridged inner edge.....***T. cristata***

ii) Inner edge of marginal scales relatively smooth.....***T. antarctica***

b) Polyps sparsely distributed, less than 25 polyps per centimetre..... ***T. koellikeri***

Subgenus *Euthouarella* Kükenthal, 1915

Diagnosis

Thouarella with polyps placed in pairs or whorls.

Geographical and bathymetrical distribution

Pacific Ocean, western from Indonesia to Japan and mid-northern between Marshall Islands and Johnston Atoll, Hawaiian Islands and North Atlantic Ocean between 250 and 6400 m.

Etymology

The subgeneric name combines the name of the genus *Thouarella* with the Greek prefix *eu-* which means normal, good.

Type species

Thouarella hilgendorfi (Studer, 1878).

Thouarella (*Euthouarella*) *laxa* Versluys, 1906

(Figures 2.60-2.63)

? *Hookerella pulchella* Gray, 1870:45.
Thouarella laxa Versluys, 1906:30–32, pl. 1, fig. 5; pl. 3, fig. 8; text figs. 28–33.—Kükenthal and Grozawsky, 1908:36–37, pl. 2, fig. 13.
Thouarella (*Euthouarella*) *laxa*, Kükenthal, 1915:150.—Kükenthal, 1919:417.—Kükenthal, 1924:293–294, text fig. 164.—Cairns and Bayer, 2009:28 (listed).—Zapata-Guardiola and López-González, 2010c:171.
Thouarella regularis Kükenthal, 1907:206-207.

Examined material

Holotype: ZMA (COEL 03576), Siboga Expedition, stn 88, 0°34.60'N, 119°08.50'E, Strait of Makassar, Sulawesi, Indonesia, 1301 m depth, 20 June 1899.

Additional material: ZSM (20080407), ZSM (20080408) and ZSM (20080409), Steam Vessel ''Zuso Maru'', Sagami Bay, Okinose, 300–700 m depth, 8–15 November 1904, Doflein Collection 1904–1905.

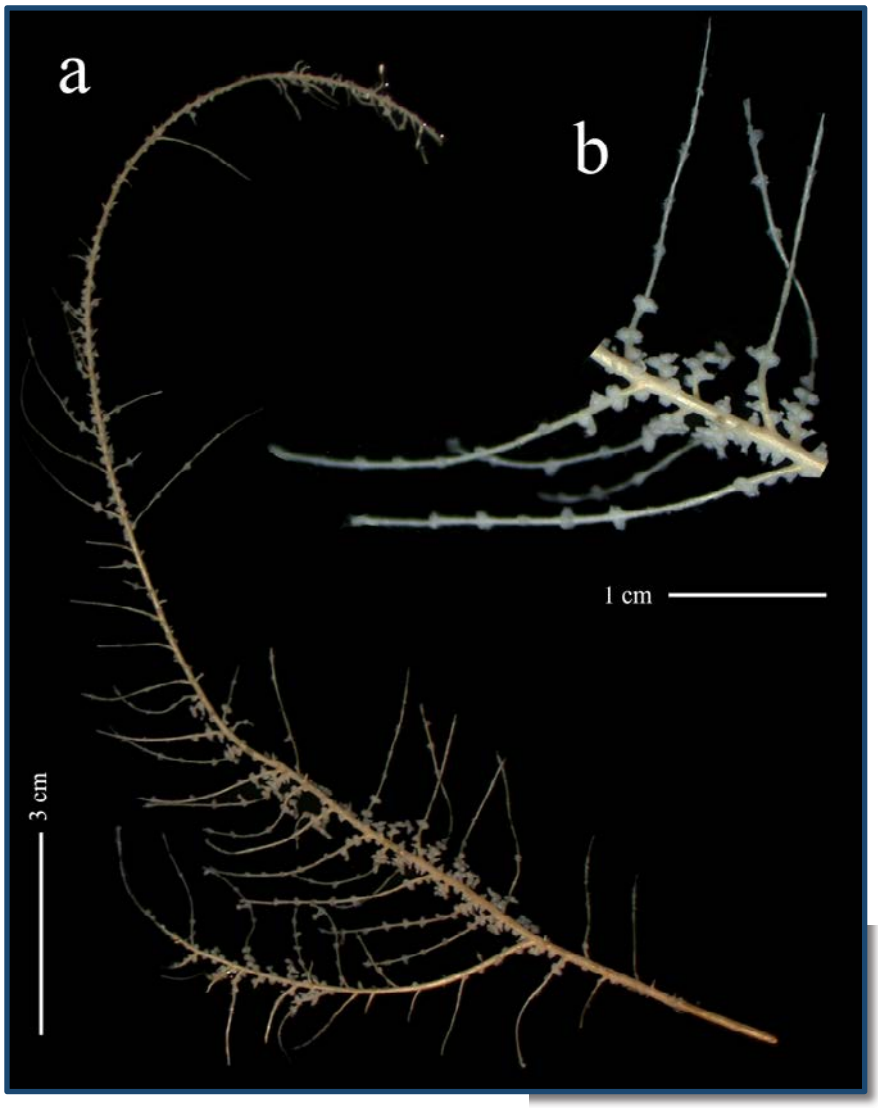


Figure 2.60.- *Thouarella laxa* Versluys, 1906, holotype (COEL03576): **a**, whole colony; **b**, detail of branchlets.

Description of the holotype

Fragment examined (Fig. 2.60a) 22 cm in length, in one plane, feather-like, with simple branchlets up to 22 mm in length (Fig. 2.60b). One main lateral branch of 70 mm in length also bearing simple branchlets up to 28 mm in length. Axis ochre coloured, slender and graceful.

Polyps perpendicular to branchlets (Fig. 2.61b), in pairs, 4 pairs per cm. Polyps singly placed on stem. Polyps (Fig. 2.61) cylindrical, funnel shaped distally, about 1.2–1.4 mm in height and about 0.8 mm in diameter in their basal portion; polyp diameter reduced at its mid length, wider distally. Operculum small (Fig. 2.61c), delicate and easily breakable. Distal part of the polyp directed inwards towards the branchlet (Figs. 2.61b,d). Polyps with 7 longitudinal rows of scales, 6–7 transverse rows of scales on each longitudinal abaxial row overlapping one

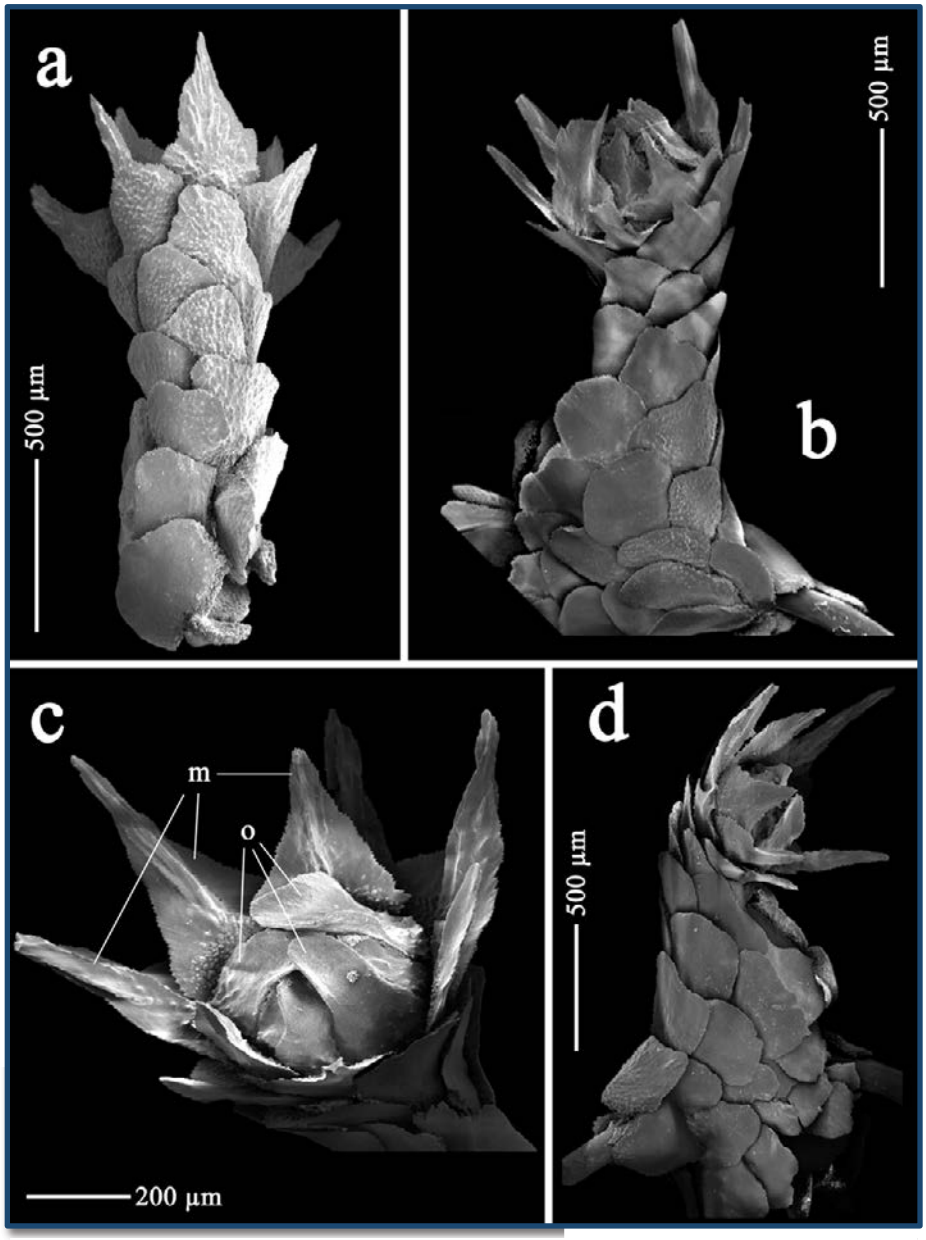


Figure 2.61.- *Thouarella laxa* Versluys, 1906, holotype (COEL03576): **a**, polyp on abaxial view; **b**, polyp on lateral adaxial view; **c**, polyp on oral view; **d**, polyp on lateral view. Abbreviations: **o,** opercular scales; **m**, marginal scales.

another (Fig. 2.61a). Opercular scales, arranged in 2 alternate cycles of 4 scales each, 0.17–0.49 mm 9 0.07–0.27 mm. Inner cycle (Fig. 2.62a) small, narrow and lancet shaped; outer cycle (Fig. 2.62b) large, concave with blunt and square tip. Inner proximal surface tuberculate, inner distal surface smooth and lacking a keel. Outer proximal surface with few granules and distal outer surface smooth. Basal margin with digitate processes, free margin smooth.

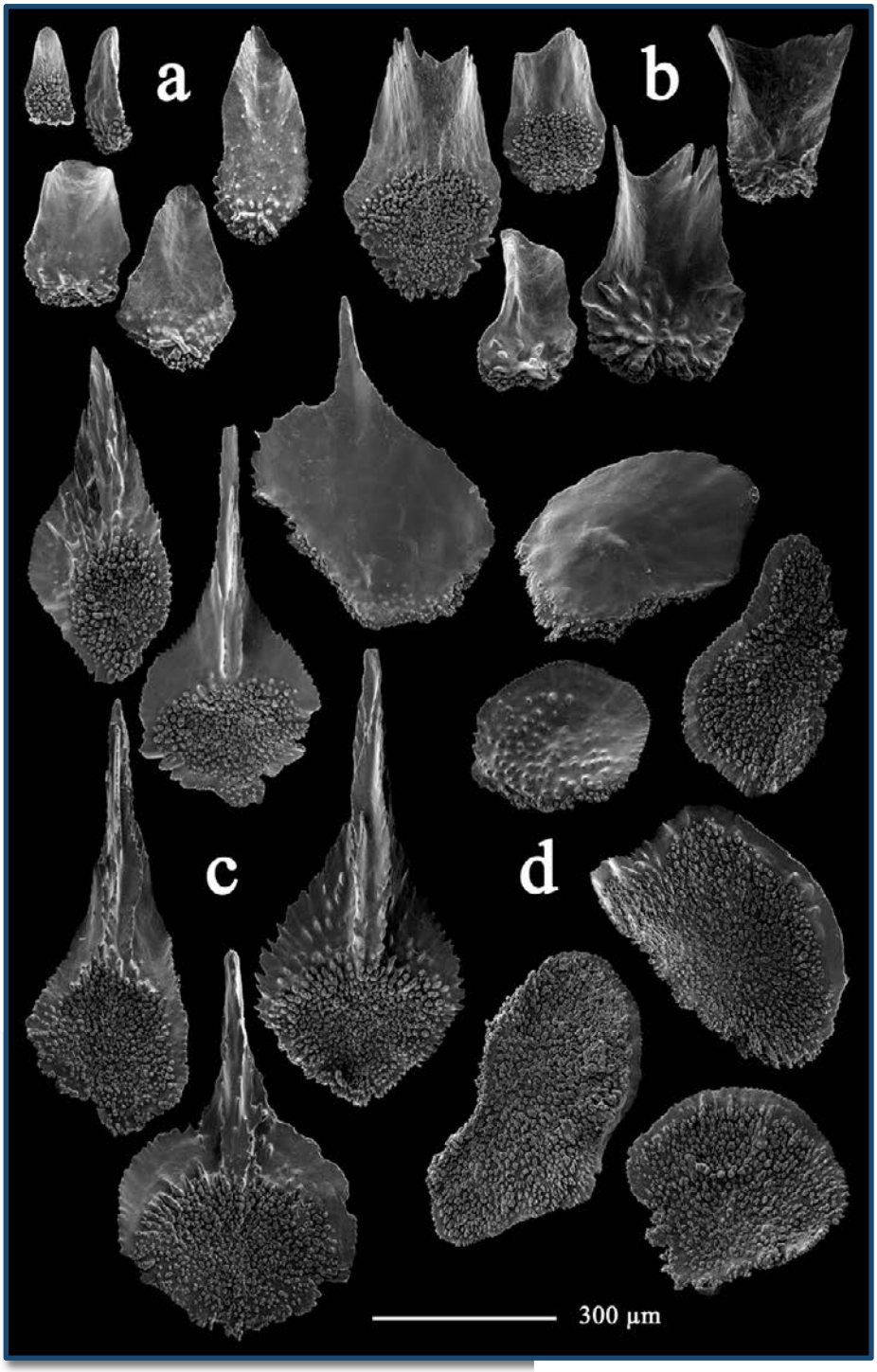


Figure 2.62.- *Thouarella laxa* Versluys, 1906, holotype (COEL03576): Opercular scales from inner (**a**) and outer (**b**) alternate cycles; **c**, marginal scales; **d**, body scales.

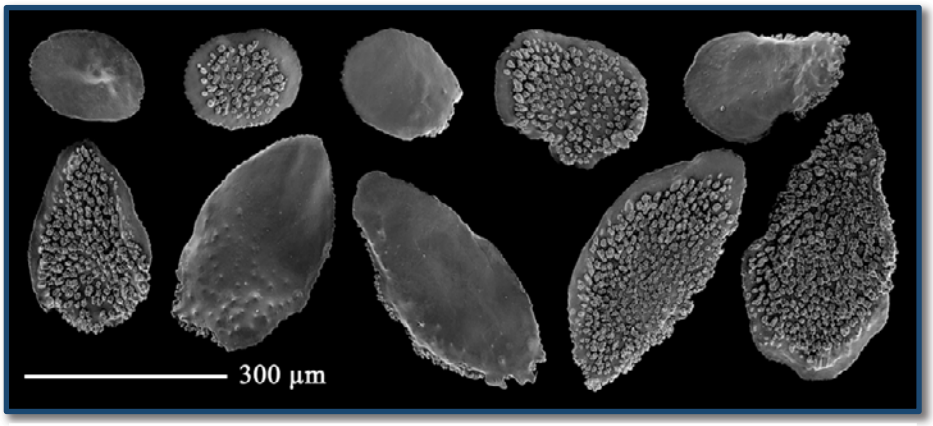


Figure 2.63.- *Thouarella laxa* Versluys, 1906, holotype (COEL03576): Coenenchymal scales.

Marginal scales (Fig. 2.62c), 0.53–0.81 x 0.24–0.40 mm, arranged in 2 alternate cycles of 4 scales each, more or less triangular with long ridged spine up to 2/3 of total scale length. Outer surface smooth, 1/3 of inner proximal surface densely tuberculate, remaining base-lateral surface of spine smooth, granulated or ridged. Basal margin with digitate processes, free margin serrated. Body scales (Fig. 2.62d) round and more or less oval, 0.26–0.64 mm in diameter. Outer surface smooth or granulated, inner surface tuberculate and with inner face of the distal margin smooth. Basal margin with digitate processes, free margin serrated. Coenenchymal scales (Fig. 2.63) similar in shape to body scales but smaller, 0.15–0.45 mm in maximum length.

Variations from holotype

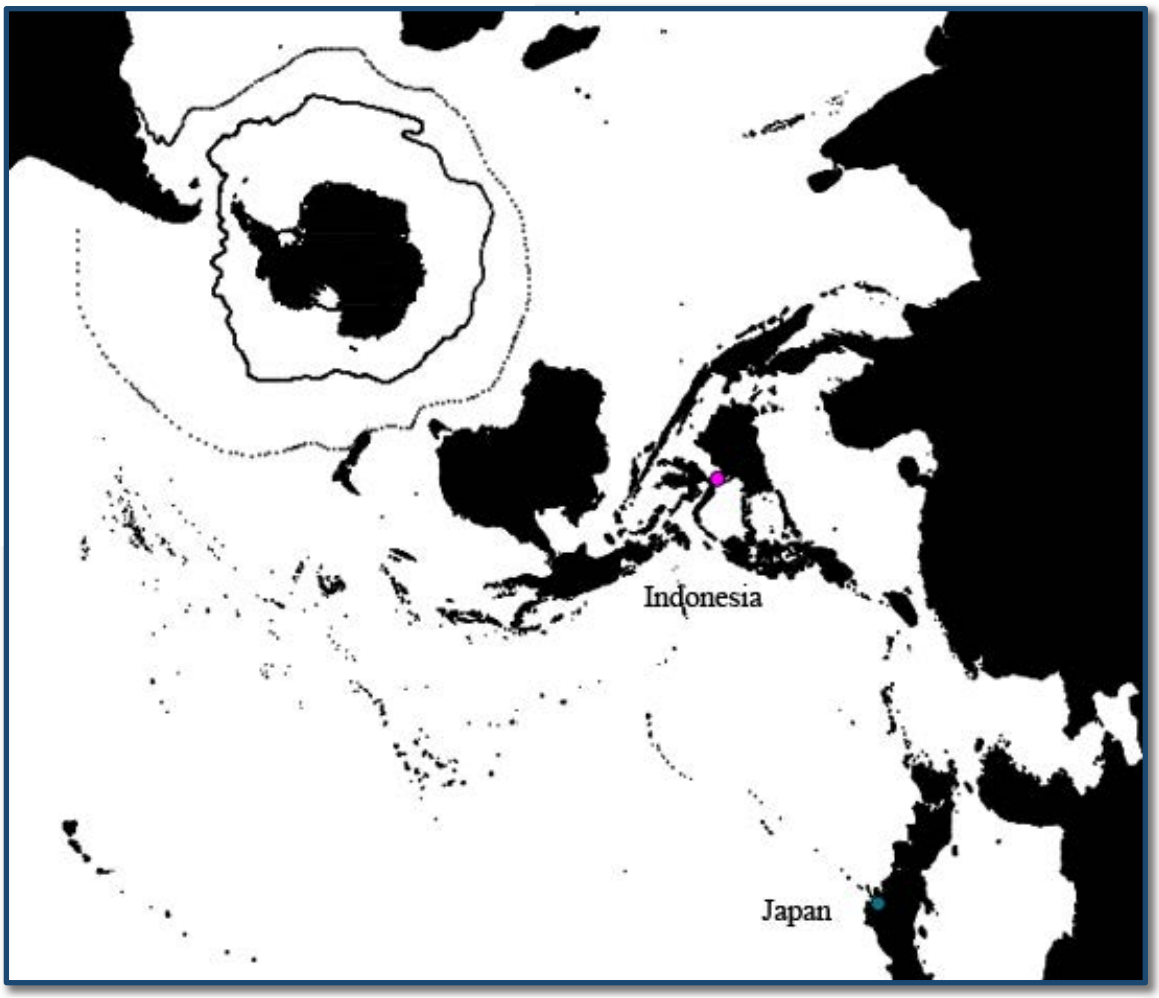
Material examined is comprised of small colony fragments of 2.6–4 cm in length; presumable side branches belong to larger colonies. These fragments show a similar branching to the holotype, having small branchlets from 0.75 to 2.8 cm in length. The polyps are 1.2 to 1.7 cm in height and are arranged in whorls of two or three on branchlets; being singly placed on stems. The number of whorls per centimetre varies from 4 to 7. The sclerites from the polyps are as in the holotype, varying slightly in sizes: opercular scales, 0.16–0.55 x 0.05–0.27 mm; marginal scales, 0.36–0.81 x 0.14–0.4 mm; and body scales, 0.24–0.64 mm in maximum length.

Geographical and bathymetrical distribution

At present, *Thouarella laxa* is known from the West Pacific Ocean, from Indonesia to Japan (Map 2.25). The bathymetric distribution of *T. laxa* varies between 400 and 1301 m.

Etymology

In Latin "*laxa"* means wide and loose and could refer to the long flexible branchlets of this species.



Map 2.25.- *Thouarella* (*Euthouarella*) *laxa* Versluys, 1906. Species examined distribution map. Pink circle, holotype.

Thouarella (*Euthouarella*) *vitjaz* Zapata-Guardiola and López-González, 2012

(Figures 2.65-2.67)

Thouarella abietina, Pasternak, 1981: 49-50.
Thouarella vitjaz Zapata-Guardiola and López-González, 2012:11.

Examined material

Holotype: IORAS IV-9-Alc-010-002, *RV Vitjaz* Cruise 48, stn 6275, 12°12'N, 179°49'E, 6400 m depth, two branchlets.

Paratype: IORAS IV-9-Alc-010-001, *RV Vitjaz* Cruise 48, stn 6265, 26°56.1'N, 178°38'E, 3200 m depth, 23 May 1970, two fragments.

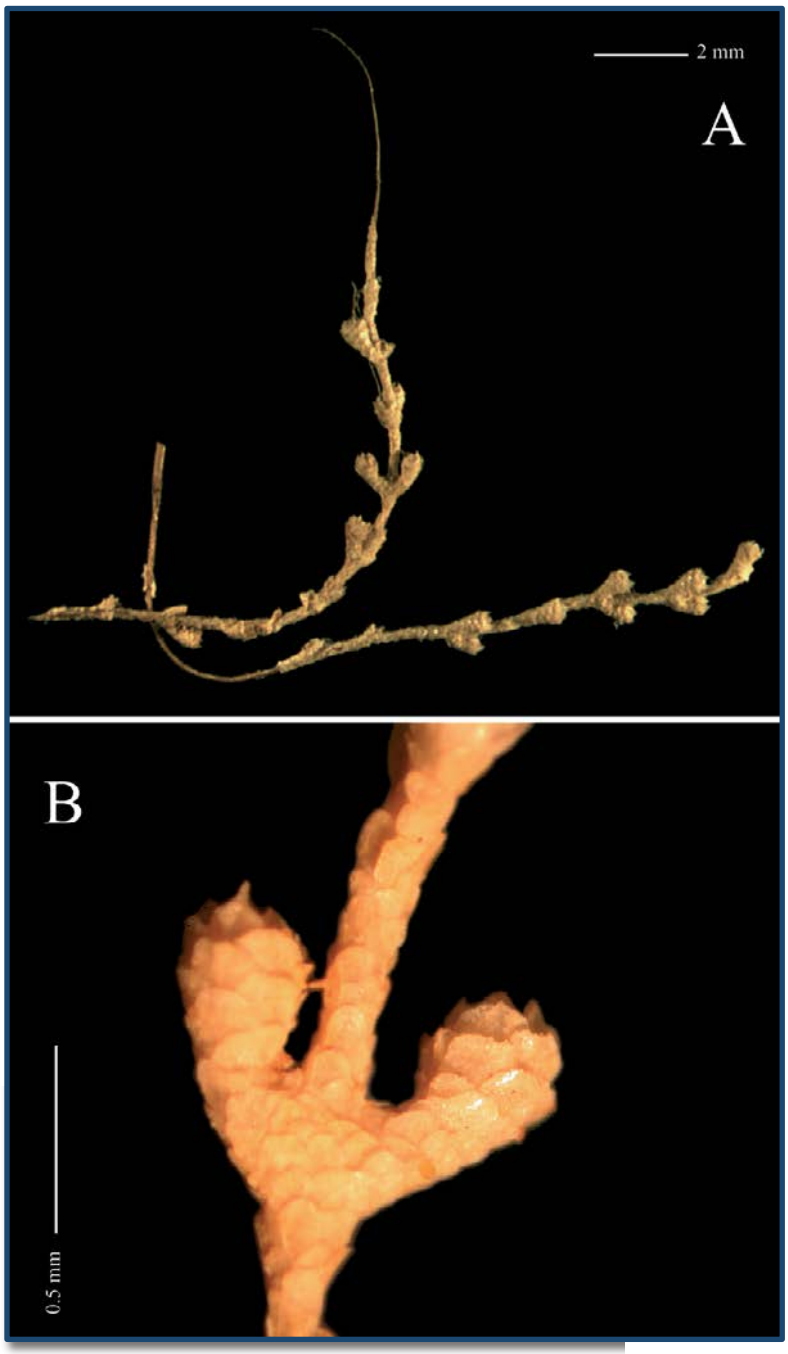


Figure 2.65.- *Thouarella vitjaz*, holotype (IORAS IV-9- Alc-010-002): **a**, detail of branchlet; **b**, detail of a whorl of two polyps.

Description of the holotype

Two small, delicate dry branchlets, 1.3 and 1.4 cm long (Fig. 2.65a). Polyps small, curved upward to branchlets at 45 (Fig. 2.65b), placed in pairs about 0.5 mm apart, 7 pairs per cm. They are trumpet-shaped (Fig. 2.66), about 0.74-1.1 mm tall and 0.39-0.57 mm greatest diameter with a low operculum and body scales in 8 longitudinal rows. Each abaxial row has 5-6 scales (Fig. 2.66a) and 3 in each adaxial row (Fig. 2.66b). Eight opercular scales (Fig. 2.67a), 0.14-0.21 x 0.06-0.11 mm, tongue-shaped. Proximal inner surface tuberculate covering up to a third of the length, distal surface with several longitudinal ridges. Outer surface granular, free margin finely serrated. Eight marginal scales (Fig. 2.67b), 0.23–0.27 mm tall and 0.12–0.16 mm wide, lancet-shaped with an acute apex; adaxials reduced. Inner proximal surface tuberculate, covering less than a half of their length, distal portion from smooth to ridged, with a modest medial keel. Outer surface granular. Basal margin with small granular processes, free margin finely serrated. Upper cycle of body scales, or submarginals, (Fig. 2.67c:sms), roughly triangular-shaped, 0.25-0.30 mm tall and 0.18–0.24 mm wide. Inner proximal surface tuberculate, covering up to half of the length, long ridges distally and a modest medial keel. Remaining body scales (Fig. 2.67c), round to fan-shaped, 0.11-0.24 mm tall and 0.16–0.28 mm wide. Inner surface tuberculate covering at least 75% of the scale, with long radial ridges distally decreasing in size in the more basal scales. Outer surface granular. Basal margin with small granular processes, free margin finely serrated. Coenenchymal scales (Fig. 2.67d) round to oval-shaped, 0.07–0.20 mm maximum length. Inner surface tuberculate, outer surface granular. Basal margin with small granular processes, free margin finely serrated.

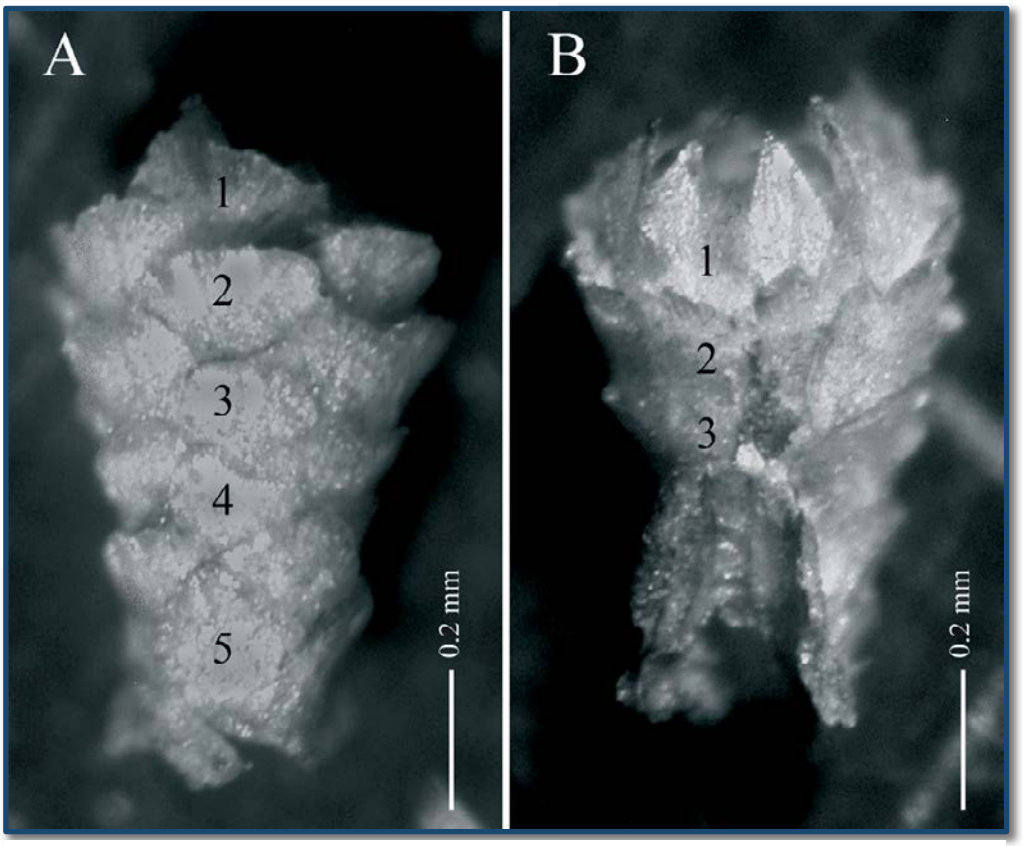


Figure 2.66.- *Thouarella vitjaz*, holotype (IORAS IV-9- Alc-010-002): **a**, polyp on abaxial view; **b**, polyp on adaxial view.

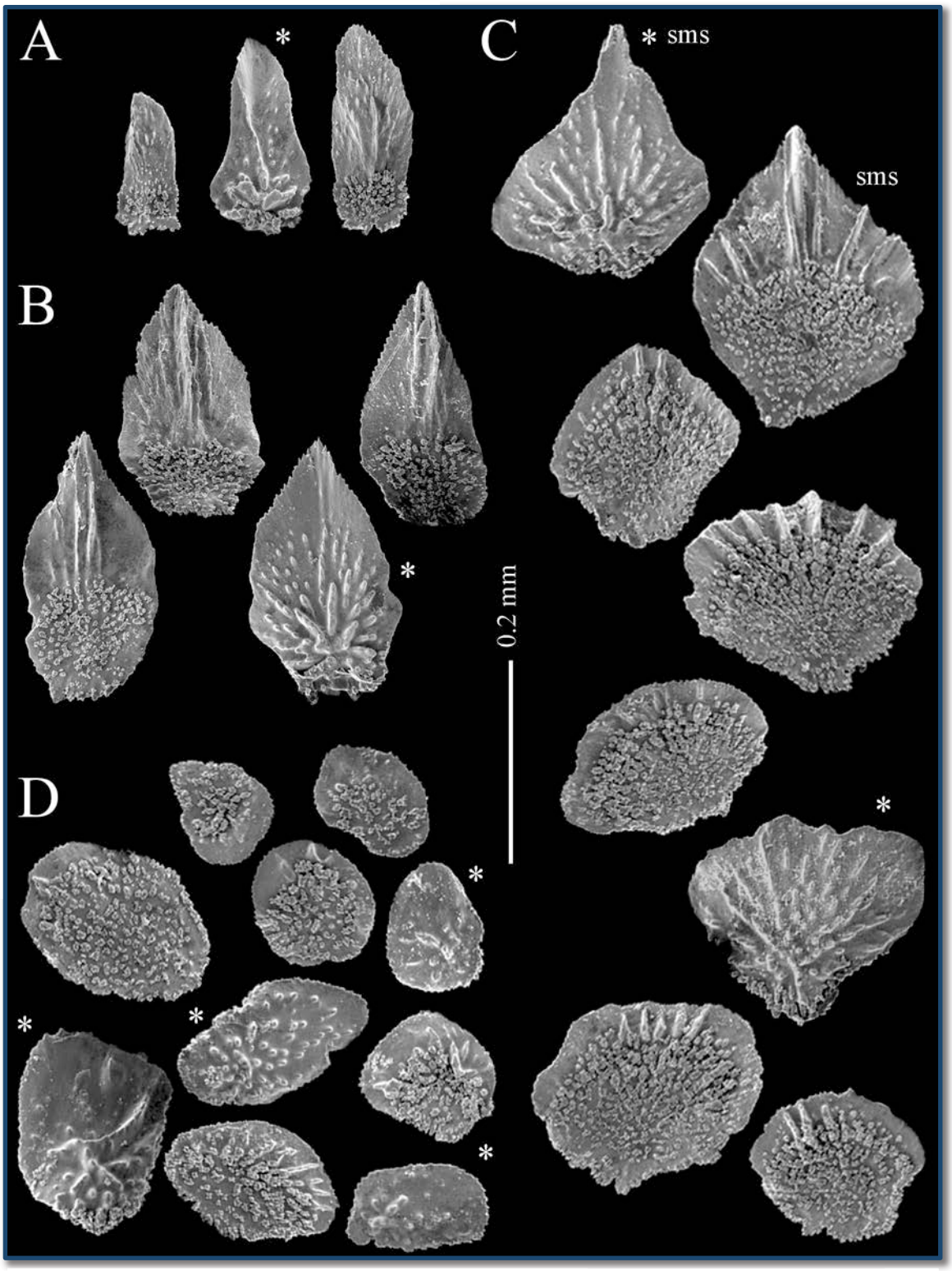


Figure 2.67.- *Thouarella vitjaz*, holotype (IORAS IV-9-Alc-010-002): **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal sclerites. [*outer surface view; **sms**=submarginal scales].

Variations from holotype

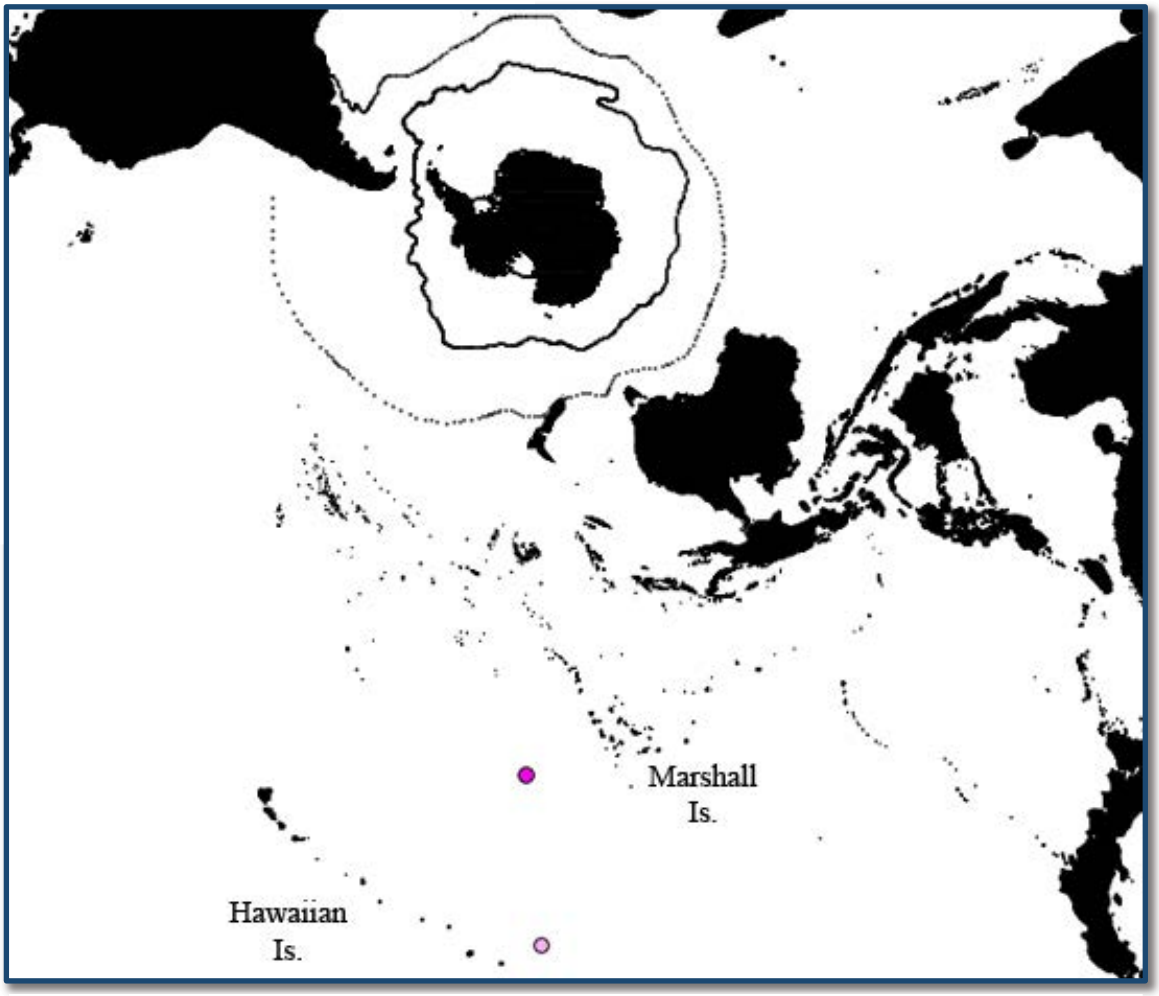
The paratype consists of 2 small dry fragments, of which the largest is 1.8 cm long. The shortest is a branchlet of 1.2 cm. The polyps' shape and arrangement and the shape of the sclerites are similar to those in the holotype.

Geographical and bathymetrical distribution.

Thouarella vitjaz is only known from the middle of the northern Pacific Ocean, between the Marshall Islands and Johnston Atoll and from the south west of Green Island, the Midway Islands, the Hawaiian archipelago, Pacific Ocean (Map 2.26), at 3200 and 6400 m depth.

Etymology

The species name, *vitjaz*, refers to the Soviet Union's research vessel "Витязь" (=knight), from which the new species was collected. The name is treated as a noun in apposition.



Map 2.26.- *Thouarella* (*Euthouarella*) *vitjaz* Zapata-Guardiola and López-González, 2012. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Subgenus *Epithouarella* Kükenthal, 1915

Diagnosis

Thouarella with single placed polyps. Marginal scales pointed, ridged or with spinose projections, without bearing real thorns.

Geographical and bathymetrical distribution

From the South Atlantic Ocean to Antarctica and Subantarctica between 100 and 2450 m in depth.

Etymology

The subgeneric name combines the name of the genus *Thouarella* with the Greek prefix *epi-* which means over, above, in addition to.

Type species

Thouarella crenelata Kükenthal, 1907.

Thouarella (*Epithouarella*) *dispersa* Kükenthal, 1912

(Figures 2.68-2.70)

Thouarella dispersa Kükenthal, 1912:307-309; pl. 20, fig. 4; text figs. 13-16.—Zapata-Guardiola and López-González, 2012.
Thouarella (*Amphilaphis*) *dispersa*, Kükenthal, 1915:149.—Kükenthal, 1919:411-412; pl. 31, fig. 11.—Kükenthal, 1924:290-291.
Amphilaphis dispersa, Cairns and Bayer, 2009:28.

Examined material

Holotype: ZMB Cni 5468, Deutsche Südpolar-Expedition, Antarctica, 2450 m depth, 1 March 1903.

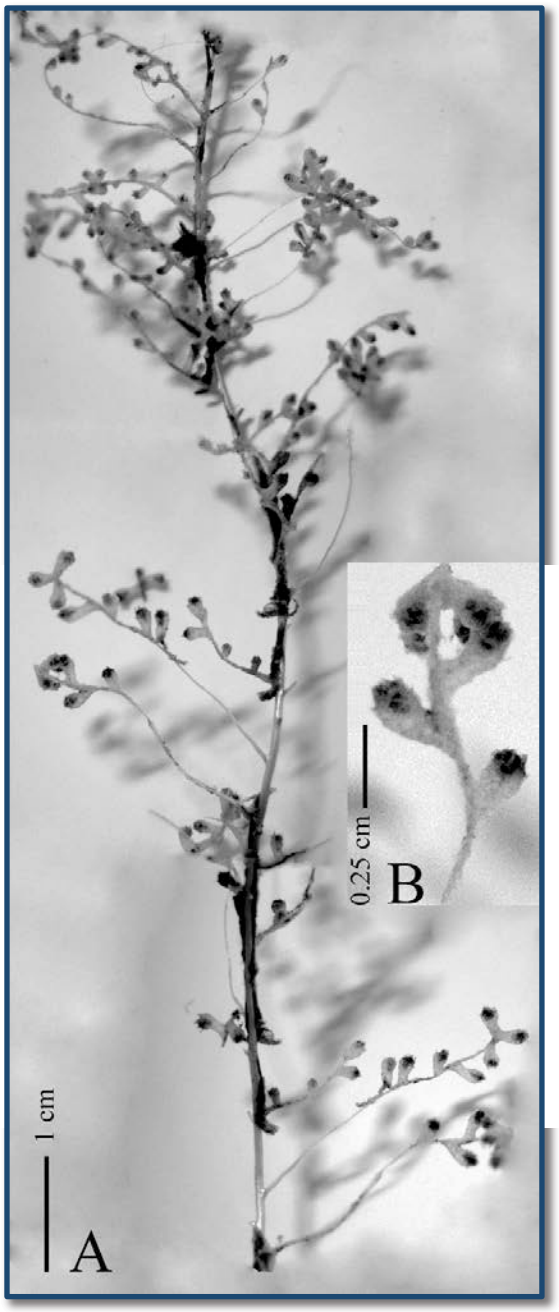


Figure 2.68.- *Thouarella dispersa* Kükenthal, 1912, holotype (ZMB Cni 5468): **a**, whole colony; **b**, detail of branchlet. [Photo: Carsten Lueter, ZMB, Berlin].

Description of the holotype

Slender bottlebrush colony (Fig. 2.68a), of 18 cm in length, and 5.3 cm in width. Simple slender branchlets, up to 3.5 cm in length, sparsely arranged, four branchlets per centimetre, in acute angles upward to stem. Axis pearl in colour, slender and graceful, without holdfast. Basal axis diameter of 1.6 mm. Polyps (Fig. 2.68b) inclined upward to branchlets, singly placed and occasionally biserial, present on main stem and branchlets. Arranged in two sides, 6 polyps per cm. Polyps (Fig. 2.69) relatively elongated, funnel-shaped, up to 1.9 mm in height and 0.8-1 mm in diameter, conical operculum. Polyp body with 8 longitudinal rows of scales, adaxial scales smaller, 5 scales on each longitudinal abaxial row, and 3-4 scales on each adaxial row (Fig. 2.69b).

Opercular scales (Fig. 2.70a) eight in number, 0.64-0.99 x 0.22-0.40 mm, stick shaped wider basally, round tip and bilobed base. Proximal inner surface tuberculate covering up to half of their length; sclerites convex, with longitudinal ridges forming an incipient distal keel. Outer surface smooth or radially granular. Free margin finely serrated. Marginal scales (Fig. 2.70b) eight in number, 0.63-0.85 x 0.32-0.52 mm, triangular shaped with acute tip. Proximal inner surface tuberculate covering about half of their length, multi keeled distally. Outer surface almost radially granular. Basal margin with irregular processes, free margin finely serrated. Body scales (Fig. 2.70c) roughly round in shape, 0.21-0.53 mm in maximum length. Inner surface almost completely tuberculate, outer surface almost smooth with some granules. Basal margin with tuberculate processes, free margin finely serrated. Coenenchymal sclerites (Fig. 2.70d) round in shape, 0.12-0.28 mm in maximum length. Inner surface with scattered tubercles, outer surface quite smooth. Margin finely serrated.

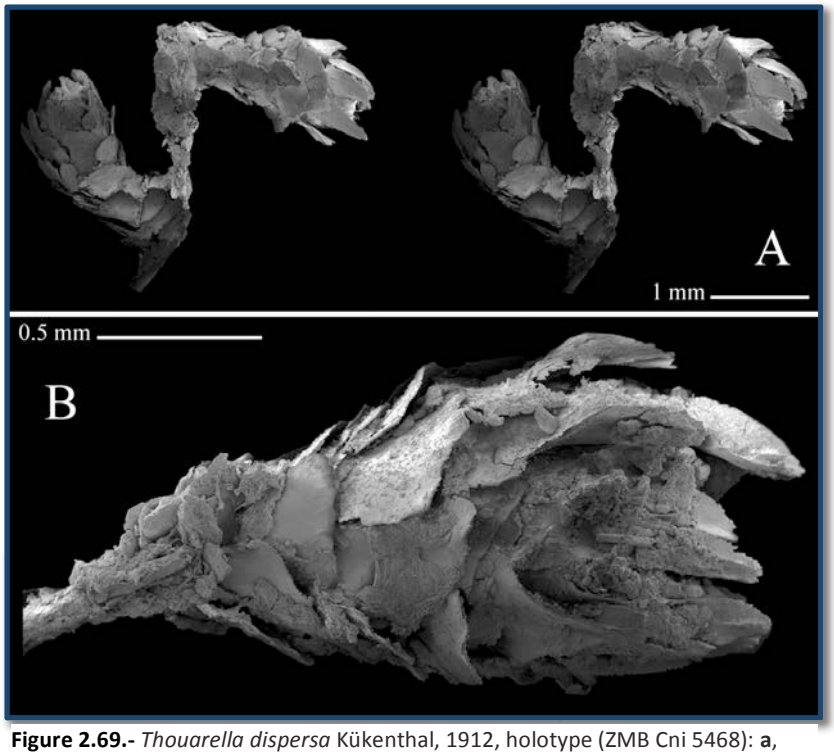


Figure 2.69.- *Thouarella dispersa* Kükenthal, 1912, holotype (ZMB Cni 5468): **a**, polyps on lateral view, stereo pair; **b**, polyp on latero-adaxial view.

Geographical and bathymetrical distribution

Thouarella dispersa is only known from the type locality, an unknown location in Antarctic waters at 2450 m in depth.

Etymology

The specific name *dispersa* was chosen by Prof. W. Kükenthal, because of the arrangement very scattered of its polyps on the branchlets.

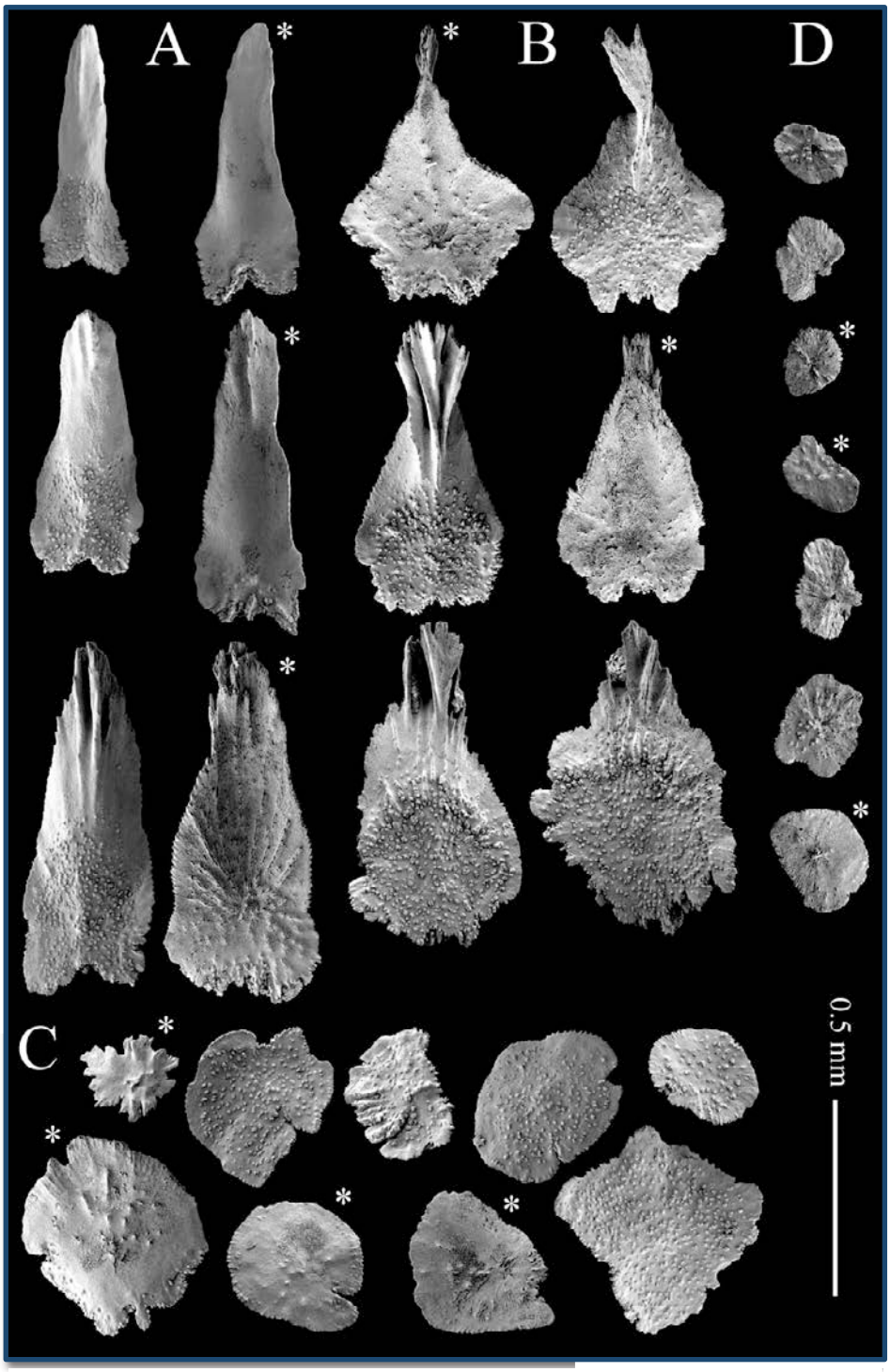


Figure 2.70.- *Thouarella dispersa* Kükenthal, 1912, holotype (ZMB Cni 5468): **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal sclerites. [* outer surface view].

Thouarella (*Epithouarella*) *grandiflora* Kükenthal, 1912

(Figures 2.71-2.75)

Thouarella grandiflora Kükenthal, 1912:309-310; pl. 21, fig. 6; text figs. 17-20.—Zapata-Guardiola and López-González, 2012.
Thouarella (*Amphilaphis*) *grandiflora*, Kükenthal, 1915:149.—Kükenthal, 1919:413; text fig. 180.—Kükenthal, 1924:291.
Amphilaphis grandiflora, Cairns and Bayer, 2009:28.

Examined material

Holotype: ZMB Cni 5469 and MPUW 44, Deutsche Südpolar-Expedition, Gauss-station, Antarctica, 385 m depth, 7 February 1903.

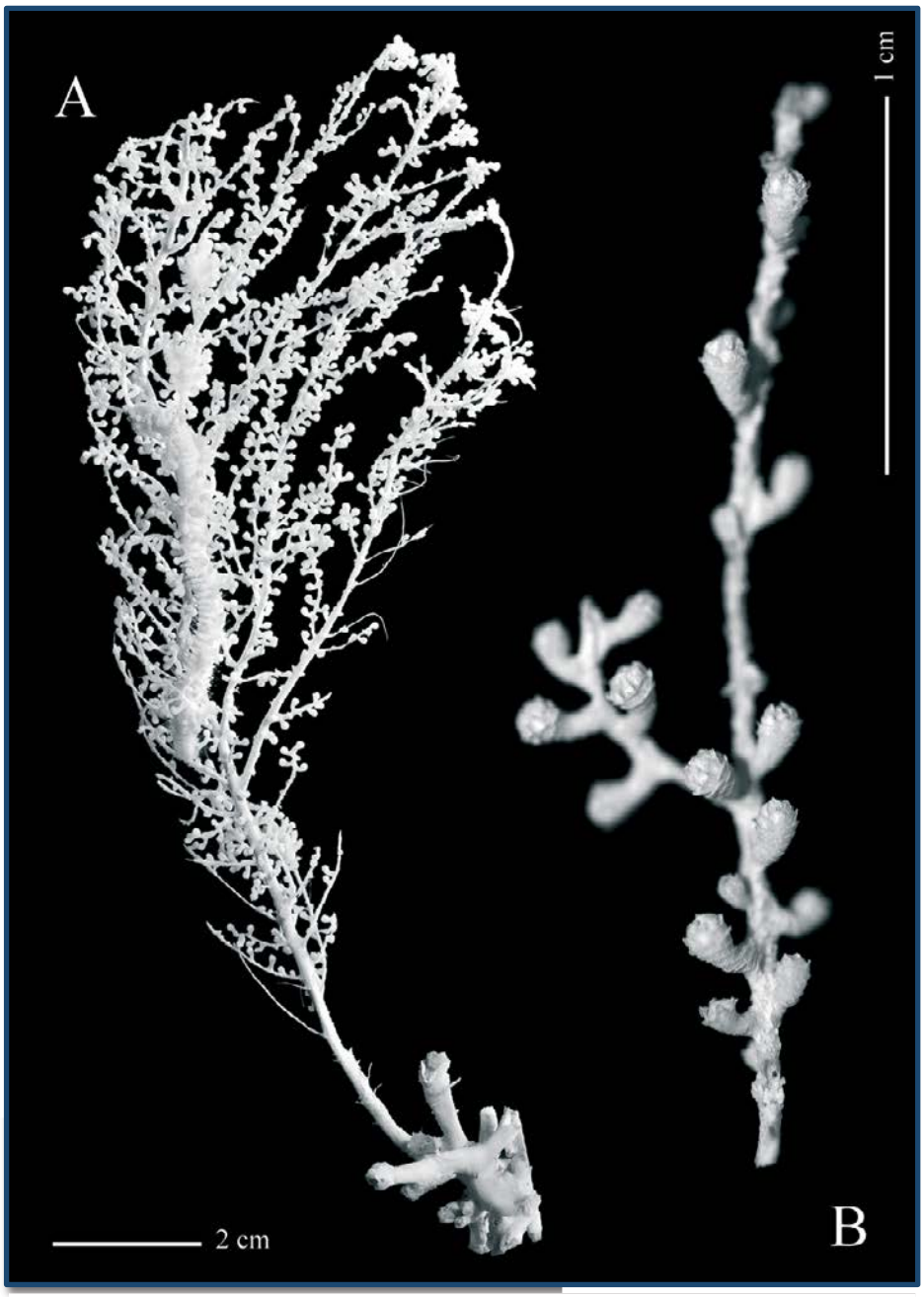


Figure 2.71.- *Thouarella grandiflora* Kükenthal, 1912: **a**, holotype (ZMB Cni 5469), whole colony, [photo: Carola Radken, ZMB, Berlin]; **b**, paralectotype (MPUW 44), detail of branchlet.

Description of the holotype

Bottlebrush colony (Fig. 2.71a), of 18 cm in length and 7.6 cm in width. Colony ramified up to the second order, branchlets up to 3.7 cm in length. Axis ochre in colour, with holdfast and attached to a hard substrate. Basal axis diameter of 2.4 mm. Polyps almost perpendicular and slightly bent upward to stem and branchlets (Figs. 2.71b, 2.72a), singly placed, arranged in spirals, about 8 polyps per cm.

Polyps (Figs. 2.72, 2.73) club-shaped, about 1.5-2.4 mm in height and 0.4-1.2 mm in diameter. Polyps with 8 longitudinal rows of scales, adaxial scales reduced in size and number, 6-8 scales on each longitudinal abaxial row (Fig. 2.73b) and four scales on adaxial row (Fig. 2.73c).

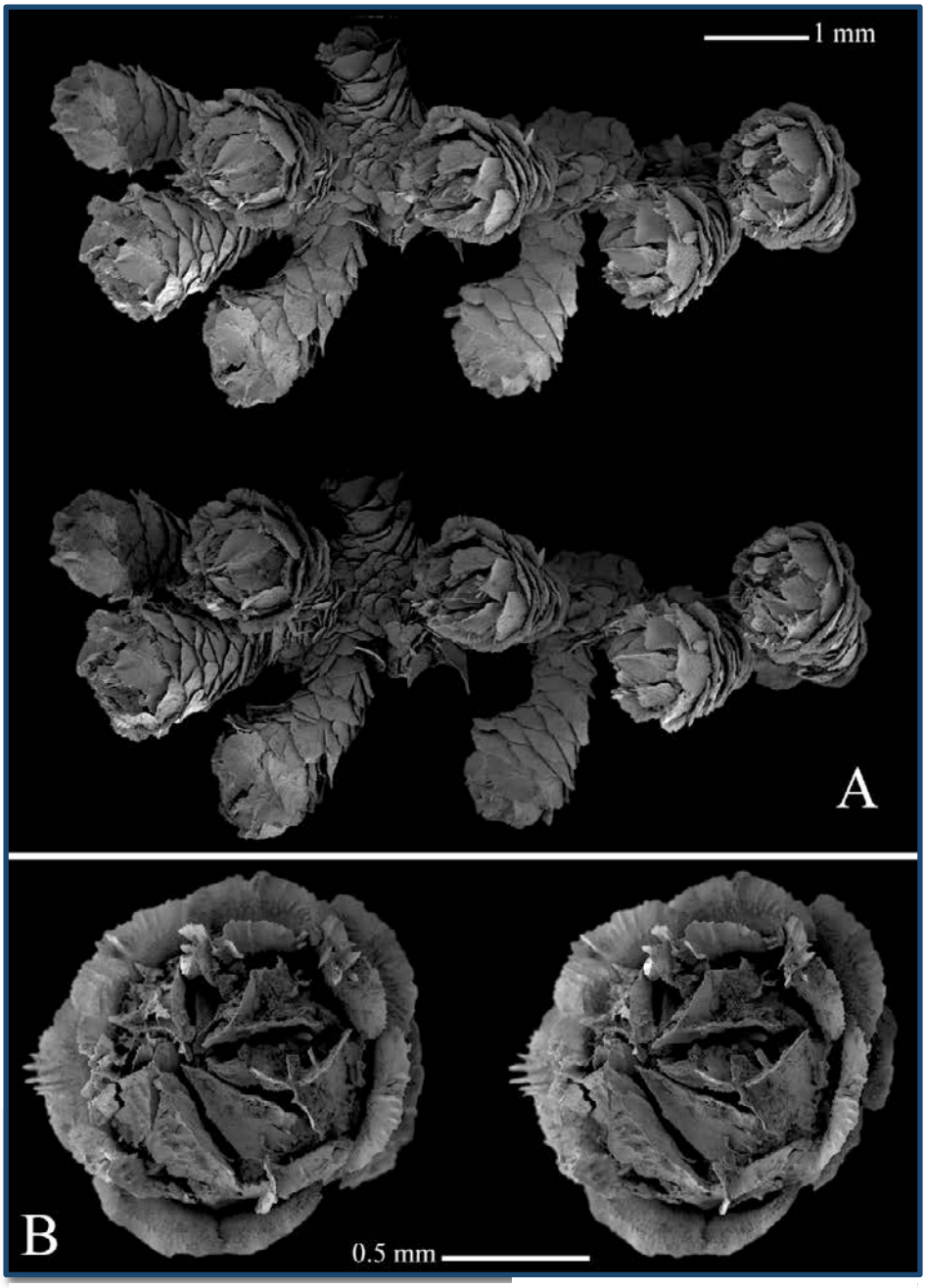


Figure 2.72.- *Thouarella grandiflora* Kükenthal, 1912, holotype (MPUW 44): **a**, detail of branchlet, stereo pair; **b**, polyp on oral view, stereo pair.

Opercular scales (Fig. 2.72b, 2.74a) eight in number, 0.42-0.58 x 0.15-0.29 mm, spade-shaped or stick-shaped with acute tips and bilobed base. Proximal inner surface sparsely tuberculate up to half of scale length; distal part with strong-ridged keel. Outer surface radially granular forming ridges, concave surface deeper distally. Free margin finely serrated, basal margin with tuberculate processes. Marginal scales (Fig. 2.74b) eight in number, pentagonal-shaped, 0.44-0.54 x 0.31-0.46 mm. Inner surface sparsely tuberculate covering up to 75% of the scale, with middle longitudinal prominent multi-keels distally. Outer surface radially granular, forming ridges. Free margin finely serrated, basal margin with irregular processes. Body wall scales (Fig. 2.75a) fan-shaped, 0.26-0.48 x 0.26-0.56 mm. Inner surface almost completely sparsely tuberculate with or without middle longitudinal fine crests distally. Outer surface granular. Free margin finely serrated, basal margin with irregular processes. Coenenchymal sclerites (Fig. 2.75b) round or oval-shaped, 0.18-0.47 mm in maximum length. Inner surface completely tuberculate, outer surface with few granules. Margin finely serrated, with tubercles.

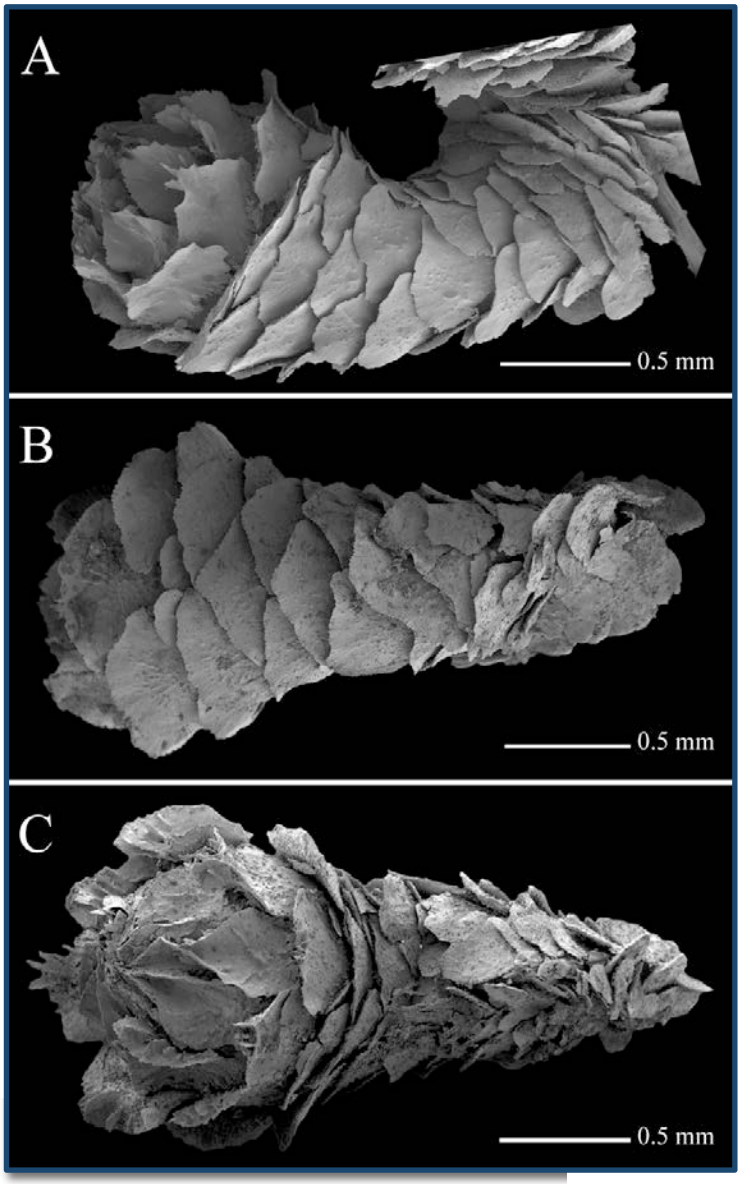


Figure 2.73.- *Thouarella grandiflora* Kükenthal, 1912: **a**, holotype (ZMB Cni 5469), polyp on lateral view; **b**, paralectotype (MPUW 44), polyp on abaxial view; **c**, paralectotype (MPUW 44), polyp on adaxial view.

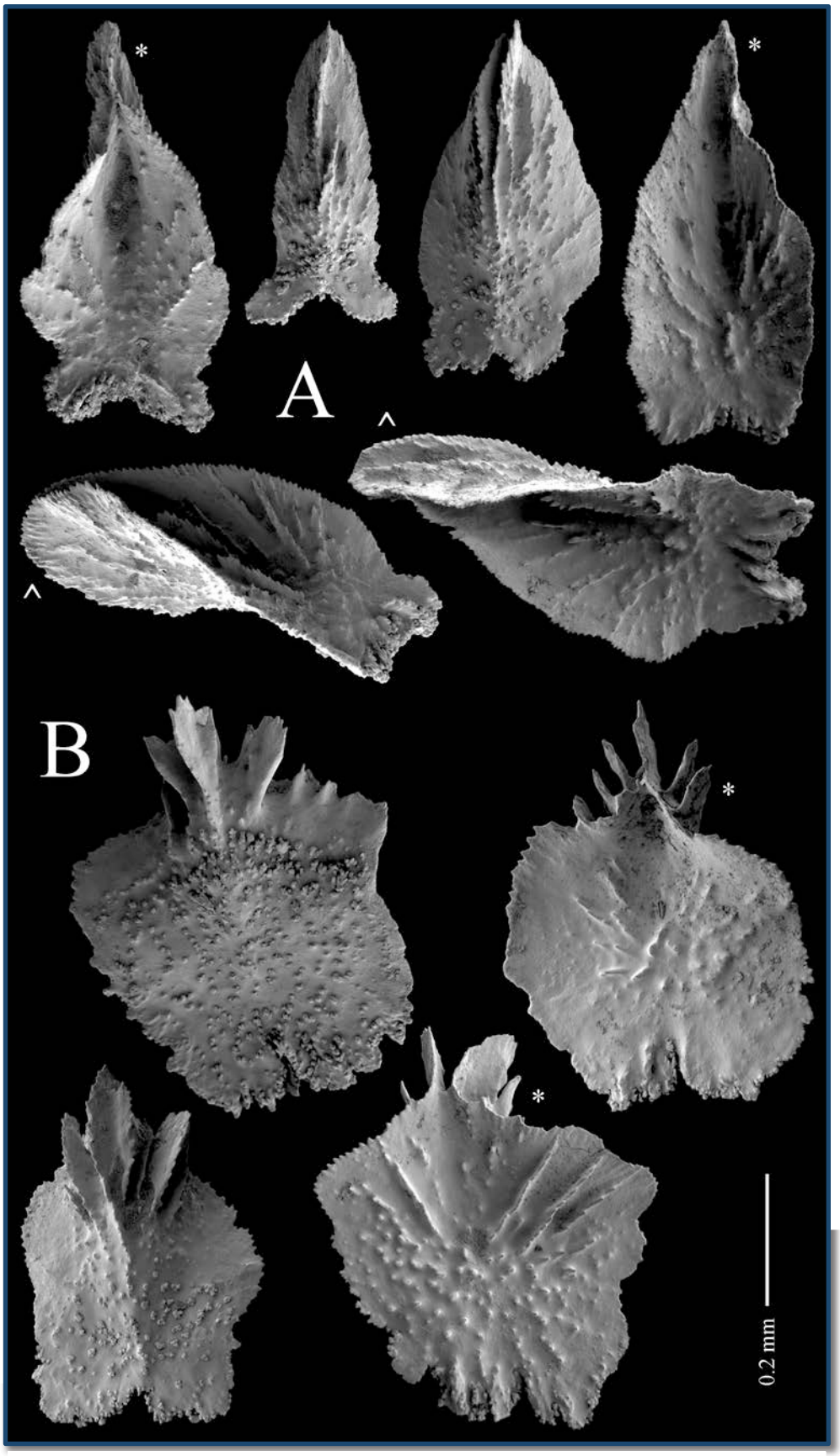


Figure 2.74.- *Thouarella grandiflora* Kükenthal, 1912, holotype (MPUW 44): **a**, opercular scales; **b**, marginal scales. [* outer surface view; ^ lateral view].

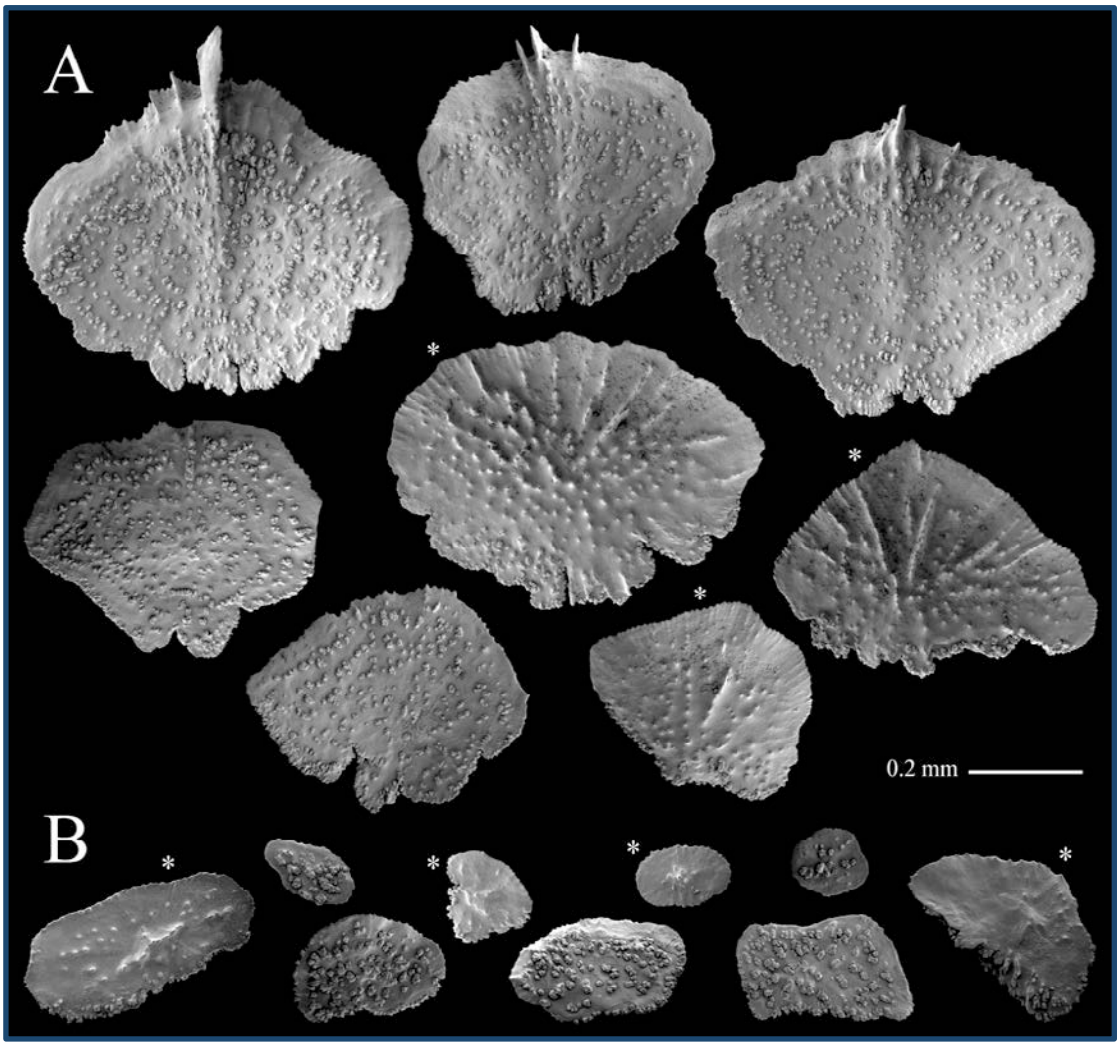


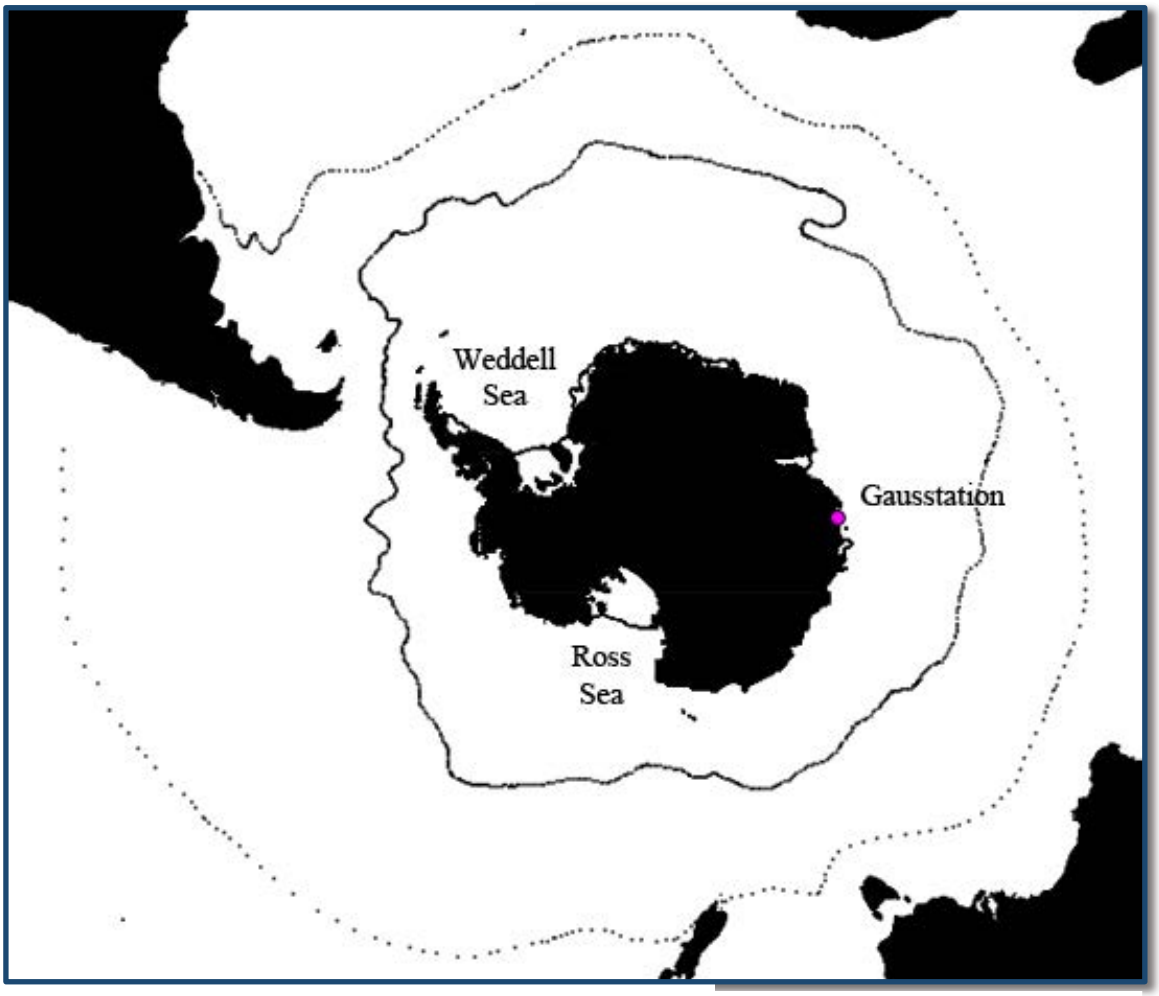
Figure 2.75.- *Thouarella grandiflora* Kükenthal, 1912, holotype (MPUW 44): **a**, body scales; **b**, coenenchymal sclerites. [* outer surface view].

Geographical and bathymetrical distribution

Thouarella grandiflora is only known from the holotype locality of Gauss-station (Map 2.27), Antarctica at 385 m in depth.

Etymology

From the modern Latin the adjective *grandiflora* combines *grandis*- that means large and *–flos* that means flower, in reference to the large polyps of this species.



Map 2.27.- *Thouarella* (*Epithouarella*) *grandiflora* Kükenthal, 1912. Species examined distribution map. Pink circle, holotype.

Thouarella (*Epithouarella*) *regularis* (Wright and Studer, 1889)

(Figures 2.76-2.80)

Amphilaphis regularis Wright and Studer, 1889:71-72; pl. 15, figs. 1, 1a; pl. 21, fig. 7.—Versluys, 1906:22.—Thomson and Ritchie, 1906:854.—Cairns and Bayer, 2009:28, 38; fig. 10 j-q.
Thouarella regularis, Kükenthal, 1912:307.—Zapata-Guardiola and López-González, 2012:3.
Thouarella (*Amphilaphis*) *regularis*, Kükenthal, 1915:149.—Kükenthal, 1919:409-410.—Kükenthal, 1924:289.—Parrish and Baco, 2007:192.
not *Thouarella regularis* Kükenthal, 1907:206-207.
not *Amphilaphis regularis,* Nutting, 1908:573-574.

Examined material

Lectotype: BMNH 1889.5.27.60, in label: "Challenger Expedition, stn 135a, 37°16.83'S, 12°45.25'W, off Inaccessible Island, Tristan da Cunha, Subantarctica, 137 m depth, 16 October 1873; stn 135c, 37°25.5'S, 12°28.5'W, off Nightingale Island, Tristan da Cunha, Subantarctica, 183-274 m depth, 17 October 1873".

Paralectotypes: BMNH 1932.12.8.7, Challenger Expedition, stn 135, 37°1.83'S, 12°19.16'W, off Tristan da Cunha, Subantarctica, 658 m depth, 15 October 1873; BMNH 1889.7.5.11.15, Challenger Expedition, stn 135a, 37°16.83'S, 12°45.25'W, off Inaccessible Island, Tristan da Cunha, Subantarctica, 137 m depth, 16 October 1873.

Additional material: BM 1889.7.5.17, Challenger Expedition, stn 135a, 37°16.83'S, 12°45.25'W, off Inaccessible Island, Tristan da Cunha, Subantarctica, 137 m depth, 16 October 1873; BM 89.7.5.18 and BM 89.7.5.21, Challenger Expedition, stn 135b, 37°22.8'S, 12°33'W, Nightingale Island, South Atlantic Ocean, 132-851 m depth, October 1873; ZMA COEL04937, Scottish National Antarctic Expedition, Saint Helena, Subantarctica, 1904.

Remarks on the type locality

According to the information on the label and that in the archives of the BMNH, two close localities are indicated for the lectotype material here designated (stns. 135a and 135c) (Emma Sherlock, pers. comm.).

Description of the lectotype

Colony fan-shaped, pinnately branched in a plane (Fig. 2.76a), of 25 cm in total length and 13 cm in width. Simple or pinnately ramified branchlets up to second order inclined upward, terminal branchlets up to 9 cm in length. Axis yellow brownish in colour. Basal axis diameter of 4 mm, with holdfast, attached to hard substrate (coral). Polyps (Fig. 2.76b, 2.77a) inclined upward to branchlets, singly placed, present on main stem, branches and branchlets. Regularly arranged in spirals, or biserially, 11-14 polyps per cm. Polyps (Fig. 2.77b,c) cylindrical to club-shaped, about 0.9-1.8 mm in height and 0.54-1.02 mm in diameter.

Polyp body with 8 longitudinal rows of scales, adaxial body rows slightly disorganized (Fig. 2.78a). Each longitudinal abaxial row with 7-9 scales (Fig. 2.77b) and 3-4 scales on each adaxial row (Fig. 2.78a).

Figure 2.76.- *Thouarella regularis* (Wright and Studer, 1889), lectotype (NHMUK 1889.5.27.60): **a**, whole colony; **b**, detail of branchlet.

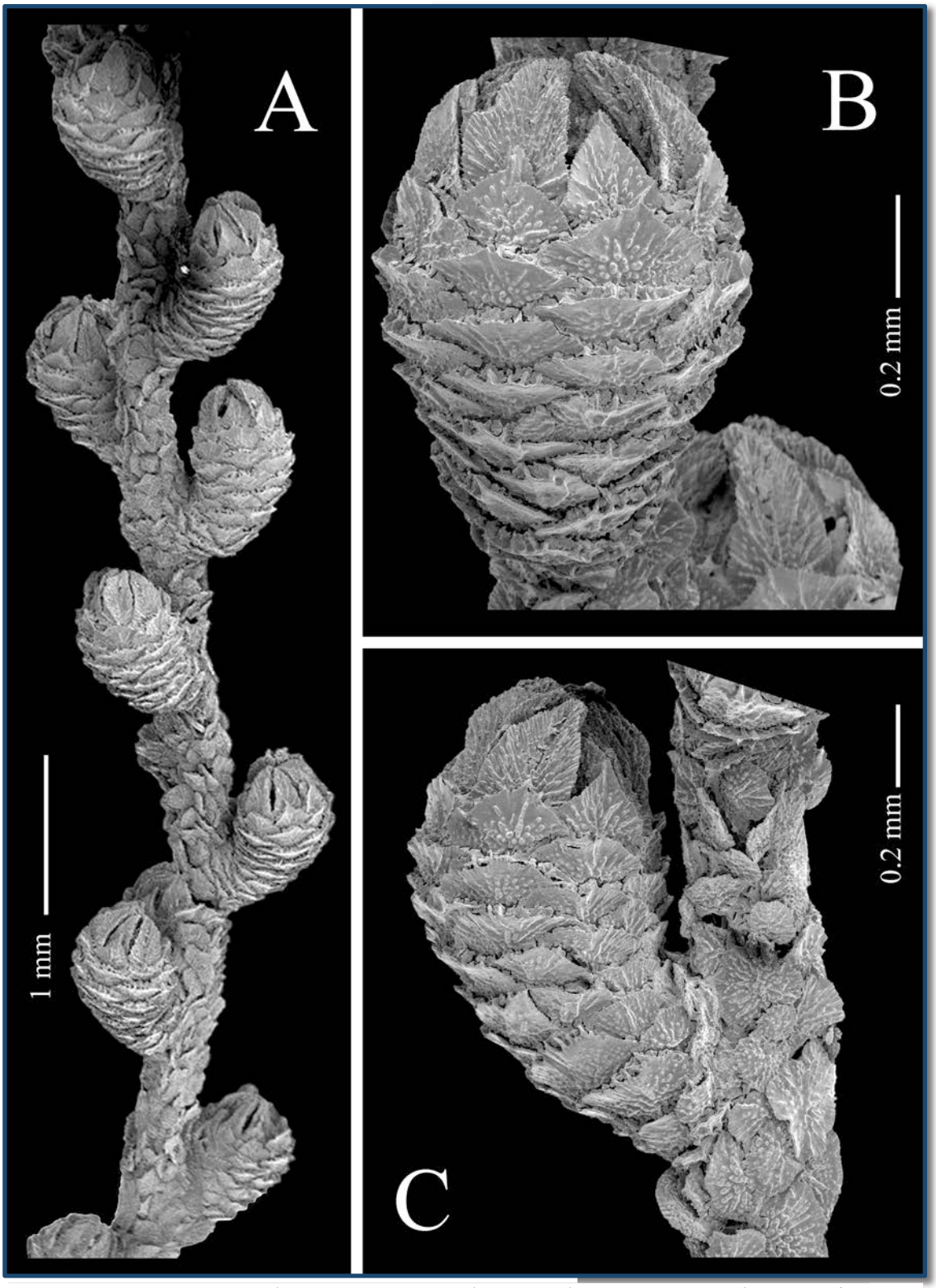


Figure 2.77.- *Thouarella regularis* (Wright and Studer, 1889), lectotype (NHMUK 1889.5.27.60): **a**, detail of branchlet; **b**, polyp on abaxial view; **c**, polyp on lateral view.

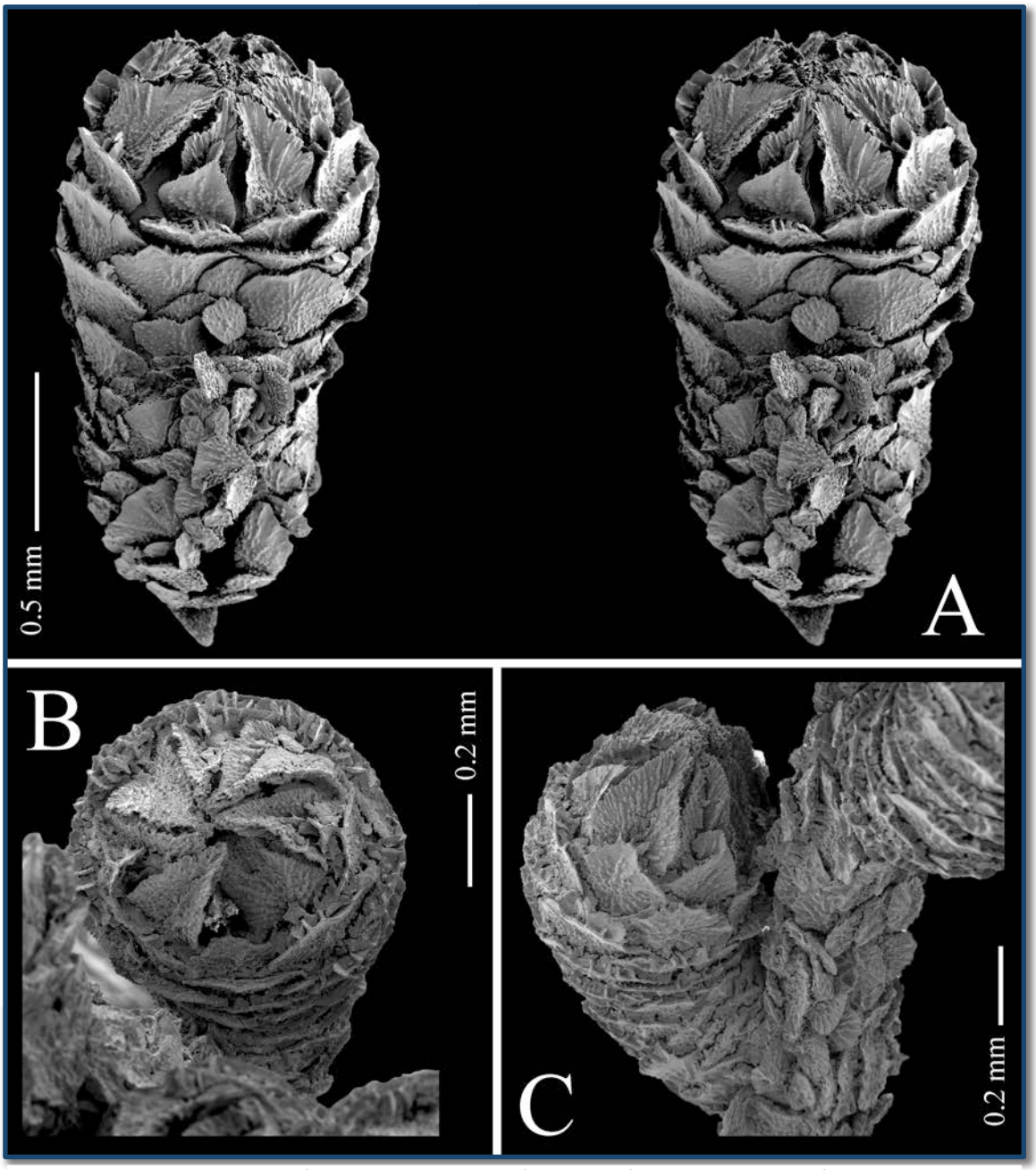


Figure 2.78.- *Thouarella regularis* (Wright and Studer, 1889), lectotype (NHMUK 1889.5.27.60): **a**, polyp on adaxial view, stereo pair; **b**, polyp on oral view; **c**, polyp on latero-adaxial view.

Opercular scales (Fig. 2.78b, 2.79a) eight in number, 0.38-0.52 x 0.19-0.3 mm, triangle-shaped, slightly bilobed basally and with acute tip. Proximal inner surface tuberculate covering up to half of their length. Prominent apical keel with multi lateral ridges. Distal inner surface of scale also with longitudinal ridges. Outer surface radially granular from basal nucleus. Basal margin with irregular processes, free margin finely serrated. Marginal scales (Fig. 2.79b) eight in number, 0.32-0.41 x 0.27-0.34 mm, pentagonal-shaped, with acute tip. Inner surface tuberculate, covering up to about 80% of their length and at least the proximal half. Prominent apical keel with multi lateral ridges, lateral inner distal surface almost smooth. Outer surface radially granular from basal nucleus. Basal margin with irregular tuberculate processes, free

margin finely serrated. Body scales (Fig. 2.80a) 0.23-0.31 x 0.29-0.49 mm, fan shaped. Inner surface almost completely tuberculate, short middle ridges distally decreasing in number and size from distal to basal scales; outer surface radially granular. Adaxials with tendency to be reduced. Basal margin with irregular tuberculate processes, free margin finely serrated. Coenenchymal sclerites (Fig. 2.80b) round to oval-shaped, 0.07-0.3 mm in maximum length. Inner surface completely tuberculate, outer surface granulate forming ridges. Margin similar to that of body scales. Presence of inner layer with tuberculate irregular sclerites.

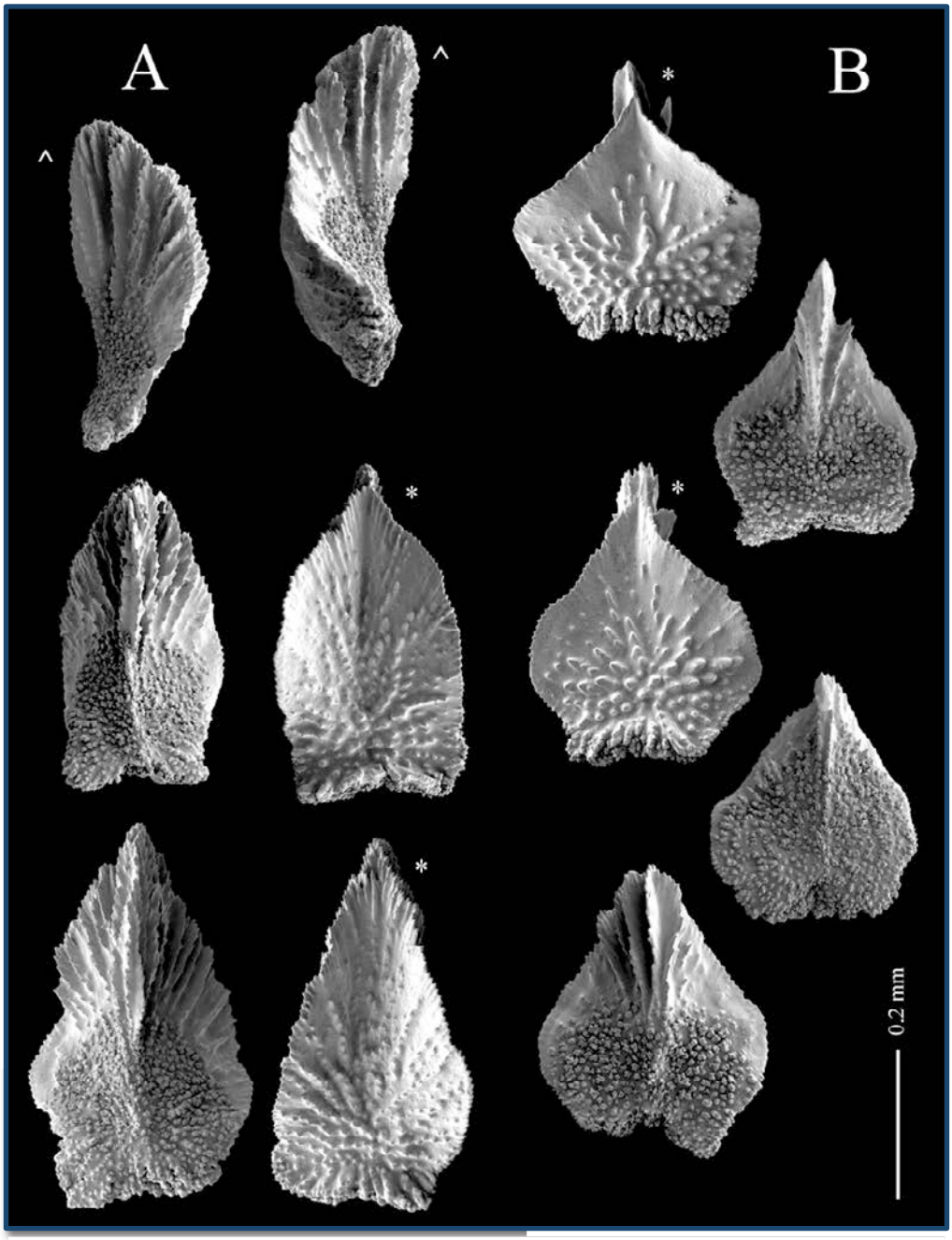


Figure 2.79.- *Thouarella regularis* (Wright and Studer, 1889), lectotype (NHMUK 1889.5.27.60): **a**, opercular scales; **b**, marginal scales. [*outer surface view; ^ lateral view].

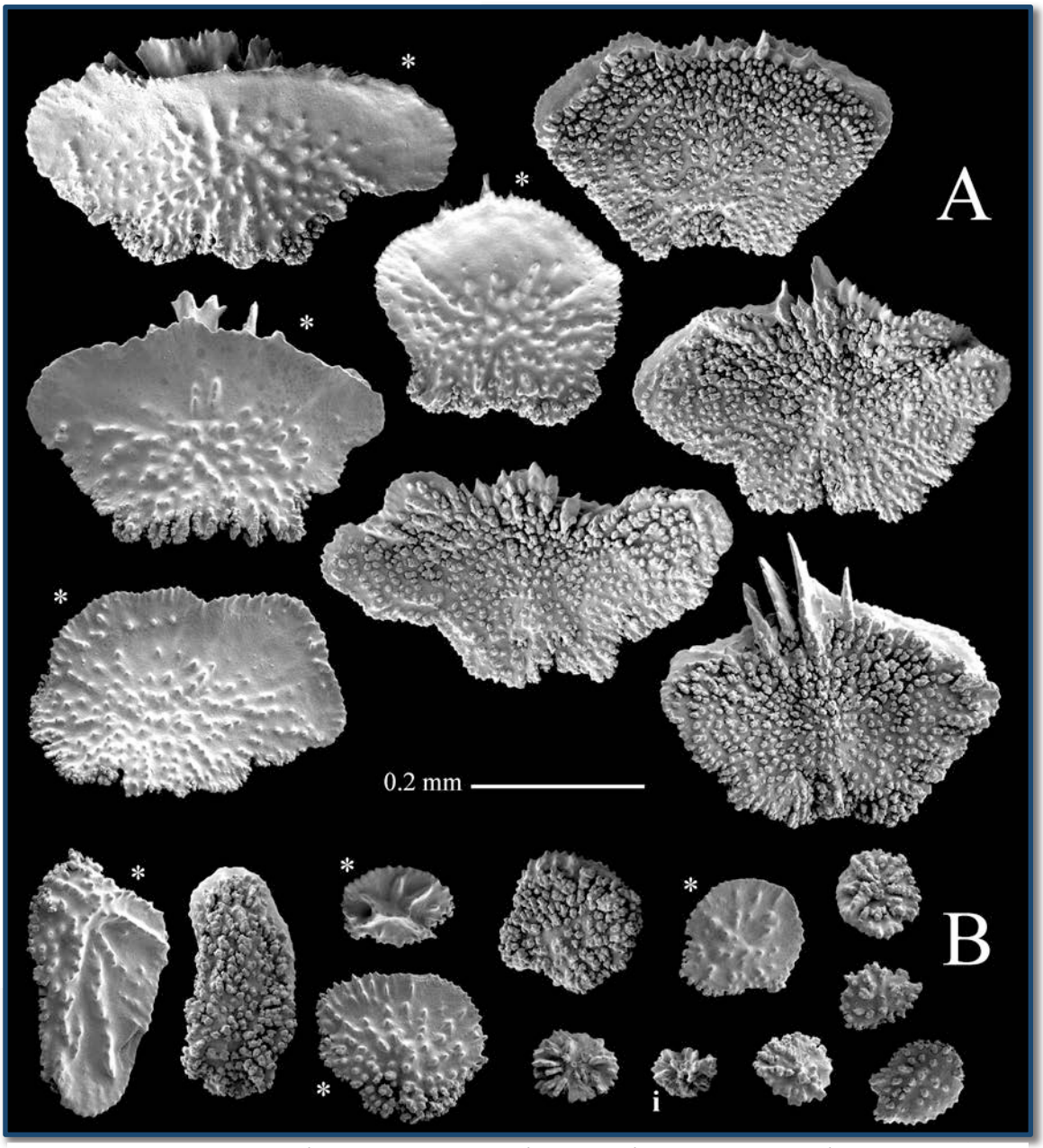


Figure 2.80.- *Thouarella regularis* (Wright and Studer, 1889), lectotype (NHMUK 1889.5.27.60): **a**, body scales; **b**, coenenchymal sclerites. [* outer surface view; **i** sclerite from inner layer].

