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Variabilidad oceanográfica espacial y su impacto sobre la estructura comunitaria, filogeografía y biogeoquímica del zooplancton pelágico en el Pacífico Suroriental

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La Tesis de “Doctorado en Oceanografía” titulada “Variabilidad oceanográfica espacial y su impacto sobre la estructura comunitaria, filogeografía y biogeoquímica del zooplancton pelágico en el Pacífico Sur Oriental”, del Srta. CAROLINA GONZÁLEZ y realizada bajo la Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, ha sido aprobada por la siguiente Comisión de Evaluación:

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A mis padres y familia

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PUBLICACIONES

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EXPERIENCIA DOCENTE

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RESUMEN

Variabilidad oceanográfica espacial y su impacto sobre la estructura comunitaria, filogeografía y biogeoquímica del zooplancton pelágico en el Pacífico Suroriental.

Carolina Andrea González Espinoza
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El zooplancton se caracteriza por ser un enlace clave en las tramas tróficas marinas, mediando los flujos biogeoquímicos en la columna de agua, y por exhibir tiempos generacionales cortos (<1 año). Este diverso grupo exhibe una alta sensibilidad a los cambios ambientales debido a su limitada capacidad migratoria, de modo que las variaciones en su abundancia y distribución pueden ser usadas como indicadores de la respuesta biológica del ecosistema a los cambios físicos y químicos ambientales. Una de las regiones con mayor heterogeneidad en las condiciones oceanográficas, es el Pacifico Suroriental (PSO), capaz de servir como laboratorio natural para entender el efecto de variabilidad ambiental sobre las comunidades pelágicas. Esta región se caracteriza por poseer un fuerte gradiente costero-oceánico, desde una zona de surgencia altamente productiva hacia la región ultra-oligotrófica en el giro del Pacifico Suroriental (GPSO), capaz de influir en la distribución de los organismos marinos. Sin embargo, en el PSO no existe suficiente información sobre como la variabilidad oceanográfica determina la estructura comunitaria, filogeografía y biogeoquímica del zooplancton pelágico.

Este estudio plantea como hipótesis general que la heterogeneidad oceanográfica del Pacifico Suroriental genera una zonación ecológica, genética y biogeoquímica en el zooplancton desde la zona de afloramiento costero hasta el giro central del Pacífico sur.

La área de estudio se dividió en cuatro zonas según los rangos de clorofila-a observados en la superficie: eutrófico (zona de surgencia chilena), mesotrófico (zona de transición costera), oligotrófico (océano abierto) y ultra-oligotrófico (giro central del Pacífico sur). Sobre esta área de estudio se utilizó información oceanográfica y muestras de zooplancton obtenidas en cruceros de gran extensión espacial, realizados durante la primavera de los años 2015 y 2016, correspondientes a la campañas oceanográficas

CIMAR-21 y CIMAR-22 a bordo del B/O Cabo de Hornos. Además, se complementó esta información con series de tiempo costeras y otros muestreos hechos en océano abierto durante el periodo de 1986 al 2016 para aumentar la cobertura espacial. La información oceanográfica *in-situ* (salinidad, temperatura, oxígeno disuelto), se complementó con datos satelitales de temperatura, clorofila-a, viento, luz PAR y circulación geostrófica. Las muestras de zooplancton se analizaron mediante identificación morfológica por microscopia tradicional y análisis automatizado con ZooScan. En muestras fraccionadas por tamaño se estimó la composición de isótopos estables de C y N, para identificar las potenciales fuentes de estos elementos en el zooplancton y examinar sus relaciones tróficas. En el análisis de diversidad genética se utilizó como modelo de estudio el copépodo cosmopolita *Pleuromamma abdominalis*, para lo cual se extrajo ADN de especímenes obtenidos sobre un gradiente costa-océano. Este ADN fue analizado mediante marcadores mitocondriales (COI-Cytb) y nucleares (28S-ITS), los cuales permitieron identificar y delimitar los diferentes linajes evolutivos dentro de la especie

Los resultados revelaron marcadas diferencias entre zonas en las condiciones oceanográficas, estructura comunitaria y composición bioquímica del zooplancton. Nuestros hallazgos sugieren un fuerte acoplamiento entre la diversidad taxonómica del zooplancton y las fuentes de nutrientes que sustentan su alimentación. Aunque múltiples factores y procesos pueden modular la composición bioquímica del zooplancton, este estudio revela que los cambios en la estructura de la comunidad están vinculados a diferentes regiones biogeoquímicas, proporcionando la base para la zonificación ecológica asociada con la utilización de nutrientes en los niveles tróficos inferiores.

Uno de los principales Ordenes dentro del zooplancton son los copépodos calanoideos que dominan el ambiente pelágico. El análisis de la estructura comunitaria de este orden reveló que tanto a nivel de familia como especies presentan una estructuración significativa en un gradiente costero-oceánico y latitudinal en el PSO. Además, los patrones de distribución de familias en ambos gradientes mostraron la presencia de zonas de transición. La temperatura superficial del mar explico hasta un 41% de la riqueza tanto de familias y especies dentro de esta región. Nuestros hallazgos sugieren que el zooplancton se encuentra altamente estructurado en el océano y que los gradientes ambientes pueden ayudar al mantenimiento de los patrones de diversidad.

Existen pocas especies de amplia distribución y abundantes dentro del orden Calanoida en el PSO. Una de las pocas especies que cumple con estos requisitos es *Pleuromamma abdominalis*. Los análisis filogenéticos de esta especie revelaron la presencia de 8 linajes divergentes que ocurren a través de un gradiente costero-oceánico. Varios nuevos linajes revelaron un inesperado alto nivel de endemismo dentro del Sistema de Corriente de Humboldt (SCH). Además, análisis multivariados indicaron una fuerte correlación entre la variación genética con los valores de clorofila-a y salinidad, lo que sugiere un importante papel de los gradientes oceanográficos en el mantenimiento de la diversidad genética del zooplancton.

Las observaciones, análisis y resultados obtenidos permiten aceptar la hipótesis general planteada, con énfasis en que la variabilidad oceanográfica posee un papel clave en la regulación de la diversidad y bioquímica de organismos pelágicos. Tal aseveración, demuestra la importancia de la heterogeneidad ambiental para procesos asociados a la estructuración y funcionamiento de grandes ecosistemas marinos.

ABSTRACT

Spatial oceanographic variability and its impact on the community structure, phylogeography and biogeochemistry of the pelagic zooplankton in the Southeast Pacific

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The zooplankton is characterized by being a key link in marine food webs, mediating biogeochemical flows in the water column, and by exhibiting short generational times (<1 year). This diverse group exhibits high sensitivity to environmental changes due to its limited migration capacity, so that variation in their abundance and distribution can be used as indicators of biological response of the ecosystem to physical and chemical changes of the environment. One of the regions with a extremely high heterogeneity in oceanographic conditions, is the Southeast Pacific (SEP), which it can also serve as a natural laboratory to study and understand the effect of environmental variability on pelagic communities. This region is characterized by having a strong coastal-oceanic gradient, from a highly productive upwelling zone towards the ultra-oligotrophic region in the Southeast Pacific Central Gyre (GSEP), influencing the distribution of marine organisms. For this region, however, there is very limited information on how oceanographic variability can affect the community structure, phylogeography and biogeochemistry of the pelagic zooplankton.

This study states the general hypothesis that oceanographic heterogeneity of the Southeast Pacific can generate an ecological zonation, a genetic structuring and modulate the biogeochemistry of zooplankton across the gradient from the coastal upwelling area to the central gyre South Pacific.

The study area was divided into four major zones in according to the chlorophyll-a ranges observed on the surface by satellite: eutrophic (Chilean upwelling zone), mesotrophic (coastal transition zone), oligotrophic (open ocean water) and ultra-oligotrophic zone (at the central gyre of the South Pacific). In this area, oceanographic information and zooplankton samples were obtained from large-spatial scale cruises, carried out during the spring of 2015 and 2016 corresponding to CIMAR-21 and CIMAR-

22 cruises onboard the R/V Cabo de Hornos, and also from spatial and time series studies performed in the coastal zone during the period 1986 to 2016 as to increase the coverage for the upwelling zone mainly. The in situ oceanographic information (salinity, temperature, dissolved oxygen) was complemented with satellite data of sea surface temperature, Chlorophyll-a, winds, PAR radiation and geostrophic circulation. Zooplankton samples were analyzed by traditional methods for morphological identification of species under the microscope, using automated analysis by ZooScan and by size-fractioned samples which were assessed for isotopes composition of C and N. Isotopic signals of C and n were used to assess the sources of these elements for zooplankton as well as their trophic relationships. The genetic diversity was studied using as a model the cosmopolitan copepod Pleuromamma abdominalis for which the DNA was extracted from specimens obtained over the coastal-oceanic gradient. This DNA was amplified (PCR) for mitochondrial (COI and Cytb) and nuclear (28s and ITS) molecular markers. These genes were then used to perform the phylogeography of the species and identification and delimitation of its lineages.

With respect to results, the zones showed marked differences in oceanographic conditions, community structure and biochemical composition of zooplankton. Our findings suggested a strong coupling between taxonomic diversity zooplankton and the sources of nutrients from their food supply, sustaining the zooplankton. Although multiple factors and processes can modulate the biochemical composition of zooplankton, this study revealed that changes in community structure are linked to different biogeochemical regions, providing the basis for ecological zonation associated with nutrient utilization at lower trophic levels.

One of the main groups within the zooplankton is comprised by the Calanoid copepods which dominate the pelagic environment. The analysis of the community structure of this order revealed that both at the family and species level there is a significant structuring over a coastal-oceanic and latitudinal gradient in the SEP. In addition, family distribution patterns in both gradients showed the presence of transition zones. The sea surface temperature explained up to 41% of richness both of families and species within this region. Our findings also suggeted that zooplankton are found highly structured in the ocean and that environmental gradients can help maintaining diversity patterns.

There were few species of wide and abundant distribution within the order Calanoida in the SEP. One of them meeting these requirements is *Pleuromamma abdominalis*. The phylogenetic analyzes of this species revealed the presence of 8 divergent lineages that occurred across a coastal-oceanic gradient. Several new lineages revealed an unexpected high level of endemism within the Humboldt Current System (HCS). In addition, multivariate analyzes indicated a strong correlation between genetic variation with chlorophyll-a and salinity values, which suggested an important role of oceanographic gradients in maintaining the genetic diversity of zooplankton.

The observations, analysis and results obtained allowed me to accept the general stated hypothesis, stressing the view that oceanographic variability has a key role in the regulation of diversity and biochemical characteristics of pelagic organisms. This view also emphasizes the key importance of environmental heterogeneity on processes linked to the structure and functioning of large marine ecosystems.

1. INTRODUCCION

1.1. Características de los ecosistemas pelágicos

Los ecosistemas se constituyen por las comunidades de seres vivos y los factores físicos y químicos en que viven. Estos sistemas suelen poseer una serie de cadenas de organismos interdependientes, principalmente debido a sus flujos de energía y nutrientes. Es común observar al interior de los ecosistemas ciertas discontinuidades físicas y químicas, que dan lugar a una segmentación, donde las condiciones son distintas respecto a las adyacentes, y más o menos uniformes al interior. Entre los distintos tipos de ecosistemas en la tierra, el marino alcanza la mayor amplitud, abarcando casi un 75% de la biósfera, en donde se destaca el ambiente pelágico por su gran extensión. Este ambiente muestra una alta diversidad de organismos que coexisten dentro de una compleja red trófica (Dunne *et al.,* 2004). Uno de los grupos principales dentro de la comunidad pelágica es el zooplancton, caracterizado por tener ciclos de vida cortos (por lo general menores a un año) y por ser un enlace clave en la trama trófica. Este grupo exhibe una alta diversidad biológica, con 12 grupos taxónomicos descritos y todos ellos muy sensibles a los cambios ambientales (Pino-Pinuer *et al.*, 2014; Peijnenburg y Goetze, 2013), de modo que las variaciones en su abundancia y distribución pueden ser usados como indicadores de la respuesta biológica del ecosistema a los cambios físicos y químicos ambientales (Richardson, 2008; Beaugrand *et al.* 2003).

1.2. Variabilidad oceanográfica del Pacifico Suroriental.

Una de las regiones con mayor heterogeneidad en las condiciones oceanográficas, capaz de servir como laboratorio natural para entender el efecto de variabilidad ambiental sobre las comunidades pelágicas, es el Pacifico Suroriental (PSO). Esta región es importante debido a su papel en el control del clima regional y sus tele-conexiones atmosféricas y oceánicas a escala global (Fiedler y Talley, 2006; Schneider *et al.*, 2007). El PSO se caracteriza por un fuerte gradiente costero-oceánico desde la zona de surgencia altamente productiva (eutrófica) hacia la región oligotrófica, e incluso ultra-oligotrófica en el giro del Pacifico Suroriental (GPSO) (Moutin *et al.*, 2017; Raimbault y García, 2008; Von Dassow y Collado-Fabbri, 2014). Además, dentro de esta misma región existe un predominio de diferentes masas de agua, tanto en gradientes longitudinales y latitudinales que promueven una variabilidad oceanográfica aún mayor en este vasto ecosistema (Silva *et al.*, 2009; Silva y Palma, 2006). Así, en el norte de Chile hasta 28°S, es posible observar el predominio en el borde oriental entre la superficie y hasta 100 m del agua subantártica (SAAW), con bajos valores de temperatura (11.5°C) y salinidad (33.8). En cambio, región oceánica está bajo la influencia del agua subtropical (STW), mostrando cerca de la superficie valores más altos de temperatura (20.0°C) y salinidad (35.2), así como bajas concentraciones de nutrientes, típico de los giros anticiclónicos. Además, cerca de la zona costera es posible observar también la presencia del agua ecuatorial subsuperficial (ESSW) hasta los 42°S, entre los 100 y 400 m de profundidad, caracterizándose por una alta salinidad (34.9), y una zona de mínima de oxígeno (< 1mL L^{-1}) (ZMO). Bajo todas estas masas de agua hasta los 28°S, se encuentra el agua intermedia antártica (AAIW) que se caracteriza por poseer los valores más bajos de salinidad (34.0) y una alta concentración de oxígeno (< 6 mL L^{-1}) (Reid, 1973; Strub *et al.*, 1998).

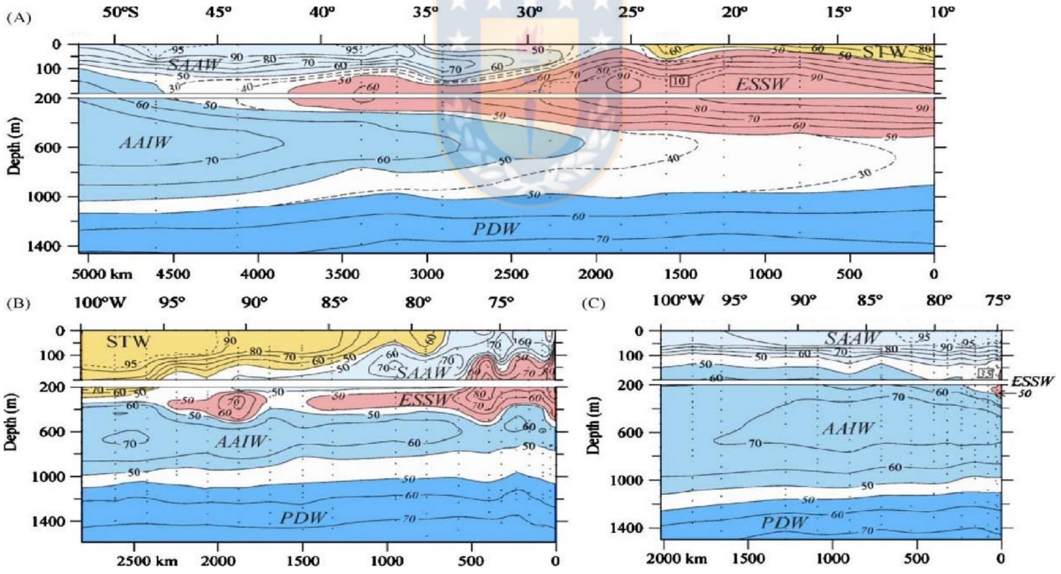

Figura 1. Distribución vertical porcentual de las distintas masas de agua presentes en el Pacífico Suroriental. (A) Sección latitudinal entre Perú y Chile (10–52°S), (B) sección longitudinal en los 28°S y (C) longitudinal sección a los 43°S. (STW = Agua Subtropical; SAAW=Agua Subantártica; ESSW = Agua Ecuatorial Subsuperficial; AAIW= Agua Intermedia Antártica; PDW = Agua Profunda del Pacifico). Extraído desde Silva *et al.*, 2009.

La zona costera del PSO se caracteriza por su alta productividad biológica, impulsada por la presencia de ESSW, asociada con altas concentraciones de nutrientes (NO_3^-= 20-28 µm, PO_4^{3-}= 1.8-2.6 µm) capaz de extender su productividad hasta la región de transición costera (Morales *et al.,* 2007; Palma *et al.,* 2009). Por otro lado, en las zonas más oligotróficas se observa el predominio de STW que provee una menor concentración de nutrientes y como consecuencia una menor productividad (NO_3^-= 1-4 µm, PO_4^{3-}= 0.25- 0.5 µm) (Reid, 1973). Sin embargo, al interior de la zona costera existe una gran variabilidad en la productividad debido a los distintos regímenes de surgencia a lo largo de Chile, desde un afloramiento intermitente durante todo el año en el norte (18°–30°S) (Thomas *et al.,* 2001), hasta un afloramiento fuertemente estacional concentrado durante la primavera-verano austral en el centro/sur (30°-40°S) (Sobarzo *et al.,* 2007). Esta variación del afloramiento puede modificar sustancialmente las condiciones físicas y químicas de la columna de agua durante el año (Ej. estratificación, salinidad, clorofila-a y oxígeno) (González *et al.*, 2015). En contraste con las zonas más oligotróficas del giro anticiclónico, donde los ciclos estacionales son débiles, por lo que las condiciones oceanográficas son más estables temporalmente al interior de esta estructura (Pennington *et al.*, 2006).

La diferenciación entre las zonas costera y oligotrófica del PSO ha sido sugerida en diferentes estudios, que proponen la existencia de dos provincias biogeoquímicas, donde los procesos físicos y químicos son únicos y distinguibles a nivel global, en las que se destaca la provincia de la Corriente costera de Humboldt y el Giro del Pacifico Sur (Oliver e Irwin, 2008; Longhurst, 2010; Reygondeau *et al.*, 2013). Por ejemplo, el nitrógeno disuelto superficial posee un rol clave dentro de esta región en promover diferencias en la productividad biológica y procesos biogeoquímicos entre estas dos zonas. En general, en áreas normóxicas que involucran la mayor parte del océano global, el nitrógeno se encuentra en forma de nitrato. En cambio, en áreas casi subóxicas o anóxicas como es la zona costera de Chile la baja concentración de oxígeno permite que el nitrato sea utilizado como aceptor final de electrones en la oxidación de la materia orgánica reduciéndolo a nitrógeno elemental (Thomas y Hopfield, 1966; Codispoti y Christensen, 1985). La dificultad para obtener este nutriente dentro de zonas oligotróficas se dificulta aún más por los procesos de desnitrificación y oxidación anaeróbica de amonio (anammox), originados en la costa, lo que sumado a la presencia de una gran estratificación en el océano abierto, complica aún más el aporte de nitrógeno desde aguas costeras o desde mayor profundidad (Raimbault y García, 2008). Todos estos factores provocan que la región oligotrófica sea sustentada por nutrientes regenerados y fijación de N atmosférico (Raimbault y García, 2008), o eventualmente por nutrientes alóctonos, provenientes de remolinos de mesoescala originados en la zona costera de Chile (Andrade *et al.*, 2014; Yáñez *et al.*, 2009).

1.3. Efecto de la variabilidad oceanográfica en la estructuración comunitaria en el Pacifico Suroriental

En el PSO se ha reportado una fuerte correlación entre la variabilidad oceanográfica y los cambios en la abundancia, biomasa y distribución del zooplancton (Gonzalez *et al.*, 2015; Palma y Silva, 2006; Pino-Pinuer *et al.*, 2014), mostrando incluso una disminución de hasta tres órdenes de magnitud en la biomasa y abundancia del zooplancton desde la zona costera hasta el GPSO (Carlotti *et al.*, 2018; Gonzalez *et al.*, 2018). Dentro del giro central, los copépodos de menor tamaño dominan entre los organismos pequeños (<500 μm), representando numéricamente hasta el 65% del zooplancton, mientras que otras fracciones de tamaño están compuestas por quetognatos, eufáusidos y sifonóforos constituyendo una parte considerable de la comunidad (Gonzalez *et al.*, 2015; Carlotti *et al.*, 2018; von Dassow y Collado-Fabbri, 2014). Por el contrario, en la zona costera los copépodos de mayor tamaño a menudo dominan en abundancia numérica (Escribano *et al.*, 2007). Sin embargo, dentro de la misma región también se ha reportado un aumento en la diversidad y riqueza de especies en grupos específicos de zooplancton como sifonóforos, tintínidos y eufáusidos hacia el centro del GPSO (Palma y Silva, 2006; von Dassow y Collado-Fabbri, 2014; Riquelme-Bugueño *et al.*, 2012). Además, se ha observado dentro de la misma área un remplazo de especies endémicas dentro de la corriente de Humboldt por especies oceánicas (Fagetti y Fischer, 1964; Riquelme-Bugueño *et al.*, 2012). Por ejemplo, el copépodo endémico *Calanus chilensis* de la Corriente de Humboldt (Marín *et al.*, 1994) parece ser sustituido por *Calanus australis* de distribución más amplia hacia aguas oceánicas (Nuñez *et al.*, 2009). Un patrón similar parece ocurrir con la especie endémica *Euphausia mucronata* de la corriente Humboldt, que puede ser reemplazada por otros eufáusidos en el océano abierto (Riquelme-Bugueño *et al.*, 2012). Estos cambios en distribución y diversidad del zooplancton revelan la fuerte dependencia con las condiciones oceanográficas del PSO, debido al efecto que estos pueden causar en su disponibilidad de alimento y tolerancia fisiológica, producto de su limitada capacidad de disperción en la columna de agua (McClain y Barry, 2010).

El zooplancton del PSO está sujeto a cambios graduales, y a veces abruptos, de las concidiones ocenográficas, y posiblemente a una variedad de procesos ecológicos y biogeoquímicos, tales como producción primaria, diazotrofia y desnitrificación, que pueden modular las fuentes de nutrientes que apoyan la producción de C y N orgánico (Raimbault y García, 2008; Fernández *et al.*, 2011; Farías *et al.*, 2013). El estudio de la composición de isótopos estables en el zooplancton puede proporcionar información muy valisosa para determinar las fuentes de C y N que sustentan a la comunidad pelágica (Peterson y Fry, 1987). Además, estos análisis se usan ampliamente para representar la red y posición trófica de los organismos, debido al enriquecimiento de isótopos pesados en el tejido de los depredadores con respecto a sus presas, ya que los isótopos ligeros se movilizan con mayor velocidad en las reacciones químicas (Post, 2002; El-Sabaawi *et al.*, 2013; Wang *et al.*, 2014). Una ventaja importante de los análisis isotópicos es que también pueden proporcionar una caracterización cuantitativa del nicho trófico de especies, comunidades y ecosistemas en general (Layman *et al.*, 2007, 2012). Investigaciones recientes, señalan que los cambios en la estructura comunitaria del zooplancton están vinculados a diferentes regiones biogeoquímicas, indicando que la zonación ecológica está asociada con la utilización de nutrientes de los niveles tróficos inferiores (Gonzalez *et al.*, 2018)

1.4. Efecto de la variabilidad oceanográfica en la estructuración genética en el Pacifico Suroriental

Uno de los principales grupos taxonómicos dentro del zooplancton son los copépodos, que representan casi el 80% de su abundancia (Escribano e Hidalgo, 2000; Hidalgo *et al.*, 2010, 2012). Dentro de este grupo el orden Calanoida domina el ambiente pelágico con aproximadamente 2000 especies descritas (Huys y Boxshall, 1991). La taxonomía de este orden ha sido bien definida desde hace muchos años (Ej. Giesbrecht, 1892; Sars, 1895), pero su subdivisión ha sido problemática producto a la gran diversidad de caracteres (Bradford-Grieve *et al.*, 2010). Actualmente, se reconocen entre 43 ó 44 familias que conforman un grupo monofilético (Bradford-Grieve *et al.*, 2010; Fosshagen y Iliffe, 1985), pudiéndose apreciar este orden desde ecosistemas altamente productivos como los sistemas de surgencia y hasta los giros ultra-oligotróficos del océano subtropical (Mauchline, 1998).

Las especies que componen el grupo Copepoda se caracterizan por presentar un gran tamaño poblacional, una alta capacidad de dispersión, un alto potencial evolutivo, y en algunos casos, una amplia distribución geográfica (Norris, 2000; Peijnenbur y Goetze, 2013). Estos caracteres, sumados a la continuidad del hábitat marino, llevan a pensar que estas especies deberían exhibir una baja o nula estructuración genética, con un alto flujo de genes entre sus poblaciones dentro de toda su área de distribución. Esta posibilidad se opone a los procesos de aislamiento y diversificación de especies (Peijnenburg y Goetze, 2013). Sin embargo, existe evidencia de que los copépodos ostentan una gran diversidad en un ambiente aparentemente homogéneo, como el océano abierto, y con un gran número de especies crípticas (Ej. Cornils *et al.*, 2014; Goetze, 2003; Bucklin *et al.*, 2018). Además, numerosos estudios realizados en distintas especies evidencian una fuerte estructura genética y un limitado flujo de genes en poblaciones con distribución desde zonas costeras hacia el océano abierto (Peijnenburg y Goetze, 2013). Por ejemplo, en los copépodos cosmopolitas *Eucalanus hyalinus, E. spinifer, Pleuromamma xiphias* y *Haloptilus longicornis* (Goetze, 2005, 2011; Norton y Goetze, 2013) se ha reportado que los fuertes gradientes oceanográficos observados en las regiones oceánicas pueden actuar como barreras ecológicas para la dispersión, causando fuertes rupturas genéticas entre poblaciones de distintos giros subtropicales y/o entre cuencas oceánicas. Esto mayormente debido a que las características bióticas y abióticas de las regiones con fuertes gradientes pueden afectar el desarrollo o la supervivencia de las poblaciones (Goetze e*t al.,* 2015), pudiendo actuar como barreras para su dispersión. Las respuestas a estos gradientes oceanográficos pueden ser específicas de la especie (respuestas idiosincrásicas), debido a los variables requisitos ecológicos de cada especie (Peijnenburg y Goetze, 2013). Dado que la divergencia genética generalmente se desacopla de la divergencia morfológica en los copépodos (Lee y Frost, 2002; Rocha-Olivares *et al.*, 2001), y que las especies de amplia distribución pueden incluir linajes con diferentes trayectorias evolutivas (Goetze, 2003), la detección de la diversidad críptica en este grupo es bastante común.

En el PSO hay pocas especies de copépodos que muestren una amplia distribución y una alta abundancia a lo largo de un gradiente costero-oceánico. Como resultado, es difícil identificar las especies objetivo, adecuadas para estudios que pretendan dilucidar el efecto de la variabilidad oceanográfica en la estructuración genética a nivel de especies dentro de esta región. Una de las pocas especies que cumple con estos requisitos es *Pleuromamma abdominalis*, que posee una distribución cosmopolita y presenta una gran abundancia en las zonas mesopelágica y epipelágica, con un comportamiento migratorio activo (Al-Mutairi y Landry, 2001; Andersen *et al.*, 2004; Hays *et al.*, 1997; Razouls *et al.*, 2015; Steinberg *et al.*, 2002; Wilson, 2011). Trabajos previos realizados sobre esta especie, describen una gran variación intraespecífica en los caracteres morfológicos y genéticos (Cuoc *et al.*, 1997; Fornshell y Ferrari, 2010; Hirai *et al.*, 2015). Además, investigaciones recientes han revelado la presencia de clados endémicos en ciertas provincias oceanográficas, incluidas las aguas subtropicales del Pacífico Sur (Hirai *et al.*, 2015).

A la fecha no existen publicaciones científicas que den cuenta de los patrones biogeográficos y biogeoquímicos de la comunidad planctónica| en el PSO. Por ejemplo, la interacción entre el gradiente físico/biogeoquímico y la estructura de la comunidad zooplanctónica es desconocida para esta gran región que cubre desde la zona de surgencia costera hasta el centro del giro del Pacífico Sur. Comprender dicha interacción puede proporcionar información sobre los procesos ecológicos y evolutivos que controlan la diversidad y la adaptabilidad del plancton a un océano cambiante, tanto en el espacio como en el tiempo. No obstante, se vislumbra la necesidad de contar con una mayor cobertura y resolución tanto espacial como temporal que permita aumentar la precisión y exactitud en la detención las discontinuidades ecológicas en esta región. En el presente proyecto se abordará esta temática con una gran cantidad de condiciones de la columna de agua, incorporando además análisis biogeoquímicos, taxonómicos y genéticos del zooplancton desde la zona de surgencia costera hasta región ultra-oligotrófica del PSO.

El presente estudio se enfocará entender como la variabilidad oceanográfica afecta la estructuración ecológica y biogeoquímica del zooplancton en el PSO. Tal línea de investigación no ha sido desarrollada en esta región y constituye la base motivacional de este trabajo.

1.5. Hipótesis

- Hipótesis 1: La heterogeneidad oceanográfica del gradiente zonal en el Pacífico Suroriental genera una zonación ecológica y biogeoquímica desde la zona de afloramiento hasta el giro central del Pacífico sur.
- Hipótesis 2: El efecto de los gradientes oceanográficos y zonación observados en el Pacifico Suroriental se manifiesta en la conformación de patrones biogeográficos significativos tanto en familias y especies de copépodos del orden Calanoida.
- Hipótesis 3: La zonación ecológica observada a través del gradiente zonal en el Pacífico Suroriental determina la estructuración y diversidad genética de poblaciones de copépodos Calanoida de amplia distribución.

1.6. Objetivo general

Comprender la influencia de la variabilidad espacial oceanográfica sobre los procesos que determinan los patrones biogeográficos, biogeoquímicos y estructuración genética del zooplancton en el Pacífico Suroriental.

1.7. Objetivos Específicos

- Objetivo 1: Comprender la relación entre procesos biogeoquímicos y la conformación de eco-regiones del zooplancton a través del gradiente zonal del Pacífico Suroriental.
- Objetivo 2: Identificar los patrones biogeográficos de familias y especies de copépodos del orden Calanoida y su asociación a la variabilidad oceanográfica y zonación latitudinal y longitudinal del Pacifico Suroriental.
- Objetivo 3: Determinar el efecto de la zonación ecológica y ambiental sobre la diversidad y estructuración genética de un copépodo Calanoida, *Pleurommama abdominales,* de amplia distribución en el Pacifico Sur.

2. MATERIALES Y METODOS

2.1. Características del área de estudio.

Una de las regiones más grandes y menos exploradas del océano abierto mundial es el Pacífico Suroriental (PSO). Esta región muestra una gran heterogeneidad en las condiciones oceanográficas, principalmente a lo largo de un gradiente longitudinal desde la zona costera hasta el océano abierto, con un aumento de la profundidad de la capa de mezcla (40-150 m), temperatura superficial (14-20°C), salinidad superficial (34.8-35.8) y concentración superficial de oxígeno disuelto (1.5-4.5 ml L-1) (Gonzalez *et al.*, 2018; Palma y Silva, 2006). Sin embargo, dentro de un gradiente latitudinal también es posible observar fuertes diferencias en temperatura (12-17°C), salinidad (33.7- 35.0) y niveles de oxigenación (0-6 ml L^1) en aguas superficiales (Silva *et al.*, 2009). Junto con marcados cambios de estacionalidad desde áreas de baja latitud, con un ciclo anual más estable, hasta áreas de más alta con una fuerte estacionalidad (Strub *et al.*, 1998).

La gran variabilidad del PSO también se manifiesta claramente en la productividad de las aguas superficiales, desde la zona de surgencia costera altamente productiva (corriente de Humboldt), hasta el giro central ultra-oligotrófico del Pacífico Suroriental (GPSO) (Bonnet *et al.*, 2008; Davenport *et al.*, 1992; Morel *et al.*, 2010; Kletou y Hall-Spencer, 2012). Además, dentro de los gradientes tanto longitudinales como latitudinales hay un predominio de diferentes masas de agua que promueven una mayor variabilidad oceanográfica en este vasto ecosistema (Palma and Silva, 2006; Palma *et al.*, 2009). El GPSO es el giro subtropical más grande, con una extensión de área de $18x10^6$ km^2 (von Dassow y Collado Fabbri, 2014), y posee las tasas de producción primaria más bajas informadas (1-2 µg C L^{-1} day^{-1}) (Raimbault *et al.*, 2008). La condición ultra-oligotrófica del GPSO contrasta con la muy alta productividad del sistema de surgencia frente a las costas de Chile, Perú y Ecuador, también conocido como Sistema de Corriente de Humboldt (SCH), que es uno de los más productivos ecosistemas de borde oriental en el mundo (Davenport *et al.*, 1992; Thiel *et al.*, 2007). La producción primaria en la zona de surgencia alcanza hasta 19.9 g C m^{-1} d^{-1} (Daneri *et al.*, 2000). Esta producción impulsada por la surgencia se extiende longitudinalmente hasta la región mesotrófica, también conocida como zona de transición costera (Yuras *et al.*, 2015), que constituye una región intermedia entre el sistema de surgencia costera y la región oligotrófica del PSO.

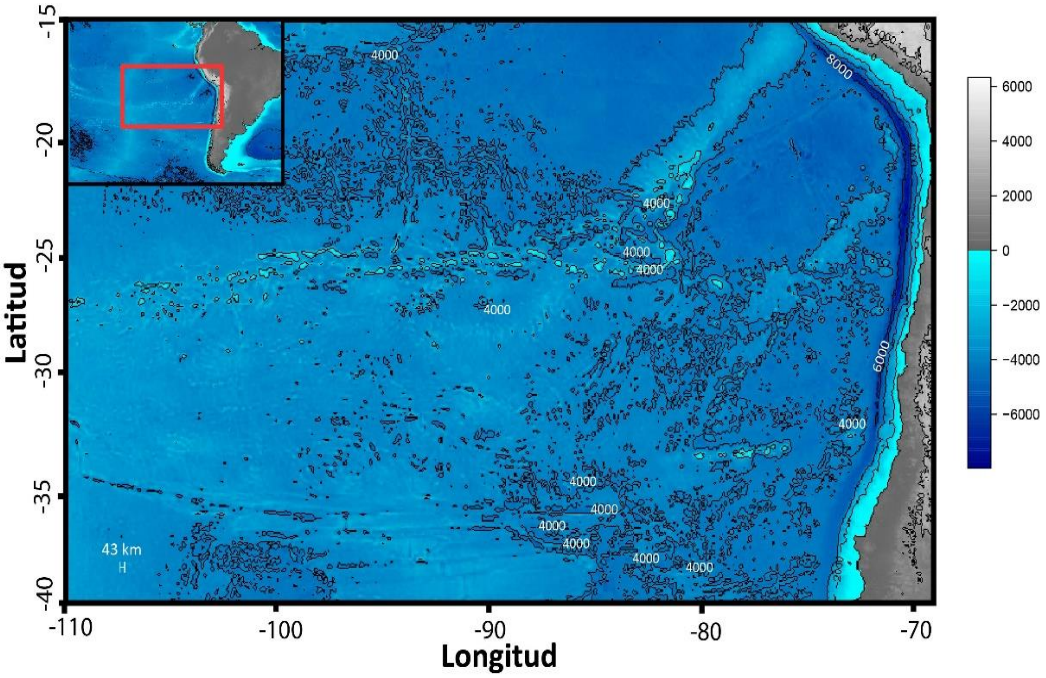

Figura 2. Región del Pacífico Suroriental donde se realizaron muestreos de zooplancton desde la zona de afloramiento costero hasta el giro central del Pacifico Sur. La batimetría de la región es indicada por su color y las isobaras de 4000, 6000 y 8000 m.

2.2. Diseño de muestreo y obtención de muestras

Las muestras utilizadas en este estudio provienen de diversas fuentes. Para el objetivo 1, las muestras fueron obtenidas a partir del crucero CIMAR-21 que se realizó en octubre del 2015 a bordo buque chileno Cabo de Hornos. El muestreo de zooplancton se hizo mediante el uso de una red Hydrobios Multinet tipo Midi con una boca de apertura de 0.25 m^2 y tamaño de malla de 200 μm. Se seleccionaron para este objetivo dos estratos superiores, es decir, 0–100 y 100–200 m. Una fracción de estos estratos fue congelada y posteriormente mezclada para aumentar la cantidad de zooplancton para los análisis isotópicos y de composición elemental. La otra fracción se conservó en formalina para los análisis taxonómicos.

En el objetivo 2, la mayoría de las bases de datos disponibles se centraron en los 200 m superiores, aunque algunas de ellas se obtuvieron hasta 2000 m sobre la trinchera o fuera de la zona costera. El período de muestreo para este objetivo abarcó desde 1985 hasta 2016, los cuales se centraron en el estudio del mesozooplancton (200 - 2000 µm), que está compuesto mayoritariamente por copépodos. Las muestras provinieron de dos series de tiempo (Mejillones y Concepción) y otras siete campañas oceanográficas desplegadas a lo largo del Pacifico Suroriental. Se hizo con uso de diferentes redes, según la disponibilidad o logística, tales como Tucker Trawl (1y 8 m^2 de abertura y malla de 200 y 300 µm), Multinet (0.5 m^2, 200 µm) Bongo (60 cm diámetro, 300 µm) y WP2 (0,25 m^2, 200 µm). Para cada uno de los muestreos disponible se seleccionaron las familias y especies pertenecientes al orden Calanoida con su respectiva abundancia. Paralelamente, los datos taxonómicos fueron complementados con secuencias del citocromo c oxidasa subunidad I (COI) y la subunidad grande del ribosoma (28S) de las especies más abundantes reportadas en el Pacifico Suroriental.

En el objetivo 3, los individuos de *Pleuromamma abodminalis* fueron recolectados durante dos cruceros oceanográficos realizados en el Pacifico Suroriental. El primer crucero se centró en un transecto entre Caldera (27°S, 70°50'W) e Isla de Pascua (27° 10'S, 109°30'W). El segundo crucero abarco dos transectos zonales a 26.5°S y 33°S, y un transecto meridional, entre las Islas Desventuradas y el Archipiélago de Juan Fernández. Ambos cruceros se realizaron a bordo buque chileno Cabo de Hornos. Las muestras se recolectaron en un total de 13 sitios durante los cruceros CIMAR-21 y CIMAR-22. El mesozooplancton se recolectó con una red de arrastre Tucker de 1 m^2 (malla de 200 µm) en ambos cruceros, y una red de arrastre Tucker de 8 m^2 (tamaño de malla de 300 µm) en CIMAR-22. Se seleccionaron hembras adultas de muestras de mesozooplancton y se identificaron como *Pleuromamma abdominalis* con la ayuda de claves taxonómicas. Los individuos seleccionados fueron preservados en etanol y se almacenaron a -20 °C para sus posteriores análisis genéticos.

La información disponible para los tres objetivos de esta investigación abarca un gran gradiente oceanográfico, por lo que se diferenció la región utilizando datos satelitales de clorofila-a (Chla), con observaciones durante los cruceros CIMAR-21 y CIMAR-22 (https://coastwatch.pfeg.noaa.gov). Se distinguieron cuatro zonas según los rangos de Chla observados en la superficie: una zona eutrófica correspondiente a la región de surgencia costera con Chla> 0.5 mg m^{-3} (CUP-Z), zona de transición costera mesotrófica (CTZ) que mostró una rango de Chla entre 0.1-0.5 mg m^{-3}, zona oligotrófica (OLIGO-Z) con una variación entre 0.05 - 0.10 mg m^{-3}, y finalmente una zona ultra-oligotrófica (U-OLIGO-Z) que se encuentra en la región central del giro del

Pacífico Sur con concentraciones de Chla <0.05 mg $m^{-3.}$ Zonaciones similares han sido reportadas en trabajos anteriores (González *et al.*, 2018; Raimbault *et al.*, 2008; von Dassow y Collado Fabbri, 2014).

2.3. Zonación ecológica y biogeoquímica del zooplancton

Las muestras congeladas de zooplancton se descongelaron para diluirlas con agua de mar filtrada (filtro GF / C) y posteriormente fraccionarlas en cinco clases de tamaños utilizando tamices. Los tamices fueron 2,000, 1,000, 500, 200 y 60 μm, por lo que se definieron las siguientes clases de tamaño: <200 μm, 200–500 μm, 500–1,000 μm, 1,000–2,000 μm,> 2,000 μm. Todas las muestras fraccionadas se concentraron en filtros GF/C previamente pesados y luego se dejaron secar en un horno a 70°C durante 48 h. Cada muestra sobre el filtro se retiró y homogenizo para tener un peso seco de 1mg para determinar su composición elemental (C: N) y abundancia de isotopos estables (δ^{15}N - δ^{13}C). Los análisis fueron realizados en el Laboratorio LABASI de Isótopos Estables y Biogeoquímica de la Pontificia Universidad Católica (Chile). Se obtuvo una alta variación en el contenido de lípidos en las muestras, como se muestra en el valor de la razón C: N> 3.5. Por lo tanto, nosotros normalizamos los valores de δ^{13}C utilizando una relación empírica con el valor de la muestra C: N (en peso) según lo determinado por el metaanálisis con datos de animales acuáticos (Post *et al.*, 2007): $\delta^{13}Ccorr = \delta^{13}C -3.32 + 0.99$ C: N.

