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# *Candida fragi* and *C. valdiviana*, two newly recorded yeast species in Taiwan

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**ABSTRACT**

Six strains of ascomycetous yeasts belonging to two yeast species of the genus *Candida* were isolated from fruiting bodies of mushrooms in Taiwan. All of the strains examined had typical morphological, physiological, and molecular characteristics of *Candida fragi* and *C. valdiviana*, two newly recorded yeast species in Taiwan.

**Key words**: new record, fruiting bodies of mushrooms, Taiwan

## Introduction

Yeasts of no distinctive cellular morphology that lack a known sexual cycle and propagate by multilateral buddings have been assigned to the genus *Candida* (Daniel *et al*. 2014), which contains approximately 300 species (Lachance *et al*. 2011). With suggestions from multi-gene sequence analyses, the taxonomy of *Candida* is increasing over time to be a reclassification of some *Candida* clades e.g., *Kurtzmaniella* Lachance & Starmer (Lachance and Starmer, 2008) and *Sugiyamaella* Kurtzman & Robnett (Kurtzman and Robnett, 2007).

During a survey on the yeast diversity associated with fruiting bodies of mushrooms in Taiwan, six strains representing two yeast species were isolated and identified as *C. fragi* and *C. valdiviana*, which have not been reported in Taiwan previously. In this study, morphological and physiological characteristics are described, and the D1/D2 domain of the large ribosomal subunit (LSU) rDNA, the internal transcribed spacer (ITS) region and electrophoretic karyotypes of these two species were analyzed.

## Materials and Methods

To isolate the yeasts from the mushroom fruiting bodies (Hsieh *et al*. 2010), approximately 1.0 g of samples was placed into a tube containing 9 mL of yeast extract–malt extract broth (YMB;

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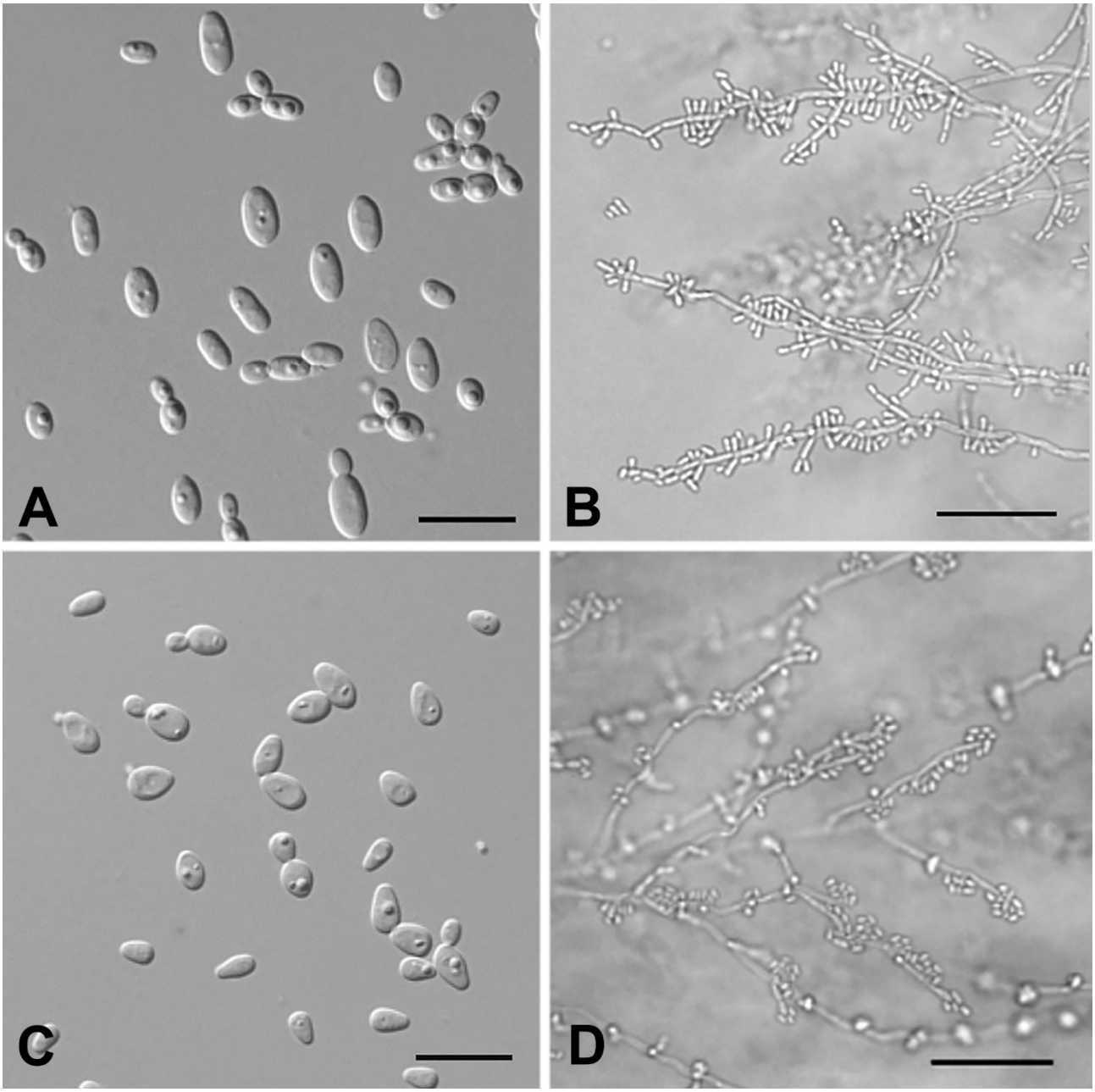



