Universidad de Concepción
Dirección de Postgrado
Facultad de Ciencias Naturales y Oceanográficas-Programa de Doctorado en Oceanografía

Diversidad, viabilidad e implicancias tróficas y biogeoquímicas de hongos aislados desde el Océano Pacífico Sur-Oriental

Tesis para optar al grado de Doctor en Oceanografía

JEANETT ALEJANDRA VERA ESCALONA
CONCEPCIÓN-CHILE
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La Tesis de "*Doctorado en Oceanografía*" titulada "*Diversidad, viabilidad e implicancias tróficas y biogeoquímicas de hongos aislados desde el Océano Pacífico Sur-Oriental"*, de la Srta. *"Jeanett Alejandra Vera Escalona"* 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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Leonardi M, Tarifeño E, **Vera J** (2008). Diseases of the Chilean flounder *Paralichthys adspersus* (Steindachner, 1867), as a Biomarkers of Marine Coastal Pollution Near the Itata River (Chile) Part. II. Histopathological Lesions. Archives of Environmental Contamination and Toxicology. 56(3): 546-566.

Leonardi M, **Vera J**, Tarifeño E, Puchi M, Morín V (2009). Vitellogenin of the Chilean flounder *Paralichthys adspersus* as a biomarker of endocrine disruption along the marine coast of the South Pacific. Part I: Induction, Purification, and Identification. Fish physiology biochemistry. 36(3): 757-765.

Vera J, Gutiérrez M, Palfner G, Pantoja S (2017). Diversity of culturable filamentous Ascomycetes in the eastern South Pacific Ocean off Chile. World Journal of Microbiology and Biotechnology. DOI: 10.1007/s11274-017-2321-7.

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RESUMEN

Los hongos se encuentran ampliamente distribuidos en el ecosistema marino, pero su rol ecológico no está definido claramente ya que aún no son considerados absolutamente como un componente microbiano activo de los océanos. Conocer la diversidad de hongos, la viabilidad de sus esporas en agua de mar y su aporte como reservorio de carbono y nitrógeno para las redes tróficas en el océano y los ciclos biogeoquímicos, nos permite explicar y evaluar su rol en el océano y fortalecer la integración del componente fúngico en el ambiente marino. Esta tesis doctoral aporta con nueva información sobre diversidad de hongos marinos cultivables aislados desde distintos ambientes fisicoquímicos del Océano Pacífico Sur-Oriental de Chile, la influencia de la disponibilidad de sustrato orgánico en su viabilidad y su potencial rol en la red trófica y los ciclos biogeoquímicos marinos. Para ello se realizaron análisis de inferencia filogenética de cepas aisladas, utilizando el código de barras de ADN de hongos (El espaciador interno transcrito; ITS), y se evaluó la capacidad de viabilidad (germinación, crecimiento de micelio y esporulación) de esporas fúngicas en agua de mar y agua dulce con diferencias en la disponibilidad de sustrato orgánico. También se cuantificó el contenido energético, porcentaje de carbono orgánico y nitrógeno, aminoácidos hidrolizables totales (THAA), ésteres metílicos de ácidos grasos (FAMEs), esteroles y lípidos polares intactos. Las cepas de hongos aisladas correspondieron a especies de los phyla: Ascomycota, Basidiomycota y Zygomycota, altamente representadas por especies del Genero *Penicillium* del Orden Eurotiales. Once secuencias no se ajustaron con las especies existentes en GenBank (< 99% identidad), lo que sugiere la presencia de nuevos taxones de hongos. *Rhodotorula mucilaginosa*, una levadura marina aislada desde el giro subtropical del Pacífico Sur (400 m) con valores considerables de carbono y nitrógeno (31,2% C; 4,4% N) respecto al zooplancton y fitoplancton fue la cepa analizada con mayor contenido energético (4,2 kcal gdw^{-1}). La señal bioquímica de las cepas indica que los aminoácidos hidrolizables totales representaron la fracción más alta del tejido fúngico, seguidos de lípidos polares intactos y ácidos grasos, mientras que los esteroles representaron solo una pequeña fracción. Específicamente, las cepas de hongos marinos aquí estudiadas son ricas en aminoácidos esenciales (histidina, treonina, lisina y leucina) y lípidos como fosfatidilcolina, ácido linoleico y ergosterol. Nuestros resultados sugieren que las comunidades fúngicas en el Océano Pacífico Sur frente a Chile parecen prosperar en una amplia gama de condiciones ambientales en el océano y que la

disponibilidad del sustrato es un factor que influye en su viabilidad. Proponemos que los hongos marinos son una fuente potencial de alimento para niveles tróficos superiores y que desempeñan un papel potencial en la movilización de carbono y nutrientes en el océano.

ABSTRACT

Fungi are widely distributed in the marine ecosystem, but their ecological role is not clearly defined since they are not yet fully considered as an active microbial component of the oceans. Knowing the diversity of fungi, the viability of their spores in seawater and their contribution as a reservoir of carbon and nitrogen for trophic networks in the ocean and biogeochemical cycles, can help to explain and evaluate their role in the oceans and strengthen the integration of the fungal component in the marine environment. This doctoral thesis provides new information on (i) the diversity of cultivable marine fungi isolated from different physicochemical environments of Eastern South Pacific Ocean of Chile, (ii) the influence of the availability of organic substrate on the viability of cultivable marine fungi, and (iii) its potential role in the trophic network and marine biogeochemical cycles. For this purpose, phylogenetic inference analyzes of isolated strains (using fungal DNA barcoding, i.e. Transcribed Internal Spacer, ITS) were performed along with the assessment of the viability (germination, mycelial growth and sporulation) of fungal spores in sea water and fresh water with differences in the availability of organic substrate. Energy content, percentage of organic carbon and nitrogen, total hydrolysable amino acids (THAA), methyl esters of fatty acids (FAMEs), sterols and intact polar lipids were also quantified. Strains of isolated fungi corresponded to species of the phyla: Ascomycota, Basidiomycota and Zygomycota, highly represented by species of *Penicillium* genus of the Eurotiales order. Eleven sequences did not match with existing species in GenBank (< 99% identity), suggesting the presence of new fungal taxa. *Rhodotorula mucilaginosa*, a marine yeast isolated from the subtropical gyre of the South Pacific (400 m) with considerable values of carbon and nitrogen (31.2% C, 4.4% N) with respect to zooplankton and phytoplankton was the analyzed strain with the highest energy content (4.2 kcal gdw-1). The biochemical signal of the strains indicated that the total hydrolysable amino acids represented the highest fraction of the fungal tissue, followed by intact polar lipids and fatty acids, while sterols represented only a small fraction. Specifically, the strains of marine fungi studied were rich in essential amino acids such (histidine, threonine, lysine and leucine) and lipids such as phosphatidylcholine, linoleic acid and ergosterol. Our results suggest that the fungal communities in the South Pacific Ocean of Chile seem to thrive in a wide range of environmental conditions in the ocean and that the availability of the substrate is a factor that influences their viability. We propose that marine

fungi are a potential source of food for higher trophic levels, playing a potential role in the mobilization of carbon and nutrients in the ocean.

1.- INTRODUCCIÓN

1.1. Hongos en el ambiente marino

Múltiples exploraciones marinas han conducido a un gran aumento de descubrimientos sobre los diversos grupos microbianos en el océano (Giovannoni et al. 1990, 2005; Karner et al. 2001; Giovannoni and Stingl 2007; Rusch et al. 2007; Andreakis et al. 2015). Las bacterias, son un grupo importante de microorganismos reconocidos por su alta abundancia y su alta diversidad en el océano (Azam and Worden 2004), considerándose la canalización de materia orgánica disuelta vía anillo microbiano el principal flujo de materia orgánica (Azam 1998). Por otra parte, el dominio Archaea es un grupo fenotípicamente diverso (metanógenos, halófilos extremos, reductores de sulfato y termófilos extremos) y abundante en el océano subsuperficial (Karner et al. 2001) y costero (Quiñones et al. 2009), mientras que los virus son considerados las entidades biológicas más abundantes (10^{10}/L en aguas superficiales), influyendo en varios procesos biogeoquímicos y ecológicos (Fuhrman 1999). Por último, otro grupo de microorganismos presentes en el océano es el de los hongos, los cuales han sido documentados en diversos hábitats como esponjas marinas (Gao et al. 2008; Wang et al. 2008), aguas empobrecidas en oxígeno (Jebaraj and Raghukumar 2009; Jebaraj et al. 2010), sedimentos marinos de aguas profundas del Océano Pacífico Occidental (Lai et al. 2007), sedimentos subsuperficiales de aguas profundas del Océano Pacífico Sur-Oriental (Edgcomb et al. 2011), en las aguas de la Patagonia chilena (Gutiérrez et al. 2015), y en el ecosistema de surgencia costera de Chile centro-sur (Gutiérrez et al. 2010, 2011, 2016a).

1.2. Clasificación y diversidad de los hongos en el ambiente marino

El dominio Eukaryota está conformado por cinco reinos: Animalia, Plantae, Chromista (Stramenopila), Protozoa y Fungi (Fig. 1; Carlile and Watkinson 2000). Los hongos son aquellos organismos que componen el Reino Fungi (Webster and Weber 2007). Se estima que existen alrededor de 1,5 millones de especies de hongos en el planeta (Hawksworth 2001) pero hasta la fecha solo han sido descritas 80.000-120.000 especies aproximadamente (Webster and

Weber 2007), considerándose una de las fuentes de biodiversidad menos exploradas del planeta (Carlile and Watkinson 2000).

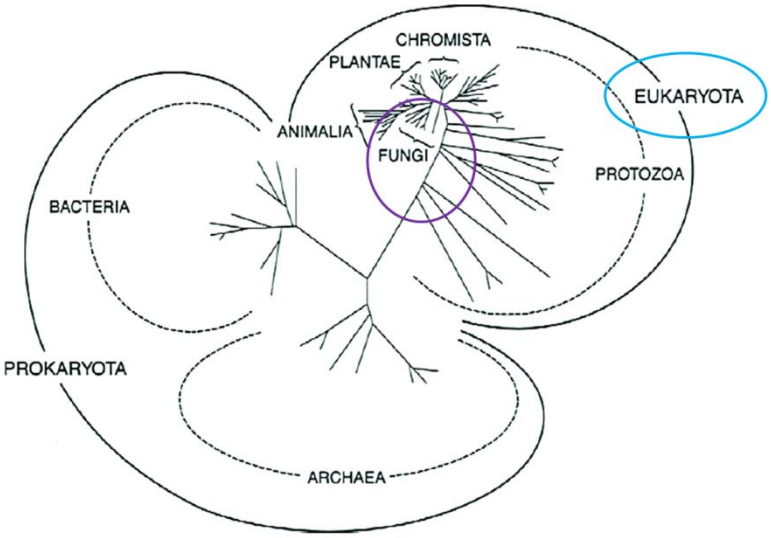

Figura 1.- Árbol filogenético que indica las relaciones entre los dos reinos procariotas y los cinco reinos eucariontes. Extraído de Carlile and Watkinson (2000).

Los hongos marinos son un grupo ecológico de microbios pertenecientes al Reino Fungi, definido sesgadamente en función de su hábitat (Andreakis et al. 2015; Jones et al. 2015). La primera definición de hongos marinos fue publicada por Johnson and Sparrow (1961) y complementada por Kohlmeyer and Kohlmeyer (1979), quiénes los clasificaron en hongos marinos obligados y facultativos. Ambas definiciones hechas antes de la incorporación de estudios moleculares (Jones et al. 2015). Los hongos marinos obligados son aquellos que crecen y se reproducen exclusivamente en ambientes marinos y los hongos marinos facultativos son aquellos de agua dulce o medios terrestres que también pueden desarrollarse en el ambiente marino (Kohlmeyer and Kohlmeyer 1979).

La mayoría de los hongos albergan niveles muy altos de diversidad críptica, indistinguible utilizando microscopía. Además, morfotipos fúngicos similares (levaduras y zoosporas flageladas) se ramifican en posiciones lejanas y parafiléticas en el árbol fúngico de

la vida (James et al. 2006; Liu et al. 2009; Richards et al. 2012), dificultando clasificaciones basadas en observaciones de caracteres morfológicos generales.

Métodos moleculares como la reacción en cadena de la polimerasa (PCR), la amplificación de marcadores de genes taxonómicamente informativos combinados con la construcción de bibliotecas de clones, la secuenciación y los análisis filogenéticos han demostrado que la diversidad microbiana es mucho más diversa de lo que se pensaba (Olsen 1986; Giovannoni et al. 1990; Pace 1997; Moon-van der Staay et al. 2001; López-García et al. 2002; Richards et al. 2012). Recientemente se ha observado un aumento impresionante de datos filogenéticos y moleculares disponibles para hongos marinos, existiendo hasta la fecha 1.112 especies aceptadas, las cuales se clasifican dentro de las divisiones: Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota y Blastocladiomycota. Estos hongos pertenecen a 129 familias y 65 órdenes. Halosphaeriaceae es la familia más grande de hongos marinos con 141 especies y 59 géneros. Los géneros con más especies son *Aspergillus*, *Penicillium* y *Candida* (Jones et al. 2015). Específicamente, hongos que se han logrado aislar desde el ambiente marino son miembros de las divisiones: Ascomycota, Basidiomycota y Zygomycota (Steele 1967; Nagahama et al. 2001; Gadanho and Sampaio 2005; Wang et al. 2008; Jebaraj and Raghukumar 2009; Jebaraj et al. 2010; Li et al. 2014; Andreakis et al. 2015).

Técnicas moleculares, en particular el análisis de secuencias de nucleótidos y enfoques filogenéticos han influido mucho en la sistemática fúngica (Guarro et al. 1999). Códigos de barras de ADN han proporcionado métodos estandarizados, fiables y rentables para la identificación de especies fúngicas marinas y terrestres (Andreakis et al. 2015). El espaciador interno transcrito (ITS) del ADN nuclear ha sido recientemente aceptado como el marcador adecuado para los hongos y se ha utilizado con éxito para la identificación de especies de hongos e inferencia filogenética. Esta aceptación se debe a que la región ITS tiene la mayor probabilidad de identificación exitosa para la gama más amplia de hongos, con la mayor definición entre la variación inter e intraespecífica (Schoch et al. 2012).

1.3. Características generales de los hongos

Los hongos son organismos eucariontes heterótrofos (setas, mohos y levaduras; Fig. 2) que se alimentan mediante la absorción de nutrientes orgánicos. Estructuralmente pueden ser unicelulares conformados por células discretas de levaduras o multicelulares conformados por un sistema de filamentos ramificados llamados hifas que en conjunto forman un micelio (Fig.3). Las hifas, presentan una pared celular compuesta por glucanos y quitina, con la presencia o ausencia de paredes transversales llamadas septos (Webster and Weber 2007). Especies pertenecientes a la división Zigomycota generalmente tienen hifas sin septos, en las cuales los núcleos se encuentran en una masa común de citoplasma, condición denominada cenocítica (Fig. 4a). En contraste, Ascomicetes y Basidiomicetes generalmente tienen hifas con septos (Fig. 4b). Algunas células de levaduras pueden crecer adheridas y formar pseudohifas (Fig. 4e). La reproducción de los hongos puede ser sexual, parasexual o asexual (fisión o gemación; Fig. 2c,d). Sus propágulos de reproducción son esporas microscópicas producidas en gran número para la dispersión y supervivencia de éstos. Las esporas de algunas especies pueden permanecer en estado de latencia por muchos años, especialmente bajo condiciones de frío y calor intensas (Webster and Weber 2007).

Figura 2.- Diferentes morfotipos de hongos: Setas (*Amanita muscaria*), mohos (P*enicillium* sp.) y levaduras (*Rhodotorula mucilaginosa*).Colección personal.

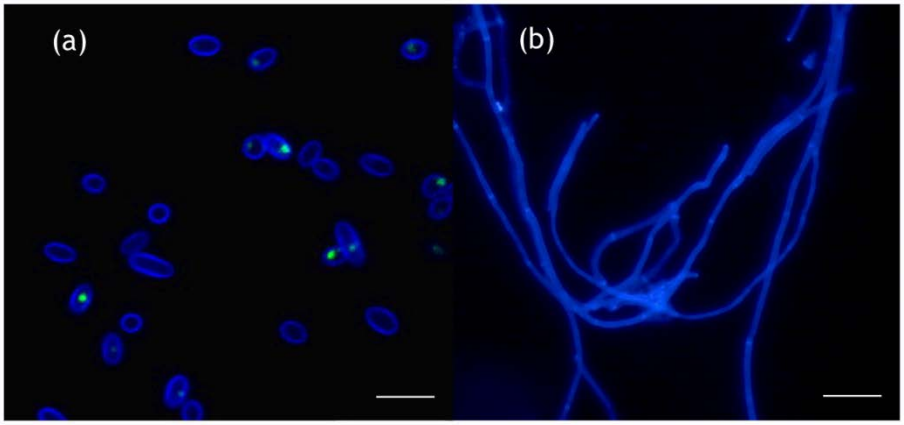

Figura 3.- (a) Levaduras de *Rhodotorula mucilaginosa* (Basidiomycota; barra = 15µm). (b) Hifas con septos de *Penicillium* sp. (Ascomycota; barra = 4µm). Colección Laboratorio de Geoquímica Orgánica. Universidad de Concepción

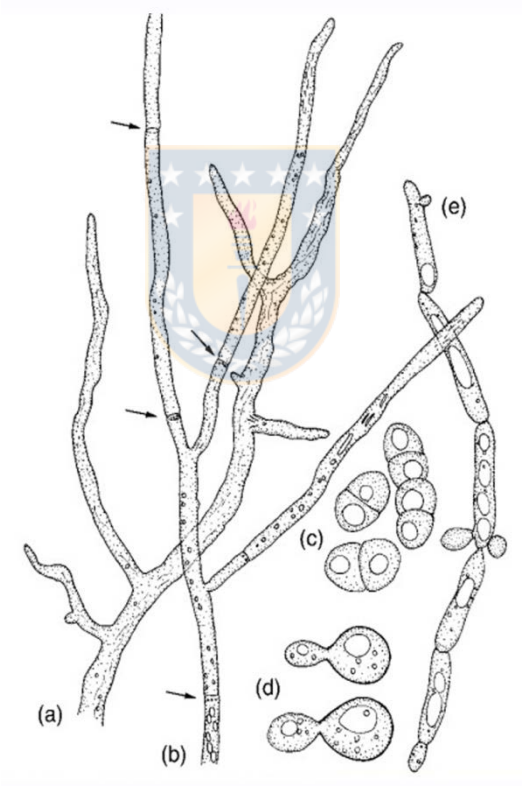

Figura 4.- Diferentes formas de crecimiento de hongos. (a) Hifas sin septos de *Mucor mucedo* (Zygomycota). (b) Ramificaciones de hifas con septos de *Trichoderma viride* (Ascomycota). (c) levaduras de *Schizosaccharomyces pombe* (Ascomycota) dividiéndose por fisión binaria. (d) Levaduras de *Dioszegia takashimae* (Basidiomycota) dividiéndose por gemación. (e) Pseudo-hifas de *Candida parapsilosis* (Ascomycota) consideradas como un estado intermedio entre hifas verdaderas y células de levaduras. Extraído de Webster and Weber (2007).

1.4. Viabilidad e implicancias tróficas y biogeoquímicas de los hongos en el ambiente marino

Tradicionalmente los hongos presentes en el ambiente marino fueron considerados como taxones terrestres arrastrados al mar (Morrison-Gardiner 2002; Kis-Papo 2005) por lo que una de las preguntas en la investigación de hongos en el océano es si su ocurrencia es producto del transporte de propágulos desde el continente y permanencia como organismos latentes en el medio marino (Hagler et al. 1982; Araujo and Hagler 2011; Fell 2012; Jones et al. 2015). Esta incertidumbre sobre el origen de los hongos presentes en el océano ha contribuido a que su rol ecológico aun no esté definido claramente, y que este Reino aun no sea considerado plenamente como un componente microbiano activo de los ecosistemas marinos. Debido a que el agua de mar inhibe la germinación de esporas de hongos terrestres (Kohlmeyer and Kohlmeyer 1979), un criterio válido para evaluar el rol ecológico de hongos aislados desde el ambiente marino, es realizar pruebas de viabilidad (capacidad de germinación, crecimiento de nuevo micelio, y esporulación) de esporas en agua de mar (Kohlmeyer and Kohlmeyer 1979).

Los hongos se consideran saprófitos, parásitos o simbiontes, obteniendo nutrientes exclusivamente por absorción. Este proceso implica la secreción de enzimas despolimerizantes, seguido del transporte de nutrientes (generalmente monómeros) hacia la célula. Como consecuencia de este estilo de vida los hongos no pueden engullir y digerir presas de la misma forma que muchos otros eucariotas. Esta dependencia al intercambio de sustancias entre el interior celular y el exterior determina la ecología de los hongos y su prosperidad en ambientes nutricionalmente enriquecidos (Richards et al. 2012).

En el océano costero, los hongos se caracterizan por su importante papel en el procesamiento de la materia orgánica detrítica de plantas terrestres (biopolímeros complejos), ya que muchos tienen la capacidad de descomponer lignocelulosa (Pointing and Hyde 2009). En los ecosistemas de manglar se ha encontrado evidencia de degradación de lignocelulosa por parte de hongos marinos aislados (a través de la producción de endoglucanasa) en más de 30

cepas filogenéticamente diversas (Hyde et al. 1998). Gutiérrez et al. (2010, 2011) informan por primera vez la presencia de hongos filamentosos en la columna de agua del ecosistema de surgencia costera frente a Chile central, evidenciando una biomasa de hongos tan alta como la de procariontes durante primavera y el verano austral del año 2009. Estos altos valores de biomasa fúngica, acoplados con el incremento de biomasa fitoplanctónica, y con mayores tasas de hidrólisis enzimática extracelular configuran un nuevo escenario ecológico marino. En este nuevo modelo (Fig. 5) los hongos están incorporados en el paradigma del anillo microbiano de los ecosistemas de surgencia costera, participando activamente en el procesamiento de biopolímeros mediante la hidrólisis enzimática de sustratos durante períodos de alta productividad (Gutiérrez et al. 2011). También se ha observado que los hongos tienen el potencial de contribuir significativamente en los ciclos biogeoquímicos mediante la degradación de la materia orgánica particulada en las profundidades marinas, ya que son parte importante de la composición comunitaria de la nieve marina batipelágica (Bochdansky et al. 2017).

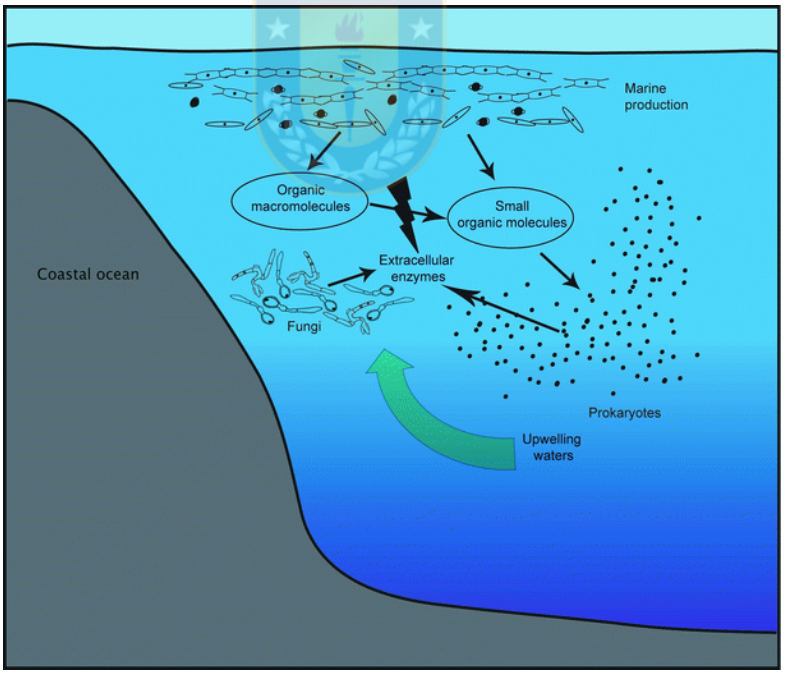

Figura 5.- Modelo conceptual propuesto para la degradación de materia orgánica. Incluye la degradación de polímeros orgánicos por hongos en el ecosistema de surgencia costera del centro-sur de Chile. Extraído de Gutiérrez et al. (2011).

Además de su papel como saprofitos, en ambientes acuáticos los hongos poseen un rol como fuente de alimento, transfiriendo nutrientes mediante el pastoreo de copépodos sobre zoosporas (Kagami et al. 2007; Sime-Ngando 2012). También se ha observado que en ecosistemas marinos, los hongos podrían tener un rol critico durante el procesamiento de detrito (Mann 1988; Raghukumar 2004; Richards et al. 2012), proporcionando nutrientes esenciales como aminoácidos (lisina y metionina), diversas vitaminas, ácidos grasos poliinsaturados y esteroles (precursores de colesterol en animales marinos; Phillips 1984) a niveles tróficos superiores. Estas vías, a través de las cuales la materia orgánica vuelve a entrar en la red alimentaria, son vitales para la supervivencia de los animales incapaces de sintetizar por sí mismos dichos compuestos (Raghukumar 2004; Richards et al. 2012). Por ejemplo, algunos crustáceos requieren de ácido docosahexaenoico para el crecimiento (Harrison 1990), el cual es proporcionado a las redes alimentarias bentónicas mediante microbios detríticos (Raghukumar 2004). Debido a que no está claro si los hongos marinos están implicados en este proceso y su rol como fuente alimenticia es desconocido, el estudio de las características energéticas y bioquímicas de hongos marinos es necesario.

En este ámbito, esta investigación determina la diversidad, viabilidad, composición elemental y características bioquímicas y energéticas de hongos aislados desde distintos ambientes fisicoquímicos del Océano Pacífico Sur-Oriental de Chile. Esta información es esencial para evaluar el rol ecológico y biogeoquímico de los hongos y fortalecer su integración como participante activo en el ambiente marino.

2.- HIPÓTESIS Y OBJETIVOS

2.1. Hipótesis

H1: La diversidad de hongos marinos cultivables está representada por el phylum Ascomycota

Fundamento de la hipótesis: Más de mil especies de hongos marinos han sido aceptadas hasta la fecha, las cuales se clasifican dentro de las divisiones Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota y Blastocladiomycota. Los géneros con más especies (805) son *Aspergillus*, *Penicillium* y *Candida*, pertenecientes al phylum Ascomycota (Jones et al. 2015).

H2: Esporas de hongos aislados desde la columna de agua del Océano Pacífico Sur-Oriental son viables en agua de mar

Fundamento de la hipótesis: Los hongos aún no han sido considerados plenamente como un componente microbiano activo de los océanos, y una de las razones es que su ocurrencia en el medio marino podría ser el resultado de su transporte desde el continente (Hagler et al. 1982; Araujo and Hagler 2011; Fell 2012; Jones et al. 2015). Esta incertidumbre sobre el origen de los hongos en el océano ha dificultado a que su rol ecológico esté definido claramente. Producto que el agua de mar inhibe la germinación de esporas terrestres (Kohlmeyer and Kohlmeyer 1979), un criterio válido para contribuir a determinar el rol ecológico de hongos aislados desde el ambiente marino, es realizar pruebas de viabilidad (capacidad de germinación, crecimiento de nuevo micelio, y esporulación) de esporas en agua de mar (Kohlmeyer and Kohlmeyer 1979).

H3: Hongos aisladas desde el Océano Pacífico Sur-Oriental tienen una composición bioquímica comparable a otros organismos planctónicos

Fundamento de la hipótesis: En ambientes terrestres, los hongos contribuyen con un 35 a 76% de la biomasa microbiana en los suelos (Joergensen and Wichern 2008) y su papel

en el ciclo de nutrientes y carbono es ampliamente reconocido (Gadd 2006; Crowther et al. 2012; Paul 2014). En ambientes acuáticos continentales (lagos) se ha observado que los hongos poseen un rol como fuente de alimento, transfiriendo nutrientes mediante el pastoreo del zooplancton sobre esporas de quitridios (Kagami et al. 2007, 2011; Sime-Ngando 2012). Mediante esta vía, la materia orgánica vuelve a entrar en la red alimentaria proporcionando nutrientes esenciales (aminoácidos, vitaminas, ácidos grasos poliinsaturados y esteroles) a niveles tróficos superiores, permitiendo la supervivencia de animales incapaces de sintetizar estos compuestos por sí mismos (Raghukumar 2004; Richards et al. 2012). Si bien, hallazgos previos apoyan la inclusión de hongos en el modelo del anillo microbiano marino y el ciclo del carbono oceánico (Gutiérrez et al. 2011, 2016; Jephcott et al. 2017), importantes vacíos en nuestro conocimiento impiden una completa comprensión del papel de los hongos en el funcionamiento de los ecosistemas marinos. En particular, debido a la falta de información sobre la composición bioquímica los hongos marinos no se consideran en los modelos actuales de redes tróficas y flujos de materia en el océano.

2.2. Objetivo general

Evaluar la diversidad, viabilidad y composición bioquímica de hongos aislados desde distintos ambientes fisicoquímicos del Océano Pacífico Sur-Oriental de Chile, fortaleciendo la integración del componente fúngico en el funcionamiento del ambiente marino.

2.3. Objetivos específicos

Objetivo específico 1. Determinar la diversidad cultivable de hongos aislados desde el Océano Pacífico Sur-Oriental

Objetivo específico 2. Determinar la viabilidad de esporas de hongos aislados desde el Océano Pacífico Sur-Oriental mediante un test de viabilidad en agua de mar

Objetivo específico 3. Determinar y comparar con organismos planctónicos la composición bioquímica de hongos aislados desde el Océano Pacífico Sur-Oriental

3.- MATERIAL Y MÉTODOS

3.1. Áreas de estudio en el Océano Pacífico Sur-Oriental

3.1.1. Gradiente desde el océano costero hasta el océano abierto (Fig. 6). Área que abarca tres zonas oceanográficas contrastantes: aguas costeras altamente productivas (Montecino et al. 2006) con una Zona de Mínimo Oxígeno somera (Pantoja et al. 2009) frente al norte de Chile (Iquique; 20,1°S-70,8°W), zona de transición y aguas oceánicas oligotróficas (Pennington et al. 2006) cerca de Isla de Pascua (26,2°S-104,0°W). El muestreo se realizó a bordo del R/V Melville (Scripps Oceanographic Institution, Universidad de California San Diego) en noviembre-diciembre de 2010 durante el crucero BiG-RAPA 2010 (http://hahana.soest.hawaii.edu/cmorebigrapa/bigrapa.html).

3.1.2. Ecosistema de surgencia costera de Chile central (Fig. 6; 36.5°S). Una de las zonas más productivas del océano mundial (Montero et al. 2007), oxigenadas durante el otoño e invierno y subóxicas durante la primavera y el verano (Sobarzo et al. 2007). Se realizó un muestreo estacional entre los años 2011 y 2012 en la Estación Oceanográfica costera (Estación 18; 36,5°S-73,1°W) de la serie de tiempo del Centro de Investigación Oceanográfica del Pacífico Sur-Oriental (http://www.copas.udec.cl/eng/research/serie/), a bordo del R/V Kay-Kay II (Departamento de Oceanografía, Universidad de Concepción).

3.1.3. Océano costero frente a la Patagonia chilena (Fig. 6). El área costera del sur de Chile se caracteriza por un complejo sistema de fiordos y canales, particularmente vulnerables a la influencia humana y con glaciares en retirada que derraman agua dulce en el océano costero (Silva and Vargas 2014). Muestras de agua superficial del Canal Baker (47,9°S-73,9°W) se obtuvieron en marzo de 2011 a bordo de la embarcación “sur-austral” del Centro COPAS Sur-Austral de la Universidad de Concepción (www.sur-austral.udec.cl).

