

Phylogenetic analysis of ascomycete yeasts that form coenzyme Q-9 and the proposal of the new genera *Babjeviella*, *Meyerozyma*, *Millerozyma*, *Priceomyces*, and *Scheffersomyces*

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Abstract Species assigned to the genera *Debaryomyces*, *Lodderomyces*, *Spathaspora*, and *Yamadazyma*, as well as selected species of *Pichia* and *Candida* that also form coenzyme Q-9, were phylogenetically analyzed from the combined sequences of the D1/D2 domains of the large subunit and the nearly complete small subunit rRNA genes. Species assigned to *Debaryomyces* partitioned into three clades and species assigned to *Pichia* were distributed among six clades. These well-supported clades were interpreted as genera, and from this analysis, the following new genera are proposed: *Babjeviella*, *Meyerozyma*, *Millerozyma*, *Priceomyces*, and *Scheffersomyces*. The genus *Schwanniomyces* was reinstated and emended, and the genus *Yamadazyma* was phylogenetically defined. From this study, 23 new combinations and 3 new ranks are proposed. The preceding genera are members of a single, large clade, and it is proposed to delineate this clade as the new family Debaryomycetaceae.

Keywords Ribosomal RNA phylogeny · Yeast classification

Introduction

Phylogenetic analyses of nucleotide sequences from single genes and from multigene datasets have shown that the budding ascomycetous yeasts, including certain arthroconidial taxa such as *Dipodascus* de Lagerheim, are members of a single large clade, the Saccharomycetales (Kurtzman and Robnett 1994, 1998; Fitzpatrick et al. 2006; James et al. 2006). Gene sequence analyses have further revealed that many genera assigned to the Saccharomycetales are not phylogenetically circumscribed, but recent studies have begun to remedy the misclassification. For example, Kurtzman and Robnett (2003, 2007) and Kurtzman et al. (2007) used multigene datasets to phylogenetically circumscribe genera assigned to the families Saccharomycetaceae, Trichomonascaceae, and Lipomycetaceae.

Pichia E.C. Hansen represents a large, polyphyletic genus that has been only partly resolved from sequence analysis. Yamada et al. (1994), from analyses of partial large subunit (LSU) and small subunit (SSU) rRNA sequences, proposed the genus *Ogataea* Y. Yamada, K. Maeda & Mikata for methanol-assimilating yeasts in the *Picea angusta* (Teun., H.H. Hall & Wick.) Kurtzman/*Hansenula polymorpha* Morais & M.H. Maia clade, and the genus *Komagataella* Y. Yamada, M. Matsuda, K. Maeda & Mikata for *P. pastoris* (Guillierm.) Phaff (Yamada et al. 1995a), a methanol-assimilating yeast not closely related to the *Ogataea* clade. Other new genera derived from *Pichia* that were proposed by Yamada and colleagues from sequence analyses included *Kuraishia* Y. Yamada, K. Maeda & Mikata for *P. capsulata* (Wick.) Kurtzman (Yamada et al. 1994), *Nakazawaea* Y. Yamada, K. Maeda & Mikata for *P. holstii* (Wick.) Kurtzman (Yamada et al. 1994), and *Kodamaea* Y. Yamada, T. Suzuki, M. Matsuda & Mikata for *P. ohmeri* (Etchells & T.A. Bell)

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Kreger-van Rij (Yamada et al. 1995b). More recently, *Kregervanrija* Kurtzman was described to accommodate *P. fluxuum* (Phaff & E.P. Knapp) Kreger-van Rij and related species (Kurtzman 2006), and reclassification of species in the *P. anomala* (E.C. Hansen) Kurtzman and *P. americana* (Wick.) Kurtzman clades has been proposed elsewhere (Kurtzman et al. 2008).

In the present study, we analyzed D1/D2 LSU and SSU rRNA gene sequences to determine the phylogenetic placement of those species of *Pichia*, as well as species from neighboring genera, that form coenzyme Q-9. Excluded from this study were comparisons of the CoQ-9 forming species assigned to the genera *Sporopachydermia* Rod. Mir., *Sugiyamaella* Kurtzman & Robnett, *Trichomonascus* H.S. Jack., *Wickerhamiella* van der Walt, and

Zygoascus M.T. Sm. and their anamorphs, which were previously examined from multigene analysis (Kurtzman and Robnett 2007). From our present study, we propose five new genera and emendation of one previously described genus. These proposed changes and those cited above will circumscribe the genus *Pichia* on the *P. membranifaciens* (E.C. Hansen) E.C. Hansen clade.

Materials and methods

Taxa examined

The species compared are given in Table 1 with culture collection and GenBank accession numbers.

Table 1 Species compared, culture collection strain numbers, and GenBank gene sequence accession numbers

Species	Strain no. ^a			GenBank no. ^b	
	NRRL	CBS	JCM	D1/D2	SSU
<i>Candida aaseri</i>	YB-3897	1913	1689	U45802	AB013564
<i>C. albicans</i>	Y-12983	562	1542	U45776	EU348783 ^c
<i>C. atlantica</i>	Y-17759	5263	9548	U45799	AB013527
<i>C. atmosphaerica</i>	Y-17642	4547	9549	U45779	AB013526
<i>C. boleticola</i>	Y-17080	6420	1500	U45777	AB013532
<i>C. buinensis</i>	Y-11706	6796	9453	U45778	AB013577
<i>C. coipomoensis</i>	Y-17651	8178	8916	U45747	AB013561
<i>C. conglobata</i>	Y-1504	2018	2373	U45789	AB013515
<i>C. dendronema</i>	Y-7781	6270	1803	U45751	AB013530
<i>C. diddensiae</i>	Y-7589	2214	9598	U45750	AB013508
<i>C. dubliniensis</i>	Y-17841	7987		U57685	AY497766
<i>C. ergastensis</i>	Y-17652	6248	9599	U45746	AB013524
<i>C. fermenticarens</i>	Y-17321	7040	9589	U45756	AB013525
<i>C. fluviatilis</i>	Y-7711	6776	9552	U45717	AB013521
<i>C. fragi</i>	Y-17910	7702	1791	U71071	AB013573
<i>C. friedrichii</i>	Y-17653	4114	9553	U45781	AB013531
<i>C. glabrosa</i>	Y-6949	5691	1590	U45757	AB013512 ^d
<i>C. glucosophila</i>	Y-17781	7349	9440	U45849	AB013519
<i>C. insectamans</i>	Y-7786	6033	9611	U45753	AB013518
<i>C. insectorum</i>	Y-7787	6213	9457	U45791	AB013565
<i>C. jeffriesii</i>	Y-27738	9898		AY520415	AY520287
<i>C. lodderae</i>	Y-17317	1924	1601	U45755	AB013533
<i>C. lyxosophila</i>	Y-17539	8194	7532	U76204	AB013522
<i>C. maltosa</i>	Y-17677	5611	1504	U45745	EU348784 ^c
<i>C. membranifaciens</i>	Y-2089	1952	9450	U45792	AB013551
<i>C. multigemis</i>	Y-17659	6524	9559	U45782	AB013535
<i>C. natalensis</i>	Y-17680	2935	1445	U45818	AB013541
<i>C. neerlandica</i>	Y-27057	434		AF245404	EF120593
<i>C. oleophila</i>	Y-2317	2219	1620	U45793	AB013534
<i>C. palmioleophila</i>	Y-17323	7418	5218	U45758	AB013520

