Antonie van Leeuwenhoek (2012) 102:45–51
DOI 10.1007/s10482-012-9711-5

ORIGINAL PAPER

Udeniomyces kanasensis sp. nov., a ballistoconidium-forming yeast species in the Cystofilobasidiales

Pei-Jie Han · Jun-Zhi Qiu · Qi-Ming Wang · Feng-Yan Bai

Received: 10 November 2011 / Accepted: 4 February 2012 / Published online: 22 February 2012

Abstract In a survey of ballistoconidium-forming yeast diversity in the phyllosphere, five strains from wilting plant leaves collected from Kanas Nature Reserve in Xinjiang province, China were selected based on morphological comparison. These strains formed pinkish-white colonies and large bilaterally symmetrical ballistoconidia. Molecular phylogenetic analyses based on the 26S rRNA D1/D2 domain and ITS region sequences showed that these strains belonged to the *Udeniomyces* clade in the Cystofilobasidiales. They differ from the described *Udeniomyces* species significantly in the rRNA sequences as well as physiological criteria. Therefore, a new species *Udeniomyces kanasensis* sp. nov. (type strain XJ 6E2^{T}=CGMCC 2.02627 ^{T}=CBS 12488 ^{T}) is proposed to accommodate these strains. The MycoBank number of the new species is MB 563659.

Keywords *Udeniomyces kanasensis* sp. nov. · Basidiomycetous yeasts · Molecular phylogeny

P.-J. Han · Q.-M. Wang (✉) · F.-Y. Bai
State Key Laboratory of Mycology, Institute of Microbiology, The Chinese Academy of Sciences, Beijing 100101, China
e-mail: wangqm@im.ac.cn

P.-J. Han · J.-Z. Qiu
College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, China

Abbreviation
ITS Internal transcribed spacer

Introduction

The genus *Udeniomyces* was proposed for threes species, *Udeniomyces megalosporus*, *Udeniomyces puniceus* and *Udeniomyces pyricola* (Nakase and Takematsu 1992), which were classified previously in the 'pyricola group' of the genus *Bullera* (Nakase 1987) and characterized by forming large bilaterally symmetrical ballistoconidia and pinkish-white to pale pink colonies (Nakase 1989). Niwata et al. (2002) described *Udeniomyces pannonicus* on the basis of its morphological and chemotaxonomic characteristics. However, this species is phylogenetically more closely related to *Itersonilia perplexans* than to other species of *Udeniomyces* (Niwata et al. 2002; Takashima and Nakase 2011) and will be excluded from the genus in the future.

An investigation of the diversity of the ballistoconidium-forming phyllosphere yeasts in Kanas Nature Reserve (coordinates: 48°49′N, 87°2′E; altitude: 1,340 m) located in the Altai Mountains in Xinjiang province of China was carried out in July 2004. Among the strains isolated, five with pinkish-white colonies and large bilaterally symmetrical ballistoconidia were classified into one group. Molecular analyses based on 26S rRNA D1/D2 and ITS sequences indicated that the five strains represent a new *Udeniomyces* species, for which the name *U. kanasensis* sp. nov. is proposed.

Materials and methods

Fifteen wilting plant leaf samples were collected along the downstream river of Kanas Lake within a distance of approximately 10 km. Yeasts were isolated from the wilting leaves using the ballistoconidia-fall method described by Nakase and Takashima (1993). Morphological, physiological and biochemical characteristics were examined according to standard methods (Kurtzman et al. 2011). Assimilation of nitrogen compounds was investigated on solid media with starved inoculums as described by Nakase and Suzuki (1986). Extraction, purification and identification of ubiquinones were carried out according to Yamada and Kondo (1973).

Nuclear DNA was extracted using the method of Makimura et al. (1994). The DNA fragments covering the 26S rRNA D1/D2 domains and ITS region were amplified and sequenced as described by Bai et al. (2002). The sequences determined in this study and the reference sequences retrieved from GenBank were aligned with the Clustal X program (Thompson et al. 1997). The phylogenetic trees were constructed from the evolutionary distance data calculated from Kimura's two-parameter model (Kimura 1980) using the neighbor-joining method (Saitou and Nei 1987). Bootstrap analyses (Felsenstein 1985) were performed on 1,000 random resampling.

