Species reassignment of Geotrichum bryndzae, Geotrichum phurueaensis, Geotrichum silvicola and Geotrichum vulgare based on phylogenetic analyses and mating compatibility

Marizeth Groenewald,1 Teresa Coutinho,2 Maudy Th. Smith1 and J. P. van der Walt2†

1CBS-KNAW Fungal Biodiversity Centre, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
2Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa

The present classification of Galactomyces and its anamorph, Geotrichum, is based on various studies that used morphology, ecology, biochemistry, DNA–DNA reassociation comparisons and gene sequencing. In this study, the identities of strains of the Centraalbureau voor Schimmelcultures yeast collection, as well as seven strains from South Africa, were examined by analyses of the nucleotide divergence in the internal transcribed spacer regions of the nuclear rRNA gene (nrRNA) operon, the D1/D2 domains of the 26S rRNA gene and partial actin gene sequences as well as compatibility studies. The South African strains were assigned to species in the genus Galactomyces. The phylogenetic analyses and mating studies revealed that Geotrichum silvicola and Geotrichum bryndzae are synonyms of Galactomyces candidus and that Geotrichum vulgare is a synonym of Galactomyces pseudocandidus.

INTRODUCTION

The present classification of the arthroconidial teleomorph genus Galactomyces and its anamorph Geotrichum (de Hoog & Smith, 2011a, 2011b) is a result of previous studies dealing with morphology and ecology (Redhead & Malloch, 1977; von Arx, 1977), biochemistry (Weijman, 1979), DNA–DNA association comparisons (Guého et al., 1985; Smith et al., 1995), and more recently with gene sequencing (Kurtzman & Robnett, 1995; Ueda-Nishimura et al., 1995; Ueda-Nishimura & Mikata, 2000; de Hoog & Smith, 2004). Both heterothallic and homothallic species are present in Galactomyces. To date, the only exclusively homothallic species is Galactomyces reessii; Butler & Petersen (1972) reported that self-sterile forms of this species do not exist in nature. The first report on the occurrence of heterothallism in Galactomyces was by Butler & Petersen (1970). Two self-fertile cultures, Pr-11T (=CBS 772.71T) and Pr-47 (=CBS 773.71), showing typical morphology of the anamorph Geotrichum candidum, were isolated from soil in which unidentified grasses were growing. From these two self-fertile species, the authors isolated compatible self-sterile clones labelled Pr-11A1, Pr-11A2 and Pr-47A1, Pr-47A2, respectively, reporting that Pr-11A1 was compatible with Pr-47A2 (= CBS 775.71) and Pr-11A2 with Pr-47A1 (= CBS 774.71). Butler & Petersen (1972) described this teleomorph state of Geo. candidum as Endomyces geotrichum. However, on the basis of morphological features, Redhead & Malloch (1977) designated this species as the type species of the novel genus Galactomyces.

Revising the genus Geotrichum and its teleomorphs and using strains CBS 774.71 and CBS 775.71 of Galactomyces geotrichum as test strains, de Hoog et al. (1986) determined the mating types of nine strains identified as Geo. candidum. However, in that study, the authors used a different mating type nomenclature from Butler et al. (1988), listing CBS 774.71 as mating type A (mt A) and CBS 775.71 as mating type α (mt α). Four strains had the latter mating type and five strains had mating type A. A second heterothallic species described was Galactomyces citri-aurantii (Butler et al., 1988). This species was introduced to accommodate Geo. candidum-like isolates that cause sour rot of citrus species. Determining the mating types within these Geo. candidum-like isolates, Butler et al. (1988) assigned mt A1 to strain 220 (=CBS 175.89T) and mt A2 to strain 178 (=CBS 176.89T) of

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

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are JN974262–JN974293 and JQ793921–JQ793944 (see supplementary material for further details).

A supplementary figure and supplementary material are available with the online version of this paper.

†Johannes P. van der Walt passed away during the review process of this manuscript. Due to his original contribution to this manuscript we still acknowledge him as one of the co-authors.
that species. Examining the sexuality of the two species, the authors noted that Gal. geotrichum Pr-47A₁ (=CBS 774.71) mated with A₂ mating type strains of Gal. citri-aureantii and that Gal. geotrichum Pr-47A₂ (=CBS 775.71) mated with A₁ mating type strains of that species, resulting in the formation of ascii with a few ascospores or of gametangium-like structures, respectively.

