

# Reassignment of the basidiomycetous yeasts *Trichosporon pullulans* to *Guehomyces pullulans* gen. nov., comb. nov. and *Hyalodendron lignicola* to *Trichosporon lignicola* comb. nov.

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Nucleotide sequence analyses of the hymenomycetous yeasts demonstrated that *Hyalodendron lignicola* should be considered as a member of the genus *Trichosporon* within the Trichosporonales and that *Trichosporon pullulans* is associated closely with the Cystofilobasidiales, rather than the Trichosporonales. Accordingly, the following proposals are made: *Trichosporon lignicola* comb. nov. and *Guehomyces* gen. nov., to accommodate *Guehomyces pullulans* comb. nov. in the Cystofilobasidiales.

The hymenomycetous yeasts are classified in four orders (Tremellales, Trichosporonales, Filobasidiales and Cystofilobasidiales), based on phylogenetic analyses of nucleotide sequences of the internal transcribed spacer (ITS) and the D1/D2 regions of the rDNA (Scorzetti *et al.*, 2002). Analysis of the genus *Trichosporon* (Guého *et al.*, 1989, 1992), which is the major component of the Trichosporonales, demonstrated incorrect systematic placements for *Trichosporon pullulans* (Lindner) Diddens & Lodder (1942) and *Hyalodendron lignicola* Diddens (1934). The relationship of these two species within the hymenomycetous orders Cystofilobasidiales and Trichosporonales is demonstrated (Fig. 1) by D1/D2 maximum-likelihood phylogenetic analysis (PAUP 4.0b10) with Bayesian Markov chain Monte Carlo (MCMC) analysis credibility values (MRBAYES: Huelsenbeck & Ronquist, 2001). Bootstrap values represent 1000 replicates from a parsimony analysis (PAUP 4.0b10).

The Trichosporonales can be separated into four clusters with the informal names of Gracile, Porosum, Cutaneum and Ovoides (Middelhoven *et al.*, 2004). *Hyalodendron* resides in the Porosum cluster, whereas *T. pullulans* is within the Cystofilobasidiales. The present communication proposes to correct the systematic positions of these two species by creation of *Guehomyces* gen. nov. in the Cystofilobasidiales, with the species *Guehomyces pullulans* comb. nov., and by transfer of *H. lignicola* to the genus *Trichosporon*.

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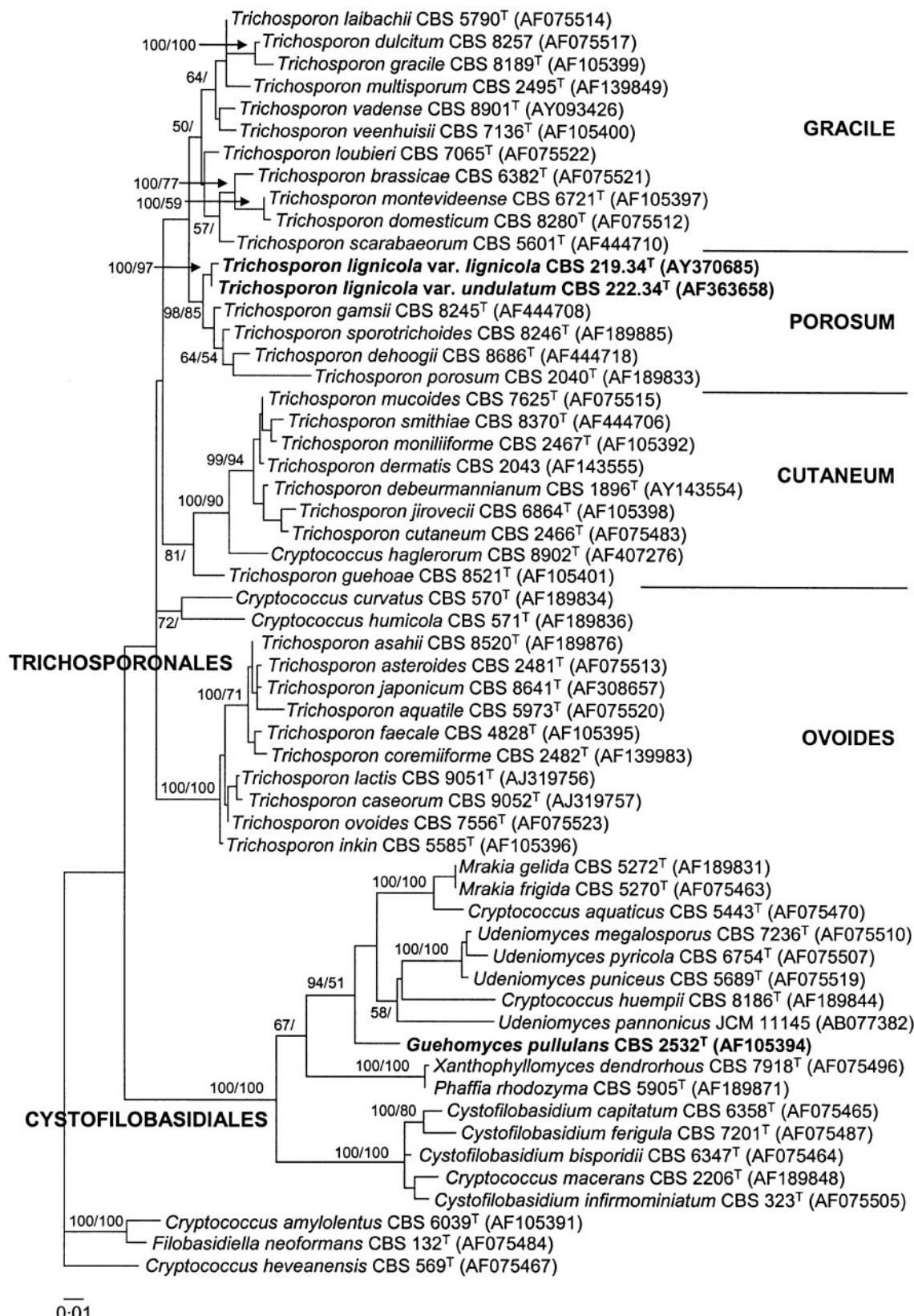
Abbreviations: ITS, internal transcribed spacer; LSU, large-subunit; MCMC, Markov chain Monte Carlo.

