Meyers et al. (1970) in their study of the oyster grass (Spartina alterniflora Loisel.) marshes in the Barataria Bay region of southeastern Louisiana found the marsh sediment to have up to 90,000 viable yeast cells per cm³. Predominant in this strikingly high yeast population was a new species, Pichia spartinae Ahearn, Yarrow et Meyers. In the species description, ascospores were reported to be spheroidal or infrequently oval with a slight hemispherical ledge (Ahearn et al., 1970). Unfortunately, the strains of P. spartinae available from that work no longer sporulate.

During the spring of 1974, cultures of P. spartinae were again isolated from oyster grass around Barataria Bay (Meyers et al., 1975). The carbon assimilation and fermentation patterns of these new isolates were identical with previously isolated P. spartinae cultures, but in contrast, all produced hat-shaped ascospores. One of these cultures, NRRL Y-7665, sporulated better than the others and was chosen for further study. Since ascospores were formed most abundantly on 5% malt extract agar after 5 to 7 da at 25 C, this medium was used in all subsequent mating and sporulation tests.

Ascus formation was preceded by either isogamous or heterogamous conjugation. Occasionally one, but usually two, hat-shaped spores formed in one of the conjugants (Fig. 1). Asci ruptured on maturity, liberating the spores. Poorly formed spores were common in all the freshly isolated cultures. They were spheroidal to ellipsoidal with a somewhat indistinct wall and had either no ledge or only a poorly formed ledge. As shown by scanning electron microscopy (SEM), well-formed spores are definitely hat-shaped (Fig. 2). Techniques for SEM have been previously reported (Kurtzman et al., 1974).

Single spores (27 of 28 were viable) isolated by micromanipulation from NRRL Y-7665 produced asporogenous colonies, but appropriate
pairs conjugated and formed hat-shaped spores. Not all pairs were equally fertile, and one of the better sporulating pairs, NRRL Y-7665–1 and NRRL Y-7665–3, was selected for subsequent study. This pair gave 1% conjugation, and about half of the conjugants formed spores. The type strain of *P. spartinae* NRRL Y-7322 (FST 119, CBS 6059) failed to sporulate on a variety of media but conjugated with NRRL Y-7665–3 to form hat-shaped ascospores. There was no conjugation or sporulation when NRRL Y-7322 was mixed with NRRL Y-7665–1, the opposite mating type. Evidently one mating type predominated to the exclusion of the other during laboratory cultivation, and the type strain became haploid. This phenomenon appears to be common and also has been observed in *Saccharomyces lipolytica* (Wickerham, Kurtzman et Herman) Yarrow, *Pichia angophorae* Miller et Barker, and *P. terricola* van der Walt (Kurtzman, Kurtzman and Smiley, unpublished data). Wickerham and Burton (1954) found that when certain haploid strains of *Pichia ohmeri* (Etchells et Bell) Kreger-van Rij were mated, hat-shaped ascospores formed, but other mating types produced spheroidal ascospores without ledges. However, there is no evidence to suggest that this also happens in *P. spartinae*.

Spencer et al. (1974) reported isolating *P. spartinae* from lakes and rivers in Saskatchewan. We obtained five of their strains (NRRL Y-7689 through NRRL Y-7693) and found them to be identical to *P. spartinae* in carbon assimilation and fermentation, but in contrast, they failed to grow on osmotic medium or at 37°C. We did not observe any of the five to sporulate when grown alone or in mixtures with one another. Neither conjugation nor sporulation occurred when the five were paired with NRRL Y-7665–1, NRRL Y-7665–3, or NRRL Y-7322. Since these five cultures also differ somewhat in morphology and physiology from other known species of *Pichia*, they may represent a new species, but this will remain uncertain until sporulation is achieved.

The physiological characteristics of several heterothallic species of *Pichia* and certain species of *Candida* are similar to *P. spartinae*. However, there was no mating response between NRRL Y-7665–1 or NRRL-7665–3 and the mating types of *P. angophorae*, *P. rhodanensis* (Ramirez et Boidin) Phaff, *P. wickerhamii* (van der Walt) Kreger-van Rij, or *P. veronae* Kodama. *Pichia spartinae* also failed to mate with *Candida freyschussii* Buckley et van Uden, *C. maritima* (Siepmann) van Uden et Buckley, *C. obtusa* (Dietrichson) van Uden et do Carmo-Sousa ex van Uden et Buckley, and *C. oregonensis* Phaff et do Carmo-Sousa.

Our data show *P. spartinae* to be a heterothallic yeast to date found only with oyster grass in the Louisiana marshes. When mature the
ascospores of this species are hat-shaped. However, the ascospores frequently do not mature properly, and this led to the misinterpretation of spore shape given in the original species description.

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LITERATURE CITED


