Universidad de Concepción
Dirección de Postgrado
Facultad de Ciencias Naturales y Oceanográficas
Programa de Doctorado en Sistemática y Biodiversidad

Patrones genómicos de divergencia en el complejo *Anthothoe* del Hemisferio Sur: Implicancias para la delimitación de especies en las anémonas de mar (Orden Actiniaria)

Tesis para optar al grado de Doctor en Sistemática y Biodiversidad

CARLOS ALBERTO SPANO PÉREZ

CONCEPCION - CHILE

2018

Profesor Guía: Dr. Daniel Gómez Uchida
Dpto. de Zoología, Facultad de Ciencias Naturales y Oceanográficas
Universidad de Concepción
Profesor Co-Guía: Dra. Vreni Häussermann
Escuela de Ciencias del Mar, Facultad de Recursos Naturales
Pontificia Universidad Católica de Valparaíso

Para mi esposa Gianinna Clementi.

Todo el tiempo que destine a esta tesis fue tiempo que permanecí alejado de ella. Que este trabajo sea siempre un recordatorio de nuestra lucha por estar juntos

AGRADECIMIENTOS

Mi primer y más sentido agradecimiento es a Dios. Todo lo que soy y he podido construir hasta ahora ha sido solo posible gracias al apoyo de mi familia. Ellos siempre han sido mi mayor bendición.

Agradezco profundamente al programa de Doctorado en Sistemática y Biodiversidad, a todos mis compañeros y profesores, y en especial a mi tutor Daniel Gómez Uchida. Su acogida, respaldo y amistad han sido fundamentales en el arduo camino al grado Doctoral. Nunca olvidare su ejemplo como profesor y, sobretodo, como persona.

Debo incontables gracias a mi extraordinaria comisión de tesis: Vreni Häussermann, Lisa Seeb, Angie Díaz, Pedro Victoriano y Cristián Hernández. Ellos no solo me corrigieron cuando hizo falta sino que también me ayudaron con ánimo y paciencia cada vez que lo necesite. Esta tesis también es fruto de su trabajo académico.

Gracias de corazón a cada amistad que forme durante estos últimos cuatro años en Concepción. Mención especial a todo el equipo del GEEC lab: Selim, Pablo, Mauricio, Diego, Mónica, Camila, Celia y Andrea; ellos dieron la cuota de alegría diaria que se hace necesaria para surcar un postgrado. Por la valiosa relación que entablamos, no puedo dejar de nombrar a Cristian Canales, Fernanda Oyarzún y Alejandra Panzera. Curioso es que en tan poco tiempo sienta tanto el aprecio de una amistad. Les agradezco profundamente sea así.

Muchas gracias al Departamento de Zoología de la Universidad de Concepción así como a la Dirección de Postgrado por todo el apoyo brindado durante el transcurso del Doctorado. De la misma manera, agradezco al Seeb Lab de la Universidad de Washington, y en especial a Jim Seeb, Garrett McKinney y Carita Pascal. La inolvidable experiencia de mi Pasantía Doctoral fue lo que fue debido a ellos. Finalmente, gracias a CONICYT por haberme adjudicado la beca que hizo todo esto posible.

Universidad de Concepción

RESUMEN

Patrones genómicos de divergencia en el complejo *Anthothoe* del Hemisferio Sur: Implicancias para la delimitación de especies en las anémonas de mar (Orden Actiniaria)

Carlos Alberto Spano Pérez

Las anémonas de mar (Orden Actiniaria) constituyen un grupo relativamente pequeño de invertebrados marinos caracterizados habitualmente como pólipos solitarios de cuerpo blando y llamativos colores. Si bien son miembros conspicuos del intermareal rocoso, aún es muy poco lo que se sabe respecto a los procesos biológicos que subyacen a su extraordinaria diversidad. En la primera mitad de la presente Tesis Doctoral se repasan las principales causas históricas y contemporáneas de esta situación, así como potenciales alternativas para abordar la delimitación de especies en Actiniaria. Mientras que las convergencias morfológicas y la falta de conocimiento sobre la estructura y evolución de los caracteres diagnósticos conforman algunos de los problemas centrales en este contexto, la distribución geográfica ha ido surgiendo recientemente como una poderosa variable explicativa ante los resultados genéticos. Esta inferencia se evaluó explícitamente en la segunda mitad de la presente tesis, poniendo a prueba la hipótesis de especie para los representantes del género *Anthothoe* del Hemisferio Sur. Información proveniente de todo el genoma demostró una alta diferenciación entre poblaciones, la cual estuvo mucho más asociada a la localidad de su procedencia que a variedades morfológicas intraespecificas. Procesos vicariantes históricos mediados por eventos dispersivos de larga distancia serían los responsables más probables de la divergencia de los linajes de *Anthothoe*. Nuestros resultados indican consistentemente que el aislamiento geográfico, incluso a pequeña escala, estarían determinando la formación de especies en anémonas de mar, aún cuando esta diferenciación no sea detectable a través de su apariencia externa.

TABLA DE CONTENIDOS

INDICE DE TABLAS

CAPÍTULO 1

CAPÍTULO 2

INDICE DE FIGURAS

CAPÍTULO 1

CAPÍTULO 2

INTRODUCCION GENERAL

La especiación constituye el puente que vincula a la evolución de las poblaciones con la evolución de los linajes filogenéticos (Futuyma 2009). Este proceso no solo describe como se originan las especies sino que además determina como nosotros comprendemos y medimos la biodiversidad que nos rodea. Debido al importante rol que desempeña dentro de la biología evolutiva, diversos aspectos acerca de la especiación han sido extensamente debatidos en la literatura (e.g. Cracraft 1983, Agapow et al. 2004, De Queiroz 2007). Quizás uno de los más controversiales en este contexto guarda relación con la definición de "especie". Mayden (1997) identificó 22 conceptos distintos de especies, habiendo incluso más si se consideran todas las variaciones gramaticales para el mismo grupo de definiciones. Gran parte del problema se inició cuando el procedimiento para identificar especies fue elevado a un carácter más bien conceptual, muchas veces alejándose bastante de la idea evolutiva subyacente (Hey 2006). En las últimas décadas esta confusión ha sido gradualmente reenfocada a la luz de nuevos antecedentes, virando la discusión de cómo definir una especie a como establecer que es distinta de una sub-especies o población.

En los últimos 30 años la biología molecular ha revolucionado el campo de la ecología y evolución. Entre otras cosas, los datos genéticos han permitido cuantificar la diversidad poblacional, estudiar el movimiento de individuos entre localidades, medir la endogamia e hibridación, caracterizar nuevas especies y rastrear patrones históricos de dispersión (Freeland et al. 2011). Las limitaciones iniciales en torno al número de genes capaces de ser examinados han sido gradualmente resueltas gracias al advenimiento de la secuenciación masiva (NGS). Estas nuevas tecnologías han abierto la posibilidad de analizar el genoma en su totalidad para revelar los procesos evolutivos que dan forma a la variación genética entre poblaciones y linajes. En particular, marcadores genéticos como los *restriction site-associated DNA tag* (RAD tag; Miller et al. 2007, Baird et al. 2008) han reducido substancialmente los costos de obtener datos genómicos, haciendo de este tipo de estudios una alternativa viable de investigación incluso en organismos no modelos (Hohenlohe et al. 2012). En este contexto podemos no solo examinar cómo opera la especiación en una mayor variedad de taxa, sino también tener una mejor resolución de las singularidades y diferencias que caracterizan a los linajes evolutivos.

Delimitación de especies: nuevas propuestas para antiguos problemas

A principios de este siglo hubo un intenso debate sobre el futuro rol de la taxonomía en las estimaciones de la biodiversidad. Clasificaciones que habían descansado tradicionalmente en caracteres morfológicos han sido objeto recurrente de cuestionamiento debido a la diversidad criptica hallada frecuentemente por medio de la evidencia molecular (e.g. Rodríguez et al. 2014). Las secuencias de ADN se han convertido en el principal recurso de los estudios filogenéticos ya que estos suelen ser más comparables e informativos que cualquier otro rasgo morfológico. No obstante lo anterior, la divergencia molecular puede variar considerablemente entre grupos emparentados y algunas de las especies recientemente formadas son difícilmente distinguibles en todos los marcadores moleculares. De hecho, algunos alelos pueden aparecer más estrechamente relacionados entre especies hermanas que dentro de poblaciones divergentes; haciendo por lo tanto que la filogenia derivada de algunos genes no siempre coincida con la filogenia de la especie que porta esos genes (Maddison 1997, Nichols 2001, Degnan & Rosenberg 2009).

Esta situación ocurre típicamente cuando existe una repartición incompleta de linajes, o cuando aún existe un activo flujo génico entre poblaciones (ver Maddison 1997). En ambos casos, los taxa emparentados pueden parecer polifileticos pese a que ya hayan dado señales de adaptación en algún otro rasgo (e.g. Moran & Kornfield 1993). Tradicionalmente, la diversidad críptica ha sido abordada mediante la comparación de aloenzimas o a través de códigos de barras genéticos (Highton 2000, Hebert et al. 2003). Si bien útil en mucho casos, estos métodos también presentan restricciones importantes para identificar especies, especialmente esta última en invertebrados marinos tales como cnidarios antozoos (Shearer & Coffroth 2008, McFadden et al. 2010). El mayor problema recae en la complejidad de establecer valores umbrales apropiados para discriminar entre variaciones intraespecíficas y divergencia interespecífica; la cual en algunos casos puede ser extremadamente baja o incluso inexistente (Shearer & Coffroth 2008). Una solución habitual a este problema ha sido concatenar múltiples loci en una única matriz para luego aplicar diferentes procedimientos de inferencia filogenética que permitan recuperar una visión más completa de la historia evolutiva de la especie. Esta aproximación, sin embargo, no necesariamente resuelve la discordancia del árbol de genes y numerosas simulaciones han indicado que datos provenientes de múltiples loci pueden producir variaciones substanciales entre reconstrucciones filogenéticas dependiendo del número de taxa y los marcadores seleccionados (Kubatko & Degnan 2007, Degnan & Rosenberg 2009).

Integrar datos de diversas fuentes de información ha sido usualmente la forma más directa de discernir el número de especies en un área dada (Padial et al. 2010). Sin embargo, esto trae consigo nuevamente el problema inicial de la definición de especies, por lo tanto, recientemente se ha ido estableciendo la idea de que la única propiedad necesaria que define a una especie es que estas conformen linajes de metapoblaciones que están evolucionando separadamente (De Queiroz 2007). Toda información adicional es entonces entendida como “criterios secundarios de especies”, o evidencia empírica de la divergencia efectiva de dos linajes (e.g. aislamiento reproductivo, identidad morfológica, monofilia reciproca). Dado que estos rasgos se desarrollan evolutivamente a distintos tiempos, surge entonces la pregunta de dónde se distinguen especies distintas a través de este continuo de divergencia. De Queiroz (2007) enfatizó que el punto aquí no es que la presencia de un único carácter garantice que las poblaciones que porten esa propiedad representen linajes distintos (i. e. especies) sino más bien que la presencia de una sola propiedad constituye evidencia—la cual es siempre falseable—en favor de esa hipótesis particular. Aun así, si aceptamos la idea de que la evolución solo ocurre cuando existen cambios significativos en las frecuencias alélicas entre dos o más poblaciones (sensu Dobzhansky 1937), el proceso de especiación debiese estar primariamente modulado a escala microevolutiva por variaciones en el genoma. Usando estos caracteres como punto de partida de la divergencia intraespecífica, herramientas como los RAD tags son excepcionalmente útiles para determinar grupos genotípicos en linajes estrechamente emparentados (ver también Mallet 1995). Pante et al. (2014), por ejemplo, demostró que la información RAD ayudó a clarificar interpretaciones previas de marcadores mitocondriales al descubrir una gran diversidad oculta en octocorales de aguas profundas. Más aun, métodos basados en la teoría coalescente han sido exitosos no solo para delimitar especies en ausencia de monofilia recíproca, sino también integrando modelos probabilísticos durante la evaluación de diversas hipótesis en competencia (Knowles & Carstens 2007, Yang & Rannala 2010, Fujita et al. 2012, Leache et al. 2014). Estas nuevas aproximaciones están entregando una visión cada vez más completa de la especiación, siendo una opción interesante para estudiar los ‘límites’ entre especies bajo un marco taxonómico integrativo.

Evaluación de la biodiversidad en Chile

Estudios que permitan estimar la riqueza de especies son esenciales bajo la acelerada pérdida de biodiversidad actual (Singh 2002). Esto es especialmente importante en países como

Chile donde dentro del territorio se cuentan con altos niveles de endemismo dada la extraordinaria heterogeneidad ambiental (Simonetti 1999, Lazo et al. 2008). De acuerdo con los últimos informes de desempeño ambiental realizado por la Organización para la Cooperación y el Desarrollo Económico (OCDE), uno de los mayores problemas para la conservación de la diversidad biológica de Chile es la falta de información con respecto a las especies, su estado de vulnerabilidad y función ecosistémica. Para enfrentar este problema, recientemente fue aprobado el proyecto que crea el Servicio de Biodiversidad y Áreas Protegidas, el cual tiene por objetivo coordinar el esfuerzo conjunto de agencias gubernamentales e instituciones académicas a fin de levantar una línea base de conocimiento científico sobre esta materia (incluyendo el desarrollo de metodologías efectivas para definir poblaciones naturales y la elaboración de un completo inventario nacional de especies).

Los invertebrados representan probablemente el mayor desafío en esta tarea, especialmente aquellos que viven en ambientes acuáticos. Lazo et al. (2008) estimó que aproximadamente existen 170.000 nuevas especies por describir en Chile y más de la mitad de ellas serian artrópodos. En tales grupos es particularmente complejo determinar la riqueza de especies ya que los criterios taxonómicos empleados habitualmente para distinguir especies dependen de la experiencia de unos pocos especialistas. Más aun, poco esfuerzo ha sido destinado a investigar los patrones y procesos que dan origen a la diversidad de invertebrados. Eventos de divergencia reciente son apenas rastreables por medio de caracteres morfológicos y de hecho estos difícilmente sirven para detectar especies cripticas. Estudios genéticos han identificado, por ejemplo, altos niveles de divergencia en poblaciones de esponjas marinas y crustáceos aun cuando *a priori* no existe una clara diferenciación fenotípica en sus linajes (Silva de Paula et al. 2012, Varela & Haye 2012). Estos tipos de análisis en Chile han ido surgiendo solo recientemente, por lo tanto no son muchos los publicados específicamente para delimitar especies. Esto se vuelve aún más crítico en organismos no-comerciales, para los cuales estudios sobre biología evolutiva siguen siendo extremadamente escasos, sobretodo en animales marinos.

¿Qué es una especie en anémonas de mar?

De los 25 phyla de invertebrados que habitan en las costas chilenas, los cnidarios están entre los menos estudiados en términos de investigación científica. Este grupo se caracteriza fundamentalmente por producir unas capsulas celulares altamente especializadas denominadas cnidocistos (Fautin 1982); e incluyen cinco clases (Anthozoa, Cubozoa, Hydrozoa, Scyphozoa y Staurozoa) más dos clados parásitos (Polypodiozoa and Myxozoa) cuya ubicación filogenética está siendo actualmente discutida. El gran polimorfismo de los cnidarios ha obstaculizado tradicionalmente la determinación taxonómica de su diversidad biológica. Estimaciones recientes indican que más de la mitad de los cnidarios se encuentran como pólipos sésiles (e.g. anémonas de mar y corales), cerca del 18% como parásitos acuáticos y solo el 2% como medusas de vida libre (Crowther, 2011; Okamura et al., 2015). Específicamente dentro del grupo de los hexacorales de cuerpo blando, las anémonas de mar (orden Actiniaria) son excepcionalmente diversas y pueden ser halladas en todos los océanos y hábitats, desde aguas profundas hasta estuarios (Häussermann & Försterra 2009). Existen aproximadamente unas 60 especies descritas para Chile continental, no obstante, dos de ellas representan aparentemente registros no válidos y 19 están actualmente en estado de incerteza taxonómica (Häussermann 2006, Häussermann & Försterra 2009). Por otra parte, un alto porcentaje de las especies reportadas en la literatura no han sido encontradas regularmente o solo han sido descritas para aguas profundas. En general, se desconoce mucho de la actinofauna de Chile y la revisión de varios géneros y familias es bastante necesaria para poder avanzar hacia estimaciones más robustas de la riqueza de especies dentro del territorio nacional (Häussermann & Försterra 2009).

Las anémonas de mar han sido históricamente difíciles de estudiar debido a su relativamente simple anatomía y aparente plasticidad fenotípica. Algunos de estos problemas se han arrastrado hasta el día de hoy y varias características morfológicas que han sostenido tradicionalmente las diferencias entre especies y géneros han sido extensamente cuestionadas a raíz de su notable variabilidad anatómica (e.g. Spano et al. 2013). Las filogenias moleculares han reforzado recientemente esta idea, típicamente recuperando grupos poli- y parafileticos bajo la clasificación taxonómica actual para numerosas familias de actiniarios (Daly et al. 2008, Rodriguez et al. 2014). A pesar de las recurrentes convergencias morfológicas, la evolución molecular en antozoos es por el contrario bastante conservativa (al menos en secuencias de ADN). Estudios en corales no solo han demostrado que los genes mitocondriales evolucionan mucho más lento que los nucleares, sino que también las tasas de sustitución nucleotídica es cientos de veces más lenta que en la mayoría de los animales bilaterales (Hellberg 2006). Bajas tasas de sustitución nucleotídica se traducen en bajos niveles de variación intraespecífica; por lo tanto, estudios filogenéticos basados en pocos genes estarían restringidos para poder delimitar linajes de anémonas estrechamente relacionadas. Asumiendo que la especiación se da mayoritariamente por deriva génica, la ausencia de monofilias reciprocas podría deberse a la repartición incompleta de linajes cuya evolución es bastante lenta. Desde esta perspectiva, incluso con poco flujo génico, la retención de polimorfismos ancestrales podría explicar en buena medida la falta de diferenciación entre genotipos específicos (Freeland et al. 2011).

La estructuración geográfica ha demostrado ser uno de los patrones más consistentes de las ultimas filogenias moleculares en Actiniaria (e.g. Larson & Daly 2016). A diferencia de la mayoría de los rasgos diagnósticos externos, la proximidad espacial tiende a verse reflejada en mayor o menor medida en varios grupos de anémonas. Por ejemplo, Daly (2004) reveló que el género *Anthopleura* no es monofiletico con respecto a *Bunodosoma* pero si con respecto a su distribución, recuperando un clado con especies propias del Hemisferio Norte y otro clado con especies cosmopolitas y propias del Hemisferio Sur. La identificación de estos géneros depende casi exclusivamente de una serie de protrusiones en la columna, característica que ha sido reiteradamente discutida en relación a su estructura y posible función ecológica (ver Häussermann 2004). Esto es aún más controversial a nivel de especies a medida que las características diagnósticas parecen volverse más lábiles. La pertinencia de diferentes rasgos en la clasificación de Actiniaria representa el marco teórico de este estudio y será particularmente tratado en el primer capítulo de la presente Tesis Doctoral. Con este objetivo, las especies cripticas serán consideradas *a priori* como metapoblaciones para así poder estudiar sus cambios evolutivos desde un punto de vista sub-especifico mientras que se mantiene la noción de que las poblaciones podrían estar interactuando entre sí (Hanski & Gaggiotti 2004). El efecto de la dinámica local en la estructura genética metapoblacional y por tanto, en el proceso de especiación, puede ser de hecho bastante importante dependiendo del nivel de flujo génico. En este sentido, una aproximación metapoblacional—así como fue aplicada originalmente en el concepto unificado de especies—puede proveer un marco más apropiados para estudiar los cambios microevolutivos que determinan la divergencia de los linajes, y a partir de allí evaluar, por ejemplo, cuan genéticamente relacionados están las poblaciones locales o si acaso sus diferencias son neutrales o no.

El problema a estudiar

Los complejos de especies presentan un buen modelo de estudio para evaluar los cambios que ocurren durante la separación de dos linajes. Este es precisamente el contexto en el cual se desenvuelve la presente Tesis Doctoral y más específicamente en torno a los criterios taxonómicos que debiesen ser considerados para establecer especies diferentes en Actiniaria (ver también Padial et al. 2010). Knowlton (1993) señaló que los complejos de especies no solo son particularmente profusos en el océano sino que también en la especiación intervienen más factores debido a las propiedades del ambiente marino (e.g. dispersión en tres ejes). En la mayoría de los casos los complejos de especies surgen cuando las especies comprendidas en el grupo difícilmente son distinguibles basándose exclusivamente en caracteres morfológicos (Mayr & Ashlock 1991). En las anémonas de mar, por ejemplo, esto se explica en buena medida por su particular anatomía (i.e. ausencia de estructura solidas como esqueleto, espinas o espículas); aunque también los artefactos producto de la preservación son relativamente comunes entre las descripciones de especies. Por otra parte, muchos de los criterios empleados para la identificación de especies aparecen gradualmente a través del tiempo y las tasas relativas a las cuales ocurren estos cambios son heterogéneas y clado-especificas (e.g. Schlick-Steiner et al. 2010). Siguiendo la analogía del continuo de divergencia entre dos linajes, antes de la adquisición del primer atributo diferencial, todos estarían de acuerdo con que existe una sola especie, y después de la adquisición del último atributo diferencial, todos estarían de acuerdo que hay dos especies (de Queiroz 2007). Es justo entremedio donde van a haber desacuerdos y es precisamente allí donde la delimitación de especies se complejiza.

El género de anémonas *Anthothoe* pertenece a la familia Sagartiidae y comprende 12 especies, ocho de las cuales viven exclusivamente en el Hemisferio Sur (Excoffon et al. 1997, Fautin 2013). De ellas, *Anthothoe chilensis* (Lesson, 1830) ha sido particularmente indicada como un complejo de especies de amplia distribución en Sudamérica. Esta abundante especies cubre densamente grandes áreas desde Perú (12°S) hasta la Península de Taitao (46°S), pudiendo ser observada desde pozas intermareales hasta los 60 metros de profundidad (Paredes et al. 1999, Häussermann & Försterra 2009). De acuerdo con su historia sinonímica, *A. chilensis* también ha sido reportada en varias localidades del Océano Atlántico Austral, incluyendo las islas de South Georgia, Mar del Plata, Rio de Janeiro y Sudáfrica (Excoffon et al. 1997, Häussermann & Försterra 2009). Por otra parte, *A. chilensis* también es morfológicamente variable, aunque algunos autores han sugerido que los especímenes del Arco de Escocia y Argentina posiblemente pertenezcan a una especie diferente (Excoffon et al. 1997, Häussermann & Försterra 2009). Esto también podría aplicarse a los especímenes de Sudáfrica, los cuales fueron originalmente descritos como *Anthothoe stimpsoni* (Verrill, 1869) para luego ser sinonimizados por Excoffon et al. (1997) basándose en las exiguas diferencias observadas entre el material tipo y *A. chilensis*. Otras especies del Pacífico Sur tales como *Anthothoe albocincta* (Hutton, 1879), *A. vagrans* (Stuckey, 1909) y *A. australiensis* Carlgren, 1950 también han sido descritas con muy pocas diferencias (a veces incluso sin ninguna aparente), sin embargo, estas siguen siendo consideradas validas dado que en muchos casos los registros taxonómicos son extremadamente pobres.

El segundo capítulo de la presente Tesis Doctoral examinara precisamente los patrones de divergencia genómica en las especies de *Anthothoe* del Hemisferio Sur. Estos resultados serán contrastados con las clasificaciones taxonómicas actuales las cuales están basadas principalmente en rasgos morfológicos externos tales como los patrones de coloración. La distribución de los linajes así como la topología del árbol de especies serán analizadas posteriormente a la luz de distintos procesos biogeográficos, tanto históricos como contemporáneos. De esta manera el presente estudio no solo pondrá a prueba las hipótesis de especies en el complejo *Anthothoe*, sino además evaluara distintas hipótesis biogeográficas que explicarían potencialmente el patrón de divergencia para este grupo de anémonas de mar. Finalmente, se examinara si la diferenciación genética entre los linajes de *Anthothoe* responde o no a procesos de adaptación local, alineando aquellos genes putativamente bajo selección con los genomas de referencia disponibles y caracterizando las funciones biológicas a las cuales estarían asociados.

Referencias

Agapow PM, ORP Bininda-Emonds, KA Crandall, JL Gittleman, GM Mace, JC Marshall & A Purvis. 2004. The impact of species concept on biodiversity studies. The Quarterly Review of Biology 79(2): 161-179.

Allendorf FW, PA Hohenlohe & G Luikart. 2010. Genomics and the future of conservation genetics. Nature Reviews 11: 697-709.

Baird NA, PD Etter , TS Atwood, MC Currey, AL Shiver, ZA Lewis, EU Selker, WA Cresko & EA Johnson. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3:e3376.

Cracraft J. 1983. Species concepts and speciation analysis. Current Ornithology 1:159–187.

Crowther AL. 2011. Class Anthozoa Ehrenberg, 1834. En: Zhang ZQ (ed). Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness, pp. 19-23. Magnolia Press, Zootaxa.

Daly M. 2004. Phylogeny and biogeography of *Anthopleura* in the North Atlantic Ocean. Hydrobiologia. 530/531: 241-248.

Daly M, A Chaudhuri, L Gusmão & E Rodriguez. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). Molecular Phylogenetics and Evolution 48: 292–301.

De Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology 56(6):879–886.

Degnan JH & NA Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends in Ecology and Evolution 24 (6): 332-340.

Dobzhansky T. 1937. Genetics and the origin of species, 364pp. Columbia University Press, Columbia.

Excoffon AC, MJC Belém, MA Zamponi & E Schlenz. 1997. The validity of *Anthothoe chilensis* (Actiniaria, Sagartiidae) and its distribution in Southern Hemisphere. Iheringia 82: 107-108.

Freeland JR, SD Petersen & H Kirk. 2011. Molecular ecology, 464 pp. Wiley-Blackwell, The Atrium, Southern Gate, Chichester, West Sussex.

Fautin D. 1982. Cnidaria: En: Parker SP (ed). Synopsis and classification of living organism, pp. 669-706. McGraw-Hill, New York.

Fautin DG. 2013. Hexacorallians of the World. Available from:
http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm (accessed 25 april 2016).

Fujita MK, AD Leache, FT Burbrink, JA McGuire & C Moritz. 2012. Coalescent-based species delimitation in an integrative taxonomy. Trends in Ecology and Evolution 27(9): 480-488.

Futuyma DJ. 2009. Evolution, 656pp. Sinauer Associates Inc, Sunderland.

Gili JM, WE Arntz, A Palanques, C Orejas, A Clarke, PK Dayton, E Isla, N Teixido, S Rossi, PJ Lopez-Gonzalez. 2006. A unique assemblage of epibenthic sessile suspension feeders with archaic features in the High Antarctic. Deep-Sea Research Part II 53, 1029–1052.

Hanski I & OE Gaggiotti. 2004. Metapopulation biology: Past, present, and future. In: Hanski I & OE Gaggiotti (eds). Ecology, genetics, and evolution of metapopulations, pp 3-22. Elsevier, Amsterdam.

Häussermann V. 2004. Re-description of *Phymactis papillosa* (Lesson, 1830) and *Phymanthea pluvia* (Drayton in Dana, 1846) (Cnidaria: Anthozoa), two common actiniid sea anemones from the south east Pacific with a discussion of related genera. Zoologische Mededelingen 78(23): 345-381.

Häussermann V. 2006. Biodiversity of Chilean sea anemones (Cnidaria: Anthozoa): distribution patterns and biogeographic implications; including new records for the fjord region. Investigaciones Marinas 34: 23-35.

Häussermann V & G Forsterra. 2005. Distribution patterns of Chilean shallow-water sea anemones (Cnidaria: Anthozoa: Actiniaria, Corallimorpharia); with a discussion of the taxonomic and zoogeographic relationships between the actinofauna of the South East Pacific, the South West Atlantic and Antarctica. Scientia Marina 69: 91-102.

Häussermann V & G Forsterra. 2009. Marine Benthic Fauna of Chilean Patagonia, 1000 pp. Nature in Focus, Puerto Montt.

Head M. 2005. Towards a panbiogeography of the seas. Biological Journal of the Linnean Society 84: 675-723.

Hebert PDN, A Cywinska, SL Ball and JR deWaard. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270: 313-321.

Hellberg ME. 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evolutionary Biology 6: 24.

Hey J. 2006. On the failure of modern species concepts. Trends in Ecology and Evolution 21(8): 447-450.

Highton R. 2000. Detecting cryptic species using allozyme data. In: Bruce RC, RG Jaeger & LD Houck (eds). The biology of plethodontid salamanders, pp 215-241. Kluwer Academic/Plenum Publishers, New York.

Hohenlohe PA, J Catchen & WA Cresko. 2012. Population genomic analysis of model and nonmodel organisms using sequenced RAD tags. In: Pompanon F & A Bonin (eds). Data production and analysis in population genomics: methods and protocols, pp 235-260. Human Press, New York

Knowles LL & BC Carstens. 2007. Delimiting species without monophyletic gene trees. Systematic Biology 56(6): 887-895.

Knowlton N. 1993. Sibling species in the sea. Annual Review of Ecology and Systematics 24: 189-216.

Kubatko LS & JH Degnan. 2007. Inconsistency of phylogenetic estimates from concatenated data under coalescence. Systematic Biology 56(1): 17-24.

Larson PG & M Daly. 2016. Phylogenetic analysis reveals an evolutionary transition from internal to external brooding in Epiactis Verrill (Cnidaria: Anthozoa: Actiniaria) and rejects the validity of the genus *Cnidopus* Carlgren. Molecular Phylogenetics and Evolution 94: 548–558.

Lazo I, R Gibocchio, H. Cofré, Y Vilina & A Iriarte. 2008. Nuestra diversidad biológica. En: Rovira J, J Ugalde & M. Stutzin (eds). Biodiversidad de Chile: patrimonio y desafíos, pp. 49-55. CONAMA, Santiago.

Leache AD, MK Fujita, VN Minin & RR Bouckaert. 2014. Species delimitation using genome-wide SNP data. Systematic Biology 63(4): 534-542.

Leprieur F, P Descombes, T Gaboriau, PF Cowman, V Parravicini, M Kulbicki, CJ Melian, CN de Santana, C Heine, D Mouillot, DR Bellwood & L Pellissier. 2016. Plate tectonics drive tropical reef biodiversity dynamics. Nature Communications |7:11461| DOI: 10.1038/ncomms11461.

Maddison WP. 1997. Gene trees in species trees. Systematic Biology 46(3): 523-536.

Mallet J. 1995. A species definition for the modern synthesis. Trends in Ecology and Evolution 10: 294–299.

Mayden RL. 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge MF, HA Dawah & MR Wilson (eds). Species: the units of biodiversity, pp 381-424. Chapman & Hall, London.

Mayr E & Ashlock PD. 1991. Principles of Systematic Zoology, 416 pp. McGraw-Hill, New York.

McFadden CS, Y Benayahu, E Pante, JN Thoma, PA Nevarez & SC France. 2010. Limitations of mitochondrial gene barcoding in Octocorallia. Molecular Ecology Resources doi: 10.1111/j.1755-0998.2010.02875.x.

Miller MR, JP Dunham, A Amores, WA Cresko & EA Johnson. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. Genome Research 17: 240–248.

Moran P & I Kornfield. 1993. Retention of an ancestral polymorphism in the Mbuna species flock (Teleostei: Cichlidae) of Lake Malawi. Molecular Biology and Evolution 10(5): 1015-1029.

Nichols R. 2001. Gene trees and species trees are not the same. Trends in Ecology & Evolution 16(7): 358-364.

Okamura B, A Gruhl & JL Bartholomew. 2015. An introduction to myxozoan evolution, ecology and development. In: Okamura B, A Gruhl & JL Bartholomew (eds). Myxozoan evolution, ecology and development, pp 1-20. Springer International Publishing, Switzerland.

Padial JM, A Miralles, I De la Riva & M Vences. 2010. The integrative future of taxonomy. Frontiers in Zoology 7:16.

Pante E, J Abdelkrim, A Viricel, D Gey, SC France, MC Boisselier & S Samadi. 2014. Use of RAD sequencing for delimiting species. Heredity: 1-10.

Paredes C, F Cardoso & J Tarazona. 1999. Invertebrados del intermareal rocoso del departamento de Lima, Perú: una lista comentada de especies. Revista Peruana de Biología 6(2): 143-151.

Rodrıguez E, PJ Lopez-Gonzalez, JM Gili. 2007. Biogeography of Antarctic sea anemones (Anthozoa, Actiniaria): What do they tell us about the origin of the Antarctic benthic fauna? Deep-Sea Research II 54: 1876–1904.

Rodriguez E, M Barbeitos, M Daly, LC Gusmao & V Häussermann. 2012. Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). Cladistics 28: 375-392.

Rodriguez E, MS Barbeitos, MR Brugler, LM Crowley, A Grajales, L Gusmao, V Häussermann, A Reft & M Daly. 2014. Hidden among sea anemones: the first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. PLoS ONE 9(5): e96998.

Schlick-Steiner BC, FM Steiner, B Seifert, C Stauffer, E Christian, RH Crozier. 2010. Integrative taxonomy: a multisource approach to exploring Biodiversity. Annual Review of Entomology 55: 421-438.

Shearer TL & MA Coffroth. 2008. Barcoding corals: limited by interspecific divergence, not intraspecific variation. Molecular Ecology Resources 8: 247–255.

Silva de Paula T, C Zilberberg, E Hajdu & G Lôbo-Hajdu. 2012. Morphology and molecules on opposite sides of the diversity gradient: Four cryptic species of the *Cliona celata* (Porifera, Demospongiae) complex in South America revealed by mitochondrial and nuclear markers. Molecular Phylogenetics and Evolution 62: 529–541.

Simonetti JA. 1999. Diversity and conservation of terrestrial vertebrates in Mediterranean Chile. Revista Chilena de Historia Natural 72: 493-500.

Singh JS. 2002. The biodiversity crisis: a multifaceted review. Current Science 82(6): 638-647.

Spalding MD, HE Fox, GR Allen, N Davidson, ZA Ferdaña, M Finlayson, BS Halpern, MA Jorge, A Lombana, SA Lourie, KD Martin, E McManus, J Molnar, CA Recchia & J Robertson. 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. BioScience 57(7): 573-583.

Spano C, N Rozbaczylo, V Häussermann & R Bravo. 2013. Redescription of the sea anemones *Anthopleura hermaphroditica* and *Bunodactis hermafroditica* (Cnidaria: Anthozoa: Actiniaria) from Chile. Revista de Biologia Marina y Oceanografia 48(3): 521-534.

Varela AI & PA Haye. 2012. The marine brooder *Excirolana braziliensis* (Crustacea: Isopoda) is also a complex of cryptic species on the coast of Chile. Revista Chilena de Historia Natural 85: 495-502.

Yang Z & B Rannala. 2010. Bayesian species delimitation using multilocus sequence data. PNAS 107(20): 9264-9269.

CAPÍTULO 1

Species delimitation in sea anemones (Anthozoa: Actiniaria): Present perspective of an old problem

Species delimitation in sea anemones (Anthozoa: Actiniaria): Present perspective of an old problem

Carlos A. Spano[1], Cristian B. Canales-Aguirre[2, 3], Selim S. Musleh[1, 3], Vreni Häussermann[4, 5] and Daniel Gomez-Uchida[1, 3]

[1] Genomics in Ecology, Evolution, and Conservation Laboratory, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, P.O. Box 160-C, Concepción, Chile.

[2] Centro i~mar, Universidad de Los Lagos, Camino a Chinquihue km. 6, Puerto Montt, Chile

[3] Nucleo Milenio de Salmonidos Invasores (INVASAL), Concepción, Chile

[4] Huinay Scientific Field Station, P.O. Box 462, Puerto Montt, Chile

[5] Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Avda. Brasil 2950, Valparaíso, Chile

(Submitted to Invertebrate Systematics)

Abstract

Sea anemones (order Actiniaria) constitute a relatively well defined group within the phylum Cnidaria and are characterized as soft-bodied, generally solitary polyps that inhabit marine environments worldwide. During the last two centuries, morphology-based taxonomy permitted the proliferation of species descriptions given the conspicuous phenotypic variability of these seemingly simple animals. The molecular revolution and the debate over the species concept led in recent decades to re-evaluate the species hypothesis within many taxa, including Actiniaria. The problem in this group, however, is accentuated by the inadequacy of most taxonomic descriptions, high levels of morphological convergences, and the lack of knowledge about the structure and evolution of the diagnostic features presently used for taxa determination. Furthermore, signs of morphological variability at micro-scale has been accumulating for many of the characteristics frequently required to distinguish anemone species. Spatial distribution has been emerging instead as a more reliable variable to explain cryptic

relationships, although it remains unclear how this may fit into species delimitation. Since the modern evolutionary synthesis, integrative methods and statistical model-based techniques have been attempting to disclose the issue through the analysis of the microevolutionary processes behind lineages divergence. Combined with the ever-increasing genomic data and different means to test biogeographic hypotheses, here we offer some guidelines on how these recent advancements may help disentangle historical questions about sea anemones diversity, both for the systematic of the group as well as for the complex issue of their species delimitation.

Introduction

In many aspects sea anemones are quite similar to the terrestrial flowers of which they received their name. The stunning range of shapes and colours makes them attractive not only as an ornamental treat but also has inspired for centuries the imagination of naturalists all around the globe. Since the time of Aristotle, the nature and properties of these polypoid creatures have been repeatedly studied in the light of its extraordinary diversity (Hyman, 1940). Taxonomists soon realized that sea anemones were not plants, but rather, one of the simplest and most ancestral forms of metazoan animals (Linnaeus, 1758; Cuvier, 1817). Most of the body organs that appear later in evolution can be initially depicted on their tissue grade of construction, displaying a remarkable cellular totipotentiality compared to bilaterian organisms (Extavour et al., 2005; Frank et al., 2009). The growing interest to understand the genomic mechanisms underlying the origin of developmental processes has been indeed one of the main drivers of the past decade’s studies on such basal lineages (Ball et al., 2004; Putnam et al., 2007; Genikhovich and Technau, 2009; Sinigaglia et al., 2013). These methodological advances, however, have permeated much more slowly into the systematics of the group, especially concerning the question about what is a species in sea anemones? (i.e. as an evolutionary unit, with its own separate and self-determining role and tendencies; Simpson, 1951). The advent of new bioinformatic and genomic tools may change this before long, enabling us to examine with an unprecedented resolution the evolutionary processes that shaped over time the current biological diversity.

The polymorphic clade Cnidaria

Sea anemones *sensu stricto* belong to the anthozoan order Actiniaria. Although it is a relatively small taxon, they are among the most ecologically successful members of Cnidaria (Daly et al., 2008). This phylum is characterized by the possession of cnidae, organelle-like capsules with eversible tubules used for movement, defence and prey capture (Dunn, 1982; Daly et al., 2007). All and exclusively cnidarians produce cnidae, even though some predators such as nudibranchs may have the ability to acquire and store them within their own cells (Greenwood, 2009). There are several types of cnidae, however, nematocysts are the most common and the only one present in all classes (Mariscal, 1974, 1984; Fautin and Mariscal, 1991). Even though other features have also been proposed as diagnostic (e.g. radial symmetry, planula larva, and polyp stages in development), they all have been ineffective to clearly define the clade Cnidaria (Daly et al., 2007). This is largely caused due to the notable polymorphism of the phylum, i.e. the variety of forms species may present (Hyman, 1940). More than half of cnidarian diversity is found as sessile polyps (e.g. sea anemones, corals, and sea fans); some as a free-swimming medusa (e.g. jellyfish and box jelly); around 18% as parasites (e.g. Myxozoa) and many others showing alternation of generations between corporal phenotypes (e.g. hydrozoans) (Crowther, 2011; Okamura et al., 2015). While phylogenetic studies based on ribosomal RNA genes usually support the subdivision between exclusively polypoid species

(Anthozoa) and those with a medusa stage (Medusozoa) (Daly et al., 2007; Collins, 2009), mitochondrial protein genes have consistently proved otherwise (Kayal et al., 2013). Despite these genetic discrepancies which have lingered over time as an ongoing debate in phylogenetics (Daly et al., 2010; Pratlong et al., 2017), the radiation of the phylum is fairly well established, and some of these results have gradually been integrated into latest classifications (e.g. Ruggiero et al., 2015).

Molecular analyses have been increasingly supporting the basal state of the polypoid form within Cnidaria phylogeny (e.g. Medina et al., 2001; Collins, 2002; Marques and Collins, 2004; Collins et al., 2006). The composition and anatomical layout of polyp' tissues constitutes the first set of diagnostic apomorphies that broadly distinguish the members of the class Anthozoa (e.g. mesenteries, actinopharynx, and siphonoglyphs; Table 1). For instance, the arrangement of the mesenteries initially determines whether polyps belong to subclass Octocorallia (with eight complete mesenteries) or Hexacorallia (usually with a multiple of six paired mesenteries). Order assignments depend afterward on a mosaic of anatomical and biological attributes that in hexacorallians includes exoskeleton formation, growth form, and types of tentacles, among others (Daly et al., 2003). While morphological features are readily recognizable in nearly all cases, they are not mutually exclusive and are typically shared among diagnoses. Yet, most phylogenetic analyses agree that sea anemones (order Actiniaria), stony corals (order Scleractinia), corallimorpharians (order Corallimorpharia), black corals (order Antipatharia) and zoanthids (order Zoanthidea) constitute monophyletic groups, with the tube-dwelling anemones (order Ceriantharia) as the most basal lineage of Hexacorallia (Berntson et al., 1999; Won et al., 2001; Daly et al., 2003; Zapata et al., 2015).

The species problem in sea anemones

Actiniaria comprises most of the soft-bodied, solitary polyps within Hexacorallia. It contains about 1100 valid species which live in benthic environments across all depths and latitudes worldwide (Fautin, 2016). Characterizing the group as well as its members has become more and more difficult to achieve given the common independent evolution of similar features throughout sea anemones phylogeny (Rodríguez et al., 2014). Perhaps the most extreme example of such morphological convergences is presented by the cryptogenic anemone *Relicanthus daphneae*, which unresolved position currently rejects the ultrastructure of the apical end of the nematocysts as an evolutionary novelty (Rodríguez et al., 2014), so far the only synapomorphy proposed for Actiniaria (Reft et al., 2009; Reft and Daly, 2012). Distinctions between taxa become even more complex at lower levels as traditional classifications based exclusively on morphological characters (such as the ones from Stephenson, 1922; and Carlgren, 1949) are usually weakly supported by more recent phylogenetic studies (Rodríguez et al., 2012; Rodríguez et al., 2014; Daly et al., 2017). Most family descriptions built on the absence of features rather than shared derived characters tend to be recovered as non-monophyletic groups and many seemingly close species are currently placed under multiple genera (e.g. Daly et al., 2008; Rodríguez et al., 2008; Rodríguez et al., 2012; Larson and Daly, 2016; Daly et al., 2017). Furthermore, several body traits used as diagnostic features have proven to be highly homoplasious (Rodríguez et al., 2012; Daly et al., 2017), and their expression might be environment-dependent (e.g. Spano et al., 2013). Laboratory experiments have even shown consistent individual differences in behaviour to exogenous factors such as risk levels or social context (Briffa and Greenaway, 2011; Hensley et al., 2012; Rudin and Briffa, 2012). Although highly repeatable responses have also been demonstrated in single celled prokaryotes, sea anemones are so far the simplest Neuralia animals capable to manifest discrete personalities (sensu Briffa and Greenaway, 2011). These differences have been linked to habitat selection, which in turn suggests that these variations could lead to reproductive isolation and, in the long term, the formation of new species by sympatric speciation (Hensley et al., 2012).

