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## Fungi ahoy! Diversity on marine wooden substrata in the high North



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### ABSTRACT

Marine fungi are severely understudied in the polar regions. We used molecularly identified cultures to study fungi inhabiting 50 intertidal and sea-floor logs along the North Norwegian coast. The aim was to explore the taxonomic and ecological diversity and to examine factors shaping the marine wood-inhabiting fungal communities. The 577 pure cultures analyzed clustered into 147 operational taxonomic units (OTUs) based on 97% ITS sequence similarity. Ascomycota dominated, but OTUs belonging to Basidiomycota, Mucromycotina and Chytridiomycota were also isolated. Nine OTUs could not be assigned to any fungal phylum. Almost half of the OTUs were considered non-marine. The western and eastern part of the Norwegian Barents Sea coast hosted different communities. Geography, substratum and site level variables contributed to shaping these communities. We characterized a previously overlooked fungal community in a poorly studied area, discovered high diversity and report many taxa for the first time from the marine environment.

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### Introduction

Marine fungi form an ecologically heterogeneous assembly of species growing and sporulating in marine, intertidal or estuarine habitats (Kohlmeyer and Kohlmeyer, 1979). They

appear as parasites on algae and animals, as mutualistic symbionts, and as saprotrophs, and play functional roles in nutrient recycling, biogeochemical processes and food web dynamics of the oceans. Most studies of marine fungi to date have been based on morphological characterization of fruit

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bodies, other structures and isolated cultures. More recently, Sanger sequencing, DNA fingerprinting and high throughput sequencing have provided new insights into the diversity and systematics of marine fungi (Stoeck and Epstein, 2003; Zuccaro et al., 2008; Amend et al., 2012). Recently, a high diversity of poorly known fungal lineages was recovered in analyses of rDNA SSU sequences from the marine environment (Richards et al., 2012). The majority of the unexplored diversity was among the early diverging lineages of Kingdom Fungi (Chytridiomycota and Zygomycota) and the Basidiomycota yeasts. When it comes to better studied marine fungi, the vast majority of them are Ascomycota, primarily belonging to Halosphaeriaceae (Microascales, Sordariomycetes) and Lulworthiales (Sordariomycetes), which are both derived from terrestrial ancestors (Spatafora et al., 1998).

Some terrestrial or freshwater taxa are frequently isolated from marine environments, and to the ones that can grow there the term facultative marine fungi has been applied (Kohlmeyer, 1974). The ecological role of putatively terrestrial species in marine habitats has been debated since the beginning of their discovery (Sparrow, 1937; Johnson, 1967; Shearer, 1972; Raghukumar and Raghukumar, 1999). Despite an abundance of terrestrial species in marine studies (e.g. Johnson, 1967), mycologists have tended to focus on obligate marine fungi, overlooking isolates of non-obligate species which have often been considered contaminants or originating from resting structures. This conservative study tradition in marine mycology contradicts the idea that extensive fungal plasticity and metabolic versatility enables fungi to adapt to different ecological niches (Slepecky and Starmer, 2009; Wittenberg et al., 2009). Indeed, as Richards et al. (2012) show, terrestrial to marine transitions and *vice versa* have occurred frequently and apparently are small evolutionary steps for fungi.

Few surveys of marine wood-inhabiting fungi have been conducted in northern waters, and these report species typical for temperate oceans (Jones et al., 1972; Schaumann, 1975; Pang et al., 2011). A study from the arctic waters of Svalbard documented a relatively high number of new and unidentified species, and indicated the occurrence of a distinctive wood-inhabiting arctic mycota (Pang et al., 2011). In two studies from other arctic seas focusing on algae and sediment fungi, respectively, several culture isolates could not be identified to species level, suggesting these might represent new taxa (Bubnova and Kireev, 2009; Bubnova, 2010).

Water temperature and salinity are the two main ecological drivers affecting the distribution of marine wood-inhabiting fungi at a global scale (Booth and Kenkel, 1986; Hughes, 1986). At a local and substratum scale, environmental parameters having an influence on the occurrence of marine fungi include, among others, habitat, zonation in relation to water level and its fluctuations, substratum type and cover of marine organisms, which indicates the duration of presence in the marine environment (Kohlmeyer and Kohlmeyer, 1979). However, the importance of some characteristics of the wooden substrata such as diameter and decay stage has barely been studied in marine mycology, even though these factors are important for terrestrial fungi (Juutilainen et al., 2011; Nordén et al., 2013). The geographic source of driftwood may play a role in structuring wood-inhabiting marine fungal communities, but is difficult to

control for in surveys based on natural substrata. The main source of shore-cast wood in the North Norwegian coast is Siberia (Johansen and Hytteborn, 2001), from which the wood is transported by the great Siberian rivers and polar ocean currents. During parts of the journey the wood drifts fixed in sea ice before it is released due to melting of the ice as it travels south. A large part of the wooden material ends up on the coast of Svalbard, but some drifts further south and comes ashore on the North Norwegian coast (Johansen and Hytteborn, 2001; Hellmann et al., 2013).

The aims of our study were: (i) to explore the taxonomic, phylogenetic and ecological diversity of wood-inhabiting marine fungi in northern waters focusing on Ascomycota and Basidiomycota; and (ii) to elucidate the environmental factors shaping the fungal communities along the long coastline of Northern Norway. Due to different large and small-scale environmental factors, we expect differences in the marine wood-inhabiting mycobiota between the eastern and western part of the study area. We selected a culture-based approach in order to obtain study material from which we could sequence multiple genetic markers (ITS and LSU). Moreover, the fungal cultures provide opportunities for later, more in-depth taxonomic studies.