Variations from holotype

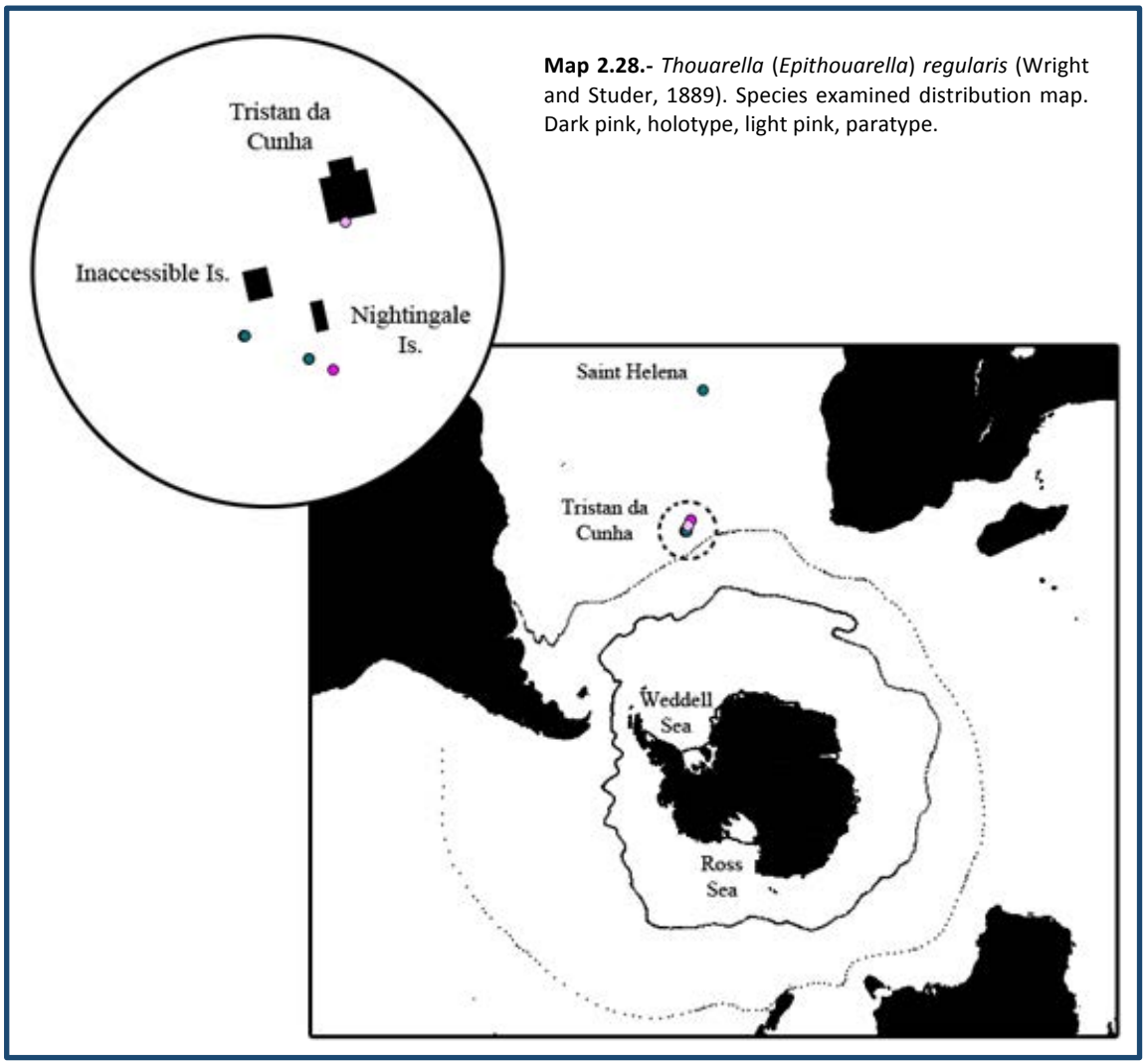
The paralectotypes and additional material here examined are pinnate colonies branched in a plane, branchlets ramified up to the second order, up to 9 cm in length. The maximum height of the colonies examined is 25 cm and width 13 cm, both measurements belonging to the lectotype. The number of polyps per centimetre can vary from 7 to 14. The polyps' shape, their arrangement and the shape of the sclerites are similar to those in the lectotype.

Geographical and bathymetrical distribution

Thouarella regularis is known from Inaccessible and Nightingale Island from the archipelago of Tristan da Cunha, and from Saint Helena, Subantarctica (Map 2.28), between 137 and 658 m in depth.

Etymology

The specific name *regularis* was presumably chosen in reference of the regular arrangement of branchlets on main stem.



Map 2.28.- *Thouarella* (*Epithouarella*) *regularis* (Wright and Studer, 1889). Species examined distribution map. Dark pink, holotype, light pink, paratype.

Thouarella (*Epithouarella*) *viridis* Zapata-Guardiola and López-González, 2010c

(Figures 2.81-2.84)

Thouarella (*Epithouarella*) *viridis* Zapata-Guardiola and López-González, 2010c:174.

Examined material

Holotype: ZIZMH C11744, ANT XIX/5, stn PS61/164-01, 53°23.80'S, 42°42.03'W, west of South Georgia, Antarctica, 312.5–321.6 m depth, 9 April 2002.

Paratypes: ZIZMH C11745, with the same sampling data as the holotype, four fragments; CRO-0038, ANT XIX/5, stn PS61/150-01, 54°30.22'S, 56°08.20'W, south east of Falkland Islands, Antarctica, 286.3–291.6 m depth, 6 April 2002, 1 fragmented colony; USNM 1128949, CRO-0039, ANT XIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, west of South Georgia, Antarctica, 306.0–342.7 m depth, 9 April 2002, two fragments and one colony and various fragments; CRO-0040, ANT XIX/5, stn PS61/174-01, 54°24.47'S, 35°36.81'W, north east of South Georgia, Antarctica, 278.3–279.8 m depth, 11 April 2002, four fragments; CRO-0041, ANT XIX/5, stn PS61/182-01, 54°27.63'S, 35°41.33'W, north east of South Georgia, Antarctica, 249.3–256.0 m depth, 12 April 2002, two fragments.

Additional material: US 6406, ANT XIX/5, stn PS61/150-01, 54°30.22'S, 56°08.20'W, south east of Falkland Islands, Antarctica, 286.3–291.6 m depth, 6 April 2002, one colony; US 6443, ANTXIX/5, PS61/160-01, 53°23.75'S, 44°45.12'W, Shag Rocks, Antarctica, 434 m depths, 9 April 2002, one colony; US 1301 and US 1337, ANT XIX/5, stn PS61/164-01, 53°23.82'S, 42°42.46'W, north west South Georgia, Antarctica, 312-322 m depth, 9 April 2002, 10 colonies, eight fragments and one colony; US 1364 and US 1415, ANT XIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, west of South Georgia, Antarctica, 306.0–342.7 m depth, 9 April 2002, various fragments.

Description of the holotype

Fragment of a larger colony (Fig. 2.81a) of 10.2 cm in total height and 5.4 cm in width, with two short lateral branches of 2.3 and 3.5 cm in length. Main stem and lateral branches with simple branchlets (Fig. 2.81b), occasionally ramified up to two orders, in a bottlebrush branching pattern.

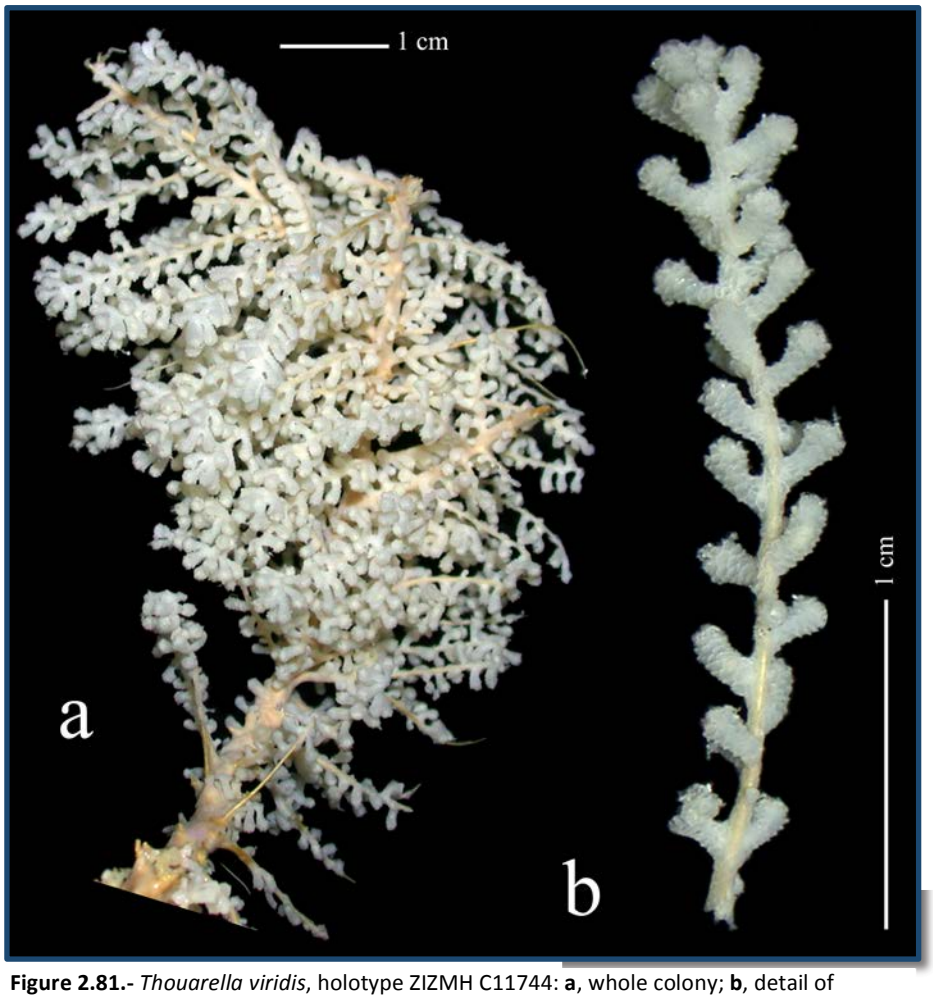


Figure 2.81.- *Thouarella viridis*, holotype ZIZMH C11744: **a**, whole colony; **b**, detail of branchlet.

Branchlets up to 3 cm in length, from seven to eight branchlets per cm. Living specimens green in colour, preserved material whitish. Axis ochre in colour, stiff and thick, basal axis diameter of 4 mm. Polyps (Figs. 2.81b, 2.82e) straight and directed upward to stem, singly placed, present on main stem, branches and branchlets. Regularly arranged in spirals and more crowded distally, while in the basal portion the polyps are occasionally nearly opposite, with no tendency towards pairs or whorls, 14–15 polyps per cm. Polyps relatively elongated, cylindrical to club-shaped distally, with a conical operculum (Figs. 2.82a–d); about 2.1–2.5 mm in height and 0.73–0.85 mm in diameter. Polyp body with 8 longitudinal rows of scales, 6–7 scales on each longitudinal abaxial row (Fig. 2.82b) overlapping one another and slightly reducing in size basally.

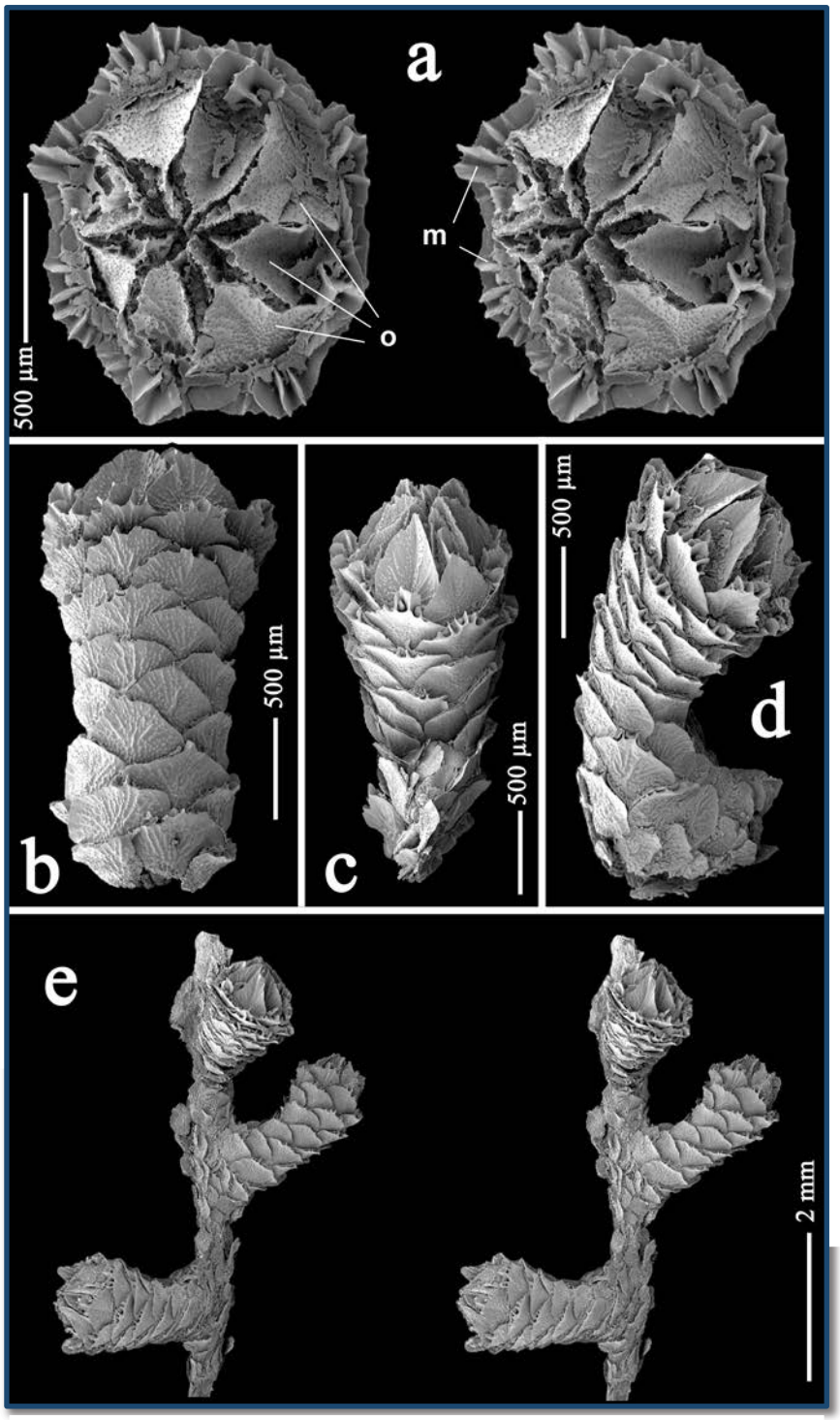


Figure 2.82.- *Thouarella viridis*, holotype ZIZMH C11744: **a**, polyp in oral view, stereo pair; **b**, polyp in abaxial view; **c** polyp in adaxial view; **d** polyp in lateral view; **e** detail of branchlets, stereo pair. Abbreviations: **o** opercular scales; **m** marginal scales.

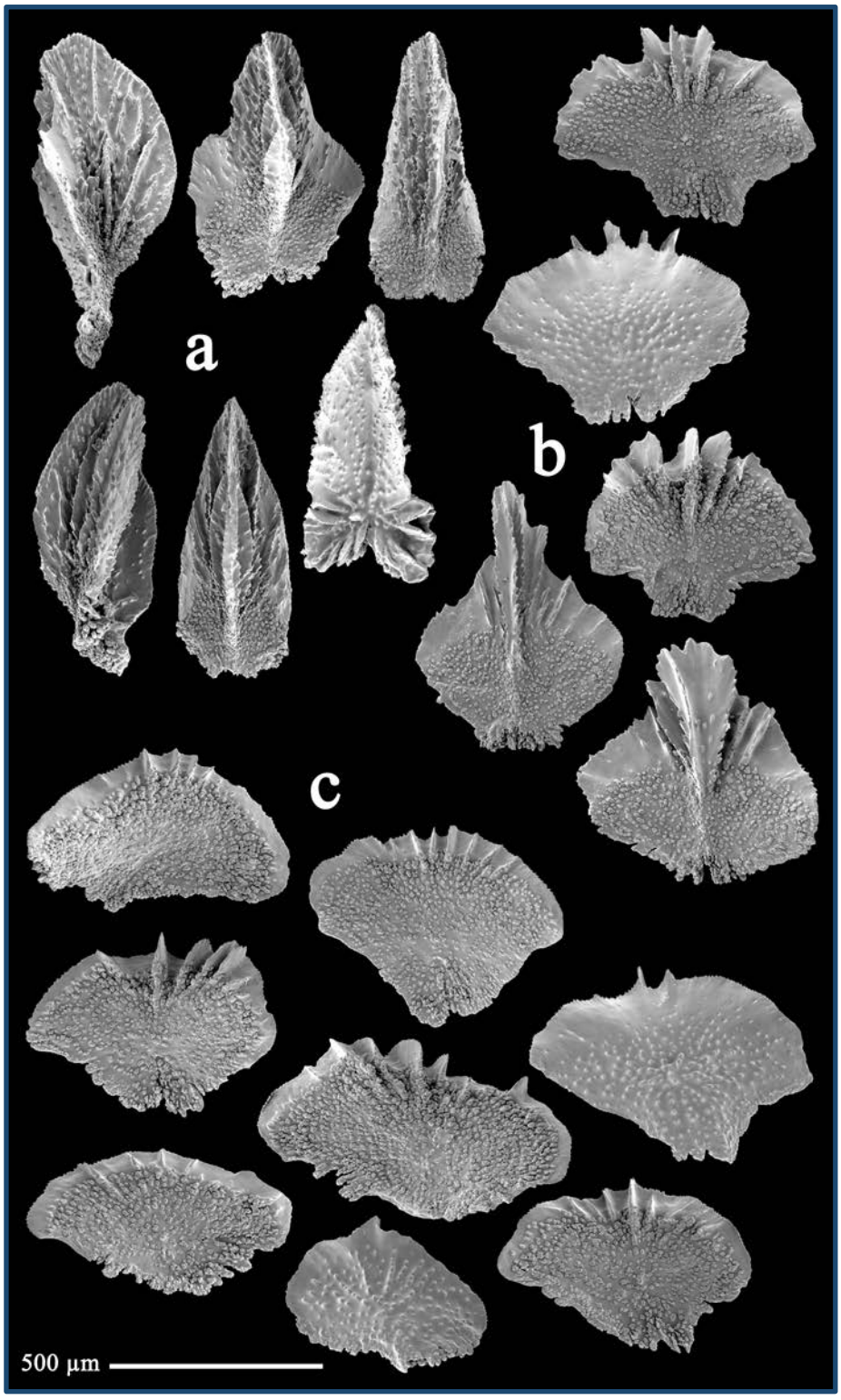


Figure 2.83.- *Thouarella viridis*, holotype ZIZMH C11744: **a**, opercular scales; **b**, marginal scales**; c**, body scales.

Opercular scales (Fig. 2.83a) large, eight in number, 0.62–0.78 mm x 0.24–0.42 mm, isosceles shaped with bilobed base and acute tip. Proximal inner surface tuberculate, covering more than one-third of their length. Prominent, strong, apical keel, often bearing additional lateral ridges. Distal inner surface of scale also with longitudinal ridges. Outer surface granulate, with a longitudinal valley corresponding to inner keel. Basal margin with digitate processes, free margin finely serrated. Eight marginal scales (Fig. 2.83b) 0.45–0.63 mm x 0.47–0.64 mm, pentagonal shaped. Proximal inner surface tuberculate, covering up to about 80% of their length of scales with 3–4 short middle ridges and without a spine; other scales with a small blunt spine sculpted by longitudinal ridges have tubercles covering up to half of their length. Lateral inner distal surface smooth, without granules or ridges. Outer surface granulate. Basal margin with digitate processes, free margin finely serrated. Body scales (Fig. 2.83c) like marginal scales in shape with tendency to be fan shape, 0.27–0.51 mm x 0.34–0.74 mm, without spines and often decreasing in number and size from distal to basal scales. Adaxials with tendency to be reduced. Inner side with short ridges and up to about 80% tuberculate. Outer surface granulate. Free margin finely serrated, basal margin with digitate processes. Coenenchymal scales (Fig. 2.84) round to oval shaped, 0.13 mm–0.36 mm in maximum length. Inner surface tuberculate, outer surface granulate forming ridges. Margin irregular with warts proximally, and the remainder finely serrated.

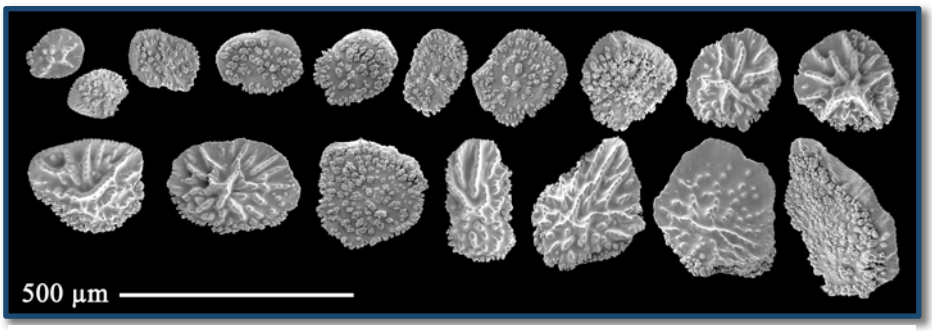


Figure 2.84.- *Thouarella viridis*, holotype ZIZMH C11744: Coenenchymal scales.

Variations from holotype

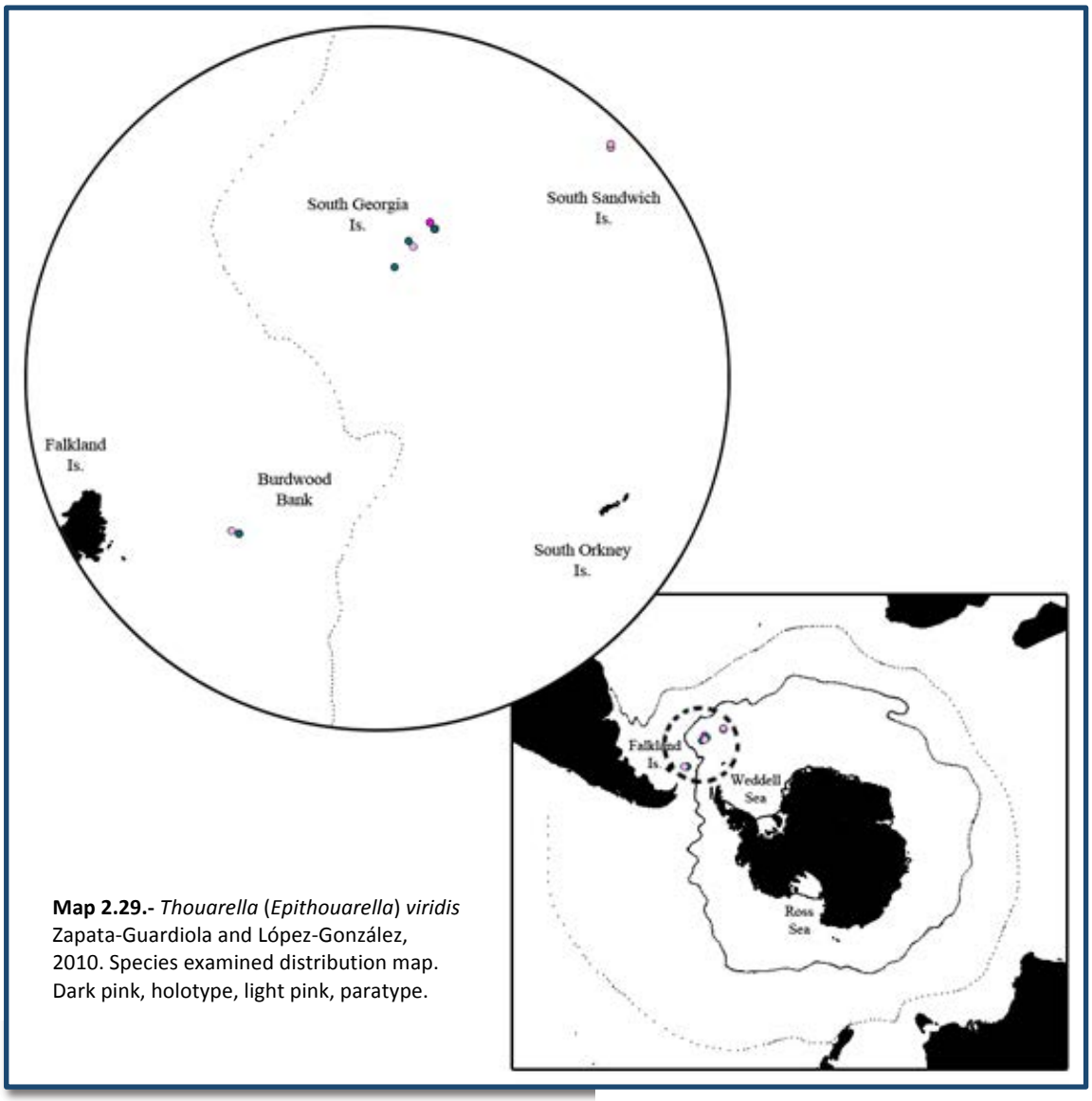
The general colonial and polyp structure of the paratypes and additional examined material are quite similar to that of the holotype. The branches and branchlets arise from all around the main stem, from seven to nine branchlets per centimetre in number. Sometimes, the colony shows the branchlets bent towards one side of the colony, giving the false impression of being disposed in a plane. The lateral branches can reach up to 9.3 cm in length, and branchlets up to 4 cm, sometimes ramified up to three orders. The number of polyps per centimetre can vary from 10 to 15 on branchlets. The distribution and form of the sclerites from polyps and coenenchyme are as in the holotype.

Geographical and bathymetrical distribution

At present, *Thouarella viridis* is only known from South Georgia Island area and south east of Falkland Islands, Antarctica (Map 2.29), between 249 and 342 m in depth.

Etymology

The specific epithet *viridis* comes from one of the most distinctive features shown by this species. When sorting the fresh material collected during the catches, its olive green colour was very evident, while most of the Antarctic primnoid gorgonians vary in colour from whitish or yellowish to orange.



Map 2.29.- *Thouarella* (*Epithouarella*) *viridis* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Subgenus *Thouarella* Gray, 1870

Diagnosis

Thouarella with polyps placed singly. Marginal scales bearing thorns.

Geographical and bathymetrical distribution

It has a circum Antarctic distribution, it has been also reported in Subantarctic waters and at the west Atlantic and north Pacific Oceans between 60 and 1005 m in depth.

Etymology

The subgeneric name uses the name of the genus *Thouarella*.

Type species

Thouarella antarctica (Valenciennes, 1846).

Thouarella (*Thouarella*) *andeep* Zapata-Guardiola and López-González, 2010a

(Figures 2.85-2.88)

Thouarella (*Thouarella*) *andeep* Zapata-Guardiola and López-González, 2010a:142.

Examined material

Holotype: ZIZMH C11744, ANTXIV/2, stn PS71/048-01, 70°24'S, 08°19.72'W, off Atka Bay, Antarctica, 601.8 m depth, 12 January 2008.

Paratypes: ZIZMH C11745, with the same sampling data as the holotype, two colonies. USNM 1123418, with the same sampling data as the holotype, two colonies.

Additional material: US 137, ANTXV-3, stn PS48/154, 74°38.7'S, 26°59.3'W, Halley Bay, east Weddell Sea, Antarctica, 569 m depth, 11 February 1998, one colony; US 174, ANTXVII-3, stn 166-01, 63°1.2'S, 59°9.2'W, Bransfield Strait, Antarctica, 666-673 m depth, 28 April 2000, two colonies; US 6423, ANTXVII-3, stn 177-01, 62°50.4'S, 60°51.6'W, west of Deception Island, Peninsula Antarctica, Antarctica, 200 m depth, 1 May 2000, one fragment; US 6427, ANTXIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, north west South Georgia, Antarctica, 306-343 m depth, 9 April 2002, one colony; US 6424, ANTXIX/5, stn PS61/253-01, 61°24.03'S, 55°24.72'W, south Elephant Island, Antarctica, 276-282 m depth, 25 April 2002, two colonies; US 2788, ANTXXI/2, PS65/292-01, 72°51.43'S, 19°38.62'W, Austasen, easter Weddell Sea, Antarctica, 596 m depth, 31 December 2003, one fragment; BEIM CRO-028, CRO43 and CRO44, ANTXIV/2, stn PS71/048-01, 70°24'S, 08°19.72'W, off Atka Bay, Antarctica, 601.8 m depth, 12 January 2008, four colonies.

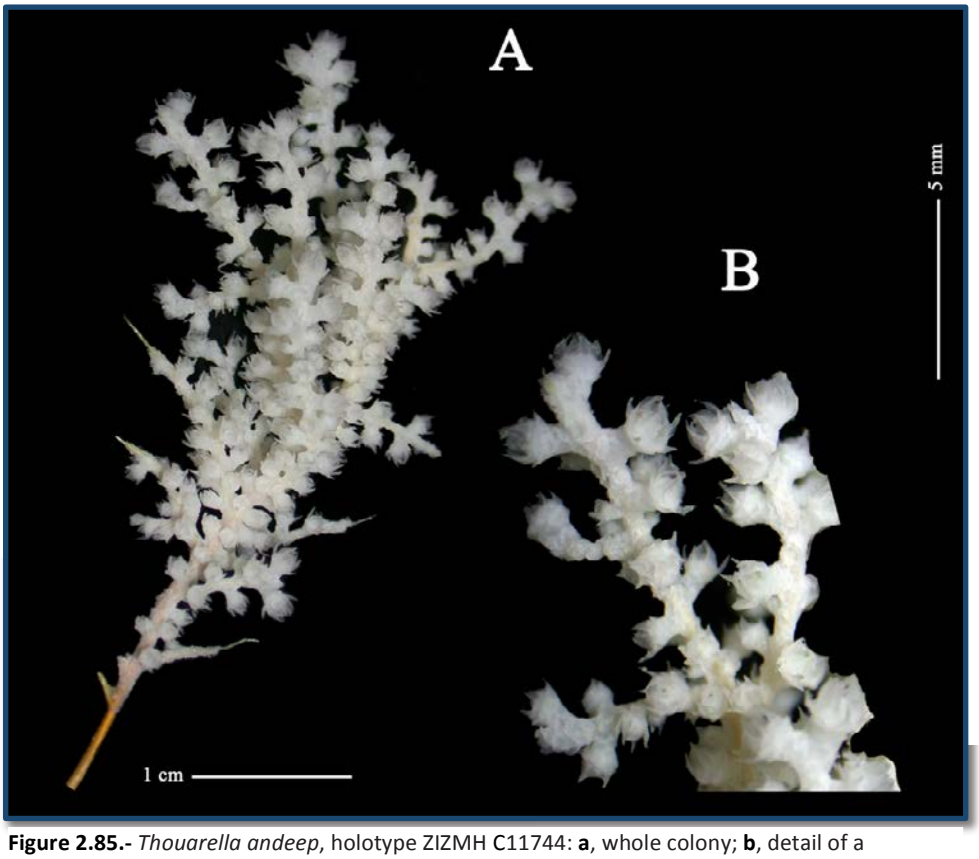


Figure 2.85.- *Thouarella andeep*, holotype ZIZMH C11744: **a**, whole colony; **b**, detail of a branchlet.

Description of the holotype

Fragment of a bottlebrush colony (Fig. 2.85a), without holdfast, 5.9 cm in height and 3.2 cm in width; branchlets stiff, in acute angles, simple or bifurcate on the base, bent towards one side, about 1-2.2 mm in length. Axis bronze in colour, stiff, basal axis diameter of 0.5 mm, and 11 mm height until the first branchlets. Colour of colony light pink. Polyps almost perpendicular to stem (Fig. 2.85b), singly placed, present on main stem and branchlets, arranged in a spiral, with no tendency toward pairs or whorls, in clumps on branchlet tips, 10-11 polyps per cm.

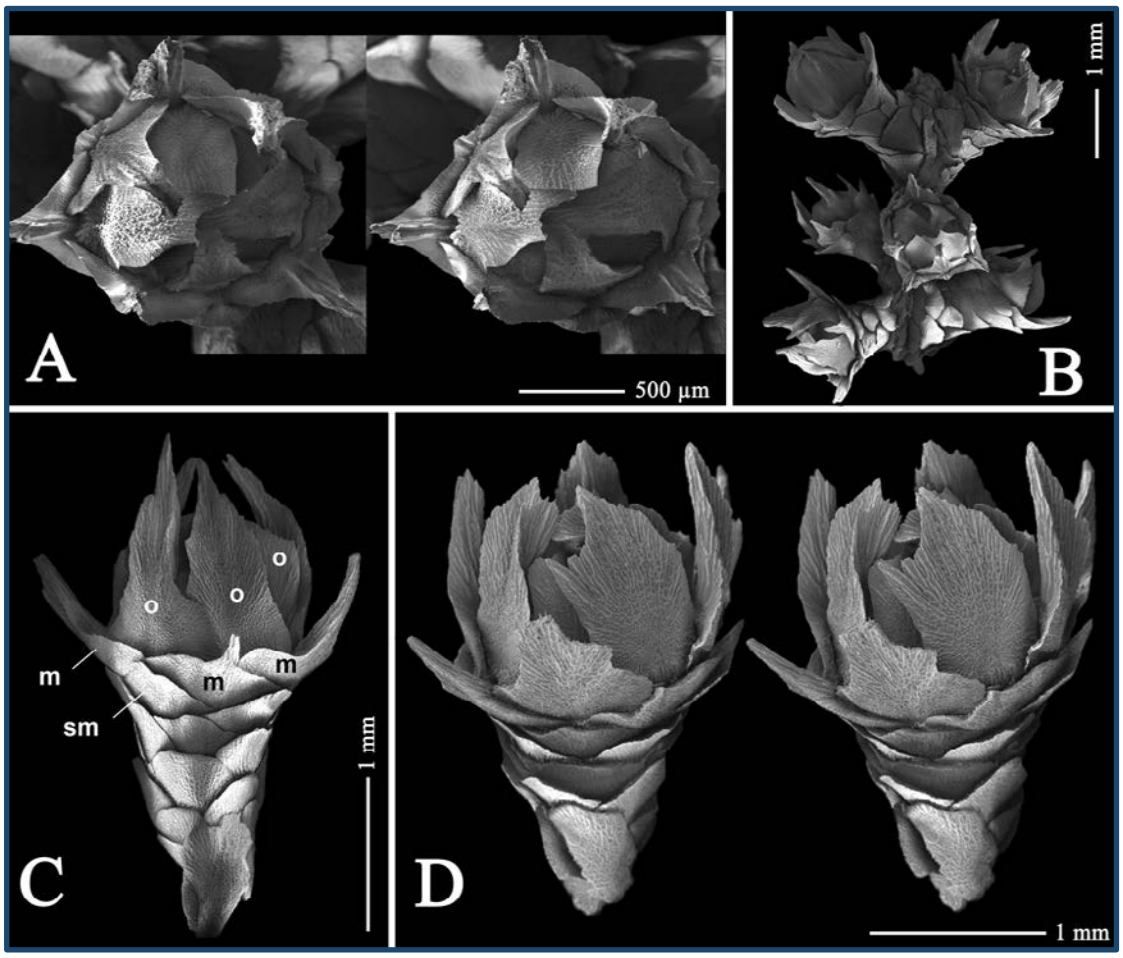


Figure 2.86.- *Thouarella andeep*, holotype ZIZMH C11744: **a**, polyps in oral view, stereo pair; **b**, detail of branchlet; **c**, polyps in latero-abaxial view; **d**, polyps in adaxial view, stereo pair. Abbreviations: **o**, opercular scales; **m**, marginal scales; **sm**, submarginal scales.

Polyps (Fig. 2.86) relatively short, club-shaped; about 1.9-3.4 mm in height and 0.70-0.95 mm in diameter. Polyp body with 7 longitudinal rows of scales, 4-5 scales on each longitudinal abaxial row overlapping one another. Accessory opercular scales (Fig. 2.87a) in numbers of five-six, small, higher than broad, 0.24-0.42 x 0.07-0.30 mm. Proximal half of inner surface tuberculate, distal inner surface smooth, without keel. Outer surface quite smooth. Basal margin with a notch medially, free margin smooth. Opercular scales, 0.51-1.1 x 0.34-0.52 mm, arranged in two alternate cycles of four scales: inner cycle (Fig. 2.87b) with rounded tips and square base. Inner surface like that in accessory opercular scales; outer surface with radial granules from nucleus on proximal portion. Free margin quite straight. Outer cycle (Fig. 2.87c)

elongated distinctly concave longitudinally, with rhombus-base-shape and truncated or pointed tips. Proximal half of inner surface tuberculate, distal inner surface with longitudinal ridges, without becoming a distinct keel; outer surface with radial smooth granules from nucleus on proximal portion. Free margin finely serrated. Marginal scales (Fig. 2.87d) eight in number, basal part of scale diamond-shaped, 0.95-1.32 x 0.74-0.99 mm (including thorn), thorn about two third (or less) of total sclerite length. Thorn with numerous longitudinal ridges on all sides. Inner surface tuberculate covering part of thorn base, with distal smooth areas. Outer surface covered totally by granules. Free margin finely serrated, proximal margin with digitate processes. Body scales (Fig. 2.87e), fan-shaped, with tendency to square or oval shape, 0.54-0.84 mm in maximum length. Inner surface completely tuberculate, outer surface covered with smooth granules. Free margin finely serrated, basal margin with digitate processes. Coenenchymal scales (Fig. 2.88) more diverse in shape, from circular to irregular elongated ovals, 0.16-0.93 mm in maximum length. Surface with similar characteristics to body scales. In some scales granules as prominent wrinkle-like. Margin finely serrated, irregular with warts proximally.

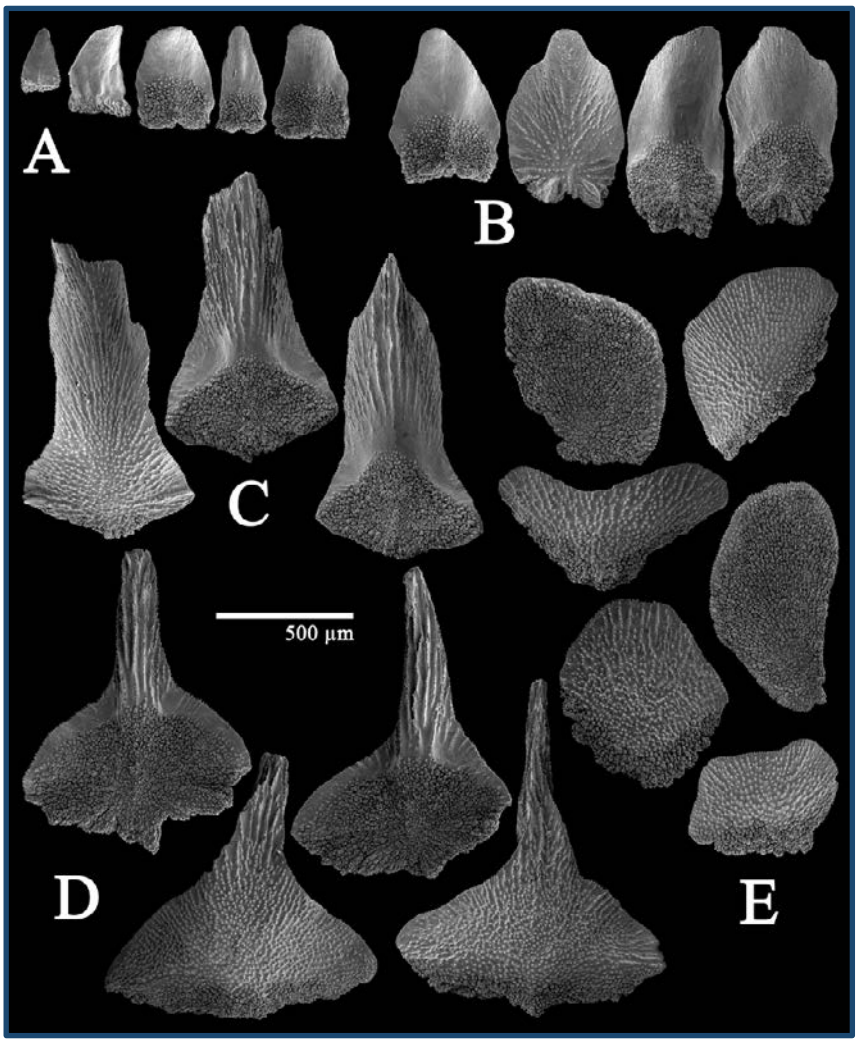


Figure 2.87.- *Thouarella andeep*, holotype ZIZMH C11744: **a**, accessory opercular scales; opercular scales from inner (**b**) and outer (**c**) alternate cycle; **d**, marginal scales; **e**, body scales.

Variations from the holotype

The general colonial structure of the paratypes and additional examined material is quite similar to that of the holotype. Colonies are more flattened in shape, from 3 to 8.5 cm in height and from 3 to 5.6 cm in width. Branchlets reaching up to 2.8 cm in length. Colonies do not present a holdfast like the holotype. The number of polyps per centimetre can reach up to 12 on branchlets. The polyp and sclerite form and distribution are as in the holotype. Accessory opercular scales are small from 3 to 8 (usually 4) in number.

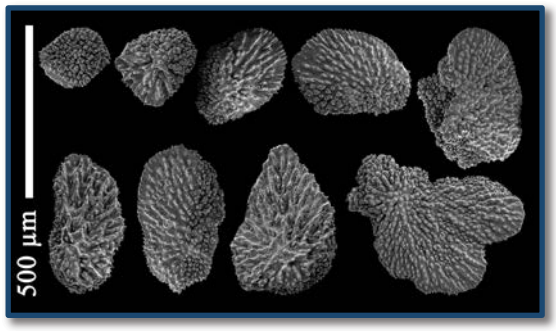


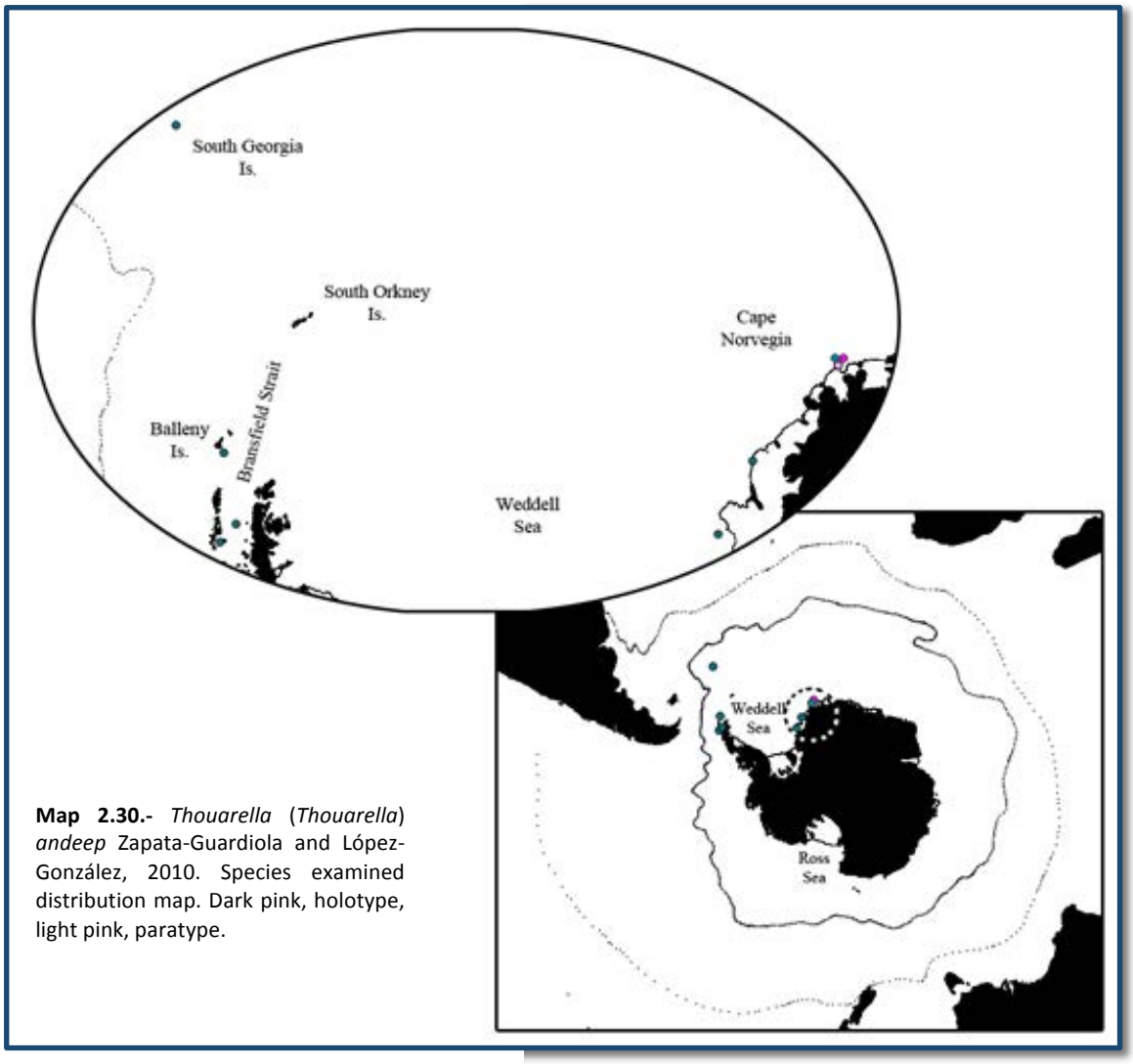
Figure 2.88.- *Thouarella andeep*, holotype ZIZMH C11744: Coenenchymal scales.

Geographical and bathymetrical distribution

At present, *Thouarella andeep* is known only from off Atka Bay, Antarctica (Map 2.30), 601.8 m in depth.

Etymology

The species name is dedicated to people belonging to the project Andeep-Systco, as thanks to their insistence we finally had an extra station, which was the locality where the new species was found. Name considered as a noun in apposition.



Map 2.30.- *Thouarella* (*Thouarella*) *andeep* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Thouarella (*Thouarella*) *antarctica* (Valenciennes, 1846)

(Figures 2.89-2.92)

Primnoa antarctica Valenciennes, 1846:pl.12, figs. 2, 2a (no text, only images).—Milne Edwards, 1857:140.—Gray, 1857:286; 1859:483.—Kölliker, 1865:135.—Bayer, 1956:F220 (list).
Thouarella antarctica, Gray, 1870:69.—?Wright and Studer, 1889:65–66, pl. 21 fig.1.—Thomson and Henderson, 1906:38 (list).—Gravier, 1914:48–56, pl. 7 figs. 31–34, pl.10 figs. 52–55.—Molander, 1929:75.
Thouarella (*Thouarella*) *antarctica,* Cairns and Bayer, 2009:27 (listed), 33–34, fig. 6, g–l.
Thouarella (*Parathouarella*) *antarctica*, Kükenthal, 1915:150 (key); 1919:433–435; 1924:299.
not *Thouarella antarctica,* Hickson, 1907:9–10, pl.2, figs. 19, 24.
not *Thouarella* (*Euthouarella*) *antarctica,* Broch, 1965:24–26, pl. 2, figs. 5–7. (= *Thouarella pendulina*).

Type material: MNHN.Oct.0000-208, Falkland Islands, Subantarctica.

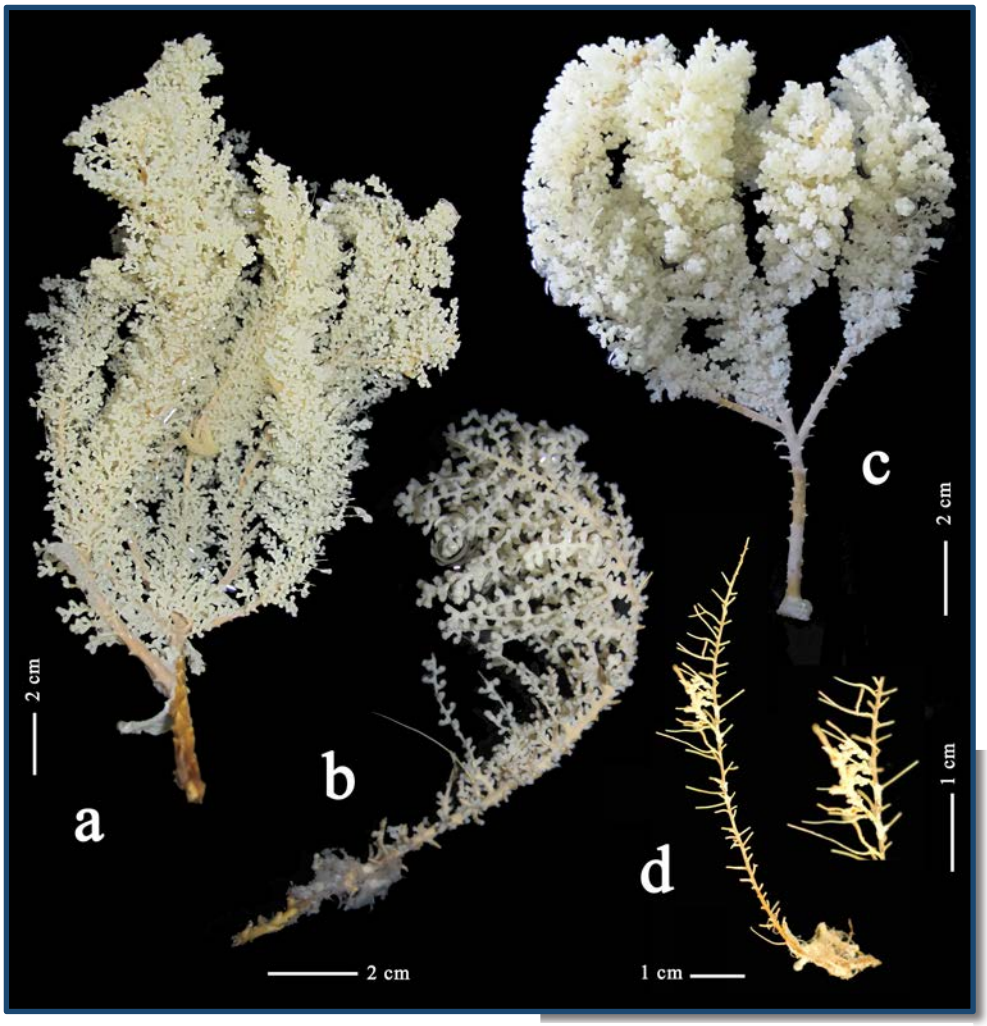


Figure 2.89.- Bottlebruch colony shape variability in *Thouarella antarctica*: **a**, US 1327; **b**, US 1660; **c**, US 1349; **d**, type NMNH-Oct-0000-208, photo: A. Andouche MNHN.

Examined material

Additional material: US 1327, US 1349, US 1387, US 1399, US 1592, US 6244, US 6245, US 6246, US 6247, ANT XIX-5, stn PS61/145-01, 54°1.36'S, 62°1.3'W, west Burdwood Bank, Subantarctica, 272 m depth, 05 April 2002, one colony each; US 1371, ANT XIX-5, stn PS61/150-01, 54°29.63'S, 56°08.13'W, east Burdwood Bank, Subantarctica, 286-290 m depth, 06 April 2002; US 1660, US 1381, US 6063, ANT XIX-5, stn PS61/164-01, 53°23.816'S, 42°42.46'W, north west South Georgia, Antarctica, 312-322 m depth, 09 April 2002, one colony and 9 colonies and 4 fragments.

Figure 2.90.- *Thouarella antarctica*, US 1660, detail of a branchlet, stereo pair.

Description of the additional material

Bottlebrush colonies (Fig. 2.89), with a holdfast, divided basally giving up to 5 main side branches of up to 20 cm height and 3,5 cm width each, giving a total colony height of 25 cm and a total width of 11.5 cm. Branchlets stiff, perpendicular to stem, simple or bifurcate on the base, up to 3.2 cm in length. Axis light brown in colour, stiff, basal axis diameter of 0.15 mm. Polyps inclined upward but almost perpendicular to stem (Fig. 2.90), singly placed, present on main stem and branchlets, arranged in a spiral, with no tendency toward pairs or whorls, in clumps on branchlet tips, 9-12 polyps per cm. Polyps (Fig. 2.91) relatively elongated, cylindrical; about 2.1-2.7 mm in height and 0.70-0.93 mm in diameter. Polyp body with 8 longitudinal rows of scales, 5 scales on each longitudinal abaxial row and 3 scales on adaxial row. Opercular scales large (Fig. 2.92a), eight in number, 0.41-0.70 x 0.23-0.55 mm, isosceles shape with bilobed base and acute tip. Proximal inner surface tuberculate, covering more than one third of their length. Prominent, strong, apical medial keel bearing lateral ridges. Distal inner surface of scale quite smooth. Outer surface granulate. Basal margin with digitate

processes, free margin finely serrated. Marginal scales (Fig. 2.92b) eight in number, pentagonal-shaped, 0.51-0.68 x 0.60-0.72 mm. Distal inner surface with a central multi-keel tuft, lateral inner surface smooth, without granules or ridges. Proximal inner surface tuberculate covering more than a half of scale length. Outer surface granulate forming blunt ridges distally. Free margin finely serrated, proximal margin with digitate processes. Body scales (Fig. 2.92c), fan-shaped, 0.67-0.85 x 0.38-0.56 mm. Inner surface completely tuberculate, a very reduced central tuft may be present, outer surface covered with granules forming ridges. Free margin finely serrated, basal margin with digitate processes. Coenenchymal scales (Fig. 2.92d) in two layers: outer layer with round to oval shaped sclerites, 0.17-0.53 mm in maximum length, inner surface tuberculate, outer surface granular, free margin scalloped; inner layer with irregular tuberculate sclerites, 0.04-0.1 mm in maximum length.

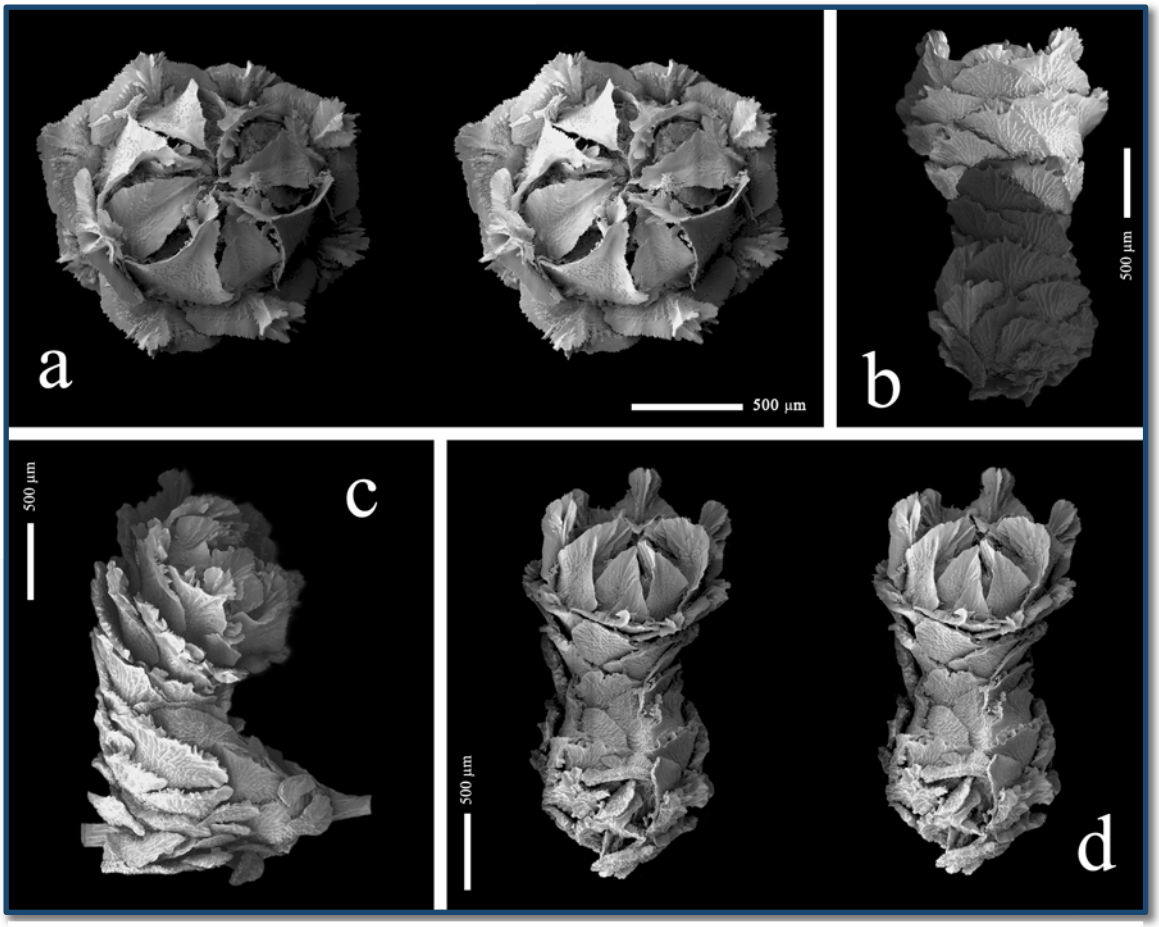


Figure 2.91.- *Thouarella antarctica,* US 1660: **a**, polyp on oral view, stereo pair; **b**, polyp on abaxial view; **c**, polyp on outer lateral view; **d**, polyp on adaxial view, stereo pair.

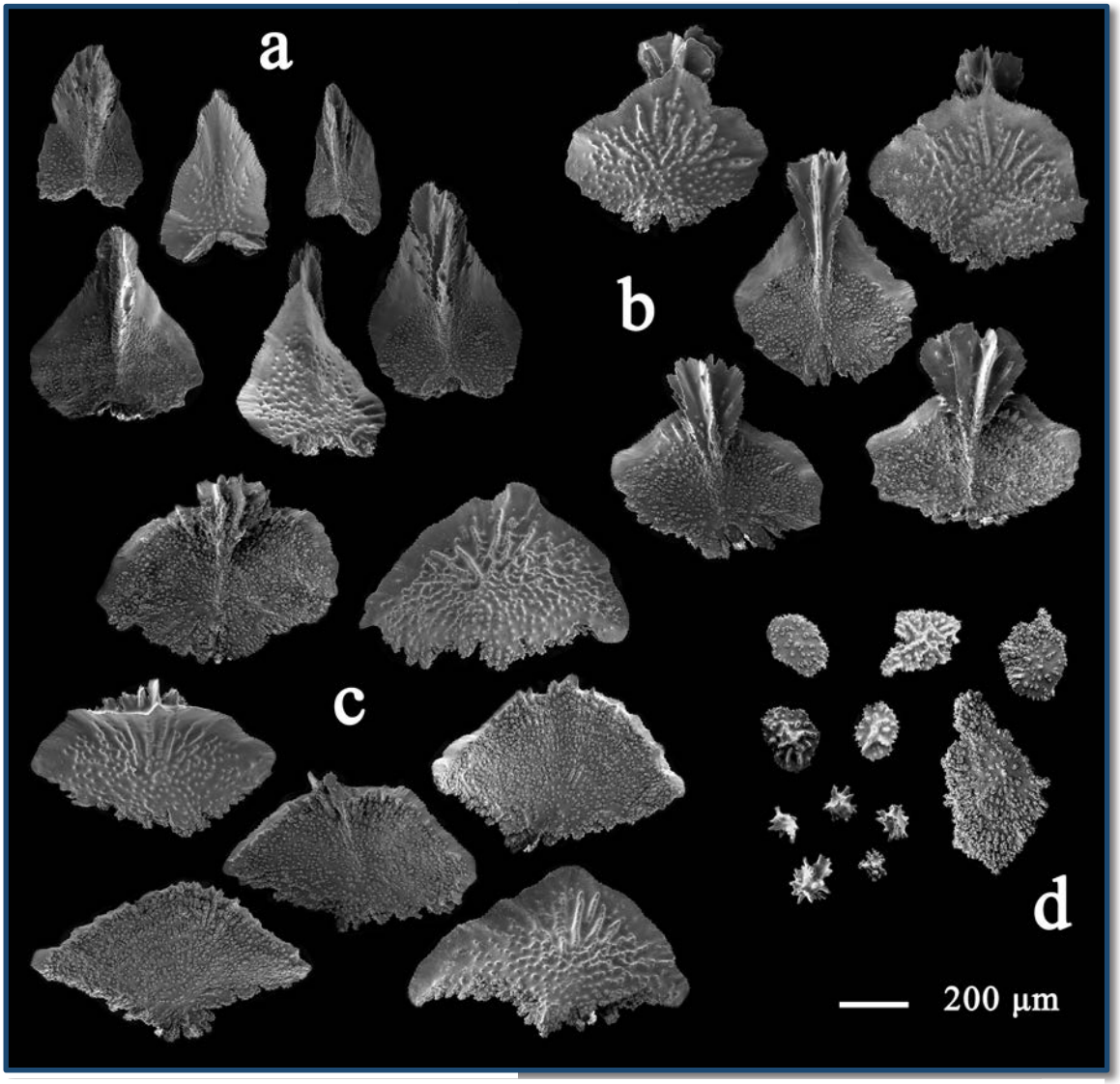


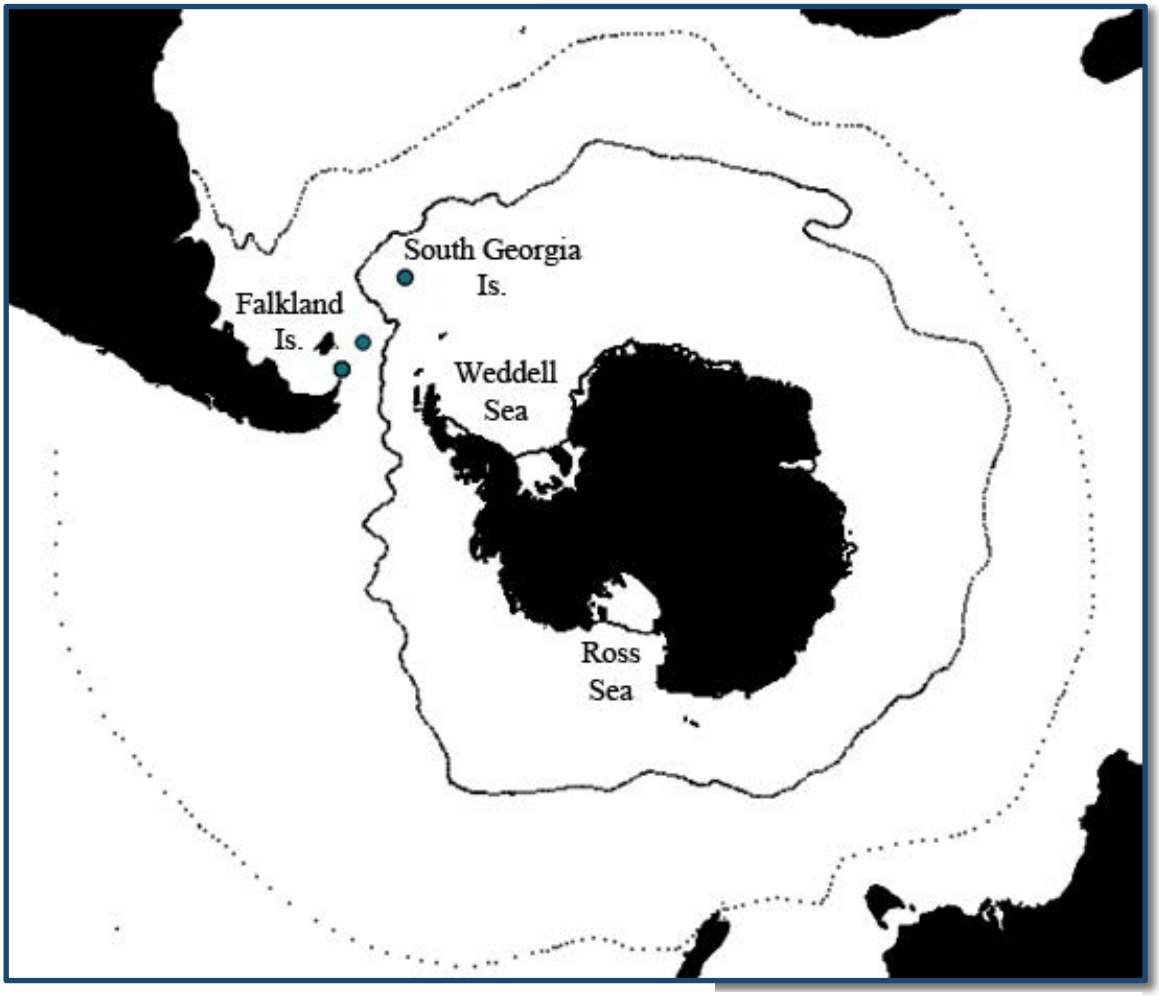
Figure 2.92.- *Thouarella antarctica,* US 1660: **a**, opercular scales; **b**, marginal scales; **c**, body Wall scales; **d**, coenenchymal scales.

Geographical and bathymetrical distribution

At present, *Thouarella antarctica* is known from Antarctic and Subantarctic waters. Latitudinally from north of the Falkland Islands to the south tip of South America and longitudinally from Burdwood Bank to South Georgia (Map 2.31), between 200-480m in depth. A sample from Crozet Island (1005 m depth) was described by Wright and Studer (1889) but I did not have the possibility to examine it.

Etymology

The specific name *antarctica* refers to the geographic area where the species was found.



Map 2.31.- *Thouarella* (*Thouarella*) *antarctica* (Valenciennes, 1846). Species examined distribution map.

Thouarella (*Thouarella*) *brucei* Thomson and Ritchie, 1906

(Figures 2.93–2.97)

Thouarella brucei Thomson and Ritchie, 1906:852–854, pl. 1, fig.1, pl. 2, fig. 1.—Kükenthal, 1919:439; 1924: 301.
not *Thouarella brucei*, Broch, 1965:27–28, pl. 4, figs. 11–13.

Examined material

Lectotype: NMS.Z.1921.143.1298 "Scottish Antarctic Expedition (1902-1904), Burdwood Bank, Gough Island (St. Helena)", one colony.

Paralectotypes: NMS.Z.2010.038.1 and BM 1912.11.9.2, Scottish Antarctic Expedition (1902-1904), stn. 346, 54°25'S, 57°32'W, Burdwood Bank, 102.4 m depth, 1 December 1903, one colony each; NMS.Z.2010.038.2, same data as in the lectotype; ZMA COEL3574, Scottish Antarctic Expedition (1902-1904), South Atlantic Ocean, fragment of a colony.

Additional material: US 6132, ANT XIX/5, stn PS61/164-01, 53°23.82'S, 42°42.47'W, north west South Georgia, Antarctica, 312-322 m depth, 9 April 2002; ZMH C11748, ANT XIX/5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, west of South Georgia Island, 308.1–334.5 m depth, 9 April 2002, five fragments. US 1310, ANT XIX/5, stn PS61/214-01, 59°42.62'S, 27°57.68'W, south South Sandwich Islands, Antarctica, 332-340 m depth, 17 April 2002.

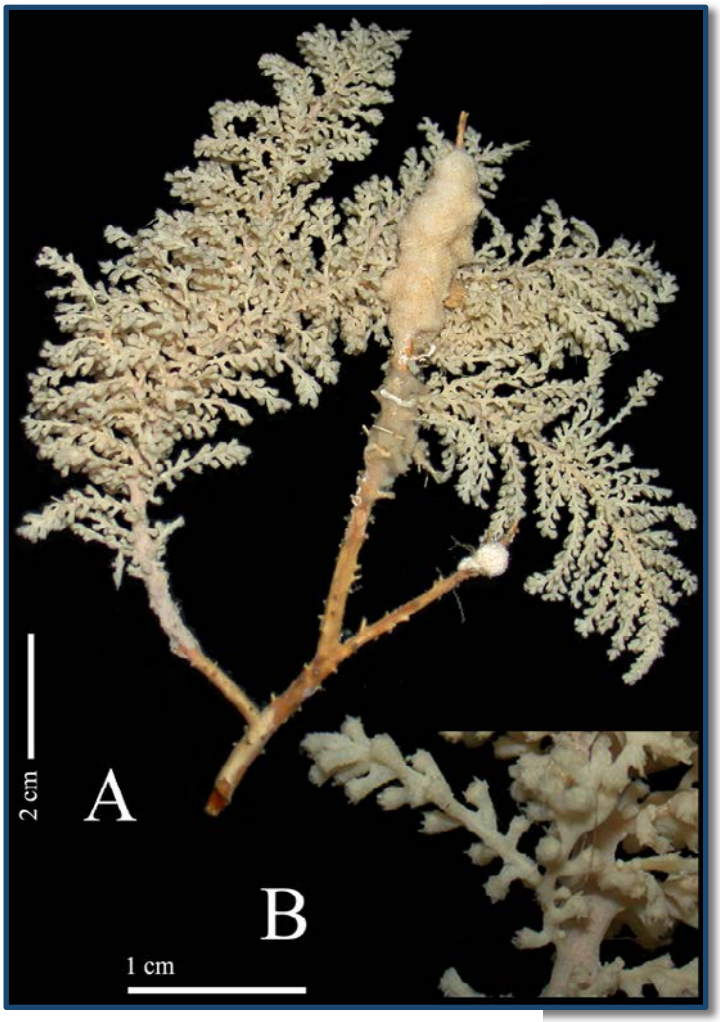


Figure 2.93.- *Thouarella brucei* Thomson and Ritchie, 1906, lectotype (NMS.Z.1921.143.1298): **a**, whole colony; **b**, detail of a branchlet.

Description of the holotype

The specimen is only a fragment of the parent colony (Fig. 2.93a), 11 cm in total height and about 8.6 cm in width. Main stem ramified up to third order giving simple branchlets (Fig. 2.93b) up to 2 cm in length, distributed all around, up to 8 branchlets per centimetre. Axis brown, broken at its proximal portion, without holdfast. Basal axis diameter 2.8 mm.

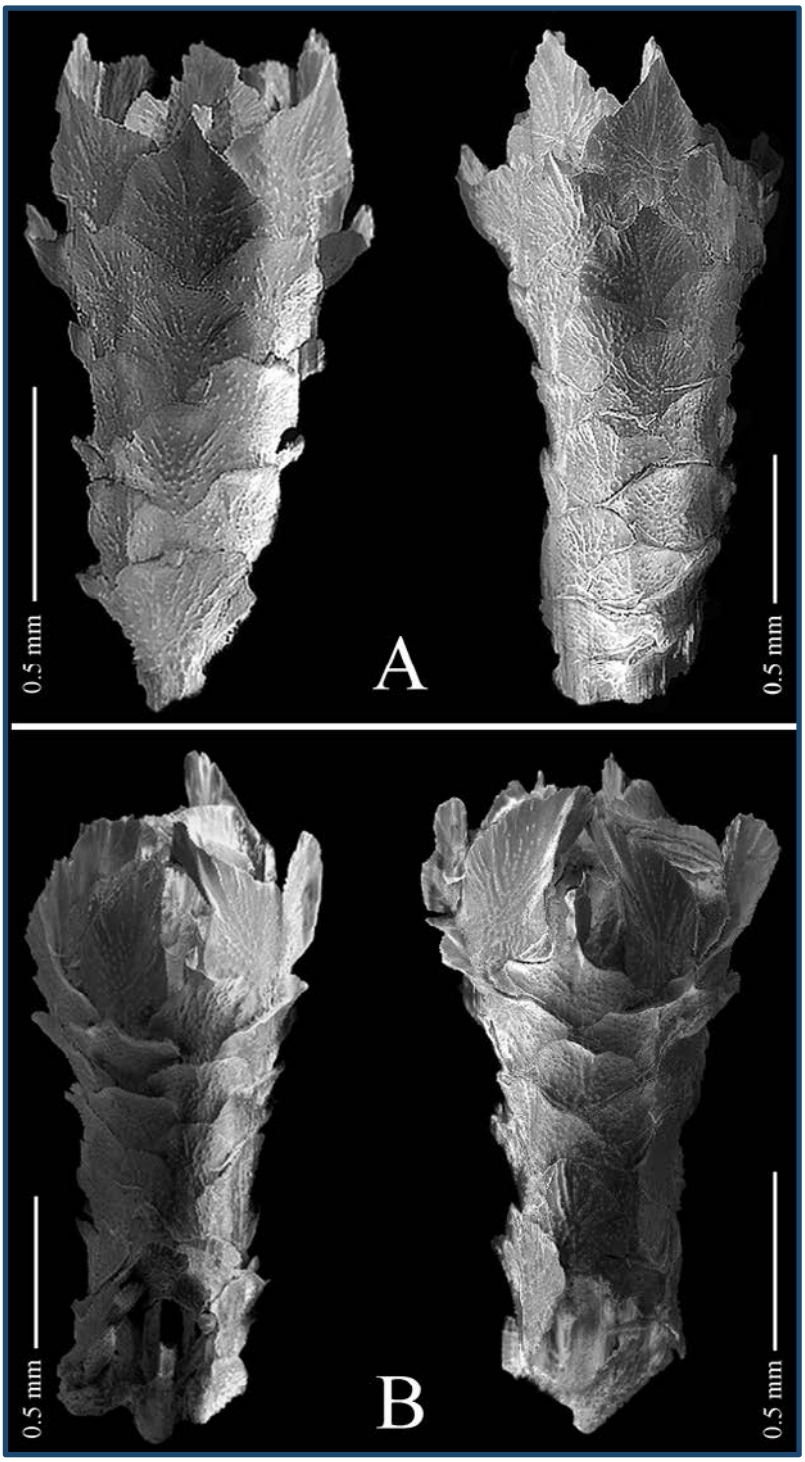


Figure 2.94.- *Thouarella brucei* Thomson and Ritchie, 1906, lectotype (NMS.Z.1921.143.1298): **a**, polyps, abaxial view; **b**, polyps, adaxial view.

Individual polyps (Fig. 2.93b) slightly bent upward and arranged in spirals around branchlets; 10–12 polyps per cm. Polyps also present on main stem. Polyps (Fig. 2.94) clavate, about 1.5–2.1 mm in height and 0.6–0.8 mm in diameter with a low operculum. Polyp body with 8 longitudinal rows of scales overlapping one another, 4–5 scales on each longitudinal abaxial row (Fig. 2.94a) and 3 scales on each adaxial row (Fig. 2.94b).

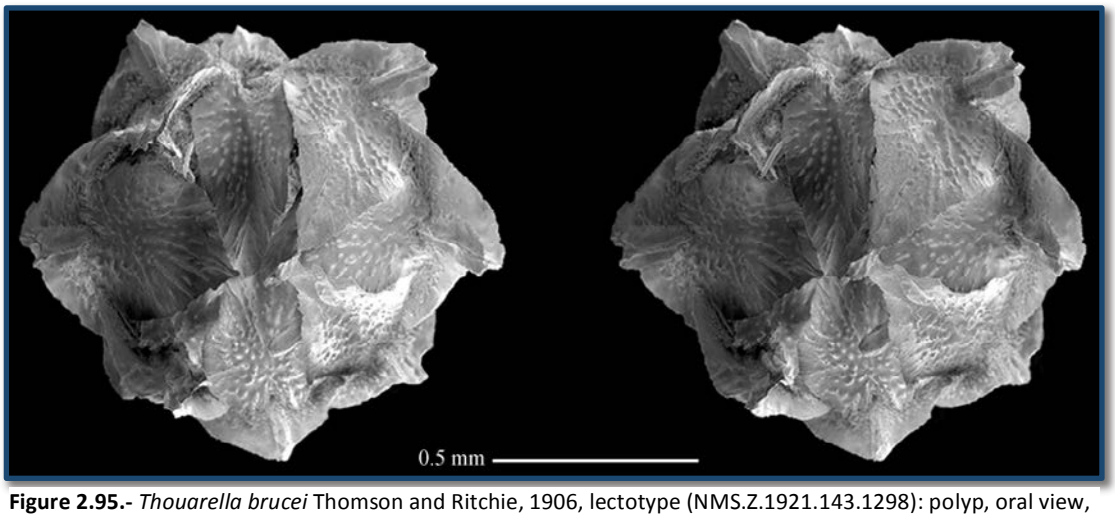


Figure 2.95.- *Thouarella brucei* Thomson and Ritchie, 1906, lectotype (NMS.Z.1921.143.1298): polyp, oral view, stereo pair.

Opercular scales (Figs. 2.95, 2.96a), 0.38–0.66 mm in height and 0.23–0.50 mm in width, arranged in two cycles of four: inner cycle concave isosceles-shaped with bilobed base and rounded tip with an incipient keel; outer cycle larger, isosceles-triangle-shaped with distal inner surface multi-keeled. Proximal inner surface tuberculate, covering about half of their length. Outer surface radially granular. Basal margin irregular. Free margin finely serrated. Marginal scales (Fig. 2.96b) eight in number, 0.41–0.66 mm in height and 0.47–0.54 mm in width, roughly rhomboidal in shape with a complex tip; adaxials reduced. Inner proximal surface tuberculate, covering about half of their length, distal surface smooth with medial process multi-keeled. Outer surface granular. Basal margin with small granular processes, free margin finely serrated. Body scales (Fig. 2.97a) irregular-fan shaped, 0.28–0.49 mm in height and 0.32–0.50 mm in width. Inner surface almost completely tuberculate, upper body scales with short keel. Outer surface granular. Free margin finely serrated. Coenenchymal scales (Fig. 2.97b) roughly round to oval-shaped, 0.14–0.43 mm in maximum length; inner surface tuberculate, outer surface granulate forming ridges, free margin irregular.

Variations from holotype

The paralectotypes and the additional material examined have a similar bottlebrush colonial structure to that of the lectotype. The main stem can be unbranched or ramified up to third order. The polyps have a wider range in size, from 1.3 to 2.4 mm in height and from 0.46 to 0.84 mm in diameter. The opercular scales can vary from 0.28 to 0.77 mm in height and from 0.10 to 0.50 mm in width. The marginal scales vary from 0.42 to 0.78 mm in height and from 0.34 to 0.58 mm in width. The body scales vary from 0.24 to 0.54 mm in height and from 0.28 to 0.67 mm in width. The coenenchymal scales vary from 0.06 to 0.46 mm in maximum length. Distribution and form of the sclerites from polyps and coenenchyme are similar to that of the lectotype.

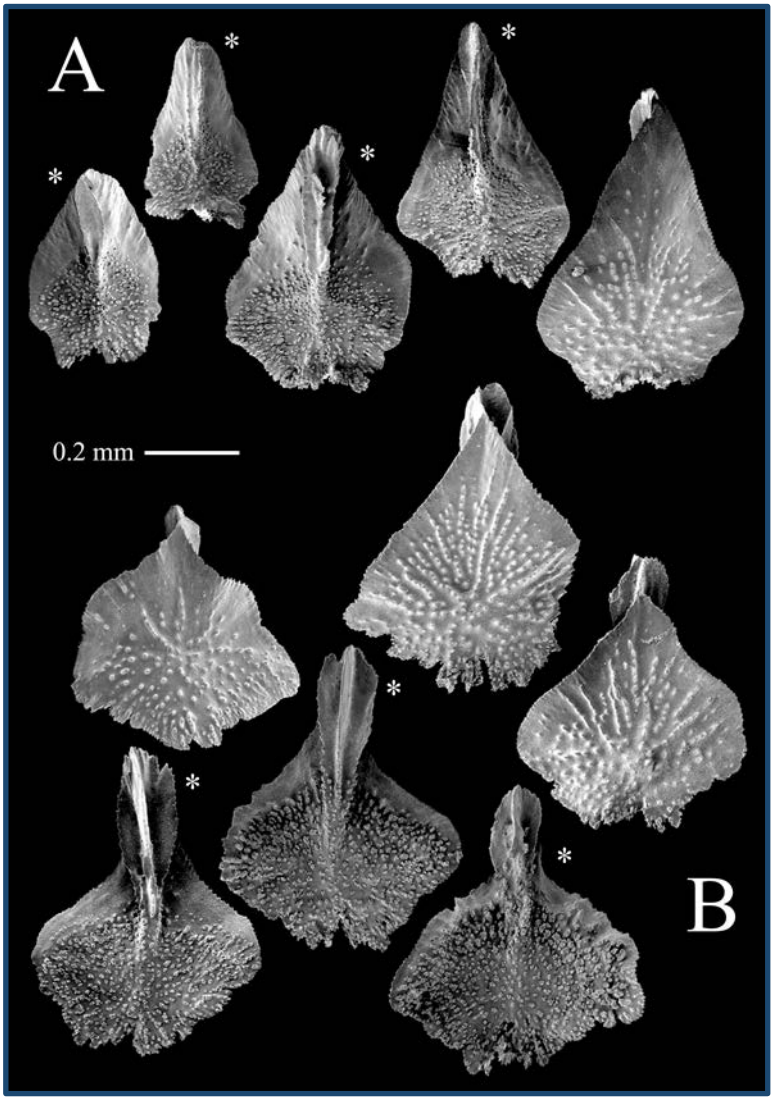


Figure 2.96.- *Thouarella brucei* Thomson and Ritchie, 1906, lectotype (NMS.Z.1921.143.1298): **a**, opercular scales; **b**, marginal scales. * inner surface view.

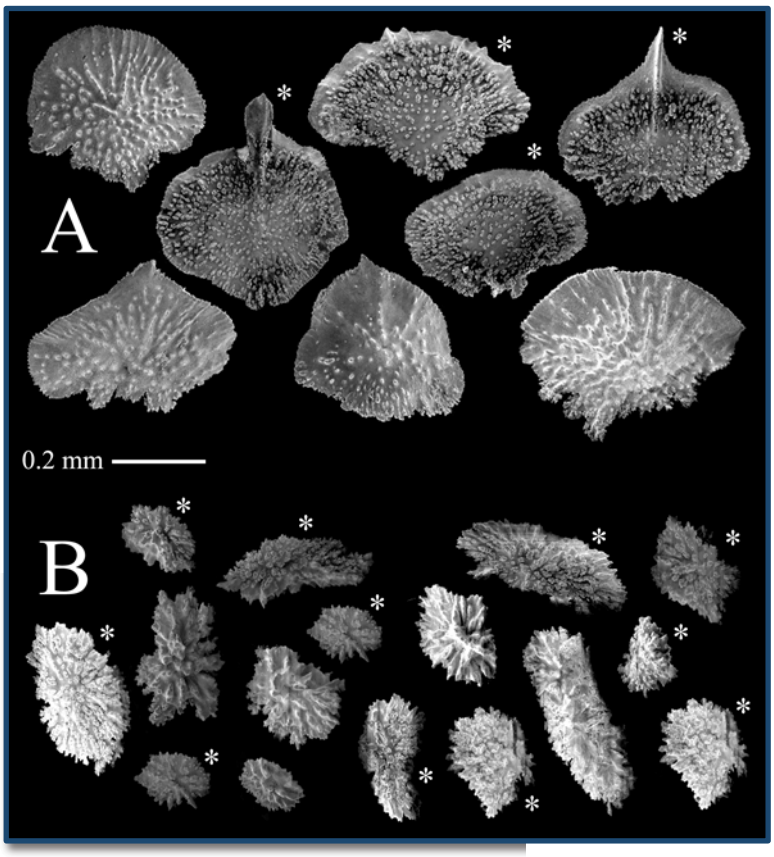


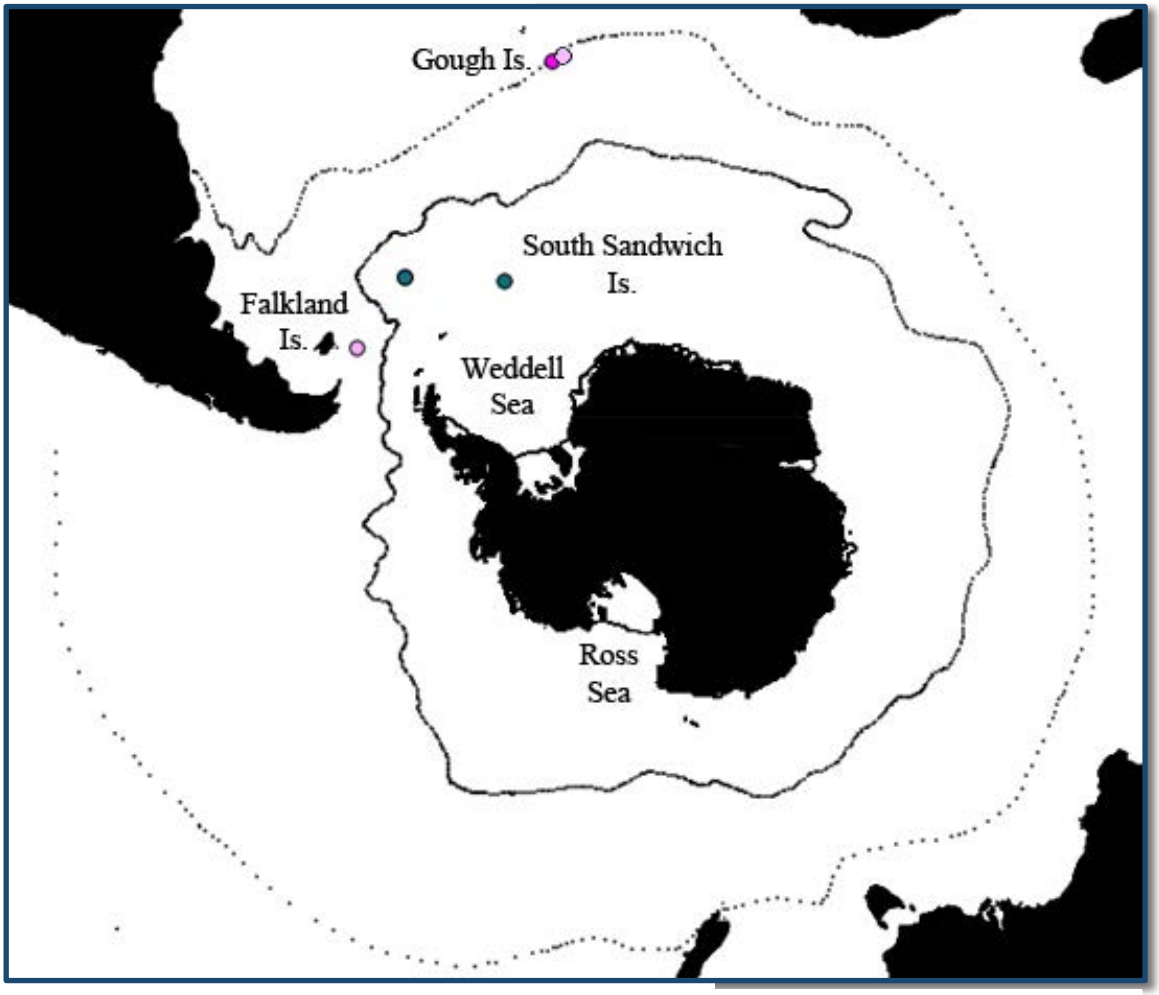
Figure 2.97.- *Thouarella brucei* Thomson and Ritchie, 1906, lectotype (NMS.Z.1921.143.1298): **a**, body scales; **b**, coenenchymal scales.
***** inner surface view.

Geographical and bathymetrical distribution

The species is known from Burdwood Bank and Gough Island (Saint Helena), Subantarctica, and from west of South Georgia Island, (Map 2.32), between 100 and 334.5 m in depth.

Etymology

The species name *brucei* was chosen in honour of Mr. W. S. Bruce, the leader of the *Scotia* expedition, from which this species was collected.



Map 2.32.- *Thouarella* (*Thouarella*) *brucei* Thomson and Ritchie, 1906. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Thouarella (*Thouarella*) *minuta* Zapata-Guardiola and López-González, 2010c

(Figures 2.98-2.100)

Thouarella (*Thouarella*) *minuta* Zapata-Guardiola and López-González, 2010c:177.

Examined material

Holotype: ZIZMH C11742, ANT XXI-2, stn PS65-166-01, 70°56.83'S, 10°32.61'W, Austasen, Antarctica, 253.2–338.0 depth, 15 December 2003.

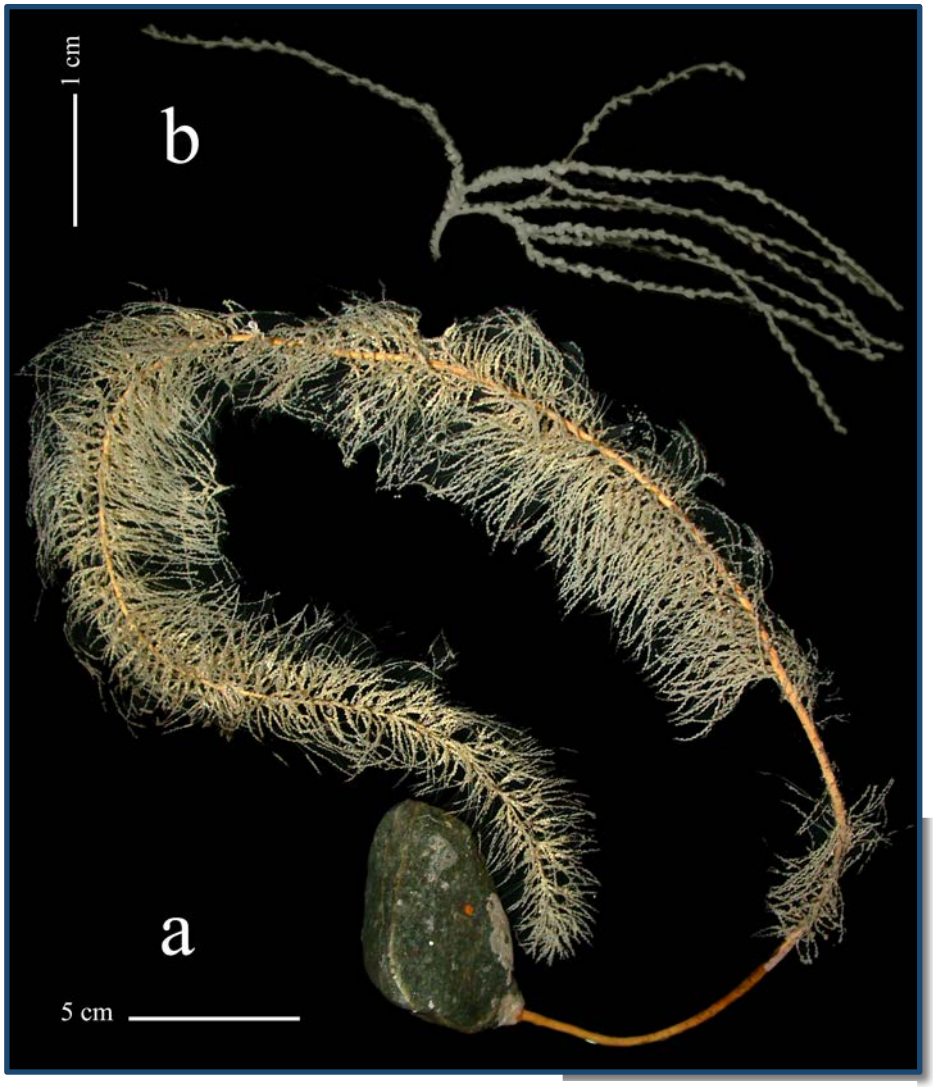


Figure 2.98.- *Thouarella minuta*, holotype ZIZMH C11742: **a**, whole colony; **b**, detail of branchlets.

Paratypes: ZIZMH C11743, USNM 1128948 and CRO-0042, with the same sampling data as the holotype, five fragmented colonies and one fragment, four colonies and one colony, one broken colony and two naked stems, respectively; CRO-0031, ANT XVII-3, stn 085-01, 71°11.30'S, 12°15.40'W, Austasen, Antarctica, 309–318 m depth, 2 April 2000, two colony fragmented; CRO-0032, ANT XVII-3, stn 119-01, 70°50.40'S, 10°35.20'W, Austasen, Antarctica, 226–266 depth, 7 April 2000, one colony and two fragments; CRO-0033, ANT XXI-2, stn PS65-174-01, 70°56.57'S, 10°31.86'W, Austasen, Antarctica, 253.2–296.0 m depth, 16 December

2003, four fragmented colonies and two fragments; CRO-0034, ANT XXI-2, stn PS65-175-01, 70°56.52'S, 10°31.78'W, Austasen, Antarctica, 288.8–337.2 m depth, 16 December 2003, two colonies without holdfast; CRO-0035, ANT XXI-2, stn PS65-265-01, 70°52.74'S, 10°52.72'W, Austasen, Antarctica, 285.6–294.8 m depth, 27 December 2003, four colonies, one of them broken; CRO-0036, ANT XXI-2, stn PS65-274-01, 70°52.16'S, 10°43.69'W, Austasen, Antarctica, 288.0–290.8 m depth, 28 December 2003, one colony and four fragmented colonies; CRO-0037, ANT XXI-2, stn PS65-292-01, 72°51.43'S, 19°38.62'W, Austasen, Antarctica, 596.4–597.6 m depth, 31 December 2003, one colony without holdfast.

Additional material: US 490, ANTXVII-3, stn 85-01, 71°12.2'S, 12°19'W, Cape Norvegia, Antarctica, 309-318 m depth, 2 April 2000, one colony; US 332, ANTXVII-3, stn 102-01, 71°11.44'S, 12°19.2'W, Cape Norvegia, Antarctica, 312-323 m depth, 3 April 2000, one colony; US 456 and US 6430, ANT XVII-3, stn 109-01, 71°11.9'S, 12°20.7'W, Cape Norvegia, Antarctica, 311-316 m depth, 4 April 2000, one colony and one fragment; US 479, US 149 and US 437, ANTXVII-3, stn 119-01, 70°51.2'S, 10°35.1'W, Austasen, Antarctica, 226-266 m depth, 7 April 2000, one colony fragmented and two colonies; US 212, ANT XVII-3, stn 124-01, 70°50.6'S, 10°35.4'W, Austasen, Antarctica, 247 m depth, 9 April 2000; US 2854, ANTXXI/2, stn PS65/121-01, 70°50.08'S, 10°34.76'W, Austasen, Antarctica, 268-274 m depth, 11 December 2003, 11 colonies, three fragmented.

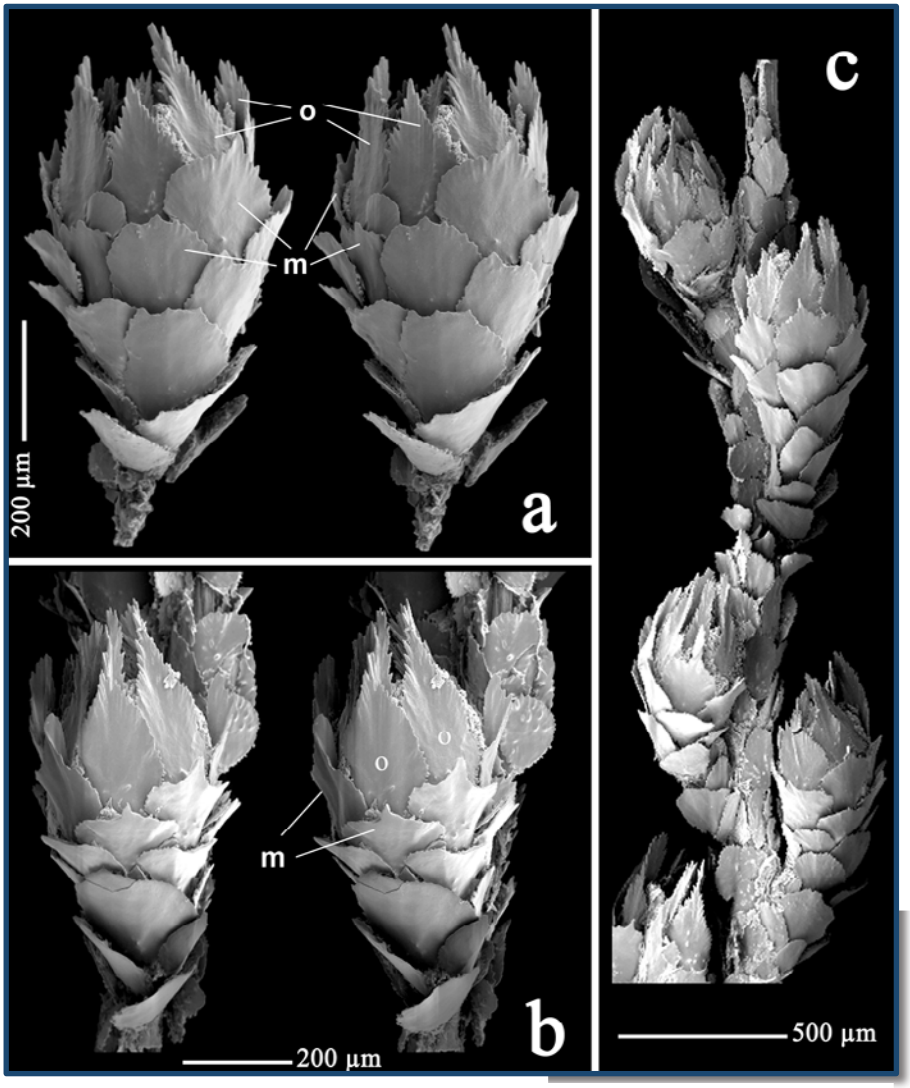


Figure 2.99.- *Thouarella minuta*, holotype ZIZMH C11742: **a**, polyps in lateral abaxial view, stereo pair; **b**, polyps in abaxial view, stereo pair; **c**, detail of branchlets. Abbreviations: **o** opercular scales; **m** marginal scales.

Description of the holotype

Bottlebrush colony (Fig. 2.98a) of 66 cm in height and 5.5 cm in width, main stem without lateral branches. First 9 cm of the main stem without branchlets, on the remain main stem branchlets all around, up to 4.5 cm long, simple at basal portion or after the first 5 mm ramified up to fourth order (usually 2, exceptionally 3 terminal branchlets starting from the same point), terminal branchlets up to 3.5 cm long (Fig. 2.98b). Axis light brown in colour, stiff and firmly attached to a rock by a greyish, calcareous, discoidal holdfast of 1.3 cm diameter, basal axis diameter of 3 mm. Polyps appressed to main stem and branchlets (Fig. 2.99c), directed upward, arranged singly, apparently alternate to loosely spiral, 11–18 polyps per cm.

Polyps (Fig. 2.99) small, cone shaped; about 0.71–0.96 mm in height and 0.31–0.44 mm in width at marginal scale level. Polyp body with 5 longitudinal rows of scales, 3–4 scales (excluding operculars) on each longitudinal abaxial row (Figs. 2.99a,b) overlapping one another. Opercular scales (Fig. 2.100a) eight in number, small, 0.25–0.43 mm x 0.07–0.16 mm, isosceles shaped or spoon shaped, adaxial scales reduced. Proximal inner surface slightly tuberculate, covering less than half of the length (about 2/3 of the basal part), with an apical serrated spine as a continuation of well-developed keel. Outer surface quite smooth. Basal margin with digitate processes, with tendency to square shape, free margin strongly serrated. Marginal scales (Fig. 2.100b) in numbers of eight, small, 0.21–0.28 mm x 0.16–0.23 mm, round to rhomboidal shaped, adaxials reduced. Proximal inner surface tuberculate, covering up to about 75% of their longitude. Distal inner surface smooth, with prominent keel projecting to form a short spine distally. Outer surface smooth. Basal margin with digitate processes, free margin serrated. Body scales (Fig. 2.100c) circular shaped, like marginal scales in size, 0.22–0.28 mm in maximum length, without keel or spine. Adaxials with tendency to be reduced. Inner surface tuberculate, outer surface smooth. Free margin quite entire, basal margin irregular due to the presence of tubercles and processes. Coenenchymal scales (Fig. 2.100d) round to oval shaped, 0.12–0.22 mm in maximum diameter. Inner surface sparsely tuberculate, outer surface smooth with some granules or short ridges. Irregular margin due to the presence of tubercles and serrations.

