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Seagrass-associated fungal communities follow Wallace's line, but host genotype does not structure fungal community

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Abstract

Aim: To test whether or not fungal communities associated with the widespread seagrass, *Syringodium isoetifolium* can be differentiated on either side of Wallace's line, a boundary line separating Asian and Australasian fauna. Additionally, we examine whether host multilocus genotype predicts fungal community composition.

Location: A total of 77 samples were collected from 14 sampling sites spanning the Indonesian archipelago.

Methods: We sequenced the fungal ITS1 gene using Illumia MiSeq technology and used a clustering-free Divisive Amplicon Denoising Algorithm to infer ribosomal sequence variants. Data were analysed via non-metric multidimensional scaling, Mantel tests and permutational multivariate analysis of variance. Binary and quantitative null models were used to determine whether results significantly deviated from random. Host genotype was determined by genotyping at 18 microsatellite loci and standard genetic analysis was performed in the R package APE.

Results: Significant differences in fungal community composition were detected on either side of Wallace's line ($p = <.001$ $R^2 = .040$). A significant distance decay of similarity pattern was observed between ribosomal sequence variants and geographical distance ($p = .001$ $R^2 = .227$) and several fungal ribosomal sequence variants were significantly associated with sampling sites found either east or west of Wallace's line.

Main conclusions: Fungi are generally considered to have excellent dispersal potentials and marine fungi have the potential to disperse far and wide in an environment that has no obvious barriers to dispersal. Despite this assumed excellent dispersal potential, we show that fungal communities on either side of Wallace's line are significantly different from one another. We speculate that limited dispersal and differences in habitat type are responsible for the observed pattern. Work examining biogeographical patterns in marine fungi is still in its infancy and further research is required to fully understand marine fungal biogeography.

KEYWORDS
biogeography, Coral Triangle, dispersal, Indonesia, ITS, marine fungi, seagrass, Sunda Shelf, Wallace's line, Wallacea

1 | INTRODUCTION

The celebrated British naturalist, Alfred Russel Wallace is widely regarded as the "Father of Biogeography" on account of his work throughout the Malay Archipelago between 1854 and 1862 (McGlynn, 2010; Metcalfe, 2006). In 1858, Wallace proposed a boundary line that splits Indonesia in half, running from the Pacific ocean between the islands of Borneo and Sulawesi and entering the Indian ocean via the Lombok Strait between the Islands of Bali and Lombok. This line closely follows the margin of the Sunda Shelf (Figure 1) and would later become known as Wallace's line when Thomas Huxley coined the term (Huxley, 1868). Wallace described the fauna west of this line as typically Asian in origin and east of this line Australasian in origin (Mayr, 1944). Several different taxa follow this pattern and this boundary is especially apparent in birds (Lincoln, 1975). To our knowledge, the extent to which microbes follow this pattern is unknown.

Unravelling patterns of fungal biogeography is complicated by their high dispersal potential. For example, 27% of the operational taxonomic units (OTUs) found associated with macroalgae collected on a Hawaiian mesophotic coral ecosystem were also observed in Hawaiian terrestrial environments, indicating a relatively high level of connectivity and assumed dispersal between two very different environments (Wainwright et al., 2017). This highly dispersive nature is further borne out by the observation that many fungi are present on all of the planet's habitable environments (Amend, Seifert, Samson, & Bruns, 2010). At the same time, DNA sequence-based work performed at global and continental scales is showing that fungal biogeography is influenced by factors such as climate and isolation, and regional fungal endemism may be the rule, not the exception (Peay, Kennedy, & Talbot, 2016). Marine fungal biogeography is further complicated by the apparent lack of any obvious barriers to dispersal in marine environments (Lessios, Kessing, & Robertson, 1998; Teske et al., 2008; Thurold, 2006; Waples, 1998).

The processes contributing to fungal biogeography in comparatively well-studied terrestrial environments are still little understood; in the far less-studied marine fungi (Blackwell, 2011; Richards, Jones, Leonard, & Bass, 2012) our understanding of biogeography is embryonic. Considering our residence on a planet whose surface is 71% water (96% of which is marine) there is clearly work to be done. Research by Tisthammer, Cobian, and Amend (2016) showed that on a global scale, the biogeography of marine fungi is largely shaped by the environment, but little work has examined finer scale patterns of marine fungal biogeography (Richards et al., 2015), or how they compare to patterns observed in larger organisms.