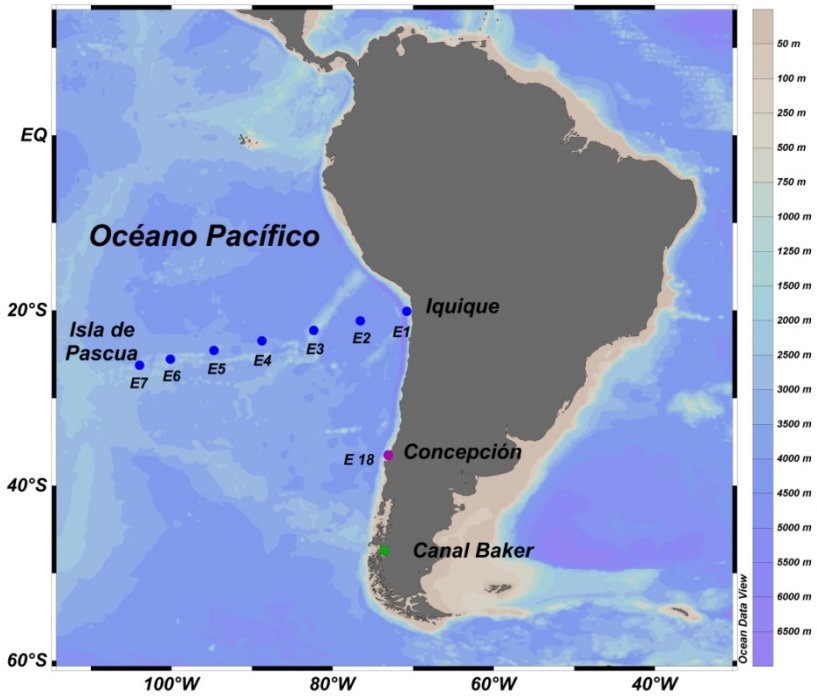

Figura 6.- Áreas de estudio en el Océano Pacífico Sur-Oriental.

3.2. Aislamiento y cultivo de hongos

Muestras de agua de mar (450 mL) y de sedimentos (3 g) fueron filtradas a través de filtros estériles (0,45 µm; MF-Millipore), los que se congelaron en nitrógeno líquido para su preservación. Posteriormente los filtros descongelados se montaron en placas Petri con medio sólido de cultivo Yeast Extract con 0,2 g/L cloranfenicol (Johnson and Sparrow 1961; Fuller and Poyton 1964; Kohlmeyer and Kohlmeyer 1979). Las placas se incubaron en oscuridad a 20 °C. Una vez que las colonias se desarrollaron completamente, estas se aislaron tras sucesivos subcultivos en medio sólido Emerson's YpSs agar (Kohlmeyer and Kohlmeyer 1979) bajo oscuridad a 4 °C. Filtros estériles fueron utilizados como controles negativos siguiendo el mismo protocolo.

3.3. Extracción, amplificación, secuenciación de ADN y análisis filogenético de la colección de cultivos

El ADN de las cepas aisladas fue extraído con el kit de aislamiento ADN Power Soil (MO BIO Laboratories US) y se utilizó como molde para amplificar el espaciador transcrito interno (ITS). Alícuotas de 1 µL de ADN molde se mezclaron con 24 µL de mezcla de PCR que contenía: 5 µL de 5x Colorless GoTaq Flexi Reaction Buffer (PROMEGA WI, USA), 1,5 µL de solución de $MgCl_2$ (PROMEGA WI, USA), 0,5 µL de un mix de desoxinucleótidos trifosfatos (50 µM de cada dNTPs en la concentración final), 0,125 µL de ADN polimerasa GoTaq (PROMEGA WI, USA), 1,25 µL (0,5 µM) del cebador fúngico específico ITS1-F (hacia adelante) 5'-CTTGGTCATTTAGAGGAAGTAA-3 'y de cebador universal ITS4 (reverso) 5' -TCCTCCGCTTATTGATATGC-3 ' (White et al. 1990; Gardes and Bruns 1993) y 14,375 µL de agua libre de nucleasas. Los ciclos de PCR se realizaron por duplicado en un termociclador TC-PRO (BOECO, Alemania) y consistieron en un ciclo inicial de 2 min a 95 °C, seguido de 35 ciclos de 30 s a 95 °C, 30 s a 55 °C y 1 min a 72 °C, con un paso final de 5 min a 72 °C. La mezcla de PCR sin ADN molde se usó como control negativo.

Los productos de PCR (ITS 1, 5,8 S y ITS 2), fueron enviados al servicio de Secuenciación de MACROGEN (http://dna.macrogen.com/eng). Géneros y especies coincidentes con las secuencias analizadas se obtuvieron mediante el alineamiento de estas con secuencias almacenadas en GenBank usando BLAST (Centro Nacional de Información Biotecnológica, NCBI). Las secuencias mayores de 600 pb con 99% de identidad de nucleótidos con secuencias de GenBank se consideraron representativas de la misma especie y las secuencias con 97% se consideraron del mismo género (Kurtzman and Robnett 1998; Landeweert et al. 2003; Redberg et al. 2003; Lyncht and Thorn 2006; Gao et al. 2008). Todas las secuencias mayores de 600 pb y sus secuencias coincidentes en GenBank se alinearon con ClustalW (Thompson et al. 1994). Grupos taxonómicos principales fueron identificadas computando un árbol filogenético (Neighbor Joining Tree) utilizando distancias genéticas por pares sobre la base de todas las sustituciones con el parámetro de distancia de Jukes-Cantor. La calidad de los patrones de ramificación se evaluó mediante bootstrap usando 1000 repeticiones. Los análisis se realizaron utilizando MEGA (Tamura et al. 2013).

Dado que la topología del árbol filogenético (NJ) exhibió bajos valores de soporte estadístico (bootstrap) para la mayoría de los clados identificados de *Penicillium* (inferior al 70%), un árbol filogenético que consideró relaciones evolutivas entre individuos se calculó utilizando análisis de inferencia Bayesiana y se implementó en MrBayes v.3.2.5 (Ronquist et al. 2012) utilizando un modelo GTR seleccionado en PhyML 3.0 (Guindon et al. 2010). Se realizó un árbol bayesiano durante 30.000.000 generaciones hasta que la desviación estándar de las frecuencias de división estuviese bajo 0,01. Se calcularon dos corridas paralelas con cuatro cadenas cada una, tomando muestras cada 200 generaciones, con diagnósticos calculados cada 1000 generaciones. Las probabilidades bayesianas se consideraron confiables cuando fueron superiores a 0,95 (Murphy et al. 2001; Wilcox et al. 2002; Alfaro and Holder 2006; Houbraken etl. 2011). El número de OTUs diferentes se estimó mediante la transferencia de matrices de distancia genética (Pairwise p-distance, Kimura 2-2, Parameter y Jukes-Cantor; MEGA) en la versión web de Automatic Barcode Gap Discovery (ABGD; http://wwwabi.snv.jussieu.fr /public/abgd/abgdweb.html; Puillandre et al. 2012) con valores intraespecíficos de divergencia por defecto como lo sugieren Andreakis et al. (2015). Las secuencias fueron subidas a GenBank con los números de acceso KY401054–KY401148 y MH231237–MH231248.

3.4. Viabilidad de cepas aisladas

Veintitrés cepas (Tabla 1) pertenecientes a los órdenes Eurotiales, Dothideales e Hypocreales, aisladas desde el océano Pacífico Sur-Oriental fueron seleccionados para evaluar su capacidad de viabilidad (germinación, crecimiento de micelio y esporulación) en agua de mar, y si la disponibilidad de sustrato orgánico afecta el desarrollo de hongos. Cuatro medios fueron utilizados: agua de mar y agua dulce para determinar viabilidad, y agua de mar y agua dulce con glucosa y Yeast extract (Sigma-Aldrich) para determinar el efecto de la disponibilidad de nutrientes. Se tomaron submuestras de cada colonia (23) con un asa de inoculación y se transfirieron a tubos con 1,5 mL de agua de mar estéril y filtrada (0,2 μm). Los tubos se agitaron fuertemente y luego fueron centrifugados durante 5 segundos a 10 rpm.

El sobrenadante (1,5 mL) se inoculo en tubos con 15 mL de: 1) agua de mar filtrada (0,2 μm) y estéril, 2) agua dulce estéril, 3) agua de mar filtrada (0,2 μm) y estéril con glucosa (1g/L) y Yeast extract (0,1g/L) y 4) agua dulce estéril con glucosa (1g/L) y extracto de levadura granulado (0,1g/L). Los tubos de incubación se mantuvieron a 10 °C durante 15 días bajo agitación suave (80 rpm). La viabilidad, se controló diariamente mediante la observación de 50 μL de suspensión de esporas bajo microscopía de contraste de fase (Axioskop 2 Plus, Zeiss). Se considera como espora germinada cuando la longitud del tubo germinal es mayor al diámetro de esporas (Bosch et al. 1995). Micrografías confocales de la cepa 24 fueron tomadas usando un microscopio confocal Zeiss LSM 780-NLO (CMA, www.cmabiobio.cl).

3.5. Contenido energético y porcentaje de carbono orgánico y nitrógeno

Calorías de 12 cepas fueron medidas usando un micro-calorímetro de bomba de Oxígeno con la metodología estándar del equipo (Parr Instrument Company; Modelo 6725). Se utilizó como control positivo una muestra liofilizada (12 h) y seca (48 h) de un hongo comestible (*Agaricus bisporus*) y de zooplancton (*Euphausia pacifica*).

Porcentaje de carbono orgánico (% C) y nitrógeno total (% N) de 12 cepas fueron determinados en la Universidad de California Davis (Stable Isotope Facility) mediante un analizador elemental.

3.6. Aminoácidos hidrolizables totales (THAA)

Aminoácidos totales hidrolizables (proteínas) fueron determinados mediante cromatografía líquida de alta presión (HPLC; Lindroth and Mopper 1979; Jones et al. 1981; Pantoja and Lee 1999). Muestras liofilizadas de hongos (0,1 g) se disolvieron con 2 mL de solución de hidrólisis (HCl 7 N, 1% fenol, ácido trifluoroacético al 10%) y purgadas durante 1 minuto bajo corriente de nitrógeno puro. Las muestras fueron hidrolizadas a 150 °C durante 1,5 h y neutralizadas con NaOH (12 M) hasta alcanzar un pH de 6,5-7,5. Finalmente, las

soluciones se derivatizaron con una mezcla de ortoftaldiaaldehído y mercaptoetanol, y se disolvieron en 50 μL de metanol. El análisis de aminoácidos fue realizado en un cromatógrafo Shimadzu (LC-10ATVP) equipado con un detector de fluorescencia (RF-10AXL), muestreador automático (SIL-10ADVP) y una bomba binaria (LC-10ATVP). La separación de cada amino ácido se hizo usando una columna Kromasil 100-5C18 (4,6 x 250 mm) a 40 °C y un flujo de 1 mL/min. El programa de elución fue: 5 min 100% de eluyente A (acetato de sodio 25 Mm, tetrahidrofurano al 5%, pH 5,7), seguido de un gradiente de eluyente B (metanol); 25% -0,01 min; 30% - 35 min; 50% - 42 min; 60% - 60 min; 100% - 72 min; 100% - 80 min; 25% - 84 min; 25% - 87 min, y luego se mantuvo al 100% de eluyente B durante 5 min. La columna se volvió a equilibrar con 100% del eluyente B a 1 mL/min durante 5 minutos entre las inyecciones. La identificación y cuantificación de aminoácidos totales hidrolizables se logró mediante la inyección conjunta de muestras con un estándar Pierce N ° 20088 THAA 2,5 μM.

3.7. Lípidos totales

Lípidos de 12 cepas de hongos (1g) fueron extraídos tres veces consecutivas (Bligh y Dyer 1959 con modificaciones) con 25 mL de diclorometano/metanol (3:1, v/v), ultrasonido (30 min) y centrifugación (3500 rpm; 5 min). La fracción orgánica se recuperó y se secó por evaporación automática (bajo una corriente de nitrógeno) almacenándose a -20 °C. Los extractos totales (hidratados con 5 mL de metanol) fueron separados para análisis de ésteres metílicos de ácidos grasos (FAMEs) y esteroles.

3.7.1. Ésteres metílicos de ácidos grasos (FAMEs)

Para el análisis de FAMEs, el 50% del extracto de lípidos de cada cepa se saponificó con 15 mL de una solución de hidróxido de potasio y metanol (0,5 N) durante 2 h 80 °C. La fracción orgánica se recuperó sucesivamente tres veces mediante agitación (10 min sonicación) con hexano (20 mL), y se hidrolizó con ácido clorhídrico (3 mL, 6 N). Finalmente, la fracción orgánica se recuperó nuevamente (x3) con hexano y se concentró por evaporación automática. Los ácidos grasos se metilaron con una solución de trifluoruro de boro-metanol (1 mL) y se calentaron a 70 °C por 1 h, se agitaron con ultrasonido por 5 min con agua destilada (1 mL) y hexano (5 mL), y secaron bajo una corriente de nitrógeno puro. El extracto se disolvió en hexano (1 mL) y se inyectó (0,2 µL) en un cromatógrafo de gases 6890 equipado con un espectrómetro de masas 5973 (GC-MS, Agilent Technologies) y columna capilar de sílice HP-5MS (30 m 0,25 mm), utilizando helio como gas portador. Las muestras se inyectaron bajo las siguientes condiciones: 120 °C durante 2 min, y luego se elevó la temperatura a 4 °C/min hasta 290 °C, donde se mantuvo durante 5 min hasta que todos los ácidos grasos de interés eluyeron. La temperatura del inyector fue de 250 °C. FAMEs se identificaron comparando sus tiempos de retención con estándares validados (Sigma-Aldrich Co., EE.UU.) y se cuantificaron usando una curva de calibración de ácido nonadecanoico.

3.7.2. Esteroles

Una segunda alícuota de lípidos se disolvió con diclorometano (500 µL) y se secó durante toda una noche en una pipeta Pasteur rellena con fibra de vidrio y silica gel (Merck; malla 600). Las muestras se fraccionaron en cuatro partes mediante elución. Fracción 1) 450 µL de hexano, Fracción 2) diclorometano (2,4 mL), Fracción 3) mezcla de diclorometano/acetato de etilo (2,4 mL; 1:1 v/v), Fracción 4) mezcla de diclorometano/metanol (2,4 mL; 7:3 v/v). La tercera fracción se derivatizó con N, O-Bis (trimetilsilil) trifluoroacetamida (45 µL), piridina (50 µL) y trimetilclorosilano (5 µL) a 70 °C durante 45 min, y se secó bajo una corriente de nitrógeno puro. Las muestras se disolvieron en

hexano (200 μL) y se inyectaron (0,2 μL) en un cromatógrafo de gases (el mismo equipo utilizado para FAMEs) bajo las siguientes condiciones: 60 ºC durante 1 min, luego se elevó la temperatura a 290 ºC a 10 ºC/min y se mantuvo durante 10 min, y aumentó a 300 ºC.

3.8. Lípidos polares intactos

Muestras de 12 cepas de hongos cultivadas y liofilizados fueron combinados con un estándar interno (Lyso C16-PAF) para su extracción con solventes orgánicos siguiendo el método de Bligh y Dyer (1959) modificado (Sturt et al. 2004). Tres extracciones fueron realizadas con una mezcla de MeOH/DCM/búfer fosfato (2:1:0,8) y una cuarta y última extracción con una mezcla de MeOH/DCM/TCA (2:1:0,8). En cada extracción, las muestras fueron sometidas a un baño ultrasónico durante 15 min y centrifugación (3500 rpm; 5 min). Finalmente, los extractos lipídicos se purificaron con DCM más agua Milli-Q. y se evaporaron bajo una corriente de nitrógeno, para ser almacenados a -20 ºC. El análisis de lípidos polares intactos (IPL) se realizó usando un sistema de cromatografía líquida de ultra alta presión (UHPLC; Dionex Ultimate 3000; Thermo Scientific, Dreieich, Alemania) acoplado a un espectrómetro de masas (qTOF) en tándem (Bruker, Bremen, Alemania) a través de una interfaz ESI (RP-LC/ESI/MSqTOF; Wörmer et al. 2013) y columna Acquity UPLC BEH C18 RP (1,7 μm, 2,1 x 150 mm, Waters Corporation, Eschborn, Alemania).

Cada extracto se disolvió en DCM/MeOH (1:9, v/v). Los eluyentes fueron: A MeOH:agua (85:15; v/v) y el eluyente B: alcohol isopropílico/MeOH (50:50; v/v), ambos con adición de HCOOH (0,04%) y de NH_3 (0,1%). El gradiente para las fases móviles se inició con 100% del solvente A durante 2 minutos y luego 85% del solvente B durante 18 minutos, para finalizar con 100% del solvente B durante 8 min y equilibrar durante 6 min. La tasa de flujo del solvente fue de 0,4 mL min^{-1}. La detección se realizó mediante ionización por electrospray (ESI). Las condiciones para la detección mediante espectrometría de masa (MS) fueron las siguientes: tasa de flujo del gas de secado del ESI, 4 L min^{-1}; temperatura del gas de secado, 200 °C; voltaje del capilar, 4500 V; ionización positiva; presión del nebulizador, 0,7

bar (ver detalles en Wörmer et al. 2013). Los espectros de masa fueron obtenidos mediante el modo auto MS-MS. Los instrumentos fueron un sistema UHPLC Dionex Ultimate 3000 acoplado a un espectrómetro de masas de tiempo de vuelo cuadrupolo Bruker maXis de resolución ultra alta (MSqTOF).

4.- RESULTADOS

4.1. Capítulo 1: Diversidad de Ascomycetes filamentosos cultivables en el Océano Pacífico Sur-Oriental de Chile

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Resumen: Este estudio informa la diversidad del mycoplancton cultivable del Océano Pacífico Sur-Oriental de Chile, contribuyendo con conocimientos nuevos sobre la taxonomía de hongos filamentosos aislados desde ambientes marinos fisicoquímicos y biológicamente diversos. Caracterizamos espacialmente la distribución de las cepas aisladas y se evaluó su viabilidad e influencia de la disponibilidad de sustrato orgánico en su desarrollo. Treinta y nueve unidades taxonómicamente operacionales fueron identificadas a partir de 99 cepas de hongos aisladas desde aguas costeras y oceánicas mediante el uso automático de Automatic Barcode Gap Discovery. Todas las Unidades taxonómicamente operacionales pertenecieron al phylum Ascomycota de los órdenes Eurotiales, Dothideales, Sordariales e Hypocreales, principalmente a *Penicillium* sp. (82%); 11 secuencias no fueron coincidentes con especies existentes en GenBank, sugiriendo que se trata de nuevos taxones de hongos. Nuestros resultados sugieren que las comunidades de hongos en el Océano Pacífico Sur-Oriental frente a Chile parecen prosperar en una amplia gama de condiciones ambientales del océano y que la disponibilidad del sustrato orgánico es un factor que influye en la viabilidad de los hongos en el océano.

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ORIGINAL PAPER

Diversity of culturable filamentous Ascomycetes in the eastern South Pacific Ocean off Chile

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Abstract Our study reports the diversity of culturable mycoplankton in the eastern South Pacific Ocean off Chile to contribute with novel knowledge on taxonomy of filamentous fungi isolated from distinct physicochemical and biological marine environments. We characterized spatial distribution of isolates, evaluated their viability and assessed the influence of organic substrate availability on fungal development. Thirty-nine Operational Taxonomic Units were identified from 99 fungal strains isolated from coastal and oceanic waters by using Automatic Barcode Gap Discovery. All Operational Taxonomic Units belonged to phylum Ascomycota and orders Eurotiales, Dothideales, Sordariales and Hypocreales, mainly *Penicillium* sp. (82%); 11 sequences did not match existing species in GenBank, suggesting occurrence of novel fungal taxa. Our results suggest that fungal communities in the South Pacific Ocean off Chile appear to thrive in a wide range of environmental conditions in the ocean and that substrate availability may be a factor influencing fungal viability in the ocean.

Keywords Ascomycota · Eastern South Pacific Ocean · Marine fungi · Mycoplankton · Penicillium

Introduction

Marine fungi have been documented in diverse habitats, such as marine sponges (Gao et al. 2008; Wang et al. 2008), oxygen-depleted waters (Jebaraj and Raghukumar 2009; Jebaraj et al. 2010), the deep-sea marine sediments of western Pacific Ocean (Lai et al. 2007; Nagahama et al. 2003; Takami et al. 1997; Takishita et al. 2006), deep-sea subsurface sediments of the eastern South Pacific Ocean (Edgcomb et al. 2011), hydrothermal vents (Bass et al. 2007), in the coastal upwelling ecosystem off central Chile (Gutiérrez et al. 2010, 2011, 2016) and in glacial coastal waters in Chilean Patagonia (Gutiérrez et al. 2015). Despite its putative ecological significance, mycoplankton (Gutiérrez et al. 2011; Wang et al. 2012) has not been fully considered as an active microbial component of marine ecosystems. Scientific understanding of the ecologic role (Jones 2011), diversity and distribution of marine filamentous fungi in the eastern South Pacific Ocean is still at early stages). Here, we contribute to knowledge of diversity of culturable fungi from distinct physicochemical environments in the eastern South Pacific Ocean off Chile, using the study of internal transcriber spacer (ITS) regions of fungal isolates from the oligotrophic ocean in the Pacific gyre off Easter Island (ca. 27°S), the coastal ocean off northern Chile (ca. 20°S) with a well developed Oxygen Minimum Zone, and the upwelling coastal ecosystem off central Chile (ca. 36°S) influenced by rivers and seasonal water column suboxia. Our results provide novel knowledge on the taxonomic diversity of culturable fungi in the ocean in contrasting physicochemical and trophic marine environments.

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Materials and methods

Study areas (Fig. 1) and sampling

1. Coastal–Open Ocean gradient off northern Chile (20.1°S 70.8°W) to Easter Island (26.2°S 104.0°W). This area encompasses high productivity coastal waters (Montecino et al. 2006), with a shallow Oxygen Minimum Zone near the coast of mainland Chile (Pantoja et al. 2004), and oligotrophic oceanic waters near Easter Island (Pennington et al. 2006). Sampling was conducted on board R/V Melville (Scripps Oceanographic Institution, University of California San Diego) in November–December 2010 during cruise BiG-RAPA 2010 (*Biogeochemical Gradients-Role in Arranging Planktonic Assemblages*; http://hahana.soest.hawaii.edu/cmorebigrapa/bigrapa.html).
2. Coastal upwelling ecosystem off central Chile (36.5°S). These waters are among the most productive in the world ocean (Montero et al. 2007), and are oxygenated during autumn and winter and suboxic during spring and summer due to circulation and high primary production (Sobarzo et al. 2007). Seasonal sampling was conducted during May 2011 (austral fall), July 2011(austral winter), September 2011 (austral spring) and January 2012 (austral summer) at the Coastal Time Series Oceanographic Station (Station 18 at 36.5°S, 73.1°W) of the Center for Oceanographic Research in the eastern South Pacific (http://www.copas.udec.cl/eng/research/serie/), aboard R/V Kay–Kay II (Department of Oceanography, University of Concepción).

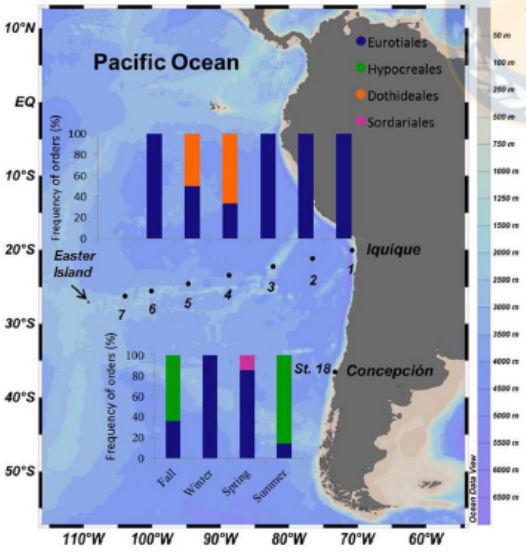

Fig. 1 Study areas and sampling sites and relative abundance of orders of Ascomycetes assigned isolated from northern Chile to Easter Island (November–December 2010) and the coastal upwelling ecosystem off central Chile (seasonal sampling 2011–2012). Ocean Data View (Schlitzer 2015)

Water samples were collected using Niskin bottles on a rosette, from surface to 1500 m depth off northern Chile to Easter Island, and from surface to 80 m depth in the coastal upwelling ecosystem off central Chile. Water samples were stored in sterile containers at in situ temperature and protected from direct sunlight until processing in the laboratory. Vertical profiles of water temperature, salinity, concentrations of chlorophyll-*a* and oxygen were determined using a CTDOF profiler (Sea-Bird).

Isolation and culture of filamentous fungi strains

Water samples (450 mL) were filtered through 0.45 µm sterile membranes filter MF-Millipore and frozen in liquid nitrogen. Filters were placed on Petri dishes with sterile solid medium of glucose-yeast extract agar with chloramphenicol (Johnson and Sparrow 1961; Kohlmeyer and Kohlmeyer 1979) that favors growth of filamentous marine fungi (Fuller and Poyton 1964). Petri dishes were incubated in darkness at 20 °C, and colonies were observed from the third day onwards. Once colonies were fully developed (about 1 week), they were isolated after successive subcultures on sterile solid medium Emerson´s YpSs agar (Kohlmeyer and Kohlmeyer 1979), and maintained in darkness at 4 °C. Sterile filters on Petri dishes with solid media were used as negative controls.

DNA extraction and amplification

DNA of cultured strains was extracted using Power Soil DNA Isolation Kit (MO BIO Laboratories US) and used as a template to amplify the internal transcriber spacer of the most sequenced region for fungi, accepted as the universal fungal DNA barcode (Schoch et al. 2012) and more unambiguous than morphology or extrolite data (Visagie et al. 2014). Aliquots of 1 µL of template DNA were combined with 24 µL PCR mix containing: 5 µL of 5× Colorless GoTaq Flexi Reaction Buffer (PROMEGA, WI USA), 1.5 µL $MgCl_2$ solution (PROMEGA WI, USA), 0.5 µL deoxynucleotide triphosphates mix (50 µM each dNTPs in the final concentration), 0.125 µL GoTaq DNA polymerase (PROMEGA WI, USA), 1.25 µL (0.5 µM) of specific fungal primer ITS1-F forward 5′-CTTGGTCATTTAGAGGAA GTAA-3′ and of universal primer ITS4 reverse 5′-TCCTCC GCTTATTGATATGC-3′ (Gardes and Bruns 1993; White et al. 1990), and 14. µL of nuclease-free water. PCR cycles

were run in duplicate on a thermal cycler TC-PRO (BOECO, Germany) and consisted of an initial 2 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, with a final step of 5 min at 72 °C to complete any partial polymerizations. PCR mix without template DNA was used as a negative control. PCR products stained with GelRed (Biotium Inc.) were run on 1.2% agarose gel and visualized under UV trans-illumination.

Sequence and phylogenetic analysis of the culture collection

PCR products including ITS 1, 5.8 S and ITS 2 regions, were sequenced at the Automated DNA Sequencing Service of MACROGEN (http://dna.macrogen.com/eng). Genera and species were identified by matching sequences with those stored in GenBank, based on previous definitions of ITS rDNA in fungi in BLAST (National Center for Biotechnology Information, NCBI). All ITS rDNA sequences larger than 600pb and their matched sequences from GenBank were aligned with ClustalW (Thompson et al. 1994). Sequences larger than 600 pb (95) with 99% nucleotide identity with sequences from GenBank were considered representative of same species and sequences with 97% were considered of same genus (Gao et al. 2008; Kurtzman and Robnett 1998; Landeweert et al. 2003; Lyncht and Thorn 2006; Redberg et al. 2003). Ninety-five species and major taxonomic groups were identified computing a Neighbor Joining Tree estimated using pairwise genetic distances on the basis of all substitutions with Jukes–Cantor distance parameter. Quality of branching patterns for neighbor joining was assessed by bootstrap using 1000 replicates. Analyses were conducted using MEGA (Tamura et al. 2013).

Since the topology of neighbor joining (NJ) tree exhibited low bootstrap support for most identified clades of *Penicillium* (lower than 70%), a second phylogenetic tree that considered an evolutionary relationship among individuals was computed using Bayesian inference analysis and was implemented in MrBayes v.3.2.5 (Ronquist et al. 2012) using a GTR model selected in PhyML 3.0 (Guindon et al. 2010). A Bayesian phylogenetic tree was conducted for 30,000,000 generations until the standard deviation of split frequencies was under 0.01. Two parallel runs were computed with four chains each, sampling every 200 generations, with diagnostics calculated every 1000 generations. Bayesian probabilities were considered reliable when higher than 0.95 (Alfaro and Holder 2006; Houbraken and Samson 2011; Murphy et al. 2001; Wilcox et al. 2002). OTUs were estimated by uploading genetic distance matrixes (Pairwise p-distance, Kimura 2–2, Parameter and Jukes–Cantor) obtained in MEGA into web version of Automatic Barcode Gap Discovery (ABGD; http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html; Puillandre et al. 2012) with divergence intraspecific values by default as suggested by Andreakis et al. (2015). ABGD prior intraspecific divergence values were set at $P = 1.67 \times e^{-0.3}$ and $P = 1.00 \times e^{-0.3}$ since both revealed 39 species, whereas the other possible value ($P = 2.78 \times e^{-0.3}$) generated artificial subdivision of species (Puillandre et al. 2012). ABGD identified 39 species, whereas with Neighbor joining and Bayesian inference only six species were distinguished (Table 1a, b). Sequences were uploaded to GenBank with Accession Number KY401054–KY401148.

Viability of isolated strains

Twenty-three strains belonging to orders Eurotiales, Dothideales and Hypocreales, isolated from the coastal ocean off northern Chile to Easter Island (strains 1, 2, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15; Table 1) and from the coastal upwelling ecosystem off central Chile (strains 16, 17, 18, 19, 20, 21, 22, 23, 24, 25; Table 1) were selected for testing germination ability. We determined (a) viability of marine fungi, i.e. whether germination, growth of new mycelium and sporulation of conidia occur exclusively in marine environments or are facultative marine fungi from freshwater or terrestrial origins that are able to grow in marine waters (Kohlmeyer and Kohlmeyer 1979), and (b) whether organic substrate availability affects fungal development. We used four different media (seawater and freshwater for determining viability of marine fungi), and (seawater and freshwater with glucose and yeast extract for determining effect of organic substrates availability). Subsamples of 100 µL of spores (conidia) were collected with an inoculating loop and transferred to tubes with 1.5 mL sterile and filtered (0.2 µm) seawater. Spores were then placed into tubes with 15 mL each of: (1) filtered sea water (0.2 µm), (2) sterile freshwater, (3) sterile and filtered sea water (0.2 µm) with glucose (1 g/L) and yeast extract granulated (0.1 g/L) and (4) sterile freshwater with glucose (1 g/L) and yeast extract granulated (0.1 g/L). Incubation tubes were maintained at 10 °C for 15 days under gentle agitation.

Viability, defined as germination, growth of new mycelium and sporulation of conidia, was monitored daily by observing 50 µL spore suspension under phase contrast microscopy (Axioskop 2 Plus, Zeiss). Confocal micrographs of strain 24 (Fig. 4) were acquired using a Zeiss LSM 780-NLO spectral confocal microscope (CMA; http://www.cmabiobio.cl). A conidia was considered germinated when germ tube length was ≥half spore diameter (Bosch et al. 1995).

