

Ascomycetous yeast communities of marine invertebrates in a Southeast Brazilian mangrove ecosystem

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Abstract

The ascomycetous yeast communities associated with 3 bivalve mollusk, and 4 crab species were studied in the mangrove at Coroa Grande on Sepetiba Bay in Rio de Janeiro, Brazil. These were made up mostly of diverse but sparse and apparently allochthonous yeast populations. The striking exception was a prevalent population of the species *Kluyveromyces aestuarii*, which predominated the yeast communities of 2 detritus feeding crabs, *Sesarma rectum* and *Uca* spp., and the shipworm *Neoteredo reynei*. However, *K. aestuarii* was absent from the omnivorous crabs *Aratus pisonii* and *Goniopsis cruentata*, and the clam *Anomalocardia brasiliana*, and was rare in the clam *Tagelus plebeius* from mostly submerged more sandy sediments. *Pichia membranaefaciens*, *Candida valida*-like, *Candida krusei*, *Candida sorbosa*, *Candida colliculosa*-like, *Candida famata*-like, *Kloeckera* spp., *Candida guilliermondii*, *Candida albicans*, *Candida silvae*, *Geotrichum* spp., *Rhodotorula* spp., *Cryptococcus* spp., and the methylotrophic yeast *Candida boidinii* were frequently isolated. The 322 ascomycetous yeast cultures representing 252 isolates from crabs and mollusks were classified as 40 species that fit standard descriptions, and 44 putative new species. The ascomycetous yeast communities of the mangrove ecosystem include many new biotypes that require better taxonomic definition.

Introduction

Many yeasts are associated with specific terrestrial environments but few associations have been reported as typical of marine habitats (Hagler & Ahearn 1987; Phaff & Starmer 1987). Among them are *Pichia spartinae* with spartina grass (Ahearn et al. 1970; Meyers et al. 1975), *Kluyveromyces aestuarii* with saline waters of the Everglades and estuaries in Florida, USA, and *Kluyveromyces drosophilorum* (Syn. *Kluyveromyces lactis*) from marshlands sediments of Louisiana, USA (Fell & van Uden 1963; Ahearn et al. 1968; Meyers et al. 1971), aquatic *Metschnikowia* species with some marine invertebrates (van Uden & Castelo Branco 1963; Fell & Hunter 1968), *Debaryomyces hansenii* and part of the *Candida parapsilosis* group with sea water (Fell & Meyer 1967; Kohlmeyer & Kohlmeyer 1979), and various basidiomycetous yeasts includ-

ing some obligate psychrophiles with sea water (Fell 1974). Yeasts can be involved in marine habitats in decomposition and nutrient cycling, biodegradation of xenobiotics such as petroleum and its derivatives, and as parasites (Meyers & Ahearn 1974; MacGillivray & Shiaris 1993).

Yeasts have been shown to be present in various marine invertebrates including oysters, clams, mussels, shrimp, and crabs; and the most frequently isolated yeasts from these organisms are generally those also prevalent in the water of their habitats (Hagler & Ahearn 1987). Counts of yeast cells can be much higher in marine invertebrates than in the water of their habitats as a result of their feeding activities and concentration of nutrients in their digestive tracts (Pagnocca et al. 1989; Kosawa da Costa et al. 1991). Yeasts may be of nutritional importance to their invertebrate hosts. Cowley & Chranowski (1980) noted an association of

Rhodotorula glutinis with the crab *Uca pugilator* and that this yeast is probably ingested rather than multiplying in the digestive tract. Opportunistic pathogenic yeasts including *C. parapsilosis*, *Candida tropicalis*, *Candida glabrata*, *Candida guilliermondii*, *Candida krusei* and *Candida albicans* were found to be present in bivalve shellfish from Long Island Sound demonstrating their presence in a valuable marine resource (Buck et al. 1977).

