Chapter 2
Yeasts in Aquatic Ecotone Habitats

Allen N. Hagler, Leda C. Mendonça-Hagler, and Fernando C. Pagnocca

Abstract Aquatic ecotone habitats, like wetlands and phytotelmata, contain higher nutrient levels than are found in open waters resulting from degradation of organic materials like leaf litter. This allows much larger autochthonous yeast populations to develop than in more traditionally studied open water habitats. The single-celled morphology of yeasts makes them naturally better adapted than filamentous fungi to fluid habitats. Heavy influence exists from the extensive phylloplane yeast populations, which have also received little study until recently, and of animals attracted to these resources. *Debaryomyces hansenii*, *Pichia membranifaciens*, *Candida* spp., *Papiliotrema laurentii*, *Naganishia albida*, and *Rhodotorula mucilaginosa* were the most common yeasts detected in these habitats. Some species have a strong association with specific types of aquatic ecotone habitat like *Kluyveromyces aestuarii* in mangroves, *Scheffersomyces spartinae* in salt marshes, and *Kazachstania bromeliacearum* in bromeliad phytotelmata. Yeast diversity in aquatic ecotones is very rich in species occurring at low frequency making these habitats good targets for bioprospecting. The studies of estuaries, mangroves, salt marshes, bogs, and phytotelmata resulted in a list of over 270 identified yeasts and many additional unidentified cultures such as those reported as *Candida* spp. and as the former polyphyletic genera *Cryptococcus* spp. and *Rhodotorula* spp., many of which have since been described as new taxa.

Keywords Yeasts • Wetlands • Mangroves • Phytotelmata • Bromeliads • Bogs • Fens • Estuaries

Contents

A.N. Hagler (✉) • L.C. Mendonça-Hagler
Univ. Fed. do Rio de Janeiro (UFRJ) IMPPG, PBV, Ilha do Fundão, CCS, bl I, Rio de Janeiro
21941-970, Rio de Janeiro, Brazil
e-mail: allenhagler@gmail.com

F.C. Pagnocca
São Paulo State University (UNESP), Institute of Biosciences, Rio Claro 13506-900,
São Paulo, Brazil

P. Buzzini et al. (eds.), *Yeasts in Natural Ecosystems: Diversity*,
DOI 10.1007/978-3-319-62683-3_2

2.1 Introduction

Yeasts are found in all kinds of aquatic habitats, but in low numbers of less than $10\,\mathrm{l}^{-1}$ in open ocean and lake waters, in larger populations up to $500\ \mathrm{l}^{-1}$ near terrestrial influence of the shore and often over $1000\,\mathrm{l}^{-1}$ in eutrophic waters such as in aquatic ecotones. Open water habitats of oceans, bays, lakes, ponds, rivers, and streams have been the subject of many studies of yeast communities or guilds (Hagler and Ahearn 1987; Nagahama 2006; Kutty and Philip 2008). The yeast diversity in freshwater of worldwide lakes and rivers and in nearshore and offshore seawater is summarized in Chap. 1 of this book. However, natural aquatic ecotones with much higher nutrient levels in their waters have received little attention in studies of yeast ecology. Yeasts survive well in salt- and freshwater in pure culture but compete in mixed cultures (Ahearn et al. 1968; Hagler and Mendonça-Hagler 1979; Starmer and Lachance 2011). They survive so well that suspensions of pure cultures in distilled water are used as a method to maintain fungi, including yeasts, for prolonged periods (McGinnis et al. 1974). The tendency to be unicellular should favor growth of yeasts over filamentous fungi in aquatic habitats (Lachance and Starmer 1998). The prevalent species in some types of aquatic ecotones are compared in Table 2.1. Estuaries are a classic example of ecotone habitats as interphases between marine and freshwaters carrying nutrients and microbial populations from terrestrial to marine ecosystems (Odum 1993).

Human-associated yeasts are frequent in most estuaries and can serve as pollution indicators (Hagler et al. 1986; Hagler 2006; Starmer and Lachance 2011). Artificial ponds for sewage treatment, recreational activities, and cultivation of aquatic life are dominated by species entering with sewage or fertilizers making them more the subject of industrial and public health microbiology and not included in this chapter. Wetland areas have relatively less volume of water and contain high amounts of degrading leaf litter and other organic materials compared with open waters (Fig. 2.1). This concentration of nutrients makes wetlands prime locations for feeding and breeding sites of many animals which can vector yeasts into the habitat. The shallow waters in these habitats are closely associated with organic and inorganic sediments supporting development of large fungal populations, including yeasts, in complex communities associated with the specialized flora and fauna of such regions (EPA 2016). Although these conditions favor diverse natural yeast communities, such habitats have not received much study. Sampling is complicated by uneven distribution in diverse microhabitats. Difficult access, unpleasant odors, mud, and insects are frequent factors. If close to human population centers, wetlands are often drained or contaminated by sewage. Smaller and somewhat ephemeral isolated volumes of water that are not wetlands, but have similar function in

Table 2.1 Prevalent yeasts in aquatic ecotones

	Estuaries	Mangroves	Bogs and fens	Phytotelmata
Yeasts	$N = 167$	$N = 234$	$N = 47$	$N = 238$
Aureobasidium pullulans	2	2	1	2
Candida aff *famata*[a]	3	17	4	8
Candida boidinii	4	8		
Candida glabrata	2	4		
Candida intermedia	4	5		10
Candida parapsilosis	3	10		3
Candida sake		5	1	3
Candida spp.	3	14	4	5
Candida tropicalis	2	15		3
Clavispora lusitaniae	2	4		1
Cyberlindnera saturnus	2	5	1	3
Cystobasidium minutum	2	1	1	2
Debaryomyces hansenii	3	6	4	5
Diutina aff *rugosa*		7		
Diutina rugosa		6		1
Hanseniaspora uvarum	3	6	2	3
Kazachstania bromeliacearum		2		7
Kazachstania exigua	1	4		1
Kluyveromyces aestuarii	1	13		3
Kodamaea ohmeri	2	5		3
Meyerozyma guilliermondii	4	12	1	4
Naganishia albida	3	1	3	8
Papiliotrema laurentii	5	1	4	7
Pichia kudriavzevii	5	11		
Pichia membranifaciens	3	14	3	7
Pichia occidentalis	1	10		3
Pichia spp.	1	3	1	2
Rhodotorula glutinis	4	2	3	4
Rhodotorula mucilaginosa	6	9	6	9
Rhodotorula spp.	5		2	1
Saccharomyces cerevisiae	3	6		5
Saturnispora silvae	1	4		2
Schwanniomyces vanrijiae		6	2	2
Sporobolomyces roseus	1		4	1
Torulaspora delbrueckii		4		4
Torulaspora sp. anamorph		7		4
Wickerhamomyces anomalus	2	6		1
Yarrowia lipolytica	2	6	1	2
Zygoascus hellenicus	1	4		1

N = total number of samples in each type of ecotone in the 30 studies noted in Tables 11.2, 11.3, 11.4, and 11.5

Nomenclature was updated using Kurtzman et al. (2011a), Species Fungorum (2016) and MycoBank Database (2016)

[a] Includes anamorphic cultures of several species phenotypically similar to *Debaryomyces hansenii*

Fig. 2.1 The Sammamish River in Redmond Washington showing typical wetland vegetation with peat bog in the willows behind them

many terrestrial habitats, are phytotelmata (plant ponds). They are aquatic ecotones involving the plants and water but not soil. Although small and ephemeral relative to humans, they are large and permanent enough to support significant microbial populations. Examples are holes in tree trunks and cups formed by leaves and flowers. Bromeliad tanks are a good example and very important in diverse neotropical habitats especially in the forest canopy. These wetland habitats and phytotelmata have in common the degradation of large amounts of lignocellulose and other plant materials in shallow waters or water-saturated sediments. The plant species in such habitats are limited in diversity by exclusion of oxygen from the roots, but wetlands can support large and diverse microbial and animal populations. Hydrothermal vents in the oceans are another notable form of aquatic ecotone but fed by chemoautotrophic bacteria rather than degrading organic matter and not included in this chapter (Gadanho and Sampaio 2005; Nagahama 2006; Le Calvez et al. 2009). The aquatic surface films, sediment-water interface, and associated benthic organisms in all aquatic habitats are ecotones and have more concentrated nutrients than the water (Hagler and Ahearn 1987; Kachalkin 2014) and are part of more conventional marine and freshwater habitats. Diverse yeast populations are consistently present in waters, sediments, and biota of aquatic ecotones.

