Chapter 9

An Overview of the Yeast Biodiversity in the Galápagos Islands and Other Ecuadorian Regions

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Additional information is available at the end of the chapter

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1. Introduction

One of the most emblematic natural regions for studies of evolution and biodiversity in the world is the Galápagos Islands, which is the inspiring environment where the naturalist Charles Darwin was moved to propose what eventually became Theory of the Origin of Species launched in the 19th Century.

This Archipelago has been formed by subaquatic volcanic activity around 5 million years ago. The plant and animal populations settled on this group of 21 islands and 107 rocks and islets were introduced mainly by the sea currents and winds that reached the emerging lands in this equatorial region of the sea.

The study of plants and endemic species of animals has fascinated biologists for decades. Giant turtles, finches, marine and terrestrial iguanas and boobies have been the center of studies, as well as other birds and flora of the region. Many adaptations and evolution evidences were found in the macrobiota adapted to the particular environments of each island in the archipelago.

However, not much attention was paid to the microorganisms and, in particular, to yeast biodiversity in the islands. In 2009 in an effort to address this scientific shortfall, a prospective study was started by Ecuadorian-Brazilian-Spanish team that visited four human-inhabited islands (i.e. Floreana, San Cristóbal, Santa Cruz and Isabela).

The substrates chosen by the researchers were mainly flowers from *Datura* and *Ipomoea* genera, as well as *Opuntia* fruits and leaves. Moreover, unique substrates like endemic tree's exudates or even giant turtle's and marine iguana's feces were also taken. Flowers, insect, fungus and rotten vegetal matter was also part of the substrates chosen by the expeditionaries.

The resulting prospection yielded more than 800 yeast isolates. Most of those yeasts have been identified by sequencing of the LSU or the 26S rDNA gene. Among the yeasts recovered, there are several novel yeast species such as *Saccharomycopsis fodiens* and *Kodamaea transpacifica*, and other hitherto non described ones.

About 31% of the yeast biota in the islands is coincident with the species found in Ecuador mainland. Most of the yeast species are hitherto not found in the mainland since 2006 when the Catholic University Yeasts Collection (CLQCA) initiated its identification, characterization and preservation activities, devoted to yeast. Currently this yeasts collection represents the most complete deposit of wild species from Ecuador.

A comparison between the yeast biodiversity in the islands with the yeasts biodiversity in Ecuadorian mainland is done in this chapter in order to draw a first line of understanding of the adaptability, biogeography and interaction of species in an insular territory located about 1000 Km from the nearest South American mainland coasts.

Moreover, an overview of the yeast biodiversity of mainland Ecuador's ecosystems is addressed in this chapter in order to establish the comparisons and the extent in which the closest mainland has had influence in current microbial (yeast) biodiversity in this relatively recently formed archipelago in the Pacific Ocean.

2. The Galápagos Islands features

As Michael H Jackson cites in his book titled Galapagos, a Natural History, "the feature that sets the Galápagos apart from all other archipelagos is its unique geographic position. Situated on the equator under the tropical sun, and yet bathed for much of the year by the cool waters of the Humboldt and Cronwell currents, the islands have a special mix of tropical and temperate environments which is reflected in the ecology of its unusual plants and animals" [1]. It is certainly something extremely unusual finding an archipelago which biota has had influences not only from the nearest American mainland but also from Australasian environments. The remarkable combination of living organisms in this group of oceanic islands and the adaptive radiation that eventually yielded such a particular group of animal and vegetal endemism makes the Galápagos Islands one of the most interesting environments in the world to study evolution, ecology and natural history.

The disharmony as one of the features of oceanic islands in the Galápagos is observed by the over-representation of certain plant groups such as Pteridophyta (ferns), Poaceae (grasses), Asteraceae (sunflower family), Amaranthaceae (pigweed family), Fabaceae (bean family), and Cyperaceae (sedge family), lichens, mosses and liverworts. On the other hand, the under or

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non-represented families of plants are: Palmaceae, Anacardiaceae, Meliaceae, Labiatae, Scrophulariaceae, Orchidiaceae, Acanthaceae, Melastomataceae and Bromeliaceae[1].