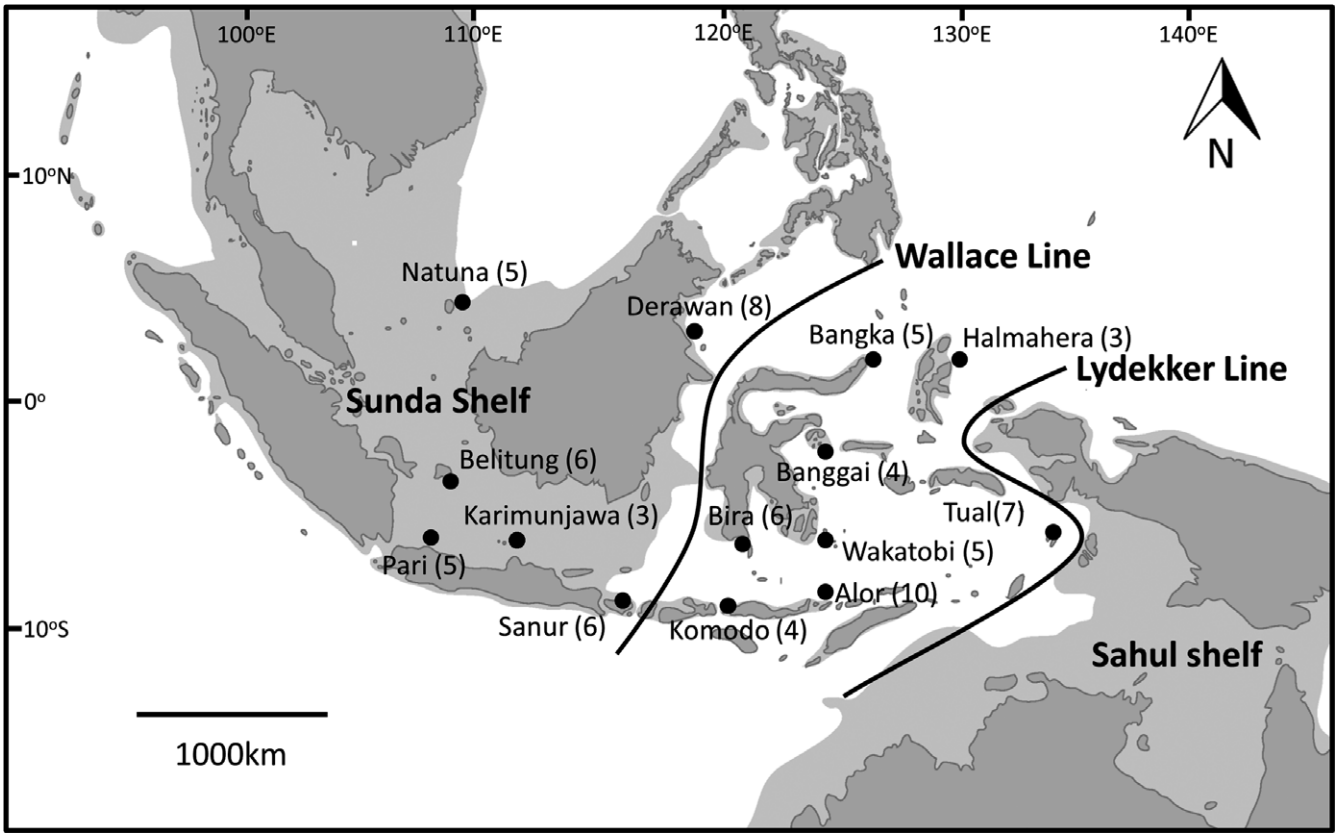

FIGURE 1 Map showing the sampling sites, numbers in parenthesis indicate sample size. Also shown are the locations of the Sunda Shelf, Wallacea and the Sahul Shelf. The light grey area indicates the maximum exposure of land during periods of glacial maxima (sea levels 120 m lower than present day). Additionally, the approximate positions of Wallace's and Lydekker's lines are indicated. Figure adapted from Voris (2000) and downloaded from the Field Museum of Natural History (http://www.fieldmuseum.org/pleistocene-sea-level-maps)