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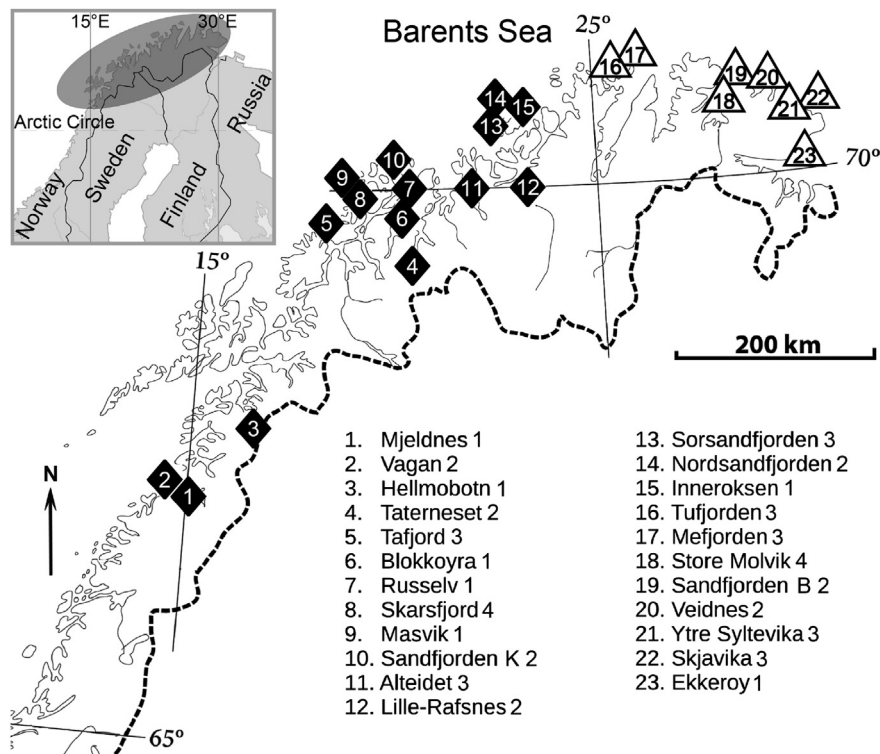
## Material and methods

### Study area

We surveyed wooden substrata, i.e. logs and pieces of wood (hereafter referred to as logs), along the North Norwegian coast between May and Oct. 2010. Of the 50 logs studied at 23 sites (Fig 1), 47 represented shore-cast intertidal and 3 represented sea bottom units (Table S1 in Supplementary data). One shore-cast, oily substratum unit was excluded from data analyses, since no isolates were recovered from it. The sites were located along the approximately 1 000 km long coastline between Bodø (67°14'82"N 015°06'12"E) in the south and Vadsø (70°04'06"N 30°06'25"E) in the northeast. The western part of the study area is slightly warmer than the eastern one (annual sea surface temperature 7 °C and 6 °C, respectively) (Locarnini et al., 2010). Salinity is 34 practical salinity units in both parts (Antonov et al., 2010). In the western part of the North Norwegian coast there is less shore-cast wood and a bigger proportion of it is broadleaved and of local origin, whereas the eastern part is rich in debris from (Siberian) coniferous tree species that do not or scarcely grow in the area (Table S1, personal observations).

### Sampling and culturing

We chose the sampled logs randomly among those showing signs of recent and long lasting presence in the sea (indicated among other features by cover of marine algae and animals). We included logs from the breaker zone only if they had recently been in the sea and if there were no intertidal units available. Shore-cast logs were sampled at low tide. Sea bottom units were caught with an Agassiz trawl. We recorded or measured ecological variables including habitat type (rocky, stony, gravelly, sandy or muddy shore), zone of sampling (tide, breaker, sublittoral or sea bottom), log attachment type (loose



**Fig 1 – Location of the study area and sampling sites on the North Norwegian coast. Diamonds represent fungal communities inhabiting logs in the western part and triangles in the eastern part of the study area. Site names are followed by numbers of logs sampled.**

or fixed), tree species (broadleaved tree or conifer), log quality (whole trunk, broken trunk, cut trunk (including pieces of boards and planks)), branch or unknown (for logs that could not be characterized), position (vertical or horizontal), compass orientation, basal and apical diameter, length, decay class based on knife penetration according to [Hottola and Siitonen \(2008\)](#) and ranging from 1 = hard to 5 = very soft, percent of marine organisms covering the log (=epiphyte cover) and percent of remaining bark cover ([Table S1](#)).

We flame sterilized a knife and forceps and used them to obtain the samples. We cut off a thin slice of the surface wood (approximately 1 mm) on the sampling point, cut out a wooden cube and placed it in a clean plastic bag which was closed air-tight. We collected 12 wooden cubes ( $5 \times 5 \times 2$  mm) on each side (upper, lower and both flanks) of the log in the basal, middle and apical part. Basal and apical cubes were taken 10 cm from each end, respectively, and middle cubes at the midpoint of the unit. When the sampling point was covered by tree bark, an additional bark cube was taken in order to capture those fungi specialized in bark on marine substrata ([Kohlmeyer and Kohlmeyer, 1979](#)). We kept the samples among ice bricks in a cold bag until plating them the same day, usually within 10 hr of sampling. Each cube was placed on a 1/5 malt extract agar plate that was prepared with filtered autoclaved seawater and amended with the antibiotics streptomycin ( $25 \text{ mg l}^{-1}$ ) and tetracycline ( $10 \text{ mg l}^{-1}$ ).

Isolation plates were incubated in the dark at  $15^\circ\text{C}$ , checked once a week until the dish was fully covered and fungi growing out of the cubes were isolated in axenic

cultures. Cubes of agar with fresh mycelium from the growing edges of the fungal colonies were cut out and frozen at  $-25^\circ\text{C}$  in 2% CTAB buffer. To detect possible aerial contamination, we positioned control dishes in the laminar flow hood and in unused plastic bags similar to the ones used in sampling. The controls either did not have any growing cultures or included fungi not detected on the focal dishes.

#### DNA extraction, amplification and sequencing

We extracted the DNA of pure culture isolates using a modified cetyltrimethyl ammonium bromide (CTAB) extraction protocol ([Murray and Thompson, 1980](#)) and amplified the target loci according to [Mysterud et al. \(2007\)](#). PCR amplification was performed on a PTC-0200 DNA engine (MJ Research, Waltham, Massachusetts, USA) using the primer pairs ITS5–ITS4 ([White et al., 1990](#)) and LR0R–LR5 ([Vilgalys and Hester, 1990](#); [Rehner and Samuels, 1994](#)) in  $0.5 \mu\text{M}$  concentration and the Illustra PuReTaq Ready-To-Go PCR beads (GE Healthcare, Buckinghamshire, UK) in a reaction volume of  $25 \mu\text{l}$ .

We cleaned the PCR products using 0.25 units of ExoSAP-IT (Affymetrix, Santa Clara, CA, USA) for  $6 \mu\text{l}$  of PCR product. Sequencing reactions were performed on an Applied Biosystems 3730 DNA analyzer in BigDye Terminator sequencing buffer using PCR primers as sequencing primers and the BigDye Cycle Sequencing kit v3.1 (Applied Biosystems, Foster City, California, USA). The samples were subjected to capillary

electrophoresis on an Applied Biosystems 3730 DNA analyzer (Foster City, CA, USA).