Molecular studies have consistently highlighted the inadequacy of the exclusive morphology-based taxonomic keys to classify sea anemones. While the genetic analysis of Rodríguez et al. (2014) rearranged higher-level taxa based on their evolutionary history (Fig. 1), molecular identification is still an unresolved issue among genera and species (Huang et al., 2008; Daly et al., 2010; Dohna and Kochzius, 2016). One of the main problems is that anthozoan cnidarians evolve extremely slow (Shearer et al., 2002; Hellberg, 2006; Huang et al., 2008; Bilewitch and Degnan, 2011); therefore, molecular markers generally become less informative in closer phylogenetic relationships. Numerous studies have overcome the ‘species problem’ in Actiniaria through different genetic approaches using allozymes (e.g. Solé-Cava and Thorpe, 1992; McFadden et al., 1997; Monteiro et al., 1997; Manchenko et al., 2000; Vianna et al., 2003; Schama et al., 2005) and DNA sequencing (e.g. Stoletzki and Schierwater, 2005; Acuña et al., 2007; Gomes et al., 2012; Pereira et al., 2014; Canales-Aguirre et al., 2015; González-Muñoz et al., 2015). More recently, next-generation sequencing tools combined with coalescent-based methods have been steadily gaining ground in species delimitation research, delivering a more comprehensive view of the evolution of genetic variation among populations (Yang and Rannala, 2010; Fujita et al., 2012; Hohenlohe et al., 2012; Leaché et al., 2014).

Here we analyze some of these latest approaches, reviewing the current context of the species problem from three fundamental topics: first, we glanced over their two hundred years of taxonomic history, focusing on when and how species of sea anemones have been described;

second, we revised the problem of species delimitation, its relation with the species concept and its explanation within sea anemones systematics; and third, we summarized recent methodological approaches that address some of these issues and highlighted future directions in which genomic resources can contribute towards a better understanding of the biological patterns that underlie the diversity of Actiniaria.

Taxonomy through time

Old notes on sea anemones show that the stinging capability of cnidarians was the main reason why some earliest zoologists decided to study these plant-like creatures. Aristotle originally named them Acalephae or Cnidae (Gk. *akalephe*, nettle; *cnidos*, thread) based precisely on the sensation that these flesh-like organisms produce upon contact. He also stated (referring to sea anemones) that even when their constitution resembles plants, their ability to detach from the substrate, catch their food and react to stimuli indicate that they must possess some sort of animal nature. Furthermore, by comparing these "intermediate" creatures Aristotle first acknowledged that "*throughout the entire animal scale there is a graduated differentiation in amount of vitality and in capacity for motion*" (Aristotle's Book VIII fide Barnes, 2014). This statement soon became one of the basic principles that ruled the classification of living things; and the reason why several sessile, soft-body animals (including sponges, molluscs and ascidians) were many years later grouped under the name Zoophyta (Gk. *zōon*, animal; *phyton*, plant).

Early classifications

With the advent of microscopy various aspects of this categorization were questioned as the complexity of nature's architecture was gradually uncovered. For example, the studies of Antony van Leeuwenhoek (1632-1723) and Luigi Ferdinando Marsigli (1638-1730) first exposed that corals were not only massive limestone constructions but they were also overwhelmed by minute organic life forms (van Leewenhoeck, 1677; Marsilius, 1725). These organisms were thoroughly examined by Jean-André Peyssonnel (1694-1759) and (on freshwater hydroids) by Abraham Trembley (1710-1784), who not only corroborated their animal nature but also came to establish the concept of polyp and some general groups based on their consistency and number of tentacles (Trembley, 1744; Vandersmissen, 2016). With this background in mind and following the Aristotelian philosophy of a *scala naturae*, Carl Linnaeus (1707-1778) placed all representatives of Cnidaria near the end of his *Systema naturae* and within the class Vermes (Lat. *vermes*, worms). This group picked up virtually all the indeterminate animals at that time, roughly classifying cnidarians based on whether they were solitary or colonial, and whether they had no skeleton, a stony skeleton or a proteinaceous skeleton (Daly et al., 2007). In an attempt to further unravel this heterogeneous cluster, Linnaeus (1758) reemerged the concept Zoophyta considering that organisms such as anthozoans (Gk. *anthos*, flower; *zōon*, animal) may coexist both in the animal as well in the vegetable kingdom. Nevertheless, the first sea anemone he described (the actiniid *Actinia equina*) was assigned at that time to the order Mollusca and characterized in the genus *Priapus* by having an oblong body, a fixed base, and a terminal "dentate" orifice.

The situation of the Vermes class was intensely debated on the coming years and new classification proposals arrive from the hand of the French naturalists Jean-Baptiste Lamarck

(1744-1829) and Georges Cuvier (1769-1832). Although they reached rather different conclusions, both broke down this group based on major distinguishing features such as the body symmetry and the lack of a bony skeletal structure. On his notes on the invertebrate animals (term originally introduced in this period), Lamarck (1801) first established the polyps as a class and assigned the sea anemone *Actinia rufa* to a minor section of members with radial naked body. Additionally, he first coined the concept Radiata (Lat. *radiatus,* ray-like) and applied it to several species with spherical body form such as medusoid cnidarians and echinoderms. On *Le Règne Animal*, Cuvier (1817) acknowledge this term but as a much higher rank, expanding it to all those animals previously known under the name of zoophytes. Unlike Lamarck, Cuvier used the Aristotelian terminology to describe sea anemones as a base-fixed Acalephes, extracting them from the Polypi class which he used mostly for corals, hydroids, and bryozoans. Numerous investigators such as Johann Friedrich Eschscholtz (1793-1831), Michael Sars (1805-1869) and George Johnston (1797-1855) subsequently addressed the Radiata division, succeeding to broadly identify each one of their main zoological components. Among these groups, Heinrich Frey (1822-1890) and Rudolf Leuckart (1822-1898) first proposed the infra-kingdom Coelenterata (Gk. *koilos*, cavity; *enteron*, intestine), in which were also included several members of Spongiaria and Ctenophora (Frey and Leuckart, 1847). Berthold Hatschek (1854-1941) extensively disclosed their anatomical differences and in 1888, he finally split them into separated diagnostically recognizable phyla (Hatschek, 1888). The term Cnidaria was first introduced in that moment and shortly after came to comprise the class Anthozoa (originally described by Christian Ehrenberg in 1834) as well as the ever-increasing group of skeleton-less, solitary polyps.

Descriptions, re-descriptions and multidisciplinarity

Most descriptions of sea anemones were submitted between 1850 and 1950, many of which were carried out through large vessel-based expeditions such as the U.S. Exploring Expedition (1838-1842), the H.M.S. Challenger voyage (1873-1876) and the Danish Ingolf-Expedition (1899-1900) (Fig. 2). The term Actiniaria (or *Actinaria* as originally spelled) was first introduced by James Dana (1813-1895) in his *Structure and Classification of Zoophytes* (1846) as a suborder of Actinoidea (alternative group of Hydroidea) and included all Anthozoa but Alcyonaria (=Octocorallia). Its definition, however, changed over time due to continuous rearrangements of several members that currently belong to the orders Staurozoa and Antipatharia (e.g. Milne-Edwards and Haime, 1857; Haeckel, 1879). It was not until Richard Hertwig's reports (1850-1937) that, based on the previous works of Gosse (1860), Verrill (1864) and Andres (1884), several conspicuous sea anemones were finally characterized under a common dichotomous key. While Hertwig (1882) only came to include thirteen families, this classification accomplished to recognize major morphological characters such as the pedal disc, acontia, and sphincter (Table 1). Perhaps even more important, throughout his work he persistently emphasized the relevance of the internal anatomy to distinguish actiniarians, providing the groundwork for future descriptions of sea anemones (e.g. Danielssen, 1890).

As new species were discovered at an increasingly accelerated rate, Hertwig's classification was eventually enhanced in Thomas Stephenson's (1898-1961) papers. On his guide of *the British Sea Anemones*, Stephenson (1935) thoroughly leads the species identification from external and internal features (e.g. tentacles arrangement and musculature morphology) down to the types of cnidae the species possess (or cnidom), aspect highly improved shortly after by the comprehensive studies of Robert Weill (1902-1980). While Weill

(1934) distrusted the usefulness of cnidae for taxonomy, the growing shortage of diagnostic characters was enough to start requesting the measurements of these microscopic capsules in species descriptions. One of the main advocates to this claim was the Swedish naturalist Oskar Carlgren (1865-1954), whose work was greatly bolstered by Weill's terminology. By mid-20th century, Carlgren (1949) finally published *A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria,* his prime work in the field of zoology and the result of over 50 years of studies in sea anemones. This paper had a massive impact on the taxonomic knowledge of Actiniaria, comprising a total of 38 families, 180 genera and being until today one of the most widely accepted classification of the order.

Beyond specific modifications, the system used to identify sea anemones has remained virtually intact since Carlgren's catalogue. Cnidae typology—which is perhaps what has drawn more attention in the past decades—has gradually been included in new descriptions, even when there is no general consensus over its nomenclature (Cutress, 1955; Schmidt, 1972; Mariscal, 1974; England, 1991; Östman, 2000). Their reliability to differentiate species has also been fairly discussed given that identifications are usually performed on inadequate conditions (often on undischarged capsules from preserved material) and their variability may respond greatly to the own biology of the specimen (Fautin, 1988; Williams, 1996, 1998, 2000; Francis, 2004). Furthermore, there is a growing debate about the suitability of several morphological traits to distinguish families and genera. Although recent studies have managed to rearrange the suborder classification according to phylogenetic reconstructions (e.g. Rodríguez et al., 2014), reciprocal monophyly in many lower-level ranks remains unclear. Widespread genera such as *Anthopleura* and *Epiactis* illustrate this point quite well, at least in a broad sense (Larson and Daly, 2016; Daly et al., 2017). In both cases species tend to gather more likely into well-established geographical groups rather than in visibly related taxonomic clades. Even if each specific member was separated by colour, number of tentacles or columnar outgrowths, there is often too little genetic differentiation to support most species hypotheses (at least based on DNA barcodes such as COI or ITS II and ribosomal markers such as 16S and 28S; Daly et al., 2010; Pereira et al., 2014; Dohna and Kochzius, 2016). This not only may entail several problems for comparing molecular and morphological information, but also raises some critical questions about the relative importance of the geographic variable for Actiniaria alpha taxonomy.

Species concepts and delimitation

It is important to keep in mind that although both aspects may involve related questions, the concept and delimitation of species are separate issues. Given their relevance in biology, the unawareness of this distinction not only has been fuelling a strong debate in the past half-century but also has derived in an unrestrained conceptualization of what species are, along with a multiplicity of approaches for inferring the boundaries between them (Mayden, 1997; Sites Jr and Marshall, 2003; reviewed by Camargo and Sites Jr, 2013). While it is well acknowledged that most of these methods are promising sources of information to cope with the increasingly recurrent cryptic diversity, it is crucial that these data are clearly separated from the species concept we intend to use (Hey, 2006). In an attempt to redress this discussion, de Queiroz (1998) described the “general lineage concept”, joining together several elements (as species definitions) from the modern evolutionary synthesis (e.g. Mayr, 1942; Simpson, 1951). His proposition supported the idea that the different species concepts should be unified under the main common property among definitions, which is basically being separately evolving metapopulation lineages. The general linage concept emphasizes the process by which species arise rather than the evolutionary outcome that we may see today (see Harrison, 1998). To be more specific here, by *lineage* de Queiroz referred to an ancestor-descendant series (not necessarily a clade or monophyletic group) and by *metapopulation* to an inclusive population made up of connected subpopulations (de Queiroz, 2007). All additional information is then understood as "secondary species criteria" as they provide the empiric evidence necessary to prove lineage splitting (e.g. intrinsic reproductive isolation, phenetic distinguishability, and reciprocal monophyly). Since this information will only test delimitation hypotheses instead of conceptual categorizations, this approach leads to a vast new set of questions but mainly around where to establish different species along the divergence continuum of lineage separation (Hey, 2006; Mallet, 2008; Padial et al., 2010).

The Actiniaria conundrum

Delimiting species of sea anemones has always been a particularly complex issue to undertake, especially by traditional means. Stephenson (1928) contended that although it might be an expert task to identify sea anemones from preserved condition, there are undoubtedly valid differences for each species. Though they may be difficult to observe, he was emphatic defending this position at the statement of some other zoologists who came to argued that the rank of genus he applied in sea anemones was likely equivalent to the species that occur in other animal groups (see preface of Carlgren, 1949). The composition and identity of actiniarians is until today an active topic of discussion, becoming an increasingly intricate issue in larger families such as Actiniidae, Actinostolidae, and Sagartiidae (Daly et al., 2007; Rodríguez et al., 2008; Rodríguez et al., 2012; Fautin, 2016). As well as the number of descriptions of new species decreased in recent times, the amount of re-descriptions have drastically augmented over the last couple decades (e.g. Edmands and Fautin, 1991; Arellano and Fautin, 2001; Häussermann, 2004b; Spano et al., 2013). Of the 1427 names of Actiniaria species that currently exist, 1101 are considered valid and nine are unavailable (Fautin, 2016). Using this same database (from Fautin, 2013), it is estimated that about 65% of all valid species contains at least a synonymous name and over 10% are homonyms. While these statistics are inevitably influenced by minor spelling errors in species names, a major portion of the synonyms can be also readily traced to morphological ambiguities in the original diagnoses. Furthermore, the number of species considered valid is likely an overestimate because there is no evidence to prove how many species names actually refer to junior synonyms (Fautin, 2016).

Because of the lack of solid structures, actiniarians taxonomy relies almost exclusively on a handful of body characters that vary in presence and form among groups. They are used nearly indistinctly from suborders to species diagnoses, showing no direct relationship between the taxonomic rank and the biological specificity of the trait (Fig. 3). Of what it is expected to be proportional (i.e. the larger the group, the more evident should be the differences that distinguish them), the current classifications of sea anemones are rather focused on the external anatomy and the arrangement and morphology of mesenteries and muscles (see also Daly et al., 2015). Along with tentacles and columnar outgrowths, these features contain most of the taxonomic information used for actiniarians identification. Nevertheless, few of these characteristics are indeed consistent or indubitably distinct between specimens. For example, both the number of tentacles and mesenteries are completed in cycles as the organism grows, therefore, its range can only be estimated from the smaller and larger polyp found for the species (Stephenson, 1921). Likewise, body structures such as marginal projections or acrorhagi not only become noticeable in adult individuals but also may vary due to environmental or ontogenetic factors (Francis, 1973, 1976; Bigger, 1980; Ayre and Grosberg, 1995, 2005). While the morphology of the musculature may seems to be less problematic, features such as the sphincter muscle has not been exempt from controversies in some critical instances (e.g. Riemann-Zürneck, 1980;

England, 1987; Rodríguez and López-González, 2008; González-Muñoz et al., 2015). Yet, the presence and nature of this trait is one of the few traditional taxonomic characters that actually has phylogenetic signal, apparently linked to its function and the evolutionary context of certain lineages (Daly et al., 2008; Rodríguez and Daly, 2010; Rodríguez et al., 2014). As some other characteristics such as acontia or adhesive verrucae (Table 1), musculature differences are fairly correlated to ecological shifts that might have occurred more than once (for example, adopting a burrowing habitat), therefore, they have been developed and lost several times in the evolutionary history of the group (Rodríguez et al., 2012; Rodríguez et al., 2014).

The key issue for all the examples presented above is that it is still unclear which characters are derived from a shared ancestry (i.e. homologies) and which ones are analogous responses from independent lineages. This distinction, which is pivotal in systematics biology, is particularly difficult to detect in sea anemones for two main reasons: the incomplete descriptions of many taxa, especially those prior to Carlgren (1949); and the lack of knowledge of the structure and evolution of the diagnostic features used. Both problems have been repeatedly highlighted throughout Actiniaria literature, from superfamily studies to genus phylogenies (e.g. Daly et al., 2007; Rodríguez et al., 2012; Daly et al., 2017). The 'description problem' usually stems from anatomical depictions that are insufficiently detailed in some determinant feature, therefore the taxonomic placement of the species bearing that trait is at the very least doubtful. While this issue is well documented, specimen-level revisions are quite difficult to find not only because there are apparently fewer specialists in sea anemones taxonomy (see Fautin, 2016), but also because many species lack of type material. The latter case is particularly critical in those species that have not been recorded ever since their description and yet are still considered valid (e.g. Spano and Häussermann, 2017; APENDICE

2). The effect of these ‘ghost’ species has not been tested neither in the perception of the taxa composition nor in the overall species richness estimated for Actiniaria (Fautin et al., 2013).

The second problem, perhaps even more critical, is related to the morphological features used to classify actiniarians. From the point of view of a systematist, it is expected that any given taxonomic character not only differs between taxa but also recapitulates to some extent the relationship between them (Mayr, 1942). Although relatively few studies have actually evaluated the evolution of diagnostic features in sea anemones, both morphological convergences and character loss seems to be recurrent phenomenon (e.g. Daly et al., 2008; Rodríguez et al., 2012; Rodríguez et al., 2014; Daly et al., 2017). Nevertheless, little is known about the underlying mechanisms behind such evolutionary changes. Phylogenetic inferences partially rely on how these characters and the relationship between them are interpreted (i.e. if they are alternate states of a single homologous structure or are distinct—evolutionarily independent—characters). The case of the acrorhagi may help illustrate this last argument: acrorhagi are prominent projections of the distal column (right below the tentacles) that are densely packed with holotrichous nematocysts. Besides being an important taxonomic feature, acrorhagi also play an important role in intra- and interspecific interactions, varying accordingly to the social setting of the individual (Francis, 1973, 1976; Bigger, 1980; Ayre and Grosberg, 1995, 2005). This character has also been historically confused with “pseudoacrorhagi”, similar structure that may co-occur in the distal column, but without the nematocysts and associated behavior seen in acrorhagi (see Daly, 2003). Since both features are key to identify members of several actiniid genera, Daly et al. (2017) evaluated the evolutionary connection between acrorhagi and pseudoacrorhagi and compared it with other columnar outgrowths such as verrucae and vesicles. They found that even though acrorhagi might be homologous, they are inappropriate for recognizing subgroups within Actiniidae since they behave more like a shared primitive feature than a uniquely derived attribute. On the other hand, this study also pointed out that all species bearing pseudoacrorhagi possess verrucae; which could suggest that pseudoacrorhagi are in fact marginal projections with enlarged verrucae rather than an alternative form of acrorhagi. The point here is not that these characters are useless in systematic, but that the validity to distinguish different taxa must be understood in the light of the evolution of the traits we intend to used. Functional and anatomical studies are deeply needed in this sense, especially because the diversity of taxonomic characters that we use today might be limited or biased in favor to those species that are more accessible to collect.

What is a species in sea anemones?

Delimitation of interspecific units has typically relied on a combination of some of the most variable features that characterize sea anemones such as coloration pattern, reproductive mode, cnidom, and geographic distribution. They have purposely been used given the wide range of character states that specimens may display in nature. Some of these forms, however, might not be a taxon specific trait but rather a recent response of certain populations to endogenous and exogenous agents. In fact, several elements have been linked as secondary sources of phenotypic variation at smaller scale, for example, geographic distribution to different physiological races (Williams, 1973); body mass to cnidae measurements (Francis, 2004); and habitat preferences to colour variability (Sebens and Paine, 1979; Häussermann, 2004b; Stoletzki and Schierwater, 2005). These differences are usually deemed as innate responses of the anemone to ecological factors such as competition for space, food availability or water loss during low tide (e.g. Hart and Crowe, 1977). Stoletzki and Schierwater (2005), for instance, found a strong association between the colour and genetic variants of *Condylactis gigantea* and their capabilities to withstand UV radiation. It is still too early to know for how many generations these changes will last, nevertheless, recent studies in corals have shown that epigenetic variations (i.e. modifications of the gene expression rather than alteration of the genetic code itself) would be at least relevant among clonal individuals (Dimond et al., 2017). The presence of mixed morphs also suggests that some of these phenotypic differences could be inherited by the offspring (e.g. Häussermann, 2004b). If they show to be improvements of a specific character (e.g. red/green morphs to exposed areas), is plausible that those polymorphisms could be maintained by heterosis and likely promote the formation of new species over time (Sebens and Paine, 1979).

The mechanisms of reproductive isolation have been poorly investigated as a leading cause of Actiniaria speciation. Comparatively much more attention has been drawn to the reproductive biology of the group, which is remarkably varied in terms of modes and behaviors (Bocharova and Kozevich, 2011; Larson, 2017). Generally speaking, sea anemones can reproduce asexually by means of pedal laceration, fission, and budding; or sexually, either with external or internal fertilization (Chia, 1976; Bocharova and Kozevich, 2011). Brooding is also possible although it is far less common compared to the free spawning norm (Larson, 2017). Chia (1976) proposed the "poor-man's game" hypothesis to explain the persistence of this rather atypical strategy in some sea anemones, arguing that the retention of the offspring may increase their survival under unfavorable environmental conditions (giving up the "luxury" of dispersal). This premise could also apply to sexual and asexual reproduction as both modes can co-occur in many species, sometimes visibly segregated by habitat (e.g. Sebens, 1983). On a broader scale, phylogenetic studies have shown that at least brooding had multiple independent derivations (Larson and Daly, 2016). The underlying mechanisms that trigger those evolutionary changes are still elusive, however they appear to be a linage-specific outcome of the tradeoff between survival and dispersion (Chia, 1976). The relevance of this hypothesis is that it predicts once again the high historical sensitivity to the variations of the environment. Evidence of sexual plasticity linked to ecological factors are indeed not rare and has been specifically tested in some widespread, potentially invasive species. For example, a study in laboratory-cultured lines of *Aiptasia diaphana* showed that the forms of reproduction that the individuals may adopt (i.e. sexual and asexual) are highly determined by the photoperiod and water temperature (Schlesinger et al., 2010). Even slight differences of sex steroids might significantly alter the breeding strategy of a particular population, allowing the co-occurrence of males, females, and hermaphrodites given the right conditions (Armoza-Zvuloni et al., 2014). Interestingly, this reproductive system has only been reported in plants (see also Dunn, 1975), where hybridization and polyploidy also play an important role in speciation.

Of all the characters mentioned above, none have been more profusely discussed than the cnidae. For some larger groups as the acontiate taxa within Metridioidea (Carlgren, 1949; Rodríguez et al., 2014), nematocysts actually represent a fundamental trait in their taxonomy, therefore comments addressing the issue are recurrent in the literature (Fautin, 1988). The main problem here has been historically related to the identity and classification of these singular, microscopic characters. Several authors have proposed different, sometimes quite conflicting, nomenclatures to account for the vast amount of morphologies nematocysts may display in Hexacorallia (e.g. Cutress, 1955; Schmidt, 1972; Mariscal, 1974; England, 1991; Östman, 2000). A nematocyst basically consists of a capsule with a tubule attached at one end that is tightly coiled inside. The apex of the capsule opens under stimulation and the tubule is rapidly everted towards the outside, typically revealing spines and releasing toxins (Mariscal, 1974; Reft and Daly, 2012). The form of the capsule, the characteristics of the tubule and the kind of spines are some examples of relevant features extracted from the nematocyst that determine to which type they belong. Nevertheless, even with a detailed map of the ultrastructure of the nematocyst, the translation from one nomenclature to another is not simple and thus, many species are currently described under different codes. A typical problem case are the mastigophores or rhabdoids, type of nematocysts in which the tubule is broader near the junction with the capsule, forming an isodiametric section called shaft (see Rodríguez et al., 2012). Mastigophores have been subdivided in multiple ways, primarily in regard to the length and spines of the shaft, and the formation of a cone-shaped invagination (V-notch) only seen while it is undischarged (e.g. Weill, 1934; Schmidt, 1969; England, 1991). Moreover, some characters such as the apical flap of the capsule or the spines of the tubule are only discernible using electron microscopy (Östman, 2000; Reft et al., 2009). This is recommended not only for one but for several specimens as measurements can vary significantly within samples, and some types might not be observed in all individuals. Whether these variations contain phylogenetic signal remains unclear; but although the results tend to be inconclusive, cnidae do seem to carry phylogenetic information at least at higher levels (Reft and Daly, 2012). Rodríguez et al. (2014) confirmed that acontiate actiniarians indeed possess a different type of *p*-rhabdoid with respect to other superfamilies like Actinoidea or Actinostoloidea. Towards closer phylogenetic relationships, however, this pattern becomes blurry as some variation of the cnidae could be heavily influenced by physiological constraints, as has been previously suggested by Robson (1988) and Francis (2004). Given the case, and especially considering all the lack of information about its morphogenesis and variability, the effectiveness of the cnidae to determine closely related species appears to be rather limited.

Genetic metrics of divergence have been helpful to partially settle these issues in diverse genera such as *Actinia* (Solé-Cava and Thorpe, 1992; Monteiro et al., 1997; Schama et al., 2005) and *Anthopleura* (McFadden et al., 1997; Manchenko et al., 2000). In the latter case, for example, significant differences in the allele frequencies have supported the existence of different species among populations with contrasting life-history phenotypes (McFadden et al., 1997). The number of nucleotide substitutions has also become a useful proxy for identifying putative phylogenetic species within cryptogenic taxa like *Anemonia alicemartinae* (Zhang et al., 2013; Canales-Aguirre et al., 2015). Whereas some of these differences are beyond the reach of most molecular markers (Daly et al., 2010), DNA sequences have been highlighting the role of geography in the genetic structure of sea anemones (Larson and Daly, 2016; Daly et al., 2017). In fact, the distribution range (including habitat and ecologies) is thus far the variable that best explains Actiniaria phylogeny in most superfamily clades (Fig. 1). The relationship between the taxonomic diversity and the spatial distribution among lineages may help understand the pattern of evolutionary radiation, giving context to the emergence of particular morphological attributes (Rodríguez et al., 2014). If confirmed not only on major groups but at species level too, this premise could also have a direct repercussion on how we determine actiniarians, especially those that cannot be reliably distinguished based on their external appearance (e.g. sibling or cryptic species). Nevertheless, inferring species boundaries from phylogenetic trees persists as a fundamental problem in evolutionary genetics and therefore, this general observation is still an untested hypothesis for this group.

Integrated approaches and future directions

No single character is able to fully distinguish closely related species because differences clearly associated with interspecific boundaries in some cases might represent intraspecific polymorphisms in others. For example, Bucklin (1985) found out that the biochemical genetic variability of the plumose anemone *Metridium senile* did not relate to geographically distinct populations but rather to what he called ecophenotypes, or morphological forms determined by habitat conditions. The main reason why these sort of variations have led to incompatible species concepts is that they naturally arise at different times during lineage splitting (de Queiroz, 1998, 2007). Gene trees (i.e. phylogenies from DNA sequences or single nucleotide polymorphisms) are especially useful to follow these divergence patterns as they contain different types of mutations—with different rates and phenotypic outcomes—that we can model mathematically. When two species (or populations) first become isolated from one another, they will retain more or less copies of the same ancestral alleles mainly due to these differences. Some gene lineages will consequently appear more closely related among sister species than within recently divergent populations; thus making the phylogeny of genes not always match the phylogeny of the species that carry those genes (reviews by Pamilo and Nei, 1988; Maddison, 1997; Degnan and Rosenberg, 2009).

The multispecies coalescent

Conflicting branching patterns between genes trees and the overall species-level phylogeny (or species tree) is commonly known as the gene tree discordance (Degnan and Rosenberg, 2009). There are many possible causes for this phenomenon including horizontal gene transfer, gene duplication, hybridization, and incomplete lineage sorting (Maddison, 1997). The latter case considers that shared polymorphisms between closely related species are mainly because the process of stochastic sorting of gene lineages (or lineage sorting; Avise et al., 1983) has not yet been completed (Funk and Omland, 2003). This hypothesis not only has been particularly studied in the past decades for its likely occurrence in nature, but also has been extensively modelled using ‘the coalescent’ (Kingman, 1982; Liu et al., 2009). Basically, the coalescent theory sustains that all selectively neutral alleles of a species should eventually lead to their most recent common ancestor by looking backwards in time to the point at which they originally coalesce (i.e. come together). The time that this takes to happen varies enormously among groups but follow an exponential distribution which is quantifiable primarily by the effective population size (Pamilo and Nei, 1988). The relationship between these parameters thus provides a direct link to the coalescent model to infer the species tree from the set of gene trees that give rise to the actual data.

Species trees are indeed the centrepiece for many species delimitation methods. Inferring the dynamics of divergence and the interplay of microevolutionary processes such as gene flow or natural selection has gradually become a crucial requirement for setting boundaries between closely related species. Coalescent theory allows to test species delimitation hypotheses by providing a way to calculate the probability of speciation under different phylogenetic scenarios (Fujita et al., 2012). The 'multispecies coalescent' (MSC) is perhaps the most popular model for this purpose; taking Wright-Fisher' principle of genetic drift and applies it to multiple populations connected by an evolutionary tree. There are several methods that currently use the MSC, which can be broadly grouped into three main categories (Liu et al., 2009; Chifman and Kubatko, 2014): i) Summary-statistics methods, which calculates the species tree by scoring each phylogeny independently for each gene (e.g. Kubatko et al., 2009; Liu et al., 2010; Mirarab et al., 2014); ii) Sequence-based methods, which uses the full data for estimation of the species tree within a Bayesian framework (e.g. Heled and Drummond, 2010; Bryant et al., 2012); and iii) Quartet inference methods, which estimates the species tree by sampling and scoring subsets of four taxa (or quartets) at random from those included in the dataset (e.g. Chifman and Kubatko, 2014). Each approach has been parameterized in several ways, covering most of the range of trade-offs between accuracy, density of markers, and time-consumption.

While many of these methods were primarily designed to harness the growing amount of genetic data (Yang and Rannala, 2010; Chifman and Kubatko, 2014; Leaché et al., 2014), current approaches are being focused on the integration of various traits in a multispecies framework (Edwards and Knowles, 2014; Solis-Lemus et al., 2015). The recent push of an 'integrative taxonomy' attempts precisely to incorporate different disciplines to face the shortcomings that each approach has, given their assumptions (e.g. neutrality or limited gene flow) or inherent subjectivity (e.g. morphological ambiguities) (Dayrat, 2005; Padial et al., 2010; Schlick-Steiner et al., 2010; Pante et al., 2014). This strategy applies to all manner of things (i.e. methods and criteria), and not only to the use of different types of characteristics to establish species boundaries (Pante et al., 2014). For example, even though MSC tools embrace most of the stochastic nature of molecular variation, it cannot fully illuminate all features of lineage divergence in specific situations or without any supporting evidence (Xu and Yang, 2016; Sukumaran and Knowles, 2017). In fact, species delimitation analyses originally arose as a validation step at the discovery of hidden diversity from intraspecific data (Ence and Carstens, 2011; Carstens et al., 2013; Table 2). Methods such as BPP (Yang and Rannala, 2010) or BFD (Leaché et al., 2014) basically provide a common statistical framework for testing delimitation hypotheses in complex groups or that are rooted solely in morphology-based classifications. Nevertheless, MSC methods alone are not designed to discriminate between population- and species-level structuring (Sukumaran and Knowles, 2017). The question of where to draw the line between both units is actually not tested, unless other sources of information are considered.

Testing biogeographic hypotheses for species delimitation

As mentioned above, molecular markers have shown that the spatial location represents a relevant variable for the delimitation of actiniarian lineages. Disjunctive distributions are currently explained by two major biogeographic paradigms: Darwin–Wallace dispersal, in which taxa evolved at a point ‘centre of origin’ and then dispersed away from the area where their ancestors originally occurred; and panbiogeography, in which ancestral taxa had a broad geographic distribution and new species are primarily develop by vicariance or allopatry (i.e. by isolation from each other) over a region (Lomolino et al., 2010). While the discussion about the relative importance of each mechanism in driving speciation is beyond the scope of this paper (but see reviews from de Queiroz, 2005; and Heads, 2005), it is worth mentioning that these processes probably took place at different geological times. On one hand, dispersalists have generally regarded the Pleistocene as the main epoch in which modern animals arose, while panbiogeographers sustain that the modernization of life happened much earlier in history, mostly during the Mesozoic Era (Heads, 2005). The latter hypothesis has been already confirmed in several marine organisms with rich fossil record such as stony corals, seagrasses, and mangroves (McCoy and Heck, 1976; Ellison et al., 1999). Furthermore, recent diversification analyses have highlighted the major role of plate tectonics in the configuration of tropical communities of wrasses and staghorn corals (Leprieur et al., 2016; Spano et al., 2016; APENDICE 1). Determining the exact origin of extant lineages of soft-bodied cnidarians is more difficult, yet phylogenetic analyses and distributional patterns may shed some lights on the most likely period when specific clades diverged. Rodríguez et al. (2007), for instance, suggested that the actiniid genus *Urticinopsis* probably originated before the Gondwanan break-up since it only comprises the species *U. crassa* from South Africa, and *U. antartica* from the Antarctic continental shelf. Moreover, the diversification of sea anemones during the Mesozoic could potentially explain the absence of endemic families in the Southern Ocean (Rodríguez et al., 2007), and the existence of several bipolar genera such as *Actinoscyphia*, *Antipodactis*, *Bolocera*, *Kadosactis*, and *Liponema* (Rodríguez et al., 2009).

There are multiple ways to test biogeographic models as a powerful line of evidence for species delimitation. Nevertheless, most of them use divergence times based on fossils, which are almost nonexistent in sea anemones (but see Han et al., 2010). Although some models may operate without any prior knowledge about historical events (e.g. Ree and Smith, 2008), temporal information other than just the phylogenetic tree and the species ranges are generally required to give reliability to the biogeographic inference. Some approaches may indirectly estimate the likelihoods for each model by associating different biogeographic processes into the delimitation hypotheses themselves; for example, evaluating species assignments that intrinsically considered geographic barriers. MSC methods have indeed shown that taxonomy-informed hypotheses that also incorporate geographic and bathymetric variables tend to have stronger support than those only based on genetic distance models (Herrera and Shank, 2016). In potentially ancient taxa such as sea anemones (Han et al., 2010; Schwaiger et al., 2014), species tree topologies could also be tested under predictions of hierarchical biogeographic patterns (Sanmartín and Ronquist, 2004). This would be the case of the southern Gondwana pattern suggested by Rodríguez et al. (2007) to account for *Urticinopsis* diversity. Event-based methods such as TreeFitter allows to compare area cladograms with organism phylogenies to estimate which biogeographic processes (i.e. vicariance, dispersal, extinction, or within-area speciation) represents the most parsimonious explanations for the co-occurring pattern (Page, 1995; Ronquist, 2003). Sanmartín and Ronquist (2004), for instance, showed that in many animals of the Southern Hemisphere, historical vicariant processes seem to be more ‘fitted’ to the topology of large, well-resolved trees. This study did focused on terrestrial taxa, so the question about whether the pattern is upheld in marine organisms such as intertidal invertebrates remains unexplored.

Levels of gene flow among divergent populations may also constitute a source of valuable information to establish species boundaries. Phylogeographic studies have significantly contributed in this line through different approaches that allow to evaluate alternative hypotheses of how speciation might have occurred. Likelihood-free techniques, and especially approximate Bayesian Computation (ABC) algorithms, have been especially utilized in the last few decades (review by Csilléry et al., 2010); whose greater advantage over other approaches is that they aim to estimate the probability of the pattern (e.g. geographic species hypotheses) based on simulations of the process that possibly underlies it (e.g. vicariance speciation). While these methods have some case-specific limitations (Oaks et al., 2012), ABC tools have been widely used in population genetics and more recently, in species delimitation analyses (Camargo et al., 2012; Turchetto et al., 2014; Resende-Moreira et al., 2017). In fact, ABC can outperform other methods in detecting speciation when there is still some gene flow between lineages (Camargo et al., 2012). Bayesian models can also be adapted to particular scenarios, allowing, for instance, to quantify the support of different biogeographic hypotheses on the allopatric isolation of marine endemic taxa (Hickerson and Meyer, 2008). These approaches have been little explored in cnidarians, however a recent study in gorgonians has shown its practicality for inferring processes of introgression in closely related species (Aurelle et al., 2017). In the same way, decrypting if genes are shared because of hybridization or incomplete lineage sorting is key for inferring the relative importance of external versus internal drivers in sea anemones evolution; therefore, methods that provide a framework to test both types of processes could potentially illuminate where to draw the species borderline (if possible) in complex taxa.

Conclusions and future perspectives

The possibility of tackling long-standing taxonomic questions by integrating multiple sources of information really makes it a very exciting time to study our planet's biodiversity. Within Actiniaria it is estimated that we currently known about 70% of all existing species, and, paradoxically, knowledge is more complete where richness is lowest (i.e. at higher latitudes) (Fautin et al., 2013). Nevertheless, these numbers might be misrepresenting sea anemones true diversity by overlooking numerous morphological ambiguities and taxonomic synonymizations. To some extent DNA may help discriminate these differences, delimiting species over a common evolutionary idea that transcends particular criteria and discipline conceptualizations. Next-generation sequencing technologies have opened the possibility of gathering huge amounts of genetic information across multiple individuals, even in organisms for which few genomic resources presently exist (Hohenlohe et al., 2012). Progress in statistical tools has been closely following this developments, remedying in several cases the gap that exists between taxonomy and phylogenetics, or between processes of genetic variation from a panmictic population to different species.

The study about the nature and identity of sea anemones certainly has come a long way since the times of Aristotle. Unlike the reality faced by earliest zoologists, today there are numerous, publicly available guidelines for the examination, analysis and description of these strikingly polymorphic animals (e.g. Häussermann, 2004a; Spano and Flores, 2013; Garese et al., 2016). Online databases such as 'Hexacorallians of the World' or 'Cnidabase' have also significantly contributed to the transmission of bibliographic, taxonomic, and spatial information worldwide (Fautin, 2013; Garese and Acuña, 2016). Some species have even their genomes sequenced (Putnam et al., 2007; Baumgarten et al., 2015), and there is currently an ongoing project that seeks to expand these types of resources throughout Anthozoa (Quattrini et al., 2017). Overall, previous impediments related to the accessibility and dissemination of knowledge have gradually been replaced by deeper evolutionary questions such as the role of cryptic species in biodiversity assessments (Fišer et al., 2018). The answer to what is a species in sea anemones may actually lay there since cryptogenic taxa seems to be a tremendously underestimated condition in Actiniaria. In fact, despite the low of correspondence with morphological attributes, population differentiation measures tend to vary from moderate to high even among individuals from nearby localities (e.g. Reitzel et al., 2013; Spano et al. 2018). If no additional data indicate that these genealogical units—typically geographically restrained—have differentiated at species level, they should be considered as deep conspecific lineages (de Queiroz, 2007; Padial et al., 2010); and given the case this a prevailing pattern among genera, they also should become over time the working species hypothesis for sea anemones taxonomy.

Acknowledgements

We are very grateful to the doctoral program of Systematics and Biodiversity (*Sistematica y Biodiversidad*) of University of Concepcion for the discussions that contributed to the development of this paper. This work was supported by a CONICYT Doctoral Fellowship to C.A.S. and a FONDECYT postdoctoral grant to C.B.C.A. The research of V.H. was funded by FONDECYT # 1131039 and D.G.U, S.S.M., and, C.B.C.A was supported by Nucleo Milenio INVASAL from Iniciativa Cientifica Milenio, Ministerio de Economia, Fomento y Turismo, Chile. This publication is drawn from the Ph.D. thesis of C.A.S. and represents publication number 152 of Huinay Scientific Field Station.

References

Acuña, F.H., Excoffon, A.C., McKinstry, S.R., Martínez, D.E., 2007. Characterization of *Aulactinia* (Actiniaria: Actiniidae) species from Mar del Plata (Argentina) using morphological and molecular data. Hydrobiologia 592, 249-256.

Andres, A., 1884. Le Attinie. Verlag von Wilhelm Engelmann, Leipzig.

Arellano, S.M., Fautin, D.G., 2001. Redescription and range extension of the sea anemone *Exocoelactis actinostoloides* (Wassilieff, 1908), with revision of genus *Exocoelactis* (Cnidaria, Anthozoa, Actiniaria). Zoosystema 23, 645-658.

Armoza-Zvuloni, R., Kramarsky-Winter, E., Loya, Y., Schlesinger, A., Rosenfeld, H., 2014. Trioecy, a unique breeding strategy in the sea anemone *Aiptasia diaphana* and its association with sex steroids. Biol. Reprod. 90, 122, 121-128.

Aurelle, D., Pivotto, I.D., Malfant, M., Topçu, N.E., Masmoudi, M.B., Chaoui, L., Kara, H.M., Coelho, M.A.G., Castilho, R., Haguenauer, A., 2017. Fuzzy species limits in Mediterranean gorgonians (Cnidaria, Octocorallia): inferences on speciation processes. Zool. Scr.

Avise, J.C., Shapira, J.F., Daniel, S.W., Aquadro, C.F., Lansman, R.A., 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. Mol. Biol. Evol. 1, 38-56.

Ayre, D.J., Grosberg, R.K., 1995. Aggression, habituation, and clonal coexistence in the sea anemone *Anthopleura elegantissima*. Am. Nat. 146, 427-453.

Ayre, D.J., Grosberg, R.K., 2005. Behind anemone lines: factors affecting division of labour in the social cnidarian Anthopleura elegantissima. Anim. Behav. 70, 97-110.

Ball, E.E., Hayward, D.C., Saint, R., Miller, D.J., 2004. A simple plan—cnidarians and the origins of developmental mechanisms. Nat. Rev. Genet. 5, 567-577.

Barnes, J., 2014. Complete Works of Aristotle, The Revised Oxford Translation. Princeton University Press.

Baumgarten, S., Simakov, O., Esherick, L.Y., Liew, Y.J., Lehnert, E.M., Michell, C.T., Li, Y., Hambleton, E.A., Guse, A., Oates, M.E., Gough, J., Weis, V.M., Aranda, M., Pringle, J.R., Voolstra, C.R., 2015. The genome of *Aiptasia*, a sea anemone model for coral symbiosis. Proceedings of the National Academy of Sciences 112, 11893-11898.

Berntson, E.A., France, S.C., Mullineaux, L.S., 1999. Phylogenetic relationships within the Class Anthozoa (Phylum Cnidaria) based on nuclear 18s rDNA sequences. Mol. Phylogen. Evol. 13, 417-433.

Bigger, C.H., 1980. Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones: a recognition of self and not-self. Biological Bulletin 159, 117-134.

Bilewitch, J.P., Degnan, S.M., 2011. A unique horizontal gene transfer event has provided the octocoral mitochondrial genome with an active mismatch repair gene that has potential for an unusual self-contained function. BMC Evol. Biol. 11, 228-228.

Bocharova, E.S., Kozevich, I.A., 2011. Modes of reproduction in sea anemones (Cnidaria, Anthozoa). Biol. Bull. 38, 849-860.

Briffa, M., Greenaway, J., 2011. High *in situ* repeatability of behaviour indicates animal personality in the beadlet anemone *Actinia equina*(Cnidaria). PLoS Biol. 6, e21963.

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N.A., RoyChoudhury, A., 2012. Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. Mol. Biol. Evol. 29, 1917-1932.

Bucklin, A., 1985. Biochemical genetic variation, growth and regeneration of the sea anemone, *Metridium*, of British shores. J. Mar. Biol. Assoc. U.K. 65, 141-157.

Camargo, A., Morando, M., Avila, L.J., Sites, J.W., 2012. Species delimitation with ABC and other coalescent-based methods: A test of accuracy with simulations and an empirical example with lizards of the *Liolaemus darwinii* complex (Squamata: Liolaemidae). Evolution 66, 2834-2849.

Camargo, A., Sites Jr, J.W., 2013. Species delimitation: a decade after the Renaissance. In: Pavlinov, I. (Ed.), The Species Problem - Ongoing Issues. INTECH Open Access Publisher, pp. 225-247.

Canales-Aguirre, C.B., Quiñones, A., Hernández, C.E., Neill, P.E., Brante, A., 2015. Population genetics of the invasive cryptogenic anemone, *Anemonia alicemartinae*, along the southeastern Pacific coast. J. Sea Res. 102, 1-9.

Carlgren, O., 1949. A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. Kungliga Svenska Vetenskapsakademiens Handlingar 1, 1-121.

Carstens, B.C., Pelletier, T.A., Reid, N.M., Satler, J.D., 2013. How to fail at species delimitation. Mol. Ecol. 22, 4369-4383.

Collins, A.G., 2002. Phylogeny of Medusozoa and the evolution of cnidarian life cycles. J. Evol. Biol. 15, 418-432.

