Sympodiomyopsis kandeliae sp. nov., a basidiomycetous anamorphic fungus from mangroves, and reclassification of *Sympodiomyopsis lanaiensis* as *Jaminaea lanaiensis* comb. nov.

Yu-Hui Wei, Guey-Yuh Liou, Hsin-Yi Liu and Fwu-Ling Lee

Correspondence

Fwu-Ling Lee
fll@firdi.org.tw

Bioresource Collection and Research Center (BCRC), Food Industry Research and Development Institute (FIRDI), PO Box 246, Hsinchu, 30062, Taiwan, ROC

Three ustilaginomycetous anamorphic strains were isolated from flowers of *Kandelia candel* in mangrove forests of Taiwan. Phylogenetic analyses based on the combined sequences of internal transcribed spacer 1 (ITS1)-5.8S-ITS2 and the D1/D2 domain of the large-subunit (LSU) rDNA indicated that the closest recognized species was *Sympodiomyopsis paphiopedili*. The results of a DNA–DNA hybridization experiment and the physiological characteristics showed that the three strains represent a novel species within the genus *Sympodiomyopsis*. The name *Sympodiomyopsis kandeliae* sp. nov. is proposed, with FIRDI 007^T (=BCRC 23165^T = CBS 11676^T) as the type strain. In addition, based on phenotypic characteristics and the phylogenetic analyses of the combined sequences of the ITS region and D1/D2 domain of the LSU rDNA, *Sympodiomyopsis lanaiensis* was clustered with the genus *Jaminaea*. A new combination, *Jaminaea lanaiensis* comb. nov. (type strain LM418^T = DSM 18755^T = ATCC MYA-4092^T = NRRL Y-48466^T = CBS 10858^T = BCRC 23177^T), is proposed.

The anamorphic genus *Sympodiomyopsis* was proposed by Sugiyama *et al.* (1991) to accommodate one species, *Sympodiomyopsis paphiopedili*, which was isolated from orchid nectar in Japan. In 2008, *Sympodiomyopsis lanaiensis*, which was isolated from driftwood in Hawai'i, was described (Mahdi *et al.*, 2008). The genus *Sympodiomyopsis* was believed to demonstrate a basidiomycetous affinity when it was proposed in 1991. The septal pore ultrastructure, chemotaxonomic characteristics and the rDNA sequence data revealed that *S. paphiopedili* belongs to the order Microstromatales (Basidiomycota, Ustilaginomycotina, Exobasidiomycetes) (de Beer *et al.*, 2006; Begerow *et al.*, 2006; Suh *et al.*, 1993; Suh & Sugiyama, 1994). During an investigation of epiphytic yeast-like fungi in Taiwan, a novel species of the genus *Sympodiomyopsis* was detected via sequencing of the internal transcribed spacer 1 (ITS1)-5.8S-ITS2 region and the D1/D2 domain of the large-subunit (LSU) rDNA and was confirmed by DNA–DNA hybridization.

Fresh and healthy flowers of *Kandelia candel* were collected in clean paper bags and transported to the laboratory. Epiphytic fungi were isolated using YM agar (Difco) with 10 % NaCl. After being incubated at 20 °C for 7–14 days, colonies were purified on YM agar with 3 % NaCl. Strains FIRDI 007^T and BCRC 23075 were isolated from a sample collected from the mangrove forest at Singfen Wetland, Hsinchu (northern Taiwan, approx. coordinates: 120° 59' E 24° 54' N), on 3 June 2004. Strain 07F0494 was isolated from a sample collected from the mangrove forest at Chiku Wetland, Tainan (southern Taiwan, approx. coordinates: 120° 05' E 23° 05' N), on 14 April 2007.

Morphological and physiological characteristics were examined using the methods described by Yarrow (1998). Nuclear DNA intended for use in PCR was extracted using the DNeasy Plant Mini kit (Qiagen).

The ITS region and D1/D2 domain of the LSU rDNA were defined by using primers ITS5/ITS4 (White *et al.*, 1990) and NL1/NL4 (O'Donnell, 1992). The reaction was performed in a GeneAmp PCR system 9700 (Applied Biosystems) using 30 cycles of the following steps: denaturation at 95 °C for 30 s, annealing at 55 °C for 1 min, and extension at 72 °C for 1 min; final extension at 72 °C for 10 min. Sequencing reactions were performed using the ABI PRISM BigDye Terminator v3.0 ready reaction cycle

Abbreviations: ITS, internal transcribed spacer; LSU, large subunit.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this study are GQ465042–GQ465045 (ITS region) and FJ426334, GU047881–GU047882 (D1/D2 domain of the LSU rDNA).

