MYCOBIOLOGY
2022, VOL. 50, NO. 6, 439–447
https://doi.org/10.1080/12298093.2022.2147135

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Description of *Vishniacozyma terrae* sp. nov. and *Dioszegia terrae* sp. nov., Two Novel Basidiomycetous Yeast Species Isolated from Soil in Korea

Soohyun Maeng[a]*, Yuna Park[a,b]*, Gi-Ho Sung[c,d], Hyang Burm Lee[e], Myung Kyum Kim[a] and Sathiyaraj Srinivasan[a]

[a]Department of Bio and Environmental Technology, College of Natural Science, Seoul Women's University, Seoul, Republic of Korea; [b]Division of Bioresources Bank, Honam National Institute of Biological Resources, Mokpo, South Korea; [c]Translational Research Division, Biomedical Institute of Mycological Resource, International St. Mary's Hospital and College of Medicine, Catholic Kwandong University, Incheon, Korea; [d]Department of Microbiology, International St. Mary's Hospital and College of Medicine, Catholic Kwandong University, Gangneung, Korea; [e]Environmental Microbiology Lab, Department of Agricultural Biological Chemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, Korea

ABSTRACT

Two strains, YP344 and YP579 were isolated from soil samples in Pocheon City, Gyeonggi Province, South Korea. The strains YP344 and YP579 belong to the genus *Vishniacozyma* and *Dioszegia*, respectively. The molecular phylogenetic analysis showed that the strain YP344 was closely related to *Vishniacozyma peneaus*. Strain YP344^T differed by four nucleotide substitutions with no gap (0.70%) in the D1/D2 domain of the LSU rRNA gene and 16 nucleotide substitutions with 8 gaps (5.76%) in the ITS region. On the other hand, the strain YP579^T varied from the type strain of the most closely related species, *Dioszegia zsoltii* var. *zsoltii*, by 6 nucleotide substitutions with four gaps (1.64%) in the D1/D2 domain of LSU rRNA gene and 26 nucleotide substitutions with 14 gaps (8.16%) in the ITS region. Therefore, the name *Vishniacozyma terrae* sp. nov. and *Dioszegia terrae* sp. nov. are proposed, with type strains YP344^T (KCTC27988^T) and YP579^T (KCTC 27998^T), respectively.

ARTICLE HISTORY
Received 26 October 2022
Accepted 9 November 2022

KEYWORDS
Two new species; basidiomycota; *Vishniacozyma*; *Dioszegia*; Pocheon city; soil; taxonomy

1. Introduction

The yeast belonging to the basidiomycetous are currently recognized in three classes of the phylum *Basidiomycota*: *Ustilaginomycetes*, *Urediniomycetes*, and *Hymenomycetes* [1]. These yeasts belong to those phyla that have considerable agricultural, medical, and economic importance. An estimate suggested that only up to 5% of the yeast species belong to the *Basidiomycota* in nature. At the time of writing (October 2022), about 500 species of basidiomycetous yeasts have been widely recognized; among them, the psychrophilic yeast belonging to the genus *Vishniacozyma* and *Rhodotorula* have been discovered in northern regions, glacial mountains, and polar habitats [2–4]. The fungi that adapted to grow in the cold environment can decompose diverse types of organic compounds that play an essential role in the nutrient cycles of polar microbial ecosystems [5–8].

Dioszegia genus forms a monophyletic group within the *Tremellales* (*Tremellomycetes*, *Agaricomycotina*) [9,10]. In 2015, Liu et al. [9] reconstructed the phylogeny of the tremellomycetous yeasts and related dimorphic and filamentous *Tremellomycetes* using sequence analysis, and proposed a novel family *Bulleribasidiaceae* for *Dioszegia* within the order *Tremellales*. As of this writing, all species characterized so far are nonfermentative, may or may not form ballistoconidia, and do not have evidence of a sexual stage. Most species of *Dioszegia* have been isolated from leaves, roots, or soil [11]. There is an accumulation of carotenoid pigments within the cells of yeasts in the genus Dioszegia, resulting in the salmon, pink or red color of their colonies [12,13].

As part of our yeast biodiversity study in Pocheon, South Korea, we isolated three strains of the genus *Vishniacozyma* and one strain of the genus *Dioszegia* from a soil sample. According to sequence analysis of the D1/D2 domain of the large subunit rRNA gene and the internal transcribed spacer (ITS) region, these yeasts are closely related to *Vishniacozyma peneaus* and *Dioszegia zsoltii* var.

CONTACT Hyang Burm Lee hblee@chonnam.ac.kr; Srinivasan Sathiyaraj drsrini@swu.ac.kr; Myung Kyum Kim biotech@swu.ac.kr

*These authors contributed equally to this work.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/12298093.2022.2147135.

zsoltii, in the *Tremellales*, *Tremellomycetes*, and *Basidiomycota*.

2. Materials and methods

2.1. Yeast isolation and phenotypic characteristics

The soil samples were collected in Pocheon city, Gyeonggi Province, South Korea (37°46′18.7″N 127°09′46.3″E, 37°51′19.2″N 127°10′56.6″E, 37°50′38.4″N 127°09′34.1″E, and 37°47′54.8″N 127°10′01.6″E) during winter (Table 1). The soil sample (1 g) was suspended in 9 ml of sterile saline and serially diluted until they were 1:10 to 1:1000, and then spread 0.1 ml of each on Yeast-Malt Agar (YMA, Difco, Detroit, USA) plates and incubated at 25 °C for 3–4 days. The strains YP344, YP155, YP333, and YP579 were isolated and purified by cross-streaking on a YM agar medium (1% yeast extract, 2% peptone, 2% glucose, and 1.5% agar). The pure culture of these strains was deposited at the Korea Collection for Type Cultures, KRIBB, Korea. The purified yeasts were maintained in 20% v/v glycerol at −80 °C.