Table 1 Information on isolated Ascomycetes from the eastern South Pacific Ocean, its taxonomic identification supported by Neighbor joining and Bayesian trees, and OTU number assigned with ABGD. **a** Northern Chile and **b** coastal upwelling ecosystem off central Chile

Area	Environment/season	Site	Strain	Depth (m)	Order	Specie	OTU
(a) Northern Chile	Coastal/spring	1	26	5	Eurotiales	*Penicillium* sp.	VIII
	Coastal/spring	1	27	5	Eurotiales	*P. brevicompactum*	IX
	Coastal/spring	1	28	30	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	36	40	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	1	40	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	2	60	Eurotiales	*P. brevicompactum*	XI
	Coastal/spring	1	29	70	Eurotiales	*Penicillium* sp.	II
	Coastal/spring	1	30	70	Eurotiales	*P. brevicompactum*	IX
	Coastal/spring	1	31	70	Eurotiales	*Penicillium* sp.	XXXV
	Coastal/spring	1	32	200	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	33	400	Eurotiales	*Penicillium* sp.	VII
	Coastal/spring	1	34	600	Eurotiales	*Penicillium* sp.	III
	Coastal/spring	1	35	600	Eurotiales	*Penicillium* sp.	XXXVI
	Coastal/spring	1	37	700	Eurotiales	*Penicillium* sp.	VIII
	Coastal/spring	1	38	800	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	39	800	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	40	900	Eurotiales	*Penicillium* sp.	III
	Coastal/spring	1	41	900	Eurotiales	*Penicillium* sp.	XII
	Coastal/spring	1	42	900	Eurotiales	*Penicillium sp*	I
	Coastal/spring	1	43	1000	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	44	1000	Eurotiales	*Penicillium* sp.	I
	Coastal/spring	1	45	1000	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	2	46	10	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	2	47	20	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	2	4	24	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	2	48	50	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	2	51	65	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	2	49	100	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	2	50	120	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	2	52	596	Eurotiales	*Penicillium* sp.	XXXVII
	Oceanic/spring	2	5	596	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	2	53	700	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	2	54	794	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	2	55	900	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	2	56	1515	Eurotiales	*Penicillium* sp.	XXXVIII
	Oceanic/spring	3	57	5	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	6	12	Eurotiales	*Penicillium* sp.	XIII
	Oceanic/spring	3	58	12	Eurotiales	*Penicillium* sp.	XIV
	Oceanic/spring	3	59	12	Eurotiales	*Penicillium* sp.	VI
	Oceanic/spring	3	60	12	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	61	23	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	62	23	Eurotiales	*Penicillium* sp.	XV
	Oceanic/spring	3	63	60	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	64	75	Eurotiales	*Penicillium* sp.	XVI
	Oceanic/spring	3	65	120	Eurotiales	*Penicillium* sp.	XXXIX
	Oceanic/spring	3	66	120	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	76	300	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	67	500	Eurotiales	*P. brevicompactum*	IX
	Oceanic/spring	3	68	500	Eurotiales	*Penicillium* sp.	I

Table 1 (continued)

Area	Environment/season	Site	Strain	Depth (m)	Order	Specie	OTU
	Oceanic/spring	3	69	600	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	70	700	Eurotiales	*Penicillium* sp.	XVII
	Oceanic/spring	3	71	800	Eurotiales	*Penicillium* sp.	VIII
	Oceanic/spring	3	7	800	Eurotiales	*Penicillium* sp.	XVIII
	Oceanic/spring	3	72	900	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	73	900	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	74	1000	Eurotiales	*Penicillium* sp.	I
	Oceanic/spring	3	75	1000	Eurotiales	*Penicillium* sp.	XIX
	Oceanic/spring	3	8	1012	Eurotiales	*Penicillium* sp.	VI
	Oceanic/spring	4	9	5	Dothideales	Order Dothideales	XX
	Oceanic/spring	4	10	5	Dothideales	Order Dothideales	XXI
	Oceanic/spring	4	11	1515	Eurotiales	*Penicillium* sp.	XXII
	Oceanic/spring	5	13	700	Eurotiales	*Penicillium* sp.	XXXIV
	Oceanic/spring	5	12	800	Dothideales	Order Dothideales	XXIII
	Oceanic/spring	6	15	500	Eurotiales	*Penicillium* sp.	III
	Oceanic/spring	6	14	1000	Eurotiales	*Penicillium* sp.	II
(b) Coastal upwelling ecosystem off central Chile	Coastal/fall	18	16	10	Eurotiales	*Penicillium* sp.	II
	Coastal/fall	18	77	10	Hypocreales	Order Hypocreales	V
	Coastal/fall	18	78	10	Hypocreales	Order Hypocreales	V
	Coastal/fall	18	79	20	Eurotiales	*Penicillium* sp.	I
	Coastal/fall	18	80	20	Hypocreales	Order Hypocreales	V
	Coastal/fall	18	17	30	Eurotiales	*Penicillium* sp.	II
	Coastal/fall	18	81	30	Hypocreales	Order Hypocreales	IV
	Coastal/fall	18	18	50	Hypocreales	Order Hypocreales	IV
	Coastal/fall	18	82	50	Eurotiales	*P. brevicompactum*	XXIV
	Coastal/fall	18	83	65	Hypocreales	Order Hypocreales	V
	Coastal/fall	18	84	80	Hypocreales	*Sarocladium strictum*	XXV
	Coastal/winter	18	85	1	Eurotiales	*Penicillium* sp.	X
	Coastal/winter	18	19	1	Eurotiales	*Penicillium* sp.	VII
	Coastal/winter	18	86	10	Eurotiales	*Penicillium* sp.	I
	Coastal/winter	18	87	20	Eurotiales	*Penicillium* sp.	X
	Coastal/winter	18	20	65	Eurotiales	*Penicillium* sp.	XXVI
	Coastal/spring	18	90	7	Sordariales	*Chaetomium* sp.	XXVII
	Coastal/spring	18	21	20	Eurotiales	*Pencillium citreonigrum*	XXVIII
	Coastal/spring	18	91	30	Eurotiales	*Penicillium* sp.	XXIX
	Coastal/spring	18	92	50	Eurotiales	*Penicillium roseopurpureum*	XXX
	Coastal/spring	18	22	65	Eurotiales	*Penicillium* sp.	XXXI
	Coastal/spring	18	23	80	Eurotiales	*Penicillium* sp.	XXXII
	Coastal/spring	18	93	80	Eurotiales	*P. brevicompactum*	IX
	Coastal/summer	18	24	1	Hypocreales	Order Hypocreales	V
	Coastal/summer	18	94	10	Hypocreales	Order Hypocreales	IV
	Coastal/summer	18	25	20	Hypocreales	Order Hypocreales	XXXIII
	Coastal/summer	18	95	30	Hypocreales	Order Hypocreales	IV
	Coastal/summer	18	96	50	Hypocreales	Order Hypocreales	V
	Coastal/summer	18	97	65	Hypocreales	Order Hypocreales	V
	Coastal/summer	18	99	80	Eurotiales	*Penicillium* sp.	VIII

Results

Isolated strains of fungi

Ninety-nine fungal strains were isolated from the water column between the coastal area of northern Chile and Easter Island (66 strains) and the coastal upwelling ecosystem off central Chile (33). Number of isolated strains from northern Chilean waters decreases significantly (Kruskal–Wallis; $p<0.05$) from continental shelf waters (23 ± 0.9) towards the subtropical gyre (0), whereas in the coastal upwelling ecosystem off central Chile (Station 18), no significant differences in number of isolated strains (8.2 ± 1.9) were observed throughout the year (Kruskal–Wallis; $p<0.05$).

Phylogenetic analysis and species identification of culture collection

The topology of the exploratory Neighbor Joining (NJ) tree revealed that all analyzed strains (n = 95) belonged to phylum Ascomycota of orders Eurotiales, Dothideales, Sordariales and Hypocreales (Fig. 2), although 11 sequences could not be assigned to any species in GenBank (<99% identity). Since some bootstrap values were lower than 70%, thus providing insufficient support to identify species (Fig. 2), a Bayesian tree was used to describe phylogenetic relations at the species level (Fig. 3a–c).

Most strains (82% n = 78) were identified as genus *Penicillium* (Fig. 3a, b) from sections Canescentia, Exilicaulis, Citrina, Fasciculata, Penicillium, Chrysogena and Brevicompacta (Houbraken and Samson 2011). Sequences of *P. brevicompactum* (Section Brevicompacta; Fig. 3b), one of the most xerophilic *Penicillium* (Pitt and Hocking 2009) often isolated from wood (Seifert and Frisvad 2000) and indoor air (Frisvad and Gravesen 1994) showed a strong relationship (probability 1.00) with strains 2, 4, 27, 30, 46, 47, 48, 49, 51, 67, 82 and 93. These strains, sorted into OTU IX using ABGD (Table 1), were isolated from suboxic waters (<22 µM) in the coastal ocean off northern and central Chile during austral spring and represented 12% of isolates, suggesting that a fraction of fungi may survive under low available O_2. Strain 21 was most likely related (probability 1.00) to *P. citreonigrum* of section Exicaulis isolated from ants (Rodrigues et al. 2011). Strain 92 was related to *Penicillium sanguifluum* (synonym of *P. roseopurpureum*) of section Citrina (probability 1.00, Fig. 3a) isolated from soil at Costa del Sol in Spain (Houbraken et al. 2011). Bayesian probabilities were high (>0.95) for sections Canescentia, Fasciculata, Penicillium and Chrysogena, but since several species were closely related, strains were identified as *Penicillium* sp..

Orders Dothideales, Sordariales and Hypocreales (Fig. 3c) were highly supported (probability 1.00), with strains 9, 10 and 12 positioned in a highly supported clade (probability 1.00) including *Aureobasidium pullulans, Yarrowia lipolytica* and *Discorea polystachya* from order

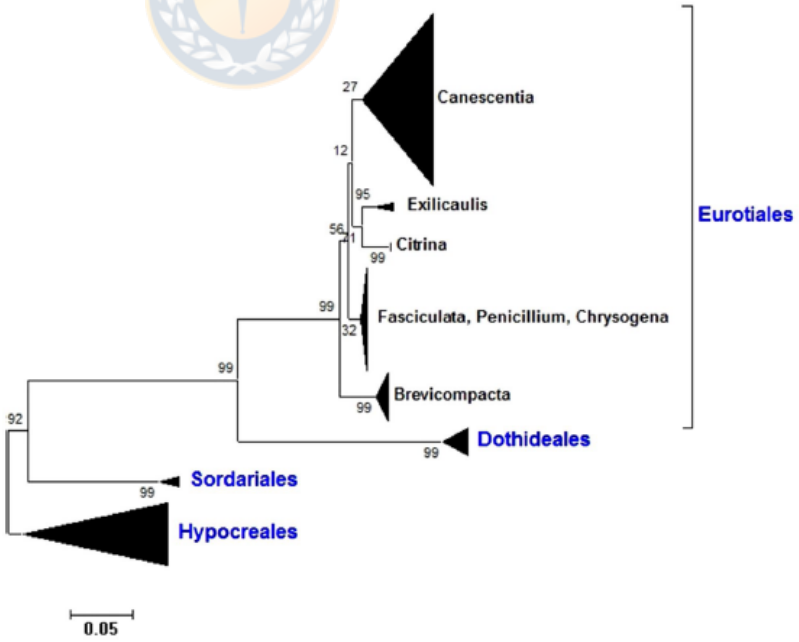

Fig. 2 Neighbor Joining tree of orders Eurotiales, Dothideales, Sordariales and Hypocreales collected from northern Chile to Easter Island and the coastal upwelling ecosystem off central Chile

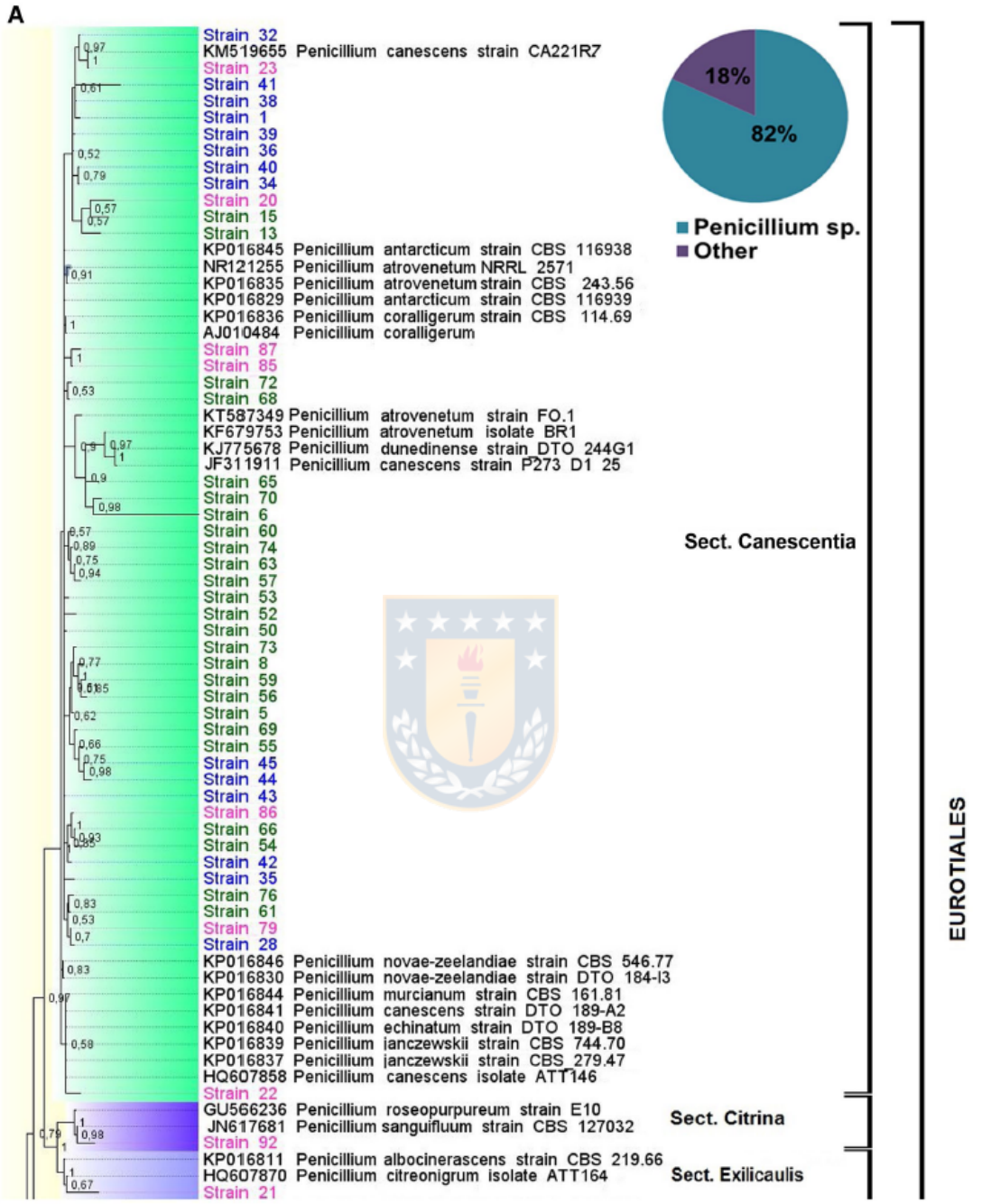

Fig. 3 Bayesian tree inferred from ITS sequences of fungal sequences collected from northern Chile to Easter Island and the coastal upwelling ecosystem off central Chile and proportion of *Penicillium* spp. The *green area* shows strains isolated from open ocean waters, *blue* from coastal waters off northern Chile, and *purple* from the coastal upwelling ecosystem off central Chile

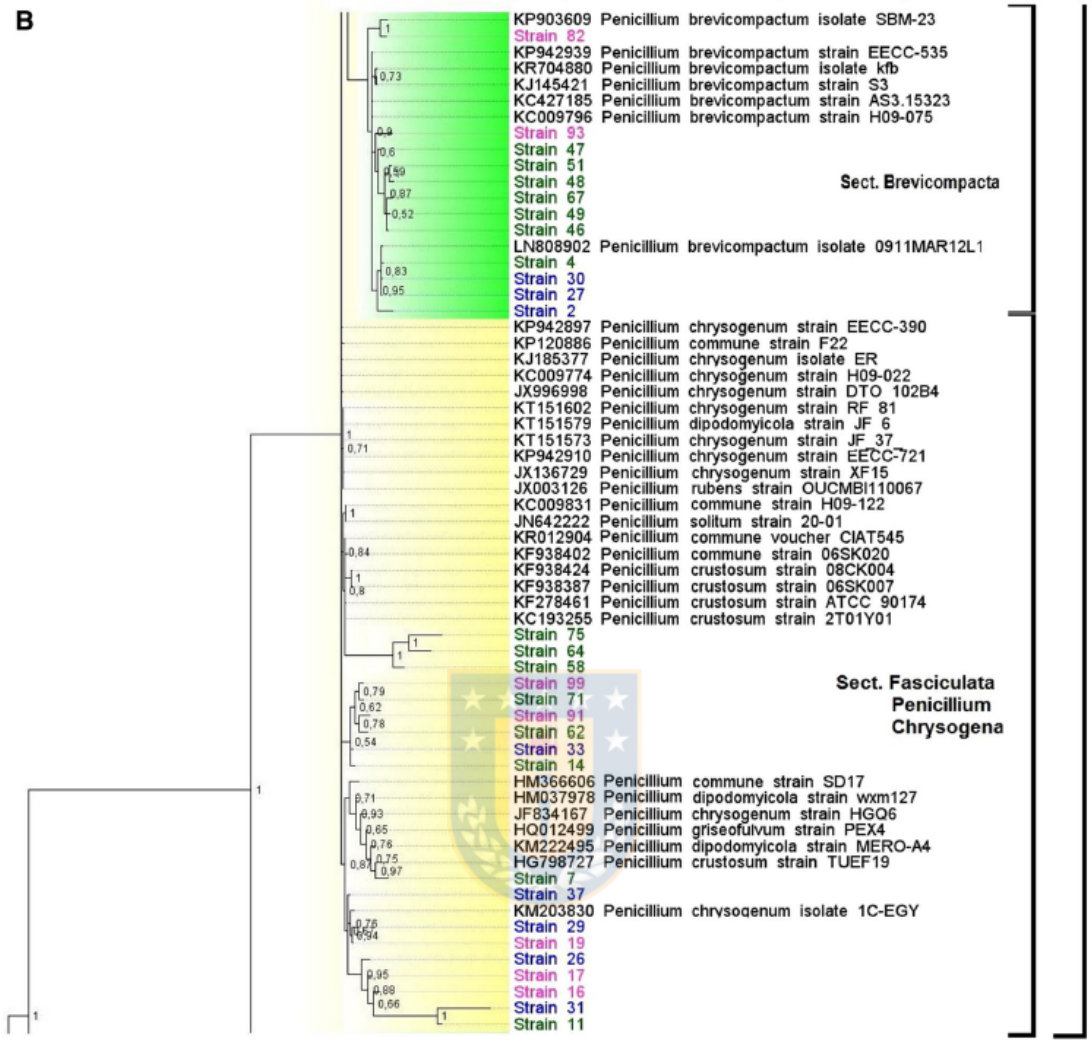

Fig. 3 (continued)

Dothideales (Fig. 3c). These three strains (9, 10 and 12) were isolated from shallow (5 m) and deep waters (800 m) of open ocean at sampling sites 4 and 5 (Fig. 1; Table 1). *A. pullulans* (Dothideales Order) was previously detected by Gao et al. (2010) in Pacific Ocean waters down to 200 m depth and it is considered a human and animal pathogenic fungi (Irinyi et al. 2015).

Strain 90 (Fig. 3c) was isolated from 7 m depth in the coastal upwelling ecosystem off central Chile and occurred in a highly supported clade (probability 1.00) including *Chaetomium* sp. (Order Sordariales). *Chaetomium* has been shown to denitrify (Shoun et al. 1992, Strains: IAM 13491, IAM 8011, IAM 8017) and it has been found in the tissues of red algae *Polysiphonia urceolata* off the coast of China (Wang et al. 2006), rhizospheres and plant leaves (Wang et al. 2016), freshwater sediments (Liu et al. 2015), and agricultural soils (Klaubauf et al. 2010).

All identified species from order *Hypocreales* (Fig. 3c) were isolated from surface waters during austral fall and summer (Table 1b; Fig. 1). Strains 18, 81, 94, 95 were associated (probability 1.00) with uncultured fungus (GenBank accession number KM032316) isolated from deep-sea sediments of eastern Pacific and south Indian Oceans. Strains 84 and 25 were related with *S. strictum* (probability 1.00)

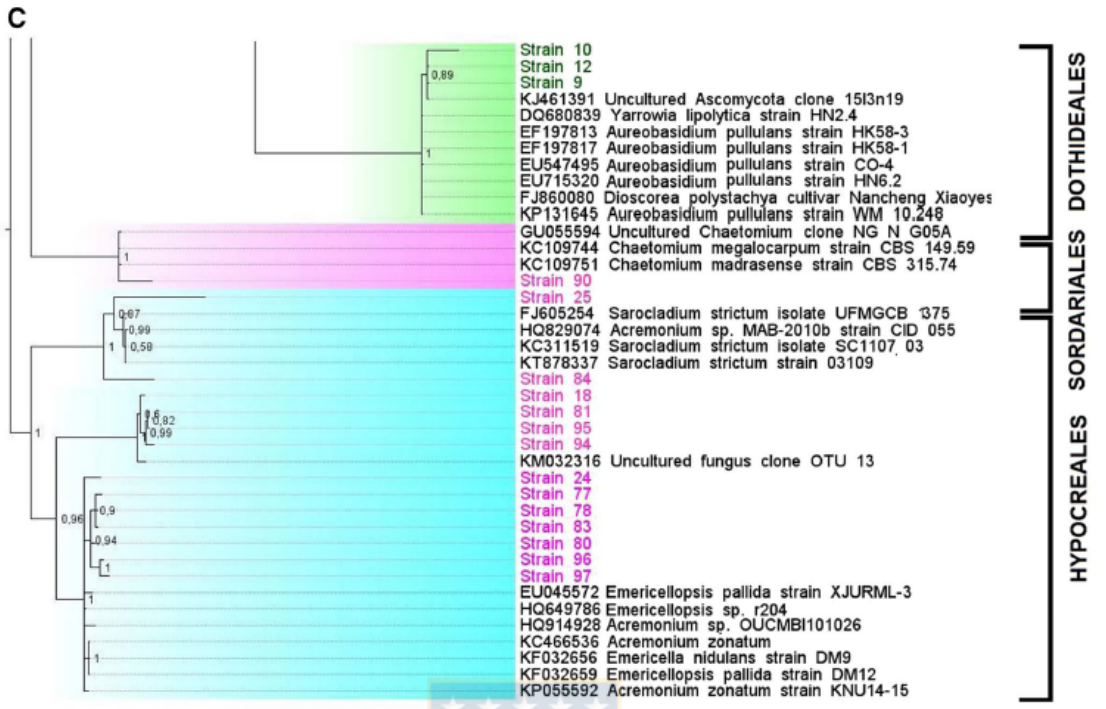

Fig. 3 (continued)

ex-type *Acremonium strictum* (Summerbell et al. 2011). Despite that strain 25 could not be assigned to any species in GenBank (98% identity; Table 2), we identified it as *S. strictum*. Strains 24, 77, 78, 80, 83, 96, 97 were related to *Emericellopsis* sp., *Acremonium* sp. and *Emiricella* sp. (probability 0.96). Species of order Hypocreales have been detected in Dead Sea waters (Oren and Gunde-Cimerman 2012), in marine sponges (Gao et al. 2008), and mangrove wood (Pang et al. 2010).

Our phylogenetic analysis and strain assignation indicate that several fungi isolated from the water column of the eastern South Pacific Ocean have also been isolated from a broad spectrum of substrates, such as air, soil, fresh and marine waters and sediments, wood, plants, insects and humans, revealing their ubiquity and likely physiological adaptability to diverse habitats.

Table 2 Fungal strains of culturable filamentous Ascomycetes in the eastern South Pacific Ocean off Chile not assigned to any species in GenBank, and its closest relative sequences

Strain	Closest relative	GenBank acc no.	% Identity
5	*Penicillium antarcticum*	KP016845	92
	Penicillium atrovenetum	KF679753	92
	Penicillium novae-zeelandiae	NR111668	92
10	*A. pullulans*	EU547495	92
21	*Penicillium restrictum*	KJ820679	96
	Penicillium albocinerascens	KP016811	96
25	*S. strictum*	EF682095	98
41	*P. atrovenetum*	NR121255	91
	Penicillium coralligerum	NR111666	91
52	*Penicillium* sp.	KJ183195	98
56	*P. atrovenetum*	KF679753	98
58	*Penicillium crustosum*	KF938426	93
62	*Penicillium commune*	HQ710540	98
64	*P. commune*	KJ820680	91
70	*P. atrovenetum*	KF679753	97

OTUs richness and distribution of major taxa

Species of order Eurotiales (78) were isolated in all sampling sites, except in oceanic waters off Easter Island (Station 7; Fig. 1). Along coastal–ocean transect off northern Chile, the frequency of species of this order decreased in open ocean waters midway to Easter Island, where species of order Dothideales accounted for more than 50% of fungal strains (Site 4; Fig. 1). In the coastal upwelling ecosystem off central Chile (Station 18), frequency of species of order

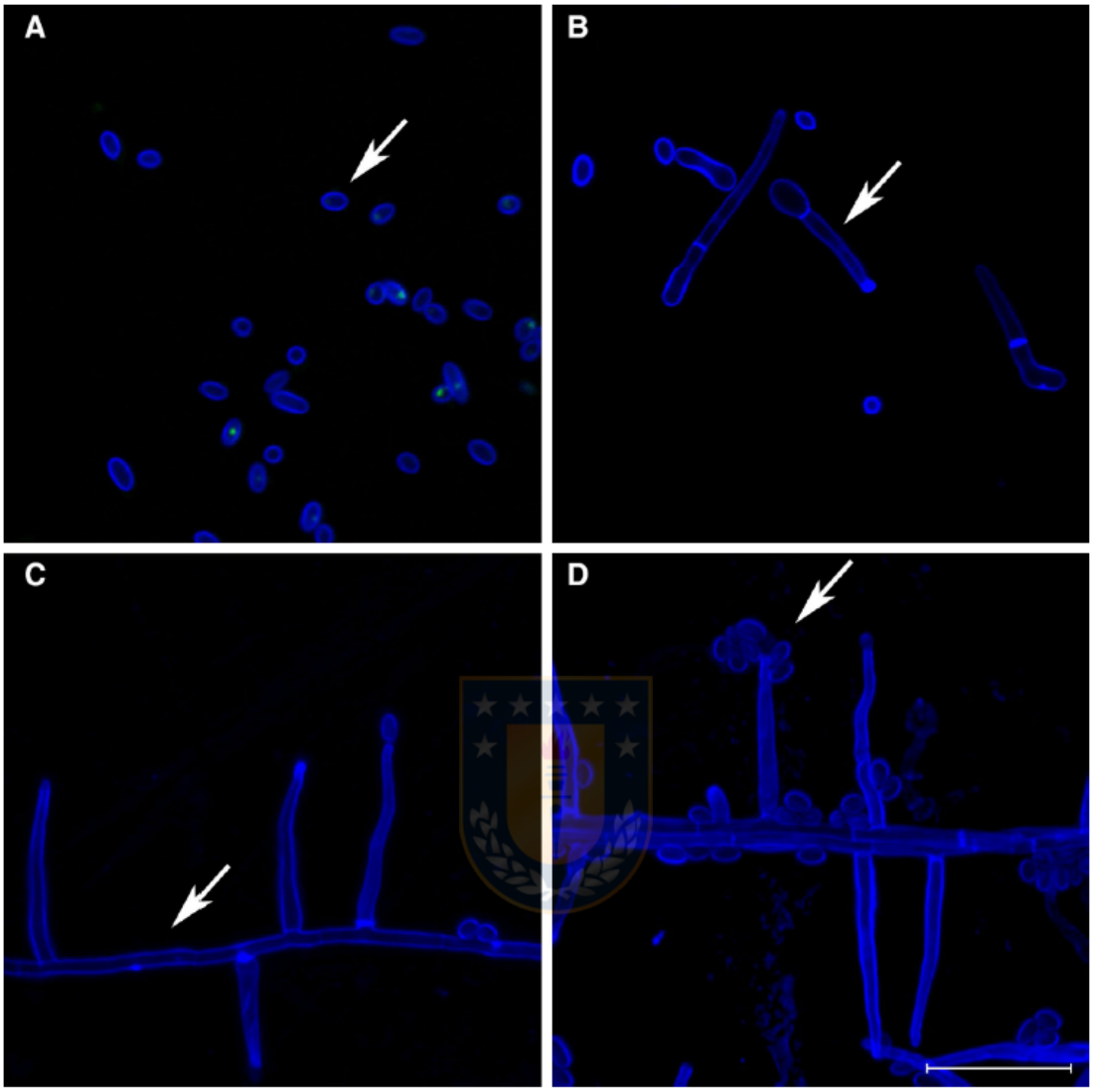

Fig. 4 Confocal micrographs (63×) of stages of strain 24 (*Hypocreales* sp.) while growing and reproducing in filtered seawater after isolation from the coastal upwelling ecosystem off central Chile. Samples were stained with *Calcofluor White Stain*, **a** Conidia, **b** Conidial germination, **c** Mycelium and **d** Conidiophores. Bar: (**a**–**d**) 20 µm

Eurotiales varied seasonally with dominance during austral winter and spring (Fig. 1). Orders Sordariales and Hypocreales were only isolated in the coastal upwelling ecosystem off central Chile (Fig. 1), with order Hypocreales accounting for over 60% of all strains in austral fall and summer, and members of order Sordariales were only present during austral spring (Fig. 1). Our results suggest seasonality and a biogeographic pattern for culturable fungi in the South Pacific Ocean off Chile, with ubiquitous taxa and others representative of productive periods in coastal upwelling waters.

Viability of isolated strains in seawater

All selected 23 strains germinated by the second day, mycelium formation was observed from the third day onward, and formation of reproductive structures (conidia) was observed

from the seventh day onwards in both sterile freshwater and filtered seawater enriched with organic nutrients (amino acids, peptides, carbohydrates and vitamins). Spore germination was not observed in sterile freshwater without added organic nutrients.

Only conidia of strains 24 and 25 of order Hypocreales germinated after 5 days in filtered seawater without added nutrients (Fig. 4). Mycelium development was detected on the seventh day, and reproductive structures (conidiophores) were observed after 10 days.

These results show that selected isolates are able to germinate, grow and generate reproductive structures, both in seawater and freshwater, and suggest that organic substrate availability may be a factor influencing fungal development in the ocean.

Discussion

Penicillium in the eastern South Pacific Ocean

Penicillium represents the dominant culturable genus in waters of the eastern South Pacific Ocean (Figs. 2, 3) constituting 82% of identified strains. *Penicillium* appears to be widely distributed in saline environments, such as the West Pacific Warm Pool (Wang et al. 2014), saltern environments around the world (Zajc et al. 2012), the oxygen-depleted waters of the Arabian Sea (Jebaraj et al. 2010), the water column of the Dead Sea (Oren and Gunde-Cimerman 2012), and colonizing sediments, algae and invertebrates in coral reefs (Morrison-Gardiner 2002). High representation of *Penicillium* in our marine-derived cultures may be resulting from its euryhaline and eurythermal capabilities (Houbraken and Samson 2011).

Significance of culturable diversity

Culturable diversity most probably only represents a small fraction of existing filamentous fungal communities in ocean water (Wang et al. 2008). In consequence, a higher in situ diversity can be expected for waters of the eastern South Pacific Ocean. Additionally, yeasts with approximately 1500 species currently described (Kurtzman and Fell 2006), and others groups of Fungi, which require different approaches to be isolated, were not considered. For instance, we excluded from our analysis symbiotic fungi, such as those from family Malasseziales of phylum Basidiomycota, whose members appear to dominate in marine sponges (Gao et al. 2008), and fungal parasites, such as Chytridiomycota, which infect marine phytoplankton (Gutiérrez et al. 2016; Wang and Johnson 2009). Thus, although culture approaches do not represent complete fungal diversity, our results provide novel knowledge on potential taxonomic diversity of culturable fungi in different environments of the ocean and supports the notion of wide distribution of fungi in the marine environment, whose viability may be a result of organic substrate availability.

Conclusions

This study is the first report of diversity and taxonomy of culturable fungi from distinct physicochemical environments of eastern South Pacific Ocean off Chile, providing evidence for distribution of fungi in the ocean, and the potential influence of substrate availability on fungal growth, an aspect of both ecological and biotechnological importance. Culturable filamentous planktonic Ascomycetes in the eastern Pacific South Ocean was composed of orders Eurotiales, Dothideales, Sordariales and Hypocreales, with a high representation of genus *Penicillium*. Our experimental results evidenced the presence of facultative fungi capable of growing in both seawater and freshwater, and that availability of organic substrates may be a factor influencing fungal development in the ocean. Isolated strains represent raw material for further research on ecology, nutrient cycling and microbial loop, as well as for production of metabolites and their use in biotechnology.