Table 1 continued

Species	Strain no. ^a			GenBank no. ^b	
	NRRL	CBS	JCM	D1/D2	SSU
<i>C. parapsilosis</i>	Y-12969	604	1785	U45754	AB013588
<i>C. pseudoglebosa</i>	Y-17911	6715	2168	U71072	AB013544
<i>C. psychrophila</i>	Y-17665	5956	2388	U45813	AB013528
<i>C. quercitrusa</i>	Y-5392	4412	9832	U45831	AB013579
<i>C. saitoana</i>	Y-17316	940	1438	U45762	AB013523
<i>C. sake</i>	Y-1622	159	2951	U45728	AB013529
<i>C. santamariae</i> var. <i>santamariae</i>	Y-6656	4515	1816	U45794	AB013536
<i>C. santamariae</i> var. <i>membranifaciens</i>	Y-17647	5838	9844	U45785	AB013581
<i>C. schatavii</i>	Y-17078	6452	1778	U45795	AB013537
<i>C. shehatae</i> var. <i>shehatae</i>	Y-17029	5813	9840	U45761	AB013582
<i>C. shehatae</i> var. <i>insectosa</i>	Y-12854	4286	9842	U45773	AB013583
<i>C. shehatae</i> var. <i>lignosa</i>	Y-12856	4705	9837	U45772	AB013584
<i>C. sojae</i>	Y-17909	7871	1644	U71070	AB013549
<i>C. sophiaereginae</i>	Y-17668	8175	8925	U45817	AB013542
<i>C. tenuis</i>	Y-1498	615	9827	U45774	AB013516
<i>C. tropicalis</i>	Y-12968	94	1541	U45749	EU348785 ^c
<i>C. viswanathii</i>	Y-6660	4024	9567	U45752	AB013589
<i>C. zeylanoides</i>	Y-1774	619	1627	U45832	AB013509
<i>Debaryomyces carsonii</i>	YB-4275	2285	8121	U45743	AB054260
<i>D. castellii</i>	Y-7423	2923	6177	U45841	AB054276
<i>D. coudertii</i>	Y-7425	5167	2387	U45846	AB054272
<i>D. etchellsii</i>	Y-7121	2011	3656	U45809	AB054258
<i>D. hansenii</i> var. <i>hansenii</i>	Y-7426	767	1990	U45808	AB013590
<i>D. hansenii</i> var. <i>fabryi</i>	Y-17914	789	2104	U94927	AJ508273
<i>D. maramus</i>	Y-2171	1958	1528	U45838	AB054261
<i>D. melissophilus</i>	Y-7585	6344	1707	U45740	AB054264
<i>D. nepalensis</i>	Y-7108	5921	2095	U45839	AB054271
<i>D. occidentalis</i> var. <i>occidentalis</i>	Y-10	819	8123	U45804	AB054262
<i>D. occidentalis</i> var. <i>persoonii</i>	Y-7400	2169	8127	U45840	AB054263
<i>D. polymorphus</i>	Y-2022	186	3647	U45836	AB054275
<i>D. polymorphus</i> var. <i>africanus</i>		6741	7443	AB054994	AB054265
<i>D. prosopidis</i>	Y-27369	8450	9913	AB054993	AB054277
<i>D. pseudopolymorphus</i>	YB-4229	2008	3652	U45845	AB054273
<i>D. robertsiae</i>	Y-6670	2934		U45805	AB054259
<i>D. udenii</i>	Y-17354	7056	7885	U45844	AB054268
<i>D. vanrijae</i> var. <i>vanrijae</i>	Y-7430	3024	3657	U45842	AB054274
<i>D. vanrijae</i> var. <i>yarrowii</i>	Y-7535	6246	6190	U45843	AB054267
<i>D. yamadae</i>	Y-11714	7035	6191	U45837	AB054266
<i>Hyphopichia burtonii</i>	Y-1933	2352	3708	U45712	AB158656
<i>Lodderomyces elongisporus</i>	YB-4239	2605	1781	U45763	X78600
<i>Pichia acaciae</i>	Y-7117	5656	10732	U45767	AB054278
<i>P. caribbica</i>	Y-27274	9966		EU348786	AJ508269 ^c
<i>P. castillae</i>	Y-7501	6053	10733	U45769	AB054290
<i>P. farinosa</i>	Y-7553	185	10734	U45739	AB054281
<i>P. guilliermondii</i>	Y-2075	2030	1539	U45709	AB013587
<i>P. haplophila</i>	Y-7860	2028	1635	U45770	AB054284
<i>P. inositovora</i>	Y-12698	8006	10736	U45848	AB054289