Two supplementary figures and two supplementary tables are available with the online version of this paper.

sequencing kit according to the manufacturer's directions. PCR products were sequenced by using an ABI PRISM 3730 DNA analyser.

Sequences of each strain were aligned by using CLUSTAL X 1.83 (Thompson *et al.*, 1997). Neighbour-joining and maximum-parsimony phylogenetic analysis was performed using the MEGA4 software package (Tamura *et al.*, 2007). Maximum-likelihood phylogenetic analysis was performed using the PHYLIP software package (Guindon & Gascuel, 2003). Confidence values for the phylogenetic trees were estimated from bootstrap analyses with 1000 replicates.

The isolation and purification of DNA for the determination of DNA base composition, expressed as DNA G + C content, and levels of DNA relatedness were performed using the Genomic DNA Buffer Set and Genomic-tip 500/G (Qiagen). The purified DNA was hydrolysed to nucleosides as described by Tamaoka & Komagata (1984), and the hydrolysate was analysed by using reversed phase HPLC to determine the DNA base composition expressed in mol% G + C. DNA–DNA relatedness values were determined using the photo-biotin-labelling microplate method (Lee *et al.*, 1993, 1998). Hybridizations were performed at 45 °C for 24 h.

Strains FIRDI 007^T and BCRC 23075 had identical LSU rDNA D1/D2 domain and ITS region sequences. The D1/

D2 domain of the LSU rDNA sequence of strains FIRDI 007^T and BCRC 07F0494 differed by three nucleotides. The ITS region sequences of strains FIRDI 007^T and BCRC 07F0494 differed by two nucleotides. Phylogenetic analyses revealed that strains FIRDI 007^T, BCRC 23075 and BCRC 07F0494 belong to the genus *Sympodiomyopsis* and are clustered within the anamorphic clade of the order *Microstromatales* (Begerow *et al.*, 2001; de Beer *et al.*, 2006). Based on combined alignment of the ITS region and the D1/D2 domain of the LSU rDNA, *S. paphiopedili* was the closest recognized species with strong bootstrap support in the neighbour-joining (Fig. 1), maximum-parsimony and maximum-likelihood trees (see Supplementary Figs S1 and S2, available in IJSEM Online). In the D1/D2 domain of the LSU rDNA, strain FIRDI 007^T exhibited 2.0 % divergence from *S. paphiopedili*. In the ITS region, the sequence of strain FIRDI 007^T differed by 10.6 % from that of *S. paphiopedili*. The result of a BLAST search through GenBank revealed a 97 % similarity between the sequences of the ITS region for strain FIRDI 007^T and the uncharacterized *Sympodiomyopsis* sp. S6A. Research has shown that the sequence difference in the ITS1 and ITS2 regions within a single yeast species is <1 % by comparing the sequence difference of the ITS region and the DNA–DNA relatedness values (Sugita *et al.*, 1999a, b; Nagahama

Fig. 1. Neighbour-joining phylogenetic tree of the genus *Sympodiomyopsis* and related genera based on the combined sequences of the ITS region and D1/D2 domain of the LSU rDNA. *Tilletiopsis derxii* and *Tilletiopsis oryzicola* were used as outgroups. Accession numbers for the sequences used in the analysis are given in parentheses (ITS/D1D2 domain of the LSU rDNA). Numbers on branches indicate confidence levels from 1000 replicate bootstrap samplings (>50 %). Bar, 0.02 substitutions per nucleotide position.

et al., 1999). A 2.4 % divergence in the ITS region of strains FIRDI 007^T and S6A was observed, which demonstrates that FIRDI 007^T is a separate species from strain S6A. The sequence analyses also support the distinction between strain FIRDI 007^T and the recognized species in the genus *Sympodiomyces*.

Colonies of strains FIRDI 007^T and BCRC 07F0494 were pale orange and velutinous, whereas colonies of strain BCRC 23075 were yellowish-white and smooth. Comparing the morphological characteristics of FIRDI 007^T with *S. paphiopedili* BCRC 23074^T, both developed conidiogenous cells from yeast cells or undifferentiated hyphae. Conidiogenous cells were polyblastic, terminal, integrated, sympodial, and sometimes geniculate or denticulate; in FIRDI 007^T, some were up to 10 µm long with successive conidia.