2.1. Climate

In the Galapagos Archipelago the diurnal temperature varies from 5°C in the windward side of the islands, to 10°C in the leeward sides [2]. The annual range is from 11 to 12°C; while during the warm season (from December to June) the temperature reaches 29°C. In August, the upper limit is about 19°C [3].

Because of the equatorial location of the islands, there are only two seasons readily distinguishable, where the rainy weather coincides with warm period (December to June) and the dry, cooler season from July to November, which is a period characterized as mostly foggy and overcasted [3].

In terms of rainfall, the whole archipelago receives less than 750 mm per year; this is due to the geographical situation of the islands which are in the dry zone of the Pacific Ocean.

2.2. Vegetation zones

The islands where the yeast expedition was carried out in October 2009 (during the dry season), are all inhabited by human populations. These four islands: Isabela, Floreana, Santa Cruz and San Cristóbal have littoral zones as well as high zones which exhibit very clear differences in plant species. The zones of the higher elevations such as Sierra Negra volcano are mostly rocky and sterile, where only a few *Opuntia* sp. individuals are seen. In contrast the higher zones of Floreana, Santa Cruz and San Cristóbal all of them exhibit a green cover and even agricultural zones where a number of introduced species are being cultivated.

The list of plants occurring in the littoral zone is small and differs less from a list of plants from a comparable mainland zone [3]. As for the vegetation in this zone, there is a lack of plant species in the sheer cliffs or basalt rocks rising from the sea to 10 or more meters. In Santa Cruz Island there is a predominant and extended area of "palo santo" *Bursera graveolens*, in a rather xerophytic vegetation environment.

There are also arid zones located immediately inland from the littoral zones, where vegetation is xerophytic which remain even up to 80 to 120 m height but sometimes much higher reaching up to 300 m. Predominant genus in this zone are *Opuntia*, *Jasminocereus* and *Brachycereus* which share the spaces with small leaved and spiny shrubs and trees. Most interestingly for yeast communities which "prefer" to degrade vegetal matter is the fact that annual herbs prosper during the wet season, providing a green cover which lasts for several weeks. After that period, the biomass from the green blanket dies and degrades: a micro ecological niche which offers a great opportunity of growth for yeasts and other microorganisms. *Datura* and *Ipomoea* species both of them introduced by human in undetermined times, are frequently found in this zone as well as in the transition zone.

In the transition zone the plant communities are frequently evergreen with ample leaves which provides with a green landscape to this zone. There is possible to find out some epiphytic plants, orchids, lichens and bryophytes. Ferns are also part of the communities: a vast forest composed by arboraceous ferns can be seen in Santa Cruz Island. The transition zone covers a large area in the Galápagos Islands, where the upper and lower limits varies considerably from one island to the other.

There is also a very particular zone inhabited mainly by an endemic species of tree, *Scalesia*. This species occurs in the range of 200 to 400 m.a.s.l. Some exudes of this species were sampled in the search of yeast species along the expedition. In this zone the trunks, branches, twigs and in some species even the leaves bear large numbers of epiphytic liverworts and mosses. Saprophytic fungi are common to abundant during the early part of the rainy season. Amanita and Agaricus-like species with caps as much as 15 cm diameter can be seen as well as several types of puffballs [3]. Additionally it is distinguishable the *Miconia* zone, another endemic plant that is catalogued as shrub which grows between 400 to 550 m.a.s.l. In the community of this zone herbaceous plants are important members: *Lycopodium* species are widely represented in the plant's community.

