The yeast genus *Tortispora* gen. nov., description of *Tortispora ganteri* sp. nov., *Tortispora mauiana* f.a., sp. nov., *Tortispora sangerardonensis* f.a., sp. nov., *Tortispora cuajiniquilana* f.a., sp. nov., *Tortispora starmeri* f.a., sp. nov. and *Tortispora phaffii* f.a., sp. nov., reassignment of *Candida caseinolytica* to *Tortispora caseinolytica* f.a., comb. nov., emendation of *Botryozyma*, and assignment of *Botryozyma, Tortispora* gen. nov. and *Trigonopsis* to the family Trigonopsidaceae fam. nov.

M.-A. Lachance¹ and C. P. Kurtzman²

¹Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7
²Bacterial Foodborne Pathogens and Mycology Research Unit, National Center for Agricultural Utilization Research, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604, USA

We describe the yeast genus *Tortispora* gen. nov., an early-diverging lineage in the Saccharomycetales that displays the formation of helical ascospores. The genus is based on 16 strains resembling *Candida caseinolytica* that were isolated from necrotic plant tissue in warm regions of the New World. Based on sequences of the D1/D2 domains of the nuclear large subunit rRNA gene, as well as other data, the strains are assigned to eight distinct species. The species are nutritionally specialized and share the unusual ability to hydrolyse casein and to grow on 1-butanol as sole carbon source. One species of the proposed new genus produces a simple ascus with a helical ascospore, whereas other species of the clade have failed to form ascospores. All species in the clade, including *C. caseinolytica*, are assigned to *Tortispora* gen. nov. The new binomials are *Tortispora ganteri* sp. nov., type species of the genus (SUB 86-469.5T = CBS 12581T = NRRL Y-17035T), *Tortispora caseinolytica* f.a., comb. nov. (UCD-FST 83-438.3T = CBS 7781T = NRRL Y-17796T), *Tortispora mauiana* f.a., sp. nov. (UWOPS 87-2430.3T = CBS 12803T = NRRL Y-48832T), *Tortispora agaves* f.a., sp. nov. (UWOPS 94-257.6T = CBS 12794T = NRRL Y-63662T), *Tortispora sangerardonensis* f.a., sp. nov. (UWOPS 00-157.1T = CBS 12795T = NRRL Y-63663T), *Tortispora cuajiniquilana* f.a., sp. nov. (UWOPS 99-344.4T = CBS 12796T = NRRL Y-63664T), *Tortispora starmeri* f.a., sp. nov. (G 91-702.5T = CBS 12793T = NRRL Y-63665T) and *Tortispora phaffii* f.a., sp. nov. (UWOPS 91-445.1T = CBS 12804T = NRRL Y-48833T). In addition, species formerly assigned to the genus *Ascobotryozyma* are reassigned to the genus *Botryozyma*. The genera *Trigonopsis, Botryozyma* and *Tortispora* are assigned to the family Trigonopsidaceae fam. nov.

Abbreviations: f.a., forma asexualis (asexual form); ITS, internal transcribed spacers; UWO, University of Western Ontario.

The MycoBank numbers of *Tortispora ganteri* sp. nov., *Tortispora cuajiniquilana* f.a., sp. nov., *Tortispora mauiana* f.a., sp. nov., *Tortispora sangerardonensis* f.a., sp. nov., *Tortispora agaves* f.a., sp. nov., *Tortispora starmeri* f.a., sp. nov., *Tortispora caseinolytica* f.a., comb. nov. and *Tortispora phaffii* f.a., sp. nov. are MB803504 to MB803511, respectively.

The GenBank/EMBL/DDBJ accession numbers of the large subunit rRNA gene sequences of the strains representing species of the genus *Tortispora* determined in this study are KC681889–KC681899.
Necrotic tissues of cactus and other plants harbour a complex community that includes a large number of unique yeast species (Lachance et al., 1988). Of particular interest is *Candida caseinolytica* (Phaff et al., 1994), an infrequently isolated species named for the yeast’s strong extracellular proteolytic activity. The yeast is noteworthy phylogenetically in that it occupies an early diverging position within the order Saccharomycotales and appears to be closely related to the genus *Botryozyma* and its ascosporic state *Ascobotryozyma* (Kurtzman, 2011a; Kurtzman & Robnett 2013). A single strain of a similar yeast, but which forms ascospores, was isolated in Baja California by P.F. Ganter in 1986 (Lachance et al., 1988). A DNA reassociation experiment performed by H.J. Phaff (Lachance et al., 2011) indicated that the ascogenous strain could be regarded as a close relative of *C. caseinolytica*, but publication of a formal description was delayed in the hope that additional strains would become available. Diligent efforts by Ganter generated other non-sporogenous strains, as did other studies, but the original ascosporic strain remains unique. We have examined in detail 16 isolates recovered in a variety of studies and concluded that they can be assigned to eight different species on the basis of variation in DNA sequences and growth profiles. We now describe these species and propose the genus *Tortispora* gen. nov. to accommodate members of the clade. The ascosporic species *Tortispora ganteri* sp. nov., the type species of the genus, is named in honour of Philip F. Ganter, in recognition of his contributions to the discovery of yeast diversity. Six other mitosporic species are also proposed and *Candida caseinolytica* is transferred to *Tortispora* gen. nov. in conformity with Article 59 of the Melbourne Code (McNeill et al., 2012).