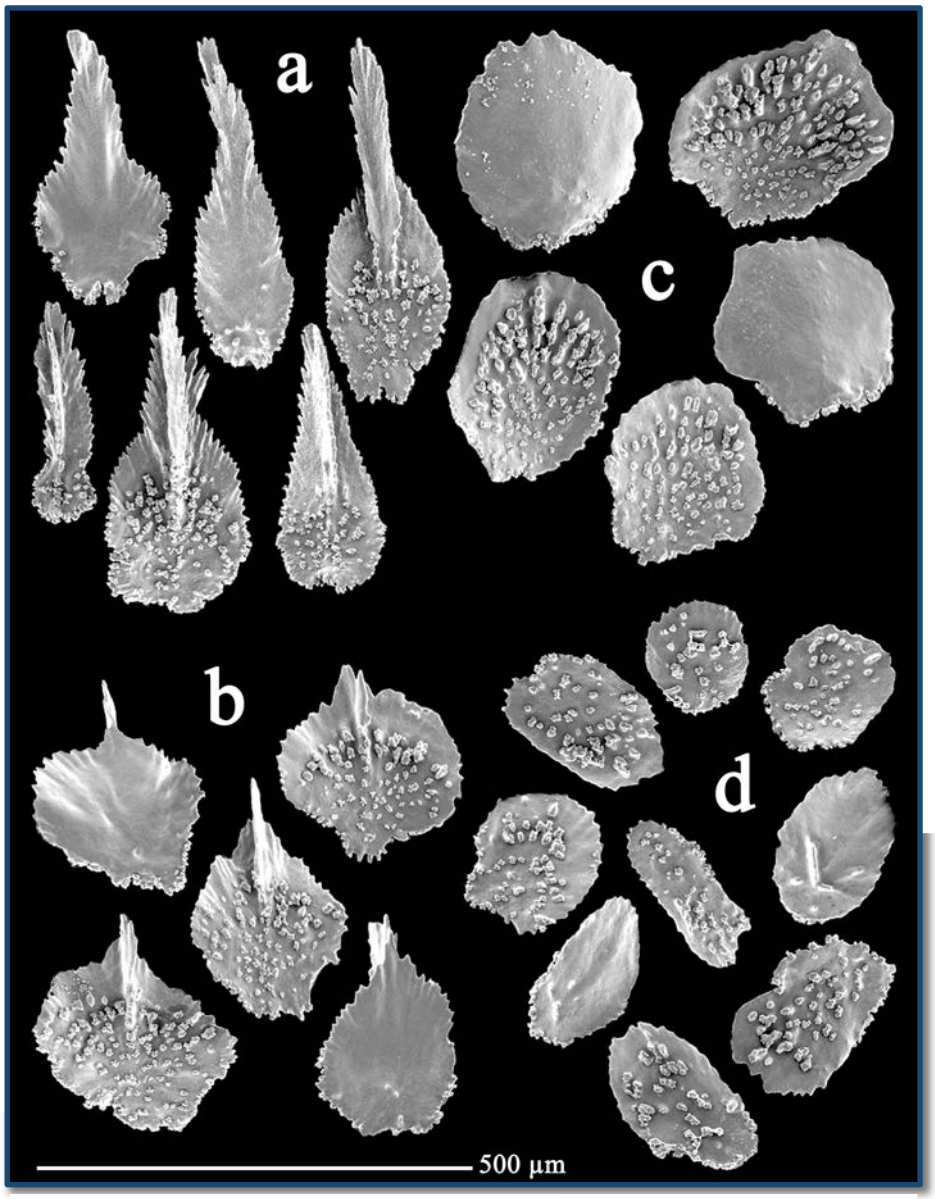


Figure 2.100.- *Thouarella minuta*, holotype ZIZMH C11742: **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal scales.

Variations from holotype

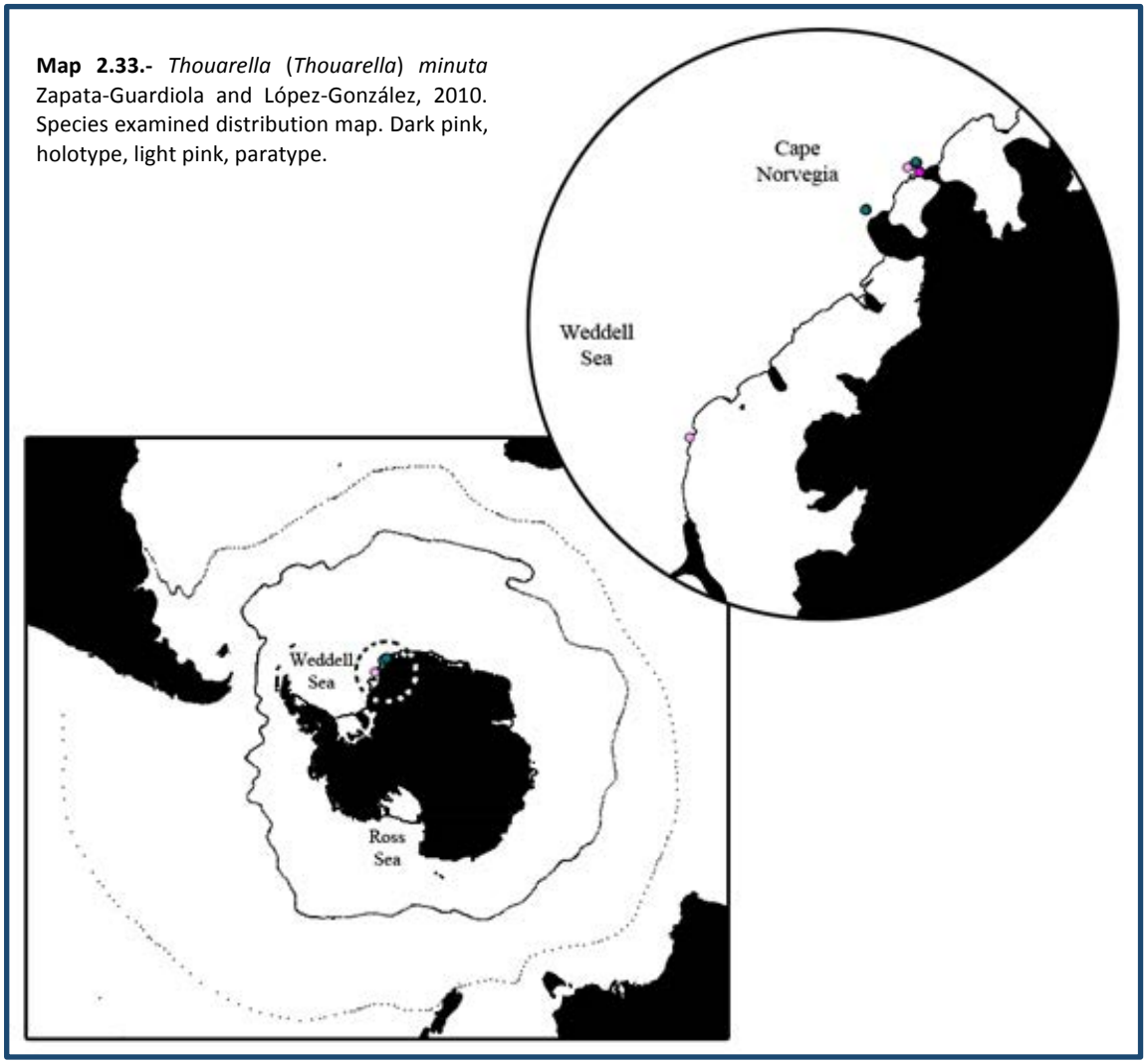
The general colonial structure of the paratypes and additional examined material are quite similar to that of the holotype. The material studied includes colonies from 11 to 66 cm in height. The branchlets arise from all around the main stem; some colonies have bend branchlets, giving a false impression of a flattened or pinnate forms, some branchlets are bent upward, up to 4.5 cm in length, simple or ramified, like those of the holotype. The polyp's proximity to the stem varies from inclined to appressed. There are from 10 to 18 polyps per centimetre on branchlets, and they are up to about 1.1 mm in height. The distribution and shape of the sclerites of the polyps and coenenchyme are as in the holotype.

Geographical and bathymetrical distribution

At present, *Thouarella minuta* is known only from, Austasen, Antarctica (Map 2.33), between 226 and 338 m in depth.

Etymology

In this species, the most interesting character is the minute size of the polyps, one of the smallest in comparison with its congeners. The specific name, *minuta*, comes from Latin, meaning small or minute.



Map 2.33.- *Thouarella* (*Thouarella*) *minuta* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Thouarella (*Thouarella*) *pendulina* (Roule, 1908)

(Figures 2.101-2.103)

Rhopalonella pendulina Roule, 1908:4, pl.1, fig. 5–8.—Gravier, 1914:70–77, text figs. 86–98, pl. 5, figs. 21–15.—Kükenthal, 1912:290.
Thouarella pendulina, Kükenthal, 1915:151; 1919:440; 1924:302.
Thouarella (*Thouarella*) *pendulina,* Cairns and Bayer, 2009:27 (listed).
Thouarella antarctica, Broch, 1965:24–26, pl.2, figs. 5–7.

Examined material

Type: MNHN-Oct.000-0211, 1ere expédition antarctique française (1903-1905), N/O *Jean Charcot*, dans des nids de cormorans, Booth-Wandel Island, Antarctica.

Syntypes: MNHN-Oct.000-0209 and MNHN-Oct.000-0210, 1ere expédition antarctique française (1903-1905), N/O *Jean Charcot*, dans des nids de cormorans, Booth-Wandel Island, Antarctica.

Additional material: US 463, ANT XVII-3, stn 183-01, 62°07.6'S, 60°23.5'W, west of Deception Island, Antarctica, 200-204 m depth, 03 May 2000, four colonies; US 1580, ANT XIX-3, stn PS61/045-01, 60°58.783'S, 55°09.16'W, north Elephant Island, Antarctica, 196-270 m depth, 29 January 2002, one colony and one fragment; US 1589, ANT XIX-3, stn PS61/051-01, 61°11.96'S, 54°53.783'W, Elephant Island, Antarctica, 62-95 m depth, 31 January 2002, one fragment; US 1559, ANT XIX-3, stn PS61/053-01, 61°20.216'S, 55°32.15'W, Elephant Island, Antarctica, 117-160 m depth, 31 January 2002; US 1532, US 6126, ANT XIX-3, stn PS61/066-01, 60°52.65'S, 55°20.76'W, Elephant Island, Antarctica, 300-440 m depth, 04 February 2002; US 1698, ANT XIX-3, stn PS61/089-01, 60°57.55'S, 55°43.783'W, Elephant Island, Antarctica, 158-190 m depth, 09 February 2002; US 6096, ANT XIX-3, stn PS61/092-01, 61°01.82'S, 55°45.63'W, Elephant Island, Antarctica, 122-158 m depth, 09 February 2002; US 1549 , ANT XIX-3, stn PS61/118-01, 62°20.56'S, 60°32.02'W, north Livingston Island, Antarctica, 119-137 m depth, 18 February 2002; US 3248, VLT ITALICA (XIX), stn H-OUT-5, 72°16.9'S, 170°17'E, cape Adare, Antarctica, 78-105 m depth, 09 February 2004, one colony; US 6365, ANT XXIII-8, stn 605-05, 61°20.26'S, 55°30.92'W, Elephant Island, Antarctica, 153 m depth, 20 December 2006, two fragments; US 6252, ANT XXIII-8, stn 611-01, 60°58.9'S, 55°11.32'W, Elephant Island, Antarctica, 215 m depth, 21 December 2006, one colony; US 6005, ANT XXIII-8, stn 613-01, 60°55.1'S, 55°25.23'W, Elephant Island, Antarctica, 112-160 m depth, 21 December 2006; US 6254, ANT XXIII-8, stn 614-01, 60°53.45'S, 55°26.13'W, Elephant Island, Antarctica, 244-250 m depth, 21 December 2006; US 6012, ANT XXIII-8, stn 621-01, 60°58.06'S, 55°53'W, Elephant Island, Antarctica, 165-197 m depth, 23 December 2006; US 6251, ANT XXIII-8, stn 630-01, 61°04.73'S, 55°49.3'W, Elephant Island, Antarctica, 115-124 m depth, 25 December 2006; US 6006, ANT XXIII-8, stn 638-01, 61°09.23'S, 56°03.55'W, Elephant Island, Antarctica, 148-170 m depth, 26 December 2006; US 6035, ANT XXIII-8, stn 728-02, 63°42.25'S, 56°02.16'W, Dundee Island, Paulet Island, Antarctica, 292-298 m depth, 24 January 2007.

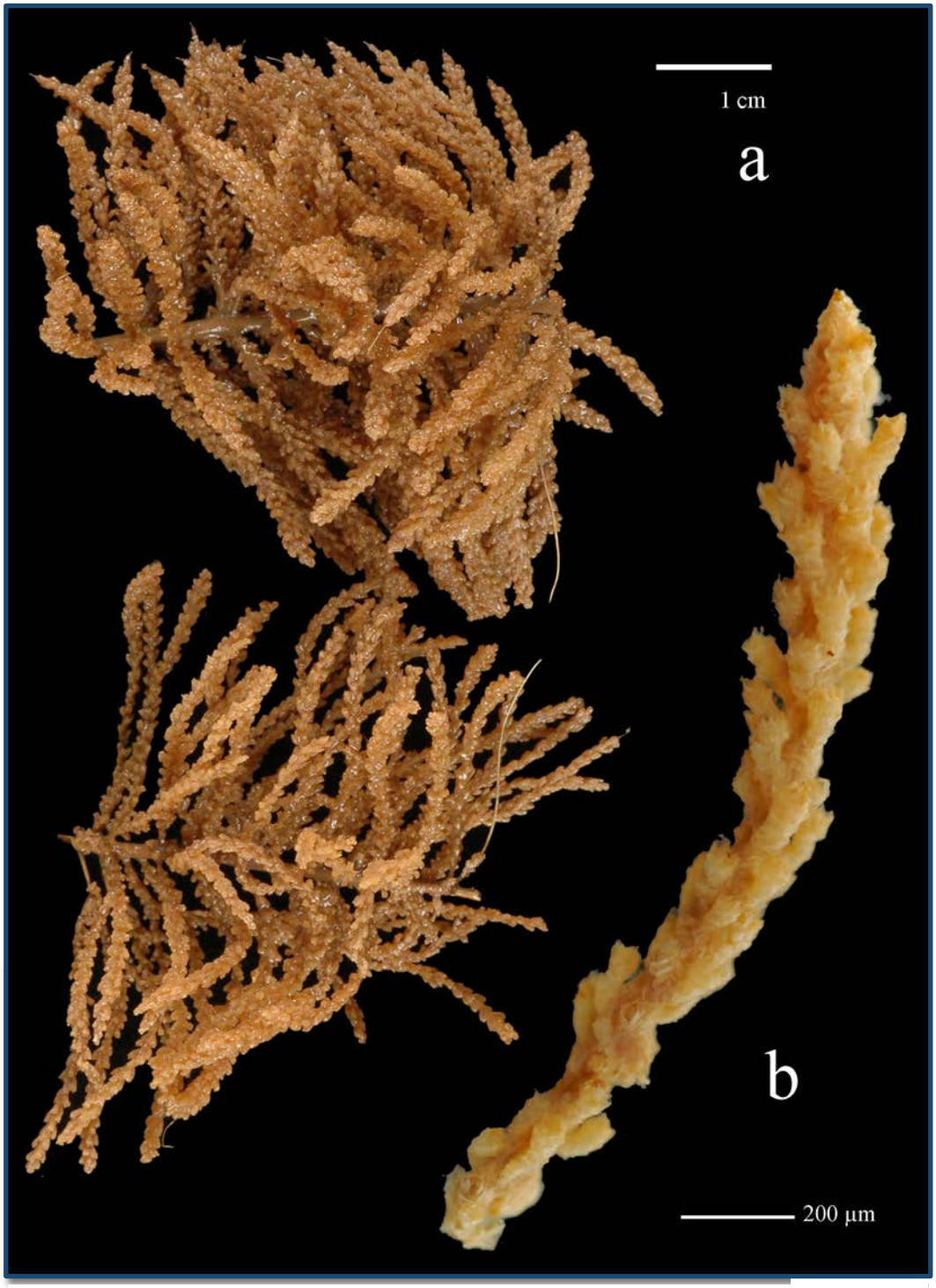


Figure 2.101.- *Thouarella pendulina*, holotype MNHN-Oct.000-0211: **a**, fragments of the colony; **b**, detail of branchlets.

Description of the type

Two fragments of a bottlebrush colony (Fig. 2.101a), of 3.7 and 4.3 cm in height and about 5.5 cm in width; simple branchlets or ramified up to third order, branchlets about 2.5-3 cm in length. Axis brown in colour, stiff and without holdfast, basal axis diameter of 0.15 mm. Colour of colony reddish. Polyps bent upward, almost appressed to branchlets (Fig. 2.101b), directed

upward, singly placed and arranged in disordered spirals, clustering on branchlet tips, 25-40 polyps per cm. Polyps (Fig. 2.102) funnel-shaped; about 1.1-1.6 mm in height and 0.34-0.55 mm in diameter. Polyp body with 8 longitudinal rows of scales, each abaxial row has 4-5 scales (Fig. 2.102b), and 2-3 scales in each adaxial row (Fig. 2-102c), reduced in size.

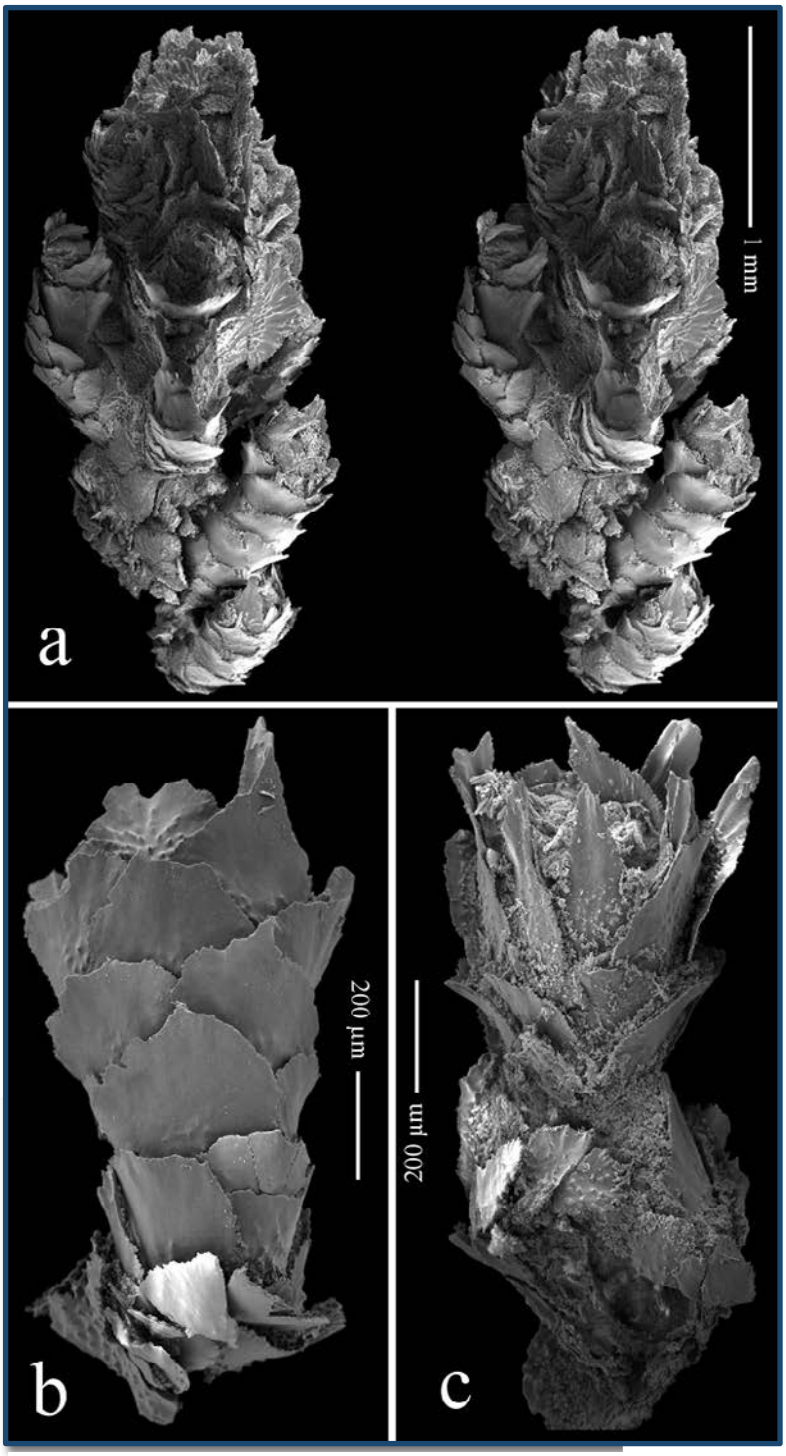


Figure 2.102.- *Thouarella pendulina*, holotype MNHN-Oct.000-0211: **a**, detail of branchlet, stereo pair; **b**, polyp on abaxial view; **c**, polyp on adaxial view.

Eight opercular scales (Fig. 2.103a), 0.22-0.54 x 0.14-0.38 mm, triangle to arrowhead-shaped with an acute tip. Proximal inner surface tuberculate, covering more than a half of the length. Larger operculars with a simple apical keel. Outer surface granular. Basal margin with irregular processes, free margin finely serrated. Marginal scales (Fig. 2.103b) eight in number, rhomboid-shaped with an acute apex, 0.24-0.52 x 0.22-0.4 mm. Inner surface tuberculate covering at least two thirds and up to 90% of the length. Simple apical keel, lateral inner distal areas smooth. Outer surface sparsely granular. Free margin finely serrated, proximal margin with digitate processes. Body scales (Fig. 2.103c), circular to oval-shaped, 0.18-0.43 mm in maximum length. Inner surface completely tuberculate, may appear a small thin apical spine. Proximal outer surface covered with granules, distally quite smooth. Free margin finely serrated, basal margin with digitate processes. Coenenchymal scales (Fig. 2.103d) irregularly circular, 0.08-0.4 mm in maximum length. Inner surface completely tuberculate, outer surface with granules sometimes forming ridges. Margin to that of body scales.

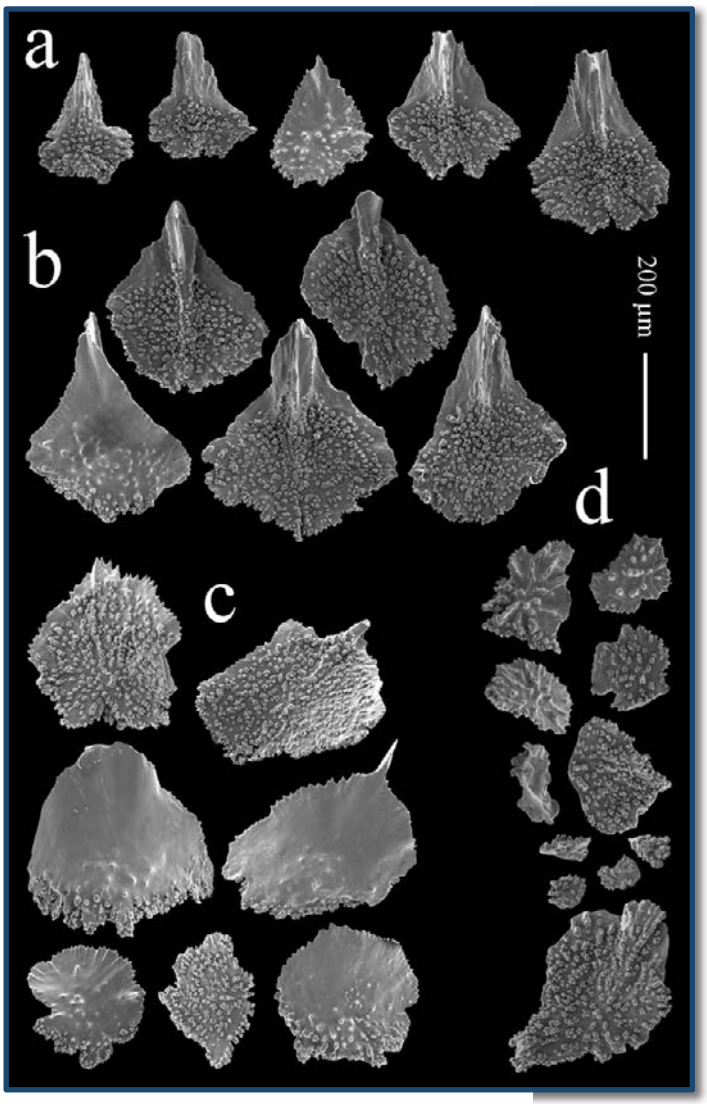
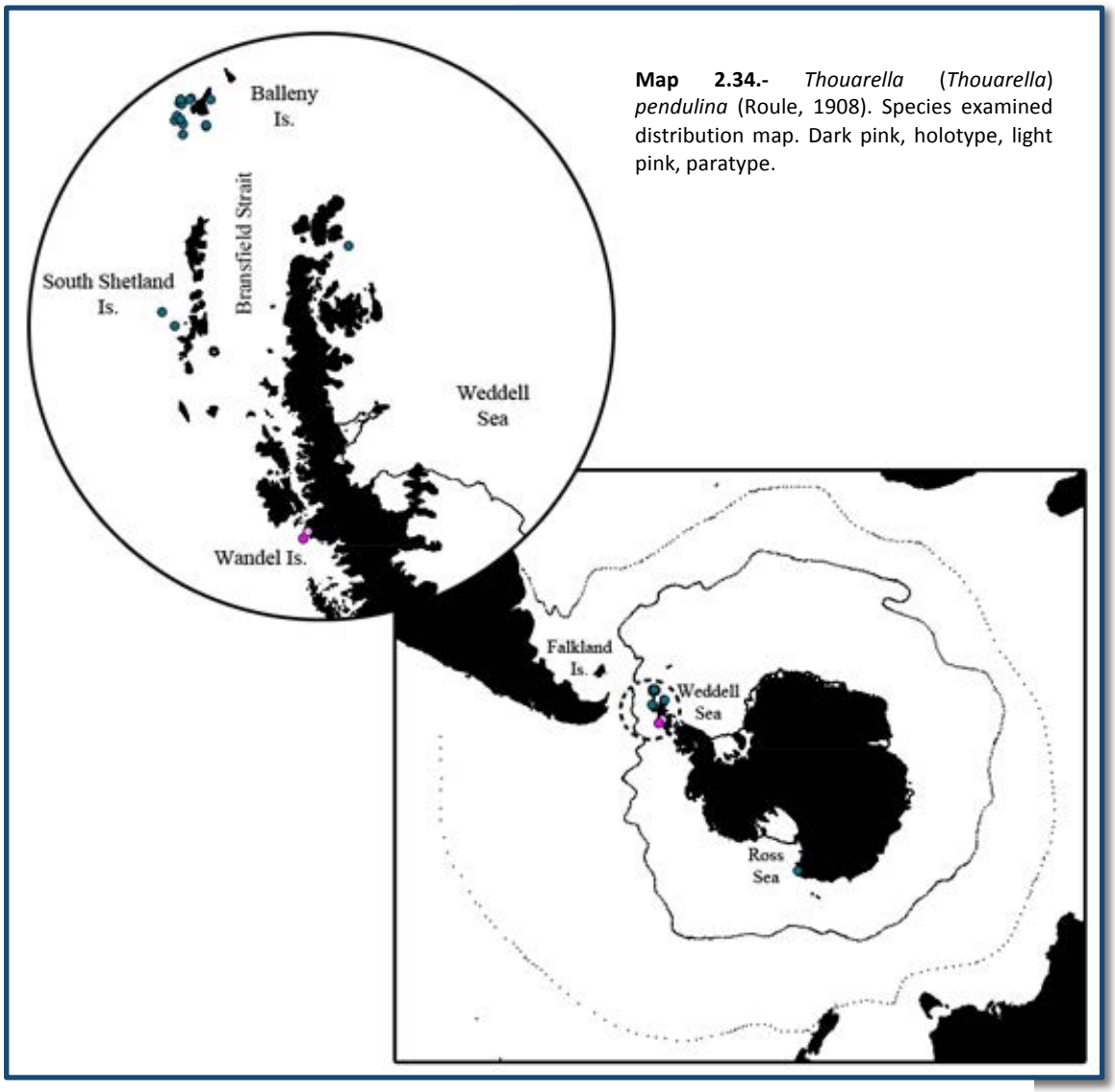


Figure 2.103.- *Thouarella pendulina*, holotype MNHN-Oct.000-0211: **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal scales.

Geographical and bathymetrical distribution

At present, *Thouarella pendulina* is known from islands around tip of Peninsula Antarctica (Wandel Island, Deception Island, Livingston Island, Dundee Island) to Elephant Island and off Cape Adare, Antarctica (Map 2.34), between 60 and 440 m in depth.



Map 2.34.- *Thouarella* (*Thouarella*) *pendulina* (Roule, 1908). Species examined distribution map. Dark pink, holotype, light pink, paratype.

Etymology

The species name is presumably derived from the Latin '*pendula'*, meaning to hang down, in reference to the drooping nature of the branchlets.

Gender: feminine

Thouarella (*Thouarella*) *variabilis* Wright and Studer, 1889

(Figures 2.104-2.106)

Thouarella variabilis var. a The type Wright and Studer, 1889:68–69, pl. 21, fig. 1.—Thomson and Henderson, 1906:40 (listed)
Thouarella variabilis var. b *brevispinosa* Wright and Studer, 1889:69.—Thomson and Henderson, 1906:38 (list).—Molander, 1929:74–5
Thouarella variabilis var. c *gracilis* Wright and Studer, 1889:70.—Thomson and Henderson, 1906:40 (list)
Thouarella variabilis Menneking, 1905:260–262, pl. 9, figs. 9, 10, 21, 22.—Versluys, 1906:37–38.—Gravier, 1914:56–61, pl. 1 fig. 6, pl. 3 fig. 13–14.—Kükenthal, 1915:150 (key).—Molander, 1929:74–5.—Broch, 1965:30–31, pl. 6, figs. 17–19.—Brito, Tyler and Clarke, 1997:63–69
Thouarella (Parathouarella) variabilis Kükenthal, 1919:428, fig. 202 (in text); 1924:297 (key).—Thomson and Rennet, 1931:27–30, pl. 9, figs. 4–5, pl. 12, fig. 3
Thouarella aff. *variabilis* Kükenthal, 1912:305–306, figs. 9–12 (in text), pl. 20 fig. 2
Thouarella (Thouarella) variabilis typica Cairns and Bayer, 2009:27 (listed)
Thouarella (Thouarella) variabilis var. *brevispinosa* Cairns and Bayer, 2009:27 (listed)
Thouarella (Thouarella) variabilis var. *gracilis* Cairns and Bayer, 2009:27 (listed)

Examined material

Holotype: NHM89.5.27.54, NHM 22.1.21.9, *Challenger Expedition*, sta. 145A, 46°41'S, 38°10'E, off Prince Edward Island, 566 m depth, 27 Dec 1873.

Syntype: NHM 89.5.27.56, *Challenger Expedition,* sta. 145, southeast of Prince Edward Island, 46˚43'S, 38˚4'30"E, 256 m depth, 27 Dec 1873.

Figure 2.104.- *Thouarella variabilis,* holotype BM 22.1.21.9: **a**, whole colony; **b**, detail of branchlet.

Additional material: ZSM 20080410, Deutsche Südpolar Expedition Valdivia, Gauss-station, 350 m depth, 08 February 1903; US 187, ANT XVII-3, stn 058-03, 70°29.5'S, 07°41.7'W, Atka

Bay, Antarctica, 302 m depth, 29 March 2000, one colony; US 482, ANT XVII-3, stn 085-01, 71°12.18'S, 12°19.02'W, Cape Norvegia, Antarctica, 309-318 m depth, 02 April 2000, one colony; US 207, ANT XVII-3, stn 102-01, 71°11.43'S, 12°19.2'W, Cape Norvegia, Antarctica, 312-323 m depth, 03 April 2000, one colony; US 142, ANT XVII-3, stn 158-01, 63°04.7'S, 57°31.6'W, Bransfield Strait, Antarctica, 94 m depth, 26 April 2000; US 462, ANT XVII-3, stn 159-01, 62°55'S, 57°39.5'W, Bransfield Strait, Antarctica, 218 m depth, 26 April 2000; US 1460, ANT XIX-3, stn PS61/052-01, 61°20.6'S, 55°10.56'W, Elephant Island, Antarctica, 264-270 m depth, 31 January 2002, two colonies; US 1644, ANT XIX-3, stn PS61/068-01, 60°52.65'S, 55°20.76'W, Elephant Island, Antarctica, 164 m depth, 04 February 2002, one colony; US 6440, ANT XIX-3, stn PS61/107-01, 61°50.36'S, 57°20.92'W, King George Island, South Shetland Islands, Antarctica, 256-278 m depth, 14 February 2002, two colonies; US 6433, ANT XIX-3, stn PS61/110-01, 61°39.13'S, 58°48.55'W, South Shetland Islands, Antarctica, 371-408 m depth, 16 February 2002, one colony; US 1338, US 6412, ANT XIX-5, stn PS61/167-01, 53°23.68'S, 42°42.23'W, north west South Georgia Islands, Antarctica, 306-343 m depth, 09 April 2002, one colony; US 1347, ANT XIX-5, stn PS61/194-01, 57°40.7'S, 26°26.08'W, central South Sandwich Islands, Antarctica, 278-309 m depth, 15 April 2002, one colony; US 1277, US 1311, ANT XIX-5, stn PS61/223-01, 60°08.43'S, 34°54.96'W, Discovery Bank, Scotia Sea, Antarctica, 374-384 m depth, 21 April 2002, one colony; US 3214, US 3216, VLT ITALICA (XIX), stn H-OUT-3, 72°17.9'S, 170°26'E, Cape Hallett, Victoria Land, Antarctica, 230-246 m depth, 04 February 2004, one colony each; US 3207, US 3209, VLT ITALICA (XIX), stn H-OUT-4, 72°17.2'S, 170°23.9'E, Cape Hallett, Victoria Land, Antarctica, 204-208 m depth, 04 February 2004, one colony each; US 6404, TAN 0402 stn 010, 71°42.67'S, 172°02.68'E, Cape Adare, Victoria Land, Antarctica, 621-636 m depth, 05 February 2004, one colony; US 6401, TAN 0402 stn 018, 71°43.63'S, 171°46.88'E, Cape Adare, Victoria Land, Antarctica, 522-530 m depth, 05 February 2004, one colony; US 054, TAN 0402 stn 029, 71°45.36'S, 171°15.82'E, Cape Adare, Victoria Land, Antarctica, 270-275 m depth, 09 February 2004, one colony; US 064, TAN 0402 stn 031, 71°44.82'S, 171°33.3'E, Cape Adare, Victoria Land, Antarctica, 340-343 m depth, 09 February 2004, one colony; US 3256, VLT ITALICA (XIX), stn H-OUT-5A, 72°17.9'S, 170°19.7'E, Cape Hallett, Victoria Land, Antarctica, 103 m depth, 09 February 2004, one colony; US 6402, TAN 0402 stn 039, 71°45.3'S, 171°08.85'E, Cape Adare, Victoria Land, Antarctica, 250 m depth, 10 February 2004, one colony; US 3276, VLT ITALICA (XIX), stn H-OUT-2(bis), 72°17.5'S, 170°29.4'E, Cape Hallett, Victoria Land, Antarctica, 339-353 m depth, 11 February 2004, three colonies; US 6389, TAN 0402 stn 054, 72°19.48'S, 170°25.67'E, Cape Hallett, Victoria Land, Antarctica, 199-206 m depth, 13 February 2004, one colony; US 6386, TAN 0402 stn 055, 72°18.46'S, 170°21.47'E, Cape Hallett, Victoria Land, Antarctica, 123-130 m depth, 13 February 2004, one colony; US 6385, US 6387, US 6388, US 6454, TAN 0402 stn 075, 72°04.62'S, 172°56.08'E, Cape Hallett, Victoria Land, Antarctica, 525 m depth, 14 February 2004, one colony, one colony, one colony and one fragment; US 6394, US 6396, US 6397, TAN 0402 stn 082, 72°03.63'S, 172°54.23'E, Cape Hallett, Victoria Land, Antarctica, 526 m depth, 14 February 2004, one colony each; US 6395, TAN 0402 stn 084, 72°04.95'S, 173°08.33'E, Cape Hallett, Victoria Land, Antarctica, 539-542 m depth, 14 February 2004, one colony; US 3314, VLT ITALICA (XIX), stn H-in-4, 72°17.2'S, 170°13.5'E, Cape Hallett, Victoria Land, Antarctica, 196-220 m depth, 16 February 2004, one colony; US 069, TAN 0402 stn 096, 71°11.32'S, 170°58.65'E, Cape Adare, Victoria Land, Antarctica, 719-736 m depth, 18 February 2004, one colony; US 6384, TAN 0402 stn 140, 72°00.82'S, 170°46.47'E, Cape Hallett, Victoria Land, Antarctica, 231-240 m depth, 26 February 2004, one colony; US 6399, TAN 0402 stn 186, 71°30.72'S, 171°25.52'E, Cape Adare, Victoria Land, Antarctica, 390 m depth, 27 February 2004, one colony; US 09, TAN 0402 stn 188, 71°32.85'S, 171°06.67'E, Cape Adare, Victoria Land, Antarctica, 280-286 m depth, 27 February 2004, one colony; US 6219, ANT XXIII-8, stn 605-01, 61°20.35'S, 55°29.17'W, Elephant Island, Antarctica, 151 m depth, 19 December 2006, two

colonies; US 6367, US 6366, ANT XXIII-8, stn 605-05, 61°20.27'S, 55°30.92'W, Elephant Island, Antarctica, 153 m depth, 20 December 2006, one colony each; US 6370, ANT XXIII-8, stn 612-01, 60°52.62'S, 55°20.53'W, Elephant Island, Antarctica, 307-483 m depth, 21 December 2006, one colony; US 6408, ANT XXIII-8, stn 614-03, 60°52.13'S, 55°30.32'W, Elephant Island, Antarctica, 248-259 m depth, 21 December 2006, two colonies; US 6409, ANT XXIII-8, stn 619-01, 60°57.78'S, 55°45'W, Elephant Island, Antarctica, 200 m depth, 22 December 2006, three colonies; US 6250, ANT XXIII-8, stn 629-01, 60°58.6'S, 55°46.77'W, Elephant Island, Antarctica, 162-191 m depth, 24 December 2006, one colony; US 6364, US 6405, ANT XXIII-8, stn 661-02, 61°39.2'S, 57°04.75'W, South Shetland Islands, Antarctica, 466-467 m depth, 30 December 2006, one colony each; US 6208, ANT XXIII-8, stn 664-01, 61°38.78'S, 57°51.87'W, South Shetland Islands, Antarctica, 336-337 m depth, 30 December 2006, two colonies; US 6407, ANT XXIII-8, stn 667-01, 61°44.98'S, 58°30.88'W, South Shetland Islands, Antarctica, 282-288 m depth, 31 December 2006, one colony and one fragment; US 6411, ANT XXIII-8, stn 668-01, 61°50.05'S, 58°30.67'W, South Shetland Islands, Antarctica, 156-193 m depth, 31 December 2006, five colonies and one fragment; US 6004, ANT XXIII-8, stn 669-01, 61°50.02'S, 58°37.28'W, South Shetland Islands, Antarctica, 191-208 m depth, 31 December 2006, one colony; US 6369, ANT XXIII-8, stn 687-01, 62°35.95'S, 54°49.77'W, Joinville Island, Antarctica, 263 m depth, 04 January 2007, one colony; US 6372, US 6376, ANT XXIII-8, stn 697-01, 63°15.38'S, 59°03.93'W, Bransfield Strait, Antarctica, 329-408 m depth, 06 January 2007, one colony each.

Description of the holotype

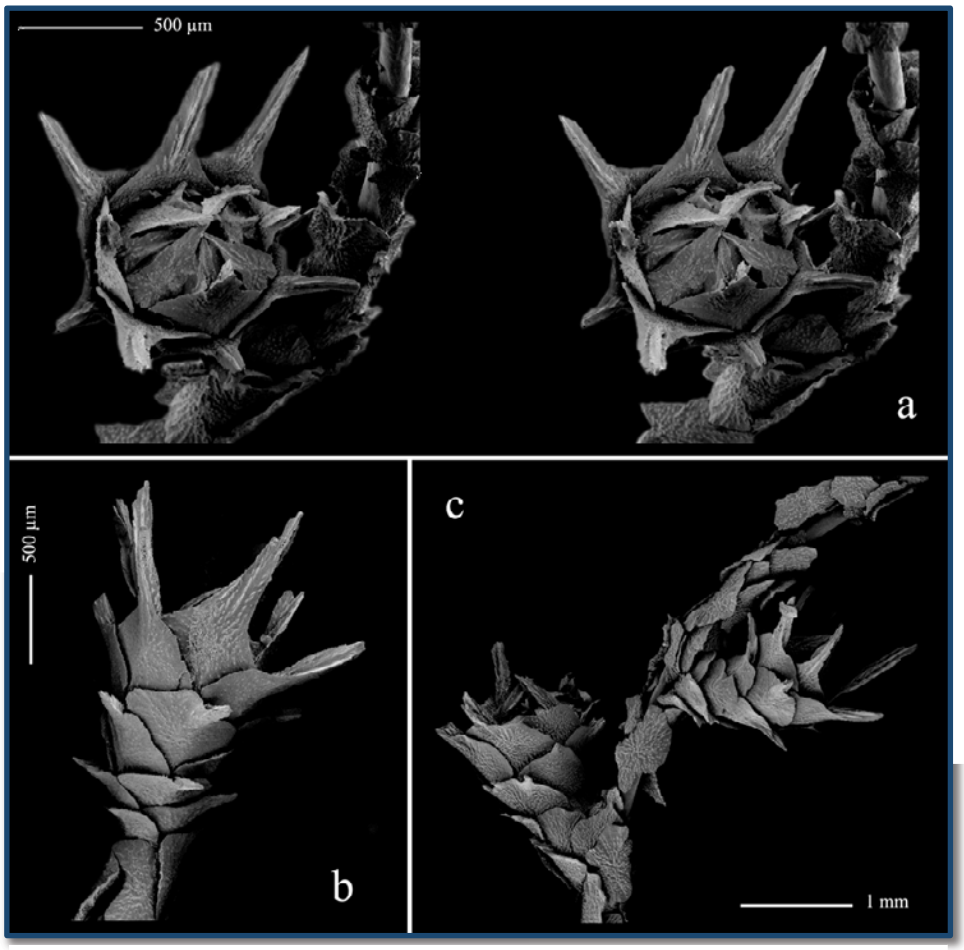


Figure 2.105.- *Thouarella variabilis,* holotype BM 22.1.21.9: **a**, polyp on oral view, stereo pair; **b**, polyp on abaxial view; **c**, detail of branchlet.

Bottlebrush colony (Fig. 2.104a), 15 cm in total height and up to 4 cm width each main branch. Simple branchlets or ramified up to third order near the base of the branchlet up to 3 cm in length, almost perpendicular to stem (Fig. 2.104b). Axis brown in colour, stiff, without a holdfast. Basal axis diameter about 3 mm. Polyps inclined upward to branchlets (Fig. 2.104b, 2.105c), singly placed, 5–10 polyps per cm. Polyps (Fig. 2.105) funnel-shaped, about 1.4-2 mm tall and 0.63–0.82 mm in diameter. Polyp body with 8 longitudinal rows of scales reduced in number at the base. Each abaxial row has 4 scales (Fig. 2.105b), and each adaxial row has 2-3 scales.

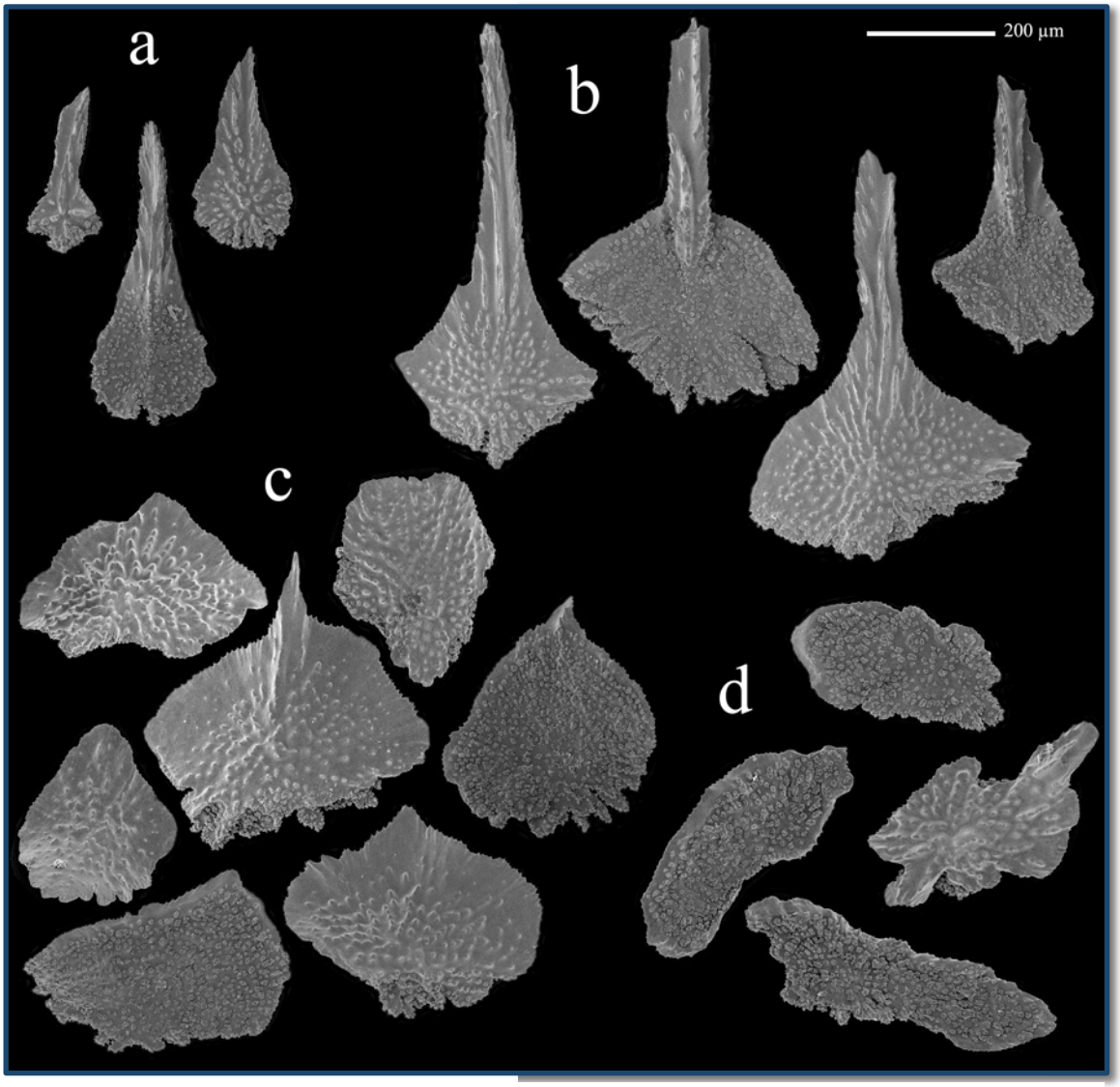


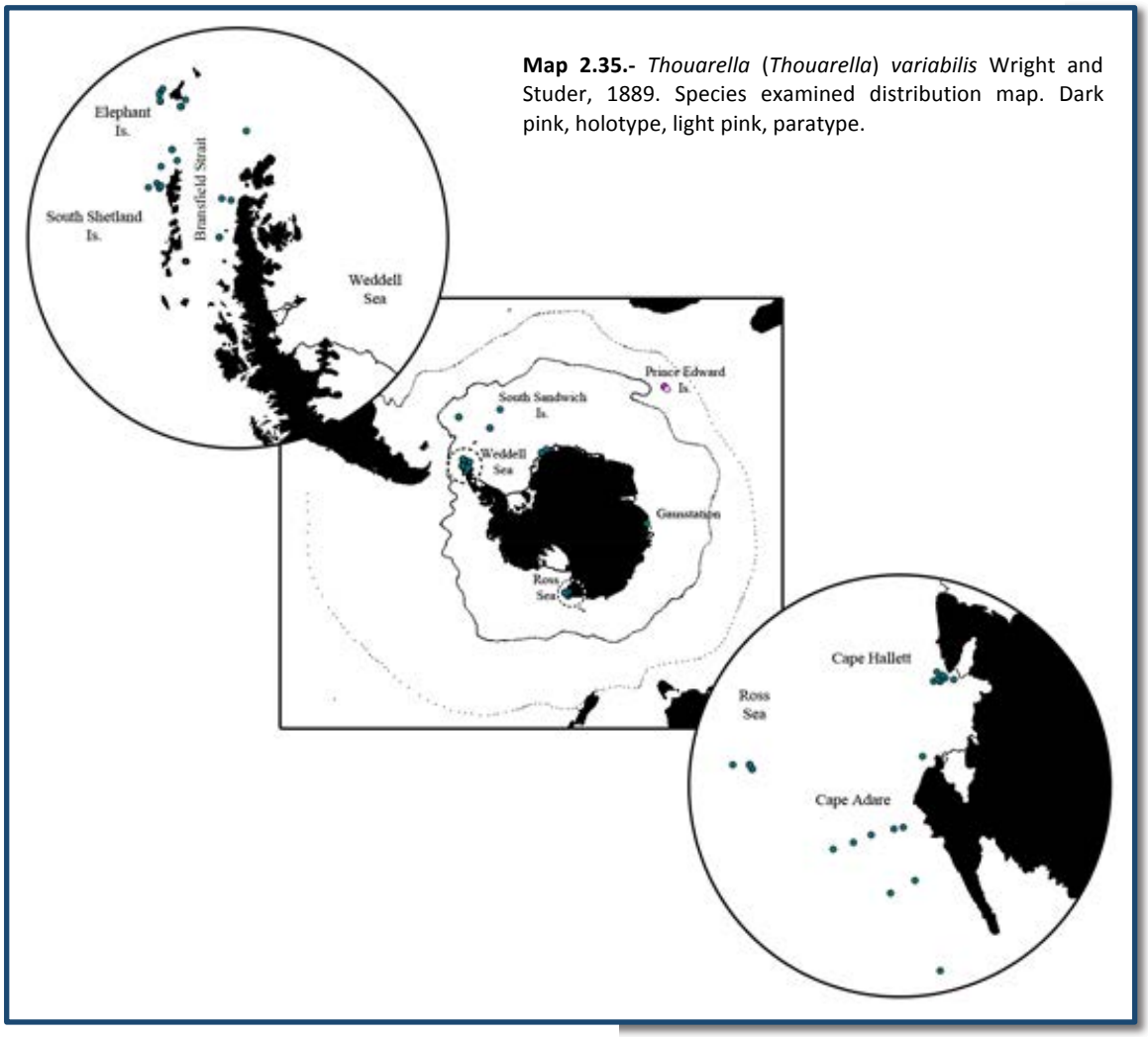
Figure 2.106.- *Thouarella variabilis,* holotype BM 22.1.21.9: **a**, opercular scales; **b**, marginal scales; **c**, body scales; **d**, coenenchymal scales.

Opercular scales (Fig. 2.106a) eight in number, 0.26–0.46 x 0.11–0.2 mm, isosceles triangle-shaped with an acute apex and rounded base. Proximal inner surface sparsely tuberculate covering up to half of their length; distal part with ridges forming an incipient or small keel. Outer surface granular. Free margin finely serrated. Marginal scales (Fig. 2.106b) eight in number, obtuse triangle-shaped with an acute base, projecting a thorn distally with

longitudinal ridges on all sides, 0.43–0.7 x 0.25–0.45 mm (including thorn). Inner surface tuberculate covering half of the scale length and the thorn base. Outer surface granular. Free margin finely serrated, proximal margin with digitate processes. Body scales (Fig. 2.106c), fan-shaped normally with an acute tip, adaxials with a blunt tip, 0.26–0.46 x 0.24-0.45. Inner surface almost completely tuberculate, outer surface covered with pointed granules. Free margin finely serrated, basal margin as in marginal scales. Coenenchymal sclerites (Fig. 2.106d) elongated oval in shape, 0.35–0.51 mm in maximum diameter. Inner and outer surface with similar characteristics to body scales. Margin also as in body scales.

Geographical and bathymetrical distribution

At present, *Thouarella variabilis***,** has a circum Antarctic distribution from Peninsula Antarctica, Weddell Sea and Ross Sea (Map 2.35), between 120 and 740 m in depth.



Map 2.35.- *Thouarella* (*Thouarella*) *variabilis* Wright and Studer, 1889. Species examined distribution map. Dark pink, holotype, light pink, paratype.

Etymology

The species name *variabilis*, refers to the variable ramification patterns found it in the type specimens, and described as different varieties by Wright and Studer (1889).

Genus *Tokoprymno* Bayer, 1996

Tokoprymno Bayer, 1996:511.—Zapata-Guardiola and López-González, 2010d:63.

Diagnosis

Primnoidae with bottlebrush colonies irregularly branched. Polyps more or less biserial but not in pairs, perpendiculars and directed toward one side of the branchlets. Vegetative polyps tall, straight, somewhat wider distally; operculum conical, usually very prominent when closed with opercular scales keeled; marginal scales 8, nearly equal in size, directly aligned with operculars; brood polyps may be present, ovate, losing opercular scales upon discharge of planulae.

Geographical and bathymetrical distribution

At present, *Tokoprymno* has been reported from a Subantarctic seamount at 549 m and off Elephant Island, Antarctica, between 2895.6 and 2896.4 m in depth.

Etymology

The generic name combines the Greek word *tokos*- meaning birth with -*prymno*, a common suffix in reference to the gorgonian family Primnoidae in allusion to the conspicuous brood polyps. Gender feminine.

Type species

Tokoprymno maia Bayer, 1996

***Key to species of the genus* Tokoprymno**

1. Opercular scales isosceles-triangle shaped; accessory opercular scales absent …….***T. maia***
2. Opercular scales duck-beak shaped; accessory opercular scales present ……***T. anatis***

Tokoprymno anatis Zapata-Guardiola and López-González, 2010b

(Figures 2.107-2.110)

Tokoprymno anatis Zapata-Guardiola and López-González, 2010d:63.

Examined material

Holotype: ZMH C11749, ANT XIX/3, stn PS61/046-08, 60°38.79'S, 53°57.42'W, north east of Elephant Island, Antarctica, 2895.6–2896.4 m depth, 2 February 2002, one colony, fragmented. Fragments of the holotype have also been deposited in USNM 1145316 and in BEIM CRO-0056.

Description of the holotype

Colony bottlebrush (Fig. 2.107a), fragmented in three main parts of 5, 7 and 9.5 cm in length, 22 cm in total length and 10.5 cm in width. Simple or scarcely ramified stiff branchlets (Fig. 2.107b) up to 6.5 cm in length, proximally almost perpendicular to stem, then curving upward. Axis bronze, stiff, broken proximally. Basal axis diameter 3 mm.

Polyps perpendicular to branchlets (Figs. 2.107b, 2.108a), absent on main stem, singly or biserial placed (Fig. 2.108a), 6–11 polyps per cm. Polyps (Fig. 2.108) relatively elongate, slightly clavate, up to 2.4 mm in height and 0.61–0.97 mm in diameter, with a conical operculum. Polyp body with 8 longitudinal rows of scales somewhat disorganized, adaxial body scales smaller (Fig. 2.108b), 4–5 transverse rows of scales in the abaxial aspect overlapping one another (Fig. 2.108c).

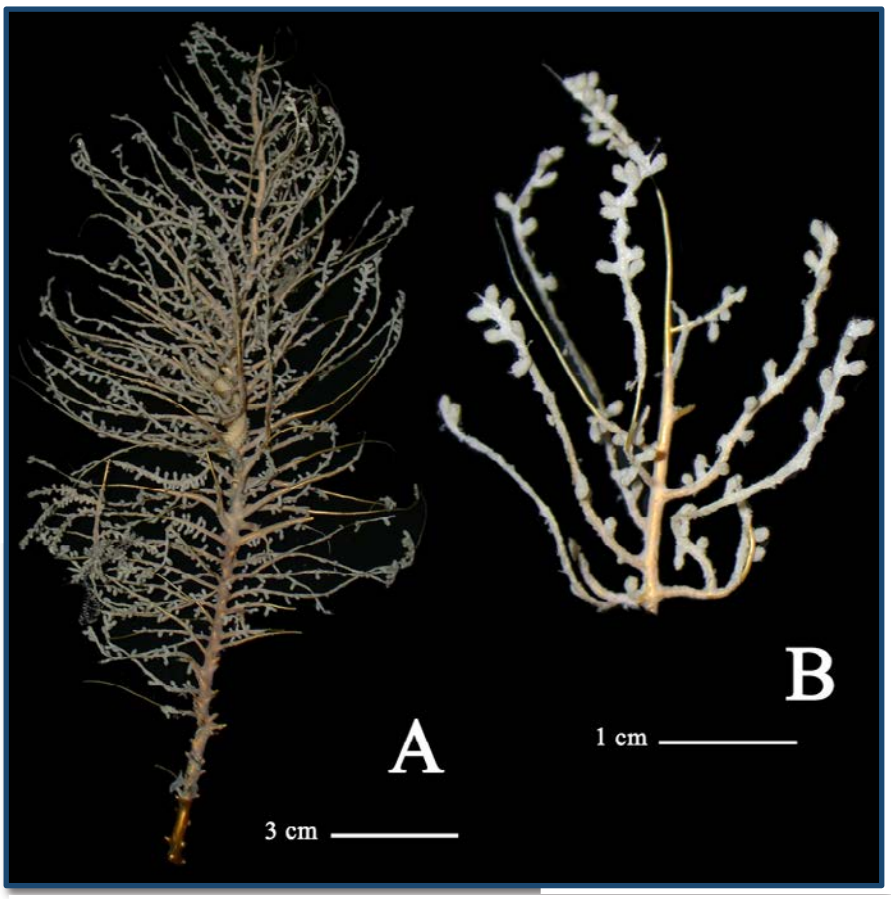


Figure 2.107.- *Tokoprymno anatis*, holotype (ZMH C11749): **a**, whole colony; **b**, detail of branchlets.

Accessory opercular scales (Fig. 2.109a), blade-shaped, variable in number from absent to two, small, about 0.49 mm in height and 0.12 mm in width. Proximal half of inner surface tuberculate, smooth distally, without keel. Margin finely serrated. Outer surface almost smooth, few granules. Opercular scales (Fig. 2.109b) eight in number, 0.58–0.84 mm in height and 0.15–0.28 mm in width, duck-beak shaped with rounded tips and square or bilobed base. Proximal inner surface tuberculate covering up to half of their length; distal part convex, with granules forming ridges or with a small keel. Outer surface almost smooth with a few granules proximally. Free margin serrated. Marginal scales (Fig. 2.109c) eight in number, more-or-less triangular, 0.52–0.91 mm in height and 0.27–0.44 mm in width. Inner surface tuberculate covering at least 75% of the scale, with distal longitudinal ridges. Outer surface almost smooth, with a few granules on proximal portion. Free margin serrated, proximal margin granular and lobed. Body wall scales (Fig. 2.110a) roughly square shaped, 0.36–0.61 mm in maximum length. Inner surface almost completely tuberculate, outer surface almost smooth and covered with granules proximally. Free margin serrated with tendency to be lobed, basal margin as in marginal scales. Coenenchymal scales (Fig. 2.110b) round in shape, 0.18–0.34 mm in maximum length. Inner surface completely tuberculate, outer surface smooth with a few granules. Margin quite smooth, granulate or finely serrated.

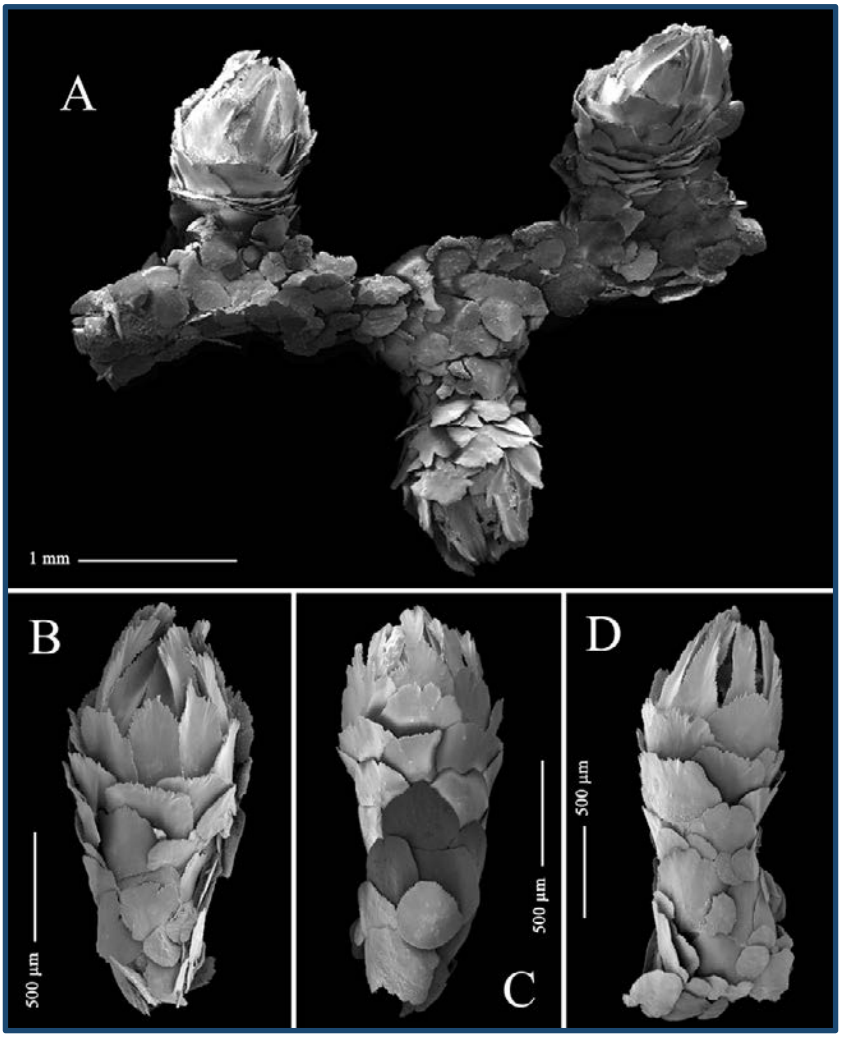


Figure 2.108.- *Tokoprymno anatis,* holotype (ZMH C11749): **a**, detail of a branchlet; **b**, polyp, adaxial view; **c**, polyp, abaxial view; **d**, polyp, lateral view.

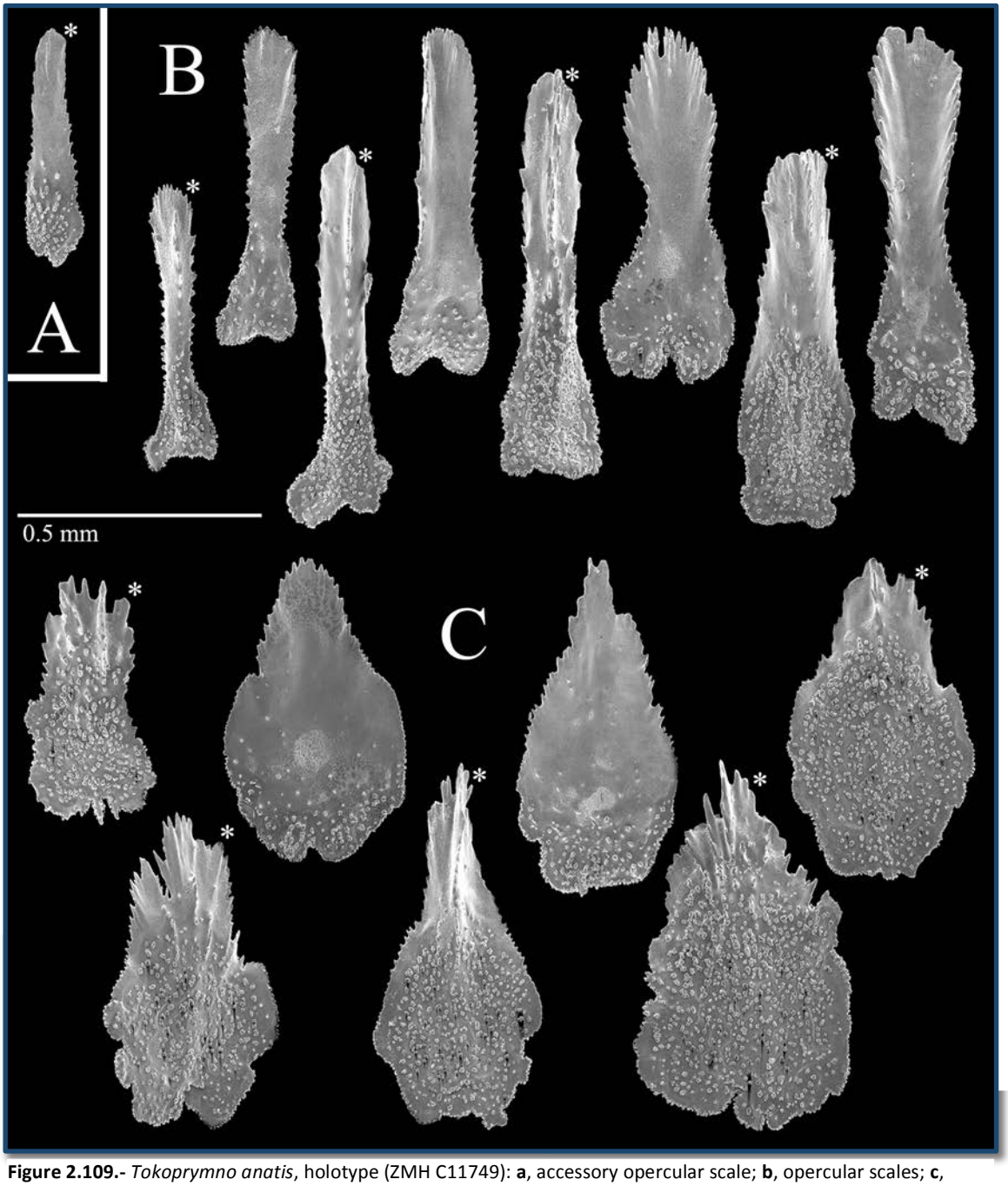


Figure 2.109.- *Tokoprymno anatis*, holotype (ZMH C11749): **a**, accessory opercular scale; **b**, opercular scales; **c**, marginal scales. * inner surface view.

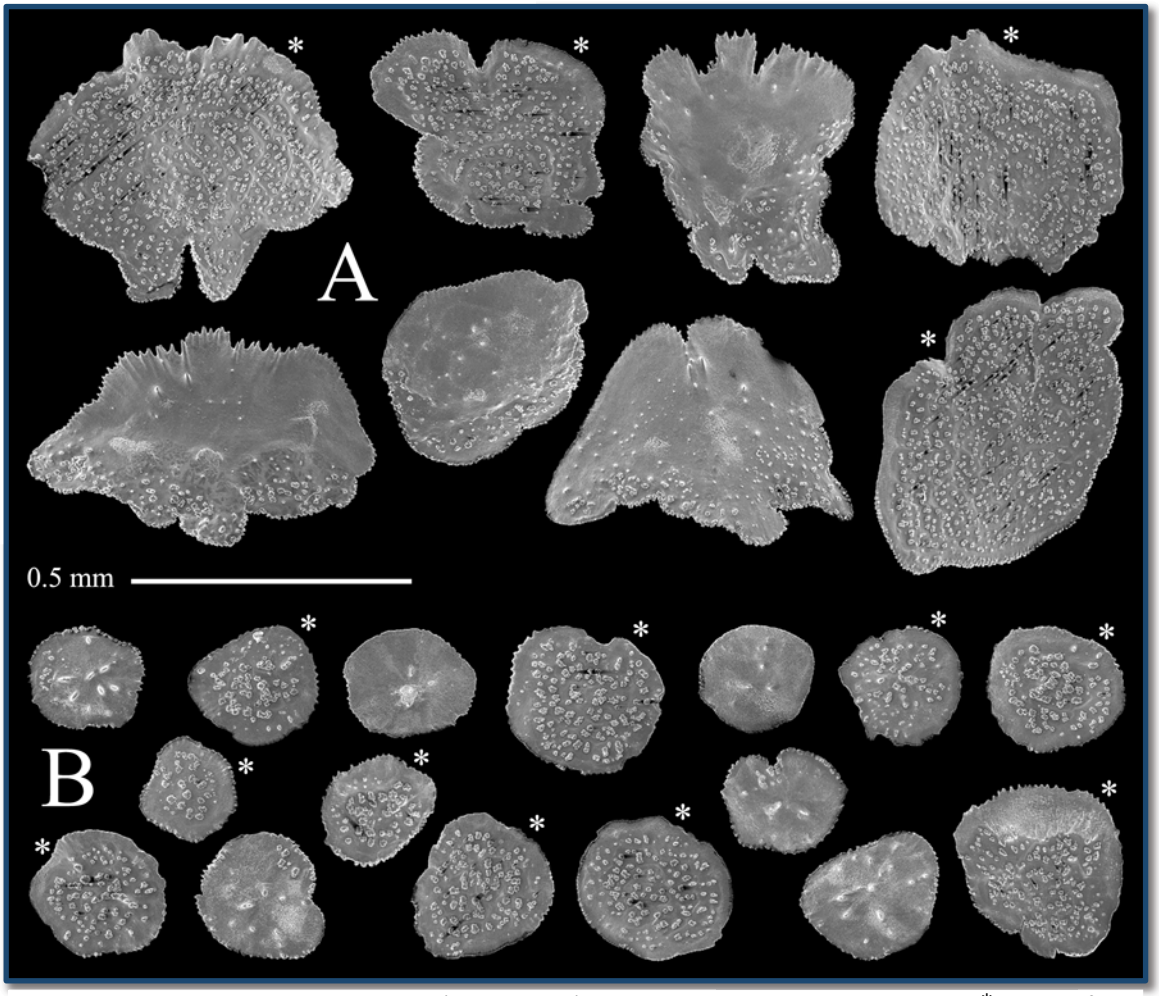


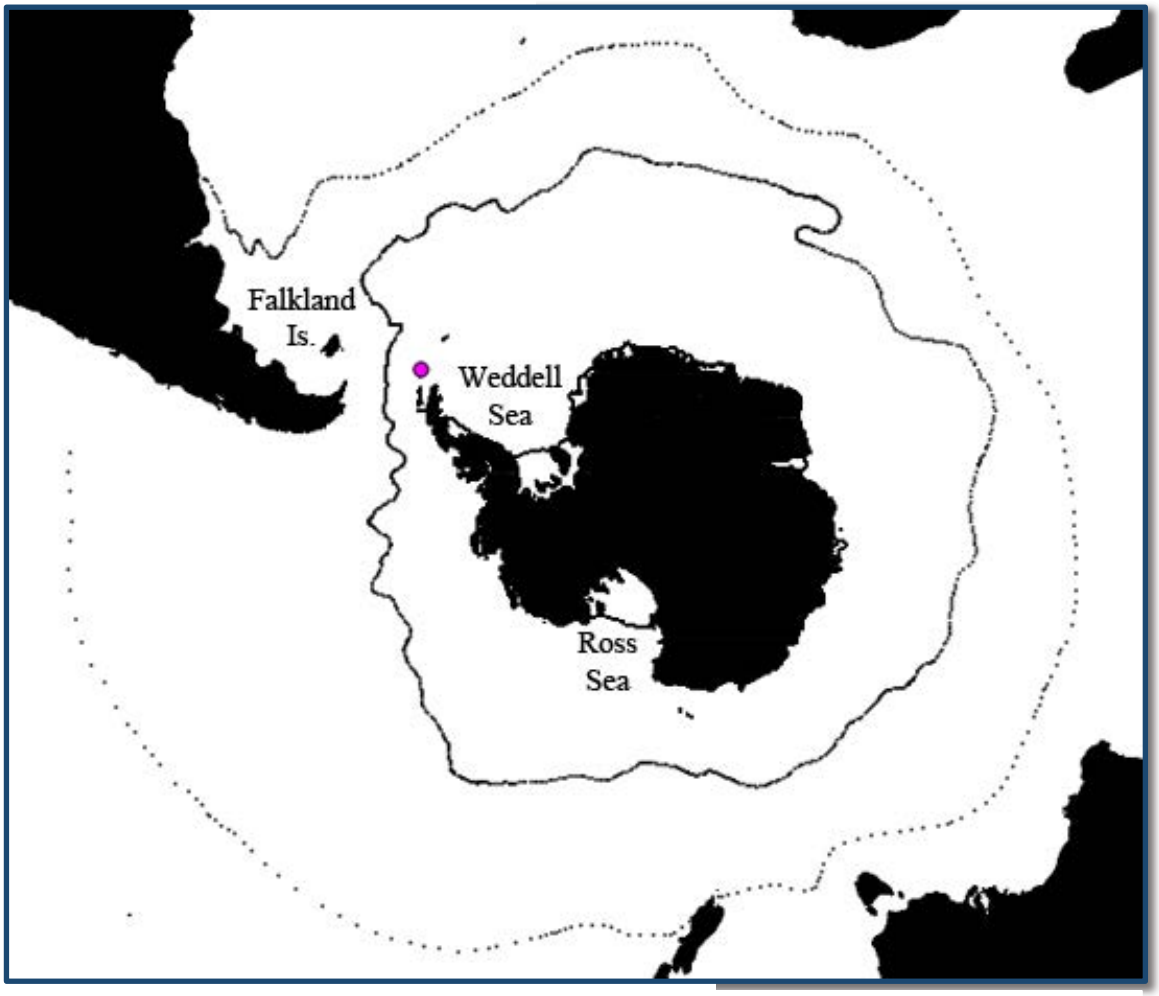
Figure 2.110.- *Tokoprymno anatis*, holotype (ZMH C11749): **a**, body scales; **b**, coenenchymal scales. * inner surface view.

Geographical and bathymetrical distribution

At present, *Tokoprymno anatis* is only known from off Elephant Island, Antarctica (Map 2.36), between 2895.6 and 2896.4 m in depth.

Etymology

The species name *anatis*, derived from the Latin and meaning duck-like, refers to the distinct shape of the opercular scales for their similarity to the beak of a duck.



Map 2.36.- *Tokoprymno anatis* Zapata-Guardiola and López-González, 2010. Species examined distribution map. Pink circle, holotype.

2.5 Discussion

The primnoid material collected from the 11 Antarctic expeditions has revealed a great hidden biodiversity. The family Primnoidae is already known as one of the richest in genera and species (Bayer 1998, Cairns & Bayer 2009) but, with the results of the present contribution, it currently includes 39 genera and more than 240 species. The first of these species was described in 1763 [*Primnoa resedaeformis typica* (Gunerus, 1763)] and since then the taxonomy of primnoids has increased considerable. Taxonomists are not only focused on describing new species, they are also constantly revising the more unclear genera (*Mirostenella* in Zapata-Guardiola, López-González & Gili 2013, *Thouarella* in Taylor *et al*. 2013). Thanks to these efforts the technology used to describe and identify species has improved, and because the level of precision is higher the assignment of the species to genera is being modified (Cairns & Bayer 2009, present work). One example found in the present work is the revision and the consequent reassignment of the species previously included in the genus *Amphilaphis* into an already existing genera (*Thouarella* and *Plumarella*) or into a new one (*Primnocapsa*) which it has made the current classification of the family Primnoidae more understandable, although we need more comprehensive contributions to find a consensus regarding the characters used to cluster species and genera.

Among the specimens studied one of the most abundant colonial shape was the bottlebrush, which was attributed to belong to *Dasystenella acanthina*, *Tokoprymno maia*, or to the genus *Thouarella* (Bayer 1981), however a deep study of the original descriptions and the observation of type specimens as well as the use of the Scanning Electro Microscope let us to propose new species and genera. Up to now the studied material has contributed to describe 4 new genera, 2 subgenera, 12 new species and designate 3 new combinations. Some taxonomic characters (*e.g*. the branching pattern of the colony or the arrangement of polyps on branchlets) have been commonly used to distinguish not only between genera such as *Plumarella* and *Parastenella* (Bayer 1981, Cairns & Bayer 2009), but also between species of the same genus as in the genus *Thouarella* (Kükenthal 1924, Cairns & Bayer 2009). Further morphological and molecular studies on species of the family could help to identify generic and specific morphologic characters, as well as a number of possible homoplasies, their evolution through the family Primnoidae and the establishment of relationships among different taxa.

CHAPTER 3

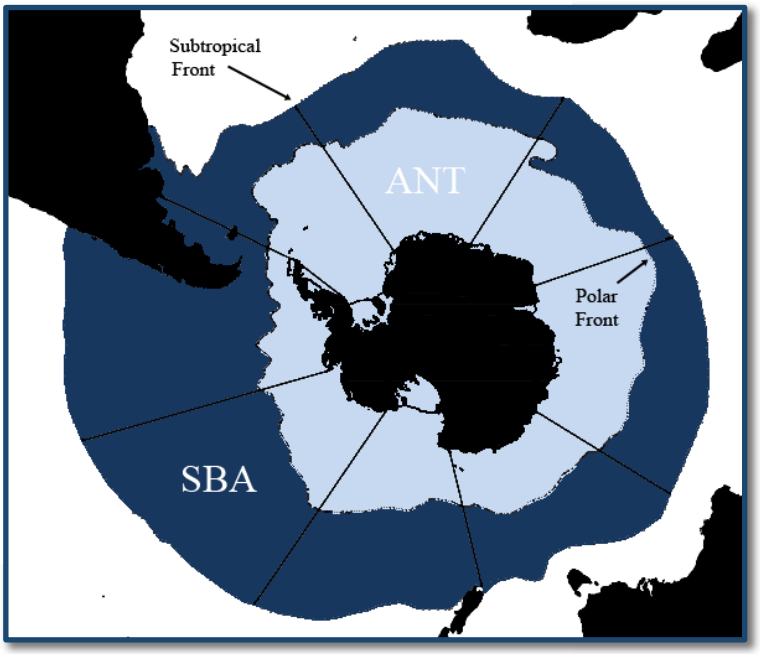
Biogeography

3.1 Introduction

3.1.1 Faunistic distribution in the Southern Ocean

3.1.1.1 Southern Ocean biogeographic subdivisions

The classical basic division of the Southern Ocean benthic fauna consists mainly in two concentric areas around the Antarctic continent, which are delimited by the continental ice sheets or land masses, and the Polar and Subtropical Fronts creating two regions known as Antarctic and Subantarctic Regions (Map 3.1).



Map 3.1.- Southern Ocean classic geographic subdivisions. **ANT**, Antarctic region; **SBA**, Subantarctic region.

Smaller size districts or sectors have also been recognized; the number and size of those districts vary depending on the fauna studied and the area of interest. In studies focusing on Antarctic molluscs and bryozoans distribution more than 29 sub-regions have been identified (Griffiths *et al.* 2009).

3.1.1.2 Distribution of Antarctic benthic organisms

General patterns of benthic distribution in the Southern Ocean have been described essentially as circumpolar (Arntz *et al.* 1994), however recent studies reinforce a distinction between Western and Eastern Antarctic fauna distribution (Rodriguez *et al.* 2007).

Studies in some groups of gastropods and bivalves (Linse *et al.* 2006) have pointed out some hot spots in South Georgia and in Weddell and Ross Seas. The availability of nutrients in the water column is seasonal dependent, and due to its high primary production this waters

become high diversity and richness. Moreover, there are diversity patterns from the Weddell Sea and Ross Sea, from the high Antarctic and Subantarctic and from the Weddell Sea, Scotia Arch and Magellan region (South America's end point, Falkland Islands and Burdwood Bank) suggesting the Weddell Sea as one of the most important components in the evolutionary history of the Antarctic benthic fauna.

In studies on peracarid crustaceans, the highest species diversity has been found in the Magellan region (De Broyer *et al.* 2004), while for isopods it is in the South Shetland Islands, for misidacea the South Georgia Island (Brandt *et al.* 1999), and for ophiuroids the East Antarctic zone, from Dronning Maud Land to Victoria Land, (Martín-Ledo & López-González in press).

3.1.1.3 Diversity of Antarctic primnoids

Since the beginning of the Southern Ocean benthic fauna exploration many primnoid genera and species have been described thanks mainly to the valuable contributions of Wright and Studer (1989), Versluys (1906), Kükenthal (1919, 1924) and Bayer (1988, 1996) among others.

During the present study new genera and species have been described (see chapter 2) other species not included in this work are still being analysed. Up to now the number of Antarctic and Subantarctic primnoids is estimated in 69 species and 27 genera (the 28% and 69% of the total described worldwide, respectively).

3.1.1.4 Previous studies on Southern Ocean primnoids distribution

Many taxonomic contributions are focused on primnoids from Tropical and Subtropical regions (*e.g.* Bayer & Stefani 1988; Cairns & Bayer 2005), however the number of contributions related to their distribution and biogeography has been almost nothing.

Wright and Studer (1889) present the geographical and bathymetrical distribution of Alcyonacea, including primnoids, however they not analyse the data in depth (*e.g.* comparisons between regions, districts or depths). Versluys (1906) in his work about the results on the Siboga Expedition, shows for the first time a small zoogeographic analysis of all known primnoids up to then, including geographical and bathymetrical distributions. More recently, López-González and Gili (2002) provided data about a high diversity in Antarctic waters for this family. These authors considered a total of 30 genera in the Primnoidae family, 17 of which are found in the Southern Ocean, and being 15 of them (the half of all primnoid genera known) exclusively from this waters. Cairns and Bayer (2009) update primnoids diversity in a global scale, they include all genera and species known up to date, however spite of they great effort, they do not undertake any posterior analysis about the possible local or regional diversity differences among these group of gorgonians.

3.1.2 Southern Ocean Protection and Management

Since 1959, the Antarctic Treaty has been the centrepiece of international cooperation to preserve the unique character of Antarctica as a natural reserve to peace and science through

exchange of information, consultation and formulation of recommendations, measures, decisions and resolutions.

Currently there are designated 71 Antarctic Specially Protected Areas (ASPAs) mainly distributed around the Antarctic Peninsula, South Shetland Islands and the Ross Sea and only seven Antarctic Specially Managed Areas (ASMAs) at the same locations where many National Antarctic Bases are located.

In the last Antarctic Treaty Consultative Meeting (XXXVI ATCM), hosted in Belgium (May 2013), a strategic work plan, which identifies priorities to be pursued under three key areas, was adopted. The strategic plan reinforces cooperation in order to ensure a robust and effective Antarctic Treaty System, where protection of the Antarctic environment, and an effective management and regulation of human activities in the continent are priorities. Moreover the Committee for Environmental Protection (CEP) identified a series of critical policy and endorsed a site clean-up manual and decided as well to develop a climate change response work plan. ATCM following CEP's advice adopted also 17 management plans for Antarctic protected areas and 16 site guidelines for visitors. (ATCM XXXVI press communication).

3.2 Objectives

It is the purpose of this chapter to get an overview and approach of the distribution of the most representative gorgonians in the Southern Ocean, the family Primnoidae. Besides to propose, or reinforce the necessity to identify and designate protected areas in the Antarctica based on its distribution to apply management plans and protection to its communities and ecosystems. Including the following specific objectives:

- Compare the primnoid fauna among and within the main biogeographic areas in the Southern Ocean.
- Compare bathymetrically the primnoid fauna between Antarctic and Subantarctic regions.
- Identify potential Antarctic Specially Protected Areas for the conservation of primnoids and its associated communities.

3.3 Material and Methods

3.3.1 Data collection

For the purpose of this chapter we have in account the same material studied in chapter 2, moreover an exhaustive bibliographic revision, including literature between 1763 and 2012, has been undertaken. Due to the lack of general revisions on family Primnoidae (the last one was Kükenthal 1924) we have considered valid the geographic and bathymetric data from species original descriptions (type species), as well those records from taxonomic revisions (*e.g.* Bayer 1998; Taylor *et al.* 2013).

3.3.2 Study area

The study area is focused on the Southern Ocean, area comprised between the Subtropical Front and the Antarctic continent.

3.3.2.1 Geographic areas

Based on distribution patterns described for benthic fauna, we used the classical geographic division of the Southern Ocean (Map 3.2a):

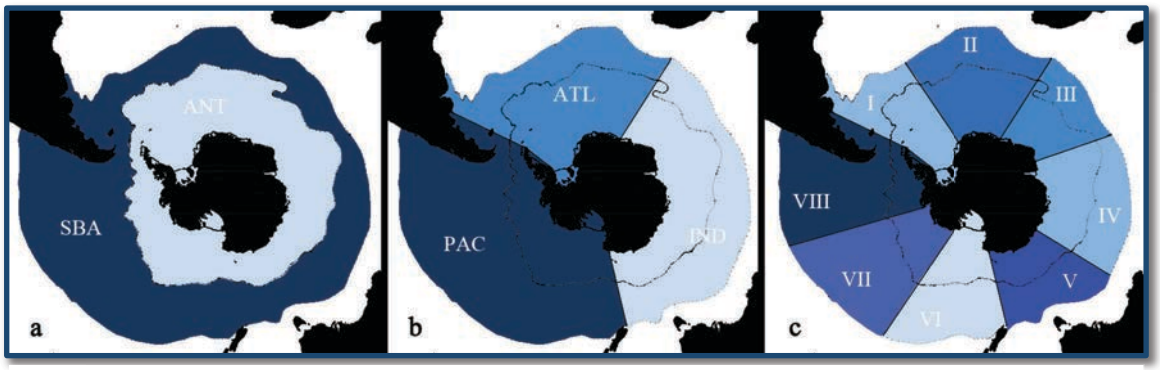
- Antarctic Region: area between the Antarctic continent and the Polar Front
- Subantarctic Region: area between the Polar Front and the Subtropical Front. The islands around the Antarctic continent and between the Polar and Subtropical fronts have been also included in this region. New Zealand Subantarctic Islands (Macquarie, Kerguelen, Heard and McDonald Islands), Crozet island, Prince Edward Island, Marion Island, as well Tristan da Cuhna and Gough Islands.

Following previous biogeographic studies in the Southern Ocean benthic fauna (Rodríguez *et al.* 2007), three categories have been identified in order to make present and future comparisons.

- ANT: species or genera present exclusively in the Antarctic Region.
- SBA: species or genera present exclusively in the Subantarctic Region.
- ANT-SBA: species or genera exclusively present in both regions Antarctica and Subantarctica.

In the same way, for comparative purposes, the study area has been divided in eight sectors (Map 3.2c), which at the same time have been gathered in three main districts (Map 3.2b).

- Atlantic district: includes sectors I and II.
- Indic district: includes sectors III, IV, and V.
- Pacific district: includes sectors VI, VII, and VIII.



Map 3.2.- Map of the Southern Ocean (SO) showing the main biogeographic subdivisions. **a**, regions of the SO, Antarctic (ANT) and Subantarctic (SBA); **b**, districts of the SO, Atlantic (ATL), Indic (IND) and Pacific (PAC); **c**, sectors of the SO numbered in roman numerals (I-VIII).

3.3.2.2 Bathymetric areas

One of the main characteristics of the Antarctic continent is its unusual depth up to 1000 m (Clarke 1996, Clarke & Johnston 2003), while the mean depth of continental shelves elsewhere is about 200 m (Walsh 1988). Meanwhile in some areas the continental shelf is quite narrow the average is almost twice that of continental shelves from other oceans (Clarke & Johnston 2003).

For the present study Antarctic and Subantarctic regions have been divided by their bathymetric features.

Antarctic Region:

- High Antarctic Area (**H**): includes the whole continental shelf, from 0 to 1000 m depth.
- Low Antarctic Area (**L**): are comprised between the continental shelf and the Polar Front.
    - Shelf Low Antarctic Area ($\mathbf{L_s}$): includes low deep shelves around Antarctic islands from 0 to 200 m depth.
    - Deep low Antarctic Area ($\mathbf{L_d}$): includes areas with depths greater than 200m.

Subantarctic Region:

- Shelf Zone ($\mathbf{S_s}$): includes shelves around Subantarctic islands.
- Deep Zone ($\mathbf{S_d}$): includes areas outside shelves and greater than 200m depth.

3.3.3 Data analysis

Statistic data and graphics about faunal composition were obtained using the Microsoft® Excel® 2011 for Mac version 14.2.4 and SPSS 15.0 (SPSS Inc.).

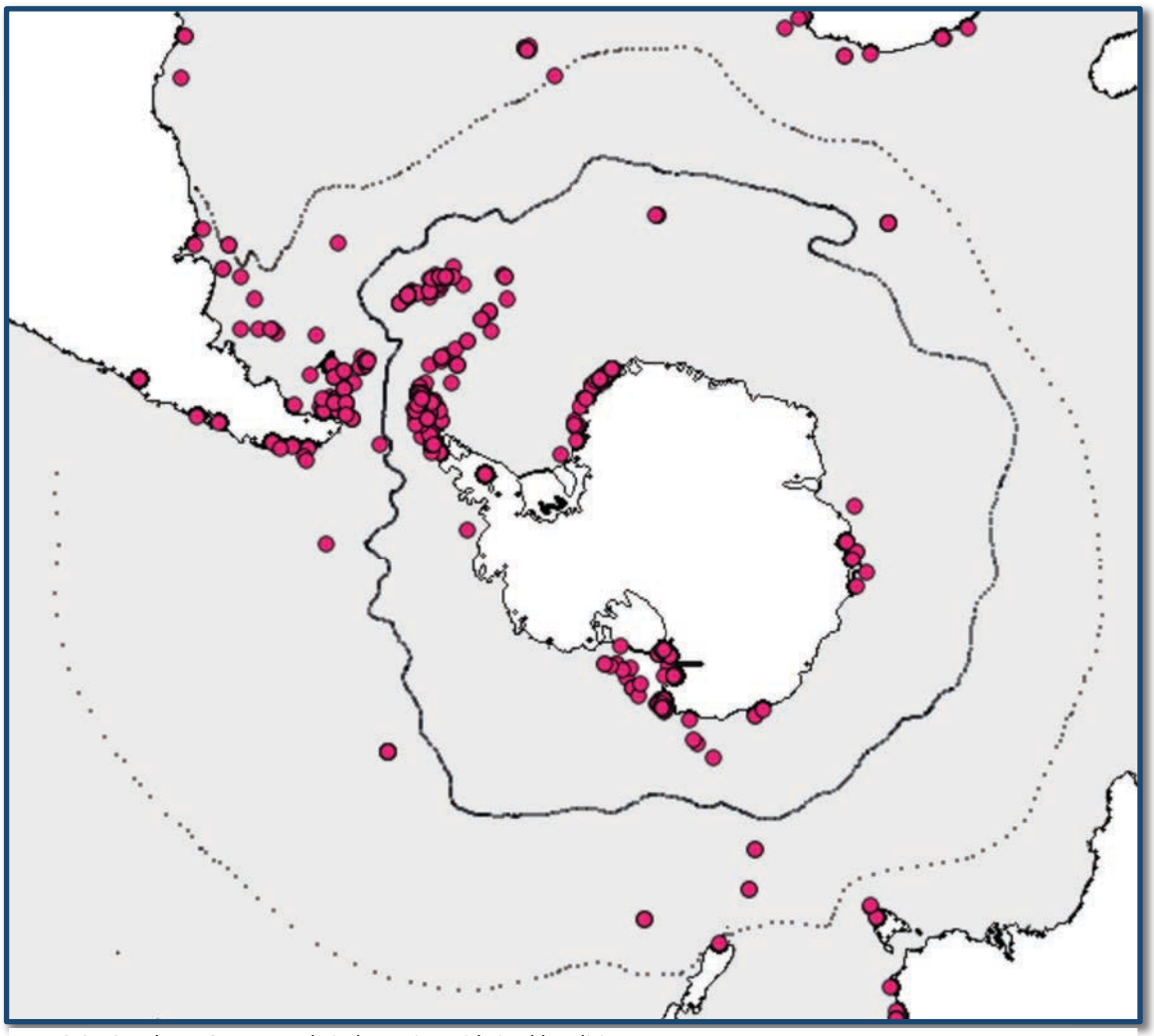
Faunistic affinities at species and genera level between regions, districts and sectors were measured using Bray-Curtis similarities index (Bray & Curtis 1957) of non-transformed presence/absence data using PRIMER version 5 (Clarke & Gorley 2006). Clusters reflect similarities between the different biogeographic areas considered.

3.4 Results

3.4.1 Faunal composition

In the present study it has been considered a total of 247 valid primnoid species and 39 valid genera. 582 cites have been taken into account for the biogeographic analyses. The majority of the cites are around Peninsula Antarctica, Ross Sea, Davis Sea, Cape Norvegia, South Shetland Island and Orkney Islands and Falkland Islands and Tierra del Fuego in South America (Map 3.3). The primnoids distribution also shows 3 main vast areas without or a few primnoid localities, from Cape Norvegia to Pritz Bay, from Budd Coast to Adelie Land, and from the Ross Sea to Peninsula Antarctica (Bellingshausen and Amundsen Sea). This lack of records may be due to the low sampling effort carried out in those areas. More sampling and accurate

identifications are needed to have a better approximation of the real primnoids distribution at the Southern Ocean.



Map 3.3.- Southern Ocean. Red circles, primnoid cited localities.

In the Southern Ocean (SO), which is the study area, 69 species (Table 3.1) and 27 genera (Table 3.2) have been reported. This numbers indicate that almost one third (28%) of worldwide primnoid species and about two thirds (69%) of worldwide primnoid genera are present in the Southern Ocean.

Fifty-six species and 15 genera have been cited exclusively in the SO, which represent more than 80% and 50% of endemicity respectively.

Nine genera present in the SO are monotypic (*Aglaoprimnoa*, *Armadillogorgia*, *Arntzia*, *Dasystenella*, *Heptaprimnoa*, *Mirostenella*, *Onogorgia*, *Pyrogorgia*, and *Tauroprimnoa*), and 4 genera (*Callozostron*, *Narella*, *Parastenella*, and *Primnoa*) have only one species cited in the Southern Ocean.

The Antarctic and Subantarctic regions share 12 species, 8 of them exclusively from the SO, and 4 also found outside the SO (Table 3.3), they also share 10 genera, 5 of them exclusively from the SO, and 5 also found outside the SO (Table 3.4).

	ATL				IND						PAC						Distribution		
	IA	IS	IIA	IIS	IIIA	IIIS	IVA	IVS	VA	VS	VIA	VIS	VIIA	VIIS	VIIIA	VIIIS	ANT	SBA	C
Aglaoprimnoa stefanii	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-
Ainigmaptilon antarcticum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Ainigmaptilon edisto	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-
Ainigmaptilon haswelli	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-	-
Ainigmaptilon virgularoides	x	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Ainigmaptilon wallini	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-
Armadillogorgia cyathella	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-
Arntzia gracilis	x	-	x	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Callozostron carlottae	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Callozostron diplodiadema	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Callozostron mirabile	x	-	-	-	-	-	x	-	x	-	-	x	-	-	-	-	x	x	x
Convexella divergens	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-
Convexella magelhaenica	-	x	-	-	-	-	x	-	x	-	-	-	-	-	-	x	x	x	x
Convexella murrayi	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Dasystenella acanthina	x	x	x	-	-	-	x	-	x	-	x	-	-	-	x	x	x	x	x
Digitogorgia brochi	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-
Digitogorgia kuekenthali	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Fanellia tuberculata	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-
Fannyella abies	x	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Fannyella kuekenthali	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Fannyella rossi	x	-	x	-	-	-	x	-	x	-	x	-	-	-	x	-	x	-	x
Fannyella spinosa	x	-	x	-	-	-	x	-	x	-	x	-	-	-	-	-	x	-	x
Heptaprimnoa patagonica	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Metafannyella aurora	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Metafannyella eos	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-
Metafannyella lepidota	x	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Metafannyella mawsoni	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Mirostenella articulata	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Narella gaussi	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-
Onogorgia nodosa	x	-	-	-	-	-	x	-	-	-	x	-	-	-	x	-	x	-	x
Ophidiogorgia kuekenthali	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Ophidiogorgia paradoxa	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Parastenella spinosa	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-
Perissogorgia vitrea	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-
Plumarella bayeri	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Plumarella castellviae	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Plumarella delicatissima	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Plumarella delicatula	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	x	-
Plumarella diadema	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-
Plumarella undulata	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Primnoa notialis	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-
Primnoeides sertularoides	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-
Primnoella antarctica	x	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-
Primnoella chilensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Primnoella divaricata	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Primnoella polita	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Primnoella scotiae	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Pyrogorgia lemnos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Scopaegorgia liouvillei	x	-	x	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Tauroprimnoa austasensis	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Thouarella affinis	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-
Thouarella andeep	x	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Thouarella antarctica	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	-
Thouarella brucei	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	x	x	x	-
Thouarella chilensis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Thouarella crenelata	x	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Thouarella dispersa	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-
Thouarella grandiflora	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-
Thouarella hicksoni	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-
Thouarella koellikeri	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	-
Thouarella minuta	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Thouarella parachilensis	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Thouarella pendulina	x	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Thouarella regularis	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-
Thouarella striata	-	-	x	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-	-
Thouarella variabilis	x	-	x	-	-	x	x	-	-	-	x	-	-	-	x	x	x	x	x
Thouarella viridis	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	x	-
Tokoprymno anatis	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Tokoprymno maia	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-

Table 3.1.- Primnoid species present in the Southern Ocean. Species in bold blue are endemic to the SO. **ATL**, Atlantic district; **IND**, Indic district; **PAC**, Pacific district. Roman numerals indicate sectors and letter **A** indicate Antarctic region and letter **S** Subantarctic region. **ANT**, Antarctic region; **SBA**, Subantarctic region; **C**, circumpolar distribution.

	ATL				IND						PAC						Distribution		
	IA	IS	IIA	IIS	IIIA	IIIS	IVA	IVS	VA	VS	VIA	VIS	VIIA	VIIS	VIIIA	VIIIS	ANT	SBA	C
Aglaoprimnoa	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-
Ainigmaptilon	x	-	-	-	-	-	x	-	x	-	x	-	-	-	x	-	x	-	x
Armadillogorgia	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-
Arntzia	x	-	x	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Callozostron	x	-	-	-	-	-	x	-	x	-	-	x	-	-	-	-	x	x	x
Convexella	-	x	-	-	-	-	x	-	x	-	x	-	-	-	-	x	x	x	x
Dasystenella	x	x	x	-	-	-	x	-	x	-	x	-	-	-	x	x	x	x	x
Digitogorgia	-	x	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-
Fanellia	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-
Fannyella	x	-	x	-	-	-	x	-	x	-	x	-	-	-	x	-	x	-	x
Heptaprimnoa	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Metafannyella	x	-	x	-	-	-	-	-	-	x	x	-	-	-	x	-	x	x	x
Mirostenella	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Narella	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	x	-	-
Onogorgia	x	-	-	-	-	-	x	-	-	-	x	-	-	-	x	-	x	-	x
Ophidiogorgia	x	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-	-
Parastenella	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-
Perissogorgia	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	-	-	x	-
Plumarella	x	x	-	-	-	-	-	-	-	x	-	-	-	-	-	x	x	x	x
Primnoa	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	-	x	-
Primnoeides	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	x	-
Primnoella	x	x	x	-	-	-	-	-	-	-	x	-	-	-	-	x	x	x	-
Pyrogorgia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	x	-
Scopaegorgia	x	-	x	-	-	-	-	-	-	-	x	-	-	-	x	-	x	-	-
Tauroprimnoa	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-
Thouarella	x	x	x	x	-	x	x	-	-	-	x	-	-	-	x	x	x	x	x
Tokoprymno	x	-	-	-	-	-	-	-	-	-	-	-	-	x	-	-	x	x	-

Table 3.2.- Primnoid genera present in the Southern Ocean. Genera in bold blue are endemic to the SO. **ATL**, Atlantic district; **IND**, Indic district; **PAC**, Pacific district. Roman numerals indicate sectors and letter **A** indicate Antarctic region and letter **S** Subantarctic region. **ANT**, Antarctic region; **SBA**, Subantarctic region; **C**, circumpolar distribution.