### Bioinformatics and statistical analyses

We automatically assembled forward and reverse sequences and manually edited assemblies in Geneious version 5.6.2. We clustered the ITS sequences using Blastclust (available at: <http://toolkit.tuebingen.mpg.de/blastclust>) with 97% sequence similarity cut-off value. For each ITS cluster, hereafter referred to as an operational taxonomic unit (OTU), we chose a full length high quality ITS sequence (i.e. no ambiguous sites) as representative sequence and sequenced the corresponding isolate for the LSU region. BLASTn searches of ITS and LSU sequences against NCBI's nr nucleotide database were used to assign taxonomy to each of the OTUs.

We used EstimateS version 8.2.0 (Colwell, 2009) to calculate expected species accumulation curves based on the Mao Tau estimator (Colwell et al., 2004) and total species richness ( $\gamma$ -diversity) (Whittaker, 1960) estimate based on the Michaelis–Menten richness estimator MMMeans (Raaijmakers, 1987; Colwell et al., 2004).

We ran maximum likelihood phylogenetic analyses in RAxML version 7.2.6 (Stamatakis, 2006) and Bayesian inferences in MrBayes version 3.2.1 (Ronquist et al., 2012). We extracted and concatenated the 5.8S region from the representative ITS sequences with the LSU sequence data. This combined 5.8S/LSU dataset was aligned with default options using the MUSCLE algorithm (Edgar, 2004) embedded in Geneious and corrected by eye. The alignment matrix measured 126 taxa by 1 389 characters having 750 unique site patterns and 28.9% gaps. We created data subsets of ITS sequences for the different fungal orders and made alignments using either MUSCLE (Edgar, 2004) or MAFFT (Katoh and Standley, 2013) algorithms with default settings. The alignments were improved by eye and analyses run in RAxML. All alignments are available from: <http://doi.org/10.5061/dryad.qg82k>.

We based the ecological annotation of detected OTUs on top LSU BLAST matches, existing literature and the WoRMS database (Appeltans et al., 2012). In addition to key taxonomic literature of marine fungi (Kohlmeyer and Kohlmeyer, 1979; Jones et al., 2009), information from reference studies of marine fungi (Henningson, 1974; Rees et al., 1979; Petersen and Koch, 1997; Tchesunov et al., 2008; Bubnova and Kireev, 2009; Bubnova, 2010; Azevedo et al., 2011; Pang et al., 2011) and other marine fungal literature were utilized. We considered an OTU to be obligate or facultative marine if the taxon (identified using BLAST matches) was known to be a marine one or was at least reported from the sea. The term 'non-marine' is used in this paper to refer to taxa previously only reported from terrestrial or freshwater habitats.

For community ecology analyses we used a community matrix where outliers, three sea-bottom logs and an additional two logs from a separate area in the south were excluded. This was done in order to focus the analyses on communities inhabiting logs in one type of main habitat (shore) in two geographical areas comparable in size: the western area, covering sites 4–14 in Fig 1 and the eastern area, covering sites 16–23. First, we performed a geographic

**Table 1 – Comparison of the wood-inhabiting fungal assemblages in the western and eastern part of the study area**

	Western part	Eastern part	Whole dataset
Number of isolates	322	255	577
Number of OTUs	105	72	147
Number of singletons (of all OTUs)	71 (68%)	49 (68%)	99 (67%)
Number of OTU occurrences	190	143	333
Sørensen index <sup>a,c</sup>	0.07 ± 0.105	0.16 ± 0.151	0.10 ± 0.121
Sørensen index <sup>b,c</sup>	0.00	0.15	0.00
PD <sup>a,d</sup>	0.46 ± 0.137	0.47 ± 0.109	0.46 ± 0.124
PD <sup>b,d</sup>	0.45	0.47	0.46
NRI <sup>a,e</sup>	−0.07 ± 1.156	−0.02 ± 1.161	−0.05 ± 1.146
NRI <sup>b,e</sup>	0.22	0.08	0.21
NTI <sup>a,f</sup>	−0.03 ± 1.267	−0.00 ± 1.060	−0.02 ± 1.167
NTI <sup>b,f</sup>	0.39	−0.03	0.34

a Means ( $\pm$ SD).

b Medians.

c The classic Sørensen index is used here to evaluate the  $\beta$ -diversity between the logs in each geographical area.

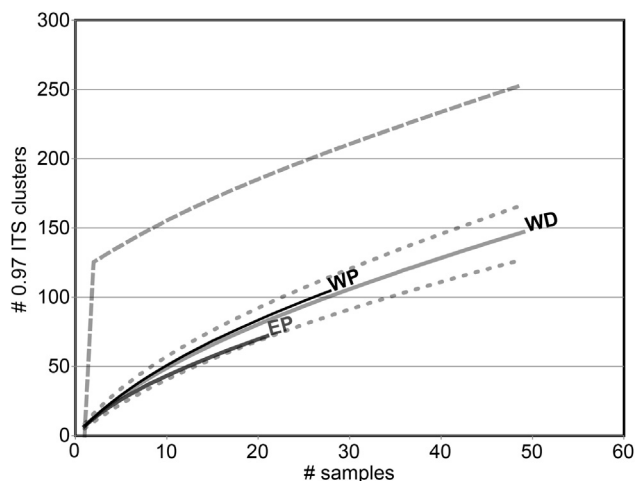
d Faith's index of phylogenetic diversity.

e Net relatedness index.