Beyond the *Miconia* zone and overlapped there is the Fern-Sedge zone that occurs from 500 to 700 m.a.s.l. This zone reaches the top of the peaks in the larger islands of the archipelago. The zone is frequently covered by clouds during the wet season, thus, the vegetation uptakes the water in the form of a fine drizzle or fog [3]. Plants growing in this zone are mostly low growing species with narrow leaves to reduce the scarce water available. This is the habitat of the endemic tree fern *Cyathea weatherbyana.*

2.3. Fauna

The fauna in the Galápagos Islands is disharmonic respect to the mainland: there is a lack of several phyla and groups of animals in the different archipelago's environments. The Class Amphibians is not present in any of the islands, islets or rocks in the Galápagos Archipelago, due to the long distance from the mainland and the scarcity of fresh water; there is no possibility to survive a trip along the 1000 km distance between the mainland and the islands for any amphibian species.

Another scarcely represented group is the mammals, from which it is possible to find three orders: Chiroptera with three endemic species of bats; Pinnipeds, represented by the Galápagos fur seals, a close relative of fur seals from the Southern Hemisphere, and sea lions which are a subspecies of the Californian sea lion. Additionally, there are six species belonging to the Order Rodentia that represent the only terrestrial endemic mammals in the islands. Nevertheless, it is important to mention that a number of mammal species were introduced in the islands namely goats, horses, pigs, dogs, donkeys, cats, rats and cattle and other Guinea pigs. These species are a serious threat to the whole environments of the islands.

Reptiles like marine and terrestrial iguanas, as well as marine and terrestrial tortoises are very representative species of the archipelago. In fact, the giant tortoises, *Geochelone elephantopus* are the animals for which the islands were named. This is a herbivorous species which represented an interesting organism to study from its gut microflora—including the yeasts—point of view. There is only one snake species endemic to the islands and seven species of lizards (lava lizard) from the genus *Tropidurus*. Finally, there are some species of gekos, especially in the inhabited islands, where they've found some empathy with human population.

Birds are fairly well represented in the islands with 108 species, of these, 89 reside and breed in the archipelago and 77 of them are endemic. The commonest and most thoroughly studied birds are the Geospizidae, or Galápagos finches, the flight less cormorant, the Galápagos penguin, the small Galápagos green heron, the endemic dove, the Galápagos hawk, the endemic duck and several subspecies of mocking birds. Additionally there are big colonies of blue footed boobies, red footed boobies and masked boobies.

All the above mentioned animals are interesting from their gut's microflora composition that becomes themselves as vectors for the introduction of yeasts species into the islands environments. The mobility of marine species and birds between the islands constitutes a factor of dispersion for yeasts as well as plant species. But there is another group of animals that are highly involved in the yeasts biodiversity: the invertebrates, and particularly the Arthropoda.

Arachnida and Chilopoda represented by spiders and scorpions are very numerous; nevertheless there are a few species in the islands. Spiders inhabit all the vegetation zones, but are less common in the fern-sedge zone before described. Centipeds are also very common and numerous.

In the case of insects, the fauna is considered relatively poor compared to the closest mainland Ecuador. This is a feature shared with other oceanic islands. "The biological paucity of diversity and abundance was noticed initially by Darwin: ''I took great pains in collecting the insects, but, excepting Tierra del Fuego, I never saw in this respect so poor a country'' [4].

There are Hymenoptera, especially bees that act as pollinators; butterflies and moths are scarcely represented with only 7 and 12 species respectively [3]. Some bees are thought to be introduced, for instance *Megachile timberlakei* that is an unexpected register in Galápagos since this bee species is common in Hawaii [4].

New studies of lice in the Galápagos report 47 genera and 104 species, 17 of them are endemic species, 79 are native and eight introduced by human agency [5]. On the other hand, the Othopteroid insects are one of the more diverse assemblages found in the archipelago, with 57 species in 37 genera in seven orders. The cricket genus *Grillus* has eight native species, nevertheless some of the crickets species are introduced [6].