Table 1 continued

Species	Strain no. ^a			GenBank no. ^b	
	NRRL	CBS	JCM	D1/D2	SSU
<i>P. media</i>	Y-7122	5521	10737	U45768	AB054291
<i>P. mexicana</i>	Y-11818	7066	1835	U45797	AB054285
<i>P. nakazawae</i> var. <i>nakazawae</i>	Y-7903	6700	7529	U45748	AB054287
<i>P. nakazawae</i> var. <i>akitaensis</i>	Y-7904	6701	10738	U45766	AB054279
<i>P. philogaea</i>	Y-7813	6696	10739	U45765	AB054283
<i>P. scolyti</i>	Y-5512	4802	3654	U45788	AB054286
<i>P. segobiensis</i>	Y-11571	6857	10740	U45742	AB054288
<i>P. spartinae</i>	Y-7322	6059	10741	U45764	AB054292
<i>P. stipitidis</i>	Y-7124	5773	10742	U45741	AB054280
<i>P. triangularis</i>	Y-5714	4094	9449	U45796	AB013575
<i>Schizosaccharomyces pombe</i>	Y-12796	356	8274	U40085	AY046272
<i>Spathaspora passalidarum</i>	Y-27907	10155		DQ109807	DQ232894

^a NRRL, ARS Culture Collection, Peoria, Illinois, USA; CBS Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; JCM Japan Collection of Microorganisms, Wako, Japan; all represent type or neotype strains unless otherwise indicated

^b D1/D2, domains 1 and 2 of the large subunit ribosomal RNA gene; SSU small subunit ribosomal RNA gene

^c Sequences new in this study

^d Sequence determined from JCM 1517

^e Sequence determined from CBS 2022

rRNA gene sequences and phylogenetic analyses

Many of the D1/D2 LSU and SSU rRNA gene sequences analyzed in this study were published earlier, and methods for their determination were reported by James et al. (1997), Kurtzman and Robnett (1998, 2003), and Suzuki et al. (1999). Sequences that are new in this study were determined by the preceding methods.

Sequences were aligned manually, and nucleotides of uncertain alignment were removed from analysis. Three datasets were analyzed: D1/D2 LSU, SSU, and a concatenation of D1/D2 and SSU. Each dataset was analyzed by maximum parsimony and by neighbor-joining analysis with the Kimura two-parameter distance correction using the programs in PAUP* 4.063a (Swofford 1998). Bootstrap values are from 1000 replicates. The aligned dataset used in the analysis has been deposited with TreeBASE (<http://www.treebase.org/>) under the accession number SN2460.

Results and discussion

As reported in earlier studies (Kurtzman and Robnett 1998; Suzuki et al. 1999), branch support in phylogenetic trees determined from analysis of either D1/D2 LSU or SSU rRNA gene sequences was generally weak, and the clades were often uncertain. Analysis of a concatenated dataset composed of these two gene sequences has provided markedly stronger branch support. Bootstrap support for

the clades that we have recognized as genera, excluding two basal species, ranged from 81 to 100% (Fig. 1). Analysis of the D1/D2 LSU + SSU rRNA dataset by both neighbor-joining and maximum parsimony analysis gave the same genus-level clades. Deeper nodes were less well supported and the more basal relationships differed somewhat, depending on whether the analysis was by maximum parsimony or neighbor joining. These deeper branches will need to be resolved from more robust datasets.

From the present analyses, 12 well-supported clades are resolved, 10 of which are ascosporic. The genera *Lodderomyces* van der Walt and *Spathaspora* N.H. Nguyen, S.O. Suh & M. Blackw. were recovered as previously defined (Kurtzman and Robnett 1998; Nguyen et al. 2006). *Debaryomyces* Lodder & Kreger-van Rij species are separated into three distinct clades, one with *D. hansenii* (Zopf) Lodder & Kreger van Rij and closely related species, a second with species closely related to *D. polymorphus* (Klöcker) Price & Phaff and *Schwanniomyces occidentalis* Klöcker, and a third that includes *D. melissophilus* (van der Walt & van der Klift) Kurtzman & Kreger-van Rij. Five of the ascosporic clades represent new genera.

From the foregoing, we propose the following new genera, an emended genus, and various new species combinations and new ranks.

Babjeviella Kurtzman et M. Suzuki, gen. nov.

Mycobank no.: MB 513455.

Asci inconjugati, evanescentes, 1–4 ascosporae petasiformes habentes. Cellulae vegetativae ellipsoideae vel



Fig. 1 Phylogenetic tree of species assigned to *Spathaspora*, *Lodderomyces*, *Debaryomyces*, *Yamadazyma*, and proposed new genera derived from neighbor-joining analysis of a concatenated dataset of gene sequences from D1/D2 large subunit (LSU) and small subunit

(SSU) rRNA. Bootstrap values $\geq 50\%$ are given at nodes and based on 1000 replicates. *Schizosaccharomyces pombe* served as the outgroup species. *Hyphopichia burtonii* is a distantly related reference species that forms CoQ-8

elongatae. Pseudohyphae et hyphae septatae non formantes. Glucosum non fermentatur. Inositol assimilantur. Systema coenzymatis Q-9 adest. Diazonium caeruleum B non respondens. Genus novum sequentibus nucleotiditis nuclei grandis parvisque submonades rRNA genorum distinguendum. Species typica: *Babjeviella inositovora* (Golubev & Blagod.) Kurtzman & M. Suzuki, comb. nov.

Type species: *Babjeviella inositovora* (Golubev & Blagod.) Kurtzman & M. Suzuki, comb. nov. MycoBank no.: MB 513461.

Basionym: *Pichia inositovora* Golubev & Blagod., Int. J. Syst. Bacteriol. 31: 91, 1981.

Ascospore formation: Asci are unconjugated and form one to four hat-shaped ascospores that are released by ascus evanescence. Golubev et al. (1981) observed from transmission electron microscopy that bulb-like structures sometimes form on the brims of the ascospores. It has not been determined whether the species is homothallic or heterothallic.

Asexual reproduction: Cell division is by multilateral budding on a narrow base. Cells are short ellipsoid to elongate. Neither pseudohyphae nor true hyphae are formed. Colonies are butyrous with a glistening surface.

Physiology/biochemistry: Sugars are not fermented, but many of the sugars and polyols in standard yeast growth tests are assimilated. The major ubiquinone formed is CoQ-9 (Billon-Grand 1985), and the mol% G + C content for the type strain is 49.9 (Kurtzman 1998). The diazonium blue B test is negative. *Babjeviella inositovora* is one of only a few ascomycetous yeasts that can utilize inositol as a sole source of carbon.

The genus is named in honor of Prof. Inna P. Babjeva, Moscow State University, Moscow, Russia, for her outstanding contributions to yeast systematics and ecology.

