Comparative Taxonomy of the Genus *Torulopsis* Berlese

By ANNA KOCKOVÁ-KRATOCHVÍLOVÁ

Institute of Chemistry of the Slovak Academy of Sciences, Bratislava, CSSR

(Received 13 March 1973)

SUMMARY

A set of 121 strains of yeasts, originally described as *Torulopsis*, was studied by means of 63 morphological, physiological and serological characters and was subjected to numerical analysis. The matching coefficient of Sokal and Michener and the average linkage cluster analysis allowed the grouping of strains into nine phenons, formed on the 80 % similarity level: *stellata*, *dattila*, *colliculosa*, *molis-chiana*, *pinus-schatavii-glabrata*, *versatilis*, *gropengiesseri*, *wickerhamii-kruisii-candida* and *holmii*. Resulting data agreed with published results of GC content in DNA.

INTRODUCTION

The genus *Torulopsis* Berlese comprises yeast-like micro-organisms with globose, ovoid to elongate cells, which reproduce by multipolar budding. Pseudomycelium is absent or rudimentary. They form neither ascospores, teliospores, ballistospores nor arthrospores. Strains are white or cream-coloured; no carotenoid pigments are present. Extracellular polysaccharides do not give the iodine–starch reaction. Inositol is not utilized as a sole carbon source. *Torulopsis colliculosa* (Hartmann) Saccardo was designated as a type species of this genus. In the taxonomic study of Lodder (1970), 36 species of this genus were described, differing in the ability to ferment and assimilate mono- and oligo-saccharides.

Recently it has been suggested that these organisms can be haploid forms of heterothallic ascosporogenous yeasts. They spend their vegetative state in the haploid phase and after isolation from their natural habitat only one sexual type becomes selected. These forms can neither diploidize nor sporulate because of the absence of the opposite mating type required for conjugation. Our attempts to mate pure cultures of *Torulopsis* species, preserved in collection, were unsuccessful. Wickerham (1965) suggested mating naturally occurring and freshly isolated strains. It also often happens that homothallic haploid cultures enter collections as *Torulopsis* species, but then diploidize and sporulate, or perhaps eventually produce pseudomycelium.

This imperfect genus is thus very heterogeneous and will be dropped when perfect forms of all its species have been discovered. These perfect forms can be predicted by the similarity of phenotype or the DNA characteristics, but the actual recognition of sexual processes can be the only irrefutable evidence. Since the recognition of perfect forms of all known *Torulopsis* species is a very tedious and complicated task, much effort has been devoted to grouping the species on the basis of their phenotype (Spencer, Gorin & Tulloch, 1970; Nakase & Komagata, 1971; Ondrušová, 1971; Campbell, 1971; etc.).

This paper presents the results of the numerical re-evaluation of 121 strains of 46 different species on the basis of 63 characters, and a comparison with other kinds of taxonomic investigations.

METHODS

Strains. The origin of strains used is given in Results. The majority were isolated from the surface of fruit bodies of various agarics, plants and other natural sources (Kocková-Kratochvílová, Petrovová, Šandula & Hronská, 1964; Kocková-Kratochvílová, Šmarda & Pokorná, 1965; Kocková-Kratochvílová & Ondrušová, 1971). Other strains were received from collections, predominantly from CBS,* as type cultures. The precise data of isolation are given in the *Catalogue of Czechoslovak Yeast Collections*, UJEP Brno, 1968.

Character evaluations. The 63 characters evaluated for each of 121 strains are mostly given in Table 1. The structure of the characters can be inferred from this Table. We used morphological, physiological, genetical and immunological methods as described in our previous paper (Kocková-Kratochvílová, Šandula, Vojtková-Lepšíková, Sedlářová & Kasmanová, 1969). Rabbit sera prepared against five different species were kindly supplied by Dr J. Šandula.

Coding of features for computation. Some features exist in two mutually exclusive (alternative, bimodal) states and were scored for computation as 1 or 0, e.g. the assimilation or fermentation of sugars; other qualitative features were divided into more than two mutually exclusive (discontinuous) states, e.g. character of giant colonies and growth in liquid media. Multistate quantitative characters were divided into states according to the frequency distribution curves, e.g. cell sizes, radial growth rate and inhibition. The non-additive code in the 0 and 1 scale was used for more than two state characters.

Computation. The similarity between strains was computed by the 'matching' coefficient method of Sokal & Michener (1958) in which both positive and negative matches were assessed as similarity. This coefficient, S_{SM} , was calculated from the formulae:

$$S_{SM} = 1 - d_{jk}^2$$

and

$$d_{jk}^2 = \frac{\sum_{i=1}^n (x_j - x_k)^2}{n}$$

(which are valid for the 0-1 scale being used) where x_j and x_k are matches for strains j and k , n is the number of matches taken into the calculation, and d_{jk} is the taxonomic distance between strains j and k . The dendrogram was prepared by the method of unweighted average linkage (Sokal & Sneath, 1963). The homogeneity of clusters was checked, using the χ^2 -test. Single phenons were characterized according to the percentage frequency of positive characters defined at the 80 % similarity level (Goodfellow, 1971).

The calculations were programmed in Algol and carried out on the Gier III computer at the Institute of Technical Cybernetics of the Slovak Academy of Sciences, Bratislava.

Guanine and cytosine (GC) content in DNA. We compared our results with the characteristics of DNA as found by Nakase & Komagata (1971). Dr T. Nakase kindly determined the GC content in DNA of twenty of our strains of species which were not investigated by Nakase & Komagata.

* Abbreviations used for sources of cultures are: BUČSAV, Biological Institute of the Czechoslovak Academy of Sciences, Prague; CBS, Centraalbureau voor Schimmelcultures, Delft; CCY, Czechoslovak Collection of Yeasts, Bratislava; IFO, Institute of Fermentation, Osaka; LVHCH, Laboratory for Vitamin and Hormone Chemistry, Prague.

RESULTS

Formation of phenons

We investigated 121 strains of the genus *Torulopsis* and classified them into nine phenons at the 80 % similarity level. Fig. 1 shows the scheme of this clustering. Phenon no. 2, *dattila*, was represented by three strains, phenon no. 4, *molischiana*, by only two strains and the first subphenon of no. 5, *pinus*, by three strains. These three minor phenons were not included in Table 1.