2.2 Estuaries

Few studies have been made of pristine estuaries. The prevalent species from some examples with different levels of urban influence increasing from left to right are presented in Table 2.2. The yeast species most typical of estuaries are *Candida* spp.

Table 2.2 Estuarine yeasts present in more than five samples

	Florida Suwannee	Florida Suwannee	Florida Biscayne Bay	Portugal Tagus	Portugal Sado	Portugal Tagus	Brazil UFRJ
	Sediment	Water	Sediment	Water	Water	Water	Water
	1	1	2	3	4	4	5
Yeasts	$N = 9$	$N = 9$	$N = 45$	$N = 84$	$N = 4$	$N = 4$	$N = 12$
Aureobasidium pullulans			2				5
Candida boidinii	1		1	1			2
Candida famata	3	1				3	
Candida intermedia			1		9	3	4
Candida parapsilosis			10	9			3
Candida sorboxylosa							7
Candida spp.	3		1				10
Candida tropicalis			19				7
Candida zeylanoides				9			1
Clavispora lusitaniae				5			1
Cutaneotrichosporon cutaneum	1	1	3			1	4
Cyberlindnera saturnus	4	1					
Cystobasidium minutum		1	9				
Debaryomyces hansenii			7	11			9
Debaryomyces spp.					20	1	
Dipodascus klebahnii	1						9
Hanseniaspora uvarum					23	2	9
Kazachstania exigua							8
Meyerozyma guilliermondii		1	2	8			4
Papiliotrema laurentii	4	1	4	2	3		
Pichia fermentans	4					2	

(continued)

Table 2.2 (continued)

	Florida Suwannee	Florida Suwannee	Florida Biscayne Bay	Portugal Tagus	Portugal Sado	Portugal Tagus	Brazil UFRJ
	Sediment	Water	Sediment	Water	Water	Water	Water
	1	1	2	3	4	4	5
Yeasts	$N = 9$	$N = 9$	$N = 45$	$N = 84$	$N = 4$	$N = 4$	$N = 12$
Pichia kudriavzevii	3	1	1	1			12
Pichia membranifaciens	4	1					4
Pichia occidentalis							11
Pichia terricola						1	7
Rhodotorula glutinis	1		9	1		3	
Rhodotorula mucilaginosa	3	1	14	25		3	1
Rhodotorula spp.	2	2	1		9	2	
Wickerhamomyces anomalus							6
Yarrowia lipolytica				1			4

N = number of samples. References: 1 = Lazarus and Koburger (1974); 2 = Fell et al. (1960); 3 = Coelho et al. (2010); 4 = Taysi and van Uden (1964); 5 = Hagler and Mendonça-Hagler (1981). Nomenclature was updated using Kurtzman et al. (2011a), Species Fungorum (2016), and MycoBank Database (2016) (especially *Candida intermedia*, *Candida parapsilosis*, and *Candida tropicalis*), *Debaryomyces hansenii*, *Pichia kudriavzevii* (*Issatchenkia orientalis* and *Candida krusei*), *Meyerozyma* (*Candida*) *guilliermondii*, *Cutaneotrichosporon* (*Trichosporon*) *cutaneum*, *Papiliotrema* (*Cryptococcus*) *laurentii*, and *Rhodotorula* spp. (especially *Rhodotorula glutinis* and *Rhodotorula mucilaginosa*). Fell et al. (1960) started a sequence of studies of yeasts in estuaries, most of them near major urban centers. Various studies have been made of the Tagus river estuary in Portugal since the 1960s (Taysi and van Uden 1964). Coelho et al. (2010) working in the same estuary, but with various cultivation methods and the identifications of cultures by rDNA gene sequences, estimated the influence from marine, terrestrial runoff, urban effluents, and resident populations from samples taken in a transect downstream from Lisbon. Some species were associated with different sources entering the estuary. *Mey. guilliermondii*, *C. parapsilosis*, and *Clavispora lusitaniae* made up most of the isolates at 37 °C, and the counts at this temperature had a high correlation with counts of the fecal indicator *Escherichia coli*. Hagler et al. (1986) also found a high correlation of 40 °C yeast counts, largely of *P. kudriavzevii* and *C. tropicalis*, with coliform counts in Rio de Janeiro. High-temperature yeast counts can serve as indicators of domestic sewage (Hagler 2006). *Rh. mucilaginosa* was found as the prevalent species by Coelho et al. (2010) and considered to be mostly from terrestrial runoff although this species is also common in seawater and lakes (Hagler and Ahearn 1987). *Deb. hansenii* was considered a marker of marine origin although it is a species associated also with diverse nonmarine habitats. Pollution from urban areas is an important factor in most estuaries, and human-associated yeasts were very evident in the heavily polluted estuary site in Guanabara Bay, Rio de Janeiro (Hagler and Mendonça-Hagler 1981), but much less present in the more pristine Suwannee river estuary (Lazarus and Koburger 1974) and Everglades (Ahearn et al. 1968). An interesting strategy was used to detect yeasts of the Tagus river estuary, Portugal, by Gadanho and Sampaio (2004). The PCR-DGGE method of Muyzer (1999) was applied to analyze two water samples directly from environmental DNA and DNA extracted after cultivation on solid and liquid enrichment media. This combined procedure increased the species richness assessed in the water samples, allowing the detection of species present in low abundance or less competitive in culture. Ascomycetous species were not detected by PCR-DGGE using environmental DNA, but were after cultivation of the samples on YM agar or broth. The prevalent species detected in this study were *Deb. hansenii*, *Rh. mucilaginosa*, and *Vanrija longa* (*Cryptococcus longus*). Sites with brackish water from ice melts and tidal action similar to an estuary were studied in the north of Russia where salinity in the Kandalaksha Gulf does not exceed 20 ppt. Kachalkin (2014) found in water, sand, silt, and a sponge in the intertidal zone some species typical of estuaries in warmer regions, but also various obligate psychrophilic species including a new species *Glaciozyma litoralis*. The largest yeast populations in these two sites were associated with healthy algae and over 10,000 CFU g^{-1} dominated by *Metschnikowia zobellii,* whereas yeast counts in water were about 100 l^{-1} and much lower than in other substrates of the intertidal zone.