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References

Alfaro M, Holder M (2006) The posterior and the prior in bayesian phylogenetics. Annu Rev Ecol Evol Syst 37:19–42. doi:10.1146/annurev.ecolsys.37.091305.110021

Andreakis N, Høj L, Kearns P et al (2015) Diversity of marine-derived fungal cultures exposed by DNA barcodes: the algorithm matters. PLoS ONE 10:1–22. doi:10.1371/journal.pone.0136130

Bass D, Howe A, Brown N, et al (2007) Yeast forms dominate fungal diversity in the deep oceans. Proc Biol Sci R Soc 274:3069–3077. doi:10.1098/rspb.2007.1067

Bosch A, Maronna RA, Yantorno OM (1995) A simple descriptive model of filamentous fungi spore germination. Process Biochem 30:599–606. doi:10.1016/0032-9592(94)00007-5

Edgcomb VP, Beaudoin D, Gast R et al (2011) Marine subsurface eukaryotes: the fungal majority. Environ Microbiol 13:172–183. doi:10.1111/j.1462-2920.2010.02318.x

Frisvad J, Gravesen S (1994) Health implications of fungi in indoor environments, air quality monographs. In: Samson R, Flannigan B, Flannigan M, et al. (eds) *Penicillium* and *Aspergillus* from Danish homes and working places with indoor air problems: identification and mycotoxin determination, 1st edn. Pergamon Press, Amsterdam, pp 281–290

Fuller MS, Poyton R (1964) A new technique for the isolation of aquatic fungi. Bioscience 14:45–46

Gao Z, Li B, Zheng C, Wang G (2008) Molecular detection of fungal communities in the hawaiian marine sponges *Suberites zeteki* and *Mycale armata*. Appl Environ Microbiol 74:6091–6101. doi:10.1128/AEM.01315-08

Gao Z, Johnson ZI, Wang G (2010) Molecular characterization of the spatial diversity and novel lineages of mycoplankton in Hawaiian coastal waters. ISME J 4:111–120. doi:10.1038/ismej.2009.87

Gardes M, Bruns TD (1993) ITS primers with enhanced specificity for basidiomycetes, application to the identification of mycorrhiza and rusts. Mol Ecol 2:113–118

Guindon S, Dufayard JF, Lefort V et al (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321. doi:10.1093/sysbio/syq010

Gutiérrez MH, Pantoja S, Quiñones RA, Gonzalez R (2010) First record of filamentous fungi in the coastal upwelling ecosystem off central Chile. Gayana 74:66–73

Gutiérrez MH, Pantoja S, Tejos E, Quiñones RA (2011) The role of fungi in processing marine organic matter in the upwelling ecosystem off Chile. Mar Biol 158:205–219. doi:10.1007/s00227-010-1552-z

Gutiérrez MH, Galand PE, Moffat C, Pantoja S (2015) Melting glacier impacts community structure of Bacteria, Archaea and Fungi in a Chilean Patagonia fjord. Environ Microbiol 17:3882–3897. doi:10.1111/1462-2920.12872

Gutiérrez MH, Jara AM, Pantoja S (2016) Fungal parasites infect marine diatoms in the upwelling ecosystem of the Humboldt current system off central Chile. Environ Microbiol 18:1–24. doi:10.1111/1462-2920.13257

Houbraken J, Samson RA (2011) Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. Stud Mycol 70:1–51. doi:10.3114/sim.2011.70.01

Houbraken J, Frisva J, Samson R (2011) Taxonomy of *Penicillium* section Citrina. Stud Mycol 70:53–158. doi:10.3114/sim.2011.70.02

Irinyi L, Serena C, Garcia-Hermoso D et al (2015) International Society of Human and Animal Mycology (ISHAM)-ITS reference DNA barcoding database—the quality controlled standard tool for routine identification of human and animal pathogenic fungi. Med Mycol 53:313–337. doi:10.1093/mmy/myv008

Jebaraj CS, Raghukumar C (2009) Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. Mycol Res 113:100–109. doi:10.1016/j.mycres.2008.08.009

Jebaraj CS, Raghukumar C, Behnke A, Stoeck T (2010) Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation. FEMS Microbiol Ecol 71:399–412. doi:10.1111/j.1574-6941.2009.00804.x

Johnson TW, Sparrow FK (1961) Fungi in oceans and estuaries. Verland, New York

Jones EBG (2011) Are there more marine fungi to be described? Bot Mar 54:343–354. doi:10.1515/BOT.2011.043

Klaubauf S, Inselsbacher E, Zechmeister-Boltenstern S, et al (2010) Molecular diversity of fungal communities in agricultural soils from lower Austria. Fungal Divers 44:65–75. doi:10.1007/s13225-010-0053-1

Kohlmeyer J, Kohlmeyer E (1979) Marine mycology: the higher fungi. Academic Press, New York

Kurtzman CP, Fell JW (2006) Yeast systematics and phylogeny-implications of molecular identification methods for studies in ecology. In: Rosa C, Péter G (eds) Biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 11–30

Kurtzman CP, Robnett CJ (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26 S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73:331–371. doi:10.1023/A:1001761008817

Lai X, Cao L, Tan H et al (2007) Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. ISME J 1:756–762. doi:10.1038/ismej.2007.51

Landeweert R, Leeflang P, Kuyper TW et al (2003) Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl Environ Microbiol 69:327–333. doi:10.1128/AEM.69.1.327-333.2003

Liu J, Wang J, Gao G et al (2015) Distribution and diversity of fungi in freshwater sediments on a river catchment scale. Front Microbiol 6:1–12. doi:10.3389/fmicb.2015.00329

Lyncht MDJ, Thorn RG (2006) Diversity of basidiomycetes in Michigan agricultural soils. Appl Environ Microbiol 72:7050–7056. doi:10.1128/AEM.00826-06

Montecino V, Paredes MA, Paolini P, Rutllant J (2006) Revisiting chlorophyll data along the coast in north-central Chile, considering multiscale environmental variability. Rev Chil Hist Nat 79:213–223. doi:10.4067/S0717-71782002030100031

Montero P, Daneri G, Cuevas LA et al (2007) Productivity cycles in the coastal upwelling area off Concepción: the importance of diatoms and bacterioplankton in the organic carbon flux. Prog Oceanogr 75:518–530. doi:10.1016/j.pocean.2007.08.013

Morrison-Gardiner S (2002) Dominant fungi from Australian coral reefs. Fungal Divers 9:105–121

Murphy WJ, Eizirik E, O'Brien SJ et al (2001) Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294:2348–2351. doi:10.1126/science.1067179

Nagahama T, Hamamoto M, Nakase T et al (2003) *Cryptococcus surugaensis* sp. nov., a novel yeast species from sediment collected on the deep-sea floor of Suruga Bay. Int J Syst Evol Microbiol 53:2095–2098. doi:10.1099/ijs.0.02712-0

Oren A, Gunde-Cimerman N (2012) Fungal life in the dead sea. In: Raghukumar C (ed) Biology of marine fungi. Progress in molecular and subcellular biology, 1st edn. Springer, Berlin, pp 115–132

Pang KL, Alias SA, Chiang MWL et al (2010) *Sedecimiella taiwanensis* gen. et sp. nov., a marine mangrove fungus in the Hypocreales (Hypocreomycetidae, Ascomycota). Bot Mar 53:493–498. doi:10.1515/BOT.2010.061

Pantoja S, Sepúlveda J, González HE (2004) Decomposition of sinking proteinaceous material during fall in the oxygen minimum zone off northern Chile. Deep Sea Res Part 1 51:55–70. doi:10.1016/j.dsr.2003.09.005

Pennington JT, Mahoney KL, Kuwahara VS et al (2006) Primary production in the eastern tropical Pacific: a review. Prog Oceanogr 69:285–317. doi:10.1016/j.pocean.2006.03.012

Pitt J, Hocking A (2009) Fungi and food spoilage. Springer, London

Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, automatic barcode gap discovery for primary species delimitation. Mol Ecol 21:1864–1877. doi:10.1111/j.1365-294X.2011.05239.x

Redberg GL, Hibbett DS, Ammirati JF, Rodriguez RJ (2003) Phylogeny and genetic diversity of *Bridgeoporus nobilissimus* inferred using mitochondrial and nuclear rDNA sequences. Mycologia 95:836–845

Rodrigues A, Mueller UG, Ishak HD et al (2011) Ecology of microfungal communities in gardens of fungus-growing ants (Hymenoptera: Formicidae): a year-long survey of three species of attine ants in Central Texas. FEMS Microbiol Ecol 78:244–255. doi:10.1111/j.1574-6941.2011.01152.x

Ronquist F, Teslenko M, Van Der Mark P et al (2012) Mrbayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. doi:10.1093/sysbio/sys029

Schlitzer R (2015) Ocean Data View. http://odv.awi.de.

Schoch CL, Seifert KA, Huhndorf S, et al (2012) From the cover: nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci 109:6241–6246. doi:10.1073/pnas.1117018109

Seifert K, Frisvad J (2000) *Penicillium* on solid wood products. In: Samson R, Pitt J (eds) Integration of modern taxonomic methods For *Penicillium* and *Aspergillus* classification. CRC Press, Amsterdam, pp 285–298

Shoun H, Kim D-H, Uchiyama H, Sugiyama J (1992) Denitrification by fungi. FEMS Microbiol Lett 94:277–282. doi:10.1016/0378-1097(92)90643-3

Sobarzo M, Shearman RK, Lentz S (2007) Near-inertial motions over the continental shelf off Concepción, central Chile. Prog Oceanogr 75:348–362. doi:10.1016/j.pocean.2007.08.021

Summerbell RC, Gueidan C, Schroers HJ et al (2011) *Acremonium* phylogenetic overview and revision of *Gliomastix*, *Sarocladium*, and *Trichothecium*. Stud Mycol 68:139–162. doi:10.3114/sim.2011.68.06

Takami H, Inoue A, Fuji F, Horikoshi K (1997) Microbial flora in the deepest sea mud of the Mariana trench. FEMS Microbiol Lett 152:279–285. doi:10.1016/S0378-1097(97)00211-5

Takishita K, Tsuchiya M, Reimer JD, Maruyama T (2006) Molecular evidence demonstrating the basidiomycetous fungus *Cryptococcus curvatus* is the dominant microbial eukaryote in sediment at the Kuroshima Knoll methane seep. Extremophiles 10:165–169. doi:10.1007/s00792-005-0495-7

Tamura K, Stecher G, Peterson D et al (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst197

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. doi:10.1093/nar/22.22.4673

Visagie CM, Houbraken J, Frisvad JC et al (2014) Identification and nomenclature of the genus *Penicillium*. Stud Mycol 78:343–371. doi:10.1016/j.simyco.2014.09.001

Wang G, Johnson ZI (2009) Impact of parasitic fungi on the diversity and functional ecology of marine phytoplankton. In: Kersey WT, Munger SP (eds) Marine Phytoplankton, 1st edn. Nova Science Publishers, pp 211–228

Wang S, Li XM, Teuscher F et al (2006) Chaetopyranin, a benzaldehyde derivative, and other related metabolites from *Chaetomium globosum*, an endophytic fungus derived from the marine red alga *Polysiphonia urceolata*. J Nat Prod 69:1622–1625. doi:10.1021/np060248n

Wang G, Li Q, Zhu P (2008) Phylogenetic diversity of culturable fungi associated with the Hawaiian Sponges *Suberites zeteki* and *Gelliodes fibrosa*. Antonie Van Leeuwenhoek 93:163–174. doi:10.1007/s10482-007-9190-2

Wang G, Wang X, Liu X, Li Q (2012) Diversity and biogeochemical funtion of planktonic fungi in the ocean. In: Raghukumar C (ed) Biology of marine fungi. Progress in molecular and subcellular biology. marine molecular biotechnology, 1st edn. Springer, Berlin, pp 71–88

Wang X, Singh P, Gao Z et al (2014) Distribution and diversity of planktonic fungi in the west pacific warm pool. PLoS ONE 9:1–7. doi:10.1371/journal.pone.0101523

Wang XW, Lombard L, Groenewald JZ, et al (2016) Phylogenetic reassessment of the *Chaetomium globosum* species complex. Persoonia 36:83–133. doi:10.3767/003158516X689657

White TJ, Bruns S, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc 38:315–322

Wilcox TP, Zwickl DJ, Heath TA, Hillis DM (2002) Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol Phylogenet Evol 25:361–371. doi:10.1016/S1055-7903(02)00244-0

Zajc J, Zalar P, Plemenitas A, Gunde-Cimerman N (2012) The mycobiota of the Salterns. In: Raghukumar C (ed) Biology of marine fungi. Progress in molecular and subcellular biology, 1st edn. Springer, Berlin, pp 133–158

4.2. Capítulo 2: Señal Bioquímica de Hongos Marinos: Implicancias para estudios Tróficos y Biogeoquímicos

Manuscrito enviado a la revista Aquatic Microbial Ecology: Marcelo H. Gutiérrez, Jeanett Vera, Benjamín Srain, Renato A. Quiñones, Lars P. Wörmer, Kai-Uwe Hinrichs, Silvio Pantoja. Biochemical Fingerprint of Marine Fungi: Implications for Trophic and Biogeochemical Studies. Aquatic Microbial Ecology (2019)

Resumen: Describimos la composición bioquímica de 14 cepas fungicas aisladas desde distintos ambientes marinos del Océano Pacífico Sur-Oriental frente a Chile. Las proteínas representaron del 3 al 21% del peso seco del micelio, con un alto contenido de aminoácidos esenciales como histidina, treonina, lisina y leucina, ácidos grasos poliinsaturados, ergosterol y fosfatidilcolina. La composición elemental y el contenido energético de los hongos de origen marino se encuentran en el rango de valores de bacterias, fitoplancton, zooplancton y otros metazoos de ambientes acuáticos, sin embargo, un patrón distinto de lípidos y proteínas fue identificado entre hongos marinos y el plancton. Estas características, junto con el alto contenido de quitina en sus paredes celulares y la composición elemental que se asemeja a una fuente planctónica marina, son potencialmente aplicables para evaluar tanto el rol de los hongos en el ciclo marino del carbono y nutrientes como su contribución a la biomasa microbiana marina y al reservorio de materia orgánica.

Biochemical Fingerprint of Marine Fungi: Implications for Trophic and Biogeochemical Studies

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Keywords

Marine fungi, biochemical composition, lipid, protein, chitin, Intact Polar Lipids

Abstract

We described the biochemical composition of 14 fungal strains isolated from distinct marine environments in the eastern South Pacific Ocean off Chile. Proteins accounted for 3 to 21% of mycelial dry weight with high contents of essential amino acids histidine, threonine, lysine and leucine, polyunsaturated fatty acids, ergosterol, and phosphatidylcholine. Elemental composition and energetic content of marine-derived fungi were in the range of values of bacteria, phytoplankton, zooplankton and other metazoans from aquatic environments, however a distinct pattern of lipids and proteins of marine fungi among plankton was identified. Those features, along with high content of chitin in their cell walls and elemental composition resembling a marine planktonic source, have potential applications to assess both contribution of fungi to marine microbial biomass and organic matter reservoir, and the role of fungi in the marine cycle of carbon and nutrients.

1. Introduction

In terrestrial environments, fungi contribute with 35 to 76% of the microbial biomass in soils (Joergensen and Wichern, 2008) and their role in nutrient and carbon cycling is widely recognized (Gadd, 2006; Crowther et al., 2012; Paul, 2014). Although less studied than their terrestrial counterpart, recent research has demonstrated presence of fungi in a variety of marine environments and unraveled novel aspects of their ecology and biogeochemical role in the ocean (Burgaud et al., 2009, 2013, Gutiérrez et al., 2010, 2011, 2016; Richards et al., 2012; Wang et al. 2014; Zhang et al., 2014; Bochdansky et al., 2016; Fuentes and Quiñones, 2016; Taylor and Cunliffe, 2016; Tisthammer et al., 2016; Cunliffe et al., 2017; Jephcott et al., 2017; Wang et al., 2017). Although prior findings support inclusion of fungi in models of marine microbial loop and oceanic carbon cycle (Gutiérrez et al., 2011, 2016; Kagami et al., 2014; Jephcott et al., 2017), important gaps in our knowledge prevent a complete understanding of the role of fungi in marine ecosystem functioning. In particular, due to lack of information on elemental and biochemical composition of marine fungi they are not considered in current models of trophic webs and carbon fluxes in the ocean.

In the marine ecosystem, transfer of carbon from primary producers to higher trophic levels is based on complex interactions, with microbes playing central roles in mobilization of dissolved and particulate organic carbon through the microbial loop (Pomeroy et al., 1974; Azam, 1998). Indeed, microbial biomass represents a major conduit for organic carbon, with prokaryotes being considered significant contributors to pelagic trophic webs and carbon fluxes in the ocean (e.g. Azam, 1998; Azam and Malfatti, 2007; Falkowski et al., 2008). The few estimates of fungal biomass in the ocean have shown that it can be comparable to that of prokaryotes in surface waters of productive coastal ecosystems (Gutiérrez et al., 2011), suggesting that they are significant contributors to living microbial carbon in the coastal ocean and a potential source of energy for marine trophic web. Terrestrial fungi are recognized producers of essential organic substrates such as proteins, lipids, vitamins and other molecules with nutritious value (Feeney et al., 2014), and in aquatic environments have been proposed as potential sources of food for benthic and planktonic organisms (Raghukumar, 2002; Kagami et

al., 2014). For example, due to presence of polyunsaturated fatty acids, zoospores of parasitic chytrids on phytoplankton could contribute to zooplankton nutrition in freshwater bodies (Kagami et al., 2011) and in the ocean (Gutiérrez et al., 2016).

In order to understand the significance of fungi as reservoirs of carbon and nitrogen and as source of energy for trophic webs in the ocean, we analyzed the elemental and biochemical composition of marine fungal isolates from distinctive coastal and oceanic environments of the eastern South Pacific Ocean off Chile. This highly heterogeneous marine environment comprises one of the most productive coastal marine ecosystems of the world (Daneri et al., 2000; Quiñones et al., 2010), is under the influence of an extensive oxygen minimum zone (Quiñones et al., 2010) and large rivers (Dávila et al., 2002), and encompasses one of the world's most extensive fjord ecosystems with glacial influence in Chilean Patagonia (Pantoja et al. ,2010). The variety of environments found in this large ecosystem of the Pacific Ocean support different communities of microorganisms (e g. Quiñones et al., 2009; Ulloa et al., 2012; Gutiérrez et al., 2018), including fungi (Gutiérrez et al., 2015, 2017; Vera et al., 2017), and thus is expected to represent a wide range of variability in elemental and biochemical composition of fungi.

2. Materials and methods

2.1. Strain collection, phylogenetic analysis, and preparation of biomass for analysis

Fourteen strains of fungi isolated from waters and one from sediment of coastal and oceanic regions of the eastern South Pacific Ocean off Chile (Fig. 1) were obtained from the culture collection of the Marine Organic Geochemistry Laboratory at University of Concepción (Table 1). We selected strains from areas with different oceanographic conditions such as surface waters of the productive coastal upwelling ecosystem of central Chile, waters of the oxygen minimum zone off northern and central Chile, oligotrophic oceanic waters near Easter Island and estuarine waters of Patagonian fjords. Isolation of fungi, DNA extraction and

amplification were described in Vera et al. (2017), based on White et al. (1990) and Gardes and Bruns (1993).

PCR products including ITS 1, 5.8 S and ITS 2 regions, were sequenced at the Automated DNA Sequencing Service of MACROGEN (http://dna.macrogen.com/eng). ITS rDNA sequences larger than 600pb and matched sequences from GenBank with 99% nucleotide identity were considered representative of same species (Kurtzman and Robnett 1998; Landeweert et al. 2003; Redberg et al. 2003; Lyncht and Thorn 2006; Gao et al. 2008). Sequences were aligned with Clustal W (Thompson et al. 1994) to run phylogenetic analyses. Bayesian tree inference was computed using MrBayes v.3.2.5 (Ronquist et al. 2012) to identify evolutionary relationship among individuals. Analysis was performed using a GTR model selected in PhyML 3.0 (Guindon et al. 2010) conducted for 30,000,000 generations until standard deviation of split frequencies was under 0.01. Two parallel runs were computed with four chains each, sampling every 200 generations, with diagnostics calculated every 1000 generations. Bayesian probabilities were considered reliable when higher than 0.95 pp (Murphy et al. 2001; Wilcox et al. 2002; Alfaro and Holder 2006; Houbraken et al. 2011a). Sequences have been submitted to the NCBA GenBank database with accession number MH231237-MH231248.

For elemental and biochemical analyses, biomass of strains was grown by inoculating fungal tissue in liquid media (Emerson´s YpSs agar, Kohlmeyer and Kohlmeyer, 1979), and incubating at 20 ºC in a shaker until mycelia were clearly distinguishable (7 days). Fungal mycelia were collected by centrifugation, freeze-dried, and biomass recorded in a micro analytical balance to be used for analyses.

2.2. Organic carbon, nitrogen and caloric content, C and N stable isotopic composition

Content of organic carbon and nitrogen and stable isotope compositions of C and N in fungal mycelia and dry culture medium were measured by continuous flow isotope ratio mass

spectrometry coupled to an on-line elemental analyzer at UC Davis Stable Isotope Facility (https://stableisotopefacility.ucdavis.edu). Caloric content of mycelia was measured in triplicate using a Parr 6725 Semi-micro Oxygen Bomb Calorimeter following manufacturer standard methodology (Parr Instrument Company). Edible fungus *Agaricus bisporus* and zooplankton *Euphausia pacifica* were used as positive controls.

2.3. Total hydrolysable amino acids (THAA)

THAA were determined based on Lindroth and Mopper (1979). One hundred mg of freeze-dried mycelia were dissolved in 2 mL of hydrolysis solution (7 N HCl, 1% phenol, 10% trifluoroacetic acid) and purged for 1 min under a pure nitrogen stream. Samples were hydrolyzed at 150 °C for 1.5 h, neutralized (6.5–7.5) with NaOH, and derivatized with ortho-phthalaldehyde and mercaptoethanol. Derivatized samples were dissolved in 50 μL methanol and analyzed by high performance liquid chromatography (HPLC) using a Shimadzu chromatograph with fluorescence detector, auto-sampler, oven, and binary pump. Amino acids were separated in a Kromasil 100-5 C18 column (4.6 x 250 mm) kept at 40 °C. Flow rate of 1 mL min^{-1} was maintained during elution of a 25 mM sodium acetate solution with 5% tetrahydrofuran for 5 min, followed by a gradient of methanol (25% for 1 min, to 30% at 35 min, to 50% at 42 min, to 60% at 60 min, to 100% at 72 min, 100% to 80 min). Column was re-equilibrated with sodium acetate/tetrahydrofuran solution for 5 min between injections. Identification and quantification of amino acids was carried out by co-injection of samples with THAA standard Pierce 20088.

2.4. Fatty acids methyl esters and sterols

Extraction of lipids from ca. 1 g dry fungal mycelia was carried out with dichloromethane and methanol (3:1) by sonication and centrifugation. Organic phase was separated by adding water and hexane to the extract and the hexane phase concentrated with a rotary evaporator (Bligh and Dyer, 1959). Lipid extracts were split for fatty acid and sterol analyses.

For analysis of fatty acids, extracts were saponified with 15 mL 0.5 N KOH:MeOH (Christie, 1989) and non-saponifiable lipids were separated with hexane. Remaining aqueous extracts were acidified with 6N HCl and fatty acids extracted with hexane. Solvent was removed by rotary evaporation and fatty acids converted to methyl esters (FAMEs) with 1 mL 10% BF_3/MeOH for 1 h at 70 ºC (Christie, 1989; Tolosa et al., 2004; Méjanelle and Laureillard, 2008). One mL Milli-Q water was added to the mixture and FAMEs were extracted with hexane and dried under a stream of nitrogen. FAME fractions redissolved in 200 µL hexane were injected into a gas chromatograph-mass spectrometer (GC-MS) with a HP5-MS column (30 m x 0.25 mm, 0.25 µm film thickness, Agilent Technologies). FAMEs were identified based on retention times (FAME mix, Supelco Analytical), by comparison with mass spectra in the internal library of the mass spectrometer and electronic data base www.lipidlibrary.co.uk/ms/arch_me/index.htm. Quantification was carried out using a calibration curve with serial dilutions of FAME standard mix. Coefficient of variation for analysis is 14%, routinely measured in five replicate analyses.

For analysis of sterols, extracts were separated into four fractions by column chromatography (10 cm length, 0.5 cm ID) filled with approximately 0.9 g deactivated silica gel. Aliphatic hydrocarbons were eluted with 40 mL hexane, ketones were eluted with 50 mL toluene/hexane (1:3 v/v), alcohols with 50 mL ethyl-acetate/hexane (1:9 v/v), and polar compounds were eluted with 35 mL ethyl acetate/methanol/hexane (4:4:1 v/v). Alcohol fractions were derivatized with 80 µL BSTFA (N,O- bis(trimethylsilyl) trifluoracetamide) and 40 µL TMCS (trimethylchlorosilane) at 70 °C for 1 h before analysis. Derivatized solutions were injected (1 µL) on a GC-MS equipped with a HP5-MS chromatographic column (30 x 0.2 mm, 0.25 µm film thickness, Agilent Technologies). Identification of sterols was achieved by analyzing mass spectra and comparison with available mass spectrometry data of sterols (Online lipid library; http://www.chemspider.com). Sterol concentration was determined using internal standard 1-nonadecanol (5 µg). Coefficient of variation for analysis was 12% (n=10).

2.5. Intact polar lipids (IPLs)

Freeze-dried fungal isolates were amended with deuterated internal standard Lyso C16-PAF and lipids extracted four times with a mixture of methanol, dichloromethane, and phosphate buffer at pH 7.4 (2:1:0.8 v/v) by sonication for 15 min. Phase separation was aided by adding dichloromethane and buffer to reach ratio 1:1:0.8 (Sturt et al. 2004). Combined extracts were washed with distilled water, the organic phase evaporated under a stream of nitrogen, and lipids stored at −20 °C.

IPLs were analyzed by reversed phase UHPLC and high resolution quadrupole time-of-flight mass spectrometer (Q-TOF) with electrospray ionization (Wörmer et al. 2013). Extracts were dissolved in dichloromethane/methanol (1:9 v/v) and lipids were separated using an Acquity UPLC BEH C18 RP column (1.7 µm, 2.1 x 150 mm, Waters). Eluent A was methanol:water (85:15 v/v with 0.04% HCOOH and 0.1% 14.8 M NH_{3aq}) and B isopropyl alcohol/methanol (50:50, v/v, with 0.04% HCOOH and 0.1% 14.8 M NH_{3aq}). Gradient was 100% A for 2 min and ramp to 85% B in 18 min, washing with 100% B for 8 min and equilibration for 6 min at 0.4 mL/min. A full scan at 2 scans per second was obtained, followed by subjecting the 5−10 dominant ions from the MS full scan to fragmentation to create MS2 spectra (data dependent mode, cf. Wörmer et al., 2013).

2.6. Statistical analysis

Principal component analysis for amino acid and fatty acid composition of fungi and planktonic components was carried out in R version 3.1.2 using the package ggbiplot. Mann-Whitney non-parametric U test was applied to test for statistical differences in elemental composition, energetic content and concentration of organic molecules among water layers and regions. Due to the low number of observations for some depths and regions, statistical tests were not applicable to all cases. Spearman correlation index was calculated to analyze associations between elemental composition and concentration of organic molecules.

Results

3.1. Taxonomic identification of fungal strains

Topology of Bayesian tree indicated that most strains analyzed belonged to phylum Ascomycota, whereas phyla Basidiomycota and Zygomycota included one strain each (Fig. 2). Among Ascomycota most isolates were of genus *Penicillium*, with a member recovered from subsurface suboxic waters of the coastal ocean off northern Chile identified as *Penicillium brevicompactum* (strain BR5-C1, Fig. 2) in a well-supported branch (1.00 pp). In contrast, strains CH82, CH92, CH58, CH115, BR1-C1, and BR11-C1, isolated from diverse areas and depths of eastern South Pacific Ocean, showed low probability values (< 0.95 pp), providing insufficient support to identify them at species level into de genus *Penicillium* (Fig. 2). Within Ascomycota, isolates from waters of the coastal upwelling ecosystem of central-south Chile, were also identified as *Cladosporium sphaerospermum* (strain CH131), *Fusarium sp.* (CH114) and *Lecanicillium* sp. (CH113) in clades strongly supported (1.00 pp, Fig. 2).

Among Basidiomycota and Zygomycota, strain BR74, isolated from deep waters of the South Pacific subtropical gyre near Easter Island (Fig. 1), was affiliated with the yeast *Rhodotorula mucilaginosa* in a strongly supported branch (1.00 pp), and strain CH132 from sediment of the coastal upwelling ecosystem off central Chile was associated (1.00 pp) to *Mucor circinelloides* (Fig. 2).

3.2. Carbon, nitrogen and caloric content and stable isotope compositions of C and N in fungal isolates

Carbon content of fungal strains ranged from 17 to 31% and of nitrogen between 1.2 and 4.4% (Table 1). The highest content of both carbon (31%) and nitrogen (4%,) were found in the yeast *Rhodotorula mucilaginosa* isolated from deep waters near Easter Island (Table 1, Site B in Fig. 1). In contrast, *Penicillium brevicompactum*, isolated from coastal subsurface waters of northern Chile (Site A in Fig. 1) showed the lowest contents of C and N (Table 1). Molar C to

N ratio averaged 11.7 ± 3.4, with maximum values observed for *Penicillium* isolated from waters of Patagonian fjords and minimum ratios for strains of *Rhodotorula mucilaginosa* from deep waters, *Lecanicillium* sp. and *Fusarium* sp. from coastal waters, and *Mucor circinelloides* from coastal sediments (Table 1). Carbon and nitrogen stable isotopic composition of fungal isolates averaged -25.8 ± 0.6‰ and 0.9 ± 1.6‰ (Table 1). Even though $\delta^{13}C$ and $\delta^{15}N$ of isolates were in average comparable to values of the food source (dry culture medium, $\delta^{13}C$= -25.5‰ and $\delta^{15}N$ 0.02‰), *Rhodotorula mucilaginosa* and *Cladosporium sphaerospermum* showed fractionation of 3-5 ‰, Table 1). Energetic content averaged 3.6 ± 0.3 kcal gdw^{-1} (gram dry weight), with the highest value in *Rhodotorula mucilaginosa* (Table 1).

Although variability in carbon and nitrogen contents, C/N, and energetic content was observed among depths (surface, subsurface and deep) and regions (Fig. 1), no significant differences (Mann-Whitney $p>0.05$) were found (Supplemental Information).

3.3. Amino acids, fatty acids and sterols in culturable marine fungi

Content of hydrolysable amino acids (THAA) averaged 87.1 ± 57.8 mg gdw^{-1} (Table 1) accounting for 3% (*Cladosporium sphaerospermum*) to 21% (*Lecanicillium sp.*) of mycelial dry weight. Relative molar proportions of lysine, alanine, aspartic acid, glutamine, leucine and threonine accounted for about 60% of THAA (Fig. 3A).

Concentration of fatty acid methyl ester (FAMEs) ranged from less than 0.1 to 3 mg gdw^{-1} (Table 1), with maximum values observed in *Fusarium* sp. isolated from subsurface waters off central Chile and *Penicillium* sp. isolated from surface waters of the coastal region off northern Chile (Table 1). Among individual FAMEs, C18:2ω6,9c (linoleic acid) and C18:1ω9c (oleic acid) accounted for 61% and with C16:0 (hexadecanoic acid), C18:3ω3,6,9c (□-linolenic acid) and C18:0 (octadecanoic acid) represented over 90% of FAMEs (Fig. 3B). Half of detected FAMEs are polyunsaturated FAMEs (PUFA, two or more unsaturation), with monounsaturated (MUFA) and saturated (SAT) compounds accounting for ~25% of each (Fig.

3B). Sterol contents ranged from 10 to ca. 600 µg gdw^{-1}, with higher values observed in strains isolated from waters and sediments off central Chile (Table 1, Fig. 1, Site C). Most sterols were C-28 (> 90%), with ergosterol and its isomer dehydrostellasterol accounting in average for 85% of sterols (Fig. 3C).

3.4. Abundance and composition of intact polar lipids (IPLs)

According to their headgroups, four main classes of IPLs were identified: a) glycolipids, represented by monoglycosyldiacylglycerol (MGDG) and diglycosyldiacylglycerol (DGDG), b) nitrogen bearing betaine diacylglyceryl-trimethyl-homoserine (DGTS) lipids, c) glycerophospholipids, which included phosphatidyl-ethanolamine (PE), phosphatidyl-N-methylethanolamine (PME), phosphatidyl-N-dimethylethanolamine (PDME), phosphatidyl-choline (PC), phosphatidic acid (PA), phosphatidyl-inositol (PI) phosphatidyl-glycerol (PG), and phosphatidyl-serine (PS), and d) ceramide sphingolipids (Fig. 3D). The summed fatty acid chains of IPLs averaged 35 carbon atoms and contained up to six double bonds. IPL headgroups were dominated (ca. 84%) by PC, PE, PA, betain and DGDG.