Comments on the genus: The genus *Babjeviella* is phylogenetically isolated from other species in the present study and from species assigned to the Trichomonascaceae, another major group of yeasts characterized by CoQ-9 (Kurtzman and Robnett 1998, 2007). The type species for the genus, *B. inositovora*, was described from a single strain (NRRL Y-12698) isolated from high moor peat in the Moscow region of Russia (Golubev et al. 1981). Since then, two previously unidentified isolates of this species were discovered in the ARS Culture Collection following D1/D2 sequencing of uncharacterized strains. Each had identical D1/D2 sequences but differed from the type strain at one nucleotide position. Both form hat-shaped ascospores and each assimilates inositol. NRRL YB-4776 was received in August 1959 in material collected from the stump of a balsam fir [*Abies balsamea* (L.) Mill], Matane, Quebec, Canada, and isolated by L.J. Wickerham. The second strain, NRRL YB-5280, was received in July 1960 in frass infested with ants from an unidentified tree, Coeur

d'Alene State Park, Washington State, USA, and also isolated by L.J. Wickerham. As noted above, the newly identified strains ascribed to *B. inositovora* differ from the type strain at one nucleotide position in the D1/D2 gene sequence. These strains are considered to be conspecific with the type strain, but the extent of their relatedness might be overestimated, as discussed below for *Candida shehatae* H.R. Buckley & van Uden and other species that produce CoQ-9.

Species accepted:

1. *Babjeviella inositovora* (Golubev & Blagod.) Kurtzman & M. Suzuki

Meyerozyma Kurtzman et M. Suzuki, gen. nov.

MycoBank no.: MB 513456.

Asci conjugati vel inconjugati, evanescentes vel non evanescentes, 1–4 ascosporae petasiformes vel ellipsoideae habentes. Cellulae vegetativae ovoideae vel elongatae. Pseudohyphae formantes; hyphae septatae non formantes. Sacchara fermentantur. Systema coenzymatis Q-9 adest. Diazonium caeruleum B non respondens. Genus novum sequentibus nucleotiditis nuclei grandis parvisque submonades rRNA genorum distinguendum. Species typica: *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki, comb. nov.

Type species: *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki, comb. nov. MycoBank no.: MB 513463.

Basionym: *Pichia guilliermondii* Wick., J. Bacteriol. 92: 1269, 1966.

Ascospore formation: Asci are usually unconjugated, evanescent or persistent, and form 1–4 ascospores. The spores may be hat-shaped or ovoid and possibly ornamented with a narrow ledge. Species may be heterothallic or possibly homothallic.

Asexual reproduction: Cell division is by multilateral budding on a narrow base. Cells are ovoid to elongate. Pseudohyphae are formed, often abundantly, but true hyphae are absent. *Candida elateridarum* S.O. Suh & M. Blackw., a member of the *Meyerozyma* clade, is reported to form true hyphae (Suh and Blackwell 2004).

Physiology/biochemistry: Sugars are fermented and many common hexoses, disaccharides, polyols, and organic acids are assimilated, but nitrate is not utilized as a sole source of nitrogen. Where known, the mol% G + C content of the nuclear DNA is 45–50%, and the major ubiquinone is CoQ-9.

The genus is named in honor of Prof. Sally A. Meyer, Georgia State University, Atlanta, GA, USA, for her pioneering studies in the molecular relatedness of *Candida* species.

Comments on the genus: Two ascosporic species are known for the newly proposed genus *Meyerozyma*,

M. caribbica and *M. guilliermondii*. *Candida athensensis* S.O. Suh & M. Blackw., *C. carpophila* (Phaff & M.W. Mill.) Vaughan-Mart., Kurtzman, S.A. Mey. & N.R. O'Neill, *C. elateridarum*, and *C. smithsonii* S.O. Suh & M. Blackw. are anamorphic members of the clade (Suh and Blackwell 2004; Vaughan-Martini et al. 2005).

Species accepted:

1. *Meyerozyma caribbica* (Vaughan-Mart., Kurtzman, S.A. Mey. & N.R. O'Neill) Kurtzman & M. Suzuki, comb. nov.
MycBank no.: MB 513462.
Basionym: *Pichia caribbica* Vaughan-Mart., Kurtzman, S.A. Mey. & N.R. O'Neill, FEMS Yeast Res. 5: 466, 2005.
2. *Meyerozyma guilliermondii* (Wick.) Kurtzman & M. Suzuki
Milleromyza Kurtzman et M. Suzuki, gen. nov.
MycBank no.: MB 513457.

Asci conjugati, evanescentes vel non evanescentes, 1–4 ascosporeae petasiformes vel globosae cum dorso habentes. Cellulae vegetativae globosae aut elongatae. Pseudohyphae formantes; hyphae septatae non formantes. Sacchara fermentantur. Systema coenzymatis Q-9 adest. Diazonium caeruleum B non respondens. Genus novum sequentibus nucleotiditis nuclei grandis parvisque submonades rRNA genorum distinguendum. Species typica: *Milleromyza farinosa* (Lindner) Kurtzman & M. Suzuki, comb. nov.

Type species: *Milleromyza farinosa* (Lindner) Kurtzman & M. Suzuki, comb. nov.

MycBank no.: MB 513465.

Basionym: *Saccharomyces farinosus* Lindner, Wochenschr. Brau. 11: 153, 1894.

Ascospore formation: Asci form following conjugation between a cell and its bud, or less commonly, between independent cells. Asci form 1–4 ascospores, which may be hat shaped with a narrow brim or spherical to ovoid with a subequatorial ledge. Asci may be evanescent or persistent. The two known species are homothallic.

Asexual reproduction: Cell division is by multilateral budding on a narrow base, and budded cells are spherical to elongate. Pseudohyphae are formed but true hyphae do not develop.

Physiology/biochemistry: Glucose is fermented and galactose and trehalose are sometimes fermented. Many of the sugars, polyols, and organic acids used for yeast identification are assimilated, but nitrate is not utilized. The major ubiquinone formed is CoQ-9 (Yamada et al. 1973; Billon-Grand 1989). The mol% G + C content of nuclear DNA ranges from 39 to 46%, and the diazonium blue B test is negative.

The genus is named in honor of Prof. Martin W. Miller, University of California, Davis, California, USA, for his

highly recognized research on yeast taxonomy, ecology, and food microbiology.