2.3 Mangroves

Mangroves are a type of tropical swamp with influence of tidal action and marine water, and like salt marshes are typically found in estuaries where they show a high level of microbial diversity (Ghizelini et al. 2012; Pires et al. 2012; Fig. 2.2). The yeast counts in water entering the mangrove vegetation of the Coroa Grande mangrove in Sepetiba Bay, Rio de Janeiro, had a geometric mean of 340 l^{-1}. Yeast counts were about 10 times higher in sediments than in water and 100 to 1000 times higher in the intestines of invertebrates living in this mangrove. Counts of filamentous fungi were typically one to two orders of magnitude higher than yeast counts in the same mangrove area (Araujo et al. 1995). The more frequent yeasts found in mangroves are presented in Table 2.3. Ahearn et al. (1968) made an extensive study of yeast communities in South Florida saline and freshwater habitats including the Everglades. More than 50 species were identified with the less advanced taxonomic methods of that time, and representative species were tested for survival as pure or mixed cultures in fresh- and seawater. Among them in all estuarine mangrove locations was *Kluyveromyces aestuarii* that had been described by Fell (1961) from a Miami Florida estuary. It has since been found in mangroves of Rio de Janeiro, Brazil (Araujo and Hagler 2011), in China (Chi et al. 2012), and in Thailand where it occurred with another very similar species, *Kluyveromyces siamensis*, described by Am-In et al. (2008). The consistent presence of *K. aestuarii* in this habitat and absence from others suggested its use as an indicator organism (Araujo and Hagler 2011). Urban beaches of mangrove sediments, but cleared of mangrove vegetation, had more human-associated yeasts including *Candida glabrata*, *C. tropicalis*, *C. parapsilosis*, *P. kudriavzevii*, and

Fig. 2.2 Mangrove at Mosqueiro, Aracaju, Sergipe, Brazil, on the margin of the estuary of Rio Vaza-Barris showing typical tree seedlings and aerial roots, but no smaller plants

Table 2.3 Yeasts found in more than five samples from mangroves

Substrate	Water	Water	Various	Sediment	Crabs	Crabs	Mollusks	Mollusks	Mollusks
Country	USA (a)	Thailand (b)	China (c)	Brazil RJ (d)	Brazil RJ (e)	Brazil RJ (f)	Brazil RJ (g)	Brazil RJ (h)	Brazil RJ (i)
References	1	5	4	3, 6	2	2	2	2	3
Yeast	$N = 44$	$N = 7$	$N = 6$	$N = 45$	$N = 16$	$N = 21$	$N = 18$	$N = 14$	$N = 26$
Candida aaseri			11		1				
Candida aff *famata*[a]				2	3	2	6	13	3
Candida boidinii			1	1		1	6	4	3
Candida intermedia			11	1			1	1	
Candida parapsilosis		2	6		1			1	2
Candida spp.				6	1	5	3	1	1
Candida tropicalis		1	74	4		1	1	2	3
Cystofilobasidium infirmominiatum	16								
Debaryomyces hansenii	25		12						
Kluyveromyces aestuarii	14		12	34	14		13	1	
Kluyveromyces siamensis			10						
Kodamaea ohmeri			15		1		1	2	
Meyerozyma guilliermondii		2	3		1	2	2	4	1
Naganishia albida	18								
Papiliotrema laurentii	59								
Pichia kudriavzevii	14		8				7	3	16
Pichia membranifaciens				10	3	3	14	3	8
Pichia occidentalis			1	1		1	3	3	6
Rhodotorula glutinis	21								
Rhodotorula mucilaginosa	18	2	2	3					2

(continued)

Table 2.3 (continued)

Substrate	Water	Water	Various	Sediment	Crabs	Crabs	Mollusks	Mollusks	Mollusks
Country	USA (a)	Thailand (b)	China (c)	Brazil RJ (d)	Brazil RJ (e)	Brazil RJ (f)	Brazil RJ (g)	Brazil RJ (h)	Brazil RJ (i)
References	1	5	4	3, 6	2	2	2	2	3
Yeast	$N = 44$	$N = 7$	$N = 6$	$N = 45$	$N = 16$	$N = 21$	$N = 18$	$N = 14$	$N = 26$
Torulaspora sp. anamorph				4		1	2	3	2
Wickerhamomyces anomalus			9				2	2	2
Yamadazyma triangularis	11								
Yarrowia lipolytica			6				1	3	2

N = number of samples. 1 = Ahearn et al. (1968); 2 = Araujo et al. (1995); 3 = Araujo (1999); 4 = Chi et al. (2012); 5 = Limtong et al. (2008a); 6 = Soares et al. (1997)

Origin of strains: (a) Florida, Everglades; (b) Khao Lumpee-Haad Thaimueang and Mu Ko Ra-Ko Prathong; (c) Fujian, Guangdong, and Hainan provinces; (d) sediment under mangrove vegetation; (e) detritivores crabs *Sesarma rectum* and *Uca* spp.; (f) herbivorous or omnivorous crabs *Goniopsis cruentata* and *Aratus pisonii*; (g) Rio de Janeiro *Anomalocardia brasiliana* and *Tagelus plebeius*; (h) mussel *Mytella guyanensis*; (i) shipworm *Neoteredo reynei*. RJ = Rio de Janeiro

[a] Anamorphs similar to *Debaryomyces hansenii*. Nomenclature was updated using Kurtzman et al. (2011a), Species Fungorum (2016), and MycoBank Database (2016)

Mey. guilliermondii and absence of *K. aestuarii* (Hagler et al. 1982; Soares et al. 1997). The intestines of detritus feeding crabs and a filter-feeding shipworm (mollusk) in the Coroa Grande mangrove in Rio de Janeiro were all found to contain *K. aestuarii*. However, it was not found in predatory and leaf-feeding crabs or a mussel attached to tree roots under the sediment, and it was nearly absent from clams in the mud flat of the same location (Araujo et al. 1995). *K. aestuarii* appears to be endemic to mangroves and associated with the detritus, presumably from degrading mangrove leaves at the sediment surface. The yeasts common in open waters are present in mangroves. But, there are also a striking number of diverse species isolated at low frequency, making a total of over 130 species in the work included in this review, many of which were probable new species. Many new species have been described from mangroves. These include *Candida sharkiensis*, *Candida rhizophoriensis*, *Sakaguchia* (*Rhodotorula*) *cladiensis*, *Rhodotorula evergladiensis*, and *Papiliotrema mangalensis* (*Cryptococcus mangaliensis*) (Fell et al. 2011); *Candida spencermartinsiae*, *Candida taylorii*, and *Ustilago* (*Pseudozyma*) *abaconensis* (Statzell-Tallman et al. 2011); *Candida chanthaburiensis*, *Candida kungkrabaensis*, and *Candida suratensis* (Limtong and Yongmanitchai 2010); *Candida thaimueangensis* (Limtong et al. 2007); *Geotrichum siamensis* and *Geotrichum phurueaensis* (Kaewwichian et al. 2010); *K. siamensis* (Am-In et al. 2008); *Kwoniella mangroviensis* (Statzell-Tallman et al. 2008); *Lachancea meyersii* (Fell et al. 2004); *Martiniozyma* (*Candida*) *asiatica* (Limtong et al. 2010a); *Rhodotorula paludigena* (*Rhodosporidium paludigenum*—Fell and Tallman 1980); *Saturnispora* (*Candida*) *siamensis* (Boonmak et al. 2009); *Candida phangngensis* (Limtong et al. 2008a); *Saturnispora* (*Candida*) *sanitii* and *Saturnispora* (*Candida*) *suwanaritii* (Limtong et al. 2010b); *Tetrapisispora arboricola* (Ueda-Nishimura and Mikata 1999); and *Torulaspora maleeae* (Limtong et al. 2008b).

Recent studies on fungal diversity in mangroves of New Caledonia used 454-pyrosequencing method, in which DNA was extracted directly from the environmental samples. Sequences from four regions of rDNA (ITS1, ITS2, SSU V5, and SSU V7) were obtained for fungi from submerged and aerial parts of trees. Species richness values were dependent on the gene marker used, ranging from 271 to 1001 OTUs (operational taxonomic units) and with the larger values for ITS sequences. Ascomycetes were dominant with 82% of the sequence reads, whereas Basidiomycetes represented 3%, and 15% could not be assigned to known taxa (Arfi et al. 2012a). The fungal diversity associated with anoxic-sulfidic sediments in the same mangrove was assessed by 454-pyrosequencing using the ITS1 and ITS2 regions (Arfi et al. 2012b). Over a hundred distinct OTUs were detected mostly of filamentous fungi but included the yeasts *Dipodascus australiensis*, *Galactomyces geotrichum*, and a few reads of *Malassezia* sp. and *Deb. hansenii*.

