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Genome comparisons in the genus *Dipodascus* de Lagerheim

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Abstract

Phenotypic characteristics of physiology and morphology of 71 strains belonging to the genus *Dipodascus* de Lagerheim were examined. The GC contents of genomic DNAs of 46 strains were calculated from the thermal denaturation curves using the spectrophotometric method. The first derivatives of the melting curves revealed that the DNAs of these strains are heterogeneous; four categories could be recognized. However, DNA similarity values calculated by using DNA–DNA reassociation kinetics showed that each category could be subdivided further. Two categories were separated into four subgroups each; the other two yielded five subgroups each. Strains belonging to the same subgroup exhibited high levels of DNA similarity ranging from 82 to 100%. The 18 subgroups represented 13 currently accepted *Dipodascus* species and five anamorphic *Geotrichum* species, four representing novel taxa. A phenotypic key to distinguish the taxa of *Dipodascus*, *Galactomyces* and *Geotrichum* is presented.

Keywords: *Dipodascus*; Taxonomy; Yeast; DNA heterogeneity; Genome comparison

1. Introduction

The classification of arthroconidial yeast-like strains has long been problematical. Isolates with budding cells, pseudohyphae, and true mycelium with arthroconidia were long assigned to the genus *Trichosporon* [1,2], while arthroconidial isolates lacking budding cells and pseudohyphae were assigned to the genus *Geotrichum*. However, recognizing heterogeneity in *Trichosporon*, Do Carmo-Sousa [2] distinguished three groups, one of which showed close relatedness to some species of *Geotrichum* and *Dipodascus*. Various studies had demonstrated that the type species of *Trichosporon* was of basidiomycetous affinity [3–5]. On the basis of differences in carbohydrate composition of the cell walls, Weijman [6] suggested a separation of the basidiomycetous and ascomycetous arthroconidial species as *Trichosporon* and *Geotrichum* species, respectively. The presence or absence of budding cells in individual species was considered unimportant in the context of this proposed revision. In 'The Yeasts, A Taxonomic Study, 3rd edn., 1984' [7], ascomycetous and basidiomycetous species were nonetheless still kept in *Trichosporon*. De Hoog et al. [8] accepted the scheme proposed by Weijman [6], and started a revision of *Geotrichum* and its teleomorphs *Galactomyces* and *Dipodascus*. This taxonomic revision, the first to consider all the arthroconidial ascomycetous yeast-like genera together, combined studies of morphology, physiology, molar guanine+cytosine percentage (mol% G+C), and DNA–DNA homology. On the basis of these characters, four species were recognized in *Geotrichum*, two in *Galactomyces* and 13 in *Dipodascus*. De Hoog et al. [8] noted that the biological species concepts as based on mating studies did not correspond well with DNA reassociation data. For instance, high, intermediate and low levels of DNA–DNA reassociation were found among strains which had been assigned to *Galactomyces geotrichum* on the basis of interfertility. In *Dipodascus*, however, intermediate reassociation values of 50% were considered to indicate different taxa.

Another striking observation was heterogeneity in the melting pattern of heated, double-stranded DNA as seen in the derivative graphs of the melting curves. The graphs for some species showed two peaks, a rare phenomenon in yeast-like ascomycetes. With regard to such derivative graphs, only the highest values calculated from the peaks were listed as the species-specific GC content by De Hoog et al. [8]. The peculiarities and inconsistencies seen in their

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Table 1
List of *Dipodascus* strains with their origin, their previous and present identity, and their growth rate