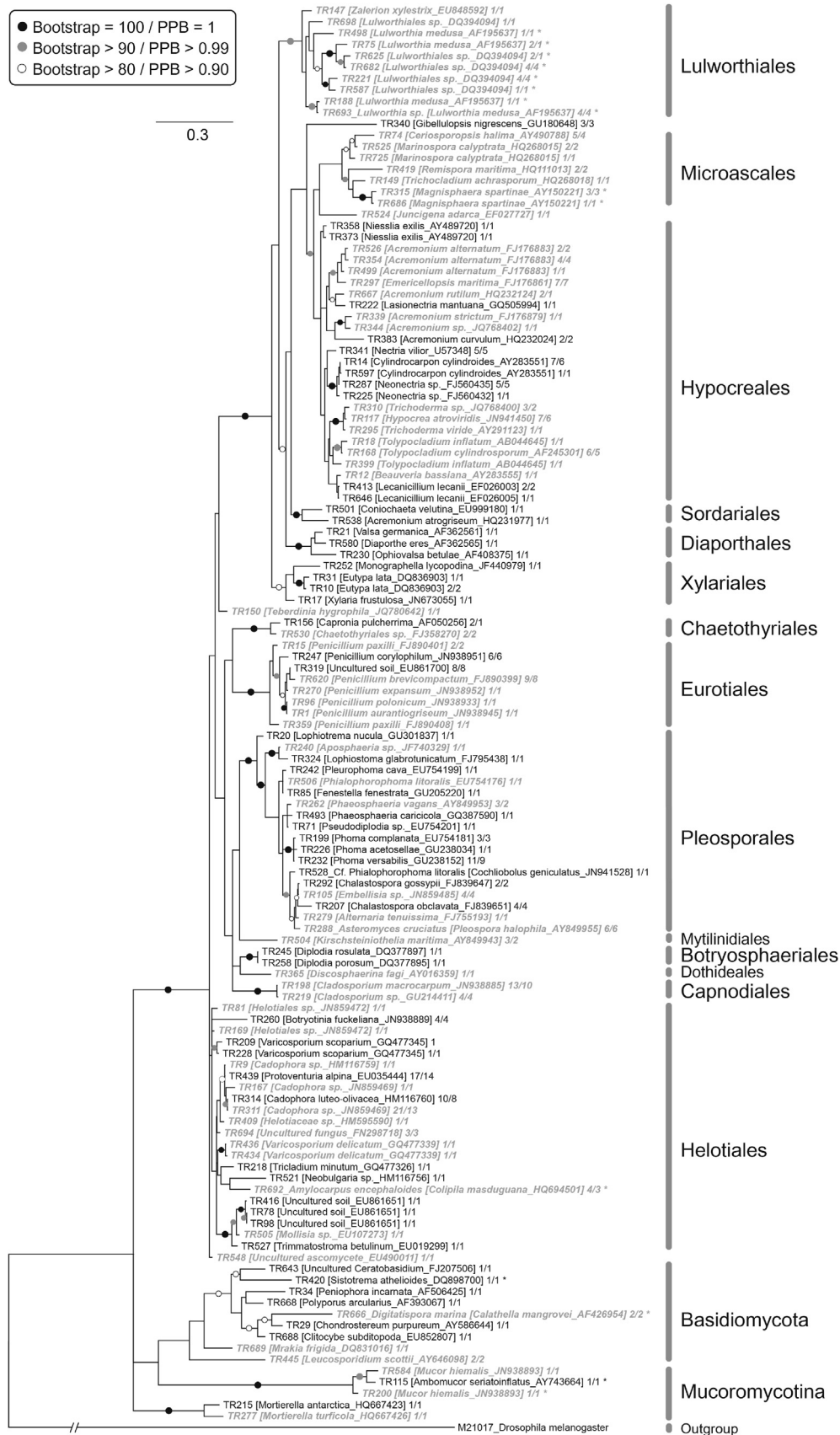
f Nearest taxon index.

comparison of OTU frequency and abundance in Qiime version 1.5.0 (Caporaso et al., 2010). G-tests and ANOVAs run with default settings were used to study whether OTUs occurred more abundantly or more frequently in the western or eastern part. Thirteen OTUs were analyzed, all of which occurred at least five times in the dataset.

We ran a non-metric multidimensional scaling (NMDS) ordination analysis in R with packages vegan and MASS (Venables and Ripley, 2002; Oksanen et al., 2013; R Core Team, 2013). Variables fitted to the ordination diagram as factors and



**Fig 2 – OTU accumulation and estimated richness. Solid lines show rarefaction curves based on Mao Tau estimator, dotted line shows 95% confidence limits and dashed line Michaelis–Menten estimate of  $\gamma$ -diversity. WP = Western part, EP = Eastern Part, WD = Whole dataset.**



**Fig 3 – Phylogenetic and ecological diversity of the 125 wood-inhabiting OTUs based on a combined 5.8S/LSU dataset. Maximum likelihood bootstrap support values > 80 and Bayesian posterior probabilities > 0.90 are shown on branches. Marine OTUs are in italics and in gray. Best LSU BLAST matches are shown in brackets and matches with < 80% sequence**

vectors included geography (western or eastern area), county and site, latitude and longitude (UTM coordinates for northern latitude and eastern longitude), sampling month and ecological variables (see [Sampling](#)). In addition, we included diversity parameters derived from the community matrix:  $\alpha$ -diversity (=OTU richness on each log) ([Whittaker, 1960](#)), the number of OTUs in each phylum and order and [Faith's \(1992\)](#) index of phylogenetic diversity (PD) of each log to account for any effect on the dispersion in the ordination space. We calculated a distance matrix from the community data matrix using Kulczynski dissimilarity index as implemented in [vegan](#) ([Oksanen et al., 2013](#)). In addition to the NMDS, separate Mantel tests were performed to compare the community matrix with Euclidean distance matrices of vectors and numerical factors.

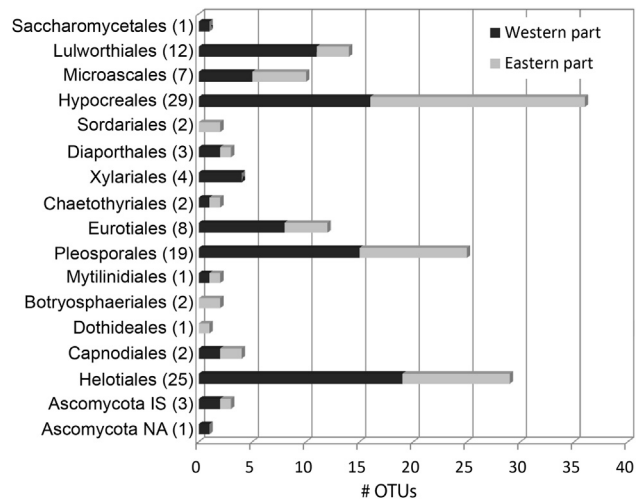
We considered the two areas to be separate habitats within the landscape and calculated a  $\beta$ -diversity ([Whittaker, 1960](#)) measure, the classic Sørensen index, using the full dataset in [EstimateS](#) ([Colwell, 2009](#)). To reveal whether fungal communities are phylogenetically clustered we calculated phylogenetic community measures, PD, net relatedness index (NRI) and nearest taxon index (NTI), in [Phylocom](#) version 4.2 ([Webb, 2000](#); [Webb et al., 2002, 2008](#)). The latter two indices count phylogenetic distances of species communities and compare them with computationally created null communities. We excluded two single-isolate logs and used logs and samples. [Phylocom](#) was run using A 5.8S tree including one study isolate per OTU per log as a phylogenetic backbone ([Fig S1](#), alignment available from: <http://doi.org/10.5061/dryad.qg82k>) and null community option -m 1 which maintains the species richness in each sample, but randomizes their identities. The number of generations used in calculations was 9 999.