Freshwater swamps and ponds near urban centers are mostly polluted showing wide fluctuations in yeast counts compared with pond and swamp waters of unpopulated regions. There were notable studies done by Ahearn et al. (1968) in the Everglades and by van Uden and Ahearn (1963) in a small unpolluted lake in Michigan that give an idea of what yeasts are present in shallow freshwaters of uninhabited and less populated areas. The human-associated yeasts present in many studies from near urban areas were not common in these waters where yeasts belonging to the former genera *Cryptococcus* and *Rhodotorula* (now classified in diverse basidiomycetous genera) were most common. Yeast counts in Everglades freshwater sites were largely in the 150–500 range and with some up to 1200 l^{-1} (Ahearn et al. 1968). Yeast counts in the sites with 0–9 ppt salinity were mostly in the 100–1000 l^{-1} range, whereas with salinity of 25 ppt, most counts were less than 100. The most common species were strictly oxidative with *Pa. laurentii* the most frequent. The principal difference from estuarine mangrove regions in the Everglades was the lack of *K. aestuarii*, lower frequency of *Cystofilobasidium infirmominiatum*, and much higher frequency of *Sporobolomyces*. The only ascomycetes frequent in the freshwater swamps were *Deb. hansenii*, *P. kudriavzevii* (*C. krusei*), *Metschnikowia reukaufii*, and *Yamadazyma triangularis*.

2.4 Salt Marshes

Salt marshes are found frequently in regions protected from the action of the surf in temperate waters of bays and estuaries. Yeasts were studied in a salt marsh in Louisiana in southern USA where a new species was prevalent, *Scheffersomyces* (*Pichia*) *spartinae*, and with concentrations as great as 9×10^7 cells g^{-1} associated with the plant culm of *Spartina alterniflora*, oyster grass, the dominant plant of the habitat. The prevalent yeasts in the sediment rhizosphere were species of the then polyphyletic genera *Trichosporon*, *Rhodotorula*, and *Rhodosporidium* and *Kluyveromyces lactis*, a species similar to *K. aestuarii* that is prevalent in mangroves (Ahearn et al. 1970; Meyers et al. 1975; Hagler and Ahearn 1987). The population of *K. lactis* was followed in 30 samples each of water and sediment using 2% galactose YNB agar with pH adjusted to 4.0 with lactic acid on which it formed deep rose- to maroon-colored colonies and was compared with the overall yeast population growing on YM agar. It was found to be consistently present as a significant portion of the total yeast population which was 10 to 100 times higher in sediments than in water (Meyers et al. 1971). A more recent study using cultivation-independent methods was unable to confirm these species in a salt marsh in Georgia, but such methods have not shown good detection of yeasts (Buchan et al. 2002). Dini-Andreote et al. (2016) reported a comprehensive study using a high-throughput sequencing method to access fungal community dynamics related to marine-terrestrial transition at a pristine salt marsh (Schiermonnikoog Island, The Netherlands). The natural sedimentation process on this island resulted in a chronosequence developed over a hundred years of terrestrial ecosystem succession. The majority of OTUs based on ITS region sequences were assigned to Ascomycota (66.8%), followed by Basidiomycota (4.3%) with Tremellomycetes yeasts mainly represented by species previously assigned to the former polyphyletic genus *Cryptococcus* found especially in the early succession stages of transition from marine to terrestrial habitats. Yeasts have important populations as part of the normal biota of salt marshes, but have not received much study in this habitat.

2.5 Bogs and Fens

Bogs and fens are the dominant wetlands of our planet and important as carbon sinks. Yeast species make up about 10% of all peatland fungi and probably use simple polymers leached from plant materials in the initial phases of decomposition (Thormann et al. 2007). Peat bogs and fens do contain yeasts, and basidiomycetous species tend to be more prevalent and increasingly so in colder climates. The yeast species found more than once in bogs and fens are noted in Table 2.4. *Candida* spp., *Deb. hansenii*, *Rh. mucilaginosa*, and *Sporobolomyces roseus* were most common, and *Goffeauzyma* (*Cryptococcus*) *gilvescens* dominated in the coldest regions. Kachalkin (2010) isolated psychrophilic yeasts *Sterigmatosporidium polymorphum* and *Phenoliferia* (*Rhodotorula*) *psychrophenolica*, and *Aureobasidium pullulans* var. *subglaciale* from the *Sphagnum* mosses and paludal vascular plants in a swamp region near Moscow. Broad assimilation spectrum fungal species, capable of utilization of organic acids and aromatic compounds, were prevalent in the moss-turf (Kurakov et al. 2008). Yeast populations were noted by Babjeva and Chernov (1995) to be lower in the litter complex at about $10^3\ g^{-1}$ compared to the 10^5–$10^6\ g^{-1}$ found in tundra epiphyte complex. A study in Canada and Siberia yielded 12 identified and 8 unidentified probable new species from 34 isolates. *Nadsonia starkeyi-henricii* was included among them and is probably a peatland specialist (Thormann et al. 2007).

2.6 Phytotelmata

Phytotelmata are formed from rainwater collected and preserved in structures of some plants including many bromeliad species. Thousands of species of bromeliads are native to diverse tropical habitats of the Americas (Fig. 2.3). These are dynamic and complex microenvironments inhabited by communities of different organisms including endemic species (Benzing 1990; Whittman 2000; Lopez et al. 2009). The phytotelmata in bromeliad leaf rosettes are a major source of nutrients for these organisms and communities associated with them (Richardson et al. 2000). Animals including insects and small mammals, known to carry yeasts, can have mutualistic relationships with tank bromeliads (Abranches et al. 1997; Pagnocca et al. 2008; Duarte et al. 2016; Leroy et al. 2016). Leaves from the bromeliad itself are in contact with the tank water, but the tanks also collect the leaf litter falling into them from surrounding vegetation. Each plant has many partitions formed by the leaf rosette making it like a circular rack of enrichment cultures around the central tank, each with different conditions for yeast growth. The leaf litter and visiting

Table 2.4 Yeasts found in bogs and fens

	Russia	Russia	Alaska	Canada-Russia	Thailand	Russia	Russia
References	1[a]	2	2	3	4[a]	5[a]	6[a]
Yeast	ND	$N = 69$	$N = 9$	$N = 9$	$N = 15$	$N = 3$	$N = 20$
Candida spp.		5		2		3	2
Cryptococcus spp.[b]		2				3	
Cystobasidium minutum	2					1	
Cystofilobasidium capitatum						1	2
Debaryomyces hansenii		4		2	2	2	
Filobasidium magnum				1			3
Goffeauzyma gilvescens	3		3				
Guehomyces pullulans	2	1		1			
Metschnikowia pulcherrima		1				2	
Mrakia frigida	3	1					
Naganishia albida		5	1	4			
Naganishia diffluens	2						1
Papiliotrema laurentii	2	2		1	1		
Pichia membranifaciens				1		1	2
Rhodotorula glutinis		2		1		2	
Rhodotorula mucilaginosa	2	5	2		3	2	3
Rhodotorula spp.		3		1			
Sporobolomyces roseus	1	5				2	2
Sugiyamaella paludigena		1		2			
Trichomonascus ciferrii					3		
Dothidella spp.							3