	WW	SO			ANT			SBA			shared ANT-SBA		
	T	T	E	+out	T	E	+out	T	E	+out	T	E	+out
spp	247	69	56	13	49	34	3	32	14	6	12	8	4
% WW		28	23		20	14		13	6				
% SO			81		71	49		46	20				
% ANT						69							
% SBA									44				

Table 3.3.- Total number and percentage of primnoid species. **WW**, worldwide; **SO**, Southern Ocean, **ANT**, Antarctic region; **SBA**, Subantarctic region; **T**, total of species; **E**, endemic species; **+out**, species also present outside the SO.

	WW	SO			ANT			SBA			shared ANT-SBA		
	T	T	E	+out	T	E	+out	T	E	+out	T	E	+out
gen	39	27	15	11	19	7	2	18	3	5	10	5	5
% WW		69	38		49	18		46	8				
% SO			56		70	26		67	11				
% ANT						37							
% SBA									17				

Table 3.4.- Total number and percentage of primnoid genera. **WW**, worldwide; **SO**, Southern Ocean, **ANT**, Antarctic region; **SBA**, Subantarctic region; **T**, total of species; **E**, endemic species; **+out**, species also present outside the SO.

The Antarctic region (ANT) includes 49 species, 19.8% of worldwide primnoid species and 70.1% of the SO primnoids. Of these 34 are endemic, representing about 69% of the total species present in ANT, and about 50% if we have in account the total species present in the SO, and more than 13% including all primnoid species worldwide (Table 3.3). There are 3

species (*Callozostron carlottae*, *Scopaegorgia liouvillei*, and *Thouarella hicksoni*) in the ANT that have been reported from other regions outside the SO but not from the Subantarctic yet (Table 3.1).

The Antarctic region primnoids comprises 19 genera, almost a half (48%) of worldwide primnoid genera and 70% of the SO primnoids. Of these 7 are endemic, representing about 37% of the total genera present in ANT, about 26% if we have in account the total genera present in the SO, and more than 17% including all primnoid genera worldwide (Table 3.4). Two genera (*Narella*, and *Scopaegorgia*) have been found in the ANT as well as in other regions outside the SO, but not in the Subantarctic region yet (Table 3.2).

The Subantarctic region (SBA) includes 32 species, 13% of worldwide primnoid species and 47% of SO primnoids. Of these 14 are endemic, representing more than 40% of the total species present in SBA, about 20% if we have in account the total species present in the SO, and a bit less than 6% including all primnoid species worldwide (Table 3.3). There are 6 species present in the SBA and also in other regions outside the SO (Table 3.1).

The Subantarctic region primnoids comprises 18 genera, almost a half (46%) of worldwide primnoid genera and 67% of SO primnoids. Of these 3 are endemic to the SBA, representing about 17% of the total genera present in SBA, about 11% if we have in account the total genera present in the SO, and more than 7% including all primnoid genera worldwide (Table 3.4). Five genera are found in the SBA and also in other regions outside the SO (Table 3.2).

3.4.1.1 Primnoid composition in longitudinal districts

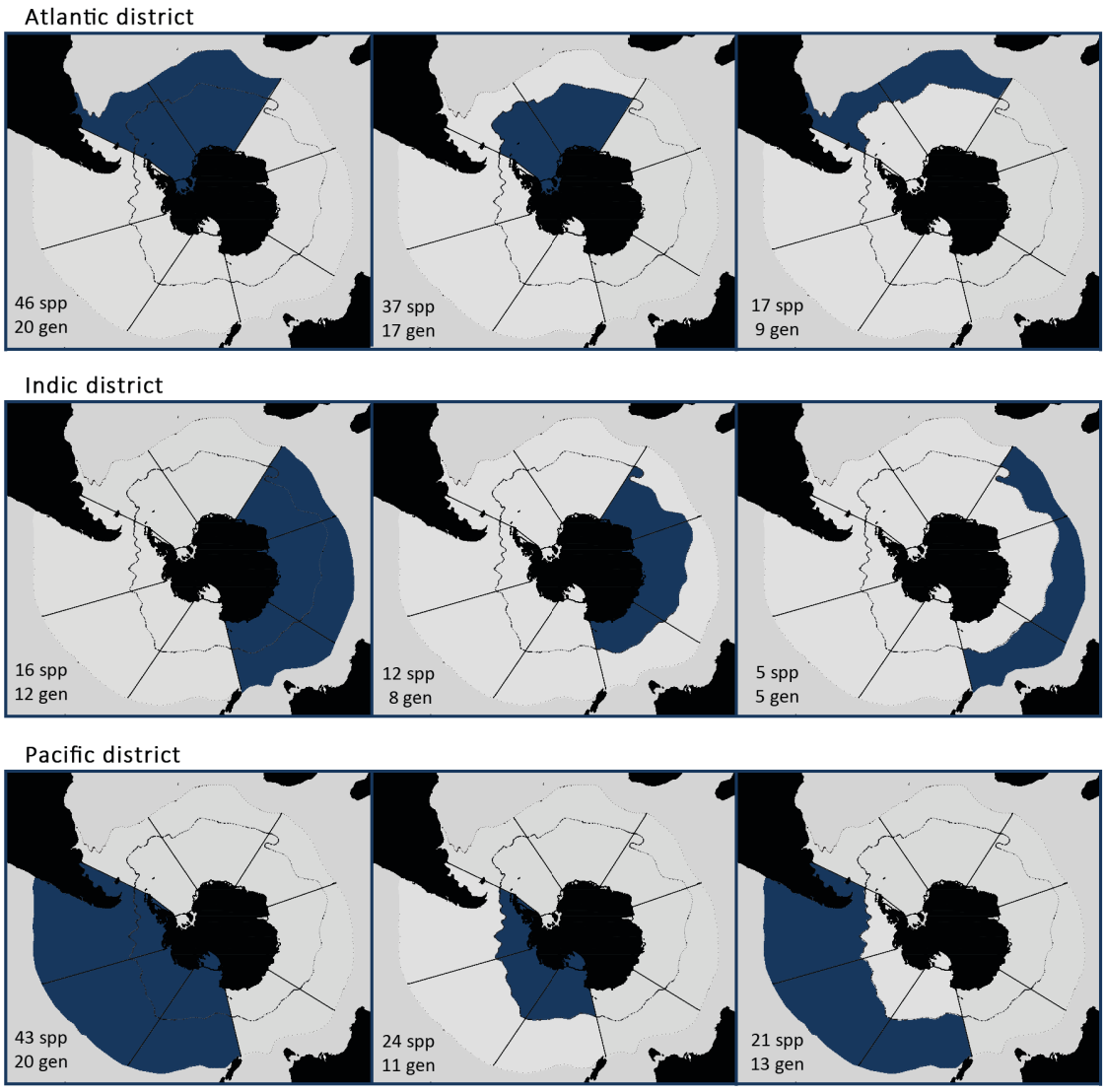
Taking into account the Southern Ocean, the more diverse district in species number is the Atlantic followed by the Pacific district, being the Indian district the least diverse. In number of genera both Atlantic and Pacific are equally diverse while Indian district is almost half of diverse. The Antarctic region follows the same pattern; the Atlantic district is the more diverse in species and genera followed by the Pacific and the Indic district. However in the Subantarctic region the more diverse district is the Pacific, followed by the Atlantic and being the Indic district the less diverse in both Antarctic and Subantarctic regions (Table 3.5).

Atlantic and Pacific districts correspond with the areas, which include more research stations in Antarctica (Peninsula Antarctica, Ross Sea, and Cape Norvegia), and thus more research expeditions are carried out in these waters.

The three districts (Atlantic, Indic and Pacific) have 7 species (10%) and 9 genera (33%) in common in the whole Southern Ocean region, which may be considered to have a circumpolar distribution. However taking into account only the Antarctic region, the three districts have only 5 species and 5 genera in common, which have a circumpolar distribution. If taking into account only the Subantarctic region, no species are shared among the three districts, but they have 2 genera in common which have a circumpolar distribution (Table 3.1 and 3.2).

	ATL				IND				PAC			
	T		E		T		E		T		E	
spp	46		13		16		8		43		10	
%	67		37		23		50		62		23	
gen	20		4		12		-		20		1	
%	74		20		44		-		74		5	
	ANT		SBA		ANT		SBA		ANT		SBA	
	T	E	T	E	T	E	T	E	T	E	T	E
spp	37	9	17	1	12	5	5	3	24	5	21	5
%	80	24	37	6	75	42	31	60	56	21	49	24
gen	17	2	9	-	8	-	5	-	11	-	13	1
%	85	12	45	-	67	-	42	-	55	-	65	

Table 3.5.- Number and percentage of primnoid species and genera in the different districts. **ATL**, Atlantic district; **IND**, Indic district; **PAC**, Pacific district; **ANT**, Antarctic region; **SBA**, Subantarctic region; **T**, total of species or genera; **E**, endemic species or genera.



Map 3.4.- Southern Ocean divided in biogeographic districts and regions. Total number of primnoid species and genera are noted for each biogeographic division.

3.4.1.2 Primnoid composition in longitudinal sectors

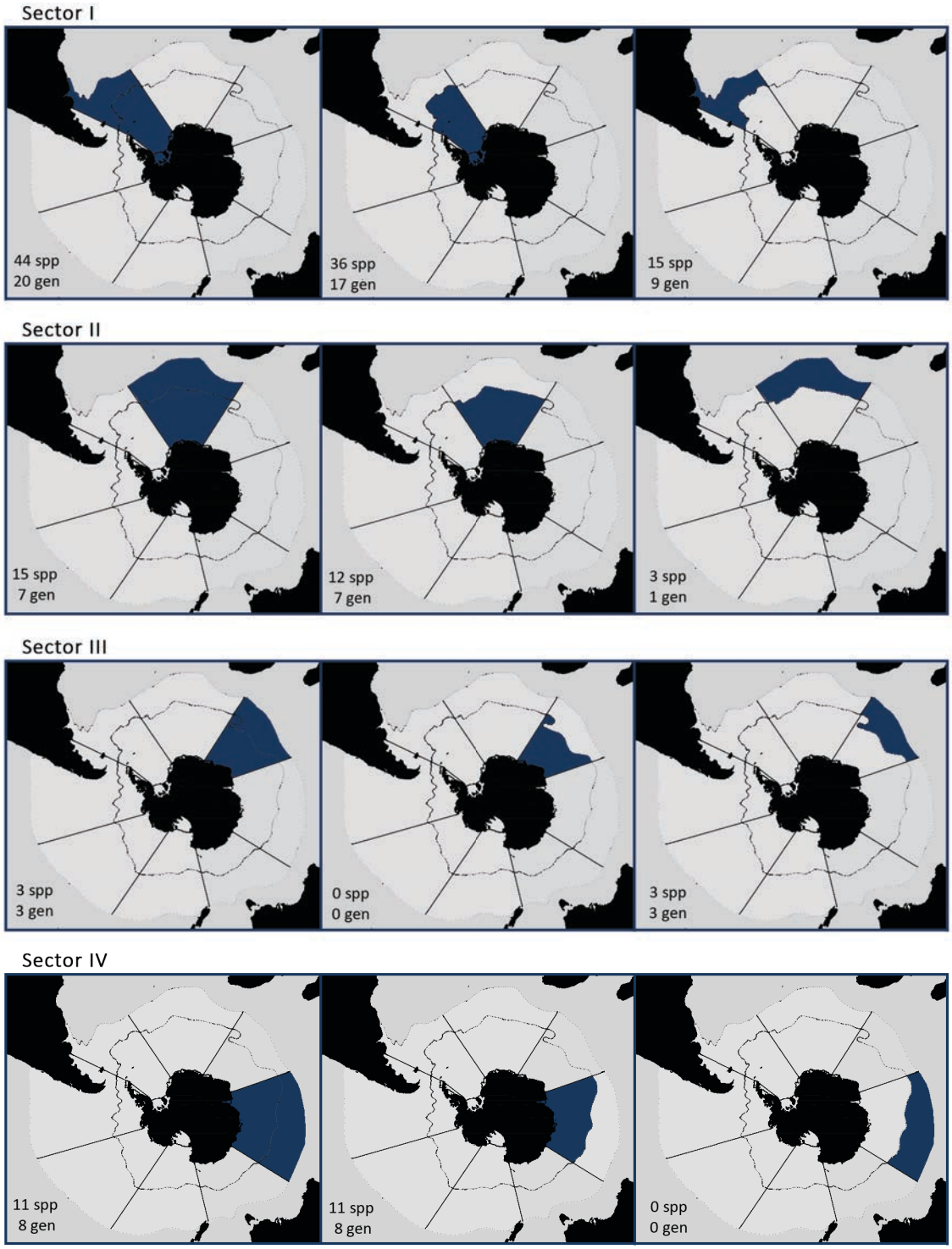
Similarly to districts, the more diverse sectors in the Southern Ocean are sectors I, VIII, and VI corresponding with east Peninsula Antarctica/Cape Norvegia, Peninsula Antarctica and Ross Sea respectively. The Antarctic and Subantarctic region follow the same diversity pattern as the Southern Ocean with sectors I, VIII and VI as the more diverse. The less diverse sectors in the Southern Ocean and in the Antarctic and Subantarctic region are sectors III, and VII (Table 3.6).

In the SO the sector I includes two thirds of total SO primnoid species (64%), and about 74% of total SO primnoid genera. 10 species and 4 genera are exclusive of this sector. There is no species or genera exclusive from sector II. And the majority of species and genera in this sector are found in the Antarctic region. The Subantarctic region includes 3 species, all of them belonging to the genus *Thouarella* which is the only genus present in this region. In sector II all species and genera are found only in the Subantarctic region, no species or genera have been found in the Antarctic. Only the species *Parastenella spinosa* is exclusively from this sector, but no genera. In sector IV four species *Ainigmaptilon edisto, Narella gaussi, Thouarella dispersa, Thouarella grandiflora* are exclusive from this sector. All the species of this sector have been found in the Antarctic region, any species have been cited in the Subantarctic yet. *Ainigmaptilon haswelli, Metafannyella eos,* and *Plumarella delicatula* are exclusive form sector V. Three species are exclusive to sector VI, *Ainigmaptilon wallini, Convexella divergens,* and *Digitogorgia brochi*. The majority of species and genera are found in the Antarctic region. Only 2 genera and species (*Primnoa notialis* and *Tokoprymno maia*) have been cited in sector VII, both species are also exclusive from the Subantarctic region of this sector. In the SO the sector VIII includes almost a half (45%) of total SO primnoid species and genera (56%). Five species are exclusive to this sector, *Ainigmaptilon antarcticum, Ophidiogorgia kuekenthali, Primnoella chilensis, Pyrogorgia lemnos,* and *Thouarella chilensis*. The genus *Pyrogorgia* is exclusive of the Subantarctic region of this sector (Table 3.1, 3.2).

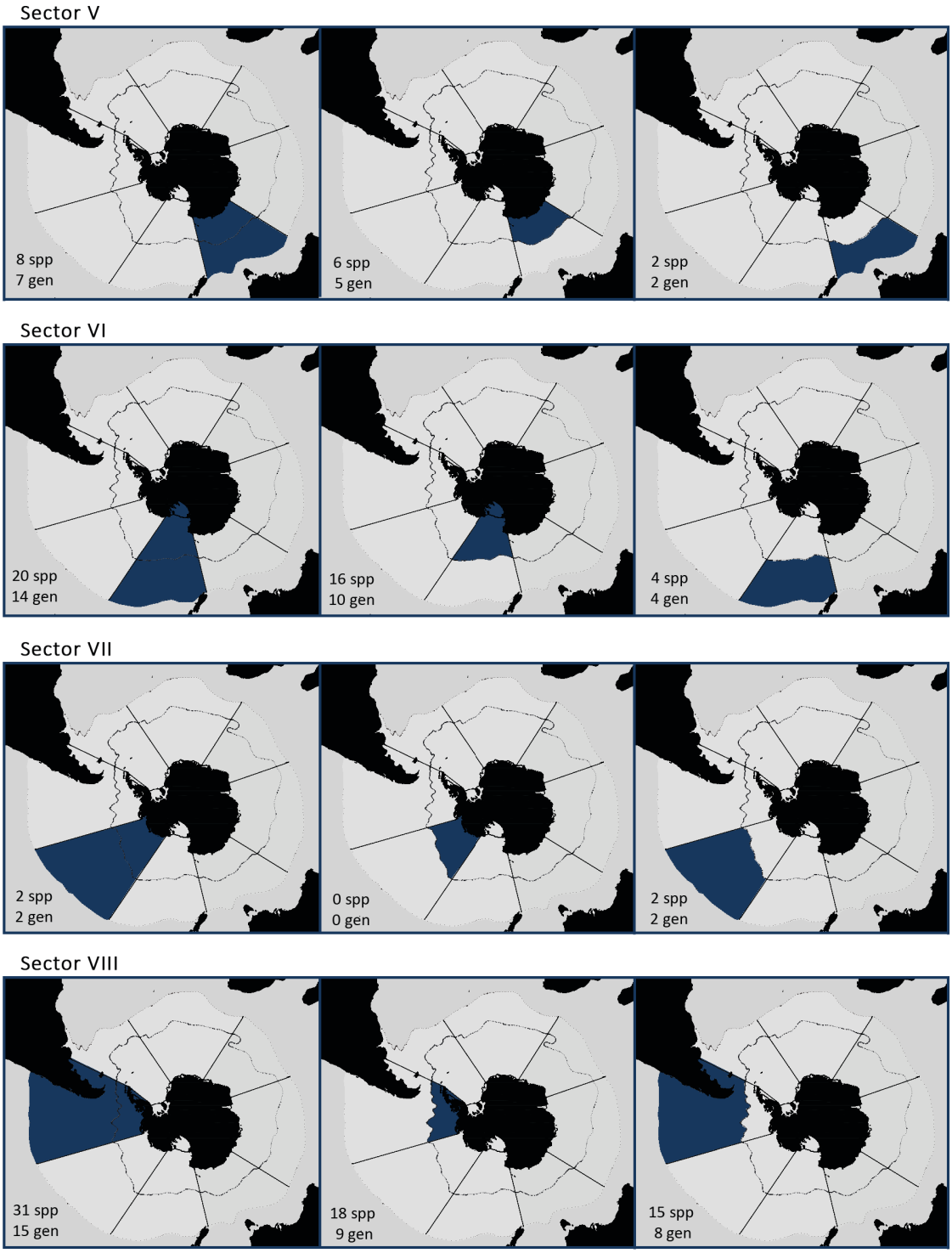
No species or genera are shared among the eight sectors in the whole SO neither taking into account only the Antarctic region nor the Subantarctic region. But three species and genera have been found in 6 of 8 sectors in the SO, two species (*Dasystenella acanthina* and *Fannyella rossii*) have been found in 6 of 8 sectors of the Antarctic region, and only *Thouarella brucei* has been found in 3 sectors and the genus *Thouarella* in 4 of 8 sectors of the Subantarctic region (Table 3.1 and 3.2).

	I		II		III		IV		V		VI		VII		VIII	
	T	E	T	E	T	E	T	E	T	E	T	E	T	E	T	E
spp	44	10	15	-	3	1	11	4	8	3	20	3	2	2	31	5
%	64	23	22	-	4	33	16	36	12	38	29	15	3	100	45	16
gen	20	4	7	-	3	-	8	-	7	-	14	-	2	-	15	1
%	74	20	26	-	11	-	30	-	26	-	52	-	7	-	56	7
	ANT	SBA	ANT	SBA	ANT	SBA	ANT	SBA	ANT	SBA	ANT	SBA	ANT	SBA	ANT	SBA
	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E	T E
spp	36 7	15 1	12 -	3 -	- -	3 1	11 4	- -	6 1	2 2	16 2	4 1	- -	2 2	18 3	15 2
%	82 19	34 7	80 -	20 -	- -	100 33	100 36	- -	75 17	25 100	80 13	20 25	- -	100 100	58 17	48 13
gen	17 2	9 -	7 -	1 -	- -	3 -	8 -	- -	5 -	2 -	10 -	4 -	- -	2 -	9 -	8 1
%	85 12	45 -	100 -	14 -	- -	100 -	100 -	- -	71 -	29 -	71 -	29 -	- -	100 -	60 -	53.3 13

Table 3.6.- Number and percentage of primnoid species and genera in the different sectors. **ANT**, Antarctic region; **SBA**, Subantarctic region; **T**, total of species or genera; **E**, endemic species or genera.



Map 3.5.- Southern Ocean divided in biogeographic sectors (I-IV) and regions (ANT-SBA). Total number of primnoid species and genera are noted for each biogeographic division.



Map 3.6.- Southern Ocean divided in biogeographic sectors (VI-VIII) and regions (ANT-SBA). Total number of primnoid species and genera are noted for each biogeographic division.

3.4.2 Faunal affinities

3.4.2.1 Southern Ocean

The Antarctic and Subantarctic primnoid fauna show 21.13% of similarity at the species level, sharing a total of 12 species (Table 3.7). Both regions have a slightly greater level of similarity at the generic level (28,98%), sharing a total of 10 genera (Table 3.7).

ANT-SBA shared species	ANT-SBA shared genera
Aglaoprimnoa stefanii	*Aglaoprimnoa*
Armadillogorgia cyathella	*Armadillogorgia*
Callozostron mirabile	*Callozostron*
Convexella magelhaenica	*Convexella*
Dasystenella acanthina	*Dasystenella*
Plumarella diadema	*Metafannyella*
Thouarella affinis	*Plumarella*
Thouarella antarctica	*Primnoella*
Thouarella brucei	*Thouarella*
Thouarella koellikeri	*Tokoprymno*
Thouarella variabilis	
Thouarella viridis	

Table 4.7.- Shared species and genera in the Antarctic and Subantarctic regions.

3.4.2.2 Faunal affinities between districts

The similarity analysis among the different districts showed that Atlantic and Pacific districts have the highest similarity at species and genus levels (Fig. 3.1, Table 3.7), sharing a total of 28 species (Table 3.8). While the Indic district shows lower values of similarity with the Atlantic and Pacific districts (Table 3.7). Only seven species are present in the three main districts, and are thus considered to have a circumpolar distribution (Table 3.1a). Same patterns are found when the faunal affinities have been analysed in more detail in the Antarctic and Subantarctic regions (Table 3.7).

IND	25.287	38.205			28.125	37.735			0	7.1429		
PAC	50	57.928	28.902	49.521	48.113	55.562	27.586	54.760	41.667	45.589	7.0175	7.1197
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
	ATL		**IND**		**ATL**		**IND**		**ATL**		**IND**	
	Southern Ocean				**Antarctica**				**Sub-Antarctica**			

Table 3.7.- Similarity matrix of primnoid species and genera between different districts. **ATL**, Atlantic district; **IND**, Indic district; **PAC**, Pacific district.

IND	7	9			6	6			0	2		
PAC	28	16	7	9	18	10	5	6	10	7	1	2
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
	ATL		**IND**		**ATL**		**IND**		**ATL**		**IND**	
	Southern Ocean				**Antarctica**				**Sub-Antarctica**			

Table 5.8.- Matrix of shared primnoid species and genera between the different districts. **ATL**, Atlantic district; **IND**, Indic district; **PAC**, Pacific district.

Under a 40% of similarity between species, the dendrogram divides fairly neatly into three clusters of species, two of these groups can be identified with two of the clusters emerged from the sample dendrogram, corresponding to Indic and Pacific districts. However the third cluster of species includes species present in the Atlantic but also species shared between Atlantic and Pacific and even present in the three districts (Fig. 3.1b). This third cluster seems to include species that are at both sides of the South American cone.

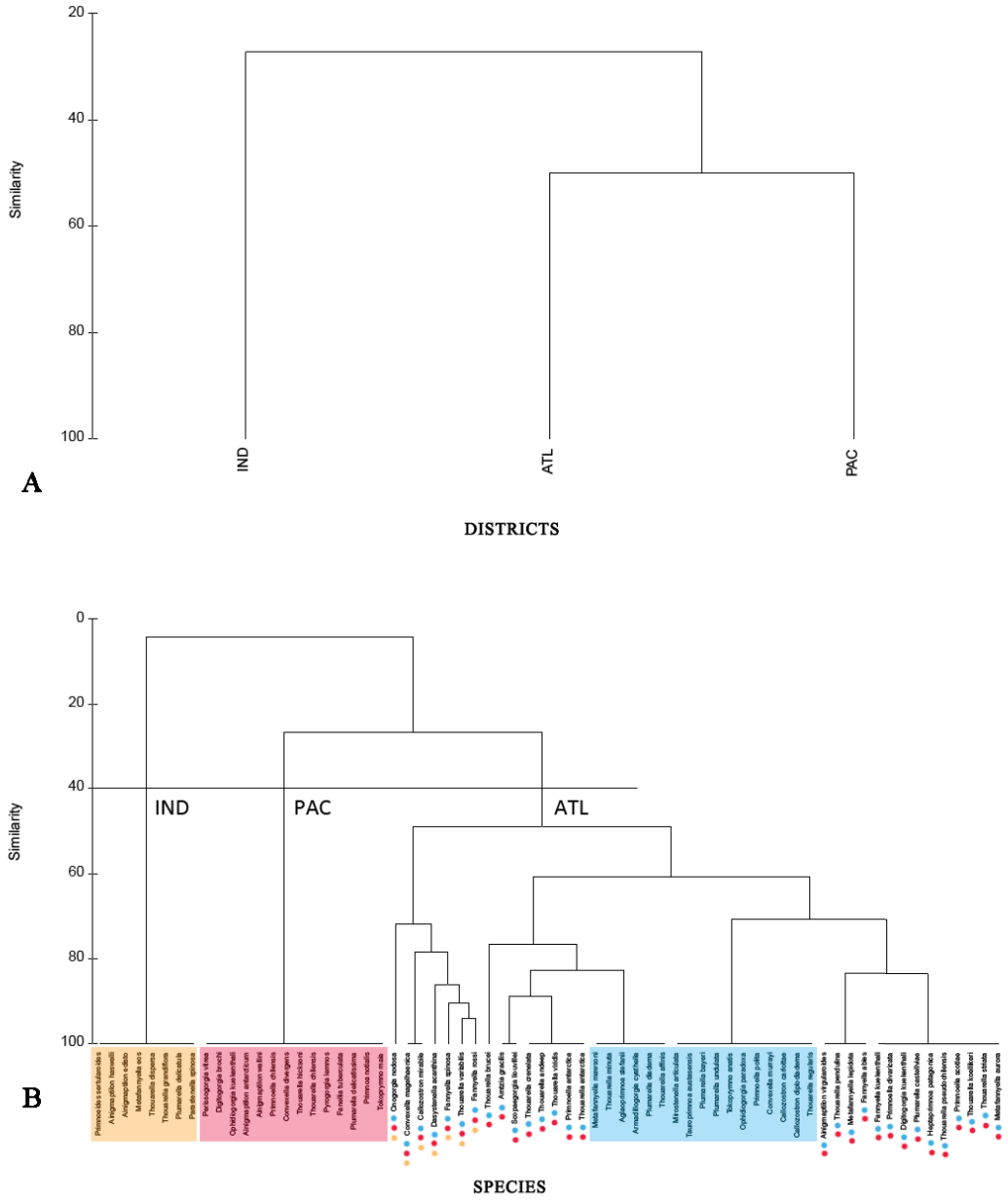


Figure 3.1.- a, similarity plot between Indic (IND), Atlantic (ATL) and Pacific (PAC) districts by species composition; **b**, similarity clusters of primnoid species by their distribution in the Southern Ocean regions. Orange, Indic distribution; Red, Pacific distribution; Blue, Atlantic distribution.

When comparing the districts in more detail based on the regions (ANT- SBA), the dendrogram (Fig. 3.2) clusters together the three Antarctic districts in one side and the three Subantarctic districts in the other one. Thus indicates that regions are more important than sectors in a biogeographic sense. Similarity values (Table 3.9) show that for each region the most similar districts are the Atlantic and Pacific, and sharing 18 and 10 species respectively (Table 3.10) for the Antarctic and Subantarctic regions, showing again a high similarity in the South American cone.

ATLs	24.242	31.755								
INDa	28.125	44.337	11.765	27.533						
INDs	3.774	3.496	0	1.709	9.524	0.886				
PACa	48.113	60.043	4.918	20.409	27.586	58.661	7.229	5.868		
PACs	15.054	14.905	41.667	48.938	17.778	22.561	7.018	2.166	7.273	16.013
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
	ATLa		**ATLs**		**INDa**		**INDs**		**PACa**	

Table 3.9.- Similarity matrix of primnoid species and genera between different districts and regions. **ATLa**, Atlantic district of the Antarctic region; **ATLs**, Atlantic district of the Subantarctic region; **INDa**, Indic district of the Antarctic region; **INDs**, Indic district of the Subantarctic region; **PACa**, Pacific district of the Antarctic region; **PACs**, Pacific district of the Subantarctic region.

ATLs	8	6								
INDa	6	6	2	3						
INDs	1	3	0	2	1	1				
PACa	18	10	1	4	5	6	1	2		
PACs	7	6	10	7	4	4	1	2	2	4
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
	ATLa		**ATLs**		**INDa**		**INDs**		**PACa**	

Table 6.10.- Matrix of shared primnoid species and genera between the different districts and regions. **ATLa**, Atlantic district of the Antarctic region; **ATLs**, Atlantic district of the Subantarctic region; **INDa**, Indic district of the Antarctic region; **INDs**, Indic district of the Subantarctic region; **PACa**, Pacific district of the Antarctic region; **PACs**, Pacific district of the Subantarctic region.

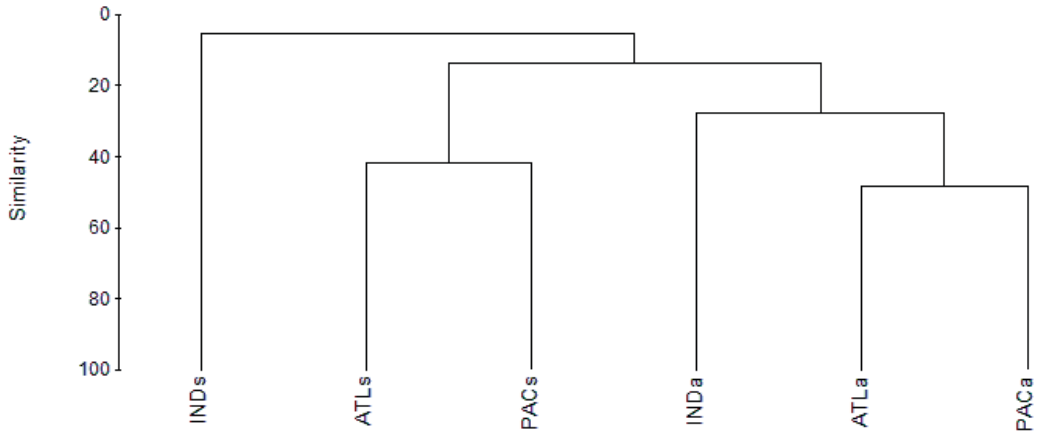


Figure 3.2.- Similarity plot between Indic Antarctic (INDa), Indic Subantarctic (INDs), Atlantic Antarctic (ATLa), Atlantic Subantarctic (ATLs), Pacific Antarctic (PACa) and Pacific Subantarctic (PACs) districts by species composition

3.4.2.3 Faunal affinities between sectors

The similarity analysis among the different sectors showed that under a 40% of similarity the dendrogram (Fig. 3.3) divides into four clusters (VII, III, I-II-VI-VIII, and IV-V). Sectors III and VII, have the least resemblance with the rest of the sectors, and also correspond with the least studied areas which may influence the low similarities (Table 3.11a). The cluster including sectors I, II, VI, and VIII correspond with the western Southern Ocean, which under a 60% the South American cone (I-VIII) is clearly defined with 24 species in common (Tables 3.12a, 3.13). The cluster of sectors IV and V are the sectors of the Australian side (Fig. 3.3), which share 5 species.

a) Southern Ocean

	I		II		III		IV		V		VI		VII	
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
II	39.394	36.847												
III	3.5088	0.9932	9.5238	2.7558										
IV	22.535	44.158	22.857	32.206	15.385	2.1001								
V	16.949	24.899	26.087	48.585	0	0	35.714	47.959						
VI	36.62	47.607	45.714	62.362	7.6923	1.7935	30	50.372	28.571	44.406				
VII	0	4.7453	0	0	0	0	0	0	0	0	0	0		
VIII	61.905	66.568	33.333	55.312	10.256	1.5005	26.415	51.864	14.634	34.153	37.736	64.852	0	0

b) Antarctica

	I		II		III		IV		V		VI		VII	
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
II	45.833	50.122												
III	0	0	0	0										
IV	26.087	33.711	36.364	38.064	0	0								
V	19.048	22.692	33.333	39.067	0	0	62.5	81.013						
VI	46.154	57.764	57.143	82.127	0	0	38.462	61.849	27.273	45.051				
VII	0	0	0	0	0	0	0	0	0	0	0	0		
VIII	55.556	58.804	46.667	70.046	0	0	28.571	55.462	16.667	36.856	58.824	82.346	0	0

c) Sub-Antarctica

	I		II		III		IV		V		VI		VII	
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
II	11.111	11.268												
III	0	3.0651	0	11.111										
IV	0	0	0	0	0	0								
V	0	5.9322	0	0	0	0	0	0						
VI	0	9.3645	0	0	0	0	0	0	0	0				
VII	0	0	0	0	0	0	0	0	0	0	0	0		
VIII	66.667	71.196	11.111	13.408	11.111	3.5242	0	0	0	6.9307	0	10.566	0	0

Table 3.11.- Similarity matrix of primnoid species and genera between different sectors. **a**, Southern Ocean; **b**, Antarctic region; **c**, Subantarctic region.

II	26	7												
III	1	1	1	1										
IV	7	7	4	3	1	1								
V	5	7	3	3	0	0	5	5						
VI	12	12	8	7	1	1	6	7	4	6				
VII	0	1	0	0	0	0	0	0	0	0	0	0		
VIII	24	14	8	7	1	1	5	6	3	5	10	11	0	0
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
a)	**I**		**II**		**III**		**IV**		**V**		**VI**		**VII**	

Southern Ocean

II	11	7												
III	0	0	0	0										
IV	6	6	4	3	0	0								
V	4	4	3	2	0	0	5	5						
VI	12	9	7	7	0	0	5	6	3	4				
VII	0	0	0	0	0	0	0	0	0	0	0	0		
VIII	15	9	7	6	0	0	4	3	2	3	10	8	0	0
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
b)	**I**		**II**		**III**		**IV**		**V**		**VI**		**VII**	

Antarctica

II	1	1												
III	0	1	0	1										
IV	0	0	0	0	0	0								
V	0	1	0	0	0	0	0	0						
VI	0	1	0	0	0	0	0	0	0	0				
VII	0	0	0	0	0	0	0	0	0	0	0	0		
VIII	10	7	1	1	1	1	0	0	0	1	0	1	0	0
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
c)	**I**		**II**		**III**		**IV**		**V**		**VI**		**VII**	

Sub-Antarctica

Table 3.12.- Matrix of shared primnoid species and genera between different sectors. **a**, Southern Ocean; **b**, Antarctic region; **c**, Subantarctic region.

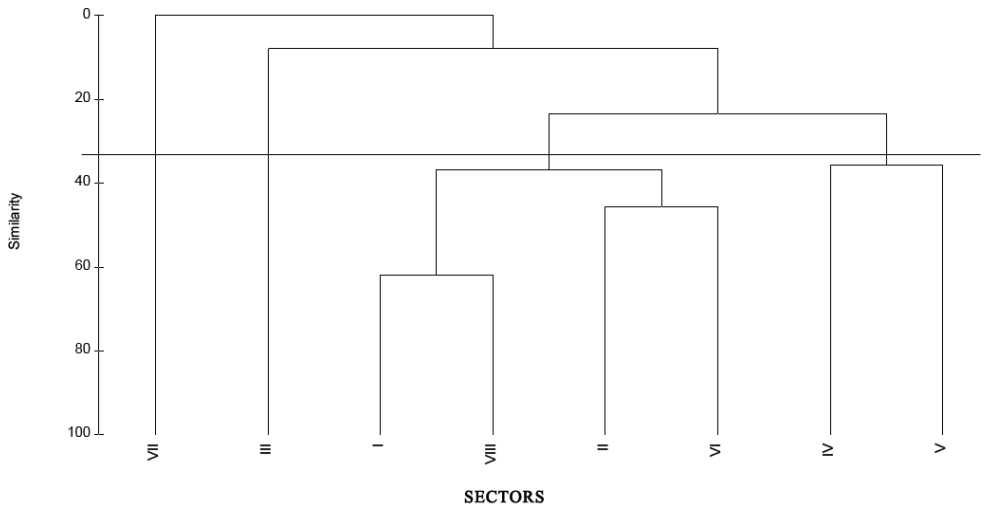


Figure 3.3.- Similarity plot between sectors (I-VIII) by species composition.

I-VIII shared spp	II-VI shared spp	IV-V shared spp
Ainigmaptilon virgularoides	*Arntzia gracilis*	*Callozostron mirabile*
Arntzia gracilis	*Dasystenella acanthina*	*Convexella magelhaenica*
Convexella magelhaenica	*Fannyella rossi*	*Dasystenella acanthina*
Dasystenella acanthina	*Fannyella spinosa*	*Fannyella rossi*
Digitogorgia kuekenthali	*Primnoella antarctica*	*Fannyella spinosa*
Fannyella abies	*Scopaegorgia liouvillei*	
Fannyella kuekenthali	*Thouarella striata*	
Heptaprimnoa patagonica	*Thouarella variabilis*	
Metafannyella aurora		
Metafannyella lepidota		
Onogorgia nodosa		
Plumarella castellviae		
Primnoella divaricata		
Primnoella scotiae		
Scopaegorgia liouvillei		
Thouarella andeep		
Thouarella antarctica		
Thouarella brucei		
Thouarella crenelata		
Thouarella koellikeri		
Thouarella parachilensis		
Thouarella pendulina		
Thouarella variabilis		
Thouarella viridis		

Table 3.13.- Shared species and genera among different sectors of the Southern Ocean.

When comparing the sectors in more detail based on the regions (ANT-SBA), the dendrogram (Fig. 3.4) clusters together all the Antarctic sectors except for the sector VII (one of the least studied) in one side and all the Subantarctic sectors in the other one. Thus indicates that regions again have more importance than sectors in the biogeography of the group. Similarity matrix (Table 3.14) shows the same pattern found when compared the sectors without taking into account the region. Under a 40% of similarity the dendrogram divides into three clusters (IVa-Va, Ia-IIa-VIa-VIIIa, and Is-VIIIs) and six independent sectors. The first one corresponds with the Australian side of the Antarctic region while the second one corresponds with the western Southern Ocean of the Antarctic region (Fig. 3.4). The third cluster corresponds to both sides of the South American cone in the Subantarctic region which cluster under 20% with the two former cluster (Antarctic sectors). Thus may suggest that the South American cone is a route for Antarctic species to spread from and towards other oceans. Number of shared species is shown in table 3.15.

	Ia		Is		IIa		IIs		IIIs		IVa		Va		Vs		VIa		VIs		VIIs		VIIIa	
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
Is	27.451	36.979																						
IIa	45.833	49.339	7.407	25.475																				
IIs	10.256	1.477	11.111	3.343	0	4.443																		
IIIs	5.128	0.453	0	0.929	13.333	1.171	0	4.795																
IVa	26.087	39.339	16.000	30.060	36.364	43.933	0	5.457	15.385	1.373														
Va	19.048	27.142	19.048	32.008	33.333	48.768	0	0	0	0	62.500	79.764												
Vs	0	4.458	0	1.016	0	11.682	0	0	0	0	0	0	0	0										
VIa	46.154	60.101	6.452	24.906	57.143	80.858	0	3.075	10.526	0.866	38.462	66.009	27.273	47.672	0	8.197								
VIs	5.000	3.777	0	15.709	0	0	0	0	0	0	14.286	11.740	20.000	15.784	0	0	0	0						
VIIs	0	7.711	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
VIIIa	55.556	66.108	6.061	18.680	46.667	75.513	0	2.831	9.524	0.808	28.571	58.310	16.667	40.086	0	7.564	58.824	90.861	0	0	0	0		
VIIIs	23.529	15.932	66.667	65.882	14.815	30.487	11.111	4.229	11.111	1.126	24.000	34.657	19.048	37.744	0	1.273	12.903	28.340	0	19.133	0	0	12.121	21.849

Table 3.14.- Similarity matrix of primnoid species and genera between different sectors and regions.

	Ia		Is		IIa		IIs		IIIs		IVa		Va		Vs		VIa		VIs		VIIs		VIIIa	
	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen	spp	gen
Is	7	6																						
IIa	11	7	1	3																				
IIs	2	1	1	1	0	1																		
IIIs	1	1	0	1	1	1	0	1																
IVa	6	6	2	3	4	3	0	1	1	1														
Va	4	4	2	2	3	2	0	0	0	0	5	5												
Vs	0	2	0	1	0	1	0	0	0	0	0	0	0	0										
VIa	12	9	1	4	8	7	0	1	1	1	5	6	3	4	0	1								
VIs	1	1	0	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0						
VIIs	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0				
VIIIa	15	9	1	2	7	6	0	1	1	1	4	5	2	3	0	1	10	8	0	0	0	0		
VIIIs	6	4	10	7	2	3	1	1	1	1	3	3	2	2	0	1	2	4	0	1	0	0	2	2

Table 3.15.- Matrix of shared primnoid species and genera between different sectors and regions.

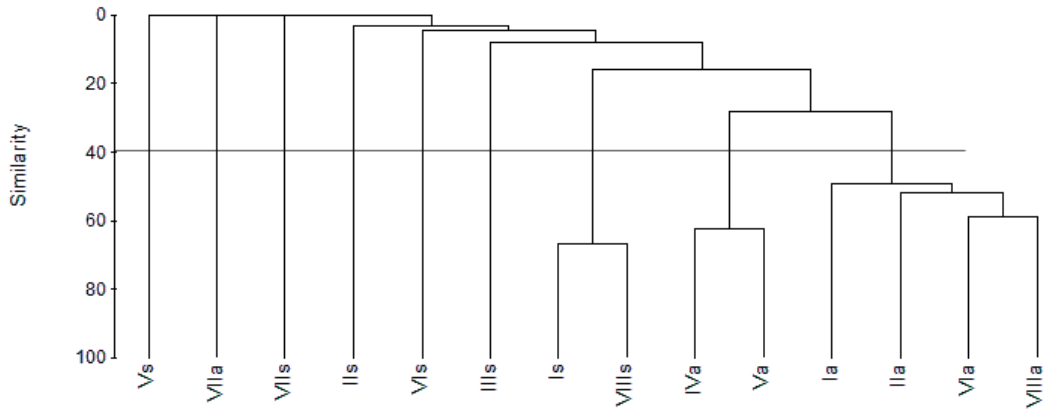


Figure 3.4.- Similarity plot between sectors (I-VIII) and regions (a-s) by species composition.

3.4.3 Bathymetric distribution

Species	Region	Depth	Dmin	Dmax	Drange	SE index	Dtolerance
Aglaoprimnoa stefanii	ANT	Shelf	659.00	686.00	27.00	3.94	S
Aglaoprimnoa stefanii	SBA	Shelf	70.00	124.00	54	43.55	S
Ainigmaptilon antarcticum	ANT	Shelf	125	125.00	0.00	0.00	S
Ainigmaptilon edisto	ANT	Shelf	183	183.00	0.00	0.00	S
Ainigmaptilon haswelli	ANT	Shelf	365.00	549.00	184.00	33.52	S
Ainigmaptilon virgularoides	ANT	Shelf	75.00	547.00	472.00	86.29	S
Ainigmaptilon wallini	ANT	Shelf	550	550.00	0.00	0.00	S
Armadillogorgia cyathella	ANT	Shelf	659.00	686.00	27.00	3.94	S
Armadillogorgia cyathella	SBA	Deep	1879.00	1886.00	7	0.37	S
Arntzia gracilis	ANT	Deep	64.00	1321.00	1257.00	95.16	E
Callozostron carlottae	ANT	Deep	2355.00	3397.00	1042.00	30.67	E
Callozostron diplodiadema	ANT	Deep	2886.00	3040.00	154.00	5.07	S
Callozostron mirabile	ANT	Deep	415.00	3876.00	3461.00	89.29	E
Callozostron mirabile	SBA	Deep	952.00	1336.00	384	28.74	S
Convexella divergens	ANT	Shelf	183	183.00	0.00	0.00	S
Convexella magelhaenica	ANT	Shelf	96	117	21.00	17.95	S
Convexella magelhaenica	SBA	Deep	51.00	2044.00	1993	97.50	E
Convexella murrayi	SBA	Deep	1097.00	1097.00	0	0.00	S
Dasystenella acanthina	ANT	Deep	204	2897.00	2693.00	92.96	E
Dasystenella acanthina	SBA	Deep	350.00	5124.00	4774	93.17	E
Digitogorgia brochi	SBA	Shelf	111.50	111.50	0	0.00	S
Digitogorgia kuekenthali	SBA	Deep	286.00	2468.00	2182	88.41	E
Fanellia tuberculata	SBA	Shelf	128.00	128.00	0	0.00	S
Fannyella abies	ANT	Shelf	94.00	515.00	421.00	81.75	S
Fannyella kuekenthali	ANT	Shelf	75.00	686.00	611.00	89.07	S
Fannyella rossii	ANT	Shelf	46.00	582.00	536.00	92.10	S
Fannyella spinosa	ANT	Shelf	55.00	485.00	430.00	88.66	S
Heptaprimnoa patagonica	SBA	Deep	265.00	1248.00	983	78.77	E
Metafannyella aurora	ANT	Shelf	311.00	549.00	238.00	43.35	S
Metafannyella eos	SBA	Shelf	333.00	371.00	38	10.24	S
Metafannyella lepidota	ANT	Deep	265.00	3514.00	3249.00	92.46	E
Metafannyella mawsoni	ANT	Deep	391	1280.00	889.00	69.45	E
Mirostenella articulata	ANT	Shelf	306	686.00	380.00	55.39	S
Narella gaussi	ANT	Deep	2450	2450.00	0.00	0.00	S
Onogorgia nodosa	ANT	Shelf	21.00	433.00	412.00	95.15	S
Ophidiogorgia kuekenthali	ANT	Shelf	53	53.00	0.00	0.00	S
Ophidiogorgia paradoxa	ANT	Shelf	55	55.00	0.00	0.00	S
Parastenella spinosa	SBA	Shelf	567.00	567.00	0	0.00	S
Perissogorgia vitrea	SBA	Shelf	128.00	128.00	0	0.00	S
Plumarella bayeri	ANT	Shelf	306.00	434	128.00	29.49	S
Plumarella castellviae	SBA	Deep	120	2044	1924	94.13	E
Plumarella delicatissima	SBA	Shelf	256.00	256.00	0	0.00	S
Plumarella delicatula	SBA	Deep	2743.00	2743.00	0	0.00	S
Plumarella diadema	ANT	Shelf	278.00	434.00	156.00	35.94	S
Plumarella diadema	SBA	Shelf	277.2	295.8	18.6	6.29	S
Plumarella undulata	ANT	Shelf	306	434	128.00	29.49	S
Primnoa notialis	SBA	Deep	549.00	1153.00	604	52.39	S
Primnoeides sertularoides	SBA	Shelf	567.00	567.00	0	0.00	S
Primnoella antarctica	ANT	Shelf	91.44	457.00	365.56	79.99	S
Primnoella chilensis	SBA	Shelf	18.00	320.00	302	94.38	S
Primnoella divaricata	SBA	Shelf	55.00	79.00	24	30.38	S
Primnoella scotiae	SBA	Shelf	95.00	171.00	76	44.44	S
Pyrogorgia lemnos	SBA	Shelf	384.00	780	396	50.77	S
Scopaegorgia liouvillei	ANT	Shelf	151.00	615.00	464.00	75.45	S
Tauroprimnoa austasensis	ANT	Shelf	406	638	232.00	36.36	S
Thouarella affinis	ANT	Shelf	306	343	37.00	10.79	S
Thouarella affinis	SBA	Shelf	132.00	132.00	0	0.00	S
Thouarella andeep	ANT	Shelf	200	673	473.00	70.28	S
Thouarella antarctica	ANT	Shelf	312.5	321.6	9.10	2.83	S
Thouarella antarctica	SBA	Shelf	118.00	290.3	172.3	59.35	S
Thouarella brucei	ANT	Shelf	306.00	343.00	37.00	10.79	S
Thouarella brucei	SBA	Shelf	102.00	341.00	239	70.09	S
Thouarella crenelata	ANT	Shelf	238.00	686.00	448.00	65.31	S
Thouarella dispersa	ANT	Deep	2450	2450.00	0.00	0.00	S
Thouarella grandiflora	ANT	Shelf	385	385.00	0.00	0.00	S
Thouarella hicksoni	ANT	Shelf	217	230	13.00	5.65	S
Thouarella koellikeri	ANT	Shelf	312.5	321.6	9.10	2.83	S
Thouarella koellikeri	SBA	Shelf	320.00	732.00	412	56.28	S
Thouarella minuta	ANT	Shelf	226	598.00	372.00	62.21	S
Thouarella pendulina	ANT	Shelf	62.4	547.00	484.60	88.59	S
Thouarella pseudochilensis	ANT	Shelf	112.5	470.1	357.60	76.07	S
Thouarella regularis	SBA	Shelf	132	851	719	84.49	S
Thouarella striata	ANT	Shelf	457.00	644	187.00	29.04	S
Thouarella variabilis	ANT	Shelf	94	736	642.00	87.23	S
Thouarella variabilis	SBA	Shelf	256.00	567.00	311	54.85	S
Thouarella viridis	ANT	Shelf	249.00	434	185.00	42.63	S
Thouarella viridis	SBA	Shelf	286.00	350.00	64	18.29	S
Tokoprymno anatis	ANT	Deep	2895.60	2896.40	0.80	0.03	S
Tokoprymno maia	SBA	Shelf	549.00	549.00	0	0.00	S

Table 3.16.- Bathymetric data of primnoid species from the Sothern Ocean. **ANT**, Antarctic region; **SBA**, Subantarctic region; **Dmin**, Minimum depth; **Dmax**, Maximum depth; **Drange**, depth range; **SE index**, index of stenobathy/eurybathy; **Dtolerance**, depth tolerance; **S**, stenobathic; **E**, eurybathic.

Records for a total of 48 primnoid species from the Antarctic and 31 from the Subantarctic have been analysed in order to discriminate possible trends in bathymetric or geographic distribution (Table 3.16). The species *Thouarella chilensis* and *Primnoella polita* have not been included in the bathymetric analysis because their depths are unknown.

A higher percentage (more than two thirds) of primnoid species are restricted to the continental shelf (<1000 m) in both Antarctic and Subantarctic regions. And only a few species are restricted to depths exceeding 1000 m, five and three respectively for Antarctic and Subantarctic regions. And only five and seven species are reported from both continental shelf and deep sea (Figs.3.5, 3.6).

Records for the 33 of the 34 endemic species in the Antarctic region and 14 in the Subantarctic have been categorized bathymetrically. Only 9 of Antarctic endemic species are restricted to the High Antarctic area (H), 9 more are restricted to the Low Antarctic area (L) specifically only one in the Low Antarctic shelf area (L_s), seven species in the Low Antarctic deep area (L_d), and one species has been found in both shelf and deep areas of the Low Antarctica (L_s-L_d). Almost a half of the species (15 spp) are present in both areas (HL), specifically seven of them are in the three areas (H- L_s-L_d), seven more are in the High Antarctic and in the deep Low Antarctic area (H-L_d), and only one species is present in the High Antarctic and in the shelf area of the Low Antarctica (H-L_s) (Table 3.17). Only 3 of the Subantarctic endemic species are restricted to the Shelf Zone of the Subantarctic area (S_s), more than a half of the species (9 spp) are restricted to the Deep Zone of the Subantarctic area (S_d), and only 2 species are present in both zones (S_s-S_d) (Table 3.18).

In the Antarctic region percentage of stenobathic and eurybathic species are greater than in the Subantarctic region, and in both regions stenobathic species are dominant than eurybathic ones (Table 3.19). However there is no significant difference in the depth tolerance of the species caused by the biogeographic distribution in regions (Table 3.20). When looking at the tolerance of the species based on their presence in the continental shelf or at deep bottoms the results show higher percentages of stenobathic and eurybathic species in shelf bottoms than in the deep sea (Table 3.21). And while at the shelf areas stenobathic species are commoner than eurybathics, at deep sea occurs the opposite, eurybathic species are a little commoner than stenobathic ones. However as occurred with regions no significant difference has been observed in the depth tolerance of species caused by their bathymetric distribution (Table 3.22).

One third of the eurybathic primnoid species found in the Antarctic region were present in the Subantarctic region, and the half of eurybathic species from the Subantarctic region were found in the Antarctic region. Similar values were found for the stenobathic species found in both regions. As the percentage of an interchange of eurybathic and stenobathic species from Antarctica and Subantarctica are the same we cannot suggest a clear predominant dispersal pathway towards or from other adjacent oceans.

High Antarctic Area (H)	**9**	27%			
Ainigmaptilon antarcticum					
Ainigmaptilon edisto					
Ainigmaptilon haswelli					
Ainigmaptilon wallini					
Convexella divergens					
Ophidiogorgia kuekenthali					
Tauroprimnoa austasensis					
Thouarella grandiflora					
Thouarella minuta					
High & Low Antarctic Area (HL)	**15**	45%			
			High & shelf Low Antarctic Area (H-Ls)	**1**	3%
			Ainigmaptilon virgularoides		
			High & deep Low Antarctic Area (H-Ld)	**7**	21%
			Fannyella abies		
			Metafannyella aurora		
			Metafannyella lepidota		
			Metafannyella mawsoni		
			Thouarella andeep		
			Thouarella crenelata		
			Thouarella striata		
			H-Ld-Ls	**7**	21%
			Arntzia gracilis		
			Fannyella kuekenthali		
			Fannyella rossii		
			Fannyella spinosa		
			Onogorgia nodosa		
			Primnoella antarctica		
			Thouarella pendulina		
Low Antarctic Area (L)	**9**	27%			
			Shelf Antarctic Area (Ls)	**1**	3%
			Ophidiogorgia paradoxa		
			Deep Antarctic Area (Ld)	**7**	21%
			Callozostron diplodiadema		
			Mirostenella articulata		
			Narella gaussi		
			Plumarella bayeri		
			Plumarella undulata		
			Thouarella dispersa		
			Tokoprymno anatis		
			Shelf & deep Antarctic Area (Ls-Ld)	**1**	3%
			Thouarella pseudochilensis		
Total	**33 spp**	100%			

Table 3.17.- Summary of bathymetric categories and subcategories of the endemic primnoid species in the ANT. Percentage related to the total endemic species in ANT.

Shelf Zone (Ss)	**3**	22%
Digitogorgia brochi		
Primnoella divaricata		
Primnoella scotiae		
Deep Zone (Sd)	**9**	64%
Convexella murrayi		
Digitogorgia kuekenthali		
Heptaprimnoa patagonica		
Metafannyella eos		
Plumarella delicatula		
Parastenella spinosa		
Primnoa notialis		
Pyrogorgia lemnos		
Tokoprymno maia		
Shelf & Deep Zone (Ss-Sd)	**2**	14%
Plumarella castellviae		
Primnoella chilensis		
Total	**14 spp**	100%

Table 3.18.- Summary of bathymetric categories of the endemic primnoid species in the SBA. Percentage related to the total endemic species in SBA.

Three of the seven circumpolar species are endemic to the Antarctic region and all of them are from the continental shelf and have an eurybathic distribution, from the non-endemic circumpolar species one is found in both regions at the shelf with an eurybathic distribution, and the remaining three are distributed at depths greater than 1000 m and with an eurybathic distribution, however only one present this pattern in both regions while one is eurybathic for the Antarctic and stenobathic for the Subantarctic and the other one is eurybathic at the deep Subantarctic and stenobathic at the shelf Antarctic region. Only three of the 18 deep primnoid species (17%) have a circumpolar distribution around the SO and are found in the ANT and SBA regions. Of the deep primnoid species eight are restricted to the deep bottoms, 5 of them are present in the Antarctic region (4 endemic), while only 3 are in the Subantarctica (2 endemic). The remaining ten species (3 from ANT, 4 from SBA and 3 circumpolar) have been also found in shelf bottoms.

			Region		Total
			ANT	SBA	ANT
SE	Stenobathic	Count	28	18	46
		% of SE	60,9%	39,1%	100,0%
	Eurybathic	Count	20	13	33
		% of SE	60,6%	39,4%	100,0%
Total		Count	48	31	79
		% of SE	60,8%	39,2%	100,0%

Table 3.19.- SE*Region crosstabulation.

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,001[b]	1	,981		
Continuity Correction[a]	,000	1	1,000		
Likelihood Ratio	,001	1	,981		
Fisher's Exact Test				1,000	,582
Linear by Linear Association	,001	1	,981		
N of Valid Cases	79				

a Calculated for a 2x2 table.

b 0 cells (,0%) have expected count less than 5. The minimum expected count is 12,95.

Table 3.20.- SE*Regions Chi-Square Test.

			Depth		Total
			Shelf	Deep	Shelf
SE	Stenobathic	Count	37	9	46
		% of SE	80,4%	19,6%	100,0%
	Eurybathic	Count	22	11	33
		% of SE	66,7%	33,3%	100,0%
Total		Count	59	20	79
		% of SE	74,7%	25,3%	100,0%

Table 3.21.- SE*Depth crosstabulation.

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1,926[b]	1	,165		
Continuity Correction[a]	1,267	1	,260		
Likelihood Ratio	1,907	1	,167		
Fisher's Exact Test				,196	,130
Linear by Linear Association	1,902	1	,168		
N of Valid Cases	79				

a Calculated for a 2x2 table.
b 0 cells (,0%) have expected count less than 5. The minimum expected count is 8,35.
Table 3.22.- SE*Depth Chi-Square Test.

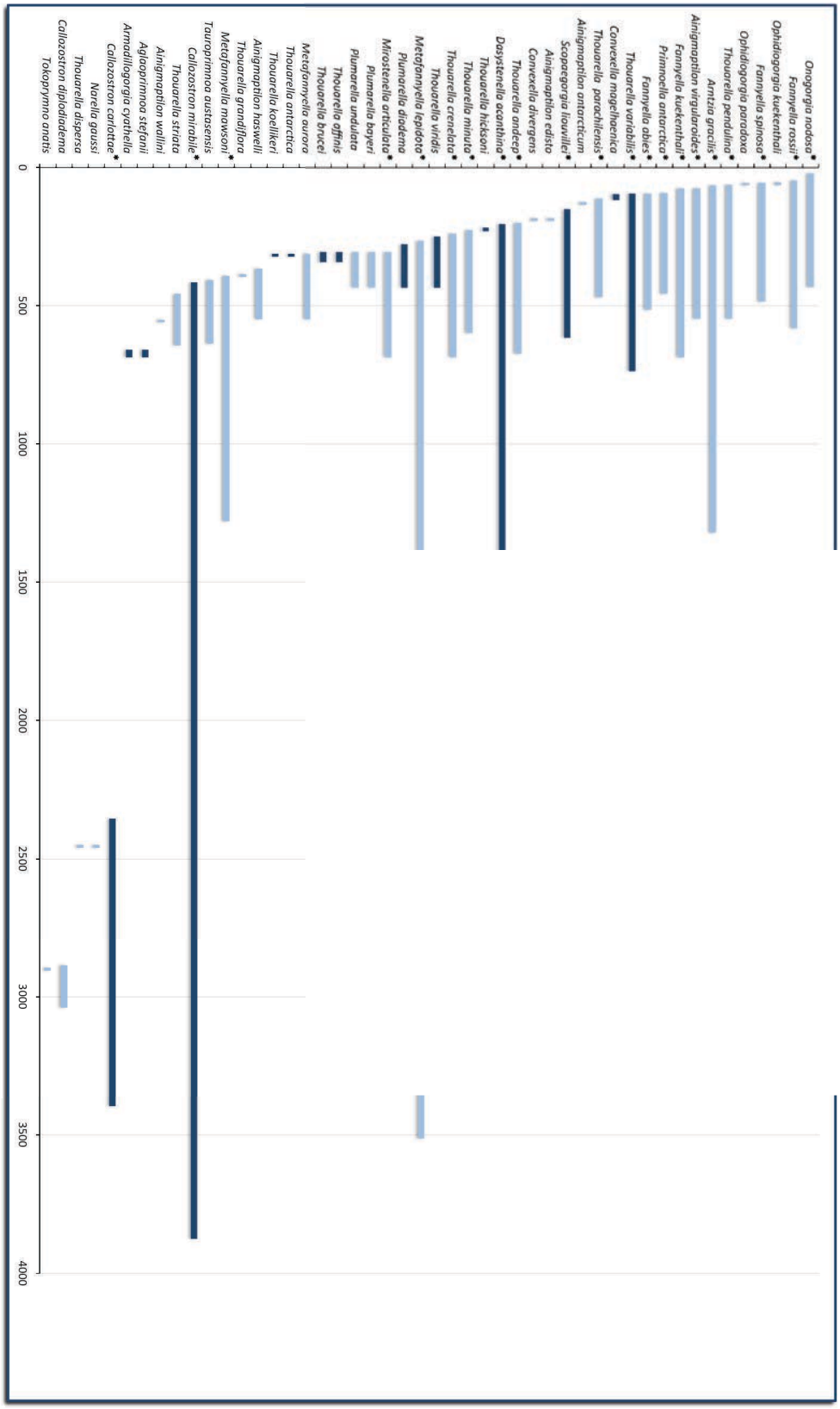


Figure 7.5.- Depth range of Antarctic primnoid species. Clear blue represent endemic species of ANT; *, eurybathic species.

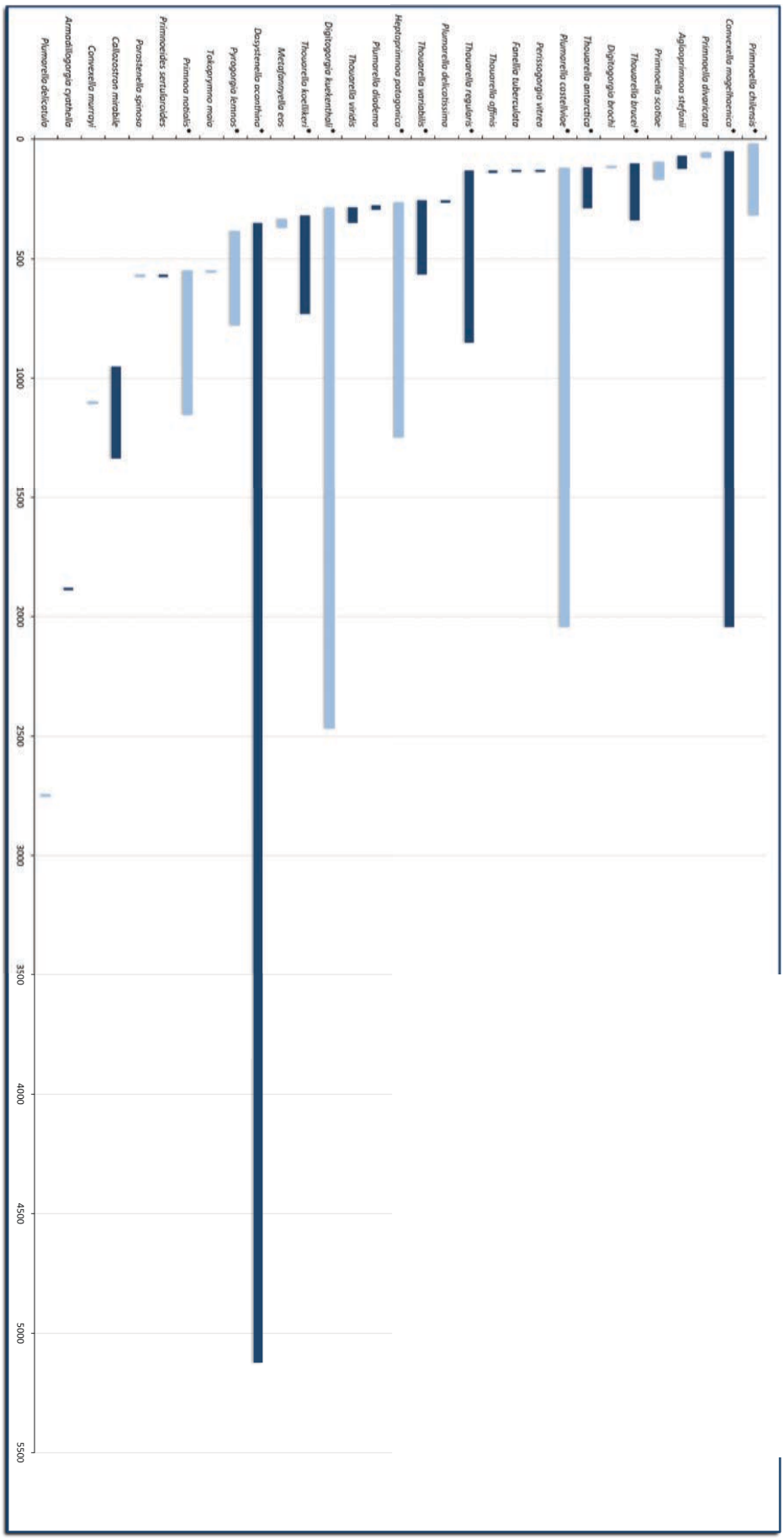


Figure 3.6.- Depth range of Subantarctic primnoid species. Clear blue represent endemic species of SBA; *, eurybathic species.

Antarctic & Subantarctic regions

For the total of 12 primnoid species shared among the Antarctic and Subantarctic regions, six species are present in lower depths in the Antarctic region than in the Subantarctic, being 3 of them also present in the highest depths in the Antarctic region (Table 3.16). The other 6 species are present in lower depths in the Subantarctic region than in the Antarctic, being one of them present also in the highest depths in the Subantarctic region. Seven species are considered eurybathic two of them being eurybathic in Antarctic as well in Subantarctic region, the remaining 5 species are considered stenobathic (Fig. 3.7).

The majority of the shared species (more than 60%) are restricted to the continental shelf (<1000 m), whereas the remaining species are reported from both continental shelf and deep sea (Fig.3.7).

Records for the 12 shared species in the Antarctic and Subantarctic region have been also categorized bathymetrically (Table 3.23). Only 2 species are present in the three areas high and low Antarctic and Subantarctic (HLS), specifically one of them is present in the High Antarctic and in the deep zones of the Antarctic and Subantarctic area (H-L_d-S_d), the other one is also present in the shelf of Antarctic area (H-L_d-L_s-S_d). More than three quarters (83% or 10 spp) are present in the Low Antarctic and Subantarctic area (LS), half of them (5 spp) are present in the deep zones of both areas (L_d-S_d).

High, Low Antarctic & Sub-Antarctic Area (HLS)	**2**	17%			
			High & deep Low ANT & Deep Zone SBA (H-Ld-Sd)	**1**	8%
			Dasystenella acanthina		
			High, Low ANT & deep SBA (H-Ld-Ls-Sd)	**1**	8%
			Thouarella koellikeri		
Low Antarctic & Sub-Antarctic Area (LS)	**10**	83%			
			Deep Low ANT & Deep Zone SBA (Ld-Sd)	**5**	42%
			Armadillogorgia cyathella		
			Callozostron mirabile		
			Plumarella diadema		
			Thouarella koellikeri		
			Thouarella viridis		
			Deep Low ANT & Shelf Zone SBA (Ld-Ss)	**2**	17%
			Aglaoprimnoa stefanii		
			Thouarella affinis		
			Deep Low ANT & SBA (Ld-Ss-Sd)	**2**	17%
			Thouarella antarctica		
			Thouarella brucei		
Total	**12 spp**	100%			

Table 3.23.- Summary of bathymetric categories of the shared primnoid species in the ANT-SBA. Percentage related to the total shared species.

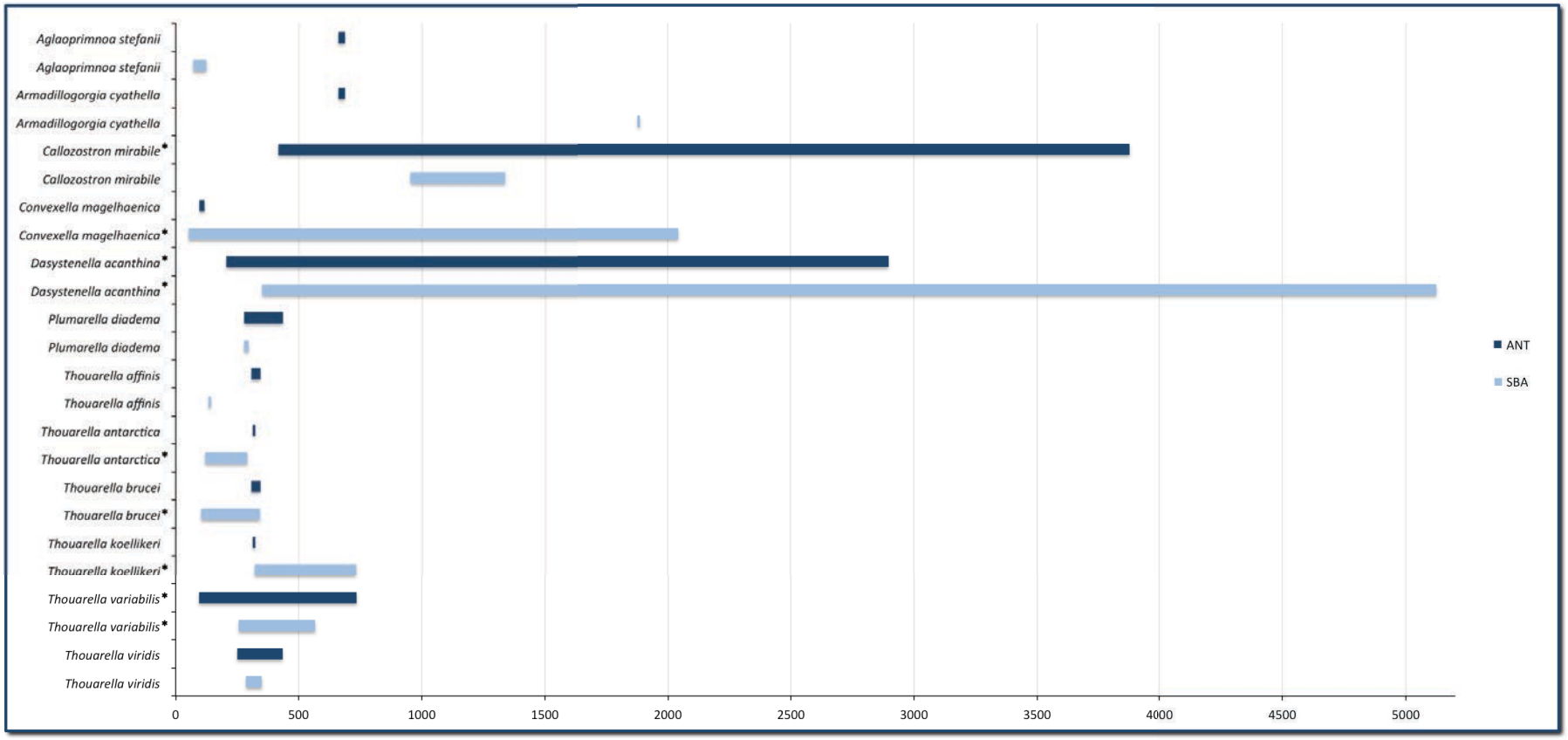
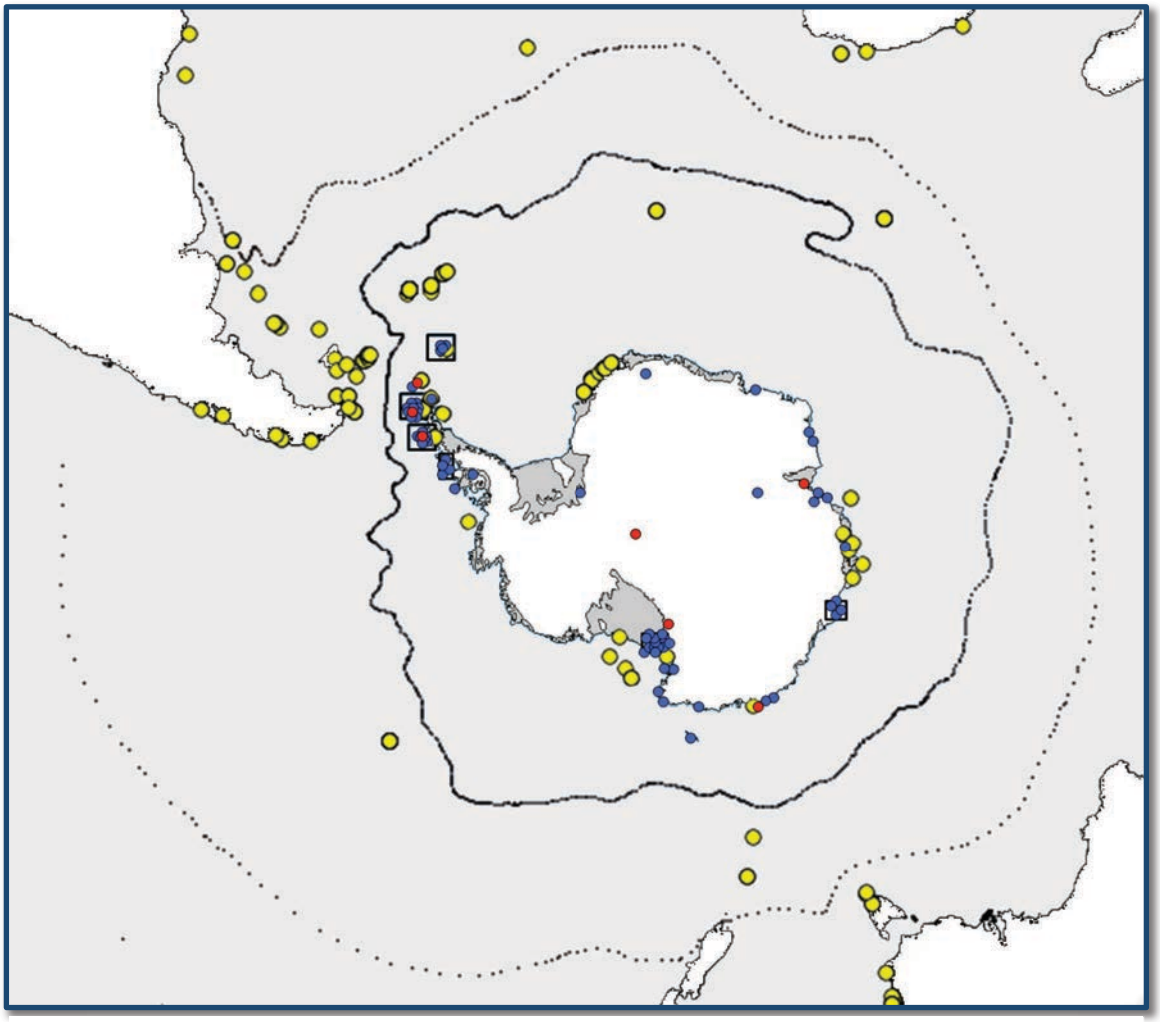


Table 3.7.- Depth range of shared species in the Antarctic and Subantarctic regions. *, eurybathic species.

3.4.4 Antarctic Protected Areas and Primnoids

Looking at the type localities of primnoid species and its distribution around the area included in the Antarctic Treaty, mainly four areas accumulate the majority of type localities. These areas are Peninsula Antarctica, Ross Sea, Davis Sea, and Cape Norvegia. It can be observed as some of those localities are included inside ASPAs (*e.g.* Orkney Islands, Antarctic Peninsula, east Ross Sea, Commonwealth Bay and Haswell Island), while other localities mainly in the centre of Ross Sea, the Davis Sea and the area around Cape Norvegia are not protected. According to article 3 (2d) from the Annex V of the Protocol on Environmental Protection of the Antarctic Treaty, those areas should be included as ASPAs. Other areas outside the Antarctic Treaty limits like the Subantarctic islands (Falkland Islands, South Georgia and South Sandwich Islands) show a great number of primnoid localities, however no protection is present in those areas (Map 3.7).



Map 3.7.- Southern Ocean. Blue circle Antarctic Specially Protected Areas (ASPAs); red circle, Antarctic Specially Managed Areas (ASMAs); yellow circle, type primnoid species.

3.5 Discussion

López-González *et al.* (2002) estimated in 17 the number of primnoid genera present in Antarctic and Subantarctic regions (=SO), and pointed out the high diversity of this gorgonian family in the Southern Ocean waters. Other information presented by the authors is the grade of endemism of primnoids in the SO, 50% from the total (15 of the 30 total genera), and about 88% if we only consider the genera present in the SO (17 gen). After several revisions (Cairns & Bayer 2009; Cairns 2011) and the addition of new material, the data presented in this study show a higher diversity, with a total of 39 genera worldwide distributed, 27 of them present in the Southern Ocean, and 15 endemic to the SO. Thus the grade of endemism is reduced at 38% of the total and about 56% if we have in account only the SO genera. At species level, it has been considered valid 247 species worldwide distributed, from them 69 species have been recorded from the SO (28%). The percentage of endemic species in the SO is about 81% (56 spp), if we consider the total of primnoid species, the percentage decrease to 23%. Comparing the previous known data on primnoid diversity and endemism at genus level (López-González & Gili 2005), we observe an increase of diversity and a decrease of endemic genera. These results may suggest a greater increase on studies, sampling and revisions on primnoid genera from tropical and sub-tropical waters (*e.g.* Studies on Western Atlantic Octocorallia by Cairns and Bayer 2003 and following) compared with studies on primnoids from the Southern Ocean waters. Although there are important collections of Antarctic expeditions, many of the material is still not identified (Smithsonian, Russia, AWI) or with imprecise or inaccurate identifications, which suggests that richness of Antarctic primnoids is underestimated.

Many authors have commented on the high degree of endemism of the SO benthic fauna with a similar degree of species endemism (81% in primnoids), in isopods (Brandt 1999) and pycnogonids (Clarke & Johnston 2003) endemicity is almost 90%, 83% in bryozoans (Barnes & De Grave 2000), and about 75% in molluscs (Linse *et al.* 2006) and sea anemones (Rodríguez *et al.* 2007). The Southern Ocean has been less prospected than other temperate and tropical oceans, however the grade of endemism at species level (81%) reveals a low dispersion grade beyond the Subtropical Front, and a high level of speciation. This phenomena could be explained by the climatic fluctuations during a long evolutionary history of the region have made benthic fauna to be concentrated into the poles, being Antarctic ecosystems comparable with those in tropical and temperate regions (Clarke & Crame 1992). The isolation, habitat heterogeneity and reproductive strategies with a non-pelagic lecitotrophic larval stage (Gili *et al.* submitted) makes the dispersion of these organisms very limited.

The Antarctic region is more diverse at species and genera level (49 and 19 respectively) than the Subantarctic region (32 and 18), and the same occurs with the primnoids level of endemism which is higher (69%) in the Antarctic region than in the Subantarctic region (40%). This higher degree of the Antarctic region points out to a high level of speciation in this region versus the Subantarctic. In a recent study (Martín-Ledo & López-González *in press*) ophiuroids present a similar pattern where the endemicity of the Antarctic region is higher than the one in the Subantarctic (58% *vs*. 22%), however in this case the Subantarctic region is more diverse at both species and genera level than the Antarctic region. In sea anemones has been observed in the other way around (Rodríguez *et al.* 2007) where the Subantarctic region shows a higher

degree of endemism possibly due to its higher area compared with the Antarctic region and the presence of more different habitats provided by groups of islands or the continental shelves of South America. However López-González *et al.* (2002) suggest that more studies focused in the Antarctic region will increase the detection of endemisms in this region.

As observed in this study for primnoids when looking at the composition of longitudinal sectors a higher diversity of sea anemones are found in sectors I, VI and VIII (Rodríguez *et al.* 2007), corresponding to Antarctic Peninsula, Scotia Arc, South Shetland Islands and Ross Sea. Because of a major sample effort these areas have shown also a high density of macrobenthic data (Gutt *et al.* 2013), and showing a cline in species number in some other areas such as sectors III and VII corresponding to Amundsen Sea and East Antarctica which are poorly sampled (Griffiths 2010), making our knowledge on Antarctic primnoids fragmentary and far to the reality. As previously mentioned the Antarctic Atlantic district showed the highest number of primnoid species, agreeing with the suggestion of the Antarctic Peninsula being a hot spot for diversification (Griffiths *et al.* 2011a). For primnoids the adjacent sectors I and VIII, corresponding to both sides of the Antarctic Peninsula and South America have been recognised to be the most resemblants, supporting the idea of the Antarctic Peninsula being a donor of species to the Weddell Sea thanks mainly to the Weddell Gyre (Arntz *et al.* 2006). Values of primnoid fauna affinity between South America and Antarctica seems to be low; at species level sector VIII (Pacific district) from Subantarctica (South American coast) and Antarctica present a similarity of 12.12%, and sector I (Atlantic district) from SBA and ANT have 27.4% of resemblance. Both sectors have a little bit higher similarities at genus level (21.85% and 37% respectively). However spite of this low values of similarity, the affinity between the Antarctic primnoid fauna and the South American fauna is the highest, as is shown in other taxa (Linse *et al.* 2006, Rodríguez *et al.* 2007, Griffiths *et al.* 2011b). It may suggest a connection between the Magellan region and Antarctica, although the direction of the linkage is still uncertain. The remaining sectors have no similarities between Antarctic and Subantarctic regions, suggesting that the Polar Front represents a biogeographic discontinuity (Clarke *et al.* 2004), and an effective barrier to gene-flow (Krabbe *et al.* 2010). Rodríguez *et al.* (2007) confirmed the hypothesis by the fact that a 32% of the sea anemone families distributed in the Southern Ocean were not present south of the Polar Front. In the same way about 30% of primnoid genera and species from the SO have not been observed at the Antarctic side of the Polar Front, suggesting that the number of species endemic to the Antarctic is likely to increase.

Previous studies where the benthic invertebrate fauna from Antarctic continental shelf is compared with other regions (*i.e.* North-west Atlantic Ocean) generally have wider depth ranges in the Antarctic species than its relatives elsewhere (Brey *et al.* 1996). This could reflect changes on the continental Antarctic ice sheet extension due to the glacial and interglacial periods and thus the adaptation of species at greater depths to survive (Clarke *et al.* 2004). Despite this the percentage of stenobathic Antarctic primnoids (58%) is higher than the eurybathic species (42%). However many of the species considered stenobathic are only known from one or very few records, and thus being overestimated the percentage of stenobathic species. The same pattern has been found for the Subantarctic primnoids. If we

take all SO species values are still similar with 54% of stenobathic species and 46% of eurybathic. This distribution pattern has been observed in some benthic groups (Rodríguez *et al.* 2007, Griffiths *et al.* 2011b). However for ophiuroids (Martin-Ledo & López-González in press), gastropods, polychaetes (Brey *et al.* 1996) and isopods (Brand *et al.* 1997) eurybathic species are predominant.

General distribution patterns for the Antarctic macrobenthic fauna have been described as predominantly circumpolar (Clarke & Johnston 2003; Gutt *et al.* 2013), thanks to the homogeneous conditions around the continent and the circumantarctic current systems (Arntz *et al.* 1994). This characteristic has been described as common for sea anemones with a 14% of species being circumpolar (Rodríguez *et al.* 2007), pycnogonid fauna (21%) (Munilla & Soler-Membrives 2009), and brittle stars (23%) (Martin-Ledo & López-González in press) among other groups. For primnoids the number of circumpolar species is quite low, only 7 species, 10% from the total in the SO. However the circumpolarity increase to 14% when is only considered species form the Antarctic region. However modern molecular techniques are revealing complex cryptic species among some circumantarctic species, which would have more restricted distributions than as thought before (Havermans *et al.* 2009).

Taking into account that the Antarctic region have a higher percentage of circumpolar species than the Subantarctic, and also has the greatest number of endemic species restricted to deep bottoms, the bathymetric patterns may suggest a previous dispersion of the species around the Antarctic region where the environmental conditions are more stable and a subsequent flux of radiation from the continental shelf to the deep, where thanks to the Antarctic Bottom Water, AABW (Orsi *et al.* 1999) the species may have dispersed to the Subantarctic region and adjacent oceans. Moreover, there is an evidence of the dispersion and evolution of the Antarctic species displacing outwards by gradations in diversity supported by molluscs fossil discoveries (Crame 1999) and phylogenetic variations in fishes (Bargelloni *et al*. 2000). These facts have made Antarctica to be proposed as a marine centre of diversity (Briggs 2003). In that way, Antarctica will produce a regular pool of successful species capable to widespread to the nearest areas, where the ancestral species will be widely distributed outward in contrast to the derived species that will form the local populations (Briggs 2003).

While in some regions such as the Mediterranean Sea the environmental management is complex due to the high number of countries who have competencies in such region (Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean 1976), the great advantage of the Antarctic Treaty is the international jointed management, which makes easier the application of protection policies. However the main restriction of the Antarctic Treaty (1959) is undoubtedly its area of application. All protection measures as well as the designation of ASPAs are restricted to territories southward of 60 degrees south. Antarctic areas including South Georgia and Sandwich Islands, and all the Subantarctic region including Falkland Islands, Tierra del Fuego, and Bouvet Island among others are excluded from the Antarctic Treaty. Some of the criterion to designate possible ASPAs by the Annex V on the Protocol on Environmental Protection to the Antarctic Treaty (Madrid, October 3rd 1991) is the type locality or the only known habitat of any species, areas with important or unusual assemblages of species, representative examples of major

ecosystems, areas free of human interference, useful for future comparisons and areas of particular interest to on going scientific research. However the type locality of a species is not always representative of the distribution of the species, is more valuable protect important aggregations or communities of marine species, areas of hotspot biodiversity that contribute to the maintenance of marine biodiversity, rare or threatened habitats and species, or even areas for key life cycle stages of mobile species including habitats important in reproduction and nursery stages and differentiating the special necessities for the protection of benthic and pelagic systems (Hyrenbach *et al.* 2000). The current designated ASPAs and ASMAs are predominantly around the national research stations and there is no connection among them. A creation of a network of marine protected areas ecologically coherent based on representation, replication, viability, adequacy, connectivity, protection and the best available evidence should be a priority for the Antarctic Treaty Secretariat.

3.6 Conclusions

- Increase of species and genus richness known in the Southern Ocean. With a total of 69 species and 27 genera for the SO, 49 species and 19 genera for the Antarctic region and 32 species and 18 genera for the Subantarctic region.
- High degree of endemism of the Southern Ocean primnoid species (81%). Values of endemism similar to other SO benthic fauna. At genus level the endemism is around 56%. These values may suggest a low dispersion beyond the Subtropical Front.
- Antarctic region is more diverse (at both species and genus level) than the Subantarctic region, furthermore primnoid endemism is higher in the Antarctic region, suggesting a high level of speciation in this region.
- Atlantic and Pacific districts are the most diverse in the Southern Ocean, and in both regions (ANT, SBA), while the Indic Ocean is the least diverse.
- Sectors I and VIII, corresponding to sectors at both sides of South America, are the most diverse in the Southern Ocean, and in both regions (ANT, SBA), suggesting a hot spot area for diversification at Antarctic Peninsula. While sectors III and VII are the least diverse.
- Atlantic and Pacific are the most similar districts. Indic district is more similar with Pacific than Atlantic district.
- Adjacent sectors seem to be more similar. Most similar sectors are I and VIII.
- Low percentage of circumpolar species (10%) in the Southern Ocean. Antarctic region has more circumpolar species than the Subantarctic region.
- Faunal affinity between Antarctic ad Subantarctic regions from the South American side are the highest in the whole Southern Ocean, suggesting a connection between Magellan and Antarctic region for evolutionary processes.
- Higher number of stenobathic than eurybathic primnoid species in Antarctic and Subantarctic regions.
- Similar percentages of eurybathic and stenobathic species present in both regions (ANT, SBA) suggests no predominant dispersal pathway towards or from other adjacent oceans.

- Only 3 of 18 deep species are circumpolar, and the Antarctic region has greater numbers of endemic species restricted to deep bottoms, suggesting a dispersion flux from continental shelves to the deep ocean.
- Off-balanced sampling efforts between the different geographic divisions (regions, districts, and sectors) may distort the values of richness, diversity, endemicity, bathymetric distribution, and faunal affinities.
- Only 2 Antarctic Specially Protected Areas include primnoids type localities.
- 2 areas (Cape Norvegia and Davis Sea) with primnoids type localities are not included in any ASPA.
- There are some ASPAs at Ross Sea, however they are mainly near the continent and research stations, leaving the middle area of the sea (with presence of primnoids type localities) without protection.
- ASPAs are restricted to the area of action of the Antarctic Treaty, territories southward of 60 degrees south, leaving areas of the Antarctic region and the whole Subantarctic region outside from the protection under the Antarctic Treaty.

CHAPTER 4

Phylogeny

4.1 Introduction

The term *phylogeny* derives from the Greek terms *phyle* (φυλή) and *phylon* (φῦλον), denoting "tribe" and "race"; and the term *geneia*, denoting "origin/relative to birth", from *genēs* (γενής) "born". The first biologist to coin the word *phylogeny* was the German entomologist Ernst Haeckel in his *Generelle Morphologie der Organismen* (1866), meaning according to him the history of evolution of phyla, a series of morphological stages that have been passed through during a given evolutionary path. However, the term *phylogeny* is currently used to designate trees, that represent the history of life (Hickman *et al.* 2008). Thus, a particular taxon will be defined by a bunch of characters, concretely by the manifestation of a state for each character (primitive or derived).

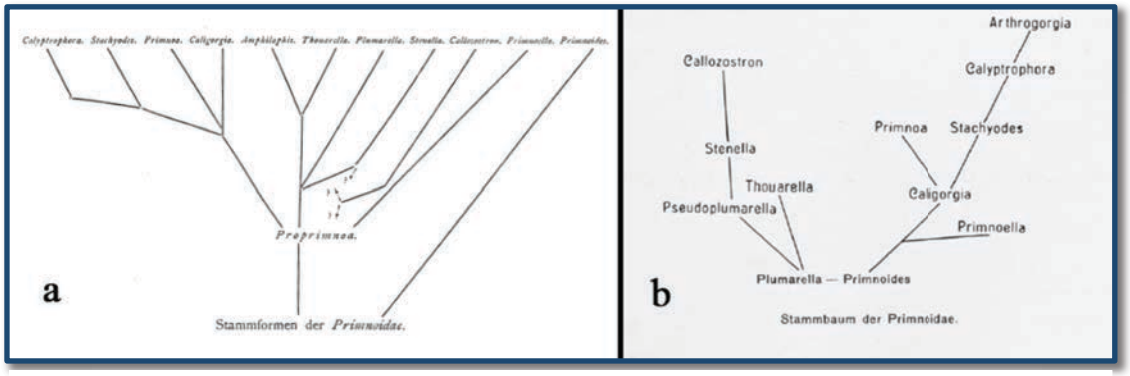


Figure 8.1.- Phylogenetic tree of family Primnoidae. **a**, from Versluys, 1906; **b**, from Kükenthal, 1919.

Versluys (1906) was one of the first authors to provide a phylogenetic approach on Primnoidae family, a first insight of the evolutionary relationship of 11 genera (3 of them are no longer valid) with *Primnoeides* as the most primitive (Fig. 4.1a). A decade later, Kükenthal (1919) proposed for some characters a different character polarization and thus new ancestral states, however the resulting primnoids phylogenetic relationship locates *Plumarella* and *Primnoides* as the most primitive primnoids (Fig. 4.1b). Cairns and Bayer (2009) presented a tree including 41 primnoids being *Primnoeides* also as one of the most primitives and concluding that characters such as the presence of the operculum, the correspondence of marginal and opercular, the number of longitudinal body wall scales as well as the number of adaxial row scales have a strong influence into the evolutionary history of primnoids (see Fig. 2 in Cairns & Bayer 2009).

Cairns (2007) also used DNA sequences analyses as an independent source of characters to classify and compare five new Alaskan species of *Narella* (Octocorallia: Primnoidae). Sequences were obtained using mitochondrial genes *ND6* and *msh1* (now renamed *mtMutS*, see Bilewitch & Degnan 2011), but little no variation was found among the five morpho species, being not variable enough to be useful for intraespecies or intrageneric differences within the octocoral family Primnoidae. The result was consistent with the known slow rate of evolution of the mitochondrial genes in Octocorallia, although *mtMutS* is considered the most

rapidly evolving mitochondrial protein-encoding gene for octocorals (France & Hoover 2002). Nevertheless *mtMutS* has been widely used for phylogenetic analyses of Octocorallia (McFadden *et al.* 2006, France 2007) and supported the monophyly of Primnoidae based on 4 genera. However for several octocoral taxa have been encountered difficulties obtaining amplification products (France & Hoover 2001) and has been hypothesized the differences in the arrangement of the mitochondrial genes in some octocoral groups as responsible for not obtaining DNA amplifications (Brugler & France 2008).

Herrera *et al.* (2010) employed nucleotide sequences of mitochondrial (*ND6*, *ND6-ND3* intergenic spacer, *ND3*, *ND2*, *COI*, *mtMutS* and *16S*) and nuclear (*28S* and *ITS2*) genomic regions from several taxa of Paragorgiidae (bubblegum corals) to infer molecular phylogenetics and to examine the correspondence of morphological features with the underlaying genetic information. They found that ND2, *mtMutS* and *ITS2* are the most variable regions for those taxa, corresponding with the ones reported for other octocorals groups (McFadden *et al.* 2006).

In this chapter information about compared morphology and molecular analysis has been used to perform a cladistic analysis of *Thouarella* species, as well as a morphological phylogenetic analysis of the family Primnoidae at genus level.

4.2 Objectives

It is the purpose of this chapter to understand the phylogenetic relationships among Antarctic gorgonians. Including the following specific objectives:

- Analyse the phylogeny of the family Primnoidae at genus level using morphological characters.
- Analyse the phylogeny of the genus *Thouarella* using the sclerites morphology as a basis.
- Correlate the results with its observed biogeographic distribution.
- Discuss the possible origin of this genus and its radiation, with special emphasis on Southern Ocean species.
- Analyse the phylogeny of the Antarctic species of the genus *Thouarella* within the family Primnoidae using molecular markers.
- Compare the results from morphological and molecular phylogenies.

4.3 Material and Methods

4.3.1 Phylogenetic hypotheses based on morphological information

Phylogeny based on morphology relies on the comparison of the different structures present on the organisms to be studied. Macroscopic and microscopic characters are defined by size, shape, colour, ornamentations, etc… and compared among the different species. Morphological phylogeny allows us an accurate identification of species in the field and laboratory.

4.3.1.1 Ingroup and outgroup taxa

Family Primnoidae

To undertake the phylogenetic analysis of the family Primnoidae, the ingroup taxa includes 39 primnoid genera (Table 4.1). The genera *Fannyella*, *Thouarella* and *Plumarella* have been analysed through their subgenera (11 in total, three, four, and four respectively). The genera have been studied using mainly the generic revision carried out by Cairns and Bayer (2009), but also information has been got through recent collected specimens, museum specimens, and looking through the literature original descriptions and redescriptions.

The outgroup taxa used is the subfamily Circinisidinae belonging to the family Isididae and probably one of the most similar subfamilies to the primnoids, by yielding a cruciform extinction pattern of its scales in polarized light. Its clear differentiation with the ingroup taxa is due their jointed axis composed of rigid calcareous nodes joined with flexible gorgonin internodes, makes Circinisidinae the perfect outgroup to avoid any close misgrouping or doubt in their classification within the family.

Genus *Thouarella*

The ingroup taxa analysed belong to the genus *Thouarella* included in one of the most specious gorgonian family named Primnoidae. Thirty-one species have been studied (Table 4.2), using recent collected specimens, museum specimens, and looking through the literature original descriptions and redescriptions.

The outgroup taxa used is the genus *Ainigmaptilon*, an Antarctic primnoid that presents a unique position of polyps on a leaf-like structure with a funnel shaped base. For its clear differentiation with the ingroup taxa, *Thouarella*, makes *Ainigmaptilon* the perfect outgroup to avoid any close misgrouping or doubt in their classification within the family.

4.3.1.2 Characters and character coding

Family Primnoidae

Code	Character	Character state
0	Colony shape	0 unbranched 1 dichotomous planar 2 dichotomous lyriform 3 dichotomous bushy 4 dichotomous sparse 5 sympodial 6 pinnate opposite 7 pinnate alternate 8 bottlebrush
1	Shape of base	0 attached 1 free
2	Branch nodes	0 present 1 absent

Code	Character	Character state
3	Basal fusion of calyces	0 not fused 1 fused
4	Coordination of polyps	0 isolated 1 spirals 2 biserial 3 paired 4 in whorls 5 on leaves
5	Proximity of calices to stem	0 inclined to perpendicular 1 appressed 2 adnate
6	Orientation of calyces	0 up 1 perpendicular 2 down
7	Operculum	0 absent 1 present
8	Distal inner surface of opercular scales	0 tuberculate 1 smooth 2 single medial keel 3 multiridged 4 spinose
9	Correspondence of opercular and marginal scales	0 correspond 1 no correspondence 2 regular offset
10	Number of marginals	0 7 1 8 2 more than 8 3 6 4 5 5 4 6 2
11	Circumoperculum	0 present 1 absent
12	Distal margin of marginal scales	0 rounded or straight 1 pectinate 2 serrate 3 pointed 4 spinose
13	Body wall sclerite imbrication	0 imbricate 1 mosaic
14	Body wall sclerite shape	0 elliptical, oval or rectangular 1 triangular 2 polygonal 3 sickle shaped 4 ascus shaped

Code	Character	Character state
15	nº longitudinal rows of body wall scales	0 not arranged in rows as adult 1 8 2 7 3 6 4 5 5 3 6 2 7 1
16	Coverage of adaxial body wall	0 naked or few vestigial sclerites 1 narrow bare trip 2 completely covered
17	Number of abaxial rows of body wall scales	0 two rows 1 one row
18	nº scales on abaxial row	0 variable 1 fixed 3 or 4 2 fixed 5 3 fixed 2
19	Fusion of body wall sclerites	0 not fused 1 fused ab- and adaxially
20	External sculpture of body wall sclerites	0 smooth 1 granular 2 longitudinal or radiating ridges 3 nodular 4 spiny 5 ascus scale 6 tuberculate ridges
21	Infrabasals	0 none 1 one pair 2 two or more pairs
22	Number of coenenchymal layers	0 two layers 1 one layer
23	Axis calcification pattern	0 radial 1 longitudinal
24	Large tentacular sclerites	0 present 1 absent
25	Operculum or anthopoma	0 eight or more scales 1 only eight scales
26	Coenenchymal sclerites	0 scales and rooted heads 1 scales and tuberculate spheroids

Table 4.1.- Characters and character states used in the phylogenetic analysis of Primnoidae family.