Las muestras conservadas en formalina fueron analizadas por ZooScan Hydroptic, para obtener imágenes digitalizadas de las muestras, que posteriormente fueron procesadas con el software ZooProcess V1.5 (Gorsky *et al.*, 2010). Este método automatizado para analizar el zooplancton se basa en el enfoque de RAPID (Benfield *et al.*, 2007) que nos permite obtener la distribución por tamaño, identificación y recuento de las principales categorías taxonómicas dominantes.

2.4. Patrones biogeográficos de copépodos del orden Calanoida

Las bases de datos de abundancia de especies del Pacifico Suroriental se utilizaron como número individuos m^3 independientemente de la profundidad de los estratos. Para cada punto de muestreo se calculó el valor promedio de la abundancia para todas las especies y familias en función del número de observaciones realizadas en cada sitio. Sin embargo, debido a la alta variabilidad estacional en la abundancia y la mayor cobertura temporal en la CUP-Z, calculamos la abundancia promedio para cada sitio de muestreo por separado para los períodos de primavera-verano (septiembre a marzo) y otoño-invierno (abril a agosto). Los cambios en las familias de copépodos se evaluaron para las diferentes regiones y estaciones utilizando descriptores comunitarios, como la riqueza de familias (R), abundancia numérica (N) y la frecuencia ponderada de ocurrencia (WFO) definidas como:

$$\mathrm{WFO} = \left(\frac{\frac{\mathrm{NF}i}{\mathrm{NS}}}{\mathrm{REF}i} \right)$$

donde NF es el número de estaciones en las que estaba presente la familia *i*, NS es el número total de estaciones muestreadas y REF es el número de especies presentes en la familia *i*. Además, nosotros modificamos la ecuación de la profundidad media ponderada sugerida por Andersen *et al.*, 2004, para estimar la abundancia promedio ponderada de las familias encontradas tanto en los gradientes longitudinales y latitudinales del Pacifico Suroriental. Para esto, estimamos la abundancia ponderada a través del gradiente costa-océano (CSWA) y latitudinal (LWA) de las familias de copépodos como:

$$\mathrm{LWA\ Or\ CSWA} = \frac{\sum(\mathrm{N}i\ \mathrm{Z}i\ \mathrm{R}i)}{\sum(\mathrm{N}i\ \mathrm{R}i)}$$

donde N*i* es la abundancia promedio estandarizada a ind.m^3 para la familia *i*, Z*i* es la distancia en km entre un punto de inicio y termino predefinidos para la zona de estudio *i* y R*i* es la mitad de la diferencia entre estos límites predefinidos de la zona *i*.

2.5. Análisis genéticos

2.5.1. Extracción y amplificación del ADN

Las muestras seleccionadas para el análisis genético se transfirieron a un tubo Eppendorf con 1 ml de agua MilliQ y se incubaron a temperatura ambiente durante 12 horas, para eliminar el etanol restante. El ADN genómico de las muestras recolectadas en los cruceros CIMAR-21 y CIMAR-22, se extrajeron con kit de ADN Mollusc y

Forense respectivamente, siguiendo los protocolos del fabricante. El ADN extraído se mantuvo a -20°C.

En los análisis filogenéticos se utilizaron fragmentos de los genes que codifican para las proteínas mitocondriales COI y citocromo b (Cytb), así como genes nucleares que codifican el 28S y regiones espaciadoras transcritas internas (ITS1-5.8SrDNA-ITS2) mediante la reacción en cadena de la polimerasa (PCR). Los productos de PCR obtenidos se enviaron a Macrogen para su posterior secuenciación.

2.5.2. Alineamiento y análisis filogenéticos

El alineamiento de las secuencias tanto para el objetivo 2 y 3 fueron construidos con MUSCLE en Geneious 7.1.3 (Duran *et al.,* 2012). Los análisis filogenéticos se realizaron utilizando los métodos de máxima verosimilitud (ML) e inferencia bayesiana (BI). Los modelos sustitución nucleotídica fueron seleccionados por el Criterio de Información Bayesiano (BIC) en Mega-X V.10 (Kumar *et al.*, 2018). Los análisis bayesianos se realizaron en MrBayes V.3.1 (Ronquist, *et al.,* 2012). En cambio, los análisis de máxima verosimilitud se ejecutaron con el modelo GTR + G en RAxML V.8.2.10 (Stamatakis *et al.*, 2014).

Las distancias genéticas fueron calculadas para los genes mitocondriales, tanto para el objetivo 2 y 3, utilizando los modelos de sustitución de nucleotídica en Mega-X V.10 (Kumar *et al.*, 2018). Paralelamente, para el objetivo 2 se utilizó Popart V 1.7 (Leigh y Bryant, 2015), para crear una presentación gráfica de una red TCS para los fragmentos 28S e ITS, que ilustra la relación entre los haplotipos nucleares y los clados mitocondriales.

2.5.3. Delimitación de especies

Se utilizaron dos enfoques analíticos para delimitar las especies de *Pleuromamma* en el Pacifico Suroriental, el método de probabilidad Generalized Mixed Yule Coalescent (GMYC) y el modelo Poisson Tree Process (PTP), utilizando los árboles de los genes COI y Cytb (Previšić *et al.*, 2016). El análisis GYMC es un método de probabilidad que permite la delimitación del número de especies según un modelo que se ajusta a los patrones de ramificación esperados dentro y entre las especies (Fujisawa *et al.,* 2013). Inferimos árboles genéticos ultraméricos para COI y Cytb utilizando BEAST 2.0 (Bouckaert *et al.,* 2014). Los archivos de salida se visualizaron en

Tracer v.1.6 (Rambaut y Drummond, 2007) para asegurar la convergencia de las cadenas. El árbol de máxima credibilidad de clade (MCC) fue determinado por Tree Annotator v.1.8 (Rambaut y Drummond, 2013). La delimitación de especies se infirió para cada árbol de genes MCC usando la función "gmyc" en el paquete ‘splits’ de Rstudio V.3.5 (Ezard *et al.*, 2009). Paralelamente, se evaluo delimitación de especies por PTP utilizando el servidor web bPTP http://species.h-its.org/ptp/. Este modelo utiliza la teoría de coalescencia y calcula la tasa de especiación a partir del número de sustituciones observadas en el árbol (Zhang *et al.*, 2013).

2.6. Data oceanográfica

Para caracterizar las condiciones oceanográficas del Pacifico Suroriental en cada uno de los objetivos planteados en esta investigación se utilizó una serie de datos satelitales y valores *in-situ*:

- Objetivo 1: se utilizaron datos provenientes de una Rosette-CTDO para obtener perfiles verticales de temperatura, salinidad y oxígeno disuelto. Además, esta información fue complementada con datos satelitales de temperatura (SST) (https://oceancolor.gsfc.nasa.gov/) y corriente geostrófica superficiales (www.aviso.altimetry.fr). Todos datos satelitales utilizados fueron calculados como promedios diarios para el periodo de muestreo.
- Objetivo 2: se usó la SST (https://coastwatch.pfeg.noaa.gov) como un indicador de las condiciones oceanográficas para el período de estudio. Para cada sitio de muestreo se utilizó la SST promedio de los últimos treinta años.
- Objetivo 3: se descargaron datos satelitales de Chla, SST y radiación fotosintéticamente activa (PAR) (https://podaac.jpl.nasa.gov), estrés del viento (WT) (https://manati.star.nesdis.noaa.gov), salinidad superficial (SSS) (https://earth.esa.int), índice de surgencia (UI) y curvatura del estrés del viento (WSC) (https://data.noaa.gov/dataset). Para cada estación de muestreo, el valor promedio se calculó durante tres días, desde un día antes hasta un día después del muestreo.

2.7. Análisis estadísticos

Se examinó para el objetivo 1 la variabilidad en la abundancia de zooplancton, biomasa, fracciones de tamaño, contenido de C y N y $\delta^{15}N$ y $\delta^{13}C$, así como para las variables oceanográficas, en las cuatro zonas, y se probaron las diferencias mediante

Kruskal-Wallis y GLM (Modelos lineales generalizados) utilizando el software SYSTAT 12 (Wilkinson, 2007). Por otro lado, se evaluó el uso de diferentes recursos por las clases de tamaño en cada zona mediante estimaciones del nicho isotópico. Para este análisis, la amplitud del nicho se calculó a partir del espacio delimitado por los valores de $\delta^{15}N$ vs $\delta^{13}C_{corr}$ con el paquete 'SIBER' desarrollado en Rstudio V.3.5 (Jackson *et al.*, 2011).

La variabilidad en la estructura de la comunitaria tanto en el objetivo 1 y 2 se evaluó mediante análisis multivariados realizados con PRIMER v.7 (Clarke y Gorley, 2006). Primero, se evaluó las similitudes dentro y entre las zonas en términos de abundancia relativa o presencia/ausencia. Se realizó un análisis de agrupamiento y escalamiento multidimensional (NMDS) con distintas transformaciones e índices de distancia dependiendo de la naturaleza de los datos. En segundo lugar, se utilizaron ANOSIM y PEMANOVA para probar la hipótesis de que la estructura comunitaria difiere entre las zonas. Finalmente, las diferencias o similitudes entre las áreas de estudio se evaluaron con SIMPER (porcentajes de similitud).

Para evaluar el efecto de la variabilidad oceanográfica sobre los objetivos 2 y 3 se emplearon una serie de análisis no paramétricos. Primero, para el objetivo 2 se utilizó el análisis de redundancia (dbRDA - análisis de redundancia basado en distancia) para examinar la influencia de la temperatura sobre la distribución de las especies y las familias dentro del orden Calanoida realizado con PRIMER v.7 (Clarke y Gorley, 2006). En segundo lugar, se evaluó una prueba Mantel para determinar si distancia genética de las especies más abundantes de cada zona estaba correlacionada con la SST mediante el paquete 'ape' de Rstudio V.3.5 (Paradis *et al.*, 2004). Finalmente, para el objetivo 3 se utilizó un análisis de análisis de correlación canónica (CCA) para evaluar la asociación entre variables oceanográficas (SSS, WT, IU, WSC y PAR) y los clados mitocondriales utilizando el paquete 'CCA' en Rstudio V.3.5 (Ter Braak, 1986).

3. RESULTADOS

3.1. Capitulo 1. Artículo publicado en Frontiers in Marine Science, doi: 10.1016/j.pocean.2011.07.003

Estructura taxonómica y trófica del zooplancton asociado a regiones biogeoquímicas en el Pacífico Suroriental

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Resumen

Las condiciones bioquímicas y la composición taxonómica del mesozooplancton fraccionado por tamaño fueron estudiados después de un crucero realizado en septiembre de 2015 entre la costa chilena (70°W) e Isla de Pascua (110°W) dentro del giro central del Pacífico sur. La taxonomía se evaluó con un método automatizado basado en el análisis de imágenes y las condiciones bioquímicas fueron evaluadas mediante el análisis de los contenidos de C y N y la composición de sus isótopos estables. Basado en los niveles superficiales de clorofila-a, cuatro regiones fueron distinguidas a través del gradiente zonal: eutrófica (zona de surgencia chilena), mesotrófica (Zona de transición costera), oligotrófica (océano abierto) y ultra-oligotrófico (centro del giro Pacífico Sur). Las zonas tuvieron marcadas diferencias en temperatura, oxígeno, salinidad y clorofila-a, y también mostraron diferencias significativas en la composición del zooplancton, la razón C/N, y $\delta^{13}C$ y $\delta^{15}N$ para todas las fracciones de tamaño del zooplancton. Variabilidad en las fuentes de C y N, vinculada a procesos biogeoquímicos, tales como producción nueva y desnitrificación en la zona de surgencia, diazotrofía potencial, presencia de C y N altamente regenerados, y oligotrofia extrema (deficiencia de N) en áreas oceánicas, se sugieren como los principales impulsores de estas diferencias. Nuestros hallazgos también sugieren un fuerte acoplamiento entre la estructura comunitaria (taxonomía y tamaño), la zooplancton-diversidad y las fuentes de nutrientes que sustentan el fitoplancton, como principal fuente de alimento para el zooplancton. Aunque múltiples factores y procesos pueden modular el C, N y la composición de isotópica del zooplancton,

nuestro estudio muestra que los cambios en la estructura de la comunidad están vinculados a diferentes regiones biogeoquímicas a través del gradiente zonal, originando la base para la zonación ecológica asociada con la utilización de nutrientes en niveles tróficos inferiores.

Zooplankton Taxonomic and Trophic Community Structure Across Biogeochemical Regions in the Eastern South Pacific

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Abstract

Biochemical conditions and taxonomic composition of size-fractioned mesozooplankton were studied after a cruise conducted in September 2015 between the Chilean coast (70°W) and Easter Island (110°W) within the central south Pacific gyre. Taxonomy was assessed with an automated method based on image analysis and biochemical conditions assessed by analyses of C and N contents and stable isotope composition. Based on surface Chlorophyll-a levels, four regions were distinguished across the zonal gradient: eutrophic (Chilean upwelling zone), mesotrophic (Coastal Transition Zone), oligotrophic (open ocean water) and ultraoligotrophic (central south Pacific gyre). The zones had marked differences in temperature, oxygen, salinity and Chlorophyll-a, and they also exhibited significant differences in zooplankton composition, C/N ratios and $\delta^{13}C$ and $\delta^{15}N$ for all size fractions of zooplankton. Variability in the sources of C and N, linked to biogeochemical processes, such as new production and denitrification in the upwelling zone, potential diazotrophy, highly regenerated C and N and extreme oligotrophy (N-deficiency) in oceanic areas, are

suggested as the key drivers of these differences. Our findings also suggest a strong coupling between taxonomic and size zooplankton-diversity and the sources of nutrients that fuel phytoplankton, the major food source for zooplankton. Although multiple factors and processes can modulate C and N and their isotopes composition of zooplankton biomass, our study shows that changes in community structure are linked to different biogeochemical regions across the zonal gradient, providing the basis for ecological zonation associated with nutrient utilization at lower trophic levels.

3.1.1. Introduction

The eastern South Pacific region is characterized by a strong coastal-offshore oceanographic gradient from the highly productive (eutrophic) coastal upwelling zone toward the oligotrophic and even ultraoligotrophic region in the central south Pacific gyre (Raimbault and Garcia, 2008; Von Dassow and Collado-Fabbri, 2014; Moutin *et al.,* 2017).

A substantial amount of research has been conducted on the functioning and structure of the eastern boundary upwelling system (EBUS) off Chile (e.g., Cury et al., 2000; Daneri *et al.,* 2000; Hormazabal *et al.,* 2001), including the mesotrophic coastal transition zone (CTZ) (Morales et al., 1999, 2010). However, the vast oceanic oligotrophic region remains largely unknown (Von Dassow and Collado-Fabbri, 2014). A few expeditions have reported the plankton composition in the region (Fagetti and Fisher, 1964; Hernández *et al.,* 2005; Palma and Silva, 2006), although there is a lack of knowledge on how these pelagic communities can be sustained in terms of their sources of organic C and N, considering the very low levels of primary production (Raimbault and Garcia, 2008).

From the highly productive Chilean coastal zone to the oceanic Easter Island (~4,500 km from shore), and over this oceanographic gradient, the plankton community is subject to gradual, and sometimes abrupt changes in temperature, oxygenation, salinity, pH, food quality and quantity, and possibly to a variety of ecological and biogeochemical processes, such as primary production, diazotrophy, denitrification among others, altogether modulating their sources of nutrients supporting the production of organic C and N (Raimbault and Garcia, 2008; Fernandez *et al.,* 2011; Farías *et al.,* 2013). However, in spite of the strong variability in oceanographic conditions observed over the zonal gradient of the eastern South Pacific, only two biogeochemical provinces have been suggested for the whole area which are characterized by globally unique and distinguishable biogeochemical processes, corresponding to the provinces of the Humboldt current coast and South Pacific gyre (Oliver and Irwin, 2008; Longhurst, 2010; Reygondeau *et al.,* 2013). Within these provinces it is then possible to distinguish ecoregions, being defined on the basis of species composition and ecological dynamics. These features within ecoregions are relatively homogeneous, and stable with respect to the adjacent regions. In the eastern

South Pacific the suggested ecoregions are Central Peru, Humboldtian, Central Chile, Araucanian, Juan Fernández and Desventuradas (Spalding *et al.,* 2007). Nevertheless, the ecological and biogeochemical partitioning of the entire zonal gradient is unclear from the point of view of the plankton community. For instance, the interaction between the physical/biogeochemical gradient and the zooplankton community structure is unknown for this large region covering the area from the coastal upwelling zone up to the central Pacific gyre. Understanding such interaction can provide insights on the ecological and evolutionary processes controlling diversity and adaptability of plankton to a changing ocean, both in space and time.

Zooplankton plays an important role in the pelagic zone because they are a key element in the transfer of organic matter from primary producers to higher levels (Saiz *et al.,* 2007). In this respect, the study of the stable isotopes composition of C and N in zooplankton can provide highly valuable information to determine the sources of C and N being transferred through the food web (Peterson and Fry, 1987). Furthermore, isotopes are widely used as markers for depicting the food web and trophic position of organisms, due to the enrichment of heavy isotopes in the tissue of predators with respect to their prey, since the light isotopes are mobilized with greater speed in chemical reactions (Post, 2002; El-Sabaawi *et al.,* 2013; Wang *et al.,* 2014). An important advantage of these analyses is that they can also provide a quantitative characterization of the trophic niche of species, communities or ecosystems (Layman *et al.,* 2007, 2012).

In this study, we assessed the mesozooplankton community structure (size and taxonomic composition) along the coastal-offshore gradient, their size-fractionated C and N contents, and corresponding ($\delta^{13}C$ and $\delta^{15}N$) in relation with the zonal variability in temperature, salinity, oxygen and Chlorophyll-a. We aimed at elucidating how changing oceanographic conditions across the coastal-offshore gradient and different biogeochemistry processes determining the sources of C and N affecting the food resource of zooplankton, can influence their biochemical conditions and community structure.

3.1.2. Materials and Methods

3.1.2.1. Oceanographic Survey

The CIMAR-21 cruise was carried out during 01–30 October 2015 onboard the Chilean R/V Cabo de Hornos. The cruise surveyed the transect between Caldera (27°S, 70°50′ W) and Easter Island (27°10′ S, 109°30′ W) covering 30 oceanographic stations (Figure 1A). We performed zooplankton sampling in 17 stations as indicated in Figure 1. At each station a Rosette-CTDO was deployed to a maximum depth of 1,500 m to obtain vertical profiles of temperature, salinity and dissolved oxygen. Water samples were also obtained at 15 depths for chemical analyses of oxygen, salinity and nutrients. At the zooplankton stations a Hydrobios Multinet Midi type of 0.25 m^2 opening mouth was vertically hauled from 800 m to surface. The Multinet had five nets 200 μm mesh-size which were electronically opened and closed at five depth strata: 0–100, 100–200, 200–400, 400–600 and 600–800 m. Hauling speed was 1 ms^{-1} and the samples were collected at different times of the day and likely different light conditions (Table 1). There were five stations sampled at nighttime conditions Once onboard, samples were collected in buckets and depending on the amount of zooplankton they were split in two fractions; one fraction was immediately preserved in 10% formalin and the second fraction was frozen at -20°C for C and N measurements. Small samples were frozen as whole.

3.1.2.2. Zooplankton Analyses

In our study, we used samples from the two upper layers, i.e., 0–100 and 100–200 m. Frozen samples from these strata were first thawed and both mixed to increase the amount of zooplankton. Half of this sample was preserved in formalin for analysis of composition. Samples were first diluted with filtered seawater (GF/C filters) and then fractioned in five size classes using sieves. The sieves were 2,000, 1,000, 500, 200 and 60 μm, so that the following size classes were defined: <200 μm, 200–500 μm, 500–1,000 μm, 1,000–2,000 μm, >2,000 μm. All fractionated samples were then received onto pre-weighed GF/C filters, dried in an oven at 70°C for 48 h. Each filter sample was removed with a spatula and homogenized with a pestle to have a dry weight of about 1 mg to feed an elemental analyzer (Flash EA2000) coupled with a mass spectrometer isotope ratio (DELTA V The IRMS). Abundance values of natural stable isotopes were reported as $\delta^{15}N$ or $\delta^{13}C$ (‰) relative to atmospheric nitrogen and Vienna Pee Dee

Belemnite, respectively (Coplen, 2011). The associated isotopic error was 0.218 and 0.43‰ for Nitrogen and Carbon, respectively. In turn, the error associated in the elemental analysis was 0.001 and 0.01 mg for Nitrogen and Carbon correspondingly, as assessed by a linear regression. The standard used for this calculation was Acetanilide, which contains 71.10% carbon and 10.36% nitrogen. For the calculation of the isotopic proportion, we used four previously standardized internal standards, Acetanilide, Atropine, Caffeine, Organic Sediment and Glutamic Acid. Determination of the isotopic composition and elemental contents were performed by LABASI Laboratory of Biogeochemistry and Applied Stable Isotopes of the Pontificia Universidad Católica (Chile). A high variation in lipid content in zooplankton samples was obtained, as shown in the value of the ratio C:N > 3.5. Therefore, $\delta^{13}C$ values were normalized ($\delta^{13}Ccorr$) using an empirical relationship with the sample C: N value (by weight) as determined by meta-analysis with data from aquatic animals (Post *et al.*, 2007): $\delta^{13}C_{corr} = \delta^{13}C - 3.32 + 0.99$ C: N.

All the samples preserved in formalin were analyzed by a ZooScan Hydroptic to obtain digitized images of samples which were thereafter processed with ZooProcess software V1.5. This automated method to analyze zooplankton is based on the RAPID approach (Benfield *et al.,* 2007) which allows us to obtain size distribution of zooplankton and identification and counting of major dominant taxonomic categories. For this, samples are scanned with a resolution of 2,400 dpi with no staining.

3.1.2.3. Data Analysis

Using satellite data of Chlorophyll-a (Chla), available for the cruise period from (https://oceancolor.gsfc.nasa.gov/), we defined four zones in according to observed ranges of surface Chla: the eutrophic zone corresponding to the coastal upwelling area off Chile with Chla >0.5 mg m^{-3} (CUP-Z), the mesotrophic CTZ with a Chla range of 0.1–0.5 mg m^{-3}, the oligotrophic area (OLIGO-Z) with a Chla range of 0.05–0.10 mg m^{-3}, and the ultra-oligotrophic region (U-OLIGO-Z) corresponding to the central south Pacific gyre with Chla concentrations <0.05 mg m^{-3} (Figure 1A). Satellite surface Chla were obtained. Complementary satellite data on sea surface temperature was obtained from the NASA's Ocean Biology Processing Group (OBPG) https://oceancolor.gsfc.nasa.gov/, and surface geostrophic current also from www.aviso.altimetry.fr (Figure 1B). All data used for the Chla, temperature and geostrophy maps were daily averages for the sampling period, 13–27 October 2015, and covered the area surrounding the oceanographic transect.

Variability in zooplankton abundance, biomass, size-fractions, C and N contents and δ^{15}N and δ^{13}C, as well as for oceanographic variables, across the four zones were examined, and differences tested by means of Kruskal–Wallis and GLM (General Linear Models) using the software SYSTAT 12 (Systat Software Inc., San Jose, CA, United States). In most cases, log-normalized variables were used for GLM and in other cases we used the non-parametric test Kruskal–Wallis, due to significant deviations from normality and homogeneity of variance, as examined by the Shapiro–Wilks and Levene tests, respectively. The post hoc tests used were those of the Pgirmess package of R, which performs multiple Tukey's range comparisons after Kruskal–Wallis test (Dickinson and Chakraborti, 2003) and the Games-Howell Test to perform paired comparisons in the case GLM.

The use of different resources by zooplankton size classes in each zone was investigated by estimations of the isotopic niche. For this analysis, the niche amplitude was calculated from the space delimited by the values of δ^{15}N vs δ^{13}Ccorr for all study areas and size classes with the SIBER package developed in the R program (Jackson *et al.*, 2011). The standard ellipses calculated could thus represent the distinct food sources and their utilization (Layman *et al.*, 2007, 2012). Layman metrics (Layman *et al.*, 2007) were estimated to describe these ellipses, as follows: NR: range of δ^{15}N (diversity of trophic levels and nitrogen sources), CR: range of $\delta^{13}C_{corr}$ (diversity of carbon sources), TA: total area of the convex hull encompassing all data points (trophic diversity and niche size), CD: centroid distance (average measure of trophic diversity among consumers), MNND: mean distance to the nearest neighbor (trophic redundancy, lower when isotopic niches are similar) and SDNND: SD of distance to the nearest neighbor (trophic equality). Maximum likelihood estimates of ellipse areas were used for graphical representation while estimates of niche overlap were made from ellipses encompassing 95% of data. Niche width was estimated using Bayesian inference of the standard ellipse areas to account for the uncertainty in sample data (Jackson *et al.*, 2011) after 10^4 estimations per ellipse.

The variability in community structure across the study area was studied from the taxonomic categories provided from the ZooScan analysis, expressed in individuals m^{-3}. The community structure was assessed by means of multivariate analyses performed with PRIMER v.7 (Clarke and Gorley, 2006). First, we evaluated similarities within and between zones in terms of relative abundance. A cluster analysis and multidimensional scaling (NMDS) were done after a fourth root transformation of data, with the Bray-Curtis index as a distance measurement. Second, ANOSIM was used to test the hypothesis that community structure and abundance of zooplankton differed among zones. Finally, the differences or similarities between the study areas were further assessed with SIMPER (percentages of similarity).

3.1.3. Results

3.1.3.1. Oceanographic Characterization

Temperature of the water column throughout the coast-offshore gradient (Figure 2A) showed that the upper layer ranging between surface and 50–300 m, depending on the region, accounted for most of the variation. This layer was approximately delimited by the 14°C isotherm. The CUP-Z had the lowest near-surface temperature (<15°C) over a narrow upper layer of less than 100 m. The CTZ showed a temperature range between 16 and 18°C within the upper 200 m with presence of a surface front at about 78°W. The OLIGO-Z was more extensive and its temperature in the upper 200 m varied between 18 and 19°C, whereas the U-OLIGO-Z exhibited a surface warming reaching down to 300 m depth, and a temperature maximum of about 21°C at the surface. Mean temperature at 10 m depth was significantly different between zones ($p < 0.001$) and post hoc tests indicated that OLIGO-Z and U-OLIGO-Z were not different in temperature (Table 2). Contrasting with temperature, salinity exhibited a greater variation, both over the horizontal and vertical gradients in the upper 1,000 m, characterized by a large and very extensive low salinity (<34.4) water mass located between ca. 300 and 1,000 m, becoming shallower toward the coast. In the upper 200 m, low salinity water (<34.6) prevailed in the CUP-Z and in the CTZ, whereas in the U-OLIGO-Za high salinity (>35.5) water mass dominated the upper 200 m intruding the western part of the OLIGO-Z (Figure 2B). Surface salinity among zones was also significantly different (test, $p < 0.001$), although post hoc tests showed no differences between OLIGO-Z and U-OLIGO-Z (Table 2). Distribution of dissolved oxygen showed a strong variation within the CUP-Z and the CTZ in the 400 m, reflecting the presence and distribution of the oxygen minimum zone (OMZ) in these areas and marking the western limit of the CTZ (Figure 2C). Mean oxygen in the upper 100 m was significantly different among zones (test, $p < 0.001$), except that CUP-Z and U-

OLIGO-Z had no difference and the same was found for OLIGO-Z and U-OLIGO-Z, in according to post hoc tests (Table 2).

Maximal zooplankton biomass was found in the upwelling zone (Table 2), decreasing abruptly toward the central gyre with extremely low values (<1% compared to the upwelling zone). Temperature, salinity and oxygen all increased toward the oceanic region, while surface Chla sharply decreased down to less than 0.1 mg m-3 in the OLIGO-Z and U-OLIGO-Z. Fractionated biomass showed that large-sized classes (>1,000 μm) had lower values than small ones (Table 3). Mean C:N ratios tended to appear higher (>7) in the small size class, but also in the largest one, whereas intermediate size classes had C: N mean ratios <6. Mean values varied in rather narrow ranges of -18.0 and -19.7 and 9.0 and 11.0, respectively (Table 3).

When including all sampling stations from the four zones, a GLM showed that zooplankton differed significantly in the C:N ratio ($p = 0.01$), $\delta \mathit{13}\mathrm{C}_{\mathrm{corr}}$ ($p = 0.01$) and δ^{15}N ($p = 0.00$) between zones (Table 4). However, size fractions of zooplankton only showed significant differences in the C:N ratio ($p < 0.01$) (Table 4). For instance, the C:N ratio strongly varied across the zonal gradient in all size classes, characterized by lower C:N ratios in the coastal zone with values between 4 and 7, and then incrementing toward offshore, reaching maximum values of ca. 15 in the OLIGO-Z in the >2,000 μm fraction, although an abrupt decrease in C:N was detected in the 1,000–2,000 μm fraction at the station near Easter Island (Figure 3A). In general, small size fractions (<500 μm) showed greater C:N ratios (7.5–14.0), whereas larger size classes (>500 μm) showed more variation between stations within zones. When comparing C:N ratios between zones, it was found that CUP-Z and CTZ were not different to each other (Test, $p > 0.05$), but CUP-Z was different from the U-OLIGO-Z (Test, p = 0.05), as well as CTZ from the U-OLIGO-Z (Test, $p < 0.05$). Pairwise comparisons between zones for isotopes composition showed that for δ^{13}C even though there significant differences among zones as found by GLM (Table 4) the Games-Howell Test was unable to detect the pairwise differences (Test, $p > 0.05$), possibly because the variance within zones was too high. In the case of δ15N the pairwise comparisons test showed that OLIGO-Z and U-OLIGO-Z did not differ (Test, $p > 0.05$), while all the others significantly differed to each other (Test, $p < 0.05$).

The distribution of δ^{13}C across the zonal gradient showed variable patterns, depending on the size fraction, although most fractions exhibited a greater value at the upwelling, a reduction in the CTZ and then higher values again toward the open ocean (Figure 3B). Different patterns were observed in the zonal distribution of $\delta^{15}N$, with a downward trend toward offshore observed in all size fractions (Figure 3C). There was a remarkable sharp decrease in $\delta^{15}N$ from the last portion of the CTZ to the OLIGO-Z and U-OLIGO-Z areas with most values of <5‰. A similar pattern through all size classes suggested the same source of N for zooplankton in these areas.

From GLM analysis differences in C:N ratios and isotopes composition of C and N size fractions were compared. Within the different size fractions, significant differences were observed only in C:N ratio ($p = 0.00$). Post hoc analyzes indicated that the fraction <200 μm was significantly different from the other four fractions (Test, $p \leq 0.05$). Also significant differences were observed between the fractions >2,000 μm and the intermediates size classes (1,000–2,000 and 500–100 μm) (Test, $p \leq 0.05$).

3.1.3.2. Isotopic Niches

The samples projected on the isospace of $\delta^{15}N$ and $\delta^{13}C$, showed distinct patterns between zones, with a greater range in δ13C in CUP-Z and U-OLIGO-Z, and a maximum of $\delta^{15}N$ in CTZ (Figure 4). In the CUP-Z and CTZ zones, values of $\delta^{15}N$ were >10‰, but in other zones, such as OLIGO-Z and U-OLIGO-Z some samples could reach values <2‰. The ellipses of the five fractions for the four zones revealed different distributions, depending on functional groups (size fractions) and the zone, but it also evidenced a large overlapping among ellipses, with the exception of the U-OLIGO-Z (Figure 4).

The Layman metrics, of the Bayesian approximation of the ellipses for the four study areas, revealed that in the OLIGO-Z and U-OLIGO-Z zones there is a potential greater number of trophic levels, and nitrogen sources when compared to the other zones (higher NR). In terms of C sources, only the region U-OLIGO-Z showed large variability (higher CR). From the TA values, the OLIGO-Z and U-OLIGO-Z zones, revealed that these regions have a large trophic diversity and niche amplitude (higher TA), and thus differing from the coastal area. The mean trophic diversity among consumers (CD) showed higher values mainly in U-OLIGO-Z, being slightly higher in the OLIGO-Z. The highest similarity in isotopic niches (low MNND) was observed in the CUP-Z and CTZ zones, while the more oceanic areas showed greater values. The distribution of trophic diversity (SDNND) was more uniform in the U-OLIGO-Z zone, and to a lesser extent in the OLIGO-Z with respect to the other ones (Table 5). As most of the variation in isotope composition was found between zones, Bayesian estimates of the ellipse areas by zones (all size-classes combined) indicated a large similarity of the isotopic niche between the coastal zones on one side and between the oligotrophic zones on the other side (Figure 5). Niche overlap was generally <20% for most zones but increased for neighboring zones, and even exceeded 50% in the oligotrophic region (Table 6).

3.1.3.3. Zooplankton Communities

When analyzing the zooplankton community by the automated approach using a ZooScan, it was found that 19 taxonomic categories could properly the composition in terms of major groups. However, 9 of them appeared as mostly controlling the total abundance for all samples. Small sized copepods (<1.5 mm), followed by large size copepods (mainly > 2 mm), were the most abundant organisms at all regions, and with greater abundances in the CUP-Z and CTZ (Figure 6). In terms of relative abundance, the prevalence of small copepods and salps increased in open ocean waters. Some taxonomic groups did not show large variations in their abundance across of the study area, such as chaetognatha, appendicularia and eggs (mainly fish eggs). While others were more abundant in specific areas, such as euphausiids in the CUP-Z region (Figure 6).

For a more detailed analysis of the zooplankton community structure, associated with the four regions, a multivariate community analysis was performed with PRIMER (V. 7). Cluster analysis (Figure 7A) showed that the CUP-Z clearly separates from the other zones, but also with strong internal variation, whereas the CTZ exhibited a more homogeneous pattern (>80% similarity). The OLIGO-Z tended to mix with CTZ and U-OLIGO-Z. The latter also formed a separate cluster, although it became mixed with the OLIGO-Z. ANOSIM showed significant segregation of the four zones (Figure 7B) with R = 0.482 and a significant level of 0.08%, indicating that the community structure between zones are different, as based on the automated taxonomic analysis.

3.1.4. Discussion

Our observations clearly showed a heterogeneous environment across the coast-offshore gradient, characterized by either gradual or abrupt changes in physical, chemical and biological variables. These results are consistent with previous observations (e.g.: Palma and Silva, 2006; Dolan *et al.*, 2007; Raimbault and Garcia, 2008; Cornejo *et al.*, 2015). It is important to consider that boundaries of our proposed zones were arbitrary and based on surface Chla levels, so that the areas occupied by each zone were very different. For instance, the CUP-Z represented only 5% of the sampled region, the CTZ 38%, the OLIGO-Z 72% and the U-OLIGO-Z 7%. This arbitrary division of zones was intended to represent potential biogeochemical regions, in the sense that distinct processes could determine the sources of C and N. These processes refer for example to nitrogen fixation (Montoya *et al*., 2004; Espinasse *et al.*, 2014; Hunt *et al.*, 2015), denitrification (Dalsgaard *et al.*, 2012), oligotrophy vs eutrophy and the ratio between new and regenerated production (Dugdale and Goering, 1967), and outgassing driven upwelling, among others.

However, variability in biochemical conditions of zooplankton may not only depend on the sources of nutrients, but also on the prevailing environmental conditions and also on some ecological processes. For example, physiological and nutritional conditions of organisms can be modified by factors, such as oxygenation (Seibel, 2011), temperature (Atkinson, 1994), pH (Yamada and Ikeda, 1999). In the same context, food conditions are critical for determining physiology and nutrition of zooplankton. Both food quantity and quality play a key role in that sense (e.g., Hirst and Bunker, 2003; Vargas *et al.*, 2006), through food availability, feeding behavior and selectivity (DeNiro and Epstein, 1978). Body composition and structure (e.g., quitinous/gelatinous) may also affect nutrient composition, including the isotopes signals (Webb *et al.*, 1998). Life cycles and ontogenia can also cause variation in physiological and nutritional conditions (Tibbets *et al.*, 2008). All these integrated processes may ultimately affect the C:N ratios and isotopes composition. However, we postulated that the main sources of C and N, which in turn depend on the above defined biogeochemical regions, are the key driving force for determining such biochemical features. As supporting this hypothesis, we found that C:N ratios, for all size fractions, were similar between CUP-Z and CTZ, but different when those of the OLIGO-Z and U-OLIGO-Z which exhibited much higher values. Elevated C:N ratios in zooplankton tissue in both oligotrophic areas may be explained by a N-deficiency upon more sable levels of C. N-deficiency is a well-known characteristic in open ocean areas of the Eastern South Pacific (Altabet et al., 2012; Stramma *et al.*, 2013; Cornejo *et al.*, 2015). However, C:N ratios >3.5 in all fractions can indicate excess of lipids in animal body (Post, 2002; Smyntek *et al.*, 2007). Lipid storage is probably a strategy against food shortages (Lee *et al.*, 2006), resulting in higher C:N ratios in oceanic regions. In both cases, N-deficiency and food shortage are processes triggered by an extreme oligotrophic condition which is one of our criteria to define biogeochemical zonation.

When comparing the zones in terms of isotopes composition, the patterns varied between δ^{13}C and δ15N. From the isospaces and Layman metrics (Figure 4 and Table 5) it was found that the range of δ^{13}C differed between zones, suggesting a variable origin of C for zooplankton biomass across the zonal gradient. A highly regenerated source of C for zooplankton biomass may be expected in the open ocean (Williams, 1981; Wollast, 1998; Del Giorgio and Duarte, 2002), compared to the upwelling zone where the major source of C is freshly uptaken from an atmospheric source (Gruber *et al.*, 1999; Mompeán *et al.*, 2013). Also in the upwelling zone greater δ^{13}C can be found after dominance of diatoms which exhibit a range between -15 and -19 compared to -21 and -25 in dinoflagellates which are more abundant in the open ocean (Nakatsuka *et al.*, 1992). The large variability in δ^{13}C (but not in δ^{15}N) observed in this zone for all size fractions can be interpreted as the consequence of the dynamics of upwelling, stimulating diatom blooms near the coast but also introducing phytoplankton species from the neighboring CTZ region, where diatom abundance is expected to be lower. Finally, higher values of CR found in the oceanic areas show a variable source of C. However, it was not possible to differentiate if the source of C was preferentially from diatoms or dinoflagellates, as in other studies (e.g., Perry *et al.*, 1999), because in the oligotrophic zone the phytoplankton may not cover the required carbon demand by zooplankton (Zhang *et al.*, 1995), and therefore zooplankton compensate this deficiency by feeding on microzooplankton (Kleppel, 1993; Calbet and Landry, 1999; Calbet and Saiz, 2005) which would translate in changes in δ^{13}C. Moreover, potential advection of organisms from coastal areas could also cause variability in the source of C by mixing processes (Gruber *et al.*, 1999).

In turn, δ^{15}N clearly decreased toward the open ocean suggesting a changing source of N for zooplankton across the zonal gradient. A variable source of N might be related to the type of food for zooplankton feeding. Even though our zooplankton size fractions may be comprised by a variety of trophic behaviors (carnivorous or herbivorous) the ultimate N source should mostly be phytoplankton, probably derived from diazotrophy, as reflected in the low values of δ^{15}N. This can thus be interpreted as evidence for presence of atmospheric nitrogen being fixed by phytoplankton and channeled to the tropic web (McClelland *et al.*, 2003; Mompeán *et al.*, 2016). There are, however, other processes potentially modifying δ^{15}N. For instance, in oligotrophic environments nitrate inputto the photic zone may occur by diffusion through the pycnocline (Mouriño-Carballido *et al.*, 2011; Fernández-Castro *et al.*, 2015) providing N for new production with low δ15N. Furthermore, diel vertical migration (DVM) of large-size zooplankton would facilitate the access to different preys causing variability in isotopic composition of zooplankton in large-size classes compared to smaller fractions (Cartes *et al.*, 2007; Agersted *et al.*, 2014). In any case, phytoplankton composition may have a key role for determining biochemical and nutritional conditions of nitrogen in zooplankton. Across the coastal-offshore gradient it is known thatthe processes that modulate the availability of nitrogen in the coastal zone differ to those of more oceanic areas, for example, presence of a shallow OMZ promotes the denitrification process in the upwelling zone (Thamdrup *et al.*, 2006; Galán *et al.*, 2009; Dalsgaard *et al.*, 2013), favoring an enrichment in δ^{15}N.

With respect to ecological zonation across the zonal gradient, we found that the zooplankton composition changes significantly from the CUP-Z toward the U-OLIGO-Z. Our automated analysis of zooplankton does not provide information on the species composition, but only on the proportion and abundance of different taxonomic categories. This proportion changes from one zone to the other and most likely the species also do so from the coastal area to the oceanic region, as seen for example in euphausiids species (Riquelme-Bugueño *et al.*, 2012) and cnidaria (Palma and Silva, 2006). Species composition may also influence the isotopic values of zooplankton due to variability in trophic position in different zooplankton assemblages (Matthews and Mazumder, 2003), and thus some of the variation in δ^{15}N may relate to changing species composition. Sampling method is another potential source of variation for the zooplankton composition. Depending on the sampling gear, mesh-size of the plankton net and light conditions, DVM cannot be depicted properly (see Harris *et al.*, 2000 for review). Our Multinet targets the mesozooplankton community which is mostly dominated by copepods. In this group DVM is mostly restricted to the upper 200 m and even shallower upon presence of the OMZ (Escribano *et al.*, 2009). More extensive

DMV is mainly performed by micronekton, including euphausiids and fish larvae (Harris *et al.*, 2000) which cannot be properly sampled by the Multinet. Therefore, within the upper 200 m we have assumed that our mesozooplankton community is adequately represented by our sampling protocol. In sum, changes in community structure can be driven by a variety of denso-dependent and denso-independent ecological processes, including potential sources of variation due to sampling methods, all of them shaping the observed spatial pattern of species distribution and diversity.

