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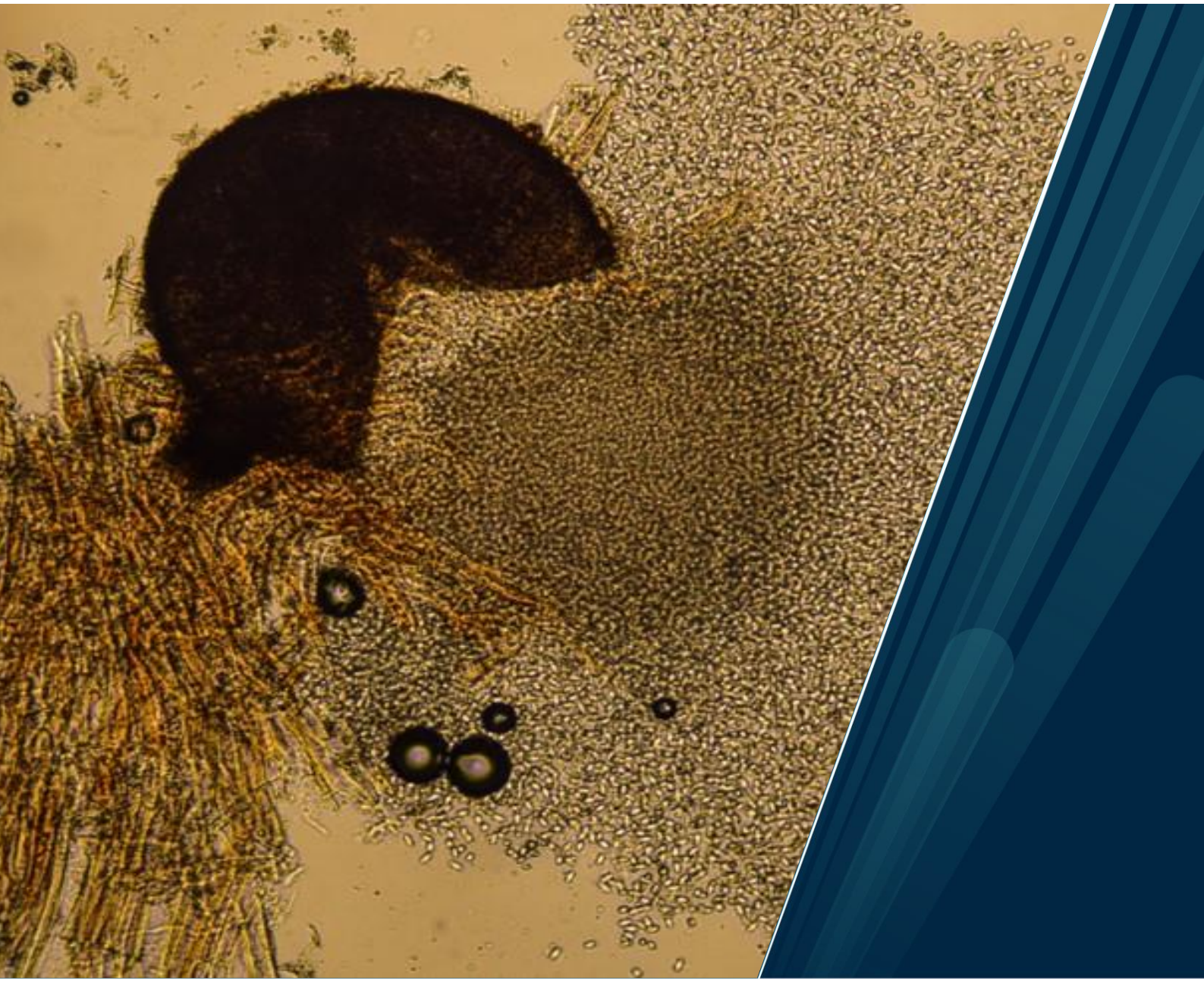
Bioprospecting of marine fungi from the High Arctic

A study of high latitude marine fungi from understudied taxa; bioactivity potential, taxonomy and genomics

Ole Christian Hagestad

A dissertation for the degree of Philosophiae Doctor

April 2021



“A true teacher would never tell you what to do. But he would give you the knowledge with which you could decide what would be best for you to do.”

— Christopher Pike, Sati

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A dissertation submitted in partial fulfilment of the requirement for the degree of Philosophiae Doctor.

Ole Christian Hagestad

Tromsø

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The work presented in this thesis was carried out at the Norwegian College of Fishery Sciences (NFH), UiT – The Arctic University of Norway from January 2017 to April 2021. The work was funded by UiT - The Arctic University of Norway as an independent PhD position.

Summary

Marine fungi comprise a group of organisms that have been overlooked for a long time. Research interest has increased with the realization of the important ecological role and rich chemistry of marine fungi. Marine fungi have yielded thousands of new natural products the last decade, but many taxa remain unstudied. Marine fungi from the Arctic have not been reported in literature in regard to bioprospecting campaigns and represent a novel source of natural products.

The aim of this thesis is to assess the potential of Arctic marine fungi to produce bioactive secondary metabolites by fermentation and genome analysis. This was achieved in three steps. First, fungi were isolated from the Svalbard archipelago. The 20 isolates obtained were characterized based on molecular markers and their antibacterial activity was tested using an agar diffusion assay (Paper 1). Secondly, three distinct marine fungi were whole genome sequenced and characterized. One of the fungi represented a putatively novel species which was circumscribed based on morphology and phylogenetic inference (Paper 2). Finally, a metabolite from one fungus among the 20 obtained around Svalbard was isolated and the bioactivities characterized (Paper 3).

In Paper 1, half of the fungal isolates showed activity against pathogenic bacteria and every third isolate represents potentially new species of fungi. Five of these isolates are strictly marine fungi belonging to the order of *Lulworthiales*. The study showed that the Arctic can yield novel marine fungal diversity that can be utilized in bioprospecting.

For Paper 2, three marine fungi were whole genome sequenced and their biosynthetic gene clusters were characterized. Mapping of the biosynthetic gene clusters (BGCs) within the *Emericellopsis* genome confirmed the detection of the secondary metabolite helvolic acid produced during fermentation. The study revealed numerous unknown biosynthetic gene clusters and a range of carbohydrate active enzymes. Each of the three genomes provides the first genome of their respective taxa and can contribute to understanding their evolutionary adaption to the marine environment.

In Paper 3, a novel compound from the fermentation broth of *Mytilinidion* sp. was isolated and its bioactivity was characterized using seven different bioactivity assays. The compound turned out to be a modified medium component with IC_{50} of 43 µM in an ACE-inhibitory assay. The compound was novel and this is the first report of its bioactivity. Molecular networking could perhaps have provided early indications that the compound was a modified medium component.

Sammendrag

Marin sopp er en gruppe av organismer som har vært oversett i lang tid. Interessen for marin sopp har steget i takt med økt forståelse for dens økologi og rike kjemi. Mange tusen nye naturprodukter har blitt beskrevet fra marin sopp de siste tiårene, men det er fremdeles mange grupper som ikke er godt studert. Marin sopp fra Arktis er ikke godt undersøkt i forbindelse med bioprospektering og representerer en ny kilde til naturprodukter.

Målet med avhandlingen var å vurdere potensialet til Arktisk marin sopp for å produsere bioaktive metabolitter ved å bruke fermentering og genomanalyse. Dette ble gjennomført i tre steg. Først ble 20 marine sopp isolert i løpet av ett forskningstokt rundt Svalbard. Disse soppene ble karakterisert ved hjelp av molekylære markører og evnen til å produsere antibakterielle forbindelser ble undersøkt ved å bruke agar-diffusjons analyse (Artikkel 1). Det neste som ble gjort var at tre forskjellige marine sopp ble helgenomsekvensert og genomene ble karakterisert. En av soppene er antageligvis ny for vitenskapen og ble beskrevet basert på morfologisk og fylogenetisk analyser (Artikkel 2). Til sist ble ett stoff fra en av soppene fra Artikkel 1 isolert og bioaktiviteten til stoffet ble beskrevet (Artikkel 3).

I den første artikkelen viste halvparten av de isolerte soppene antibakteriell aktivitet mot sykdomsfremkallende bakterier. Hvert tredje isolat representerer muligens nye arter basert på tilgjengelige referansesekvenser. Fem av de potensielt nye artene tilhører ordenen *Lulworthiales* som kun finnes i havet. Studien har vist at det er stort potensiale for å avdekke nye arter og at mange av disse viser evne til å produsere antibakterielle forbindelser. Dette viser at de kan utnyttes i bioprospektering.

I Artikkel 2 ble tre ulike marine sopper helgenomsekvenseert og genomet ble karakterisert med tanke på hvilke biosyntetiske genklynger og karbohydrat-aktive enzymer som ble detektert. Karakteriseringen av genklyngene i *Emericellopsis* bekreftet deteksjonen av metabolitten helvolsyre som soppen produserte under fermentering. Studien avslørte mange ukjente genklynger og en rekke karbohydrat-aktive enzymer. De tre genomene er de første helsekvenserte genomene i deres respektive slekter og kan bidra til å gi ny kunnskap om de evolusjonære tilpasningene de har til det marine miljø.

I den siste artikkelen ble ett ukjent stoff fra fermenteringen av *Mytilinidion* isolert. Bioaktiviteten til stoffet ble karakterisert ved hjelp av syv forskjellige bioaktivitetstester. Stoffet viste seg å være en delvis nedbrutt og modifisert mediekomponent som hadde en IC_{50} verdi på 42.3 µM i en ACE-inhiberingstest. Analyse ved hjelp av molekylært nettverk kunne kanskje avdekket at stoffet var relatert til dyrkningsmediumet.

Acknowledgements

Ever since I was a young boy I have always wanted to become a scientist and complete a PhD, mainly due to my parent's supply of Science Illustrated[1] issues. I was delighted when I got the opportunity to fulfil this dream at Marbio. I do not believe many research groups have the same team spirit on both professional and social levels as Marbio. First and foremost, I would like to express my thanks and highlight my main supervisor Dr. Teppo Rämä, who introduced me to the wonderful world of marine fungi, and was always prepared to answer questions even late in the weekend evenings. I am also deeply appreciative for my co-supervisors Prof. Dr. Jeanette H. Andersen, Prof. Dr. Espen H. Hansen and Dr. Bjørn Altermark. Your knowledge and feedback have been invaluable in pushing me forward and keeping me on track in this work. It was always possible for you to make me take a step back and look at the bigger picture when I got too hung up on details.

Secondly, I would like to express my gratitude to all of my colleagues who have contributed to my work in different ways. Thanks to Marte Albrigtsen, Kirsti Helland, Dr. Kine Ø. Hansen, Dr. Johan Isaksson, Dr. Ruth Hendus-Altenburger, Dr, Chun Li and Dr. Sara Ullsten Wahlund for their work and help on bioassays, NMR, DNA extraction and PCR. I would also like to thank everyone else at Marbio and MarBank for making these four years as enjoyable as they were. A very heartfelt thanks to the other PhD students Marte, Yannik (now Dr.), Renate and Venke that shared the PhD life with me. A special thanks to Marte and André for proofreading my thesis.

I also want to express my gratitude to the external collaborators that participated in parts of the project. A special thanks to Prof. Dr. Russell Cox and Dr. Eric Kuhnert at Leibniz Universität Hannover that allowed me to have a 3-months research stay in their group, digging into the biosynthetic potential of fungi (unfortunately cut short by corona). Thanks to Prof. Dr. Alan Dobson and his group at University College Cork for providing a deep-sea sponge isolate for our study and Lingwei Hou and Prof. Dr. Pedro Crous at Westerdijk Fungal Biodiversity Institute for providing assistance in morphological descriptions. Additionally, I would like to thank Prof. Dr. Joseph Spatafora and JGI collaborators in the 1KFG project that enabled the sequencing of the genomes.

Lastly, I would like to thank all my friends and family for checking in on me, keeping me grounded, cheering me on and giving me support and encouragement through my PhD. Thank you for letting me share my happiness and frustrations with you. Thanks to everyone who has made this journey possible. I look forward to new adventures with you in the future!

Tromsø, April 2021
Ole Christian Hagestad

[1] Science Illustrated (Norwegian: Illustrert Vitenskap) is a popular science magazine that is given out monthly covering all areas of science.

Abbreviations

18S	Small ribosomal subunit
1KFG	1000 fungal genomes project
1D/2D NMR	One and two dimensional nuclear magnetic resonance
28S	Large ribosomal subunit
ACE	Angiotensin converting enzyme
BGC	Biosynthetic gene cluster
bp	Base pair
CAZyme	Carbohydrate active enzyme
CoA	Coenzyme A
DMAT	Dimethylallyltryptophan synthase
DNA	Deoxynucleic acid
DPY	Dextrin, Peptone, Yeast extract
EtOAc	Ethyl acetate
FDA	Food and Drug Administration
HMM	Hidden Markov Model
ITS	Internal transcribed spacer
JGI	Joint Genome Institute
MS	Mass spectrometry
NCBI	National Center for Biotechnology Information
NP	Natural product
NRPS	Nonribosomal peptide synthase
OSMAC	One strain, many compounds
OTU	Operational taxonomic unit
PAINS	Pan-assay interference compounds
PhD	Philosophiae doctor
PKS	Polyketide synthase
PTP1B	Protein-tyrosine phosphatase 1B
rDNA	Ribosomal DNA
RiPP	Ribosomally synthesized and post-translationally modified peptide
RNA	Ribonucleic acid
SPE	Solid phase extraction
TCPTP	T-cell protein tyrosine phosphatase
UHPLC	Ultra high-performance liquid chromatography
WORMS	World Registry of Marine Species

List of publications

Paper 1

Hagestad OC, Andersen JH, Altermark B, Hansen E, Rämä T (2019) Cultivable marine fungi from the Arctic Archipelago of Svalbard and their antibacterial activity. Mycology:1-13. doi:10.1080/21501203.2019.1708492 (Published)

Paper 2

Hagestad OC, Hou L, Andersen JH, Hansen EH, Altermark B, Li C, Kuhnert E, Cox RJ, Crous PW, Spatafora JW, Lail K, Amirebrahimi M, Lipzen A, Pangilinan J, Andreopoulos W, Hayes RD, Ng V, Grigoriev IV, Jackson SA, Sutton TDS, Dobson ADW, Rämä T (2021) Genomic characterization of three marine fungi, including *Emericellopsis atlantica* sp. nov. with signatures of a generalist lifestyle and marine biomass degradation. IMA Fungus (In review)

Paper 3

Hagestad OC, Hanssen KØ, Isaksson J, Andersen JH, Hansen EH, Rämä T (2021) Pitfalls in biodiscovery – a case study of *Mytilinidion* sp. M16HEL1360D1-10.1, a wood-associated fungus from the marine environment in the Arctic (Manuscript)

Contributions

	Paper I	**Paper II**	**Paper III**
Concept and idea	OCH, JHA, BA, EHH, TR	OCH, JHA, BA, EHH, TR	OCH, JHA, EHH, TR
Study design and methods	OCH, JHA, BA, EHH, TR	OCH, JHA, EHH, TR, BA, LH, CL	OCH, KØH, TR, JHA, EHH
Data gathering and interpretation	OCH, TR	OCH, LH, TR, CL, EK	OCH, JI, KØH
Other contributions		TDSS, SAJ, KL, MA, JP, WA, AL, RDH, VN	
Manuscript preparation	OCH	OCH, TR, LH	OCH, KØH
Revision	OCH, JHA, BA, EHH, TR	OCH, TR, EK, JHA, EHH, BA, CL, RJC, PWC, IVG, JWS, ADWD	OCH, JHA, EHH, TR

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Cover photo:

A ruptured fruiting body of a *Halosphaeriaceae* sp. (M17HEL1517FB1) mounted on a microscope slide dispersing its spores. Field identification by Teppo Rämä. Taken on R/V Helmer Hansen during the cruise in 2017. We did not succeed in culturing the fungus.

1 Introduction

1.1 Marine environment as a source of drugs

In very broad terms, the search for natural products (NPs) is a process that has been with us humans for as long as we have recorded history (Petrovska 2012). Humans have always used natural resources around them. Bark of willow trees has been used to reduce pain and inflammation (Norn et al. 2009; Desborough & Keeling 2017). Yellow ochre has been used to alleviate internal maladies such as diarrhea since ancient Egypt (Carretero et al. 2006; Abrahams 2009). Today we actively search for new remedies in a systematic process called bioprospecting. This is done by extracting molecules produced by different organisms, testing them for biological activities and making a commercial product out of the most promising ones. This search for natural products from living beings – the isolation, screening and identification of them – can be visualized in a pipeline, Figure 1. This effort declined in the middle of the 20th century as more and more compounds were rediscovered (Kong et al. 2011) and the focus shifted towards synthetic chemistry (which ultimately failed to replicate the intricate and diverse structures produced by living organisms). Finally, in the late 20th century natural products research again blossomed due to the greater variation in chemical diversity and bioactivity compared to what synthetic chemistry was able to offer (Baker et al. 2008; Stratton et al. 2015). This development is reflected in a downward trend in natural products approved by the Food and Drug Administration (FDA). The highest point (32) occurred in 1987, and the lowest in 2006 (5), after which it stabilized at around 8 per year (Patridge et al. 2016; Newman & Cragg 2020). However, the rate of FDA drug approval in general has increased from 17 in 2002 to a record high in 2018 of 59, but this includes synthetic products (Pereira 2019; Mullard 2021).

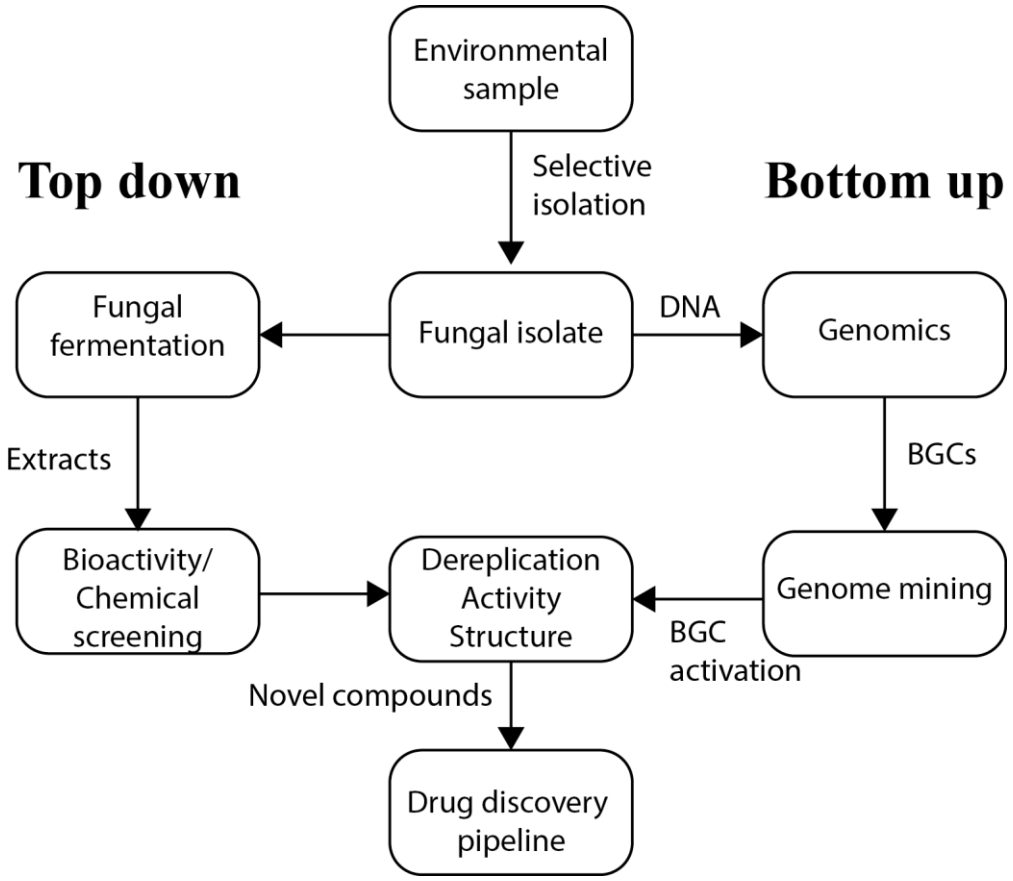


Figure 1 – The general workflow in bioprospecting for natural products for drug discovery. Top-down starts with the living organism while bottom-up starts with non-living material (DNA) after the initial sampling. Inspired from (Wikmark et al. 2016; Sekurova et al. 2019)

To combat the problem of rediscovery of known compounds, researchers looked to new niches and unexplored habitats. Especially the marine environment saw increased interest (Kong et al. 2011). The oceans make up over 99 % of the earth's biosphere (Munafo et al. 2020), containing unique habitats with vastly different environmental stresses compared to terrestrial habitats. Examples of unique habitats in the marine environment are the littoral zone, coral reefs, deep-ocean and black smokers (hydrothermal vents). One example of why the ocean is a good source to look for novel organisms can be found in the animal kingdom. One phylum is unique for the terrestrial habitats, while the marine habitats have 14 unique phyla (Heap et al. 1998). A comparison of NPs from terrestrial and marine sources, shows that the diversity and uniqueness of marine NPs is higher than those of terrestrial origin (Shang et al. 2018). The differences reflect the marine environment, where oxygen is limited and halogens are more abundant as solved ions. The high rate of novel discoveries from marine sources could be either due to a larger structural variation or higher degree of unexplored potential (Shang et al. 2018).

The marine environment has become a hotspot for NP research. Fungi, sponges, cnidarians and bacteria are the four most prolific or investigated producers of new compounds (Carroll et al. 2020). Fungi yielded the highest number of new marine compounds discovered

between 2014 and 2018 and show a rising trend in the number of compounds uncovered in this time period (Carroll et al. 2020). This indicates that marine fungi have seen an increased focus the last years and they are a good source for novel compounds. However, fungal NPs, including terrestrial sources, only make up 10-12 % of NPs (Patridge et al. 2016; Ntie-Kang & Svozil 2020). Currently, 14 marine NPs are approved as drugs and 23 NPs are in different phases of clinical trials (Ghareeb et al. 2020; Midwestern University 2020). Only one marine fungal NP is currently in development, Plinabulin in Phase III (Midwestern University 2020).

1.2 Fungi – Evolution, diversity and ecology

It is believed that fungi first evolved as aquatic organisms between 800-2,400 million years ago (James et al. 2006; Hermann & Podkovyrov 2010; Bengtson et al. 2017; Raghukumar 2017; Bonneville et al. 2020) because the basal taxa (*Opisthosporidia*, *Chytridiomycota*) are unicellular flagellated organisms. This was followed by an adaptation to life on land (as the first eukaryotes) with loss of the flagellum around the same time as plants first appeared some 460 million years ago, Figure 2 (Naranjo-Ortiz & Gabaldón 2019b).

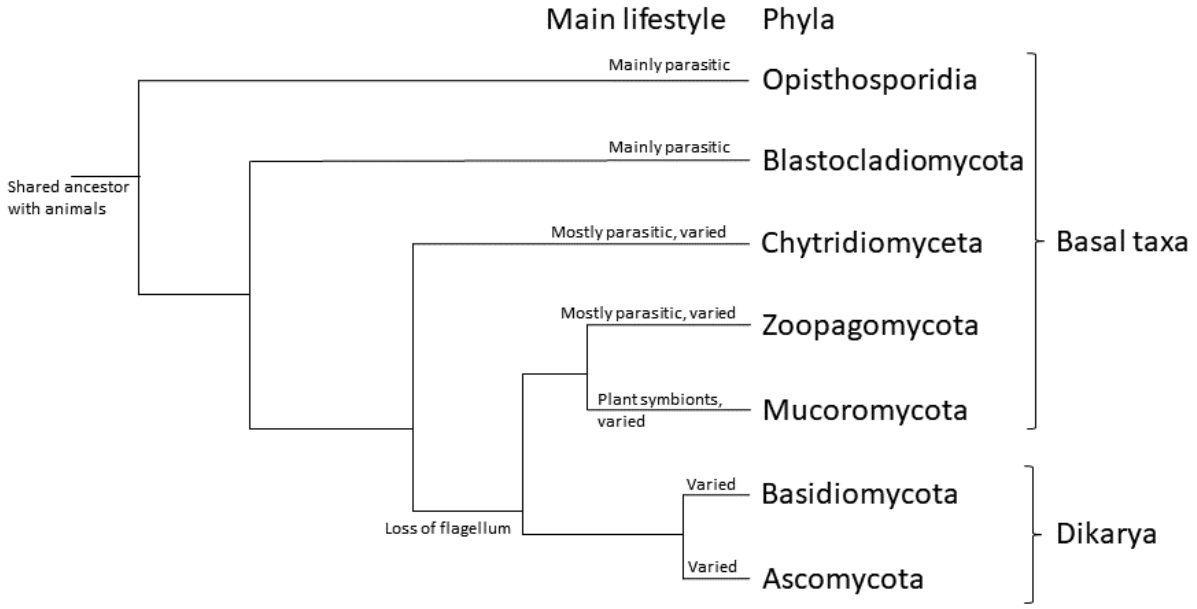


Figure 2 – Fungal tree of life showing the main divisions and ecological adaptions of fungi.
The depiction is simplified and the systematic and ecological diversity is greater. Inspired by (Guarro et al. 1999; Naranjo-Ortiz & Gabaldón 2019b, 2020; Li et al. 2021)

Fungi continued to evolve alongside other organisms and plants, forming different symbiotic relationships and adapting to different terrestrial niches. Today fungi are detected in almost every niche that has been examined (Maheshwari et al. 2000; Zhdanova et al. 2000; Dadachova & Casadevall 2008; Cantrell et al. 2011; Ciobanu et al. 2014; Wang et al. 2017). Fungi that can form multicellular macroscopic fruiting bodies can be divided into *Basidiomycota* and *Ascomycota*. The difference between these two phyla can be seen in the

septation of the mycelium and structure of the fruiting body and hymenium, Figure 3. After the colonization of the terrestrial environment, several linages in *Dikarya* (*Basidiomycota* and *Ascomycota*) have readapted to life in the marine environment. These adaptations have occurred at least 11 times since the initial terrestrial colonization (Spatafora et al. 1998; Jones et al. 2009; Jones & Pang 2012; Raghukumar 2017). The initial transition to the terrestrial environment with the loss of the flagellum might have been a larger barrier to cross than re-entry into the marine environment (Richards et al. 2011a). After the initial transition from the aquatic to the terrestrial environment, transitions in both directions have occurred many times (Richards et al. 2011a).

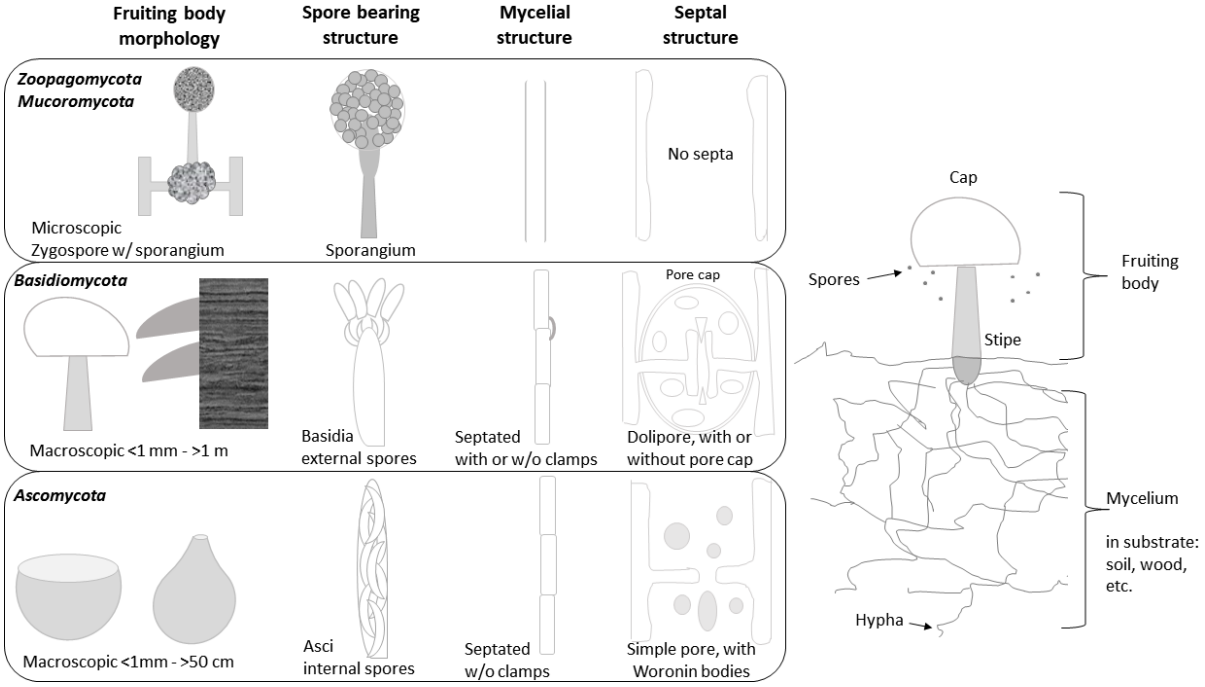


Figure 3 – Overview of the different macro- and micromorphological traits of the different higher phyla within the fungal kingdom and general morphology of fungi.
The traits shown are general examples and the diversity of structures and exceptions to these are many. Inspired from (Naranjo-Ortiz & Gabaldón 2019a, 2020)

1.2.1 Marine fungi

Fungi from the marine environment were first isolated in the middle of the 19th century from algae and plants (Montagne 1846; Shin 2020). The marine environment was not considered a major fungal habitat and the fungi were believed to be closely tied to terrestrial resources nearshore (Amend et al. 2019). This view has changed over time as we have understood the importance of fungi in the ecosystem and seen how widely distributed they are in the marine environment. Marine fungi have a wide distribution across the oceans and are found in nearly all habitats that have been examined to date, even deep beneath the ocean floor (Ciobanu et al. 2014; Ivarsson et al. 2016).

What makes a fungus in the marine environment a marine fungus? This is a central question which has been debated in the mycological community. The answer to this question seems simple; a marine fungus is a fungus that lives in the marine environment. But what is "living" in the marine environment? Is it fungi you isolate from the ocean? Metabolically active fungi in the ocean? Fungi able to reproduce in seawater? Or do they need other specific adaptations to be considered marine fungi? These are some of the questions that have been asked in the search for a definition of marine fungi. To begin with, marine fungi were characterized by the ability to grow and reproduce in marine environments or on media prepared on seawater (Johnson & Sparrow 1961; Amend et al. 2019). This definition was refined by Kohlmeyer (1974) splitting fungi into two fungal groups, the obligate marine fungi which grow and sporulate exclusively in the marine habitat, and the facultative marine fungi that are mainly freshwater or terrestrial species that are halotolerant or halophilic. This has been the dominant definition for the last 35 years (Pang et al. 2016).

The definition proposed by Kohlmeyer (1974) has excluded seemingly terrestrial fungi that are repeatedly isolated or detected in the marine environment (Jones et al. 2009). The latest definition proposed by Pang et al. (2016) suggested that marine fungi should have a broad definition and include fungi that are repeatedly isolated from the marine environment because they (1) grow or sporulate in the marine environment, (2) form symbiotic relations with other marine organisms, or (3) adapt, evolve or are metabolically active in the marine environment. Lately, a new term was introduced, marine fungi "*sensu stricto*" (*s.s.*, obligate marine fungi as Kohlmeyer (1974) defined it), these are marine fungi that occur only in the marine environment with no known species on land (Overy et al. 2014). Finally, within natural products research and bioprospecting, the term "marine-derived" is common, indicating that the fungus has been isolated from a marine source. Generally, papers on natural products research are usually focused on the secondary metabolites produced and not on the physiology or ecology of the isolated fungi (Pang et al. 2016). The relationship between these definitions of marine fungi can be seen in Figure 4.

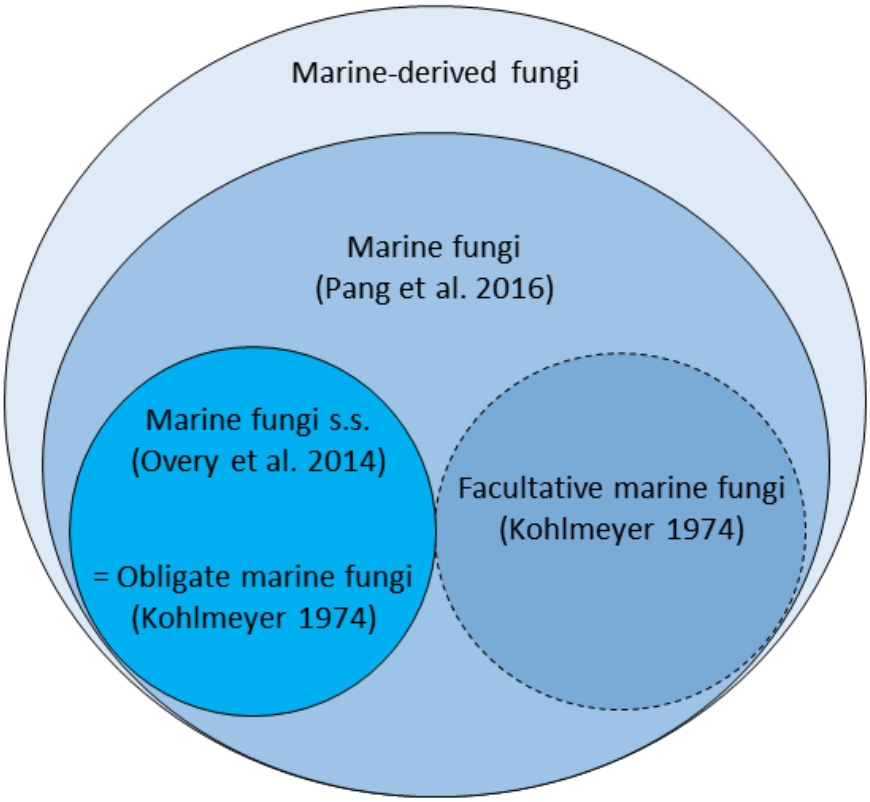


Figure 4 – Overview of the different definitions of marine fungi and how they relate to each other. Adapted from a figure provided by Teppo Rämä.

In order to check if a fungus fits the definition of Pang et al. (2016), a few methods can be used. Active mycelium and dormant spores that germinate from a sample are difficult to differentiate. Detection of active mycelium can be performed by analyzing RNA which is less stable and only present if a cell is metabolically active. This is in contrast to DNA, which is more stable, and present regardless of the state of the cell (Pang et al. 2016; Singer et al. 2017). Resting spores that are able to germinate in the presence of salt would fall into the category of being able to germinate in the marine environment and can thus be regarded as marine. It is therefore important to use salt based media for isolation of marine fungi. Isolation of marine fungi will be discussed further in chapter 1.2.3.

In this thesis, the term marine fungi will be used for fungi isolated from the marine environment using sea water based media. If a fungus is a marine fungus *sensu stricto*, it will be specified when relevant.

1.2.2 Taxonomic identification of fungi

There are several ways to identify species of fungi. Before the DNA sequencing era, fungi were classified based on morphological traits, such as the ones shown in Figure 3. Different taxa usually have markedly different macro- and microscopic traits, such as septal structure, branching of mycelium, spore shape, size and appendages, and color reactions in different

chemicals. This is sometimes referred to as classical or alpha taxonomy. Convergent evolution, where two distantly related genera have evolved similar morphological traits, can be difficult to detect and resolve solely based on alpha taxonomy. The opposite can also be a challenge, where phylogenetically closely related fungi have large morphological differences.

Alpha taxonomy has been partially replaced by molecular taxonomy. By comparing different conserved regions in the genome, it is possible to identify which taxonomic group a fungus belongs to. There are several conserved regions of the genome that are used to determine the systematic position. These genetic regions are often called barcode regions. The main fungal barcode region is the internal transcribed spacer (ITS) region (Schoch et al. 2012). There are many other areas in the genome that can be used for taxonomic placement and the selection depends to some degree on the group of fungi that is studied (Quast et al. 2013; Visagie et al. 2014; Stielow et al. 2015; Banos et al. 2018). Molecular taxonomy is only as accurate as the selection of the barcode region and availability of reference sequences. Often, isolates which have nearly identical barcode sequences (a similarity above a certain threshold) are grouped into what is called operational taxonomic units (OTUs). These are then considered to roughly represent the same species (Ryberg 2015). Convergent evolution, which can be a challenge in alpha taxonomy, can be resolved by molecular taxonomy. A complementary approach using both alpha and molecular taxonomy is usually advisable to determine the systematic placement accurately (Lücking et al. 2020).

Many fungi do not produce characteristic fruiting bodies in the laboratory and have few macro and microscopic traits that can be discerned. Fungi lacking sexual fruiting bodies are often called anamorphic fungi. Systematic placement of anamorphic fungi has been greatly aided by molecular taxonomy.

1.2.3 Current status and trends in research on marine fungi

The mycological research efforts can be classified into fundamental and applied research. Fundamental research attempts to understand the biology, physiology, evolution, ecology or biogeography of fungi. Applied research utilizes the fundamental understanding of fungi to solve specific practical issues or challenges, for example in bioremediation, bioprospecting or biocatalysis. Fundamental and applied research can overlap. Applied research is usually based on initial findings from fundamental research. The development of research on marine fungi can be viewed with these two branches of research in mind.

Marine fungi were first isolated as early as 1846 (Montagne 1846). However, attention to marine fungi as a distinctly adapted ecologically important group only began in the middle

of the 20th century (Barghoorn & Linder 1944; Johnson 1956a, 1956b). Since then a relatively small group of mycologists worked in this field and the numbers of publications generally did not exceed 10 per year until the 1990s, Figure 5. This was around the time when fungal research also started to branch more into applied mycology and screening for natural products and enzymes (Rohrmann & Molitoris 1992; Fenical & Jensen 1993; Davidson 1995). This trend can be seen in the sudden increase in publications on marine natural products in the early 90s.

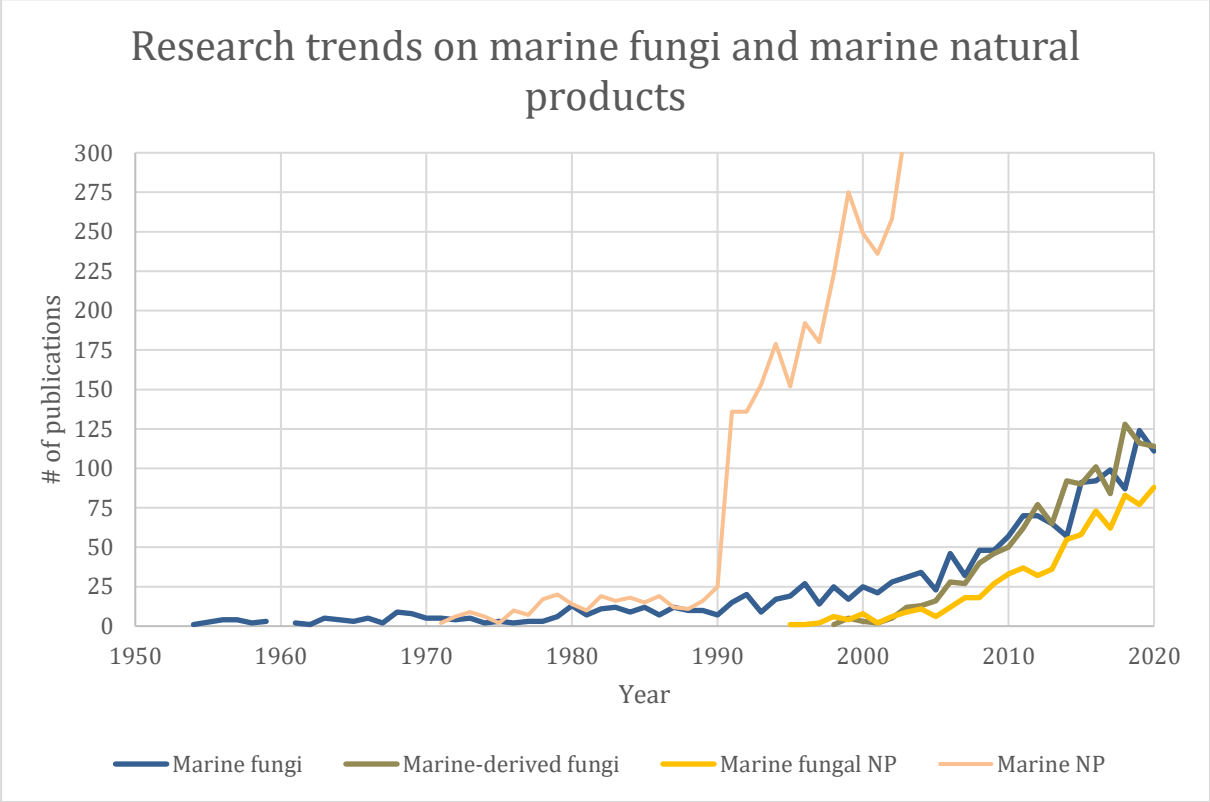


Figure 5 - Number of publications on marine NPs, fungi and fungal NPs spanning from 1950-2020. This does not reflect all publications in each field but the trend of publications as the search is limited by the keywords used. Data extracted from Clarivate Analytics (2021). The search strings used were: Marine fungi ("Marine fungi" OR "marine fungus"), Marine-derived fungi ("marine-derived fungi" OR "marine-derived fungus"), marine fungal NP (("Marine fungi" OR "Marine fungus" OR "marine-derived fungi" OR "marine-derived fungus") AND (natural product OR secondary metabolite OR bioprospecting), Marine NP (Marine AND (natural product OR secondary metabolite OR bioprospecting))

Despite the acceleration in research on marine-derived fungi and marine fungal natural products, the research on marine fungi *sensu stricto* is lagging behind (Overy et al. 2014). However, this discrepancy has been noticed and marine fungi *sensu stricto* have to a larger extent entered the spotlight the last few years (Jones 2011; Overy et al. 2014; Tasdemir 2017; Overy et al. 2019). The issues raised by these authors show the necessity of an ecological understanding in order to properly determine how different taxa relate to the marine environment.

Many microorganisms do not grow on artificial media and are therefore uncultivable. The problem of cultivability has been well established in bacteria (Rappé & Giovannoni 2003).

This is also a challenge in investigating and isolating marine fungi (Amend et al. 2019). This can be seen in the difference of detected taxa between cultivation studies and culture-independent studies (Grossart et al. 2016; Chrismas & Cunliffe 2019; Wu et al. 2019). The uncultivable diversity detected from culture-independent methods has been coined "microbial dark matter" (Garrity et al. 2013). The difference between culture and culture-independent studies is in part due to selective biases of the different methods that are used (Stefani et al. 2015; Rämä et al. 2017). Environmental DNA studies have been able to detect previously unknown fungal phyla (Amend et al. 2012; Manohar & Raghukumar 2013). Several strategies have been developed to grow fungi in laboratory conditions outside of their natural habitat. Examples of different strategies are baiting with specific substrates (Prenafeta-Boldú et al. 2001; Overy et al. 2014; Basu et al. 2015; Hassett et al. 2019), adapting cultivation by using extracts from hosts, changing temperature, pH, salinity, pressure or other environmental variables (Kohlmeyer & Kohlmeyer 1979; Redou et al. 2015), *in-situ* diffusion chambers such as the iChip or microbial traps (Nichols et al. 2010; Epstein 2013; Berdy et al. 2017; Libor et al. 2019; Overy et al. 2019; Liu et al. 2020a).

1.2.4 The adaption of fungi to the marine environment

The estimates indicate that there are as many as 2.2-3.8 million fungal species. Around 120,000 (3.2-5.5 %) of these species are described, with most of these being terrestrial (Hawksworth & Lücking 2017). Marine species are found in almost all parts of the fungal tree of life (Manohar & Raghukumar 2013; Jones et al. 2019). Currently, there are over 1,300 recognized marine fungal species (Jones et al. 2019; WoRMS Editorial Board 2021), which is about 10 % of the current estimate of the total number of marine fungal species (Jones & Pang 2012). Most of the described marine species are ascomycetes (1027) (Jones et al. 2015; WoRMS Editorial Board 2021). Within *Ascomycota* there are several classes with marine fungi *sensu stricto* orders, *Sordariomycetes* is the largest class with 7 orders, followed by two in *Dothideomycetes* and one in each of *Eurotiomycetes* and *Lecanoromycetes* (Jones & Pang 2012).

Marine fungi have morphological, physiological and ecological adaptations to the marine environment (Overy et al. 2019). Morphological adaptations include filamentous spores or appendages on the spores, sometimes with mucus, small or embedded fruiting bodies and passive spore release (Hyde et al. 1993; Jones 2000; Sridhar 2009; Jones 2011; Overy et al. 2019). The physiological adaptations of marine fungi enables them to tolerate high salt levels, low water potential, higher pH and low temperatures (Raghukumar 2008). These tolerances are conferred through a variety of molecular mechanisms, for example, ion transporters, osmolytes

and different cell membrane composition, adaptation of enzymes and substrate preference (Jennings 1983; Raghukumar 2008; Ahumada-Rudolph et al. 2014; Lee et al. 2015). The marine environment also offers different substrates compared to the terrestrial environment, which requires specialized enzymes (Balabanova et al. 2018; Pilgaard et al. 2019). This will be discussed further in section 1.5.3.

In the context of adaptation to the marine environment, the genus *Emericellopsis* (*Hypocreales, Sordariomycetes*) could provide an interesting case study. Molecular work has shown that most of the species within *Emericellopsis* form two distinct clades, one marine and one terrestrial clade, as well as a putative alkali-tolerant clade (Zuccaro et al. 2004; Grum-Grzhimaylo et al. 2013; Gonçalves et al. 2020). Lately, it has been questioned whether this actually holds true as more species are being described, especially considering the ecology of the three most recent species (Gonçalves et al. 2020). The three species are all isolated from marine algae, but each species is placed in different clades, breaking the previously established hypothesis of a marine and terrestrial clade. Similarly, *Calycina marina* (previously *Laetinaevia marina, Helotiales, Leotiomycetes*) was considered the sole member of a strictly marine genera based on ecology and morphology until a more recent molecular study placed the genus within *Calycina*, a terrestrial clade (Jones & Pang 2012; Baral & Rämä 2015). These fungi are examples of how previous assumption and knowledge is challenged when new techniques are used, and new species are isolated and described.

1.3 Natural products from fungi

Natural products (NPs) and metabolites are basically the same. They are any organic molecules or compounds produced by the enzymes of an organism. Some of the metabolites form larger macromolecules and polymers, such as cellulose, lignin, proteins, DNA and RNA. The macromolecules and large polymers are usually excluded when the metabolome is considered. Just as the genome contains all genetic information of an organism, the metabolome contains all small molecules, usually <1500 Daltons, that an organism produces (Wishart 2008; Lamichhane et al. 2018). The natural products that are investigated for pharmaceutical uses are often even smaller, <500 Daltons (Lipinski et al. 1997). There are many NPs that are larger than 1500 Da (Benet et al. 2016), an example of this is ziconotide at 2630.2 Dalton (Wang & Bowersox 2000).

The metabolites are often classified into primary metabolites and secondary metabolites. Primary metabolites are considered essential for the survival of the organism. They are usually found across the entire tree of life or large taxonomic groups, for example the nucleotides for

DNA and RNA synthesis, phospholipids in cell membranes and amino acids used in proteins. Secondary metabolites are considered nonessential metabolites that often increase the organisms' ability to survive, communicate and reproduce, or they have no known function. All secondary metabolites branch out from the primary metabolites. Amino acids can be turned into alkaloids, flavonoids or specialized peptides, acetyl-CoA are the building blocks for polyketides, terpenoids and lipids and these can be merged in any numbers of ways with other primary or secondary metabolites to form hybrid metabolites.

There are some prolific fungal clades renowned for their number of natural products. These include fungi within *Eurotiales*, such as *Penicillium*, *Aspergillus* and *Fusarium* (Wisecaver et al. 2014). However, the marine nature of many fungi within those clades are questioned (Overy et al. 2014). The genomes of these taxa show many cases of horizontal gene transfers and expansion of biosynthetic gene clusters or enzymes (Richards et al. 2011b; Fitzpatrick 2012; Chen et al. 2014; Wisecaver et al. 2014). Since the research effort is not equal on different classes of fungi it is difficult to say how marine fungi *sensu stricto* compare to the more cosmopolitan fungi. By 2014, only about 80 of more than 1000 described fungal metabolites were from marine fungi *sensu stricto* (Overy et al. 2014).

Many secondary metabolites are produced by enzymes encoded in biosynthetic gene clusters (BGCs), Figure 6. The biosynthetic gene clusters have a structure where a core gene produce an enzyme that generates the scaffold of the molecule. Then other enzymes, coded by different tailoring genes, modify this scaffold, e.g. by adding functional groups. Transporters can be present, which are responsible for the secretion of these compounds to the environment. The expression of genes within the cluster are often regulated by transcription factors that are activated based on environmental cues (Keller 2019).

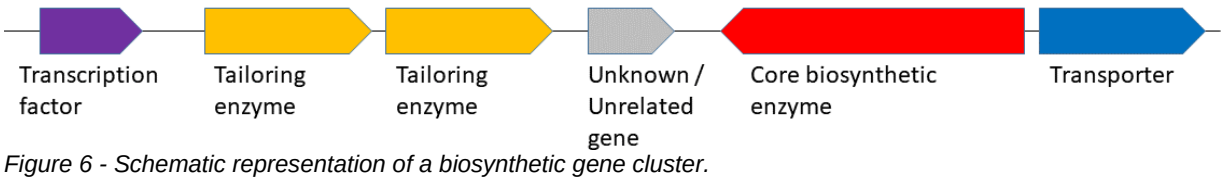


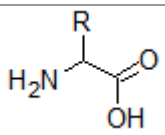
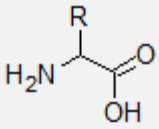
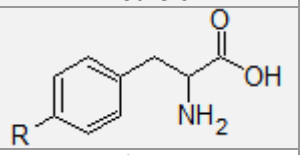
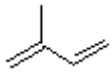
Figure 6 - Schematic representation of a biosynthetic gene cluster.
They can contain several copies of genes, especially tailoring genes. In case of hybrid clusters, there are several different types of core biosynthetic genes. A minimal biosynthetic gene cluster contains at least the core enzyme and one or more tailoring gene. Adapted from (Keller 2019)

1.3.1 Natural product classes

Natural products can be categorized in many different ways based on the chemical, structural or functional properties, biosynthetic pathways or by species taxonomy. The different classes and biosynthetic pathways that produce the natural products are numerous and diverse, due to this many NPs can fit into several classes depending on the definition that is used. Here,

they will be classified based on their constituent subunits. Following this, NPs can be classified broadly into the categories polyketides, specialized amino acids and peptides, terpenes, specialized carbohydrates and hybrids (Hanson 2003), Table 1.

Table 1 – Overview of different classes of secondary metabolites from fungi.
Alkaloids are included as a subclass of specialized amino acids. Adapted from (Hanson 2003; Keller et al. 2005; Keller 2019)

Class	Pathway	Subunit / derivative of
Polyketides and fatty acids	Polyketide synthases	
Specialized amino acids, derivatives and peptides:	Nonribosomal peptide synthases Ribosomally synthesized and post-translationally modified peptides	
• **Alkaloids**[1]	Various Dimethylallyltryptophan synthases Cyclic dipeptide oxygenase	 + others
• **Phenylpropanoids / flavonoids**[2]	Shikimate pathway Aromatic amino acids	
Terpenes:	Mevalonate pathway	
Specialized carbohydrates	Various	Various
Hybrids:	Mix of the above	Mix of the above

[1] Alkaloids are generally considered nitrogen containing compounds, they have considerable chemical diversity and comes from different pathways. True alkaloids have nitrogen that comes from amino acids. Often alkaloids are divided in three classes, true alkaloids, protoalkaloids and pseudoalkaloids (Vickery & Vickery 1981; Zhang et al. 2012).

[2] It has been claimed that fungi does not possess the enzymatic machinery for flavonoid production (Gil-Ramírez et al. 2016), but this has later been disputed (Mohanta 2020).

Polyketides are the most common class of secondary metabolites produced by fungi (Rateb & Ebel 2011; Joint Genome Institute 2021b). Polyketides are synthesized by polyketide synthases (PKSs) from acetyl-CoA, propionyl-CoA and malonyl-CoA that are condensed together. Amino acid derivative NPs are produced by, among others, nonribosomal peptide synthases (NRPSs), ribosomally synthesized and post-translationally modified peptides (RiPPs) and dimethylallyltryptophan synthases (DMATs). The compounds resulting from these pathways are built up by amino acid chains or modified amino acids. The amino acid residues can be proteogenic and/or non-proteogenic. Alkaloids are usually heavily modified amino acids, or the amine group has been donated from amino acids. Diketopiperazines are a special

class consisting of two amino acids in a fused cycle. Phenylpropanoids are derived from the shikimate or aromatic amino acid pathways. In contrast to alkaloids, the amine group of the amino acid has been removed while the aromatic ring is retained. This group of compound is not very common in fungi because the enzymatic machinery to produce the compounds are not universally distributed in the fungal kingdom (Mohanta 2020). Terpenes (or terpenoids or isoprenoids) are made from one or more isoprene units (Oldfield & Lin 2012). These are the precursors to carotenoids, sterols and rubber (Brahmkshatriya & Brahmkshatriya 2013). Terpenes are often classified based on the number of isoprene units in the skeleton (Abdallah & Quax 2017). One of the most common terpenoids (triterpenoid) is cholesterol, and its fungal equivalent ergosterol (Liu & Nes 2009). Carbohydrates rarely occur as core structures in secondary metabolites and are usually attached to other classes of NPs. Carbohydrate containing NPs are called glycosides (Weymouth-Wilson 1997).

Many secondary metabolites can seem similar to primary metabolites. However, they can have some key modifications that makes it possible to differentiate them from the primary metabolites. The modifications change the chemical properties of the secondary metabolites which can affect the selectivity, function and stability in the environment. One example of how stability is affected is the modification of the peptide bonds in many NP peptides. Usually, proteins and peptides are readily degraded by enzymes that specifically target the peptide bonds between amino acids. These enzymes are called proteinases or peptidases. By adding a methyl group to the nitrogen of the peptide bond or using non-proteinogenic amino acids, Figure 7, the proteinases and peptidases are unable to recognize the peptide bond and unable to cleave the NP which increases the NPs stability (Dong et al. 2012; Böttger et al. 2017).

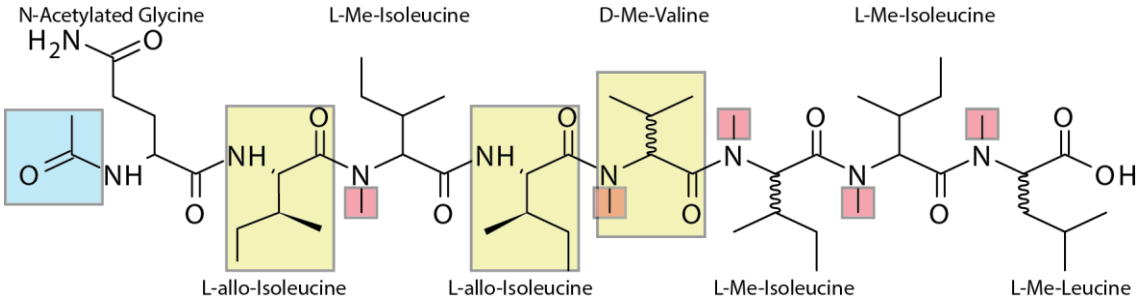


Figure 7 - Acrepeptin A, an example of a non-ribosomal peptide with modifications highlighted. Blue is N-acetylated capping of the end of the peptide. Red is N-methylated peptide bonds. Yellow is non-proteinogenic amino acids. These modifications differentiate NRPS peptides from ribosomally produced peptides and increase their structural diversity, stability and resistance to degradation. Adapted from (Hsiao et al. 2020)

1.3.2 Troublesome natural products and un-natural products

There are a few special cases of NPs that deserve mention when it comes to bioprospecting and bioactivity screening (which will be introduced in chapter 1.4). Pan-assay interference

compounds (PAINS) are compounds that are known to have many biological activities, unspecific binding, interfere with assay readout and can thus seem promising at first due to high bioactivity (Dahlin et al. 2015; Baell 2016; Pouliot & Jeanmart 2016). Because they interact and bind unspecifically to many different enzymes, receptors and proteins used in assays, they are often referred to as promiscuous compounds. PAINS should be possible to detect if a sample shows activity in multiple unrelated target assays. Many PAINS have highly reactive functional groups such as thiols and specific chemical motifs that can be recognized and used to aid in the identification of them (Baell & Holloway 2010; Baell 2016; Baell & Nissink 2018).

A second group of compounds that should be kept in mind are NP artefacts or pseudo-NPs. These compounds are derived from natural products that have been modified by spontaneous chemical reactions due to changes in pH, temperature or reactions with extraction solvents (Maltese et al. 2009; Hanson 2017; Venditti 2018). The group of artificial NPs have received attention in the last few years due to an increasing number of reports of new NPs that are in fact suspected of being artefacts from the extraction process (Venditti 2020). Many of these compounds are new, but they are reported as having a natural origin (e.g. biologically produced) instead of being derived artificially during the extraction procedure (Venditti 2020).

1.3.3 Natural environment as inspiration for expression of natural products

Secondary metabolites are produced for a multitude of reasons. They can be used to promote host growth, signaling and quorum sensing, virulence, protection against environmental stresses, defense against competitors and nutrient acquisition (Rokas et al. 2020). The production of these metabolites can be triggered by mimicking conditions under which these metabolites are naturally produced, similar to mimicking the environment for the isolation of fungi. The environment is a very important source of inspiration for different cultivation mediums. By examining the environment the fungi is isolated from, environmental triggers or competing organisms that can be used in co-culture can be detected. The addition of triggers to cultivation media can activate production of unique metabolites (Bertrand et al. 2013; Haas 2015). A technique based around this approach is the One Strain Many Compounds (OSMAC) method (Hewage et al. 2014; Gubiani et al. 2016; Romano et al. 2018). This method has been employed in numerous ways from using ground up host tissue, mimicking environments such as seawater, co-culturing to mimic competition, different sources of nutrition or lack of it (for example iron free medium for siderophore production) and varying carbon and nitrogen sources (Romano et al. 2018).

One of the many challenges with natural products research is that many secondary metabolites are not expressed under laboratory conditions (Brakhage et al. 2008; Brakhage & Schroeckh 2011). The OSMAC approach has been employed with success in this regard (Hewage et al. 2014; Fan et al. 2019; Zhang et al. 2019). However, other methods exist to enable the expression of additional metabolites. The methods include modifying the genome to overexpress global or specific regulators, modifying epigenetic regulators, heterologous expression of gene clusters or gene editing using CRISPR/Cas9 (Reen et al. 2015; Zhang et al. 2019).

1.4 Screening fungal metabolites for bioactivity

Several diseases are increasing in prevalence in today's society. Modern medicine is currently facing increasing incidences of multiresistant bacteria, cancer and diabetes (Ventola 2015; World Health Organization 2020). This means that there is an increasing need for new medicines that can treat these conditions (Lage et al. 2018). Different types of screening methods and assays are used in order to detect different activities and targets. If there is activity in different unrelated assays, especially for pure compounds, the activity could be caused by unspecific interactions caused by the aforementioned PAINS (Fu et al. 2019). This is undesired because you want the NPs to be specific in their activity. This might not be the case in complex extracts containing many different compounds. In complex mixtures compounds can interact and cause synergistic or antagonistic effects. This leads to an increase or decrease, respectively, in their activity compared to their individual effects (Li & Zhang 2008; Stefanović 2017).

1.4.1 Phenotype / cell-based assays

Phenotypic assays are screening assays that look for an observable change in cells or organisms caused by a compound. The use of a phenotypic assay allows for detection of effects on a complex system, where many different enzymes, proteins and receptors, called targets, are present. Commonly used phenotypic assays for determining the toxicity of NPs are cell based viability assays (Hansen & Bross 2010). By using the cell viability assay, it is possible to screen NPs that cause changes in the cells survival by for example colorimetric readouts, Figure 8. There are several advantages of using phenotypic assays. No prior knowledge about the target of the compound is required and novel targets can also be discovered by using phenotypic assays (Moffat et al. 2017). Another advantage is that the results you get are usually biologically relevant. It is a live system representative of the pathogen or disease (Ursu et al. 2017). If several different phenotypic assays are used, for example using different cancer cell types, fungi or

bacterial strains, it is possible to detect the specificity of the compound tested and potentially get clues of the affected target (Matano et al. 2016; Moffat et al. 2017; Lage et al. 2018). Ideally, antibacterial NPs should be harmless to human cells and only affect the bacterial cells. One disadvantage of phenotypic assays is that it can be difficult to deconvolute the target the compound interacts with and to screen out promiscuous compounds (Moffat et al. 2017; Ursu et al. 2017).

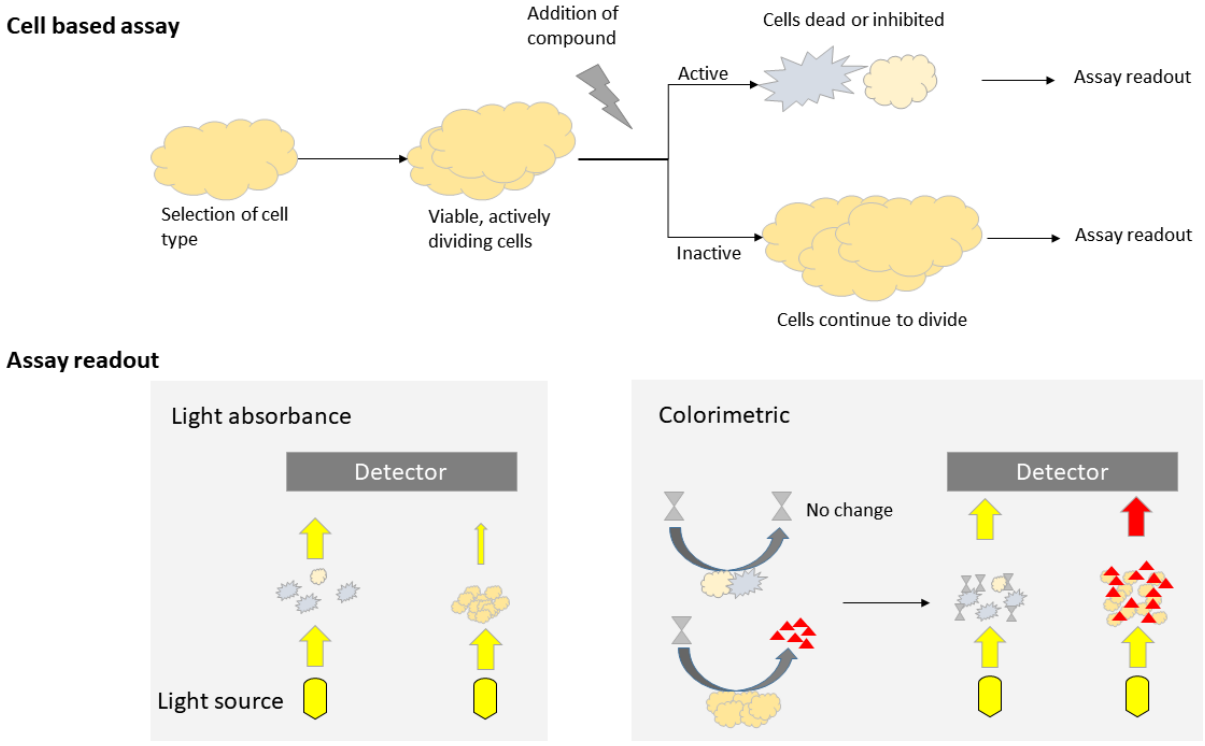


Figure 8 – An example of a phenotypic assay where a compound is added and two different methods to detect the resulting phenotypic change.

1.4.2 Biochemical or target based assays

Biochemical or target-based assays will only allow detection of compounds interacting with the specific protein, enzyme or receptor used in the assay (Fu et al. 2019). The advantage of using a target-based assay is that the target is representative for a specific disease. The interaction between compound and target can be measured directly. Target-based assays requires that the target is well characterized in terms of the disease it is involved in (Matano et al. 2016), and there are many different types of targets that can be used, Figure 9. Additionally, other similar targets or off-targets can be used as counter screens to measure specificity (Lage et al. 2018). An example of a biochemical assay is the Protein-tyrosine phosphatase 1B (PTP1B) enzyme assay, PTP1B is a negative regulator of insulin and it is a promising target for anti-diabetic therapeutics. A counter-screen with T-cell protein tyrosine phosphatase (TCPTP) is often used because TCPTP is essential for normal growth and development (Tiganis 2013).