Species accepted:

1. *Milleromyza acaciae* (van der Walt) Kurtzman & M. Suzuki, comb. nov.
MycBank no.: MB 513464.
Basionym: *Pichia acaciae* van der Walt, Antonie van Leeuwenhoek 32: 159, 1966.
2. *Milleromyza farinosa* (Lindner) Kurtzman & M. Suzuki
Priceomyces M. Suzuki et Kurtzman, gen. nov.
MycBank no.: MB 513458.

Asci conjugati vel inconjugati, evanescentes vel non evanescentes, 1–4 ascosporeae petasiformes, globosae, glabrae vel verrucosae habentes. Cellulae vegetativae ovoideae vel elongatae. Pseudohyphae formantes (variabiles); hyphae septatae non formantes. Sacchara non fermentantur. Systema coenzymatis Q-9 adest. Diazonium caeruleum B non respondens. Genus novum sequentibus nucleotiditis nuclei grandis parvisque submonades rRNA genorum distinguendum. Species typica: *Priceomyces haplophilus* (Shifrine & Phaff) M. Suzuki & Kurtzman, comb. nov.

Type species: *Priceomyces haplophilus* (Shifrine & Phaff) M. Suzuki & Kurtzman, comb. nov.

MycBank no.: MB 513468.

Basionym: *Pichia haplophila* Shrifine & Phaff, Mycologia 48: 45, 1956.

Ascospore formation: Asci may be unconjugated or form following conjugation between a cell and its bud or between independent cells. Asci, which are deliquescent or persistent, form 1–4 ascospores that may be hat shaped, spherical with a smooth surface, or spherical with a roughened surface. Currently known species are homothallic.

Asexual reproduction: Cell division is by multilateral budding on a narrow base and budded cells are spherical to elongate. Pseudohyphae are formed by some species, but true hyphae are absent.

Physiology/biochemistry: Sugars are not fermented, but many of the sugars, polyols, and organic acids used for yeast identification tests are assimilated. Nitrate is not assimilated. The major ubiquinone formed is CoQ-9 (Yamada et al. 1973; van der Walt and Johannsen 1980; Billon-Grand 1985), and the mol% G + C content of nuclear DNA for known species ranges from 37 to 41%. The diazonium blue B test is negative.

The genus is named in honor of Prof. Chester W. Price, University of California, Davis, California, USA, for his pioneering studies on molecular relatedness of many different yeast genera.

Comments on the genus: The genus *Priceomyces* is phylogenetically well supported by the present dataset (see

Fig. 1). The species do not ferment sugars and have a similar growth spectrum on standard assimilation tests. There are, however, some differences in ascospore morphology. *Priceomyces castillae*, *Pr. haplophilus*, and *Pr. medius* produce hat-shaped ascospores that are released from the ascus soon after formation. The ascospores of *Pr. carsonii* are spherical and smooth, but asci may be persistent or evanescent. In contrast, asci of *Pr. melissophilus* are persistent and form spherical ascospores that are roughened by abundant protuberances on the spore surfaces.

Species accepted:

1. *Priceomyces carsonii* (Phaff & E.P. Knapp) M. Suzuki & Kurtzman, comb. nov.
Mycobank no.: MB 513466.
Basionym: *Pichia carsonii* Phaff & E.P. Knapp, *Antonie van Leeuwenhoek* 22: 127, 1956.
2. *Priceomyces castillae* (Santa María & C. García) M. Suzuki & Kurtzman, comb. nov.
Mycobank no.: MB 513467.
Basionym: *Pichia castillae* Santa María & C. García, *Bol. Inst. Nac. Invest. Agron.* 62: 53, 1970.
3. *Priceomyces haplophilus* (Shifrine & Phaff) M. Suzuki & Kurtzman
4. *Priceomyces medius* (Boidin, Pignal, Lehodey, Vey & Abadie) M. Suzuki & Kurtzman, comb. nov.
Mycobank no.: MB 513469.
Basionym: *Pichia media* Boidin, Pignal, Lehodey, Vey & Abadie, *Bull. Soc. Mycol. France* 80: 438, 1964.
5. *Priceomyces melissophilus* (van der Walt & van der Klift) M. Suzuki & Kurtzman, comb. nov.
Mycobank no.: MB 513470.
Basionym: *Pichia melissophila* van der Walt & van der Klift, *Antonie van Leeuwenhoek* 38: 361, 1972.

Scheffersomyces Kurtzman et M. Suzuki, gen. nov.

Mycobank no.: MB 513459.

Asci conjugati, evanescentes 1–4 ascosporae petasiformes habentes. Cellulae vegetativae globosae vel elongatae. Pseudohyphae formantes; hyphae septatae non formantes. D-Xylosa (variabile) et sacchara alia fermentantur. Systema coenzymatis Q-9 adest. Diazonium caeruleum B non respondens. Genus novum sequentibus nucleotiditis nuclei grandis parvisque submonades rRNA genorum distinguendum. Species typica: *Scheffersomyces stipitis* (Pignal) Kurtzman & M. Suzuki, comb. nov.

Type species: *Scheffersomyces stipitis* (Pignal) Kurtzman & M. Suzuki, comb. nov.

Mycobank no.: MB 513473.

Basionym: *Pichia stipitis* Pignal, *Bull. Mens. Soc. Linn. Lyon.* 36: 168, 1967.

Ascospore formation: Asci form following conjugation between a cell and its bud, between independent cells, or

following pairing of complementary mating types. Thus, some species are homothallic and one species is heterothallic. Asci produce 1–2 hat-shaped ascospores that are released soon after formation.

Asexual reproduction: Cell division is by multilateral budding on a narrow base and budded cells are spherical to elongate. Pseudohyphae are formed, but true hyphae do not develop.

Physiology/biochemistry: Glucose is fermented as well as certain other sugars. Some of the species ferment D-xylose, which is important for biofuel production. Various sugars, polyols, and organic acids used for yeast identification are assimilated, but nitrate is not utilized. The major ubiquinone produced is CoQ-9 (Billon-Grand 1985, 1989). The mol% G + C content of nuclear DNA for assigned species ranges from 38 to 43%, and the diazotium blue B test is negative.

The genus is named in honor of Prof. W. Alexander Scheffers, Delft University of Technology, Delft, The Netherlands, for outstanding contributions to yeast physiology and biotechnology, especially concerning D-xylose fermentation by yeasts, and for his service to science as founding editor of the journal *FEMS Yeast Research*.

