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ORIGINAL PAPER

Cultivable yeasts associated with marine sponges in the Gulf of Thailand, South China Sea

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Abstract Marine sponges harbor numerous microorganisms, among which sponge-associated yeasts are the least explored. To gain greater knowledge of sponge-associated yeasts, an investigation was therefore performed on marine sponges in Sattahip Bay, Gulf of Thailand, South China Sea. Seventy-one (71) marine sponge samples were collected at sites near Samae-san, Mu, and Khram islands, and were subsequently identified as 17 sponge species in 14 genera. Eighty-seven (87) yeast strains were isolated from 42 samples. The identification of yeasts by similarity analysis of the D1/D2 domain sequences of the large subunit rRNA gene revealed that 64% of the yeast strains obtained belonged to the phylum Basidiomycota, while the remaining strains belonged to the phylum Ascomycota. The strains that belonged to Ascomycota comprised 11 known yeast species in five genera (*Candida*, *Kodamaea*, *Magnusiomyces*, *Meyerozyma*, and *Pichia*). The strains belonging to the phylum Basidiomycota comprised 14 known yeast species in eight genera (*Cutaneotrichosporon*, *Cystobasidium*, *Naganishia*, *Papiliotrema*, *Rhodosporidiobolus*, *Rhodotorula*, *Trichosporon*, and *Vishniacozyma*). In addition, three strains represented a potential novel species closest to *Cys. slooffiae*; one strain represented a potential novel species closest to *R. toruloides*; and one strain represented a potential novel species closest to *V. foliicola*. The species with the highest occurrence was *Rhodotorula mucilaginosa*. No marked difference was found in the principal coordinates analysis of the ordinations of yeast communities from the three sampling sites. The estimation using EstimateS software showed that the expected species richness was higher than the observed species richness. As the marine sponge–yeast association remains unclear, more systematic investigations should be carried out.

Keywords China sea · Culturable yeast · Diversity · Marine sponge · Thailand

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Introduction

Marine sponges are filter-feeding organisms belonging to the phylum Porifera (Thakur and Muller 2004). They are classified into four taxonomic classes, namely, *Calcarea* (calcareous sponges), *Hexactinellida* (glass sponges), and the demosponges, *Homoscleromorpha*, and *Demospongiae*, which collectively contain an estimated 15,000 species (Kiran et al. 2018). Sponges, as sedentary organisms, efficiently obtain food from the surrounding water by pumping and filtering a large volume (up to 24 m^3 per kg of sponge per day) of seawater (Reiswig 1974; Vogel 1977; Pile et al. 1996; Hentschel et al. 2002) through the porous internal canal system that circulates water throughout their bodies (Reiswig 1974; Trautman and Hinde 2001). During the filter feeding process, large numbers of microorganisms, such as bacteria, microalgae, fungi, and yeasts, can become residents in sponges (Vacelet 1975; Vacelet and Donadey 1977). Although these microorganisms are major components of sponges' natural diets, some microbial species may survive the sponge's digestion and immune response and have the capability to grow and reside in the microenvironment of the sponge's mesohyl (Zu et al. 2008). The diversity of bacteria, actinobacteria, archaea, and filamentous fungi in sponges has been thoroughly characterized (Jiang et al. 2007; Taylor et al. 2007; Gao et al. 2008; Li et al. 2009; Simister et al. 2012; Naim et al. 2017). However, little knowledge is available of the diversity of yeast communities living within sponges (Vaca et al. 2013; Li and Wang 2009).

Marine occurring yeasts are the yeasts isolated from marine environments: these environments include seawater, seaweeds, sea sediments, marine invertebrates, and vertebrates, as well as mangrove ecosystems (Zhang et al. 2012; Vaca et al. 2013; Chi et al. 2016; Naim et al. 2017; Paulino et al. 2017; Li 2019). Researchers have reported the isolation of yeasts in the phyla Ascomycota and Basidiomycota from the inner tissue of sponges (Li et al. 2009; Vaca et al. 2013; Laich et al. 2014; Kiran et al. 2018). The examples of ascomycetous yeast species reported were *Candida saitoana, Candida tropicalis, Debaryomyces hansenii*, *Metschnikowia australis*, *Metschnikowia bicuspidata*, *Meyerozyma guilliermondii*, *Ogataea angusta*, *Pichia membranifaciens*, and *Starmerella etchellsii* (Gao et al. 2008; Kutty and Philip 2008; Sugiyama et al. 2009; Burgaud et al. 2010; Liu et al. 2010; Duarte et al. 2013; Vaca et al. 2013; Passarini et al. 2014; Calabon et al. 2019). The examples of basidiomycetous yeast species reported were *Cystofilobasidium infirmominiatum*, *Holtermanniella festucosa*, *Malassezia restricta, Leucosporidiella creatinivora*, *Leucosporidium escuderoi, Papiliotrema laurentii*, *Papiliotrema pseudoalba* (*Bullera pseudoalba*), *Pseudozyma aphidis*, *Rhodotorula diobovata*, *Rhodotorula graminis*, *Rhodotorula mucilaginosa*, *Rhodotorula pinicola*, *Sporobolomyces roseus*, and *Trichosporon cutaneum* (Gao et al. 2008; Kutty and Philip 2008; Burgaud et al. 2010; Duarte et al. 2013; Vaca et al. 2013; Laich et al. 2014; Passarini et al. 2014; Poli et al. 2018). Moreover, only limited information is available on the ecological function of yeasts living within marine sponges, with the nature of the sponge–yeast association remaining unknown. Some investigators reported that fungi contributed to the localized lesions of sponges and thus may enhance disease development in sponges. Some fungi are opportunistic, acting as secondary colonizers to other infections or stresses (Galstoff 1942; Sparks 1985; Vacelet et al. 1994).

Yeasts associated with marine sponges have been investigated in only some marine areas, such as Fildes Bay, King George Island, Antarctica (Vaca et al. 2013; Laich et al. 2014); Punta Ulmann and Punta Plaza, Antarctica (Duarte et al. 2013); the Mid-Atlantic Ridge, South Pacific Basins, and East Pacific Rise (Burgaud et al. 2010); and Oosterschelde estuary, the Netherlands (Naim et al. 2017). However, the knowledge of sponge-associated yeasts is limited due to having been investigated the least, especially in tropical areas. Therefore, to increase the knowledge of sponge-associated yeasts, more investigation in other marine areas should be performed.

Samae-san, Mu, and Khram islands are three islands located in Sattahip Bay on the eastern side of the Gulf of Thailand, in the western part of the South China Sea. These three islands are in Chonburi Province, Thailand, and are controlled by the Royal Thai Navy. Samae-san Island is one of nine islands in the Plant Genetic Conservation Project, under the Royal Initiative of Her Royal Highness Princess Maha Chakri Sirindhorn. The objective of the project is to promote the island as a place for biodiversity learning and conservation travel. Mu Island is one of seven major islands in the Sattahip District, Chonburi Province. This island has no human activities, with

its long barrier protecting it from ocean waves. Khram Island is a protected area, used as a coral nursery and for planting corals (Viyakarn et al. 2020). In addition, as previously stated, the available information indicates that no study on sponge-associated yeasts has been carried out in this area.

The exploration of the yeast–marine sponge association in the Gulf of Thailand in the South China Sea has never been reported. In addition, some sponge-associated yeast strains obtained from this diversity study could be further explored for their potential in the production of important biotechnological products. The aim of the current study was to determine the diversity of culturable yeasts associated with marine sponges in Sattahip Bay, Gulf of Thailand, in the western part of the South China Sea.

Materials and methods

Sampling sites

Marine sponge samples were collected in Sattahip Bay (12°38′45″ N 100°54′30″ E) on the eastern side of the Gulf of Thailand. The first sampling site (S1) was at 12°34′15.1″ N, 100°56′39.7″ E, located 274 m off Samae-san Island (Fig. 1). The samples from this site were collected at depths of 5–6 m in March 2016, July 2016, and January 2017 (Table 1). The second sampling site (S2) was at 12°37′9″ N, 100°54′25″ E, located 622 m off Mu Island (Fig. 1). From this site, samples were collected at a depth of 15–20 m in March 2016, July 2016, and January 2017 (Table 1). The last sampling site (S3) was at 12°42′19″ N, 100°48′27″ E, and located 274 m off Khram Island (Fig. 1), where the samples were collected at a depth of 3 m in March 2016, July 2016, and February 2017 (Table 1).

Collection of samples

Marine sponge samples were collected by hand while scuba diving. Each collected sample was transferred directly into a new clean zip-lock plastic bag containing seawater to prevent the contact of sponge tissue with air, and immediately placed in an icebox for transportation to the laboratory.

At each of the three sampling sites, the temperature and salinity of the seawater were measured with a sound velocity probe (ATLAS 23120M, Bremen, Germany), while a pH meter (Mettler Toledo, Columbus, Ohio, USA) was used for the pH measurement.