Mangrove ecosystems are typical of tropical estuarine areas, and are important for the reproduction of many marine species including some important food resources, although they are often seriously threatened by human activities (Citron & Schaeffer-Novelli 1983). Mangroves offer numerous microhabitats with the potential of harboring yeast communities. We focused our work on 4 crabs and 3 bivalve mollusks from different regions of a mangrove area. Adults of the tree crab *Aratus pisonii* are mostly herbivorous omnivores (feeding mainly on live algae and mangrove leaves) living on branches and trunks of mangrove trees (Lacerda et al. 1991). *Goniopsis cruentata*, the red and black 'Aratu', is an omnivorous crab that includes *A. pisonii* in its diet and lives in burrows in the mud at the base of and on the mangrove trees (Jonés 1987). The 'Aratu' crab, *Sesarma rectum*, is an omnivore feeding mostly on degrading plant material and lives in burrows above the tide line. *Uca* spp. (fiddler crabs) are detritivorous crabs living in burrows in mud of the mangrove area. The small benthic clam *Anomalocardia brasiliana* lives in the top 5 cm of the sediments in shallow waters of bays and sediments (Narchi 1972). *Tagelus plebeius* is a clam living in estuaries at a depth of 40 to 50 cm in the sediment and can dig quickly (Pearse et al. 1942). The shipworm *Neoteredo reynei* is a bivalve mollusk burrowing in dead wood below the high tide line (Turner 1966). Liquid from its burrows has a strong H₂S odor and is used in the North of Brazil as a folk medicine for lung diseases (Andrade 1979). Our objective was to describe the ascomycetous yeast communities associated with some mollusks and crabs of a mangrove area and initiate taxonomic studies of the new biotypes encountered.

Materials and methods

The collection sites were about 50 km southwest of the city of Rio de Janeiro, Brazil, in the Coroa Grande mangrove area at 4 sampling sites: 79A, tide flat in front of mangrove; 79B, intertidal area under the mangrove

trees *Rhizophora mangle*, *Avicenia schaueriana* and *Laguncularia racemosa*; 79C, with the same vegetation as 79B, but shorter and separated from direct contact with the sea by an elevated strip of sand; and 79D, a sand bank along a freshwater stream at the transition of the mangrove to grassland (Fig. 1a and 1b). The invertebrates collected were the shipworm *Neoteredo reynei* (degrading wood, site 79B), and clams *Anomalocardia brasiliana* (mud flats, site 79A) and *Tagelus plebeius* (along a stream in the mud flat, site 79A), and crabs *Aratus pisonii* (limbs of trees, site 79B), *Goniopsis cruentata* (tree trunks and mud, site 79B), *Sesarma rectum* (sand bank near stream, site 79D) and *Uca* sp. (mud, site 79C). Samples were collected between September of 1991 and December of 1992. Materials were collected aseptically, placed in sterile wide mouthed bottles and returned to the lab on ice within 5 hr for processing (Hagler & Mendonça-Hagler 1981). Seawater was collected at high tide near the edge of the mangrove vegetation of site 79B and Timirim River at low tide from site 79A. Individual invertebrates were washed with sterile distilled water to remove adhered sediment or other materials. Aseptically removed viscera of the crabs and *Anomalocardia brasiliana* were weighted, 5 to 7.5 g suspended in 1 to 3 volumes of sterile 0.85% NaCl and homogenized with a hand held glass Teflon homogenizer. Pooled 40 g samples of whole *Tagelus plebeius* or *Neoteredo reynei* aseptically removed from their shells were placed in 4 volumes of sterile 0.85% NaCl and homogenized in a blender.

Total and fecal coliforms (MPN) and heterotrophic bacterial counts (CFU) were done by the American Public Health Association (1990) standard methods. Yeast and total fungal counts were done by a most probable number method (Hagler et al. 1986). Counts were made from 7 samples of separate collections made from March to December of 1992. Yeast isolations were made directly from the sample preparations on Aquatic Yeast Agar (Glucose 1%, (NH₄)₂SO₄ 0.5%, yeast extract 0.5%, NaCl 1%, NaH₂PO₄, 0.2%, agar 1.7%, Chloramphenicol 200 mg/l and pH 4.5) spread plates.