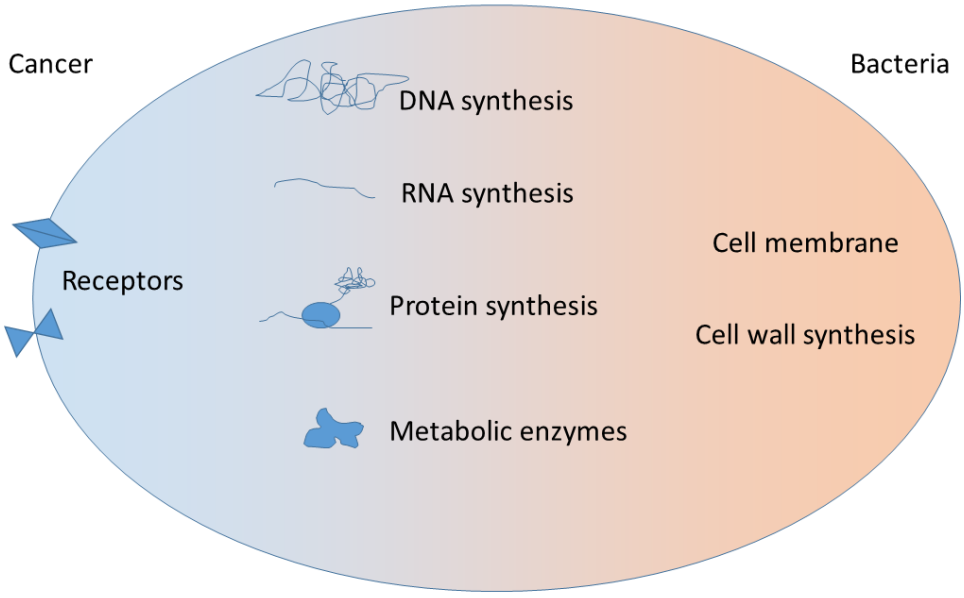


Figure 9 – Examples of different cellular target classes that can be targeted by anticancer and antibacterial compounds.
Adapted from (Chifiriuc et al. 2016; Nass & Efferth 2018)

1.4.3 Screening complex mixtures

In a complex extract, such as crude extract from fermentations, there are very many different compounds. Crude extracts contain lipids, carbohydrates, media components and salts that have unspecific effects and can interfere with the assays used to screen for bioactivity. In order to limit and decrease the interference from such compounds, it is generally advisable to pre-fractionate the extract (Hubert et al. 2017). This creates a series of fractions that are often separated based on polarity. Highly polar sugars, salts and media components are usually found in the early fractions, while very hydrophobic compounds such as lipids and oils are found in the last fractions, if reverse phase chromatography is used (Appleton et al. 2007). Carbohydrates and lipids are often unwanted because they are part of the primary metabolites and can have unspecific effects that are not possible to exploit pharmaceutically.

Other confounding effects are the synergistic and antagonistic effects between different compounds. Pre-fractionation have shown promise in reducing these interfering effects, enabling the detection of active NPs that would have been missed (Thornburg et al. 2018). This is also because the active compounds become more concentrated when the complexity of extracts are reduced. The reduced complexity again simplifies the dereplication that has to be performed to identify the active components (Thornburg et al. 2018).

1.5 Fungal genomics

In the early 2010s, the Joint Genome Institute (JGI) started a scientific community project called the 1000 fungal genomes project (1KFG) which aims to make fungal genomes available for the public (Grigoriev et al. 2011; Grigoriev et al. 2014). Only a small portion of the diversity in the fungal kingdom had been sequenced and a significant portion of the diversity had no sequencing data available.

Currently, more than 7,680 genomes belonging to 2,655 fungal species are available in GenBank. Additionally, 1,821 genomes are available on the JGI webpage of MycoCosm, although there is overlap between the databases. Fungal genomes can vary greatly in size, from 2.3 Mbp in *Encephalitozoon intestinalis* (The smallest eukaryotic genome; smaller than many bacteria) to the three orders of magnitude larger 3.6 Gbp in *Jafnea semitosta* (Over half the size of the human genome at 6.27 Gbp) (Corradi et al. 2010; Egertová & Sochor 2017; Piovesan et al. 2019). Currently, there are no searchable databases of fungal genomes where metadata of ecological niches or geographical location can be easily accessed. Only a small portion of the known fungal diversity has genomic data available. The 1KFG project is of immense importance. The genetic information it provides can give clues to evolutionary traits for specific lifestyles or adaptions and aid in phylogenetic work. In addition, it can reveal the capabilities of natural product synthesis within the vast fungal biodiversity.

1.5.1 Biosynthetic gene clusters in the fungal kingdom

Biosynthetic gene clusters (BGCs) that encode proteins involved in the synthesis of secondary metabolites, introduced in section 1.3, are not evenly spread around in the fungal kingdom. Different phyla have different abilities to produce secondary metabolites. Genome sequencing allows us to compare the potential of fungi to produce secondary metabolites based on BGCs that can be found in the genomes. With an increased amount of sequenced genomes, it is possible to pre-screen fungal strains by performing whole genome comparisons. A comparison of 101 Dothideomycetes genomes showed that the core genome consists of approximately 2,000 genes and the total number of genes varied from 10,000-22,000 genes (Haridas et al. 2020). It also revealed that the amount of repetitive elements varied tremendously. In some cases, the repetitive elements made up more than 66 % of the entire assembly (Haridas et al. 2020).

A kingdom-wide comparison of fungi in terms of genome size and metabolic gene clusters showed that fungal genomes smaller than approximately 20 Mbp contained few, if any, secondary metabolic clusters (Rokas et al. 2018). This seems to indicate that genomes need to

be of a certain size in order to harbor biosynthetic gene clusters. Larger genomes contain a larger portion of repetitive elements (Mohanta & Bae 2015; Li et al. 2018; Haridas et al. 2020), which increases the difficulty of direct comparison of genomes, Figure 10. Most of the repetitive elements are non-coding such as tandem repeats, but interspersed repeats can contain genes or pseudogenes (Muszewska et al. 2019).

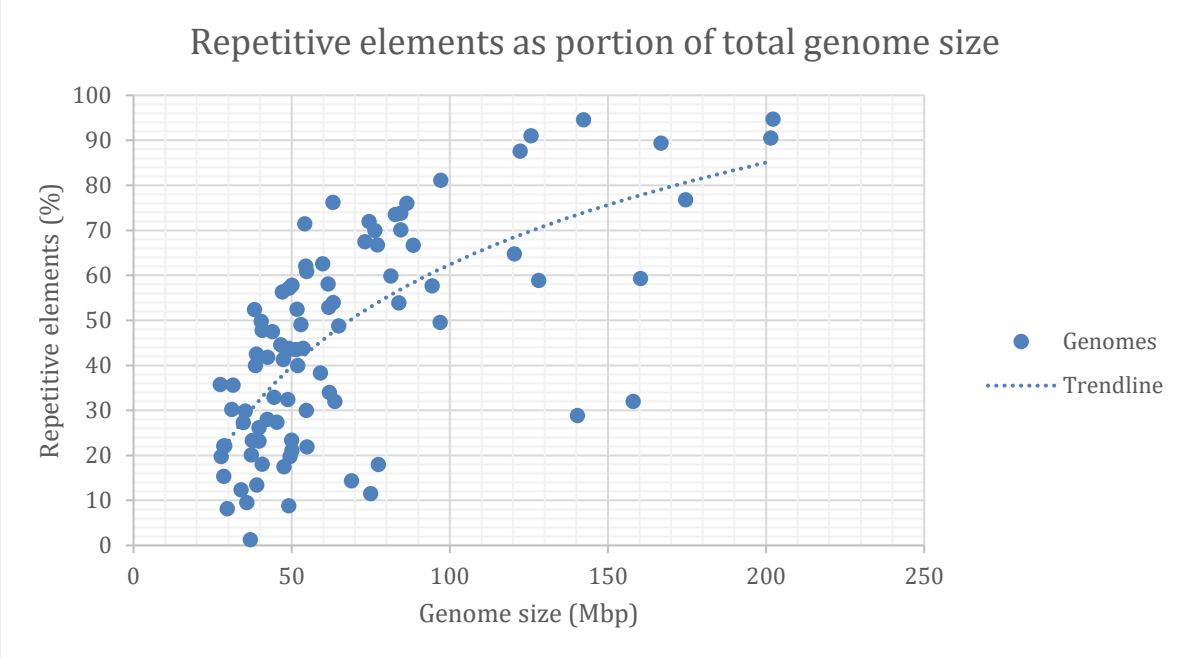


Figure 10 – Correlation between repetitive elements (%) and genome size (Mbp) in fungal genomes. The graph is based on 90 genomes with repeat content analyzed from Basidiomycota (83) and Ascomycota (7) as reported by (Li et al. 2018).

A better genome comparison can be achieved using the number of BGCs in the genomes against the gene count of each genome, Figure 11. This enables us to see the trends of the different orders of fungi. Indeed, there is a sharp decline in the number of BGCs in genomes with a low gene count. This trend can aid in the selection process in finding good candidates for bioprospecting. Few fungi have more than 100 clusters, and the *Eurotiomycetes* generally have the greatest number of BGCs in relation to total gene count. The non-dikarya (*Mucoromycota*, *Chytridiomycota*, *Cryptomycota*, *Microsporidia*, *Blastocladiomycota* and *Zoophagomycota*) and yeasts in *Ascomycota* (*Saccharomycotina* and *Taphrinomycotina*) have few BGCs compared to the rest of the fungal kingdom in relation to their gene count. There are a few exceptions to this in *Chytridiomycota* such as *Orpinomyces* sp., with 155 BGCs (Youssef et al. 2013).

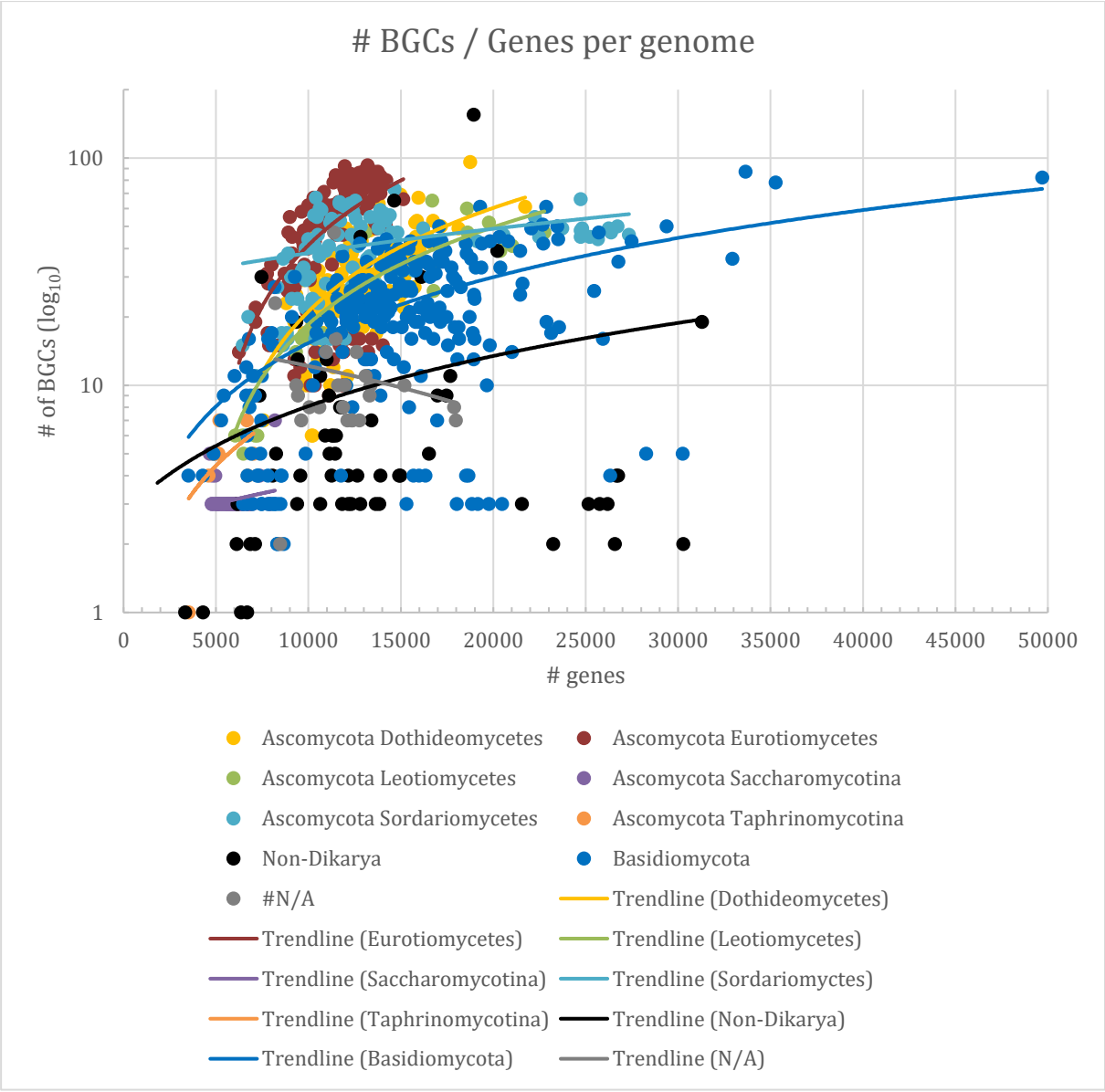


Figure 11 - The relationship between the number of biosynthetic gene clusters and gene count in fungi. Note the low number of BGCs in genomes with less than 5000-6000 genes, with a steady increase up to around 15000 genes. The group called N/A is fungi without clear phylogenetic placement (such as Tuber). The figure is based on 793 published genomes and annotations of BGCs found in Mycocosm. Data available from (Joint Genome Institute 2021a, 2021b).

Due to the continual accumulation of information regarding fungal genomes (and indeed any genomes) and characterization of new genes, reannotation of previously annotated genomes are required (Salzberg 2007; Ejigu & Jung 2020). This reduces the chance of misannotations perpetuating onto new genomes and updates the old annotation with current knowledge (Ejigu & Jung 2020). Currently, reannotation for prokaryotic genomes are performed in the RefSeq database of NCBI (O'leary et al. 2016; NCBI 2019), but it seems this function is only performed when eukaryotic genomes become updated with new assemblies or RNA sequences (NCBI 2021).

1.5.2 Sequencing technologies and processing of fungal genetic material

One key step in bioprospecting is the characterization of the organism that is examined. This information can give clues to which metabolites it can produce, how to optimize fermentation and storage, and can help during dereplication, the process of recognizing and eliminating known compounds from consideration (Ito & Masubuchi 2014). Extraction of fungal genomic DNA can usually be performed on fresh mycelium using any of the available commercial kits. However, some procedures work better than others for certain fungal groups (Fredricks et al. 2005). When it comes to sequencing, there are several technologies to choose from depending on what the aim is. Sanger sequencing is a tested method suitable for sequencing barcode regions that can help identifying the organism in phylogenetic analyses (Paul et al. 2018). However, it is becoming more and more common to sequence whole genomes, as the cost of sequencing has dropped significantly the last decades (Schwarze et al. 2018). The leading technologies at the moment is Illumina or Ion Torrent, for short reads, and PacBio or Nanopore for long reads (but alternative long read technologies exist through 10x Genomics and Illumina) (Goodwin et al. 2016). Short reads are short fragments of DNA, usually less than 1000 bp, that are sequenced. The sequence length can be artificially increased by sequencing short portion at the ends of longer fragments (called mate pairs) or extensive use of barcodes for single long DNA fragments (Wetzel et al. 2011; Amarasinghe et al. 2020; Srikanth et al. 2020). Long reads on the other hand are from 10’000 bp and up, with one reported case of 2’272’580 bp using Nanopore (Rhoads & Au 2015; Mahmoud et al. 2019; Payne et al. 2019). The quality of these longer reads is generally lower, 79-99.8 % accurate, than for the short reads that are 99.9 % accurate, depending on the technique that are used (Rhoads & Au 2015; Noakes et al. 2019; Wenger et al. 2019). One of the limitations of the short reads is that they are unable to resolve and bridge highly repetitive DNA (Jung et al. 2020). To overcome the limitation of each read

technology the solution is to combine the two methods when sequencing a genome, Figure 12 (Ma & Fedorova 2010; Jung et al. 2020; Montoliu-Nerin et al. 2020).

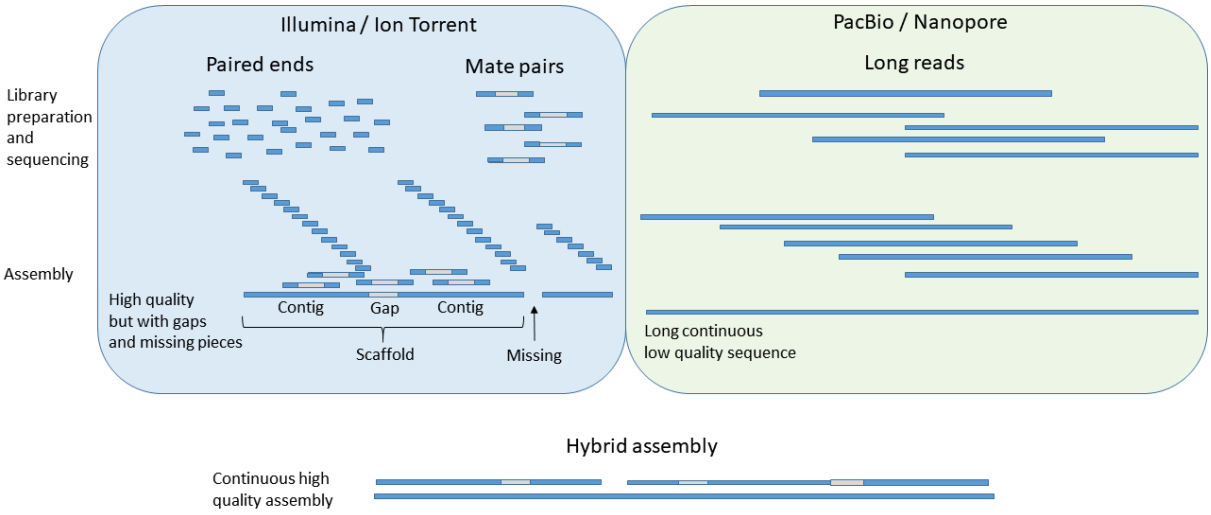


Figure 12 - Difference in sequencing technologies between short and long reads.

There are several ways to process sequences once you have had a fungus sequenced. A summary of all these steps can be found in Jung et al. (2020) and are presented here in a more condensed form. The idea is the same for all sequencing technologies, but the length of the reads, biases and software used are different. The genomic DNA is extracted, each piece of DNA is sequenced, producing a read, and then each of these reads is mapped against each other to form contigs. This is called assembly. Contigs are continuous sequences of reads that overlap. These contigs can further be assembled to scaffolds that have short gaps of undefined sequences between them. These are generally eliminated using long reads. There are many tools that can be used to assemble genomes, each with their own strengths and weaknesses (Zerbino & Birney 2008; Bankevich et al. 2012). Generally, it does not matter if the sequence is from prokaryotes or eukaryotes at this stage, as the basis of the sequence is the same, only the length is different. Once you have a set of scaffolds, this is your draft genome.

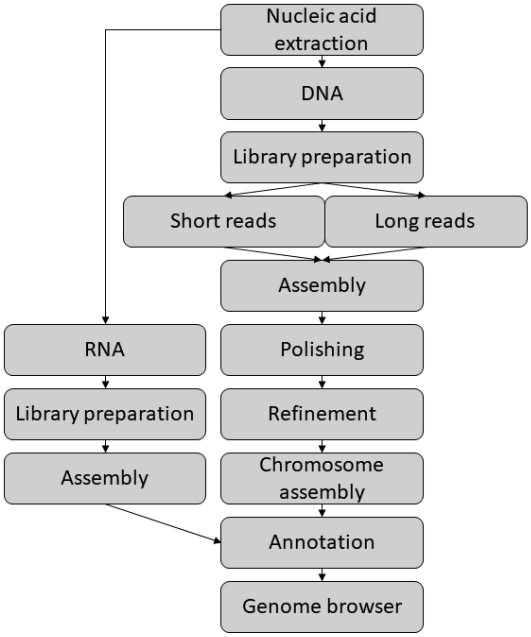


Figure 13 – Overview of the steps in a sequencing project from extraction of nucleic acids to annotation. This is a general pipeline and different technologies may have fewer or additional steps. Adapted from (Jung et al. 2020).

The next step in the process is to annotate the genome with gene features, and this paragraph is a summary of what is described in Ma and Fedorova (2010); Yandell and Ence (2012); Thibaud-Nissen et al. (2013); Jung et al. (2020). Identifying genes in eukaryotes is more challenging compared to prokaryotes due to the gene structure consisting of introns and exons. This is why eukaryotic genome sequencing projects generally require transcriptomic data to determine the intron/exon structure of genes. In addition to these issues, eukaryotes change between haploid and diploid stages, diploid genomes complicate assembly due to the similar, but not identical chromosomes (Campoy et al. 2020). By sequencing haploid stages, this issue is avoided. If there are closely related genomes available, it is possible to map and transfer annotations from one genome to the other based on homology. The annotation process generally incorporates different tools and database searches to detect and add information to the genes. These tools are often put together into an automated program sequence called a pipeline. There are many publicly available online pipelines for prokaryotic genomes (Giardine et al. 2005; Aziz et al. 2008; Seemann 2014; Tanizawa et al. 2017). There are few similar publicly available services for eukaryotic genomes (Campbell et al. 2014; Liu et al. 2019). Many pipelines require you to be part of a larger project with specialists in bioinformatics, that have large computer resources available and that are comfortable working on command line in UNIX (Haas et al. 2008; Thibaud-Nissen et al. 2013; Jung et al. 2020). In order to reduce the complexity within bioinformatics workflows, large scale collaborative platforms have been established, such as

the Galaxy platform. Here, users can utilize streamlined graphical interfaces and computer resources which simplifies genome processing (Giardine et al. 2005).

When you have an assembled an annotated genome, you can start to explore its contents. From here on it is largely project dependent where to focus the attention. In this thesis, the focus is on bioprospecting and identifying novel biosynthetic pathways or enzymes that can be of industrial use, as well as phylogenetic placement of the fungi. Fortunately, there are publicly available tools for this specific purpose available through FungiSMASH (Blin et al. 2019), a pipeline specifically developed to detect biosynthetic gene clusters of secondary metabolites in fungi. In addition to this, there are tools available for detection of carbohydrate active enzymes (CAZymes), such as dbCAN2, that are responsible for the utilization of different carbohydrate sources such as alginate, lignin, pectate, agar and cellulose (Zhang et al. 2018).

1.5.3 Enzymes with industrial potential

A field closely related to NP discovery is bioprospecting for industrially relevant enzymes. Enzymes are used in a range of different applications within textile, laundry, paper, leather, sugar, baking, dairy, brewing, juice and animal feed industries (Kumar et al. 2014), not to mention pharmaceutical, nutraceutical and laboratory applications (Watanabe et al. 2010; Fernández-Lorente et al. 2011; Ishino & Ishino 2014). Fungi have a close evolutionary relationship with plants and has developed an enzymatic machinery to break down and utilize plant biomass (Hage & Rosso 2021). One field that has received a lot of attention the last decade is biorefinery of plant biomass into feed, biofuel and chemical applications using CAZymes (Chettri et al. 2020; Contesini et al. 2021). One of the main reasons for the interest in CAZymes is to support sustainable growth and to decrease the dependency on fossil energy sources (Chettri et al. 2020).

CAZY is a database for CAZymes (Lombard et al. 2014). This database is used as a reference by dbCAN2, a web server that can automatically annotate carbohydrate active protein sequences (Zhang et al. 2018). It does this by utilizing three different tools, similar to gene prediction, two tools using Hidden Markov Models (HMMs) to predict catalytic domains and one tool utilizing conserved peptide motifs. Marine carbohydrates are often sulphated, a characteristic that differentiates them from terrestrial carbohydrates (Helbert 2017). Sulfatases have a similar diversity to CAZymes. SulfAtlas, an online database, was made with a similar classification system as CAZymes and contains an overview of the different classes of sulfatases (Barbeyron et al. 2016). Together, these can be used to look for CAZymes with

specificity towards marine sulphated substrates that can be used as an additional biomass resource (Balabanova et al. 2018; Salgado et al. 2021).

1.6 Current gaps and challenges in marine mycology and bioprospecting

The following challenges and gaps have been identified in the field of bioprospecting marine fungi:

- There is currently inconsistent representation of fungal taxa in sequence databases (Araujo & Sampaio-Maia 2018; Thines et al. 2018). Some taxa are overrepresented (*Penicillium*, *Aspergillus* and *Fusarium*) due to high research interest since they are prolific producers of metabolites. This is complicated further as many described type species are lost or have no sequence information available to compare with (Vu et al. 2019). Classification of isolates can be difficult without expertise for relevant fungal groups (Visagie et al. 2014). In many publications, incomplete or no identifications are provided (Ariffin et al. 2011; Flewelling et al. 2015).
- A large portion of the diversity detected in environmental samples are not detected in cultivation studies and a large portion of fungal diversity remains uncultivable (Tasdemir 2017; Thines et al. 2018).
- Many niches, substrates, hosts and geographical areas, such as the Arctic, are overlooked, understudied or have received little attention in regard to bioprospecting (Pang et al. 2011; Tisthammer et al. 2016; Rämä et al. 2017).
- The lack of sequenced genomes and knowledge about biosynthetic clusters present in different fungi represents a knowledge gap when it comes to NP research. There are many orphan metabolites where the clusters responsible for their production are unknown (Trautman & Crawford 2016).
- The problem of expression of metabolites from cryptic or silent gene clusters requires additional techniques to induce them (Lim et al. 2012).
- Rediscovery of known compounds requires extensive dereplication and can be time-consuming (Monciardini et al. 2014; Chávez et al. 2015; Hubert et al. 2017; Liu et al. 2020b).

1.7 Aim of the thesis

The overall aim of this thesis was to screen marine fungi from the Arctic to assess their potential to produce bioactive metabolites by combining top-down and bottom-up bioprospecting approaches.

Specific objectives were:

1. Isolate fungi from marine sources in the Arctic
    a. Identify the fungal strains by molecular phylogeny
    b. Screen their ability to produce antibacterial compounds
2. Sequence and characterize marine fungi and provide taxonomic description of new species.
    a. Assess biosynthetic potential of fungi by examining the presence of biosynthetic gene clusters and enzymes
    b. Isolate metabolites and link metabolites to gene clusters
3. Isolate and determine the bioactivities of metabolites from selected marine fungi

2 Summary of papers

2.1 Paper 1

Cultivable marine fungi from the Arctic Archipelago of Svalbard and their antibacterial activity

Ole Christian Hagestad, Jeanette H. Andersen, Bjørn Altermark, Espen Hansen, & Teppo Rämä

Mycology, 2020, 11, 3.

The study performed in Paper 1 focused on examining fungal isolates from the understudied Arctic marine environment. The Arctic has received relatively little attention from the research community, especially in regards to bioprospecting. Therefore, a study of the Arctic marine fungal diversity and their bioactivity would provide an assessment of their potential utilization in bioprospecting.

The fungi were collected using several different methods and techniques, both direct plating, microbial traps, spore suspension plating and diffusion chamber incubation. The plates used both common media such as corn meal agar and malt extract agar amended with seawater and agar based on *Ascophyllum nodosum* algae. The three selected media would select for

halotolerant species and provide different substrate capable of capturing some of the diversity present.

The isolates were phylogenetically characterized using three molecular markers. Isolates from *Ascomycota*, *Basidiomycota* and *Mucoromycota* were recovered, *Ascomycota* made up the majority of the 20 operational taxonomic units (OTUs). One third of the isolates represented putatively novel fungi based on sequence similarity alone, most of these in the marine fungi *sensu stricto* order *Lulworthiales*.

The fungi were screened using an agar plug diffusion assay. This allowed for detection of antimicrobial metabolites expressed on two different media. Half of the isolates tested were able to inhibit bacterial growth against at least one bacterial strain.

This study shows that even traditional isolation efforts yield numerous diverse isolates, many of them from poorly studied taxa. The antimicrobial assay results show that Arctic marine fungi represent a resource for bioprospecting.

2.2 Paper 2

Genomic characterization of three marine fungi, including *Emericellopsis atlantica* sp. nov. with signatures of a generalist lifestyle and marine biomass degradation

Ole Christian Hagestad, Lingwei Hou, Jeanette H. Andersen, Espen H. Hansen, Bjørn Altermark, Chun Li, Eric Kuhnert, Russell J. Cox, Pedro W. Crous, Joseph W. Spatafora, Kathleen Lail, Mojgan Amirebrahimi, Anna Lipzen, Jasmyn Pangilinan, William Andreopoulos, Richard D. Hayes, Vivian Ng, Igor V. Grigoriev, Stephen A. Jackson, Thomas D. S. Sutton, Alan D. W. Dobson, & Teppo Rämä

Submitted to IMA Fungus (under review)

One of the major shortcomings in marine fungal research is the lack of available genomic data for phylogenetic, evolutionary and comparative genomic studies. Marine fungi remain poorly covered in global genome sequencing campaigns due the ambiguity in the definition of what a marine fungus truly is. An additional shortcoming is the lack of characterized biosynthetic gene clusters (BGCs) that are linked with their respective metabolites. In order to provide additional marine fungal genomes to publicly available databases, three marine fungal genomes were sequenced, and their biosynthetic potential was evaluated. In addition, a formal description of a new species of *Emericellopsis* was provided with the genome. One last aim was to isolate metabolites and link them to their respective biosynthetic gene clusters.

The genomes of *Amylocarpus encephaloides*, *Calycina marina* and *Emericellopsis atlantica*, were sequenced and annotated at JGI. Annotations of BGCs and CAZymes were performed locally using FungiSMASH and dbCAN2 web server. The BGCs were screened for similarity in databases for clues to the metabolites and the CAZymes were compared to literature on specificity for marine substrates. All three genomes were compared in a phylogenetic analysis to determine their systematic placement. *E. atlantica* was morphologically described together with experts on *Hypocreales* from Westerdijk Institute. Fermentation of *Emericellopsis* was performed in both solid and liquid media cultures and the resulting fractions were dereplicated.

The phylogenetic analysis placed the *E. atlantica* isolate as an early branch in the marine clade. This, together with a morphological examination, indicated that it represented a novel species and a circumscription of the isolate was performed. A 15-gene phylogenetic analysis of *A. encephaloides* and *C. marina* confirmed their previous placement in their respective clades. Analysis of their genomes revealed that all three fungi contained over 20 BGCs and more than 200 CAZymes each. Culturing of *E. atlantica* in 11 different media enabled us to characterize its bioactivity and compare it to the identified BGCs, which enabled the identification of helvolic acid in fractions with antibacterial activity.

The three genomes represent the first whole genome sequenced fungi in their respective genera. This represent a source that can be used in future comparisons to detect specific adaptions of marine fungi. The presence of unknown BGCs in all three marine fungi represent a potential source for novel metabolites. The CAZyme enzymes could be explored for specific substrate interaction. No novel metabolites were isolated and the only metabolite that could be linked to its respective BGCs was helvolic acid.

2.3 Paper 3

Pitfalls in biodiscovery – a case study of *Mytilinidion* sp. M16HEL1360D1-10.1, a wood-associated fungus from the marine environment in the Arctic

Ole Christian Hagestad, Kine Ø. Hanssen, Johan Isaksson, Jeanette H. Andersen, Espen H. Hansen, & Teppo Rämä

(Manuscript)

The aim of Paper 3 was to isolate metabolites from a selected marine fungus. The candidates were the fungi isolated in Paper 1. The selection of the fungus was based on the activity detected

in the antimicrobial assay and the sequence similarity of the isolate to the closest representative in the databases. By selecting a fungus based on these criteria, the isolate had already shown capability to produce bioactive compounds and it could provide undescribed chemistry.

Based on these criteria, the fungus *Mytilinidion* sp. M16HEL1360D1-10.1 (OTU15), from Paper 1 was selected. This *Mytilinidion* sp. was isolated from a driftwood piece collected from 120 m depth outside of Kvitøya, Svalbard. The phylogenetic placement of the fungus from Paper 1 was tested using a more extensive range of genetic markers and the available sequences from closely related taxa in *Mytilinidiales*. The fungus was cultivated in a high-nutrition medium that was extracted using solid phase extraction. The extract was fractionated to reduce the complexity of the extract. The analysis of the fractions and subsequent dereplication ended with a single metabolite being isolated and characterized. The pure compound was tested in a range of different bioactivity assays. Because this compound was not found in databases, its structure was elucidated using NMR.

Based on the phylogenetic analysis, the fungus seems to be a novel species closely related to *M. mytilinellum* CBS 303.34. However, due to the lack of sequenced type specimen for the taxa and no fruiting body it was not possible to resolve its systematic placement accurately at this time. The isolated compound was only active in the angiotensin converting enzyme inhibition assay with an IC_{50} value of 42.86 µM. The compound was shown to be a modified octapeptide by NMR. Analysis of the peptide sequenced indicated that the peptide is likely a partially hydrolysed casein peptide. This is the first report of this modified peptide and its bioactivity.

3 Discussion

The search for new bioactive molecules has always been important. With the necessity to change to a greener economy and to meet the demand for new medicines, especially against the increasing incidences of multiresistant bacteria, bioprospecting activities must be fortified. The aim of this thesis was to explore marine fungi from the Arctic for their taxonomic diversity and ability to produce bioactive secondary metabolites. Fungi from the Arctic has been studied in both cultivation studies and environmental sequencing studies and there are a few cases of metabolites isolated from Arctic marine fungi (Grunwald et al. 2016; Grunwald et al. 2017; Rämä et al. 2017). This project has been one of the first (if not the first) that has screened marine fungi from diverse substrates from this area for bioactive metabolites and show that there is potential to use Arctic marine fungi in bioprospecting.

My personal knowledge about marine fungi was limited at the beginning of the PhD, it had never been mentioned in marine biology and marine bioprospecting courses, or if mentioned it was merely a curiosity which did not warrant much attention by the teachers. This lack of mentions in courses piqued my interest when I saw the PhD position on marine fungi. It was apparent that marine mycology was indeed a neglected research area, there were few researchers in the field and literature was sparse, especially for the Arctic region. This provided a unique opportunity to expand upon a comparatively small field and contribute significantly to the available knowledge.

3.1 Diversity of marine fungi in the Arctic

The fungi isolated in Paper 1 came from different marine ecological niches and areas in the Arctic. The isolates were obtained from algae, driftwood, sediments and sea water. Characterization of the fungi using the genetic markers 18S, ITS and 28S revealed that several of the 20 identified fungal OTUs were cosmopolitan genera that can be isolated in both marine and terrestrial environments, *Penicillium*, *Oidiodendron*, *Tolypocladium*, *Cosmospora* and *Geomyces*. Several species are frequently isolated from Arctic or Antarctic soils (Rice & Currah 2005; Malosso et al. 2006; Durán et al. 2019; Perini et al. 2019; Santos et al. 2020), such as halo- and psychrotolerant *Geomyces* species associated with Arctic permafrost soil (Kochkina et al. 2007; Hayes 2012) and psychrophilic aquatic anamorphs in *Tetracladium* (Roldán et al. 1989; Anderson & Shearer 2011; Wang et al. 2015). Using the DNA sequences, the identities were compared towards sequences in GenBank and the majority of the isolates were identical or had scores of >98 % identity to the reference sequences. The isolates of *Lulworthiales*, *Mytilinidion* and *Tolypocladium* had low similarity <95 % to reference sequences, indicating that these sequences could represent novel species.

The main problem using sequence similarity as an indicator of novelty is that the databases are not complete, and many described species lack representation to make a proper comparison possible. The purpose of this thesis was not to do a revision of the fungal orders detected, but to examine novel fungal diversity, which these species likely represent. Proper classification of these species may be possible in the future if experts of each fungal group are involved, type specimen of the genera becomes sequenced or fruiting bodies are produced that allows for morphological description and comparison. Considering the list of Arctic marine fungi presented in Rämä et al. (2017) and additional publications, all of the isolated genera have previously been detected in the Arctic or Antarctic (Zhang & Yao 2015; Perini et al. 2019), except *Mytilinidion*. This indicates that many of the fungi are repeatedly identified in the Arctic.

Many isolates within *Lulworthiales* are only reported to the genus level due to unresolved taxonomy (Pang et al. 2011; Rämä et al. 2014). Both previous studies and sequence databases provide indication towards undescribed species diversity (Campbell et al. 2005; Koch et al. 2007; Jones et al. 2015; Abdel-Wahab et al. 2017; Azevedo et al. 2017; Calado et al. 2019; Hyde et al. 2020; Schoch et al. 2020) and that the Arctic is a source of novel species within *Lulworthiales*.

One of the constraints of Paper 1 is the limited methods of isolation. The different substrata were inoculated on seawater-based corn meal agar, malt extract agar and *Ascophyllum nodosum* agar with antibiotics. Additionally, samples from sea-water were inoculated in diffusion chambers and incubated in a seawater pool on deck of a research vessel. The isolation plates were kept at 10 ºC. This provides a fairly strong selective screening towards fungi able to adapt to the artificial substrate and that are halotolerant and can grow at lower temperatures. The salt would prevent non-halotolerant fungi from being isolated and the low incubation temperatures would increase recovery of cold adapted and slow growing fungi, which is advantageous. The basis for the inoculum in the diffusion chambers was sea water from the Barents Sea obtained at 5m depth earlier that year. This provided a selection towards fungal spores and only *Penicillium* spp. were detected in the diffusion chambers. The limitations arose due to the limited time to process samples on board and space available to store samples properly. The fungi then had to be processed further to obtain pure cultures. If possible, future isolation efforts should employ additional techniques and if diffusion chambers are used, have additional inoculum sources.

A commonly used technique that also allows for recovery of additional algae or wood-inhabiting fungi is the damp/moist chamber technique (Overy et al. 2019). This allows the fungi to continue to grow on their natural substrata and perhaps enables them to produce fruiting bodies that makes a morphological examination possible. This technique might also allow for detection of fungi that do not grow on traditional agar plates. Domestication of these fungi could be attempted using iChip like entrapment of fungal hypha by placing the isolation device directly onto the wood, ensuring good contact with it. Additional methods such as collecting sea foam on the beaches or at sea, using dilution series plating, or utilize baiting stations would increase species recovery even more (Overy et al. 2019). Despite these limitations, about 1 of every 3 fungi isolated in Paper 1 are likely novel. This indicates a vast potential to isolate unique fungi in the Arctic, even with simple isolation efforts.

The only novel genus detected from the Arctic marine environment was *Mytilinidion*. This fungus was studied in detail in Paper 3. The submerged driftwood likely had drifted for

months and perhaps been locked in drift ice before finally sinking to the bottom of the ocean where it could have been for even longer (Hellmann et al. 2013). The driftwood may therefore be several months to several decades old depending on the original size and transportation of the driftwood (Johansen 1998; Hellmann et al. 2013). The type and size of the driftwood affects the buoyancy, transportation and final deposition depth in the sediments (Johansen 1998; Björdal & Nilsson 2008; Hellmann et al. 2013; Fojutowski et al. 2014; Charles et al. 2016). Terrestrial fungi without the ability to endure in the marine environment could still be isolated if there are viable spores. However, *Mytilinidion* was isolated from within surface sterilized wood and likely represent vegetative mycelium in the wood rather than spores. A test of other terrestrially obtained *Mytilinidion* would enable a comparison of physiological differences, such as halotolerance, between different species in the genus. This is the first account of *Mytilinidion* from the marine environment. All other isolates from this genus are terrestrial wood-associated fungi, some of them have been reported from the Arctic (GBIF Secretariat 2019).

3.2 Bioactivity potential of marine fungi

There are several ways to assess the potential of fungi to produce bioactive compounds. Both top-down and bottom-up approaches have been used in the papers presented in this thesis. The top-down approach assesses the potential bioactivity of expressed metabolites that the fungi produce. This was the purpose of the agar diffusion plug assay performed on the isolated fungi in Paper 1. By using an agar plug with growing mycelium, metabolites affecting bacterial cell growth should be detectable by visual inhibition zones around the agar plugs. The advantage of this method is that it significantly reduces the usual work of fermentation, extraction, fractionation and screening, especially for so many isolates. It provided an initial overview of the antibacterial potential of the fungal isolates and was used to select fungal strains for further processing. There are some drawbacks and limitations of using this method for screening. Fungi have very different growth requirements and growth rates. This allows some of the heat tolerant fungi to potentially respond to the bacteria it was co-cultivated with and survive the incubation at 37 °C, while other fungi would be unable to respond and perhaps succumb to the high temperatures. Some of the detected activity could have been induced by the bacterial presence. The difference in growth rate also made it more difficult to test fungi at the “same” time in their growth cycle. Finally, the agar plug diffusion assay only shows activity against bacteria, not other potential bioactivities. Despite these drawbacks, half of the screened fungi showed activity in the agar diffusion assay against bacteria. The method has been used to test bacterial

and fungal antibacterial activity (Balouiri et al. 2016; Sabdaningsih et al. 2017). There are reports on bioactivity of soil and glacial fungi from both Arctic and Antarctic, and reports of singular strains of marine fungi or substrates that have been screened (Sonjak et al. 2006; Sepcic et al. 2011; Wu et al. 2014; Giddings & Newman 2019; Ogaki et al. 2019; Tsuji & Hoshino 2019). Paper 1, to the best of my knowledge, represent the first reported screening of the antibacterial potential of Arctic marine fungi from diverse marine substrates.

Emericellopsis was fermented in both liquid and solid medium and different extraction methods were used in Paper 2. Overall, both solid and liquid fermentation using the same extraction method yielded similar observed activity in the antimicrobial assays. On the other hand, different extraction methods showed difference in the detected activity. Fractions extracted with SPE were inhibitory towards biofilm formation, while fractions from ethyl acetate extractions were inhibitory towards bacterial growth. This is likely an indication of the selective bias of the extraction method for certain compounds with different biological effects. The recovery of metabolites is known to vary depending on the extraction methods or solvents that are used (Sitnikov et al. 2016; Roopashree & Naik 2019). There were also differences in activity between each individual liquid extracts and solid extracts in Paper 2. The differences between fractions using the same extraction method are likely due to different content of the active metabolites produced in the different culture media. Small differences in culturing conditions are likely to modulate the expression pattern of metabolites which is the goal of the OSMAC approach (Romano et al. 2018). None of the fractions were active in multiple unrelated assays and it is therefore unlikely that the fractions contained PAINS. The antimicrobial and antibiofilm activity shows that *Emericellopsis* is able to produce compounds with antimicrobial effect in several different fermentation settings (Paper 2).

The second method to assess the potential of a fungi to produce bioactive metabolites is the bottom-up approach. This approach is based on extracting the genetic material from an isolate and analyzing the genome for BGCs. Initially, the idea was to sequence and analyze two fungi from Paper 1 that were novel and showed antibacterial activity. After several months of culturing, harvesting and unsuccessfully extracting DNA of sufficient quality for sequencing the work was suspended. Usually, DNA extraction is performed on fresh or newly germinated mycelium because the cell wall is not as strong and there are very few dead cells present that could contain partially degraded DNA (Van Burik et al. 1998; Haugland et al. 1999; Fredricks et al. 2005). This proved to be challenging to achieve with slow growing, non-sporulating marine fungi. Successful DNA extraction was later achieved using liquid cultures without shaking for aeration to reduce cell wall stress (results not published).

In lieu of the two marine genomes initially planned for analysis from Paper 1, one marine genome that had previously been sequenced in the 1KFG project would be used for analysis, and subsequently two others were included in Paper 2. The three genomes were *Emericellopsis atlantica*, *Amylocarpus encephaloides* and *Calycina marina*. If one compares the results of the three genomes to Figure 11, the fungi contained the expected number of BGCs given the total number of genes and fungal phyla they belonged to. Most of these BGCs were uncharacterized with no related clusters to compare them to as reported from FungiSMASH. Whether or not the uncharacterized BGCs can produce novel metabolites is not possible to say until the resulting metabolites are characterized. This shows that there is extensive need to map metabolites to their parent gene clusters. The results shows that there are opportunities for novel discoveries if these clusters are characterized, as it will bridge the current knowledge gap that they represent. However, this requires the expression and isolation of the metabolites which was not achieved (discussed in 3.3).

3.3 Dereplication and isolation of metabolites from marine fungi

One of the aims of this thesis was to isolate metabolites from the marine fungi that were examined. This was performed in Paper 2 and 3. In Paper 2, *Emericellopsis atlantica* was extensively fermented in different media and dereplication revealed that, beside lipids, the most prominent peaks were helvolic acid and its intermediates. Several minor peaks showed signatures of peptides, but the quantities were so low that additional fermentations and yield optimization would likely be required to obtain enough compound for structure elucidation, which was outside the scope of the thesis. Identification of helvolic acid was achieved by database searches of the elemental composition and was further confirmed by the presence of the BGC encoding the enzymes for its production in the genome. When there is an established link between a BGC and specific metabolite it is possible to use this to search for the specific metabolites in the fermentation media (Trivella & De Felicio 2018; Hoskisson & Seipke 2020). Search for specific metabolites encoded by the other BGCs with high similarity to characterized clusters was attempted in *Emericellopsis*, but it yielded no hits in the extracts. This is likely due to the metabolite not being expressed in the culture conditions or because the BGCs are different enough that they do not produce the same metabolite. Currently, the technology is not at a point that the structure of compounds can be accurately predicted from uncharacterized BGCs alone (Ziemert et al. 2016; Skinnider et al. 2020).

For Paper 3, the selection of the fungus was based on the initial bioactivity screening showing potential for production of antibacterial compounds and that it had low sequence

similarity to reference sequences in GenBank. Of the possible candidates, *Mytilinidion* displayed activity on both solid and liquid media (results not published for liquid media screening), and had a low similarity score using the ITS barcode region of 83.7 % to reference sequences. *Mytilinidion* also produced a strong coloration of the medium it grew on, which can be caused by different classes of secondary metabolites (Kalra et al. 2020). On solid medium, it produced a violet color after 1-2 weeks which over time slowly faded. In liquid medium, it produced a deep red color. The fractions were compared to the medium control and major peaks not present in the control were targeted for isolation. After the initial purification, each fraction was analyzed on UHPLC-MS for dereplication. One compound was isolated and bioactivity screening revealed a weak ACE-inhibition. The structure of the compound was elucidated using NMR and it turned out to be an octapeptide with an N-terminal modification of a glutamine to pyroglutamate. This is a modification that is present in some secondary metabolites and it increases the stability of the peptide. The structure was novel and did not appear in database searches. However, the lack of other modifications and only proteinogenic amino acids created doubts whether it actually was a secondary metabolite. Searches using partial sequences matched with a casein peptide fragment with the exception of the pyroglutamate modification. It is likely that the compound isolated is a modified media component since the media is made from peptone of casein.

Initially, this might seem like a failure because it is a modified media component rather than a metabolite produced by the fungus. However, without the report of this compound and the registry of it in databases, other researchers would perhaps also isolate this compound in the belief that it is novel. It is possible that many compounds like these are not published or included in databases because they are viewed as negative results, but they are essential to include for improved dereplication in the future. Potentially, the isolation of medium component derivatives can be avoided by using molecular networking and fragmentation pattern analysis, since the derivatives likely will group together peptides in the medium control (Yang et al. 2013).

4 Future prospects

The papers presented here have shown the potential of Arctic marine fungi in bioprospecting pipelines. All the isolates in Paper 1 have been fermented, extracted, fractionated and to some extent screened for bioactivity, but dereplication remains in order to determine the presence of novel metabolites.

When new research cruises are planned, additional methods for isolation should be attempted, such as damp chamber incubation and diffusion chamber cultivation in sediments. Furthermore, other habitats could be examined, such as plant and animal associated fungi, methane seeps, hydrothermal vent areas, glacial runoff areas or deep ocean habitats. This would allow for the recovery of additional diversity. Seasonal investigation could also yield different fungal diversity. The additional isolation techniques might also enable the recovery of fruiting bodies for better morphological descriptions of the isolated species.

Many taxa in *Eurotiomycetes* have been extensively investigated, and for good reason, since the order has the highest number of BGCs compared to the total gene count. The likelihood of finding novel metabolites in less explored taxa is still high since many BGCs are specific for species and genera. The genomes that have been published so far show that most orders within *Dikarya* have a number of BGCs, many of them unexplored. Because of this, research effort should be focused into underexplored marine taxa such as *Lulworthiales*.

An in-depth study of the promoters in the different BGCs could help to optimize fermentation and trigger expression of the gene clusters within *E. atlantica*, *C. marina* and *A. encephaloides*. Since the genomes for these isolates now are available, it is possible to do differential transcription analysis in order to determine the activated BGCs if metabolites are detected in specific media.

To properly assess the industrial relevance of the detected CAZymes of *E. atlantica*, specialized knowledge is needed. This would require cooperation with research groups that specialize in the characterization of CAZymes and could open up for international cooperation in the future.

5 Concluding remarks

The aim of this thesis was to assess the potential of Arctic marine fungi in bioprospecting. The diversity isolated, with a majority being Ascomycota. This is a trend that is reflected in the distribution of described marine fungi. The work presented here shows how the Arctic marine environment is a good source of both novel marine fungi and marine fungi with bioactive metabolites.

There are a few genera that has received massive attention in natural products research, but literature shows that the marine environment has unique NP diversity and many BGCs are genus and species specific. This means that there is likely a large undescribed chemical diversity in underexplored marine fungal taxa.

Several of the limitations presented in this work are an issue for marine mycology in general. Isolation work will benefit from the constant development of new isolation techniques which will enable the recovery of previously undescribed and uncultivable diversity. A standardization of how fungal species are characterized using molecular markers and the sequencing of herbarium and culture type specimens would provide a more complete reference database that will increase the accuracy of the taxonomic work. In addition to the lack of sequence representation of marine fungi for molecular taxonomy, marine fungi are also poorly represented in genome databases. The three genomes reported within this thesis will add to the number of marine fungal genomes available and may be used to understand the physiological and ecological adaptions of marine fungi.

Finally, the NP research community would greatly benefit from a more standardized and extensive database on metabolites and common media with additional metadata regarding distribution and bioactivity. Additionally, awareness of spontaneous reactions and solvolysis of NPs should be put higher on the agenda of NPs research. The understanding of the biosynthetic origin of each NP would make the detection of artefacts easier.

6 Works cited

Abdallah I, Quax W. 2017. A Glimpse into the Biosynthesis of Terpenoids. KnE Life Sciences 2017:81-98. Available from: https://doi.org/10.18502/kls.v3i5.981

Abdel-Wahab MA, Dayarathne MC, Suetrong S, Guo S-Y, Alias SA, Bahkali AH, Nagahama T, Elgorban AM, Abdel-Aziz FA, Hodhod MS et al. . 2017. New saprobic marine fungi and a new combination. Botanica Marina 60(4):469-488. Available from: https://doi.org/10.1515/bot-2016-0118

Abrahams PW. 2009. "“Earth Eaters”: Ancient and Modern Perspectives on Human Geophagy'[Internet], in: Landa ER, Feller C (ed) *Soil and Culture*. Springer Netherlands. Available from: https://doi.org/10.1007/978-90-481-2960-7_23

Ahumada-Rudolph R, Cajas-Madriaga D, Rudolph A, Reinoso R, Torres C, Silva M, Becerra J. 2014. Variation of sterols and fatty acids as an adaptive response to changes in temperature, salinity and pH of a marine fungus *Epicoccum nigrum* isolated from the Patagonian Fjords. Revista de Biología Marina y Oceanografía 49:293-305. Available from: https://doi.org/10.4067/S0718-19572014000200009

Amarasinghe SL, Su S, Dong X, Zappia L, Ritchie ME, Gouil Q. 2020. Opportunities and challenges in long-read sequencing data analysis. Genome Biology 21(1):30. Available from: https://doi.org/10.1186/s13059-020-1935-5

Amend A, Burgaud G, Cunliffe M, Edgcomb VP, Ettinger CL, Gutiérrez MH, Heitman J, Hom EFY, Ianiri G, Jones AC et al. . 2019. Fungi in the Marine Environment: Open Questions and Unsolved Problems. mBio 10:e01189-18. Available from: https://doi.org/10.1128/mBio.01189-18

Amend AS, Barshis DJ, Oliver TA. 2012. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. Isme j 6(7):1291-1301. Available from: https://doi.org/10.1038/ismej.2011.193

Anderson JL, Shearer CA. 2011. Population Genetics of the Aquatic Fungus *Tetracladium marchalianum* over Space and Time. PLoS ONE 6(1):e15908. Available from: https://doi.org/10.1371/journal.pone.0015908

Appleton D, Buss A, Butler M. 2007. A Simple Method for High-Throughput Extract Prefractionation for Biological Screening. CHIMIA International Journal for Chemistry 61:327-331. Available from: https://doi.org/10.2533/chimia.2007.327

Araujo R, Sampaio-Maia B. 2018. 'Chapter Two - Fungal Genomes and Genotyping'[Internet], in: Sariaslani S, Gadd GM (ed) *Advances in Applied Microbiology*. Academic Press. Available from: https://doi.org/10.1016/bs.aambs.2017.10.003

Ariffin SA, Davis P, Ramasamy K. 2011. Cytotoxic and antimicrobial activities of Malaysian marine endophytic fungi. Botanica Marina 54(1):95-100. Available from: https://doi.org/10.1515/bot.2011.005

Azevedo E, Barata M, Marques MI, Caeiro MF. 2017. *Lulworthia atlantica*: a new species supported by molecular phylogeny and morphological analysis. Mycologia 109(2):287-295. Available from: https://doi.org/10.1080/00275514.2017.1302255

Aziz RK, Bartels D, Best AA, Dejongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M et al. . 2008. The RAST Server: Rapid Annotations using Subsystems Technology. BMC Genomics 9(1):75. Available from: https://doi.org/10.1186/1471-2164-9-75

Baell JB. 2016. Feeling Nature's PAINS: Natural Products, Natural Product Drugs, and Pan Assay Interference Compounds (PAINS). Journal of Natural Products 79(3):616-628. Available from: https://doi.org/10.1021/acs.jnatprod.5b00947

Baell JB, Holloway GA. 2010. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. Journal of Medicinal Chemistry 53(7):2719-2740. Available from: https://doi.org/10.1021/jm901137j

Baell JB, Nissink JWM. 2018. Seven Year Itch: Pan-Assay Interference Compounds (PAINS) in 2017—Utility and Limitations. ACS chemical biology 13(1):36-44. Available from: https://doi.org/10.1021/acschembio.7b00903

Baker D, Chu M, Oza U, Rajgarhia V. 2008. The value of natural products to future pharmaceutical discovery. Natural Product Reports 24:1225-44. Available from: https://doi.org/10.1039/b602241n

Balabanova L, Slepchenko L, Son O, Tekutyeva L. 2018. Biotechnology potential of marine fungi degrading plant and algae polymeric substrates. Frontiers in Microbiology 9. Available from: https://doi.org/10.3389/fmicb.2018.01527

Balouiri M, Sadiki M, Ibnsouda SK. 2016. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis 6(2):71-79. Available from: https://doi.org/10.1016/j.jpha.2015.11.005

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD et al. . 2012. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology 19(5):455-477. Available from: https://doi.org/10.1089/cmb.2012.0021

Banos S, Lentendu G, Kopf A, Wubet T, Glöckner FO, Reich M. 2018. A comprehensive fungi-specific 18S rRNA gene sequence primer toolkit suited for diverse research issues and sequencing platforms. Bmc Microbiology 18(1):190. Available from: https://doi.org/10.1186/s12866-018-1331-4

Baral HO, Rämä T. 2015. Morphological update on *Calycina marina* (*Pezizellaceae*, *Helotiales*, *Leotiomycetes*), a new combination for *Laetinaevia marina*. Botanica Marina 58(6):523-534. Available from: https://doi.org/10.1515/bot-2015-0049

Barbeyron T, Brillet-Guéguen L, Carré W, Carrière C, Caron C, Czjzek M, Hoebeke M, Michel G. 2016. Matching the Diversity of Sulfated Biomolecules: Creation of a Classification Database for Sulfatases Reflecting Their Substrate Specificity. PLoS ONE 11(10):e0164846. Available from: https://doi.org/10.1371/journal.pone.0164846

Barghoorn E, Linder DH. 1944. Marine fungi : their taxonomy and biology. Farlowia 1:395-467. Available from:

Basu S, Bose C, Ojha N, Das N, Das J, Pal M, Khurana S. 2015. Evolution of bacterial and fungal growth media. Bioinformation 11(4):182-184. Available from: https://doi.org/10.6026/97320630011182

Benet LZ, Hosey CM, Ursu O, Oprea TI. 2016. BDDCS, the Rule of 5 and drugability. Advanced Drug Delivery Reviews 101:89-98. Available from: https://doi.org/10.1016/j.addr.2016.05.007

Bengtson S, Rasmussen B, Ivarsson M, Muhling J, Broman C, Marone F, Stampanoni M, Bekker A. 2017. Fungus-like mycelial fossils in 2.4-billion-year-old vesicular basalt. Nature Ecology & Evolution 1(6):0141. Available from: https://doi.org/10.1038/s41559-017-0141

Berdy B, Spoering AL, Ling LL, Epstein SS. 2017. In situ cultivation of previously uncultivable microorganisms using the ichip. Nature Protocols 12:2232. Available from: https://doi.org/10.1038/nprot.2017.074

Bertrand S, Schumpp O, Bohni N, Monod M, Gindro K, Wolfender J-L. 2013. De Novo Production of Metabolites by Fungal Co-culture of *Trichophyton rubrum* and *Bionectria ochroleuca*. Journal of Natural Products 76(6):1157-1165. Available from: https://doi.org/10.1021/np400258f

Björdal CG, Nilsson T. 2008. Reburial of shipwrecks in marine sediments: a long-term study on wood degradation. Journal of Archaeological Science 35(4):862-872. Available from: https://doi.org/10.1016/j.jas.2007.06.005

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Research 47(W1):W81-W87. Available from: https://doi.org/10.1093/nar/gkz310

Bonneville S, Delpomdor F, Préat A, Chevalier C, Araki T, Kazemian M, Steele A, Schreiber A, Wirth R, Benning LG. 2020. Molecular identification of fungi microfossils in a Neoproterozoic shale rock. Science Advances 6(4):eaax7599. Available from: https://doi.org/10.1126/sciadv.aax7599

Brahmkshatriya PP, Brahmkshatriya P. 2013. 'Terpenes: Chemistry, Biological Role, and Therapeutic Applications'[Internet], in: Ramawat KG, Mérillon JM (ed) *Natural Products*. Springer-Verlag. Available from: https://doi.org/10.1007/978-3-642-22144-6_120

Brakhage AA, Schroeckh V. 2011. Fungal secondary metabolites – Strategies to activate silent gene clusters. Fungal Genetics and Biology 48(1):15-22. Available from: https://doi.org/10.1016/j.fgb.2010.04.004

Brakhage AA, Schuemann J, Bergmann S, Scherlach K, Schroeckh V, Hertweck C. 2008. 'Activation of fungal silent gene clusters: A new avenue to drug discovery'[Internet], in: Petersen F, Amstutz R (ed) *Natural Compounds as Drugs: Volume II*. Birkhäuser Basel. Available from: https://doi.org/10.1007/978-3-7643-8595-8_1

Böttger R, Hoffmann R, Knappe D. 2017. Differential stability of therapeutic peptides with different proteolytic cleavage sites in blood, plasma and serum. PLoS ONE 12(6):e0178943-e0178943. Available from: https://doi.org/10.1371/journal.pone.0178943

Calado MDL, Carvalho L, Barata M, Pang K-L. 2019. Potential roles of marine fungi in the decomposition process of standing stems and leaves of *Spartina maritima*. Mycologia 111(3):371-383. Available from: https://doi.org/10.1080/00275514.2019.1571380

Campbell J, Volkmann-Kohlmeyer B, Grafenhan T, Spatafora JW, Kohlmeyer J. 2005. A re-evaluation of *Lulworthiales*: relationships based on 18S and 28S rDNA. Mycological Research 109(5):556-568. Available from: https://doi.org/10.1017/S0953756205002716

Campbell MS, Holt C, Moore B, Yandell M. 2014. Genome Annotation and Curation Using MAKER and MAKER-P. Current Protocols in Bioinformatics 48(1):4.11.1-4.11.39. Available from: https://doi.org/10.1002/0471250953.bi0411s48

Campoy JA, Sun H, Goel M, Jiao W-B, Folz-Donahue K, Wang N, Rubio M, Liu C, Kukat C, Ruiz D et al. . 2020. Gamete binning: chromosome-level and haplotype-resolved genome assembly enabled by high-throughput single-cell sequencing of gamete genomes. Genome Biology 21(1):306. Available from: https://doi.org/10.1186/s13059-020-02235-5

Cantrell SA, Dianese JC, Fell J, Gunde-Cimerman N, Zalar P. 2011. Unusual fungal niches. Mycologia 103(6):1161-1174. Available from: https://doi.org/10.3852/11-108

Carretero MI, Gomes CSF, Tateo F. 2006. 'Chapter 11.5 Clays and Human Health'[Internet], in: Bergaya F, Theng BKG, Lagaly G (ed) *Developments in Clay Science*. Elsevier. Available from: https://doi.org/10.1016/S1572-4352(05)01024-X

Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR. 2020. Marine natural products. Natural Product Reports 37(2):175-223. Available from: https://doi.org/10.1039/c9np00069k

Charles F, Coston-Guarini J, Guarini J-M, Fanfard S. 2016. Wood decay at sea. Journal of Sea Research 114:22-25. Available from: https://doi.org/10.1016/j.seares.2016.05.002

Chávez R, Fierro F, García-Rico RO, Vaca I. 2015. Filamentous fungi from extreme environments as a promising source of novel bioactive secondary metabolites. Frontiers in Microbiology 6:903. Available from: https://doi.org/10.3389/fmicb.2015.00903

Chen W, Lee M-K, Jefcoate C, Kim S-C, Chen F, Yu J-H. 2014. Fungal Cytochrome P450 Monooxygenases: Their Distribution, Structure, Functions, Family Expansion, and Evolutionary Origin. Genome biology and evolution 6(7):1620-1634. Available from: 10.1093/gbe/evu132

Chettri D, Verma AK, Verma AK. 2020. Innovations in CAZyme gene diversity and its modification for biorefinery applications. Biotechnology Reports 28:e00525. Available from: https://doi.org/10.1016/j.btre.2020.e00525

Chifiriuc MC, Holban AM, Curutiu C, Ditu L-M, Mihaescu G, Oprea AE, Grumezescu AM, Lazar V. 2016. 'Antibiotic Drug Delivery Systems for the Intracellular Targeting of Bacterial Pathogens'[Internet], in: Sezer AD (ed) *Smart Drug Delivery System*. Available from: https://doi.org/10.5772/61327

Chrismas N, Cunliffe M. 2019. Marine fungal dark matter in the global ocean. Access Microbiology 1(1A). Available from: https://doi.org/10.1099/acmi.ac2019.po0055

Ciobanu M-C, Burgaud G, Dufresne A, Breuker A, Rédou V, Ben Maamar S, Gaboyer F, Vandenabeele-Trambouze O, Lipp JS, Schippers A et al. . 2014. Microorganisms persist at record depths in the subseafloor of the Canterbury Basin. Isme j 8(7):1370-1380. Available from: https://doi.org/10.1038/ismej.2013.250

Clarivate Analytics. Web of Science [Internet]. [cited 06/03 2021]. Available from: https://apps.webofknowledge.com/RAMore.do?product=WOS&search_mode=GeneralSearch&SID=E6Eum6ktzoP6G4Hl5gE&qid=6&ra_mode=more&ra_name=PublicationYear&colName=WOS&viewType=raMore

Contesini FJ, Frandsen RJN, Damasio A. 2021. Editorial: CAZymes in Biorefinery: From Genes to Application. Frontiers in Bioengineering and Biotechnology 9(86). Available from: https://doi.org/10.3389/fbioe.2021.622817

Corradi N, Pombert J-F, Farinelli L, Didier ES, Keeling PJ. 2010. The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. Nature Communications 1(1):77. Available from: https://doi.org/10.1038/ncomms1082

Dadachova E, Casadevall A. 2008. Ionizing radiation: how fungi cope, adapt, and exploit with the help of melanin. Current Opinion in Microbiology 11(6):525-531. Available from: https://doi.org/10.1016/j.mib.2008.09.013

Dahlin JL, Nissink JWM, Strasser JM, Francis S, Higgins L, Zhou H, Zhang Z, Walters MA. 2015. PAINS in the assay: chemical mechanisms of assay interference and promiscuous enzymatic inhibition observed during a sulfhydryl-scavenging HTS. Journal of Medicinal Chemistry 58(5):2091-2113. Available from: https://doi.org/10.1021/jm5019093

Davidson BS. 1995. New dimensions in natural products research: cultured marine microorganisms. Current opinion in biotechnology 6(3):284-291. Available from: https://doi.org/10.1016/0958-1669(95)80049-2

Desborough MJR, Keeling DM. 2017. The aspirin story – from willow to wonder drug. British Journal of Haematology 177(5):674-683. Available from: https://doi.org/10.1111/bjh.14520

Dong Q-G, Zhang Y, Wang M-S, Feng J, Zhang H-H, Wu Y-G, Gu T-J, Yu X-H, Jiang C-L, Chen Y et al. . 2012. Improvement of enzymatic stability and intestinal permeability of deuterohemin-peptide conjugates by specific multi-site N-methylation. Amino Acids 43(6):2431-2441. Available from: https://doi.org/10.1007/s00726-012-1322-y

Durán P, Barra PJ, Jorquera MA, Viscardi S, Fernandez C, Paz C, Mora MDLL, Bol R. 2019. Occurrence of Soil Fungi in Antarctic Pristine Environments. Frontiers in Bioengineering and Biotechnology 7(28). Available from: https://doi.org/10.3389/fbioe.2019.00028

Egertová Z, Sochor M. 2017. The largest fungal genome discovered in *Jafnea semitosta*. Plant Systematics and Evolution 303(7):981-986. Available from: https://doi.org/10.1007/s00606-017-1424-9

Ejigu GF, Jung J. 2020. Review on the Computational Genome Annotation of Sequences Obtained by Next-Generation Sequencing. Biology 9(9):295. Available from: https://doi.org/10.3390/biology9090295

Epstein SS. 2013. The phenomenon of microbial uncultivability. Current Opinion in Microbiology 16(5):636-642. Available from: https://doi.org/10.1016/j.mib.2013.08.003

Fan B, Parrot D, Blümel M, Labes A, Tasdemir D. 2019. Influence of OSMAC-based cultivation in metabolome and anticancer activity of fungi associated with the brown alga fucus vesiculosus. Marine Drugs 17. Available from: https://doi.org/10.3390/md17010067

Fenical W, Jensen PR. 1993. 'Marine Microorganisms: A New Biomedical Resource'[Internet], in: Attaway DH, Zaborsky OR (ed) *Pharmaceutical and Bioactive Natural Products*. Springer US. Available from: https://doi.org/10.1007/978-1-4899-2391-2_12

Fernández-Lorente G, Betancor L, Carrascosa A, Guisan J. 2011. Release of Omega-3 Fatty Acids by the Hydrolysis of Fish Oil Catalyzed by Lipases Immobilized on Hydrophobic Supports. Journal of the American Oil Chemists' Society 88:1173-1178. Available from: https://doi.org/10.1007/s11746-011-1776-1

Fitzpatrick DA. 2012. Horizontal gene transfer in fungi. FEMS Microbiology Letters 329(1):1-8. Available from: https://doi.org/10.1111/j.1574-6968.2011.02465.x

Flewelling AJ, Currie J, Gray CA, Johnson JA. 2015. Endophytes from marine macroalgae: promising sources of novel natural products. Current Science 109(1):88-111. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.692.9487&rep=rep1&type=pdf

Fojutowski A, Wróblewska H, Komorowicz M, Kropacz A, Noskowiak A, Pomian I. 2014. Changes in the properties of English oak wood (*Quercus robur* L.) as a result of remaining submerged in Baltic Sea waters for two years. International Biodeterioration & Biodegradation 86:122-128. Available from: https://doi.org/10.1016/j.ibiod.2013.06.029

Fredricks DN, Smith C, Meier A. 2005. Comparison of six DNA extraction methods for recovery of fungal DNA as assessed by quantitative PCR. Journal of clinical microbiology 43(10):5122-5128. Available from: https://doi.org/10.1128/JCM.43.10.5122-5128.2005

Fu Y, Luo J, Qin J, Yang M. 2019. Screening techniques for the identification of bioactive compounds in natural products. Journal of Pharmaceutical and Biomedical Analysis 168:189-200. Available from: https://doi.org/10.1016/j.jpba.2019.02.027

Garrity GM, Banfield J, Eisen J, Van Der Lelie N, Mcmahon T, Rusch D, Delong E, Moran MA, Currie C, Furhman J et al. . 2013. Prokaryotic Super Program Advisory Committee DOE Joint Genome Institute, Walnut Creek, CA, March 27, 2013. Standards in Genomic Sciences 8(3):561-570. Available from: 10.4056/sigs.4638348

GBIF Secretariat. *Mytilinidiales* [Internet]. 'Available from:' https://doi.org/10.15468/39omei

Ghareeb MA, Tammam MA, El-Demerdash A, Atanasov AG. 2020. Insights about clinically approved and Preclinically investigated marine natural products. Current Research in Biotechnology 2:88-102. Available from: https://doi.org/10.1016/j.crbiot.2020.09.001

Giardine B, Riemer C, Hardison RC, Burhans R, Elnitski L, Shah P, Zhang Y, Blankenberg D, Albert I, Taylor J et al. . 2005. Galaxy: a platform for interactive large-scale genome analysis. Genome Research 15(10):1451-1455. Available from: https://doi.org/10.1101/gr.4086505

Giddings L-A, Newman DJ. 2019. 'Bioactive Compounds from Extremophilic Marine Fungi', in: Tiquia-Arashiro SM, Grube M (ed) *Fungi in Extreme Environments: Ecological Role and Biotechnological Significance*. ed. Cham: Springer International Publishing. 349-382.