Strain	Additional information	Designation		Growth rate (mm/7 days)
		Previous	This study	
CBS175.53[Ta]	pupal galleries of *Ips acuminatus* in *Pinus sylvestris*, Germany	*D. aggregatus*[b]	*D. aggregatus* s.s.	9.5
CBS152.57[a]	*Ips pini* frass in root of *Pinus resinosa*, USA	*D. aggregatus*	*D. aggregatus* s.s.	10.5
CBS764.85[a]	slime flux of *Pinus ponderosa*, USA	*D. aggregatus*	*D. aggregatus* s.s.	9.5
CBS284.86[a]	unknown	*D. aggregatus*	*D. aggregatus* s.s.	11.0
CBS285.86	unknown	*D. aggregatus*	*D. aggregatus* s.s.	9.0
CBS765.85	slime flux in *Pinus ponderosa*, USA	*D. aggregatus*[b]	*D. aggregatus* A	4.0
CBS625.85	*Nectria cinnabarina*, Russia	*D. aggregatus*[b]	*D. aggregatus* B	7.0
CBS766.85[T]	exudates of angiosperm tree, Japan	*D. albidus*[b]	*D. albidus*	14.0
CBS165.29	unknown	*D. armillariae*[b]	*D. armillariae*	8.0
CBS817.71[Ta]	T of *Geotrichum armillariae*, *Armillaria mellea*, Netherlands	*D. armillariae*	*D. armillariae*	6.0
CBS818.71[a]	*Armillaria mellea*, Netherlands	*D. armillariae*	*D. armillariae*	5.5
CBS834.71	gills of *Armillaria mellea*, Netherlands	*D. armillariae*	*D. armillariae*	4.5
CBS540.76	gills of *Armillaria mellea*, Netherlands	*D. armillariae*	*D. armillariae*	6.5
CBS623.82	*Armillaria* sp., Belgium	*D. armillariae*	*D. armillariae*	5.0
CBS624.82[a]	*Armillaria mellea*, Belgium	*D. armillariae*	*D. armillariae*	6.5
CBS600.83	*Armillaria* sp., Netherlands	*D. armillariae*	*D. armillariae*	5.5
CBS625.74[Ta]	decaying cladode of *Opuntia inermis*, Australia	*D. australiensis*[b]	*D. australiensis*	25.5
CBS666.79[a]	necrosis in *Opuntia* sp., South Africa	*D. australiensis*	*D. australiensis*	28.5
CBS667.79	necrosis in *Opuntia* sp., South Africa	*D. australiensis*	*D. australiensis*	25.5
CBS372.83[a]	*Euphorbia ingens*, South Africa	*D. australiensis*	*D. australiensis*	24.0
UOFSY0065	unknown	*D. australiensis*	*D. australiensis*	25.0
CBS197.35	woodpulp, Sweden, MTA	*D. capitatus*[b]	*D. capitatus*	7.0
CBS312.76	sputum of man, Germany	*D. capitatus*	*D. capitatus*	5.5
CBS162.80[a]	bovine mastitis milk, UK	*D. capitatus*	*D. capitatus*	6.5
CBS571.82[LTa]	LT of *Trichosporon capitatum*, woodpulp, Sweden	*D. capitatus*	*D. capitatus*	6.5
CBS572.82	woodpulp, Sweden	*D. capitatus*	*D. capitatus*	6.0
CBS573.82[a]	yeastcake	*D. capitatus*	*D. capitatus*	6.5
CBS574.82	sputum of man, Norway	*D. capitatus*	*D. capitatus*	6.0
CBS575.82	man, South Africa	*D. capitatus*	*D. capitatus*	7.5
CBS577.82	sputum of man, Germany	*D. capitatus*	*D. capitatus*	6.0
CBS579.82	AUT of *Geotrichum linkii*	*D. capitatus*	*D. capitatus*	5.5
CBS580.82	AUT of *Geotrichum linkii*, sputum of man, MTα	*D. capitatus*	*D. capitatus*	6.5
CBS207.83[a]	T of *Blastoschizomyces pseudotrichosporon*	*D. capitatus*	*D. capitatus*	7.5
	sputum of man, USA	*D. capitatus*	*D. capitatus*	
CBS598.83	oral infection of human patient	*D. capitatus*	*D. capitatus*	6.0
CBS716.84	digestive tube of pig, France	*D. capitatus*	*D. capitatus*	7.5
CBS327.86	blood culture of human patient, USA	*D. capitatus*	*D. capitatus*	6.5
VTTD-95458	unknown	*D. capitatus*	*D. capitatus*	6.0
UAMH375	unknown	*D. capitatus*	*D. capitatus*	8.0
CBS184.80[T]	pulp *Psidium guajava*, Maharastra, India	*D. geniculatus*[b]	*D. geniculatus*	10.5
CBS517.90[Ta]	= CBS4603, wine cellar, South Africa	*D. ingens*[b]	*D. ingens* s.s.	4.5
CBS524.90[a]	= CBS6787, T of *Pichia humboldtii*, mutant of 517.90	*D. ingens*	*D. ingens* s.s.	6.0
CBS518.90[a]	= CBS4115, phenolic waste	*D. ingens*[b]	*D. ingens* A	5.0
CBS519.90[a]	= CBS4825, wine cellar, South Africa	*D. ingens*	*D. ingens* A	6.0
CBS520.90	= CBS4826, wine cellar, South Africa	*D. ingens*	*D. ingens* A	6.0
CBS521.90	= CBS4827, wine cellar, South Africa	*D. ingens*	*D. ingens* A	4.5
CBS522.90[a]	= CBS6057, asphalt plant waste lagoon	*D. ingens*	*D. ingens* A	4.5
CBS523.90[a]	= CBS7197, unknown	*D. ingens*	*D. ingens* A	5.0
CBS101346	= CBS1971, industrial sulfite waste	*D. ingens*	*D. ingens* A	nd
CBS259.82[Ta]	*Badhamia utricularis*, slime trail plasmodium, UK	*D. macrosporus*[b]	*D. macrosporus*	3.5
CBS260.82[a]	*Badhamia utricularis*, slime trail plasmodium, UK	*D. macrosporus*	*D. macrosporus*	3.5
CBS107.12[a]	unknown	*D. magnusii*[b]	*D. magnusii*	10.0
CBS108.12	unknown	*D. magnusii*	*D. magnusii*	9.0
CBS151.30[a]	slime flux in *Quercus* sp., Germany	*D. magnusii*	*D. magnusii*	9.5
CBS234.85[a]	slime flux in *Quercus alba*, Pennsylvania, USA	*D. magnusii*	*D. magnusii*	9.5
CCY42-1-2	unknown	*D. magnusii*	*D. magnusii*	9.5
CCY42-1-3	unknown	*D. magnusii*	*D. magnusii*	9.5
CCY42-1-4	unknown	*D. magnusii*	*D. magnusii*	9.5
CCY42-1-5	unknown	*D. magnusii*	*D. magnusii*	8.5

Table 1 (*Continued*).

Strain	Additional information	Designation		Growth rate (mm/7 days)
		Previous	This study	
CBS192.55[Ta]	tannin concentrate, Spain	*D. ovetensis*[b]	*D. ovetensis* s.s.	5.0
CBS634.85[a]	slime flux in *Quercus* sp., Germany	*D. ovetensis*	*D. ovetensis* s.s.	5.5
CBS635.85[a]	unknown, Germany	*D. ovetensis*	*D. ovetensis* s.s.	6.5
CCY30-2-6	unknown	*D. ovetensis*	*D. ovetensis* s.s.	5.5
CBS749.85[a]	T of *D. ambrosiae*, insect gallery, California, USA	*D. ambrosiae*[b]	*D. ovetensis* s.s.	5.5
CBS750.85[a]	slime flux *Quercus rubra*, Ontario, Canada	*D. ovetensis*[b]	*D. ovetensis* A	7.5
CBS751.85[a]	slime flux *Quercus rubra*, Ontario, Canada	*D. ovetensis*	*D. ovetensis* A	5.5
CBS752.85[Ta]	slime flux *Quercus rubra*, Ontario, Canada	*D. ovetensis*	*D. ovetensis* A	7.0
CBS8187[T]	T of *S. chiloense*, rotten trunk of *Eucryphia cordifolia*, Chile	*S. chiloense*[c]	*Geotrichum* species[c]	2.5
CBS244.85[T]	cactus rot, Arizona, USA	*D. spicifer*	*D. spicifer*	8.5
CBS780.96[Ta]	rotting saguaro plant, Arizona, USA	*D. starmeri*[d]	*D. starmeri*	4.0
CBS781.96[a]	rotting cladode of *Opuntia ficus-indica*, Arizona, USA	*D. starmeri*	*D. starmeri*	4.0
CBS765.70[T]	wet conveyer, California, USA	*D. tetrasperma*[b]	*D. tetrasperma*	9.0

[T] = type strain; [LT] = lectotype strain.

CBS = Centraalbureau voor Schimmelcultures; CCY = Czechoslovak Collection of Yeasts, Institute of Chemistry, Slovakian Academy of Science, Bratislava, Slovak Republic; UAMH = University of Alberta Microfungus Herbarium and Collection, Edmonton, AB, Canada; UOFS = Department of Microbiology and Biochemistry, University of Orange Free State, Bloemfontein, South Africa; VTT = VTT, Biotechnology and Food Research, Espoo, Finland.

