Journal of Species Research 9(3):198-203, 2020

Isolation and characterization of unrecorded yeasts species in the family *Metschnikowiaceae* and *Bulleribasidiaceae* in Korea

Yuna Park, Soohyun Maeng and Sathiyaraj Srinivasan*

Department of Bio & Environmental Technology, College of Natural Science, Seoul Women's University, Seoul 01797, Republic of Korea

**Correspondent: drsrini@swu.ac.kr*

The goal of this study was to isolate and identify wild yeasts from soil samples. The 15 wild yeast strains were isolated from the soil samples collected in Pocheon city, Gyeonggi Province, Korea. Among them, four yeast stains were unrecorded, and 11 yeast stains were previously recorded in Korea. To identify wild yeasts, microbiological characteristics were observed by API 20C AUX kit. Pairwise sequence comparisons of the D1/D2 domain of the 26S rRNA were performed using Basic Local Alignment Search Tool (BLAST). Cell morphology of yeast strains was examined by phase contrast microscope. All strains were oval-shaped and polar budding and positive for assimilation of glucose, 2-keto-D-gluconate, *N*-acetyl-D-glucosamine, D-maltose and D-saccharose (sucrose). There is no official report that describes these four yeast species: one strain of the genus *Kodamaea* in the family *Metschnikowiaceae* and three strains of the *Hannaella* in the family *Bulleribasidiaceae*. *Kodamaea ohmeri* YI7, *Hannaella kunmingensis* YP355, *Hannaella luteola* YP230 and *Hannaella oryzae* YP366 were recorded in Korea, for the first time.

Keywords: *Bulleribasidiaceae*, *Hannaella*, *Kodamaea*, *Metschnikowiaceae*, unrecorded yeasts

DOI:10.12651/JSR.2020.9.3.198

Introduction

In 2019, we isolated unrecorded yeasts from soil collected in Pocheon city, Korea. The identified yeasts belong to the family *Metschnikowiaceae* and *Bulleribasidiaceae*. This study focusses on the isolation and description of unrecorded species in the genera *Kodamaea* and *Hannaella*.

The genus *Kodamaea* contains ascomycetous yeast in the family *Metschnikowiaceae*, the order *Saccharomycetales*, the subphylum *Saccharomycotina*, the phylum *Ascomycota*. The *Kodamaea* clade consists of 23 recognized species and has *K. ohmeri* as the type species. The *Kodamaea* clade is closely related to the genera *Clavispora* and *Metschnikowia* (Lachance and Kurtzman, 2011). Species in the clade *Kodamaea* have been isolated from various sources such as mushrooms, ephemeral flowers, insects (Freitas *et al*., 2013) and rotting wood (Gao *et al*., 2017). The members of genus *Kodamaea* are fermentative and form coenzyme Q-9 and hyphae.

The genus *Hannaella* contains basidiomycetous yeast in the family *Bulleribasidiaceae*, the order *Tremellales*, the subphylum *Agaricomycotina*, the phylum *Basidiomycota*. The genus *Hannaella* consists of 11 recognized species and has *H. sinensis* as the type species. The genus *Hannaella* is closely related to the genera *Dioszegia* and *Derxomyces* and was proposed to accommodate seven species that were transferred from the *Bullera sinensis* clade: *H. coprosmaensis*, *H. kunmingensis*, *H. luteola*, *H. oryzae*, *H. sinensis*, *H. surugaensis* and *H. zeae* (Wang & Bai, 2008). Species in the genus *Hannaella* have been isolated from various sources such as soil (Landell *et al*., 2014), plants (Surussawadee *et al*., 2015; Kaewwichian *et al*., 2015) and water (Han *et al*., 2017). The members of the genus *Hannaella* are not fermentative and form coenzyme Q-10 and hyphae.

This study focuses on the description of four yeast species belonging to genera *Kodamaea* and *Hannaella* that have not officially been reported in Korea.

