Research article

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Culturable fungi associated with the marine shallow-water hydrothermal vent crab *Xenograpsus testudinatus* at Kueishan Island, Taiwan

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Abstract: Reports on fungi occurring on marine crabs have been mostly related to those causing infections/diseases. To better understand the potential role(s) of fungi associated with marine crabs, this study investigated the culturable diversity of fungi on carapace of the marine shallow-water hydrothermal vent crab *Xenograpsus testudinatus* collected at Kueishan Island, Taiwan. By sequencing the internal transcribed spacer regions (ITS), 18S and 28S of the rDNA for identification, 12 species of fungi were isolated from 46 individuals of *X. testudinatus*: *Aspergillus penicillioides*, *Aspergillus versicolor*, *Candida parapsilosis*, *Cladosporium cladosporioides*, *Mycosphaerella* sp., *Parengyodontium album*, *Penicillium citrinum*, *Penicillium paxili*, *Stachylium bicolor*, *Zasmidium* sp. (Ascomycota), *Cystobasidium calypotogenae* and *Earliella scabrosa* (Basidiomycota). With additional data from other published reports, a total of 26 species of fungi (23 Ascomycota, three Basidiomycota) have been recorded from *X. testudinatus*. *Aspergillus* is the most speciose genus on the crab, followed by *Penicillium* and *Candida*. All but one species (*Xylaria arbuscula*) had been previously isolated from substrates in the marine environment, although many are typical terrestrial taxa. None of the

recorded fungi on *X. testudinatus* are reported pathogens of crabs, but some have caused diseases of other marine animals. Whether the crab *X. testudinatus* is a vehicle of marine fungal diseases requires further study.

Keywords: Arthropoda; asexual fungi; crustacea; Eurotiales; marine fungi.

1 Introduction

Kueishan Island (also known as Turtle Island with reference to its shape) is an active volcano situated at the northeastern end of the main Taiwan island. At one end of the island, a shallow-water hydrothermal vent system is present with roughly 50 hydrothermal vents, where hydrothermal fluids (between 48 and 116 °C) and volcanic gases (carbon dioxide and hydrogen sulfide) are constantly being emitted (Chen et al. 2005). The hydrothermal vent system of Kueishan Island is considered to be a mixed photosynthetic-chemosynthetic ecosystem in contrast to the obligate chemoautotrophic primary production in deep-sea hydrothermal vents (Chang et al. 2018).

With the possibly extreme environmental conditions (high temperature, low pH), macro- and microorganisms have been reported at/near the hydrothermal vent system of Kueishan Island, including fishes, crabs, mussels, sea anemones, snails, sipunculid worms, algae and zooplankton (Chen et al. 2005; Kâ and Hwang 2011; von Corsel 2008). In particular, the crab *Xenograpsus testudinatus* is the dominant animal species at the vents with a high population density (Tseng et al. 2020). The crab mainly consumes zooplankton killed by the plumes from the vents which eventually settle to the seafloor (Jeng et al. 2004). Algae, fishes, bivalves and anthozoans were also reported to be possible food sources for the crab (Ho et al. 2015), as well as vent particulate organic matter (Chang et al. 2018). *X. testudinatus* occupies the highest

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trophic level in the hydrothermal ecosystem of Kueishan Island (Wang et al. 2014).

Concerning microorganisms, the ϵ -Proteobacteria including the genera *Sulfurovum* and *Sulfurimonas* dominated the bacterial community in sediment samples collected near the hydrothermal vents at Kueishan Island using metabarcoding analysis of the 16S rRNA gene sequences (Wang et al. 2016). In the same study, chemoautotrophic carbon fixation genes of the ϵ -Proteobacteria were also detected, suggestive of their possible role in the primary production at the hydrothermal vent area of Kueishan Island. For fungi, species of the genus *Aspergillus* were isolated near the hydrothermal vent area of Kueishan Island (Ding et al. 2016; Jiang et al. 2013; Pan et al. 2016), but these studies mainly explored the secondary metabolites of these fungi. Pang et al. (2019) was the first comprehensive study to look into the culturable and non-culturable diversities of fungi associated with sediment, seawater, and animal samples including the crab *X. testudinatus* at/near the hydrothermal vent area of Kueishan Island. The main results of Pang et al. (2019) were: (1) a higher diversity of fungi was observed in sediment than in seawater and animal samples, (2) Ascomycota was dominant over Basidiomycota, (3) metabarcoding analysis revealed a more diverse fungal community than the culture method, and (4) both marine and terrestrial Ascomycota were recovered.

