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Gall-forming protistan parasites infect southern bull kelp across the Southern Ocean, with prevalence increasing to the south

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ABSTRACT: Protistan pathogens can have devastating effects on marine plants, yet the processes that affect their distributions and infection intensities are poorly understood. Species within the brown algal genus *Durvillaea* are major ecosystem engineers throughout the sub-Antarctic and cold-temperate Southern Hemisphere, and a newly described genus of protistan parasite, *Maullinia*, was recently found infecting *D. antarctica* in Chile. We set out to address 3 key questions. (1) Is there evidence for trans-oceanic dispersal of *Maullinia*? (2) Does *Maullinia* infect other *Durvillaea* species? (3) Does infection prevalence vary throughout the hosts' ranges? We sampled *Maullinia* on *Durvillaea* populations along coasts in Chile (*D. antarctica*, from 32° to 42° S: 8 sites), Australia (*D. potatorum* and *D. amatheiae*, from 36° to 38° S: 5 sites) and sub-Antarctic Marion Island (46° 53′ 47″ S, 37° 43′ 32″ E). We used a genetic marker (18S rRNA) to verify the presence of *Maullinia* on *Durvillaea* at all sites and visual surveys of *Maullinia* galls to assess infection prevalence in Chile and Australia. We confirm that *Maullinia* infects Australian *Durvillaea* species, but our results indicate that each host species is parasitised by a different *Maullinia* lineage. *Maullinia* infection prevalence increased with latitude. Long- and short-distance dispersal events are inferred to have occurred based on genetic patterns. We conclude that *Maullinia* protists are broadly distributed and affect multiple host species, including at least 3 *Durvillaea* species (2 in Australia, and 1 in both Chile and Marion Island), and that environmental factors influence host susceptibility to infection.

KEY WORDS: Pathogen · Macroalgae · Host-specificity · Intertidal · Dispersal · *Durvillaea* · *Maullinia*

INTRODUCTION

The infection intensities and geographic ranges of many parasitic organisms are expected to increase under forecast scenarios of environmental change (Eggert et al. 2010, Gleason et al. 2013). These increases may prove devastating where infections affect keystone species at low trophic levels, even potentially leading to ecosystem collapse (see Mouritsen et al. 2005, Collinge et al. 2008). As such, understanding the factors shaping parasite biogeography and patterns of infection is an important part of predicting how ecosystems will respond to global environmental change.

Large algae (e.g. kelps) are critical components of many shallow marine ecosystems as both food and

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habitat for diverse organisms (Jones et al. 1997). The susceptibility of marine algae to pathogen infection can be influenced by factors including population density, tissue damage and life stage (Andrews 1976). Analyses of kelp pathosystems have also revealed evidence for a molecular basis to host resistance, whereby some species or genetic lineages of marine algae are more prone to infection than others (Carius et al. 2001, Gachon et al. 2009, 2010). Marine pathogen and parasite infections may increase along environmental gradients (e.g. depth, longitude and latitude), and their distributions and intensities can indicate dispersal routes and barriers, and biogeographical regions (Rohde 2002).

Durvillaea is a brown algal genus comprising large, keystone species inhabiting rocky intertidal and shallow subtidal shores in the Southern Hemisphere. In Australia, 2 *Durvillaea* species occur: *D. amatheiae* is found along the south-eastern coasts of New South Wales, Victoria and Tasmania, and *D. potatorum* along the coasts of eastern South Australia, western Victoria and Tasmania (Fraser et al. 2009, Weber et al. 2017). *D. antarctica* is not found in Australia but has a broad, circumpolar distribution that includes the sub-Antarctic islands, New Zealand and much of the coast of Chile (Fraser et al. 2010). The species is highly buoyant and dispersive, and has previously been inferred to have transported an endophytic algal parasite, *Herpodiscus durvillaeae,* across the Pacific Ocean (Fraser & Waters 2013).

Maullinia is a recently identified genus of phytomyxean parasite that can infect several brown algal genera including *Macrocystis, Ectocarpus* and *Acinetospora* (Maier et al. 2000, Goecke et al. 2012). The microscopic organisms produce motile zoospores which disperse and infect new hosts, causing gall-like hypertrophies of host tissue (Neuhauser et al. 2011). The production of resting spores may enable the parasite to disperse long distances and survive adverse conditions (Neuhauser et al. 2011). While some research has described phytomyxids, including *Maullinia*, as parasites that infect hosts without directly leading to mortality (Neuhauser et al. 2014), on flexible species such as *D. antarctica* the formation of galls has been suggested to reduce the host's ability to survive in high-energy wave environments (Eggert et al. 2010). In laboratory cultures, *Maullinia ectocarpii* was also found to heavily infect the gametophytes of algal species such as *Macrocystis pyrifera* (Maier et al. 2000), which could reduce the reproductive potential of its host. The host range, life cycle and infection pathway of *M. ectocarpii* are relatively well characterised, but the factors shaping its virulence in natural populations are not. The only *Maullinia* recorded on *Durvillaea* appears likely to be a newly recognised species ('*Maullinia* sp.' in Goecke et al. 2012; now *M. braseltonii*: Murúa et al. 2017). Although Australian *Durvillaea* populations have not yet been confirmed to host *Maullinia*, galls resembling those of the parasite were observed at Sorrento in southern Australia several decades ago (Jahnke 1978), and samples of *M. ectocarpii* were confirmed by Maier et al. (2000) on local populations of *Ectocarpus siliculosus*.