[a] Strain used in infraspecific reassociations.

[b] Designation given in keys of De Hoog et al. [8].

[c] *S. chiloense* clustered with *Dipodascus* clade in phylogenetic study of Kurtzman and Robnett [13].

[d] Described in 1997 by Phaff et al. [15].

study led to a re-examination of the genera involved to determine whether the DNA heterogeneity was a common, consistent character within these arthroconidial genera. Concurrently the species concepts as based on DNA similarities and mating results were re-evaluated. Starting with *Galactomyces*, a series of studies was published concerning whole-genome comparisons [9], molecular differentiation [10], and speciation and life cycle [11]. The anamorph *Geotrichum* was re-examined by whole-genome comparisons only [12].

Meanwhile, various phylogenetic studies of hyphal ascomycetous yeasts and yeast-like taxa, *Galactomyces*, *Geotrichum* and *Dipodascus* included, have been published [13–16]. From determinations of the sequence divergence of the large subunit (26S) rDNA gene, it was concluded [13,14] that these genera are related to the ascomycetous yeasts and should be placed in the order Saccharomycetales. It was revealed also that species of these genera separate into two main distantly related clades, one of which includes *Galactomyces*. Therefore, there is no support for maintaining *Galactomyces* as a separate genus. A phylogenetic tree based on 18S rRNA gene sequences [16] showed approximately the same two groups as were shown by D1/D2 sequences. These groups differ in ability to survive for 6 months on yeast malt (YM) agar slants at 5°C, and in assimilation of xylose. The group able to assimilate xylose was subdivided further into three subgroups, one comprising the genus *Galactomyces* in its entirety.

The same phylogenetic studies [13–16] have suggested conspecificity between *Dipodascus ambrosiae* and *Dipodascus ovetensis* and between *Geotrichum clavatum*, *Dipodascus spicifer* and *Dipodascus capitatus*. However, conspecificity of the latter group of species was not supported by genome comparison studies [17,18], while that of the former group was confirmed [17].

The present study reports the results of genome comparisons in *Dipodascus*. In addition to the 13 species recognized in 'The Yeasts, A Taxonomic Study, 4th edn., 1998' [19], *Dipodascus starmeri* and *Schizoblastosporion chiloense* were included, since both species have been placed phylogenetically in the *Dipodascus* clade close to *Dipodascus ingens* [13,15].

2. Materials and methods

2.1. Cultures examined

Dipodascus strains examined are listed in Table 1 along with their origins and previous and present identities.

2.2. Physiology and morphology

Physiological characteristics of strains not examined by De Hoog et al. [8] were tested using standard methods [20]. Cultures were grown in liquid medium in tubes for 4 weeks at 25°C and shaken continuously at 30 rpm. Utilization of nitrogen compounds was examined auxanographically after 1 week of incubation at 25°C.

All strains listed in Table 1 were tested for their growth rate by making a small streak of a heavy inoculum of a 1–7-day-old culture onto GPYA (4% glucose+0.5% peptone+0.5% yeast autolysate+2% agar) in Petri dishes. After incubation at 25°C for 7 days, measures were taken from the center of the streak to the colony margin.

Production of ascospores was examined by inoculation of 2–7-day-old cultures on Difco malt–agar, V8 agar, potato dextrose agar or MacClary acetate agar [20] at 25°C. Each individual strain was checked for ascospore production, and when it was not observed, strains were mixed in all possible combinations and were rechecked after 7–30 days.

2.3. DNA analysis

Strains were grown for 1–2 days at 25°C on a rotary shaker at 125 rpm in 0.5 l YM broth [21] using 1-l flat-bottom flasks. Isolation and purification of DNA and determination of DNA base composition were done as previously described [9]. DNA–DNA hybridization experiments were carried out as described by Seidler and Mandel [22] and modified by Kurtzman et al. [23]. The optimal reassociation temperature was recognized as either 57°C or 59°C, based on the highest mol% G+C value calculated from the derivative curves. Reassociation experiments were performed at least twice.