Results and discussion

Phenotypic characterization

A total of 112 strains were isolated from the fifteen wilting leaf samples. Based on phenotypic properties including the color and texture of colonies, the ability to form ballistoconidia and the shape of vegetative cells and ballistoconidia if produced, the strains were classified into six groups (Table 1). Group 1 included 35 isolates characterized by forming red or orange-red, butyrous or mucoid colonies. Group 2 contained 41 strains characterized by forming white or cream, butyrous or mucoid colonies and subglobose or ellipsoidal cells. Group 3 had 12 strains characterized by forming orange-yellow colonies. Group 4, containing eight isolates, was characterized by forming cream colonies and bilaterally symmetrical ballistoconidia. Group 5, comprising 11 strains, was characterized by forming whitish or brownish-yellow colonies and fusiform cells. Group 6, consisting of five strains, was characterized by forming pinkish-white colonies, long ellipsoidal or ovoid cells and large bilaterally symmetrical ballistoconidia with a size range of (5.5–10) × (10–18) μm (Fig. 1b). The ballistoconidia of this group are morphologically similar to, but somewhat larger than, those formed by *Bensingtonia* and *Sporobolomyces* species (Nakase et al. 2011; Hamamoto et al. 2011). *Tilletiopsis* species also produce bilaterally symmetrical ballistoconidia, which, however, are usually thinner than those formed by the group 6 strains (Boekhout 2011). The ballistoconidia of *Bullera* species are usually rotationally symmetrical and nearly globose and smaller than those formed by the group 6 strains (Boekhout et al. 2011). Further characterization showed that the major ubiquinone of the strains in group 6 was Q-10. Hyphae, teliospore-like thick-walled cells and sexual structures were not observed in the cultures of single strains or pairwise mixtures of the five strains on corn meal agar, malt extract agar and sucrose-yeast extract agar media at 17°C for 2 months. The results suggest that the five strains belong to the genus *Udeniomyces* (Takashima and Nakase 2011).

Molecular phylogeny

Nineteen strains were selected from the six morphological groups for 26S rRNA gene D1/D2 domain and ITS region sequence analyses, resulting in the identification of 13 species distributed in eight genera (Table 1). The five *Udeniomyces* strains exhibited identical sequences in the D1/D2 domain and ITS region, suggesting they are conspecific. They were clustered in a clade together with *U. megalosporus*, *U. puniceus*, *U. pyricola* and several unpublished strains with strong bootstrap supports in the trees drawn from the D1/D2 domain and ITS region sequences (Fig. 2). In the D1/D2 domain, they differed from the described species *U. puniceus*, *U. megalosporus* and *U. pyricola* by 4, 4 and 11 mismatches, respectively. In the ITS region, they differed from the most closely related species *U. puniceus* by 38 substitutions and 13 indels and from the other two described *Udeniomyces* species by more than 15% mismatches in the ITS region. The above data indicated that the five strains represent a distinct novel species closely related to *U. puniceus*. The name *U. kanasensis* sp. nov. is therefore proposed.

Four undescribed strains from France, PDD-38b-3 (JN176617), PDD-32b-40 (JF706582), PDD-32b-21

Table 1 Yeast strains from wilting leaves selected for rRNA gene sequence analysis

Group	Species	Strain[b]	GenBank accession no.	Source
1	*Sporobolomyces oryzicola*	XJ 13E2=CGMCC 2.0.2628	HE650893	Unidentified plant
	Sporobolomyces roseus	XJ 4E2	HE650894	*Rubus sachalinensis*
		XJ 10B1	HE650895	*Cotoneaster* sp.
	Sporobolomyces salicinus	XJ 10B5=CGMCC 2.02629	HE650887	*Cotoneaster* sp.
			HE650896	
2	*Bulleromyces albus*	XJ 7E1	HE650881	*Betula platyphylla*
			HE650889	
		XJ 15A5	HE650882	Unidentified plant
			HE650890	
	Cryptococcus albidus	XJ 14A2=CGMCC 2.02790	HE650883	*Vitis* sp.
	Cryptococcus oeirensis	XJ 10A2=CGMCC 2.0.2791	HE650884	*Cotoneaster* sp.
	Cryptococcus victoriae	XJ 3A5=CGMCC 2.02632	HE650891	Unidentified plant
	Hannaella oryzae	XJ 12C1=CGMCC 2.02633	HE650885	Unidentified plant
			HE650892	
3	*Dioszegia butyracea*	XJ 7E2=CGMCC 2.02630	EU266508	*Betula platyphylla*
			EU266530	
	Dioszegia fristingensis	XJ 4E3=CGMCC 2.02631	EU070927	*Rubus sachalinensis*
			EU070933	
4	*Bensingtonia* sp.[a]	XJ 10A6=CGMCC 2.02634	HE650880	*Cotoneaster* sp.
			HE650888	
5	*Pseudozyma rugulosa*	XJ 4C2	HE650886	*Rubus sachalinensis*
6	*Udeniomyces kanasensis* sp. nov.	XJ 6E2^T=CGMCC 2.02627^T=CBS 12488^T	JQ002681	*Cotoneaster melanocarpus*
		XJ 6C3=CGMCC 2.02758	JQ002683	*Cotoneaster melanocarpus*
		XJ 8B3=CGMCC 2.02759	JQ002682	*Epilobium angustifolium*
		XJ 10B3=CGMCC 2.02757	JQ002684	*Cotoneaster* sp.
		XJ 10C5	JQ002685	*Cotoneaster* sp.