Lying between several continental and oceanic plates, Indonesia has an extremely complex geological, oceanographic and tectonic history (Hall, 2002). During periods of glacial maxima, sea levels throughout the region dropped by as much as 120 m entirely drying the shallow Sunda and Sahul shelves (Voris, 2000). The drying of these shelves caused the complete extirpation of all marine species on them and facilitated the spread of Asian terrestrial fauna throughout the Indonesian islands of Sumatra, Java and Borneo. These islands together with the Malay Peninsular created a landmass known as Sundaland. It is likely that this complex history has contributed, in part, to the biogeographical patterns observed throughout the region (e.g. Wallace's and Lydekker's lines).

The marine environment of Indonesia can be divided into three very broad regions based on habitat type, two of which are dominated by shallow water and the continental landmasses associated with the Sunda Shelf in western Indonesia and the Sahul Shelf along with its associated landmasses in the east (Mayer, Stacke, Stottmeister, & Pohlmann, 2015). The third sits between the Sunda and Sahul shelves and is called Wallacea, this region is characterized by deep water and volcanic islands (Van Aken, Brodjonegoro, & Jaya, 2009) (Figure 1).

Extensive seagrasses meadows are found throughout all the marine regions of Indonesia, these meadows are vital nursery habitats for many marine organisms and they play a critical role in nutrient cycling (Waycott et al., 2009). Seagrasses are globally distributed marine angiosperms that evolved from terrestrial monocotyledonous flowering plants between 70 million and 100 million years ago (Les, Cleland, & Waycott, 1997; Wissler et al., 2011). The interactions between plants and fungi are well documented in terrestrial ecosystems (Bonfante & Genre, 2010; Contreras-Cornejo, Macías-Rodríguez, Cortés-Penagos, & López-Bucio, 2009; Rodriguez, White, Arnold, & Redman, 2009) and just like their terrestrial counterparts fungal associations are ubiquitous in seagrasses; however, research into all aspects of marine fungi lags considerably in comparison to terrestrial environments. Consequently, at present, it is difficult to determine the exact nature of the seagrass fungal association, the fungal diversity contained on and within seagrasses or the biogeographical provinces that may be structuring marine fungal communities.

With this study, we investigate whether or not the fungi associated with the widespread seagrass *Syringodium isoetifolium* can be differentiated on either side of Wallace's line. Additionally, we examine whether host multilocus genotype predicts fungal community composition.

2 | MATERIALS AND METHODS

2.1 | Study sites and sample collection

All samples collected in this study came from the Indonesian archipelago and were collected between May-August 2010 and May-August 2011 (Table 1). These samples were originally collected as part of a multispecies marine genetic connectivity study throughout Indonesia, see Wainwright, Arlyza, and Karl (2013) for details. *Syringodium isoetifolium* was specifically chosen for its ease of identification; additionally, it has a widespread distribution making it easy to find throughout Indonesia.

TABLE 1 Details of sampling location and sampling date

Sample Site	Latitude	Longitude	East or West of Wallace's line	Sampling date
Belitung	−2.55878	107.6689	West	21-Jul-10
Derawan	2.28151	118.2445	West	27-Jun-10
Karimunjawa	−5.87927	110.4320	West	02-Aug-11
Natuna	3.92645	108.3829	West	07-Jun-11
Pari	−5.86356	106.6106	West	19-Jun-10
Sanur	−8.68645	115.2655	West	05-Aug-10
Alor	−8.26874	124.4013	East	18-Jun-11
Banggai	−1.90526	123.0892	East	09-Jul-10
Bangka	1.74665	125.1496	East	21-Jul-10
Bira	−5.61541	120.4592	East	05-Jul-10
Halmahera	1.74401	128.0361	East	07-Jul-11
Komodo	−8.49736	119.7589	East	25-Jun-11
Tual	−5.64577	132.6378	East	23-Jul-11
Wakatobi	−5.33832	123.5349	East	10-Aug-11

In total, 77 individual seagrass blades were collected — 33 west of Wallace's line from six sampling sites and 44 to the east from eight sampling sites. Collections from the east and west occurred over both sampling years in roughly equal proportions (i.e. not all samples from the east were collected 1 year and not all samples from the west collected the other year [Table 1]). Collected blades were immediately placed in individual sterile tubes containing heat dried silica gel and stored unopened until DNA extraction was performed.