Yeasts were characterized by conventional methods using for most of the biochemical tests a multitip inoculation device on solid medium (Kreger-van Rij 1984), and identified by keys in Kreger-van Rij (1984), and Barnett et al. (1990). Dubious test results were confirmed in individual tubes with broth medium and the DBB test done in individual tubes on YCB-2% urea agar (Hagler & Mendonça-Hagler 1991).

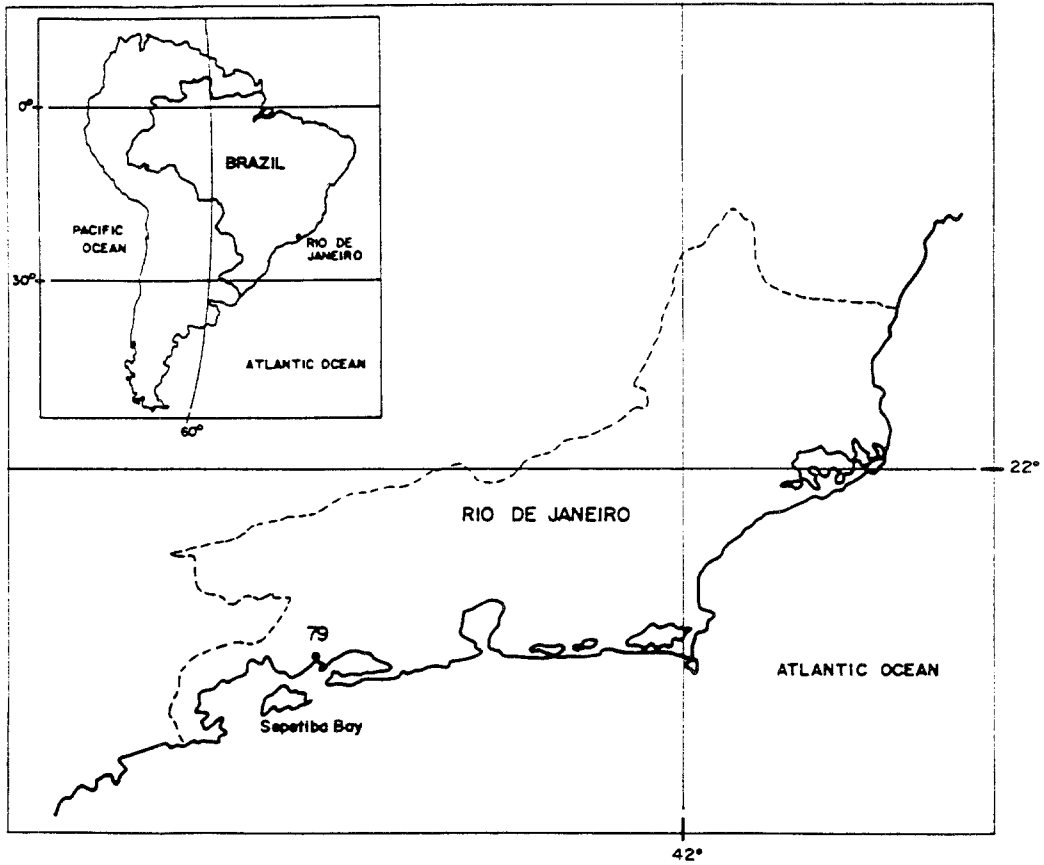


FIGURE 1A: Map of the State of Rio de Janeiro showing the position of the Coroa Grande Mangrove collection site 79.

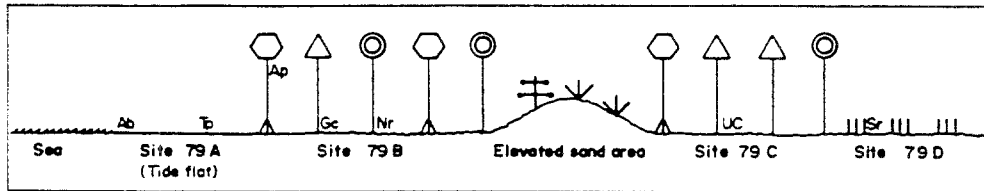


FIGURE 1B: Schematic profile of collection sites in the Coroa Grande Mangrove.