**Fig. 1.** *Candida fragi* and *C. valdiviana*. A, B. *C. fragi* EN1M03. A. Vegetative cells grown on YM agar for 3 days at 25°C. Bar, 10 μm. B. Pseudohyphae grown on corn meal agar for 2 weeks at 25°C. C, D. *C. valdiviana* GE17L11. C. Vegetative cells grown on YM agar for 3 days at 25°C. D. Pseudohyphae grown on corn meal agar for 2 weeks at 25°C. Bars in A, C = 10 μm; B, D = 25 μm.

1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, pH 5.4) supplemented with 50 μg/mL of chloramphenicol and then vortex mixed. One-tenth of a milliliter of these suspensions and appropriate decimal dilutions was spread on acidified YM agar (AYMA, with the same gradients as YMB except for adding 2.0% agar, pH 3.5) or Dichloran rose Bengal chloramphenicol agar (DRBC, Merck, Darmstadt, Germany) and the plates incubated at 24°C for 3 days.

Representative colonies were selected, and the yeasts were purified by streaking onto YMA and grown for 3 days at 24°C. All isolated strains were stored in the freezer at -70°C with 30% glycerol (w/v) added as a cryoprotectant for long-term preservation and/or remained on YMA at 4°C for short-term preservation. The morphological, physiological, and biochemical characteristics of the strains were determined as described by Kurtzman *et al*. (2011).

The yeast genomic DNA was extracted and purified using a Genome DNA Extraction kit (Biokit Co., Miaoli, Taiwan). The D1/D2 domain of the LSU rDNA, and ITS region were amplified using primers NL1 and NL4 (Kurtzman and Robnett, 1998), and ITS1 and ITS4 (White *et al*., 1990), respectively. The sequences obtained in this study were compared with those in the GenBank database using BLASTN (https://www.ncbi.nlm.nih.gov). The sequences were initially aligned using the multiple alignment program CLUSTAL X 1.83 (Thompson *et al*., 1997). A phylogenetic tree was constructed using the maximum-likelihood (ML) method with the MEGA version 7.0 software package (Kumar *et al*., 2016). The strain of *Candida glucosophila* NRRL Y-17781$^{T}$ (U45849) was used as an outgroup. Bootstrap analysis was performed from 1000 bootstrap replications (Felsenstein, 1995).