Gil-Ramírez A, Pavo-Caballero C, Baeza E, Baenas N, Garcia-Viguera C, Marín FR, Soler-Rivas C. 2016. Mushrooms do not contain flavonoids. Journal of Functional Foods 25:1-13. Available from: https://doi.org/10.1016/j.jff.2016.05.005

Gonçalves MFM, Vicente TFL, Esteves AC, Alves A. 2020. Novel halotolerant species of *Emericellopsis* and *Parasarocladium* associated with macroalgae in an estuarine environment. Mycologia 112(1):154-171. Available from: https://doi.org/10.1080/00275514.2019.1677448

Goodwin S, Mcpherson JD, Mccombie WR. 2016. Coming of age: ten years of next-generation sequencing technologies. Nature Reviews Genetics 17(6):333-351. Available from: https://doi.org/10.1038/nrg.2016.49

Grigoriev IV, Cullen D, Goodwin SB, Hibbett D, Jeffries TW, Kubicek CP, Kuske C, Magnuson JK, Martin F, Spatafora JW et al. . 2011. Fueling the future with fungal genomics. Mycology 2(3):192-209. Available from: https://doi.org/10.1080/21501203.2011.584577

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F et al. . 2014. MycoCosm portal: Gearing up for 1000 fungal genomes. Nucleic Acids Research 42:D699. Available from: https://doi.org/10.1093/nar/gkt1183

Grossart H-P, Wurzbacher C, James TY, Kagami M. 2016. Discovery of dark matter fungi in aquatic ecosystems demands a reappraisal of the phylogeny and ecology of zoosporic fungi. Fungal Ecology 19:28-38. Available from: https://doi.org/10.1016/j.funeco.2015.06.004

Grum-Grzhimaylo AA, Georgieva ML, Debets AJM, Bilanenko EN. 2013. Are alkalitolerant fungi of the *Emericellopsis* lineage (*Bionectriaceae*) of marine origin? Ima Fungus 4(2):213-228. Available from: https://doi.org/10.5598/imafungus.2013.04.02.07

Grunwald AL, Berrué F, Overy DP, Kerr RG. 2016. Isolation of iqalisetins A and B from a *Tolypocladium* sp. isolated from marine sediment from Frobisher Bay in Canada's arctic. Canadian Journal of Chemistry 94(4):444-448. Available from: https://doi.org/10.1139/cjc-2015-0439

Grunwald AL, Berrue F, Robertson AW, Overy DP, Kerr RG. 2017. Mortiamides A–D, Cyclic Heptapeptides from a Novel *Mortierella* sp. Obtained from Frobisher Bay. Journal of Natural Products 80(10):2677-2683. Available from: https://doi.org/10.1021/acs.jnatprod.7b00383

Guarro J, Genéj, Stchigel AM. 1999. Developments in Fungal Taxonomy. Clinical microbiology reviews 12:454-500. Available from: https://doi.org/10.1128/CMR.12.3.454

Gubiani JR, Habeck TR, Chapla VM, Silva GH, Bolzani VS, Araujo AR. 2016. One strain-many compounds (OSMAC) method for production of phenolic compounds using *Camarops* sp., an endophytic fungus from *Alibertia macrophylla* (*Rubiaceae*). Química Nova 39:1221–1224. Available from: https://doi.org/10.21577/0100-4042.20160169

Hage H, Rosso M-N. 2021. Evolution of Fungal Carbohydrate-Active Enzyme Portfolios and Adaptation to Plant Cell-Wall Polymers. Journal of fungi (Basel, Switzerland) 7(3):185. Available from: https://doi.org/10.3390/jof7030185

Hansen J, Bross P. 2010. 'A Cellular Viability Assay to Monitor Drug Toxicity'[Internet], in: Bross P, Gregersen N (ed) *Protein Misfolding and Cellular Stress in Disease and Aging: Concepts and Protocols*. Humana Press. Available from: https://doi.org/10.1007/978-1-60761-756-3_21

Hanson JR. 2003. 'The classes of natural product and their isolation'[Internet], in: Hanson JR (ed) *Natural Products: The Secondary Metabolites*. The Royal Society of Chemistry. Available from: https://doi.org/10.1039/9781847551535-00001

Hanson JR. 2017. Pseudo-Natural Products, Some Artefacts Formed during the Isolation of Terpenoids. Journal of Chemical Research 41(9):497-503. Available from: https://doi.org/10.3184/174751917X15021050367558

Haridas S, Albert R, Binder M, Bloem J, Labutti K, Salamov A, Andreopoulos B, Baker SE, Barry K, Bills G et al. . 2020. 101 *Dothideomycetes* genomes: A test case for predicting lifestyles and emergence of pathogens. Studies in Mycology 96:141-153. Available from: https://doi.org/10.1016/j.simyco.2020.01.003

Hassett BT, Vonnahme TR, Peng X, Jones EBG, Heuzé C. 2019. Global diversity and geography of planktonic marine fungi. Botanica Marina 63(2):121-139. Available from: https://doi.org/10.1515/bot-2018-0113

Haugland RA, Heckman JL, Wymer LJ. 1999. Evaluation of different methods for the extraction of DNA from fungal conidia by quantitative competitive PCR analysis. Journal of Microbiological Methods 37(2):165-176. Available from: https://doi.org/10.1016/S0167-7012(99)00061-5

Hawksworth DL, Lücking R. 2017. Fungal Diversity Revisited: 2.2 to 3.8 Million Species. Microbiology Spectrum 5(4). Available from: https://doi.org/10.1128/microbiolspec.FUNK-0052-2016
Hayes M. 2012. The Geomyces Fungi: Ecology and Distribution. BioScience 62:819-823. Available from: https://doi.org/10.1525/bio.2012.62.9.7
Heap C, Warwick RM, D'ozouville L. 1998. A European science plan on marine biodiversity. European Science Foundation (ESF). 'Available from:' https://researchrepository.murdoch.edu.au/id/eprint/23338/
Helbert W. 2017. Marine Polysaccharide Sulfatases. Frontiers in Marine Science 4(6). Available from: https://doi.org/10.3389/fmars.2017.00006
Hellmann L, Tegel W, Eggertsson Ó, Schweingruber FH, Blanchette R, Kirdyanov A, Gärtner H, Büntgen U. 2013. Tracing the origin of Arctic driftwood. Journal of Geophysical Research: Biogeosciences 118(1):68-76. Available from: https://doi.org/10.1002/jgrg.20022
Hermann TN, Podkovyrov VN. 2010. A discovery of riphean heterotrophs in the Lakhanda group of Siberia. Paleontological Journal 44(4):374-383. Available from: https://doi.org/10.1134/S0031030110040027
Hewage RT, Aree T, Mahidol C, Ruchirawat S, Kittakoop P. 2014. One strain-many compounds (OSMAC) method for production of polyketides, azaphilones, and an isochromanone using the endophytic fungus *Dothideomycetes* sp. Phytochemistry 108:87–94. Available from: https://doi.org/10.1016/j.phytochem.2014.09.013
Hoskisson PA, Seipke RF. 2020. Cryptic or Silent? The Known Unknowns, Unknown Knowns, and Unknown Unknowns of Secondary Metabolism. mBio 11(5):e02642-20. Available from: https://doi.org/10.1128/mBio.02642-20
Hsiao G, Wang S-W, Chiang Y-R, Chi W-C, Kuo Y-H, Phong D, Chen C-Y, Lee T-H. 2020. Anti-inflammatory effects of peptides from a marine algicolous fungus *Acremonium* sp. NTU492 in BV-2 microglial cells. Journal of Food and Drug Analysis 28(2):89-97. Available from: https://doi.org/10.38212/2224-6614.1062
Hubert J, Nuzillard J-M, Renault J-H. 2017. Dereplication strategies in natural product research: How many tools and methodologies behind the same concept? Phytochemistry Reviews 16(1):55-95. Available from: https://doi.org/10.1007/s11101-015-9448-7
Hyde KD, Greenwood R, Gareth Jones EB. 1993. Spore attachment in marine fungi. Mycological Research 97(1):7-14. Available from: https://doi.org/10.1016/S0953-7562(09)81105-2
Hyde KD, Norphanphoun C, Maharachchikumbura SSN, Bhat DJ, Jones EBG, Bundhun D, Chen YJ, Bao DF, Boonmee S, Calabon MS et al. . 2020. Refined families of *Sordariomycetes*. Mycosphere 11(1):305-1059. Available from: https://doi.org/10.5943/mycosphere/11/1/7
Haas BJ, Salzberg SL, Zhu W, Pertea M, Allen JE, Orvis J, White O, Buell CR, Wortman JR. 2008. Automated eukaryotic gene structure annotation using EVidenceModeler and the Program to Assemble Spliced Alignments. Genome Biology 9(1):R7-R7. Available from: https://doi.org/10.1186/gb-2008-9-1-r7
Haas H. 2015. How to trigger a fungal weapon. eLife 4:e10504. Available from: https://doi.org/10.7554/eLife.10504
Ishino S, Ishino Y. 2014. DNA polymerases as useful reagents for biotechnology - the history of developmental research in the field. Frontiers in Microbiology 5:465-465. Available from: https://doi.org/10.3389/fmicb.2014.00465
Ito T, Masubuchi M. 2014. Dereplication of microbial extracts and related analytical technologies. The Journal Of Antibiotics 67(5):353-360. Available from: https://doi.org/10.1038/ja.2014.12

Ivarsson M, Bengtson S, Neubeck A. 2016. The igneous oceanic crust – Earth's largest fungal habitat? Fungal Ecology 20:249-255. Available from: https://doi.org/10.1016/j.funeco.2016.01.009

James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J et al. . 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. Nature 443(7113):818-822. Available from: https://doi.org/10.1038/nature05110

Jennings DH. 1983. Some aspects of the physiology and biochemistry of marine fungi. Biological Reviews 58(3):423-459. Available from: https://doi.org/10.1111/j.1469-185X.1983.tb00395.x

Johansen S. 1998. The origin and age of driftwood on Jan Mayen. Polar Research 17(2):125-146. Available from: https://doi.org/10.1111/j.1751-8369.1998.tb00267.x

Johnson TW. 1956a. Marine Fungi. I. *Leptosphaeria* and *Pleospora*. Mycologia 48(4):495-505. Available from: https://doi.org/10.2307/3755331

Johnson TW. 1956b. Marine Fungi. II. Ascomycetes and Deuteromycetes from Submerged Wood. Mycologia 48(6):841-851. Available from: https://doi.org/10.2307/3755710

Johnson TW, Sparrow FK. 1961. *Fungi in oceans and estuaries*. Weinheim (Germany), J. Cramer; Codicote, Herts, Wheldon & Wesley, Ltd.; New York, N.Y., Hafner Publishing Co.

Joint Genome Institute. 1000 Fungal Genomes Project - Genome portal [Internet]. Joint Genome Institute of the Department of Energy; [cited 06/03 2021]. Available from: https://mycocosm.jgi.doe.gov/fungi/fungi.info.html

Joint Genome Institute. 1000 Fungal Genomes Project - Secondary metabolite clusters [Internet]. [cited 06/03 2021]. Available from: https://mycocosm.jgi.doe.gov/pages/sm-clusters-summary.jsf?organism=fungi&genomes=fungi

Jones EBG. 2000. Marine fungi: Some factors influencing biodiversity. Fungal Diversity 4:53-73. Available from: https://www.fungaldiversity.org/fdp/sfdp/FD_4_53-73.pdf

Jones EBG. 2011. Fifty years of marine mycology. Fungal Diversity 50(1):73-112. Available from: https://doi.org/10.1007/s13225-011-0119-8

Jones EBG, Pang K-L. 2012. *Marine Fungi and Fungal-like Organisms*. Göttingen: De Gruyter.

Jones EBG, Pang KL, Abdel-Wahab MA, Scholz B, Hyde KD, Boekhout T, Ebel R, Rateb ME, Henderson L, Sakayaroj J et al. . 2019. An online resource for marine fungi. Fungal Diversity 96:347-433. Available from: https://doi.org/10.1007/s13225-019-00426-5

Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL. 2009. Classification of marine *Ascomycota*, anamorphic taxa and *Basidiomycota*. Fungal Diversity 35:1-187. Available from: https://www.researchgate.net/profile/Satinee-Suetrong/publication/280797437_Classification_of_marine_Ascomycota_anamorphic_taxa_and_Basidiomycota/links/55f623f408ae6a34f66328bb/Classification-of-marine-Ascomycota-anamorphic-taxa-and-Basidiomycota.pdf

Jones EBG, Suetrong S, Sakayaroj J, Bahkali AH, Abdel-Wahab MA, Boekhout T, Pang KL. 2015. Classification of marine *Ascomycota*, *Basidiomycota*, *Blastocladiomycota* and *Chytridiomycota*. Fungal Diversity 73(1):1-72. Available from: https://doi.org/10.1007/s13225-015-0339-4

Jung H, Ventura T, Chung JS, Kim W-J, Nam B-H, Kong HJ, Kim Y-O, Jeon M-S, Eyun S-I. 2020. Twelve quick steps for genome assembly and annotation in the classroom. PLoS Computational Biology 16(11):e1008325. Available from: https://doi.org/10.1371/journal.pcbi.1008325

Kalra R, Conlan XA, Goel M. 2020. Fungi as a Potential Source of Pigments: Harnessing Filamentous Fungi. Frontiers in Chemistry 8(369). Available from: 10.3389/fchem.2020.00369

Keller NP. 2019. Fungal secondary metabolism: regulation, function and drug discovery. Nature Reviews Microbiology 17(3):167-180. Available from: https://doi.org/10.1038/s41579-018-0121-1

Keller NP, Turner G, Bennett JW. 2005. Fungal secondary metabolism - from biochemistry to genomics. Nature Reviews Microbiology 3(12):937-47. Available from: https://doi.org/10.1038/nrmicro1286

Koch J, Pang K-L, Jones EBG. 2007. *Rostrupiella danica* gen. et sp. nov., a *Lulworthia*-like marine lignicolous species from Denmark and the USA. 50(5-6):294-301. Available from: https://doi.org/10.1515/BOT.2007.034

Kochkina GA, Ivanushkina NE, Akimov VN, Gilichinskii DA, Ozerskaya SM. 2007. Halo-and psychrotolerant *Geomyces* fungi from arctic cryopegs and marine deposits. Microbiology 76(1):31-38. Available from: https://doi.org/10.1134/S0026261707010055

Kohlmeyer J. 1974. On the Definition and Taxonomy of Higher Marine Fungi. Veröffentlichungen des Instituts für Meeresforschung in Bremenhaven Supplement 5. Available from:

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

Kong D-X, Guo M-Y, Xiao Z-H, Chen L-L, Zhang H-Y. 2011. Historical Variation of Structural Novelty in a Natural Product Library. Chemistry & Biodiversity 8(11):1968-1977. Available from: https://doi.org/10.1002/cbdv.201100156

Kumar V, Sangwan P, Singh D, Kaur Gill P. 2014. 'Global scenario of industrial enzyme market'[Internet], in: (ed). Available from: https://doi.org/10.13140/2.1.3599.0083

Lage OM, Ramos MC, Calisto R, Almeida E, Vasconcelos V, Vicente F. 2018. Current Screening Methodologies in Drug Discovery for Selected Human Diseases. Marine Drugs 16(8). Available from: https://doi.org/10.3390/md16080279

Lamichhane S, Sen P, Dickens AM, Hyötyläinen T, Orešič M. 2018. 'Chapter Fourteen - An Overview of Metabolomics Data Analysis: Current Tools and Future Perspectives'[Internet], in: Jaumot J, Bedia C, Tauler R (ed) *Comprehensive Analytical Chemistry*. Elsevier. Available from: https://doi.org/10.1016/bs.coac.2018.07.001

Lee H, Lee YM, Heo YM, Lee H, Hong J-H, Jang S, Park MS, Lim YW, Kim J-J. 2015. Halo-tolerance of Marine-derived Fungi and their Enzymatic Properties. 2015 10(4):11. Available from: https://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_10_4_8450_Lee_Halo_Tolerance_Marine_Derived_Fungi

Li H, Wu S, Ma X, Chen W, Zhang J, Duan S, Gao Y, Kui L, Huang W, Wu P et al. . 2018. The Genome Sequences of 90 Mushrooms. Scientific Reports 8(1):9982. Available from: https://doi.org/10.1038/s41598-018-28303-2

Li X-J, Zhang H-Y. 2008. Synergy in natural medicines: implications for drug discovery. Trends in Pharmacological Sciences 29(7):331-332. Available from: https://doi.org/10.1016/j.tips.2008.04.002

Li Y, Steenwyk JL, Chang Y, Wang Y, James TY, Stajich JE, Spatafora JW, Groenewald M, Dunn CW, Hittinger CT et al. . 2021. A genome-scale phylogeny of the kingdom Fungi. Current Biology. Available from: https://doi.org/10.1016/j.cub.2021.01.074

Libor B, Harms H, Kehraus S, Egereva E, Crüsemann M, König GM. 2019. Isolation of fungi using the diffusion chamber device FIND technology. Beilstein Journal of Organic Chemistry 15:2191-2203. Available from: https://doi.org/10.3762/bjoc.15.216

Lim FY, Sanchez JF, Wang CCC, Keller NP. 2012. Toward awakening cryptic secondary metabolite gene clusters in filamentous fungi. Methods in Enzymology 517:303-324. Available from: https://doi.org/10.1016/B978-0-12-404634-4.00015-2

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 23(1):3-25. Available from: https://doi.org/10.1016/S0169-409X(96)00423-1

Liu H, Xue R, Wang Y, Stirling E, Ye S, Xu J, Ma B. 2020a. FACS-iChip: a high-efficiency iChip system for microbial 'dark matter' mining. Marine Life Science & Technology. Available from: https://doi.org/10.1007/s42995-020-00067-7

Liu J, Nes WD. 2009. Steroidal triterpenes: design of substrate-based inhibitors of ergosterol and sitosterol synthesis. Molecules (Basel, Switzerland) 14(11):4690-4706. Available from: https://doi.org/10.3390/molecules14114690

Liu Y, Sargent L, Leung W, Elgin SCR, Goecks J. 2019. G-OnRamp: a Galaxy-based platform for collaborative annotation of eukaryotic genomes. Bioinformatics 35(21):4422-4423. Available from: https://doi.org/10.1093/bioinformatics/btz309

Liu Z, Zhao J-Y, Sun S-F, Li Y, Liu Y-B. 2020b. Fungi: outstanding source of novel chemical scaffolds. Journal of Asian Natural Products Research 22(2):99-120. Available from: https://doi.org/10.1080/10286020.2018.1488833

Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Research 42(D1):D490-D495. Available from: https://doi.org/10.1093/nar/gkt1178

Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM et al. . 2020. Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? Ima Fungus 11(1):14. Available from: https://doi.org/10.1186/s43008-020-00033-z

Ma L-J, Fedorova ND. 2010. A practical guide to fungal genome projects: strategy, technology, cost and completion. Mycology 1(1):9-24. Available from: https://doi.org/10.1080/21501201003680943

Maheshwari R, Bharadwaj G, Bhat MK. 2000. Thermophilic fungi: their physiology and enzymes. Microbiology and molecular biology reviews : MMBR 64(3):461-488. Available from: https://doi.org/10.1128/mmbr.64.3.461-488.2000

Mahmoud M, Zywicki M, Twardowski T, Karlowski WM. 2019. Efficiency of PacBio long read correction by 2nd generation Illumina sequencing. Genomics 111(1):43-49. Available from: https://doi.org/10.1016/j.ygeno.2017.12.011

Malosso E, Waite IS, English L, Hopkins DW, O'donnell AG. 2006. Fungal diversity in maritime Antarctic soils determined using a combination of culture isolation, molecular fingerprinting and cloning techniques. Polar Biology 29(7):552-561. Available from: https://doi.org/10.1007/s00300-005-0088-z

Maltese F, Van Der Kooy F, Verpoorte R. 2009. Solvent Derived Artifacts in Natural Products Chemistry. Natural Product Communications 4(3):1934578X0900400326. Available from: https://doi.org/10.1177/1934578X0900400326

Manohar CS, Raghukumar C. 2013. Fungal diversity from various marine habitats deduced through culture-independent studies. FEMS Microbiology Letters 341(2):69-78. Available from: https://doi.org/10.1111/1574-6968.12087

Matano LM, Morris HG, Wood BM, Meredith TC, Walker S. 2016. Accelerating the discovery of antibacterial compounds using pathway-directed whole cell screening. Bioorganic & Medicinal Chemistry 24(24):6307-6314. Available from: https://doi.org/10.1016/j.bmc.2016.08.003

Midwestern University. Clinical Pipeline - Marine Pharmacology [Internet]. [cited 04/04 2021]. Available from: https://www.midwestern.edu/departments/marinepharmacology/clinical-pipeline.xml
Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M. 2017. Opportunities and challenges in phenotypic drug discovery: an industry perspective. Nature Reviews Drug Discovery 16(8):531-543. Available from: https://doi.org/10.1038/nrd.2017.111
Mohanta TK. 2020. Fungi contain genes associated with flavonoid biosynthesis pathway. Journal of Functional Foods 68:103910. Available from: https://doi.org/10.1016/j.jff.2020.103910
Mohanta TK, Bae H. 2015. The diversity of fungal genome. Biological Procedures Online 17:8. Available from: https://doi.org/10.1186/s12575-015-0020-z
Monciardini P, Iorio M, Maffioli S, Sosio M, Donadio S. 2014. Discovering new bioactive molecules from microbial sources. Microbial Biotechnology 7(3):209-220. Available from: https://doi.org/10.1111/1751-7915.12123
Montagne C. 1846. 'Flore d'Algérie. Classis I. Acotyledoneae Juss. Ordo I. Phyceae Fries', in: Durieu De Maisonneuve M, Bory De Saint-Vincent J (ed) *Exploration Scientifique de l'Algérie pendant les Années 1840, 1841, 1842[, 1843, 1844 et 1845]: publiée par ordre du gouvernement et avec le concours d'une commission académique. Sciences physiques. Botanique*. ed. Paris: Imprimerie Nationale.
Montoliu-Nerin M, Sánchez-García M, Bergin C, Grabherr M, Ellis B, Kutschera VE, Kierczak M, Johannesson H, Rosling A. 2020. Building de novo reference genome assemblies of complex eukaryotic microorganisms from single nuclei. Scientific Reports 10(1):1303. Available from: https://doi.org/10.1038/s41598-020-58025-3
Mullard A. 2021. 2020 FDA drug approvals. Nature Reviews Drug Discovery 20:85-90. Available from: https://doi.org/10.1038/d41573-021-00002-0
Munafo A, Ferri G, Jakuba M. Special Issue "Measuring, Monitoring and Exploring the Ocean: From Coast to the Deep Sea" [Internet]. [cited 29/04 2021]. Available from: https://www.mdpi.com/journal/remotesensing/special_issues/marine_sensor_RS
Muszewska A, Steczkiewicz K, Stepniewska-Dziubinska M, Ginalski K. 2019. Transposable elements contribute to fungal genes and impact fungal lifestyle. Scientific Reports 9(1):4307. Available from: https://doi.org/10.1038/s41598-019-40965-0
Naranjo-Ortiz MA, Gabaldón T. 2019a. Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. Biological Reviews 94(6):2101-2137. Available from: https://doi.org/10.1111/brv.12550
Naranjo-Ortiz MA, Gabaldón T. 2019b. Fungal evolution: major ecological adaptations and evolutionary transitions. Biological Reviews 94(4):1443-1476. Available from: https://doi.org/10.1111/brv.12510
Naranjo-Ortiz MA, Gabaldón T. 2020. Fungal evolution: cellular, genomic and metabolic complexity. Biological Reviews 95(5):1198-1232. Available from: https://doi.org/10.1111/brv.12605
Nass J, Efferth T. 2018. Drug targets and resistance mechanisms in multiple myeloma. Cancer Drug Resistance 1(2):87-117. Available from: https://doi.org/10.20517/cdr.2018.04
NCBI. Prokaryotic RefSeq Genome Re-annotation Project [Internet]. NCBI; [cited 9/4 2021]. Available from: https://www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/reannotation/
NCBI. The NCBI Eukaryotic Genome Annotation Pipeline [Internet]. NCBI; [cited 9/4 2021]. Available from: https://www.ncbi.nlm.nih.gov/genome/annotation_euk/process/#reannot
Newman DJ, Cragg GM. 2020. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. Journal of Natural Products 83(3):770-803. Available from: https://doi.org/10.1021/acs.jnatprod.9b01285

Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, Belanger A, Kanigan T, Lewis K, Epstein SS. 2010. Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. Applied and Environmental Microbiology 76(8):2445-50. Available from: https://doi.org/10.1128/aem.01754-09

Noakes MT, Brinkerhoff H, Laszlo AH, Derrington IM, Langford KW, Mount JW, Bowman JL, Baker KS, Doering KM, Tickman BI et al. . 2019. Increasing the accuracy of nanopore DNA sequencing using a time-varying cross membrane voltage. Nat Biotechnol 37(6):651-656. Available from: https://doi.org/10.1038/s41587-019-0096-0

Norn S, Permin H, Kruse PR, Kruse E. 2009. [From willow bark to acetylsalicylic acid]. Dansk Medicinhistorisk Årbog 37:79-98. Available from:

Ntie-Kang F, Svozil D. 2020. An enumeration of natural products from microbial, marine and terrestrial sources. Physical Sciences Reviews 5(8). Available from: https://doi.org/10.1515/psr-2018-0121

O'leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, Mcveigh R, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D et al. . 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Research 44(D1):D733-45. Available from: https://doi.org/10.1093/nar/gkv1189

Ogaki MB, De Paula MT, Ruas D, Pellizzari FM, García-Laviña CX, Rosa LH. 2019. 'Marine Fungi Associated with Antarctic Macroalgae', in: Castro-Sowinski S (ed) *The Ecological Role of Micro-organisms in the Antarctic Environment*. ed. Cham: Springer International Publishing. 239-255.

Oldfield E, Lin F-Y. 2012. Terpene biosynthesis: modularity rules. Angewandte Chemie (International ed. in English) 51(5):1124-1137. Available from: https://doi.org/10.1002/anie.201103110

Overy DP, Bayman P, Kerr RG, Bills GF. 2014. An assessment of natural product discovery from marine (sensu strictu) and marine-derived fungi. Mycology 5(3):145-167. Available from: https://doi.org/10.1080/21501203.2014.931308

Overy DP, Rämä T, Oosterhuis R, Walker AK, Pang KL. 2019. The neglected marine fungi, *sensu stricto*, and their isolation for natural products' discovery. Marine Drugs 17. Available from: https://doi.org/10.3390/md17010042

Pang K-L, Chow RK, Chan C-W, Vrijmoed LP. 2011. Diversity and physiology of marine lignicolous fungi in Arctic waters: a preliminary account. Polar Research 30(1):5859. Available from: https://doi.org/10.3402/polar.v30i0.5859

Pang K-L, Overy DP, Jones EBG, Calado MDL, Burgaud G, Walker AK, Johnson JA, Kerr RG, Cha H-J, Bills GF. 2016. 'Marine fungi' and 'marine-derived fungi' in natural product chemistry research: Toward a new consensual definition. Fungal Biology Reviews 30(4):163-175. Available from: https://doi.org/10.1016/j.fbr.2016.08.001

Patridge E, Gareiss P, Kinch MS, Hoyer D. 2016. An analysis of FDA-approved drugs: natural products and their derivatives. Drug Discovery Today 21(2):204-207. Available from: https://doi.org/10.1016/j.drudis.2015.01.009

Paul F, Otte J, Schmitt I, Dal Grande F. 2018. Comparing Sanger sequencing and high-throughput metabarcoding for inferring photobiont diversity in lichens. Scientific Reports 8(1):8624. Available from: https://doi.org/10.1038/s41598-018-26947-8

Payne A, Holmes N, Rakyan V, Loose M. 2019. BulkVis: a graphical viewer for Oxford nanopore bulk FAST5 files. Bioinformatics 35(13):2193-2198. Available from: https://doi.org/10.1093/bioinformatics/bty841

Pereira F. 2019. Have marine natural product drug discovery efforts been productive and how can we improve their efficiency? Expert Opinion on Drug Discovery 14(8):717-722. Available from: https://doi.org/10.1080/17460441.2019.1604675

Perini L, Gostinčar C, Gunde-Cimerman N. 2019. Fungal and bacterial diversity of Svalbard subglacial ice. Scientific Reports 9(1):20230. Available from: https://doi.org/10.1038/s41598-019-56290-5

Petrovska BB. 2012. Historical review of medicinal plants' usage. Pharmacognosy reviews 6(11):1-5. Available from: https://doi.org/10.4103/0973-7847.95849

Pilgaard B, Wilkens C, Herbst F-A, Vuillemin M, Rhein-Knudsen N, Meyer AS, Lange L. 2019. Proteomic enzyme analysis of the marine fungus *Paradendryphiella salina* reveals alginate lyase as a minimal adaptation strategy for brown algae degradation. Scientific Reports 9(1):12338. Available from: https://doi.org/10.1038/s41598-019-48823-9

Piovesan A, Pelleri MC, Antonaros F, Strippoli P, Caracausi M, Vitale L. 2019. On the length, weight and GC content of the human genome. BMC Research Notes 12(1):106. Available from: https://doi.org/10.1186/s13104-019-4137-z

Pouliot M, Jeanmart S. 2016. Pan Assay Interference Compounds (PAINS) and Other Promiscuous Compounds in Antifungal Research. Journal of Medicinal Chemistry 59(2):497-503. Available from: https://doi.org/10.1021/acs.jmedchem.5b00361

Prenafeta-Boldú FX, Kuhn A, Luykx DMaM, Anke H, Van Groenestijn JW, De Bont JaM. 2001. Isolation and characterisation of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycological Research 105(4):477-484. Available from: https://doi.org/10.1017/S0953756201003719

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41(D1):D590-D596. Available from: https://doi.org/10.1093/nar/gks1219

Raghukumar C. 2008. Marine fungal biotechnology: an ecological perspective. Fungal Diversity 31:19-35. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.464.9076&rep=rep1&type=pdf

Raghukumar S. 2017. 'Origin and Evolution of Marine Fungi'[Internet], in: Raghukumar S (ed) *Fungi in Coastal and Oceanic Marine Ecosystems: Marine Fungi*. Springer International Publishing. Available from: https://doi.org/10.1007/978-3-319-54304-8_14

Rappé MS, Giovannoni SJ. 2003. The Uncultured Microbial Majority. Annual Review of Microbiology 57(1):369-394. Available from: https://doi.org/10.1146/annurev.micro.57.030502.090759

Rateb ME, Ebel R. 2011. Secondary metabolites of fungi from marine habitats. Natural Product Reports 28(2):290-344. Available from: https://doi.org/10.1039/C0NP00061B

Redou V, Navarri M, Meslet-Cladiere L, Barbier G, Burgaud G. 2015. Species Richness and Adaptation of Marine Fungi from Deep-Subseafloor Sediments. Applied and Environmental Microbiology 81(10):3571-3583. Available from: https://doi.org/10.1128/aem.04064-14

Reen FJ, Romano S, Dobson ADW, O'gara F. 2015. The Sound of Silence: Activating Silent Biosynthetic Gene Clusters in Marine Microorganisms. Marine Drugs 13(8):4754-4783. Available from: https://doi.org/10.3390/md13084754

Rhoads A, Au KF. 2015. PacBio Sequencing and Its Applications. Genomics, Proteomics & Bioinformatics 13(5):278-289. Available from: https://doi.org/10.1016/j.gpb.2015.08.002

Rice AV, Currah RS. 2005. *Oidiodendron*: A survey of the named species and related anamorphs of *Myxotrichum*. Studies in Mycology 53:83-120. Available from: https://doi.org/10.3114/sim.53.1.83

Richards TA, Jones MDM, Leonard G, Bass D. 2011a. Marine Fungi: Their Ecology and Molecular Diversity. Annual Review of Marine Science 4(1):495–522. Available from: https://doi.org/10.1146/annurev-marine-120710-100802

Richards TA, Leonard G, Soanes DM, Talbot NJ. 2011b. Gene transfer into the fungi. Fungal Biology Reviews 25(2):98-110. Available from: https://doi.org/10.1016/j.fbr.2011.04.003

Rohrmann S, Molitoris HP. 1992. Screening for wood-degrading enzymes in marine fungi. Canadian Journal of Botany 70(10):2116-2123. Available from: https://doi.org/10.1139/b92-263

Rokas A, Mead ME, Steenwyk JL, Raja HA, Oberlies NH. 2020. Biosynthetic gene clusters and the evolution of fungal chemodiversity. Natural Product Reports 37(7):868-878. Available from: https://doi.org/10.1039/C9NP00045C

Rokas A, Wisecaver JH, Lind AL. 2018. The birth, evolution and death of metabolic gene clusters in fungi. Nature Reviews Microbiology 16(12):731-744. Available from: https://doi.org/10.1038/s41579-018-0075-3

Roldán A, Descals E, Honrubia M. 1989. Pure culture studies on *Tetracladium*. Mycological Research 93(4):452-465. Available from: https://doi.org/10.1016/S0953-7562(89)80039-5

Romano S, Jackson S, Patry S, Dobson A. 2018. Extending the "One Strain Many Compounds" (OSMAC) Principle to Marine Microorganisms. Marine Drugs 16:244. Available from: https://doi.org/10.3390/md16070244

Roopashree KM, Naik D. 2019. Advanced method of secondary metabolite extraction and quality analysis. International Journal of Pharmacognosy and Phytochemical Research 8(3):1829-1842. Available from: https://www.phytojournal.com/archives/?year=2019&vol=8&issue=3&ArticleId=8275

Ryberg M. 2015. Molecular operational taxonomic units as approximations of species in the light of evolutionary models and empirical data from Fungi. Molecular Ecology 24(23):5770-5777. Available from: https://doi.org/10.1111/mec.13444

Rämä T, Hassett B, T., Bubnova E. 2017. Arctic marine fungi: from filaments and flagella to operational taxonomic units and beyond. Botanica Marina 60(4):433. Available from: https://doi.org/10.1515/bot-2016-0104

Rämä T, Norden J, Davey ML, Mathiassen GH, Spatafora JW, Kauserud H. 2014. Fungi ahoy! Diversity on marine wooden substrata in the high North. Fungal Ecology 8:46–58. Available from: https://doi.org/10.1016/j.funeco.2013.12.002

Sabdaningsih A, Cristianawati O, Sibero MT, Nuryadi H, Radjasa OK, Sabdono A, Trianto A. 2017. 'Screening Antibacterial Agent from Crude Extract of Marine-Derived Fungi Associated with Soft Corals against MDR-*Staphylococcus haemolyticus*', in: Hadiyanto (ed) *2nd International Conference on Tropical and Coastal Region Eco Development 2016*. ed.

Salgado CL, Muñoz R, Blanco A, Lienqueo ME. 2021. Valorization and upgrading of the nutritional value of seaweed and seaweed waste using the marine fungi *Paradendryphiella salina* to produce mycoprotein. Algal Research 53:102135. Available from: https://doi.org/10.1016/j.algal.2020.102135

Salzberg SL. 2007. Genome re-annotation: a wiki solution? Genome Biology 8(1):102. Available from: https://doi.org/10.1186/gb-2007-8-1-102

Santos J, Meyer E, Sette L. 2020. Fungal Community in Antarctic Soil Along the Retreating Collins Glacier (Fildes Peninsula, King George Island). Microorganisms 8:1145. Available from: https://doi.org/10.3390/microorganisms8081145

Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D, Mcveigh R, O'neill K, Robbertse B et al. . 2020. NCBI Taxonomy: a comprehensive update on curation, resources and tools. Database (Oxford) 2020. Available from: https://doi.org/10.1093/database/baaa062

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences 109(16):6241. Available from: https://doi.org/10.1073/pnas.1117018109

Schwarze K, Buchanan J, Taylor JC, Wordsworth S. 2018. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic review of the literature. Genetics in Medicine 20(10):1122-1130. Available from: https://doi.org/10.1038/gim.2017.247

Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30(14):2068-2069. Available from: https://doi.org/10.1093/bioinformatics/btu153

Sekurova ON, Schneider O, Zotchev SB. 2019. Novel bioactive natural products from bacteria via bioprospecting, genome mining and metabolic engineering. Microbial Biotechnology 12(5):828-844. Available from: https://doi.org/10.1111/1751-7915.13398

Sepcic K, Zalar P, Gunde-Cimerman N. 2011. Low Water Activity Induces the Production of Bioactive Metabolites in Halophilic and Halotolerant Fungi. Marine Drugs 9(1):43-58. Available from: https://doi.org/10.3390/md9010043

Shang J, Hu B, Wang J, Zhu F, Kang Y, Li D, Sun H, Kong D-X, Hou T. 2018. Cheminformatic Insight into the Differences between Terrestrial and Marine Originated Natural Products. Journal of Chemical Information and Modeling 58(6):1182-1193. Available from: https://doi.org/10.1021/acs.jcim.8b00125

Shin HJ. 2020. Natural Products from Marine Fungi. Marine Drugs 18(5):230. Available from: https://doi.org/10.3390/md18050230

Singer E, Wagner M, Woyke T. 2017. Capturing the genetic makeup of the active microbiome in situ. Isme j 11(9):1949-1963. Available from: https://doi.org/10.1038/ismej.2017.59

Sitnikov DG, Monnin CS, Vuckovic D. 2016. Systematic Assessment of Seven Solvent and Solid-Phase Extraction Methods for Metabolomics Analysis of Human Plasma by LC-MS. Scientific Reports 6(1):38885. Available from: https://doi.org/10.1038/srep38885

Skinnider MA, Johnston CW, Gunabalasingam M, Merwin NJ, Kieliszek AM, Maclellan RJ, Li H, Ranieri MRM, Webster ALH, Cao MPT et al. . 2020. Comprehensive prediction of secondary metabolite structure and biological activity from microbial genome sequences. Nature Communications 11(1):6058. Available from: https://doi.org/10.1038/s41467-020-19986-1

Sonjak S, Frisvad JC, Gunde-Cimerman N. 2006. *Penicillium* Mycobiota in Arctic Subglacial Ice. Microbial Ecology 52(2):207-216. Available from: https://doi.org/10.1007/s00248-006-9086-0

Spatafora JW, Volkmann-Kohlmeyer B, Kohlmeyer J. 1998. Independent terrestrial origins of the *Halosphaeriales* (marine *Ascomycota*). American Journal of Botany 85(11):1569-1580. Available from: https://doi.org/10.2307/2446483

Sridhar K. 2009. 'Aquatic fungi – Are they planktonic?'[Internet], in: (ed). Available from: https://doi.org/10.13140/RG.2.1.5135.0166

Srikanth K, Park J-E, Lim D, Cha J, Cho S-R, Cho I-C, Park W. 2020. A Comparison between Hi-C and 10X Genomics Linked Read Sequencing for Whole Genome Phasing in Hanwoo Cattle. Genes 11(3). Available from: https://doi.org/10.3390/genes11030332

Stefani FOP, Bell TH, Marchand C, De La Providencia IE, El Yassimi A, St-Arnaud M, Hijri M. 2015. Culture-Dependent and -Independent Methods Capture Different Microbial

Community Fractions in Hydrocarbon-Contaminated Soils. PLoS ONE 10(6):e0128272. Available from: https://doi.org/10.1371/journal.pone.0128272
Stefanović OD. 2017. 'Synergistic Activity of Antibiotics and Bioactive Plant Extracts: A Study Against Gram-Positive and Gram-Negative Bacteria'[Internet], in: Kırmusaoğlu S (ed) *Bacterial Pathogenesis and Antibacterial Control*. Available from: https://doi.org/10.5772/intechopen.72026
Stielow JB, Lévesque CA, Seifert KA, Meyer W, Iriny L, Smits D, Renfurm R, Verkley GJM, Groenewald M, Chaduli D et al. . 2015. One fungus, which genes? Development and assessment of universal primers for potential secondary fungal DNA barcodes. Persoonia 35:242-263. Available from: https://doi.org/10.3767/003158515X689135
Stratton CF, Newman DJ, Tan DS. 2015. Cheminformatic comparison of approved drugs from natural product versus synthetic origins. Bioorganic & Medicinal Chemistry Letters 25(21):4802-4807. Available from: https://doi.org/10.1016/j.bmcl.2015.07.014
Tanizawa Y, Fujisawa T, Nakamura Y. 2017. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34(6):1037-1039. Available from: https://doi.org/10.1093/bioinformatics/btx713
Tasdemir D. 2017. Marine fungi in the spotlight: opportunities and challenges for marine fungal natural product discovery and biotechnology. Fungal Biology and Biotechnology 4(1):5. Available from: https://doi.org/10.1186/s40694-017-0034-1
Thibaud-Nissen F, Souvorov A, Murphy T, Et Al. 2013. 'Eukaryotic Genome Annotation Pipeline.', in: (ed) *The NCBI Handbook [Internet]*. 2 ed. Bethesda (MD): National Center for Biotechnology Information (US).
Thines M, Crous PW, Aime MC, Aoki T, Cai L, Hyde KD, Miller AN, Zhang N, Stadler M. 2018. Ten reasons why a sequence-based nomenclature is not useful for fungi anytime soon. Ima Fungus 9(1):177-183. Available from: https://doi.org/10.5598/imafungus.2018.09.01.11
Thornburg CC, Britt JR, Evans JR, Akee RK, Whitt JA, Trinh SK, Harris MJ, Thompson JR, Ewing TL, Shipley SM et al. . 2018. NCI Program for Natural Product Discovery: A Publicly-Accessible Library of Natural Product Fractions for High-Throughput Screening. ACS chemical biology 13(9):2484-2497. Available from: https://doi.org/10.1021/acschembio.8b00389
Tiganis T. 2013. PTP1B and TCPTP – nonredundant phosphatases in insulin signaling and glucose homeostasis. The FEBS Journal 280(2):445-458. Available from: https://doi.org/10.1111/j.1742-4658.2012.08563.x
Tisthammer KH, Cobian GM, Amend AS. 2016. Global biogeography of marine fungi is shaped by the environment. Fungal Ecology 19:39–46. Available from: https://doi.org/10.1016/j.funeco.2015.09.003
Trautman EP, Crawford JM. 2016. Linking Biosynthetic Gene Clusters to their Metabolites via Pathway- Targeted Molecular Networking. Current Topics in Medicinal Chemistry 16(15):1705-1716. Available from: https://doi.org/10.2174/1568026616666151012111046
Trivella DBB, De Felicio R. 2018. The Tripod for Bacterial Natural Product Discovery: Genome Mining, Silent Pathway Induction, and Mass Spectrometry-Based Molecular Networking. mSystems 3(2):e00160-17. Available from: https://doi.org/10.1128/mSystems.00160-17
Tsuji M, Hoshino T. 2019. *Fungi in Polar Regions*. CRC Press.
Ursu A, Schöler HR, Waldmann H. 2017. Small-molecule phenotypic screening with stem cells. Nature Chemical Biology 13(6):560-563. Available from: https://doi.org/10.1038/nchembio.2383

Van Burik JA, Schreckhise RW, White TC, Bowden RA, Myerson D. 1998. Comparison of six extraction techniques for isolation of DNA from filamentous fungi. Medical Mycology 36(5):299-303. Available from: https://academic.oup.com/mmy/article-pdf/36/5/299/2793057/36-5-299.pdf

Venditti A. 2018. Artifacts in natural products studies. An old and underestimated re-emerging problem. Natural Product Research 32(7):(i)-(ii). Available from: https://doi.org/10.1080/14786419.2018.1443421

Venditti A. 2020. What is and what should never be: artifacts, improbable phytochemicals, contaminants and natural products. Natural Product Research 34(7):1014-1031. Available from: https://doi.org/10.1080/14786419.2018.1543674

Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. P & T : a peer-reviewed journal for formulary management 40(4):277-283. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4378521/

Vickery ML, Vickery B. 1981. 'Alkaloids'[Internet], in: Vickery ML, Vickery B (ed) *Secondary Plant Metabolism*. Macmillan Education UK. Available from: https://doi.org/10.1007/978-1-349-86109-5_9

Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CHW, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA. 2014. Identification and nomenclature of the genus *Penicillium*. Studies in Mycology 78:343-371. Available from: https://doi.org/10.1016/j.simyco.2014.09.001

Vu D, Groenewald M, De Vries M, Gehrmann T, Stielow B, Eberhardt U, Al-Hatmi A, Groenewald JZ, Cardinali G, Houbraken J et al. . 2019. Large-scale generation and analysis of filamentous fungal DNA barcodes boosts coverage for kingdom fungi and reveals thresholds for fungal species and higher taxon delimitation. Studies in Mycology 92:135-154. Available from: https://doi.org/10.1016/j.simyco.2018.05.001

Wang M, Jiang X, Wu W, Hao Y, Su Y, Cai L, Xiang M, Liu X. 2015. Psychrophilic fungi from the world's roof. Persoonia 34:100-112. Available from: https://doi.org/10.3767/003158515x685878

Wang M, Tian J, Xiang M, Liu X. 2017. Living strategy of cold-adapted fungi with the reference to several representative species. Mycology 8(3):178-188. Available from: https://doi.org/10.1080/21501203.2017.1370429

Wang Y-X, Bowersox SS. 2000. Analgesic Properties of Ziconotide, a Selective Blocker of N-Type Neuronal Calcium Channels. CNS Drug Reviews 6(1):1-20. Available from: https://doi.org/10.1111/j.1527-3458.2000.tb00134.x

Watanabe K, Emoto N, Sunohara M, Kawakami M, Kage H, Nagase T, Ohishi N, Takai D. 2010. Treatment of PCR products with exonuclease I and heat-labile alkaline phosphatase improves the visibility of combined bisulfite restriction analysis. Biochem Biophys Res Commun 399(3):422-4. Available from: https://doi.org/10.1016/j.bbrc.2010.07.093

Wenger AM, Peluso P, Rowell WJ, Chang P-C, Hall RJ, Concepcion GT, Ebler J, Fungtammasan A, Kolesnikov A, Olson ND et al. . 2019. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nat Biotechnol 37(10):1155-1162. Available from: https://doi.org/10.1038/s41587-019-0217-9

Wetzel J, Kingsford C, Pop M. 2011. Assessing the benefits of using mate-pairs to resolve repeats in de novo short-read prokaryotic assemblies. BMC Bioinformatics 12(1):95. Available from: https://doi.org/10.1186/1471-2105-12-95

Weymouth-Wilson AC. 1997. The role of carbohydrates in biologically active natural products. Natural Product Reports 14(2):99-110. Available from: https://doi.org/10.1039/NP9971400099

Wikmark O-G, Brautaset T, Z. Agapito-Tenfen S, Okoli A, Myhr A, Binimelis R, Ching L. 2016. Synthetic biology: biosafety and contribution to addressing societal challenges. 'Available from:' https://doi.org/10.13140/RG.2.2.29987.25121

Wisecaver JH, Slot JC, Rokas A. 2014. The Evolution of Fungal Metabolic Pathways. PLOS Genetics 10(12):e1004816. Available from: https://doi.org/10.1371/journal.pgen.1004816

Wishart DS. 2008. Metabolomics: applications to food science and nutrition research. Trends in Food Science & Technology 19(9):482-493. Available from: https://doi.org/10.1016/j.tifs.2008.03.003

World Health Organization. The top 10 causes of death [Internet]. [cited 29/04 2021]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

WoRMS Editorial Board. World Register of Marine Species (WoRMS) [Internet]. VLIZ. 'Available from:' https://doi.org/10.14284/170

Wu B, Hussain M, Zhang W, Stadler M, Liu X, Xiang M. 2019. Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. Mycology 10(3):127-140. Available from: https://doi.org/10.1080/21501203.2019.1614106

Wu B, Oesker V, Wiese J, Schmaljohann R, Imhoff J. 2014. Two New Antibiotic Pyridones Produced by a Marine Fungus, *Trichoderma* sp. Strain MF106. Marine Drugs 12(3):1208. Available from: https://doi.org/10.3390/md12031208

Yandell M, Ence D. 2012. A beginner's guide to eukaryotic genome annotation. Nature Reviews Genetics 13:329-42. Available from: https://doi.org/10.1038/nrg3174

Yang JY, Sanchez LM, Rath CM, Liu X, Boudreau PD, Bruns N, Glukhov E, Wodtke A, De Felicio R, Fenner A et al. . 2013. Molecular networking as a dereplication strategy. Journal of Natural Products 76(9):1686-1699. Available from: https://doi.org/10.1021/np400413s

Youssef NH, Couger MB, Struchtemeyer CG, Liggenstoffer AS, Prade RA, Najar FZ, Atiyeh HK, Wilkins MR, Elshahed MS. 2013. The genome of the anaerobic fungus *Orpinomyces* sp. strain C1A reveals the unique evolutionary history of a remarkable plant biomass degrader. Applied and Environmental Microbiology 79(15):4620-34. Available from: https://doi.org/10.1128/aem.00821-13

Zerbino DR, Birney E. 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Research 18(5):821-829. Available from: https://doi.org/10.1101/gr.074492.107

Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y. 2018. DbCAN2: A meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Research 46:W95-W101. Available from: https://doi.org/10.1093/nar/gky418

Zhang T, Yao Y-F. 2015. Endophytic Fungal Communities Associated with Vascular Plants in the High Arctic Zone Are Highly Diverse and Host-Plant Specific. PLoS ONE 10(6):e0130051. Available from: https://doi.org/10.1371/journal.pone.0130051

Zhang X, Hindra, Elliot MA. 2019. Unlocking the trove of metabolic treasures: activating silent biosynthetic gene clusters in bacteria and fungi. Current Opinion in Microbiology 51:9-15. Available from: https://doi.org/10.1016/j.mib.2019.03.003

Zhang Y, Han T, Ming Q, Wu L, Rahman K, Qin L. 2012. Alkaloids produced by endophytic fungi: a review. Natural Product Communications 7(7):963-8. Available from: https://doi.org/10.1177/1934578X1200700742

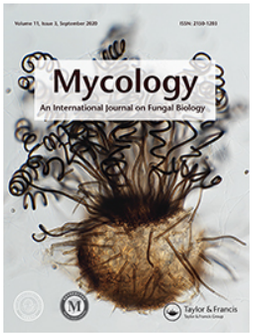
Zhdanova NN, Zakharchenko VA, Vember VV, Nakonechnaya LT. 2000. Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged

nuclear reactor. Mycological Research 104(12):1421-1426. Available from: https://doi.org/10.1017/S0953756200002756

Ziemert N, Alanjary M, Weber T. 2016. The evolution of genome mining in microbes - a review. Natural Product Reports 33(8):988-1005. Available from: https://doi.org/10.1039/c6np00025h

Zuccaro A, Summerbell RC, Gams W, Schroers H-J, Mitchell JI. 2004. A new *Acremonium* species associated with *Fucus* spp., and its affinity with a phylogenetically distinct marine *Emericellopsis* clade. Studies in Mycology 50:283-297. Available from: https://www.studiesinmycology.org/sim/Sim50/027-A_new_Acremonium_species_associated_with_Fucus_spp._and_its_affinity_with_a_phylogenetically_distinct_marine_Emericellopsis_clade.pdf

Paper 1



Mycology
An International Journal on Fungal Biology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tmyc20

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To cite this article: Ole Christian Hagestad , Jeanette H. Andersen , Bjørn Altermark , Espen Hansen & Teppo Rämä (2020) Cultivable marine fungi from the Arctic Archipelago of Svalbard and their antibacterial activity, Mycology, 11:3, 230-242, DOI: 10.1080/21501203.2019.1708492

To link to this article: https://doi.org/10.1080/21501203.2019.1708492

MYCOLOGY
2020, VOL. 11, NO. 3, 230–242
https://doi.org/10.1080/21501203.2019.1708492

Cultivable marine fungi from the Arctic Archipelago of Svalbard and their antibacterial activity

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ABSTRACT

During a research cruise in 2016, we isolated fungi from sediments, seawater, driftwood, fruiting bodies, and macroalgae using three different media to assess species richness and potential bioactivity of cultivable marine fungi in the High Arctic region. Ten stations from the Svalbard archipelago (73–80 °N, 18–31 °E) were investigated and 33 fungal isolates were obtained. These grouped into 22 operational taxonomic units (OTUs) using nuc rDNA internal transcribed spacer regions (ITS1-5.8S-ITS2 = ITS) with acut-off set at 98% similarity. The taxonomic analysis showed that 17 OTUs belonged to Ascomycota, one to Basidiomycota, two to Mucoromycota and two were fungal-like organisms. The nuc rDNA V1-V5 regions of 18S (18S) and D1-D3 regions of 28S (28S) were sequenced from representative isolates of each OTU for comparison to GenBank sequences. Isolates of Lulworthiales and Eurotiales were the most abundant, with seven isolates each. Among the 22 OTUs, nine represent potentially undescribed species based on low similarity to GenBank sequences and 10 isolates showed inhibitory activity against Gram-positive bacteria in an agar diffusion plug assay. These results show promise for the Arctic region as asource of novel marine fungi with the ability to produce bioactive secondary metabolites with antibacterial properties.

ARTICLE HISTORY
Received 7 August 2019
Accepted 1 December 2019

KEYWORDS
Agar plug diffusion assay; Barents Sea; bioactivity; DNA barcoding; Lulworthiales; molecular phylogeny

Introduction

Terrestrial habitats have been extensively explored for fungi, resulting in discoveries of species able to produce antibiotic, anticancer, antifungal, and immunomodulating compounds, among others (Bills and Gloer 2016). However, marine fungi have, to a large degree been neglected by bioprospectors and remain a potential source of novel compounds (Imhoff 2016). This is reflected by the large number of undescribed species that frequently appear in environmental samples and the chemical diversity that is still being uncovered (Richards et al. 2011, 2015; Jeffries Thomas et al. 2016; Ji and Wang 2016; Rämä et al. 2016; Hassett et al. 2017; Reich and Labes 2017). Studies on bioactive compounds from marine fungi have mainly focused on fungi from tropic and temperate regions. These fungi are most frequently isolated from specific hosts, such as mangrove trees, sponges, algae, and corals (Debbab et al. 2010; Jones and Pang 2012; Thatoi et al. 2013; Yarden 2014; Bajpai 2016; Pang et al. 2016; Sridhar 2017). However, the Arctic remains underexplored compared to other regions with a relatively low number of studies on marine fungi (Shearer et al. 2007; Imhoff 2016; Tisthammer et al. 2016; Rämä et al. 2017; Hassett et al. 2019).

The low number of studies on Arctic marine fungi might be explained by varying sea ice conditions and remoteness of the Arctic making it difficult and expensive to access study locations. A couple of studies have explored the fungal diversity in the polar regions using both cultivation and metagenomics approaches. Metabarcoding studies show a large marine fungal diversity, reporting hundreds to thousands of OTUs (Zhang et al. 2015; Comeau et al. 2016; Rämä et al. 2016; Tisthammer et al. 2016). Of these, 10–30% remain unidentified to order level within the Fungi kingdom, indicating a high-level richness of putatively undescribed species in the Arctic. Comeau et al. (2016) were also able to show that the frequency of certain fungal sequences increased from temperate to Arctic waters, indicating that some species or strains are specifically adapted to the Arctic environment.

Even though metabarcoding is an efficient tool for studying taxonomic diversity, it reveals nothing about

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Supplemental data for this article can be accessed here.

Published online 27 Dec 2019

the ability of a fungi to produce secondary metabolites. A review of studies on marine fungi in the Arctic found that only 13 studies investigated cultivable marine fungi from the Arctic, none of these investigated potential secondary metabolites from the fungi (Rämä et al. 2017). A review of bioactive fungal isolates from polar regions found that most studies were from soil samples, freshwater ponds and lakes, or exclusively Antarctic marine sources; no papers were found reporting bioactivity from any Arctic marine fungal sources (Lo Giudice and Fani 2016). We have found three papers reporting metabolites from Arctic marine fungi, all of them isolated from marine sediments. *Trichoderma* sp. strain MF106 from the Greenland Sea showed activity towards *Staphylococcus epidermidis* (Wu et al. 2014). Metabolites isolated from *Tolypocladium* sp. and *Mortierella* sp. from Frobisher Bay, Nunavut, Canada did not exhibit any significant antibacterial or cytotoxic activity (Grunwald et al. 2016, 2017). These are the only papers reporting of bioactivity from Arctic marine fungal sources. With results from only three isolates published in scientific articles, there is a severe lack of knowledge about bioactivity of Arctic marine fungi.

The aim of this study was to isolate and identify fungi from different substrates in the Arctic. In addition, the potential of the isolates to produce secondary metabolites was assessed by examining their antibacterial activity against five common human pathogens in an agar plug diffusion assay.

Materials and methods

Sampling and isolation of marine fungi

Samples were collected during a bioprospecting cruise with R/V Helmer Hansen around Svalbard in September 2016 (see Supplementary figure and Table 1 for exact locations and metadata). In addition to fruiting bodies, four different microhabitats were sampled: marine macroalgae, driftwood, sediments, and seawater. Marine macroalgae were collected from intertidal sites and were handpicked into sterile bags. Algal samples were surface sterilised by cutting the material into pieces of approximately 1 × 1 cm and dipping them into 70% ethanol for 30 seconds before rinsing them in autoclaved MilliQ water. Two to three pieces were then plated onto one 0.4% malt extract agar prepared with artificial seawater (ASMEA), corn meal agar in artificial seawater (ASCMA) and 1% dried and homogenised *Ascophyllum nodosum* in artificial seawater (1.0ASAsco) plates containing 30 µg/mL streptomycin and 30 µg/mL tetracycline. Driftwood samples, obtained from the intertidal zone or bottom trawl, were prepared following the procedure from Rämä et al. (2014) by removing the surface of the wood with a sterile knife and then cutting out 0.5 × 1 cm pieces. Two to three pieces were plated on each type of plate. Fruiting bodies (sporocarps) from driftwood were moistened with seawater and let dry until spores were discharged and then transferred to agar plates. Sediment samples were collected using a Van Veen grab. The surface of the material in the grab was removed using a sterile knife and sediment was scooped using a sterilised knife into a Petri dish that was closed immediately afterwards. An inoculation loop was used to streak out the sediment onto the agar plates. *In-situ* culturing plates were made by pouring inoculated agar into a mould and covering it with barrier membranes which only allows small molecules to pass through. These plates were inoculated in a seawater pool with constant seawater flow on the deck of the boat during the cruise. The cell suspension for the *in-situ* agar inoculum was sampled in a sterile Duran flask on a cruise to approximately the same area (76°15′27.5″N, 29°51′35.0″E and 5 m depth) in May 2016, was stored at +2°C and used undiluted in September. This method is inspired by the technique described by Nichols et al. (2010) and Berdy et al. (2017).

Once fungal mycelium was visible and had grown out from the substrate it was transferred to a new agar plate until an axenic culture was obtained. The subculture-media did not contain antibiotics. All incubations were done at 4–10.