Because of the large difference in productivity and resources between the zones identified, the size of the isotopic niche was also very variable. Relatively small niches were obtained for the plankton communities samples in the coastal zones, as the availability of feeding resources will allow for specialized feeding on prey of similar isotopic composition. Grazing on phytoplankton is a dominant process in these zones and this is indicated by the composition of the copepod community (Morales *et al.*, 2010). Indeed, our results showed a relatively large homogeneity in composition for the CTZ. In contrast, oligotrophic zones had large isotopic niches, mainly due to a large variability in δ^{13}C, and resulted also more variable in taxonomic composition than the coastal zones. Opportunistic feeding on prey that are scarce (i.e., low plankton biomass) and diverse would cause large variations in the isotopic composition of both prey and consumers, and therefore would produce large isotopic niches. Similar results were found in plankton from deep layers of the Atlantic, where the communities living in tropical, oligotrophic zones showed isotopic niches of large size (Bode and Hernández-León, 2018). From an ecological perspective, it can be suggested that the U-OLIGO-Z, representing a special ecosystem of the South Pacific central gyre, may be less sensitive to a changing environment upon a greater plasticity of species to exploit highly diverse resources.

In spite of the recognition that multiple factors and processes can modulate C and N and their isotopes composition of the zooplankton community, it is relevant to show that a changing community structure and isotopic niche characteristics appear linked to different biogeochemical zones, providing the basis for ecological zonation and revealing that basic processes governing nutrient recycling and availability can modulate the food web at least at lower trophic levels. Lower availability of prey and the prevalence of regenerated or atmospheric nitrogen sources in the open ocean would likely explain these differences in isotopic niches, and that would reflect mostly the opportunistic feeding of zooplankton in oligotrophic regions. The proposed trophic zones identified by both the isotopic and taxonomic approach could serve as a framework for comparison of pelagic food webs across large ocean regions.

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Figure legends

Figure 1.The eastern south Pacific region where the CIMAR-21 cruise was carried out in October 2015: (A) Representation of the sampling stations across the four study zones defined from satellite chlorophyll-a levels as: coastal upwelling area(CUP-Z),the mesotrophic coastal transition zone (CTZ),the oligotrophic area (OLIGO-Z) and the ultra-oligotrophic region (U-OLIGO-Z), (B) Satellite surface temperature and geostrophic surface currents during the cruise.

Figure 2. Hydrographic conditions of the water column across the zonal gradient from the Chilean coast to Easter Island from CTDO profiles to 1000 m: (A) temperature, (B) salinity and (C) dissolved oxygen (mlL-1).

Figure 3. Biochemical variables of size-fractioned zooplankton across the zonal gradient: (A) C:N ratio, (B) stable carbon isotopes ($\delta^{13}C$), (C) stable nitrogen isotopes (δ ^{15}N).

Figure 4. Layer isospaces of five size fractions for δ ^{13}C and δ ^{15}N, including the individual measurements of the samples for each zone: (A) CUP-Z, (b) CTZ, (C) OLIGO-Z and (D) U-OLIGO-Z. The maximum likelihood calculated of standard ellipse areas for each size fraction (continuous line).

Figure 5. The standard ellipse areas (SEAB) for the plankton communities considered in this study and computed from the Bayesian multivariate distributions fitted to the original isotope data for each zone (all size-classes combined). The modes (white dots) and the intervals encompassing 50%, 95%, and 99% of the estimations (shaded boxes) are indicated.

Figure 6. Variation in relative abundance (%) of the most abundant taxonomic categories sampled during the cruise CIMAR-21 from the Chilean coast to Easter Island for a depth strata 0-200 m. The taxonomic categories were assessed by automated analysis (ZooScan). Four study zone were defined: CUP-Z, CTZ, OLIGO-Z and U-OLIGO-Z.

Figure 7. Cluster analysis of taxonomic groups of zooplankton from the upper 0-200 m over the zonal gradient from the Chilean coast to Easter Island (A) similarity distance was Bray Curtis. Non-metric multidimensional scaling graph (NMDS)based on the

Bray-Curtis distance index of $\sqrt[4]{x}$ transformed from the abundance data of the taxonomic groups (B). Both analyses were associated with 4 zones across the zonal gradient: Eutrophic (CUP-Z), Mesotrophic (CTZ), Oligotrophic (OLIGO-Z) and Ultra-oligotrophic (U-OLIGO-Z).

Table 1. Description of the sampling stations used for the study from the coast to open ocean waters. The transect was performed at the latitude 27°S and both zooplankton and CTD profiles were obtained at each station.

Station	Longitude	Distance from shore (km)	Zone	Depth (m)	Date	Time
1	70°52’	4.4	CUP-Z	130	13-Oct-2015	20:50
4	71°12’	104.5	CUP-Z	2900	14-Oct-2015	14:00
5	71°46’	211.1	CUP-Z	6700	15-10-2015	1:15
8	74°35’	614.5	CTZ	4250	16-10-2015	4:10
9	76°05’	757.8	CUP-Z	4041	16-10-2015	19:20
10	77°34’	947.9	CUP-Z	2981	17-10-2015	8:00
11	79°04’	1091.2	CUP-Z	1944	17-10-2015	23:30
12	80°35’	1275.7	CUP-Z	3980	18-10-2015	14:00
13	82°05’	1386.8	CUP-Z	4200	19-10-2015	3:00
14	83°32’	1609.0	CUP-Z	3700	19-10-2015	16:11
16	86°32’	1942.4	OLIGO-Z	3700	20-10-2015	13:40
18	89°34’	2275.8	OLIGO-Z	3700	21-10-2015	12:13
20	92°36’	2609.1	OLIGO-Z	3700	22-10-2015	12:20
24	98°38’	3053.6	OLIGO-Z	3600	24-10-2015	7:38
28	104°36’	3942.5	U-OLIGO-Z	2720	26-10-2015	17:03
30	107°34’	4164.8	U-OLIGO-Z	2356	27-10-2015	13:30
64	105°49’	4220.3	U-OLIGO-Z	1440	01-11-2015	11:30

Table 2. Mesozooplankton biomass (mean ± SD) and hydrographic variables in the four study regions. Mesozooplankton biomass is from the upper 200 m. n=samples size, T-10 and S-10 are temperature and salinity at 10 m depth, DO is the mean dissolved oxygen in the upper 100 m, and Chla-Sis surface Chlorophyll-a. Post hocs tests were performed using the Tukey's range test for each variable analyzed. The combination of letters for each variable by zone indicates significant differences vs non-significant differences.

Zone	Biomass (mg C m-3)	n	T-10 (°C)	Sal-10	DO (0-100 m) (mL L-1)	Chla-S (mg m3)
CUP-Z	4.84±4.72(b)	15	17.88±1.70(b)	35.03±0.43(b)	5.03±1.00(a)	0.70±1.97(b)
CTZ	0.58±0.32(c)	35	17.91±1.63(c)	35.01±0.42(c)	5.13±0.89(c)	0.56±1.74(c)
OLIGO-Z	0.18±0.10(a)	20	18.02±1.65(a)	35.05±0.42(a)	5.11±0.90(b)	0.57±1.77(a)
U-OLIGO-Z	0.09±0.05(a)	15	18.07±1.71(a)	35.07±0.45(a)	5.10±0.91(a,b)	0.58±1.78(a)

Table 3. Size-fractioned zooplankton biomass (mean± SD) and their isotopes composition of C and N. Biomass is in terms of C, the C/N ratio and isotopic composition of δ^{13}Cand δ^{15}N. n= sample size.

Size fraction (µm)	Biomass (mg C m^{-3})	C: N	δ^{13}Ccorr	δ^{15}N	n
<200	0.26±0.61	9.10±2.98	-17.98±2.91	8.96±3.77	17
200-500	0.21±0.51	5.89±1.14	-19.58±2.40	10.15±2.98	17
500-1000	0.17±0.38	5.37±1.08	-19.68±2.09	11.04±2.58	17
1000-2000	0.14±0.22	4.93±0.94	-19.67±1.45	10.82±3.86	17
>2000	0.11±0.13	7.90±3.14	-19.04±2.22	9.64±5.06	17

Table 4.A Generalized Linear Model (GLM) to test the effect of size fractions and zone on C:N ratio and isotopes composition of C and N of mesozooplankton across a zonal gradient in the eastern south Pacific. ***=highly significant effects.

Source and Variation	Dependent	D.F.	F-ratio	P
Fraction	C: N	4	12.09	0.00***
	δ^{13}Ccorr	4	1.76	0.15
	δ^{15}N	4	1.43	0.23
Zone	C: N	3	6.37	0.01
	δ^{13}Ccorr	3	4.09	0.01
	δ^{15}N	3	28.61	0.00***

Table 5. Layman metrics (mean ± SE) estimated by a Bayesian approach from the area defined by δ^{15}N and δ^{13}C of mesoozooplankton for four study zones (all size-clases combined). NR (‰): δ^{15}N range, CR (‰): δ^{13}C range, TA (‰): total area of the convex hull encompassing all data points, CD (‰): centroid distance, MNND (‰): mean distance to the nearest neighbor, SDNND (‰): standard deviation of the distance to the nearest neighbor.

Zones	CUP-Z		CTZ		OLIGO-Z		U-OLIGO-Z	
Metric	Mean	SE	Mean	SE	Mean	SE	Mean	SE
NR	2.47	0.019	2.70	0.009	5.98	0.014	5.30	0.020
CR	2.66	0.019	1.46	0.008	3.28	0.015	6.20	0.020
TA	2.84	0.038	1.99	0.013	9.24	0.055	12.26	0.080
CD	0.95	0.007	1.04	0.003	2.50	0.005	3.16	0.007
MNND	0.96	0.006	0.73	0.003	1.99	0.007	2.30	0.008
SDNND	0.45	0.008	0.18	0.003	0.73	0.007	1.12	0.008

Table 6. Mean ± SE overlap (expressed as a fraction (%) of the non-overlapping area) between estimates of ellipses encompassing 95% of isotope data (all size-classes combined) of the different zones showed in Fig. 4.

	CTZ	CUP-Z	OLIGO-Z	U-OLIGO-Z
CTZ	---			
CUP-Z	20.07±0.03	---		
OLIGO-Z	24.17±0.05	17.07±0.03	---	
U-OLIGO-Z	12.81±0.03	15.02±0.03	54.42±0.10	---

Fig.1.

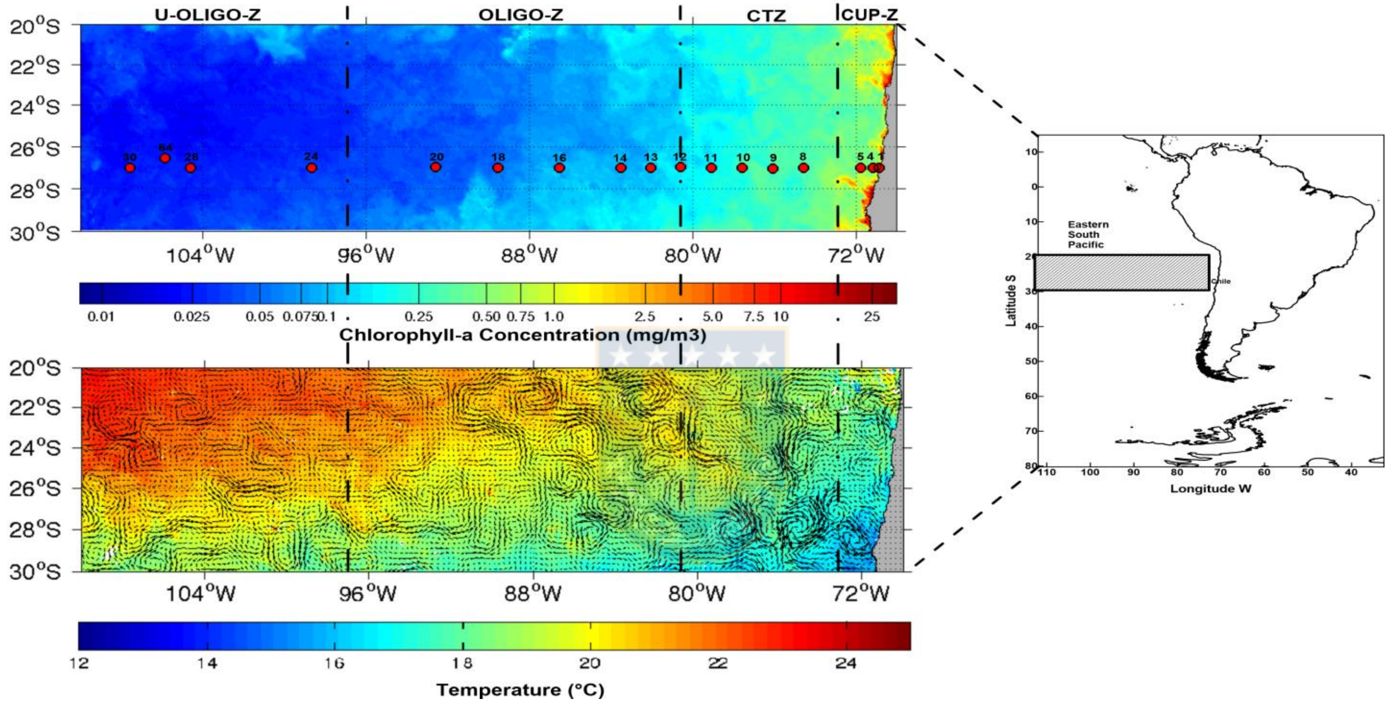

U-OLIGO-Z
OLIGO-Z
CTZ
CUP-Z
20°S
22°S
24°S
26°S
28°S
30°S
30
64
28
24
20
18
16
14
13
12
11
10
9
8
5
4
1
104°W
96°W
88°W
80°W
72°W
0.01
0.025
0.05
0.075
0.1
0.25
0.50
0.75
1.0
2.5
5.0
7.5
10
25
Chlorophyll-a Concentration (mg/m3)
12
14
16
18
20
22
24
Temperature (°C)
Eastern
South
Pacific
Chile
Latitude S
Longitude W
10
0
10
20
30
40
50
60
70
80
110
100
90
80
70
60
50
40

Fig.2.

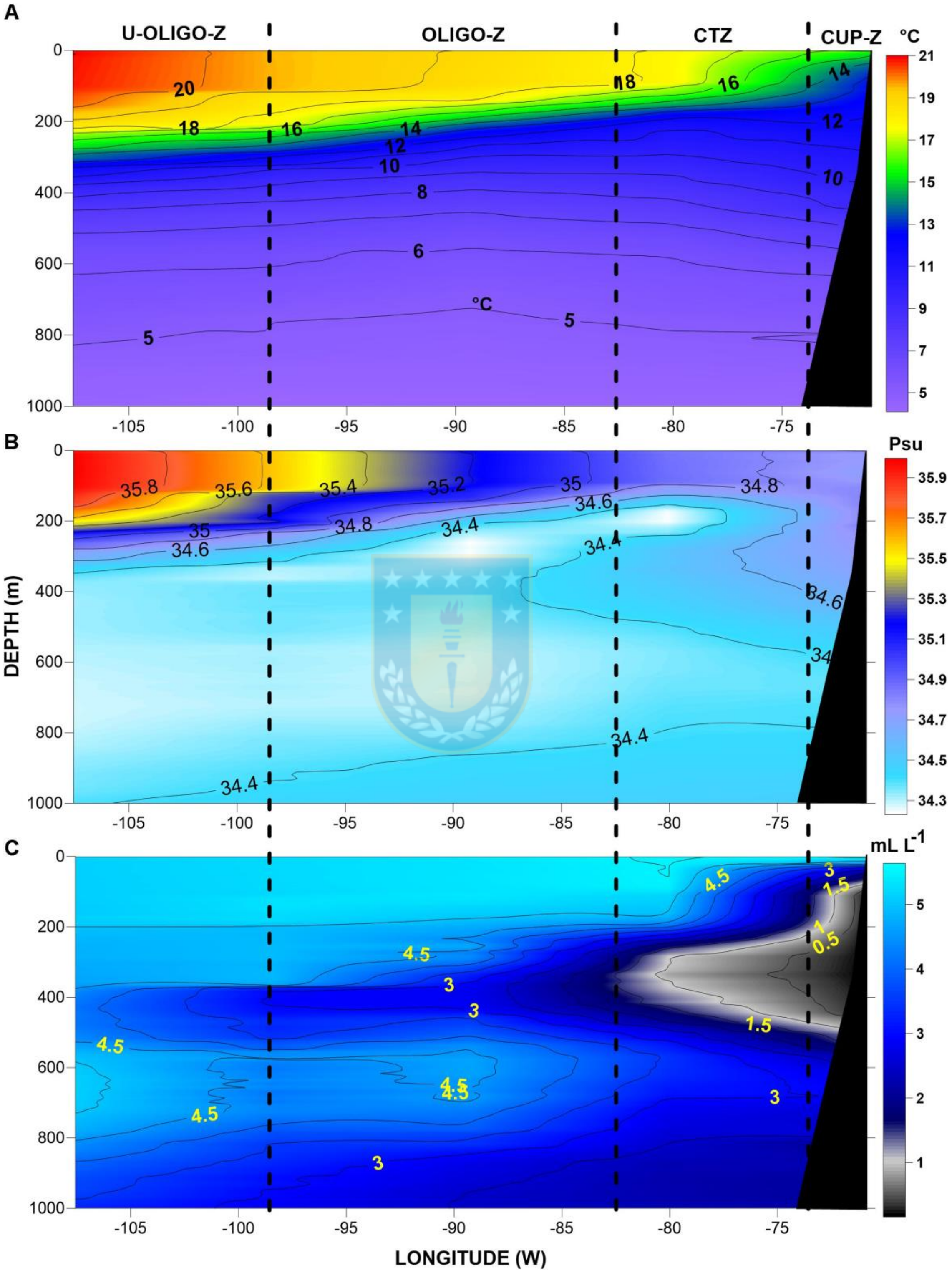
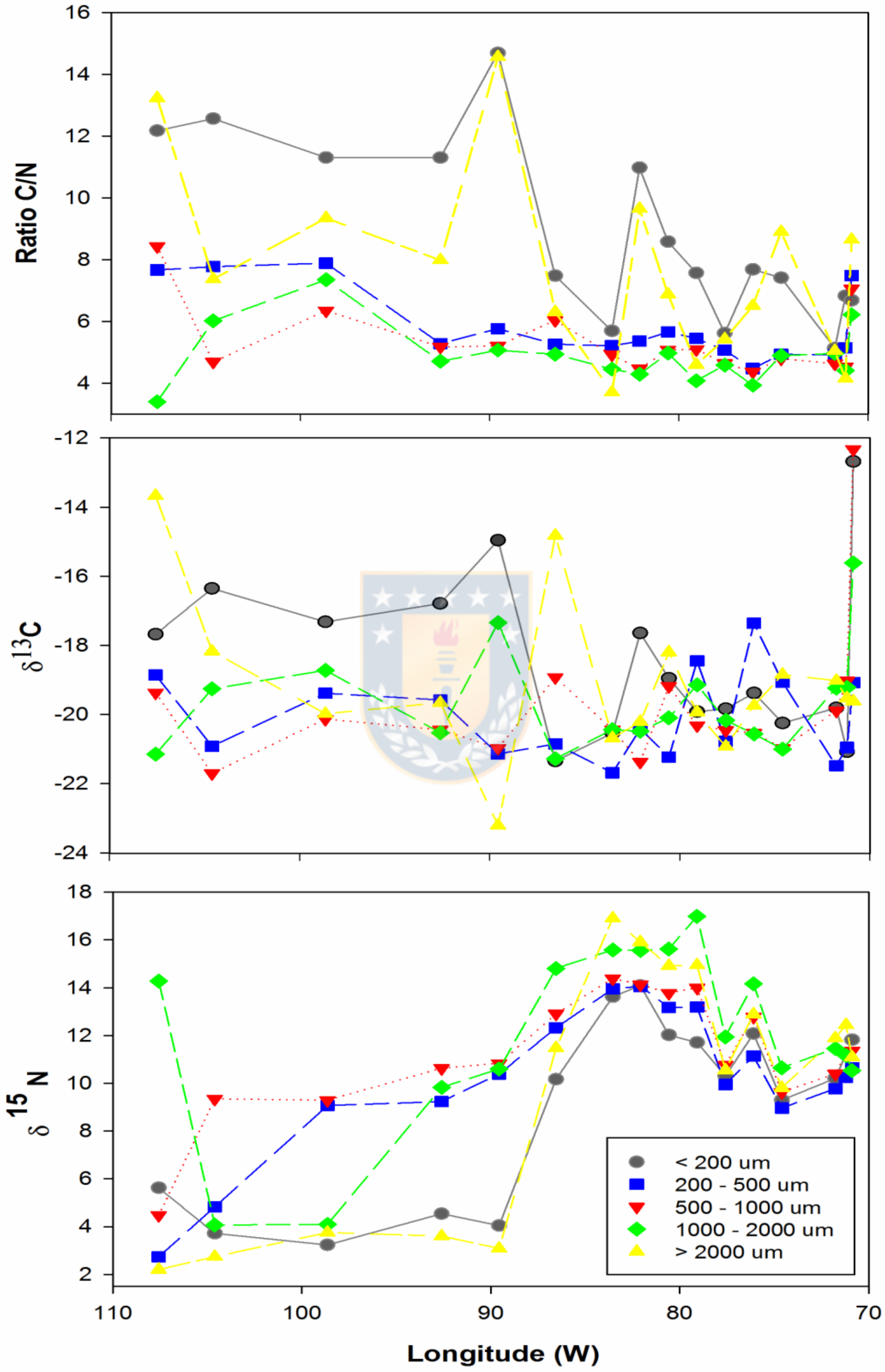

A
U-OLIGO-Z
OLIGO-Z
CTZ
CUP-Z
°C
20
18
16
14
12
10
8
6
5
°C
5
21
19
17
15
13
11
9
7
5
B
Psu
35.8
35.6
35.4
35.2
35
34.8
34.6
34.4
35.9
35.7
35.5
35.3
35.1
34.9
34.7
34.5
34.3
C
mL L^{-1}
4.5
3
1.5
1
0.5
5
4
3
2
1
DEPTH (m)
0
200
400
600
800
1000
-105
-100
-95
-90
-85
-80
-75
LONGITUDE (W)

Fig.3.



Ratio C/N
16
14
12
10
8
6
4
$\delta^{13}C$
-12
-14
-16
-18
-20
-22
-24
$\delta^{15}N$
18
16
14
12
10
8
6
4
2
110
100
90
80
70
Longitude (W)
< 200 um
200 - 500 um
500 - 1000 um
1000 - 2000 um
> 2000 um

Fig.4.

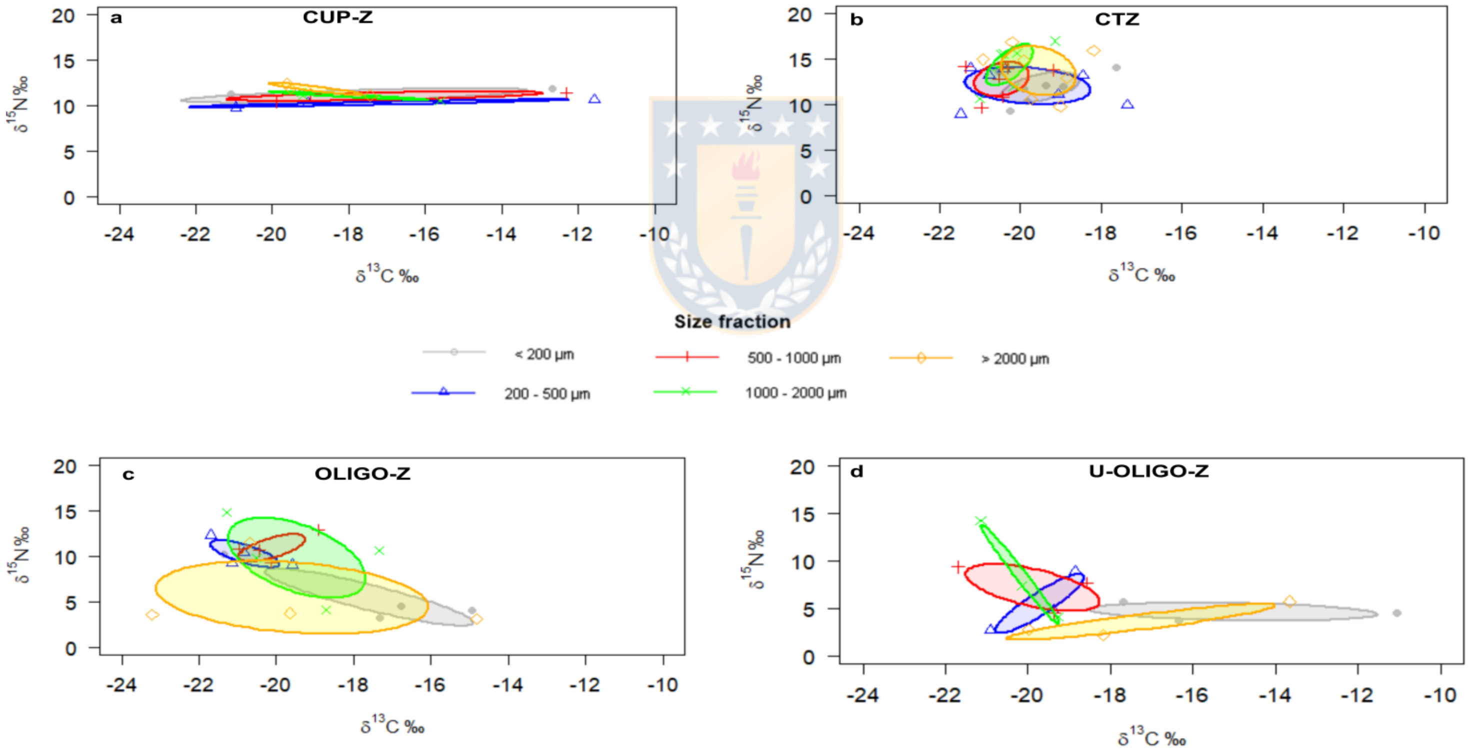

a
CUP-Z
b
CTZ
c
OLIGO-Z
d
U-OLIGO-Z
$\delta^{15}N$ ‰
$\delta^{13}C$ ‰
20
15
10
5
0
-24
-22
-20
-18
-16
-14
-12
-10
Size fraction
< 200 µm
200 - 500 µm
500 - 1000 µm
1000 - 2000 µm
> 2000 µm

Fig.5.

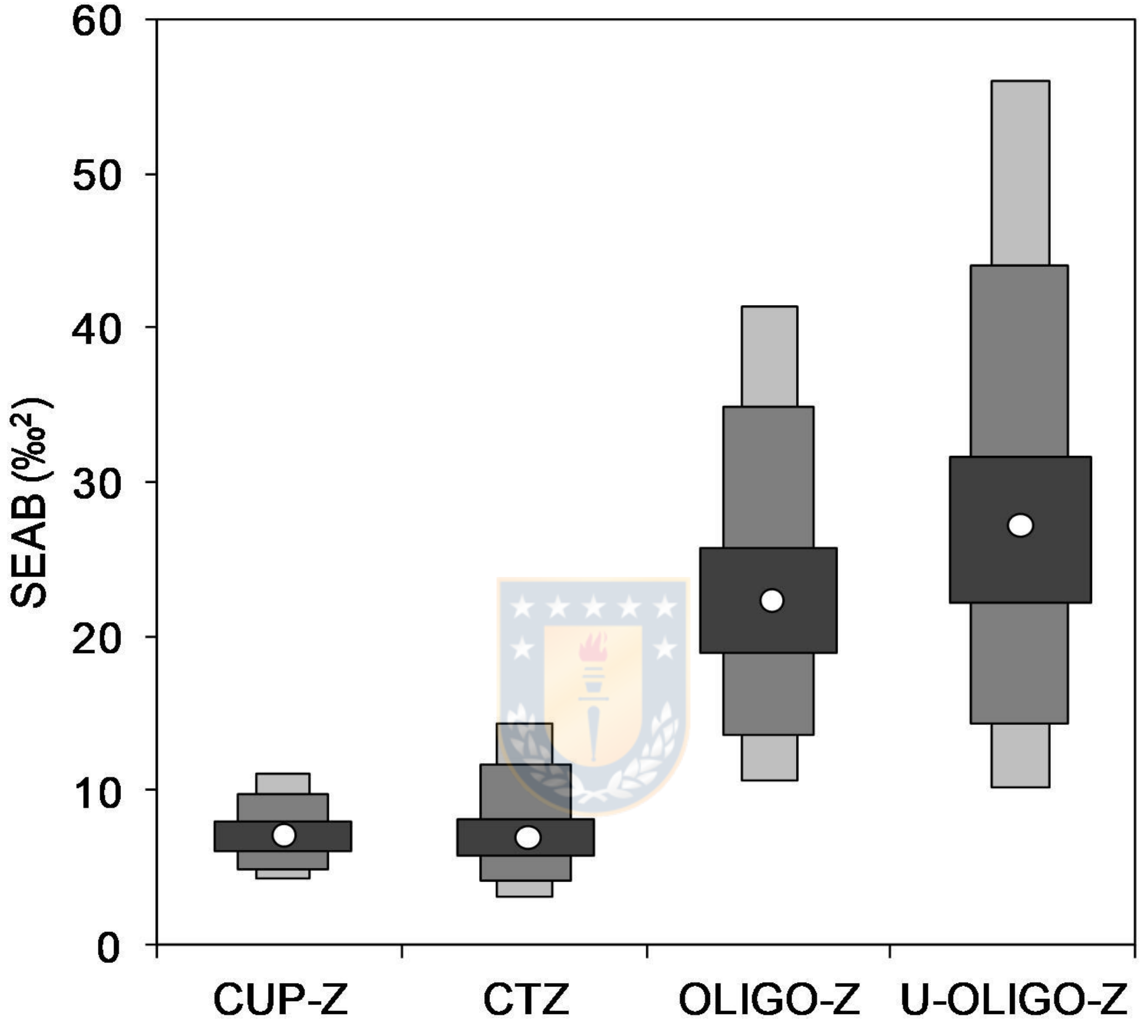


Fig.6.

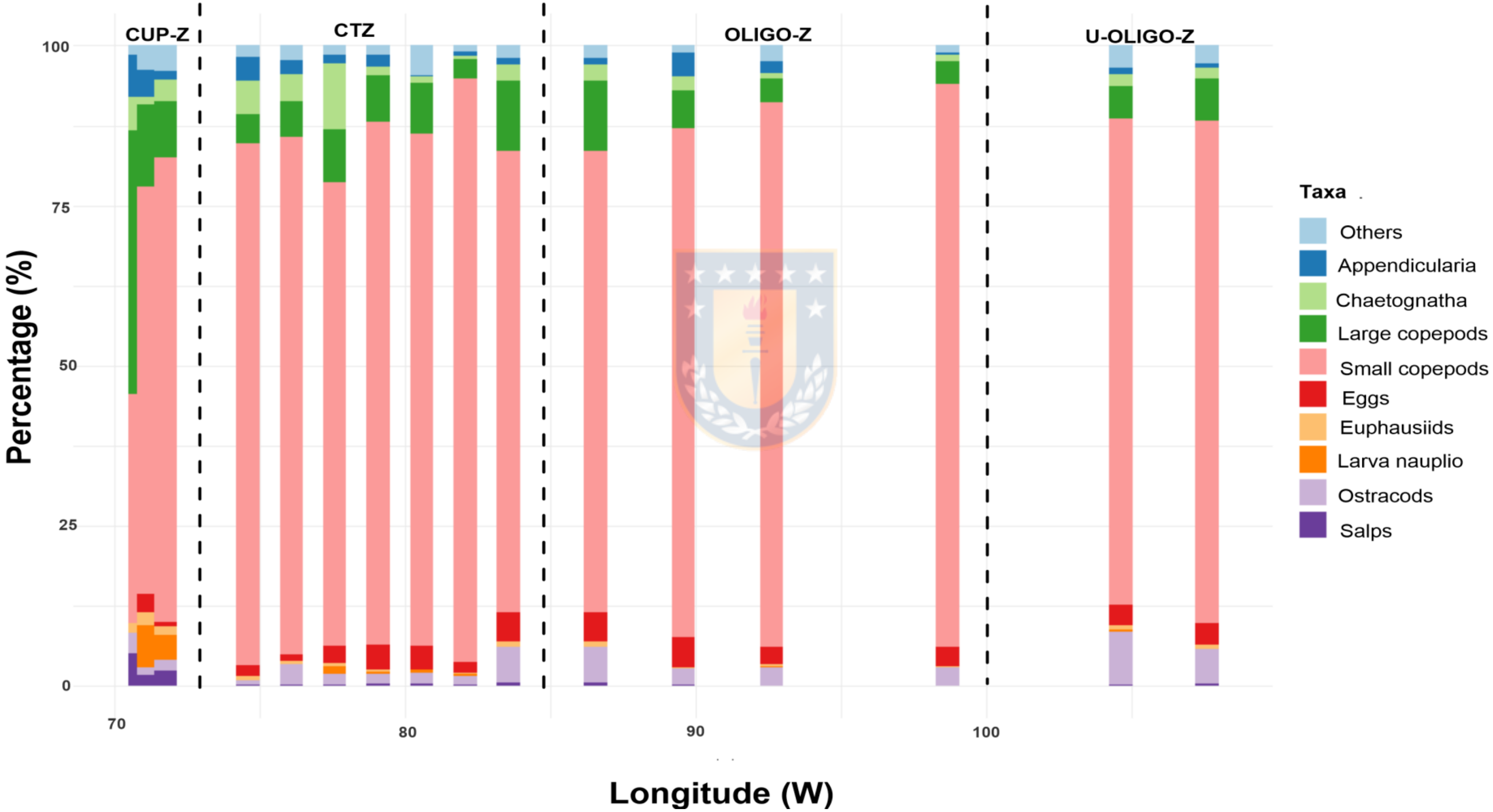
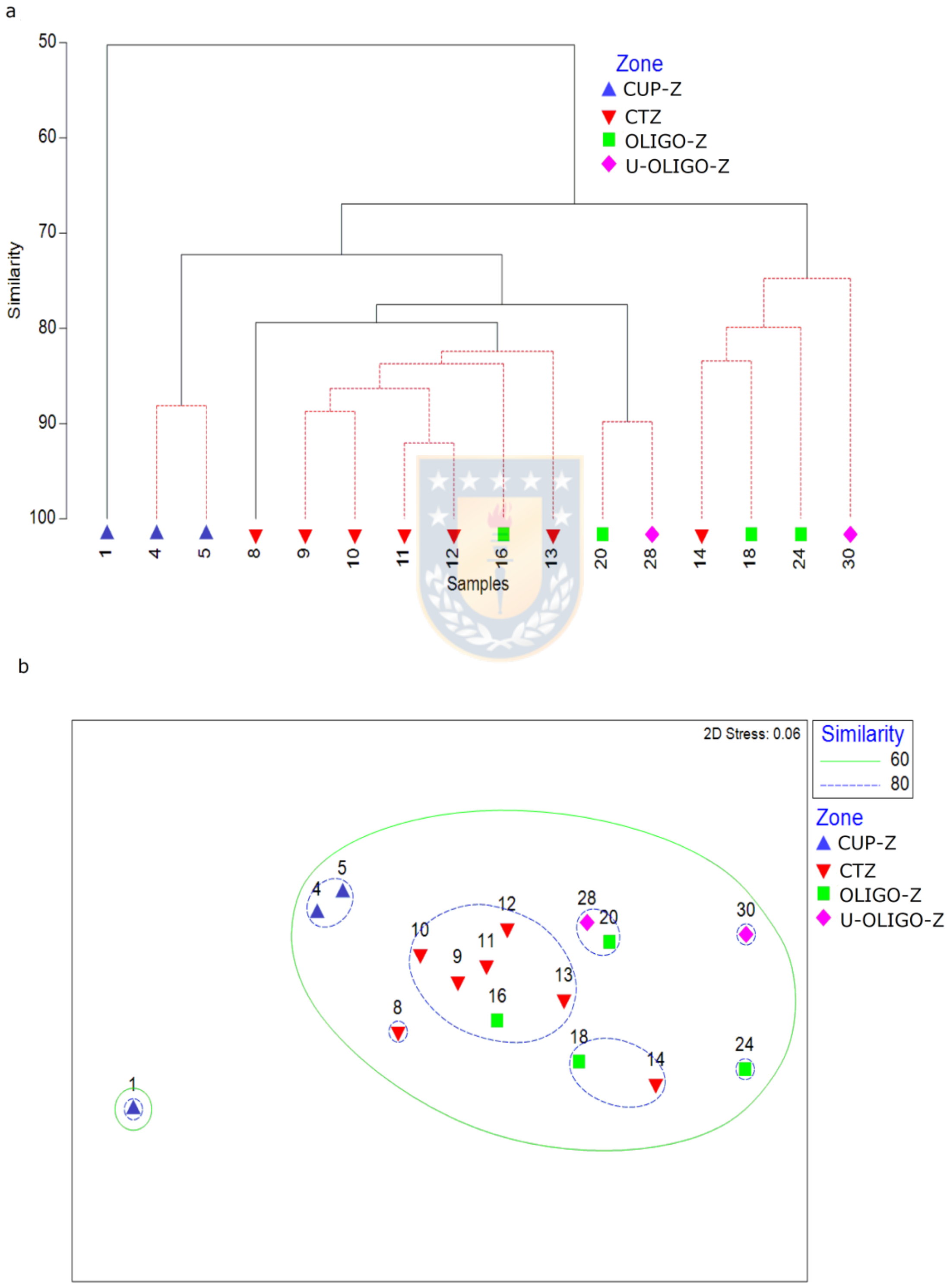

CUP-Z
CTZ
OLIGO-Z
U-OLIGO-Z
100
75
50
25
0
Percentage (%)
70
80
90
100
Longitude (W)
Taxa
Others
Appendicularia
Chaetognatha
Large copepods
Small copepods
Eggs
Euphausiids
Larva nauplio
Ostracods
Salps

Fig.7.


a
50
60
70
80
90
100
Similarity
Zone
▲ CUP-Z
▼ CTZ
■ OLIGO-Z
◆ U-OLIGO-Z
1
4
5
8
9
10
11
12
16
13
20
28
14
18
24
30
Samples
b
2D Stress: 0.06
Similarity
60
80
Zone
▲ CUP-Z
▼ CTZ
■ OLIGO-Z
◆ U-OLIGO-Z
5
4
12
28
20
30
10
9
11
13
16
8
18
14
24
1

3.2. Capitulo 2. Artículo en preparación

Gradientes ambientales y patrones espaciales de los copépodos calanoides en el Pacífico sudoriental

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Resumen

La comprensión de los patrones de diversidad espacial en los grandes sistemas marinos se ha convertido en un tema crítico en el estudio de las comunidades de plancton sometidas a un océano cambiante impulsado por la variabilidad climática. Aquí, utilizamos datos sobre la distribución de los copépodos calanoides, un grupo ampliamente distribuido dentro del zooplancton pelágico, para evaluar la influencia de los gradientes costeros-oceánicos y latitudinales en su estructura comunitaria. Se analizó la abundancia y ocurrencia tanto de familias como especies desde 1986 hasta 2016, a partir de una base de datos que cubre la región entre la zona de surgencia costera y el océano abierto oligotrófico. Nosotros encontramos un total 181 especies distribuidas en 20 familias a treves del área de estudio. Las familias y especies se estructuraron significativamente en ambos gradientes (costero- oceánico y latitudinales), revelando el hábitat dominante para cada una de las familias con efectos estacionales significativos dentro de la zona de surgencia. Los patrones espaciales revelaron la presencia de zonas de transición compuestas por taxones mixtos. Sobre el gradiente latitudinal, la zona de transición estaba vinculada a la región convergencia subtropical (30 ° S). La temperatura de la superficie del mar explicó hasta un 41 % de variación en la riqueza de especies sobre un gradiente costa-océano, aunque no se observaron efectos significativos sobre los gradientes latitudinales. Un análisis filogenético de las especies dominantes también reveló una estructuración genética significativa sobre el gradiente costa-océano, lo que sugiere que los procesos históricos también tienen un papel importante en los patrones actuales. Nosotros llegamos a la conclusión que la zonificación ambiental forzada por los gradientes de temperatura son un factor clave para mantener la diversidad de las comunidades planctónicas, aunque la diversidad genética puede estar sujeta a otros procesos a lo largo de escalas evolutivas.

Palabras claves: Zooplancton, Patrones de Distribución, Copépodos Calanoides, Gradiente Oceanográfico, Pacífico Sudoriental.

Environmental gradients and spatial patterns of Calanoid copepods in the Southeast Pacific

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Abstract

Understanding patterns of spatial diversity in large marine systems has become a critical issue in the study of plankton communities subjected to a changing ocean driven by climate variability. Here, we used data on distribution of Calanoid copepods, a widely distributed group of zooplankton in the pelagic realm, to assess the influence of cross-shelf and latitudinal gradients on their community structure at family and species levels. Families and species abundances and occurrence for the period 1986 and 2016 were assessed from a data base covering the region between the coastal upwelling zone and the offshore region off Chile at subtropical and temperate areas. We analyzed 20 families comprising 181 species distributed in 3 predefined zones over the cross-shelf gradient, the coastal upwelling, the coastal transition and the oligotrophic zones, whereas over the latitudinal gradient, same families were majorly linked to the northern and southern portions of the sampled area (20° S to 40°S). Families and species were significantly structured over both gradients (cross-shelf and latitudinal), revealing the dominant habitat for each of the families with significant seasonal effects within the more variable upwelling zone. Spatial patterns revealed presence of transitional zones comprised by mixed taxa. Over the latitudinal gradient this transition zone was linked to the subtropical convergence (30°S). Sea surface temperature as an environmental proxy explained ca.