N = number of samples, ND = number of samples unknown

[a] Relative abundance data expressed as scale of three levels rather than frequency of occurrence 1 = minimal level detected; 2 = intermediate level; 3 = prevalent. References: 1 = Bab'eva and Chernov (1995); 2 = Polyakova et al. (2001); 3 = Thormann et al. (2007); 4 = Jaiboon et al. (2016); 5 = Kachalkin et al. (2008); 6 = Kachalkin and Yurkov (2012)

[b] Can include other Tremellomycetes formerly in the polyphyletic genus *Cryptococcus*. Nomenclature was updated using Kurtzman et al. (2011a), Species Fungorum (2016), and MycoBank Database (2016)

Fig. 2.3 Bromeliads. **A**, rupestrian bromeliads; **B**, bromeliads and other epiphytes on a tree; **C**, bromeliad growing on soil showing various phytotelmata surrounding central tank and leaf litter in the center; **D**, unshaded bromeliad with photoautotrophic growth in its tank

animals vector diverse species to form metapopulations in an extensive matrix of natural aquatic microcosms. Heavy rains can flood the tanks and wash out the existing nutrients and microbial populations, and during prolonged dry periods, the tanks can dry out (Araujo et al. 1998; Araujo 1999; Garcia 2007). More than 112 yeast species have been found in phytotelmata, and the prevalent yeasts in them are presented in Table 2.5. Phytotelmata in direct sunlight have strong algal growth, rather than degradation of organic materials, as a principal source of organic nutrients for microbial growth. Their yeast community is dominated by basidiomycetous species, whereas shaded plants are also rich in ascomycetous species. The species *Kazachstania bromeliacearum* appears to be endemic to bromeliad phytotelmata, and *Kazachstania rupicola* has been found in rupestrian bromeliads. *C. intermedia* was frequent in the phytotelmata and was the dominant

Table 2.5 Yeasts found in more than five samples of phytotelmata

	Coroa G	Bracui	P. Antas	Marica	MG	MG	SP
	Qq	Vp and Na	Np and Qq	Nc	Vm dry	Vm rain	Cs
References	1, 2	1	1	1, 3	4	4	5
Yeast	$N = 50$	$N = 38$	$N = 43$	$N = 22$	$N = 30$	$N = 30$	$N = 11$
Anomalomyces panici					3	6	
Aureobasidium pullulans					9	4	
Candida aff *famata*			3	5			
Candida intermedia	12	10	9	9		1	2
Candida spp.	2	2		1			2
Candida tropicalis	4	2		1			
Cryptococcus spp.[a]				1	5	8	
Cyberlindnera saturnus	3		3				
Debaryomyces hansenii	6	3	17	1			3
Kazachstania bromeliacearum	4	10	15	4			
Metschnikowia spp.	1			1	1		5
Meyerozyma guilliermondii	4			6		4	
Myriangiales spp.					12	22	
Naganishia albida	10	3	2	9			
Occultifur brasiliensis					13	10	
Papiliotrema laurentii	6		6	9	5		
Rhodotorula glutinis	4	1		1			
Rhodotorula mucilaginosa	8	1	4	3	1	1	
Saccharomyces cerevisiae		6	4	1			
Saitozyma podzolica					9	7	
Saturnispora silvae						12	
Schwanniomyces occidentalis	1		3	5			
Schwanniomyces polymorphus	1		6				
Schwanniomyces vanrijiae			9				
Torulaspora delbrueckii	7	2	2				

N = number of samples. Collection sites in Brazil: Coroa G = sand dune in mangrove, Coroa Grande, Itaguaí, RJ; Bracuí = mangrove epiphytes, Ilha do Jorge, Bracui, RJ; P. Antas = swamp, Poço das Antas Biological Reserve, RJ; Maricá = Restinga da Barra de Maricá, Rio de Janeiro, Brazil; MG = Serra da Piedade, Caeté, Minas Gerais; SP = Picinguaba área, an Atlantic rain forest site at the "Serra do Mar" State Park in São Paulo, Brazil

Plant species: An = *Aechmeae nudicaulis*; Cs = *Canistropsis seidelii*; Nc = *Neoregelia cruenta*; Np = *Nidularium procerum*; Qq = *Quesnelia quesneliana*; Vm = *Vriesea minarum*; Vp = *Vriesea procera*.

References: 1 = Araujo (1999); 2 = Hagler et al. (1993); 3 = Garcia (2007); 4 = Gomes et al. (2015); 5 = Ruivo (2005)

[a] Can include other Tremellomycetes formerly in the polyphyletic genus *Cryptococcus*. Nomenclature was updated using Kurtzman et al. (2011a), Species Fungorum (2016), and MycoBank Database (2016)

yeast in fruits of the bromelias *Quesnelia quesneliana*, *Vriesea procera*, and *Aechmea nudicaulis*. *Deb. hansenii*, its anamorph *Candida famata*, and similar species including *Schwanniomyces occidentalis*, *Schwanniomyces polymorphus*, *Schwanniomyces vanrijiae* and *Mey. guilliermondii* were prevalent especially in the shaded plants. An example of phytotelma other than bromeliad tanks is in the more ephemeral flower structures of the wild banana-like plant *Heliconia velloziana*. The 15 ascomycetous yeasts isolated from 14 phytotelmata of *Hel. velloziana* with more than one isolate were four cultures of *Candida heliconiae*, three each of *Candida picinguabensis* and *Metschnikowia* spp. and two each of *Candida apis*, *Candida pseudointermedia*, *Candida restingae*, *Candida saopaulonensis*, and *Debaryomyces* sp. (Ruivo 2005). The more common basidiomycetous species of the phylloplane were also among the prevalent phytotelmata yeasts (Fonseca and Inácio 2006). Phytotelmata are "in situ" enrichment cultures for yeasts making them a good natural source to tap the species richness of the phylloplane. Gomes et al. (2016) screened enzymes produced by yeasts from *Vriesea minarum* phytotelmata. These enzymes would allow them to participate in the degradation of plant and animal materials falling into the tanks. Phytotelmata yeasts have been the source of various new species: *Carlosrosaea* (*Bullera*) *vrieseae* (Landell et al. 2015); *Candida aechmeae* and *Candida vrieseae* (Landell et al. 2010); *Candida bromeliacearum* and *Candida ubatubensis* (Ruivo et al. 2005); *Candida heliconiae*, *C. picinguabensis*, and *C. saopaulonensis* (Ruivo et al. 2006); *Hagleromyces aurorensis* (Sousa et al. 2014); *Hannaella pagnoccae* (Landell et al. 2014); *Kaz. bromeliacearum* (Araujo et al. 2012); *Kaz. rupicola* (Safar et al. 2013); *Kockovaella libkindii* (Gomes et al. 2016); and *Occultifur brasiliensis* (Gomes et al. 2015).