Sanger sequencing and OTU clustering

A small piece of agar containing mycelium was transferred to an Eppendorf tube and 100 µl of autoclaved MilliQ water was added, followed by vigorous vortexing. For the PCR reaction, 1 µl of this material was used as a template. DreamTaq Green PCR Master Mix (2X) was used together with the different primer sets (Supplementary table 2). The barcode region sequenced was the nuc internal transcribed spacer

rDNA (ITS1-5.8S-ITS2 = ITS) using primers ITS5/ITS4 (White et al. 1990), with a backup set ITS3(White et al. 1990)/LR0Ri (Inverted LR0R sequence) for ITS2.

Successful PCRs were purified using either QIAquick PCR purification kit, ExoSAP-IT or A'SAP PCR clean-up treatment according to the manufacturer's manual. The purified PCR products were then prepared for two directional Sanger sequencing reaction using BigDye3.1 and a PCR program of 30 cycles at 95 °C for 30 seconds, 47 °C for 10 seconds and 72 °C for 1 minute. The sequencing was performed by the sequencing platform at the University Hospital of North Norway utilising Applied Biosystems 3130xl Genetic Analyser (Life Technologies/Applied Biosystems). The returned chromatograms were imported into Geneious v10.2.3 (https://www.geneious.com/), trimmed to 0.05 error probability, primer regions removed, assembled into consensus sequences and proofread according to guidelines proposed by Nilsson et al. (2012).

ITS sequences were trimmed to the same start and end before they were aligned using MAFFT plugin v7.388 in Geneious v10.2.3 using E-INS-I algorithm with scoring matrix PAM200 (Katoh et al. 2002; Katoh and Standley 2013). The aligned sequences were then used to cluster sequences into OTUs using MOTHUR v.1.35.1 at 98% ITS similarity cut-off (Schloss et al. 2009). The full length sequences of each cluster were queried against Rfam for intron detection (Bateman et al. 2017; Kalvari et al. 2018). A list of the isolates and OTU division can be found in Supplementary table 3.

Representative sequences for each OTU were sequenced for two additional barcode regions. The nuc rDNA covering V1-V5 regions of 18S (18S) using primers NS1/NS4 (White et al. 1990), and nuc rDNA covering D1-D3 regions of 28S (28S) using primers LR0R/LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1994).

The PCR reaction parameters for ITS and 28S were an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 47 °C for 30 seconds, elongation at 72 °C for 1 minute. A final elongation step of 72 °C for 10 minutes was carried out followed by a hold at 4 °C indefinitely. 18S amplification only differed in annealing temperature of 42 °C. Success of the amplification was checked by gel electrophoresis of the samples on 1% Tris-Borate-EDTA agar gel infused with 0.1‰ GelRed. A list of accession numbers for sequences generated in this study can be found in Supplementary table 4.

OTU identification and phylogenetic analyses

Representative sequences of each OTU were submitted to GenBank for a BLAST search with an ENTREZ query string limiting hits to fungal and fungal-like origin and excluding uncultured, metagenomic, and environmental samples to limit the numbers of hits to sequences of known origin of relative high quality (Souvorov et al. 2006; Raja et al. 2017). Sequences for each locus were downloaded from GenBank and aligned. If available, type strains with sequences from all marker regions (18S, ITS and 28S) were preferably downloaded. Strains were only included if two of the three loci were available in GenBank. A list of reference sequence accession numbers is listed in Supplementary table 5.

Alignment of the sequences were done in Geneious 10.2.3, using MAFFT v.7.388 for each separate barcode region before they were concatenated to a single sequence. The sequences were aligned using different algorithms for different markers, E-INS-I for ITS and 28S, and G-INS-I for 18S. E-INS-I considers multiple conserved domains interspaced with long gaps or regions harder to align. G-INS-I considers a global homology between the sequences. The scoring matrices used were PAM200 for ITS, and PAM100 for 18S and 28S to account for difference in sequence similarity.

The multiloci dataset of 3148 nt was partitioned using PartitionFinder v2.1.1 (Lanfear et al. 2017) and jModelTest v2.1.10 (Posada 2008) was used to find the best substitution model and priors selected by the corrected Akaike information criterion (AICc) and Bayesian information criterion (BIC). The three suggested partitions were 18S and 5.8S, ITS1 and ITS2, and 28S. The suggested model GTR+I + G for 18S and 28S, and SYM+I + G for ITS was changed to GTR+G to avoid simultaneous optimisation of both gamma and the proportion of invariant sites as these parameters correlate strongly (Jia et al. 2014). Instead, six gamma categories was used to accommodate for slowly

evolving sites. A prior mean branch length, $\overline{brl}$ was first estimated using maximum likelihood. The prior mean was used to set the parameters for the exponential prior exp (λ) where $\lambda = \frac{1}{\overline{brl}}$.

The resulting alignments, partitions and models were used for Maximum likelihood and Bayesian tree construction using RAxML v8.2.11 in Geneious (Stamatakis 2014) and MrBayes v.3.2.6 (MPI parallel version with Beagle) (Ronquist et al. 2004, 2012). RAxML was run with the following settings: Substitution model GTR gamma, rapid bootstrapping and search for best scoring ML tree with 2000 bootstrap replicates.

The nexus file with alignments and MrBayes command block can be found in the supplementary data. Tracer v1.7.1 was used to assess the convergence of the runs (Rambaut et al. 2018). The optimisation of proposal mechanisms was done according to Ronquist et al. (2009). The analysis was run until the standard deviation of the split frequency stabilised at a value or dropped below 0.05. The command blocks used and resulting trees have been deposited to Mendeley Data and are available at http://dx.doi.org/10.17632/52dkhy5xsb.1 (Hagestad et al. 2019).

Agar plug diffusion assay

For the diffusion assay, 5 or 6 agar plugs (diameter 8 mm) from different samples with actively growing mycelium were placed evenly spaced in an empty petri dish. Mueller-Hinton (MH) or brain heart infusion (BHI)-agar was infused with overnight culture (100 µL in 100 mL) of *Staphylococcus aureus* (ATCC 25923), *Streptococcus agalactiae* (ATCC 12386), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853) or *Escherichia coli* (ATCC 25922), the latter two using BHI-agar. The inoculated agar was poured on the petri dishes until the agar reached the height of the plugs. The plates were then incubated for approximately 24 hours at 37 °C and the diameter of inhibition zones was measured.

Results

Richness and phylogenetic diversity of isolated fungi

A total of 197 isolates were analysed from algae, sediment, driftwood, fruiting bodies, and seawater. Sequences that failed to assemble or were truncated were excluded from further analysis. Most of the sequences had >80% high quality base calls. In cases where samples had identical sequences and came from the same isolation plate, only one was selected as an isolate to prevent isolation of the same fungus several times. This finally resulted in 33 different isolates. Eleven isolates came from littoral macroalgae, followed by benthic driftwood (9), benthic sediments (6), fungal fruiting bodies from benthic driftwood (4), and *in-situ* cultures from seawater (3) (Figure 1(a)).

Clustering of similar ITS sequences grouped the isolates into 22 unique OTUs (Figure 1(a)). Seven OTUs were found in algae, eight in driftwood, six in sediments, four in fruiting bodies, and three in water. The OTUs were generally found in only one sample and only one substrate with a few exceptions. *Penicillium* sp. OTU3 and *Mortierella* sp. OTU7 were found in algae, driftwood, fruiting bodies, and sediments, indicating a wide substrate preference. The distribution of the specific OTUs and isolates among the substrates can be seen in Figure 2. The most speciose substrate was driftwood, which yielded 8 OTUs. Some OTUs were found in multiple locations, e.g. Lulworthiaceae sp. OTU1 and *Geomyces* sp. OTU4. A complete list of representative isolates is shown in Supplementary table 4.

The isolates had some identifiable morphological traits. All but one OTU were filamentous. One basidiomycete, *Glaciozyma* sp. OTU17, was able to change between a filamentous and a unicellular yeast form. *Mucor, Penicillium* spp., *Oidiodendron*, and *Tolypocladium* produced spores, presumably conidiospores. *Lulworthia* sp. OTU14 and OTU21 were confirmed to belong to *Lulworthia* based on isolated ascospores from fruiting bodies on driftwood that germinated and were used to establish mycelial cultures. Isolates were observed to have different morphological characteristics (colour, growth pattern) on different media during cultivation (Figure 1(c)).

The isolates spanned four divisions (three fungal), seven classes, 10 orders, 14 families, and 15 genera. The pairwise identity with reference sequences from GenBank ranged from 78.9–100%, with identity being highest in species also known from terrestrial sources (*Penicillium, Mucor, Geomyces* and *Mortierella*) and lowest in marine species and poorly studied genera (Lulworthiales and Oomycota). Based on identity in GenBank, nine isolates seemed to represent

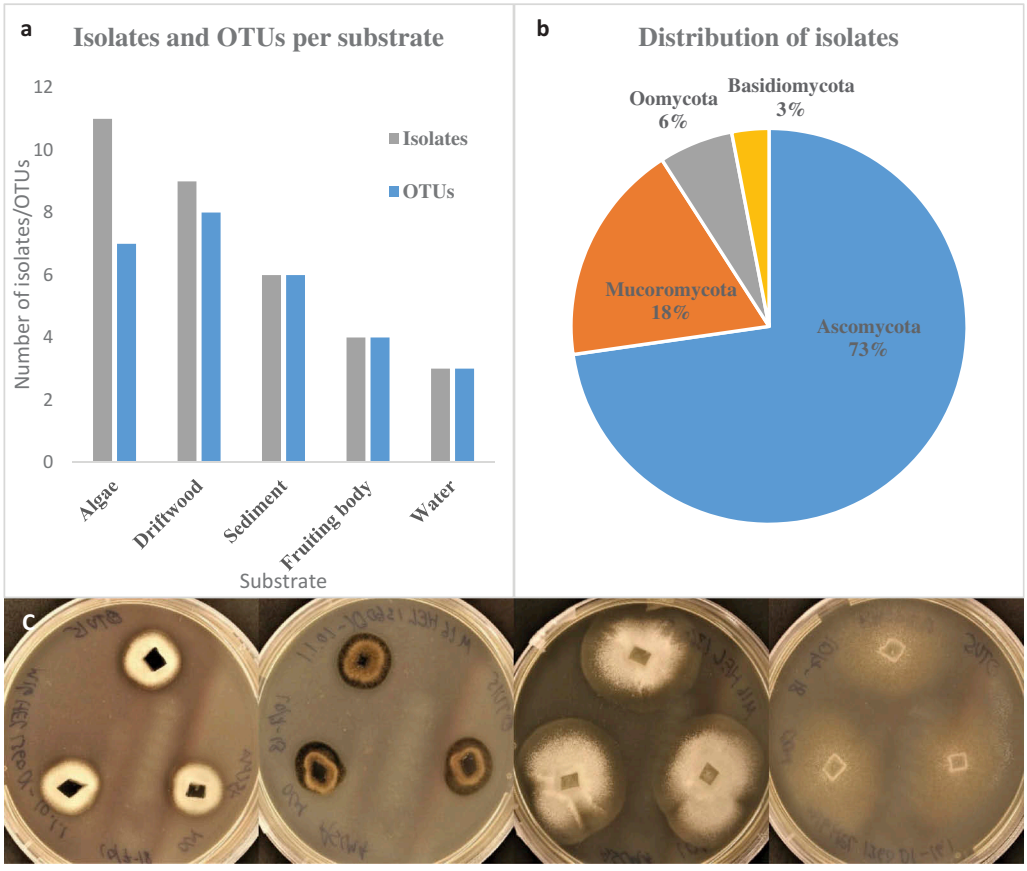

Figure 1. (a) Overview over substrate source and number of isolates and OTUs. (b) Phylum level distribution of isolates. (c) Illustration of two different isolates on two media. From right: *Mytilinidion* sp. OTU15 on ASCMA and ASMEA, *Pseudeurotium* sp. OTU5 on ASCMA and ASMEA.

undescribed species with ITS similarity of less than 96% and LSU similarity ranging from 89–99% (Supplementary Table 4). Most of the isolates belonged to Ascomycota (73%), followed by Mucoromycota (18%), fungal-like organisms (Oomycota) (6%), and finally Basidiomycota (3%) (Figure 1(b)).

Both maximum likelihood and Bayesian inference arrived at similar topologies and relative branch lengths using all three sequenced loci, contrasting in nodes at the class level and placement of terminal nodes in *Oidiodendron, Penicillium, Cosmospora*, and *Tolypocladium* (Figures 3 and 4). The deep nodes were generally well supported as well as nodes closer to the terminal branches.

In most cases the OTUs were placed within monophyletic clades, but there were a few exceptions. The fungal-like organisms in this study were placed outside the referenced genera within Saprolegniales. Lulworthiaceae sp. OTU19, Lulworthiaceae sp. OTU10, and Lulworthiaceae sp. OTU1 were placed outside of the genus *Lulworthia*, together with sequences of *Lulworthia, Zalerion, Lulwoana*, and *Haloguignardia* indicating that more thorough work on phylogeny of marine fungi within Lulworthiales is needed for proper placement.

Antibacterial activity

Ten of the 20 tested isolates showed activities against at least one human pathogenic bacterium (Table 1). Activities were observed against the Gram-positive bacteria, *S. aureus, E. faecalis*, and *S. agalactiae*. Eurotiomycetes and Leotiomycetes gave the strongest antibacterial activity. *Acremonium* sp. OTU5 and *Mortierella* sp. OTU7 of Sordariomycetes and Mortierellomycetes, did also give strong antibacterial activity. Most of the growth inhibition was observed against *S. aureus* and *S. agalactiae*. However, *Pseudeurotium* sp. OTU5 and *Geomyces* sp. OTU4 were active against *E. faecalis*. *Penicillium* sp. OTU13 was noted for having high and specific activity against *S. agalactiae*,

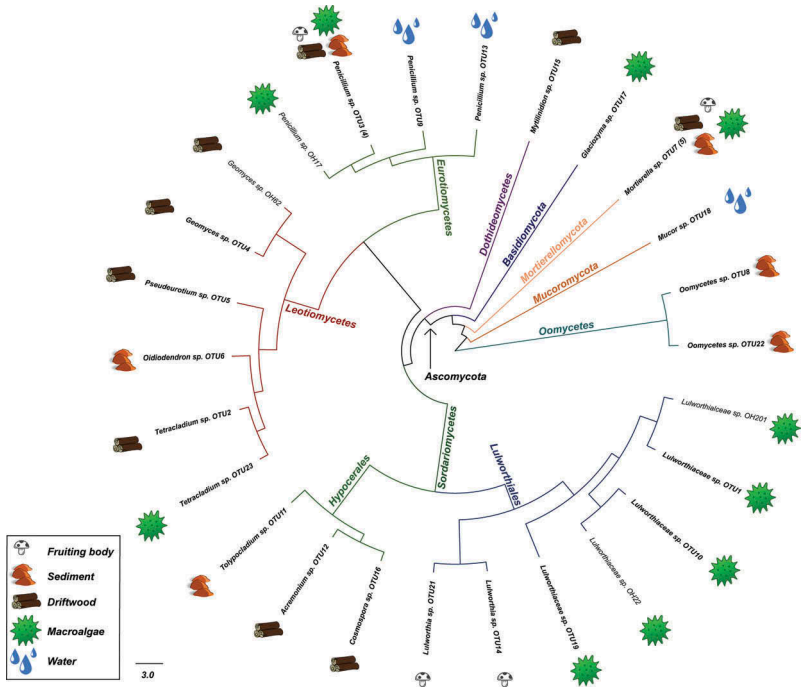

Figure 2. Graphical representation of the 33 isolates in this study based on a Maximum likelihood tree constructed from the ITS sequences. Taxonomic ranks are colour coded in branches. Substrates are marked with icons along the edge after isolate name. Representative isolates names are in bold and numbers of identical sequences represented by the entry in brackets. Scale bar show nucleotide substitutions per site.

many times higher than any other isolate. *Mortierella* sp. OTU7 was the only isolate in this study outside of Ascomycota with activity towards the pathogenic bacteria. The activity against *S. agalactiae* and *E. faecalis* were only present when cultured on ASCMA, with the exception of *Penicillium* sp. OTU13 which had activity towards *S. agalactiae* when cultured on ASMEA.

Discussion

Fungi remain one of the most understudied group of microbial organisms in the ocean and they represent a good source for novel biochemistry (Liberra and Lindequist 1995; Richards et al. 2011; Berlinck 2013; Wang et al. 2015). The relatively few studies on cultivable fungi undertaken in the Arctic underlines the importance of mapping and exploring the species richness and capabilities of marine fungi in this region (Rämä et al. 2017; Luo et al. 2019). The present study provides the first insight into the bioactivity of cultivable marine fungi isolated from diverse substrates from cold waters of the Arctic region.

We found 22 unique OTUs (98% ITS similarity) from the 33 isolates originating from substrates collected at 10 stations. Ascomycota was the most abundant division, accounting for 73% of the isolates. The relative abundance of Ascomycota compared to other divisions is similar to what has been described in other culture-dependent studies on marine fungi from for example driftwood and sediments (Bubnova 2010; Pang et al. 2011; Nagano and Nagahama 2012; Rämä et al. 2014; Blanchette et al. 2016; Bubnova and Nikitin 2017). The number of isolates obtained in culture dependent studies varies considerably, from as low as eight to as high as 577 (Singh et al. 2012; Rämä et al. 2014; Blanchette et al. 2016). However, direct comparison of the different articles is difficult due to differences in study focus, methods, isolation substrate, media, and geographical area.

It is interesting to note that although fruiting bodies collected from driftwood belonged to the Lulworthiales, none of the driftwood samples themselves yielded Lulworthiales isolates. This could be due to low number of driftwood samples, or that other species outgrew Lulworthiales on the isolation plate. Generally, isolates of Lulworthiales grew slowly compared to other isolates.

Despite the modest number of isolates described in this study, the species richness is high, spanning four different phyla and 10 different orders, providing

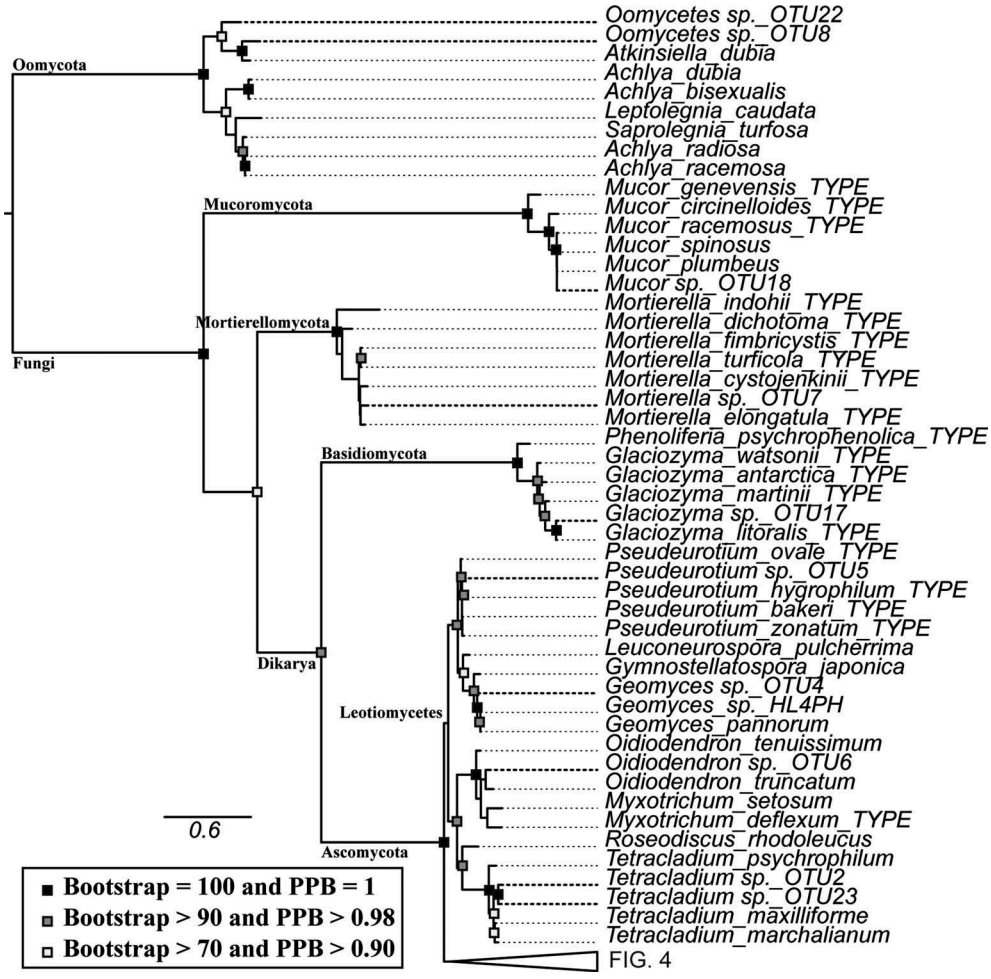

Figure 3. Taxonomic placement of representative isolates used in the study (bold), showing portion close to the base of the tree. RAxML tree with bootstrap and posterior probability support values where nodes are identical with Bayesian analysis marked with filled boxes. Phyla, order, class and family is noted on respective branches. Accession numbers of reference sequences can be found in Supplemental Table 5.

isolates with both high and low similarities to known sequences in GenBank. Based on sequence similarity compared to GenBank sequences of the three loci, nine of the isolates seem to be novel species. Whether the novel sequences represent novel species remains unclear, as many species and old type specimens are not properly sequenced for the different rDNA loci used in this paper. Most of the reference sequences in GenBank have been deposited after 2007 and most species have been added after 2014. Curated fungal databases have few sequences of marine origin, and only 12% of all marine fungal genera are represented in RefSeq (Hassett et al. 2019). Other databases have similar deficiencies of marine representation and provides an equally poor source of identification and comparison of the isolates. When isolates have a poor hit score, identification becomes much more difficult as it is hard to determine the proper taxa. However, with multiple loci it is possible to ascertain a proper taxonomic placement, at least to family or genus level by comparing the similarity of the loci to reference sequences.

During manual OTU control after running MOTHUR it was seen that *Tetracladium* OTU2 and OTU23 were included in the same OTU, despite a 285 bp insert at the end of 18S rDNA in OTU23. This insert differentiated OTU23 markedly from OTU2, although the ITS sequences themselves had only five parsimony-informative sites over 556 bp, making them more than 99.1% similar. A similar insertion of approximately 335 bp was found in *Mytilinidion* sp. OTU15 in the same region. These 18S rDNA inserts of about 300 bp were determined to be group 1 introns by Rfam and are known to occur at the end of 18S rDNA

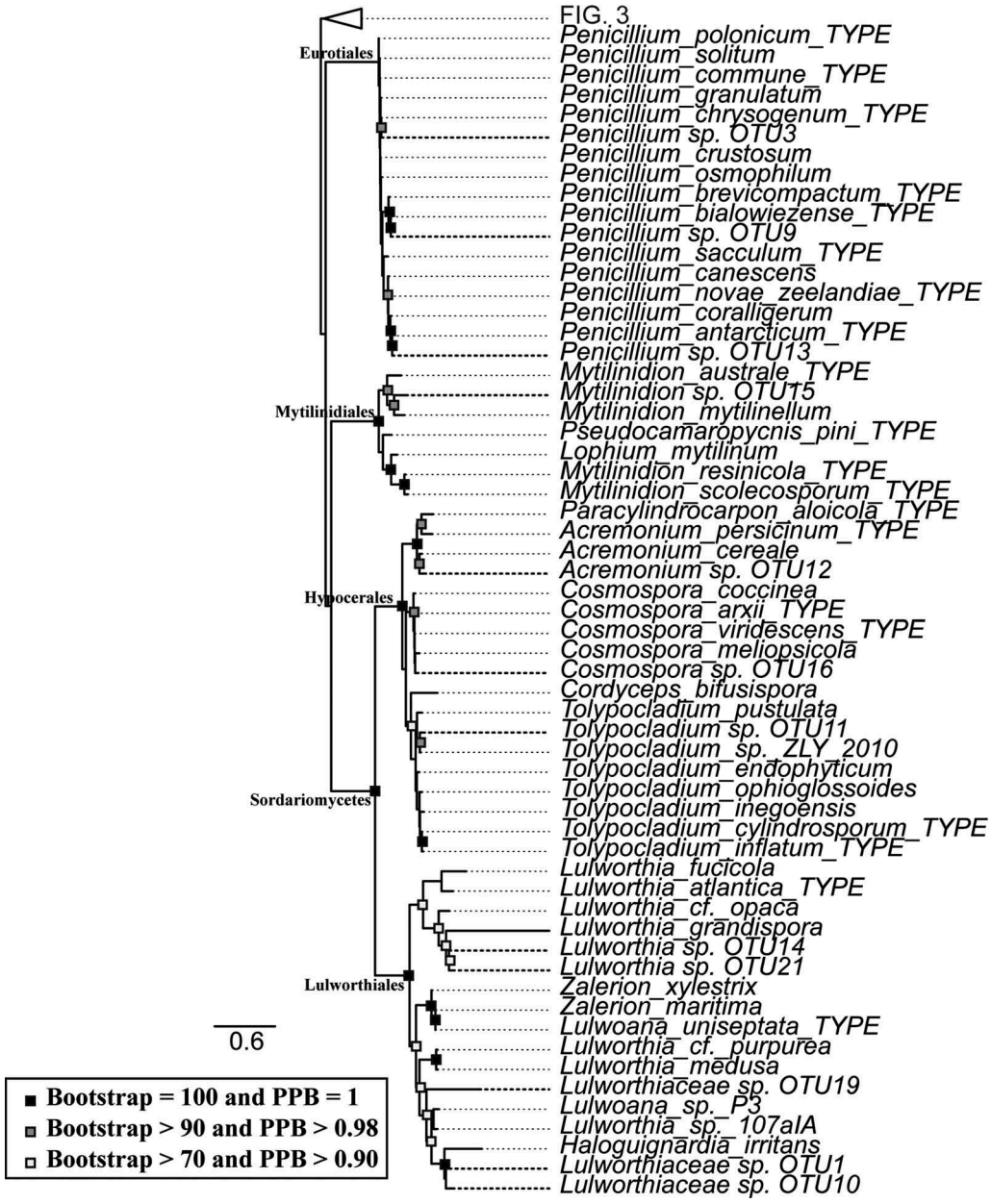

Figure 4. Taxonomic placement of representative isolates used in the study (bold), with base of the tree collapsed. RAxML tree with bootstrap and posterior probability support values where nodes are identical with Bayesian analysis marked with filled boxes. Phyla, order, class and family is noted on respective branches. Accession numbers of reference sequences can be found in Supplemental Table 5.

(Rogers et al. 1993; Gargas et al. 1995; Hibbett 1996; Taylor et al. 2016). The ITS sequences of *Lulworthia* sp. OTU14 and OTU21 were only 96.3% similar, but they had the same sequence gaps, and differed only in some G-A and C-T conversions that are common polymerase errors and could represent the same OTU when 18S and 28S rDNA sequences are considered (Potapov and Ong 2017). In addition to this, the ribosomal DNA exists in multiple copies with inherent sequence variations and can also lead to ITS sequence variation within a single specimen (Simon and Weiß 2008). Our observations support the conclusion that ITS is not a perfect barcoding region alone and highlights that ITS similarity and dissimilarity can be misleading and that manual control is important where applicable (Schoch et al. 2012).

Both maximum likelihood and Bayesian phylogenetic trees provided a consensus for the classification of the sequences to the determined level of taxonomy. The long terminal branches within

Table 1. Results from agar plug diffusion assay given as average inhibition zone diameter in mm. Average is based only on active plugs. Plugs used were 8 mm in diameter. Numbers in superscript are numbers of active plugs out of total plugs tested for a specific isolate on a specific media. There was no activity against gram negative bacteria (not shown).

	ASCMA[a]			ASMEA[b]		
Name	*S. aureus*	*E. faecalis*	*S. agalactiae*	*S. aureus*	*E. faecalis*	*S. agalactiae*
Lulworthiaceae sp. OTU1	–	–	–	–	–	–
Lulworthiaceae sp. OTU10	–	–	–	–	–	–
Lulworthia sp. OTU14	–	–	–	–	–	–
Lulworthia sp. OTU21	–	–	–	–	–	–
Lulworthiaceae sp. OTU19	–	–	–	n.d.	n.d.	n.d.
Acremonium sp. OTU12	–	–	$9^{1/3}$	$10.5^{2/4}$	–	–
Cosmospora sp. OTU16	–	–	–	$9^{1/4}$	–	–
Tolypocladium sp. OTU11	–	–	–	–	–	–
Oidiodendron sp. OTU6	–	–	–	–	–	–
Pseudeurotium sp. OTU5	$12^{1/3}$	$10^{1/3}$	–	–	–	–
Tetracladium sp. OTU23	–	–	–	–	–	–
Tetracladium sp. OTU2	–	–	–	$9^{2/5}$	–	–
Geomyces sp. OTU4	$14^{3/4}$	$12^{1/4}$	$10^{1/4}$	$12.3^{3/3}$	–	–
Penicillium sp. OTU3	$17^{1/4}$	–	$13^{2/4}$	$9^{3/3}$	–	–
Penicillium sp. OTU9	$11^{1/3}$	–	–	$11.7^{3/3}$	–	–
Penicillium sp. OTU13	$12^{1/3}$	–	$12.5^{2/3}$	$9^{3/3}$	–	$28^{3/3}$
Mytilinidion sp. OTU15	–	–	–	$11.3^{3/3}$	–	–
Glaciozyma sp. OTU17	–	–	–	–	–	–
Mortierella sp. OTU7	$12.5^{2/3}$	–	–	–	–	–
Mucor sp. OTU18	–	–	–	–	–	–

[a]Corn meal agar in artificial seawater
[b]0.4% Malt extract agar in artificial seawater
n.d. = no data | – = no activity

Lulworthiales, Mytilinidiales and fungal-like organism clades indicate novel species of poorly studied families or poor availability of reference sequences.

There were some higher rank taxa that were not recovered in this culture-dependent study, such as the phylum Chytridiomycota and yeast-like fungi in Ascomycota (Saccharomycetales), although these are known to frequently occur in the Arctic marine environment (Zhang et al. 2015; Hassett et al. 2017). The reason why these groups remained unrecovered is likely methodological, as different kinds of isolation and culturing techniques or media are needed to culture these (Sparrow 1960; Kutty and Philip 2008). We used methods that are known to capture a broad diversity of filamentous fungi in Dikarya and other taxa such as some fungal-like Oomycota. Testing the recovered diversity in antibacterial bioassays allowed us to give a preliminary account of the antibacterial activity of Arctic marine fungi.

Antibacterial activity

The agar plug diffusion assay was chosen for bioactivity screening as it is a relatively simple method to perform and can give an indication of isolates producing antibacterial compounds. In addition, fungi growing on solid media has been shown to produce higher amounts and more diverse bioactive molecules compared to liquid cultures (VanderMolen et al. 2013). The incubation period for the agar plug diffusion assay was relatively short, only 24 hours, possibly not allowing all fungi to properly respond to the presence of the bacteria. The incubation temperature (37 °C) was also high compared to the temperatures these fungi exist at in nature (less than 10 °C). At lower temperatures, such as room temperature, fast growing fungi quickly overgrew the plate before the pathogenic bacteria could grow. However, the agar plug diffusion assay provides a way to detect constitutively expressed molecules or molecules that are expressed during stress.

The only isolate with activity against all Gram-positive bacteria tested was *Geomyces* sp. OTU4 when cultured on ASCMA. The *Penicillium* OTUs on the other hand, had the overall strongest antimicrobial activity, with activity towards *S. aureus* and *S. agalactiae*, supporting the evidence of some genera in Eurotiales as potent producers of antimicrobial compounds (Richards 2011). The different *Penicillium* OTUs also had different antimicrobial activities. Species from the same genus within *Penicillium, Aspergillus*, and *Fusarium* are known to have widely different metabolic profiles (Bladt et al. 2013; Frisvad 2014; Nesic et al. 2014; Hasan et al. 2015; Wang et al. 2015). Ten different

Penicillium species have previously been challenged with different growth conditions in order to examine shifts in their metabolic profiles. The study showed that *Penicillium* species have highly variable secondary metabolite production, ranging from producing 4 to 34 different identified secondary metabolites. These metabolites were often species specific, among the identified metabolites only six were shared between some of the species, none were shared between all (Grijseels et al. 2017).

Another observation we made was that bioactivity changed over time. Even though a single isolate tested several times the same day provided the same inhibition zone against the same bacteria, the activity varied if tested a week, a month or several months later indicating that the expression changed over time. This difference in activity could also be due to difference in fungal biomass on the plugs as they had different times to grow between the samplings. This time-dependent difference in metabolism has been shown for different *Penicillium* species before (Khalil et al. 2014; Roullier et al. 2016).

Several of the tested OTUs showed altered antibacterial activity on the two different media. This was especially apparent in *Penicillium* sp. OTU13 that had an inhibition zone against *S. agalactiae* of 12.5 mm when cultured on ASCMA and 28 mm on ASMEA. On the other hand, the inhibition zone towards *S. aureus* decreased from 12 mm on ASCMA to 9 mm on ASMEA indicating that the activity towards *S. agalactiae* was specific. For some fungi, the alteration of growth media produced different selectivities in antibacterial activity, as seen with *Acremonium* sp. OTU12, with activity against *S. aureus* on ASCMA and on *S. agalactiae* on ASMEA. Grijseels et al. (2017) also reported that the species altered expression patterns when cultured in different culture media.

Bioactivity can be dependent on many factors such as culture medium, temperature, water activity, light, pH, salt content, culture time, and presence of small molecules (Sepcic et al. 2011; Hewage et al. 2014; Gubiani et al. 2016). Our results illustrate medium-dependent changes of bioactivity. This has also previously been shown by Overy et al. (2017) in a comprehensive study where the same ex-type strain of *Aspergillus aculeatus*, a halotolerant terrestrial fungus, produced distinct culture media-specific metabolites when grown with different osmotic pressures as well as laboratory (site) specific metabolites.

Some of the species isolated in this study have likely been isolated before in other studies if sequence similarity is considered. However, this does not mean that new discoveries from these isolates cannot be made. A different species, *Aspergillus flavus*, is a cosmopolitan species present all over the globe, and a thorough examination by Ramírez-Camejo et al. (2012) showed that the terrestrial and marine isolates of this species composed a single population. This, together with the results of Overy et al. (2017) shows that isolation of completely novel species is not necessary to uncover unique secondary metabolites, as these may be expressed just by culturing in a different laboratory environment. This highlights the importance of using the one strain, many compounds (OSMAC) approach to express secondary metabolites from cultured strains, and to use parameters from different habitats as inspiration for culture conditions.

This study shows that there is potential to make discoveries in the Arctic and that the research community should put more effort into identifying and characterising marine fungi and fungal metabolites from the Arctic to reduce the current knowledge gap.

Author Contributions

All authors conceived and designed the study. JH acquired funding. TR made initial isolations of fungal material. OH isolated axenic cultures with contributions from TR. OH carried out the experiments and analysis. OH interpreted the results with contributions from TR. BA, EH, JH and TR provided supervision. OH led the manuscript writing process. All authors provided critical feedback, helped shape the research and manuscript and approved the final version.

Disclosure statement

No potential conflict of interest was reported by the authors.

Geolocation information

The approximate midpoint of the stations in this study is 78° 13′ 57.6″N, 27° 46′ 11.3″Ø. The individual stations can be seen in Supplementary Figure 1 and location data can be found in Supplementary Table 1.

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References

Bajpai VK. 2016. Antimicrobial secondary metabolites from marine fungi: a mini review. Indian J Geo-Marine Sci. 45 (9):1067–1075.

Bateman A, Kalvari I, Argasinska J, Finn RD, Petrov AI, Quinones-Olvera N, Nawrocki EP, Rivas E, Eddy SR. 2017. Rfam 13.0: shifting to a genome-centric resource for non-coding RNA families. Nucleic Acids Res. 46(D1):D335–D342.

Berdy B, Spoering AL, Ling LL, Epstein SS. 2017. In situ cultivation of previously uncultivable microorganisms using the ichip. Nat Protoc. 12:2232.

Berlinck RGS. 2013. Review of marine fungi and fungal-like organisms. J Nat Prod. 76(10):2004–2004.

Bills GF, Gloer JB. 2016. Biologically active secondary metabolites from the fungi. Microbiol Spectr. 4(6):1087–1119.

Bladt T, Frisvad J, Knudsen P, Larsen T. 2013. Anticancer and antifungal compounds from *Aspergillus, Penicillium* and other filamentous fungi. Molecules. 18(9):11338.

Blanchette RA, Held BW, Hellmann L, Millman L, Büntgen U. 2016. Arctic driftwood reveals unexpectedly rich fungal diversity. Fungal Ecol. 23:58–65.

Bubnova EN. 2010. Fungal diversity in bottom sediments of the Kara Sea. Botanica Marina. 53(6):595.

Bubnova EN, Nikitin DA. 2017. Fungi in bottom sediments of the barents and Kara seas. Russ J Marine Biol. 43(5):400–406.

Comeau AM, Vincent WF, Bernier L, Lovejoy C. 2016. Novel chytrid lineages dominate fungal sequences in diverse marine and freshwater habitats [Article]. Sci Rep. 6:30120.

Debbab A, Aly AH, Lin WH, Proksch P. 2010. Bioactive compounds from marine bacteria and fungi. Microb Biotechnol. 3(5):544–563.

Frisvad JC. 2014. Taxonomy, chemodiversity, and chemoconsistency of *Aspergillus, Penicillium*, and *Talaromyces* species. Front Microbiol. 5:773.

Gargas A, DePriest PT, Taylor JW. 1995. Positions of multiple insertions in SSU rDNA of lichen-forming fungi. Mol Biol Evol. 12(2):208–218.

Grijseels S, Nielsen JC, Nielsen J, Larsen TO, Frisvad JC, Nielsen KF, Frandsen RJN, Workman M. 2017. Physiological characterization of secondary metabolite producing *Penicillium* cell factories. Fungal Biol Biotechnol. 4:8.

Grunwald AL, Berrué F, Overy DP, Kerr RG. 2016. Isolation of iqalisetins A and B from a Tolypocladium sp. isolated from marine sediment from Frobisher Bay in Canada's arctic. Can J Chem. 94(4):444–448.

Grunwald AL, Berrue F, Robertson AW, Overy DP, Kerr RG. 2017. Mortiamides A–D, Cyclic Heptapeptides from a Novel Mortierella sp. obtained from Frobisher Bay. J Nat Prod. 80 (10):2677–2683.

Gubiani JR, Habeck TR, Chapla VM, Silva GH, Bolzani VS, Araujo AR. 2016. One strain-many compounds (OSMAC) method for production of phenolic compounds using *Camarops* sp., an endophytic fungus from *Alibertia macrophylla* (Rubiaceae). Química Nova. 39:1221–1224.

Hagestad OC, Andersen JH, Altermark B, Hansen E, Rämä T. 2019. Data for: cultivable marine fungi from the Arctic Archipelago of Svalbard and their antibacterial activity. Mendeley Data. doi:10.17632/52dkhy5xsb.1.

Hasan S, Ansari MI, Ahmad A, Mishra M. 2015. Major bioactive metabolites from marine fungi: a review. Bioinformation. 11 (4):176–181.

Hassett BT, Ducluzeau A-L-L, Collins RE, Gradinger R. 2017. Spatial distribution of aquatic marine fungi across the western Arctic and sub-arctic. Environ Microbiol. 19(2):475–484.

Hassett BT, Vonnahme TR, Peng X, Jones EBG, Heuzé C. 2019. Global diversity and geography of planktonic marine fungi. Botanica Marina. doi:10.1515/bot-2018-0113.

Hewage RT, Aree T, Mahidol C, Ruchirawat S, Kittakoop P. 2014. One strain-many compounds (OSMAC) method for production of polyketides, azaphilones, and an isochromanone using the endophytic fungus Dothideomycete sp. Phytochemistry. 108:87–94. eng.

Hibbett DS. 1996. Phylogenetic evidence for horizontal transmission of group I introns in the nuclear ribosomal DNA of mushroom-forming fungi. Mol Biol Evol. 13 (7):903–917. eng.

Imhoff JF. 2016. Natural products from marine fungi—still an underrepresented resource. Mar Drugs. 14(1):19.

Jeffries Thomas C, Curlevski Nathalie J, Brown Mark V, Harrison Daniel P, Doblin Martina A, Petrou K, Ralph Peter J, Seymour Justin R. 2016. Partitioning of fungal assemblages across different marine habitats. Environ Microbiol Rep. 8 (2):235–238.

Ji NY, Wang BG. 2016. Mycochemistry of marine algicolous fungi. Fungal Divers. 80(1):301–342.

Jia F, Lo N, Ho SYW. 2014. The impact of modelling rate heterogeneity among sites on phylogenetic estimates of intraspecific evolutionary rates and timescales. PLoS One. 9 (5):e95722.

Jones EBG, Pang K-L. 2012. Tropical aquatic fungi. Biodivers Conserv. 21(9):2403–2423.

Kalvari I, Nawrocki EP, Argasinska J, Quinones-Olvera N, Finn RD, Bateman A, Petrov AI. 2018. Non-coding RNA analysis using the Rfam database. Curr Protocols in Bioinf. 62(1): e51.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30(14):3059–3066.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780.

Khalil ZG, Kalansuriya P, Capon RJ. 2014. Lipopolysaccharide (LPS) stimulation of fungal secondary metabolism. Mycology. 5(3):168–178.

Kutty SN, Philip R. 2008. Marine yeasts—a review. Yeast. 25 (7):465–483.

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol Biol Evol. 34(3):772–773. eng.

Liberra K, Lindequist U. 1995. Marine fungi–a prolific resource of biologically active natural products? Pharmazie. 50 (9):583–588. eng.

Lo Giudice A, Fani R. 2016. Antimicrobial potential of cold-adapted bacteria and fungi from polar regions. In: Rampelotto PH, editor. Biotechnology of extremophiles: advances and challenges. Cham: Springer International Publishing; p. 83–115.

Luo Y, Xu W, Luo Z-H, Pang K-L. 2019. Diversity and temperature adaptability of cultivable fungi in marine sediments from the Chukchi Sea. Botanica Marina. doi:10.1515/bot-2018-0119

Nagano Y, Nagahama T. 2012. Fungal diversity in deep-sea extreme environments. Fungal Ecol. 5(4):463–471.

Nesic K, Ivanovic S, Nesic V. 2014. Fusarial toxins: secondary metabolites of *Fusarium* fungi. Rev Environ Contam Toxicol. 228:101–120. eng.

Nichols D, Cahoon N, Trakhtenberg EM, Pham L, Mehta A, Belanger A, Kanigan T, Lewis K, Epstein SS. 2010. Use of ichip for high-throughput in situ cultivation of "uncultivable" microbial species. Appl Environ Microbiol. 76 (8):2445–2450. eng.

Nilsson RH, Tedersoo L, Abarenkov K, Ryberg M, Kristiansson E, Hartmann M, Schoch CL, Nylander JAA, Bergsten J, Porter TM, et al. 2012. Five simple guidelines for establishing basic authenticity and reliability of newly generated fungal ITS sequences. MycoKeys. 4:37–63. doi:10.3897/mycokeys.4.3606.

Overy D, Correa H, Roullier C, Chi W-C, Pang K-L, Rateb M, Ebel R, Shang Z, Capon R, Bills G, et al. 2017. Does osmotic stress affect natural product expression in fungi? Mar Drugs. 15(8):254.

Pang K-L, Chow RK, Chan C-W, Vrijmoed LP. 2011. Diversity and physiology of marine lignicolous fungi in Arctic waters: a preliminary account. Polar Res. 30(1):5859.

Pang K-L, Overy DP, Jones EBG, Calado M, Burgaud G, Walker AK, Johnson JA, Kerr RG, Cha H-J, Bills GF. 2016. 'Marine fungi' and 'marine-derived fungi' in natural product chemistry research: toward a new consensual definition. Fungal Biol Rev. 30(4):163–175.

Posada D. 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol. 25(7):1253–1256.

Potapov V, Ong JL. 2017. Examining sources of error in PCR by single-molecule sequencing. PLoS One. 12(1):e0169774.

Raja HA, Miller AN, Pearce CJ, Oberlies NH. 2017. Fungal identification using molecular tools: a primer for the natural products research community. J Nat Prod. 80(3):756–770.

Rämä T, Davey ML, Nordén J, Halvorsen R, Blaalid R, Mathiassen GH, Alsos IG, Kauserud H. 2016. Fungi sailing the Arctic Ocean: speciose communities in North Atlantic driftwood as revealed by high-throughput amplicon sequencing. Microb Ecol. 72(2):295–304.

Rämä T, Hassett BT, Bubnova E. 2017. Arctic marine fungi: from filaments and flagella to operational taxonomic units and beyond. Botanica Marina. 60(4):433.

Rämä T, Norden J, Davey ML, Mathiassen GH, Spatafora JW, Kauserud H. 2014. Fungi ahoy! Diversity on marine wooden substrata in the high North. Fungal Ecol. 8:46–58.

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in Bayesian phylogenetics using tracer 1.7. Syst Biol. 67(5):901–904.

Ramírez-Camejo LA, Zuluaga-Montero A, Lázaro-Escudero M, Hernández-Kendall V, Bayman P. 2012. Phylogeography of the cosmopolitan fungus *Aspergillus flavus*: is everything everywhere? Fungal Biol. 116(3):452–463.

Rehner SA, Samuels GJ. 1994. Taxonomy and phylogeny of Gliocladium analysed from nuclear large subunit ribosomal DNA sequences. Mycol Res. 98(6):625–634.

Reich M, Labes A. 2017. How to boost marine fungal research: a first step towards a multidisciplinary approach by combining molecular fungal ecology and natural products chemistry. Mar Genomics. 36:57–75.

Richards TA. 2011. Genome evolution: horizontal movements in the fungi. Curr Biol. 21(4):R166–R168.

Richards TA, Jones MDM, Leonard G, Bass D. 2011. Marine fungi: their ecology and molecular diversity. Ann Rev Mar Sci. 4(1):495–522.

Richards TA, Leonard G, Mahé F, Del Campo J, Romac S, Jones MDM, Maguire F, Dunthorn M, De Vargas C, Massana R, et al. 2015. Molecular diversity and distribution of marine fungi across 130 European environmental samples. Proc R Soc B Biol Sci. 282(1819):20152243.

Rogers SO, Yan ZH, Shinohara M, LoBuglio KF, Wang CJ. 1993. Messenger RNA intron in the nuclear 18s ribosomal RNA gene of deuteromycetes. Curr Genet. 23(4):338–342. eng.

Ronquist F, Altekar G, Huelsenbeck JP, Dwarkadas S. 2004. Parallel metropolis coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. Bioinformatics. 20 (3):407–415.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol. 61 (3):539–542. eng.

Ronquist F, Van der Mark PHuelsenbeck J. 2009. Bayesian phylogenetic analysis using MRBAYES. In Lemey P, Salemi M, Vandamme A-M, editors. The Phylogenetic handbook: a practical approach to phylogenetic analysis and hypothesis testing. Cambridge: Cambridge University Press; p. 210–266. doi:10.1017/CBO9780511819049.009

Roullier C, Bertrand S, Blanchet E, Peigné M, Robiou Du Pont T, Guitton Y, Pouchus YF, Grovel O. 2016. Time dependency of chemodiversity and biosynthetic pathways: an LC-MS

metabolomic study of marine-sourced *Penicillium*. Mar Drugs. 14(5):103.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 75(23):7537–7541.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci. 109(16):6241. doi:10.1073/pnas.1117018109.

Sepcic K, Zalar P, Gunde-Cimerman N. 2011. Low water activity induces the production of bioactive metabolites in halophilic and halotolerant fungi. Mar Drugs. 9(1):43–58.

Shearer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett D, Porter D, Raja HA, Schmit JP, Thorton HA, et al. 2007. Fungal biodiversity in aquatic habitats. Biodivers Conserv. 16(1):49–67.

Simon UK, Weiß M. 2008. Intragenomic variation of fungal ribosomal genes is higher than previously thought. Mol Biol Evol. 25(11):2251–2254.

Singh P, Wang X, Leng K, Wang G. 2012. Chapter 19, Diversity and ecology of marine-derived fungi. In: Jones EBG, Pang KL, editors. Marine fungi and fungal like organisms. Berlin: De Gruyter; p. 383–408.

Souvorov A, Landsman D, Lipman DJ, Church DM, Benson DA, Maglott DR, Sequeira E, Yaschenko E, Schuler GD, Starchenko G, et al. 2006. Database resources of the national center for biotechnology information. Nucleic Acids Res. 35 (suppl_1):D5–D12.

Sparrow FK. 1960. Aquatic Phycomycetes. 2d rev ed. Ann Arbor: University of Michigan Press.

Sridhar KR. 2017. Marine filamentous fungi: diversity, distribution and bioprospecting. In: Satyanarayana T, Deshmukh SK, Johri BN, editors. Developments in fungal biology and applied mycology. Singapore: Springer Singapore; p. 59–73.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.

Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T. 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for illumina amplicon sequencing. Appl Environ Microbiol. 82(24):7217.

Thatoi H, Behera BC, Mishra RR. 2013. Ecological role and biotechnological potential of mangrove fungi: a review. Mycology. 4(1):54–71.

Tisthammer KH, Cobian GM, Amend AS. 2016. Global biogeography of marine fungi is shaped by the environment. Fungal Ecol. 19:39–46.

VanderMolen KM, Raja HA, El-Elimat T, Oberlies NH. 2013. Evaluation of culture media for the production of secondary metabolites in a natural products screening program. AMB Express. 3:71–71.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol. 172 (8):4238–4246.

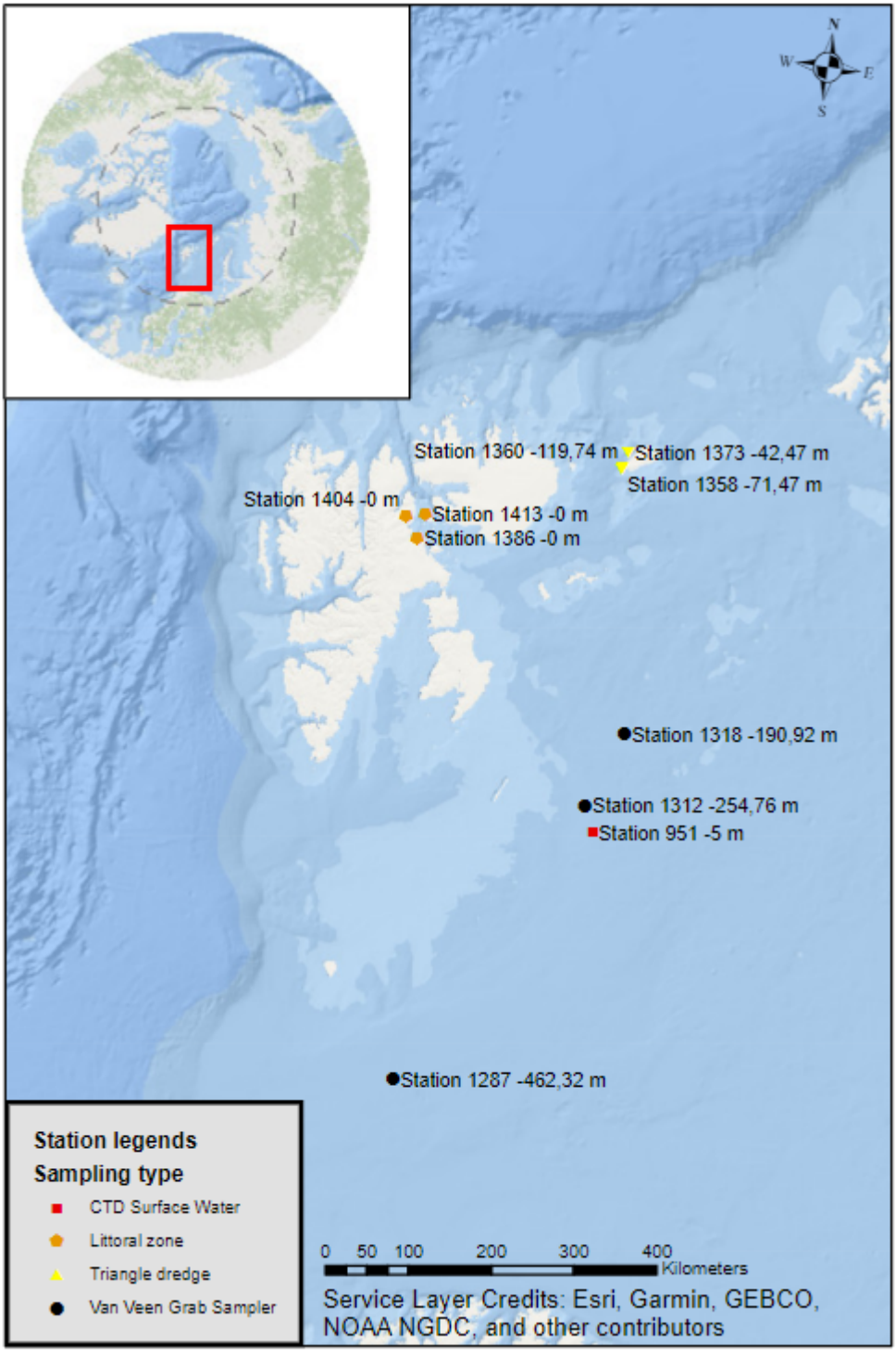
Wang YT, Xue YR, Liu CH. 2015. A brief review of bioactive metabolites derived from deep-sea fungi. Mar Drugs. 13 (8):4594–4616.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: A guide to methods and applications. California: Academic Press; p. 315–322.

Wu B, Oesker V, Wiese J, Schmaljohann R, Imhoff J. 2014. Two new antibiotic pyridones produced by a marine fungus, *Trichoderma* sp. Strain MF106. Mar Drugs. 12(3):1208.

Yarden O. 2014. Fungal association with sessile marine invertebrates. Front Microbiol. 5:228.

Zhang T, Fei Wang N, Qin Zhang Y, Yu Liu H, Yan YL. 2015. Diversity and distribution of fungal communities in the marine sediments of Kongsfjorden, Svalbard (High Arctic). Sci Rep. 5:14524.


N
W
E
S
Station 1360 -119,74 m
Station 1373 -42,47 m
Station 1358 -71,47 m
Station 1404 -0 m
Station 1413 -0 m
Station 1386 -0 m
Station 1318 -190,92 m
Station 1312 -254,76 m
Station 951 -5 m
Station 1287 -462,32 m
Station legends
Sampling type
CTD Surface Water
Littoral zone
Triangle dredge
Van Veen Grab Sampler
0 50 100 200 300 400
Kilometers
Service Layer Credits: Esri, Garmin, GEBCO, NOAA NGDC, and other contributors

Supplementary Tables

Supplementary Table 1. Location and metadata of stations sampled in this study.

Supplementary Table 2. Primer sequences and references used in this study

Supplementary Table 3. Isolates obtained in this study with clustering to OTUs

Supplementary Table 4. Representative isolates with accession numbers, isolate ID, Rfam accession and pairwise similarity to GenBank sequences.

Supplementary Table 5. Reference sequences with accession used in alignment.

Supplementary Table 1

Object-ID	Station	Lat	Long	Depth (m)	Sampling type
1	**951**	**76.15459000**	**29.51583000**	**5**	**CTD Surface Water**
2	**1287**	**73.37032090**	**22.12993333**	**462**	**Van Veen Grab Sampler**
3	**1312**	**76.43991036**	**29.14506487**	**255**	**Van Veen Grab Sampler**
4	**1318**	**77.20864592**	**31.00135609**	**191**	**Van Veen Grab Sampler**
5	**1358**	**80.06359641**	**31.12167571**	**71**	**Triangle dredge**
6	**1360**	**80.08007713**	**31.20546539**	**120**	**Triangle dredge**
7	**1373**	**80.24265730**	**31.57104469**	**42**	**Triangle dredge**
8	**1386**	**79.12958237**	**19.28680541**	**0**	**Littoral zone**
9	**1404**	**79.33720822**	**18.38804856**	**0**	**Littoral zone**
10	**1413**	**79.39730700**	**19.48133027**	**0**	**Littoral zone**

Supplementary Table 2

Primer	Sequence	Target	Length	T_m (°C)	GC (%)	Ref
ITS5	5’-GGAAGTAAAAGTCGTAACAAGG-3’	ITS1-5.8S-ITS2	500+	52.4	40.9	(White et al. 1990)
ITS4	5’-TCCTCCGCTTATTGATATGC-3’			53.5	45.0	(White et al. 1990)
ITS3	5’-GCATCGATGAAGAACGCAGC-3’	ITS2	350	68.2	55.0	(White et al. 1990)
LR0Ri	5’-GCTTAAGTTCAGCGGGT-3’			57.9	52.9	Inverted sequence of LR0R
NS1	5’-GTAGTCATATGCTTGTCTC-3’	SSU (V1-V5)	1100	48.9	42.1	(White et al. 1990)
NS4	5’-CTTCCGTCAATTCCTTTAAG-3’			57.9	40.0	(White et al. 1990)
LR0R	5’-ACCCGCTGAACTTAAGC-3’	LSU (D1-D3)	900	57.9	52.9	(Rehner and Samuels 1994, Moncalvo, Wang, and Hseu 1995)
LR5	5’-TCCTGAGGGAAACTTCG-3’			58.8	52.9	(Vilgalys and Hester 1990)

Supplementary Table 3

Isolate	Source	Kingdom	Division	Genus
1358A1-02	Algae	Fungi	Ascomycota	Lulworthiaceae
1373A1-20.1	Algae	Fungi	Ascomycota	Lulworthiaceae
1360D1-02.M	Driftwood	Fungi	Ascomycota	Tetracladium
1373A1-05.F2	Algae	Fungi	Ascomycota	Penicillium
1413A2-01	Algae	Fungi	Ascomycota	Penicillium
1360D1-12.2	Driftwood	Fungi	Ascomycota	Penicillium
Tra3202.IV.2	Fruiting Body	Fungi	Ascomycota	Penicillium
1287S1-05.2	Sediments	Fungi	Ascomycota	Penicillium
1360D1-09	Driftwood	Fungi	Ascomycota	Geomyces
1386D1-04.1	Driftwood	Fungi	Ascomycota	Geomyces
1360D1-16	Driftwood	Fungi	Ascomycota	Pseudorotium
1287S1-05.1.2	Sediments	Fungi	Ascomycota	Oidiodendron
1386A4-02	Algae	Fungi	Mucoromycota	Mortierella
1413A2-02	Algae	Fungi	Mucoromycota	Mortierella
1360D1-03.F	Driftwood	Fungi	Mucoromycota	Mortierella
Tra3202.III.2	Fruiting Body	Fungi	Mucoromycota	Mortierella
1287S1-05.1	Sediments	Fungi	Mucoromycota	Mortierella
1318S1-08	Sediments	Chromista	Oomycota	Oomycete
PIIIWF2.2.1	Water	Fungi	Ascomycota	Penicillium
1358A1-08.2	Algae	Fungi	Ascomycota	Lulworthiaceae
1373A1-03.F	Algae	Fungi	Ascomycota	Lulworthiaceae
1318S1-10	Sediments	Fungi	Ascomycota	Tolypocladium
1360D1-08	Driftwood	Fungi	Ascomycota	Acremonium
PVIWA5.2	Water	Fungi	Ascomycota	Penicillum
TRa3202.II.1	Fruiting Body	Fungi	Ascomycota	Lulworthia
1360D1-10.1	Driftwood	Fungi	Ascomycota	Mytilinidion
1360D1-09.1	Driftwood	Fungi	Ascomycota	Cosmospora
1404A1-01.1	Algae	Fungi	Basidiomycota	Glaciozyma
PVWE4.1	Water	Fungi	Mucoromycota	Mucor
1413A3-02	Algae	Fungi	Ascomycota	Lulworthiaceae
TRa3202.VI.1	Fruiting Body	Fungi	Ascomycota	Lulworthia
1312S1-05.2	Sediments	Chromista	Oomycota	Oomycete
1386A3-02	Algae	Fungi	Ascomycota	Tetracladium

Supplementary Table 4

		Accession numbers				Closest GenBank similarity (%)		
Isolate_ID	**Genus**	**18S rDNA**	**ITS**	**28S rDNA**	**Rfam**	**18S rDNA**	**ITS**	**28S rDNA**
M16HEL1358A1-02	**Lulworthiaceae sp. OTU1**		MK543196	MK531712		na	85.6	98.9
M16HEL1360D1-02	**Tetracladium sp. OTU2**	MK531782	MK543198	MK531714		99.9	99.8	99.3
M16HEL1360D1-12.2	**Penicillium sp. OTU3**	MK531786	MK543203	MK531718		100.0	100.0	100.0
M16HEL1386D1-04.1	**Geomyces sp. OTU4**		MK543206	MK531720		na	100.0	100.0
M16HEL1360D1-16	**Pseudorotium sp. OTU5**	MK531787	MK543204	MK531719		100.0	100.0	100.0
M16HEL1287S1-05.1.2	**Oidiodendron sp. OTU6**	MK531778	MK543192	MK531709		99.4	99.8	98.4
M16HEL1360D1-03	**Mortierella sp. OTU7**	MK531783	MK543199	MK531715		99.9	99.8	97.8
M16HEL1318S1-08	**Oomycete sp. OTU8**	MK531779	MK543194	MK531710		98.2	79.1	89.6
M16HELPIIIWF2.2.1	**Penicillium sp. OTU9**	MK531773	MK543187	MK531704		100.0	100.0	100.0
M16HEL1358A1-08.2	**Lulworthiaceae sp. OTU10**	MK531781	MK543197	MK531713		98.1	83.4	98.7
M16HEL1318S1-10	**Tolypocladium sp. OTU11**	MK531780	MK543195	MK531711		99.7	95.1	98.8
M16HEL1360D1-08	**Acremonium sp. OTU12**		MK543200			na	99.8	na
M16HELPVIWA5.2	**Penicillum sp. OTU13**	MK531774	MK543188	MK531705		99.9	100.0	100.0
M16HELTRa3202.II.1	**Lulworthia sp. OTU14**	MK531776	MK543190	MK531707		98.9	82.9	94.2
M16HEL1360D1-10.1	**Mytilinidion sp. OTU15**	MK531785	MK543202	MK531717	RF00028	99.4	83.7	96.9
M16HEL1360D1-09.1	**Cosmospora sp. OTU16**	MK531784	MK543201	MK531716		100.0	100.0	99.1
M16HEL1404A1-01.1	**Glaciozyma sp. OTU17**	MK531789	MK543207	MK531721		99.5	98.7	97.5
M16HELPVWE4.1	**Mucor sp. OTU18**	MK531775	MK543189	MK531706		100.0	99.9	99.8
M16HEL1413A3-02	**Lulworthiaceae sp. OTU19**	MK531790	MK543208	MK531722		95.4	81.6	92.6
M16HELTRa3202.VI.1	**Lulworthia sp. OTU21**	MK531777	MK543191	MK531708		98.8	81.5	94.1
M16HEL1312S1-05.2	**Oomycete sp. OTU22**		MK543193			na	80.7	na
M16HEL1386A3-02	**Tetracladium sp. OTU23**	MK531788	MK543205		RF00028	99.9	99.2	na
	Missing locus	**4**	**0**	**3**				

Supplementary Table 5

Name	TYPE	18S	ITS	28S
Achlya bisexualis	FALSE		MH685199	MH685378
Achlya dubia	FALSE		HQ643093	AF119578
Achlya racemosa	FALSE		HQ643105	JX115214
Achlya radiosa	FALSE		HQ643106	JX115215
Acremonium cereale	FALSE		AB540571	AB540497
Acremonium persicinum TYPE	TRUE		NR_131260	AB540501
Atkinsiella dubia	FALSE	AB284575		AB285221
Cordyceps bifusispora	FALSE	HQ680636	AY245627	
Cosmospora arxii TYPE	TRUE		NR_145062	NG_058892
Cosmospora coccinea	FALSE		FJ474072	GQ505990
Cosmospora meliopsicola	FALSE		HM054159	HM042406
Cosmospora viridescens TYPE	TRUE		NR_154791	NG_060412
Geomyces pannorum	FALSE		MH859889	MH871677
Geomyces sp. HL4PH	FALSE		KU612398	KU612427
Glaciozyma antarctica TYPE	TRUE		AF444529	NG_057664
Glaciozyma litoralis TYPE	TRUE		NR_155110	HF934009
Glaciozyma martinii TYPE	TRUE		NR_132821	NG_058293
Glaciozyma watsonii TYPE	TRUE		NR_155146	NG_058294
Gymnostellatospora japonica	FALSE		MF375781	MF375781
Haloguignardia irritans	FALSE	AY566252	AY581940	
Leptolegnia caudata	FALSE	KP098370	KP098340	KP098357
Leuconeurospora pulcherrima	FALSE	AF096178	KJ755518	AF096193
Lophium mytilinum	FALSE	EF596818	EF596819	EF596819
Lulwoana sp. P3	FALSE		KY465973	KY486880
Lulwoana uniseptata TYPE	TRUE	AY879031	LC146746	LC146746
Lulworthia atlantica TYPE	TRUE		NR_148084	NG_060278
Lulworthia cf. opaca	FALSE	AY879003		AY878961
Lulworthia cf. purpurea	FALSE	KT347201	KT347219	JN886824
Lulworthia fucicola TYPE	TRUE	AY879007		AY878965
Lulworthia grandispora	FALSE	AF047582		AF047583
Lulworthia medusa	FALSE	AF195636		AF195637
Lulworthia sp. 107aIA	FALSE		KM272368	KM272360
Mortierella cystojenkinii TYPE	TRUE	HQ667504	NR_111581	NG_042564
Mortierella dichotoma TYPE	TRUE	HQ667477	NR_111568	NG_042552
Mortierella elongatula TYPE	TRUE	HQ667505	NR_111582	NG_042565
Mortierella fimbricystis TYPE	TRUE	GU559980	NR_152949	NG_057967
Mortierella indohii TYPE	TRUE	HQ667461	NR_111561	NG_042545
Mortierella turficola TYPE	TRUE	HQ667506	NR_111583	NG_042566
Mucor circinelloides TYPE	TRUE	JF723652	NR_126116	NG_055735
Mucor genevensis TYPE	TRUE	HM623319	NR_103632	NG_057971
Mucor plumbeus	FALSE	JN939014	JN942877	JN938896
Mucor racemosus TYPE	TRUE	JF723672	NR_126135	NG_055727
Mucor spinosus	FALSE	JF723692	JF723577	JF723763
Mytilinidion australe TYPE	TRUE		MH855533	NG_057806
Mytilinidion mytilinellum	FALSE	FJ161144	HM163570	FJ161184
Mytilinidion resinicola TYPE	TRUE	NG_016511	MH855535	NG_057807
Mytilinidion scolecosporum TYPE	TRUE	NG_016510	MH855536	NG_057808
Myxotrichum deflexum TYPE	TRUE	AB015777	NR_156338	NG_057619
Myxotrichum setosum	FALSE		MH857025	MH868555

Supplementary Table 5

Name	TYPE	18S	ITS	28S
Oidiodendron tenuissimum TYPE	TRUE	AB015787		AB040706
Oidiodendron truncatum	FALSE		MH858549	MH870187
Paracylindrocarpon aloicola TYPE	TRUE		NR_154346	NG_058238
Penicillium antarcticum TYPE	TRUE		NR_137880	MH874309
Penicillium bialowiezense TYPE	TRUE		NR_111323	MH866464
Penicillium brevicompactum TYPE	TRUE		KF465776	MH866525
Penicillium canescens	FALSE		MH856353	MH867903
Penicillium chrysogenum TYPE	TRUE		MH856357	MH867907
Penicillium commune TYPE	TRUE		NR_111143	MH867909
Penicillium coralligerum	FALSE		MH860682	MH872388
Penicillium crustosum	FALSE	JN939046	JN942857	JN938953
Penicillium granulatum	FALSE		MH865458	MH876918
Penicillium novae-zeelandiae TYPE	TRUE		NR_111668	MH867584
Penicillium osmophilum	FALSE	GU733358	MH860734	MH872446
Penicillium polonicum TYPE	TRUE	JN939264	NR_103687	JN939272
Penicillium sacculum TYPE	TRUE		NR_156551	MH869599
Penicillium solitum	FALSE		MH860945	MH872705
Phenoliferia psychrophenolica TYPE	TRUE	NG_061175	NR_154289	KY108774
Pseudeurotium bakeri TYPE	TRUE		NR_145345	MH872136
Pseudeurotium hygrophilum TYPE	TRUE	AY129282	NR_111128	MH874401
Pseudeurotium ovale TYPE	TRUE		MH857368	MH868913
Pseudeurotium zonatum TYPE	TRUE	AF096183	NR_111127	MH867318
Pseudocamaropycnis pini TYPE	TRUE		NR_153459	NG_058211
Roseodiscus rhodoleucus	FALSE	KT972703	KT972704	KT972705
Saprolegnia turfosa	FALSE		HQ644012	HQ665210
Tetracladium marchalianum	FALSE	EU883417	EU883417	EU883417
Tetracladium maxilliforme	FALSE	EU883429	EU883429	EU883429
Tetracladium psychrophilum	FALSE	JX029138	JX029129	
Tolypocladium cylindrosporum TYPE	TRUE		MH859917	MH871712
Tolypocladium endophyticum	FALSE	KF747321	KF747245	KF747152
Tolypocladium inegoensis	FALSE		AB027368	AB027368
Tolypocladium inflatum TYPE	TRUE		MH859963	MH871762
Tolypocladium ophioglossoides	FALSE	AB027321	AB027367	AB027367
Tolypocladium pustulata	FALSE		AF389189	AF389190
Tolypocladium sp. ZLY-2010	FALSE		HM595510	HM595578
Zalerion maritima	FALSE	KT347203	KT347216	JN886806
Zalerion xylestrix	FALSE	EU848591		EU848592
Missing reference locus		**44**	**7**	**3**

Paper 2

Genomic characterization of three marine fungi, including *Emericellopsis atlantica* sp. nov. with signatures of a generalist lifestyle and marine biomass degradation

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Genomic characterization of three marine fungi, including *Emericellopsis atlantica* sp. nov. with signatures of a generalist lifestyle and marine biomass degradation

Marine fungi remain poorly covered in global genome sequencing campaigns; the 1000 fungal genomes (1KFG) project attempts to shed light on the diversity, ecology and potential industrial use of overlooked and poorly resolved fungal taxa. This study characterizes the genomes of three marine fungi. The generalist *Emericellopsis* TS7, the saprotrophic *Amylocarpus encephaloides* and the algae-associated *Calycina marina* were genome sequenced to study their genomic features and phylogenetic placement using multilocus data. *A. encephaloides* and *C. marina* were placed in the *Helotiaceae* and *Pezizellaceae*, respectively, within *Helotiales* based on a 15-gene phylogenetic analysis. These two genomes had fewer biosynthetic gene clusters (BGCs) and carbohydrate active enzymes (CAZymes) than *Emericellopsis* TS7. *Emericellopsis* TS7 isolate (*Hypocreales*, *Ascomycota*) was isolated from the sponge *Stelletta normani* and whole genome sequenced to study the antibacterial activity observed in initial screenings and the systematic position within the *Emericellopsis*. A six-gene phylogenetic analysis placed the isolate TS7 in the marine *Emericellopsis* clade and morphological examination confirmed that the isolate represents a new species, described here as *Emericellopsis atlantica*. Analysis of its CAZyme repertoire indicated that *E. atlantica* is a generalist fungus. FungiSmash revealed the presence of 35 BGCs including, eight non-ribosomal peptide synthases (NRPSs), six NRPS-like, six polyketide synthases, nine terpenes and six hybrid, mixed or other clusters. Of these BGCs, only five were homologs with characterized BGCs. *E. atlantica* was grown in 11 different media that were extracted, pre-fractioned and tested for bioactivity. Several fractions showed antibacterial, anti-biofilm or immunomodulatory activities. Accurate mass and mass spectrometry fragmentation pattern indicated the presence of helvolic acid in the antibacterial fractions. This observation was supported by the presence of the helvolic acid gene cluster. We provide phylogenetic and genome-based evidence for the first genome-sequenced *Emericellopsis* species, *E. atlantica*, being a sponge-associated fungus that has potential to degrade several types of marine biomass. The presence of unknown BGCs and peptide signatures in MS from extracts sets the stage for further investigations of the NRPS peptides produced by *E. atlantica*. In addition, we provide the first sequenced representative of

Pezizellaceae and the first sequenced fungi in the genus *Amylocarpus* and resolve their phylogeny using multilocus data.