Comments on the genus: The genus *Scheffersomyces*, including the phylogenetically related *Candida shehatae* species complex, has a majority of the D-xylose-fermenting yeasts that are of interest to biotechnology (Toivola et al. 1984). Other significant D-xylose-fermenting yeasts are *Pachysolen tannophilus* Boidin & Adzet (Kurtzman et al. 1982) and members of the *Spathaspora* clade that include *S. passalidarum* N.H. Nguyen, S.O. Suh & M. Blackw., *C. jeffriesii* N.H. Nguyen, S.O. Suh & M. Blackw., and *C. lyxosophila* van der Walt, N.P. Ferreira & Steyn (Nguyen et al. 2006). *Scheffersomyces* and *Spathaspora* are relatively closely related (see Fig. 1), but *Pachysolen* Boidin & Adzet is not a member of this group (Kurtzman 1990). *Scheffersomyces spartinae*, which does not ferment D-xylose, is the basal species in the genus *Scheffersomyces*, and its placement is weakly supported. The addition of new species to the clade may strengthen placement of *S. spartinae* in the genus or provide evidence that it is a member of an undescribed sister genus.

Species accepted:

1. *Scheffersomyces segobiensis* (Santa María & C. García) Kurtzman & M. Suzuki, comb. nov.
Mycobank no.: MB 513471.
Basionym: *Pichia segobiensis* Santa María & C. García, *An. Inst. Nac. Invest. Agron., Serie General* 5: 45, 1977.
2. *Scheffersomyces spartinae* (Ahearn, Yarrow & Meyers) Kurtzman & M. Suzuki, comb. nov.
Mycobank no.: MB 513472.

Basionym: *Pichia spartinae* Ahearn, Yarrow & Meyers, *Antonie van Leeuwenhoek* 36: 505, 1970.

3. *Scheffersomyces stipitis* (Pignal) Kurtzman & M. Suzuki.

Elevation of two varieties of *Candida shehatae* to species status

1. *Candida insectosa* (Kurtzman) Kurtzman, stat. nov.
MycBank no.: MB 513475.
Basionym: *Candida shehatae* H.R. Buckley & van Uden var. *insectosa* Kurtzman, *Antonie van Leeuwenhoek* 57: 218, 1990.
2. *Candida lignosa* (Kurtzman) Kurtzman, stat. nov.
MycBank no.: MB 513476.
Basionym: *Candida shehatae* H.R. Buckley & van Uden var. *lignosa* Kurtzman, *Antonie van Leeuwenhoek* 57: 218, 1990.

The preceding new ranks, *C. insectosa* and *C. lignosa*, were originally described as varieties of *C. shehatae* (Kurtzman 1990). The varieties showed approximately 50% nuclear DNA reassociation with each other and with *C. shehatae* var. *shehatae*. The varieties *shehatae* and *insectosa* differ by 1 nucleotide in their D1/D2 LSU rRNA gene sequences, but varieties *shehatae* and *lignosa* show no differences (Kurtzman and Robnett 1998). There are no differences among the three varieties of *C. shehatae* for the SSU rRNA gene sequence. Interestingly, Passoth et al. (1992) reported some differences in the electrokaryotypes of the three varieties. The early interpretation of varietal circumscription, which was based on DNA relatedness, now appears to have been too inclusive. With the elevation of varieties *insectosa* and *lignosa* to species status, the variety *shehatae* is no longer maintained for *C. shehatae*.

Since the description of the *C. shehatae* varieties, other closely related taxa have been discovered, such as *Meyerozyma guilliermondii* and *M. caribbica*, which also showed much reduced nuclear DNA relatedness but little D1/D2 divergence (Bai 1996; Vaughan-Martini et al. 2005). On the basis of reduced nuclear DNA relatedness and divergent electrokaryotypes, *M. guilliermondii* and *M. caribbica* were considered separate species, even though quite closely related. This same interpretation has been applied to elevate the *C. shehatae* varieties to species level. A similar relationship exists between *Debaryomyces hansenii* var. *hansenii* and *D. hansenii* var. *fabryi* (M. Ota) Nakase & M. Suzuki. Nakase and Suzuki (1985) demonstrated 52% nuclear DNA relatedness between the two taxa but maintained their designation as varieties. Prillinger et al. (1999) compared the RAPD patterns of *D. hansenii* and its variety *fabryi* and proposed that the two represented separate species. In view of the genetic divergence shown

in both studies, we accept *D. hansenii* and *D. fabryi* as separate but closely related species.

Schwanniomyces Klöcker emend. M. Suzuki & Kurtzman.

MycBank no.: MB 513460.

Kurtzman and Robnett (1991) examined the relatedness of a limited number of *Debaryomyces* and *Schwanniomyces* species from partial SSU and LSU rRNA sequences and noted that species of *Schwanniomyces* clustered with certain *Debaryomyces* species, such as *D. polymorphus*. From this analysis, they proposed the transfer of *Schwanniomyces* species to *Debaryomyces*, the genus of taxonomic priority. Later, when Kurtzman and Robnett (1998) analyzed a much larger number of species from D1/D2 LSU rRNA gene sequence divergence, it was seen that *Debaryomyces hansenii* and closely related species were separate from the *D. polymorphus* clade. Because bootstrap support for these two groups was weak, no taxonomic changes were proposed.

In the present study, bootstrap support for the two groups is much stronger, showing that the *D. hansenii* clade is clearly separate from the *D. polymorphus* clade, which includes *Schwanniomyces occidentalis*. Because *Schwanniomyces* is a valid name, we propose transfer of species in the *D. polymorphus* clade to the genus *Schwanniomyces*, and retention of *Debaryomyces* for the *D. hansenii* clade (Nakase et al. 1998). Included in the proposed transfer of species is *Debaryomyces etchellsii*, which is provisionally placed in the genus *Schwanniomyces*. *Debaryomyces etchellsii* is the basal species in the *Schwanniomyces* clade and its placement is only weakly supported. However, it is not sufficiently isolated in the present dataset to support its placement in a new, monotypic genus, but this placement may change as new species are described.