D. antarctica is able to raft long distances and has even been known to wash up on Australian shores, 1000s of km from the nearest source population (Moore & Cribb 1952). We hypothesised that *Maullinia* would thus have a broad distribution and that distant populations — possibly even those affecting other host species — would show evidence of connectivity. We further hypothesised that infection prevalence would vary along latitudinal gradients, with the greatest infection prevalence toward the northern limits of the hosts' ranges, where kelps are most physiologically stressed (e.g. Tala et al. 2016). We tested these hypotheses using ecological and genetic surveys of infected *Durvillaea* along the southern Australian and southern-central Chilean coasts.

MATERIALS AND METHODS

Sample collection

Sampling was conducted in Australia, Chile and sub-Antarctic Marion Island (Fig. 1). Australian sampling and surveys were carried out at 5 sites: Tathra, City Rock, Mallacoota, Cape Conran and Cape Schanck. In Chile, intertidal sampling was carried out at 8 sites: Pichicuy, Quintay, Pichilemu, Curanipe, Queule, Chaihuin, Hua Huar and Pumillahue. Infected tissue from Marion Island was collected opportunistically from 3 sites: Macaroni Bay, Trypot Beach and Rockhopper Bay. All sampling occurred in late spring and summer 2015/16. For visual quantification of infections in the field (Australia and Chile only), morphological identification of galls was based on descriptions in Aguilera et al. (1988), Jahnke (1978) and Goecke et al. (2012). Infection prevalence was quantified along a series of 10 m transects. Numbers of kelp transect^{-1} varied within and between sites, leading to differing sample sizes at each location (Table 1). Three transects site^{-1} were surveyed in Australia, and 3 to 5 transects site^{-1} were surveyed in Chile, depending on the extent and accessibility of

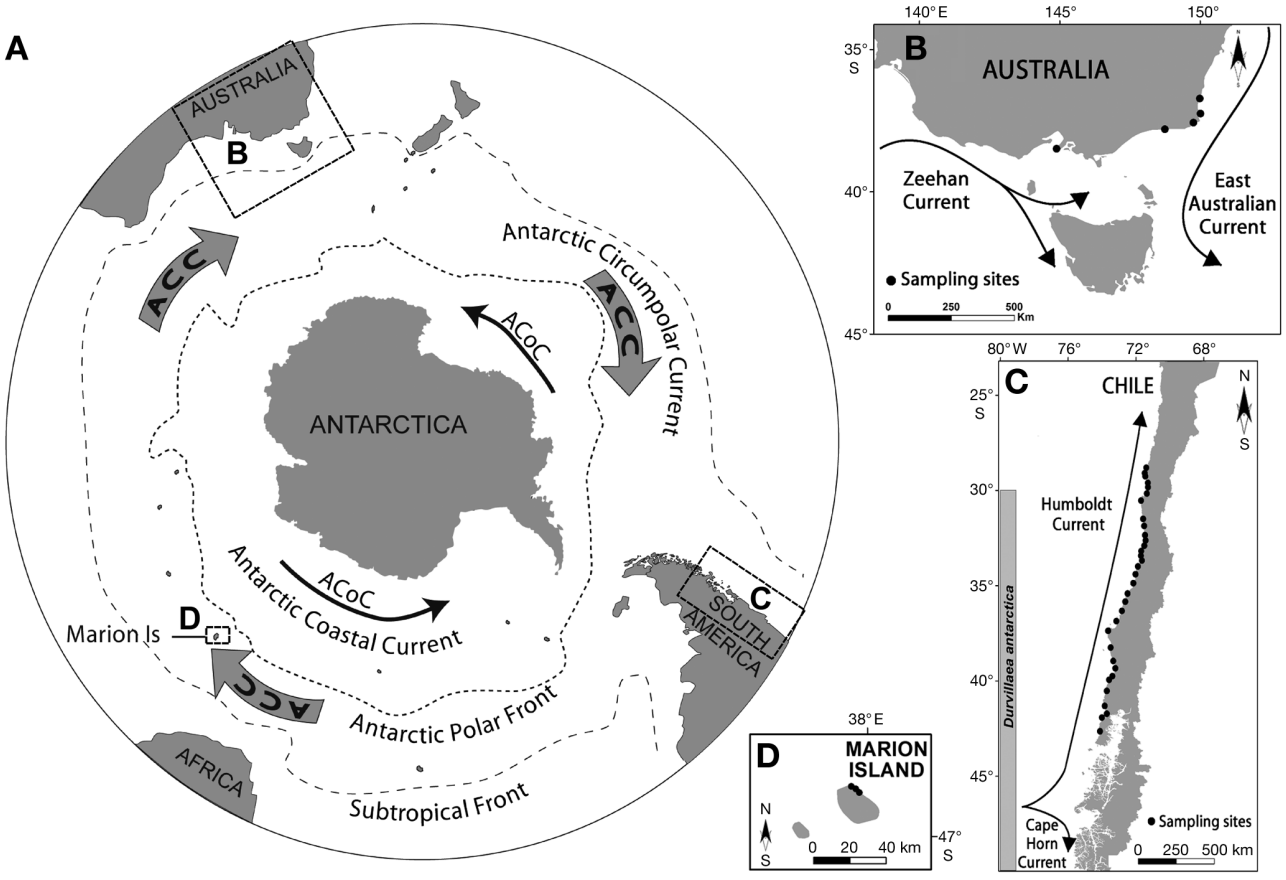

Fig. 1. (A) Global projection of Antarctica and sub-Antarctic areas, showing the main marine currents and the location of sub-Antarctic Marion Island. The study areas on (B) the Australian coast, (C) the Chilean coast and (D) Marion Island and their local marine currents are also indicated. Black dots indicate sampling and survey sites. The geographic distribution of *Durvillaea antarctica* along the Chilean coast is indicated

