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RESEARCH ARTICLE

Using point data to assess biogeographical signal, endemicity and factors associated with macrofungal diversity in the data-poor Pacific oceanic island bioregion

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Abstract

Aim: Oceanic hotspot archipelagos are important systems for studying evolutionary and ecological processes; however, studies of macrofungi in these habitats are extremely limited. We assessed diversity across habitat types, using DNA point data to draw inferences about biogeographical signal, endemicity and potential radiation for fungi of a representative isolated island (Moorea, French Polynesia) within the poorly known South Pacific bioregion, comparing resulting patterns to those previously inferred for more comprehensively sampled plants and animals.

Location: Moorea and Tahiti, French Polynesia.

Taxon: Fungi (Basidiomycota, Ascomycota).

Methods: Morphological characters and similarity assessment of rDNA-ITS sequence data were used to measure diversity and habitat associations. A Monte Carlo simulation method was developed to detect biogeographical signal and assess its significance.

Results: A total of 553 specimens yielded 205 morphospecies; 433 sequenced specimens yielded a molecular:morphological richness ratio of 1.24:1. Approximately 58% of sequences lacked a close (≥98%) GenBank match. Community composition differed by vegetation type, with highest similarity between forest types occupying similar low-elevation habitats. A predominant Australian/Oceanean biogeographical signal was detected.

Main conclusions: Moorea fungi exhibit a strong Australian biogeographical affinity as reported for other taxa, supplemented by more recent introductions. Like Society Islands plants and animals, macrofungi may have high endemism. Morphological evidence does not indicate that intra-island radiation has occurred, though molecular evidence suggests possible cryptic speciation. Habitat types differ in their biogeographical affinities, but habitat does not appear to be driving radiation. This study introduces a robust method for inferring biogeographical signal using sequence database comparisons, applicable to other taxa and systems for which only point data are available.

KEYWORDS
French Polynesia, fungi, island biogeography, ITS sequences, Monte Carlo simulation, Moorea Biocode Project, mushroom diversity, null models, Society Islands, Tahiti

1 | INTRODUCTION

Due to their isolation and chronological spatial arrangement, Pacific hotspot archipelagos such as the Hawaiian and Galápagos Islands have provided important insights into the roles of dispersal, evolutionary radiation and species interactions in shaping biotas (Losos & Ricklefs, 2009; Wagner & Funk, 1995; Whittaker et al., 2008). Although less intensively studied, the French Polynesian hotspot archipelagos (Society, Marquesas and Austral islands) have important potential for comparative studies due to their greater diversity in age of formation, greater isolation and a biogeographical history involving less affinity with the Americas (Gillespie, 2002; Gillespie et al., 2008).

Except for a few studies with limited taxonomic scope (e.g. Olive, 1957, 1958a, 1958b), published biodiversity data are scant for fungi of insular Oceania (Cooper, 2011; Reid et al., 1980). Although fungarium collection records have recently (most since mid-2017) become available through the Mycology Collections data Portal (MyCoPortal; http://mycoportal.org/portal/index.php), French Polynesian fungi, like fungi worldwide, have poorly known diversity and distributions (Mueller et al., 2007). Therefore, whether the biogeographical patterns exhibited by Pacific island plants and animals also hold true for fungi remains an open question. Dispersed via microscopic spores, and often associated with specific plant or animal hosts, fungi are likely to exhibit biogeographical patterns shaped by their dispersal capacity and degree of habitat and host specificity, geologic forces, habitat availability and size, distance between habitats, and interspecific interactions (Halling et al., 2008; Peay et al., 2007; Vilgalys & Sun, 1994; Wu et al., 2000). Understanding the relevance of each of these factors in shaping fungal distributions, and uncovering any key differences in dispersal potential between fungi and other organisms, has important implications for conservation and biodiversity estimation.

Phylogeographical studies have shed considerable light on the biogeography of plants and animals on Pacific island archipelagos by identifying likely source regions, distinguishing single from multiple colonization events, identifying radiations, inferring dispersal patterns, differentiating intra- versus inter-island speciation events and providing time estimates for these processes (Butaud et al., 2005; Cibois et al., 2011; Gillespie et al., 2008; Hamilton et al., 2010; Hembry & Balukjian, 2016; Moritz et al., 1993). However, the lack of available DNA sequences—even for the more comprehensively documented Hawaiian mycota (Hemmes & Desjardin, 2001, 2002)—precludes such analyses for Pacific fungi. In contrast, efforts in DNA barcoding and molecular identification have added numerous fungal rDNA-ITS sequences from continental regions to global sequence databases. These databases are a valuable resource for inferring biogeographical affinity using sequence similarity comparisons; however, their uneven geographical coverage can produce a biased picture of biogeographical signal unless coverage is accounted for in analyses.

The Moorea Biocode Project (MBP) is the first effort to exhaustively characterize the macroscopic biodiversity of an entire island combining biological collections and DNA sequencing (Check, 2006; Eichenseher, 2011). The MBP enabled intensive collection, description and sequencing of macrofungi (fungi with visible assimilative or reproductive structures including mycelia, stroma and sporocarps) on Moorea, Society Islands archipelago, French Polynesia. In the current study, we use MBP field surveys and DNA sequence data to examine macrofungal diversity across habitats and to develop an unbiased approach for assessing island- and habitat-scale biogeographical signal using sequence comparisons to a global reference database.

1.1 | Biogeographical signal: What does it look like, and how can it be detected using point data?

Since the Moorea sequence dataset cannot be incorporated into a formal phylogeographical framework, it is necessary to treat these as point data and infer biogeographical signal by comparing genetic relatedness between Moorea sequences and sequences from more comprehensively sampled continental biotas available in global databases. Here, we define biogeographical signal as a predominant pattern of genetic similarity between Moorea fungi and fungi from other geographical regions that indicates an overall historical association between their biotas. If association with a particular region is strong—for example, in the case of a predominant mainland source—then the genetically closest relatives for many Moorea species should be found among sequence database records from that source region. In contrast, if dispersal limitation is not a factor, we would not expect to see a predominant biogeographical signal. A significant impediment to using sequence databases such as GenBank for this purpose is that their geographical coverage is uneven. Therefore, for a given level of similarity to a query sequence, matches are more likely to come simply by chance from a region that is better represented in the database. To address this issue, we developed a Monte Carlo simulation approach to detect statistically significant biogeographical signal, distinguishing it from noise caused by underlying geographical coverage bias in the reference database.

1.2 | Hypotheses and objectives

In this study, we address the following hypotheses: (1) Due to both isolation and lack of prior studies in the region, Moorea harbours undescribed biodiversity; (2) Due to dispersal limitation (Peay et al., 2010), a dominant biogeographical signal will be detectable; this signal is likely to be Australasian (i.e. Australian/East Palearctic/Oceanean), based on the patterns observed in plants and animals; (3) Exceptions to the dominant biogeographical signal will be habitat dependent, with native and early Polynesian forest types exhibiting the dominant Australasian origin pattern, and with human-dominated habitats of recent origin containing more instances of human-mediated long-distance dispersal. In addition to addressing these hypotheses, we examine the similarity of fungal

communities in the context of similarity in vegetation type, host arrival history and elevation.