Fungi of the marine environment have been mainly reported from plant-based substrata (Jones et al. 2019). Little is known on the mycota associated with marine animals, except those that cause diseases (Pang et al. 2021). For crustaceans, fungi are known to cause diseases of aquacultured animals, such as the black gill disease caused by *Fusarium* spp., leading to significant mortalities of the giant tiger prawn (*Penaeus monodon*; Khoa et al. 2004) and the Kuruma prawn (*Marsupenaeus japonicus*; Khoa and Hatai 2005, Khoa et al. 2005). For crabs, the best known is the lethargic crab disease of the mangrove land crab *Ucides cordatus* in Brazil, caused by *Exophiala cancerae* and *Fonsecaea brasiliensis* (Boeger et al. 2007; Vicente et al. 2012). The Microsporidia, instead of the Ascomycota, is the main fungal group causing diseases of marine crabs (Pang et al. 2021).

Pang et al. (2019) isolated 13 species of fungi from 10 individuals of *X. testudinatus*: *Hortaea werneckii*, *Aspergillus sydowii*, *Aspergillus terreus*, *Aspergillus unguis*, *Penicillium citreosulfuratum*, *Hypocreales* sp., *Parengyodontium album*, *Microascus brevicaulis*, *Candida oceani*, *Hypoxyton monticulosum*, *Peroneutypa scoparia*, *Xylaria* sp. and *Chondrostereum* sp. While some of these fungi are common in the marine environment as saprobes and symbionts, some are

potential pathogens of marine animals (Pang et al. 2021). In order to have a better understanding of the potential role(s) of fungi associated with *X. testudinatus*, a larger number of specimens of this species was collected in the hydrothermal vent area of Kueishan Island, and this study investigated the culturable diversity of fungi on the carapace of this crab based on molecular identification using three nuclear ribosomal genes (18S, ITS, 28S).

2 Materials and methods

2.1 Sample collection

The crab *X. testudinatus* Ng, Huang et Ho, 2000 was collected from the shallow hydrothermal vents of Kueishan Island, Yilan, Taiwan (Figure 1) on 13 August 2018 and 2 September 2018. The crabs were collected by a rectangular cage trap and brought up to the fishing boat by SCUBA divers. Crabs were immediately put into sterile ziplock plastic bags on board, placed in a cool box during transportation to the laboratory, and kept at 4 °C before isolation (time from collection to isolation < 12 h).

2.2 Fungal isolation

A total of 46 crabs were rinsed twice with 0.1% of Tween 80 in sterile natural seawater and once with sterile natural seawater. For each crab, a flame-sterilized spatula was used to scrape the surface of the carapace and the resulting biofilm was suspended in 1 ml of sterile natural seawater. Aliquots of the biofilm suspension (100 μ l) were spread-plated (triplicate plates) onto two media: (1) marine agar (MA) (Himedia, Dindhori, Nashik, India), and (2) glucose (Bioshop, Burlington, Canada)-yeast extract (Oxoid, Basingstoke, UK)-peptone (Oxoid, Basingstoke, UK) seawater agar (Bioshop, Burlington, Canada) (GYPS; 1% glucose, 1% yeast extract, 1% peptone in natural seawater) supplemented with 0.5 g l⁻¹ each of Penicillin G sodium salt (Bioshop, Burlington, Canada) and streptomycin sulfate (Bioshop, Burlington, Canada). The plates were incubated at 25 °C and checked for fungal growth for up to one month. Fungi grown on these plates were sub-cultured as pure cultures onto cornmeal seawater agar (CMAS) (Himedia, Dindhori, Nashik, India) made with natural seawater.