Marine sponge identification

Underwater photographs of marine sponges were taken in situ to assist in identification. Laboratory preparation, with the tangential and perpendicular histological sectioning of sponges, spicule preparation, spicule type, and measurement were carried out, following the method described by Putchakarn (2007). The tangential and perpendicular histological sections of sponges were cut into small pieces (approximately 1 mm thick) with a scalpel. These sections were dried on a hotplate, subsequently mounted in Canada balsam, and examined under bright field microscopy with 100–400× magnification. Spicule preparation was performed by cutting a small fragment (approximately $5 \times 5 \times 5$ mm^3) from the sponge. The sponge fragment was boiled in concentrated nitric acid solution, washed and centrifuged three times in distilled water and three times in 95% alcohol, and suspended in 95% alcohol. The spicule suspension was pipetted onto glass microscope slides, dried, and mounted in Canada balsam for bright field microscopy. Spicule-size data were based on 25 measurements of randomly chosen spicules for each category. The identification of marine sponges at species level was based on the World Porifera Database (van Soest et al. 2020).

Yeast isolation

The yeasts were isolated upon arrival in the laboratory using Li and Wang's (2009) method with slight modification. Briefly, the sponges were rinsed twice with sterile 0.85% sodium chloride (NaCl) to remove surface microorganisms. The surface of the sample was disinfected with 70% ethanol. Samples were then cut into five small pieces (approximately 2.5 cm^3) and homogenized using a blender with 5 ml of sterile 0.85% NaCl in an aseptic condition. After homogenization, two procedures for yeast isolation were used in parallel. In the first procedure, 100 µl of the homogenate sample were spread directly onto the surface of yeast extract malt extract (YM) agar prepared with artificial seawater (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g dextrose, and 15 g

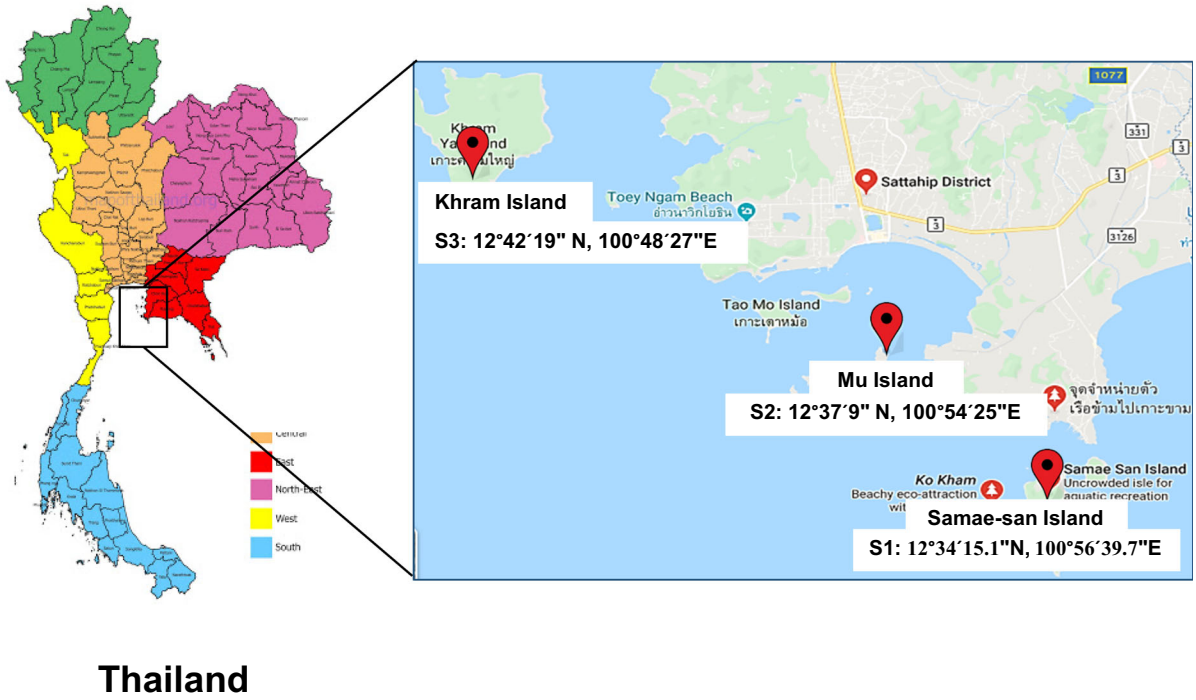


Fig. 1 Map of the Samae-san, Mu and Khram islands

agar in 1 L artificial seawater) supplemented with 300 mg l^{-1} penicillin, 300 mg l^{-1} streptomycin, and 250 mg l^{-1} sodium propionate in a Petri dish. Two replicates were performed. In the second procedure, the remaining homogenate sample was added to a 250 ml Erlenmeyer flask, containing 50 ml of sterile 0.85% NaCl, which was then shaken on a rotary shaker at 150 rpm and 20 °C for 1 h. The precipitate was collected by centrifugation at 1,800*g* for 5 min, with 0.1 ml of the precipitate spread onto the surface of the same medium used in the first procedure. Two replicates were performed. The Petri dishes were incubated at 20 °C for 5–7 days. Colonies of yeasts with different morphologies in each sample were picked and purified by cross-streaking on YM agar prepared with artificial seawater. The purified yeast strains were preserved in YM broth supplemented with 10% (v/v) glycerol and maintained at −80 °C.

DNA extraction

Genomic DNA was extracted from yeast cells using the method described by Limtong et al. (2007). Briefly, cells grown on YM agar at 30 °C for 24–48 h were suspended in 50 µl of sterile distilled water contained in a 1.5 ml microfuge tube. The cell suspension in the microfuge tube was kept at − 20 °C for 30 min before being put in boiling water for 15 min, and then returned to − 20 °C for 30 min, before the microfuge tube was subjected to centrifuge at a speed of 12,000*g* for 5 min. The supernatant containing genomic DNA was used as the DNA template for amplification. If genomic DNA could not be successfully extracted using the described method, extraction was undertaken with a solvent mixture of chloroform and isoamyl alcohol (24:1), as described by Ruiz-Barba et al. (2005), with slight modification. Briefly, 50 µl of the solvent mixture was added to 50 µl of cell suspension in a 1.5 ml microfuge tube and then vigorously vortexed for 5 min. The microfuge tube was centrifuged at 12,000*g* for 5 min. The supernatant containing genomic DNA was used as the DNA template.

Polymerase chain reaction (PCR)

Amplification of the D1/D2 domain of the large subunit (LSU) rRNA gene and the internal transcribed

Table 1 Yeast strains isolated from marine sponges of each sampling site and their accession numbers of the D1/D2 domain of the LSU rRNA gene

Sampling site and Collection date	No. of sponge samples	Marine sponge	Yeast strain (DMKU)	DDBJ Accession number	Yeast species
Sampling site S1 (12°34′15.1″N, 100°56′39.7″E)					
6 March 2016 (pH 8.03, 27.0 °C, 32.7 psu)	5	*Monanchora unguiculata*	–	–	–
		Ircinia mutans	313-1	LC413273	*Meyerozyma caribbica*
			313-2	LC529381	*Cystobasidium minutum*
		Echinodictyum sp.	314-1	LC413274	*Meyerozyma caribbica*
			C314-1	LC413275	*Naganishia albida*
			C314-2	LC413276	*Cystobasidium minutum*
		Iotrochota purpurea	315-1	LC413277	*Meyerozyma caribbica*
		Xestospongia testudinaria	316-1	LC413278	*Candida parapsilosis*
			316-2	LC413279	*Meyerozyma caribbica*
			C316-1	LC413280	*Meyerozyma guilliermondii*
31 July 2016 (pH 8.35, 32.4 °C, 32.7 psu)	4	*Monanchora unguiculata*	–	–	–
21 January 2017 (pH 8.37, 21.0 °C, 32.0)	6	*Clathria (Thalysias) reinwardti*	JC1-2	LC430997	*Papiliotrema laurentii*
		Xestospongia testudinaria	JC2-1	LC430998	*Papiliotrema laurentii*
			JC2-2	LC430999	*Kodamaea ohmeri*
		Monanchora unguiculata	–	–	–
		Xestospongia testudinaria	–	–	–
		Clathria (Thalysias) reinwardti	JC5-1	LC431000	*Rhodotorula mucilaginosa*
			JC5-2	LC431001	*Papiliotrema laurentii*
		Clathria (Thalysias) reinwardti	J6-2	LC431002	*Papiliotrema laurentii*
			J6-3	LC431003	*Meyerozyma caribbica*
			JC6-1	LC431004	*Rhodotorula diobovata*
Sampling site S2 (12°37′9″N, 100°54′25″E)					
6 March 2016 (pH 8.17, 29.4 °C, 33.2 psu)	4	*Clathria (Thalysias) reinwardti*	317-1	LC415302	*Meyerozyma caribbica*
			C317-1	LC216896	Potential novel species closest to *Cystobasidium slooffiae*
		Ircinia mutans	–	–	–
		Xestospongia testudinaria	–	–	–
		Ircinia mutans	–	–	–
31 July 2016 (pH 8.37, 30.8 °C, 32.7 psu)	8	*Clathria (Thalysias) reinwardti*	711-1	LC430100	*Cystobasidium calyptogenae*