2.2. DNA sequencing and phylogenetic analysis

The four yeast strains (YP344^{T}, YP155, YP333, and YP579^{T}) were identified by analysis of D1/D2 domain of the LSU rRNA gene and ITS region. The genomic DNA was amplified by PCR with NL1/NL4 [14] and ITS1/ITS4 primers [15], respectively. In order to assemble the contigs, the SeqMan program version 7.1.0 was used. The BLAST search of pairwise sequences [16] was conducted and alignments were performed with Clustal X 2.0 [17] to match sequences from related species. Based on the combined sequences of the ITS region and the D1/D2 domains of the LSU rRNA gene, phylogenetic trees were constructed using the MEGA X program [18–20]. The evolutionary distances were calculated by the general time reversible (GTR) and Kimura two-parameter model for the maximum-likelihood and neighbor-joining analyses, respectively [21,22]. A bootstrap analysis was also conducted using 1000 replicates [23]. The BLAST tool software (https://blast.ncbi.nlm.nih.gov), was used to calculate sequence similarities and nucleotide substitutions in ITS and D1/D2 regions between newly isolated strains and closely related species.

2.3. Phenotypic characterization

For the microscopic examination, four strains were cultivated on YM agar at 15 °C and examined using a phase-contrast microscope (DM500, LEICA, Wetzlar, Germany). The strains were phenotypically characterized by the methods reported by Kurtzman et al. [24]. For the physical examination, strains were cultivated on YM agar at 15 °C and examined with a phase-contrast microscope (DM500). To stimulate the sexual reproductive phase and spore formation of the yeast cells, single

Table 1. List of the yeast strains of *Vishniacozyma terrae* sp. nov. examined in the present study and related species.

					GenBank accession numbers	
Genus	Species	Strain ID	Isolation source	Location	D1/D2	ITS
Vishniacozyma	*treae*	YP344	Soils	Pocheon City, Korea	MZ734225	MZ734447
	alagoana	CBS15966	A tropical rainforest/a seasonally dry tropical forest	Northeast and Southeastern Brazil	MH909005	MH885328
	carnescens	CBS 973	Muscatel grape	USA	KY105817	NR130695
	changhuana	HM6L11	Mangrove forests	Taiwan	MT906468	MT906456
	dimennae	CBS 5770	Di Menna from pasture plants	New Zealand	AF075489	NR144808
	ellesmerensis	JCM 32573	Sediments and soil at the front of a retreating glacier	Canadian Arctic	LC335796	LC335796
	europaea	CGMCC 2.3099	Phylloplane	Germany	MK050335	MK050335
	foliicola	CBS 9920	Plant leaves	Shennongjia, Hubei province, China	AY557599	NR144809
	globispora	CBS 6981	Plant	–	AF075509	NR073235
	heimaeyensis	CBS 8933	Soil	Iceland	DQ000317	NR077070
	kurtzmanii	CBS 12229	The surface of maize kernels	Minnesota, USA	MH718303	MH718303
	melezitolytica	CBS15490	Phylloplane	Hebei province, China	MK050330	MK050330
	nebularis	CBS 12283	–	–	EU266921	EU266921
	peneaus	CBS 2409	Surface washing of shrimp	Texas, the gulf of Mexico	AB035051	NR165987
	phoenicis	KBP Y-6564	Fruit	Moscow, Russia	MN449981	MN449981
	pseudopenaeus	CGMCC2.3165	Phylloplane	Germany	MK050333	MK050333
	psychrotolerans	CBS 12690	Subglacial ice	Austre lovénbreen glaciers, Norway	JN193445	JN193464
	taibaiensis	CBS 9919	Plant leaves	Tabai mountains, China	NG058434	NR144810
	taiwanica	HM5L06	Mangrove forests	Taiwan	MT906477	MT906464
	tephrensis	CBS 8935	Soil	Iceland	KX507032	NR144812
	victoriae	CBS 8685	Soil	Antarctica	AF363647	NR073260

or mixed cultures of the both strains were incubated at 10 °C for 2 months on YM agar. Pseudohyphae and true hyphae have been observed weekly during cell culture on YM agar at 10 °C for up to 2 months. Basidiospore formation was confirmed by growing the individual strains on corn meal agar (2% corn meal infusion and 2% agar), 5 % malt extract agar (5 % malt extract and 1.5 % agar), potato dextrose agar (PDA, Difco, Detroit, USA), YM agar, and yeast extract–peptone glucose (YPD, Difco, Detroit, USA) at 25 °C for 2 months. The color reaction with diazonium blue B (DBB, Sigma-Aldrich, Darmstadt, Germany) was performed by dropping the DBB reagent into the 3 days incubated colonies grown on YM agar medium and observing the color development after 2 min. Growth was accessed at various temperatures (4 °C, 10 °C, 15 °C, 25 °C, 30 °C, 35 °C, 37 °C, 42 °C, and 45 °C) and determined by cultivation on PDA, YPD agar, and YMA for 15 days. Growth in YM broth with different NaCl concentrations (0%–10% in 1% intervals, w/v) was studied for 1–5 days. Ubiquinone was prepared, extracted and analyzed as described by Prillinger et al. [25].

3. Results and discussion

3.1. Species identification and delineation

In the study, a total of 472 yeast strains were isolated from 35 soil samples collected in the city of Pocheon, Gyeonggi Province, South Korea. Of these strains, 11 were classified as *Vishniacozyma* (taxonomy: *Basidiomycota*, *Agaricomycotina*, *Tremellomycetes*, *Tremellales*, *Bulleribasidiaceae*) and one were classified as *Dioszegia* (taxonomy: *Basidiomycota*, *Agaricomycotina*, *Tremellomycetes*, *Tremellales*, *Bulleribasidiaceae*) by analyzing sequences of the ITS and the D1/D2 domain of the LSU rRNA gene. As a result, three strains (YP344, YP155 and YP333) were classified as the new *Vishniacozyma* species and one strain (YP579) was classified as the new *Dioszegia* species. MycoBank numbers of the strains YP344 and YP579 are MB841262 and MB841263, respectively.