Collins, A.G., 2009. Recent insights into cnidarian phylogeny. Smithsonian Contributions to Marine Sciences 38, 139-149.

Collins, A.G., Schuchert, P., Marques, A.C., Jankowski, T., Medina, M., Schierwater, B., 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. Syst. Biol. 55, 97-115.

Crowther, A.L., 2011. Class Anthozoa Ehrenberg, 1834. In: Zhang, Z.Q. (Ed.), Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. Zootaxa, Magnolia Press, pp. 19-23.

Csilléry, K., Blum, M.G.B., Gaggiotti, O., François, O., 2010. Approximate Bayesian computation (ABC) in practice. Trends Ecol. Evol. 25, 410-418.

Cutress, C.E., 1955. An interpretation of the structure and distribution of cnidae in Anthozoa. Systematic Zoology 4, 120-137.

Cuvier, G., 1817. Le Règne Animal. Déterville, Paris.

Chia, F.S., 1976. Sea anemone reproduction: patterns and adaptive radiations. In: Mackie, G.O. (Ed.), Coelenterate Ecology and Behavior. Springer US, Boston, pp. 261-270.

Chifman, J., Kubatko, L., 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30, 3317-3324.

Daly, M., 2003. The anatomy, terminology, and homology of acrorhagi and pseudoacrorhagi in sea anemones. Zoologische Verhandelingen 345, 89-102.

Daly, M., Brugler, M.R., Cartwright, P., Collins, A.G., Dawson, M.N., Fautin, D.G., France, S.C., McFadden, C., Opresko, D.M., Rodríguez, E., 2007. The phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. Zootaxa 1668, 127-182.

Daly, M., Crowley, L.M., Larson, P., Rodríguez, E., Heestand Saucier, E., Fautin, D.G., 2017. Anthopleura and the phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). Organisms Diversity & Evolution 17, 545-564.

Daly, M., Chaudhuri, A., Gusmão, L., Rodríguez, E., 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). Mol. Phylogen. Evol. 48, 292-301.

Daly, M., Endara, L.A., Burleigh, J.G., 2015. Peeking behind the page: using natural language processing to identify and explore the characters used to classify sea anemones. Zool. Anz. 256, 54-60.

Daly, M., Fautin, D.G., Cappola, V.A., 2003. Systematics of the Hexacorallia (Cnidaria: Anthozoa). Zool. J. Linn. Soc. 139, 419-437.

Daly, M., Gusmão, L.C., Reft, A.J., Rodríguez, E., 2010. Phylogenetic signal in mitochondrial and nuclear markers in sea anemones (Cnidaria, Actiniaria). Integr. Comp. Biol. 50, 371-388.

Dana, J.D., 1846. Structure and Classification of Zoophytes Lea and Blanchard, Philadelphia.

Danielssen, D.C., 1890. Actinida. Den Norske Nodrhavs Expedition 1876–1878: Zoologi. Grøndahl and Søn, Christiania, p. 184.

Dayrat, B., 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. 85, 407-415.

de Queiroz, A., 2005. The resurrection of oceanic dispersal in historical biogeography. Trends Ecol. Evol. 20, 68-73.

de Queiroz, K., 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species And Speciation. Oxford University Press, New York, pp. 57-75.

de Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879-886.

Degnan, J.H., Rosenberg, N.A., 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. Trends Ecol. Evol. 24, 332-340.

Dimond, J.L., Gamblewood, S.K., Roberts, S.B., 2017. Genetic and epigenetic insight into morphospecies in a reef coral. Mol. Ecol. 26, 5031–5042.

Dohna, T.A., Kochzius, M., 2016. Obstacles to molecular species identification in sea anemones (Hexacorallia: Actiniaria) with COI, a COI intron, and ITS II. Marine Biodiv. 46, 291-297.

Dunn, D.F., 1975. Gynodioecy in an animal. Nature 253, 528-529.

Dunn, D.F., 1982. Cnidaria. In: Parker, S.P. (Ed.), Synopsis and classification of living organisms. . McGraw-Hill Bokk Company, New York, pp. 669-706.

Edmands, S., Fautin, D.G., 1991. Redescription of *Aulactinia veratra* n. comb. (=*Cnidopus veratra*) (Coelenterata: Actiniaria) from Australia. Rec. West. Aust. Mus. 15, 59-68.

Edwards, D.L., Knowles, L.L., 2014. Species detection and individual assignment in species delimitation: can integrative data increase efficacy? Proc. R. Soc. Lond., Ser. B: Biol. Sci. 281, 20132765.

Ehrenberg, C.G., 1834. Beiträge zur physiologischen Kenntniss der Corallenthiere im allgemeinen, und besonders des Rothen Meeres, nebst einem Versuche zur physiologischen Systematik derselben. Abhandlungen der Königlichen Akademie der Wissenschaften zu Berlin, 225-380.

Ellison, A.M., Farnsworth, E.J., Merkt, R.E., 1999. Origins of mangrove ecosystems and the mangrove biodiversity anomaly. Global Ecol. Biogeogr. 8, 95-115.

Ence, D.D., Carstens, B.C., 2011. SpedeSTEM: a rapid and accurate method for species delimitation. Mol Ecol Resour 11, 473-480.

England, K.W., 1987. Certain Actiniaria (Cnidaria, Anthozoa) from the Red Sea and tropical Indo-Pacific Ocean. Bulletin of the British Museum (Natural History) 53, 205-292.

England, K.W., 1991. Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. Hydrobiologia 216, 691-697.

Extavour, C.G., Pang, K., Matus, D.Q., Martindale, M.Q., 2005. Vasa and nanos expression patterns in a sea anemone and the evolution of bilaterian germ cell specification mechanisms. Evol. Dev. 7, 201-215.

Fautin, D.G., 1988. Importance of nematocysts to actinian taxonomy. In: Lenhoff, H.M. (Ed.), The Biology of Nematocysts. Academic Press, San Diego, pp. 487-500.

Fautin, D.G., 2013. Hexacorallians of the World.

Fautin, D.G., 2016. Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). Zootaxa 4145, 449.

Fautin, D.G., Malarky, L., Soberón, J., 2013. Latitudinal diversity of sea anemones (Cnidaria: Actiniaria). The Biological Bulletin 224, 89-98.

Fautin, D.G., Mariscal, R.N., 1991. Cnidaria: Anthozoa. In: Harrison, F.G., Westfall, J.A., Liss, A. (Eds.), Microscopic Anatomy of Invertebrates. Wiley-Liss, pp. 267-358.

Fišer, C., Robinson, C.T., Malard, F., 2018. Cryptic species as a window into the paradigm shift of the species concept. Mol. Ecol. 27, 613-635.

Francis, L., 1973. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. The Biological Bulletin 144, 73-92.

Francis, L., 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. The Biological Bulletin 150, 361-376.

Francis, L., 2004. Microscaling: why larger anemones have longer cnidae. The Biological Bulletin 207, 116-129.

Frank, U., Plickert, G., Müller, W.A., 2009. Cnidarian interstitial cells: the dawn of stem cell research. Stem Cells in Marine Organisms. Springer, pp. 33-59.

Frey, H., Leuckart, R., 1847. Beiträge zur kenntniss wirbelloser thiere mit besonderer berücksichtigung der fauna des norddeutschen meeres. Friedrich Vieweg & Sohn, Braunschweig.

Fujita, M.K., Leache, A.D., Burbrink, F.T., McGuire, J.A., Moritz, C., 2012. Coalescent-based species delimitation in an integrative taxonomy. Trends Ecol. Evol. 27, 480-488.

Funk, D.J., Omland, K.E., 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics 34, 397-423.

Garese, A., Acuña, F.H., 2016. Cnidabase: Database of the cnidae of sea anemones and corallimorpharians. http://labic.mdp.edu.ar/cnidabase/.

Garese, A., Carrizo, S., Acuña, F.H., 2016. Biometry of sea anemone and corallimorpharian cnidae: statistical distribution and suitable tools for analysis. Zoomorphology 135, 395-404.

Genikhovich, G., Technau, U., 2009. The starlet sea anemone *Nematostella vectensis*: an anthozoan model organism for studies in comparative genomics and functional evolutionary developmental biology. Cold Spring Harbor Monogr. Ser. 2009, pdb. emo129.

Gomes, P.B., Schama, R., Sole-Cava, A.M., 2012. Molecular and morphological evidence that *Phymactis papillos*a from Argentina is, in fact, a new species of the genus *Bunodosoma* (Cnidaria: Actiniidae). J. Mar. Biol. Assoc. U.K. 92, 895-910.

González-Muñoz, R., Simões, N., Mascaró, M., Tello-Musi, J.L., Brugler, M.R., Rodríguez, E., 2015. Morphological and molecular variability of the sea anemone *Phymanthus crucifer* (Cnidaria, Anthozoa, Actiniaria, Actinoidea). J. Mar. Biol. Assoc. U.K. 95, 69-79.

Gosse, P.H., 1860. A History of the British Sea-Anemones and Corals. Van Voorst, London.

Greenwood, P.G., 2009. Acquisition and use of nematocysts by cnidarian predators. Toxicon 54, 1065-1070.

Haeckel, E., 1879. Das System der Medusen. Gustav Fisher, Jena.

Han, J., Kubota, S., Uchida, H., Stanley, G.D.J., Yao, X., Shu, D., Li, Y., Yasui, K., 2010. Tiny sea anemone from the lower Cambrian of China. PLoS ONE 5, e13276.

Harrison, R.G., 1998. Linking evolutionary pattern and process: the relevance of species concept for the study of speciation. In: Howard, D.J., Berlocher, S.H. (Eds.), Endless Forms: Species And Speciation. Oxford University Press, New York, pp. 19-31.

Hart, C.E., Crowe, J.H., 1977. The effect of attached gravel on survival of intertidal anemones. Transactions of the American Microscopical Society 96, 28-41.

Hatschek, B., 1888. Lehrbuch der Zoologie: eine morphologische Übersicht des Thierreiches zur Einführung in das Studium dieser Wissenschaft. Gustav Fischer, Jena.

Häussermann, V., 2004a. Identification and taxonomy of soft-bodied hexacorals exemplified by Chilean sea anemones; including guidelines for sampling, preservation and examination. J. Mar. Biol. Assoc. U.K. 84, 931-936.

Häussermann, V., 2004b. Redescription of *Phymactis papillosa* (Lesson, 1830) and *Phymanthea pluvia* (Drayton in Dana, 1846)(Cnidaria: Anthozoa), two actiniid sea anemones from the south east Pacific with a discussion of the genera *Phymactis, Bunodosoma* and *Phymanthea*. Zoologische Mededelingen 78, 345-381.

Heads, M., 2005. Towards a panbiogeography of the seas. Biol. J. Linn. Soc. 84, 675-723.

Heled, J., Drummond, A.J., 2010. Bayesian inference of species trees from multilocus data. Mol. Biol. Evol. 27, 570-580.

Hellberg, M.E., 2006. No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evol. Biol. 6, 24-24.

Hensley, N.M., Cook, T.C., Lang, M., Petelle, M.B., Blumstein, D.T., 2012. Personality and habitat segregation in giant sea anemones (*Condylactis gigantea*). J. Exp. Mar. Biol. Ecol. 426, 1-4.

Herrera, S., Shank, T.M., 2016. RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. Mol. Phylogen. Evol. 100, 70-79.

Hertwig, R., 1882. Report on the Actiniaria dredged by H.M.S. Challenger during the years 1873-1876. Report on the Scientific Results of the Voyage of the H.M.S. Challenger during the years 1873-76 (Zoology) 6, 1-136.

Hey, J., 2006. On the failure of modern species concepts. Trends Ecol. Evol. 21, 447-450.

Hickerson, M.J., Meyer, C.P., 2008. Testing comparative phylogeographic models of marine vicariance and dispersal using a hierarchical Bayesian approach. BMC Evol. Biol. 8, 322.

Hohenlohe, P.A., Catchen, J., Cresko, W.A., 2012. Population genomic analysis of model and nonmodel organisms using sequenced RAD tags. In: Pompanon, F., Bonin, A. (Eds.), Data Production and Analysis in Population Genomics: Methods and Protocols. Humana Press, Totowa, NJ, pp. 235-260.

Huang, D., Meier, R., Todd, P.A., Chou, L.M., 2008. Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J. Mol. Evol. 66, 167-174.

Hyman, L.H., 1940. Protozoa through Ctenophora. The Invertebrates. McGraw-Hill, New York.

Kayal, E., Roure, B., Philippe, H., Collins, A.G., Lavrov, D.V., 2013. Cnidarian phylogenetic relationships as revealed by mitogenomics. BMC Evol. Biol. 13, 5.

Kingman, J.F.C., 1982. The coalescent. Stochastic Processes and their Applications 13, 235-248.

Kubatko, L., Carstens, B.C., Knowles, L.L., 2009. STEM: species tree estimation using maximum likelihood for gene trees under coalescence. Bioinformatics 25, 971-973.

Lamarck, J.B.P., 1801. Système des Animaux Sans Vertèbres. Deterville, Paris.

Larson, P., 2017. Brooding sea anemones (Cnidaria: Anthozoa: Actiniaria): paragons of diversity in mode, morphology, and maternity. Invertebr. Biol. 136, 92-112.

Larson, P.G., Daly, M., 2016. Phylogenetic analysis reveals an evolutionary transition from internal to external brooding in *Epiactis* Verrill (Cnidaria: Anthozoa: Actiniaria) and rejects the validity of the genus *Cnidopus* Carlgren. Mol. Phylogen. Evol. 94 (B), 548-558.

Leaché, A.D., Fujita, M.K., Minin, V.N., Bouckaert, R.R., 2014. Species delimitation using genome-wide SNP data. Syst. Biol. 63, 534-542.

Leprieur, F., Descombes, P., Gaboriau, T., Cowman, P.F., Parravicini, V., Kulbicki, M., Melian, C.J., de Santana, C.N., Heine, C., Mouillot, D., Bellwood, D.R., Pellissier, L., 2016. Plate tectonics drive tropical reef biodiversity dynamics. Nature Communications 7, 11461.

Linnaeus, C., 1758. Systema Naturae. Regnum Animale. Cura Societatis Zoologiae Germaniae, Leipzig.

Liu, L., Yu, L., Edwards, S.V., 2010. A maximum pseudo-likelihood approach for estimating species trees under the coalescent model. BMC Evol. Biol. 10, 302.

Liu, L., Yu, L., Kubatko, L., Pearl, D.K., Edwards, S.V., 2009. Coalescent methods for estimating phylogenetic trees. Mol. Phylogen. Evol. 53, 320-328.

Lomolino, M.V., Riddle, B.R., Whittaker, R.J., Brown, J.H., 2010. Reconstructing the history of biotas. In: Lomolino, M.V., Riddle, B.R., Whittaker, R.J., Brown, J.H. (Eds.), Biogeography. Sinauer Associates, Inc., Sunderland, USA, pp. 457-507.

Maddison, W.P., 1997. Gene trees in species trees. Syst. Biol. 46, 523-536.

Mallet, J., 2008. Hybridization, ecological races and the nature of species: empirical evidence for the ease of speciation. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 363, 2971-2986.

Manchenko, G.P., Dautova, T.N., Latypov, Y.Y., 2000. High level of genetic divergence between sympatric color morphs of the littoral sea anemone *Anthopleura orientalis* (Anthozoa: Actiniaria). Biochem. Syst. Ecol. 28, 737-750.

Mariscal, R.N., 1974. Nematocysts. In: Muscatine, L., Lenhoff, H.M. (Eds.), Coelenterate Biology: Reviews and New Perspectives. Academic Press, New York, pp. 129-178.

Mariscal, R.N., 1984. Cnidaria: Cnidae. In: Jürgen, R.H., Gedeon, M.A., Sylvia, R.K. (Eds.), Biology of the Integument: Invertebrates. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 57-68.

Marques, A.C., Collins, A.G., 2004. Cladistic analysis of Medusozoa and cnidarian evolution. Invertebr. Biol. 123, 23-42.

Marsilius, L.F., 1725. Histoire physique de la mer par Louis Ferdinand, Comte de Marsilli. Aux De' Pens de la Compagnie, Amsterdam.

Mayden, R.L., 1997. A hierarchy of species concepts: the denouement in the saga of the species problem. In: Claridge, M.F., Dawah, H.A., Wilson, M.R. (Eds.), Species: The Units of Biodiversity. Chapman & Hall, pp. 381-424.

Mayr, E., 1942. Systematics and the origin of species, from the viewpoint of a zoologist. Harvard University Press, Cambridge.

McCoy, E.D., Heck, K.L., 1976. Biogeography of corals, seagrasses, and mangroves: an alternative to the center of origin concept. Syst. Biol. 25, 201-210.

McFadden, S.C., Grosberg, K.R., Cameron, B.B., Karlton, P.D., Secord, D., 1997. Genetic relationships within and between clonal and solitary forms of the sea anemone *Anthopleura elegantissima* revisited: evidence for the existence of two species. Mar. Biol. 128, 127-139.

Medina, M., Collins, A.G., Silberman, J.D., Sogin, M.L., 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. Proc. Natl. Acad. Sci. USA 98, 9707-9712.

Milne-Edwards, H., Haime, J., 1857. Histoire naturelle des Coralliaires ou Polypes proprement dits. Librairie encyclopédique de Roret, Paris.

Mirarab, S., Reaz, R., Bayzid, M.S., Zimmermann, T., Swenson, M.S., Warnow, T., 2014. ASTRAL: genome-scale coalescent-based species tree estimation. Bioinformatics 30, i541-i548.

Monteiro, A.F., Sole-Cava, M.A., Thorpe, P.J., 1997. Extensive genetic divergence between populations of the common intertidal sea anemone *Actinia equina* from Britain, the Mediterranean and the Cape Verde Islands. Mar. Biol. 129, 425-433.

Oaks, J.R., Sukumaran, J., Esselstyn, J.A., Linkem, C.W., Siler, C.D., Holder, M.T., Brown, R.M., 2012. Evidence for climate-driven diversification? A caution for interpreting ABC inferences of simultaneous historical events. Evolution 67, 991-1010.

Okamura, B., Gruhl, A., Bartholomew, J.L., 2015. An introduction to myxozoan evolution, ecology and development. In: Okamura, B., Gruhl, A., Bartholomew, L.J. (Eds.), Myxozoan Evolution, Ecology and Development. Springer International Publishing, Cham, pp. 1-20.

Östman, C., 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. Scientia Marina 64, 31-46.

Padial, J.M., Miralles, A., De la Riva, I., Vences, M., 2010. The integrative future of taxonomy. Frontiers in Zoology 7, 1-14.

Page, R.D.M., 1995. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10, 155-173.

Pamilo, P., Nei, M., 1988. Relationships between gene trees and species trees. Mol. Biol. Evol. 5, 568-583.

Pante, E., Schoelinck, C., Puillandre, N., 2014. From integrative taxonomy to species description: One step beyond. Syst. Biol. 64, 152-160.

Pereira, A.M., Brito, C., Sanches, J., Sousa-Santos, C., Robalo, J.I., 2014. Absence of consistent genetic differentiation among several morphs of *Actinia* (Actiniaria: Actiniidae) occurring in the Portuguese coast. Zootaxa 3893, 595-600.

Pratlong, M., Rancurel, C., Pontarotti, P., Aurelle, D., 2017. Monophyly of Anthozoa (Cnidaria): why do nuclear and mitochondrial phylogenies disagree? Zool. Scr. 46, 363-371.

Putnam, N.H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V.V., 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 317, 86-94.

Quattrini, A., Faircloth, B.C., Duenas, L., Bridge, T., Brugler, M.R., Calixto-Botia, I., DeLeo, D., Foret, S., Herrera, S., Lee, S., Miller, D.J., Prada, C., Radis-Baptista, G., Ramirez-Portilla, C., Sanchez, J., Rodriguez, E., McFadden, C., 2017. Universal target-enrichment baits for anthozoan (Cnidaria) phylogenomics: new approaches to long-standing problems. Mol Ecol Resour.

Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. Syst. Biol. 57, 4-14.

Reft, A.J., Daly, M., 2012. Morphology, distribution, and evolution of apical structure of nematocysts in Hexacorallia. J. Morphol. 273, 121-136.

Reft, A.J., Westfall, J.A., Fautin, D.G., 2009. Formation of the apical flaps in nematocysts of sea anemones (Cnidaria: Actiniaria). The Biological Bulletin 217, 25-34.

Reitzel, A.M., Herrera, S., Layden, M.J., Martindale, M.Q., Shank, T.M., 2013. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Mol. Ecol. 22, 2953-2970.

Resende-Moreira, L.C., Ramos, A.C.S., Scliar, M.O., Silva, R.M., Azevedo, V.C.R., Ciampi, A.Y., Lemos-Filho, J.P., Lovato, M.B., 2017. Gene flow between vicariant tree species: insights into savanna-forest evolutionary relationships. Tree Genet. Genom. 13, 36.

Riemann-Zürneck, K., 1980. Actiniaria des Südwestatlantik V. *Bolocera*, *Isotealia*, *Isosicyonis* (Actiniidae). Mitteilungen aus dem Hamburgischen Zoologischen Museum und Institut 77, 19-33.

Robson, E.A., 1988. Problems of supply and demand for cnidae in Anthozoa. In: Lenhoff, H.M. (Ed.), The Biology of Nematocysts. Academic Press, San Diego, pp. 179–207.

Rodríguez, E., Barbeitos, M.S., Brugler, M.R., Crowley, L.M., Grajales, A., Gusmão, L., Häussermann, V., Reft, A.J., Daly, M., 2014. Hidden among sea anemones: The first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. PLoS Biol. 9, e96998.

Rodríguez, E., Barbeitos, M.S., Daly, M., Gusmão, L.C., Häussermann, V., 2012. Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). Cladistics 28, 375-392.

Rodríguez, E., Castorani, C.N., Daly, M., 2008. Morphological phylogeny of the family Actinostolidae (Anthozoa : Actiniaria) with description of a new genus and species of hydrothermal vent sea anemone redefining the family Actinoscyphiidae. Invertebr. Syst. 22, 439-452.

Rodríguez, E., Daly, M., 2010. Phylogenetic relationships among deep-sea and chemosynthetic sea anemones: Actinoscyphiidae and Actinostolidae (Actiniaria: Mesomyaria). PLoS ONE 5, e10958.

Rodríguez, E., López-González, P.J., 2008. The gastropod-symbiotic sea anemone genus *Isosicyonis* Carlgren, 1927 (Actiniaria: Actiniidae): a new species from the Weddell Sea (Antarctica) that clarifies the taxonomic position of the genus. Scientia Marina 72, 73-86.

Rodríguez, E., López-González, P.J., Daly, M., 2009. New family of sea anemones (Actiniaria, Acontiaria) from deep polar seas. Polar Biol. 32, 703-717.

Rodríguez, E., López-González, P.J., Gili, J.M., 2007. Biogeography of Antarctic sea anemones (Anthozoa, Actiniaria): what do they tell us about the origin of the Antarctic benthic fauna? Deep Sea Research Part II: Topical Studies in Oceanography 54, 1876-1904.

Ronquist, F., 2003. Parsimony analysis of coevolving species associations. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation and Coevolution. University of Chicago Press, Chicago, IL, pp. 22-64.

Rudin, F.S., Briffa, M., 2012. Is boldness a resource-holding potential trait? Fighting prowess and changes in startle response in the sea anemone, Actinia equina. Proc. R. Soc. Lond., Ser. B: Biol. Sci. 279, 1904-1910.

Ruggiero, M.A., Gordon, D.P., Orrell, T.M., Bailly, N., Bourgoin, T., Brusca, R.C., Cavalier-Smith, T., Guiry, M.D., Kirk, P.M., 2015. A higher level classification of all living organisms. PLoS Biol. 10, e0119248.

Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst. Biol. 53, 216-243.

Schama, R., Sole-Cava, A.M., Thorpe, J.P., 2005. Genetic divergence between east and west Atlantic populations of *Actinia* spp. sea anemones (Cnidaria: Actiniidae). Mar. Biol. 146, 435-443.

Schlesinger, A., Kramarsky-Winter, E., Rosenfeld, H., Armoza-Zvoloni, R., Loya, Y., 2010. Sexual plasticity and self-fertilization in the sea anemone *Aiptasia diaphana*. PLoS ONE 5, e11874.

Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E., Crozier, R.H., 2010. Integrative taxonomy: a multisource approach to exploring biodiversity. Annu. Rev. Entomol. 55, 421-438.

Schmidt, H., 1969. Die Nesselkapseln der Aktinien und ihre differentialdiagnostishce Bedeutung. Helgoländer Wissenschaftliche Meeresuntersuchungen 19, 284-317.

Schmidt, H., 1972. Die Nesselkapseln der Anthozoen und ihre Bedeutung fur die phylogenetische Systematik. Helgoländer Wissenschaftliche Meeresuntersuchungen 23, 422-458.

Schwaiger, M., Schönauer, A., Rendeiro, A.F., Pribitzer, C., Schauer, A., Gilles, A.F., Schinko, J.B., Renfer, E., Fredman, D., Technau, U., 2014. Evolutionary conservation of the eumetazoan gene regulatory landscape. Genome Res. 24, 639-650.

Sebens, K.P., 1983. Population dynamics and habitat suitability of the intertidal sea anemones *Anthopleura elegantissima* and *A. xanthogrammica*. Ecol. Monogr. 53, 405-433.

Sebens, K.P., Paine, R.T., 1979. Biogeography of anthozoans along the west coast of South America: habitat, disturbance, and prey availability. Proceedings of the International Symposium of Marine Biogeography and Evolution in the Southern Hemisphere. N. Z. D. S. I. R., Auckland, New Zealand, pp. 219-237.

Shearer, T.L., van Oppen, M.J.H., Romano, S.L., Wörheide, G., 2002. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol. Ecol. 11, 2475-2487.

Simpson, G.G., 1951. The species concept. Evolution 5, 285-298.

Sinigaglia, C., Busengdal, H., Leclere, L., Technau, U., Rentzsch, F., 2013. The bilaterian head patterning gene six3/6 controls aboral domain development in a cnidarian. PLoS Biol. 11, e1001488.

Sites Jr, J.W., Marshall, J.C., 2003. Delimiting species: a Renaissance issue in systematic biology. Trends Ecol. Evol. 18, 462-470.

Solé-Cava, A.M., Thorpe, J.P., 1992. Genetic divergence between colour morphs in populations of the common intertidal sea anemones *Actinia equina* and *A. prasina* (Anthozoa: Actiniaria) in the Isle of Man. Mar. Biol. 112, 243-252.

Solis-Lemus, C., Knowles, L.L., Ane, C., 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. Evolution 69, 492-507.

Spano, C.A., Flores, V., 2013. Staining protocol for the histological study of sea anemones (Anthozoa: Actiniaria) with recommendations for anesthesia and fixation of specimens. Latin American Journal of Aquatic Research 41, 1019-1024.

Spano, C.A., Häussermann, V., 2017. *Anthopleura radians*, a new species of sea anemone (Cnidaria: Actiniaria: Actiniidae) from northern Chile, with comments on other species of the genus from the South Pacific Ocean. Biodiversity and Natural History 3, 1-11.

Spano, C.A., Hernández, C.E., Rivadeneira, M.M., 2016. Evolutionary dispersal drives the latitudinal diversity gradient of stony corals. Ecography 39, 836-843.

Spano, C.A., Rozbaczylo, N., Häussermann, V., Bravo, R., 2013. Redescription of the sea anemones *Anthopleura hermaphroditica* and *Bunodactis hermafroditica* (Cnidaria: Anthozoa: Actiniaria) from Chile. Rev. Biol. Mar. Oceanog. 48, 521-534.

Stephenson, T.A., 1921. On the classification of Actiniaria. Part II. - Consideration of the whole group and its relationships, with special reference to forms not treated in Part I. Quarterly Journal of Microscopical Science 65, 493-576.

Stephenson, T.A., 1922. On the classification of Actiniaria. Part III. - Definitions connected with the forms dealt with in Part II. Quarterly Journal of Microscopical Science 66, 247-319.

Stephenson, T.A., 1928. The British Sea Anemones. The Ray Society, London.

Stephenson, T.A., 1935. The British Sea Anemones. The Ray Society, London.

Stoletzki, N., Schierwater, B., 2005. Genetic and color morph differentiation in the Caribbean sea anemone *Condylactis gigantea*. Mar. Biol. 147, 747-754.

Sukumaran, J., Knowles, L.L., 2017. Multispecies coalescent delimits structure, not species. Proceedings of the National Academy of Sciences 114, 1607–1612.

Trembley, A., 1744. Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce, à bras en forme de cornes. Chez Jean & Herman Verbeek.

Turchetto, C., Fagundes, N.J.R., Segatto, A.L.A., Kuhlemeier, C., Solís Neffa, V.G., Speranza, P.R., Bonatto, S.L., Freitas, L.B., 2014. Diversification in the South American Pampas: the genetic and morphological variation of the widespread *Petunia axillaris* complex (Solanaceae). Mol. Ecol. 23, 374-389.

van Leewenhoeck, A., 1677. Observations, communicated to the publisher by mr. Antony van Leewenhoeck, in a Dutch letter of the 9th of Octob. 1676. Here English'd: Concerning little animals by him observed in rain-well-sea. And snow water; as also in water wherein pepper had lain infused. Philosophical Transactions (1665-1678) 12, 821-831.

Vandersmissen, J., 2016. Experiments and evolving frameworks of scientific exploration: Jean-André Peyssonnel’s work on coral. In: Klemun, M., Spring, U. (Eds.), Expeditions as Experiments: Practising Observation and Documentation. Palgrave Macmillan UK, London, pp. 51-72.

Verrill, A.E., 1864. Revision of the Polypi of the eastern coast of the United States. Memoirs of the Boston Society of Natural History 1, 1-45.

Vianna, P., Schama, R., Russo, C.A.M., 2003. Genetic divergence and isolation by distance in the West Atlantic sea anemone *Actinia bermudensis* (McMurrich, 1889). J. Exp. Mar. Biol. Ecol. 297, 19-30.

Weill, R., 1934. Contribution a l'Étude des Cnidaires et de leurs Nématocystes. Les Presses Universitaires de France, Paris.

Williams, R.B., 1973. Are there physiological races of the sea anemone *Diadumene luciae*? Mar. Biol. 21, 327-330.

Williams, R.B., 1996. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria): statistical parameters and taxonomic relevance. Scientia Marina 60, 339-351.

Williams, R.B., 1998. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance. Scientia Marina 62, 361-372.

Williams, R.B., 2000. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. Scientia Marina 64, 49-68.

Won, J., Rho, B., Song, J., 2001. A phylogenetic study of the Anthozoa (phylum Cnidaria) based on morphological and molecular characters. Coral Reefs 20, 39-50.

Xu, B., Yang, Z., 2016. Challenges in species tree estimation under the multispecies coalescent model. Genetics 204, 1353-1368.

Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. Proc. Natl. Acad. Sci. USA 107, 9264–9269.

Zapata, F., Goetz, F.E., Smith, S.A., Howison, M., Siebert, S., Church, S.H., Sanders, S.M., Ames, C.L., McFadden, C., France, S.C., Daly, M., Collins, A.G., Haddock, S.H.D., Dunn, C.W., Cartwright, P., 2015. Phylogenomic analyses support traditional relationships within Cnidaria. PLoS ONE 10, e0139068.

Zhang, J., Kapli, P., Pavlidis, P., Stamatakis, A., 2013. A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29, 2869-2876.

Table 1.1. Glossary of anatomical terms of taxonomic importance in sea anemones.

Acontium (pl. acontia)	Thin threads attached to one end of the mesenteries that are densely laden with nematocysts
Acrorhagus (pl. acrorhagi)	Marginal structure in which the epidermis contains holotrichous nematocysts
Actinopharynx	Ectodermal channel that connects the mouth to the gastrovascular cavity
Cnida (pl. cnidae)	Intracellular capsules with an attached tubule that characterize the phylum Cnidaria. Cnidae can be grouped into three broad groups: spirocysts (only Anthozoa), nematocysts (all Cnidaria) and ptychocysts (only Ceriantharia)
Marginal projection	Protrusion of the distal part of the body, outside the tentacle crown
Mesentery (pl. mesenteries)	Longitudinal sheets of tissue that extend radially from the body wall (=column) and sub-divide the gastrovascular cavity
Siphonoglyph	Anatomically differentiated, ciliated grooves running down the actinopharynx from the mouth to its proximal end
Pedal disc	Most common form of base of the body which consists of thin plate of tissue with which the animal adheres to stones or other surfaces
Sphincter	Concentration of the circular muscles of the column at or near the margin
Tubercle	Solid, rounded, conical mounds of mesoglea covered by epidermis and often by cuticle. Not adherent
Verruca (pl. verrucae)	More or less ampullaceous, hollow, adhesive evaginations of the body, without nematocyst in their central part

Table 1.2. Species delimitation studies developed in the last decade on some well-known species complexes in Cnidaria sorted by year of appearance.

Species complex	Nº of loci	Discovery approach	Specie tree inference method	Validation approach	Nº of species after delimitation	Reference
Aulactinia marplatensis and *A. reynaudi*	5^{N}	Phylogenetic analysis	None	None	1 species out of 2 species	Acuña et al. (2007)
Symbiotic zoanthids from the wider Caribbean region	$1^{N, M}$	Phylogenetic analysis	None	None	9 species out of 6 species	Swain (2009)
Genus *Pocillopora* from the Tropical Eastern Pacific	$9^{N, M, SSR}$	Structure, Structurama	None	Symbiosis specificity	3 clades out of 8 species	Pinzón and LaJeunesse (2011)
Genus *Stylophora* from the Indo-Pacific	$3^{N, M}$	Phylogenetic analysis, Haploweb	None	Morphology comparison	Unstated (4 clades total)	Flot et al. (2011)
Genus *Physalia* in New Zealand and Australia	$2^{N, M}$	Phylogenetic analysis	BEAST	None	3 clans out of 1 species	Pontin and Cruickshank (2012)
Genus *Porites* from the Caribbean	$10^{N, M}$	Phylogenetic analysis	step up STEM, BEAST	BPP	1 species out of 3 species	Prada et al. (2014)
Genus *Chrysogorgia*	$>10K^{M, SNP}$	Phylogenetic analysis, DAPC	None	None	6 clades out of 9 species	Pante et al. (2014)
Seriatopora hystrix from the Great Barrier Reef	10^{SSR}	Structure	None	Good and Wake (1992) method	4 genetic clusters out of 1 species	Warner et al. (2015)
Genus *Goniopora* from the Saudi Arabian Red Sea	$4^{N, M}$	ABGD, PTP, GMYC	None	Morphology comparison	5 species out of 9 species	Terraneo et al. (2016)
Family Aglaopheniidae	$\sim 30^{N, M, SSR}$	ABGD, PTP, GMYC, DAPC, Structure	None	None	Unstated (but higher than previously known)	Postaire et al. (2016)
Genera *Echinophyllia* and *Oxypora*	$4^{N, M}$	ABGD, PTP, GMYC	BEAST	Morphology comparison	7 species out of 6 species	Arrigoni et al. (2016)
Anthomastus-Corallium clade (genus *Paragorgia*)	$\sim 6K^{M, SNP}$	Phylogenetic analysis	SNAPP	BFD*	9 species out of 12 species	Herrera and Shank (2016)

^{N} Nuclear genes; ^{M} Mitochondrial genes; ^{SSR} Simple sequence repeats, usually microsatellites; ^{SNP} Single nucleotide polymorphisms; *with genomic data

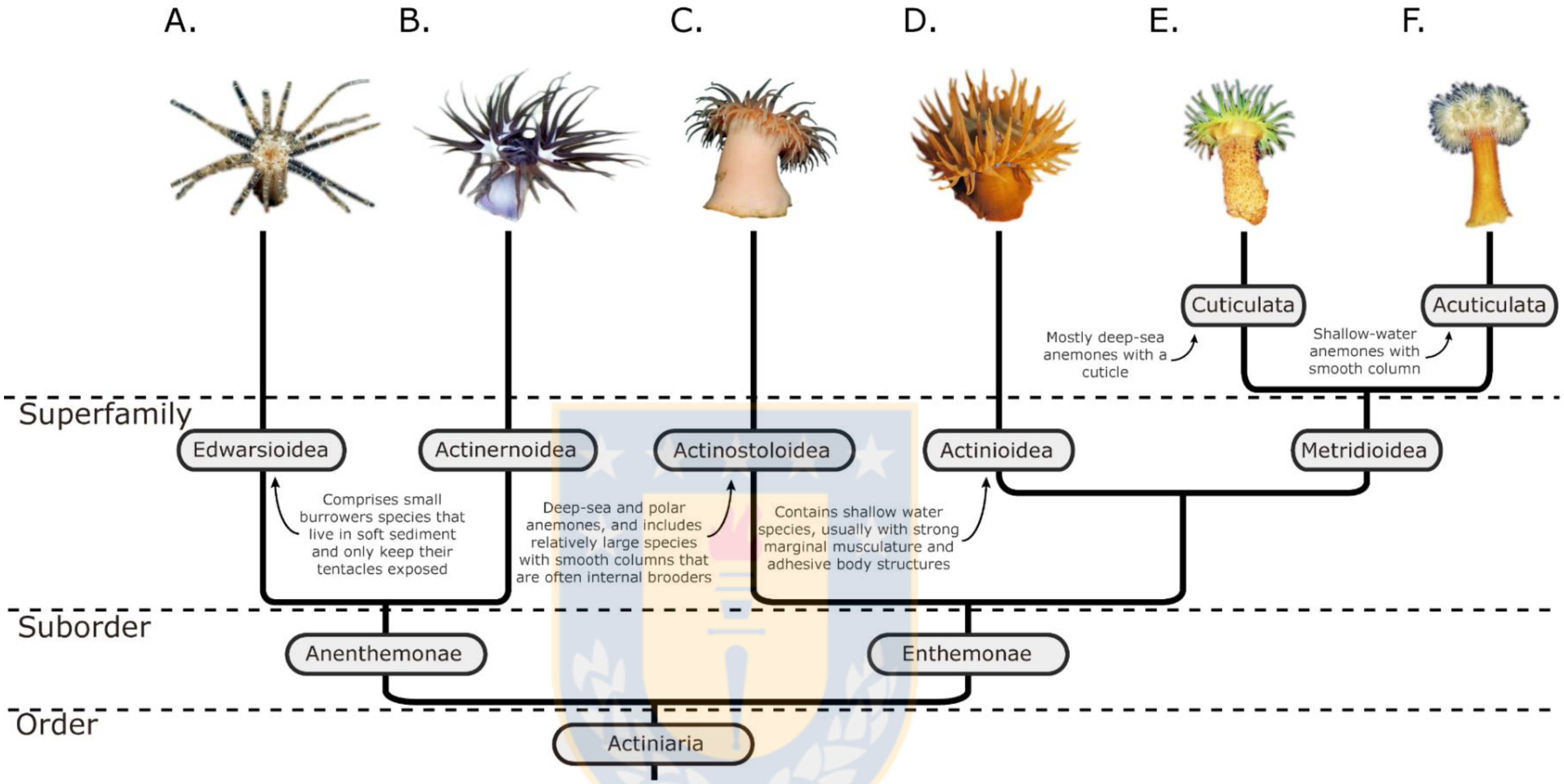


Figure 1.1. Higher-level phylogeny of Actiniaria based on the concatenated genes 12S, 16S, 18S, 28S, and COIII. Figure and text modified from Rodríguez et al. (2014). Descriptions represent a generalization of the group main ecological and morphological attributes; exceptions are expected. Sea anemone pictures: A, *Edwardsia claparedii* (Panceri, 1869); B, *Actinernus michaelsarsi* Carlgren, 1918; C, *Actinostola chilensis* McMurrich, 1904; D, *Actinia equina* (Linnaeus, 1758); E, *Phellia exlex* (McMurrich, 1904); and F, *Metridium senile lobatum* (Carlgren, 1899). Photos: Bernard Picton (A and D), Andrew Gates from the SERPENT Project, National Oceanography Centre (B), and Vreni Häussermann (C, E, and F).

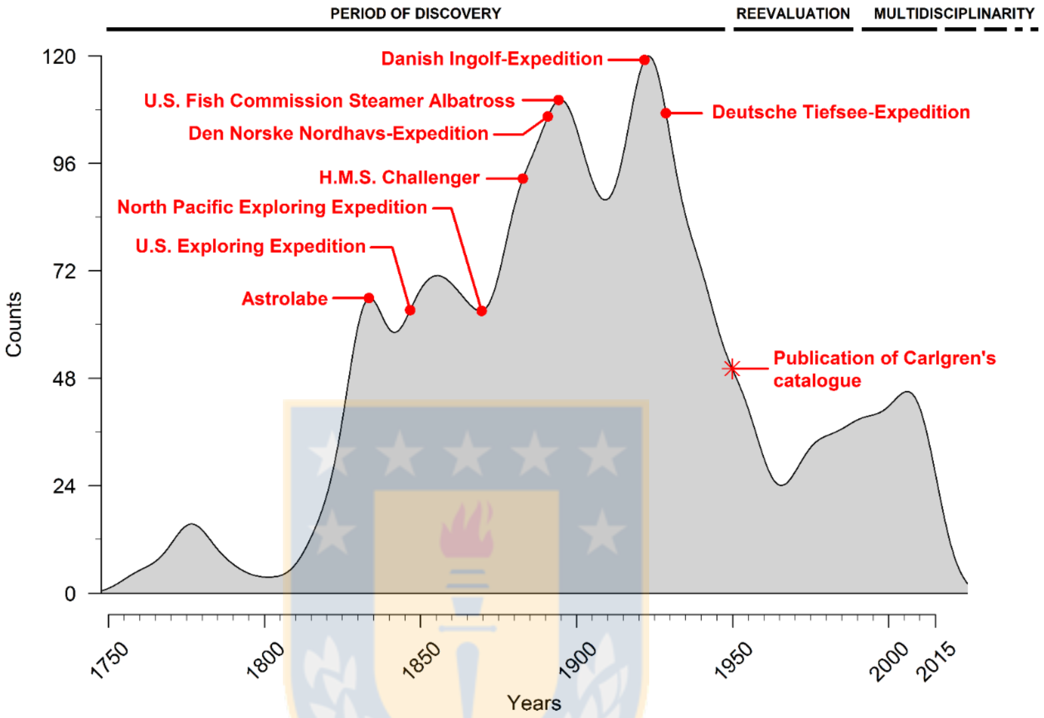
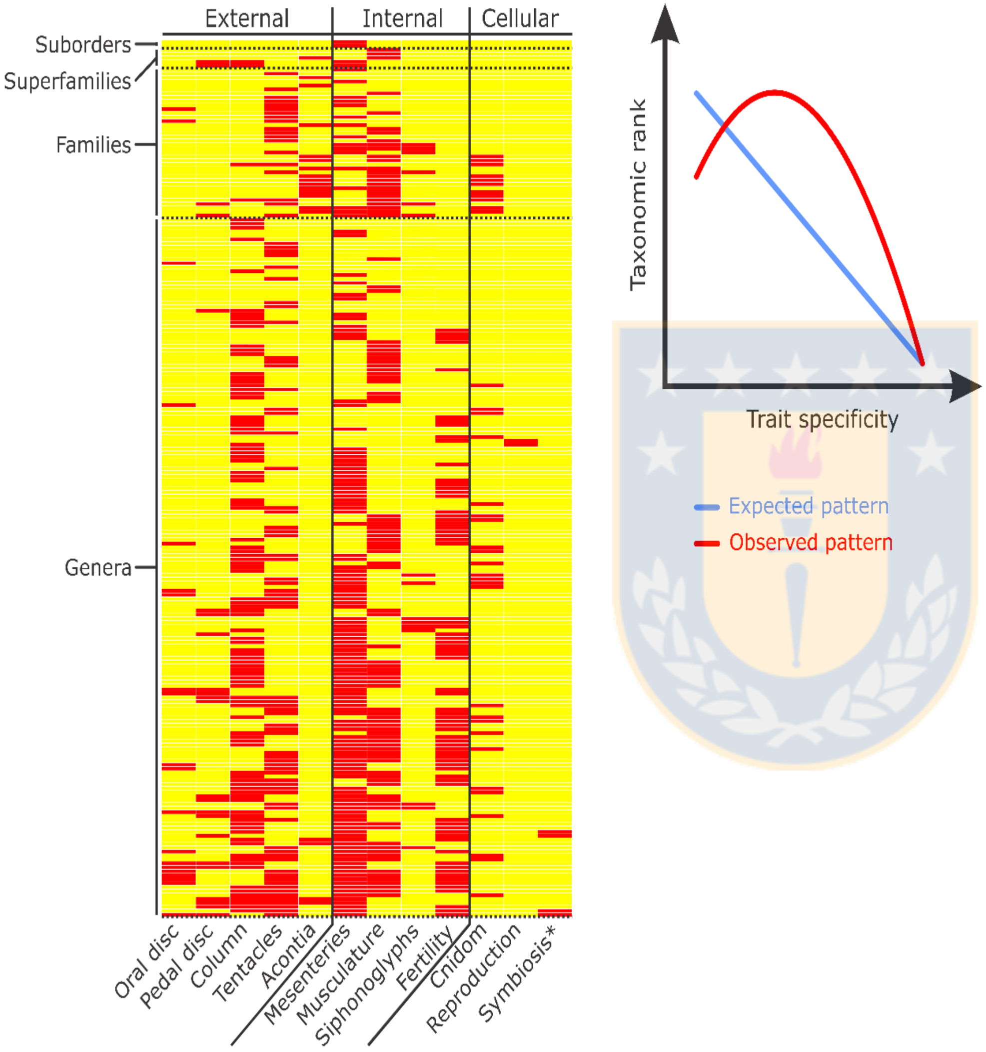


Figure 1.2. Kernel density estimation of actiniarians descriptions through time. Oceanographic expeditions where several species of sea anemones were originally described (≥ 20 species) are indicated next to the year of the publication of the expedition results. The year of the publication of a survey of the Ptychodactiaria, Corallimorpharia and Actiniaria by Carlgren (1949) is also signposted as the culmination milestone of the discovery period. The subsequent use of Carlgren's classification is compressed in a period of taxonomic reevaluation, in which the overall number of new species is reduced at least based on the number of published descriptions. Methodological advances in molecular techniques during the early 90's opened the possibility to use genetic tools to evaluate the species hypothesis in complex taxa. This multidisciplinary trend has been growing over time and is showing no signs to change soon.