Table 1 continued

Sampling site and Collection date	No. of sponge samples	Marine sponge	Yeast strain (DMKU)	DDBJ Accession number	Yeast species
		Xestospongia testudinaria	C712-1	LC430101	*Meyerozyma guilliermondii*
		Cacospongia sp.	714-1	LC430102	*Rhodotorula mucilaginosa*
		Clathria (Thalysias) reinwardti	C715-1	LC430103	*Rhodotorula mucilaginosa*
		Xestospongia testudinaria	C716-1	LC430104	*Rhodotorula mucilaginosa*
			C716-2	LC430105	*Cystobasidium minutum*
		Clathria (Thalysias) reinwardti	–	–	–
		Xestospongia testudinaria	C719-1	LC430106	*Rhodotorula mucilaginosa*
		Gelliodes petrosioides	7110-1	LC496532	Potential novel species closest to *Cystobasidium slooffiae*
21 January 2017 (pH 8.37, 19.7 °C, 31.7 psu)	11	*Pseudoceratina purpurea*	JC10-1	LC431011	*Candida tropicalis*
			JC10-3	LC431012	*Papiliotrema laurentii*
		Pseudoceratina purpurea	J12-1	LC431013	*Candida nonsorbophila*
			JC12-1	LC431014	*Papiliotrema laurentii*
			JC12-2	LC431015	*Candida orthopsilosis*
		Clathria (Thalysias) reinwardti	–	–	–
		Neopetrosia sp. "blue"	–	–	–
		Neopetrosia sp. "blue"	–	–	–
		Clathria (Thalysias) reinwardti	JC36-1	LC497443	*Candida orthopsilosis*
		Pseudoceratina purpurea	JC38-1	LC497444	*Magnusiomyces capitatus*
			JC38-2	LC497445	*Candida parapsilosis*
		Pseudoceratina purpurea	J39-2	LC497446	*Rhodotorula mucilaginosa*
			JC39-1	LC497447	*Candida tropicalis*
			JC39-3	LC497448	*Candida parapsilosis*
		Xestospongia testudinaria	JC44-1	LC529382	*Rhodotorula mucilaginosa*
			JC44-2	LC529383	*Pichia kudriavzevii*
		Clathria sp.	JC45-1	LC529384	*Rhodosporidiobolus fluvialis*
			JC45-2	LC529385	*Papiliotrema laurentii*
			JC45-3	LC529386	*Rhodotorula diobovata*
			JC45-5	LC529387	*Kodamaea ohmeri*

Table 1 continued

Sampling site and Collection date	No. of sponge samples	Marine sponge	Yeast strain (DMKU)	DDBJ Accession number	Yeast species
		Clathria (Thalysias) reinwardti	J47-1	LC497438	Potential novel species closest to *Rhodotorula toruloides*
			JC47-1	LC529388	*Candida tropicalis*
			JC47-2	LC529389	*Candida orthopsilosis*
Sampling site S3 (12°42′19″ N, 100°48′27″E)					
26 March 2016 (pH 7.94, 27.1 °C, 31.9 psu)	8	*Lamellodysidea herbacea*	–	–	–
		Xestospongia testudinaria	326-1	LC415309	*Rhodotorula mucilaginosa*
			326-2	LC415310	*Meyerozyma caribbica*
			326-3	LC529390	*Naganishia liquefaciens*
		Ircinia mutans	3214-1	LC415318	*Naganishia albida*
			3214-2	LC415319	*Rhodotorula mucilaginosa*
		Ircinia mutans	C3215-1	LC529391	*Rhodotorula mucilaginosa*
			C3215-2	LC529392	*Naganishia albida*
			C3215-3	LC529393	*Naganishia liquefaciens*
		Pseudoceratina purpurea	–	–	–
		Cacospongia sp.	3231-1	LC529394	*Naganishia liquefaciens*
			3231-2	LC529395	*Rhodotorula mucilaginosa*
		Lamellodysidea herbacea	–	–	–
		Pseudoceratina purpurea	3235-1	LC529396	*Rhodotorula mucilaginosa*
30 July 2016 (pH 8.13, 28.6 °C, 31.9 psu)	19	*Lamellodysidea herbacea*	–	–	–
		Haliclona (Soestella) sp.	–	–	–
		Xestospongia testudinaria	724-1	LC430108	*Cystobasidium calyptogenae*
		Xestospongia testudinaria	–	–	–
		Haliclona (Soestella) sp.	–	–	–
		Cacospongia sp.	–	–	–
		Biemna tubulata	7212-1	LC430112	*Rhodotorula mucilaginosa*
			7212-3	LC430113	*Trichosporon japonicum*

Table 1 continued

Sampling site and Collection date	No. of sponge samples	Marine sponge	Yeast strain (DMKU)	DDBJ Accession number	Yeast species
		Haliclona (Soestella) sp.	–	–	–
		Lamellodysidea herbacea	–	–	–
		Xestospongia testudinaria	7218-1	LC529397	Potential novel species closest to *Vishniacozyma foliicola*
			7218-3	LC529398	*Cystobasidium minutum*
		Haliclona (Soestella) sp.	–	–	–
		Siphonodictyon mucosum	C7221-1	LC430114	*Vishniacozyma victoriae*
			C7221-2	LC430115	*Rhodotorula mucilaginosa*
			C7221-3	LC496531	Potential novel species closest to *Cystobasidium slooffiae*
			C7221-4	LC430578	*Cystobasidium slooffiae*
		Siphonodictyon mucosum	C7224-1	LC430579	*Rhodotorula mucilaginosa*
		Xestospongia testudinaria	C7230-1	LC430580	*Candida sake*
		Iotrochota purpurea	C7232-1	LC430582	*Rhodotorula mucilaginosa*
			C7232-2	LC430583	*Rhodotorula taiwanensis*
			C7232-4	LC430584	*Vishniacozyma victoriae*
		Clathria (Thalysias) reinwardti	7236-1	LC430592	*Candida tropicalis*
			C7236-1	LC430593	*Meyerozyma guilliermondii*
			C7236-2	LC430594	*Cystobasidium minutum*
			C7236-5	LC430595	*Cutaneotrichosporon dermatis*
		Siphonodictyon mucosum	–	–	–
		Cacospongia sp.	7239-1	LC430597	*Rhodotorula mucilaginosa*
		Haliclona (Reniera) sp.	7240-1	LC430598	*Rhodotorula mucilaginosa*
18 February 2017 (pH 8.40, 20.5 °C, 31.2 psu)	6	*Xestospongia testudinaria*	F2-1	LC529399	*Rhodotorula mucilaginosa*
		Clathria (Thalysias) reinwardti	–	–	–

Table 1 continued

Sampling site and Collection date	No. of sponge samples	Marine sponge	Yeast strain (DMKU)	DDBJ Accession number	Yeast species
		Clathria sp.	FC10-1	LC529400	*Meyerozyma caribbica*
			FC10-2	LC529401	*Candida conglobata*
			FC10-3	LC529402	*Rhodotorula mucilaginosa*
			FC10-4	LC529403	*Papiliotrema laurentii*
			FC10-5	LC529404	*Candida parapsilosis*
		Neopetrosia sp. "blue"	–	–	–
		Ircinia mutans	F16-1	LC529405	*Candida conglobata*
			F16-2	LC529406	*Rhodotorula diobovata*
			FC16-2	LC529407	*Candida parapsilosis*
		Biemna fortis	–	–	–

spacer (ITS) region from the genomic DNA by the PCR technique was performed using primers NL1 (5′-GCATATCAATAAGCG GAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (Kurtzman and Robnett 1998), and universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al. 1990), respectively. The reaction mixture contained 3 µl of 10 × PCR buffer; 2.4 µl of 25 mM $MgCl_2$; 2.4 µl of 2.5 mM dNTP; 0.9 µl of 10 pmol forward primer; 0.9 µl of 10 pmol reverse primer; 3 µl of yeast genomic DNA; and 0.15 µl of Taq DNA polymerase (Thermo Fisher Scientific Balties UAB, Vilnius, Lithuania), with the total volume adjusted to 30 µl with sterile distilled water. The PCR condition for the D1/D2 domain of the LSU rRNA gene comprised predenaturation (94 °C for 5 min); 35 cycles of denaturation (94 °C for 1 min); primer annealing (55 °C for 1 min); and an extension (72 °C for 1 min); with a final extension at 72 °C for 10 min. Furthermore, the PCR condition for the ITS region comprised predenaturation (94 °C for 3 min); 30 cycles of denaturation (94 °C for 0.30 min); primer annealing (57 °C for 0.30 min); and an extension (72 °C for 0.45 min); with a final extension at 72 °C for 10 min. The PCR product was confirmed by 1% agarose gel electrophoresis prepared by dissolving 1 g agarose in a 1 × Tris base/boric acid/ ethylenediaminetetraacetic acid (EDTA) (TBE) buffer, with a final volume of 100 ml. A 1 × TBE buffer was prepared by dilution of a 10 × TBE buffer consisting of a 10.8 g Tris base, 5.5 g boric acid, 4 ml of 0.5 mM EDTA, at a pH of 8.0, in a 100 ml of sterile distilled water. The PCR product was purified with a TIANquick Midi Purification kit (Tiangen Biotech, Beijing, China), in accordance with the manufacturer's protocol.

