**Bensingtonia changbaiensis** sp. nov. and **Bensingtonia sorbi** sp. nov., novel ballistoconidium-forming yeast species from plant leaves

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Six ballistoconidium-forming yeast strains that were isolated from plant leaves collected on Changbai Mountain, north-east China, were assigned to the genus *Bensingtonia* Ingold emend. Nakase & Boekhout due to the formation of asymmetrical ballistoconidia, cream-coloured colonies and Q-9 as the major ubiquinone. Two separate groups, representing two novel *Bensingtonia* species, were recognized among these yeasts by 26S rDNA D1/D2 domain, internal transcribed spacer (ITS) region and 18S rDNA sequence analyses. The names *Bensingtonia changbaiensis* sp. nov. (type strain, CB 346T = AS 2.2310T = CBS 9497T) and *Bensingtonia sorbi* sp. nov. (type strain, CB 286T = AS 2.2303T = CBS 9498T) are proposed for these two species.

In a survey of ballistoconidium-forming yeast diversity of the phyllosphere in north-east China, approximately 250 yeast strains were isolated from 39 leaf samples of various plants collected on Changbai Mountain, Jilin Province, north-east China. Among the yeasts, six strains were classified into one group by conventional and chemotaxonomic characterization. Molecular phylogenetic analyses based on the large-subunit (26S) rDNA D1/D2 domain, the internal transcribed spacer (ITS) region and the small-subunit (18S) rDNA indicated that these strains represent two undescribed species in the genus *Bensingtonia* Ingold emend. Nakase & Boekhout [see Nakase & Boekhout (1988)]. They are hereby described as *Bensingtonia changbaiensis* sp. nov. and *Bensingtonia sorbi* sp. nov.

The six strains studied were isolated from wilting leaves of *Aconitum coreanum* (CB 228), *Betula ermanii* (CB 235 and CB 346T) and *Sorbus pohuashanensis* (CB 284, CB 286T and CB 287) by using the improved ballistoconidia-fall method (Nakase & Takashima, 1993). Leaf samples were collected on Changbai Mountain in October 1998. Morphological, physiological and biochemical characteristics were examined according to standard methods (Yarrow, 1998). Extraction, purification and identification of ubiquinones were carried out according to Yamada & Kondo (1973). Assimilation of nitrogen compounds was investigated on solid media with starved inocula (Nakase & Suzuki, 1986).

Nuclear DNA was extracted by the method of Makimura *et al.* (1994). The ITS (including 5.8S rDNA) and 26S rDNA D1/D2 domain sequences were determined by a method described previously (Bai *et al.*, 2002). 18S rDNA sequences were determined according to Sugita & Nakase (1999) with the following modifications: cycle sequencing was performed by using an ABI BigDye Cycle Sequencing kit and electrophoresis and data collection were performed on an ABI PRISM 3100 genetic analyser. Sequences were aligned with the CLUSTAL_X program (Thompson *et al*., 1997). Phylogenetic trees were constructed from evolutionary distance data calculated with Kimura’s two-parameter model (Kimura, 1980) by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (Felsenstein, 1985) was performed from 1000 random resamplings.

**Morphology and chemotaxonomy**

The six yeast strains (CB 228, CB 235, CB 284, CB 286T, CB 287 and CB 346T) were classified together by their formation of asymmetrical ballistoconidia and cream-coloured colonies. Their major ubiquinone was Q-9. Sexual structures were not observed in cultures of single strains or in mating tests. According to the current taxonomy of basidiomycetous yeasts (Boekhout, 1998; Boekhout & Nakase, 1998), these strains could be assigned to the genus *Bensingtonia.*
Molecular phylogenetic analysis

Two groups were recognized among the six strains by ITS and D1/D2 sequence comparison. Strains CB 286\(^T\) and CB 287 had identical sequences in both regions. CB 228, CB 255, CB 284 and CB 346\(^T\) were classified into another group that also had identical sequences in both regions except for CB 284, which differed from the other three strains by 1 nt in the D1/D2 region. The two groups differed from each other by 34 nt (5\%) in the D1/D2 region and 143 nt (~25\%) in the ITS–5\(S\) rDNA region.