Material and Methods

Soil samples were collected in Pocheon city, Gyeonggi Province, Korea and serially diluted in distilled water. The aliquot was spread onto YM agar incubated at 25°C for 3−4 days. The strain IDs, isolation sources, taxonomic

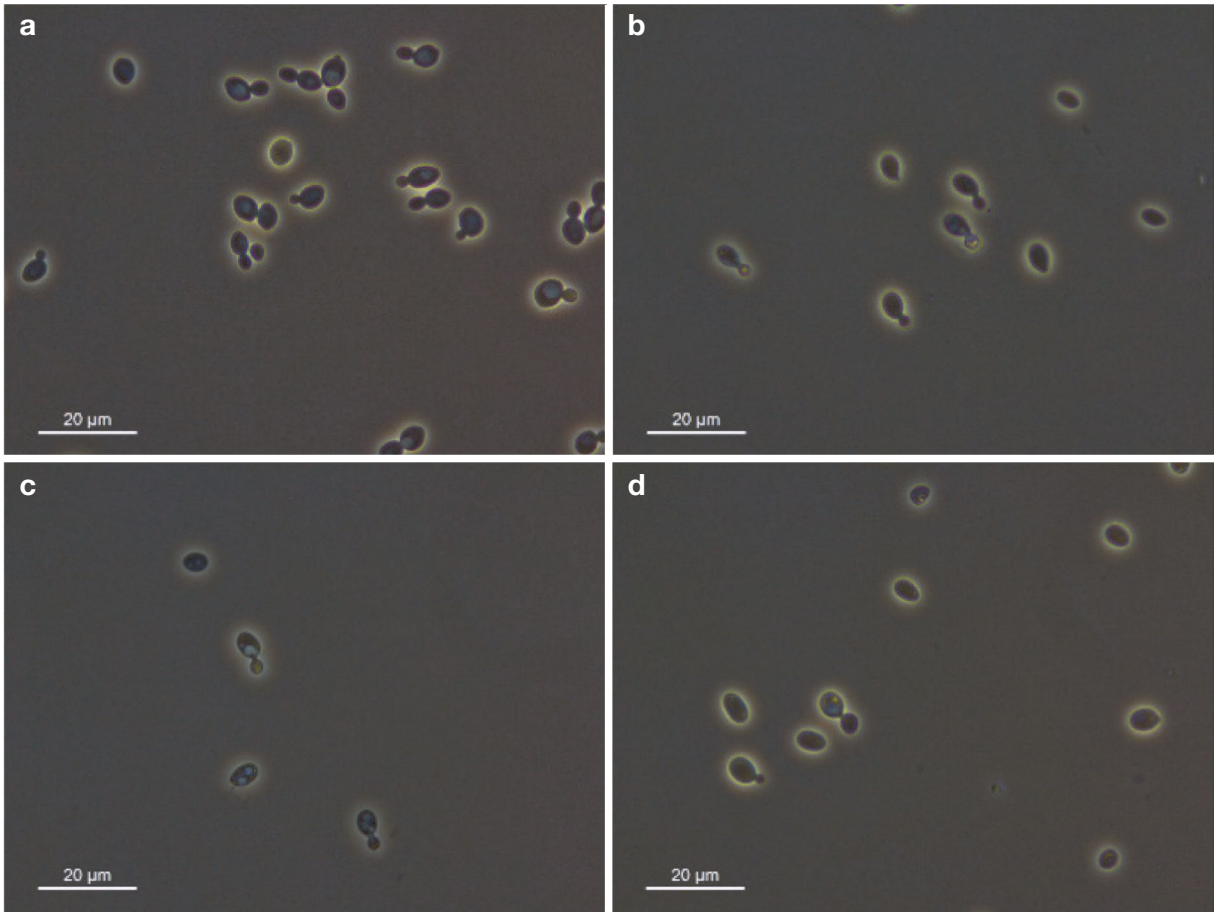

Fig. 1. Photomicrographs showing budding cells of strains YI7 (a); YP355 (b); YP366 (c); YP320 (d). All strains were grown on YM agar for 3 days. Bars, 20 μm.

composition and identification results were summarized in Table 1.

Cell morphology of strains was examined by phase contrast microscope (LEICA, DM500) using cells grown for 3−4 days on YM agar. Photomicrographs of the strains YI7, YP335, YP230 and YP366 are shown in Fig. 1. Biochemical characteristics were established using API 20C AUX (bioMérieux) according to the manufacturer's instructions.

All yeast strains were cultivated on YM agar for 3 days. Then, genomic DNA was extracted and the D1/D2 domain of the LSU rRNA gene was amplified by PCR with NL1 and NL4 primers (Kurtzman and Robnett, 1998). Pairwise sequence comparisons were made using Basic Local Alignment Search Tool (BLAST) search (Altschul *et al*., 1997) and aligned with the sequences of related species retrieved from GenBank by using the multiple alignment program Clustal X 2.0 (Larkin *et al*., 2007). A phylogenetic tree based on the D1/D2 domains of the LSU rRNA gene sequences was reconstructed using the neighbor-joining method in MEGA 7.0 (Saitou and Nei, 1987). The evolutionary distances were calculated using the two-parameter model of Kimura (Kimura, 1980) for the neighbor-joining analyses. The confidence levels of the clades were estimated through bootstrap analysis (1,000 replicates) (Felsenstein, 1985). Reference sequences were retrieved from GenBank under the accession numbers indicated on the tree.