2.2 | DNA extraction, PCR amplification and Illumina sequencing

DNA was extracted from an entire blade that contained no visible epiphytes and measured between 10 and 20 cm. DNA extraction was performed with a Machery Nagel GmbH and Co. (Bethlehem, PA, USA) NucleoSpin® Plant II kit following the manufacture's CTAB protocol, once DNA had been extracted it was stored at −80°C. Because the mass of host DNA will be several orders of magnitude greater than that of fungal template, DNA concentration was not quantified. Fungal DNA amplification of the ITS1 region was performed using the ITS1F primer (CTTGGTCATTTAGAGGAAGTAA; Gardes & Bruns, 1993) and the ITS2 primer (GCTGCGTTCTTCATCGATGC; White, Bruns, Lee, & Taylor, 1990). Forward and reverse primers were modified to include Illumina adaptors, a linker and a unique barcode (see Smith and Peay (2014) for additional details including custom sequencing primers). Each reaction was performed in a total volume of 25 μl, containing 9 μl of template DNA diluted 1:5, with final concentrations of 0.25 U of KAPA 3G Enzyme (Kapa Biosystems, Inc, Wilmington, MA, USA), 0.3 μM of each primer,

1.5 mg/ml of BSA and KAPA Plant PCR Buffer to a final concentration of 1×. PCR cycling protocol was 95°C for 3 min, followed by 35 cycles of 95°C for 20 s, 53°C for 15 s, 72°C for 20 s with a final extension at 72°C for 60 s. Negative controls were included to identify any possible contamination issues.

PCR products were visualized on a 1% SB buffer agarose gel and cleaned with AMpure beads. Normalization of PCR products was performed with a just-a-plate™ 96 PCR purification and normalization kit (Charm Biotech, San Diego, CA, USA). Cleaned and normalized PCR products were quantified using a Qubit® 2.0 Fluorometer following the hs-DS-DNA protocol (Invitrogen, Carlsbad, CA, USA), pooled into equimolar amounts and submitted for sequencing on the Illumina MiSeq platform (600 cycles, V3 chemistry, 300 bp paired end reads) at the Hawai'i Institute of Marine Biology sequencing core facility.

2.3 | Host genotyping

Seagrass blades were genotyped at 18 microsatellite loci (Wainwright et al., 2013). PCR products were resolved with an ABI 3730 Genetic Analyser and sized with GENEIOUS 6.1.6 (Biomatters, San Francisco, CA, USA; http://geneious.com). Pairwise genetic distances were calculated using the dist.gene function in the Analyses of Phylogenetics and Evolution (APE) package (Paradis, Claude, & Strimmer, 2004).

2.4 | Bioinformatics

All sequences have been deposited in the NCBI Sequence Read Archive under accession number PRJNA387470. Sequencing reads were demultiplexed and barcode sequences were removed by the sequencing centre. Quality filtration and bioinformatics were performed in R. We utilized a clustering-free Divisive Amplicon Denoising Algorithm (DADA2) to infer ribosomal sequence variants (RSVs) generated by dividing amplicon reads into partitions consistent with a quality-aware Illumina error model (Callahan et al., 2016). This approach allows exact inference of sample sequences without coarsely binning reads into operational taxonomic units (OTUs), thus preserving granular biological information that is potentially excluded via commonly used clustering algorithms, see Callahan, McMurdie, and Holmes (2017) for a comprehensive discussion on the merits of RSVs versus OTUs. Briefly, advances in Illumina DNA sequencing technology mean it is now possible to resolve single nucleotide differences in DNA sequences with a high degree of certainty; each unique fungal RSV can be considered a single species. RSVs can be used in much the same way that DNA barcoding is used in species identification. OTUs group DNA sequences that are 97% similar together, consequently informative biological information can be lost when using OTUs. Another benefit of this approach is that unlike OTUs, RSVs are directly comparable between studies as long as the read filtration parameters are consistent.

