


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

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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 zsoitii* var. *zsoitii*, 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.

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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 zsoitii* var.

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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.

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

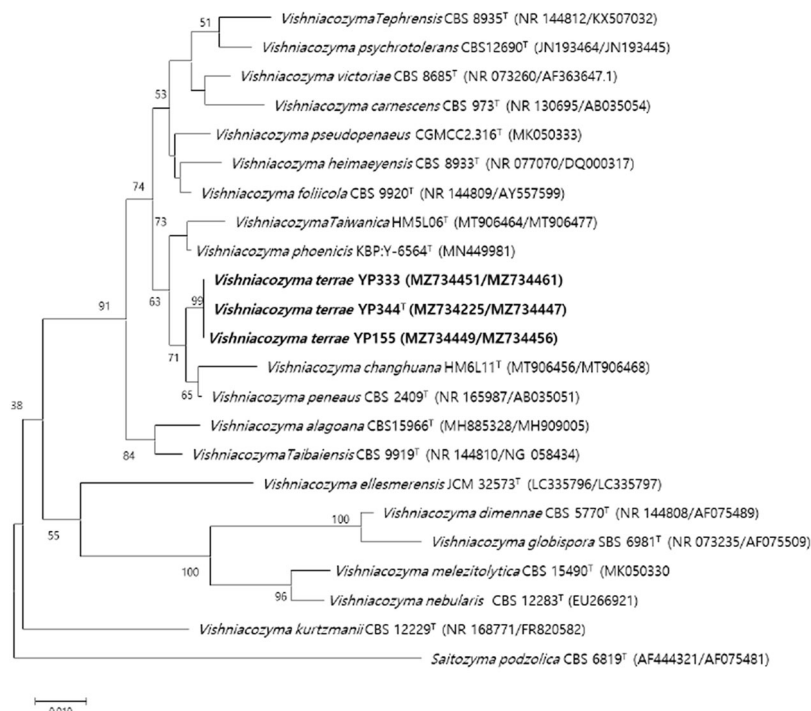


Figure 1. Phylogenetic tree based on the concatenated sequences of the D1/D2 region of the LSU rRNA gene and ITS regions and constructed by the neighbor-joining method, shows relationships between strains of a novel species (YP344, YP155, and YP333) and closely related species. The novel species described 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.

| Genus | Species | Strain ID | Isolation source | Location | GenBank accession numbers | |
|----------------------------|------------------------|--------------|--|-----------------------------------|---------------------------|----------|
| | | | | | D1/D2 | ITS |
| <i>Dioszegia</i> | <i>trreae</i> | YP579 | Soils | Pocheon City, Korea | MZ734403 | MZ734406 |
| | <i>anctartica</i> | CBS 10920 | The culturable soil fungal population | Taylor Valley, Antarctica | FJ640575 | DQ402529 |
| | <i>athyri</i> | AS 2.2559 | Senescent leaves | different regions of China | EU070931 | EU070926 |
| | <i>aurantiaca</i> | CBS 6980 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | AB104689 | AB049613 |
| | <i>buhagiarii</i> | CBS 10054 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | AY562151 | AY885687 |
| | <i>butyracea</i> | AS 2.2600 | Senescent leaves | different regions of China | EU070929 | EU070924 |
| | <i>catarinonii</i> | CBS 10051 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | AY562142 | AY562154 |
| | <i>changbaiensis</i> | CBS 9608 | Basidiomycetous yeasts | Northeast China | NG059069 | NR136964 |
| | | CBS 6714 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | AF075508 | NR155062 |
| | <i>cryoxerica</i> | ANT-03-071 | The culturable soil fungal population | Taylor Valley, Antarctica | FJ640562 | FJ640565 |
| | <i>dumuzii</i> | CBS 12501 | Forest soils | Germany | – | LT548261 |
| | <i>fristingensis</i> | CBS 10052 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | NG070549 | NR136970 |
| | | CBS 4214 | A birch leaf | Northern Portugal | AF075503 | NR073227 |
| | <i>rishiriensis</i> | CBS 11844 | A soil sample collected on Rishiri Island | Hokkaido, Japan | AB545810 | NR157461 |
| | | CBS 8925 | Soils | Antarctic | AY029341 | AY029342 |
| | <i>statzeliiae</i> | CBS 10053 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | AY562149 | NR136971 |
| | | CBS 10053 | Leaves of selected Mediterranean plant species | Portugal (Arra'bida Natural Park) | AY562149 | NR136971 |
| | <i>xingshanensis</i> | AS 2.2481 | Senescent leaves | different regions of China | EU070928 | EU070923 |
| | <i>zsoltii</i> | CBS 9128 | Plant leaves | Yunnan, China | AF544246 | NR156190 |
| | <i>var.yunnanensis</i> | | | | | |
| <i>zsoltii var.zsoltii</i> | 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 × 1.8–2 μm). 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 *Vishniacozyma* 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) | 50(2) | 57(4) | 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) | 8(0) | 7(0) | 8(0) | 11(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) | 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) | – | – | – | 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) | – |

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.