Elimination of the dual nomenclature traditionally used for fungi will hopefully be of benefit in the long-term. However, a less desirable outcome is a potential decline in the emphasis placed on sexual life cycles in yeast systematics and as a consequence, a reduction in the quality and information content of species descriptions (Lachance, 2012). With this in mind, in the interest of making it clear to readers that the mitosporic species of *Tortispora* gen. nov. are described based on asexual forms (anamorphs), the abbreviation ‘f.a.’, for *forma asexualis*, asexual form, has been added to the relevant names in the title and abstract of this publication. Article 4.1 of the Melbourne Code (McNeill et al., 2012) recognizes the *forma specialis*, assigned to certain parasitic fungi, but states in 4.4, Note 4, that the details of its usage are not governed by provisions of the Code. Thus, we treat the asexual form in a manner analogous to the special form but without a subspecific epithet. Consistent with this, the mention f.a. is not meant to affect typification of the species and the current typification applies to both the sexual and the asexual form, as required by Article 59. We do not anticipate that the eventual discovery of the sexual form will require any special action, as the mention f.a. is meant to concern the original species description only. Groenewald & Smith (2013) have taken the lead with the implementation of the asexual form in their reassignment of *Candida yakushimensis* to *Yarrowia yakushimensis* f.a., comb. nov.

**Yeast cultures and characterization**

Yeast isolates were obtained from the authors’ collections [Northern Regional Research Laboratory (NRRL) and University of Western Ontario Department of Plant Sciences (UWOPS)], where they had been deposited following various biodiversity research programs as detailed in Table 1. The prefixes ‘G’ and ‘SUB’ are used to denote strains received from P.F. Ganter, Biological Science Department, Tennessee State University, Memphis TN or W.T. Starmer, Department of Biology, Syracuse University, Syracuse NY, respectively. Sequencing followed direct PCR amplification of DNA from whole cells using various combinations of the primers NS7A, NL1, NL4 and NL5A (Kurtzman & Robnett, 2003) as well as IT1 and IT2 (Lachance et al., 2001). Alternatively, cells were freeze-dried, broken by shaking with glass beads and the DNA was then extracted (Kurtzman & Robnett, 2003).

Growth abilities were determined by replica plating in accordance with standard methods (Kurtzman et al., 2011). Growth on 1-butanol was assessed on yeast nitrogen base agar with 0.15% 1-butanol added immediately before pouring the plates. Casein hydrolysis was determined using a modification (Phaff et al., 1994) of the medium proposed by Ahearn et al. (1968). The medium contained 2.4% skim milk powder, 0.3% beef extract, 0.5% tryptone, 0.1% glucose and 1.5% agar. Dalmau plates were prepared with yeast carbon base agar supplemented with 0.01% yeast extract (YCBB). Cardinal growth temperatures were determined in the Microbiology Unit of the Biotron Institute for Experimental Climate Change Research of the University of Western Ontario (UWO). Replica plates (YM agar) of all isolates were incubated at temperatures from 30 to 44 °C (± 0.2) at one-degree intervals and at every even-numbered temperature from 4 to 12 °C. Growth was evaluated over 1 month. Light micrographs were taken with either bright-field or phase-contrast illumination on living material. Scanning electron microscopy was performed on cells deposited on a Cyclopore membrane (Whatman) with 0.4 μm pores that were later used for scale. The membrane was floated on a 2.5% glutaraldehyde solution in 0.3 M sodium cacodylate buffer, pH 7.0, for 15 min and rinsed twice in cacodylate buffer. The fixed material was then dehydrated in an ethanol series, critical-point dried and examined in a 3400 N Variable Pressure Scanning Electron Microscope (Hitachi; also at the Biotron, UWO) at full vacuum and potentials ranging from 10 000 to 20 000 V as required to obtain the best resolution. To determine whether ascosporic strains are homothallic or heterothallic, single ascospore isolations were made with a de Fontbrune micromanipulator.