The new phenetic re-evaluation of some strains was not in agreement with the standard description of the genus *Torulopsis* mentioned in the introduction. Some of them produced ascospores or pseudomycelium (e.g. the group C), or chlamydospores (e.g. *Torulopsis burgeffiana*). The perfect forms were completely excluded; others were included in the clustering, but their relations to other genera were designated as A, B, C and D in Fig. 1. All classified strains were similar on the common level 0.758. The phenons were named after the most frequently included or centroidal strains.

*Characteristics of phenons*1. *Phenon stellata*

The strains assigned to the phenon *stellata* were: CCY 26-13-1, *Torulopsis bacillaris* (CBS, 1947); CCY 26-13-2, *T. bacillaris* (CBS 843, 1964); CCY 26-10-3, *T. stellata* var. *cambresieri* (South Slovakia, 1958); CCY 26-10-6, *T. stellata* var. *cambresieri* (CBS 158, 1964); CCY 26-19-2, *T. famata* (BU ČSAV 623, 1958); and CCY 26-11-1, *T. kefyi* (LVHCH, Prague, 1943).

Cells were elongate with a surface/volume (S/V) quotient typical for haploid genotypes. They formed a sediment in liquid media. Giant colonies were smooth to semi-dull and their growth rate was high. Growth temperature range was 5 to 42 °C. Some strains required external vitamins for growth. Sedimentation and autolysis were slow. Strains were mostly resistant to 0.1 µg actidione/disc and 2 % (w/v) lactic acid, but strongly inhibited by 2 % (w/v) tartaric acid. The TTC test was positive in the presence of succinate. Catalase activity was high. Strains exhibited different agglutination with the antisera used. Glucose and sucrose were fermented. Assimilation of KNO₃ was negative.

2. *Phenon dattila*

The strains assigned to the phenon *dattila* were: CCY 26-6-1, *Torulopsis dattila* (CBS, 1947); CCY 26-6-2, *T. dattila* (CBS 137, 1964); and CCY 26-5-1, *T. rosei* (CBS, 1947).

Cells were globose, ovoid to elongate; S/V quotients were typical for polyploid cells. Only sediment was formed in liquid media. Giant colonies were smooth, glistening or semi-dull; their growth rate was moderate. Strains grew well at 5 °C, but not at 42 °C. They tolerated 1 to 12 % (w/v) ethanol in the medium. Osmophily was high. Sedimentation and autolysis were slow. Strains needed external vitamins for growth. Growth was not inhibited by 0.1 µg actidione/disc, 2 % (w/v) lactic acid and 2 % (w/v) tartaric acid. The TTC test was positive in the presence of succinate, lactate and acetate. Catalase activity was moderate. Arbutin was split. All strains agglutinated with serum prepared against *Torulopsis globosa*. Glucose and sucrose were fermented; KNO₃ was not assimilated.

3. *Phenon colliculosa*

The strains assigned to the phenon *colliculosa* were: CCY 26-7-1, *Torulopsis colliculosa* (CBS, 1947); CCY 26-7-3, *T. colliculosa* (Evonymus, South Slovakia, 1957); CCY 26-7-4,

Fig. 1. Dendrogram of strains of the genus *Torulopsis*.

Table 1. Percentage frequency of positive characters found in major phenons defined at the 80 % similarity level

Character	Phenons											
	1	3	5			6	7	8			9	
			Subphenons:					Subphenons:				
			(ii)	(iii)				(i)	(ii)	(iii)		
<i>Stellata</i>	<i>Colliculosa</i>	<i>Schatavii</i>	<i>Glabrata</i>	<i>Versatilis</i>	<i>Gropen- giesseri</i>	<i>Wickerhamii</i>	<i>Kruisii</i>	<i>Candida</i>	<i>Holmii</i>			
Cell morphology												
Cell lengths												
<3.9 μ m	0	12	0	33	12	25	16	30	0	0		
3.9-6.1 μ m	100	88	100	67	62	75	84	70	87	25		
>6.1 μ m	0	0	0	0	26	0	0	0	13	75		
Cell widths												
<3.0 μ m	0	0	0	11	34	12	12	16	0	0		
3.0-4.7 μ m	100	84	100	89	56	88	88	84	100	75		
>4.7 μ m	0	16	0	0	10	0	0	0	0	25		
Length:width												
<1.20	16	84	0	33	37	37	83	8	12	25		
1.20-1.40	68	16	93	66	37	37	17	69	76	25		
>1.40	16	0	7	11	26	26	0	23	12	50		
Surface/volume												
<1.0	0	16	0	0	25	0	0	0	0	0		
1.0-1.6	50	34	82	67	50	37	33	15	75	75		
>1.6	50	50	18	33	25	63	67	85	25	25		
Correlation coefficient												
<0.500	32	0	14	11	50	25	0	23	0	0		
0.500-0.750	52	50	72	55	50	50	16	47	37	50		
>0.750	16	50	14	43	0	25	84	30	63	50		
Culture morphology												
Pseudomycelium												
Absent	100	100	92	100	100	87	100	61	87	75		
Rudimentary	0	0	8	0	0	13	0	39	13	25		
Growth in liquid media												
Sediment only	33	33	0	0	37	11	16	15	0	75		
Islets on the surface	66	66	100	100	63	89	84	85	100	25		

Table 1 (cont.)

Character	Phenons									
	1	3			5			7		
		<i>Stellata</i>	<i>Colliculosa</i>	Subphenons:		<i>Versatilis</i>	<i>Gropen-giesseri</i>	Subphenons:		
				(ii) <i>Schatavii</i>	(iii) <i>Glabrata</i>			(i) <i>Wickerhamii</i>	(ii) <i>Krüsii</i>	(iii) <i>Candida</i>
Giant colonies										
Smooth	50	84		7	11	62	75	84	15	76
Semi-rough	50	16		93	89	38	25	16	85	12
Rough	0	0		0	0	0	0	0	0	12
Radial growth rate (mm/24 h)										
< 0.93	16	16		0	0	50	50	0	8	12
0.94–1.53	52	84		93	100	38	50	84	62	63
> 1.53	32	0		7	0	12	0	16	30	25
Growth at the temperature										
5 °C	83	100		100	100	37	50	100	100	100
42 °C	66	0		92	90	37	0	0	30	12
Chlamydo-spores present	0	0		0	0	0	0	0	0	12
Maximal level of tolerance to ethanol										
1–4 %	33	17		23	50	0	0	17	69	50
8 %	33	50		23	24	24	50	17	31	17
12 %	17	33		20	12	12	25	16	0	33
16 %	17	0		32	15	64	25	50	0	0
Osmophily										
Growth < 70 %	50	0		30	56	25	12	34	0	25
Growth 70–100 %	50	100		70	44	75	88	66	100	75
Vitamin requirement	33	0		38	100	75	100	100	25	25
Sedimentation in 30 min										
< 30 %	100	100		100	75	88	38	100	83	100
> 30 %	0	0		0	25	12	62	0	17	0

Table I (cont.)

