

DIVERSIDAD DE LOS POLIPLACÓFOROS COSTEROS DEL PACÍFICO SURESTE: DESENMASCARANDO UN PATRÓN LATITUDINAL INVERSO DE RIQUEZA DE ESPECIES

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DEDICATORIA

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LISTA DE ABREBIATURAS

16S rRNA	Ribosomal Ribonucleic Acid Subunit 16S
18S rRNA	Ribosomal Ribonucleic Acid Subunit 18S
ANOVA	Analysis of Variance
COI	Cytochrome oxidase subunit I
CONICYT	Comisión Nacional de Investigación Científica y Tecnológica
DNA	Deoxyribonucleic Acid
FONDECYT	Fondo Nacional de Desarrollo Científico y Tecnológico
gDNA	Total Deoxyribonucleic Acid
GLM	Generalized Linear Model
ICZN	International Commission on Zoological Nomenclature
LGR	Latittudinal Gradient of Species Richness
LIT	Literature (data review)
MDE	Mid-domain effect
MCMC	Markov Chain Monte Carlo
MNHNCL	Museo Nacional de Historia Natural de Chile
nMDS	Non-metric multidimensional scaling
OLS	Ordinary Least Square model
OTUs	Operational Taxonomic Units
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDp	proportional Phylogenetic Diversity index
PERMANOVA	Permutational multivariate analysis of variance
rRNA	Ribosomal Ribonucleic Acid
SAR	Simultaneous Autoregressive model
SBMNH	Santa Barbara Museum of Natural History, Santa Barbara, US
SCBUCN	Sala de Colecciones Biológicas Universidad Católica del Norte,
	Coquimbo, Chile
SEP	Southeast Pacific
SSUC	Collection of Flora and Fauna, Professor Patricio Sánchez from
	Pontificia Universidad Católica de Chile, Santiago, Chile
UMIP	Instituto de la Patagonia from Universidad de Magallanes, Punta
	Arenas, Chile
ZISP	Zoological Institute of the Russian Academy of Sciences, St. Petersburg,
	Kussia

RESUMEN

El conocimiento actual de los patrones de diversidad biológica se basa principalmente en la evaluación de la riqueza de especies a distintas escalas. Es reconocido ampliamente que esta medida de diversidad no se distribuye al azar, donde el patrón más recurrente y documentado a escala global es el marcado aumento de riqueza de especies hacia menores latitudes (i.e. hacia los trópicos). El reconocimiento de este patrón, tanto en especies terrestres como marinas, ha motivado la formulación hipótesis que proponen mecanismos causales tras esta estructuración espacial de la diversidad, donde puede estar asociada a gradientes en las condiciones físicoambientales y/o a características o restricciones propias de los organismos. Sin embargo desde ya hace un par de décadas se reconoce la necesidad de cambiar el foco tradicional del estudio de los patrones de riqueza de especies, resaltando la importancia de utilizar medidas de diversidad complementarias en la búsqueda de los procesos que determinan tales patrones. A pesar que el patrón de aumento del número de especies hacia los trópicos es ampliamente reconocido, existen zonas como la costa del Pacífico sureste donde una cantidad inusual de taxa, tanto de algas como de animales, se desvían del patrón general, lo que sugiere la complejidad de los procesos que determina los patrones de diversidad. Dentro de estos taxa destacan los poliplacóforos, los que presentan un patrón de riqueza de especies inverso de aumento con la latitud, y el cual se explicaría por condiciones ambientales como la extensión

de la plataforma continental, o los avances y retrocesos glaciares que han afectado principalmente a la zona sur desde el Eoceno, favoreciendo la diversificación de los moluscos en esta zona. Esta tesis tuvo como objetivo buscar los mecanismos tras la conformación del patrón de riqueza registrado para los poliplacóforos de la costa del Pacífico Sureste. Para esto se recopiló información de literatura, se revisó ejemplares de colecciones biológicas y se colecto individuos en terreno entre los años 2011 a 2016. Con los datos obtenidos se reevaluó el patrón de riqueza de especies y su asociación con variables ambientales como el oxígeno, disuelto, la temperatura, el pH., entre otras. Además se determinó la diversidad morfológica y filogenética de los poliplacóforos en el gradiente latitudinal, evaluando su asociación en la búsqueda de los procesos tras la conformación de los patrones de diversidad de este grupo. El primer resultado muestra que al graficar nuestros datos obtenidos desde la literatura se confirma el patrón previamente descrito de aumento de la riqueza con la latitud. Sin embargo, al analizar los datos obtenidos desde colecciones biológicas y muestro directo observamos que el patrón de los poliplacóforos registra máximos a latitudes intermedias. El patrón de riqueza de especies se relacionó con la concentración de oxígeno disuelto, mientras que la diversidad morfológica se relacionó con el rango del oxígeno disuelto y con los niveles de pH. De forma similar, la diversidad morfológica se relacionó positivamente con la diversidad filogenética, destacando la importancia de los procesos histórico-evolutivos en la conformación del patrón de diversidad morfológica de los poliplacóforos en el Pacífico sureste.

Este trabajo de tesis aporta al conocimiento del poco estudiado grupo de los poliplacóforos en el Pacífico sureste, re-describiendo el patrón de riqueza de especies y señalando la importancia de los procesos histórico-evolutivos en su conformación. Además, reconoce que variables ambientales como el oxígeno disuelto y el pH se relacionan con la diversidad de este grupo, y

por lo cual podrían ser de gran relevancia en el escenario actual de cambo global acelerado, y que pronostica variaciones importantes en estos parámetros ambientales en la costa del Pacífico sureste. En términos biogeográficos, este trabajo actualiza la distribución de los poliplacóforos, sumándose a los patrones ya descritos para organismos marinos del Pacífico sureste, zona de alto interés biogeográfico por la particular configuración que permite evaluar el gradiente latitudinal, y por ser reconocida como una zona con una cantidad inusual de organismos marinos que no presentan el patrón clásico global de aumento de especies hacia el Ecuador.

ABSTRACT

Current knowledge of patterns of biological diversity is based primarily on the assessment of species richness at different scales. It is widely recognized that this measure of diversity is not randomly distributed, where the most recurrent and documented pattern at a global scale is the marked increase in species richness towards lower latitudes (i.e. towards the tropics). The recognition of this pattern, both in terrestrial and marine species, has motivated the formulation of hypotheses that propose causal mechanisms behind this spatial structuring of diversity, where it may be associated to gradients in the physical-environmental conditions and/or to characteristics or restrictions of the organisms themselves. However, for a couple of decades, the need to change the traditional focus of the study of species richness patterns has been recognized, highlighting the importance of using complementary diversity measures in the search for the processes that determine such patterns. Although the pattern of species increase towards the tropics is widely recognized, there are zones such as the southeast Pacific coast where an unusual amount of taxa, both algae and animals, deviate from the general pattern, suggesting the complexity of the processes that determine diversity patterns. Within these taxa, polyplacophorans stand out, those that present a pattern of species richness that increases with latitude, and which would be explained by environmental conditions such as the extension of the continental platform, or the glacial advances and retreats that have mainly

affected the southern zone since the Eocene, favoring the diversification of mollusks in this zone. The objective of this thesis was to search for the mechanisms behind the conformation of the richness pattern registered for the polyplacophorans of the Southeast Pacific coast. For this purpose, information from literature was collected, specimens from biological collections were reviewed, and individuals were collected in the field between 2011 and 2016. With the data obtained, we re-evaluated the pattern of species richness and its association with environmental variables such as oxygen, dissolved, temperature, and pH, among others. In addition, the morphological and phylogenetic diversity of polyplacophorans in the latitudinal gradient was determined, evaluating their association in the search for processes after the formation of the diversity patterns of this group. The first result shows that when graphing our data obtained from the literature, the previously described pattern of increasing richness with latitude is confirmed. However, when analyzing the data obtained from biological collections and direct sampling we observe that the pattern of polyplacophorans registers maximums at intermediate latitudes. The species richness pattern is related to dissolved oxygen concentration, while morphological diversity is related to dissolved oxygen range and pH levels. Similarly, morphological diversity was positively related to phylogenetic diversity, highlighting the importance of historical-evolutionary processes in shaping the pattern of morphological diversity of polyplacophorans in the southeast Pacific.

This thesis contributes to the knowledge of the little studied group of polyplacophorans in the Southeast Pacific, re-describing the pattern of species richness and pointing out the importance of historical-evolutionary processes in their conformation. In addition, it recognizes that environmental variables such as dissolved oxygen and pH are related to the diversity of this group, and therefore could be of great relevance in the current scenario of accelerated global change, and that it predicts important variations in these environmental parameters in the southeast Pacific coast. In biogeographic terms, this work updates the distribution of polyplacophorans, adding to the patterns already described for marine organisms of the Southeast Pacific, an zone of high biogeographic interest due to the particular configuration that allows the evaluation of the latitudinal gradient, and for being recognized as an zone with an unusual amount of marine organisms that do not present the classic global pattern of species increase towards the Equator.

INTRODUCCIÓN GENERAL

Gradiente de diversidad biológica

La diversidad biológica reúne el conjunto de aspectos relacionados con la "variedad de la vida" (Magurran, 2004; Roy et al., 2004) y puede ser evaluada en todos sus niveles de organización jerárquica (Swingland, 2001) a través del uso de diversas medidas e índices que buscan reflejar su estructura, dinámica y complejidad (Magurran, 2004; 2011). Sin embargo, el conocimiento actual de los cambios en la diversidad biológica está basado principalmente en mediciones de la riqueza taxonómica (Willing et al., 2003; Roy et al., 2004), la cual cuantifica el número de especies, solo como categorías discretas, registradas en un lugar y tiempo determinado (Marquet et al. 2004), siendo escasos los que han intentado usar medidas complementarias de diversidad (Magurran y McGill, 2011). Actualmente biólogos de diferentes disciplinas han comenzado a utilizar variados índices (e.g. ecológicos, fenotípicos, filogenéticos) para cuantificar la biodiversidad en el tiempo y en el espacio (Magurran y McGill, 2011), lo que refleja la idea creciente de que para realizar una adecuada evaluación de la diversidad biológica de un grupo taxonómico particular no deberían ser utilizadas solo la riqueza de especies y sus respectivas abundancias, sino que también debieran incluir información adicional como, por ejemplo de las relaciones taxonómicas entre las especies en estudio (Clarke y Warwick, 1988).

El hecho que la diversidad biológica no se distribuya al azar, lo cual es aún más evidente a escalas espaciales amplias (Gaston, 2000; Marquet et al., 2004; Terribile et al., 2009), genera uno de los patrones globales más reportados, y que describe una disminución gradual del número de especies desde los trópicos hacia los polos (Rosenzweig, 1995; Roy et al., 1998; 2000; 2004; Ojeda et al., 2000; Macpherson, 2002; Willig et al., 2003; Mittelbach et al., 2007; Ibáñez et al., 2009; Rivadeneira et al., 2011), conocido como Gradiente Latitudinal en Riqueza de Especies (LGR). No obstante, a pesar del alto número de estudios que describen este patrón tanto en ambientes terrestres como acuáticos, pocos lo intentan explicar, y en los casos en que se proponen hipótesis estas dependen en gran medida del taxa o grupo de organismos que se utilice como modelo de estudio para ponerlas a prueba. Esta falta de explicaciones causales a los patrones de diversidad observados ha llevado a que estudios ecológicos y biogeográficos actuales busquen explicar estos cambios en diversidad. Lo anterior ha fomentado la formulación de variadas hipótesis que proponen mecanismos causales tras los patrones observados (Willig et al., 2003; Marquet et al., 2004; Mittelbach et al., 2007; Lomolino et al., 2010), planteando que las variaciones en la diversidad biológica pueden estar asociadas principalmente a gradientes en las condiciones físco-ambientales y/o a características o restricciones inherentes de los organismos (Brown, 1998).

Desde hace ya un par de décadas varios autores han señalado la necesidad de cambiar el foco tradicional de estudio de los patrones de riqueza de especies (e.g. Harrison *et al.*, 1992; Blackburn y Gaston, 1996; Koleff y Gaston, 2002; Rodríguez y Arita, 2004; Qian *et al.*, 2005; Soininen *et al.*, 2007), resaltando la importancia de estudiar adicionalmente, por ejemplo, su recambio específico o más conocido como diversidad beta (Whittaker, 1972). Estos señalan que el análisis complementario de riqueza y recambio de especies ayuda a entender mejor las dinámicas de la diversidad entre comunidades (Soininen *et al.*, 2007), lo que favorece el comprender e identificar los procesos que determinan los patrones de diversidad a distintas escalas espaciales, así como también promueve el desarrollo de hipótesis relacionadas con los patrones generales de diversidad (Ricklefs, 1987; Castilla *et al.*, 2004).

Hace ya más de una década, Willig *et al.* (2003) y Hillebrand (2004a, b) confirman la amplia generalidad del LGR mediante la realización de meta-análisis, sugiriendo que éste patrón no difiere entre hemisferios, ni funcionalmente entre grupos terrestres o marinos, ni entre organismos con dispersión activa o pasiva, así como tampoco entre organismos endo o ectotérmicos (Hillebrand, 2004a). A pesar del gran respaldo de este patrón, se ha registrado que no todos los taxa se ajustan a lo esperado (Willing *et al.*, 2003; Rivadeneira *et al.*, 2011), o incluso que el mismo tipo de organismos presenten diferencias en sus patrones al compararlos entre hemisferios (Rivadeneira, 2005), lo que dificulta el alcanzar un consenso en él o los mecanismos generales que actúan en su conformación.

Sistema de estudio

La costa temperada del Pacífico sureste (SEP) presenta una inusual cantidad de taxa marinos que se desvían del patrón general de riqueza de especies (i.e. LGR), mostrando un LGR inverso, o con máximos de riquezas a latitudes intermedias. Grupos tan distintos entre sí como macroalgas, moluscos y peracáridos presentan un LGR inverso con un marcado aumento en riqueza de especies a mayores latitudes, principalmente al sur de los 42°S (Santelices y Marquet, 1998; Lancellotti y Vásquez, 2000; Valdovinos et al., 2003; Rivadeneira, 2005; González et al., 2008; Fernández et al., 2009; Rivadeneira et al., 2011). Explicaciones a este patrón plantean, por ejemplo, que el aumento de la diversidad en esta zona (i.e. al sur de 42° S) respondería al aislamiento geográfico generado por la separación de la corriente de Deriva del Oeste alrededor de los 42°S (i.e. sistema de Humboldt hacia el norte de los 42°S y la corriente de Cabo de Hornos hacia el sur de los 42°S) y la presencia de refugios durante las glaciaciones sucesivas que han afectado la zona austral de Sudamérica desde hace 40 millones de años (Valdovinos et al., 2003; Hernández et al., 2005). Estos avances y retrocesos glaciares habrían fragmentado y aislado los rangos distribucionales de los distintos taxa de moluscos en la zona, favoreciendo la diversificación de especies de este grupo en los fiordos Magallánicos (Crame, 1997, Valdovinos et al., 2003). También se propone la importancia de procesos histórico-evolutivos en la conformación de un patrón inverso de riqueza de especies en peracáridos (Rivadeneira et al., 2011), donde la región sur podría ser una fuente de novedades evolutivas y/o exhibir altas tasas de especiación, acompañado además de la posible limitación en el rango de expansión de este grupo debido al conservadurismo filogenético del rango de distribución latitudinal sus especies. Por otra parte poliquetos, peracáridos intermareales y peces intermareales, también se desvían del patrón típico en LGR, registrando máximos de riqueza en latitudes intermedias (Thiel et al., 2002; Hernández et al., 2005; Moreno et al., 2006; Navarrete et al, 2014), proponiendo como posibles causas de este patrón la presencia de zonas con alto endemismo de especies a latitudes intermedias y restricciones geométricas asociadas al rango de distribución las especies.

Las excepciones al LGR registradas en las costas del SEP la convierten en un área de gran interés, dado que hipótesis ecológicas relacionadas con el gradiente energético asociado a la latitud, que por ejemplo explican de buena forma la diversidad de moluscos en las costas del Pacífico y Atlántico de Norteamérica (Roy et al., 1998; 2000; 2004), no son capaces de explicar por sí solas los cambios en riqueza en las costas del SEP (Fernández et al. 2009), fomentando el desarrollo de nuevas perspectivas de análisis con el fin de comprender los procesos que subyacen a los patrones de diversidad de los grupos marinos en esta zona. Más aún, la existencia de diferencias inter-hemisféricas en el patrón del LGR para un mismo taxa marino (e.g. bivalvos. Rivadeneira; 2005), da señales de una posible multicausalidad en la generación del patrón, así como de la relevancia de las condicionantes locales-regionales (i.e. ambientales, ecológicas, histórico-evolutivas) en su conformación. Esta multicausalidad se encuentra implícita en la medida "riqueza de especies", ya que al identificar una especie, reconocemos aquellos atributos particulares que en conjunto la caracterizan y a la vez diferencian de otras. Sin embargo, esta categorización de "especie" esconde la magnitud de la diferencia detrás de las diversas propiedades biológicas que nos conducen a su categorización. Tales atributos particulares y diferenciadores de las especies han sido constantemente ignorados al momento analizar los datos de riqueza de especies en busca de los mecanismos causales de los patrones observados, ya que se considera al conjunto de especies como equivalentes (en términos numéricos), obviando sus aspectos ecológicos, biológicos y

evolutivos particulares. Por ejemplo, Ojeda *et al.* (2000) registran el patrón clásico de disminución de especies de peces con el aumento de latitud en las costas de Chile (i.e. en peces óseos y cartilaginosos), sin embargo, Navarrete *et al.* (2014) al analizar el patrón de riqueza de peces en distintos hábitats en el mismo rango geográfico (i.e. intermareales vs submareales) registraron un patrón de riqueza con máximos a latitudes intermedias para peces de hábitats intermareales, a la vez que confirman el patrón de disminución de especies con la latitud en peces de hábitats submareales someros. Lo anterior demuestra la importancia que puede tener el considerar características tanto ecológicas como de historia de vida de los taxa para observar posibles desviaciones al patrón clásico del LGR, lo que promueve la formulación de nuevas hipótesis relacionadas con los mecanismos tras las variaciones de diversidad.