Quality control on the resulting forward reads for each sample consisted of trimming the first 12 bases, truncating each read at the first quality score of 2 (a quality score of 2 indicates a portion of the sequence that contains mostly low quality reads of Q15 or less), and removing any reads with ambiguous base calls or higher than a single expected error (Expected Errors = sum(10^(-QualScore/10))). Reads shorter than 125 bases after quality trimming were removed. Due to lower quality, reverse reads were not used. This workflow was performed with the DADA2 package in R (Callahan et al., 2016), and the code used is provided in the supporting information. To maximize the number of samples used, while ensuring the overwhelming majority of RSVs were recovered samples were rarefied to 208 sequences per sample (Figure S1) using the rarefy function in the Vegan R package (Okansen et al., 2017). After construction of the RSV table, taxonomy was assigned using the RDP naïve Bayesian Classifier (Wang, Garrity, Tiedje, & Cole, 2007) with Unite's dynamic ITS database (release 11.20.2016) modified to include non-fungal taxa potentially detected in our dataset. All RSVs that were not assigned to Kingdom Fungi were removed from subsequent analysis. (Table S1).

2.5 | Analysis

Non-metric multidimensional scaling (NMDS) analysis was performed with the metaMDS function in the vegan package using a Bray–Curtis dissimilarity index on a rarefied, Hellinger-transformed contingency table. Permutational multivariate analysis of variance (PerMANOVA) used a Bray–Curtis dissimilarity index and was performed using the adonis function with 999 permutations, and included sampling year and region as model terms. The commsim command within vegan was used to develop a distribution of 1,000 randomized communities against which our data were compared, see Hardy (2008) for a review of the use of null models in ecological applications. Briefly, we tested whether the number of RSVs unique to either the east or west of Wallace's line is greater than random expectations given RSV rank abundance distributions. Two null models were used; one binary using a curveball randomization (Strona, Nappo, Boccacci, Fattorini, & San-Miguel-Ayanz, 2014), the other was quantitative and used the r2dtable randomization (Patefield, 1981). All previously listed tests were performed in the R package vegan.

The indicspecies package (De Caceres & Legendre, 2009) was used to infer associations of fungal species to either side of Wallace's line. Geographical distance between sampling sites was calculated using the earth.dist function in the R package fossil (Vavrek, 2011).

A Mantel test was used to establish whether or not fungal community composition (Bray–Curtis index) correlated with host genotype or geographical distance.

See Figure 2 for a summary of fungal rank abundance and taxonomy of RSVs comprising >.005% of dataset.

3 | RESULTS

Our results suggest that marine fungal communities associated with the seagrass *S. isoetifolium*, similar to macro-organisms, are divided by Wallace's line, with fungal communities from the east of the