DNA–DNA reassociation studies showed that Gal. geotrichum was heterogeneous (de Hoog et al., 1986), an observation supported by Smith et al. (1995). The latter authors distinguished four reassociation groups, where one represented Gal. geotrichum sensu stricto and three were considered to be novel species. The same authors also demonstrated that the first derivative graph of the nuclear DNA melting curve showed two peaks that were highly characteristic of the groups. This phenomenon was observed by Smith et al. (1995). By sequencing the internal transcribed spacer (ITS) regions of the rRNA (nrRNA) operon, de Hoog & Smith (2004) confirmed the existence of three novel species. The same authors also distinguished four reassociation groups, where one represented Gal. candidus, Gal. geotrichum CBS 774.71 and Gal. geotrichum CBS 775.71. Since the study of de Hoog & Smith (2004), nine novel anamorphic Geotrichum species have been described of which four species, Geotrichum silvicola (Pimenta et al., 2005), Geotrichum vulgare (Wuczkowski et al., 2006), Geotrichum bryndzae (Sulo et al., 2009) and Geotrichum phurueanaensis (Kaewwichian et al., 2010), are phylogenetically closely related to Gal. geotrichum. However, strains of Gal. candidus, Gal. pseudocandidus and Geo. europaeum species that are also phylogenetically close to Gal. geotrichum were not included in the phylogenetic analyses in these publications. In a recent overview (de Hoog & Smith, 2011b), it was suggested that, phenotypically, Geo. silvicola was identical to Gal. candidus and that Geo. vulgare was identical to Gal. pseudocandidus. In light of these observations, the present study was initiated to examine the relationships among these species based on phylogeny and mating compatibility of all Galactomyces species and closely related Geotrichum species known from culture. The mating type of their type strains was determined in a standard fashion using test strains of Gal. geotrichum. In addition, seven strains isolated from grass, lichen, moss and soil in South Africa were characterized on the basis of morphology and molecular phylogeny.

**METHODS**

**Strain information.** The strains used in this study are listed in Table 1. G indicates strains used in the genotypic characterization and M indicates strains examined in mating experiments.

**Sequence analyses.** Genomic DNA was extracted from cultures grown on GPYA medium (4 % glucose, 0.5 % peptone, 0.5 % yeast autolysate, 2 % agar) for 3 days using the FastDNA kit (Bio 101) with the FastPrep Instrument (Q-Biogene). Primers V9G (de Hoog & Gerrits van den Ende, 1998) and LR5 (Vilgalys & Hester, 1990) were used to amplify the region of the nrRNA gene operon that includes the 3' end of the small-subunit rRNA gene, the ITS regions (ITS 1, ITS 2 and the intervening 5S rRNA gene), and the D1/D2 domain of the large-subunit (LSU) rRNA gene, as described by Knutsen et al. (2007). Primers CA14 and CASR (Daniel et al., 2001) were used to amplify part of the actin gene using the method described by Daniel et al. (2001). The PCR products were separated by electrophoresis at 80 V for 40 min on a 0.8 % (w/v) agarose gel containing 0.1 µg ethidium bromide ml⁻¹ in 1 x TAE buffer (0.4 M Tris, 0.05 M sodium acetate, and 0.01 M EDTA, pH 7.85) and examined under UV light. The amplicons were sequenced in both directions using the primers LR0R (Vilgalys & Hester, 1990) and LR5 for the D1/D2 domain, whereas the primers V9G and ITS4 (White et al., 1990) were used for the ITS regions and the primers CA14 and CASR for the partial actin gene. The BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems) was used according to the manufacturer’s recommendations and the products were analysed on an ABI Prism 3730XL DNA Sequencer (PerkinElmer). A consensus sequence was computed from the forward and reverse sequences with SeqMan version 8 from the Lasergene package (DNASTAR). All sequences were assembled and aligned using the online version of MAFFT (version 6, http://mafft.cbrc.jp/alignment/server/index.html; Katoh et al., 2002).

The sequence data were analysed by phylogenetic analysis using parsimony (PAUP) v4.0b10 (Swofford, 2003) and the resulting trees were printed as described by Groeneveld et al. (2008). Maximum parsimony analyses were performed using the heuristic search option with 1000 random taxon additions and the robustness of the trees was evaluated by 1000 bootstrap replicates. Gaps were treated as fifth characters (‘new state’) and TBR was used as branch swapping algorithm. Other measures calculated included tree length, consistency index, retention index and rescaled consistency index (TL, CI, RI and RC, respectively). Neighbour-joining analyses using uncorrected ‘p’ and two different substitution models (HK85 and Kimura two-parameter) were also performed. All ties were broken randomly when encountered. The sequences were deposited in GenBank (Figs 1 and S1).