38% of variation in species richness over the cross-shelf, although with no significant effect over the latitudinal gradients. A phylogenetic analysis of the MtCOI of dominant species from the pre-defined zones also revealed a significant genetic structuring over the cross-shelf gradient, suggesting that historical processes are also underlying observed distributional patterns. We concluded that environmental zonation forced by temperature gradients is a key factor for maintaining taxonomic of plankton communities in ocean basins, although genetic diversity may be subject to other processes over perhaps evolutionary scales.

Keywords: Zooplankton, Distribution Patterns, Calanoid Copepods, Oceanographic Gradient, Southeast Pacific.

3.2.1.Introduction

The marine pelagic environment presents a high diversity of organisms coexisting within a complex trophic food web (Lenz, 2012). One of the main groups within the pelagic community is zooplankton characterized by having short life cycles (< 1 year), and being a key trophic link in the pelagic food web. This group exhibits a high taxonomic diversity with 12 phyla described and all of them highly sensitive to environmental changes (Pino-Pinuer *et al.*, 2014; Peijnenburg and Goetze, 2013), such that their changes in abundance and distribution can be used as indicators of ecosystem response to climate driven variation (Richardson, 2008; Beaugrand *et al.*2003).

In the marine zooplankton, planktonic copepods and in particular the species comprising the order Calanoida represent almost 80% of the abundance of zooplankton in the Southeast Pacific (SEP) (Escribano and Hidalgo, 2000; Hidalgo *et al.*, 2010, 2012). In offshore areas of this region, studies on copepods are scarce and of limited geographic range and most expeditions and samplings have focused in obtaining general patterns of distribution and diversity (Fagetti and Fisher, 1964; Petrillo *et al.*, 2005; Pierrot-Bults and Spoel, 2003; Heinrich, 1973; Escribano *et al.*, 2003). Furthermore, research efforts have mainly focused on the upwelling zone (e.g. Escribano *et al.*, 2007; Hidalgo *et al.*, 2010), or in the coastal transition zone (eg. Morales *et al.*, 2010), but a lack of knowledge on the community structure of copepods for the open ocean system of the SEP has become evident. In general terms, it is known that copepods in this region are mainly composed by sub-antarctic species and from the equatorial region (Escribano *et al.*, 2003).Also it has been reported for the SEP that the greatest abundance of copepods has been registered in the coastal zone (upwelling zone), and that their diversity tends to increase towards the open ocean, in agreement with a the suggested negative relationship between abundance and biodiversity (Angel, 1993; Escribano *et al.*, 2003; Irigoien *et al.*, 2004). These patterns may be affected by changes in oceanographic conditions, such as temperature, oxygenation, salinity and stratification as it has been demonstrated in other regions of the world ocean (Peterson *et al.*,2006; Aronés *et al.*, 2009; Beaugrand *et al.*, 2002, 2003;Richardson and Schoeman, 2004; Gewin, 2010; Seibel, 2011), revealing the strong dependence of these organisms with oceanographic conditions, because of the effects that hydrographic factors can have on their distribution and their prey providing their limited migration capacity (McClain and Barry, 2010).

The SEP shows a great heterogeneity in hydrographic conditions from the coastal area towards the open ocean evidenced by a progressive increase of the depth of the mixing layer, dissolved oxygen, temperature and surface salinity (Gonzalez *et al.,* 2018; Palma and Silva, 2006). Also, within the same gradient it is possible to observe drastic changes in biological productivity being high in the coastal zone (Humboldt Current) and extremely low in the ultra-oligotrophic central gyre (Bonnet *et al.*, 2008; Mann and Lazier, 2013; Morel *et al.,* 2010; Kletou *et al.*, 2010). Across the latitudinal gradient strong differences in temperature, salinity and levels of oxygenation of surface waters can occur. These changes are also accompanied by variable seasonal regimes from low to high latitudes (Strub *et al.*, 1998).

The strong hydrographic variability observed in the SEP over both zonal and meridional gradients allows us to distinguish distinct ecoregions, where the environmental characteristics are relatively stable and homogeneous with respect to the adjacent ones, such effects are also reflected in distinct communities between these ecoregions. For instance, along the coastal zone and oceanic areas of the SEP five ecoregions have been suggested: Central Peru, Humboldtian, Central Chile, Araucanian, Juan Fernandez and Desventuradas (Spalding *et al.*, 2007). Although, a recent work based on ecological and biogeochemical division of zooplankton, proposed the existence of four regions over the zonal gradient in according to surface chlorophyll-a (Gonzalez *et al.,* 2018). Same work suggested that changes in the community structure of zooplankton in the SEP could be linked to the factors or processes that modulate the availability of nutrients at lower trophic levels (Gonzalez et al., 2018).

Changes in the community structure of planktonic organisms do not only respond to factors acting over the spatial scales, but also over temporal scales (Mann and Lazier 2013; Angel, 1997; Haury and McGowan 1998). For example, geological events have modified the distribution of the continents, opening or closing sea lanes and causing changes in the thermohaline circulation. These processes have led to speciation in the zooplankton. Also, changes in temperature and sea level have modified the geographical range of some species of copepods (Gee, 1991; Crame, 1993; Molfino, 1994), also affecting their evolutionary history (Fleminger, 1986; Cronin and Schneider 1990). On a smaller scale, ecological factors influencing the physiology of the species can modify the spatial-temporal distribution of species, depending on their tolerance and optimal ranges of their populations, and so regulating diversity patterns. Such spatial-temporal variability, from microscale to large scale, has contributed synergistically to promote speciation events, and hence shaping up the actual patterns of biodiversity of pelagic organisms. However, the mechanisms that regulate biogeographical patterns need to be elucidated and understood to gain sufficient knowledge and tools allowing us to predict the variation in the distribution of species, under the effect of natural or anthropogenic impact. Under this view, the pelagic ecosystem of the Southeast Pacific may constitute a suitable model to enhance our understanding of underlying mechanisms modulating zooplankton distribution patterns and other higher trophic levels. With these ultimate goals in mind, in this work we assessed the coastal-oceanic (zonal) and alongshore (meridional) distribution of pelagic Calanoid families and their species over pre-defined zones to test the hypothesis that ecological zonation is determined by environmental gradients of which the temperature gradient can play a unique role. We then aimed at understanding the underlying mechanisms influencing the large-scale patterns of species distribution over ocean basins.

3.2.2. Methods

3.2.2.1. Study area and data bases

The Southeast Pacific region off Chile has been focus of zooplankton studies and sampling mainly focused on the coastal zone, also known as the southern portion of the Humboldt Current system covering the area between 18°S and 40°S. A few oceanographic cruises have also been carried out in the offshore region, even reaching the area near Easter Island (ca. 110° W). Fig. 1 illustrates our study area and the sites from which zooplankton samples have been obtained and used in this study. González *et al.*,(2018) distinguished four ecological zones in this region, as based on the surface Chlorophyll concentration from the highly productive upwelling area towards the central south Pacific gyre. This study found significant changes in zooplankton community structure among these zones, defined as: the coastal upwelling zone (CUP-Z), the coastal transition zone (CTZ), the oligotrophic zone (OLIGO-Z) and the ultra-oligotrophic zone (U-OLIGO-Z) which are illustrated in Fig. 1. Here, we used these pre-defined zones to allocate all the sampling sites, although the OLIGO-Z and the U-OLIGO-Z were considered only as OLIGO-Z, because of too few samples to separate them.

Although most samples have been performed in the upper 200 m, some of them were obtained down to 2000 m over the trench or in the offshore region. The sampling period for our study is 1985 to 2016 and variable seasonal periods, depth strata and sampling gears have been applied (Supplemental Material 1), although in all cases the target community has been mesozooplankton (200 µm to 2000 µm) which is the zooplankton mostly comprised by copepods in the ocean. In the coastal zone two sites are part of time series studies so that they have a much better temporal resolution and replication in comparison with all the other for which in some cases a single sampling was done (see Supplemental Material 1).

3.2.2.2. Data analyses

Sampling methods have been described in previous studies (Supplemental Material 1). Data on species abundance from all sampling sites were used as number of individual m^{-3}, regardless the depth strata and so representing the species found at each site. For each sampling point we calculated the average value of abundance for all species and families based on the number of observations made at each site. However, because of the high seasonal variability in abundance and a larger temporal coverage in the CUP-Z, we calculated the mean abundance for each sampling site separately for spring-summer (September to March) and autumn-winter (April to August) periods. Changes in copepod families were evaluated for the different zones and seasons using community descriptors, such as families richness (R) which is the number of species per family, numerical abundance (N) and weighed frequency of occurrence (WFO) defined as:

$$\mathrm{WFO} = \left(\frac{\frac{\mathrm{NF}i}{\mathrm{NS}}}{\mathrm{REF}i} \right)$$

where NF is the number of stations in which the *I* family was present, NS is total number of sampled stations and REF is the number of species present in the *I* family. Also, we modified the equation of weighted mean depth suggested by Andersen *et al.*, 2004, as to estimate the average weighted abundance of families found across the meridional and zonal gradients of the SEP. For this, we estimated the weighted abundance across the cross-shelf (CSWA) and latitudinal (LWA) gradients of the copepod families as:

$$\mathrm{LWA\ Or\ CSWA} = \frac{\sum(\mathrm{N}i\ \mathrm{Z}i\ \mathrm{R}i)}{\sum(\mathrm{N}i\ \mathrm{R}i)}$$

where N*i* is standardized average abundance to ind.m^{-3} for family *i*, Z*i* is the distance in km between a predefined starting point and end point of a given study area *i* and *Ri* is half the difference between these predefined boundaries of their *i* zone.

Satellite sea surface temperature (SST) was used as an indicator of oceanographic conditions for the study period (January 1986 until December 2016).For each sampling site, we used the mean SST of the last thirty years, from monthly data with a spatial resolution of 0.042 degrees. Satellite SST were obtained from AVHRR Pathfinder Version 5.3 (PFV53) produced by the National Centers of Environmental Information (NOCE) from NOAA (https://coastwatch.pfeg.noaa.gov).

3.2.2.3. Statistical analysis

We assessed similarities in distribution of families and species of the Calanoid copepods within and between zones in terms of relative abundance across of latitudinal and longitudinal transects. The structure of the community was evaluated by multivariate analyzes performed with PRIMER v.7 (Clarke and Gorley, 2006). For this, we grouped the different samples made a longitudinal gradient according to their location as CUP-Z, CTZ and OLIGO-Z. Samples from the latitudinal gradient of the CUP-Z were split into north and south with respect to the subtropical convergence (ca. 30ºS). For evaluating similarity in composition and abundance within zones, we applied a cluster analysis and multidimensional scaling (MDS) with log-transformed data (Log (x + 1)) for families and presence/absence for species. We used presence and absence data for species, because in some cases only genera are reported and also to reduce extremely high variance of abundance due to presence of dominant and rare species. The similarity index used for abundance data of families was the Bray-Curtis coefficient and for species we used the Jaccard index. Two factors were examined: temporary (season) only in the latitudinal gradient and spatial for both transects (Latitudinal: North and South; Longitudinal: CUP-Z, CTZ and OLIZGO-Z).The differences or similarities between the zones and seasons of study were evaluated with SIMPER (percentages of similarity). PERMANOVA analyzes were applied to test these effects, under the hypothesis that abundance at the level of species and families of copepods of the order Calanoida do not differ significantly between seasons and zones. Additionally, we evaluate the interaction between station and zone for a latitudinal gradient product of a greater temporary coverage and seasonal variability of coastal zone (Escribano *et al.*, 2007; Letelier *et al.*, 2009). The matrices of similarities were built for families from the Bray-curtis index and for species with Jaccard distance. All analyzes of PERMANOVA were made with 9999 permutations.

We evaluate the effect of the mean sea surface temperature (SST) about variability of the community structure in families and species within of the order Calanoid. The SST was selected as predictive variable because shows a high correlation with other hydrographic variables and a great dependence with physiology, development and behavior of the copepods. For this, was used the DISTLM analysis (linear model based on distance) both for latitudinal and longitudinal gradients. Environmental and abundance of families data were log-transformed (Log (x + 1) and species data to presence/absence. At the family level were evaluated its mean abundance and richness; however, for species its presence/absence and richness to test the effect of SST about response variables. Redundancy analysis (dbRDA - distance-based redundancy analysis) was used to examine the influence of temperature about the distribution of the species and families. The similarity indices used were Jaccard (presence / absence), Bray-Curtis (abundance) and Euclidean (richness) performed with 9999 permutations. All statistical analyzes were performed in PRIMER 7 with the complementary statistical package PERMANOVA + add-on (PRIMER-E, Plymouth Marine Laboratory, UK).

3.2.3. Results

Copepod families and species are listed in Supplemental Material 2: Species list. From the whole data base, 19 Calanoid families were found for a total number of 186 species (Supplemental Material 2). Among them, the familiy Aetideidae was the most represented by 24 species, followed by Calanidae with 18 species and then Clausocalanidae with 17 species. The least represented families were Rhyncalanidae and Temoridae with 3 species and Nullosetigeridae with a single unidentified species. Paracalanidae, Acartidae and Calanidae were the most abundant and commons species, although mainly present within the coastal upwelling zone, although their abundances also varied substantially depending on seasonal periods, as seen during the time series samplings in the coastal zone. Rare families in terms of occurrence and abundance were Calocalanidade and Nullosetigeridae both found only in the oceanic zone and with low abundances. Other rare families were Temoridae and Rhincalanidae both present only in the CUZ and CTZ.

When examining the community descriptors abundance (N), species richness (R) and weighed frequency of occurrence (WFO) for each predefined zone (Table 1), it was found that much greater abundances occurred in the CUZ both during the upwelling (Spring-Summer) and non-upwelling (Autumn-Winter) seasons, while in the OLIGO-Z abundances were commonly 1 order of magnitude lower when compared same families. Species richness tended to increase in the OLIGO-Z in the families containing more species. This was the case for Clausocalanidae (15 species) and Aetideidae (14 species). In most cases Families had fewer species in the CUZ, although in this zone R tended to increase in the Spring-Summer period.

3.2.3.1. Cross-shelf distribution

In the analysis of zonal distribution, we used the cross-shelf weighed abundance (CSWA) of the families to assess their dominant habitat across the coastal-offshore gradient. This distributional pattern is shown in Fig. 2. It was found that many families can occupy the entire zonal range, although a few appeared restricted to the coastal area and only Calocalanidae was absent from this area. It was also found that CSWA values were aggregated within the first 2000 km from the coast and these varied considerably among families, although with a tendency towards the coastal zone. In any case, CSWA's could help us to allocate dominant habitat of families, such that Augaptilidae, Clausocalanidae and Lucicutidae which seemed to have more oceanic habitats compared to the abundant Paracalanidae, Acartidae, Calanidae and Centropagidae whose habitat seem centered close to shore (Fig. 2). Some dominant families in the CTZ were Aetideidae, Candacidae, Clausocalanidae, Heterohabbidae, Nullosetigeridae and Phaennidae.

In terms of absolute abundance across the cross-shelf gradient, Fig. 3 represents how families are distributed. Calanidae and Paracalanidae were the families more widely distributed (with more homogeneous distribution) across the zonal gradient and along with Acartidae and Centropagidae they are the most abundant within the CUP-Z. By contrast, Aetideidae, Calocalanidae and Candacidae are represented with much more restricted distribution (Fig. 3).

Changes in community structure at the family level across the zonal gradient became evident after Cluster analysis using the Bray-Curtis similarity index of distance. This cluster analysis (Fig. 4A) shows that families within the upwelling zone (CUP-Z) became grouped together and the OLIGO-Z had a similar separated group, while the CTZ exhibited more mixing, especially with the CUP-Z. NMDS analysis revealed that two major clusters can be arranged at 20% of similarity and significant grouping may occur at 40% of similarity (Fig. 4B). Despite mixing and high stress PERMANOVA test was highly significant (*Pseudo-F*= 8.02, *P-value*<0.01) and indicated that zone factor explained 32.26% of the variation in families. Pair-wise tests by PERMANOVA indicated that all the zones are significantly different to each other in terms of families composition (*P-value* <0.01).

A similar analysis to that of families was applied to the community structure at the species level. In this case however, using presence or absence since abundance was too heterogeneous for comparisons. The Jaccard index of distance yielded a cluster array shown in Fig. 5A. Grouping according to zonation was more heterogeneous than in families, although structuring of species assemblages was also evident after presence of a CUP-Z group, another of the CTZ mixed with the CUP-Z, and a group mostly represented by the OLIGO-Z (Fig. 5A). NMDS analysis revealed a low level of similarity (20%) for grouping (Fig. 5B), although PERMANOVA was still significant (*Pseudo-F*= 3.36, *P-value*<0.05), while zone factor explained only 25.98% of the variation of the species. Pair-wise tests by PERMANOVA indicated that the CTZ and OLIGO-Z zones had no significant differences in terms of species composition (*P-value*> 0.05).

3.2.3.2. Latitudinal distribution

When looking at the latitudinal distribution of copepod families, we used a latitudinal weighed abundance (LWA), designed in according to our sampling area. This allowed us to allocate the dominant habitat of each family over the latitudinal axis within the CUP-Z. This alongshore pattern is shown in Fig. 6. It can be seen that most families are widely distributed over the region, except for Phaennidae inhabiting the northern area. Then two major groups can be distinguished located either in the southern or northern portions of the study area. In the southern area most representative families are Rhincalanidae, Lucicutidae, Heterohabbidae and Clausocalanidae, whereas in the northern portion Acartidade, Augaptilidae, Candacidae, Eucalanidae, Scocitricellidae and Temoridae are well represented. Other families, such as Metrinidae, Euchaetidae, Calanidae and Aetididae also tend to predominate in the southern area (Fig. 6). A shaded plot (Fig. 7) shows that Paracalanidae and Calanidae are widely distributed families along the upwelling zone, also representing the most abundant copepods in the region. In a lesser extent Acartidae and Metrinidae also appeared as widely distributed whereas other families exhibit more restricted distributions concentrated either at the south or northern portions of the study area. Another distinct group represented by Paracalanidae, Metrinidae, Calanidae and Clausocalanidae appeared aggregated in a transition zone around 30°S (Fig. 7).

Cluster analysis based on Bray-Curtis distance resulted in a highly structured community at the family level (Fig. 8A) providing three major groups associated with the southern, northern and transitional areas (around 30° S). NMDS shows that at 20% level of similarity two major groups emerge, one resulting from the mixing of the southern and northern portions and a second comprised by the transition zone (Fig. 8B). However, at 40% similarity level the three groups observed in the cluster can be found (Fig. 8B). PERMANOVA test detected significant differences between zones (*Pseudo-F*=5.04, *P-value*<0.05), although with a significant interaction with season (*Pseudo-F*= 2.63,*P-value*<0.05),and significant seasonal effects (*Pseudo-F*= 0.68, *P-value*>0.05). Both factors explained 47.57% of the variation in terms of family composition.

The analysis of the community structure at the species level alongshore also revealed structured patterns in according to clustering, although with more mixing and at a lower level of similarity (Fig. 9A). NMDS analysis revealed the presence of several groups of species assemblages which became structured at a 40% of similarity (Fig. 9B). PERMANOVA test in this case were also significant both for zones (*Pseudo-F*=4.48,*P-value*<0.05), season(*Pseudo-F*= 2.29,*P-value*<0.05) and their interaction (*Pseudo-F*= 2.09,*P-value*<0.05), explaining these factors xx% of the variation in terms of species composition

3.2.3.3. Environmental correlates

In order to examine environmental correlates of the copepod community with habitat conditions, we used sea surface temperature (SST). Mean SST distribution (1986-2016) is shown in Fig. 10, reflecting the horizontal gradients of SST, both over the cross-shelf and latitudinal dimensions. In the CUP-Z a warmer SST in the range of 16°C-22°C prevails in the northern portion,while a range of 12°C-16°C prevails in the southern portion of this CUP-Z. The CTZ exhibits a similar northern-southern pattern and SST range as that of the CUP-Z, whereas the OLIGO-Z shows a gradual increase in SST from about 16°C up to 26 °C at the western portion of the study area (Fig. 10).

Mean SST was correlated to copepod families and species by redundance analysis (RDA) using family species richness and abundance, and species richness and presence/absence of species. RDA was based on different distances indices (see Methods). Over the cross-shelf gradient it was found that at family level a 41.5% of variation in richness and a 21.1 % in abundance can be explained by SST as shown by the dislink plots (Fig. 11).These effects as tested by RDA were highly significant (*P-value*<0.01). Over a latitudinal gradient we found that at family level a 21.1% of variation on richness and 19.4% of the abundance can be explained by SST. These two effects were significant (*P-value*<0.05).

At species level we found that a 37.5% of variation on richness and 12.5% of presence/absence can be explained by the SST (Fig. 12). Both effects were highly significant (*P-value*<0.01). Over the latitudinal gradient, it was found that a 16.2% of variation of presence/absence of species could be explained by SST (*P-value*<0.01) (Fig. 12B), although SST effect on species richness was not significant over this gradient (*P-value*> 0.05).

3.2.4. Discussion

Biological diversity of zooplankton is modulated by environmental gradients which can modify the spatial patterns of species and communities (Lawton, 1999; Hillebrand y Azzsky, 2001; Rex *et al.,* 2001). Although, the partition of the habitat over the horizontal plane of zooplankton communities has been reported in some works (e.g. Heinrich, 1973; Gonzalez and Marín, 1998; Escribano and Hidalgo, 2000; Linacre and Palma, 2004), there have been difficulties for identifying and understanding the mechanisms that modulate differential distributions. Despite the fact that our data validate a high structuring of the order Calanoida, with totally different zones both in latitudinal and longitudinal gradients, there were differences in the sampling methods that may have introduced some biases. For example, different sampling depths can modify the community of copepods (e.g. Judkins, 1980). Furthermore, most of the samplings were made in the coastal upwelling zone targeting the upper 200 m, which is the zone where most species of copepods concentrate due to vertical restrictions imposed by presence of a shallow oxygen minimum zone (Escribano *et al.,* 2009). Only two samples from the coastal upwelling zone were deep (ca. 2000 m) and these may have sampled some rare species that inhabit the oxygen minimum zone, such as *Eucalanus inermis* (Hidalgo *et al.,* 2005). By contrast, for the coastal transition and the oligotrophic zones most of the samplings were centered in the upper 500 m, upon absence of a shallow oxygen minimum zone and then less vertical restriction for the species. Another additional eventual source of variation could be caused by the use of different sampling gear. In our case, we used the same mesh size for the zooplankton nets, but different opening diameter of nets and variable towing speed. A comparison of the MOCNESS and WP2 nets mentions that biomasses of zooplankton were different between these nets, although the largest fractions and contributions are represented mostly by euphausiids and amphipods, and there is no evidence for a significant effect of net opening diameter in the estimates of abundance of smaller organisms, such as copepods (Gjøsaeter *et al.*, 2000)

Samples analyzed in this study were obtained in different years and seasons. The coastal transition and oligotrophic zones were sampled during spring-summer, unlike the coastal region (upwelling zone) which was sampled throughout the year, such that it was then possible to assess the effect of seasonality on the species composition and families. Even although, for the coastal zone it has been reported an effect of seasonal variability on the composition of species (Hidalgo and Escribano, 2001; Escribano *et al.*, 2007), it was not possible to see such effect when evaluating composition of families over the latitudinal gradient. This lack of seasonality effect could be explained by continued presence of copepod families in the upwelling zone, although eventually represented by different species, or different stages of same species, so that showing a lack of variability in the total abundance and diversity at different times of the year (Escribano and McLaren, 1999, Hidalgo and Escribano, 2007).

With respect to zonation for the cross-shelf gradient, as found in the composition of families and species of the order Calanoida, observed changes were highly significant from coastal upwelling to oligotrophic zones. Other works in the same area also reported changes in species composition; for example in euphausiids (Riquelme-Bugueño *et al.*, 2012) and cnidarias (Palma and Silva, 2006).One of the main explanations for this pattern is attributed to the presence of different water masses, although variable sources of food and nutrient over this gradient has also been suggested, providing distinctive characteristics to each of the study zones (González et al., 2018) .The costal upwelling zone is mainly influenced by Equatorial Subsurface Water, characterized by a high concentration of nutrients and source of fresh carbon from the atmosphere that is rapidly transferred to copepods (Gruber *et al.*, 1999; Mompeán *et al.*, 2013; Morales *et al.*, 1996; Daneri *et al.*, 2000; Silva and Valdenegro, 2003). However, mesotrophic coastal transition and oligotrophic zones are composed by Subantarctic Water and Subtropical Water, respectively, which present lower concentrations of nutrients and more regenerated carbon sources compared to the coastal zone (Nakatsuka *et al.*, 1992; Reid, 1973). All these factors along with presence of the anticyclonic gyre in oceanic region can isolates species assemblages from the adjacent areas, and so promoting a semi-closed ecosystem regulated by in situ processes (McGowan, 1971, 1974, 19779). Over a longer time scale, samples of marine microfossils collected during the Pleistocene indicate that the longitudinal zonation observed in Southern Pacific persisted allowing to observe the structuring patterns at the level of family and species of calanoid copepods (Riedel and Funnell, 1964).

Latitudinal gradient showed a high structuring and spatial agreement between the analyzes done both at level of species as families, showing the presence of three groups north, south and transition. Other works done about the same gradient have also reported the presence of these biogeographic breaks in other organisms such as kelps, molluscs and bivalves (Gonzalez *et al.*, 2012; Cardenas *et al.*, 2009; Broitman *et al.*, 2001).The boundaries of these biogeographic regions are not fixed and can vary seasonally and between years (Escribano *et al.*, 2003). Despite that the limits between the different regions are not clear,has been reported that the transition region shows a high correlation with a gradient of surface temperature (Broitman *et al.*, 2001), irregular patterns of circulation (Hormazabal, 2004) and a sharp narrowing of the continental shelf (Strub *et al.*, 1998).Furthermore, the presence and interaction of different water masses such as: equatorial subsurface water and intermediate antarctic water provide equatorial species in the north region and polar/ sub-Antarctic in the south, possibly generating a transition zone between 30ºS corresponding to tropical convergence (Escribano *et al.*, 2003; Hidalgo *et al.*, 2010). Over longer time scales, climate change of the Pleistocene had a great impact in the present marine diversity. Such phenomenon has been reported in many species and oceans (e.g, North Atlantic. Maggs *et al.*, 2008). At the end of the Pleistocene, coastal upwelling zone showed presented a warming of surface seawater, sea level rise, and lowering of productivity probably restricting even more the zone of mixture of species of the north and south (Von Dassow and Collado-Fabbri, 2014; Zacas *et al.*, 2009).

In this study we report a strong dependence of community indices with temperature both at family and species levels. In this respect, it is known that temperature can modify the rates of various biological processes in copepods, such as growth, productivity and mortality, but also influencing physical conditions of the water column, such as stratification and availability of nutrients and indirectly affecting the ecology of copepods (Hirst and Kiørboe, 2008). Along the cross-shelf gradient it was observed a notorious increase in temperature from the coastal zone to the open ocean associated with increasing richness of copepod families. In fact, temperature was strongly correlated (> 40%) with richness both of families and species levels. In several regions of the world a positive correlation between temperature and richness of aquatic zooplankton species has been reported, accompanied by a decrease in abundance (Matsubara, 1993; Castro *et al.,* 2005; Hessen *et al.,* 2007). Although, abundance of families and presence / absence of species showed a lower correlation value (<21%) probably due to the nature of the indexes used that homogenized the biological matrix. Regarding the latitudinal gradient and the community indices, we observed that presence/absence of species and family richness presented the highest values but strongly variable and this can be associated with rapid and abrupt changes of temperature in the coastal zone due to upwelling variation (e.g. Sobarzo et al., 2007).

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Competing interests

The authors declare no conflict of financial and non-financial interests.

Author contributions

All co-authors have contributed to the work. CG contributed to data analysis and writing. RE integrated all results and developed the direction of the manuscript. JM and RE participated in discussion and data analyses and commented on the manuscript.

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Figure legends

Figure 1. The Southeast Pacific region where the samplings were made between the years 1986-2016. The sampling stations are indicated by a yellow circle. The study area was divided in three zones according to surface chlorophyll-a values: coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic zone (OLIGO-Z) (Gonzalez *et al.*, 2019). The bathymetry of each zone is indicated by its color and the isobars of 2000, 4000, 6000 and 8000 m.

Figure 2. Mean distribution of families presents along a longitudinal gradient from the coastal zone of Chile to Easter Island between the years 1986-2016. For each family shows the site of Cross-shelf weighed abundance (CSWA: 🔴), greatest abundance (⬛), lowest abundance (🟩) and range of distribution (🟨).

Figure 3. Shade pot of mean abundances transformed by log (x + 1) of the 19 families reported along a longitudinal gradient from 1986 to 2016for the Southeast Pacific. Oceanographic zones are specified as: coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic zone (OLIGO-Z). The color scale represents abundance transformed each family.

Figure 4. Multivariate analysis of families of the order Calanoida found along a longitudinal gradient defined as: coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic zone (OLIGO-Z) of the Southeast Pacific. (A) Conglomerate Analysiscalculated through the Bray-Curtis distance index from the transformation of abundance data with log (x + 1) of the different families present. (B) Non-metric multidimensional scaling diagram (NMDS) for the first two axes of family abundance present across the three zones of the longitudinal gradient (stress = 0.1).

Figure 5. Multivariate analysis of the species of the order Calanoida found along of a longitudinal gradient defined as: coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic zone (OLIGO-Z) of the Southeast Pacific. (A) The Cluster Analysis calculated using the Jaccard distance index from the transformation of the abundance data in presence / absence of the different species present. (B) Non-

metric multidimensional scaling diagram (NMDS) for the first two axes of the species present across the three zones of the longitudinal gradient (stress = 0.12).

Figure 6. Mean distribution of families presents across latitudinal gradient from the coastal zones of Antofagasta to Concepción between 1986-2016.For each family is observed latitudinal weighed abundance (LWA: 🔴), range of distribution (⬛), site of greatest abundance (🟩) and lowest abundance (🟨).

Figure 7. Shade plot of Mean abundances transformed by log (x + 1) of the18 families found in the north and south zones coastal upwelling zone (CUP-Z) from 1986 to 2016 of the Southeast Pacific. The color scale represents abundance transformed each family.

Figure 8. Multivariate analysis of families of the order Calanoida found both in the north and south of coastal upwelling zone (CUP-Z) of the Southeast Pacific.(A) The Cluster Analysis was calculated using the Bray-Curtis distance index through transformation of abundance data with log (x + 1)of different families.(B) Non-metric multidimensional scaling diagram (NMDS) for the first two axes for the mean abundance families across the Chilean coast (stress = 0.07).

Figure 9.Multivariate analysis of species of the order Calanoida found in both the north and south zones of coastal upwelling zone(CUP-Z) of the Southeast Pacific.(A) The Cluster Analysis was calculated using the Jaccard distance index through transformation of the abundance data in presence / absence of the different species presents.(B) Non-metric multidimensional scaling diagram (NMDS) for the first two axes for the families present in the north and south zones of the coastal region of Chile (stress = 0.12).

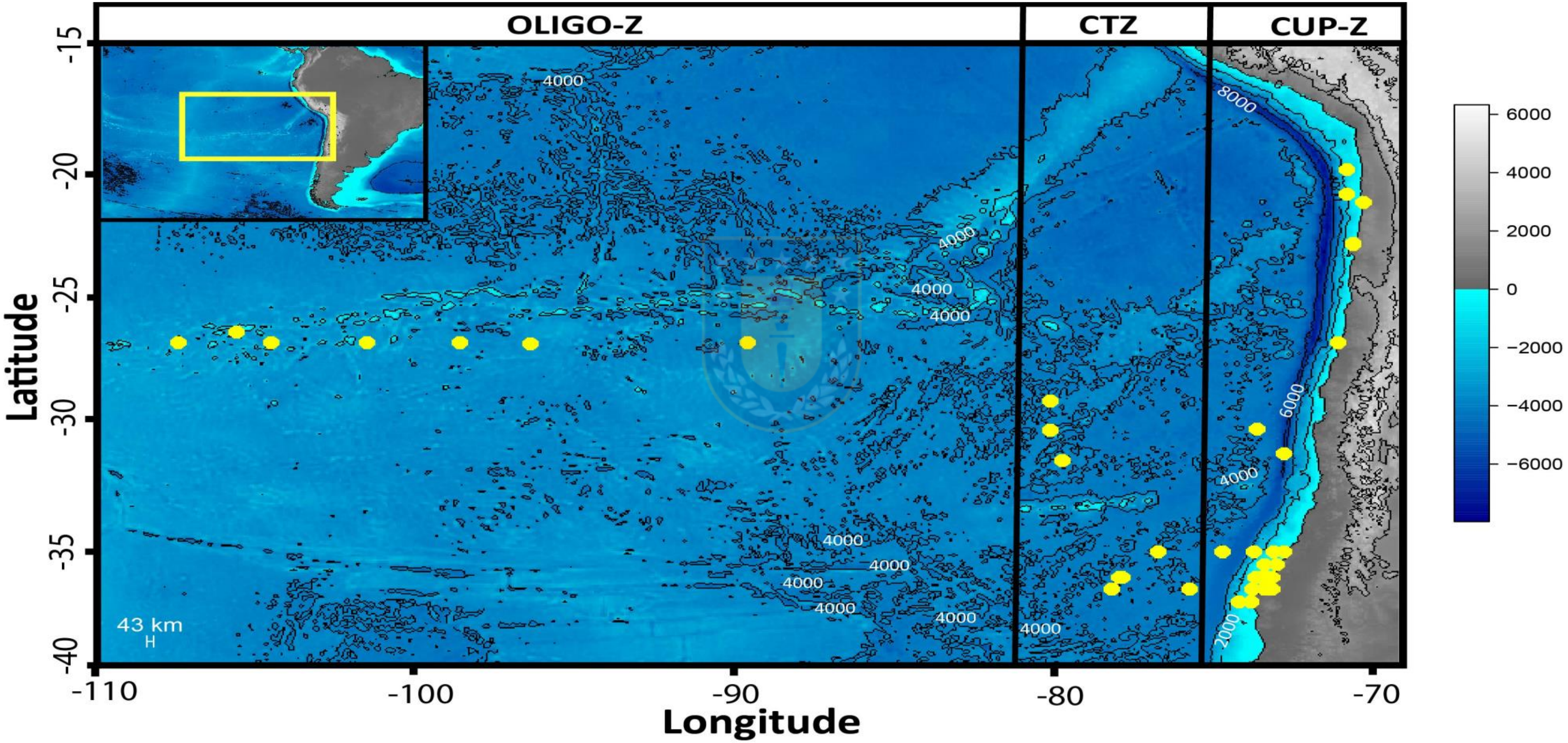
Figure 10.Southeast Pacific Region where was collected the copepod database of the order Calanoida from 1986 to 2016.Sea surface temperature averaged for entire sampling periodin the three study areas defined as: coastal upwelling area (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic area (OLIGO-Z)

Figure 11. Distance-based redundancy analysis (dbRDA) plot of the DistLM based on the mean temperature of the sea fitted to community structure of families the order Calanoida in Southeast Pacific between the years 1986-2016. At the family level were related temperature with its abundance and richness.