Contents of IPLs among fungal strains ranged from 4 to 32 mg gdw^{-1} (Table 1), with PC accounting for 65% of IPL, followed by Lyso-PC (17%) and betaine-DGTS (9%) (Fig. 3D). The highest IPL content was found in a *Penicillium* strain isolated from coastal waters off northern Chile (Table 1, Site A in Fig. 1). Relative abundance of IPLs appeared variable among sampling regions, however, no significant differences (Mann-Whitney $p > 0.05$) were detected with this sample size (Fig. 1C Supplemental Information).

4. Discussion

4.1. Taxonomy of marine-derived fungi

Out of 14 isolates of marine fungi, 7 strains from upwelling and fjord regions of the eastern South Pacific Ocean off Chile were Ascomycota and identified as *Penicillium*, *Lecanicillium*

sp., *Fusarium sp.*, and *Cladosporium sphaerospermum.* Basidiomycetes were represented with *Mucor circinelloides*, isolated from surface sediments off central Chile, and *Rhodotorula mucilaginosa*, along with an uncharacterized yeast, recovered from deep waters off northern Chile. These results are consistent with previous findings of wide representation of genus *Penicillium* in isolates from waters of coastal and oceanic regions off Chile (Vera et al., 2017), and a high diversity of culturable (Vera et al., 2017) and ambient (Gutiérrez et al., 2017) taxa in central Chile, likely associated to high environmental heterogeneity of this environment. *Rhodotorula* yeasts frequently appear in marine samples (e.g. Wirth and Goldani, 2012), filamentous fungi *Lecanicillium* sp. has been previously recovered from hydrothermal vents (Burgaud et al., 2009), and *Cladosporium* and *Mucor* have been found in coastal environments and associated with marine sponges (Batista-García et al., 2017).

4.2. Elemental composition and organic compounds in marine fungal mycelia

Organic carbon and nitrogen contents in fungal mycelia were comparable with that of planktonic organisms although C to N molar ratio almost doubled Redfield ratio of marine plankton (Table 2). Organic carbon and energetic content of fungal isolates showed low variability among taxonomic groups (coefficient of variation <20%), with relatively high carbon and caloric contents in unrelated taxa such as basidiomycete *Rhodotorula* and ascomycete *Penicillium*. Organic carbon contents reported here for marine fungi are lower than those of fungi isolated from terrestrial environments (38 –57%, Zhang and Elser, 2017), although nitrogen content of marine fungi was within the range of those of terrestrial fungi (0.23 – 15%, Zhang and Elser, 2017), with *Rhodotorula mucilaginosa and Lecanicillium sp.* having the highest nitrogen content (≥ 4%). Average elemental composition and energetic content of marine-derived fungi were in the range of values of bacteria, phytoplankton, zooplankton and other metazoans from aquatic environments (Table 2). Caloric content of marine fungi was lower than of krill *Euphausia pacifica* and *Thysanoessa inermis* and higher than zooplanktonic Cnidaria, Ctenophora and Gastropoda (Table 2). Particularly, energetic content in *Rhodotorula mucilaginosa* was higher than in phytoplankton (Tables 1 and 2).

Considering that biomass of filamentous fungi can be as high as that of prokaryotes as shown for the coastal upwelling ecosystem off central Chile (Gutiérrez et al., 2011), fungal mycelia could represent a sizable reservoir of extant carbon and nitrogen in the pelagic ecosystem and as such marine fungi play a potential role in organic carbon and nutrient mobilization in the ocean. In order to reduce the uncertainty of estimates of biomass in the planet (Bar-On et al., 2018), and verify the traditional view of fungi as a minor contributor of biomass in the ocean (Bar-On et al., 2018) it is necessary to improve quantitation methods of detection and expand current coverage of measurements of marine fungi.

Regarding elemental stoichiometry, a novel aspect understudied in marine fungi, our results showed C to N molar ratios (C/N ~12) lower than those of terrestrial fungi (C/N ~16, Zhang and Elser, 2017) and within the ample range of what has been reported for fungi isolated from freshwater environments (C/N ~ 7-31, Danger et al., 2016). Our average C/N values were nearly twice that of planktonic Redfield ratio (Redfield, 1958), but in 3 isolates from diverse environments: upwelling coastal water and sediment, and deep oceanic waters they approached the planktonic value (C/N ~ 8, Table 1). It has been shown that aquatic hyphomycete fungi are not homeostatic for elemental composition unless unlimited nutrient condition occurs (Danger and Chauvet, 2013; Danger et al., 2016). Since C/N among our studied strains is rather homogeneous (coefficient of variation of 29%) for replete culture conditions, we hypothesize that our C/N values accounted for intrinsic properties of the analyzed strains. Differences observed in elemental composition of strains among depths and regions of collection may indicate a potential environmental effect, however, analysis of a larger suite of strains is required to give statistical support to our observations. In particular, natural variability of elemental ratios among mycoplankton species would help assessing the role of fungi in C to N ratio modification during diagenesis in the ocean, by increasing or maintaining planktonic Redfield ratio depending on fungal contribution to reworking of planktonic organic matter.

Composition of individual organic molecules in mycelia evidenced several essential amino acids, and predominance of polyunsaturated fatty acids, ergosterol in the sterol fraction, and phosphatydylcoline in the fraction of intact polar lipids. Protein (as THAA) accounted for ca. 9% of fungal mycelia, 0.1% fatty acids, 1.3% intact polar lipid (that includes head group plus core fatty acids) and 0.02% sterols. Contents of total hydrolysable amino acids (protein) of fungal mycelia (3 – 21% per dry weight) were lower than in plankton (20-40%; Lee 1988), freshwater zooplankton (>50%; Dabrowsky and Rusiecki, 1983), marine phytoplankton (12-35%; Brown, 1991), zooplankton (4-55%, Raymont et al., 1973), and marine bacteria (~60%; Simon and Azam, 1989). These results suggest that marine fungal proteins have lower trophic value than those from other planktonic organisms. However, since marine fungal strains were rich in essential amino acids histidine, threonine, lysine and leucine (Fig. 3A), with even higher contents than in soil fungi (Wallis et al., 2012), they may be a source of critical molecules for diet of metazoans in the marine environment.

Hydrolyzable amino acids (protein) account for as low as 20 and up to 78% of fungal nitrogen, indicating that up to 80% nitrogen (e.g., in yeast *Cladosporium sphaerospermum*, Table 1) is stored in other molecules in fungal mycelia. Chitin, a main polymer of fungi cell walls (Latgé, 2017), is a large reservoir of carbon and nitrogen in the ocean (Souza et al., 2011) produced by crustacean at a rate of ca. 2 million metric tons per year (Jeuniaux and Voss-Foucart, 1991). Cell wall of marine fungi could be an important previously unaccounted source and carrier of chitin, and as such of nitrogen in the marine environment, thus playing a role in nutrient cycling. In support of our hypothesis, fungal remains have been documented in sediment traps in association with sinking chitin fluxes in the subarctic Pacific Ocean (Montgomery et al., 1990).

Fatty acid composition showed a predominance of C-18 molecules, with C18:2ω6,9 accounting for ca. 38% and C18:1□,9c for 23% of fatty acids. Consistently, fatty acid 18:2ω6,9 (linoleic acid) is considered a fungal marker in terrestrial environments (Vestal and White, 1989; Frostegard and Baath, 1996; Olsson, 1999; Boschker and Middelburg, 2002;

Kaur et al., 2005), has been reported as a major constituent (11-37%) of fatty acids of marine fungi (Cooney et al., 1993; Devi et al., 2006; Das et al., 2007), and covaries with abundance of fungal filaments in the coastal upwelling ecosystem off Chile (Gutiérrez et al., 2011). PUFAs account for half of fatty acids, suggesting that along with other planktonic organisms, fungi can be source of nutritious lipids for pelagic organisms. In support of this idea, proportion of PUFAs in marine-derived fungi (this study and Das et al., 2007) is in the upper range of that reported for marine phytoplankton (Lewis, 1969; Zhukova and Aizdaicher, 1995; Arendt et al., 2005;), zooplankton (Najdek, 1997; Escribano and Pérez, 2010) and bacteria (Russell and Nichols, 1999; Das et al., 2007).

Ergosterol, and its isomer dehydrostellasterol ($C_{28}\Delta^{5,7,22}$), was the dominant sterol in all analyzed marine strains, consistent with its known predominance in most terrestrial ascomycetes and basidiomycetes (Weete et al., 2010), and has been used to estimate fungal biomass in soils (Montgomery et al., 2000; Joergensen and Wirchen, 2008), rivers (Jørgensen and Stepanauskas, 2009), salt and freshwater marsh (Newell et al., 2000; Buesing and Gessner, 2006) and wetlands (Verma et al., 2003). Considering that ergosterol and dehydrostellasterol accounted for more than 80% of sterols in all fungal strains, we posit that ambient ergosterol concentration in particulate organic matter could be an appropriate proxy for extant biomass of fungi in the ocean.

Among intact polar lipids, consistent with being a major phospholipid of cell membranes, phosphatidylcholine (PC) was the dominant type in marine-derived fungi. Phospholipids, particularly PC, appear to be beneficial in the diet of several species of freshwater and marine fish and crustaceans by improving survival, growth and resistance to stress (Coutteau et al., 1997, 2000; Wang et al., 2016) and kept as storage lipid in some species of marine zooplankton (Hagen et al., 1996; Lee et al., 2006). Considering that abundance of marine fungi could be as high that of prokaryotes in the ocean (Gutiérrez et al., 2011), marine fungi-derived phospholipids could be transferred through diet of marine organisms and thus play a role transferring energy via storage molecules trough marine trophic webs.

4.3. The biochemical signature of fungi in the marine ecosystem

The composition of marine organic matter is controlled by the accumulation of organic components of a variety of biological sources. Recognizing patterns of individual components of autochthonous and allochthonous organic matter is key to understand their contribution to biogeochemical cycling and to trace their fate and trophic interactions in marine ecosystems. The composition of amino acids and fatty acids of marine-derived fungi differs from that of prokaryotes, phytoplankton, zooplankton and fish, with lysine, histidine, threonine and alanine and mono- and di-unsaturated C18 fatty acids being the differentiating compounds (Fig. 4A,B).

We compared carbon and nitrogen stable isotope composition of the substrate for fungi growth and of resulting biomass in order to learn about fractionation by marine fungi during heterotrophic growth and to identify potentially diagnostic signals for geochemical studies in the ocean. Placed in the general scheme of Meyers (1994), the pattern of marine- fungi derived C/N and $\delta^{13}C$ (Fig. 5) is consistent with an expected heterotrophic role proposed for marine fungi as degraders of planktonic detritus in the coastal ocean (Gutiérrez et al., 2011; Cunlife et al., 2017). Caution must be observed since $\delta^{13}C$ is determined by that of substrate for fungi growth ($\delta^{13}C$ = -25.5‰, C/N = 6.5, a mix of terrestrial $\delta^{13}C$ and marine C/N). In spite of that, marine-derived fungi are 0.3 ‰ depleted in ^{13}C relative to the substrate, close to carbon isotopic fractionation of 0.6‰ determined for heterotrophic microbial glucose oxidation by *Escherichia coli* (Blair et al., 1985). Defining the biochemical signature of marine fungi certainly merits future research since it will help explaining stable isotope fractionation of organic molecules in the ocean.

In conclusion, our findings strongly suggest that marine fungi play a role in carbon and nutrient cycling in the ocean and that their nutritional value is at least comparable to that of other planktonic organisms. We also demonstrated that marine fungi have a distinctive pattern

of lipids and proteins, and their elemental composition is consistent with a marine source and heterotrophic uptake of phytoplankton-derived organic matter. These findings open new perspectives to understand the contribution of fungi to microbial biomass and marine carbon and nutrient cycling and open new avenues for detection and study of fungi in the ocean.

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References

Alfaro, M., Holder, M., 2006. The Posterior and the Prior in Bayesian Phylogenetics. Annu. Rev. Ecol. Evol. Syst. 37, 19–42. doi:10.1146/annurev.ecolsys.37.091305.110021

Arendt, K.E., Jónasdóttir, S.H., Hansen, P.J., Gärtner, S., 2005. Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. Mar. Biol. 146, 513–530. doi:10.1007/s00227-004-1457-9

Azam, F., 1998. Microbial Control of Oceanic Carbon Flux: The Plot Thickens. Science 694–696.

Azam, F., Malfatti, F., 2007. Microbial structuring of marine ecosystems. Nat. Rev. Microbiol. 5, 782–791. doi:10.1038/nrmicro1747

Bar-on, Y.M., Phillips, R., Milo, R., 2018. The biomass distribution on Earth. Proc. Natl. Acad. Sci. 115, 6506–6511. doi:10.1073/pnas.1711842115

Batista-García, R.A., Sutton, T., Jackson, S.A., Tovar-Herrera, O.E., Balcazar-López, E., Sánchez-Carbente, M., Sánchez-Reyes, A., Dobson, A.D.W., Folch-Mallol, J.L., 2017. Characterization of lignocellulolytic activities from fungi isolated from the deep-sea sponge *Stelletta normani*. PLoS One 12, 1–30.

Blair, N., Leu, A., Muñoz, E., Olsen, J., Kwong, E., Des Marais, D., 1985. Carbon Isotopic Fractionation in Heterotrophic Microbial Metabolism. Appl. Environ. Microbiol. 50, 996–1001.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917.

Bochdansky, A.B., Clouse, M.A., Herndl, G.J., 2016. Dragon kings of the deep sea: marine particles deviate markedly from the common number-size spectrum. Sci. Rep. 6, 1–7. doi:10.1038/srep22633

Boschker, H.T.S., Middelburg, J.J., 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol. Ecol. 40, 85–95.

Brown, P.C., Painting, S.J., Cochrane, K.L., 1991. Estimates of phytoplankton and bacterial biomass and production in the northern and southern Benguela ecosystems. South African J. Mar. Sci. 11, 537–564. doi:10.2989/025776191784287673

Buesing, N., Gessner, M.O., 2006. Benthic Bacterial and Fungal Productivity and Carbon Turnover in a Freshwater Marsh. Appl. Environ. Microbiol. 72, 596–605. doi:10.1128/AEM.72.1.596

Burgaud, G., Le Calvez, T., Arzur, D., Vandenkoornhuyse, P., Barbier, G., 2009. Diversity of culturable marine filamentous fungi from deep-sea hydrothermal vents. Environ. Microbiol. 11, 1588–1600. doi:10.1111/j.1462-2920.2009.01886.x

Burgaud, G., Woehlke, S., Rédou, V., Orsi, W., Beaudoin, D., Barbier, G., Biddle, J.F., Edgcomb, V.P., 2013. Deciphering the presence and activity of fungal communities in marine sediments using a model estuarine system. Aquat. Microb. Ecol. 70, 45–62. doi:10.3354/ame01638

Christie, W.W., 1998. Gas Chromatography – Mass Spectrometry Methods for Structural Analysis of Fatty Acids. Lipids 33, 343–353.

Chuecas, B.L., Riley, J.P., 1969. Component fatty acids of the total lipids of some marine phytoplankton. J. Mar. Biol. Assoc. United Kingdom 2, 97–116.

Cooney, J.J., Doolittle, M.M., Grahl-Nielsen, O., Haaland, I.M., Kirk, P.W., 1993. Comparison of fatty acids of marine fungi using multivariate statistical analysis. J. Ind. Microbiol. 12, 373–374.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholopids in fish and crustacean larviculture. Aquaculture 155, 149–164.

Coutteau, P., Kontara, E.K.M., Sorgeloos, P., 2000. Comparison of phosphatidylcholine purified from soybean and marine fish roe in the diet of postlarval *Penaeus vannamei* Boone. Aquaculture 181, 331–345.

Crowther, T.W., Boddy, L., Jones, T.H., 2012. Functional and ecological consequences of saprotrophic fungus–grazer interactions. ISME J. 6, 1992–2001. doi:10.1038/ismej.2012.53

Cunliffe, M., Hollingsworth, A., Bain, C., Sharma, V., Taylor, J.D., 2017. Algal polysaccharide utilisation by saprotrophic planktonic marine fungi. Fungal Ecol. 30, 135–138. doi:10.1016/j.funeco.2017.08.009

Dabrowski, K., Rusiecki, M., 1983. Content of total and free amino acids in zooplanktonic food of fish larvae. Aquaculture 30, 31–42.

Daneri, G., Dellarossa, V., Quiñones, R., Jacob, B., Montero, P., Ulloa, O., 2000. Primary production and community respiration in the Humboldt Current System off Chile and associated oceanic areas. Mar. Ecol. Prog. Ser. 197, 41–49.

Danger, M., Chauvet, E., 2013. Elemental composition and degree of homeostasis of fungi: are aquatic hyphomycetes more like metazoans, bacteria or plants ? Fungal Ecol. 6, 453–457. doi:10.1016/j.funeco.2013.05.007

Danger, M., Gessner, M.O., Barlocher, F.B., 2016. Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. Fungal Ecol. 19, 100–111. doi:10.1016/j.funeco.2015.09.004

Das, S., Lyla, P.S., Khan, S.A., 2007. Fatty Acid Profiles of Marine Benthic Microorganisms Isolated from the Continental Slope of Bay of Bengal: A Possible Implications in the Benthic Food Web. Ocean Sci. J. 42, 247–254.

Dávila, P., Figueroa, D., Müller, E., 2002. Freshwater input into the coastal ocean and its relation with the salinity distribution off austral Chile (35–55°S). Cont. Shelf Res. 22, 521–534. doi:10.1016/S0278-4343(01)00072-3

Devi, P., Shridhar, M.P.D., Souza, L.D., Naik, C.G., 2006. Cellular fatty acid composition of marine-derived fungi. Indian J. Mar. Sci. 35, 359–363.

Escribano, R., Perez, C., 2010. Variability in fatty acids of two marine copepods upon changing food supply in the coastal upwelling zone off Chile: importance of the picoplankton and nanoplankton fractions. J. Mar. Biol. Assoc. United Kingdom 90, 301–313. doi:10.1017/S002531540999083X

Falkowski, P.G., Fenchel, T., Delong, E.F., 2008. The Microbial Engines That Drive Earth's Biogeochemical Cycles. Science 320, 1034–1039. doi:10.1126/science.1153213

Feeney, M.J., Dwyer, J., Hasler-lewis, C.M., Milner, J.A., Noakes, M., Rowe, S., Wach, M., Beelman, R.B., Caldwell, J., Cantorna, M.T., Castlebury, L.A., Chang, S., Cheskin, L.J., Clemens, R., Drescher, G., Iii, V.L.F., Haytowitz, D.B., Hubbard, V.S., Law, D., Miller, A.M., Minor, B., Percival, S.S., Riscuta, G., Schneeman, B., Thornsbury, S., Toner,

C.D., Woteki, C.E., Wu, D., 2014. Mushrooms and Health Summit Proceedings. J. Nutr. 144, 1128S–1136S. doi:10.3945/jn.114.190728.topics

Frostegard, A., Baath, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–60.

Fuentes, M., Quiñones, R.A., Gutiérrez, M.H., Pantoja, S., 2015. Fuentes, M., Quiñones, R.A., Gutiérrez, M.H., Pantoja, S., 2015. Effects of temperature and glucose concentration on the growth and respiration of fungal species isolated from a highly productive coastal upwelling ecosystem. Fungal Ecol. 13, 135–149.Effec. Fungal Ecol. 13, 135–149.

Gadd, G., 2006. Fungi in biogeochemical cycles. Cambridge university press, New York.

Gao, Z., Li, B., Zheng, C., Wang, G., 2008. Molecular detection of fungal communities in the hawaiian marine sponges *Suberites zeteki* and *Mycale armata*. Appl. Environ. Microbiol. 74, 6091–6101. doi:10.1128/AEM.01315-08

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes, application to the identification of mycorrihiza and rusts. Mol. Ecol. 2, 113–118. doi:Doi 10.1111/J.1365-294x.1993.Tb00005.X

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., Gascuel, O., 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst. Biol. 59, 307–321. doi:10.1093/sysbio/syq010

Gutiérrez, M.H., Galand, P.E., Moffat, C., Pantoja, S., 2015. Melting glacier impacts community structure of Bacteria, Archaea and Fungi in a Chilean Patagonia fjord. Environ. Microbiol. 17, 3882–3897. doi:10.1111/1462-2920.12872

Gutiérrez, M.H., Garcés, D. V, Pantoja, S., González, R., Quiñones, R.A., 2017. Environmental fungal diversity in the upwelling ecosystem off central Chile and potential contribution to enzymatic hydrolysis of macromolecules in coastal ecotones. Fungal Ecol. 29, 90–95. doi:10.1016/j.funeco.2017.07.002

Gutiérrez, M.H., Jara, A.M., Pantoja, S., 2016. Fungal parasites infect marine diatoms in the upwelling ecosystem of the Humboldt Current System off central Chile. Environ. Microbiol. 18, 1–24. doi:10.1111/1462-2920.13257

Gutiérrez, M.H., Narváez, D., Daneri, G., Montero, P., 2018. Linking Seasonal Reduction of Microbial Diversity to Increase in Winter Temperature of Waters of a Chilean Patagonia Fjord. Front. Mar. Sci. 5. doi:10.3389/fmars.2018.00277

Gutiérrez, M.H., Pantoja, S., Quiñones, R.A., Gonzalez, R., 2010. First record of filamentous fungi in the coastal upwelling ecosystem off central Chile. Gayana 74, 66–73.

Gutiérrez, M.H., Pantoja, S., Tejos, E., Quiñones, R.A., 2011. The role of fungi in processing marine organic matter in the upwelling ecosystem off Chile. Mar. Biol. 158, 205–219. doi:10.1007/s00227-010-1552-z

Hagen, W., Schnack-Schiel, S., 1996. Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction ? Deep. Res. Part I Oceanogr. Res. Pap. 43, 139–158.

Houbraken, J., Frisva, J., Samson, R., 2011. Taxonomy of *Penicillium* section Citrina. Stud. Mycol. 70, 53–158. doi:doi:10.3114/sim.2011.70.02

Jeffries, H.P., 1970. Seasonal composition of temperate plankton comunities: Fatty acids. Limnol. Oceanogr. 15, 419–426.

Jephcott, T.G., Van Ogtrop, F.F., Gleason, F.H., Macarthur, D.J., Scholz, B., 2017. The ecology of chytrid and aphelid parasites of phytoplankton, in: Dighton, J., White, J.F., Oudemans, P. (Eds.), In The Fungal Community. Boca Raton, FL: CRC Press, pp. 239–256.

Jeuniaux, C., Voss-foucart, M.F.O., 1991. Chitin Biomass and Production in the Marine Environment. Biochem. Syst. Ecol. 19, 347–356.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. 40, 2977–2991. doi:10.1016/j.soilbio.2008.08.017

Jørgensen, N.O.G., Stepanauskas, R., 2009. Biomass of pelagic fungi in Baltic rivers. Hyrobiologia 623, 105–112. doi:10.1007/s10750-008-9651-2

Kagami, M., Helmsing, N.R., Donk, E. Van, 2011. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hyrobiologia 659, 49–54. doi:10.1007/s10750-010-0274-z

Kagami, M., Miki, T., Takimoto, G., 2014. Mycoloop: chytrids in aquatic food webs. Front. Microbiol. 5, 1–9. doi:10.3389/fmicb.2014.00166

Kaur, A., Chaudhary, A., Kaur, A., Choudhary, R., Kaushik, R., 2005. Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. Curr. Sci. 89, 1103–1112. doi:10.2307/24110962

Kohlmeyer, J., Kohlmeyer, E., 1979. Marine Mycology: The Higher Fungi. Academic Press, New York.

Kurtzman, C.P., Robnett, C.J., 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek 73, 331–371. doi:10.1023/A:1001761008817

Landeweert, R., Leeflang, P., Kuyper, T.W., Hoffland, E., Rosling, A., Wernars, K., Smit, E., 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl. Environ. Microbiol. 69, 327–333. doi:10.1128/AEM.69.1.327-333.2003

Latgé, J., 2007. MicroReview The cell wall: a carbohydrate armour for the fungal cell. Mol. Microbiol. 66, 279–290. doi:10.1111/j.1365-2958.2007.05872.x

Lee, C., Wakeham, S.G., Hedges, J.I., 1988. The Measurement of Oceanic Particle Flux-are "Swimmers" A Problem? Oceanography 1, 34–36.

Lee, R.F., Hagen, W., Kattner, G., 2006. Lipid storage in marine zooplankton. Mar. Ecol. Prog. Ser. 307, 273–306.

Lewis, R.W., 1969. The fatty acid composition of arctic marine phytoplankton and zooplankton with special reference to minor acids. Limnol. Oceanogr. 14, 35–40.

Lindroth, P., Mopper, K., 1979. High Performance Liquid Chromatographic Determination of Subpicomole Amounts of Amino Acids by Precolumn Fluorescence Derivatization with o-Phthaldialdehyde. Anal. Chem. 51, 1667–1674. doi:10.1021/ac50047a019

Lyncht, M.D.J., Thorn, R.G., 2006. Diversity of basidiomycetes in Michigan agricultural soils. Appl. Environ. Microbiol. 72, 7050–7056. doi:10.1128/AEM.00826-06

Medina, G., Castro, L., Pantoja, S., 2014. Fatty acids in *Merluccius australis* tissues, a comparison between females from inshore and offshore spawning areas in the Chilean Patagonia. Fish. Res. 160, 41–49. doi:10.1016/j.fishres.2013.11.005

Méjanelle, L., Laureillard, J., 2008. Lipid biomarker record in surface sediments at three sites of contrasting productivity in the tropical North Eastern Atlantic. Mar. Chem. 108, 59–76. doi:10.1016/j.marchem.2007.10.002

Meyers, P., 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. 114, 289–302.

Montgomery, H.J., Monreal, C.M., Young, J.C., Seifert, K.A., 2000. Determinination of soil fungal biomass from soil ergosterol analyses. Soil Biol. Biochem. 32, 1207–1217.

Montgomeryl, M.T., Nicholas, A., David, L., 1990. A simple assay for chitin: application to sediment trap samples from the subarctic Pacific. Mar. Ecol. Prog. Ser. 64, 301–308. doi:10.3354/meps064301

Murphy, W.J., Eizirik, E., O'Brien, S.J., Madsen, O., Scally, M., Douady, C.J., Teeling, E., Ryder, O. a, Stanhope, M.J., de Jong, W.W., Springer, M.S., 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 294, 2348–2351. doi:10.1126/science.1067179

Najdek, M., 1997. Unusual changes of zooplankton fatty acid composition in the northern Adriatic during the 1991 mucilage event. Mar. Ecol. Prog. Ser. 159, 143–150.

Newell, S.Y., Blum, L.K., Crawford, R.E., Dai, T., Dionne, M., 2000. Autumnal Biomass and Potential Productivity of Salt Marsh Fungi from 29° to 43° North Latitude along the United States Atlantic Coast. Appl. Environ. Microbiol. 66, 180–185.

Nichols, D.S., Nichols, P.D., Mcmeekin, T.A., 1993. Polyunsaturated fatty acids in Antarctic bacteria. Antarct. Sci. 5, 149–160.

Olsson, A., 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. 29, 303–310.

Oren, A., Mana, L., 2002. Amino acid composition of bulk protein and salt relationships of selected enzymes of *Salinibacter ruber*, an extremely halophilic bacterium. Extremophiles 6, 217–223.

Pantoja, S., Iriarte, L., Daneri, G., 2011. Oceanography of the Chilean Patagonia. Cont. Shelf Res. 31, 149–153. doi:10.1016/j.csr.2010.10.013

Paul, E.., 2014. Soil microbiology, ecology and biochemistry. Academic press.

Pomeroy, 1974. The Ocean's Food web, A Changing Paradigm. Bioscience 24, 499–504.

Quiñones, R., Gutiérrez, M., Daneri, G., Gutiérrez, D., González, H., Chávez, F., 2010. Pelagic carbon fluxes in the Humboldt Current System, in: Liu, K., Atkinson, L., Quiñones, A., Talaue-McManus, L. (Eds.), Carbon and Nutrient Fluxes in Global Continental Margins: A Global Synthesis. IGBP Series Book, Springer-Verlag New York., pp. 44–65.

Quiñones, R.A., Levipan, H., Urritia, H., 2009. Spatial and temporal variability of planktonic archaeal abundance in the Humboldt Current System off Chile. Deep. Res. Part II Top. Stud. Oceanogr. 56, 1073–1082. doi:10.1016/j.dsr2.2008.09.012

Raghukumar, S., 2002. Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids). Eur. J. Protistol. 145, 127–145.

Raymont, J.E.G., Ferguson, C.F., Raymont, J.K.B., 1973. Biogeochemical studies on marine zooplankton the aminoacid composition of some local species. Spec. Publ. Mar. Bol. Ass. India 60, 91–99.

Redberg, G.L., Hibbett, D.S., Ammirati, J.F., Rodriguez, R.J., 2003. Phylogeny and genetic diversity of *Bridgeoporus nobilissimus* inferred using mitochondrial and nuclear rDNA sequences. Mycologia 95, 836–845.

Redfield, A.C., 1958. The biological control of chemical factors in the environment. Am. Sci. 46, 205–221.

Richards, T.A., Jones, M.D.M., Leonard, G., Bass, D., 2012. Marine Fungi: Their Ecology and Molecular Diversity. Ann. Rev. Mar. Sci. 4, 495–522. doi:10.1146/annurev-marine-120710-100802

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A., Huelsenbeck, J.P., 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542. doi:10.1093/sysbio/sys029

Russell, N.J., Nichols, D.S., 1999. Polyunsaturated fatty acids in marine bacteria-a dogma rewritten. Microbial 145, 767–779.

Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., and Fungal Barcoding Consortium., 2012. From the Cover: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. 109, 6241–6246. doi:10.1073/pnas.1117018109

Simon, M., Azam, F., 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. 51, 201–213.

Souza, C.P., Almeida, B.C., Colwell, R.R., Rivera, I.N.G., 2011. The Importance of Chitin in the Marine Environment. Mar. Biotechnol. 13, 823–830.

Sturt, H.F., Summons, R.E., Smith, K., Elvert, M., Hinrichs, K.U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry - new biomarkers for biogeochemistry and microbial ecology. Rapid Commun. Mass Spectrom. 18, 617–628. doi:10.1002/rcm.1378

Taylor, J., Parkes, R.J., 1983. The Cellular Fatty Acids of the Sulphate-reducing Bacteria, *Desulfobacter* sp., *Desulfobulbus* sp. and *Desulfovibvio* desulfuvican. J. Gen. Microbiol. 129, 3303–3309.

Taylor, J.D., Cunliffe, M., 2016. Multi-year assessment of coastal planktonic fungi reveals environmental drivers of diversity and abundance. ISME J. 10, 2118–2128. doi:10.1038/ismej.2016.24

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680. doi:10.1093/nar/22.22.4673

Tisthammer, K.H., Manuel, G., Stuart, A., 2016. ScienceDirect Global biogeography of marine fungi is shaped by the environment. Fungal Ecol. 19, 39–46. doi:10.1016/j.funeco.2015.09.003

Tolosa, I., Vescovali, I., Leblond, N., Marty, J., Mora, S. De, Prieur, L., 2004. Distribution of pigments and fatty acid biomarkers in particulate matter from the frontal structure of the Alboran Sea (SW Mediterranean Sea). Mar. Chem. 88, 103–125. doi:10.1016/j.marchem.2004.03.005

Ulloa, O., Delong, E.F., Letelier, R.M., Stewart, F.J., 2012. Microbial oceanography of anoxic oxygen minimum zones. Proc. Natl. Acad. Sci. 109, 15996–16003. doi:10.1073/pnas.1205009109

Vera, J., Gutiérrez, M.H., Palfner, G., Pantoja, S., 2017. Diversity of culturable filamentous Ascomycetes in the eastern South Pacific Ocean off Chile. World J. Microbiol. Biotechnol. 33. doi:10.1007/s11274-017-2321-7

Verma, B., Robarts, R.D., Headley, J. V, 2003. Seasonal Changes in Fungal Production and Biomass on Standing Dead Scirpus lacustris Litter in a Northern Prairie Wetland. Appl. Environ. Microbiol. 69, 1043–1050. doi:10.1128/AEM.69.2.1043

Vestal, R., White, D., 1989. Lipid Analysis in Microbial Ecology. Quantitative approaches to the study of microbial communities. Oxford Journals 39, 535–541.