Table 1. Sampling and sequencing information for intertidal sites visited

Country	Site	Individuals surveyed	Samples collected	Samples amplified	Samples sequenced	Number of unique sequences
Australia	Tathra	63	8	4	4	2
	City Rock	63	2	2	2	1
	Mallacoota	79	9	9	9	1
	Cape Conran	64	7	4	3	1
	Cape Schanck	125	12	7	4	2
Chile	Pichicuy	112	4	1	1	1
	Quintay	133	17	8	4	2
	Pichilemu	158	10	4	4	4
	Curanipe	76	4	3	2	1
	Queule	164	2	2	2	2
	Chaihuin	144	24	14	5	3
	Hua Huar	317	24	13	7	4
	Pumillahue	145	32	18	7	4

kelp beds. Transect lines were laid on rocky platforms partially or completely emergent at low tide. As *Durvillaea* species often inhabit fragmented rocky platforms composed of a number of reefs separated by water, it was not always possible to lay transects parallel to one another. Rather, they were laid on the edges of such platforms, separated by at least 2 m if on the same platform, where kelp are likely to experience similar exposure to environmental factors between sites. The number of healthy and diseased

kelp were counted on each transect, with only those plants whose stipe intercepted a transect being considered. To confirm the presence of *Maullinia* at each site (as other organisms are known to produce gall-like structures on *D. antarctica* in Chile; Saavedra 2011), and to enable phylogeographic analyses, tissue samples for genetic analysis were collected from galls from every third infected kelp. Samples were placed in a 50 ml Eppendorf tube filled with 70% ethanol, with the alcohol replaced once after several hours. After 24–48 h, samples were either air-dried on clean paper towel or in an oven at 60°C for several hours, then stored with silica gel.

In order to determine the rafting potential of *Maullinia*, we examined recently stranded specimens of *D. antarctica* on 33 sandy and boulder beaches along the Chilean coast (28–42° S) during austral winters and summers of 2 consecutive years (2014–2015) across the benthic and pelagic geographical range of the continental clade of *D. antarctica* (Fig. 1C). Within each beach, recently stranded individuals of *D. antarctica* were collected on foot following the coastline along the most recent flotsam lines (i.e. the last 2–3 high tides). For each complete kelp individual (including holdfast and fronds), the presence of galls of *Maullinia* was determined, and the frequency of infected specimens was calculated. The details of the sampling protocols used for collection and measurement of stranded specimens of *D. antarctica* are described in López et al. (2017a).

Genetic analyses

Small (<2 mm) pieces of infected, dried kelp tissue were excised using a scalpel sterilised with alcohol and flame, and DNA was extracted following the standard Chelex© protocol (Walsh et al. 1991). Extractions were diluted 1:100 in MilliQ water to reduce the possibility of alginates blocking PCR processes (see Wilson et al. 2016). PCR amplification was conducted in a 20 µl solution, comprising 12.5 µl MilliQ water, 0.2 µl each of forward and reverse 10 mM primers, 4 µl of 1 mM dNTPS, 0.1 µl of PerfectTaq polymerase, 2 µl of PerfectTaq buffer (5Prime) and 1 µl of diluted DNA extraction. *Maullinia*-specific primers that would not amplify host DNA were used: Mau2F (5′ ACGGGTACGAGGGACGTGGG) and Mau9R (5′ TGCATCAGTGTAGCGAGCGT) (Goecke et al. 2012). These primers amplified part of the 18S nuclear ribosomal gene, which is used for taxonomic identifications, descriptions of variation between populations of differing geographical origin and analyses of protist phylogenetic relationships (Pawlowski et al. 2012, Hadziavdic et al. 2014, Wang et al. 2014). PCRs were run in an Eppendorf Mastercycler (Epgradient S) using the *Maullinia* Touchdown protocol of Goecke et al. (2012). PCR products were purified using a QIAQuick PCR Purification Kit (Qiagen) to be sent for sequencing at the University of Otago's Genetic Analysis Services (Otago, New Zealand), using an Applied Biosystems 3730xl capillary sequencer (Thermo Fisher Scientific).

Phylogenetic analyses

Sequences were aligned and trimmed, and ambiguities were corrected in Geneious 6.1.8 (Kearse et al. 2012). Two published sequences, i.e. 1 from *M. ectocarpii* on *Ectocarpus* alga in Chile and 1 from *Maullinia* sp. (now *M. braseltonii*) on *D. antarctica* in Chile, were included in the alignment alongside related outgroup sequences from GenBank (*Phagomyxa odontellae* AF310904; *Spongospora subterranea* AF310899). Haplotype networks were created using TCS 1.21 (Clement et al. 2000).

The most appropriate model of DNA evolution was determined using jModeltest2 (Darriba et al. 2012) according to Akaike's information criterion adjusted for small sample sizes (AICc). Model parameters were: TrN+G (gamma shape 0.4240, proportion inv sites 0). The maximum likelihood (ML) phylogenetic tree was constructed in PhyML 3.0 (Guindon et al. 2010) with support for nodes determined using 1000 bootstrap iterations. The Bayesian phylogenetic tree was constructed in MrBayes 3.2.0 (Ronquist & Huelsenbeck 2003) with Markov chain Monte Carlo (MCMC) searches of 4 chains and burn-in of 10 000 trees. Trees were sampled every 100 generations for a total of 5 000 000 generations.

Ecological analyses

Testing for latitudinal effects in infection prevalence using a binomial model showed over-dispersion, so a negative binomial distribution with a logarithm link function (Lawless 1987) was used instead. Modelling was based on the number of infected kelps transect^{-1} by latitude, offset by the natural log of total kelp due to variability in total numbers of kelp transect^{-1}. Data from Chile and Australia were tested separately. Likewise, to verify the relationship of the frequency of stranded individuals of *D. antarctica* with *M. braseltonii* and latitude, analyses were per-

formed using generalised linear models (GLMs) for the total and for each field survey separately, offset by the natural log of the total number of sampled kelps. The model used was:

model =
glm.nb (count of infected individuals ~ latitude +
offset (log(total individuals per beach)), data = data).