**Sequence analyses and species delineation**

Amplification of DNA in the region spanning the internal transcribed spacers (ITS), the 5.8S rRNA gene and the
D1/D2 domains of the large subunit rRNA gene was complicated by the fact that primer IT1 (Lachance et al., 2001), which has been used successfully to amplify the ITS/5.8S rRNA gene region of vast numbers of yeast species, did not anneal to the corresponding site in any of the strains now assigned to the genus Tortispora. Although primer IT1 matches perfectly its intended binding site at the far 3’ end of the small-subunit rRNA gene of such diverse species as Botryozyma nematodophila or Schizosaccharomyces pombe, the amount of divergence observed at the various Tortispora spp. strains was nothing short of phenomenal, with as many as 11 substitutions in 24 nt. The use of primer NS7A resolved the problem and further allowed the discovery of a unique 322 nt intron in the small-subunit rRNA gene of strain UWOPS 94-257.6. Fortunately, the four strains with incomplete sequences can be assigned unambiguously to one or the other of two phylogenetic species based solely on D1/D2 sequences. The 16 isolates can be assigned to eight phylotypes (Fig. 1). Analysis of the combined ITS and D1/D2 dataset (not shown) identified minor polymorphisms in the ITS sequences of strains assigned to T. caseinolytica comb. nov. but confirmed the presence of the same eight distinct phylotypes. Representative sequences of every variant have been deposited in GenBank.

The species assigned to Tortispora gen. nov. form a monophyletic assemblage, at least to the extent of the phylogenetic signal contained in D1/D2 sequences. Individual BLAST searches (Altschul et al., 1990) of the GenBank database generally identified members of the genera Botryoascus or Trigonopsis as the nearest matches, but always at lower identities than observed for comparisons among Tortispora species. Phylogenetic analyses that included representatives of these and other genera always yielded the same topology for Tortispora species as shown in Fig. 1.

Whether or not species delineation is warranted by a small amount of sequence divergence is the subject of ongoing debate. Minor polymorphisms do occur in barcoding sequences such that observed discontinuities in sequence space may be the result of insufficient sampling and not
always a sign of speciation. The smallest degree of divergence observed in the present D1/D2 dataset was three substitutions separating a five-strain clade that included the type strain of *Candida caseinolytica* from a two-strain clade that contains the only known ascosporic strain in the complex. This sits at the limit between what can be interpreted as polymorphism and what should be treated as indirect evidence of reproductive isolation (e.g. Lachance et al., 2005). Four additional sources of evidence bear on the present case. One is the fact that all five isolates of *Tortispora caseinolytica* and both isolates of *Tortispora ganteri* sp. nov., respectively, are monomorphic in their D1/D2 sequences. Another element in support of separate species is the eight substitutions and five indels observed between the ITS sequences of the types of the two species (the two strains assigned to *T. ganteri* sp. nov. had identical ITS sequences and the four *C. caseinolytica* strains for which sequences in that region were available had two polymorphic sites). Also in favour of delineating two species is the approximately 50% DNA reassociation observed by H.J. Phaff (Lachance et al., 2011) between the ascogenous strain and the type of *C. caseinolytica*. Last is the remarkable correlation observed between phylotypes and growth responses, and keeping in mind that the strains utilize a relatively low number of carbon compounds, we feel that in the present case, it is appropriate to regard the two clades as distinct species.

Another matter of contention, regarding which the authors of this paper have openly expressed differing views (Fell et al., 2000; Kurtzman, 2010; Lachance, 2011), is whether it is desirable or even appropriate to describe species on the basis of a single isolate. Such a consideration indeed played a significant role in the fact that the genus *Tortispora* was not described at the time of its discovery. The delay was predicated on the hope that due diligence would result in the isolation of additional ascogenous strains, which would provide a better indication of the distribution and habitat specificity of the species. Added to this is the already mentioned desirability of identifying discontinuities in sequence space, which would have benefited from better sampling. However, due diligence has now been exercised and it is unlikely that additional efforts in the foreseeable future will alter the present findings. The result is that seven novel phylotypes, some of which represent relatively rare species related to *C. caseinolytica*, have been discovered and that further delays in publishing their descriptions would constitute a clear disservice to our collective knowledge of yeast biodiversity.
Table 2. Properties of species of the genus Tortispora gen. nov. based on all strains listed in Table 1

