

# Molecular identification of *Pseudozyma aphidis* (Henninger & Windisch) Boekhout: first record from a Brazilian mangrove swamp

Marcela Castilho Boro<sup>1,2</sup>, Ana Lucia de Jesus<sup>1</sup>, José Ivanildo de Souza (*in memoriam*)<sup>1</sup>, Agostina Virginia Marano<sup>1</sup> and Carmen Lidia Amorim Pires-Zottarelli<sup>1</sup>

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**ABSTRACT** - (Molecular identification of *Pseudozyma aphidis* (Henninger & Windisch) Boekhout: first record from a Brazilian mangrove swamp). *Pseudozyma aphidis* (Henninger & Windisch) Boekhout is an anamorph yeast-like basidiomycete commonly found as saprotroph in vegetable debris, such as leaves and flowers. However, there are also reports that this species is pathogenic in humans, usually occurring in patients with some type of immunosuppression, which predisposes to opportunistic infections. Specimens of *P. aphidis* were collected from water samples and *Rhizophora mangle* L. leaves at different salinities along the Perequê River, located in a mangrove swamp area at Parque Estadual da Ilha do Cardoso, Cananéia municipality, São Paulo State, Brazil. Aliquots of these samples were spread in Petri dishes. The samples baited with cataphylls of *Allium cepa* L. were incubated for seven days at 21 °C. After incubation, the baits were observed under microscope and the specimens were isolated and purified. The identification of the specimens was made through the phylogenetic analysis of the ITS rDNA region. This is the first record of *P. aphidis* in São Paulo State.

**Keywords:** Atlantic rainforest, Basidiomycota, ITS, yeast-like fungi

**RESUMO** - (Identificação molecular de *Pseudozyma aphidis* (Henninger & Windisch) Boekhout: primeira citação em um manguezal brasileiro). *Pseudozyma aphidis* (Henninger & Windisch) Boekhout é um basidiomiceto anamórfico leveduriforme comumente encontrado como sapróbio em detritos vegetais, como folhas e flores. No entanto, há relatos de que esta espécie é patogênica em seres humanos, geralmente ocorrendo em pacientes com algum tipo de imunossupressão que predispõe infecções oportunistas. Espécimes de *P. aphidis* foram coletados a partir de amostras de água e de folhas de *Rhizophora mangle* L. em diferentes salinidades ao longo do rio Perequê, localizado em um manguezal no Parque Estadual da Ilha do Cardoso, município de Cananéia, Estado de São Paulo, Brasil. No laboratório, alíquotas destas amostras foram colocadas separadamente em placas de Petri, iscadas com catafilos de *Allium cepa* L. e incubadas por sete dias a 21 °C. Após incubação, as iscas foram observadas ao microscópio e os espécimes foram isolados e purificados. A identificação dos espécimes foi realizada através da análise filogenética da região ITS rDNA, sendo este o primeiro registro de *P. aphidis* no Estado de São Paulo.

**Palavras-chave:** Basidiomycota, fungo leveduriforme, ITS, Mata Atlântica

## Introduction

*Pseudozyma* Bandoni emend. Boekhout *pro tempore* comprises approximately 25 distinct anamorphic yeast species from the Ustilaginaceae, Basidiomycota (Index Fungorum 2016). Although a reliable connection with their teleomorph stages has not been established for many of these species (Boekhout 2011), recent studies have proposed to transfer anamorphic yeast species to their corresponding teleomorphic genera based on strongly supported phylogenetic affinities (Piatek *et al.* 2015, Wang *et al.* 2015). From these studies, many species of the

genus *Pseudozyma* have been transferred to the genus *Moesziomyces*, as is the case of *Pseudozyma aphidis* (= *Moesziomyces aphidis*) and the use of the “*pro tempore*” was suggested by the authors to indicate that in *Pseudozyma* species names are temporarily remained. The results of these studies allowed the proposition of 16 new combinations for *Pseudozyma* (Index Fungorum 2016), however, the taxonomic status of five species still remains to be determined because of their uncertain phylogenetic positions (Bandoni 1985, Boekhout 1995, Sugita *et al.* 2003, Wang *et al.* 2006, Liou *et al.* 2009).