Strain YP344 was most closely related to *V. peneaus* with 94.2% sequence identity, against which 4 nt substitutions were observed in the D1/D2 domains and 16 nt substitutions in the ITS regions of YP344 were replaced by 16 nt substitutions. Phylogenetic trees constructed by maximum-likelihood and neighbor-joining methods showed that strains YP344, YP155 and YP333 were grouped with members of the genus *Vishniacozyma* (Figure 1 and Supplementary Figure S1).

The strain YP579 was most closely related to *D. zsoltii* var. *zsoltii* with sequence identities of 91.84%, against which 6 nt substitutions were observed in the D1/D2 domain (Table 2) and 26 nt the sequence of the ITS region. The phylogenetic trees generated by maximum-likelihood and neighbor-joining

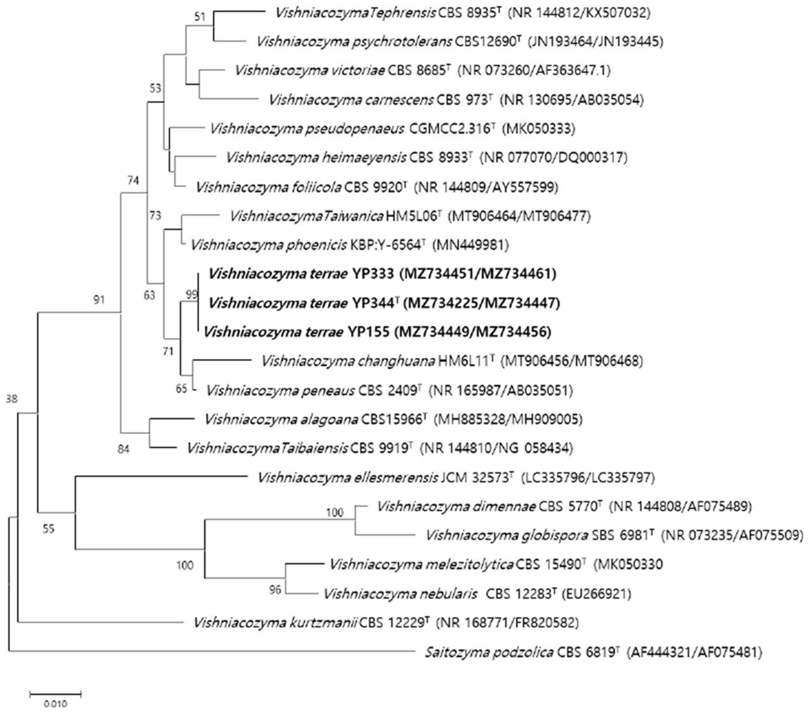

Figure 1. Phylogenetic tree based on the concatenated sequences of the D1/D2 region of the LSU rRNA gene and ITS regions and constructed by the neighbor-joining method, shows relationships between strains of a novel species (YP344, YP155, and YP333) and closely related species. The novel species described in this manuscript are highlighted in bold. *Saitozyma podzolica* CBS 6819^T was used as outgroup. Bootstrap values >50% (% of 1000 replications) were shown at branch points. Accession numbers were shown in parentheses. Bar, 0.01 substitutions per nucleotide position.

Table 2. List of the yeast strains of *Dioszegia terrae* sp. nov. examined in the present study and related species.

					GenBank accession numbers	
Genus	Species	Strain ID	Isolation source	Location	D1/D2	ITS
Dioszegia	*trreae*	YP579	Soils	Pocheon City, Korea	MZ734403	MZ734406
	anctartica	CBS 10920	The culturable soil fungal population	Taylor Valley, Antarctica	FJ640575	DQ402529
	athyri	AS 2.2559	Senescent leaves	different regions of China	EU070931	EU070926
	aurantiaca	CBS 6980	Leaves of selected Mediterranean plant species	Portugal (Arra'bida Natural Park)	AB104689	AB049613
	buhagiarii	CBS 10054	Leaves of selected Mediterranean plant species	Portugal (Arra'bida Natural Park)	AY562151	AY885687
	butyracea	AS 2.2600	Senescent leaves	different regions of China	EU070929	EU070924
	catarinonii	CBS 10051	Leaves of selected Mediterranean plant species	Portugal (Arra'bida Natural Park)	AY562142	AY562154
	changbaiensis	CBS 9608	Basidiomycetous yeasts	Northeast China	NG059069	NR136964
	crocea	CBS 6714	Leaves of selected Mediterranean plant species	Portugal (Arra'bida Natural Park)	AF075508	NR155062
	cryoxerica	ANT-03-071	The culturable soil fungal population	Taylor Valley, Antarctica	FJ640562	FJ640565
	dumuzii	CBS 12501	Forest soils	Germany	–	LT548261
	fristingensis	CBS 10052	Leaves of selected Mediterranean plant species	Portugal (Arra'bida Natural Park)	NG070549	NR136970
	hungarica	CBS 4214	A birch leaf	Northern Portugal	AF075503	NR073227
	rishiriensis	CBS 11844	A soil sample collected on Rishiri Island	Hokkaido, Japan	AB545810	NR157461
	statzeliiae	CBS 8925	Soils	Antarctic	AY029341	AY029342
	takashimae	CBS 10053	Leaves of selected Mediterranean plant species	Portugal (Arra'bida Natural Park)	AY562149	NR136971
	xingshanensis	AS 2.2481	Senescent leaves	different regions of China	EU070928	EU070923
	zsoltii var.yunnanensis	CBS 9128	Plant leaves	Yunnan, China	AF544246	NR156190
	zsoltii var.zsoltii	AS 2.2089	Plant leaves	Yunnan, China	AF544245	AF385445

methods showed that strain YP579 was grouped with members of the genus *Dioszegia* (Figure 1 and Supplementary Figure S1). The type strain YP344^{T} did not assimilate glycerol and could not grow at 10% NaCl but *V. peneaus* assimilated glycerol and was able to grow at 10% NaCl (Tables 3 and 4). Ballistoconidia are produced on YM agar. Basidiospore formation is not observed on corn meal agar, PDA, YPD agar, 5 % malt extract agar, and YM agar at 15 and 25 °C for 2 months. The respiratory quinone is Q-10.