All statistical tests were done with R 3.4., using the 'MASS' and 'visreg' packages (R Development Core Team 2017).

RESULTS

Infections of *Maullinia braseltonii* in Chile and Marion Island were similar in shape, size and colour to those described by Goecke et al. (2012) and Aguilera et al. (1988); gall diameters ranged from 2 to 15 cm. Infections in Australia were smaller than their Chilean counterparts, ranging in diameter from 1 to 5 cm. Infected tissue had a warty appearance, raised and tougher than surrounding areas and often showing concentric rings of discolouration.

Sequencing confirmed *Maullinia* infections on *Durvillaea* at all intertidal sites visited in Australia (Fig. 2) and Chile (Fig. 3), as well as from the 3 Mar-

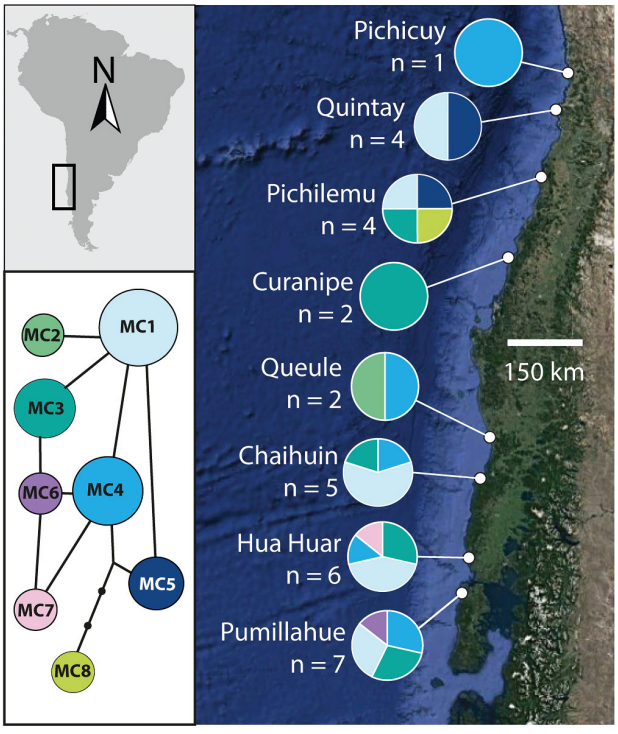

Fig. 3. Sampling locations in Chile. The proportion of *Maullinia* haplotypes at each sampled site is shown. Haplotype network at left shows the relative prevalence of *Maullinia* haplotypes (circle size), with black dots representing hypothetical undetected haplotypes

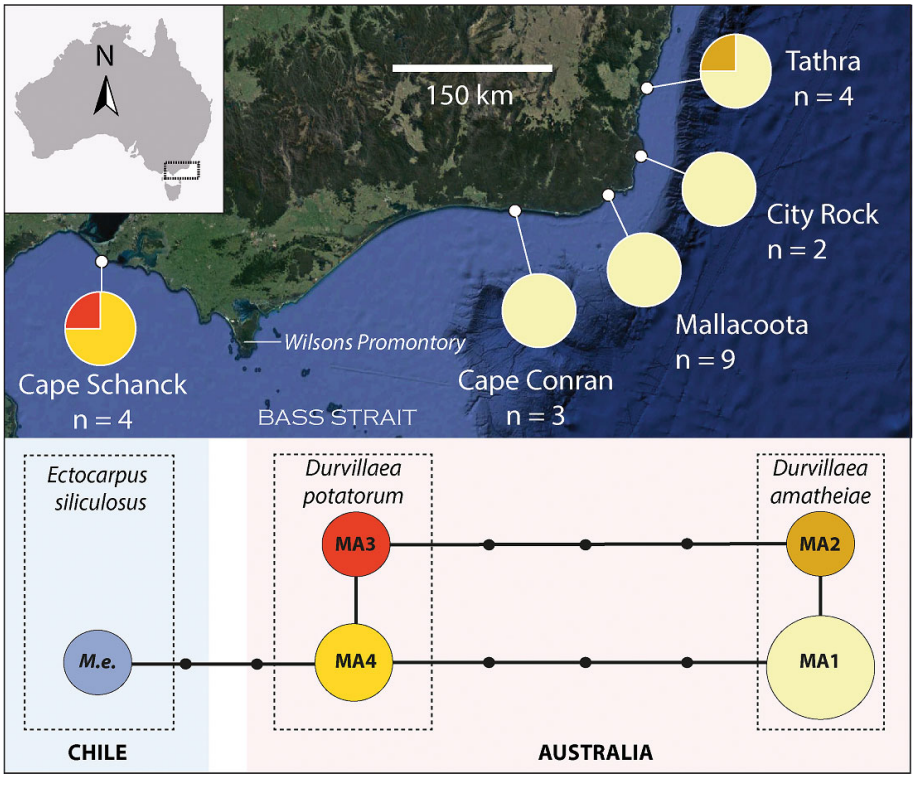

Fig. 2. Sampling locations in Australia. The proportion of *Maullinia* haplotypes at each sampled site is shown. Haplotype network below shows host species (dashed boxes), and relative prevalence of *Maullinia* haplotypes (circle size); black dots represent hypothetical undetected haplotypes. Previously published sequences of *M. ectocarpii* (*M.e.*) from *Ectocarpus siliculosus* in Chile from Maier et al. (2000) were closely related to Australian *Maullinia* samples from *Durvillaea*