Character	Phenons									
	1	3			5			7		
		<i>Stellata</i>	<i>Colliculosa</i>	Subphenons:		<i>Schatavii</i>	<i>Glabrata</i>	<i>Versatilis</i>	<i>Gropen- giesseri</i>	Subphenons:
				(ii)	(iii)					(i) (ii) (iii)
				<i>Schlatavii</i>	<i>Glabrata</i>			<i>Wickerhamii</i>	<i>Kruisii</i>	<i>Candida</i>
Autolysis rate (mg amino-nitrogen evolved from 100 mg dry wt)										
< 5	100	100	100	100	100	100	100	100	100	100
> 5	0	0	0	0	0	0	0	0	0	0
Assimilation of:										
Galactose	33	66	66	84	0	62	62	100	100	100
Sucrose	66	100	0	0	25	100	100	83	100	100
Maltose	66	100	15	28	62	37	37	100	100	25
Lactose	0	0	0	0	0	25	25	0	0	37
Melibiose	0	0	0	0	0	0	0	0	0	50
Trehalose	0	33	100	100	12	12	12	66	76	100
Cellobiose	0	0	0	0	0	0	0	100	100	25
Melzitose	66	33	7	0	0	25	25	0	8	25
Raffinose, 1/3	66	100	0	0	37	87	87	0	0	100
Raffinose, 3/3	34	0	0	0	12	0	0	16	7	0
Maltotriose	0	83	0	0	62	0	0	0	0	0
Maltotetraose	0	16	0	0	62	0	0	0	0	0
L-Sorbose	16	0	0	0	0	50	50	0	0	25
D-Xylose	0	0	15	0	0	0	0	83	92	87
D-Fucose	33	16	0	0	0	0	0	0	0	0
Erythrose	0	66	0	0	0	0	0	0	0	0
Inulin	0	0	0	0	0	12	12	0	0	0
D-Mannitol	16	66	93	0	12	62	62	100	100	25
Galactitol	0	0	0	0	0	16	16	16	0	0
Ribitol	33	50	39	42	12	37	37	16	61	12
D-Arabitol	83	16	0	0	12	25	25	0	31	0
Assimilation of:										
Ethanol	75	58	100	100	80	12	12	88	80	35
Ethylene-glycol	25	33	0	0	12	0	0	0	50	0
Glycerol	100	75	100	77	37	100	100	50	80	50
KNO ₃	0	0	0	0	75	12	12	16	0	0
Lysine	33	100	100	100	25	62	62	100	100	0

Table I (cont.)

Character	Phenons											
	1	3			5		6	7	8			9
		<i>Stellata</i>	<i>Colliculosa</i>	(ii) <i>Schatavii</i>	(iii) <i>Glabrata</i>	<i>Versatilis</i>			<i>Gropen- giesseri</i>	(i) <i>Wickerhamii</i>	(ii) <i>Kruisii</i>	
Fermentation of:												
Glucose	83	100	100	15	100	90	100	100	100	87	100	
Maltose	0	100	0	0	0	66	16	90	100	0	0	
Sucrose	83	100	0	0	0	83	75	16	0	0	25	
Galactose	0	0	0	0	0	44	16	80	100	62	100	
The influence of substances:												
0.1 μ g Actidione/disc												
Inhibition	34	50	7	11	100	68	50	30	63	0		
No inhibition	66	50	93	89	0	37	50	70	37	100		
2 % (w/v) lactic acid:												
Inhibition > 50 %	0	0	0	33	88	12	66	0	0	0		
< 50 %	0	100	15	11	0	76	34	40	36	0		
No inhibition	100	0	85	56	12	12	0	60	64	100		
2 % (w/v) tartaric acid:												
Inhibition > 50 %	17	100	11	11	52	52	66	0	64	0		
< 50 %	66	0	19	33	0	36	34	24	36	0		
No inhibition	17	0	70	56	48	12	0	76	0	100		
TTC test												
In the presence of ethanol:												
abs. < 1 %	33	17	7	11	24	64	0	8	0	25		
1-20 %	67	33	93	89	64	36	83	92	100	75		
20-40 %	0	33	0	0	12	0	17	0	0	0		
> 40 %	0	17	0	0	0	0	0	0	0	0		
In the presence of succinate												
abs. < 1 %	0	0	0	11	12	76	0	24	0	0		
1-20 %	100	100	93	89	88	24	100	76	100	100		
20-40 %	0	0	7	0	0	0	0	0	0	0		

Table 1 (cont.)

Character	Phenons										
	1	3	5			6	7	8			9
			Subphenons:		Versatilis			Gropen- gresseri	Subphenons:		
<i>Stellata</i>	<i>Colliculosa</i>	(ii) <i>Schatavii</i>	(iii) <i>Glabrata</i>	<i>Versatilis</i>		<i>Gropen- gresseri</i>	(i) <i>Wickerhamii</i>		(ii) <i>Kruisii</i>	(iii) <i>Candida</i>	<i>Holmii</i>
In the presence of lactate:											
abs. < 1 %	33	17	7	11	0	63	0	0	16	0	
1-20 %	67	33	46	67	37	37	66	68	68	50	
20-40 %	0	33	46	33	63	0	34	16	16	50	
> 40 %	0	17	0	0	0	0	0	0	0	0	
In the presence of acetate:											
abs. < 1 %	0	33	54	33	88	76	34	68	0	25	
1-20 %	100	33	46	67	12	24	66	32	100	75	
20-40 %	0	33	0	0	0	0	0	0	0	0	
Activity of catalase											
< 10 %	0	0	0	10	0	0	0	0	0	0	
10-40 %	0	0	0	12	0	0	0	0	0	0	
40-75 %	16	100	7	56	63	37	50	86	0	0	
> 75 %	84	0	93	22	37	67	50	14	100	100	
Iodine-starch reaction of extracellular polysaccharides											
No reaction	100	100	100	78	88	100	84	100	88	100	
Red-brown colour	0	0	0	22	12	0	16	0	12	0	
Splitting of arbutin											
Positive	67	0	0	22	50	25	100	100	75	25	
Negative	33	100	100	78	50	75	0	0	25	75	
Test with phosphomolybdic acid											
White	33	50	0	22	75	50	50	100	11	0	
Brown	67	0	100	78	25	50	50	0	89	25	
Dark blue	0	50	0	0	0	0	0	0	0	75	

Table I (cont.)