Wallis, I.R., Claridge, A.W., Trappe, J.M., 2012. Nitrogen content, amino acid composition and digestibility of fungi from a nutritional perspective in animal mycophagy. Fungal Biol. 116, 590–602. doi:10.1016/j.funbio.2012.02.007

Wang, J.T., Han, T., Li, X.Y., Hu, S.X., Jiang, Y.D., Wang, C.L., 2016. Effects of dietary phosphatidylcholine (PC) levels on the growth, molt performance and fatty acid composition of juvenile swimming crab, *Portunus trituberculatus*. Anim. Feed Sci. Technol. 216, 225–233. doi:10.1016/j.anifeedsci.2016.03.023

Wang, T., Tong, S., Liu, N., Li, F., Wells, M.L., Gao, K., 2017. The fatty acid content of plankton is changing in subtropical coastal waters as a result of OA: results from a mesocosm study. Mar. Environ. 132, 51–62.

Wang, X., Singh, P., Gao, Z., Zhang, X., Johnson, Z.I., Wang, G., 2014. Distribution and diversity of planktonic fungi in the west pacific warm pool. PLoS One 9, 1–7. doi:10.1371/journal.pone.0101523

Weete, J.D., Abril, M., Blackwell, M., 2010. Phylogenetic Distribution of Fungal Sterols. PLoS One 5, 3–8. doi:10.1371/journal.pone.0010899

White, T.J., Bruns, S., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc. A Guid. to Methods Appl. 38, 315–322. doi:citeulike-article-id:671166

Wilcox, T.P., Zwickl, D.J., Heath, T.A., Hillis, D.M., 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. 25, 361–371. doi:10.1016/S1055-7903(02)00244-0

Wirth, F., Goldani, L.Z., 2012. Epidemiology of *Rhodotorula*: An Emerging Pathogen. Interdiscip. Perspect. Infect. Dis. 2012. doi:10.1155/2012/465717

Wörmer, L., Lipp, J.S., Schröder, J.M., Hinrichs, K.U., 2013. Application of two new LC-ESI-MS methods for improved detection of intact polar lipids (IPLs) in environmental samples. Org. Geochem. 59, 10–21. doi:10.1016/j.orggeochem.2013.03.004

Zhang, J., Elser, J.J., 2017. Carbon:Nitrogen:Phosphorus Stoichiometry in Fungi: A Meta-Analysis. Front. Microbiol. 8, 1–9. doi:10.3389/fmicb.2017.01281

Zhukova, N. V, Aizdaicher, N.A., 1995. Fatty acid composition of 15 species of marine microalgae. Phytochemistry 39, 351–356.

Table and Figure Captions

Table 1. Elemental and C and N stable isotopic composition, caloric content and abundance of major classes of organic molecules in marine fungal strains

Table 2. Comparison of carbon, nitrogen and caloric content per dry weight (gdw) of marine organisms

Figure 1. Sampling areas in the eastern South Pacific Ocean off Chile. A) Oxygen Minimum Zone off Iquique, B) South Pacific subtropical gyre near Easter Island, C) Coastal upwelling ecosystem of central Chile off Concepción, and D) Baker fjord in Chilean Patagonia

Figure 2. Bayesian tree inferred from ITS sequences of fungi isolated from eastern South Pacific Ocean of Chile

Figure 3. Average composition of amino acids (A), fatty acids (B), sterols (C) and intact polar lipids (D) in marine fungal strains

Figure 4. Principal component analysis showing singular signature of A) amino acids and B) fatty acids of marine-derived fungi compared with prokaryote, phytoplankton and zooplankton (Chuecas and Riley, 1969; Lewis, 1969; Jeffries, 1970; Raymont et al., 1973; Taylor and Parkes, 1983; Lee, 1988; Simon and Azam, 1989; Brown, 1991; Nichols et al., 1993; Zhukova and Aizdaicher, 1995; Russell and Nichols, 1999; Oren and Mana 2002; Das et al. 2007; Escribano and Pérez, 2010; Medina et al. 2014; Wang et a. 2017). Arrows indicate amino acids and fatty acids that explain singular signatures of each group.

Figure 5. Chemical and stable isotopic composition of carbon and nitrogen of marine fungi in the framework of Meyer´s classification of terrestrial and marine organic matter. Values were taken from Table 1 of Meyer (1994).

Figure 1

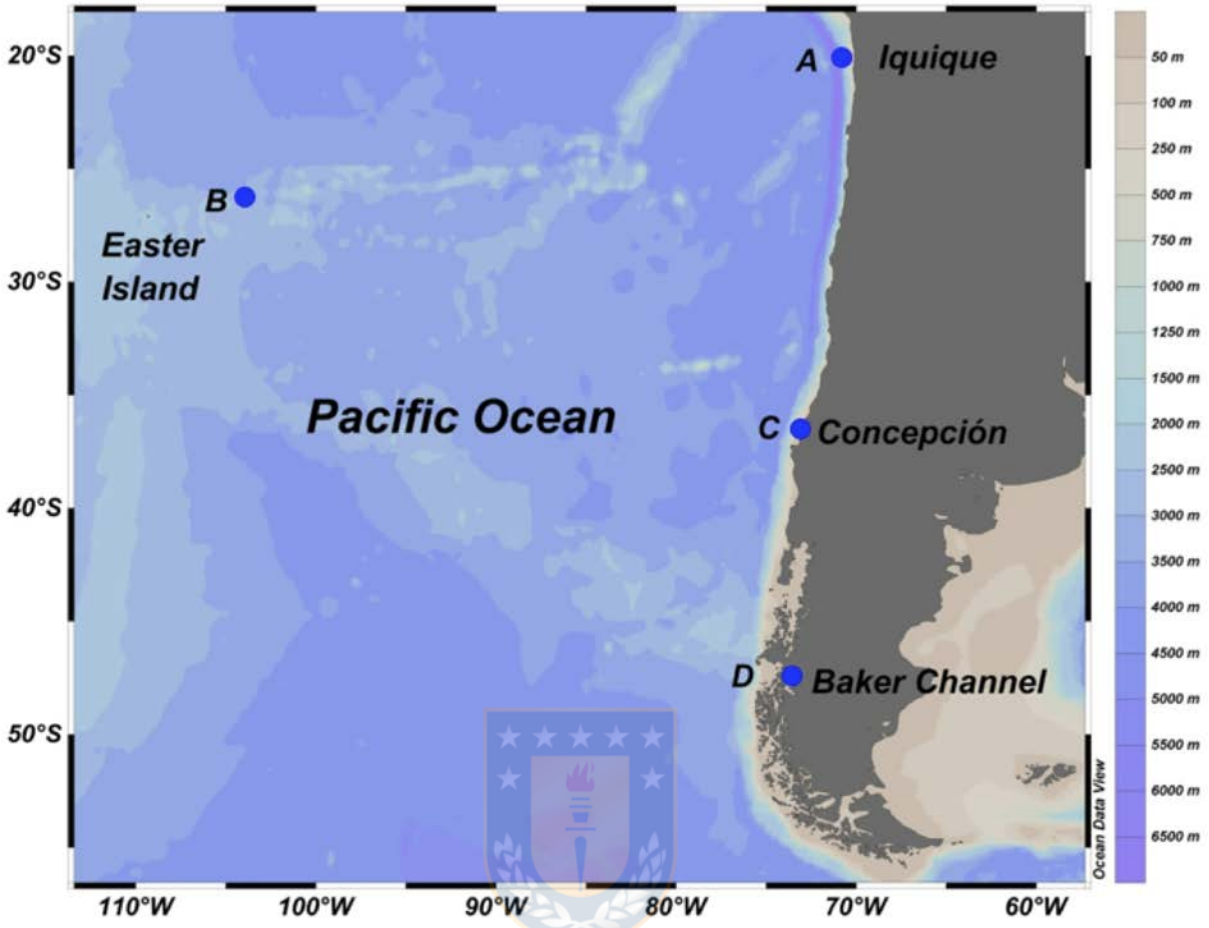
20°S
30°S
40°S
50°S
110°W
100°W
90°W
80°W
70°W
60°W
A
Iquique
B
Easter
Island
Pacific Ocean
C
Concepción
D
Baker Channel
50 m
100 m
250 m
500 m
750 m
1000 m
1250 m
1500 m
2000 m
2500 m
3000 m
3500 m
4000 m
4500 m
5000 m
5500 m
6000 m
6500 m
Ocean Data View

Figure 2

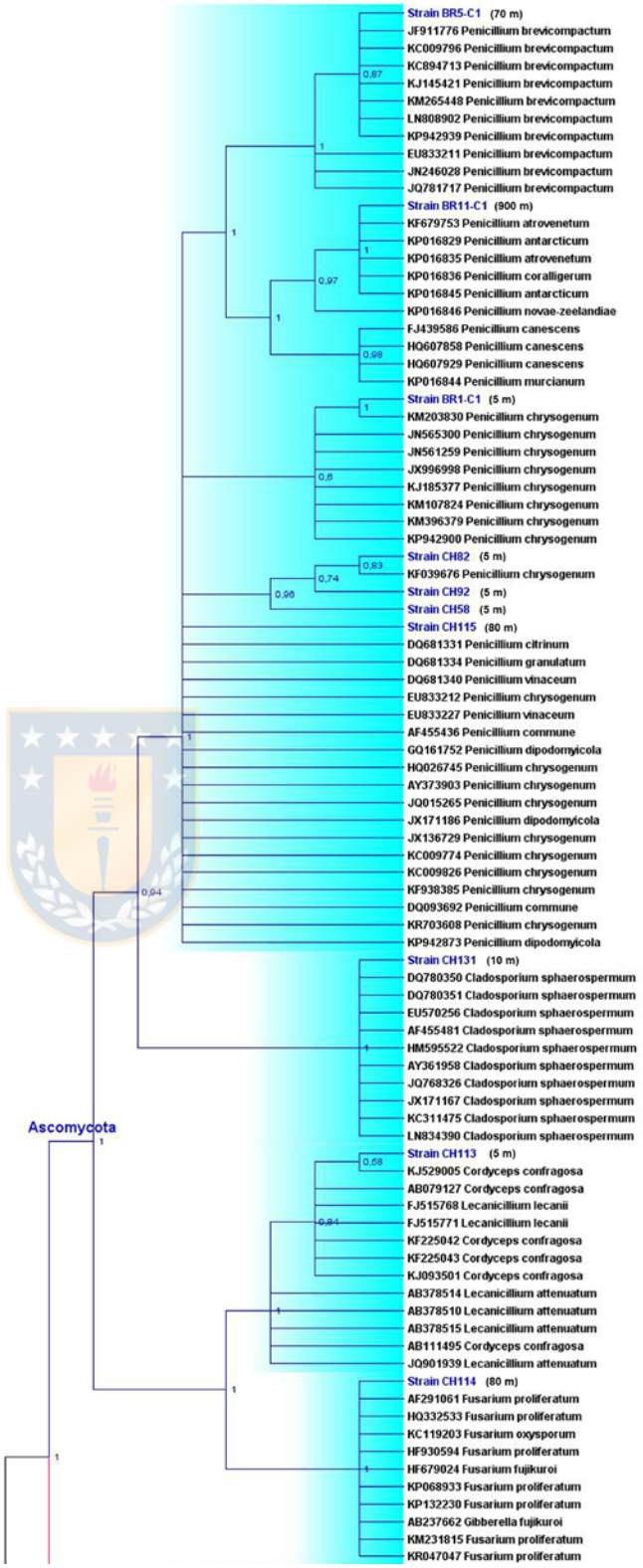
Strain BR5-C1 (70 m)
JF911776 Penicillium brevicompactum
KC009796 Penicillium brevicompactum
KC894713 Penicillium brevicompactum
KJ145421 Penicillium brevicompactum
KM265448 Penicillium brevicompactum
LN808902 Penicillium brevicompactum
KP942939 Penicillium brevicompactum
EU833211 Penicillium brevicompactum
JN246028 Penicillium brevicompactum
JQ781717 Penicillium brevicompactum
Strain BR11-C1 (900 m)
KF679753 Penicillium atrovenetum
KP016829 Penicillium antarcticum
KP016835 Penicillium atrovenetum
KP016836 Penicillium coralligerum
KP016845 Penicillium antarcticum
KP016846 Penicillium novae-zeelandiae
FJ439586 Penicillium canescens
HQ607858 Penicillium canescens
HQ607929 Penicillium canescens
KP016844 Penicillium murcianum
Strain BR1-C1 (5 m)
KM203830 Penicillium chrysogenum
JN565300 Penicillium chrysogenum
JN561259 Penicillium chrysogenum
JX996998 Penicillium chrysogenum
KJ185377 Penicillium chrysogenum
KM107824 Penicillium chrysogenum
KM396379 Penicillium chrysogenum
KP942900 Penicillium chrysogenum
Strain CH82 (5 m)
KF039676 Penicillium chrysogenum
Strain CH92 (5 m)
Strain CH58 (5 m)
Strain CH115 (80 m)
DQ681331 Penicillium citrinum
DQ681334 Penicillium granulatum
DQ681340 Penicillium vinaceum
EU833212 Penicillium chrysogenum
EU833227 Penicillium vinaceum
AF455436 Penicillium commune
GQ161752 Penicillium dipodomyicola
HQ026745 Penicillium chrysogenum
AY373903 Penicillium chrysogenum
JQ015265 Penicillium chrysogenum
JX171186 Penicillium dipodomyicola
JX136729 Penicillium chrysogenum
KC009774 Penicillium chrysogenum
KC009826 Penicillium chrysogenum
KF938385 Penicillium chrysogenum
DQ093692 Penicillium commune
KR703608 Penicillium chrysogenum
KP942873 Penicillium dipodomyicola
Strain CH131 (10 m)
DQ780350 Cladosporium sphaerospermum
DQ780351 Cladosporium sphaerospermum
EU570256 Cladosporium sphaerospermum
AF455481 Cladosporium sphaerospermum
HM595522 Cladosporium sphaerospermum
AY361958 Cladosporium sphaerospermum
JQ768326 Cladosporium sphaerospermum
JX171167 Cladosporium sphaerospermum
KC311475 Cladosporium sphaerospermum
LN834390 Cladosporium sphaerospermum
Ascomycota
Strain CH113 (5 m)
KJ529005 Cordyceps confragosa
AB079127 Cordyceps confragosa
FJ515768 Lecanicillium lecanii
FJ515771 Lecanicillium lecanii
KF225042 Cordyceps confragosa
KF225043 Cordyceps confragosa
KJ093501 Cordyceps confragosa
AB378514 Lecanicillium attenuatum
AB378510 Lecanicillium attenuatum
AB378515 Lecanicillium attenuatum
AB111495 Cordyceps confragosa
JQ901939 Lecanicillium attenuatum
Strain CH114 (80 m)
AF291061 Fusarium proliferatum
HQ332533 Fusarium proliferatum
KC119203 Fusarium oxysporum
HF930594 Fusarium proliferatum
HF679024 Fusarium fujikuroi
KP068933 Fusarium proliferatum
KP132230 Fusarium proliferatum
AB237662 Gibberella fujikuroi
KM231815 Fusarium proliferatum
KR047047 Fusarium proliferatum

Figure 2 continued

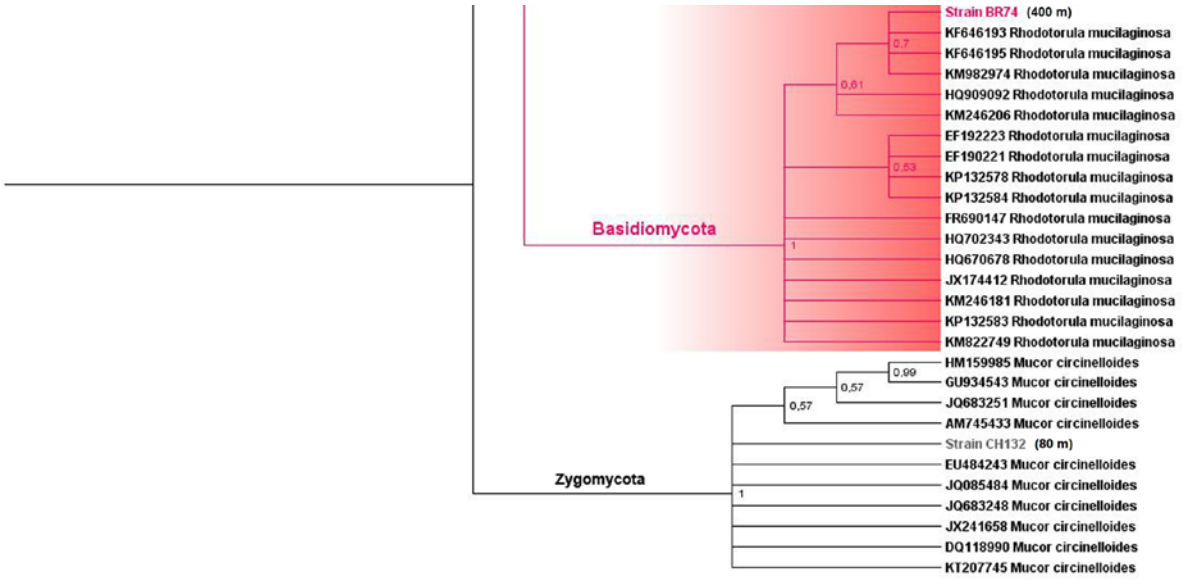

Figure 3

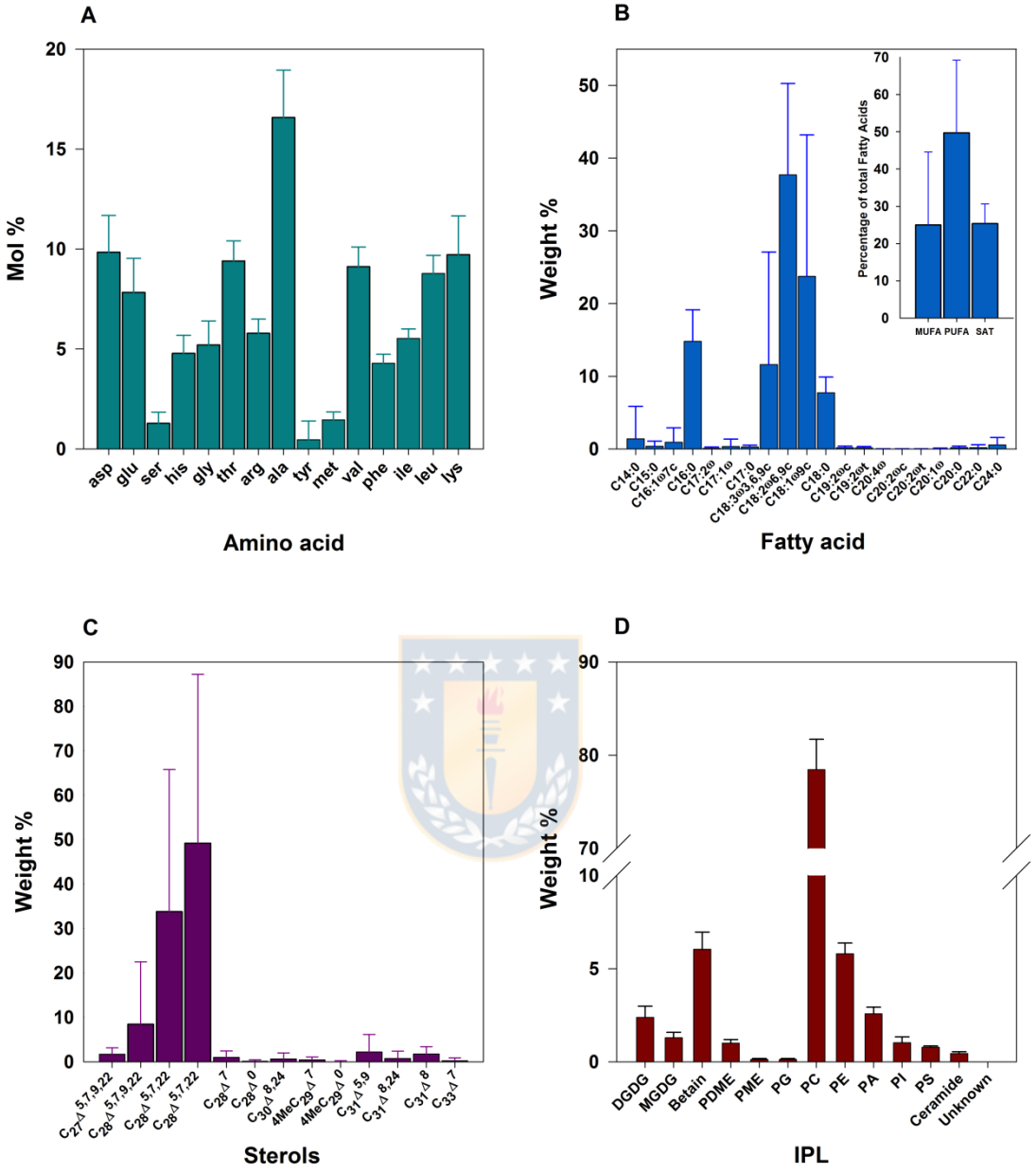
A
Mol %
Amino acid
asp glu ser his gly thr arg ala tyr met val phe ile leu lys
B
Weight %
Fatty acid
Percentage of total Fatty Acids
MUFA PUFA SAT
C
Weight %
Sterols
D
Weight %
IPL
DGDG MGDG Betain PDME PME PG PC PE PA PI PS Ceramide Unknown

Figure 4

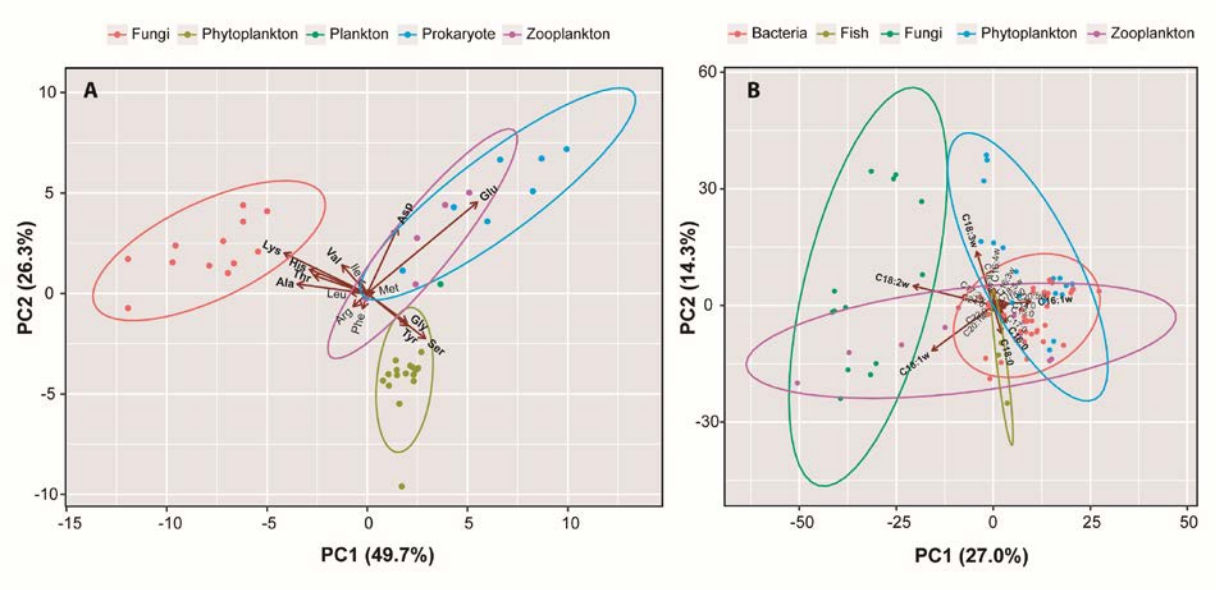
Fungi
Phytoplankton
Plankton
Prokaryote
Zooplankton
A
PC2 (26.3%)
PC1 (49.7%)
Lys
His
Thr
Ala
Val
Ile
Leu
Arg
Phe
Met
Asp
Glu
Gly
Tyr
Ser
Bacteria
Fish
Fungi
Phytoplankton
Zooplankton
B
PC2 (14.3%)
PC1 (27.0%)
C18:3w
C18:2w
C16:1w
C14:1w
C16:0

Figure 5

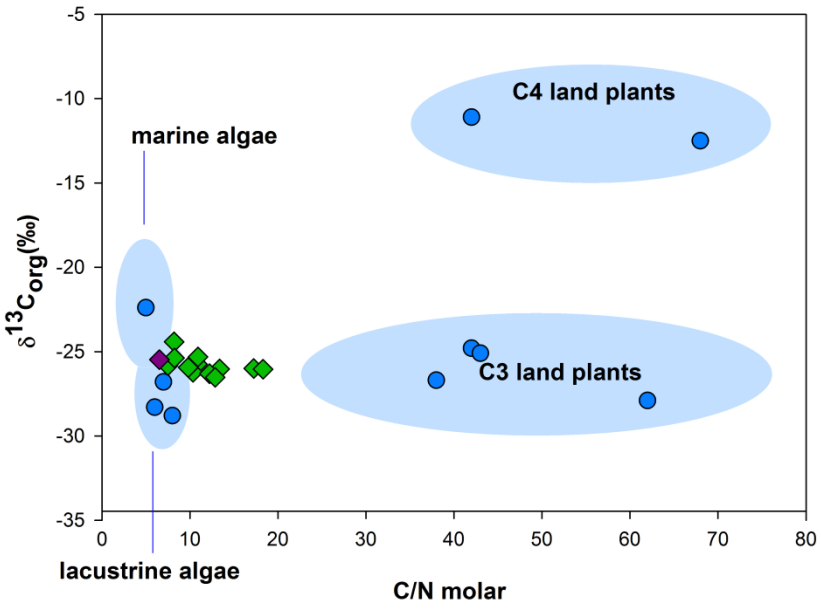
C4 land plants
marine algae
C3 land plants
lacustrine algae
C/N molar
δ13Corg(‰)
-5
-10
-15
-20
-25
-30
-35
0
10
20
30
40
50
60
70
80

Figure 1. Supplemental information

A. Distribution of elemental composition, caloric content and proteins and fatty acids of fungal strains among water depths
B. Distribution of elemental composition, caloric content and proteins and fatty acids of fungal strains among zones of collection.
C. Distribution of head groups of intact polar lipids of fungal strains among depths and zones of collection

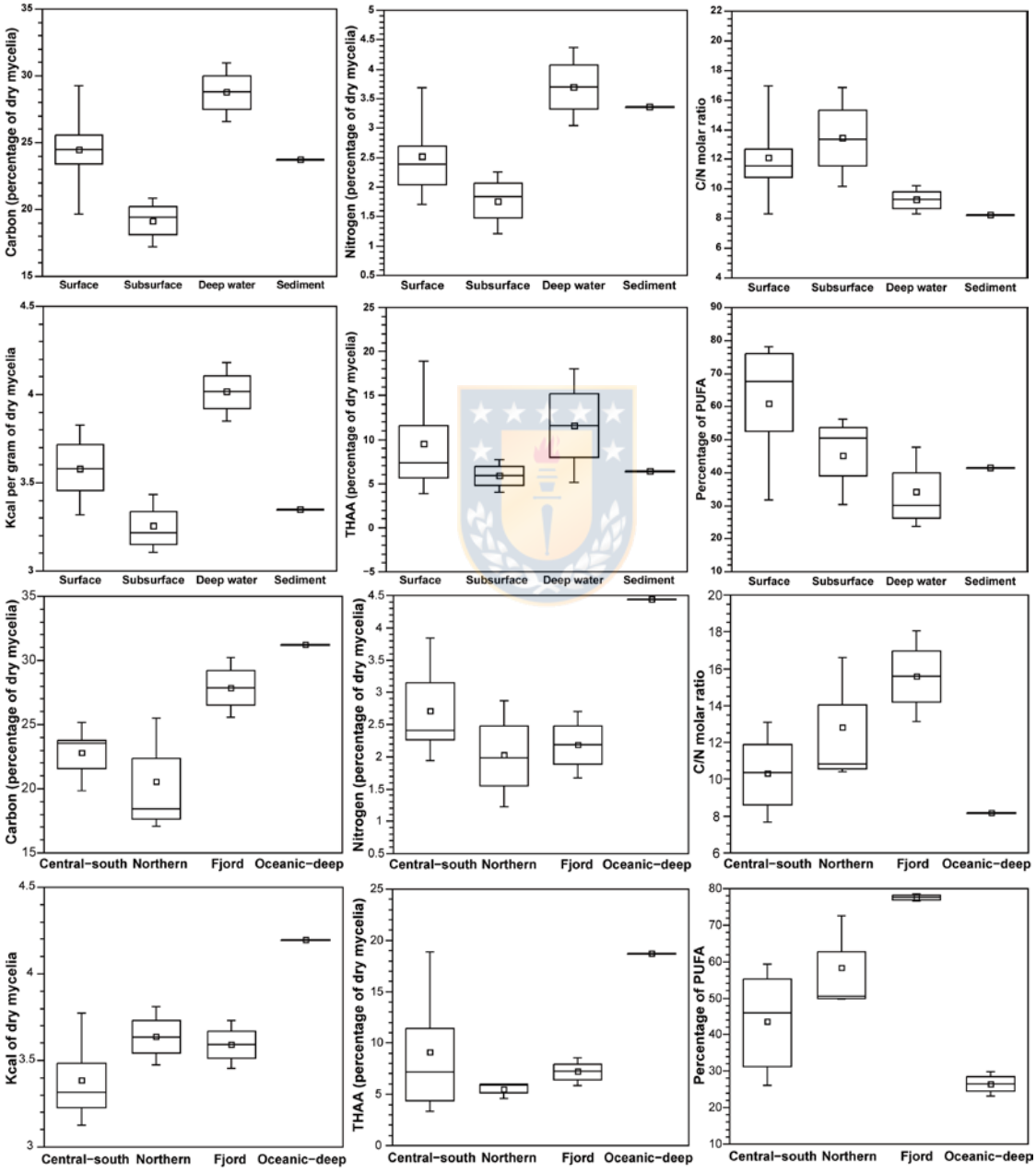

Figure 1. Supplemental information continued

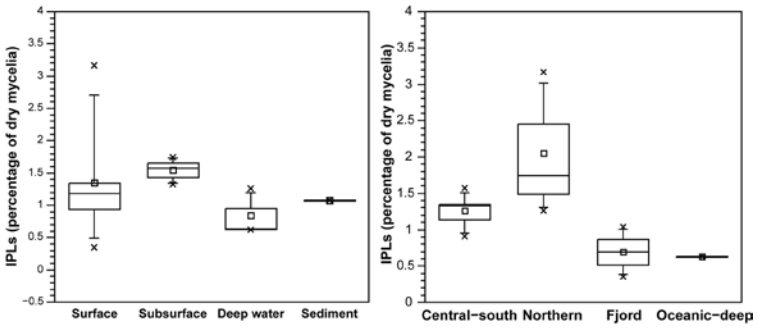

Figure 2 Supplemental information

Average mol% amino acid of marine fungi from this study, and bacteria (*Salinibacter ruber*, *Halanaerobium praevalens*), archaea (*Holoarcula marismortoui*, *Halobacterium salinarum*) from Ren and Mana (2002). Values reported by Ren and Mana (2002) were corrected to account for our lack of detection of proline in our procedure.