<table>
<thead>
<tr>
<th>Growth test</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>s</td>
<td>w</td>
<td>s</td>
<td>vw</td>
<td>s</td>
<td>w</td>
<td>vw/s</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>s</td>
<td>+</td>
<td>s</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sorbose</td>
<td>–/w/s</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xylose</td>
<td>s</td>
<td>s</td>
<td>w</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>–</td>
</tr>
<tr>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>+</td>
<td>s</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>–</td>
<td>s</td>
<td>s</td>
<td>vw</td>
<td>s</td>
<td>–</td>
<td>–</td>
<td>s</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>w/vw</td>
<td>w</td>
<td>–</td>
<td>s</td>
<td>w</td>
<td>s</td>
<td>vw/w</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>– (vw)</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>s</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mannitol</td>
<td>vw</td>
<td>vw</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>s</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glucitol</td>
<td>vw/w</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>s/w</td>
<td>–</td>
<td>s</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>+</td>
<td>+</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>–</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>Malic acid</td>
<td>+</td>
<td>s</td>
<td>+</td>
<td>s</td>
<td>–</td>
<td>–</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>D-Gluconic acid</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>vw</td>
</tr>
<tr>
<td>D-Gluconol-( \alpha )-lactone</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>NaCl (5 %)</td>
<td>s</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cycloheximide (10 mg l(^{-1}))</td>
<td>+</td>
<td>s</td>
<td>+</td>
<td>s</td>
<td>s</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cycloheximide (100 mg l(^{-1}))</td>
<td>s</td>
<td>–</td>
<td>s</td>
<td>vw</td>
<td>vw</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cycloheximide (1000 mg l(^{-1}))</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>s</td>
<td>s</td>
</tr>
<tr>
<td>CTAB (10 mg l(^{-1}))</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ethanol (6 %)</td>
<td>–</td>
<td>s</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>s (+, w)</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>s</td>
<td>s</td>
<td>s</td>
<td>–</td>
<td>–</td>
<td>s</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cadaverine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>s</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Phenylalanine as C and N source</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Minimum growth temperature (( ^\circ C ))</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Maximum growth temperature (( ^\circ C ))</td>
<td>40</td>
<td>42</td>
<td>38</td>
<td>40</td>
<td>32</td>
<td>37</td>
<td>42</td>
<td>41</td>
</tr>
</tbody>
</table>

Growth characteristics, ecology and phylogenetic affinities

Members of Tortispora gen. nov. share a distinctive growth profile. Sugar utilization is weak and restricted to galactose, trehalose, sorbose or xylose. Alditol utilization is also weak and is limited to mannitol or glucitol growth in some species. All strains examined utilized 2-ketogluconic acid. Most noteworthy however was the fact that all strains grew abundantly at the expense of 1-butanol and were able to hydrolyze casein. Although neither of these is a standard test used in yeast species descriptions, both are used routinely by some workers and thousands of yeast strains have been examined for these properties. An informal estimate is that 1-butanol is assimilated by less than 5 % of ascomycetous yeast species. Strong utilization of volatile compounds is common in the predominant yeast species found in cactus tissue or in sap fluxes (Lachance et al., 1988). The most frequently isolated cactophilic yeast species, Pichia cactophila, Candida sonorensis and Sporopachyderma spp., share a narrow, specialized carbon utilization profile combined with strong growth on ethanol in the first species, methanol in the second, or an diverse array of volatiles that include 1-propanol, 2-propanol, 1-butanol, acetone and ethyl acetate in the third. Cactophilic yeast species now reassigned to the genus Magnusiomyces also are avid utilizers of these compounds. Species of the achlorophyllous alga genus Prototheca, which are also regularly isolated from necrotic plant tissue of various kinds, superficially mirror species of the genus Tortispora by combining a strong utilization of 1-butanol with a sparse assimilation of carbohydrates. Volatile compounds are presumed to arise as end products of plant decay caused by bacterial agents (Fogleman & Foster, 1989).

The production of extracellular proteases that solubilize casein is characteristic of the Tortispora clade and was the basis for the specific epithet of its first described member
(Phaff et al. 1994), which contributed to the misidentification of some of the isolates included in the original description of C. caseinolytica. According to Poza et al. (2001), excretion of the enzyme is induced by the presence of casein, but not albumins, and is repressed in the presence of high concentrations (>1 %) of assimilable sugars (e.g. glucose, galactose, trehalose, sorbose or xylose). The enzyme has an unusually broad pH range of activity and apparently occurs as three ionic isoforms.