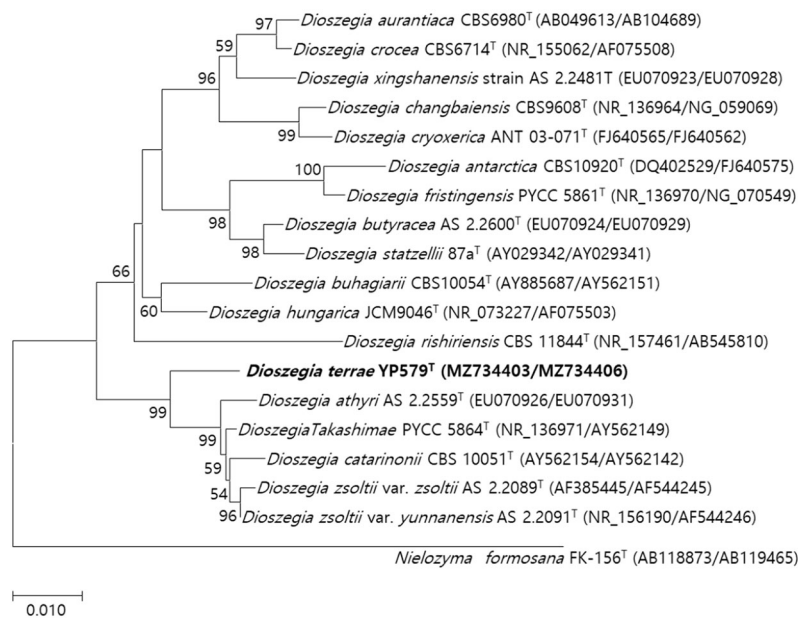


Figure 2. Phylogenetic tree based on the concatenated sequences of the D1/D2 region of the LSU rRNA gene and ITS regions and constructed by the maximum-likelihood method, shows relationships between strains of a novel species (YP579^T) and closely related species. The novel species described in this manuscript are highlighted in bold. *Niellozyma 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. zoltii* 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-α-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. zoltii* 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-α-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.

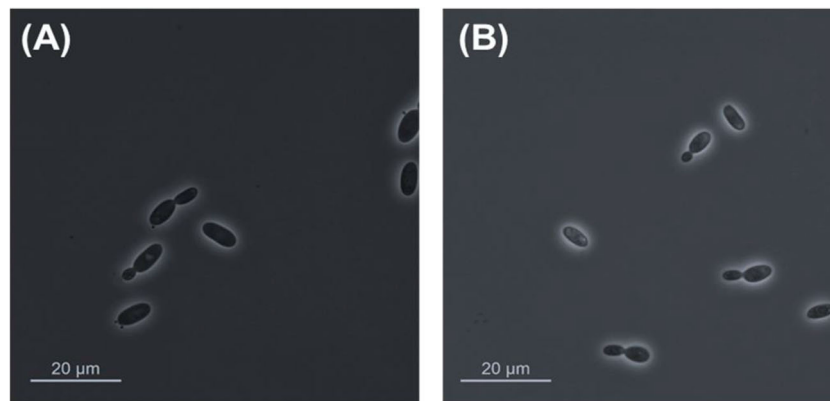


Figure 3. *Vishniacozyma terrae* sp. nov. YP344^T and *Dioszegia terrae* sp. nov. YP579^T (A) The polar budding cells of *V. terrae* YP344^T and (B) *D. terrae* YP579^T on YM agar after 3 days at 10 °C.

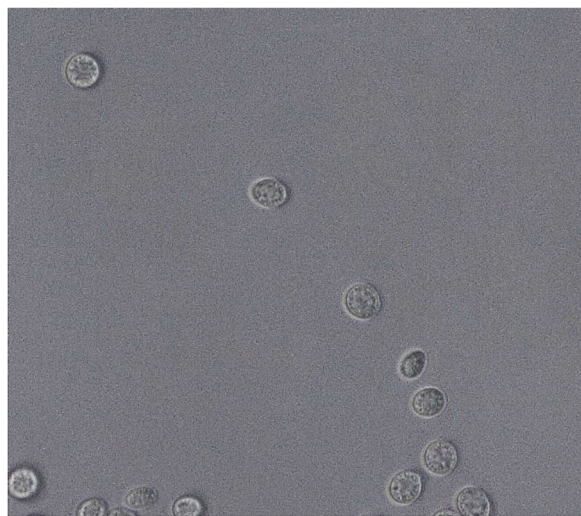


Figure 4. *Dioszegia terrae* sp. nov. YP579^T ballistoconidia produced on YM agar after 53 days at 10 °C. Bars, 10 µm.

Disclosure statement

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

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