**Mating type determination and intra- and interspecific compatibility.** The mating type designation of Butler & Petersen (1970), who first reported heterothallism in Galactomyces, is followed in this study. The reference strains Gal. geotrichum CBS 774.71 (mt A₁) and CBS 464.89 (mt A₂) were used to standardize mating type nomenclature of the type strains of Geo. bryndzae, Geo. phurueanaensis, Geo. silvicola and Geo. vulgare. Sexual compatibilities were also used to validate the delineations of Galactomyces and Geotrichum species.

The degree of sexual compatibility was evaluated as follows: 1, ascii are present of which the majority contain mature ascospores that are broadly ellipsoidal, echinulate, with an irregular exosporium with an equatorial furrow (Fig. 2a, b); 2, ascii are present that contain mainly immature ascospores that lack spines and an irregular exosporium with an equatorial furrow plus very rarely mature ascospores (Fig. 2c); 3, ascii are present without ascospores (Fig. 2d); and 4, ascii are not formed. Compatibility tests were performed at least three times.

Media tested for compatibility assays included 5 % Difco malt (DM), potato dextrose (PDA) and yeast malt (YM) agars (Yarrow, 1998). Single and mixed cultures on ascosporeulation media were incubated at 25 °C and examined at 3–7 day intervals over 1 month.
RESULTS AND DISCUSSION

Sequence comparison

Multiple peaks at a single position were found in numerous regions of the electropherograms of the ITS sequences for the majority of the strains tested. Due to this, the sequences in these regions were difficult to interpret and it was not possible to use the ITS sequences to determine the precise identity of the strains or to use these sequences in the phylogenetic analyses (data not shown).

Although many of the ITS sequences could not be determined unambiguously, some key strains of this study, Gal.
In view of the above, D1/D2 and actin (ACT) sequences were used in our phylogenetic analyses. The D1/D2 sequence alignment for 32 strains, including the outgroup, had a total length of 506 positions, of which 412 were invariant and 39 were parsimony-informative. The parsimony analysis resulted in two equally most parsimonious trees, the first of which is shown in Fig. 1. The two trees only differed in the position of Geo. europaeum CBS 866.68, which was positioned as a sister taxon to either the Geo. vulgare/Geo. pseudocandidus clade (Fig. 1) or the Geo. geotrichum clade (data not shown). Tree topologies similar to that in Fig. 1 were found when gaps were treated as missing data or in trees inferred by neighbour-joining with different substitution models (data not shown).

The combined D1/D2 and ACT sequence alignment, containing 25 strains including the outgroup, had 1184 positions, of which 1000 were invariant and 94 were parsimony-informative. The parsimony analysis resulted in 180 equally most parsimonious trees, one of which is shown in Fig. S1. The trees only differed in the position of the strains within the clade containing the Geo. candidus strains. Similar tree topologies to the one shown in Fig. S1 were found when gaps were treated as missing data or using neighbour-joining (data not shown).

The phylogenetic trees obtained from the D1/D2 (Fig. 1) as well as the combined D1/D2 and ACT (Fig. S1) sequence alignments show four relatively well-supported clades, one containing Geo. candidus, Geo. silvicola and Geo. bryndzae strains (bootstrap support values of 93% and 100%, respectively), a second clade containing strains of Geo. vulgare and Gal. pseudocandidus (bootstrap values of 79% and 98%, respectively), a third containing all Geo. geotrichum strains (bootstrap supports of 98% and 100%, respectively) and a fourth consisting of the two Geo. citri-aurentii isolates (bootstrap support value of 100% in both analyses). Strains of Geo. phureaeae (CBS 11418T), Geo. europaeum (CBS 866.68T) and Gal. reessii (CBS 179.60T) could not be assigned to these clades (Figs 1 and S1), lending support to the view that these strains belong to three distinct species.