In terms of physiological characteristics, strains FIRDI 007^T, BCRC 23075 and BCRC 07F0494 differed in their ability to assimilate melibiose, ribitol and nitrite. Strains FIRDI 007^T, BCRC 23075 and BCRC 07F0494 differed from *S. paphiopedili* in the assimilation of galactose, lactose, myo-inositol, glucuronate and D-galactonate and the ability to grow in the presence of 50 % glucose. Furthermore, the DNA G+C contents of strains FIRDI 007^T, BCRC 23075 and BCRC 07F0494 were 47.5–49.4 mol%, which are quite different as compared with that of *S. paphiopedili* (55.1 %).

To evaluate the taxonomic status of strains FIRDI 007^T, BCRC 07F0494 and *S. paphiopedili* BCRC 23074^T, DNA–DNA hybridization experiments were performed (Supplementary Table S1). Based on the study of nuclear DNA hybridization among yeasts, strains showing DNA–DNA hybridization values of 70 % or more are conspecific (Kurtzman, 1998). Strain BCRC 07F0494 was conspecific with FIRDI 007^T because their DNA–DNA hybridization value (71 %) was within the range for members of the same species. Low DNA–DNA hybridization values (13–23 %) were observed between *S. paphiopedili* BCRC 23074^T and strains FIRDI 007^T and BCRC 07F0494. Therefore, strain FIRDI 007^T is considered to represent a novel species in the genus *Sympodiomyces*.

Sympodiomyces lanaiensis was described in 2008. In the ITS tree, *S. lanaiensis* was clustered with *S. paphiopedili* but with little bootstrap support; however, the D1/D2 tree placed *S. lanaiensis* and *S. paphiopedili* on separate branches (Mahdi *et al.*, 2008). In 2009, the genus *Jaminaea* was proposed. A phylogenetic analysis based on the D1/D2 domain of the LSU rDNA indicated that *Jaminaea angkorensis* and *S. lanaiensis* LM418^T were related; this finding was supported with high bootstrap values (Sipiczki & Kajdacs, 2009) and confirmed by the phylogenetic tree based on the combined sequences of the ITS region and D1/D2 domain of the LSU rDNA (Fig. 1) in this study. Nucleotide divergence in the ITS region is concordant with that in the D1/D2 domain of the LSU rDNA and further suggests that *S. lanaiensis* and

J. angkorensis are closely related to each other (Supplementary Table S2). Furthermore, the morphological characteristics of *S. lanaiensis* LM418^T coincide with the original description of the genus *Jaminaea* (Sipiczki & Kajdacs, 2009). On the basis of the phylogenetic analysis and phenotypic characteristics, we suggest that *S. lanaiensis* should be transferred to the genus *Jaminaea*, and we propose a new combination.

**Description of *Jaminaea lanaiensis* (Mahdi, Statzell-Tallman, Fell, Brown & Donachie)
G.-Y. Liou, Y.-H. Wei & F.-L. Lee comb. nov.**

Basionym: *Sympodiomyces lanaiensis* Mahdi, Statzell-Tallman, Fell, Brown & Donachie. FEMS Yeast Res. 8 (2008), 1359.

The type strain is LM418^T (=DSM 18755^T =ATCC MYA-4092^T =NRRL Y-48466^T =CBS 10858^T =BCRC 23177^T).