*Typically with hermit crabs, although the symbiosis with zooxanthellae is more frequent below the genus level.

Figure 1.3. Heat map showing the use of different diagnostic features for distinguishing Actiniaria taxa. Yellow cells do not indicate that the character is absent but rather that is not determinant for identifying that particular taxon. The data matrix is based on Fautin (2013) and Rodríguez et al. (2014) and considers 2 suborders, 5 superfamilies, 38 families and 177 genera; 12 monotypic genera were discarded from the analysis. Taxonomic classification was based on Carlgren (1949). The upper right plot illustrates the expected vs. observed pattern of trait specificity against taxonomic rank. While it is expected that towards higher ranks, the specificity of the trait diminishes (i.e. the differences become less evident and more taxon-specific), sea anemones taxonomy is strongly concentrated on the organization of the body wall (=column) and tentacles, and the internal arrangement of the mesenteries and muscles.

CAPÍTULO 2

Hierarchical biogeographical processes largely explain the genomic divergence pattern in a species complex of sea anemones (Metridioidea: Sagartiidae: *Anthothoe*)

Hierarchical biogeographical processes largely explain the genomic divergence pattern in a species complex of sea anemones (Metridioidea: Sagartiidae: *Anthothoe*)

Carlos A. Spano[1], Vreni Häussermann[2, 3], Fabián H. Acuña[4], Charles Griffiths[5], Lisa W. Seeb[6] and Daniel Gomez-Uchida[1, 7]

[1] Genomics in Ecology, Evolution, and Conservation Laboratory, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, P.O. Box 160-C, Concepción, Chile

[2] Huinay Scientific Field Station, P.O. Box 462, Puerto Montt, Chile

[3] Escuela de Ciencias del Mar, Pontificia Universidad Católica de Valparaíso, Avda. Brasil 2950, Valparaíso, Chile

[4] Instituto de Investigaciones Marinas y Costeras, CONICET - Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata, Funes 3250, 7600 Mar del Plata, Argentina

[5] Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa

[6] School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat Street, Box 355020, Seattle WA 98195-5020,USA

[7] Nucleo Milenio de Salmonidos Invasores (INVASAL), Concepción, Chile

(Under review in Molecular Phylogenetics and Evolution)

Abstract

The phylogenetic resolution provided by genome-wide data has demonstrated the usefulness of RAD-tag sequencing to tackle long-standing taxonomic questions. Cnidarians have recently become a model group in this regard, yet species delimitation analyses have been mostly performed in octocorals. In this study, we use RAD tags to test the species hypotheses in a wide-spread complex of sea anemones (genus *Anthothoe*), contrasting this new line of evidence with their current classification. The alternative hypotheses were tested using a Bayes Factors delimitation method and the most probable species tree was then evaluated under different biogeographic scenarios. Our results decisively reject the current morphology-

informed delimitation model and infer the presence of several cryptic species associated with distinct marine ecoregions. This spatial pattern was remarkably consistent throughout the study, highlighting the role of geographic distribution as a powerful explanatory variable of lineages diversification. The southern Gondwana pattern with episodic, jump dispersal events is the biogeographic historical representation that best fits the *Anthothoe* species tree. The high population differentiation possibly amplified by the occurrence of asexual reproduction makes it difficult to identify genes responsible for local adaptation, however, these seem to be mainly associated with cellular and metabolic processes. We propose a new set of species hypotheses for the Southern Hemispheric *Anthothoe* clade, based on the pronounced genomic divergence observed among lineages. Although the link between the genetic and phenotypic differentiation remains elusive, newer sequencing technologies are bringing us closer to understanding the evolution of sea anemone diversity and, therefore, how to appropriately classify them.

Introduction

One of the most promising applications of genomic data has been the possibility of delimiting species in borderline cases. These are generally characterized as unresolved groups compounded by sibling species that cannot be reliably distinguished based on their external appearance. Whether there is an incomplete knowledge of their morphological features or the diversification of lineages is not accompanied by clearly visible diagnostic differences, species complexes are quite pervasive among marine taxa (Knowlton, 1993; Appeltans et al., 2012). Next-generation sequencing tools have been delivering unparalleled opportunities to inquire into the evolutionary processes that underlie speciation (see Seehausen et al., 2014), giving access to the causes of these disparities, even in organisms for which few genomic resources presently exist.

Recognizing different species along the divergence continuum of two or more lineages is not trivial. Perhaps the most fundamental reason is that the properties used to distinguish taxa (e.g. reciprocal monophyly, reproductive incompatibility, ecological specialization) often arise at different times during speciation (de Queiroz, 2007). There is a wide variety of methods available for inferring both the species-level phylogeny as well as the lineages that comprise it (e.g. Bryant et al., 2012; Leaché et al., 2014; Yang, 2015); yet they do not always accurately discriminate between species and populations (Sukumaran and Knowles, 2017). In fact, establishing boundaries between these biological units might only be attained by assessing different lines of evidence (Padial et al., 2010), especially in closely related species. Integrating these diverse sources of information is still challenging; however, recent advances in this direction have shown noteworthy improvements in the inferences drawn from genetic data (Solis-Lemus et al., 2015).

Sea anemones (Order Actiniaria) have proven to be an interesting group for molecular biology and genomic research. A successful example of this is the starlet anemone *Nematostella vectensis*, which since the publication of its genome, has been used to examine the evolution of bilaterian development up to the patterning mechanisms during regeneration (Layden et al., 2016). While population genomic studies are less frequent, the benefits of employing high-throughput sequencing technologies have already been demonstrated within Actiniaria (Reitzel et al., 2013). One of the most popular approaches for non-model organisms has been restriction site-associated DNA tags (RAD tags; Baird et al., 2008). By sequencing thousands of loci across the genome, this method is particularly useful for resolving shallow divergences and otherwise intractable relationships.

Generally speaking, actiniarians are soft-bodied, solitary polyps; therefore, unlike corals, they lack any solid structure that could be used to aid their taxonomic determination. Despite this, Actiniaria constitute a morphologically diverse clade. The issue about species delimitation has been growing because many of the characteristics traditionally employed as diagnostic features are not reflected in molecular phylogenies (Daly et al., 2008; Rodríguez et al., 2014). In fact, analyses based on DNA sequences usually recover non-monophyletic groups below the family rank, and many seemingly close species are currently placed under multiple genera (e.g. Rodríguez et al., 2012; Larson and Daly, 2016; Daly et al., 2017). Several factors may be converging to cause this pattern, such as parallel evolution, morphological convergences, and even the fact that many species were insufficiently described, and no type material was deposited for further examination (Spano et al., *submitted*). Interestingly, species relationships based on molecular markers seem to be better explained by geographic patterns (Larson and Daly, 2016; Daly et al., 2017), thus raising the question about the validity of several morphological attributes for taxonomic determination.

Knowing which biogeographic processes generates the diversification of anemone lineages can help clarify the species delimitation in many borderline cases. We focus this study on the sea anemone genus *Anthothoe* and particularly on the species complex of *A. chilensis* (Lesson, 1830). This abundant sagartiid species densely covers large, often disturbed areas from Peru (12°S) to the northern Patagonian zone (47°S), and can be collected from exposed tide pools down to 60 m depth (Paredes et al., 1999; Häussermann and Försterra, 2009). *A. chilensis* has also been reported in several other localities throughout the South Atlantic Ocean, including

South Georgia Island, Mar del Plata, Rio de Janeiro, and South Africa (Excoffon et al., 1997; Häussermann and Försterra, 2009). Some color morphs have been mentioned in the literature and although they are not geographically restricted, it has been suggested that specimens from the Scotia Arc and Argentina might belong to a different species (Häussermann and Försterra, 2005). This hypothesis could also apply to the specimens from South Africa, which were originally described as *A. stimpsoni* (Verrill, 1869), but were years later synonymized by Excoffon et al. (1997) based on the scarce differences seen between its type material and *A. chilensis*. The majority of the remaining *Anthothoe* species are only reported from few localities of Australia and New Zealand; and while they have also been characterized by very slight differences, they are still considered valid since in most cases taxonomic records are extremely poor and no recent revisions exist.

In this study, we utilized RAD tags to characterize the divergence pattern within the *Anthothoe chilensis* complex. Phylogenetic relationships, possibly under incomplete lineage sorting, were inferred using a coalescent-based approach. In addition to the morphology-informed species hypotheses, we also tested different delimitation models founded on historical and contemporary biogeographic processes. The relative probability of each model was evaluated under a Bayesian inference framework, and the biogeographical scenario during which the diversification of *Anthothoe* lineages occurred was assessed according to the number of species and the topology of the tree. Population structure was estimated within candidate species, and loci potentially differentiated due to spatially heterogeneous selection were identified and mapped against the reference genomes available within the order Actiniaria. Finally, the genomic differentiation across geographic scales was discussed to provide a new point of reference for future studies on the species delimitation of sea anemones.

Material and Methods

Specimen collection and DNA sequencing

Anthothoe specimens were collected from 18 localities distributed along the South Atlantic and South Pacific coasts (85 individuals in total; see Table S1 for details). Genomic DNA was extracted from tissue samples taken from the lower column using the NucleoSpin Tissue Kit (Macherey-Nagel). The quality and concentration of the DNA was checked with a ND-2000 Nanodrop spectrophotometer (Nanodrop Technologies) prior to library preparation. Paired-end libraries were produced from DNA digested with the 6-cutter restriction enzyme *PstI* following the Rapture protocol of Ali et al. (2016). Libraries barcoded with 10-base pair sequence tags were sequenced on two lanes of an Illumina HiSeq 4000 (150 bp) in the Genomics and Cell Characterization Core Facility of the University of Oregon. Raw HiSeq output was de-multiplexed and quality filtered using the *process_radtags* module (default quality settings; reads truncated to 140 bp) from the STACKS v1.46 pipeline (Catchen et al., 2013). Additionally, identical sequences (as PCR clones) were identified and removed from the paired-end RAD data using the *clone_filter* module. Reads quality (as measured by Phred score and percentage of sequence overrepresentation) were checked before and after *process_radtags* using the FastQC v0.11.5 software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Demultiplexed RADseq reads were deposited in the NCBI Short Read Archive.

Determination of the divergence parameters

We set a minimum depth of four reads per stack and eight per locus using the research on *Nematostella vectensis* as reference (Reitzel et al., 2013). The intra- and inter-individual divergence parameters were increased together from 2 to 8 and the combination where the number of single-nucleotide polymorphisms (SNPs) was maximized without decreasing the percentage of heterozygotes was chosen. Both the deleveraging algorithm and the gapped alignment option were implemented in all scenarios and the maximum distance used to align secondary reads to primary stacks was kept to default values. To assess the effect of the divergence parameters on the genomic differentiation among individuals, a series of principal component analyses (PCA) were conducted using the adegenet R package (Jombart, 2008; R Development Core Team, 2017). Mantel statistics based on Pearson's product-moment correlation were also used to compare STACKS outputs for each pair of batch runs (Legendre and Legendre, 2012).

Lineage tree inference

The phylogenetic relationship among individual lineages was estimated from a sub-sample of loci that were shared throughout the *Anthothoe* complex. To avoid reducing this number below 1000 loci, individuals with a percentage of missing genotypes higher than 50% were discarded; therefore, 63 out of the 85 individuals moved to the downstream analyses. The matrix of unlinked SNPs was constructed allowing no missing data and selecting only one SNP per locus based on their minor allele frequency (for a total of 3606 aligned nucleotides positions). The phylogeny was inferred under the multispecies coalescent model using SVDquartets implemented in the software PAUP* 4.0a158 (Swofford, 2002; Chifman and Kubatko, 2014). We evaluated all possible quartets and performed a bootstrap analysis of 100 replicates. This procedure was repeated with a second matrix that allowed 20% of missing data (for a total of 53,984 aligned nucleotides positions), and both resulting trees were compared in terms of their topology and bootstrap support.

Species delimitation and species tree reconstruction

The comparison of different species hypotheses within the *Anthothoe* complex was carried out using the Bayes Factor Delimitation method (BFD*) of Leaché et al. (2014). We randomly selected one individual per locality from the previous SNP matrix to prevent the overrepresentation of intraspecific differences due to highly similar individuals from potentially clonal populations. The marginal likelihoods were calculated for each species delimitation model and then compared to the currently accepted species hypothesis (NULL), which recognizes *Anthothoe chilensis* as the only species of the genus in South America and South Africa (Excoffon et al., 1997). The estimates of model support were computed using the implementation of BFD* in the SNAPP plug-in for BEAST v2.4.6 in a path-sampling process of 48 steps (Bryant et al., 2012; Bouckaert et al., 2014). A Markov chain Monte Carlo (MCMC) chain length of 100,000 with a 10% pre-burnin was sufficient to achieve most effective sample sizes well above 100 and in apparent stationarity. For the few steps where this basal situation was not reached, the chains were lengthened accordingly to the variability of the likelihood estimates. Bayes Factors (BF) were calculated for each competing model and compared under the hypothesis testing framework of Kass and Raftery (1995).

We evaluated eight species delimitation models, four based on morphological characteristics and four based on eco-geographic criteria. Including NULL, the other morphology-informed models were: i) ‘ASTI’: NULL model but rejecting the synonymization of *A. stimpsoni* with *A. chilensis* (Excoffon et al., 1997); ii) ‘AMOR’: Model in which each color morphotype within the *Anthothoe* complex represents distinct species (Häussermann and Försterra, 2005; 2009; Fig. 1B-F); and iii) ‘ASTI+AMOR’: NULL model that both rejects the synonymization of *A. stimpsoni* and recognizes each color morphotype of *A. chilensis* as distinct species. Alternatively, to assess the effect of the spatial distribution on the divergence pattern of *Anthothoe* lineages, the following four eco-geographic hypotheses were also tested: iv) 'HIST': Model that assigns as distinct species the groups of specimens distributed in different oceanic regions (i.e. Southeast Pacific, Southwest Pacific, Southeast Atlantic and Southwest Atlantic); v) 'CONT': Model that assigns as distinct species the groups of specimens distributed in different biogeographic provinces; vi) 'ECOL': Model that assigns as distinct species the groups of specimens distributed in different marine ecoregions ; and vii) 'HABT': Model that assigns as distinct species the groups of specimens distributed in different littoral zones (i.e. intertidal and subtidal). Both CONT and ECOL models were based on the bioregionalization of coastal and shelf areas from Spalding et al. (2007).

The species tree was inferred from the delimitation model with the strongest support. The phylogeny was reconstructed in SNAPP through three independent runs of 10,000,000 MCMC length, sampling every 1000 and with a 10% burnin. Mutation rates were estimated during the MCMC, and priors for coalescence rate and ancestral population size were kept at their default values. The set of species trees with their posterior distribution for each topology were summarized using DensiTree v2.2.6 (Bouckaert and Heled, 2014).

Evaluation of biogeographic hypotheses

We used a parsimony-based tree fitting method to test which biogeographic scenario could better explain the topology of the species tree. This analysis originally implemented by Ronquist (2003) for coevolution patterns in the software TreeFitter, employs a four-event model (Page, 1995). In a biogeographic context, these possible events are geographical vicariance, speciation within an area (e.g. sympatric speciation), local extinction and dispersal. The cost of each of these events during the analysis was kept by default, that is: 0.01 for vicariance and within-area speciation, 1.0 for extinction, 2.0 for dispersal. Three area cladograms were built as hierarchical representations for some of the most frequently cited biogeographic hypotheses for southern organisms (see Sanmartín and Ronquist, 2004): i) the southern Gondwana pattern (SGP), which essentially reflects the breakup sequence of the Southern temperate Gondwana; ii) the inverted southern pattern (ISP), which is based on similar vicariant processes, but including dispersive events during the Cenozoic; and iii) the west wind dispersal (WWD), which considers the West Wind Drift as the leading driver of the present distribution pattern (Fig. S1). To measure the significance of the fit of the species tree with each of the area cladograms, randomization tests of 1000 permutations were carried out in TreeFitter v1.3 for both areas and species terminals. The frequency of the four different events necessary to explain the hierarchical biogeographic pattern was calculated for the most parsimonious cladogram given the species phylogeny of *Anthothoe*.

Population structure and detection of candidate loci for local adaptation

Demographic statistics were inferred based on the candidate species revealed by previous analyses. We employed the matrix of unlinked SNPs that allowed 20% of missing data for a total of 26,992 aligned nucleotide positions. One individual from pairs that contained more than 99% of genotypes matching were removed as being potential clones. Genetic differentiation among populations was measured using the F_{ST} estimator of Weir and Cockerham (1984) in the software Genepop v4.7 (Rousset, 2008). Heterozygosities were then used to identify paralogous loci that may affect genotype calculations within our dataset. Gene duplication events have been reported for several taxa, yet there is no evidence of such phenomenon in sea anemones. We assessed the untested occurrence of tetrasomic and disomic loci (as duplicates and diverged duplicates, respectively) using the *HDplot* R code of McKinney et al. (2016). The number of gene pools (K) within each candidate species was estimated from the resulting matrices that comprised more than three populations from different localities. The optimal value of K was assessed using the *find.clusters* function from the *adegenet* R package (Jombart, 2008; R Development Core Team, 2017), and the choice was made based on the Bayesian Information Criterion (BIC). The composition and probabilities of individual assignment to the genetic clusters was inferred through a Discriminant Analysis of Principal Components (DAPC, Jombart et al., 2010).

To identify loci potentially responsible for local adaptation, we used the OutFLANK approach of Whitlock and Lotterhos (2015). This outlier method trims the distribution of F_{ST} among loci to reduce the false positives caused by selective pressures other than those likely generated by spatial heterogeneity. Since loci with extremely low heterozygosities may affect the estimation of the F_{ST} distribution, a minimum threshold of 0.1 was established. Results were compared to the genome scan from a *pcadapt* analysis (Luu et al., 2016), which tests for selection based on a measure of Mahalanobis distance. Both analyses considered a false discovery rate of 5%, and were performed in R in their latest versions (accessed on October 2017). Sequences that contained outlier SNPs were subsequently mapped to the reference genomes of *Nematostella vectensis* and *Exaiptasia pallida* (Putnam et al., 2007; Baumgarten et al., 2015). The search of the query sequences on the U.S. National Center for Biotechnology Information (NCBI) database was conducted using the BlastN algorithm (Altschul et al., 1997), with an E-value threshold of 1×10^{-10} and a word size of 15. Contigs with a sequence homology of more than 70% were finally annotated and searched in terms of Gene Ontology (GO).

Results

Divergence patterns and lineages tree inference

Approximately 200 million reads per lane were retained from *process_radtags*, and individual values ranged between 17,386 and 13,622,356 reads. After PCR clones were removed from paired-end reads, concatenated sequences yielded an average per individual of 1,543,240 (95% CI ± 285,964; SD = 1,345,157; *n* = 85) and 1,608,657 (95% CI ± 297,930; SD = 1,401,441; *n* = 85) reads for each lane. The sum of both Illumina outputs gave an approximate average of 3.15 million reads per individual (Table S1), with a mean depth of coverage of 24 reads per RAD marker. The number of SNPs recovered for the same filtering steps, but with different divergence parameters, ranged from 23,112 to 28,498 (Fig. S2). These differences carried no substantial variations in the overall relatedness pattern observed among individual PCAs. In all cases, the first component tends to separate the Chilean samples from everything else, while the second component differentiates the specimens from South America with those from South Africa and Oceania (Fig. S3). Correlation between datasets was also persistent and significant among batch runs (Table S2).

Most individual lineages were grouped by location and tended to coalesce into larger geographical regions (Fig. 1). The inference drawn from the matrix that allowed 20% of missing data yielded much higher bootstrap support than the matrix that filtered out all loci with missing information. Polytomies only occurred in the latter case and specifically in those individuals that were later discarded as possible clones. Reciprocal monophyly for the specimens from Argentina, Australia, New Zealand, and Chile had bootstrap support of 100% in both datasets. Furthermore, the topology of the trees was remarkably similar despite the large difference in the amount of SNPs analyzed. The main disparities arose within the samples from Chile, and typically in specimens from geographically close populations (e.g. HU/LIL, LH/MON).

Species delimitation and species tree reconstruction

The null hypothesis was decisively rejected against most alternative models (Fig. 2). ECOL—which identified those populations from different marine ecoregions as distinct species—was the species hypothesis that obtained stronger support from Bayes Factors. This model proposed nine species among the examined specimens, seven of which would be cryptic for *Anthothoe chilensis*. No relationship was found between the proposed species by BFD* and the different morphotypes observed in the field. Preliminary analyses of the internal anatomy also did not provide any diagnostic trait according to ECOL subdivisions. Furthermore, the ecogeography-informed models had an overall stronger support than those based exclusively on external morphology.

The cloudgram built from 10 million iterations displayed well-supported nodes with highly differentiated lineages (Fig. 3). The New Zealand species emerged as the basal-most *Anthothoe* taxon, from which the other lineages began to diverge primarily according to their oceanic distribution. Species from the biogeographic provinces of South America and South Africa diverged shortly after the lineages separated from the Southeast Australian Shelf (Cape Howe ecoregion in this case). The *Anthothoe* members from the South American ecoregions began to diverge more recently in the Southeastern Pacific; first from the extreme ends of their distribution range in Chile (i.e. Humboldtian and Chiloense) and later from the south central region (i.e. Araucanian and Central Chile). Some other alternative topologies also revealed a closer relationship among species from the South American provinces of the Southeastern Pacific and Southwestern Atlantic, although with a much lower frequency.

Evaluation of biogeographic hypotheses

The species tree of *Anthothoe* proved to be significantly fitted to the SGP and ISP biogeographic scenarios ($p < 0.01$). Among them, the SGP hypothesis (Fig. S1A) was the most parsimonious pattern for the topology of the ECOL model (cost = 5.06); being predominantly represented by vicariant processes occasionally mediated by long-distance dispersal events. The latter case would be particularly responsible for the origin of the South African species from the South American populations. Jump dispersal is also the minimum-cost explanation for the recent connection between the lineages from the Humboldtian and Chiloense ecoregions. A single case of local extinction would have contributed to the genetic break in Central Chile and no within-area speciation event was reported for the ECOL model.

Population structure and detection of candidate loci for local adaptation

There were 19 individuals identified as possible clones by presenting over 99% of the same genotypes for the 26,992 SNP matrix. They belonged to eight different populations: five from Chile (12 ind), and one each from Argentina (4 ind), Australia (2 ind) and South Africa (1 ind). Not a single potential clone pair shared identical genotypes, there being a minimum difference of 36 SNPs among the most similar individuals. The pairwise comparison of F_{ST} values showed an overall strong population differentiation (Table 1). These estimates ranged between 0.012 (among populations within ecoregions) and 0.749 (among populations from distant oceanic provinces). The most differentiated populations compared to the rest ($F_{ST\ average}$ > 0.5) were those from Tathra (TA) in Australia, Punta Cantera (PTC) in Argentina and Strand (ST) in South Africa; the latter also showing one of the highest variations in terms of standard deviation. Genetic diversity calculated from the observed heterozygosities among individuals of the same local population was, on the contrary, moderately to low (in reference to other cnidarians; Solé-Cava and Thorpe, 1991); on average 0.173 (95% CI ± 0.032; SD = 0.057) over all loci. No pattern of gene duplication was identified, either for the entire *Anthothoe* sample, or for the population subsamples within the candidate species (Fig. S4). In both cases, the number of possible paralogs represented less than 1% of the total loci assessed.

The population analyses focused on the Southeastern Pacific region for two main reason: i) there were enough populations for most of the species derived from the ECOL model; and ii) the recent divergence inferred from the species tree might suggest that these populations have not been isolated long enough to reach a complete differentiation of their lineages. The resulting matrix (after removing the paralogous loci) contained 19,169 SNPs for a sample of 22 individuals. The *find.clusters* function consistently recovered two gene pools within this dataset. One of them assigned the individuals from the Humboldtian and Chiloense ecoregions and the other one from the Araucanian ecoregion (Fig. 4). The individuals from Central Chile were distributed in both clusters, although they were mostly assigned to the Araucanian group. Based on the *Anthothoe* phylogeny (Fig. 1), this also constitutes the ancestral gene pool from which the lineages of the extreme ends of Chile diverged, being the latter completely nested within the central-region clade.

The number of loci potentially under selection varied substantially between the two outlier methods. While OutFLANK identified 654 putatively adaptive markers (3.4% of total SNPs), the *pcadapt* analysis found over 2800 (14.6% of total SNPs); 319 of which were shared among methods (1.7% of total SNPs). We trimmed out 16% of both tails of the F_{ST} distribution before being able to detect outlier SNPs with OutFLANK. Approximately 2 and 20% of them belonged to contigs that positively aligned against the reference genomes of *Nematostella* *vectensis* and *Exaiptasia pallida*, respectively. Seven of them appeared in both genomes, five had been annotated to protein sequences and three were only reported in cnidarians species. These had an identity between the query and the hit of over 80%, an E-value smaller than 10^{-20}, and they were all detected both by OutFLANK and *pcadapt*. The first one, the SNP *7093_139*, was situated in the genes *5506328* of *N. vectensis* and *110233792* of *E. pallida*, which are involved in the ubiquitin-protein ligase activity, possibly encoding for a Kelch-like protein. The SNP *21785_102* aligned to the gene *110242064* of *E. pallida*, which had been predicted to encode for a Diacylglycerol kinase delta-like protein, a signaling molecule involved in endocytosis processes and cell growth. Finally, the SNP 71849_127 was situated in the genes *5511148* of *N. vectensis* and *110243008* of *E. pallida*, which are involved in metalloendopeptidase activity and ion transport, possibly encoding for a mitochondrial-processing peptidase subunit beta-like protein. Despite the lack of experimental evidence for these predictions among the actiniarian species, all the molecular products for the five shared genes were consistently recovered under the GO categories of cellular and metabolic processes in the Uniprot database.

Discussion

Genomic divergence in the southern Anthothoe complex

All molecular analyzes produced highly resolved, well-supported trees for the *Anthothoe* phylogeny. Most lineages, however, were only reciprocal monophyletic to the geographic distribution, rejecting all previous species hypotheses that were exclusively based on morphology. This pattern was especially noticeable at broader spatial scales (>1000 km), being almost unaltered throughout different divergence parameters, proportions of missing data and number of loci. Isolation by distance and high levels of genetic differentiation have actually been reported within several species of sea anemones using allozymes (e.g. McFadden et al., 1997; Manchenko et al., 2000; Vianna et al., 2003). They are usually complemented by some other morphological variation of their color or nematocyst measurements, although this is not always the case (Douek et al., 2002). In the *Anthothoe* complex for example, it is not the lack of these differences, but rather the low correspondence between the morphotypes and the genetic breaks observed among groups.

Species delimitation analysis highlighted the role of ecological factors to outline boundaries in closely related lineages. Moreover, geography largely seem to explain the divergence pattern of the *Anthothoe* clade in the Southern Hemisphere, agreeing with other recent studies on sea anemones that were based on DNA sequences (Larson and Daly, 2016; Daly et al., 2017). Ecoregions are not only characterized by their ecosystemic cohesion, but because they are also the smallest-scale units in the current global system for coastal and shelf areas (Spalding et al., 2007). Consequently, the multispecies coalescent model posits nine candidate species hidden within the *Anthothoe* complex, of which at least seven would be new to science. Previous analyzes on RAD-seq data yielded similar results on octocorals, concurring on the importance of environmental variables to delimit cryptic species (Herrera and Shank, 2016). Although we observed that the BFD* method generally favored hypotheses that recognize more taxa, above nine species, the tree tended to lose support and resolution among terminal branches (particularly those derived from the same ecoregion). High levels of differentiation might also be causing the model to identify each divergent population as a distinct species (Sukumaran and Knowles, 2017), which could explain the incongruity between the number of gene pools detected in Chile and the number of ecoregions it comprises. This is still reconcilable under a unified species concept (de Queiroz, 2007), yet it remains fairly impractical without any supporting evidence that may be employed as diagnostic. Therefore, the validity of the seven new *Anthothoe* species can still be disproved (as any hypothesis), especially in terms of the subsisting connectivity among the described lineages (Pante et al., 2015).

The biogeography of speciation

The species tree fitted significantly well to some of the hierarchical biogeographic patterns that had been previously reported for Southern Hemisphere terrestrial organisms (Sanmartín and Ronquist, 2004). The resemblance of these trends even despite the different environments may be explained by the predominantly sessile lifestyle of *Anthothoe* and the historical connection to the coastal zone. Furthermore, sea anemones are seemingly less prone to short-term environmental changes compared to other marine invertebrates, making them a suitable group to evaluate general biogeographic patterns (Riemann-Zürneck, 1986; Häussermann and Försterra, 2005). Among them, the southern Gondwana pattern was the most supported hypothesis, even though the ancestral divergence towards Oceania suggests a more recent origin of the *Anthothoe* clade (Sanmartín and Ronquist, 2004; Fig. S1A). Paleogeographic reconstructions show that the separation of New Zealand landmasses from Antarctica and South America happened about 80 million years ago, during the formation of the Tasman Sea in the late Cretaceous (Coleman, 1980). Australia, on the other hand, remained connected to South America at least until the early Eocene (ca. 51-54 Ma), when a shallow marine seaway began to form with East Antarctica, that later gave way to the South Tasman Sea (Coleman, 1980; Woodburne and Case, 1996). It is quite possible that the *Anthothoe* genealogy was initially shaped by these two vicariant events that happened long before the current configuration of the coastline was reached. Although we cannot fully rule out other hypotheses, given the lack of fossil record, the overall difference between the F_{ST} values from Oceania and all the other populations does point to a deeper genetic break, likely due to a longer period of isolation.

Our results also emphasized the occurrence of long-distance dispersal events to explain the divergence pattern of the lineages from South Africa and Chile. Studies that explicitly measure larval dispersal in sea anemones are practically non-existent, yet molecular markers have allowed us to get an idea of how much they might move among distant locations. Billingham and Ayre (1996) for instance, found that the populations of *Anthothoe albocincta* were strongly differentiated among neighboring areas, consequently obtaining low levels of gene flow within the whole region. Nevertheless, they concluded that while the sexual production of genotypically diverse larvae is the primary source of widely dispersed colonists, the upkeep of local populations by asexual reproduction might be influencing the inferences of genetic differentiation (Billingham and Ayre, 1996; Billingham and Ayre, 1997). These findings agree with previous observations in *Anthothoe chilensis*, where it seems that intertidal individuals reproduce mostly by longitudinal fission, since they usually do not reach the minimum size required for gonadal development (Excoffon and Acuña, 1998).

The transportation of adult individuals across large oceanic distances is also feasible through rafting dispersal (Thiel and Haye, 2006). The sporadic connection between South America and South Africa through floating debris has already been hypothesized for the disjoint distribution of other marine invertebrates, such as the Chilean abalone and the isopod *Sphaeroma terebrans* (Castilla and Guiñez, 2000; Baratti et al., 2005). Recent anthropogenic introductions may also be strongly contributing to this pattern, as has been shown in numerous invasive taxa like the wide-spread anemone *Nematostella vectensis* (Reitzel et al., 2008). Likewise, there have been reports of *Anthothoe* specimens adhered to ship hulls in Australia and some of the largest populations observed in this study were indeed found within port areas. Unlike the case of transoceanic dispersal with South Africa, there are important barriers in Chile that limits the natural transportation of marine organisms from the fjord region to the north. There is, however, an 'intermediate area' described for including mixed components of both biota and would be comprised between the Araucanian and Central Chile ecoregions (Camus, 2001; Häussermann and Försterra, 2005). This biogeographic pattern not only matches with the transitional zone observed in the northern portion of Central Chile (Fig. 4), but also the phylogeographic discontinuity around 30ºS (or between the MOB and LH populations) agrees with previous reports on other low disperser invertebrates (Haye et al., 2014). The extent to which the two gene pools might differentiate between the four ecoregions essentially depends on the capabilities of *Anthothoe* individuals to move across these barriers through external agents, phenomenon largely unknown in sea anemones.

Signals of selection

The usefulness of genomic scans has been extensively discussed due to its limitations and potentialities for RAD-seq data (e.g. Lowry et al., 2017; McKinney et al., 2017). In all cases authors agreed that markers density and levels of linkage disequilibrium are critical for differentiation tests that aim to detect loci that may be involved in adaptive divergence. Nevertheless, when these variables are controlled, the cofounding effect of the population structure and demographic history may turn especially relevant, although usually by increasing the number of false positives (Whitlock and Lotterhos, 2015). The frequency and distribution of related individuals can significantly influence the performance of F_{ST} outlier methods that are based on univariate statistics, such as the Mahalanobis distance (Verity et al., 2017). In *Anthothoe* about one-third of the sample corresponded to clonal individuals and up to a half presented a pairwise genotypic similarity of over 90%. The overall fit of the data to the distribution models appeared to be strongly linked to the mode of reproduction, especially in terms of neutral expectations (see also Solé-Cava and Thorpe, 1991). This pattern of high population differentiation is indeed pervasive in sea anemones (McFadden et al., 1997; Manchenko et al., 2000; Vianna et al., 2003), and almost irrespective of the metrics used or even if clones were removed or not from the analysis (Reitzel et al., 2013). Isolating signals of selection from the background differentiation noise is quite challenging in the present scenario; although the relevance of the specimens location points towards the importance of spatial heterogeneity. Furthermore, the high similarity between the results of this study and those based on traditional molecular markers, such as allozymes, also deserves some attention. It is quite possible that a considerable portion of the differences we see at population scale are primarily due to the evolutionary forces that are acting on the markers themselves. So far, these would be mostly represented in non-synonymous substitutions associated with cellular and metabolic processes, as it was also demonstrated in *Nematostella vectensis* (Reitzel et al., 2013).

Taxonomic considerations and future direction

There are four key features that characterized the genus *Anthothoe* following Carlgren's (1949) classification: a smooth column, presence of acontia, a mesogleal sphincter, and the first cycle of mesenteries being sterile. Even though these features were all present in the nine candidate species, the sphincter characteristics and the fertility of the mesenteries could only partially be confirmed given the anatomical deformations produced by asexual reproduction and the contraction of preserved specimens. Excoffon et al. (1997) reported similar observations in *A. chilensis,* finding gonads inconspicuously and specifically in the third cycle of mesenteries. We occasionally found fertile mesenteries down to the second cycle, but mainly in the proximal end, which would suggest that they might be growing from the pedal disc upward. Whether or not the first cycle of mesenteries is always sterile ultimately defines the separation between *Anthothoe* and *Actinothoe*; genera that otherwise are practically indistinguishable (Rodríguez et al., 2012).

Species identification within the genus *Anthothoe* are mainly based on the coloration pattern of the body and the development of the muscles (Table 2). Nonetheless, the descriptions of *Anthothoe australiae, A. australiensis, A. similis,* and *A. neozelanica* were carried out only on one or two specimens, therefore their diagnosis needs to be revised. Other species like *A. albens* were actually never fully characterized due to the "delicacy" of their tissues for preservation (Parry, 1952). While our results show a high genomic differentiation among lineages, this had little to do with previous species hypothesis based exclusively on their morphology. No substantial differences were found in the cnidom either, at least beyond some disparities in the relative frequency of certain types of nematocysts in particular localities (for example, low number of basitrichs in the samples from Oceania). It is worth mentioning that the differences in the cnidae nomenclature could also have led to confusion, since it seems that the basitrichs that Carlgren (1950; b) used for the description of *A. australiensis* and *A. albocincta* are apparently the same as the *b*-rhabdoids that Excoffon et al. (1997) used in the synonimization of *A. stimpsoni* and *A. chilensis*. We thus recommend the re-evaluation of the diversity of the Southern Hemispheric *Anthothoe* complex, taking the nine candidate species as a new set of hypotheses. This would integrate the evolutionary process assessed in the present analysis, while giving the chance to search for any morphological trait that would allow us to characterized these cryptic species (and which were not tested in this study, for example the ultrastructure of the cnidae). If there are no additional data showing any signs of differentiation, these nine genealogical units might well be flagged as deep conspecific lineages following the terminology of Padial et al. (2010). The longer period of isolation suggests to start this examination with the specimens from Australia and New Zealand, where coincidentally there are a larger number of 'ghost' species (i.e. considered valid even though they lack type material and have not been reported ever since their description; Spano *et al. submitted*). The high differentiation of the Argentine population and its traceable relationship to the Southeastern Pacific population also calls for attention, especially as a possible case of founder effect speciation. Overall, the genomic divergence across the clade seems to be strongly outlined by geographic isolation and probably natural selection, making speciation likely, regardless of whether it agrees with the phenotypic character systems that we are using today for sea anemones identification.

Acknowledgements

We are very grateful to Carita Pascal, Garrett McKinney, and Jim Seeb for all their assistance generating the RAD-seq dataset. Special thanks to Cristian Hernandez, Angie Diaz, Pedro Victoriano, and the whole crew of the GEEC lab for providing constructive comments throughout the elaboration of the study. For enabling access to key specimens we thank Sadie Mills and Malcolm Francis (National Institute of Water and Atmospheric Research), Stephen Keable (Australian Museum Research Institute), and Paula Gonzalez (Centro de Buceo Prosub). This research was funded by a CONICYT Doctoral Fellowship and represents publication number 157 of Huinay Scientific Field Station.

References

Ali, O.A., O'Rourke, S.M., Amish, S.J., Meek, M.H., Luikart, G., Jeffres, C., Miller, M.R., 2016. RAD capture (Rapture): flexible and efficient sequence-based genotyping. Genetics 202, 389-400.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389-3402.

Appeltans, W., Ahyong, S.T., Anderson, G., Angel, M.V., Artois, T., Bailly, N., Bamber, R., Barber, A., Bartsch, I., Berta, A., Błażewicz-Paszkowycz, M., Bock, P., Boxshall, G., Boyko, C.B., Brandão, S.N., Bray, R.A., Bruce, N.L., Cairns, S.D., Chan, T.Y., Cheng, L., Collins, A.G., Cribb, T., Curini-Galletti, M., Dahdouh-Guebas, F., Davie, P.J.F., Dawson, M.N., De Clerck, O., Decock, W., De Grave, S., de Voogd, N.J., Domning, D.P., Emig, C.C., Erséus, C., Eschmeyer, W., Fauchald, K., Fautin, D.G., Feist, S.W., Fransen, C.H.J.M., Furuya, H., Garcia-Alvarez, O., Gerken, S., Gibson, D., Gittenberger, A., Gofas, S., Gómez-Daglio, L., Gordon, D.P., Guiry, M.D., Hernandez, F., Hoeksema, B.W., Hopcroft, R.R., Jaume, D., Kirk, P.M., Koedam, N., Koenemann, S., Kolb, J.B., Kristensen, R.M., Kroh, A., Lambert, G., Lazarus, D.B., Lemaitre, R., Longshaw, M., Lowry, J., Macpherson, E., Madin, L.P., Mah, C., Mapstone, G., McLaughlin, P.A., Mees, J., Meland, K., Messing, C.G., Mills, C.E., Molodtsova, T.N., Mooi, R., Neuhaus, B., Ng, P.K.L., Nielsen, C., Norenburg, J., Opresko, D.M., Osawa, M., Paulay, G., Perrin, W., Pilger, J.F., Poore, G.C.B., Pugh, P., Read, G.B., Reimer, J.D., Rius, M., Rocha, R.M., Saiz-Salinas, J.I., Scarabino, V., Schierwater, B., Schmidt-Rhaesa, A., Schnabel, K.E., Schotte, M., Schuchert, P., Schwabe, E., Segers, H., Self-Sullivan, C., Shenkar, N., Siegel, V., Sterrer, W., Stöhr, S., Swalla, B., Tasker, M.L., Thuesen, E.V., Timm, T., Todaro, M.A., Turon, X., Tyler, S., Uetz, P., van der Land, J., Vanhoorne, B., van Ofwegen, L.P., van Soest, R.W.M., Vanaverbeke, J., Walker-Smith, G., Walter, T.C., Warren, A., Williams, G.C., Wilson, S.P., Costello, M.J., 2012. The magnitude of global marine species diversity. Curr. Biol. 22, 2189-2202.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., Selker, E.U., Cresko, W.A., Johnson, E.A., 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS Biol. 3, e3376.

Baratti, M., Goti, E., Messana, G., 2005. High level of genetic differentiation in the marine isopod *Sphaeroma terebrans* (Crustacea Isopoda Sphaeromatidae) as inferred by mitochondrial DNA analysis. J. Exp. Mar. Biol. Ecol. 315, 225-234.

Baumgarten, S., Simakov, O., Esherick, L.Y., Liew, Y.J., Lehnert, E.M., Michell, C.T., Li, Y., Hambleton, E.A., Guse, A., Oates, M.E., Gough, J., Weis, V.M., Aranda, M., Pringle, J.R., Voolstra, C.R., 2015. The genome of *Aiptasia*, a sea anemone model for coral symbiosis. Proceedings of the National Academy of Sciences 112, 11893-11898.

Billingham, M., Ayre, D.J., 1996. Genetic subdivision in the subtidal, clonal sea anemone *Anthothoe albocincta*. Mar. Biol. 125, 153-163.

Billingham, M., Ayre, D.J., 1997. Asexual reproduction and genetic determination of colour patterns within populations of the subtidal sea anemone *Anthothoe albocincta*. Mar. Ecol. Prog. Ser. 156, 121-130.

Bouckaert, R., Heled, J., 2014. DensiTree 2: seeing trees through the forest. bioRxiv, p. 012401.

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.H., Xie, D., Suchard, M.A., Rambaut, A., Drummond, A.J., 2014. BEAST 2: a software platform for bayesian evolutionary analysis. PLoS Comp. Biol. 10, e1003537.

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N.A., RoyChoudhury, A., 2012. Inferring species trees directly from biallelic genetic markers: bypassing gene trees in a full coalescent analysis. Mol. Biol. Evol. 29, 1917-1932.

Camus, P.A., 2001. Biogeografía marina de Chile continental. Rev. Chil. Hist. Nat. 74, 587-617.