3. Results

3.1. Physiology and morphology

All species assimilated D-glucose, D-galactose, glycerol,

Table 2
Phenotypic key characters of *Dipodascus* and closely related species

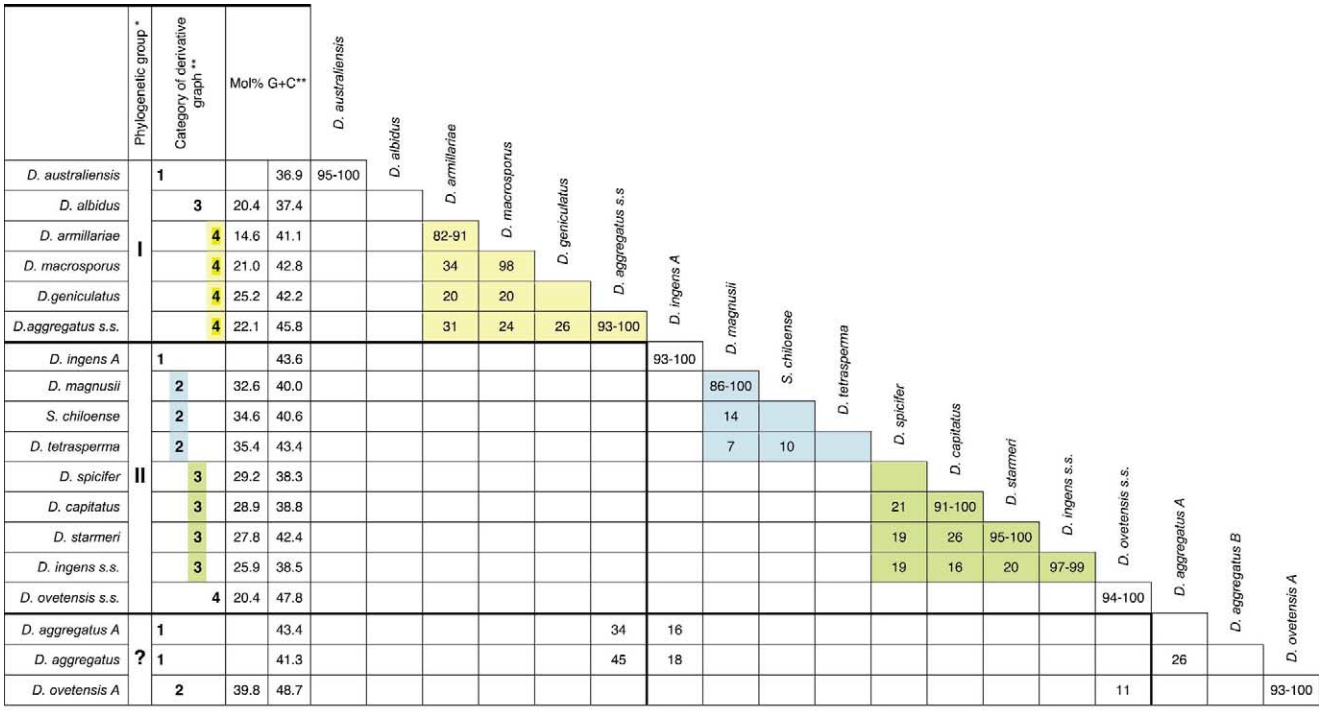
	D. aggr s.s.	*D. aggr* A	*D. aggr* B	*D. alb*	*D. arm*	*D. aus*	*D. cap*	*Sch. chil*	*D. gen*	*D. ing* s.s.	*D. ing* A	*D. mag*	*D. macr*	*D. ovet* s.s.	*D. ovet* A	*D. spic*	*D. starm*	*D. tetra*
Number of strains tested in physiology	5	1	1	1	8	5	17	1	1	2	7	8	2	4 1[a]	3	1	2	1
reassociations	4	1	1	1	3	3	4	1	1	2	4	3	2	3 1	3	1	2	1
Growth on:																		
D-Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+ +	+	+	+	+
L-Sorbose	+	+	+	+	−	+	v	+	+	−	+	+	+	v v	v	+	+	+
D-Ribose	v	−	v	−	v	−	−	−	−	−	−	−	−	− −	−	−	−	−
D-Xylose	+	+	−	+	+	+	−	−	+	−	−	−	+	− −	−	+	−	−
Sucrose	−	−	−	−	−	−	−	−	−	−	−	+	−	− −	−	−	−	−
Maltose	−	−	−	−	−	−	−	−	−	−	−	−	−	− −	−	−	−	−
α,α-Trehalose	−	−	−	−	−	−	−	w	−	−	−	−	−	v v	−	−	−	−
Cellobiose	−	−	−	−	+/w	−	−	−	−	−	−	−	+/w	− −	−	+	−	−
Salicin	−	−	−	−	−	−	−	−	−	−	−	−	−	− −	−	+	−	−
Arbutin	−	−	−	−	−	−	−	−	−	−	−	−	−	− −	−	+	−	−
Raffinose	−	−	−	−	−	−	−	−	−	−	−	+	−	− −	−	−	−	−
Soluble starch	−	−	−	−	−	w/−	−	−	+	−	−	−	−	− −	−	−	−	−
Ribitol	v	+	+	−	−	v	−	−	−	−	−	−	v	− −	−	−	−	−
D-Glucitol	+	+	+	+	v	+	−	−	+	−	−	+	+/w	− +	−	−	−	+
D-Mannitol	+	+	+	+	+	+	−	−	+	−	−	+	w	− +	−	−	−	−
Glucono-δ-lactone	−	+	+	−	−	−	−	−	−	−	−	−	+/w	v +	−	−	−	+
DL-Lactate	v	+	+	+	v	+	+	+	w	+	+	v	+/w	+ +	+	+	−	+
Citrate	+	+	+	−	v	−	v	−	w	−	−	−	+/w	− −	−	+	−	+
Growth without vitamins	−	−	−	−	+	−	−	+	−	+	+	−	+	− −	+	−	−	−
Growth at:																		
25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+ +	+	+	+	+
30°C	+	−	+	−	−	+	+	−	+	+	+	+	−	+ +	+	+	+	+
35°C	−	−	+	−	−	+	+	−	+/w	+	+	−	−	− +	−	+	+	+
37°C	−	−	−	−	−	+	+	−	−	+	+	−	−	− −	−	+	+	+
40°C	−	−	−	−	−	+	+	−	−	−	−	−	−	− −	−	+	+	+
Range of growth (mm/7 days)	9–11	3–5	7–8	3–15	4–9	24–29	5–10	2–3	10–11	3–7	3–7	8–11	3–4	4–8, 6	5–5–9	8–9	4–5	8–10
Mating[b]	S	−	−	S	−	S	mt	−	S	−	mt	S	S	S	−	S	mt	S

v = variable; w = weak.

D. aggr = *Dipodascus aggregatus*; *D. alb* = *Dipodascus albidus*; *D. arm* = *Dipodascus armillariae*; *D. aus* = *Dipodascus australiensis*; *D. cap* = *Dipodascus capitatus*; *D. gen* = *Dipodacus genidulatus*; *D. ing* = *Dipodacus ingens*; *D. mag* = *Dipodascus magnussii*; *D. macr* = *Dipodascus macrosporus*; *D. ovet* = *Dipodascus ovetensis*; *D. spic* = *Dipodascus spicifer*; *D. starm* = *Dipodacus starmeri*; *D. tetra* = *Dipodascus tetrasperma*; *Sch. chil* = *Schizoblastosporion chiloense*.

[a] Physiological key characters of *D. ambrosiae* which is synonymous with *D. ovetensis* s.s.

[b] S = self-sporulation; mt = mating types; − = ascopores and mating not observed.



	Phylogenetic group *	Category of derivative graph **	Mol% G+C**		D. australiensis	D. albidus	D. armillariae	D. macrosporus	D. geniculatus	D. aggregatus s.s	D. ingens A	D. magnusii	S. chiloense	D. tetrasperma	D. spicifer	D. capitatus	D. starmeri	D. ingens s.s.	D. ovetensis s.s.	D. aggregatus A	D. aggregatus B	D. ovetensis A
D. australiensis	I	1		36.9	95-100																	
D. albidus		3	20.4	37.4																		
D. armillariae		4	14.6	41.1			82-91															
D. macrosporus		4	21.0	42.8			34	98														
D.geniculatus		4	25.2	42.2			20	20														
D.aggregatus s.s.		4	22.1	45.8			31	24	26	93-100												
D. ingens A	II	1		43.6							93-100											
D. magnusii		2	32.6	40.0								86-100										
S. chiloense		2	34.6	40.6								14										
D. tetrasperma		2	35.4	43.4								7	10									
D. spicifer		3	29.2	38.3																		
D. capitatus		3	28.9	38.8											21	91-100						
D. starmeri		3	27.8	42.4											19	26	95-100					
D. ingens s.s.		3	25.9	38.5											19	16	20	97-99				
D. ovetensis s.s.		4	20.4	47.8															94-100			
D. aggregatus A	?	1		43.4						34	16											
D. aggregatus		1		41.3						45	18									26		
D. ovetensis A		2	39.8	48.7															11			93-100

* Phylogeny group taken from [13]and [16]; ? = Not yet determined.
** Categories and mol% G+C taken from Table 3.