1. Instituto de Botânica, Núcleo de Pesquisa em Micologia, Av. Miguel Stéfano 3687, 04301-012 São Paulo, SP, Brazil

2. Corresponding Author: marcelaboro@gmail.com

This genus has been isolated from different types of materials such as leaves (Golubev *et al.* 2007, Chamnanpa *et al.* 2013), flowers (Wei *et al.* 2005, Liou *et al.* 2009), blood samples (Sugita *et al.* 2003, Mekha *et al.* 2014) and even associated with coral reefs and insects (Henninger & Windisch 1975, Statzell-Tallman *et al.* 2010, Oliveira *et al.* 2014). *Pseudozyma aphidis* (Henninger & Windisch) Boekhout was firstly described as *Sterigmatomyces aphidis* Henninger & Windisch in Germany and isolated from Aphididae secretions on leaves of *Solanum pseudocapsicum* (Henninger & Windisch 1975). This species is commonly found as a saprotroph on leaves and flowers (Han *et al.* 2015, Limtong & Kaewwichian 2015, Nasanit *et al.* 2015, Tantrungkij *et al.* 2015), although it is not pathogenic to plants. However, there are reports of this species as pathogenic in humans (Prakash *et al.* 2014, Herb *et al.* 2015, Joo *et al.* 2015, Orecchini *et al.* 2015), usually occurring in patients with some type of immunosuppression that predisposes opportunistic infections.

In Latin America, the first report of *P. aphidis* was in Brazil when Costa (2006) reported the presence of this species, through molecular identification of the region D1/D2 of 26S rDNA, in semi-arid soil from Mucugê municipality, Bahia State. Tristão *et al.* (2012) reported an association of *P. aphidis* with pineapple fruits sampled from the Central Region of Tocantins State. As a human pathogen, the first case was reported from a public pediatric oncology center in Recife, Pernambuco State, causing pulmonary infection in a patient with Burkitt lymphoma (Parahym *et al.* 2013).

The occurrence and activity of yeasts in marine waters have been observed worldwide since first reported by Fischer & Brebeck (1894) and is well established due to their role in the decomposition of organic substrates, nutrient cycling, hydrocarbon biodegradation, and as pathogenic for a variety of marine organisms (Vogel *et al.* 2007, Kutty & Philip 2008). Species of *Pseudozyma*, including *P. aphidis*, have commonly been reported in marine and mangrove environments (Gadanhó *et al.* 2003, Statzell-Tallman *et al.* 2010, Fell *et al.* 2011).

Several papers have also shown the potential of this species for inducing systemic resistance and as a biocontrol agent against plant pathogens of different crops (Tristão *et al.* 2012, Buxdorf *et al.* 2013a, b, Gafni *et al.* 2015). They are also reported as a potential source of squalene (Chang *et al.* 2008), lipase (Dimitrijevic *et al.* 2011) and biosurfactant

mannosylerythritol lipids (Morita *et al.* 2007, Fan *et al.* 2014, Lorenz *et al.* 2014, Gunther *et al.* 2015).

Molecular methods are useful tools for the accurate positioning of yeasts within the Kingdom Fungi, being the classic methods of limited value due to the low morphological variation between species and the existence of a significant yet undescribed diversity (Hoog *et al.* 1998). The use of nucleotide sequences of internal transcribed spacer (ITS) of the rDNA region is considered the best tool for rapid and accurate identification of yeast isolates (White *et al.* 1990).

During a survey at the Parque Estadual da Ilha do Cardoso (PEIC), we identified four isolates of *P. aphidis* based on the phylogenetic analysis of the ITS rDNA region. Thus, the aim of this study is to present the phylogeny of the genus with the inclusion of specimens collected in this mangrove area of Brazilian Atlantic Rainforest.

## Material and methods

The *Pseudozyma* specimens used in this study were isolated from water samples and *Rhizophora mangle* L. leaves collected (in sterile plastic bottle) in the months of August and November 2012 at different salinities (1.5 to 3%) along the Perequê River. The study area is located in a mangrove swamp from Parque Estadual da Ilha do Cardoso (PEIC), 25°03'05"-25°18'18"S; 47°53'48"-48°05'42"W, situated in Cananéia municipality, São Paulo State, Brazil. This island is a preserved fragment of Atlantic Rainforest that contains different types of vegetation, including tropical forest, "restinga", dunes and mangrove swamps, with several water bodies like waterfalls, streams and rivers (Barros *et al.* 1991). During the sampling, the surface water temperature of the estuary was lower in August, about 20 °C and higher in November, about 27 °C.

Aliquots (30 mL) of water samples and disks (1 cm diameter) of *R. mangle* leaves (washed in sterilized seawater) together with 30 mL of a dilution of 50% reverse osmosis and 50% seawater, were placed separately in Petri dishes. These samples were baited with cataphylls of *Allium cepa* L. (Sparrow 1960, Milanez 1989) and incubated for seven days at 21 °C. After incubation, the baits were observed under microscope and the specimens were isolated and purified onto PYGs culture medium (Fuller & Jaworski 1987) prepared with a dilution of 50% sterile seawater, penicillin G 0.4 g L<sup>-1</sup> and streptomycin sulphate 0.4 g L<sup>-1</sup>.