Carlgren, O., 1949. A survey of the Ptychodactiaria, Corallimorpharia and Actiniaria. Kungliga Svenska Vetenskapsakademiens Handlingar 1, 1-121.

Carlgren, O., 1950. Actiniaria and Corallimorpharia. Scientific Reports of the Great Barrier Reef Expedition 1928-29 5, 427-457.

Castilla, J.C., Guiñez, R., 2000. Disjoint geographical distribution of intertidal and nearshore benthic invertebrates in the Southern Hemisphere. Rev. Chil. Hist. Nat. 73, 1-23.

Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124-3140.

Coleman, P.J., 1980. Plate tectonics background to biogeographic development in the southwest Pacific over the last 100 million years. Palaeogeogr., Palaeoclimatol., Palaeoecol. 31, 105-121.

Chifman, J., Kubatko, L., 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30, 3317-3324.

Daly, M., Crowley, L.M., Larson, P., Rodríguez, E., Heestand Saucier, E., Fautin, D.G., 2017. Anthopleura and the phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). Organisms Diversity & Evolution 17, 545-564.

Daly, M., Chaudhuri, A., Gusmão, L., Rodríguez, E., 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). Mol. Phylogen. Evol. 48, 292-301.

de Queiroz, K., 2007. Species concepts and species delimitation. Syst. Biol. 56, 879-886.

Douek, J., Barki, Y., Gateño, D., Rinkevich, B., 2002. Possible cryptic speciation within the sea anemone *Actinia equina* complex detected by AFLP markers. Zool. J. Linn. Soc. 136, 315-320.

Excoffon, A.C., Acuña, F.H., 1998. Dinámica reproductiva y aspectos poblacionales de un clon de *Anthothoe chilensis* (Lesson, 1830)(Cnidaria, Anthozoa) del intermareal de Mar del Plata. Frente Marit. 17, 107-111.

Excoffon, A.C., Belem, M.J.C., Zamponi, M.O., Schlenz, E., 1997. The validity of *Anthothoe chilensis* (Actiniaria, Sagartiidae) and its distribution in Southern Hemisphere Iheringia, Ser. Zool. 82, 107-118.

Häussermann, V., Försterra, G., 2005. Distribution patterns of Chilean shallow-water sea anemones (Cnidaria: Anthozoa: Actiniaria, Corallimorpharia); with a discussion of the taxonomic and zoogeographic relationships between the actinofauna of the South East Pacific, the South West Atlantic and the Antarctic. Scientia Marina 69, 91-102.

Häussermann, V., Försterra, G., 2009. Actiniaria - Sea Anemones. In: Häussermann, V., Försterra, G. (Eds.), Marine Benthic Fauna of Chilean Patagonia. Nature in Focus, Santiago, p. 1000.

Haye, P.A., Segovia, N.I., Muñoz-Herrera, N.C., Gálvez, F.E., Martínez, A., Meynard, A., Pardo-Gandarillas, M.C., Poulin, E., Faugeron, S., 2014. Phylogeographic structure in benthic marine invertebrates of the Southeast Pacific coast of Chile with differing dispersal potential. PLOS ONE 9, e88613.

Herrera, S., Shank, T.M., 2016. RAD sequencing enables unprecedented phylogenetic resolution and objective species delimitation in recalcitrant divergent taxa. Mol. Phylogen. Evol. 100, 70-79.

Jombart, T., 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24, 1403-1405.

Jombart, T., Devillard, S., Balloux, F., 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11, 94.

Kass, R.E., Raftery, A.E., 1995. Bayes factors. Journal of the American Statistical Association 90, 773-795.

Knowlton, N., 1993. Sibling species in the sea. Annu. Rev. Ecol. Syst. 24, 189-216.

Larson, P.G., Daly, M., 2016. Phylogenetic analysis reveals an evolutionary transition from internal to external brooding in *Epiactis* Verrill (Cnidaria: Anthozoa: Actiniaria) and rejects the validity of the genus *Cnidopus* Carlgren. Mol. Phylogen. Evol. 94 (B), 548-558.

Layden, M.J., Rentzsch, F., Röttinger, E., 2016. The rise of the starlet sea anemone *Nematostella vectensis* as a model system to investigate development and regeneration. Wiley Interdisciplinary Reviews. Developmental Biology 5, 408-428.

Leaché, A.D., Fujita, M.K., Minin, V.N., Bouckaert, R.R., 2014. Species delimitation using genome-wide SNP data. Syst. Biol. 63, 534-542.

Legendre, P., Legendre, L.F.J., 2012. Numerical Ecology. Elsevier.

Lowry, D.B., Hoban, S., Kelley, J.L., Lotterhos, K.E., Reed, L.K., Antolin, M.F., Storfer, A., 2017. Breaking RAD: an evaluation of the utility of restriction site-associated DNA sequencing for genome scans of adaptation. Mol Ecol Resour 17, 142-152.

Luu, K., Bazin, E., Blum, M.G.B., 2016. pcadapt: an R package to perform genome scans for selection based on principal component analysis. Mol Ecol Resour 17, 67-77.

Manchenko, G.P., Dautova, T.N., Latypov, Y.Y., 2000. High level of genetic divergence between sympatric color morphs of the littoral sea anemone *Anthopleura orientalis* (Anthozoa: Actiniaria). Biochem. Syst. Ecol. 28, 737-750.

McFadden, S.C., Grosberg, K.R., Cameron, B.B., Karlton, P.D., Secord, D., 1997. Genetic relationships within and between clonal and solitary forms of the sea anemone *Anthopleura elegantissima* revisited: evidence for the existence of two species. Mar. Biol. 128, 127-139.

McKinney, G.J., Larson, W.A., Seeb, L.W., Seeb, J.E., 2017. RADseq provides unprecedented insights into molecular ecology and evolutionary genetics: comment on Breaking RAD by Lowry et al. (2016). Mol Ecol Resour 17, 356-361.

McKinney, G.J., Waples, R.K., Seeb, L.W., Seeb, J.E., 2016. Paralogs are revealed by proportion of heterozygotes and deviations in read ratios in genotyping-by-sequencing data from natural populations. Mol Ecol Resour 17, 656-669.

Padial, J.M., Miralles, A., De la Riva, I., Vences, M., 2010. The integrative future of taxonomy. Frontiers in Zoology 7, 1-14.

Page, R.D.M., 1995. Parallel phylogenies: reconstructing the history of host-parasite assemblages. Cladistics 10, 155-173.

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F., Samadi, S., 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. Mol. Ecol. 24, 525-544.

Paredes, C., Cardoso, F., Tarazona, J., 1999. Invertebrados del intermareal rocoso del Departamento de Lima, Perú: una lista comentada de especies. Rev. Peru. Biol. 6, 143-151.

Parry, G., 1952. The Actiniaria of New Zealand. A check-list of recorded and new species, a review of the literature, and a key to the commoner forms. Part II. Records of the Canterbury Museum 6, 121-141.

Putnam, N.H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V.V., 2007. Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science 317, 86-94.

Reitzel, A.M., Darling, J.A., Sullivan, J.C., Finnerty, J.R., 2008. Global population genetic structure of the starlet anemone *Nematostella vectensis*: multiple introductions and implications for conservation policy. Biol. Invasions 10, 1197-1213.

Reitzel, A.M., Herrera, S., Layden, M.J., Martindale, M.Q., Shank, T.M., 2013. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Mol. Ecol. 22, 2953-2970.

Riemann-Zürneck, K., 1986. Zur biogeographie des siidwestatlantik mit besonderer beriicksichtigung der seeanemonen (Coelenterata: Actiniaria). Helgoländer Meeresuntersuchungen 40, 91-149.

Rodríguez, E., Barbeitos, M.S., Brugler, M.R., Crowley, L.M., Grajales, A., Gusmão, L., Häussermann, V., Reft, A.J., Daly, M., 2014. Hidden among sea anemones: The first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. PLoS Biol. 9, e96998.

Rodríguez, E., Barbeitos, M.S., Daly, M., Gusmão, L.C., Häussermann, V., 2012. Toward a natural classification: phylogeny of acontiate sea anemones (Cnidaria, Anthozoa, Actiniaria). Cladistics 28, 375-392.

Ronquist, F., 2003. Parsimony analysis of coevolving species associations. In: Page, R.D.M. (Ed.), Tangled Trees: Phylogeny, Cospeciation and Coevolution. University of Chicago Press, Chicago, IL, pp. 22-64.

Rousset, F., 2008. genepop’007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8, 103-106.

Sanmartín, I., Ronquist, F., 2004. Southern Hemisphere biogeography inferred by event-based models: plant versus animal patterns. Syst. Biol. 53, 216-243.

Seehausen, O., Butlin, R.K., Keller, I., Wagner, C.E., Boughman, J.W., Hohenlohe, P.A., Peichel, C.L., Saetre, G.P., Bank, C., Brannstrom, A., Brelsford, A., Clarkson, C.S., Eroukhmanoff, F., Feder, J.L., Fischer, M.C., Foote, A.D., Franchini, P., Jiggins, C.D., Jones, F.C., Lindholm, A.K., Lucek, K., Maan, M.E., Marques, D.A., Martin, S.H., Matthews, B., Meier, J.I., Most, M., Nachman, M.W., Nonaka, E., Rennison, D.J., Schwarzer, J., Watson, E.T., Westram, A.M., Widmer, A., 2014. Genomics and the origin of species. Nat. Rev. Genet. 15, 176-192.

Solé-Cava, A.M., Thorpe, J.P., 1991. High levels of genetic variation in natural populations of marine lower invertebrates. Biol. J. Linn. Soc. 44, 65-80.

Solis-Lemus, C., Knowles, L.L., Ane, C., 2015. Bayesian species delimitation combining multiple genes and traits in a unified framework. Evolution 69, 492-507.

Spalding, M.D., Fox, H.E., Allen, G.R., Davidson, N., Ferdaña, Z.A., Finlayson, M., Halpern, B.S., Jorge, M.A., Lombana, A., Lourie, S.A., Martin, K.D., McManus, E., Molnar, J., Recchia, C.A., Robertson, J., 2007. Marine ecoregions of the world: a bioregionalization of coastal and shelf areas. Bioscience 57, 573-583.

Sukumaran, J., Knowles, L.L., 2017. Multispecies coalescent delimits structure, not species. Proceedings of the National Academy of Sciences 114, 1607–1612.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Team, R.D.C., 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.

Thiel, M., Haye, P.A., 2006. The ecology of rafting in the marine environment. III. Biogeographical and evolutionary consequences. Oceanogr. Mar. Biol. Annu. Rev. 44, 323-428.

Verity, R., Collins, C., Card, D.C., Schaal, S.M., Wang, L., Lotterhos, K.E., 2017. MINOTAUR: A platform for the analysis and visualization of multivariate results from genome scans with R Shiny. Mol Ecol Resour 17, 33-43.

Vianna, P., Schama, R., Russo, C.A.M., 2003. Genetic divergence and isolation by distance in the West Atlantic sea anemone *Actinia bermudensis* (McMurrich, 1889). J. Exp. Mar. Biol. Ecol. 297, 19-30.

Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358-1370.

Whitlock, M.C., Lotterhos, K.E., 2015. Reliable detection of loci responsible for local adaptation: inference of a null model through trimming the distribution of F_{ST}. The American Naturalist 186, S24-S36.

Woodburne, M.O., Case, J.A., 1996. Dispersal, vicariance, and the Late Cretaceous to early tertiary land mammal biogeography from South America to Australia. J. Mamm. Evol. 3, 121-161.

Yang, Z., 2015. The BPP program for species tree estimation and species delimitation. Curr. Zoo. 61, 854-865.

Table 2.1. Genetic differentiation among local populations of *Anthothoe* based on Weir and Cockerham (1984) F_{ST} estimator. Thicker lines of the grid indicate the separation between ecoregions. Warmer colors indicate higher values of differentiation while cooler colors indicate lower values.

		New Zealand	**Australia**	**Chile**										**Argentina**	**South Africa**			
	Population	**BH**	**TAT**	**IQ**	**CA**	**LH**	**MOB**	**MON**	**CO**	**LIR**	**TAL**	**LIL**	**HU**	**PTC**	**LA**	**SP**	**FSH**	**ST**
Australia	**TAT**	0.5062																
Chile	**IQ**	0.5759	0.6449															
	CA	0.5449	0.6348	0.4618														
	LH	0.3719	0.4603	0.1768	0.0514													
	MOB	0.3557	0.4568	0.1555	0.0183	0.012												
	MON	0.508	0.5709	0.5032	0.5137	0.1063	0.1511											
	CO	0.4876	0.5762	0.5166	0.5424	0.0345	0.0435	0.2798										
	LIR	0.4557	0.55	0.4063	0.5382	0.0488	0.0591	0.3572	0.4063									
	TAL	0.5357	0.572	0.5258	0.5284	0.1715	0.1211	0.3791	0.2972	0.4123								
	LIL	0.4772	0.5731	0.3424	0.2319	0.1319	0.0879	0.3894	0.2254	0.2717	0.3715							
	HU	0.4508	0.5456	0.3078	0.2274	0.1904	0.1501	0.3559	0.2247	0.2828	0.3261	0.0214						
Argentina	**PTC**	0.5891	0.6275	0.6299	0.6059	0.4711	0.4669	0.5442	0.5495	0.5165	0.5869	0.5861	0.5692					
South Africa	**LA**	0.432	0.4853	0.4704	0.4197	0.298	0.2946	0.3173	0.2967	0.2234	0.399	0.4215	0.4545	0.4598				
	SP	0.5293	0.5968	0.6969	0.7289	0.28	0.2659	0.5241	0.6158	0.5537	0.6174	0.4851	0.4841	0.5458	0.1769			
	FSH	0.3893	0.4379	0.4362	0.2969	0.2514	0.2461	0.3494	0.1853	0.1018	0.3891	0.3491	0.4418	0.4997	0.2029	0.0452		
	ST	0.5221	0.572	0.7186	0.7489	0.3174	0.3059	0.5742	0.6459	0.595	0.6429	0.5173	0.5037	0.588	0.308	0.644	0.031	
	Average	**0.4832**	**0.5506**	**0.4731**	**0.4433**	**0.2065**	**0.1866**	**0.4015**	**0.3607**	**0.3538**	**0.4298**	**0.3427**	**0.3460**	**0.5523**	**0.3538**	**0.4869**	**0.2869**	**0.5108**
	SD (±)	**0.0698**	**0.0645**	**0.1673**	**0.2202**	**0.1533**	**0.1644**	**0.1431**	**0.2169**	**0.1953**	**0.1538**	**0.1701**	**0.1567**	**0.0556**	**0.1012**	**0.1944**	**0.1543**	**0.1981**

Table 2.2. Comparison of the diagnostic features of *Anthothoe* species from the Southern Hemisphere. Coloration is indicated separately for the column (C), oral disc (OD) and tentacles (T).

Species	N° of tentacles	Coloration	Mesenteries arrangement	Musculature	Reproduction	Cnidae	Distribution and habitat	Abundance	Main references*
Anthothoe albens (Stuckey 1909)	Up to 4 cycles (96 tentacles)	C: Opaque-white OD: Idem as column T: Opaque-white, with an incomplete ring of yellow pigment at the base of each tentacle	Not reported	Sphincter mesogleal, strong	Not reported	Not reported	New Zealand; In chinks among *Lessonia* holdfast or underneath large boulders	Rare	Stuckey (1909b)
Anthothoe albocincta (Hutton, 1879)	In 5-6 cycles (200 tentacles)	C: With alternating vertical stripes, pink and white, pellucid brown and white, brown and yellow, or green and yellow OD: Bright orange, sometimes with radial markings T: Opaque-white	Hexamerously arrange in 5 cycles; >15 pairs perfect	Sphincter mesogleal, strong; Retractors diffuse to diffuse-restricted, strong	Gonochoric and longitudinal fission	Spirocysts, Basitrichs, microbasic *p*-mastigophores and microbasic amastigophores	Australia and New Zealand; Under wharf piles, in rock pools and on open surfaces of rocks and reefs	Abundant	Stuckey (1909a) Carlgren (1950b)
Anthothoe australiae (Haddon and Duerden, 1896)	Not reported	C: Translucent buff OD: Translucent with insertions of mesenteries show as light lines Tentacles: Orangish	Not reported	Sphincter mesogleal, strong; Retractors diffuse, weak	Gonochoric	Not reported	Australia	Rare	Haddon and Duerden (1896)
Anthothoe australiensis Carlgren, 1950	Up to 5 cycles (130 tentacles)	Not reported	Hexamerously arrange in 4 cycles; 12 pairs perfect	Sphincter mesogleal, reticulated, strong; Retractors diffuse, weak	Not reported	Basitrichs, microbasic *p*-mastigophores and microbasic amastigophores	Australia; On shell with hermit	Rare	Carlgren (1950a)
Anthothoe chilensis (Lesson, 1830)	In 5-6 cycles (200 tentacles)	C: White, with 12-40 yellow-orange longitudinal stripes, rarely bluish or brownish OD: Transparent white, yellow, orange or rarely green-blue T: White, upper half may be orange	Hexamerously arrange in 5 cycles; 6-24 pairs perfect	Sphincter mesogleal, strong; Retractors diffuse, strong	Gonochoric and longitudinal fission	Spirocysts, basitrichs, *b*- mastigophores, microbasic *p*-mastigophores and microbasic amastigophores	Peru, Chile, South Georgia Island, Argentina, Southern Brazil and South Africa; in rocky substratum, in crevices, under overhangs or on biogenic substrata	Abundant	Excoffon et al. (1997)
Anthothoe neozelanica (Carlgren, 1924)	Up to 4 cycles (60 tentacles)	Not reported	Pentamerously arrange; ~10 pairs perfect	Sphincter mesogleal, strong; Retractors diffuse, strong	Not reported	Only nematocysts present	New Zealand	Rare	Carlgren (1924)
Anthothoe similis (Haddon and Duerden, 1896)	Not reported	C: Deep orange, almost a terra-cota tint OD: Idem as column T: Paler than column, a sort of flesh-color	Irregularly arrange in 3-4 cycles; 13 pairs perfect	Sphincter mesogleal; Retractors diffuse, weak	Not reported	Not reported	Australia	Rare	Haddon and Duerden (1896)
Anthothoe vagrans (Stuckey, 1909)	Up to 5 cycles (192 tentacles)	C: Dirty white and olive-brown in alternate longitudinal lines, or dirty white, gray or even pink-colored O: Olive-brown T: Salmon-pink, sometimes white	Hexamerously arrange in 5 cycles; 1-4 cycles perfect	Sphincter mesogleal, strong; Retractors diffuse, strong	Gonochoric	Only nematocysts present	New Zealand; At or below low water, adherent to stones, wharf piles and shells of mussels	Frequent	Carlgren (1924)

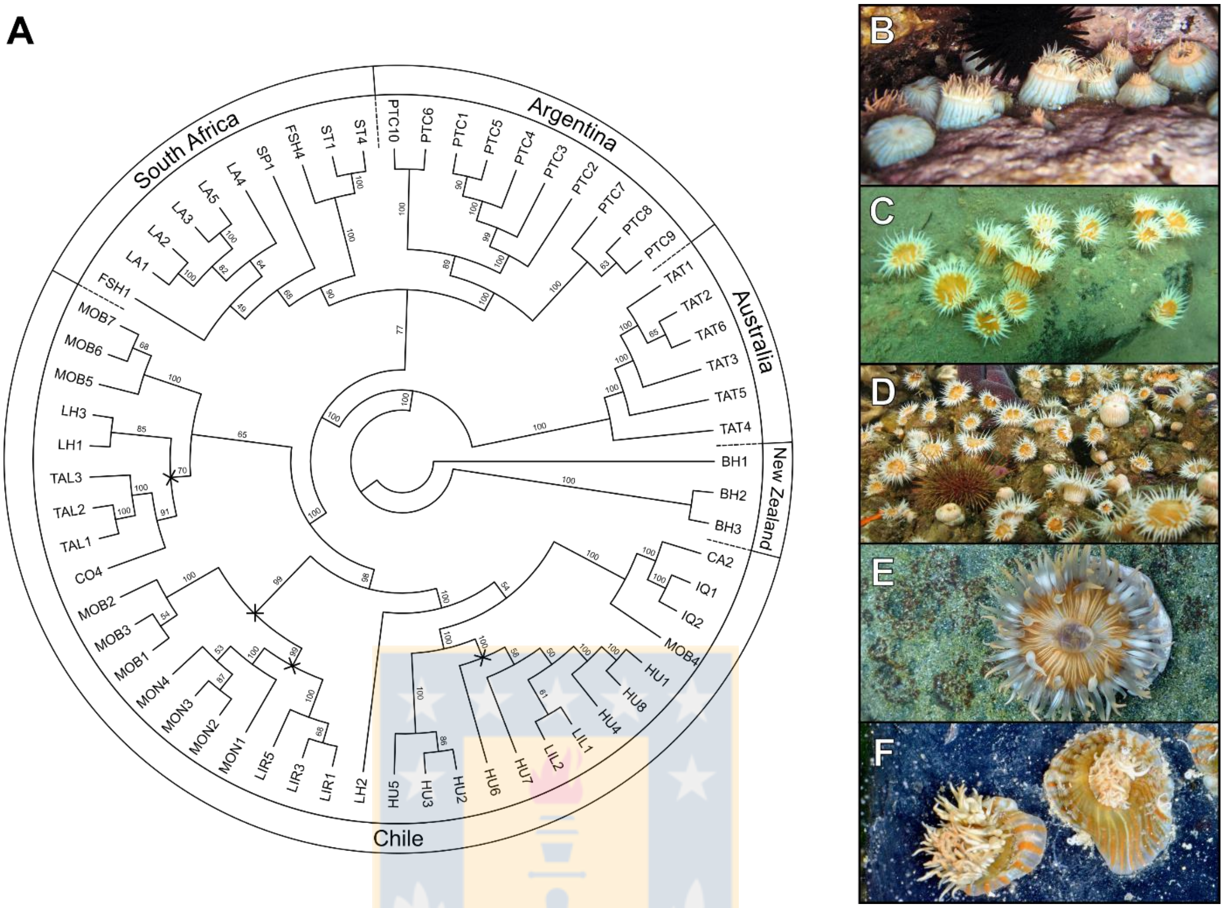
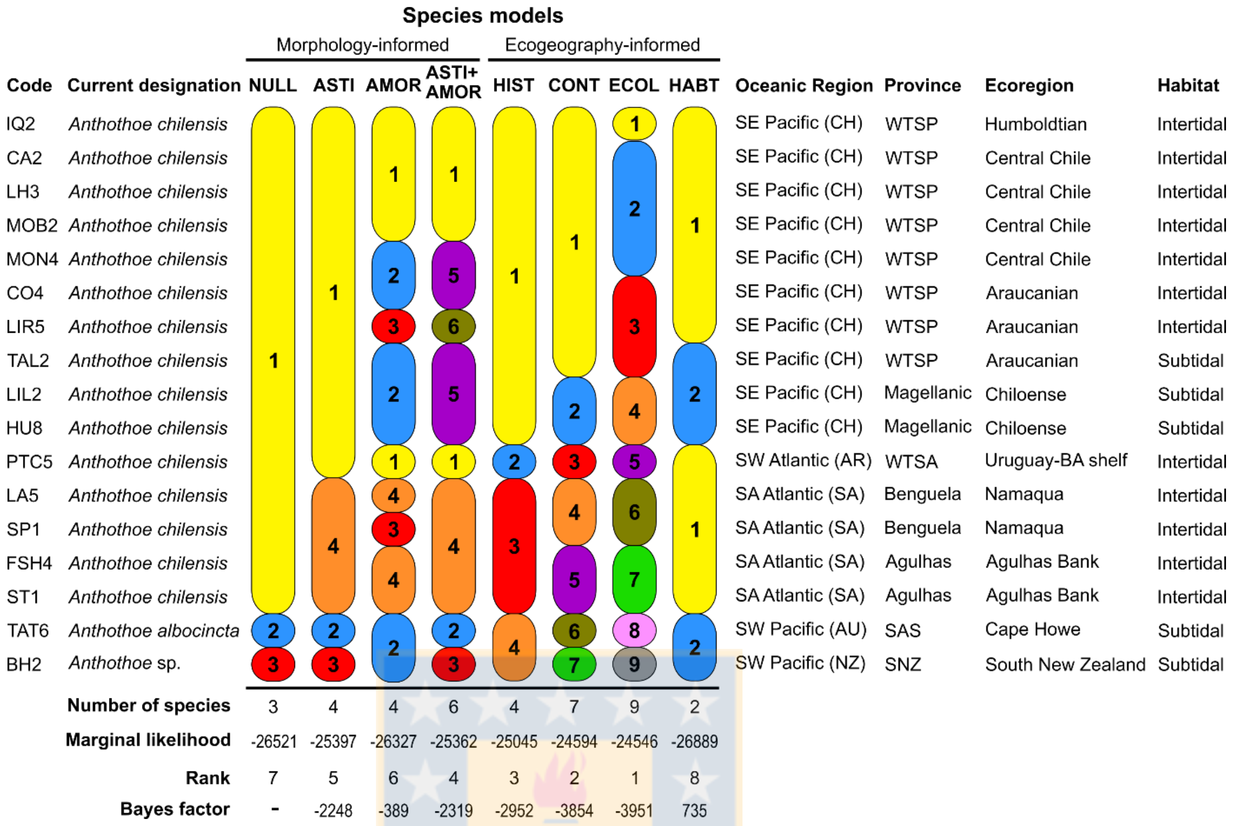


Figure 2.1. Phylogenetic reconstruction of *Anthothoe* lineages from the Southern Hemisphere based on RAD-seq data (A), with in situ photographs of the morphotypes included in this study (B-F). The tree was inferred with SVDquartets from the SNPs matrix that allowed 20% of missing data. Numbers above the branches are bootstrap support values expressed as percentage, and trees depicted are majority-rule consensus trees over 100 bootstrap samples; + indicates differences of topology with respect to the tree generated from the SNPs matrix with no missing data. The places where the pictures were taken are: B) Caldera (CA), Chile; C) Bluff Harbour (BF), New Zealand; D) Lilihuapi (LIL), Chile; E) Lirquen (LIR), Chile; F) Langebaan (LA), South Africa.



		Species models											
		Morphology-informed				Ecogeography-informed							
Code	**Current designation**	**NULL**	**ASTI**	**AMOR**	**ASTI+ AMOR**	**HIST**	**CONT**	**ECOL**	**HABT**	**Oceanic Region**	**Province**	**Ecoregion**	**Habitat**
IQ2	*Anthothoe chilensis*	1	1	1	1	1	1	1	1	SE Pacific (CH)	WTSP	Humboldtian	Intertidal
CA2	*Anthothoe chilensis*	1	1	1	1	1	1	2	1	SE Pacific (CH)	WTSP	Central Chile	Intertidal
LH3	*Anthothoe chilensis*	1	1	1	1	1	1	2	1	SE Pacific (CH)	WTSP	Central Chile	Intertidal
MOB2	*Anthothoe chilensis*	1	1	1	1	1	1	2	1	SE Pacific (CH)	WTSP	Central Chile	Intertidal
MON4	*Anthothoe chilensis*	1	1	2	5	1	1	2	1	SE Pacific (CH)	WTSP	Central Chile	Intertidal
CO4	*Anthothoe chilensis*	1	1	2	5	1	1	3	1	SE Pacific (CH)	WTSP	Araucanian	Intertidal
LIR5	*Anthothoe chilensis*	1	1	3	6	1	1	3	1	SE Pacific (CH)	WTSP	Araucanian	Intertidal
TAL2	*Anthothoe chilensis*	1	1	2	5	1	1	3	2	SE Pacific (CH)	WTSP	Araucanian	Subtidal
LIL2	*Anthothoe chilensis*	1	1	2	5	1	2	4	2	SE Pacific (CH)	Magellanic	Chiloense	Subtidal
HU8	*Anthothoe chilensis*	1	1	2	5	1	2	4	2	SE Pacific (CH)	Magellanic	Chiloense	Subtidal
PTC5	*Anthothoe chilensis*	1	1	1	1	2	3	5	1	SW Atlantic (AR)	WTSA	Uruguay-BA shelf	Intertidal
LA5	*Anthothoe chilensis*	1	4	4	4	3	4	6	1	SA Atlantic (SA)	Benguela	Namaqua	Intertidal
SP1	*Anthothoe chilensis*	1	4	3	4	3	4	6	1	SA Atlantic (SA)	Benguela	Namaqua	Intertidal
FSH4	*Anthothoe chilensis*	1	4	4	4	3	5	7	1	SA Atlantic (SA)	Agulhas	Agulhas Bank	Intertidal
ST1	*Anthothoe chilensis*	1	4	4	4	3	5	7	1	SA Atlantic (SA)	Agulhas	Agulhas Bank	Intertidal
TAT6	*Anthothoe albocincta*	2	2	2	2	4	6	8	2	SW Pacific (AU)	SAS	Cape Howe	Subtidal
BH2	*Anthothoe* sp.	3	3	2	3	4	7	9	2	SW Pacific (NZ)	SNZ	South New Zealand	Subtidal
	Number of species	3	4	4	6	4	7	9	2				
	Marginal likelihood	-26521	-25397	-26327	-25362	-25045	-24594	-24546	-26889				
	Rank	7	5	6	4	3	2	1	8				
	Bayes factor	-	-2248	-389	-2319	-2952	-3854	-3951	735				

Figure 2.2. Species delimitation analysis for *Anthothoe* specimens from the Southern Hemisphere. Different assignment models evaluated with BFD* are depicted as stacked bars for each one of the species hypotheses indicated in the top row. Bottom rows show the total number of species proposed, the marginal likelihood estimate (calculated from the matrix with no missing data, which contains 1803 SNPs for the 17 individuals), rank for each model, and Bayes Factor comparisons calculated with respect to the NULL model. The morphotypes referred on the AMOR model are: 1) white-bluish morph (Fig. 1B); 2) orange morph (Fig. 1C-D); 3) grayish morph (Fig. 1E), and 4) goldenrod morph (Fig. 1F). In the "Oceanic Region" column, CH refers to Chile, AR to Argentina, SA to South Africa, AU to Australia, and NZ to New Zealand. In the "Province" column, WTSP refers to Warm Temperate Southeastern Pacific, WTSA to Warm Temperate Southwestern Atlantic, SAS to Southeast Australian Shelf, and SNZ to Southern New Zealand.

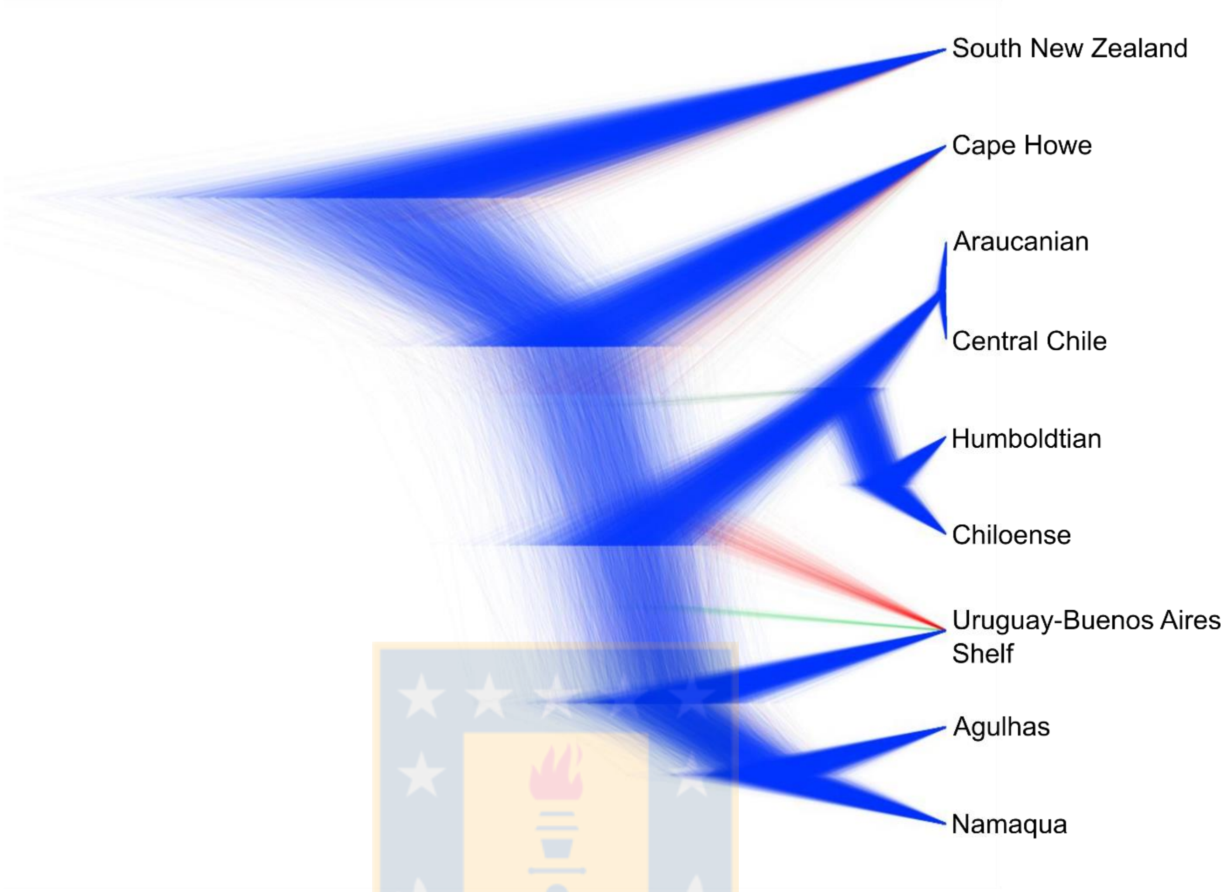


Figure 2.3. Species tree of the genus *Anthothoe* from the Southern Hemisphere. This claudogram illustrates the posterior distribution of the species trees inferred with SNAPP based on the best delimitation model ECOL. Each tip of the tree shows the ecoregion to which the different *Anthothoe* lineages belong. Color density indicates the level of topology agreement among trees, with different colors representing alternative topologies. All interior branches had posterior probabilities equal or close 1.0, excepting the one leading to the Southern Atlantic Ocean clade (Uruguay-Buenos Aires Shelf + Agulhas + Namaqua), which had a posterior probability of 0.94.

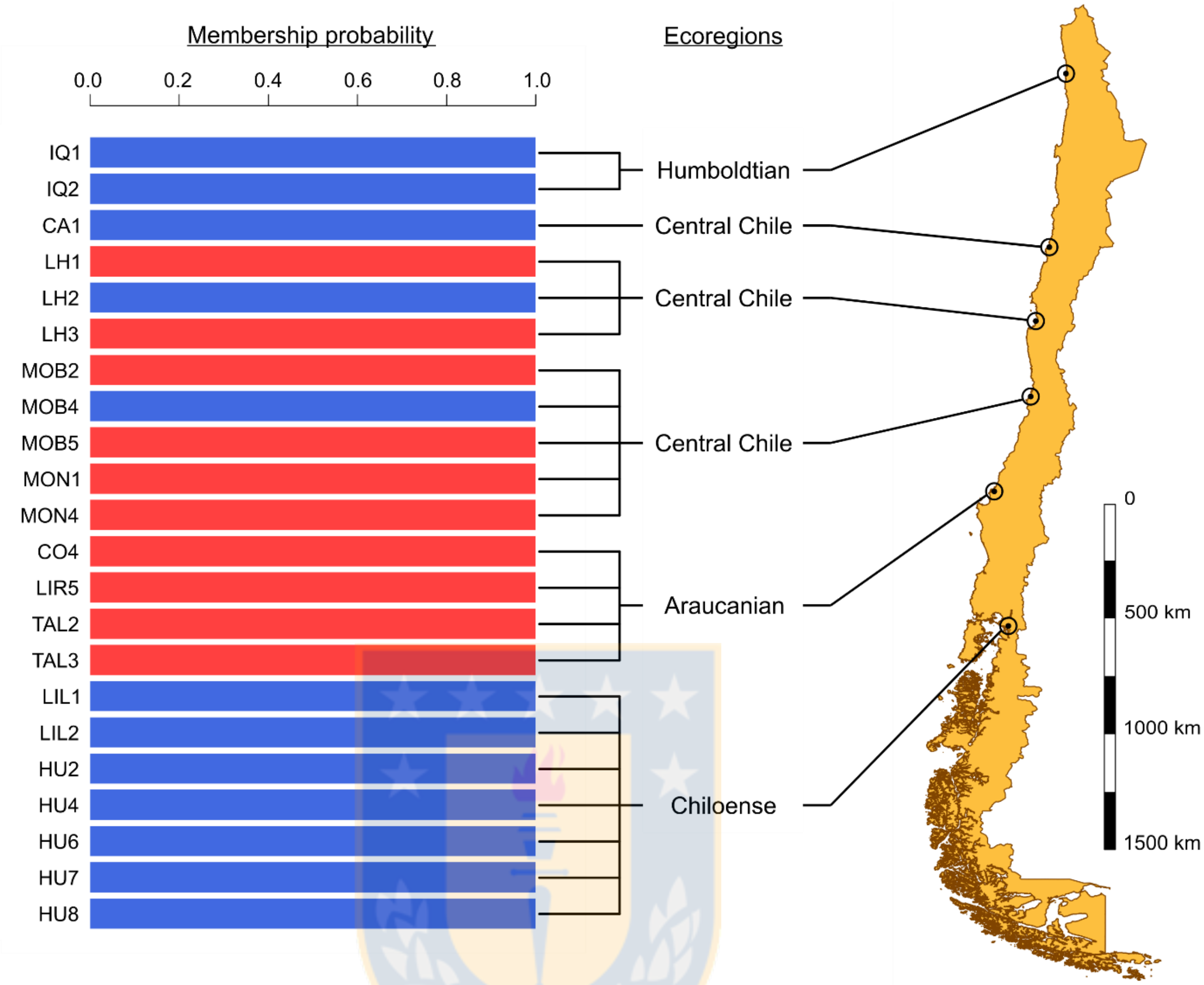


Figure 2.4. Individual assignment of Chilean populations to the genetic clusters identified by the DAPC analysis (K = 2). The area where the specimens were collected together with the ecoregion to which they belong is indicated for each *Anthothoe* individual.

Table 2.S1. Specimens included in this study, with voucher location and accession numbers. Quality-filtered reads with an asterisk (*) indicate the samples that were removed from downstream analyses.