Fig. 1. Matrix of infra- and interspecific DNA–DNA similarities among *Dipodascus* and closely related species. Colors refer to reassociations among taxa of the same DNA chemistry. The standard deviations for levels of infraspecific reassociations were $<5\%$. The standard deviations for levels of interspecific reassociations are $<10\%$. Values are averages of at least two determinations.

succinate, ethanol (except for *D. armillariae*), propane-1,2-diol, butane-2,3-diol, ethylamine, L-lysine and cadaverine. No species utilized L-arabinose, D-arabinose, L-rhamnose, methyl-α-D-glucoside, melibiose, lactose, melezitose, inulin, erythritol, xylitol (except for *D. aggregatus* B), L-arabinitol, galactitol, inositol, 2-keto-D-gluconate, D-gluconate, D-glucuronate, D-galacturonate, methanol, or nitrate. Table 2 lists the physiological characteristics that proved useful for distinguishing the taxa shown to be distinct in DNA similarity comparisons.

Growth rates are shown in Tables 1 and 2. All *Dipodascus* strains grew <15 mm/7 days, except *D. australiensis*, all strains of which grew >20 mm/7 days.

Ascospores were observed in the homothallic *D. aggregatus* s.s., *D. albidus*, *D. australiensis*, *D. geniculatus*, *D. macrosporus*, *D. magnusii*, *D. spicifer*, *D. tetrasperma* and *D. ovetensis* s.s. Ascospores were obtained after mating in the named heterothallic species *D. capitatus* and *D. starmeri* as well as in the as yet undescribed taxon labelled *D. ingens* A. No ascospore production was observed in *D. aggregatus* A (represented only by CBS765.85), *D. aggregatus* B (CBS625.85), *D. ingens* s.s., *D. ovetensis A* and *S. chiloense* (Table 2). Ascospore production by *D. armillariae*, though expected, was not confirmed.

3.2. Nuclear DNA base composition

Mol% G+C determinations were obtained for 46 *Dipodascus* strains. Following previous procedures [9], the mol% G+C was calculated from the derivative graphs of the melting curves instead of directly from the melting curve. This method allows calculation of the G+C content of the individual peaks, which represent major fractions of the DNA examined. As in *Galactomyces* [9] and *Geotrichum* [12], most *Dipodascus* strains yielded heterogeneous derivative graphs. In Table 3 the strains are arranged by the shape of the derivative curves and the taxa are based on the present classification. Twelve strains, including the type of *D. australiensis*, produced derivative graphs with a single broad peak. The calculated G+C content of this fraction ranged from 36.9 to 43.6 mol%. This group of strains is referred to as Category 1. Of the remaining strains characterized by derivative graphs with two peaks, 18 strains showed peaks of equal height and 17 strains had peaks of unequal height. Detailed examination revealed that the 18 strains with derivative graphs with peaks of equal height could be subdivided into two groups by differences in G+C content of the two fractions. Eight strains, including the types of *S. chiloense*, *D. magnussii* and *D. tetrasperma*, had two fractions with respective G+C contents of roughly 33–40 mol% and 40–49 mol%. The strains showing this property are referred to as Category 2. Ten strains assigned to Category 3, including the types of *D. spicifer*, *D. capitatus*, *D. ingens*, *D. starmeri* and *D. albidus*, had two fractions with respective G+C values of roughly 20–29 mol% and 37–42

mol%. The 16 strains showing derivative graphs with peaks of unequal height are referred to as Category 4 and include the types of *D. aggregatus*, *D. armillariae*, *D. geniculatus*, *D. macrosporus* and *D. ovetensis*. The range of the G+C content in the two DNA fractions was roughly 14–25 mol% and 41–48 mol%, respectively.

3.3. Infraspecific DNA relatedness

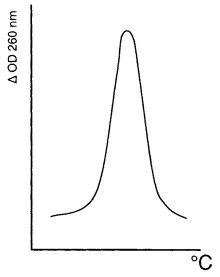
In Table 1 the strains used in the infraspecific reassociations are indicated and in Fig. 1 the range of infraspecific DNA similarities calculated from reassociation rates are shown. The taxa are arranged in the two main groups after their phylogenetic relatedness [13,16]. Taxa of which the phylogenetic position is unknown are arranged apart. The order of the taxa within the groups is according to the observed derivative category (Table 3). Reassociations were performed among strains of the same category differing <4 mol% in their G+C values.

Category 1. Strains showing T_m derivative graphs with one broad peak

Reassociation values of 95–100% were observed among three selected strains having an average G+C value of 36.9%. This group of strains was considered to represent *D. australiensis* since it included the type strain of this species. Six selected strains showing an average G+C content ranging from 41 to 44% could be divided into three homology groups. Four strains showed 93–100% mutual DNA similarity. This group was referred to as *D. ingens* A, because these strains were formerly identified as *D. ingens* but were not conspecific with the type. Two entities remained, each represented by a single strain. CBS765.85 is referred to as *D. aggregatus* A, and

Table 3
Mol% G+C of *Dipodascus* and closely related species estimated using first-order derivatives of double-stranded DNA melting graphs