[a] Differing from the closest relative *Bensingtonia yuccicola* by 10 mismatches in the 26S rRNA gene D1/D2 domain

[b] Numerals after the abbreviation XJ (standing for Xinjiang province) in strain numbers represent sample numbers

(JF706580) and PDD-32b-71 (JN176594) isolated from cloud water (puy de Dome summit, 1465 m a.s.l., France), were closely related with the five Chinese strain in the D1/D2 tree (Fig. 2a). They differed from the Chinese strains by only 1–3 indels in the D1/D2 domain, suggesting that these French strains may be conspecific with the Chinese strains. However, their taxonomic relationship need be confirmed by ITS sequence analysis. Strains with similar D1/D2 sequences may have quite different ITS sequences in this group. For example, another Chinese strain isolated by us, HS 11.1 (AY841862), possessed identical D1/D2 sequence with that of the type strain of *U. pyricola* (Fig. 2a); however, it differed from the latter by 15 mismatches (nine substitutions and six indels) in the ITS region (Fig. 2b), indicating strain HS 11.1 may not belong to *U. pyricola*.

Ecological distribution

The strains of *U. kanasensis* sp. nov. studied were isolated from wilting leaves of herbaceous and woody plants (Table 1), implying that this species may occur in the phyllosphere of various plants. Number of this species or closely related species may occur in other types of substrates. The four undescribed France strains conspecific or closely related with *U. kanasensis* sp. nov. were isolated from cloud water. *U. puniceus*, the close relative of the new species, was first isolated from a frozen fish in Japan and was

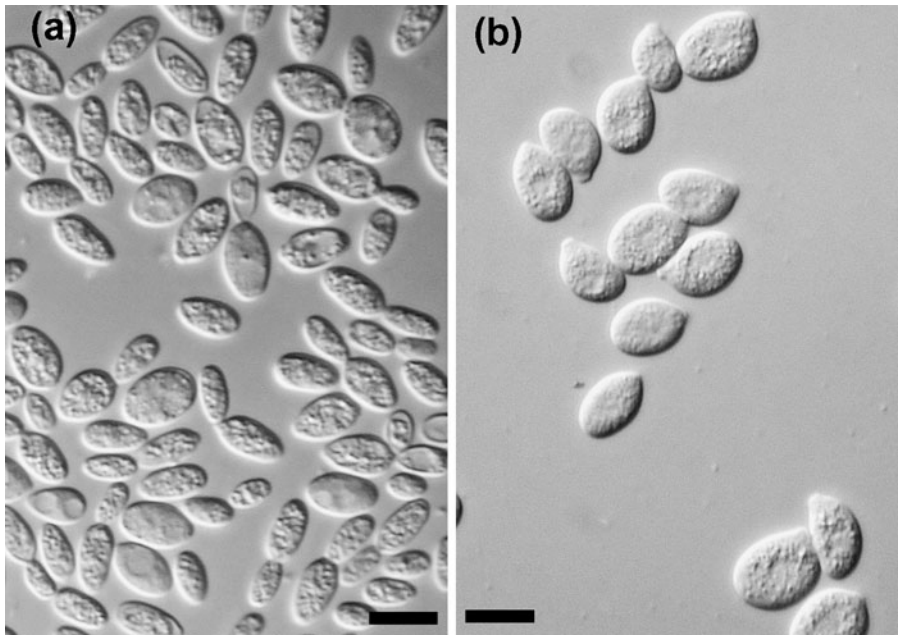

Fig. 1 *U. kanasensis* sp. nov. (CGMCC 2.02627^{T}=CBS 12488^{T}) vegetative cells grown in YM broth for 7 days at 17°C (**a**) and ballistoconidia produced on corn meal agar after 5 days at 17°C (**b**). *Bars*, 10 μm