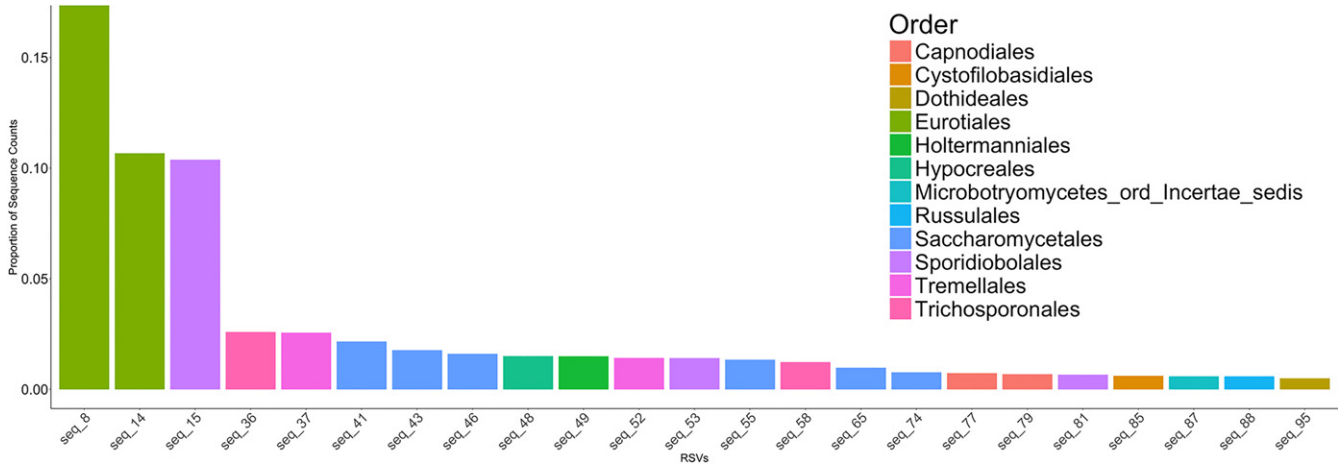

FIGURE 2 Rank abundance and taxonomy of RSVs comprising >.005% of dataset

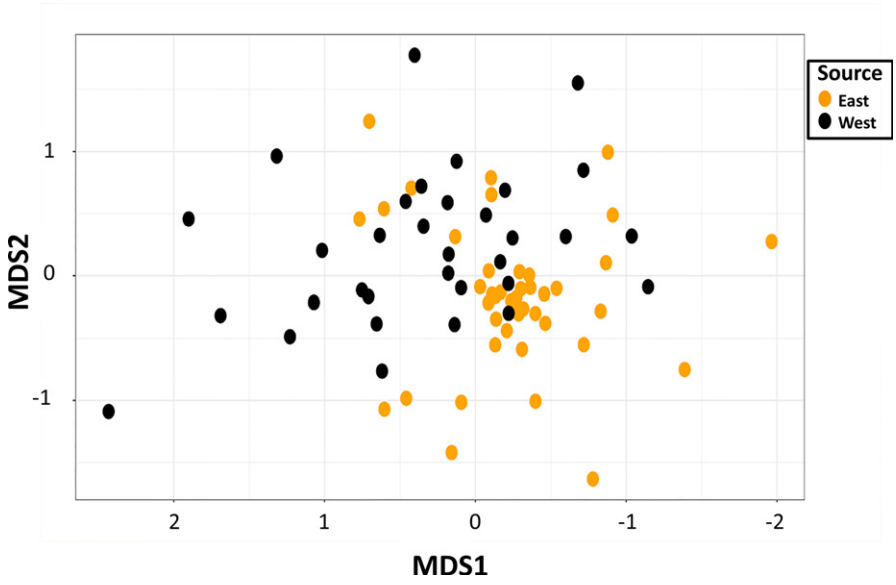

FIGURE 3 NMDS plot of fungal community similarity, coloured by location, east or west of Wallace's line. Non-metric fit, R^2 = .946 & Linear fit, R^2 = .798. Stress value = 0.2321175

archipelago more similar to themselves than those from the west and vice versa (Figure 3). PerMANOVA tests revealed that location, east or west of Wallace's line had a significant effect on fungal community (p = <.001, R^2 = .040), after accounting for variance contributed by sampling year.

Individual RSVs are statistically associated with one of the two regions, as demonstrated by the indicspecies analysis. A total of 660 unique RSVs were recovered from the Indonesian archipelago, of these three were significantly associated with samples collected from the west of Wallace's line and six showed significant associations with sites east of Wallace's line (Table S2). The results of both the quantitative- and incidence-based null models indicated that more RSVs are found in a single region than random expectation would predict. Quantitative r2dtable randomization (p = <.001) and binary curveball randomization (p = .01) indicating that our results are unlikely to be the result of chance alone (Table S3).

After accounting for Wallace's line, site location was a strong predictor of fungal community composition (ADONIS; p < .001, R^2 = .316). Mantel tests revealed a significant distance decay of similarity relationship between RSV similarity and geographical distance over the entire region (p = .001, r = .227). Sites associated with only the Sunda Shelf did not show a significant pattern of distance decay (p = .093, r = .066) and sites only found within Wallacea did show a significant pattern of distance decay (p = .017, r = .1694). No significant associations between host genotype and fungal RSV were observed (p = .46, r = .0005).

RSV richness was higher in Wallacea (565 RSVs) compared with the Sunda Shelf (419 RSVs), although there were no significant differences among individuals between regions (ANOVA; df = 1, F = 0.01, p = .92).