The intact chromosomal DNAs used for Pulsed-field gel electrophoresis (PFGE) were prepared as described by Lee *et al*. (2009a, b). A Rotaphor 6.0 rotating field electrophoresis apparatus (Biometra, Taipei, Taiwan) was used to separate chromosomal DNA. The conditions of PFGE were modified by the method described by to Lee *et al*. (2009a, b). The electrophoresis program was completed in 3 steps with a total run time of 72 hr: Step 1, field switch time of 90-120 sec for 15 hr at 180V; Step 2, switch time of 120-360 sec for 18 hr at 120V; Step 3, switch time of 360-1200 sec for 39 hr at 80V. The agarose gel was 1% and the temperature of the running buffer was maintained at 13ºC throughout the electrophoresis.

## Taxonomy

***Candida fragi*** M. Suzuki, T. Nakase & Y. Fukazawa, J Gen Appl Microbiol 37: 423–429. 1991.

After growth in YM broth at 25ºC for 3 days, streak cultures are cream colored, smooth, convex, butyrous, and entire margin. Cells are ellipsoidal to ovoid, 2-4 × 3-6 μm, and occur singly or in pair (Fig. 1a). Mycelium and pseudomycelium were found on cornmeal agar (Fig. 1b). No sexual activity and ballistospores are observed on cornmeal agar or YM agar. Glucose is fermented. D-Glucose, D-galactose, L-sorbose, D-xylose, sucrose, maltose, salicin, arbutin, melezitose, glycerol, ribitol, sorbitol, D-mannitol, D-glucono-1,5-lactone, 2-keto-D-gluconate, D-glucuronate, ethanol and N-acetyl-glucosamine are assimilated, but D-glucosamine, D-ribose, L-arabinose, D-arabinose, L-rhamnose, α,α-trehalose, methyl-α-D-glucoside, cellobiose, melibiose, lactose, raffinose, inulin, starch, erythritol, xylitol, L-arabinitol, galactitol, myo-inositol, 5-keto-D-gluconate, D-gluconate, D-galacturonic acid, DL-lactate, succinate, citrate, methanol, propane-1,2-diol and butane-2,3-diol are not assimilated. Ethylamine, L-lysine and cadaverine are assimilated. Growth in vitamin-free medium is positive. Growth in 0.01% cycloheximide is negative. Growth in 50% D-glucose is negative.