SYMBOLS

- | | | | |
|---|--------------------------------|----|------------------------------------|
| ○ | - <i>Rhizophora mangle</i> | Ap | - <i>Aratus pisonii</i> |
| ⊙ | - <i>Laguncularia racemosa</i> | Gc | - <i>Goniopsis cruentata</i> |
| △ | - <i>Avicennia shaueriana</i> | Sr | - <i>Sesarma rectum</i> |
| ⊕ | - <i>Hibiscus sp.</i> | UC | - <i>Uca sp.</i> |
| ∇ | - bromeliad | Ab | - <i>Anomalocardia brasiliiana</i> |
| | - grass | Tp | - <i>Tagelus plebeius</i> |
| | | Nr | - <i>Neoteredo reynei</i> |

Fig. 1. A: Map of the State of Rio de Janeiro showing the position of the Coroa Grande Mangrove collection site 79. B: Schematic profile of collection sites in the Coroa Grande Mangrove.

The similarity between the different yeast communities was calculated as the Pearson's product-moment correlation coefficient (r) and clustering was done by the unweighted pair-group arithmetic average (UPGMA) procedure (Legendre & Legendre 1983). Correlations were calculated from the proportional yeast species composition (number of isolates of a species/total number of isolates for that source) of each community. Two or more cultures identified as the same species from a single sample were considered to be one isolate in Table 1. Yeast proportions were arcsine square root transformed before the correlation coefficient calculations.

Results

Microbial counts of the different substrates are presented in Table 1. Mean fecal coliform counts were only 39 per 100 ml in the seawater but around 100 per 100 g in the crabs, and above 1000 per 100 ml in the fresh water stream and bivalves. Total coliforms were highest in *T. plebeius*, *N. reynei*, and *S. rectum* at about 10^5 per 100 ml. The microbial counts were mostly higher in the filter feeding bivalves than in other microhabitats. Yeast counts were generally low with less than 1000 per 100 ml, but they were consistently present and highest in the filter feeding bivalves, especially in *N. reynei* with 12,000 per 100 g.

The 322 ascomycetous yeast cultures and anamorphs of ascomycetes obtained from crabs and mollusks represented 252 isolates (Table 2). These were classified as 40 species that fit standard descriptions, and 44 putative new species listed as being like the closest description and including 7 identified only as *Candida* spp. Most species were represented by only a single or a few isolates. None of the yeast species was found in all of the microhabitats sampled. Various species occurred in all of the bivalves studied but none occurred in all of the crabs. The most frequently isolated genus was *Candida* followed by *Pichia*, *Kluyveromyces*, and *Kloeckera*; and the most frequently isolated species occurring in 28 samples was *K. aestuarii* with 82 cultures studied. A large population of *K. aestuarii* was found in the 2 detritus feeding crabs and *N. reynei*, but not in the omnivorous crabs feeding mostly on fresh materials within the mangrove and was mostly absent from clams of the tide flat in front of the mangrove area. *Candida famata*-like, *P. membranaefaciens*, *Candida valida*-like, and *Candida boidinii* were also frequently isolated species. The most widely dis-

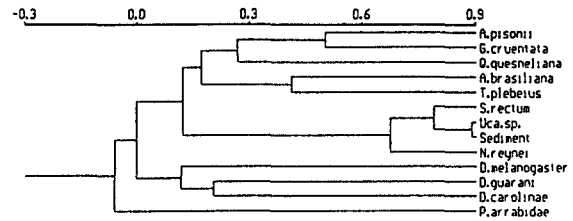


Fig. 2. Dendrogram representing similarity (r) between different yeast communities of the mangrove ecosystem and representative yeast communities from other environments: *Aratus pisonii* (tree crab), *Goniopsis cruentata* (Red and black Aratu crab), *Sesarma rectum* (Aratu crab), *Uca* spp. (fiddler crab), *Neoteredo reynei* (shipworm), *Anomalocardia brasiliana* (clam from mud flats, site 79A), *Tagelus plebeius* (clam from along stream in mud flat), sediments from mangrove area (site 79B, C & D), *Quesnelia quesneliana* (bromeliad), *Drosophila melanogaster* from Ilha do Fundão, *Drosophila guarani* group from Pau de Fome Forest, *Drosophila carolinae* from Ilha Grande Forest, *Pilosocereus arrabidae* cactus from the Maricá sand dunes.