Medidas complementarias de diversidad biológica

Además de la importancia que puede tener el utilizar caracteres particulares de las especies al evaluar los patrones latitudinales de riqueza de especies, también resulta relevante utilizar otras medidas de diversidad de forma complementaria con el fin de explorar los posibles mecanismos tras los patrones observados. Desde esta perspectiva, el aumento de la riqueza de especies en función de la latitud (LGR inverso) podría estar acompañado de otros gradientes de diversidad (e.g. morfológicos, ecológicos, filogenéticos, funcionales), que podrían ser el resultado, por ejemplo, de una mayor diversificación del grupo de estudio a mayores latitudes. De hecho, varias teorías evolutivas proponen una relación positiva entre la diversidad

taxonómica de un grupo y su diversidad fenotípica (e.g. radiación adaptativa, equilibrio puntuado, entre otras. Ver Adams *et al.*, 2009), por lo que patrones particulares en riqueza de especies dentro de un grupo taxonómico podrían estar acompañados de patrones morfológicos concomitantes, pudiendo aportar señales de los procesos tras la conformación de tales patrones de riqueza. Diversos estudios biogeográficos realizados en ambientes terrestres y también marinos han encontrado correlaciones entre el número de especies y la diversidad fenotípica tanto en vertebrados como en invertebrados (Futuyma, 2009; Adams *et al.*, 2009; Rabosky y Adams, 2012). Esta diversidad fenotípica (e.g. tamaño corporal y morfología) se correlaciona con las tasas de diversificación en los clados filogenéticos (Adams *et al.*, 2009; Rabosky y Adams, 2012), por lo que diferencias morfológicas entre clados relacionados podrían, por ejemplo, ser resultado de procesos de diversificación diferencial o de distintas edades de origen en el caso que la tasa de diversificación no difiera entre clados.

En este contexto, una medida de diversidad que resulta de importancia al intentar inferir acerca de los procesos tras los patrones de diversidad biológica es la diversidad filogenética (Faith, 1992). La diversidad filogenética reconoce que distintas especies no son solo unidades numéricamente distintas y equivalentes (i.e. medida de riqueza de especies), sino que reconoce además que tales diferencias (entre especies) son el resultado de sus relaciones evolutivas, y que la magnitud (i.e. distancia) de estas diferencias puede variar enormemente dependiendo del grupo de especies que se comparen. Esta medida de diversidad filogenética ayuda a distinguir entre clados que en términos de riqueza taxonómica pudieran ser equivalentes, pero en términos de su historia evolutiva particular (i.e. suma de los largos de rama de cada taxa del clado) pudieran diferir. Esto ayudaría a generar hipótesis sobre los mecanismos tras la conformación de la diversidad de cada clado en particular, así como evaluar si tales mecanismos pueden ser comunes entre clados distanciados geográficamente.

Modelo de estudio: Poliplacóforos

Para este trabajo se utilizó como grupo de estudio la Clase Polyplacophora, perteneciente al Filo Mollusca, presentes en las costas someras del SEP. Históricamente este grupo en las costas del SEP ha sido poco investigado en comparación a otras clases de moluscos (i.e. Bivalvia, Gastropoda), y sus estudios se han enfocado principalmente en la ecología trófica, los patrones de distribución latitudinal y batimétrica, morfometría y su taxonomía (e.g. Boudet, 1944; Otaíza y Santelices, 1985; Otaíza, 1986; Schwabe y Sellanes, 2004; 2010; Aguilera, 2005a; 2005b; Schwabe *et al.*, 2006; Aguilera y Navarrete, 2007; Sirenko, 2007; Camus *et al.*, 2008; Sanhueza *et al.*, 2008; Camus *et al.*, 2009; Camus *et al.*, 2012; Camus *et al.*, 2013; Tobar-Villa y Ibáñez, 2013; Araya y Araya, 2015; 2017; Sirenko, 2015a; 2015b; Sanhueza y Ibáñez, 2016; Sirenko y Sellanes, 2017; Ibáñez *et al.*, 2016; 2018).

Se ha descrito que algunos grupos de moluscos presentan un LGR inverso en las costas del SEP, con niveles de riqueza relativamente bajos y constantes desde los 10°S a *ca* 42°S, punto desde el cual el número de especies aumenta notoriamente hasta los 55°S (Valdovinos *et al.*, 2003). Dentro de los moluscos, este patrón es compartido por bivalvos, gastrópodos, poliplacóforos y prosobranquios (Crame, 1997; Valdovinos *et al.*, 2003; Rivadeneira, 2005). Sin embargo, Kiel y Nielsen (2010) no encuentran evidencia de una acumulación de especies

de bivalvos y gasterópodos fósiles desde el Eoceno en las costas del SEP, proponiendo que el origen del actual patrón inverso de riqueza de moluscos en esta zona puede ser aún más reciente, durante el cuaternario, y debido a procesos de colonización y adaptación de taxa ya presentes a lo largo de la costa del SEP después del retroceso de los glaciares en el Pleistoceno tardío. Lamentablemente los registros fósiles de la Clase Polyplacophora en las costas del SEP no permiten realizar inferencias sobre sus riquezas pasadas, por lo que no hay evidencia que los mecanismos que configuran el patrón actual de riqueza de poliplacóforos sean los mismos que pueden haber configurado la riqueza de otros moluscos, por lo cual resulta necesario aproximarse desde otra perspectiva al entendimiento del patrón de riqueza actual de los poliplacóforos en las costas del SEP. Lo anterior convierte a los poliplacóforos en un modelo de estudio interesante para avanzar en el entendimiento de los mecanismos generales que pueden ser relevantes en la conformación de los patrones de riqueza de especies (e.g. LGR, LGR inverso, máximos de riqueza intermedios) registrados en la costa sudamericana del SEP. Si bien es posible que el patrón inverso de riqueza descrito para los poliplacóforos en las costas del SEP pueda ser el resultado de procesos de diversificación y/o extinción diferencial de los taxa en el gradiente latitudinal, tales procesos no son apreciables al cuantificar solo la riqueza actual de especies. Es por esto que el uso de medidas complementarias de diversidad (i.e. taxonómica, filogenética, morfológica) pueden ayudar a entender la importancia relativa de estos procesos en la conformación de patrón actual.

HIPÓTESIS GENERAL

La conformación y mantención de los patrones de diversidad de poliplacóforos en el Pacífico sureste está determinado por procesos ecológicos e histórico-evolutivos, los cuales reflejan tanto las restricciones ambientales actuales como los procesos de diversificación ancestrales que subyacen a los patrones de diversidad observados.

Capítulo 1: Objetivo

Tomando en cuenta los recientes y continuos cambios en la clasificación taxonómica de los poloplacóforos de pacífico sur este, donde se han descrito nuevas especies y sinonimizado otras, el objetivo de este capítulo de tesis es redescribir el patrón de riqueza de especies de poliplacóforos intermareales presentes en el Pacífico sureste (a partir de una revisión exaustiva de literatura, y de ejemplares de coleciones biológicas y muestreo en trereno, dilucidando genéticamente especies conflictivas).

Capítulo 2: Predicciones

Predicción 1

El patrón actual de riqueza de los poliplacóforos en el Pacífico sureste es influenciado por las condiciones ambientales que varían en el gradiente latitudinal, por lo que se espera una relación positiva con variables como la concentración de oxígeno disuelto, la concentración de clorofila y con la salinidad; mientras se espera una relación negativa con la temperatura superficial del mar.

Predicción 2

El patrón actual de riqueza de especies de poliplacóforos en la costa del Pacífico sureste está determinado por la amplitud y sorbreposición de los rangos de distribución de sus especies (los cuales presentan restricciones espaciales), acumulando mayor cantidad de especies en latitudes intermedias del rango de distribución de este grupo (mid-domain effect MDE).

Capítulo 3. Predicción

Predicción 3

El patrón actual de diversidad morfológica de los poliplacóforos en el Pacífico sureste ha sido influenciado por procesos de diversificación diferencial y dispersión de las especies en el gradiente latitudinal, por lo que se espera que la diversidad morfológica se relacione significativamente con variables ambientales, y que también se relacione de forma positiva con la diversidad filogenética.

OBJETIVO GENERAL

Este estudio busca avanzar en el entendimiento de los procesos ecológicos e históricoevolutivos que son relevantes en la conformación y en el mantenimiento del patrón de riqueza de especies de los poliplacóforos en las costas del Pacífico sureste, estimando su relación con variables ambientales en el gradiente, además de determinar adicionalmente patrones de diversidad morfológica y filogenética del grupo en el gradiente latitudinal.

CAPÍTULO 1: LATITUDINAL DISTRIBUTION OF POLYPLACOPHORANS ALONG THE SOUTH-EASTERN PACIFIC COAST: UNRAVELLING BIASES IN GEOGRAPHICAL DIVERSITY PATTERNS

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Abstract

Latitudinal diversity patterns in marine species are commonly estimated from literature records, which at times are incomplete and/or biased. Advances in molecular phylogenetics have contributed to avoid this bias, clarifying the identity of the species, improving our knowledge of species diversity and distribution. With the aim to identify biogeographic biases, we compiled and compared range distribution data of polyplacophorans along the South-eastern Pacific (SEP) coast (0°–56° S) generated from: (i) literature review (LIT dataset) and (ii) Operational Taxonomic Units (OTUs dataset), based on the analysis of 8949 individuals obtained from field sampling and biological collections. Cytochrome oxidase I (COI) and 16S rRNA of 104 specimens were used for genetic identification of conflictive morphospecies.

Multivariate analysis (nMDS, PERMANOVA) were applied to test differences between datasets (LIT, OTUs) and also between biogeographic ecoregions. Just like prior studies based on literature reviews, the richness of LIT species showed an increase with latitude. Contrastingly, OTUs' richness peaked at intermediate latitudes showing a bell-shaped distribution, indicating that the LIT dataset was flawed by inaccuracies in the identification and location of polyplacophoran species on the South-eastern Pacific, causing an overestimation of their geographic ranges. Our results contrast with the previous richness patterns described for the SEP polyplacophorans, where species richness was reported to increase with latitude. Both an overestimation of geographic ranges and inaccuracies in the identification of species cause these differences. Biogeographical studies should be conducted on the basis of a comprehensive review of specimens with verifiable occurrences, and incorporate as far as possible genetic analysis to define the identity of conflicting morphospecies, in order to improve the estimation of species richness and the understanding of marine biodiversity.

Keywords Chitons . Species richness . Biogeography . Latitudinal diversity gradient . Conflicting morphospecies

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research was developed under the ethical guidelines of Universidad Andres Bello, Universidad de Chile and FONDECYT, via the fishing permit # 1554 provided by the Chilean Government.

Sampling and field studies Field studies and sampling permits have been provided by the Undersecretary of Fisheries and Aquaculture (SUBPESCA, fishing permit #1554), by the Chilean government. Individuals registered and stored in biological collections and their respective GenBank and repository codes are detailed in the Online Table S1.

Data Availability All data generated or analysed during this study are included in this published article, in its supplementary information file and are available in the Genebank repository https://www.ncbi.nlm.nih.gov/genbank/

Author contribution AHN, CMI and JS contributed to the study conception. AHN, CMI, MCP-G and JS contributed to the study design. Data collection was performed by CMI, MCP-G, AHN, JS, BS and DJE. Species identification was conducted by BS, CMI, DE and AHN.

Statistical analysis was performed by CMI and AHN. The first draft of the manuscript was written by AHN and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Introduction

Large-scale studies in ecology, biogeography and conservation biology are commonly based on data collected from published or unpublished sources, which at times are incomplete and can be biased (Kodric-Brown and Brown 1993). This problem may include marine studies and, as such, claims of large-scale patterns should be evaluated carefully. Specifically, biases can exist due to the collection methodologies used (Camus 2001; Navarrete *et al.* 2014), which can lead to artefactual trends in diversity reported for certain taxonomic groups.

Biogeographic studies in the South-eastern Pacific (hereafter SEP) have described a classic pattern of decreasing diversity toward higher latitudes for several marine taxa such as chondrichthyans, teleost fishes, decapod crustaceans, prosobranchs, nudibranchs, cephalopods, and nematodes (Lancellotti and Vásquez 2000; Ojeda *et al.* 2000; Astorga *et al.* 2003; Ibáñez *et al.* 2009; Lee and Riveros 2012; Navarrete *et al.* 2014). However, there are several studies that also report the opposite pattern in groups such as seaweeds, polychaetes, amphipods, isopods, echinoderms, polyplacophorans and bivalves (Santelices and Marquet 1998; Lancellotti and Vásquez 2000; Valdovinos *et al.* 2003; Hernández *et al.* 2005; Rivadeneira *et al.* 2011), which exhibit an increasing species diversity toward high latitudes. Studies mentioned above were performed compiling information from literature, and not using, or partially including, empirical data. Therefore all of them should be interpreted carefully due to

two reasons: i) the bias caused by the use of different approaches and collection methods to get samples (Camus 2001; Navarrete *et al.* 2014) might lead to erroneous interpretations about diversity for certain groups; ii) the effects of taxonomic accuracy and synonymy over the species number in some places has seemingly generated an increase in the entities during the last two centuries (e.g. Rivadeneira *et al.* 2011), but the underlying reason is that many of these "new" taxa are just synonyms of the same taxonomic entity, artificially increasing the species richness value, and eventually producing a latitudinal bias. In this sense, advances in molecular biology and phylogenetic analysis in the last decades have revealed ecosystems with under- or overestimation of species diversity (Knowlton 2000; Fouquet *et al.* 2007; Fonseca *et al.* 2010; Leray and Knowlton 2015; Tedersoo *et al.* 2014).

Marine molluscs have been frequently the focus of biogeographical studies, given that they are fundamental parts of marine systems, perform key ecological roles, and are economically important (Pérez-Matus *et al.* 2017; Wanninger and Wollesen 2018). Among molluscs, polyplacophorans have been included on studies using data from literature to determine biogeographic patterns (e.g. Viviani 1979; Brattström and Johanssen 1983; Lancellotti and Vásquez 2000; Valdovinos *et al.* 2003; Fernández *et al.* 2009; Pappalardo and Fernández 2014). Despite the ecological importance of polyplacophorans in rocky shore ecosystems of the SEP coast (Pérez-Matus *et al.* 2017), the taxonomic identification of species in this group have remained problematic. The most complete taxonomical review of Chilean polyplacophorans by Valdovinos (1999) reported at least 60 species of chitons belonging to six families. Nevertheless, recent studies have significantly refined and reduced the taxonomic list to 45 species, currently suggesting an increase of chiton species diversity towards the poles (Valdovinos *et al.* 2003; Sirenko and Gallardo 2005; Fernández *et al.* 2009; Schwabe and Sellanes 2010; Pappalardo and Fernández 2014; Sirenko 2015; Sirenko and Sellanes 2016).

In this study we compare the richness, composition, and latitudinal distribution of polyplacophoran species along the SEP coast, considering the high variability of biogeographical patterns of marine invertebrate taxa documented for the area, and the incompleteness and biases of the datasets used to build large-scale distribution patterns. To do so, we analyse two datasets compiled from: (i) literature reviews (hereafter LIT), and (ii) direct identification of morphospecies (field sampling and museum collections) aided with DNA barcoding genetic identification for conflictive species (hereafter OTUs), comparing the richness of each dataset between biogeographic ecoregions. Here we sought to determine the latitudinal pattern of polyplacophoran species richness along the SEP coast and to quantify biases associated with data collection in order to identify sources that built variance to the observed patterns.

Materials and methods

Datasets

To improve the estimated pattern in polyplacophoran species richness along the SEP coast, we compiled two datasets (Table 1). Together, the datasets include reports and field samplings of coastal chiton species occurrences between 0° and 56° S, covering more than 5000 km of coastline and 56 latitudinal bands (Fig. 1). The first dataset was obtained from a literature review (LIT) compiled from published studies of the SEP region. All chiton species records included in this study are available in Kaas and Van Belle (1987), Bullock (1988a, b), Valdovinos (1999), Sirenko (2006), Kaas *et al.* (2006), Schwabe *et al.* (2006), Schwabe

(2009) and Ibáñez et al. (2016). Our second dataset included identifications using morphological species (MS) aided with genetic data (determining Operational Taxonomic Units, OTUs). For this, an exhaustive sampling was performed between 2011 and 2016 along the SEP coast (Fig. 1) collecting a total of 6123 chitons belonging to 35 morphospecies. For each sample site, individual chitons were collected from intertidal (highest tidal level) and shallow subtidal (10-m depth) zones by hand during low tide via snorkelling and SCUBA diving. All of the specimens collected were identified to species-level following Leloup (1956), Bullock (1988a, b), Kaas et al. (2006), Schwabe et al. (2006), Sirenko (2006) and Schwabe (2009). Some voucher specimens were properly registered and stored at the Museo Nacional de Historia Natural de Chile (MNHNCL) and in the Sala de Colecciones Biológicas Universidad Católica del Norte, (SCBUCN) Coquimbo, Chile (Online Table S1). Additionally, we reviewed 2826 specimens from: Museo Nacional de Historia Natural de Chile, Santiago, Chile (MNHNCL); Collection of Flora and Fauna, Professor Patricio Sánchez from Pontificia Universidad Católica de Chile, Santiago, Chile (SSUC); Instituto de la Patagonia Universidad de Magallanes, Punta Arenas, Chile (UMIP); Santa Barbara Museum of Natural History, Santa Barbara, USA (SBMNH); and the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZISP) (Fig. 1). Combining data from all collections and those collected from field sampling, a total of 8949 chitons belonging to 41 morphospecies were utilized to estimate the geographic distribution range of all species (Fig. 1).

To estimate the representativeness of the presence-absence matrix of species distribution and species richness, a species accumulation curve (Colwell *et al.* 2004) was performed using the software PAST v3.25 (Hammer *et al.* 2001). The expected number of

species was estimated using the Chao 2 algorithm (Colwell *et al.* 2004), based on the number of latitudinal bands (Fig. 2).