Table 1. Taxa, strain numbers and GenBank accession numbers of the species considered in this study.

Taxa	Strain number	GenBank accession number
<i>Cintractia axicola</i>	HUV17460	AY344967
<i>Trichocintractia utriculicola</i>	MP 2075	DQ875354
<i>Pseudozyma abaconensis</i>	CBS8380	FJ008053
<i>Pseudozyma alboarmeniaca</i>	DMST17135	AB117961
<i>Pseudozyma antarctica</i>	JCM10317	AB089358
<i>Pseudozyma antarctica</i>	JCM3941	JN942669
<i>Pseudozyma aphidis</i> *	CCIBt 4253	KY288985
<i>Pseudozyma aphidis</i> *	CCIBt 4254	KY288982
<i>Pseudozyma aphidis</i> *	CCIBt 4255	KY288981
<i>Pseudozyma aphidis</i> *	CCIBt 4256	KY288984
<i>Pseudozyma aphidis</i>	CBS51783	AF294699
<i>Pseudozyma aphidis</i>	IMUFRJ51941	FN424100
<i>Pseudozyma aphidis</i>	JCM10318	AB089362
<i>Pseudozyma aphidis</i>	LH59	HQ832804
<i>Pseudozyma brasiliensis</i>	GHG001	KF737866
<i>Pseudozyma churashimaensis</i>	OK96	AB548947
<i>Pseudozyma churashimaensis</i>	OK99	AB548950
<i>Pseudozyma crassa</i>	DMST17136	AB117062
<i>Pseudozyma flocculosa</i>	JCM10321	AB089364
<i>Pseudozyma fusiformata</i>	JCM3931	AB089366
<i>Pseudozyma fusiformata</i>	AP6	FJ919774
<i>Pseudozyma graminicola</i>	LI20	AB180728
<i>Pseudozyma hubeiensis</i>	AS2.2493	DQ008954
<i>Pseudozyma hubeiensis</i>	LH146	HQ832814
<i>Pseudozyma jejuensis</i>	CBS10454	EF079966
<i>Pseudozyma jejuensis</i>	IMUFRJ52021	FN428892
<i>Pseudozyma parantarctica</i>	JCM11752	NR130693
<i>Pseudozyma parantarctica</i>	M9932	AB089356
<i>Pseudozyma prolifica</i>	JCM10319	AB089368
<i>Pseudozyma prolifica</i>	CBS31987	AF294700
<i>Pseudozyma pruni</i>	BCRC34227	EU379942
<i>Pseudozyma rugulosa</i>	M12	FJ464594
<i>Pseudozyma rugulosa</i>	JCM10323	AB089370
<i>Pseudozyma rugulosa</i>	CBS17088	AF294697
<i>Pseudozyma shanxiensis</i>	AS2.2523	DQ008956
<i>Pseudozyma shanxiensis</i>	SN37	FJ515182
<i>Pseudozyma siamensis</i>	DMST17137	AB117963
<i>Pseudozyma thailandica</i>	M9933	AB089354
<i>Pseudozyma tsukubaensis</i>	JCM10324	AB089372
<i>Pseudozyma tsukubaensis</i>	KM160	AB855769
<i>Pseudozyma vetiver</i>	DMKU-LV99	AB809652
<i>Pseudozyma vetiver</i>	DMKU-LV90	AB809651

\* Taxa, strain numbers and GenBank accession numbers of the isolates of *Pseudozyma aphidis* sequenced in this study.

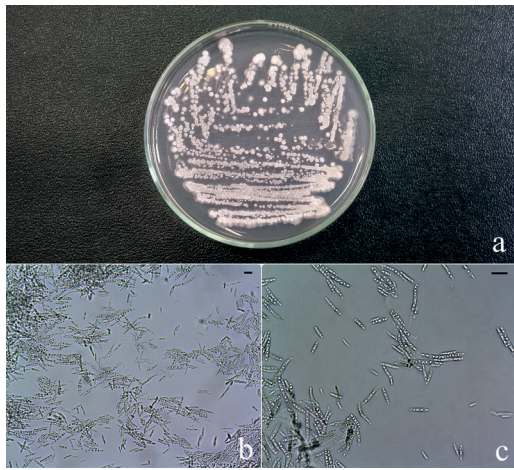


Figure 1. a-c. *Pseudozyma aphidis*. a. One-week old culture onto PYGs culture medium. b-c. Yeast-like fungi cells obtained from colonies growing onto PYGs culture medium. Bars = 10 µm.