tributed species in the mangrove microhabitats were *C. guilliermondii* and *C. famata*. Basidiomycetous yeasts including 22 isolates of *Cryptococcus* spp., 26 isolates of *Rhodotorula* spp., 2 isolates of *Tremella* spp., and 2 isolates of *Trichosporon cutaneum*-like, and yeast-like organisms including 7 isolates of black yeasts and 17 isolates of *Geotrichum* species were present although not studied extensively in this project.

The similarities between different invertebrate associated yeast communities in the mangrove ecosystem, mangrove sediment (Soares et al. unpublished), the mangrove bromeliad *Quesnelia quesneliana* (Hagler et al. 1993), the restinga cactus *Pilosocereus arrabidae* (Rosa et al. 1994), and representative rain forest *Drosophila* groups *melanogaster* and *guarani* (Morais et al. 1992) and *carolinae* (Morais et al. 1995) are presented in Fig. 2. Yeast communities of the omnivorous crabs and detritus feeding crabs formed two separate groups. The yeast communities of the detritus feeding crabs were grouped with those of the sediment and *N. reynei*, but the omnivorous crab yeast communities were linked to that of the bromeliad *Q. quesneliana* and more distantly with those of the clams *A. brasiliana* and *T. plebeius*. The yeast communities from rain forest drosophilids and the cactus *P. arrabidae* representing other littoral ecosystems of the southeastern region of Brazil were distant from those of the mangrove community. The cactophilic yeast community was distinct from all the other yeast commu-

Table 1. Geometric means of microbiological counts per 100 ml of water or 100 g of invertebrates.

Substrats	Total coliforms (MPN) ¹	Fecal coliforms (MPN)	Total fungi (MPN)	Yeasts (MPN)	Heterotrophic bacteria (CFU) ²
Water (marine) ³	2.8×10^2	3.9×10^1	1.4×10^3	3.4×10^1	1.8×10^5
Water (Rio Timirim) ⁴	2.3×10^4	4.0×10^3	2.0×10^3	1.3×10^2	6.5×10^5
<i>T. plebeius</i>	2.0×10^5	2.8×10^4	1.2×10^5	4.2×10^3	7.1×10^6
<i>N. reynei</i>	9.9×10^4	1.0×10^4	9.2×10^4	1.2×10^4	3.4×10^7
<i>A. brasiliiana</i>	6.7×10^3	1.1×10^3	1.1×10^5	2.6×10^3	4.0×10^6
<i>G. cruentata</i>	2.3×10^3	1.0×10^2	9.6×10^4	1.6×10^2	6.2×10^7
<i>A. pisonii</i>	1.7×10^3	7.8×10^1	1.0×10^5	3.9×10^2	1.3×10^8
<i>Uca</i> sp.	2.0×10^3	7.8×10^1	1.0×10^5	5.0×10^2	4.1×10^7
<i>S. rectum</i>	7.9×10^4	1.7×10^2	7.2×10^4	1.0×10^3	2.6×10^8

¹ MPN – Most Probable Number. ² CFU – Colony Forming Unit. ³ Salinity $23 \pm 8\%$, Temperature $27.5 + 3.4^\circ$ C. ⁴ Salinity $2.6 \pm 1.9\%$.

nities, and the three *Drosophila* associated yeast communities formed a separate cluster.