One insect species (*Halogates robustus*) from the Hemiptera order occupies a somewhat uncommon habitat in the islands. This is a flightless marine insect which occurs in the surface of the coastal waters, associated to mangrove and lava edges [7].

The Thysanoptera in the Galápagos Islands accounts for 77 species of thrips which belong to 42 genera and four families. This group of insects has been registered in 17 islands. At least nine of the existing species are considered serious pests [8], owing to their herbivorous habits that causes serious damage to crops.

The islands harbor also endemic cockroaches' species which may be of high interest in terms of the gut's microbiota. Eighteen species are reported to occur in the archipelago, where five are endemic [9].

Beetles species are relatively more abundant, occurring in all the vegetation zones. Some of them are found around flowers of cacti, *Ipomoea* sp., *Datura* sp., and a number of other plant species. It has been reported a number of about 200 beetles species [10].

Additionally there is a number of introduced species of insects such as cockroaches, *Drosophila* sp, ants and flies, among the most conspicuous. The changes in the insect biota composition in the islands occurred with the introduction of foreign species by aboriginal peoples and later by colonization activities of Europeans [11]. These insects also play a fairly important role in dispersion and introduction of yeast species into the islands.

3. On the Galápagos biogeography

The Galápagos Islands represent a strategic geographic zone in the planet to carry out studies on biodiversity, speciation, adaptation, ecology and dispersion of species belonging to different kingdoms. In an attempt to explain the current biogeography of the islands, a number of studies have been done since the Darwin's times in the 19th Century [12-14].

It is well known that the islands geological origin is oceanic, while the origin of its biota is continental [15]. The studies on the Galapagos Islands' biogeography and evolution were predominantly (if not exclusively) focused on macro organisms: there is a marked shortfall in the knowledge of microbial populations, communities and biodiversity in the Galapagos Islands.

Standard biogeographic tracks link the archipelago of Galápagos with Central America, western North and South America, the Caribbean, Asia and Australasia [14]. The Galápagos Islands community characteristics share the common features of the biota occurring in oceanic islands such as: disharmony, endemism and relictualism [16].

The archipelago of Galápagos is composed by oceanic islands where the terrestrial colonists such as plants, animals, and part of the microorganisms, should have crossed an oceanic barrier to reach the land. It means that the origin of the biota in this group of islands is entirely explained by the dispersal with no vicariant component, since the islands were never connected to the mainland by any land bridge or island chain [14]. Furthermore, since the Galápagos Islands are separated from the centers of origin of species in the mainland, there are abundant "empty" ecological niche spaces [17].

The colonization of the varied environments within the islands by yeast species must be primarily explained by the occurrence of colonizing terrestrial species of plants and animals that arrived to the islands as a sweepstake along the natural history of the archipelago. The sea water and marine fauna must have been other sources of colonizing yeasts species. Finally, the yeasts flora inhabiting the gut of insects associated to plants as well as birds and terrestrial vertebrates must have completed the cast of yeast diversity in the Islands.

Undoubtedly the strong association of plants and microorganisms must be regarded as a dispersion factor for microorganisms, including yeasts in the Galápagos. In 1976 [18] determined that 236 species of vascular plants are endemic (representing 45% of total species); 155 species are from Neotropical origin (which represents 30% of the total species); 62 different species of vascular plants are from Pantropical origin (12% of total species); 61 species are originated in the Andes (representing 12% of total species); while only 4 and 2 species correspond to Mexico and Central America and non-tropical South America, respectively (2% of total species). This study takes into account the indigenous species of the archipelago, namely, those species that were not introduced by man's activities [18].

4. The expedition to Galápagos in the search of yeasts

In the past, the studies of microorganisms in the Galápagos Islands were focused on topics such as the bacterial dynamics around the islands [19] some studies referring to entomogenous fungi found in the Galápagos Islands [20]; and probably some other works devoted to punctual groups of moulds, etc.