Specimen ID	Barcode	Collection date	Quality-filtered reads (M)	Latitude	Longitude	Sampling Location	Habitat	Voucher location	Accession number
IQ1	GGCACCTTAC	23/05/2006	0.95	-20,1309	-70,0922	Iquique, Chile	Intertidal	MZUC-UCC	
IQ2	GGCCATCCTC	23/05/2006	1.09	-20,1309	-70,0922	Iquique, Chile	Intertidal	MZUC-UCC	
CA1	GGCCTCCTGA	17/11/2006	0.14*	-27,0346	-70,4926	Caldera, Chile	Intertidal	MZUC-UCC	
CA2	GGCGAACTTA	17/11/2006	6.43	-27,0346	-70,4926	Caldera, Chile	Intertidal	MZUC-UCC	
CA3	GGCGACTGGA	17/11/2006	0.02*	-27,0346	-70,4926	Caldera, Chile	Intertidal	MZUC-UCC	
LH1	GGACAAGCTA	28/05/2015	5.46	-29,5859	-71,2137	La Herradura, Chile	Intertidal	MZUC-UCC	
LH2	GGAAACATCG	28/05/2015	9.61	-29,5859	-71,2137	La Herradura, Chile	Intertidal	MZUC-UCC	
LH3	GGACATTGGC	28/05/2015	5.29	-29,5859	-71,2137	La Herradura, Chile	Intertidal	MZUC-UCC	
MON1	GGCAATGGAA	04/06/2016	2.57	-32,5725	-71,3303	Montemar, Chile	Intertidal	MZUC-UCC	
MON2	GGCACTTCGA	04/06/2016	3.40	-32,5725	-71,3303	Montemar, Chile	Intertidal	MZUC-UCC	
MON3	GGCAGCGTTA	04/06/2016	6.93	-32,5725	-71,3303	Montemar, Chile	Intertidal	MZUC-UCC	
MON4	GGCATACCAA	04/06/2016	3.01	-32,5725	-71,3303	Montemar, Chile	Intertidal	MZUC-UCC	
MOB1	GGAGATCGCA	04/06/2016	4.32	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
MOB2	GGAGCAGGAA	04/06/2016	4.50	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
MOB3	GGAGTCACTA	04/06/2016	6.72	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
MOB4	GGATCCTGTA	04/06/2016	5.79	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
MOB5	GGATTGAGGA	04/06/2016	4.48	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
MOB6	GGCAACCACA	04/06/2016	6.87	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
MOB7	GGCAAGACTA	04/06/2016	5.02	-32,5723	-71,3302	Montemar, Chile	Intertidal	MZUC-UCC	
CO1	GGACCACTGT	11/02/2016	0.08*	-36,3558	-72,5839	Cocholgüe, Chile	Intertidal	MZUC-UCC	
CO2	GGAACGTGAT	11/02/2016	0.31*	-36,3558	-72,5839	Cocholgüe, Chile	Intertidal	MZUC-UCC	
CO3	GGCGCTGATC	11/02/2016	0.05*	-36,3556	-72,5838	Cocholgüe, Chile	Intertidal	MZUC-UCC	
CO4	GGCAGATCTG	11/02/2016	4.06	-36,3556	-72,5838	Cocholgüe, Chile	Intertidal	MZUC-UCC	
CO5	GGATGCCTAA	11/02/2016	0.12*	-36,3556	-72,5838	Cocholgüe, Chile	Intertidal	MZUC-UCC	
LIR4	GGAGTGGTCA	11/02/2016	0.39*	-36,4208	-72,5839	Lirquen, Chile	Intertidal	MZUC-UCC	
LIR5	GGAACAACCA	11/02/2016	6.31	-36,4208	-72,5839	Lirquen, Chile	Intertidal	MZUC-UCC	

LIR1	GGAACGAACG	11/02/2016	6.17	-36,4210	-72,5839	Lirquen, Chile	Intertidal	MZUC-UCC
LIR2	GGAGTACAAG	11/02/2016	0.21*	-36,4210	-72,5839	Lirquen, Chile	Intertidal	MZUC-UCC
LIR3	GGCATCAAGT	11/02/2016	6.54	-36,4210	-72,5839	Lirquen, Chile	Intertidal	MZUC-UCC
TAL1	GGCCAGTTCA	04/06/2016	6.07	-36,4227	-73,0445	Talcahuano, Chile	Subtidal	MZUC-UCC
TAL2	GGCCGAAGTA	04/06/2016	5.86	-36,4227	-73,0445	Talcahuano, Chile	Subtidal	MZUC-UCC
TAL3	GGCCGTGAGA	04/06/2016	1.30	-36,4227	-73,0445	Talcahuano, Chile	Subtidal	MZUC-UCC
LIL1	GGAACCGAGA	01/04/2016	4.69	-42,0946	-72,3537	Lilihuapi, Chile	Subtidal	MZUC-UCC
LIL2	GGAACGCTTA	01/04/2016	1.01	-42,0946	-72,3537	Lilihuapi, Chile	Subtidal	MZUC-UCC
LIL3	GGAAGACGGA	01/04/2016	0.16*	-42,0946	-72,3537	Lilihuapi, Chile	Subtidal	MZUC-UCC
HU7	GGACTATGCA	15/04/2016	3.47	-42,2253	-72,2527	Huinay, Chile	Subtidal	MZUC-UCC
HU8	GGAGAGTCAA	15/04/2016	4.74	-42,2253	-72,2527	Huinay, Chile	Subtidal	MZUC-UCC
HU1	GGAAGGTACA	21/07/2015	7.54	-42,2327	-72,2521	Huinay, Chile	Subtidal	MZUC-UCC
HU2	GGACACAGAA	21/07/2015	5.34	-42,2327	-72,2521	Huinay, Chile	Subtidal	MZUC-UCC
HU3	GGACAGCAGA	21/07/2015	10.55	-42,2327	-72,2521	Huinay, Chile	Subtidal	MZUC-UCC
HU4	GGACCTCCAA	21/07/2015	2.01	-42,2327	-72,2521	Huinay, Chile	Subtidal	MZUC-UCC
HU5	GGACGCTCGA	21/07/2015	5.36	-42,2327	-72,2521	Huinay, Chile	Subtidal	MZUC-UCC
HU6	GGACGTATCA	21/07/2015	12.82	-42,2327	-72,2521	Huinay, Chile	Subtidal	MZUC-UCC
PTC1	GGCGCATACA	12/05/2016	6.74	-38,0542	-57,3229	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC2	GGCTCAATGA	12/05/2016	0.94	-38,0542	-57,3229	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC3	GGCTGAGCCA	12/05/2016	1.29	-38,0542	-57,3229	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC4	GGCTGGCATA	12/05/2016	2.77	-38,0542	-57,3229	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC5	GGGAATCTGA	12/05/2016	2.68	-38,0542	-57,3229	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC6	GGGACTAGTA	13/06/2016	1.66	-38,0539	-57,3228	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC7	GGGAGCTGAA	13/06/2016	2.76	-38,0539	-57,3228	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC8	GGGATAGACA	13/06/2016	5.99	-38,0539	-57,3228	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC9	GGGCCACATA	13/06/2016	3.69	-38,0539	-57,3228	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
PTC10	GGGCGAGTAA	13/06/2016	4.65	-38,0539	-57,3228	Pta. Cantera, Argentina	Intertidal	MZUC-UCC
LA1	GGGTCGTAGA	21/02/2016	3.25	-33,0526	18,0141	Langebaan, South Africa	Intertidal	MZUC-UCC
LA2	GGGTCTGTCA	21/02/2016	1.65	-33,0526	18,0141	Langebaan, South Africa	Intertidal	MZUC-UCC
LA3	GGGTGTTCTA	21/02/2016	1.01	-33,0526	18,0141	Langebaan, South Africa	Intertidal	MZUC-UCC
LA4	GGTAGGATGA	21/02/2016	2.35	-33,0526	18,0141	Langebaan, South Africa	Intertidal	MZUC-UCC
LA5	GGTATCAGCA	21/02/2016	4.26	-33,0526	18,0141	Langebaan, South Africa	Intertidal	MZUC-UCC
SP1	GGTCCGTCTA	25/01/2016	1.66	-33,5449	18,2313	Sea Point, South Africa	Intertidal	MZUC-UCC

SP2	GGTCTTCACA	25/01/2016	0.35*	-33,5449	18,2313	Sea Point, South Africa	Intertidal	MZUC-UCC
SP3	GGTGAAGAGA	25/01/2016	0.58*	-33,5449	18,2313	Sea Point, South Africa	Intertidal	MZUC-UCC
SP4	GGTGGAACAA	25/01/2016	0.14*	-33,5449	18,2313	Sea Point, South Africa	Intertidal	MZUC-UCC
SP5	GGTGGCTTCA	25/01/2016	0.55*	-33,5449	18,2313	Sea Point, South Africa	Intertidal	MZUC-UCC
FSH1	GGTGGTGGTA	09/02/2016	1.40	-34,0814	18,2615	Fishoek, South Africa	Intertidal	MZUC-UCC
FSH2	GGTTCACGCA	09/02/2016	0.21*	-34,0814	18,2615	Fishoek, South Africa	Intertidal	MZUC-UCC
FSH3	GGACACGAGA	09/02/2016	0.88*	-34,0814	18,2615	Fishoek, South Africa	Intertidal	MZUC-UCC
FSH4	GGAAGAGATC	09/02/2016	3.30	-34,0814	18,2615	Fishoek, South Africa	Intertidal	MZUC-UCC
KB1	GGAAGGACAC	09/02/2016	0.32*	-34,0736	18,2702	Kalk Bay, South Africa	Intertidal	MZUC-UCC
ST1	GGAATCCGTC	07/03/2016	4.89	-34,0703	18,4922	Strand, South Africa	Intertidal	MZUC-UCC
ST2	GGAATGTTGC	07/03/2016	0.20*	-34,0703	18,4922	Strand, South Africa	Intertidal	MZUC-UCC
ST3	GGACACTGAC	07/03/2016	0.14*	-34,0703	18,4922	Strand, South Africa	Intertidal	MZUC-UCC
ST4	GGACAGATTC	07/03/2016	7.96	-34,0703	18,4922	Strand, South Africa	Intertidal	MZUC-UCC
ST5	GGAGATGTAC	07/03/2016	1.57*	-34,0703	18,4922	Strand, South Africa	Intertidal	MZUC-UCC
TAT1	GGAGCACCTC	06/04/2008	2.64	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
TAT2	GGAGCCATGC	06/04/2008	2.68	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
TAT3	GGAGGCTAAC	06/04/2008	2.34	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
TAT4	GGATAGCGAC	06/04/2008	0.98	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
TAT5	GGACGACAAG	06/04/2008	1.12	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
TAT6	GGATTGGCTC	06/04/2008	3.41	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
TAT7	GGCAAGGAGC	06/04/2008	0.61*	-36,4657	149,5824	Tathra, Australia	Subtidal	AMRI
FI1	GGGGTGCGAA	14/02/2016	0.04*	-45,2400	166,5200	Fiordland, New Zealand	Subtidal	MZUC-UCC
FI2	GGGTACGCAA	14/02/2016	0.11*	-45,2400	166,5200	Fiordland, New Zealand	Subtidal	MZUC-UCC
BH1	GGGCTAACGA	01/02/2016	1.47	-46,3600	168,2103	Bluff Harbour, New Zealand	Subtidal	MZUC-UCC
BH2	GGGCTCGGTA	01/02/2016	2.51	-46,3600	168,2103	Bluff Harbour, New Zealand	Subtidal	MZUC-UCC
BH3	GGGGAGAACA	01/02/2016	2.37	-46,3600	168,2103	Bluff Harbour, New Zealand	Subtidal	MZUC-UCC

Table 2.S2. Mantel test based on Pearson's product-moment correlation from a series of 10,000 permutations for each batch run of the *Anthothoe* RAD-seq data. All correlation statistics were significant among pairwise comparison ($p < 0.001$).

***r* (PCA)**	**Batch1M2n2**	**Batch2M3n3**	**Batch3M4n4**	**Batch4M5n5**	**Batch5M6n6**	**Batch6M7n7**	**Batch7M8n8**
Batch1M2n2							
Batch2M3n3	0,9988*						
Batch3M4n4	0,9973*	0,9996*					
Batch4M5n5	0,9964*	0,9992*	0,9999*				
Batch5M6n6	0,9960*	0,9990*	0,9998*	0,9999*			
Batch6M7n7	0,9958*	0,9989*	0,9997*	0,9999*	1,0000*		
Batch7M8n8	0,9955*	0,9988*	0,9996*	0,9998*	0,9999*	1,0000*	

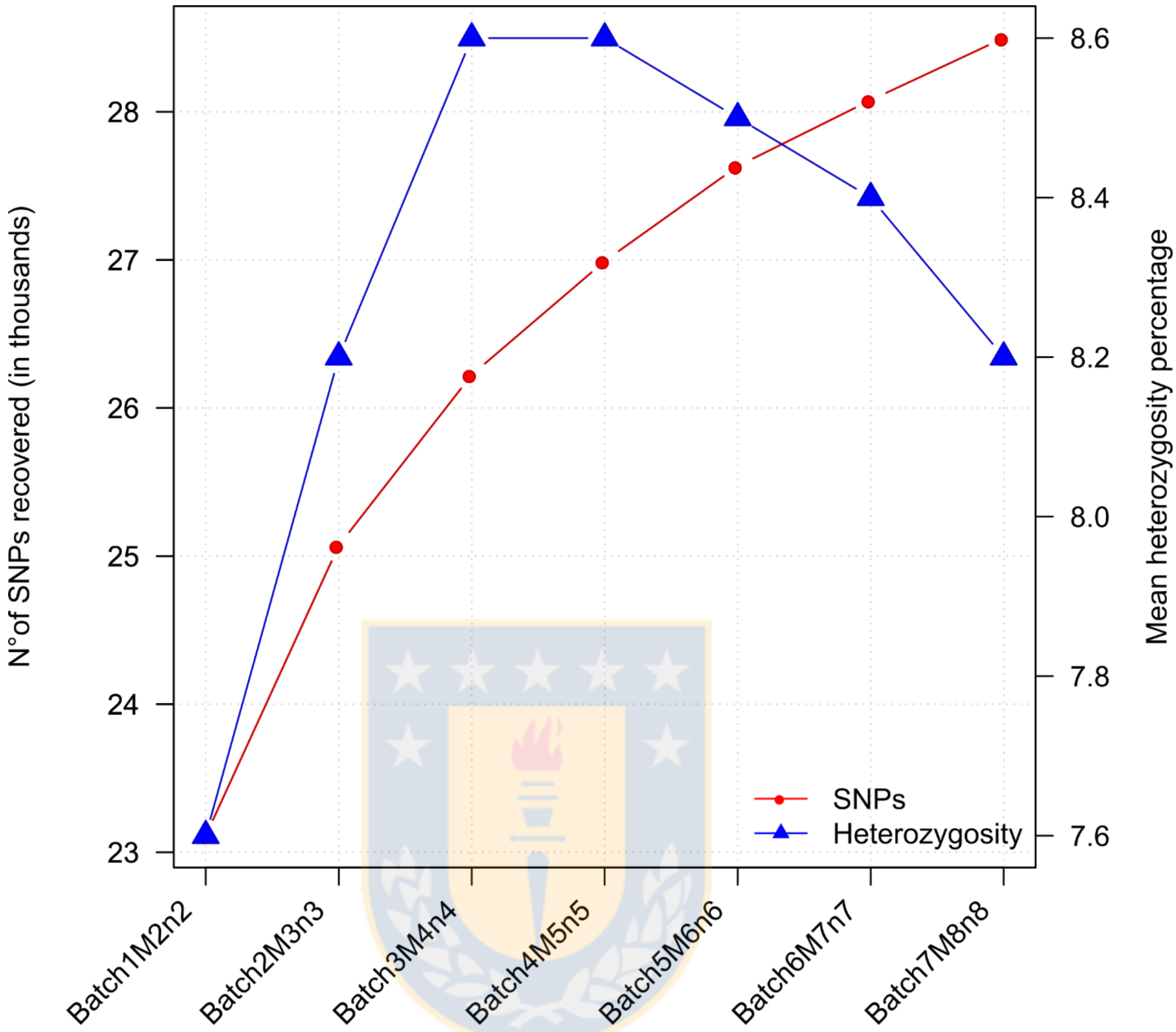


Figure 2.S1. Number of SNPs recovered and mean heterozygosity percentage for each batch run with different divergence parameters (i.e. *M* and *n*; for details see main text). The plot shows the results from the final post-processing stage, considering a filtering threshold of 50% of missing data per individual, 20% missing data per loci and a single SNP per locus chosen according to their minor allele frequency.

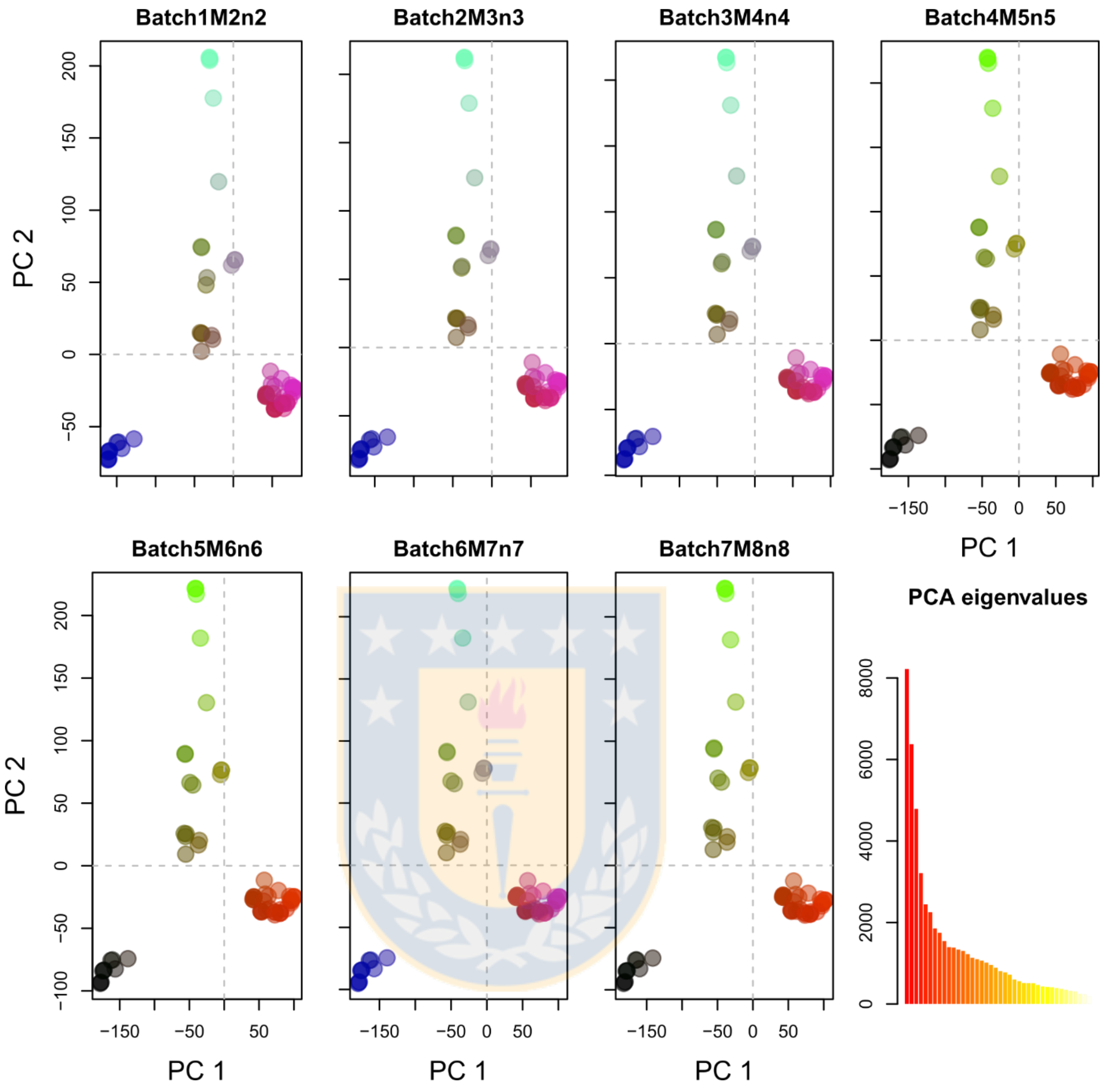


Figure 2.S2. Principal component analyses (PCA) from each batch run with different divergence parameters (i.e. *M* and *n*; for details see main text). All plots are based on the first two components which explain more than 25% of the variance (see PCA eigenvalues). Note that they all show the same pattern even though the colors associated to the axes layout may vary in some cases.

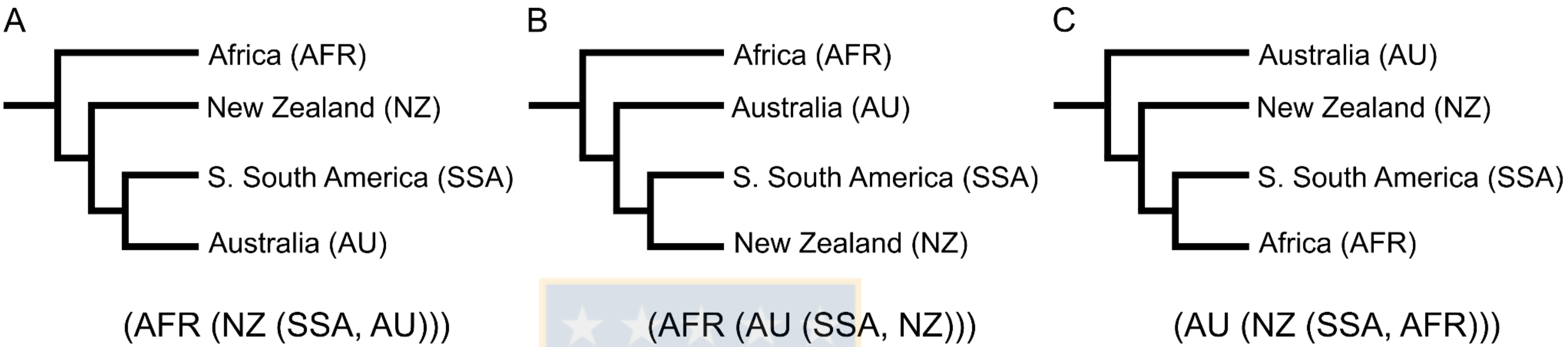


Figure 2.S3. Area cladograms representing well documented hierarchical biogeographic patterns that could explain the current southern distribution of *Anthothoe* lineages: A) Southern Gondwana pattern; B) inverted southern pattern; and C) west wind dispersal. The basic translation for TreeFitter appears below each area cladogram.

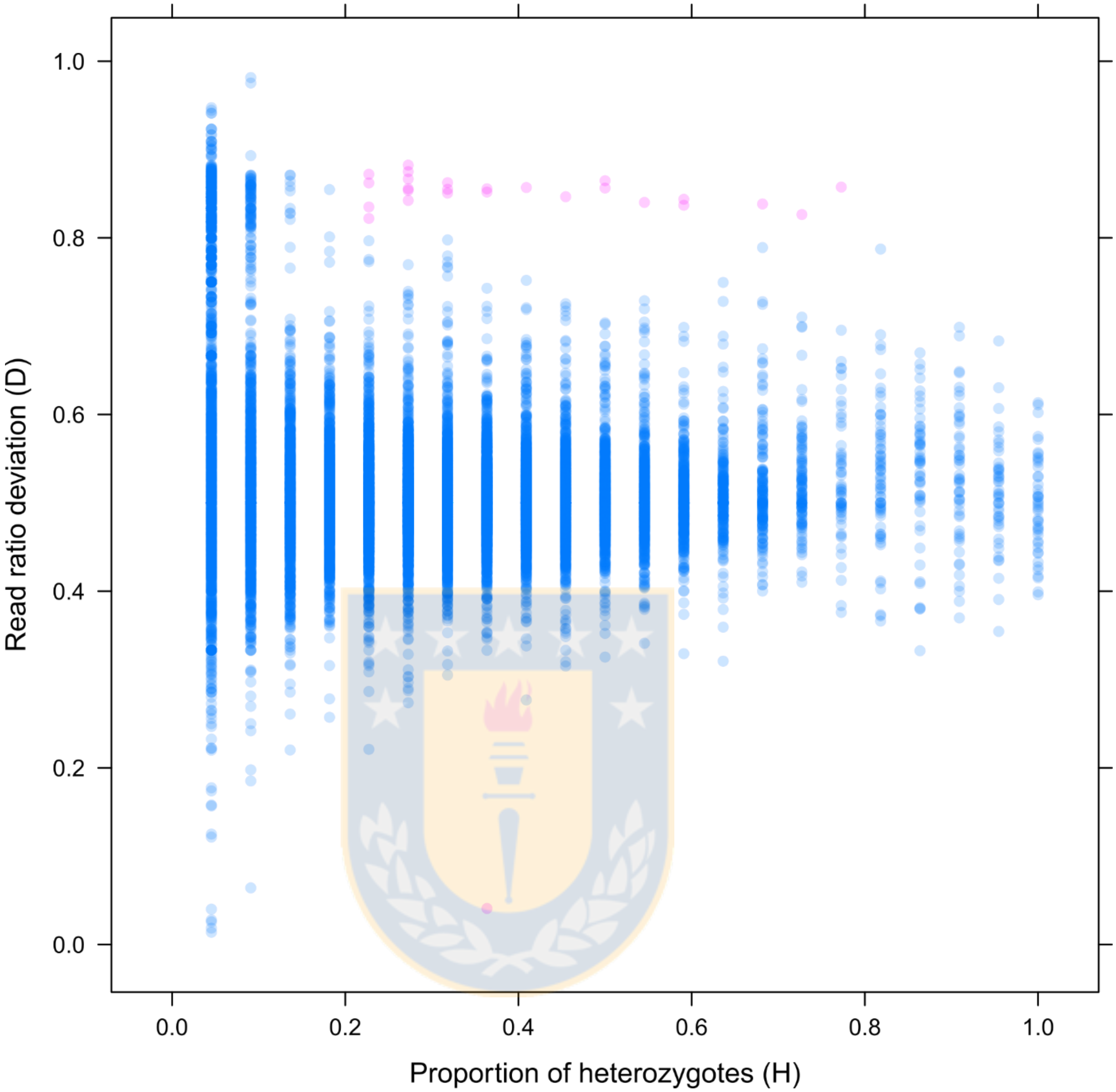


Figure 2.S4. Read-ratio deviation z-score (*D*, *y*-axis) plotted against proportion of heterozygotes (*H*, *x*-axis) for each locus in the dataset of the Chilean populations (see *HDplot* method of McKinney et al. (2016)). Presumed singletons form the dense cloud between 0.2 and 0.8 on the *y*-axis for *D*. Loci outside these thresholds and with *H* > 0.2 are highlighted in pink as possible duplicate loci.

CONCLUSION GENERAL

La presente Tesis Doctoral constituye la primera aproximación genómica poblacional ante el recurrente problema de la delimitación de especies en anémonas de mar. Los altos niveles de información proporcionados por las herramientas de secuenciación masiva permitieron alcanzar una resolución sin precedentes en un grupo de organismos que ha demostrado ser molecularmente muy conservado. Este trabajo no solo contextualiza con múltiples ejemplos las limitaciones de las clasificaciones taxonómicas exclusivamente basadas en la morfología, sino que además provee por primera vez una evaluación directa de la geografía tanto en las hipótesis de especies como en los posibles procesos biogeográficos asociados a la divergencia de los linajes.

La extensa revisión bibliográfica del primer capítulo comienza por describir las principales características de las unidades taxonómicas en donde anida el grupo de las anémonas de mar. Entender el alto grado de polimorfismos y, a su vez, la ausencia de caracteres sólidos como huesos o espinas, es clave para explicar porque estos animales suelen ser tan difíciles identificar (Häussermann 2004). Por otra parte, las descripciones de un número importante de especies se ha llevado a cabo sobre material preservado, frecuentemente de pocos individuos y que en condiciones inadecuadas han permitido perpetuar largas historias sinonímicas. Muchas especies cripticas emergen a partir de estas circunstancias, tal como ocurre con el complejo *Anthothoe*. Hasta mediados del siglo pasado, la labor de clasificar a las anémonas marinas ha dependido de la experiencia de unos pocos especialistas, quienes generalmente ponderaban de distinta manera los rasgos que poseen las especies para ser caracterizadas. Los avances de la biología molecular han ayudado significativamente a la sistemática del grupo (e.g. Rodríguez et al. 2014), sin embargo, la lenta evolución observada en secuencias de ADN han confinado bastante estas herramientas a estudios más bien macroevolutivos.

Gran parte de las descripciones del último tiempo demuestran que la determinación de nuevas especies apelan típicamente a un conjunto de características altamente variables tales como los patrones de coloración y las medidas de los cnidocistos. Esto se ha estado haciendo regularmente pese a la falta de conocimiento sobre la estructura y evolabilidad de los rasgos diagnósticos. De hecho, estudios en las últimas décadas han ido demostrando cómo muchos de estos atributos pueden estar fuertemente influenciados por factores endógenos y exógenos, en algunos casos presentando notorias diferencias en función del contexto social de los individuos (Francis, 2004; Stoletzki and Schierwater, 2005; Schlesinger et al., 2010). Por otra parte, análisis aloenzimáticos han contribuido a delimitar especies de anémonas ya que a diferencia de otros marcadores, estos han revelado altos niveles de diferenciación poblacional (e.g. Schama 2005). Interesantemente, en múltiples estudios estos han revelado estar mucho más relacionados con la distribución geográfica que a características estrictamente morfológicas (e.g. Larson & Daly 2016), habiendo, por el contrario, una relativa baja correspondencia entre las clasificaciones taxonómicas tradicionales y las filogenias recientes. Estos resultados no solo relevan la importancia del espacio en la divergencia de las anémonas de mar, sino, además, rechazan la validez de varios rasgos diagnósticos, al menos para entender las relaciones entre especies. Si efectivamente este es un patrón generalizado y los linajes de anémonas tienen a divergir en ausencia de cambios fenotípicos, nosotros proponemos que se trabaje basalmente con una hipótesis de especie que considere a los taxa crípticos como linajes conespecíficos profundos (de Queiroz, 2007; Padial et al., 2010).

El segundo capítulo evalúa estas propuestas en un complejo de especies del género *Anthothoe* ampliamente distribuido en el Hemisferio Sur. De acuerdo a la base de datos de Fautin (2013), este grupo está compuesto mayoritariamente por la especie *Anthothoe chilensis*, sin embargo, la calidad de las descripciones de las otras especies hace prácticamente imposible poder distinguirlas unas de otras. Esto fue confirmado en terreno, identificando al menos cuatro morfotipos ligeramente distintos entre especímenes procedentes de Australia, Nueva Zelanda, Chile, Perú, Argentina y Sudáfrica. Debido a las limitaciones de los marcadores tradicionales para resolver relaciones filogenéticas a escala microevolutiva, este estudio incorporó por primera vez herramientas de secuenciación masiva (*RAD-tags*) para la delimitación de especies de anémonas de mar. Asimismo, dada las bajas tasas de sustitución nucleotídica y la posible divergencia reciente de las poblaciones es que se emplearon métodos coalescentes para modelar la probable repartición incompleta de los linajes crípticos incluidos en la muestra.

Los análisis genómicos revelaron una baja correspondencia entre los patrones de divergencia y las variedades morfológicas del complejo *Anthothoe*. Al igual que en estudios previos en otras especies de anémonas (e.g. Daly et al. 2017), los linajes tienden estar mucho más relacionados en función de donde viven que a como lucen externamente. La hipótesis que utiliza las ecoregiones como unidades de asignación de especies fue la que recibió un mayor soporte de probabilidad, lo cual es concordante con los altos niveles de diferenciación poblacional observados entre localidades cercanas. Precisamente debido a esta gran diferenciación “de fondo” es que no fue posible estimar con exactitud qué tan preponderante es la selección natural en la divergencia de los linajes, sin embargo, parece ser al menos relevante en la adecuación biológica de los procesos metabólicos y celulares de los organismos (ver también Reitzel et al. 2013). Finalmente, se proponen nueve especies candidatas propias del complejo *Anthothoe* para el Hemisferio Sur, las cuales, de acuerdo a las conclsuiones del capítulo anterior, debiesen ser interpretadas inicialmente como linajes conespecíficos profundos. Debido a que cada una presenta distintos niveles de diferenciación (o en otras palabras, se hallan en distintas etapas dentro del continuo de divergencia), estas deberán ser validadas sobre algún otro conjunto de caracteres taxonómicos como por ejemplo, la ultraestructura de los nematocistos.

La exigua relación observada entre la variación genómica y la variación morfológica plantea nuevas preguntas sobre la naturaleza de los cambios fenotípicos que ocurren durante la especiación. Una posibilidad, por ejemplo, es que la diferenciación genética esté ocurriendo discontinuamente a lo largo del genoma, habiendo genes particulares apostados en ciertas regiones cromosómicas que están divergiendo conforme lo van expresando los organismos (Nosil & Freder 2012). Estas regiones son popularmente denominadas como "islas genómicas de especiación" y si bien aún no hay consenso respecto a que tan efectivas son en realidad, algunos estudios recientes han dado pistas sobre su existencia al comparar poblaciones simpátricas y alopátricas (Marques et al. 2016). Otra posible explicación es que buena parte de las variaciones morfológicas observadas son más bien respuestas fenotípicas ante las condiciones del ambiente. Estas pueden deberse a modificaciones en la expresión de los genes en lugar de cambios directos sobre las secuencias nucleotídicas (e.g. Duncan et al. 2014). Esta hipótesis es particularmente atractiva considerando el alto grado de totipotencialidad de los cnidarios y el amplio espectro de estrategias reproductivas que pueden adoptar incluso dentro de una misma especie.

Cada una de estas hipótesis proyecta nuevas líneas de investigación en torno al estudio de los procesos microevolutivos que dan forma a la biodiversidad. Establecer límites entre especies desconociendo los mecanismos que subyacen a la diferenciación entre linajes tiende a culminar en una determinación practicamente arbitraria respecto al número de especies en un área dada (Willis 2017). Si bien nuestro estudio se desarrolló sobre un complejo de especies particular, antecedentes en otras especies sugieren que los fenómenos aquí descritos son más bien patrones generalizados en las anémonas de mar (e.g. Vianna et al. 2003). De ser así, los resultados de esta investigación relevan sustancialmente la importancia del aislamiento geográfico en la divergencia de los linajes y, por lo tanto, en la delimitación de especies. Más aun, tanto por el tipo de preguntas como por la aproximación metodológica, la presente Tesis Doctoral consigue expandir nuestro conocimiento sobre los mecanismos que estarían detrás del extraordinario polimorfismo de este ancestral orden de invertebrados.

Referencias

Daly M, LM Crowley, P Larson, E Rodriguez, E Heestand & DG Fautin. 2017. *Anthopleura* and the phylogeny of Actinioidea (Cnidaria: Anthozoa: Actiniaria). Organisms Diversity & Evolution 17: 545-564.

de Queiroz K. 2007. Species concepts and species delimitation. Systematic Biology 56: 879-886.

Duncan EJ, PD Gluckman & PK Dearden. 2014. Epigenetics, plasticity, and evolution: How do we link epigenetic change to phenotype? Journal of Experimental Zoology 322(4):208-20.

Fautin DG. 2013. Hexacorallians of the World. Available from: http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm.

Francis L. 2004. Microscaling: why larger anemones have longer cnidae. The Biological Bulletin 207: 116-129.

Häussermann V. 2004 Identification and taxonomy of soft-bodied hexacorals exemplified by Chilean sea anemones; including guidelines for sampling, preservation and examination. Journal of the Marine Biological Association of the United Kingdom 84: 931-936.

Larson PG & M Daly. 2016. Phylogenetic analysis reveals an evolutionary transition from internal to external brooding in Epiactis Verrill (Cnidaria: Anthozoa: Actiniaria) and rejects the validity of the genus *Cnidopus* Carlgren. Molecular Phylogenetics and Evolution 94: 548-558.

Marques DA, K Lucek, JI Meier, S Mwaiko, CE Wagner, L Excoffier & O Seehausen. 2016. Genomics of rapid incipient speciation in sympatric threespine stickleback. PLoS Genet 12(2): e1005887.

Nosil P & JL Feder. 2012. Genomic divergence during speciation: causes and consequences. Philosophical Transaction of the Royal Society (B) 367: 332-342.

Padial, JM., A Miralles, I de la Riva & M Vences. 2010. The integrative future of taxonomy. Frontiers in Zoology 7, 1-14.

Reitzel AM, S Herrera, MJ Layden, MQ Martindale & TM Shank. 2013. Going where traditional markers have not gone before: utility of and promise for RAD sequencing in marine invertebrate phylogeography and population genomics. Molecular Ecology 22: 2953-2970.

Rodriguez E, MS Barbeitos, MR Brugler, LM Crowley, A Grajales, L Gusmao, V Häussermann, A Reft & M Daly. 2014. Hidden among sea anemones: the first comprehensive phylogenetic reconstruction of the order Actiniaria (Cnidaria, Anthozoa, Hexacorallia) reveals a novel group of hexacorals. PLoS ONE 9(5): e96998.

Schama R, AM Sole-Cava & JP Thorpe. 2005. Genetic divergence between east and west Atlantic populations of *Actinia* spp. sea anemones (Cnidaria: Actiniidae). Marine Biology 146: 435-443.

Schlesinger A., E Kramarsky-Winter, H Rosenfeld, R Armoza-Zvoloni & Y Loya. 2010. Sexual plasticity and self-fertilization in the sea anemone *Aiptasia diaphana*. PLoS ONE 5: e11874.

Stoletzki, N & B Schierwater. 2005. Genetic and color morph differentiation in the Caribbean sea anemone *Condylactis gigantea*. Marine Biology 147: 747-754.

Vianna P, R Schama & CAM Russo. 2003. Genetic divergence and isolation by distance in the West Atlantic sea anemone *Actinia bermudensis* (McMurrich, 1889). Journal of Experimental Marine Biology and Ecology 297: 19-30.

Willis SC. 2017. One species or four? Yes!...and, no. Or, arbitrary assignment of lineages to species obscures the diversification processes of Neotropical fishes. PLoS ONE 12(2): e0172349.

APENDICE 1

Evolutionary dispersal drives the latitudinal diversity gradient of stony corals

Carlos A. Spano[1], Cristián E. Hernández[2] and Marcelo M. Rivadeneira[3]

[1] Programa Doctorado de Sistemática y Biodiversidad, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

[2] Laboratorio de Ecología Evolutiva y Filoinformática, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas Universidad de Concepción, Barrio Universitario s/n, Casilla 160-C, Concepción, Chile

[3] Laboratorio de Paleobiología, Centro de Estudios Avanzados en Zonas Áridas (CEAZA) and Universidad Católica del Norte, Av. Bernardo Ossandón 877, C.P. 1781681, Coquimbo, Chile

(Published in Ecography)

Abstract

The diversity of stony corals displays one of the most exemplary latitudinal gradients on the planet, yet the evolutionary dynamics that produced this pattern remains unclear. Using both paleontological and distributional data, we compare the origination, extinction and immigration levels between low and high latitudes since the earliest proliferation of the group during the mid-Triassic. Altogether, first and last occurrence localities in the fossil record do not support a positive preference towards either latitudinal bin. Nonetheless, considering past and present scleractinian fauna, the process of extinction has been apparently more pronounced at higher latitudes based on face values and correlation coefficients. Far above these differences, immigration of extant taxa has been substantially higher towards the tropics than to temperate regions. While the net dispersal toward low latitudes persists in all temporal intervals, the

gradient of diversity was largely built up during the Cenozoic Era and only becomes significantly steep from the Neogene Period onwards. This dynamic supports the “into the tropical museum” model, which suggests that tropics have historically acted as a center of accumulation for marine biodiversity.

Introduction

The pronounced upsurge in species richness from the poles to the tropics is one of the oldest and yet most famous large-scale pattern in ecology (Pianka 1966, Hawkins 2001). With few exceptions, the latitudinal diversity gradient (LDG) is strikingly persistent regardless of the biota’s taxonomic affiliation, geographic context or even time domain (Willig et al. 2003, Hillebrand 2004). A consequence of this same universality is that in recent decades numerous studies have accumulated a vast variety of hypotheses addressing the primary causes underlying this pattern (see Krug et al 2009). Thus far, consensus remains elusive among ecologists about the actual mechanism that gave rise to the modern distribution of biodiversity.

In this context it is undoubtedly apparent that evolution of lineages must have played a pivotal role to set and shape the present-day LDG (Mittelbach et al. 2007). As we gather more information about the past, evolutionary and historical explanations have been expanding the traditional ecological framework to a more long-term progression of natural events over the diversification rates. Currently, most evolutionary hypotheses ascribe the higher number of taxa in the tropics to a higher rate of speciation or a lower extinction rate compared to temperate zones (Mittelbach et al. 2007). While these simplified scenarios may occur, this dichotomy is misleading and contradicts most of the biogeographic data that show large distributional changes over time (Jablonski et al. 2006, Roy and Goldberg 2007). In marine bivalves, for instance, more than half of the genera that originated in the tropics have extend their geographic range towards higher latitudes, evidence used by Jablonski et al. (2006) to suggest an “out of the tropics” (OTT) dynamic. Linking evolutionary and ecological processes through a spatially explicit approach, this model pose that ‘taxa preferentially originate in the tropics and expand toward the poles but without losing their tropical presence’. Although the OTT model was tested in marine organisms, phylogenetic information on terrestrial clades also confirmed these predictions over the tropical conservatism hypothesis (Jansson et al. 2013). In fact, dynamic model simulations of range shifts and diversification processes have shown that extratropical regions may act not only as a macroevolutionary sink but as a dispersal source as well (Goldberg et al. 2005, Arita and Vázquez-Domínguez 2008). Either way, results consistently indicate that speciation and extinction cannot fully explain the canonical LDG, thus it seems quite likely that historical dispersion plays a central role in the current distribution of taxa (Goldberg et al. 2005, Jablonski et al. 2006, Roy and Goldberg 2007, Arita and Vázquez-Domínguez 2008, Escarguel et al. 2008, Soria-Carrasco and Castresana 2012).

Stony corals (Order Scleractinia) are the world’s primary reef-builders, one of the most productive and biologically diverse ecosystems on the planet (Moberg and Folke 1999). Given that not all species form massive calcareous skeletons, genera are often characterized based on whether or not they contribute to the framework of reefs (hermatypic and ahermatypic corals, respectively) or interact with zooxanthellae (Schuhmacher and Zibrowius 1985). This remarkable diversification in response to different ecological requirements is well imprinted on its modern distribution, scleractinian representatives being found from shallow tropical waters to the great depths near the poles (Keller 1976). The vast majority of stony corals, however, are currently located in tropical latitudes and, more precisely, between the 30° north and south of the equator (Stehli and Wells 1971, Veron 1995, Keller 1998, Cairns 2007). Since Darwin’s first monograph about coral atolls, this particular LDG has caught the attention of marine biogeographers for years and it has been even used as an historical indicator of tropical conditions (Jablonski 1993, Bellwood and Hughes 2001, Hughes et al. 2002, Connolly et al. 2003, Bellwood et al. 2005). Veron (1995) explained this spatial pattern as a 'progression of layers of detail', where the family-level distribution determined by major global and climatological changes such as the continental movements and extinction events, is passed down as a template to generic groups defined by more recent geological events, especially the obliteration of the Tethys Sea and the closure of the Central American Seaway. As an outcome of the post-Pliocene climate, the highest species richness is nowadays concentrated in the Indonesian/Philippines archipelago due to a combination of environmental and geometric constraints (Kleypas et al. 1999, Bellwood and Hughes 2001, Connolly et al. 2003, Bellwood et al. 2005). This area commonly known as the "Coral Triangle" comprises thirty-two functional seascapes, eleven different ecoregions and is today acknowledged as the global center of marine biodiversity (Green and Mous 2008).

While most Scleractinia ancestors date back from the Middle Triassic (Veron 1995), the historical variability of the gradient slope as well as the evolutionary dynamic that built up their present distribution remains largely untested (but see Kiessling et al. 2012). As a group, stony corals fulfill all the criteria to address each of the OTT predictions directly (i.e. possesses a distinguishable LDG, a fossil record fairly sampled and its taxonomy is well standardized across groups). Like no other major type of ecosystem, coral reefs imprint paleoclimatic events through massive ramparts of rock which are a direct product of the environmental conditions (Veron 2008). Although we cannot completely rule out a spatial bias, these geological structures have been sampled nearly worldwide (including non-reef-building corals). Given its ecological and socioeconomic importance (see Moberg and Folke 1999), coral taxonomy has been comprehensively reviewed in popular textbooks (e.g. Veron 2000), specialized journals (e.g. *Coral Reefs*) and online projects (http://www.corallosphere.org/index). Scleractinia phylogeny is also currently undergoing an extensive revision that has frequently revealed little coincidence between the skeleton morphology and the evolutionary history of its major clades (Romano and Palumbi 1996, Huang 2011, Budd 2012). Though results from molecular techniques are similarly under intense debate (e.g. Kitahara et al. 2014), we acknowledge some of the taxonomic limitations the fossil record might have. Still, the present study assesses the latitudinal diversity gradient of stony corals throughout geologic time. Following Jablonski et al. (2006, 2013)' approach, here we: (i) test the fidelity and sampling bias of the fossil record, (ii) estimate the origination, extinction and migration levels on a spatially explicit scenario, using modern latitudinal distribution and paleontological data of the first and last occurrence of corals genera, (iii) evaluate the historical strength of the canonical LDG, and (iv) discuss the role of low and high latitudes in the light of the spatiotemporal dynamic underlying the modern scleractinians diversity gradient.

Material and Methods

Paleontological and distributional data

As most paleontological studies, we used the genera level (henceforth termed taxa) as the unit of analysis owing its representativeness to macroecological processes, completeness of fossil record and robustness against sampling artifacts common at lower levels (Campbell and Valentine 1977, Jablonski and Finarelli 2009). All living stony corals were identified through the World Register of Marine Species (WoRMS, September 2014) and subsequently verified in the *Corallosphere* web application (http://www.corallosphere.org/, September 2014). To determine the first and last occurrence in the fossil record (the latter only for extinct taxa), about 32 000 occurrences data of Scleractinia were downloaded from the Paleobiology Database (PBDB, accessed on 26 August 2014). Of the 30 extant families, only one completely lacks a fossil record (Coscinaraeidae), therefore, a total of 29 families and 223 genera were finally included in the comparative analysis between past and present distributions. Modern geographic ranges were projected through the Ocean Biogeographic Information System (OBIS, September 2014) and refined using the *Corals of the World* information base (Veron, 2000).

Sampling bias and fidelity of the fossil record

Spatial bias occurs, among other reasons (see Vilhena and Smith 2013), due to sampling effort varying non-randomly between geographic locations. Even though this study considers spatial bins large enough to minimize this effect, fossil records tend to be more frequent in temperate latitudes and sometimes higher from what is expected from the canonical LDG (Krug et al 2009, Rivadeneira et al. 2015). In order to assess this deviation, we used three different approaches: First, we evaluate some conditions as possible predictors for the absence of genera in the fossil record (hereafter *missing taxa*). Usually they do not represent a random sample of the clade but rather tend to be related to certain ecological and life-history traits mirroring the rarity of the taxa (Valentine et al 2006, Cooper et al. 2006). Features such as date of description, geographic range and taxon’s size were assessed based on their representativeness within each genus without a fossil record, broadly weighing the level of bias forced by the accessibility of the samples. Secondly, we use the proportion of living genera known from the fossil record within each family as a sampling gauge. In case there was no substantial bias, the relationship between the proportion of living taxa that first occur at low latitudes and the taxa known from the fossil record ought to be positive as a direct result from the latitudinal gradient (‘The better the fossil record of a family, the higher the proportion of its genera that first occurs in the tropics'; Jablonski et al. 2006). Finally, the aggregation of the occurrence data within spatial bins was quantified through Green's coefficient of dispersion (Kiessling et al. 2007). This variance to mean ratio measures the sampling distribution, indicating an overall equitable allocation with low values or a possible spatial bias toward a particular geographic bin when over 0.5 (Kiessling et al. 2007, Clapham et al. 2009).

Diversification dynamics

The rates of origination, extinction and immigration were estimated in a two-box model, using the 30° N and S of the equator as the foremost boundary between the regions of low and high latitudes (based on the post-Neogene tropical and extratropical categorization; see also Supplementary material Appendix 1, Fig. A1). In order to quantify the evolutionary dynamic, we use all records of stony corals that originated or went extinct in the last 247 million years (i.e. since the Middle Triassic). First, the origination rates were determined based on the place of first occurrence for each taxon in the fossil record. Paleocoordinates and localities assignation in one of the two spatial bins was carried out through the GPlates rotation model implemented in the PBDB Navigator (Wright et al. 2013). Due to lack of evidence to prove local extinction processes, the last occurrence data were used to estimate the fluctuation of global extinction in Scleractinia as well as the temporal strength of the LDG based on the latitudinal generic richness ratio (L/H ratio). In this case we also included extinct families, adding a total of 356 taxa according to the Global Biodiversity Information Facility (GBIF, October 2014). Biogeographic data was then incorporated to measure the extent of historical immigration between latitudinal bins through the present-day maximum range limits (i.e. degree of dispersal of tropical taxa in the extratropics, and vice versa). Categorization of modern distribution between tropical and extratropical regions was defined using the 95% of the genus occurrence records. Finally, all statistical analyses were performed in R (R Development Core Team 2014).