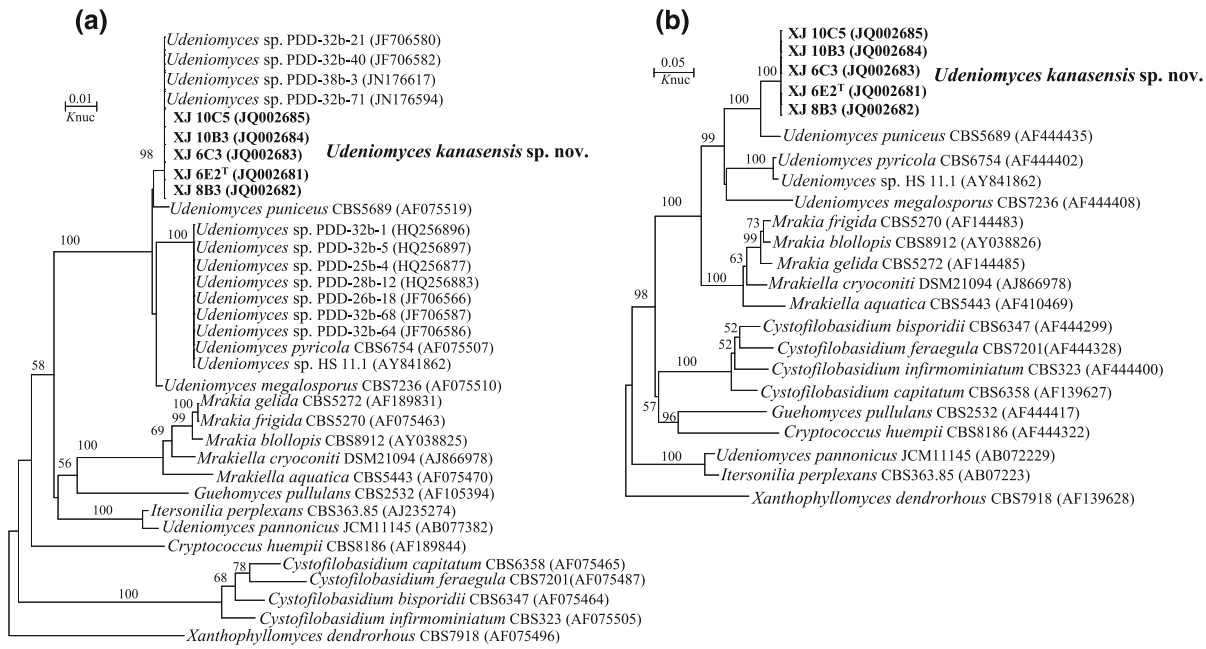

Fig. 2 Phylogenetic trees drawn from neighbor-joining analysis based on sequences of **a** the 26S rDNA D1/D2 domain and **b** the ITS region (including 5.8S rDNA), depicting the relationships of the species *U. kanasensis* sp. nov. with closely related taxa. Bootstrap percentages over 50% from 1,000 bootstrap replicates are shown. Reference sequences were retrieved from GenBank under the accession numbers indicated. The *scale bar* indicates 1 and 5% sequence divergence for D1/D2 and ITS tree, respectively. *Xanthophyllomyces dendrorhous* was set as the outgroup

also isolated from seawater of the Pacific Ocean off the west coast of Baja California, Mexico (Takashima and Nakase 2011). The other described species of the genus usually occur in plant material (Takashima and Nakase 2011). Though *Udeniomyces* species may occur in different substrates, they are usually psychrophilic. The Kanas Nature Reserve from where the new species was isolated is located in the northern temperate climate zone in China, with the average annual temperature and annual precipitation of −1.0°C and 550–600 mm, respectively. July is the hottest month of 1 year with an average temperature of 16.5°C (Wang et al. 2007). *U. kanasensis* sp. nov. does not grow at the temperature above 22°C, being consistent with the psychrophilic nature of other four described *Udeniomyces* species (Nakase 2000; Takashima and Nakase 2011). This indicates that members of the genus *Udeniomyces* may usually be found in low temperature environments.