Discussion

The coliform counts showed a low level of recent fecal pollution in the seawater in front of and entering the Coroa Grande mangrove area. This water is of bathing quality but is not fit for shellfish harvest based on United States standards. However the water of the Timirim River which flows along the mangrove is notably more polluted and failed to pass bathing standards although it had a clean appearance. The higher microbial counts in the filter feeding bivalves, which concentrate microorganisms along with other suspended solids from water, were consistent with previous data for the mussel *Perna perna* (Araujo et al. 1991; Kosawa da Costa et al. 1991). The high coliform counts of *N. reynei* may result from a favorable environment for enteric microbes within its burrows, whereas those from *S. rectum* (influenced by a very high count in one sample) and *T. plebeius* probably reflect the proximity of their habitat to the Timirim River.

The yeasts associated with each invertebrate reflected its habitat and feeding habits. *N. reynei* contained more yeasts considered to be opportunist pathogens, including *C. albicans*, *C. krusei*, *C. guilliermondii*, and *C. tropicalis* (Hurley et al. 1987), than the other invertebrates. This should reflect its filter feeding activity and the unusual environmental conditions rich in reduced sulfur compounds that should favor more

fermentative yeasts within the burrows of this organism. *T. plebeius* also contained a number of opportunistic pathogenic yeasts which were probably filtered from the polluted waters of its habitat along the mouth of the Timirim River. The most frequent genus was the form genus *Candida*, which is the imperfect states of diverse ascomycetous genera and does not represent a natural group. The second most isolated genus was *Pichia* which is now the largest ascomycetous genus but appears to be polyphyletic in its origin and eventually will need to be split into several genera (Kurtzman 1984). Both *Pichia* and *Candida* species are also frequent isolates from aquatic and forest environments (Hagler & Ahearn 1987; Morais et al. 1992, 1995). These two genera probably are not as dominant as they appear now because they should each represent various genera when their taxonomic situations are better resolved. *Candida sorbosa* and *Candida silvae* were frequent isolates from the bivalves and are typical of the yeasts isolated from polluted estuarine waters. The genus *Kloeckera* was frequent in the clams and may enter with water from the Timirim River since this genus is associated with polluted estuarine waters, ripe and spoiling fruits and insect vectors (Hagler & Mendonça-Hagler 1981; Phaff & Starmer 1987; Morais et al. 1992). *Candida colliculosa* and its teleomorph *Torulasporea delbrueckii* were frequent isolates from the mangrove invertebrates and were also frequent in studies of forest drosophiloids (Morais et al. 1992, 1995). *C. famata*, the anamorph of *D. hanseni*, and *P. membranaefaciens* and its anamorph *C. valida* are generalist species that were frequently isolated in

Table 2. Ascomycetous yeasts and anamorphs from invertebrates of the mangrove at Coroa Grande, RJ, Brazil.