2 | MATERIALS AND METHODS

2.1 | Study site

Moorea is located near the southeastern end of the Society archipelago, *c*. 20 km northwest of Tahiti. Moorea is a volcanic high island *c*. 2.15–1.36 Ma in age, characterized by steep topography comprising elevations from 0 to 1207 m within a 134 km^2 area (Guillou et al., 2005; Neall & Trewick, 2008; Uto et al., 2007). Vegetation varies strongly with altitude, with a xeric coastal zone, a low- and mid-elevation mesic zone, and a high-elevation wet zone dominated by montane cloud forest (Meyer, 2004). The Society Islands are hotspot islands that were never connected to a continental land mass; therefore, fungal colonization must result from (1) natural colonization via dispersal by aquatic or aerial transport of spores, substrate material or animal vectors; (2) 'historic' introduction via Polynesian human colonization *c*. 2.9–1.0 kyr (Kirch, 2000; Kirch & Green, 2001); or (3) modern human introduction resulting from trade and tourism. Plant and animal distributions at low- and mid-elevations exhibit high human impact, including recent invasive and agricultural species; higher elevation habitats are less impacted, with a higher proportion of endemic and indigenous taxa (Craig et al., 2001; Gillespie et al., 2008; Meyer, 2004). Moorea is located approximately 4400 km south of Hawaii, 7900 km west of Chile and 5700 km east of Australia; this exceptional isolation is likely to shape the island's biodiversity, though tourism and agriculture provide potential conduits for long-distance, human-mediated dispersal.

2.2 | Specimen collection

Macrofungi producing a differentiated thallus (fruiting body) ≥2 mm diameter, including crusts formed by clonally reproducing mitosporic fungi, were surveyed in 2008 (late September–late November) and 2009 (early July–late September). Specimens were collected using opportunistic and transect sampling encompassing the greatest geographical coverage and habitat diversity possible (Figure 1), including (1) human-dominated habitats (lawns, pastures, ornamental trees); (2) introduced tree plantations (Caribbean pine, *Pinus caribaea*; she-oak, *Casaurina equisetifolia*; and semi-invasive *Falcataria moluccana* forests); (3) sandy coastlines dominated by coconut palm (*Cocos nucifera*); (4) lowland forests representing diverse arrival histories: native (e.g. *Hibiscus tiliaceus*), Polynesian introductions (e.g. Tahitian Chestnut, *Inocarpus fagifer*) and recent introductions; and (5) montane wet forests dominated by native woody plants including *Metrosideros collina*, *Fagraea berteroana*, *Pandanus tectorius*, *Pittosporum* spp., *Pouteria grayana*, *Weinmannia* spp. and *Wikstroemia coriacea*. Low- and mid-elevation forests were moderately to heavily invaded by the non-native plant *Miconia calvescens*. Transect

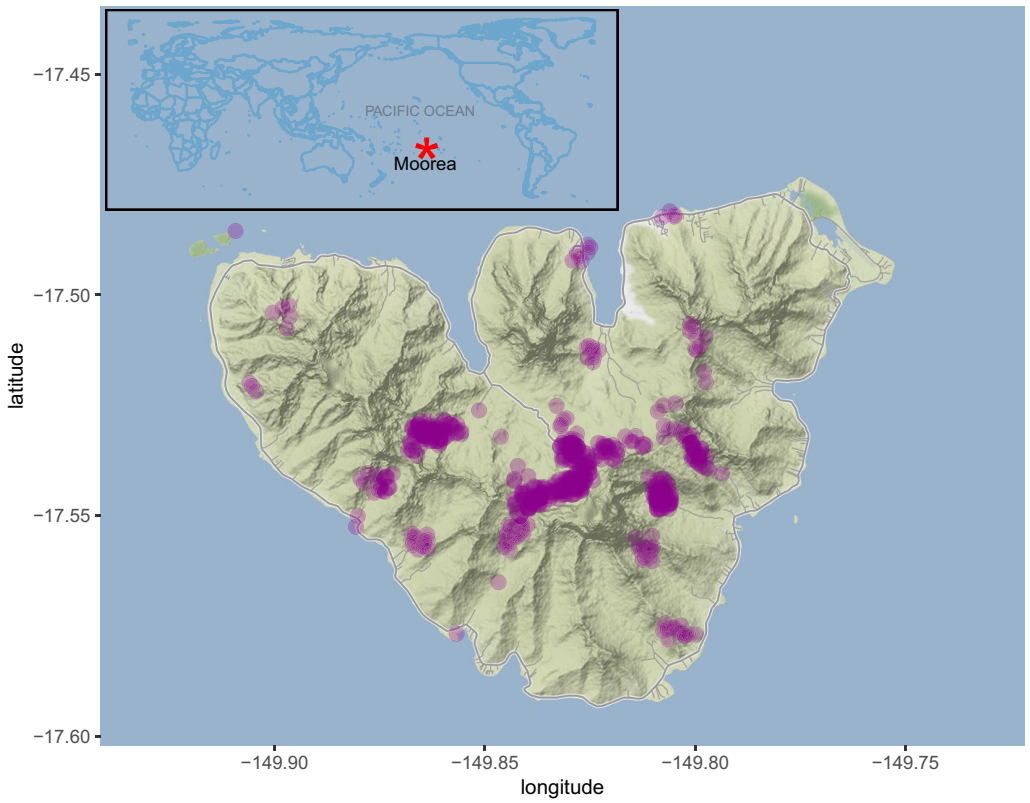

FIGURE 1 Map of Moorea, French Polynesia showing locations of sites surveyed for macrofungi. Main map: Mercator projection. Inset map: equirectangular projection. Map data ©2022 Google

sampling effort was uniform across four habitat types (*Inocarpus/Hibiscus*, *Pinus*, *Cocos* and *Casaurina*); opportunistic collecting was non-uniform in design (Appendix S1).

Field characters were described from fresh specimens; tissue samples (8–64 mm^3) were then lyophilized for DNA extraction, and remaining material was dehydrated for fungarium vouchering. Collection data (Appendix S2), specimen descriptions and photographs are available through the MBP database (https://n2t.net/ark:/21547/CYQ2). Vouchers are housed in the University Herbarium, University of California, Berkeley.

2.3 | Molecular sampling

DNA extraction followed Ivors et al. (2004) or Wang et al. (1993), as amended by Osmundson, Robert, et al. (2013) and (Osmundson, Eyre, et al. (2013), respectively. PCR was conducted using primers ITS1F (Gardes & Bruns, 1993) and ITS4 or TW13 (White et al., 1990), amplifying the nuclear ribosomal ITS1+5.8S+ITS2 (rDNA-ITS) region (~700 bp). PCR followed Osmundson, Eyre, et al. (2013), and sequencing employed the same primers as PCR. Contig assembly/editing used Geneious v5 (Biomatters Ltd.). Genbank accession numbers for newly generated sequences appear in Appendix S2.