Keywords: *Amylocarpus encephaloides*; *Calycina marina*; carbohydrate active enzymes; gene cluster; genome; marine fungi; *Emericellopsis*; 1 new taxon

Introduction

The first genome of a fungus, *Saccharomyces cerevisiae*, was sequenced in 1996 (Goffeau et al. 1996). Subsequent developments in technology have made sequencing much more affordable, and the number of fungal genome and transcriptome sequencing projects has increased exponentially and in 2020 1886 genomes were released (Grigoriev et al. 2014; Sharma 2015; NCBI 2021). Most of the early sequencing efforts were focused on terrestrial ecologically or economically significant fungi, crop-pathogens, or fungi related to human health (Sharma 2015). One problem in studying fungi using comparative genomics, is the lack of available genomic data and proper taxonomic representation of the known taxa (Naranjo-Ortiz and Gabaldón 2019; Lücking et al. 2020). This is especially prominent among marine fungi, where few genomes are available compared to terrestrial fungi. There is also debate about the definition of the characteristics of marine fungi that has been ongoing for at least half a century (Kohlmeyer and Kohlmeyer 1979; Raghukumar and Raghukumar 1999; Pang et al. 2016; Reich and Labes 2017). The 1000 fungal genomes (1KFG) project is addressing ecologically and taxonomically overlooked fungi like marine fungi in poorly resolved taxa, such as *Helotiales* (*Leotiomycetes*). By making their genomes publicly available, 1KFG contributes to better elucidate the general features of marine fungi (Grigoriev et al. 2011; Grigoriev et al. 2014).

The marine environment is vastly different from terrestrial environments, leading to distinct adaptions by the organisms living there. Such adaptions may take the form of unique enzymes that withstand low or high temperature, pressure or salt

concentration, and high potency of signaling molecules and sensitive receptors, specific pigments, and other unique metabolites (van Noort et al. 2013; Kis-Papo et al. 2014; Rédou et al. 2015; Oey 2016; Fouillaud et al. 2017; Huang et al. 2017; Trincone 2018). There are many substrates available in the marine environment that are different compared to terrestrial substrates. Such substrates include polysaccharides such as laminarin, carrageenan, fucoidan, alginate, ulvan, galactans, porphyrin, agarose, chitin among others that do not occur in terrestrial sources or have different modifications such as sulfation (Barbosa et al. 2019). Fungal enzymes utilizing specific marine polysaccharides, such as glycoside hydrolase family 29 (GH29) linked to degradation of algal fucoidan, GH107 for sulfated fucans, GH78 and GH105 for ulvan and GH18 and GH82 for carrageenan are of interest for industrial processing. These enzymes make sugars bioavailable and usable in feed for aquaculture and agriculture, in the production of specific polysaccharides for pharmaceutical purposes or as a carbon source for bioenergy production. Marine microorganisms also have to communicate with each other and protect themselves using secondary metabolites. Because the water dilutes any secreted molecules, the secondary metabolites have to be potent and they are therefore of special pharmaceutical interest as potential drugs (Berteau et al. 2002; Haefner 2003; Michel et al. 2006; Collén et al. 2014; Vickers et al. 2018; Reisky et al. 2019; Carroll et al. 2020; Dobrinčić et al. 2020).

Some of the fungi frequently observed in the marine environment include acremonium-like fungi that are a polyphyletic assembly of mostly indistinct, hyaline, simple, asexual fungi. Binomially named *Acremonium* fungi are found within *Glomerellales*, *Hypocreales*, *Sordariales*, *Cephalothecales* (*Cephalothecaceae)* and *Leotiomycetes* showing how *Acremonium* is used collectively on phylogenetically distinct, but often morphologically indistinct fungi (Summerbell et al. 2011). Many of

these fungi have close sequence similarity to sexual reproductive morph of described species and likely represent the asexual morph of these species (Summerbell et al. 2011). Some of the acremonium-like taxa within the *Emericellopsis* clade are marine, specifically those closely related to *E. maritima* and *A. fuci,* whereas terrestrial isolates form a distinct clade (Zuccaro et al. 2004). Alkali-tolerant soda soil fungi seem to have derived from the marine lineage and are nested in their own clade within the marine clade (Grum-Grzhimaylo et al. 2013). This concept is challenged by recent research based on rDNA ITS1-5.8S-ITS2 region (ITS) and β-tubulin (*TUB*2) phylogeny and should be retested with multilocus gene phylogenies when new species are described (Gonçalves et al. 2020).

Despite frequent phylogenetic studies and descriptions of new species, relatively few acremonium-like fungi have had their genome sequenced. For the genus *Emericellopsis*, there are no reference genomes available (Grigoriev et al. 2014; NCBI Resource Coordinators 2018). This means that there is no genomic information available on the potential of *Emericellopsis* species to produce secondary metabolites. From chemical studies, we know that species within the genus of *Acremonium* and *Emericellopsis* can produce a range of known bioactive metabolites (Argoudelis et al. 1974; Rogozhin et al. 2018; Hsiao et al. 2020). Several terpenes and non-ribosomal peptide synthase (NRPS) peptides have been reported from *Emericellopsis* isolates (Argoudelis and Johnson 1974; Argoudelis et al. 1975; Pinheiro et al. 2012). Those secondary metabolites have shown antibacterial, antibiofilm, antifungal and anticancer activities (Argoudelis and Johnson 1974; Ishiyama et al. 2000; Pinheiro et al. 2012; Arai et al. 2013; Rogozhin et al. 2018). Despite evidence of secondary metabolite production, our understanding of the full biosynthetic potential of *Emericellopsis* fungi remain unknown.

Here, we provide a thorough taxonomic and genomic description of the first fully sequenced *Emericellopsis* species along with an investigation of its bioactivities under different growth conditions. Using the genome sequence, we attempt to identify likely candidate molecules responsible for the observed bioactivities and find evidence for the truly marine nature of the TS7 isolate. To further contribute to the knowledge of marine fungi, we include a description of the two marine fungi, *Calycina marina* and *Amylocarpus encephaloides* (*Helotiales, Ascomycota*), and resolve their phylogeny based on multilocus data extracted from genome sequences.

Materials and Methods

In this manuscript we adhere to italicizing Latin names of organisms and higher order taxonomic ranks as given in Thines et al. (2020). Several of the methods used here have previously been published and will only be briefly described here. For full, in depth methods, see Supplementary data 1 – methods.

Sampling and isolate information

The isolation method of the isolate TS7 was previously described in Batista-García et al. (2017). *Emericellopsis* sp. (Class *Sordariomycetes*, Order *Hypocreales*, Family *Hypocreales incertae sedis*) was obtained from the sponge *Stelletta normani* (Class *Demospongiae*, Order *Astrophorida*, Family *Ancorinidae*) collected on 16th June 2010 from 1350 m depth in the Atlantic Ocean (54.0613° N, 12.5518° W), off the west coast of Ireland using a remote operated vehicle (R.O.V) *Holland I* on board the *R.V. Explorer* (Kennedy et al. 2014). Briefly, one mL of the macerated sponge material was serially diluted and 100 µL of each dilution was inoculated on agar plates with either Malt extract agar-ASW or Potato dextrose agar-ASW (DIFCO). Axenic cultures were obtained after two passages from the primary isolation. The fungus is accessible in the

fungal collection of the School of Microbiology at University College Cork, under accession code TS7, and the Westerdijk Fungal Biodiversity Institute (CBS-KNAW) under the accession CBS 147198. *Emericellopsis* isolate TS7 was selected for full genome sequencing in the 1KFG project due to the lack of sequenced *Emericellopsis* species, its marine origin, promising antibacterial activity against gram-negative bacteria in initial bioactivity testing and as a putative novel species (Jackson et al. 2016).

Isolation of *Calycina marina* TRa3180A (Class *Leotiomycetes*, Order *Helotiales*, Family *Pezizellaceae*) was described in Baral and Rämä (2015). Spores from apothecia growing on decaying *Ascophyllum nodosum* (Class *Phaeophyceae*, Order *Fucales*, Family *Fucaceae*) at the entrance to Portsmouth Harbor, Portsmouth, Hampshire, England, were inoculated and isolated on 0.2SeaMEA (4 g/L malt extract agar with sterile filtered seawater) with antibiotics. The fungus was deposited into the Norwegian marine biobank (Marbank) with the accession number M16FUN0001.

Isolation of *Amylocarpus encephaloides* TRa018bII (Class *Leotiomycetes*, Order *Helotiales*, Family *Helotiaceae*) was described in Rämä et al. (2014). Spores from a cleistothecium on decaying *Betula* sp. (Class *Magnoliopsida*, Order *Fagales*, Family *Betulaceae*) at 70.22874993° N, 19.68153674° E, Troms, Norway, were isolated on 0.2SeaMEA. The fungus was deposited into the Norwegian marine biobank (Marbank) with the accession number M15FUN0043.

Morphology

Emericellopsis TS7 was incubated on oatmeal agar (OA), potato dextrose agar (PDA) and malt extract agar (MEA) (recipes in Crous et al. (2019)) for 21 days at 25 °C. The cultures where then examined using a dissecting and compound light microscope

equipped with differential interference contrast. Morphological characteristics were described and compared to closely related species.

Cultivation for nucleic acid extraction, nucleic acid extraction, sequencing and annotation

Cultivation

For DNA and RNA extractions, mycelium from liquid seed cultures of *Emericellopsis* TS7, *Amylocarpus encephaloides* and *Calycina marina* in 0.2ASME medium (4 g/L malt extract, 40 g/L artificial sea salts (Sigma), MilliQ-water – hereafter MilliQ) were inoculated in 250 mL of the same medium in 1000-mL baffled culture flasks. The media constituents were dissolved in MilliQ. All media were autoclaved at 121 °C for 30 min before inoculation. Incubations were performed at 10-16 °C at 140 rpm (shaking for liquid cultures only). After 13 days the culture was harvested by vacuum filtration through Miracloth (Merck) and the mycelium was subsequently placed in aluminum foil and stored at -80 °C until processing.

Isolation of nucleic acids

Genomic DNA from *Emericellopsis* TS7, *Amylocarpus encephaloides* and *Calycina marina* mycelium was isolated using Quick-DNA Fungal/bacterial Miniprep Kit (Zymo Research) kit according to supplier's instructions. The DNA quality was checked by three methods: To check for DNA degradation a gel electrophoresis on 1 % TBE (Life technologies) UltraPure agarose (Life technologies) gel stained by GelRed (BioTium) was run at 180 V for 20 min after loading the samples using Agarose gel loading dye (Amresco). Samples were compared to GeneRuler High Range DNA ladder (ThermoFisher). NanoVue Plus (GE healthcare) measurement of wavelength ratio was used to control for contamination and estimate concentration. Finally, Qubit

(Invitrogen) measurement using Qubit dsDNA BR Assay Kit (Invitrogen) was used for accurate concentration determination. The DNA sample was stored at -80 °C.

Total RNA from *Emericellopsis* TS7, *A. encephaloides* and *C. marina* mycelium was isolated using Quick-RNA Fungal/Bacterial Miniprep Kit (Zymo Research) according to the supplier's protocol. All MilliQ used for RNA extraction were treated with diethyl pyrocarbonate (DEPC - Sigma). Quality control was performed using the same methods as for DNA with the exception of using RiboRuler High Range RNA ladder (ThermoFisher) for gel electrophoresis and Qubit RNA BR Assay Kit (Invitrogen) for concentration determination.

DNA sequencing and assembly

The draft genomes of *Emericellopsis* TS7, *Calycina marina* and *Amylocarpus encephaloides* were sequenced at the DOE Joint Genome Institute (JGI) using Illumina technology. For genome sequencing, 100 ng of DNA was sheared to 300 bp using the Covaris LE220 and size selected using SPRI beads (Beckman Coulter). The fragments were treated with end-repair, A-tailing, and ligation of Illumina compatible adapters (IDT, Inc) using the KAPA-Illumina library creation kit (KAPA biosystems). Illumina Regular Fragment, 300 bp, standard shotgun library (STD) and long insert, 3000 bp, mate pair library (sLMP) were constructed and sequenced using Illumina NovaSeq. All raw Illumina sequence data were filtered for artifact/process contamination using the JGI QC pipeline (Supplementary data 2-4 - sequencing). An automated attempt was made to reassemble any potential organelle (mitochondrion) from the filtered reads and remove any organelle-matching reads with kmer matching against the resulting contigs with an in-house tool (Supplementary data 5 – sequencing (mitochondrion)). An assembly of the target genome was generated using the resulting non-Organelle reads

with SPAdes v3.12.0 (Bankevich et al. 2012) using the following parameters [--phred-offset 33 --cov-cutoff auto -t 16 -m 115 –k 25,55,95 --careful]. Similar methodology (Supplementary data 5 – sequencing (mitochondrion)), employing the UNITE rDNA database (Kõljalg et al. 2013), was used to reassemble the ribosomal DNA from the filtered reads.

Completeness of the euchromatic portion of the genome assemblies were assessed by aligning assembled consensus RNA sequence data with bbtools v38.31 bbmap.sh [k=13 maxindel=100000 customtag ordered nodisk] and bbest.sh [fraction=85] (Bushnell 2014). This was a routine test by JGI to determine whether significant portions of the genomes were missing.

RNA library creation, read processing and De novo assembly

For transcriptomics, plate-based RNA sample prep was performed on the PerkinElmer Sciclone NGS robotic liquid handling system using Illumina's TruSeq Stranded mRNA HT sample prep kit utilizing poly-A selection of mRNA following the protocol outlined by Illumina in their user guide: https://support.illumina.com/sequencing/sequencing_kits/truseq-stranded-mrna.html, and with the following conditions: total RNA starting material was 1 µg per sample and 8 cycles of PCR was used for library amplification. The prepared libraries were then quantified using KAPA Biosystem’s next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified libraries were then multiplexed with other libraries, and the pool of libraries was then prepared for sequencing on the Illumina NovaSeq 6000 sequencing platform using NovaSeq XP v1 reagent kits, S4 flow cell, following a 2x150 indexed run recipe.

Raw reads were filtered and trimmed using the JGI QC pipeline resulting in the filtered fastq file (*.filter-RNA.fastq.gz files). Using BBDuk (Bushnell 2014), raw reads were evaluated for artifact sequence by kmer matching (kmer=25), allowing 1 mismatch and detected artifact was trimmed from the 3' end of the reads. RNA spike-in reads, PhiX reads and reads containing any Ns were removed. Quality trimming was performed using the phred trimming method set at Q6. Finally, following trimming, reads under the length threshold were removed (minimum length 25 bases or 1/3 of the original read length - whichever is longer).

Filtered fastq files were used as input for de novo assembly of RNA contigs. Reads were assembled into consensus sequences using Trinity (v2.3.2) (Grabherr et al. 2011). Trinity was run with the --normalize_reads (In-silico normalization routine) and --jaccard_clip (Minimizing fusion transcripts derived from gene dense genomes) options.

Genome annotation and functional annotation

The genome was processed through the JGI Fungal Annotation Pipeline according to the Fungal Genome Annotation Standard Operating Procedure available at https://mycocosm.jgi.doe.gov/programs/fungi/FungalGenomeAnnotationSOP.pdf (Grigoriev et al. 2014). Briefly, gene models are iteratively improved using several gene-predicting tools and comparing it to the RNA transcriptome. Functional annotation using SignalP (Petersen et al. 2011), TMHMM (Krogh et al. 2001), InterProScan (Hunter et al. 2009), SwissProt (UniProt Consortium 2013) and KOG (Koonin et al. 2004) follows. Finally, KEGG (Kanehisa et al. 2012) hits are used for EC numbers and map to metabolic pathways, while Intepro and SwissProt are used to map gene ontology (GO) terms. Core Eukaryotic Genes Mapping Approach (CEGMA) was used to make a

set of reliable genes and determine the completeness of the gene annotation (Parra et al. 2007; Parra et al. 2009).

In addition to the annotations done by JGI, a functional annotation of the Carbohydrate Active Enzymes was performed using the dbCAN2 meta server (Zhang et al. 2018). Annotations ran using HMMER (Eddy 2020), Hotpep (Busk et al. 2017) and DIAMOND (Buchfink et al. 2015) on protein FASTA sequence. Domains were assigned by HMMER or if HMMER had no results, by HotPep and DIAMOND as long as both did predicted the same domains. Only genes where two tools had hits were included as recommended on by dbCAN2s manual. For comparison with other fungi, two terrestrial and one marine genome were downloaded, *Acremonium chrysogenum* ATCC 11550 (Terfehr et al. 2014) (Accession GCA_000769265.1), *Aspergillus niger* (Andersen et al. 2011) (Accession GCA_000230395.2) and *Sarocladium schorii* (Schor et al. 2018) (Accession GCA_900290465.1). The genome of *S. schorii* only had the assembly available on NCBI, and the annotation was received from the authors (Schor et al. 2018). Furthermore, the CAZyme amino acid sequences were extracted and searched against the SulfAtlas database (http://abims.sb-roscoff.fr/sulfatlas/) and the catalytic domain pattern of sulfatases using PROSITE (Sigrist et al. 2002; de Castro et al. 2006; Barbeyron et al. 2016).

The annotated genomes were also uploaded on antiSMASH fungal version (v5.0) to detect biosynthetic gene clusters and assess the biosynthetic potential of the isolates (Blin et al. 2019). Border prediction was manually adjusted; genes with homology to biosynthetic genes or putative tailoring genes were included in the clusters and the clusters were compared to previously published clusters using clinker (Gilchrist and Chooi 2020).

Finally, the amount of short simple repeats (SSR) was checked using the Repeat Finder v1.0.1 plugin within Geneious.

Phylogeny

For *Emericellopsis* isolate TS7: 27 reference sequences including 19 sequences from ex-type strains or cultures were included in the phylogenetic analyses (Supplementary data 6 – 6-gene phylogeny). Sequences for each gene were aligned individually using the E-INS-I and G-INS-I algorithms with PAM100 of MAFFT v7.388 (Katoh et al. 2002; Katoh and Standley 2013) in Geneious Prime v11.0.4 followed by manual adjustment of alignments. The dataset was concatenated in Geneious. PartitionFinder v2.1.1 (Lanfear et al. 2017) was run with the concatenated dataset consisting of the rDNA genes, 18S, ITS barcode region (ITS1-5.8S-ITS2) and 28S, and the protein coding genes RNA polymerase II subunit 2 (*RPB*2), transcription elongation factor 1 alpha (*TEF*1α) and β-tubulin (*TUB*2) with a single intron. For the protein coding regions, each position of the codon was split to different partitions. The PartitionFinder analyses were run with: models MrBayes, linked branchlengths, greedy search, and AICc and BIC model selection criterion (Lanfear et al. 2012). This suggested 12 partitions (using AICc), of varying models (Supplementary data 6 – 6-gene phylogeny models). Parallel-MPI MrBayes v3.2.7a with beagle was run for 5.000.000 generations or until average standard deviation of split frequencies was below 0.01 with sampling each 2500 generations with the 12 partitions as suggested by ModelFinder (Ronquist et al. 2012). In addition, PhyML 3.0 was run from the webserver as a single partition with smart model selection (SMS) using AIC, SPR tree search improvement and aBayes and aLRT SH-like fast likelihood-based branch support search (Anisimova and Gascuel 2006; Guindon et al. 2010; Anisimova et al. 2011; Lefort et al. 2017). The model

selected was GTR+I+G. The ML tree using aBayes can be found in supplementary data 7 – PhyML-tree.

For *Calycina marina* and *Amylocarpus encephaloides*: The 15 gene datasets from Johnston et al. (2019) containing 265 taxa were downloaded and the genes from *C. marina* and *A. encephaloides* were aligned to each individual gene alignment before it was concatenated to a single multilocus dataset. The dataset from Johnston et al. (2019) was modified slightly by removing a few introns from protein coding genes and cutting edge alignments only present in a minority of sequences. The alignment was loaded into IQ-TREE v1.6.12, each gene with its own partition (Nguyen et al. 2014). IQ-TREE was run with the parameters [–m MFP –bb 10000 –alrt 10000 –nt AUTO], such that it selected the best model for each partition using ModelFinder (Kalyaanamoorthy et al. 2017), performed 10000 ultrafast bootstraps (Minh et al. 2013) and 10000 SH-aLRT branchtests (Guindon et al. 2010).

Cultivation for metabolite extraction, metabolite extraction and dereplication

The methods for extraction of metabolites, fractionation, UHPLC-HR-MS and dereplication have previously been described in Schneider et al. (2019).

Extraction of metabolites

To prepare biomass for extraction of metabolites, mycelium of *Emericellopsis* TS7 was inoculated in 250 mL media in 1000 mL baffled culture flasks containing YES-ASW, MPM-ASW, DMB, SAPM-ASW, SDPM-ASW, F1, AF or DPY (Supplementary data 8 – media composition). Solid culture fermentations were performed with BRFT, Verm or MWM (Supplementary data 8 – media composition). Liquid fermentations of *Emericellopsis* TS7 in YES-ASW, MPM-ASW, DMB, SAPM-ASW and SDPM-ASW was extracted by solid phase extraction (SPE) using Diaion®HP-20 resin (Supelco

Analytica, Bellefonte, PA, USA). The resin was activated before 40 g of resin was added to 1 L of culture 3-7 days before extraction. The resin was separated from the media and washed with MilliQ to remove remnants from the culture media. Methanol was used to elute metabolites from the resin. Extraction of metabolites from the resin was repeated once and the extract dried under reduced pressure at 40 °C. The dried extracts were dissolved in DMSO and stored at -20 °C.

Liquid-liquid and liquid-solid media extraction was performed using 1:1 ethyl acetate (EtOAc - Sigma) and liquid or solid fermentation culture. The solid culture was cut into pieces in the culturing flasks before addition of EtOAc. The fermentations of DPY, F1, AF, BRFT, Verm and MWM was extracted two times, and the organic phase was separated using a separation funnel or filter before the organic phase was evaporated to dryness under reduced pressure at 40 °C.

Flash chromatography of extracts

Extracts from both liquid and solid cultures were fractioned into eight fractions using reversed phase flash liquid chromatography. The dried extracts were dissolved in 90% MeOH and 2 g of Diaion HP-20ss resin were added before it was dried under reduced pressure. Fractionation of the loaded resin was performed using a Biotage SP4 system with a stepwise gradient of MilliQ water to MeOH followed by MeOH:acetone (1:1) to 100% Acetone. The eight resulting fractions were dried under reduced pressure. Fractions were then dissolved in DMSO and stored at -20 °C.

Mass spectrometry and dereplication

Fractions were diluted in methanol for analysis on an Acquity I-class UHPLC (Waters, Milford, MA, USA) coupled to a PDA detector and a Vion IMS QToF-MS (Waters). Separation of the samples was performed on an Acquity C-18 UPLC column (Waters).

The separation gradient was 10-90 % MilliQ to acetonitrile (HiPerSolv, VWR) with 0.1 % formic acid (Sigma) during 12 min at a flow rate of 0.45 mL/min. Samples were run in ESI+ or ESI- mode, with the following settings: mass window 150-2000 Da, capillary voltage 0.8 kV, cone voltage 30 V, Source offset 50V, source temperature 120 °C, desolvation-gas N_2, desolvation-gas temperature 350 °C, desolvation-gas flow 600 L/h, cone gas flow rate 50 L/h and analyzed using the software UNIFI v1.9.4 (Waters).

Bioactivity

Bioactivity assays were performed as described in Lind et al. (2013), Lauritano et al. (2016) and Schneider et al. (2020).

Growth inhibition assay

Briefly, the human pathogens *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 259233) and *Pseudomonas aeruginosa* (ATCC 27853) from precultures in Muller Hinton Broth (MH, Becton, Dickinson and Company) and *Enterococcus faecalis* (ATCC 29122) and *Streptococcus agalactiae* (ATCC 12386) from precultures in Brain Heart Infusion Broth (BHI, Sigma-Aldrich) were grown to exponential growth phase, and a total of 1500-15000 CFU were added to each well of a 96-well plate (Nunclon, Thermo Scientific) with a total volume of 100 µL/well. Flash fractions were added to the wells with a final concentration of 100 µg/mL. The plates were incubated at 37 °C overnight before measuring the absorbance at 600 nm with a 1420 Multilabel Counter Victor^{3}™ (Perkin Elmer, Waltham, MA, USA). Bacterial suspension diluted with MilliQ (1:1) acted as growth control, growth medium without bacteria as negative growth control. A dilution series of gentamycin was used as a positive assay control. Active fractions were tested at a serial dilution from 100 to 0.8 µg/mL.

Biofilm inhibition assay

To test for biofilm formation inhibition, a Tryptic Soy Broth (TSB, Merck, Kenilworth, NJ, USA) preculture of *Staphylococcus epidermidis* (ATCC 35984) was diluted in media containing 1 % glucose before being transferred to a 96-well microtiter plate at 50 µL/well. Fifty microliters of each flash fraction were added to separate wells and incubated overnight. The bacteria were then removed and the plate washed in tap water. The biofilm was fixed before crystal violet was added to the wells followed by a 10 min incubation. Excess crystal violet solution was removed and the plate was dried, then 70 % EtOH was added and the plate incubated on a shaker for 10 min. To assess biofilm inhibition absorbance at 600 nm (OD600) was detected using a 1420 Multilabel Counter Victor3 plate reader. *S. haemolyticus* (clinical isolate 8-7A, University hospital, UNN, Tromsø, Norway), a non-biofilm forming clinical isolate, was used as a control and 50 µL TSB with Milli-Q (1:1) was used as media blank control. If the compounds tested inhibited bacterial growth such that the bacterial suspension was clear before removal, this was noted as bacterial inhibition rather than antibiofilm activity.

Removal of established biofilm was tested in a similar way as described above. *Staphylococcus epidermidis* (ATCC 35984) was incubated in TSB with 1 % glucose overnight at 37 °C. The next day the bacterial suspension was removed and washed carefully with PBS before the sample was added. After one day of incubation, the sample was removed and the biofilm was colored using crystal violet. The OD600 was measured using 1420 Multilabel Counter Victor3 plate reader. *S. haemolyticus* was used as a non-biofilm producing bacterial control.

Cell proliferation assay

Toxicity of the fractions were assessed using a cell viability assay. Fractions were tested

at 50 µg/mL final concentration in an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) *in vitro* cell proliferation assay against A2058 human melanoma cancer cell line (ATCC CRL-11147). Final measurement of absorbance at 485 nm was performed on a DTX 880 multimode detector (Beckman Coulter) and cell viability was calculated. Growth media with and without 10 % DMSO was used as positive and negative control respectively.

Anti-inflammatory and immunostimulatory assay

Anti-inflammatory: Prepared THP-1 cells (ATCC TIB-202) was seeded in 96-well plates at 10^6 cells/ml and incubated for 48 h. The cells were checked and fresh media added, followed by 24 h incubation. Then, fresh media and 10 µL of sample (100 µg/mL final concentration) was added to each well. After 1 h of incubation 1 ng/mL of LPS (final concentration, Sigma) was added, followed by a 6 h incubation at 37 ºC. The reaction was stopped by freezing the plates at -80 °C immediately after incubation in preparation of Enzyme-linked immunosorbent assay (ELISA) assay. Positive and cell control is with and without LPS addition, respectively.

Immunostimulatory: This assay generally followed the exact same procedure as the anti-inflammatory assay, except after cell differentiation 90 µL fresh media and 10 µL of sample (100 µg/mL final concentration) was added to each well (omitting LPS addition). The reaction was stopped by freezing the plates at -80 °C immediately after incubation in preparation of ELISA. Positive and cell control is with and without LPS addition, respectively.

The secretion of TNFα was measured using ELISA. Between each of the following steps, the plates were washed with washing buffer (TBS with 0.05 % Tween-20, Sigma). All incubations were performed in room temperature in a shaking incubator. Samples were diluted 1:20 using assay diluent (Tris buffered saline - TBS with 1 %

BSA) in the plate and a TNFα-standard dilution series was added to a prepared plate before incubation for 2 h. Biotin coupled anti-human antibody was added to each well followed by a 1 h incubation before diluted ExtrAvidin-Alkaline Phosphatease (Sigma) was added followed by 30 min incubation. Finally, 100 µL pNPP substrate (Sigma, 1 M dietanolamin, pH 9.8) was added to each well, incubated for 45 min and the plates were read in DTX 880 plate reader at 405 nm.

Results

Genome features

The *Emericellopsis* sp. genome was assembled into 114 scaffolds, with a total size of 27.3 Mbp, Table 1. The three largest scaffolds were 1.47, 1.29 and 1.25 Mbps, respectively. The number of scaffolds needed to contain half the assembly (N50) was 14 and the L50 was 0.76 Mbp. There were 22 bridged gaps in the assembly of negligible size, and the G+C content was 54.2 %. The average read coverage depth was 225.6x and the genome contained 9964 predicted gene models with 2.59 exons on average. Mapping of RNA-Seq reads and de novo assembled contigs revealed that 99.2 % and 97.3 %, respectively, mapped back to the genome. The mitochondrial genome was separately assembled into a single scaffold of 25,688 bp and is likely to be circular. The genome characteristics of *Amylocarpus encephaloides* and *Calycina marina* are presented at the end of the results section.

Table 1 - Overview of genome assembly and gene statistics for *Emericellopsis* TS7, *Calycina marina* and *Amylocarpus encephaloides*.

Isolates	*Emericellopsis* sp. TS7	*C. marina*	*A. encephaloides*
Genome statistics			
Genome assembly size (Mbp)	27.3	34.21	46.29
Coverage	225.6	185.26	127.83

# of scaffolds	114	1318	2381
# of scaffolds >= 2k	105	1168	1600
Scaffold N50	14	173	168
Scaffold L50 (Mbp)	0.76	0.05	0.07
# of gaps	22	37	68
% of scaffold length in gaps	0.0	0.0	0.0
Largest scaffold	1.47	0.38	0.42
% GC	54.2	47.6	44.9
Transcriptome and gene models			
EST mapped to genome (%)	99.2	98.8	99.0
Average gene length	1832	1758	1770
exons per gene	2.59	3.13	3
# of gene models	9964	9558	11869
Genes/Mbp	364.98	279.39	256.41
CEGMA (%)	99.34	99.34	99.54
BGCs	36	21	34
CAZyme genes	396	217	356
KOG annotated	5201	4723	5413
KEGG annotated	1969	1670	2041

Gene features and functional annotation of Emericellopsis TS7

The 9964 predicted gene models gave a gene density of 365 genes/Mbp. CEGMA estimated that 99.34 % of the core genes were present, which indicate a nearly complete genome. There were 162 tRNAs and a single complete rDNA region in the assembly. A total of 4331 (43 %) genes were generically annotated with hypothetical (3252) or expressed (1079) proteins. The MAT-1-1 mating locus was also identified via BLAST in the assembly.

A total of 5201 (52 %) genes were recognized as orthologous genes based on hits in the KOG database (Table 1), of these 1317 (25 %) received general functional predictions or were conserved genes with unknown functions (Supplementary data 9 – KEGG-KOG). This indicates that 4763 of 9964 (47.8 %) of the predicted genes do not have characterized orthologs or are lineage specific genes. A small portion of these genes could be pseudogenes that are not functional or genes that have been incorrectly predicted from the annotation pipeline. The largest group of identified orthologs

belonged to the posttranslational modification, protein turnover and chaperones category (483). Signal transduction (377), energy production and conversions (323), carbohydrate transport and metabolism (318) and translation, ribosomal structures and biogenesis (317) were the next four highly represented categories. Secondary metabolite biosynthesis, transport and catabolism (268) made up 2.5 % of the functionally annotated orthologs.

Of the 9964 genes, only 1969 were classified based on the KEGG database, Table 1. The largest group of these were enzymes with known function but undetermined pathways (688) (Supplementary data 9 – KEGG-KOG). This was followed by enzymes involved in amino acid metabolism (618), carbohydrate metabolism (433), metabolism of complex carbohydrates (314), and biodegradation of xenobiotics (298). Pathways associated with biosynthesis of secondary metabolites had 99 enzymes assigned to it.

Phylogenetic placement

Preliminary ITS analysis indicated that *Emericellopsis* TS7 was likely to be a new species and for this reason, a thorough multigene phylogenetic analysis was performed. A concatenation of nuclear rDNA 18S, ITS barcode region and 28S, and the protein coding genes *RPB*2, *TEF1α* and *TUB*2 were made and run through MrBayes using 12 partitions with different models (Supplementary data 6 – 6-gene phylogeny) as suggested by PartitionFinder and PhyML using the smart model selection. The *Acremonium/Emericellopsis* species split into three clades; terrestrial soil, marine, and alkaline or "soda soil" (Figure 1) as previously reported by Grum-Grzhimaylo et al. (2013). *Emericellopsis* TS7 was grouped in the marine clade as an early branch, close to *E. pallida* with branch support based on posterior probability and high bootstrap values.

All three major clades have support in both Bayesian and maximum likelihood models, while individual taxon and branches in some cases have different branching in Bayesian and maximum likelihood trees. There is no support for *Acremonium* sp. A106 branching close to the soda soil clade. The terrestrial clade and outgroup have long branches and polytomy, but it is also the clade with the largest portion of missing data (70.1 % - missing 18s, *RPB2* and *TEF1α*) compared to the marine and alkaline clade (20.1 %). The alkaline clade contains *E. cladophorae* that was isolated from marine algae and not from alkaline soil. *E. donezkii* was isolated from fresh water and *E. enteromorphae* was isolate from marine algae, not from a terrestrial source. However, these isolates all miss data from several of the loci used in this analysis.

Figure 1 - Phylogenetic tree from MrBayes of the genus *Emericellopsis* based on a six gene multilocus alignment of available ex-type and representative sequences. Branch support values are from Bayesian posterior probability (top) and Maximum Likelihood aBayes support test (bottom). Branch length represent substitutions per sequence site. The taxon in bold is the studied fungus. The bold letter T denotes sequences of ex-type cultures. Sequence accession numbers for each isolate are found in supplementary data 6 – 6 gene-phylogeny, PhyML tree can be found in supplementary data 7 – PhyML-tree.

CAZymes and other industrially relevant genes

Fungi are known to secrete a wide array of carbohydrate-active enzymes (CAZymes), which often reflects their specialized habitat-related substrate utilization. These CAZymes are responsible for the breakdown, biosynthesis or modification of different types of carbohydrate substrates. The number of CAZymes in *Emericellopsis* TS7 was 396 (3.97 % of total genes), of which 149 possessed secretory peptide signal indicating that they are likely to be secreted into the external environment or across other membranes (38 % of CAZymes). A comparison of *Emericellopsis* TS7, *Amylocarpus encephaloides* and *Calycina marina* with three other fungal genomes, namely

Aspergillus niger, *Sarocladium schorii* and *Acremonium chrysogenum* (two terrestrial/pathogens and one from sewage water outlet to the sea) indicated that *Emericellopsis* TS7 had the second highest number of CAZyme genes (Figure 2). A relatively high number of CAZymes in *Emericellopsis* TS7 and *A. chrysogenum* had a secretory signal compared to the other species, 38 % compared to 23-33 %. *Emericellopsis* TS7 had a higher number of polysaccharide lyase (PL), glycosyl transferase (GT) and glycoside hydrolase (GH) domains compared to the other marine isolates. *A. encephaloides* on the other hand contained the highest number of carboxyl esterases (CEs), carbohydrate binding modules (CBMs) and auxiliary activity (AA) domains of the marine fungi. CAZyme genes are often modular with many genes containing one or more enzymatic domains along with CBMs that bind to substrates and have no catalytic function. Examples of this are the putatively secreted CAZyme gene 217297 in *Emericellopsis* TS7 with the domain GH18 and CBM18 (putative chitinase) or 546426 (putative cellulase) with the domains CBM1, AA3_1 and AA8 (Figure 3).

Figure 2 - Overview of the distribution of CAZymes in *Emericellopsis* TS7 and five other fungi. The lines indicate the number of genes and number of genes with putative secretion signal and uses the secondary Y-axis.

Figure 3 – Examples of putatively secreted modular CAZymes from *Emericellopsis atlantica*, *Amylocarpus encephaloides* and *Calycina marina*. The illustration is not to scale. SP – Secretion signal peptide, GH – Glycoside hydrolase, CBM – Carbohydrate binding module, AA – Auxiliary activity, CE – Carboxyl esterase. Number indicates enzyme class. Number in brackets is protein identifier.

The different classes of CAZymes followed a similar putative secretion signal pattern in the fungi compared here (Supplementary data 10 - CAZymes). Generally, few genes (4-6 %) with predicted GT activity contained putative signal peptide for secretion, but these are often involved in intracellular synthesis. Genes with polysaccharide lyase

(PL) activity contained secretion signal in 80-88 % of cases, with the exception in *Calycina marina* and *Sarocladium schorii* that only had signal in 50 % and 60 % of genes, respectively. *Amylocarpus encephaloides* had the highest ratio of CBM containing genes with secretion signal (66.7 %) and *C. marina* had the lowest ratio of genes with secretion signal for all classes except GHs. For example, *Emericellopsis* TS7 genes with AA had secretion signal in 42.3 % of cases, CBM in 55.0 %, CE in 66.7 %, GH in 42.5%, GT in 6.7 % and PL in 88.2 %.

The domains that occurred in the highest numbers across the six genomes analyzed were associated with cellulose, hemicellulose, xylan, mannose, fucose, pectate, and chitin. In the secreted enzymes mainly cellulose-, chitin- and xylan-interacting domains were abundant. The unclassified domain GH0 was found in *Emericellopsis* TS7 (1), *Calycina marina* (1) and *Amylocarpus encephaloides* (2). In total, *Emericellopsis* TS7 had 176 different classes of CAZymes (Supplementary data 10 - CAZymes).

Emericellopsis TS7 does not appear to possess genes encoding polyphenol oxidases or fucoidanase, but does have genes encoding fucosidase (GH29 and GH95), a fucose transporter and a few GTs that could have fucose activity (GT1 and GT31). In addition, *Emericellopsis* TS7 also contains seven potential sulfatase genes based on the sulfatase catalytic site pattern (Barbeyron et al. 2016).

The gene for the industrially relevant enzyme phytase was also found (Lei et al. 2013) in *Emericellopsis atlantica*, *Calycina marina* and *Amylocarpus encephaloides*, along with histidine acid phosphatases that share the same enzyme classification (EC 3.1.3.8) with phytase.

Biosynthetic gene clusters of Emericellopsis TS7

A total of 35 biosynthetic gene clusters (BGCs) were predicted using antiSMASH, with 27 of these gene clusters being shown in Figure 4. Eight are not included in the figure because they were solitary core genes not surrounded by other tailoring, transport or transcription genes or they were likely precursor genes in sterol synthesis such as squalene and lanosterol synthase (Supplementary data 11 – BGCs of *E. atlantica*). The clusters contained a range of oxidoreductases, transcription factors, tailoring genes and transporters together with core biosynthetic gene(s). These BGCs included, eight NRPS clusters, six NRPS-like clusters, nine terpene clusters, six PKS clusters, three mixed NRPS-PKS clusters, one hybrid NRPS-PKS cluster, one phosphonate cluster and one indole cluster.

Figure 4 - Overview of BGC structure of the predicted clusters in *Emericellopsis* TS7 with functionally coded coloring. Clusters marked in red were on the end of scaffolds and may be incomplete. The leucinostatin-like cluster was split in two, but is presented as one cluster with a gap. Helvolic acid, produced by the cluster in bold, was detected in MS analysis.

Several of the clusters had homology to known clusters according to KnownClusterBlast, these were further investigated by a synteny analysis using clinker (Gilchrist and Chooi 2020). Only the BGC for ascochlorin (Araki et al. 2019), leucinostatin A/B (Wang et al. 2016), botrydial (Pinedo et al. 2008), cephalosporin C (Terfehr et al. 2014) and helvolic acid (Mitsuguchi et al. 2009) showed a high degree of conserved genes in the *Emericellopsis* clusters (Supplementary data 12 – clinker). Several of the NRPS-genes without homologous hits had a configuration of 4-13 modules according to antiSMASH.

Bioactivity and dereplication of extracts from Emericellopsis TS7

Emericellopsis TS7 cultures were grown for 3-6 weeks before being extracted using liquid-liquid or liquid-solid extraction with ethyl acetate, or SPE of liquid cultures with HP-20 resin followed by methanol elution of the extracted metabolites. The extracts were fractioned into eight fractions using flash chromatography. The bioactivity in the fractions from different cultures varied, indicating the likelihood of expression of potentially different active compounds. A total of 11 different extracts and eight different fractions from each extract (except F1, Verm and MWM) were tested. Fractions from fermentations in MPM-ASW, DMB and SAPM-ASW-media were active against biofilm formation (25 µg/mL) while fermentations in F1, AF, DPY, BRFT, Verm and MWM showed anti-bacterial activity to varying degree, Table 2. The highest antibacterial activity was observed in fermentations using AF and DPY. Fermentation in media containing both yeast extract and peptone such as DPY and AF showed activity against *E. faecium*. None of the fractions showed toxic activity against A2058 melanoma cells. Fractions from the DMB-fermentation displayed an immunostimulatory effect down to a level of 10 µg/mL in a dose dependent manner.

Table 2 - Bioactivity from the fractions of the *Emericellopsis* TS7 fermentations.

Frac. no. – Fraction number, Ext. met. – Extraction method, IS – Immunostimulatory, AIF – Anti-inflammatory, Form. – Biofilm formation, Estab. – Established biofilm, Growth inh. – Growth inhibition, EF – *Enterococcus faecium,* EC – *Escherichia coli,* PA – *Pseudomonas aeruginosa,* SA – *Staphylococcus aureus,* Str.B – *Streptococcus agalactiae*

Dereplication was performed using UHPLC-QToF-MS where the fractions were compared against each other and media controls. The second highest peak in the active fraction 5 from the AF and DPY fermentations was not present in the inactive fractions. The peak had a retention time of 7.45 min and a *m/z* of 567.2883 $[M-H]^-$ in ESI- and a

calculated elemental composition of $C_{33}H_{43}O_8$ (calculated deprotonated monoisotopic mass: 567.2958 Da). A search in ChemSpider indicated that this compound could be helvolic acid (Fumigacin). To confirm the identity, we compared the endogenous compound with a commercial standard of helvolic acid (Sigma) on the UHPLC-QToF-MS. Both compounds had identical retention times, accurate mass and fragmentation pattern. The presence of the gene cluster of helvolic acid further supported the correct identification. The helvolic acid peak was followed by a smaller peak at m/z 569.3010 Da. This compound had a similar fragmentation pattern as helvolic acid (fragments were consistently 2 Da larger with few exceptions), and this was most likely 1,2-dihydrohelvolic acid.

The third most abundant peak in the fermentation using DMB had a *m/z* of 1050.7529 Da $[M+H]^+$ with a calculated elemental composition of $C_{54}H_{100}N_9O_{11}$ (Calculated protonated monoisotopic mass: 1050.7542 Da). The fragmentation pattern indicated that the compound was a peptide, and a search in ChemSpider matched the elemental composition of the peptide acrepeptin C. However, the fragmentation pattern did not match with the amino acid composition of this peptide. Several other probable peptides (based on fragmentation) in the range of 800-1100 Da were also observed among the most abundant ions in the DMB sample, and these signals were not detected in the media control. However, none of the calculated elemental compositions matched known peptides in the ChemSpider database.

Genome description of Calycina marina

The genome assembly of *Calycina marina* was more fragmented when compared to *Emericellopsis* TS7. The assembly statistics reveal that the N50 was 173 with an L50 of 50 kbp and the final assembly consisted of 1318 scaffolds with a total length of 34.2

Mbp. The number of predicted genes in *C. marina* was 9558, which was slightly fewer than in *Emericellopsis* TS7 despite the fact that *C. marina* has a larger genome. *C. marina* distinguished itself from the other genomes analyzed in having comparatively few CAZyme genes, totaling 217; and the lowest proportion of potentially secreted CAZyme genes at 51 (24 % of CAZymes). The genome contained 21 potential BGCs distributed as nine NRPS/NRPS-like, five PKS (including two type 3), three terpene, one indole, one hybrid, one aromatic prenyltransferases (PTase) and one ribosomally synthesized and post-translationally modified peptide (RiPP, for predicted BGCs see Supplementary data 13 – BGCs for *C. marina*).

Genome description of Amylocarpus encephaloides

The genome assembly of *Amylocarpus encephaloides* was also more fragmented than *Emericellopsis* TS7 with an N50 value of 168 and L50 of 74 kbp. The genome assembly consisted of 2381 scaffolds with a total length of 46.3 Mbp, which was larger than that for *Emericellopsis* TS7 and *Calycina marina.* The total number of predicted genes was 11869, which was again more than both *Emericellopsis* TS7 and *C. marina*. Despite being fragmented, the genome was complete in terms of core gene presence with a CEGMA value of 99.56 %. *A. encephaloides* had 356 CAZyme genes, of which 115 are potentially secreted. The genome showed a higher portion of CAZyme genes with CBM1 (Cellulose binding) modules and secretion of these (15 genes, 80 % secreted). *A. encephaloides* also had the largest portion of CBM containing CAZymes with secretion signal (66.7 %). A total of 34 BGCs were detected in the genome, distributed as 14 PKS (one type 3), 10 NRPS/NRPS-like, five terpene, four hybrid clusters and one RiPP (Supplementary data 14 – BGCs of *A. encephaloides*).

Helotiales phylogeny of Amylocarpus encephaloides and Calycina marina

A 15-gene multilocus phylogenetic analysis was performed using a slightly modified dataset that was first published by Johnston et al. (2019) examining the phylogenetic placement of the two fungi within *Leotiomycetes*. Johnston et al. (2019) only looked at *Amylocarpus encephaloides* in the ITS tree because there were no available sequences for the multilocus phylogenetic analysis, while the isolates of *Calycina* included lacked several of the loci examined. *C. marina* was placed together with the rest of *Calycina* within *Pezizellaceae*, where it formed a monophyletic clade (Figure 5). *A. encephaloides* was placed within *Helotiaceae* on a branch with *"Hymenoscyphus" repandus*. *H. repandus* was not placed together with the rest of the *Hymenoscyphus* that formed a distinct monophyletic clade. Both of these clades were within *Helotiales*.

Figure 5 –Phylogeny of *Helotiales* using a 15 gene multilocus dataset for analysis. *Amylocarpus encephaloides* was placed in the *Helotiaceae* and *Calycina marina* within *Pezizellaceae* within *Helotiales*. The support values are from the ultrafast bootstrap in IQ-TREE. The bold letter T denotes ex-type sequences. *Xylaria hypoxylon* was used as an outgroup.

Taxonomy

Emericellopsis atlantica L.W. Hou, Crous, Rämä & Hagestad, ***sp. nov.***

MycoBank MB838493

Figure 6

Figure 6 - *Emericellopsis atlantica* (ex-type CBS 147198). Colonies on OA (A), MEA (B) and PDA (C) after 21 d at 25 C. D–F, I. Monophialides. G–H. Branched conidiophores. J. Conidia. Scale bars = 10 μm.

***Etymology*:** *atlantica*, referring to the Atlantic Ocean where the fungus was isolated.

Diagnosis: *Emericellopsis atlantica* can be distinguished by the production of conidia

with irregular-shaped guttules, and longer phialides measuring 24.5–50(–64) µm. Furthermore, *E. atlantica* occasionally produces polyphialides with two conidiogenous loci. *E. atlantica* differed by its longer conidiogenous cells, which were 19.0 ± 7.5 × 1.5 ± 0.5 µm in *E. enteromorphae*. Colonies of *E. atlantica* also grew faster than the three other marine species (Gonçalves et al. 2020).

Typus: Ireland, from 1350 m depth in the Atlantic Ocean (54.0613N, 12.5518W), from the sponge *Stelletta normani*, 16 June 2010, T.D.S. Sutton (holotype CBS H-24579, ex-type living culture TS7 = CBS 147198).

Description: After 21 d at 25 °C: On OA reaching 65 mm diam., flat, entire margin, dusty and rosy buff at centre, dirty white at periphery, reverse ochreous. On MEA reaching 70 mm diam., flat, entire margin, felty, pale ochreous at centre, dirty at periphery, reverse ochreous. On PDA reaching 80 mm diam., flat, entire margin, cottony, rosy buff at centre, buff at periphery, reverse buff. *Mycelium* consisting of branched, septate, hyaline, smooth- and thin-walled hyphae, up to 2 µm wide. *Conidiophores* arising from submerged or superficial hyphae, sometimes radiating out from sterile coils formed by the mycelium, (sub-)erect or slightly curved, simple or poorly branched, ca. up to 66 µm long, 1.5–3 µm wide at the base, hyaline, smooth-walled, with cell walls usually thicker than those of the vegetative hyphae. *Conidiogenous cells* mono- or polyphialidic, terminal, lateral, straight to slightly flexuose, cylindrical, 24.5–50(–64) µm long, 1.5–2.5 µm wide at the base, with inconspicuous collarette and periclinal thickening at the conidiogenous locus, hyaline, thick- and smooth-walled, polyphialides with up to two conidiogenous loci. *Conidia* formed in globose slimy heads at the apex of phialides, obovoid or ellipsoidal with truncate base, aseptate, hyaline, thin- and smooth-walled, 3–6(–9) × 2–2.5 µm, with 1–2

irregular shaped guttules. *Chlamydospores* and sexual morph not observed.

Habitat/host: Isolated from marine environment, from the host sponge *Stelletta normani*.

Distribution: Currently unknown.

Notes: *Emericellopsis atlantica* was represented by a single isolate which clusters on a solitary branch basal to the clade containing the "Marine clade" and "Soda soil" clade of *Emericellopsis*. Morphologically, *E. atlantica* was similar to other species in having comparable conidiophores and conidia, but it can be distinguished by the production of conidia with irregular-shaped guttules, and longer phialides measuring 24.5–50(–64) µm. The closely related species "*Acremonium*" *fuci*, *E. alkalina*, *E. maritima*, *E. minima*, *E. pallida* and *E. stolkiae* that were described based on characters of both sexual and asexual morphs had phialides as follows: 20–35 µm long in *E. alkalina*, 7.5–23.5 µm in "*Acremonium*" *fuci*, (17–)20–26(–29) µm in *E. maritima*, 20–30 µm in *E. minima*, 25–45 µm in *E. pallida* and 18–40 µm in *E. stolkiae* (Stolk 1955; Davidson and Christensen 1971; Beliakova 1974; Zuccaro et al. 2004; Grum-Grzhimaylo et al. 2013). *E. phycophila* and *E. cladophorae* that were described as asexual morphs from macroalgae collected in an estuarine environment had phialides measuring 15.0 ± 4.0 µm and 21.0 ± 5.0 µm, respectively (Gonçalves et al. 2020). Furthermore, *E. atlantica* also differed from the other species by occasionally producing polyphialides with two conidiogenous loci, while the other species were mostly producing monophialides that were simple or poorly branched. Compared to the other recently described marine species *E. enteromorphae*, *E. atlantica* differed by its longer conidiogenous cells, which were 19.0 ± 7.5 × 1.5 ± 0.5 µm in *E. enteromorphae*. Colonies of *E. atlantica* also grew faster than the three marine species (Gonçalves et al. 2020).

Material examined: Ireland, from 1350 m depth in the Atlantic Ocean (54.0613N, 12.5518W), from the sponge *Stelletta normani*, 16 June 2010, T.D.S. Sutton (holotype CBS H-24579, ex-type living culture TS7 = CBS 147198).

Discussion

The driftwood inhabiting Amylocarpus encephaloides and brown seaweed endophyte Calycina marina

The genome assemblies of *Amylocarpus encephaloides* and *Calycina marina* were significantly more fragmented than *Emericellopsis atlantica*; this is potentially due to a larger portion of repetitive elements which can complicate genome assemblies (Sotero-Caio et al. 2017; Tørresen et al. 2019). CEGMA analysis indicated that nearly all core genes were accounted for, so the genome can be considered complete with respect to gene content. However, the many small contigs made it more difficult to identify complete BGCs. *A. encephaloides* had the largest genome of the three genomes presented here, with about 2000 more genes than the two other fungal genomes. Despite the high gene count, the number of CAZymes was lower than in *E. atlantica*, but the number of secreted CAZymes containing CBMs in *A. encephaloides* outnumbered the other genomes. CBMs are important for binding to insoluble substrates such as cellulose (Boraston et al. 2004; Zhao et al. 2014). In addition, *A. encephaloides* had a higher number of CEs and AAs than the two other genomes. Specifically, it had seven AA1 laccases acting on phenolic substrates and can be involved in lignin degradation. AAs are often associated with degradation of lignin and the high amounts of CBMs, AAs and CEs shows an adaption towards woody substrates that sporocarps of *A. encephaloides* are exclusively found on.

Calycina marina had the lowest number of genes, despite having a larger genome compare to *Emericellopsis atlantica*. *C. marina* also had the fewest BGCs and a

significantly smaller amount of CAZymes. This requires further investigation to determine if this is due to dependency on its algal host as an endophyte, a notion suggested in Baral and Rämä (2015).

The genome of Emericellopsis atlantica

The size of the genome assembly of *Emericellopsis atlantica* was 27.3 Mbp. The assembly was approximately 3 Mbp smaller than the average genome size of karyotyped *Acremonium* (Walz and Kück 1991) and 1.3 Mbp smaller than the sequenced *A. chrysogenum* ATCC 11550 (Terfehr et al. 2014). However, they are not part of the *Emericellopsis* clade. Unfortunately, there is no information on the number of chromosomes in *Emericellopsis* in order to assess the fragmentation, but fungi in *Ascomycota* that has been investigated had between 4-17 chromosomes (Wieloch 2006). *E. atlantica* has a smaller genome than *Calycina marina*, but contains 15 more BGCs. However, this could be due to missed genes caused by the fragmentation of the *C. marina* genome. Genome size in prokaryotes are proportionally linked with the number of secondary metabolite genes (Konstantinidis and Tiedje 2004), and in fungi this is at least partially true (Rokas et al. 2018). Fungal genomes smaller than 20 Mbp harbored few if any BGCs (Rokas et al. 2018). The fungi examined here all contain a number of BGCs, but the fragmentation of both *C. marina* and *A. encephaloides* could reduce the number of BGCs detected. The findings still support that fungal genomes above 20 Mbp can have diverse and numerous BGCs.

The G+C content of *Emericellopsis atlantica* was 54.2 %, which is higher than the median (48.9 %) for *Pezizomycotina* (Storck 1966; Nishida 2015) and the average for *Ascomycota* (>50%) (Li and Du 2014). High GC content has been indicated to play a role in complex environmental adaption and horizontal gene transfer (Mann and Chen

2010) and is linked with halotolerance in prokaryotes (Jacob 2012). A slight positive correlation between chromosome length and GC content was also found by Li and Du (2014) in fungi. High GC content has also been linked with thermal stability of the DNA through base pair stacking (Yakovchuk et al. 2006), higher affinity of the histones (Nishida 2015), and lower occurrence of transposable elements (TEs) (Muszewska et al. 2017), while high AT content has been linked to anaerobic fungi (Wilken et al. 2020). This indicates that *E. atlantica* is adapted to an environment with high salt content or an environment with active exchange of genes, increasing the GC content, which in turn decreased the portion of TEs or indicates that it has fewer but longer chromosomes. The amount of short simple repeats was only about 1.16 % in *E. atlantica*, 4.32 % in *Amylocarpus encephaloides* and 1.48 % in *Calycina marina*, but only the assembly of *E. atlantica* contained long scaffolds. This indicates a low portion of repetitive elements in *E. atlantica* compared to the other species described here.

Biosystematics and sexual reproduction

Morphologically, *Emericellopsis atlantica* is differentiated from the other *Emericellopsis* species in the marine and alkaline clade by irregularly shaped guttules, longer phialides and the occasional production of polyphialides with two conidiogenous loci, which were absent in the other species. The distinct morphology supported the phylogenetic placement on a separate branch within the marine clade of *Emericellopsis*, closely related to *E. pallida* and *E. phycophila* that were morphologically different. The major branches in the three clades of “terrestrial”, marine and “soda soil” *Emericellopsis* were supported in both Bayesian and maximum likelihood models. As Gonçalves et al. (2020) noted, the clades do not contain species with the same traits and isolation locality. *E. cladophorae* and *E. enteromorphae* were isolated from algae in

estuarine environments and were placed in the "alkaline soda soil" and "terrestrial" clade, respectively. The long branches of the terrestrial clade were likely induced by missing data in three to four of the six loci in terrestrial isolates (Wiens 2006; Darriba et al. 2016) and in the three newly described species in Gonçalves et al. (2020). Only one of the terrestrial sequences, *E. minima* CBS871.68, contained all six loci. The close relation of these species makes it difficult to establish proper phylogenetic relations without sequence data from several loci. The type species of the genus is *E. terricola* and it was placed in the terrestrial clade (Grum-Grzhimaylo et al. 2013; Gonçalves et al. 2020). The lack of sequence data, together with "*Acremonium*" species in each of the three clades, as well as the placement of the algae associated *E. cladophorae* and *E. enteromorphae* outside of the marine clade shows the necessity of a taxonomic revision and addition of sequences of *Emericellopsis* and closely related genera supporting the conclusions of Gonçalves et al. (2020).