Results

Of the 223 extant genera considered in the comparative analysis, almost one-third has no fossil record (Table 1). Generally, missing taxa vary around 20% of the family and only in Coscinaraeidae and Stenocyathidae outnumbered the genera with fossil record. Following the three-step approach to assess the spatial bias, our analyses revealed that: First, 26% of all missing taxa have a restricted geographic range (i.e. confined to one biogeographic province) and only 15% have been recently described (i.e. after the inventory of Veron 2000). Yet, 35 out of the 66 genera lacking a fossil record proved to be monotypic, which means that 53% of all missing taxa contain single species. Secondly, the relationship between living corals originated at low latitudes and the quality of the fossil record was directly proportional (Fig. 1). In general terms, this tendency would indicate that there is no significant evidence to support a strong bias toward temperate areas because of its heavier sampling effort. Finally, the spatial independence of the fossil record was upheld in most intervals and only at the edges of the timeline (i.e. Middle Triassic, Pliocene and Pleistocene) Green's coefficients become critical (Fig. 2). Even so, the mean range was relatively low (~0.2), suggesting that data aggregation would not generate a major bias in our analysis.

The origination distribution of stony corals has been very variable over time (Table 2). Considering only living genera, 83 and 74 first occurrences took place at low and high latitudes respectively. Overall, there is no significant difference in the origination rate between both spatial bins (*P* = 0.7546; One-way ANOVA). By including extinct families the variance considerably increases as origination differences get even larger. Two of the four temporal intervals with significant differences between latitudinal regions match the epochs where the fossil record seems to be more aggregated according to Green's coefficient (i.e. Middle Triassic and Pliocene; Table 2). This correlation remains true for the subset of extant taxa, with geographic significant differences noticed only during the Quaternary (Fig. 3E). Without ignoring that these data are likely undersampled, the remarkable variability of the location of first occurrence implies that there is no substantial origination preference toward any particular climatic region.

Global records of last occurrence frequencies outline a bimodal curve apparently defined by the end Triassic (205 mya) and end Cretaceous (65 mya) mass extinctions (Supplementary material Appendix 1, Fig. A2). This last interval brings together 24% of all extinct taxa and nearly 50% when considering the entire Cretaceous period. No epoch showed significant differences in the proportion of extinctions between spatial bins (Table 2). Assuming face values though, 50 exclusively low-latitude genera went extinct in comparison with the 92 genera that were only found above the 30º boundary. When comparing local origination trends with global extinction, there is also a greater correlation between these historical processes at higher latitudes (r = 0.35, n = 430, P = 0.2278) than in the equatorial zone (r = 0.02, n = 439, P = 0.9558). Taking into account the same cautions as for the previous results, some of these differences suggest that ultimately the extinction may have played a more preponderant role than origination in order to passively shape the scleractinians LDG.

Contrary to the predictions of the OTT model, biogeographic data show a far greater tendency to immigrate toward the tropics than temperate regions. Even considering pre-Neogene conditions, most high-latitude genera expanded their geographic distribution by several degrees of latitude toward the equator (Fig. 3). Conversely, the vast majority of the taxa originated in this region did not spread beyond the 35° latitude, exhibiting a significantly lower propensity to immigrate compared to extratropical corals ($P < 0.0001$; Pearson's X^2 test). Summing over the entire 247-million-year interval, only 23 (28%) of the 83 low-latitude genera expanded their geographic range toward temperate regions compared to 70 (95%) of the 74 high-latitude genera that are currently also distributed in the tropics. While the maximum origination levels varied among spatial bins, this immigration drift toward low latitudes persisted through all geological periods, being even more evident in older taxa and especially during the Cretaceous-Paleogene (K-Pg) boundary. Furthermore, the L/H ratio shows that the modern LDG strengthens in the Cenozoic and becomes significantly pronounced from the Miocene onwards (Fig. 2). Considering the 30° N and S of the equator as boundaries, our estimations indicate that during this period the number of tropical stony corals started to double those in extratropical latitudes.

Discussion

Fossil record reveals that despite its early Mesozoic origin, the canonical LDG in Scleractinia is actually not that old. In fact, the diversity gradient was largely built up after the end of the Cretaceous mass extinction and only became significantly steep from the Neogene period (see also Buzas et al. 2002). The evolutionary dynamic that induced this pattern also does not meet the OTT model in at least two of its three main predictions (i.e. higher tropical origination, lower extratropical extinction and a net immigration towards the tropics). Although we cannot overlook the impact that sampling restrictions may have in these conclusions, the fossil record in Scleractinia not only is fairly complete but consistent enough to firmly reject the origination and dispersion premises (Fig. 3). Even assuming the underestimation certainty of the paleontological data, the results were upheld throughout different sampling quality segments, showing no substantial spatial bias and a "pull of the Recent" influence of less than 10% (see Jablonski et al. 2003). Quite the opposite, Green's coefficient indicates that geographic sampling distribution tends to be poorer after the Pliocene interval (Fig. 2). In fact, more than half of the taxa without a fossil record are comprised by single species, many of which are derived from other genera and are likely from a recent origin. Overall, stony corals proved to be a suitable model system to test evolutionary hypotheses and thus evaluate the spatiotemporal dynamic that shaped the macroecological patterns of their current biodiversity.

The high biotic richness in the tropics has traditionally portrayed this region as a "cradle" or a "museum" of taxa depending on whether this condition is attributed to a higher origination or a lower extinction rate, respectively (Chown and Gaston 2000). The main goal of Jablonski's et al. (2006, 2013) alternative model was not to propose yet another hypothesis to explain the LDG, but rather argue that this exclusion paradigm is not coherent with paleontological data or present-day distribution patterns. Even though the OTT dynamic does not always apply in relation to the diversification rates (Mannion et al. 2014, and references therein), phylogenetic reconstructions and simulation studies have repeatedly recovered the strong influence of geographic range shifts in the modern latitudinal gradient (Arita and Vázquez-Domínguez 2008, Escarguel et al. 2008, Soria-Carrasco and Castresana 2012). In benthic Foraminifera, for instance, origination occurs worldwide and, therefore, differences in the species richness seem to be primarily caused by a temperate-tropical interchange in favor of the latter (Buzas and Culver 2009). While this may be also the case in stony corals, our results additionally reveal a somewhat preponderance of extinction process at higher latitudes (Table 2). Wallace (1878) initially reasoned that this contrast presumably caused by the exposure to climatic fluctuations ultimately made the difference in the diversification process, with the equatorial zone relatively less vulnerable to natural disasters than temperate regions (as is presumed from the correlation coefficients). The substrate stability not only would have been crucial for the proliferation of shallow-water corals but could also explain the high diversity of deep-water azooxanthellate Scleractinia at lower latitudes (see Cairns 2007). Accordingly to the fossil record, the combined effect of high immigration with low rates of extinction has led scleractinians to accumulate in the tropics in a more or less uninterrupted fashion. Unlike marine bivalves, this variant to the "out of the tropics" model has been recently reported for rocky intertidal gastropods under the "into the tropical museum" (ITM) hypothesis (Rivadeneira et al. 2015). To date, stony corals represent the first taxon to explicitly exhibit this evolutionary trend as the potential leading cause of its modern geographic distribution.

While the diversity gradient of stony corals appears to be fairly pronounced, the generality of this pattern often depends on the scale at which it is observed (Hillebrand 2004). Indeed, recent studies have demonstrated that the canonical LDG is mostly recognized at large geographic scales (e.g. at tropical vs. extratropical areas; Rivadeneira et al. 2015) and especially during the last 30 million years (Mannion et al. 2014). As in other marine invertebrates, the slope of the latitudinal differences in scleractinians has also undergone severe changes over time presumably due to major oceanographic shifts (Buzas et al. 2002, Crame 2002, Powell 2007, Kiessling et al. 2012). For example, Schwartz et al. (2012) showed that the rapid ecological radiation of Caribbean "faviid" corals coincided with high productivity periods of the water column during the late Miocene and early Pliocene (see also Fig. 2). Assuming that tropical genera are less prone to extinction, it is expected that the strengthened of the LDG was largely determined in the Cenozoic by the accumulation of older taxa around the equator given the more stable suitable-conditions. Average ages, however, seems to decrease with diversity (Stehli and

Wells 1971) even though most Mesozoic corals live in the tropics and molecular phylogenies indicate that the soft-bodied ancestor came from somewhere off the Tethys Ocean (Stanley 2003, Stolarski et al. 2011, Kitahara et al. 2014). Due to the autocorrelation restrictions of age-frequency distributions, indirect tests may provide a more dependable way to respond some of these predictions and thus have better resolution of the evolutionary dynamic of individual taxa (Jablonski et al. 2006). Currently, one of the most promising approaches to do so is through model-based historical biogeographic inferences that rely on Maximum-likelihood (Ree et al. 2005, Ree and Smith 2008). This emerging field provides a powerful alternative to assess the ancestral geographic ranges from an evolutionary perspective, progressively integrating the entire diversification process (i.e. dispersal-extinction-cladogenesis model) with both paleontological and neontological data along each divergence event (Ree and Smith 2008, Pyron 2011, Ronquist et al. 2012).

Unlike the OTT dynamic, the ITM model does not predict that endemism should increase toward the equator. On the contrary, biogeographic data from living taxa strongly suggest that the tropics act more as a natural reservoir than as source of species for temperate regions (Veron 2000, Hughes et al. 2002). According to the World Wide Fund for Nature (WWF), almost 80% of the world’s coral species are found in the Coral Triangle but only 2% of them are regionally endemic. Interestingly, this pattern is nearly transversal whether or not they are zooxanthellate (Cairns 2007). Several hypotheses have been proposed to explain the causal mechanism (see Bowen et al. 2013), however, the noted contribution of peripheral habitats to biodiversity hotspots particularly supports the ‘Center of Accumulation’ model. Concomitant with our results, Jokiel and Martinelli (1992) ascribed the high diversity of coral reefs to a net dispersal of taxa caused by prevailing currents toward tropical latitudes, which may occur via “bridge species” or isolated deep-water colonies (Roberts et al. 2006, Jablonski et al. 2013). Moreover, this hypothesis has not only been put forward for stony corals but also for reef associated organisms such as the ember parrotfish, the yellow tang, the tropical sea cucumber and the holocentrid fishes (Fitzpatrick et al. 2011, Eble et al. 2011, Skillings et al. 2011, Dornburg et al. 2014).

As Hughes et al. (2002) pointed out, centering most conservation efforts on localized hotspots for immediate reasons may be ineffective in the mid and long term. For this purpose, being able to understand what shapes macroecological patterns as the latitudinal diversity gradient is crucial if we want to determine the actual role of climatic regions and thus model the biotic responses to future changes. Recent analyses have highlighted that far more than interspecific interactions, the environmental filtering is the most relevant factor for the community organization of scleractinian corals (Sommer et al. 2014). More and more evidence is revealing how these populations and especially reef-building species are being increasingly endangered by human impact, one-third of them currently facing an elevated risk of extinction (Carpenter et al. 2008). Even independently of local anthropogenic disturbances, the projected ocean acidification has by itself the potential to trigger a mass extinction event by the end of this century (Veron 2008, Kiessling and Simpson 2011). Additionally, several models also alert that the rise of the global temperature over the next 40 years significantly exceed the conditions under which most corals have flourished over the past half-million years (Hughes et al. 2003, Hoegh-Guldberg, 2007). These very rapid changes not only appear to surpass their adaptive capacity but also seem to have a multiplicative deleterious effect in the early planktonic stages (Anlauf et al. 2011). Yet, the part that dispersion will play in a globally threatened marine environment is extremely important, especially when the fossil record suggests it is one of the main drivers of the diversification process (see also Tager et al. 2010). Contrary to most post-Paleozoic marine invertebrates (Jablonski 1993), evolutionary novelties in corals are indeed more concentrated at the edge of their distributions than within the diversity hotspots (Budd and Pandolfi 2010). These range boundaries are likely to change over time, however, past references and environmental predictors indicate that they will be far more restricted than today (Kiessling et al. 2012, Muir et al. 2015). As the tropics progressively become a less suitable place to preserve marine biodiversity, fossil data forecasts that future climate change will have a direct impact on the corals evolutionary trajectory.

Acknowledgements

We deeply appreciate the comments of Morana Mihaljević and two anonymous reviewers which helped improve this manuscript. We are also very grateful to Celia Lima for her assistance with the translation of this paper. The research of C.E.H. was funded by FONDECYT # 1140692 and 1130868; and M.M.R. was funded by FONDECYT # 1140841 and 1150664. C. A. Spano was supported by a CONICYT Doctoral Fellowship. This is Palaeobiology Database publication number 242.

References

Anlauf, H. et al. 2011. A corrosive concoction: The combined effects of ocean warming and acidification on the early growth of a stony coral are multiplicative. - J. Exp. Mar. Biol. Ecol. 397: 13-20.

Arita, H. T. and Vázquez-Domínguez, E. 2008. The tropics: cradle, museum or casino? A dynamic null model for latitudinal gradients of species diversity. - Ecol. Lett. 11: 653-663.

Bellwood, D. R. and Hughes, T. P. 2001. Regional-scale assembly rules and biodiversity of coral reefs. - Science 292: 1532-1534.

Bellwood, D. et al. 2005. Environmental and geometric constraints on Indo-Pacific coral reef biodiversity. - Ecol. Lett. 8: 643-651.

Bowen, B. W. et al. 2013. The origins of tropical marine biodiversity. - Trends Ecol. Evol. 28: 359-366.

Budd, A. F. and Pandolfi, J. M. 2010. Evolutionary novelty is concentrated at the edge of coral species distributions. - Science 328: 1558-1561.

Budd, A. F. et al. 2012. Taxonomic classification of the reef coral family Mussidae (Cnidaria: Anthozoa: Scleractinia). - Zool. J. Linn. Soc-Lond. 166: 465-529.

Buzas, M. A. et al. 2002. Latitudinal difference in biodiversity caused by higher tropical rate of increase. - PNAS 99: 7841-7843.

Buzas, M. A. and Culver, S. J. 2009. Geographic origin of species: The temperate-tropical interchange. - Geology 37: 879-881.

Carins, S. D. 2007. Deep-water corals: An overview with special reference to diversity and distribution of deep-water scleractinian corals. - B. Mar. Sci. 81(3): 311-322.

Campbell, C. A. and Valentine, J. W. 1977. Comparability of modern and ancient marine faunal provinces. - Paleobiology 3: 49-57.

Carpenter, K. E. et al. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. - Science 321: 560-563.

Chown, S. L. and Gaston, K. J. 2000. Areas, cradles and museums: the latitudinal gradient in species richness. - Trends Ecol. Evol. 15: 311-315.

Clapham, M. E. et al. 2009. The double mass extinction revisited: reassessing the severity, selectivity, and causes of the end-Guadalupian biotic crisis (Late Permian). - Paleobiology 35: 32-50.

Connolly, S. R. et al. 2003. Indo-Pacific biodiversity of coral reefs: deviations from a mid-domain model. - Ecology 84: 2178-2190.

Cooper, R. A. et al. 2006. Completeness of the fossil record: Estimating losses due to small body size. - Geology 34: 241-244.

Crame, J. A. 2002. Evolution of taxonomic diversity gradients in the marine realm: a comparison of Late Jurassic and Recent bivalve faunas. - Paleobiology 28: 184-207.

Dornburg, A. et al. 2014. The impact of shifts in marine biodiversity hotspots on patterns of range evolution: Evidence from the Holocentridae (squirrelfishes and soldierfishes). - Evolution 69: 146-161.

Eble, J. A. et al. 2011. Escaping paradise: larval export from Hawaii in an Indo-Pacific reef fish, the yellow tang (*Zebrasoma flavescens*). - Mar. Ecol. Prog. Ser. 428: 245-258.

Escarguel, G. et al. 2007. Evolutionary rates do not drive latitudinal diversity gradients. - J. Zool. Syst. Evol. Res. 46: 82-86.

Fitzpatrick, J. M. et al. 2011. The West Pacific diversity hotspot as a source or sink for new species? Population genetic insights from the Indo-Pacific parrotfish *Scarus rubroviolaceus*. - Mol. Ecol. 20: 219-234.

Goldberg, E. E. et al. 2005. Diversity, endemism, and age distributions in macroevolutionary sources and sinks. - Am. Nat. 165: 623-633.

Green, A. L. and Mous, P. J. 2008. Delineating the Coral Triangle, its ecoregions and functional seascapes (Version 5.0). - TNC Coral Triangle program report.

Hawkins, B.A. 2001. Ecology’s oldest pattern?. - Trends Ecol. Evol. 16: 470.

Hillebrand, H. 2004. On the generality of the latitudinal diversity gradient. - Am. Nat. 163: 192-211.

Hoegh-Guldberg, O. et al. 2007. Coral reefs under rapid climate change and ocean acidification. -Science 318: 1737-1742.

Huang, D. et al. 2011. Cleaning up the ‘Bigmessidae’: Molecular phylogeny of scleractinian corals from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae. - BMC. Evol. Biol. 11: 37.

Hughes, T. P. et al. 2002. Biodiversity hotspots, centres of endemicity, and the conservation of coral reefs. - Ecol. Lett. 5: 775-784.

Hughes, T. P. et al. 2003. Climate change, human impacts, and the resilience of coral reefs. - Science 301: 929-933.

Jablonski, D. 1993. The tropics as a source of evolutionary novelty through geological time. - Nature 364: 142-144.

Jablonski, D. and Finarelli, J.A. 2009. Congruence of morphologically-defined genera with molecular phylogenies. - PNAS 106: 8262-8266.

Jablonski, D. et al. 2003. The impact of the pull of the recent on the history of marine diversity. - Science 300: 1133-1135.

Jablonski, D. et al. 2006. Out of the tropics: evolutionary dynamics of the latitudinal diversity gradient. - Science 314: 102-106.

Jablonski, D. et al. 2013. Out of the tropics, but how? Fossils, bridge species, and thermal ranges in the dynamics of the marine latitudinal diversity gradient. - PNAS 110: 10487-10494.

Jansson, R. et al. 2013. What can multiple phylogenies say about the latitudinal diversity gradient? A new look at the tropical conservatism, out of the tropics, and diversification rate hypotheses. - Evolution 67: 1741-1755.

Jokiel, P. and Martinelli, F. J. 1992. The vortex model of coral reef biogeography. - J. Biogeogr. 19: 449-458.

Keller, N. B. 1976. The deep-sea madreporarian corals of the genus *Fungiacyathus* from the Kurile-Kamchatka, Aleutian Trenches and other regions of the world oceans. Trudy Instit. Okean. - 99:31-44.

Keller, N. B. 1998. Spatial distribution of the azooxanthellate Scleractinia (Cnidaria, Anthozoa). - Okeanologiya 38: 227-232.

Kiessling, W. et al. 2007. Extinction trajectories of benthic organisms across the Triassic–Jurassic boundary. - Palaeogeogr. Palaeocl. 244: 201-222.

Kiessling, W. and Simpson, C. 2011. On the potential for ocean acidification to be a general cause of ancient reef crises. - Global Change Biol. 17: 56-67.

Kitahara, M. V. et al. 2014. The ‘‘Naked Coral’’ Hypothesis revisited - Evidence for and against scleractinian monophyly. - PLoS ONE 9(4): e94774. doi:10.1371/journal.pone.0094774.

Kleypas, J. A. et al. 1999. Environmental limits to coral reef development: where do we draw the line?. - Am. Zool. 39: 146-159.

Krug, A. Z. et al. 2009. Generation of Earth’s first-order biodiversity pattern. - Astrobiology 9: 113-124.

Mannion, P. D. et al. 2014. The latitudinal biodiversity gradient through deep time. - Trends Ecol. Evol. 29: 42-50.

Mittelbach, G. G. et al. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. - Ecol. Lett. 10: 315-331.

Moberg, F. and Folke, C. 1999. Ecological goods and services of coral reef ecosystems. - Ecol. Econ. 29: 215-233.

Muir, P. R. et al. 2015. Limited scope for latitudinal extension of reef corals. - Science 348: 1135-1138.

Pianka, E. R. 1966. Latitudinal gradients in species diversity: A review of concepts. - Am. Nat. 100: 33-46.

Powell, M. G. 2007. Latitudinal diversity gradients for brachiopod genera during late Palaeozoic time: links between climate, biogeography and evolutionary rates. - Global Ecol. Biogeogr. 16: 519-528.

Pyron, R. A. 2011. Divergence time estimation using fossils as terminal taxa and the origins of Lissamphibia. - Syst. Biol. 60: 466-481.

R Development Core Team. 2014. R: A language and environment for statistical computing. - ISBN 3-900051-07-0, URL http://www.R-project.org.

Ree, R. H. et al. 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. - Evolution 59: 2299-2311.

Ree, R. H. and Smith, S. A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. - Syst. Biol. 57: 4-14.

Rivadeneira, M. M. et al. 2015. Geographic patterns of diversification and the latitudinal gradient of richness of rocky intertidal gastropods: the ‘into the tropical museum’ hypothesis. - Global Ecol. Biogeogr. (*in press*).

Roberts, J. M. et al. 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. - Science 312: 543-547.

Romano, S. l. and Palumbi, S. R. 1996. Evolution of scleractinian corals inferred from molecular systematics. - Science 271: 640-642.

Ronquist, F. S. et al. 2012. A total-evidence approach to dating with fossils, applied to the early radiation of the Hymenoptera. - Syst. Biol. 61: 973-999.

Roy, K. and Goldberg, E. E. 2007. Origination, extinction, and dispersal: Integrative models for understanding present-day diversity gradients. - Am. Nat. 170: S71-S85.

Schuhmacher, H. and Zibrowius, H. 1985. What is hermatypic? A redefinition of ecological groups in corals and other organisms. - Coral Reefs 4: 1-9.

Schwartz, S. A. et al. 2012. Molecules and fossils reveal punctuated diversification in Caribbean “faviid” corals. - BMC Evol. Biol. 12:123.

Skillings, D. et al. 2011. Gateways to Hawai’i: genetic population structure of the tropical sea cucumber *Holothuria atra*. - J. Mar. Biol. 2011: 783030.

Sommer , B. et al. 2014. Trait-mediated environmental filtering drives assembly at biogeographic transition zones. - Ecology 95(4): 1000-1009.

Soria-Carrasco, V. and Castresana, J. 2012. Diversification rates and the latitudinal gradient of diversity in mammals. - Proc. R. Soc. B. 279: 4148-4155.

Stanley, G. D. 2003. The evolution of modern corals and their early history. - Earth-Sci. Rev. 60: 195-225.

Stehli, F. G. and Wells, J. W. 1971. Diversity and age patterns in hermatypic corals. - Syst. Zool. 20: 115-126.

Stolarski, J. et al. 2011. The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. - BMC Evol. Biol. 11: 316.

Tager, D. et al. 2010. Community dynamics of Pleistocene coral reefs during alternative climatic regimes. - Ecology 91(1): 191-200.

Team, R. D. C. 2014. R: A language and environment for statistical computing.

Valentine, J. W. et al. 2006. Assessing the fidelity of the fossil record by using marine bivalves. - PNAS 103: 6599-6604.

Veron, J. E. N. 1995. Corals in space and time: the biogeography and evolution of the Scleractinia. - Cornell Univ. Press.

Veron, J. E. N. 2000. Corals of the world. - Australian Institute of Marine Science.

Veron, J. E. N. 2008. Mass extinctions and ocean acidification: biological constraints on geological dilemmas. - Coral Reefs 27: 459-472.

Vilhena, D. A. and Smith, A. B. 2013. Spatial bias in the marine fossil record. - PLoS One 8: e74470.

Wallace, A. R. 1878. Tropical nature and other essays. - Macmillan and Co.

Willig, M. R. et al. 2003. Latitudinal gradients of biodiversity: pattern, process, scale, and synthesis. - Annu. Rev. Ecol. Evol. Syst. 34: 273-309.

Wright, N. S. et al. 2013. Towards community-driven paleogeographic reconstructions: integrating open-access paleogeographic and paleobiology data with plate tectonics. -Biogeosciences 10: 1529-1541.

Table 1. Families of Scleractinia with their respective number of genera and the percentage without fossil record. Classification according to WoRMS database; the recently described family Coscinaraeidae Benzoni, F., Arrigoni, R., Stefani, F., Stolarski, J., 2012, is not included.

Family	No. genera	Missing genera, %
Acroporidae	7	29
Agariciidae	7	14
Anthemiphylliidae	1	0
Astrocoeniidae	4	0
Caryophylliidae	41	41
Deltocyathidae	1	0
Dendrophylliidae	21	29
Diploastreidae	1	0
Euphylliidae	7	29
Flabellidae	10	30
Fungiacyathidae	1	0
Fungiidae	15	33
Gardineriidae	2	50
Guyniidae	1	0
Lobophylliidae	11	18
Meandrinidae	4	0
Merulinidae	24	33
Micrabaciidae	4	50
Montastraeidae	1	0
Mussidae	10	10
Oculinidae	7	14
Pocilloporidae	3	0
Poritidae	4	50
Psammocoridae	1	0
Rhizangiidae	4	0
Schizocyathidae	3	33
Siderastreidae	2	0
Stenocyathidae	3	67
Turbinoliidae	23	43
Total	**223**	**30**

Table 2. Latitudinal differences in the percentages of origination and extinction of stony coral genera. Significant X^2 values ($P < 0.01$) are highlighted in bold.

		Origination			Extinction		
Period	Epoch	Low latitudes	High latitudes	X^2	Low latitudes	High latitudes	X^2
Triassic	Middle	88	12	**12.30**	75	25	1.32
	Late	78	22	**10.74**	56	44	2.23
Jurassic	Early	61	39	1.03	45	55	0.02
	Middle	51	49	0.24	47	53	0.00
	Late	33	67	**12.09**	42	58	0.41
Cretaceous	Early	50	50	0.51	43	57	0.49
	Late	48	52	0.73	46	54	0.01
Paleogene	Paleocene	57	43	0.07	44	56	0.05
	Eocene	36	64	4.22	44	56	0.04
	Oligocene	31	69	2.87	44	56	0.01
Neogene	Miocene	52	48	0.10	52	48	0.34
	Pliocene	100	0	**6.76**	50	50	0.01
Quaternary	Pleistocene	80	20	4.02	50	50	0.03

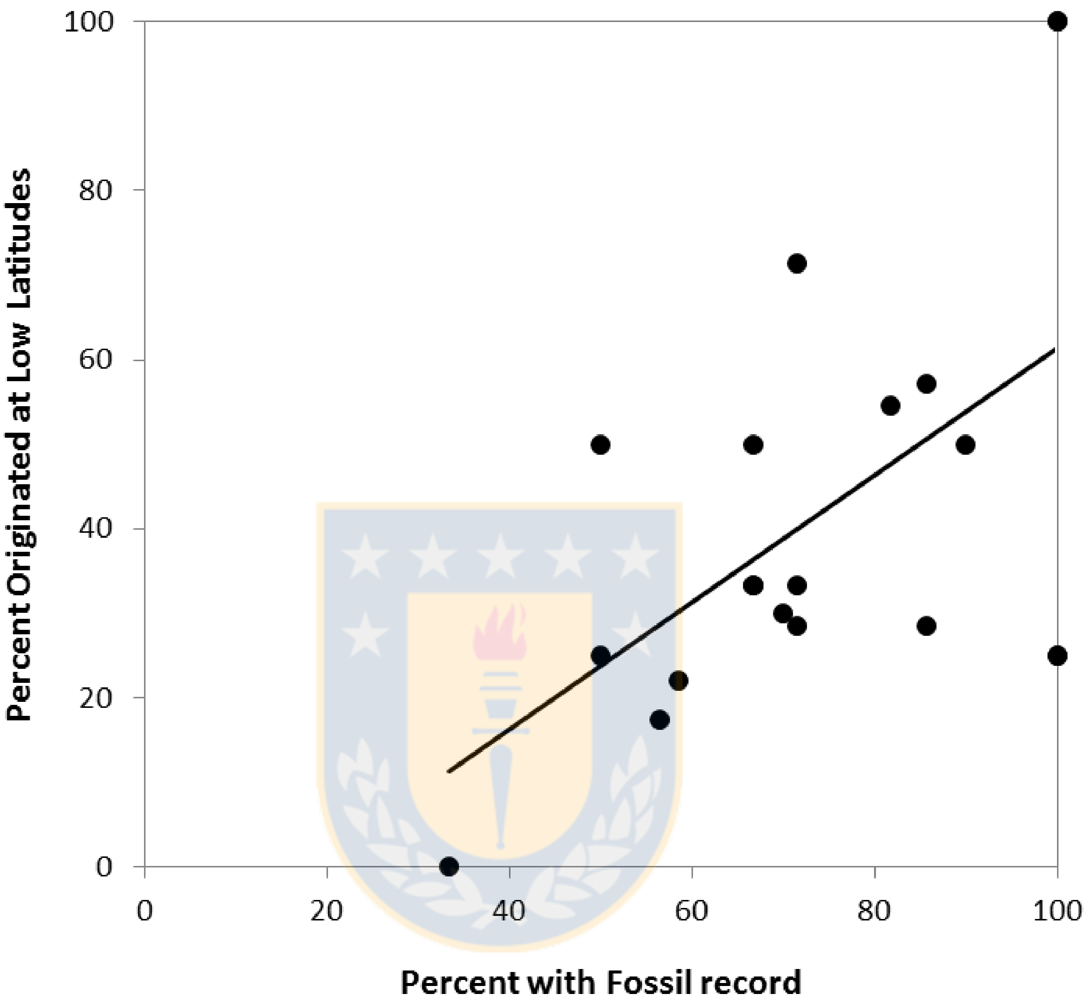


Figure 1. Sampling quality at low latitudes in stony coral families since the start of the Middle Triassic (ca. 240 million years ago); only extant families having three or more first occurrences within that interval were consider in this analysis ($R^2 = 0.309$, $P = 0.0018$; OLS regression).

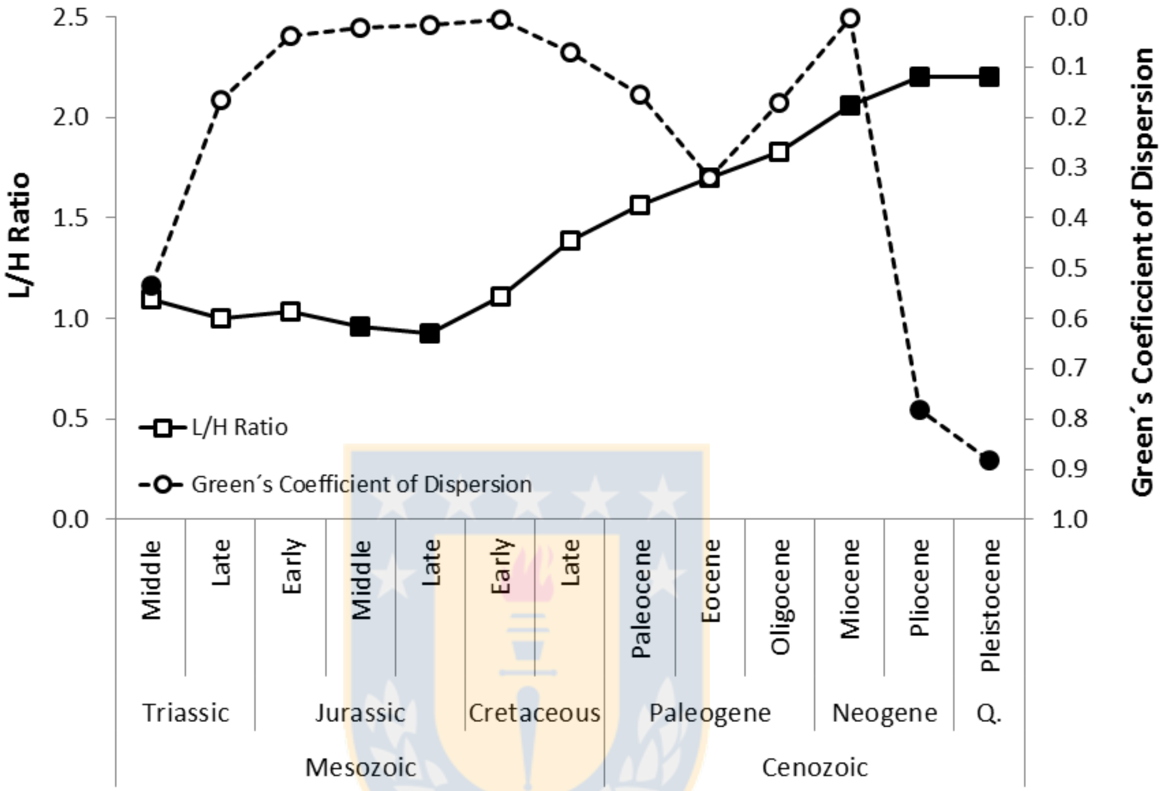


Figure 2. Temporal trends in the data aggregation (Green´s coefficient of dispersion for occurrences) and the strength of the LDG (L/H ratio); filled-in squares indicate significant chi-square values (*P* < 0.01) and filled-in circles indicate coefficients above 0.5. Because smaller values of the Green's coefficient indicate less data aggregation, the axis is inverted to display smaller values at the top.

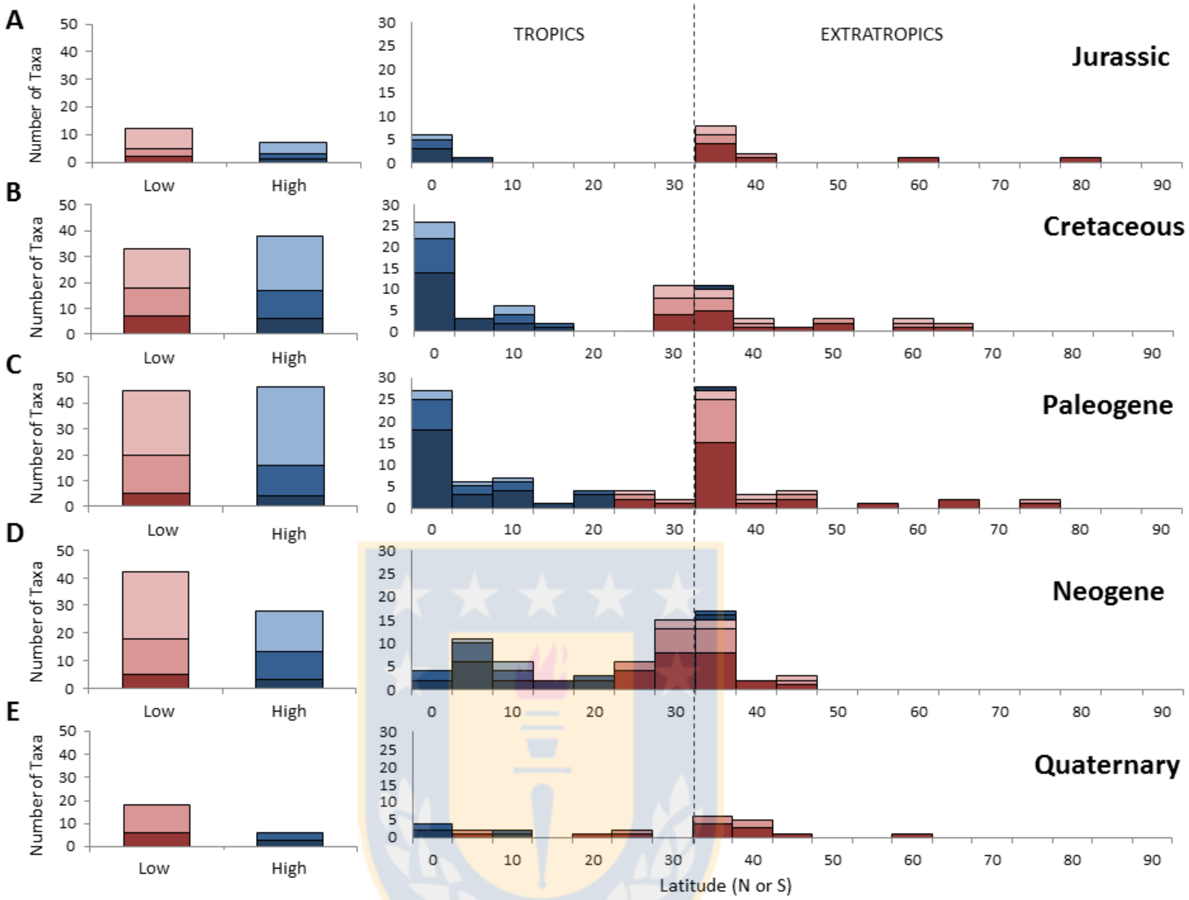


Figure 3. Spatial distribution of the first occurrences of stony corals genera in the fossil record (left) and their corresponding present-day geographic range limits (right). Bars of taxa originated at low (red) and high (blue) latitudes are subdivided according to the quality of the fossil record, showing from the bottom up families with ≥90% (dark colored), ≥70% (normal color) and ≥50% (light colored) of their living taxa known as fossils. With the exception of the Quaternary, there is no significant difference between the frequencies of first occurrence by spatial category [(A), *P* = 0.18; (B), *P* = 0.76; (C), *P* = 0.58; (D), *P* = 0.06; (E), *P* = 0.01; exact binomial test].

APENDICE 2

Anthopleura radians, a new species of sea anemone (Cnidaria: Actiniaria: Actiniidae) from northern Chile, with comments on other species of the genus from the South Pacific Ocean

Carlos A. Spano[1] and Vreni Häussermann[2]

[1] Genomics in Ecology, Evolution and Conservation Laboratory, Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Barrio Universitario s/n Casilla 160-C, Concepción, Chile.

[2] Huinay Scientific Field Station, Chile, and Pontificia Universidad Católica de Valparaíso, Facultad de Recursos Naturales, Escuela de Ciencias del Mar, Avda. Brazil 2950, Valparaíso, Chile.

(Published in Biodiversity and Natural History)

Abstract

A new species of sea anemone, *Anthopleura radians* n. sp, is described from the intertidal zone of northern Chile and the taxonomic status of the other *Anthopleura* species from the South Pacific are discussed. *A. radians* n. sp. is characterized by a yellow-whitish and brown checkerboard-like pattern on the oral disc, adhesive verrucae along the entire column and a series of marginal projections, each bearing a brightly-colored acrorhagus on the oral surface. This is the seventh species of *Anthopleura* described from the South Pacific Ocean; each one distinguished by a particular combination of differences related to their coloration pattern, presence of zooxanthellae, cnidae, and mode of reproduction. Some of these species have not been reported since their original description and thus require to be

taxonomically validated. *A. hermaphroditica* and *A. aureoradiata* are synonyms considering the lack of differences seen between live specimens, museum collections and published records. *A. radians* could also be a junior synonym of *A. minima*, however, no type material was found for testing this hypothesis. Furthermore, it is crucial to designate neotypes for *A. inconspicua*, *A. rosea* and *A. minima* since there are no name-bearing types reported for these species.

Introduction

The genus *Anthopleura* Duchassaing & Michelotti, 1860 comprises some of the most familiar and well-known sea anemones from the intertidal and shallow subtidal ecosystems. This group is present in all oceans except polar seas, and some species can be locally abundant in hard-bottom communities (Daly, 2004). Through different body features such as adhesive verrucae or acrorhagi, members of *Anthopleura* have been able to colonize diverse habitats by better withstanding desiccation, predation and competition for space (Hart & Crowe, 1977; Bigger 1980, 1982). In fact, the development of these structures seems to respond to environmental condition and thus, the taxonomic use of such diagnostic features has been extensively debated in the past decades (e.g. Belem & Pinto, 1990; Daly & den Hartog 2004; Spano *et al.*, 2013).

There are currently 45 described species of *Anthopleura*, and it is the second largest genus of the family Actiniidae after *Actinia* (Fautin, 2016). Most members of *Anthopleura* are especially abundant in temperate regions, even though phylogenetic studies suggest a tropical origin of the clade (Daly, 2004). Carlgren (1949) listed four species for the South

Pacific: *Anthopleura kohli* Carlgren, 1930, *Anthopleura aureoradiata* (Stuckey, 1909) and *Anthopleura inconspicua* (Hutton, 1879) from New Zealand and *Anthopleura hermaphroditica* (Carlgren, 1899) from the coast of Chile. Additionally, Stuckey & Walton (1910) described *Anthopleura rosea* and *Anthopleura minima* from Manukau Harbour in New Zealand. Most of these descriptions were later completed by Parry (1951), who added information on size, coloration, cnidae and habitat of the species. Since then, few reports have reassessed the diversity of *Anthopleura* fauna in the South Pacific, of which the vast majority seems to be endemic of New Zealand (Dawson, 1992).

In the present article a new species of sea anemone—*Anthopleura radians* n. sp.—is described from the intertidal of northern Chile. While in several aspects it resembles some of the species from New Zealand, *A. radians* can be distinguished by the coloration pattern and body dimensions. In addition, the diversity and taxonomic validity of the other *Anthopleura* species described from the South Pacific Ocean are further discussed.

Materials and Methods

In 1997, V. Häussermann and G. Försterra collected a few specimens of an unknown morph of *Anthopleura* at Pan de Azucar (26°11'S; 70°39'W) and Totoralillo (30°3'S; 71°24'W), in northern Chile. They originally listed them as a morphological variation of *A. hermaphroditica* (Häussermann, 1998), assignment that persisted since then (e.g. Yanagi & Daly, 2004). More recently, many more individuals were found in Coquimbo, being especially abundant in sector Bucanero of La Herradura (29º58’S; 71º21’W). This material was reexamined by Spano *et al.* (2013), who detailed its description and expanded the anatomical differences between this morph and the *Anthopleura* species already known. Several dozen specimens have been photographed in the field during this time, from which approximately 35 were collected, relaxed with menthol crystal, and fixed in 8-10% seawater formalin for morphological analysis. For histological examination, longitudinal and cross-sectional segments of 10 specimens were embedded in paraffin, sectioned at 5-9 µm, and stained with Masson's trichrome modified by Spano & Flores (2013).

Fired and unfired cnidae from living and preserved specimens were observed, photographed and measured per type (i.e. of cnidae) and per tissue (i.e. tentacle, column, acrorhagi, actinopharynx and mesenterial filament) using a light microscope (1000X oil immersion) connected to an image analysis software. The largest and smallest cnidae of a particular type were sought for each tissue sample (Hand, 1955; England, 1987). Nematocyst terminology follows that of Schmidt (1972) and England (1991).

Type and voucher specimens were deposited at the Museo Nacional de Historia Natural (MNHNCL), Sala de Colecciones Biológicas de la Universidad Católica del Norte (SCBUCN), Museo de Zoología de la Universidad de Concepción (MZUC-UCCC) and the Zoologische Staatssammlung München (ZSM).

Results

Suborder Enthemonae Rodríguez & Daly, 2014

Superfamily Actinioidea (Rafinesque, 1815)

Family Actiniidae Rafinesque, 1815

Genus *Anthopleura* Duchassaing & Michelotti, 1860

Anthopleura radians n. sp.

Material examined (all from intertidal, preserved in formalin and stored in 70% ethanol): *Holotype*: 1 specimen (MNHNCL CNID-15008), Playa La Herradura (29º58'S; 71º21'W), 1 May 2012, Coll. C. Spano; *Paratypes*: 3 specimens (MZUC-UCCC 45078), Escollo El Pulpo (27º01'S; 70º49'W), 18 May 2012, Coll. C. Spano; 2 specimens (MZUC-UCCC 45079), Punta Teatinos (29º49'S; 71º17'W), 30 April 2012, Coll. C. Spano; 4 specimens (MZUC-UCCC 45080), Playa La Herradura (29º58'S; 71º21'W), 1 May 2012, Coll. C. Spano; 2 specimens (SCBUCN-4117), Playa La Herradura (29º58'S; 71º21'W), 28 May 2015; 1 specimen (ZSM 20060554), Playa Totoralillo (30º03'S; 71º24'W), 30 December 1997, Coll. V. Häussermann; 3 specimens (MZUC-UCCC 45081), Puerto Aldea (30º17'S; 71º36'W), 28 April 2012, Coll. C. Spano.

Diagnosis: Orange to pink, small cylinder-shaped actiniid with marginal projections, acrorhagi and endocoelic adhesive verrucae fully covering the column with particles from surrounding environment. Oral disc with yellow-whitish and brown checkerboard-like pattern radiating from the mouth towards the base of the tentacles, which typically present a row of white spots on oral surface. Marginal sphincter circumscript, retractor muscles diffuse and mesenteries more numerous proximally than distally. Gonochoric; azooxanthellate.