In the phylogenetic tree drawn from D1/D2 sequences (Fig. 1), the two groups were located in the Kondoa clade, Agaricostilbum lineage of urediniomycetous yeasts (Scorzetti et al., 2002). They clustered together with Bensingtonia yuccicola and an undescribed Bensingtonia species represented by strain PYCC 5562 (Fig. 1). The Changbai strains differed from the latter two taxa by approximately 3–4\% of nucleotides in the D1/D2 region. More than 20\% nucleotide difference was found in the ITS region among closely related Bensingtonia taxa.

Phylogenetic positions of the two groups depicted in the D1/D2 tree (Fig. 1) were different from those depicted in the ITS tree. Such discordances have been reported in other groups of basidiomycetous yeasts (Bai et al., 2002; Scorzetti et al., 2002). In order to confirm the phylogenetic positions of the two groups, 18S rDNA sequences of the representative strains CB 286\(^T\) and CB 346\(^T\) were determined and aligned with those of reference taxa, including all described Bensingtonia species and related urediniomycetous yeast taxa. The two strains clustered together with five Bensingtonia and one Kondoa species in the Agaricostilbum/Bensingtonia lineage (Hamamoto & Nakase, 2000) with 100\% bootstrap support. CB 286\(^T\) was located with Bensingtonia miscanthi, whereas CB 346\(^T\) showed a close relationship to B. yuccicola.

The ITS and 18S rDNA trees are available as supplementary data in IJSEM Online.

The results suggest that the two yeast groups from Changbai Mountain represent two novel Bensingtonia species, for which the names Bensingtonia changbaiensis sp. nov. and Bensingtonia sorbi sp. nov. are proposed.

Latin diagnosis of Bensingtonia changbaiensis

F.-Y. Bai et Q.-M. Wang sp. nov.

In YM (Difco) liquido post dies 5 ad 17 \(^\circ\)C, cellulae vegetatiae ellipsoidae, 2.0–3.6 × 4.5–8.6 \(\mu\)m, singulae. Sedimentum formantur. Post unum mensem ad 17 \(^\circ\)C, annulus et sedimen-
tum formantur. In agaro YM post unum mensem ad 17 \(^\circ\)C, cultura bruneusea-cremea, glabra aut rugosa, nitida aut non-nitida, butyacea, margine glabra. Pseudomycelium non formantur. Ballistosporae reniformia vel allantoidia, 2.7–4.1 × 5.6–10.9 \(\mu\)m. Fermentatio nulla. Glucosum, saccharosum, maltosum, cellobiosum (lente), trehalosum, raffi-
nosum (lente), inulinum (lente et exigue), amyllum solubile (lente), D-xylosum (lente et exigue), glycerolum, ribitolum (lente et exigue), D-mannitolum et D-glucitolum (lente) assim-
lantur at non galactosum, L-sorbosum, lactosum, melibio-
sum, melezitosum, L-arabinosum, D-arabinosum, D-ribose, L-
hamnosum, D-glucosaminum, methanolum, ethanolum, erithritolum, galactitolum, methyl-\(\alpha\)-D-glucosidum, salici-
um, acidum DL-lacticum, acidum succinicum, acidum citricum, inositolum nec hexadecanum. Ammonium sulphatum, kalium nitricum, natrium nitrosum et L-lysinum assimi-

Typus: isolatus ex folio Betula ermanii, CB 346\(^T\), depositus in...
collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2310T).

Description of Bensingtonia changbaiensis F.-Y. Bai & Q.-M. Wang sp. nov.

Bensingtonia changbaiensis (chang.bai.en’sis. N.L. fem. adj. changbaiensis referring to the geographical origin of the type strain of the species).

In YM broth after 5 days at 17 °C, cells are ellipsoidal, 2·0–3·6 × 4·5–8·6 μm (Fig. 2), single, budding is polar and sediment is formed. After 1 month at 17 °C, sediment and a ring are present. On YM agar after 1 month at 17 °C, the streak culture is brownish-cream, butyrous, smooth or slightly wrinkled and shining or dull. The margin is entire. In Dalmau plate culture on corn-meal agar, pseudohyphae are not formed. Ballistoconidia are produced on sterigmata of CB 346T vegetative cells grown in YM broth for 5 days at 17 °C (a) and ballistoconidia produced on corn-meal agar after 5 days at 20 °C (b). Bars, 10 μm.