Yeast Species	crabs				mollusks			total
	Ap	Gc	Sr	UC	Nr	Ab	Tp	
	(8)	(13)	(8)	(8)	(18)	(8)	(6)	(51)
<i>Candida apis</i> var. <i>galacta</i> (Lavie ex van Uden & Vidal-Leiria)								
Meyer & Yarrow							1	1
<i>Candida azyma</i> -like		1						1
<i>Candida albicans</i> (Robin) Berkhout			1		3			4
<i>Candida boidinii</i> Ramirez	1				6	2	2	11
<i>Candida butyri</i> Nakase			1					1
<i>Candida butyri</i> -like			2					2
<i>Candida castellii</i> -like						1	1	2
<i>Candida catenulata</i> Diddens & Lodder							2	2
<i>Candida colliculosa</i> -like		1			2		3	6
<i>Candida diddensiae</i> (Phaff et al.) Fell & Meyer						1		1
<i>Candida diversa</i> -like						1		1
<i>Candida edax</i> -like	1							1
<i>Candida entomaea</i> van der Walt.					1			1
<i>Candida famata</i> (Harrison) Meyer et Yarrow		1	1	1	1	1	4	9
<i>Candida famata</i> -like	1		1		5	3	5	15
<i>Candida fennica</i> -like					1			1
<i>Candida freyschussii</i> Buckley e van Uden							1	1
<i>Candida guilliermondii</i> (Castellani) Langeron et Guerra	1	1		1	2	1	3	9
<i>Candida homilentoma</i> van der Walt & Nakase							1	1
<i>Candida homilentoma</i> -like	1							1
<i>Candida incommunis</i> -like							1	1
<i>Candida inconspicua</i> -like	1							1
<i>Candida intermedia</i> (Ciferri & Ashford) Langeron & Guerra					1		1	2
<i>Candida ishiwadae</i> Sugiyama & Goto							1	1
<i>Candida krusei</i> (Castellani) Berkhout					7	1	2	10
<i>Candida krusei</i> -like						2		2
<i>Candida lipolytica</i> (Harrison) Diddens et Lodder					1		2	3
<i>Candida lusitanae</i> van Uden et Do Carmo Sousa						1		1
<i>Candida melibiosica</i> Buckley & van Uden					1			1
<i>Candida milleri</i> Yarrow					1		1	2
<i>Candida oleophila</i> Montrocher							1	1
<i>Candida parapsilosis</i> (Ashford) Langeron & Talice				1				1
<i>Candida parapsilosis</i> -like	1	1			1			3
<i>Candida pseudolambica</i> -like					2			2
<i>Candida rugosa</i> -like		1	1		1	1	1	5
<i>Candida sake</i> (Saito & Ota) van Uden & Buckley	1	1			1		1	4
<i>Candida silvae</i> Vidal-Leiria & van Uden					2		3	5
<i>Candida sorbosa</i> Kurtzman, Smiley et Johnson		1			3	1	2	7
<i>Candida sorboxylosa</i> -like		1						1
<i>Candida steatolytica</i> -like							2	2
<i>Candida tenuis</i> -like	2	1						3
<i>Candida terebra</i> Sugiyama & Goto	1			2				3
<i>Candida tropicalis</i> (Castellani) Berkhout		1			1	1	1	4
<i>Candida tropicalis</i> -like			1					1

Table 2. Continued

Yeast Species	crabs				mollusks			total
	Ap	Gc	Sr	UC	Nr	Ab	Tp	
	(8)	(13)	(8)	(8)	(18)	(8)	(6)	(51)
<i>Candida valida</i> -like		1			8	1	1	11
<i>Candida vinaria</i> -like				1				1
<i>Candida viswanathii</i> -like					1			1
<i>Candida zeylanoides</i> -like							1	1
<i>Candida</i> spp.	2	3	1		3		1	10
<i>Debaryomyces vanrijae</i> -like		1		1				2
<i>Debaryomyces vanrijae</i> (van der Walt & Tscheuschner) Abadie et al.	1	1						2
<i>Endomycopsella crataegensis</i> -like anomorph					1			1
<i>Issatchenkia scutulata</i> -like anomorph					2		1	3
<i>Kloeckera africana</i> (Klocker) Janke						2		2
<i>Kloeckera apiculata</i> (Reess emend. Klocker) Janke					1	1	4	6
<i>Kloeckera apis</i> Lavis ex Smith, Simione et Meyer					1		1	2
<i>Kloeckera corticis</i> (Klocker) Jonke						1		1
<i>Kluyveromyces aestuarii</i> (Fell) van der Walt			6	8	13		1	28
<i>Pichia amethionina</i> -like anomorph		1						1
<i>Pichia angusta</i> (Tennison et al.) Kurtzman				1				1
<i>Pichia angusta</i> -like							1	1
<i>Pichia anomala</i> Sydow & Sydow					2	1	1	4
<i>Pichia carsonii</i> -like					1			1
<i>Pichia cifferri</i> -like			1					1
<i>Pichia fabianii</i> -like anomorph					1		1	2
<i>Pichia kluyveri</i> -like					1		2	3
<i>Pichia media</i> -like anomorph					1			1
<i>Pichia membranaefaciens</i> Hansen	1			3	6	2		12
<i>Pichia ohmeri</i> (Etchells & Bell) Kreger van Rij			1		1	1	1	4
<i>Pichia ohmeri</i> -like							1	1
<i>Pichia pipperi</i> van der Walt & Tscheuschner							1	1
<i>Pichia rabaulensis</i> Soneda & Uchida							1	1
<i>Pichia spartinae</i> -like anomorph						1		1
<i>Pichia</i> sp.							1	1
<i>Saccharomyces cerevisiae</i> Meyen ex Hansen				1	1		1	3
<i>Saccharomyces exiguus</i> Rees ex Hansen					1			1
<i>Schizoblastosporon</i> sp.						1		1
<i>Torulaspota delbrueckii</i> (Lindner) Lindner	1			1	1			3
Total	16	18	17	21	89	28	63	252