Genus *Thouarella*

Code	Character	Character state
0	Accessory operculars	0 absent 1 present
1	Central keel on opercular scales	0 absent 1 present
2	Outer surface of opercular scales	0 smooth 1 granular
3	Opercular scales 3 times longer than wider	0 no 1 yes
4	Central keel on marginal scales	0 absent 1 present
5	Outer surface of marginal scales	0 smooth 1 ornamented
6	Apical shape of marginal scales	0 rounded 1 pointed
7	Shape of marginal scales	0 oval 1 pentagonal 2 triangular
8	Margin of marginal scales	0 pectinated 1 serrated
9	Marginal thorn	0 absent 1 present smooth 2 present ridged
10	Platforms on marginals keels	0 absent 1 present
11	Distal inner surface of operculars	0 smooth 1 ridged
12	Distal inner surface of marginals	0 smooth 1 ridged
13	Shape of abaxial body scales	0 round 1 pointed
14	Bilobed base of marginal scales	0 no 1 yes
15	nº abaxial scales	0 3 1 4 2 5 3 6 4 7 5 8 6 9 7 10 8 11 9 12
16	Op bilobed base	0 no 1 yes

Code	Character	Character state
17	nº adaxial scales	0 2 1 3 2 4 3 5 4 6 5 7
18	warts	0 sparsely 1 densely
19	Op concave	0 no 1 yes
20	Op dimorphism	0 no 1 yes
21	Polyp length	0 0.83 1 1.204 2 1.578 3 1.952 4 2.326 5 2.7 6 3.074 7 3.448 8 3.822 9 4.2
22	Polyp wide	0 0.29 1 0.424 2 0.558 3 0.692 4 0.826 5 0.96 6 1.094 7 1.228 8 1.362 9 1.5
23	Singly polyps	0 no 1 yes
24	Polyp orientation	0 inclined 1 perpendicular
25	nº polyps/cm	0 ≤4 1 5 2 6 3 7 4 8 5 9 6 10-15 7 16-20 8 21-30 9 >30
26	Bottlebrush colony shape	0 no 1 yes
27	Op apex truncated	0 no 1 yes

Code	Character	Character state
28	Bs crests	0 absent 1 present
29	Mg spine	0 absent 1 present
30	Mg crests	0 absent 1 present

Table 4.2.- Characters and character states used in the phylogenetic analysis of *Thouarella* species.

In this study it is proposed for the first time a phylogeny based on morphological information for the genus *Thouarella*. Because of that the characters used and the states considered for each character are described and illustrated below.

0-Accessory operculars.- The accessory operculars of primnoids consist of a maximum of eight minute, from narrow tongue-shape to arrowhead-like scales, arranged in two alternate cycles of four scales each, distally to opercular scales (Zapata-Guardiola & López-González 2010a) and laid against the polyp tentacles. (Fig. 4.2A, B).

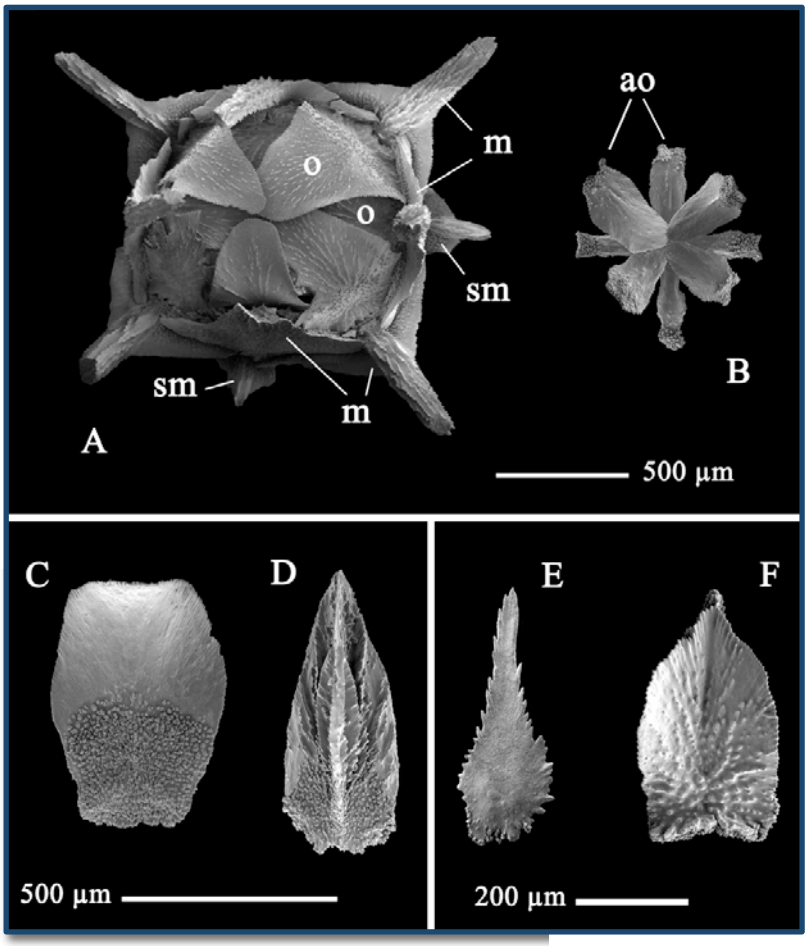


Figure 4.2.- A, B, C: *Thouarella bayeri*, holotype ZIZMH C11738; **A**, polyp in oral view, with visible opercular scales; **B**, two cycles of accessory opercular scales, not visible in polyp oral view; **C**, opercular scale without keel; **D**, *Thouarella viridis*, holotype ZIZMH C11744, opercular scale with medial keel; **E**, *Thouarella alternata*, syntype USNM 30097, opercular scale with smooth outer surface; **F**, *Thouarella regularis*, lectotype NHMUK 1889.5.27.60, opercular scale with granulated outer surface; **ao**, accessory opercular scales; **o**, opercular scales; **m**, marginal scales; **sm**, submarginal scales.

1-Central keel on opercular scales.- The presence of a keel in the inner surface of opercular scales (state 1) is the commonest ornamentation, however a smooth inner surface (state 0) is found in few species. (Fig. 4.2C, D).

2-Outer surface of opercular scales.- The commonest outer surface of opercular scales tends to be ornamented with granules (state 1), but a few species present a smooth outer surface (state 0). (Fig. 4.2E, F).

3-Opercular scales 3 times longer than wider.- As opercular scales shape and size can be very variable, the relation between its height and wide gives a complementary approach to distinguish scales with a high H:W relation (3 times higher than wider, state 1). (Fig. 4.3A, B).

4-Central keel on marginal scales.- The presence of a simple or multi keels in the inner surface of marginal scales (state 1) is the commonest ornamentation, and the presence of keels along the spines of some *Thouarella* species are now not considered as a proper keel and thus that species will be included in the genus *Plumarella* (Cairns 2010, Taylor *et al.* 2013). (Fig. 4.3C, D).

5-Outer surface of marginal scales.- The commonest outer surface of marginal scales tends to be ornamented with granules (state 1), but a few species present a smooth outer surface (state 0). (Fig. 4.3E, F).

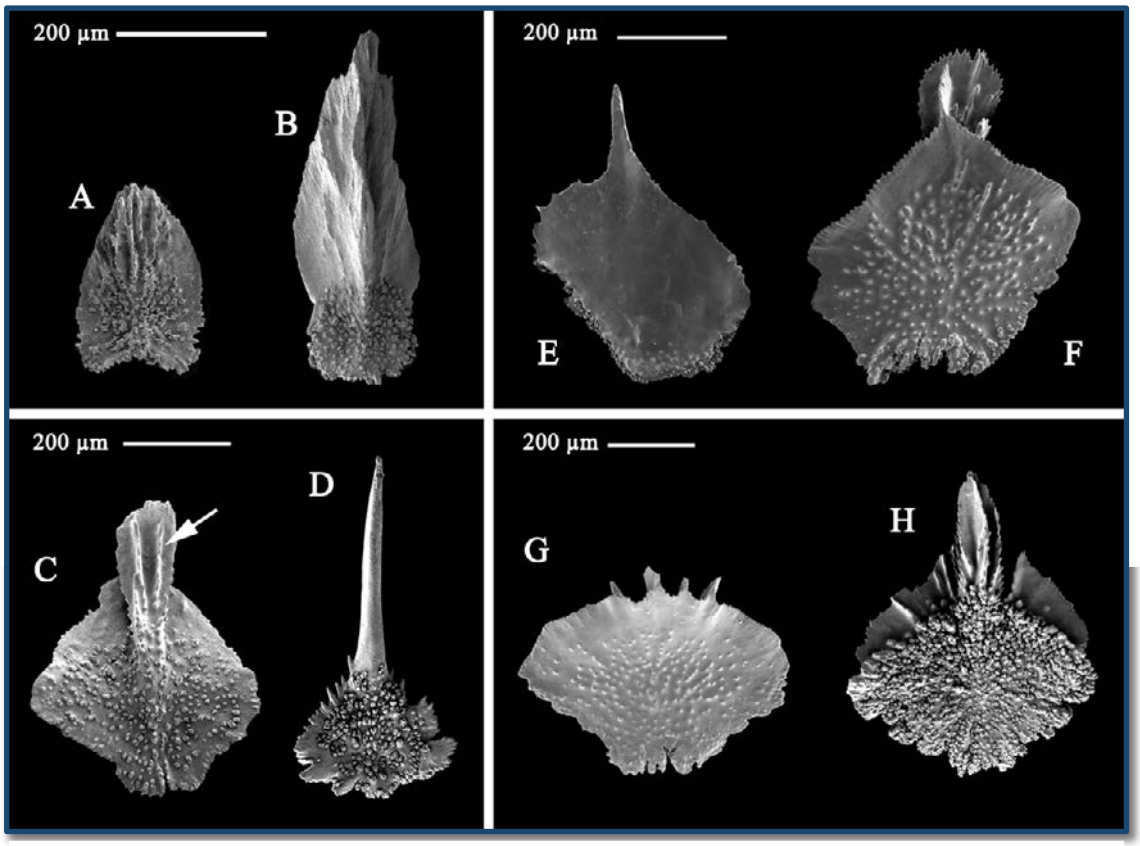


Figure 4.3.- Opercular scales: **A**, *Thouarella crenelata*, type ZMB Cni 6081, low H:W relation; **B**, *Thouarella moseleyi*, holotype BM 1889.5.27.39, high H:W relation. Marginal scales: **C**, *Thouarella brucei*, lectotype NMS.Z.1921.143.1298, the arrow points the central keel; **D**, *Thouarella alternata*, syntype USNM 30097, without keel. Outer surface of marginal scales: **E**, *Thouarella laxa*, holotype COEL03576, smooth; **F**, *Thouarella antarctica*, US 1660, with granules. Marginal scales: **G**, *Thouarella viridis*, holotype ZIZMH C11744, round apical shape; **H**, *Thouarella striata*, type ZMB Cni 8594 , pointed apical shape.

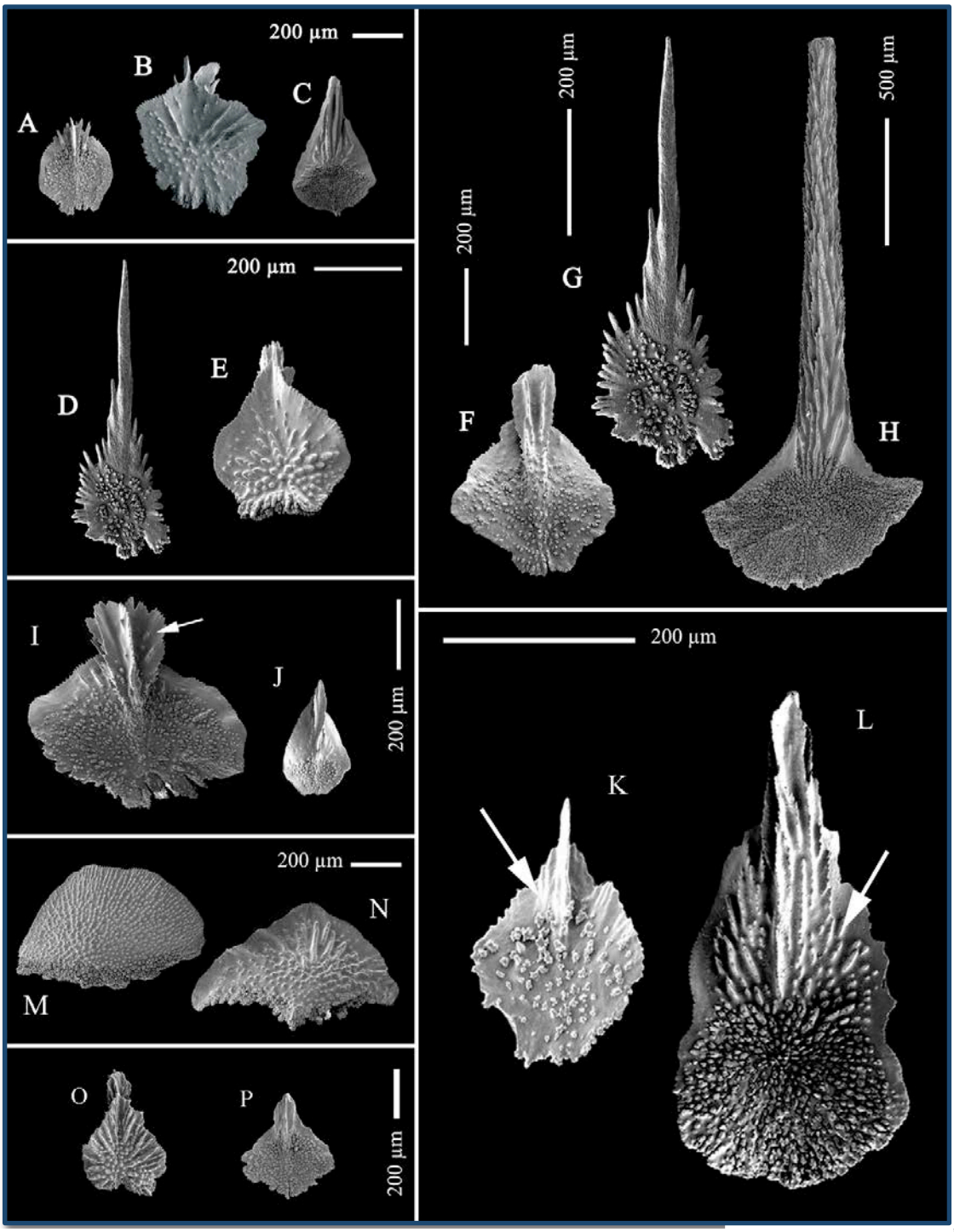


Figure 4.4.- Marginal scales: **A**, *Thouarella crenelata*, type ZMB Cni 6081, oval shape; **B**, *T. grandiflora*, holotype MPUW 44, pentagonal shape; **C**, *T. coronata*, holotype G224, triangular shape; **D**, *T. recta*, holotype USNM 30040, pectinate margin; **E**, *T. regularis*, lectotype NHMUK 1889.5.27.60, serrate margin; **F**, *T. brucei*, lectotype NMS.Z.1921.143.1298, without thorn; **G**, *T. recta*, holotype USNM 30040, with smooth thorn; **H**, *Plumarella diadema*, holotype ZIZMH C11740, with ridged thorn; **I**, *T. antarctica*, US 1660, the arrow points a lateral platform on the keel; **J**, *T. hilgendorfi*, holotype USNM 50123, central keel without lateral platforms; **K**, *T. minuta*, holotype ZIZMH C11742, the arrow points the smooth distal inner surface; **L**, *T. biserialis*, holotype USNM 22583, the arrow points the ridged distal inner surface. Abaxial body scales: **M**, *T. andeep*, holotype ZIZMH C11744, rounded; **N**, *T. antarctica*, US 1660, pointed. Marginal scales: **O**, *T. chilensis*, type ZMB Cni 6079, bilobed scale's base; **P**, *T. pendulina*, paratype MNHN-Oct.000-0211, continue base of scale.

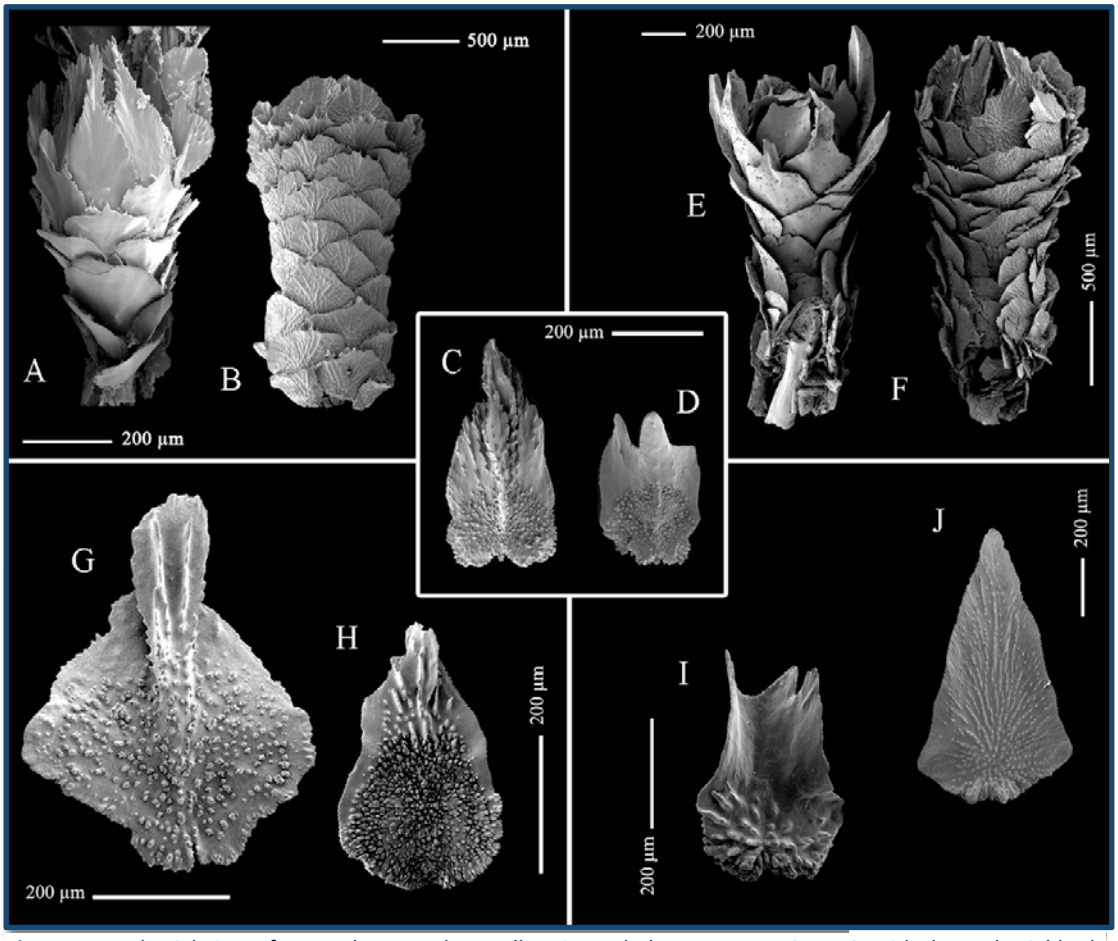


Figure 4.5.- Abaxial view of two polyps: **A**, *Thouarella minuta*, holotype ZIZMH C11742, with three abaxial body scales; **B**, *Thouarella viridis*, holotype ZIZMH C11744, with seven abaxial body scales. Opercular scales: **C**, *Thouarella koellikeri*, syntype USNM 1002247, bilobed scale's base; **D**, *Thouarella carinata*, type ZMB Cni 6078, with continue base of scale. Adaxial view of two polyps: **E**, *Thouarella carinata*, type ZMB Cni 6078, with four adaxial body scales; **F**, *Thouarella koellikeri*, syntype USNM 1002247, with six adaxial body scales. Difference in warts density on the inner surface of marginal scales of two *Thouarella* species: **G**, *Thouarella brucei*, lectotype NMS.Z.1921.143.1298; **H**, *Thouarella biserialis*, holotype USNM 22583. **I**, *Thouarella laxa*, holotype COEL03576, concave opercular scale; **J**, *Thouarella bayeri*, holotype ZIZMH C11738, flat opercular scale.

6-Apical shape of marginal scales.- The apical shape of marginal scales in *Thouarella* species can vary from rounded (state 0), pointed (state1). (Fig. 4.3G, H).

7-Shape of marginal scales.- The shape of marginal scales could be oval(state 0), pentagonal (state 1) or triangular (state 2). (Fig. 4.4A, B, C).

8-Margin of marginal scales.- The margin is usually serrated (state 1), with a toothed edge, but we can also find it a pectinate margin in some species (state 0). (Fig. 4.4D, E).

9-Marginal thorn.-Some species may present a stiff, sharp-pointed projection in the apical free margin of the marginal scales. That projection could be ridged (Fig. 4.4H) or smooth (Fig. 4.4G).

10-Platforms on marginal keels.- The keel present on some marginal scales may have lateral platforms. (Fig. 4.4I, J).

11-Distal inner surface of operculars.-The distal inner surface of opercular scales could be smooth or ridged. (Fig. 4.4K, L).

12-Distal inner surface of marginals.- The distal inner surface of marginal scales could be smooth or ridged. (Fig. 4.4K, L).

13-Shape of abaxial body scales.- The shape of abaxial body scales may be different among species, being more rounded rather than pointed. (Fig. 4.4M, N).

14-Bilobed base of marginal scales.- The base of marginal scales may have a bilobed (Fig.4.4O) or a continue (Fig. 4.4P) shape.

15-Number of abaxial body scales.- Number of scales covering the polyp body beneath the operculum to the polyp base in the abaxial row. Abaxial body wall scales are usually larger than those adaxially. This character has been considered as a continuous character. (Fig. 4.5A, B).

16-Bilobed base of opercular scales.- The base of opercular scales may have a bilobed (Fig. 4.5C) or a continue (Fig. 4.5D) shape.

17-Number of adaxial body scales.- Number of scales covering the polyp body beneath the operculum to the polyp base in the adaxial row. Adaxial body wall scales are usually smaller than those abaxially. This character has been considered as a continuous character. (Fig. 4.5E, F).

18-Warts.- Scales usually have warts in their inner surface, however they can be densely or more sparsely arranged. (Fig. 4.5G, H).

19-Opercular scales concave.- Many *Thouarella* species have opercular scales with a concave surface. (Fig. 4.5I, J).

20-Opercular scales dimorphism.- Opercular scales are usually arranged in two alternate cycles of 4 scales each. The scales of each cycle can vary in size and shape, having a dimorphism in opercular scales. (Fig. 4.6).

21-Polyp length.- The polyp length is the length measured from polyp tip to polyp base (Fig.4.7A, B). This character has been considered as a continuous character.

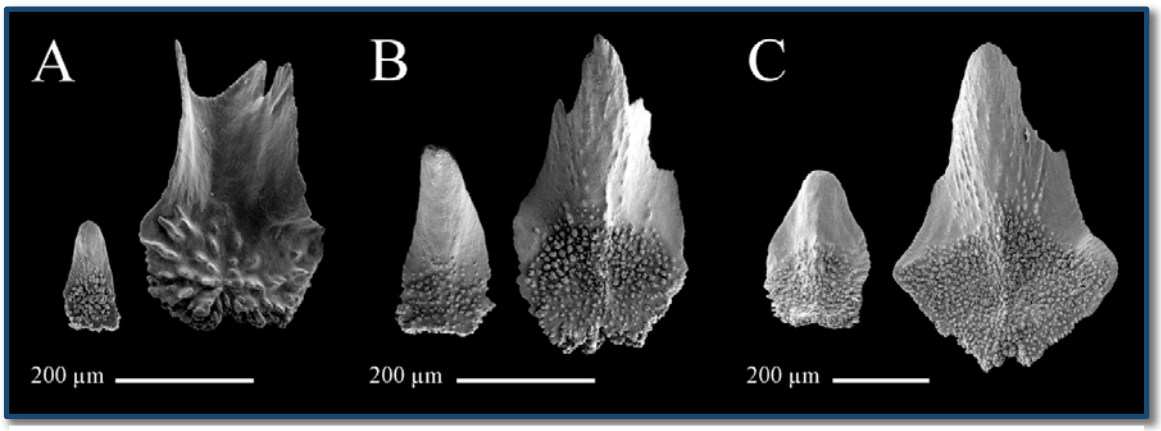


Figure 4.6.- Difference in opercular scales of different cycles in the same *Thouarella* species. **A**, *Thouarella laxa*, holotype COEL03576; **B**, *Thouarella flabellata*, type ZMB Cni 6083; C, *Thouarella undulata*, ZIZMH C11742.

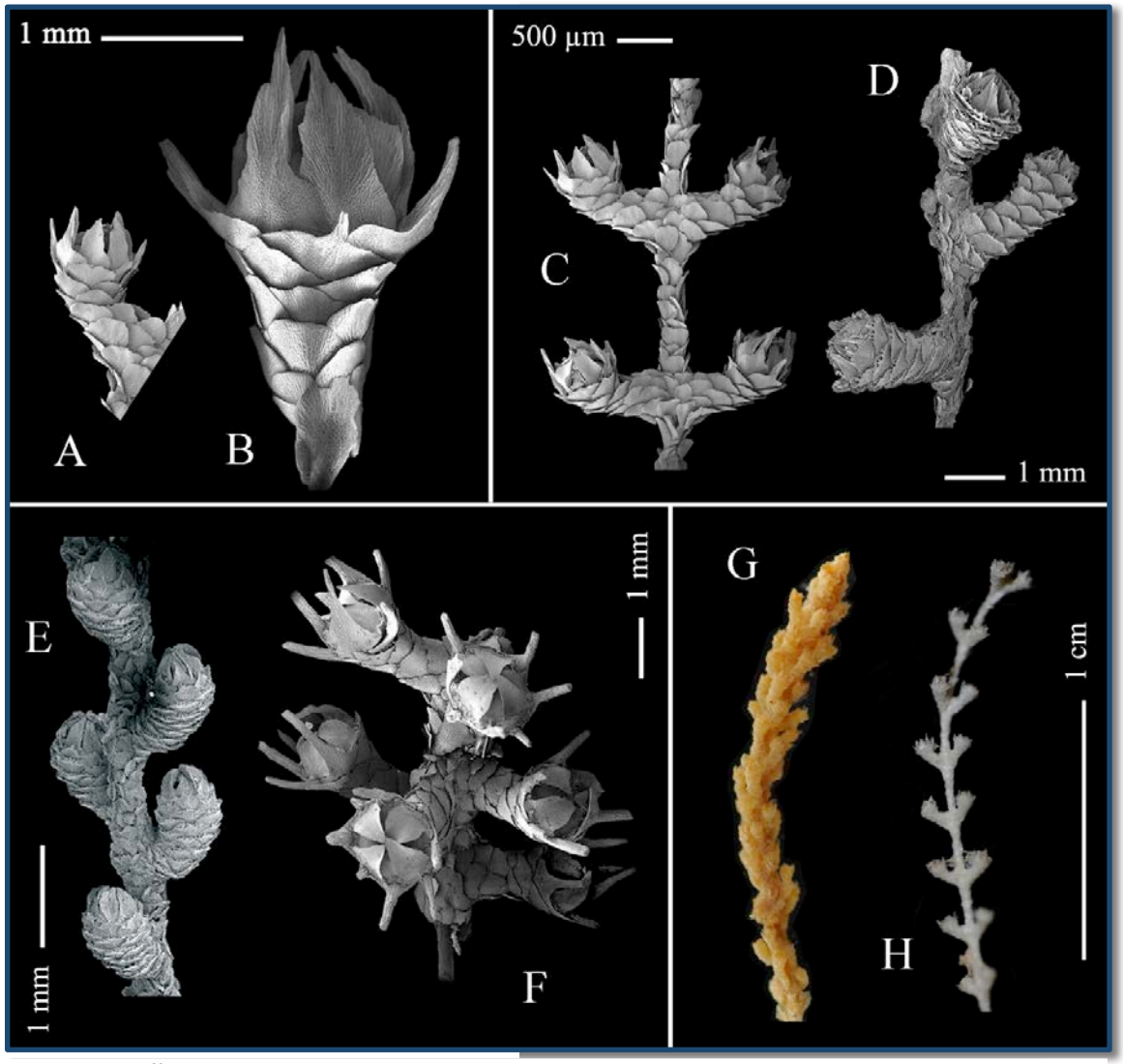


Figure 4.7.- Differences in polyp length and wide in two *Thouarella* species: **A**, *Thouarella tenuisquamis*, syntype MPUW 42; **B**, *Thouarella andeep*, holotype ZIZMH C11744. Detail of branchlets: **C**, *Thouarella tenuisquamis*, syntype MPUW 42, biserial arrangement of polyps; **D**, *Thouarella viridis*, holotype ZIZMH C11744, single arrangement of polyps. Detail of branchlets: **E**, *Thouarella regularis*, lectotype NHMUK 1889.5.27.60, polyps inclined; **F**, *Thouarella sardana*, holotype ZIZMH C11740, polyps perpendicular to stem. Difference in polyps density per centimetre on branchlets: **G**, *Thouarella pendulina*, paratype MNHN-Oct.000-0211; **H**, *Thouarella flabellata*, type ZMB Cni 6083.

22-Polyp wide.- The polyp wide is the wide measured at the upper part of the polyp body (Fig. 4.7A, B) under the head of polyp (operculum). This character has been considered as a continuous character.

23-Singly arrangement of polyps.- The polyps of *Thouarella* species may be arranged in different configurations from isolated (state 1, including irregular or spiral arrangements) to pairs or whorls. (Fig. 4.7C, D).

24-Orientation of polyps.- The polyps of some species stand perpendicular (state1) to the branch or are slightly inclined (state 0) distally, such polyps have usually adaxial body scales reduced in size and/or in number due to the lean. (Fig. 4.7E, F).

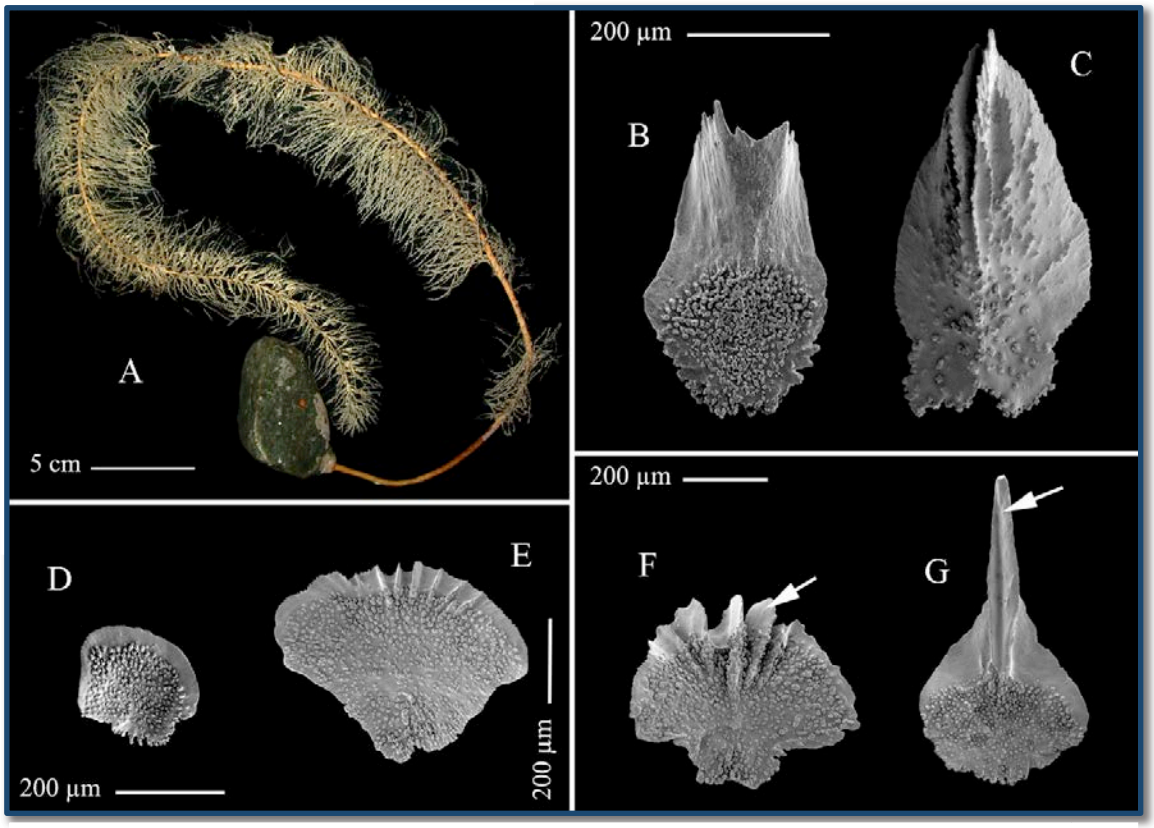


Figure 4.8.- A, Bottlebrush colony shape of *Thouarella minuta*, holotype ZIZMH C11742. Opercular scales: **B**, *Thouarella laxa*, holotype COEL03576, apex truncated; **C**, *Thouarella grandiflora* MPUW44, pointed apex. Body scales: **D**, *Thouarella hilgendorfi*, type BM 1889.5.27.40, free margin smooth; **E**, *Thouarella viridis*, holotype ZIZMH C11744, arrow points small crests. Difference on marginal scales ornamentation on the free margin: **F**, *Thouarella viridis*, holotype ZIZMH C11744, arrow points small crests; **G**, *Thouarella carinata*, type ZMB Cni 6078, arrow points a central spine.

25-Number of polyps/whorls per cm.- The density of polyps and whorls per centimetre is sometimes a useful character in species identification. This character has been considered as a continuous character. (Fig. 4.7G, H).

26-Bottlebrush colony shape.- The commonest branching structure of *Thouarella* species is the bottlebrush (state 1), with branchlets arranged on all sides of the main stem, in at least three directions. However it can also be found other branching structures like pinnate, where branchlets are arranged on each side of the branch as a feather, or dichotomous where branchlets divide into two. (Fig. 4.8A).

27-Opercular scales apex truncated.- Some species present a truncated apex in opercular scales (state 1) while the majority have an entire pointed or rounded apex (Fig. 4.8B, C).

28-Crests on body scales.- Upper body scales may have small crests around the free margin (state 1). (Fig. 4.8D, E).

29-Spine on marginal scales.- The presence of a spine (state 1) is defined as a prickle or hard pointed projection (Fig. 4.8F, G).

30-Crests on marginal scales.- Marginal scales may have small crests around the free margin (state 1). (Fig. 4.8F, G).

4.3.1.3 Data analysis

Family Primnoidae

The phylogenetic relationships within Primnoidae were assessed using the program NONA version 2.0 (Goloboff 1999) under WinClada version 1.0 (Nixon 1999). A matrix (Table 4.3) consisting of 27 discrete morphological characters used to define and distinguish genera was used to deduce a maximum parsimony phylogenetic hypothesis of the family. All characters were scaled prior to the phylogenetic analysis in order to avoid undesired overweighting of transformation series with more character states and to balance the influence of non-additive binary coded characters. We chose a base value of 90 for binary (0, 1) transformation series in order to avoid fractional weights for other characters in the matrix after scaling (not allowed by NONA). We set NONA to perform 1000 independent replicates using the tree bisection-reconnection algorithm (tbr; option mult*max*), and to save all the best trees found during the heuristic search. Several trees were found and a consensus strict compromise was performed in order to get the single most parsimonious phylogenetic tree for the family *Primnoidae*, consistency (CI) and retention (RI) indices were also calculated.

Genus *Thouarella*

The phylogenetic relationships within *Thouarella* were assessed using the program NONA version 2.0 (Goloboff 1999) under WINCLADA version 1.0 (Nixon 1999). A matrix (Table 4.4) consisting of 30 morphological characters (25 discrete and 5 continuous) covering all recognized *Thouarella* species was used to deduce a maximum parsimony phylogenetic hypothesis of the genus. Continuous characters were coded using Gap-Weighting (Thiele 1993) and treated as ordered transformation series. All characters were scaled prior to the phylogenetic analysis in order to avoid undesired overweighting of transformation series with more character states and to balance the influence of non-additive binary coded characters. We chose a base value of 90 for binary (0, 1) transformation series in order to avoid fractional weights for other characters in the matrix after scaling (not allowed by NONA). We set NONA to perform 1000 independent replicates using the tree bisection-reconnection algorithm (tbr; option mult*max*), and to save all the best trees found during the heuristic search. Several trees were found and a consensus strict compromise was performed in order to get the single most parsimonious phylogenetic tree for the genus *Thouarella*, consistency (CI) and retention (RI) indices were also calculated.

		Taxon	Characters																										
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
0	CIRC	*Circinisidinae* (outgroup)	0,1,2,3,4,5	0	0	0	0	0,2	0,1,2	0,1	0,1	0	0,1	0,1,2	0,1	0,2	0	0	0,2	0,1,2	0	0	0	0,1,2	0	0	0	0	0
1	PRIS	*Primnoeides*	6	0	1	0	4	0	0	0	1	0	1	0	0	0	0	0	2	-	0	0	0	0	0	1	1	1	1
2	OPHI	*Ophidiogorgia*	0	0	1	1	4	1	0	0	-	-	2	1	0	0	0	0	1	-	0	0	1	0	0	1	1	1	1
3	AGLA	*Aglaoprimnoa*	4	0	1	0	4	1	0	0	2	0	2	0	0	0	0	0	2	-	0	0	2	0	0	1	1	1	1
4	ARMA	*Armadillogorgia*	4	0	1	0	4	2	0	0	-	-	-	-	-	0	3	0	2	0	0	0	0	0	0	1	1	1	1
5	AINI	*Ainigmaptilon*	0	1	1	0	5	1	0	1	1	0	1	1	0	0	1	0	2	-	0	0	0	0,4	1	1	1	1	1
6	PRIA	*Primnoella*	0	0	1	0	4	1	0	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1
7	CONV	*Convexella*	0	0	1	0	4	0	0	1	1	0	1	0	3	0	0	1	2	0	0	0	0	0	0	1	1	1	1
8	HEPT	*Heptaprimnoa*	2	0	1	0	4	1	0	1	1	0	1	0	0	0	0	2	2	1	0	0	0	0	0	1	1	1	1
9	CALN	*Callozostron*	0	0	1	1	4	0	1	1	1	0	1	1	4	0	0	1	2	0	0	0	0	0	1	1	1	1	1
10	ARNT	*Arntzia*	0	0	1	1	4	0	1	1	3	0	1	1	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1
11	THOU	*Thouarella* (*Thouarella*)	8	0	1	0	0	0	0	1	3	0	1	0	4	0	0	3	2	0	0	0	2	0	0	1	1	1	1
12	EUTH	*Thouarella* (*Euthouarella*)	7	0	1	0	4	0	0	1	2	0	1	0	4	0	0	3	2	0	0	0	2	0	0	1	1	1	1
13	DIPL	*Thouarella* (*Diplocalyptra*)	1	0	1	0	4	0	0	1	2	0	1	0	4	0	0	3	2	0	0	0	0	0	0	1	1	1	1
14	EPIT	*Thouarella* (*Epithouarella*)	8	0	1	0	0	0	0	1	2	0	1	0	3	0	0	3	2	0	0	0	2	0	0	1	1	1	1
15	META	*Metafannyella*	1	0	1	0	4	1	0	1	2	0	1	0	0	0	4	1	2	0	0	0	0	0	0	1	1	1	1
16	FANN	*Fannyella* (*Fannyella*)	2	0	1	0	4	1	0	1	3	0	1	0	3	0	4	3	0	0	0	0	5	0	0	1	1	1	1
17	SCYP	*Fannyella* (*Scyphogorgia*)	8	0	1	0	4	1	0	1	3	0	1	0	4	0	4	1	2	0	0	0	5	0	0	1	1	1	1
18	CYAT	*Fannyella* (*Cyathogorgia*)	1	0	1	0	4	1	0	1	3	0	1	0	4	0	4	1	2	0	0	0	5	0	0	1	1	1	1
19	ONOG	*Onogorgia*	0	0	1	1	4	1	0	1	3	0	1	0	3	0	4	1	2	0	0	0	5	0	0	1	1	1	1
20	PYRO	*Pyrogorgia*	2	0	1	0	4	1	0	1	3	0	1	0	0	0	0	1	2	0	0	0	2	0	0	1	1	1	1
21	MIRO	*Mirostenella*	1	0	0	0	4	0	0	1	0	0	1	1	4	0	0	1	2	0	0	0	0	0	1	1	1	1	1
22	ACAN	*Acanthoprimnoa*	7	0	1	0	2	0	0	1	4	0	1	1	4	0	0	1	2	0	0	0	3	0	1	1	1	1	1
23	PLUM	*Plumarella* (*Plumarella*)	7	0	1	0	2	1	0	1	1	0	1	1	2	0	0	1	2	0	0	0	1	0	1	1	1	1	1
24	CALA	*Callogorgia*	7	0	1	0	4	1	0	1	3	0	1	1	0	0	0	1	0	0	0	0	1	0	1	1	1	1	1
25	FANE	*Fanellia*	1	0	1	0	4	1	0	1	3	0	1	1	0	0	0	1	0	0	0	0	6	0	0	1	1	1	1
26	PALA	*Paranarella*	1	0	1	0	4	1	?	1	2	0	1	1	0	0	0	6	0	0	2	0	1	0	1	1	1	1	1
27	PROA	*Primnoa*	3	0	1	0	0	1	2	1	2	0	1	0	0	0	0	3	0	0	0	0	1	0	1	1	1	1	1
28	AUST	*Australogorgia*	1	?	1	0	0	1	2	1	2	1	3	1	0	0	0	6	0	0	1	0	1	0	1	1	1	1	1
29	NARE	*Narella*	1	0	1	0	4	1	2	1	2	1	5	1	0	0	0	6	0	0	1	0	4	0	1	1	1	1	1
30	ARTH	*Arthrogorgia*	7	0	1	0	4	1	2	1	2	1	6	1	4	0	0	6	0	0	3	0	1	2	1	1	1	1	1
31	PORA	*Paracalyptrophora*	1	0	1	0	4	1	2	1	2	1	6	1	4	0	0	6	0	0	3	0	0	1	1	1	1	1	1
32	CALY	*Calyptrophora*	1	0	1	0	4	1	0	1	2	1	6	1	4	0	0	6	0	0	3	1	0	1	1	1	1	1	1
33	TOKO	*Tokoprymno*	3	0	1	0	2	0	1	1	2	0	1	1	0	0	0	1	2	0	0	0	2	0	1	1	1	1	1
34	PAST	*Parastenella*	1	0	1	0	4	0	1	1	2	2	1	1	4	0	0	4	2	0	0	0	1	0	1	1	1	1	1
35	CAND	*Candidella*	1	0	1	0	4	0	1	1	2	1	5	1	0	0	0	0	2	-	-	0	1	0	1	1	1	1	1
36	MICR	*Microprimnoa*	4	0	1	0	3	0	1	1	1	1	5	1	0	1	2	0	2	-	-	0	2	0	1	1	1	1	1
37	PTER	*Pterostenella*	7	0	1	0	4	0	0	1	2	1	4	1	3	0	0	2	2	0	0	0	1	0	1	1	1	1	1
38	PERI	*Perissogorgia*	0	0	1	0	4	1	0	1	2	1	4	1	2	0	0	7	0	1	0	0	4	0	1	1	1	1	1
39	DASY	*Dasystenella*	8	0	1	0	4	0	0	1	2	1	4	1	4	0	0	4	2	1	0	0	1	0	1	1	1	1	1
40	PSEU	*Pseudoplumarella*	7	0	1	0	2	2	0	1	2	1	4	1	0	0	0	5	2	1	0	0	0	0	0	1	1	1	1
41	TAUR	*Tauroprimnoa*	8	0	1	0	4	0	0	1	2	1	5	1	4	0	0	4	2	1	1	0	1	0	1	1	1	1	1
42	SCOP	*Scopaegorgia*	8	0	1	0	4	0	0	1	2	1	0	0	2	0	0	2	2	0	0	0	0	0	0	1	1	1	1
43	PRSA	*Primnocapsa*	1	0	1	0	1	0	0	1	2	2	1	1	2	0	0	1	2	0	0	0	1	0	1	1	1	1	1
44	DIGI	*Digitogorgia*	8	0	1	0	4	0	0	1	1	0	1	0	2	0	0	1	2	0	0	0	1	0	1	1	1	1	1
45	FAXI	*Plumarella* (*Faxiella*)	7	0	1	0	3	0	0	1	3	0	1	0	2	0	0	1	2	0	0	0	1	0	1	1	1	1	1
46	DICH	*Plumarella* (*Dicholaphis*)	8	0	1	0	0	0	1	1	1	0	1	0	4	0	0	1	2	0	0	0	1	0	1	1	1	1	1
47	VERT	*Plumarella* (*Verticillata*)	7	0	1	0	4	0	1	1	1	0	1	0	4	0	0	1	2	0	0	0	1	0	1	1	1	1	1

Table 4.3.- Character matrix used in phylogenetic analysis as defined by Table 4.1. A question mark indicates the state was unknown; a dash indicates the character was inapplicable.

		Taxon	Characters																												
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
0	AED	*Ainigmaptilon edisto*	0	0	0	0	0	0	0	0	0	0	-	0	0	0	0	?	0	?	1	0	0	5	1	0	0	0	0	0	0	0	0
1	AFF	*Thouarella affinis*	0	1	1	0	1	1	1	2	1	0	0	1	0	1	0	3,4	1	3	0	1	0	2	2	1	0	3,4,5	1	0	0	0	0
2	AND	*Thouarella andeep*	1	0	1	0	0	1	1	2	1	2	-	1	0	0	0	1,2	0	1	1	1	1	5	4	1	1	6	1	0	0	0	0
3	ANT	*Thouarella antarctica*	0	1	1	0	1	1	1	2	1	0	1	2	0	1	0	2,3,4	1	?	1	1	0	3	6	1	0	7	1	0	0	0	0
4	BIP	*Thouarella bipinnata*	0	1	1	0	1	1	1	2	1	0	1	0	0	1	0	0,1,2	1	0	1	1	0	4	6	1	1	4	0	0	0	0	0
5	BIS	*Thouarella biserialis*	0	0	1	0	1	1	1	2	1	0	0	0	1	0	0	3,4	0	0	1	0	0	1	2	0	0	1	0	0	1	0	0
6	BRE	*Thouarella brevispinosa*	0	1	1	0	1	1	1	2	1	0	1	0	0	1	0	3,4	1	?	0	1	0	5	5	1	0	3,4,5,6	1	0	0	1	0
7	BRU	*Thouarella brucei*	0	1	1	0	1	1	1	2	1	0	1	0	0	1	0	1,2	1	0,1	0	1	1	2	3	1	0	6	1	0	0	0	0
8	CHI	*Thouarella chilensis*	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	3,4,5	1	2	1	0	0	5	6	1	0	6,7	1	0	0	0	0
9	CLA	*Thouarella clavata*	0	1	0	0	1	1	1	2	1	0	0	1	?	0	0	3,4	1	2	1	1	0	2	4	1	0	3,4	1	0	0	0	0
10	COR	*Thouarella coronata*	1	0	1	0	1	1	1	2	1	0	0	0	1	1	0	2,3	0	1	1	0	0	3	1	0	1	1,2,3	0	0	0	1	0
11	CRE	*Thouarella crenelata*	0	1	1	0	1	1	0	0	0	0	1	2	1	0	1	3,4,5,6,7	1	4.5	1	0	0	5	7	1	0	4,5,6	1	0	1	0	0
12	CRI	*Thouarella cristata*	0	0	1	0	1	1	1	2	1	0	1	1	1	1	0	3,4,5,6	0	1,2	1	0	0	4	3	1	0	8	0	0	0	1	1
13	DIS	*Thouarella dispersa*	0	1	0	1	1	1	1	2	1	0	0	0	0	0	0	2	1	1.2	0	1	0	3	5	1	0	2	1	0	0	0	0
14	GRN	*Thouarella grandiflora*	0	1	1	0	1	1	0	1	1	0	0	1	0	0	0	3,4,5	1	2	0	1	0	3	4	1	1	4	1	0	0	0	1
15	GRA	*Thouarella grasshoffi*	0	1	1	0	1	1	1	1	1	0	0	0	0	1	0	2,3,4	1	1	1	0	0	1	3	0	0	3	1	0	0	0	0
16	HIC	*Thouarella hicksoni*	1	1	1	0	1	1	1	2	1	0	1	0	0	1	0	1,2	1	0.1	0	1	1	1	2	1	0	6,7	1	0	0	0	0
17	HIL	*Thouarella hilgendorfi*	0	0	0	0	1	1	1	2	1	0	1	0	0	1	0	3,4	0	0	1	1	1	1	2	0	0	2,3	1	0	0	1	0
18	KOE	*Thouarella koellikeri*	0	1	1	0	1	1	1	2	1	0	0	2	0	1	0	4,5,6,7	1	3,4,5	1	1	0	3	4	1	0	6	1	0	0	0	0
19	LAX	*Thouarella laxa*	0	0	0	0	1	0	1	2	1	0	1	0	0	0	0	2,3	0	1,2	1	1	1	1	0	0	1	1,2,3	1	1	0	1	0
20	MIN	*Thouarella minuta*	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0,1	0	0	0	0	0	0	0	1	0	6	1	0	0	1	0
21	MOS	*Thouarella moseleyi*	0	1	1	1	1	1	1	2	1	0	0	0	1	0	0	1,2	1	1,2	1	1	0	2	1	0	0	1	0	0	0	0	0
22	PCH	*Thouarella parachilensis*	0	1	1	0	1	1	0	1	0	0	0	1	0	1	1	5,6,7,8,9	1	4.5	1	0	0	5	8	1	0	6,7	1	0	0	0	0
23	PAR	*Thouarella parva*	1	0	1	0	1	?	0	2	1	0	0	?	1	0	0	2,3	0	2	1	1	0	0	1	0	0	2	0	0	1	0	0
24	PEN	*Thouarella pendulina*	0	1	1	0	1	1	1	2	1	0	0	0	0	1	0	1,2	0	0.1	1	0	0	0	3	1	0	7	1	0	0	0	0
25	REG	*Thouarella regularis*	0	1	1	0	1	1	1	2	0	0	1	0	1	1	0	4,5,6	0	1.2	1	0	0	1	3	1	0	6	1	0	0	0	0
26	STR	*Thouarella striata*	0	1	1	1	1	1	1	2	1	0	1	1	1	1	0	1,2,3	0	1,2	1	1	0	2	3	1	0	6,7	1	0	0	0	0
27	TRI	*Thouarella trilineata*	0	0	1	0	0	1	1	2	1	0	0	1	1	1	0	2,3	0	1	1	0	0	2	3	1	0	9	0	0	1	1	1
28	TYD	*Thouarella tydemani*	0	0	0	0	1	0	1	2	1	0	1	0	0	0	0	2,3	0	1,2	1	1	1	1	1	0	1	2	1	1	0	1	0
29	VAR	*Thouarella variabilis*	0	1	1	0	1	1	1	2	1	0	1	0	0	1	0	1,2	0	0.1	1	0	0	2	4	1	0	1,2,3,4,5,6	1	0	0	1	0
30	VIR	*Thouarella viridis*	0	1	1	0	0	1	0	1	1	0	0	1	0	0	0	3,4	1	1.2	1	0	0	4	3	1	0	6	1	0	1	0	1
31	VIT	*Thouarella vitjaz*	0	0	1	0	1	1	1	2	1	0	0	0	0	1	0	2,3	0	1	1	0	0	0	1	0	0	3	0	0	0	0	0

Table 4.4.- Character matrix used in phylogenetic analysis as defined by Table 4.2. A question mark indicates the state was unknown; a dash indicates the character was inapplicable.

4.3.2 Phylogenetic hypotheses based on molecular (DNA) information

Phylogeny based on molecular information relies on the comparison of the sequences of aminoacids of proteins and nucleotides sequences of DNA to infer evolutionary relationships.

4.3.2.1 DNA extraction, amplification, and sequencing

Octocoral specimens used in this study include ethanol and formalin preserved material collected on the *R/V Polarstern* cruises ANT XIII/4 (EASIZ II, Ecology of the Antarctic Sea Ice Zone, 17 March-20 May 1996), ANT XVII/3 (EASIZ III, Ecology of the Antarctic Sea Ice Zone, 18 March–11 May 2000), ANT XIX/5 (LAMPOS, Latin American Polarstern Study, 3 April–5 May 2002), ANT XXI/2 (BENDEX, BENthos Disturbance Experiment, 17 November 2003–18 January 2004), ANT XXIII/8 (23 November 2006–30 January 2007), ANT XXIV/2 (ANDEEP-SYSTCO, Antarctic Benthic Deep-Sea Biodiversity System Coupling, 28 November 2007–4 February 2008) and from cruises on board the *R/V Tangaroa* (Ross Sea 2004, TAN0402, 26 January–5 March 2004), and *R/V Italica* (VLT-2004,*Italica* XIX, 1 February–5 March 2004) by the members of the research group "Biodiversidad y Ecología de Invertebrados Marinos (BEIM)" of the University of Seville, Spain.

Molecular analyses were performed on 30 coral specimens (Table 4.5) belonging to 16 of 39 recognized genera of the family Primnoidae, and 16 of 27 genera present in Antarctic and Sub

Antarctic waters. Specimens were previously identified using the methodology described by various authors (Bayer & Stefani 1988; Alderslade 1998).

Species	Locality	Latitude	Longitude	Depth	Vessel	Expedition	Station	Gear	Date	Catalog number	Fixative	WEG
Ainigmaptilon antarcticum	south east South Georgia, Subantarctica	-54.4605	-35.68883	249-256	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-182-01	AGT	12 Apr 2002	6500	Ethanol	9
Armadillogorgia cyathella	north west South Georgia, Subantarctica	-53.39467	-42.70383	306-343	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-167-01	BT	09 Apr 2002	1309	Ethanol	10
Arntzia gracilis	Austasen, Eastern Weddell Sea, Antarctica	-72.857	-19.644	596-598	R/V Polarstern	ANT XXI-2 BENDEX	PS65-292-01	BT	31 Dec 2003	2987	Ethanol	11
Convexella magelhaenica	west Burdwood Bank, Subantarctic	-54.023	-62.022	271	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-145-01	AGT	05 Apr 2002	1403	Ethanol	12
Dasystenella acanthina	off Atka Bay, Antarctica	-70.398	-8.311	595-602	R/V Polarstern	ANT XXIV-2	PS71-048-01	AGT	12 Jan 2008	6274	Ethanol	1
Digitogorgia kuekenthali	south east of Isla Nueva, Subantarctica	-55.55667	-65.91	2468	R/V Polarstern	ANT XIII-4	PS40-114-01	AGT	18 May 1996	CRO-30	Formaline	25
Fannyella abies	North of Joinville Island, Antarctica	-62.431	-55.585	243	R/V Polarstern	ANT XXIII-8 CLIMANT	693-01	BT	05 Jan 2007	CRO-48	Ethanol	7
Fannyella kuekenthali	north west South Georgia, Subantarctica	-53.397	-42.708	312-322	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-164-01	AGT	09 Apr 2002	1306	Ethanol	13
Fannyella rossi	Cape Adare, Antarctica	-72.031	170.905	270-280	R/V Tangaroa	TAN 0402	145	SEL	26 Feb 2004	6451	Ethanol	4
Fannyella rossi	Cape Hallett, Victoria Land, Antarctica	-72.308	170.447	234-235	R/V Italica	VLT (Italica XIX)	H-out-4(bis)	AGT	12 Feb 2004	3278	Ethanol	8
Fannyella spinosa	Cape Adare, Antarctica	-71.778	170.964	219-230	R/V Tangaroa	TAN 0402	026	ORH	09 Feb 2004	6450	Ethanol	14
Metafannyella mawsoni	Shag Rocks, South Georgia, Subantarctica	-53.396	-44.752	434	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-160-01	AGT	09 Apr 2002	1361	Ethanol	17
Mirostenella articulata	north west South Georgia, Subantarctica	-53.397	-42.708	312-322	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-164-01	AGT	09 Apr 2002	6062	Formaline	15
Ophidiogorgia paradoxa	Cape Hallett, Victoria Land, Antarctica	-72.283	170.180	408-414	R/V Italica	VLT (Italica XIX)	H-in-2??	AGT	11 Feb 2004	3265	Ethanol	3
Plumarella bayeri	north west South Georgia, Subantarctica	-53.395	-42.704	306-343	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-167-01	BT	09 Apr 2002	6192	Formaline	16
Plumarella castellviae	SE Isla Nueva, Tierra del fuego, Subantarctica	-55.480	-66.075	1147	R/V Polarstern	ANT XIII-4	PS40-111-01	AGT	17-may-96	62	Formaline	28
Plumarella diadema	Shag Rocks, South Georgia, Subantarctica	-53.396	-44.752	434	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-160-01	AGT	09 Apr 2002	6065	Formaline	30
Plumarella undulata	north west South Georgia, Subantarctica	-53.395	-42.704	306-343	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-167-01	BT	09 Apr 2002	6303	Formaline	29
Primnoella chilensis	Isla Bridges, off Baliza Cap. Iturrieta, Subantarctica	-54.883	-68.217	25	-	-	-	SCUBA	28 Dec 2010	ARG005	Ethanol	*
Primnoidae gen. nov.	north west South Georgia, Subantarctica	-53.395	-42.704	306-343	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-167-01	BT	09 Apr 2002	6501	Ethanol	22
Scopaegorgia liouvillei	south Elephant Island, Antarctica	-61.391	-55.447	285-288	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-252-01	AGT	25 Apr 2002	CRO-59	Ethanol	18
Tauroprimnoa austasensis	Halley Bay, Eastern Weddell Sea, Antarctica	-75.062	-27.345	406	R/V Polarstern	ANT XV-3 EASIZ II	PS48-167	GSN	12 Feb 1998	6413	Formaline	31
Thouarella andeep	off Atka Bay, Antarctica	-70.398	-8.311	595-602	R/V Polarstern	ANT XXIV-2	PS71-048-01	AGT	12 Jan 2008	CRO-28	Ethanol	5
Thouarella antarctica	north west South Georgia, Subantarctica	-53.397	-42.708	312-322	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-164-01	AGT	09 Apr 2002	1660	Ethanol	19
Thouarella minuta	Cape Norvegia, Antarctica	-71.191	-12.320	312-323	R/V Polarstern	ANT XVII-3 EASIZ III	102-01	GSN	03 Apr 2000	332	Ethanol	20
Thouarella pendulina	Elephant Island, Antartica	-60.982	-55.189	215	R/V Polarstern	ANT XXIII-8 CLIMANT	611-01	BT	21 Dec 2006	6252	Ethanol	6
Thouarella parachilensis	?	?	?	?	?	?	?	?	?	6502	Ethanol	23
Thouarella variabilis	Cape Adare, Antarctica	-72.298	170.328	103	R/V Italica	VLT ITALICA (XIX)	H-OUT-5A	BENNA	09 Feb 2004	3256	Ethanol	2
Thouarella viridis	north west South Georgia, Subantarctica	-53.395	-42.704	306-343	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-167-01	BT	09 Apr 2002	1415	Ethanol	21
Tokoprymno anatis	north east Elephant Island, Antarctica	-60.647	-53.957	2896	R/V Polarstern	ANT XIX-3 ANDEEP I	PS61-046-08	AGT	02 Feb 2002	1600	Formaline	27

Table 4.5.- Specimens used in this study with data collection. WEG, well at DNA extraction gel. **-**, data not applicable; **?**, data unknown; *****, DNA already extracted.

DNA from samples preserved in ethanol and a pair of samples preserved in formalin (Table 4.5) were extracted from 20 to 30 mg of tissue previously grinded to powder in liquid N_2. We use as many pestles and mortars as samples we have. We put a sample of 0.5 cm in the mortar, which is inside a cork box, we add liquid N_2 and carefully grind the material until liquid N_2 is completely evaporated. With helping of a spatula, the powder is placed in a 1.5 ml microcentrifuge tube with 180µl of Buffer ATL. Following the Spin-column protocol of Qiagen DNeasy Blood & Tissue Kit, firstly we add 20 µl of proteinase K at each tube and mix them thoroughly by vortexing, and let a completely lysis of the tissues by incubate them at 56°C for 1-3 hours. We need to vortex the tubes occasionally during the incubation to disperse the sample. 200 µl of Buffer AL were added to the sample and mixed by vortexing, immediately after 200 µl ethanol (96-100%) were added and mixed again thoroughly by vortexing. The resulting mixture was pipetted into the DNeasy Mini spin column previously placed in a 2ml collection tube, and centrifuged to retain the DNA in the membrane of the column and discard the flow-through. A couple of washing-DNA steps were conducted using 500 µl of Buffer AW1 and 500 µl of Buffer AW2, then Mini spin column was centrifuged for 3 minutes at maximum speed to dry the DNeasy membrane to ensure that no residual ethanol will be carried out during the DNA elution and thus interfere with subsequent reactions. To elude the DNA, the Mini spin column was placed in a new clean 1.5 ml microcentrifuge tube, and 200 µl of Buffer AE was added directly onto the membrane, incubated at room temperature for 1 minute and centrifuged to elute the DNA.

DNA from samples preserved in formalin (Table 4.5) was extracted using a CTAB extraction protocol. To remove excess of EtOH we blot the sample, then the tissue is placed in 1.0 ml 2x

CTAB in a 1.5 ml microfuge tube and let soak for 24 hours on a Nutator™ rotatory platform to mix samples gently. During this period CTAB buffer was changed several times. In a clean1.5 ml eppendorf tube, 0.2% (1.0 µl) β-mercaptoethanol (βME) is added to 500 µl 2x CTAB and incubated at 55°C for 5 minutes. Then, we remove the sample from CTAB, blot it to remove excess liquid, place it in a ceramic mortar and cover it immediately with liquid nitrogen. We use a pestle to pulverize the tissue to a fine powder while it is still frozen. Immediately we scrape powdered tissue into the tube containing warm CTAB/βME. To this tube we add 5 µl of proteinase K and mix gently by inverting the tube several times, and let a completely lysis of the tissue by incubate it at 65°C for 4 hours (or at 55°C from 24 hours for very old specimens up to 3 days to help break DNA protein crosslinks). Tubes need to be flicked periodically to mix. After lysis, we centrifuge the tube for several seconds and gently transfer liquid supernatant to a clean 1.5 ml tube and discard the pellet. 600 µl PCI (25:24:1 Phenol:Chloroform:Isoamyl alcohol) were added and mixed gently for 10 minutes, followed by 5 min of centrifugation at top speed (13,000 rpm) at 4°C. The supernatant (aqueous top layer) was transferred to a clean 1.5 ml tube, being careful not to disturb the interface (if the interface is very thick or poorly formed, add 500 µl PCI more to the mixture, mix gently again for 10 min and centrifuge 5 min at 4°C). Let the sample chill on ice for 20 minutes. Then, 500 µl of cold chloroform and 100 µl of Phytopure resin were added and mixed gently for 10 min, followed by 1o min of centrifugation at 1,200 rpm at 4°C. The supernatant is transferred again to a clean 1.5 ml tube, and an equal volume of cold isopropanol was added. Chill the mixture in -20°C freezer overnight (samples can be left in the freezer indefinitely at this stage). Centrifuge at top speed for 30 min at 4°C and drain off EtOH inverting the tube or using a pipette. Add 1.0 ml of cold 70% EtOH and centrifuge 10 min at 4°C, drain off EtOH again, and repeat it once. The pellet was dried in a vacuum centrifuge for 25 min with no heat or air dry by suspending the tube upside down. The DNA pellet was finally re-suspended in 25µlof sterile TE buffer by flicking and inverting the tube and 1.5 µl RNAse (1.0 mg/ml stock solution; final concentration in reaction should be 50 µg/µl) added and incubated at 37°C for 30 minutes.

DNA extracted samples were loaded into mini-gel wells (see 4.3.2.2 Gel electrophoresis) and viewed through the gel imaging system Molecular Imager® Gel Doc™ XR connected to a System with Image Lab™ Software (UV transilluminator) to check DNA extractions. DNA extracted samples were then kept in the fridge at 4°C until amplification.

Two mitochondrial genes (*mtMutS* and *COI*) and two nuclear regions (*28S* and DNA fragments containing the transcribed spacers *ITS1* and *ITS2*, including gene *5.8S*) were amplified (Fig. 4.9) using primers previously designed by different authors (Table 4.6). Template DNA for sequencing was obtained from the combination of 1 µl of DNA template (1:1 or 1:10 dilutions of genomic DNA extractions) and 49 µl of a cocktail (Table 4.7) containing the reagents and enzymes to run the polymerase chain reaction (PCR). Negative controls were run in every experiment to test for contamination. The PCR conditions (Table 4.8) for all sequenced genes include an initial period of denaturation where DNA fragments are heated at high temperatures (94-95°C), reducing DNA double helix to single strands accessible to primers. Followed by a cooling period called annealing (50-58°C), where primers anneal to the complementary regions in the DNA template strands, and double strands are formed again

between primers and complementary sequences, and a final extension (72°C) where DNA polymerase synthesises a complementary strand. The enzyme reads the opposing strand sequence and extends the primers by adding nucleotides in the order in which they can pair. The 3-step process (a cycle) is repeated 30-35 times. The reactions were carried out in MJ Research Thermocyclers PTC-225 (GMI, Inc.) and in MJ Mini™ Gradient Personal Thermal Cycler (Bio-Rad Laboratories, Inc.). PCR products were loaded into mini-gel wells (see 4.3.2.2 Gel electrophoresis) and viewed in the UV transilluminator to check amplifications. For specimens that yielded no visible PCR product, we ran a second PCR reaction (nested PCR) using an internal forward primer and 1 µl of the original product as template (Berntson & France 2001). Negative (no DNA) controls from the first reaction were re-amplified in the second reaction to check for sample contamination.

Gene	Primer name	Primer lenght (bp)	Primer sequence (5'-3')	Published Reference
mtMutS				
	ND4L-2475F	19	TAGTTTTACTGGCCTCTAC	Brugler & France 2008
	ND4L-2599F	21	GCCATTATGGTTAACTATTAC	France & Hoover 2002
	ND4L-2625F	18	TACGTGGYACAATTGCTG	McFadden *et al.* 2006
	msh1-2761F			unpub
	msh1-2768F	24	TTTTACCGGGGGAGGTTATTCTCA	unpub
	msh1-2932R	24	TTTGGCATGAGCCTGATGTTCCTA	unpub
	msh1-3055R	20	GGAGAATAAACCTGAYAC	Brugler & France 2008
	msh1-3270R			unpub
	Mut-3458R	18	TSGAGCAAAAGCCACTCC	Sánchez *et al.* 2003
COI				
	COII-8068F	22	CCATAACAGGACTAGCAGCATC	McFadden *et al.* 2004
	COII-8068xF		CCATAACAGGRCTWGCAGCATC	McFadden *et al.* 2011
	COI-AN-F	20	CCAGGTAGTATGTTAGGRGA	A. Nevarez, unpub.
	COI-OCT-R	20	ATCATAGCATAGACCATACC	McFadden *et al.* 2011
	HCO-2198R	26	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* 1994
28S				
	28S-F	24	CACGAGACCGATAGCGAACAAGTA	A. Reynolds, unpub.
	28S-R	21	TCATTTCGACCCTAAGACCTC	A. Reynolds, unpub.
ITS1-ITS2				
	ITS1wa-F	20	GATTGAATGGTTTAGTGAGG	McFadden & Hutchinson 2004
	ITS2w-R	21	ATTGCCACGTACGGGGTTGTC	McFadden *et al.* 2001
ITS1				
	ITS-2-R	20	GCTGCGTTCTTCATCGATGC	White *et al.* 1990
ITS2				
	ITS-5-R	22	GGAAGTAAAAGTCGTAACAAGG	White *et al.* 1990

Table 4.6.- Primers used in this study.

The PCR product was purified using a PEG-precipitation, where the product was precipitated with an equal volume of PEG (20% w/vPEG 8000 in 2.5 M NaCl), briefly (15 min) incubated at 37°C, and centrifuged at 14,000 rpm (15 min) to precipitate DNA. Supernatant was discarded, and the DNA pellet was washed with 200 µl of ice cold 80% ethanol, followed by 8 min of centrifugation at 14,000 rpm and at 4°C; the supernatant was again discarded, and the pellet was washed with 200 µl of ice cold 95% ethanol, followed by 8 min of centrifugation at 14,000 rpm and at 4°C; the supernatant was again discarded, and the pellet was dried in a vacuum centrifuge for 15-30 min with no heat. The pellet was finally re-suspended in 10-30 µl (depending on brightness of PCR product on gel) of sterile water.

If when visualizing PCR product appears more than a single band, the PCR product needs to be purified by cutting out the bands to get the two sequences separately. First, we run a gel a

little bit thicker than usual, 0.4 g of agarose are dissolved in 40 ml TBE rather than 0.35 g in 35 ml. We must visualise the band, in an ethidium bromide stained gel, in a darkroom on a UV light-box while we cut the band. As UV is dangerous we need to proceed carefully, we need to wear gloves, long-sleeves, face protection and never look at UV with unprotected eyes. If possible we should minimise the amount of time the DNA is exposed. This is because the UV mutagenises the DNA at a measurable rate. A scalpel blade is used to cut around the band of interest; it is good to trim off as much empty agarose as possible. Then the excised band is placed in a 1.5 ml microfuge tube and resuspended in sterile water.

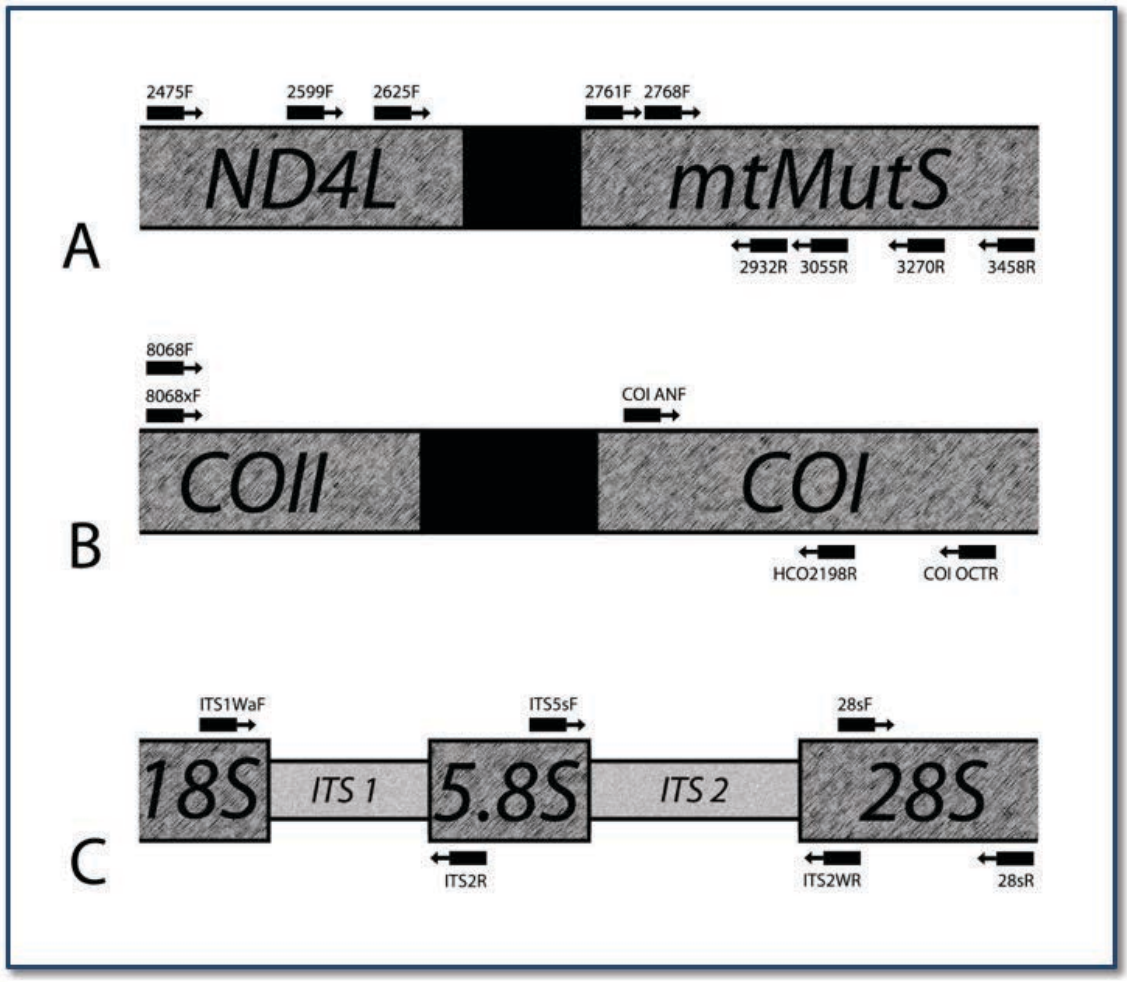


Figure 4.9.- Scheme of the primers location in amplified genes. **A**, location of primers used to amplify *mtMutS*; **B**, location of primers used to amplify *COI* and *COII*; **C**, location of primers used to amplify the Internal Transcribed Spacer and *28S* gene.

A full spectrum UV/Vis spectrophotometer (NanoDrop ND-100) was used to measure the DNA concentration (ng/µl) of the purified PCR products. To sequence the purified PCR products we need them at 10 ng/µl in a total volume of 10-12 µl and primers used in the amplification at 3 ng/µl in a total volume of 10-12 µl. We used dH_2O to dilute the purified PCR products and primers to get the final concentration needed for sequencing at University of Washington High Throughput Sequencing Lab.

Reagents and enzymes	*mtMutS*	*COI*	*28S*	UCD	*ITS*
dH_2O	33.8	34.3	34.3	36.3	36.3
PCR Buffer[1]	5	5	5	5	5
$MgCl_2$[2]	1.5	1	1	1	1
Primer-F[3]	2	2	2	1	1
Primer-R[3]	2	2	2	1	1
dNTP[4]	4	4	4	4	4
BSA	0.5	0.5	0.5	0.5	0.5
TaKaRa Taq™ [5]	0.2	0.2	0.2	0.2	0.2

Table 4.7.- Cocktail content (in µl)of reaction tubes for PCR amplification.
[1]10x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl) (*Takara Bio Inc.*)
[2] 25mM (*Promega*)
[3] 10 µM
[4] 2.5mM (*Takara Bio Inc.*)
[5] 5 units/µl (*Takara Bio Inc.*)

	mtMutS		*COI*		*28S*		UCD		*ITS*	
step	temperature	time	temperature	time	temperature	time	temperature	time	temperature	time
1	95ºC	3'	95ºC	10'	95ºC	2'	94ºC	5'	94ºC	2'
2	95ºC	1' 30"	94ºC	1' 30"	94ºC	30"	94ºC	30"	94ºC	30"
3	50ºC	1' 30"	57.7ºC	1' 30"	50ºC	30"	52ºC	1'	52ºC	1'
4	72ºC	1'	72ºC	1'	72ºC	1'	72ºC	1' 30"	72ºC	1' 30"
5	go to nº2	34 times	go to nº2	34 times	go to nº2	34 times	go to nº2	34 times	go to nº2	29 times
6	72ºC	5'	72ºC	5'	72ºC	5'	72ºC	5'	72ºC	5'
7	4ºC	forever	4ºC	forever	4ºC	forever	35ºC	3'	4ºC	forever
8							4ºC	forever		

Table 4.8.- PCR protocols used in this study

4.3.2.2 Gel electrophoresis

Agarose gel electrophoresis is the easiest and the most common way to separate and analyse DNA. The DNA is visualised in the gel by addition of ethidium bromide (EtBr). This binds strongly to DNA by intercalating between the bases, EtBr is also fluorescent meaning that it absorbs invisible UV light and transmits the energy as visible light. Thus, DNA might be quantified or a particular band isolated.

- *0.7% Agarose gel*: shows a good separation of large DNA fragments. Used to quantify DNA after extraction.
- *1% Agarose gel*: shows a good separation of small DNA fragments. Used to quantify DNA after amplification.

Protocol to make Agarose mini-gels:

1. Dissolve agarose (0.245gfor 0.7% gel and0.350g for 1% gel) in 35ml of 0.5x TBE in an Erlenmeyer flask.
2. Heat solution to boiling point, stirring continuously with a magnetic stirrer.
3. When agarose is completely dissolved, remove from heat and let cool to 65°C.
4. Place a clean, dry gel mould on level surface and make sure end gates are raised up

and clamped. Set the comb straight in slot.

5. Add 35μl of EtBr to the cooled agarose (final EtBr concentration in gel should be 0.5μg/ml, so for each 1 ml of TBE we add 1 μl of EtBr), swirl gently to mix, and pour into gel mould. Check for bubbles and use a spatula or pipette to remove any large ones.
6. When gel mould is cool to the touch and agarose has turned opaque (30 min. should be enough), gently remove the comb.
7. Lower end gates and clamp in lowered position and place gel on platform in mini gel box with wells at anodal end.
8. Add 0.5x TBE to the box until buffer floods over top of gel (gel should be covered by ~ 2 mm of buffer). Mini-gel boxes hold ~ 300 ml buffer.
9. Load 10 μl of DNA into wells (Table 4.9).
10. Add 300 μl (the same amount of TBE you added in 8) EtBr to gel box (~150 μl in each end).
11. Close lid and plug in leads. Turn power supply on and raise voltage to ~ 100 V.
12. When dye front has advanced to end of gel, turn voltage down to 0. Turn off power supply and remove gel.
13. View gel using an UV transilluminator.

Reagents	Extracted DNA	Marker eDNA	Amplified DNA	Marker aDNA
Buffer TE	8	9.5	5	9.5
Loading dye	2	2	2	2
DNA	2		5	
Ladder 1000 bp		0.5		
Ladder 100 bp				0.5
TOTAL μl	**12**	**12**	**12**	**12**

Table 4.9.- Preparation of DNA samples for gel electrophoresis. Volumes in μl. Marker eDNA, DNA ladder of 1kB used in gel electrophoresis to check extracted DNA; Marker aDNA, DNA ladder of 100bp used in gel electrophoresis to check PCR products.

4.3.2.3 Data analysis

LaserGene software (DNASTAR) was used to edit and assembly nucleotide sequences and translate them using the cnidarian mitochondrial genetic code. SeqMan Pro™ (LaserGene) was used to assemble the two DNA sequences provided by High Throughput Sequencing at University of Washington from the two amplified strands, into contigs. SeqMan Pro™ was also used to view, edit the chromatograms and get their consensus sequence. MegAlign™ (LaserGene) was used to align the consensus sequences using the Clustal W Method and get a preliminary and simple phylogenetic tree. MegAlign™ was a quick, helpful and easy tool to modify the alignment after getting the phylogenetic tree by removing sequences from the alignment or adding new ones. EditSeq™ (LaserGene) was used to get the sequences in different formats (.seq, .fas) and also to get the reverse complement sequence when the reverse primer was the only one that was well sequenced.

The aminoacid sequences were aligned using MUSCLE v.3.6 (Edgar 2004), and translated protein-coding regions were adjusted by eye to conform to amino acid alignment using MacClade 4.08 (Maddison & Maddison 2005). The sequences obtained of *Ainigmaptilon antarcticum* were used as an outgroup for the genes analysed (Figure 4.9). Phylogenetic trees were constructed using corresponding nucleotide alignments for each gene separately as well as for the combined dataset.

Maximum likelihood analyses were conducted using PhyML 3.0 (Guindon *et al.* 2010), which uses a HKY85 as a substitution model. PAUP* v. 4.0b10 (Swofford 2003) was used to analyse the maximum parsimony (MP). For maximum parsimony, we used a heuristic search with TBR (tree-bisection-reconnection) branch-swapping algorithm where branches collapsed and create polytomies if the maximum branch length was zero; due to computational and time constraints, we ran 100 bootstrap replicates with a maximum of 1000 trees saved per replicate. Gaps were treated initially as missing data but additional MP analyses were run with gap coded as fifth nucleotide.

Bayesian phylogenetic analyses were conducted using MrBayes v. 3.04 (Huelsenbeck & Ronquist 2001) which uses Markov Chain Monte Carlo methods to approximate the posterior probabilities of trees with a GTR (General Time Reversible) model run for $2.52x10^6$ generations (burnin=2520 generations) for *28S*, $2.016x10^6$ generations (burnin=2016 generations) for ITS, $1.53x10^7$ generations (burnin=$1.53x10^4$ generations) for *mtMutS*, and $3.6x10^7$generations (burnin=$3.6x10^4$generations) for *COI* and separate data partitions were conducted for $3.15x10^6$ generations (burnin=3150 generations) for *28S* and *ITS,* $8.4x10^6$ generations (burnin=8400 generations) for *mtMutS* and *COI* and for$2.4x10^6$ generations (burnin=2400 generations) for the four genes together in a combined analysis. FigTree v1.3.1 was used to display, modify and present the phylogenetic resulted trees.

4.4 Results

4.4.1 Morphological phylogeny

Family Primnoidae

650 trees were found and a consensus strict compromise was performed in order to get the single most parsimonious phylogenetic tree (L=4684; CI=45; RI=70) for the family *Primnoidae*.

In the resulting most parsimonious cladogram (Fig. 4.10) 6 genera group together in a polychotomy, with sister group 1 which includes 4 genera and sister group 1' which includes 29 genera (74% of the primnoids). Most of the clades are characterized by the homoplasic expression of the characters.

The sister group 2' of the second clade is characterized by the synapomorphy "ascus-shaped scales" which includes the genera *Metafannyella*, *Onogorgia* and all *Fannyella* subgenera.

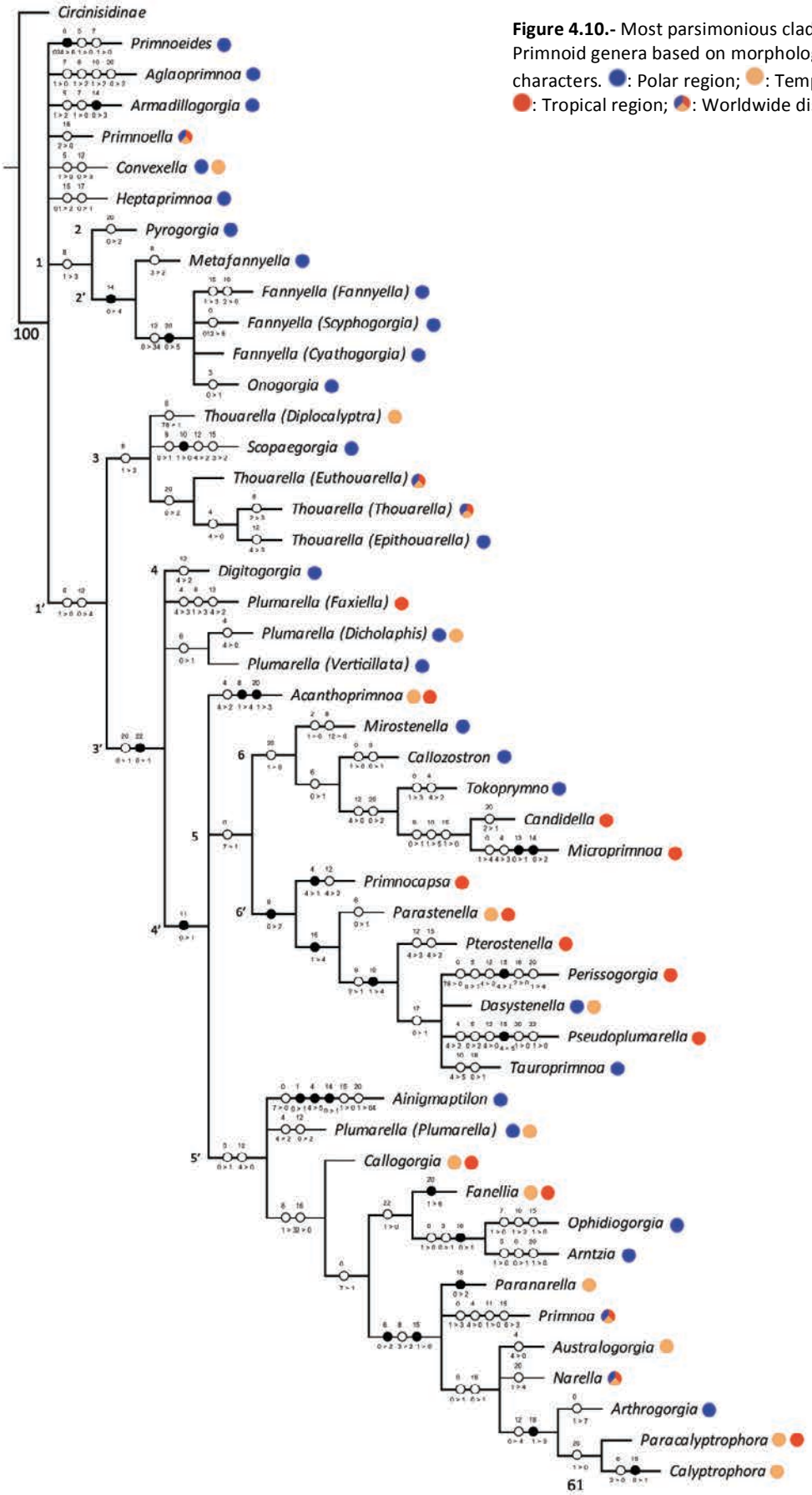


Figure 4.10.- Most parsimonious cladogram of Primnoid genera based on morphological characters. : Polar region; : Temperate region; : Tropical region; : Worldwide distribution.

In the third clade, it can be clearly distinguished the sister group 3 which includes all *Thouarella* subgenera and the genus *Scopaegorgia* separated by their sister group 3' by homoplasic expressions of the external sculpture of the body wall scales and characterised by the synapomorphy of having one single layer of coenenchymal scales. However the state of this character it is reverted in genera such as *Digitogorgia*, *Pseudoplumarella* or *Fanellia* among others.

In the fourth clade, a synapomorphy characterizes the sister group 4' by the absence of a circumoperculum, while *Plumarella* subgenera and *Digitogorgia* are grouped together in a polychotomy. The fifth clade isolates the genus *Acanthoprimnoa* by the synapomorphies of the distal inner surface of opercular scales and the external sculpture of body wall scales and the clade also clearly distinguishes the sister group 5 which includes genera with a planar dichotomous branching patterns whose polyps do not have a correspondence between marginals and operculars (sister group 6') and body wall scales are not ornamented (sister group 6), while the sister group 5' includes genera with polyps appressed to the stem, which have few rows of body scales and polyps facing downward.

Genus *Thouarella*

Five trees were found and a consensus strict compromise was performed in order to get the single most parsimonious phylogenetic tree (L=9393; CI=28; RI=59) for the genus *Thouarella*.

In the resulting most parsimonious cladogram (Fig. 4.11) synapomorphies and homoplasies divide the species in a series of clades, in which some sister groups are represented by a single species or a few species. In the first Clade, the sister group 1 is characterized by the homoplasic expressions of the characters "bottlebrush colony shape" and "presence of spine in marginal scales" (*T. minuta*, *T. hilgendorfi*, *T. laxa* and *T. tydemani*), while the rest of the species are in the sister group 1' which is characterized by the synapomorphy "granular outer surface of opercular scales" and "marginals with a serrated margin".

In the second clade, one of the sister groups is only represented by a single species (*T. biserialis*) separated by their sister group 2' by homoplasic expressions of a set of characters (polyp width and presence of crests on body scales).

The third clade separate a group of two temperate species from the Pacific Ocean (*T. moseleyi* and *T. parva*) from the rest of the species by homoplasic expressions of the character "concave operculars" (sister group 3), while the remaining *Thouarella* species are also grouped by homoplasies in the sister group 3'.

The fourth clade separate a group of three *Thouarella* species from Japan and the Aleutian Islands (*T. coronata*, *T. cristata*, and *T. trilineata*) (again by homoplasic expressions of a set of characters such as presence of a spine in marginal scales, and the polyp length) from the remaining *Thouarella* species, in this case the sister group 4' is grouped by the homoplasy "smooth distal inner surface of marginal scales".

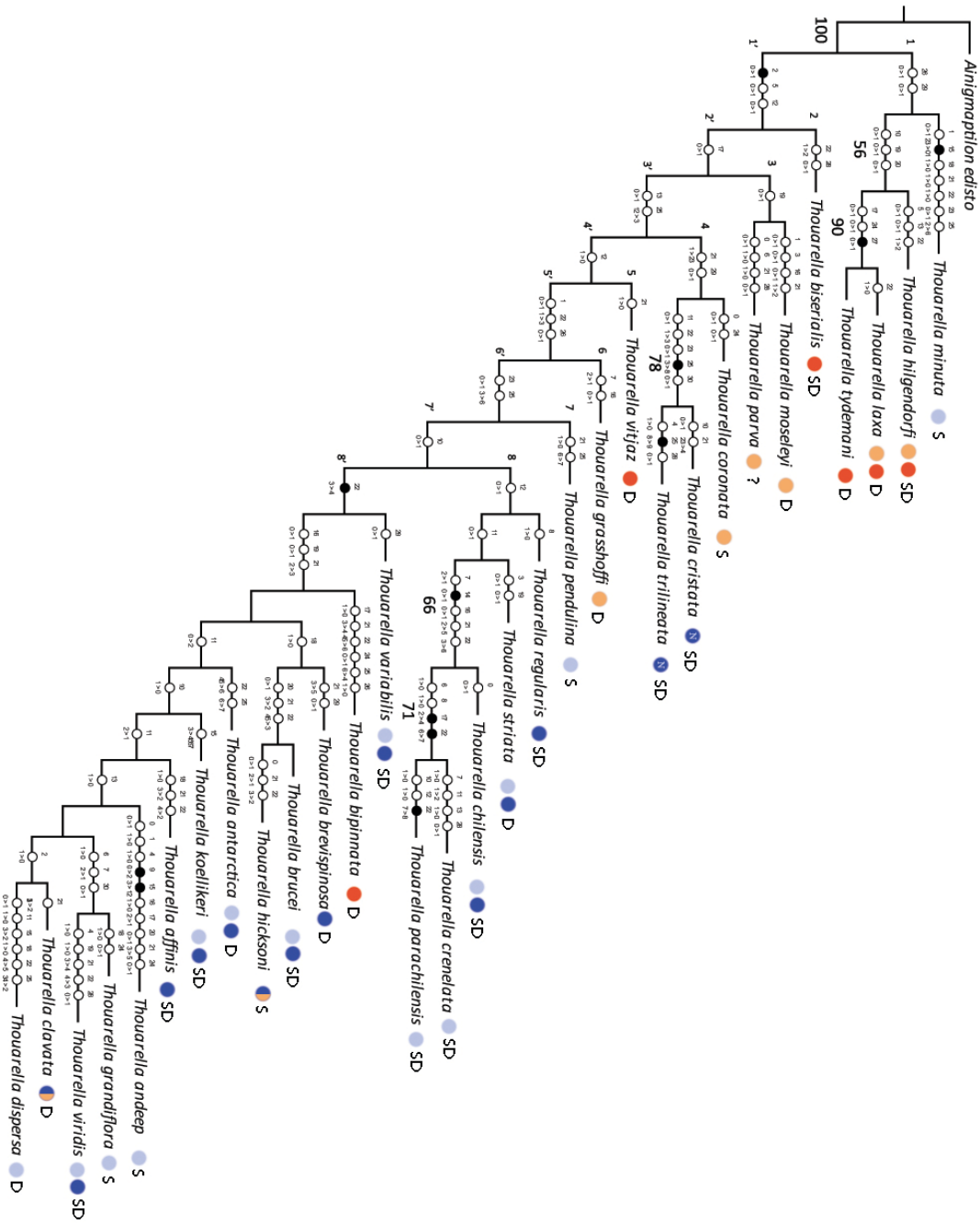


Figure 4.11.- Most parsimonious cladogram of *Thouarella* species based on morphological characters. D: deep-sea; S: shallow-waters; : Antarctic region; : Sub-antarctic region; : South Africa, border with Subantarctic and Temperate region; : Temperate region; : Tropical region; : Subarctic region.

In the fifth clade, one of the sister groups is only represented by the deepest *Thouarella* species (*T. vitjaz*) separated by their sister group 5' by the homoplasic expression of the character polyp length.

In the sixth and seventh clade, a similar situation is showed, *T. grasshofi* is isolated from the rest of the species by homoplasic expressions of characters shape of marginal scales and bilobed shaped of operculars base (sister group 6), and *T. pendulina* by the density of polyps on branches (sister group 7) while the remaining *Thouarella* species are also grouped by homoplasies in the sister group 6' and 7'.

Finally the eighth clade includes the sister group 8 characterized by Antarctic and Subantarctic species having scales with more granules and ridges on their surfaces, resulting in more ornamented sclerites and the sister group 8' characterized by the synapomorphy "wide of polyps".

This sister group 6', together with *T. minuta* include all species present in the Southern Ocean, while the remaining clades include species from temperate and tropical waters. *Thouarella bipinnata* is from tropical waters, but in the cladogram is in the clade with all polar species.

A preliminary version of the phylogeny based on morphological characters showed above were presented at the second International Congress of Invertebrate Morphology (ICIM) hosted by the Museum of Comparative Zoology, the Department of Organismic and Evolutionary Biology, and the Harvard Museum of Natural History, at Harvard University, Cambridge, MA, from June 19th to 24th of 2011. The congress strived to interconnect researchers from the International Society for Invertebrate Morphology and other scientists interested in the morphology and evolution of invertebrate animals.

4.4.2 Molecular phylogeny

4.4.2.1 DNA extraction

22 extracted DNA samples from 30 gorgonian samples were obtained to conduct subsequent PCR analyses. It was not possible to extract DNA from formalin fixed specimens neither with the regular extraction protocol (wells 15, 16 in figure 4.12) nor CTAB extraction protocol (wells 24-31 in figure 4.12).

Extracted DNA from *Thouarella pendulina* (6252) and *Fannyella abies* (CRO-48) (*i.e.* wells 6, 7 respectively), show an increased intensity (meaning more mass of DNA) at the upper side of the electrophoresis gel, which means fragments of larger size, around 3 kb or more, and thus a good DNA quality for amplifications. On the other hand, extracted DNA from *Dasystenella acanthina* (6274) and *Thouarella andeep* (CRO-28) (*i.e.* wells 1, 5), show a higher intensity at the lower side of the electrophoresis gel, which means fragments of smaller size, around 1 kb or less, and thus a degraded DNA which is not so good for PCR analysis. DNA extraction of *Armadillogorgia cyathella* (1309), *Thouarella minuta* (332) and *Primnoidae* gen. nov. (6501) (*i.e.* well 10, 20, 22) appears as a very weak band almost indiscernible, however their PCR amplification were a succeed (for *ITS* see Fig.4.15G).

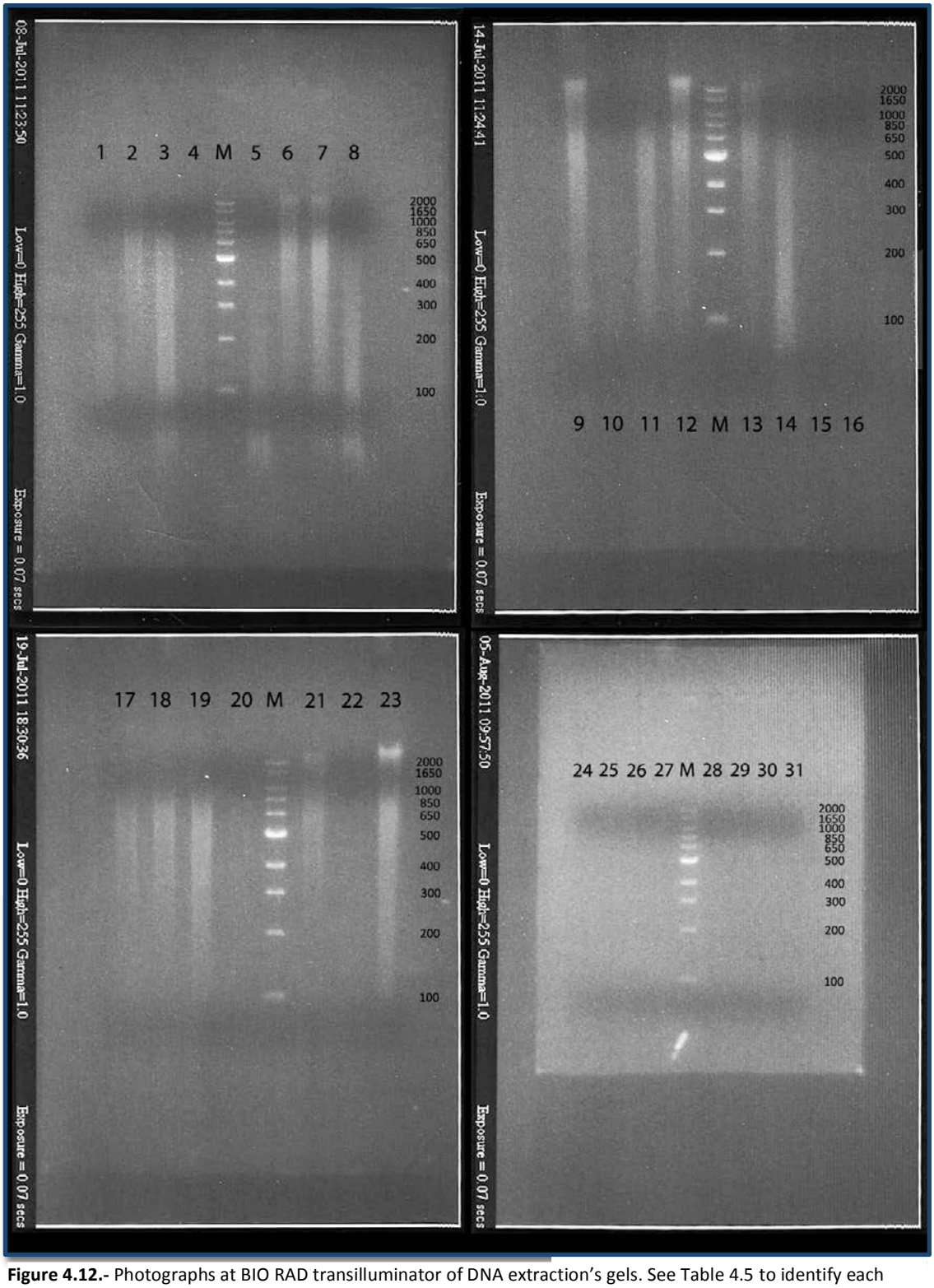


Figure 4.12.- Photographs at BIO RAD transilluminator of DNA extraction's gels. See Table 4.5 to identify each extracted sample.