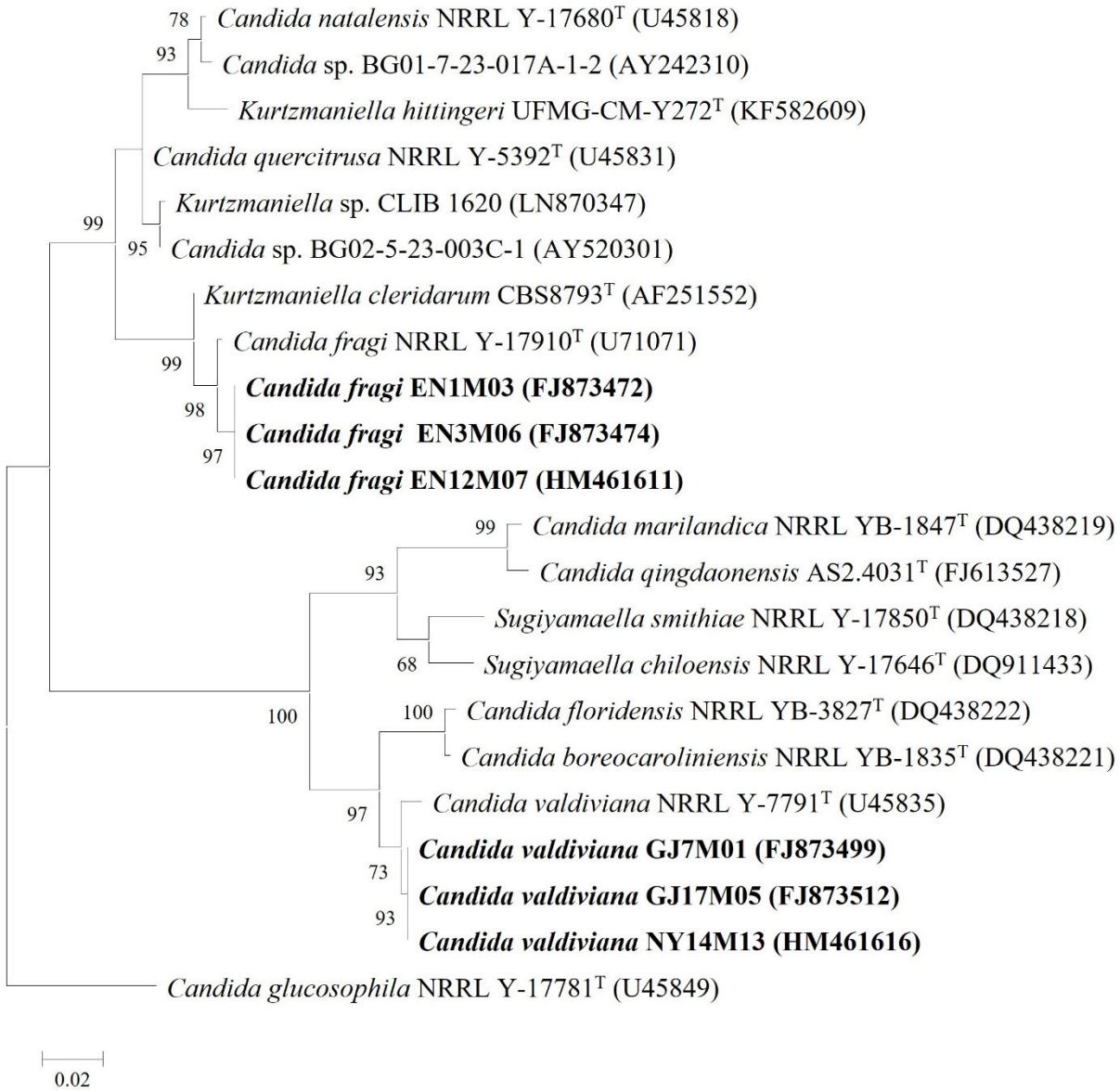
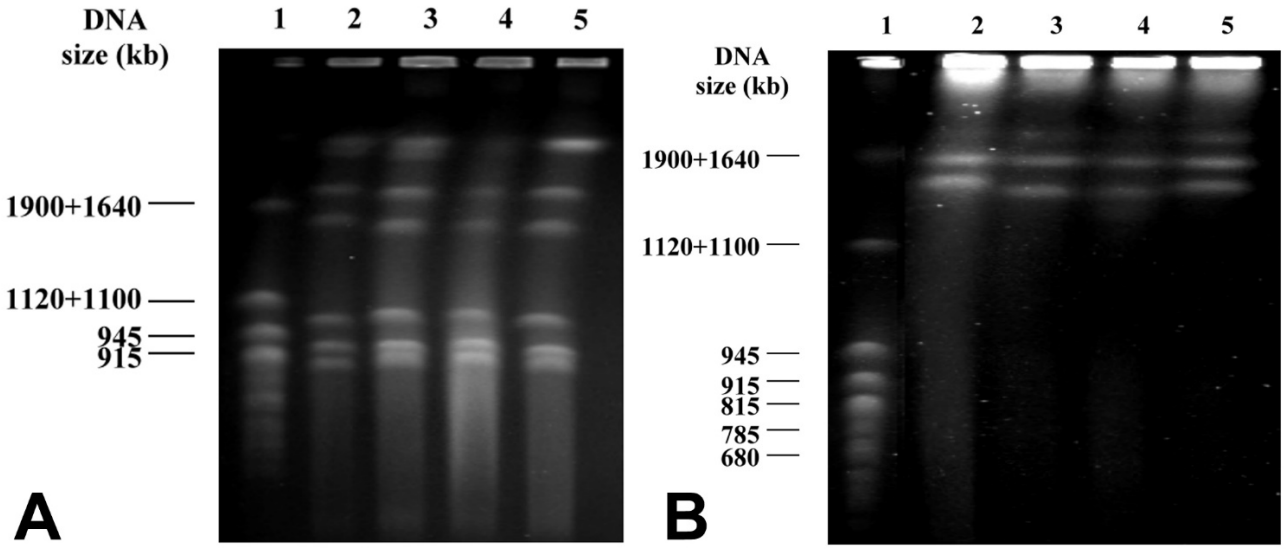