Finally, two absence–presence matrices with a resolution of one degree of latitude were built from the obtained datasets of LIT and OTUs (Table 1).

Phylogenetic analysis

Portions of mitochondrial genes were sequenced, and OTUs were determined. For this, the cytochrome oxidase subunit I (COI) gene was sequenced for 104 specimens belonging to 35 morphological species. In addition, sequences of *Hemiarthrum setulosum* (KJ574095), *Leptochiton medinae* (HQ907865) and *L. kerguelensis* (HQ907864) were obtained from GenBank and incorporated in our analysis. Additionally, the 16S rRNA ribosomal genes (16S) of 45 specimens belonging to 31 morphological species were sequenced.

Total DNA (gDNA) was extracted considering the saline extraction protocol following Aljanabi and Martinez (1997). The polymerase chain reaction (PCR) amplifications were performed using a total reaction volume of 25 µl, with 2.5 µl of Buffer 10x (200 mM Tris-HCL using pH = 8.4, 500 mM KCL), 2.0 µl of dNTPs [2.5 mM], 1.0 µl MgCl2 [50 mM], 0.3 µl of InvitrogenTM PlatinumTM Taq DNA polymerase (now part of ThermoFisher Scientific), 0.1 µl ofDNA and 0.25 µl of each primer [10 pmol] (COI primer pair LCO1490–HCO 2198 in Folmer *et al.* 1994; 16S rRNA primer pair 16Sa–16Sb in Okusu *et al.* 2003). For amplification, the optimum condition had an initial denaturation at 94 °C for 5min, followed by 30 cycles of 94 °C (60 s), 50 °C (60 s), and 72 °C (60 s); followed by a final extension at 72 °C during 10 min, using a thermal cycler. Double-stranded PCR products were purified and
sequenced in both directions using an abi3730 automatic sequencer (Macrogen, Inc., Seoul, Korea). Sequences were edited and aligned using the MUSCLE software (Edgar 2004a, b) implemented in MEGA 7.0 (Kumar *et al.* 2016). Sequences extracted in this survey were stored at the GenBank database (Online Table S1).

Bayesian inference was applied to evaluate the phylogenetic relationships among polyplacophorans from the SEP using rRNA 16S and COI separately in the software BayesPhylogenies v1.1 (Pagel and Meade 2004). Three independent analyses were ran using four Markov Chain Monte Carlo (MCMC), each including 5,000,000 generations, and to assure that successive samples were independent, sampling was done every 1000 trees. To assess the stationary distribution of the MCMC we visually inspected the log-likelihood values of the iterations until they reached convergence, removing all of them prior to this burn-in point. Also, a sampling size above 500 was verified. This was done using the software Tracer v1.5 (Rambaut and Drummond 2009). Trees that did not reach convergence (20%) were discarded. Among the remaining trees, 4001 were selected to reconstruct a majority rule consensus tree collapsing nodes above 0.7 posterior probability in the BayesTrees v1.3 software (Meade 2011). We rooted the trees using *Hanleyella oldroydi* (Dall 1919) (16S KJ574077 and COI HQ907874) as outgroup.

Comparison between datasets

To detect differences in species compositions along the SEP coast between the LIT and OTUs datasets, a two-way permutational multivariate analyses of variance (PERMANOVA+: Anderson 2001; Anderson *et al.* 2008) with 10,000 permutations was performed to test for differences between datasets and ecoregions (after Spalding *et al.* 2007) along the SEP coast.

Ordination analyses were based on the Jaccard similarity index (Jindex) calculated for all species within each 1° latitude bin. Non-metric multidimensional scaling (nMDS) was performed (Clarke 1993) to visualize potentially distinct clusters along the latitudinal gradient and ecoregions (Spalding *et al.* 2007). All analyses were performed using the Plymouth Routines in Multivariate Ecological Research (PRIMER v6) software (Clarke and Gorley 2006).

To evaluate the replacement degree in species composition between localities and ecoregions, beta diversity was estimated using the Williams index (Koleff *et al.* 2003). This analysis was performed only for the OTUs data base.

Results

Morphospecies and OTUs identification

We identified 37 morphospecies from biological collections and field sampling. Not all species were sequenced for COI and 16S, but our results helped improve the taxonomic resolution of the dataset. In relation to genetic results, it was possible to identify 31 OTUs using 104 COI sequences derived from 35 morphospecies with 88.6% certainty. Using the 16S subunit, we identified 26 OTUs from 30 morphospecies with 86.6% certainty. By combining results from both genetic markers (COI and 16S), we identified 32 OTUs from 37 morphospecies sequenced with 86.5% certainty (Fig. 3). The Bayesian phylogenetic trees based on the COI and 16S sequences showed cohesive clusters for most of the species (Fig. 3). The sequences of both genes (COI and 16S) revealed that particular pairs of morphospecies of the genera *Chiton* or *Tonicia* were genetically indistinguishable (i.e. correspond to synonymies) (Fig. 3), where individuals of *Chiton bowenii* were not genetically different with

C. magnificus, individuals of *T. elegans* were not genetically different with *T. calbucensis*, individuals of *T. smithi* were not genetically different with *T. disjuncta* and individuals of *T. atrata* was not genetically different with *T. chilensis*.

Latitudinal diversity

Each dataset displayed a distinct latitudinal pattern of species richness. For example, species richness increased poleward according to the LIT dataset. Specifically, 12 species were found near the Equator, and a maximum of 26 species were found at ~ 55° S (Fig. 4). The trend was not monotonic as two significant drops in species richness were recoded along thelatitudinal gradient. The first drop was observed between ~ 5° S and ~ 10° S with ~ 10 species while the second drop was observed at ~ 38° S with ~ 13 species (Fig. 4). Species richness compiled from OTUs datasets varied significantly along the latitudinal gradient (Fig. 4), tended to increase slightly southwards, and exhibited a bell-shape pattern with species richness reaching a maximum at 33° S. Eight species were found near the Equator, and 12 species were found at ~ 55° S, whereas a maximum of 14 species were found at intermediate latitudes (33° S). Two significant drops in species richness were recoded along the latitudinal gradient. The first drop was observed between ~ 5° S and ~ 11° S (with 2-4 species) while the second drop was observed between ~ 5° S and 51° S (with 7-8 species) (Fig. 4).

Differences of species ranges between both data sets were around 58%, and one of the main differences was the decrease in range distribution of 23 species (Table 1). Other eight species increased their distribution, and only three had the same distribution in both data sets (Table 1).

Significant differences in species composition were found when we compared the two datasets (Fig. 5). Aligned along a latitudinal gradient two distinctive clusters were identified from the nMDS ordination, one for each dataset (Fig. 5a), and significant differences were found between each group according to the PERMANOVA (F=90.32, $p_{perm} < 0.001$) (Table 1). Pairwise comparisons between the LIT and OTUs datasets indicated similarities of ~64.94% (J_{index}).

The nMDS ordination by ecoregions showed gradual changes in species composition along the SEP coast (Fig. 5b). Each cluster arranged along the latitudinal gradient constituted an ecoregion, and each biogeographic unit was significantly distinguishable from the others (Table 1). The interaction term of the PERMANOVA analysis (Table 2) reflected changes in composition along the latitudinal gradient, but also reflected uncertainty in species composition given by the exaggerated distribution range of some chiton species (Table 1). Each of the paired comparisons of species composition between LIT and OTUs databases showed significant differences for each ecoregion (Table 2).

Beta diversity was relatively higher among Guayaquil-Central Peru and Central Chile-Araucanian ecoregions (0.27–0.17, respectively), suggesting an important turnover of species among these biogeographic units (Table 2). Beta diversity among Humboldtian-Central Chile, Araucanian-Chiloense and Chiloense-Channels and Fjords ecoregions showed intermediate values (0.012, 0.084, 0.075, respectively), meanwhile beta diversity between the Central Peru-Humboldtian ecoregions was the lowest (0.010), suggesting a low turnover among these biogeographic units (Table 3).

Discussion

Our results highlight the value of exhaustive studies that include multiple sources of evidence for describing the patterns and processes that affect latitudinal gradients in species richness. The re-evaluated diversity of polyplacophoran species along the SEP coast (i.e. OTUs dataset) exhibited a bell-shape pattern with species richness reaching a maximum at intermediate latitudes. Our results are in contrast with previous richness patterns described for polyplacophorans (e.g. Valdovinos *et al.* 2003; Fernández *et al.* 2009) as well as for other invertebrate taxa such as sponges, bryozoans, polychaetes, peracarids, and gastropods (Desqueyroux and Moyano 1987; Moyano 1991; Valdovinos *et al.* 2003; Hernández *et al.* 2005; Rivadeneira *et al.* 2011), which describe a steady poleward increase of species richness.

The database created from the literature records provides evidence that the taxonomic richness of polyplacophorans has been greatly overestimated. For instance, 24% (12 out of 50) of the described species include unverified occurrences, and 12% of the occurrences in the literature are synonyms, according to the genetic information provided here. This implies that 46% of all species described in previous studies are invalid (Reid and Osorio 2000; Aldea and Valdovinos 2005; Schwabe *et al.* 2006; Schwabe 2009; Araya and Araya 2015).

A thorough examination of the literature indicates that inaccuracies in distribution ranges and species richness estimates are mainly due to uneven sampling efforts along the SEP coast. For instance, so far there is only one study describing the composition of polyplacophorans in both the Guayaquil (Ibáñez *et al.* 2016) and Central Peru (Uribe *et al.* 2013) ecoregions. Conversely, the sampling effort in the Chiloense and the Channels and Fjords ecoregions has been high (Reid and Osorio 2000; Aldea and Valdovinos 2005; Schwabe *et al.* 2006; Sirenko 2006; Rosenfeld *et al.* 2015), although taxonomic inconsistencies in these regions are still frequent. Overall, inconsistencies could be due to the high intraspecific phenotypic variability associated with habitat heterogeneity and the subsequent assignment of different names to the same phenotypes (e.g. Schwabe 2009). On the other hand, differences in species composition between datasets were low for the Central Chile, Humboldtian and Araucanian ecoregions. This could be due to greater sampling effort, and the occurrence of many research centers in these areas (Camus 2001).

Unravelling biogeographic patterns mainly conducted through a comprehensive review of specimens (i.e. from field sampling and from biological collections) and aided with genetic analysis allowed us to improve taxonomic identifications. With robust estimates of the number of species in a particular area, evolutionary hypotheses related to gradientsof species diversity can be thoroughly tested. For example, several studies have used genetic barcoding to verify species diversity and have also found biases associated with either overestimation due to poor identification or underestimation due to cryptic species (Fouquet *et al.* 2007; Fonseca *et al.* 2010; Chen *et al.* 2011; Tedersoo *et al.* 2014; Leray and Knowlton 2015).

The quantitative results in this study reject the previously reported inverse pattern of latitudinal richness (e.g.Valdovinos *et al.* 2003; Fernández *et al.* 2009; Pappalardo and Fernández 2014), mostly due to the previous lack of taxonomic resolution. The improved accuracy achieved in this study, product of 5 years of fieldwork and the re-identification of many specimens deposited during the last century in biological collections (i.e. SSUC,UMIP, MNHNCL, SBMNH and ZISP), has given us some confidence that we have sampled most species present along the SEP coast.

Our genetic results suggest that some genera are in urgent need of taxonomic revision (e.g. *Ischnochiton, Chiton* and *Tonicia*). Some taxonomic names of chitons (*Tonicia elegans*,

T. lineolata) were invalidated by Frembly (1827) because these were used beforehand. Recently, Ibáñez et al. (2019) synonymized T. elegans with T. calbucensis and clarified the taxonomic position of T. lineolata as T. fremblyana. Two other Tonicia species were synonymized as well, T. smithi with T. disjuncta and T. atrata with T. chilensis (Ibáñez et al. 2019), while other species names (e.g. Chiton granosus, C. cumingsii) are classified as nomen dubium because they were proposed before 1931 (ICZN 1999). In the absence of a taxonomic solution, we used these names provisionally, since they have been frequently used in chiton literature (e.g. Bullock 1988a). Taxonomic problems in marine molluscs related to shell morphology and coloration have been resolved by genetic studies, resulting in the synonymization of species (Knowlton 2000; González-Wevar et al. 2010). Our genetic distance estimates with barcoding allowed a rapid differentiation at species, genus and family level. Similar COI distances have been previously reported for chitons in the USA and China (Kelly et al. 2007; Chen and Sun 2013). This approach is useful to identify species, but for phylogenetic relationships it is necessary to include more mitochondrial and nuclear genes. In our phylogenetic tree, several groups (Ischnochiton, Plaxiphora and Stenoplax) do not result in monophyletic groups (according to taxonomy), probably due to gene saturation. The genus *Ischnochiton*, for example, has resulted to be polyphyletic in other studies with the barcoding approach, even when using more genes (Okusu et al. 2003; Chen and Sun 2013), suggesting more than one subgroup inside this genus. At the genus level, only four genera (e.g. Acanthochitona, Chaetopleura, Callistochiton and Tonicia) represented monophyletic groups. All these taxonomic conflicts, together with the difficulty to identify morphologically several chitons at the species level, have produced a significant bias in the diversity gradient along SEP caused by incorrect geographical distributions

The geographical bands displaying the highest species richness (often associated with a higher number of genera) of polyplacophorans were located between 20 and 42° S (10–14 species), forming a bell-shaped distribution along the SEP coast, which has also been previously described for organisms such as polychaetes (Hernández et al. 2005), nematodes (Lee and Riveros 2012) and intertidal fishes (Navarrete *et al.* 2014). This range lies within the Intermediate Area (after Camus 2001), flanked by lower-diversity transitional zones that include mixed components of biota from adjacent provinces. Climatic shifts related to quaternary glacial/tectonic events are expected to have produced biotic shifts in latitude for both the intermediate area and associated transition zones (Camus 2001). In the case of chitons, 11 species, including the monotypic genus Gallardoia, are endemic to this region. Similar patterns have been recorded for the northeastern Pacific Ocean, especially as discussed for the California Transition Zone (Briggs and Bowen 2012). Biogeographic transition zones are defined as overlapping geographical areas, with a gradient of substitution and partial segregation between biotic components (sets of taxa sharing a similar geographical distribution as result of a common history) (Ferro and Morrone 2014). In the case of the SEP chitons, the transition zone extends from 20° S to 42° S, since in this range we found the highest diversity and distributional overlap. As evidenced here, nearby localities tended to have similar species, with the exception of the high turnover across the biogeographical break between the Panamian and Peruvian provinces (Guayaquil-Central Peru ecoregions) where species composition varied significantly as the distance between localities increased. The same pattern has been observed by Ibáñez et al. (2016) and by Fenberg and Rivadeneira (2019) suggesting that the type of habitat and environmental influence would be key in explaining differences in composition and diversity. Along the Peruvian and Chilean ecoregions, species replacement did not show a latitudinal pattern since few chitons had small geographical ranges, and the dissimilarity between close localities remained low through the latitudinal gradient with a moderate increment in the Araucanian ecoregion.

Differences in the geographic distribution of chitons revealed by this study are related to errors in the distribution records of some species in the literature (e.g. *Tonicia lebruni, T. atrata, T. smithi, Chiton bowenii*) (Valdovinos 1999; Schwabe 2009; Araya and Araya 2015). For instance, while *Plaxiphora aurata* is reported to inhabit from 34-55° S (Schwabe 2009), in this study we propose it would have a wider distribution, ranging from 23-55° S. The opposite was found for *Chiton barnesii*, which in this study was shown to have a much narrower distribution ($27 - 30^{\circ}$ S) than that reported in the literature (27° S - 45° S: Schwabe 2009).

Our work shows that the exhaustive analysis of specimens for the identification of morphospecies can improve the recorded richness patterns, and where the rapid evolution of the field of molecular biology can help to improve the resolution of conflicting morphospecies. Furthermore, biodiversity should not "have to be just about the number of a species in an ecosystem", and its study needs a revolution (Cernansky 2017). Not only the variety of sizes, shapes and functional traits of organisms, but also different approaches (e.g. genetic, ecological, physiological) need to be employed to achieve a more comprehensive estimation of biodiversity in all ecosystems.