2.7 Concluding Remarks

Yeasts in aquatic ecotones are rich in species diversity and with high population levels compared with open waters. More than 270 yeast species and many other unidentified yeasts, often representing new taxa, were reported from aquatic ecotones covered in this chapter. Cultivation of yeasts from these waters is complicated by large populations of filamentous fungi and competition between different yeasts while growing on culture media. Using various enrichment cultures and solid media with and without different antifungal antibiotics to inhibit parts of the fungal populations, including some yeasts, should improve isolation of yeasts from aquatic ecotones. No medium, even if all yeasts can grow on it in pure culture, will allow cultivation of all yeasts in the mixed populations of an environmental sample. The methods of cultivation, isolation, and enumeration of yeasts have been reviewed by Boundy-Mills (2006) and Kurtzman et al. (2011b). A single nutrient-rich medium favors fast-growing species that can later inhibit development of others on the isolation medium. Various media with inhibitors and nutrients favoring different species should be used, and the frequency of presence in various samples, rather than counts, used to indicate the relative importance of different species. Indicator dyes, such as bromocresol green at pH 4, added to media can inhibit some species and assist in selection of colonies for further study. Culture-independent methods with DNA analysis can detect the species present and their relative population (Xu 2006; Abarenkov et al. 2010), although this methodology does not yield cultures for further studies or applications. Culture-independent methods have detected yeasts in the presence of large populations of filamentous fungi, but yeasts known to be abundant by cultivation methods are often not detected as prevalent by these methods. The ITS region has been proposed as the universal bar code for next-generation sequencing methodology applied in fungal diversity studies and has shown detection of yeasts (Arfi et al. 2012a, b; Schoch et al. 2011). The detection of yeast taxa could be improved when sequences of large subunit rDNA (D1/D2 region) are used, probably due to the large database available on this region for yeasts (Bokulich et al. 2014). A polyphasic approach of cultivation and culture-independent methods should provide better information on yeast communities in aquatic ecotones. The exceptional species richness found, especially for mangroves and phytotelmata, yielded many new species descriptions. This should encourage further studies of yeast ecology and bioprospecting for yeasts in aquatic ecotone habitats.

References

Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt V, Erland S, HØiland K, KjØller R, Larsson E, Pemmansen T, Sen R, Taylor AFS, Tedersoo L, Ursing M, Vralstad T, Liimatainen K, Peintner U, Kõljalg U (2010) The UNITE database for molecular identification of fungi – recent updates and future perspectives. New Phytol 186:281–285

Abranches J, Valente P, Nóbrega HN, Fernandez FAS, Mendonça-Hagler LC, Hagler AN (1997) Yeast diversity and killer activity dispersed by fecal pellets from marsupials and rodents in a southeast Brazilian tropical habitat mosaic. FEMS Microbiol Ecol 26:27–33

Ahearn DG, Roth FJ, Meyers SP (1968) Ecology and characterization of yeasts from aquatic regions of south Florida. Mar Biol 1:291–308

Ahearn DG, Yarrow D, Meyers SP (1970) *Pichia spartinae* sp. nov. from Louisiana marshland habitats. Antonie Van Leeuwenhoek 36:503–508

Am-In S, Yongmanitchai W, Limtong S (2008) *Kluyveromyces siamensis* sp. nov., an ascomycetous yeast isolated from water in a mangrove forest in Ranong Province, Thailand. FEMS Yeast Res 8:823–828

Araujo, FV (1999) Comunidades de leveduras associadas a sedimentos de manguezais e a bromélias em ecossistemas costeiros do Rio de Janeiro, Brasil. D.Sc Thesis, Inst. Microbiol. PPG, UFRJ. pp 183

Araujo FV, Hagler AN (2011) *Kluyveromyces aestuarii*, a potential environmental quality indicator yeast for mangroves in the State of Rio de Janeiro, Brazil. Braz J Microbiol 42:954–958

Araujo FV, Soares CAG, Hagler AN, Mendonça-Hagler LC (1995) Ascomycetous yeast communities of marine invertebrates in a southeast Brazilian mangrove ecosystem. Antonie Van Leeuwenhoek 68:91–99

Araujo FV, Medeiros RJ, Mendonça-Hagler LC, Hagler AN (1998) A preliminary note on yeast communities of bromeliad-tank waters of Rio de Janeiro, Brazil. Rev Microbiol 29:118–121

Araújo FV, Rosa CA, Freitas LFD, Lachance M-A, Vaughan-Martini A, Mendonça-Hagler LC, Hagler AN (2012) *Kazachstania bromeliacearum* sp. nov., a yeast species from water tanks of bromeliads. Int J Syst Evol Microbiol 62:1002–1006

Arfi Y, Buée M, Marchand C, Devasseur A, Record E (2012a) Multiple markers pyrosequencing reveals highly diverse and host specific fungal communities on the mangrove trees *Avicenia marina* and *Rhizophora stylosa*. FEMS Microbiol Ecol 79:433–444

Arfi Y, Marchand C, Wartel M, Record E (2012b) Fungal diversity in anoxic-sulfidic sediments in a mangrove soil. Fungal Ecol 5:282–285

Babjeva IP, Chernov IY (1995) Geographic aspects of yeast ecology. Physiol Gen Biol Rev 9:1–54

Benzing DH (1990) Vascular epiphytes. General biology and related biota. Cambridge University Press, Cambridge

Bokulich NA, Thomgate JH, Richardson PM, Mills DA (2014) Microbial biography of wine grapes is conditioned by cultivar, vintage, and climate. Proc Natl Acad Sci U S A 111:E139–E148

Boonmak C, Jindamorakot S, Kawasaki H, Yongmanitchai W, Suwanarit P, Nakase T, Limtong S (2009) *Candida siamensis* sp. nov., an anamorphic yeast species in the *Saturnispora* clade isolated in Thailand. FEMS Yeast Res 9:668–672

Boundy-Mills K (2006) Methods for investigating yeast biodiversity. In: Rosa CA, Gabor P (eds) Biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 67–100

Buchan A, Newell SY, Moreta JI, Moran MA (2002) Analysis of internal transcribed spacer (ITS) regions of rRNA genes in fungal communities in a southeastern U.S. salt marsh. Microb Ecol 43:329–340

Chi Z-M, Liu T-T, Chi Z, Liu G-L, Wang Z-P (2012) Occurrence and diversity of yeasts in the mangrove ecosystems in Fujian, Guangdong and Hainan provinces of China. Indian J Microbiol 52:346–353

Coelho MA, Almeida JMF, Martins IM, da Silva AJ, Sampaio JP (2010) The dynamics of the yeast community of the Tagus river estuary: testing the hypothesis of the multiple origins of estuarine yeasts. Antonie Van Leeuwenhoek 98:331–342

Dini-Andreote F, Pylro VS, Baldrian P, van Elsas JD, Salles JF (2016) Ecological succession reveals potential signatures on marine-terrestrial transition in salt marsh fungal communities. ISMEJ 10:1–14

Duarte APM, Ferro M, Rodrigues A, Bacci M Jr, Nagamoto NS, Forti LC, Pagnocca FC (2016) Prevalence of the genus *Cladosporium* on the integument of leaf-cutting ants characterized by 454 pyrosequencing. Antonie Van Leeuwenhoek 109:1235–1243

EPA (2016) Wetlands classification and types in wetlands protection and restoration. https://www.epa.gov/wetlands. Accessed on Nov. 18, 2016

Fell JW (1961) A new species of *Saccharomyces* isolated from a subtropical estuary. Antonie Van Leeuwenhoek 27:27–30

Fell JW, Tallman AS (1980) *Rhodosporidium paludigenum* sp. nov., a basidiomycetous yeast from intertidal waters of south Florida. Int J Syst Evol Microbiol 30:658–659

Fell JW, Ahearn DG, Meyers SP, Roth FJ (1960) Isolation of yeasts from Biscayne Bay, Florida, and adjacent benthic areas. Limnol Oceanogr 5:366–371

Fell JW, Statzell-Tallman A, Kurtzman CP (2004) *Lachancea meyersii* sp. nov., an ascosporogenous yeast from mangrove regions in the Bahama Islands. Stud Mycol 50:359–363

Fell JW, Statzell-Tallman A, Scorzetti G, Gutierrez MH (2011) Five new species of yeasts from fresh water and marine habitats in the Florida Everglades. Antonie Van Leeuwenhoek 99:533–549

Fonseca A, Inácio J (2006) Phylloplane yeasts. In: Rosa CA, Gabor P (eds) Biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 263–301