To the best of our knowledge there is not any yeast collection from the Galápagos Islands or any other study involving yeasts in the past. Based upon this, we can say that this is likely the first study focused on the biodiversity of yeasts carried out in this zone of the planet.

The yeast biodiversity in four populated islands is the first approach that we've done in order to try shortening the lack of previous knowledge of the yeasts biodiversity in this archipelago. Nevertheless it is to say that inhabited islands' environments present certain degrees of disturb and where human mediated introduction of certain yeast species is a real issue. The islands herein reported as the localities for our collections were explored for logistical reasons, since those islands that are mostly pristine, represent a major logistic challenge that could be faced in future expeditions.

There's interesting islands to be explored in future works, such as Española, Pinta, Genovesa, Marchena, etc. Additionally there are rock shelters that could be explored in the search of substrates for yeasts isolation. In these environments no fresh water is available, moreover, most of these islets or rocks are inaccessible since their precipitous cliffs, rising directly out of the water present hazards to landing that almost no one has attempted to collect any living organism from those inaccessible and still pristine environments [3].

In this expedition we were able to focus our attention in collecting yeasts from several substrates, so we can draw a general overview of the yeasts biodiversity in the islands. Nevertheless, there is still much work to be done in future expedition in order to refine the current data of the yeasts biodiversity in the archipelago, since it is one of the still unexplored regions in the planet [21].

5. Collection methodologies

In October 2009, from 19th to 23rd, an expedition composed by 5 researchers from Ecuador, Brazil and Spain went to the Galapagos Islands in order to start a pioneer study mostly on yeasts biodiversity within the still natural environments of four inhabited islands. This chapter is the first report of such expedition that explored Santa Cruz, Isabela, Floreana and San Cristóbal Islands, where a number of substrates were sampled by using different culture media and techniques.

Yeast sampling in substrates like flowers, fruits, excrement or fugus was carried out using sterile cotton wool swabs, to inoculate in liquid and solid YM media. In the case of sampling the insect's gut content a technique of catching the living insect in plastic bags for further inoculation by the living insect walking on the surface of Petri dishes containing YM agar medium. Eventually the insects were liberated alive. Additionally a number of substrates were collected in plastic sterile tubes for further culturing in selective culture broths such as YNB-CMC, YNB-D-xylose; YNB-xylan; YNB-L-arabinose, and YNB-raffinose the CLQCA laboratory in Quito, Ecuador. The selective culture media were used especially in the search of yeast strains that exhibit some biotechnological potential use in xylose fermentation as well as cellulose degradation/fermentation.

6. Yeast species identification

Macroscopic and microscopic identification of yeasts is frequently inaccurate due to the high similarity that yeast may show at a glance, either in colony or in a microscopic field. The best way to identify and differentiate yeast species as well as strains is by molecular techniques that are being used since 2000. A variety of techniques have been developed and this fact has greatly boosted the number of new species of yeasts identified. Molecular analyses of the variable D1/D2 regions of the 26S rDNA, 18S, 5.8S and mitochondrial small subunit rDNAs gene, as well as ITS sequencing and RFLP-ITS are very useful ways to identify yeast species and invaluable tools for phylogenetic studies [21, 22].

The D1/D2 domain of the LSU rRNA gene was PCR amplified directly from whole yeast cell suspensions [23], and using the primers NL1 and NL4 [24]. Initial amplification reactions were carried out in Ecuador (The Catholic University Yeast Collection-CLQCA) and Brazil (Collection of Microorganisms and Cells of UFMG). Amplified fragments were checked by agarose gel electrophoresis. The PCR purified products, were further sequenced with the external amplification primers NL1 and NL4. Finally, a sequence similarity search was conducted using NCBI Blast tool. Yeasts with more than 99% identity were considered members of the same species.