In the first clade, strains of Geo. silvicola, Geo. bryndzae and Gal. candidus were intermingled (Figs 1 and S1). Up to 10 nt differences in the D1/D2 regions and up to 5 nt differences in the ACT region were observed in the Gal. candidus/Geo. bryndzae/Geo. silvicola clade. Pimenta et al. (2005) did not include strains of Gal. candidus in the analysis that led to the description of Geo. silvicola, and therefore did not evaluate the possibility that these species might be conspecific. The authors reported polymorphisms within the D1/D2 region as well as different PCR fingerprint profiles among Geo. silvicola strains, suggesting a degree of polymorphism comparable to that of Gal. candidus (de Hoog & Smith, 2004). Sulo et al. (2009) showed that Geo. bryndzae is closely related to Geo. silvicola, although they did not include strains of Gal. candidus in their study. Consequently, the relative
phylogenetic positions of Geo. bryndzae, Geo. silvicola and Gal. candidus were not evaluated. DNA–DNA reassociation values (de Hoog et al., 1986) among strains of Gal. candidus ranged from 90.2 to 100%, indicating their conspecificity. This included two strains that appear as outliers in the Gal. candidus clade within which strains of Geo. bryndzae and Geo. silvicola are nested (Fig. 1). On this basis, the possibility that Geo. silvicola, Gal. candida and Geo. bryndzae are conspecific should be examined further. Six of the strains isolated in South Africa, CBS 11605, CBS 11607, CBS 11616, CBS 11620, CBS 11627 and CBS 11628, were well nested within Gal. candidus (Fig. 1) and hereby assigned to that species. Strains CBS 10073 T and CBS 626.83 T, the two type strains of Geo. vulgare and Gal. pseudocandidus, respectively, contained within the well-supported Gal. pseudocandidus/Geo. vulgare clade, differed by two nucleotide substitutions in the D1/D2 domain and one substitution in the ACT gene. These data indicate that Gal. pseudocandidus and Geo. vulgare are conspecific (Figs 1 and S1). One of the strains

---

**Fig. 1.** The first of two equally most parsimonious trees obtained from a heuristic search with 1000 random taxon additions of the D1/D2 sequence alignment (TL = 143 steps; CI = 0.783; RI = 0.863; RC = 0.676). The D1/D2 GenBank accession numbers are indicated after the strain names. Bar, 2 changes; bootstrap support values (>49%) from 1000 replicates are shown as percentages at nodes. The strains obtained from South Africa are indicated in bold. Mating types assigned are indicated for each strain, if available. On the right, intra-genomic DNA–DNA reassociation values are given for some Gal. candidus strains (Smith et al., 1995; de Hoog et al., 1986). Thickened lines indicate branches present in the strict consensus tree.
isolated in South Africa, CBS 11392, also belonged to the *Gal. pseudocandidus* clade (Fig. 1).

All strains of *Gal. geotrichum* had identical D1/D2 sequences and no more than one substitution in ACT gene sequences (Figs 1 and S1). No differences in either region were found between the *Gal. citri-aurantii* strains (Fig. 1).

Kurtzman & Robnett (1998) observed that, in the vast majority of cases, conspecific strains exhibit fewer than three substitutions in the D1/D2 region of the LSU rRNA gene and that strains that are otherwise known to represent separate species generally differ by 1% or more substitutions. Here a maximum divergence of 10 substitutions was found within the *Geo. silvicola/Geo. bryndzae/Gal. candidus*, the *Gal. pseudocandidus/Geo. vulgar* clades, respectively.

**Mating type determination and, intra- and interspecific compatibility**

The standardized mating types of strains of *Geotrichum* and *Galactomyces* species are presented in Table 1 and a summary of their mating compatibilities are shown in Table 2.

**Mating type determination.** Of the three media tested, PDA was found to be the most effective for inducing mating reactions. On this medium, none of the type strains of *Geo. bryndzae, Geo. europaeum, Geo. phurueaensis, Geo. silvicola* or *Geo. vulgar* were found to be self-fertile.