**Fig. 2.** Maximum likelihood phylogenetic tree based on the D1/D2 domain of the LSU rDNA sequences showing the relationship of *Candida* species. The *Candida* strains investigated in this study are boldfaced. Bootstrap values based on 1000 replicates are given at branch nodes. Bar = 0.02 substitutions per nucleotide position. T = Type strain.

Growth in 10% NaCl are negative. Growth occurs at 25ºC but not at 30ºC. Production of starch-like compounds and acetic acid are negative. Diazonium blue B reaction and urease activity are negative.

**Strains examined**. The strain EN1M03 was isolated from a fruiting body of *Russula* sp. collected in Jianshin, Hsinchu, Taiwan in 2007. The strain EN3M06 was isolated from a fruiting body of *Flamella* sp. collected in Jianshin, Hsinchu, Taiwan in 2007. The strain EN12M07 was isolated from a fruiting body of *Tricholoma* sp. collected in Jianshin, Hsinchu, Tainan, Taiwan in 2007. The GenBank/EMBL/DDBJ accession




Fug 3. A. Electrophoresis karyotypes of three isolates and the type strain of *C. fragi*. Lane 1, Marker (*Saccharomyces cerevisiae* YPH 80); Lane 2, *C. fragi* BCRC 22806$^T$; Lane 3, EN1M03; Lane 4, EN3M06; Lane 5, EN12M07. B. Electrophoresis karyotypes of three isolates and the type strain of *C. valdiviana*. Lane 1, Marker (*Saccharomyces cerevisiae* YPH 80); Lane 2, *C. valdiviana* BCRC 22646$^T$; Lane 3, GJ7M01T; Lane 4, GJ17M05; Lane 5, NY14M13.

numbers for the D1/D2 domain of the LSU rDNA sequences obtained in this study are FJ873472, FJ873474 and HM461611, and those for the ITS regions are HM461638, HM461639 and HM461640.

**Notes.** The three strains that we isolated exhibited identical nucleotide sequences in the D1/D2 domain of the LSU rDNA and similar phenotypic characteristics, indicating that they are conspecific. The sequences of the D1/D2 domain of the LSU rDNA of the three strains differed from the type strain of *C. fragi* BCRC 22806$^T$ by 4 nucleotide substitutions (0 gap), equating to sequence similarities of 99.36% (Fig. 2). The ITS sequences of these three strains differed from that of *C. fragi* BCRC 22806$^T$ by 6-7 nucleotide substitutions (2-3 gap), equating to sequence similarities of 98.42–98.77%. To further elucidate the taxonomic relationships of the three strains with *C. fragi*, the electrophoretic karyotype of each strain was determined and compared with that of *C. fragi* BCRC 22806$^T$ (Fig 3a). The three strains were found to be the same to *C. fragi* BCRC 22806$^T$ in terms of electrophoretic karyotypes, with six chromosomal bands ranging from 915 to 1900 kb (Fig. 3a). Molecular and physiological analysis demonstrated that these strains are *C. fragi*.