## Systematic placement of *Trichosporon pullulans* (Lindner) Diddens & Lodder 1942

The specific epithet *pullulans* was originally employed by Lindner (1895) in the genus *Oidium* for a yeast-like fungus that was isolated from an experimental brewery. Lindau (1907) brought the species into the genus *Oospora*. Subsequently, Diddens & Lodder (1942) placed the species in the genus *Trichosporon*. Guého *et al.* (1989, 1992) examined the rRNA D2 region and reported that *T. pullulans* was phylogenetically distinct from other members of the genus *Trichosporon*. This finding was confirmed by sequence analyses of the small-subunit rDNA (Sugita & Nakase, 1998), the D1/D2 region of the large-subunit (LSU) rDNA (Fell *et al.*, 1995, 2000; Sugita *et al.*, 2001) and the ITS (Scorzetti *et al.*, 2002). All these studies demonstrated the phylogenetic relationship of *T. pullulans* to members of the Cystofilobasidiales. The latter order consists of a diversity of genera, including the teleomorphs *Mrakia*, *Cystofilobasidium* and *Xanthophyllomyces* and the anamorphs *Phaffia*, *Udeniomyces* and several species assigned to the genus *Cryptococcus* (Fig. 1).

*Mrakia* and *Cystofilobasidium* are teliospore-forming genera, whereas *Xanthophyllomyces* reproduces sexually via cell-bud conjugation. *Phaffia* is an anamorphic representative of *Xanthophyllomyces*, whereas the anamorphic genus *Udeniomyces* produces large, asymmetrical ballistoconidia. *T. pullulans* does not share these generic characteristics. In addition, its branch position (Fig. 1) suggests that *T. pullulans* represents a separate cluster within the Cystofilobasidiales.

A point that has been established is the absence of a close relationship of *T. pullulans* to the Trichosporonales; the



**Fig. 1.** D1/D2 rDNA maximum-likelihood analysis (PAUP 4.0b10) of the Trichosporonales and Cystofilobasidiales. Numbers on branches represent Bayesian MCMC analysis credibility values/bootstrap values (1000 replicates from parsimony analysis). Values of <50 % (e.g. 58/) are not reported. Bar, 0.01 substitutions per site.

semblance of similarity is based on the presence of disarticulating hyphae (arthroconidia). Arthric conidio-genesis occurs in phylogenetically diverse genera, such as *Malbranchea*, *Coccidioides*, *Dipodascus* and *Galactomyces*. Consequently, the presence of this type of conidium does not, as a sole character, link *T. pullulans* to the Trichosporonales. As a result, *Guehomycetes* gen. nov. is proposed, in order to recognize this phylogenetically distinct species within the Cystofilobasidiales.

### **Latin diagnosis of *Guehomycetes* Fell et Scorzetti gen. nov.**

*Reproductio vegetativa gemmatione enteroblastica videtur. Pseudomycelium et arthroconidia formantur. Ureum finditur, diazonium caeruleum B positivum. Reproductio sexualis nulla. Genus pertinet ad Cystofilobasidiales.*

*Typus:* *Guehomycetes pullulans* (Lindner) Fell et Scorzetti.

### **Description of *Guehomycetes* Fell & Scorzetti gen. nov.**

*Guehomycetes* (gu.e.ho.my'ces. N.L. masc. n. *Guehomycetes* to recognize the outstanding taxonomic yeast research of Eveline Guého).

Budding cells that may be enteroblastic are present. Disarticulate hyphae develop arthroconidia. Tests for urease and diazonium blue B are positive. Sexual reproduction is not observed. Based on rDNA analysis, the genus is related to members of the Cystofilobasidiales. The type species is *Guehomycetes pullulans* (Lindner) Fell & Scorzetti comb. nov.