Latin diagnosis of *U. kanasensis* Wang, Bai, Qiu and Han sp. nov

In YM (Difco) liquido post dies 7 ad 17°C, cellulae vegetativae ellipsoideae elongatae aut ovoideae (4.5–8) × (10–16) μm, singulae aut binae. Sedimentum formatur. In agaro YM post unum mensem ad 17°C, cultura roseus-album, glabra, butyracea, margine glabra. Pseudomycelium non formatur. Ballistosporae ellipsoideae vel pyriformes (5.5–10) × (10–18) μm. Fermentatio nulla. Glucosum, saccharosum, maltosum, cellobiosum, trehalosum, raffinosum, melezitosum, amylum solubile (exigue), D-xylosum (exigue), L-arabinosum, D-mannitolum et glucitolum (exigue) assimilantur at non galactosum, L-sorbosum, lactosum, melibiosum, inulin, L-rhamnosum, D-glucosaminum, methanolum, ethanolum, glycerolum, erythritolum, galactitolum, methyl α-D-glucosidum, acidum citricum, acidum DL-lacticum, acidum succinicum, inositolum nec hexadecanum at variabile D-arabinosum, D-ribosum, ribitolum nec salicinum. Ammonium sulfatum, kalium nitricum et natrium nitrosum assimilantur at non L-lysinum, cadaverinum et ethylaminum. Vitaminae externae ad crescentiam necessaria snut. Maxima temperatura crescentiae: 22°C. Materia amyloidea iodophila non formatur. Urea finditur. Diazonium caeruleum B positivum. Ubiquinonum majus: Q-10. Typus: Isolatus ex folio *Cotoneaster melanocarpus* Lodd., XJ 6E2^T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (CGMCC 2.02627^T=CBS 12488 ^{T}).

Description of *U. kanasensis* Wang, Bai, Qiu and Han sp. nov

In YM broth, after 7 days at 17°C, cells are long ellipsoidal or ovoid (4.5–8) × (10–16) μm (Fig. 1), single or in pairs. Budding is polar. Sediment is formed. After 1 month at 17°C, a ring and sediment are present. On YM agar, after 1 month at 17°C, the streak culture is pinkish-white, smooth. The margin is entire. In Dalmau plate culture on corn meal agar, pseudomycelium is not formed. Ballistoconidia are ellipsoidal or pyriform (5.5–10) × (10–18) μm. Fermentation of glucose is negative. Glucose, sucrose, maltose, cellobiose, trehalose, raffinose, melezitose, soluble starch (weak), D-xylose (weak), L-arabinose,

Table 2 Physiological characteristics that distinguish *U. kanasensis* sp. nov. from described *Udeniomyces* species

Growth	*U. kanasensis*	*U. puniceus*[a]	*U. megalosporus*[a]	*U. pyricola*[a]	*U. pannonicus*[a]
Galactose	−	−	−	+	−
L-Sorbose	−	w	v	+	−
Lactose	−	−	−	+	−/s/w
Melibiose	−	−	−	+	+
D-Xylose	w	−	v	+	+
D-Arabinose	−/w	+	−	+	−
Ethanol	−	+	v	+	+
Glycerol	−	+	v	+	+
Galactitol	−	+	v	+	s/w
Succinic acid	−	+	+	+	+
Citric acid	−	+	+	+	+

+ Positive, − negative, *s* slowly positive, *w* weak, *v* variable

[a] Data from Takashima and Nakase (2011)

D-mannitol and D-glucitol (weak) are assimilated. Galactose, L-sorbose, lactose, melibiose, inulin, L-rhamnose, D-glucosamine, methanol, ethanol, glycerol, erythritol, galactitol, methyl α-D-glucoside, critic acid, DL-lactic acid, succinic acid, inositol and hexadecane are not assimilated. D-arabinose, D-ribose, ribitol and salicin are variably assimilated. Ammonium sulfate, potassium nitrate and sodium nitrite are assimilated. L-Lysine, ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 22°C. Growth in vitamin-free medium is negative. Starch-like substances are not produced. Growth on 50% (w/w) glucose-yeast extract agar is negative. Urease activity is positive. Diazonium Blue B reaction is positive. The major ubiquinone is Q-10. The type strain, XJ 6E2 ^{T}, was isolated from a leaf of *Cotoneaster melanocarpus* Lodd. collected from Kanas Lake in Xinjiang, China in July, 2004. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as CGMCC 2.02627^{T}, and in the Centraalbureau voor Schimmelcultures, Uppsalalaan 83584 CT Utrecht, The Netherlands, as CBS 12488 ^{T}. The Mycobank deposit number is MB 563659.