Character	Phenons										
	1	3	5			6	7	8			9
			Subphenons:					Subphenons:			
	<i>Stellata</i>	<i>Colliculosa</i>	(ii) <i>Schatavii</i>	(iii) <i>Glabrata</i>	<i>Versatilis</i>	<i>Gropen- giesseri</i>	(i) <i>Wickerhamii</i>	(ii) <i>Kruisii</i>	(iii) <i>Candida</i>	<i>Holmii</i>	
Agglutination with rabbit sera:											
AS 27-7-1											
(<i>T. colliculosa</i>)											
No agglutination	33	0	77	89	64	88	17	0	50	50	
D.T. 40-160	67	67	23	11	24	12	66	46	50	50	
320-1280	0	33	0	0	12	0	17	54	0	0	
AS 26-9-9											
(<i>T. candida</i>)											
No agglutination	33	50	31	78	75	63	0	0	63	75	
D.T. 40-160	50	50	62	22	25	37	100	85	25	25	
320-1280	17	0	7	0	0	0	0	15	12	0	
AS 26-12-4											
(<i>T. sphaerica</i>)											
No agglutination	50	50	38	67	75	73	100	72	75	75	
D.T. 40-160	50	50	62	33	25	37	0	21	25	25	
320-1280	0	0	0	0	0	0	0	7	0	0	
AS 26-20-1											
(<i>T. glabrata</i>)											
No agglutination	33	0	0	0	12	37	0	0	64	0	
D.T. 40-160	67	100	100	100	88	63	83	79	36	100	
320-1280	0	0	0	0	0	0	17	21	0	0	
AS 26-37-1											
(<i>T. globosa</i>)											
No agglutination	16	0	38	11	25	75	0	21	37	75	
D.T. 40-160	68	100	62	89	75	25	67	58	37	25	
320-1280	16	0	0	0	0	0	33	21	26	0	

AS, antiserum; D.T., dilution titre.

T. colliculosa (Rosa, South Slovakia, 1958); CCY 26-7-5, *T. colliculosa* (BU ČSAV, 626, 1958); and CCY 26-7-6, *T. colliculosa* (CBS 133, 1964).

Cells were globose to short-oval; S/V quotients were typical for haploid or diploid cells. Sediment was produced in liquid media. Giant colonies were smooth, glistening or semi-dull; the growth rate was moderate. Strains grew well at 5 °C, but not at 42 °C. They tolerated 1 to 12 % (w/v) ethanol in the medium. Osmophily was high. They did not require external vitamins for growth. Sedimentation and autolysis were slow. The growth inhibition by 0.1 µg actidione/disc was variable: 2 % (w/v) lactic acid inhibited the growth strongly, 2 % (w/v) tartaric acid weakly. The TTC test was positive in the presence of succinate. Catalase activity was moderate. The phosphomolybdic acid test gave a dark blue colour. All strains agglutinated with the serum prepared against *Torulopsis colliculosa* and *T. glabrata*. Glucose, sucrose and maltose were fermented; KNO₃ was not assimilated.

4. *Phenon molischiana*

The strains assigned to the phenon *molischiana* were: CCY 26-8-1, *Torulopsis molischiana* (BU ČSAV 628, 1958); and CCY 26-8-2, *T. molischiana* (CBS 136, 1964).

Cells were ovoid, and S/V quotients were typical for haploid cells. Sediment and islets were produced in liquid media. Fast-growing giant colonies were smooth to mucous. The growth temperature range was 5 to 42 °C. Strains tolerated 8 % (w/v) ethanol in the medium. Osmophily was low. They required vitamins for growth. Sedimentation and autolysis were slow. Strains were inhibited by 0.1 µg actidione/disc and weakly inhibited by 2 % (w/v) tartaric acid, but no inhibition was observed in the presence of 2 % (w/v) lactic acid. The TTC test was positive in the presence of succinate. Catalase activity was moderate. Arbutin was split. The strains agglutinated with sera prepared against *Torulopsis candida* and *T. glabrata*. Glucose was fermented; KNO₃ was assimilated.

5. *Phenon pinus-schatavii-glabrata*

The strains assigned to the phenon *pinus-schatavii-glabrata* were: (i) CCY 26-24-1, *Torulopsis pinus* (IFO 0741, 1958); CCY 26-24-2, *T. pinus* (CBS 970, 1964); and CCY 26-31-1, *T. buffoni* (CBS 2838, 1964). (ii) CCY 26-26-1, *T. schatavii* (*Grifola montana*; Dobroč, 1962); CCY 26-26-2, *T. schatavii* (*Russula cyanocantha*; Dobroč, 1962); CCY 26-26-4, *T. schatavii* (*Polyporus varius*; Dobroč, 1962); CCY 26-26-5, *T. schatavii* (*Fomitopsis pinicola*; Dobroč, 1962); CCY 26-26-6, *T. schatavii* (*Grifola montana*; Dobroč, 1962); CCY 26-26-7, *T. schatavii* (*Grifola sulphurea*; Dobroč, 1962); CCA 26-26-8, *T. schatavii* (*Russula integra*; Dobroč, 1962); CCY 26-26-9, *T. schatavii* (*Amylocystis lapponica*; Dobroč, 1962); CCY 26-21-7, *T. schatavii* (originally as *T. burgeffiana*); CCY 26-20-1, *T. glabrata* (*T. tsuchiya*, Japan; no. 4-002, 1962); CCY 26-20-4, *T. glabrata* (*Fomitopsis pinicola*; Dobroč, 1962); CCY 26-20-8, *T. glabrata* (*Lactarius flexuosus*; Kuřim, 1962); and CCY 26-20-13, *T. glabrata* (*Flammula muricata*; Omice-Střelice, 1962). (iii) CCY 26-20-2, *T. glabrata* (*Amylocystis lapponica*; Dobroč, 1962); CCY 26-20-3, *T. glabrata* (*Trametes abietina*; Dobroč, 1962); CCY 26-20-5, *T. glabrata* (*Fomitopsis pinicola*; Dobroč, 1962); CCY 26-20-6, *T. glabrata* (*Phellinus hartigii*; Dobroč, 1962); CCY 26-20-9, *T. glabrata* (*Lactarius flexuosus*; Kuřim, 1962); CCY 26-20-10, *T. glabrata* (*Lactarius turpis*; Kuřim, 1962); and CCY 26-20-11, *T. glabrata* (*Amanita muscaria*; Kuřim, 1962).