6.1. An overview of the yeast species in four Islands of the Galápagos Archipelago

As a result of the collections carried out in Santa Cruz, Isabela, Floreana, and San Cristóbal Islands 881 isolates were recovered from a wide variety of substrates. Currently we can report 614 yeast isolates already identified, while 267 isolates are in process of identification.

The number of yeast isolates collected per island is shown in Table 1.

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Santa Cruz Island	Isabela Island	Floreana Island	San Cristóbal Island	Total Isolates
321	269	177	114	**881**

Table 1. Number of yeast isolates collected in four islands of the Galápagos Archipelago during an expedition carried out in October 2009

The difference in the abundance of yeast isolates collected in each island is due to different conditions of time and logistics of the expedition and does not have any relationship with the abundance or diversity of yeasts in each island.

The present chapter reports the isolates and biodiversity recovered in a number of substrates including: flowers, rotten wood, excrement, insects, fruits, exudates, leafs, one sugar cane mill (Santa Cruz Island) and others. Figure 1 shows the shares of yeast isolates by substrate we have preserved in the Catholic University Yeasts Collection (CLQCA).

6.2. Yeast species in Ecuador Mainland and Galápagos Islands

The Catholic University Yeasts Collection in Quito, in its database presents 118 yeast species belonging to the Ecuador mainland and the Galápagos Islands as shown in Table 4. By establishing a comparison between these two regions, there is not a big difference in number of species registered in the mainland and the islands (82 and 78 respectively). It is important to remark that the yeasts registers in this work were taken only from natural environments and substrates (no clinical or industrial samples are taken into account).

In Mainland Ecuador, about 50% of the characterized isolates preserved in the CLQCA (c.a. 250 yeast isolates) belong to four species: *Candida tropicalis, Meyerozyma guilliermondii, Kodamaea ohmeri,* and *Pichia kudriavzevii.* In contrast, the more represented isolates from the Galápagos Islands in the CLQCA (c.a. 615 yeast isolates) correspond to the species: *Candida tropicalis, Hanseniaspora* sp., *Pichia norvegensis, Candida parazyma, Kodamaea transpacifica, Hanseniaspora uvarum, Barnettozyma californica, Candida intermedia,* and *Galactomyces geotrichum*.

Candida tropicalis in both cases is the most abundant yeast species registered in CLQCA: in mainland it is about 21% of the total identified isolates, while in the Galápagos Islands it represents about 18%. *C. tropicalis* is a cosmopolite yeast species that is ubiquitous in a wide range of substrates: from beetles to fermented beverages, but predominantly it is found in rotten vegetal matter, flowers and excrements.

Between the three regions of mainland there are also registers of 10 species that have been collected from different substrates. These species are quite adaptable to a wide range of climatic conditions and substrates. Table 2 shows the species that are ubiquitous in Amazonia, Andes and Pacific Coast.

Matching the coincidences of yeast species between any individual mainland region with the Galápagos Islands yeast isolates it is noticeable an increase in the number of coincident yeast species if compared to the matching between the three regions within the mainland (Table 3).

N°	YEASTS SPECIES
1	*Candida intermedia*
2	*Candida tropicalis*
3	*Galactomyces geotrichum*
4	*Hanseniaspora* sp.
5	*Kodamaea ohmeri*
6	*Pichia kluyveri*
7	*Pichia kudriavzevii*
8	*Pichia manshurica*
9	*Saccharomyces cerevisiae*
10	*Torulaspora delbrueckii*

Table 2. Ubiquitous yeast species within Ecuadorian mainland

This increase represents almost four times the amount of the yeasts species shown in Table 2 (i.e. 38 yeast species) according to updated CLQCA registers.

Despite the fact that it is really difficult to address an accurate center of origin of the yeast species occurring in the natural zones of the Galápagos Islands (the islands are now visited by thousands of tourists along the year), we must assume that the Ecuadorian mainland must be the center of origin of most of the yeast species occurring in the Galápagos based upon the Porter's report [18] where about 30% of the vegetal species in the Galápagos Islands have had a Neotropical origin. From our data, about 31% of yeast species have been found both in Ecuador Mainland and the Galápagos Islands. These figures reveal the disharmony of taxa between mainland and oceanic islands [16].