Diazonium blue B and urease reactions are positive, consistent with the characteristics of the genus *Dioszegia* [24,26]. In addition, the type strain YP579 did not assimilate galactitol and D-mannitol. In contrast, *D. zsoltii* var. *zsoltii* did assimilate galactitol and D-mannitol. Ballistoconidia are produced on YM agar after 53 days of incubation. Basidiospore formation is not observed on PDA, corn meal agar, 5 % malt extract agar, YPD agar, and YM agar at 15 and 25 °C for 2 months. The respiratory quinone is Q-10. Based on these results, YP344 and YP579 should be considered a new species, for which the name *Vishniacozyma terrae* (*terrae* of the soil) and *Dioszegia terrae* (*terrae* of the soil) is proposed.

3.2. Description of V. terrae *Park, Maeng, and Sathiyaraj sp. nov.*

V. terrae (ter′rae. L. gen. n. *terrae* of the soil, referring to the isolation source of the type strain)

Novel yeast species belonging to phylum *Basidiomycota*, subphylum *Agaricomycotina*, class *Tremellomycetes*, order *Tremellales*, family *Bulleribasidiaceae*.

Yeast cells after three days on YM agar at 15 °C are ovoid ($6–6.5 \times 1.8–2\,\mu m$). Budding is polar budding (Figure 2). Streak culture on YM agar for 1 week at 15 °C produces cream–color, convex, round, shiny, and slimy colonies. Pseudohyphae and true hyphae are not formed after 53 days of incubation on YMA, PDA, and CMA. Ballistoconidia are not formed after 53 days of incubation on YMA, PDA, and CMA. Basidiospore formation is not formed after 53 days of incubation on YMA, PDA, and CMA.

Table 3. Nucleotide substitutions in the sequences of the D1/D2 domain of the LSU rRNA gene and ITS region of *Vishniacozyma terrae* sp. nov. (YP344^{T}) and *Vishiniacozyma* species.

	YP155	YP344	YP333	13	14	15	18	7	3	9	17	5	16	1	2	20	19	10	6	12	11	4	8
YP155	–	0(0)	0(0)	4(0)	5(0)	6(3)	9(0)	10(0)	10(1)	11(1)	12(0)	12(3)	14(0)	16(0)	16(0)	16(0)	19(3)	30(11)	37(2)	43(5)	47(2)	51(2)	56(4)
YP344	0(5)	–	0(0)	4(0)	5(0)	6(3)	9(0)	10(0)	10(1)	11(1)	12(0)	12(3)	14(0)	16(0)	16(0)	16(0)	19(3)	30(11)	37(2)	43(5)	47(2)	51(2)	56(4)
YP333	0(3)	0(5)	–	4(0)	5(0)	8(3)	9(0)	12(0)	10(1)	11(1)	14(1)	14(3)	14(0)	16(0)	16(0)	16(1)	19(4)	32(12)	37(2)	43(5)	49(2)	53(3)	58(4)
13	16(5)	16(7)	16(5)	–	6(0)	8(3)	11(0)	12(0)	7(1)	13(0)	13(0)	18(4)	13(0)	18(0)	18(0)	18(0)	19(1)	42(14)	37(2)	47(6)	46(2)	50(2)	57(4)
14	15(7)	15(8)	15(7)	19(5)	–	22(6)	4(0)	6(0)	11(1)	12(1)	14(0)	37(9)	12(0)	16(0)	13(0)	11(0)	16(3)	36(11)	127(42)	33(2)	112(52)	38(2)	44(3)
15	22(3)	22(4)	22(3)	16(3)	22(6)	–	12(0)	8(0)	17(5)	10(1)	18(0)	88(29)	14(0)	18(0)	15(0)	15(0)	18(4)	44(8)	110(36)	48(4)	204(63)	52(2)	58(4)
18	27(9)	27(10)	27(9)	23(5)	13(7)	23(5)	–	10(0)	13(1)	13(0)	18(0)	17(0)	16(0)	18(0)	17(0)	19(0)	20(1)	31(10)	29(2)	42(4)	41(4)	46(2)	51(4)
7	24(13)	24(15)	24(10)	15(3)	16(8)	22(2)	25(7)	–	19(1)	7(0)	18(0)	7(0)	8(0)	18(0)	11(0)	11(0)	14(2)	29(8)	36(2)	44(4)	47(4)	49(2)	52(4)
3	24(8)	24(10)	24(8)	15(10)	15(6)	19(8)	19(6)	13(5)	–	19(1)	17(1)	16(4)	19(1)	22(1)	25(1)	24(1)	24(2)	29(14)	34(5)	41(6)	44(3)	51(3)	53(5)
9	26(13)	26(15)	26(10)	15(3)	12(6)	19(2)	19(8)	11(0)	20(6)	–	13(1)	11(1)	9(0)	17(0)	18(0)	12(1)	15(3)	32(8)	40(0)	42(4)	41(4)	48(2)	55(4)
17	38(18)	38(18)	39(15)	26(8)	27(5)	34(4)	27(6)	31(14)	32(12)	28(12)	–	19(0)	17(0)	8(0)	25(0)	19(0)	21(4)	39(12)	38(0)	40(4)	40(4)	64(4)	54(4)
5	25(11)	26(13)	25(8)	17(1)	28(7)	59(12)	23(5)	13(2)	25(4)	14(2)	23(13)	–	13(0)	20(0)	18(0)	16(0)	18(4)	28(8)	81(31)	46(4)	51(3)	50(2)	52(4)
16	21(13)	21(15)	21(10)	11(2)	12(7)	13(4)	19(6)	6(0)	16(5)	11(0)	11(2)	11(2)	–	20(0)	17(0)	11(0)	8(0)	26(5)	28(4)	39(2)	33(0)	38(0)	42(3)
1	29(8)	29(9)	29(8)	25(6)	20(8)	28(4)	27(8)	30(12)	36(11)	28(10)	17(6)	29(11)	25(12)	–	27(0)	23(0)	24(3)	34(5)	35(2)	40(4)	41(4)	49(2)	55(4)
2	23(4)	23(6)	42(13)	16(2)	20(12)	22(4)	25(5)	29(13)	24(5)	33(13)	23(12)	31(11)	26(11)	23(12)	–	10(0)	13(1)	39(4)	40(0)	46(6)	48(7)	43(4)	48(6)
20	22(3)	22(5)	22(3)	15(2)	16(10)	21(3)	24(5)	33(13)	23(5)	36(12)	21(10)	34(11)	31(12)	22(11)	18(9)	–	9(3)	31(8)	44(0)	43(4)	45(5)	45(2)	50(4)
19	41(15)	42(19)	41(13)	19(4)	19(12)	37(17)	25(4)	31(12)	27(6)	34(12)	45(19)	35(10)	24(12)	25(11)	19(4)	26(11)	–	30(13)	41(5)	43(5)	45(8)	45(4)	49(8)
10	22(16)	22(16)	22(16)	12(13)	25(8)	88(42)	23(17)	25(16)	14(10)	13(12)	12(17)	53(27)	13(12)	22(19)	15(10)	15(10)	12(11)	–	43(6)	47(9)	47(13)	65(8)	60(7)
6	20(12)	20(12)	20(12)	33(19)	87(32)	76(26)	44(13)	48(33)	31(19)	46(30)	53(34)	81(31)	32(20)	33(28)	31(16)	46(32)	52(29)	79(29)	–	43(8)	85(26)	44(8)	41(8)
12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	11(0)	34(0)	40(2)
11	46(24)	36(23)	46(24)	20(14)	60(36)	141(43)	20(13)	36(26)	24(14)	30(28)	31(32)	51(3)	17(16)	27(28)	17(14)	36(25)	39(25)	99(36)	85(26)	–	–	29(0)	35(2)
4	13(12)	25(21)	25(21)	23(16)	34(24)	29(23)	29(11)	27(24)	25(12)	28(22)	32(32)	34(24)	9(13)	25(26)	27(15)	38(23)	38(22)	43(26)	48(14)	–	34(11)	–	9(2)
8	40(25)	27(21)	40(25)	21(21)	38(24)	50(35)	24(10)	33(19)	26(12)	30(22)	34(31)	35(24)	10(13)	38(28)	23(13)	39(23)	40(22)	39(30)	40(18)	–	39(12)	15(4)	–