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Tables

Table 1. Distributional range $(0-56^{\circ} \text{ south})$ of polyplacophoran species along the Southeastern Pacific included in the literature richness data (LIT) and operational taxonomic unit's data (OTUs)

Snecies	Range	Range	Range
species		OTUs	difference
Acanthochitona arragonites (Carpenter, 1857)	0-2 (1)		
Acanthochitona ferreirai Lyons, 1988	0-5 (8)	0-4 (3)	1
Acanthochitona hirudiniformis (Sowerby I, 1832)	0-5 (7)	0-1 (1)	4
Acanthopleura echinata (Barnes, 1824)	4-36 (17)	4-37 ⁽⁹⁾	1
Callistochiton expressus (Carpenter, 1865)	0-3 (2)	0-4 (4)	1
Callistochiton pulchellus (Gray, 1828)	0-33 (12)	17-20 (17)	30
Callochiton gaussi Thiele, 1908	50-55 ⁽⁴⁸⁾		
Callochiton puniceus (Gould, 1846)	41-55 ⁽³⁷⁾	53-55 ⁽³³⁾	12
Callochiton steinenii (Pfeffer, 1886)	45-55 ⁽⁴¹⁾	54-55 ⁽³⁶⁾	9
Calloplax vivípara (Plate, 1899)	20-35 (27)	20-33 (19)	2
Chaetopleura angulata (Spengler, 1797)	45-55 (42)		
Chaetopleura benaventei Plate, 1899	15-37 (24)	35-46 (28)	11
Chaetopleura brucei Iredale in Melvill & Standen, 1912	45-55 (43)		
Chaetopleura hanselmani (Ferreira, 1982)	0-6 (10)		
Chaetopleura hennahi (Gray, 1828)	11-27 (21)	12-21 (14)	7
Chaetopleura lurida (G. B. Sowerby I, 1832)	0-18 (11)		
Chaetopleura peruviana (Lamarck, 1819)	1-55 (13)	12-41 (16)	25
Chaetopleura roddai Ferreira, 1983	0-3 (3)	0-4 (5)	1
Chiton barnesii Gray, 1828	26-45 (28)	27-39 (22)	7
Chiton bowenii King, 1832 ^a	42-55 (39)		
Chiton cumingsii Frembly, 1827	5-45 (18)	5-41 (10)	4
Chiton granosus Frembly, 1827	5-55 ⁽¹⁹⁾	8-45 (11)	13
Chiton magnificus Deshayes, 1827	12-55 (22)	27-55 (23)	15
Chiton stokesii Broderip, 1832	0-5 (9)	0-4 (6)	1
Enoplochiton niger (Barnes, 1824)	4-30 (16)	12-30 (15)	8
Gallardoia valdiviensis Sirenko, 2007	39-39 ⁽³⁴⁾	39-39 ⁽²⁹⁾	0
Hemiarthrum setulosum Carpenter in Dall, 1876	53-55 ⁽⁴⁹⁾	53-55 ⁽³⁵⁾	0
Ischnochiton dispar(Sowerby in Broderip & Sowerby, 1832)	0-3 (4)	0-4 (7)	1
Ischnochiton dorsuosus Haddon, 1886	45-55 ⁽⁴⁴⁾		
Ischnochiton punctulatissimus (Sowerby, 1832)	3-55 (14)		
Ischnochiton pusillus (Sowerby, 1832)	7-20 (20)		
Ischnochiton pusio (Sowerby I, 1832)	3-55 (15)	20-41 (20)	31

Species	Range LIT	Range OTUs	Range difference
Ischnochiton stramineus (G. B. Sowerby I, 1832)	15-55 (26)	33-53 ⁽²⁶⁾	20
Ischnochiton viridulus (Gould, 1846)	54-55 (50)		
Lepidopleurus cullierti Rochebrune, 1889	45-55 (45)		
Leptochiton kerguelensis Haddon, 1886	45-55 (46)	53-55 ⁽³⁴⁾	8
Leptochiton lascrusesi Sirenko, 2015	33-33 ⁽³²⁾	33-33 ⁽²⁵⁾	0
Leptochiton medinae (Plate, 1899)	40-55 (35)	42-55 (30)	2
Nuttallochiton martiali (Rochebrune, 1889)	42-55 (40)	52-55 (31)	10
Plaxiphora aurata (Spalowsky, 1795)	26-55 (29)	23-55 (21)	3
Stenoplax limaciformis (Sowerby, 1832)	0-3 (5)	0-2 (2)	1
Stenoplax rugulata (G.B. Sowerby I, 1832)	0-3 (6)	0-5 (8)	2
Stenosemus exaratus (Sars G. O., 1878)	45-55 (47)		
Tonicia atrata (G.B. Sowerby II, 1840) ^b	27-55 (30)		
Tonicia calbucensis Plate, 1897	41-55 (38)	18-55 (18)	23
Tonicia chilensis (Frembly, 1827)	12-55 (23)	33-55 (27)	21
Tonicia disjuncta (Frembly, 1827)	30-49 (31)	27-55 (24)	9
<i>Tonicia elegans</i> (Frembly, 1827) ^{<i>c</i>}	15-45 (25)		
Tonicia fremblyana (Kass, 1957)		11-33 (12)	
Tonicia lebruni Rochebrune, 1884	40-55 (36)	52-55 ⁽³²⁾	12
Tonicia smithi Leloup, 1980 ^d	36-55 (33)		
Tonicia swainsoni (Sowerby in Broderip & Sowerby, 1832)		12-18 (13)	
Total richness	50	36	

Superscript numbers in parentheses for each database refer to the distribution range represented in Fig. 1. Species with no occurrence in the OTUs data correspond to species with incorrect records from the LIT database

^asynonymy of *Chiton magnificus* following genetic distances. ^bsynonymy of *Tonicia chilensis* following genetic distances. ^csynonymy of *Tonicia calbucensis* following genetic distances.

^dsynonymy of *Tonicia disjuncta* following genetic distances.

Table 2. Results of the PERMANOVA analysis comparing the composition of polyplacophoran species along the South-eastern Pacific coast between datasets (LIT: Literature; OTUs: Operative Taxonomic Units) and ecoregions (after Spalding *et al.* 2007) based on Jaccard dissimilarity.

Source	df	MSq	F	$p_{\rm perm}$
Dataset (D)	1	35162	90.32	0.001
Ecoregion (E)	6	29133	7483	0.001
D x E	6	8571.8	22.02	0.001
Residuals	111	389.3		

Post hoc following PERMANOVA between LIT and OTUs datasets across different ecoregions

		LIT vs OTUs
Ecoregions	Latitudinal extent (°S)	<i>t</i> value
Guayaquil (GUA)	0-5	2.84*
Central Peru (CPE)	6-12	5.39*
Humboldtian (HUM)	13-25	7.37*
Central Chile (CCH)	26-33	8.78*
Araucanian (ARA)	34-41	5.71*
Chiloense (CH)	42-46	7.78*
Channels and Fjords (CF)	47-55	7.60*

df degrees of freedom, *MSq* mean square, p_{perm} Monte Carlo permutation significance *p < 0.05

Ecoregions	Range	Mean	S.D.
Guayaquil/Central Peru	0.00 - 0.50	0.27	0.11
Central Peru/Humboldtian	0.00 - 0.18	0.01	0.04
Humboldtian/Central Chile	0.00 - 0.25	0.12	0.07
Central Chile/Araucanian	0.00 - 0.27	0.17	0.05
Araucanian/Chiloense	0.07 - 0.14	0.08	0.02
Chiloense/Channels and Fjords	0.00 - 0.20	0.07	0.09

Table 3. Williams' Beta diversity results comparing polyplacophoran species turnover of operational taxonomic unit's data (OTUs) along the South-eastern Pacific coast.

Figures



Figure 1. Latitudinal distribution ranges of polyplacophorans species along the South-eastern Pacific coast recorded from a literature reviews (LIT) and b Operational Taxonomic Units (OTUs). The numbers over the latitudinal distribution ranges for each database identify each species included in Table 1. Filled dots drawn along the Southeastern Pacific coast represent sampled localities, and open dots represent records from biological collections.



Figure 2. Species accumulation curve of the number of coastal polyplacophorans reordered for every band of 1° of latitude along the South-eastern Pacific coast. Solid lines correspond to the average richness of Literature (LIT in red) and Operational Taxonomic Units (OTUs in green), while dashed lines represent the 95% confidence interval.



Figure 4. Bayesian phylogenetic tree of polyplacophorans from the South-eastern Pacific coast. a) COI sequences, b) 16S sequences. Node values are posterior probabilities. Coloured boxes indicated species with taxonomic problems.



Figure 5. Latitudinal gradient of polyplacophoran species richness along the South-eastern Pacific coast. Each curve is based on information from two different sources. LIT: Literature (red dots; OTUs: Operative Taxonomic Units (green triangle).





Coordinate 1

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Figure 6. Non-metric Multidimensional Scaling plot of polyplacophoran species composition along the South-eastern Pacific coast. Ordination space was built based on Jaccard similarity index. Panel a) shows the polyplacophoran species composition based on two sources of information. LIT: Literature OTUs: Operative Taxonomic Units. Panel b) shows distinctive clusters (ecoregions) ordered along the South-eastern Pacific coast. GUA= Guayaquil; CPE= Central Peru; HUM= Humboldtian; CCH= Central Chile; ARA= Araucanian; CH= Chiloense; CF= Channels and Fjords. Thick back arrow (\rightarrow) indicates latitudinal gradient along the South-eastern Pacific coast.

Supplementary material

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Latitudinal distribution of polyplacophorans along the South-eastern Pacific coast: unravelling biases in geographical diversity patterns

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Online Resource: South-eastern Pacific littoral chitons morpho-species individuals in collection data and respective GenBank codes.

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	168
Leptochiton	-	-	Antarctica	-	-	-	-	HQ907865	HQ907865
medinae									
Leptochiton	-	-	Antarctica	-	-	-	-	HQ907864	HQ907864
kerguelensis									
Hemiarthrum	-	-	Antarctica	-	-	-	-	KJ574095	KJ574095
setulosum									
Acanthochitona	AFPOP6	MNHNCL 401390	Pocitas, Peru	C.M. Ibáñez - M.C. Pardo	03-01-2014	-4.116	-81.084	MK016365	-
ferreirai									
Acanthochitona	AFPOP21	-	Pocitas, Peru	C.M. Ibáñez - M.C. Pardo	03-01-2014	-4.116	-81.084	MK016366	-
ferreirai									
Acanthochitona	AFPOP19	SCBUCN-3787	Pocitas, Perú	C.M. Ibáñez - M.C. Pardo	03-01-2014	-4.116	-81.084	MK016367	MK097372
ferreirai									
Acanthochitona	AHME10	SCBUCN-3776	Montañita,	C.M. Ibáñez - M.C. Pardo	24-09-2013	-1.819	-80.759	MK016368	-
hirudiniformis			Ecuador						
Acanthochitona	AHME11	SCBUCN-3776	Montañita,	C.M. Ibáñez - M.C. Pardo	24-09-2013	-1.819	-80.759	MK016369	-
hirudiniformis			Ecuador						
Acanthochitona	AHME12	SCBUCN-3776	Montañita,	C.M. Ibáñez - M.C. Pardo	24-09-2013	-1.819	-80.759	MK016370	-
hirudiniformis			Ecuador						
Gallardoia	GVVPP1	SCBUCN-5394	Pilolcura,	B. Sirenko	09-11-2008	-39.667	-73.400	MK016371	-
valdiviensis			Valdivia, Chile						
Chiton magnificus	CMSC1	MNHNCL 400845	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J.	17-08-2013	-30.519	-71.694	MK016372	-
				Sellanes, G. Zapata- Hernández					
Chiton magnificus	CMV22	CBUCN 4600	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016373	-
Chiton magnificus	CMPT30	-	Punta de Tralca,	C.M. Ibáñez - M.C. Pardo - C.	26-05-2014	-33.426	-71.704	MK016374	-
			Chile	Tobar - A.H. Navarrete		0 0 5 0 (
Chiton magnificus	CMV21	CBUCN 4599	Valdıvıa, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016375	-
Chiton magnificus	CMCV19	MNHNCL 400804	Cascabeles, Los	C.M. Ibáñez - M.C. Pardo -	01-09-2013	-31.979	-71.499	MK016376	-
Chiton magnificus	CMSC14	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J.	17-08-2013	-30.519	-71.694	MK016405	-
				Sellanes					

Table S1. Littoral chitons morpho-species from the South-eastern Pacific Ocean, collection data and GenBank codes.

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	168
Chiton magnificus	CMCV6	-	Cascabeles, Los Vilos, Chile	C.M. Ibáñez - M.C. Pardo - A.H Navarrete	01-09-2013	-31.979	-71.499	MK016406	-
Chiton magnificus	CMV12	CBUCN 4597	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016407	-
Chiton magnificus	CMV20	CBUCN 4598	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016408	-
Chiton magnificus	CMV11	CBUCN 4596	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016409	-
Chiton magnificus	CMSC19	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes	17-08-2013	-30.519	-71.694	MK016410	-
Callistochiton pulchellus	CPUGI11	-	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	MK016378	-
Callistochiton pulchelllus	CPUGI12	-	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	MK016377	-
Callistochiton pulchellus	CPUGI17	-	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	MK016379	-
Callistochiton pulchellus	CPUGI18	-	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	-	MK097386
Callochiton puniceus	CPPA1	SCBUCN-4347	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016380	-
Callochiton puniceus	CPPA2	SCBUCN-4348	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016381	MK097384
Callochiton puniceus	CPPA3	SCBUCN-4349	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016382	-
Calloplax vivipara	CVVP2	-	Ventanas, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	25-05-2014	-32.743	-71.497	MK016383	-
Calloplax vivipara	CVVP3	-	Ventanas, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	25-05-2014	-32.743	-71.497	-	MK097389
Calloplax vivipara	CVVP7	-	Ventanas, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	25-05-2014	-32.743	-71.497	MK016384	-
Chaetopleura benaventei	CBV1	MNHNCL 400921	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016385	MK097377
Chaetopleura hennahi	PSCA1	MNHNCL 401382	Callao, Peru	C.M. Ibáñez - M.C. Pardo	18-10-2013	-12.067	-77.159	MK016386	-

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	16S
Chaetopleura	PSCA2	-	Callao, Peru	C.M. Ibáñez - M.C. Pardo	19-10-2013	-12.067	-77.159	MK016387	-
hennahi									
Chaetopleura	RBSC9	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo	17-08-2013	-30.519	-71.694	-	MK097397
hennahi									
Chaetopleura	CPSC11	MNHNCL 400960	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J.	17-08-2013	-30.519	-71.694	MK016388	MK097385
peruviana				Sellanes, G. Zapata- Hernández					
Chaetopleura	CPSC14	MNHNCL 400965	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J.	17-08-2013	-30.519	-71.694	MK016389	-
peruviana				Sellanes, G. Zapata-					
Chaetonleura	CPSC17	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J.	17-08-2013	-30.519	-71.694	MK016390	-
neruviana				Sellanes, G. Zapata-					
	CSDI D1	SCDUCN 5201	Labitas Dam	Hernández	06.01.2014	4 452	01 200	MK016201	ME007297
Chaetopleura	CSPLP1	SCBUCIN-3591	Lobitos, Peru	C.M. IDanez - M.C. Pardo	00-01-2014	-4.432	-01.200	WIK010591	MK09/38/
roddai	CCDI DO	SCRUCN 5202	Labitan Dama	CM Ibáña MC Daula	06.01.2014	4 450	01 200	ME01(202	
Chaetopleura	CSPLP2	SCBUCN-5392	Lobitos, Peru	C.M. Idanez - M.C. Pardo	06-01-2014	-4.452	-81.288	MK010392	-
roddai	CODI DO		L L' D		06.01.0014	4 450	01.200	M K016202	
Chaetopleura	CSPLP3	MNHNCL 401389	Lobitos, Peru	C.M. Ibanez - M.C. Pardo	06-01-2014	-4.452	-81.288	MK016393	-
roddai	ID CD 10				22.00.2012	0.011	00.046	N H101 (201	1.0005201
Chaetopleura	IDSR49	MNHNCL 401388	Santa Rosa, Salinas	C.M. Ibánez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016394	MK097391
roddai			Ecuador						
Chiton bowenii	CBPA1	SCBUCN-4341	Puerto del	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016395	MK097376
			Hambre, Punta Arenas Chile						
Chiton bowenii	CBPA4	SCBUCN-4344	Puerto del	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016396	-
			Hambre, Punta	-					
Chitan hawanii	CBPA6	SCBUCN-4346	Arenas, Chile Puerto del	R D. Semílveda	01-12-2013	-53 612	-70 929	MK016397	
Chilon bowenii	CDI NO	Sebeen-4540	Hambre, Punta	R.D. Sepurveda	01-12-2015	-55.012	-10.929	WIR010377	
			Arenas, Chile						
Chiton cumingsii	CCCO5	MNHNCL 401391	Coloso Sur,	C.M. Ibáñez - M.C. Pardo - C. Tobar, G. Zapata-Hernández	01-06-2013	-23.774	-70.477	MK016398	-
			Chile	100ar, O. Zapata-Hernandez					
Chiton cumingsii	CCCO6	-	Coloso Sur,	C.M. Ibáñez - M.C. Pardo - C.	01-06-2013	-23.774	-70.477	MK016399	MK097378
			Antofagasta,	Tobar, G. Zapata-Hernández					
Chiton cumingsii	CCCO7	MNHNCL 401392	Coloso Sur,	C.M. Ibáñez - M.C. Pardo - C.	01-06-2013	-23.774	-70.477	MK016400	-

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	16S
			Antofagasta, Chile	Tobar, G. Zapata-Hernández					
Chiton granosus	CGRS1	MNHNCL 401393	Río Seco, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	26-01-2014	-21.001	-70.165	MK016401	-
Chiton granosus	CGRS4	-	Río Seco, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	26-01-2014	-21.001	-70.165	MK016402	MK097382
Chiton granosus	CGRS5	-	Río Seco, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	26-01-2014	-21.001	-70.165	MK016403	-
Chiton granosus	CGRS2	-	Río Seco, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	26-01-2014	-21.001	-70.165	MK016404	-
Chiton magnificus	CMSC14	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	MK016405	-
Chiton magnificus	CMCV6	SCBUCN-3714	Cascabeles, Los Vilos, Chile	C.M. Ibáñez - M.C. Pardo - A.H. Navarrete	01-09-2013	-31.979	-71.499	MK016406	-
Chiton magnificus	CMV12	SCBUCN-4597	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016407	-
Chiton magnificus	CMV20	SCBUCN-4598	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016408	MK097383
Chiton magnificus	CMV11	SCBUCN-4596	Valdivia, Chile	R.D. Sepúlveda	15-03-2014	-39.796	-73.401	MK016409	-
Chiton magnificus	CMSC19	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	MK016410	-
Chiton stokesii	CSSR1	MNHNCL 401377	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016411	MK097388
Chiton stokesii	CSSR3	MNHNCL 401378	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016412	-
Chiton stokesii	CSSR11	MNHNCL 401376	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016413	-
Enoplochiton niger	ENTI21	-	Tres Islas, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	25-01-2014	-20.309	-70.136	MK016414	-
Enoplochiton niger	ENTI22	-	Tres Islas, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	25-01-2014	-20.309	-70.136	MK016415	MK097390