The API 20C AUX, a commercial yeast characterization kit (bioMérieux), was used according to the manufacturer's instructions for yeast identification on the basis of metabolic/physiological features.

Results and Discussion

The four yeast strains were distributed in two kingdoms: *Ascomycota* and *Basidiomycota*. Based on D1/D2 domain of the LSU rRNA gene sequence analyses and phylogenies, strain YI7 was assigned to the phylum *Ascomycota* and strains YP335, YP230 and YP366 were assigned to the phylum *Basidiomycota*. The photomicro-

Table 1. Yeasts isolated from soils of Korea.

Phylum	Class	Order	Family	Strain ID	Most closely related species	D1/D2 similarity (%)	Record in Korea
Ascomycota	*Dothideomycetes*	*Dothideales*	*Saccotheciaceae*	YA46	*Aureobasidium namibiae*	582/584 (99)	Reported
	Saccharomycetes	*Saccharomycetales*	*Metschnikowiaceae*	YI7	*Kodamaea ohmeri*	533/534 (99)	Unreported
Basidiomycota	*Tremellomycetes*	*Filobasidiales*	*Filobasidiaceae*	YP182	*Filobasidium magnum*	614/614 (100)	Reported
				YP191	*Naganishia globosa*	612/612 (100)	Reported
		Tremellales	*Bulleraceae*	YP597	*Bullera alba*	625/627 (99)	Reported
			Bulleribasidiaceae	YP355	*Hannaella kunmingensis*	611/611 (100)	Unreported
				YP230	*Hannaella luteola*	608/608 (100)	Unreported
				YP366	*Hannaella oryzae*	608/609 (99)	Unreported
			Trimorphomycetaceae	YO19	*Saitozyma podzolica*	613/616 (99)	Reported
				YO28		615/618 (99)	Reported
				YO42		608/611 (99)	Reported
		Trichosporonales	*Trichosporonaceae*	YO23	*Trichosporon moniliiforme*	614/614 (100)	Reported
	Microbotryomycetes	*Leucosporidiales*	*Leucosporidiaceae*	YP69	*Leucosporidium intermedium*	601/601 (100)	Reported
		–	*Chrysozymaceae*	YP301	*Sampaiozyma ingeniosa*	608/608 (100)	Reported
				YP266	*Sampaiozyma vanillica*	606/607 (99)	Reported