Values above the diagonal are number of nucleotide substitutions in the D1/D2 domain of the LSU rRNA gene. Values below the diagonal are number of nucleotide substitutions and sequence similarity (%, in parentheses) in the sequences of the ITS region.

Table 4. Nucleotide substitutions in the sequences of the D1/D2 domain of the LSU rRNA gene and ITS region of *Dioszegia terrae* sp. nov. (YP579^T) and *Dioszegia* species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	–	6(0)	6(0)	8(1)	10(0)	10(0)	5(0)	11(2)	14(0)	12(3)	13(3)	16(1)	14(3)	19(2)	20(1)	19(2)	15(1)	24(2)	24(1)
2	22(13)	–	0(0)	4(1)	14(0)	4(0)	1(0)	13(2)	20(0)	18(3)	17(3)	18(1)	20(3)	21(2)	22(1)	21(2)	21(1)	26(2)	23(1)
3	23(13)	3(0)	–	4(1)	14(0)	4(0)	1(0)	13(2)	20(0)	18(3)	17(3)	18(1)	20(3)	21(2)	22(1)	21(2)	21(1)	26(2)	23(1)
4	18(13)	11(3)	3(1)	–	16(1)	6(1)	3(0)	15(3)	22(1)	20(4)	16(4)	18(2)	20(4)	23(2)	22(1)	23(2)	21(1)	26(2)	25(1)
5	22(22)	23(20)	24(20)	21(18)	–	18(0)	13(0)	6(0)	12(0)	14(3)	11(3)	12(1)	11(3)	19(2)	14(1)	19(2)	13(1)	16(2)	16(1)
6	28(28)	5(3)	0(0)	10(4)	25(18)	–	5(0)	17(2)	23(0)	21(3)	21(3)	21(2)	21(3)	24(2)	23(1)	24(2)	23(1)	27(2)	24(1)
7	19(10)	8(6)	9(6)	8(4)	23(17)	6(6)	–	12(2)	19(0)	17(2)	16(3)	17(1)	19(3)	20(2)	21(1)	20(2)	20(1)	25(2)	24(1)
8	28(28)	19(12)	20(12)	21(12)	27(16)	20(12)	21(10)	–	16(0)	15(4)	12(5)	12(1)	14(5)	17(2)	10(3)	17(2)	16(3)	14(4)	14(3)
9	24(21)	23(23)	23(24)	24(26)	17(17)	22(27)	23(17)	16(14)	–	10(3)	11(3)	19(1)	8(3)	17(2)	17(1)	17(2)	12(1)	21(2)	21(1)
10	23(21)	25(21)	27(21)	21(20)	18(10)	23(23)	21(21)	19(9)	23(18)	–	3(2)	20(2)	10(4)	7(1)	14(1)	7(0)	11(1)	16(3)	18(1)
11	22(26)	23(25)	25(25)	20(25)	11(9)	23(26)	22(22)	18(8)	19(18)	9(3)	–	17(2)	11(4)	8(1)	13(2)	8(1)	10(2)	15(3)	17(2)
12	28(28)	5(3)	6(3)	10(4)	25(18)	17(24)	6(6)	14(15)	22(27)	23(23)	13(12)	–	15(12)	21(1)	14(0)	21(1)	17(0)	18(1)	18(0)
13	19(18)	17(16)	0(0)	20(21)	12(11)	18(20)	18(12)	13(12)	10(9)	14(14)	13(13)	10(9)	–	17(3)	11(2)	17(3)	5(2)	13(3)	13(2)
14	26(30)	25(29)	27(29)	27(26)	24(10)	29(31)	26(25)	27(12)	21(21)	23(10)	17(11)	21(21)	14(1)	–	15(1)	0(0)	18(1)	17(2)	19(1)
15	19(13)	21(11)	23(11)	29(24)	20(12)	29(23)	22(9)	19(11)	16(8)	22(14)	25(13)	16(8)	14(5)	31(14)	–	15(1)	13(0)	4(0)	4(0)
16	22(27)	24(25)	26(25)	20(25)	22(9)	24(26)	21(24)	23(11)	14(15)	21(6)	21(4)	14(15)	20(8)	11(6)	19(12)	–	18(1)	17(2)	19(1)
17	21(19)	16(20)	18(20)	18(27)	12(12)	17(24)	18(17)	13(14)	12(9)	11(14)	13(12)	12(9)	0(3)	24(14)	15(4)	18(11)	–	13(0)	13(0)
18	23(28)	16(11)	17(11)	22(27)	17(15)	23(26)	21(15)	14(13)	10(7)	23(19)	19(17)	10(7)	14(8)	25(21)	13(3)	14(13)	11(9)	–	4(0)
19	28(22)	21(11)	24(11)	23(12)	23(12)	23(11)	23(9)	19(12)	20(7)	25(13)	27(12)	20(7)	19(5)	36(13)	10(0)	23(11)	18(4)	16(3)	–