ion Island sites. Successful amplification of tissue collected from galls was not always achieved (~60% success in Chile, ~70% in Australia, ~50% Marion Island; Table 1), but some amplification failure is common in PCR, particularly for algal extractions that include inhibitors (Wilson et al. 2016). Furthermore, the primers we used were developed for *M. ectocarpii*, and although they also amplify *M. braseltonii,* there might be other *Maullinia* or related phytomyxean species affecting these kelp populations that our primers could not detect. In an attempt to improve our capacity to detect other species and resolve fine-scale structure for *Maullinia*, we designed and assayed a range of other primers for alternative markers based on available sequences from other genera (though few such sequences were available), but none performed well and all were therefore discarded (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m583p095_supp.pdf). New sequences recently published for *M. ectocarpii* (see Schwelm et al. 2016) may assist in future primer development. Based on the morphological similarity of amplified and non-amplified infections, and the confirmation of *Maullinia* at all sites, we considered visual field identifications adequate for analyses of infection prevalence. Each unique sequence generated during this research was deposited in GenBank (accessions MF872442–MF872453).

Phylogenetic analyses

We obtained *Maullinia* sequences from 54 samples from 8 intertidal sites in Chile and 5 intertidal sites in Australia, with 12 distinct sequences detected (8 sequences from 32 samples in Chile, and 4 sequences from 22 samples in Australia). Ten samples were sequenced from Marion Island. The 18S ML and Bayesian phylogenetic trees showed strong consistency in overall topology and branch support (Fig. 4). No sequences were shared between Australia and Chile, although the published *M. ectocarpii* sequence of Maier et al. (2000) from Chile was closely related to Australian *Maullinia* (Fig. 2). All sequences from Marion Island were identical to sequence MC4 from *M. braseltonii*, which is widespread in Chile (Figs. 3 & 4). There was up to 0.3% divergence (uncorrected P distance) within each country, and 2.4–2.6% between the 2 countries.

Australian sequences appeared to show separation into eastern and western groups, with sequence MA1 being present at all eastern sites and MA4 being most abundant at Cape Schanck (Fig. 2). No seuences were shared between eastern and western areas, corresponding to the geographical distributions of different host species described by Weber et al. (2017); however, a greater number of samples would be needed to confirm if this trend was not simply a result of the relatively small number of pathogen sequences.

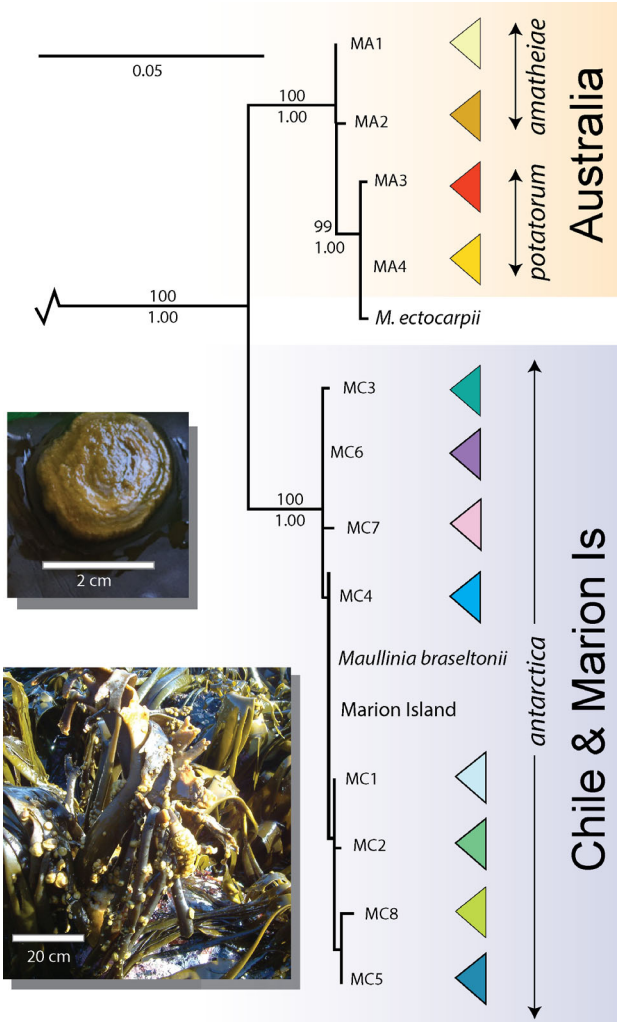

Fig. 4. Maximum likelihood phylogeny of *Maullinia* samples collected in Australia, Chile and Marion Island. Haplotype colours correspond to those in Figs. 2 & 3. Bootstrap values >75% are shown above major branches and Bayesian posterior probabilities below. Outgroups have been trimmed for clarity. Two previously published sequences of *Maullinia* (*M. ectocarpii* from an *Ectocarpus* host in Chile, Maier et al. 2000; and *M. braseltonii* from a *Durvillaea* host in Chile, Goecke et al. 2012) were included. To the right of the tree, vertical text indicates host *Durvillaea* species and geographic region. Top inset photo: relatively small *Maullinia* gall on *D. antarctica* tissue from Quintay, Chile. Bottom inset photo: galls on *D. antarctica* at Marion Island in 2007 (observed a decade prior to this genetic research)

In Chile, the 3 most abundant sequences (MC1, MC3 and MC4) were found across large parts of the host's range, from the southernmost to northern sampled regions. Sequence MC4 exactly matched the published sequence of *Maullinia* sp. (Goecke et al. 2012; now *M. braseltonii*: Murúa et al. 2017) and sequences from Marion Island (Fig. 4). There was greater overall diversity in the Chilean than the Australian samples, but there was also a strong positive relationship between number of unique sequences detected and number of samples sequenced at sites.