Figure 12. Distance-based redundancy analysis (dbRDA) plot of the DistLM based on the mean temperature of the sea fitted to community structure of species of the order

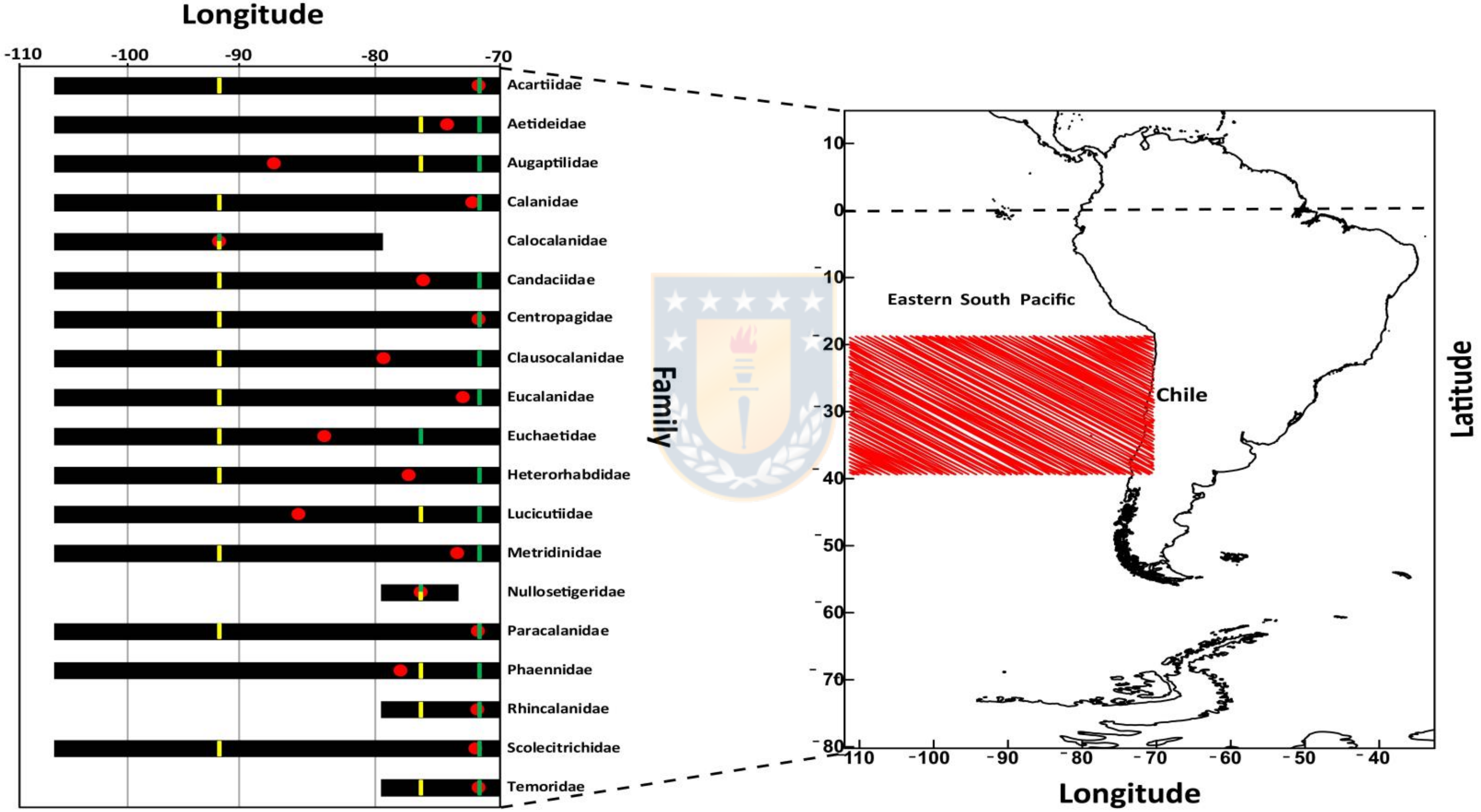
Calanoida in Southeast Pacific between the years 1986-2016. At the species level were related temperature with its presence/absence and richness

Fig.1



OLIGO-Z
CTZ
CUP-Z
Latitude
-15
-20
-25
-30
-35
-40
Longitude
-110
-100
-90
-80
-70
4000
8000
6000
2000
43 km
6000
4000
2000
0
−2000
−4000
−6000

Fig.2


Longitude
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-100
-90
-80
-70
Acartiidae
Aetideidae
Augaptilidae
Calanidae
Calocalanidae
Candaciidae
Centropagidae
Clausocalanidae
Eucalanidae
Euchaetidae
Heterorhabdidae
Lucicutiidae
Metridinidae
Nullosetigeridae
Paracalanidae
Phaennidae
Rhincalanidae
Scolecitrichidae
Temoridae
Family
10
0
-10
-20
-30
-40
-50
-60
-70
-80
Eastern South Pacific
Chile
Latitude
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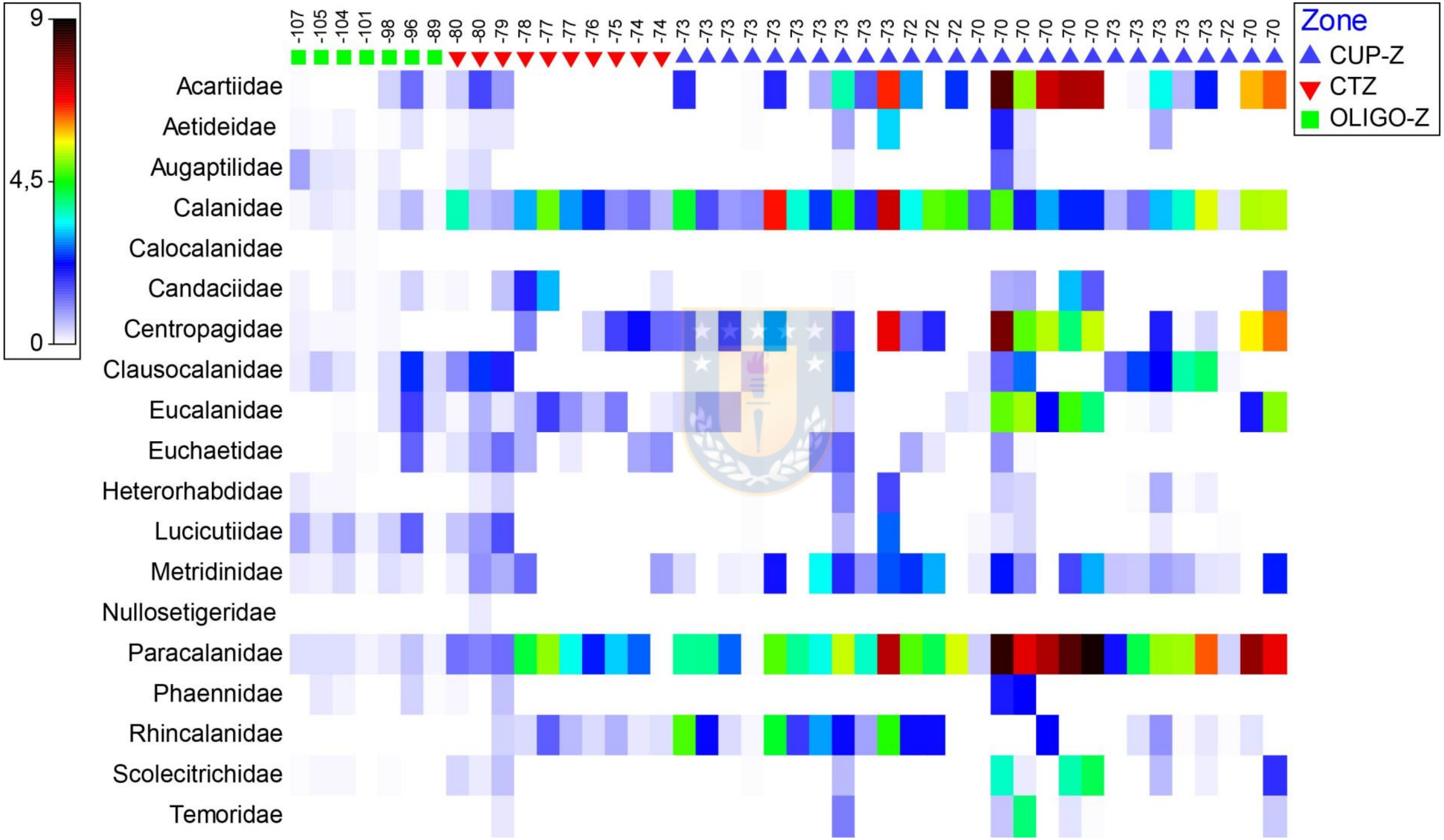
-100
-90
-80
-70
-60
-50
-40
Longitude

Fig.3



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-70
-70
Acartiidae
Aetideidae
Augaptilidae
Calanidae
Calocalanidae
Candaciidae
Centropagidae
Clausocalanidae
Eucalanidae
Euchaetidae
Heterorhabdidae
Lucicutiidae
Metridinidae
Nullosetigeridae
Paracalanidae
Phaennidae
Rhincalanidae
Scolecitrichidae
Temoridae
Zone
CUP-Z
CTZ
OLIGO-Z

Fig.4

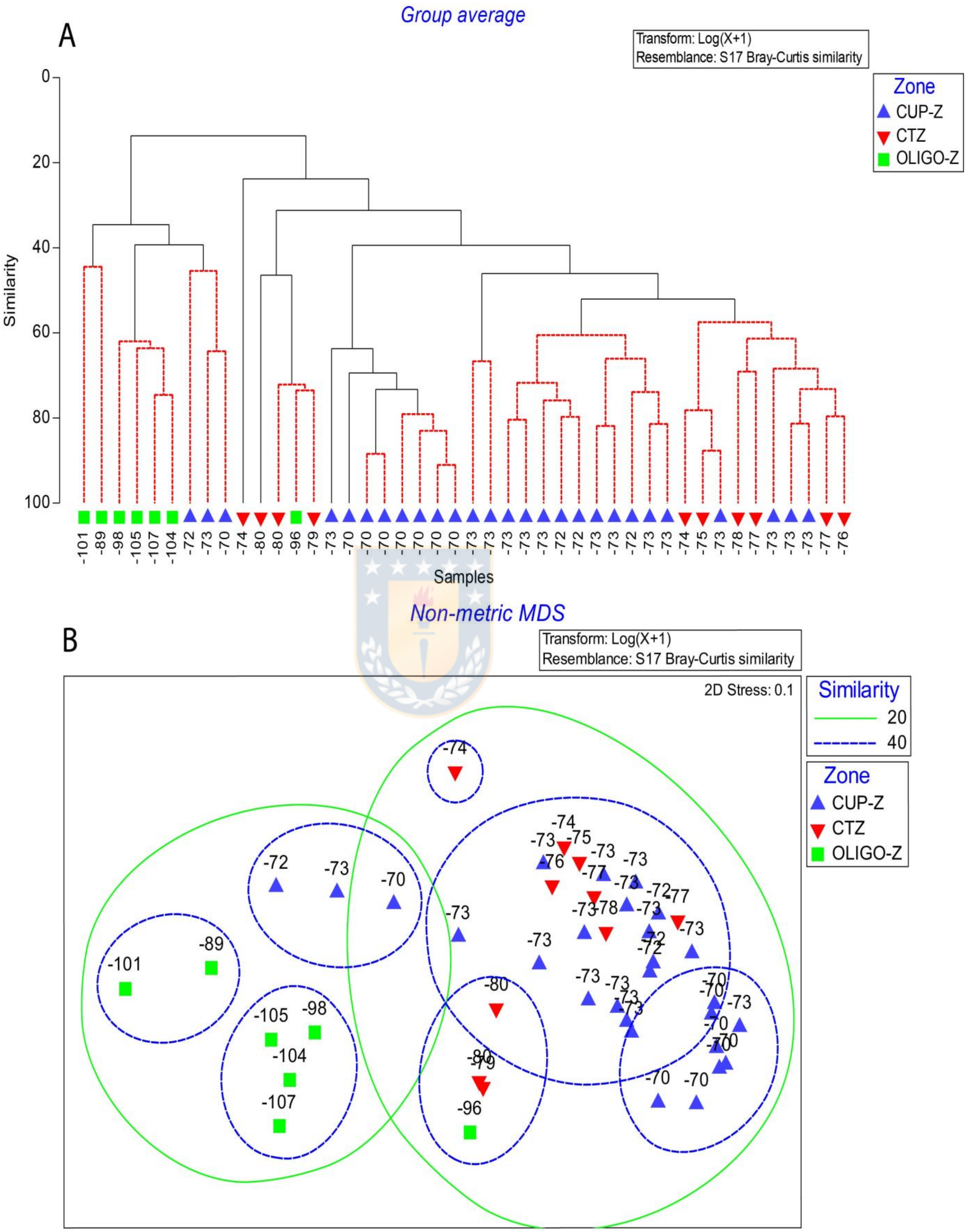
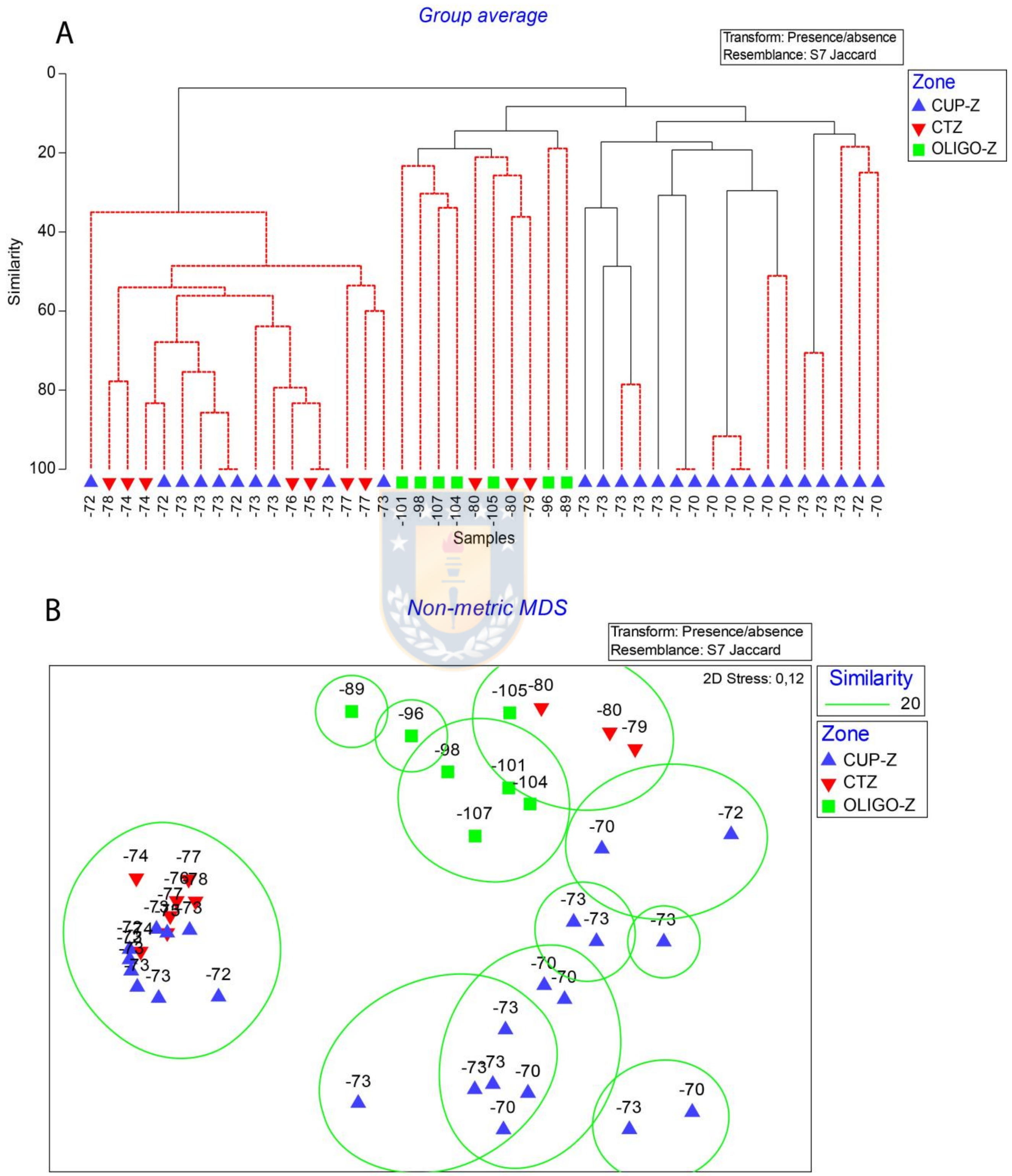

A
Group average
Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
Zone
CUP-Z
CTZ
OLIGO-Z
Similarity
0
20
40
60
80
100
-101 -89 -98 -105 -107 -104 -72 -73 -70 -74 -80 -80 -96 -79 -73 -70 -70 -70 -70 -70 -70 -70 -73 -73 -73 -73 -73 -72 -72 -73 -73 -72 -73 -73 -74 -75 -73 -78 -77 -73 -73 -73 -77 -76
Samples
B
Non-metric MDS
Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0.1
Similarity
20
40
Zone
CUP-Z
CTZ
OLIGO-Z

Fig.5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Group average
Transform: Presence/absence
Resemblance: S7 Jaccard
Zone
CUP-Z
CTZ
OLIGO-Z
Similarity
0
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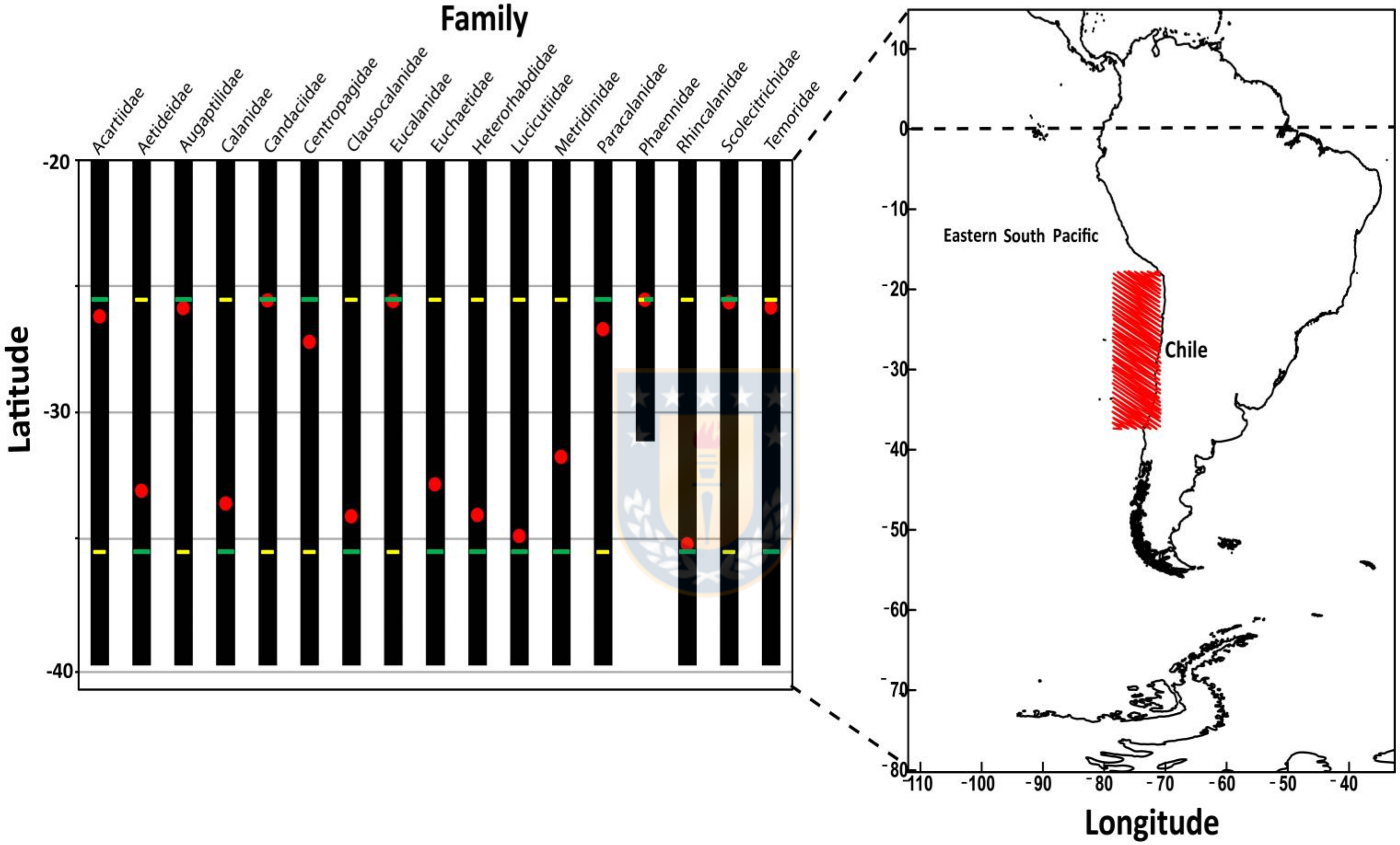
100
-72 -78 -74 -74 -72 -73 -73 -73 -72 -73 -73 -76 -75 -73 -77 -77 -73 -101 -98 -107 -104 -80 -105 -80 -79 -96 -89 -73 -73 -73 -73 -73 -70 -70 -70 -70 -70 -70 -70 -73 -73 -73 -72 -70
Samples
B
Non-metric MDS
Transform: Presence/absence
Resemblance: S7 Jaccard
2D Stress: 0,12
Similarity
20
Zone
CUP-Z
CTZ
OLIGO-Z

Fig.6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Aetideidae
Augaptilidae
Calanidae
Candaciidae
Centropagidae
Clausocalanidae
Eucalanidae
Euchaetidae
Heterorhabdidae
Lucicutiidae
Metridinidae
Paracalanidae
Phaennidae
Rhincalanidae
Scolecitrichidae
Temoridae
Latitude
-20
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-40
10
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-40
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Eastern South Pacific
Chile
-110
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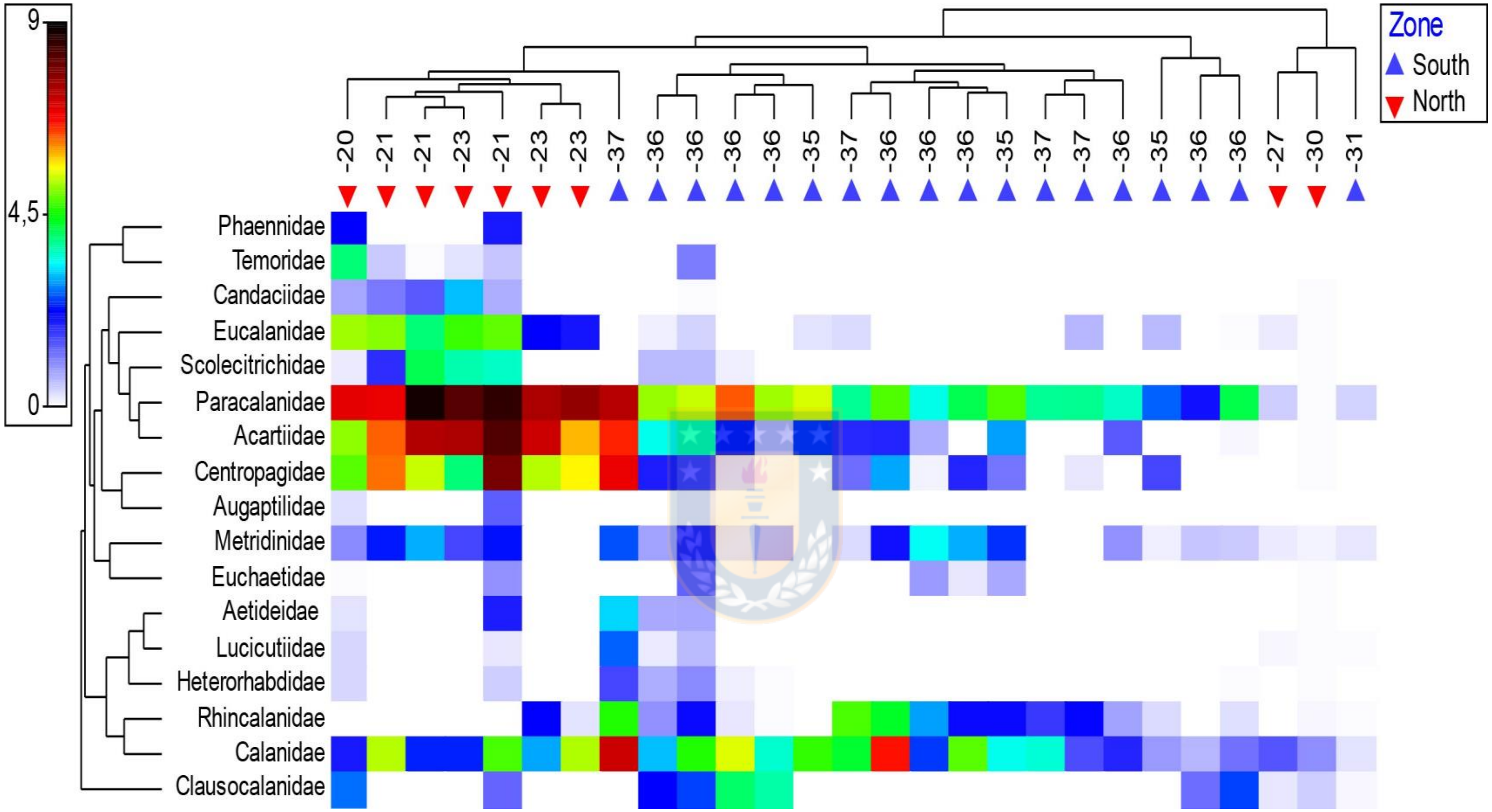
-40
Longitude

Fig.7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-27
-30
-31
Zone
South
North
Phaennidae
Temoridae
Candaciidae
Eucalanidae
Scolecitrichidae
Paracalanidae
Acartiidae
Centropagidae
Augaptilidae
Metridinidae
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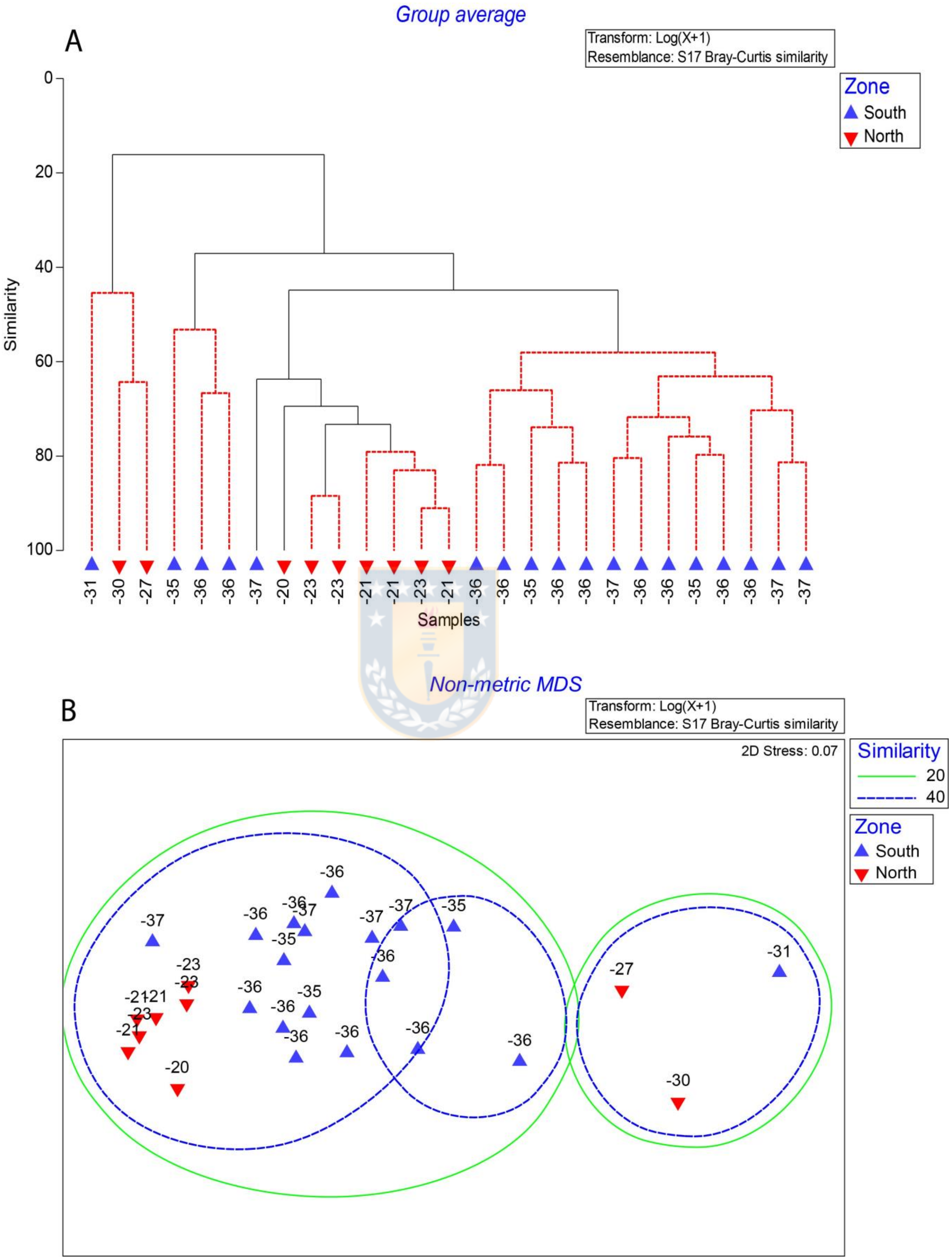
Aetideidae
Lucicutiidae
Heterorhabdidae
Rhincalanidae
Calanidae
Clausocalanidae

Fig.8


Group average
A
Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
Zone
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▼ North
Similarity
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-37
-37
Samples
Non-metric MDS
B
Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
2D Stress: 0.07
Similarity
20
40
Zone
▲ South
▼ North
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-36
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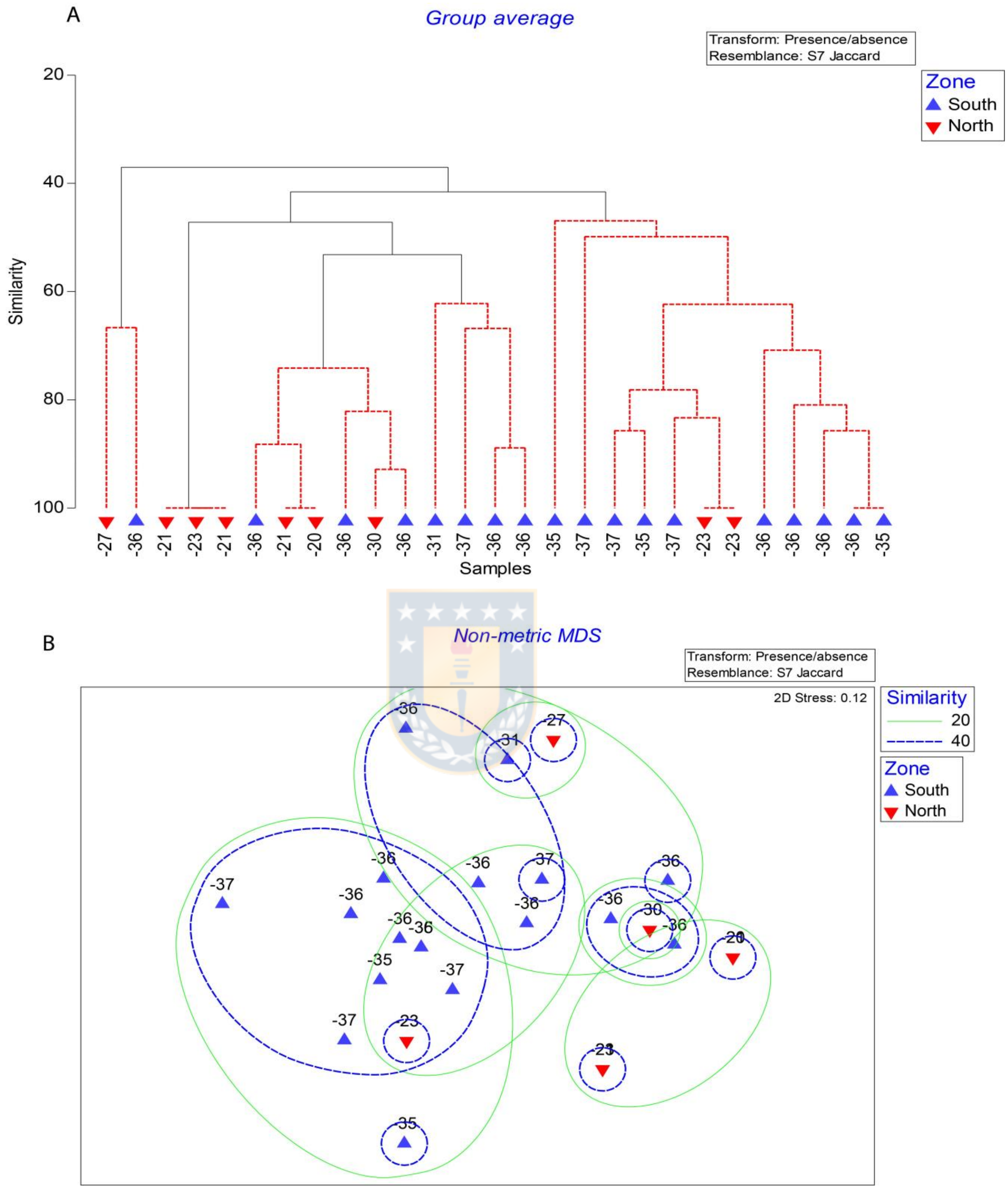
-30

Fig.9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Resemblance: S7 Jaccard
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North
Similarity
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Samples
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Non-metric MDS
Transform: Presence/absence
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2D Stress: 0.12
Similarity
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Zone
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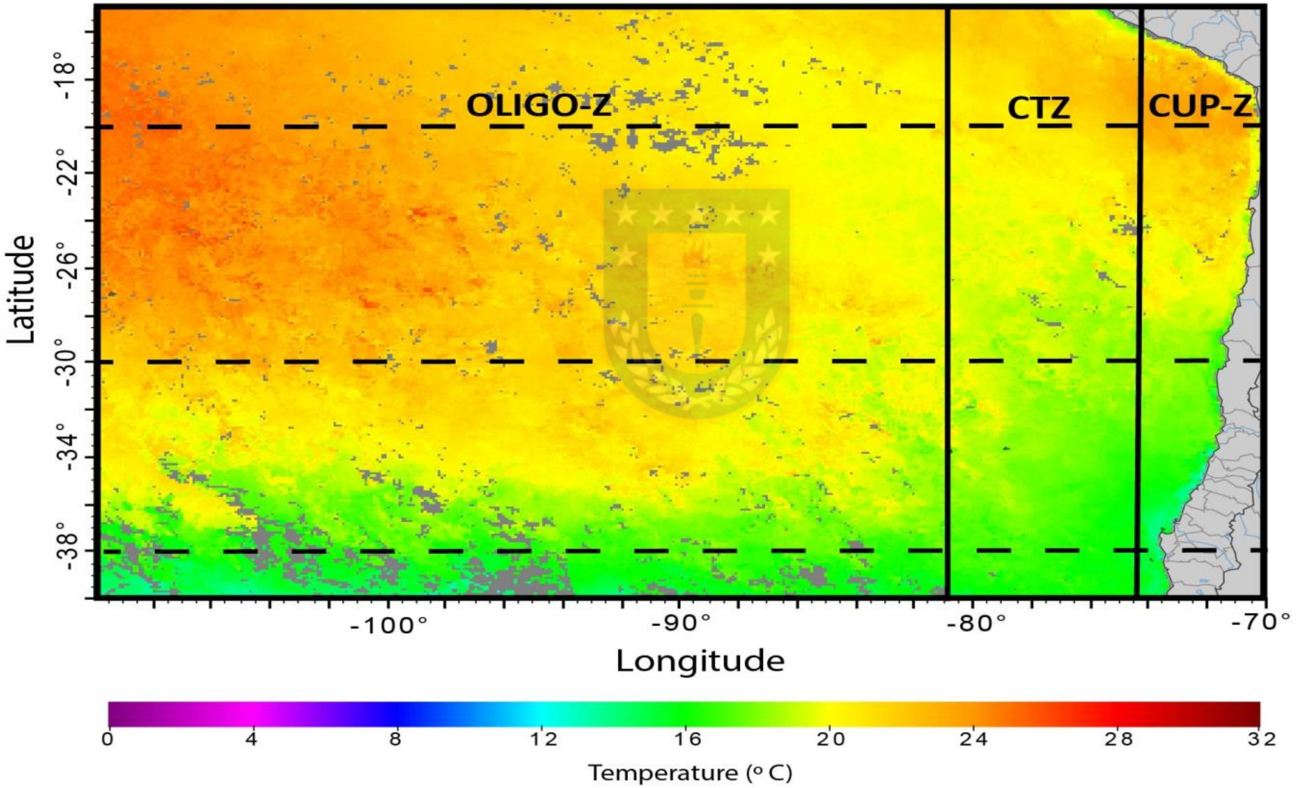
Fig.10


OLIGO-Z
CTZ
CUP-Z
Latitude
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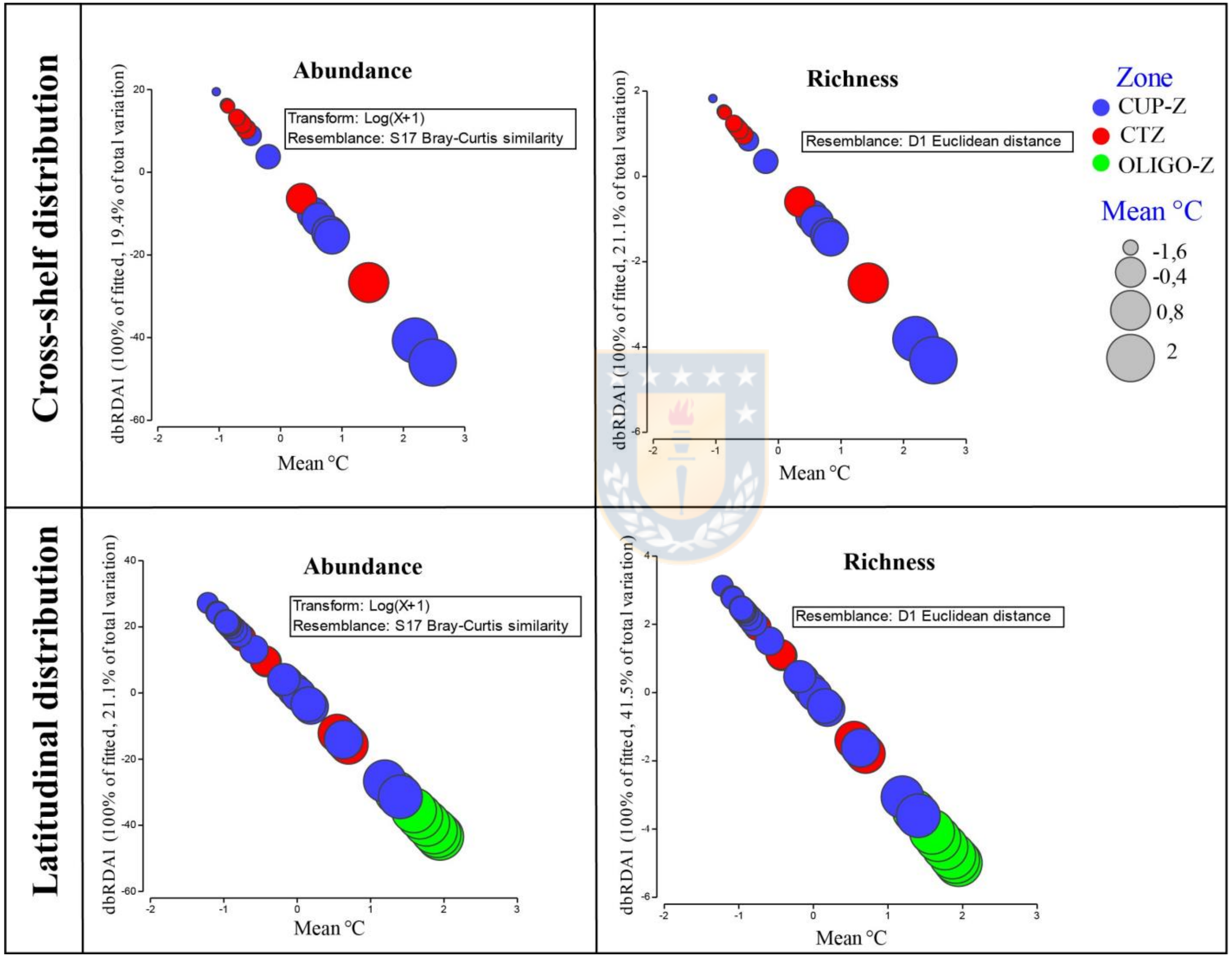
-22°
-26°
-30°
-34°
-38°
-100°
-90°
-80°
-70°
Longitude
0
4
8
12
16
20
24
28
32
Temperature (° C)

Fig.11


Cross-shelf distribution
Latitudinal distribution
Abundance
Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
dbRDA1 (100% of fitted, 19.4% of total variation)
Mean °C
Richness
Resemblance: D1 Euclidean distance
dbRDA1 (100% of fitted, 21.1% of total variation)
Zone
CUP-Z
CTZ
OLIGO-Z
Mean °C
-1,6
-0,4
0,8
2
Abundance
Transform: Log(X+1)
Resemblance: S17 Bray-Curtis similarity
dbRDA1 (100% of fitted, 21.1% of total variation)
Mean °C
Richness
Resemblance: D1 Euclidean distance
dbRDA1 (100% of fitted, 41.5% of total variation)
Mean °C
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0
-20
-40
-60
40
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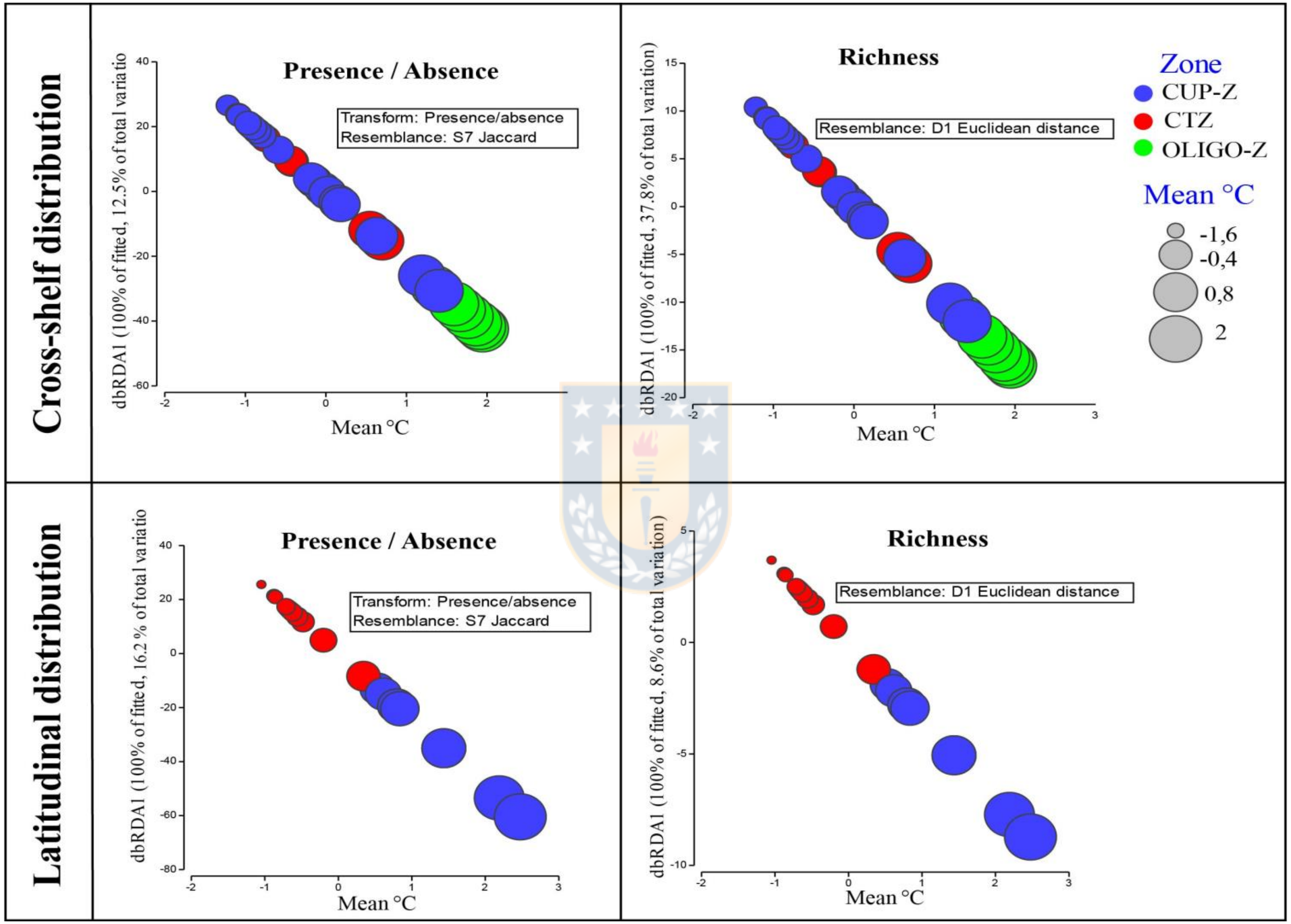
4
-2
-4
-6
-1
1
3

Fig.12.


Cross-shelf distribution
Latitudinal distribution
Presence / Absence
Transform: Presence/absence
Resemblance: S7 Jaccard
dbRDA1 (100% of fitted, 12.5% of total variatio
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2
Mean °C
Richness
Resemblance: D1 Euclidean distance
dbRDA1 (100% of fitted, 37.8% of total variation)
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-5
-10
-15
-20
-2
-1
0
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3
Mean °C
Zone
CUP-Z
CTZ
OLIGO-Z
Mean °C
-1,6
-0,4
0,8
2
Presence / Absence
Transform: Presence/absence
Resemblance: S7 Jaccard
dbRDA1 (100% of fitted, 16.2 % of total variatio
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-20
-40
-60
-80
-2
-1
0
1
2
3
Mean °C
Richness
Resemblance: D1 Euclidean distance
dbRDA1 (100% of fitted, 8.6% of total variation)
5
0
-5
-10
-2
-1
0
1
2
3
Mean °C

Supplementary table 1. Sampling stations from the coastal region to the open ocean waters of Southeast Pacific from 1986 to 2016. For each station is informed the longitude, latitude, distance from the coast, depth, year and sampling equipment used. Oceanographic zones are specified as: coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic zone (OLIGO-Z). The seasons of the year were divided two periods one from September to March (S-S: Spring-Summer) and another from August to April (A-W: Autumn-Winter). The databases used for this analysis most have been reported in previous publications (Time Series St18: Escribano *et al.*, (2007), Time Series Mejillones (Escribano et al.,2012), CENSOR (Hidalgo *et al.*, 2010), FIP (Escribano *et al.*, 2016), ZOMEI (Hidalgo *et al.*, 2010), Castro *et al.*, 2007,MOPEX (Unpublished data), FONDECYT (Unpublished data), CIMAR-21 (González *et al.*, 2019), CIMAR-22 (Unpublished data).