Ren A, L Mana. 2002. Amino acid composition of bulk protein and salt relationships ofselected enzymes of *Salinibacter ruber*, an extremely halophilic bacterium

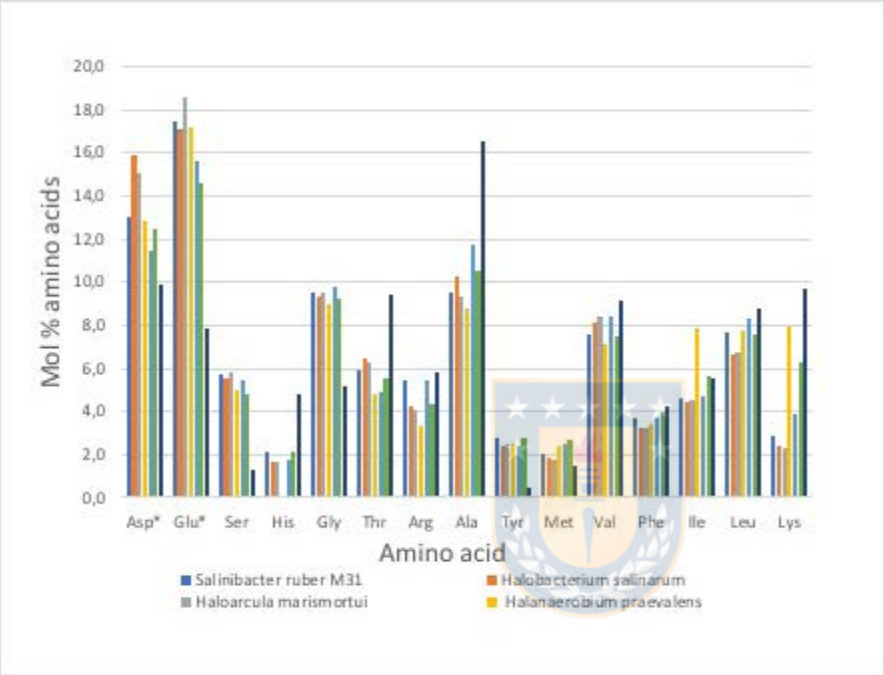

Table 1

Strain GOM ID	Taxonomy	Latitude	Longitude	Sampling site	Depth of collection (m)	Organic carbon (%)	$\delta^{13}C$	Nitrogen (%)	$\delta^{15}N$	C/N molar	kcal gdw^{-1}	THAA (mg gdw^{-1})	FAMEs (mg gdw^{-1})	Sterols (mg gdw^{-1})	IPLs (mg gdw^{-1})
BR1	*Penicillium sp.*	20,83	70,80	Coastal Upwelling northern Chile	5	18.4	-25.7	2.0	0.20	10.8	3.6	60.2	0.43	0.07	31.6
BR11	*Penicillium sp.*	20,83	70,80	Coastal Upwelling northern Chile	900	26.3	-26.2	3.0	0.25	10.3	3.8	44.6	2.77	0.11	12.6
BR5	*Penicillium brevicompactum*	20,83	70,80	Coastal Upwelling northern Chile	70	16.9	-26.0	1.1	0.02	17.3	3.5	59.4	1.14	0.02	17.5
BR71	Not identified yeast	26,14	103,57	Oligotrophic ocean near Easter Island	400	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.4
BR74	*Rhodotorula mucilaginosa*	26,14	103,57	Oligotrophic ocean near Easter Island	400	31.2	-24.4	4.4	4.88	8.2	4.2	187.4	0.61	0.10	6.3
CH113	*Lecanicillium sp.*	36,50	73,10	Coastal Upwelling central Chile	5	25.7	-25.8	4.0	-0.04	7.5	3.9	209.5	0.39	0.50	13.3
CH114	*Fusarium sp.*	36,50	73,10	Coastal Upwelling central Chile	80	19.4	-25.9	2.3	0.91	9.8	3.1	79.6	3.00	0.12	15.7

Table 1 continued

CH115	*Penicillium*	36,50	73,10	Coastal Upwelling central Chile	80	21.0	-26.0	1.8	0.10	13.4	3.2	37.9	1.03	0.59	13.2
CH131	*Cladosporium sphaerospermum*	36,38	72,89	Coastal Upwelling central Chile	10	23.4	-25.3	2.5	3.23	10.9	3.5	32.3	0.78	0.09	13.5
CH132	*Mucor circinelloides*	36,50	73,10	Coastal Upwelling central Chile	Surface sediment	23.8	-25.4	3.4	0.47	8.3	3.4	64.0	1.51	0.33	10.8
CH58	*Penicillium sp.*	36,50	73,10	Coastal Upwelling central Chile	5	23.7	-26.3	2.3	-0.15	12.2	3.3	125.8	0.15	0.37	9.1
CH92	*Penicillium sp.*	47,41	73,57	Chilean Patagonian fjord	0	30.5	-26.5	2.8	0.27	12.9	3.7	57.0	0.03	0.01	3.5
CH96	Not identified yeast	47,41	73,57	Chilean Patagonian fjord	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.4
CH82	*Penicillium sp.*	47,41	73,57	Chilean Patagonian fjord	0	25.3	-26.0	1.6	0.18	18	3.4	87.2	0.28	0.06	
Average						**23.8**	**-25.8**	**2.6**	**0.9**	**12**	**3.6**	**87.1**	**1.01**	**0.20**	**12.6**
SD						**4.4**	**0.6**	**1.0**	**1.6**	**3**	**0.3**	**57.8**	**0.98**	**0.20**	**6.9**
Dry culture medium						40.3	-25.5	7.2	0.02	6.5					

gdw = grams per dry weight, ND = No data

Table 2

Group of organisms	Carbon (% dw)	Nitrogen (% dw)	Caloric content (kcal gdw^{-1})	References
Phytoplankton	34.1 -– 50.8* 16.5 – 34.7	4.6-6.9 5.1 – 11.6* 1.1 – 6.3	2.5 – 4.8 2.1 – 3.7	Whyte, 1987 Peltomaa et al. 2017* Platt and Irwin, 1973
Copepods Euphausiids/Mysiids Chaetognatos Fish/Fish Larvae Polychaetes Siphonophores Hydromedusae Pteropods Ctenophores	25.9 – 67.5 34.8 – 54.0 21.9 – 47.7 32.6 –46.5 15.9 – 43.9 3.0 – 16.0 5.4 – 10.4 17.0 – 29.0	4.4 –14.9 7.3 – 12.1 6.3 –12.6 8.27 – 10.6 4.37 – 11.2 0.98 – 4.4 1.34 – 6.9 1.5 – 4.2	5.6 – 7.2 0.9 – 6.0 1.2 – 6.7 3.3 – 9.3 0.7 – 3.5	Beers 1966; Ventura 2006; Omori 1969; Davis 1993; Murphy, 2001; Schaafsma et al. 2018
Crustacean zooplankton	42.5 – 64.2	5.2 – 12.7		Walve and Larson 1999
Mixed net plankton	14.0 – 21.5	2.38 – 4.1	2.7	Hedges et al. 2002
Mixed zooplankton				
Bacteria	29.0–50.4	4.0 – 12.9	4.6 – 6.3***	Bratbak and Dundas 1984; Nagata 1986**; Vrede et al. 2002; Prochazka et al. 1970
Terrestrial Fungi	38 –57	0.23 – 15	No data	Zhang and Elser 2017
Marine Fungi	23.8 ± 4.4	2.6 ± 1 3.6	± 0.3	this study

5.- DISCUSIÓN

5.1. Diversidad cultivable

Los hongos marinos se clasifican dentro de las divisiones; Ascomycota, Basidiomycota, Chytridiomycota, Zygomycota y Blastocladiomycota (Jones et al. 2015). Nuestros resultados proporcionan nuevo conocimiento sobre la diversidad taxonómica de hongos cultivables en diferentes ambientes fisicoquímicos del Océano Pacífico Sur-Oriental, la cual estuvo representada por las divisiones Ascomycota, Basidiomycota y Zygomycota. Basándonos en la variación espacial (geográfica y en profundidad) de los diversos taxones aislados desde el Océano Pacífico Sur-Oriental, y en la variación estacional de la frecuencia de los diferentes órdenes del phylum Ascomycota en el ecosistema de surgencia costera de Chile central (Estación 18), sugerimos que la diversidad de hongos marinos cultivables del Océano Pacífico Sur Oriental tiene un patrón biogeográfico y temporal con especies ubicuas y otras representativas de diferentes condiciones oceanográficas como: la disponibilidad de nutrientes, concentraciones oxígeno disuelto, salinidad y diferencias de presión. Por ejemplo, *Penicillium* es un género ubicuo, altamente frecuente en nuestros cultivos que tiene una amplia gama de adaptaciones fisiológicas y puede crecer en diferentes condiciones de temperatura y salinidad (Houbraken et al. 2011a), por otra parte el género *Rhodotorula* del phylum Basidiomycota está conformado por levaduras extremofílicas que se encuentran con frecuencia en los hábitats árticos, antárticos y alpinos y que en este caso, fue aislado específicamente desde aguas oligotróficas frías (8 °C) a 400 m de profundidad cerca Isla de Pascua. Las levaduras basidiomycetes tienen la capacidad de captar una amplia gama de nutrientes presentes en bajas concentraciones (Lachance 2011) y a menudo representan la mayoría de las poblaciones totales de levaduras en el aguas oceánicas oligotróficas (Navarrete and Tovar-Ramírez 2014). Para una mayor comprensión de las poblaciones de hongos en el Pacífico Sur-Oriental, sugerimos la realización de análisis posteriores de similitud y frecuencia con los diferentes taxones identificados.

Taxones similares han sido observados por otros autores utilizando métodos dependientes de cultivos (Steele 1967; Nagahama et al. 2001; Gadanho and Sampaio 2005; Gao et al. 2008;

Kutty and Philip 2008; Jebaraj and Raghukumar 2009; Jebaraj et al. 2010; Li et al. 2014; Andreakis et al. 2015). Por ejemplo, el género *Penicillium* (un hongo osmotolerante) ha sido repetidamente aislado en ambientes marinos (Overy et al. 2014). *Cladosporium* ha sido aislado desde agua de mar y sedimentos del Océano Pacífico, estudiado por su capacidad para producir antibióticos, antiincrustantes y por su actividad antiviral (Xiong et al. 2009; Wu et al. 2014). Se ha informado sobre el hongo desnitrificante *Fusarium* sp. (Shoun et al. 1992) en aguas con bajas concentraciones de oxígeno del Mar Arábigo (Jebaraj et al. 2010) y descrito por Hatai et al. (1986) y Khoa et al. (2005) como patógeno de peces (*Pagrus major*) y camarones marinos (*Penaeus japonicas*). *Rhodotorula mucilaginosa* se ha aislado a partir de muestras de agua de mar, camarones y mejillones de sistemas hidrotermales de aguas profundas (Gadanho and Sampaio 2005; Burgaud et al. 2010), animales bentónicos y sedimentos del fondo marino en diversas áreas del noroeste del Océano Pacífico (Nagahama et al. 2001). Esta levadura (*R. mucilaginosa*) también se ha observado en asociación con algas de la Antártica (*Adenocystis utricularis*, *Desmarestia anceps* y *Palmaria decipiens*; Loque et al. 2010) y el pez *Synechogobius hasta* recolectado en el Mar Amarillo (Li et al. 2014). El género *Mucor* ha sido aislado desde esponjas marinas del océano Índico (Mohapatra et al. 1998) y utilizado en la producción de biodiesel (Vicente et al. 2009) y etanol (Lübbehüsen et al. 2004).

A pesar que la definición ecológica de "hongo marino" continúa siendo objeto de debate (Kohlmeyer and Kohlmeyer 1979; Overy et al. 2014; Jones et al. 2015), tres especies aisladas e identificadas en este estudio: *Penicillium brevicompactum*, *Cladosporium sphaerospermum* y *R. mucilaginosa* han sido aceptadas como hongos marinos debido a su frecuente detección en los océanos (Jones et al. 2015).

Considerando que la diversidad cultivable solo representa una pequeña fracción de las comunidades fúngicas existentes en el océano (Wang et al. 2008) y hongos con diferentes enfoques para ser aislados, no fueron considerados en este estudio, nosotros esperamos una mayor diversidad *in situ* en el Océano Pacífico Sur-Oriental. Por ejemplo, excluimos de nuestros análisis hongos simbióticos, como los de la familia Malasseziales del phylum Basidiomycota, cuyos miembros parecen dominar en esponjas marinas (Gao et al. 2008) y

hongos parásitos, como Chytridiomycetes, que infectan el fitoplancton marino (Wang and Johnson 2009; Gutiérrez et al. 2016a).

5.2. *Penicillium* en el Océano Pacífico Sur-Oriental

Penicillium es un género importante del phylum Ascomycota, que se encuentra en el medio natural y en alimentos. Algunos miembros del género producen penicilina, una molécula utilizada como antibiótico que mata o detiene el crecimiento de ciertasbacterias dentro del cuerpo humano. El género *Penicillium* es ubicuo y uno de los hongos más comunes que se encuentran en una gran variedad de hábitats, incluidos suelo, aire y varios productos alimenticios (Yadav et al. 2018). Su función principal en la naturaleza es la descomposición de materia orgánica, donde algunas especies causan pudriciones devastadoras como patógenos de cultivos alimentarios (Frisvad and Samson 2004).

Penicillium parece estar ampliamente distribuido en ambientes con alta salinidad como la piscina cálida del Océano Pacífico occidental (Wang et al. 2014) y salares de todo el mundo (Zajc et al. 2012). Se ha encontrado también en aguas empobrecidas en oxígeno del Mar Arábigo (Jebaraj et al. 2010), en la columna de agua del Mar Muerto (Oren and Gunde-Cimerman 2012), colonizando sedimentos, algas e invertebrados en los arrecifes de coral (Morrison-Gardiner 2002) y asociados regularmente con esponjas marinas (Li and Wang 2009; Richards et al. 2012) Los frecuentes cultivos marinos de *Penicillium* sp., un género con características eurihalinas y euritérmicas (Houbraken et al. 2011), sugieren que se trataría de un género común en los océanos (Overy et al. 2014; Vera et al. 2017).

Penicillium representa el género cultivable dominante en las aguas del Océano Pacífico Sur-Oriental, constituyendo el 82% de las cepas identificadas. Secuencias de *Penicillium* sp. coincidentes (≥97% de similitud, GenBank) provienen de diversos hábitats terrestres (frutas, hojas, tallos, suelos cultivables, polvo, setas, cuevas, semillas, madera sumergida, tumbas) y marinos (esponjas, algas, sedimentos, corales). Estos resultados evidencian que la mayoría de

los taxones fúngicos aislados en este estudio corresponden a hongos marinos facultativos capaces de crecer en ambos ambientes.

Particularmente, podemos observar que las secuencias coincidentes (≥97% de similitud, GenBank) de *P. brevicompactum* fueron aisladas desde fuentes terrestres (frutos, plantas y hongos) y las secuencias de esta investigación fueron aisladas principalmente desde aguas suboxicas en el océano costero del norte de Chile y Chile central, y un hongo marino común en salares del mar Adriático (Jones et al. 2015; Yadav et al. 2018). Una diferencia entre hongos marinos y terrestres es que los marinos están adaptados para crecer en un medio marino (Pawar and Thirumalachar 1966; Zhang et al. 2015). Se ha observado que algunas especies de hongos podrían producir proteínas del estrés para adaptarse a las altas concentración de sodio. Por ejemplo, la proteína ribosomal L44 (RPL44) obtenida a partir de especies de *Aspergillus* se encuentra asociada con la resistencia a altas salinidades (Liu et al. 2014; Zhang et al. 2015). El aumento en las concentraciones intracelulares de trehalosa, polioles y lípidos de membrana insaturados, así como la secreción de proteínas y enzimas anticongelantes activas a bajas temperaturas son adaptaciones de hongos para tolerar ambientes fríos (Robinson 2001). Muchos hongos también tienen adaptaciones fisiológicas que les permiten prosperar en ambientes con concentraciones bajas de oxígeno (Zhou et al. 2002) capaces de utilizar nitrato y/o nitrito como alternativa a este (Kurakov et al. 2008) y otros como la levadura *Saccharomyces cerevisiae* puede modificar su composición de membrana para tolerar una alta presión hidrostática (Simonato et al. 2006; Rédou et al. 2015). Bajo este contexto podemos especular que los hongos detectados en este estudio tienen el potencial para adaptarse a las condiciones de salinidad de los océanos y en algunos casos a las altas presiones, a las bajas temperaturas y a las bajas concentraciones de oxígeno disuelto.

5.3. Viabilidad de hongos asilados desde el Océano Pacífico Sur-Oriental

En el ambiente marino los hongos son aislados con poca frecuencia y generalmente son considerados taxones terrestres arrastrados al mar (Morrison-Gardiner 2002; Kis-Papo 2005; Jones et al. 2015), por lo que no son considerados verdaderamente marinos, cuestionándose su rol ecológico y adaptación en este ambiente (Jones et al. 2009). Nuestros experimentos de viabilidad probaron que todas las cepas analizadas (23) germinaron, crecieron y formaron estructuras reproductivas en ambos sustratos; agua de mar y agua dulce, siendo la disponibilidad de sustrato orgánico el factor que estaría determinando su germinación. Estos resultados evidencian que las cepas experimentales aisladas desde diferentes ambientes fisicoquímicos del Océano Pacífico Sur-Oriental, corresponden a especies marinas facultativas, que pueden desarrollarse en el ambiente marino como también en agua dulce o medios terrestres, adaptadas a un amplio rango de salinidad y por lo tanto a la vida en los océanos. La relación observada entre el desarrollo de hongos aislados desde el Océano Pacífico Sur-Oriental y la disponibilidad de sustrato orgánico es el reflejo del su mecanismo de nutrición (digestión externa por enzimas secretadas al ambiente), el cuál determina la ecología de los hongos marinos y su prosperidad en entornos nutricionalmente ricos (Richards et al. 2012). Estas características ecológicas pueden explicar en parte por qué los hongos se encuentran altamente representados (valores de carbono fúngico dos veces más alto que la biomasa procarionte) durante periodos con alta biomasa fotoautótrofa en el ecosistema de surgencia costera del centro-sur de Chile y poco abundantes en muchas muestras marinas con bajas concentraciones de nutrientes (Kis-Papo 2005; Richards and Bass 2005; Massana and Pedrós-Alió 2008; Richards et al. 2012).

5.4. Composición elemental, y moléculas orgánicas en micelios de hongos marinos: Implicancias biogeoquímicas

Estudios específicos sobre la influencia de los hongos en los ciclos biogeoquímicos marinos son escasos respecto a otros grupos microbianos, a pesar que existe evidencia creciente de sus múltiples y complejas formas de impactar la biogeoquímica de los océanos

(Amend et al. 2019). Cuantificar la biomasa de los hongos, su actividad y variabilidad (espacial, temporal, metabólica) es una herramienta necesaria para una mejor comprensión del componente fúngico en los océanos, como también su composición elemental y bioquímica para estimar en detalle los flujos de carbono y nitrógeno, y su impacto en los ciclos biogeoquímicos

Considerando que la biomasa de hongos filamentosos puede ser tan alta como la de los procariotas en el ecosistema de surgencia costera en el centro de Chile (Gutiérrez et al. 2011) y que el contenido de carbono orgánico y nitrógeno determinado en micelios fúngicos en este estudio fue comparable con valores observados en organismos planctónicos marinos, sugerimos que los hongos podrían representar un depósito considerable de carbono y nitrógeno en el ecosistema marino y como tales, desempeñar un papel potencial en la movilización de carbono orgánico y nutrientes en el océano. Por ejemplo, en el Atlántico Norte la biomasa fúngica es dominante en partículas de nieve marina en el ambiente pelágico, sugiriendo que los hongos contribuyen al transporte de carbono y otros nutrientes hacia el océano profundo (Bochdansky et al. 2017; Amend et al. 2019). Por otra parte, la levadura *R. mucilaginosa* es la especie con el mayor contenido calórico, de carbono y nitrógeno (31,2% C; 4,4% N; 4,2 kcal gdw^{-1}) observado en esta investigación y comparable al fitoplancton (Whyte 1987) y fue aislada desde aguas oligotróficas cercanas a Isla de Pascua donde podría estar potencialmente cumpliendo un rol importante como fuente de materia y energía.

La constitución de la materia orgánica marina está controlada por la acumulación de componentes orgánicos de una variedad de fuentes biológicas. Reconocer patrones de componentes individuales de materia orgánica autóctona y alóctona también es clave para comprender su contribución a los ciclos biogeoquímicos y para rastrear su destino y sus interacciones tróficas en los ecosistemas marinos. Nosotros comparamos la composición de isótopos estables (carbono y nitrógeno) entre la biomasa hongos marinos y su medio de cultivo para aprender sobre el fraccionamiento de hongos marinos durante el crecimiento heterotrófico. Al ubicar los patrones de C/N y $\delta^{13}C$ en el esquema general de Meyers (1994),

el resultado es consistente con un papel heterótrofo derivado del fitoplancton (Gutiérrez et al. 2011; Cunliffe et al. 2017). A pesar que se debe tener precaución con estos resultados ya que $\delta^{13}C$ está determinado por el sustrato de crecimiento ($\delta^{13}C$ = -25,5 C/N = 6.5, una mezcla de $\delta^{13}C$ terrestre y C/N marina), los hongos de origen marino se encuentran 0,3‰ agotados en ^{13}C respecto al sustrato, cerca del fraccionamiento isotópico de carbono 0,6 ‰ determinado para la oxidación de glucosa microbiana heterotrófica por *Escherichia coli* (Blair et al. 1985). Definir la señal biogeoquímica de los hongos marinos ciertamente merece una investigación futura, ya que ayudará a explicar el fraccionamiento de isótopos estables de las moléculas orgánicas en el océano.

El contenido de aminoácidos hidrolizables totales; proteínas (3-21% por peso seco) fue menor que en el plancton (20-40%; Lee et al. 1988), zooplancton de agua dulce (> 50%; Dabrowski and Rusiecki 1983), fitoplancton marino (12-35%; Brown et al. 1991), zooplancton marino (4-55%; Raymont et al. 1973) y bacterias marinas (~ 60%; Simon and Azam 1989). Estos resultados sugieren que las especies fúngicas marinas tienen un valor trófico menor en proteínas que las de otros organismos planctónicos. Sin embargo, dado que las cepas de hongos marinos aquí estudiadas son ricas en aminoácidos esenciales como histidina, treonina, lisina y leucina, proponemos que pueden ser una fuente de moléculas críticas para la dieta de los metazoos en el medio marino. Aminoácidos hidrolizables representaron tan solo el 20 y hasta 78% del nitrógeno fúngico, lo que indica que hasta un 80% del nitrógeno (ej. *Cladosporium sphaerospermum*) se almacena en otras moléculas. La quitina, un polímero producido por crustáceos (Jeuniaux and Voss-foucart 1991) y gran reservorio de carbono y nitrógeno en el océano (Souza et al. 2011), también forma parte de las paredes celulares de los hongos (Latgé 2007). Por lo tanto, los hongos también podrían ser una importante fuente de quitina sin contabilizar, y como tal de nitrógeno en el ambiente marino, desempeñando así un papel en el ciclo de los nutrientes.

La composición de ácidos grasos mostró un predominio de las moléculas C-18, con 38% de ácido linoleico (C18:2ω6,9) y 23% de ácido oleico (C18:1ω,9c). Consistentemente, el

ácido linoleico se considera un marcador fúngico en ambientes terrestres (Vestal and White 1989; Frostegard and Baath 1996; Olsson 1999; Boschker and Middelburg 2002; Kaur et al. 2005), se ha informado como el principal componente (11-37%) de los ácidos grasos de hongos marinos (Cooney et al. 1993; Devi et al. 2006; Das et al. 2007) y varia junto a la abundancia de hongos filamentos en el ecosistema de surgencia costera de Chile (Gutiérrez et al. 2011). Los ácidos grasos poliinsaturados (PUFA) representan la mitad de los ácidos grasos totales, lo que sugiere que, junto con otros organismos planctónicos, los hongos pueden ser fuente importante de lípidos esenciales para los organismos pelágicos. En respaldo a esta idea, la proporción de PUFA en hongos de origen marino (este estudio y Das et al. 2007) se encuentra en el rango superior al reportado para el fitoplancton marino (Lewis 1969; Zhukova and Aizdaicher 1995; Arendt et al. 2005) y bacterias (Russell and Nichols 1999; Das et al. 2007). En comparación con otros organismos, la composición de aminoácidos y ácidos grasos de hongos de origen marino, difiere de la de los procariotas, fitoplancton, zooplancton y peces, siendo la lisina, histidina, treonina, alanina, y los ácidos grasos C18 (linoleico y oleico) los compuestos diferenciadores.

Ergosterol, y su isómero dehydrostellasterol (C28Δ5,7,22), es el esterol dominante en todas las cepas marinas analizadas, y se ha utilizado para estimar la biomasa fúngica en suelos (Montgomery et al. 2000; Joergensen and Wichern 2008), ríos (Jørgensen and Stepanauskas 2009), marismas de agua dulce y salada (Newell et al. 2000; Buesing and Gessner 2006) y humedales (Verma et al. 2003). Teniendo en cuenta que el ergosterol y el dehydrostellasterol representaron más del 80% de los esteroles en todas las cepas de hongos, afirmamos que la concentración ambiental de ergosterol en la materia orgánica en partículas podría ser un sustituto apropiado para determinar la biomasa existente de hongos en el océano. Entre los lípidos polares intactos, la fosfatidilcolina (PC) el fosfolípido principal de las membranas celulares, fue el tipo dominante en los hongos de origen marino. Los fosfolípidos, particularmente la PC, parecen ser beneficiosos en la dieta de varias especies de crustáceos, peces marinos y de agua dulce al mejorar la supervivencia, el crecimiento y la resistencia al estrés (Coutteau et al. 1997, 2000; Wang et al. 2016) conservándose como lípido de

almacenamiento en algunas especies de zooplancton marino (Hagen and Schnack-Schiel 1996; Lee et al. 2006).

Durante los últimos años se ha desarrollado investigación que ha permitido sugerir la incorporación de los hongos marinos en el mundo microbiano de bacterias y arqueas del océano, pero claramente se requiere un entendimiento más profundo del control fúngico sobre los flujos de nutrientes en el océano para generar mejores modelos asociados a cambio climático (Amend et al. 2019). Tanto en el ambiente terrestre como en el océano los hongos son importantes como degradadores de materia orgánica (Hyde et al. 1998; Gadd 2006; Wang and Johnson 2009; Pointing and Hyde 2009; Gutiérrez et al. 2011; Crowther et al. 2012; Raghukumar 2012, 2017; Paul 2014; Fuentes et al. 2015; Fuentes and Quiñones 2016) e infectando células del fitoplancton (Richards et al. 2012; Gutiérrez et al. 2016b; Taylor and Cunliffe 2016). Adicionalmente se ha observado que pueden ser parte de la dieta del zooplancton (Kagami et al. 2007, 2011b; Sime-Ngando 2012; Maloy et al. 2013; Hu et al. 2015) y como mostramos en esta investigación potenciales fuentes de moléculas esenciales (aminoácidos y lípidos) para niveles tróficos superiores. El impacto de los hongos en el océano puede ser significativo en la liberación de partículas y carbono orgánico disuelto, modificando la composición química de la nieve marina y posterior funcionamiento de la bomba biológica de carbono (Amend et al. 2019). La cuantificación detallada y la caracterización temporal, espacial y taxonómica de la biomasa fúngica, su actividad metabólica y la experimentación con modelos aislados como los obtenidos en esta tesis son necesarias para la comprensión futura e integración definitiva de los hongos en los ciclos biogeoquímicos marinos.

6.- CONCLUSIONES

1. Cincuenta y una especies de hongos filamentosos planctónicos fueron identificadas en el Océano Pacífico Sur-Oriental

2. Once secuencias fúngicas no coinciden con especies existentes en Genbank y podrían tratarse de nuevas especies de hongos

3. La diversidad de hongos filamentosos planctónicos aislados desde distintos ambientes fisicoquímicos del Océano Pacífico Sur de Chile está representada por los Phyla Ascomycota Basidiomycota y Zygomycota

4. Ascomicetes planctónicos filamentosos cultivables en el Océano Pacífico Sur-Oriental se clasifican dentro de los órdenes Eurotiales, Dothideales, Sordariales e Hypocreales.

5. *Penicillium* es el género de hongos planctónicos filamentosos cultivables en el Océano Pacífico Sur-Oriental con la mayor representación.

6. Nuestros resultados experimentales evidenciaron la presencia de hongos filamentosos facultativos en Océano Pacífico Sur-Oriental, capaces de crecer tanto en agua de mar como en agua dulce

7. La producción de esporas de hongos filamentoso en el océano podría estar influenciada por la disponibilidad de sustratos orgánicos

8. El valor nutricional de hongos marinos es comparable al de otros organismos planctónicos.

9. Hongos marinos como *Rhodotorula mucilaginosa* y algunas cepas de *Penicillium* sp. con alto contenido energético y altas proporciones de carbono, nitrógeno y macromoléculas

esenciales (ácidos grasos esenciales y fosfatidilcolina), respecto del fitoplancton y zooplancton son una fuente potencial de alimento para otros organismos de marinos.

10. Los hongos marinos tienen un patrón distintivo de lípidos y proteínas

11. La composición elemental de los hongos marinos es consistente con una fuente marina y una captación heterotrófica de materia orgánica derivada de fitoplancton.

7.- REFERENCIAS

Alfaro, M., and M. Holder. 2006. The Posterior and the Prior in Bayesian Phylogenetics. Annu. Rev. Ecol. Evol. Syst. **37**: 19–42. doi:10.1146/annurev.ecolsys.37.091305.110021

Amend, A., G. Burgaud, M. Cunliffe, and others. 2019. Fungi in the Marine Environment: Open Questions and Unsolved Problems. Am. Soc. Microbiol. **10**: 1–15.

Andreakis, N., L. Høj, P. Kearns, and others. 2015. Diversity of marine-derived fungal cultures exposed by DNA barcodes: The algorithm matters. PLoS One **10**: 1–22. doi:10.1371/journal.pone.0136130

Araujo, F. V., and A. N. Hagler. 2011. Kluyveromyces aestuarii, a potential environmental quality indicator yeast for mangroves in the state of rio de janeiro, brazil. Brazilian J. Microbiol. **42**: 954–958.

Arendt, K. E., S. H. Jónasdóttir, P. J. Hansen, and S. Gärtner. 2005. Effects of dietary fatty acids on the reproductive success of the calanoid copepod Temora longicornis. Mar. Biol. **146**: 513–530. doi:10.1007/s00227-004-1457-9

Azam, F. 1998. Microbial Control of Oceanic Carbon Flux: The Plot Thickens. Science (80-.). 694–696.

Azam, F., and A. Worden. 2004. Microbes, Molecules, and Marine Ecosystems. Science (80-.). **303**: 1622–1624. doi:10.1126/science.1093892

Blair, N., A. Leu, E. Muñoz, J. Olsen, E. Kwong, and D. Des Marais. 1985. Carbon Isotopic Fractionation in Heterotrophic Microbial Metabolism. Appl. Environ. Microbiol. **50**: 996–1001.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911–917.

Bochdansky, A. B., M. A. Clouse, and G. J. Herndl. 2017. Eukaryotic microbes, principally fungi and labyrinthulomycetes, dominate biomass on bathypelagic marine snow. ISME J. **11**: 362–373. doi:10.1038/ismej.2016.113

Bosch, A., R. A. Maronna, and O. M. Yantorno. 1995. A simple descriptive model of filamentous fungi spore germination. Process Biochem. **30**: 599–606. doi:10.1016/0032-9592(94)00007-5

Boschker, H. T. S., and J. J. Middelburg. 2002. Stable isotopes and biomarkers in microbial ecology. FEMS Microbiol. Ecol. **40**: 85–95.

Brown, P. C., S. J. Painting, and K. L. Cochrane. 1991. Estimates of phytoplankton and bacterial biomass and production in the northern and southern Benguela ecosystems. South African J. Mar. Sci. **11**: 537–564. doi:10.2989/025776191784287673

Buesing, N., and M. O. Gessner. 2006. Benthic Bacterial and Fungal Productivity and Carbon Turnover in a Freshwater Marsh. Appl. Environ. Microbiol. **72**: 596–605. doi:10.1128/AEM.72.1.596

Burgaud, G., D. Arzur, L. Durand, M. A. Cambon-Bonavita, and G. Barbier. 2010. Marine culturable yeasts in deep-sea hydrothermal vents: Species richness and association with fauna. FEMS Microbiol. Ecol. **73**: 121–133. doi:10.1111/j.1574-6941.2010.00881.x

Carlile, M., and S. Watkinson. 2000. The Fungi, 2nd ed. Academic Press.