Members of the Tortispora clade are only infrequently isolated, which makes it difficult to draw generalizations on habitat and biogeography. This may be due in part to their weak growth on common isolation media, which could cause them to be overlooked in samples that abound in other, more vigorously growing, yeasts. All isolates originate in tropical to subtropical localities and were recovered from necrotic tissue of various plant species, but principally cacti. The strong proteolytic and lipolytic activity raises the possibility of a pathogenic adaptation, although we have no direct evidence of this and it is equally possible that the yeasts are saprophytes. The nearest relatives, members of the Botryozyma clade, share some similarities in their responses on standard growth tests (Kerrigan & Smith 2011a,b), but the single strain of Botryozyma nematodophila examined for the assimilation of 1-butanol and the hydrolysis of casein gave negative responses for both. The four known species of the clade were isolated in close association with nematodes found in necrotic tissue of poplars or rotting grapes and are thought to be dispersed by insects such as drosophilids or nitidulid beetles that feed and breed in this kind of substrate and often carry nematodes. Unlike members of the Tortispora clade, which reproduce by simple cell budding, species of the genera Ascobotryozyma and Botryozyma form a specialized thallus that consists of a short chain of elongated cells and most intriguingly, a T-shaped foot cell that is involved in adhesion to the cuticle of nematodes. Kerrigan & Rogers (2013) examined these structures in detail and concluded that they serve purely for attachment, that the adhesion is host-specific and that the yeasts’ relationship with the nematodes is commensalistic. The fungus Enteroramus dimorphus, now identified as Scheffersomyces stipitis, is also known to form attachment cells in guts of beetles, but these cells are absent in agar culture (Suh et al. 2004). S. stipitis is not closely related to Botryozyma and so these observations suggest that attachment cells may be more common than suspected among yeasts that interact with small invertebrates.

Assuredly the most interesting feature linking the Tortispora and Botryozyma clades is the remarkable similarity of their ascospore morphology. As shown in Fig. 2, Tortispora ganteri sp. nov. forms helical ascospores that show an astounding resemblance to those of Ascobotryozyma americana (Kerrigan & Smith 2011a). In the latter, however, typical ascii contain four ascospores, whereas T. ganteri forms only a single spore. The helical shape is unique amongst ascosporogenous yeasts and may be indicative not only of the kinship of the two clades, but also of a common adaptation. A recent analysis (Kurtzman & Robnett, 2013) of phylogenetic relationships among genera of Saccharomyces suggested the possibility of an affinity between C. caseinolytica, Botryozyma nematodophila and Trigonopsis variabilis, each acting as a representative of their respective clade. The low statistical support for such a clade, however, would suggest prudence in inferring monophyly without additional corroborating evidence. This prompted us to examine further the growth characteristics of members of the Trigonopsis clade. These include the four species included in the genus by Kurtzman (2011b) plus two isolates of an undescribed species related to Trigonopsis cantarellii and eight isolates of three undescribed species related to Trigonopsis vinaria, recovered from a variety of plant substrates and preserved in the UWOPS culture collection. We observed that members of the three clades share strikingly similar growth response profiles, characterized by a restricted range of sugar utilization that may include galactose, trehalose, sorbose and xylose, as well as the frequent utilization of 2-ketogluconic acid. All tested strains in the Trigonopsis clade utilized 1-butanol as did those assigned to Tortispora gen. nov., although, as mentioned already, this last characteristic was absent in the single strain of the genus Botryozyma examined. The utilization of 1-butanol occurs in some members of Kurtzman & Robnett’s (2013) clade 9, but the rest of the assimilation profiles are not similar to those shared by the genera Tortispora gen. nov., Botryozyma and Trigonopsis. Also of interest is that most species of in the Trigonopsis clade form relatively small cells. In particular, Trigonopsis vinaria (Kurtzman, 2011b) has cell sizes and shapes that are well in line with those observed in species of Tortispora gen. nov. (Fig. 3) Together, these observations lead us to propose the assignment of these taxa to a single family.

Taxonomy

Description of Tortispora Lachance & Kurtzman gen. nov.

Tortispora [tor.ti.spo’ ra. L. nom. f. n., from tortus, adjective meaning twisted (participle of torquer) and spora, spore]. Note that the name was created by the late H. J. Phaff (Lachance et al., 1988).

Growth is by multilateral budding of typically small cells. Species typically utilize 1-butanol as sole carbon source and produce extracellular proteases. Nitrate is not utilized as sole nitrogen source. Helical ascospores may be formed singly in an apical bud.

The type species of the genus is Tortispora ganteri Lachance & Kurtzman.

The MycoBank number is MB803503.

Description of Tortispora ganteri Lachance & Kurtzman sp. nov.