Values above the diagonal are number of nucleotide substitutions in the D1/D2 domain of the LSU rRNA gene. Values below the diagonal are number of nucleotide substitutions and sequence similarity (%, in parentheses) in the sequences of the ITS region.

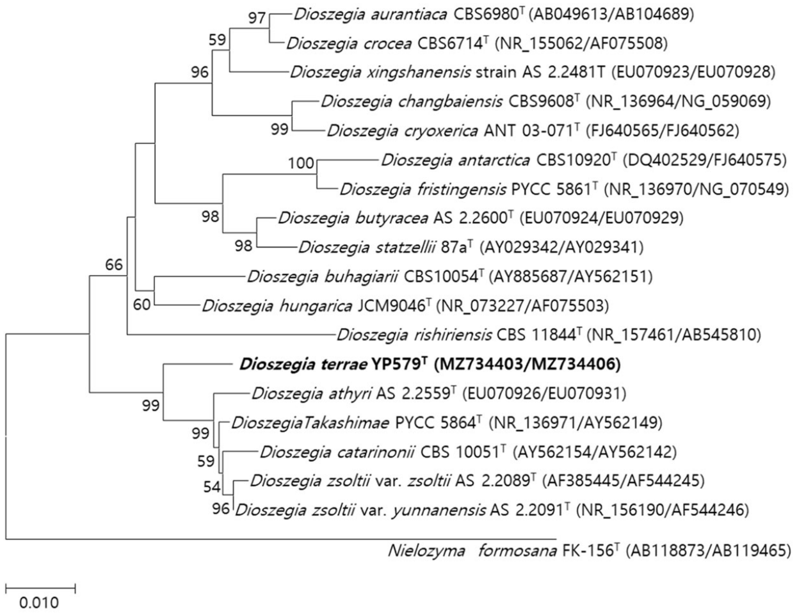

Figure 2. Phylogenetic tree based on the concatenated sequences of the D1/D2 region of the LSU rRNA gene and ITS regions and constructed by the maximum-likelihood method, shows relationships between strains of a novel species (YP579^T) and closely related species. The novel species described in this manuscript are highlighted in bold. *Nielozyma formosana* FK-156^T was used as outgroup. Bootstrap values >50% (% of 1000 replications) were shown at branch points. Accession numbers were shown in parentheses. Bar, 0.01 substitutions per nucleotide position.

Glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, D-galactose, inositol, D-sorbitol, *N*-acetyl-D-glucosamine, D-cellobiose, D-lactose (bovine origin), D-maltose, D-saccharose (sucrose), D-melezitose, adonitol, D-trehalose, and D-raffinose are assimilated, but glycerol, xylitol, and d-methyl-D-glucoside are not assimilated. Growth occurs at 15 °C–37 °C (optimum 25 °C) and cells can tolerate up to 6% NaCl in YM broth. Growth occurs on YM agar, YPD agar, and 50% glucose medium. Growth with 0.01% of cycloheximide is positive. Production of starch and diazonium blue B reaction and urea hydrolysis are negative. The respiratory quinone is Q-10 (Tables 5 and 6).

The holotype, YP344^T, was isolated from the soil sample in Pocheon city, Gyeonggi Province in Korea, and is preserved in a metabolically inactive state at the Korea Collection for Type Cultures, KRIBB, Korea as KCTC 27988^T. The GenBank accession numbers for the D1/D2 domain of the LSU rRNA gene and ITS region for YP344^T are MZ734225 and MZ734447, respectively. The MycoBank accession number is MB 841262.

Table 5. Phenotypic characteristics that differentiate *Vishniacozyma terrae* sp. nov. and related species, *V. peneaus* and *V. phoenicis.*

	1	2	3	4	5
Colony color	Pale yellow	Pale yellow	Pale yellow	Yellow	Pale yellow brown to cream
Growth at/with					
0.01% Cycloheximide	+	+	+	w	w
50% D-Glucose	+	+	+	+	−
10% NaCl	−	−	−	+	−
Assimilation of					
Glycerol	−	−	−	+	v
Adonitol	w	w	w	n	n
Xylitol	−	−	−	+	n
D-galactose	+	+	+	+	w
D-sorbitol	+	+	+	n	n
D-methyl-D-glucoside	−	−	−	+	+
N-acetyl-D-glucosamine	+	+	+	w	+
D-cellobiose	+	+	+	+	n
D-lactose (bovine origin)	+	+	+	w	w
D-maltose	+	+	+	+	+
D-trehalose	w	w	+	+	+

Growth reactions: +, positive; w, weak positive; −, negative; v, variable; n, no data.
Strains: 1, *V. terrae* YP 344^{T}; 2, *V. terrae* YP155; 3, *V. terrae* YP333; 4, *V. peneaus* CBS 2409^{T}; 5, *V. phoenicis* CBS 16172^{T}.
All strains were positive for growth at 30 °C, glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, inositol, D-maltose, Sucrose, D-melezitose, and D-raffinose.
Data for species 1–3 are from the present study, for species 4 and 5 are from previous studies. [27–30].