Latitudinal effects

For Australia, GLMs supported latitude as being a significant predictor of infection prevalence ($p = 0.015$), with numbers of infected kelp increasing towards higher latitudes (Fig. 5). For Chile, infection prevalence also appeared to increase with latitude, but the relationship was not significant. Data from Quintay, however, had a marked influence on the strength of the relationship, and when Quintay data were excluded, latitude was a strong predictor ($p < 0.001$) of infection prevalence.

In the case of recently stranded individuals of *D. antarctica* from continental Chile, a positive and significant relationship between the frequency of individuals infected with *M. braseltonii* and latitude was observed for the total of all surveys ($p < 0.001$), as well as for each individual survey (winter 2014, $p = 0.002$; summer 2014/2015, $p < 0.001$; winter 2015, $p < 0.001$; summer 2015/2016, $p = 0.005$; Fig. 6).

DISCUSSION

Our results confirm, for the first time, infections of *Maullinia ectocarpii* on populations of *Durvillaea* (*D. potatorum* and *D. amatheiae*) in Australia. Different parasite lineages were found associated with each algal host species, suggesting some host specificity. Infections of *M. braseltonii* were found on *D. antarctica* throughout central Chile and at Marion Island more than 8000 km away, indicating that recent long-distance dispersal of the parasite has occurred, presumably by dispersal with its buoyant algal host.

Our hypothesis that infections would be greatest toward the northern range limits of the hosts was not supported by our results. Environmental gradients do appear to influence infection levels, however, as we observed a pattern of increased parasite prevalence with increasing latitude.

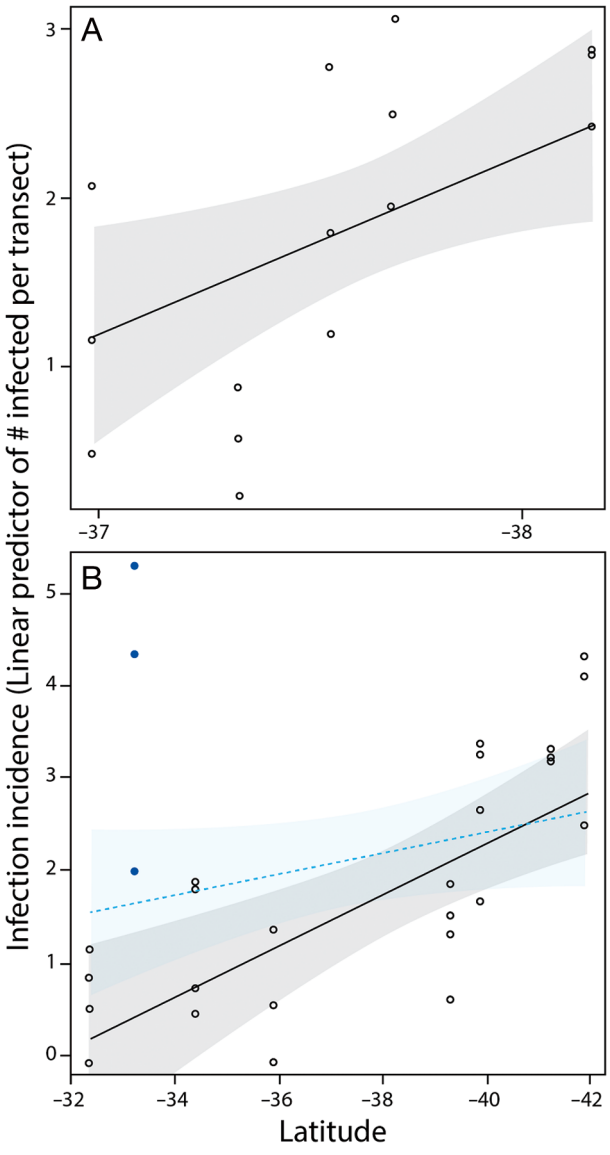

Fig. 5. Relationship of latitude to infection prevalence (number of infected kelp transect^{-1}, offset by the natural log of total kelp) in (A) Australia and (B) Chile. Trendlines are presented with 95% confidence intervals (shaded). For Chile, infections recorded from transects at a single site, Quintay (indicated by filled blue dots), had a strong influence on the trend (dashed blue line). When Quintay data were removed from analyses, the general trend of decreasing infection with decreasing latitude was strongly emphasised (black line)

Genetic trends for *Maullinia*

The 2 deeply divergent (2.4–2.6%, Fig. 4) clades appear to represent distinct species: *M. ectocarpii* in Australia and *M. braseltonii* in Chile and Marion Island.

Within-country genetic patterns, with identical sequences detected at sites separated by hundreds of