Tortispora ganteri (gan’ter.i. N.L. gen. m. n. ganteri of Ganter, in honour of Professor Philip F. Ganter, who
Fig. 3. Phase-contrast micrographs of budding cells of six new species of *Tortispora* gen. nov. after 3 days at 25 °C on YM agar. (a) *T. cuajiniquilana*; (b) *T. mauiana*; (c) *T. sangerardonensis*; (d) *T. agaves*; (e) *T. starmeri*; (f) *T. phaffii*. Bar, 5 μm.

Fig. 2. Scanning electron (a–d), bright-field (e, h, i) and phase-contrast (f, g, j) micrographs of *Tortispora ganteri* sp. nov. NRRL Y-17035 T after 3–5 days on YM agar at 25 °C. (a) Budding cells (bc) and mature asci (ma); (b) mature asci, including a deliquesced ascus (da); (c) older cell with bud scars near the apices; (d, e) free ascospores (as); (f) germination of ascospores by budding (ba) or formation of a conjugation tube (ct); (g) isogamous conjugation; (h) conjugation between complementary mating types (NRRL Y-17035-3 and NRRL Y-17035-7); (i, j) young asci. Bars, 1 μm (a, b–d); 5 μm (e–j).
collected the first isolate of the species and later made many attempts to recover additional sporulating isolates).

After 3 days on YM agar at 25 °C, the cells are ovoid to short cylindroid, 1–2 × 2–5 μm and occur singly, in pairs, or in short chains (Fig. 2a,e–j). Budding is multilateral but concentrated near the apices of the cell (Fig. 2c). The streak culture is white, smooth, glossy, butyrous, convex and slow-growing. Colonies are approximately 1 mm or less in diameter. Sporulating colonies are irregular and brown-coloured. After 10 days the Dalmau plate culture on YCB agar shows no signs of pseudohyphae or true hyphae. Ascospores (Fig. 2d,e: as) are twisted into a short helix with tapered ends, 1 × 5–7 μm, and germinate by swelling followed by budding (Fig. 2f) or the production of a conjugation tube (Fig. 2f) and zygote formation (Fig. 2g,h). Asci are induced by the transformation of a bud that can be elongated due to the presence of a spore (Fig. 2i) or remain spheroidal (Fig. 2j). Ascospores are released in the medium by ascus deliquescence (Fig. 2b). Sporulation is sparse at first in 3–5 day-old cultures on YM agar at 17 and 25 °C. Ascosporangia also occurs on 1 % malt extract, restricted growth (RG) and YCBAS (yeast carbon base with 0.01 % ammonium sulfate) agar media at 17 and 25 °C. Transfer of ascosporangia colonies to fresh medium can give rise to uniformly abundant ascus formation, but in some cases, serial transfer can cause loss of ascospore formation. Only the type strain (NRRL Y-17035T) is ascosporangious. In order to determine if the species is heterothallic or homothallic, single ascospores were isolated by micro-manipulation. Selection of 60 random spores gave four that were viable. Of these, three represented the h+ mating type (NRRL Y-17035-1, -2 and -3) and the fourth was the complementary h− mating type (NRRL Y-17035-7). The mating type designations h+ and h− are arbitrary. Isolates -3 and -7 represented the most fertile pair. Because ascospore viability was so low, it seemed possible that viability might be greater if spores of the complementary mating type were in contact as has been demonstrated previously for Saccharomyces ludwigii (Winge & Laustsen 1937). Growth resulted from three of four groups containing four random spores. Two of the resulting cultures showed neither ascosporangulation nor mating response, whereas growth from the third culture represented the h+ mating type. These results suggest the possibility of a lethal gene being present in some of the ascospores and that survival depends on circumventing the lethality through mating. In mixes of complementary mating types, conjugation was common, but the conjugants did not produce ascis with ascospores. DNA sequences of D1/D2, ITS and EF1-α were identical for NRRL Y-17035 and haploid strains -3 and -7. To a limited extent, these results rule out the possibility that NRRL Y-17035 is a hybrid. Two strains of the closely related T. caseinolytica were tested for mating response. NRRL Y-17796 represents the h+ mating type, but did not form ascospores, and NRRL Y-48831 showed neither mating response nor ascospore formation. These results are not in conflict with our proposal that the strains represent separate species, as conjugation among closely related yeast species is not uncommon (Kurtzman et al., 1980; Naumov et al., 2000; Lachance et al., 2005). The responses to growth tests are given in Table 2. The habitat is necrotic tissue of columnar cacti in Baja California.

The type strain, SUB 86-469.5T (CBS 12581T=NRRL Y-17035T), is the only sporulating strain and was isolated from a stem rot of Stenocereus gummosus (pitaya agria cactus) in Baja California by P.F. Ganter. Complementary mating types are NRRL Y-17035-3 (=CBS 12582) and NRRL Y-17035-7 (=CBS 12583).