4.4.2.2 PCR amplification

A total of 29 PCR were conducted to amplify *mtMutS*, *COI*, *28S* and ITS regions on the 22 extracted DNA samples.

Every PCR analysis includes several controls to verify assay performance; positive controls assure that all reactants are performing correctly and negative controls consisting of all reactants except the sample help to identify the cause of background if it is a problem. No PCR contamination was found in any of the analysis conducted.

MtMutS

Because of in PCR of gel A (Fig. 4.13) three samples (3256, 6451 and 6252) did not show any amplification for *mtMutS* (using primers ND4L-2599F and Mut-3458R), a second PCR (Fig. 4.13B), with the same parameters and primers than the first PCR, was conducted. However sample 6252 still did not show any amplification and tried to dilute the sample 1:10 in PCR of gel C without any result.

In samples 6062 and 6192 (gel C), DNA was not amplified because of the chemical (formalin) used to fix and preserve them. All DNA samples of PCR gel D (Fig. 4.13D) were amplified successfully, although the yield of sample 6502 was a little weak and thus a dilution of 1:10 was used to amplify the remaining genes for this sample.

In gels from E to H, PCR analysis were conducted in order to get *mtMutS* amplified from sample 6252 (*Thouarella pendulina*), in E we tried a different forward primer (*mtMutS*-2761F), and in the four first wells of gel F we also changed the reverse primer (*mtMutS*-2761F and *mtMutS*-3270R) but no amplification was observed and in the four last wells of gel F we used ND4L-2475F and 3055R primers and finally we get some amplification, however reverse primer used was very close to the beginning to the gene *mtMutS* (Fig. 4.9) and thus we tried to get a larger DNA piece by using reverse primers further from the origin. Knowing that the last combination of primers was successful, in PCR of gel G (Fig. 4.13) we used in the first four wells, the same forward primer and Mut-3458R as a reverse (the furthest reverse primer for *mtMutS*) and in the four last wells we used the last reverse primer to use, the *mtMutS*-3270R, however there was no results and seems to indicate that reverse primers are the problem, and in the last attempt we tried in gel H forward primer ND4L-2599F and reverse primer *mtMutS*-3270R with no amplification. In every PCR we used samples 1:1 and 1:10 dilutions.

For formalin-fixed samples, we tried to do nested PCR's (Fig. 4.13I-L) in case that in fact the regular amplification works very weakly and then increase the signal by a second PCR using the products of the first one. Moreover, in order to amplify smaller DNA fragments, which usually are more accessible to PCR analysis than the larger ones, a couple of new primers were designed (*mtMutS*-104F and *mtMutS*-268R). However none of the reactions worked, and the only sample with amplification was actually a contamination by the positive control in a nested PCR (Fig. 4.13J), which is more sensitive to contaminations next-to.

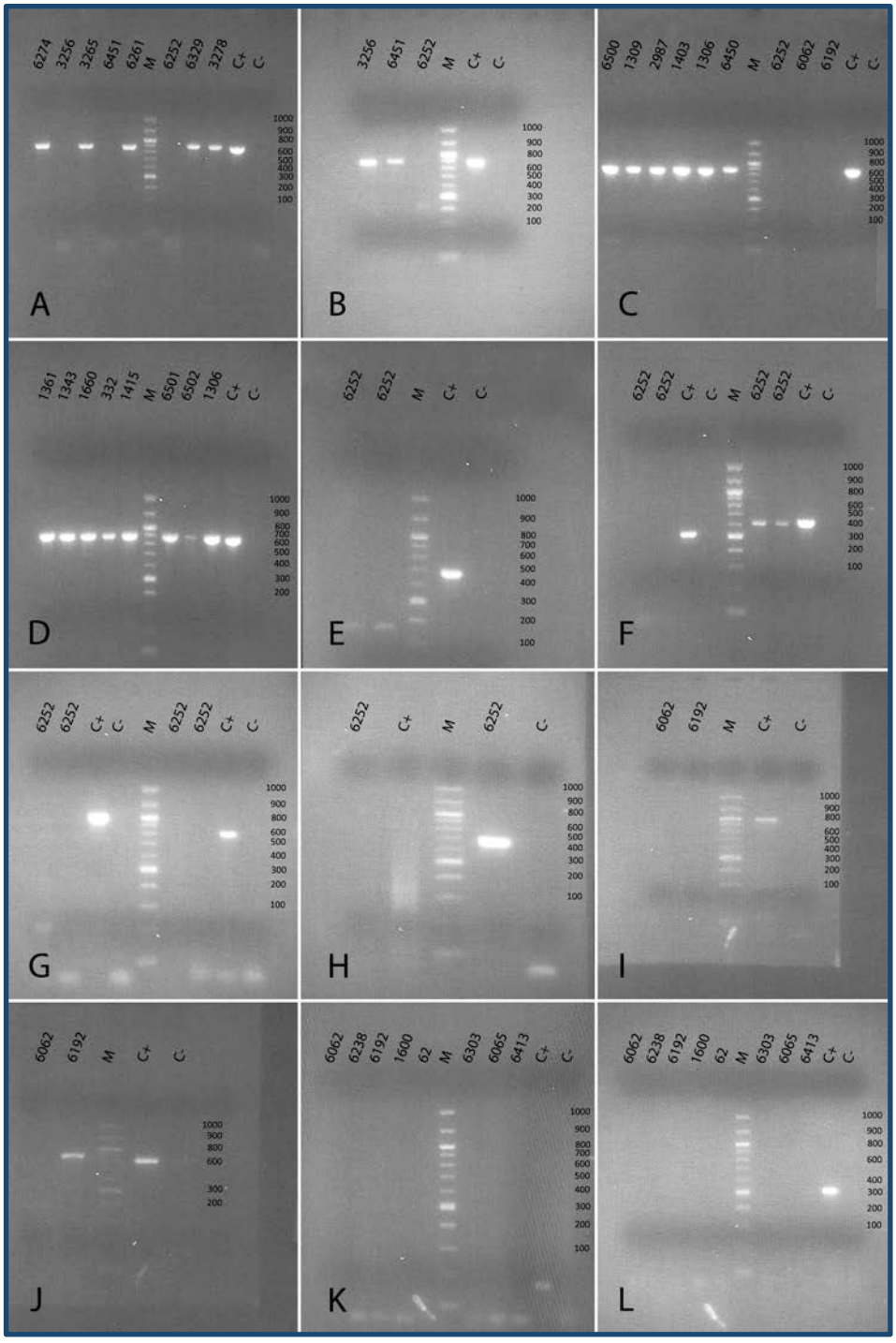


Figure 4.13.- Gel electrophoresis of PCR products for gene *mtMutS*.

COI

PCR products run in gels B, D and E (Fig. 4.14) are from the same set of samples. In gel B, there was amplification only in sample 3278 and the positive control. What happened here was that when the Taq polymerase was added during the PCR cocktail preparation we did not mix well, thus Taq polymerase which is more dense went to the bottom of the eppendorf tube, and only in the two last samples Taq polymerase was not able to sink completely and PCR was carried

out. In gel D (Fig. 4.14), there is no amplification of any sample due to an error of the own thermocycler, to make sure that PCR fails we checked the PCR products with a gel electrophoresis. Finally, in gel E (Fig. 4.14) almost all samples were amplified successfully except for *Thouarella pendulina* (6252). PCR products loaded in wells of first row were carried out using *COII*-8068F and *COI*-OCT-R primers and products loaded in wells of second row were carried out using *COII*-8068xF forward primer.

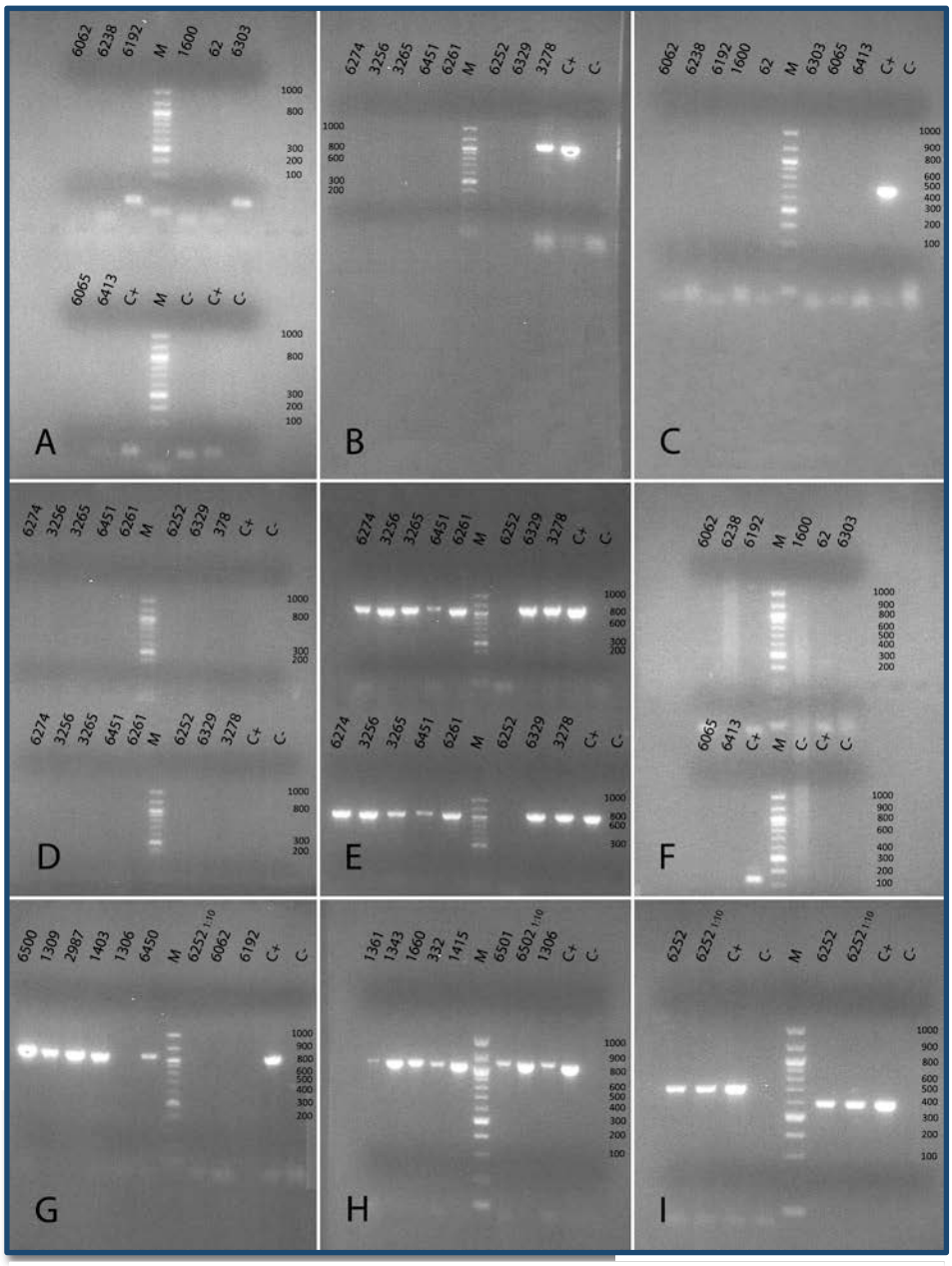


Figure 4.14.- Gel electrophoresis of PCR products. A, amplification of gene *mtMutS*; B-I, amplification of gene *COI*.

PCR products of gels G and H were obtained using *COII*-8068F and *COI*-OCT-R primers. In gel G, sample 1306 was not amplified but it did in a second PCR loaded in gel H. Samples 6062 and 6192 are formalin fixed, thus no reaction was carried out, and a PCR reaction was conducted again for sample 6252 diluted 1:10 (gel G), but did not work and in gel I (Fig. 4.14) we can see

amplification for 6252, where the four first yields were obtained using a different forward primer (*COI*-AN-F), and the four lasts using as well a different reverse primer (HCO-2198R). In that case seems that regular forward primer out from *COI* region did not work and the primer included in the amplified gen did. To be sure that no DNA was extracted from formalin fixed samples we run a nested PCR (C and F in fig. 4.14), no amplification was observed.

28s

All three PCR reactions were a complete succeed (Fig. 4.15A-C), only the two formalin fixed samples (6062 and 6192) failed.

ITS

PCR products loaded in gels D-G have amplified the complete Internal Transcriber Spacer, while PCR products in gel H correspond only to *ITS1* and PCR products in gel I to *ITS2* (Fig. 4.15).

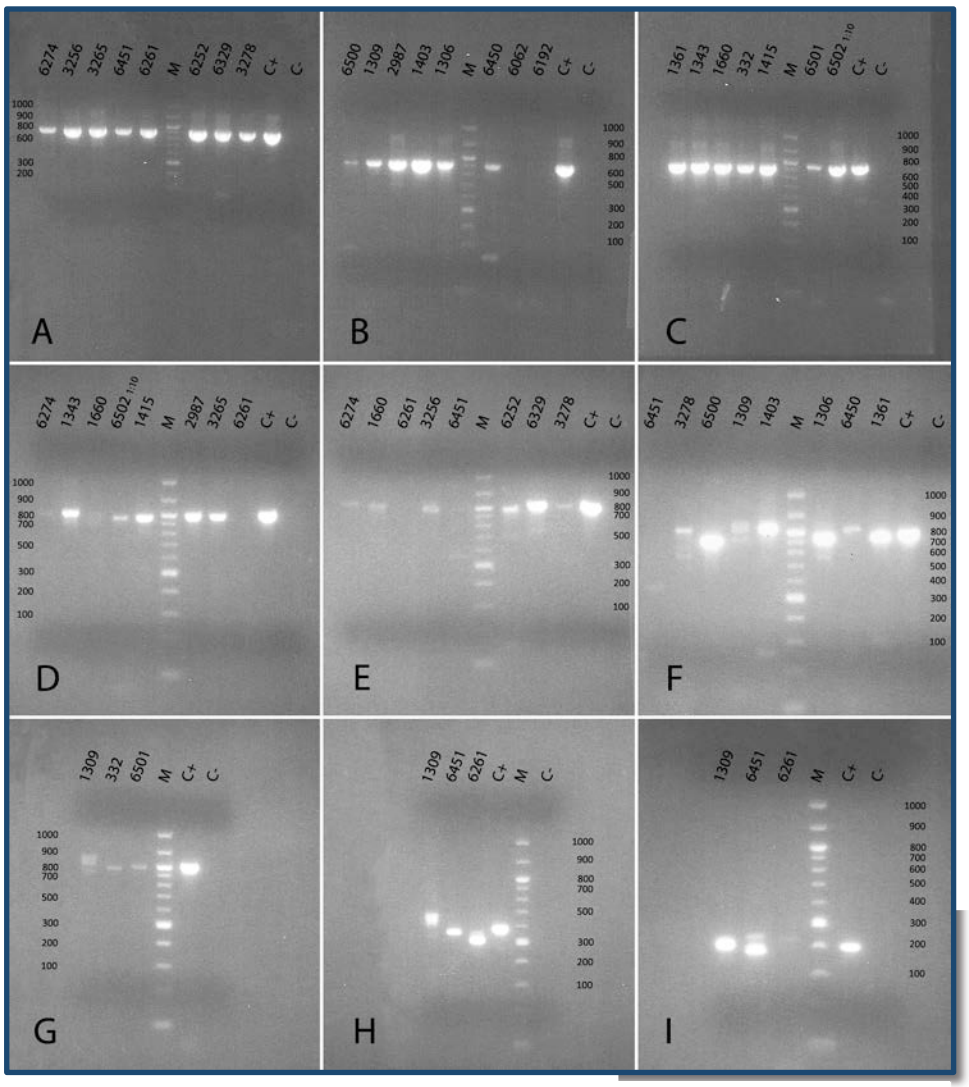


Figure 4.15.- Gel electrophoresis of PCR products. A-C, amplification of gene *28S*; D-I, amplification of *ITS*.

Samples 6274 and 1660 in gel electrophoresis D (Fig. 4.15) have a weak signal, thus the PCR was done over again, and in gel E the resulting DNA amplified was larger. Samples 6261 and 6451 did not show any sign of amplification, thus we tried to amplify the complete region *ITS* separately, running a first PCR to amplify *ITS1* (Fig. 4.15H) and a second one to amplify *ITS2* (Fig. 4.15I). ITS amplification of sample 1309 was also obtained by this method, as the first PCR (Fig. 4.15G) was not very convincing.

4.4.2.3 DNA purification

93 DNA samples have been purified using PEG precipitation and 2 samples by cutting out the band of the agarose gel. To sequence the PCR products the ideal concentration of DNA would be 10 ng/µl, so we check their concentration with NanoDrop (Fig. 4.16) before sending samples to the sequencing lab. After purification seven of our samples gave DNA concentration lower than 10 ng/µl but apparently enough quantity to get an acceptable sequence, only one of them (1306 for *mtMutS*) seems to be lost during the delicate PEG process as its concentration measured by the spectrophotometer is abnormally low 0.35 ng/µl (Table 4.10). In that case, a PCR analysis was run again for the lost sample.

The spectrophotometer also gives a couple of measurements related to nucleic acid purity, ratios of absorbance at 260/280 and 260/230 (Table 4.10). A ratio of absorbance at 260 and 280 nm of 1.8 is generally accepted as "pure" DNA. If the ratio is appreciably lower it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. A second measurement of purity is the 260/230 values, which are often higher than the respective 260/280 values. They are commonly in the range of 1.8-2.2. If the ratio is appreciable lower, this may indicate the presence of co-purified contaminants.

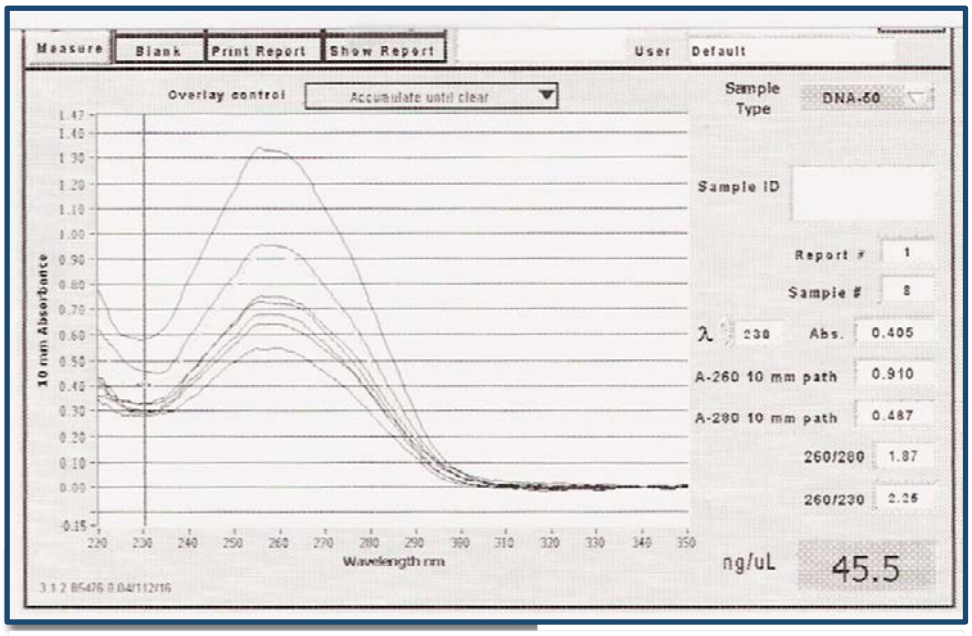


Figure 4.16.- Example of spectrum given by NanoDrop ND-100 displaying a set of seven plots corresponding to *mtMutS* DNA purified samples.

Sample ID	ng/ µL	A260	260/230	260/280	Const.	RA
6274	47.61	0.952	2.08	1.81	50	*MtMutS*
3265	33.98	0.68	2.25	1.87	50	*MtMutS*
6261	37.56	0.751	2.26	1.73	50	*MtMutS*
6329	36.29	0.726	2.21	1.83	50	*MtMutS*
3278	32.04	0.641	2.2	1.73	50	*MtMutS*
3256	66.25	1.325	2.27	1.84	50	*MtMutS*
6451	27.21	0.544	1.92	1.87	50	*MtMutS*
3278	45.49	0.91	2.25	1.87	50	*COI*
6274	59.32	1.186	2.41	1.87	50	*COI*
3256	93.05	1.861	2.26	1.86	50	*COI*
3265	80.05	1.601	2.33	1.88	50	*COI*
6451	15.41	0.308	2.03	1.87	50	*COI*
6261	83.62	1.672	2.34	1.86	50	*COI*
6329	80.5	1.61	2.3	1.85	50	*COI*
6274	85.73	1.715	2.19	1.82	50	*28S*
3256	161	3.22	2.26	1.88	50	*28S*
3265	178.89	3.578	2.18	1.9	50	*28S*
6451	81.12	1.622	2.19	1.84	50	*28S*
6261	92.83	1.857	2.34	1.87	50	*28S*
6252	120.04	2.401	2.33	1.86	50	*28S*
6329	155.69	3.114	2.3	1.93	50	*28S*
3278	93.46	1.969	2.26	1.82	50	*28S*
6500	176.08	3.522	2.34	1.86	50	*MtMutS*
1309	76.63	1.533	2.08	1.82	50	*MtMutS*
2987	109.27	2.185	2.23	1.88	50	*MtMutS*
1403	197.54	3.951	1.16	1.74	50	*MtMutS*
1306	0.53	0.011	1.01	1.76	50	*MtMutS*
6450	104.7	2.094	2.34	1.84	50	*MtMutS*
ARG005	258.61	5.172	2.18	1.89	50	*28S*
6500	85.29	1.706	2.29	1.88	50	*COI*
1309	73.79	1.476	3.9	2.09	50	*COI*
2987	84.56	1.691	2.31	1.8	50	*COI*
1403	94.52	1.89	2.31	1.87	50	*COI*
6450	18.22	0.364	3.57	1.99	50	*COI*
6500	33.55	0.671	2.27	1.8	50	*28S*
1309	72.79	1.456	2.55	1.88	50	*28S*
2987	197.98	3.96	2.31	1.91	50	*28S*
1403	238.16	4.763	2.32	1.9	50	*28S*
1306	122.94	2.459	2.41	1.87	50	*28S*
6450	79.71	1.594	2.3	1.85	50	*28S*
1361	69.09	1.382	2.24	1.82	50	*MtMutS*
1343	76.33	1.527	2.25	1.83	50	*MtMutS*
1660	56.94	1.139	2.3	1.84	50	*MtMutS*
332	33.98	0.68	2.21	1.71	50	*MtMutS*
1415	66.08	1.322	2.93	1.85	50	*MtMutS*
6501	70.87	1.417	2.48	1.86	50	*MtMutS*
6502	9.05	0.181	26.1	1.88	50	*MtMutS*
1306	82.33	1.647	2.23	1.82	50	*MtMutS*
6252	19.64	0.393	1.57	1.63	50	*MtMutS*
1361	9.15	0.183	1.47	1.83	50	*COI*
1343	60.82	1.216	2.35	1.85	50	*COI*
1660	52.74	1.055	2.35	1.83	50	*COI*
332	22.63	0.453	1.93	1.95	50	*COI*
1415	88.75	1.775	1.63	1.79	50	*COI*
6501	32.65	0.653	2.29	1.88	50	*COI*
6502	102.56	2.051	2.25	1.87	50	*COI*
1306	30.88	0.618	2.53	1.85	50	*COI*
6252	23.13	0.463	2.09	1.73	50	*COI*
6252	76.09	1.522	2	1.81	50	*MtMutS*

Sample ID	ng/ µL	A260	260/230	260/280	Const.	RA
1361	141.31	2.826	1.95	1.86	50	*28S*
1343	191.31	3.826	2.14	1.9	50	*28S*
1660	184.41	3.688	2.09	1.86	50	*28S*
332	22.63	0.453	1.93	1.95	50	*COI*
1415	88.75	1.775	1.63	1.79	50	*COI*
6501	32.65	0.653	2.29	1.88	50	*COI*
6502	102.56	2.051	2.25	1.87	50	*COI*
1306	30.88	0.618	2.53	1.85	50	*COI*
6252	23.13	0.463	2.09	1.73	50	*COI*
6252	76.09	1.522	2	1.81	50	*MtMutS*
1361	141.31	2.826	1.95	1.86	50	*28S*
1343	191.31	3.826	2.14	1.9	50	*28S*
1660	184.41	3.688	2.09	1.86	50	*28S*
332	124.96	2.499	2.11	1.91	50	*28S*
1415	185.34	3.707	2.15	1.88	50	*28S*
6501	34.75	0.695	2.07	1.89	50	*28S*
6502	178	3.56	2.14	1.89	50	*28S*
6192	75.03	1.501	1.32	1.76	50	*MtMutS*
6192	22.35	0.447	1.65	1.74	50	*MtMutS*
6303	17.97	0.359	1.74	1.67	50	*MtMutS*
6413	4.01	0.08	1.81	1.64	50	*MtMutS*
1309	7.1	0.142	0.64	1.85	50	*ITS 2*
6451	5.55	0.111	0.67	2.28	50	*ITS 1*
6451	10.87	0.217	0.96	1.83	50	*ITS 1*
6274	13.57	0.271	1.15	1.51	50	*ITS1-2*
1343	62.22	1.244	1.98	1.84	50	*ITS1-2*
1660	50.98	1.02	1.69	1.78	50	*ITS1-2*
6502	16.68	0.334	1.95	1.84	50	*ITS1-2*
1415	62.4	1.248	2.04	1.82	50	*ITS1-2*
2987	58.66	1.173	2.05	1.84	50	*ITS1-2*
3265	46.8	0.936	2.09	1.77	50	*ITS1-2*
ARG005	86.02	1.72	2.08	1.86	50	*ITS1-2*
6252	22.41	0.448	2.16	1.78	50	*ITS1-2*
6329	51.7	1.034	2.03	1.86	50	*ITS1-2*
3256	53.23	1.065	2.13	1.8	50	*ITS1-2*
3278	26.99	0.54	1.81	1.77	50	*ITS1-2*
6500	130.67	2.613	1.59	1.83	50	*ITS1-2*
1403	91.93	1.839	2.11	1.84	50	*ITS1-2*
1306	73.77	1.475	2.15	1.83	50	*ITS1-2*
6450	13.33	0.267	1.62	1.64	50	*ITS1-2*
1361	44.11	0.882	2.15	1.74	50	*ITS1-2*
332	11	0.22	1.93	1.74	50	*ITS1-2*
6501	6.78	0.136	2.38	1.75	50	*ITS1-2*
6261	60.25	1.205	2.22	1.86	50	*ITS 2*
6451	22.77	0.455	2.76	1.82	50	*ITS 2*
1309	41.64	0.833	2.47	1.79	50	*ITS 1*
6261	15.52	0.31	2.02	1.65	50	*ITS 1*

Table 4.10- Data from the UV/Vis spectrophotometer (NanoDrop ND-100) used to measure the DNA concentration (ng/µl) of the purified PCR products. ng/µl, sample concentration based on absorbance at 260 nm; A260, absorbance of the sample at 260 nm; 260/230, ratio of sample absorbance at 260 and 230 nm; 260/280, ratio of sample absorbance at 260 and 280 nm; Const., selected analysis constant; RA, region of amplified DNA.

4.4.2.4 Data analysis

A maximum likelihood (ML), maximum parsimony (MP) and Bayesian analysis (B) were carried out for each gene or cluster of genes in order to compare the phylogenetic trees obtained by each method.

MtMutS

Nucleotide sequences of 700-720 bp in length were obtained from the *mtMutS* gene of the 22 Primnoid specimens. To confirm the metazoan nature of our sequences we performed a BLAST search in GenBank. The best matches we obtained were with *Thouarella grasshoffi* and other primnoid genera like *Plumarella* or *Parastenella*. The 22 sequences were aligned to produce a matrix of 732 characters, which were treated as unordered and equal weighted; 529 characters were constant, 124 variable and parsimony uninformative and 79 were parsimony informative characters.

The three phylogenetic analyses (ML, MP, B) show the same phylogenetic relationships for *Thouarella* and *Fannyella* species (Fig. 4.17). They group *Thouarella* species (pink) in a monophyletic clade supported through high values of bootstrap and clade credibility, 86% (ML), 97% (MP) and 1 (B). A second clade includes three of the four *Fannyella* species studied (blue) supported by values of 79% (ML), 100% (MP) and 1 (B), but the fourth species is cladded with genus *Metafannyella* (green), meaning a polyphyletic origin of genus *Fannyella*, or if a wrong classification of this species (as both genera are very close related, and originally *Metafannyella* species was considered as a *Fannyella* species) occurred a monophyletic origin could be hypothesised. The highest supportive value comes from the Bayesian analysis.

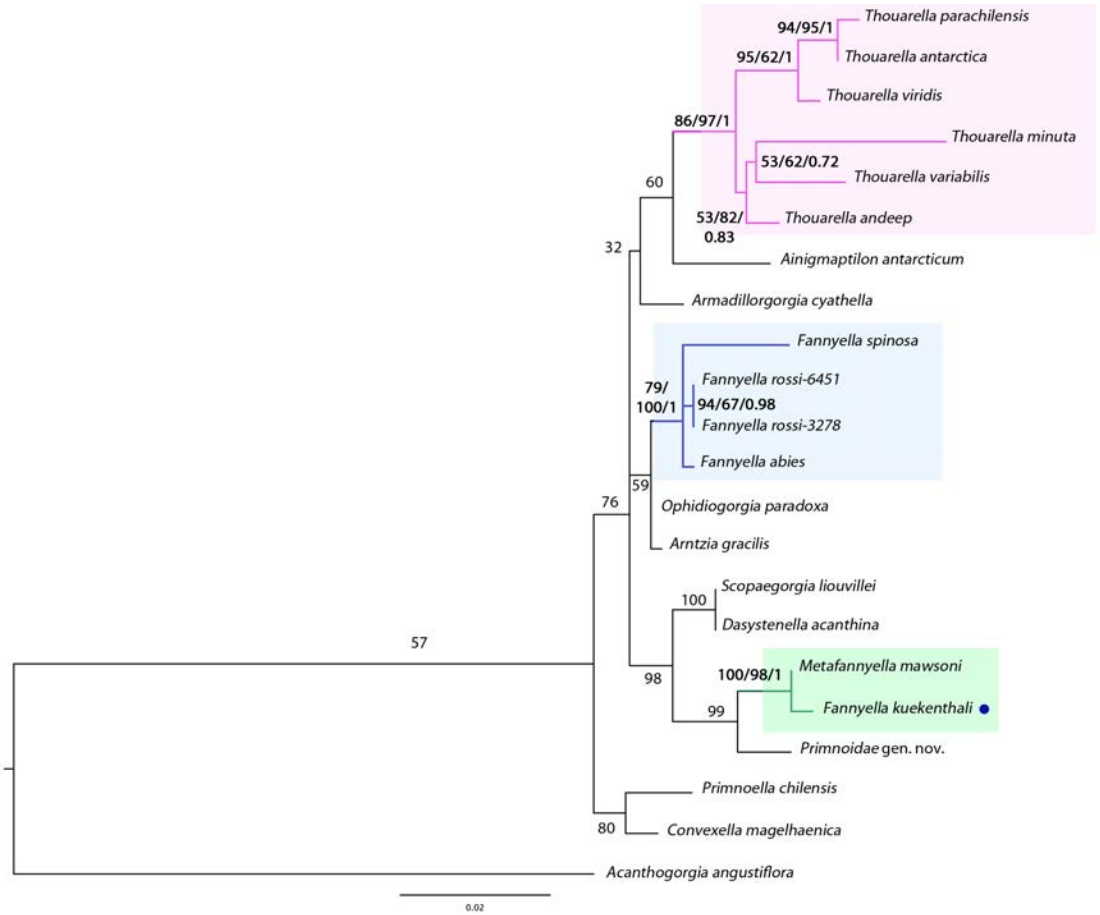


Figure 4.17.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids form *mtMutS* region. The bootstrap support values (BS) equal or above 50% are indicated at each node, in bolt BS values of maximum likelihood / maximum parsimony / Bayesian analysis.

COI

Nucleotide sequences of 880-940 bp in length were obtained from the *COI* gene (including the adjacent intergenic region IGR1) of the 22 Primnoid specimens. To confirm the metazoan nature of our sequences we performed a BLAST search in GenBank. The best matches we obtained were with *Thouarella grasshoffi* and other primnoid genera like *Primnoa* or *Narella*. The 22 sequences were aligned to produce a matrix of 964 characters, which were treated as unordered and equal weighted; 780 characters were constant, 92 variable and parsimony uninformative and 92 were parsimony informative characters.

The three different phylogenetic analyses show, like in *MtMutS*, the same phylogenetic relationships for *Thouarella* species (Figs. 4.18-4.20). *Thouarella* species (pink) are present in different evolutionary moments through the tree supporting a polyphyletic origin of the genus, however in the three analyses they appear in different moments and clustered with different species. All the analysis (ML, MP, B) suggest the polyphyletic origin of genus *Fannyella* (blue dots), however if a wrong classification of this species occurred (as both genera are very close related, and originally *Metafannyella* species where considered as *Fannyella* species) ML and MP would suggest a monophyletic origin while the Bayesian analysis would hypothesise on a paraphyletic origin with a clade credibility of 0.62.

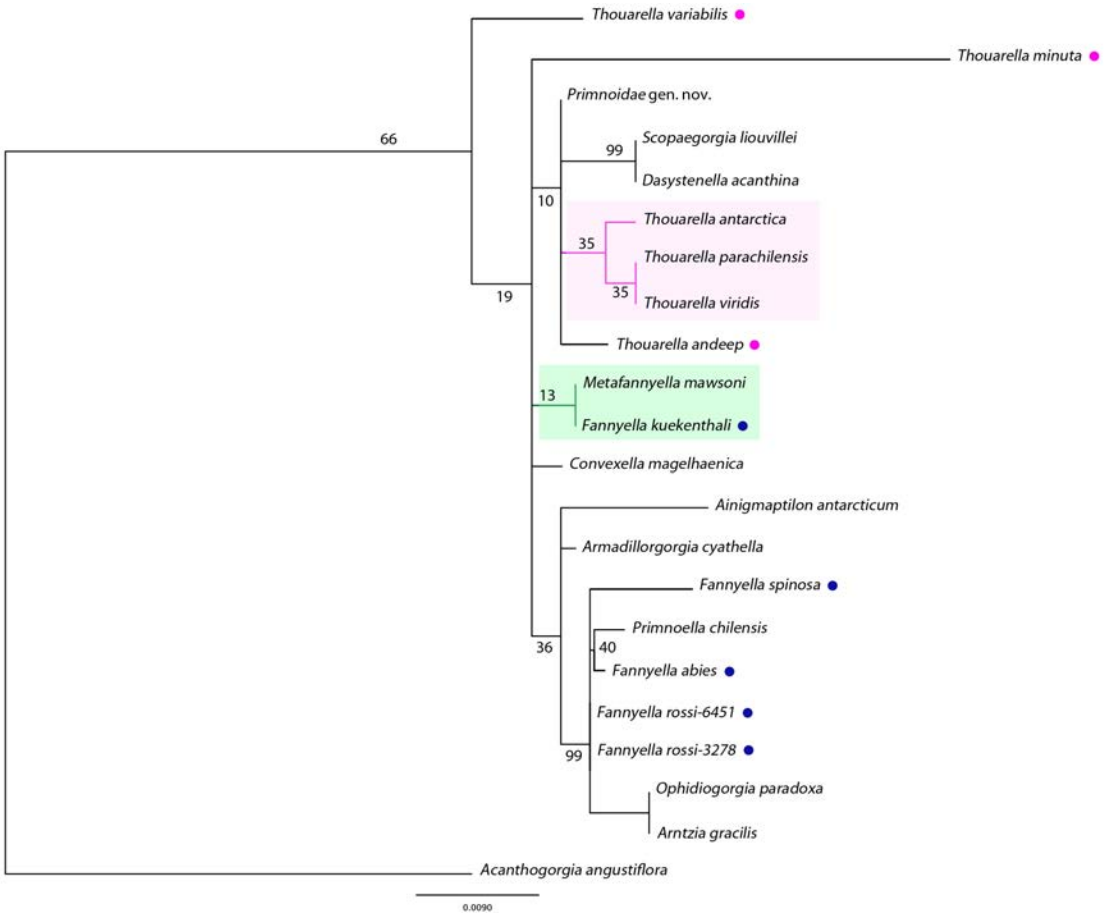


Figure 4.18.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids for *COI* region. The bootstrap support values (BS) equal or above 50% are indicated at each node.

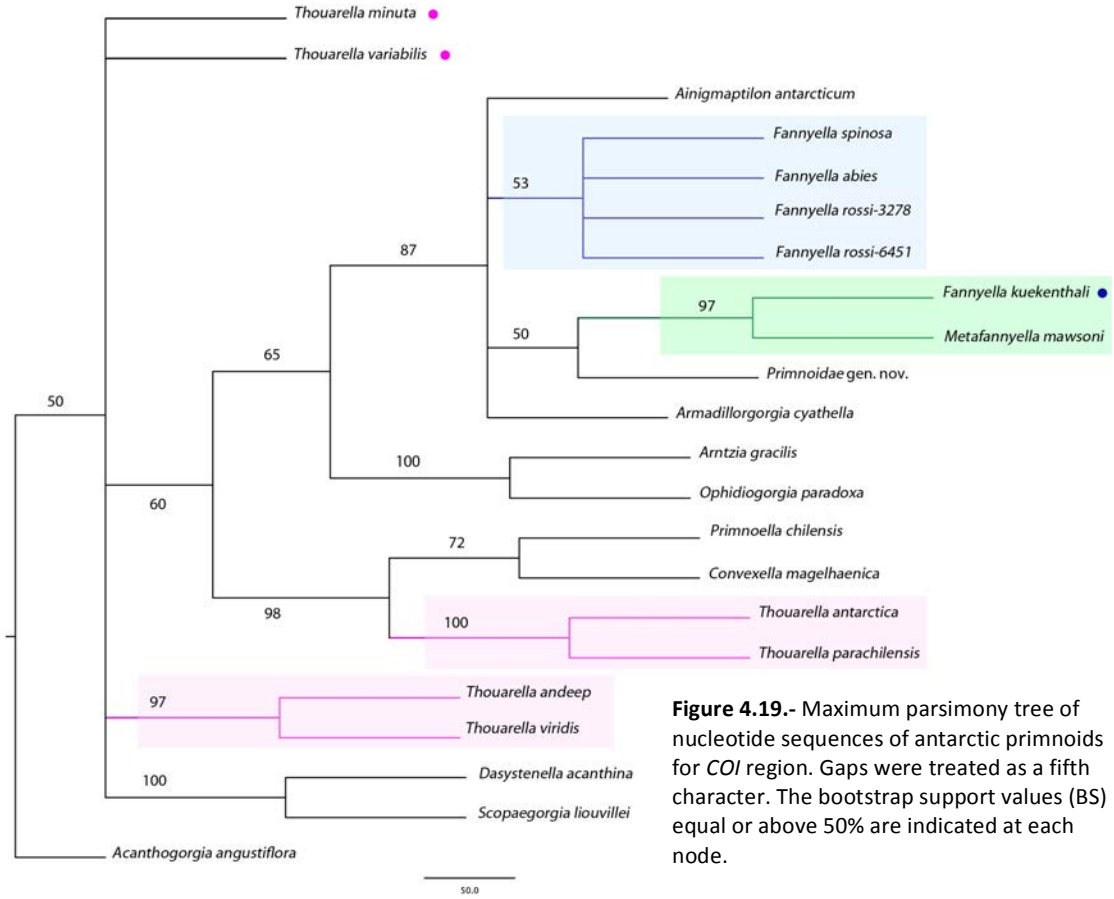


Figure 4.19.- Maximum parsimony tree of nucleotide sequences of antarctic primnoids for *COI* region. Gaps were treated as a fifth character. The bootstrap support values (BS) equal or above 50% are indicated at each node.

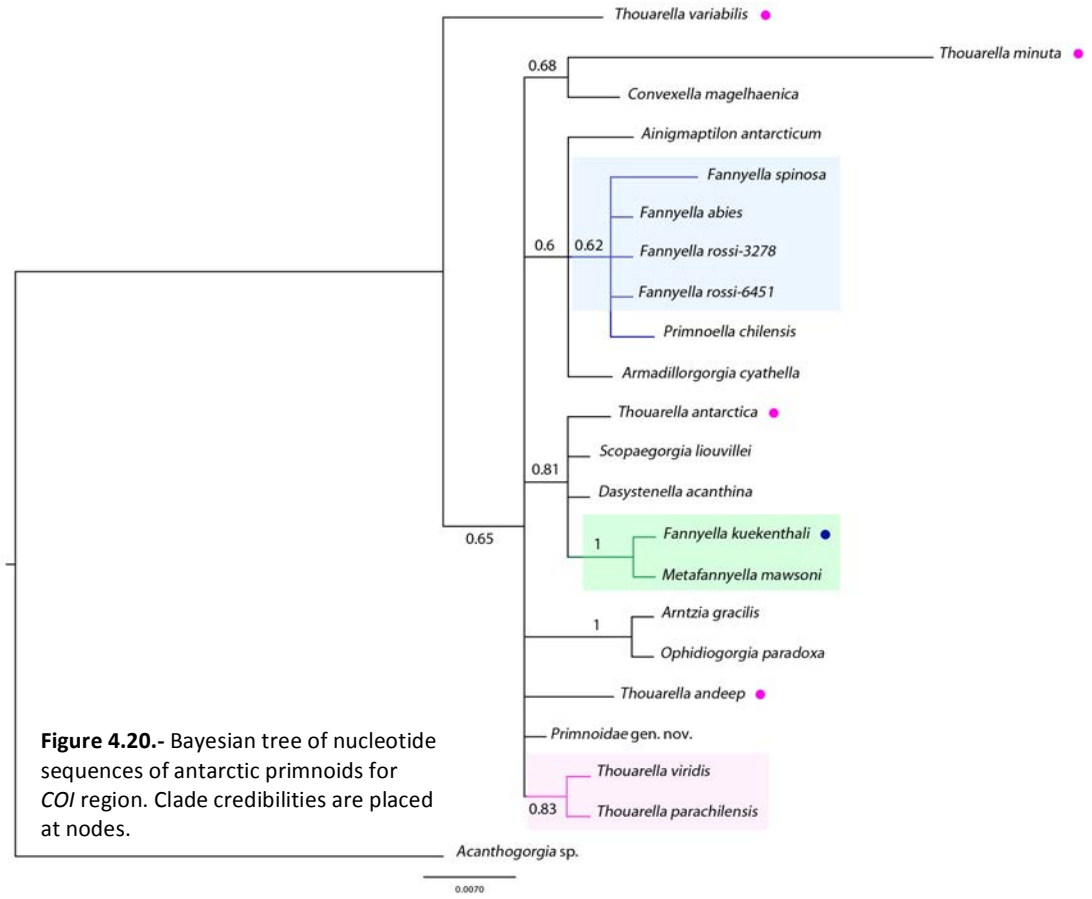


Figure 4.20.- Bayesian tree of nucleotide sequences of antarctic primnoids for *COI* region. Clade credibilities are placed at nodes.

MtMutS and COI

Nucleotide sequences of 1490-1650 bp in length were obtained from the combined nucleotide dataset of *mtMutS* and *COI* (including IGR1). The 22 sequences were aligned to produce a matrix of 1679 characters, which were treated as unordered and equal weighted; 1509 characters were constant, 93 variable and parsimony uninformative and 77 were parsimony informative characters.

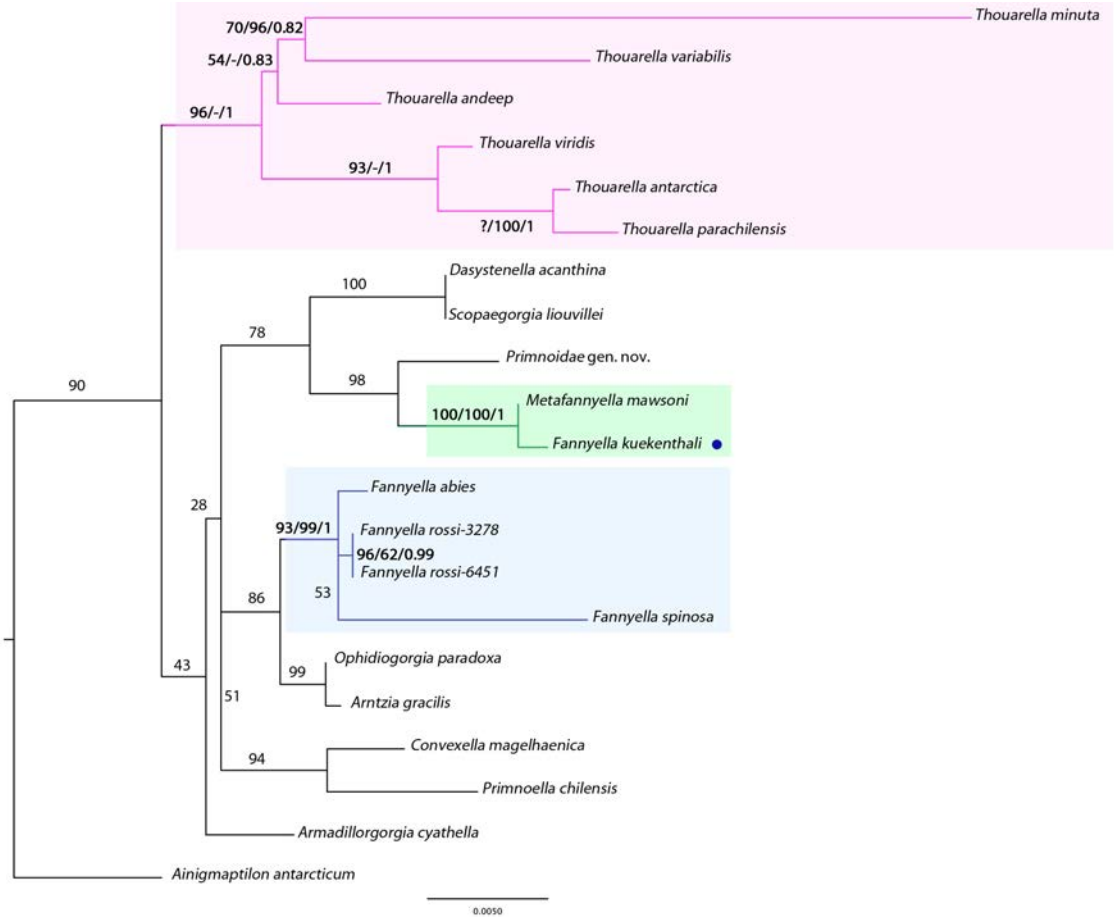


Figure 4.21.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids for the *mtMutS* and *COI* regions. The bootstrap support values (BS) equal or above 50% are indicated at each node; in bolt BS values of maximum likelihood / maximum parsimony / Bayesian analysis; **?**, BS value unknown; **-**, BS value does not apply.

The estimated maximum likelihood tree (Fig. 4.21), the best tree found by maximum parsimony (Fig. 4.22) and the consensus tree got from the Bayesian analysis (very similar to ML tree) show the same phylogenetic relationship for *Fannyella* species. They group most examined *Fannyella* species in a monophyletic clade supported through high values of bootstrap and clade credibility, 93% (ML), 99% (MP) and 1 (B), the highest supportive value comes from the Bayesian analysis. However one of the four species is cladded with genus *Metafannyella* (green), meaning a polyphyletic origin of the current conception of the genus *Fannyella*, or if a wrong classification of this species (as both genera are very close related, and originally *Metafannyella* species where considered as *Fannyella* species, or if *F. kuekenthali* should belong to *Metafannyella* instead of *Fannyella* genus) occurred a monophyletic origin could be hypothesised. In two of the analysis (ML and B) the genus *Thouarella* is also grouped

in a monophyletic clade supported by values of 96% (ML) and 1 (B), while the maximum parsimony shows a para- or polyphyletic origin (Fig. 4.22).

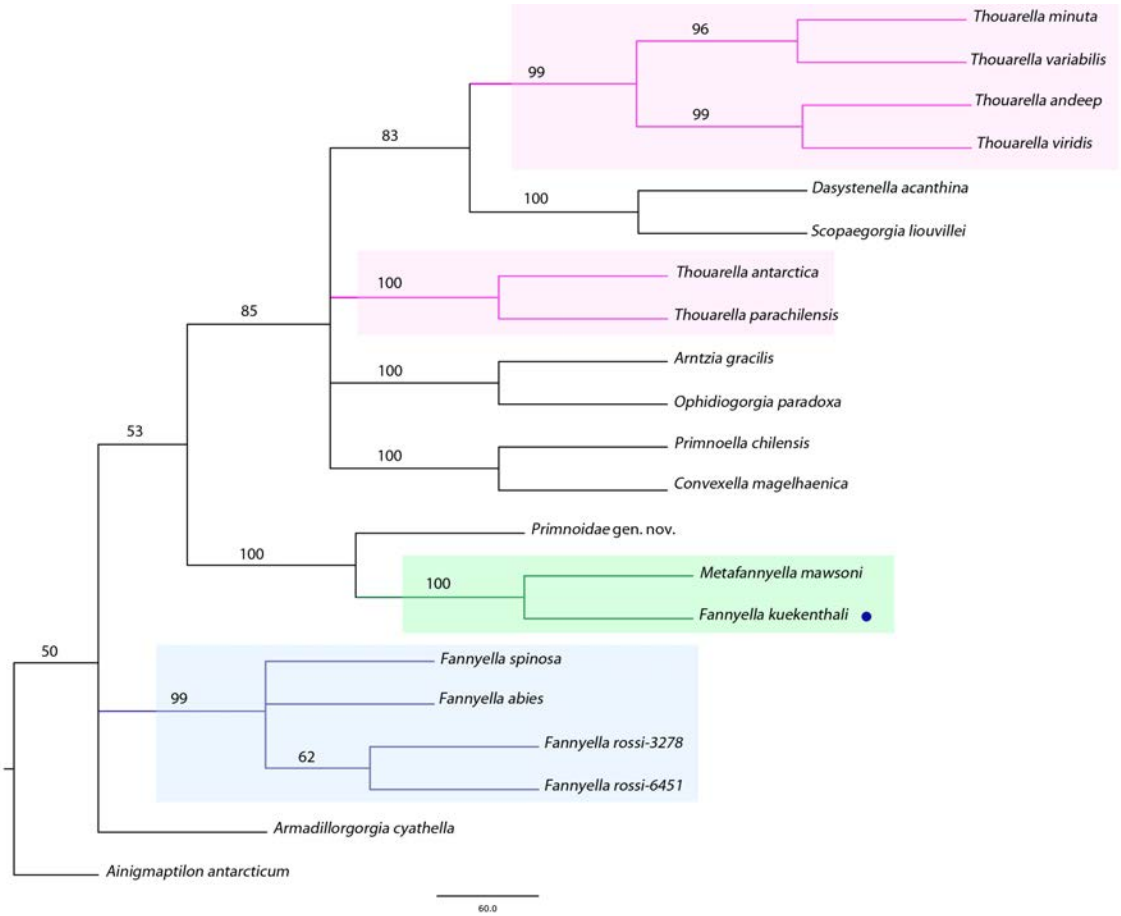


Figure 4.22.- Maximum parsimony tree of nucleotide sequences of antarctic primnoids for *mtMutS* and *COI* regions. Gaps were treated as a fifth character. The bootstrap support values (BS) equal or above 50% are indicated at each node.

28S

Nucleotide sequences of 800-830 bp in length were obtained from the *28S* gene of the 23 Primnoid specimens. To confirm the metazoan nature of our sequences we performed a BLAST search in GenBank. The best matches we obtained were with some genera from families Gorgoniidae and Acanthogorgiidae. The 22 sequences were aligned to produce a matrix of 896 characters, which were treated as unordered and equal weighted; 379 characters were constant, 210 variable and parsimony uninformative and 307 were parsimony informative characters.

The three phylogenetic analyses (ML, MP, B) show the same phylogenetic relationships for *Thouarella* and *Fannyella* species (Fig. 4.23). They group *Thouarella* species in a monophyletic clade supported through high values of bootstrap and clade credibility, 100% (ML), 100% (MP) and 1 (B). The only difference is found in the species *T. andeep*, where ML and MP analysis cluster it with *T. pendulina*, *T. minuta* and *T. variabilis* (BS values of 47 and 51) while the Bayesian analysis clusters *T. andeep* with *T. antarctica*, *T. parachilensis* and *T. viridis* with a

0.76 of credibility. Three of the four *Fannyella* species studied (blue) are also grouped in a monophyletic clade supported by values of 100% (ML) and 83% (MP), the fifth species is cladded with genus *Metafannyella* (green), meaning a polyphyletic origin of genus *Fannyella*, or if a wrong classification of this species (as both genera are very close related, and originally *Metafannyella* species where considered as *Fannyella* species) occurred a monophyletic origin could be hypothesised.

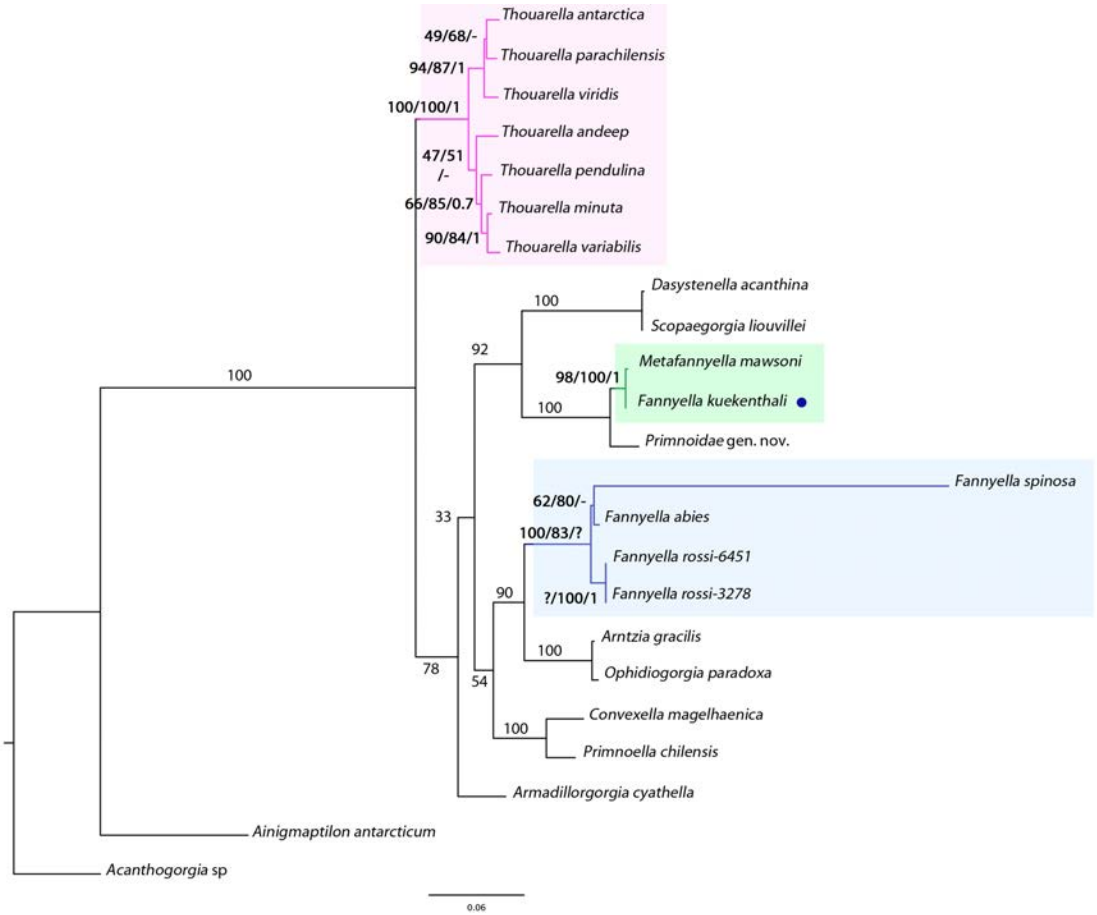


Figure 4.23.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids for *28S* region. The bootstrap support values (BS) equal or above 50% are indicated at each node. In bolt BS values of maximum likelihood / maximum parsimony / Bayesian analysis; ?, BS value unknown; -, BS value does not apply.

ITS

Nucleotide sequences of 600-730 bp in length were obtained from the *ITS* region (including gene *5.8S*) of the 22 Primnoid specimens. To confirm the metazoan nature of our sequences we performed a BLAST search in GenBank. The best matches we obtained were with octocorals *Heliopora* and *Alcyonium*. The 22 sequences were aligned to produce a matrix of 1035 characters, which were treated as unordered and equal weighted; 223 characters were constant, 206 variable and parsimony uninformative and 606 were parsimony informative characters.

The phylogenetic analyses carried out (ML, MP, B) show the same phylogenetic relationships for *Thouarella* species. They group *Thouarella* species in a monophyletic clade (Fig. 4.24) supported through high values of bootstrap and clade credibility, 77% (ML), 90% (MP) and 1

(B), however the species are related from each other differently in each analyses. In two of the analysis (ML and B) four of the five *Fannyella* sequences (three species) studied (blue) are cladded together, supported by values of 100% (ML) and 1 (B), while the fifth sequence is cladded with genus *Metafannyella* (green), meaning a polyphyletic origin of genus *Fannyella*, or if a wrong classification of this species (as both genera are very close related, and originally *Metafannyella* species where considered as *Fannyella* species) occurred a monophyletic origin could be hypothesised. The maximum parsimony analysis group together the group of four *Fannyella* sequences with *Armadillogorgia cyathella*, in that case if *F. kuekenthali* should be included in *Metafannyella*, *Fannyella* genus will be paraphyletic supported by 90%.

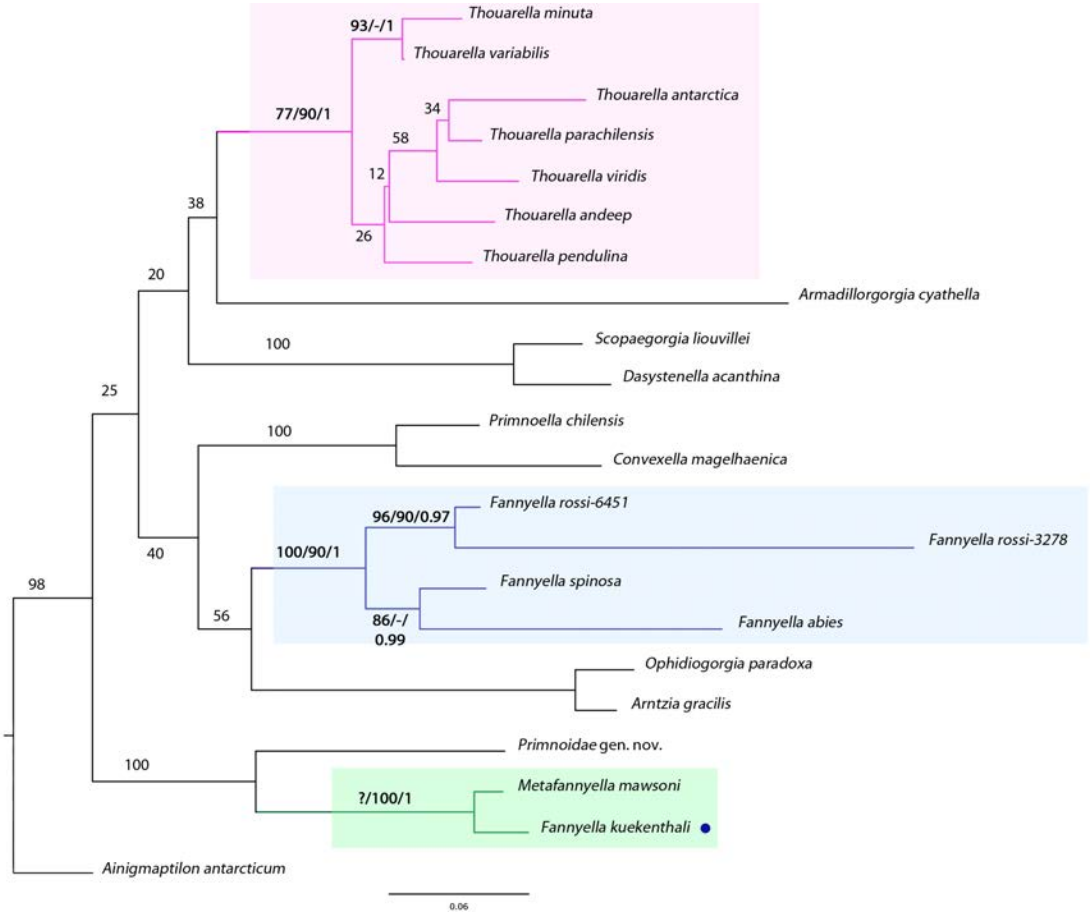


Figure 4.24.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids for *ITS* region. The bootstrap support values (BS) equal or above 50% are indicated at each node; in bolt BS values of maximum likelihood / maximum parsimony / Bayesian analysis; **?**, BS value unknown; **-**, BS value does not apply.

28S and ITS

Nucleotide sequences of 1380-1560 bp in length were obtained from the combined nucleotide dataset of *28S* and *ITS*. The 22 sequences were aligned to produce a matrix of 1940 characters, which were treated as unordered and equal weighted; 729 characters were constant, 455 variable and parsimony uninformative and 756 were parsimony informative characters.

The estimated maximum likelihood tree (Fig. 4.25), the best tree found by maximum parsimony and the consensus tree got from the Bayesian analysis show the same phylogenetic relationships for *Thouarella* and *Fannyella* species. They group *Thouarella* species in a

monophyletic clade supported through the highest values of bootstrap and clade credibility, 100% (ML), 100% (MP) and 1 (B), while ML and B cluster all species in the exact same clades, MP cluster in an unresolved polychotomy *T. viridis*, *T andeep* and *T. antarctica* with the clade formed by *T. minuta*, *T. variabilis* and *T. pendulina; T. parachilensis* is found at the base of the *Thouarella* monophyletic clade. In the case of *Fannyella* species, three of the four species studied are cladded together and the fourth species is cladded with genus *Metafannyella,* meaning a polyphyletic origin of genus *Fannyella*, or if a wrong classification of this species (as both genera are very close related, and originally *Metafannyella* species where considered as *Fannyella* species) occurred a monophyletic origin could be and supported by values of 100% (ML), 100% (MP) and 1 (B).

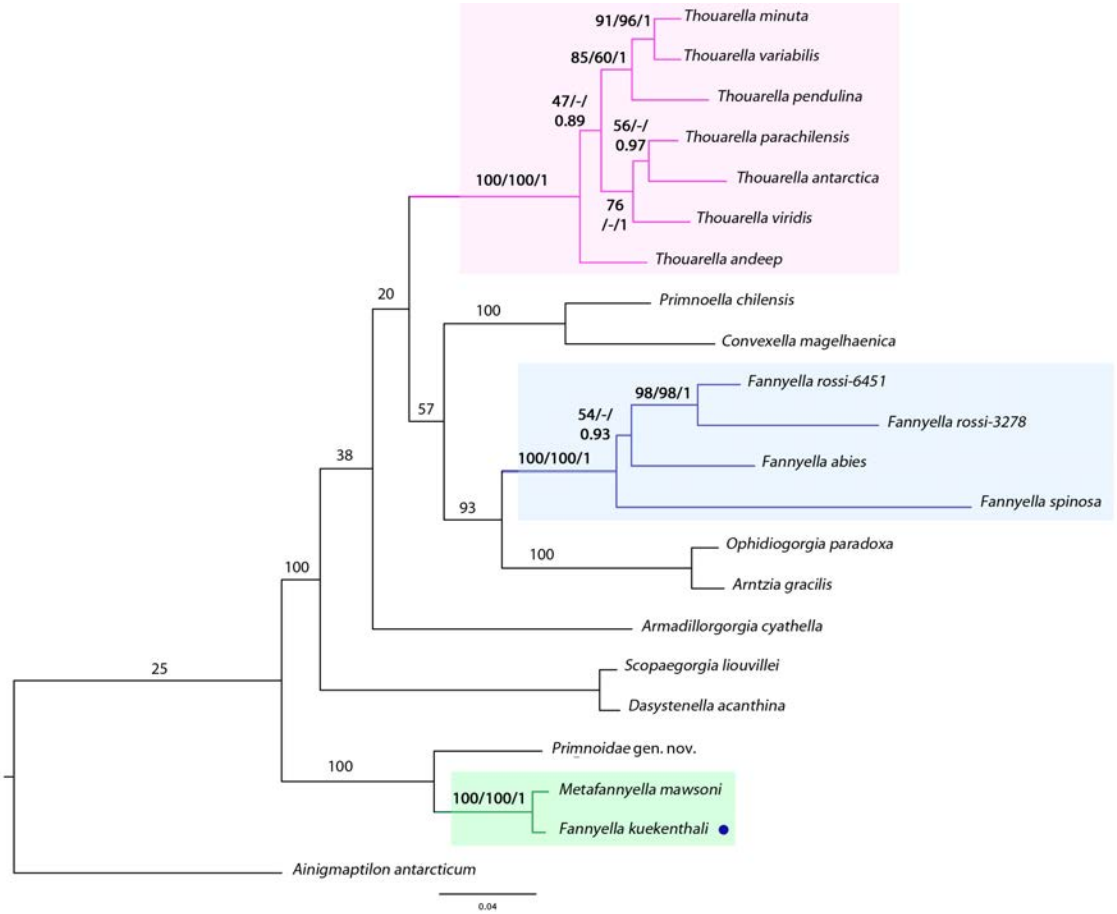


Figure 4.25.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids for the *28S* and ITS regions. The bootstrap support values (BS) equal or above 50% are indicated at each node; in bolt BS values of maximum likelihood / maximum parsimony / Bayesian analysis; **-**, BS value does not apply.

MtMutS, COI, 28S and ITS

Nucleotide sequences of 2950-3180 bp in length were obtained from the combined nucleotide dataset of *mtMutS*, *COI*, *28S* and ITS. The 22 sequences were aligned to produce a matrix of 3622 characters, which were treated as unordered and equal weighted; 1920 characters were constant, 719 variable and parsimony uninformative and 983 were parsimony informative characters.

When all the studied genes are analysed together using the different phylogenetic analyses (Fig. 4.26), all of them show the same phylogenetic relationships for *Thouarella* species. They group *Thouarella* species in a monophyletic clade supported through the values of bootstrap and clade credibility, 57% (ML), 100% (MP) and 1 (B). However for *Fannyella* species is showed a polyphyletic origin of genus and as occurred when analysing ITS, the maximum parsimony analysis group together the group of four *Fannyella* sequences with *Armadillogorgia cyathella*, in that case if *F. kuekenthali* is included in *Metafannyella*, *Fannyella* genus will be paraphyletic supported by 94%. For the maximum parsimony and Bayesian analysis *Fannyella* species could be hypothesised to be monophyletic, supported by values of 100% (ML) and 1 (B).

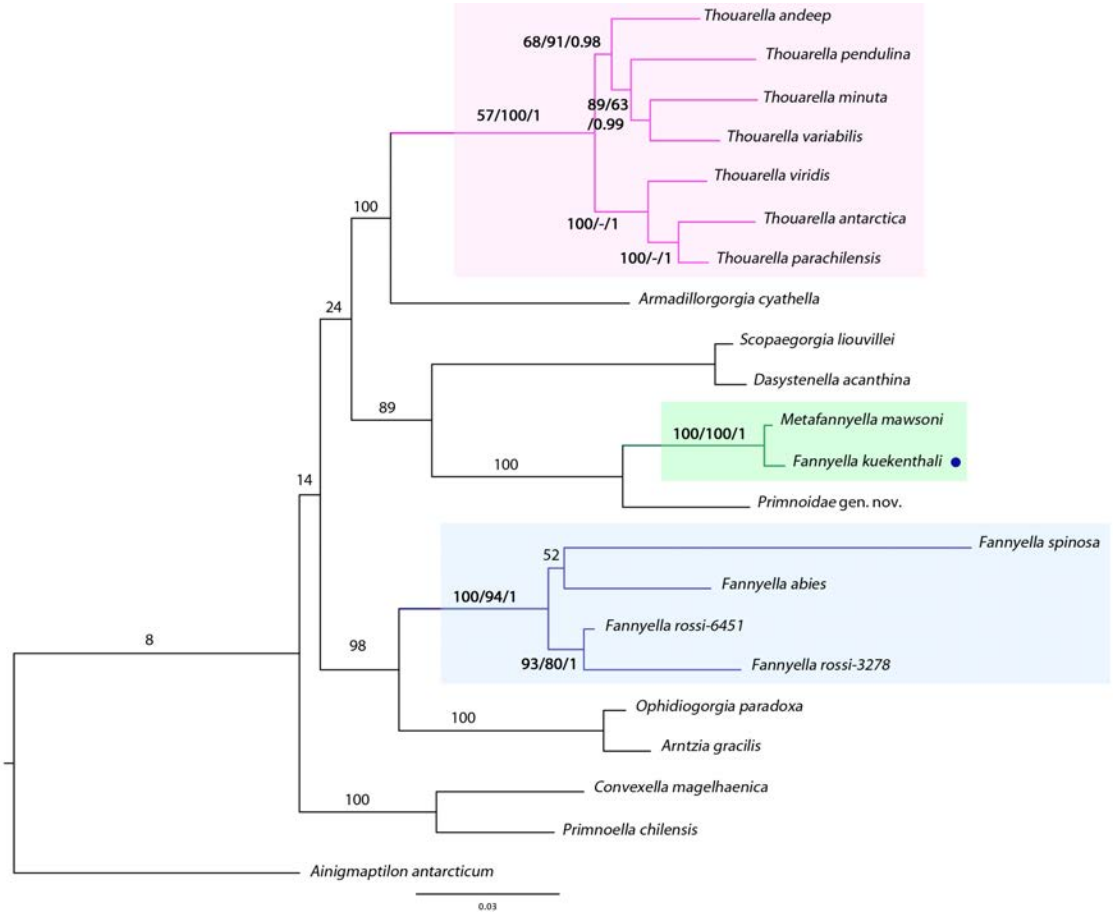


Figure 4.26.- Maximum likelihood tree of nucleotide sequences of antarctic primnoids for the *mtMutS*, *COI*, *28S* and ITS regions. The bootstrap support values (BS) equal or above 50% are indicated at each node; in bolt BS values of maximum likelihood / maximum parsimony / Bayesian analysis; **-**, BS value does not apply.

4.5 Discussion

4.5.1 Morphological phylogeny

Family Primnoidae

The most primitive genus has been attributed to *Primnoeides* (Wright & Studer 1889; Versluys 1906; Kükenthal 1919) and Cairns and Bayer (2009) also found this genus to be at their basal tree together with *Armadillogorgia*, *Aglaoprimnoa* and *Ophidiogorgia*. The consensus tree obtained in this study shows a basal polycothomy formed by seven genera (*Primnoeides*,

Aglaoprimnoa, *Armadillogorgia*, *Primnoella*, *Convexella* and *Heptaprimnoa*), three of them coinciding with the results of Cairns and Bayer (2009), however our results also show *Primnoella* as one of the primitive genera which agrees with Versluys (1906). *Convexella* which is closely related to *Primnoella* (Bayer 1996a), and the new described genus *Heptaprimnoa* (Cairns 2012) have also been found to be as one of the primitive genera.

The clade 1 (Fig. 4.10) includes an unresolved clade with all *Fannyella* subgenera and the genus *Onogorgia*, which type species was previously included in *Fannyella* (Bayer 1998). *Fannyella* subgenera are differentiated mainly by their colony shape (Cairns & Bayer 2009): *Scyphogorgia* (bottlebrush), *Cyathogorgia* (dichotomous almost pinnate) and *Fannyella* (lyriform). *Onogorgia* was described noting the close resemblance with *Fannyella* species (Cairns & Bayer 2009), and also their differences such as the colony shape (flagelliform), eight longitudinal rows of body scales, and a completely covered polyp. However subgenus *Cyathogorgia* also has eight longitudinal rows of scales which completely cover the polyps, and as noted before colony shape was a character used to differentiate *Fannyella* subgenera, which may suggest, together with the results of our consensus tree, that *Onogorgia* should be included in *Fannyella* as a subgenus, and thus support a monophyletic origin of the genus. The *Fannyella* clade is grouped with *Metafannyella*, which has also resemblances with *Fannyella* such as the polyp distribution (in whorls) and proximity (appressed) to branches and differs in having a primitive ascus-scale (Cairns & Bayer 2009). *Pyrogorgia*, which is at the base of clade 1, does not show the ascus-scale shape but a completely ridged outer surface (Cairns & Bayer 2009), and its body scales which cover completely the polyps are irregularly arranged at the lower half of the polyp (primitive character shown in *Primnoeides* and *Aglaoprimnoa*), suggesting *Pyrogorgia* as an intermediate genus towards the appearance of the derivate state character of ascus-scale shape.

Clade 3 includes all *Thouarella* subgenera and the genus *Scopaegorgia,* which is at the base in an unresolved clade with subgenus *Diplocalyptra*. The main difference between *Scopaegorgia* and all *Thouarella* subgenera is the number of marginals (Zapata-Guardiola & López-González 2010e), which is reduced in the former. Before the description of the genus *Scopaegorgia* a phylogenetic analyses proposed the origin of *Thouarella* as monophyletic (Cairns & Bayer 2009). However after our morphological analyses much information about the characters interpretation would be needed to hypothesize a monophyletic origin for genus *Thouarella*.

Genus *Plumarella* has been recently divided in subgenera based on the arrangement of polyps on branchlets (Cairns 2011; Zapata-Guardiola *et al.* 2012). Three of its four subgenera are placed at the base of clade 3' and *Plumarella s.s.* which has polyps occurring on alternating sides of polyps (biserial) is cladded in a more derived position in the tree. Based on our results *Plumarella* cannot be considered monophyletic but polyphyletic, however the placement of subgenus *Plumarella s.s* with species characterised by having polyps arranged in whorls and having a naked or with some vestigial sclerites on the adaxial body side suggests that the interpretation of characters should be revised.

Clade 6 group together genera where the planar dichotomous colony shape is the predominant and whose polyps are placed perpendicular to the branches (Bayer 1996a;

Zapata-Guardiola *et al.* 2013). At the base of the clade are placed the genera from the polar latitudes while the more recent are from tropical areas suggesting a polar origin of the family (Cairns & Bayer 2009).

In clade 6' the divergence starts on tropical latitudes with genera with a planar colony shape and finishes on polar latitudes with *Dasystenella* and *Tauroprimnoa,* both with a bottlebrush colony shape (Cairns 2006; Zapata-Guardiola & López-González 2010b). This clade tends to group together the genera with less than 8 rows of body scales, which leads to have less than 8 marginal scales and thus to not have a correspondence between opercular and marginal scales. Cairns and Bayer (2009) found a similar clade characterised by the non-correspondence of those scales, however the composition of the genera are slightly different in both clades. *Microprimnoa* and *Candidella* included by Cairns and Bayer (2009) are cladded here with the group of having polyps arranged perpendicular to the stem.

Clade 5' includes genera from all latitudes, even a couple of them are cosmopolitan (*Narella* and *Primnoa*). In this clade seven genera correspond to the clade D (Cairns & Bayer 2009) characterised by having only 2 rows of body scales and polyps facing downward. Cairns and Bayer (2009) cladded this group of genera with *Callogorgia* and *Fanellia*, however in our analysis we relate also *Fanellia* with *Ophidiogorgia* and *Arntzia* becoming a sister group of the clade D. In Cairns and Bayer (2009) these last two genera are not close related, not even in sister groups, they are in different clades.

All assumed primitive genera are present in the Southern Ocean, four of them are restricted to that ocean, while one of them is also found in temperate waters (*Convexella*) and another one has a worldwide distribution (*Primnoella*). Moreover all genera from clade 2 are restricted to the Southern Ocean. In our consensus tree we can also observe a slight tendency where temperate and tropical genera are located in more recent clades. This tendency might suggest, as previously noted by Cairns and Bayer (2009), that the origin of the family Primnoidae have been from the Southern Ocean.

Genus *Thouarella*

One of the major problems to deduce octocoral phylogenies is the existence of several continuous character used in its taxonomy. The presence of a high rate of homoplasy could be due that the available characters are not conservative or that *Thouarella* species are quite convergent, developing the same characters states many times and even reversing them in their evolutionary history.

According to the current morphologic analysis, and taking into account the amount of homoplasic expressions of characters, we could tentatively discuss that the distribution pattern in the cladogram seems to point out an origin outside the Southern Ocean which at the same time act as a biodiversity hotspot for this gorgonian genus.

Most of the basal positions of the cladogram (Fig. 4.11) are occupied by species from tropical and temperate waters (sister groups 1, 2, 3, 4, 5 and 6). Although in sister group 1 appears *Thouarella minuta* a recent described Antarctic species from shallow waters and in sister group

4 are included two species from the Aleutian Islands (Cairns 2011) most of the basal speciation processes occurred from temperate and tropical waters in different events (and widely separate scenario after this morphological approach) the colonization of polar and deep-sea waters, and vice versa, as the sister group 5', includes a couple of species in the proximities of the S.O. (South Africa) and a species from the Caribbean (*T. bipinnata*).

It's also remarkable the distribution of the last clade from sister group 5 (*T. crenelata* and *T. parachilenesis*) both from Antarctic waters, while the species which diverged earlier are also present in the Subantarctic. The same occurs with more diverged species in sister group 5', where four of the last five diverged species are present in the Antarctica (and three are restricted to that area), while the species diverged earlier at least appear in the Subantarctic region.

The results of this analysis seems to point out a primitive origin of the genus in tropical and temperate waters which supports the hypothesis of colonization of the Southern Ocean through the deep-sea waters.

Due to the homoplasic nature in most of the morphological characters used in this approach, we highly recommend a combined study with molecular data in order to get stronger phylogenies.

4.5.2 Molecular phylogeny

In general phylogenetic analyses of sequences from the *mtMutS* (mitochondrial), *28S* (nuclear) and *ITS* (nuclear) produced well-supported phylogenetic relationships for Antarctic primnoid gorgonians. The analyses from *COI* (mitochondrial) sequences are the ones with a lower supported phylogenetic relationships (Figs. 4.18-20). The three analyses (maximum-parsimony, maximum-likelihood and Bayesian) produced very similar results, with high supporting values for most nodes. The analyses mainly supported the monophyletic origin of genus *Thouarella* (Figs. 4.17, 21, 23-26) and for some DNA sequences analysed *Thouarella* genus is one of the two main groups supported (Figs. 4.21, 23). In other cases, genus *Thouarella* still performs a monophyletic group which is in one evolutionary side of the tree (Figs. 4.17, 24-26).

Species included in *Thouarella* clade have in common the arrangement of polyps on their branches, single placed in spirals or without any order, while the remaining primnoid species have their polyps in whorls from 3 to more than 20 polyps each. In this last group its possible to observe four clades, two of them have unbranched colony shaped (*Primnoella*, *Convexella*, *Arntzia*, and *Ophidiogorgia*) while the other two clades have species from uniplanar dichotomous to bottlebrush shape, which includes the clade of genus *Fannyella*, characterised by the presence of ascus body scales. However the phylogenetic relationships among this clades is not the same through all analyses, the tendency is to closely relate genus *Fannyella* with *Ophidiogorgia* and *Arntzia* clade (Fig. 4.17) and both clades closely related with the other unbranched species (Figs. 4.18, 20-21, 23-26).

Four *Fannyella* species have been analysed and in all scenarios *F. kuekenthali* is cladded with the *Metafannyella* species analysed suggesting a polyphyletic origin of the genus (Fig. 4.17).

Fannyella lepidota Bayer, 1998 (Cairns & Bayer 2009) was designated as the type species for genus *Metafannyella*, revealing a close relationship between *Fannyella* and *Metafannyella* genera. In that context if *Fannyella kuekenthali* is included in the genus *Metafannyella* a monophyletic origin of genus *Fannyella* will be hypothesised.

If we look closer the *Thouarella* clade we can see that maximum likelihood and parsimony of *28S* includes *Thouarella andeep* in a clade with *T. minuta*, *T. variabilis* and *T. pendulina*, however the Bayesian analysis includes *T. andeep* in the sister group with *T. viridis*, *T. antarctica* and *T. parachilensis.* The values that support one emplacement or another of this species is higher in the Bayesian analysis with a 0.76. However the analysis of *mtMutS*, shows for the three analyses the same relation between *Thouarella* species, which includes *T. andeep* in the group with *T. minuta* and *T. variabilis*, the supporting values are higher in the three cases than values found in *28S* analyses. ITS does not show the same phylogenetic relationship among the three analysis but put together *T. parachilensis*, *T. antarctica* and *T. viridis* and also *T. minuta* with *T. variabilis*, while *T. pendulina* and *T. andeep* are in the middle of the two "groups". The combination of *28S* and ITS shows two main clades, the first with *T. minuta*, *T. variabilis* and *T. pendulina* and the second with *T. parachilensis*, *T. antarctica*, and *T. viridis*. The combination of the 4 genes also creates two main clades, one with *T. minuta*, *T. variabilis*, *T. pendulina*, *T. andeep*, and another with *T. parachilensis*, *T. antarctica* and *T. viridis*, the same as observed for *28S* and *mtMutS* independently.

According to the current molecular analysis, and taking into account the results of the different DNA regions analysed with different programs, we could tentatively discuss that the number of abaxial scales seems to be responsible in part of the phylogenetic relationships in the genus *Thouarella*, which its tendency is to group species with 6 or more abaxial scales together (*T. viridis*, *T. antarctica,* and *T. parachilensis*) and group in another clade species up to 5 abaxial scales (*T. minuta*, *T. variabilis*, *T. pendulina*, and *T. andeep*).

4.5.3 Morphological *vs.* Molecular phylogeny

The comparison of morphological and molecular phylogeny for the seven *Thouarella* species common in both phylogenies shows few similarities. In the molecular analyses we observed two groups one including *T. minuta*, *T. variabilis* and *T. pendulina* (when available) which in the condensed morphological phylogeny (Fig. 4.27) these species appears in the basal half of the tree, while from the other group including *T. antarctica*, *T. viridis* and *T. parachilensis* only the two formers appear in the upper half of the morphological tree (Fig. 4.27). In the morphological analyses *T. parachilensis* appears more closely related to the first group, while *T. andeep* which in molecular analyses does not have a consistent placement among all the genes analysed appears close related with *T. viridis* and *T. antarctica*.

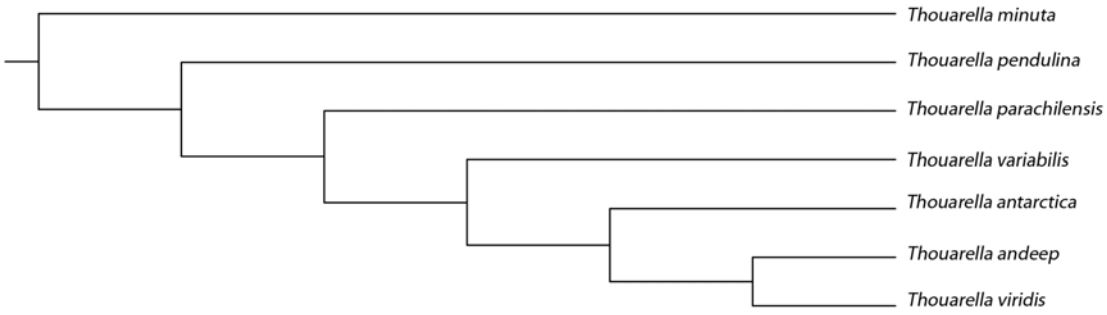


Figure 4.27.- Condensed morphological phylogeny of *Thouarella* species

These differences could be due on one hand to the homoplasic characters present in the morphological study that misrepresent patterns of relationships, as well the complexity to define states for some characters (*e.g.* sclerite shape). And on the other hand the few DNA material of *Thouarella* species accessible for molecular analyses.

When looking at the information revealed for Primnoids genera even with differences on their phylogenetic relationships a tendency to group closely some genera is present in both molecular and morphological analyses. Nuclear and mitochondrial genes and morphological characters as well place *Ophidiogorgia* and *Arntzia* as a very close related taxa; a similar situation occurs with the clade formed by *Convexella* and *Primnoella* however in the morphology approach they are also grouped with other genera in an unresolved clade. In the molecular analyses *Dasystenella*, *Scopaegorgia* and *Metafannyella* appear in the same clade except when analysing the ITS, however the morphological analyses seems to agree with the close relation found between these genera although the phylogenetic events are slightly different.

4.6 Conclusion

Three different phylogenetic analyses have been carried out to the sequences of coding (*mtMutS* and *COI*) and non-coding (*28S* and *ITS*) DNA regions. The best-supported trees are those belonging to the Bayesian analysis results, followed by the maximum parsimony and the maximum likelihood analysis. Generally the three tools gave the same results for the genera *Thouarella* and *Fannyella*, however for some DNA regions (combination of *mtMutS* and *COI*, *ITS* and, combination of the four regions) the maximum parsimony analysis includes another species in the clades, making them as a paraphyletic groups.

Although mitochondrial genes are nearly invariable in octocorals, and other anthozoans (Shearer *et al.* 2002), *mtMutS* is the most rapidly evolving protein-coding region in the octocoral mtDNA (van der Ham *et al.* 2009) but it is often no more effective than *COI* (McFadden personal comment) at distinguishing species. However among the deep-sea calcaxonian taxa, some morphospecies that shared *COI* haplotypes did differ at *mtMutS* (McFadden *et al.* 2011).

Although intragenomic variation (*i.e*. polymorphisms) occurs frequently in *ITS* sequences and has been considered troublesome for phylogenetic studies (McFadden *et al.* 2010), *ITS* sequences have been very useful to compare alcyoniid (McFadden *et al.* 2001) and gorgoniid

octocorals (Aguilar & Sánchez 2007) showing a considerable variation among genera and sometimes among species. Perhaps being *ITS* among the most variable genomic regions in these organisms where mitochondrial DNA and other sequences are much conserved (Berntson *et al.* 2001, Shearer *et al.* 2002).

In our study the best-supported Bayesian tree belongs to the combined analyses of non-coding regions, *28S* and *ITS*. However, the results given only by *28S* are quite similar, making *28S* a great systematic tool with moderate effort (fast and straightforward PCR and sequencing).

The results based on the morphological phylogeny suggest that the origin of the family Primnoidae may have been from the Southern Ocean. And while the morphologic antarctic *Thouarella* species seem evolved from a common ancestor, until to have DNA material of *Thouarella* species, from other regions outside of Antarctic waters, accessible for molecular analyses, we cannot support the results suggested by the morphological phylogeny. This phylogeny pointed out the ancestor origin in tropical and temperate waters, suggesting a subsequent colonization of the Southern Ocean. The primitive Primnoid genera would have been originated from the Antarctica and spread across other regions, then diversificate and subsequently some species/genera (*i.e. Thouarella*) would have come back to the Southern Ocean.

CHAPTER 5

Growth Rings

5.1 Introduction

One of the main disturbances in Antarctic benthic sessile communities is the effect of the scouring by icebergs and glaciers. Studies based on age and growth patterns are useful to determine rates and time of restoration of habitats forming deep-sea coral stands, being a very attractive field to be studied in polar ecosystems. Octocoral studies on growth rings have been recently increased, however the number of contributions is still scant and many of them are focused on temperate and tropical waters. Even when studies on polar waters are taking the initiative there is no available information on age and growth rings of Antarctic gorgonians.

Octocoral growth rings were first studied in the earlier 1970s. Grigg (1974) compared the number of growth rings present in the skeleton of two *Muricea* species from tropical waters with estimates of their age based on observed growth rates, the results suggest that the periodicity of growth rings formation is annual.

Primnoid gorgonians (Fig.1) show an axial microstructure composed of concentric rings, alternating organic gorgonin rings (proteinaceous) with different calcareous impregnation (inorganic). Alternating concentric rings can be easily seen in transversal sections of the axis (Fig.2). In deep-sea corals it have been demonstrated that these rings are deposited annually, and they are able to record, in the organic rings, environmental data as well as reflect the surface water productivity (Sherwood *et al.* 2005). Thus, hypothetically, in a gorgonian age known, techniques on trace elements and stable isotopes can be used to identify the climate conditions during its growing process, however is still unclear in which percentage geochemical variation on coral axis is due to environmental changes or biological processes.

In the present work we use several techniques to have a first approach into the dating process, from visually age estimation to radiometric analysis using ^{210}Pb. An effectiveness protocol is also designed to estimate the age and growth rate of Antarctic gorgonians. Our results are compared with those found in other polar organisms as well as with those in gorgonian species from other latitudes. As a preliminary conclusion we have found a slower growth rate in height and diameter gorgonians from polar than in temperate regions, the same pattern that has been found in bryozoans or molluscs.

5.2 Objectives

It is the purpose of this chapter to get an overview and approach of the dating estimations among Antarctic gorgonians. Including the following specific objectives:

- Approach into dating processes.
- Correlate visual age estimation with Scanning Electron Microscopy images.
- Correlate age estimations with radiometric analysis using ^{210}Pb.

- Design an effectiveness protocol to estimate age and growth rate of Antarctic gorgonians.

5.3 Materials and Methods

5.3.1. Material origin

For the purpose of this chapter the most abundant primnoid species in Antarctic waters, *Thouarella variabilis* Wright and Studer, 1889 has been examined. Specimens were collected during sampling efforts on board of the New Zealand research vessel *R/V Tangaroa* during the cruise TAN 0402, station number 75 at 525 m depth in the Ross Sea (72.077ºS 172.934ºE) the 14th February of 2004.

5.3.2. Material preparation

5.3.2.1. Sectioning and cutting

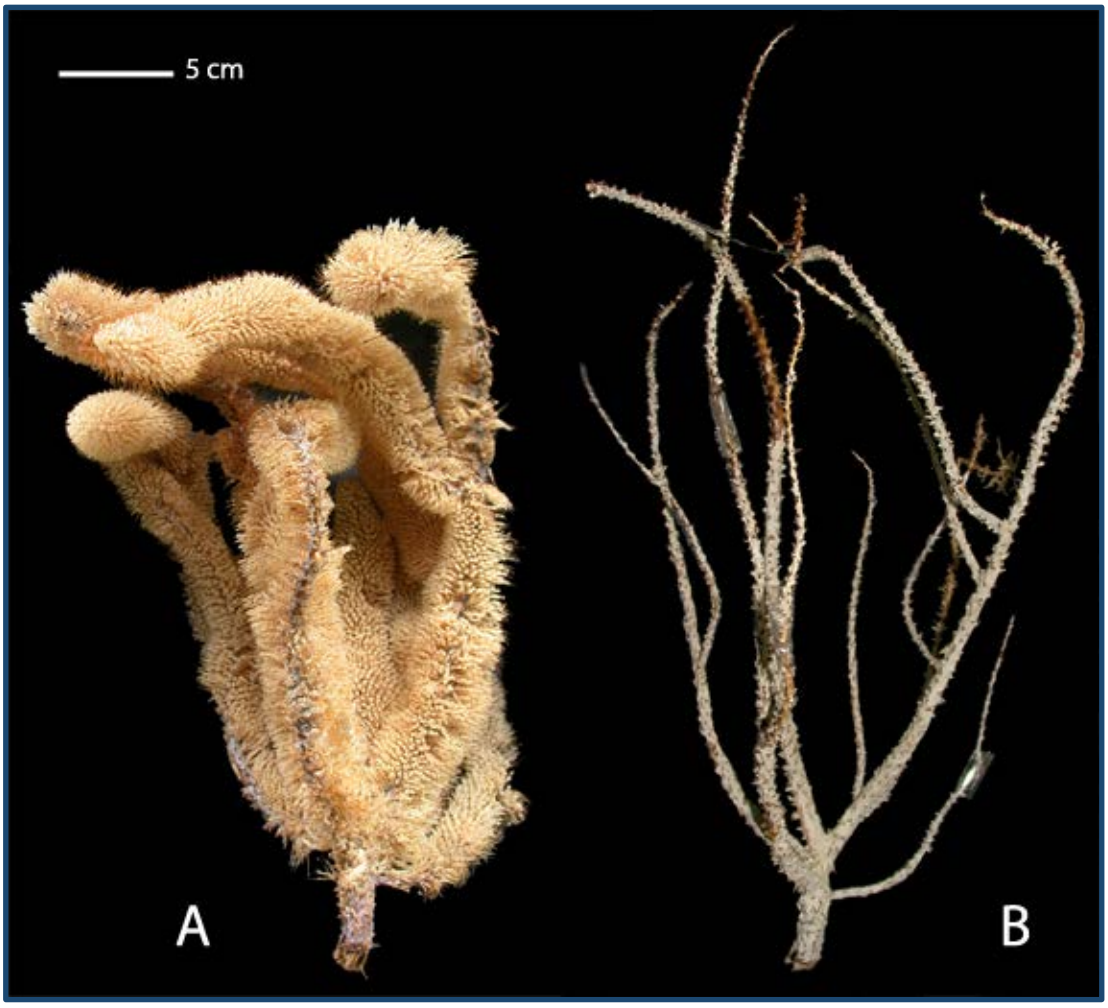


Figure 5.1.- *Thouarella variabilis*. **A**, before cleaning; **B**, without branchlets nor coenenchyma.

The studied specimen was firstly cleaned using a bottlebrush. Branchlets and coenenchyma were removed from the main branches leaving gorgonians an appearance of small trees (Fig. 5.1). The entire colony was cut using a Dremel® in segments of 1 cm in length, segments were kept in separate eppendorfs in order to let the pieces dry completely. To differentiate the basal from the upper part of each segment a small distinguishing mark was done (Fig. 5.2).

For the present study only the segment of the basal part of the colony and thus the thickest part was used, the remaining segments were stored for future growing analyses.

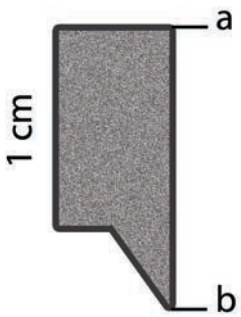


Figure 5.2.- Diagram of branch section of 1 cm. A, apical side; B, basal side.

Cross-sections were cut from the thickest pieces, which belong to the base of the colony adjacent to the holdfast using a Dremel® (Fig. 5.3). Each section was approximately 3 mm thick.

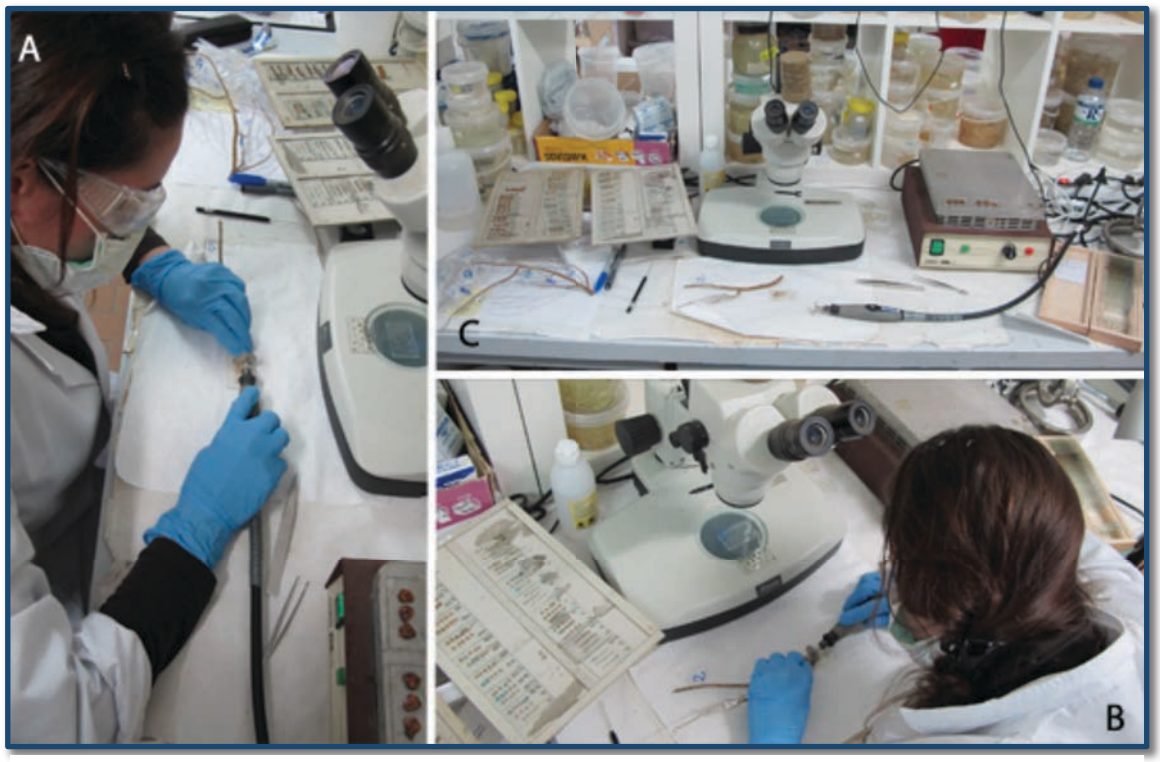


Figure 5.3.- Sectioning and cutting process.

5.3.2.2. Mounting

Sections were mounted on slides by casting a thermostable resin at high temperature on a thermostatic hot plate. When the resin was completely melted, the cross-sections were embedded in the resin and let the resin solidify at room temperature. Slides were labelled by scratching the glass using a Dremel® to avoid the future removal of the label by water (Fig. 5.4).

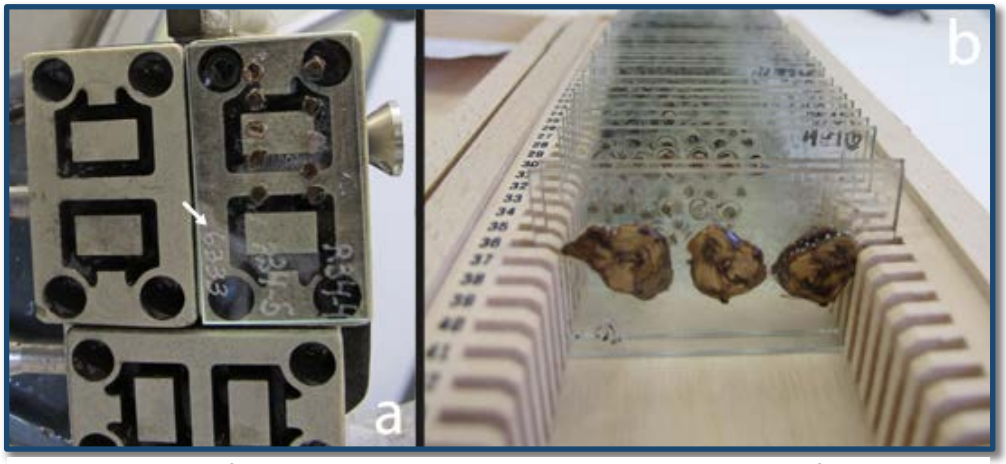


Figure 5.4.- A, detail of slide labelled pointed out by the white arrow; **B**, storage of gorgonian cross-sections mounted on slides**.**

5.3.2.3. Planar grinding

Grinding is required to planarize the specimen and to reduce the damage created by sectioning (Fig. 5.5). The planar grinding step is accomplished by sequentially abrasion to obtain surface flatness. Care must be taken to avoid being too abrasive in this step. The sample is moved slightly forward by hand and it is checked regularly under the light microscope to avoid lose the sample.