2.3 Molecular identification of fungi

Mycelia on CMAS were transferred to a mortar and pestle, and ground into fine powder in liquid nitrogen. Total genomic DNA was extracted using the DNeasy Plant DNA Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Three nuclear ribosomal genes (18S, ITS, 28S) were amplified by PCR using the primer sets NS1(5'-GTAGTCATATGCTTGTCTC-3')/NS4 (5'-CTTCCGTCATTCCTT-TAAG-3'), ITS4 (5'-TCCTCCGCTTATTGATATGC-3')/ITS5 (5'-GGAAGT-AAAAGTCGTAACAAGG-3') (White et al. 1990) and LROR (5'-ACCC-GCTGAACCTTAAGC-3')/LR6 (5'-CGCCAGTCTGCTTACC-3') (Vilgalys and Hester 1990), respectively. PCR reactions were performed in 25 μ l volumes containing 1 μ l genomic DNA, 0.2 μ M of each primer, 12.5 μ l Gran Turismo PreMix (Ten Giga BioTech, Taiwan). The amplification

Figure 1: (A) Location of Kueishan Island (box) at the northeastern end of the main Taiwan Island. (B) Kueishan Island showing the vent and sampling locations. (C) *Xenograpsus testudinatus*, the hydrothermal vent crab.

cycle consisted of an initial denaturation step of 95 °C for 2 min, followed by 35 cycles of (a) denaturation (95 °C for 30 s), (b) annealing (54 °C for 30 s) and (c) elongation (72 °C for 30 s) and a final 10 min elongation step at 72 °C. The PCR products were analyzed by agarose gel electrophoresis (Bioman Scientific CO, LTD, Taipei, Taiwan) and sent to Genomics (Taiwan) for sequencing with the same PCR primers. The sequences obtained were checked for ambiguity, assembled and submitted to the National Center for Biotechnology Information (NCBI) for a nucleotide BLAST search.

3 Results

A total of 23 colony morphotypes were isolated on the two media from the carapace of the crab *X. testudinatus* collected at the shallow hydrothermal vents of Kueishan Island, Yilan, Taiwan. These colony morphotypes were identified based on the nucleotide BLAST search of the 18S, ITS and 28S rDNA in NCBI with the highest sequence coverage and similarity (Table 1). Twelve different fungal species were identified: *Aspergillus penicillioides*, *Aspergillus versicolor*, *Candida parapsilosis*, *Cladosporium cladosporioides*, *Mycosphaerella* sp., *Parengyodontium album*, *Penicillium citrinum*, *Penicillium paxili*, *Stachylium bicolor*, *Zasmidium* sp. (Ascomycota), *Cystobasidium calyptogenae*

and *Earliella scabrosa* (Basidiomycota). For most species, the top matches for all three gene regions agreed on one fungal name; for *A. penicillioides* and *Pa. album*, two gene regions agreed on one name. For *Mycosphaerella* sp. and *Zasmidium* sp., the BLAST results of the three gene regions could refer these two species only to the genus level. For *P. paxili* NTOU5880, the 28S rDNA phylogenetic tree grouped this isolate with *P. paxili* MH876591 downloaded from GenBank (results not shown). Based on richness, 10 species of the Ascomycota were isolated (83.3%), in comparison to only two species belonging to the Basidiomycota (16.7%) (Figure 2). The species mostly belonged to the Eurotiales of the Eurotiomycetes (33.3%), and the Capnoidiales of the Dothideomycetes (25.0%). These 12 species could be referred to seven different families: Aspergillaceae (33.3%), Mycosphaerellaceae (16.7%), Saccharomycetidae, Cladosporiaceae, Cystobasidiaceae, Polyporaceae, Cordycipitaceae, and *incertae sedis* (8.3% for the latter five families). Two species of *Aspergillus* and *Penicillium* were isolated from the crab while the rest were only represented by one species, including *Candida*, *Cladosporium*, *Cystobasidium*, *Mycosphaerella*, *Parengyodontium*, *Stachylium* and *Zasmidium*.

Table 1: BLAST search results of the 18S, ITS (internal transcribed spacer regions) and 28S rDNA of the fungi isolated from the carapace of *Xenograpsus testudinatus* collected at the hydrothermal vent area of Kueishan Island, Taiwan in National Center for Biotechnology Information (NCBI).