Long.	Lat.	Distance from Cost (km)	Season	Zone	Depth	Database	Date	Sampling gear
-107.57	-27.01	4166.86	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-105.70	-26.57	3958.91	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-104.61	-27.00	3837.69	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-101.60	-27.00	3502.95	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-98.65	-27.01	3174.24	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-96.43	-27.04	2927.77	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-89.57	-27.02	2163.47	S-S	OLIGO-Z	0-450	CIMAR21	2015	1 m^2 Tucker trawl net
-80.03	-30.55	1101.58	S-S	CTZ	0-500	CIMAR 22	2016	1 m^2 Tucker trawl net
-80.00	-29.37	1098.74	S-S	CTZ	0-500	CIMAR 22	2016	1 m^2 Tucker trawl net
-79.66	-31.79	1060.50	S-S	CTZ	0-500	CIMAR 22	2016	1 m^2 Tucker traw net
-78.07	-37.00	883.18	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net
-77.85	-36.50	859.14	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net
-77.74	-36.50	846.66	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net
-76.63	-35.50	723.74	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net
-75.60	-37.00	608.76	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net
-74.59	-35.50	495.84	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net
-74.05	-37.50	436.26	S-S	CTZ	0-200	FIP	2005	8 m^2 Tucker trawl net

-73.69	-37.50	395.37	S-S	CUP-Z	0-50	FIP	2005	8 m^2 Tucker trawl net
-73.63	-37.00	389.23	S-S	CUP-Z	0-50	FIP	2005	8 m^2 Tucker trawl net
-73.56	-35.50	381.90	S-S	CUP-Z	0-200	FIP	2005	8 m^2 Tucker trawl net
-73.50	-30.50	374.77	S-S	CUP-Z	0-2000	MOPEX	2016	8 m^2 Tucker trawl net
-73.38	-36.50	361.97	S-S	CUP-Z	0-100	FIP	2005	8 m^2 Tucker trawl net
-73.26	-37.00	348.07	S-S	CUP-Z	0-35	FIP	2005	8 m^2 Tucker trawl net
-73.24	-36.00	346.29	S-S	CUP-Z	0-200	FIP	2005	8 m^2 Tucker trawl net
-73.13	-36.51	333.49	S-S	CUP-Z	0-80	Time series St18	2002-2009	1 m^2 Tucker trawl net
-73.01	-36.50	320.54	S-S	CUP-Z	0-35	FIP	2005	8 m^2 Tucker trawl net
-73.00	-37.00	319.11	S-S	CUP-Z	0-20	Castro *et al.*, 2007	1985	Centrifuge pump
-72.95	-35.50	313.53	S-S	CUP-Z	0-120	FIP	2005	8 m^2 Tucker trawl net
-72.87	-36.00	304.60	S-S	CUP-Z	0-120	FIP	2005	8 m^2 Tucker trawl net
-72.64	-35.50	279.35	S-S	CUP-Z	0-30	FIP	2005	8 m^2 Tucker trawl net
-70.94	-27.00	89.83	S-S	CUP-Z	0-180	CIMAR21	20015	1 m^2 Tucker trawl net
-70.67	-21.00	59.37	S-S	CUP-Z	0-100	ZOMEI	2005	0.5 m^2 Multinet
-70.63	-20.00	55.66	S-S	CUP-Z	0-100	ZOMEI	2005	0.5 m^2 Multinet
-70.47	-23.01	37.48	S-S	CUP-Z	0-50	Time series Mejillones	1993-2008	Bongo net
-70.45	-23.00	35.25	S-S	CUP-Z	0-100	CENSOR	2006-2007	WP2 net
-70.13	-21.32	1.00	S-S	CUP-Z	0-100	CENSOR	2005-2007	WP2 net
-73.54	-36.50	379.23	A-W	CUP-Z	0-200	FONDECYT	2008	0.5 m^2 Multinet
-73.34	-36.50	356.96	A-W	CUP-Z	0-200	FONDECYT	2008	0.5 m^2 Multinet
-73.13	-36.51	333.49	A-W	CUP-Z	0-80	Time series St18	2002-2008	1 m^2 Tucker trawl net
-73.12	-36.50	332.47	A-W	CUP-Z	0-80	FONDECYT	2008	0.5 m^2 Multinet
-73.02	-36.50	321.34	A-W	CUP-Z	0-100	FONDECYT	2008	0.5 m^2 Multinet
-72.63	-31.50	278.15	A-W	CUP-Z	0-2000	MOPEX	2016	8 m^2 Tucker trawl net
-70.47	-23.01	37.48	A-W	CUP-Z	0-34	Time series Mejillones	1993-2008	WP2 net
-70.13	-21.32	1.00	A-W	CUP-Z	0-100	CENSOR	2005-2006	WP2 net

Supplementary table 2. Community descriptors for copepod families of the order Calanoida in the Southeast Pacific during 1986 until 2016. Richness (R), mean abundance (N) with its standard deviation (±SD) and weighed frequency of occurrence (WHO) were estimated divided by zone and station only for coastal region. Oceanographic zones are specified as: coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ) and oligotrophic zone (OLIGO-Z). The seasons of the year were divided two periods one from September to March (Spring-Summer) and another from August to April (Autumn-Winter).

	Zones											
Family	**CUP-Z (Spring-Summer)**			**CUP-Z (Autumn-Winter)**			**CTZ (Spring-Summer)**			**OLIGO-Z (Spring-Summer)**		
	R	**WHO**	**N (±SD)**	**R**	**WHO**	**N (±SD)**	**R**	**WHO**	**N (±SD)**	**R**	**WHO**	**N (±SD)**
Acartiidae	4	0.18	9447.29 ± 967.507	1	0.75	942.89 ± 197.530	4	0.08	5.97 ± 1.223	4	0.22	3.19 ± 0.883
Aetideidae	9	0.03	30.70 ± 5.226	5	0.05	1.09 ± 0.360	9	0.03	0.53 ± 0.090	14	0.05	0.56 ± 0.091
Augaptilidae	5	0.03	3.52 ± 0.683	-	-	-	2	0.10	0.55 ± 0.115	6	0.13	2.03 ± 0.417
Calanidae	8	0.13	2745.54 ± 323.878	5	0.20	673.23 ± 95.241	9	0.11	227.37 ± 39.630	13	0.07	1.74 ± 0.247
Calocalanidae	-	-	-	-	-	-	-	-	-	3	0.17	0.11 ± 0.020
Candaciidae	3	0.11	25.97 ± 4.576	2	0.13	2.24 ± 0.736	3	0.17	26.56 ± 5.913	8	0.09	0.91 ± 0.151
Centropagidae	3	0.26	270.27 ± 610.800	1	0.63	785.19 ± 176.678	1	0.50	16.72 ± 2.373	4	0.19	0.46 ± 0.047
Clausocalanidae	8	0.03	28.56 ± 3.858	12	0.06	117.71 ± 19.834	5	0.06	17.98 ± 3.336	15	0.06	11.87 ± 3.379
Eucalanidae	6	0.11	479.82 ± 51.867	5	0.10	166.50 ± 52.507	4	0.23	11.22 ± 1.300	2	0.31	5.10 ± 1.508
Euchaetidae	5	0.07	7.24 ± 0.769	3	0.04	0.01 ± 0.001	5	0.14	8.02 ± 0.826	3	0.21	3.07 ± 0.995
Heterorhabdidae	7	0.04	6.746 ± 0.949	5	0.13	1.25 ± 0.323	2	0.10	0.64 ± 0.144	2	0.19	0.39 ± 0.079
Lucicutiidae	5	0.06	14.63 ± 2.890	2	0.19	0.21 ± 0.066	3	0.10	5.77 ± 1.142	5	0.18	6.35 ± 0.964
Metridinidae	11	0.07	117.26 ± 7.903	6	0.15	12.92 ± 2.826	12	0.04	6.75 ± 0.884	10	0.09	1.30 ± 0.128
Nullosetigeridae	-	-	-	-	-	-	1	0.10	0.18 ± 0.053	-	-	-
Paracalanidae	8	0.125	20629.82 ± 1872.850	8	0.13	4054.62 ± 680.684	7	0.13	309.48 ± 47.606	10	0.09	2.14 ± 0.170
Phaennidae	2	0.05	14.42 ±2.228	-	-	-	1	0.20	0.78 ± 0.209	3	0.21	0.82 ± 0.164
Rhincalanidae	2	0.34	350.98 ± 34.792	2	0.38	2.55 ± 0.496	2	0.40	6.63 ± 0.863	-	-	-
Scolecitrichidae	10	0.03	136.00 ± 16.970	6	0.08	6.26 ±1.733	6	0.05	1.28 ± 0.228	10	0.06	0.22 ± 0.027
Temoridae	2	0.01	53.39 ± 11.227	1	0.13	0.60 ± 0.198	1	0.10	0.23 ± 0.070	-	-	-

Supplementary table 3. Families and species of copepods of the order Calanoida identified in samples from 1986 to 2016 in the Southeast Pacific. A total of 186 species are reported distributed in 19 families.

Acartiidae
Acartia (Acartia) danae Giesbrecht, *1889*
Acartia (Odontacartia) lilljeborgi Giesbrecht, *1889*
Acartia (Acartia) negligens Dana, 1849
Acartia (Acartiura) omorii Bradford, 1976
Acartia (Acanthacartia) plumosa Scott T., 1894
Acartia sp.
Acartia (Acanthacartia) tonsa Dana, 1849

Aetideidae
Aetideus acutus Farran, 1929
Aetideus acutus Farran, 1929
Aetideus armatus (Boeck, 1872)
Aetideus giesbrechti Cleve, 1904
Aetideus sp.
Chiridiella sp.
Chiridius gracilis Farran, 1908
Chiridius poppei Giesbrecht, 1893
Chirundina streetsii Giesbrecht, 1895
Euchirella amoena Giesbrecht, 1888
Euchirella curticauda Giesbrecht, 1888
Euchirella maxima Wolfenden, 1905
Euchirella pulchra (Lubbock, 1856)
Euchirella similis Wolfenden, 1911
Euchirella sp.
Gaetanus brevicornis Esterly, 1906
Gaetanus kruppii Giesbrecht, 1903
Gaetanus miles Giesbrecht, 1888
Gaetanus minor Farran, 1905
Gaetanus sp.
Gaetanus tenuispinus (Sars G.O., 1900)
Undeuchaeta major Giesbrecht, 1888
Undeuchaeta plumosa (Lubbock, 1856)
Valdiviella insignis Farran, 1908

Augaptilidae
Augaptilus sp.
Euaugaptilus longimanus (Sars G.O., 1905)
Euaugaptilus magnus (Wolfenden, 1904)
Euaugaptilus sp.
Haloptilus chierchiae (Giesbrecht, 1889)
Haloptilus longicornis (Claus, 1863)
Haloptilus oxycephalus (Giesbrecht, 1889)
Haloptilus plumosus (Claus, 1863)
Haloptilus sp.

Calanidae
Calanoides carinatus (Krøyer, 1849)
Calanoides patagoniensis Brady, 1883
Calanus australis Brodsky, 1959
Calanus chilensis Brodsky, 1959
Calanus jashnovi Hulsemann, 1994
Calanus sp.
Cosmocalanus darwinii (Lubbock, 1860)
Mesocalanus lighti (Bowman, 1955)
Mesocalanus tenuicornis (Dana, 1849)
Nannocalanus elegans Andronov, 2001
Nannocalanus minor (Claus, 1863)
Nannocalanus minor (Claus, 1863)
Neocalanus cristatus (Krøyer, 1848)
Neocalanus cristatus (Krøyer, 1848)
Neocalanus gracilis (Dana, 1852)
Neocalanus robustior (Giesbrecht, 1888)
Neocalanus sp.
Neocalanus tonsus (Brady, 1883)

Calocalanidae
Calocalanus contractus Farran, 1926
Calocalanus pavoninus Farran, 1936
Calocalanus sp.

Candaciidae
Candacia armata Boeck, 1872
Candacia bipinnata (Giesbrecht, 1889)
Candacia bispinosa (Claus, 1863)
Candacia catula (Giesbrecht, 1889)
Candacia cheirura Cleve, 1904
Candacia longimana (Claus, 1863)
Candacia sp
Candacia tenuimana (Giesbrecht, 1889)
Candacia varicans (Giesbrecht, 1893)
Candacia worthingtoni (Grice, 1981)

Centropagidae
Centropages brachiatus (Dana, 1849)

Centropages elegans Giesbrecht, 1895
Centropages elongatus Giesbrecht, 1896
Centropages gracilis (Dana, 1849)
Centropages sp.
Centropages typicus Krøyer, 1849

Clausocalanidae

Clausocalanus arcuicornis (Dana, 1849)
Clausocalanus furcatus (Brady, 1883)
Clausocalanus ingens Frost & Fleminger, 1968
Clausocalanus jobei Frost & Fleminger, 1968
Clausocalanus laticeps Farran, 1929
Clausocalanus lividus Frost & Fleminger, 1968
Clausocalanus mastigophorus (Claus, 1863)
Clausocalanus minor Sewell, 1929
Clausocalanus parapergens Frost &Fleminger,1968
Clausocalanus paululus Farran, 1926
Clausocalanus pergens Farran, 1926
Clausocalanus sp.
Ctenocalanus citer Heron & Bowman, 1971
Ctenocalanus sp.
Ctenocalanus vanus Giesbrecht, 1888
Drepanopus forcipatus Giesbrecht, 1888
Microcalanus pygmaeus (Sars G.O., 1900)

Eucalanidae

Eucalanus hyalinus (Claus, 1866)
Eucalanus inermis Giesbrecht, 1893
Eucalanus sp.
Paraeucalanus sp.
Subeucalanus crassus (Giesbrecht, 1888)
Subeucalanus crassus (Giesbrecht, 1888)
Subeucalanus subtenuis (Giesbrecht, 1888)

Euchaetidae

Euchaeta media Giesbrecht, 1888
Euchaeta rimana Bradford, 1974
Euchaeta sp.
Euchirella pulchra (Lubbock, 1856)
Paraeuchaeta aequatorialis Tanaka, 1958
Paraeuchaeta scotti (Farran, 1908)
Paraeuchaeta sp.
Paraeuchaeta weberi Scott A., 1909

Heterorhabdidae

Haloptilus acutifrons (Giesbrecht, 1893)
Heterorhabdus abyssalis (Giesbrecht, 188
Heterorhabdus clausi (Giesbrecht, 1889)
Heterorhabdus lobatus Bradford, 1971
Heterorhabdus norvegicus (Boeck, 1872)
Heterorhabdus papilliger (Claus, 1863)
Heterorhabdus sp.
Heterorhabdus spinifer Park, 1970
Heterorhabdus spinifrons (Claus, 1863)
Paraheterorhabdus vipera (Giesbrecht, 1889)

Lucicutiidae

Lucicutia clausi (Giesbrecht, 1889)
Lucicutia flavicornis (Claus, 1863)
Lucicutia gaussae Grice, *1963*
Lucicutia gemina Farran, 1926
Lucicutia grandis (Giesbrecht, 1895)
Lucicutia longicornis (Giesbrecht, 1889)
Lucicutia longiserrata (Giesbrecht, 1889)
Lucicutia ovalis (Giesbrecht, 1889)
Lucicutia sp.

Metridinidae

Metridia brevicauda Giesbrecht, 1889
Metridia curticauda Giesbrecht, 1889
Metridia gerlachei Giesbrecht, 1902
Metridia longa (Lubbock, 1854)
Metridia lucens lucens Boeck, 1865
Metridia princeps Giesbrecht, 1889
Metridia sp.
Metridia venusta Giesbrecht, 1889
Pleuromamma abdominalis (Lubbock, 1856
Pleuromamma borealis Dahl F., 1893
Pleuromamma gracilis Claus, 1863
Pleuromamma piseki Farran, 1929
Pleuromamma quadrungulata (Dahl F., 189
Pleuromamma robusta (Dahl F., 1893
Pleuromamma sp.
Pleuromamma xiphias (Giesbrecht, 1889)

Nullosetigeridae
Nullosetigera sp.

Paracalanidae
Calocalanus contractus Farran, 1926
Calocalanus pavo (Dana, 1852)
Calocalanus sp.
Calocalanus styliremis Giesbrecht, 1888
Calocalanus tenuis Farran, 1926
Delibus nudus (Sewell, 1929)
Mecynocera clausi Thompson I.C., 1888
Paracalanus aculeatus Giesbrecht, 1888
Paracalanus denudatus Sewell, 1929
Paracalanus indicus Wolfenden, 1905
Paracalanus nanus Sars G.O., 1925
Paracalanus parvus parvus (Claus, 1863)
Paracalanus quasimodo Bowman, 1971
Paracalanus sp.

Phaennidae
Onchocalanus subcristatus Wolfenden, 1911
Phaenna sp.
Phaenna spinifera Claus, 1863
Xanthocalanus sp.

Rhincalanidae
Rhincalanus cornutus (Dana, 1849)
Rhincalanus nasutus Giesbrecht, 1888
Rhincalanus sp.

Scolecitrichidae
Amallothrix sp.
Amallothrix tenuiserrata (Giesbrecht, 1893)
Lophothrix frontalis Giesbrecht, 1895
Lophothrix latipes (Scott T., 1894)
Pseudoamallothrix emarginata (Farran, 1905)
Pseudoamallothrix ovata (Farran, 1905)
Pseudoamallothrix profunda (Brodsky, 1950)
Pseudoamallothrix profunda (Brodsky, 1950)
Scaphocalanus curtus (Farran, 1926)
Scaphocalanus echinatus (Farran, 1905)
Scaphocalanus elongatus Scott A., 1909
Scolecithricella abyssalis (Giesbrecht, 1888)
Scolecithricella pacifica Chiba, 1956
Scolecithricella sp.
Scolecithricella vittata (Giesbrecht, 1893)
Scolecithrix bradyi Giesbrecht, 1888
Scolecithrix danae (Lubbock, 1856)
Scolecithrix sp.
Scottocalanus sp.

Temoridae
Temora discaudata Giesbrecht, 1889
Temora sp.
Temora stylifera (Dana, 1849)

3.3. Capitulo 3. Articulo enviado a Scientific Reports – Nature Nº MS SREP-19-10336

Diversidad genética y nuevos linajes en el copépodo cosmopolita *Pleuromamma abdominalis* en el Pacífico sudoriental

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Resumen

A través de corrientes de márgenes continentales, el zooplancton está sujeto a fuertes gradientes oceanográficos y por lo tanto fuertes presiones selectivas. Cómo interactúan tales gradientes con el proceso de especiación de organismos pelágicos es todavía poco entendido en el reino del océano abierto. Aquí nosotros informamos sobre la diversidad genética dentro del copépodo pelágico *Pleuromamma abdominalis* en la poco conocida región del Pacífico sudoriental, con muestras que abarcan un gradiente oceánico desde la costa de sugerencia hasta el giro oligotrófico subtropical del Pacífico Sur. Evaluamos la variación en los fragmentos de los genes mitocondriales (mt) citocromo c oxidasa subunidad I (COI) y citocromo b, así como la región nuclear espaciadora interna transcrita interna (ITS) y el ARNr 28S. Los análisis filogenéticos revelaron la presencia de 8 linajes divergentes ocurriendo a través del gradiente con distancias genéticas en el rango de 0.036 y 0.95 (genes mt), y métodos de delimitación de especies GMYC apoyan su inferencia como especies distintas (no descritas). Varios nuevos linajes genéticos que ocurren a través del gradiente zonal mostraron una fuerte estructuración genética y la presencia de nuevos linajes dentro de la zona de sugerencia costera reveló un inesperado alto nivel de endemismo dentro del Sistema de Corriente de Humboldt. Los análisis multivariados encontraron una fuerte correlación entre la variación genética con los valores superficiales de clorofila-a y salinidad, sugiriendo un papel importante de los gradientes hidrográficos en el mantenimiento de la diversidad genética. Sin embargo, la presencia de linajes crípticos dentro de la zona de surgencia no puede explicarse fácilmente por la heterogeneidad ambiental y plantea preguntas desafiantes para comprender el proceso de especiación del zooplancton oceánico.

Palabras claves: Zooplancton, Estructura Genética, Especies Crípticas, Endemismo, Gradiente Oceanográfico, COI, Cytb, Pacífico Suroriental, Coalescencia General Mixta Yule.

Genetic diversity and novel lineages in the cosmopolitan copepod *Pleuromamma abdominalis* in the Southeast Pacific

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Abstract

Across boundary currents, zooplankton are subject to strong oceanographic gradients and hence strong selective pressures. How such gradients interact with the speciation process of pelagic organisms is still poorly understood in the open ocean realm. Here we report on genetic diversity within the pelagic copepod *Pleuromamma abdominalis* in the poorly known Southeast Pacific region, with samples spanning an ocean gradient from coastal upwelling to the oligotrophic South Pacific Subtropical Gyre. We assessed variation in fragments of the mitochondrial (mt) genes cytochrome c oxidase subunit I (COI) and Cytochrome b as well as in the nuclear internal transcribed spacer (ITS) region and 28S rRNA. Phylogenetic analyses revealed the presence of 8 divergent lineages occurring across the gradient with genetic distances in the range of 0.036 and 0.95 (mt genes), and GMYC species delimitation methods support their inference as distinct (undescribed) species. Several new genetic lineages occurring across the zonal gradient showed a strong genetic structuring and presence of new lineages within the coastal upwelling zone revealed an unexpectedly high level of endemism within the Humboldt Current System. Multivariate analyses found strong correlation between genetic variation and surface Chlorophyll-a and salinity, suggesting an important role for hydrographic gradients in maintaining genetic diversity. However, the presence of cryptic lineages within the upwelling zone cannot be easily accounted for by environmental heterogeneity and poses challenging questions for understanding the speciation process for oceanic zooplankton.

Keywords: Zooplankton, Genetic Structure, Cryptic Species, Endemism, Oceanographic Gradient, COI, Cytb, Southeast Pacific, Generalized Mixed Yule Coalescent.

3.3.1. Introduction

. One of the largest and least explored regions of the open ocean is the Southeast Pacific (SEP). This region is globally important due to its role in controlling regional climate, its atmospheric and oceanic teleconnections at a global scale [1,2], and due to the presence of one of the strongest and shallowest oxygen minimum zones in the global ocean [3,4]. This region exhibits high heterogeneity in hydrographic conditions, primarily along a zonal gradient from the coastal zone to the open ocean, with increasing mixed layer depth (40-150 m), sea surface temperature (14-20°C), sea surface salinity (34.8-35.8) and surface dissolved oxygen concentration (1.5-4.5 mlL^{-1}) [5,6]. This high zonal variability is also clearly manifested in productivity of surface waters from the highly productive coastal upwelling zone (Humboldt Current), across to the large ultra-oligotrophic South Pacific Subtropical Gyre (SPSG) [7–10]. Moreover, within this zonal gradient there is a changing predominance of different water masses promoting even greater hydrographic variability in this vast ecosystem. The SPSG is the largest subtropical gyre, with an areal extent of $18x10^6$ km^2 [11], and it has the lowest reported primary production rates (1-2 $\mu g\ C\ L^{-1}\ day^{-1}$)[12]. The ultra-oligotrophic condition of the eastern SPSG contrasts with the very high productivity of the SEP upwelling system off the coast of Chile, Peru and Ecuador, also known as the Humboldt Current System (HCS), which is one of the most productive eastern boundary current ecosystems in the world [8,13] . Primary production in the upwelling zone reaches up to 19.9 $g\ C\ m^{-1}\ d^{-1}$ [14]. Upwelling-driven production extends zonally into a mesotrophic region, also known as the coastal transition zone [15], which constitutes an intermediate region between the upwelling system and the oligotrophic region of the SPSG.

Oceanographic variability across the SEP zonal gradient is strongly correlated with changes in zooplankton abundance, biomass and distribution. Zooplankton biomass and abundance can decline by up to 3 orders of magnitude from the coastal zone to the SPSG [16]. Within the central gyre, small organisms (<500 μm),which numerically represent up to 65% of the zooplankton, are dominated by copepods, whereas other size fractions composed of chaetognaths, euphausiids and siphonophores make up a considerable part of the community [11,16]. In the coastal zone in contrast, larger-sized copepods often dominate numerical abundance [17]. In specific groups of zooplankton, for example siphonophores, tintinnids, and euphausiids, there is an increase in diversity and species richness towards the center of the gyre [6]. There also are endemic species within the Humboldt Current that are replaced by different species in the oceanic zone [18–20]. For example, the endemic copepod *Calanus chilensis* of the Humboldt Current [21] appears to be replaced by the more widely distributed *Calanus australis* towards open ocean waters [19]. A similar pattern seems to occur with the endemic Humboldt Current *Euphausia mucronata*, which may be replaced by other euphausiids in the oligotrophic zone [20]. How the apparent environmental barriers across the zonal gradient, from the Humboldt Current to the SPSG, have influenced species distributions, population structure and genetic diversity within zooplankton species are poorly known for this ocean basin.

Planktonic copepods are the most species-rich taxonomic group of the mesozooplankton, with a large number of cryptic species reported [22–25]. Strong genetic structure among populations within plankton species also has been shown to occur from coastal to oceanic environments[26] . For example, in the cosmopolitan copepods *Eucalanus hyalinus, E. spinifer*, *Pleuromamma xiphias* and *Haloptilus longicornis* [27–29] ocean regions with strong hydrographic gradients can act as ecological barriers to dispersal, causing strong genetic breaks among populations in distinct subtropical gyres and/or among ocean basins. Because biotic and abiotic characteristics of regions with strong zonal gradients may affect the development or survivorship of populations [22], they can act as barriers to dispersal. Responses to these ocean gradients can be species-specific (idiosyncratic responses), due to the variable ecological requirements of each species [26]. Given that genetic divergence is commonly decoupled from morphological divergence in copepods [30,31], and that broadly-distributed species may include lineages with different evolutionary trajectories [25], detection of cryptic diversity in this group is fairly common.

Within the SEP, there are few copepods species that show broad distribution and high abundance across the zonal gradient. As a result, it is difficult to identify appropriate target species for studies aiming to elucidate the effect of hydrographic variability on genetic structuring within species. One of the few species that meets these requirements is *Pleuromamma abdominalis*, which has been described to inhabit preferably oceanic environments, has a cosmopolitan distribution in subtropical and tropical waters worldwide, and occurs in high abundance across the mesopelagic and epipelagic zones with active diel migratory behavior [32–37]. Within the SEP, seven species of *Pleuromamma* co-occur, including *P. borealis*, *P. gracilis*, *P. piseki*, *P. quadrungulata*, *P. robusta* and *P. xiphias* [35]. *Pleuromamma abdominalis* can be clearly distinguished from other co-distributed species by the shape of the rostrum, the presence and number of denticles on proximal segments of the A1, body size, and in adult females by the 5th swimming leg segmentation[38]. In recent studies conducted on this species, large intraspecific variation was reported in both morphological and genetic characters [39–41] . Prior work has reported the presence of endemic clades in certain oceanographic provinces, including subtropical waters of the South Pacific [41].

In this work, we conducted a molecular study of the evolutionary lineages present within the nominal species *Pleuromamma abdominalis* in the Southeast Pacific. Our objectives were to (1) identify the evolutionary lineages present in the SEP region, (2) evaluate whether they may be distinct, currently undescribed species, using multiple independently inherited markers, and (3) determine the potential effects of hydrographic variability across the SEP region on the distribution of these distinct evolutionary lineages. For this, we used samples collected across a zonal transect of the SEP (13 sites; Fig. 1, Table 1), and study genetic diversity within *Pleuromamma abdominalis* using sequences from the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and cytochrome b (Cytb), as well as nuclear 28S rRNA and the internal transcribed spacer (ITS) region.

3.3.2. Results

3.3.2.1. Phylogenetic inferences

Phylogenetic analyses of Southeast Pacific specimens using COI and Cytb gene fragments resulted in trees that include eight primary clades within *P. abdominalis* (Fig. 2). Of the eight clades reported for the region, five were previously described (by Hirai *et al.*, 2015) [41] (see Suppl. Fig. S1 for global *P. abdominalis* phylogeny). In order to facilitate comparison of our results with those from other ocean regions, we identify the 8 clades present in the SEP by the clade identifiers originally reported by Hirai and authors (5 clades), and in the cases where the clades have not been previously detected, we give them a new identifier and flag them as new to the literature (e.g., 2s – NEW).

The eight clades were split into two genetically very divergent clades, PLAB2 *Hirai* and PLAB1 *Hirai*, which showed high nodal support values [100% Maximum

Likelihood (ML), 100% Bayesian Posterior Probability (BPP)] for both mitochondrial genes (Fig. 2). PLAB2 *Hirai* was more diverse than PLAB1 in the SEP and included seven clades (2a-2t) located across the four oceanographic study zones for the two mitochondrial markers. PLAB1 included only the clade 1a_1 *Hirai*, which included haplotypes located mainly in the coastal upwelling zone. Within PLAB2 *Hirai*, seven clades were observed, which were divided into two large groups with a high support value in both gene trees (100% ML, 100% BPP). The first group, including clades 2c *Hirai*, 2d *Hirai*, and 2a *Hirai*, showed variation in tree topology depending on the gene. In the Cytb tree, clade 2c *Hirai* was sister to the pair of clades 2d *Hirai* and 2a *Hirai*, while for the COI gene, the clades 2c *Hirai* and 2d *Hirai* make up a group with a sister clade 2a *Hirai.* The second PLAB2 *Hirai* group, including four clades, also had high nodal support (cytb: 89% BPP, COI:100% BPP, 93% ML) and variation in topology depending on the gene. In the Cytb tree we observed two groups, one formed by the clades 2r-NEW and 2s-NEW and another by the clades 2f *Hirai* and 2t-NEW; however, in the COI tree the clade 2s-NEW was placed with clades 2f *Hirai* and 2t *Hirai*.

Most of the eight primary clades were well supported as reciprocally monophyletic groups in both mitochondrial gene trees (>75% node support, ML/BPP). Nodal support values for each clade were high in Bayesian analyses (cytb: 53-100% BPP, COI: 54-100% BPP), with 6 or more clades with > 99% BPP support (both genes). ML bootstrap support values were lower, but still supported reciprocal monophyly (> 75% ML) in the majority of clades in the cytb gene tree, with lower ML bootstrap values in the COI tree. In addition, placement of animals into clades was entirely congruent across the two mitochondrial trees, with animals present in both trees always occurring in the same mitochondrial clade (animals marked by a square in Fig. 2).In terms of composition, previously reported clades 2c *Hirai* and 2a *Hirai* mainly consisted of haplotypes from the transition and oligotrophic zones, while clade 2d *Hirai* included only individuals sampled in the ultra-oligotrophic zone. Clade 2f *Hirai* consisted of individuals from the coastal upwelling and transition zones, and clade 1a_1 *Hirai* included specimens from the coastal upwelling zone and a single individual from the oligotrophic zone (COI).

Three clades primarily sampled in the coastal upwelling and transition zones have not been found or reported in any prior work, and are newly described in this study: They are the clades 2r-NEW, 2s-NEW, and 2t-NEW. All three were well supported as reciprocally monophyletic in Bayesian analyses (both genes, 89-100% BPP), with more moderate support in ML results, as was observed in the other 5 previously described clades. The clade 2r-NEW included haplotypes from the coastal upwelling, transition and oligotrophic zones, clade 2s-NEWoccurred in the coastal upwelling and oligotrophic zones, and finally clade 2t-NEW included only coastal upwelling zone clades.

The eight *P. abdominalis* mitochondrial clades reported from the Southeast Pacific in the present study could be distinguished from other clades reported in a COI gene tree that included all available sequences from *P. abdominalis* from the South Pacific, covering the equatorial divergence region, oligotrophic zones and even the coastal transition zone off South America (Fig. 3; Hirai *et al.*, 2015 data) [41]. This analysis revealed that the sampling performed in this study included all clades reported for the South Pacific with the exception of the clades 2e *Hirai*, 2k *Hirai* and 2m *Hirai*, which occur in the equatorial region (Fig. 3), and 1b-1 *Hirai* which occurs in the western South Pacific.

Genetic distances (D) calculated using the T92 substitution model for eight mitochondrial clades were higher for the Cytb fragment (D = 0.056 - 0.495), with lower maximum genetic distances among clades observed at COI (D = 0.036 - 0.183) (Table 2). The highest genetic distance values for both genes were observed in comparisons between the clades PLAB1 and PLAB2. For Cytb the highest values were observed between clades 1a_1 Hirai and 2s-New (D = 0.4952); however, for the COI gene, the maximum value was observed between the clades 1a_1 *Hirai* and 2c *Hirai* (D = 0.1831) (Table 2). Genetic distances observed between sister clades were generally between 0.04 and 0.07, for example between the clades 2t-NEW and 2f *Hirai* (D <0.056) and 2a *Hirai* with 2d *Hirai* (D <0.0688), for both genes.

The 28s and ITS gene fragments had low polymorphism, with 3 and 4 variable nucleotide sites in 28S and ITS, respectively (Fig. 4). Analysis of the nuclear sequences for individuals for which we also have mitochondrial sequence found concordant genetic differences in both genomes between clades in many cases. Three informative sites were observed for the 28S gene, located at bases 46, 122 and 655 (Fig. 4; Suppl. alignments S1-S4). Focusing on clades with N>5 animals sequenced at 28S rRNA, analysis of these polymorphic sites revealed shifts in nucleotide composition between the closely-related clades 2c *Hirai*/2d *Hirai*/ 2a *Hirai* and 2r-NEW/2f-NEW, with a higher frequency of Y at all 3 sites for the latter clade pair. The 28S TCS haplotype network had an abundant central haplotype separated from another far less abundant haplotype that included only individuals belonging to clade 1a_1 *Hirai* (note that sites with ambiguous bases are masked in this analysis).

Within the ITS gene fragment, we observed four polymorphic sites located at bases 126, 361, 481 and 510. Analysis of the relative frequency of the clades 2a *Hirai* and 2r-NEW revealed changes only in the first site, observing a change in the relative frequency of base R for the first clade with 16.7% and 76.9% for the second. On the other hand, within the clades 2d *Hirai* and 2c *Hirai* the penultimate site (481) showed changing frequency between the haplogroups, in the first clade a lower frequency of Y (5%) with the second one at 58.3%. Finally, clade 2f *Hirai* was the only haplogroup with apparently fixed frequencies for all four polymorphic sites. The haplotype network for ITS revealed the presence of two abundant haplotypes separated by two mutational steps (Fig. 5). All animals from mitochondrial clade 2d *Hirai* and nearly all of the animals from closely-related 2c *Hirai* had one nuclear haplotype, while animals from the second major group of clades within PLAB2, including 2r-NEW, 2s-NEW, 2t-NEW, 2f *Hirai*, all had nuclear haplotypes from the rest of the haplotype network (Fig. 5).

3.3.2.2. Species Delimitation

Two analytical approaches were used to delimit *Pleuromamma* species in our study region, the Generalized Mixed Yule Coalescent (GMYC) likelihood method and the Poisson Tree Process (PTP) model, using single marker gene trees for both COI and Cytb [42]. GMYC and PTP analyses for both mitochondrial genes supported the inference of several species being present within the nominal species *P. abdominalis*. In the GMYC results, the number of distinct entities (clusters) associated with the highest likelihood score for the COI gene was 7 (confidence interval 7-9; $p < 0.001$), with 9 entities identified for Cytb (confidence interval 8-9; $p < 0.001$). GMYC support values for most clades were high at both mitochondrial genes (>0.6; Table 3), confirming the phylogenetic results reported above. The groups identified by GMYC analyses were concordant with the eight mitochondrial clades previously described (Fig. 2). Clade 2f *Hira*i had relatively low support (0.11) at the COI gene, but was well supported by analyses of Cytb. PTP analysis indicated the presence of 11 species within the study sequences for both COI and Cytb genes. This analysis showed concordance with the six mitochondrial clades described above with high support values (> 0.5 BPP). However, PTP analyses also indicated greater splitting, for example both mitochondrial genes indicated that clade 2d *Hirai* was composed of 3 species and clade 2c *Hirai* by 2 species.

Because greater congruence was observed between GMYC and phylogenetic results (above), we place greater emphasis on these inferences.

3.3.2.3. Oceanic biogeography of mitochondrial clades

When analyzing *P. abdominalis* mitochondrial clades present in the Southeast Pacific, high variability was found in their relative abundance across the four study zones (Fig. 6a). In the ultra-oligotrophic zone near Easter Island, all animals sampled were from clade 2d *Hirai* (100% relative abundance). In the oligotrophic and transition zones, four shared clades were observed, including 2a *Hirai*, 2c *Hirai*, 2f *Hirai* and 2r-New. Within these two regions, clade 2c *Hirai* showed the highest relative abundance (>50%) with a second clade 2a *Hirai* also occurring at high frequency (>8%). Within the oligotrophic zone, three clades occurred with low relative abundance (<5%) that were not shared with the transition zone, including clade 2d *Hirai,* with overlapping distribution in the ultra-oligotrophic zone, and 2s-New and 1a_1 *Hirai* that also were present in the coastal upwelling zone. The coastal upwelling zone was dominated by the newly discovered mitochondrial clades 2r-NEW (> 50%) and 2t-NEW (<30%), the latter being exclusive to this zone. Some haplo groups showed a decrease in their relative abundance from the upwelling to oligotrophic zones as observed in clade 2r-NEW and 1a_1 *Hirai*; however, others remained relatively constant across zones, as in the rarer clades 2f *Hirai* and 2s-NEW (Fig. 6a).

The CCA between abundance of the eight mitochondrial clades and the hydrographic variables indicated that the most parsimonious and significant model (*p-value*<0.05), comprised of Chla, salinity, wind stress and PAR, explained 94% of the variability (Suppl. Table S1). The first canonical axis (CCA1) captured 83% of the variance and covaried primarily with Chla, while the second axis (CCA2) explained 12%

of the variance and was primarily capturing variation in salinity. The clades located in the coastal upwelling zone,1a_1 *Hirai*, 2t-NEW and 2r-NEW, were observed in regions of high Chla, showing a strong negative correlation with CCA1 axis. Clades 2a *Hirai,* 2c *Hirai* and 2f *Hirai*, which occurred across the coastal transition and oligotrophic zones, were located near 0.0 of the CCA plot indicating that the hydrographic variables of the model were not good predictors of the distribution of these clades, suggesting less dependency with the hydrographic regime. These clades were observed in environments that were intermediate or variable in hydrographic conditions (Chla, Salinity). Finally, clade 2d *Hirai*, the only clade present in the ultra-oligotrophic zone, showed strong association with high SSS environments and had a strong positive correlation with the CCA2 axis (high salinity, low Chla) (Fig. 6b).

3.3.3. Discussion

Molecular analysis in zooplankton species with cosmopolitan distributions has revealed a variable number of genetic lineages in organisms that were ascribed as single species using traditional taxonomy [43–45]. These distinct lineages commonly exhibit patterns of distribution associated with variable oceanographic conditions [22]. Our study constitutes a first step to disentangle the genetic diversity within *Pleurommama abdominalis* in the Southeast Pacific. Phylogenetic analysis of mitochondrial and nuclear genes of the single species *Pleurommama abdominalis* showed a complex of genetic entities not described morphologically, suggesting a process of cryptic or pseudocryptic speciation. To assess this possibility, we delimited putative species using a Generalized Mixed Yule Coalescent approach, finding that these 8 clades likely correspond to different species. Subsequently, we investigated the distribution of these cryptic or pseudocryptic species and found them linked to oceanographic variability. Phylogenetic analysis of COI and Cytb gene fragments indicated this nominal species as having high diversity over a rather limited geographic range (4000 km), in comparison with some other oceanic copepods of cosmopolitan distribution [44,46]. The different mitochondrial lineages observed within *Pleurommama abdominalis* may thus represent distinct species [41], or ancient mitochondrial lineages that persist within large populations of oceanic plankton. The *P. abdominalis* lineages detected and reported here extend the distribution of material examined in Hirai *et al.*, (2015) [41], and suggest that a number of additional genetic lineages within the nominal species may yet be found across the circumglobal distribution of this species.

Our study further suggests that different clades are adapted to specific oceanographic conditions. Some mitochondrial lineages showed a wide distribution in the SEP, from the upwelling zone to the oligotrophic gyre (e.g. clades 2f *Hirai*, 2s NEW, 2r-NEW, 1a_1 *Hirai*) (Fig.2). In contrast, other clades had a more limited distribution within the coastal transition and oligotrophic zones (e.g. clades 2a *Hirai*, 2c *Hirai*), while the remaining clades showed an unexpectedly high level of endemism: one in the ultra-oligotrophic zone (clade 2d *Hirai*), and the other in the coastal upwelling zone (clade 2t-NEW) (Fig. 2). The latter represents a new undescribed lineage of *P. abdominalis*, comparable to other endemic species of zooplankton known from the Humboldt Current, such as the calanoid copepod *Calanus chilensis* and euphausiid *Euphausia mucronata* , both of which are restricted to the upwelling zone [20],[21]. Our phylogenetic analysis included all lineages already reported for distinct regions of the South Pacific and therefore it became clear that our new clades had not been described previously, reflecting a hidden speciation process within the SEP basin.