Cooney, J. J., M. M. Doolittle, O. Grahl-Nielsen, I. M. Haaland, and P. W. J. Kirk. 1993. Comparison of fatty acids of marine fungi using multivariate statistical analysis. J. Ind.

Microbiol. **12**: 373–374.
Coutteau, P., I. Geurden, M. R. Camara, P. Bergot, and P. Sorgeloos. 1997. Review on the dietary effects of phospholopids in fish and crustacean larviculture. Aquaculture **155**: 149–164.
Coutteau, P., E. K. M. Kontara, and P. Sorgeloos. 2000. Comparison of phosphatidylcholine purified from soybean and marine fish roe in the diet of postlarval Penaeus vannamei Boone. Aquaculture **181**: 331–345.
Crowther, T. W., L. Boddy, and T. H. Jones. 2012. Functional and ecological consequences of saprotrophic fungus–grazer interactions. ISME J. **6**: 1992–2001. doi:10.1038/ismej.2012.53
Cunliffe, M., A. Hollingsworth, C. Bain, V. Sharma, and J. D. Taylor. 2017. Algal polysaccharide utilisation by saprotrophic planktonic marine fungi. Fungal Ecol. **30**: 135–138. doi:10.1016/j.funeco.2017.08.009
Dabrowski, K., and M. Rusiecki. 1983. Content of total and free amino acids in zooplanktonic food of fish larvae. Aquaculture **30**: 31–42.
Das, S., P. S. Lyla, and S. A. Khan. 2007. Fatty Acid Profiles of Marine Benthic Microorganisms Isolated from the Continental Slope of Bay of Bengal: A Possible Implications in the Benthic Food Web. Ocean Sci. J. **42**: 247–254.
Devi, P., M. P. D. Shridhar, L. D. Souza, and C. G. Naik. 2006. Cellular fatty acid composition of marine-derived fungi. Indian J. Mar. Sci. **35**: 359–363.
Edgcomb, V. P., D. Beaudoin, R. Gast, J. F. Biddle, and A. Teske. 2011. Marine subsurface eukaryotes: The fungal majority. Environ. Microbiol. **13**: 172–183. doi:10.1111/j.1462-2920.2010.02318.x
Fell, J. W. 2012. Yeasts in marine environments, p. 91–102. *In* E.B.G. Jones and K. Pang [eds.], Marine fungi and fungal-like organisms. Walter de Gruyter.
Frisvad, J., and R. Samson. 2004. Penicillium subgenus Penicillium - A guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud. Mycol. **49**: 1–173.
Frostegard, A., and E. Baath. 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils **22**: 59–60.
Fuentes, M. E., and R. A. Quiñones. 2016. Carbon utilization profile of the filamentous fungal species Fusarium fujikuroi, Penicillium decumbens , and Sarocladium strictum isolated from marine coastal environments. Mycologia **108**: 1069–1081. doi:10.3852/15-338
Fuentes, M., R. A. Quiñones, M. H. Gutiérrez, and S. Pantoja. 2015. Effects of temperature and glucose concentration on the growth and respiration of fungal species isolated from a highly productive coastal upwelling ecosystem. Fungal Ecol. **13**: 135–149.
Fuhrman, J. A. 1999. Marine viruses and their biogeochemica and ecological effects. Nature **399**: 541–548.
Fuller, M. S., and R. Poyton. 1964. A New Technique for the Isolation of Aquatic Fungi. **14**: 45–46.
Gadanho, M., and J. P. Sampaio. 2005. Occurrence and diversity of yeasts in the mid-atlantic ridge hydrothermal fields near the Azores Archipelago. Microb. Ecol. **50**: 408–417. doi:10.1007/s00248-005-0195-y
Gadd, G. 2006. Fungi in biogeochemical cycles., Cambridge university press.

Gao, Z., B. Li, C. Zheng, and G. Wang. 2008. Molecular detection of fungal communities in the hawaiian marine sponges Suberites zeteki and Mycale armata. Appl. Environ. Microbiol. **74**: 6091–6101. doi:10.1128/AEM.01315-08

Gardes, M., and T. D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes, application to the identification of mycorrihiza and rusts. Mol. Ecol. **2**: 113–118. doi:Doi 10.1111/J.1365-294x.1993.Tb00005.X

Giovannoni, S. J., T. B. Britschgi, C. L. Moyer, and K. G. Field. 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature **345**: 60–63. doi:10.1038/345060a0

Giovannoni, S. J., H. J. Tripp, S. Givan, and others. 2005. Genome streamlining in a cosmopolitan oceanic bacterium. Science **309**: 1242–1245. doi:10.1126/science.1114057

Giovannoni, S., and U. Stingl. 2007. The importance of culturing bacterioplankton in the "omics" age. Nat. Rev. Microbiol. **5**: 820–826. doi:10.1038/nrmicro1752

Guarro, J., J. Gené, and A. M. Stchigel. 1999. Developments in fungal taxonomy. Clin. Microbiol. Rev. **12**: 454–500.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. Syst. Biol. **59**: 307–321. doi:10.1093/sysbio/syq010

Gutiérrez, M. H., P. E. Galand, C. Moffat, and S. Pantoja. 2015. Melting glacier impacts community structure of Bacteria, Archaea and Fungi in a Chilean Patagonia fjord. Environ. Microbiol. **17**: 3882–3897. doi:10.1111/1462-2920.12872

Gutiérrez, M. H., A. M. Jara, and S. Pantoja. 2016a. Fungal parasites infect marine diatoms in the upwelling ecosystem of the Humboldt Current System off central Chile. Environ. Microbiol. **18**: 1–24. doi:10.1111/1462-2920.13257

Gutiérrez, M. H., A. M. Jara, and S. Pantoja. 2016b. Fungal parasites infect marine diatoms in the upwelling ecosystem of the Humboldt current system off central Chile. Environ. Microbiol. **18**: 1646–1653. doi:10.1111/1462-2920.13257

Gutiérrez, M. H., S. Pantoja, R. A. Quiñones, and R. Gonzalez. 2010. First record of filamentous fungi in the coastal upwelling ecosystem off central Chile. Gayana **74**: 66–73.

Gutiérrez, M. H., S. Pantoja, E. Tejos, and R. A. Quiñones. 2011. The role of fungi in processing marine organic matter in the upwelling ecosystem off Chile. Mar. Biol. **158**: 205–219. doi:10.1007/s00227-010-1552-z

Hagen, W., and S. Schnack-Schiel. 1996. Seasonal lipid dynamics in dominant Antarctic copepods: Energy for overwintering or reproduction ? Deep. Res. Part I Oceanogr. Res. Pap. **43**: 139–158.

Hagler, A. N., R. B. De Oliveira, and L. C. Mendonca Hagler. 1982. Yeasts in the intertidal sediments of a polluted estuary in Rio de Janeiro, Brazil. **48**: 53–54.

Harrison, K. E. 1990. The role nutrition in maturation, reproduction and embryonic development of decapod crustaccens: a review. J. Shellfish Res. **9**: 1–28.

Hatai, K., S. S. Kubota, N. Kida, and S. Udagawa. 1986. Fusarium oxysporum in Red Sea Bream (Pagrus sp.). **22**: 570–571.

Hawksworth, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. Mycol. Res. **105**: 1422–1432. doi:10.1017/S0953756201004725

Houbraken, J., J. Frisva, and R. Samson. 2011a. Taxonomy of Penicillium section Citrina.

Stud. Mycol. **70**: 53–158. doi:doi:10.3114/sim.2011.70.02
Houbraken, J., J. C. Frisvad, and R. A. Samson. 2011b. Taxonomy of Penicillium section Citrina. Stud. Mycol. **70**: 53–138. doi:10.3114/sim.2011.70.02
Hu, S., Z. Guo, T. Li, C. Xu, H. Huang, S. Liu, and S. Lin. 2015. Molecular analysis of in situ diets of coral reef copepods: evidence of terrestrial plant detritus as a food source in Sanya, China. J. Plankt. Res. **37**: 1–9. doi:10.1093/plankt/fbv014
Hyde, K. D., E. B. G. Jones, E. Leaño, S. B. Pointing, A. D. Poonyth, and L. L. P. Vrijmoed. 1998. Role of fungi in marine ecosystems. Biodivers. Conserv. **7**: 1147–1161. doi:10.1023/A:1008823515157
James, T. Y., P. M. Letcher, J. E. Longcore, S. E. Mozley-Standridge, D. Porter, M. J. Powell, G. W. Griffith, and R. Vilgalys. 2006. A molecular phylogeny of the flagellated fungi (Chytridiomycota) and description of a new phylum (Blastocladiomycota). Mycologia **98**: 860–871. doi:10.3852/mycologia.98.6.860
Jebaraj, C. S., and C. Raghukumar. 2009. Anaerobic denitrification in fungi from the coastal marine sediments off Goa, India. Mycol. Res. **113**: 100–109. doi:10.1016/j.mycres.2008.08.009
Jebaraj, C. S., C. Raghukumar, A. Behnke, and T. Stoeck. 2010. Fungal diversity in oxygen-depleted regions of the Arabian Sea revealed by targeted environmental sequencing combined with cultivation. FEMS Microbiol. Ecol. **71**: 399–412. doi:10.1111/j.1574-6941.2009.00804.x
Jeuniaux, C., and M. F. O. Voss-foucart. 1991. Chitin Biomass and Production in the Marine Environment. Biochem. Syst. Ecol. **19**: 347–356.
Joergensen, R. G., and F. Wichern. 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biol. Biochem. **40**: 2977–2991. doi:10.1016/j.soilbio.2008.08.017
Johnson, T. W., and F. K. Sparrow. 1961. Fungi in Oceans and Estuaries, 1st ed. Weinheim.
Jones, B. N., S. Pääbo, and S. Stein. 1981. Amino acid analysis and enzymatic sequence determination of peptides by an improved o-phthaldialdehyde precolumn labeling procedure. J. Liq. Chromatogr. **4**: 565–586. doi:10.1080/01483918108059956
Jones, E. B. G., J. Sakayaroj, S. Suetrong, S. Somrithipol, and K. L. Pang. 2009. Classification of marine Ascomycota, anamorphic taxa and Basidiomycota. Fungal Divers. **35**: 1–187.
Jones, E. B. G., S. Suetrong, J. Sakayaroj, A. H. Bahkali, M. A. Abdel-Wahab, T. Boekhout, and K. L. Pang. 2015. Classification of marine Ascomycota, Basidiomycota, Blastocladiomycota and Chytridiomycota. Fungal Divers. **73**: 1–72. doi:10.1007/s13225-015-0339-4
Jørgensen, N. O. G., and R. Stepanauskas. 2009. Biomass of pelagic fungi in Baltic rivers. Hyrobiologia **623**: 105–112. doi:10.1007/s10750-008-9651-2
Kagami, M., E. von Elert, B. W. Ibelings, A. de Bruin, and E. van Donk. 2007. The parasitic chytrid, Zygorhizidium, facilitates the growth of the cladoceran zooplankter, Daphnia, in cultures of the inedible alga, Asterionella. Proc Biol Sci **274**: 1561–1566. doi:566800241L66NNJP [pii]\r10.1098/rspb.2007.0425
Kagami, M., N. Helmsing, and E. van Donk. 2011a. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hyrobiologia **659**: 49–54. doi:10.1007/s10750-010-0274-z

Kagami, M., N. R. Helmsing, and E. Van Donk. 2011b. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. Hyrobiologia **659**: 49–54. doi:10.1007/s10750-010-0274-z

Karner, M. B., E. F. DeLong, and D. M. Karl. 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature **409**: 507–510. doi:10.1038/35054051

Kaur, A., A. Chaudhary, A. Kaur, R. Choudhary, and R. Kaushik. 2005. Phospholipid fatty acid - A bioindicator of environment monitoring and assessment in soil ecosystem. Curr. Sci. **89**: 1103–1112. doi:10.2307/24110962

Khoa, L. Van, K. Hatai, A. Yuasa, and K. Sawada. 2005. Morphology and Molecular Phylogeny of Fusarium solani Isolated from Kuruma Prawn Penaeus japonicus with Black Gills. Fish Pathol. **40**: 103–109.

Kis-Papo, T. 2005. Marine fungal communities, p. 61–92. *In* J. Dighton, J. White, and P. Oudemans [eds.], The Fungal Community: Its Organisation and Role in the Ecosystem. Taylor and Francis.

Kohlmeyer, J., and E. Kohlmeyer. 1979. Marine Mycology: The Higher Fungi, Academic Press.

Kurakov, A. V, R. B. Lavrent, T. Y. Nechitailo, P. N. Golyshin, and D. G. Zvyagintsev. 2008. Diversity of Facultatively Anaerobic Microscopic Mycelial Fungi in Soils. Microbiology **77**: 90–98. doi:10.1134/S002626170801013X

Kurtzman, C. P., and C. J. Robnett. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek **73**: 331–371. doi:10.1023/A:1001761008817

Kutty, S. N., and R. Philip. 2008. Marine yeasts—a review. Yeast **25**: 465–483. doi:10.1002/yea.1599

Lachance, M. 2011. Yeasts, 5th ed. Chichester.

Lai, X., L. Cao, H. Tan, S. Fang, Y. Huang, and S. Zhou. 2007. Fungal communities from methane hydrate-bearing deep-sea marine sediments in South China Sea. ISME J. **1**: 756–762. doi:10.1038/ismej.2007.51

Landeweert, R., P. Leeflang, T. W. Kuyper, E. Hoffland, A. Rosling, K. Wernars, and E. Smit. 2003. Molecular identification of ectomycorrhizal mycelium in soil horizons. Appl. Environ. Microbiol. **69**: 327–333. doi:10.1128/AEM.69.1.327-333.2003

Latgé, J. 2007. MicroReview The cell wall: a carbohydrate armour for the fungal cell. Mol. Microbiol. **66**: 279–290. doi:10.1111/j.1365-2958.2007.05872.x

Lee, C., S. G. Wakeham, and J. I. Hedges. 1988. The Measurement of Oceanic Particle Flux-are “Swimmers” A Problem? Oceanography **1**: 34–36.

Lee, R. F., W. Hagen, and G. Kattner. 2006. Lipid storage in marine zooplankton. Mar. Ecol. Prog. Ser. **307**: 273–306.

Lewis, R. W. 1969. The fatty acid composition of arctic marine phytoplankton and zooplankton with special reference to minor acids. Limnol. Oceanogr. **14**: 35–40.

Li, L., P. Singh, Y. Liu, S. Pan, and G. Wang. 2014. Diversity and biochemical features of culturable fungi from the coastal waters of Southern China. Appl. Microbiol. Biotechnol. **60**: 1–13. doi:10.1007/s00253-013-5391-y

Li, Q., and G. Wang. 2009. Diversity of fungal isolates from three Hawaiian marine sponges. Microb. Res. **164**: 233–241. doi:10.1016/j.micres.2007.07.002

Lindroth, P., and K. Mopper. 1979. High Performance Liquid Chromatographic Determination of Subpicomole Amounts of Amino Acids by Precolumn Fluorescence Derivatization with o-Phthaldialdehyde. Anal. Chem. **51**: 1667–1674. doi:10.1021/ac50047a019

Liu, X., L. Xie, Y. Wei, X. Zhou, B. Jia, and J. Liu. 2014. Abiotic Stress Resistance , a Novel Moonlighting Function of Ribosomal Protein RPL44 in the Halophilic Fungus Aspergillus glaucus. Appl. Environ. Microbiol. **80**: 4294–4300. doi:10.1128/AEM.00292-14

Liu, Y., E. T. Steenkamp, H. Brinkmann, L. Forget, H. Philippe, and B. F. Lang. 2009. Phylogenomic analyses predict sistergroup relationship of nucleariids and Fungi and paraphyly of zygomycetes with significant support. BMC Evol. Biol. **9**: 1–11. doi:10.1186/1471-2148-9-272

López-García, P., F. Rodrguez-Valera, C. Pedrós-Alió, and D. Moreira. 2002. Unexpected Diversity of Smal Eukaryotes in Deep-Sea Antartic Plankton. Nature **409**: 603–606.

Loque, C. P., A. O. Medeiros, F. M. Pellizzari, E. C. Oliveira, C. A. Rosa, and L. H. Rosa. 2010. Fungal community associated with marine macroalgae from Antarctica. Polar Biol. **33**: 641–648. doi:Doi 10.1007/S00300-009-0740-0

Lübbehüsen, T. L., J. Nielsen, and M. McIntyre. 2004. Aerobic and anaerobic ethanol production by Mucor circinelloides during submerged growth. Appl. Microbiol. Biotechnol. **63**: 543–548. doi:10.1007/s00253-003-1394-4

Lyncht, M. D. J., and R. G. Thorn. 2006. Diversity of basidiomycetes in Michigan agricultural soils. Appl. Environ. Microbiol. **72**: 7050–7056. doi:10.1128/AEM.00826-06

Maloy, A. P., U. S. Fish, W. Service, S. C. Culloty, and J. W. Slater. 2013. Dietary analysis of small planktonic consumers : a case study with marine bivalve larvae. J. Plankt. Res. doi:10.1093/plankt/fbt027

Mann, K. H. 1988. Production and use of detritus in various freshwateer, estuarine, and coastal marine systems. Limnol. Oceanogr. **33**: 910–930.

Massana, R., and C. Pedrós-Alió. 2008. Unveiling new microbial eukaryotes in the surface ocean. Curr. Opin. Microbiol. **11**: 213–218. doi:10.1016/j.mib.2008.04.004

Meyers, P. 1994. Preservation of elemental and isotopic source identification of sedimentary organic matter. Chem. Geol. **114**: 289–302.

Montecino, V., M. A. Paredes, P. Paolini, and J. Rutllant. 2006. Revisiting chlorophyll data along the coast in north-central Chile, considering multiscale environmental variability. Rev. Chil. Hist. Nat. **79**: 213–223. doi:10.4067/S0717-71782002030100031

Montero, P., G. Daneri, L. A. Cuevas, H. E. Gonza, and L. Liza. 2007. Productivity cycles in the coastal upwelling area off Concepción: The importance of diatoms and bacterioplankton in the organic carbon flux. **75**: 518–530. doi:10.1016/j.pocean.2007.08.013

Montgomery, H. J., C. M. Monreal, J. C. Young, and K. A. Seifert. 2000. Determinination of soil fungal biomass from soil ergosterol analyses. Soil Biol. Biochem. **32**: 1207–1217.

Moon-van der Staay, S. Y., R. De Wachter, and D. Vaulot. 2001. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature **409**: 607–610. doi:10.1038/35054541

Morrison-Gardiner, S. 2002. Dominant fungi from Australian coral reefs. Fungal Divers. **9**: 105–121.

Murphy, W., E. Eizirik, S. O'Brien, and others. 2001. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science **294**: 2348–2351. doi:10.1126/science.1067179

Nagahama, T., M. Hamamoto, T. Nakase, H. Takami, and K. Horikoshi. 2001. Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific Ocean. Antonie van Leeuwenhoek, Int. J. Gen. Mol. Microbiol. **80**: 101–110. doi:10.1023/A:1012270503751

Navarrete, P., and D. Tovar-ramírez. 2014. Use of Yeasts as Probiotics in Fish Aquaculture, p. 135–172. *In* M. Hernandez-Vergara and C. Pérez-Rostro [eds.], Sustainable Aquaculture Techniques. InTech.

Newell, S. Y., L. K. Blum, R. E. Crawford, T. Dai, and M. Dionne. 2000. Autumnal Biomass and Potential Productivity of Salt Marsh Fungi from 29° to 43° North Latitude along the United States Atlantic Coast. Appl. Environ. Microbiol. **66**: 180–185.

Olsen, G. J. 1986. Microbial Ecology and evolution: a ribosomal RNA approach. Annu. Rev. Microbiol. **40**: 337–365. doi:10.1146/annurev.mi.40.100186.002005

Olsson, A. 1999. Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol. Ecol. **29**: 303–310.

Oren, A., and N. Gunde-Cimerman. 2012. Fungal Life in the Dead Sea, p. 115–132. *In* C. Raghukumar [ed.], Biology of Marine Fungi. Progress in Molecular and Subcellular Biology. Springer-Verlag.

Overy, D. P., P. Bayman, R. G. Kerr, and G. F. Bills. 2014. An assessment of natural product discovery from marine (sensu strictu) and marine-derived fungi. Mycology **5**: 145–167. doi:10.1080/21501203.2014.931308

Pace, N. R. 1997. A molecular view of microbial diversity and the biosphere. Science (80-.). **276**: 734–740. doi:10.1126/science.276.5313.734

Pantoja, S., and C. Lee. 1999. Molecular weight distribution of proteinaceous material in Long Island Sound sediments. Limnol. Oceanogr. **44**: 1323–1330.

Pantoja, S., P. Rossel, R. Castro, L. A. Cuevas, G. Daneri, and C. Córdova. 2009. Microbial degradation rates of small peptides and amino acids in the oxygen minimum zone of Chilean coastal waters. Deep. Res. Part II Top. Stud. Oceanogr. **56**: 1019–1026. doi:10.1016/j.dsr2.2008.09.007

Paul, E. . 2014. Soil microbiology, ecology and biochemistry, Academic press.

Pawar, V. H., and M. J. Thirumalachar. 1966. Studies on halophilic soil fumgi of Bombay. Nov. Hedwigia **12**: 497–508.

Pennington, J. T., K. L. Mahoney, V. S. Kuwahara, D. D. Kolber, R. Calienes, and F. P. Chavez. 2006. Primary production in the eastern tropical Pacific: A review. Prog. Oceanogr. **69**: 285–317. doi:10.1016/j.pocean.2006.03.012

Phillips, N. W. 1984. Role of different microbes and substrates as potential suppliers of specific, essential nutrients to marine detritivores. Bull. Mar. Sci. **35**: 283–298.

Pointing, S. B., and K. D. Hyde. 2009. Lignocellulose - degrading marine fungi Lignocellulose-degrading Marine Fungi. **7014**. doi:10.1080/08927010009386312

Puillandre, N., A. Lambert, S. Brouillet, and G. Achaz. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Mol. Ecol. **21**: 1864–1877. doi:10.1111/j.1365-294X.2011.05239.x

Quiñones, R. A., H. Levipan, and H. Urritia. 2009. Spatial and temporal variability of planktonic archaeal abundance in the Humboldt Current System off Chile. Deep. Res. Part II Top. Stud. Oceanogr. **56**: 1073–1082. doi:10.1016/j.dsr2.2008.09.012

Raghukumar, C. 2012. Biology of marine fungy, 1st ed. Chandralata Raghukumar [ed.]. Springer-Verlag Berlin Heidelberg.

Raghukumar, S. 2004. The Role of Fungi in Marine Detrital Processes, p. 91–101. *In* N. Ramaiah [ed.], Marine Microbiology: Facets & Opportunities. National Institute of Oceanography.

Raghukumar, S. 2017. Fungi in Coastal and Oceanic Marine Ecosystems: Marine Fungi, 1st ed. Springer.

Raymont, J. E. G., C. F. Ferguson, and J. K. B. Raymont. 1973. Biogeochemical studies on marine zooplankton the aminoacid composition of some local species. Spec. Publ. Mar. Bol. Ass. India **60**: 91–99.

Redberg, G. L., D. S. Hibbett, J. F. Ammirati, and R. J. Rodriguez. 2003. Phylogeny and genetic diversity of Bridgeoporus nobilissimus inferred using mitochondrial and nuclear rDNA sequences. Mycologia **95**: 836–845.

Rédou, V., M. Navarri, L. Meslet-cladière, G. Barbier, and G. Burgaud. 2015. Species Richness and Adaptation of Marine Fungi from Deep-Subseafloor Sediments. Appl. Environ. Microbiol. **81**: 3571–3583. doi:10.1128/AEM.04064-14

Richards, T. A., and D. Bass. 2005. Molecular screening of free-living microbial eukaryotes: Diversity and distribution using a meta-analysis. Curr. Opin. Microbiol. **8**: 240–252. doi:10.1016/j.mib.2005.04.010

Richards, T. A., M. D. M. Jones, G. Leonard, and D. Bass. 2012. Marine Fungi: Their Ecology and Molecular Diversity. Ann. Rev. Mar. Sci. **4**: 495–522. doi:10.1146/annurev-marine-120710-100802

Robinson, C. H. 2001. Cold adaptation in Arctic and Antarctic fungi. New Phytol. **151**: 341–353.

Ronquist, F., M. Teslenko, P. Van Der Mark, and others. 2012. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. **61**: 539–542. doi:10.1093/sysbio/sys029

Rusch, D. B., A. L. Halpern, G. Sutton, and others. 2007. The Sorcerer II Global Ocean Sampling expedition: Northwest Atlantic through eastern tropical Pacific. PLoS Biol. **5**: 0398–0431. doi:10.1371/journal.pbio.0050077

Russell, N. J., and D. S. Nichols. 1999. Polyunsaturated fatty acids in marine bacteria-a dogma rewritten. Microbial **145**: 767–779.

Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, and Fungal Barcoding Consortium. 2012. From the Cover: Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc. Natl. Acad. Sci. **109**: 6241–6246. doi:10.1073/pnas.1117018109

Shoun, H., D.-H. Kim, H. Uchiyama, and J. Sugiyama. 1992. Denitrification by fungi. FEMS Microbiol. Lett. **94**: 277–282.

Silva, N., and C. A. Vargas. 2014. Hypoxia in Chilean Patagonian Fjords. Prog. Oceanogr. **129**: 62–74. doi:10.1016/j.pocean.2014.05.016

Sime-Ngando, T. 2012. Phytoplankton chytridiomycosis: Fungal parasites of phytoplankton

and their imprints on the food web dynamics. Front. Microbiol. **3**: 1–13. doi:10.3389/fmicb.2012.00361
Simon, M., and F. Azam. 1989. Protein content and protein synthesis rates of planktonic marine bacteria. Mar. Ecol. Prog. Ser. **51**: 201–213.
Simonato, F., S. Campanaro, F. M. Lauro, A. Vezzi, M. D. Angelo, N. Vitulo, G. Valle, and D. H. Bartlett. 2006. Piezophilic adaptation: a genomic point of view. J. Biotechnol. **126**: 11–25. doi:10.1016/j.jbiotec.2006.03.038
Sobarzo, M., R. K. Shearman, and S. Lentz. 2007. Near-inertial motions over the continental shelf off Concepción, central Chile. Prog. Oceanogr. **75**: 348–362. doi:10.1016/j.pocean.2007.08.021
Souza, C. P., B. C. Almeida, R. R. Colwell, and I. N. G. Rivera. 2011. The Importance of Chitin in the Marine Environment. Mar. Biotechnol. **13**: 823–830.
Steele, C. W. 1967. Fungus Populations in Marine Waters and Coastal Sands of the Hawaiian, Line, and Phoenix Islands. Pacific Sci. **21**: 317–331.
Sturt, H. F., R. E. Summons, K. Smith, M. Elvert, and K. U. Hinrichs. 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry - new biomarkers for biogeochemistry and microbial ecology. Rapid Commun. Mass Spectrom. **18**: 617–628. doi:10.1002/rcm.1378
Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. **30**: 2725–2729. doi:10.1093/molbev/mst197
Taylor, J. D., and M. Cunliffe. 2016. Multi-year assessment of coastal planktonic fungi reveals environmental drivers of diversity and abundance. ISME J. **10**: 2118–2128. doi:10.1038/ismej.2016.24
Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**: 4673–4680. doi:10.1093/nar/22.22.4673
Vera, J., M. H. Gutiérrez, G. Palfner, and S. Pantoja. 2017. Diversity of culturable filamentous Ascomycetes in the eastern South Pacific Ocean off Chile. World J. Microbiol. Biotechnol. **33**. doi:10.1007/s11274-017-2321-7
Verma, B., R. D. Robarts, and J. V Headley. 2003. Seasonal Changes in Fungal Production and Biomass on Standing Dead Scirpus lacustris Litter in a Northern Prairie Wetland. Appl. Environ. Microbiol. **69**: 1043–1050. doi:10.1128/AEM.69.2.1043
Vestal, R., and D. White. 1989. Lipid Analysis in Microbial Ecology. Quantitative approaches to the study of microbial communities. Oxford Journals **39**: 535–541.
Vicente, G., L. F. Bautista, R. Rodríguez, F. J. Gutiérrez, I. Sádaba, R. M. Ruiz-vázquez, S. Torres-martínez, and V. Garre. 2009. Biodiesel production from biomass of an oleaginous fungus. **48**: 22–27. doi:10.1016/j.bej.2009.07.014
Wang, G., and Z. I. Johnson. 2009. Impact of Parasitic Fungi on the Diversity and Functional Ecology of Marine Phytoplankton, p. 211–228. *In* W.T. Kersey and S.P. Munger [eds.], Marine Phytoplankton. Nova Science Publishers, Inc.
Wang, G., Q. Li, and P. Zhu. 2008. Phylogenetic diversity of culturable fungi associated with

the Hawaiian Sponges Suberites zeteki and Gelliodes fibrosa. Antonie Van Leeuwenhoek **93**: 163–174. doi:10.1007/s10482-007-9190-2

Wang, J. T., T. Han, X. Y. Li, S. X. Hu, Y. D. Jiang, and C. L. Wang. 2016. Effects of dietary phosphatidylcholine (PC) levels on the growth, molt performance and fatty acid composition of juvenile swimming crab, Portunus trituberculatus. Anim. Feed Sci. Technol. **216**: 225–233. doi:10.1016/j.anifeedsci.2016.03.023

Wang, X., P. Singh, Z. Gao, X. Zhang, Z. I. Johnson, and G. Wang. 2014. Distribution and diversity of planktonic fungi in the west pacific warm pool. PLoS One **9**: 1–7. doi:10.1371/journal.pone.0101523

Webster, J., and R. Weber. 2007. Introduction to Fungi, 3rd ed. Cambridge University Press.

White, T. J., S. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protoc. A Guid. to Methods Appl. **38**: 315–322. doi:citeulike-article-id:671166

Whyte, J. N. C. 1987. Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture **60**: 231–241.

Wilcox, T. P., D. J. Zwickl, T. A. Heath, and D. M. Hillis. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. Mol. Phylogenet. Evol. **25**: 361–371. doi:10.1016/S1055-7903(02)00244-0

Wörmer, L., J. S. Lipp, J. M. Schröder, and K. U. Hinrichs. 2013. Application of two new LC-ESI-MS methods for improved detection of intact polar lipids (IPLs) in environmental samples. Org. Geochem. **59**: 10–21. doi:10.1016/j.orggeochem.2013.03.004

Wu, G., X. Sun, G. Yu, W. Wang, T. Zhu, Q. Gu, and D. Li. 2014. Cladosins A-E, hybrid polyketides from a deep-sea-derived fungus, Cladosporium sphaerospermum. J. Nat. Prod. **77**: 270–275. doi:10.1021/np400833x

Xiong, H., S. Qi, Y. Xu, L. Miao, and P. Qian. 2009. Antibiotic and antifouling compound production by the marine-derived fungus Cladosporium sp . F14. J. Hydro-Environment Res. **2**: 264–270. doi:10.1016/j.jher.2008.12.002

Yadav, A. N., P. Verma, V. Kumar, P. Sangwan, S. Mishra, N. Panjiar, V. K. Gupta, and A. K. Saxena. 2018. Biodiversity of the Genus Penicillium in Different Habitats, p. 1–18. *In* New and Future Developments in Microbial Biotechnology and Bioengineering: Penicillum System Properties and Applications. Elsevier B.V.

Zajc, J., P. Zalar, A. Plemenitâs, and N. Gunde-Cimerman. 2012. The Mycobiota of the Salterns, *In* Biology of Marine Fungi. Progress in Molecular and Subcellular Biology.

Zhang, T., N. F. Wang, Y. Q. Zhang, H. Y. Liu, and L. Y. Yu. 2015. Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). Nat. Publ. Gr. 1–11. doi:10.1038/srep14524

Zhou, Z., N. Takaya, A. Nakamura, M. Yamaguchi, K. Takeo, and H. Shoun. 2002. Ammonia Fermentation , a Novel Anoxic Metabolism of Nitrate by Fungi Ammonia Fermentation , a Novel Anoxic Metabolism of Nitrate by Fungi *. J. Biol. Chemestry **277**: 1892–1896. doi:10.1074/jbc.M109096200

Zhukova, N. V, and N. A. Aizdaicher. 1995. Fatty acid composition of 15 species of marine microalgae. Phytochemistry **39**: 351–356.