Sequencing

The purified products were submitted to First BASE Laboratories (Apical Scientific Sdn. Bhd. Company, Selangor, Malaysia) for sequencing of the D1/D2 domain and the ITS region using the same primers used for amplification. The sequences obtained for the D1/D2 domain of the LSU rRNA gene and the ITS region were deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers shown in Table S1.

Yeast identification was performed by similarity analysis of the D1/D2 domain of the LSU rRNA gene sequences with available type strain sequences in the GenBank database using the BLASTn search. For the

identification of ascomycetous yeasts in this study, the strains which differed by 0–3 nucleotide differences in the D1/D2 domain of the LSU rRNA gene were considered to be conspecific strains, while the strains showing greater than 1% nucleotide substitutions (six nucleotide substitutions in ca. 600 nucleotides) were regarded as a different species (Kurtzman and Robnett 1998). For basidiomycetous yeasts, the strains that differed by two or more nucleotides were taken to be different taxa (Fell et al. 2000). Analysis of the ITS sequence similarity was performed when the nucleotide substitutions of ascomycetous and basidiomycetous yeasts were more than six, and two or more than two nucleotide substitutions, respectively. The term "potential novel species" was used for the strain for which no further investigation was made to propose as the novel species.

Phylogenetic analysis

Phylogenetic analysis, undertaken based on the D1/D2 domain sequences of the LSU rRNA gene, was carried out, as described by Kaewkrajay et al. (2020), to determine the evolutionary development of each species. The phylogenetic positions of strains could confirm identification by sequence similarity analysis. The MUSCLE program in Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0 (Kumar et al. 2016) was used for multiple alignments of the D1/D2 domain sequences. A phylogenetic tree was constructed from the evolutionary distance data using the maximum-likelihood (ML) algorithm, included in MEGA software version 7.0, with Kimura's two-parameter correction (Kimura 1980). Confidence levels of the clades were estimated from bootstrap analysis with 1000 replicates (Felsenstein 1985).

Biodiversity analysis

The classical Jaccard similarity coefficient of PAST software version 3.25 (Hammer et al. 2001) was used to calculate the similarity of yeast communities in marine sponges collected at the three sites off Samae-san, Mu, and Khram islands. The principal coordinates analysis (PCoA) of the ordinations of yeast communities in the 71 samples from the three sampling sites was based on the Jaccard similarity indices and employed PAST software version 3.25 (Hammer et al. 2001). When a particular species was observed, the number of samples of that species as a proportion of the total number of samples was used to calculate the frequency of occurrence (FO), expressed as a percentage. The study employed EstimateS software using 100 randomizations to calculate species accumulation curves. The total number of species observed in a sample, or in a set of samples, was used to calculate the observed species richness (S_{obs}). Estimated species richness (S_{est}) is the estimated number of species in the assemblage represented by the sample, or by the set of samples, where *est* is replaced by the name of an estimator (Gotelli and Colwell 2011). Three species richness estimators, namely, Chao 1, incidence-based coverage (ICE), and bootstrap estimators with sample-based abundance data were used (Colwell 2006).

Results and discussion

Collection and identification of marine sponges

Marine sponges were collected from three sampling sites off Samae-san (S1), Mu (S2), and Khram islands (S3) in 2016 and 2017. The marine sponges from site S2 were collected at a greater depth than at sites S1 and S3. On each sampling date at each site, seawater was measured for pH, temperature, and salinity. The pH levels of seawater at every site were found to be weakly basic (7.94–8.40), salinity values were in the range of 31.2–33.2 practical salinity units (PSUs), and temperatures were in the range of 19.7–32.4 °C (Table 1). Each parameter affected yeast diversity. Some yeast species are recognized for their tolerance to high salt concentrations, such as *Torulopsis famata*, *Rhodotorula rubra*, *Pichia etchelsii*, *Candida parapsilosis*, and *Debaryomyces hansenii*, which can grow at salt concentrations above 10–15% NaCl (Lages et al. 1999; Samson et al. 2000). Some yeast species, such as *Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa*, are dominant yeasts in hypersaline wastewater, growing in a wide range of salinity and pH levels (Lahav et al. 2002). In addition, some species (e.g., *Debaryomyces hansenii*) have been found mainly in cold sea water (Norkrans 1966; Butinar et al. 2005). Butinar et al. (2005) studied yeast diversity in diverse hypersaline habitats, obtaining several hundred yeast cells l^{-1}. The work of van Uden

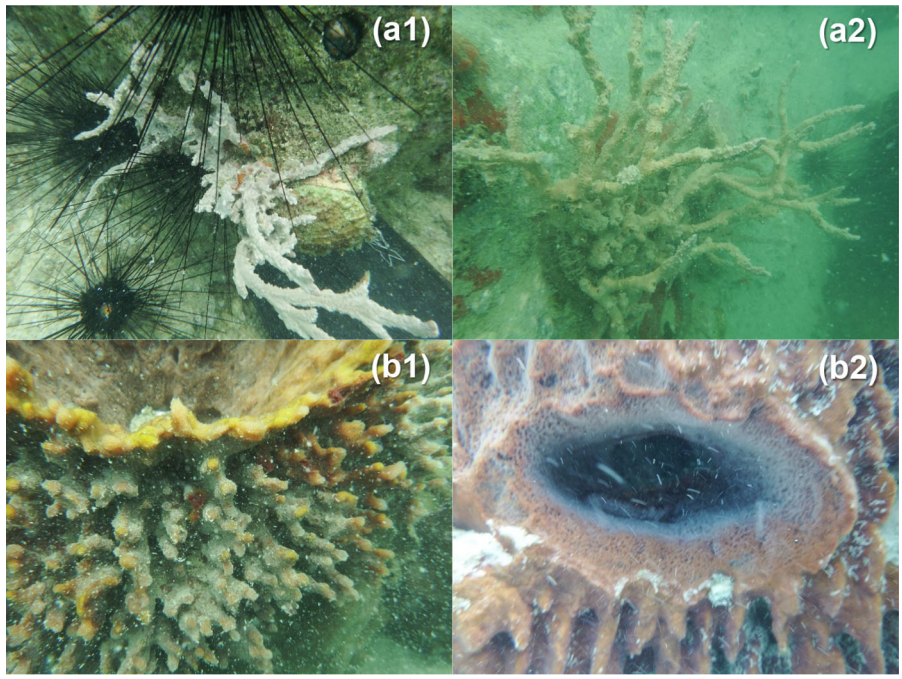


Fig. 2 Underwater photographs of *Clathria (Thalysias) reinwardti* (**a1**, **a2**) and *Xestospongia testudinaria* (**b1**, **b2**)

and Fell (1968) reported that a low yeast count, about 10 cells l^{-1}, was typical in open ocean water.

In the current study, samples were collected from each sampling site in March (summer), July (rainy season), and January or February (winter). Marine sponge samples collected from sites S1, S2, and S3 numbered 15, 23, and 33, respectively. The marine sponges were identified as 17 species in 14 genera (Table 1), namely, *Biemna fortis* (1 sample), *Biemna tubulata* (1 sample), *Cacospongia* sp. (4 samples), *Clathria* sp. (2 samples), *Clathria* (*Thalysias*) *reinwardti* (12 samples) (Fig. 2a), *Echinodictyum* sp. (1 sample), *Gelliodes petrosioides* (1 sample), *Haliclona* (*Reniera*) sp. (1 sample), *Haliclona* (*Soestella*) sp. (4 samples), *Iotrochota purpurea* (2 samples), *Ircinia mutans* (6 samples), *Lamellodysidea herbacea* (4 samples), *Monanchora unguiculata* (6 samples), *Neopetrosia* sp. (blue) (3 samples), *Pseudoceratina purpurea* (6 samples), *Siphonodictyon mucosum* (3 samples), and *Xestospongia testudinaria* (14 samples) (Fig. 2b). Differences in the number of marine sponges collected at each sampling site may have been the result of different environmental factors.