In general terms, the genus *Pleurommama* is considered omnivorous and this generalist feeding behavior may allow a wider spatial distribution [47]. However, we observed that the 8 clades were highly correlated with Chla and salinity allowing us to establish distributional limits (Fig. 6, Suppl. Table S1). The lineages found primarily within the coastal upwelling and in some cases extending into the transition and oligotrophic zones were generally distributed in areas with higher Chla (e.g., 2r-NEW), and these clades are possibly associated with nutrient-rich waters in the coastal upwelling zone and also in oligotrophic areas subjected to mesoscale eddies that can transport upwelled waters from the coastal zone into the oceanic region [48, 49]. On the other hand, the lineages primarily within the coastal transition and oligotrophic zones exhibited a correlation to low Chlorophyll-a areas (e.g., 2c *Hirai*), and they might also be associated with other oceanographic factors not considered in our study. Weak correlations with the predictors of our study suggest this may be the case. Finally, the lineage located in the ultra-oligotrophic zone showed strong correlation to high salinity (2d *Hirai*), which is likely a water mass tracer for the subtropical gyre (Fig. 6b). Hirai *et al.,* (2015) [41] also reports this lineage extending to the west of our sampling domain, but not beyond the ultra-oligotrophic conditions of the eastern limb of the South Pacific Subtropical Gyre. Adaptation to specific ranges of temperature, salinity, or other oceanographic parameters has been reported as an important driver for genetic diversification in several planktonic organisms, such as chaetognaths and calanoid copepods [50,51], jellyfishes [52], and estuarine copepods[53].

The high level of endemism found, in particular in the upwelling and in the ultra-oligotrophic zones, may be a response to both present and historical physical processes. In the pelagic environment there are several physical barriers, such as land masses and oceanic currents, that can influence dispersal and connectivity among populations and act to promote species diversity[28,54–57]. The upwelling zone shows strong interactions among oceanographic forces, for example upwelling fronts, eddies, and tidal currents. These interactions may exert subtle effects on pelagic organisms over short time scales, but over large scales they may have profound effects on population structure [58–60]. For instance, the ultra-oligotrophic zone has been described as a region where populations exhibit different genetic composition[61–63]. This is because the subtropical gyres act as retention areas, and so allow genetic structuring to develop and persist [27,29,61,28,41,64]. Over longer time scales, it has been suggested that during the last maximum glacial (LGM, 19,000 to 23,000 years ago) the productivity in the coastal zone was much higher [65], while the subtropical gyre had lower productivity [66]. This contrast may have acted as an allopatric barrier for dispersal between the zones, explaining the present divergence of the ultra-oligotrophic area (clade 2d *Hirai*) with respect to the other zones. Later, by the end of the Pleistocene, warming of surface seawater, sea level rise, and lowering of productivity in the coastal zone may have weakened the oceanographic gradient and dispersal barriers between the oligotrophic and upwelling zones, and thus allowing an expansion and possibly secondary contact between zones generating the current parapatric pattern [11,67]. In any case, it has been well documented that distributional ranges can change rapidly, as evidenced presently due to climate change (e.g. change shifts of ~ 23 km/year) [68].

The GMYC analyses of mitochondrial genes clearly indicated that *Pleurommama abdominalis* is likely a complex of species comprised of genetically divergent clades that are reproductively isolated but difficult to distinguish morphologically. Our results suggest the presence of at least 7 species, within which two appear highly specialized in two particular zones, the upwelling and the ultra-oligotrophic ones. Other studies focused on cosmopolitan species within the same genus, such as *P. xiphias, P. gracilis,* and/or *P. piseki*, also have described the presence of several lineages, suggesting that a number of species in this genus are in fact cryptic species complexes [29, 44]. The prior study by Hirai *et al.*, (2015) [41] inferred with certainty that *P. abdominalis* was comprised of at least two species coinciding with the clades PLAB1 and PLAB2, given concordant patterns of genetic variation between mitochondrial and nuclear markers. However, there was an open possibility for many more species, because the observed divergence within both clades was greater than 4.3 % [41]. In the present study, values of divergence greater than 30% were reported among the 8 clades, yet both mitochondrial genes also showed that the greatest genetic distances were observed among the clades forming the haplogroups PLAB2 and PLAB1. In general terms, many described species of marine copepods exhibit COI divergences in the range of 8 to 9% [69, 70], while other work suggests that intraspecific variation of copepod lineages occurs within the range of 1 and 4 % [71, 72.]

The 28S and ITS genes were less polymorphic and less informative than mitochondrial genes in detecting species limits within *P. abdominalis*. In the haplotype networks, both nuclear genes failed to show clear patterns with respect to the pre-established mitochondrial clades (Fig. 5). However, these genes showed differences in nucleotide frequencies at polymorphic sites, allowing us to distinguish several of the 8 clades by means of the percentage of the nucleotide bases (Fig. 4). The lack of clear concordance between our mitochondrial and nuclear markers can be explained by greater sensitivity of mitochondrial markers to genetic drift than nuclear markers, due to haploid uniparental heredity and smaller effective population size [73–75]. Work conducted in other copepod species has reported that the 28s gene shows a slower rate of evolution than that of the COI gene [56,76,77] and incomplete lineage sorting may occur. Furthermore, it is well known that in order to effectively delimit species it is necessary to analyze multiple loci [78, 79,53,80]. This is because single locus analysis can either underestimate or overestimate the number of species due to the presence of pseudogenes [81], ancient mitochondrial lineages that are present within interbreeding populations (single species), and introgression among closely related species as well as other processes that may obscure species boundaries. Several studies have shown that the use of mitochondrial markers may be more efficient for initially detecting cryptic species [46], with subsequent studies using a combination of markers for consistent species delimitation (e.g., copepod *Haloptilus longicornis*) [55]. In any case, the high level of genetic diversity and endemism observed here seems unexpected within an ocean basin that is subject to both meridional and zonal advection and water mixing, suggesting that evolution and speciation processes can occur more rapidly than traditionally thought in the open ocean for short-lived species like pelagic copepods.

3.3.4. Methods

3.3.4.1. Oceanographic Survey and Samples

Zooplankton samples were collected during two oceanographic cruises conducted in the Southeast Pacific (Fig. 1, Table 1). The first cruise, CIMAR-21, sampled during 01-30 October 2015 and the second cruise, CIMAR-22, from 14 October to 13 November 2016. The first cruise focused on a transect between Caldera (27°S, 70°50'W) and Easter Island (27°10' S, 109°30'W), covering a total of 30 oceanographic stations. The second cruise sampled 25 oceanographic stations along two zonal transects at 26.5 °S and 33°S, and one meridional transect, between the Desventuradas Islands and the Juan Fernández Archipelago.

The cruises spanned a large oceanographic gradient, and we differentiated the study region using satellite-derived Chlorophyll-a (Chla), with observations during the two cruise periods (https://coastwatch.pfeg.noaa.gov/erddap/index.html). We differentiated four zones according to the observed near-surface Chla ranges: a eutrophic zone corresponding to the coastal upwelling region of Chile with Chla >0.5 mg m^{-3} (CUP-Z), the mesotrophic coastal transition zone (CTZ) that shows a range of Chla between 0.1-0.5 mg m^{-3}, the oligotrophic zone (OLIGO-Z) with variation between 0.05 - 0.10 mg m^{-3} , and finally an ultraoligotrophic zone(U-OLIGO-Z) that is located in the central region of the South Pacific gyre with concentrations of Chla <0.05 mg m^{-3} (as shown in Fig. 1). Similar zonation has been reported in previous works[5, 12, 11].

Samples were collected at a total of 13 sites during the CIMAR-21 and CIMAR-22 cruises: 25 within the CUP-Z, 34 in the CTZ, 24 in OLIGO-Z and 49 in U-OLIGO-Z (Table 1). Mesozooplankton were collected with a 1 m^2 Tucker trawl (200 µm mesh) on both cruises, and an 8 m^2 Tucker trawl net (300 µm mesh size) on CIMAR-22 (Table 1). Most samples were collected at night and covered a wide depth strata (>100 m in most cases). The number of specimens per station, coordinates of the sampling sites, date, time, maximum depth of the plankton net, cruise and gear used at each station are reported in Table 1. Plankton samples for genetic analysis of the four study zones were frozen at -20°C at sea. In the laboratory, samples were thawed and screened with a 200 µm sieve to eliminate excess water and were subsequently preserved with 95% ethanol. The ethanol was replaced within 24 hours of initial preservation and samples subsequently stored at -20°C. Adult female specimens were sorted from bulk plankton samples and identified as *Pleuromamma abdominalis* with the aid of taxonomic keys by Bradford-Grieve (1999) [38]. All specimens of the target species found in the CUP-Z and CTZ areas were selected; however, for the most oceanic areas (OLIGO-Z and U-OLIGO-Z), with greater abundance of the target species, individuals were chosen randomly.

3.3.4.2. DNA extraction and Polymerase Chain Reaction (PCR)Amplification

Specimens selected for genetic analysis were transferred to an Eppendorf tube with 1ml of MilliQ water, and incubated at room temperature for 12 hours, in order to eliminate any remaining ethanol. Genomic DNA from specimens collected on the CIMARR 22 cruise were extracted with the Mollusc DNA Kit (Omega) following manufacturer protocols. Specimens collected on CIMAR 21 were extracted using the Forensic DNA Kit (Omega) following manufacturer protocols, with the exception of incubating the sample with the lysis buffer and proteinase K for only 1 hour. Extracted DNA was maintained at -20°C.

Fragments of the genes encoding for mitochondrial proteins cytochrome *c* oxidase subunit I (COI) and cytochrome *b* (Cytb) as well as nuclear genes encoding the large subunit of the ribosome (28S) and internal transcribed spacer regions (ITS1-5.8SrDNA-ITS2) were used for phylogenetic analyses. The primers used to amplify each gene fragment, the length of the amplified fragment, and the annealing temperatures used during PCR amplification are specified in Table 4. PCR amplifications were performed in a total volume of 28 µl for 35 cycles, including 4 µl of 5X Green GoTaq G2 Flexi Buffer, 4.48 µl of 25Mm MgCl2 25, 2 µl of dNTP (final concentration of each 0.25 Mm), 2 µl of each primer (0.35 mM), 0.045 units of the GoTaq G2 Flexi DNA Polymerase (Promega) and 5 µl of the DNA template. PCR products were visualized by 1% agarose gel electrophoresis and sent to Macrogen for sequencing. Sequencing of PCR products was performed primarily along the sense (forward) strand; both strands were sequenced if the forward reads were ambiguous at some nucleotide sites (forward and reverse).

3.3.4.3. Sequence Alignment and Phylogenetic analyses

Sequences from both strands of the same specimen were aligned, base calls confirmed and edited manually, and consensus sequences produced using Geneious 7.1.3 [82]. A BLAST search was conducted on confirmed and consensus sequences in order to verify the absence of contamination (correct organism) [83]. Cytb sequence amino acid translations contained no stop codons. Within COI, a total of 6 individuals were found to have a 1-baseindel, which would result in a frame-shift mutation. These sequences were inferred to derive from a COI pseudogene and these individuals were removed from all analyses. All included animals have COI sequence translations with no stop codons. Final alignments were created for each gene fragment using MUSCLE within Geneious 7.1.3 [82]. A total of 55 sequences were aligned for COI, 75 for Cytb, 102 for 28S rRNA and 80 for ITS. The length of the alignment obtained for each gene fragment is reported in Table 4 (Suppl. alignments S1-S4).

Phylogenetic analyses were performed using both maximum likelihood (ML) and Bayesian inference (BI) methods for both mitochondrial genes. The T92+I and T92+ G models were selected as the best-fit nucleotide substitution models by the Bayesian Information Criterion (BIC), as performed in Mega-X V.10 [84] for mtCOI and Cytb, respectively. Bayesian analyses were carried out in MrBayes V.3.1 [85]. The Markov Chain Monte Carlo chains were executed in three simultaneous runs with default heating, selected the appropriate models of sequence evolution obtained from MEGA-X V.10 [84], over 1.0 x 10^6 generations, and trees were sampled every 1000 generations for both mitochondrial genes. The first 25% of the trees generated were eliminated (as burn-in). Stationarity was estimated by standard deviation of the three runs (> 0.001) and by observation of stationarity when the curve of the log-likelihood scores against generation time in Tracer was non-trending [86]. Maximum likelihood analyses were performed with the GTR + G model and with 5000 bootstrap replicates to calculate the support for each node using RAxML V.8.2.10 [87]. ML and BI trees were inferred for all newly obtained sequences from the SEP for both COI and cytb gene fragments (separately), as well as including all COI sequences reported from the South Pacific in Hirai *et al.*, (2015) [41]. In the latter tree, the analysis included 179 sequences of *P. abdominalis* from the Hirai *et* *al.*, study [GenBank access numbers: KT320612.1- KT320869.1 [41]] in addition to the 54 sequences newly reported here. A global tree for *P. abdominalis* also was inferred with 944 sequences from Hirai *et al.*, 2015 [GenBank accessions: KT319926.1- KT320869.1 [41]] and including sequences new to this study, in order to assign mitochondrial clades in the present study to existing names or identify them as new lineages that had not been previously reported. We also included COI sequence from the sympatric species *P. xiphias* [GenBank access numbers: JN574427 [29]] as an outgroup in phylogenetic analyses.

We used Popart V 1.7[88] to create a graph presentation of a TCS network for the 28s and ITS gene fragments, which illustrates the relationship between nuclear haplotypes and mitochondrial clades [89]. In these TCS networks, nucleotide sites with > 5% ambiguities were masked (not included). In doing this, in the case of 28S, much of the observed variation was thus masked. Genetic distances between mitochondrial clades were calculated as the net average between clades using final alignments for each gene, with 10,000 bootstrap replicates, using the Tamura 3-parameter (T92) nucleotide substitution model (Tamura, 92) and uniform rates between sites for COI and gamma-distributed rate variation for Cytb (using Mega-X V.10 [84]).

3.3.4.4. Species Delimitation

Two analytical approaches were used to delimit *Pleuromamma* species in our study region, the Generalized Mixed Yule Coalescent (GMYC) likelihood method and the Poisson Tree Process (PTP) model. The GYMC analysis is a likelihood method that enables delimitation of the number of species based on model fitting the branching patterns expected within and between species [90]. We inferred ultrameric gene trees for the COI and Cytb alignments using BEAST 2.0 [91]. Parameters selected for analysis included the HKY nucleotide substitution model, 6 categories for gamma-distributed rate variation, a relaxed lognormal clock, and a Yule model to express the branching pattern of the tree for both genes. Two independent runs were performed to ensure convergence, each run for 50 million generations and with sampling every 10,000 generations. Output files were visualized in Tracer v1.6 [86] to ensure convergence of the chains. The maximum clade credibility (MCC) tree was determined by Tree Annotator v1.8 [92] after removing the first 25% of trees (as a burnin).The number of delimited species was inferred for each MCC gene tree using the “gmyc” function in the splits R package [93], with the single threshold option selected, as recommended by Fujisawa and Barraclough (2013) [90]. The GMYC analysis compares a null model (without a threshold) and an alternative hypothesis (with a threshold) in order to evaluate whether all samples constitute a single species (null) against the alternative hypothesis of the existence of more than one species in the data. A likelihood ratio test (LRT) is used to evaluate the model results, with significance calculated using the chi-square test with two degrees of freedom. The analysis also gives the number of genetic entities with their confidence intervals (IC), and the sequences that are associated with each group.

We also performed a PTP delimitation of species using the bPTP web server http://species.h-its.org/ptp/.This model uses coalescence theory and estimates the speciation rate from the number of substitutions observed across the tree. As the input phylogeny, we used the COI and Cytb Bayesian phylogenies. We ran the PTP analysis with 5 x10^5 generations of MCMC sampling, with a thinning value of 100 and a burnin of 25%. MCMC convergence was visually confirmed as recommended by Zhang *et al.*, (2013) [94].

3.3.4.5. Biogeography

A canonical correlation analysis (CCA [95]) was performed to assess the association between hydrographic variables and mitochondrial clades occurring at each station. The model was constructed with eight response variables corresponding to the eight genetic clades, and as predictor variables, we selected sea surface values of chlorophyll-a (Chla), temperature (SST), photosynthetically active radiation (PAR), salinity (SSS), wind stress (WT) at the sea surface , an upwelling index (UI), which is an estimate of water mass being transported offshore by the alongshore wind [96], and wind stress curl (WSC) representing the curvature of the tension of the surface wind [97] . CCA analysis incorporates both ordination and multiple regression techniques for the analysis of relationships among multivariate data, and can be an efficient tool to relate categorical and environmental predictive variables [98–100]Satellite data were used to describe oceanographic conditions during the CIMAR 21 and CIMAR 22 cruises. For each sampling station, the average value was calculated over three days, from one day before to one day after sampling, with a spatial resolution of 0.025 degrees (Table 1). Chla, SST and PAR values were obtained from the MODIS satellite (https://podaac.jpl.nasa.gov/datasetlist?search=MODIS),WT was obtained by a sensor aboard the EUMETSAT METOP satellite (https://manati.star.nesdis.noaa.gov/products/ASCAT.php), SSS was downloaded from the SMOS radio telescope (https://earth.esa.int/web/guest/missions/esa-operational-eo-missions/smos),and finally values of UI and WSC were obtained by the instrument Advanced Scatterometer (ASCAT) from NOAA Coast Watch (https://data.noaa.gov/dataset/dataset/wind-stress-metop-ascat-0-25-degrees-global-near-real-time-curl3).

The CCA was performed using the CCA package in Rstudio V.3.5 [101]. To determine which variables to include in this analysis, a correlation analysis among the hydrographic variables was carried out. SST was found to have high correlation (>62%, *p-value* < 0.05) with Chla and salinity, and it was therefore eliminated from the analysis in order to minimize collinearity of hydrographic variables. The CCA was conducted with two contingency tables based on 1000 Monte Carlo permutations: one (X) represented the number of animals within each mitochondrial clade observed at each station and the second (Y) included values of Chla, SSS, WT, IU, WSC and PAR values for each station. The hydrographic variables that explained the largest component of variation in the distribution of the different mitochondrial clades were used to calculate the two most parsimonious canonical components. To evaluate significance of the most parsimonious model the Wilks' lambda likelihood test was used, which indicates the variance not accounted for by the independent variables, and chi-square tests were used to evaluate the significance of lambda [102].

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Author Contributions

CG and RE conducted fieldwork on two cruises. CG generated the sequence data. CG and EG conducted data analyses, and CG, EG and RE contributed significantly to writing of the manuscript. OU and PV participated in discussion and data analyses and commented on the manuscript. RE provided grant and equipment support for the research.

Competing Interests

The authors declare no conflict of interests.

Data accessibility

Sequence data reported in this publication are available through NCBI under accession numbers: MN045402 - MN045456 (COI), MN045457- MN045531 (Cytb), MN069652 - MN069731 (ITS), and MN069732 - MN069833 (28S rRNA). Alignments of the nuclear sequences are available in Supplementary alignments S1-S2.

Table 1. Sampling stations for the four study areas of the Southeast Pacific. Reported for each station are the number of *P. abdominalis* individuals (N), the longitude, latitude, date, time, sampling depth, cruise and the sampling gear. The oceanographic zones are specified as: eutrophic coastal upwelling zone (CUP-Z), mesotrophic coastal transition zone (CTZ), oligotrophic zone (OLIGO-Z) and ultra-oligotrophic zone (U-OLIGO-Z). Sampling time is specified as night (N) or day (D).

Zone	Station	N	Latitude	Longitude	Date	Time	Depth (m)	Cruise	Sampling gear
CUP-Z	1	10	27°04’	70°57’	10-14- 2016	23:13 (N)	0-100	CIMAR22	1 m^2 Tucker trawl net
	4	2	27°00’	71°12’	10-15- 2016	08:51 (D)	0-1500	CIMAR22	8 m^2 Tucker trawl net
	6	13	26°59’	72°38’	10-16- 2016	09:31 (D)	0-1600	CIMAR22	8 m^2 Tucker trawl net
CTZ	16	7	25°15’	80°16’	10-25-2016	19:26 (N)	0-1000	CIMAR22	1 m^2 Tucker trawl net
	17	3	26°15’	80°07’	10-23-2016	22:46 (N)	0-50	CIMAR22	1 m^2 Tucker trawl net
	22	16	26°19’	79°56’	10-22-2016	21.45 (N)	0-150	CIMAR22	1 m^2 Tucker trawl net
	23	1	29°22’	80°00’	11-02-2016	20:46 (N)	0-1000	CIMAR22	1 m^2 Tucker trawl net
	24	2	30°33’	80°01’	11-03-2016	08:48 (D)	0-1000	CIMAR22	1 m^2 Tucker trawl net
	25	4	31°47’	79°39’	11-04-2016	02:44 (N)	0-1000	CIMAR22	1 m^2 Tucker trawl net
	34	1	33°35’	79°12’	11-04-2016	21:54 (N)	0-1000	CIMAR22	1 m^2 Tucker trawl net
OLIGO-Z	13	14	26°59’	81°56’	10-20-2016	05:12 (N)	0-3000	CIMAR22	8 m^2 Tucker trawl net
	18	10	27°01’	89°34’	10-21-2015	20:19 (N)	0-900	CIMAR21	1 m^2 Tucker trawl net
U-OLIGO-Z	30	44	27°00’	107°34’	10-28-2015	02:29 (N)	0-900	CIMAR21	1 m^2 Tucker trawl net
	64	5	26°33’	105°49’	11-01-2015	21:57 (N)	0-900	CIMAR21	1 m^2 Tucker trawl net

Table 2. Average T92 genetic distances among eight primary mitochondrial clades based on COI and Cytb alignments. Distances for Cytb are reported below the diagonal, and COI values are above the diagonal.

	2c *Hirai*	**2d *Hirai***	**2a *Hirai***	**2r-New**	**2s-New**	**2f *Hirai***	**2t-New**	**1a_1 *Hirai***
2c *Hirai*	--	0.044	0.052	0.121	0.135	0.138	0.132	0.183
2d *Hirai*	0.136	--	0.050	0.114	0.126	0.131	0.131	0.174
2a *Hirai*	0.095	0.069	--	0.106	0.125	0.127	0.124	0.170
2r-New	0.142	0.274	0.285	--	0.087	0.094	0.084	0.176
2s-New	0.419	0.443	0.414	0.142	--	0.093	0.088	0.173
2f *Hirai*	0.271	0.199	0.195	0.138	0.153	--	0.036	0.176
2t- New	0.287	0.187	0.222	0.181	0.161	0.056	--	0.166
1a_1 *Hirai*	0.372	0.379	0.374	0.279	0.495	0.389	0.395	--

Table 3. GMYC support values for each mitochondrial clade based on COI and Cytb gene fragments. Clades with support values closer to 1.0 have a higher probability of being a distinct, currently undescribed species.

		Support values from GMYC analyses	
Clade	Zone	COI	Cytb
2c *Hirai*	OLIGO-Z CTZ	0.77	0.88
2d *Hirai*	U-OLIGO-Z	0.66	0.95
2a *Hirai*	OLIGO-Z CTZ	0.89	0.97
2r-NEW	CUP-Z CTZ OLIGO-Z	0.88	0.97
2s-NEW	OLIGO-Z CTZ	0.98	1.00
2f *Hirai*	CUP-Z CTZ	0.11	0.74
2t-NEW	CUP-Z	0.89	0.77
1a_1 *Hirai*	CUP-Z OLIGO-Z	1.00	1.00

Table 4. Names of the primers used, sequences [5’ to 3’], literature sources and the annealing temperature used in PCR. The expected length of the PCR product in base pairs (bp) for each gene fragment is indicated, together with the length of the alignments used in phylogenetic analyses (in parentheses).

Gene	Primer name, sequence	Product (Alignment)	AT
COI	COI (F), GGTCATGTAATCATAAAGATATTGG [74]	~800bp(639bp)	47°C
	PLAB-R(R), GTKGTAAAATATGCYCGTGTGTC [41]		
Cytb	UCYTB151F, TGTGGRGCNACYGTWATYACTAA [102]	~350bp (281bp)	45°C
	UCYTB270R, AANAGGAARTAYCAYTCNGGYTG [102]		
28S	LSU Cop-D1F, GCGGAGGAAAAGAAAACAAC [103]	~800bp (754bp)	55°C
	LSU Cop-D3R, CGATTAGTCTTTCGCCCCT [103]		
ITS	LR1(F), GGTTGGTTTCTTTTCCT [104]	~800bp (687bp)	50°C
	SR6R (R), AAGWAAAAGTCGTAACAAGG [105].		

Figure legends

Figure 1. The Southeast Pacific where the CIMAR21 and CIMAR22 cruises were carried out in the years 2015-2016. Sampling stations with numbering as shown. The four oceanographic zones were defined by surface chlorophyll-a values: coastal upwelling zone (CUP-Z), the mesotrophic coastal transition zone (CTZ), the oligotrophic zone (OLIGO-Z) and the ultra-oligotrophic zone (U-OLIGO-Z). Colors shown for each zone are included in other figures to indicate these ocean regions.

Figure 2. Bayesian phylogeny of *P. abdominalis* specimens from the Southeast Pacific inferred from partial sequences of the COI (right) and Cytb (left) genes (55 COI, 75 CtyB sequences). Numbers at the nodes indicate the Bayesian probability (above) and maximum likelihood (below) support values. Mitochondrial clades are identified by letters (a-s) and colors. For each individual, the oceanographic zone of collection is indicated by its color (CUP-Z, CTZ, OLIGO-Z and U-OLIGO-Z; legend upper left). Individuals with both COI and Cytb sequences included are indicated by squares, and individuals represented by a single sequence are marked by a circle. Clades identified with a 'Hirai' label are consistent with those reported in Hirai *et al*., (2015) [41]: NEW clades are first reported here.

Figure 3. Bayesian phylogeny of 235 *P. abdominalis* COI sequences from the Southeast Pacific, as reported in this study and in Hirai *et al.*, (2015) [41]. Numbers at the nodes represent the Bayesian posterior probability (upper) and maximum likelihood (lower) support values. Mitochondrial clades are labelled by letters (a-s) and colors. The tree includes 179 *P. abdominalis* COI sequences from GenBank (accession numbers: KT320612.1- KT320869.1; Hirai *et al.*, 2015) [41] and also a *P. xiphias* sequence (access numbers: JN574427) that was used to root the tree. Higher level clades are labelled and referred to in the text (PLAB1, PLAB2).

Figure 4. Nucleotide proportions at variable sites in the nuclear genes 28S and ITS for each of the mitochondrial clades. Three polymorphic sites at positions 56, 122 and 655 are shown for the 28S gene. In the ITS gene, four polymorphic sites were observed at positions 126, 361, 481 and 510.

Figure 5. *Pleuromamma abdominalis* statistical parsimony haplotype networks for the nuclear genes 28S and ITS, with color indicating correspondence with mitochondrial clades. Each branch and hash mark represents a mutational step. The size of the circles is proportional to the number of individuals sampled with that haplotype.

Figure 6. Oceanographic distribution of mitochondrial clades and their correlation with hydrographic variables in the Southeast Pacific. (a) Variation in relative abundance (%) of mitochondrial clades observed from the Chilean coast (right) to Easter Island (left), (b) Biplot of CCA results for mitochondrial clades and hydrographic variables at each station. Results shown for the most parsimonious model. Length of the arrows indicates the correlation between hydrographic variables and ordination axes. Numbers within the diagram represent the different sampling stations where individuals were collected.

Fig 1.

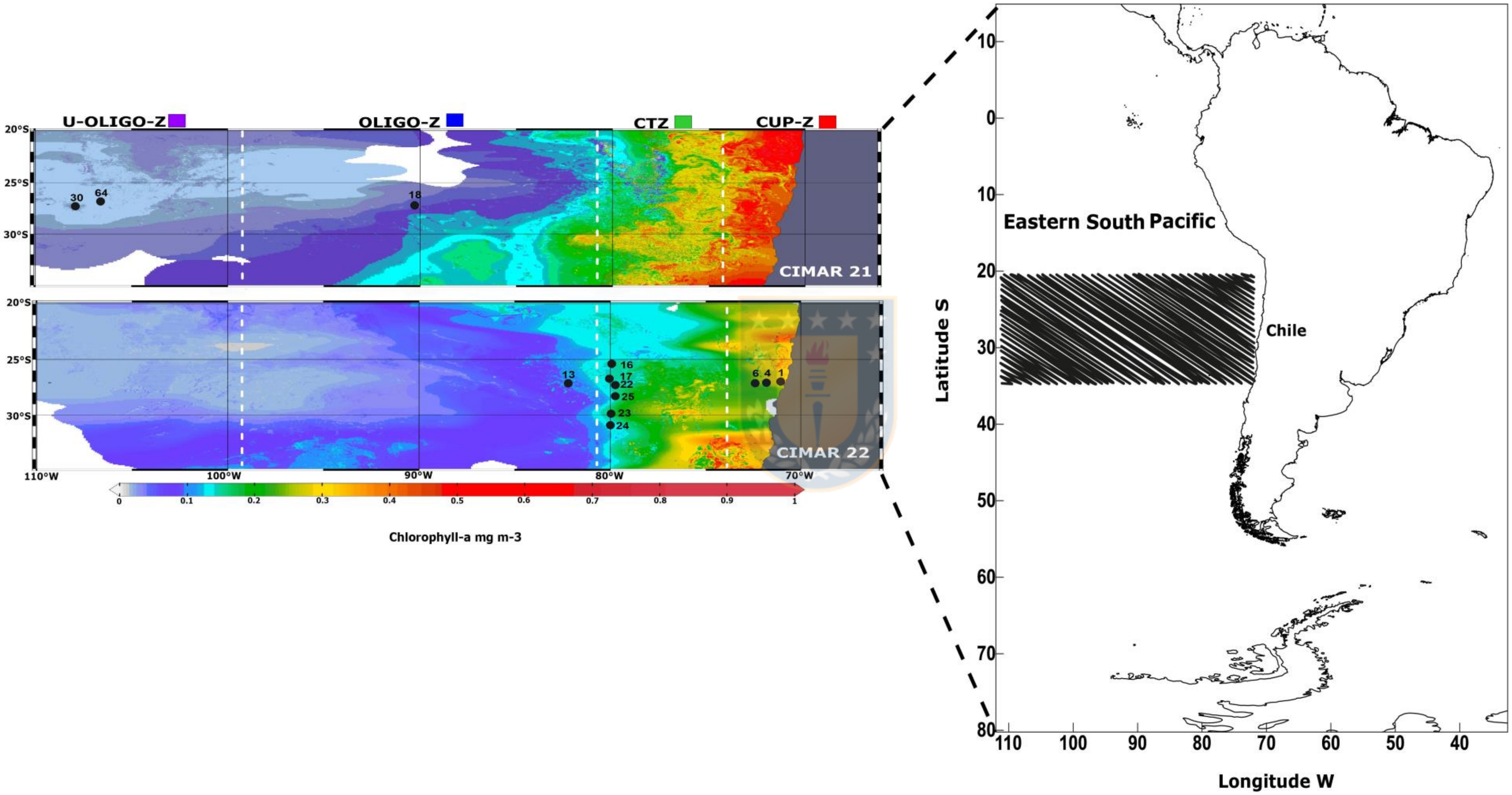

U-OLIGO-Z
OLIGO-Z
CTZ
CUP-Z
20°S
25°S
30°S
30
64
18
CIMAR 21
20°S
25°S
30°S
16
13
17
22
25
23
24
6
4
1
CIMAR 22
110°W
100°W
90°W
80°W
70°W
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Chlorophyll-a mg m-3
10
0
10
20
30
40
50
60
70
80
Eastern South Pacific
Chile
Latitude S
110
100
90
80
70
60
50
40
Longitude W

Fig.2

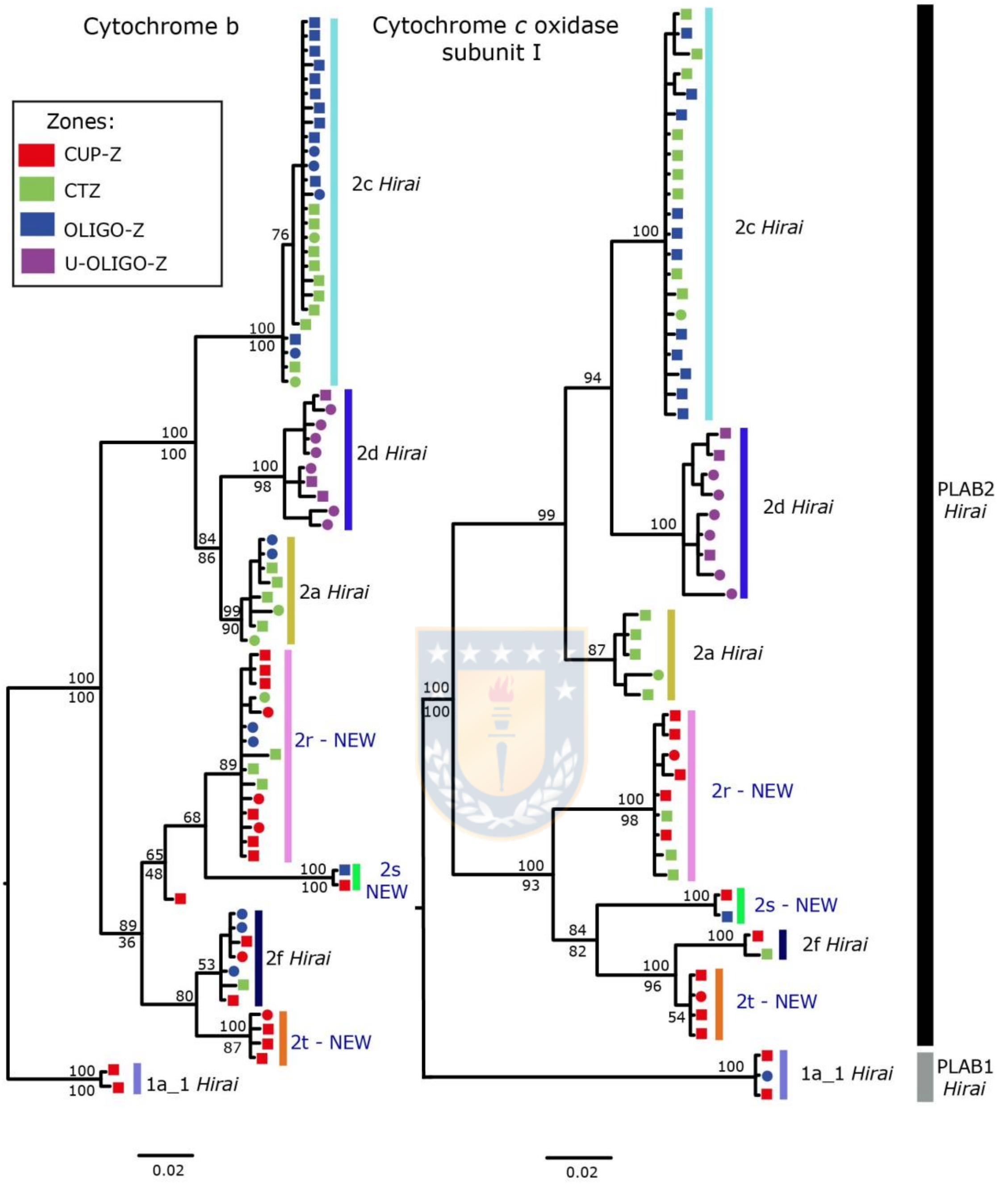

Cytochrome b
Zones:
CUP-Z
CTZ
OLIGO-Z
U-OLIGO-Z
2c Hirai
2d Hirai
2a Hirai
2r - NEW
2s NEW
2f Hirai
2t - NEW
1a_1 Hirai
76
100
100
100
100
100
98
84
86
99
90
100
100
89
68
65
48
100
100
89
36
53
80
100
87
100
100
0.02
Cytochrome c oxidase subunit I
2c Hirai
2d Hirai
2a Hirai
2r - NEW
2s - NEW
2f Hirai
2t - NEW
1a_1 Hirai
100
94
99
100
87
100
100
100
98
100
93
100
84
82
100
100
96
54
100
0.02
PLAB2 Hirai
PLAB1 Hirai

Fig.3

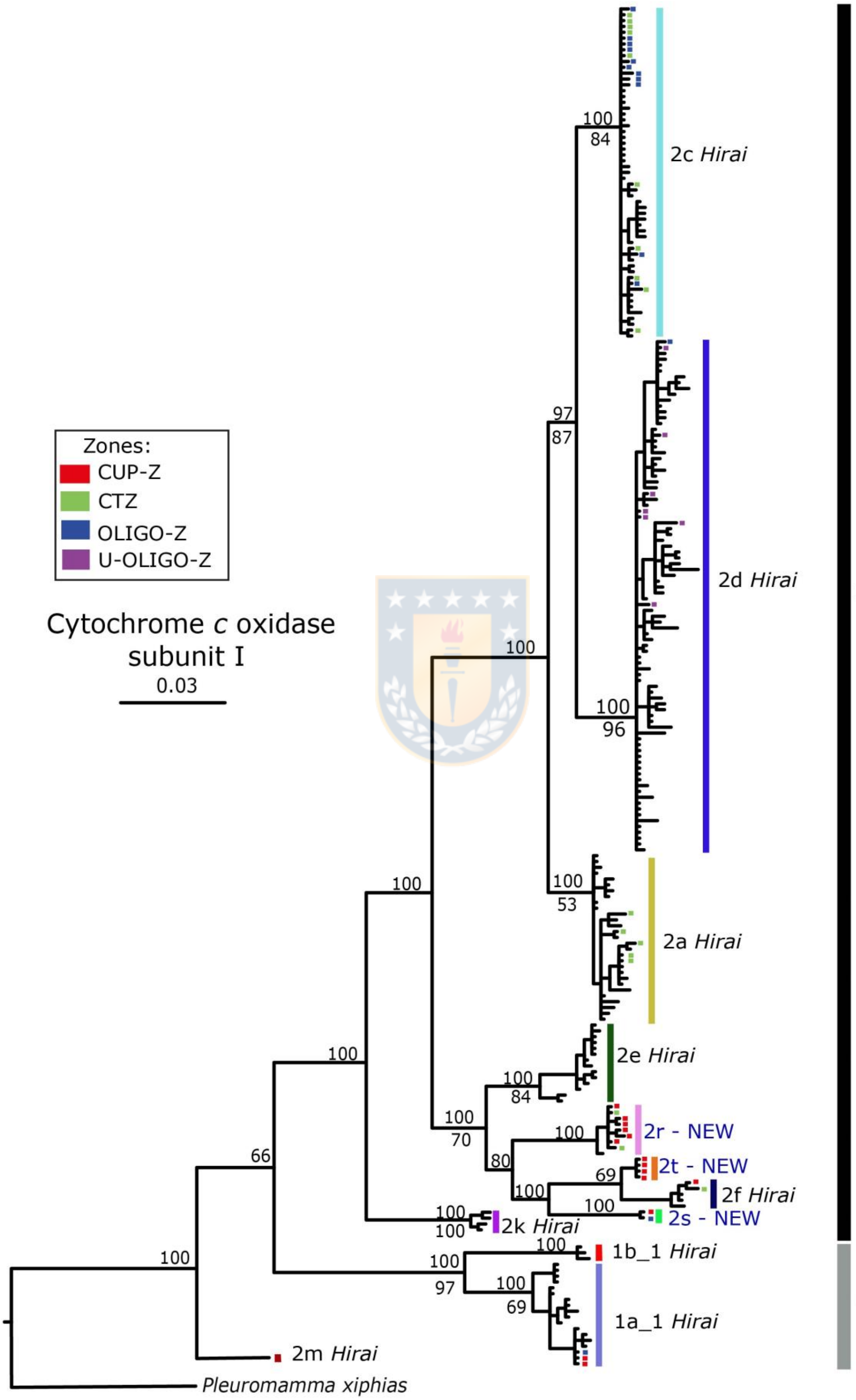

Zones:
🟥 CUP-Z
🟩 CTZ
🟦 OLIGO-Z
🟪 U-OLIGO-Z
Cytochrome *c* oxidase subunit I
0.03
2c *Hirai*
2d *Hirai*
2a *Hirai*
2e *Hirai*
2r - NEW
2t - NEW
2f *Hirai*
2s - NEW
2k *Hirai*
1b_1 *Hirai*
1a_1 *Hirai*
2m *Hirai*
Pleuromamma xiphias
PLAB2 *Hirai*
PLAB1 *Hirai*
100
84
97
87
100
96
100
100
53
100
100
84
100
70
100
80
69
100
100
100
100
66
100
100
97
100
69
100
100