The specimens were preserved by the Castellani's method (50% sterile reverse osmosis water and 50% sterile seawater) and posteriorly deposited at the CCIBt culture collection (Coleção de Culturas de Algas, Cianobactérias e Fungos do Instituto de Botânica, São Paulo, SP, Brazil).

Biomass for DNA extraction was obtained by cultivating each isolate onto PYGs culture medium, prepared with a dilution of 50% sterile seawater, penicillin G 0.4 g L<sup>-1</sup> and streptomycin sulphate 0.4 g L<sup>-1</sup>, for seven days at 21 °C. After incubation, the cells were harvested using a platinum loop. The DNA extraction followed the protocol described in the PureLink Genomic DNA kit (Invitrogen®). The ITS1-5.8S-ITS2 region was amplified using the primers UN-up18S42 and UN-lo28S22 (Robideau *et al.* 2011). PCR amplification was performed with

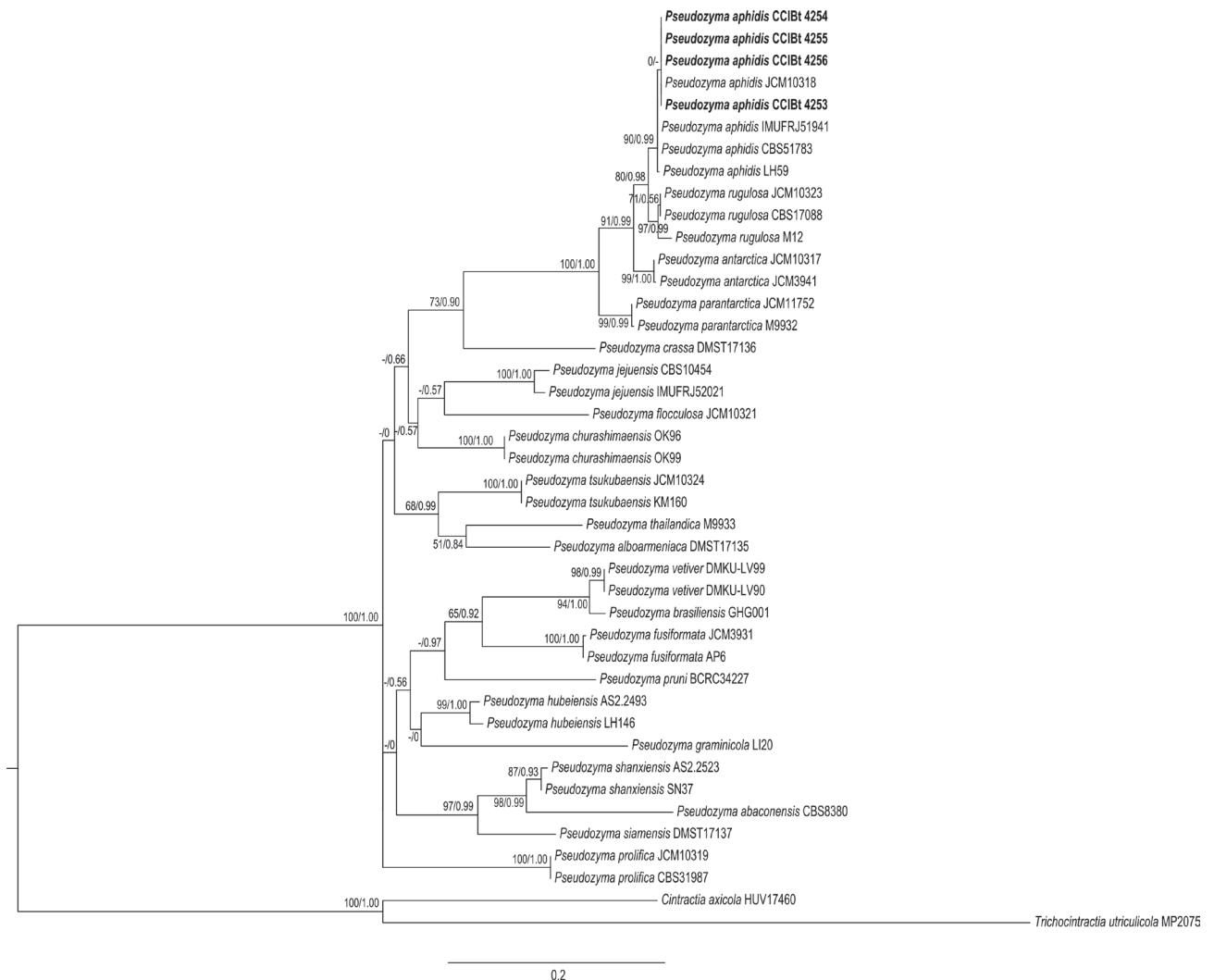


Figure 2. Maximum likelihood tree of internal transcribed spacer (ITS) rDNA sequences of *Pseudozyma* spp. Maximum likelihood bootstrap support and posterior probability values large than 50% are indicated numerically, those under 50% are marked with (-). Those clades that were not present in the Bayesian trees are marked as (0). The scale bar represent the average number of substitutions per site