2.4 | Morphological and molecular richness estimation

Specimens were grouped into morphological operational taxonomic units (OTUs, or morphospecies) via rapid identifications based on photographs and field descriptions. Specimens were assigned to molecular OTUs (MOTUs) by clustering using CD-HIT-EST (Huang et al., 2010) at a 98% similarity threshold, a level previously shown to minimize erroneous species assignments across a broad taxonomic range when ITS sequences include the highly conserved (and uninformative for species identification) 5.8S gene in addition to the more informative ITS1 and ITS2 regions (Osmundson, Robert, et al., 2013). OTU and MOTU counts were compared, and event-based species accumulation curves were generated for both.

2.5 | Habitat-level diversity and community similarity comparisons

Accumulation curves were constructed for major habitat types to assess sampling saturation. To determine, given available data, whether diversity is more likely to be partitioned by forest type or by other factors (e.g. physical proximity, elevation, microclimate), taxonomic similarity between habitat types was calculated using Sørensen's index, $= \frac{2c}{s_1 + s_2}$ where s_1 represents OTU or MOTU richness in community 1, s_2 represents richness in community 2 and c represents OTUs or MOTUs common to both communities.

2.6 | Biogeographical signal detection

Because DNA sequence data are not available for other Pacific islands, we determined biogeographical affinity to continental sources by determining the collection location of the best-matching sequence in a global DNA sequence reference database. When a database hit exhibits high (species level or species group level) similarity to the query sequence, a location geographically close to Moorea suggests regional dispersal, whereas a distant location suggests long-distance dispersal. Individual specimens' affinities can be aggregated to compare the amount of signal present for different biogeographical regions. However, this approach suffers from two major deficiencies. First, database matches at lower than species group-level similarity do not capture underlying biogeographical processes (evolutionary radiation, long-distance dispersal, island hopping, etc.), and cannot confidently identify evolutionary or biogeographical affinity, since many distantly related taxa can share the same low similarity to a given target sequence. Second, geographical signals can be obscured by the underlying geographical bias in the reference sequence database. To address these deficiencies, we developed a method that treats species- (or species group-) level matches and non-species-level matches separately for comparison, and uses a Monte Carlo simulation method to detect statistically significant biogeographical signals. We conducted the method in three steps as described below.

2.6.1 | Determine biogeographical affinity and highest match similarity for each query sequence

Moorea macrofungal sequences were compared to the NCBI nucleotide database using a BLASTn search, and the 10 highest scoring hits were retrieved for each query sequence. Results were filtered to retain the best hit (highest BLAST bitscore) and any additional hits having a bitscore within 75% and percent identity within 0.5% of those for the best hit. Geographical data were retrieved for each retained hit using a bash command to perform an NCBI eFetch operation and parse contents of the '/country' feature annotation. The geographical locality of the top hit was assumed to share biogeographical affinity to the sample. If geographical data were lacking for the top hit, a manual search of the GenBank record and other sources (e.g. publications, MyCoPortal records, herbarium records) was performed to locate the geographical origin. If unsuccessful, the next highest retained hit was substituted; otherwise, the sample was omitted in subsequent analyses. Country names were converted to biogeographical regions following the IUCN classification by Udvardy (1975), with two modifications: division of the Palearctic region into eastern and western components (Morse, 2020), and inclusion of New Zealand in the Oceanian rather than the Antarctic region. Records from Hawaii were placed within the Oceanian region rather than with other United States records (Nearctic region). Results were divided into

two groups prior to further analyses: (1) sequences for which the best BLAST hit exhibits ≥98% similarity—that is, roughly species-level (Osmundson, Robert, et al., 2013)—and ones with <98% sequence similarity.

2.6.2 | Determine biogeographical signal across the dataset

For each data partition (≥98% and <98%), the observed aggregate signal was determined for each of eight global biogeographical regions (Afrotropical, Australian, Indomalayan, Nearctic, Neotropical, Oceanian, East Palearctic and West Palearctic) as the total number of sequences exhibiting biogeographical affinity to that region. Only samples with an unambiguous signal corresponding to terrestrial habitats within a single biogeographical region were retained, resulting in a total of 394 of the 433 sequenced samples (229 and 165 for the <98% and ≥98% partitions, respectively). As no Moorea sequences had a top BLAST hit corresponding to the Antarctic region, this region was excluded from further analyses.

2.6.3 | Determine statistical significance of biogeographical signal

Signal significance was determined for each biogeographical region by comparing the observed signal to a null distribution of signals determined from random samples of sequences from the reference database using a Monte Carlo approach. For the reference database, records for rDNA-ITS sequences 400–2000 bp in length from the major fungal groups that contain macrofungi (phylum Ascomycota, subphylum Pezizomycotina, and phylum Basidiomycota, subphylum Agaricomycotina) were obtained from Genbank in XML format using an NCBI esearch operation. The search returned 546,195 sequence records. Records containing a country feature annotation were retained, resulting in a set of 362,364 records. To eliminate bias resulting from the same species being sequenced multiple times from a single source (e.g. population genetics studies), records with identical DNA sequences—including nested sequences and sequences differing only in ambiguous nucleotide positions—were reduced to one per country, resulting in a final database of 265,401 records. Country names were converted to biogeographical regions as described above. For Monte Carlo simulations, 10,000 random samples equal in size to the number of sequences in the corresponding Moorea datasets (229 and 165 for the <98% and ≥98% subsets, respectively) were drawn without replacement. The occurrence count for each biogeographical region was calculated for each replicate, and two-tailed 95% confidence intervals were calculated for each simulation. A biogeographical region was considered to be 'overrepresented'—that is, a significant biogeographical signal from that region was detected—when the observed number of Moorea specimens with top hits collected from that region occurred at or above the 97.5th percentile of the null distribution. A biogeographical region was considered to be 'underrepresented'—that is, a signal from that region was significantly absent—when the number of Moorea specimens with top hits collected from that region occurred at or below the 2.5th percentile of the null distribution. Simulations were conducted using R 4.0.3.

Molecular assessments of biogeographical signal were then compared to those derived from fungarium records stored in MyCoPortal and accessible via iDigBio (https://www.idigbio.org). A search of MyCoPortal records was conducted using the criterion 'country = French Polynesia'. Records produced in the present study, not containing a genus and specific epithet, or belonging to microfungi were removed, yielding a list of 200 unique Latin binomials (Appendix S4). Other countries from which each binomial has been reported were obtained using the R package 'ridigbio' (Michonneau et al., 2016). Country names were converted to biogeographical regions, and the number of taxa shared with French Polynesia was determined for each region.

3 | RESULTS

3.1 | Moorea data augment knowledge of Pacific macrofungal diversity and reveal potential endemism

A total of 205 morphospecies (OTUs) were identified from 553 vouchered specimens collected in 52 collecting events (Appendix S2). DNA sequences were obtained for 433 collections, representing 175 of the OTUs. CD-HIT-EST 98% similarity clustering yielded 218 MOTUs, resulting in a MOTU: sequenced OTU ratio of 1.24:1 (Figure 2a).

The Moorea dataset contains many taxa not currently represented in GenBank. In BLAST comparisons of 433 Moorea sequences following the match criteria described in Materials and Methods, only 10 (2.3%) exhibited 100% similarity to an existing fungal ITS sequence in GenBank. A majority (182, or 58%) of the sequences did not match a GenBank sequence at approximate species- or species group-level (98%) similarity (Figure 2b).