Further details of the methods and analyses can be found in [Supplement 1](#).

## Results

### Fungal diversity

ITS sequences were obtained from 577 pure cultures and clustered into 147 OTUs ([Table 1](#), [Table S2](#)). More than half of the OTUs were singletons ([Table 1](#)). More OTUs were found in the western than eastern area, but the average  $\alpha$ -diversity was similar (on average 6.55 for the western, 6.81 for the eastern part and 6.66 for the whole dataset). OTU accumulation curves show that sampling was insufficient in both parts, as well as the whole study area, since the curves do not reach an asymptote ([Fig 2](#)). The Michaelis–Menten richness estimate suggests 254 OTUs to exist on coastal marine wood in the whole study area, which is almost twice as high as the observed  $\gamma$ -diversity. A representative partial LSU sequence was successfully generated for 125 (85%) of the ITS based OTUs.



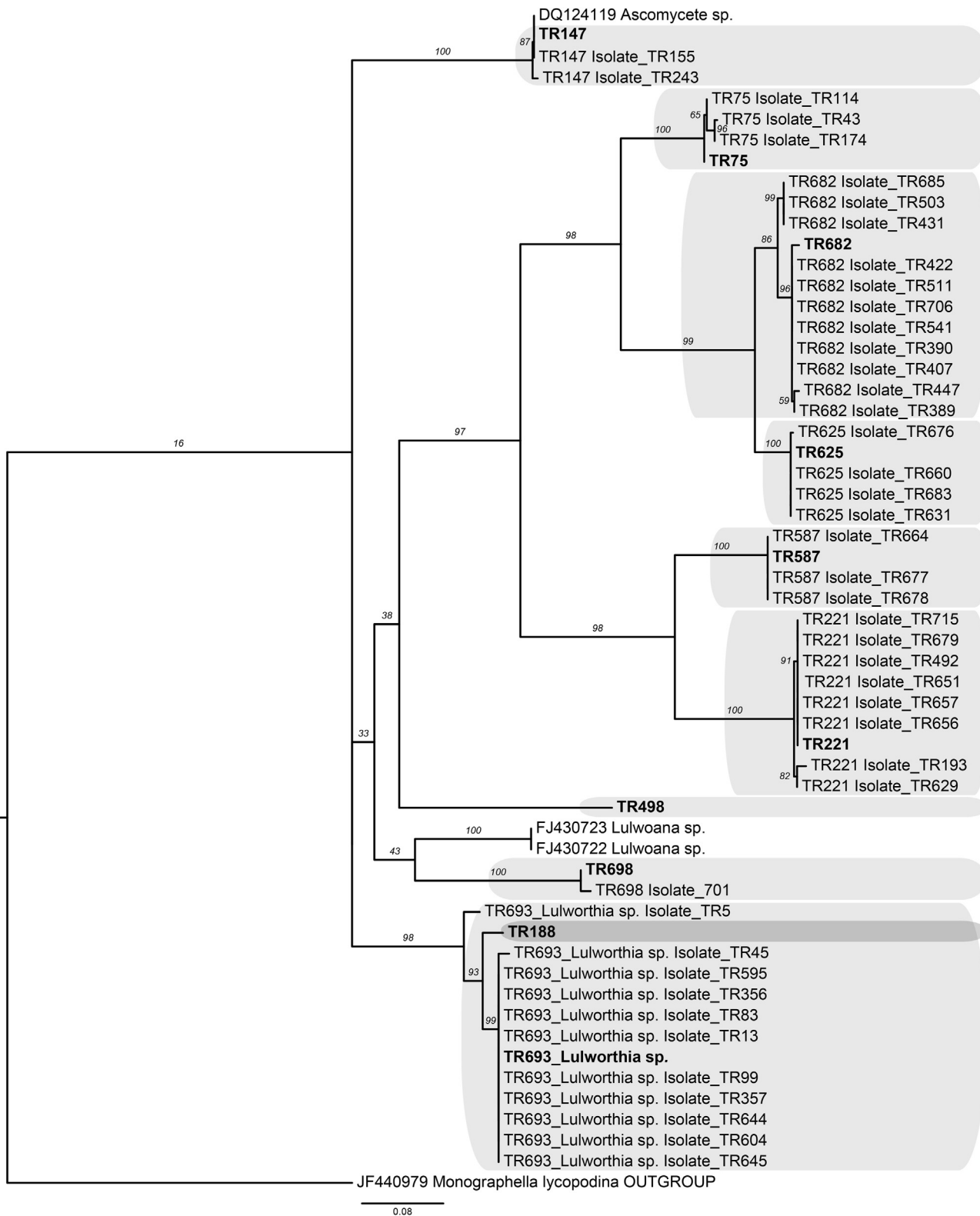
**Fig 4 – Ascomycetous OTUs detected based on LSU and ITS top hits in the two sea areas. Taxonomic annotation is followed by the total number of OTUs in parentheses. Number of ascomycetous OTUs in the western part was 88, in the eastern part 63 and in the whole dataset 122.**

### Taxonomy and phylogeny

Ascomycota clearly dominated the recovered community with 122 (83%) of the OTUs assigned to the phylum ([Fig 3](#), [Table S2](#)). Only ten OTUs belonged to Basidiomycota, five to subphylum Mucoromycotina and one to Chytridiomycota. Nine OTUs only represented by ITS sequences could not be assigned to any fungal phylum. The five most frequent orders were Hypocreales, Helotiales, Pleosporales, Lulworthiales and Eurotiales, and altogether Ascomycota was represented by 15 orders. At the taxonomic level of order, the communities recovered in the two sea areas were similar, although some orders were more abundant in the western part, and some were missing in one of the sea areas ([Fig 4](#)). In Basidiomycota, Agaricales and Cantharellales occurred most frequently with four and two OTUs, respectively ([Supplementary Table S2](#)). At the genus level the most frequent OTUs had affinities to ascomycete anamorphs such as *Cadophora*, *Cladosporium* and *Penicillium* ([Fig 3](#)). Five OTUs fruiting in culture were identified morphologically as *Asteromyces cruciatus*, *Amylocarpus encephaloides*, *Digitatispora marina*, *Lulworthia* sp. and cf. *Phialophorophoma litoralis*. Only the first mentioned was likely represented by ITS or LSU sequence data in GenBank.