the PCR SuperMix kit (Invitrogen®) for a final volume of 25 µl in a C1000 Touch™ Thermal Cycler Bio-Rad following the conditions described by Marano *et al.* (2014). Amplicons were purified with AxyPrep PCR Clean-up kit (Axygen®). Sequencing was performed using the same PCR primers in an ABI 3730 DNA Analyser (Life Technologies™). Assembly of contigs and correction of ambiguous bases were manually edited using Sequencher™ version 4.1.4.

The ITS sequences obtained were compared against BLASTn (Basic Local Alignment Search Tool). For phylogenetic reconstruction, we used ITS rDNA sequences of our isolates and of the other isolates of *Pseudozyma* available at GenBank (table 1). We analysed 42 sequences from 23 species, using *Cintractia axicola* (Berk.) Cornu and *Trichocintractia utriculicola* (Henn.) M. Piepenbr. as outgroups. Sequences were aligned using MAFFT version 7 (Kazutaka & Daron 2013), the ambiguously aligned characters were removed manually using Geneious version 8 (Kearse *et al.* 2012) and the alignment was deposited in the Treebase (Boettinger & Lang 2011). The best fitting model of evolution was selected using the Akaike Information Criterion in jModeltest version 0.1.1 (Posada 2008). The Maximum Likelihood (ML) phylogenies were reconstructed with PhyML version 3.1 (Guindon & Gascuel 2003) using the TPm2uf + G substitution model, branch swapping by best of NNI and SPR, and support for nodes obtained by 1,000 bootstrap pseudo-replicates. Bayesian inferences (BI) were generated using MrBayes version 3.2.1 (Ronquist & Huelsenbeck 2003) with Markov Chain Monte Carlo (MCMC) methodology to calculate posterior probabilities of the phylogenetic trees. The program was run for 5 millions generations with the TPm2uf + G substitution model. The first 10% of the interactions were discarded as burn-in.

## Results and Discussion

The specimens grew abundantly on *Allium cepa* L. used as bait and later onto PYGs (figure 1a), with a great number of cells produced in one week (figures 1b, 1c).

The BLASTn query showed that these specimens belong to *Pseudozyma aphidis* (alignment in the Treebase, <http://purl.org/phylo/treebase/phyloids/study/TB2:S21546>). Our isolates of *P. aphidis* are well supported and clustered together with other isolates of the same species (90% of bootstrap support with high

values of posterior probability (0.98), as shown in figure 2. This species has a close phylogenetic affinity with *P. rugulosa* (80% of bootstrap support and 0.98 of posterior probability), as previously demonstrated (Chang *et al.* 2008).

The culture collection numbers of the isolates obtained in this study and their respective sequences deposited in GenBank database are shown in table 1.

Although *P. aphidis* has been already reported in Brazil (Costa 2006, Tristão *et al.* 2012, Parahym *et al.* 2013) and worldwide, this is the first report from a mangrove area in São Paulo State.

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## Literature cited

- Bandoni, R.J.** 1985. On an undescribed, pleomorphic hyphomycete from litter. *Botanical Journal of the Linnean Society* 91: 37-43.
- Barros, F., Melo, M.M.R.F., Chiea, S.A.C., Kirizawa, M., Wanderley, M.G.L. & Jung-Mendaçolli, S.L.** 1991. Flora fanerogâmica da Ilha do Cardoso: caracterização geral da vegetação e listagem das espécies ocorrentes. *Boletim do Instituto de Botânica* 1: 1-184.
- Boekhout, T.** 1995. *Pseudozyma* Bandoni emend. Boekhout, a genus for yeast-like anamorphs of Ustilaginales. *Journal of General and Applied Microbiology* 41: 359-366.
- Boekhout, T., Fonseca, A., Sampaio, J.P., Bandoni, R.J., Fell, J.W. & Kwong-Chung.** 2011. Discussion of teleomorphic and anamorphic basidiomycetous yeast. *In: C.P. Kurtzman, J.W. Fell & T. Boekhout (eds.). The yeast, a taxonomic study.* Elsevier, Amsterdam, pp. 1339-1372.
- Boettinger, C. & Lang, D.T.** 2012. Treebase: an R package for discovery, access and manipulation of online phylogenies. *Methods in Ecology and Evolution* 3: 1060-1066.
- Buxdorf, K., Rahat, I. & Levy, M.** 2013a. *Pseudozyma aphidis* induces ethylene independent resistance in plants. *Plant Signaling & Behavior* 8: e26273.