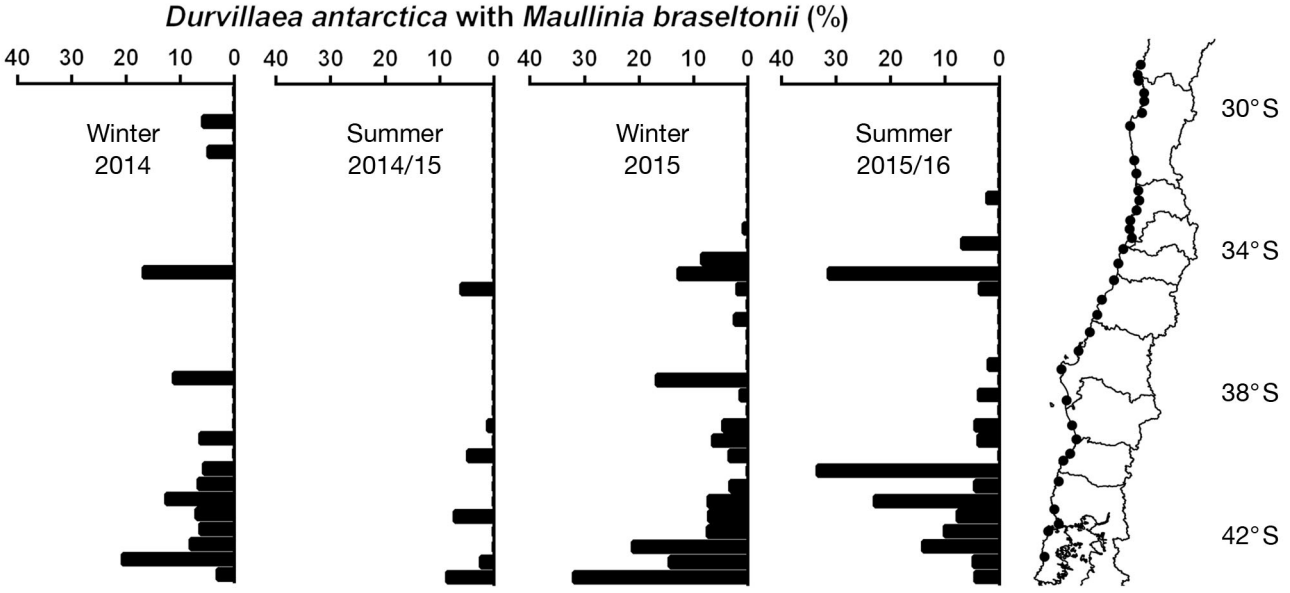

Fig. 6. Percentages of stranded individuals of *Durvillaea antarctica* with *Maullinia braseltonii* found in surveys on sandy and boulder beaches from 28° to 42° S along the Chilean coast, during winters and summers of 2014–2016

kilometres (Figs. 2 & 3), indicate that coastal dispersal of this parasite probably occurs, perhaps through both rafting of infected tissue and larval/zoosporic stages. The Chilean coast is affected by a number of oceanic current systems, in particular the Humboldt Current, which transports cool water towards the equator, and the Peru Countercurrent, which transports warmer subtropical water to the south (Silva et al. 2009). These currents affect a range of organisms along the Chilean coast (Peters & Breeman 1993), including kelp rafts (Rothäusler et al. 2011), with similar currents affecting organismal larval stages (Gaylord & Gaines 2000). As such, the lack of strong phylogeographic structure in Chile across more than 1000 km of coastline (Fig. 3) may be attributable to passive dispersal mechanisms (see also Haye et al. 2012). Likewise, a recent study on the red seaweed *Gelidium lingulatum*, which is frequently found growing in *D. antarctica* holdfasts, showed that some haplotypes were common in southern as well in northern localities (López et al. 2017b).

In contrast, coastal current systems in south-eastern Australia are likely to maintain the separation of pathogen populations to the east and west of southern Victoria. Indeed, our results show considerable (0.3%, 18S) divergence among eastern and western populations of *M. ectocarpii* in Australia. The eastern and western lineages appear to align with the disjunct distributions of hosts *D. potatorum* and *D. amatheiae* (Fig. 2), suggesting there may be host-specificity and/or other processes maintaining biogeographic structure in both hosts and pathogens. Although our sample sizes and sampling range were too limited in this study to allow detailed analysis of such fine-scale structure, a biogeographic break across Wilson's Promontory in southern Victoria would be consistent with patterns observed in a wide range of other taxa (O'Hara & Poore 2000, Waters 2008), including the host genus *Durvillaea* (Fraser et al. 2009, Weber et al. 2017). While this biogeographic pattern is broadly thought to be a historical outcome of vicariant processes related to the Bassian Isthmus land bridge during the Last Glacial Maximum (Fraser et al. 2009), it is thought to have been maintained through modern oceanographic systems and through density-dependent ecological processes (Waters et al. 2005, 2013).

Identical *M. braseltonii* sequences were obtained from Chile and from Marion Island (more than 8000 km away), suggesting that long-distance dispersal of the parasite has recently occurred. Furthermore, although *Maullinia* collected from *Durvillaea* in Australia and in Chile formed distinct geographic clades, the similarity of *M. ectocarpii* sequences from *Durvillaea* in Australia, and from *Ectocarpus* in Chile, also supports long-distance dispersal of *Maullinia*. The Antarctic Circumpolar Current connects the sub-Antarctic islands and the major continents of the Southern Hemisphere (Fig. 1), and detached macroalgae floating eastward in the path of this ocean highway have been inferred to have transported a range of rafting organisms (including another algal

parasite, *Herpodiscus durvillaeae*: Fraser & Waters 2013) among distant landmasses (e.g. Helmuth et al. 1994, Nikula et al. 2010, 2013, Fraser et al. 2011, Cumming et al. 2014). Interestingly, the strong link between *M. braseltonii* from both central Chile and the sub-Antarctic (Marion Island) is not reflected in the kelp genetics, as these 2 areas have distinct clades of *D. antarctica* (Fraser et al. 2010). That we nonetheless here infer movement of the parasite with its buoyant host between the sub-Antarctic and central Chile supports suggestions that phylogeographic structure in *D. antarctica* results from density-dependent processes that maintain structure even with frequent dispersal (Waters et al. 2013).

Despite some evidence for host-specificity (with different *Maullinia* lineages detected on each *Durvillaea* species), our data suggest this parasite readily shifts hosts, much like other phytomyxids (Neuhauser et al. 2014). Indeed, infections of *M. ectocarpii* in Australia have previously been recorded on the alga *E. siliculosus* (Maier et al. 2000). Host shifting may allow parasites to survive for long time periods in environments with ephemeral hosts or adverse environmental conditions (Neuhauser et al. 2014), as infections on one species can supplement those on another.