Cells were small, globose to oval, and S/V quotients were typical for haploid strains. Sediment and islets were produced in liquid media. Growth temperature range was 5 to 42 °C with the exception of subphenon *pinus*, strains of which did not grow at 42 °C. These strains tolerated various percentages of ethanol in the media: in the subphenon *pinus*, up to

16 % (w/v); in *glabrata*, 4 % (w/v) only. The majority of these strains required vitamins in the media for growth. Sedimentation and autolysis were slow. Neither 0.1 µg actidione/disc, nor 2 % (w/v) lactic and tartaric acid inhibited growth. The TTC test was positive in the presence of ethanol and succinate. Only some strains split arbutin. All strains of the subphenons *glabrata* and *schatavii* agglutinated with the serum prepared against *Torulopsis glabrata*, those of subphenon *pinus* with serum against *T. colliculosa*, *T. sphaerica*, *T. candida* and *T. globosa*. Strains of subphenon *pinus* did not ferment sugars; some strains of *schatavii* fermented glucose weakly, but all strains of *glabrata* fermented glucose. KNO₃ was not assimilated. A detailed description of *T. schatavii* is given in our previous paper (Kocková-Kratochvílová & Ondrušová, 1971).

6. *Phenon versatilis*

The strains assigned to the phenon *versatilis* were: CCY 26-46-1, *Torulopsis osloensis* (CBS 2752, 1964); CCY 26-42-1, *T. miso alfa* (CBS 2032, 1964); CCY 26-50-1, *T. versatilis* (CBS 1752, 1964); CCY 26-33-1, *T. citrus* (CBS 2987, 1964); CCY 26-35-1, *T. ernobii* (CBS 1737, 1964); CCY 26-27-1, *T. anomala* (CBS 1731, 1964); and CCY 26-38-1, *T. halophilus* (CBS 4019, 1964).

Cells were globose, ovoid or elongate; S/V quotients were variable. Sediment or islets were formed in liquid media. Giant colonies were smooth or semi-rough, growing fast. Some strains grew at 5 °C, some at 42 °C, but mostly the temperature range for growth was narrow. Strains tolerated 16 % (w/v) ethanol in the medium; osmophily was high. The majority of strains needed external vitamins for growth. Sedimentation and autolysis were slow. Growth was inhibited by 0.1 µg actidione/disc, and 2 % (w/v) lactic and tartaric acids were weakly inhibitory. The TTC test was usually positive in the presence of succinate and acetate. Catalase activity was moderate. Serological reactions were variable. Fermentation of sugars was also variable; KNO₃ was assimilated by the majority of strains.

7. *Phenon gropengiesseri*

The strains assigned to the phenon *gropengiesseri* were: CCY 26-28-1, *Torulopsis apis* (CBS 2674, 1964); CCY 26-29-1, *T. apicola* (CBS 2868, 1964); CCY 26-44-1, *T. nodaensis* (CBS 3094, 1964); CCY 26-25-1, *T. gropengiesseri* (BUČSAV 0626, 1958); CCY 26-25-2, *T. gropengiesseri* (CBS 156, 1964); CCY 26-10-1, *T. stellata* var. *cambresieri* (South Slovakia, JS241, 1958); CCY 26-10-2, *T. stellata* var. *cambresieri* (South Slovakia, JS240, 1958); and CCY 26-40-1, *T. magnoliae* (CBS 166, 1964).

Cells were globose, ovoid or elongate; S/V quotients were mostly similar to haploid strains. Pseudomycelium was absent or rudimentary. Sediment or islets were formed on the surface of liquid media. Giant colonies were smooth to semi-dull, growing slowly. These strains grew well at 28 °C, half of them grew at 5 °C, but none at 42 °C. They all tolerated 8 % (w/v) ethanol in the medium, and some single strains up to 16 % (w/v). The osmophily was high. They needed vitamins for growth. Sedimentation was fast, autolysis slow. Growth was strongly inhibited by 0.1 µg actidione/disc, and by 2 % (w/v) lactic and tartaric acid. Catalase activity was high. Arbutin was split. Serological reactions were variable. External polysaccharides were characterized as heteropolysaccharides, mostly galactomannans (Spencer, Gorin & Tulloch, 1970). KNO₃ was not assimilated. Glucose and sucrose were fermented, and some strains fermented maltose and galactose.

8. *Phenon wickerhamii-kruisii-candida*

The strains assigned to the phenon *wickerhamii-kruisii-candida* were: (i) CCY 26-51-1, *Torulopsis wickerhamii* (CBS 2928, 1964); CCY 29-9-10, *T. candida* (*Russula veternosa*; Kuřim, 1962); CCY 26-49-1, *T. saccharini* (CBS 4514, 1964); CCY 26-49-2, *T. saccharini* (Santa Maria Az8, 1964); CCY 26-17-1, *T. minor* (CBS, 1947); and CCY 26-35-2, *T. ernobii* (*Boletus satanas*; Kuřim, 1962). (ii) CCY 26-19-3, *T. kruisii* (*Amanita muscaria*; Kuřim, 1962); CCY 26-19-4, *T. kruisii* (*Boletus satanas*; Kuřim, 1962); CCY 26-19-5, *T. kruisii* (*Lactarius vellereus*; Kuřim, 1962); CCY 26-19-6, *T. kruisii* (*L. vellereus*; Kuřim, 1962); CCY 26-19-7, *T. kruisii* (*B. purpureus*; Kuřim, 1962); CCY 26-19-8, *T. kruisii* (*Russula chloroides*; Kuřim, 1962); CCY 26-19-9, *T. kruisii* (*R. lepida*; Kuřim, 1962); CCY 26-19-10, *T. kruisii* (*R. vesca*; Kuřim, 1962); CCY 26-19-11, *T. kruisii* (*R. chloroides*; Kuřim, 1962); CCY 26-19-12, *T. kruisii* (*Paxillus atramentosus*; Kuřim, 1962); CCY 26-19-13, *T. kruisii* (*A. porphyrea*; Kuřim, 1962); CCY 26-19-14, *T. kruisii* (*R. veternosa*; Kuřim, 1962); CCY 26-19-15, *T. kruisii* (*Crepidotus mollis*; Kuřim, 1962); and CCY 26-19-1, *T. famata* (CBS, 1947). (iii) CCY 26-9-1, *T. candida* (acorns; South Slovakia, 1958); CCY 26-9-2, *T. candida* (grapes; South Slovakia, 1958); CCY 26-9-3, *T. candida* (CBS, 1947); CCY 26-9-4, *T. candida* (South Slovakia, 1959); CCY 26-9-5, *T. candida* (South Slovakia, 1959); CCY 26-9-6, *T. candida* (South Slovakia, 1959); CCY 26-9-8, *T. candida* (BUČSAV 621/1, 1958); and CCY 26-9-9, *T. candida* (CBS 940, 1964).