- Buxdorf, K., Rahat, I., Gafni, A. & Levy, M.** 2013b. The epiphytic fungus *Pseudozyma aphidis* induces jasmonic acid and salicylic acid/nonexpressor of PR1-independent local and systemic resistance. *Plant Physiology* 161: 2014-2022.
- Chamnanpa, T., Limtong, P., Srisuk, N. & Limtong, S.** 2013. *Pseudozyma vetiver* sp. nov., a novel anamorphic ustilaginomycetous yeast species isolated from the phylloplane in Thailand. *Antonie van Leeuwenhoek* 104: 637-644.
- Chang, M.H., Kim, H.J., Jahng, K.Y. & Hong, S.C.** 2008. The isolation and characterization of *Pseudozyma* sp. JCC 207, a novel producer of squalene. *Applied Microbiology and Biotechnology* 78: 963-972.
- Costa, M.S.F.** 2006. Diversidade de leveduras do solo semiárido da Bahia, Brasil. Tese de Doutorado, Universidade Federal de Pernambuco, Recife.
- Dimitrijević, A., Velicković, D., Bezbradica, D., Bihelović, F., Jankov, R. & Milosavić, N.** 2011. Production of lipase from *Pseudozyma aphidis* and determination of the activity and stability of the crude lipase preparation in polar organic solvents. *Journal of the Serbian Chemical Society* 76: 1081-1092.
- Fan, L.L., Dong, Y.C., Fan, Y.F., Zhang, J. & Chen, Q.H.** 2014. Production and identification of mannosylerythritol lipid-A homologs from the ustilaginomycetous yeast *Pseudozyma aphidis* ZJUDM34. *Carbohydrate Research* 392: 1-6.
- Fell, J.W., Statzell-Tallman, A., Scorzetti, G. & Gutierrez, M.H.** 2011. Five new species of yeasts from fresh water and marine habitats in the Florida Everglades. *Antonie van Leeuwenhoek* 99: 533-549.
- Fischer, B. & Brebeck, C.** 1984. Zur Morphologie, Biologie und Systematik der Kahmpilze, der *Monilia candida* Hansen und des Soorerregers. G. Fischer Jena: 1-52.
- Fuller, M.S. & Jaworski, A.** 1987. Zoosporic Fungi in teaching and research. Palfrey Contributions in Botany 3. Southeastern Publishing. Athens, Georgia.
- Gadanhó, M., Almeida, J.M.G.C.F. & Sampaio, J.P.** 2003. Assessment of yeast diversity in a marine environment in the south of Portugal by microsatellite-primed PCR. *Antonie van Leeuwenhoek* 84: 217-227.
- Gafni, A., Caldero, C.E., Harris, R., Buxdorf, K., Dafa-Berger, A., Zeilinger-Reichert, E. & Levy, M.** 2015. Biological control of the cucurbit powdery mildew pathogen *Podosphaera xanthii* by means of the epiphytic fungus *Pseudozyma aphidis* and parasitism as a mode of action. *Frontier in Plant Science* 6: 1-11.
- Golubev, W., Sugita, T. & Golubev, N.** 2007. An ustilaginomycetous yeast, *Pseudozyma graminicola* sp. nov., isolated from the leaves of pasture plants. *Mycoscience* 48: 29-33.
- Guindon, S. & Gascuel, O.** 2003. A Simple, fast, and accurate algorithm to estimate large phylogenies by Maximum Likelihood. *Systematic Biology* 52: 679-704.
- Günther, M., Grumaz, C., Lorenz, S., Stevens, P., Lindemann, E., Hirth, T., Sohn, K., Zibek, S. & Rupp, S.** 2015. The transcriptomic profile of *Pseudozyma aphidis* during production of mannosylerythritol lipids. *Applied Microbiology and Biotechnology* 99: 1375-1388.
- Han, S.M., Hyun, S.H., Lee, H.B., Lee, H.W., Kim, H.K. & Lee, J.S.** 2015. Isolation and Identification of Yeasts from Wild Flowers Collected around Jangseong Lake in Jeollanam-do, Republic of Korea, and Characterization of the Unrecorded Yeast *Bullera coprosmaensis*. *Mycobiology* 43: 266-271.
- Henninger, W. & Windisch, S.** 1975. A new yeast of Sterigmatomyces, *S. aphidis* sp. n. *Archive Microbiology* 105: 49-50.
- Herb, A., Sabou, M., Delhorme, J.B., Pessaux, P., Mutter, D., Candolfi, E. & Letscher-Bru, V.** 2015. *Pseudozyma aphidis* fungemia after abdominal surgery: First adult case. *Medical Mycology* 8: 37-39.
- Hoog, G.S., Bowman, B. & Graser, Y.** 1998. Molecular phylogeny and taxonomy of medically important: Fungi. *Medical Mycology* 36: 52-56.
- Index Fungorum.** 2016. Index Fungorum. Available in <http://www.indexfungorum.org/> (accessed in 07-XI-2016).
- Joo, H., Choi, Y.G., Cho, S.Y., Choi, J.K., Lee, D.G., Kim, H.J., Jo, I., Park, Y.J. & Lee, K.Y.** 2015. *Pseudozyma aphidis* fungemia with invasive fungal pneumonia in a patient with acute myeloid leukaemia: case report and literature review. *Mycoses: Diagnosis, Therapy and Prophylaxis of Fungal Diseases* 59: 56-61.
- Kazutaka, K. & Daron, M.** 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Available in <http://mafft.cbrc.jp/alignment/server> (accessed in 10-VI-2016).
- Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes P. & Drummond A.** 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28: 1647-1649.
- Kutty, S.N. & Philip, R.** 2008. Marine yeasts - a review. *Yeast* 25: 465-483.
- Limtong, S. & Kaewwichian, R.** 2015. The diversity of culturable yeasts in the phylloplane of rice in Thailand. *Annals of Microbiology* 65: 667-675.
- Liou, G.Y., Wei, Y.H., Lin, S.J., Wen, C.Y. & Lee, F.L.** 2009 *Pseudozyma pruni* sp. nov., a novel ustilaginomycetous anamorphic fungus from flowers in Taiwan. *International Journal of Systematic and Evolutionary Microbiology* 59: 1813-1817.