Gadanho M, Sampaio JP (2004) Application of temperature gradient gel electrophoresis to the study of yeast diversity in the estuary of the Tagus River, Portugal. FEMS Yeast Res 5:253–261

Gadanho M, Sampaio JP (2005) Occurrence and diversity of yeasts in the Mid-Atlantic Ridge hydrothermal fields near the Azores archipelago. Microb Ecol 50:408–417

Garcia, KM (2007) Meios diferenciais para isolamento e triagem de leveduras endofíticas da bromélia *Neoregelia cruenta*. Dissertação, Programa de Pós Graduação em Biotecnologia Vegetal-UFRJ pp 100

Ghizelini AM, Mendonça-Hagler LCS, Macrae A (2012) Microbial diversity in Brazilian mangrove sediments – a mini review. Braz J Microbiol 43:1242–1254

Gomes FCO, Safar SVB, Marques AR, Medeiros AO, Santos ARO, Carvalho C, Lachance MA, Sampaio JP, Rosa CA (2015) The diversity and extracellular enzymatic activities of yeasts isolated from water tanks of *Vriesea minarum,* an endangered bromeliad species in Brazil, and the description of *Occultifur brasiliensis* f.a., sp. nov. Antonie Van Leeuwenhoek 107:597–611

Gomes FCO, Safar SVB, Santos ARO, Lachance M-A, Rosa CA (2016) *Kockovaella libkindii* sp. nov., a yeast species isolated from water tanks of bromeliad. Int J Syst Evol Microbiol 66:5066–5069

Hagler AN (2006) Yeasts as indicators of environmental quality. In: Rosa CA, Gabor P (eds) Biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 519–536

Hagler AN, Ahearn DG (1987) Ecology of aquatic yeasts. In: Rose AH, Harrison JS (eds) The yeasts, vol 1, 2nd edn. Academic Press, London, pp 181–206

Hagler AN, Mendonça-Hagler LC (1979) Sodium chloride tolerance of yeasts from a polluted estuary in Rio de Janeiro. Rev Microbiol 10:30–33

Hagler AN, Mendonça-Hagler LC (1981) Yeasts from marine and estuarine waters with different levels of pollution in the state of Rio de Janeiro, Brazil. Appl Environ Microbiol 41:173–178

Hagler AN, de Oliveira RB, Mendonça-Hagler LC (1982) Yeasts in the intertidal sediments of a polluted estuary in Rio de Janeiro, Brazil. Antonie Van Leeuwenhoek 48:53–56

Hagler AN, Mendonça-Hagler LC, Silva JB, Santos EA, Farage S, Shrank A, de Oliveira RB (1986) Evaluation of microbial pollution indicators in Brazilian tropical and subtropical marine surface waters. Sci Total Environ 58:151–160

Hagler AN, Rosa CA, Morais PB, Mendonça-Hagler LC, Franco GMO, Araujo FV, Soares CAG (1993) Yeasts and coliform bacteria of water accumulated in bromeliads of mangrove and sand dune ecosystems in the southeast Brazil. Can J Microbiol 39:973–977

Jaiboon K, Lertwattanasakul N, Limtong P, Limtong S (2016) Yeasts from peat in a tropical peat swamp forest in Thailand and their ability to produce ethanol, indole-3-acetic acid and extracellular enzymes. Mycol Prog 15:755–770

Kachalkin AV (2010) New data on the distribution of certain psychrophilic yeasts in Moscow oblast. Microbiology 79:840–844

Kachalkin AV (2014) Yeasts of the White Sea intertidal zone and description of *Glaciozyma litorale* sp. nov. Antonie Van Leeuwenhoek 105:1073–1083

Kachalkin AV, Yurkov AM (2012) Yeast communities in *Sphagnum* phyllosphere along the temperature-moisture ecocline in the boreal forest-swamp ecosystem and description of *Candida sphagnicola* sp. nov. Antonie Van Leeuwenhoek 102:29–43

Kachalkin AV, Glushakova AM, Yurkov AM, Chernov IY (2008) Characterization of yeast groupings in the phyllosphere of *Sphagnum* mosses. Mikrobiologiya 77:533–541

Kaewwichian R, Yongmanitchai W, Srisuk N, Fujiyama K, Limtong S (2010) *Geotrichum siamensis* sp. nov. and *Geotrichum phurueaensis* sp. nov., two asexual arthroconidial yeast species isolated in Thailand. FEMS Yeast Res 10:214–220

Kurakov AV, Lavrent'ev RB, Nechitailo TY, Golyshin PN, Zvyagintsev DG (2008) Diversity of facultatively anaerobic microscopic mycelial fungi in soils. Microbiology 77:90–98

Kurtzman CP, Fell JW, Boekhout T (2011a) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam

Kurtzman CP, Fell JW, Boekhout T, Robert V (2011b) Methods for isolation, phenotypic characterization and maintenance of yeasts. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 97–107

Kutty SN, Philip R (2008) Marine yeasts – a review. Yeast 25:465–483

Lachance MA, Starmer WT (1998) Ecology and yeasts. In: Kurtzman CP, Fell JW (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 21–30

Landell MF, Billodre R, Ramos JP, Leoncini O, Vainstein MH, Valente P (2010) *Candida aechmeae* sp. nov. and *Candida vrieseae* sp. nov., novel yeast species isolated from the phylloplane of bromeliads in southern Brazil. Int J Syst Evol Microbiol 60:244–248

Landell MF, Brandão LR, Barbosa AC, Ramos JP, Safar SV, Gomes FCO, Sousa FM, Morais PB, Broetto L, Leoncini O, Ribeiro JR, Fungsin B, Takashima M, Nakase T, Lee CF, Vainstein MH, Fell J, Scorzetti G, Vishniac H, Rosa CA, Valente P (2014) *Hannaella pagnoccae* sp. nov., a tremellaceous yeast species isolated from plants and soil. Int J Syst Evol Microbiol 64:1970–1977

Landell MF, Brandão LR, Safar SVB, Gomes FCO, Félix CR, Santos ARO, Pagani DM, Ramos JP, Broetto L, Mott T, Vainstein MH, Valente P, Rosa CA (2015) *Bullera vrieseae* sp. nov., a tremellaceous yeast species isolated from bromeliads. Int J Syst Evol Microbiol 65:2466–2471

Lazarus CR, Koburger JA (1974) Identification of yeasts from the Suwannee River Florida estuary. Appl Microbiol 27:1108–1111

Le Calvez T, Borgaud G, Mahé S, Barbier G, Vandenkoornhuyse P (2009) Fungal diversity in deep-sea hydrothermal ecosystems. Appl Environ Microbiol 75:6415–6421

Leroy C, Petitclerc F, Orivel J, Corbara B, Carrias JF, Dejean A, Cereghino R (2016) The influence of light, substrate and seed origin on the germination and establishment of an ant-garden bromeliad. Plant Biol 19:70–78

Limtong S, Yongmanitchai W (2010) *Candida chanthaburiensis* sp. nov., *Candida kungkrabaensis* sp. nov. and *Candida suratensis* sp. nov., three novel yeast species from decaying plant materials submerged in water of mangrove forests. Antonie Van Leeuwenhoek 98:379–388

Limtong S, Yongmanitchai W, Kawasaki H, Seki T (2007) *Candida thaimueangensis* sp. nov., an anamorphic yeast species from estuarine water in a mangrove forest in Thailand. Int J Syst Evol Microbiol 57:650–653

Limtong S, Yongmanitchai W, Kawasaki H, Seki T (2008a) *Candida phangngensis* sp. nov., an anamorphic yeast species in the *Yarrowia* clade, isolated from water in mangrove forest in Phang-Nga Province, Thailand. Int J Syst Evol Microbiol 58:515–519