Physiologically, *U. kanasensis* differs from the four previously described *Udeniomyces* species by being unable to produce starch-like substances. In addition, *U. kanasensis* differs from the closely related species *U. puniceus* in its negative assimilation reactions of ethanol, glycerol, galactitol, succinic acid and citric acid (Table 2).

Etymology

The specific epithet *kanasensis* (ka.nas.en'sis N.L. fem. adj.) refers to the geographic origin of the type strain of the species.

Acknowledgment This study was supported by Grants No. 30700001, No. 30825002 and No. 30970013 from the National Natural Science Foundation of China (NSFC) and KSCX2-YW-Z-0936 from the Knowledge Innovation Program of the Chinese Academy of Sciences.

References

Bai FY, Zhao JH, Takashima M, Jia JH, Boekhout T, Nakase T (2002) Reclassification of the *Sporobolomyces roseus* and the *Sporidiobolus pararoseus* complexes, with the description of *Sporobolomyces phaffii* sp. nov. Int J Syst Evol Microbiol 52:2309–2314

Boekhout T (2011) *Tilletiopsis* Derx ex Derx (1930). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 2003–2014

Boekhout T, Bai FY, Nakase T (2011) *Bullera* Derx (1930). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 1623–1695

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

Hamamoto M, Boekhout T, Nakase T (2011) *Sporobolomyces* Kluyver & van Niel (1924). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 1929–1990

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

Kurtzman CP, Fell JW, Boekhout T, Robert V (2011) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 87–110

Makimura K, MurayamaYS Yamaguchi H (1994) Detection of a wide range of medically important fungi by the polymerase chain reaction. J Med Microbiol 40:358–364

Nakase T (1987) Isolation and maintenance of ballistospore-forming yeasts. Stud Mycol 30:375–387

Nakase T (1989) Classification of ballistosporous yeasts. Yeast 5:S511–S516

Nakase T (2000) Expanding world of ballistosporous yeasts: distribution in the phyllosphere, systematics and phylogeny. J Gen Appl Microbiol 46:189–216

Nakase T, Suzuki M (1986) *Bullera megalospora*, a new species of yeast forming large ballistospores isolated from dead leaves of *Oryza sativa*, *Miscanthus sinensis,* and *Sasa* sp. in Japan. J Gen Appl Microbiol 32:225–240

Nakase T, Takashima M (1993) A simple procedure for the high frequency isolation of new taxa of ballistosporous yeasts living on the surfaces of plants. RIKEN Rev 3:33–34

Nakase T, Takematsu A (1992) *Udeniomyces*, a new ballistosporous anamorphic yeast genus in the Cryptococcaceae proposed for three *Bullera* species which produce large bilaterally symmetrical ballistospores. FEMS Mircrobiol Lett 100:497–502

Nakase T, Bai FY, Boekhout T (2011) *Bensingtonia* Ingold emend (Nakase & Boekhout). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 1607–1622

Niwata Y, Takashima M, Tornai-Lehoczki J, Deak T, Nakase T (2002) *Udeniomyces pannonicus* sp. nov., a ballistoconidium-forming yeast isolated from leaves of plants in Hungary. Int J Syst Evol Microbiol 52:1887–1892

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

Takashima M, Nakase T (2011) *Udeniomyces* Nakase & Takematsu (1992). In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts: a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 2063–2068

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 24:4876–4882

Wang XP, Yu SL, Chen HW (2007) Exploitation and conservation of ecological and tourism resources in Kanasi reserve, Xinjiang. Chin J Wildl 28:34–39

Yamada Y, Kondo K (1973) Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus* and the yeast like genera *Sporobolomyces* and *Rhodosporidium*. J Gen Appl Microbiol 19:59–77