Glucose, sucrose, maltose, cellobiose (delayed), trehalose, raffinose (delayed), inulin (delayed and weak), soluble starch (delayed), D-xylose (delayed and weak), glycerol, ribitol (delayed and weak), D-mannitol and D-glucitol (delayed) are assimilated. Galactose, L-sorbitose, lactose, melibiose, melezitose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, erythritol, galactitol, methyl α-D-glucoside, salicin, DL-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, potassium nitrate, sodium nitrite and L-lysine are assimilated. Ethylamine hydrochloride and cadaverine dihydrochloride are not assimilated. Maximum growth temperature is 27 °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. Major ubiquinone is Q-9.

The type strain of Bensingtonia changbaiensis, CB 346T, was isolated from a wilting leaf of Betula ermanii Cham collected on Changbai Mountain, north-east China, in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2310T (=CBS 9497T).

Latin diagnosis of Bensingtonia sorbi F.-Y. Bai et Q.-M. Wang sp. nov.


Typus isolatus ex folio Sorbus pohuashanensis, CB 286T, depositus in collectione China General Microbiological Culture Collection Center, Academia Sinica (AS 2.2303T).
**Description of Bensingtonia sorbi** F.-Y. Bai & Q.-M. Wang sp. nov.

*Bensingtonia sorbi* (sor’bi. N.L. gen. adj. sorbi of Sorbus, referring to the genus name of the plant from which the type strain was isolated).

In YM broth after 5 days at 17 °C, cells are ovoidal or globose, 2·7–6·4 × 2·7–8·6 μm (Fig. 3), single, budding is polar, sediment and a ring are formed. After 1 month at 17 °C, sediment and a ring are present. On YM agar after 1 month at 17 °C, the streak culture is brownish-cream, butyrous, smooth or slightly wrinkled and shining or semi-dull. The margin is entire. In Dalmau plate culture on corn-meal agar, pseudohyphae are not formed. Ballistoconidia are produced on corn-meal agar, ovoidal, 2·7–4·5 × 4·1–7·3 μm (Fig. 3). Fermentation is negative. Glucose, L-sorbose, galactose (delayed), sucrose, maltose, trehalose, raffinose, D-xylose, L-arabinose (delayed and weak), glycerol, ribitol (delayed and weak), D-mannitol and D-glucitol are assimilated. Cellobiose, lactose, melibiose, melezitose, inulin, soluble starch, D-arabinose, D-ribose, L-rhamnose, D-glucosamine, methanol, ethanol, erythritol, galactitol, methyl 2-D-glucoside, salicin, D-lactic acid, succinic acid, citric acid, inositol and hexadecane are not assimilated. Ammonium sulfate, L-lysine and cadaverine dihydrochloride are assimilated. Potassium nitrate, sodium nitrite and ethylamine hydrochloride are not assimilated. Maximum growth temperature is 27 °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. Major ubiquinone is Q-9.

The type strain of *Bensingtonia sorbi*, CB 286T, was isolated from a wilting leaf of *Sorbus pohuashanensis* (Hance) Hedl collected on Changbai Mountain, north-east China, in October 1998. This strain has been deposited in the China General Microbiological Culture Collection Center (CGMCC), Academia Sinica, Beijing, China, as AS 2.2303T (= CBS 9498T).

In addition to differences in molecular data, the two novel *Bensingtonia* species are distinguishable from each other and from other described species of the genus by some physiological properties, as shown in Table 1. With the inclusion of the recently described species *Bensingtonia thailandica* Fungsin et al. (2001) and the anamorph of *Mastigobasidium intermedium* Golubev (1999) (*Bensingtonia intermedia*), 14 species are now included in the genus *Bensingtonia* (Table 1). Although the heterogeneity of this genus, as well as a number of other basidiomycetous yeast

![Fig. 3. Bensingtonia sorbi sp. nov. CB 286T vegetative cells grown in YM broth for 5 days at 17 °C (a) and ballistoconidia produced on corn-meal agar after 5 days at 20 °C (b). Bars, 10 μm.](image)

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genera, has been demonstrated by rRNA gene sequence analyses (Takashima et al., 1995; Fell et al., 2000; Hamamoto & Nakase, 2000; Scorzetti et al., 2002), redefinitions of these genera would be premature at present. Relationships among lineages and clades of these yeasts should become clearer as novel species are added to the phylogenetic trees, which is hoped to make the demarcation of genera more reliable.

Acknowledgements
This study was supported by grants no. 30170002 from the National Natural Science Foundation of China (NSFC) and no. KSCX2-SW-101C from the Chinese Academy of Sciences.

References