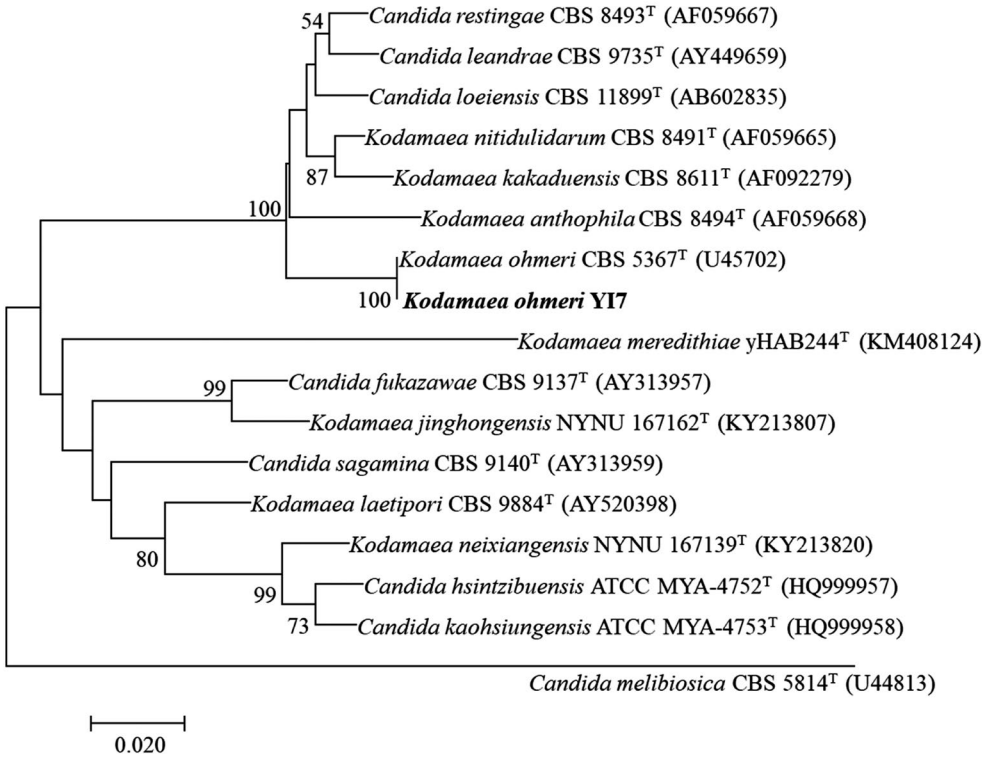

Fig. 2. Phylogenetic tree derived from neighbor-joining analysis based on the D1/D2 domains of the LSU rRNA gene sequences, showing the placement of strain YI7 in the *Kodamaea* clade. *Candida melibiosica* CBS 5814^T was used as outgroup. Bootstrap values of above 50% are given at nodes based on 1,000 replicates. Bar, 0.02 substitutions per site.

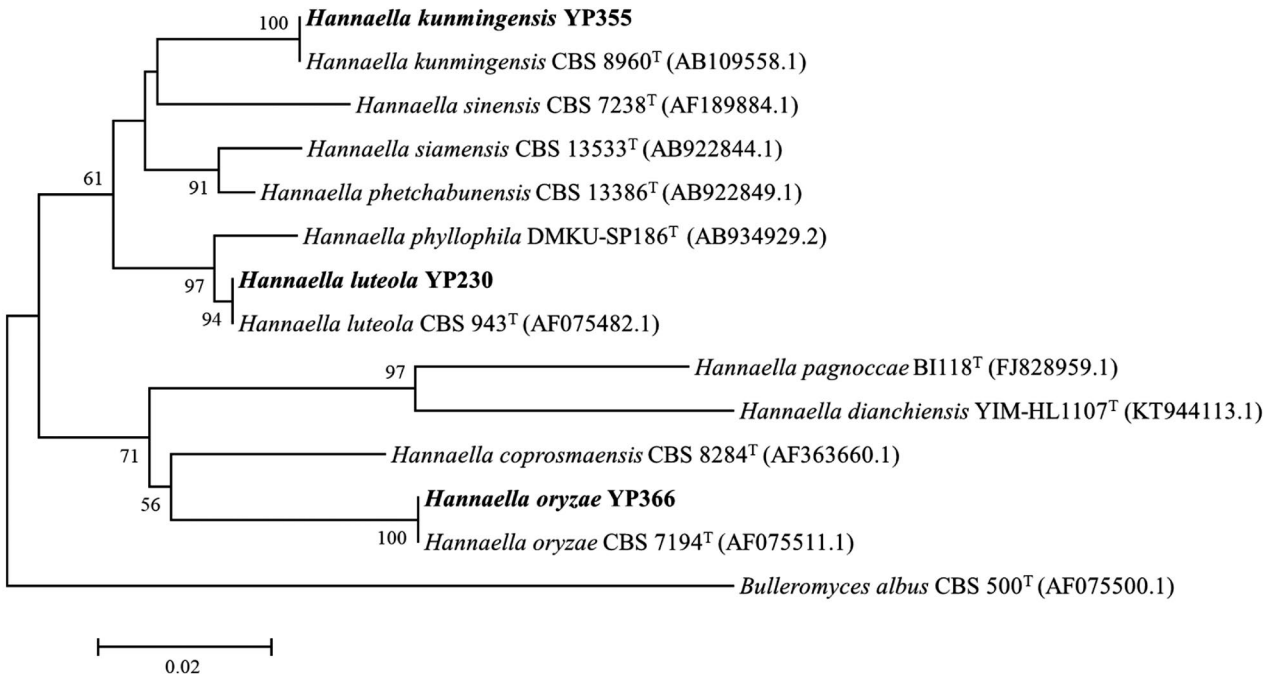

Fig. 3. Phylogenetic tree derived from neighbor-joining analysis based on the D1/D2 domains of the LSU rRNA gene sequences, showing the placement of strains YP355, YP230 and YP366 in the genus *Hannaella*. *Bulleromyces albus* CBS 500^T was used as outgroup. Bootstrap values of above 50% are given at nodes based on 1,000 replicates. Bar, 0.02 substitutions per site.

graphs showing budding cells of four strains are given in the Fig. 1. The detailed morphological and physiological characteristics were given in the strain descriptions.