3.2 | Macrofungal diversity differs by habitat type

Most Moorea macrofungi are wood or litter saprotrophs, though forest pathogenic fungi are also present. Only one ectomycorrhizal taxon (*Rhizopogon* sp.) was encountered, associated with introduced *P. caribaea*. Although uneven sampling effort precludes direct richness comparisons between habitats, the mean number of morphological OTUs per collection foray is considerably higher for lowland forests dominated by *Inocarpus fagifer* and *Hibiscus tiliaceus* (11.1 OTUs/foray) than in introduced *Falcataria moluccana* stands, coastal *Cocos nucifera* habitats, introduced *Casuarina equisetifolia* stands, introduced *P. caribaea* plantations, upland mixed native forests, and from lawns, pastures and ornamental trees (1–5 OTUs/foray; Appendix S1). Only lowland forests showed evidence of saturated

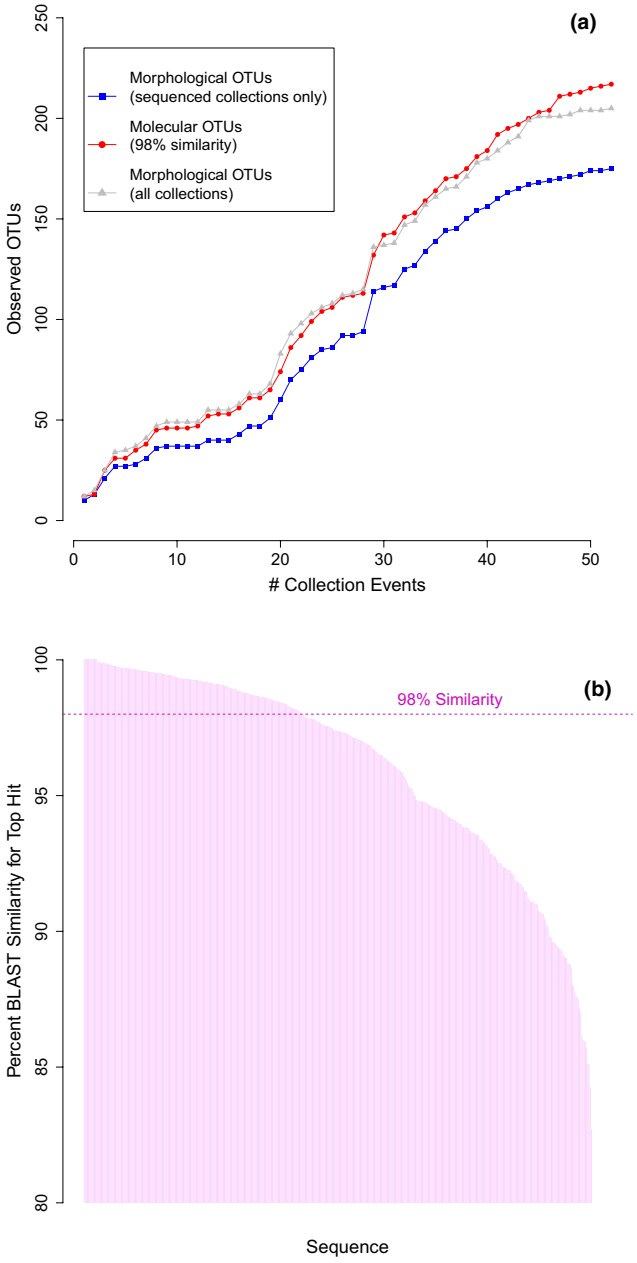

FIGURE 2 Diversity and sequence novelty of macrofungi on the island of Moorea, French Polynesia. (a). Accumulation curves comparing morphological and molecular OTU richness of macrofungi on Moorea over 52 collection forays. (b) Rank-ordered distribution of highest percent match to a fungal ITS sequence in GenBank for macrofungal specimens from Moorea. Pink dotted horizontal line denotes a 98% similarity threshold

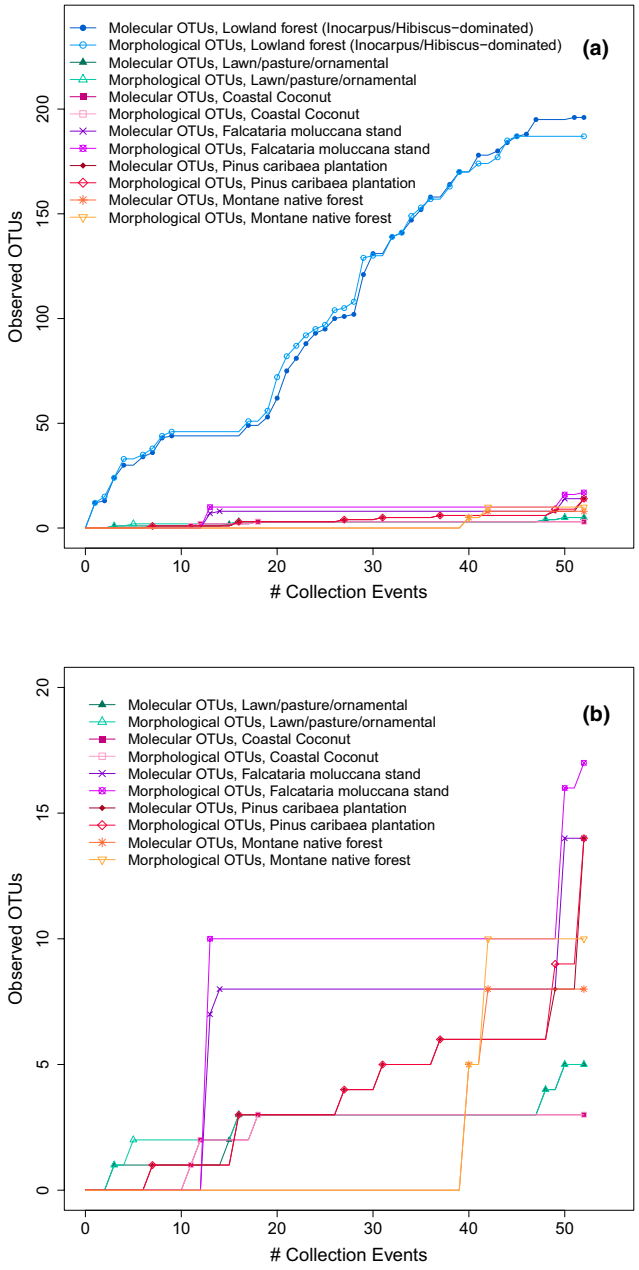

FIGURE 3 Accumulation curves comparing morphological and molecular OTU richness of macrofungi between major habitat types on the island of Moorea, French Polynesia. (a). All habitat types included. (b). Lowland forest habitat type excluded for the purpose of allowing the *y*-axis to be scaled to show patterns for other habitat types more clearly

sampling for either morphological or molecular OTUs (Figure 3). The highest Sørensen similarities were observed between habitats occurring in close proximity, such as between lowland forests and *Falcataria moluccana* and *P. caribaea* plantations occupying similar lowland regions, and between lawns and coconut stands found in low-lying regions near the coast (Table 1). However, this result must be interpreted with some caution due to apparent undersampling of most habitats.