Culture no. (NTOU)	18S sequence length (bp)	Top BLAST results (highest score)	Query coverage (%)	Sequence similarity (%)	Accession no.	GenBank accession no.
5893	955	<i>Aspergillus penicillioides</i>	100	100	DQ985958	MW879366
		<i>Aspergillus penicillioides</i>	100	100	AF548066	
		<i>Aspergillus penicillioides</i>	100	100	AB004330	
5888	955	<i>Aspergillus versicolor</i>	100	99.90	KR233971	MW879367
		<i>Aspergillus versicolor</i>	100	99.90	KM096354	
		<i>Aspergillus versicolor</i>	100	99.90	KM096344	
5943	940	<i>Candida parapsilosis</i>	100	99.90	KY118177	MW879368
		<i>Candida parapsilosis</i>	100	99.90	KY118176	
		<i>Candida parapsilosis</i>	100	99.90	KT229545	
5932	957	<i>Cladosporium</i>	100	99.90	MK123409	MW879369
		<i>cladosporioides</i>				
		<i>Cladosporium</i>	100	99.90	MG769026	
		<i>cladosporioides</i>				
		<i>Cladosporium</i> sp.	100	99.90	LT860211	
5890	958	<i>Cystobasidium</i> sp.	100	99.48	AB055191	MW879370
		<i>Cystobasidium</i> sp.	100	99.48	EU723505	
		<i>Cystobasidium</i>	100	99.48	AB126648	
		<i>calyptogenae</i>				
5882, 5883, 5884	953–954	<i>Neofomitella guangxiensis</i>	100	99.69	MK192496	MW879371, MW879372,
		<i>Neofomitella guangxiensis</i>	100	99.69	MK192495	MW879373
		<i>Earliella scabrosa</i>	100	99.58	AY336766	
5926	957	<i>Mycosphaerella graminicola</i>	100	99.58	AY251117	MW879374
		<i>Mycosphaerella</i> sp.	100	99.58	AY251116	
		<i>Mycosphaerella</i>	100	99.58	AY490775	
		<i>punctiformis</i>				
5875, 5877, 5879	954–964	<i>Parengyodontium album</i>	100	100	KU747081	MW879375, MW879376, MW879377
5881, 5885, 5889		<i>Halophytophthora vesicula</i>	100	100	KT582539	MW879378, MW879379, MW879380
5924, 5931, 5933		<i>Engyodontium</i> sp.	100	100	KM096193	MW879381, MW879382, MW879383
5927, 5934	954	<i>Penicillium citrinum</i>	100	99.69	MK615877	MW879384, MW879385
		<i>Penicillium citrinum</i>	100	99.69	MG456717	
		<i>Penicillium citrinum</i>	100	99.69	MH392275	
5880	956	<i>Penicillium</i> sp.	100	99.69	JX910356	MW879386
		<i>Penicillium chrysogenum</i>	99.00	99.48	KM222292	
		<i>Penicillium javanicum</i>	100	99.48	KT582512	
5886	957	<i>Chordomyces antarcticum</i>	100	99.58	MN475234	MW879387
		<i>Stachylidium bicolor</i>	100	99.48	GU180616	
		<i>Chordomyces antarcticum</i>	100	99.27	MT328164	
5923	956	<i>Zasmidium cellare</i>	100	99.90	EF137361	MW879388
		Fungal sp.	100	99.69	KY576834	
		<i>Zasmidium daviesiae</i>	100	99.69	GU214620	

Table 1: (continued)