Strain	Value(s)[b]		Difference[c]	Diagram of first derivative graph of the melting curve of nDNA
Category 1				
D. australiensis				
CBS 625.74[T a *]		36.7		
CBS 666.79 [a]		36.8		
CBS 667.79		36.7		
CBS 372.83 [a *]		36.8		
UOFS Y 0065		37.7		
		36.9 ± 0.4		
D. aggregatus A				
CBS 765.85 *		43.4		
		43.4 ± 0.5		
D. aggregatus B				
CBS 625.85 *		41.3		
		41.3 ± 0.0		
D. ingens A				
CBS 518.90 [a]		43.0		
CBS 519.90 [a]		43.5		
CBS 521.90		44.0		
CBS 522.90 [a *]		43.7		
CBS 523.90 [a *]		44.0		
		43.6 ± 0.4		
Category 2				
Sch. chiloense				
CBS 8187 [T *]	34.6	40.6	6.0	
	34.6 ± 0.0	**40.6 ± 0.2**	**6.0 ± 0.0**	
D. magnusii				
CBS 107.12 [a *]	32.7	40.3	7.6	
CBS 151.30 [a *]	33.8	41.6	7.8	
CBS 234.85 [a *]	31.4	38.8	7.4	
	32.6 ± 1.7	**40.0 ± 1.3**	**7.6 ± 0.6**	
D. tetrasperma				
CBS 765.70[T *]	35.4	43.4	8.0	
	35.4 ± 0.9	**43.4 ± 1.2**	**8.1 ± 0.4**	
D. ovetensis A				
CBS 750.85 [a *]	39.9	48.0	8.1	
CBS 751.85 [a *]	39.1	48.5	9.4	
CBS 752.85 [a *]	40.0	50.0	10.0	
	39.8 ± 0.9	**48.7 ± 1.2**	**9.0 ± 1.1**	



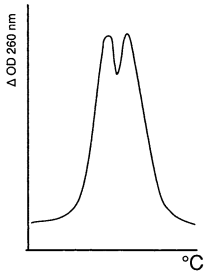
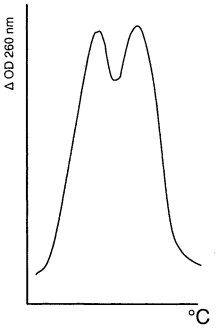
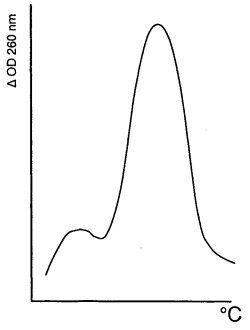


Table 3 (*Continued*).

Strain	Value(s)[b]		Difference[c]	Diagram of first derivative graph of the melting curve of nDNA
Category 3				
D. spicifer				
CBS 244.85 [T]*	29.2	38.3	9.1	
	29.2 ± 0.4	**38.3 ± 0.2**	**9.2 ± 0.4**	
D. capitatus				
CBS 162.80 [Ta]*	26.9	38.7	11.8	
CBS 571.82 [a]	30.0	39.3	9.2	
CBS 573.82 [a]	28.7	37.9	9.2	
CBS 207.83 [a]	30.0	39.4	9.4	
	28.9 ± 1.3	**38.8 ± 0.6**	**9.9 ± 1.1**	
D. ingens s.s.				
CBS 517.90 [Ta]*	25.9	39.7	13.8	
CBS 524.90 [a]*	25.9	37.4	11.5	
	25.9 ± 1.3	**38.5 ± 1.5**	**12.6 ± 1.3**	
D. starmeri				
CBS 780.96 [Ta]*	27.9	42.5	14.6	
CBS 781.96 [Ta]*	27.6	42.3	14.7	
	27.8 ± 0.9	**42.4 ± 0.9**	**14.7 ± 0.1**	
D. albidus				
CBS 766.85 [T]*	20.4	37.4	17.0	
	20.4 ± 0.0	**37.4 ± 1.5**	**17.0 ± 1.5**	
Category 4				
D. aggregatus s.s.				
CBS 175.53 [Ta]	22.1	44.7	22.6	
CBS 152.57 [a]*	22.1	46.0	23.9	
CBS 764.85 [a]*	22.0	47.2	25.2	
CBS 284.86 [a]*	22.2	45.2	23.0	
	22.1 ± 0.1	**45.8 ± 0.9**	**23.7 ± 1.0**	
D. armillariae				
CBS 817.71 [Ta]	13.5	41.7	26.2	
CBS 818.71 [a]*	15.7	36.5	20.8	
CBS 834.71	14.3	41.6	27.3	
CBS 624.82 [a]	15.4	43.6	28.2	
CBS 600.83	14.2	42.1	27.9	
	14.6 ± 0.8	**41.1 ± 2.4**	**26.1 ± 2.7**	
D. geniculatus				
CBS 184.80 [T]*	25,2	42,2	16,3	
	25,2 ± 0.0	**42.2 ± 1.0**	**16,3 ±0,1**	
D. macrosporus				
CBS 259.82 [Ta]*	20.5	42.8	22.3	
CBS 260.82 [a]	21.4	42.7	21.3	
	21.0 ± 0.5	**42.8 ± 0.1**	**21.8 ± 0.4**	
D. ovetensis s.s.				
CBS 192.55 [Ta]	21.5	47.5	26.0	
CBS 634.85 [a]	19.0	46.9	27.9	
CBS 635.85 [a]	20.9	48.8	27.9	
CBS 749.85 [Ta]*	19.4	47.5	28.1	
CCY 30-2-6	22.0	49.1	27.1	
	20.4 ± 1.4	**47.8 ± 1.6**	**27.4 ± 1.4**	





CBS = Centraalbureau voor Schimmelcultures; CCY = Czechoslovak Collection of Yeasts, Institute of Chemistry, Slovakian Academy of Science, Bratislava, Slovak Republic; UOFS = Department of Microbiology and Biochemistry, University of Orange Free State, Bloemfontein, South Africa.

[a]Strains used in infraspecific reassociations.

[b]Values and standard deviations of species in bold; standard deviations of individual strains were ≤ 1.5 mol% ($n = 2$–4).

[c]Differences between the peaks in the derivative of the DNA T_m profiles.

*Strains used in interspecific reassociations.

CBS625.85 is referred to as *D. aggregatus* B. Both isolates were formerly identified as *D. aggregatus*.

Category 2. Strains showing T_m derivative graphs with two equal peaks 6–9 mol% G+C apart

The eight Category 2 strains comprised four distinct groups. One group of three strains is identified as *D. magnusii*, since it included the type strain of this species. It exhibited DNA homology values of 86–100%. Another

group, *D. ovetensis* A, consists of three strains with 93–100% DNA similarity. These three strains were formerly identified as *D. ovetensis*. There were also two genetically distinct single strains, CBS765.70, the type strain of *D. tetrasperma*, and CBS8187, the type strain of *S. chiloense*.