3.3 | Moorea macrofungi exhibit a predominant Australian-Oceanean biogeographical signal

In the ≥98% similarity subset, a strong southwestern Pacific Rim pattern was evident: the Australian and Oceanian regions were overrepresented, whereas the Nearctic and Western Palearctic regions were underrepresented, relative to the null distribution (Table 2; Figure 4). The Australian region is also overrepresented in the <98% similarity subset; this overrepresentation ceases at levels below 90%

TABLE 1 Sørensen similarity coefficients showing macrofungal community similarity between major habitat types on Moorea, French Polynesia based on presence/absence of morphological and molecular operational taxonomic units (OTUs)

Comparison	Similarity (morphological OTUs)	Similarity (molecular OTUs)
Lowland/Lawn	0.031 (3)	0.02 (2)
Lowland/Coconut	0.032 (3)	0.02 (2)
Lowland/Falcataria	0.137 (14)	0.094 (10)
Lowland/Pine	0.07 (7)	0.085 (9)
Lowland/Montane	0.03 (3)	0.019 (2)
Lawn/Coconut	0 (0)	0.4 (2)
Lawn/Falcataria	0.091 (1)	0.095 (1)
Lawn/Pine	0 (0)	0.095 (1)
Lawn/Montane	0 (0)	0.133 (1)
Coconut/Falcataria	0.2 (2)	0.105 (1)
Coconut/Pine	0.118 (1)	0.211 (2)
Coconut/Montane	0 (0)	0.154 (1)
Falcataria/Pine	0.194 (3)	0.333 (5)
Falcataria/Montane	0.148 (2)	0.167 (2)
Pine/Montane	0 (0)	0.083 (1)

Number of shared OTUs appear in parentheses.

TABLE 2 Level of observed biogeographical representation of the closest BLAST hits for the Moorea sporocarp sequence dataset, partitioned by level of sequence similarity to the closest BLAST hit

Biogeographical region	Level of sequence similarity to top BLAST hit	
	<98%	≥98%
Afrotropical	11 (4–15)	7 (2–12)
Australian	68 (2–12) [a]Over	29 (1–9) [a]Over
Indomalayan	20 (16–35)	26 (11–26)
Nearctic	51 (37–61)	9 (25–46) [a]Under
Neotropical	19 (13–30)	20 (8–22)
Oceanian	0 (1–10) [a]Under	28 (0–7) [a]Over
Eastern Palearctic	19 (38–62) [a]Under	34 (26–46)
Western Palearctic	40 (51–77) [a]Under	12 (35–57) [a]Under

[a]Result significant at $p = 0.05$. 'Over' = region overrepresented compared to jackknife-simulated dataset; 'Under' = region underrepresented compared to jackknife-simulated dataset. Biogeographical over- or under-representation was assessed by Monte Carlo simulation; 95% confidence intervals appear in parentheses.

similarity. The Oceanian, Eastern Palearctic and Western Palearctic regions were underrepresented in the <98% similarity subset (Figure 5).

In the fungarium data, 50 names were not reported outside of French Polynesia. For the remaining 150 names, the Neotropical, Nearctic, Indomalayan and Oceanian regions (in descending order) were most frequently represented (Appendix S5).

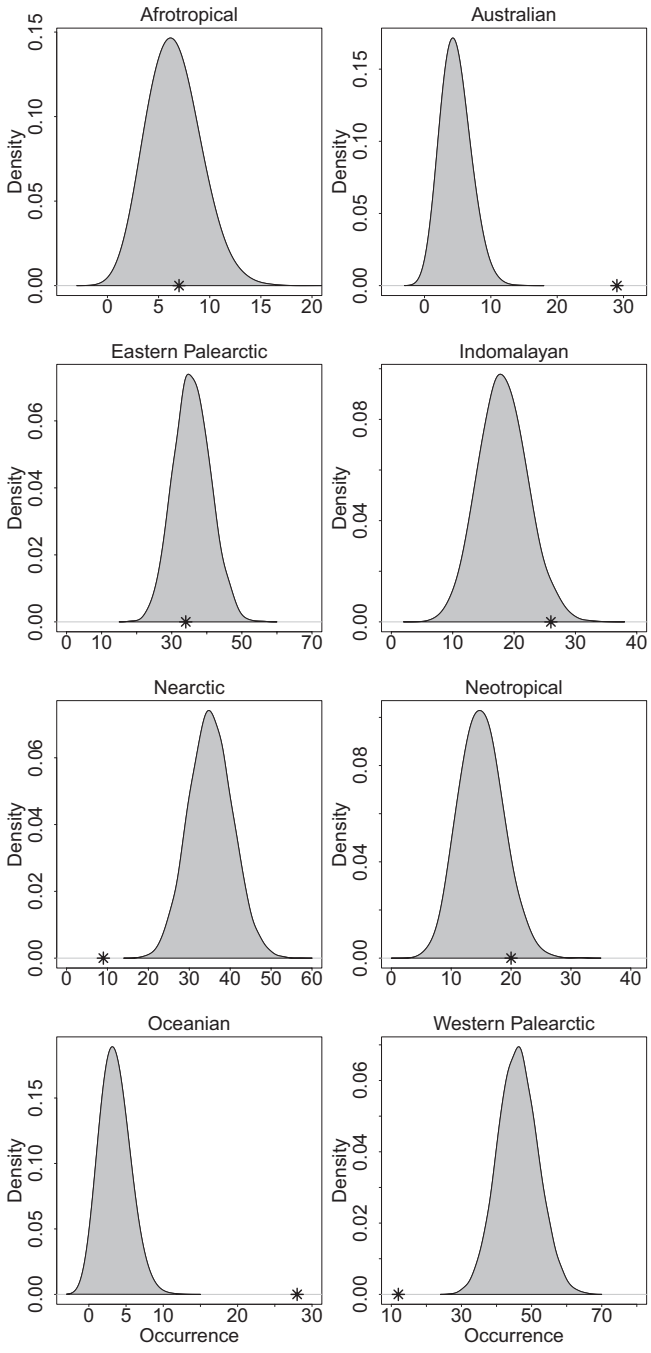

FIGURE 4 Kernel density plots for simulations of biogeographical representation among macrofungi of Moorea, French Polynesia, based on the 165-sequence subset exhibiting ≥98% sequence similarity to the best BLAST hit in GenBank. The observed number of occurrences for each biogeographical region (represented by asterisks along the *x*-axis) was compared to the null distribution of occurrences of that region in 10,000 Monte Carlo draws of 165 randomly sampled records from the reference database

3.4 | Biogeographical signal varies by habitat type

Biogeographical affinities differed between habitat types. Native montane forests exhibited an exclusively Pacific rim signature, though from the Neotropical and East Palearctic regions. Although the more