Cells were globose, oval to elongate; S/V quotients were similar to haploid strains, except for the subphenon *candida* where they were similar to diploid cells. Pseudomycelium was absent or rudimentary. Islets were formed on liquid media. Giant colonies were smooth and fast growing, except in the *candida* group where they were semi-dull to rough. These strains grew well at 5 °C and 28 °C but not at 42 °C; some exceptions were again found in the *candida* group. They varied in their tolerance of ethanol in the medium: maximal concentration was 16 % (w/v) in the group *wickerhamii*, 12 % (w/v) in *candida* and 8 % (w/v) in the *kruisii* group. Osmophily was high. Most strains needed vitamins for growth. Sedimentation and autolysis were slow. Some chlamydospores similar to those of *Candida pulcherrima* were found in the *candida* group. The sensitivity to actidione, lactic acid and tartaric acid was variable. The TTC test was positive in the presence of succinate in the subphenon *wickerhamii*, in the presence of ethanol in the *kruisii* group, and of ethanol, succinate and acetate in the *candida* subphenon. Catalase activity was high. Arbutin was usually split. All strains of the subphenon *wickerhamii* agglutinated with sera prepared against *Torulopsis candida* or *T. glabrata*, none reacted with the serum against *T. sphaerica*. All strains of the subphenon *kruisii* agglutinated with sera prepared against *T. colliculosa*, *T. candida* and *T. glabrata*. The agglutination of the group *candida* with all investigated sera was variable. The subphenon *wickerhamii* fermented glucose, some strains fermented galactose, maltose and sucrose, and other subphenons only glucose and galactose. With the exception of some strains in the *wickerhamii* subphenon, KNO₃ was not assimilated. A detailed description of the phenon *kruisii* is given in our previous paper (Kocková-Kratochvílová & Ondrušová, 1971).

9. *Phenon holmii*

The strains assigned to the phenon *holmii* were: CCY 26-16-1, *Torulopsis holmii* (CBS, 1947); CCY 26-16-2, *T. holmii* (BUČSAV 627, 1958); CCY 26-16-3, *T. holmii* (CBS 135, 1964); and CCY 26-15-2, *T. sake* (CBS 159, 1964).

Cells were large and mostly elongate; S/V quotients were typical for diploid strains. Pseudomycelium was absent or rudimentary. The only sediment existed in liquid media.

Giant colonies were semi-dull to rough and growth rate was slow. They grew well at 28 °C but no growth was observed at 5 or 42 °C. These strains tolerated 8 % (w/v) ethanol in the medium; osmophily was high. They required vitamins for growth. Growth was not inhibited by 0.1 µg actidione/disc, or by 2 % (w/v) lactic and tartaric acid; the TTC test was positive in the presence of succinate and lactate. Catalase activity was high. Splitting of arbutin was variable. The phosphomolybdc acid test gave a dark blue colour. All strains were agglutinated with the serum prepared against *Torulopsis glabrata*. They fermented glucose, galactose and sucrose, and did not assimilate KNO₃.

The intermediate and marginal strains

There are five individual strains: CCY 26-3-1, *Torulopsis etchellsii* (CBS 1751, BUČSAV 0624, 1958); CCY 26-22-1, *T. ingensiosa* (di Menna, 1959); CCY 26-26-11, *T. inconspicua* (CBS 180, 1964); CCY 26-43-1, *T. nitratoiphila* (CBS 2027, 1964); and CCY 26-45-1, *T. norvegica* (CBS 4239, 1964).

The positions of these strains in the dendrogram were as follows: *Torulopsis norvegica* between the phenons *schatavii* and *glabrata*; *T. inconspicua* between the phenons *C* and *versatilis*; *T. etchellsii* between the phenons *versatilis* and *gropengiesseri*; *T. nitratoiphila* between *wickerhamii* and *kruisii*; *T. ingensiosa* near *T. pseudoaeria*, which was excluded by transfer to *Cryptococcus*. Two strains of *T. aeria*, CCY 26-23-1 and CCY 26-23-2, were also transferred to the genus *Cryptococcus*. These two strains and *T. ingensiosa* were the only strains showing urease activity.

The excluded strains

These strains, whose description did not agree with the definition of the genus *Torulopsis*, formed four groups, designated A, B, C and D (Fig. 1).

The strains assigned to group A were: CCY 26-6-3, *Torulopsis dattila* var. *rohrbachense* (CBS 2254, 1964); CCY 26-15-1, *T. sake* (BUČSAV 625, 1958); CCY 26-14-1, *T. xylinusa* (BUČSAV 0627, 1958); CCY 26-10-4, *T. stellata* var. *cambresieri* (no. XII, South Slovakia, 1958); CCY 26-18-1, *T. lipofera* (CBS, 1947); CCY 26-1-1, *T. utilis* (LVHCH, 1943); CCY 26-1-2, *T. utilis* (LVHCH, 1943); and CCY 26-1-4, *T. utilis* (LVHCH, 1943). These strains formed rudimentary or well-developed pseudomycelium, and were therefore thought to be *Candida* spp.

The strains assigned to group B were: CCY 26-2-1, *Torulopsis casei* (LVHCH, 1943); CCY 26-2-2, *T. casei* (LVHCH, 1943); CCY 26-2-3, *T. casei* (LVHCH, 1943); CCY 26-12-1, *T. sphaerica* (CBS, 1947); CCY 26-12-2, *T. sphaerica* (BUČSAV 622/2, 1958); CCY 26-12-3, *T. sphaerica* (BUČSAV 622/1, 1958); and CCY 26-12-4, *T. sphaerica* (CBS 141, 1964). These strains were thought to be related to the genus *Kluyveromyces*. Their detailed description is given in our previous paper (Kocková-Kratochvílová, Blagodatskaja & Hronská, 1972).