Culture no. (NTOU)	ITS sequence length (bp)	Top BLAST results (highest score)	Query coverage (%)	Sequence similarity (%)	Accession no.	GenBank accession no.
5893	625	Uncultured fungus	98.00	99.52	HQ436053	MW940752
		Uncultured fungus	100	99.20	JX240404	
		Uncultured <i>Aspergillus</i>	100	95.61	KC785540	
5888	561	<i>Aspergillus versicolor</i>	100	99.29	MT560203	MW940753
		<i>Aspergillus versicolor</i>	100	99.29	MT541876	
		<i>Aspergillus amoenus</i>	100	99.29	MN944896	
5943	512	<i>Candida parapsilosis</i>	100	98.65	MH545914	MW940754
		<i>Candida parapsilosis</i>	100	98.65	MN450875	
		<i>Candida parapsilosis</i>	100	98.65	MK859898	
5932	543	<i>Cladosporium</i>	100	99.27	MT258647	MW940755
		<i>cladosporioides</i>				
		<i>Cladosporium</i> sp.	100	99.27	MK336600	
		<i>Cladosporium</i>	100	99.27	MK732115	
		<i>xanthochromaticum</i>				
5890	572	<i>Cystobasidium</i>	98.00	97.03	EU669878	MW940756
		<i>calyptogenae</i>				
		<i>Cystobasidium</i>	100	96.86	FJ515190	
		<i>calyptogenae</i>				
		<i>Cystobasidium</i>	100	96.82	FJ515209	
		<i>calyptogenae</i>				
5882, 5883, 5884	628–667	<i>Earliella scabrosa</i>	100	99.76	MN892530	MW940757, MW940758,
		<i>Earliella scabrosa</i>	98.00	99.76	MN592930	MW940759
		<i>Earliella scabrosa</i>	100	99.52	JN165008	
5926	577	<i>Mycosphaerella</i> sp.	100	99.30	JQ732916	MW940760
		<i>Mycosphaerella</i> sp.	100	99.30	JQ732904	
		<i>Phaeophleospora</i>	99.00	99.30	NR145123	
		<i>eucalypticola</i>				
5875, 5877, 5879	543–596	<i>Parengyodontium album</i>	100	99.83	MT626052	MW940761, MW940762, MW940763
5881, 5885, 5889		<i>Parengyodontium album</i>	100	99.83	MT102852	MW940764, MW940765, MW940766
5924, 5931, 5933		<i>Parengyodontium album</i>	100	99.83	MK834516	MW940767, MW940768, MW940769
5927, 5934	547	<i>Penicillium citrinum</i>	100	99.45	MT529486	MW940770, MW940771
		<i>Penicillium citrinum</i>	100	99.45	MT529471	
		<i>Penicillium citrinum</i>	100	99.45	MT529135	
5880	578	<i>Penicillium paxilli</i>	100	99.31	MH856391	MW940772
		<i>Penicillium paxilli</i>	100	99.31	JN617709	
		<i>Penicillium paxilli</i>	98.00	99.30	KU204492	
5886	957	<i>Stachylidium bicolor</i>	94.00	88.41	MF803167	MW940773
		<i>Stachylidium bicolor</i>	94.00	88.37	MF803162	
		<i>Stachylidium bicolor</i>	87.00	89.89	MF803166	
5923	543	<i>Zasmidium syzygii</i>	99.00	97.23	NR111826	MW940774
		<i>Ramichloridium</i> sp.	98.00	96.83	JQ768795	
		<i>Zasmidium</i> sp.	97.00	96.12	KT203781	

Table 1: (continued)

Culture no. (NTOU)	28S sequence length (bp)	Top BLAST results (highest score)	Query coverage (%)	Sequence similarity (%)	Accession no.	GenBank accession no.	Proposed taxa
5893	952	<i>Aspergillus penicillioides</i>	100	98.65	GU017535	MW881463	<i>Aspergillus penicillioides</i> Speg.
		<i>Aspergillus penicillioides</i>	100	98.32	GU01754		
		<i>Aspergillus penicillioides</i>	100	97.40	GU017537		
5888	954	<i>Aspergillus versicolor</i>	100	99.79	KX958051	MW881464	<i>Aspergillus versicolor</i> (Vuill.) Tirab.
		<i>Aspergillus versicolor</i>	100	99.79	KX958049		
		<i>Aspergillus protuberus</i>	100	99.58	FJ176897		
5943	944	<i>Candida parapsilosis</i>	100	99.80	MK638869	MW881465	<i>Candida parapsilosis</i> (Ashford) Langeron et Talice
		<i>Candida parapsilosis</i>	100	99.80	HE605209		
		<i>Candida parapsilosis</i>	99.00	99.80	KT282393		
5932	945	<i>Cladosporium cladosporioides</i>	100	99.89	MK116414	MW881466	<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries
		<i>Cladosporium cladosporioides</i>	100	99.89	KX958034		
		<i>Cladosporium tenuissimum</i>	100	99.47	MH398553		
5890	980	<i>Cystobasidium laryngis</i>	100	96.84	MH047192	MW881467	<i>Cystobasidium calyptogenae</i> (Nagah., Hamam., Nakase et Hori-khosi) Yurkov, Kachalkin, H.M
		<i>Cystobasidium laryngis</i>	100	96.54	MK131277		Daniel, M. Groenew., Libkind, V. de
		<i>Cystobasidium calyptogenae</i>	100	99.88	KY107431		Garcia, Zalar, Gouliam., Boekhout et Begerow
5882,	984	<i>Earliella scabrosa</i>	100	100	JN164793	MW881468,	<i>Earliella scabrosa</i> (Pers.) Gilb. et
5883,		<i>Earliella scabrosa</i>	100	99.89	KC867484	MW881469,	Ryvarden
5884		<i>Corioloopsis aspera</i>	100	98.93	KC867472	MW881470	
5926	940	<i>Mycosphaerella</i> sp.	100	100	JQ732965	MW881471	<i>Mycosphaerella</i> sp.
		<i>Mycosphaerella</i> sp.	100	100	JQ732958		
		<i>Mycosphaerella</i> sp.	100	100	JQ732957		
5875,	942–945	<i>Acremonium</i> sp.	100	99.73	MK913356	MW881472,	<i>Parengyodontium album</i> (Limber)
5877,						MW881473,	C.C. Tsang, J.F.W. Chan, W.M. Pong,
5879						MW881474	J.H.K. Chen, A.H.Y. Ngan, Cheung,
5881,		<i>Lecanicillium</i> sp.	100	99.73	KU342077	MW881475,	C.K.C. Lai, D.N.C. Tsang, S.K.P. Lau
5885,						MW881476,	et P.C.Y. Woo
5889						MW881477	
5924,		<i>Lecanicillium</i> sp.	100	99.73	KU342075	MW881478,	
5931,						MW881479,	
5933						MW881480	
5927,	952	<i>Penicillium citrinum</i>	100	99.58	MH398532	MW881481,	<i>Penicillium citrinum</i> Thom
5934		<i>Penicillium citrinum</i>	100	99.58	KX958075	MW881482	
		<i>Penicillium citrinum</i>	100	99.58	KX958073		