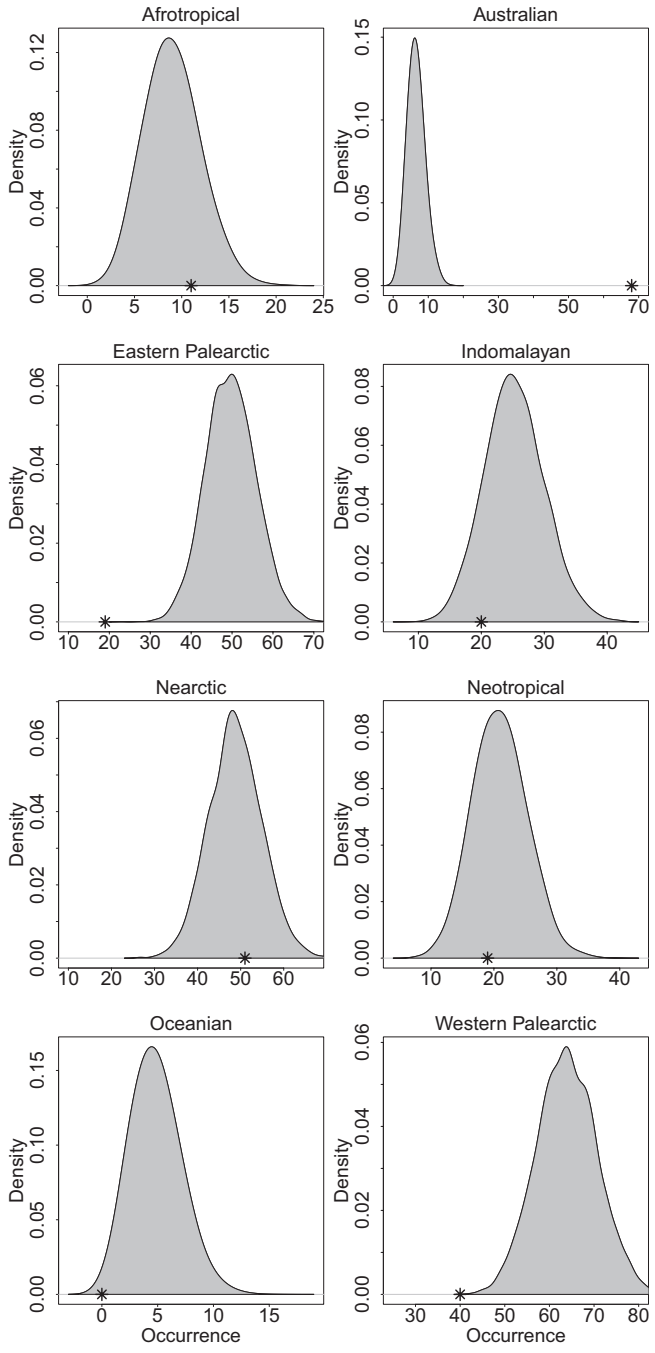

FIGURE 5 Kernel density plots for simulations of biogeographical representation among macrofungi of Moorea, French Polynesia, based on the 229-sequence subset exhibiting <98% sequence similarity to the best BLAST hit in GenBank. The observed number of occurrences for each biogeographical region (represented by asterisks along the x-axis) was compared to the null distribution of occurrences of that region in 10,000 Monte Carlo draws of 229 randomly sampled records from the reference database

human-dominated, lower-elevation forests and pine plantations exhibited a more cosmopolitan pattern that includes both Pacific Rim and non-Pacific Rim affinities, the habitats that contain more Australasian floristic elements (*Falcataria moluccana* stands and lowland forests) have strong Australian biogeographical signals (Table 3; Figure 6).

TABLE 3 Biogeographical affinities of Moorea macrofungi by habitat type. Cell values represent the proportion of sequences with affinity to each major biogeographical province (Morse, 2020; Udvardy, 1975), assigned according to the geographical origin of the highest GenBank species-level (≥98% similarity) match for each Moorea sequence. *N* = number of samples

	Biogeographical region							
Habitat	**Afrotropical**	**Australian**	**Indomalayan**	**Nearctic**	**Neotropical**	**Oceanian**	**East palearctic**	**West palearctic**	***N***
Lowland forest	0.05	0.19	0.15	0.06	0.11	0.18	0.21	0.06	140
Lawn	0	0	0.2	0	0.2	0.2	0.2	0.2	5
Coastal coconut	0	0	0	0	0	0	1	0	2
Falcataria moluccana stand	0	0.43	0.29	0	0	0.14	0	0.14	7
Pine plantation	0	0	0.29	0.14	0.14	0.14	0.14	0.14	7
Montane forest	0	0	0	0	0.75	0	0.25	0	4