Latitudinal effects on infection prevalence

Our hypothesis that infection prevalence would increase towards the hosts' northern latitudinal range limits due to increasing physiological stress was not supported. However, the infection prevalence was instead found to increase towards higher latitudes, or the southern range limit of the host (Fig. 5). For Chile, this effect was strongest when data from Quintay, where prevalence was remarkably high, were removed. Quintay might have unusually high infection levels due to an environmental stressor, such as pollution; pollution has increasingly been implicated in outbreaks of marine disease (Li et al. 2010, Saavedra 2011), including for algae (Buschmann et al. 2014), and Quintay was the closest site to Valparaiso, the most densely populated coastal city within our sampled range in Chile. The observed trend in multi-year samplings of stranded individuals of *D. antarctica* confirms the pattern that *Maullinia* infection rates tend to increase with latitude, although there may be some temporal variability (Fig. 6).

Our observations of increased *Maullinia* infections at higher latitudes are consistent with the suggestion by Aguilera et al. (1988) that infections within this pathosystem might intensify towards the south in Chile. Despite our low sample numbers and limited sampling range, geographic variability in infection prevalence suggests parasite susceptibility to local conditions (Poulin et al. 2012), and several possibilities could explain the observed patterns, as follows.

Population densities

Pathogen transmission can increase with higher densities and greater connectivity between hosts (Poulin et al. 2012, Izhar & Ben-Ami 2015). *Durvillaea* species in Australia are known to transition from being a relatively rare intertidal element at Tathra (the northern range limit) to a dominant habitat-forming species on rocky intertidal platforms in Tasmania (southern part of the species' range) (Millar 2007). *D. antarctica* in Chile experiences competition from other algal species including *Lessonia* spp. towards its northern latitudinal range limit, reducing its density and prevalence, but is more abundant toward the south (Santelices et al. 1980), where abundant rafting populations of large adult plants have also been observed (López et al. 2017a). In this research, we sampled southward from the northern range limits of *Durvillaea* in Chile and Australia, such that more southern populations also had higher host densities, which could have led to increased pathogen transmission, and thus infection levels, at high latitudes.

Temperature effects

Although we hypothesised that hosts would prove most susceptible to infection towards their range limits due to higher physiological stress, these hosts might actually have greater resistance due to their ability to survive in marginal conditions. Although ecotype effects have not been demonstrated previously for *Durvillaea*, they have been inferred for other algal species including the kelp *Undaria pinnatifida* (Gao et al. 2013), for which populations varied in temperature resistance depending on the latitude from which samples were collected. Such ecotypes often develop due to strong environmental patterns, the most pervasive of which are temperature gradients in the marine intertidal realm (Cruces et al. 2013). Along the Chilean coast, mean surface water temperatures range from around 18°C in the north to 6–8°C in the far south (Locarnini et al. 2013), and within the region that we sampled, temperatures

range from around 14°C (winter) to 19°C (summer) in the north to 12°C (winter) to 17°C (summer) in the south (Tala et al. 2016). Such latitudinal temperature changes can present different physiological challenges for intertidal taxa (Tala et al. 2016), and thus might also lead to geographic genetic variation in the host kelp.

Our detection of identical sequences of *M. braseltonii* in both central Chile and in the sub-Antarctic suggests that at least some lineages of the parasite can survive at a range of temperatures, but their virulence might increase, and/or host resistance might decrease, in cooler waters. The relationship between latitude and water temperature is well-recognised along a range of coastlines (Tuya et al. 2012), with both Australia and Chile showing a decrease in mean water temperature towards higher latitudes. Temperature effects have been shown in a range of terrestrial and marine pathosystems (Case et al. 2011), acting on both host and parasite. For *Durvillaea* species, increasing temperature has been associated with increased physiological stress (Cruces et al. 2013), and while such effects remain unquantified for *Maullinia* species (Neuhauser et al. 2011), the parasite might be similarly affected. For some marine species, larvae have been found to persist for shorter periods at sea when exposed to higher temperatures (Bradbury et al. 2008, Cowen & Sponaugle 2009), limiting their ability to disperse and, in the case of parasites, infect new hosts. As such, at cooler temperatures *Maullinia* might be more virulent and have improved dispersal and survival of zoospores, as also suggested by the tendency for slightly higher infestation rates during winter months (see Fig. 6). In the face of warming sea temperatures, this may thus counteract the virulence of the parasite, pushing its range south to cooler areas.

CONCLUSIONS

Understanding the factors underpinning outbreaks of marine disease is essential for appropriate monitoring and management of marine ecosystems into the future. Our research emphasises the value of combining molecular and ecological approaches in order to describe pathosystem dynamics, particularly for micro-organisms whose visual identification can prove challenging.

Previous research had only shown *M. braseltonii* infecting *Durvillaea* hosts in central Chile. We have demonstrated that the parasitic genus infects multiple host species within the genus *Durvillaea* in Chile, Marion Island and Australia. Further, these populations are probably connected via dispersal of the parasite with floating macroalgal hosts. The presence of this parasite on brown algal hosts across the Pacific and Indian Oceans would suggest a high likelihood of the pathosystem extending to other locations. Variable prevalence over latitudinal ranges suggests that environmental or ecological factors shape infection patterns, and these data may assist in understanding how the pathogen will respond to a changing climate. A broader study that assesses the phylogeography of *Maullinia* from other landmasses with *D. antarctica* populations (such as New Zealand and other sub-Antarctic islands) would help to provide a clearer picture of the dispersal potential of this pathogen. Molecular analyses using a greater number of markers, and modelling of a wide range of environmental factors, could also provide greater insights into how *Maullinia* spp. infections will change under future climate scenarios.

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