Table 1: (continued)

Culture no. (NTOU)	28S sequence length (bp)	Top BLAST results (highest score)	Query coverage (%)	Sequence similarity (%)	Accession no.	GenBank accession no.	Proposed taxa
5880	951	<i>Penicillium westlingii</i>	100	99.06	MT226560	MW881483	<i>Penicillium paxilli</i> G. Bainier
		<i>Penicillium sanguifluum</i>	100	98.85	MG976982		
		<i>Digitaria exilis</i>	100	98.64	LR792838		
5886	948	<i>Stachylidium bicolor</i>	100	98.50	GU180651	MW881484	<i>Stachylidium bicolor</i> Link
		<i>Gibellulopsis nigrescens</i>	100	96.80	KP671747		
		<i>Gibellulopsis nigrescens</i>	100	96.80	GU180648		
5923	933	<i>Zasmidium</i> sp.	100	98.70	MT712180	MW881485	<i>Zasmidium</i> sp.
		<i>Zasmidium cerophillum</i>	100	97.90	NG057852		
		<i>Zasmidium anthuriicola</i>	100	97.70	FJ839662

Figure 2: Taxonomic classification of the fungi isolated from the carapace of the crab *Xenograpsus testudinatus* collected in the hydrothermal vent area of Kueishan Island, Taiwan.

Figure 3 shows the taxonomic classification of the 26 species of fungi reported from *X. testudinatus* based on the results of this study and others (Ding et al. 2016; Jiang et al. 2013; Pan et al. 2017; Pang et al. 2019). Twenty-three species belong to the Ascomycota while

there are only three basidiomycetes. *Aspergillus* is the most speciose genus on the crab, followed by *Penicillium* (both Eurotiomycetes) and *Candida* (Saccharomycetes); other genera are only represented by one species.

Figure 3: Taxonomic classification of all fungi reported (by this study and previously published work) on the crab *Xenograpsus testudinatus*.

Among the 26 species documented for *X. testudinatus*, eight species (Ascomycota: *Aspergillus* sp., *A. terreus*, *H. werneckii*, *Parengyodontium album*, *P. citrinum*, *P. scoparia*; Basidiomycota: *Chondrostereum* sp., *E. scabrosa*) were also isolated from the sediment samples (either from black or yellow sediment types or both) collected at the hydrothermal areas of Kueishan Island (Figure 4).