The phylogenetic diversity of the fungal communities based on 5.8S sequences was similar in both sea areas ([Table 1](#)). No phylogenetic clustering of fungal assemblages was detected when comparing phylogenetic distances of observed and null communities using NRI and NTI, as indicated by negative or close to zero values ([Table 1](#)). Separate

similarity are marked with an asterisk (\*). Number of logs/sites each OTU occurred in is given after BLAST match identities. Scale bar shows nucleotide substitutions per site. Maximum likelihood and Bayesian analyses produced highly similar trees, and the topology of the latter is shown here. Differences were related to some low supported nodes of the Bayesian tree shown as polytomies in the maximum likelihood tree.



**Fig 5 – ITS phylogeny among Lulworthiales isolates found in the present study. Gray boxes represent OTUs and their representative isolates are in bold. Taxa labeled with GenBank accession numbers represent best BLAST matches of the study isolates. RAxML standard bootstrap values are shown above branches or at nodes. Scale bar shows nucleotide substitutions per site.**

order-level phylogenies using ITS data from all the 577 isolates, revealed that several of the OTUs putatively included more than one species, as indicated by well-supported subclades (Fig 5 and Supplementary Fig S2).

### Ecology

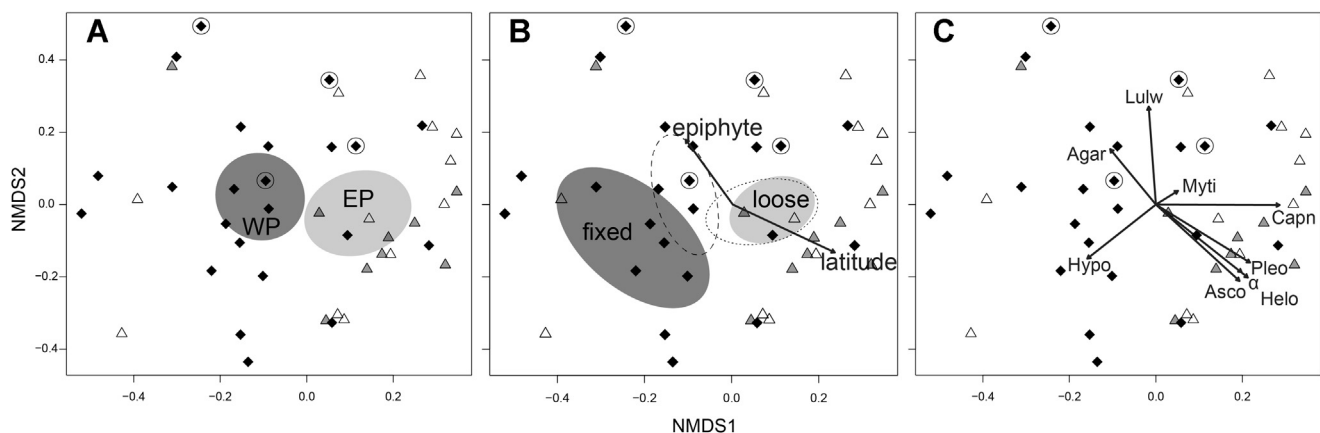
Marine taxa were present in every phylum throughout the 5.8S/LSU tree (Fig 3). Sixty-seven of the 125 OTUs having LSU sequences were classified as marine based on LSU BLAST match and literature, whereas 58 OTUs (46%) were judged as non-marine. The primarily marine taxa Lulworthiales and Halosphaeriaceae (Microascales) included ten and seven OTUs, respectively. Numerous OTUs classified as marine were also present in Eurotiales (6 out of 8 OTUs marine), Hypocreales (14/25), Helotiales (10/22) and Pleosporales (7/19). Xylariales included four OTUs, all of which were categorized as non-marine. In Basidiomycota three out of nine OTUs were classified as marine. Two of these are yeasts and one a filamentous fungus, *D. marina*. In Mucoromycotina three out of five OTUs were categorized as marine. Six out of the 19 most frequent OTUs were obligate marine, namely Lulworthiaceae sp., *Emericellopsis maritima*, *A. cruciatus*, Halosphaeriaceae sp., *Lulworthia* sp. and *A. encephaloides* (Supplementary Table S3). The primarily marine taxa Lulworthiales and Halosphaeriaceae (Microascales) had fewer and poorer (=low sequence similarity) BLAST matches than primarily non-marine orders (Figs 3; 5 and Supplementary Fig S2 and Table S2). Among the ten OTUs (encompassing 53 isolates) recovered from the marine order Lulworthiales, a majority had low sequence similarity BLAST matches and were not considered conspecific with any GenBank reference sequence (Fig 5). The trend observed across all orders was that BLAST

hits of marine OTUs had lower sequence similarity than terrestrial ones (Supplementary Figs S3 and S4).

### Fungal communities

The fungal community composition differed between the sea areas. The number of shared OTUs found in both areas was 30 out of 147 and the  $\beta$ -diversity between the western and eastern area was 0.339, which means the two communities were more different from than similar to each other. The logs in the western part were slightly more heterogeneous in fungal community composition compared to the eastern part, as indicated by mean and median Sørensen index values closer to zero (Table 1). None of the most frequent OTUs were associated with a particular sea area according to ANOVA and g-tests.

The NMDS ordination demonstrates a compositional difference in the fungal communities in the western and eastern part of our study area (Fig 6). Logs from a particular site were often far apart in the ordination space (result not shown), indicating high local heterogeneity. Several factors had strong relationships with the compositional variation in the ordination space. Taxonomic parameters that explained most of the dispersion in the ordination space were the number of OTUs in Helotiales, Capnodiales, Pleosporales, Ascomycota, Lulworthiales, Hypocreales, Mytilinidiales, Agaricales, and  $\alpha$ -diversity (Table 2, Fig 6C). Variables that co-varied with the compositional variation were latitude, epiphyte cover, month, log attachment, habitat type, orientation, county, zone of sampling, geography, log position and tree type (Table 2, Fig 6B). The ordination shows that geography divides the communities (Fig 6A), and logs having different attachment (fixed or loose) and tree type host distinct communities