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	16S
Enoplochiton niger	ENTI37	-	Tres Islas, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	25-01-2014	-20.309	-70.136	MK016416	-
Ischnochiton dispar	IDSR39	MNHNCL 401384	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016417	-
Ischnochiton dispar	IDSR41	SCBUCN-4312	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016418	-
Ischnochiton pusio	IPPGQ1	SCBUCN-5363	Playa Grande, Quintay, Chile	C.M. Ibáñez - M.C. Pardo - V. Sanhueza, A.H. Navarrete	04-07-2015	-33.183	-71.686	MK016419	-
Ischnochiton pusio	IPPGQ2	SCBUCN-5364	Playa Grande, Quintay, Chile	C.M. Ibáñez - M.C. Pardo - V. Sanhueza, A.H. Navarrete	04-07-2015	-33.183	-71.686	MK016420	-
Ischnochiton punctulatissimus	IPTCO1	-	Coloso Sur, Antofagasta, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar, G. Zapata-Hernández	01-06-2013	-23.774	-70.477	MK016421	-
Ischnochiton punctulatissimus	IPTGI4	-	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	MK016422	MK097392
Ischnochiton punctulatissimus	IPGI2	SCBUCN-4274	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	MK016423	-
Ischnochiton punctulatissimus	IPGI3	SCBUCN-4275	Golf, Iquique, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	27-01-2014	-20.333	-70.149	MK016424	-
Ischnochiton stramineus	ISPA7	SCBUCN-4324	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016425	-
Ischnochiton stramineus	ISPA3	SCBUCN-4614	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016426	-
Ischnochiton stramineus	ISPA17	-	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	MK016427	-
Ischnochiton stramineus	ISSC1	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	-	MK097394
Ischnochiton stramineus	ISSC2	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	MK016428	-
Ischnochiton	ISSC3	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J.	17-08-2013	-30.519	-71.694	MK016429	-

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	16S
stramineus				Sellanes, G. Zapata- Hernández					
Plaxiphora aurata	PAPH1	SCBUCN-4731	Punta Huinay, Chile	C.M. Ibáñez - M.C. Pardo	01-02-2013	-42.375	-72.428	MK016430	MK097395
Plaxiphora aurata	PAPH2	SCBUCN-4732	Punta Huinay, Chile	C.M. Ibáñez - M.C. Pardo	01-02-2013	-42.375	-72.428	MK016431	-
Plaxiphora aurata	PAPH4	SCBUCN-4734	Punta Huinay, Chile	C.M. Ibáñez - M.C. Pardo	01-02-2013	-42.375	-72.428	MK016432	-
Chiton barnesii	SLRS34	-	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	-	MK097398
Chiton barnesii	RBSC9	MNHNCL 401383	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	MK016433	-
Chiton barnesii	RBSC22	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	MK016434	-
Chiton barnesii	RBSC26	-	El Sauce, Chile	C.M. Ibáñez - M.C. Pardo - J. Sellanes, G. Zapata- Hernández	17-08-2013	-30.519	-71.694	MK016435	-
Tonicia atrata	TAEH14	-	Estación Huinay, Chile	C.M. Ibáñez - M.C. Pardo	02-03-2013	-42.375	-72.428	MK016436	-
Tonicia atrata	TAEH15	-	Estación Huinay, Chile	C.M. Ibáñez - M.C. Pardo	02-03-2013	-42.375	-72.428	MK016437	-
Tonicia atrata	TAEH17	-	Estación Huinay, Chile	C.M. Ibáñez - M.C. Pardo	02-03-2013	-42.375	-72.428	MK016438	-
Tonicia calbucensis	TCSL4	-	Isla San Lorenzo, Peru	C.M. Ibáñez - M.C. Pardo	22-10-2013	- 12.090	- 77.224	-	MK097403
Tonicia calbucensis	TCAHU30	SCBUCN-4663	X-Huinay, Chile	J. Sellanes, G. Zapata- Hernández	03-03-2013	-42.391	-72.457	MK016439	-
Tonicia calbucensis	TCLFSA2	MNHNCL 401385	Fuerte San Antonio, Ancud, Chile	C.M. Ibáñez - M.C. Pardo	07-02-2015	-41.860	-73.831	MK016440	-
Tonicia disjuncta	TSHU1	-	X-Huinay, Chile	C.M. Ibáñez - M.C. Pardo	03-03-2013	-42.391	-72.457	-	MK097423
Tonicia disjuncta	TDP01	-	Puerto Oscuro, Los Vilos, Chile	C.M. Ibáñez - M.C. Pardo	31-08-2013	- 31.423	- 71.593	-	MK097406
Tonicia elegans	TECPT24	-	Punta de Tralca, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	26-05-2014	-33.426	-71.704	MK016441	MK097408
Tonicia elegans	TEGPT22	CBUCN 4357	Punta de Tralca,	C.M. Ibáñez - M.C. Pardo - C.	26-05-2014	-33.426	-71.704	MK016442	-

Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	16S
			Chile	Tobar - A.H. Navarrete					
Tonicia elegans	TEGPT7	-	Punta de Tralca, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A H. Navarrete	26-05-2014	-33.426	-71.704	-	MK097409
Tonicia elegans	TEGPT8	-	Punta de Tralca, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	26-05-2014	-33.426	-71.704	MK016443	MK097410
Tonicia fremblyana	PSCA1	-	Callao, Peru	C.M. Ibáñez - M.C. Pardo	10-10-2013	- 12.067	- 77.159	-	MK097396
Tonicia swainsoni	TCSL4	MNHNCL 401387	Isla San Lorenzo, Peru	C.M. Ibáñez - M.C. Pardo	22-10-2013	-12.090	-77.224	MK016444	MK097403
Tonicia swainsoni	TCSL6	-	Isla San Lorenzo, Peru	C.M. Ibáñez - M.C. Pardo	22-10-2013	-12.090	-77.224	MK016445	MK097404
Tonicia swainsoni	TCSL7	-	Isla San Lorenzo, Peru	C.M. Ibáñez - M.C. Pardo	22-10-2013	-12.090	-77.224	MK016446	MK097405
Tonicia fremblyana	TELMA26	MNHNCL 401386	Balneario Municipal, Antofagasta, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar, G. Zapata-Hernández	01-06-2013	-23.670	-70.408	MK016447	MK097412
Tonicia fremblyana	TELMA24	_	Balneario Municipal, Antofagasta, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar, G. Zapata-Hernández	01-06-2013	-23.670	-70.408	MK016449	MK097411
Tonicia fremblyana	TEC3	-	Corazones, Arica, Chile	C.M. Ibáñez - M.C. Pardo	30-01-2014	-18.528	-70.323	MK016450	MK097407
Tonicia atrata	TAEH15	-	Estación Huinay, Chile	C.M. Ibáñez - M.C. Pardo	02-03-2013	- 42.375	- 72.428	-	MK097400
Tonicia atrata	TAEH17	-	Estación Huinay, Chile	C.M. Ibáñez - M.C. Pardo	02-03-2013	- 42.375	- 72.428	-	MK097401
Tonicia atrata	TCAHU19	-	X-Huinay, Chile	C.M. Ibáñez - M.C. Pardo	03-03-2013	-42.391	-72.457	-	MK097402
Tonicia atrata	TLPA10	-	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	-	MK097417
Tonicia atrata	TLPA11	-	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	-	MK097418
Tonicia atrata	TLPA12	-	Puerto del Hambre, Punta Arenas, Chile	R.D. Sepúlveda	01-12-2013	-53.612	-70.929	-	MK097419
Tonicia chilensis	TLVP9	-	Ventanas, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	25-05-2014	-32.743	-71.497	MK016456	MK097422
Species	Code	Repository	Locality	Collected by	Collection date	Latitude	Longitude	COI	168
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Tonicia chilensis	TLVP13	-	Ventanas, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	25-05-2014	-32.743	-71.497	MK016457	MK097420
Tonicia chilensis	TLVP16	MNHNCL 400563	Ventanas, Chile	C.M. Ibáñez - M.C. Pardo - C. Tobar - A.H. Navarrete	25-05-2014	-32.743	-71.497	MK016458	MK097421
Acanthopleura echinata	AEPP1	-	Paracas, Peru	C.M. Ibáñez	03-07-2011	-13.849	-76.282	MK016459	-
Acanthopleura echinata	AEPP2	-	Paracas, Peru	C.M. Ibáñez	03-07-2011	-13.849	-76.282	MK016460	-
Acanthopleura echinata	AEPP3	-	Paracas, Peru	C.M. Ibáñez	03-07-2011	-13.849	-76.282	MK016461	-
Stenoplax limaciformis	SLRS6	-	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016462	-
Stenoplax limaciformis	SLRS26	-	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016463	-
Stenoplax limaciformis	SLRS27	-	Santa Rosa, Salinas, Ecuador	C.M. Ibáñez - M.C. Pardo	22-09-2013	-2.211	-80.946	MK016464	-
Stenoplax limaciformis	TAEH14	-	Estación Huinay, Chile	C.M. Ibáñez - M.C. Pardo	02-03-2013	- 42.375	- 72.428	-	MK097399
Stenoplax rugulata	ISPBP3	-	Bonanza, Peru	C.M. Ibáñez - M.C. Pardo	31-12-2013	-3.716	-80.745	-	MK097393
Stenoplax rugulata	ISPBP5	-	Bonanza, Peru	C.M. Ibáñez - M.C. Pardo	31-12-2013	-3.716	-80.745	MK016465	-

CAPÍTULO 2: ECOLOGICAL FACTORS AND GEOMETRIC CONSTRAINS: EFFECT ON THE POLYPLACOPHORAN DIVERSITY ALONG THE SOUTH-EAST PACIFIC COAST

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Abstract

We investigated the latitudinal pattern of polyplacophoran species richness in the Southeast Pacific coast (SEP), evaluating the importance of ecological and geometric constrains on diversity. The study area covered more than 5,000 km along the SEP coast, between 0° S and 56° S. We compiled diversity data from sampling collections and museum specimens. The relationship between species richness and environmental variables was analysed using simultaneous autoregressive (SAR) models. Additionally, the mid-domaind effect (MDE) null model was tested to explain the empirical pattern of polyplaciphoran richness. The richness of species and genera showed a bell-shaped distribution at intermediate latitudes. The only significant relationship was between species richness and oxygen, however, when data was corrected by spatial regression, none of the environmental variables were associated to diversity. The mid-domain effect (MDE) was a explanatory variable in intermediate latitude, but not at the extremes of the distribution range were empirical richness was above that expected by the model

Keywords: Diversity, biogeography, latitudinal gradient, mid-domain effect, environmental variables.

Introduction

The occurrence of latitudinal gradients of species richness from tropical to polar zones is the most exciting and transcendental pattern in biogeography and macroecology (Roy *et al.*, 1998; Mittelbach *et al.*, 2007; Lomolino *et al.*, 2010). The most widely documented pattern, known as the Latitudinal Gradient in Species Richness (LGR), describes a gradual decrease in the

number of species from the tropics to the poles (Rosenzweig, 1995; Roy *et al.*, 1998; 2000; 2004; Macpherson, 2002; Willig *et al.*, 2003; Mittelbach *et al.*, 2007). However, patterns of species richness seem to be highly variable among taxa from marine systems. For instance, many biogeographical studies of the South-eastern Pacific (SEP) described patterns of increasing species diversity toward higher latitudes (i.e., inverse LGR) in several marine groups including seaweeds, polychaetes, amphipods, isopods, echinoderms and molluscs (polyplacophorans and bivalves) (Santelices & Marquet, 1998; Lancellotti & Vásquez, 2000; Valdovinos *et al.*, 2003; Hernández *et al.*, 2005; Rivadeneira *et al.*, 2011). Conversely, a poleward decrease in the number of species is also a commonly reported pattern in some taxa such as: cartilaginous and teleost fishes, decapod crustaceans, "prosobranch" gastropods, nudibranchs, cephalopods, and nematodes (Lancellotti & Vásquez, 2000; Ojeda *et al.*, 2000; Astorga *et al.*, 2003; Valdovinos *et al.*, 2003; Ibáñez *et al.*, 2003; Ibáñez *et al.*, 2009; Lee & Riveros, 2012; Navarrete *et al.*, 2014, Ibáñez *et al.*, 2019).

Given that molluscs are fundamental parts of marine systems as they perform key ecological roles and are economically important, these organisms are frequently aimed in biogeographical studies (Perez-Matus *et al.*, 2017; Wanninger & Wollesen, 2018). The majority of these researches detected that species richness increased according to latitude within the SEP (Valdovinos *et al.*, 2003; Fernandez *et al.*, 2009; Pappalardo & Fernández, 2014). Several hypotheses have been formulated to explain this pattern of latitudinal richness in SEP molluscs and other marine invertebrates, and among the determinant factors are environmental variability (e.g. continental shelf area, sea surface temperature, primary productivity), higher speciation rates at southern ecoregions, and restricted geographic range in southern species (Astorga *et al.*, 2003; Valdovinos *et al.*, 2003; Hernández *et al.*, 2005;

Fernández *et al.*, 2009; Rivadeneira *et al.*, 2011; Lee & Riveros, 2012). Alternatively, paleontological evidence suggests that lower richness at the northern SEP has resulted from higher extinction rates during the Neogene (Kiel & Nielsen, 2010). Among molluscs, several biogeographic studies focused on polyplacophorans (e.g. Viviani, 1979; Brattström & Johanssen, 1983; Lancellotti & Vásquez, 2000), and suggest that diversity of genera increases southwards SEP (Valdovinos *et al.*, 2003; Fernandez *et al.*, 2009; Pappalardo & Fernández, 2014), which has been related to the latitudinal increment of continental shelf area. However, a recent study by Navarrete *et al.* (2020), based on exhaustive sampling, revision of museum specimens and molecular analyses to determine the identity of problematic species, evidenced that the maximum species richness is reached at intermediate latitudes, which is a shared pattern among other marine animal groups such as polychaetes (Hernández *et al.*, 2005), nematodes (Lee & Riveros, 2012) and intertidal fishes (Navarrete *et al.*, 2014). Therefore, causal links and hypotheses that have been proposed to explain latitudinal gradients in polyplacophorans along SEP need to be reviewed.

Considering the high variability of biogeographical patterns of marine invertebrate taxa along SEP, in this study we assessed the importance of: (1) environmental variables (temperature, salinity, chlorophyll-*a*, and oxygen) (2) and the potential influence of geographic restrictions and distributional range species through the mid-domain effect (MDE) in the observed polyplacophoran richness pattern along SEP.

Materials and Methods

Richness estimates and relationship with environmental variables

To evaluate the relation between polyplacophoran species richness and environmental variables along the SEP coast, we used the dataset of Navarrete et al. (2020) that included species identifications with genetic data. Navarrete et al. (2020) carried out an exhaustive sampling between 2011 and 2016 along the SEP coast (Fig. 1, Fig. 2). For each sampling site, individual chitons were collected from intertidal (highest tidal level) and shallow subtidal (10 m depth) zones by hand during low tide via snorkelling and SCUBA diving. All of the specimens collected were identified to species-level following Leloup (1956), Bullock (1988a, 1988b), Kaas et al. (2006), Schwabe et al. (2006), Sirenko (2006), and Schwabe (2009). Some voucher specimens were registered and stored at Museo Nacional de Historia Natural de Chile (MNHNCL) and in Sala de Colecciones Biológicas Universidad Católica del Norte (SCBUCN) Coquimbo, Chile. We reviewed additional biological material deposited in Museo Nacional de Historia Natural de Chile, Santiago, Chile (MNHNCL); Collection of Flora and Fauna, Professor Patricio Sánchez from Pontificia Universidad Católica de Chile, Santiago, Chile (SSUC); Instituto de la Patagonia from Universidad de Magallanes, Punta Arenas, Chile (UMIP); Santa Barbara Museum of Natural History, Santa Barbara, USA (SBMNH); and the Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia (ZISP) (Fig. 1).

To determine the potential causal factors of the latitudinal patterns in polyplacophoran species richness along the SEP coast, we explored the association between chiton richness and the following environmental variables: temperature, chlorophyll-*a*, dissolved oxygen, and salinity. These environmental variables were selected given that temperature and chlorophyll-

a represent proxies of productivity and can be used as alternative measurements of energy within ecosystems. Therefore, these two variables can be used to test the species-energy hypothesis (Evans *et al.*, 2005). Additionally, sea surface salinity and sea surface dissolved oxygen were used to evaluate niche variability or the level of tolerance of the species. The relationship between species richness and environmental variables was explored through Simultaneous Autoregressive model (SAR). The SAR analyses were performed spatially using the Spatial Analysis in Macroecology (SAM) (Rangel *et al.*, 2010). All of the analyses were developed following the protocols detailed by Dormann *et al.* (2007). Chlorophyll-*a* (mg/m³, monthly averages 2002-2009), sea-surface temperature (°C, monthly averages 2002-2009), salinity (UPS, DIVA interpolation 2009) and dissolved oxygen (ml/l, DIVA interpolation 2009) were obtained from the Bio-Oracle data base (Tyberghein *et al.*, 2012; www.oracle.ugent.be/) through DIVA-GIS software (Hijmans *et al.*, 2001).

Richness pattern and geometric constrains

The potential influence of geometric constraints on the latitudinal pattern of species richness, excluding any environmental and evolutionary influence, was tested through the evaluation of the mid-domain effect (MDE) null model hypothesis (Colwell *et al.*, 2004). The Range-Model software version 5 (Colwell, 2006) was used for the simulation. The simulation process was repeated 25,000 times, and we used expected mean richness and its 95% confidence intervals to assess the effects of geometric constraints on the latitudinal gradient of species richness of SEP polyplacophorans. Pearson moment correlation was used to evaluate the relationship between empirical richness, and the richness predicted by the null model.