4 Discussion

Twelve species of fungi were isolated from 46 individuals of the hydrothermal vent crab *X. testudinatus* collected at Kueishan Island, Taiwan with 10 ascomycetes and two basidiomycetes. In our previous study (Pang et al. 2019), 13 species were isolated from 10 individuals of the same crab species at the same area. The low fungal species richness isolated in this study may be related to the fact that the crabs were collected in different months/years in these two studies, i.e. Pang et al. (2019): October 2015, March 2017, June 2017;

this study: August 2018, September 2018. Also different methods of isolation were used between the two studies; the biofilm on carapaces was inoculated in this study but whole crabs were crushed and inoculated in Pang et al. (2019).

Twenty-six fungi have been recorded from *X. testudinatus* based on the results of this study and others; the majority of the fungi belong to the Ascomycota (23 species), with *Aspergillus*, *Penicillium* and *Candida* being the most speciose genera, and these are also three of the most speciose genera in the marine environment (Jones et al. 2015). All but one ascomycete, *Xylaria arbuscula*, were previously reported from substrates of the marine environment (Jones et al. 2015). In fact, some species appear to be very common, for example, *H. werneckii* from seawater, sediment and sponges (Anteneh et al. 2019; Leo et al. 2018; Singh et al. 2012), and *Parengyodontium album* from seawater, sediment, sponges and corals (Huang et al. 2018; Khusnullina et al. 2018; Leo et al. 2018; Proksch et al. 2008). It is worth noting that some of these species are regarded as terrestrial species, including *H. monticulosum* and *X. arbuscula*, and their ecological association with *X. testudinatus* is unknown.

Figure 4: Comparison of fungal diversity on the vent crab *Xenograpsus testudinatus*, in yellow sediment (with sulfur granules) and in black sediment (with no sulfur granules) collected in the hydrothermal vent area of Kueishan Island, Taiwan.

Among the three species of the Basidiomycota, *Cystobasidium* was previously reported from the marine environment (Jones et al. 2015). The polypore *E. scabrosa* and the gilled mushroom *Chondrostereum* sp. (both Agaricomycetes) were isolated not only from *X. testudinatus*, but also from the sediment samples at the hydrothermal vent area of Kueishan Island (Figure 4). *E. scabrosa* was previously isolated from the sea fan coral *Pacifigorgia* cf. *eximia* (Barrero-Canosa et al. 2013). *Chondrostereum* spp. have been commonly isolated from various substrata in the marine environment. For example, one *Chondrostereum* sp. was isolated from the soft coral *Sarcophyton tortuosum* (Li et al. 2020), and another from the red alga *Pterocladia capillacea* (Hsiao et al. 2017). Agaricomycetes is the dominant class of Basidiomycota in air over coasts and seas (Fröhlich-Nowoisky et al. 2012), which provides a constant supply of terrestrial fungal propagules for inoculation of marine substrates, including seawater, sediment, marine animals and macroalgae. Moreover, many typical terrestrial ascomycetes isolated from substrates of the hydrothermal area of Kueishan Island were able to grow in seawater (Pang et al. 2020). Further research is

required to investigate if *Chondrostereum* spp., *E. scabrosa* and other filamentous basidiomycetes can grow and play a role in the marine environment.

The ecological relationships between *X. testudinatus* and the fungi associated with it are not known. In the hydrothermal ecosystem of Kueishan Island, green and red algae, epibiotic biofilms on crustacean surfaces, and zooplankton form the base of the trophic system based on a stable isotope analysis (Wang et al. 2014), but Chang et al. (2018), using the same technique, discovered that biofilms on biotic surfaces made little contribution to other benthic consumers. None of the recorded fungi on the crab are reported pathogens of crabs, but some of them are known pathogens of other marine animals (Pang et al. 2021). *A. terreus* was reported to cause pneumonia and severe otitis media in the harbor porpoise *Phocoena phocoena* (Jepson et al. 2000; Prah et al. 2011). *A. sydowii* causes diseases of corals (Smith et al. 1996). *A. versicolor* formed white masses on the sea fan *Annella* sp. and caused tissue disintegration (Phongpaichit et al. 2006). Granulomas in the swim bladder of the humpback grouper *Cromileptes altivelis* were reported to be caused by *C. cladosporioides*

(Bowater et al. 2003). Whether the crab *X. testudinatus* is a vehicle of fungal diseases requires further study.

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