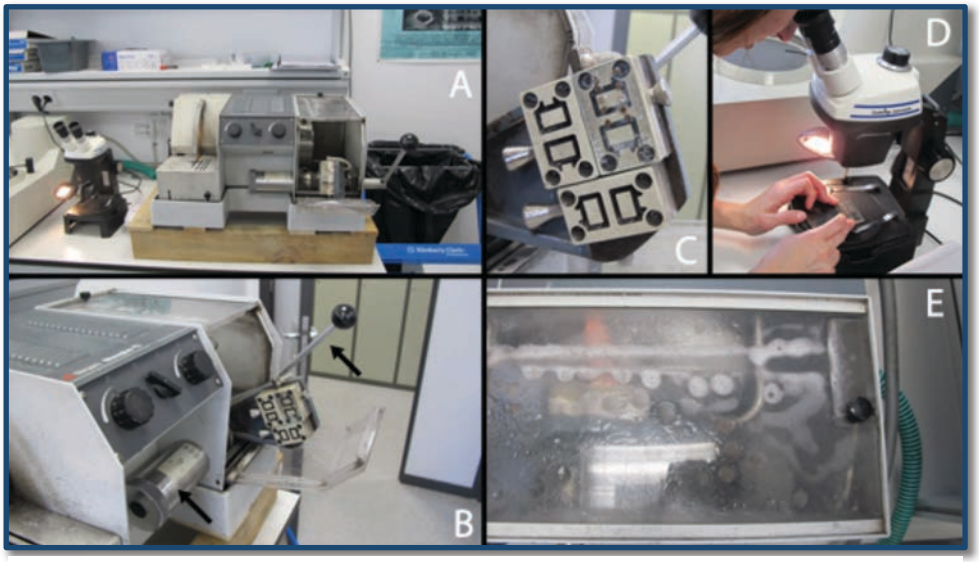


Figure 5.5.- Planar grinding. **A**, Planar grinding machine and light microscope; **B**, detail of the planar grinding, arrows point out the lever and the micrometre to control the amount of gridding; **C**, detail of the vacuum system that holds the slide; **D**, observation of growth rings under light microscope; **E,** frontal cover of planar grinding avoiding water spills.

5.3.3. Microscopy

5.3.3.1. Light microscopy

To obtain count rings, slides with embedded cross-sections in resin were observed under the light microscope.

5.3.3.2. Scanning Electron Microscopy

Cross-sections were removed from the thermostable resin by casting it at high temperature. To avoid resin to remain in the polished surface, samples were submerged in acetone ($(CH_3)_2CO$). In order to improve the appearance of ring couplets and make it easier to distinguish the rings for counting under the SEM, sections were submerged in 5% formic acid (HCO2H) for 6-12" to dissolve partially the calcareous fraction (inorganic matter) and transferred to water. Samples were air-dried and mounted in SEM stubs. For each section, SEM images were taken along its radius at 1300x, resulting images were assembled in Photoshop® CS5.1 to count rings.

5.3.4. Electron Probe Microanalysis (EPMA)

For chemical analysis the cross-sections not treated with formic acid, were mounted in HITACHI S-3500N stubs and coated with Au-Pd. The energy-dispersive x-ray spectrometer (EDS) was used to characterize and quantify the presence of elements at ring couplets. Analysis in spot-mode, where individual sites of interest on a sample were analysed, and transverse-mode, continuous sampling along a defined transect, were conducted.

5.3.5. Radiometric Analysis

Radial sampling of cross-sections was conducted using a Dremel®. Initially three serial samples were extracted from the exterior edge to the centre, due to the small axial radius of the sections; only two (exterior edge and centre) were finally extracted. To increase the sample size and get enough material to measure the ^{210}Pb activity, serial samples of successive cross-sections were combined. The Radioisotopes Service of the University of Seville measured preliminary ^{210}Pb activity.

5.4 Results

5.4.1. Microscopy

5.4.1.1. Light microscopy

As observed in Figures 5.6 and 5.7, wide couplets seem to be easy to differentiate while thinner couplets are often indistinguishable between them, make counting difficult. The rings counted at the most basal cross-section of the specimen analysed reveal an average count of 100 rings and 50 couplets. If each couplet observed is considered being formed annually then the specimen of 37 cm height and 10 mm of basal diameter would be about 50 years, with a radial growth rate of 0.2 mm/yr and an axial growth rate of 0.74 cm/yr.

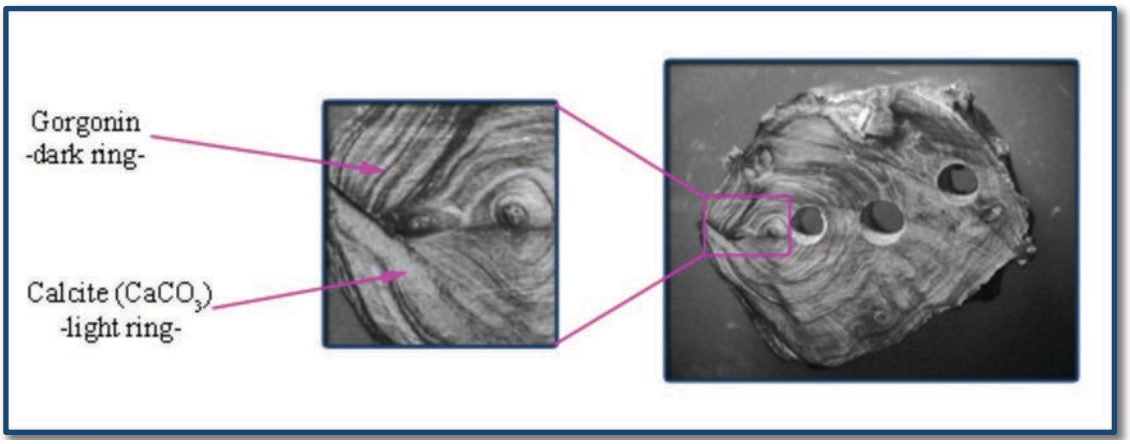


Figure 5.6.- Cross section of the basal axis of *Thouarella variabilis* Wright and Studer, 1889.

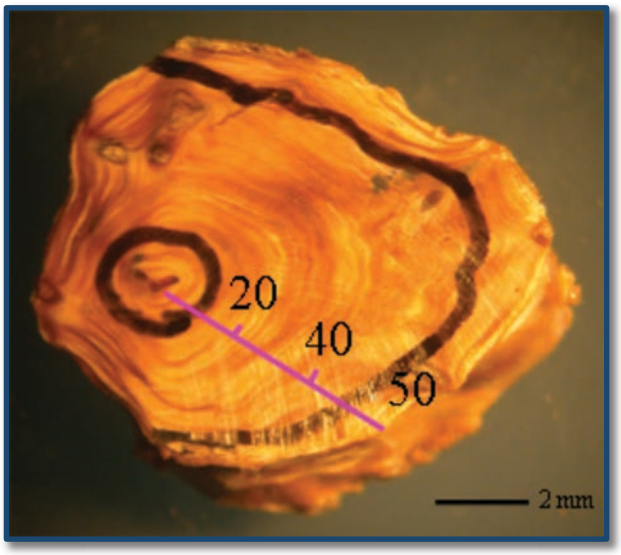


Figure 5.7.- Light microscope cross-section photography. Black lines mark the areas of central core and periphery.

5.4.1.2. Scanning Electron Microscopy

Images under SEM, with the secondary detector, have a higher definition letting the counter differentiate better the borders and revealing rings that are invisible by naked eye (Fig. 5.8), and multiply more than 7 times the number of rings counted using light microscopy (Fig. 5.7). The specimen of *Thouarella variabilis* analysed revealed more than 300 rings, 150 couplets. Some rings are wider (28-63 µm) than others (3-14 µm), and we can find about 10±12.55 small rings between the larger ones. If each couplet is considered being formed annually then the specimen of 37 cm height and 10 mm of basal diameter would be about 180 years old, with a radial growth rate of 0.05 mm/yr and longitudinal axial growth rate of 0.21 cm/yr.

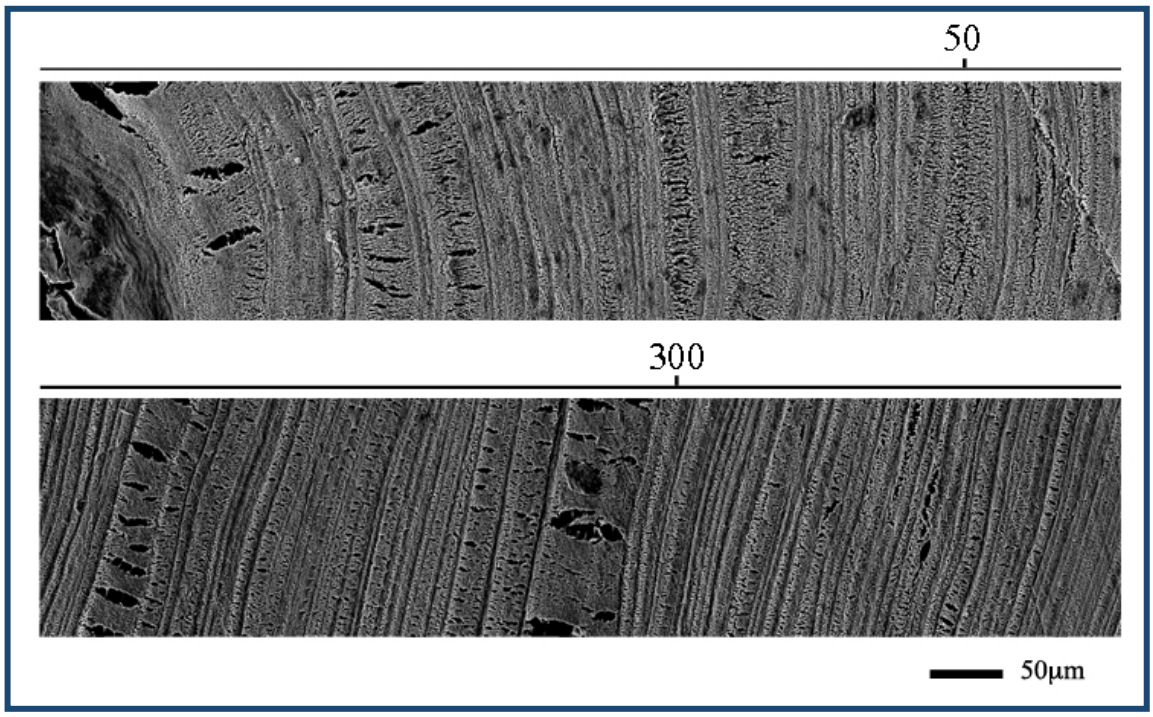


Figure 5.8.- SEM images of a partial cross-section of *Thouarella variabilis* of 367 growth rings.

5.4.2. Electron Probe Microanalysis (EPMA)

The microanalysis of the cross-sections for magnesium (Mg), calcium (Ca) and sulphur (S) allows the observation of a slight relation between Ca and the dark bands showed by the SEM images. Darkest bands correspond with those rings with higher amount of gorgonin, where the concentration of Ca decreases (Fig. 5.9 and 5.10). A SEM image (Fig. 5.11) of the same analysed transects, at higher magnification shows the presence of additional rings inside the light (where inorganic matter dominate) bands. The number of the inner rings is not constant for these last bands analysed. A slight anti-correlation seems to be present between Ca and S while Mg and Ca seem to have a very slightly positive correlation (Fig. 5.12).

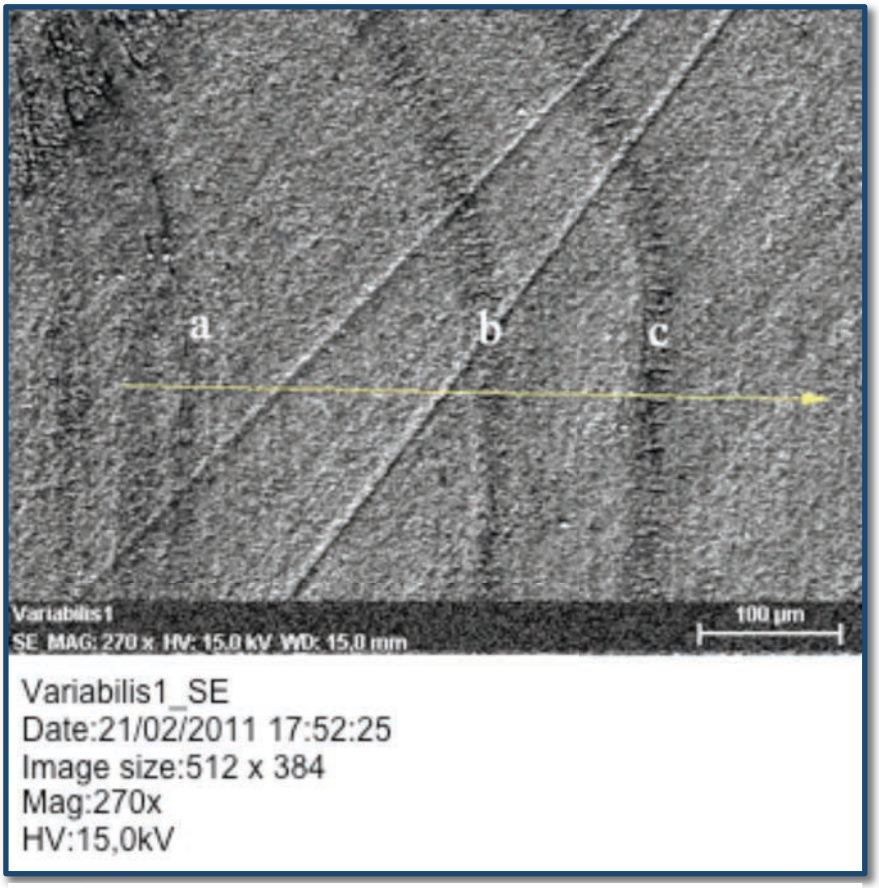


Figure 5.9.- SEM image of an axis section of *Thouarella variabilis*. Yellow line shows the transect of the microanalyses carried out.

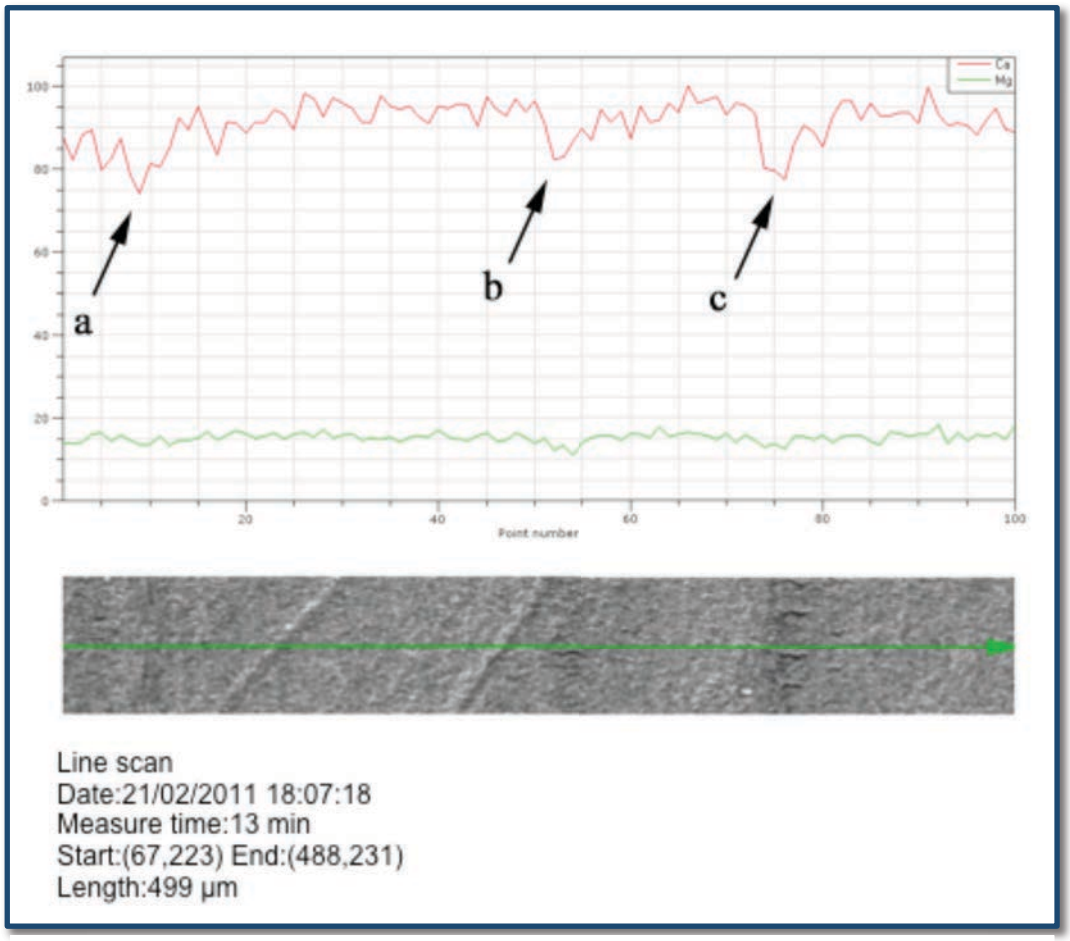


Figure 5.10.- Plot of Ca (red) and Mg (green) levels obtained along the transverse transect of Figure 5.9.

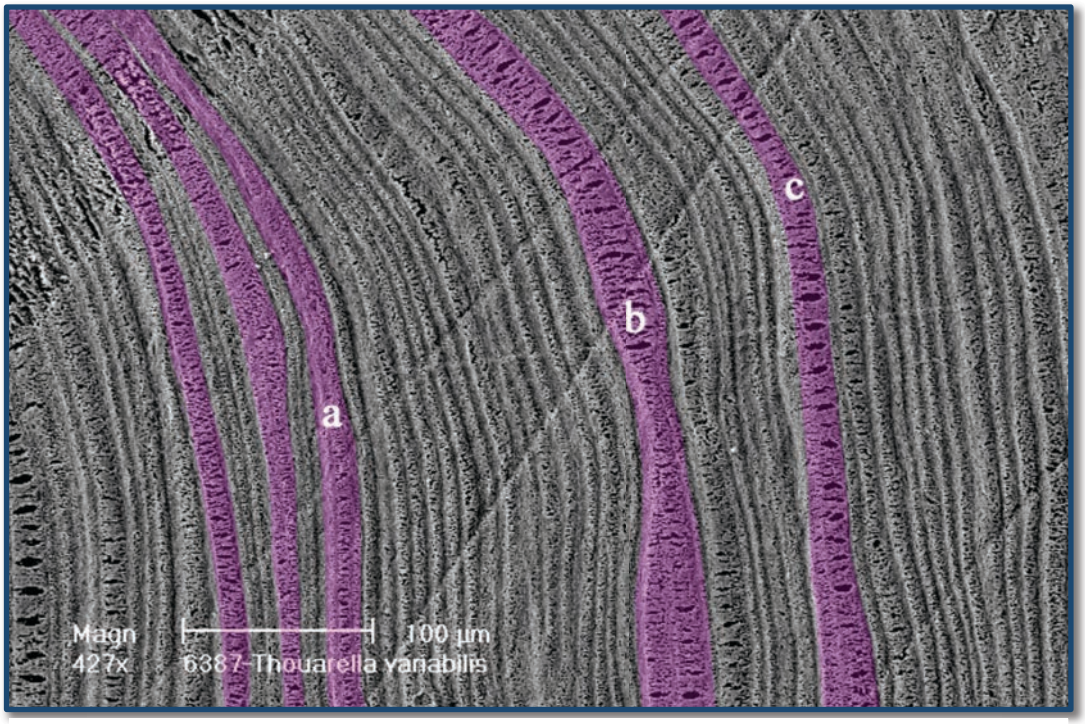


Figure 5.11.- SEM image of the axis section in figure 5.9 at high magnification. Small rings are visible between larger dark bands (coloured in pink).

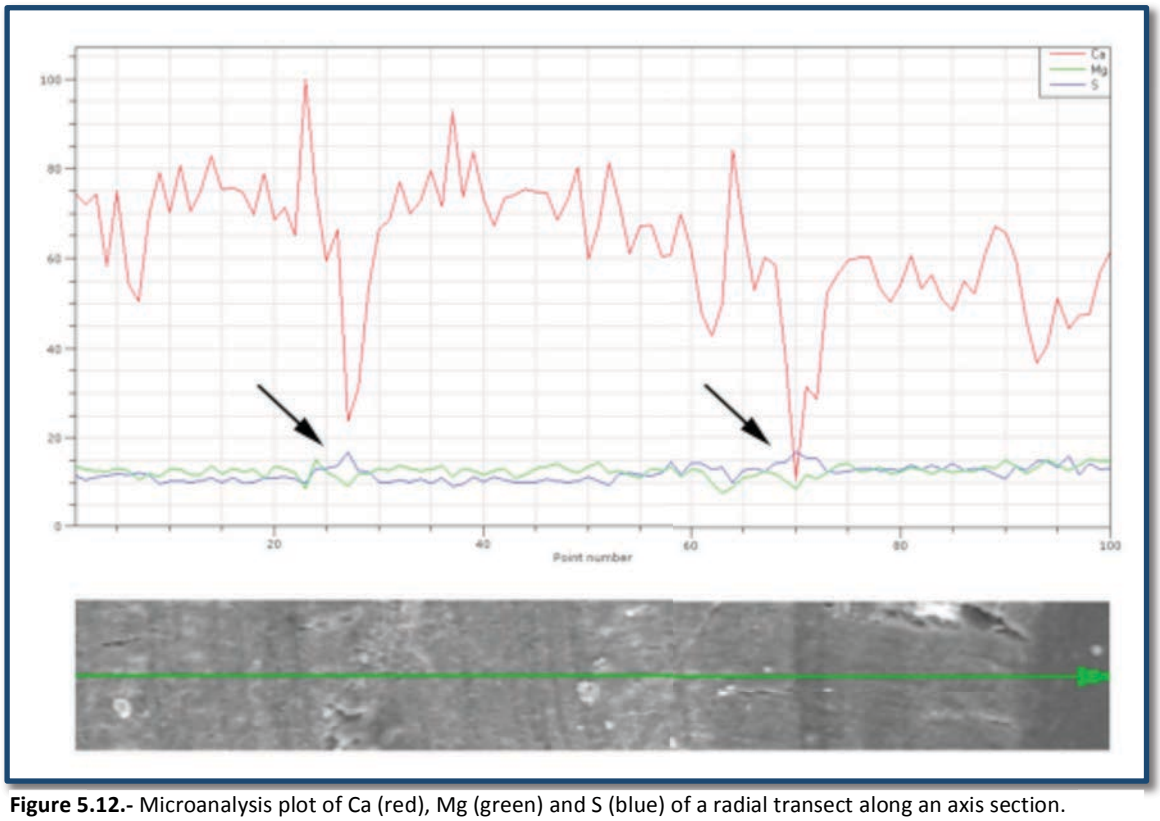


Figure 5.12.- Microanalysis plot of Ca (red), Mg (green) and S (blue) of a radial transect along an axis section.

5.4.3. Radiometric Analysis

The preliminary results of the measurements of the ^{210}Pb activity for one of the specimens estimate a radial growth rate of 0.17mm/year for a 10mm axial diameter. The estimated age for the specimen of *Thouarella variabilis* analysed, with a total height of 37cm, is about 60 years, resulting in an axial growth rate of 0.62 cm/year.

	Rings	Couplets	RGR	AGR	Est. Age
Light Microscopy	100	50	0.2 mm/yr	0.74 cm/yr	50
SEM	367	183	0.05 mm/yr	0.21 cm/yr	180
210Pb			0.17 mm/yr	0.62 cm/yr	60

Table 5.1.- Summary of techniques used to estimate the radial growth rate (**RGR**), the axial growth rate (**AGR**) and age of Thouarella variabilis specimen of 37 cm height and 10 mm of basal diameter. **Est. Age**, estimated age.

5.5 Discussion

The counts of number of growth rings of the hard parts of the organisms have been reported since the 1930s in a wide range of organisms, such as corals (Ma 1937), molluscs (Clarke 1968), echinoderms (Dahm & Brey 1998), fishes (Pannella 1971), or trees (Fritts 1966). However their observation under a light microscopy is sometimes not clear and requires thin cross-sections for a better light contrast to recognize individual growth rings as well different counters due to

the difficulty to discern some rings. To accurate the counts and make them reproducible a higher magnification and resolution has been used, however SEM images revealed a higher number of rings like in bamboo corals (Sánchez *et al.* 2004) which lead to the necessity of validate the annual periodicity of growth rings to understand the origin of the elevated number of rings.

Several radiometric methods have been used to confirm growth ring counts to establish growth rates and to date corals (Bender 1973, Druffel *et al.* 1990). The dating range in natural systems of ^{210}Pb can vary from less than 50 years up to 150 years (Goldberg 1963), and it has provided a good age and growth estimations for bamboo corals from the Pacific Ocean (Andrews *et al.* 2009). However the precision of this method could be questionable. To determine the ^{210}Pb is necessary a certain weight per sample (0.02g) and for colonies with a small basal diameter a thicker section (or several thin sections) is needed. When a sample (a hole) is extracted from a thicker section different age rings might be sampled and the same will occur when combining samples of successive cross-sections. Moreover the drilling size is also important, as we are talking of micron-wide rings, and thus sampling a bunch of rings in one sample.

In the deep-sea primnoid *Primnoa resedaeformis* Sherwood *et al.* (2005) isolated the gorgonin fraction to obtain separated rings to analyse the activity of ^{14}C. In this study the activity of ^{14}C hasn't been analysed as the number of rings counted by naked eye revealed an estimated age of 50 years old and the analysis of ^{14}C wouldn't be able to date the specimen. ^{14}C cannot be used to date modern specimens as it uses as a reference dating point the nuclear bomb testing of late 1950s (Bowman 1990).

After determining the age and growth rates for the *Thouarella variabilis* specimen using the three different methodologies, light microscopy, scanning electron microscopy and radiometric analysis of ^{210}Pb, we observe similar results for two of the techniques used (see Table 5.1).

Under light microscope an approximated 50 couplets have been counted for an estimated 60 years old gorgonian colony using the ^{210}Pb method. Annual band formation has been validated previously in deep-sea primnoids using ^{210}Pb (Andrews *et al.* 2002) and ^{14}C (Sherwood *et al.* 2005). Therefore we consider that those couplets of rings counted in this study might be also produced annually. In the presumed annually formed couplets, the microanalysis shows a correlation of the dark rings with a lower concentration of Ca, while in the light rings Ca also fluctuates but keeping higher values. Light microscopy and radiometric analyses of ^{210}Pb display similar values of growth rates, radial from 0.17 to 0.2 mm/yr and axial from 0.62 to 0.74 cm/yr. These values correspond with those found previously for some deep-sea gorgonians species (Table 5.2 and 5.3) for radial growth rates like in the bamboo corals *Lepidisis* with a rate of 0.18 mm/yr determined using ^{210}Pb analysis (Tracey *et al*. 2007) and *Isidella tentaculum* with a rate of 0.10-0.17 mm/yr determined with ^{226}Ra and also with ^{210}Pb (Andrews *et al*. 2009) in these last cases, the authors established 43 and 53±10 years old for their respective studied species. For axial growth rates *Thouarella variabilis* has similar values than *Keratoisis* with a growth rate of 0.7 cm/year with an estimated age of 98± 9 years

(Andrews *et al*. 2009). A recent study on the *in situ* length measurements of the Antarctic primnoid *Primnoella scotiae* reveals a high range of axial growth mean rates, from 0.096 to 5.53 cm/yr which differs significantly depending on the site, season and years of sampling (Peck & Brockington 2013). Deep-sea species seem to present slow growth rates than the shallow water species (Cordes *et al.* 2001, Andrews *et al.* 2009, see Table 5.1 and 5.2). Studies on Antarctic molluscs (Andrew *et al*. 2004) and bryozoans (Barnes 1995) show a slow growth rate compared with the temperate species, and the same pattern is observed here for octocorals (see Table 5.2 and 5.3). The data seems to corroborate that growth rates decrease towards high latitudes and deep-sea.

In gorgonians maximum longevity has been reported for the temperate bamboo coral *Keratoisis* using the ^{210}Pb activity (400 years old) (Thresher *et al.* 2004). Other methods like the combination of ^{14}C with growth rings counts also estimated ages of centuries, the deep-sea gorgonian *Primnoa resedaeformis* was estimated to be 320 years old (Risk *et al.* 2002). Field measurements have been mainly used to estimate growth rates and only few works have estimated ages, Brazeau & Lasker (1992) estimated the age of the Caribbean gorgonian *Briareum asbestinum* in 50 years old by *in situ* length measures.

A high magnification SEM images show that the light rings of the couplets are actually formed by several rings, in a variable number. A total of more than 350 rings (180 couplets) have been counted for the same gorgonian using SEM images. Similar patterns have been found in deep-sea bamboo corals (Sánchez *et al.* 2004) suggesting an influence of lunar cycles or occurrences of export of particulate matter (Tracey *et al.* 2007).

The electron probe microanalysis showed that in the dark areas (organic) observed by the back-scattered SEM mode levels of Ca decrease however while anti-correlation between Mg and Ca was found by other authors in red coral (Vielzeuf *et al.* 2008) it was not observed in our samples, making Mg usefulness for dating. The EMP analysis has been performed only in a small transect, in order to determine a correlation between number of rings observed by naked eye and those counted by Ca ratio the whole diameter should be analysed. However as small rings have been observed in the light areas where Ca is higher, the total count of couplets should be similar than those counted by naked eye. The EMP also showed small intravariations in the light areas revealing an irregular deposition of Ca which could be related with the continuous feeding of antarctic gorgonians by resuspension and advection processes, producing the small rings observed by SEM but not by naked eye.

Taking into account that estimated age using ^{210}Pb have the inconvenience of the accuracy of sampling, we can propose two hypothesis:

1) These extra inner rings are actually formed annually and would mean an estimated age of 180 yrs with lower growth rates than obtained by light microscope and ^{210}Pb radiometric analyses, radial of 0.05 mm/yr and axial of 0.21 cm/yr (similar values have been observed for the deep-sea bamboo coral *Keratoisis* by Andrews et al. (2009) and gorgonian *Primnoa resedaeformis* by Risk *et al.* (2002).

2) These extra inner rings are not formed annually, and might correspond to intraannual variations due to environmental fluctuations.

Independently of which technic we use to determine age and growth rates, the primnoid gorgonian here studied shows low growth rates and a high longevity when compared with other gorgonians from shallow and temperate waters (see Table 5.2 and 5.3). Recovery time for this species might be around the order of a century. When compared with the red coral (*Corallium rubrum*) we observe a radial growth rate for the Antarctic species 2-3 times slower, while the longitudinal axial growth for the Antarctic species is 3-4 times faster than in red coral (Bramanti *et al.* 2005), however that study focused on a 4 year old colonies which doubled their size between age 3 and 4.

Antarctic ecosystems are particularly vulnerable and sensitive to the growing impact of the climate change and other anthropogenic influences (Ingels *et al.* 2011). Continued warming together with increasing CO_2 concentrations in the SO is causing a cascade of environmental effects with broad consequences for the benthic fauna (Barnes & Peck 2008). One of the most serious changes is assumed to be the increasing of iceberg scouring rates due to the retreat of the maritime glaciers and ice shelves which increase the number of floating icebergs, which causes considerable damage to benthic communities (Gutt *et al*. 1996, Teixidó *et al.* 2004). Although to fully understand the recovery potential of Antarctic gorgonians more information is needed in terms of patterns of fecundity, recruitment and sexual *vs*. asexual reproduction patterns, and an accurate determination of growth rates and age populations, due to the slow growth of Antarctic species (Barnes 1995, Clarke *et al.* 2004) including gorgonians, conservation measures are needed to protect fragile habitat-forming members of the Antarctic ecosystem.

Species	Type	R	Locality	Method	Radial growth (mm/y)	Axial growth (cm/y)	Age	Reference
Acanella arbuscula	bamboo coral	Ds	Newfouland Labrador	14 C/Growth rings	0.020 - 0.070	0.3 - 1	<100	Sherwood et al, 2009
Briareum asbestinum	gorgonian	C	San Blas Islands, Panam	increment of heigh		16.6 cm/y--1.2 br/y	10.6 to 50.1 yrs	Brazeau and Lasker, 1992
Colpophyllia natans	Hexacoral	A	Jamaica	X-radiographic measurements annual density bands		0.3 - 1.05		Huston, 1985
Corallium niobe	coral	Ds	Little Bahama Banks	210 Pb	0.11±0.02		180±40 yrs	Druffel et al, 1990
Corallium rubrum	coral	M	Tuscany, Italy	artificial settlement plates	0.62±0.19	0.183 ± 0.015	4 yrs for 7,34 mm heigh	Bramanti et al, 2005
Corallium rubrum	coral	M	Marseilles/Medes Islanc	Growth rings	0.35±0.15		30 to 40 yrs for 7mm basal diam	Maschal et al, 2004
Enicea laxispica	gorgonian	C	Puerto Rico	monitored		2.21±1.68		Yoshioka and Yoshioka, 1991
Eunicea tourneforti	gorgonian	C	Puerto Rico	monitored		2.06±1.71		Yoshioka and Yoshioka, 1991
Eunicella cavolini	gorgonian	M				1.14±0.44		Velimirov, 1973
Eunicella cavolini	gorgonian	M				0.52 - 2.15		Velimirov, 1975
Eunicella cavolini	gorgonian	M				0.85±0.46		Weinbauer and Vellmirov, 1995
Euricea succinea	gorgonian	C	Puerto Rico	monitored		1.36±1.86		Yoshioka and Yoshioka, 1991
Gorgonia flagellum	gorgonian	T				0 - 8.3		Cary, 1914
Gorgonia ventalina	gorgonian	C	Puerto Rico	monitored		1.92±2.02 - 2.34±2.52		Yoshioka and Yoshioka, 1991
Halipteris willemoesi	sea pen	A	Bering Sea	210 Pb 226 Ra/Growth riings	0.145	3.9±0.2	7.1 yrs for a 25-29 cm heigh	Wilson et al, 2002
						6.1±0.3	19.3 yrs for a 97-130 cm heigh	
						3.6±0.1	44.3 yrs for a 152-167 cm heigh	
Isidella tentaculum	bamboo coral	Ds	Gulf of Alaska	210 Pb 226 Ra	0.10 - 0.17	1.4	53±10 yrs	Andrews et al, 2009
Isididae	bamboo coral	fossil	New Zealand	radiocarbon	0.4 (0.23 - 0.64)		305±30 yrs	Noe and Dullo, 2006
Keratoisis	bamboo coral	Ds	Davidson Seamount	210 Pb 226 Ra	0.055	0.7	98±9 yrs	Andrews et al, 2009
Keratoisis	bamboo coral	Ds	Davidson Seamount	210 Pb 226 Ra	0.039	0.14 - 0.28	>145-282 yrs	Andrews et al, 2009
Keratoisis	bamboo coral	Ds	Gulf of Alaska	210 Pb 226 Ra	0.056	1	116±29 yrs	Andrews et al, 2009
Keratoisis	bamboo coral		Tasmania	210 Pb	0.05 - 0.1		400 yrs	Thresher et al, 2004
Keratoisis ornata	bamboo coral	Ds	Newfouland Labrador	14 C/Growth rings	0.053±0.009 - 0.075±0.011	0.93±0.08	200±30 yrs	Sherwood et al, 2009
Leiopathes glaberrima	antipatharian		Hawaii		0.01		>2000 yrs	Roark et al, 2006
Leiopathes glaberrima	antipatharian	A	Florida, USA		0.015			Williams et al, 2006
Lepidisis	bamboo coral	Ds	New Zealand	210 Pb	0.18 ± 0,02			Tracey et al, 2007
Leptogorgia	gorgonian					4.5	10 yrs	Mitchell et al 1993
Montastrea annularis	Hexacoral	A	Jamaica	X-radiographic measurements annual density bands		0.1 - 1.22		Huston, 1985
Montastrea cavernosa	Hexacoral	A	Jamaica	X-radiographic measurements annual density bands		0.2 - 1.09		Huston, 1985
Muricea californica	gorgonian	Ts	California	increment of heigh/compare known substrata		0.61±2		Grigg, 1974
Muricea fruticosa	gorgonian	Ts	California	increment of heigh/compare known substrata		1.69	20 yrs for a 30 cm heigh	Grigg, 1974
Muriceopsis flavida	gorgonian	C	Puerto Rico	monitored		1.85±1.61		Yoshioka and Yoshioka, 1991
Paragorgia arborea	gorgonian	Ds	Atl Canada	Growth rings		1	180 yrs	Mortensen and Buhl-Mortensen, 2005
Paragorgia arborea	gorgonian	Ds	Norway	photographic time series		2.2 - 4		Mortensen and Buhl-Mortensen, 2005
Paragorgia arborea	gorgonian	Ds	New Zealand	radiocarbon		0.8 - 1.3	400±100 yrs	D. Gordon pers. Comm
Paragorgia arborea	gorgonian	Ds	Newfouland Labrador	14 C/Growth rings	0.830 ± 0.12	1.62±0.22	80±11 yrs	Sherwood et al, 2009
Paragorgia arborea	gorgonian	Ds	New Zealand			0.8 - 4		Tracey et al, 2003

Table 8.1.- Growth rates and estimated ages previously reported for various species of octo- and hexacorals. **R,** region; **Ds**, Deep Sea; **C**, Caribbean; **A**, Atlantic; **M**, Mediterranean Sea; **T**, Tropical; **Ts**, Temperate Sea.

Species	Type	R	Locality	Method	Radial growth (mm/y)	Axial growth (cm/y)	Age	Reference
Paramuricea clavata	gorgonian	M	Medes Islands	monitored photographically		1.8 (0.2 - 6.4)	31 yrs for a 55 cm heigh	Coma et al, 1998
Paramuricea clavata	gorgonian	M	Tyrrhenian Sea	Growth rings 4 age, field measurements		2.7±1.6 - 3±0.3	15 yrs for a 32 cm heigh	Mistri and Ceccherelli, 1994
Paramuricea clavata	gorgonian	M				1.8 (1.6 - 3.7)		
Paramuricea spp.	gorgonian	Ds	Newfouland Labrador	14 C/Growth rings	0.092±0.018 - 0.205±0.02	0.56±0.05 - 0.58±0.08	71±6 - 103±14 yrs	Sherwood et al, 2009
Plexaura	gorgonian					2 - 20		Kim and Lasker, 1997
Plexaura A	gorgonian	T	Bahamas	monitored photographically		3.8 - 3.9		Lasker 1990
Plexaura dichotoma	gorgonian	C	Puerto Rico	monitored		0.80±1.94		Yoshioka and Yoshioka, 1991
Plexaura flexuosa	gorgonian	T				0.5 - 5.5		Cary, 1914
Plexaura flexuosa	gorgonian	T				2.15 - 1.77		Cary, 1914
Plexaura flexuosa	gorgonian	C	Puerto Rico	monitored		1.77±1.68 - 2.15±1.27		Yoshioka and Yoshioka, 1991
Plexaura homomalla	gorgonian	T				1.99		Kinzie 1973
Plexaura homomalla	gorgonian	T				2 (0.13 - 4.2)		Kinzie 1973
Plexaura homomalla	gorgonian	C	Puerto Rico	monitored		1.99±1.29		Yoshioka and Yoshioka, 1991
Plexaura homomalla f. kukentha	gorgonian	T				1.18		Kinzie 1973
Plexaura homomalla f. kukentha	gorgonian	C	Puerto Rico	monitored		1.18±1.10		Yoshioka and Yoshioka, 1991
Porites astreoides	Hexacoral	A	Jamaica	X-radiographic measurements annual density bands		0.19 - 0.63		Huston, 1985
Porites porites	Hexacoral	A	Jamaica	X-radiographic measurements annual density bands		0.7 - 2.1		Huston, 1985
Primnoa resedaeformis	gorgonian	Ds	Gulf of Alaska	210 Pb/Growth rings	0.36	1.6 - 2.32	112 yrs	Andrews et al, 2002
Primnoa resedaeformis	gorgonian	Ds	Atl Canada	Growth rings		1.7	61 yrs	Mortensen and Buhl-Mortensen, 2005
Primnoa resedaeformis	gorgonian	Ds	Atlantic Ocean			0.15 - 0.25	320 yrs	Risk et al, 2002
Primnoa resedaeformis	gorgonian	Ds	Nova Scotia	14 C/Growth rings			24 to 78 yrs	Sherwood et al, 2005
Primnoa resedaeformis	gorgonian	Ds	Newfouland Labrador	14 C/Growth rings	0.083±0.006 - 0.215±0.037	1±0.09 - 2.61±0.45	18±4 - 100±9 yrs	Sherwood et al, 2009
Pseudoplexaura porosa	gorgonian	C	Puerto Rico	monitored		1.98±1.52 - 2.22±1.96		Yoshioka and Yoshioka, 1991
Pseudoplexaura wagenaari	gorgonian	C	Puerto Rico	monitored		2.13±2.16 - 2.57±1.88		Yoshioka and Yoshioka, 1991
Pseudopterogorgia acerosa	gorgonian	C	Puerto Rico	monitored		2.12±3.03 - 4.03±3.14		Yoshioka and Yoshioka, 1991
Pseudopterogorgia americana	gorgonian	C	Puerto Rico	monitored		3.44±3.12 - 4.48±2.82		Yoshioka and Yoshioka, 1991
Siderastrea siderea	Hexacoral	A	Jamaica	X-radiographic measurements annual density bands		0.27 - 0.93		Huston, 1985
Stauropathes arctica	antipatharian	Ds	Newfouland Labrador	14 C/Growth rings	0.033±0.011 - 0.066±0.011	1.22±0.46 - 1.36±0.2	55±8 - 82±31 yrs	Sherwood et al, 2009
Thouarella variabilis	**gorgonian**	**Ds**	**Antarctica**	**Growth rings @ Light Microscope**	**0.2**	**0.74**	**50 yrs**	**Present Study**
				Growth rings @ SEM	**0.05**	**0.21**	**180 yrs**	**Present Study**
				210 Pb	**0.17**	**0.62**	**60 yrs**	**Present Study**
Bamboo coral	bamboo coral		California		0.05 - 0.11			Andrews et al, 2005
Bamboo coral	bamboo coral		Warwick Seamount	bomb radiocarbon	0.05±0.01 - 0.16±0.01		64±4 - 208±42 yrs	Roark et al, 2005
Bamboo coral	bamboo coral		New Zealand		0.13 - 0.29			Tracey et al 2007

Table 5.2.- Continuation. Growth rates and estimated ages previously reported for various species of octo- and hexacorals. **R,** region; **Ds**, Deep Sea; **C**, Caribbean; **A**, Atlantic; **M**, Mediterranean Sea; **T**, Tropical; **Ts**, Temperate Sea.

CHAPTER 6

Reproductive Patterns on *Thouarella variabilis*

6.1 Introduction

Gorgonians reproductive biology is poorly known in most species compared with soft corals or scleractinians, and the great majority of the studies are mostly based in tropical and temperate species (Grigg 1988, Brazeau & Lasker 1990, Gori *et al.* 2007), which are essential to understand patterns in other latitudes. However reproductive biology on deep-sea species are still unknown. Primnoid gorgonian species are the main and more representative gorgonian taxa in benthic Antarctic ecosystems, however the current knowledge is based on a few descriptions on reproductive morphology (Kükenthal 1919) and surprisingly just a couple of studies on reproductive biology are based on them (Brito *et al*. 1997, Orejas *et al.* 2007).

The data based on these studies reveals gonochorism associated to brooding as the trend in Antarctic and deep-sea corals, being the distance between colonies of both sex a critic reproductive factor (Coma & Lasker 1997). Brito (1997) suggests that the non-pelagic, non-feeding lecitotrophic larvae of primnoids might settle soon after its release, and thus a low dispersal capacity leading to a patchy distribution like occurs with other Antarctic invertebrates. However has been observed in this study that some primnoid species have a wide distribution in the Southern Ocean, almost circumpolar, suggesting the possibility that other factors might be influencing the distribution of these patches (Gutt, 2000). It has been also suggested by Orejas *et al.* (2007) that the colony shape might have influence on feeding (prey capture) and therefore to be a key role in the reproductive strategy chosen by these organisms.

The release of one larva per female polyp and season at a time has been proposed for those species that present two or more oocyte size classes corresponding to different stages of development and thus to different reproduction cycles in the same polyp. However, a continuous gametogenesis instead and a larval release all year around could be also feasible (Brito 1997) as demonstrated for other octocorals (Benayahu *et al.* 1989).

Although some studies have been focused on the characterization of the gametogenesis process on Antarctic gorgonians (Brito 1997, Orejas *et al.* 2002, 2007), information about fertilization, planulation, larval settlement and/or recruitment important to give explanation to spatial distributions and community structure patterns are still unknown.

Moreover, gorgonians are also clonal marine species that clonal at two levels. First, a colony (ramet) is a group of genetically identical and physiologically integrated modules (polyps) that is generated by the replication of modules. Second, genetically identical ramets that are physiologically and ecologically independent entities arise through a variety of processes such as fragmentation. All those ramets that are genetically identical are named genet.

Clonal reproduction can be considered as an alternative life cycle that allows persistence of the species in the absence of the ability to complete normal life cycle and in the purpose of

avoiding risks. This mechanism produces genetically identical but physiologically distinct individuals. Lasker (1990) suggest that clonal propagation is a growth strategy that increases genet's fitness, but no evidence of trade-off between larval reproduction and clonal propagation has been observed. Clonal growth strategies can affect the tempo and mode of evolution by increasing generation times and altering both genetic diversity and effective population size, which will retard population responses when climate change effects reductions in reproduction and larval recruitment. Extended survival of populations may provide time for propagules to become established in new habitats, but many species may persist only as relict populations, in which the great longevity of colonies and clones masks the environmental effects (Lasker & Coffroth 1999).

6.2 Objectives

It is the purpose of this chapter to increase the knowledge on the reproductive patterns of Antarctic gorgonians. Including the following specific objectives:

- Identify patterns on gametogenesis
- Identify the developmental stages of the sexual products
- Estimate the reduction of sexual products after dehydration processes
- Estimate the gonadal volume

6.3 Material and Methods

6.3.1 Material origin

6.3.1.1 Research Expeditions

For the purpose of this chapter major sampling effort was carried out on board of the German research vessel *R/V Polarstern* during the cruises ANT XVII-3 (EASIZ III, Ecology of the Antarctic Sea Ice Zone, 18 March to 11 May 2000), ANT XIX-3 (ANDEEP I, Antarctic Benthic Deep-Sea Biodiversity, 23 January 2002 to 26 February 2002), ANT XIX-5 (LAMPOS, Latin American Polarstern Study, 3 April to 5 MAY 2002) and ANT XXIII-8 (23 November 2006 to 30 January 2007) sponsored by the Alfred Wegener Institute für Polar und Meeresforschung (Bremerhaven). Additional sampling was carried out on board *R/V Tangaroa* during the Ross Sea 2004 cruise (26 January to 5 March 2004).

Octocoral colonies were collected using trawls (Agassiz, Bottom and Beam trawls), the Epibenthic Sledge, and Rauschert dredge. The octocorals from *Polarstern* cruises were fixed in buffered formalin 10% and then transferred to ethanol 70%, the colonies from *Tangaroa* cruise were all fixed in pure ethanol.

6.3.1.2 Studied species

The Antarctic gorgonian species, *Thouarella variabilis* Wright and Studer, 1889 (Fig. 6.1), has been used to carry out this study. The species belongs to the family Primnoidae, the dominant and more specious octocoral family in the Southern Ocean benthic communities. *Thouarella*

variabilis has been recently re-described (Taylor *et al*, 2013), it shows a typical bottlebrush colony shape, with isolated polyps standing all around the branchlets. The species, with a circumpolar distribution is endemic of the Southern Ocean waters.

It has been analysed 43 colonies (21 males and 22 females) of *Thouarella variabilis*, 19 of them were from spring (9 males and 10 females), 20 from summer (10 males and 10 females) and 4 colonies were from autumn (2 males and 2 females) (Table 6.1).

Id	Sex	Season	Date	Latitude	Longitude	Depth	Vessel	Cruise	Station	Location
1644	♂	SUMMER	04 Feb 2002	-60,88600	-55,60450	164	R/V Polarstern	ANT XIX-3 ANDEEP I	PS61-068-01	Elephant Island
6367	♂	SUMMER	20 Dec 2006	-61,33783	-55,51533	-	R/V Polarstern	ANT XXIII-8 CLIMANT		Elephant Island
69	♂	SUMMER	18 Feb 2004	-71,18867	170,97717	719-736	R/V Tangaroa	TAN 0402	096	Ross Sea
6401	♂	SUMMER	05 Feb 2004	-71,72717	171,78133	522-530	R/V Tangaroa	TAN 0402	018	Ross Sea
6387	♂	SUMMER	14 Feb 2004	-72,07700	172,93483	525-526	R/V Tangaroa	TAN 0402	075	Ross Sea
6395	♂	SUMMER	14 Feb 2004	-72,08250	173,13883	539-542	R/V Tangaroa	TAN 0402	084	Ross Sea
6394	♂	SUMMER	14 Feb 2004	-72,06050	172,90383	526-527	R/V Tangaroa	TAN 0402	082	Ross Sea
6384	♂	SUMMER	26 Feb 2004	-72,01350	170,77450	231-240	R/V Tangaroa	TAN 0402	140	Ross Sea
6396	♂	SUMMER	14 Feb 2004	-72,06050	172,90383	526-527	R/V Tangaroa	TAN 0402	082	Ross Sea
6397	♂	SUMMER	14 Feb 2004	-72,06050	172,90383	526-527	R/V Tangaroa	TAN 0402	082	Ross Sea
6004	♂	SPRING	31 Dec 2006	-61,83350	-58,62150	191-208	R/V Polarstern	ANT XXIII-8 CLIMANT	669-01	South Shetland Islands
6366	♂	SPRING	20 Dec 2006	-61,33783	-55,51533	153	R/V Polarstern	ANT XXIII-8 CLIMANT	605-05	Elephant Island
6208A	♂	SPRING	30 Dec 2006	-61,64633	-57,86433	336-337	R/V Polarstern	ANT XXIII-8 CLIMANT	664-01	South Shetland Islands
6208B	♂	SPRING	30 Dec 2006	-61,64633	-57,86433	336-337	R/V Polarstern	ANT XXIII-8 CLIMANT	664-01	South Shetland Islands
6411A	♂	SPRING	31 Dec 2006	-61,83417	-58,51117	151-193	R/V Polarstern	ANT XXIII-8 CLIMANT	668-01	South Shetland Islands
6411C	♂	SPRING	31 Dec 2006	-61,83417	-58,51117	151-193	R/V Polarstern	ANT XXIII-8 CLIMANT	668-01	South Shetland Islands
6411F	♂	SPRING	31 Dec 2006	-61,83417	-58,51117	151-193	R/V Polarstern	ANT XXIII-8 CLIMANT	668-01	South Shetland Islands
6411G	♂	SPRING	31 Dec 2006	-61,83417	-58,51117	151-193	R/V Polarstern	ANT XXIII-8 CLIMANT	668-01	South Shetland Islands
6411H	♂	SPRING	31 Dec 2006	-61,83417	-58,51117	151-193	R/V Polarstern	ANT XXIII-8 CLIMANT	668-01	South Shetland Islands
142	♂	AUTUMN	26 Apr 2000	-63,07833	-57,52667	94-95	R/V Polarstern	ANT XVII-3 EASIZ III	158-01	Bransfield Strait
1347	♂	AUTUMN	15 Apr 2002	-57,67833	-26,43483	278-309	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-194-01	South Sandwich Islands
6385	♀	SUMMER	14 Feb 2004	-72,07700	172,93483	525-526	R/V Tangaroa	TAN 0402	075	Ross Sea
6386	♀	SUMMER	13 Feb 2004	-72,30783	170,35767	123-130	R/V Tangaroa	TAN 0402	055	Ross Sea
6388	♀	SUMMER	14 Feb 2004	-72,07700	172,93483	525-526	R/V Tangaroa	TAN 0402	075	Ross Sea
54	♀	SUMMER	09 Feb 2004	-71,75600	171,26350	270-275	R/V Tangaroa	TAN 0402	029	Ross Sea
9	♀	SUMMER	27 Feb 2004	-71,54750	171,11117	280-286	R/V Tangaroa	TAN 0402	188	Ross Sea
64	♀	SUMMER	09 Feb 2004	-71,74683	171,55500	340-343	R/V Tangaroa	TAN 0402	031	Ross Sea
6399	♀	SUMMER	27 Feb 2004	-71,51200	171,42517	389-390	R/V Tangaroa	TAN 0402	186	Ross Sea
6402	♀	SUMMER	10 Feb 2004	-71,75500	171,14750	250	R/V Tangaroa	TAN 0402	039	Ross Sea
6404	♀	SUMMER	05 Feb 2004	-71,71100	172,04467	621-636	R/V Tangaroa	TAN 0402	010	Ross Sea
6389	♀	SUMMER	13 Feb 2004	-72,32483	170,42767	199-206	R/V Tangaroa	TAN 0402	054	Ross Sea
6250	♀	SPRING	24 Dec 2006	-60,97667	-55,77933	162-191	R/V Polarstern	ANT XXIII-8 CLIMANT	629-01	Elephant Island
6409	♀	SPRING	22 Dec 2006	-60,96300	-55,75000	199-201	R/V Polarstern	ANT XXIII-8 CLIMANT	619-01	Elephant Island
6370	♀	SPRING	21 Dec 2006	-60,87683	-55,34217	307-483	R/V Polarstern	ANT XXIII-8 CLIMANT	612-01	Elephant Island
6405	♀	SPRING	30 Dec 2006	-61,65333	-57,07917	466-467	R/V Polarstern	ANT XXIII-8 CLIMANT	661-02	South Shetland Islands
6407	♀	SPRING	31 Dec 2006	-61,74967	-58,51483	282-288	R/V Polarstern	ANT XXIII-8 CLIMANT	667-01	South Shetland Islands
6364	♀	SPRING	30 Dec 2006	-61,65333	-57,07917	466-467	R/V Polarstern	ANT XXIII-8 CLIMANT	661-02	South Shetland Islands
6408A	♀	SPRING	21 Dec 2006	-60,86883	-55,50517	248-259	R/V Polarstern	ANT XXIII-8 CLIMANT	614-03	Elephant Island
6408B	♀	SPRING	21 Dec 2006	-60,86883	-55,50517	248-259	R/V Polarstern	ANT XXIII-8 CLIMANT	614-03	Elephant Island
6219A	♀	SPRING	19 Dec 2006	-61,33917	-55,48600	151	R/V Polarstern	ANT XXIII-8 CLIMANT	605-01	Elephant Island
6219B	♀	SPRING	19 Dec 2006	-61,33917	-55,48600	151	R/V Polarstern	ANT XXIII-8 CLIMANT	605-01	Elephant Island
462	♀	AUTUMN	26 Apr 2000	-62,91667	-57,65833	218	R/V Polarstern	ANT XVII-3 EASIZ III	159-01	Bransfield Strait
6412	♀	AUTUMN	09 Apr 2000	-53,39467	-42,70383	306-343	R/V Polarstern	ANT XIX-5 LAMPOS	PS61-167-01	South Georgia Island

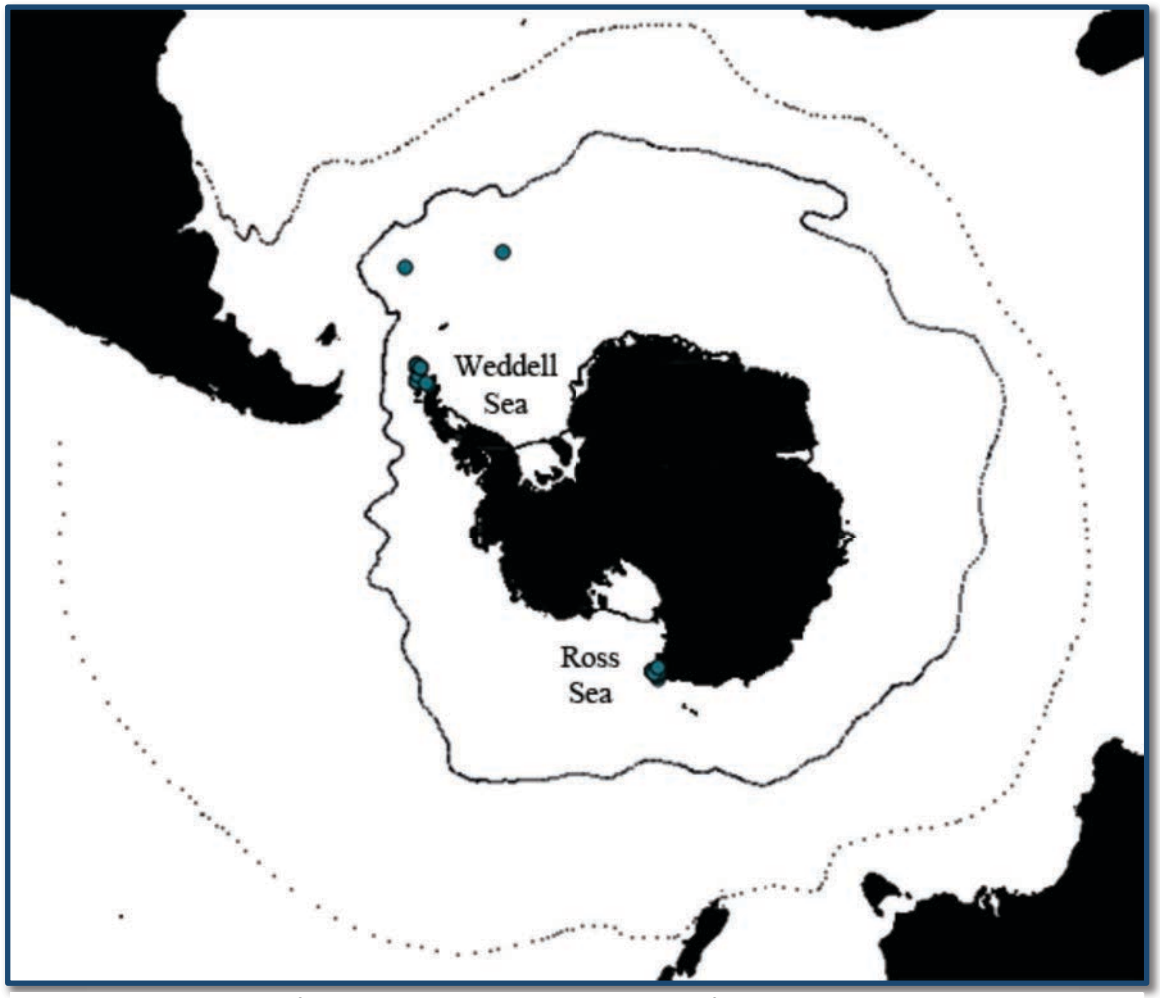
Table 6.1.- Colonies of *Thouarella variabilis* studied.

6.3.1.3 Storage material

The material here studied has been deposited at the National Institute of Water & Atmospheric Research, Wellington, New Zealand (NIWA) and in the octocoral reference collection of the research group "Biodiversidad y Ecología de Invertebrados Marinos" of the University of Seville, Spain (BEIM-CRO).

6.3.2 Study area

The material studied has been collected on the Southern Ocean, along South Shetland Islands, Elephant Island and Bransfield Strait (Peninsula Antarctica) in the west Antarctica and Cape Adare (Eastern Ross Sea) in the east Antarctica, from 94 to 736 m depth (Map 6.1).



Map 6.1.- Distribution map of *Thouarella variabilis* specimens analysed for reproductive studies.

It has not been possible study the annual reproduction cycle of *Thouarella variabilis*, due to the access limitations to the Antarctic bases. Because of that the opportunity to participate in several Antarctic expeditions on board oceanographic research vessels was taken. The problem of doing research in vessels is the access to the Antarctic waters as it is limited from mid-November to April, when the sea ice around Antarctica is the lowest. Moreover, in a research vessel there are several projects going on, a tight schedule, and usually no revisits are

made at the same stations. The lack of continuity in sampling and great difficulties in performing in situ experiments, and the restriction to use the traditional sampling techniques not allow the possibility of pick just a small piece of the gorgonian, measure it or even tag it (depths from 100 m to 800 m). Because all of that the studied specimens cover three seasons from different locations. These limitations make difficult to compare the results with those made in other areas more accessible and in shallow waters where a continuous sampling can be made, however the study let us obtain valuable information and an important approach about the reproductive patterns in the Antarctic benthos.

6.3.3 Laboratory methods

6.3.3.1 Gonad number and size

Number and size of the sexual products (oocytes and spermatic cysts) and larvae, randomly selected in each colony, were studied following the methodology described for octocorals by several authors (Brazeau & Lasker 1990; Coma *et al.* 1995; Brito *et al.* 1997; Orejas *et al.* 2007).

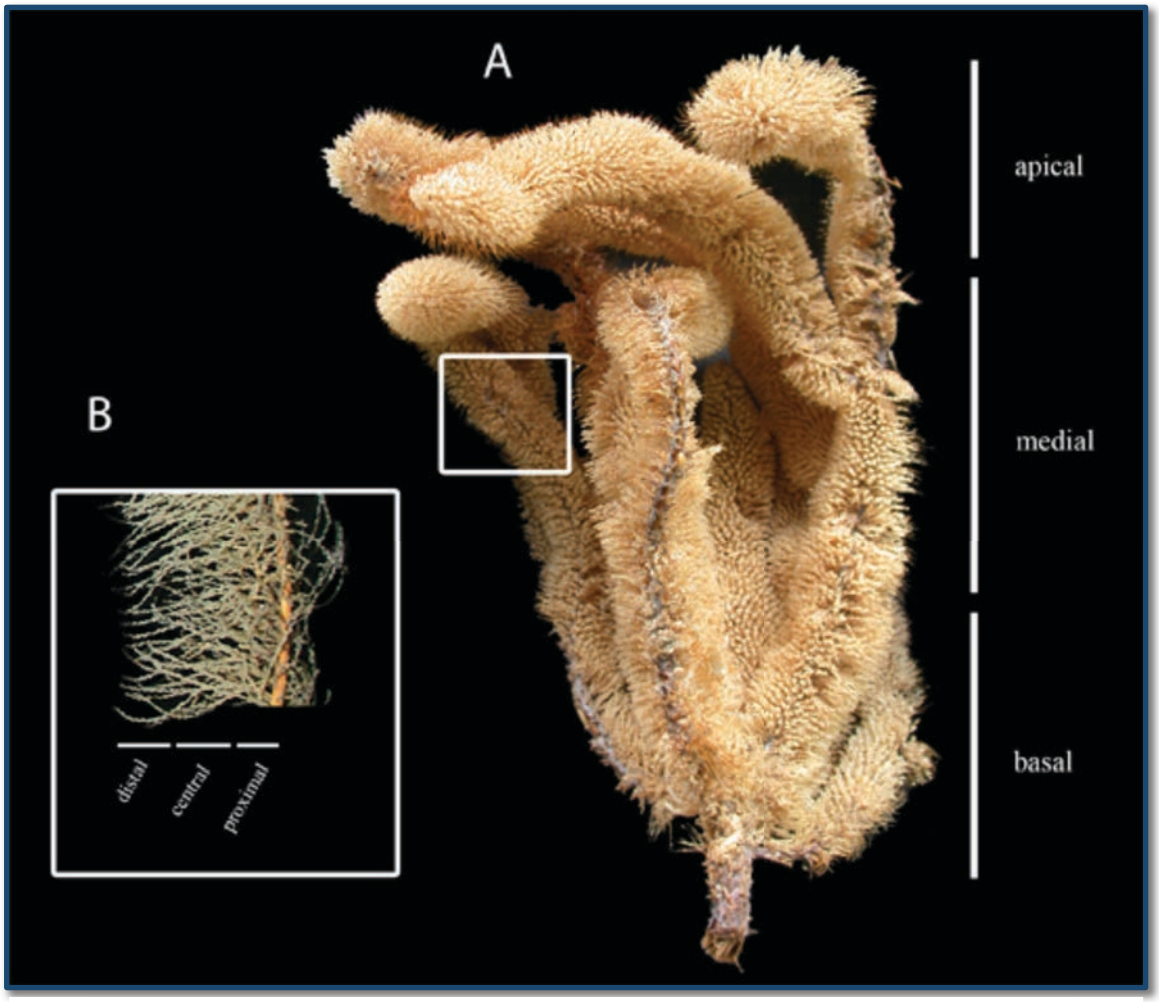


Figure 6.1.- Illustration of the divisions made in the colony. **A**, colony divided in apical, medial and basal zones; **B**, branchlets divided in distal, central and proximal zones.

Each colony was divided in 3 colony zones (basal, medial and apical) and at the same time in 3 branch zones (proximal, central and distal) (Fig. 6.1). From each branch zone 5 polyps were dissected, resulting in 15 polyps from each colony zone and in a total of 45 polyps dissected from each colony. A total of 1935 polyps were examined (945 from males and 990 from female colonies).

The dissection of specimens was done under the stereomicroscope MOTIC SMZ-140 series; sexual products and larvae were immersed in lactic acid and measured with a light microscope using an eyepiece calibrated with a micrometre scale.

6.3.3.2 Histology

For histological studies fragments of branchlets, (of 1 cm) bearing polyps on it, were decalcified in formic acid 10%, then washed in water and dehydrated in xylol in a vacuum pump, finally the material was embedded in paraffin wax. Histological sections of 6-8 μm thick were made using an R Jung AG Heidelberg microtome, slices were mounted and stained with Ramón y Cajal's Triple Stain (Gabe 1968) (Fig. 6.2). Histological preparations were used to confirm and identify the developmental stages of the sexual products.

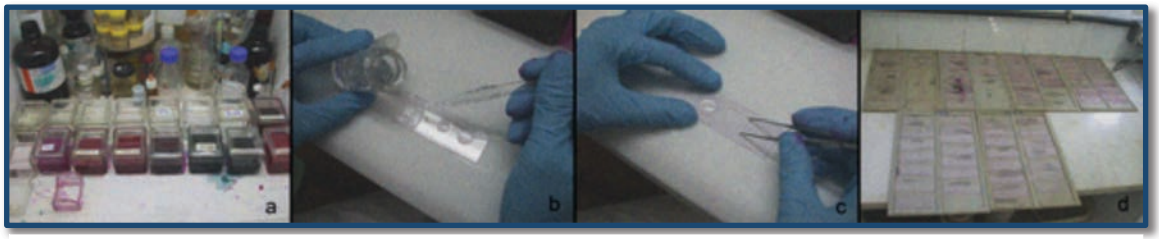


Figure 6.2.- Staining process of histological sections. **a,** staining jars and racks of Ramón y Cajal's Triple Stain method; **b** and **c,** careful process of covering stained slides; **d**, drying up of the final slides.

6.3.3.3 Scanning Electron Microscope

Selected sexual products were washed in water, dehydrated in an alcoholic series from 70 to 100%, and subsequently critical-point-dried with hexamethyldisilazane, were mounted on stubs by a carbon-impregnated film, coated with gold-palladium target in a Edwards Scancoat six SEM sputter coater, and finally observed with a Philips XL30 scanning electron microscope to confirm and identify together with histology the developmental stages of the sexual products.

6.3.3.4 Study of dehydration effect

To estimate de reduction suffered by the sexual products after dehydration processes we used polyps of two colonies (one male, and one female) and we compare their size ~~of~~ in hydrated and dehydrated conditions. We measured 18 male and 25 female sexual products hydrated and after dehydration, the same sexual products were measured again. Dehydration process consisted in an alcoholic series from 70 to 100% with a last xylol step of 1 hour each.

6.3.3.5 Gonadal volume

To estimate the percentage of gonadal volume per polyp, diameters (d) of the sexual products of 15 male polyps and 15 female polyps, were previously transformed into volume by the formula $V = 4/3\pi(d/2)^3$. The volume of the gastrovascular cavity of the polyps was estimated by the frustum volume formula $V = 1/3\pi h(R^2+r^2+R.r)$, where h is the height, R the major and r de minor radius. To calculate the height we use the formula:

$h = \sqrt{(g^2-(R-r)^2)}$

where g is the generatrix of the frustum.

6.3.3 Data analysis

The analyses of sexual products have been done separately for males and females. To evaluate individual effects and interactions of the factor season, colony zone and branch zone, and their influence on the variability in number and size of sexual products, we applied a one-way ANOVA. In case of significant differences, a Bunferroni/Dunn post hoc analysis of means was performed. For statistical analyses, we used the data analysis software system STATISTICA version 6 (StatSoft, Inc. 2001).

6.4 Results

6.4.1. Gonad number frequency distribution

6.4.1.1. Female polyps

Of the total amount of female polyps dissected (990), around 60% (587) were on a reproductive stage, a quarter per cent of these last polyps contained larvae, and around 98% contained at least one oocyte (half of the polyps contained 2 oocytes and one third only one oocyte). A total number of 1048 oocytes and 147 larvae were counted. The average number of oocytes per polyp (in polyps with oocytes) was 1.82 ± 0.74 (Table 6.2). The number of larvae per polyp (in polyps with larvae) was always 1.

There is not a clear season with more reproductive female polyps than other, although spring has a high percentage, and larvae appear to be more abundant in summer. The colony zone with less percentage of reproductive polyps is the basal zone while the medial and apical zone present a very similar percentage (~60%) of reproductive polyps. The medial colony zone has more polyps with larvae than basal and apical zones. The distal part of the branches are almost lack of polyps on reproduction (less than 10%), the most reproductive zone appears to be the proximal branch zone (more than 90%). The majority of larvae were found at proximal and central branch zone, and almost no larvae were found at the distal part (Table 6.3). The half per cent of times that larva appears inside the cavity of a polyp goes together with one oocyte and the 35% of the times with 2 oocytes.

The generalized linear model (GLM) showed statistically significant differences ($p < 0.05$) in oocyte number for the branch zone (proximal, central and distal) and the interaction between season, colony zone and branch zone (Table 6.4).

		Female polyps			
		Oocytes			Larvae
		N	$\bar{x}$	±SE	N
Season	Spring	291	1,72	0,64	56
	Summer	236	1,90	0,82	81
	Autumn	49	2,02	0,83	10
Colony	Basal	160	1,78	0,73	31
	Medial	211	1,82	0,82	78
	Apical	205	1,85	0,67	38
Branch	Proximal	307	1,90	0,75	81
	Central	247	1,77	0,73	60
	Distal	22	1,36	0,58	6
Total		**576**	**1,82**	**0,74**	**147**

Table 6.2.- Descriptives of reproductive female polyps. **N**, Number of observed female reproductive polyps, containing oocytes and/or larvae; $\bar{x}$, average number of oocytes per polyp.

nº oocytes per polyp	Season			Colony			Branch		
	Sp	S	A	B	M	A	P	C	D
0	35.33	47.56	45.56	51.52	36.06	37.88	6.97	25.15	93.33
1	23.78	17.11	15.56	17.58	24.55	17.88	26.36	29.09	4.55
2	36.22	26.22	24.44	25.45	29.39	36.97	53.94	36.06	1.82
3	3.56	7.11	12.22	4.24	7.58	6.06	9.70	7.88	0.30
4	1.11	1.33	2.22	0.91	1.82	1.21	2.12	1.82	0
5	0	0.67	0	0.30	0.61	0	0.91	0	0
Total	100	100	100	100	100	100	100	100	100
Larvae	12.44	18.00	11.11	9.39	23.64	11.52	24.55	18.18	1.82

Table 6.3.- Percentage of female polyps with a determined number of oocytes and larvae based on season, colony and branch zone. **Sp**, spring, **S**, summer; **A**, autumn; **B**, basal; **M**, medial; **A**, apical; **P**, proximal; **C**, central; **D**, distal.

	SS	Degr. of Freedom	MS	F	p
Intercept	13,55714	1	13,55714	88,56970	0,000000
SEA	0,09210	2	0,04605	0,30086	0,740306
COL_ZONE	0,47720	2	0,23860	1,55880	0,211316
BR_ZONE	1,56025	2	0,78012	5,09660	0,006409
SEA*COL_ZONE	0,59649	4	0,14912	0,97422	0,421068
SEA*BR_ZONE	1,07887	4	0,26972	1,76209	0,135028
COL_ZONE*BR_ZONE	0,49786	4	0,12447	0,81314	0,517073
SEA*COL_ZONE*BR_ZONE	2,04035	6	0,34006	2,22162	0,039691
Error	84,34017	551	0,15307		

Table 6.4.- GLM comparing numbers of oocytes for the factors season (SEA), colony zone (COL_ZONE), and branch zone (BR_ZONE).

6.4.1.2. Male polyps

Of the total amount of male polyps dissected (945), around 69% (648) were on a reproductive stage, containing at least one spermatic cyst; more than a half (62%) of these polyps contained 2 spermatic cysts. A total number of 1388 spermatic cysts were counted. The average number of spermatic cyst per polyp (in reproduction) was 2.06 ± 0.8 (Table 6.5).

		Male polyps		
		Spermatic cysts		
		N	$\bar{x}$	$\pm SE$
Season	Spring	312	2,04	0,70
	Summer	284	2,07	0,84
	Autumn	52	2,19	1,07
Colony	Basal	185	1,98	0,88
	Medial	234	2,06	0,74
	Apical	229	2,14	0,78
Branch	Proximal	275	2,07	0,78
	Central	242	2,20	0,86
	Distal	131	1,80	0,65
Total		**648**	**2,06**	**0,80**

Table 6.5.- Descriptives of reproductive male polyps. **N**, Number of observed male reproductive polyps, containing cysts; $\bar{x}$, average number of cysts per polyp.

The season with more reproductive male polyps is spring, where more than 75% of the polyps present spermatic cysts. The medial colony zone slightly has more reproductive polyps however it is the apical zone, which has more spermatic cysts in total number (more cysts/polyp). Distal parts of the branches show clearly less reproduction events, only 42% of its polyps are on reproduction. The most reproductive branch zone appears to be the proximal with almost 90% of polyps dissected on reproduction (Table 6.6).

The generalized linear model (GLM) showed statistically significant differences ($p < 0.05$) in cyst number for the colony zone (between basal and apical), the branch zone (distal part) and the interaction of both factors (Table 6.7).

nº cysts per polyp	Season			Colony			Branch		
	Sp	S	A	B	M	A	P	C	D
0	22.96	36.89	42.22	41.27	25.71	27.30	12.70	23.17	58.41
1	13.33	13.33	15.56	16.51	13.33	10.79	16.19	12.06	12.38
2	50.37	39.11	26.67	32.70	48.25	47.30	55.56	46.35	26.35
3	10.62	4.00	5.56	4.76	7.94	8.25	8.89	10.48	1.59
4	2.47	6.44	8.89	4.13	4.76	6.03	6.35	7.30	1.27
5	0	0.22	1.11	0.32	0	0.32	0.32	0.32	0
6	0.25	0	0	0.32	0	0	0	0.32	0
TOTAL	100	100	100	100	100	100	100	100	100

Table 6.6.- Percentage of male polyps with a determined number of cysts based on season, colony and branch zone. **Sp**, spring, **S**, summer; **A**, autumn; **B**, basal; **M**, medial; **A**, apical; **P**, proximal; **C**, central; **D**, distal.

	SS	Degr. of Freedom	MS	F	p
Intercept	119,0546	1	119,0546	833,9529	0,000000
SEA	0,2840	2	0,1420	0,9946	0,370457
COL_ZONE	1,2108	2	0,6054	4,2407	0,014817
BR_ZONE	1,7318	2	0,8659	6,0654	0,002462
SEA*COL_ZONE	0,4032	4	0,1008	0,7062	0,587905
SEA*BR_ZONE	0,3883	4	0,0971	0,6800	0,605977
COL_ZONE*BR_ZONE	1,3950	4	0,3488	2,4429	0,045583
SEA*COL_ZONE*BR_ZONE	1,6985	8	0,2123	1,4872	0,158398
Error	88,6535	621	0,1428		

Table 6.7.- GLM comparing numbers of sperm cysts for the factors season (SEA), colony zone (COL_ZONE), and branch zone (BR_ZONE).

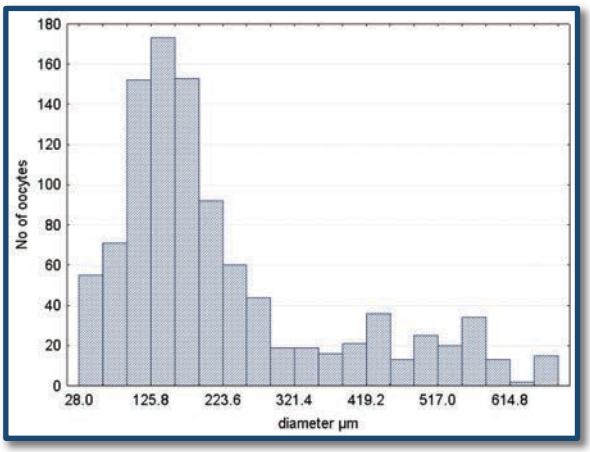
6.4.2. Gonad size frequency distribution

6.4.2.1. Female polyps

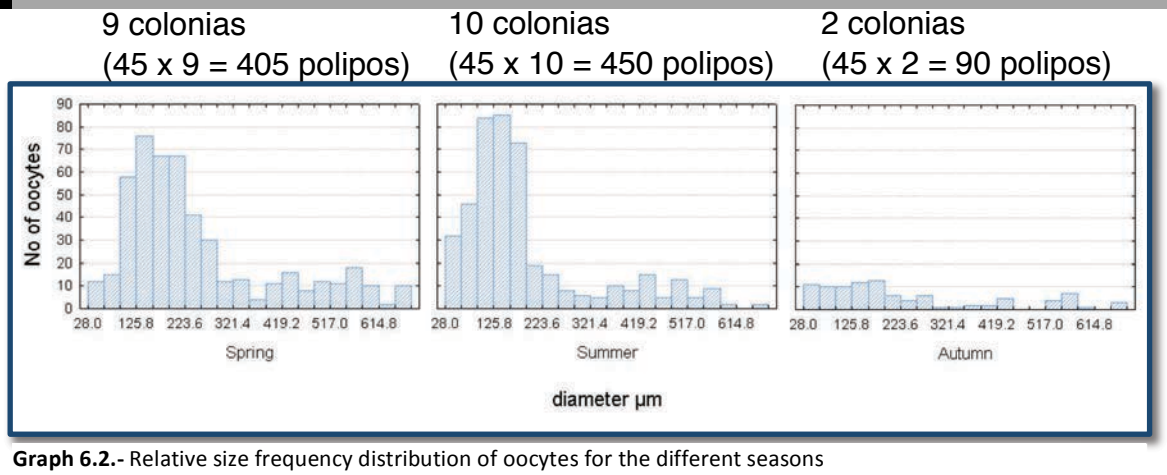
		Female polyps					
		Oocytes			Larvae		
		N	$\bar{x}$	±SE	N	$\bar{x}$	±SE
Season	Spring	493	249,37	152,15	80	851,69	116,46
	Summer	442	192,63	135,77	53	764,15	118,47
	Autumn	98	238,02	179,24	10	812,00	64,60
Colony	Basal	277	215,20	152,44	31	826,61	131,31
	Medial	383	230,45	151,91	76	818,68	126,44
	Apical	373	223,96	147,94	36	803,06	100,99
Branch	Proximal	563	232,38	156,20	78	837,95	123,91
	Central	433	216,58	144,63	58	786,29	107,86
	Distal	37	183,81	121,95	7	827,14	157,24
Total		**1033**	**224,02**	**150,60**	**143**	**816,47**	**121,13**

Table 6.10.- Descriptives of reproductive female polyps. **N**, Number of measured oocytes and larvae; $\bar{x}$, average size of oocytes and larvae.

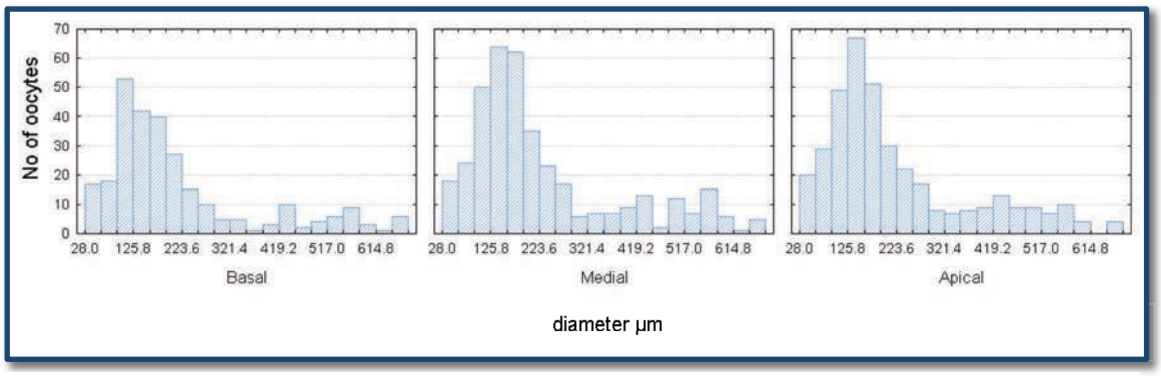
The mean (±SE) size of oocytes was 224.02 ± 150.6 μm (Table 6.10). The largest oocyte measured about 680 μm and the smallest 28 μm. Frequency distribution of oocyte size (Grf. 6.1) showed a bimodal type graph with a peak at approximately 142 μm and a second one at 436 μm, indicating two overlapping cohorts of oocyte. The relative oocyte size frequency distribution has been also represented separately (Gr. 6.2-4) in order to emphasize statistically significant differences due to seasons, colony zones, or branch zones. The oocyte size distribution by seasons (Gr. 6.2) seems to follow the same bimodal pattern for the three seasons analysed, and also for the different colony zones (Gr. 6.3), however in the distal branch oocytes are mainly restricted to small sizes (Gr. 6.4). Statistically significance differences ($p < 0.05$) were found in oocyte size among the different branch zones (Table 6.11).



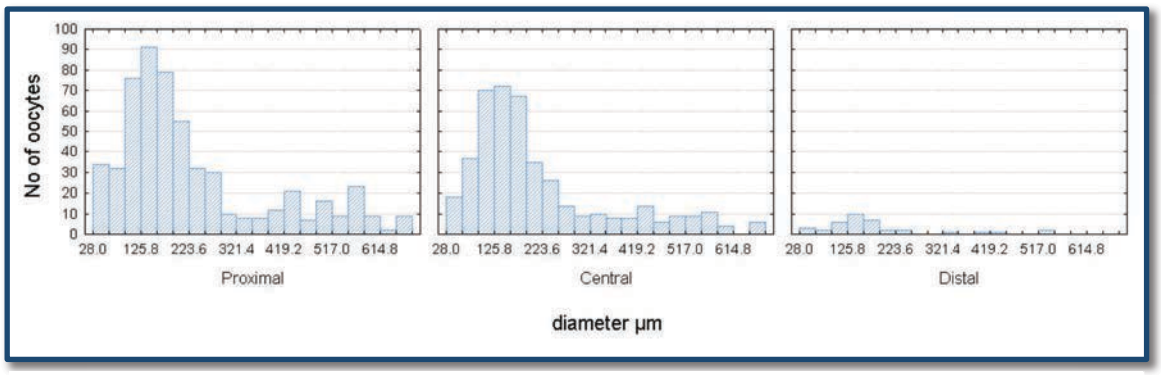
Graph 6.1.- Relative size frequency distribution of oocytes.



Graph 6.2.- Relative size frequency distribution of oocytes for the different seasons

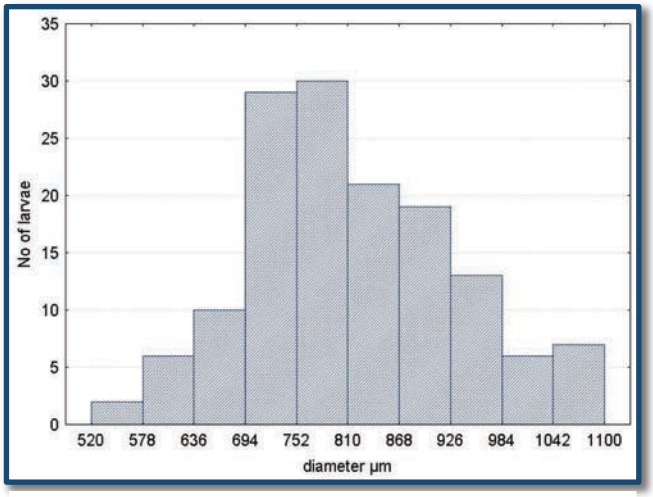


Graph 6.3.- Relative size frequency distribution of oocytes for the three colony zones.

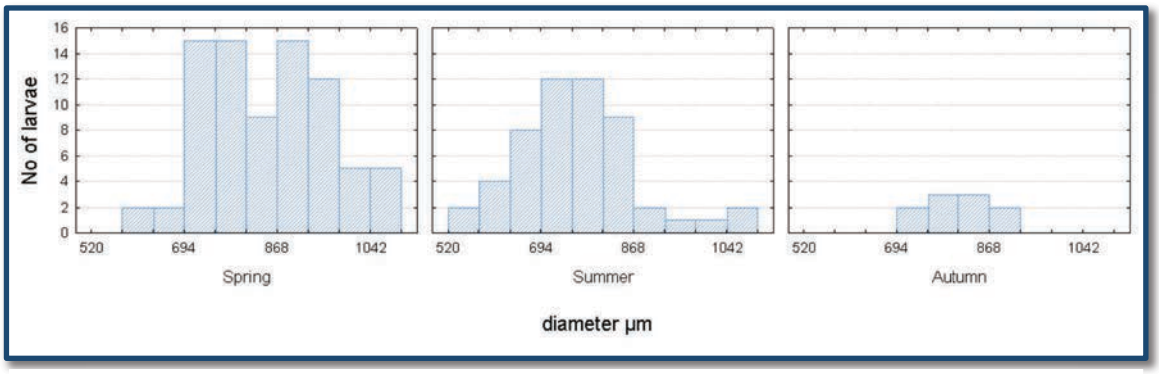


Graph 6.4.- Relative size frequency distribution for the three branch zones.

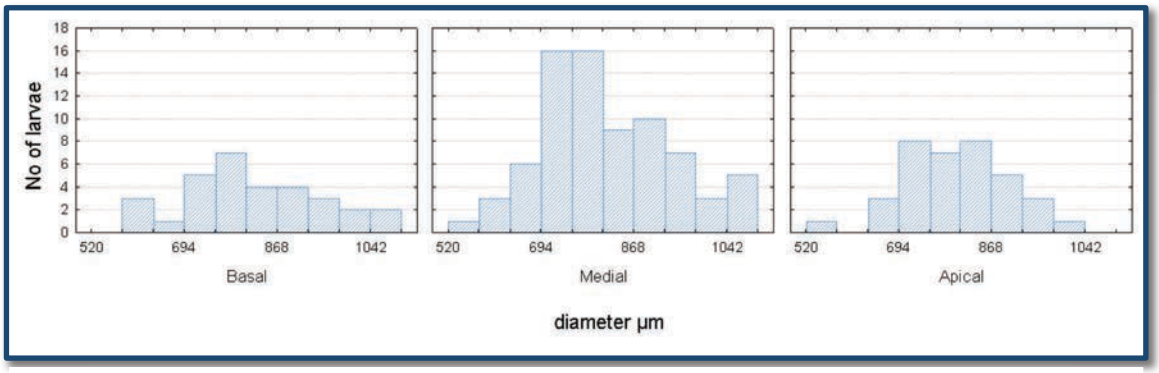
The mean (±SE) size of larvae was 816.46 ± 121.12 µm (Table 6.10). The largest larva measured about 1100 µm in maximum diameter and the smallest one 520 µm. Frequency distribution of larva size (Grf. 6.2) showed a clear peak at approximately 796 µm. The relative larvae size frequency distribution has been also represented separately (Gr. 6.6-8) in order to emphasize statistically significant differences due to seasons, colony zones, or branch zones. The larva size distribution by seasons (Gr. 6.6) seems to follow the same unimodal pattern for the three seasons analysed, and also for the different colony zones (Gr. 6.7), however in the distal branch zone due to the few larvae analysed a clear pattern is not observed (Gr. 6.8). Statistically significance differences ($p < 0.05$) were found in larva size among the interaction between season and branch zones factors (Table 6.12).



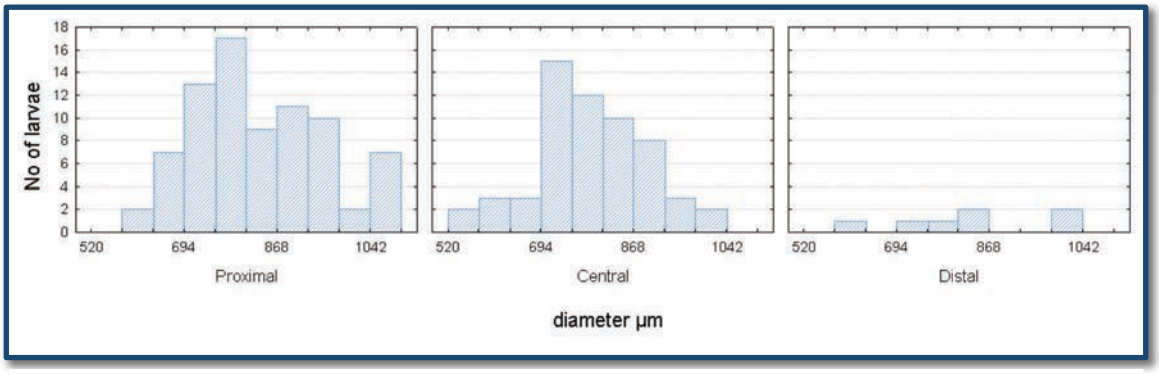
Graph 6.5.- Relative size frequency distribution of larvae.



Graph 6.6.- Relative size frequency distribution of larvae for the different seasons.



Graph 6.7.- Relative size frequency distribution of larvae for the three colony zones.



Graph 6.8.- Relative size frequency distribution of larvae for the three branch zones.

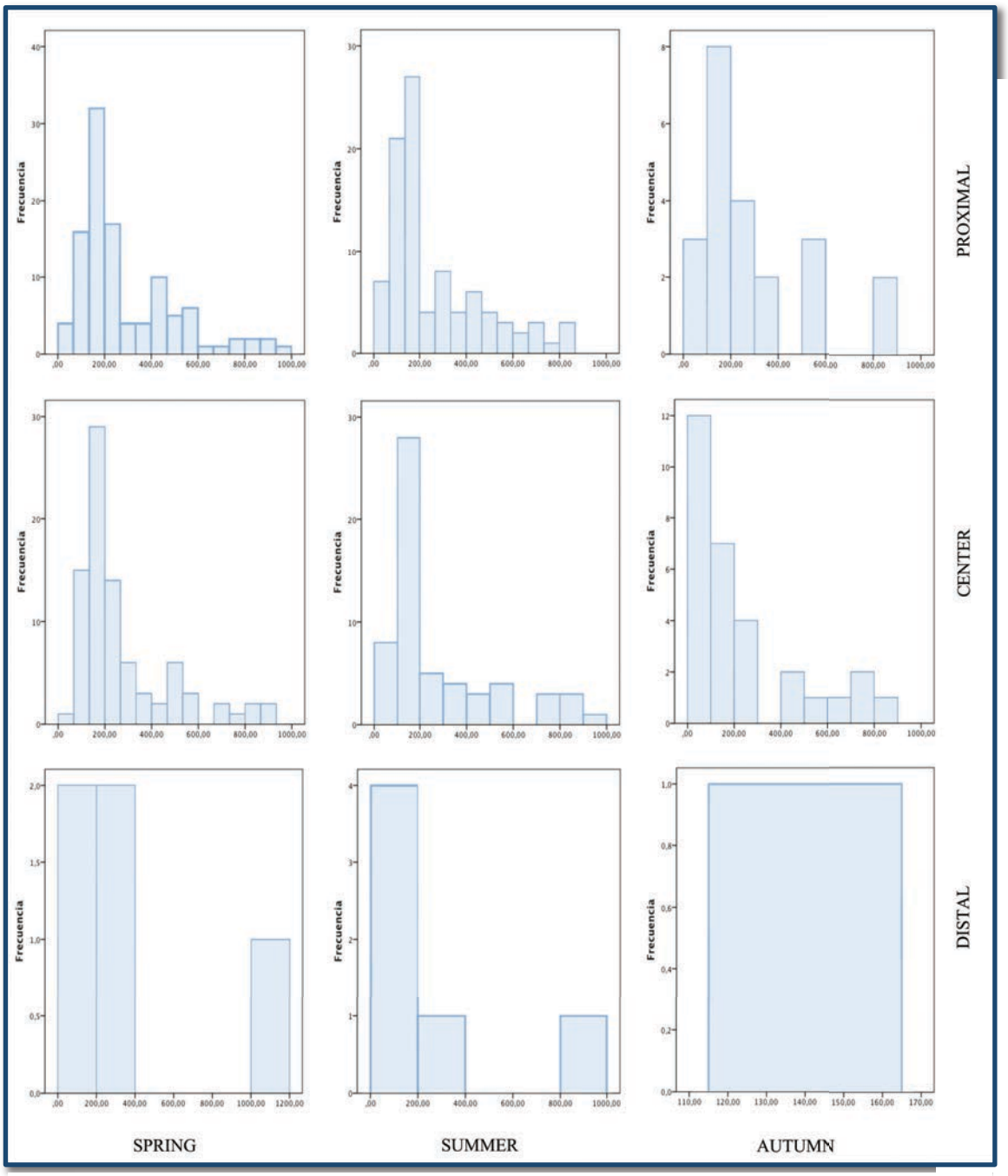
	SS	Degr. of Freedom	MS	F	p
Intercept	-	0	-	-	-
SEA	-	0	-	-	-
COL_ZONE	0,1831	1	0,183109	0,472362	0,492061
BR_ZONE	1,9416	1	1,941616	5,008751	0,025437
SEA*COL_ZONE	0,1714	2	0,085704	0,221090	0,801684
SEA*BR_ZONE	0,9107	2	0,455336	1,174621	0,309358
COL_ZONE*BR_ZONE	0,8465	3	0,282155	0,727871	0,535437
SEA*COL_ZONE*BR_ZONE	1,8247	6	0,304118	0,784529	0,582098
Error	390,7459	1008	0,387645	-	-

Table 6.11.- GML comparing the oocytes size for the factors season (SEA), colony zone (COL_ZONE), and branch zone (BR_ZONE).

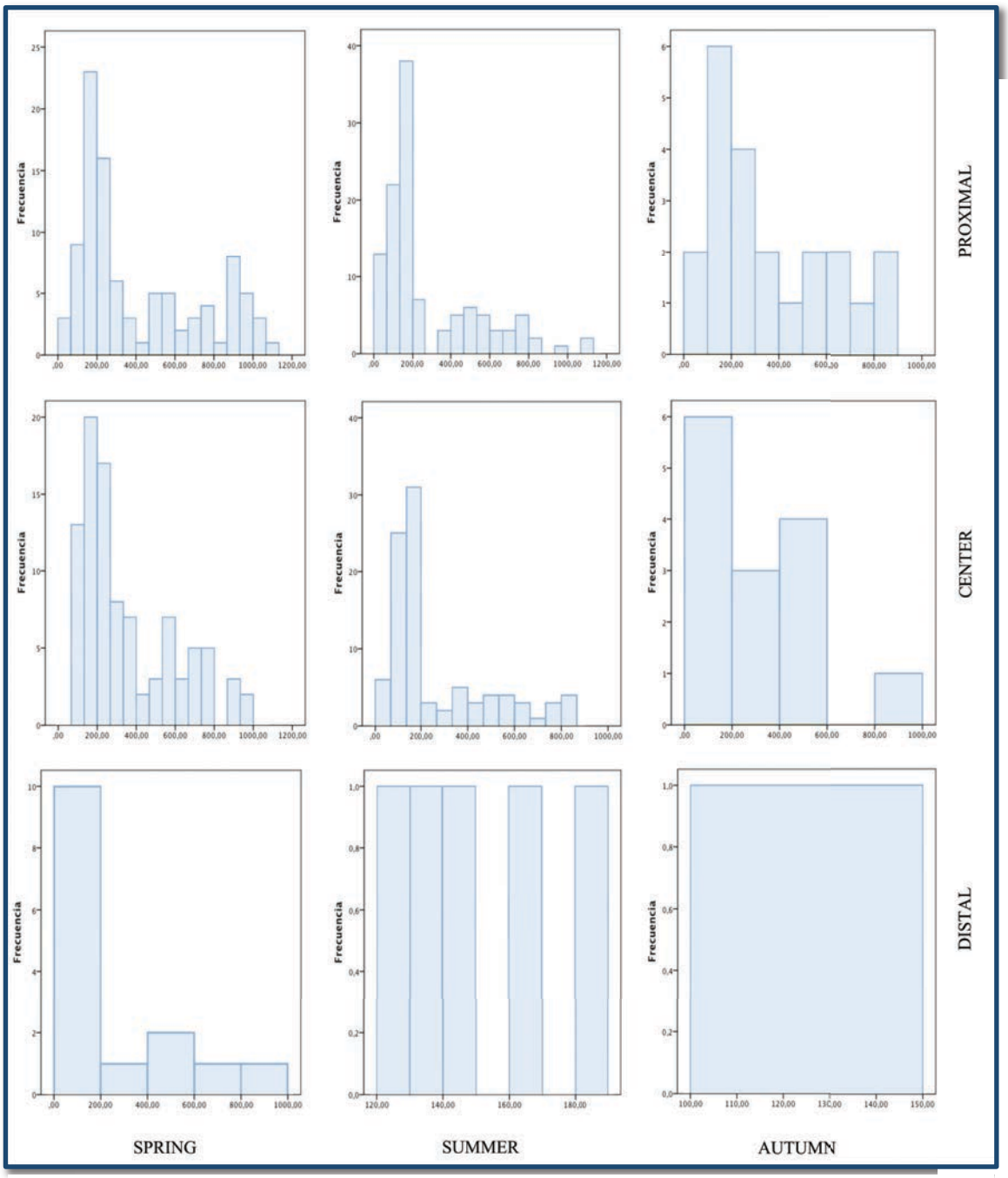
	SS	Degr. of Freedom	MS	F	p
Intercept	-	0	-	-	-
SEA	-	0	-	-	-
COL_ZONE	-	0	-	-	-
BR_ZONE	-	0	-	-	-
SEA*COL_ZONE	-	0	-	-	-
SEA*BR_ZONE	0,112724	1	0,112724	6,099089	0,014908
COL_ZONE*BR_ZONE	0,000024	1	0,000024	0,001293	0,971374
SEA*COL_ZONE*BR_ZONE	0,124884	3	0,041628	2,252330	0,085678
Error	2,254826	122	0,018482	-	-

Table 6.12.- GML comparing the larvae size for the factors season (SEA), colony zone (COL_ZONE), and branch zone (BR_ZONE).