4 | DISCUSSION

In comparison to terrestrial environments, biogeographical patterns in the marine environment are less clear, the result of an apparent

absence of any obvious barriers to dispersal (Lessios et al., 1998; Teske et al., 2008; Thurold, 2006; Waples, 1998). Despite this perceived lack of barriers in marine environments, genetic differences on either side of Wallace's line have been documented in a number of marine species (e.g. seahorses [Lourie & Vincent, 2004], three species of Spanish mackerels [Helfman, Collette, & Facey, 1997; Sulaiman & Ovenden, 2009] and marine molluscs [Reid et al., 2006, 2013]). Here, we show that fungal communities associated with a widespread seagrass are significantly different on either side of this line.

A distance decay of similarity pattern was observed over the entire region. This is unsurprising given that sample sites span a distance of approximately 3,000 km in an east to west orientation, with markedly different habitats types as you move from the deep, fast flowing water of Wallacea into the more enclosed and much shallower waters of the Sunda Shelf (average water depth ~48 m [Mayer et al., 2015]). The islands enclosing the Sunda Shelf are continental in origin. In comparison, Wallacea has some of the deepest water on the planet, with depths in the Weber Basin exceeding 7,000 m and average water depths in the region are approximately 2,500 m (van Aken et al., 2009).

Aside from differences in community composition between regions, there were also differences in the spatial structure of community turnover. No significant pattern of distance decay was observed among Sunda Shelf samples, whereas a significant pattern of distance decay was observed among Wallacea samples. We propose that environmental differences, differences in dispersal potential and differences in host genotype may serve as three non-mutually exclusive factors driving these differences.

Several environmental factors differentiate these two regions, which may explain differences in fungal community composition and dynamics between them. The Sunda Shelf presents a comparatively uniform habitat, characterized by shallow depths and warm tropical water. Sunda, is further characterized by relatively heavy anthropogenic stresses associated with the densely populated island of Java and the heavily deforested Islands of Sumatra and Borneo (Mayer et al., 2015). In contrast, Wallacea is characterized by deep water, many islands of different ages and sizes creating a heterogeneous habitat that differs markedly from that of the Sunda Shelf. As water moves past each of these islands, eddies of differing sizes and intensities are created, fungal spore advection by these eddies could be facilitating fungal recruitment within Wallacea, similar phenomenon has been shown to shape microbial assemblages in the ocean (Wilkins, van Sebille, Rintoul, Lauro, & Cavicchioli, 2013). Furthermore, atmospheric eddies influence aerobiota in much the same way (Isard & Irwin, 1993).

Host genetics can be a strong determinant of fungal composition on plant leaves (Bálint et al., 2013; Hunter, Pink, & Bending, 2015; Sapkota, Knorr, Jørgensen, O'Hanlon, & Nicolaisen, 2015). Consequently, regional differences between host genetics could structure (or at least correlate with) fungal communities. In fact, a related seagrass *Thalassia hemprichii* shows distinct population genetic structure associated with the Sunda Shelf (Hernawan et al., 2017), and microsatellite analysis shows a similar pattern in *S. isoetifolium*, with support for two distinct populations roughly corresponding to regions east and west of Wallace's line (Wainwright in review) giving rise to the possibility that fungal communities could be differentiated by host genotype. We genotyped seagrass hosts at 18 presumably neutral microsatellite loci and did not observe a significant relationship between host genotype and fungal community ($p = .46$, $r = .0005$). Notably, although neutral markers are likely to detect genetic drift, markers linked to selective differences among populations were not examined here, and they may correlate better with fungal communities.

Last, the possibility exists that dispersal limitation could influence fungal community composition on the Sunda Shelf and across Wallace's line. The Indonesian throughflow (ITF) is a formidable water current that moves such a large volume of water in a constant north to south direction (Pacific to Indian Ocean) through Wallacea that Norwegian scientist Harold Sverdrup developed a new unit of measurement, the Sverdrup, to measure it. One Sverdrup = one million cubic metres of water per second, the volume of water moved by the ITF varies between 10–22 Severdrups or on average 10.5×10^6 m^3s^{-1} (Pandey & Pandey, 2006). The ITF forms the only low latitude, warm water connection between the Pacific and Indian oceans, and this current flows directly through the centre of Indonesia (Sprintall et al., 2014). Because the overwhelming majority of spores disperse passively, entrainment in the ITF is a real possibility unless those spores encounter suitable habitat around one of the many islands within Wallacea or make it onto the Sunda Shelf, leading, potentially to spatial structure in the former but not the latter (Figures S2 & S3).