---

**Fig. 2.** Asci with ascospores in the genus *Galactomyces*: (a), asci with mature ascospores (black arrowheads) with the focus point on the echinulate outer layer of the ascus; (b), asci with mature ascospores (black arrowheads) with the focus point on the inner part of the ascus; (c), sterile asci (white arrowheads) and asci with immature ascospores (black arrowheads) without the echinulate outer layer; (d), Sterile asci (black arrowheads). Bars, 5 μm.
Because it has been shown previously that closely related heterothallic species of a genus may share the same mating type system (Pitt & Miller, 1970; Naumov, 1987; Naumov et al., 1997; Lachance et al., 1998; Smith et al., 2005), compatibility tests were performed across all strains. Positive mating reactions resulting in ascospores containing mainly immature or very rarely mature ascospores (Fig. 2c) were observed in crosses of the type strain Gal. geotrichum CBS 774.71 (mt A1) with Geo. silvicola CBS 9194\textsuperscript{T}, Geo. vulgare CBS 10073\textsuperscript{T} and Geo. bryndzae CBS 11176\textsuperscript{T}, respectively. Consequently, mating type A2 could be assigned to these three strains, although the mating reactions did not result in ascospores with mature ascospores.

Mating reactions resulting in ascospores (Fig. 2d) were obtained between the type strain of Geo. phurueaensis, CBS 11418\textsuperscript{T}, and the test strain CBS 464.93 (mt A2) of Gal. geotrichum, allowing the assignment of mating type A1 to strain CBS 11418\textsuperscript{T}.

Mating was not observed between the type strain of Geo. europaeum, CBS 866.68\textsuperscript{T}, and either test strain of Gal. geotrichum [CBS 774.71 (mt A1) and CBS 464.93 (mt A2)].

Intra- and interspecific compatibility. The type strains of Geo. silvicola (CBS 9194\textsuperscript{T}) and Geo. bryndzae (CBS 11176\textsuperscript{T}) formed a single clade (Figs 1 and S1) with the self-fertile type strain of Gal. candidus (CBS 178.71\textsuperscript{T}), as well as eight additional Gal. candidus strains of known mating types (Table 1) and six South African strains (CBS 11605, CBS 11607, CBS 11616, CBS 11620, CBS 11627 and CBS 11628) of unknown mating type. Compatibility was observed between the last six self-sterile cultures and the type strain of Geo. silvicola (CBS 9194\textsuperscript{T} (mt A2), resulting in the formation of ascospores with mature ascospores (Fig. 2a, b). The six strains were therefore assigned mating type A1. Asci with mature ascospores were also found in the crosses between Geo. bryndzae CBS 11176\textsuperscript{T} (mt A2) and two Gal. candidus strains, CBS 557.83 and CBS 11607 (mt A1). The formation of asci with mature ascospores by strains of this clade provides strong evidence that the strains represent the same species, in agreement with the high DNA–DNA reassociation values (90.2–100 \%) observed among strains of this clade (Fig. 1) that were previously examined (de Hoog et al., 1986). Reduced fertility was found for strains of this clade when crossed with the test strains of Gal. geotrichum. Asci containing mainly immature ascospores and rarely mature ascospores were observed (Fig. 2c). Strains of this clade failed to mate with strains of Geo. phurueaensis, Geo. europaeum, Geo. vulgare, Gal. pseudocandidus and Gal. citri-aurantii.

Within the second clade (Figs 1 and S1), mating of Geo. vulgare CBS 10073\textsuperscript{T} (mt A2) with CBS 10072 and CBS 11392 gave asci with mature ascospores, as did the latter strain when crossed with CBS 626.83\textsuperscript{T}. In addition to allowing the assignment of mating type A1 to strains CBS 10072 and CBS 11392 and mating type A2 to strains CBS 10073 and CBS 626.83, these results demonstrate that Geo. vulgare and Gal. pseudocandidus should be considered conspecific. Reduced compatibility was recorded between strains of this clade and strains of Geo. phurueaensis, Gal. geotrichum and Gal. citri-aurantii where crosses resulted in the formation of ascospores without ascospores (Fig. 2d). No mating was detected between strains of any clade and the type strain of Geo. europaeum (CBS 866.68\textsuperscript{T}).

The third and fourth clades (Figs 1 and S1) included strains of Gal. geotrichum and Gal. citri-aurantii, respectively, whose mating types were known from previous publications (Butler & Petersen, 1972; Butler et al., 1988). Reduced compatibility was observed when mating type A2 strains of either species were crossed with Geo. phurueaensis strain CBS 11418\textsuperscript{T} (mt A1), resulting in the formation of ascospores without ascospores (Fig. 2d), and no mating occurred in crosses with Geo. europaeum strain CBS 866.68\textsuperscript{T}.