Despite the lack of sporocarps during isolation and morphological studies, *Emericellopsis atlantica* contained a complete MAT1-1 mating locus (SLA2, MatA-3, MatA-2, MatA-1, APN1, COX6a) on scaffold_14 and pheromone sensing protein. This indicates that sexual reproduction and sporocarp formation could be possible in the species if MAT1-2 exists (Klix et al. 2010). However, one can ask the question whether sexual reproduction would take place in the sponge-host in deep-sea environment. It is possible that the fungus is present elsewhere in other marine substrates or habitats that could function as suitable places for sexual reproduction. So far, we have no evidence for this. Information about the distribution and ecology of *E. atlantica* remains to be further elucidated in future environmental sequencing studies where the fungus can be identified after our thorough characterization and submission of marker gene sequences to NCBI.

A generalist fungus with symbiotic lifestyle

The adaption of *Emericellopsis atlantica* to the marine environment is supported by the presence of CAZyme classes relating to utilization of marine polysaccharides such as fucose, carrageenan and laminarin. However, no modular sulfatases with a CAZyme domain as described in Helbert (2017) were predicted from the JGI annotation. A manual search using the SulfAtlas database (Barbeyron et al. 2016) revealed several CAZymes with low E score against putative sulfatases and sulfatase domains. However, they did not contain the conserved peptide pattern of the catalytic site. Many marine polysaccharides have attached sulfate groups and removal of these are necessary for utilization of the sugars (Schultz-Johansen et al. 2018; Kappelmann et al. 2019). *E. atlantica* contained six putative sulfatases annotated by JGI in the genome, which is three times more than *Amylocarpus encephaloides* and *Calycina marina*, but less than the terrestrial species (11 and 22 genes). Similar to the other marine clade *Emericellopsis*, *E. atlantica* lacked genes for polyphenol oxidase, of which the activity has been observed in the terrestrial clade of *Emericellopsis* (Zuccaro et al. 2004; Grum-Grzhimaylo et al. 2013). The absence of polyphenol oxidase and the presence of fucosinase (GH29/95/141) is in line with the detected phenotype of the marine clade of *Emericellopsis* (Zuccaro et al. 2004). The absence of polyphenol oxidase indicates that *E. atlantica* does not degrade gallotannins and ellagitannins from terrestrial sources (Cammann et al. 1989; Salminen et al. 2002; Zuccaro et al. 2004). There are other types of tannins in for example brown algae, such as phlorotannins, which might require other enzymes to degrade it (Jormalainen et al. 2003; Zuccaro et al. 2004). Loss of the ability to break down gallotannins and ellagitannins is likely a specialization to the available substrates in the marine environment (Zuccaro et al. 2004).

Generalists tend to have a higher amount of CAZymes than specialists do in order to utilize a wider range of substrates (Zhao et al. 2014). *Emericellopsis atlantica* showed a wider range of enzymatic classes than the other fungi in this study (176 different classes). Considering that *E. atlantica* did not have the highest number of genes, but still had the highest diversity of enzymatic classes indicates an adaption to utilize a wide diversity of substrates available in water. The ability to process any source of nutrients efficiently would be beneficial in a nutrient poor ocean environment (Turley 2000). Sponges are natural filters for organic matter such as marine snow and naturally increases the availability of different nutrients, therefore fungi may exploit this by living within the sponge and adapt specifically to that environment (Anteneh et al. 2019).

Polysaccharide lyases occurred in fewer numbers in terrestrial saprophytic and facultative parasitic fungi, where some even lacked PLs altogether (Soanes et al. 2008; Zhao et al. 2014). PLs have been shown to be related to breakdown of pectins from cell walls in marine diatoms and seagrasses (Desikachary and Dweltz 1961; Ovodova et al. 1968; Hehemann et al. 2017; Hobbs et al. 2019). *Emericellopsis atlantica* had almost twice the number of PLs (17) compared to the other fungi in the CAZyme analysis. Furthermore, the relative number of CAZymes with secretory signal was also high (38 %). This indicates that it can break down cell remnants in marine snow or pectin rich substrates in marine sediments or within the sponge host (Smith et al. 1992).

The presence of DNA photolyases in the genome could indicate that the species is not specifically adapted to dark deep-sea environments (Núñez-Pons et al. 2018). Partial loss of photolyases have previously been reported in white-nose fungi from bats as an adaption to the darkness (Palmer et al. 2018). The host sponge *Stelletta normani* was collected from 1350 meters depth, but the type specimen of the species was

collected from 330 meters depth in Southern Norway (Sollas 1880). Other sources report specimens collected from the twilight (dysphotic) zone with small amounts of light penetration (Murillo et al. 2012). The sponge occurrence is not restricted to the deep-sea environment, which makes it logical that the associated *Emericellopsis atlantica* has not lost its photolyases and is not an obligate deep-sea dweller.

Biosynthesis potential and bioactive secondary metabolites

The antibacterial activity observed in fractions of *Emericellopsis atlantica* cultivated in AF and DPY is likely explained by helvolic acid. Helvolic acid has previously been reported with antibacterial activity against *Escherichia coli, Bacillus subtilis, Micrococcus lysodeikticus,* and *Staphylococcus aureus* down to 0.78 µg/mL (Lee et al. 2008; Xu et al. 2019), but we could not confirm this activity in our assays. This may be due to different composition of the fractions and the fact that we did not test purified compounds. There were several smaller peaks in the chromatogram of fractions from the extracts of the DPY and AF fermentations with masses corresponding to different intermediates in the helvolic acid biosynthesis pathway and fragmentation similar to helvolic acid (Lv et al. 2017). These intermediates (such as 3,7-diketo-cephalosporin P1 and helvolinic acid) have previously been reported as antibacterial, especially against *S. aureus,* and in some cases with higher activity than helvolic acid (Lv et al. 2017). The fractions containing helvolic acid only showed activity against *S. epidermis*, *Enterococcus faecium* and *Streptococcus agalactiae* that are gram-positive. No activity against gram-negative bacteria was observed, despite *E. atlantica* showing activity in the initial screening against gram-negative bacteria. None of the other molecules predicted from the homologous clusters were detected from the fermentations using monoisotopic mass searches.

Compounds such as acrepeptins, ascochlorin, cephalosporins, emericellipsins, emericellopsins, emerimicins (later zervamicin), helvolic acid, heptaibin and leucinostatins have previously been isolated from acremonium-like or *Emericellopsis* species (Cole and Rolinson 1961; Argoudelis et al. 1974; Argoudelis and Johnson 1974; Argoudelis et al. 1975; Strobel et al. 1997; Ishiyama et al. 2000; Degenkolb et al. 2003; Bills et al. 2004; Degenkolb and Brückner 2008; Summerbell et al. 2011; Khan 2017; Rogozhin et al. 2018; Araki et al. 2019; Hsiao et al. 2020). Many of these compounds are modified peptides and peptaibols, likely produced by NRPSs. Of the different gene clusters detected in *Emericellopsis atlantica* with known products, only botrydial has not been described from *Acremonium* or *Emericellopsis*. The total number of BGCs detected by antiSMASH were 35 clusters, slightly lower than average for *Sordariomycetes* (Rokas et al. 2018; Robey et al. 2020), but higher than for the two other marine fungi in this study. Compared to *Calycina marina* and *Amylocarpus encephaloides* with 9 and 10 NRPS and NRPS-like clusters, respectively, *E. atlantica* has 16. Isolates within *Emericellopsis* are capable of producing a range of NRPS derived peptides (Cole and Rolinson 1961; Argoudelis et al. 1974; Ishiyama et al. 2000; Rogozhin et al. 2018; Baranova et al. 2019). This together with the detection of molecules with peptide-like fragmentation pattern indicate that that *E. atlantica* is a promising source of potentially novel NRPS produced peptides. However, application of the OSMAC approach in culturing or heterologous expression and gene-knockout experiments may be needed to produce these putatively novel NRPS-peptides and characterize the gene clusters (de Mattos-Shipley et al. 2018).

Emericellopsis atlantica is the first genome sequenced *Emericellopsis* species and a distinct species of marine fungi showing adaptions to utilize a range of different substrates in the marine environment. The nature of the relationship to the host sponge

cannot be determined based on our study. The function of a large portion of the genes (61 %) remains unknown or have a general function prediction, which underlines the need to sequence the genomes of more marine fungi and characterize their genes, and elucidate the produced metabolites. This is necessary to improve our understanding of the unique adaptions in marine fungi using comparative genomics studies. The *E. atlantica* genome also contained several unknown NRPS clusters and enzymes that warrant future research and may be of biotechnological and industrial interest. Together with the two other marine fungal genomes analysed, we contribute to the increase of marine fungal genomes and shedding light on the characteristics of marine fungi.

Declarations

Ethics approval and consent to participate

Not applicable

Adherence to national and international regulations

Genetic resources used in this study adhere to national and international regulations (Nagoya Protocol of the Convention on Biological Diversity), as the UK materials used were accessed in accordance with applicable legislation of the providing country (sampled before 12 October 2015).

Consent for publication

Not applicable

Availability of data and materials

The trees generated and/or analysed during the current study are available in the

Treebase repository, http://purl.org/phylo/treebase/phylows/study/TB2:S27616. Genomes and annotations are submitted to NCBI/GenBank under the accession XXXX and is also available at MycoCosm. All data generated or analysed during this study are included in this published article and its supplementary information files. Raw data used in the study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

Funding

Digibiotics (Digital Life Norway) and Centre for New Antibacterial Strategies at UiT The Arctic University of Norway, through the Research Council of Norway (Project 269425) provided funding for Teppo Rämä. This research was performed within the Community Sequence Program "1KFG" conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, which was supported by the Office of Science of the U.S. Department of Energy under contract DE-AC02-05CH11231. This work benefitted from the sharing of expertise within the DFG priority program "Taxon-Omics: New Approaches for Discovering and Naming Biodiversity" (SPP 1991) to RJC and EK.

Authors' contributions

OCH, TR, JHA, EHH and BA conceived the idea, aims and designed the experiments. TDSS isolated *E. atlantica*, TR isolated *C. marina* and *A. encephaloides*. SAJ and ADWD did initial screening of *E. atlantica*. CL optimized DNA and RNA extraction protocols. OCH cultured and extracted metabolites, analysed genomic and MS data, performed phylogenetic analysis and visualized the findings. KL and MA prepared the

libraries and sequenced the fungi, JP and WA assembled the genomes, AL assembled the transcriptomes and RH annotated them. The sequencing effort at JGI was coordinated by VN. JWS has been the PI for the sequencing of the three genomes. JHA, EHH, TR, BA and EK supervised and mentored OCH. LH compared, visualized and wrote the description of the morphology of *E. atlantica*. JHA, IVG, RJC, PWC and ADWD have provided funding and materials for the completion of this project. OCH and TR wrote the first draft of the manuscript. OCH, TR, EK, JHA, EHH, BA, CL, RJC, PWC, IVG, JWS and ADWD have reviewed and commented the manuscript. All authors have read through and accepted the manuscript for publication.

Acknowledgements

Bernard Picton (Ulster Museum) and Christine Morrow (Queens University Belfast) performed the identification of the host sponge. Ronald de Vries and Ad Wiebenga (Westerdijk Institute) provided extraction protocols. Marte Jenssen (Marbio) cultured *Emericellopsis atlantica* for DNA and RNA extraction. Marte Albrigtsen and Kirsti Helland (Marbio) ran the bioassays.

Literature cited

Andersen MR, Salazar MP, Schaap PJ, van de Vondervoort PJ, Culley D, Thykaer J, Frisvad JC, Nielsen KF, Albang R, Albermann K, Berka RM, Braus GH, Braus-Stromeyer SA, Corrochano LM, Dai Z, van Dijck PW, Hofmann G, Lasure LL, Magnuson JK, Menke H, Meijer M, Meijer SL, Nielsen JB, Nielsen ML, van Ooyen AJ, Pel HJ, Poulsen L, Samson RA, Stam H, Tsang A, van den Brink JM, Atkins A, Aerts A, Shapiro H, Pangilinan J, Salamov A, Lou Y, Lindquist E, Lucas S, Grimwood J, Grigoriev IV, Kubicek CP, Martinez D, van Peij NN, Roubos JA, Nielsen J, Baker SE (2011) Comparative genomics of citric-acid-producing Aspergillus niger ATCC 1015 versus enzyme-producing CBS 513.88. Genome Research 21 (6):885-897. doi:10.1101/gr.112169.110

Anisimova M, Gascuel O (2006) Approximate Likelihood-Ratio Test for Branches: A Fast, Accurate, and Powerful Alternative. Systematic Biology 55 (4):539-552. doi:10.1080/10635150600755453

Anisimova M, Gil M, Dufayard J-F, Dessimoz C, Gascuel O (2011) Survey of Branch Support Methods Demonstrates Accuracy, Power, and Robustness of Fast

Likelihood-based Approximation Schemes. Systematic Biology 60 (5):685-699. doi:10.1093/sysbio/syr041

Anteneh YS, Brown MH, Franco CMM (2019) Characterization of a Halotolerant Fungus from a Marine Sponge. BioMed Research International 2019:3456164. doi:10.1155/2019/3456164

Arai M, Niikawa H, Kobayashi M (2013) Marine-derived fungal sesterterpenes, ophiobolins, inhibit biofilm formation of Mycobacterium species. Journal of Natural Medicines 67 (2):271-275. doi:10.1007/s11418-012-0676-5

Araki Y, Awakawa T, Matsuzaki M, Cho R, Matsuda Y, Hoshino S, Shinohara Y, Yamamoto M, Kido Y, Inaoka DK, Nagamune K, Ito K, Abe I, Kita K (2019) Complete biosynthetic pathways of ascofuranone and ascochlorin in Acremonium egyptiacum. Proceedings of the National Academy of Sciences 116 (17):8269. doi:10.1073/pnas.1819254116

Argoudelis AD, Dietz A, Johnson LE (1974) Zervamicins I and II, polypeptide antibiotics produced by Emericellopsis salmosynnemata. The Journal of Antibiotics 27 (5):321-328. doi:10.7164/antibiotics.27.321

Argoudelis AD, Johnson LE (1974) Emerimicins II, 3 and IV, antibiotics produced by Emericellopsis microspora in media supplemented with trans-4-n-propyl-L-proline. The Journal of Antibiotics 27 (4):274-282. doi:10.7164/antibiotics.27.274

Argoudelis AD, Mizsak SA, Baczynskyj L (1975) N-acetyl-L-phenylalanyl-L-phenylalaninol a metabolite of Emericellopsis salmosynnemata. The Journal of Antibiotics 28 (10):733-736. doi:10.7164/antibiotics.28.733

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. Journal of Computational Biology 19 (5):455-477. doi:10.1089/cmb.2012.0021

Baral HO, Rämä T (2015) Morphological update on Calycina marina (Pezizellaceae, Helotiales, Leotiomycetes), a new combination for Laetinaevia marina. Botanica Marina 58 (6):523-534. doi:10.1515/bot-2015-0049

Baranova AA, Rogozhin EA, Georgieva ML, Bilanenko EN, Kul'ko AB, Yakushev AV, Alferova VA, Sadykova VS (2019) Antimicrobial Peptides Produced by Alkaliphilic Fungi Emericellopsis alkalina: Biosynthesis and Biological Activity Against Pathogenic Multidrug-Resistant Fungi. Applied Biochemistry and Microbiology 55 (2):145-151. doi:10.1134/S0003683819020030

Barbeyron T, Brillet-Guéguen L, Carré W, Carrière C, Caron C, Czjzek M, Hoebeke M, Michel G (2016) Matching the Diversity of Sulfated Biomolecules: Creation of a Classification Database for Sulfatases Reflecting Their Substrate Specificity. PLoS ONE 11 (10):e0164846. doi:10.1371/journal.pone.0164846

Barbosa AI, Coutinho AJ, Costa Lima SA, Reis S (2019) Marine Polysaccharides in Pharmaceutical Applications: Fucoidan and Chitosan as Key Players in the Drug Delivery Match Field. Marine Drugs 17 (12). doi:10.3390/md17120654

Batista-García RA, Sutton T, Jackson SA, Tovar-Herrera OE, Balcázar-López E, Sánchez-Carbente MDR, Sánchez-Reyes A, Dobson ADW, Folch-Mallol JL (2017) Characterization of lignocellulolytic activities from fungi isolated from the deep-sea sponge Stelletta normani. PLoS ONE 12 (3):e0173750. doi:10.1371/journal.pone.0173750

Beliakova L (1974) Genus Emericellopsis van Beyma (Eurotiaceae). Mikologiia i Fitopatologiia 8:385-394

Berteau O, McCort I, Goasdoué N, Tissot B, Daniel R (2002) Characterization of a new alpha-L-fucosidase isolated from the marine mollusk Pecten maximus that catalyzes the hydrolysis of alpha-L-fucose from algal fucoidan (Ascophyllum nodosum). Glycobiology 12 (4):273-282. doi:10.1093/glycob/12.4.273

Bills GF, Platas G, Gams W (2004) Conspecificity of the cerulenin and helvolic acid producing 'Cephalosporium caerulens', and the hypocrealean fungus Sarocladium oryzae. Mycological Research 108 (Pt 11):1291-1300. doi:10.1017/s0953756204001297

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T (2019) antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Research 47 (W1):W81-W87. doi:10.1093/nar/gkz310

Boraston AB, Bolam DN, Gilbert HJ, Davies GJ (2004) Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem J 382 (Pt 3):769-781. doi:10.1042/BJ20040892

Buchfink B, Xie C, Huson DH (2015) Fast and sensitive protein alignment using DIAMOND. Nature Methods 12 (1):59-60. doi:10.1038/nmeth.3176

Bushnell B (2014) BBTools software package.

Busk PK, Pilgaard B, Lezyk MJ, Meyer AS, Lange L (2017) Homology to peptide pattern for annotation of carbohydrate-active enzymes and prediction of function. BMC Bioinformatics 18 (1):214. doi:10.1186/s12859-017-1625-9

Cammann J, Denzel K, Schilling G, Gross GG (1989) Biosynthesis of gallotannins: β-Glucogallin-dependent formation of 1,2,3,4,6-pentagalloylglucose by enzymatic galloylation of 1,2,3,6-tetragalloylglucose. Archives of Biochemistry and Biophysics 273 (1):58-63. doi:https://doi.org/10.1016/0003-9861(89)90161-6

Carroll AR, Copp BR, Davis RA, Keyzers RA, Prinsep MR (2020) Marine natural products. Natural Product Reports 37 (2):175-223. doi:10.1039/c9np00069k

Cole M, Rolinson GN (1961) 6-Aminopenicillanic acid. II. Formation of 6-aminopenicillanic acid by Emericellopsis minima (Stolk) and related fungi. Proceedings of the Royal Society B: Biological Sciences 154:490-497. doi:10.1098/rspb.1961.0046

Collén PN, Jeudy A, Sassi J-F, Groisillier A, Czjzek M, Coutinho PM, Helbert W (2014) A novel unsaturated β-glucuronyl hydrolase involved in ulvan degradation unveils the versatility of stereochemistry requirements in family GH105. The Journal of biological chemistry 289 (9):6199-6211. doi:10.1074/jbc.M113.537480

Crous PW, Groenewald JZ, Verkley GJM, Houbraken J, Institute WFB, Cock AWAM, Aime C, Centre C-KFB (2019) Fungal Biodiversity. Westerdijk Fungal Biodiversity Institute,

Darriba D, Weiß M, Stamatakis A (2016) Prediction of missing sequences and branch lengths in phylogenomic data. Bioinformatics 32 (9):1331-1337. doi:10.1093/bioinformatics/btv768

Davidson DE, Christensen M (1971) Emericellopsis stolkiae sp.nov. from saline soils in Wyoming. Transactions of the British Mycological Society 57 (3):385-IN383. doi:https://doi.org/10.1016/S0007-1536(71)80053-0

de Castro E, Sigrist CJ, Gattiker A, Bulliard V, Langendijk-Genevaux PS, Gasteiger E, Bairoch A, Hulo N (2006) ScanProsite: detection of PROSITE signature matches and ProRule-associated functional and structural residues in proteins. Nucleic Acids Research 34 (Web Server issue):W362-365. doi:10.1093/nar/gkl124

de Mattos-Shipley Kate MJ, Greco C, Heard DM, Hough G, Mulholland NP, Vincent JL, Micklefield J, Simpson TJ, Willis CL, Cox RJ, Bailey AM (2018) The cycloaspeptides: uncovering a new model for methylated nonribosomal peptide biosynthesis. Chemical Science 9 (17):4109-4117. doi:10.1039/C8SC00717A

Degenkolb T, Berg A, Gams W, Schlegel B, Gräfe U (2003) The occurrence of peptaibols and structurally related peptaibiotics in fungi and their mass spectrometric identification via diagnostic fragment ions. Journal of Peptide Science 9 (11- 12):666-678. doi:https://doi.org/10.1002/psc.497

Degenkolb T, Brückner H (2008) Peptaibiomics: Towards a Myriad of Bioactive Peptides Containing Cα-Dialkylamino Acids? Chemistry & Biodiversity 5 (9):1817-1843. doi:https://doi.org/10.1002/cbdv.200890171

Desikachary TV, Dweltz N The chemical composition of the diatom frustule. In: Proceedings of the Indian Academy of Sciences-Section B, 1961. vol 4. Springer, pp 157-165

Dobrinčić A, Balbino S, Zorić Z, Pedisić S, Bursać Kovačević D, Elez Garofulić I, Dragović-Uzelac V (2020) Advanced Technologies for the Extraction of Marine Brown Algal Polysaccharides. Marine Drugs 18 (3). doi:10.3390/md18030168

Eddy SR (2020) HMMER: biosequence analysis using profile hidden Markov models. HMMER development team. http://hmmer.org/. 2020

Fouillaud M, Venkatachalam M, Llorente M, Magalon H, Cuet P, Dufossé L (2017) Biodiversity of Pigmented Fungi Isolated from Marine Environment in La Réunion Island, Indian Ocean: New Resources for Colored Metabolites. Journal of Fungi 3:36. doi:10.3390/jof3030036

Gilchrist CLM, Chooi Y-H (2020) clinker and clustermap.js: Automatic generation of gene cluster comparison figures. bioRxiv:2020.2011.2008.370650. doi:10.1101/2020.11.08.370650

Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG (1996) Life with 6000 Genes. Science 274 (5287):546. doi:10.1126/science.274.5287.546

Gonçalves MFM, Vicente TFL, Esteves AC, Alves A (2020) Novel halotolerant species of Emericellopsis and Parasarocladium associated with macroalgae in an estuarine environment. Mycologia 112 (1):154-171. doi:10.1080/00275514.2019.1677448

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature biotechnology 29 (7):644-652. doi:10.1038/nbt.1883

Grigoriev IV, Cullen D, Goodwin SB, Hibbett D, Jeffries TW, Kubicek CP, Kuske C, Magnuson JK, Martin F, Spatafora JW, Tsang A, Baker SE (2011) Fueling the future with fungal genomics. Mycology 2 (3):192-209. doi:10.1080/21501203.2011.584577

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F, Smirnova T, Nordberg H, Dubchak I, Shabalov I (2014) MycoCosm portal: Gearing up for 1000 fungal genomes. Nucleic Acids Research 42:D699. doi:10.1093/nar/gkt1183

Grum-Grzhimaylo AA, Georgieva ML, Debets AJM, Bilanenko EN (2013) Are alkalitolerant fungi of the Emericellopsis lineage (Bionectriaceae) of marine origin? IMA Fungus 4 (2):213-228. doi:10.5598/imafungus.2013.04.02.07

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. Systematic Biology 59 (3):307-321. doi:10.1093/sysbio/syq010

Haefner B (2003) Drugs from the deep: marine natural products as drug candidates. Drug Discovery Today 8 (12):536-544. doi:https://doi.org/10.1016/S1359-6446(03)02713-2

Hehemann J-H, Truong LV, Unfried F, Welsch N, Kabisch J, Heiden SE, Junker S, Becher D, Thürmer A, Daniel R, Amann R, Schweder T (2017) Aquatic adaptation of a laterally acquired pectin degradation pathway in marine gammaproteobacteria. Environmental Microbiology 19 (6):2320-2333. doi:10.1111/1462-2920.13726

Helbert W (2017) Marine Polysaccharide Sulfatases. Frontiers in Marine Science 4 (6). doi:10.3389/fmars.2017.00006

Hobbs JK, Hettle AG, Vickers C, Boraston AB (2019) Biochemical Reconstruction of a Metabolic Pathway from a Marine Bacterium Reveals Its Mechanism of Pectin Depolymerization. Applied and Environmental Microbiology 85 (1):e02114-02118. doi:10.1128/AEM.02114-18

Hsiao G, Wang S-W, Chiang Y-R, Chi W-C, Kuo Y-H, Phong D, Chen C-Y, Lee T-H (2020) Anti-inflammatory effects of peptides from a marine algicolous fungus Acremonium sp. NTU492 in BV-2 microglial cells. Journal of Food and Drug Analysis 28 (2):89-97. doi:10.38212/2224-6614.1062

Huang Q, Rodgers JM, Hemley RJ, Ichiye T (2017) Extreme biophysics: Enzymes under pressure. J Comput Chem 38 (15):1174-1182. doi:10.1002/jcc.24737

Hunter S, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, Bork P, Das U, Daugherty L, Duquenne L, Finn RD, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Laugraud A, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, Mistry J, Mitchell A, Mulder N, Natale D, Orengo C, Quinn AF, Selengut JD, Sigrist CJ, Thimma M, Thomas PD, Valentin F, Wilson D, Wu CH, Yeats C (2009) InterPro: the integrative protein signature database. Nucleic Acids Research 37 (Database issue):D211-215. doi:10.1093/nar/gkn785

Ishiyama D, Satou T, Senda H, Fujimaki T, Honda R, Kanazawa S (2000) Heptaibin, a Novel Antifungal Peptaibol Antibiotic from Emericellopsis sp. BAUA8289. The Journal of Antibiotics 53:728-732. doi:10.7164/antibiotics.53.728

Jackson SA, Sutton T, Bromley CL, Young RM, O'Gara F, Baker BJ, Dobson ADW (2016) Potent antibacterial fungi isolated from a deep-sea sponge. In: 11th International Marine Biotechnology Conference, BALTIMORE, MARYLAND, 2016. vol 1. International Marine Biotechnology Association and Institute of Marine and Environmental Technology, p 68

Jacob J (2012) Classification of Halophilic Heterotrophic Bacteria Thriving in the Jordanian Dead Sea Littoral Zone. Journal of Biological Sciences 12:246-252. doi:10.3923/jbs.2012.246.252

Johnston PR, Quijada L, Smith CA, Baral H-O, Hosoya T, Baschien C, Pärtel K, Zhuang W-Y, Haelewaters D, Park D, Carl S, López-Giráldez F, Wang Z, Townsend JP (2019) A multigene phylogeny toward a new phylogenetic classification of Leotiomycetes. IMA Fungus 10 (1):1. doi:10.1186/s43008-019-0002-x

Jormalainen V, Honkanen T, Koivikko R, Eränen J (2003) Induction of phlorotannin production in a brown alga: Defense or resource dynamics? Oikos 103:640-650. doi:10.1034/j.1600-0706.2003.12635.x

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14 (6):587-589. doi:10.1038/nmeth.4285

Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M (2012) KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Research 40 (Database issue):D109-114. doi:10.1093/nar/gkr988

Kappelmann L, Krüger K, Hehemann J-H, Harder J, Markert S, Unfried F, Becher D, Shapiro N, Schweder T, Amann RI, Teeling H (2019) Polysaccharide utilization loci of North Sea Flavobacteriia as basis for using SusC/D-protein expression for predicting major phytoplankton glycans. The ISME journal 13 (1):76-91. doi:10.1038/s41396-018-0242-6

Katoh K, Misawa K, Kuma Ki, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30 (14):3059-3066. doi:10.1093/nar/gkf436

Katoh K, Standley DM (2013) MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30 (4):772–780. doi:10.1093/molbev/mst010

Kennedy J, Flemer B, Jackson SA, Morrissey JP, O'Gara F, Dobson ADW (2014) Evidence of a Putative Deep Sea Specific Microbiome in Marine Sponges. PLoS ONE 9 (3):e91092. doi:10.1371/journal.pone.0091092

Khan NT (2017) Cephalosporin C Production from Acremonium chrysogenum. Enzyme Engineering 6 (1). doi:10.4172/2329-6674.1000159

Kis-Papo T, Weig AR, Riley R, Peršoh D, Salamov A, Sun H, Lipzen A, Wasser SP, Rambold G, Grigoriev IV, Nevo E (2014) Genomic adaptations of the halophilic Dead Sea filamentous fungus Eurotium rubrum. Nature Communications 5 (1):3745. doi:10.1038/ncomms4745

Klix V, Nowrousian M, Ringelberg C, Loros JJ, Dunlap JC, Pöggeler S (2010) Functional characterization of MAT1-1-specific mating-type genes in the homothallic ascomycete Sordaria macrospora provides new insights into essential and nonessential sexual regulators. Eukaryotic Cell 9 (6):894-905. doi:10.1128/EC.00019-10

Kohlmeyer J, Kohlmeyer E (1979) Marine Mycology, the Higher Fungi. Marine Ecology, 1 edn. Academic Press, New York

Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson K-H (2013) Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22 (21):5271-5277. doi:10.1111/mec.12481

Konstantinidis KT, Tiedje JM (2004) Trends between gene content and genome size in prokaryotic species with larger genomes. Proceedings of the National Academy of Sciences of the United States of America 101 (9):3160-3165. doi:10.1073/pnas.0308653100

Koonin EV, Fedorova ND, Jackson JD, Jacobs AR, Krylov DM, Makarova KS, Mazumder R, Mekhedov SL, Nikolskaya AN, Rao BS, Rogozin IB, Smirnov S, Sorokin AV, Sverdlov AV, Vasudevan S, Wolf YI, Yin JJ, Natale DA (2004) A

comprehensive evolutionary classification of proteins encoded in complete eukaryotic genomes. Genome Biology 5 (2):R7. doi:10.1186/gb-2004-5-2-r7

Krogh A, Larsson B, von Heijne G, Sonnhammer EL (2001) Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. Journal of molecular biology 305 (3):567-580. doi:10.1006/jmbi.2000.4315

Lanfear R, Calcott B, Ho SY, Guindon S (2012) Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Molecular Biology and Evolution 29 (6):1695-1701. doi:10.1093/molbev/mss020

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: New Methods for Selecting Partitioned Models of Evolution for Molecular and Morphological Phylogenetic Analyses. Molecular Biology and Evolution 34 (3):772–773. doi:10.1093/molbev/msw260

Lauritano C, Andersen J, Hansen E, Albrigtsen M, Escalera L, Esposito F, Helland K, Hanssen K, Romano G, Ianora A (2016) Bioactivity Screening of Microalgae for Antioxidant, Anti-Inflammatory, Anticancer, Anti-Diabetes, and Antibacterial Activities. Frontiers in Marine Science 3. doi:10.3389/fmars.2016.00068

Lee S-Y, Kinoshita H, Ihara F, Igarashi Y, Nihira T (2008) Identification of novel derivative of helvolic acid from Metarhizium anisopliae grown in medium with insect component. Journal of Bioscience and Bioengineering 105 (5):476-480. doi:https://doi.org/10.1263/jbb.105.476

Lefort V, Longueville J-E, Gascuel O (2017) SMS: Smart Model Selection in PhyML. Molecular Biology and Evolution 34 (9):2422-2424. doi:10.1093/molbev/msx149

Lei XG, Weaver JD, Mullaney E, Ullah AH, Azain MJ (2013) Phytase, a New Life for an "Old" Enzyme. Annual Review of Animal Biosciences 1 (1):283-309. doi:10.1146/annurev-animal-031412-103717

Li X-Q, Du D (2014) Variation, Evolution, and Correlation Analysis of C+G Content and Genome or Chromosome Size in Different Kingdoms and Phyla. PLoS ONE 9 (2):e88339. doi:10.1371/journal.pone.0088339

Lind KF, Hansen E, Østerud B, Eilertsen K-E, Bayer A, Engqvist M, Leszczak K, Jørgensen TØ, Andersen JH (2013) Antioxidant and Anti-Inflammatory Activities of Barettin. Marine Drugs 11 (7). doi:10.3390/md11072655

Lv J-M, Hu D, Gao H, Kushiro T, Awakawa T, Chen G-D, Wang C-X, Abe I, Yao X-S (2017) Biosynthesis of helvolic acid and identification of an unusual C-4-demethylation process distinct from sterol biosynthesis. Nature Communications 8 (1):1644. doi:10.1038/s41467-017-01813-9

Lücking R, Aime MC, Robbertse B, Miller AN, Ariyawansa HA, Aoki T, Cardinali G, Crous PW, Druzhinina IS, Geiser DM, Hawksworth DL, Hyde KD, Irinyi L, Jeewon R, Johnston PR, Kirk PM, Malosso E, May TW, Meyer W, Öpik M, Robert V, Stadler M, Thines M, Vu D, Yurkov AM, Zhang N, Schoch CL (2020) Unambiguous identification of fungi: where do we stand and how accurate and precise is fungal DNA barcoding? IMA Fungus 11 (1):14. doi:10.1186/s43008-020-00033-z

Mann S, Chen YP (2010) Bacterial genomic G+C composition-eliciting environmental adaptation. Genomics 95 (1):7-15. doi:10.1016/j.ygeno.2009.09.002

Michel G, Nyval-Collen P, Barbeyron T, Czjzek M, Helbert W (2006) Bioconversion of red seaweed galactans: a focus on bacterial agarases and carrageenases. Applied microbiology and biotechnology 71 (1):23-33. doi:10.1007/s00253-006-0377-7

Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast Approximation for Phylogenetic Bootstrap. Molecular Biology and Evolution 30 (5):1188-1195. doi:10.1093/molbev/mst024

Mitsuguchi H, Seshime Y, Fujii I, Shibuya M, Ebizuka Y, Kushiro T (2009) Biosynthesis of steroidal antibiotic fusidanes: functional analysis of oxidosqualene cyclase and subsequent tailoring enzymes from Aspergillus fumigatus. Journal of the American Chemical Society 131 (18):6402-6411. doi:10.1021/ja8095976

Murillo FJ, Muñoz PD, Cristobo J, Ríos P, González C, Kenchington E, Serrano A (2012) Deep-sea sponge grounds of the Flemish Cap, Flemish Pass and the Grand Banks of Newfoundland (Northwest Atlantic Ocean): Distribution and species composition. Marine Biology Research 8 (9):842-854. doi:10.1080/17451000.2012.682583

Muszewska A, Steczkiewicz K, Stepniewska-Dziubinska M, Ginalski K (2017) Cut-and-Paste Transposons in Fungi with Diverse Lifestyles. Genome Biol Evol 9 (12):3463-3477. doi:10.1093/gbe/evx261

Naranjo-Ortiz MA, Gabaldón T (2019) Fungal evolution: major ecological adaptations and evolutionary transitions. Biological Reviews 94 (4):1443-1476. doi:10.1111/brv.12510

NCBI (2021) Genome List - Genome - NCBI. National Library of Medicine (US), National Center for Biotechnology Information. https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/. Accessed 7/1-21 2021

NCBI Resource Coordinators (2018) Database resources of the National Center for Biotechnology Information. Nucleic Acids Research 46 (D1):D8-d13. doi:10.1093/nar/gkx1095

Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2014) IQ-TREE: A Fast and Effective Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular Biology and Evolution 32 (1):268-274. doi:10.1093/molbev/msu300

Nishida H (2015) Relationship between chromosomal GC content and isoelectric points of histones in fungi. The Journal of General and Applied Microbiology 61 (1):24-26. doi:10.2323/jgam.61.24

Núñez-Pons L, Avila C, Romano G, Verde C, Giordano D (2018) UV-Protective Compounds in Marine Organisms from the Southern Ocean. Marine Drugs 16 (9):336. doi:10.3390/md16090336

Oey I (2016) Effects of High Pressure on Enzymes. In: Balasubramaniam VM, Barbosa-Cánovas GV, Lelieveld HLM (eds) High Pressure Processing of Food: Principles, Technology and Applications. Springer New York, New York, NY, pp 391-431. doi:10.1007/978-1-4939-3234-4_19

Ovodova RG, Vaskovsky VE, Ovodov YS (1968) The pectic substances of zosteraceae. Carbohydrate Research 6 (3):328-332. doi:https://doi.org/10.1016/S0008-6215(00)81454-8

Palmer JM, Drees KP, Foster JT, Lindner DL (2018) Extreme sensitivity to ultraviolet light in the fungal pathogen causing white-nose syndrome of bats. Nature Communications 9 (1):35. doi:10.1038/s41467-017-02441-z

Pang K-L, Overy DP, Jones EBG, Calado MdL, Burgaud G, Walker AK, Johnson JA, Kerr RG, Cha H-J, Bills GF (2016) 'Marine fungi' and 'marine-derived fungi' in natural product chemistry research: Toward a new consensual definition. Fungal Biology Reviews 30 (4):163-175. doi:10.1016/j.fbr.2016.08.001

Parra G, Bradnam K, Korf I (2007) CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23 (9):1061-1067. doi:10.1093/bioinformatics/btm071

Parra G, Bradnam K, Ning Z, Keane T, Korf I (2009) Assessing the gene space in draft genomes. Nucleic Acids Research 37 (1):289-297. doi:10.1093/nar/gkn916

Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nature Methods 8 (10):785-786. doi:10.1038/nmeth.1701

Pinedo C, Wang CM, Pradier JM, Dalmais B, Choquer M, Le Pêcheur P, Morgant G, Collado IG, Cane DE, Viaud M (2008) Sesquiterpene synthase from the botrydial biosynthetic gene cluster of the phytopathogen Botrytis cinerea. ACS Chemical Biology 3 (12):791-801. doi:10.1021/cb800225v

Pinheiro Â, Dethoup T, Bessa J, Silva AMS, Kijjoa A (2012) A new bicyclic sesquiterpene from the marine sponge associated fungus Emericellopsis minima. Phytochemistry Letters 5 (1):68-70. doi:https://doi.org/10.1016/j.phytol.2011.10.002

Raghukumar S, Raghukumar C (1999) Marine fungi: A critique. Aquatic Microbiology Newsletter 38:26-27

Rédou V, Navarri M, Meslet-Cladière L, Barbier G, Burgaud G (2015) Species Richness and Adaptation of Marine Fungi from Deep-Subseafloor Sediments. Applied and Environmental Microbiology 81 (10):3571-3583. doi:10.1128/AEM.04064-14

Reich M, Labes A (2017) How to boost marine fungal research: A first step towards a multidisciplinary approach by combining molecular fungal ecology and natural products chemistry. Marine Genomics 36:57–75. doi:10.1016/j.margen.2017.09.007

Reisky L, Préchoux A, Zühlke M-K, Bäumgen M, Robb CS, Gerlach N, Roret T, Stanetty C, Larocque R, Michel G, Song T, Markert S, Unfried F, Mihovilovic MD, Trautwein-Schult A, Becher D, Schweder T, Bornscheuer UT, Hehemann J-H (2019) A marine bacterial enzymatic cascade degrades the algal polysaccharide ulvan. Nature Chemical Biology 15 (8):803-812. doi:10.1038/s41589-019-0311-9

Robey MT, Caesar LK, Drott MT, Keller NP, Kelleher NL (2020) An Interpreted Atlas of Biosynthetic Gene Clusters from 1000 Fungal Genomes. bioRxiv:2020.2009.2021.307157. doi:10.1101/2020.09.21.307157

Rogozhin EA, Sadykova VS, Baranova AA, Vasilchenko AS, Lushpa VA, Mineev KS, Georgieva ML, Kul'ko AB, Krasheninnikov ME, Lyundup AV, Vasilchenko AV, Andreev YA (2018) A Novel Lipopeptaibol Emericellipsin A with Antimicrobial and Antitumor Activity Produced by the Extremophilic Fungus Emericellopsis alkalina. Molecules (Basel, Switzerland) 23 (11):2785. doi:10.3390/molecules23112785

Rokas A, Wisecaver JH, Lind AL (2018) The birth, evolution and death of metabolic gene clusters in fungi. Nature Reviews Microbiology 16 (12):731-744. doi:10.1038/s41579-018-0075-3

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61 (3):539–542. doi:10.1093/sysbio/sys029

Rämä T, Mathiassen G, Kauserud H (2014) Marine fungi new to Norway, with an outlook to the overall diversity. Agarica 35:35-47

Salminen J-P, Ossipov V, Pihlaja K (2002) Distribution Of Hydrolysable Tannins In The Foliage Of Finnish Birch Species. Zeitschrift für Naturforschung C, Journal of Biosciences 57:248-256. doi:10.1515/znc-2002-3-409

Schneider Y, Jenssen M, Isaksson J, Hansen KØ, Andersen JH, Hansen EH (2020) Bioactivity of Serratiochelin A, a Siderophore Isolated from a Co-Culture of Serratia sp. and Shewanella sp. Microorganisms 8 (7). doi:10.3390/microorganisms8071042

Schneider YKH, Hansen KØ, Isaksson J, Ullsten S, Hansen EH, Andersen JH (2019) Anti-Bacterial Effect and Cytotoxicity Assessment of Lipid 430 Isolated from Algibacter sp. Molecules 24 (21). doi:10.3390/molecules24213991

Schor R, Schotte C, Wibberg D, Kalinowski J, Cox RJ (2018) Three previously unrecognised classes of biosynthetic enzymes revealed during the production of xenovulene A. Nature Communications 9 (1):1963-1963. doi:10.1038/s41467-018-04364-9

Schultz-Johansen M, Bech PK, Hennessy RC, Glaring MA, Barbeyron T, Czjzek M, Stougaard P (2018) A Novel Enzyme Portfolio for Red Algal Polysaccharide Degradation in the Marine Bacterium Paraglaciecola hydrolytica S66T Encoded in a Sizeable Polysaccharide Utilization Locus. Frontiers in Microbiology 9 (839). doi:10.3389/fmicb.2018.00839

Sharma K (2015) Fungal genome sequencing: basic biology to biotechnology. Critical Reviews in Biotechnology 36 (4):743-759. doi:10.3109/07388551.2015.1015959

Sigrist CJ, Cerutti L, Hulo N, Gattiker A, Falquet L, Pagni M, Bairoch A, Bucher P (2002) PROSITE: a documented database using patterns and profiles as motif descriptors. Brief Bioinform 3 (3):265-274. doi:10.1093/bib/3.3.265

Smith DC, Simon M, Alldredge AL, Azam F (1992) Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. Nature 359 (6391):139-142. doi:10.1038/359139a0

Soanes DM, Alam I, Cornell M, Wong HM, Hedeler C, Paton NW, Rattray M, Hubbard SJ, Oliver SG, Talbot NJ (2008) Comparative Genome Analysis of Filamentous Fungi Reveals Gene Family Expansions Associated with Fungal Pathogenesis. PLoS ONE 3 (6):e2300. doi:10.1371/journal.pone.0002300

Sollas WJ (1880) The sponge-fauna of Norway; a Report on the Rev. A.M. Norman' s Collection of Sponges from the Norwegian Coast. The Annals and magazine of natural history; zoology, botany, and geology 5 (27):130-144

Sotero-Caio CG, Platt RN, Suh A, Ray DA (2017) Evolution and Diversity of Transposable Elements in Vertebrate Genomes. Genome Biol Evol 9 (1):161-177. doi:10.1093/gbe/evw264

Stolk AC (1955) Emericellopsis minima sp.nov. and Westerdykella ornata gen.nov., sp.nov. Transactions of the British Mycological Society 38 (4):419-IN412. doi:https://doi.org/10.1016/S0007-1536(55)80046-0

Storck R (1966) Nucleotide composition of nucleic acids of fungi. II. Deoxyribonucleic acids. Journal of Bacteriology 91 (1):227-230. doi:10.1128/JB.91.1.227-230.1966

Strobel GA, Torczynski R, Bollon A (1997) Acremonium sp.—a leucinostatin A producing endophyte of European yew (Taxus baccata). Plant Science 128 (1):97-108. doi:https://doi.org/10.1016/S0168-9452(97)00131-3

Summerbell RC, Gueidan C, Schroers HJ, de Hoog GS, Starink M, Rosete YA, Guarro J, Scott JA (2011) Acremonium phylogenetic overview and revision of

Gliomastix, Sarocladium, and Trichothecium. Studies in Mycology 68:139-162. doi:10.3114/sim.2011.68.06

Terfehr D, Dahlmann TA, Specht T, Zadra I, Kürnsteiner H, Kück U (2014) Genome Sequence and Annotation of Acremonium chrysogenum, Producer of the β-Lactam Antibiotic Cephalosporin C. Genome Announc 2 (5):e00948-00914. doi:10.1128/genomeA.00948-14

Thines M, Aoki T, Crous PW, Hyde KD, Lücking R, Malosso E, May TW, Miller AN, Redhead SA, Yurkov AM, Hawksworth DL (2020) Setting scientific names at all taxonomic ranks in italics facilitates their quick recognition in scientific papers. IMA Fungus 11 (1):25. doi:10.1186/s43008-020-00048-6

Trincone A (2018) Update on Marine Carbohydrate Hydrolyzing Enzymes: Biotechnological Applications. Molecules 23 (4). doi:10.3390/molecules23040901

Turley C (2000) Bacteria in the cold deep-sea benthic boundary layer and sediment—water interface of the NE Atlantic. FEMS Microbiology Ecology 33 (2):89-99. doi:10.1111/j.1574-6941.2000.tb00731.x

Tørresen OK, Star B, Mier P, Andrade-Navarro MA, Bateman A, Jarnot P, Gruca A, Grynberg M, Kajava AV, Promponas VJ, Anisimova M, Jakobsen KS, Linke D (2019) Tandem repeats lead to sequence assembly errors and impose multi-level challenges for genome and protein databases. Nucleic Acids Research 47 (21):10994-11006. doi:10.1093/nar/gkz841

UniProt Consortium (2013) Update on activities at the Universal Protein Resource (UniProt) in 2013. Nucleic Acids Research 41 (Database issue):D43-47. doi:10.1093/nar/gks1068

van Noort V, Bradatsch B, Arumugam M, Amlacher S, Bange G, Creevey C, Falk S, Mende DR, Sinning I, Hurt E, Bork P (2013) Consistent mutational paths predict eukaryotic thermostability. BMC Evolutionary Biology 13 (1):7. doi:10.1186/1471-2148-13-7

Vickers C, Liu F, Abe K, Salama-Alber O, Jenkins M, Springate CMK, Burke JE, Withers SG, Boraston AB (2018) Endo-fucoidan hydrolases from glycoside hydrolase family 107 (GH107) display structural and mechanistic similarities to α-l-fucosidases from GH29. The Journal of biological chemistry 293 (47):18296-18308. doi:10.1074/jbc.RA118.005134

Walz M, Kück U (1991) Polymorphic karyotypes in related Acremonium strains. Current genetics 19 (2):73-76. doi:10.1007/BF00326285

Wang G, Liu Z, Lin R, Li E, Mao Z, Ling J, Yang Y, Yin WB, Xie B (2016) Biosynthesis of Antibiotic Leucinostatins in Bio-control Fungus Purpureocillium lilacinum and Their Inhibition on Phytophthora Revealed by Genome Mining. PLoS Pathog 12 (7):e1005685. doi:10.1371/journal.ppat.1005685

Wieloch W (2006) Chromosome visualisation in filamentous fungi. Journal of Microbiological Methods 67 (1):1-8. doi:https://doi.org/10.1016/j.mimet.2006.05.022

Wiens JJ (2006) Missing data and the design of phylogenetic analyses. Journal of Biomedical Informatics 39 (1):34-42. doi:https://doi.org/10.1016/j.jbi.2005.04.001

Wilken SE, Seppälä S, Lankiewicz TS, Saxena M, Henske JK, Salamov AA, Grigoriev IV, O'Malley MA (2020) Genomic and proteomic biases inform metabolic engineering strategies for anaerobic fungi. Metabolic Engineering Communications 10:e00107. doi:https://doi.org/10.1016/j.mec.2019.e00107

Xu X, Han J, Wang Y, Lin R, Yang H, Li J, Wei S, Polyak S, Song F (2019) Two New Spiro-Heterocyclic γ-Lactams from A Marine-Derived Aspergillus fumigatus Strain CUGBMF170049. Marine Drugs 17:289. doi:10.3390/md17050289

Yakovchuk P, Protozanova E, Frank-Kamenetskii MD (2006) Base-stacking and base-pairing contributions into thermal stability of the DNA double helix. Nucleic Acids Research 34 (2):564-574. doi:10.1093/nar/gkj454

Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y (2018) DbCAN2: A meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Research 46:W95-W101. doi:10.1093/nar/gky418

Zhao Z, Liu H, Wang C, Xu J-R (2014) Correction: Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. BMC Genomics 15:6-6. doi:10.1186/1471-2164-15-6

Zuccaro A, Summerbell RC, Gams W, Schroers HJ, Mitchell JI (2004) A new Acremonium species associated with Fucus spp., and its affinity with a phylogenetically distinct marine Emericellopsis clade. Studies in Mycology 50 (1):283-297

Additional files

Supplementary data 1 – methods.docx

Full detailed description of all methods, including previously published methods.

Supplementary data 2 – sequencing (E. atlantica).rtf

Sequencing details and statistics regarding library and method for assembly for *E. atlantica*.

Supplementary data 3 – sequencing (C. marina).rtf

Sequencing details and statistics regarding library and method for assembly for *C. marina*.

Supplementary data 4 – sequencing (A. encephaloides).rtf

Sequencing details and statistics regarding library and method for assembly for *A. encephaloides*.

Supplementary data 5 – sequencing (mitochondrion).docx

Assembly details for organelles.

Supplementary data 6 – 6-gene phylogeny.xlsx

Overview of fungal accessions used with type status, isolation source and model selection for phylogenetic analysis.

Supplementary data 7 – PhyML-tree.pdf

Phylogenetic tree produced by PhyML webpage used as supporting data for figure 1.

Supplementary data 8 – media composition.xlsx

Overview of media components in the different media used in this study.

Supplementary data 9 – KEGG-KOG.xlsx

Overview of functional and enzymatic classes of genes

Supplementary data 10 – CAZymes.xlsx

Overview of detected classes, numbers of each class, number of genes with secretion signal.

Supplementary data 11 – BGCs of E. atlantica.rar

BGCs from *E. atlantica* as genbank files.

Supplementary data 12 – clinker.rar

Graphic output from synteny analysis of BGCs in *E. atlantica*.

Supplementary data 13 – BGCs for C. marina.rar

BGCs from *C. marina* as genbank files.

Supplementary data 14 – BGCs for A. encephaloides.rar

BGCs from *E. atlantica* as genbank files.

The supplementary files for Paper 2 can be accessed on this link before publication:

https://data.mendeley.com/datasets/xzhk7vnhdb/draft?a=90a7af97-678b-48b2-875b-d7410ab93a5b

If the link does not work, contact ole.c.hagestad@uit.no

Table 2

State	Medium	Frac. no.	Ext. met.	Cell prolif.	Immuno		Biofilm inhibition			Growth inhib. assay				
					IS	AIF	Form.	Estab.	Growth inh.	EF	EC	PA	SA	Str.B
Liquid	**YES**	5	SPE	-	-	-	-	-	-	-	-	-	-	-
Liquid	**MPM**	5	SPE	-	-	-	25	-	-	-	-	-	-	-
Liquid	**DMB**	5	SPE	-	10	-	25	-	-	-	-	-	-	-
Liquid	**SAPM**	5	SPE	-	-	-	25	-	-	-	-	-	-	-
Liquid	**SDPM**	5	SPE	-	-	-	-	-	-	-	-	-	-	-
Liquid	**F1**	-	EtOAc	-			-	n.t.	-	-	-	-	-	25
Liquid	**AF**	5	EtOAc	-	-	-	-	-	50	12.5	-	-	-	6.3
Liquid	**DPY**	5	EtOAc	-	-	-	-	-	50	25	-	-	-	6.3
Solid	**BRFT**	5	EtOAc	-	-	-	-	-	-	-	-	-	-	-
Solid	**Verm**	-	EtOAc	-	n.t.	n.t.	-	-	50	-	-	-	-	6.3
Solid	**MWM**	-	EtOAc	-	n.t.	n.t.	-	-	-	-	-	-	-	25

Figure 1

Click here to access/download;Figure;Figure 1.pdf

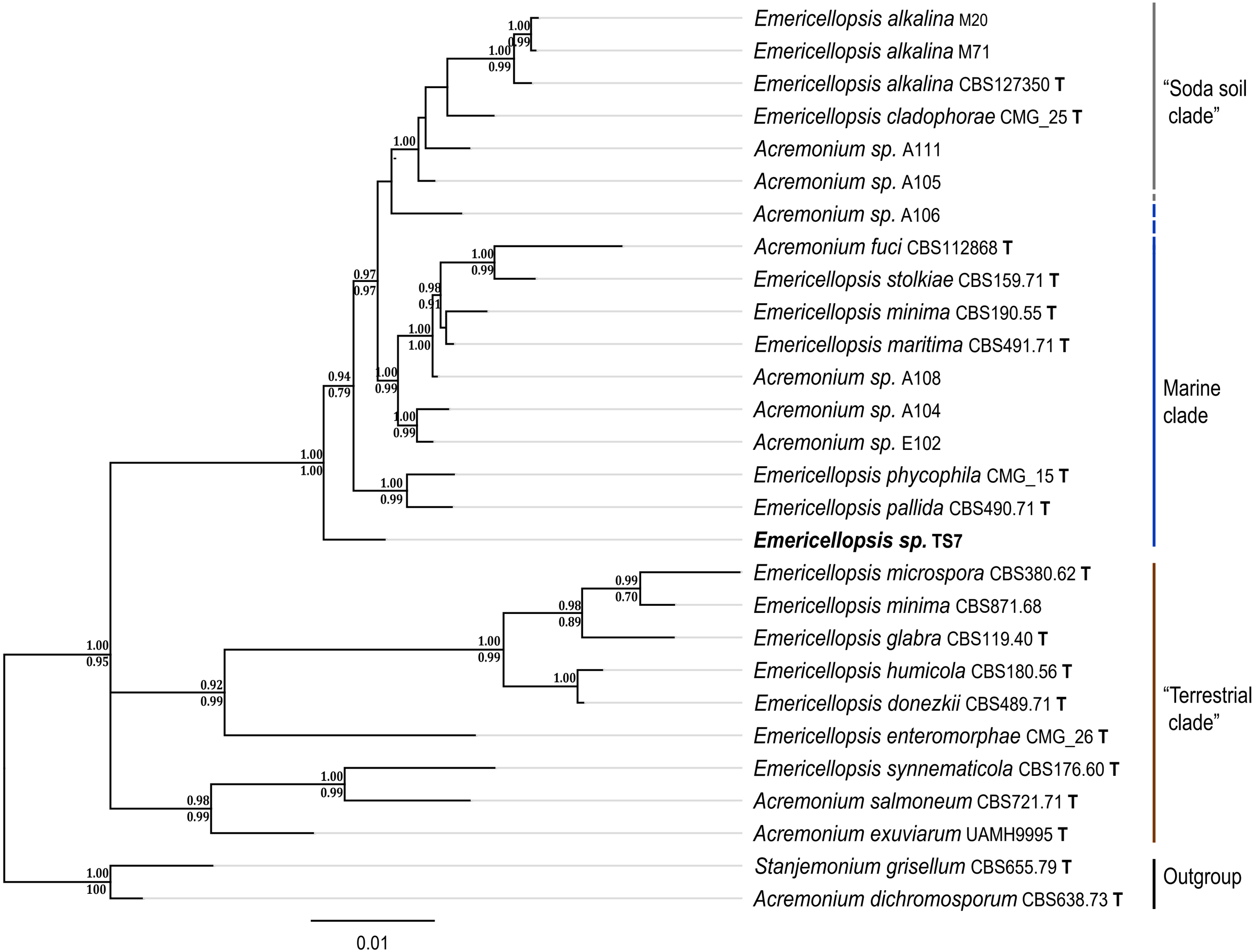
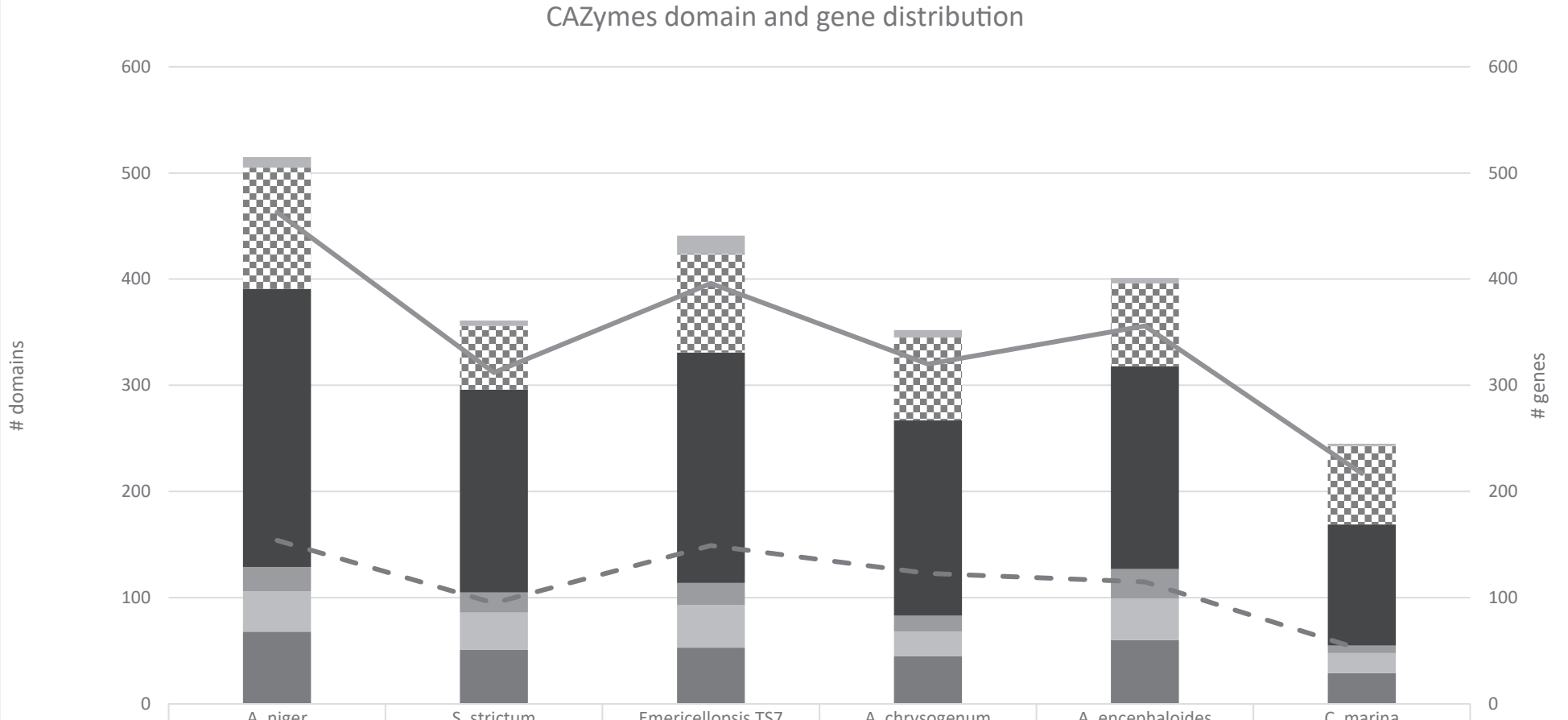


Figure 2

Click here to access/download;Figure;Figure 2.pdf



	A. niger	S. strictum	Emericellopsis TS7	A. chrysogenum	A. encephaloides	C. marina
PL	10	5	17	7	5	2
GT	114	60	93	78	78	74
GH	262	191	217	184	191	114
CE	23	19	21	15	28	7
CBM	38	35	40	23	39	19
AA	68	51	53	45	60	29
Genes	463	312	396	320	356	217
Secreted	154	95	149	123	115	51

Figure 3

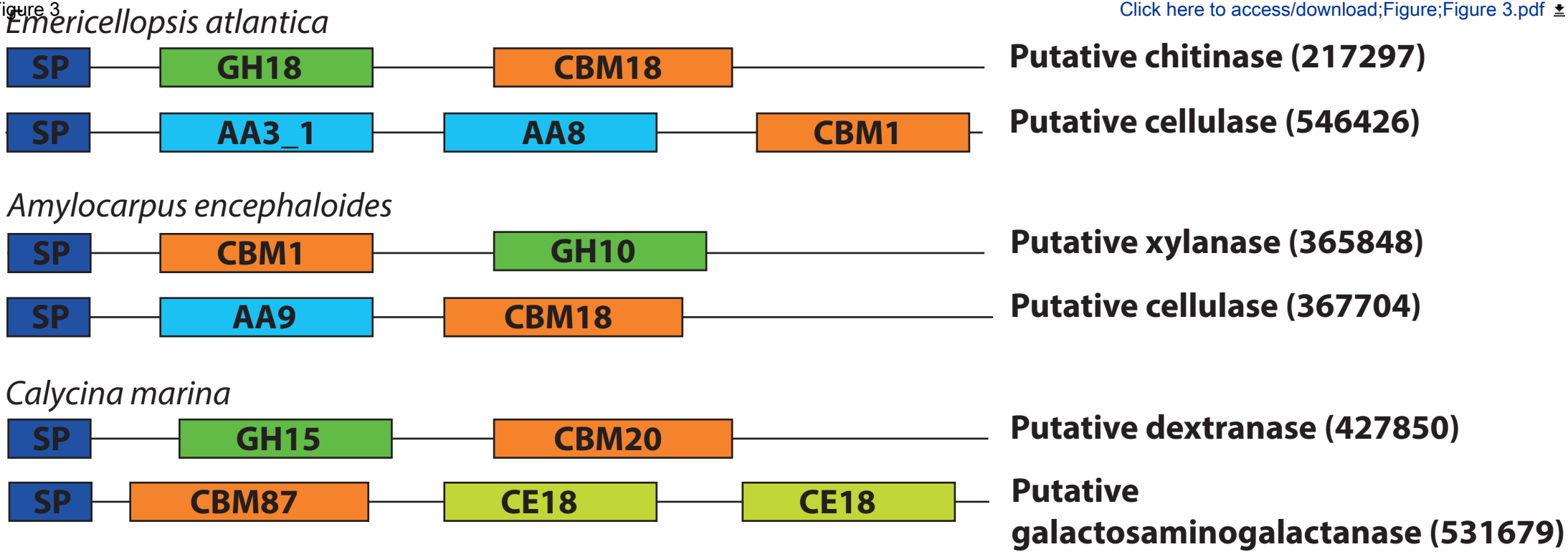


Figure 4

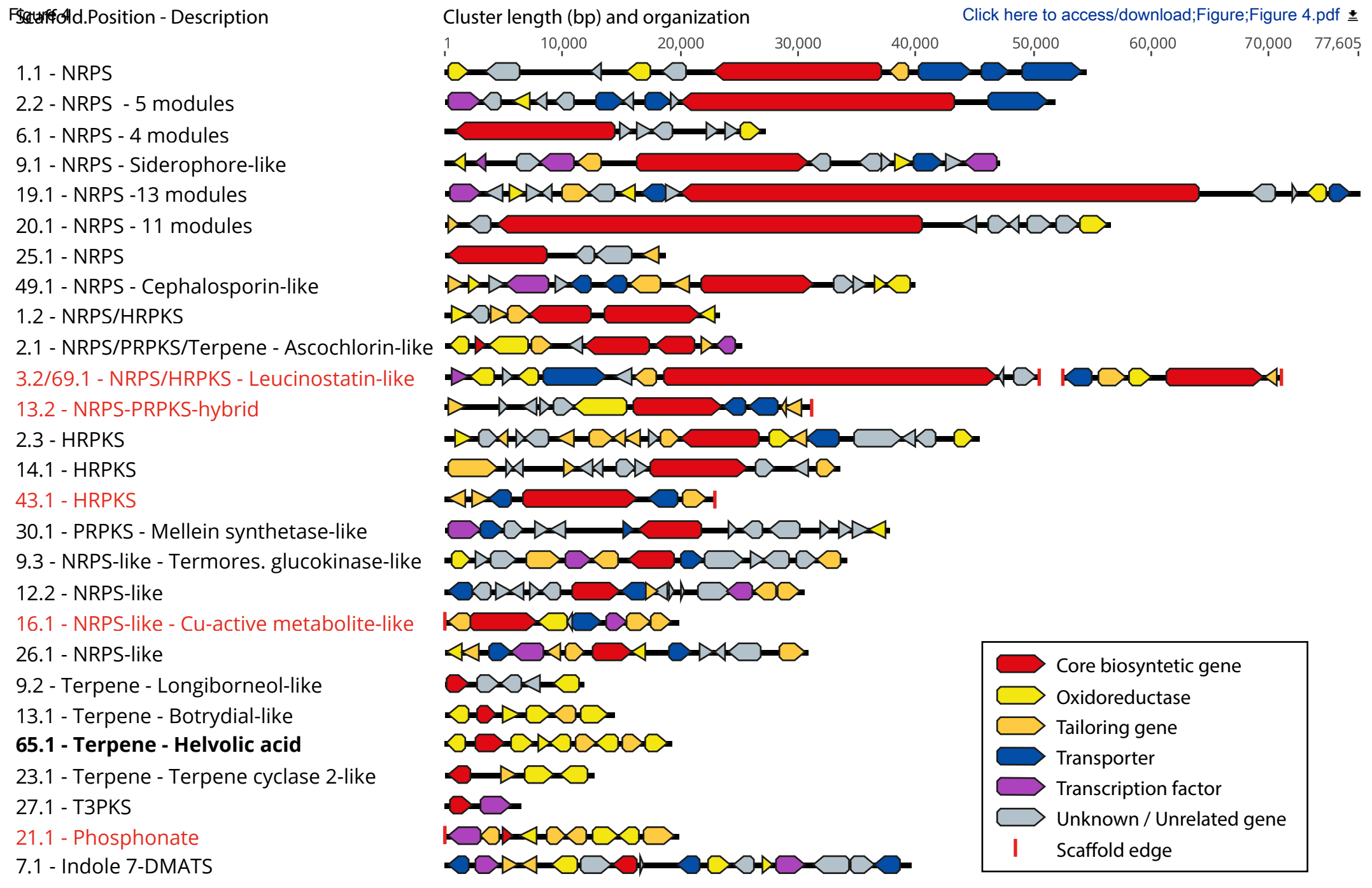

Scaffold.Position - Description
Cluster length (bp) and organization
Click here to access/download;Figure;Figure 4.pdf
1
10,000
20,000
30,000
40,000
50,000
60,000
70,000
77,605
1.1 - NRPS
2.2 - NRPS - 5 modules
6.1 - NRPS - 4 modules
9.1 - NRPS - Siderophore-like
19.1 - NRPS -13 modules
20.1 - NRPS - 11 modules
25.1 - NRPS
49.1 - NRPS - Cephalosporin-like
1.2 - NRPS/HRPKS
2.1 - NRPS/PRPKS/Terpene - Ascochlorin-like
3.2/69.1 - NRPS/HRPKS - Leucinostatin-like
13.2 - NRPS-PRPKS-hybrid
2.3 - HRPKS
14.1 - HRPKS
43.1 - HRPKS
30.1 - PRPKS - Mellein synthetase-like
9.3 - NRPS-like - Termores. glucokinase-like
12.2 - NRPS-like
16.1 - NRPS-like - Cu-active metabolite-like
26.1 - NRPS-like
9.2 - Terpene - Longiborneol-like
13.1 - Terpene - Botrydial-like
65.1 - Terpene - Helvolic acid
23.1 - Terpene - Terpene cyclase 2-like
27.1 - T3PKS
21.1 - Phosphonate
7.1 - Indole 7-DMATS
Core biosyntetic gene
Oxidoreductase
Tailoring gene
Transporter
Transcription factor
Unknown / Unrelated gene
Scaffold edge