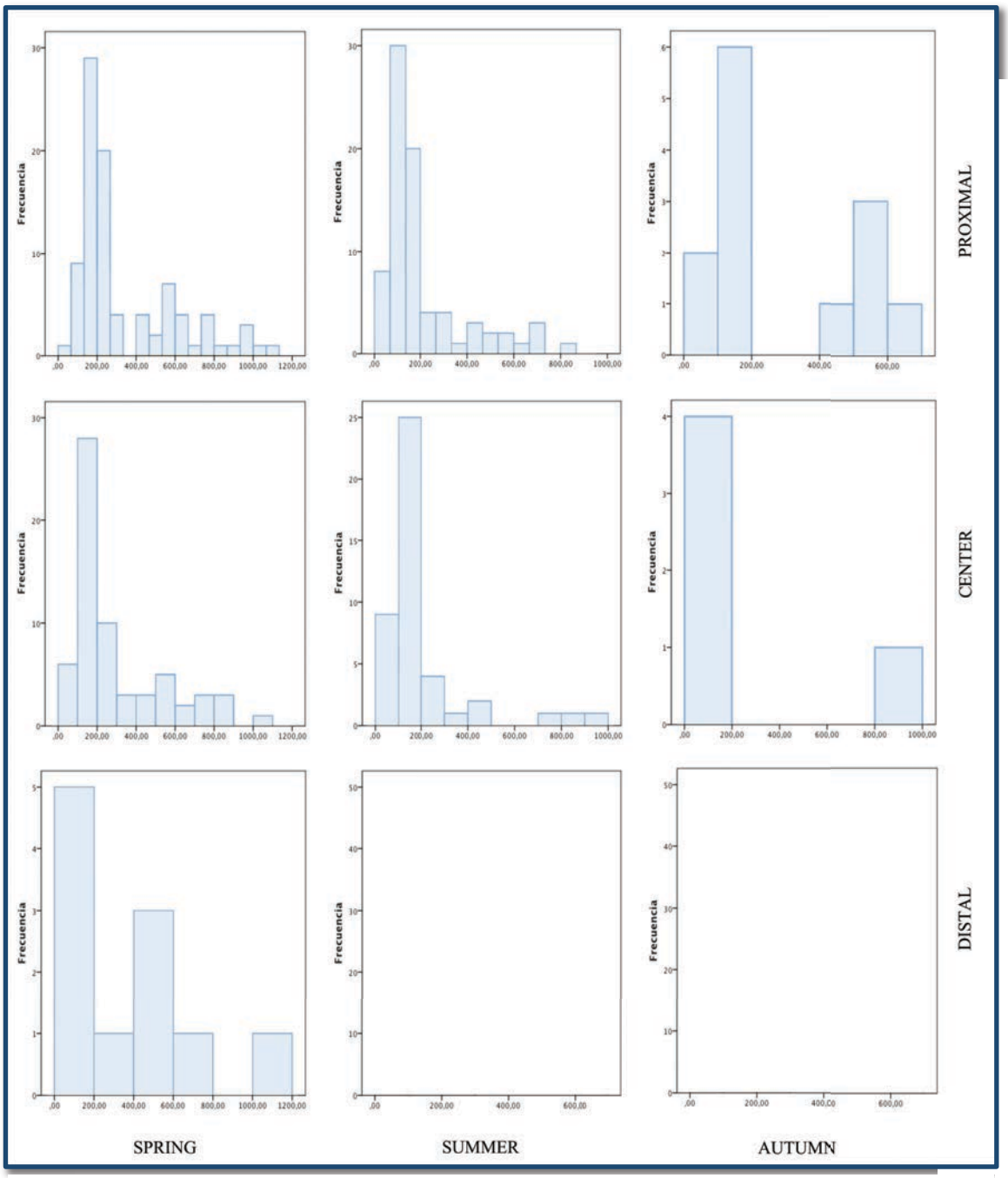
In order to observe de differences between the factors (colony zone and branch zone) and each season the relative size frequency of female reproductive products is represented in graphics 6.9-11. The results show patterns very similar among each season; even with the low number of individuals studied for autumn the size distribution pattern seems to follow the same as observed for spring and summer. Except for the distal branch zone in each of the colony zones, all colony seems to follow the same size distribution pattern with two clear peaks, corresponding to immature and mature oocytes and a third peak less differentiated belonging to larval stage.



Graph 6.9.- Relative size frequency distribution of female sexual products for the apical colony zone represented separately for season and branch zone.



Graph 6.10.- Relative size frequency distribution of female sexual products for the medial colony zone represented separately for season and branch zone.



Graph 6.11.- Relative size frequency distribution of female sexual products for the basal colony zone represented separately for season and branch zone.

6.4.2.2. Male polyps

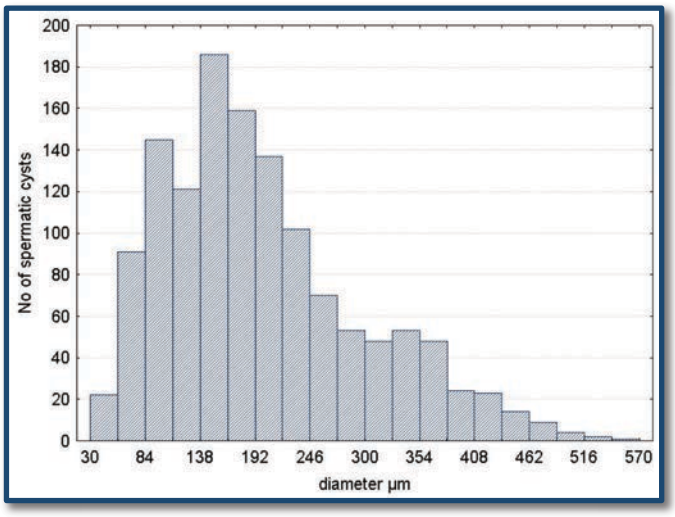
The mean (±SE) size of spermatic cysts was 200.71 ± 99.06 μm (Table 6.13). The largest spermatic cyst measured about 570 μm and the smallest 30 μm. Frequency distribution of spermatic cysts (Gr. 6.12) showed a bimodal type graph with a peak at 152 μm and a second one at approximately at 341 μm, indicating two overlapping cohorts of spermatic cysts.

The relative cyst size frequency distribution has been also represented separately (Gr. 6.13-15) in order to emphasize statistically significant differences due to seasons, colony zones, or

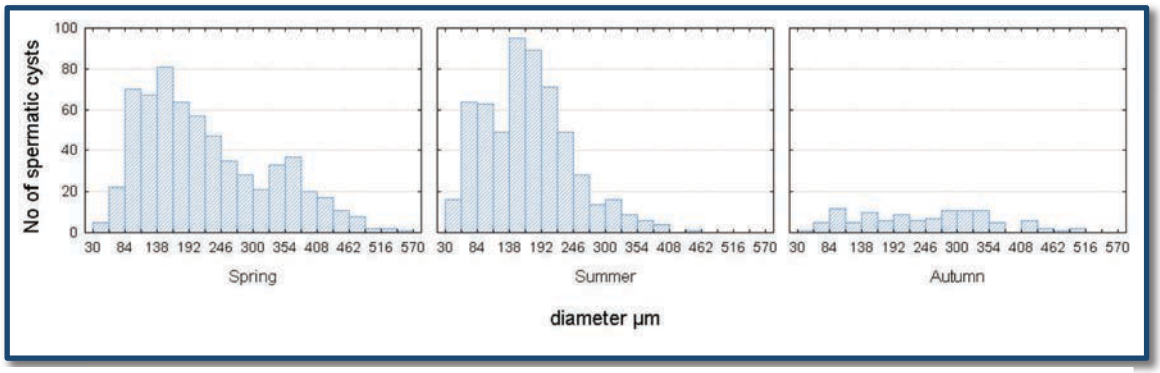
branch zones. The cyst size distribution by seasons (Gr. 6.13) shows a bimodal pattern in spring where the biggest cysts, while in summer the second peak represent smaller cysts, in autumn not a clear pattern is observed, the distribution of cysts in the colony zone clearly follow a similar bimodal distribution (Gr. 6.14), as well as for the branch zones cysts distribution (Gr. 6.15). Statistically significance differences ($p < 0.05$) were found in cyst size among the seasons (spring, summer and autumn), the colony zone (basal part), the branch zone (distal part) and the interaction between the season and colony zone factors (Table 6.14).

		Male polyps		
		Spermatic cysts		
		N	$\bar{x}$	±SE
Season	Spring	628	220,85	108,38
	Summer	574	169,94	73,78
	Autumn	110	246,34	111,46
Colony	Basal	362	183,33	95,12
	Medial	468	210,32	100,81
	Apical	482	204,44	98,79
Branch	Proximal	558	204,46	98,60
	Central	522	204,47	102,99
	Distal	232	183,28	89,23
Total		**1312**	**200,71**	**99,06**

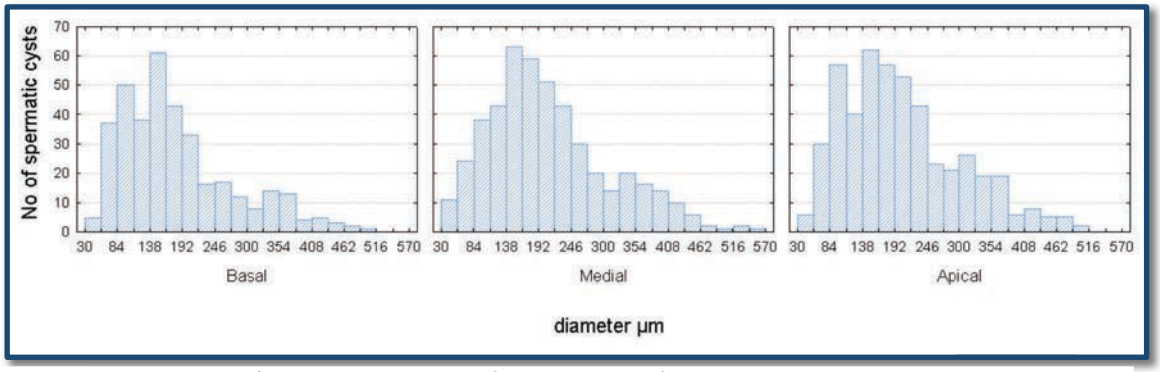
Table 6.13.- Descriptives of reproductive male polyps. N, Number of measured cysts; $\bar{x}$, average cyst size.



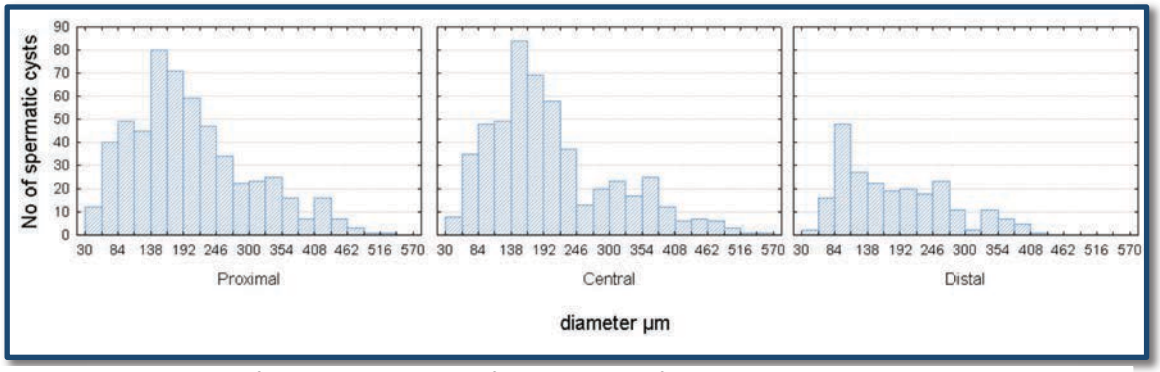
Graph 6.12.- Relative size frequency distribution of male sexual products.



Graph 6.13.- Relative size frequency distribution of spermatic cysts for the different seasons.



Graph 6.14.- Relative size frequency distribution of spermatic cysts for the three colony zones.

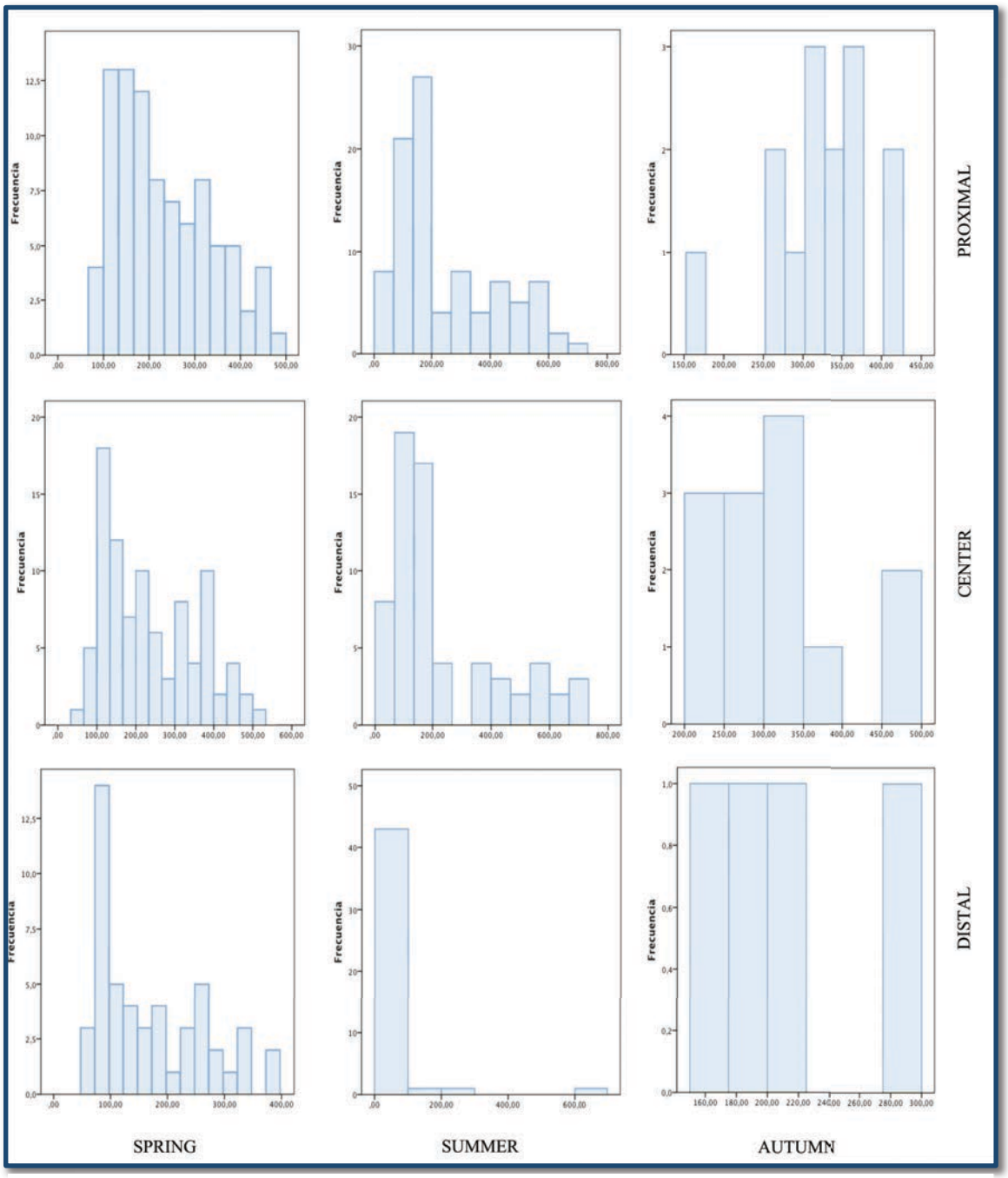


Graph 6.15.- Relative size frequency distribution of spermatic cysts for the three branch zones.

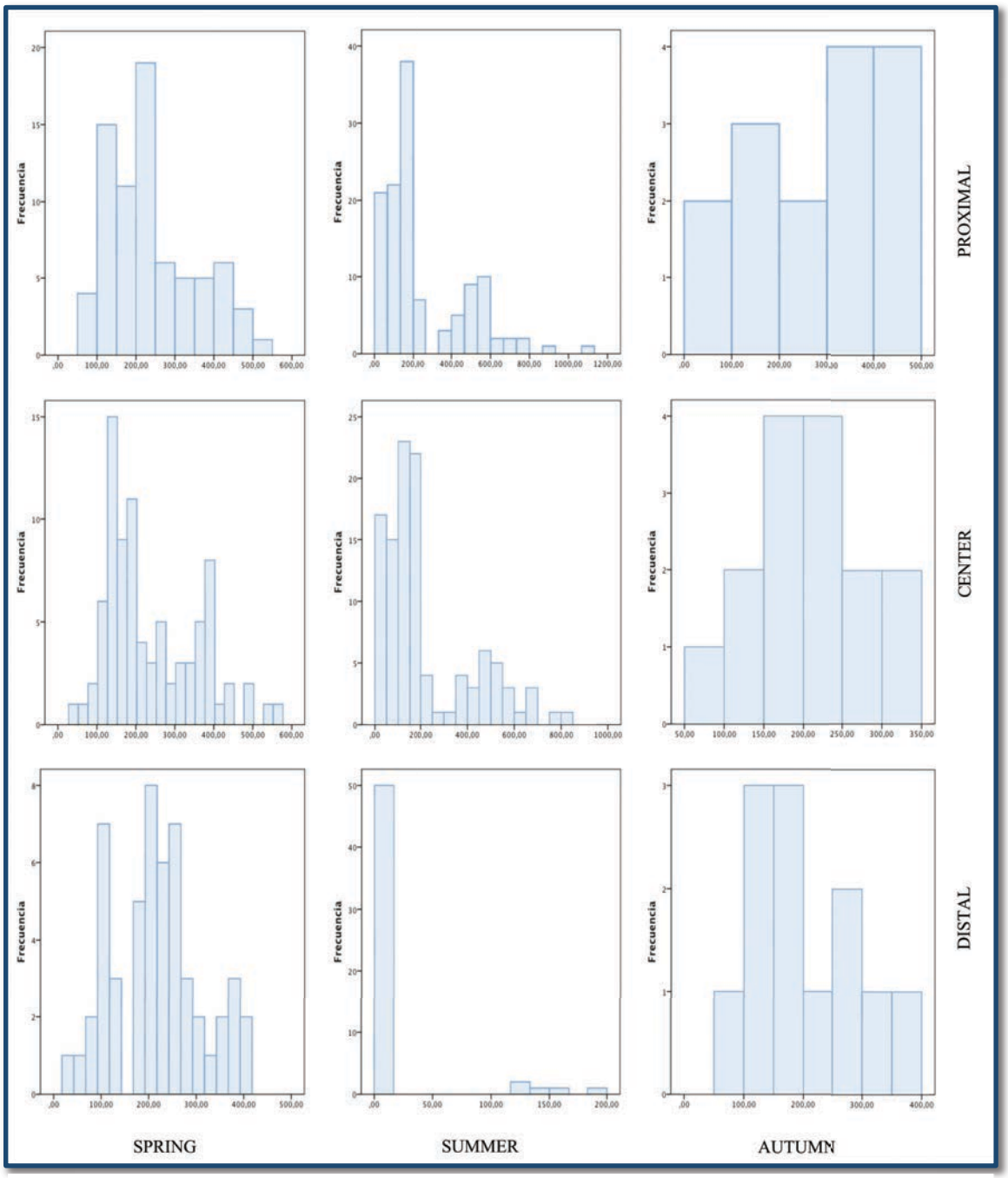
	SS	Degr. of Freedom	MS	F	p
Intercept	15608,58	1	15608,58	66067,72	0,000000
SEA	17,41	2	8,70	36,84	0,000000
COL_ZONE	4,74	2	2,37	10,02	0,000048
BR_ZONE	3,49	2	1,75	7,39	0,000643
SEA*COL_ZONE	3,01	4	0,75	3,19	0,012878
SEA*BR_ZONE	1,13	4	0,28	1,19	0,312806
COL_ZONE*BR_ZONE	1,46	4	0,36	1,54	0,187749
SEA*COL_ZONE*BR_ZONE	1,37	8	0,17	0,72	0,670678
Error	303,58	1285	0,24	-	-

Table 6.14.- GML comparing the spermatic cysts size for the factors season (SEA), colony zone (COL_ZONE), and branch zone (BR_ZONE).

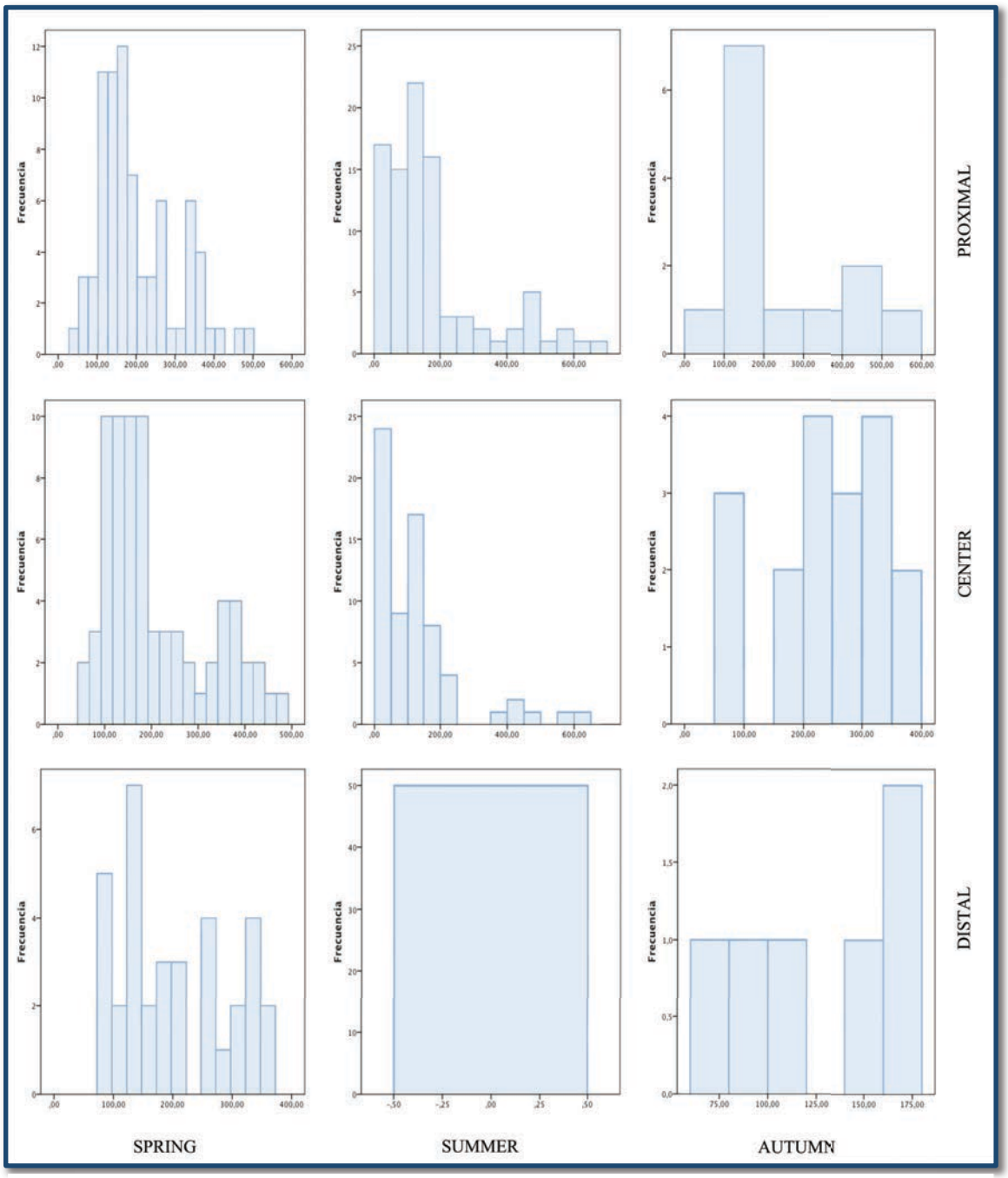
In order to observe de differences between the factors (colony zone and branch zone) and each season the relative size frequency of male reproductive products is represented in graphics 6.16-18. The results show patterns very similar among each season; even with the low number of individuals studied for autumn the size distribution pattern seems to follow the same as observed for spring and summer. Except for the distal branch zone in summer, all colonies seems to follow the same size distribution pattern with two clear peaks, corresponding to immature and mature cysts.



Graph 6.16.- Relative size frequency distribution of male sexual products for the apical colony zone represented separately for season and branch zone.



Graph 6.17.- Relative size frequency distribution of male sexual products for the medial colony zone represented separately for season and branch zone.



Graph 6.18.- Relative size frequency distribution of male sexual products for the basal colony zone represented separately for season and branch zone.

6.4.3. Development of sexual products

The colonies of *Thouarella variabilis* are presumably gonochoric as no female and male sexual products have been observed in the same colony or in the same polyp. All the colonies studied were in a reproductive phase, however not all polyps were breeding. Sexual dimorphism has not been observed for this species, thus to correctly sex the colonies reproductive polyps were dissected to observe their sexual products under a light microscope (Fig. 6.2.). 43 colonies were analysed, 21 males and 22 females.

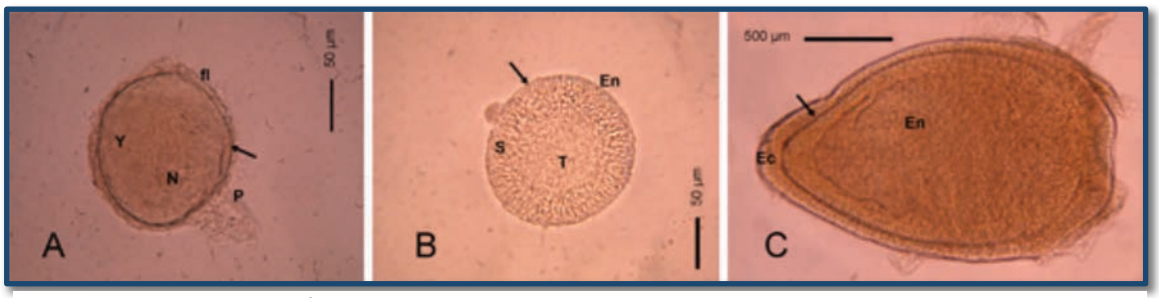


Figure 6.2.- Sexual products of *Thouarella variabilis* observed under light microscopy. **A**, Oocyte, *Y*: yolk; *N*: nucleus; *P*: pedicel; *fl*: follicular layer; **B**, Sperm cyst, *S*: sperm; *T*: tails; *En*: endoderm; **C**, planula larva, *Ec*: Ectoderm, *En*: endoderm. Arrow points out the mesoglea.

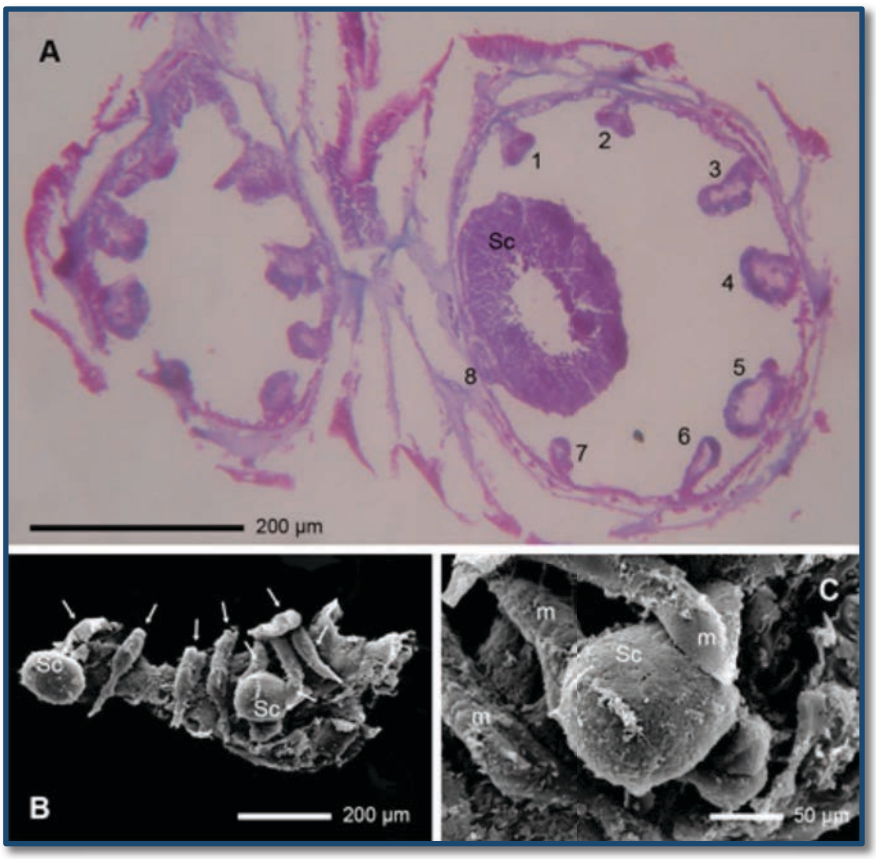


Figure 6.3.- A, Histological section of two male polyps in horizontal plane, the eight mesenteries of one of them are numbered, *Sc*: Sperm cyst; **B**, SEM of the basal portion of male polyp, *Sc*: Sperm cyst, white arrows point out the mesenteries; **C**, SEM of a detail of an spermatic cyst attached to the mesentery, *Sc*: Sperm cyst, *m*: mesentery.

Male polyps usually present only two spermatic cysts attached to the mesenteries at each side of the polyp cavity (Fig. 6.3., 6.4), which are in the most cases in the same developmental stage. The presence of larvae inside female polyp cavities indicates that fertilization is internal and the reproductive mode is brooding. The fecundity is very low as only one mature larvae is

brooded and released per female polyp at a time, however different stages of oocyte development has been observed within the same polyp, even sharing the polyp cavity with the larval stage (Fig. 6.4).

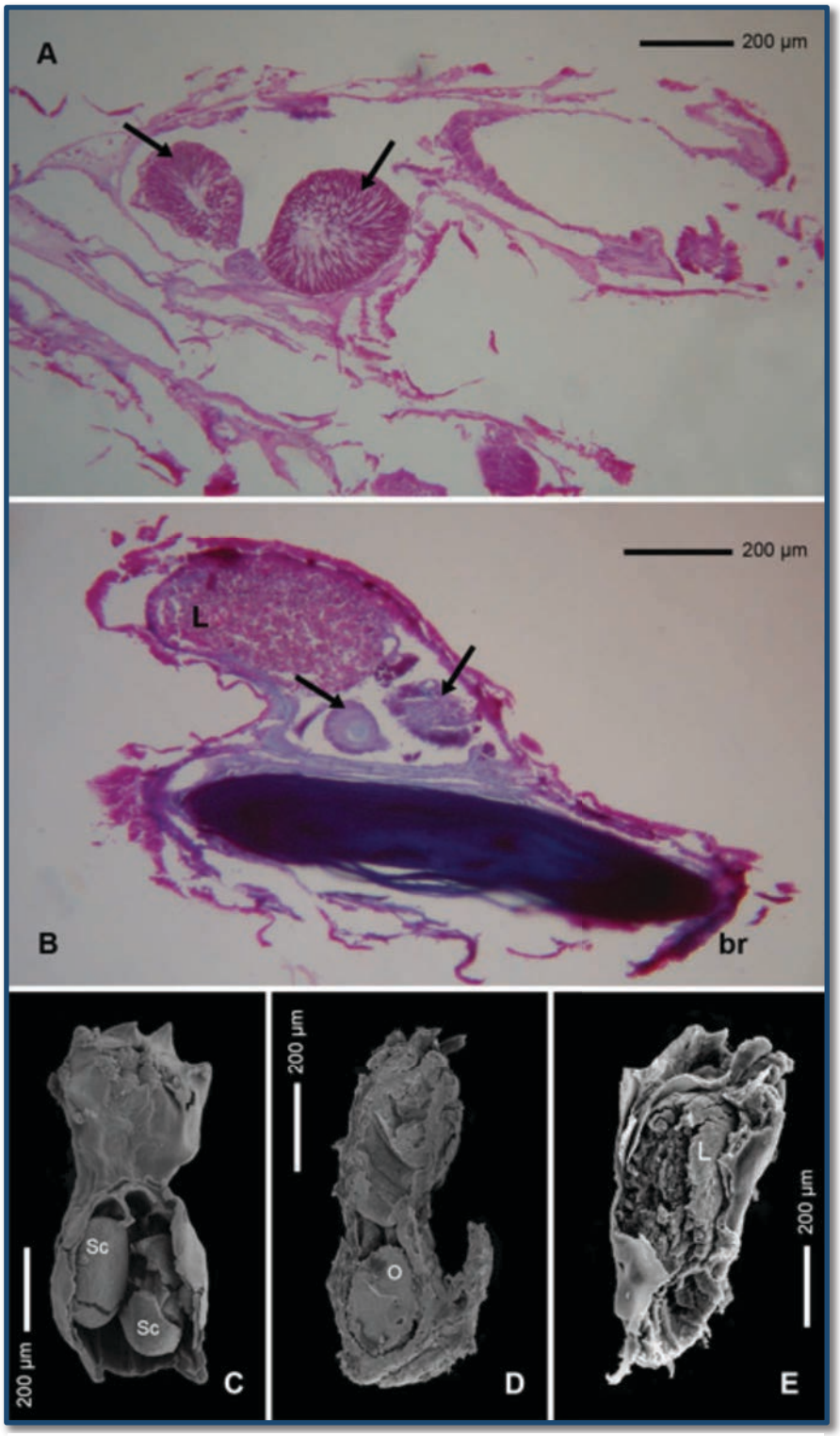


Figure 6.3.- A, Histological section of a male polyp in a sagittal plane, black arrows point out the sexual products (spermatic cysts); **B**, Histological section of a female polyp in a sagittal plane, black arrows point out the sexual products (oocytes), *L*: larva; *br*: branchlet; **C**, SEM of a male polyp in a frontal plane, *Sc*: Sperm cyst; **D**, SEM of a female polyp in a sagittal plane, *O*: oocyte; **E**, SEM of a female polyp in a sagittal plane, *L*: larva.

6.4.3.1. Oogenesis

Oocytes at different stages of development were observed in female polyps, however not all stages were identifiable at light microscope, and only when histological samples were observed the earlier stages were identified.

Oogonia: the earliest oocyte stage is transparent in colour, rounded and with a large and centred nucleus; the size diameter is about 10 µm (fig. 6.6a).

Previtellogenic: oocytes diameter around 50 µm, opaque in colour and without yolk granules and a thin oolemma can be slightly observed (fig. 6.6b)

Vitellogenic: oocytes oval or elongated in shape, diameter ranged between 70 and 250 µm. At this stage nuclei is located to the periphery, some oocytes showed more than one nucleolus (fig. 6.6a, c). Oocytes filled with yolk granules are surrounded by the mesoglea and a wrinkled follicular endoderm.

Mature: round oocyte densely packed with yolk, up to 500 µm in diameter, oocyte is ready to be fertilized.

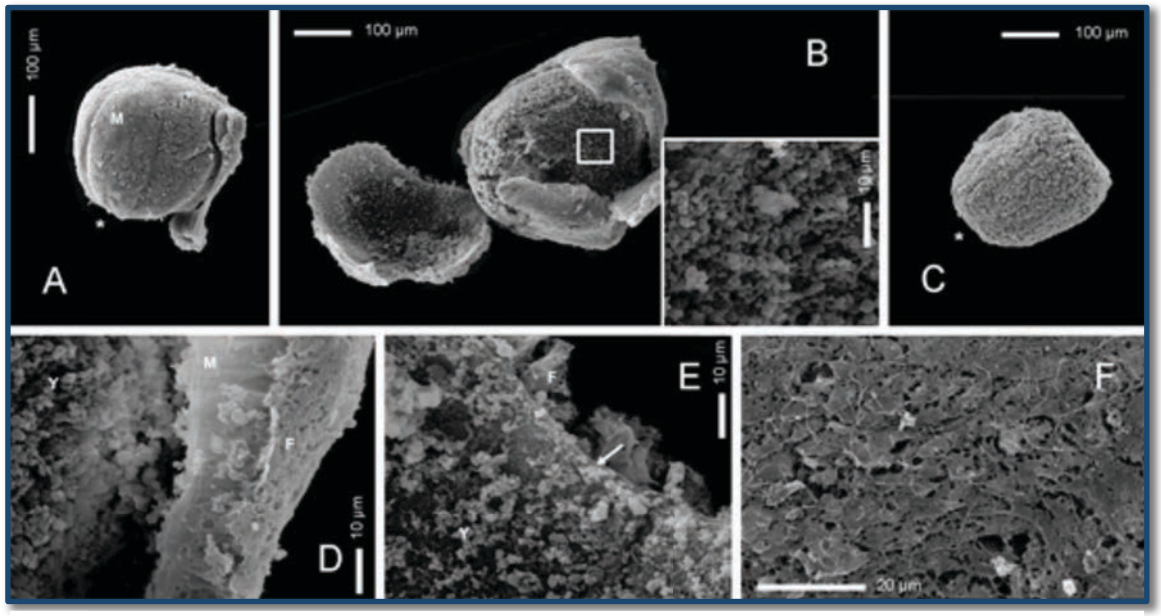


Figure 6.5.- SEM of female sexual products**. A,** vitellogenic oocyte; **B,** fractured oocyte, high magnification of the yolky material; **C,** vitellogenic oocyte without mesogleal coat; **D,** section of oocyte; **E**, detail of oocyte layers; **F,** detail of oocyte surface. *F*: follicular layer, *M*: mesogleal coat, *Y*: yolk, *: oral pole, white arrow points out the mesogleal coat.

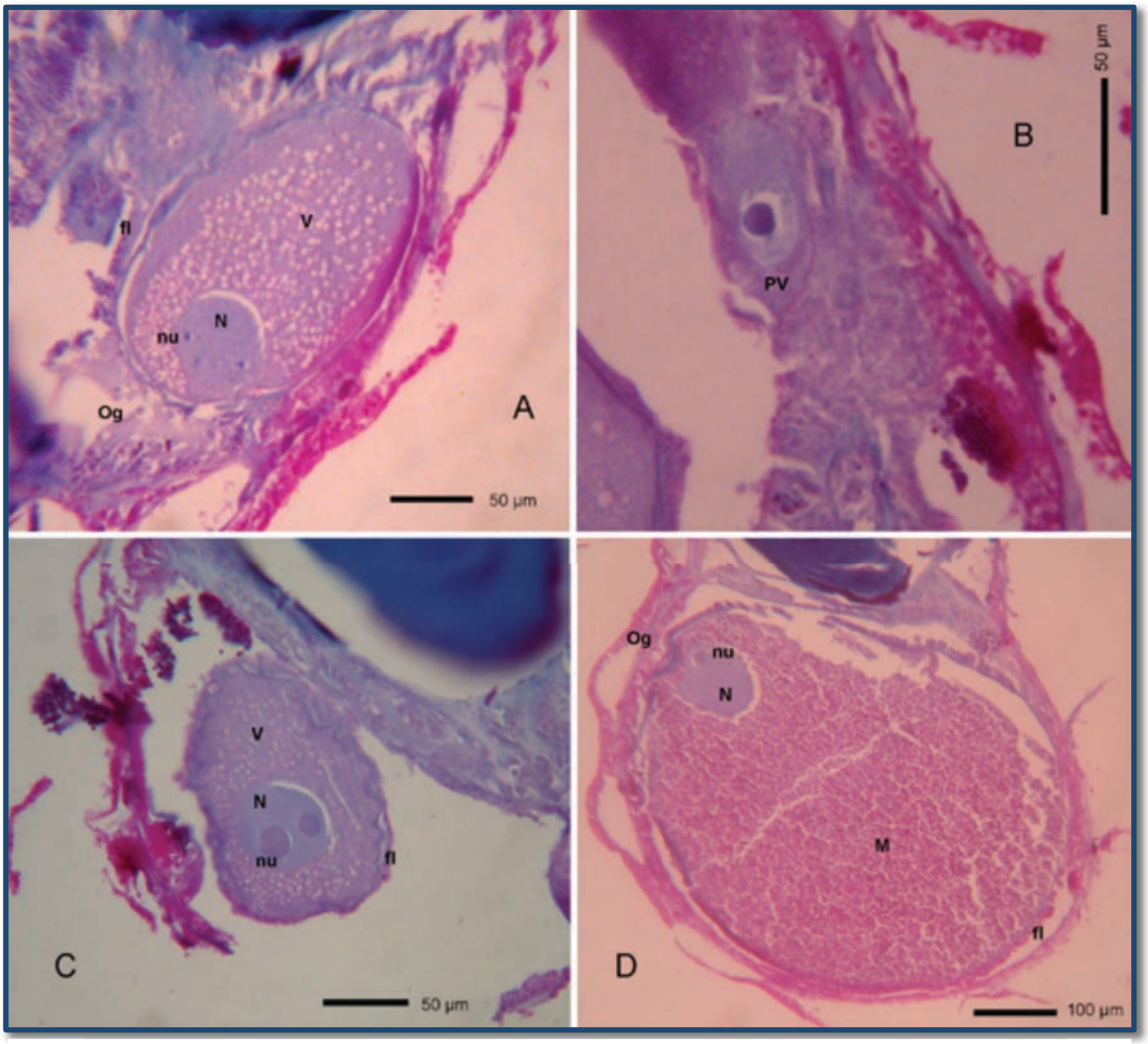


Figure 6.6.- Histological sections of oocytes development in *Thouarella variabilis*. **A,** vitellogenic oocyte with a cluster of primary genital cells at the base (Og); **B**, previtellogenic oocyte embedded in the mesentery; **C**, vitellogenic oocyte with two nuclei; **D**, mature oocyte. *fl*: follicular layer, *M*: mature, *N*: nucleus, *nu*: nucleolus, *Og*: oogonia, *PV*: previtellogenic, *V*: vitellogenic.

6.4.3.2. Spermatogenesis

Spermaries at different stages of development were observed in male polyps, however not all stages were identifiable at light microscope, unfortunately when histological samples were examined the earlier stages were not observed.

Spermatogonia (S1): the earliest sperm cysts should be observed in the mesoglea mesenteries forming clusters of sprematogonias.

Spermatocytes (S2): spermaries up to 100 µm (fig. 6.8a).

Spermatids (S3): spermatocytes began to develop into spermatids, which are arranged at the periphery of the sperm sac. As a result of this accumulation the central space change to opaque white. Spermaries reach up to 300 µm (fig. 6.8b).

Spermatozoa (S4): spermaries have metamorphosed spermatozoa radially arranged with their tails projecting toward the centre of sperm sacs. About 400 µm in diameter (fig. 6.8c).

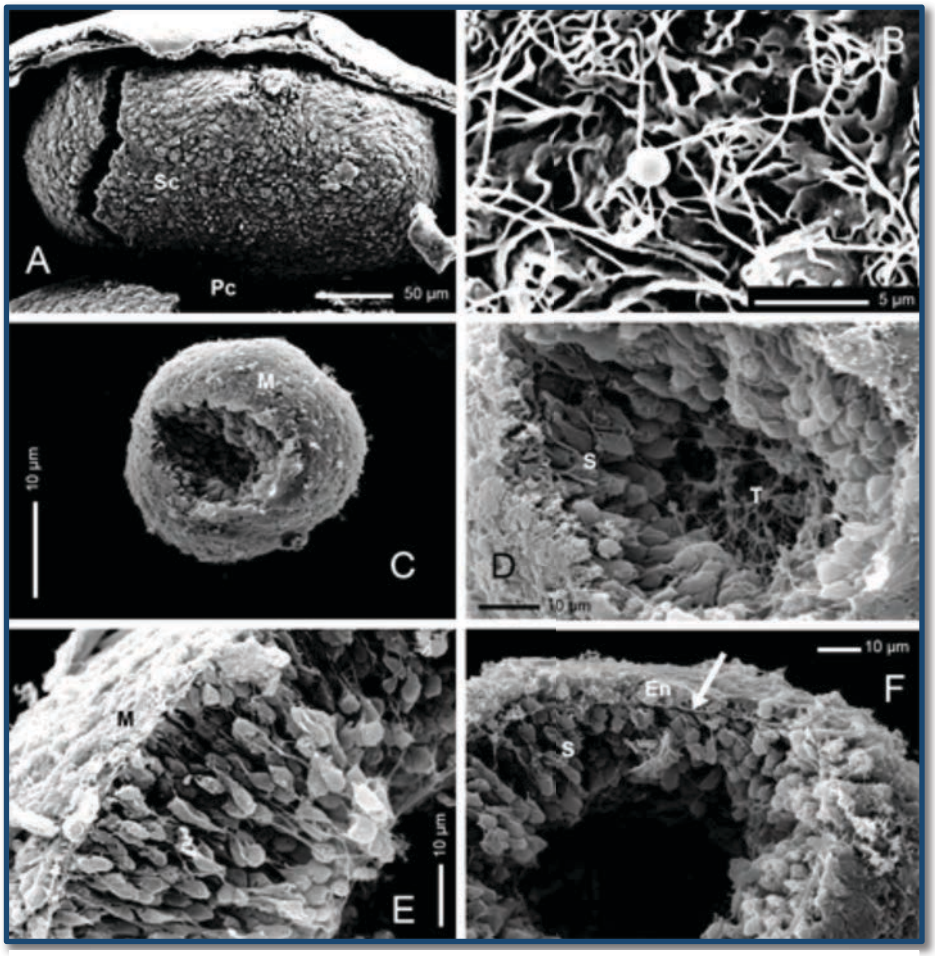


Figure 6.7.- SEM of male sexual products**. A,** sperm cyst; **B,** detail of the surface of a sperm cyst; **C,** fractured sperm cyst; **D,** detail of the interior of a sperm cyst; **E**, detail of the sperm; **F,** detail of the section oocyte surface. *En*: endoderm, *M*: mesogleal coat, *Pc*: polyp cavity, *S*: sperm, *Sc*: Spermatic cyst, *T*: tails.

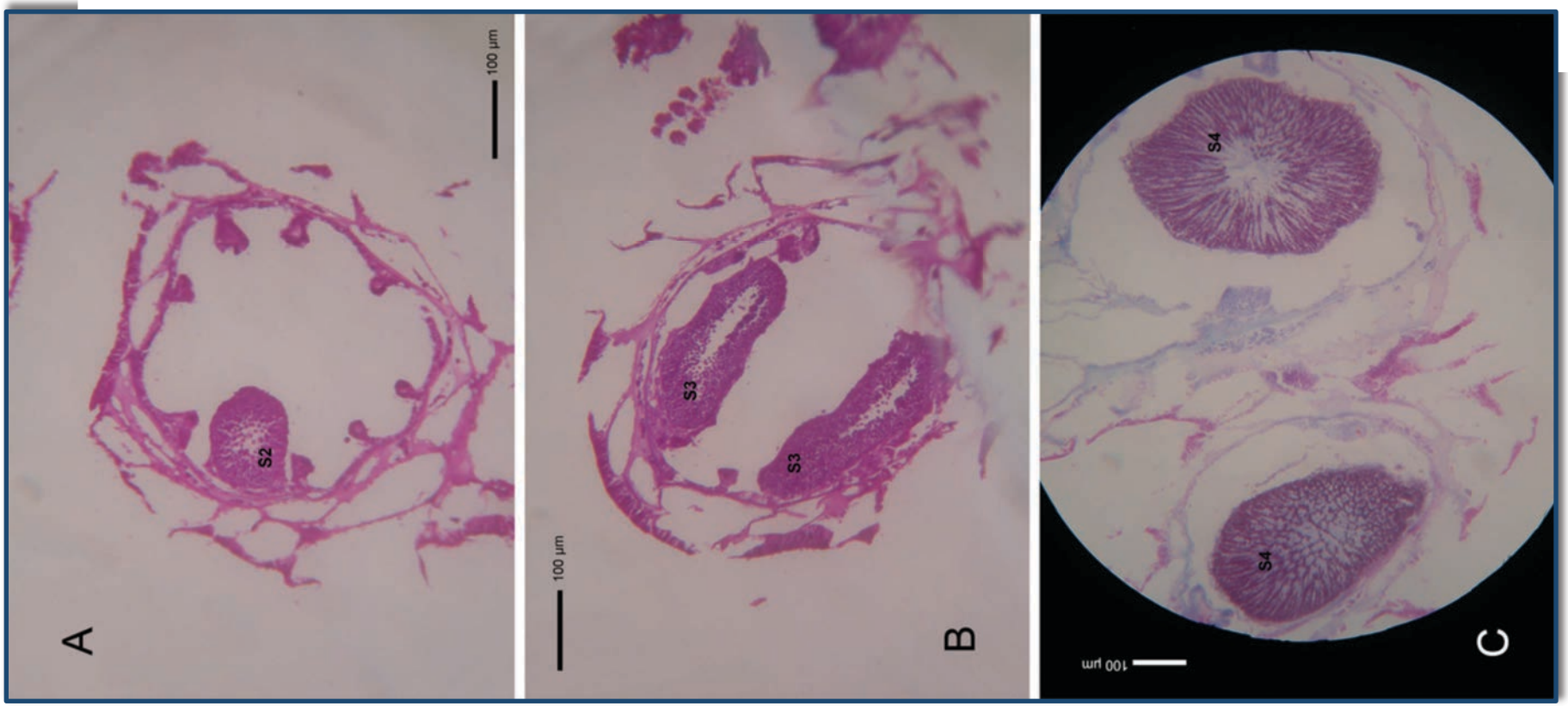


Figure 6.8.- Histological sections of male polyps in horizontal plane showing sperm cysts development in *Thouarella variabilis*. **A,** spermary in stage 2 of development; **B**, two spermaries in stage 3 of development; **C**, two spermaries in stage 4 of development.

6.4.3.3. Larvae

Thouarella variabilis broods their fertilized eggs to planulae in the gastrovascular polyp cavity. After fertilization eggs are located in the uppermost zone of the polyp cavity where the larva develops. At the same time oocytes in earlier stages of development can be found at the lowermost zone of the polyp cavity near the mesenteries. The larvae consist of an endoderm, an internal layer, which is continuous with the central yolk material, a thin mesoglea, and a columnar external layer, the ectoderm (fig. 6.9). The maximum length of larva planula observed was 1100 µm.

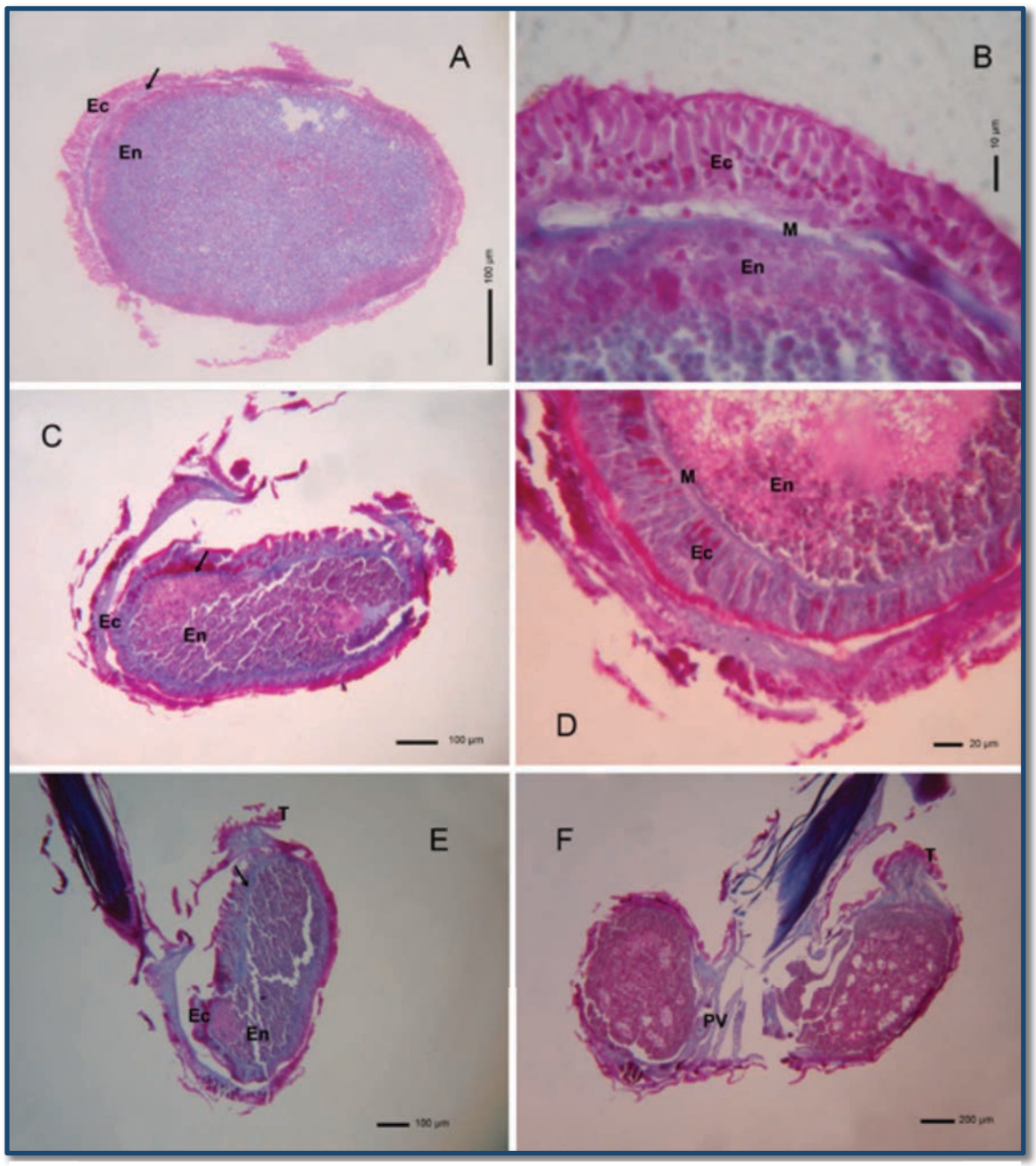


Figure 6.9.- Histological sections of larvae. **A, C,** larva differentiated into ectoderm, mesoglea and inner yolky endoderm; **B**, **D**, detail of the two germ layers; **E**, sagittal plane of female polyp with a larva inside; **F**, two female polyps sectioned in horizontal and sagittal planes with mature larvae inside. *Ec*: ectoderm, *En*: endoderm, *M*: mesogleal coat, *PV*: previtellogenic, black arrow points out the mesogleal coat.

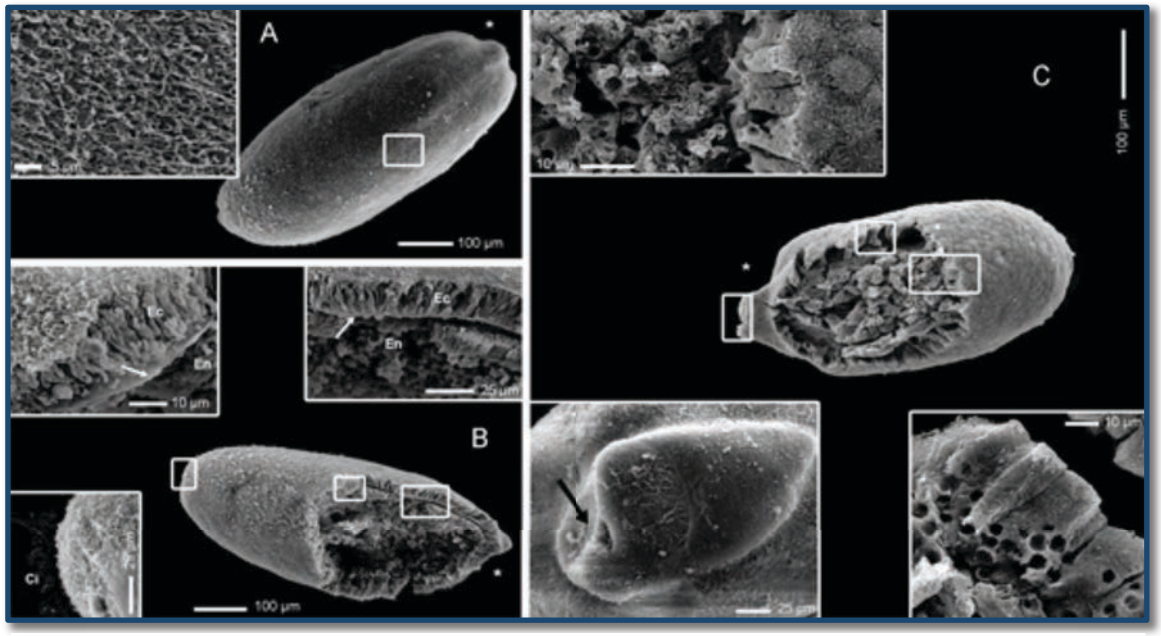
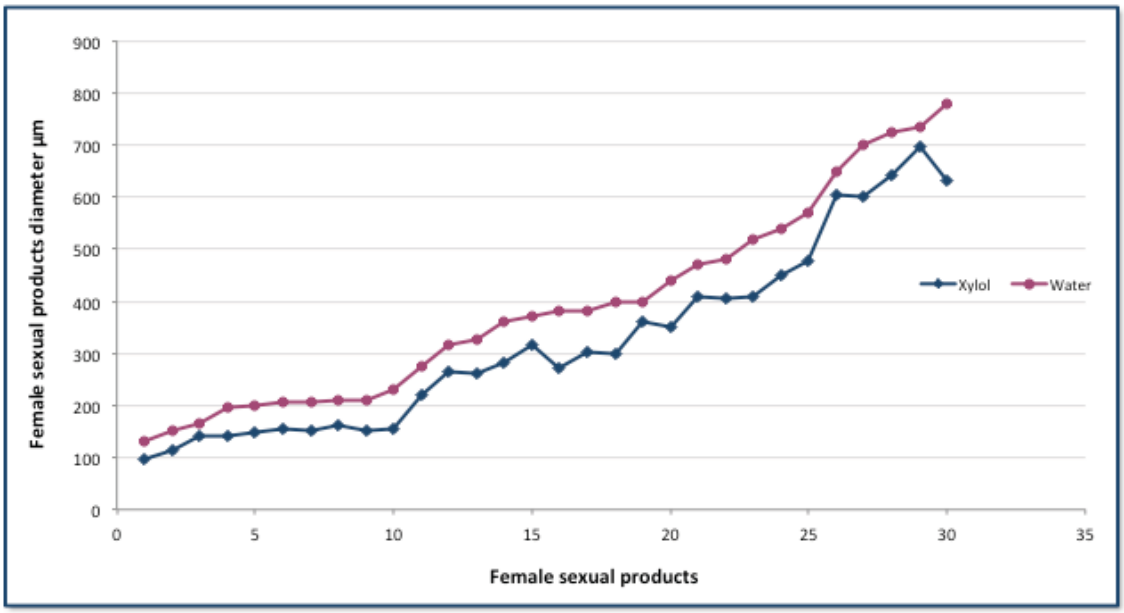


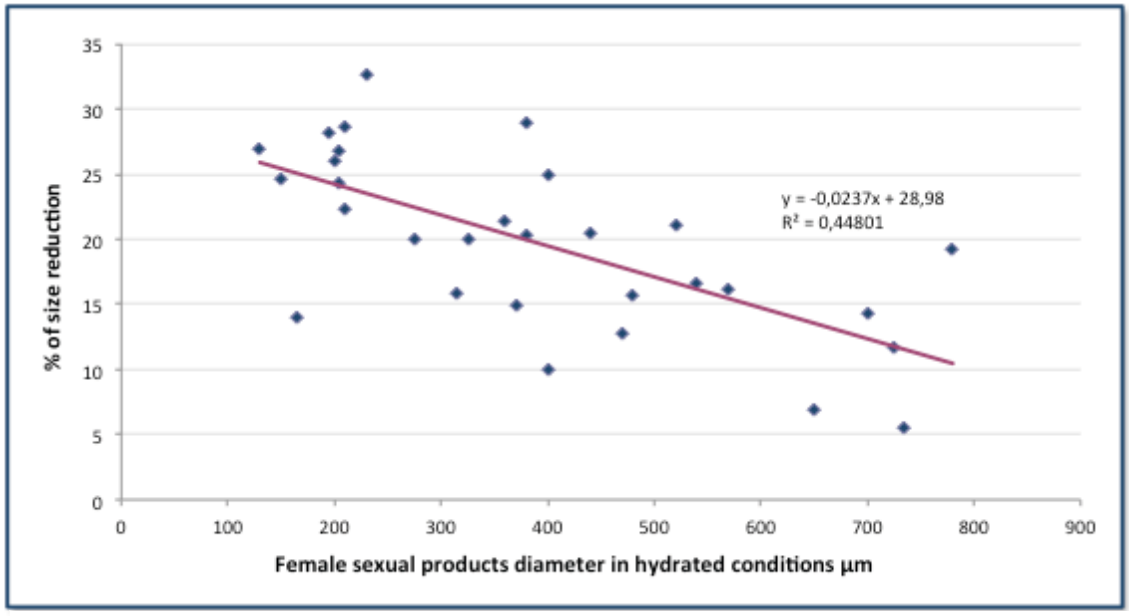
Figure 6.10.- SEM of planula larvae**. A,** vitellogenic oocyte, high magnification of the ciliated larvae surface; **B,** fractured larva, high magnification of the germ layers, white arrows points out the mesogleal coat; **C,** fractured mature larva, high magnification of the ectoderm and oral pole, black arrow points out the stomodeum. *Ec:* ectoderm, *En*: endoderm, *: oral pole.

6.4.4. Dehydration effect

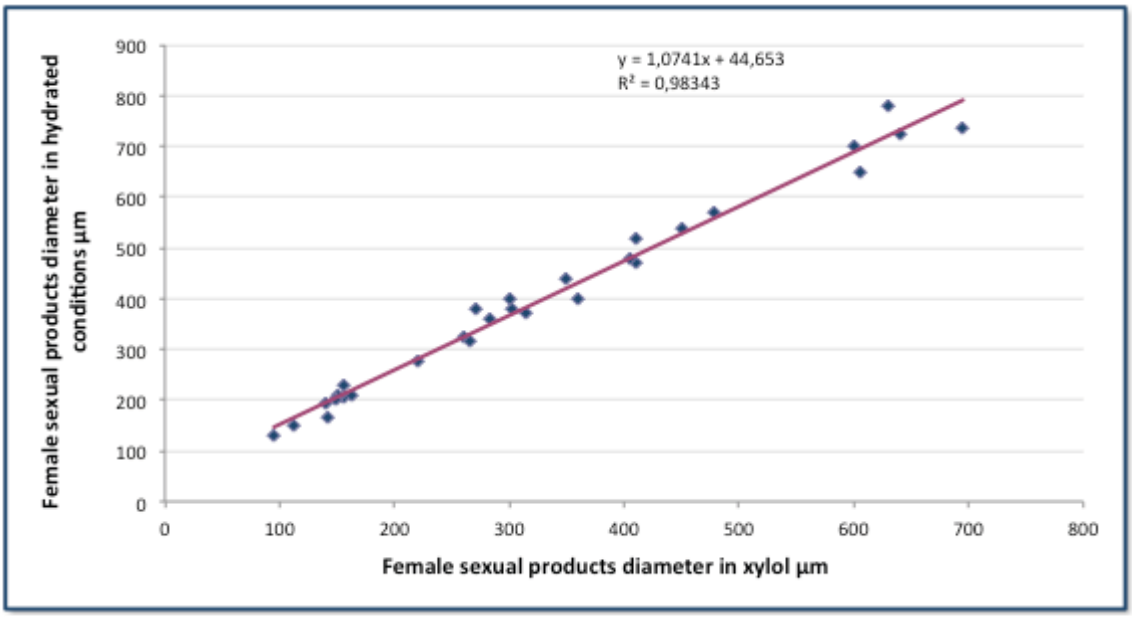
After the measurement of female sexual products (oocytes and larvae) in hydrated conditions first and then, measured after dehydration in xylol, we observed a reduction of their diameters after their dehydration (Gr. 6.19). The mean (±SE) size reduction was 68.5 ± 27.98 µm; about 20% of their size is reduced after dehydration. The largest reduction was 150 µm (19.2% reduced) and the smallest 23 µm (13.9% reduced). The maximum percentage of reduction was 32.6% (75 µm reduced) and the minimum 5.4% (40 µm). If we analyse the percentage of size reduction for different diameters, we see a general tendency where diameter and percentage of size reduction are inversely related, if diameter increases the percentage decreases (Gr. 6.20). If we compare diameters measured in hydrated conditions with diameters measured after dehydration we obtain the linear regression line (Gr. 6.21) that permit us extrapolate measurements and compare our data with data from other works.



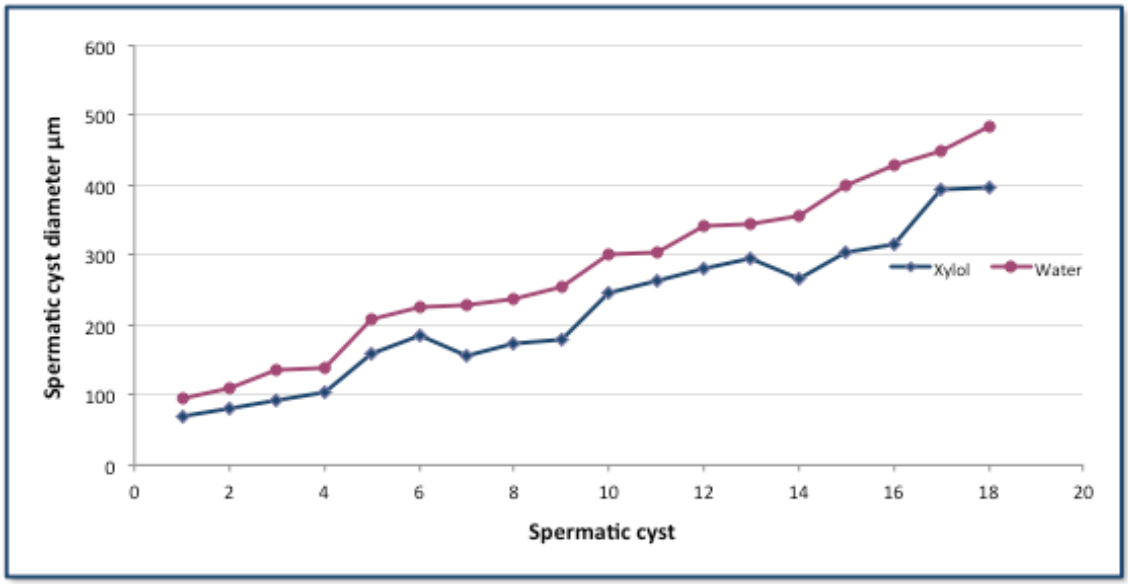
Graph 6.19.- Representation of 30 sexual female products size in hydrated and dehydrated conditions.



Graph 6.20.- Relation of the diameter of female sexual products in hydrated conditions and their percentage of size reduction after dehydration.



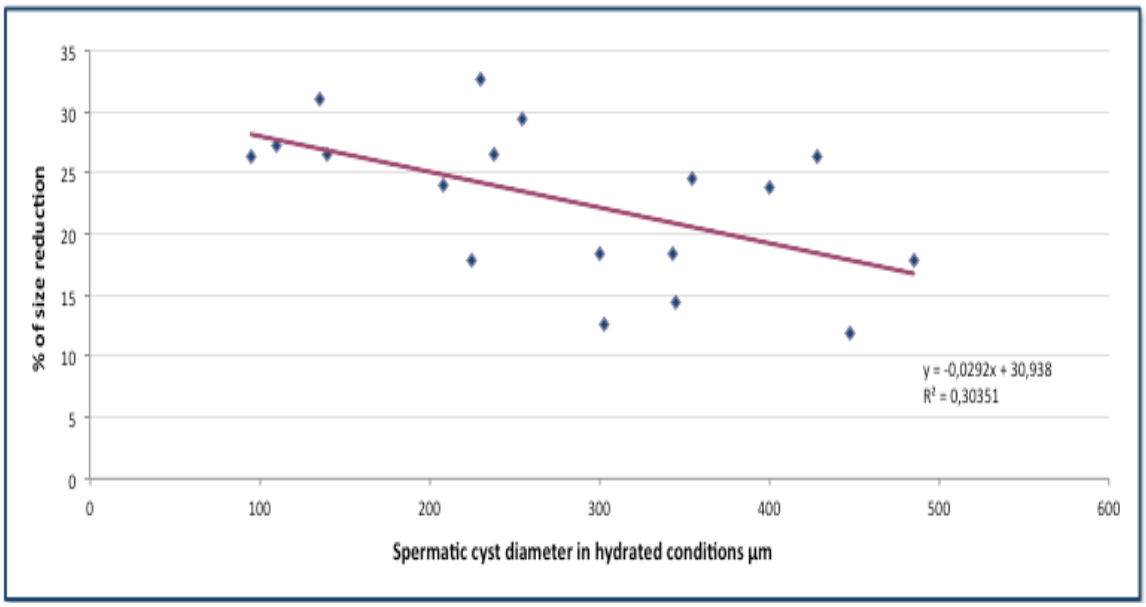
Graph 6.21.- Comparison of female sexual products in hydrated and dehydrated conditions.



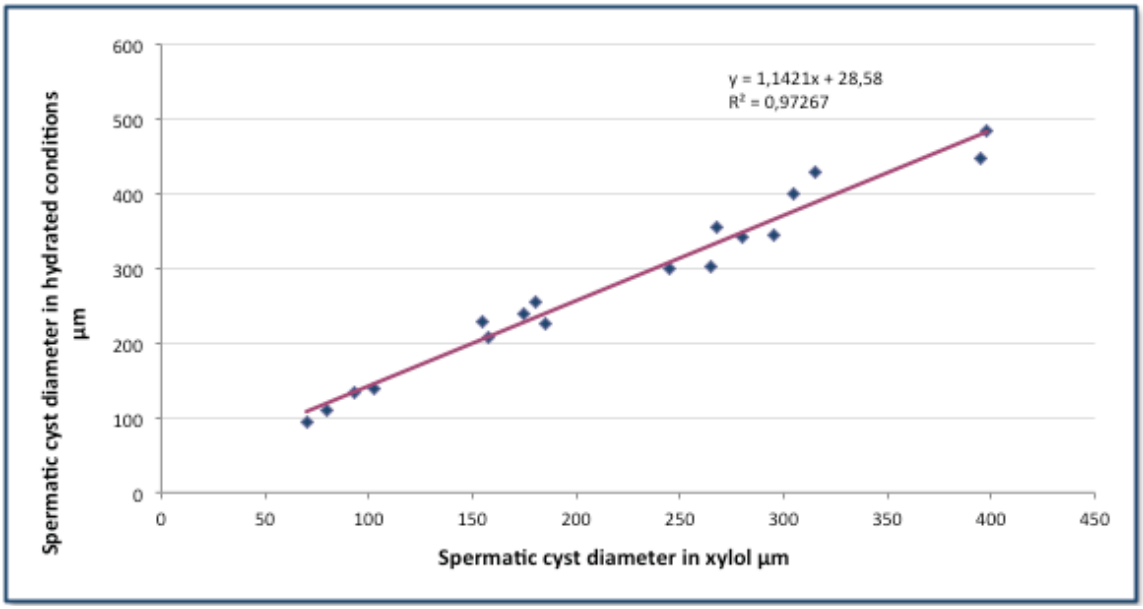
Graph 6.22.- Representation of 30 sexual male products size in hydrated and dehydrated conditions.

After the measurement of male sexual products (spermatic cysts) in hydrated conditions first and then, measured after dehydration in xylol, we observe the same pattern as observed for females, a reduction of their diameters after this dehydration (Gr. 6.22). The mean (±SE) size reduction was 59.8 ± 24.36 µm, about 23% of their size is reduced after dehydration. The largest reduction was 113 µm (26.4% reduced) and the smallest 25 µm (26.3% reduced). The maximum percentage of reduction was 32.6% (75 µm reduced) and the minimum 11.8% (53 µm). If we analyse the percentage of size reduction for different diameters, we see again, as in females, a general tendency where diameter and percentage of size reduction are inversely related, if diameter increases the percentage decreases (Gr. 6.23). If we compare diameters measured in hydrated conditions with diameters measured after dehydration we obtain the

linear regression line (Gr. 6.24) that permit us extrapolate measurements and compare our data with data from other works.



Graph 6.23.- Relation of the diameter of male sexual products in hydrated conditions and their percentage of size reduction after dehydration.

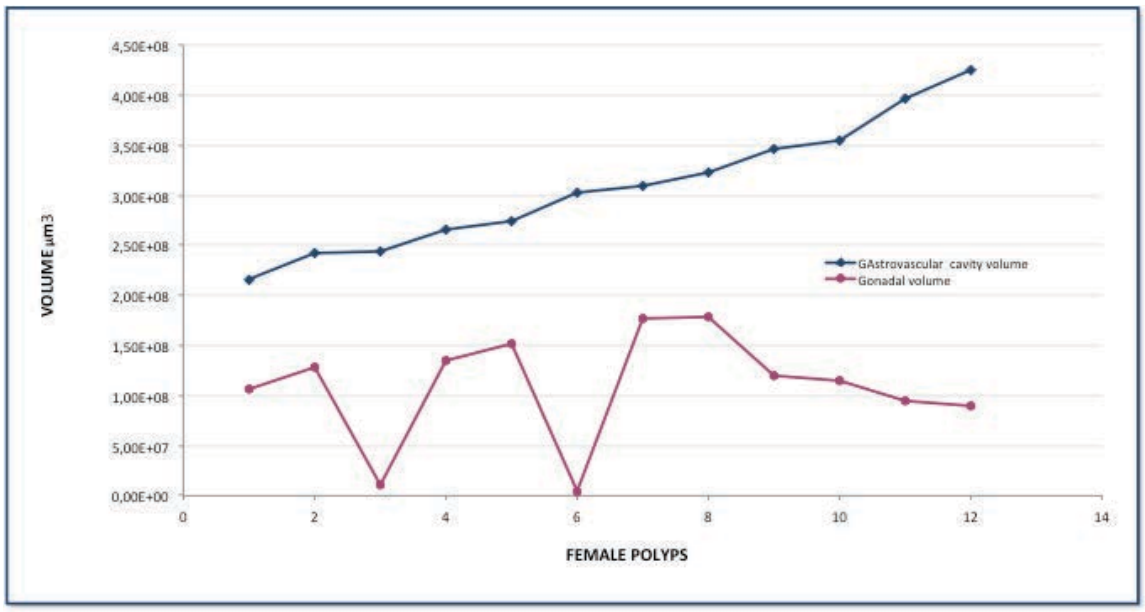


Graph 6.24.- Comparison of male sexual products in hydrated and dehydrated conditions.

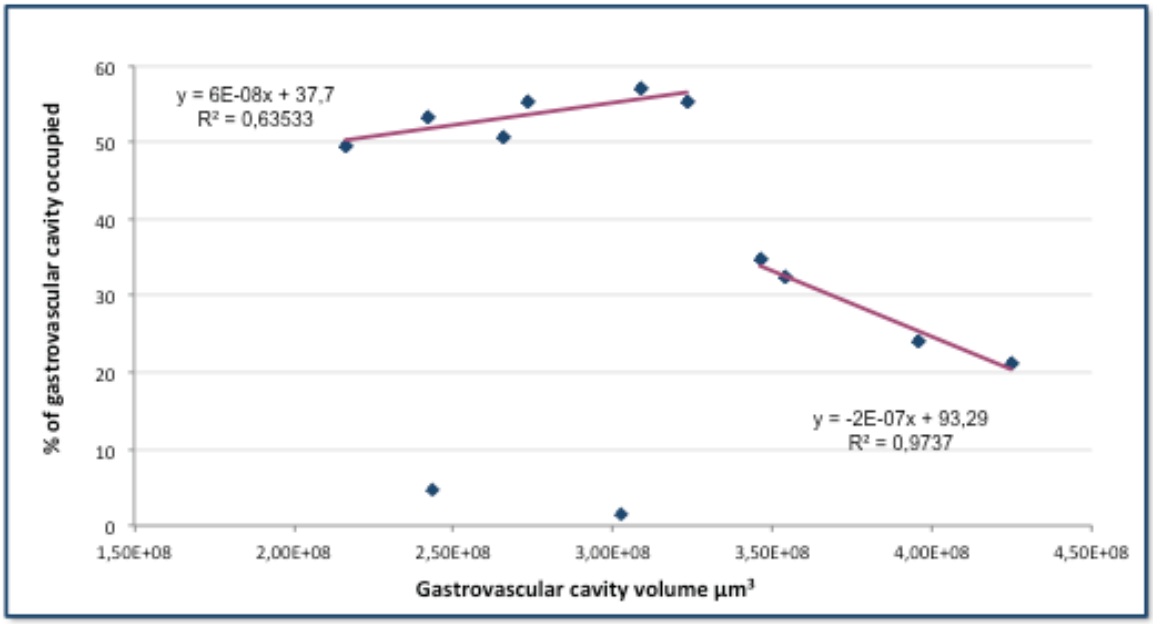
6.4.5. Gonadal volume

We observed no clear relationship between the total volume of the gastrovascular cavity in female polyps and their gonadal volume (Gr. 6.25). If we compare the percentage of GC occupied in relation to the total volume GC, we can distinguish two groups of polyps (Gr. 6.26). Polyps from group *a*, have percentages of occupation more than 50%, while polyps on group *b*,

have an occupation about 20-30%. Only two polyps have less than 5% of GC occupation. If we look carefully (Table 6.15), we can observe that the group of polyps on the left contain one larva with at least one more oocyte, while the group of polyps on the right are characterized to have two oocytes, one in a mature stage (about 550 μm) and another one in an early stage (about 200 μm). Polyps with the fewest gonadal volume have all their oocytes in early stages.



Graph 6.25.- Representation of the gastrovascular cavity and gonadal volume for 12 female polyps.

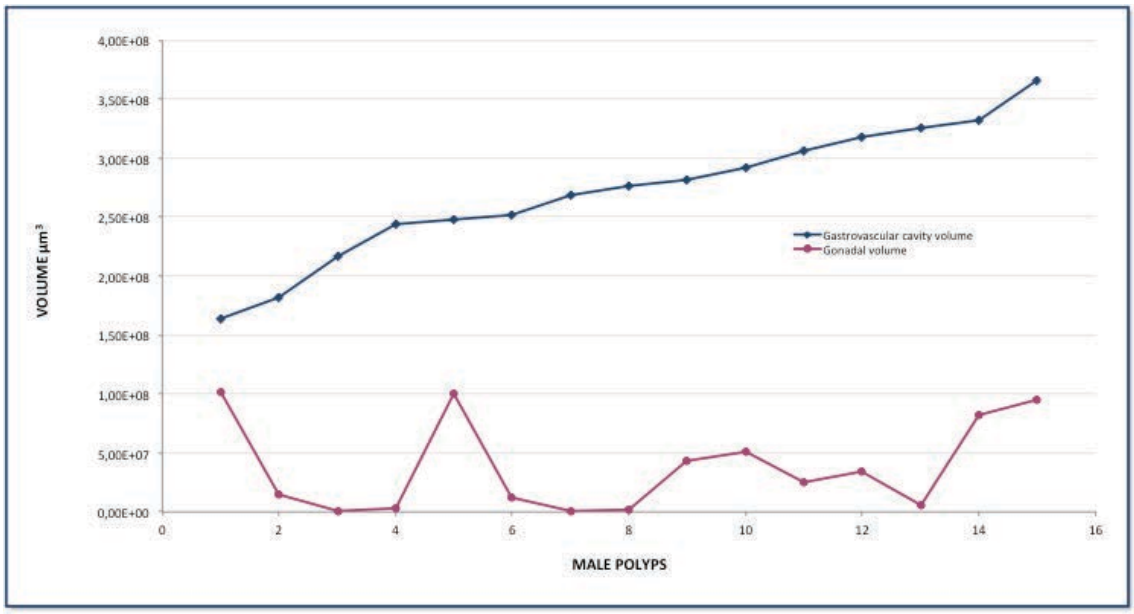


Graph 6.26.- Relation of the percentage of gastrovascular cavity occupied with the total gastrovascular cavity volume.

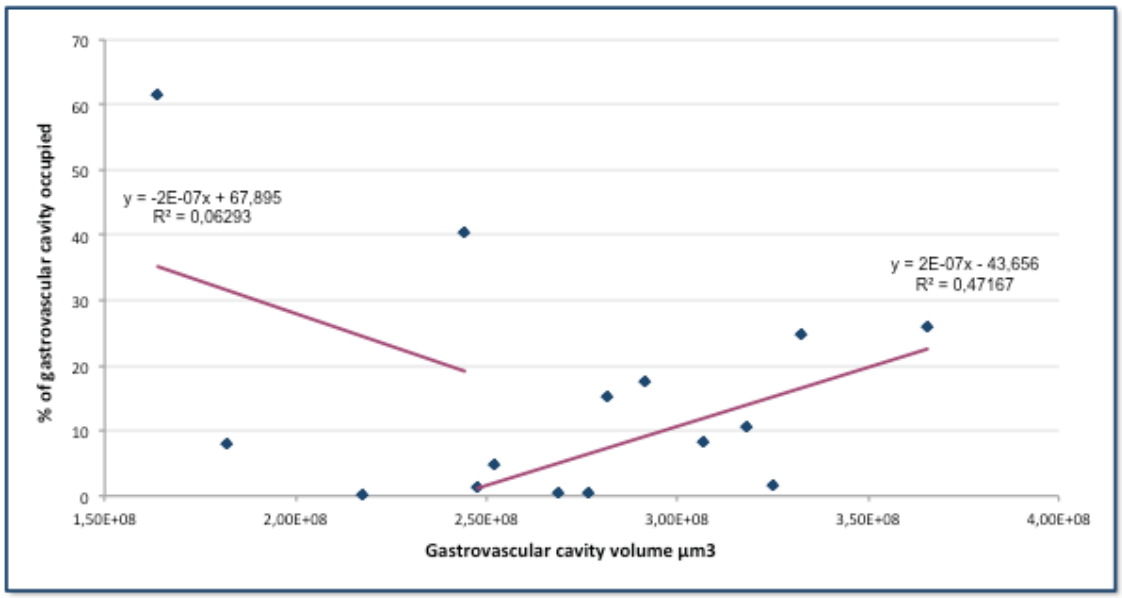
Polyp	Oocyte	Diameter µm	Polyp	Oocyte	Diameter µm
1	1	272,5	8	19_L	654,0
1	2	120,0	8	20	200,0
1	3	30,0	8	21	90,0
2	4	65,0	9	22_L	592,5
2	5	105,0	9	23	130,0
2	6	45,0	9	24	330,0
2	7	45,0	10	25_L	689,4
2	8	195,0	10	26	220,0
3	9	595,0	10	27	40,0
3	10	197,5	10	28	125,0
4	11	185,0	10	29	80,0
4	12	550,0	11	30_L	601,2
5	13	685,0	11	31	272,5
5	14	250,0	11	32	120,0
6	15	540,0	11	33	42.5
6	16	287,5	11	34	260,0
7	17	605,0	12	35_L	581,4
7	18	195,0	12	36	202,5

Table 6.15.- Size of the sexual products from the twelve female polyps analysed. L: larva.

In male polyps we observed the same pattern as in females, no relationship between the total volume of the gastrovascular cavity and their gonadal volume (Gr. 6.27). If we compare the percentage of GC occupied in relation to the total volume GC (Gr. 6.28), and the data concerning the spermatic cysts of the studied polyps (Table 6.16) we can distinguish that polyps with a percentage greater than 24%, have their spermatic cysts in mature stages. Polyps with less than 5% of GC occupations have all their spermatic cysts in earlier stages, where polyps with a percentage of GC occupation is from 8 to 20% have spermatic cysts in an intermediate stage between early and mature stages.



Graph 6.27.- Representation of the gastrovascular cavity and gonadal volume for 15 male polyps.



Graph 6.28.- Relation of the percentage of gastrovascular cavity occupied with the total gastrovascular cavity volume.

Polyp	Spermatic cyst	Diameter µm	Polyp	Spermatic cyst	Diameter µm
1	1	80,0	8	17	242,5
1	2	95,0	9	18	130,0
2	3	62,5	9	19	397,5
2	4	115,0	10	20	442,5
3	5	160,0	10	21	177,5
3	6	135,0	10	22	180,0
4	7	185,0	11	23	455,0
4	8	137,5	11	24	460,0
4	9	117,5	12	25	540,0
5	10	110,0	13	26	455,0
5	11	105,0	13	27	462,5
6	12	290,0	14	28	384,0
6	13	291,0	14	29	292,0
7	14	245,0	15	30	525,0
7	15	235,0	15	31	320,0
8	16	205,0	15	32	160,0

Table 6.16.- Size of the sexual products from the fifteen male polyps analysed.

6.5 Discussion

The histological sections of *Thouarella variabilis* indicate that sexual products are produced in the mesenteries where they are attached, then migrate into the polyp cavity and as maturation approaches they move to the upper part of the cavity, near polyp mouth, the same as observed in the shallow water temperate (southern Korea) species *Dendronephthya gigantea* (Hwang & Song 2007).

Oocyte production in numbers found in this study for *Thouarella variabilis* ranges from a minimum of 1,36 ± 0,58 in distal polyps to a maximum of 2,02 ± 0,83 per female polyps in autumn, with a medium production of 1,82 ± 0,74 for the 576 polyps analysed. Orejas *et al.* (2007) observed an oocyte production per polyp much lower 1.1 ± 0,1 in *Thouarella* sp. The oocyte size observed ranges from 183,81 ± 121,95 µm to 249,37 ± 152,15 µm, with a medium size of 224,02 ± 150,60 µm for 1033 oocytes measured. Orejas *et al.* (2007) observed a medium oocyte size higher for *Thouarella* sp. of 261,3 ± 15,6 µm. The high difference in standard deviation on oocyte size between results from both studies may suggest that Orejas *et al.* (2007) only observed polyps with oocytes in the same developmental stage, with very similar sizes as the deviation is only of 15,6 compared with the deviation found in this study of 150,60 which indicates that we observed polyps with oocytes from a wide variety of sizes from 28 µm to 680 µm. This fact also agree with the oocyte production observed by Orejas *et al.* (2007) of an average of 1 oocyte per polyp suggesting that smallest oocytes were not seen.

In soft corals the number of visible oocytes contained by female polyps ranges from 5.3 in the deep-sea *Anthomastus ritteri* (Cordes *et al.* 2001) to hundreds of eggs per polyp in some *Xenia* species from shallow waters at Red Sea (Benayahu 1991). The shallow Mediterranean Sea holaxonian gorgonians *Paramuricea clavata* can release 13 mature eggs per polyp (Coma *et al.* 1995), while *Eunicella singularis* (Gori *et al.* 2007) presents a low number of female products per polyp (from 1,7 to 4,5). Both Mediterranean species have shown monthly changes of the mean number of total oocytes per polyp, which may double its numbers in just of a couple of months. However in our study we did not find any statistical difference on number of female sexual products among seasons. Moreover, *E. singularis* like *T. variabilis* are both brooder species, which may lead to produce fewer sexual products per polyp than broadcasting species (Gori *et al.* 2007) due to internal space constriction and larvae protection. However, in *Leptogorgia sarmentosa*, a gorgonian species from the shallow Mediterranean Sea which produces a fewer number of eggs per polyp (1.4-4.3), there is no evidence of an internal brooding, neither surface brooding nor external fertilization (Rossi & Gili 2009).

The largest mature oocytes observed vary in size on different species of shallow waters soft corals, from 473 μm in the subtropical species *Scleronepthya gracillimum* (Hwang & Song 2009), 620 μm in *Sarcophyton elegans* from the Great Coral Reef (Hellström *et al.* 2010), 700 μm in *Parerythropodium fulvum* (Benayahu & Loya 1983) to 900 μm in *Heteroxenia fuscescens* (Benayahu *et al.* 1989) both from the Read Sea. Similar size values have been observed for gorgonians from temperate shallow water ~~species~~ ranging from 500 μm (Rossi & Gili 2009), 700 μm (Excoffon *et al.* 2004) to 900 μm (Gori *et al.* 2007) and in the deep-sea *Ainigmaptilon antarcticum* (700 μm) from the Southern Ocean (Orejas *et al.* 2002). The results of *Thouarella variabilis* are quite similar with a maximum oocyte diameter around 700 μm as in the surface brooding species *P. fulvum* or the internal brooder *T. clavaria* which may suggest that size of mature oocytes are not dependant on gastrovascular cavity space.

The production in number of male sexual products is almost the same as for females with a mean of 2.06 ± 0.8 spermatic cysts per polyp, which vary significantly depending on the branch and colony zone, with a lowest production rate for distal polyps 1.80 ± 0.65 and the highest 2.20 ± 0.86 for polyps at central zones of branchlets. However our results are slightly smaller than the previous noted by Orejas *et al.* (2007) for a *Thouarella* sp., in that case showed a mean of 3.0 ± 0.2 spermatic cysts per polyp. The sperm cyst size observed ranges from 169.94 ± 73.78 μm to 246.34 ± 111.46 μm, with a medium size of 200.71 ± 99.06 μm for 1312 sperm cysts measured. Again our results are slightly smaller than the previously noted for *Thouarella* sp. which sizes range from 199.7 ± 19.1 μm to 292.1 ± 12.4 μm (Orejas *et al.* 2007). As occurring with oocytes the great difference between standard deviation in both studies might suggest that Orejas *et al.* (2007) only observed polyps with sperm cysts in the same developmental stage, with very similar sizes as the deviation is around 15 compared with the deviation found in this study around 100 which indicates that we observed polyps with sperm cysts from a wide variety of sizes from 30 μm to 570 μm. Also the maximum sperm cyst diameter observed in this study for *T. variabilis* is larger than the previous noted by Orejas *et al.* (2007) for a *Thouarella* sp. (400 μm).

The number of spermatic cysts observed per polyp in the Antarctic gorgonian species *Fannyella rossii*, *F. spinosa* and *Dasystenella acanthina* showed higher mean values ranging from 2.6 to 5.0 spermatic cysts per polyp (Orejas *et al.* 2007). The same occurs for the shallow Mediterranean *L. sarmentosa* where it is possible to find from 2.5 o 7.0 sperm cysts per male polyp (Rossi & Gili 2009), and *E. singularis* where 4.4 to 20.8 sperm cysts per polyp can be found (Gori *et al.* 2007). In the Mediterranean *Corallium rubrum* the number of sexual products found inside polyps where significantly correlated with the size of the colony, where the spermatic cyst mean number was higher in large colonies (2.42 ± 0.166) than in small ones (1.74 ± 0.18) (Tsounis *et al.* 2006).

Smaller sperm cysts diameters are found in the Antarctic *Fannyella* species with 200-300 μm (Orejas *et al.* 2007) and in the subtropical and tropical species *S. gracillimum*, *S. elegans* and *P. fulvum* with 281 μm, 450 μm and 480 μm respectively (Hwang & Song 2009; Hellström *et al.* 2010; Benayahu & Loya 1983). While larger sizes are found in the Antarctic gorgonians *A. antarcticum,* up to 1000 μm, and *Dasystenella acanthina,* up to 750 μm, (Orejas *et al.* 2002, 2007). The same occurs when comparing with Mediterranean species *P. clavata* and *E. singularis* have maximum diameters around 800 μm (Coma *et al.* 1995; Gori *et al.* 2007) and species such as *Corallium rubrum* or *Trilapea clavaria* have mature sperm cysts of 900 μm (Tsounis *et al.* 2006; Excoffon *et al.* 2004). The Mediterranean *L. sarmentosa* has a maximum sperm size of 550 μm (Rossi & Gili 2009) very close to which has been found in T. variabilis (570 μm). The data reflects a tendency where tropical and subtropical species have smaller mature sperm cysts compared with temperate species, but in the polar region we find both small and large sizes.

Thouarella variabilis exhibits a reproductive strategy characterised by a low number of oocytes per polyp, which is compensated by a high number of polyps in the colony and a high percentage of polyps on reproduction (60%). However, it shows a low fecundity, only one larva (up to 1100 μm), has been observed at the same time in a particular polyp, but larvae can share the gastrovascular cavity with small oocytes. Planula larvae have been found inside female polyps agreeing with Brito (1997) about the internal fertilization as the mode of reproduction in *T. variabilis*, where also its fertilized eggs are internally brooded as other Antarctic species (Orejas *et al.* 2007).

The apparently presence of two oocyte and sperm cysts size classes simultaneously (Gr. 6.1 and Gr. 6.13) point out the presence of two generation of sexual products. Two hypothesis have been proposed (Brito 1997): A two year cycle, where the oocytes reach maturation in two years time and thus one larva is released per summer; and a continuous gametogenesis where larva is released during all year around. Our results, contrary to Orejas *et al.* (2007), agree with patterns found in Xeniidae soft corals (Benayahu 1991), where larvae are found in all seasons analysed, supporting the hypothesis of a continuous gametogenesis. However, recruitment and settlement studies suggest that released planulae are more competent to settle and metamorphose in periods, which coincides with maximum primary production in the water column (Ben-David-Zaslow *et al.* 1999). In the Southern Ocean there is a marked spring peak in primary production (Turner *et al.* 2009) and the subsequent sedimenting particulate matter in

the bottom would allow to planula larvae take advantage from the planktonic blooms (Arntz & Gili 2001). Thanks to the continuous resuspension of the organic matter sunk during spring, the benthic trophic conditions for suspension feeders are almost constant all year round (Smith *et al.* 2006) allowing a continuous gametogenesis.

Our results might also suggest a two spawning events per year: in spring we find mature sperm cysts that would be released at the end of the season, then mature oocytes found in female colonies would be fertilized, and polyps with mature oocytes, fertilized ones, and larvae would be found at the same colony but not in the same polyp. At the end of summer and at the beginning of autumn mature larvae would be released. In summer sperm cysts are smaller and immature, as well the oocytes, larvae are also observed as they could be released at the end of summer. In autumn mature sperm cysts would be released at the end of the season, where oocytes are larger and ready to be fertilized, larvae and mature oocytes would be found again in the same colony at the end of the season and in winter.

Unfortunately we do not have any data from winter and nothing is known about the fate (timing of free phase, settlement, and metamorphosis to reach the first functional polyp) of these larvae and therefore their best fitting conditions.

In *T. variabilis* both oogenesis and spermatogenesis are rather overlapping compared to other gorgonian species. In many corals the development cycle of the sexual products consists on a short period of spermatogenesis compared to that of oogenesis (Benayahu & Loya 1983; Brazeau & Lasker 1990; Coma *et al.* 1995; Gori *et al.* 2007), although sperm cysts are present together all year round in some soft corals (Fan *et al.* 2005) and in the present study. In the Mediterranean species *Paramuricea clavata* and *Eunicella singularis* there is an overlapping oogenesis with 2 cohorts of oocytes while no overlapping exists for spermatogenesis (Coma *et al.* 1995; Gori *et al.* 2007). The same occurs for the subtropical species *Sarcophyton elegans* (Hellström *et al.* 2010).

Previous studies on *Thouarella variabilis* and other Antarctic primnoids show no statistical differences in oocyte number in the different colony zones, branch zones, or the interaction of them (Orejas *et al.* 2007). However, in this study we have found differences in the branch zone, pattern also observed in the primnoid *Dasystenella acanthina* (Orejas *et al.* 2007). For male products we also found differences in number in the colony zone, and their interaction with branch zone factor, pattern not registered in previous primnoid studies (Orejas *et al.* 2007), but observed for both males and females in the Mediterranean *Paramuricea clavata* where in the basal parts of the colony the number of sexual products decreases and its maximum at the distal part (Coma *et al.* 2005) and *Leptogorgia sarmentosa* where the medial parts of the colony are the ones with a maximum number of sexual products per polyp (Rossi & Gili 2009). Furthermore larvae appear to be more abundant in medial areas of the colony and at proximal and central parts of the branches.

These results support the suggestion made by Brito (1997) that polyps located at the periphery of the colony, which are found often to be non-reproductive, are mainly dedicated to feed investing more in growth than reproduction. Polyps located more internally show an increased

reproductive effort, these protected parts of the colony (medial colony zone and proximal branch zone) where predation pressure is lower, are considered the safest tissues for reproduction (Hugues & Jackson 1980). The larger sexual products of both males and females were also located in the medial colony zone, pattern also found in flagelliform gorgonians (Orejas *et al.* 2002) and among gorgonians from other latitudes (Szmant-Froehlich 1985) and in some hard corals (Wallace 1985).

While for soft corals coenenchyme thickness and gonad production seems to be related (Benayahu 1991), in *T. variabilis* we have not found any relation between the gastrovascular cavity and the gonadal volume, perhaps because of the variability of polyp size within the same colony and among colonies of the same species. Although the gastric cavity is limited by the calcareous sclerites that surround the polyp body walls as a protective armature, it has been observed a capacity of plasticity and expansion in polyps in late stages of gametogenesis.

The results point out that gonadal volumes are related to maturation stage of sexual products. Polyps with high gonadal volumes present an internal larva, followed by polyps with oocytes or sperm cysts in late stages of maturation and finally polyps with sexual products in early stages of development are the ones with a least gonadal volume.

After the study of dehydration effect on the measurement of sexual products we have to stress the importance of comparing sizes using different techniques where products are dehydrated or not, studies based on light microscopy measures are not equivalent to measures made after histological techniques, with a reduction of size up to 33%. That is really important when developmental stages have been categorized by the size of sexual products after histological techniques were developmental features such as the presence of lipids, membranes or other structures can be seen. For smaller sizes the reduction after dehydration is higher which may lead to include oocytes or sperm cysts of the earlier stages in one class higher of development erroneously when we sort them under the light microscope.

To conclude *Thouarella variabilis* exhibits a reproductive pattern similar to those observed in other Antarctic gorgonians (Orejas *et al.* 2002, 2007), as well as among gorgonians from other latitudes (Grig 1977; Brazeau & Lasker 1990) characterized by a low number of sexual products per polyp. Studies have been aimed to characterize the process of gametogenesis (number of oocytes and spermaries per polyp, size and distribution through the colony of the sexual products) (Brito 1997; Orejas *et al.* 2002, 2007; present study). Unfortunately gaps in information still exist, and so much is needed to understand the Antarctic gorgonian reproductive strategies and their implications on their distribution and dispersion patterns and their relationships with the Antarctic benthic communities. Thus it's highly recommended to carry out studies focusing on fertilization, planulation, larval settlement and recruitment on Antarctic gorgonians.

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