**Latin diagnosis of *Sympodiomyces kandeliae*
G.-Y. Liou, Y.-H. Wei & F.-L. Lee sp. nov.**

In agar malti post dies 3 ad 25 °C, cellulae subglobus, ellipsodeus, cylindratae aut fusus, 3.4–16.8 × 2.1–4.2 µm. Pseudomycelia et mycelia formantur. Fermentatio nulla. Assimilantur glucosum, galactosum (vel lente), L-sorbosum, D-ribosum (vel lente), D-xylosum (vel lente), L-arabiosum, D-arabiosum (vel lente), sucrosus, maltosus, trehalosus, methyl α-D-glucosidus, cellobiosus (vel lente), salicinum (infirmum), arbutinum (vel exiguum), melibiosus (variabile), lactosus (vel lente), raffinosis, melezitosis, inulinum (infirmum vel nullum), amyllum solubile (infirmum vel nullum), glycerolum, erythritolum, ribitolum (variabile), xylitolum (vel lente), L-arabinitolum (vel nullum), D-glucitolum, D-mannitolum, galactitolum (infirmum), myo-inositolum (infirmum vel nullum), D-gluconatum (lente vel infirmum), D-glucuronatum (infirmum vel nullum), DL-succinatum (vel infirmum), citratum (infirmum), ethanolum (vel lente), propenum 1,2 diolum (lente vel infirmum), D-galactonatum, kalium nitricum (vel infirmum), natrium nitrosus (variabile), ethylaminum (vel infirmum), L-lysinum (vel infirmum), cadaverinum (lente vel infirmum), creatinum (infirmum), creatininum (infirmum), glucosaminum (infirmum), imidazolium (infirmum), ad crescentiam vitaminum non necessarium est. Non assimilantur D-glucosaminum, L-rhamnosus, D-glucono-δ-lactonum, galacturonatum, DL-lactatum, methanolum, butane 2,3 diolum, quinquatum, saccharatum. In medio 0.01 % cycloheximidio addito non crescit. In medio 50 % glucosum continente crescit. Augmentum fiunt in temperatura 30 °C. Amyllum non formatur. Urea finditur. Diazonium caeruleum positivum. Proportio molaris guani-ni + cytosini in acido deoxyribonucleico: 47.5–49.4 mol% (per HPLC).

Typus: isolatus ex flos Kandelia candel (L.) Druce ad Singfen Wetland, Hsinchu, Taiwan, FIRDI 007^T (BCRC 23165^T =CBS 11676^T), depositus in BCRC, FIRDI, Hsinchu, Taiwan, ROC.

Description of *Sympodiomyopsis kandeliae* sp. nov.

Sympodiomyopsis kandeliae (kan.de'li.æ. N.L. n. *kandelia* a botanical genus name; N.L. gen. n. *kandeliae* of the plant *Kandelia*, from which the type strain was isolated).

After 3 days of growth on 5 % malt extract agar at 25 °C, colonies are smooth to velutinous, wrinkled, yellowish-white to pastel red and have entire or eroded margins. Cells are subglobose, cylindrical or fusiform, and variable in size (3.4–16.8 × 2.1–4.2 µm) (Fig. 2). Pseudohyphae and hyphae are produced. Fermentation is negative. The following compounds are assimilated: glucose, galactose (or delayed), L-sorbose, D-ribose (or delayed), D-xylose (or delayed), L-arabinose, D-arabinose (or delayed), sucrose, maltose, trehalose, methyl α-D-glucoside, cellobiose (or delayed), salicin (weak), arbutin (or weak), melibiose (variable), lactose (or delayed), raffinose, melezitose, inulin (weak or negative), soluble starch (weak or negative), glycerol, erythritol, ribitol (variable), xylitol (or weak), L-arabinitol, D-glucitol, D-mannitol, galactitol (weak), myo-inositol (weak or negative), D-gluconate (delayed or weak), D-glucuronate (weak or negative), DL-succinate (or weak), citrate (weak), ethanol (or delayed), propane-1,2-diol (delayed or weak), D-galactonate, potassium nitrate (or weak), sodium nitrite (variable), ethylamine (or weak), L-lysine (or weak), cadaverine (delayed or weak), creatine (weak), creatinine (weak), glucosamine (weak), imidazole (weak) and 0.01 % cycloheximide. Growth is observed in vitamin-free medium. D-Glucosamine, L-rhamnose, glucono-δ-lactone, D-galacturonate, DL-lactate, methanol, butane-2,3-diol, quinate and saccharate are not assimilated.

Fig. 2. Cells of strain FIRDI 007^T grown on 5 % malt extract agar at 25 °C for 5 days. (a, b) Blastoconidia and conidiogenous cells; (c, d) scanning electron micrographs of blastoconidia and conidiogenous cells. Bars, 10 µm (a, b), 4 µm (c) and 5 µm (d).

lated. Growth is observed in the presence of 50 % glucose. Maximum growth temperature is 30 °C. Urease activity is positive. Starch-like substance is not produced. Diazonium blue B reaction is positive. G + C content of nuclear DNA is 47.5–49.4 mol% (by HPLC).

The type strain, FIRDI 007^T (=BCRC 23165^T =CBS 11676^T), was isolated from flowers of *Kandelia candel* (L.) Druce that were collected in a mangrove forest, Singfen Wetland, Hsinchu, Taiwan, ROC, on 3 June 2004.