**Fig 6 – The NMDS ordination of fungal communities inhabiting 44 logs and important ecological and taxonomic parameters influencing the dispersion of OTUs in the ordination space: (A) geography; (B) attachment type, tree type, epiphyte cover and latitude; and (C) taxonomic parameters. Diamonds represent fungal communities inhabiting logs in the western part and triangles in the eastern part of the study area. Circled diamonds are sublittoral logs by the shore and filled triangles logs in the breaker zone. The 95% confidence ellipsoids are drawn based on standard errors of the averages of the points for attachment (filled ellipsoids) and tree type (dashed for broadleaved and dotted for coniferous logs). Agar = Agaricales, Asco = Ascomycota, Capn = Capnodiales, Helo = Helotiales, Hypo = Hypocreales, Lulw = Lulworthiales, Myti = Mytilinidiales, Pleo = Pleosporales and  $\alpha$  =  $\alpha$ -diversity. The two first dimensions of the three studied are plotted. Four of the statistically significant parameters (zone, habitat type, orientation and log position) are not shown, since these included parameter categories with only a few observations making it difficult to evaluate their importance.**



**Table 2 – Results of the three-dimensional NMDS ordination and the Mantel tests: statistically significant parameters ( $P \leq 0.05$ ) are in bold**

NMDS ordination					
Diversity parameters	$r^2$	$P$	Variable	$r^2$	$P$
Helotiales	0.510	0.001	Latitude	0.272	0.007
Capnodiales	0.481	0.001	Epiphyte	0.182	0.031
Pleosporales	0.425	0.001	Month	0.180	0.003
Ascomycota	0.302	0.004	Attachment	0.154	0.001
Lulworthiales	0.281	0.001	Orientation	0.149	0.002
$\alpha$ -Diversity	0.275	0.001	Habitat type	0.131	0.043
Hypocreales	0.201	0.027	County	0.130	0.001
Mytilinidiales	0.188	0.024	Zone	0.100	0.032
Agaricales	0.171	0.036	Geography	0.086	0.007
Microascales	0.168	0.064	Position	0.081	0.004
Ascomycota IS <sup>a</sup>	0.083	0.351	Tree type	0.077	0.018
Leucosporidiales	0.075	0.358	Site	0.452	0.283
PD	0.072	0.407	Longitude	0.179	0.057
Xylariales	0.061	0.547	Bark	0.116	0.171
Basidiomycota	0.059	0.524	Substrate type	0.103	0.362
Chaetothyriales	0.055	0.520	Length	0.101	0.229
Eurotiales	0.053	0.522	Decay	0.097	0.154
			Apical diameter	0.086	0.294
			Basal diameter	0.073	0.367

Mantel test		
Variable	$r^b$	$P$
Latitude	0.153	0.004
Month	0.135	0.008
Epiphyte	0.117	0.049
Decay	0.113	0.048
Latitude & longitude	0.108	0.006
Longitude	0.090	0.015
Bark	0.032	0.314
Length	0.023	0.341
Basal diameter	0.003	0.475

a IS = Incertae sedis.  
b Spearman correlation coefficient.

(Fig 6B). Loose coniferous logs and logs in the breaker zone are associated with higher  $\alpha$ -diversity, whereas broadleaved logs that have more epiphytes are associated with decreased  $\alpha$ -diversity and increased number of Agaricales and Lulworthiales OTUs (Fig 6B and C). Mantel tests, where differences in community composition and geographic distances were related, indicated significant relationship between fungal community composition and geographic distance (Table 2). In addition, month, epiphyte cover and decay stage correlated with community composition.

## Discussion

### Taxonomy and phylogeny

Wood-inhabiting species are the most studied among marine fungi (Barghoorn and Linder, 1944; Jones, 2011b). Nevertheless, the present study, based on almost 600 axenic cultures provided new information about their taxonomic diversity, highlighting that this group of fungi is still poorly

known at regional and global scales. It was estimated that the logs would host up to 250 OTUs. However, we think the true diversity is likely to be even higher, considering that (i) we studied only a small fraction of the surface wood of the 50 logs, (ii) not all fungi grow in culture, and (iii) several of the OTUs consisted of two or more well-supported sub-groups that might represent different species. The majority of the OTUs were members of Ascomycota representing a total of fifteen orders that were relatively evenly distributed between the two geographic regions. Some less diverse orders were absent in one or the other area, e.g. Xylariales and Sordariales, which is most likely a sampling effect, not a real distribution pattern, given the unsaturated species accumulation curves for the dataset.

Out of the 19 most frequent taxa discovered in this study, eight have been recovered in four other culture based reference studies made in West-Eurasian temperate and arctic seas, whereas only two were found in non-culture reference studies (Supplementary Table S3). The higher taxonomic overlap between culture based plating surveys (Henningsson, 1974; Rees et al., 1979; Bubnova and Kireev, 2009; Bubnova, 2010) compared to fruit body based ones (Petersen and Koch, 1997; Azevedo et al., 2011; Pang et al., 2011) was expected, since it is well known that different study methods favor the discovery of certain taxa at the expense of others. Only two cosmopolitan taxa of obligate marine fungi were isolated in this study, namely *Ceriosporopsis halima* and *Lulwoana uni-septata* (= *Zalerion maritimum*) (Hughes, 1986; Jones, 1993). The surprising absence of cosmopolitan taxa in our study, such as the genus *Corollospora* or the species *Humicola alopallonella*, can partly be explained by our sampling method. Mycelia of marine fungi, known to prefer the surface layers of the wood (Kohlmeyer and Kohlmeyer, 1979), may have been excluded in the mechanical surface sterilization of the sampling points. Secondly, the cool incubation temperature used is not optimal for the growth of several cosmopolitans (Panebianco, 1994), and hence they might have been outcompeted by faster growing fungi. And finally, the lack of publicly available reference sequences for marine fungi (discussed below) must have contributed to the apparent absence of some cosmopolitan marine fungi.

### Facultative marine fungi

In this first larger scale culturing study on wood-inhabiting marine fungi in the northern seas we detected many taxa that have not been considered obligately marine. Only about 30 (20%) of the taxa detected in the present study are reported in key taxonomic literature of marine fungi (Kohlmeyer and Kohlmeyer, 1979; Jones et al., 2009) and considered obligately marine. However, many of the OTUs have affinities to taxa reported from marine environments, which underlines the need to explore the marine wood-inhabiting mycobiota outside the framework set by traditional taxonomic literature of marine mycology. For example, *Tolypocladium cylindrosporium* (TR169) was the eleventh most common OTU in the present study and identified with 100% ITS and LSU sequence similarity. It has been found in culturing studies from arctic waters (Bubnova and Kireev, 2009; Bubnova, 2010), but is not found in key taxonomic literature of marine fungi.