- Lorenz, S., Günther, M., Grumaz, C., Rupp, S., Zibek, S. & Sohn, K. 2014. Genome Sequence of the Basidiomycetous Fungus *Pseudozyma aphidis* DSM70725, an Efficient Producer of Biosurfactant Mannosylerythritol Lipids. *Genome Announcements* 2: e00053-14.
- Marano, A.V., Jesus, A.L., de Souza, J.I., Leão, E.M., James, T.Y., Jerônimo, G.H., de Cock, A.W.A.W. & Pires-Zottarelli, C.L.A. 2014. A new combination in *Phytophthium*: *P. kandeliae* (Oomycetes, Straminipila). *Mycosphere* 5: 510-522.
- Mekha, N., Takashima, M., Boon-Long, J., Cho, O. & Sugita, T. 2014. Three new basidiomycetous yeasts, *Pseudozyma alboarmeniaca* sp. nov., *Pseudozyma crassa* sp. nov. and *Pseudozyma siamensis* sp. nov. isolated from Thai patients. *Microbiology and Immunology* 58: 9-14.
- Milanez, A.I. 1989. Fungos de águas continentais. In: O. Fidalgo, V.L. Bononi (coords.). Técnicas de coleta, preservação e herborização de material botânico. Série Documentos Instituto de Botânica, São Paulo, pp. 17-20.
- Morita, T., Konishi, M., Fukuoka, T., Imura, T. & Kitamoto, D. 2007. Physiological differences in the formation of the glycolipid biosurfactants, mannosylerythritol lipids, between *Pseudozyma antarctica* and *Pseudozyma aphidis*. *Applied Microbiology and Biotechnology* 74: 307-315.
- Nasanit, R., Tangwong-o-Thai, A., Tantirungkij, M. & Limtong, S. 2015. The assessment of epiphytic yeast diversity in sugarcane phyllosphere in Thailand by culture-independent method. *Fungal Biology* 119: 1145-1157.
- Oliveira, J.V.C., Borges, T.A., dos Santos, R.A.C., Freitas, L.F.D., Rosa, C.A., Goldman, G.H. & Riaño-Pachón, D.M. 2014. *Pseudozyma brasiliensis* sp. nov., a xylanolytic, ustilaginomycetous yeast species isolated from an insect pest of sugarcane roots. *International Journal of Systematic and Evolutionary Microbiology* 64: 2159-2168.
- Orecchini, L.A., Olmos, E., Taverna, C.G., Murisengo, O.A., Szuzs, W., Vivot, W., Córdoba, S., Bosco-Borgeat, M.E. & Montanaro, P.C. 2015. First Case of fungemia due to *Pseudozyma aphidis* in a pediatric patient with osteosarcoma in Latin America. *Journal of Clinical Microbiology* 53: 3691-3694.
- Parahym, A.M.R.C., da Silva, C.M., Domingos, I.F., Gonçalves, S.S., Rodrigues, M.M., de Moraes, V.L.L. & Neves, R.P. 2013. Pulmonary infection due to *Pseudozyma aphidis* in a patient with burkitt lymphoma: first case report. *Diagnostic Microbiology and Infectious Disease* 75: 104-106.
- Piatek, M., Lutz, M. & Yorou, N.S. 2015. A molecular phylogenetic framework for *Anthracoystis* (Ustilaginales), including five new combinations (inter alia for the asexual *Pseudozyma flocculosa*), and description of *Anthracoystis grodzinskae* sp. nov. *Mycological Progress* 14: 1-15.
- Posada, D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology Evolution* 25: 1253-1256.
- Prakash, A., Wankhede, S., Singh, P.K., Agarwal, K., Kathuria, S., Sengupta, S., Barman, P., Meis, J.F. & Chowdhary, A. 2014. First neonatal case of fungaemia due to *Pseudozyma aphidis* and a global literature review. *Mycoses* 57: 64-68.
- Robideau, G.P., de Cock, A.W.A.M., Coffey, M.D., Voglmayr, H., Brouwer, H., Bala, K., Chitty, D.W., Désaulniers, N., Eggertson, Q.A., Gachon, C.M.M., Hu, C.H., Küpper, F.C., Rintoul, T.L., Sarhan, E., Verstappen, E.C.P., Zhang, Y., Bonants, P.J.M., Ristaino, J.B. & Lévesque, A.C. 2011. DNA barcoding of oomycetes with cytochrome c oxidase subunit I and internal transcribed spacer. *Molecular Ecology Resources* 11: 1002-1011.
- Ronquist, F., Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- Sparrow Jr., F.K. 1960. Aquatic Phycomycetes. Ambassador Books (2th revised edition), Toronto, pp. 1187.
- Statzell-Tallman, A., Scorzetti, G. & Fell, J.W. 2010. *Candida spencermartinsiae* sp. nov., *Candida taylorii* sp. nov. and *Pseudozyma abaconensis* sp. nov., novel yeasts from mangrove and coral reef ecosystems. *International Journal of Systematic and Evolutionary Microbiology* 60: 1978-1984.
- Sugita, T., Takashima, M., Poonwan, N., Mekha, N., Malaithao, K., Thungmuthasawat, B., Prasarn, S., Luangsook, P. & Kudo, T. 2003. The First Isolation of Ustilaginomycetous Anamorphic Yeasts, *Pseudozyma* Species, from Patients' Blood and a Description of Two New Species: *P. parantarctica* and *P. thailandica*. *Microbiology and Immunology* 47: 183-190.
- Tantirungkij, M., Nasanit, R. & Limtong, S. 2015. Assessment of endophytic yeast diversity in rice leaves by a culture-independent approach. *Antonie van Leeuwenhoek* 108: 633-647.
- Tristão, G.B., Malta, C.M., Borges, A.K.P., de Moraes, P.B., da Silva, J.F.M. & Pimenta, R.S. 2012. Leveduras associadas a frutos de abacaxi (*Ananas comosus*) e sua utilização como agente de controle biológico. *Engenharia Ambiental - Espírito Santo do Pinhal* 9: 85-93.