Fig.4

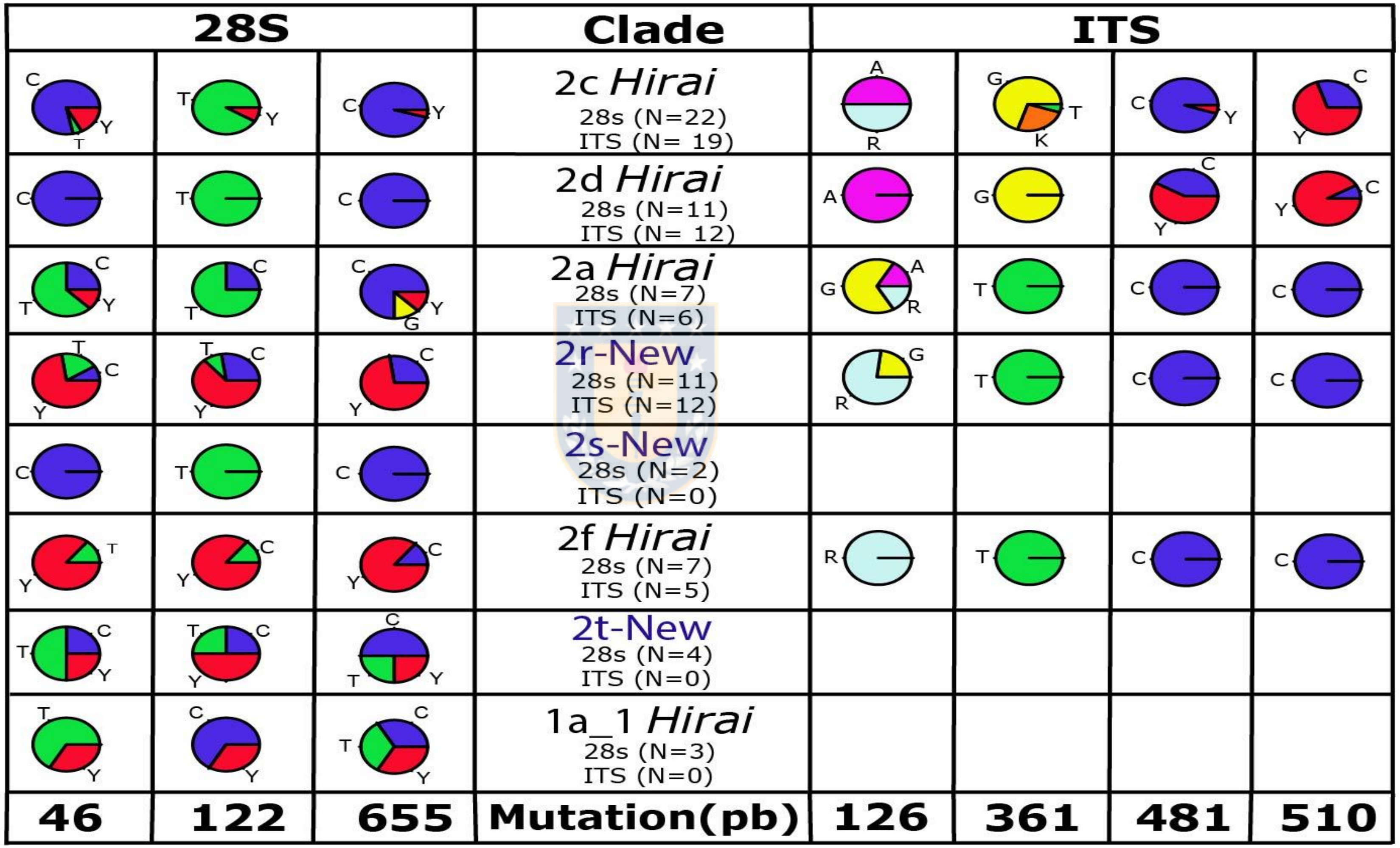

28S
Clade
ITS
C T Y
T Y
C Y
2c Hirai
28s (N=22)
ITS (N= 19)
A R
G T K
C Y
C Y
C
T
C
2d Hirai
28s (N=11)
ITS (N= 12)
A
G
C Y
Y C
C T Y
C T
C Y G
2a Hirai
28s (N=7)
ITS (N=6)
G A R
T
C
C
T C Y
T C Y
C Y
2r-New
28s (N=11)
ITS (N=12)
G R
T
C
C
C
T
C
2s-New
28s (N=2)
ITS (N=0)
T Y
C Y
C Y
2f Hirai
28s (N=7)
ITS (N=5)
R
T
C
C
C T Y
T C Y
C T Y
2t-New
28s (N=4)
ITS (N=0)
T Y
C Y
T C Y
1a_1 Hirai
28s (N=3)
ITS (N=0)
46
122
655
Mutation(pb)
126
361
481
510

Fig.5

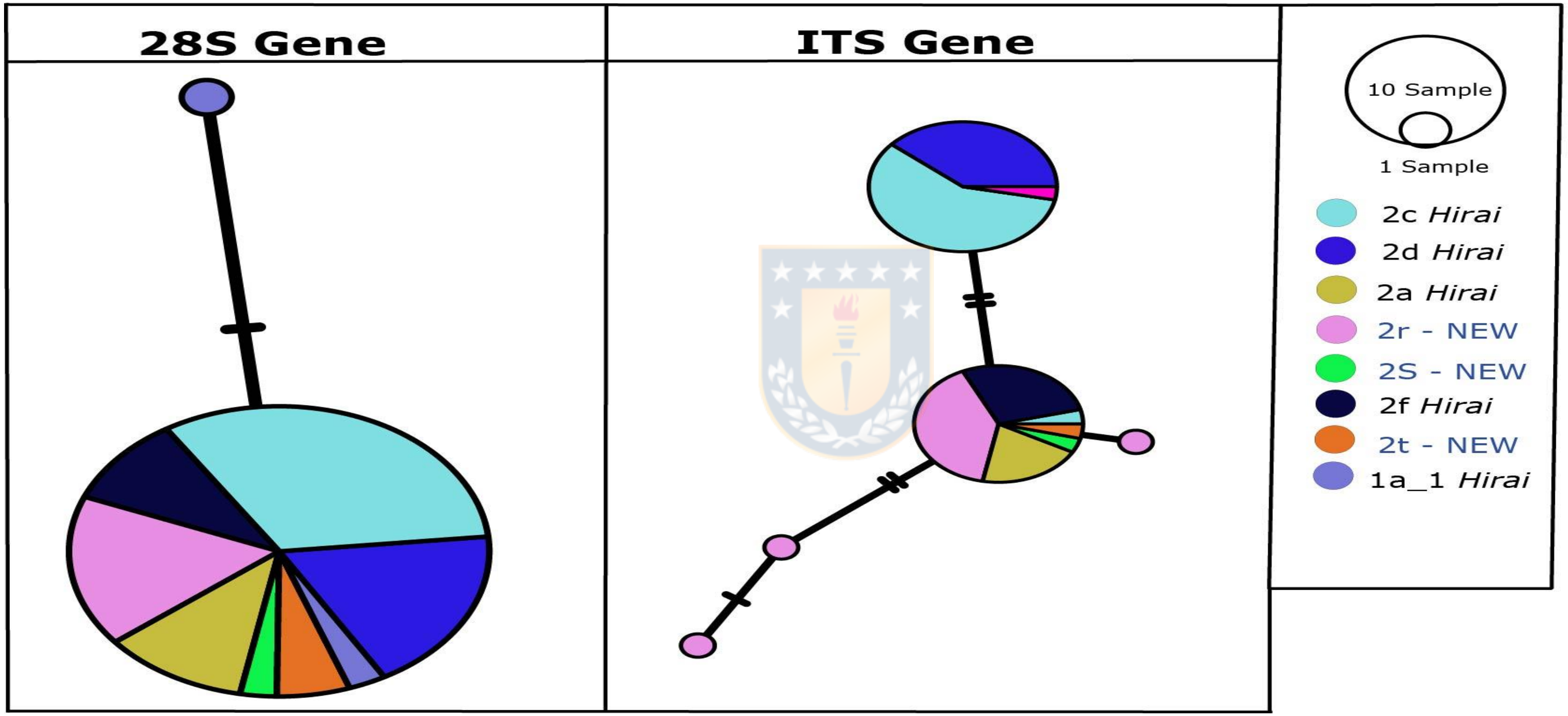
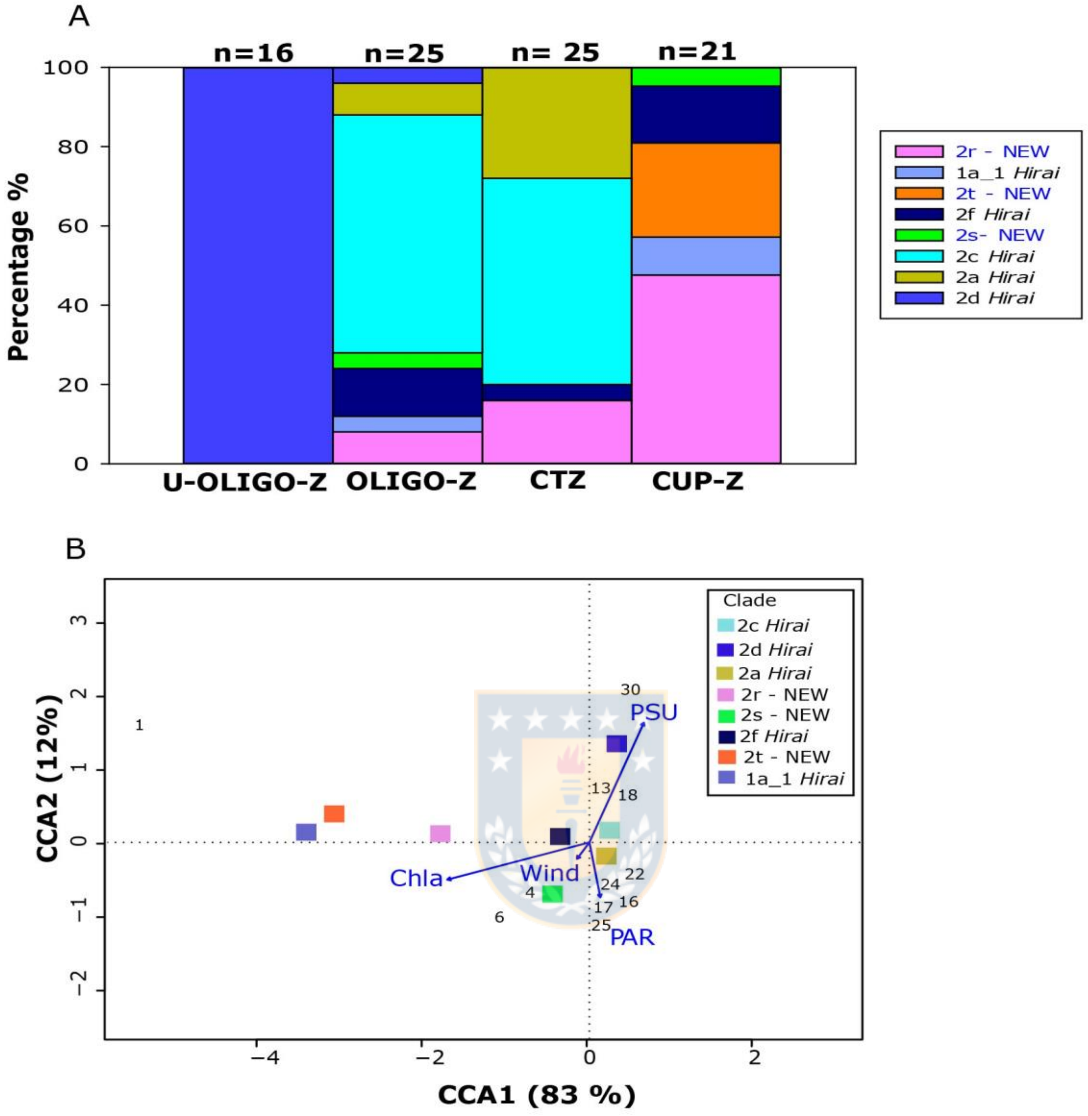

28S Gene
ITS Gene
10 Sample
1 Sample
2c Hirai
2d Hirai
2a Hirai
2r - NEW
2S - NEW
2f Hirai
2t - NEW
1a_1 Hirai

Fig.6


A
n=16
n=25
n= 25
n=21
Percentage %
100
80
60
40
20
0
U-OLIGO-Z
OLIGO-Z
CTZ
CUP-Z
2r - NEW
1a_1 *Hirai*
2t - NEW
2f *Hirai*
2s- NEW
2c *Hirai*
2a *Hirai*
2d *Hirai*
B
Clade
2c *Hirai*
2d *Hirai*
2a *Hirai*
2r - NEW
2s - NEW
2f *Hirai*
2t - NEW
1a_1 *Hirai*
PSU
Chla
Wind
PAR
1
4
6
13
16
17
18
22
24
25
30
CCA2 (12%)
CCA1 (83 %)
−4
−2
0
2
3
2
1
0
−1
−2

Supplementary Information

Genetic diversity and novel lineages in the cosmopolitan copepod *Pleuromamma abdominalis* in the Southeast Pacific

Carolina E. González, Erica Goetze, Rubén Escribano, Osvaldo Ulloa and Pedro Victoriano.

Supplementary table S1. Results of Canonical Correlation Analysis (CCA) for mitochondrial clades and hydrographic variables. Correlation of ordination axes with variables, eigenvalues and percentage variances explained. Significance of the permutation test for CCA between genetic and hydrographic variables as indicated (*p-value*). The hydrographic variables which contributed to the most parsimonious model were surface chlorophyll-a (Chla), sea surface temperature (SST), surface salinity (SSS) and wind stress (WT)

Variables	Axis 1	Axis2	*p-value*
Chla	**-0.9547**	-0.281	0.009***
PAR	0.0737	-0.414	
SSS	0.3662	**0.903**	
WT	-0.0833	-0.124	
Cumulative percentage of variance explained	0.8267	0.943	

4. DISCUSIÓN

Los resultados de la presente tesis muestran claramente que el Pacifico Suroriental es un sistema ambientalmente heterogéneo reflejado en los gradientes longitudinales y latitudinales y caracterizados por cambios graduales o abruptos en variables físicas, químicas y biológicas. Estos resultados son consistentes con observaciones anteriores (Ej. Palma y Silva, 2006; Dolan *et al.*, 2007; Raimbault y Garcia, 2008; Silva *et al*., 2009; Cornejo *et al.*, 2015; Escribano *et al.*, 2003; Hidalgo *et al.*, 2010). Sin embargo, es importante destacar que los límites de las zonas del gradiente longitudinal fueron arbitrarias, y se basaron exclusivamente en los niveles de Clorofila-a superficiales y cobertura espacial de las muestras, por lo que las áreas ocupadas por cada zona son muy diferentes. En cambio, para el gradiente latitudinal la subdivisión se hizo a los 30°S, ya que este sitio ha sido reportado como un lugar de quiebre biogeográfico en otros organismos marinos (Ej. Algas: Gonzalez *et al.*, 2012; moluscos: Cárdenas *et al.*, 2009; bivalvos: Broitman *et al.*, 2001). Por lo tanto, esta división arbitraria pretende representar la zonación ecológica y biogeoquímica del zooplancton en el Pacifico Suroriental.

La variabilidad en las condiciones bioquímicas del zooplancton, tales como sus contenidos de C y N, puede depender no sólo de las fuentes de nutrientes, sino también de las variables ambientales prevalecientes y algunos procesos ecológicos. Por ejemplo, las condiciones fisiológicas y nutricionales de los organismos pueden modificarse por factores como la oxigenación (Seibel, 2011), temperatura (Atkinson, 1994) y pH (Yamada e Ikeda, 1999). En el mismo contexto, las condiciones de los alimentos son fundamentales para determinar la fisiología y nutrición del zooplancton. Tanto la cantidad como calidad de los alimentos desempeñan un papel clave en este sentido (Ej. Hirst y Bunker, 2003; Vargas *et al.*, 2006), a través de la disponibilidad, conducta alimenticia y selectividad (DeNiro y Epstein, 1978). Todos estos procesos pueden afectar en última instancia las relaciones C: N y la composición de los isótopos de C y N del zooplancton. Sin embargo, el estudio postula que las principales fuentes de C y N, que a su vez dependen de la zonación definida anteriormente, son la fuerza impulsora clave para determinar tales características bioquímicas. A modo de ejemplo, la elevada razón de C: N observada en el zooplancton en áreas oligotróficas pueden explicarse por una deficiencia de N en altos niveles de C. La deficiencia de N es una característica bien conocida en el océano abierto del Pacífico Suroriental (Altabet *et al.*, 2012; Stramma *et* *al.*, 2013; Cornejo *et al.*, 2015). Sin embargo, las razones C: N> 3.5 observadas en todas las fracciones de tamaño y zonas pueden indicar también un exceso de lípidos en el zooplancton (Post, 2002; Smyntek *et al.*, 2007). El almacenamiento de lípidos es probablemente una estrategia contra la escasez de alimentos (Lee *et al.*, 2006), que resulta en una mayor proporción de C: N en las regiones oceánicas. En ambos casos, la deficiencia de N y la escasez de alimentos son procesos desencadenados por una condición oligotrófica extrema, que es uno de los criterios propuestos para definir la zonación biogeoquímica.

Al comparar las zonas en términos de composición isotópica, los patrones variaron entre $\delta^{13}C$ y $\delta^{15}N$. Los isoespacios y métricas Layman indicaron que el rango de $\delta^{13}C$ difería entre las zonas, lo que sugiere un origen variable de C para el zooplancton a través del gradiente zonal. Es de esperar una fuente altamente regenerada de C para la biomasa del zooplancton en el océano abierto (Williams, 1981; Wollast, 1998; Del Giorgio y Duarte, 2002), en comparación con la zona de surgencia donde la fuente principal de C se ha recuperado recientemente desde la atmósfera (Gruber *et al.*, 1999; Mompeán *et al*., 2013). Además, en la zona de surgencia se observó un altor valor de $\delta^{13}C$ probablemente debido al predominio de diatomeas, que exhiben una mayor razón ($\delta^{13}C$: $^{-}21$-$^{-}25$) que los dinoflagelados ($\delta^{13}C$: $^{-}21$-$^{-}25$), siendo estos últimos más abundantes en el océano abierto (Nakatsuka *et al.*, 1992). Sin embargo, debido a la gran variabilidad observada de $\delta^{13}C$ a través del gradiente zonal, no fue posible diferenciar si la fuente de C provenía de diatomeas o dinoflagelados, como en otros estudios (Ej. Perry *et al.*, 1999), puesto que en las zonas oligotróficas el fitoplancton puede no cubrir la demanda de carbono requerida por el zooplancton (Zhang *et al.*, 1995). Por lo tanto, estos organismos compensan esta deficiencia alimentándose de microzooplancton (Kleppel, 1993; Calbet y Landry, 1999; Calbet y Saiz, 2005) traduciéndose este proceso en cambios de $\delta^{13}C$ en su biomasa.

A su vez, $\delta^{15}N$ disminuyó claramente hacia el océano abierto, lo que sugiere una fuente cambiante de N para el zooplancton a través del gradiente zonal. Se conoce que los procesos que modulan la disponibilidad de N en la zona costera difieren a aquellos de áreas más oceánicas, por ejemplo la presencia de una zona de mínimo oxigeno promueve el proceso de desnitrificación en la zona de surgencia (Thamdrup *et al.*, 2006; Galán *et al.*, 2009; Dalsgaard *et al.*, 2013), favoreciendo un enriquecimiento en $\delta^{15}N$. Otra posible fuente de variación podría estar relacionada con el tipo de alimento para zooplancton en el océano abierto. A pesar de que todas las fracciones de tamaño analizadas pueden estar compuestas por una variedad de niveles tróficos (carnívoros o herbívoros), la fuente principal de N debe ser fitoplancton, probablemente derivado de la diazotrofía como se refleja en los bajos valores de δ^{15}N en ambientes oligotróficos. Sin embargo, existen otros procesos potenciales que podrían modificar el δ^{15}N. Por ejemplo, en ambientes oligotróficos la entrada de nitrato a la zona fótica puede ocurrir por difusión a través de la picnoclina (Mouriño-Carballido *et al.*, 2011; Fernández-Castro *et al.*, 2015) proporcionando un N con un bajo valor de δ^{15}N. Por otro lado, la migración vertical del zooplancton de gran tamaño facilita el acceso a diferentes presas que promueven la variabilidad en su composición isotópica (Cartes *et al.*, 2007; Agersted *et al.*, 2014). En cualquier caso, la composición del fitoplancton posee un papel clave para determinar las condiciones bioquímicas y nutricionales del nitrógeno en el zooplancton.

Con respecto a la zonificación ecológica a través del gradiente zonal, se encontró que la composición del zooplancton cambia significativamente desde la zona costera hasta el océano abierto. El análisis automatizado del zooplancton no proporciona información sobre la composición de las especies, sino sólo sobre la proporción y abundancia de diferentes categorías taxonómicas equivalentes a taxa de nivel superior a especies. Esta proporción cambia de una zona a otra y lo más probable es que las especies también lo hagan desde la zona costera hasta la región oceánica como ha sido reportado en otros trabajos en el Pacifico Suroriental (Riquelme-Bugueño *et al*., 2012; Palama y Silva, 2006; Morales *et al*., 2010). La composición de las especies también puede influir en los valores isotópicos del zooplancton debido a la variabilidad en las posiciones tróficas en los diferentes grupos del zooplancton (Matthews y Mazumder, 2003) y, por lo tanto, algunas de las variaciones en δ^{15}N pueden estar relacionadas con la composición cambiante de la especies.

Debido a la gran diferencia en productividad y recursos entre las zonas identificadas, el tamaño del nicho isotópico también fue muy variable. Se obtuvieron nichos relativamente pequeños para muestras provenientes de la zona de surgencia y transición costera, ya que la gran disponibilidad de recursos permite una alimentación especializada de presas con una composición isotópica similar. En contraste, con las zonas oligotróficas que exhibieron nichos isotópicos más amplios, principalmente debido a una gran variabilidad en δ^{13}C, y que resultaron también ser más variables en la composición taxonómica que en las zonas costeras. Bajo este mismo contexto, se ha reportado que una alimentación oportunista en el zooplancton basada en fitoplancton, detritos y otros organismos puede ampliar enormemente su nicho isotópico (Bode *et al.,* 2015; Teuber, *et al.,* 2014). Otros trabajos han encontrado resultados similares en el plancton de las capas profundas del Atlántico, donde las comunidades que viven en zonas tropicales y oligotróficas mostraron nichos isotópicos de gran tamaño producto de una alimentación tipo oportunista (Bode y Hernández-León, 2018).

Los gradientes oceanográficos no sólo poseen un rol importante en la zonación bioquímica del zooplancton, sino que también en la estructuración de los patrones temporales y espaciales de sus comunidades (Lawton, 1999; Hillebrand y Azzsky, 2001; Rex *et al.*, 20001). Las muestras analizadas para determinar los patrones biogeográficos de los copépodos del orden Calanoida a nivel de familia y especies, se obtuvieron desde diferentes años y estaciones, por lo que fue posible evaluar el efecto de la estacionalidad sólo en la zona de surgencia debido a una mayor cobertura temporal. A pesar, de que en la zona costera se ha informado el efecto de la variabilidad estacional en la composición de las especies (Hidalgo y Escribano, 2001; Escribano *et al.*, 2007), no fue posible observarlo al evaluar la composición de familias dentro de un gradiente latitudinal. Esta falta de estacionalidad podría ser explicada por continua presencia de las familias al interior de la zona de surgencia, pudiendo incluso mostrar una falta de variabilidad en sus abundancia y diversidades en diferentes épocas del año, producto de su reproducción continua durante el ciclo anual (Escribano y McLaren, 1999, Hidalgo y Escribano, 2007).

Con respecto al gradiente costa-océano del Pacifico Suroriental se encontró que la composición de familias y especies del orden Calanoida cambia significativamente desde la zona de surgencia costera hasta las áreas oligotróficas. Otros trabajos realizados en la misma área de estudio también informan de un cambio en la composición de las especies como se observa, por ejemplo en eufáusidos (Riquelme-Bugueño *et al.*, 2012) y cnidarios (Palma y Silva, 2006). Entre las posibles explicaciones de este patrón, se destaca la presencia de diferentes masas de agua y fuentes de nutrientes al interior de este gradiente, que proporcionarían características distintivas para cada una de las zonas de estudio. La zona de surgencia costera se encuentra influenciada principalmente por el agua ecuatorial subsuperficial, caracterizada por una alta concentración de nutrientes que promueve un rápido traspaso de carbono atmosférico hacia los copépodos (Gruber *et al.,* 1999; Mompeán *et al.*, 2013; Daneri *et al.*, 2000; Silva y Valdenegro, 2003). Sin embargo, las zonas de transición costera y oligotróficas están compuestas por las aguas subantárticas y subtropicales respectivamente, las cuales presentan concentraciones más bajas de nutrientes y carbono más regenerado en comparación con la zona costera (Nakatsuka *et al.,* 1992; Reid, 1973). Todo esto, sumado a la presencia del giro anticiclónico en el océano abierto, que aísla la región de surgencia costera de las adyacentes, promoviendo un ecosistema semi-cerrado regulado por procesos *in-situ* (McGowan, 1971, 1974, 1977). Sin embargo, gracias a la evidencia de microfósiles se ha reportado que durante el Pleistoceno la zonación costa-océano persistió, lo que ha permitido a través el tiempo observar un mayor nivel de estructuración de los copépodos calanoides en el Pacifico Suroriental (Riedel y Funnell, 1964).

En cuanto al gradiente latitudinal, éste mostró una alta estructuración y concordancia espacial entre los análisis realizados, tanto a nivel de especies como de familias. Exhibiendo la presencia de tres grupos: norte, sur y transición. Investigaciones previas hechas sobre el mismo gradiente también informaron la presencia de estas rupturas biogeográficas en otros organismos como algas, moluscos y bivalvos (Gonzalez *et al.,* 2012; Cardenas *et al.*, 2009; Broitman *et al.,* 2001). Los límites de estas regiones biogeográficas no son fijos, pudiendo variar según la estación y año (Escribano *et al.*, 2003). A pesar, de que sus límites no están claros, se ha informado que la región de transición muestra una alta correlación con un gradiente de temperatura superficial (Broitman *et al.*, 2001), patrones de circulación (Hormazabal, 2004) y estrechamiento de la plataforma continental (Strub *et al.*, 1998). Además, la presencia e interacción de diferentes masas de aguas como ecuatorial subsuperficial e intermedia antártica, proporcionan especies de origen ecuatorial en la región norte y polar/subantártica en la sur, generando posiblemente una zona de transición entre 30ºS concordante con la zona de convergencia subtropical (Escribano *et al.*, 2003; Hidalgo *et al.*, 2010). En escalas de tiempo más largas, el cambio del clima durante el Pleistoceno tuvo un gran impacto en la diversidad costera actual. Tal fenómeno, ha sido reportado en muchas especies y océanos del todo el mundo (Maggs *et al.*, 2008; Papadopoulos *et al.*, 2005; Provan *et al.*, 2008). Al final de este periodo, la zona de surgencia costera mostró un calentamiento del agua superficial, aumento del nivel del mar y disminución de la productividad que probablemente favorecieron a la aparición de una zona de transición entre las especies del norte y sur (Escribano *et al.,* 2003; Hidalgo *et al.,* 2010).

Existe una gran dependencia de la temperatura superficial del mar con la estructura comunitaria del orden Calanoida, tanto nivel de familias y especies, en el Pacifico Suroriental. Se conoce que la temperatura modifica las tasas de diversos procesos biológicos en los copépodos, como su crecimiento, productividad y mortalidad (Hirst y Kiørboe, 2008). A lo largo del gradiente costa-océano se observó un notorio aumento de la temperatura desde la zona costera hasta el océano abierto, el cual está fuertemente correlacionado (> 40%) con la riqueza tanto de las familias como de las especies dentro de este orden. En varias regiones del mundo se ha reportado una correlación positiva entre la temperatura y la riqueza de las especies de zooplancton acompañado de una disminución en su abundancia (Matsubara, 1993; Castro *et al.*, 2005; Hessen *et al.,* 2007). Sin embargo, en este trabajo los datos de abundancia de las familias y presencia/ausencia de las especies mostraron un bajo valor de correlación (<21%), probablemente debido a la naturaleza de los índices utilizados que tendieron a homogenizar la matriz biológica ocupada para estos análisis. Con respecto al gradiente latitudinal, en general se observa que existe una mayor correlación con la temperatura a nivel de familias (> 20%), debido a que algunas especies de copépodos acoplan su ciclo de vida con los pulsos de surgencia otorgándole mayor variabilidad al sistema (Hidalgo y Escribano, 2007).

Los copépodos calanoideos están sujetos a fuertes gradientes oceanográficos en el Pacifico Suroriental y, por lo tanto, a fuertes presiones selectivas que juegan un rol fundamental en la estructuración de sus comunidades a nivel de especie. La forma en que tales gradientes interactúan con el proceso de especiación de los organismos pelágicos todavía no se conoce bien para esta vasta región. Los análisis moleculares han revelado que especies de copépodos con distribuciones cosmopolitas muestran un número variable de linajes genéticos, en organismos que se atribuyeron como una sola especie usando la taxonomía tradicional (Halbert *et al.,* 2013; Goetze, 2003; Cornils y Held, 2014). Estos distintos linajes exhiben comúnmente patrones de distribución asociados con condiciones oceanográficas variables (Goetze *et al.,* 2015). El presente estudio constituye un primer paso para desenredar la diversidad genética dentro de *Pleurommama abdominalis,* un copépodo ampliamente distribuido en el Pacifico Suroriental. El análisis filogenético de los genes mitocondriales y nucleares de

Pleurommama abdominalis, mostró un complejo de entidades genéticas no descritas morfológicamente, lo que sugiere un proceso de especiación críptica o pseudocríptica ocurre a lo largo de esta región.

En términos generales, el género *Pleurommama* se considera omnívoro y este comportamiento generalista le permite a las especies dentro del género ocupar una distribución espacial más amplia (Benedetti *et al*., 2018). Sin embargo, los 8 clados observados estaban altamente correlacionados con la clorofila-a y salinidad, lo que permite establecer límites de su distribución. Los linajes fueron encontrados principalmente en el área surgencia costera y, en algunos casos, se extendieron hasta las zonas de transición y oligotrófica (Ej.2r-NEW). Estos clados posiblemente están asociados con aguas ricas en nutrientes de la zona de surgencia costera y también áreas oligotróficas sometidas a remolinos de mesoescala que pueden transportar aguas de surgencia a la región oceánica (Andrade *et al.*, 2014; Hormazabal *et al.*, 2013). Por otra lado, los linajes observados en las zonas de transición costera y oligotrófica mostraron una correlación con áreas de baja concentración de clorofila-a (Ej. 2c *Hirai*). Finalmente, el linaje ubicado en la zona ultra-oligotrófica mostró una fuerte correlación con zonas de alta salinidad (2d *Hirai*), probablemente este clado sea un indicador de la masa de agua subtropical del giro Pacífico Sur (Hirai *et al.*, 2015; Silva *et al.*, 2009). Se ha informado que la adaptación a rangos específicos de temperatura, salinidad u otros parámetros oceanográficos son importantes impulsores de la diversificación genética en varios organismos planctónicos, como quetognatos (Peijnenburg *et al*., 2014), medusas (Schroth *et al*., 2002) y copépodos calanoideos (Yebra *et al*., 2011)

El alto nivel de endemismo encontrado, en particular las zonas de surgencia costera y ultra-oligotrófica, pueden ser una respuesta tanto a los procesos físicos actuales como a los históricos. En el ambiente pelágico hay varias barreras físicas, como las masas de tierra y corrientes oceánicas, que pueden influir en la dispersión y conectividad entre las poblaciones promoviendo la diversidad de especies (Norton y Goetze, 2013; Weiner *et al.*, 2012; Miyamoto *et al*., 2012; Peijnenburg *et al*., 2006). La zona costera muestra fuertes interacciones entre distintas fuerzas oceanográficas, por ejemplo los frentes de surgencia, remolinos y corrientes de marea. Estas interacciones pueden ejercer efectos sutiles en los organismos pelágicos en escalas de tiempo cortas, pero a largas escalas pueden tener profundos efectos en la estructura de la población (Marko, 2004; Wares, 2001,2002). Por ejemplo, la zona ultra-oligotrófica se ha descrito como una región donde las poblaciones exhiben una composición genética diferente (Papetti *et al.*, 2005; Norton y Goetze, 2013; Bucklin *et al.,* 2000). Esto se debe a que los giros subtropicales actúan como áreas de retención y, por lo tanto, permite una alta estructuración genética se desarrolle y persista (Goetze, 2003, 2011; Papetti *et al*.,2005; Norton y Goetze, 2013; Hirai *et al*., 2015; Iacchei *et al*., 2017). En escalas de tiempo más largas, se ha sugerido que durante el último máximo glaciar (LGM, 19,000 a 23,000 años atrás) la productividad en la zona costera fue mucho mayor (Kohfeld *et al*., 2013), mientras que el giro subtropical tuvo una menor productividad (Kumar *et al.*, 1993). Este contraste puede haber actuado como una barrera alopátrica para la dispersión entre las zonas, explicando divergencia actual del área ultra-oligotrófica (clado 2d *Hirai*) con respecto a las otras zonas. Más tarde, al final del Pleistoceno, el calentamiento de la superficie del mar, aumento del nivel del mar y la disminución de la productividad en la zona costera pueden haber debilitado el gradiente oceanográfico y las barreras de dispersión entre las zonas oligotrófica y de surgencia, permitiendo así una expansión y posiblemente un contacto secundario entre las zonas generando el actual patrón parapátrico (von Dassow y Collado Fabbri, 2014; Zakas *et al*., 2009). En cualquier caso, está bien documentado que los rangos de distribución de los copépodos pueden cambiar rápidamente, como se ha evidenciado actualmente debido al cambio climático (~ 23 km/año) (Beaugrand *et al*., 2002)

Los análisis GMYC con los genes mitocondriales indicaron claramente que *Pleurommama abdominal* es probablemente un complejo de especies compuesto por clados genéticamente divergentes que son difíciles de distinguir morfológicamente. Los resultados sugieren la presencia de al menos 7 especies, dentro de las cuales dos aparecen altamente especializadas en las zonas de surgencia costera y ultra-oligotrófica. Otros estudios centrados en especies cosmopolitas dentro del mismo género, como *P. xiphias*, *P. gracilis* y *P. piseki* también han descrito la presencia de varios linajes, lo que sugiere que varias especies de este género son un complejo de especies cripticas (Goetze, 2011; Halbert *et al.,* 2013). En el estudio de Hirai *et al.,* (2015) infirió con certeza que *P. abdominalis* estaba compuesto por al menos dos especies que coincidían con los clados PLAB1 y PLAB2, dado a los patrones concordantes de variación genética entre marcadores mitocondrial y nuclear. Sin embargo, se dejó abierta la posibilidad de la presencia de más especies, porque la divergencia observada dentro de ambos clados fue mayor que 4.3% (Hirai *et al.,* 2015). En el presente estudio, se reportaron valores de divergencia mayores al 30% entre los 8 clados, aunque ambos genes mitocondriales también mostraron que las mayores distancias genéticas se observaron entre los clados que forman los haplogrupos PLAB2 y PLAB1. En términos generales, muchas especies descritas de copépodos marinos exhiben divergencias COI en el rango de 8 a 9% (Bucklin *et al.,* 2003; Costa *et al.,* 2007), mientras que otros trabajos sugieren que la variación intraespecífica de copépodos ocurre dentro del rango de 1 y 4% (Andrews *et al.,* 2014; Bucklin y Frost, 2009).

Los genes 28S e ITS fueron menos polimórficos e informativos que los marcadores mitocondriales en la detección de límites de especies dentro de *P. abdominalis*. En las redes de haplotipos, ambos genes nucleares no mostraron patrones claros con respecto a los clados mitocondriales preestablecidos. Sin embargo, estos genes mostraron diferencias en las frecuencias de nucleótidos en los sitios polimórficos, lo que nos permitió distinguir varios de los 8 clados, por medio del porcentaje de las bases nucleotídicas. La clara falta de concordancia entre marcadores mitocondriales y nucleares puede explicarse, por una mayor sensibilidad de los marcadores mitocondriales a la deriva genética, debido a la herencia uniparental haploide y el tamaño efectivo más pequeño (Avise, 2012; Birky *et al.,* 1983; Wilson y Hubschman, 1985). Algunos trabajos realizado en otras especies de copépodos, han informado que el gen 28S muestra una tasa de evolución más lenta que el gen COI (Miyamoto *et al.,* 2012; Blanco-Bercial *et al.,* 2011; Machida *et al*., 2004) y puede ocurrir una clasificación incompleta del linaje. Además, es bien conocido que para delimitar eficazmente especies es necesario analizar múltiples loci (Boissin *et al.,* 2008; Casteleyn *et al.,* 2010; Chen y Hare, 2011; Gaither *et al.,* 2011). Esto se debe a que el análisis de un sólo locus puede subestimar o sobreestimar el número de especies debido a la presencia de pseudogenes (Song *et al.,* 2008), linajes mitocondriales antiguos que están presentes dentro de poblaciones entrecruzadas e introgresión entre especies estrechamente relacionadas, así como otros procesos que pueden oscurecer los límites de las especies. Varios estudios han demostrado que el uso de marcadores mitocondriales puede ser más eficiente para detectar inicialmente especies crípticas (Goetze, 2010), pero trabajos posteriores demuestran que una combinación de marcadores permite una delimitación más consistente (Ej. *Haloptilus longicornis*)(Goetze *et al*., 2015).

5. CONCLUSIONES

A partir de los análisis y discusión expuesta en este trabajo, el estudio apoya las hipótesis planteadas y además se concluye lo siguiente:

1. El Pacifico Suroriental se manifiesta como un ecosistema altamente heterogéneo, caracterizado por cambios graduales y abruptos en variables oceanográficas superficiales, como la temperatura, la salinidad, el oxígeno disuelto y la concentración de fitoplancton estimada como clorofila-a.
2. Las razones Carbono: Nitrógeno > 3.5 observadas en todas las fracciones de tamaño del mesozooplancton y zonas estudiadas pueden reflejar un alto contenido de lípidos en el zooplancton, como una potencial estrategia frente la escasez de alimentos. Sin embargo, en áreas oligotróficas la razón C: N puede ser aún mayor, asociada a una deficiencia de N en altos niveles de C.
3. La variabilidad de la señal isotópica ($\delta^{13}C$) entre zonas analizadas sugiere un origen variable de las fuentes de C para el zooplancton a través de un gradiente costa-océano, con un C altamente regenerado en el océano abierto y C de fijación más reciente desde la atmósfera en la zona costera. Además, el alto valor de $\delta^{13}C$ observado en la zona de surgencia, comparado con aquel de la región oceánica, puede estar asociado diferentes fuentes de alimentos. En la zona costera el zooplancton probablemente se alimenta de diatomeas formadores de cademas, que exhiben una razón isotópica mayor que las fuentes potenciales del océano abierto, como dinoflagelados y diatomeas de menor tamaño.
4. La señal isotópica del N ($\delta^{15}N$) disminuyó abruptamente hacia el océano abierto, lo que sugiere una fuente cambiante de N para el zooplancton a través de un gradiente costa-océano. Existen diferentes procesos que promueven la diferenciación entre estas dos zonas, tales como la desnitrificación en la área costera que favorece el enriquecimiento de $\delta^{15}N$ y la diazotrofía en el océano abierto que promueve bajos valores de las señal $\delta^{15}N$.
5. La abundancia relativa de las diferentes categorías taxonómicas del zooplancton cambia significativa desde la zona costera hasta el océano abierto que se manifiesta en una zonación ecológica significativa, originado ecoregiones sobre el gradiente zonal.
6. El tamaño del nicho isotópico para las distintas fracciones de tamaño del zooplancton aumentó en amplitud desde la zona costera hacia el océano abierto, sugiriendo cambios en la estrategia de alimentación de los organismos que componen

las distintas fracciones de tamaño, desde una alimentación especialista en la zona de surgencia hasta una oportunista en áreas oligotróficas.

7. Las especies de copépodos pertenecientes al orden Calanoida muestran una variabilidad estacional a lo largo de la costa chilena, a diferencia de las familias de este orden que exhiben una falta de estacionalidad, probablemente por su continua presencia al interior de la zona de surgencia producto de sus ciclos de vida multigeneraciones sobre el ciclo anual.
8. La composición de familias y especies del orden Calanoida cambia significativamente desde la zona de surgencia costera hasta áreas oligotróficas, debido a la presencia de la zonación ecológica descrita previamente. dEl giro anticiclónico del Pacifico Sur posiblemente promueve tambien la diferenciación entres estas zonas.
9. El gradiente latitudinal mostró una alta estructuración y concordancia tanto a nivel de especies como de familias. Exhibiendo la presencia de tres grupos: norte, sur y transición. Esta estructuración podría ser explicada por las diferentes masas de agua y patrones de circulación. Otros factores no analizados en este estudio pueden ser el ancho de la plataforma continental y el calentamiento durante el holoceno.
10. La temperatura superficial del mar muestra una alta correlación principalmente con la riqueza (> 40 %) del orden Calanoida, tanto nivel de familias y especies. Existe un efecto ecológico de la la temperatura modifica las tasas de diversos procesos biológicos en copépodos, pero también puede indicar las características de diferentes masas de agua.
11. El análisis genético de *Pleurommama abdominalis* mostró un complejo de entidades genéticas no descritas morfológicamente, sugiriendo que el proceso de especiación críptica o pseudocríptica puede estar asociado a la gran variabilidad oceanográfica exhibida en el Pacifico Suroriental. No obstante, el alto nivel de endemismo encontrado, en particular las zonas de surgencia costera y ultra-oligotrófica, pueden ser una respuesta tanto a los procesos oceanográficos actuales como a los históricos.
12. La falta de concordancia entre marcadores mitocondriales y nucleares puede explicarse, por una mayor sensibilidad de los marcadores mitocondriales a la deriva génica, debido a la herencia uniparental haploide, un tamaño efectivo más pequeño y una tasa de evolución más rápida pudiendo promover una clasificación incompleta de linaje.

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7. ANEXO

Supplementary figure S1. Bayesian phylogeny of 999 *P. abdominalis* COI sequences reported in this study and at level global in Hirai et al., (2015). Numbers at the nodes represent the Bayesian posterior probability (upper) and maximum likelihood (lower) support values. Mitochondrial clades are labelled by letters (a-s) and colors. The tree includes 944 *P. abdominalis* COI sequences from GenBank (accession numbers: KT319926.1- KT320869.1; Hirai et al., 2015) and also a *P. xiphias* sequence (access numbers: JN574427) that was used to root the tree. Higher level clades are labelled and referred to in the text (PLAB1, PLAB2).