Differences were found in the depth at each site and may therefore have influenced the number and species of sponges. At site S1, sponges were collected at a depth of 5–6 m, with six sponge species obtained. Among these six species, two were only found at this site, namely, *Echinodictyum* sp. and *Monanchora unguiculata*. At site S2, eight sponge species were collected at a depth of 15–20 m. The sponge species, *Gelliodes petrosioides*, was only found at this site. The depth of site S3 was 3 m with 14 sponge species collected. Among these species, six were only obtained from this site, comprising: *Biemna furtis*, *Biemna tubulta*, *Haliclona* (*Reniera*) sp., *Haliclona* (*Soestella*) sp., *Lamellodysidea herbacea*, and *Siphonodictyon mucosum*. However, three of the 14 sponge species viz. *Clathria* (*Thalysias*) *reinwardti*, *Ircinia mutans*, and *Xestospongia testudinaria* were found at every site. These results implied that the depth of the water affected the sponge species. Bell and Barnes (2000) reported that depth significantly influenced marine sponge diversity, richness, evenness, and density. At sites with turbulent and fast flow conditions, sponge diversity and richness were lowest, with the highest values being found at sites with moderate to high levels of sedimentation. Wilborn et al. (2018) reported that rocky substrate, tidal currents, and depth affected the density and diversity

Table 2 Frequency of occurrence of each yeast species isolated from marine sponges

Phylum and subphylum	Species	Number of samples contained	FO (%)[a]
Ascomycota (31 strains)	*Candida conglobata*	2	2.8
Saccharomycotina	*Candida nonsorbophila*	1	1.4
	Candida orthopsilosis	3	4.2
	Candida parapsilosis	5	7.0
	Candida sake	1	1.4
	Candida tropicalis	4	5.6
	Kodamaea ohmeri	2	2.8
	Magnusiomyces capitatus	1	1.4
	Meyerozyma caribbica	8	11.3
	Meyerozyma guilliermondii	3	4.2
	Pichia kudriavzevii	1	1.4
Basidiomycota (56 strains)	*Cutaneotrichosporon dermatis*	1	1.4
Agaricomycotina	*Naganishia albida*	3	4.2
	Naganishia liquefaciens	3	4.2
	Papiliotrema laurentii	8	11.3
	Trichosporon japonicum	1	1.4
	Vishniacozyma victoriae	2	2.8
	Potential novel species closest to *Vishniacozyma foliicola*	1	1.4
Pucciniomycotina	*Cystobasidium calyptogenae*	2	2.8
	Cystobasidium minutum	5	7.0
	Cystobasidium slooffiae	1	1.4
	Potential novel species closest to *Cystobasidium slooffiae*	3	4.2
	Rhodosporidiobolus fluvialis	1	1.4
	Rhodotorula diobovata	3	4.2
	Rhodotorula mucilaginosa	20	28.2
	Rhodotorula taiwanensis	1	1.4
	Potential novel species closest to *Rhodotorula toruloides*	1	1.4
	Total number of sample	87	

[a]FO; Frequency of occurrence (%) = number of samples, where a particular species was observed, as a proportion of the total number of samples

of sponges. Moreover, a towed camera survey revealed marine sponges of the species *Craniella longipilis* were numerically dominant across all depths, but other species showed significant changes in abundance with changes in depth. At shallower depths, *Pheronema carpenteri* was more prevalent, whereas the encrusting species *Hexadella* sp. increased in frequency with increased depth (McIntyre et al. 2016). In addition, the different numbers and species of marine sponges collected at each sampling date resulted from differences in physical properties, such as temperature, pH, and salinity of the seawater.

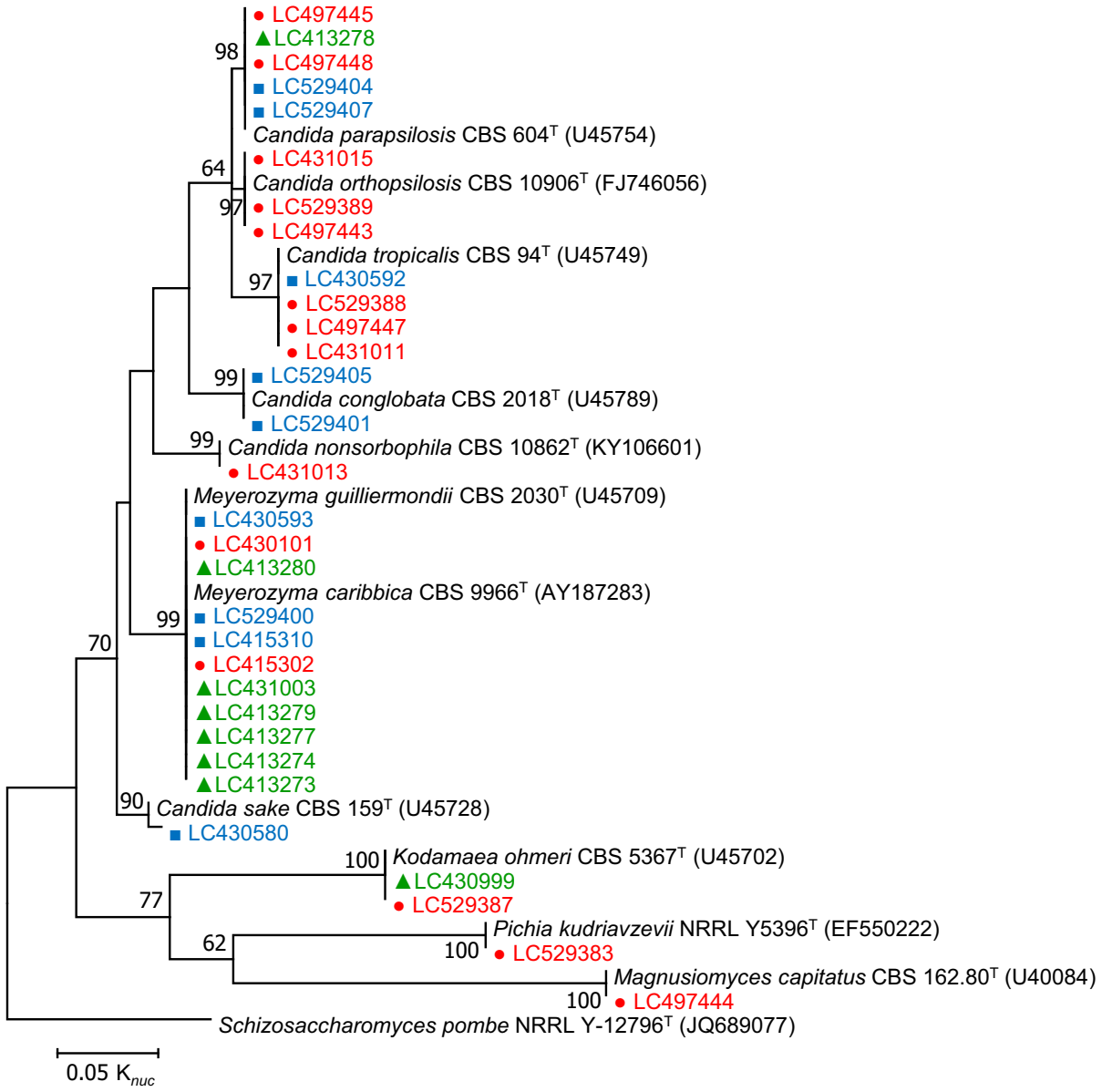
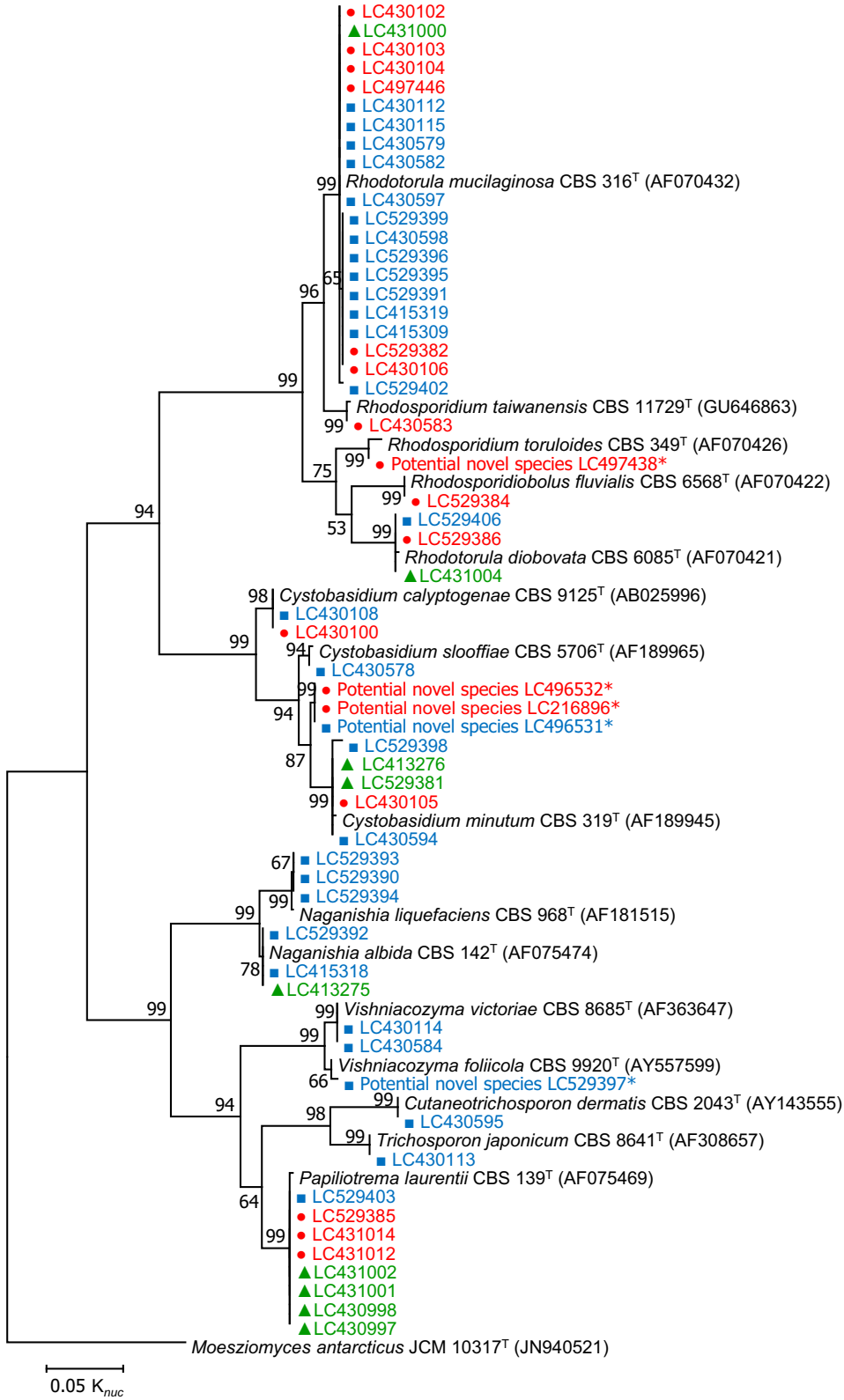