Table 6. Phenotypic characteristics that differentiate *Dioszegia terrae* sp. nov. and related species, *D. zsoltii* and *D. catarinonii.*

	1	2	3
Colony color	Light orange	Orange	Orange
Ballistoconidia	+	+	−
Growth on/at			
Temp. 30 °C	+	−	−
Growth glucose 50%	−	−	n
Cycloheximide 0.001%	+	n	+
Cycloheximide 0.01%	−	n	−
Assimilation of			
Galactose	+	+	w
Lactose	−	v	+
Methyl-a-D-glucoside	−	v	v
Soluble starch	+	+	v
L-Sorbose	−	v	v
L-Arabinose	+	+	d
D-Arabinose	v	v	v
D-Ribose	v	w	w
Erythritol	−	−	v
Xylitol	+	d	v
Galactitol	−	w	v
D-Mannitol	−	w	v
DL-Lactate	−	v	v
Citrate	−	v	v
Potassium nitrate	v	−	v
L-Lysine	v	+	+
Production of starch	+	w	+

Strains: 1, *D. terrae* YP579^{T}; 2, *D. zsoltii* CBS 9128^{T}; 3, *D. catarinonii* CBS 10051^{T}.
All strains were positive for glucose, sucrose, raffinose, melibiose, trehalose, maltose, melezitose, cellobiose, L-rhamnose, and D-xylose but negative for inulin, methanol, ethanol, glycerol, ribitol, and D-glucitol.
Data for species 1 are from the present study, for species 2 and 3 are from previous studies [31–33].
Growth reactions: +, positive; w, weak positive; −, negative; v, variable; d, delayed positive; n, no data.

3.3. Description of D. terrae *Park, Maeng, and Sathiyaraj sp. nov.*

D. terrae (ter′rae. L. gen. n. *terrae* of the soil, referring to the isolation source of the type strain)

Novel yeast species belonging to phylum *Basidiomycota*, subphylum *Agaricomycotina*, class *Tremellomycetes*, order *Tremellales*, family *Bulleribasidiaceae*.

Yeast cells after 3 days on YM agar at 15 °C are ovoid (6–6.5 × 1.8–2 μm). Budding is polar budding (Figures 2 and 3). Pseudohyphae and true hyphae are not formed after 53 days of incubation. Streak culture are on YM agar for 1 week at 15 °C and produces colonies that are orange–color, convex, round, and slimy. Ballistoconidia is formed after 53 days of incubation but basidiospore is not formed (Figure 4).

Glucose, sucrose, raffinose, melibiose, galactose, trehalose, maltose, melezitose, soluble starch, cellobiose, L-rhamnose, D-xylose, L-arabinose, D-arabinose, D-ribose, xylitol, D-gluconate, D-glucosamine, *N*-acetyl-D-glucosamine, potassium nitrate, cadaverine dihydrochloride, and L-lysine are assimilated. Inulin, lactose, methyl-a-D-glucoside, L-sorbose, methanol, ethanol, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, myo-inositol, DL-lactate, citrate, gluconolactone, and sodium nitrate are not assimilated.

Growth occurs at 10 °C–30 °C (optimum 18 °C) and cells can tolerate up to 4% NaCl in YM broth. Growth occurs on YM agar, YPD agar, and PDA medium but not on 50% glucose medium. Growth in the presence of 0.01% of cycloheximide is positive. Production of starch and diazonium blue B reaction and urea hydrolysis are positive. The respiratory quinone is Q-10.

The holotype, YP579^{T}, was isolated from the soil sample in Pocheon city, Gyeonggi province, South Korea, and is preserved in a metabolically inactive state at the Korea Collection for Type Cultures, KRIBB, Korea as KCTC 27998^{T}. The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain of the LSU rRNA gene and ITS region for YP579^{T} are MZ734403 and MZ734406, respectively. The MycoBank accession number is MB 841263.

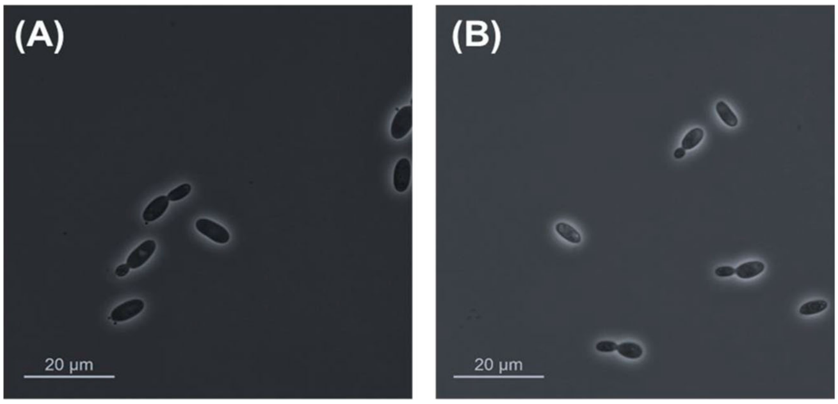

Figure 3. *Vishniacozyma terrae* sp. nov. YP344^T and *Dioszegia terrae* sp. nov. YP579^T (A) The polar budding cells of *V. terrae* YP344^T and (B) *D. terrae* YP579^T on YM agar after 3 days at 10 °C.

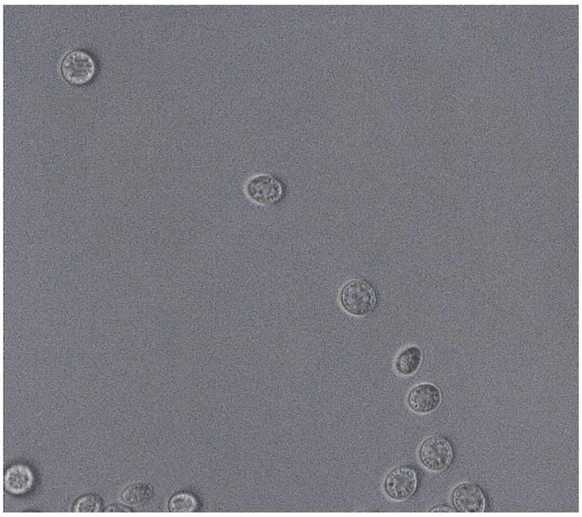

Figure 4. *Dioszegia terrae* sp. nov. YP579^T ballistoconidia produced on YM agar after 53 days at 10 °C. Bars, 10 μm.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was in part supported by the Nakdonggang National Institute of Biological Resources (NNIBR), Ministry of Environment, Korea [NNIBR202101203], and by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea [NIBR202130202] and was also supported in part by National Research Foundation of Korea (NRF) funded by the MSIT (2022M3H9A1082984).