Results

Latitudinal diversity and environmental variables

Species richness presented a tendency to slightly increase southwards and exhibited a bell shape pattern (quadratic fit: $R^2 = 0.49$; F=25.74; p < 0.00001), the maximum value was reached at 33° S. Eight species were found at ~0° S, 12 species were found at ~ 55° S , and the maximum number of species was 14 at intermediate latitude (i.e., 33° S). Two significant declines in species richness were recoded along the latitudinal gradient, the first was observed between ~ 5° S and ~ 11° S (with 2-4 species), whereas the second decline was observed between ~ 45° S and 51° S (with 6-7 species) (Fig. 2). The number of genera showed a similar pattern of richness in regard to latitude (quadratic fit $R^2 = 0.46$; F=23.04; p < 0.00001). Genera presented a slight increase southwards, with the maximum value at intermediate latitude, between 20°S and 33°S, and at the southernmost localities between 53°S and 55°S (Fig. 2). Similar to the latitudinal pattern of species richness observed, two marked declines in the number of genera were detected according to the latitudinal gradient. The first was recorded between 5°S and 10°S with only two genera, and the second between 47°S and 51°S with four genera (Fig. 2). Although the number of species and the number of genera showed a similar pattern in the latitudinal gradient, the species/gene ratio was not significant in the gradient (llinear fit $R^2 = 0.051$; F = 2.89; p = 0.094) (Fig. 2).

The Ordinary least square (OLS) model did not show a significant relationship between richness and temperature (Fig. 3A), chlorophyll-*a* (Fig. 3B) and salinity (Fig. 3D). In contrast, dissolved oxygen was positively and significantly correlated with species richness (Fig. 3C)

(Table 1). However, SAR indicated that the environmental variables were not related to species richness (Table 1).

Richness pattern and mid-domain effect (MDE)

The MDE null model showed a deviation of the empirical species richness from simulated richness. The analysis revealed that more than 17% of the points fell outside the 95% confidence interval, where the points from 0° to 4° and those from 51° to 55° of latitude were above ahat was expected by the MDE null model (Fig. 4). The relationship between empirical and predicted polyplacophorans richness was correlated significantly (Pearson r = 0.55; t = 4.86; p = 0.00001) (Fig. 4).

Discussion

Biogeographic patterns

The highest number of chiton species and genera was found in the transition zone between 20° S and 42° S, this high diversity is probably due to an overlap of species from adjacent tropical and Sub-Antarctic regions (see Camus, 2001). Moreover, bell-shaped distributions along the SEP coast were described for several animal taxa such as polychaetes (Hernández *et al.*, 2005), intertidal fishes (Navarrete *et al.*, 2014), and nematodes (Lee & Riveros, 2012). Overall, it was suggested that this pattern resulted from repeated isolation events followed by invasions between the Peruvian and Magellan provinces during the Pleistocene glacial-interglacial cycles (Kiel & Nielsen, 2010). This bell-shaped richness pattern could be associated with the "Centre of Overlap Hypothesis" (Woodland, 1983; Gaither & Rocha, 2013), which suggests that the high species diversity within a region is due in part to the

overlap of distinct fauna from adjacent regions. The MDE hypothesis predicts well the observed richness between 5 and 50 degrees latitude. However, both extremes showed observed values of richness that exceed those predicted by the model. Benthic polychaete, like the polyplacophores, show an observed richness above that expected by the MDE null model from ~52 to 56 °S, but also show an intermediate peak at about 41°S (Hernández *et al.*, 2005). These results suggest that the southern tip of South America may not be a geographic barrier to influence the richness pattern of these species in the SEP.

Alternatively, Moreno *et al.* (2006) indicated that the latitudinal endemism along the SEP plus the high species richness at mid latitudes could generate non-linear species distributions delimited by geography and environmental conditions intrinsic of the Peruvian and Magellan provinces. In addition, it is relevant to note that we were able to obtain these results regardless of the relatively few (< 10) chiton species recognized for Sub-Antarctic waters (Schwabe, 2009), and the lower number of chiton species that occur in shallow water compared with other marine molluscan taxa.

Relationship with environmental variables

Our results indicate that the environmental variables evaluated could not explain patterns of polyplacophoran species richness along the SEP coast. Although dissolved oxygen showed a relationship with chiton richness (i.e. OLS) prior to spatial structure is included in the model. Thus, this result partially supports hypotheses related to physiological tolerance to abiotic conditions (climatic variability) and does not provide evidence to endorse the hypothesis related to energy-productivity (Wright, 1983; Lomolino *et al.*, 2010). This lack of relationship between richness and environmental variables (sea surface temperature, sea surface salinity,

and Chlorophyl-a) could be related to the bell-shaped richness pattern and the absence of productivity gradient along the Humboldt Current System, which derives from the high heterogeneity of upwelling regimens that combine zones of seasonal and permanent upwelling (Thiel et al., 2007). However, as mentioned earlier, the slope in OLS analysis was significant for dissolved oxygen, which indicates that polyplacophorans are adapted to oxygen-saturated environments. Polyplacophorans present relatively high metabolic rates for ectotherms that require high oxygen levels to compensate their physiological responses (Carey *et al.*, 2013). Therefore, it is highly likely that chiton species prefer well-oxygenated environments, although some species (mainly intertidal) present physiological traits specialized to cope with variations in dissolved oxygen (Murdoch & Shumway, 1980). The ecological traits such as long lifespan, relatively slow growth rates, short pelagic development and marked seasonal reproduction (sensu Eernisse, 2007) found in chitons, suggest that these species are adapted to cold currents along the SEP coast. In this sense, the Plio-Pleistocene extinction event and recurrent glaciations in southern Chile during the Neogene could have influenced the speciation of this group (Kiel & Nielsen, 2010), which would confirm that polyplacophoran diversity patterns are determined by the complex interaction between historical and ecophysiological factors.

The diversity of invertebrates along the SEP is influenced by a complex mixture of environmental factors, and chiton species are no exception to this statement. Our results suggest the biogeography of chiton is determined by the intra-specific physiological tolerance level and by MDE effect, both of which combined contribute to constrain and drive the modern richness pattern observed in polyplacophorans from the SEP. The Mallegan ancestral chitons and their subsequent speciation, dispersion and changes in the distributional range of these organisms throughout the SEP are reinforced by repeated events of successive glaciations. This alternate cooling and warming period forced the advance and retreat of the flora and fauna in marine and terrestrial habitats of the southern end of the South American cone from the Neogene, and perhaps favoured the overlapping of the distribution ranges at intermediate latitudes, modulating the bell-shaped wealth pattern observed today.

Our results partialy support the hypothesis of tolerance to abiotic variables and were the MDE explain the richness pattern at intermediate latitude, suggesting that the biogeographical patterns of shallow-coast polyplacophorans along the SEP are built by historical and ecological factors.

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Tables

Table 1. Summary of OLS (Ordinary Least Square Regression) and SAR (Spatial imultaneous Autoregressive Model) regression analyses for the relationships between polyplacophoran species richness and the environmental variables along SEP coast.

		OLS		SAR			
Species richness	b	R^2	p-value	b	R^2	p-value	
Chlorophyll- <i>a</i> (mg/m ³)	0.066	0.004	0.63	-0.032	0.01	0.682	
Dissolved Oxygen (ml/l)	1.803	0.176	0.001	1.956	0.166	0.42	
Salinity (UPS)	-0.131	0.004	0.604	0.304	0.008	0.378	



Figure 1. Latitudinal distribution ranges of polyplacophoran species along the South-eastern Pacific coast. Filled dots represent sampled localities, and open dots represent records from biological collections.



Figure 2. Diversity patterns of polyplacophorans along the South-eastern Pacific coast. Filled dots represent species richness at each latitudinal bin and solid black line its respective quadratic fit. Open dots represent genera richness at each latitudinal bin and solid grey line its respective quadratic fit. Gray dots represent species/genera (Sp/G) ratio at each latitudinal bin, and dashed line its respective liear fit.



Figure 3. Latitudinal gradients in species richness of polyplacophorans and environmental variables along the South-eastern Pacific coast. A) \log_{10} relationship between richness and temperature, B) \log_{10} relationship between richness and chlorophyll-*a*, C) \log_{10} relationship between richness and oxygen, D) \log_{10} relationship between richness and salinity.



Figure 4. Empirical (black dots) and predicted (gray line) richness of polyplacophorans species along the South-eastern Pacific coast (domain). Dashed lines represent the 95% confidence intervals for the predicted mid-domain effect (MDE).



Figure 5. Relationship (Pearson correlation) between Empirical and Predicted species richness of polyplacophorans along the South-eastern Pacific coast.

CAPÍTULO 3: MORPHOLOGICAL DIVERSITY OF POLYPLACOPHORANS AND THEIR RELATIONSHIP WITH ENVIRONMENTAL VARIABLES, AND PHYLOGENETIC DIVERSITY ALONG THE SOUTH EAST PACIFIC COAST.

MS in preparation

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Abstract

Morphological diversity, or morphodisparity, is generally measured as the variance of a set of morphometric measures obtained from a group of organisms, and has become a useful tool for ecologists and evolutionary biologists to structure biotic patterns. In fact, recent studies propose the existence of a correlation between morphology and species diversity due to diversification processes. In this research, the effect of environmental variables and phylogenetic diversity occurring along the latitudinal gradient was evaluated on the morphological diversity pattern of polyplacophorans from the Southeast Pacific (SEP) coast. For this purpose, 2548 specimens were used, belonging to 7 families and 27 species of chitons, collected from Ecuador (~ 2°S) to Puerto Williams (~ 55°S) between 2011 and 2017. On each specimen, 15 morphometric measurements were taken and then standardized by body size, thus avoiding possible allometric effects associated with ontogeny. Principal Component Analysis (PCA) was used to obtain the morphospace and its variance across the latitude gradient, using it as a measure of morphological disparity (PC1 variance). Additionally, an index of phylogenetic diversity (PDp) was calculated from a Bayesian phylogenetic tree constructed by concatenated genes 16S+COI+18S, and its relationship with the morphological diversity was evaluated. The results show that polyplacophoran families have a differentiated morphospace, being Chitonidae and Mopaliidae those with a greater morphological variability, compared to Acanthochitonidae, Ischnochitonidae, Chaetopleuridae and Callochitonidae, which have a reduced disparity. Morphological diversity is associated with latitude, showing greater morphometric variability near the equator. However, when the effect of latitude is extracted, morphodisparity shows an inverse relationship with both oxygen range, and pH. Morphodisparity was not related to richness, but it was positively related to phylogenetic diversity, suggesting that the morphological diversity pattern matches the underlying diversification processes. This association between morphodisparity and phylogenetic diversity points out the importance of the diversification processes that result in the morphological differentiation and it is reflected in the phylogenetical relationships of the component species in the localities studied along the SEP coast.

Keywords: Morphodisparity, Phylogenetic diversity, polyplacophoran, Southeast Pacific.

Introduction

Species diversity patterns have been broadly associated with both environmental variation and evolutionary history of the lineages (Mittelbach *et al.*, 2007; Lomolino *et al.*, 2010). However, linking these correlative approaches directly with the ecological, evolutionary, and biogeographical processes that generate and maintain biodiversity has been difficult (Kerkhoff *et al.*, 2014). Due to these difficulties to describe the relative importance of the processes that shape the current species richness patterns, since two decades several authors have pointed out the need to change the traditional focus of its study (e.g. Harrison *et al.*, 1992; Blackburn & Gaston, 1996; Koleff & Gaston, 2002; Rodríguez & Arita, 2004; Soininen *et al.*, 2007), highlighting the importance of studying complementary measures of diversity, in order to try to infer about processes behind this emerging feature of diversity across the broad spatial scales. When the species richness is evaluated, it is important to keep in mind that species are different from one another not only in a categorical way, the magnitude of these differences varies tremendously depending on the set of species in question, and in the particular traits analysed (Scherson & Fait, 2018), therefore changes in species richness of a particular group

as a function of latitude could be accompanied by other gradients of biodiversity measures that reflect its morphological variation as well as its evolutionary relationships (Benitez *et al.*, 2014; Lemic *et al.*, 2016).

The morphological diversity of a set of species corresponds to "morphodisparity", which can be understood as a representation of a biotic epiphenomenon reflected as a variation in their body plans, represented in a multidimensional space such as the morphospace occupied by each group (Gould, 1991; Foote, 1992; Pavlinov, 2011). Another measure of diversity corresponds to the measure of evolutionary relationships between a species group (i.e. clade), the phylogenetic diversity (Faith, 1992). Phylogenetic diversity allows, for example, to distinguish between clades that in terms of taxonomic richness could be equivalent (e.g. equal richness), but which could differ in terms of their particular evolutionary history (i.e. sum of the branch lengths of each clade taxa), helping to generate hypotheses about the mechanisms behind the diversity of each clade in particular. Since both morphological and phylogenetical diversity arise from biological processes, they would be related because the phylogenetic distance between two species estimates the amount of time to the most recent common ancestor of both species, in other words, the time that each has evolved independently of the other. Thus, phylogenetic distance represents a proxy for the magnitude of phenotypic differences expected between any two species (Cavender-Bares et al., 2009).

When analysing the diversification process that generates a net increase in species, an increase in the sum of their phylogenetic relationships should also be observed (i.e. new branches in the clade), as well as in their morphological variants. Recent studies have shown a relationship between disparity and diversity through time, which goes in line with the

processes of adaptive diversification (Foote, 1997; Neige, 2003; Dera *et al.*, 2016), where diversification processes increase not only the number of species, but morphological, phylogenetical, and other features of biological diversity too, helping to accelerate the evolution of the species. Therefore, it is expected that during the diversification processes, not only species number increases, but also the morphological diversity, which helps to speed up the phenotypic evolution of the lineages. On the other hand, when diversification is smaller or null, the species richness decreases, and the disparity could do the same in a gradual and similar way through the loss of morphs.

Species diversity generally distributes forming patterns over broad spatial scales, and the most recurrent is an increase towards the equator in the latitudinal gradient (Rosenzweig, 1995; Roy *et al.*, 1998; 2000; 2004; Ojeda *et al.*, 2000; Macpherson, 2002; Willig *et al.*, 2003, Mittelbach *et al.*, 2007; Ibáñez *et al.*, 2009; Lomolino *et al.*, 2010; Rivadeneira *et al.*, 2011; Ibáñez *et al.*, 2019a). Therefore, in a group of species that shows a particular latitudinal pattern of diversity, it is expected that both morphological disparity (Collins *et al.*, 2019) and phylogenetic diversity would be found to vary concomitantly along the gradient.

The focus group in this study is the chitons, a cosmopolitan and exclusively marine molluscan group belonging to the class Polyplacophora, whose morphology is relatively constrained among extant taxa (Sirenko, 2006; Sigwart, 2009). Chitons usually attach to hard substrata with their muscular foot, which is protected by their characteristic articulating eight-part shell armour. The first (anterior: head) and the last (posterior: tail) plates are approximately semi-circular, their breadths are usually smaller than the intermediate plates, which influence the general oval shape of the body (Schwabe, 2010). The six intermediate plates are similar in shape, though shell plate II (immediately behind the head) is anterio-

posteriorly elongated compared to the others, and in many species of different genera (e.g. *Lepidochitona, Tonicella, Chiton, Enoplochiton, Tonicia*), there is a clear difference in widths among plates in a single animal (Baxter, 1982; Baxter & Jones, 1986; Connors *et al.*, 2012; Ibáñez *et al.*, 2018). These plates provide protection while still allowing flexibility during locomotion over uneven and rough surfaces, as well as when rolling defensively into a ball-like conformation when dislodged from a substrate (Connors *et al.*, 2012; Sigwart *et al.*, 2015; Ibáñez *et al.*, 2018). In chitons, armature is a complex multi-element combination of hard and soft structures, with shell plates surrounded by a flexible girdle (i.e. perinotum), therefore these structures have the potential to present inter-specific variability in their growth (Baxter & Jones, 1986; Avila-Poveda & Abadia-Chanona, 2013).

Former studies on polyplacophorans biogeography in the southeast Pacific (SEP) have reported an inverse richness pattern with increasing species diversity southwards, which has been related to increasing continental shelf area (Valdovinos *et al.*, 2003; Fernández *et al.*, 2009; Pappalardo & Fernández, 2014). However, a recent study by Navarrete *et al.* (2020), where they performed molecular analyses to accurately determine doubtful species identity, showed that the inverse richness pattern previously proposed for polyplacophorans is invalid, finding maximum of species richness at intermediate latitudes, a pattern shared with other marine animal groups such as polychaetes (Hernández *et al.*, 2005), nematodes (Lee & Riveros, 2012) and intertidal fish (Navarrete *et al.*, 2014) in SEP coast.

Based on the richness pattern recorded by Navarrete *et al.* (2020), the morphologic and phylogenetic diversity was used in order to shed light over the subjacent processes that conform the richness pattern of polyplacophorans along de SEP coast. In this context, the morphological and phylogenetic diversity concepts add new dimensions toward the

understanding of diversity, through the estimation of both the potential adaptive variation in shape (McClain, 2004; Pavlinov, 2011) and the amount of phylogenetical relatedness (Scherson & Fait, 2018) of the group. Bell-shaped richness in SEP polyplacophorans (Navarrete *et al.*, 2020) could be reflected in its morphological disparity and its phylogenetic diversity, therefore the hypothesis subject to testing is that the morphodisparity and phylogenetic diversity of the SEP polyplacophorans are related, also evaluating the importance of environmental variability to predict the morphodisparity pattern recorded for this group along the SEP coast.

Materials and methods

Study areas and sample collection

Chiton specimens were collected on intertidal rocky shores and subtidal shallow waters until five meters depth at 23 locations along the Southeast Pacific (SEP) coast, encompassing from 1.8°S to 56°S latitude over more than 4,500 km of coastline (Fig. 1). A total of 2,548 specimens belonging to 7 families, 12 genera and 27 species of intertidal chitons were obtained through the original fieldwork between years 2011 and 2017 (Fig. 1). Conspecific individuals from all localities were combined at different taxonomic levels for morphometric analyses, aiming at including all shape and size variation along the latitudinal gradient. All specimens measured in this study were deposited at the Museo Nacional de Historia Natural, Santiago, Chile (MNHNCL) and Biological Collection of Universidad Católica del Norte, Coquimbo, Chile (SCUCN).

Morphometric analysis

To analyse the morphological variation of each chiton species, the following 15 distance variables were measured through a digital calliper (precision: ± 1 mm) on ethanol preserved specimens: total length (TL), total width (TW), length of plate I (L-I) and plate VIII (L-VIII) (anterior and terminal shell plates, respectively), widths of each shell plate (I to VIII) and lengths of anterior (APL), lateral (LPL) and posterior perinotum (PPL) (Fig.2). To avoid morphometric bias or skew, only flattened specimens were used and no measurements from curled specimens were taken.