Acknowledgements

This work was supported by a grant from the Ministry of Economic Affairs, ROC (Contract no. 08-EC-17-A-01-04-0525). We thank Dr Sung-Oui Suh, ATCC, for valuable suggestions. Additionally, we thank Dr Sung-Yuan Hsieh, FIRDI, for sample collection and Dr Li-Ling Liaw, FIRDI, for assistance with DNA sequencing.

References

- Begerow, D., Bauer, R. & Oberwinkler, F. (2001). *Muribasidiopsis*: *Microstromatales* or *Exobasidiales*? *Mycol Res* **105**, 798–810.
- Begerow, D., Stoll, M. & Bauer, R. (2006). A phylogenetic hypothesis of *Ustilaginomycotina* based on multiple gene analyses and morphological data. *Mycologia* **98**, 906–916.
- de Beer, Z. W., Begerow, D., Bauer, R., Pegg, G. S., Crous, P. W. & Wingfield, M. J. (2006). Phylogeny of the *Quambalaria* fam. nov., including important *Eucalyptus* pathogens in South Africa and Australia. *Stud Mycol* **55**, 289–298.
- Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.
- Kurtzman, C. P. (1998). Nuclear DNA hybridization: quantitation of close genetic relationships. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 63–68. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.
- Lee, F.-L., Lee, C.-F., Okada, S., Uchimura, T., Komagata, K. & Kozaki, M. (1993). *Candida galacta* comb. nov., a new combination for *Candida apis* var. *galacta*. *Int J Syst Bacteriol* **43**, 183–184.
- Lee, F.-L., Fu, H.-M. & Hsu, W.-H. (1998). DNA hybridization and electrokaryotype study of some *Candida* species. *Int J Syst Bacteriol* **48**, 1463–1466.
- Mahdi, L. E., Statzell-Tallman, A., Fell, J. W., Brown, M. V. & Donachie, S. P. (2008). *Sympodiomyopsis lanaiensis* sp. nov., a basidiomycetous yeast (Ustilaginomycotina: Microstromatales) from marine driftwood in Hawai'i. *FEMS Yeast Res* **8**, 1357–1363.
- Nagahama, T., Hamamoto, M., Nakase, T. & Horikoshi, K. (1999). *Kluyveromyces nonfermentans* sp. nov., a new yeast species isolated from the deep sea. *Int J Syst Bacteriol* **49**, 1899–1905.
- O'Donnell, K. (1992). Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Curr Genet* **22**, 213–220.
- Sipiczki, M. & Kajdacs, E. (2009). *Jaminia angkorensis* gen. nov., sp. nov., a novel anamorphic fungus containing an S943 nuclear small-subunit rRNA group IB intron represents a basal branch of Microstromatales. *Int J Syst Evol Microbiol* **59**, 914–920.
- Sugita, T., Nishikawa, A., Ikeda, R. & Shinoda, T. (1999a). Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a

database for *Trichosporon* identification. *J Clin Microbiol* 37, 1985–1993.

Sugita, T., Cañete-Gibas, C., Takashima, M. & Nakase, T. (1999b). Three new species of *Bullera* isolated from leaves in the Ogasawara Islands. *Mycoscience* 40, 491–501.

Sugiyama, J., Tokuoka, K., Suh, S.-O., Hirata, A. & Komagata, K. (1991). *Sympodiomyopsis*: a new yeast-like anamorph genus with basidiomycetous nature from orchid nectar. *Antonie van Leeuwenhoek* 59, 95–108.

Suh, S.-O. & Sugiyama, J. (1994). Phylogenetic placement of the basidiomycetous yeasts *Kondoa malvinella* and *Rhodospiridium dacryoidum*, and the anamorphic yeast *Sympodiomyopsis paphiopedili* by means of 18S rRNA gene sequence analysis. *Mycoscience* 35, 367–375.

Suh, S.-O., Hirata, A., Sugiyama, J. & Komagata, K. (1993). Septal ultrastructure of basidiomycetous yeasts and their taxonomic implications with observations on the ultrastructure of *Erythrobasidium hasegawianum* and *Sympodiomyopsis paphiopedili*. *Mycologia* 85, 30–37.

Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 25, 125–128.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24, 1596–1599.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.

White, T. J., Bruns, T., Lee, S. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a Guide to Methods and Applications*, pp. 315–322. Edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White. San Diego: Academic Press.

Yarrow, D. (1998). Methods for the isolation, maintenance and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 4th edn, pp. 77–100. Edited by C. P. Kurtzman & J. W. Fell. Amsterdam: Elsevier.