The strains YI, YP335, YP230 and YP366 were most closely related to *K. ohmeri* (CBS5367^T; 99% D1/D2 domain of LSU sequence similarity), *H. kunmingensis* (CBS8960^T; 100%), *H. luteola* (CBS943^T; 100%) and *H. oryzae* (CBS7194^T; 99%), respectively. The D1/D2 domain of the LSU rRNA gene similarities of the four strains also formed a robust phylogenetic clade with the most closely related species (Figs. 2 and 3).

Based on phylogenetic analysis, we concluded that strain YI7 was a member of the genus *Kodamaea* in the family *Metschnikowiaceae* and that strains YP335, YP230 and YP366 were members of the genus *Hannaella* in the family *Bulleribasidiaceae*.

Description of *Kodamaea ohmeri* YI7

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, dull and white colored after 3 days of incubation on YM agar at 25°C. In the API 20C AUX, strain YI7 is positive for assimilation of L-arabinose (w, weak), adonitol (w), D-lactose (bovine origin) (w), D-melezitose (w), glucose, glycerol, 2-keto-D-gluconate, D-galactose, D-sorbitol, d-methyl-D-glucoside, *N*-acetyl-D-glucosamine, D-cellobiose, D-maltose, D-saccharose (sucrose), D-trehalose and D-raffinose; but negative for D-xylose, xylitol and inositol. Strain YI7 was isolated from a soil sample collected in Pocheon city, Gyeonggi Province, Korea.

Description of *Hannaella kunmingensis* YP335

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, shiny and beige colored after 3 days of incubation on YM agar at 25°C. In the API 20C AUX, strain YP335 is positive for assimilation of L-arabinose (w), D-xylose (w), D-galactose (w), D-sorbitol (w), D-cellobiose (w), D-raffinose (w), glucose, 2-keto-D-gluconate, *N*-acetyl-D-glucosamine, D-maltose, D-saccharose (sucrose) and D-melezitose; but negative for glycerol, adonitol, xylitol, inositol, d-methyl-D-glucoside, D-lactose (bovine origin) and D-trehalose. Strain YP335 was isolated from a soil sample collected in Pocheon city Gyeonggi Province, Korea.

Table 2. Characteristics of the unrecorded yeasts from soil in Korea.

Strain ID	1	2	3	4
Morphological characteristics				
Shape	Oval	Oval	Oval	Oval
Reproduction	Budding	Budding	Budding	Budding
API 20C AUX				
Glucose	+	+	+	+
Glycerol	+	−	w	−
2-Keto-D-Gluconate	+	+	+	+
L-Arabinose	w	w	−	+
D-Xylose	−	w	−	+
Adonitol	w	−	w	−
Xylitol	−	−	w	−
D-Galactose	+	w	+	+
Inositol	−	−	+	+
D-Sorbitol	+	w	w	−
d-Methyl-D-Glucoside	+	−	w	+
N-Acetyl-D-Glucosamine	+	+	+	+
D-Cellobiose	+	w	+	w
D-Lactose (bovine origin)	w	−	w	−
D-Maltose	+	+	+	+
D-Saccharose (Sucrose)	+	+	+	+
D-Trehalose	+	−	+	−
D-Melezitose	w	+	+	+
D-Raffinose	+	w	+	+

Taxa: 1, *Kodamaea ohmeri* YI7; 2, *Hannaella kunmingensis* YP355; 3, *H. luteola* YP230; 4, *H. oryzae* YP366
All data were obtained in this study. +, positive; w, weakly positive; −, negative.

Description of *Hannaella luteola* YP230

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, shiny and beige colored after 3 days of incubation on YM agar at 25°C. In the API 20C AUX, strain YP230 is positive for assimilation of glycerol (w), adonitol (w), xylitol (w), D-sorbitol (w), d-methyl-D-glucoside (w), D-lactose (bovine origin) (w), glucose, 2-keto-D-gluconate, D-galactose, inositol, *N*-acetyl-D-glucosamine, D-cellobiose, D-maltose, D-saccharose (sucrose), D-trehalose, D-melezitose and D-raffinose; but negative for L-arabinose and D-xylose. Strain YP230 was isolated from a soil sample collected in Pocheon city, Gyeonggi Province, Korea.