The MycoBank number is MB803504.

**Description of Tortispora cuajiniquilana**

*Tortispora cuajiniquilana* (cu.ai.ni.qui.la’na. N.L. nom. f. adj. cuajiniquilana of Cuajiniquil Beach, Guanacaste Province, Costa Rica, after the locality where the only known isolate was recovered).

Growth on YM agar: After 3 days on YM agar at 25 °C, the cells are ovoid, 1–1.5 × 2–3 μm and occur singly or in pairs (Fig. 3a). The streak culture is white, smooth, semi-glossy, butyrous, convex and very slow-growing. Colonies are approximately 0.5 mm or less in diameter. After 10 days, the Dalmau plate culture on YCBY agar shows no signs of pseudohyphae or true hyphae. Ascis are not produced on common sporulation media. The responses to growth tests are given in Table 2. The habitat is necrotic tissue of columnar cactus in Costa Rica.

The type strain, UWOPS 99-344.4T (=CBS 12796T=NRRL Y-63664T), was isolated from liquid rot of a dead, unidentified columnar cactus on Cuajiniquil Beach, Guanacaste Province, Costa Rica, by M.A. Lachance.

The MycoBank number is MB803505.

**Description of Tortispora mauiana**

*Tortispora mauiana* (ma.u.i.a’na. N.L. nom. f. adj. mauiana of Maui Island, Hawaii, USA, after the locality where the only known isolate was recovered).

Growth on YM agar: After 3 days on YM agar at 25 °C, the cells are ovoid, 1–1.5 × 2–3 μm and occur singly or in pairs (Fig. 3b). The streak culture is white, smooth, semi-glossy, butyrous, convex and slow-growing. Colonies are approximately 1 mm or less in diameter. After 10 days, the Dalmau plate culture on YCBY agar shows no signs of pseudohyphae or true hyphae. Ascis are not produced on common sporulation media. The responses to growth tests are given in Table 2. The habitat is necrotic tissue of Opuntia ficus-indica in Maui.

The type strain, UWOPS 87-2430.3T (=CBS 12803T=NRRL Y-48832T), was isolated from a rotting cladode of Opuntia ficus-indica.

The MycoBank number is MB803506.

Description of Tortispora sangerardonensis Lachance & Kurtzman sp. nov.

Tortispora sangerardonensis (san.ge.rar.do.nen’sis N.L. fem. adj. sangerardonensis of or pertaining to San Gerardo, Costa Rica, after Estación San Gerardo of the Area de Conservación Guanacaste, where the only known isolate was recovered).

Growth on YM agar: After 3 days on YM agar at 25 °C, the cells are ovoid, 1.5–2 × 2.5–3 μm and occur singly or in pairs (Fig. 3c). The streak culture is white, smooth, semi-glossy, butyrous, convex and slow-growing. Colonies are approximately 1 mm in diameter. After 10 days the Dalmau plate culture on YCBY agar shows no signs of pseudohyphae or true hyphae. Asci are not produced on common sporulation media. The responses to growth tests are given in Table 2. The habitat is necrotic tissue of cacti in the Sonoran Desert of Arizona and Mexico.

The type strain, G91-702.5T (=CBS 12793T=NRRL Y-63665T), was isolated from necrotic tissue of Stenocereus thurberi (organ pipe cactus) in Arizona, USA, by P.F. Ganter.

The MycoBank number is MB803509.

Description of Tortispora phaffii Lachance & Kurtzman sp. nov.

Tortispora phaffii (phaf’ii.i. N.L. gen. m. n. phaffii of Phaff, in honour of the late Professor Herman Jan Phaff, yeast hunter par excellence, who participated in the discovery of this species and contributed to the characterization of the genus).

Growth on YM agar: After 3 days on YM agar at 25 °C, the cells are ellipsoid to elongate, 1 × 2.5–3 μm and occur singly or in pairs (Fig. 3f). Colonies are approximately 1 mm in diameter. The streak culture is white, smooth, semi-glossy, butyrous, convex and slow-growing. After 10 days the Dalmau plate culture on YCBY agar shows no signs of pseudohyphae or true hyphae. Asci are not produced on common sporulation media. The responses to growth tests are given in Table 2. The habitat is necrotic tissue of prickly pear cacti in Argentina.

The type strain, UWOPS 91-445.1T (=CBS 12804T=NRRL Y-48833T), was isolated from the necrotic cladode of Opuntia bonaerensis in Tucumán, Argentina, by M.A. Lachance and H.J. Phaff.