Fig. 3 Phylogenetic relationship based on the sequence of the D1/D2 domain of known yeast species isolated from marine sponges sampling site S1 (green triangle), site S2 (red circle) and site S3 (blue square) in the phylum Ascomycota, and their closely related yeast species retrieved from the GenBank databases. The tree was constructed with the maximum-likelihood method and the general time reversible (GTR) evolutionary model in the MEGA software version 7.0. Numbers at nodes indicate the bootstrap percentage (> 50%) derived from 1000 datasets. *Schizosaccharomyces pombe* NRRL Y-12796 ^{T} (JQ689077) was used as the outgroup. The scale bar indicates an evolutionary distance of 0.05 K_{nuc}. (colour figure online)

Yeast isolation and identification

From the samples collected at site S1, 17 yeast strains were isolated from eight of the 15 marine sponge samples, indicating that yeasts were isolated from 53.3% of the samples. From the 23 marine sponge samples collected at site S2, yeasts could be isolated from 16 samples (69.6%) and 30 yeast strains were


LC430102
LC431000
LC430103
LC430104
LC497446
LC430112
LC430115
LC430579
LC430582
Rhodotorula mucilaginosa CBS 316T (AF070432)
LC430597
LC529399
LC430598
LC529396
LC529395
LC529391
LC415319
LC415309
LC529382
LC430106
LC529402
Rhodosporidium taiwanensis CBS 11729T (GU646863)
LC430583
Rhodosporidium toruloides CBS 349T (AF070426)
Potential novel species LC497438*
Rhodosporidiobolus fluvialis CBS 6568T (AF070422)
LC529384
LC529406
LC529386
Rhodotorula diobovata CBS 6085T (AF070421)
LC431004
Cystobasidium calyptogenae CBS 9125T (AB025996)
LC430108
LC430100
Cystobasidium slooffiae CBS 5706T (AF189965)
LC430578
Potential novel species LC496532*
Potential novel species LC216896*
Potential novel species LC496531*
LC529398
LC413276
LC529381
LC430105
Cystobasidium minutum CBS 319T (AF189945)
LC430594
LC529393
LC529390
LC529394
Naganishia liquefaciens CBS 968T (AF181515)
LC529392
Naganishia albida CBS 142T (AF075474)
LC415318
LC413275
Vishniacozyma victoriae CBS 8685T (AF363647)
LC430114
LC430584
Vishniacozyma foliicola CBS 9920T (AY557599)
Potential novel species LC529397*
Cutaneotrichosporon dermatis CBS 2043T (AY143555)
LC430595
Trichosporon japonicum CBS 8641T (AF308657)
LC430113
Papiliotrema laurentii CBS 139T (AF075469)
LC529403
LC529385
LC431014
LC431012
LC431002
LC431001
LC430998
LC430997
Moesziomyces antarcticus JCM 10317T (JN940521)
0.05 Knuc

◀**Fig. 4** Phylogenetic relationship based on the sequence of the D1/D2 domain of known yeast species and potential novel species isolated from marine sponges in sampling site S1 (green triangle), site S2 (red circle) and site S3 (blue square) in the phylum Basidiomycota, and their closely related yeast species retrieved from the GenBank databases. The tree was constructed with the maximum-likelihood method and the GTR evolutionary model in the MEGA software version 7.0. Numbers at nodes indicate the bootstrap percentage (> 50%) derived from 1000 datasets. *Moesziomyces antarcticus* JCM 10317^T (JN940521) was used as the outgroup. The scale bar indicates an evolutionary distance of 0.05 K_{nuc}. (colour figure online)

detected. Forty (40) yeast strains were isolated from 18 (54.5%) of the 33 marine sponge samples collected at site S3.

In total, 87 yeast strains isolated from 42 marine sponge samples were identified based on the similarity of the D1/D2 domain sequences of the LSU rRNA gene (Table S1). The results revealed that 31 strains (36%) belonged to 11 known yeast species in five genera of the subphylum Saccharomycotina, phylum Ascomycota, while 56 strains (64%) were identified as 13 known yeast species and three potential novel species belonging to eight genera of the two subphyla: Agaricomycotina (6 species, 19 strains) and Pucciniomycotina (7 species, 37 strains) in the phylum Basidiomycota (Table 2; Figs. 3, 4, 5).

The current study obtained a higher number of yeast strains in the phylum Basidiomycota than in the phylum Ascomycota. The results showed both agreement with and contrast to results of other investigations on yeasts associated with marine invertebrates. In the case of investigations with which the current study's results agreed, marine occurring yeasts isolated from marine sponges collected from the North Sea and the Mediterranean mostly belonged to the order Malasseziales of the phylum Basidiomycota (Naim et al. 2017). Of marine yeast strains isolated from shrimp (*Peneaus setiferus*) collected from the Gulf of Mexico, more were in the phylum Basidiomycota than in the phylum Ascomycota (Phaff et al. 1952). Moreover, marine occurring yeasts isolated from corals and zoanthids in the Gulf of Thailand mainly belonged to the phylum Basidiomycota (Kaewkrajay et al. 2020). Conversely, the results of the following investigations contrasted with the current study's results. Vaca et al. (2013) reported that the marine ascomycetous yeast was the predominant yeast in marine sponges collected near King George Island in Antarctica. Culturable marine yeasts associated with zoanthids collected at Maceió in northeast Brazil mostly belonged to the phylum Ascomycota (Paulino et al. 2017). Li and Wang (2009) also reported that the cultivated fungi isolated from marine sponges belonged to at least 25 genera of the phylum Ascomycota and one genus of the phylum Basidiomycota. Marine environmental conditions, such as depth, salinity, pH, and temperature, and substrates, such as marine sponges, shrimps, corals, and zoanthids from which yeasts are isolated, therefore might influence the composition of yeast species and communities.