Category 3. Strains showing T_m derivative graphs with two equal peaks 9–17 mol% G+C apart

The 10 strains in Category 3 could be divided into five groups. Two strains, the type strain of *D. ingens* included, exhibited 97–99% DNA homology. The type and isotype strains of *D. starmeri* showed 95–100% DNA similarity. Four *D. capitatus* strains, including the type, showed 91–100% DNA similarity. There were also two genetically distinct single strains, the type of *D. spicifer*, CBS244.85, and the type of *D. albidus*, CBS766.85.

Category 4. Strains showing T_m derivative graphs with two unequal peaks

Thirteen Category 4 strains could be divided into five groups. Four *D. aggregatus* s.s. strains, including the type, exhibited 93–100% DNA homology. Three *D. armillariae* strains, the type included, gave DNA similarity values of 82–91%. Two *D. macrosporus* strains, including the type, had 98% mutual DNA similarity. The type strains of *D. ovetensis* and *D. ambrosiae* along with two other strains grouped together with similarity values of 94–100%. This species is referred to as *D. ovetensis* s.s., because this name has priority over *D. ambrosiae*. The remaining strain, CBS184.80, is the type of *D. geniculatus*.

3.4. Interspecific DNA relatedness

In Fig. 1 DNA similarity values of some interspecific reassociations are presented. The strains used for interspecific reassociations are marked with an asterisk in Table 3. Reassociations were performed among taxa of the same DNA chemistry belonging to the same phylogenetic group [13,16].

Phylogenetic group 1: This group included four members of Category 4, *D. armillariae*, *D. macrosporus*, *D. geniculatus* and *D. aggregatus* s.s., showing mutual DNA similarities ranging from 20 to 34%, and two single taxa, *D. australiensis* from Category 1 and *D. albidus* from Category 3.

Phylogenetic group 2: This group included three members of Category 2, *D. magnusii*, *S. chiloense* and *D. tetrasperma*, showing mutual DNA similarities ranging from 7 to 14%; four members of Category 3, *D. spicifer*, *D. capitatus*, *D. starmeri* and *D. ingens* s.s., showed mutual DNA similarities of 19–26%, and two single taxa, *D. ingens* A (Category 1) and *D. ovetensis* s.s. (Category 4).

Three taxa remained of which to date the phylogenetic relatedness has not been determined. One taxon, *D. ovetensis* A, showed a Category 2 derivative graph, and two taxa, *D. aggregatus* A and *D. aggregatus* B, showed Category 1 derivative graphs, sharing 26% of their total nDNA. Homology values of the last named pair of taxa with *D. ingens* A, which shares the same DNA chemistry, were 16% and 18%, respectively. These segregates of *D. aggregatus* s.l. demonstrated similarity values of 34% and 45%, respectively, with *D. aggregatus* s.s. Similarly, *D. ovetensis* A, previously identified as *D. ovetensis*, showed 11% similarity with the type of *D. ovetensis*.

4. Discussion

In the revision by De Hoog et al. [8] of *Geotrichum*, *Galactomyces* and *Dipodascus*, morphology, physiology, mating, mol% G+C and genome comparisons have been combined, and on this basis two interesting observations were made. Firstly, the heterogeneous character of the nDNA, seen as the appearance of a shoulder in the DNA thermal denaturation graph or as a second peak in the derivative graph of the melting curve, was noted. Secondly, the conflict between species concepts based on mating results and those based on whole-genome comparisons became apparent. These observations have triggered a re-examination of this complex beginning with *Galactomyces* [9]. On the basis of genome comparisons six groups have been recognized, four of which showed mutual DNA homology ranging from 40 to 60%. The remaining mutual DNA similarities were $<27\%$. This grouping was supported by later studies [10,24]. From analyses of restriction patterns of mtDNA (cytoplasmic origin) and RAPD patterns (nuclear origin) of natural isolates as well as of the offspring of laboratory crosses, it has been concluded that hybridization among the closely related groups does not occur in nature, though mating in the laboratory is possible [24]. By PCR fingerprinting, multilocus enzyme electrophoresis and molecular karyotyping, the same six groups were recognized [10]. Naumov et al. [11] have discussed the molecular relatedness and interfertility genetics of *Galactomyces* species with special attention directed to the four groups sharing 40–60% DNA similarity. These authors have compared a similar situation in the well-studied genus *Saccharomyces*, where hybridization occurs between *S. cerevisiae* and *S. paradoxus*, species having 51–58% of their total DNA in common [25]. Normal recombination is impossible between these species in that the hybrids are invariably sterile [26]. Similarly, in the *Hansenula polymorpha* complex, Naumov et al. [27] have reported the presence of sibling species pairs in which interfertility did occur, but hybrids showed low ascospore viability and an irregular meiotic segregation in the F2 generation. In these examples the DNA relatedness was around 65%. In view of these observations, it has been suggested that the four *Galactomyces* groups with 40–60% DNA communality could be considered separate species [11]. Kurtzman [28], discussing the interpretation of DNA relatedness in ascomycetous and basidiomycetous yeasts, has suggested that strains showing intermediate

DNA homology values (40–70%) should be considered varieties unless genetic crosses demonstrate a genetic barrier preventing interfertility. In most comparisons of mating reactions and DNA homologies in yeasts, mating was established only by observing the occurrence of conjugation [29–33]. In cases where production of ascospores or basidiospores was examined, only the viability of these spores was recorded [23,30,34]. No determination was made of their fertility or of recombinants in the F2 generation. From the genetic studies to date, one may conclude that species sharing intermediate genome similarity are distinct. The presence of mating reactions among species indicates that they have common mating type systems [35,36]. The assignment of the species to the same genus is justified, but the finding does not automatically mean that they are conspecific.

The presence of DNA heterogeneity noted by De Hoog et al. [8] has been reported in various other groups of fungi and yeasts [37–44] as well as in bacteria [45]. Explanations of this phenomenon vary. Some authors have attributed the presence of heterogeneous material to rDNA [46], mtDNA [37,39,47,48], or satellite DNA [44,45]. By using CsCl ultracentrifugation to collect the nDNA fraction free from mtDNA and by applying restriction analysis, the possibility of repetitive DNA (ribosomal DNA) was eliminated (unpublished data, cf. [9]). The nuclear DNA obtained by these methods still showed two peaks of equal height in the derivative graph of the melting curves.