Description of *Hannaella oryzae* YP366

Cells are oval shaped, and budding is polar (Fig. 1). Colonies are convex, smooth, dull and yellow colored after 3 days of incubation on YM agar at 25°C. In the API 20C AUX, strain YP366 is positive for assimilation of D-cellobiose (w), glucose, 2-keto-D-gluconate, L-arabinose, D-xylose, D-galactose, inositol, d-methyl-D-glucoside, *N*-acetyl-D-glucosamine, D-maltose, D-saccharose (sucrose), D-melezitose and D-raffinose; but negative for glycerol, adonitol, xylitol, D-sorbitol, D-lactose (bovine origin) and D-trehalose. Strain YP366 was isolated from a soil sample collected in Pocheon city, Gyeonggi Province, Korea.

Acknowledgements

This work was supported by a research grant from Seoul Women's University (2020) and by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201928201).

References

Altschul, S.F., T.L. Madden, A.A. Schäffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25(17):3389-3402.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4):783-791.

Freitas, L.F., E.J. Carvajal Barriga, P.P. Barahona, M.A. Lachance and C.A. Rosa. 2013. *Kodamaea transpacifica* f.a., sp. nov., a yeast species isolated from ephemeral flowers and insects in the Galapagos Islands and Malaysia: further evidence for ancient human transpacific contacts. Int J Syst Evol Microbiol 63(Pt 11):4324-4329.

Gao, W.L., T.T. Liu, J. Zheng and F.L. Hui 2017. *Kodamaea neixiangensis* f.a., sp. nov. and *Kodamaea jinghongensis* f.a., sp. nov., two yeast species isolated from rotting wood. Int J Syst Evol Microbiol 67(9):3358-3362.

Han, L., Z.Y. Li, X.F. Guo, J.L. Tan, S.Z. He, X.L. Cui and S.L. Li. 2017. *Hannaella dianchiensis* sp. nov., a basidiomycetous yeast species isolated from lake water. Int J Syst Evol Microbiol 67(6):2014-2018.

Kaewwichian, R., S. Jindamorakot, S. Am-In, M. Sipiczki and S. Limtong. 2015. *Hannaella siamensis* sp. nov. and *Hannaella phetchabunensis* sp. nov., two new anamorphic basidiomycetous yeast species isolated from plants. Int J Syst Evol Microbiol 65(Pt 4):1297-1303.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16(2):111-120.

Kurtzman, C.P. and C.J. Robnett. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek 73(4):331-371.

Lachance, M. and C.P. Kurtzman. 2011. Chapter 36 - *Kodamaea* Y. Yamada, T. Suzuki, Matsuda & Mikata emend. Rosa, Lachance, Starmer, Barker, Bowles & Schlag-Edler (1999). The Yeasts (Fifth Edition). pp. 483-490.

Landell, M.F., L.R. Brandão, A.C. Barbosa, J.P. Ramos, S.V. Safar, F.C. Gomes, F.M. Sousa, P.B. Morais, L. Broetto, O. Leoncini, J.R. Ribeiro, B. Fungsin, M. Takashima, T. Nakase, C.F. Lee, M.H. Vainstein, J.W. Fell, G. Scorzetti, H.S. Vishniac, C.A. Rosa and P. Valente. 2014. *Hannaella pagnoccae* sp. nov., a tremellaceous yeast species isolated from plants and soil. Int J Syst Evol Microbiol. 64(Pt 6): 1970-1977.

Larkin, M.A., G. Blackshields, N.P. Brown, R. Chenna, P.A.H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson and D.G. Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947-2948.

Saitou, N. and M. Nei. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406-425.

Surussawadee, J., S. Jindamorakot, T. Nakase, C.F. Lee and S. Limtong. 2015. *Hannaella phyllophila* sp. nov., a basidiomycetous yeast species associated with plants in Thailand and Taiwan. Int J Syst Evol Microbiol 65(7):2135-2140.

Wang, Q.M. and F.Y. Bai. 2008. Molecular phylogeny of basidiomycetous yeasts in the *Cryptococcus luteolus* lineage (*Tremellales*) based on nuclear rRNA and mitochondrial cytochrome b gene sequence analyses: proposal of *Derxomyces* gen. nov. and *Hannaella* gen. nov., and description of eight novel *Derxomyces* species. FEMS Yeast Res 8(5):799-814.

Submitted: April 23, 2020
Revised: May 20, 2020
Accepted: May 20, 2020