Table 3 shows the species that matched at least once between any single regions of Ecuador's mainland with yeast species found in anyone of the four islands explored.

N°	SPECIES FOUND IN MAINLAND AND THE ISLANDS
1	*Aureobasidium pullulans*
2	*Barnettozyma californica*
3	*Candida carvajalis*
4	*Candida humilis*
5	*Candida intermedia*
6	*Candida oleophila*
7	*Candida orthopsilosis*
8	*Candida parapsilosis*

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N°	SPECIES FOUND IN MAINLAND AND THE ISLANDS
9	*Candida pseudointermedia*
10	*Candida quercitrusa*
11	*Candida saopaulonensis*
12	*Candida sinolaborantium*
13	*Candida theae*
14	*Candida tropicalis*
15	*Cryptococcus humícola*
16	*Cryptococcus laurentii*
17	*Debaryomyces hansenii*
18	*Debaryomyces nepalensis*
19	*Galactomyces geotrichum*
20	*Hanseniaspora sp.*
21	*Kazachstania exigua*
22	*Kodamaea ohmeri*
23	*Meyerozyma guilliermondii*
24	*Pichia fermentans*
25	*Pichia kluyveri*
26	*Pichia kudriavzevii*
27	*Pichia manshurica*
28	*Pichia occidentalis*
29	*Pichia terricola*
30	*Rhodosporidium paludigenum*
31	*Rhodotorula glutinis*
32	*Rhodotorula mucilaginosa*
33	*Saccharomyces cerevisiae*
34	*Torulaspora delbrueckii*
35	*Trichosporon asahii*
36	*Trichosporon coremiiforme*
37	*Wickerhamomyces anomalus*
38	*Yamadazyma mexicana*

Table 3. Yeast species represented both in mainland and Galápagos Islands

On the other hand, mainland Ecuador is one of the richest biodiversity zones in the world [21], due to the varied environments as consequence of the topography that provokes the occurrence of uncountable micro-ecosystems as a consequence of a number of biotic and abiotic factors such as the altitude variation (from 0 to more than 6000 m.a.s.l), geographic location, marine currents, as well as the natural history involving the Neotropic which provides biogeographic unique features to this region of the planet [21].

The total yeast biodiversity currently registered both in Ecuadorian mainland and the Galápagos Islands (i.e. 118 yeast species identified) is shown in Table 4. The shaded cases represent the register of occurrence of the species either in the mainland or in the Galápagos Islands.

N°	YEAST SPECIES	ECUADOR MAINLAND	GALAPAGOS ISLANDS
1	*Aureobasidium pullulans*		
2	*Barnettozyma californica*		
3	*Candida albicans*		
4	*Candida apicola*		
5	*Candida asparagi*		
6	*Candida boidinii*		
7	*Candida boleticola*		
8	*Candida bombi*		
9	*Candida carpophila*		
10	*Candida carvajalis*		
11	*Candida cylindracea*		
12	*Candida dendronema*		
13	*Candida ecuadorensis*		
14	*Candida gigantensis*		
15	*Candida glabrata*		
16	*Candida humilis*		
17	*Candida intermedia*		
18	*Candida leandrae*		
19	*Candida naeodendra*		
20	*Candida natalensis*		
21	*Candida oleophila*		