Figure 5

Click here to access/download;Figure;Figure 5.pdf

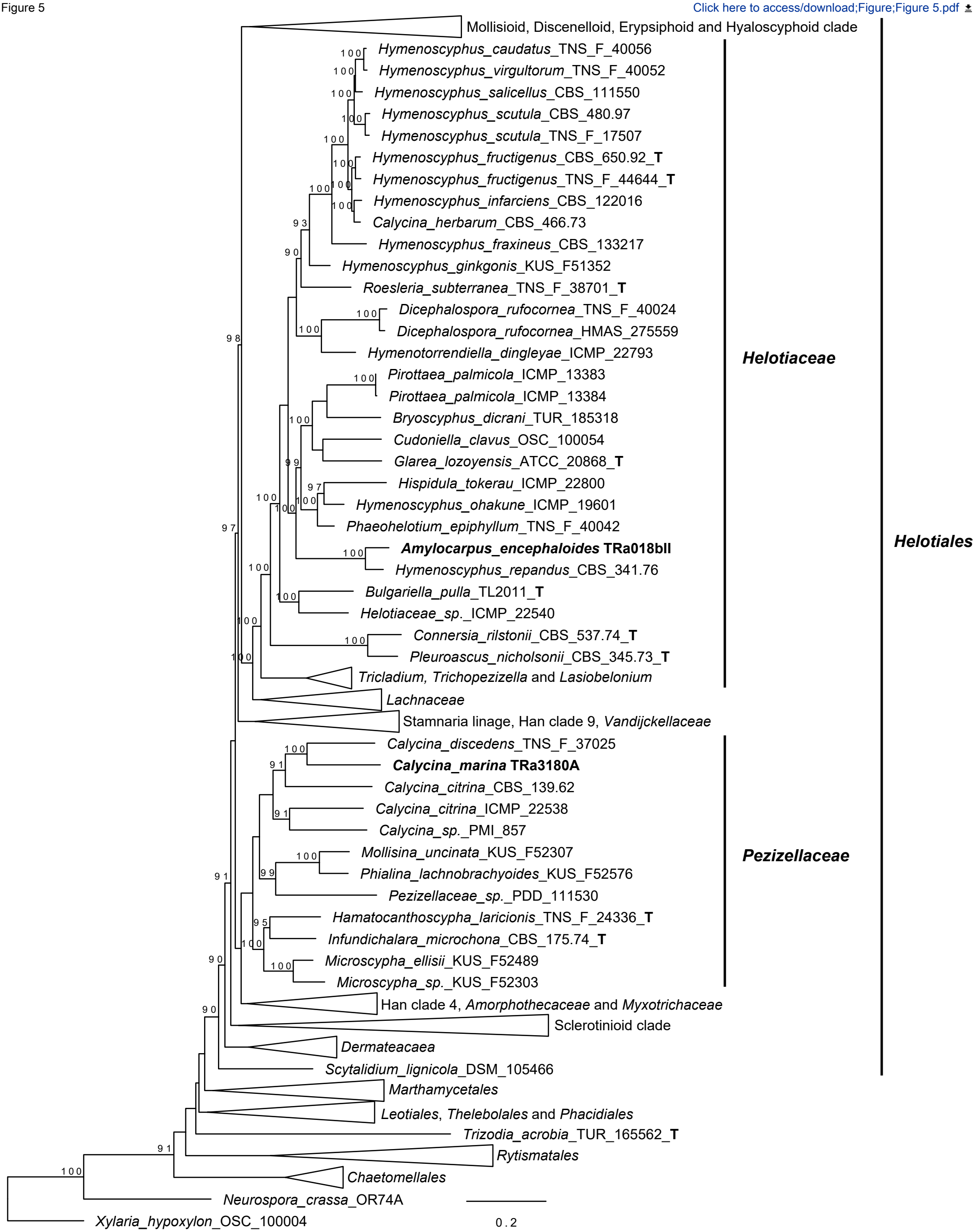
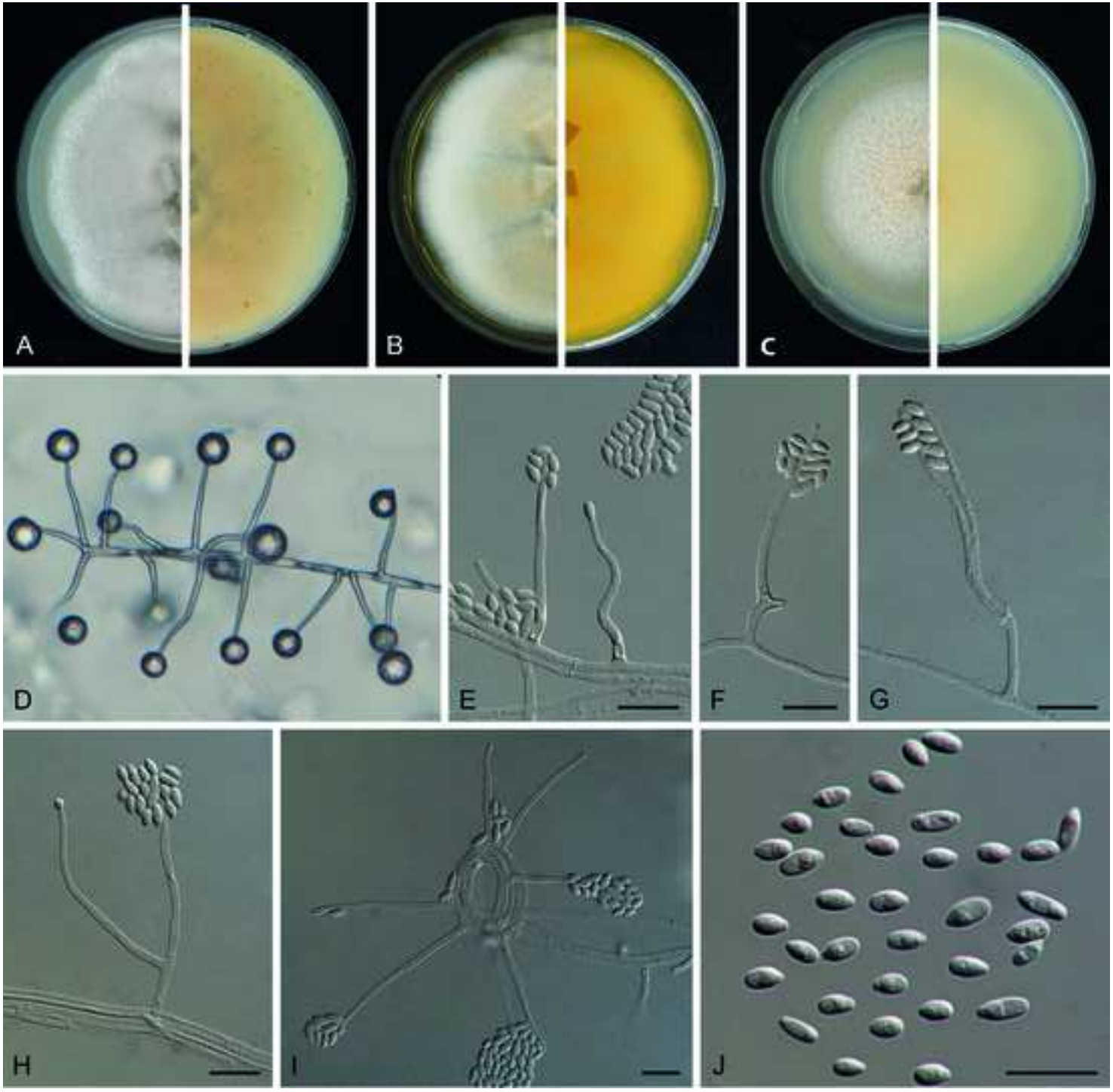


Figure 6

Click here to access/download;Figure;Figure 6.jpg



Paper 3

Pitfalls in biodiscovery – a case study of *Mytilinidion* sp. M16HEL1360D1-10.1, a wood-associated fungus from the marine environment in the Arctic

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Pitfalls in biodiscovery – a case study of *Mytilinidion* sp. M16HEL1360D1-10.1, a wood-associated fungus from the marine environment in the Arctic

An ascomycete fungus M16HEL1360D1-10.1 was isolated from a waterlogged piece of driftwood collected at 120 m depth 4 km off the west coast of Kvitøya (White Island), Svalbard. The identity of the isolate established in an earlier study was confirmed through a nine gene multilocus phylogenetic analysis. Based on the phylogenetic analysis, the fungus was placed on a sister branch to *M. mytilinellum* CBS 303.34. *Mytilinidion* sp. M16HEL1360D1-10.1 was selected for fermentation based on antibacterial activity in an initial screening. Fermentation was performed in DPY medium. In search of new secondary metabolites secreted by the fungus, the fermentation broth was extracted and fractionated, and the fractions were analyzed by UHPLC-HR-MS and compared to media controls. Compounds that were exclusively present in the fermentation broth were dereplicated by searching for their elemental compositions in relevant databases. A potential novel compound remained after the dereplication, and it was isolated using preparative HPLC. The structure of the isolated compound was elucidated using 2-D NMR which revealed a modified octapeptide (**1**) with a pyroglutamate at the N-terminal end. Comparison of the partial peptide sequence to peptide databases indicated similarity to bovine casein. The modified peptide was tested for antimicrobial, anticancer, antibiofilm, anti-inflammatory and ACE-inhibition activities as well as modulation of cytokine-induced cell death. The screening revealed that the compound has a weak activity against ACE with an IC_{50} of 42.86 µM. Compound **1** could potentially be a modified media component, but represents a novel chemical entity and is reported here with results on its bioactivity.

Keywords: ACE inhibitor, Dereplication, Marine fungi, Modified peptide, Natural product artefact

Introduction

Mytilinidiales (Subclass: *Pleosporomycetidae*, Class: *Dothideomycetes*, Subphylum: *Pezizomycotina*, Phylum: *Ascomycota*), according to NCBI taxonomy , is an order of fungi that contains two families, *Mytilinidiaceae* and *Argynnaceae* (Schoch et al. 2020). *Mytilinidiaceae* contains three genera, *Lophium*, *Mytilinidion* and *Pseudocamaropycnis*, all of them wood-associated. In contrast, literature states that the order only contains a single family

Mytilinidiaceae that consist of *Actidium*, *Lophium*, *Mytilinidion*, *Ostreola*, *Peyronelia* (anamorphs), *Pseudocamaropycnis*, *Quasiconcha*, *Slimacomyces*, *Septonema* (anamorphs) and *Zoggium* (Schoch et al. 2009; Spatafora et al. 2012; Hyde et al. 2013; Wijayawardene et al. 2014; Delgado et al. 2019; Hongsanan et al. 2020). *Actidium*, *Ostreola*, *Peyronelia* and *Zoggium* does not have sequence data associated with them and it is therefore not possible to compare them in molecular studies. The same is true for the type specimen of *Mytilinidion, M. aggregatum* (Mathiassen et al. 2015). There are two genera *incertae sedis* in *Mytilinidiales*, *Halokirschsteiniothelia* and *Quasiconcha* (Schoch et al. 2020). Furthermore, there are four families and genera *incertae sedis (Gloniaceae*, *Halojulellaceae*, *Neodactylariaceae* and *Pseudoberkleasmium)* within *Pleosporomycetidae* (Hyde et al. 2013; Qiao et al. 2020; Schoch et al. 2020). The obvious taxonomic uncertainties is due to the lack of reference sequences and type species of the different genera associated with the order *Mytilinidiales*.

Mytilinidion species are regarded as terrestrial by world registry of marine species (WoRMS Editorial Board 2021). Encyclopedia of Life and Global Biodiversity Information Facility shows that all registered *Mytilinidion* species have been detected in terrestrial environments and occur across the globe on all continents except Antarctica (Parr et al. 2014; GBIF Secretariat 2019). Most papers on *Mytilinidiales* are phylogenetic investigations or descriptions of new species. *Mytilinidion* species are typically gymnosperm-associated fungi and have been isolated from e.g. *Pinus*, *Cupressus*, *Eucalyptus*, and coniferous litter (Minter 2007; Boehm et al. 2009; Jayasiri et al. 2018; Lacerda et al. 2018; Koukol et al. 2020). The main challenge in characterizing isolates within *Mytilinidiales* is that sequence data is sparse and patchy. Some species have only one locus sequenced, while other species have different loci sequenced, which makes it impossible to accurately determine and compare species relations from sequences.

There are few studies examining *Mytilinidiales* isolates for their ability to produce secondary metabolites. Two metabolites, ascochital and ascochitine, have been isolated from the marine fungus *Halokirschsteiniothelia maritima*. These metabolites had MICs of 0.5 µg/mL and 0.1 µg/mL against *Bacillus subtilis*, respectively, and asochital had cytotoxic activity (95-100% cell death) against human amniotic epithelial cells (FL cells) at 15 µg/mL (Kusnick et al. 2002; Von Woedtke et al. 2002). To our knowledge, there are no reports of secondary metabolites isolated from other genera in *Mytilinidiaceae*. There might be several reasons why *Mytilinidion* spp. have not been screened for secondary metabolites previously. The fruiting bodies are small, the lack of sequences makes species determination difficult and some of these fungi are rare and not often detected in metagenomic or culturing studies. The only report of bioactivity from *Mytilinidion* is in Hagestad et al. (2019). That was also the first report of a *Mytilinidion* isolated from the marine environment.

We conducted an in depth study of the fungus since it can produce antibacterial metabolites, there are no reported metabolites from *Mytilinidion*, and Hagestad et al. (2019) indicated that the isolate M16HEL1360D1-10.1 is a putative novel species. The aim of this paper is to perform an in depth phylogenetic analysis to test the initial systematic placement of the fungus and to isolate potential bioactive secondary metabolites it secrete into the fermentation broth.

Methods and materials

Fungal isolate and cultivation

Mytilinidion sp. M16HEL1360D1-10.1 was isolated from a piece of driftwood collected at 120 m depth 4 km of the coast off Kvitøya (White Island), Svalbard (80.08007713 °N 31.20546539 °E) using a triangle dredge as described in Hagestad et al. (2019). The isolate was cultured in DPY (250 mL culture medium consisting of 20 g/L dextrin (MP Biomedicals), 10 g/L peptone,

5 g/L yeast extracts, 5 g/L KH_2PO_4, 0.5 g/L $MgSO_4$ $7H_2O$ in 1 L baffled aerated culture flasks) by cutting up agar with mycelium and adding the agar pieces to the culture medium. The fermentation was incubated at 13 °C at 130 rpm for 25 days before extraction.

PCR, sequencing and phylogenetic analysis

Genomic DNA from fresh mycelium was isolated using Quick-DNA Fungal/bacterial Miniprep Kit according to supplier's instructions (Zymo Research, CA, USA). The gDNA was used as template for a PCR reaction with primers for targeted loci (TUB2, TEF1, MCM7, ACT, RPB1, RPB2 and MCM7 – see Table 1 for primers) in DreamTaq Green PCR Master Mix. This was used for a template in the sequencing reaction with BigDye 3.1. The amplicons were sent to the sequencing lab at the University Hospital of North Norway utilizing Applied Biosystems 3130xl Genetic Analyzer (Life Technologies/Applied Biosystems).

Table 1 – Overview of the primers used in this study.

Locus	Primer name	Sequence	Reference
18S	NS1 NS4	GTAGTCATATGCTTGTCTC CTTCCGTCAATTCCTTTAAG	(White et al. 1990)
ITS[1]	ITS5 LR0Ri	GGAAGTAAAAGTCGTAACAAGG GCTTAAGTTCAGCGGGT	(White et al. 1990) (Hagestad et al. 2019)
28S	LR0R LR5	ACCCGCTGAACTTAAGC TCCTGAGGGAAACTTCG	(Vilgalys & Hester 1990; Rehner & Samuels 1994; Moncalvo et al. 1995)
TEF1	EF1-983F EF1-1620R	GCYCCYGGHCAYCGTGAYTTYAT GACGTTGAADCCRACRTTGTC	(Rehner & Buckley 2005; Stielow et al. 2015)
RPB1	RPB1-Af RPB1-Cr	GARTGYCCDGGDCAYTTYGG CCNGCDATNTCRTTRTCCATRTA	(Bischoff et al. 2006; Carvajal-Campos et al. 2017)
RPB2	fRPB2-5f fRPB2-7cR	GAYGAYMGWGATCAYTTYGG ATGGGYAARCAAGCYATGGG	(Liu et al. 1999)
TUB2	Btub2Fd Btub4Rd	GTBCACCTYCARACCGGYCARTG CCRGAYTGRCCRAARACRAAGTTGTC	(Woudenberg et al. 2009)
ACT	ACT512f ACT783r	ATGTGCAAGGCCGGTTTCG TACGAGTCCTTCTGGCCCAT	(Carbone & Kohn 1999)
MCM7	Mcm7-709for Mcm7-1348rev	ACIMGIGTITCVGAYGTHAARCC GAYTTDGCIACICCIGGRTCWCCCAT	(Schmitt et al. 2009)

[1] The *Mytilinidion* in this study cannot be amplified using ITS5/4 primer pair

Sequences were manually controlled for errors and the two directional sequencing assemblies provided high sequence quality. Individual loci were aligned using MAFFT v7.450 [Algorithm AUTO, Pam 200, Gap 1.53, Offset 0.123] in Geneious v10.2.6 and manually controlled before concatenating the sequences (Katoh et al. 2002; Kearse et al. 2012; Katoh & Standley 2013). IQ-Tree v1.6.12 was used for tree construction with the following commands [-m MFP -bb 10,000 -nt AUTO] (Minh et al. 2013; Nguyen et al. 2014; Kalyaanamoorthy et al. 2017; Hoang et al. 2018). Each gene had separate partitions, 18S, ITS, 28S, RPB1, RPB2, TUB2, TEF1, ACT and MCM7 (ITS was split into ITS1, 5.8S and ITS2), for a total of 11 partitions. Total amount of missing data was 67 %. Accession numbers of all sequences used in this study can be found in Supplementary table S1.

Metabolite extraction and flash fractioning

The liquid fermentation medium of *Mytilinidion* sp. was extracted using solid phase extraction (SPE) with Diaion®HP-20 resin (13607, Supelco Analytica, Bellefonte, PA, USA). The resin was activated in MeOH (PrepSolv, VWR, Radnor, Penns., USA) for 30 minutes before being washed in MilliQ for 15 minutes. The SPE resin (40 g/L) was added to the fermentation broth 3-7 days before extraction. The resin was separated from the medium by vacuum filtration through a cheesecloth mesh (1057, Dansk Hjemmeproduktion, Ejstrupholm, Danmark) and subsequently washed with MilliQ to remove remnants from the culture media. Metabolites were eluted from the resin by washing with 400 mL MeOH per 40 g of resin and vacuum filtration through a Whatman No. 3 filterpaper (Whatman plc, Buckinghamshire, UK). The elution from the resin was repeated one more time and the extract dried under reduced pressure at 40 °C. The dried extract was dissolved in DMSO and stored at -20 °C.

The extract was fractioned into eight fractions using reversed phase flash liquid chromatography. The dried extract was dissolved in 10 mL 90 % MeOH, then 2 g of Diaion® HP-20ss resin were added and the mixture was dried under reduced pressure. Separation

columns were prepared by activating 6.5 g of Diaion® HP-20ss resin in 75 mL MeOH for 30 min before washing it with Milli-Q water for 15 min. The resin was packed into a flash cartridge (Biotage® SNAP Ultra, Biotage, Uppsala, Sweden). The column was equilibrated with 5% MeOH in Milli-Q before the resin/extract was loaded on top. Fractionation was performed using a Biotage SP4™ system with a flowrate of 12 mL/min and a stepwise gradient of 5, 20, 40, 60, 80, 100 % MeOH in Milli-Q over 32 min and MeOH:acetone (1:1) to 100 % acetone over 18 min. The eight resulting fractions were dried under reduced pressure at 40 °C. Fractions were then stored at 4 °C.

Purification and isolation of metabolites

Purification was performed by preparative HPLC-MS using a 600 HPLC pump, a 3100 mass spectrometer, a 2996 photodiode array detector and a 2767 sample manager (Waters, MA, USA). For infusion of the eluents into the ESI-quadrupole-MS, a 515 HPLC pump (Waters) and a flow splitter were used and 80% methanol in MilliQ (v/v) acidified with 0.2% formic acid (Sigma) as make-up solution at a flow rate of 0.7 mL/min. The columns used for the isolation and purification were Atlantis (Prep dC18 10 µm 10x250 mm Column, Waters) and XSELECT CSH (Prep Phenyl-Hexyl 5µm 10x250 mm Column, Waters). The mobile phases for the gradients were A (MilliQ with 0.1% (v/v) formic acid) and B (acetonitrile with 0.1% (v/v) formic acid) and the flow rate was set to 6 mL/min. The gradient was 10-80 % B over 10 minutes, followed by 80-100 % B over 2.5 minutes. The fraction trigger was set activate on detection of the mass 818.8 m/z. The isolated compound was dried at 40 °C in reduced pressure and freeze dried in an 8 L laboratory freeze dryer (Labconco, Fort Scott, KS, USA).

UHPLC-HR-MS and Dereplication

The fractions (dereplication analysis) and purified compound (purity control) was diluted in methanol for analysis on an Acquity I-class UHPLC (Waters, Milford, MA, USA) coupled to a

PDA detector and a Vion IMS QToF-MS (Waters). Separation was performed on an Acquity C-18 UPLC column (Waters). The separation gradient was 10-90 % acetonitrile (HiPerSolv, VWR) with 0.1 % formic acid (Sigma) in Milli-Q during 12 min at a flow rate of 0.45 mL/min. Samples were run in ESI+ and ESI- mode, with the following settings: mass window 150-2,000 Da, capillary voltage 0.8 kV, cone voltage 30 V, Source offset 50 V, source temperature 120 °C, desolvation-gas N2, desolvation-gas temperature 350 °C, desolvation-gas flow 600 L/h, cone gas flow rate 50 L/h and analysed using the software UNIFI v1.9.4 (Waters).

NMR

NMR spectra were acquired in DMSO-d_6 on a Bruker Avance III HD spectrometer (Bruker, Billerica, MA, USA) operating at 600 MHz for protons, equipped with an inverse TCI cryo probe enhanced for ^{1}H, ^{13}C, and ^{2}H. All NMR spectra were acquired at 298 K, in 3-mm solvent-matched Shigemi tubes using standard pulse programs for proton, carbon, HSQC, HMBC, COSY, and ROESY with gradient selection and adiabatic versions where applicable. $^{1}H/^{13}C$ chemical shifts were referenced to the residual solvent peak (DMSO-d_6: $\delta_H = 2.50$, $\delta_C = 39.51$).

Bioactivity

Growth inhibition assay

Briefly, the human pathogens *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 259233) and *Pseudomonas aeruginosa* (ATCC 27853) from precultures in Muller Hinton Broth (MH, Becton, Dickinson and Company) and *Enterococcus faecalis* (ATCC 29122) and *Streptococcus agalactiae* (ATCC 12386) from precultures in Brain Heart Infusion Broth (BHI, Sigma-Aldrich) were grown to exponential growth phase, and a total of 50 µL (1,500-15,000 colony forming units) were added to each well of a 96-well plate (Nunclon, Thermo Scientific). The purified compound was added to the wells for a total of 100 µL/well with a final

concentration of 50 µM. The plates were incubated at 37 °C overnight before measuring the absorbance at 600 nm with a 1420 Multilabel Counter Victor^{3}™ (Perkin Elmer, Waltham, MA, USA). Bacterial suspension diluted with MilliQ (1:1) acted as a growth control, growth medium without bacteria as a negative growth control. A dilution series of gentamycin was used as a positive assay control.

Biofilm inhibition assay

To test for biofilm formation inhibition, a Tryptic Soy Broth (TSB, Merck, Kenilworth, NJ, USA) preculture of *Staphylococcus epidermidis* (ATCC 35984) was diluted in media containing 1 % glucose before being transferred to a 96-well microtiter plate at 50 µL/well. Fifty microliters of the purified compound (final concentration of 50 µM) was added to wells and incubated overnight. The bacteria were then removed and the plate washed in tap water. The biofilm was fixed before crystal violet was added to the wells followed by a 10 min incubation. Excess crystal violet solution was removed and the plate was dried, then 70 % EtOH was added and the plate incubated on a shaker for 10 min. To assess biofilm inhibition, absorbance at 600 nm (OD600) was detected using a 1420 Multilabel Counter Victor3 plate reader. *S. haemolyticus* (clinical isolate 8-7A, University hospital, UNN, Tromsø, Norway), a non-biofilm forming clinical isolate, was used as a control and 50 µL TSB with Milli-Q (1:1) was used as a media blank control.

Cell proliferation assay

Toxicity of the purified compound was assessed using a cell viability assay. The compound was tested at 50 µM final concentration in an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) *in vitro* cell proliferation assay against A2058 human melanoma (ATCC CRL-11147), MRC-5 (ATCC CCL-171) and MDA-MB-468 (ATCC HTB-132) cancer cell line. The final measurement of absorbance at 485 nm

was performed on a DTX 880 multimode detector (Beckman Coulter, Wals, Austria) and cell viability was calculated. Growth media (D-MEM – A2059, MEM-Eagle – MRC5, Leibovitz L15 – MDA-MB-468 all with 10 % FBA and 1 % glutamine) with and without 10 % DMSO was used as positive and negative controls respectively.

Cytokine induced apoptosis assay (RIN)

RIN-m5f cells were seeded at 10,000 cells/well in 100 uL culture medium (RPMI with 10 % FBS, 2 mM L-glutamine, 1 mM sodium pyruvate) in 96-well plates. After 72 h of incubation, culture medium was replaced with 90 µL culture medium and 10 µL purified compound (50 µM final concentration). Cells were incubated for 24 h before 5 µL cytokine mix (1:1:48 human IL-1β, IFN-γ and 0.1 % BSA in PBS) was added to every well. After 72 h, 10 µL AQueous One Solution was added. Absorbance was measured after 90 min incubation using a DTX 880 multimode detector (Beckman Coulter).

Angiotensin I Converting Enzyme (ACE)-Inhibition

The ACE inhibitory activity of the purified compound was measured based on the method of Cushman and Cheung (1971) and modified as described in Dragnes et al. (2009). This method is based on the quantitative HPLC measurement of hippuryl-histidine-leucine (HHL) converted to hippuric acid (HA) by ACE and was performed at Nofima (Tromsø, Norway).

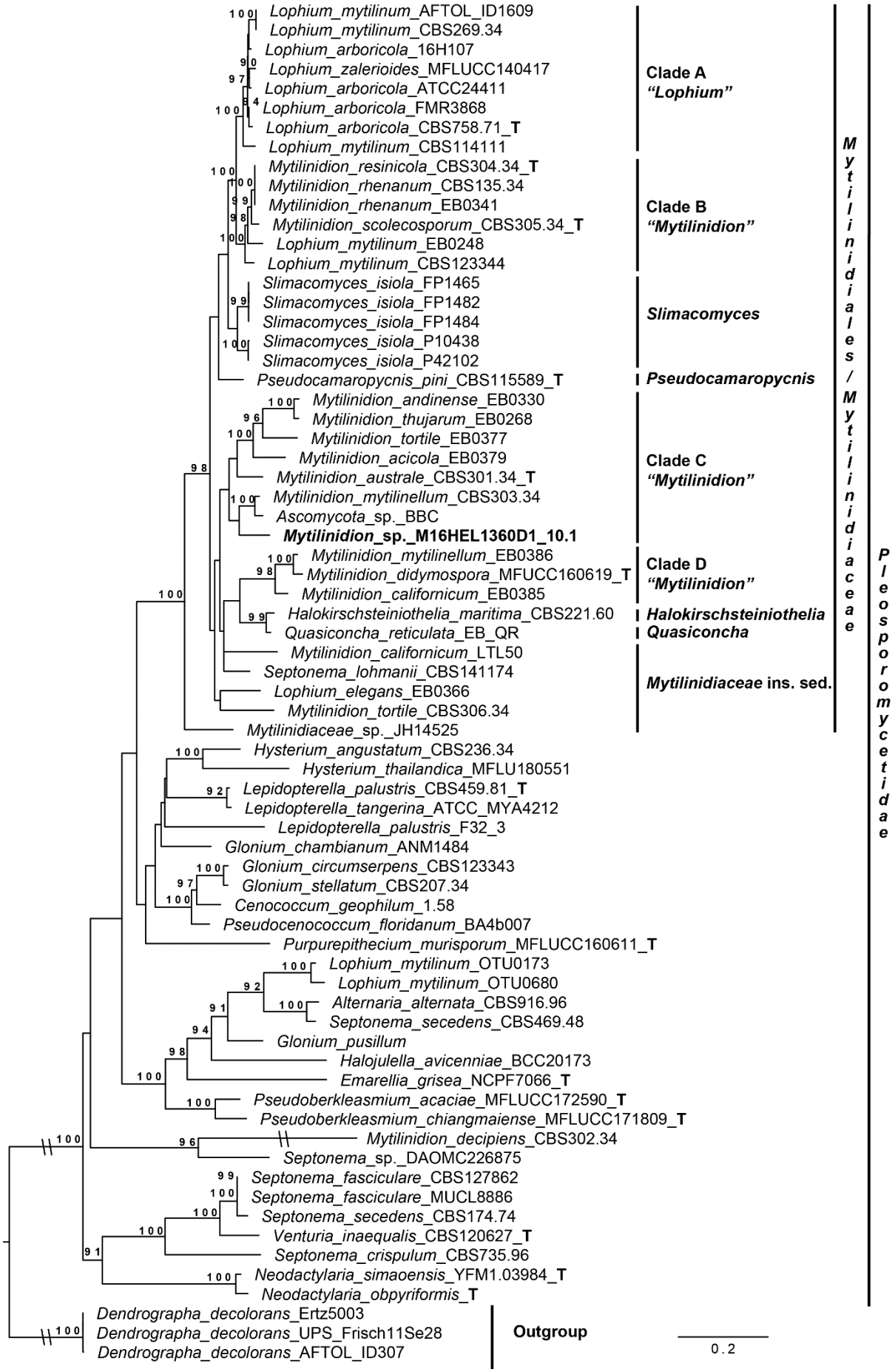
In a typical run, 300 µL of volume reaction mixture, containing 2 mM HHL (Sigma H 1635) and 42.86 µM purified compound (final concentration) in 100 mM sodium borate buffer, pH 8.3, was incubated at 37 °C for 10 min. The reaction was initiated by the addition of 50 µL of ACE (10 mU, Sigma A 6778), followed by incubation at 37 °C for 30 min at a final volume of 350 µL. The reaction was stopped by the addition of 430 µL 1 M HCl. This was followed by Quantitative HPLC analysis on a Waters 2695 Separations Module equipped with a Waters 2996 photodiode array detector (Waters Corporation, MA, USA), using a SymmetryShield

reverse phase C-18 Intelligent Speed column (Waters, 20 mm × 3.0 mm id, 3.5 μm). The HA was eluted at ambient temperature using a mobile phase that consisted of 0.05 % aqueous trifluoroacetic acid and HPLC-grade acetonitrile. A 2-min linear gradient was used, beginning after 0.5 min isocratic elution with 5 % acetonitrile and ending with 90 % acetonitrile. The flow-rate was maintained at 2 mL min^{-1} and the UV absorption was measured at 228 nm. The concentration of an inhibitor that was required to inhibit 50 % of ACE activity was defined as the IC_{50} value.

Results and discussion

Phylogeny

Previous phylogenetic analysis using three nuclear rDNA genes (18S-SSU, ITS, 28S-LSU) revealed that the isolate *Mytilinidion* sp. M16HEL1360D1-10.1 is most closely related to *M. mytilinellum* CBS 303.34, but distinct from it (Hagestad et al. 2019). To promote production of sexual fruiting bodies, the fungus has been cultured on several different media (DPY, 0.2ASMEA, FMAP, 0.2ASCMA and birch seawater media (Kirk 1969)). Despite these efforts the fungus has remained in an asexual state. An extensive phylogenetic analysis, Figure 1, with available sequences from all species within *Mytilinidiales* and some sequences within *Pleosporomycetidae* was conducted to retest the results from the previous study with a dataset that include additional loci (TEF1, TUB2, RPB1, RPB2, ACT and MCM7).


Lophium_mytilinum_AFTOL_ID1609
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Lophium_arboricola_ATCC24411
Lophium_arboricola_FMR3868
Lophium_arboricola_CBS758.71_T
Lophium_mytilinum_CBS114111
Mytilinidion_resinicola_CBS304.34_T
Mytilinidion_rhenanum_CBS135.34
Mytilinidion_rhenanum_EB0341
Mytilinidion_scolecosporum_CBS305.34_T
Lophium_mytilinum_EB0248
Lophium_mytilinum_CBS123344
Slimacomyces_isiola_FP1465
Slimacomyces_isiola_FP1482
Slimacomyces_isiola_FP1484
Slimacomyces_isiola_P10438
Slimacomyces_isiola_P42102
Pseudocamaropycnis_pini_CBS115589_T
Mytilinidion_andinense_EB0330
Mytilinidion_thujarum_EB0268
Mytilinidion_tortile_EB0377
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Mytilinidion_australe_CBS301.34_T
Mytilinidion_mytilinellum_CBS303.34
Ascomycota_sp._BBC
Mytilinidion_sp._M16HEL1360D1_10.1
Mytilinidion_mytilinellum_EB0386
Mytilinidion_didymospora_MFUCC160619_T
Mytilinidion_californicum_EB0385
Halokirschsteiniothelia_maritima_CBS221.60
Quasiconcha_reticulata_EB_QR
Mytilinidion_californicum_LTL50
Septonema_lohmanii_CBS141174
Lophium_elegans_EB0366
Mytilinidion_tortile_CBS306.34
Mytilinidiaceae_sp._JH14525
Hysterium_angustatum_CBS236.34
Hysterium_thailandica_MFLU180551
Lepidopterella_palustris_CBS459.81_T
Lepidopterella_tangerina_ATCC_MYA4212
Lepidopterella_palustris_F32_3
Glonium_chambianum_ANM1484
Glonium_circumserpens_CBS123343
Glonium_stellatum_CBS207.34
Cenococcum_geophilum_1.58
Pseudocenococcum_floridanum_BA4b007
Purpurepithecium_murisporum_MFLUCC160611_T
Lophium_mytilinum_OTU0173
Lophium_mytilinum_OTU0680
Alternaria_alternata_CBS916.96
Septonema_secedens_CBS469.48
Glonium_pusillum
Halojulella_avicenniae_BCC20173
Emarellia_grisea_NCPF7066_T
Pseudoberkleasmium_acaciae_MFLUCC172590_T
Pseudoberkleasmium_chiangmaiense_MFLUCC171809_T
Mytilinidion_decipiens_CBS302.34
Septonema_sp._DAOMC226875
Septonema_fasciculare_CBS127862
Septonema_fasciculare_MUCL8886
Septonema_secedens_CBS174.74
Venturia_inaequalis_CBS120627_T
Septonema_crispulum_CBS735.96
Neodactylaria_simaoensis_YFM1.03984_T
Neodactylaria_obpyriformis_T
Dendrographa_decolorans_Ertz5003
Dendrographa_decolorans_UPS_Frisch11Se28
Dendrographa_decolorans_AFTOL_ID307
Clade A "Lophium"
Clade B "Mytilinidion"
Slimacomyces
Pseudocamaropycnis
Clade C "Mytilinidion"
Clade D "Mytilinidion"
Halokirschsteiniothelia
Quasiconcha
Mytilinidiaceae ins. sed.
Outgroup
0.2
Mytilinidiales / Mytilinidiaceae
Pleosporomycetidae

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Figure 1 – Phylogenetic tree of *Mytilinidiales* with groups from *Pleosporomycetidae* based on nine genes (individual taxa are represented by 1-9 genes). Clade names are based on sequenced types within the clades. Support values (>90) from ultrafast bootstrap test are presented by the branch (Minh et al. 2013; Hoang et al. 2018). The study isolate is in bold font. Bold **T** denotes type, holotype and epitypes. "\\" indicate branches of half length. The type of the genus *Mytilinidion* has not been sequenced. The outgroup belongs to *Arthoniomycetes, Dothideomyceta*.

Mytilinidiales splits into several clades with varying support, Figure 1, designated clade A-D. The clades have received names based on the sequenced type(s) in the clade. The branching of clade A and B and the deeper branch between clade A-B and C-D have high support. The lack of support for many branches is likely due to the high amount of missing sequence data (67 %). *Mytilinidion* sp. M16HEL1360D1-10.1 branch out with *M. mytilinellum* CBS 303.34 on an unsupported branch in clade C. The lack of the sequence for the original type specimen (*M. aggregatum* and *L. mytilinum*) and the sequence of the neotype specimen (*M. mytilinellum*) means that the polyphyletic *Mytilinidion*, *Lophium* and *Septonema* remains unresolved. Description of new species is difficult without morphological data as there are so many species without sequence data. *Mytilinidion* sp. M16HEL1360D1-10.1 does not cluster close enough to any sequenced species to believe this is an already described sequenced species.

The isolate M16HEL1360D1-10.1 originated from a waterlogged piece of unidentified wood collected at 120 m depth off the coast of Svalbard. This is the first report of a *Mytilinidion* isolated in the marine environment on driftwood. The only species previously detected in driftwood within *Mytilinidiales* is *Halokirschsteiniothelia* (Siepmann & Johnson 1960; Rees & Jones 1985; Prasannarai & Sridhar 1997; Rämä et al. 2014; Garzoli et al. 2015; Blanchette et al. 2016; Kunttu et al. 2020). Driftwood generally stay afloat for an average of 6-10 months before sinking (Hellmann et al. 2013). In addition, Arctic driftwood can stay trapped in ice for long periods before finally sinking (Hellmann et al. 2013). Since this is not a cosmopolitan species, it is doubtful this is an airborne contamination (Bridge & Spooner 2012). There is one

case of *Mytilinidion mytilinellum* detected on whale bone on a beach in South Georgia (British Antarctic Survey 1978), which is within the Antarctic polar front. The isolate in this paper and *M. mytilinellum* from whale bone are isolated outside of the normal habitat of *Mytilinidion*. Species within *Mytilinidiales* have been isolated from Islands around the world, but not directly tied to the marine environment (GBIF Secretariat 2019). Isolation from the marine habitat increases the likelihood that this is a novel species, but the lack of sequenced types and morphological characters prevents further description at this time.

Chemical and bioactivity investigation of Mytilinidion sp.

Antibacterial characterization of isolates in Hagestad et al. (2019) revealed that *Mytilinidion* sp. M16HEL1360D1-10.1 produced metabolites able to inhibit *Streptomyces aureus*. The *Mytilinidion* isolate was selected for a more in-depth study since the family is understudied, the isolate had a significant ITS sequence difference to known isolates and it showed promising bioactivity in an initial screening on solid medium. In order to isolate secondary metabolites from the fungi, the fermentation broth was extracted, fractionated and the fractions were analysed on a UHPLC-HR-MS. Compounds that were exclusive to the fermentation broth were dereplicated by searching with their elemental composition in relevant databases. The 10 largest non-lipid peaks without hits in databases or where the instrument were unable to get a molecular formula were isolated in two steps. First, the individual peaks were isolated using an Atlantis (C_{18}) column before further purification on an XSELECT (Phenyl-hexyl) column, these columns provided the best peak resolution for each isolation step. The purity of the compounds were then controlled on the UHPLC-HR-MS system and only one compound was deemed of high enough purity and amount to perform further analysis on. The pure compound was tested in several bioassays. A single compound with the neutral mass 818.4539 u ($C_{39}H_{62}N_8O_{11}$ – calculated monoisotopic mass: 818.4538 u) was isolated. The peak of this compound was one of the smaller peaks of the 10 initially targeted and eluted at 6.7 min on Atlantis and 6.6 min

on XSELECT. The elemental composition suggested a peptide-like compound.

Compound **1** was tested in antimicrobial, anticancer, antibiofilm, anti-inflammatory, Angiotensin converting enzyme-inhibition and modulation of cytokine-induced cell death assays. It had weak activity in ACE-inhibition assay with an IC_{50} of 42.85 µM, but no activity in the other assays. Due to no hits in the databases for this molecule and the activity in the ACE-assay, the structure of the peptide-like compound was determined using 1D (^{1}H and ^{13}C) and 2D (HSQC, HMBC, COSY, TOCSY, ROESY, HSQCTOCSY, H2BC and ^{15}N-HSQC) NMR experiments (Table 1, Supplementary figures S1-S12). It turned out to be a linear octapeptide with a modified N-terminal glutamine (pyroglutamate). The peptide sequence was determined to be pGln-Thr-Pro-Val-Val-Val-Pro-Pro ($C_{39}H_{62}N_8O_{11}$), Figure 2.

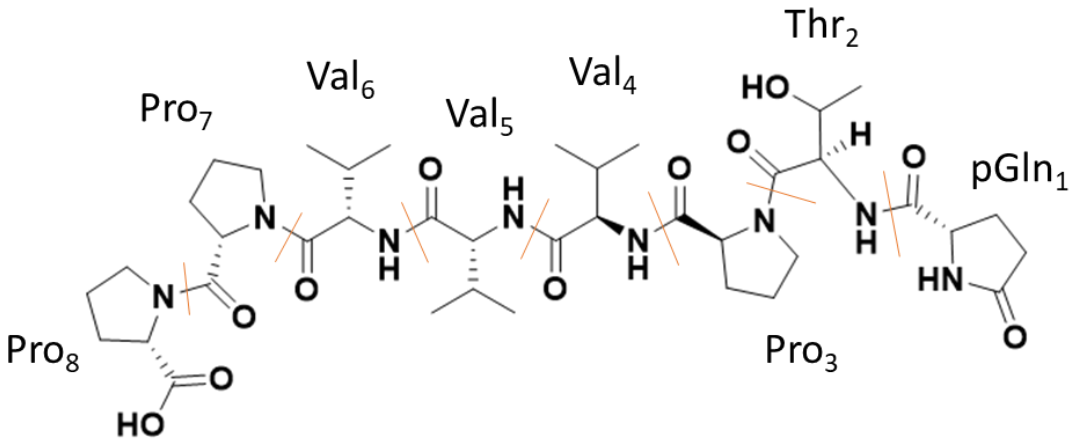

Figure 2 – Compound **1**, the structure was determined to be an octapeptide, pGln-Thr-Pro-Val-Val-Val-Pro-Pro (ZTPVVVPP).

The individual amino acids were assembled based on correlations found in the HSQC, HMBC, COSY, TOCSY and H2BC spectra (Figures S3 – S8 and S11). Modification of the N-terminal amino acid was supported by detection on non-equivalent protons on carbon atoms $pGln_1\beta$ and $pGln_1\gamma$, which would not be the case for the corresponding CH_2 groups in glutamine. This structural modification also matched the calculated elemental composition of the compound (Figure S13). The amino assay sequence was assembled based on ROSEY data (Figure 3 and S9). Consequently, the structure of the peptide was assigned.

Table 1. ^{1}H, ^{13}C and ^{15}N NMR Assignments for the peptide.

Position		δ_C, type	δ_H (*J* in Hz)	Position		δ_C, type	δ_H (*J* in Hz)
pGln$_1$	C=O	172.3, C		**Val$_5$**	C=O	170.6, C	
	NH	121.1, HN	7.84, s		NH	117.1, NH	7.75, d (8.8)
	α	55.2, CH	4.12, m		α	57.4, CH	4.20, m*
	β'	25.0, CH_2	1.84, m*		β	30.0, CH	1.93, m*
	β"		2.22, m		γ'	18.0, CH_3	0.79, m*
	γ'	29.2, CH_2	2.07, m		γ"	19.1, CH_3	0.80, m*
	γ"		2.12, m*	**Val$_6$**	C=O	169.1, C	
	δ	177.4, C			NH	119.8	7.81, d
Thr$_2$	C=O	168.6, C			α	55.5, CH	4.31, t (8.4)
	NH	115.6, NH	7.99, d (7.5)		β	30.5, CH	1.93, m*
	α	56.5, CH	4.43, m*		γ'	18.3, CH_3	0.84, m*
	β	67.0, CH	3.83, t (6.5)		γ"	19.0, CH_3	0.89, m*
	γ	19.2, CH_3	1.12, d (6.3)	**Pro$_7$**	C=O	179.6, C	
Pro$_3$	C=O	171.4, C			α	57.4, CH	4.52, dd (8.4, 5.0)
	α	59.2, CH	4.42, m*		β'	27.8, CH_2	1.75, m*
	β'	29.1, CH_2	1,78, m*		β"		2.13, m*
	β"		2.02, m		γ	24.5/24.4, CH_2"	1.91 - 1.82, m"
	γ	24.5/24.4, CH_2"	1.91 - 1.82, m"		δa	47.0, CH_2	3.76, m
	δa	47.3, CH_2	3,71, m		δb		3.53, m*
	δb		3.66, m*	**Pro$_8$**	COOH	173.3, C	
Val$_4$	C=O	170.8, C			α	58.4, CH	4.20, m*
	NH	115.6, NH	7.90, d (8.9)		β'	28.4, CH_2	1.83, m*
	α	57.8, CH	4.15, m		β"		2.13, m*
	β	30.3, CH	1.94, m*		γ	24.5/24.4, CH_2"	1.91 - 1.82, m"
	γ'	18.2, CH_3	0.83, m*		δa	46.2, CH_2	3.63, m*
	γ"	19.2, CH_3	0.83, m*		δb		3.52, m*

*Signals are overlapping, ‘’Peaks are indistinguishable

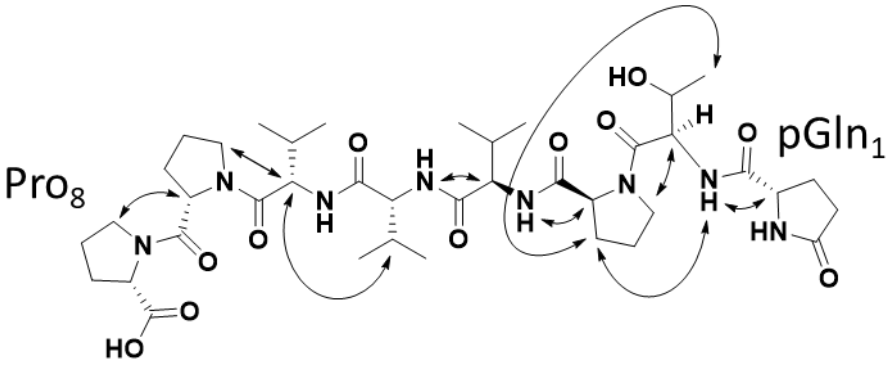

Figure 3 – Key ROESY correlations (black arrows) used to assemble the amino acid sequence.

To the best of our knowledge this novel modified peptide has not been examined for bioactivity previously. The N-terminal pyroglutamate, the high amount of proline and valine (6/8 residues) and proline motif at the C-terminal end of the peptide is similar to what is found in many ACE-inhibitory peptides and drugs (Fitzgerald et al. 2004; Lee et al. 2006; Morais et al. 2013; Pasqualoto et al. 2018). Specifically the tripeptide VPP from whey and milk has been studied for its hypotensive effects, reducing blood pressure up to 32 mm Hg in hypertensive rats (Masuda et al. 1996; Abubakar et al. 1998; Fitzgerald et al. 2004). Nakamura et al. (1995) reported an IC_{50} of 9 µM for VPP which is 4 times lower than the IC_{50} of **1**. The peptide TPVVVPPFLEP had a considerable higher IC_{50} of 749 µM (Abubakar et al. 1998), suggesting a possible relation to peptide length and activity. Direct comparison of the IC_{50} values of VPP, TPVVVPPFLEP and **1** is not possible since the tests are performed using different protocols, but all IC_{50} values were based on biochemical assays. The ACE-inhibitory activity of peptides observed in biochemical assays and rats have not always been successfully reproduced in human trials because peptides have poor absorption and rapid elimination (Wuerzner et al. 2009). However, more recent studies on marine protein hydrolysates have shown efficacy in human trials (Musa-Veloso et al. 2019). Larger and shorter peptides containing an almost identical peptide sequence of **1**, QTPVVVPP, were examined in fermentations of *Lactococcus lactis* (Huang & Kok 2020). The hydrolysate was based on enzymatic digests of β-casein and hydrolysates of casein has been extensively studied (Silva & Malcata 2005; Irshad et al. 2015; Mohanty et al. 2016; Huang & Kok 2020).

Peptone from casein (Sigma 82303-5kg-F), used here as a media component, is produced by proteolytic cleavage of casein by pepsin. Pepsin has a broad substrate preference (or cleavage site preference), but generally favors cleavage at Phe or Leu followed by Met. It also readily accepts Tyr, Trp, Cys and Glu in the P1 position. His, Lys, Arg and Pro in P1 are inhibitory to the peptidase activity, as well as Pro in P2 and to a certain extent in P2' and P3'

position (Ehren et al. 2008; Ahn et al. 2013). Proline containing peptides are fairly resistant to degradation by ordinary peptidases (Hausch et al. 2002; Shan et al. 2002).

There are two fungi within *Mytilinidiales* that has been whole genome sequenced, *Lophium mytilinum* CBS 269.34 and *Mytilinidion resinicola* CBS 304.34 (Haridas et al. 2020). Because of the available genomes, it is possible to say something about their biosynthetic potential and this is to some extent indicative for the potential of the genus as a whole. *L. mytilinum* contains 22 biosynthetic gene clusters and *M. resinicola* contains 32 biosynthetic gene clusters, responsible for secondary metabolite production (Grigoriev et al. 2014; Joint Genome Institute 2021). Nonribosomal peptide synthases are responsible for the production of peptide-like secondary metabolites and *L. mytilinum* and *M. resinicola* contain 4 and 7 NRPSs, respectively (Joint Genome Institute 2021). This indicate that species within this genus has some capabilities to produce NP peptides. However, the high similarity to peptide fragments from casein, whey and milk (Abubakar et al. 1998; Fitzgerald et al. 2004; Duchrow et al. 2009), the absence of N-methylation of peptide bonds (Velkov et al. 2011), no non-proteinogenic amino acids and the linearity of the peptide; it was questioned whether it actually originated from the fungus studied here. The only difference between the isolated peptide, ZTPVVVPP, and the peptide sequence in bovine β-casein, QTPVVVPP, is the cyclized glutamine. The amount of proline and the pyroglutamate modification increases the stability and resistance to degradation of the peptide, which could explain why the fungus had not degraded and utilized the peptide further (Rink et al. 2010).

The modification of the N-terminal glutamine can be catalyzed by the enzymes glutaminyl cyclase or glutamyl-peptide cyclotransferase (Schilling et al. 2008; Wu et al. 2017) or by spontaneous cyclization (Gazme et al. 2019). The enzyme glutaminyl cyclase has been characterized from *Saccharomyces cerevisiae* (Boswell et al. 2007) and homologs have been detected in most fungi using protein queries (Wu et al. 2017). There are also homologs

identified as putative glutaminyl cyclase in the sequenced genomes within *Mytilinidiales* (Haridas et al. 2020). In *Neurospora crassa*, glutaminyl cyclase is secreted into the endoplasmic reticulum (Wu et al. 2017), and could in some cases be released to the extracellular environment. The spontaneous cyclization reaction can occur in alkaline and acidic solutions or if heat and pressure is used when handling peptides or proteins containing glutamic acid or glutamine (Gazme et al. 2019). The cyclization of the pyroglutamate is known to change retention time of proteins since it changes the functional groups that can interact with the stationary phase of the isolation column (Liu et al. 2019), increasing the difficulty of identifying modified media components.

Modifications of compounds during extraction have been documented for several natural products, which include amine groups reacting to silanol groups in glassware (Nielsen et al. 2015), formation of artificial compounds by condensation with solvents or changes or degradation of compounds due to high or low pH or temperature (Maltese et al. 2009; Salim et al. 2020). These modifications can happen to media components as well as natural products and introduces a confounding factor in dereplication of extracts, and increases the difficulty to identify actual metabolites of the organism that is studied. This problem has been somewhat neglected and underreported by scientists and reviewers (Bianco et al. 2014; Venditti 2018; Capon 2020), and may be a larger issue than many researchers think. This means that all novel compounds should undergo careful consideration as being artefacts. In addition, selection of fermentation media, extraction method and solvents, as well as which modifications and artefacts can occur when using a specific set of these methods has to be considered. One method which could have provided an earlier indication that the compound was a medium component is molecular networking (Purves et al. 2016). Compound **1** would likely have grouped together with peptide fragments of casein and allowed for an earlier comparison and putative identification as a medium component (Zdouc et al. 2021).

In order to determine if **1** is spontaneously or enzymatically modified the medium control and fermentation medium should be compared directly before extraction. The pH of the fermentation medium and the control should also be measured to check if the fermentation had significant effect on pH. This was not done during the extraction described here. A small sample for MS analysis should be taken directly from the fermentation medium before extraction to compare to the later extracts to serve as a control against solvolysis or changes due to the repeated heating, freezing and general processing of the extract (Hanson 2017; Capon 2020).

Conclusion

The order of *Mytilinidiales* requires revision using both morphological and molecular markers. Currently, the morphological and the molecular taxonomy are disconnected due to lacking types and types with available sequences. Currently, molecular phylogeny yields clades containing mixed genera despite support of branching clades. Available herbarium specimens of identified samples and types should be sequenced and a revision of the clade would provide a basis for future description of new species within the order.

The search for natural product from microorganisms is a challenging exercise with many stumbling blocks. Novel compounds can turn out to be artefacts due to modifications of the chemical structure resulting from reactions with media, solvents and acid/bases rather than a metabolite of the organisms that is examined. The fermentation of this fungus from a poorly explored order and subsequent isolation of a new chemical entity resulted in the identification of a casein peptide that has been partially hydrolyzed and modified. This study shows the importance of consider all possible origins for novel compounds, especially when they differ from or lack modification common to other NPs. A comprehensive database of medium components, chemical groups that can undergo spontaneous reactions and common solvolysis groups could be introduced to more easily identify artefacts. Compounds, such as the one

isolated here, should be reported despite not being a metabolite of the organism originally studied to establish them in databases and aid in future dereplication.

Despite likely not originating from the fungus, compound **1** is a novel compound with bioactivity that has not been characterized previously. It has an IC_{50} against ACE of 42.86 µM, which is approximately four times higher than for the partial peptide of VPP (Nakamura et al. 1995).

Data Availability Statement

The raw data supporting the conclusions of this article will be available upon request.

Author Contributions

OCH, TR, JHA and EHH conceived the idea, aims and designed of the study. OCH and TR isolated and cultured the fungus. OCH extracted and isolated metabolites, analysed MS data, performed phylogenetic analysis and visualized the findings. JI performed the NMR experiment. KØH wrote the NMR description of the compounds. KØH managed the screening activity. OCH wrote the first draft of the manuscript. JHA, EHH, TR and KØH supervised and mentored OCH. OCH, TR, JHA, EHH, JI and KØH have reviewed and commented the manuscript. All authors have read through and accepted the manuscript for publication.

Funding

Digibiotics (Digital Life Norway) and Centre for New Antibacterial Strategies at UiT The Arctic University of Norway, through the Research Council of Norway (Project 269425) provided funding for Teppo Rämä.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgement

Marte Albrigtsen, Kirsti Helland, Sara Ullsten-Wahlund, Njål Rauø and Ruth Hendus-Altenburger performed the bioassays. Nofima performed ACE-inhibition assay. Jostein Johansen did additional MS analysis before NMR. Chun Li provided help with PCR of the fungus.