First, differences were explored in total length (TL) of pooled chiton among ecoregions in the latitudinal gradient using PERMANOVA analyses. Afterwards, a standardization of each length and width obtained for individual chiton was performed, dividing each one by total length (TL) to avoid allometric effects across species (Ibáñez *et al.*, 2018). Standardized measurements were used in multivariate analyses to explore potential shifts in shape-space over the latitudinal gradient within each family, using Principal Component Analysis (PCA). In a dataset comprising multiple data sets (i.e. families, or localities), the first component (PC1) reflects variation in shape trajectories (Shea, 1985). The standardized data for 14 measures were subjected to a PCA specifying a variance-covariance matrix. Therefore, the variance of PC1 scores for each locality was estimated as a proxy of morphodisparity. Morphospace was estimated as the area of the convex hull, which is the smallest convex polygon enclosing the points of PC1 and PC2 for each chiton family. To compare morphology between genera, families and ecoregions, PERMANOVA tests were performed in PAST ver. 3.25 using Euclidean distances (Hammer *et al.*, 2001). The association of morphodisparity with latitude and environmental variables as temperature, salinity, pH, chlorophyll-*a*, oxygen was evaluated. Environmental data was extracted from Bioracle v.20 using DIVAGIS 7.5 software (Hijmans, 2012). A Generalized Linear Model (GLM) was applied to explore significant association between morphodisparity and environmental gradients, and between morphodisparity and phylogenetic diversity using R v3.5.3 (R development core team, 2019). Significance of GLM coefficients were evaluated by Analysis of Variance (ANOVA) in R.

Phylogenetic tree and phylogenetic diversity index

To obtain the hypothetical phylogenetic tree of the SEP polyplacophorans, the sequence alignments were performed with ClustalW (implemented in MEGA 6.0) (Tamura *et al.*, 2013) for the multiple alignment of DNA sequences. A matrix with three concatenated genes (16S+COI+18S) was used and the best substitution model (e.g. GTR+G+I) was selected on jModelTest v. 0.1.1 (Posada, 2008). The phylogenetic reconstructions were performed in MrBayes ver. 3.2 (Ronquist *et al.*, 2012). This software was used to build the phylogenetic relationships through Bayesian inference, using Markov Chain Monte Carlo (MCMC). Four "warm chains" were implemented, sampling every 1000 generations, for 10 million generations. *Nierstraszella lineata* was used as outgroups, since Lepidopleurida order has been previously described as a sister group of the Chitonida order (Okusu *et al.*, 2003; Sigwart *et al.*, 2013). On the phylogenetic tree obtained, the software Biodiverse v.1 (Laffan *et al.*, 2010) was used to calculate phylogenetic diversity index as the proportion of the total tree length (PDp) at each locality, which was used as a predictor for diversification of polyplacoporans along the SEP coast.

Results

The total length (TL) of pooled chiton was significantly variable among ecoregions (PERMANOVA: F = 21.5, P= 0.0001). The mean TL of chitons is smaller in Guayaquil ecoregion, and tends to increase steadily southward through Central Peru, Humboldtian, reaching a maximum in the Central Chile ecoregion (Fig.3). The mean LT of chitons decreases in the Araucanian, Chiloense, and Channels and Fjords, reaching similar values to those recorded in Central Peru and Humboldtian ecoregions (Fig. 3). Largest chitons were found in the Humboldtian, Central Chile and Araucanian ecoregion (mean > 44 mm), while the smallest chitons were found in the Guayaquil ecoregion (mean ~25 mm) (Table S1). The TL range size of pooled chitons varied between 3.6 and 193 mm, being the larger species (>30 mm) *Achantopleura echinata* (mean 86.9 mm) and *Enoplochiton niger* (mean 81.8 mm), while the smallest (\leq 15 mm) are *Calloplax vivipara* (mean 9.5 mm), and *Ischnochiton stramineus* (mean 8.5 mm) (Table S1).

In PCA analyses of standardized data, the first component (PC1) in species-specific analyses accounted for more than 78.1% of variation and the second component (PC2) reached 11.6% of the total variation. The signs of PC1 loadings were distributed specimens according to plate's width (I-VIII). In all species, as in all typical chitons, the anterior and terminal shell plates were the narrowest, and the central shell plates (IV, V and VI) were wider, though the widths of various features generally differed among species. The first component of PCA (PC1) reflects changes in shape according to total width and perinotum lengths (APL, LPL, PPL), where perinotum gets narrower when the plates are wider (Table 1). Morphospace size was variable among families of polyplacophorans, being Chitonidae the biggest (1.32), followed by Mopaliidae (0.63), Chaetopleuridae (0.32), and Ischnochitonidae (0.30). The remaining families (i.e. Callistoplacidae, Callochitonidae and Acanthochitonidae) showed small morphospaces (0.14, 0.05, and 0.03, respectively) (Fig. 4).

The pooled morphodisparity at each ecoregion was variable along the latitudinal gradient, with the maximum values recorded in Guayaquil and Central Chile ecoregions (Fig. 5). Meanwhile, at the genus level, the morphodisparity was highly variable in the latitudinal gradient among ecoregions (Fig.6). In the genus with the largest latitudinal distribution, Tonicia occurs between Central Peru and Channels and Fjord, and shows a general increment of morphodisparity southward, with a drop in the Central Chile ecoregion (Fig.6). The genus Chaetopleura is distributed between Guayaquil and Chiloense ecoregions with the greatest morphodisparity value in Central Peru, showing decreased and similar values southward until Chiloense (Fig. 6). The genus *Chiton* was the only distributed all along the study area, and showed a relatively constant morphodisparity along the latitudinal gradient, with a slight increment in Central Chile ecoregion. The remaining genera of polyplacophora have a narrower distribution in the SEP, where *Enoplochiton* shows a slight increase of morphodisparity southward from Central Peru to Central Chile ecoregion, whereas Achantopleura and Plaxiphora show a steady morphodisparity, from Guayaquil to Araucanian, and from Araucanian to Chanel and Fjords ecoregions, respectively (Fig. 6).

Significant differences in morphology, using Euclidean distances, were found in each taxonomic factor evaluated (i.e. Family, Genus, Species and Ecoregion), and also in the ecoregion-species interaction (Table 2), suggesting that taxonomic classifications and biogeographic subdivisions can affect morphodisparity. Morphodisparity was inversely

associated with latitude, oxygen range and pH (Table 3) (Fig.7). The ANOVA of GLM coefficients shows a high deviation explained by latitude in morphodisparity (31.19%), but when corrected by latitudinal gradient only the oxygen range and pH have a significant effect (Table 3) (Fig. 7). Morphodisparity was positively associated with phylogenetic diversity (Fig.7), where deviance explains 26.56% of variation (Table 4).

Discussion

This study gives us evidence of morphological differences of polyplacophorans along the SEP coast. Such differences show patterns mainly associated with the latitude gradient, however when the latitude is extracted, environmental factors and phylogenetic diversity show importance to explain the observed patterns in morphological diversity of polyplacophorans. Brayard *et al.* (2005) state that there is a direct relationship between latitudinal gradients and species richness in most of the studies about marine taxa, supporting the hypothesis that species evolved through adaptation to temperature in the tropical borders. However, our results correlated different environmental patterns regardless of temperature (i.e. O² and pH) with morphological disparity patterns.

The general shape of chitons is more or less oval among the ~930 living species (and \geq 430 extinct) (Schwabe, 2008; 2010), but presents a wide variation from broad oval to worm like (i.e. vermiform) body shape since Jurassic (Sirenko, 2006). Shape allometry, changes in the contour shape during adult growth, varies among species and can potentially vary in separate populations (Emam & Ismail, 1993). This variation is in part related to niche specification, both in specific adaptations of overall body size and in terms of shell construction and material strength (Sigwart *et al.*, 2015). Our first analyses showed both total
length and morphological diversity (i.e. morphodisparity) peaked at central Chile ecoregion, matching with the species richness peak described recently; nevertheless, the recorded drops in length and morphological diversity do not match with that species richness reported by Navarrete *et al.* (2020). Smallest chitons are in Guayaquil ecoregion, while the largest are in Central Chile ecoregion, whereas the morphological diversity was higher in Guayaquil and Central Chile ecoregions. These results suggest species richness does not represent a good predictor for length or morphological diversity patterns of polyplacophorans along the SEP coast.

Among the families of polyplacophorans inhabiting the SEP coast, Chitonidae was the only distributed overall the range, showing a twice bigger morphospace than following Mopalidae, with a southern bounded distribution. The contribution of each genus to the morphological diversity in each ecoregion was variable, where for example *Tonicia* seems to variate its morphodisparity according to the latitudinal gradient increasing southwards, *Cheatopleura* genus has a great proportion of local morphodisparity in only one ecoregion (i.e. Central Peru), and *Chiton* contributes similarly to the total morphodisparity in each ecoregion. This variability in the contribution to morphological diversity of each genus in the latitudinal gradient reflects the complexity of underlying processes that results in the emerging biological features of polyplacophorans in the south Pacific coast of South America.

Within the environmental variables, only the range of dissolved oxygen and the average pH were related to morphological diversity. This can be due to the physiological tolerance of this group, which inhabits the intermareal and shallow subtidal habitats, where less variation of oxygen (narrower ranges) seems to be related with more morphological diversity. The Humboldt Current System is characterized by coastal upwelling in Peru and Chile, and it is tightly related to the spatial and temporal dynamics of coastal environments (Thiel et al., 2007). However, chitons abundance and diversity are related to the local availability of favourable habitats (i.e. rocky shores) and food, rather than environmental variables (Ibáñez et al., 2016; 2019b). The positive relationship of morphodisparity and phylogenetic diversity can be explained because phylogenetic distance represents an estimate for the magnitude of phenotypic differences (Cavender-Bares et al., 2009). In a local clade, the extinction and diversification processes determine the removal of branches through the (local) extinction of species, or the occurrence of new tips through the speciation or immigration (and establishment) of new species in the locality. Such processes determine the phylogenetic diversity, and this may reflect the morphological adaptive response of new species to physical and biological conditions that they must overcome in the environment to persist. If new tips in a clade are generated by speciation processes, its contribution to the local phylogenetic and morphological diversity could be low, because this new branch is short in terms of age in the clade (less differentiation since the split). On the other hand, if a new tip in a local clade is formed due to the immigration of new species through a dispersion process, their contribution to the local phylogenetic and morphologic diversity will depend on the degree of phylogenetic relationship with the local group of species, and may be low or high if their phylogenetic relationships are recent or ancient, respectively. In polyplacophorans along SEP coast, a positive relationship is observed between morphodisparity and phylogenetic diversity, which suggests that causes to shape local clades respond to diversification processes that have been accompanied by morphological adaptive differentiation. It is worth noting that the relationships between morphological disparity and environmental variability are directly associated to the theory of ecological opportunity, which predicts a link between the processes that generate species diversity (environment and its relations) and morphological differentiation. However, the results show that variation in morphology is independent from the spatial dynamics of diversification, meaning that other processes apart from speciation may be promoting or, conversely, restricting evolution of shape in the Chitons of the SEP coast.

Yoder *et al.* (2010) explain in their theory of ecological opportunity a relation between distribution (spatial variation) of taxonomic diversity and morphological disparity. This may be particularly associated with colonization of new niches and, consequently, with a high variance in the patterns of phenotypic adaptation with the purpose of colonizing adequately, resulting these future speciation patterns. Therefore, our results support the importance of historical-evolutionary processes and the constraints imposed by the environment to shape the patterns of morphological diversity, as well as the importance of taking additional measures of diversity, such phylogenetic, to explain the determinants under the observed diversity patterns of polyplacophorans along the SEP coast.

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Tables

Table 1. PCA loadings of the first four principal components (PC) of 14 morphometric measurements standardized by total length (LT) of the chitons from Southeastern Pacific coast. Total width (TW), length of plate I (L-I) length of plate VIII (L-VIII), widths of each shell plate (I to VIII), length of anterior perinotum (APL), length of lateral perinotum (LPL) and length of posgterior perinotum (PPL).

Measure	PC 1	PC 2	PC 3	PC 4
L-I	0.093	0.007	0.290	0.279
L-VIII	0.115	-0.057	0.291	0.619
TW	0.267	0.870	-0.357	0.209
Ι	0.304	-0.125	-0.029	0.025
II	0.355	-0.107	-0.045	-0.070
III	0.367	-0.076	-0.019	-0.188
IV	0.366	-0.049	-0.001	-0.278
V	0.359	-0.033	0.024	-0.243
VI	0.340	-0.033	0.080	-0.125
VII	0.312	-0.071	0.132	0.099
VIII	0.276	-0.150	0.083	0.428
LPL	-0.045	0.342	0.624	-0.301
APL	-0.011	0.191	0.401	-0.131
PPL	-0.001	0.149	0.346	0.043

Factor	SS	df	MS	F	Р
Family (F)	12812	6	21354	39959	0.0001
Genus (G)	86366	11	78515	37151	0.0001
Species (S)	101.79	26	3915	23017	0.0001
Ecoregion (E)	72048	6	12008	2247	0.0001
E x F	-1170.9	36	-32524	-60861	1.0
E x G	-429.7	66	-65106	-30806	1.0
E x S	-325.63	156	-20873	-12272	0.0061

Table 2. Results of two-way PERMANOVA comparing morphology between genera, families and ecoregions using Euclidean distances.

Predictor	F	Р	% Deviance
Latitude	7.731	0.003	31.19
Oxy_range	8.405	0.011	11.86
pН	6.450	0.023	10.95
Temp_range	0.277	0.606	8.36
Salinity_range	0.953	0.344	0.38
Chl_range	0.427	0.524	2.88

Table 3. Analysis of variance of GLM coefficients of the association between morphodisparity and environmental gradients.

Predictor	F	Р	% Deviance	
Richness	3.346	0.087	10.57	
Family	0.769	0.394	2.43	
PDp	8.403	0.011	26.56	

Table 4: Analysis of variance for GLM coefficients of the association between morphodisparity and the community descriptors Species richness (Richness), Family number (Family), and proportional phylogenetic diversity index (PDp).

Figures



Figure 1. Sampling sites along the Southeastern Pacific coast. Along the latitudinal gradient each colour represents different Ecoregions 1 gradient. Green: Guayaquil, red: Humboldtian; blue: Central Peru, pink: Central Chile, orange: Araucanian, yellow; Chiloense, and light-blue: Channels and Fjords.



Figure 2. Picture of *Achantopleura echinata* showing the measures realized to obtain data for morphometric analyses. Roman numerals denote the eight chiton's plates (I to VIII), were I is the cephalic, and VIII is the posterior plate. White segmented lines represent the total length (TL horizontal line) and the total width (TW vertical line) of the organism. White solid lines represent the widths measured in each plate. Red solid lines represent the length measured to plate I (L-I) and VIII (L-VIII) Yellow solid lines represent the length measured on perinotun realized in the anterior (APL), lateral (LPL) and posterior (PPL) margin of the organism.



Figure 3. Mean of total length (LT) (mm) of pooled polyplacophorans in each ecoregion along the SEP coast. Guayaquil = GUA, Central Peru = CPE, Humboldtian = HUM, Central Chile = CCH, Araucanian = ARA, Chiloense = CH, and Channels and Fjords = CF. Vertical bars denote the 95% confidence intervals.



Figure 4. Principal component analysis (PCA) ordination of morphometric measures of chitons standardized by total length. Coloured convex hulls represent the morphospace of the seven chiton families studied along the SEP coast.



Figure 5. Morphodisparity of pooled polyplacophorans in each ecoregion along the SEP coast. Guayaquil = GUA, Central Peru = CPE, Humboldtian = HUM, Central Chile = CCH, Araucanian = ARA, Chiloense = CH, and Channels and Fjords = CF.



Figure 6. Morphodisparity of polyplacoporan genus at each ecoregions along the latitudinal gradient in SEP coast. Horizontal dashed lines represent ecoregions of Guayaquíl: GUA, Central Peru, CPE, Humboldtian: HUM, Central Chile: CCH, Araucanian: ARA, Chiloense: CHI, and Channels and Fjords: CF.



Figure 7. Association between morphodisparity, environmental and evolutionary predictors. A) Latitudinal gradient, environmental predictors B) pH, C) Oxygen range, and to the D) Phylogenetic Diversity (PDp)..

Supplementary material

Table S1. Mean chiton's total length (mm) by species and ecoregions along Southeastern Pacific. Values on parenthesis are minimum and maximum TL. GUA= Guayaquil; CPE= Central Peru; HUM= Humboldtian; CCH= Central Chile; ARA= Araucanian; CH= Chiloense; CF= Channels and Fjords.