The strains assigned to group C were: CCY 26-20-7, *Torulopsis glabrata* (*Amanita caesarea*; Kuřim, 1962); CCY 26-20-12, *T. glabrata* (*Flammula muricata*; Omice-Střelice, 1962); CCY 26-20-14, *T. glabrata* (*Flammula muricata*; Omice-Střelice, 1962); CCY 26-20-15, *T. glabrata* (*Lacrymaria velutina*; Omice-Střelice, 1962); CCY 26-20-16, *T. glabrata* (*Hydnum coralloides*; Žďár, 1962); CCY 26-20-17, *T. glabrata* (*Anthrordra mollis*; Žďár, 1962); CCY 26-20-18, *T. glabrata* (*Anthrordra mollis*; Žďár, 1962); CCY 26-20-19, *T. glabrata* (*Anthrordra mollis*; Žďár, 1962); and CCY 26-21-3, *T. burgeffiana* (Benda, Würzburg, 1963). They formed pseudomycelium and were thought to be *Candida* spp.

The strains assigned to the group D were: CCY 26-21-1, *Torulopsis burgeffiana* (Benda; Würzburg, no. b1, 1963); CCY 26-21-2, *T. burgeffiana* (Benda; Würzburg, no. b2, 1963);

CCY 26-21-4, *T. burgeffiana* (Benda; Würzburg, no. b4, 1963); CCY 26-21-5, *T. burgeffiana* (Benda; Würzburg, no. b5, 1963); and CCY 26-21-6, *T. burgeffiana* (Benda; Würzburg, no. b6, 1963). These strains produced chlamydospores similar to those of *Candida pulcherrima*. We transferred these strains to the species *C. pulcherrima* or to its perfect form *Metschnikowia pulcherrima*.

DISCUSSION

The strains used were received from other collections or were isolated from a variety of natural habitats and identified. Some of these strains had been maintained in pure culture in our collection for a long time, e.g. six strains since 1943 and seven since 1947. Consequently, some characters had altered and after the re-evaluation did not agree with the original description or with the standard description given by Lodder (1970). However, the names under which they were described or received originally were retained.

Among the 118 strains included in Fig. 1, 94 produced budding cells, but none formed sexual spores or pseudomycelium; these strains are therefore valid strains of the genus *Torulopsis*. The recent identification of species of this genus is based on differences in fermentation and assimilation abilities of various sugars (Lodder, 1970). We proposed (Kocková-Kratochvílová & Vojtková-Lepšíková, 1961) sorting the yeast strains into four types according to their ability to ferment maltose and sucrose. Table 2 shows how the nine phenons studied are classified into these fermentation types.

We have computed the frequency of occurrence of individual fermentation types and their abilities to assimilate various sugars, for the whole set studied (Table 3). We have done likewise for the set of 36 species accepted by Lodder (1970), and similar results were obtained. We therefore consider that the set of strains chosen for our investigation adequately represents the whole genus *Torulopsis*. According to the frequencies with which the assimilation of sugars occurs in this genus, sugars can be sorted into five groups. (1) Frequency = 75 to 100 %: glucose (100 %), galactose (79.3 %) and mannitol (73.7 %). (2) Frequency = 50 to 75 %: maltose (67.9 %), trehalose (64.5 %) and sucrose (62.8 %). (3) Frequency = 25 to 50 %: xylose (41.5 %), cellobiose (37.7 %) and ribitol (34.3 %). (4) Frequency = 10 to 25 %: melizitose (18.7 %), sorbose (18.2 %) and arabitol (18.0 %). (5) Frequency < 10 %: melibiose (6.5 %) and lactose (5.3 %).

We have compared this sorting with that of fermentation types, taking into consideration the phylogenetical development:

According to both assimilative and fermentative expressions, four different phylogenetic lines could be constructed: (A) Strains not fermenting galactose, i.e. (1) phenons no. 1, 2, 3, 4, and (2) phenon no. 5; and (B) Strains fermenting galactose, i.e. (3) phenon no. 6, 7, and (4) phenons no. 8, 9 (see Table 3).

Table 2. Survey of fermentation types in individual phenons

	1	2	3	4	5	6	7	8	9
					subphenons:			subphenons:	
					(i) Pinus (ii) Schatavii (iii) Glabrata	Versatilis	Gropen- giesseri	(i) Wicker- hamii (ii) Kruisii (iii) Candida	
		Dattila	Colli- culosa	Molis- chiana					Holmii
Saccharide	+	+	+	+	- , ±	+	+	+	+
Glucose	-	-	-	-	-	±	±	+	+
Galactose	+	+	+	-	-	±	+	+	±
Maltose	-	±	+	-	-	+	±	±	-
Phospho- molybdic acid test	-	-	+	-	-	-	-	-	+
Fermenta- tion type	III	II-III	II	IVa	IVb	II	II-III	I	III-IVa
Phylogenic line	I	I	I	I-2	2	3	3	4	4

Table 3. Frequency of natural occurrence of *Torulopsis* strains of various fermentation types and their assimilation

Fermentation type	I Maltose + Sucrose -	II Maltose + Sucrose +	IIIa Maltose - Sucrose + Lactose -	IV Maltose - Sucrose -		Total frequency of occurrence (%)
				(a) Glucose -	(b) Glucose +	
Occurrence in %	29.0	11.4	20.4	18.8	20.4	100
Assimilation in %						
Galactose	100	71	64	76	74	79.3
Maltose	100	100	52	52	35	67.9
Sucrose	54	100	100	44	35	62.8
Lactose	0	0	0	0	26	5.3
Melibiose	0	0	4	12	17	6.5
Trehalose	71	57	28	84	78	64.5
Cellobiose	57	21	24	36	35	37.7
Melzitose	9	35	36	12	13	18.7
Xylose	62	28	36	32	35	41.5
Sorbose	6	7	40	12	26	18.2
Mannitol	85	64	48	76	87	73.7
Ribitol	42	42	40	20	26	34.3
Arabitol	28	14	32	0	9	18.0