- Vogel, C., Rogerson, A., Scatz, S., Laubach, H., Tallman, A. & Fell, J.W.** 2007. Prevalence of yeast in beach sand at three bathing beaches in South Florida. *Water Research* 41: 1915-1930.
- Wang, Q.M., Jia, J.H. & Bai, F.Y.** 2006. *Pseudozyma hubeiensis* sp. nov. and *Pseudozyma shanxiensis* sp. nov., novel ustilaginomycetous anamorphic yeast species from plant leaves. *International Journal of Systematic and Evolutionary Microbiology* 56: 289-293.
- Wang, Q.M., Begerow, D., Groenewald, M., Liu, X.Z., Theelen, B., Bai, F.Y. & Boekhout, T.** 2015. Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. *Studies in Mycology* 81: 55-83.
- Wei, Y.H., Lee, F.L., Hsu, W.H., Chen, S.R., Chen, C.C., Wen, C.Y., Lin, S.J., Chu, W.S., Yuan, G.F. & Liou, G.Y.** 2005. *Pseudozyma antarctica* in Taiwan: a description based on morphological, physiological and molecular characteristics. *Botanical Bulletin of Academia Sinica* 46: 223-229.
- White, T.J., Bruns, T., Lee, S. & Taylor, J.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: M.A. Innis, D.H. Gelfand, J.J. Sninsky & T.J. White (eds.). *PCR Protocols: A Guide to Methods and Applications*. San Diego, CA: Academic Press, pp. 315-324.