***Candida valdiviana*** J. Grinbergs, D. Yarrow, Antonie Van Leeuwenhoek 36: 143–148. 1970.

After growth in YM broth at 25ºC for 3 days, streak cultures are cream colored, smooth, convex, butyrous, and entire margin. Cells are ellipsoidal to ovoid, 2-3 × 4-6 μm, and occur singly or in pair (Fig. 1c). Mycelium and pseudomycelium were found on cornmeal agar (Fig. 1d). No sexual activity and ballistospores are observed on cornmeal agar or YM agar. Glucose and D-

galactose (delayed) are fermented. D-Glucose, D-galactose, L-sorbose (weak), D-glucosamine (weak), D-ribose, D-xylose, L-arabinose, D-arabinose, L-rhamnose, sucrose, maltose, α,α-trehalose, methyl-α-D-glucoside, cellobiose, salicin, arbutin, melibiose (weak), melezitose, glycerol, erythritol, ribitol, xylitol, L-arabinitol, sorbitol, D-mannitol, D-glucono-1,5-lactone (weak), D-gluconate, succinate (weak), citrate (weak), ethanol, propane-1,2-diol (weak) and N-acetyl-glucosamine are assimilated, but lactose, raffinose, inulin, starch, galactitol, myo-inositol, 2-keto-D-gluconate, 5-keto-D-gluconate, D-glucuronate, D-galacturonic acid, DL-lactate, methanol, and butane-2,3-diol are not assimilated. Nitrite, ethylamine, L-lysine, and cadaverine are assimilated. Growth in vitamin-free medium is positive (weak). Growth in 0.01% cycloheximide is negative. Growth in 50% D-glucose is negative. Growth in 10% NaCl are positive. Growth occurs at 30ºC and 35ºC (variable) but not at 37ºC. Production of starch-like compounds and acetic acid are negative. Diazonium blue B reaction and urease activity are negative.

**Strains examined**. The strain GJ7M01 was isolated from a fruit body of unidentified wild mushroom collected in Sandimen, Pingtung, Taiwan in 2007. The strain GJ17M05 was isolated from a fruiting body of *Lentinus* sp. collected in Wutai, Pingtung, Taiwan in 2007. The strain NY14M13 was isolated from a fruiting body of *Filoboletus manipularis* collected in Sinyi, Nantou, Taiwan in 2007. The GenBank/EMBL/DDBJ accession numbers for the D1/D2 domain of the LSU rDNA sequences obtained in this study are FJ873499, FJ873512 and HM461616, and those for the ITS regions are FJ873580, FJ873587 and HM461647.

**Notes.** The three strains that we isolated exhibited identical nucleotide sequences in the D1/D2 domain of the LSU rDNA and similar phenotypic characteristics, indicating that they are conspecific. Phylogenetic analysis of the sequences of the D1/D2 domain of the LSU rDNA revealed that the three strains differed from the type strain of *C. valdiviana* BCRC 22646$^T$ by 6 nucleotide substitutions (0 gap), equating to sequence similarities of 98.89% (Fig. 2). The ITS sequences of these three strains differed from that of *C. valdiviana* BCRC 22806$^T$ by 10-17 nucleotide substitutions (2-5 gap), equating to sequence similarities of 97.55-98.10%. To further elucidate the taxonomic relationships of the three strains with *C. valdiviana*, the electrophoretic karyotype of each strain was determined and compared with that of *C. valdiviana* BCRC 22646$^T$ (Fig 3b). The three strains were found to be the same to *C. valdiviana* BCRC 22646$^T$ in terms of electrophoretic karyotypes, with 3 chromosomal bands about 1900 kb (Fig. 3b). Molecular and physiological analysis demonstrated that these strains are *C. valdiviana*.

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# 臺灣新紀錄種酵母菌 *Candida fragi* 與 *C. valdiviana*

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## 摘　　要

由臺灣山區野生菇類子實體分離出 6 株屬於 *Candida* 屬的酵母菌菌株。形態學、生理學與分子生物學特性的測試結果顯示，所有菌株分別屬於 *Candida fragi* 與 *C. valdiviana* 的臺灣新紀錄酵母菌菌種。

**關鍵詞：**新紀錄種、菇類子實體、臺灣