In light of the results obtained with members of the four clades, the distinct phylogenetic positions (Figs 1 and S1) of strains of Geo. phurueaensis (CBS 11418\textsuperscript{T}), Geo. europaeum

---

**Table 2. Sexual compatibility among Geotrichum and Galactomyces species**

*Geo. europaeum* is excluded from the table as it did not show any mating reaction. The degree of sexual compatibility is defined as follows: 1, ascospores (see Fig. 2a, b); 2, ascospores, mostly sterile (Fig. 2c); 3, ascospores, sterile (Fig. 2d); 4, incompatible (see text for complete definitions).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mating type</th>
<th>Geo. phurueaensis</th>
<th>Gal. geotrichum</th>
<th>Gal. pseudocandidus</th>
<th>Gal. candidus</th>
<th>Gal. citri-aurantii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1</td>
<td>A2</td>
<td>A1</td>
<td>A2</td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>Gal. geotrichum</td>
<td></td>
<td></td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Gal. pseudocandidus</td>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gal. candidus</td>
<td></td>
<td></td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Gal. citri-aurantii</td>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>
(CBS 866.68\textsuperscript{3}) and \textit{Gal. reessii} (CBS 179.60\textsuperscript{3}) support their assignment to three separate species. \textit{Galactomyces reessii} is homothallic, \textit{Geo. phurueaensis} is represented by a strain of mating type A\textsubscript{1}, and \textit{Geo. europaeum} lacks a sexual cycle.

**TAXONOMY**

On basis of the phylogenetic analysis, \textit{Geotrichum phurueaensis} is recognized as a valid anamorphic species of mating type A\textsubscript{1}. The species \textit{Geo. silvicola} and \textit{Geo. bryndzae} are considered synonyms of \textit{Gal. candidus}, and \textit{Geo. vulgare} is treated as a synonym of \textit{Gal. pseudocandidus}. The mating type of the three type strains is mt A\textsubscript{2}.


An important matter that has to be taken into account in the near future is the Amsterdam declaration on fungal nomenclature, which addresses the very important issue of how the current system of naming pleomorphic fungi will change as a result of the availability of DNA sequence data (Hawksworth \textit{et al.}, 2011). This declaration proposes that, in principle, priority should be given to the oldest described name, with some exceptions (Rossman & Seifert, 2011). This proposal has been accommodated in the International Code of Nomenclature for algae, fungi, and plants (ICN), the Melbourne Code (Knapp \textit{et al.}, 2011), which will require pleomorphic fungi to bear a single name. Nucleotide sequence data and inferred phylogenies will play a significant role in the new naming system.

Here, we have shown that the phylogeny derived from sequences of the D1/D2 region of the LSU rRNA gene and partial sequences of the actin gene (Fig. S1) generates, for species of \textit{Galactomyces} and \textit{Geotrichum}, a species circumcision that is compatible with boundaries suggested by mating success among heterothallic strains. As the anamorphic \textit{Geotrichum} species are linked to two teleomorphic genera, \textit{Galactomyces} and \textit{Dipodascus} (de Hoog & Smith, 2004), a comprehensive study including all species in these three genera will contribute to our knowledge of the relationships among the closely related yeast species in these two phylogenetically separate groups. In light of the Amsterdam declaration, the present study and others (Smith \textit{et al.}, 1995; de Hoog & Smith, 2004) paved the way to the future, where all described \textit{Galactomyces} species can be reassigned to \textit{Geotrichum} as the oldest name available for this phylogenetic clade. The reassignment of the additional \textit{Geotrichum} species, \textit{Geo. carabidarum}, \textit{Geo. cucujoidarum}, \textit{Geo. fermentans}, \textit{Geo. hantanense}, \textit{Geo. histeridarum}, \textit{Geo. klebahnii}, \textit{Geo. restrictum} and \textit{Geo. siamensis}, which were not included in this study but were shown to belong to the \textit{Dipodascus} clade (de Hoog & Smith, 2004; Suh & Blackwell, 2006; Kaewwichian \textit{et al.}, 2010; Nielsen \textit{et al.}, 2010), is necessary. A comprehensive study on all \textit{Galactomyces}, \textit{Geotrichum} and \textit{Dipodascus} species should therefore be conducted to clarify the affinity of species within these three genera.

**ACKNOWLEDGEMENTS**

We thank Wendy Epping for her technical assistance and J. Z. (Ewald) Groenewald and André Lachance for critical reading of the manuscript.

**REFERENCES**