*Cadophora* (Leotiomyces) was frequently isolated and it seems to represent a previously overlooked dominant component of the marine wood-inhabiting mycota. Five OTUs had close affinity to this genus and these include the most, second and fifth most frequent OTU detected in this study. The genus has been found in the marine environment only recently (Gunde-Cimerman et al., 2005; Burgaud et al., 2009; Almeida et al., 2010), however, older records of the genus *Phialophora* might be considered to represent *Cadophora* due to nomenclatural recombinations (Gams, 2000; Harrington and McNew, 2003). *Cadophora* is an anamorphic genus in Helotiales, morphologically similar to *Phialophora* but molecularly distinct from this genus (Harrington and McNew, 2003). *Phialophora* is considered a plant symbiont that has also been found inhabiting marine wooden substrata with medium and low frequency of occurrence in the North Sea and the Baltic Sea (Henningsson, 1974; Rees et al., 1979). However, Kohlmeyer and Kohlmeyer (1979) did not mention either genera, and in Jones et al. (2009) *Phialophora* is mentioned as an anamorph of *Gaeumannomyces* (Sordariomycetes). Contamination is unlikely to explain the high frequency of *Cadophora*, especially since contamination controls were either negative or included taxa phylogenetically distinct from *Cadophora*. What roles the species of *Cadophora* play in marine ecosystems remains unknown.

The occurrence of terrestrial species in marine environments is not a new discovery (Elliott, 1930; Sparrow, 1937), but the great number of them found in the present and other studies underlines our limited knowledge of these fungi and their ecology. One bottleneck in molecular surveys of fungi, as in the present study, is often too low resolution in species identification. In the present study the primarily marine taxa, Halosphaeriaceae (Microascales) and Lulworthiales included most of the OTUs having poor BLAST matches across the ITS region. Furthermore, only one morphologically identified culture had >97% similar ITS BLAST match. The ITS region has barely been used in marine mycology and the recent barcoding effort of marine fungi is therefore of significant importance (Velmurugan et al., 2013). Since the barcoding efforts for marine fungi are still in their infancy, there is obvious uncertainty in the taxonomic assignments of the detected OTUs. Thus, we cannot conclude whether putative terrestrial taxa found in the present study represent facultative marine fungi or new lineages that are specialized to a marine habitat.

### Community ecology

The fungal communities were different between the western and eastern part of the North Norwegian coast, and this was due to geography and several site and substratum level variables. The most important variable affecting community composition was the latitude. The logs included in the analysis were within a 150 km latitudinal and 500 km longitudinal range. The importance of the latitude might, in addition to geographical variation, reflect the distribution of the sites in inner parts of the fjords and by the open sea. Sites by the open sea were more in the northern parts of the studied area. If latitude reflected the distribution of sites, different fungal communities would dwell in logs in the inner parts vs. by the

open sea, which is an interesting topic to address in the future research of marine wood-inhabiting fungi.

Epiphyte cover was the most important substratum level variable influencing the communities. This suggests that fungal communities on driftwood change along the duration of presence in marine habitat, which is in line with an earlier finding by Tan et al. (1989). The  $\alpha$ -diversity decreased with epiphyte cover. This result likely reflects the vast difference in the numbers of marine and non-marine fungi (Blackwell, 2011; Jones, 2011a); a small fraction of all fungal species is able to exist in wood which has been long in the marine realm. In contrast to  $\alpha$ -diversity, the number of Agaricales and Lulworthiales OTUs increased with epiphyte cover. This is to be expected considering that Lulworthiales is a marine order and the only Agaricales included in the ordination was the obligate marine *D. marina* (TR666).

Attachment and tree type were important and reflected the geographic distribution of the communities. More OTUs were isolated from loose than fixed logs (on average 7.70 vs. 6.30, respectively). One might think that the increased  $\alpha$ -diversity in loose logs is due to recolonization of the logs in the breaker zone by airborne fungi. However, this was not the case, since higher  $\alpha$ -diversity was detected in loose logs also in the intertidal zone (on average 7.47 vs. 6.30). Coniferous logs hosted more culturable fungal species than broadleaved logs, but the difference was minimal (on average 4.85 vs. 4.76 respectively) and unlikely to explain the increase in  $\alpha$ -diversity. Habitat type and zone of sampling were of importance in shaping the communities which is in line with previous studies (e.g. Hyde, 1989; Petersen and Koch, 1997). However, their confidence ellipsoids were largely overlapping in the ordination space (results not shown). Sampling month seemed to have a major impact on the fungal communities, but sampling was biased on a temporal scale as the western part was sampled during May–Jul. and the eastern mostly from Aug. to Sep.. Marine fungal communities on wood are not known to change drastically during the summer (cf. Tan et al., 1989), and this result likely represents an artifact in our study caused by biased sampling. Other insufficiently sampled variables include orientation and position, and thus their importance cannot be justified.

Some ecological variables difficult to measure representatively were not taken into account in this study, including site water temperature, salinity and origin of the shore-cast wood. Differences in temperature and salinity are known to be important on a global scale (Booth and Kenkel, 1986; Hughes, 1986). In the present study in a limited geographic area with overlapping seasonal fluctuations, differences in mean surface water temperature and salinity are small (Sælen, 1950; Eilertsen and Skarøhamar, 2006) and unlikely to explain the different communities between the western and eastern part of Norwegian Barents Sea coast. At site level differences in these variables are likely notable due to local hydrographic variation, but this information could not have been caught at sufficient resolution with field measurements, especially when sampling in different localities at different times of the season. The origin of logs might be one of the key factors shaping the fungal communities, and explaining the differences in community composition and diversity. The logs originating from Siberia found in the east may have been

initially inoculated with a different fungal community than logs of (mostly) local origin in the western part of the study area.

## Conclusions

Northern marine wooden substrata host species-rich fungal communities consisting of both obligate and facultative marine species. Diverse communities with previously unknown dominant taxa can be revealed with culturing methods, but additional and more effective sampling and identification methods (i.e. high throughput sequencing) are needed to reveal the true diversity and ecological preferences of marine-wood inhabiting fungi in the northern seas. Geography and ecological factors such as length of submersion and log attachment type shape the fungal communities on driftwood. Many of the taxa we report for the first time from marine wooden material could not be identified to species because of lack of barcode sequences. It is, therefore, not known whether these taxa represent facultative marine fungi or new lineages that are specialists for a marine habitat. For more than a half century marine mycological research has explored the morphological diversity of obligate marine fungi on driftwood. It is time to move beyond fruit bodies towards an integrated approach to gain a more holistic view of the fungal communities drifting around the world's oceans.

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## Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.funeco.2013.12.002>.

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