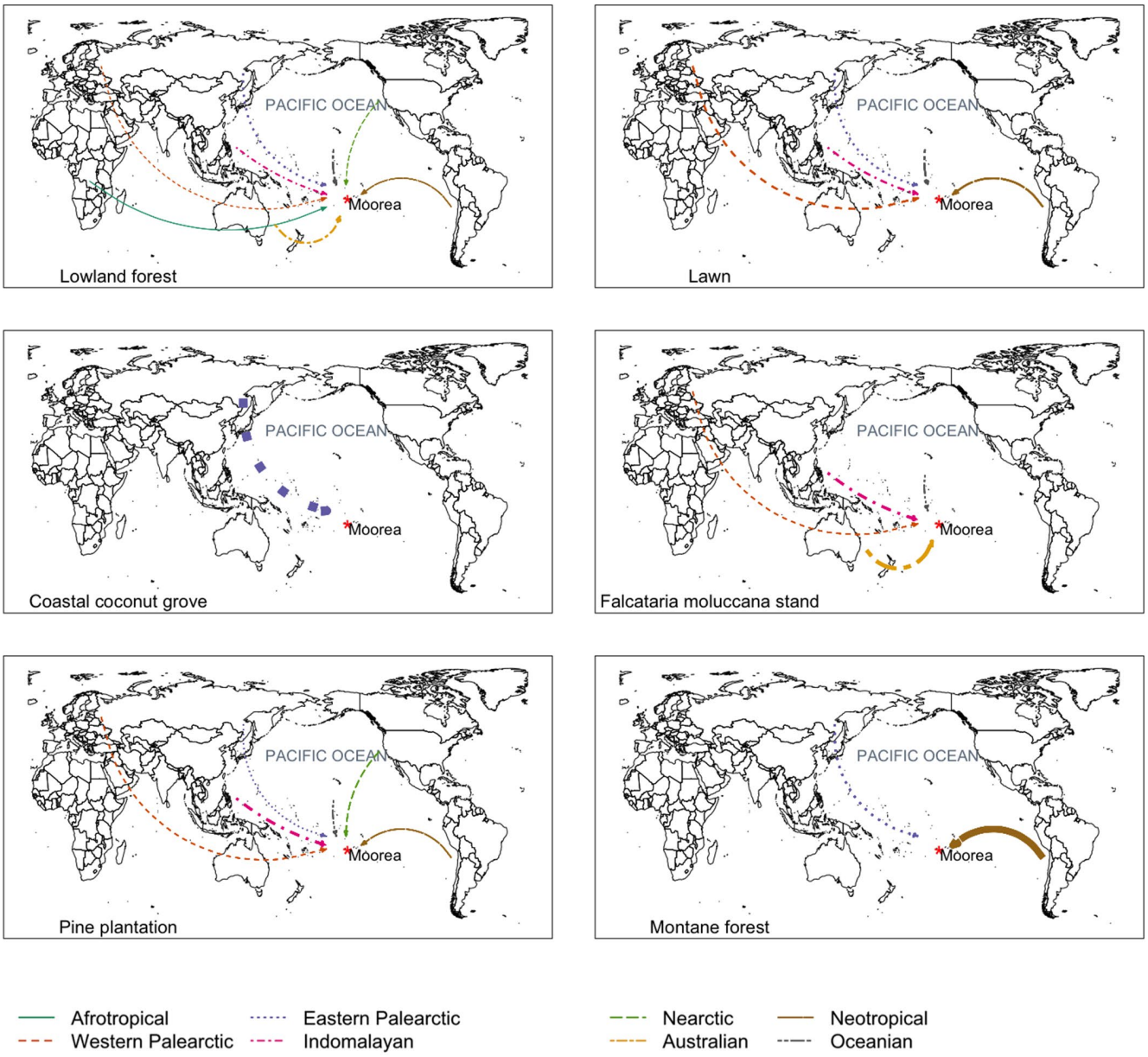

FIGURE 6 Conceptual representation of biogeographical affinities of macrofungi in major habitat types on Moorea, French Polynesia, as inferred by the origin of GenBank sequences with the highest percent similarity matches (≥98%) to Moorea sequences. Arrow thickness is scaled to the proportion of samples from that habitat with highest matches to each of the eight biogeographical regions (omitting Antarctic) delimited by Udvardy (1975) and Morse (2020)

4 | DISCUSSION

The Moorea Biocode Project provides a first picture of macrofungal diversity on a French Polynesian island using DNA sequences, increasing geographically annotated rDNA-ITS sequence coverage for French Polynesian fungi in GenBank by over 43X. We examined these data to infer the likely source regions, potential for endemicity and potential for intra-island radiations for Moorea macrofungi; to infer the potential role of habitat in influencing intra-island variation in community composition and biogeographical signal; and to assess whether fungi follow the biogeographical trends previously observed for more comprehensively sampled Society Islands plants and animals.

4.1 | Diversity of Moorea macrofungi

Over 200 species were recorded based on field surveys and macromorphological assessments. Based on available data, diversity and community composition appear to differ by habitat type. Lowland *Inocarpus/Hibiscus* forests yielded the greatest number of taxa overall and in terms of mean number of OTUs obtained per collecting event. Although community dissimilarity suggests that specific host and/or edaphic factors are important in partitioning diversity, the observation that adjacent habitats have higher levels of similarity also indicates an important role of climate and/or local dispersal in shaping fungal communities. As many habitats appear to be

undersampled, these hypotheses should be further tested when more data are available.

As expected, diversity of ectomyorrhizal fungi was low, as the introduced pine tree *P. caribaea* is the only known ectomycorrhizal host plant on Moorea. Although only a single ectomycorrhizal sporocarp collection was obtained, DNA sequencing of *P. caribaea* mycorrhizal roots from soil cores detected additional ectomycorrhizal fungi; these consisted predominantly of fungi that produce inconspicuous reproductive structures, including *Tomentella* and *Wilcoxina* spp. The introduced tree *Casuarina equisetifolia* can form ectomycorrhizae in vitro (Dell et al., 1994; Theodorou & Reddell, 1991; Thoen et al., 1990); however, both sporocarp and root tip surveys suggest that *C. equisetifolia* is non-ectomycorrhizal on Moorea (Osmundson et al., unpublished).

4.2 | Biogeographical signal

A review of phylogeographical studies by Hembry and Balukjian (2016) indicates that Society Island animals and plants have a predominantly Australian source with eastward migration and subsequent colonization between islands. A western Pacific Rim pattern for fungi would be consistent with spore transport via storm and prevailing winds and/or high-altitude jet streams, both of which move predominantly west to east in the southern Pacific (Gillespie et al., 2012). Results of our null model simulation for samples with species-level or near-species-level matches in the reference database similarly revealed a strong Australian and Oceanian biogeographical signal, with the Australian signal persisting in the subset of more divergent sequences down to a level of between 90% and 91% similarity, and the Oceanian region underrepresented in the <98% similarity subset. This combination of results suggests a long and consistent biogeographical connection between the Australian region and Pacific islands, and a more recent one within Oceania, supporting the hypothesis of an Australian source with subsequent inter-island migration. Biogeographical affinity to other Pacific islands is further supported by the observation that matches to Hawaiian sequences only occur within the ≥98% similarity dataset (Appendix S3). Other regions are either underrepresented or do not exhibit a significant signal in either the ≥98% and <98% similarity subset, indicating a weaker biogeographical connection.

From these results, it can be inferred that the Moorea mycobiota originated predominantly, though not exclusively, via dispersal from adjacent biogeographical regions, naturally and/or linked to historical human migration. A strong genetic signature of long-distance dispersal (i.e. greater-than-expected representation of closely related sequences from distant biogeographical provinces) is mostly lacking in the dataset; however, individual counts for the ≥98% similarity subset (Table 2) show that each biogeographical region contains species- or species-group similarity to at least several collections from Moorea, and the presence of fungal specialists on non-native substrates (e.g. *Rhizopogon* on pine roots; *Panaeolus* on horse dung) demonstrates that some human-mediated transport of fungi has occurred. Incomplete species representation in GenBank may obscure some other, less obvious, cases of human-mediated transport. Land use appears to play an important role in the distribution of fungal taxa, as lower elevation, human-dominated habitats were associated with more instances of apparent long-distance dispersal than native montane forests; this observation mirrors the pattern observed in terrestrial invertebrates, in which low-elevation habitats contain more cosmopolitan (probably recently introduced) taxa, whereas higher elevations contain a higher proportion of native species (Gillespie et al., 2008).

The survey of fungarium records also indicated a Pacific Rim biogeographical signal, but with the eastern portion (Nearctic and Neotropical regions) more highly represented. A fungarium label-based approach has several drawbacks, including geographical collection bias, synonomy and misidentification. As genetic data provide a more robust metric of relatedness and the Monte Carlo approach presented in this study accounts for geographical collection bias, we maintain that the approach introduced in this study provides a more accurate measure of biogeographical signal compared to fungarium records.

4.3 | Endemism

The Society Islands contain high endemism in several plant and animal groups (Cibois et al., 2004; Gillespie, 2002; Gillespie et al., 2008; Hembry & Balukjian, 2016). Because microscopic fungal spores are theoretically more vagile than the propagules or adults of other organisms, one might hypothesize that fungi exhibit lower endemism than these other organisms. However, our results suggest this is not the case. Few Moorea sequences have 100% similarity matches in the reference database, suggesting that consistent and/or contemporary migration from a continental source is unlikely; instead, dispersal followed by divergence appears to be the general rule. A detailed study of Moorea Entolomataceae supports this conclusion, finding that Moorea collections appear to be endemic to French Polynesia and are distinct from, but most closely related to, Australian species (Bergemann et al., in prep.).