### **Description of *Guehomycetes pullulans* (Lindner) Fell & Scorzetti comb. nov.**

Basonym: *Trichosporon pullulans* (Lindner) Diddens, H. A. & Lodder, J. *Die Anaskosporogenen Hefen, Zweite Hälfte*, North Holland, Amsterdam 410 (1942).

*Guehomycetes pullulans* (pul'l'u.lans. N.L. part. adj. *pullulans* degrading pullulan).

Synonyms: *Oidium pullulans* Lindner (1895); *Oospora pullulans* (Lindner) Lindau (1907); *Oosporidium fuscans* Stautz (1931); *Monilia pullulans* (Lindner) Langeron & Talice (1932); *Trichosporon fuscans* (Stautz) Buchwald (1939); *Basidiotrichosporon pullulans* (Lindner) Kocková-Kratochvílová, Slavíkova and Zemek (1977).

Detailed descriptions of the species were presented by Guého *et al.* (1992, 1998) and Barnett *et al.* (2000). The synonymy of *Oosporidium fuscans* was determined by DNA-DNA reassociation (Guého *et al.*, 1992).

The type strain is CBS 2532<sup>T</sup>.

### **Transfer of *H. lignicola* to *Trichosporon***

*H. lignicola* was described by Diddens (1934) with two varieties (var. *undulatum* and var. *simplex*) from strains

isolated from wood pulp in Sweden. This monotypic genus was regarded as a hyphomycetous fungus, due to the formation of dry colonies with true, disarticulating hyphae and a paucity of budding cells (de Hoog & Smith, 1998). Typical basidiomycetous characteristics were present, i.e. positive diazonium blue B and urease reactions and the presence of xylose in cell walls and dolipore septa (Martínez, 1979; Guého *et al.*, 1992; de Hoog & Smith, 1998). The relationship of *H. lignicola* to members of the genus *Trichosporon* was recorded by Guého *et al.* (1992) with an examination of the D2 domain of the LSU rRNA. This observation was confirmed by D1/D2 and ITS rDNA analyses (Fell *et al.*, 2000; Scorzetti *et al.*, 2002). The relationship of *H. lignicola* to the genus *Trichosporon*, based on classical taxonomy and sequence evidence, was summarized by de Hoog & Smith (1998). Consequently, it is necessary to transfer the species to the genus *Trichosporon*.

Classical taxonomic description of the species (de Hoog & Smith, 1998) concluded that the three varieties (*lignicola*, *simplex* and *undulatum*) were synonyms. Our analysis of the D1/D2 and ITS regions of the strains *lignicola* (CBS 219.34<sup>T</sup>; GenBank accession numbers: ITS, AY370684; D1/D2, AY370685) and *simplex* (CBS 220.34; ITS, AF444481; D1/D2, AF363657) demonstrated identical sequences for both strains. The sequence of the strain *undulatum* (CBS 222.34; ITS, AF444482; D1/D2, AF363658) differed from the other two strains at two nucleotide locations [transitions : transversions (Ti:Tv), 2:0] in the D1/D2 region and in the ITS region by 1 nt (Ti:Tv, 1:0). The lack of significant phenotypic and sequence differences suggests that formal acceptance of separate varieties is not warranted at this time.

### **Description of *Trichosporon lignicola* (Diddens) Fell & Scorzetti comb. nov.**

Basonym: *Hyalodendron lignicola* H. Diddens. Eine neue Pilzgattung, *Hyalodendron*. Zentbl Bakteriol Parasitenkd Abt 2 **90**, 317 (1934).

*Trichosporon lignicola* (lig.ni'co.la. L. n. *lignum* wood; L. suff. -cola inhabitant, dweller; L. masc. n. *lignicola* wood-dweller).

Synonyms: *Hyalodendron lignicola* Diddens var. *lignicola* Diddens 1934; *Hyalodendron lignicola* Diddens var. *simplex* Diddens 1934; *Hyalodendron lignicola* Diddens var. *undulatum* Diddens 1934.

The type strain is CBS 219.34<sup>T</sup>.

There are several species of *Cryptococcus* among the Trichosporonales: *Cryptococcus haglerorum* (Middelhoven *et al.*, 2003) in the Cutaneum cluster and *Cryptococcus curvatus* and *Cryptococcus humicola*, which are represented as a separate cluster. Additionally, Takashima *et al.* (2001) demonstrated that *Cryptococcus musae*, *Cryptococcus ramirez-gomezianus*, *Cryptococcus longus* and *Cryptococcus pseudolongus* are similar phenotypically to *Cryptococcus humicola*.

and represent a species complex within the Trichosporonales. The analysis by Takashima *et al.* (2001) was based on 18S rDNA. As a result, ITS and D1/D2 sequences are not available for inclusion in our study. Those authors also reported *Cryptococcus daszewskae* to be related to *C. curvatus*. Consequently, a systematic evaluation of *Cryptococcus* spp. within the Trichosporonales is required.

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