ORCID

Srinivasan Sathiyaraj http://orcid.org/0000-0001-6019-7421

References

[1] Kurtzman CP, Fell JW, Boekhout T. The yeasts. 5th ed. Burlington: Elsevier; 2011. p. 1339–1372.

[2] Sibanda EP, Mabandla M, Chisango T, et al. Endophytic fungi isolated from the medicinal plants *Kigelia africana* and *Warburgia salutaris*. CBIOT. 2018;7(4):323–328.

[3] Ogaki MB, Teixeira DR, Vieira R, et al. Diversity and bioprospecting of cultivable fungal assemblages in sediments of Lakes in the antarctic peninsula. Fungal Biol. 2020;124(6):601–611.

[4] Aliyu H, Gorte O, Zhou X, et al. In silico proteomic analysis provides insights into phylogenomics and plant biomass deconstruction potentials of the *Tremelalles*. Front Bioeng Biotechnol. 2020;8:226.

[5] Buzzini P, Turchetti B, Yurkov A. Extremophilic yeasts: the toughest yeasts around? Yeast 2018; 35(8):487–497.

[6] Vincent WF. Microbial ecosystems of Antarctica. Cambridge: University Press; 2004.

[7] Welander U. Microbial degradation of organic pollutants in soil in a cold climate. Soil Sediment Contam Int J. 2005;14(3):281–291.

[8] Streletskii RA, Kachalkin AV, Glushakova AM, et al. Yeasts producing zeatin. PeerJ. 2019;7:e6474.

[9] Liu XZ, Wang QM, Theelen B, et al. Phylogeny of tremellomycetous yeasts and related dimorphic and filamentous basidiomycetes reconstructed from multiple gene sequence analyses. Stud Mycol. 2015;81:1–26.

[10] Kirk PM, Cannon PE, Minter DW, et al. 2008. Ainsworth & bisby's dictionary of the fungi. 10th ed. Wallingford: CAB International.

[11] Takashima M, Nakase T. Dioszegia zsolt emend. In: Kurtzman CP, Fell JW, Boekhout T, editors. The yeasts, a taxonomic study. Amsterdam: Elsevier; 2001.

[12] Villarreal P, Carrasco M, Barahona S, et al. Tolerance to ultraviolet radiation of psychrotolerant yeasts and analysis of their carotenoid, mycosporine, and ergosterol content. Curr Microbiol. 2016;72(1):94–101.

[13] Madhour A, Anke H, Mucci A, et al. Biosynthesis of the xanthophyll plectaniaxanthin as a stress response in the red yeast dioszegia (Tremellales, Heterobasidiomycetes, Fungi). Phytochemistry. 2005;66(22):2617–2626.

[14] Kurtzman CP, Robnett CJ. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie Van Leeuwenhoek. 1998;73(4):331–371.

[15] White TJ, Bruns T, Lee S, et al. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. editors. PCR protocols: a guide to methods and applications. New York: Academic Press; 1990. p. 315–322.

[16] Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997;25(17):3389–3402.

[17] Larkin MA, Blackshields G, Brown NP, et al. Clustal W and clustal X version 2.0. Bioinformatics. 2007;23(21):2947–2948.

[18] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406–425.

[19] Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol. 1981;17(6):368–376.

[20] Kumar S, Stecher G, Li M, et al. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35(6):1547–1549.

[21] Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. New York: Oxford University Press.

[22] Kimura MA. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16(2):111–120.

[23] Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985; 39(4):783–791.

[24] Kurtzman CP, Fell JW, Boekhout T, et al. 2011. The yeast, a taxonomic study. 5th ed. Netherlands: Elsevier. p. 87–110.

[25] Prillinger H, Lopandic K, Suzuki M, et al. 2011. The yeasts. 5th ed. Netherlands: Elsevier. p. 129–136.

[26] Fell JW. 2011. The yeasts. 5th ed. Netherlands: Elsevier

[27] Phaff HJ, Mrak EM, Williams OB. Yeasts isolated from shrimp. Mycologia. 1952;44(4):431–451.

[28] Crous PW, Wingfield MJ, Chooi YH, et al. Fungal planet description sheets: 1042-1111. Persoonia. 2020;44:301–459.

[29] Takashima M, Sugita T, Shinoda T, et al. Three new combinations from the *Cryptococcus laurentii* complex: *cryptococcus aureus*, *Cryptococcus carnescens* and *Cryptococcus peneaus*. Int J Syst Evol Microbiol. 2003;53(Pt 4):1187–1194.

[30] Chang CF, Huang SY, Lee CF. *Vishniacozyma changhuana* sp. nov., and *Vishniacozyma taiwanica* sp. nov., two novel yeast species isolated from mangrove forests in Taiwan. Int J Syst Evol Microbiol. 2019;(3):71.

[31] Bai FY, Takashima M, Jia JH, et al. *Dioszegia zsoltii* sp. nov., a new ballistoconidium-forming yeast species with two varieties. J Gen Appl Microbiol. 2002;48(1):17–23.

[32] Inácio J, Portugal L, Spencer-Martins I, et al. Phylloplane yeasts from Portugal: seven novel anamorphic species in the *Tremellales* lineage of the *Hymenomycetes* (Basidiomycota) producing orange-coloured colonies. FEMS Yeast Res. 2005;5(12):1167–1183.

[33] Takashima M, Nakase T. 2011. The yeasts. Netherlands: Elsevier. p. 1747–1757.