In view of the proposed species reassignments, the following emendation is provided: *Schwanniomyces* Klöcker emend. M. Suzuki & Kurtzman. Species form 1–4 globose ascospores that may be glabrous, roughened, or roughened with an equatorial ledge. Asci may be unconjugated or show conjugation between a cell and its bud or between independent cells. Cells divide by multilateral budding and are spherical to elongate. Pseudohyphae may form, but septate hyphae are absent. Sugars may be fermented and various sugars, polyols, and organic acids are assimilated, but nitrate is not utilized. The major ubiquinone is CoQ-9. Species are negative on the diazonium blue B test.

1. *Schwanniomyces capriottii* M. Suzuki & Kurtzman, comb. et nom. nov.

MycBank no.: MB 513474.

Basionym: *Debaryomyces castellii* Capr., *Arch. Mikrobiol.* 28: 348, 1958. The species epithet ‘*castellii*’

- was previously used for the validly described species *Schwanniomyces castellii* Capr., now recognized as a synonym of *Schwanniomyces occidentalis* var. *occidentalis* from the nuclear DNA reassociation studies of Price et al. (1978). In view of this prior usage, we have proposed the new species epithet ‘*capriottii*’ in honor of Prof. Augusto Capriotti, who described *S. castellii*.
2. *Schwanniomyces etchellsii* (Kreger-van Rij) M. Suzuki & Kurtzman, comb. nov.
MycBank no.: MB 513477.
Basionym: *Pichia etchellsii* Kreger-van Rij, Antonie van Leeuwenhoek 30: 431, 1964.
 3. *Schwanniomyces occidentalis* Klöcker var. *occidentalis* Meddn Carlsberg Lab. 7, 1909.
This species is the type species of the genus *Schwanniomyces*.
 4. *Schwanniomyces occidentalis* Klöcker var. *personii* (van der Walt) Phaff & M.W. Mill. The Yeasts: A Taxonomic Study, 3rd edn.: 424, 1984.
 5. *Schwanniomyces polymorphus* (Klöcker) M. Suzuki & Kurtzman var. *polymorphus*, comb. nov.
MycBank no.: MB 513478.
Basionym: *Pichia polymorpha* Klöcker. Zentralbl. Bakteriologie. Parasitenkd., Abt. II, 35: 369, 1912.
≡ *Debaryomyces polymorphus* (Klöcker) Price & Phaff var. *polymorphus* (Klöcker) Price & Phaff, Mycologia 71: 445, 1979.
 6. *Schwanniomyces polymorphus* (Klöcker) M. Suzuki & Kurtzman var. *africanus* (van der Walt, Nakase & M. Suzuki) M. Suzuki & Kurtzman, comb. nov.
MycBank no.: MB 513479.
Basionym: *Debaryomyces polymorphus* (Klöcker) Price & Phaff var. *africanus* van der Walt, Nakase & M. Suzuki, J. Gen. Appl. Microbiol. 40: 333, 1994.
 7. *Schwanniomyces pseudopolymorphus* (C. Ramírez & Boidin) M. Suzuki & Kurtzman, comb. nov.
MycBank no.: MB 513480.
Basionym: *Pichia pseudopolymorpha* C. Ramírez & Boidin, Rev. Mycol. 18: 149, 1953.
 8. *Schwanniomyces vanrijiae* (van der Walt & Tscheuschner) M. Suzuki & Kurtzman var. *vanrijiae*, comb. nov.
MycBank no.: MB 513481.
Basionym: *Pichia vanrijiae* van der Walt & Tscheuschner, J. Gen. Microbiol. 15: 460, 1956.
≡ *Debaryomyces vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob var. *vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob, Bull. trimest. Soc. Mycol. Fr. 79: 36, 1963.
 9. *Schwanniomyces vanrijiae* (van der Walt & Tscheuschner) M. Suzuki & Kurtzman var. *yarrowii* (Santa María & C. García) M. Suzuki & Kurtzman, comb. nov.
MycBank no.: MB 513482.
Basionym: *Debaryomyces vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob var. *yarrowii* (Santa María & C. García) Kreger-van Rij, The Yeasts: A Taxonomic Study, 3rd edn., 143, 1984.
≡ *Debaryomyces yarrowii* Santa María & C. García, An. Inst. Nac. Invest. Agron., Serie General 1: 89, 1971.
 10. *Schwanniomyces yamadae* (van der Walt & E. Johannsen) M. Suzuki & Kurtzman, comb. nov.
MycBank no.: MB 513483.
Basionym: *Debaryozyma yamadae* van der Walt & E. Johannsen, J. Gen. Appl. Microbiol. 26: 219, 1980.

Yamadazyma Billon-Grand

The genus *Yamadazyma* was described by Billon-Grand (1989) for species assigned to the genus *Pichia* that formed CoQ-9 as their major ubiquinone. Other characters included in the genus description were presence of pseudohyphae, formation of hat-shaped ascospores that were usually liberated from the ascus at maturity, ability to ferment sugars, and absence of growth in vitamin-free media. Billon-Grand (1989) transferred the following 16 species to *Yamadazyma*: *Y. acaciae* (van der Walt) Billon-Grand, *Y. besseyi* (Kurtzman & Wick.) Billon-Grand, *Y. castillae* (Santa María & C. García) Billon-Grand, *Y. farinosa* (Lindner) Billon-Grand, *Y. guilliermondii* (Wick.) Billon-Grand, *Y. haplophila* (Shifrine & Phaff) Billon-Grand, *Y. inositovora* (Golubev & Blagod.) Billon-Grand, *Y. media* (Boidin, Pignal, Lehody, Vey & Abadie) Billon-Grand, *Y. mexicana* (M. Miranda, Holzschu, Phaff & Starmer) Billon-Grand, *Y. nakazawae* (K. Kodama) Billon-Grand, *Y. ohmeri* (Etchells & T.A. Bell) Billon-Grand, *Y. philogaea* (van der Walt & E. Johannsen) Billon-Grand (type species of the genus), *Y. scolyti* (Phaff & Yoney.) Billon-Grand, *Y. segobiensis* (Santa María & C. García) Billon-Grand, *Y. spartinae* (Ahearn, Yarrow & Meyers) Billon-Grand, and *Y. stipitis* (Pignal) Billon-Grand. However, the polyphyletic nature of *Yamadazyma* became evident from D1/D2 LSU rRNA gene sequence analysis (Kurtzman and Robnett 1998), and the genus was not generally accepted as proposed.