01 0111010	GUA	CPE	HUM	CCH	ARA	CH	CF
Acanthochitona	11.6						
ferreirai	(8.9-13.8)						
Acanthochitona	15.8						
hirudiniformis	(11.6-22)						
Acanthopleura	52.2	79.7	79	109.4	84.6		
echinata	(25.2-73)	(68-100)	(23-151)	(29-193)	(24-137)		
Callochiton							16
puniceus							(9.6-21)
Calloplax				8.2	11.6		
vivípara				(6.3-10.3)	(9.6-13.7)		
Chaetopleura					53.9	33.7	
benaventei					(23-89)	(25-53)	
Chaetopleura		44					
hennahi							
Chaetopleura		22.5	16.5	22.4	32.5	33.7	
peruviana		(13-32)	(5.7-37)	(5.6-58)	(11-57)	(23-46)	
Chaetopleura	32						
roddai	(23-41)						
Chiton barnesii			11.1	14.4	9.1		
			(10-12.3)	(3.8-54)	(9.1-9.1)		
Chiton	41	29	29.8	43	37.1	31.3	
cumingsii	(36-46)	(11.5-46)	(11.3-47)	(16-69)	(15-60)	(24-38)	
Chiton		35.2	32.5	50	44.2	59.2	63.5
granosus		(10-66)	(14-61)	(18-79)	(14.1-79)	(24-82)	(57-70)
Chiton				45.7	50.1	42.1	34.5
magnificus				(11-102)	(6.1-108)	(8.7-72)	(21-57)
Chiton stokesii	33.8						
	(11-67)						
Enoplochiton		51.2	76.1	98.4			
niger		(29-66)	(32-130)	(28-137)			
Gallardoia					15		
valdiviensis	10.0						
Ischnochiton	13.8						
dispar	(7.4-18)						
Ischnochiton			4.3	9.9		11.3	
pusio			(4.3-4.3)	(8.5-11.5)		(6.5-18.9)	
Ischnochiton				6.7			8.8
stramineus				(5.5-7.6)			(3.6-13.3)
Plaxiphora					26	52.6	55.9
aurata					(16-34)	(30-81)	(22-97)
Stenoplax	21.6						
limaciformis	(12.8-27)						
Tonicia		32	22	35.9	34.2	32.2	25.5
calbucensis		(32-32)	8.1-42)	(15.5-48)	(9.9-86)	(21-42)	(12-36)
Tonicia		31.3	26.6	37.4	43.4	43.3	39.2

chilensis	(26-37)	(24-29)	(20-59)	(7.2-91)	(23-67)	(21-77)
Tonicia			48	84	56	65
disjuncta			(39-57)	(84-84)	(56-56)	(40-90)
Tonicia	29.9	25.6		21.9	41	
fremblyana	(22-38)	(19-34)		(12.7-28)	(41-41)	
Tonicia lebruni			20	45		28.8
			(20-20)	(45-45)		(17.3-44)
Tonicia	34	18.1				
swainsoni	(34-34)	(4.1-33)				



Figure S1: Bayesian phylogenetic tree of polyplacophorans from the South-eastern Pacific coast construct from concatenated genes 16S+COI+18S, used to calculate the phylogenetic diversity in Biodiverse v.1 software.

DISCUSIÓN

Los resultados iniciales de esta tesis resaltan la importancia de realizar estudios exhaustivos que incluyan múltiples fuentes de información y evidencia para describir los patrones y procesos que afectan los gradientes latitudinales de riqueza de especies.

Lo anterior se sustenta en la comparación entre las dos bases de datos que se obtuvieron en esta tesis, donde se analizaron los patrones de riqueza de poliplacóforos a lo largo de la costa del SEP, evidenciando que patrones obtenidos desde de literatura para esta clase de moluscos tienden a sobreestimar en gran medida los rangos de distribución y la ocurrencia de las especies en el gradiente latitudinal. El hecho que más del 20% de los registros de literatura no puedan ser verificadas por la ausencia de ejemplares en colecciones biológicas representa una dificultad insoslayable para los investigadores rigurosos que buscan confirmar, o incluso complementar a la información de ocurrencia analizando dichos ejemplares. El criterio en este trabajo fue riguroso, por lo que las muestras que no señalaron de forma comprobable que están depositadas en colecciones biológicas no fueron consideradas. Adicionalmente, a partir de la información genética que se obtuvo en este trabajo se pudo determinar que más de un 10% de las ocurrencias registradas en la literatura corresponden a sinonimias. Estos resultados implican que del total de especies utilizadas en estudios previos alrededor del 46% son inválidas (Reid y Osorio, 2000; Aldea y Valdovinos, 2005; Schwabe *et al.*, 2006; Schwabe, 2009; Araya and Araya, 2015).

El objetivo que dió inicio este trabajo fue reevaluar el patrón de riqueza de especies de poliplacóforos a lo largo del SEP, analizando en primera instancia, y de forma exploratoria, los datos obtenidos a partir de la literatura disponible sobre la ocurrencia de poliplacóforos en el área de estudio. A partir de este análisis se registró un claro patrón de aumento de especies con la latitud, y que concuerda con lo planteado en estudios previos que incluyen a esta clase de moluscos (e.g. Valdovinos et al., 2003; Fernández et al., 2009). Sin embargo, este patrón que resulta en principio evidente desde información de literatura, pierde sustento al considerar de forma adicional la información recopilada desde la revisión (y en algunos casos redescripción) directa de ejemplares correctamente depositados en colecciones biológicas y de ejemplares colectados en terreno (Proyecto FONDECYT 1130266). Al representar el patrón de riqueza con los datos revisados, y en algunos casos validados con DNA, mostraron un patrón latitudinal en forma de campana con máximos de riqueza a latitudes intermedias (i.e. 33°S), y donde incluso es posible observar zonas en que los niveles de riqueza caen notoriamente. Tales variaciones de riqueza en el gradiente latitudinal parecen estar asociadas a límites y transiciones de unidades biogeográficas descritas para organismos marinos en la costa del pacífico sudamericano (Camus, 2001; Spalding et al., 2007).

Nuestra revisión exhaustiva de los poliplacóforos ayuda a establecer de forma más rigurosa el patrón de este grupo en la costa del SEP. Además esta información aporta a los estudios biogeográficos de las especies marinas de esta zona del pacífico, donde se registra una inusual cantidad de taxa marinos, tanto de algas (Santelices y Marquet, 1998) como de animales (Lancellotti y Vásquez, 2000; Valdovinos *et al.*, 2003; Hernández *et al.*, 2005;

Rivadeneira *et al.* 2011; Lee & Riveros, 2012; Navarrete *et al.*, 2014) que se desvían del patrón global de aumento de riqueza de especies hacia menores latitudes (Rosenzweig, 1995; Roy *et al.*, 1998; 2000; 2004; Macpherson, 2002; Willig *et al.*, 2003; Mittelbach *et al.*, 2007).

A los problemas inherentes al uso de literatura no verificada y a la identificación poco rigurosa de los ejemplares en el establecimiento del rango de ocupación de las especies en el ambiente, debemos sumar los grandes problemas logísticos que muchas veces imponen los estudios que buscan abarcar escalas espaciales extensas. Estas limitaciones resultan la mayoría de las veces en que el esfuerzo de muestreo histórico sea bastante desigual entre localidades, viéndose favorecidas, por ejemplo, aquellas con mayores facilidades de acceso. Inncluso, localidades que pudieran no estar tan distanciadas entre sí, y que frecuentemente presentan distintas metodologías de muestreo hacen difícil el uso directo y homologación de los datos (Camus, 2001; Navarrete *et al.*, 2014). Por lo tanto, zonas geográficas de fácil acceso estarán mejor representadas en el registro que aquellas que presentan dificultades mayores.

En términos de la verificación mediante DNA, nuestros resultados genéticos sugieren que géneros como *Ischnochiton*, *Chiton* and *Tonicia* necesitan una revisión urgente. De hecho, en el reciente trabajo Ibáñez *et al.* (2019) que analiza 9 de las 11 morfoespecies descritas del género *Tonicia* en la costa del SEP, resuelven solo 6 especies en base a una filogenia molecular y a un análisis de delimitación de especies. Esto demuestra que las especies del género *Tonicia* pueden presentar alta variabilidad en los caracteres que usualmente se utilizan para su identificación, donde el uso de la coloración como criterio de identificación debe ser reexaminado (Ibáñez *et al.*, 2019). En el contexto que se plantean resultados de Ibáñez *et al.* (2019) respecto al género *Tonicia*, nuestros resultados también señalan la necesidad de realizar una revisión detallada de los géneros *Ischnochiton* y *Chiton* del SEP. El hecho que de las variables ambientales analizadas solo la concentración de oxígeno disuelto haya mostrado algún grado de asociación con el patrón de riqueza registrado en el gradiente latitudinal, da sustento parcial a hipótesis relacionadas con la variación de productividad-energía del ambiente. Lo anterior podría sugerir de alguna forma (o en algún grado) que los poliplacóforos tengan afinidad por ambientes oxigenados de aguas frías y con mayores niveles de saturación de oxígeno, como por ejemplo los asociados al sistema de corrientes de Humboldt (Thiel *et al.*, 2007). Más aun, el hecho que los poliplacóforos presenten caracteres ecológicos particulares como tasas de crecimiento relativamente bajas y larvas con corto desarrollo pelágico (*sensu* Eernisse, 2007) sugiere que estas especies podrían estar adaptadas a las condiciones particulares de la corriente fría del sistema de corrientes de Humboldt. Si bien la relación del oxígeno con la riqueza de quitones podría ser bastante intuitiva, es necesario tomar esta evidencia de forma cautelosa, dado que al incluir la estructura espacial en el análisis de la riqueza con las variables ambientales, la significancia de la concentración de oxígeno desaparece.

La diversidad morfológica de los poliplacóforos varió a distintos niveles en el gradiente latitudinal del SEP. A nivel taxonómico la diversidad morfológica varió tanto entre familias, géneros y especies, lo que responde los procesos divergencia de los representantes de este grupo y se ve reflejado en las variaciones morfológicas registradas. Por ejemplo, la familia con mayor rango de distribución en el área de estudio, Chitonidae, (i.e. se distribuyó en todo el rango estudiado) es la que presenta una mayor variación en forma (i.e. mayor mosfoespacio), lo que puede responder a procesos de diferenciación por distanciamiento geográfico y/o adaptaciones particulares a lo largo del borde costero del SEP. En este punto es importante recordar que este estudio abarca más de 5000 kilómetros lineales de costa, la cual

presenta marcadas variaciones en el gradiente latitudinal. Por ejemplo, en la zona norte el aporte de agua dulce al mar es escaso y la extensión de la playas de arena es mayor (Thiel *et al.*, 2007; Acuña y Jaramillo, 2015), mientras que desde Chiloé (*ca.* 41°S) hasta el Cabo de Hornos (*ca.* 56°S) hay un gran aporte de agua dulce al mar y se configura una alta fragmentación de la línea de costa, formando una gran cantidad de islas y canales protegidos (Ahumada *et al.*, 2000; Ojeda *et al.*, 2000; Camus 2001), lo cual impone a los quitones del SEP una gran variabilidad de habitas en el gradiente latitudinal.

A pesar que la diversidad morfológica registró un aumento hacia el Ecuador, el gradiente latitudinal por sí mismo no es lo que modula los patrones bilógicos que comúnmente se registran en este gradiente, siendo procesos histórico-evolutivos y ecológicos, y que generalmente se asocian con la latitud, los responsables de tal variación. Del conjunto de variables ambientales que se evaluaron en este trabajo, se registró que solo el pH y la variación local en las condiciones de oxígeno disuelto (i.e. rango de oxígeno) se relacionaron con el patrón de diversidad morfológica en el gradiente latitudinal. Estos resultados toman mayor relevancia en el escenario actual de cambio global acelerado que enfrentamos, y donde se pronostica un aumento en la acidificación del océano (Gattuso y Hansson, 2011; Vargas et al., 2017), donde los poliplacóforos podrían verse particularmente afectados, dado que es reconocido que la disminución del pH afecta tanto la disponibilidad de las distintas formas de carbonato de calcio, como también al proceso de calcificación de las placas. Dada la relación registrada en este estudio entre la diversidad morfológica, filogenética y el pH, los cambios globales proyectados en el pH también podrían afectar estas medidas de diversidad en los poliplacóforos del SEP, a través de influir en la función protectora de las placas calcáreas en los poliplacóforos (Sigwart et al., 2015). Estos cambios potenciales en la diversidad morfológica podrían resultar de plasticidad morfológica de este grupo modulada por el ambiente, situación que ha sido descrita para cambios en la morfología de la concha en otros moluscos (Knowlton, 2000; Esteban-Delgado, 2006; Puillandre *et al.*, 2012; Sepúlveda y Ibáñez, 2012; Sigwart *et al.*, 2015)

El hecho que la morfología de los poliplacóforos se relacione a la variación de oxígeno disuelto y no al valor medio de oxígeno registrado a lo largo de la costa del SEP, nos sugiere que los quitones pueden ser sensibles a las variaciones importantes las condiciones ambientales. Tal efecto ante la variación de oxígeno local, y al igual que lo discutido para el pH, toma relevancia en un escenario de cambio global acelerado, donde para la costa norte del sistema de corriente de Humboldt se proyectan cambios importantes de la temperatura superficial del mar, que van en la dirección opuesta a la tendencia global de aumento (Falvey y Garreaud, 2009). Esta baja en la temperatura superficial del mar en la costa centro-norte y norte de Chile (17°S-37°S) es el resultado de la intensificación de los eventos de surgencia en esta zona, debido reforzamiento del anticiclón del Pacifico sur causado por un progresivo aumento del gradiente de presión entre el océano y la masa continental (Falvey y Garreaud, 2009). Por lo tanto, la baja en la temperatura en esa zona costera es consecuencia de la intensificación de los eventos de surgencia que llevan agua con un alto contenido de nutrientes, baja temperatura y bajo oxígeno disuelto a la zona eufótica (Escribano et al., 2004; Sobarzo et al., 2007; Thiel et al., 2007). Es precisamente esta condición de baja de oxígeno en el agua en periodo de surgencia verus condiciones de oxígeno disuelto mayores en periodos de relajación lo que determina variaciones importantes en las condiciones de oxígeno disuelto locales, pudiendo afectar el patrón de diversidad morfológica de los poliplacóforos de la costa SEP.

Nuestros resultados muestran que la diversidad morfológica de los poliplacóforos no está relacionada con la riqueza de especies en el SEP. Esto resulta interesante, dado que, al pensar en el proceso de especiación, de forma intuitiva se tiende a asociar que las nuevas especies reflejarán diferencias morfológicas que evidenciarán tal separación. Nuestros resultados nos muestran que a pesar de registrase distinto número de especies a escala local en el gradiente estudiado, tal diferencia en riqueza no se ve reflejada de forma directa en el aumento de la diversidad morfológica. A pesar de la falta de asociación entre la diversidad morfológica y la riqueza de especies anterior, sí se registró una relación entre la diversidad morfológica y la filogenética, donde los clados locales con mayor diversidad morfológica son los que comparten relaciones filogenéticas más diversas (mayor suma en distancia evolutiva entre el grupo de especies). En un clado local los procesos de extinción, diversificación de especies, así como la llegada de nuevas especies por inmigración, determinan el número de tips que componen el clado, mediante la remoción (extinción local) o adición (especiación e inmigración) especies al clado. Estos procesos determinan la diversidad filogenética local, y actúan de forma conjunta en los resultados que obtuvimos en este trabajo. Aunque resulta difícil evaluar el aporte de cada proceso en la conformación del patrón de diversidad morfológica de los poliplacóforos, es frecuente que las especies componentes del clado aporten de manera diferencial a la diversidad del clado. Por ejemplo, pueden existir especies que aportan mayor variación (diversidad), tanto en la morfología y en las relaciones filogenéticas, pudiendo ser aquellas con relación filogenética más lejana y originadas en zonas alejadas, y que se dispersaron a la localidad en estudio acumulando modificaciones morfológicas. En la configuración de estas medidas de diversidad también es importante la extinción de especies, sin embargo resulta difícil establecer una tasa en poliplacóforos dado el escaso registro fósil existente de esta clase de molusco en el SEP. En conjunto, estos resultados refuerzan la importancia de procesos de diversificación, extinción, y dispersión en la conformación del patrón morfológico registrado en los quitones del SEP.

La Clase Polyplacophora es una de las más antiguas del Filo Mollusca, con registros fósiles de hace aproximadamente 500 Ma (Eernisse, 2007), y aunque su plan corporal es conservativo, al menos desde el Jurásico se registran variaciones en su forma general desde formas ovales a otras vermiformes (Sirenko, 2006). Esta gran conservación en su plan corporal básico es una señal de su éxito en el ambiente, llegando a ser uno de los representantes de mayor tamaño de los moluscos, y de gran relevancia ecológica en ambientes intermareales y submareales someros alrededor del mundo (Otaiza, 1986; Eernisse, 2007; Sanhueza *et al.*, 2008; Camus *et al.*, 2012; Camus *et al.*, 2013).

CONCLUSIÓN GENERAL

- En conjunto, los resultados de esta tesis dan sustento parcial a la hipótesis general, donde solo algunas variables ambientales, y la relación de los patrones de diversidad morfológica y filogenética en el gradiente latitudinal, sugieren la importancia de factores ecológicos e histórico evolutivos particulares en la conformación del patrón de diversidad registrado para los polipacóforos en la costa del Pacífico sureste.
- El patrón latitudinal de riqueza de especies de los poliplacóforos que habitan la costa del Pacífico Sureste presenta máximos a latitudes intermedias, así como también disminuciones notables de la riqueza en dos zonas próximas al extremo norte y sur del rango de distribución estudiado. Además, pone en evidencia los riesgos de utilizar información bibliográfica no verificable para establecer las ocurrencias de especies a grandes escalas espaciales.
- El hecho que el pH se relacionara con la diversidad morfológica, y que oxígeno se relacionara tanto con la riqueza de especies, como con la diversidad morfológica, señala a estas variables como las principales en modular los patrones de diversidad de poliplacóforos en el SEP, las cuales son altamente sensibles en un escenario de cambio global acelerado.

- La diversidad morfológica de los poliplacóforos varió dentro de cada jerarquía taxonómica, representando las respectivas diferencias en su clasificación, y donde las jerarquías que presentarón mayor rango de sistribuciín presentaron tambien la mayor proporción de morfoespacio.
- En el gradiente latitudinal la diversidad morfológica tiende a aumentar hacia el ecuador, lo que no se condice con el máximo de riqueza registrado en latitudes intermedias. Este resultado sumado a la relación registrada entre la diversidad morfológica y la filogenética resaltan la importancia de los procesos de extinción, diversificación y migración de especies en la configuración local de las distintas medidas de diversidad de los poliplacóforos en la costa del SEP. En términos biogeográficos, este trabajo actualiza distribución de los poliplacóforos, sumándose a los patrones ya descritos para organismos marinos del Pacífico sureste, zona de alto interés biogeográfico por la particular configuración que permite evaluar el gradiente latitudinal, y por ser reconocida como una zona con una cantidad inusual de organismos marinos que no presentan el patrón clásico global de aumento de especies hacia el Ecuador.

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