Ap = *Aratus pisonii* (tree crab). Gc = *Goniopsis cruentata* (Red Aratu crab). Sr = *Sesarma rectum* (Aratu crab). UC = *Uca* sp. (fiddler crab). Nr = *Neoteredo reynei* (shipworm). Ab = *Anomalocardia brasiliana* (clam from mud flats, site 79A). Tp = *Tagelus plebeius* (clam from along stream in mud flat). -like indicates closest species description to a probable undescribed species.

this study. Caution should be taken in drawing conclusions on frequency and distribution of *C. famata* and *P. membranaefaciens* isolates because these species descriptions include many variable results and may include many undescribed species. An example of this

occurred in the early work on cactophilic yeasts with various cactophilic *Pichia* species being included in *P. membranaefaciens* (Phaff & Starmer 1987). *Candida boidinii* and other methylotrophic yeasts that were isolated with moderate frequency in this study may be

using methanol formed by other microbes involved in metabolism of C1 compounds in the mangrove environment. The large proportion of yeasts including *Candida lipolytica* capable of assimilating hexadecane suggests that hydrocarbon substrates should be common in this ecosystem. The frequent isolation of *Cryptococcus* and *Rhodotorula* species is consistent with the importance of these groups in the phyllosphere and in unpolluted sediments and water (Ruinen 1963; Hagler & Ahearn 1987).

K. aestuarii appears to be strongly associated with some detrital feeding invertebrates and sediments within mangrove vegetation areas (Table 2, Soares et al. unpublished). Crabs could serve as vectors for *K. aestuarii*, but are apparently not essential in this role since this yeast was frequent in *N. reynei* and sediment in site 79B but not present in the two dominant crabs of this site. It is possible that the tidal action of site 79B was sufficient to disperse the yeasts in this region of the mangrove. The only other study noting extensive isolations of this species was Ahearn et al. (1968) where 14 strains were isolated from the saline regions of the everglades in Florida, USA. Occasional isolates including that which was the basis of the original description (Fell 1961) have been made from shallow sediments of South Florida and the Bahamas. *K. drosophilorum* (Syn. *K. lactis*) has been isolated in high numbers from rhizosphere sediments of spartina grass dominated marshlands. *K. aestuarii* and *K. lactis* have been found to be closely related to each other and also to *Kluyveromyces marxianus*, a yeast associated with the drosophiloids of forests in both temperate and tropical regions (Phaff & Starmer 1987; Morais et al. 1992, 1995; Peng Shen & Molina 1994). This suggests a possible evolution of the mangrove inhabiting species *K. aestuarii* from the marshland occurring species *K. lactis* or vice versa, and these may have originated from *K. marxianus* or all from a common ancestor.

Many of the yeasts appear to represent transient allochthonous species in the invertebrates studied because they were represented by only one or a few isolates. These may have their origins in the Atlantic forests behind the mangrove area and enter with runoff. However, there was a strong separation between the yeast communities associated with invertebrates of the mangrove ecosystem and those representing the coastal forest ecosystem of this region (Fig. 2). The Atlantic Forest *Drosophila* associated yeast communities were more similar to the yeast community of the dominant cactus species of the region than to those of the mangrove invertebrates. This suggested that many of the

yeasts isolated may have originated from yet unstudied sources such as other microhabitats of the mangrove area like the fruits, flowers, and fluxes of mangrove plants, or insects. The extreme diversity complicated taxonomic studies of the community because few species were represented by enough isolates to permit an idea of normal phenotypic variations, and many may be 'atypical' biotypes of known or new species. Further studies including application of molecular genetic methods will be necessary for an adequate treatment of these isolates.

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