In the current study, nine of the 11 ascomycetous yeast species, namely, *Candida nonsorbophila*, *Candida orthopsilosis, Candida parapsilosis, Candida sake, C. tropicalis, Meyerozyma caribbica, M. guilliermondii, Kodamaea ohmeri*, and *Pichia kudriavzevii* were reported to have previously been found in marine environments. These environments included seawater, sea sediments, beach sand, sea stars, seaweeds, sea isopods, sea snails, shrimp, corals, zoanthids, and mangrove forest (Seshadri and Sieburth 1971; Pagnocca et al. 1989; Prabhakaran and Gupta 1991; Papadakis et al. 1997; Gadanho and Sampaio 2005; Loureiro et al. 2005; Vogel et al. 2007; Chen et al. 2009; Nakase et al. 2009; Singh et al. 2012; Duarte et al. 2013; Zhang et al. 2014; Rédou et al. 2015; Kunthiphun et al. 2018; Kaewkrajay et al. 2020). Among these yeast species, *C. tropicalis* and *M. guilliermondii* were previously reported as having been isolated from marine sponges (Gao et al. 2008; Burgaud et al. 2010). Two ascomycetous yeast species, *Candida conglobata* and *Magnusiomyces capitatus*, obtained in the current study had not previously been reported in marine environments. These yeast species were reported as having been isolated from terrestrial habitats. *C. conglobata* was reported as having been obtained from a tubercular lung (Kurtzman et al. 2011) and bloodstream (Sweih et al. 2017), while *Mag. capitatus* was isolated from dishwashers (Zalar et al. 2011; Gümral et al. 2016). In addition, 11 of the 13 basidiomycetous yeast species detected in this study, namely, *Cutaneotrichosporon dermatis*, *Cystobasidium calyptogenae*, *Cystobasidium minutum*, *Cystobasidium slooffiae*, *Naganishia albida*, *Naganishia liquefaciens*, *P. laurentii*, *Rhodosporidiobolus fluvialis, R. diobovata*, *R. mucilaginosa*, and *Vishniacozyma victoriae* were previously isolated from marine environments, such as seawater, sea sediment, beach sand, shrimp, sea fish, sea urchins,

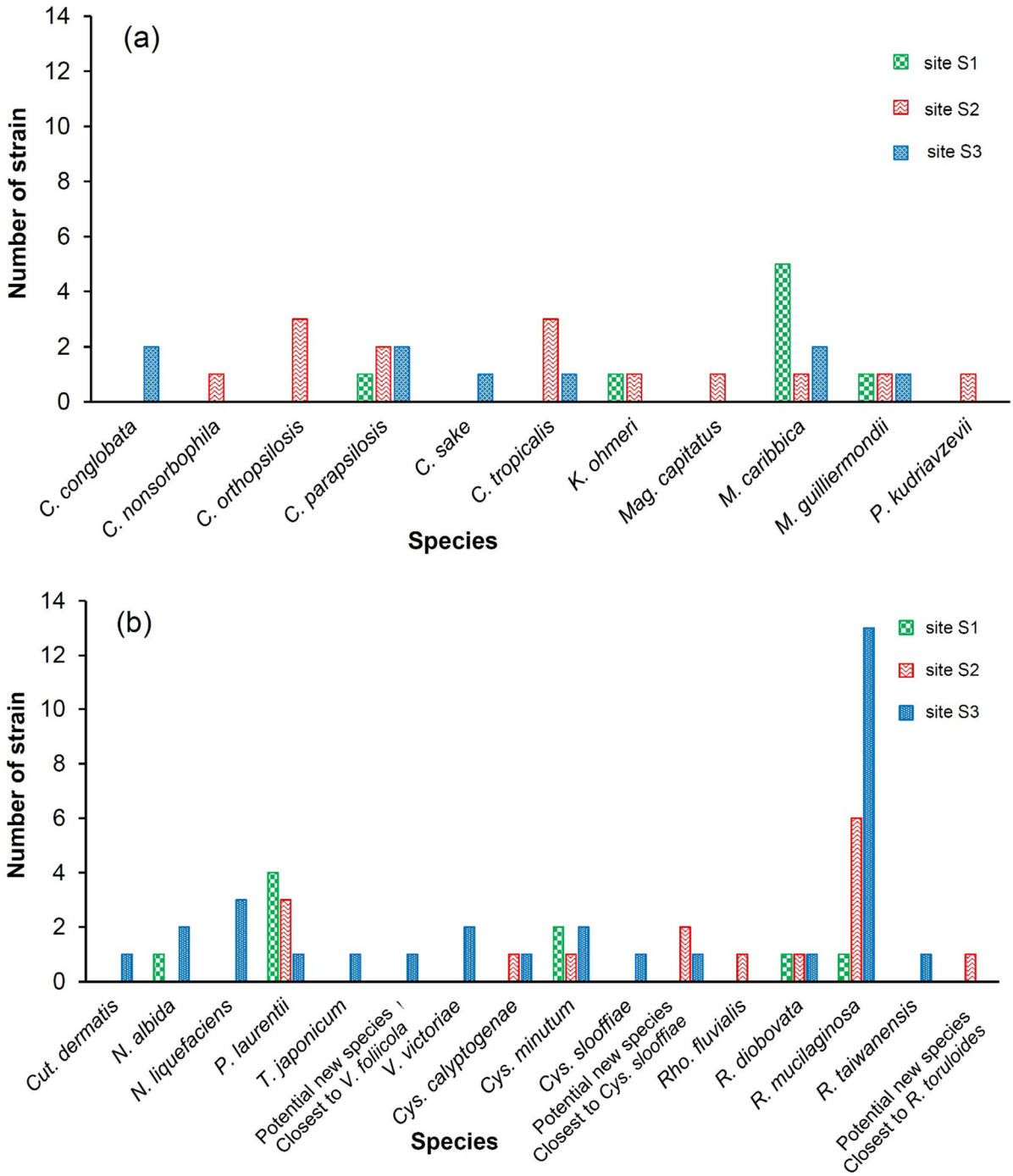


Fig. 5 Number of yeast strains of each yeast species collected at site S1, S2 and S3. **a** ascomycetous yeast, and **b** basidiomycetous yeast

algae, sea squirts, corals, and zoanthids (Gadanho et al. 2003; Loureiro et al. 2005; Nagano et al. 2010; Yang et al. 2011; Singh et al. 2012; Duarte et al. 2013; Xu et al. 2014; Rédou et al. 2015; Kaewkrajay et al. 2020). Among these basidiomycetous species, *P. laurentii*, *R. diobovata*, and *R. mucilaginosa* had

previously been isolated from marine sponges (Burgaud et al. 2010; Duarte et al. 2013). Two basidiomycetous yeast species isolated in the current study, namely, *Trichosporon japonicum* and *Rhodotorula taiwanensis*, had not previously been reported in marine environments, but they had been isolated from terrestrial habitats. *R. taiwanensis* was reported as having been obtained from plant leaves (Huang et al. 2011; Limtong et al. 2014) and peat soil in peat swamp forests (Satianpakiranakorn et al. 2020), while *T. japonicum* was isolated from the air (Sugita and Nakase 1998).

In the current study, ascomycetous yeast species (except for *C. conglobata* and *Mag. capitatus*) and basidiomycetous yeast species (except for *R. taiwanensis* and *T. japonicum*) were found in marine sponges and in other marine substrates, as reported by other investigators. *C. parapsilosis* was isolated from sponges collected from all three sampling sites, whereas *C. tropicalis* were found at sites S2 and S3 and *Cut. dermatis* and *T. japonicum* we detected at site S3. This result indicated that all three sites may be affected by terrestrial run-off and human activities (Paulino et al. 2017).

Yeast diversity

In the current study, yeasts were isolated from 42 (59%) of the 71 marine sponge samples. Of the sponge samples with yeasts, *R. mucilaginosa* was isolated from 20 samples (28.2%); therefore, this species had the highest occurrence. This was followed by *M. caribbica* and *P. laurentii* which were isolated from eight samples (11.3%), and *C. parapsilosis* and *Cys. minutum* which were isolated from five samples (7.0%) (Table 2). These five yeast species were isolated from samples collected at all three sampling sites. The remaining species were isolated from one to four samples. Five yeast species were isolated from only one marine sponge sample, whereas four yeast species were isolated from three samples. One, two, and three yeast species were obtained from 16, 12, and 10 marine sponge samples, respectively. Interestingly, among these yeast species, *C. parapsilosis* and *M. caribbica* were generally distributed at all sampling sites in the summer and winter; however, they could not be isolated in the rainy season. *P. laurentii* was also distributed at all sampling sites, but only in the winter season, while *Cys. minutum* could not be detected in the winter season (Table 1).