Literature Cited

Abubakar A, Saito T, Kitazawa H, Kawai Y, Itoh T. 1998. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. Journal of Dairy Science 81(12):3131-8. Available from: https://doi.org/10.3168/jds.S0022-0302(98)75878-3

Ahn J, Cao M-J, Yu YQ, Engen JR. 2013. Accessing the reproducibility and specificity of pepsin and other aspartic proteases. Biochimica et biophysica acta 1834(6):1222-1229. Available from: https://doi.org/10.1016/j.bbapap.2012.10.003

Bianco A, Venditti A, Foddai S, Toniolo C, Nicoletti M. 2014. A new problem. Contamination of botanicals by phthalates. Rapid detection tests. Natural Product Research 28(2):134-137. Available from: https://doi.org/10.1080/14786419.2013.842997

Bischoff JF, Rehner SA, Humber RA. 2006. *Metarhizium frigidum* sp. nov.: a cryptic species of *M. anisopliae* and a member of the *M. flavoviride* complex. Mycologia 98(5):737-745. Available from: https://doi.org/10.1080/15572536.2006.11832645

Blanchette RA, Held BW, Hellmann L, Millman L, Büntgen U. 2016. Arctic driftwood reveals unexpectedly rich fungal diversity. Fungal Ecology 23:58–65. Available from: https://doi.org/10.1016/j.funeco.2016.06.001

Boehm E, Mugambi GK, Miller A, Huhndorf SM, Marincowitz S, Spatafora J, Schoch C. 2009. A molecular phylogenetic reappraisal of the *Hysteriaceae, Mytilinidiaceae* and *Gloniaceae* (*Pleosporomycetidae*, *Dothideomycetes*) with keys to world species. Studies in Mycology 64:49-83S3. Available from: https://doi.org/10.3114/sim.2009.64.03

Boswell M, Terrell D, Booth RE. 2007. Isolation of Glutaminyl Cyclase from *Saccharomyces cerevisiae*. The FASEB Journal 21(6):A1022-A1023. Available from: https://doi.org/10.1096/fasebj.21.6.A1022-d

Bridge PD, Spooner BM. 2012. Non-lichenized Antarctic fungi: transient visitors or members of a cryptic ecosystem? Fungal Ecology 5(4):381-394. Available from: https://doi.org/10.1016/j.funeco.2012.01.007

British Antarctic Survey. 1978. Occurrence dataset. Antarctic plant database. Available from: https://doi.org/10.15468/6dgnjf

Capon RJ. 2020. Extracting value: mechanistic insights into the formation of natural product artifacts – case studies in marine natural products. Natural Product Reports 37(1):55-79. Available from: https://doi.org/10.1039/C9NP00013E

Carbone I, Kohn LM. 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91(3):553-556. Available from: https://doi.org/10.1080/00275514.1999.12061051

Carvajal-Campos A, Manizan AL, Tadrist S, Akaki DK, Koffi-Nevry R, Moore GG, Fapohunda SO, Bailly S, Montet D, Oswald IP et al. . 2017. *Aspergillus korhogoensis*, a Novel Aflatoxin Producing Species from the Côte d'Ivoire. Toxins 9(11). Available from: https://doi.org/10.3390/toxins9110353

Cushman DW, Cheung HS. 1971. Concentrations of angiotensin-converting enzyme in tissues of the rat. Biochimica et Biophysica Acta (BBA) - Enzymology 250(1):261-265. Available from: https://doi.org/10.1016/0005-2744(71)90142-2

Delgado G, Koukol O, Miller A, Piepenbring M. 2019. *Septonema lohmanii* G. Delgado & O. Koukol, sp. nov., a New Species in *Mytilinidiales* (*Dothideomycetes*) and the Phylogenetic Position of *S. fasciculare* (Corda) S. Hughes. Cryptogamie Mycologie 40:3-21. Available from: https://doi.org/10.5252/cryptogamie-mycologie2019v40a2

Dragnes BT, Stormo SK, Larsen R, Ernstsen HH, Elvevoll EO. 2009. Utilisation of fish industry residuals: Screening the taurine concentration and angiotensin converting enzyme inhibition potential in cod and salmon. Journal of Food Composition and Analysis 22(7):714-717. Available from: https://doi.org/10.1016/j.jfca.2009.01.020

Duchrow T, Shtatland T, Guettler D, Pivovarov M, Kramer S, Weissleder R. 2009. Enhancing navigation in biomedical databases by community voting and database-driven text classification. BMC Bioinformatics 10:317. Available from: https://doi.org/10.1186/1471-2105-10-317

Ehren J, Govindarajan S, Morón B, Minshull J, Khosla C. 2008. Protein engineering of improved prolyl endopeptidases for celiac sprue therapy. Protein Engineering, Design and Selection 21(12):699-707. Available from: https://doi.org/10.1093/protein/gzn050

Fitzgerald RJ, Murray BA, Walsh DJ. 2004. Hypotensive Peptides from Milk Proteins. The Journal of Nutrition 134(4):980S-988S. Available from: https://doi.org/10.1093/jn/134.4.980S

Garzoli L, Gnavi G, Tamma F, Tosi S, Varese GC, Picco AM. 2015. Sink or swim: Updated knowledge on marine fungi associated with wood substrates in the Mediterranean Sea and hints about their potential to remediate hydrocarbons. Progress in Oceanography 137:140-148. Available from: https://doi.org/10.1016/j.pocean.2015.05.028

Gazme B, Boachie RT, Tsopmo A, Udenigwe CC. 2019. Occurrence, properties and biological significance of pyroglutamyl peptides derived from different food sources. Food Science and Human Wellness 8(3):268-274. Available from: https://doi.org/10.1016/j.fshw.2019.05.002

GBIF Secretariat. GBIF Backbone Taxonomy [Internet]. 'Available from:' https://doi.org/10.15468/39omei

Grigoriev IV, Nikitin R, Haridas S, Kuo A, Ohm R, Otillar R, Riley R, Salamov A, Zhao X, Korzeniewski F et al. . 2014. MycoCosm portal: Gearing up for 1000 fungal genomes. Nucleic Acids Research 42:D699. Available from: https://doi.org/10.1093/nar/gkt1183

Hagestad OC, Andersen JH, Altermark B, Hansen E, Rämä T. 2019. Cultivable marine fungi from the Arctic Archipelago of Svalbard and their antibacterial activity. Mycology:1-13. Available from: https://doi.org/10.1080/21501203.2019.1708492

Hanson JR. 2017. Pseudo-Natural Products, Some Artefacts Formed during the Isolation of Terpenoids. Journal of Chemical Research 41(9):497-503. Available from: https://doi.org/10.3184/174751917X15021050367558

Haridas S, Albert R, Binder M, Bloem J, Labutti K, Salamov A, Andreopoulos B, Baker SE, Barry K, Bills G et al. . 2020. 101 *Dothideomycetes* genomes: A test case for predicting lifestyles and emergence of pathogens. Studies in Mycology 96:141-153. Available from: https://doi.org/10.1016/j.simyco.2020.01.003

Hausch F, Shan L, Santiago NA, Gray GM, Khosla C. 2002. Intestinal digestive resistance of immunodominant gliadin peptides. American Journal of Physiology-Gastrointestinal

and Liver Physiology 283(4):G996-G1003. Available from: https://doi.org/10.1152/ajpgi.00136.2002
Hellmann L, Tegel W, Eggertsson Ó, Schweingruber FH, Blanchette R, Kirdyanov A, Gärtner H, Büntgen U. 2013. Tracing the origin of Arctic driftwood. Journal of Geophysical Research: Biogeosciences 118(1):68-76. Available from: https://doi.org/10.1002/jgrg.20022
Hoang DT, Chernomor O, Von Haeseler A, Minh BQ, Vinh LS. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Molecular Biology and Evolution 35(2):518-522. Available from: https://doi.org/10.1093/molbev/msx281
Hongsanan S, Hyde K, Phookamsak R, Wanasinghe D, Mckenzie E, V S, Boonmee S, Jeewon R, M N, Wijayawardene N et al. . 2020. Refined families of *Dothideomycetes*: *Dothideomycetidae* and *Pleosporomycetidae*. Mycosphere 11:2107. Available from: https://doi.org/10.5943/mycosphere/11/1/13
Huang C, Kok J. 2020. Editing of the Proteolytic System of *Lactococcus lactis* Increases Its Bioactive Potential. Applied and Environmental Microbiology 86(18):e01319-20. Available from: https://doi.org/10.1128/AEM.01319-20
Hyde KD, Jones EBG, Liu J-K, Ariyawansa H, Boehm E, Boonmee S, Braun U, Chomnunti P, Crous PW, Dai D-Q et al. . 2013. Families of *Dothideomycetes*. Fungal Diversity 63(1):1-313. Available from: https://doi.org/10.1007/s13225-013-0263-4
Irshad I, Kanekanian A, Peters A, Masud T. 2015. Antioxidant activity of bioactive peptides derived from bovine casein hydrolysate fractions. Journal of Food Science and Technology 52(1):231-239. Available from: https://doi.org/10.1007/s13197-012-0920-8
Jayasiri S, Hyde K, Ebg J, Persoh D, Erio C, Kang J-C. 2018. Taxonomic novelties of hysteriform *Dothideomycetes*. Mycosphere 9:803-837. Available from: https://doi.org/10.5943/mycosphere/9/4/8
Joint Genome Institute. 1000 Fungal Genomes Project - Secondary metabolite clusters [Internet]. [cited 06/03 2021]. Available from: https://mycocosm.jgi.doe.gov/pages/sm-clusters-summary.jsf?organism=fungi&genomes=fungi
Kalyaanamoorthy S, Minh BQ, Wong TKF, Von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14(6):587-589. Available from: https://doi.org/10.1038/nmeth.4285
Katoh K, Misawa K, Kuma KI, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Research 30(14):3059–3066. Available from: https://doi.org/10.1093/nar/gkf436
Katoh K, Standley DM. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Molecular Biology and Evolution 30(4):772–780. Available from: https://doi.org/10.1093/molbev/mst010
Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C et al. . 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647-9. Available from: https://doi.org/10.1093/bioinformatics/bts199
Kirk PW. 1969. Isolation and Culture of Lignicolous Marine Fungi. Mycologia 61(1):174-177. Available from: https://doi.org/10.1080/00275514.1969.12018712
Koukol O, Halasů V, Janošík L, Mlčoch P, Polhorský A, Šandová M, Zíbarová L. 2020. Citizen science facilitates phylogenetic placement of old species of non-lichenised *Pezizomycotina* based on newly collected material. Czech Mycology 72:263-280. Available from: https://doi.org/10.33585/cmy.72207

582 Kunttu P, Pasanen H, Rämä T, Kulju M, Kunttu S-M, Kotiranta H. 2020. Diversity and
583 ecology of aphyllophoroid fungi on driftwood logs on the shores of the Baltic Sea.
584 Nordic Journal of Botany 38(4). Available from: https://doi.org/10.1111/njb.02735
585 Kusnick C, Jansen R, Liberra K, Lindequist U. 2002. Ascochital, a new metabolite from the
586 marine ascomycete *Kirschsteiniothelia maritima*. Pharmazie 57(7):510-2. Available
587 from: https://doi.org/10.24355/dbbs.084-201812041521-0
588 Lacerda LT, Gusmão LFP, Rodrigues A. 2018. Diversity of endophytic fungi in Eucalyptus
589 microcorys assessed by complementary isolation methods. Mycological Progress
590 17(6):719-727. Available from: https://doi.org/10.1007/s11557-018-1385-6
591 Lee N-Y, Cheng J-T, Enomoto T, Nakamura I. 2006. Essential of Proline and Valine Residues
592 in the Peptide Derived from Lactoferrin for Angiotensin Converting Enzyme
593 Inhibition. Journal of the Chinese Chemical Society 53(3):515-518. Available from:
594 https://doi.org/10.1002/jccs.200600068
595 Liu YJ, Whelen S, Hall BD. 1999. Phylogenetic relationships among ascomycetes: evidence
596 from an RNA polymerse II subunit. Molecular Biology and Evolution 16(12):1799-
597 808. Available from: https://doi.org/10.1093/oxfordjournals.molbev.a026092
598 Liu Z, Valente J, Lin S, Chennamsetty N, Qiu D, Bolgar M. 2019. Cyclization of N-Terminal
599 Glutamic Acid to pyro-Glutamic Acid Impacts Monoclonal Antibody Charge
600 Heterogeneity Despite Its Appearance as a Neutral Transformation. Journal of
601 Pharmaceutical Sciences 108(10):3194-3200. Available from:
602 https://doi.org/10.1016/j.xphs.2019.05.023
603 Maltese F, Van Der Kooy F, Verpoorte R. 2009. Solvent Derived Artifacts in Natural
604 Products Chemistry. Natural Product Communications 4(3):1934578X0900400326.
605 Available from: https://doi.org/10.1177/1934578X0900400326
606 Masuda O, Nakamura Y, Takano T. 1996. Antihypertensive Peptides Are Present in Aorta
607 after Oral Administration of Sour Milk Containing These Peptides to Spontaneously
608 Hypertensive Rats. The Journal of Nutrition 126(12):3063-3068. Available from:
609 https://doi.org/10.1093/jn/126.12.3063
610 Mathiassen G, Granmo A, Rämä T. 2015. *Lophium elegans* (*Ascomycota*), a rare European
611 species. Mycotaxon 129(2):433-438. Available from: https://doi.org/10.5248/129.433
612 Minh BQ, Nguyen MaT, Von Haeseler A. 2013. Ultrafast Approximation for Phylogenetic
613 Bootstrap. Molecular Biology and Evolution 30(5):1188-1195. Available from:
614 https://doi.org/10.1093/molbev/mst024
615 Minter DW. 2007. *Mytilinidion mytilinellum*. [Descriptions of Fungi and Bacteria]. IMI
616 Descriptions of Fungi and Bacteria:Sheet 1738. Available from:
617 https://doi.org/10.1079/DFB/20083005531
618 Mohanty DP, Mohapatra S, Misra S, Sahu PS. 2016. Milk derived bioactive peptides and their
619 impact on human health - A review. Saudi journal of biological sciences 23(5):577-
620 583. Available from: https://doi.org/10.1016/j.sjbs.2015.06.005
621 Moncalvo J-M, Wang H-H, Hseu R-S. 1995. Phylogenetic Relationships in *Ganoderma*
622 Inferred from the Internal Transcribed Spacers and 25S Ribosomal DNA Sequences.
623 Mycologia 87(2):223-238. Available from: https://doi.org/10.2307/3760908
624 Morais KLP, Ianzer D, Miranda JRR, Melo RL, Guerreiro JR, Santos RaS, Ulrich H, Lameu
625 C. 2013. Proline rich-oligopeptides: Diverse mechanisms for antihypertensive action.
626 Peptides 48:124-133. Available from: https://doi.org/10.1016/j.peptides.2013.07.016
627 Musa-Veloso K, Paulionis L, Pelipyagina T, Evans M. 2019. A Randomized, Double-Blind,
628 Placebo-Controlled, Multicentre Trial of the Effects of a Shrimp Protein Hydrolysate
629 on Blood Pressure. International Journal of Hypertension 2019:2345042. Available
630 from: https://doi.org/10.1155/2019/2345042

631 Nakamura Y, Yamamoto N, Sakai K, Okubo A, Yamazaki S, Takano T. 1995. Purification
632 and Characterization of Angiotensin I-Converting Enzyme Inhibitors from Sour Milk.
633 Journal of Dairy Science 78(4):777-783. Available from:
634 https://doi.org/10.3168/jds.S0022-0302(95)76689-9
635 Nguyen L-T, Schmidt HA, Von Haeseler A, Minh BQ. 2014. IQ-TREE: A Fast and Effective
636 Stochastic Algorithm for Estimating Maximum-Likelihood Phylogenies. Molecular
637 Biology and Evolution 32(1):268-274. Available from:
638 https://doi.org/10.1093/molbev/msu300
639 Nielsen KF, Ngemela AF, Jensen LB, De Medeiros LS, Rasmussen PH. 2015. UHPLC-
640 MS/MS Determination of Ochratoxin A and Fumonisins in Coffee Using QuEChERS
641 Extraction Combined with Mixed-Mode SPE Purification. Journal of Agricultural and
642 Food Chemistry 63(3):1029-1034. Available from: https://doi.org/10.1021/jf504254q
643 Parr CS, Wilson N, Leary P, Schulz KS, Lans K, Walley L, Hammock JA, Goddard A, Rice J,
644 Studer M et al. . 2014. The Encyclopedia of Life v2: Providing Global Access to
645 Knowledge About Life on Earth. Biodiversity Data Journal 2:e1079. Available from:
646 https://doi.org/10.3897/BDJ.2.e1079
647 Pasqualoto K, Da Silva BaVG, Kodama RT, Portaro FCV. 2018. Exploring the C-domain
648 inhibition of angiotensin converting enzyme through novel bradykinin potentiating
649 peptides. Pharmacy & Pharmacology International Journal 6(6):408-416. Available
650 from: https://doi.org/10.15406/ppij.2018.06.00210
651 Prasannarai K, Sridhar K. 1997. Effect of incubation period of driftwood on the occurrence of
652 marine fungi. Indian Journal of Marine Sciences 26:380-382. Available from:
653 http://nopr.niscair.res.in/bitstream/123456789/36374/1/IJMS%2026%284%29%20380
654 -382.pdf
655 Purves K, Macintyre L, Brennan D, Hreggviðsson GÓ, Kuttner E, Ásgeirsdóttir ME, Young
656 LC, Green DH, Edrada-Ebel R, Duncan KR. 2016. Using Molecular Networking for
657 Microbial Secondary Metabolite Bioprospecting. Metabolites 6(1):2. Available from:
658 https://doi.org/10.3390/metabo6010002
659 Qiao M, Zheng H, Lv R, Yu Z. 2020. *Neodactylariales*, *Neodactylariaceae*
660 (*Dothideomycetes*, *Ascomycota*): new order and family, with a new species from
661 China. MycoKeys 73:69-85. Available from:
662 https://doi.org/10.3897/mycokeys.73.54054
663 Rees G, Jones EBG. 1985. The Fungi of a Coastal Sand Dune System. Botanica Marina
664 28(5):213-220. Available from: https://doi.org/10.1515/botm.1985.28.5.213
665 Rehner SA, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-
666 alpha sequences: evidence for cryptic diversification and links to *Cordyceps*
667 teleomorphs. Mycologia 97(1):84-98. Available from:
668 https://doi.org/10.3852/mycologia.97.1.84
669 Rehner SA, Samuels GJ. 1994. Taxonomy and phylogeny of *Gliocladium* analysed from
670 nuclear large subunit ribosomal DNA sequences. Mycological Research 98(6):625–
671 634. Available from: https://doi.org/10.1016/S0953-7562(09)80409-7
672 Rink R, Arkema-Meter A, Baudoin I, Post E, Kuipers A, Nelemans SA, Akanbi MHJ, Moll
673 GN. 2010. To protect peptide pharmaceuticals against peptidases. Journal of
674 Pharmacological and Toxicological Methods 61(2):210-218. Available from:
675 https://doi.org/10.1016/j.vascn.2010.02.010
676 Rämä T, Norden J, Davey ML, Mathiassen GH, Spatafora JW, Kauserud H. 2014. Fungi
677 ahoy! Diversity on marine wooden substrata in the high North. Fungal Ecology 8:46–
678 58. Available from: https://doi.org/10.1016/j.funeco.2013.12.002

679 Salim AA, Samarasekera K, Khalil ZG, Capon RJ. 2020. Exploring Natural Product Artifacts:
680 The Polyketide Enterocin Warms to a Ballet of Isomers. Organic Letters 22(12):4828-
681 4832. Available from: https://doi.org/10.1021/acs.orglett.0c01629
682 Schilling S, Wasternack C, Demuth H-U. 2008. Glutaminyl cyclases from animals and plants:
683 a case of functionally convergent protein evolution. Biological Chemistry 389(8):983-
684 991. Available from: https://doi.org/10.1515/BC.2008.111
685 Schmitt I, Crespo A, Divakar P, Fankhauser JD, Herman-Sackett E, Kalb K, Nelsen M,
686 Nelson NA, Rivas Plata E, Shimp AD et al. . 2009. New primers for promising single-
687 copy genes in fungal phylogenetics and systematics. Persoonia 23:35-40. Available
688 from: https://doi.org/10.3767/003158509X470602
689 Schoch C, Spatafora J, Lumbsch T, Huhndorf SM, Hyde K, Groenewald JZ, Crous P. 2009. A
690 phylogenetic re-evaluation of *Dothideomycetes*. Studies in Mycology 64. Available
691 from: https://www.studiesinmycology.org/sim/Sim64/sim64.pdf
692 Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, Khovanskaya R, Leipe D,
693 Mcveigh R, O'neill K, Robbertse B et al. . 2020. NCBI Taxonomy: a comprehensive
694 update on curation, resources and tools. Database (Oxford) 2020. Available from:
695 https://doi.org/10.1093/database/baaa062
696 Shan L, Molberg Ø, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C. 2002.
697 Structural Basis for Gluten Intolerance in Celiac Sprue. Science 297(5590):2275.
698 Available from: https://doi.org/10.1126/science.1074129
699 Siepmann R, Johnson TW. 1960. Isolation and Culture of Fungi From Wood Submerged in
700 Saline and Fresh Waters. Journal of the Elisha Mitchell Scientific Society 76(1):150-
701 154. Available from: http://www.jstor.org/stable/24334439
702 Silva SV, Malcata FX. 2005. Caseins as source of bioactive peptides. International Dairy
703 Journal 15(1):1-15. Available from: https://doi.org/10.1016/j.idairyj.2004.04.009
704 Spatafora J, Owensby C, Douhan G, Boehm E, Schoch C. 2012. Phylogenetic placement of
705 the ectomycorrhizal genus *Cenococcum* in *Gloniaceae* (*Dothideomycetes*). Mycologia
706 104:758-65. Available from: https://doi.org/10.3852/11-233
707 Stielow JB, Lévesque CA, Seifert KA, Meyer W, Iriny L, Smits D, Renfurm R, Verkley GJM,
708 Groenewald M, Chaduli D et al. . 2015. One fungus, which genes? Development and
709 assessment of universal primers for potential secondary fungal DNA barcodes.
710 Persoonia 35:242-263. Available from: https://doi.org/10.3767/003158515X689135
711 Velkov T, Horne J, Scanlon Martin j, Capuano B, Yuriev E, Lawen A. 2011. Characterization
712 of the N-Methyltransferase Activities of the Multifunctional Polypeptide Cyclosporin
713 Synthetase. Chemistry & Biology 18(4):464-475. Available from:
714 https://doi.org/10.1016/j.chembiol.2011.01.017
715 Venditti A. 2018. Artifacts in natural products studies. An old and underestimated re-
716 emerging problem. Natural Product Research 32(7):(i)-(ii). Available from:
717 https://doi.org/10.1080/14786419.2018.1443421
718 Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically
719 amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology
720 172(8):4238–4246. Available from: https://doi.org/10.1128/jb.172.8.4238-4246.1990
721 Von Woedtke T, Lindequist U, Alhitari N, Kusnick C, Jülich WD, Abel PU. 2002. The
722 usefulness of a biosensor controlled perfusion cell culture for the investigation of new
723 drugs demonstrated with the marine fungus *Kirschsteiniothelia maritima*. Pharmazie
724 57(4):270-4. Available from: https://publikationsserver.tu-
725 braunschweig.de/rsc/viewer/dbbs_derivate_00044861/images/00000308.jpg
726 White TJ, Bruns T, Lee S, Taylor J. 1990. 'Amplification and direct sequencing of fungal
727 ribosomal RNA Genes for phylogenetics', in: (ed) *PCR - Protocols and Applications -*
728 *A Laboratory Manual*. ed.: Academic Press. 315–322.

729 Wijayawardene NN, Crous PW, Kirk PM, Hawksworth DL, Boonmee S, Braun U, Dai D-Q,
730 D'souza MJ, Diederich P, Dissanayake A et al. . 2014. Naming and outline of
731 *Dothideomycetes*–2014 including proposals for the protection or suppression of
732 generic names. Fungal Diversity 69(1):1-55. Available from:
733 https://doi.org/10.1007/s13225-014-0309-2
734 WoRMS Editorial Board. World Register of Marine Species [Internet]. VLIZ. 'Available
735 from:' https://doi.org/10.14284/170
736 Woudenberg JHC, Aveskamp MM, De Gruyter J, Spiers AG, Crous PW. 2009. Multiple
737 *Didymella* teleomorphs are linked to the *Phoma clematidina* morphotype. Persoonia
738 22:56-62. Available from: https://doi.org/10.3767/003158509X427808
739 Wu VW, Dana CM, Iavarone AT, Clark DS, Glass NL. 2017. Identification of Glutaminyl
740 Cyclase Genes Involved in Pyroglutamate Modification of Fungal Lignocellulolytic
741 Enzymes. mBio 8(1):e02231-16. Available from: https://doi.org/10.1128/mBio.02231-
742 16
743 Wuerzner G, Peyrard S, Blanchard A, Lalanne F, Azizi M. 2009. The lactotripeptides
744 isoleucine-proline-proline and valine-proline-proline do not inhibit the N-terminal or
745 C-terminal angiotensin converting enzyme active sites in humans. Journal of
746 Hypertension 27(7). Available from: https://doi.org/10.1097/HJH.0b013e32832b4759
747 Zdouc MM, Iorio M, Maffioli SI, Crüsemann M, Donadio S, Sosio M. 2021.
748 *Planomonospora*: A Metabolomics Perspective on an Underexplored *Actinobacteria*
749 Genus. Journal of Natural Products 84(2):204-219. Available from:
750 https://doi.org/10.1021/acs.jnatprod.0c00807
751

Pitfalls in biodiscovery – a case study of Mytilinidion sp. M16HEL1360D1-10.1, a wood-associated fungus from the marine environment in the Arctic

Ole Christian Hagestad, Kine Ø. Hansen, Johan Isaksson, Jeanette H. Andersen, Espen H. Hansen and Teppo Rämä

Supplementary table S1 - Accession number of sequences

	Locus									
Sequence name	18S	ITS	28S	TEF1	RPB1	RPB2	TUB2	ACT	MCM7	Genome accession
Alternaria_alternata_CBS916.96	KC584507	KF465761				KC584375			JN672967	
Ascomycota_sp._BBC		GU453169								
Cenococcum_geophilum_1.58	Genome	Genome	Genome	Genome	Genome		Genome	Genome		GCA_001692895.1
Dendrographa_decolorans_AFTOL_ID307				DQ883725	DQ883718	DQ883715				
Dendrographa_decolorans_Ertz5003	AY548809	AY548808	AY548815							
Dendrographa_decolorans_UPS			KJ851054			KJ851141				
Emarellia_grisea		LT160922	LT160923	LT160934		LT160933		LT160932		
Glonium_chambianum_ANM1484			GQ221883							
Glonium_circumserpens_CBS123343	FJ161160		FJ161200	FJ161108	GU371806	FJ161126				
Glonium_pusillum_			EU552134							
Glonium_pusillum_fung28		MT635300								
Glonium_pusillum_GA_S10			KX661381							
Glonium_pusillum_RP390_11		KX067812								
Glonium_stellatum_CBS207.34	Genome	Genome	Genome	Genome	Genome	Genome	Genome	Genome		GCA_001692915.1
Halojulella_avicenniae_BCC20173	GU371830		GU371822	GU371815		GU371786				
Halokirschsteiniothelia_maritima_CBS221.60			GU323203	GU349001						
Hysterium_angustatum_CBS_236.34	GU397359		FJ161180	FJ161096	GU456341	FJ161117				
Hysterium_thailandica_MFLU180551	MN017900		MN017832	MN077055		MN077077				
Lepidopterella_palustris_CBS459.81	GU266227	NR_160162	NG_066253	Genome		Genome	Genome	Genome		GCA_001692735.1
Lepidopterella_palustris_F32_3										
Lepidopterella_tangerina_strain_ATCC_MYA4212		FJ172274								
Lophium_arboricola_16H107		MK159395								
Lophium_arboricola_ATCC24411		NR_153447								
Lophium_arboricola_CBS758.71			NG_064094							
Lophium_arboricola_FMR3868		KU705825	KU705842							
Lophium_elegans_EB0366	GU323184		GU323210							
Lophium_mytilinum_AFTOL_ID1609	DQ678030		DQ678081	DQ677926		DQ677979				
Lophium_mytilinum_CBS114111	EF596818-19	EF596819	EF596819							
Lophium_mytilinum_CBS123344			FJ161203							
Lophium_mytilinum_CBS269.34	Genome	Genome	Genome		Genome	Genome	Genome	Genome		GCA_010093605.1
Lophium_mytilinum_EB0248	FJ161163			FJ161110		FJ161128				
Lophium_mytilinum_OTU0173										
Lophium_mytilinum_OTU0680		MT925006								
Lophium_zalerioides_MFLUCC14_0417	MF621592	MF621583	MF621587							
Mytilinidiaceae_sp._JH14525			MN384597							
Mytilinidion_acicola_EB0349	GU323185		GU323209			GU371757				
Mytilinidion_acicola_EB0379	GU397362		GU397346			GU397355				
Mytilinidion_andinense_EB0330	FJ161159			FJ161107						
Mytilinidion_australe_CBS301.34		NR_160067	FJ161183							
Mytilinidion_californicum_EB0385	GU323186		GU323208							
Mytilinidion_californicum_LTL50		MF663546								

Supplementary table S1 - Accession number of sequences

	Locus									
Sequence name	18S	ITS	28S	TEF1	RPB1	RPB2	TUB2	ACT	MCM7	Genome accession
Mytilinidion_decipiens_CBS302.34		MH855534	MH867036							
Mytilinidion_didymospora_MFUCC160619	MH535891		MH535902			MH535877				
Mytilinidion_mytilinellum_CBS303.34	FJ161144	HM163570		FJ161100	GU357810	FJ161119				
Mytilinidion_mytilinellum_EB0386	GU397363		GU397347			GU397356			JN673016	
Mytilinidion_resinicola_CBS304.34	FJ161145	MH855535	FJ161185	Genome	Genome	Genome	Genome	Genome	Genome	GCA_010093595.1
Mytilinidion_rhenanum_CBS135.34	FJ161136		FJ161175	FJ161092		FJ161115				
Mytilinidion_rhenanum_EB0341	GU323187		GU323207							
Mytilinidion_scolecosporum_CBS305.34	FJ161146	MH855536	FJ161186	FJ161102	GU357811	FJ161121				
Mytilinidion_sp._M16HEL1360D1_10.1	MK531785	MK543202	MK531717	This study	This study		This study	This study	This study	
Mytilinidion_thujarum_EB0268	GU323188		GU323206							
Mytilinidion_tortile_CBS306.34	FJ161147	MH855537	FJ161187							
Mytilinidion_tortile_EB0377	GU323189		GU323205							
Neodactylaria_obpyriformis		NR_154267	NG_060361							
Neodactylaria_simaoensis_YFM1.03984	MK562747	MH379209	MH379210	MK562748		MK562749				
Pseudoberkleasmium_acaciae_MFLUCC172590	NG_065782	NR_163343	NG_066316	MK360073						
Pseudoberkleasmium_chiangmaiense_MFLUCC171809		MK131259	MK131260	MK131261						
Pseudocamaropycnis_pini_CBS115589		NR_153459	NG_058211	KU728594			KU728632			
Pseudocenococcum_floridanum_BA4b007	LC095448	LC095213	LC095431	LC095383	LC095414	LC095399				
Purpurepithecium_murisporum	NG_065680		NG_059797	KY887666		KY799176				
Quasiconcha_reticulata_EB_QR			GU397349							
Septonema_crispulum_CBS735.96		MH862607	NG_068576			MK442679				
Septonema_fasciculare		LS998794								
Septonema_fasciculare_CBS127862		MH864666	MH876104							
Septonema_fasciculare_MUCL8886		LS998795		LS998798						
Septonema_secedens_CBS174.74			MH878272							
Septonema_secedens_CBS469.48		MH856437	MH867983							
Septonema_sp._DAOMC226875		MT026473								
Septonema_sp._NK413__PRC		LS998796	LS998796	LS998799						
Septonema_sp._NK413_BPI		LS998797	LS998797	LS998800						
Slimacomyces_isiola		KP739877								
Slimacomyces_isiola_FP1465	AB597227	AB597207	AB597217							
Slimacomyces_isiola_FP1482	AB597225	AB597216	AB597218							
Slimacomyces_isiola_FP1484	AB620068	AB597208	AB618625							
Slimacomyces_isiola_P10433	AB620069	AB597209	AB618626							
Slimacomyces_isiola_P10438	AB597228	AB597211	AB597224							
Slimacomyces_isiola_P42102	AB597226	AB597210	AB597222							
Venturia_inaequalis_CBS_120627		MK810983	MK810868	MK888804		MK887865	MK926538			

Missing loci 67.24 %

Supplementary

Pitfalls in biodiscovery – a case study of *Mytilinidion* sp. M16HEL1360D1-10.1, a wood-associated fungus from the marine environment in the Arctic

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CONTACT Ole Christian Hagestad ole.c.hagestad@uit.no

NMR Spectroscopic Data for the peptide

Figure S1 ^{1}H NMR spectrum

Figure S2 ^{13}C spectrum

Figure S3 HSQC + HMBC spectrum

Figure S4 Zoom in HSQC spectrum

Figure S5 Zoom in HSQC spectrum

Figure S6 Zoom in HMBC spectrum

Figure S7 COSY spectrum

Figure S8 TOCSY spectrum

Figure S9 ROESY spectrum

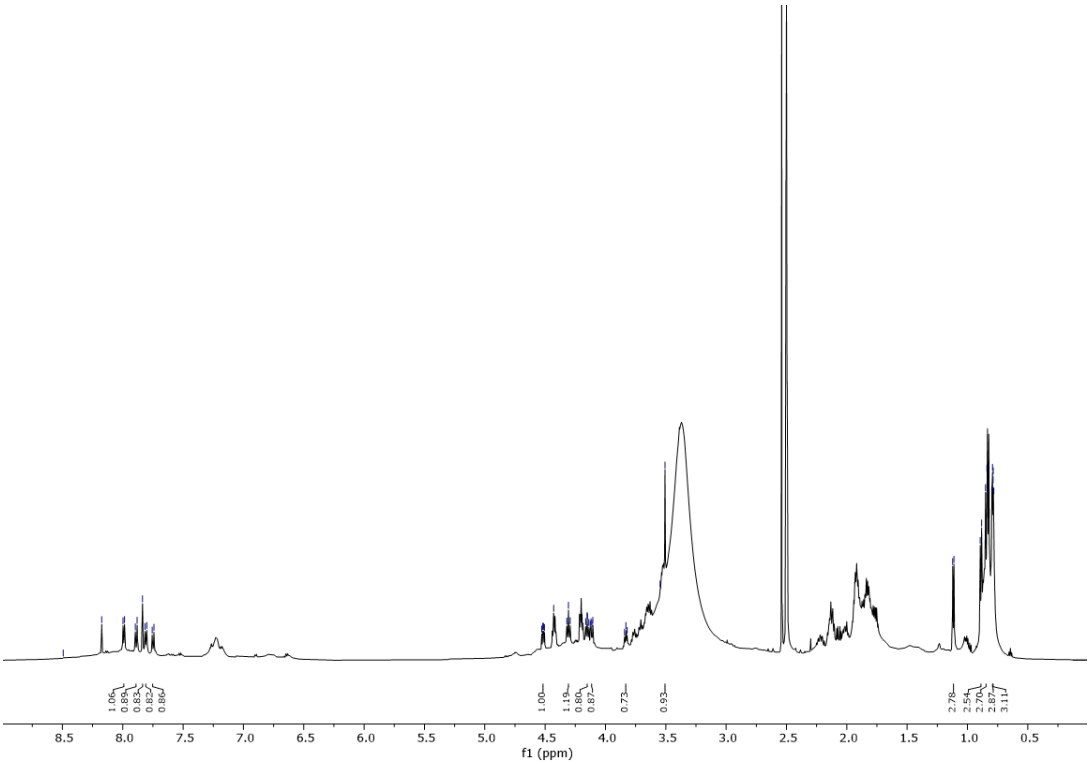
Figure S10 HSQCTOCSY spectrum

Figure S11 H2BC spectrum

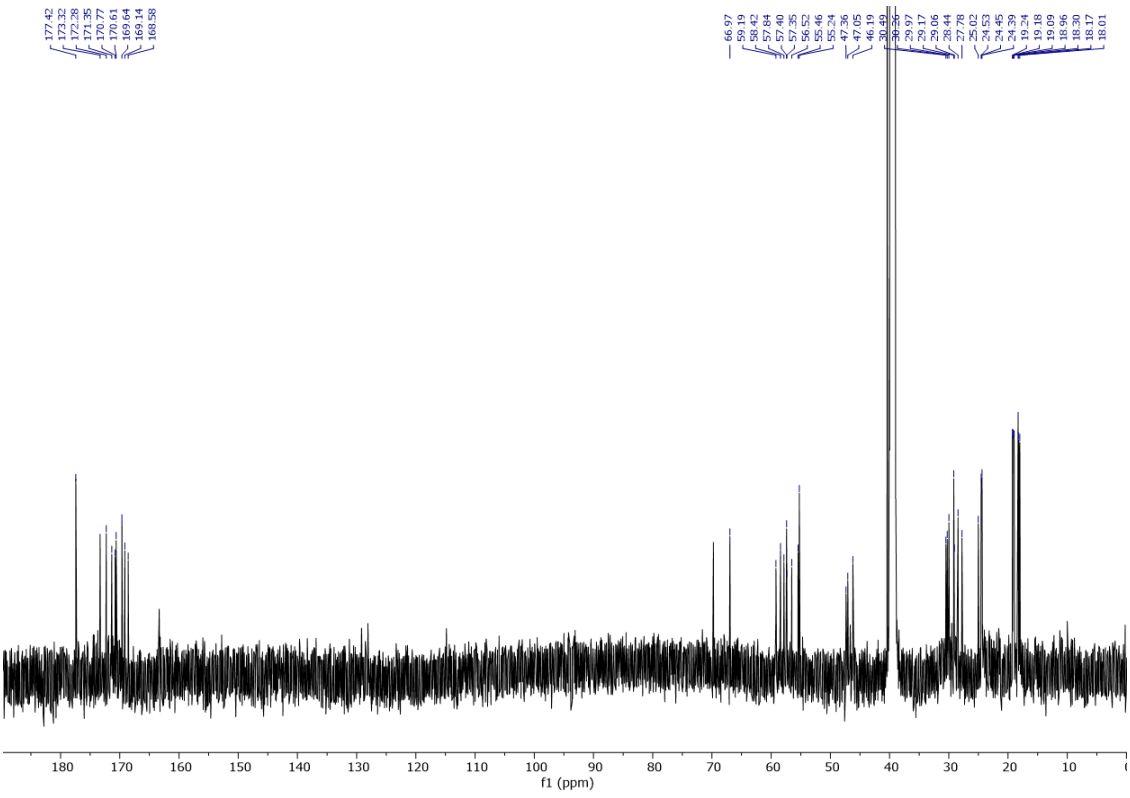
Figure S12 ^{15}N-HSQC

Figure S13 Comparison of predicted chemical shifts for pGln and Gln

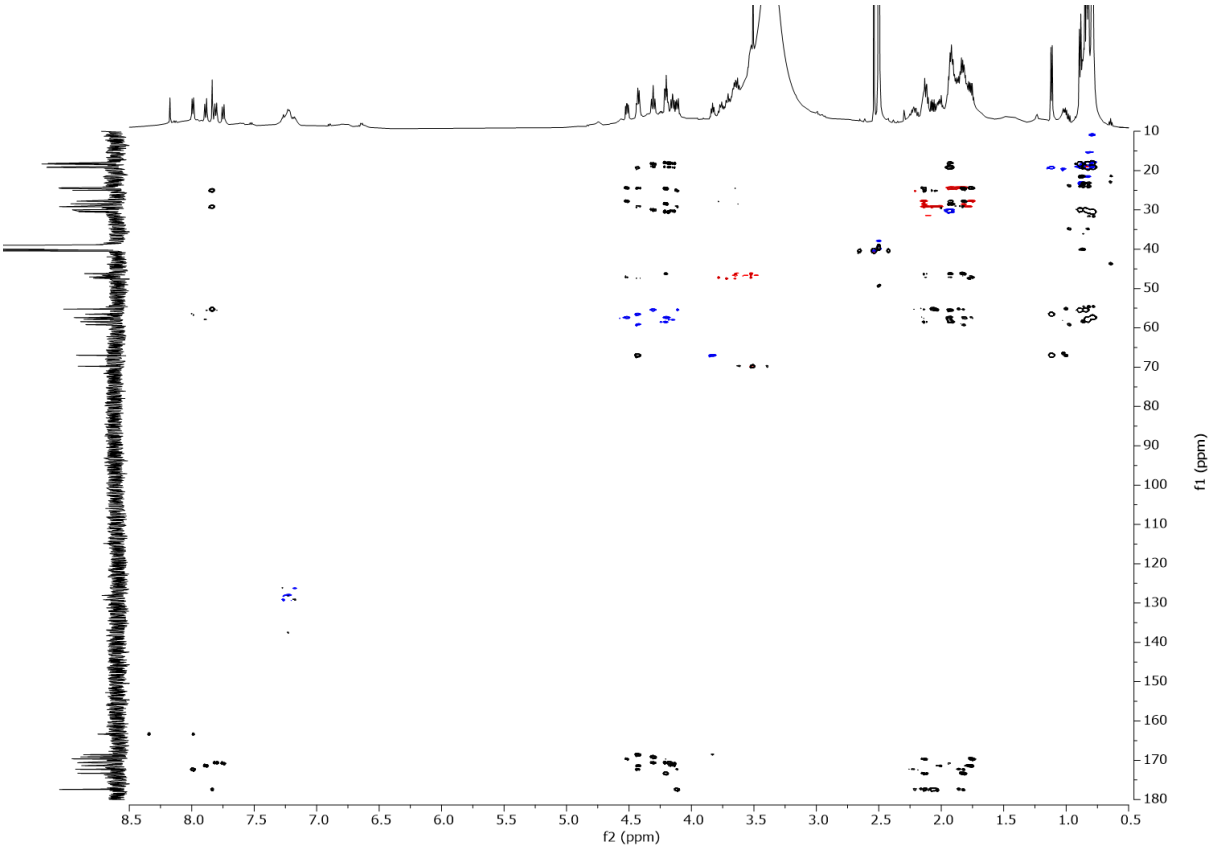
S1. ^{1}H NMR spectrum



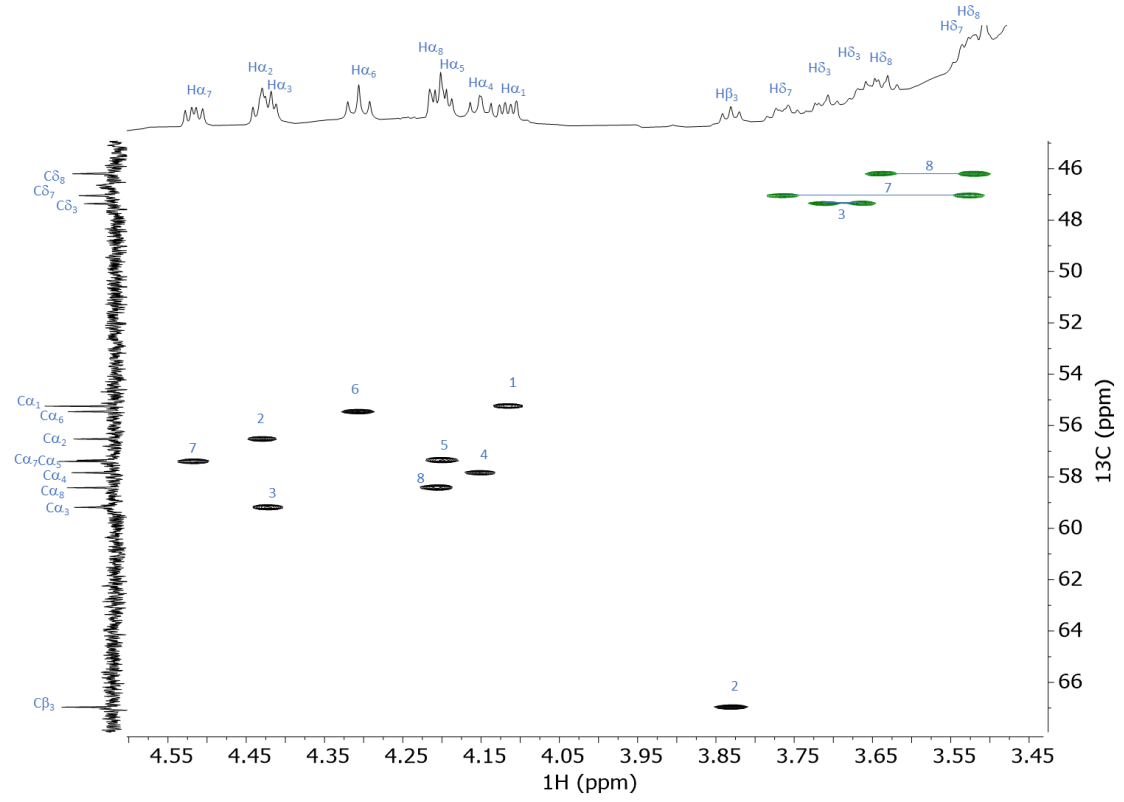
S2. ^{13}C spectrum



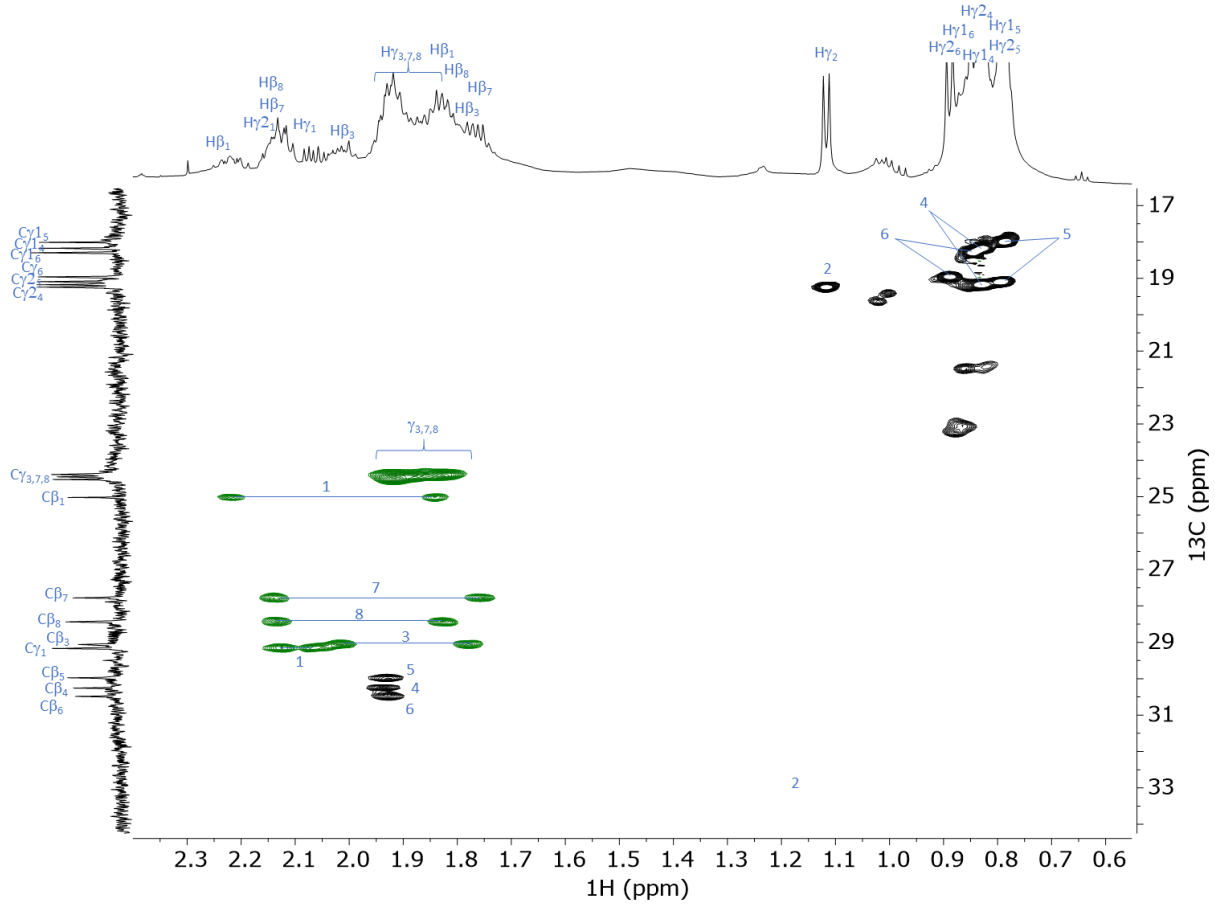
S3. HSQC + HMBC spectrum



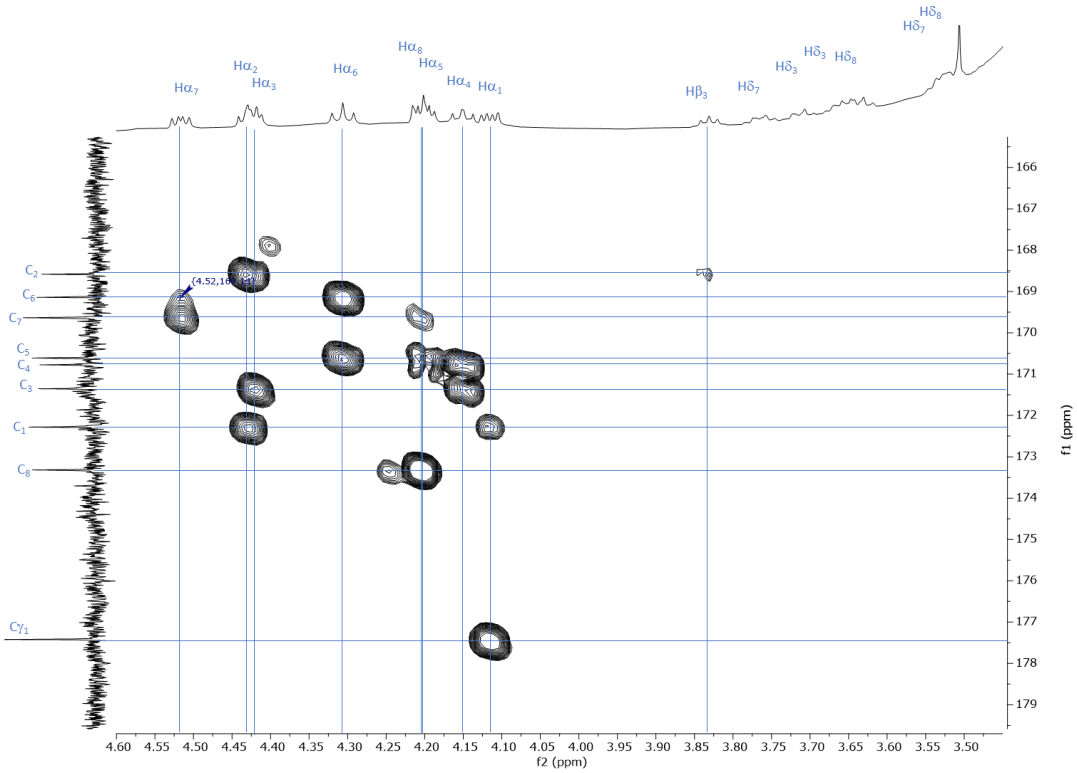
S4. Zoom in HSQC spectrum



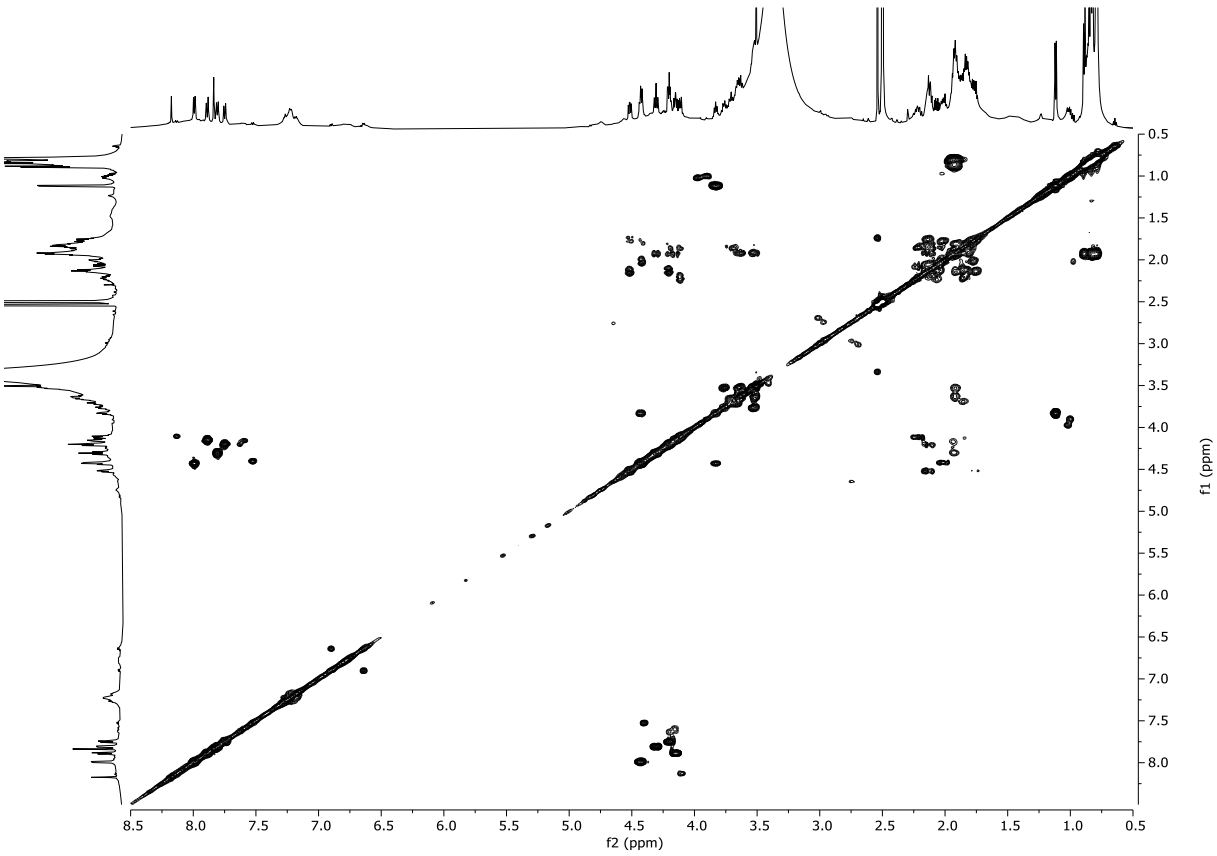
S5. Zoom in HSQC spectrum



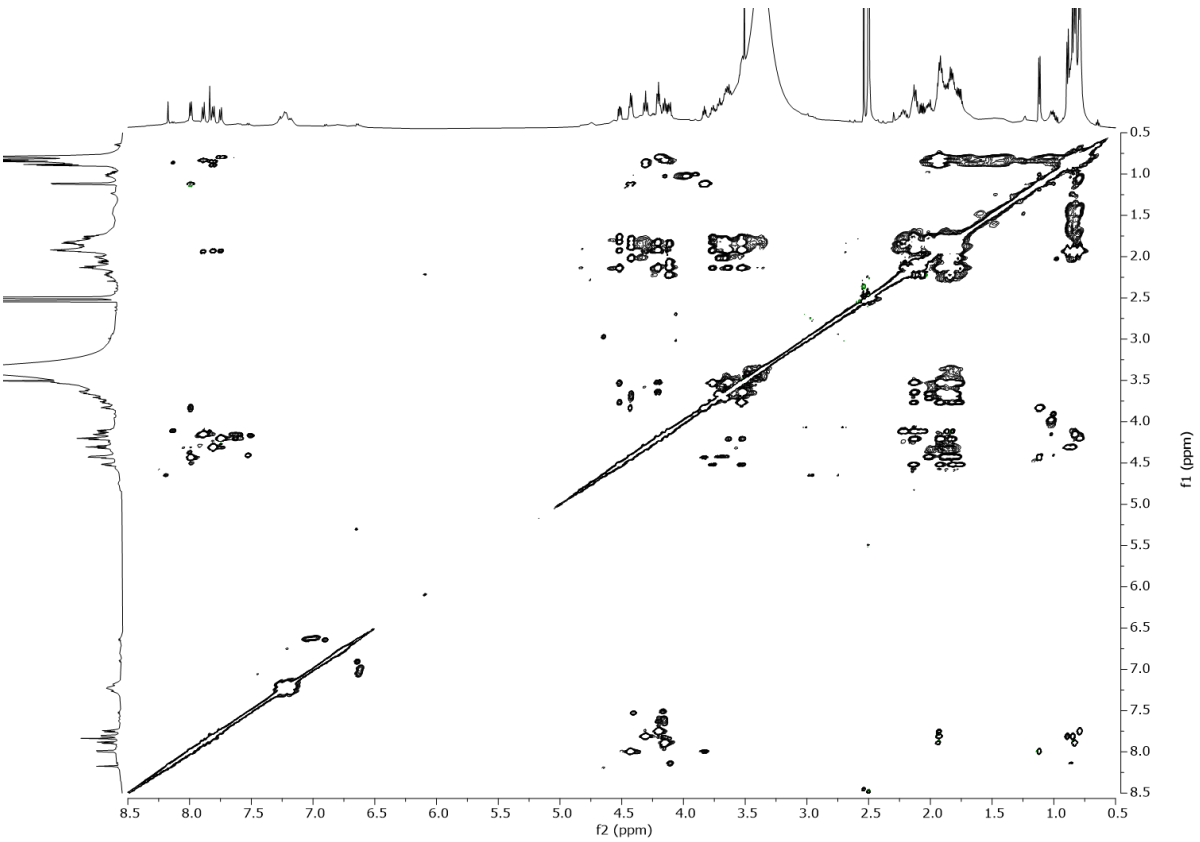
S6. Zoom in HMBC spectrum



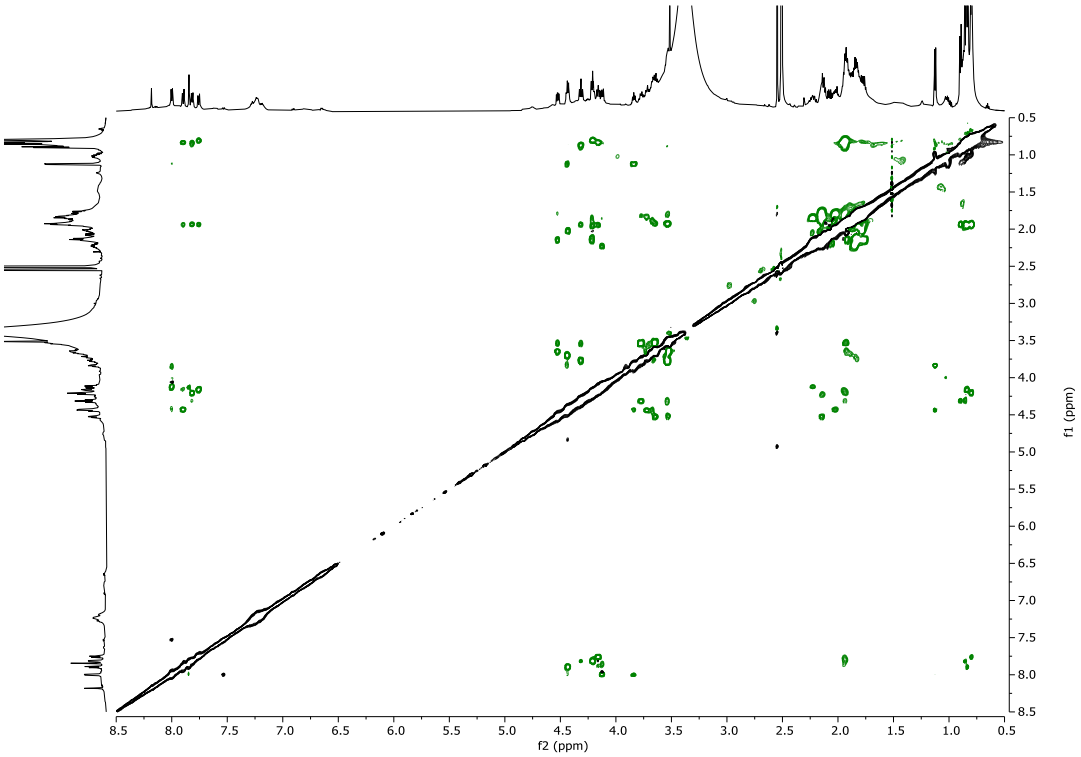
S7. COSY spectrum



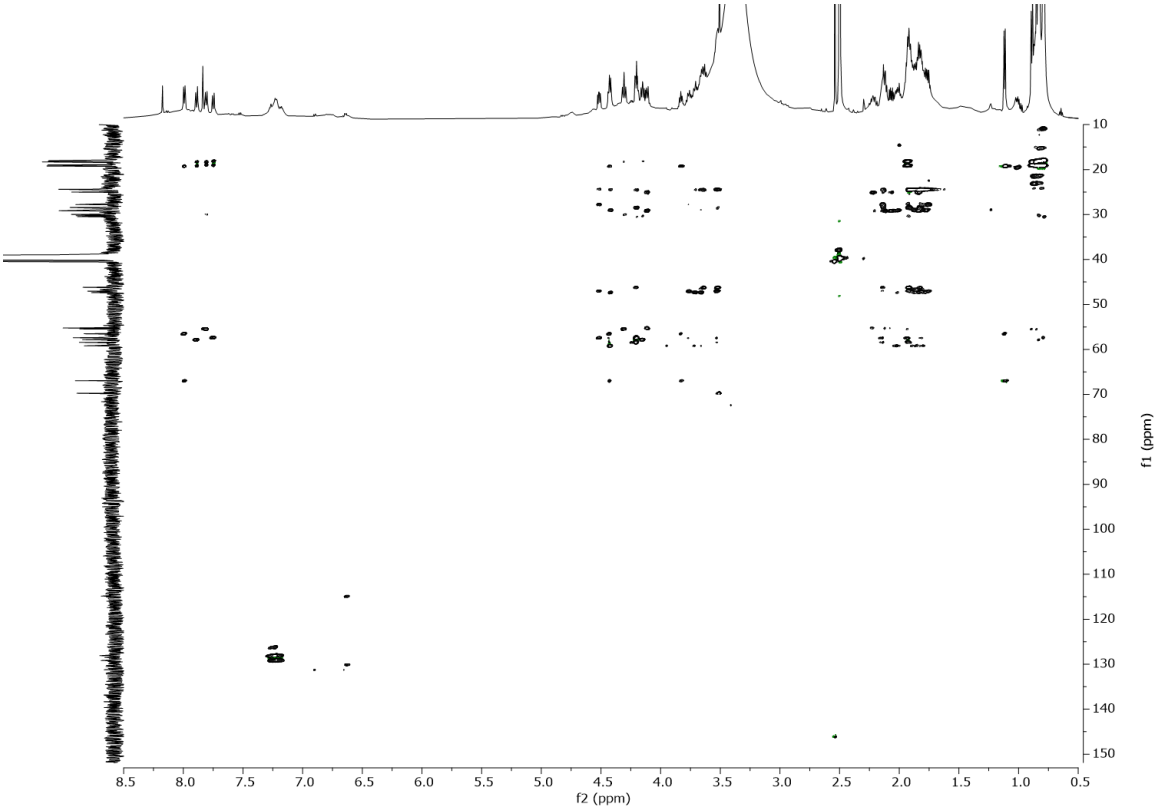
S8. TOCSY spectrum



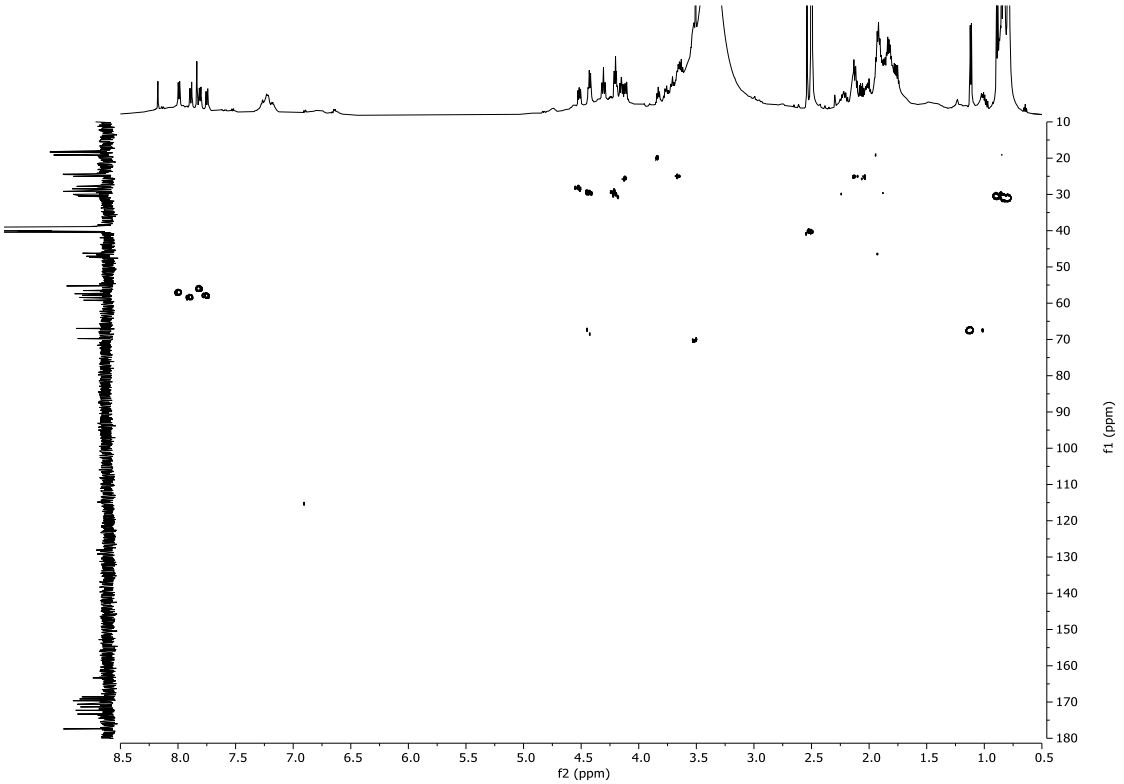
S9. ROESY spectrum



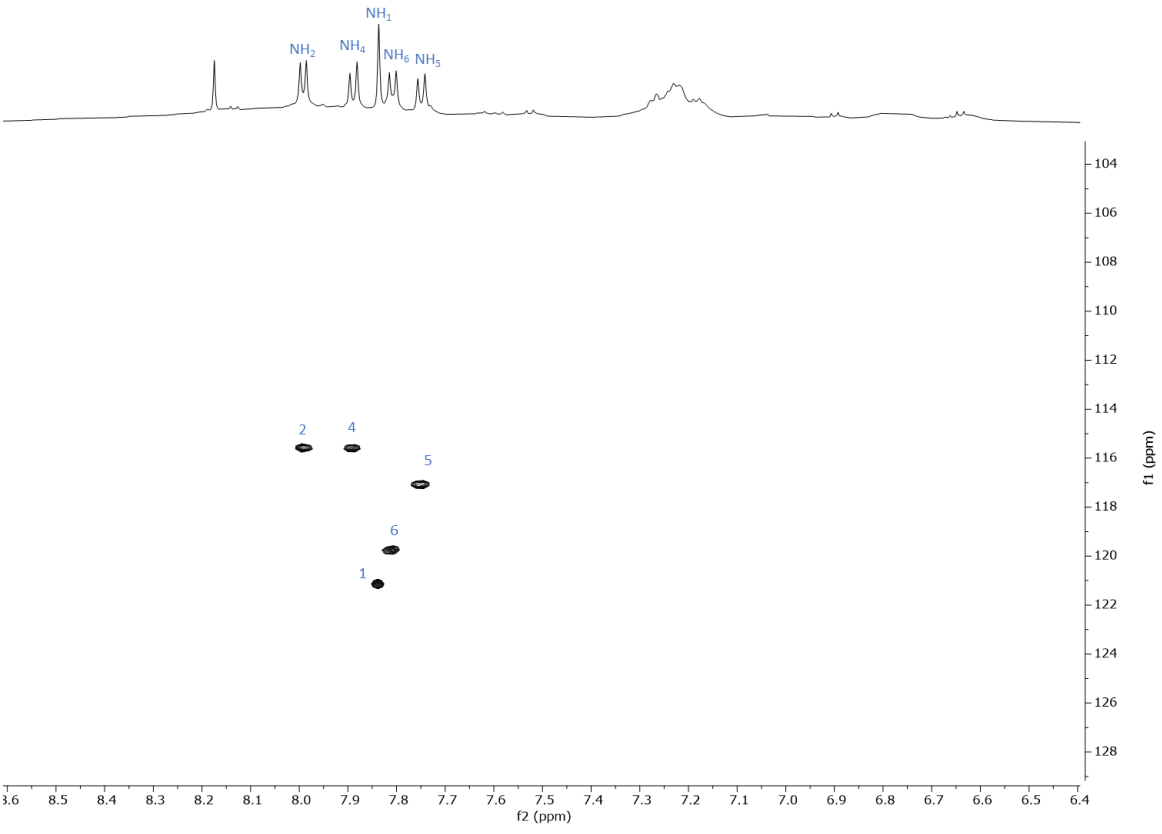
S10. HSQCTOCSY spectrum



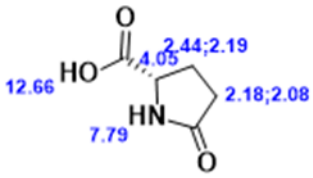
S11. H2BC spectrum



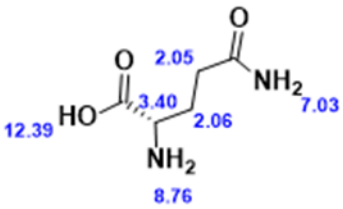
S12. ^{15}N-HSQC



S13. Comparison of predicted chemical shifts for pGln and Gln. Calculated using ChemDraw professional.



pGln



Gln