The present analysis placed the type species, *Y. philogaea*, as well as *Y. mexicana*, *Y. nakazawae* var. *nakazawae*, *P. nakazawae* K. Kodama var. *akitaensis* K. Kodama, *Y. scolyti*, *P. triangularis* M.T. Sm. & Bat. Vegte, and 11 *Candida* species in the *Yamadazyma* clade (see Fig. 1). Earlier, on the basis of gene sequence analysis, *Y. besseyi* was transferred to the genus *Saturnispora* Z.W. Liu & Kurtzman (Kurtzman 2006). It was also determined that *S. besseyi*, typical of other *Saturnispora* species, forms

CoQ-7, not CoQ-9 (Y. Yamada, personal communication). As seen from Fig. 1, the remaining *Yamadazyma* species are distributed among five other clades. However, the reduced size of *Yamadazyma*, as defined from sequence analysis, has not substantially changed the broad genus description given by Billon-Grand (1989), and an emendation seems unnecessary.

Pichia nakazawae was among species originally transferred to *Yamadazyma*, but its variety *akitaensis* was described later and was not transferred (Kodama 1975). In the present study, we propose to transfer var. *akitaensis* to *Yamadazyma* as a distinct species. Kurtzman (1984) determined from reassociation experiments that var. *nakazawae* and var. *akitaensis* had 40% nuclear DNA relatedness. In keeping with earlier interpretations of DNA relatedness values, the two taxa were regarded as varieties. However, as already discussed regarding the *C. shehatae* varieties, this low level of DNA relatedness seems to indicate that the taxa are separate species. These two species differ at only two nucleotide positions in their D1/D2 LSU rRNA gene sequences, further indicating their close relationship (Kurtzman and Robnett 1998).

Species accepted:

1. *Yamadazyma akitaensis* (K. Kodama) Kurtzman & M. Suzuki, comb. et stat. nov.
MycBank no.: MB 513484.
Basionym: *Pichia nakazawae* K. Kodama var. *akitaensis* K. Kodama, J. Ferment. Technol. 53: 627, 1975.
2. *Yamadazyma mexicana* (M. Miranda, Holzschu, Phaff & Starmer) Billon-Grand, Mycotaxon 35: 203, 1989.
3. *Yamadazyma nakazawae* (K. Kodama) Billon-Grand, Mycotaxon 35: 203, 1989.
4. *Yamadazyma philogaea* (van der Walt & E. Johannsen) Billon-Grand, Mycotaxon 35:203, 1989.
This species is the type species of the genus *Yamadazyma*.
5. *Yamadazyma scolyti* (Phaff & Yoney.) Billon-Grand, Mycotaxon 35: 203, 1989.
6. *Yamadazyma triangularis* (M.T. Sm. & Bat.Vegte) Kurtzman & M. Suzuki, comb. nov.
MycBank no.: MB 513485.
Basionym: *Pichia triangularis* M.T. Sm. & Bat.Vegte. Antonie van Leeuwenhoek 52: 438, 1986.

As shown in Fig. 1, the genera *Spathospora* and *Babjeviella* delineate a clade that has high bootstrap support (98%) and which is interpreted as a family. In view of this, the following new family is proposed and typified on the genus *Debaryomyces*.

Debaryomycetaceae Kurtzman et M. Suzuki, fam. nov.
MycBank no.: MB 513454.

Cellulae globosae vel cylindricae, propagatione per gemmationem multilateralem; pseudohyphae et hyphae septatae (raro) praesentes. Asci ovoidei vel elongati, conjugati vel non conjugati, persistentes vel deliquescentes; 1–4 ascosporae, globosae, ellipsoideae, elongatae vel petasi-formes. Saccharas fermentantur aut non fermentantur. Systema coenzymatis Q-9 adest. Diazonium caeruleum B non respondens. Familia nova sequentibus nucleotiditis nuclei grandis parvisque submonades rRNA genorum distinguenda. Genus typicum: *Debaryomyces* Klöcker.

As noted above, the family Debaryomycetaceae, typified by *Debaryomyces* Klöcker, is delimited by the genera *Spathospora* and *Babjeviella*, has high bootstrap support (98%) in the present analysis, and represents a monophyletic lineage. Because prior analyses have also shown that species in this clade are separate from other Co-9-forming species, as well as other yeasts (Kurtzman 2003; Kurtzman and Robnett 1998, 2007; Kurtzman et al. 2008; Suzuki et al. 1999), we propose that the included genera are members of a single family. The proposal parallels family designations in other groups of yeasts (Kurtzman 2003; Kurtzman and Robnett 2007; Kurtzman et al. 2008). The genera delimited by *Spathospora* and *Yamadazyma* appear to have similar divergence from one another. However, *Babjeviella* is basal to this group of genera (see Fig. 1), and its assignment to the Debaryomycetaceae is provisional. Future studies with expanded datasets will help resolve the issue of whether *Babjeviella* is a member of the Debaryomycetaceae or should be assigned to a new family.

Conclusions

The number of isoprene units in the side chain of coenzyme Q is generally shared by members of large phylogenetic groups, but the CoQ number is not genus specific. In the present study, our phylogenetic analysis of rRNA gene sequences has resolved one of these large groups into 12 well-supported clades, and these clades are interpreted as genera.

Despite strong bootstrap support for the proposed and emended ascosporic genera examined in this study, noticeable phenotypic differences are seen among species of some clades. For example, D-xylose is fermented only by the four species in the *S. stipitis* subclade of *Scheffersomyces*, and ascospore morphology is quite different among some species of *Priceomyces*. In this latter genus, *Pr. melissophilus* forms roughened, spherical ascospores, *Pr. carsonii* has smooth, spherical ascospores, but the other three assigned species, *Pr. castillae*, *Pr. haplophilus*, and *Pr. medius*, have hat-shaped ascospores. Consequently, these results support conclusions drawn from many earlier

phylogenetic studies, i.e., phenotype may not be predictive of genotype. For this reason, species assignments in many yeast genera defined from phenotype are now being markedly changed following phylogenetic studies based on gene sequence analyses. These findings also demonstrate that gene sequence analysis is now often necessary to recognize genera. As more species are discovered, some of these newly circumscribed genera may also change. In particular, weakly supported basal species may become the type species of sister genera. *Scheffersomyces spartinae*, which is only weakly associated with other members of *Scheffersomyces*, may prove to be an example of this possibility.

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