External anatomy: *Size.* Pedal disc diameter up to 17 mm, typically 9 mm (S.D = 3; n = 30); Oral disc diameter up to 16 mm, typically 9 mm (S.D = 3; n = 30). Column about a centimeter long and contracted specimens generally dome-shaped. Tentacles *in vivo* usually as long as oral disc diameter; about 8 mm long in preserved specimens.

Coloration. Column very variable in color, ranging from grayish or purple ochre to brick orange or light pink. Tentacles paler than column, usually in more purplish tones towards inner cycles. Oral disc with a very noticeable yellow-whitish and brown checkerboard-like pattern radiating from a typically pink mouth towards the dark brown-base of the tentacles. Most specimens also present a straight row of white spots on the oral surface of some or all of the tentacles (Fig. 1A). In preservation, column turns pale yellow or light brown and tentacles usually translucent gray with white marks.

Column. Covered from margin to just above the limbus with numerous small, endocoelic, adhesive verrucae, generally arranged in several fairly distinguishable longitudinal rows (Fig. 1A). Verrucae may be more or less developed and either distinctly pigmented or slightly paler in color than the column; holding small stones and shell debris in life. Each row ends distally in a marginal projection that can bear up to four verrucae on its outer surface and a single swollen brightly-colored acrorhagus on the inner edge (Figs. 1B, 2A). On larger specimens, the acrorhagus can be very prominent (over 2 mm in diameter), widely protruding outside the denticulate margin even under contraction.

Oral disc and tentacles. Mouth prominent, rounded, and may be elevated on oral cone in center of disc. In life, tentacles slender, conical with rounded and, occasionally, perforated tip. Tentacles number 40—70 depending on size of animal, usually hexamerously arranged in 4 to 5 cycles (last one never complete). Inner tentacles longer than outer one, typically held erect and all can be fully covered by the column during contraction (Fig. 1C).

Pedal disc. Distinct limbus; usually arcuate inwards in preserved organisms. Pedal disc roughly circular, adherent and well-developed.

Internal anatomy: *General*. Actinopharynx no longer than half length of the column, deeply sulcated, with two well-developed aborally prolonged siphonoglyphs, each attached to a pair of directives. Marginal stoma slightly larger than oral stoma. Mesenteries hexamerously arranged in three perfect cycles; occasionally individuals with less than 24 pairs or with a fourth cycle in early development were found (Fig. 2C). Mesenteries grow from the pedal disc upward (with more mesenteries proximally than distally). Gonochoric; Reproductive tissue only observed in perfect mesenteries (Fig. 2E). Azooxanthellate.

Musculature. Marginal sphincter muscle endodermal, circumscribed and generally palmate; with numerous secondary mesogleal branches (Fig. 2B). Mesoglea of the body wall at least as thick as the epidermis and circular endodermal musculature frequently visible. Longitudinal muscles of tentacles ectodermal (Fig. 2D). Retractor muscles diffuse, highly branched and occasionally reniform. Parietal muscle may span as much as half the distance between the column and the free edge of the mesentery. Basilar and parietobasilar muscles well-defined and usually strong (Fig. 2F).

Cnidom: Spirocysts, basitrichs, rod-like basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores (Fig. 3). See Table 1 for size and distribution of cnidae.

Distribution and habitat: *Anthopleura radians* can be found in the rocky intertidal of protected or semi-protected beaches from Pan de Azucar (26º11'S; 70º39'W) to Puerto Aldea (30º17'S; 71º36'W). This species typically lives in rock crevices and buried in gravel, with only the tentacles visible during high tide. It can be locally abundant, apparently in clonal populations (Fig. 1D). Individuals usually live in close physical proximity and may share habitat with other sea anemones such as *Anthopleura hermaphroditica* (Carlgren, 1899) and *Anemonia alicemartinae* Häussermann & Försterra, 2001.

Etymology: *Anthopleura radians* receive its name based on the radiated pattern of the oral disc. The epithet *radians* means sun in Latin and refers to the concentric warm-colored marks around the mouth.

Remarks: The species described here possess some similarities to other intertidal sea anemones; however, *Anthopleura radians* n. sp. can be readily identified by both external and internal features. The only congeneric species which occurs in the same habitat across its distribution is *A. hermaphroditica*, a widespread intertidal and shallow subtidal species in the southeastern Pacific coast (Spano *et al.*, 2013). In the field, *A. radians* can be distinguished from *A. hermaphroditica* by coloration, especially by the particular checkerboard-like pattern of its oral disc. *A. radians* also typically has a thicker musculature with a distinctive parietal muscle, whereas *A. hermaphroditica* tends to present thinner longitudinal muscles and the coelenteron usually contains one or several embryos. Additionally, unlike *A. radians*, *A. hermaphroditica* is zooxanthellate.

Further north from the current distribution limits of *Anthopleura radians* is *A. mariscali* Daly & Fautin 2004. This species was originally described from the Galapagos Islands, but recent surveys found it up to Puntarenas in Costa Rica (Quesada *et al*., 2016). While similar in size and habitat, *A. mariscali* can be distinguished by its coloration pattern and especially the frosted white markings in the distal column (Daly & Fautin 2004). *A. mariscali* has also been described with a much larger number of tentacles and acrorhagus holotrichs that can be twice as long as in *A. radians*. This type of variation also distinguishes *A. radians* from other South Pacific *Anthopleura* species (Table 2); nevertheless, since several of these diagnostic differences are currently under debate and there is a noticeable similarity between some species descriptions from Australia and New Zealand to those from Chile, this comparison will be further detailed in the following discussion.

Discussion

Anthopleura radians shows all features that characterize the genus (see Spano *et al.*, 2013 for revised diagnosis). Unfortunately this cannot be said for many other *Anthopleura* species since this taxon has undergone several taxonomic rearrangements that have made it increasingly difficult to determine specific membership in the genus. The generic definition is mainly debated nowadays based upon the acrorhagi and holotrich cnidae, whose presence coarsely brings together all *Anthopleura* species and distinguish the genus from *Bunodactis* Verrill, 1899, *Aulactinia* Verrill, 1864, and *Gyractis* Boveri, 1893 (Daly & den Hartog, 2004). Some of these characters, however, are highly variable and quite inconsistent among populations (see, for instance, Fautin, 1988). This problem (which is not exclusive of *Anthopleura* or cnidarians for that matter) has been resolved either by splitting the description to new taxa (e.g. England, 1987) or by expanding the original diagnosis to include all morphological variations (e.g. Dunn *et al.*, 1980). Each approach leads to rather different conclusions about generic diversity, for which lower-level phylogenies are still unresolved at least based on current taxonomic classifications (Daly *et al.*, 2008). Progressively more traits are needed in an integrated framework not only for discovering new species but first and foremost, to demonstrate that they have not been already described.

The main characteristics that distinguish the species of *Anthopleura* in the South Pacific are summarized in Table 2. Only *Anthopleura aureoradiata* and *A. hermaphroditica* have been reported frequently since their description, although Parry (1951) mentioned that the other species from New Zealand are also common 'if one knows its usual environment'. They were all depicted with typically large marginal spherules on the edge of the oral disc, supporting their original assignment to the genus *Bunodes* Gosse, 1855 and later to *Anthopleura*. Nevertheless, this wide-ranging character (as originally defined) does not necessarily correspond with the acrorhagial structure that currently characterizes the genus. Daly (2003) analyzed different marginal structures and concluded that they are basically composed of two parts: a projection of the column and a holotrich-dense pad (= acrorhagus) that is histologically differentiated from the surrounding tissues. From this perspective, the affiliation of *A. inconspicua* and *A. rosea* has yet to be confirmed.

It is noteworthy that not all specimens may exhibit fully developed acrorhagi (e.g. *Anthopleura hermaphroditica*; Schories *et al.*, 2011). Larger individuals of *A. radians* typically showed this feature, probably because they were often found in very dense populations. Francis (1973, 1976) established that competition for space is possibly the main cause for the acrorhagial response of actiniid species. In *A. elegantissima* (Brandt, 1835), for example, differences in the number and size of acrorhagi have been related to the division of labour in clonal aggregations, being far more developed in those polyps that live along the boundaries of the population (Ayre & Grosberg, 2005). Although no such pattern nor clonal division was observed in *A. radians*, its gregarious nature together with sporadic siphonoglyph asymmetries and column scars does suggest that this species might reproduce asexually. This was already confirmed for *A. rosea* by Stuckey & Walton (1910) and has been also suggested by Parry (1951) for *A. minima*.

Anthopleura hermaphroditica and *A. aureoradiata.*

One of the first things that emerges from the species comparison in Table 2 is the striking similarity between *Anthopleura hermaphroditica* and *A. aureoradiata*. They are not only the most abundant members of the genus in the South Pacific Ocean but also inhabit virtually the same environments (see also Schories *et al.*, 2011). Moreover, *A. hermaphroditica* and *A. aureoradiata* are the only southern *Anthopleura* species that have been reported to brood their young internally (Carlgren, 1899; Carlgren, 1950), being one of the few cases that expresses this reproductive mode within the genus (the others are *A. atodai* Yanagi & Daly, 2004, from Japan and *A. handi* Dunn, 1978, from the Philippines, Hong Kong and Malaysia).

The body size as well as the coloration pattern are also extremely similar. Stuckey (1909a: 369) characterized *Anthopleura aureoradiata* by having a ‘broken circle of yellow around the peristome, from which extend 6 groups, each consisting of 3 radiating yellow lines with a shorter yellow line between each 2 groups’. This “stellate pattern” is commonly observed in *A. hermaphroditica* (see Fig. 2A in Spano *et al.*, 2013), although it should be noted that it is not always present and appears independently from the silvery white patches of the tentacles that were included in Parry's (1951) description (i.e. specimens may either show an oral stellate pattern with white patches on their tentacles or only the pattern or tentacles markings).

Examination of preserved and *in situ* material from both species revealed no differences that could distinguish them beyond what it is expected from intraspecific variability. We verify the cnidom of *Anthopleura aureoradiata* and additionally found spirocysts and microbasic *b*-mastigophores profusely spread in the tentacles and mesenterial filaments, respectively. No rod-like basitrichs were seen, however, that does not mean that they are absent given the scarce occurrence of this type of nematocyst in *A. hermaphroditica*. Cnidae measurements were relatively similar and in both cases zooxanthellae were visibly present throughout the body. Carlgren (1924) indicated that *A. aureoradiata* is dioecious, which so far would be the only remaining difference between the two species. We do have seen some degree of asynchrony in the gonadal development of *A. hermaphroditica* (see also Jennison, 1979); therefore, when gametogenic follicles rip out during spawning, individuals are found with a lower proportion of residual, usually single-sex gametes. Furthermore, the occurrence of males, females and hermaphrodites within a local population is not rare (e.g. Dunn *et al.*, 1980; Jennison, 1981; Van Praët, 1990; Rodríguez *et al.*, 2013) and has been recently described as 'trioecy' in the sea anemone *Aiptasia diaphana* (Armoza-Zvuloni *et al.*, 2014). Whether or not this is the case here has yet to be demonstrated, but overall we did not find sufficient evidence to argue that *A. aureoradiata* and *A. hermaphroditica* are different species and hence, we suggest that they should be formally synonymized.

Anthopleura minima and A. radians

Perhaps the most distinctive similarity between these two species is the coloration pattern of the oral disc (Fig. 1A, Table 2). Stuckey & Walton (1910: 543) originally described *Anthopleura minima* with a rose-color center surrounded by a ‘complicated pattern of light and dark olive-green and gray’. Parry (1951) further detailed this specific feature, including a diagram of the oral disc in her check-list of New Zealand actiniarians (see Fig. 7 in that paper). Although the distribution of the colors within this 'complicated pattern' is still unclear in Parry's description, at least the oral pattern outlined there for *A. minima* fits quite well with that of *A. radians.*

The similarity between both descriptions is also evident in the characterizations of the tentacles and column, although there are considerable differences in some measurements. *Anthopleura minima* was described as usually having 36 tentacles, few non-prominent verrucae, and only 14 pairs of mesenteries, unevenly distributed with 9 pairs on one side and 5 pairs on the other. Based on these differences, this species looks more like a juvenile of *A. radians* rather than a different taxon. Individuals of *A. minima* were commonly found in large numbers in mussel beds or among *Pomatoceros* tubes, possibly in clonal populations (Parry, 1951). Even though *A. minima* individuals seems to be smaller in size (especially if they reproduce asexually), surprisingly the overall body dimensions are larger (i.e. 2–3 cm in height and 1–1.5 cm in diameter) while the tentacles are somewhat shorter (i.e. 0.5 cm long). Furthermore, the cnidom of *A. minima* has yet to be revised, especially considering the oddly absence of any basitrich nematocysts in its description. Since no type specimen (or any specimen at all) could be found for *A. minima*, the decision whether or not it is a senior synonym of *A. radians* remains pending until new samples from its original locality are examined.

Anthopleura inconspicua, A. rosea and *A. kohli*

The lack of type material is a major problem for species that are rare or have incomplete descriptions. Many sea anemone species have been determined based on their similarities with other species rather than on shared derived characters (Daly *et al.*, 2008). For example, all descriptions of *Anthopleura inconspicua* stressed how similar it is to *A. aureoradiata*, but the former differs by a larger number of tentacles (as indicated by Stuckey, 1909b), less prominent verrucae and generally larger sizes (Parry, 1951). This also applies to *A. kohli*, which is endemic to Stewart Island in New Zealand. In fact, Carlgren initially thought that this species was a young form of *A. inconspicua* or *A. rosea*, but later decided to give it a different name as they were all too 'imperfectly known', particularly regarding their nematocysts (Carlgren, 1924, 1930). He was a keen proponent of the cnidom as taxonomic character, especially to distinguish closely related species (Carlgren, 1940). Today this trait is essential in sea anemones descriptions; however, its usefulness is largely discussed and should be handled carefully (Fautin, 1988). In *A. rosea*, for instance, we were able to confirm (from museum collections: NIWA 35279) much of the cnidom indicated by Parry (1951); however, we also found many basitrichs and some other basitrich-like nematocysts that could have been previously classified as microbasic *p*-mastigophores (no V-shaped notch was clearly distinguishable at the base of the unfired shafts). While the differences here were qualitative, even disparities among cnidae measurements are commonly used to identify and re-describe species (but see Williams, 1996, 1998, 2000). Even though we could verify some of the external features of *A. rosea*, accurately characterizing its cnidom was impractical since we could only examine one poorly preserved specimen whose anatomy was heavily deteriorated due to fixative solution.

Conclusions

The determination of the diversity of *Anthopleura* poses a challenging topic within sea anemone taxonomy considering the large number of species that have been historically assigned to this genus. Perhaps the most straightforward way to untangle this problem is by addressing species complexes regionally (e.g. Daly & den Hartog, 2004). We agree with the current diagnosis of *Anthopleura* Duchassaing and Michelotti, 1860 for the South Pacific species but do suggest that *A. hermaphroditica* and *A. aureoradiata* are synonyms. Because *A. hermaphroditica* (Carlgren, 1899) has priority over *A. aureoradiata* (Stuckey, 1909), the former should be kept as the valid name (Art. 23: International Commission on Zoological Nomenclature, 1999). With the newly described species and the synonymy of *A. aureoradiata*, the genus *Anthopleura* would retain 45 nominal species. The lack of name-bearing types prevents the verification of the validity of *A. inconspicua* (Hutton, 1879), *A. rosea* (Stuckey & Walton, 1910) and *A. minima* (Stuckey & Walton, 1910). If no such material exists, a neotype for each species must be fixed (Art. 72.2: International Commission on Zoological Nomenclature, 1999). Furthermore, the validity of several diagnostic features that have been traditionally used to recognize *Anthopleura* members has yet to be resolved. Most recent proposals point to the nematocyst ultrastructure to define the genus *Anthopleura* (England, 1987; Belem & Pinto, 1990); and a combination of other characteristics such as coloration pattern and cnidae dimensions are generally used to identify species. Nevertheless, ascertaining which variations represent phenotypic plasticity (i.e. changes of the phenotype in response to changes in the environment; e.g. Francis, 2004; Price, 2006) rather than evolutionary novelties is crucial if we want to settle species designations in Actiniaria. The lack of synapomorphies (or occurrence of character convergences; Daly, 2004) on neutral genetic markers might be highlighting the importance of local adaptations to explain the current taxonomic diversity. Only by knowing the nature of these differences will we be able to finally estimate the true richness of sea anemones.

Acknowledgments

We are grateful to Jim and Lisa Seeb, Garrett McKinney, and two anonymous reviewer for their detailed and constructive suggestions on previous versions of the manuscript. We also would like to acknowledge Victor Castelletto, Veronica Flores, and Jeanette Santana for all their technical and bibliographic support. Many thanks to Sadie Mills of the National Institute of Water and Atmospheric Research (NIWA) for loan of *Anthopleura* specimens from New Zealand. We are particularly grateful to Günter Försterra, Marta Luz Pérez, Jose Luis Spano, and Rossana Spano for their support and help collecting specimens during several surveys. The research of VH was funded by FONDECYT # 1131039 and CS was supported by a CONICYT Doctoral Fellowship. This is publication number 143 of Huinay Scientific Field Station.

References

Armoza-Zvuloni, R., Kramarsky-Winter, E., Loya, Y., Schlesinger, A., Rosenfeld, H. 2014. Trioecy, a unique breeding strategy in the sea anemone *Aiptasia diaphana* and its association with sex steroids. Biology of Reproduction 90: 122.

Ayre, D.J., Grosberg, R.K. 2005. Behind anemone lines: factors affecting division of labour in the social cnidarian *Anthopleura elegantissima*. Animal Behaviour 70(2): 97-110.

Belem, M.J.C., Pinto, S.M. 1990. Mophological and microanatomical study of *Anthopleura krebsi* Duchassaing & Michelotti, 1860 (Cnidaria, Anthozoa, Actiniidae), a new record in Brazil. Anais da Academia Brasileira de Ciências 62(2): 183-192.

Bigger, C.H. 1980. Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones: a recognition of self and not-self. The Biological Bulletin 159: 117-134.

Bigger, C.H. 1982. The cellular basis of the aggressive acrorhagial response of sea anemones. Journal of Morphology 173: 259-278.

Carlgren, O. 1899. Zoantharien. Hamburger Magelhaenische Sammelreise 4: 1-48.

Carlgren, O. 1924. Actiniaria from New Zealand and its Subantarctic Islands [article XXI in Papers from Dr. Th. Mortensen's Pacific Expedition 1914-16]. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening (Copenhagen) 77: 179-261.

Carlgren, O. 1930. Antarctic and Subantarctic Actiniaria. Scientific Results of the Norwegian Antarctic Expeditions 1927-1928 and 1928-1929 (Norske Videnskaps-Akademi i Oslo) 7: 1-6.

Carlgren, O. 1940. A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. Kungliga Fysiografiska Sällskapets Handlingar 51(3): 1-62.

Carlgren, O. 1949. A survey of the Ptychodactaria, Corallimorpharia and Actiniaria. Kungliga Svenska Vetenskaps Akademiens Handlingar, Fjärde Serien 1: 1-121.

Carlgren, O. 1950. Actiniaria and Zoantharia from South Australia. Arkiv für Zoologi 20: 121-135.

Daly, M. 2003. The anatomy, terminology, and homology of acrorhagi and pseudoacrorhagi in sea anemones. Zoologische Verhandelingen 345: 89-101.

Daly, M. 2004. Phylogeny and biogeography of *Anthopleura* in the North Atlantic Ocean. Hydrobiologia 530/531: 241-248.

Daly, M., Fautin, D.G. 2004. *Anthopleura mariscali*, a new species of sea anemone (Cnidaria: Anthozoa: Actiniaria) from the Galápagos Islands. Zootaxa 416: 1-8.

Daly, M., den Hartog, J.C. 2004. Taxonomy, circumscription, and usage in *Anthopleura* (Cnidaria: Anthozoa: Actiniaria) from the Gulf of Mexico and Caribbean. Bulletin of Marine Science 74(2): 401-421.

Daly, M., Chaudhuri, A., Gusmão, L., Rodríguez, E. 2008. Phylogenetic relationships among sea anemones (Cnidaria: Anthozoa: Actiniaria). Molecular Phylogenetic and Evolution 48: 292-301.

Dawson, E.W. 1992. The Coelenterata of the New Zealand region: a handlist for curators, students and ecologists. Occasional Papers of the Hutton Foundation, Wellington, New Zealand.

Dunn, D.F., Chia, F.S., Levine, R. 1980. Nomenclature of *Aulactinia* (= *Bunodactis*), with description of *Aulactinia incubans* n. sp. (Coelenterata: Actiniaria), an internally brooding sea anemone from Puget Sound. Canadian Journal of Zoology 58: 2071-2080.

England, K.W. 1987. Actiniaria from the Red Sea and tropical Indo-Pacific. Bulletin of the British Museum of Natural History 53: 205-292.

England, K.W. 1991. Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. Hydrobiologia, 216/217: 691-697.

Fautin, D.G. 1988. Importance of nematocysts to actinian taxonomy. In: Hessinger, D.A., Lenhoff, H. (Eds.). The Biology of nematocysts, first edition. Academic Press, San Diego, California, USA. pp. 487-500.

Fautin, D.G. 2016. Catalog to families, genera, and species of orders Actiniaria and Corallimorpharia (Cnidaria: Anthozoa). Zootaxa 4145(1): 1-449.

Francis, L. 1973. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. The Biological Bulletin 144: 73-92.

Francis, L. 1976. Social organization within clones of the sea anemone *Anthopleura elegantissima*. The Biological Bulletin 150: 361-376.

Francis, L. 2004. Microscaling: Why larger anemones have longer cnidae. The Biological Bulletin 207: 116-129.

Hand, C. 1955. The sea anemones of central California, part II. Wasmann Journal of Biology 13: 37-99.

Hart, C.E., Crowe, J.H. 1977. The effect of attached gravel on survival of intertidal anemones. Transactions of the American Microscopical Society 96(1): 28-41.

Häussermann, V. 1998. Beiträge zur Aktinienfauna Chiles (Cnidaria, Anthozoa), Teil 2. Diplomarbeit, Zoologisches Institut, Universität München, Martinsried, 157 pp.

International Commission on Zoological Nomenclature. 1999. International code of zoological nomenclature. International Trust for Zoological Nomenclature, London.

Jennison, B.L. 1979. Gametogenesis and reproductive cycles in the sea anemone *Anthopleura elegantissima* (Brandt, 1835). Canadian Journal of Zoology 57: 403-411.

Jennison, B.L. 1981. Reproduction in three species of sea anemones from Key West, Florida. Canadian Journal of Zoology 59: 1708-1719.

Mariscal, R.N. 1974. Nematocysts. In: Muscatine, L., Lenhoff, H. (Eds.). Coelenterate biology. Reviews and new perspectives. Academic Press, New York, USA. pp. 129-178.

Parry, G. 1951. The Actiniaria of New Zealand. A check-list of recorded and new species a review of the literature and a key to the commoner forms Part I. Records of the Canterbury Museum 6(1): 83-119.

Price, T.D. 2006. Phenotypic plasticity, sexual selection and the evolution of colour patterns. Journal of Experimental Biology 209: 2368-2376.

Quesada, A.J., Acuña, F.H., Cortés, J. 2016. First record of the sea anemone *Anthopleura mariscali* outside the Galápagos Archipelago. Marine Biodiversity doi: 10.1007/s12526-016-0445-x.

Rodríguez, E., Orejas, C., López-González, P.J., Gili, J.M. 2013. Reproduction in the externally brooding sea anemone *Epiactis georgiana* in the Antarctic Peninsula and the Weddell Sea. Marine Biology 160: 67-80.

Schmidt, H. 1972. Die Nesselkapseln der Anthozoen und ihre Bedeutung fur die phylogenetische Systematik. Helgoländer Wissenschaftliche Meeresuntersuchungen 23: 422-458.

Schories, D., Reise, K., Sanamyan, K., Sanamyan, N., Clasing, E., Reise, A. 2011. Actinian dominated intertidal mudflats: A new case of an extraordinary rare phenomenon from Southern Chile. Journal of Sea Research 65: 304-314.

Spano, C., Flores, V. 2013. Staining protocol for the histological study of sea anemones (Anthozoa: Actinaria) with recommendations for anesthesia and fixation of specimens. Latin American Journal of Aquatic Research 41(5): 1019-1024.

Spano, C., Rozbaczylo, N., Häussermann, V., Bravo, R. 2013. Redescription of the sea anemones *Anthopleura hermaphroditica* and *Bunodactis hermafroditica* (Cnidaria: Anthozoa: Actiniaria) from Chile. Revista de Biología Marina y Oceanografía 48(3): 521-534.

Stuckey, F.G.A. 1909a. Notes on a New Zealand actinian, *Bunodes aureoradiata*. Transactions of the New Zealand Institute 41: 367-369.

Stuckey, F.G.A. 1909b. A review of the New Zealand Actiniaria known to science, together with a description of twelve new species. Transactions of the New Zealand Institute 41: 374-398.

Stuckey, F.G.A., Walton, C.L. 1910. Notes on a collection of sea-anemones. Transactions of the New Zealand Institute 42: 541-543.

Van Praët, M. 1990. Gametogenesis and the reproductive cycle in the deep-sea anemone *Paracalliactis stephensoni* (Cnidaria: Actiniara). Journal of the Marine Biological Association of the United Kingdom 70: 163-172.

Williams, R.B. 1996. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria): statistical parameters and taxonomic relevance. Scientia Marina 60: 339-351.

Williams, R.B. 1998. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance. Scientia Marina 62: 361-372.

Williams, R.B. 2000. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. Scientia Marina 64: 49-68.

Yanagi, K., Daly, M. 2004. The hermaphroditic sea anemone *Anthopleura atodai* n. sp. (Anthozoa: Actiniaria: Actiniidae) from Japan, with a redescription of *A. hermaphroditica*. Proceedings of the Biological Society of Washington 117(3): 422-436.

Table 1. Capsules size and distribution of cnidae of *Anthopleura radians*. ‘m_l’ and ‘m_w’ are the means, ‘d_l’ and ‘d_w’ are the standard deviations (all in µm), ‘*N*’ is the proportion of animals examined with respective type of cnidae present and ‘*n*’ is the number of capsules measured.

Tissue	Cnidae	Length	m_l	d_l	Width	m_w	d_w	*N*	*n*
Tentacle	Spirocyst (A)	9.2–24.0	17.3	3.3	1.4–3.0	2.3	0.3	9/9	220
	Basitrich 2 (B)	11.2–20.6	17.0	1.4	1.5–3.2	2.2	0.3	9/9	280
Acrorhagus	Holotrich (C)	22.3–38.4	32.1	3.2	2.8–6.2	4.5	0.6	4/4	220
Column	Basitrich 1 (D)	8.3–10.0	9.3	0.4	1.4–2.1	1.7	0.2	4/10	11
	Basitrich 2 (E)	10.5–20.4	14.6	1.6	1.5–2.6	2.1	0.2	10/10	340
	Holotrich (F)	11.7–26.7	15.8	2.5	1.6-5.0	4.0	0.4	10/10	220
Actinopharynx	Basitrich 2 (G)	11.6–23.9	19.7	2.1	1.7–3.1	2.4	0.3	6/6	220
	Microbasic *p*-mastigophore A2 (H)	13.3–21.1	17.9	1.8	3.0–5.6	4.1	0.5	5/6	56
Filament	Basitrich 1 (I)	8.2–12.5	10.4	0.9	1.6–2.9	2.3	0.2	3/6	110
	Basitrich 2 (J)	11.9–23.3	18.7	3.1	1.8–3.3	2.5	0.3	4/6	48
	Rod-like basitrich (K)	17.5–42.0	26.6	9.8	1.2–2.2	1.6	0.4	4/6	8
	Microbasic *b*-mastigophore (L)	18.0–35.0	24.6	3.5	2.6–6.3	4.6	0.7	6/6	185
	Microbasic *p*-mastigophore A1 (M)	12.3–17.9	14.9	0.9	2.1–3.7	2.8	0.3	6/6	100
	Microbasic *p*-mastigophore A2 (N)	14.0–22.4	18.2	1.6	2.7–6.8	4.3	0.7	6/6	240

Table 2. Comparison of the diagnostic features of *Anthopleura* species from the South Pacific Ocean based on the literature. Nematocysts terminology from early references was updated based on Mariscal (1974) and was consistent with Bigger's (1982) observation about acrorhagial "atrichs". *Abundance is taken from author's description.

Species	N° of tentacles	Coloration	Verrucae arrangement	Zooxanthellae	Reproduction	Cnidae	Distribution and habitat	Abundance	Main references
Anthopleura inconspicua (Hutton, 1879)	Up to 4 cycles (120 tentacles)	**Column:** Olive brown to yellowish white **Oral Disc:** Marked with darker lines along the mesenteries **Tentacles:** Olive brown margined with white, and often white-spotted	In rows, from margin to mid-column; Non-prominent	Present	Not reported	Spirocysts, basitrichs, holotrichs and microbasic *p*-mastigophores	New Zealand; In mudflats	Occasional*	Stuckey 1909b, Parry 1951
Anthopleura hermaphroditica (Carlgren, 1899)	Up to 4 cycles (68 tentacles)	**Column:** Brown, from olive green to grayish ochre **Oral Disc:** With opaque marks in a stellate pattern of radial stripes around mouth **Tentacles:** Idem as column, with greenish-white spots	In rows, from margin to limbus; Distally prominent	Present	Hermaphrodite; Brood internally	Spirocysts, basitrichs, rod-like basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores	Chile; In rocky intertidal, shallow subtidal and mudflats	Abundant	Spano *et al.* 2013
Anthopleura aureoradiata (Stuckey, 1909)	Up to 4 cycles (70 tentacles)	**Column:** Brown, yellow and white **Oral Disc:** With yellow mark on the peristome, corresponding to the primary endocoels **Tentacles:** Brown, mottled with irregular patches of silvery white	In rows, from margin to limbus; Distally prominent	Present	Gonochoric; Brood internally	Basitrichs, holotrichs and microbasic *p*-mastigophores	South Australia New Zealand; In rocky intertidal and mudflats	Abundant	Carlgren 1924, Parry 1951
Anthopleura minima (Stuckey and Walton, 1910)	In 3 cycles (36 tentacles)	**Column:** Dark pink or red, to orange or brick **Oral Disc:** With radiating gold marks running from the peristome towards the bases of the tentacles **Tentacles:** Mottled with silver and brown in a complex pattern	Not reported; Non-prominent	Absent	Not reported	Spirocysts, holotrichs and microbasic *p*-mastigophores	New Zealand; In mussel beds and serpulid tubes	Common*	Parry 1951
Anthopleura rosea (Stuckey and Walton, 1910)	In 3 cycles (50 tentacles)	**Column:** From white or grey to bright orange or olive green **Oral Disc:** Creamy white with 12 dark brown rays running out from the peristome **Tentacles:** Rosy pink and marked with brown and white spots	In rows, from margin to limbus	Absent	Gonochoric	Spirocysts, basitrichs and microbasic *p*-mastigophores	New Zealand; Under stones and buried in gravel or mud	Common	Parry 1951
Anthopleura kohli Carlgren, 1930	Up to 4 cycles (54 tentacles)	Reddish or white (in alcohol)	Not reported	Present	Not reported	Spirocysts and nematocysts	New Zealand; Under stones	Common*	Carlgren 1924 (as *Anthopleura* sp.?)
Anthopleura radians n. sp.	Up to 4 cycles (70 tentacles)	**Column:** From grayish or purple ocher to brick orange or light pink **Oral Disc:** With yellow-white checkerboard-like pattern running from the peristome towards the base of the tentacles **Tentacles:** Paler than column, with white spots on upper surface	In rows, from margin to limbus; Distally prominent	Absent	Gonochoric	Spirocysts, basitrichs, rod-like basitrichs, holotrichs, microbasic *b*-mastigophores and microbasic *p*-mastigophores	Chile; In rocky intertidal and buried in gravel	Common	This paper

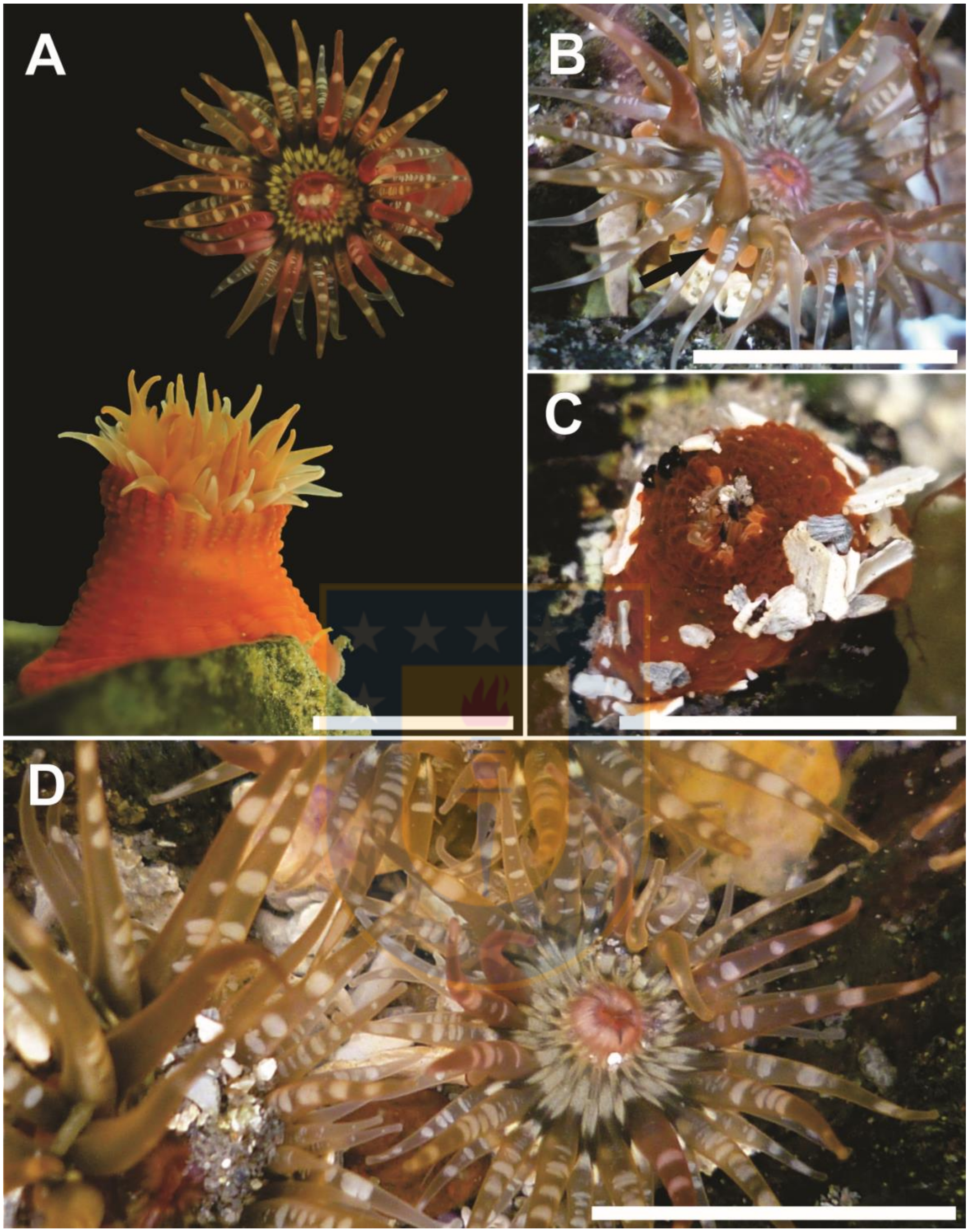


Figure 1. *Anthopleura radians*. A) Oral and lateral view; B) Specimen displaying its acrorhagi outside tentacle crown (see arrow); C) Oral view of contracted specimen; D) *In situ* photograph of several specimens from La Herradura, Chile. Scale bar: 1 cm.

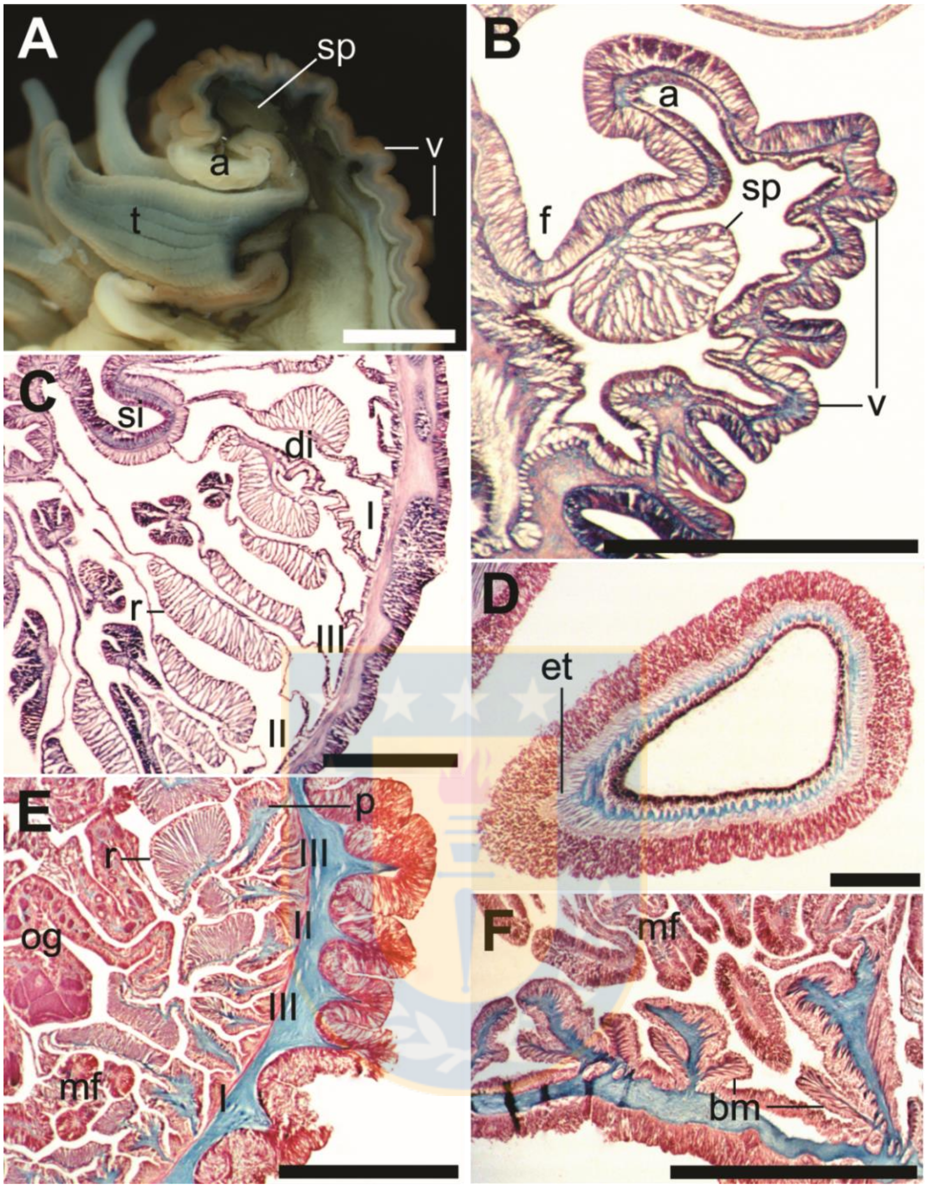


Figure 2. *Anthopleura radians* anatomy. A-B) Longitudinal section of margin, scale: 1 mm; C) Cross section of upper column, scale: 1 mm; D) Cross section of tentacle, scale: 0.1 mm; E) Cross section of lower column, scale: 1 mm; F) Longitudinal section of pedal disc, scale: 0.5 mm. I, II and III: 1st to 3th cycle of mesenteries, a: acrorhagus, bm: basilar muscle, di: directives, et: ectodermal longitudinal muscle of tentacle, f: fosse, mf: mesenterial filaments, og: oogonia, p: parietal muscle, r: retractor muscle, si: siphonoglyph, sp: sphincter, v: verruca.

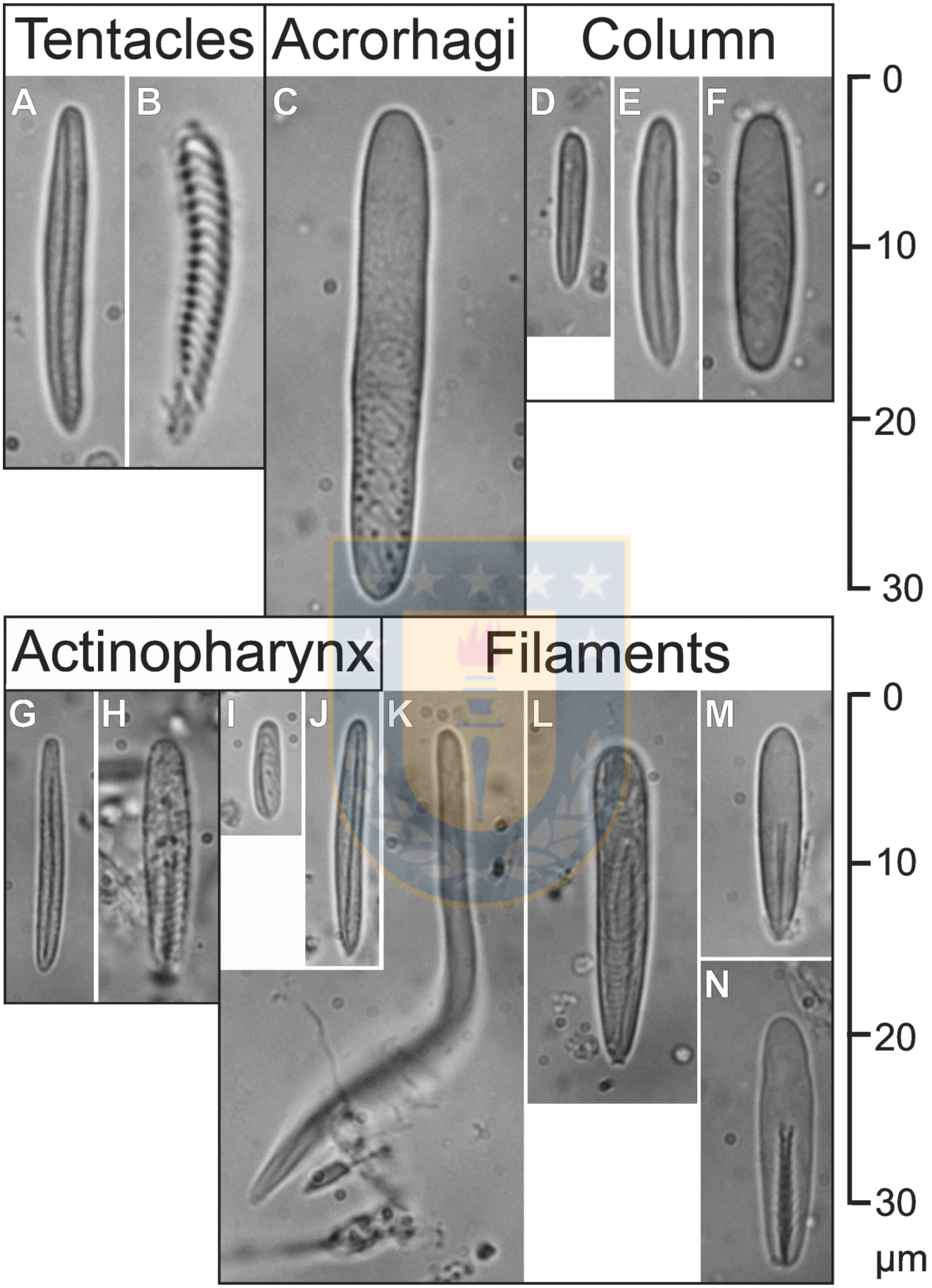


Figure 3. Cnidae of *Anthopleura radians.* Letters A-N refer to Table 1.