The islands of Wallacea may present a major opportunity for spores to land on suitable habitat and establish. Indonesia acts like a funnel concentrating water flowing from the Pacific to the Indian Ocean through a relatively small channel. Indonesia could conceivably receive marine fungal spore inputs from throughput the Pacific and possibly from across the globe as a result of ocean circulation patterns, particularly the great ocean conveyor, reviewed by Broecker (1991). These inputs of spores via ocean circulation coupled with the acknowledged highly heterogeneous marine environment could make Indonesia and the neighbouring countries home to the highest marine fungal diversity on the planet. Indonesia contains the largest portion of the Coral Triangle, a marine region that is known to harbour the highest biodiversity of any marine system (Allen, 2008; Briggs, 2005; Hughes, Bellwood, & Connolly, 2002; Roberts et al., 2002; Veron et al., 2009). In a similar fashion to what is observed in Hawaiian macroalgae (Wainwright et al., 2017), it is conceivable that each of the many species found in the Coral Triangle has its own unique microbiome composition determined by its ecological niche. If each of these species contains only one or two fungi that are unique to them, the possibility that the Coral Triangle is a hotspot of marine fungal diversity is plausible. Future research could examine this idea in greater depth with more specialized sampling strategies designed to specifically address questions relating to hotspots of marine fungal diversity.

Similar to what has already been documented in fish and corals (Allen, 2008; Hughes et al., 2002), the highest diversity of RSVs was observed east of Wallace's line (565 RSVs). Three RSVs were significantly associated with sampling sites west of Wallace's line and six were significantly associated with sites east of Wallace's line. This difference maybe a consequence of the almost exclusively passive nature of spore dispersal, it is probable that once entrained in the ITF there are few opportunities to escape. Spore entrainment in the ITF gives rise to the possibility of limited dispersal onto the Sunda Shelf. If dispersal onto the Sunda Shelf is limited, we could expect to observe fewer RSVs on the shelf and this is what we do see; 419 RSVs and 565 RSVs were recorded the Sunda Shelf and Wallacea respectively.

There is unlikely one unifying process that is entirely responsible for creating the biogeographical patterns and fungal diversity we observed in this survey. The Coral Triangle, the region where the majority of this work was performed, is considered the most biodiverse marine environment on the planet and several hypotheses have been developed to explain this remarkable diversity (Center of Origin, Center of Accumulation and Center of Speciation, see Bowen, Rocha, Toonen, Karl, and ToBo Laboratory (2013) for a summary of each). It is now generally considered that each hypothesis has contributed to the biodiversity of the Coral Triangle. Similar to this, we suggest that fungal biogeography is shaped by many variables (e.g. habitat type, environmental regimes and dispersal limitations, amongst other factors) all working in union contributing to the biogeographical patterns we observed.

With this work, we were able to detect significant differences in fungal community composition on either side of Wallace's line and we postulate that these differences are a consequence of limited dispersal onto the Sunda Shelf, coupled with strongly divergent habitats on either side of Wallace's line.

Future research could take advantage of the large repositories of well-preserved tissue that have already been collected by laboratories or is stored in DNA banks throughout the world (Datlof et al., 2017). If tissue has been collected and suitably preserved, it is not difficult to envision future fungal projects that could take advantage. Given our extremely limited knowledge of marine fungal diversity, these could be worthy and rewarding endeavours, describing fungal diversity and uncovering biogeographical patterns at the same time.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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BIOSKETCH

Ben Wainwright's research is primarily motivated by the conservation of biodiversity and understanding the mechanisms that create and maintain it. He has a particular interest in the marine regions of S.E. Asia, especially the Coral Triangle.

Author contributions: B.J.W. and A.S.A conceived the idea; B.J.W. performed fieldwork; B.J.W., G.L.Z & A.S.A analysed data; B.J.W led the writing; all authors reviewed drafts and made valuable comments.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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