The anamorph *Geotrichum* [12] and the teleomorph *Dipodascus* (present revision) have been re-examined by genome comparisons as well as by physiology and morphology. It has been found that *Geotrichum* showed less DNA heterogeneity than *Galactomyces* [9] and *Dipodascus* by having only two types of derivative graphs instead of four. By genome comparisons six *Geotrichum* species were recognized, of which only *Geo. gigas* and *Geotrichum* species A (=CBS164.32) share as much as 50% of their DNA. The remaining mutual similarities were $<30\%$.

In the present study, *Dipodascus* could be subdivided into 18 different taxa of which 13 are validly described *Dipodascus* species. Two strains previously identified as *D. aggregatus* differed from this species by their DNA chemistry as well as by low DNA similarity values. In showing a mutual homology value of 26%, they can be considered two separate taxa. One strain, CBS521.90, previously identified as *D. ingens*, differed from this species by its DNA chemistry and by a low DNA homology of 22%. This strain as well as the type strain of *D. ingens* were included in the phylogenetic study of Kurtzman and Robnett [13]. They differed in three nucleotides in the D1/D2 domain and were placed in the same phylogenetic clade. Another group of three isolates formerly determined as *D. ovetensis* was different from this species in its DNA chemistry and in its low homology value of 11%. Therefore, it represents a taxon distinct from *D. ovetensis* s.s. Since the sexual life cycle was not observed in any of the above-mentioned novel taxa or in *S. chiloense*, these species should be assigned to the anamorph genus *Geotrichum*. The redisposition of *S. chiloense* has been suggested before by Kurtzman and Robnett [13]. However, before novel taxa of *Dipodascus* and *Geotrichum* are introduced, the phylogenetic relatedness of the species involved will have to be determined.

Identification of most taxa of the revised genera is possible with physiological characteristics. There are some exceptions. *Galactomyces geotrichum* cluster B and *Gal. geotrichum* cluster C cannot be distinguished, nor can some strains of *Geotrichum fragrans* and *Geo. gigas*, or some strains of *Geo. fragrans* and *Geotrichum* species A (=CBS164.32). For the present, until a molecular identification system has been developed, a phenotypic key is presented which allows the distinction of *Geotrichum*, *Galactomyces* and *Dipodascus* taxa recognized by the present authors on basis of genome comparisons.

1.	a. Growth on D-xylose	+	2
	b. Growth on D-xylose	−	17
2.	a. Growth at 40°C	+	3
	b. Growth at 40°C	−	4
3.	a. Growth on cellobiose, salicin and arbutin	+	*D. spicifer*
	b. Growth on cellobiose, salicin and arbutin	−	*D. australiensis*
4.	a. Growth on maltose and soluble starch	+	*D. geniculatus*
	b. Growth on maltose and soluble starch	−	5
5.	a. Growth on L-sorbose	+	6
	b. Growth on L-sorbose	−	*D. armillariae (Geo. decipiens)*
6.	a. Growth on cellobiose	+	7
	b. Growth on cellobiose	−	8
7.	a. Growth at 35°C	+	*Geo. fermentans*
	b. Growth at 35°C	−	*D. macrosporus*
8.	a. Expansion growth >15 mm/7 days	9	
	b. Expansion growth <15 mm/7 days		13
9.	a. Growth on mannitol	+	10
	b. Growth on mannitol	−	12
10.	a. Growth without vitamins	+	11
	b. Growth without vitamins	−	*Gal. citri-aurantii (Geo. citri-aurantii)*

11.	a. Growth at 35°C	+	*Gal. geotrichum A*
	b. Growth at 35°C	−	*Gal. geotrichum* s.s. (*Geo. candidum*)
12.	a. Growth without vitamins	+	*Gal. geotrichum* B or *Gal. geotrichum* C
	b. Growth without vitamins	−	*Gal. reessii*
13.	a. Growth without vitamins	+	*Geo. klebahnii*
	b. Growth without vitamins	−	14
14.	a. Growth at 30°C	+	*D. aggregatus* s.s.
	b. Growth at 30°C	−	15
15.	a. Growth on ribitol	+	16
	b. Growth on ribitol	−	*D. albidus*
16.	a. Growth at 35°C	+	*D. aggregatus* B (=CBS625.85)
	b. Growth at 35°C	−	*D. aggregatus* A (=CBS765.85)
17.	a. Growth at 40°C	+	18
	b. Growth at 40°C	−	21
18.	a. Growth on cellobiose, salicin and arbutin	+	*Geo. clavatum*
	b. Growth on cellobiose, salicin and arbutin	−	19
19.	a. Growth on D-sorbitol and glucono-δ-lactone	+	*D. tetrasperma*
	b. Growth on D-sorbitol and glucono-δ-lactone	−	20
20.	a. Expansion growth >5 mm/7 days		*D. capitatus (Geo. capitatum)*
	b. Expansion growth <5 mm/7 days		*D. starmeri*
21.	a. Growth at 37°C	+	22
	b. Growth at 37°C	−	23
22.	a. Growth on L-sorbose	+	*D. ingens A*
	b. Growth on L-sorbose	−	*D. ingens* s.s.
23.	a. Growth at 30°C	+	24
	b. Growth at 30°C	−	*Schizoblastosporion chiloense*
24.	a. Growth on sucrose and raffinose	+	*D. magnusii (Geo. ludwigii)*
	b. Growth on sucrose and raffinose	−	25
25.	a. Growth on D-glucitol and D-mannitol	+	26
	b. Growth on D-glucitol and D-mannitol	−	29
26.	a. Growth on glucono-δ-lactone	+	27
	b. Growth on glucono-δ-lactone	−	*Geo. fragrans* or *Geotrichum* sp. A (=CBS164.32)
27.	a. Expansion growth <7 mm/7days		*D. ovetensis* s.s.
	b. Expansion growth >7 mm/7 days		28
28.	a. Growth at 35°C	+	*Geo. fragrans* or *Geo. gigas*
	b. Growth at 35°C	−	*Geotrichum* sp. B (=CBS100158)
29.	a. Growth at 35°C	+	*Geo. fragrans*
	b. Growth at 35°C	−	30
30.	a. Growth without vitamins	+	*D. ovetensis* A
	b. Growth without vitamins	−	*D. ovetensis* s.s. (*Geo. sericeum*)

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