The Mycobank number is MB803511.

At the time of going to press, we were informed by Prof. C.A. Rosa (Federal University of Minas Gerais, Belo Horizonte, Brazil) of the recent recovery of three isolates of T. phaffii from lepidopteran larvae found in cactus tissue in the state of Tocantins, Brazil, adding support to its status as a South American endemic.

Emended description of Tortispora caseinolytica (Phaff, Starmer, Lachance & Ganter) Lachance & Kurtzman comb. nov.


Type: UCD-FST 83-438.3T (=CBS 7781T=NRRL Y-17796T)

Some strains are capable of mating with strains of Tortispora ganteri, but the zygotes do not bear asci. In

The MycoBank number is MB803508.

Description of Tortispora starmeri Lachance & Kurtzman sp. nov.

Tortispora starmeri (star’me.ri. N.L. gen. m. n. starmeri of Starmer, referring to Professor William T. Starmer, who spearheaded the study of cactophilic yeasts of the Sonoran Desert of Arizona and Mexico).
view of small differences in growth responses between authentic strains of the species and the results given in the original description, the description of growth characteristics is amended as detailed in Table 2.

The MycoBank number is MB803510.

At the time of going to press, we were informed by Prof. C.A. Rosa (Federal University of Minas Gerais, Belo Horizonte, Brazil) of the recent recovery of three isolates of T. phaffii from lepidopteran larvae found in cactus tissue in the state of Tocantins, Brazil, adding support to its status as a South American endemic.

Emended description of Botryozyma Shann & M.Th. Smith emend. Lachance & Kurtzman


Phylogenetic analysis of D1/D2 LSU rRNA gene sequences demonstrated that the two described species of Botryozyma are quite closely related to one another as well as to the two known species of the genus Ascobotryozyma (Kerrigan & Smith, 2011a), and thus represent a single genus in the new single nomenclature system. The Melbourne Code assigns equal weight to anamorphic and teleomorphic genus names. Because the genus Botryozyma (1992) has priority of date over Ascobotryozyma (2001), we propose assignment of species of this clade to the genus Botryozyma. In the original descriptions of Ascobotryozyma americana and Ascobotryozyma cognata, the anamorphic states Botryozyma americana and Botryozyma cognata were also described and so these names are available for use.

The original description is emended to include the fusion of thallus cells followed by the formation of a spheroid bud that becomes an ascus contains up to four helical ascospores.

Type species: Botryozyma nematodophila Shann & M.Th. Smith

Species included:


Botryozyma nematodophila Shann & M. Th. Smith (1992)


Description of Trigonopsidaceae Lachance & Kurtzman fam. nov.

Inclusion of the three genera Trigonopsis, Botryozyma and Tortispora gen. nov. in the family Trigonopsidaceae fam. nov. is warranted by the inferred monophyly of these genera (Kurtzman & Robnett, 2013), as well as a number of shared morphological or physiological characteristics. The family occupies an early-diverging position among genera of the Saccharomycetales. In ascosporic species, the spores are helical with tapering ends and arise in a bud formed by cells following isogamous conjugation. The growth profile is characterized by a narrow range of sugar utilization that may include galactose, trehalose, sorbose or xylose, the frequent assimilation of 2-ketogluconate or 1-butanol and absence of growth on nitrate as sole nitrogen source. Alditol utilization may include that of mannitol, glucitol and in some cases erythritol. Most simple carboxylic acids commonly tested are utilized. The cells are either relatively small, with diameters that can reach less than 1 μm, or they can be arranged in thalli with a T-shaped cell that has the ability to adhere to the cuticle of nematodes. Mycobank number: MB803502.

Type species: Trigonopsis variabilis Schachner (1929)

Genera included:

Trigonopsis Schachner emend. Kurtzman & Robnett

Botryozyma Shann & M.Th. Smith emend. Lachance & Kurtzman

Tortispora Lachance & Kurtzman

Acknowledgements

We acknowledge funding by the Natural Science and Engineering Council of Canada to MAL. Field assistance by Jane Bowles (University of Western Ontario), Dan Janzen (University of Pennsylvania), Tom Starmer (Syracuse University), and the personnel of Tequila Herradura SA de CV are gratefully acknowledged. The late Herman Phaff, Dorothy Spencer and Frank Spencer also contributed to the collections. We thank Christie Robnett (ARS-URDA, Peoria, Illinois) for technical assistance and Richard Gardner (Biotron, University of Western Ontario), for help with scanning electron microscopy. We are most indebted to Phil Gaither (Tennessee State University) for the gift of strains. The mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The USDA is an equal opportunity provider and employer.

References