Among the 71 marine sponge samples, yeasts could not be isolated from some sponge species, namely, *Monanchora unguiculata* (6 samples), *Neopetrosia* sp. "blue" (3 samples), *Lamellodysidea herbacea* (4 samples), *Haliclona* (*Soestella*) sp. (4 samples), and *Biemna fortis* (1 sample). Some marine sponge genera produced secondary metabolites that could inhibit microorganisms. During the sponge feeding process, the currents driven by sponges have been reported to continuously swirl microorganisms, including yeasts, from the environment, with most of these microorganisms retained in the bodies of sponges (Selvin et al. 2010). However, some marine sponge genera have been found to produce secondary metabolites that could inhibit some microorganisms. Demerdash et al. (2018), in the summary of their literature survey, reported that sponges in the genera *Batzella*, *Crambe*, and *Monanchora* are rich sources of bioactive compounds. These compounds include highly physiologically active pyrroloquinoline and guanidine-derived alkaloids, revealing the vast scope of biological potentiality, such as cytotoxic and antimicrobial activity, and enzyme inhibition. Thus, some sponge genera do not contain yeast. Research has found that associations between sponges and microbes were widely distributed across sponge taxa and, in some cases, were unique to sponges (Hentschel et al. 2002, 2006). Maldonado et al. (2005) proposed that

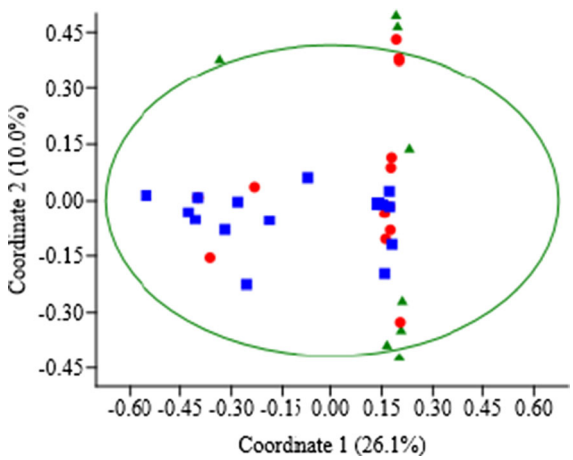


Fig. 6 Principle Coordinate Analysis (PCoA) plots of marine occurring yeast communities in 71 marine sponge samples of site S1 (filled green triangle), S2 (filled red circle) and S3 (filled blue square) using the Jaccard similarity coefficient

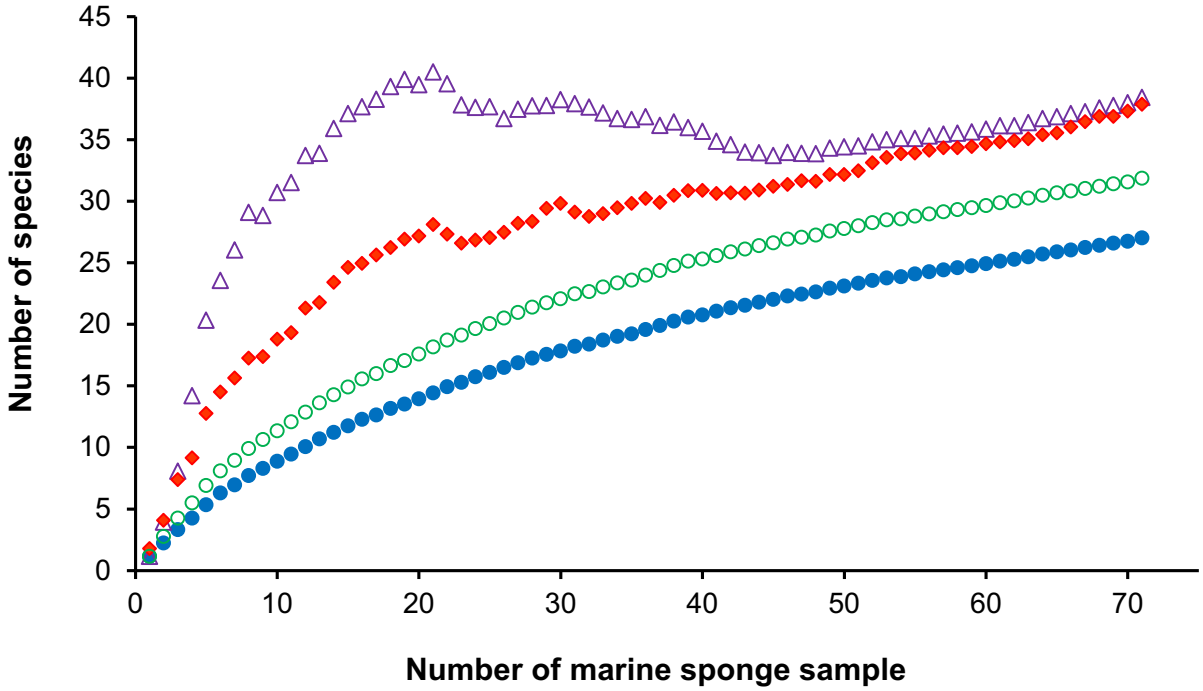


Fig. 7 The number of species observed (filled blue circle) and estimated species richness of marine sponge yeast using Bootstrap (unfilled green circle), Chao 1 (filled red diamond) and ICE (unfilled purple triangle)

symbiotic yeast cells isolated from demosponges of the genus *Chondrilla* were transmitted from the soma through the oocytes to the fertilized eggs, and that these yeasts were not present in other demosponge genera. The term "sponge-specific microorganisms" was introduced for all microbes repeatedly detected in sponges from different seas and oceans around the world (Hentschel et al. 2002; Taylor et al. 2007). In addition, Godinho et al. (2019) studied the diversity of cultivable fungi associated with marine animals (Phylum Mollusca, Nemertea, Chordata, Cnidaria, Annelida, Echinodermata, Arthopoda, and Platyhelmintes). The results indicated that the diversity, richness, and dominance of fungi differed among the hosts.

To find out if relationships existed between sponge species and yeast species, the current study checked the yeast species in two sponge species, namely, *X. testudinaria* (14 samples) and *C. reinwardti* (12 samples), for both of which high numbers were collected. From the 14 samples of *X. testudinaria*, yeasts could be isolated from 11 samples and 11 yeast species were isolated. Each sample contained one to three yeast species. *R. mucilaginosa* was present in five sponge samples, representing a frequency of occurrence (FO) of 35.7%, whereas *M. caribbica*, *M. guilliermondii*, and *Cys. minutum* were each isolated from two sponge samples. Each of the other yeast species was detected in one sample. Therefore, in the marine species, *X. testudinaria*, the species *R. mucilaginosa* was the yeast species with the highest occurrence. At site S3, the yeast species seemed to be different in each sample, although four samples of the *X. testudinaria* sponge were collected on the same date. In addition, in this sponge species, the yeast species obtained seemed to be different on each sampling date (Table 1). Of the 12 samples of the *C. reinwardti* sponge, nine samples yielded yeasts, with 12 yeast species identified. Each sample yielded one to four yeast species. Among the 12 yeast species, *P. laurentii* was detected in three samples collected at site S1 site on the same date (21 January 2017). Each of the species, *C. orthopsilosis*, *C tropicalis*, *M. caribbica*, and *R. mucilaginosa*, was present in two samples. Each of the other yeast species was isolated from only one sponge sample. The yeast species were different on three *C. reinwardti* samples collected at site S2 on 21 January 2017, when compared to the

yeast species obtained from seven *C. reinwardti* samples collected at site S2 on different dates. The result also showed different yeast species (Table 1). In addition, the low number of yeast strains isolated could affect this analysis.

The classical Jaccard similarity coefficient was used to compare the similarity of the yeast communities from each sampling site. The similarity coefficient values were in the range of 0.37–0.44. The average value was 0.40 which meant that each sampling site shared 40% of the species with the other sites. A comparatively higher similarity was observed between sampling sites S1 and S2. In contrast, comparison of the similarity of sampling sites S2 and S3 showed that they had the lowest similarity index score. However, the yeast communities of 71 samples collected from the three sampling sites showed no marked differences in similarity when using the PCoA plot base on the Jaccard similarity indices (Fig. 6). This result was consistent with our prior investigation which showed that yeast communities associated with corals and zoanthids collected from the Gulf of Thailand were not markedly different (Kaewkrajay et al. 2020). Furthermore, all zoanthid species from a Brazilian reef had associated yeast communities that were relatively homogeneous (Paulino et al. 2017).

Estimation of the expected species richness of the current study's sampling efforts, by bootstrap, Chao 1, and ICE estimators, showed a higher than observed species richness (Fig. 7). This was calculated with species accumulation curves by employing the number of each yeast species of each sample in EstimateS software. These results indicated that some yeast species remained unobserved and unculturable: the results were also in agreement with findings of other investigations (Khunnamwong et al. 2018; Srisuk et al. 2019; Into et al. 2020). Therefore, in future research on yeast communities associated with marine sponges, culture-independent methods, such as using next-generation sequencing (NGS), should be used along with the culture-dependent approach to obtain the complete information.

Conclusions

The marine occurring yeasts obtained in the current study by isolation from marine sponges mostly belonged to the phylum Basidiomycota. The species with the highest occurrence was *R. mucilaginosa*. The classical Jaccard similarity coefficient showed the similarity of the yeast communities, with each sampling site sharing 40% of the species with the other sites. The yeast communities of 71 samples from the three sampling sites showed no marked differences in similarity when using principal coordinates analysis (PCoA). However, the marine sponge–yeast association is still unclear. More systematic investigations need to be carried out of the association of marine occurring yeast with marine sponges.

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Authors' contribution C.K.: Performed research and wrote the paper, S.P.: Performed research, S.L.: Designed study, data discussion and wrote the paper.

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Compliance with ethical standards

Conflict of Interest The authors declare that there are no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors. The Thailand Animal Science Act 2015 defines animals as not including marine sponges.

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