Limtong S, Imanishi Y, Jindamorakot S, Ninomiya S, Yongmanitchai W, Nakase T (2008b) *Torulaspora maleeae* sp. nov., a novel ascomycetous yeast species from Japan and Thailand. FEMS Yeast Res 8:337–343

Limtong S, Kaewwichian R, Am-In S, Nakase T, Lee C, Yongmanitchai W (2010a) *Candida asiatica* sp. nov., an anamorphic ascomycetous yeast species isolated from natural samples from Thailand, Taiwan, and Japan. Antonie Van Leeuwenhoek 98:475–481

Limtong S, Kaewwichian R, Am-In S, Boonmak C, Jindamorakot S, Yongmanitchai W, Srisuk N, Kawasaki H, Nakase T (2010b) Three anamorphic yeast species *Candida sanitii* sp. nov., *Candida sekii* sp. nov. and *Candida suwanaritii*, three novel yeasts in the *Saturnispora* clade isolated in Thailand. FEMS Yeast Res 10:114–122

Lopez LCS, Alves RR, Rios RI (2009) Micro-environmental factors and the endemism of bromeliad aquatic fauna. Hydrobiologia 625:151–156

McGinnis MR, Padhye AA, Ajello L (1974) Storage of stock cultures of filamentous fungi, yeasts, and some aerobic Actinomycetes in sterile distilled water. Appl Microbiol 28:218–222

Meyers SP, Ahearn DG, Miles PC (1971) Characterization of yeasts in Barataria Bay. LSU Coastal Stud Bull 6:7–17

Meyers SP, Ahearn DG, Alexander SK, Cook WL (1975) *Pichia spartinae* a dominant yeast of the *Spartina* salt marsh. Dev Ind Microbiol 16:262–267

Muyzer G (1999) DGGE/TGGE: a method for identifying genes from natural ecosystems. Curr Opin Microbiol 2:317–322

Mycobank (2016) Mycobank database. http://www.mycobank.org/quicksearch.aspx. Accessed on 15 Mar 2017

Nagahama T (2006) Yeast biodiversity in freshwater, marine, and deep-sea environments. In: Rosa CA, Gabor P (eds) Biodiversity and ecophysiology of yeasts. Springer, Berlin, pp 241–262

Odum EP (1993) Ecology and our endangered life-support systems. Sinauer Associates, Sunderland, MA

Pagnocca FC, Rodrigues A, Nagamoto N, Bacci M (2008) Yeasts and filamentous fungi carried by the gynes of leaf-cutting ants. Antonie Van Leeuwenhoek 94:517–526

Pires ACC, Cleary DFR, Almeida A, Cunha A, Dealtry S, Mendonça-Hagler L, Smalla K, Gomes NCM (2012) Denaturing gradient gel electrophoresis and barcoded pyrosequencing reveal unprecedented Archaeal diversity in mangroves sediments and rhizosphere samples. Appl Environ Microbiol 78:5520–5528

Polyakova AV, Chernov IY, Panikov NS (2001) Yeast diversity in hydromorphic soils with reference to a grass-*Sphagnum* wetland in western Siberia and a hummocky tundra region at Cape Barrow (Alaska). Microbiology 70:714–720

Richardson BA, Richardson MJ, Scatena FN, McDowell WH (2000) Effects of nutrient availability and other elevational changes on bromeliad populations and their invertebrate communities in a humid tropical forest in Puerto Rico. J Trop Ecol 16:167–188

Ruivo CCC (2005) Ocorrência de leveduras em espécies vegetais nativas da Mata Atlântica, Parque Estadual da Serra do Mar – Núcleo Picinguaba, São Paulo. Thesis UNESP Rio Claro. http://repositorio.unesp.br/handle/11449/103947. Accessed 12 Nov 2016

Ruivo CCC, Lachance M-A, Rosa CA, Bacci M, Pagnocca FC (2005) *Candida bromeliacearum* sp. nov. and *Candida ubatubensis* sp. nov., two yeast species isolated from the water tanks of *Canistropsis seidelii* (Bromeliaceae). Int J Syst Evol Microbiol 55:2213–2217

Ruivo CCC, Lachance MA, Rosa CA, Bacci M, Pagnocca FC (2006) *Candida heliconiae* sp. nov., *Candida picinguabensis* sp. nov. and *Candida saopaulonensis* sp. nov., three ascomycetous yeasts from *Heliconia velloziana* (Heliconiaceae). Int J Syst Evol Microbiol 56:1147–1151

Safar SVB, Gomes FCO, Marques AR, Lachance MA, Rosa CA (2013) *Kazachstania rupicola* sp. nov., a yeast species isolated from water tanks of a bromeliad in Brazil. Int J Syst Evol Microbiol 63:1165–1168

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Lavesque CA, Chen W, Fungal barcoding consortium (2011) Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. Proc Natl Acad Sci U S A 109:6241–6246

Soares CA, Maury M, Pagnocca FC, Araujo FA, Hagler AN, Mendonça-Hagler LC (1997) Yeast communities in dark muddy intertidal estuarine sediments of Rio de Janeiro, Brazil. J Gen Appl Microbiol 43:265–272

Sousa FMP, Morais PB, Lachance MA, Rosa CA (2014) *Hagleromyces* gen. nov., a yeast genus in the Saccharomycetaceae, and description of *Hagleromyces aurorensis* sp. nov., isolated from water tanks of bromeliads. Int J Syst Evol Microbiol 64:2915–2919

Species Fungorum (2016) CABI databases. www.speciesfungorum.org/names/names.asp. Accessed 15 Mar 2017

Starmer WT, Lachance MA (2011) Yeast ecology. In: Kurtzman CP, Fell JW, Boekhout T (eds) The yeasts, a taxonomic study, 5th edn. Elsevier, Amsterdam, pp 65–83

Statzell-Tallman A, Belloch C, Fell JW (2008) *Kwoniella mangroviensis* gen. nov., sp. nov. (Tremellales, Basidiomycota), a teleomorphic yeast from mangrove habitats in the Florida Everglades and Bahamas. FEMS Yeast Res 8:103–113

Statzell-Tallman A, Scorzetti G, Fell JW (2011) *Candida spencermartinsiae* sp. nov., *Candida taylorii* sp. nov. and *Pseudozyma abaconensis* sp. nov., novel yeasts from mangrove and coral reef ecosystems. Int J Syst Evol Microbiol 60:1978–1984

Taysi I, van Uden N (1964) Occurrence and population densities of yeast species in an estuarine-marine area. Limnol Oceanogr 9:42–45

Thormann MN, Rice AV, Beilman DW (2007) Yeasts in peatlands: a review of richness and roles in peat decomposition. Wetlands 27:761–773

Ueda-Nishimura K, Mikata K (1999) A new yeast genus, *Tetrapisispora* gen. nov.: *Tetrapisispora iriomotensis* sp. nov., *Tetrapisispora nanseiensis* sp. nov. and *Tetrapisispora arboricola* sp. nov., from the Nansei Islands, and reclassification of *Kluyveromyces phaffii* (van der Walt) van der Walt as *Tetrapisispora phaffii* comb. nov. Int J Syst Evol Microbiol 49:1915–1924

Van Uden N, Ahearn DG (1963) Occurrence and population densities of yeast species in a freshwater lake. Antonie Van Leeuwenhoek 29:308–312

Whittman PK (2000) The animal community associated with canopy bromeliads of the lowland Peruvian Amazon rain forest. Selbyana 21:48–51

Xu J (2006) Microbial ecology in the age of genomics and metagenomics: concepts, tools, and recent advances. Mol Ecol 5:1713–1731