Table 4. GC content in DNA of individual phenons

No. of the group	GC content (mol %)	Name and no. of the phenon	Name of individual species
(1)	< 35	<i>Holmii</i> (9)	
(2)	35-9	<i>Wickerhamii-kruisii-candida</i> (8) <i>Pinus-schatavii-glabrata</i> (5)	<i>Torulopsis nitratophila</i> (8) <i>T. norvegica</i> (5)
(3)	39-44	<i>Stellata</i> (1) <i>Dattila</i> (2) <i>Colliculosa</i> (3) <i>Molischiana</i> (4)	
(4)	44-50	<i>Versatilis</i> (6)	<i>T. inconspicua</i> (5-6)
(5)	> 50	<i>Gropengiesseri</i> (7)	<i>T. etchellsii</i> (6-7)

We have compared our results with those of Nakase & Komagata (1971), obtained from analyses of GC content in DNA. Altogether, the 68 strains of the genus *Torulopsis* examined formed a heterogeneous set. Thus the frequency distribution gave a histogram with five peaks, which divided the observed phenons into five groups (see Table 4). These five groups may represent monophyletic groups, which could be very helpful in the search for perfect forms among Ascomycetes and Basidiomycetes. In our previous paper (Kocková-Kratochvílová & Nakase, 1971) we compared species of the genus *Saccharomyces*, the frequency distribution of which was quite normal with a mean mol % of GC 36.75 and a s.d. of 1.96. Numerical classification of the genus *Saccharomyces* revealed two different clusters; the first belongs to the genus *Saccharomyces sensu stricto* and the second to species originally described as *Zygosaccharomyces*. The range of GC contents of *Saccharomyces sensu stricto* was 36.1 to 38.0, while for the second cluster it was 36.6 to 42.0. Thus the range of GC content of the genus *Saccharomyces* slightly overlaps with that of the genus *Torulopsis*, for which it is 32.4 to 60.0. Taking into consideration the fermentation ability of both genera, the possibility of common phylogenetical origin becomes slight. In contrast, a common

origin for *Torulopsis* species and the second cluster, *Zygosaccharomyces*, becomes more likely. The comparison with other perfect genera will be discussed in our future studies.

After we had completed this study some new species were described: *Torulopsis dendrica*, *T. philyla*, *T. silvatica*, *T. insectalens* and *T. nemodendra* by van der Walt, Scott & Van der Klift (1971) and *T. fragaria* by Barnett & Buhagiar (1971), which could be included into the heterogeneous phenon *pinus-schatavii-glabrata*. *T. bombicola*, described by Spencer *et al.* (1970) is similar to the phenon *gropengiesseri*, characterized by the presence of heteropolysaccharides.

In conclusion, it can be emphasized that the grouping of species on the basis of their phenetic similarities can considerably facilitate future search for perfect forms of *Torulopsis* species.

REFERENCES

- BARNETT, J. A. & BUHAGIAR, R. W. M. (1971). *Torulopsis fragaria* species nova, a yeast from fruit. *Journal of General Microbiology* **67**, 233–238.
- CAMPBELL, I. (1971). Numerical taxonomy of various genera of yeasts. *Journal of General Microbiology* **67**, 223–231.
- GOODFELLOW, M. (1971). Numerical taxonomy of some Nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.
- KOCKOVÁ-KRATOCHVÍLOVÁ, A., BLAGODATSKAJA, V. & HRONSKÁ, L. (1972). *The Grouping of the Species within the Genus Kluyveromyces van der Walt. Yeasts, Models in Science and Technics*. Bratislava: Slovak Academy of Science.
- KOCKOVÁ-KRATOCHVÍLOVÁ, A. & NAKASE, T. (1971). Vergleichende Taxonomie der Gattung *Saccharomyces* (Meyen) Reess. *Zeitschrift für allgemeine Mikrobiologie* **11**, 35–38.
- KOCKOVÁ-KRATOCHVÍLOVÁ, A. & ONDRUŠOVÁ, D. (1971). *Torulopsis*arten aus den Oberflächen höherer Pilze. *Biológia* **26**, 477–485.
- KOCKOVÁ-KRATOCHVÍLOVÁ, A., PETROVÁ, T., ŠANDULA, J. & HRONSKÁ, L. (1964). A contribution to the ecology of yeast-like microorganisms. Yeast-like microorganisms from the surface of agarics from the virgin forest in Dobroč. *Česká mykologie* **18**, 91–98 (in Slovak).
- KOCKOVÁ-KRATOCHVÍLOVÁ, A., ŠANDULA, J., VOJTKOVÁ, LEPŠÍKOVÁ, A., SEDLÁROVÁ, L. & KASMANOVÁ, M. (1969). *A taxometric Study of the Genus Saccharomyces (Meyen) Reess. II. Saccharomyces cerevisiae Hansen and Related Species*. Biologické práce. Bratislava: Slovak Academy of Science.
- KOCKOVÁ-KRATOCHVÍLOVÁ, A., ŠMARD, F. & POKORNÁ, M. (1965). A contribution to the ecology of yeast-like microorganisms. Yeast-like microorganisms from the surface of agarics from Bohemian-Moravian highland. *Česká mykologie* **19**, 114–120 (in Slovak).
- KOCKOVÁ-KRATOCHVÍLOVÁ, A. & VOJTKOVÁ-LEPŠÍKOVÁ, A. (1961). Über die Bedeutung von vier Gärungstypen für die Identifizierung der Hefen. *Brauwissenschaft* **14**, 210–215.
- LODDER, J. (1970). *The Yeasts, a Taxonomic Study*. Amsterdam: North-Holland Publishing.
- NAKASE, T. & KOMAGATA, K. (1971). Significance of DNA base composition in the classification of yeast genus *Torulopsis*. *Journal of General and Applied Microbiology* **17**, 161–166.
- ONDRUŠOVÁ, D. (1971). *Numerical taxonomy of the genus Torulopsis*. Thesis, Comenius University, Bratislava (in Slovak).
- SPENCER, J. F. T., GORIN, A. P. J. & TULLOCH, A. P. (1970). *Torulopsis bombicola* species nova. *Antonie van Leeuwenhoek* **36**, 129–133.
- SOKAL, R. R. & MICHENER, C. D. (1958). A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* **38**, 1409–1438.
- SOKAL, R. R. & SNEATH, P. H. A. (1963). *Principles of Numerical Taxonomy*. San Francisco and London: W. H. Freeman.
- VAN DER WALT, J. P., SCOTT, D. B. & VAN DER KLIFT, W. C. (1971). Five new *Torulopsis* species from South African insect sources. *Antonie van Leeuwenhoek* **37**, 461–471.
- WICKERHAM, L. (1965). Opposite sexes as type specimen for heterothallic haploid yeasts. *Taxon* **14**, 187–188.