The question remains of whether endemism occurs between Pacific islands, or whether fungal species are distributed across islands or even archipelagos. Species lists (Cooper, 2011) and fungarium records (mycoportal.org) suggest that many French Polynesian species are found on other archipelagos and/or continental land masses. However, this question should be more rigorously examined using molecular data. A preliminary comparison of Moorea sequences with data obtained from the Cook Islands (~1000 km southwest) revealed a high proportion of genetically similar taxa, suggesting that species are shared between archipelagos and that stepping-stone dispersal plays an important role in Pacific macrofungal biogeography (R. Fuller et al., in revision).

4.4 | Radiation

Except for a few notable examples of radiations between and even within islands, there is little evidence of radiation within the

Society Islands for plants and animals (Hembry & Balukjian, 2016). Similarly, our results do not detect readily apparent fungal radiations within Moorea, as most genera contained only one morphospecies. To determine whether cryptic speciation may have occurred, we compared morphological to molecular operational taxonomic units (OTUs and MOTUs, respectively). The MOTU:OTU ratio of 1.24:1 and the observation that 59 of the 175 sequenced morphospecies corresponded to more than one MOTU—ranging from 2 to 5 MOTUS per morphospecies—suggest that cryptic speciation may have occurred. These MOTUs did not correlate with habitat type, suggesting that ecology is not the major factor driving or maintaining molecular divergence in Moorea fungi.

Although we previously demonstrated that the 98% similarity criterion used in this study reasonably delimits many species of macrofungi (Osmundson, Robert, et al., 2013), any arbitrary similarity threshold could inflate molecular diversity estimates if set above the lower boundary of intraspecific variation for a taxon (Meyer & Paulay, 2005). Though ITS sequences have shown potential to inflate diversity estimates due to intraspecific or intra-individual heterogeneity (Lindner & Banik, 2011) or, conversely, deflate estimates due to interspecific homogeneity (O'Donnell et al., 2011), there is currently little evidence that either factor is widespread in macrofungi. Nonetheless, sequencing of additional loci could more accurately delimit possible cryptic species in targeted taxa. Since the 59 morphospecies corresponding to >1 MOTU represent 80% of the morphospecies with more than one sequenced representative, the number of MOTUs could rise considerably with additional ITS sequencing effort. Detailed morphological and molecular taxonomic studies of Moorea macrofungi are ongoing; these will allow better estimates of diversity and potential radiation of this biota.

5 | CONCLUSIONS AND FUTURE RESEARCH

The Moorea Biocode Project has produced the most comprehensive molecular picture of a Pacific island macrofungal biota to-date. We used these point data to draw inferences about the biogeographical and ecological factors that influence fungal diversity on a representative isolated island within the poorly known South Pacific bioregion, comparing patterns inferred for fungi to those previously inferred for more comprehensively sampled plant and animal taxa. We applied a null model approach for detecting significant biogeographical signal that may prove useful for other studies involving biogeographical point data. This approach is robust to the addition of sequences—from inside or outside of the dominant source region—to the reference database, since the null distribution of biogeographical signal is built upon a series of random samples from the database; it is also robust to the addition of multiple sequences of a single species, as a single exemplar of each unique sequence per country is selected randomly to assemble a nonredundant reference database. Finally, although there is a limit of regional bias at which this method will fail to detect a true biogeographical signal (take, as an extreme case, a region composing the entire reference database), it is robust against detecting false signals.

Society Islands plant and animal distributions show an overall pattern suggesting a dominant Australasian origin, with multiple colonization events for many clades. Moorea macrofungi appear similarly to have a strong Australasian affinity, supplemented by more recent, human-mediated introductions. Like Society Islands plants and animals, macrofungi have potentially high endemism. There is little morphological evidence to suggest that intra-island radiation has occurred, though molecular evidence leaves open the possibility of cryptic speciation. Habitat types differ in the biogeographical affinities of their taxa, but habitat does not appear to be a strong factor driving speciation.

Further biogeographical understanding of Moorea and south Pacific macrofungi would benefit greatly from sampling additional islands, allowing the following questions to be addressed:

1. In clades represented by multiple species, are distributions shaped by radiation, multiple independent colonization events, or both?
2. Do endemic species occur at the scale of individual islands and/or archipelagos, or is there a pan-Pacific island macrofungal biota?
3. Is colonization best described by a stepping-stone model, or by multiple, frequent colonization events from a continental source?
4. Is the pattern of divergence in biogeographical signal between habitat types on Moorea corroborated by studies of other islands?
5. Are certain taxa more dispersive than others (Gillespie & Roderick, 2002)?
6. Are fungi more vagile (i.e. less biogeographically partitioned) than plants or animals?
7. Within the Society Islands, do fungi exhibit the Windward/Leeward break observed for other taxa (Hembry & Balukjian, 2016)?

Increased representation of higher elevation habitats dominated by native plant species could also provide important biogeographical insights. As high-elevation massifs and mountains are important in allopatric speciation of plants and animals, these are important locations to look for evidence of radiations and endemicity; such evidence is more likely to be found in native taxa due to their older age. Due to low sporocarp occurrence and inaccessibility of steep volcanic terrain, upland habitats were not well-enough sampled in the present study to characterize their diversity. However, most of the species found in these and similar habitats in neighbouring Tahiti were not found in the much better-sampled lowland habitats, suggesting that upland habitats are important from a biodiversity standpoint, as previously noted for plants and insects (Gillespie et al., 2008; Hembry & Balukjian, 2016).

A growing consensus based on local, regional and intercontinental scales concludes that fungi, despite having microscopic propagules, do exhibit dispersal limitation and biogeographical structure (O'Donnell et al., 2011; Ordynets et al., 2018; Peay et al., 2010; Taylor et al., 2006; Vilgalys & Sun, 1994). However, true oceanic

island biogeographical model systems are mostly lacking for macrofungi (Tanesaka, 2012). Building upon the Moorea data with collections from additional Pacific islands will improve knowledge of island biogeography for fungi, provide additional insights into distributions and endemicity that will benefit conservation, and improve understanding of historical and modern colonization pathways relevant to biocontrol, plant pathology and invasion biology.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All DNA sequence data have been submitted to the DDBJ/EMBL/GenBank databases under accession numbers listed in Appendix S2. Morphological and molecular operational taxonomic unit designations and habitat associations for all samples used in community and diversity analyses are listed in Appendix S2. BLAST results and biogeographical associations for samples used in the biogeographical signal analyses are listed in Appendix S3. Scripts and data used for analyses are available in Appendix S6 and via GitHub (https://github.com/toddosmu/moorea_sporocarp_diversity). Specimens are housed at the University Herbarium, University of California, Berkeley. Collection data are available via the Moorea Biocode Project database (https://n2t.net/ark:/21547/CYQ2) and the Mycology Collections Portal (MyCoPortal; http://mycoportal.org).

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BIOSKETCH

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Authors' contributions: Todd W. Osmundson, Sarah E. Bergemann and Matteo M. Garbelotto developed the protocols, conducted the fieldwork and compiled the data; Todd W. Osmundson and Rikke Rasmussen developed the laboratory protocols and conducted the labwork; Todd W. Osmundson analysed the data; and Todd W. Osmundson and Matteo M. Garbelotto led the writing with assistance from Sarah E. Bergemann.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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