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N°	YEAST SPECIES	ECUADOR MAINLAND	GALAPAGOS ISLANDS
22	*Candida orthopsilosis*	■	■
23	*Candida parapsilosis*	■	■
24	*Candida parazyma*		■
25	*Candida pomicola*	■	
26	*Candida pseudointermedia*	■	■
27	*Candida pseudolambica*	■	
28	*Candida quercitrusa*	■	■
29	*Candida rugosa*		■
30	*Candida saopaulonensis*	■	■
31	*Candida silvae*		■
32	*Candida sinolaborantium*	■	■
33	*Candida sonorensis*		■
34	*Candida sorbosivorans*		■
35	*Candida sorboxylosa*	■	
36	*Candida stellimalicola*	■	
37	*Candida tallmaniae*	■	
38	*Candida theae*	■	■
39	*Candida tropicalis*	■	■
40	*Candida trypodendroni*		■
41	*Candida xylopsoci*	■	
42	*Candida zeylanoides*		■
43	*Clavispora lusitaniae*	■	
44	*Clavispora opuntiae*		■
45	*Cryptococcus albidus*	■	
46	*Cryptococcus flavescens*		■
47	*Cryptococcus flavus*		■
48	*Cryptococcus humicola*	■	■
49	*Cryptococcus laurentii*	■	■
50	*Cryptococcus rajastharensis*		■
51	*Cryptococcus saitoi*	■	

N°	YEAST SPECIES	ECUADOR MAINLAND	GALAPAGOS ISLANDS
52	*Debaryomyces hansenii*		
53	*Debaryomyces nepalensis*		
54	*Dekkera anomala*		
55	*Dekkera bruxellensis*		
56	*Filobasidium uniguttulatum*		
57	*Galactomyces geotrichum*		
58	*Geotrichum silvicola*		
59	*Hanseniaspora guilliermondii*		
60	*Hanseniaspora meyeri*		
61	*Hanseniaspora opuntiae*		
62	*Hanseniaspora* sp.		
63	*Hanseniaspora uvarum*		
64	*Hanseniaspora valbyensis*		
65	*Kazachstania exigua*		
66	*Kazachstania unispora*		
67	*Kluyveromyces lactis*		
68	*Kluyveromyces marxianus*		
69	*Kodamaea ohmeri*		
70	*Kodamaea transpacifica*		
71	*Kurtzmaniella zeylanoides*		
72	*Kwoniella mangrovensis*		
73	*Lindnera* sp.		
74	*Lindnera fabianii*		
75	*Lindnera jadinii*		
76	*Lindnera saturnus*		
77	*Metschnikowia kipukae*		
78	*Metschnikowia koreensis*		
79	*Metschnikowia reukaufii*		
80	*Meyerozyma guilliermondii*		
81	*Pichia fabianii*		

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N°	YEAST SPECIES	ECUADOR MAINLAND	GALAPAGOS ISLANDS
82	*Pichia fermentans*		
83	*Pichia kluyveri*		
84	*Pichia kudriavzevii*		
85	*Pichia manshurica*		
86	*Pichia nakasei*		
87	*Pichia norvegensis*		
88	*Pichia occidentalis*		
89	*Pichia terricola*		
90	*Rhodosporidium babjevae*		
91	*Rhodosporidium paludigenum*		
92	*Rhodotorula glutinis*		
93	*Rhodotorula minuta*		
94	*Rhodotorula mucilaginosa*		
95	*Rhodotorula slooffiae*		
96	*Rhodotorula* sp.		
97	*Saccharomyces cerevisiae*		
98	*Saccharomycodes ludwigii*		
99	*Saccharomycopsis fodiens*		
100	*Saccharomycopsis vini*		
101	*Saturnispora quitensis*		
102	*Scheffersomyces stipitis*		
103	*Sporidiobolus ruineniae*		
104	*Sporopachydermia* sp.		
105	*Torulaspora delbrueckii*		
106	*Trichosporon asahii*		
107	*Trichosporon coremiiforme*		
108	*Trichosporon dermatis*		
109	*Trichosporon insectorum*		
110	*Trichosporon jirovecii*		
111	*Trichosporon laibachii*		

N°	YEAST SPECIES	ECUADOR MAINLAND	GALAPAGOS ISLANDS
112	*Trichosporon multisporon*		
113	*Wickerhamiella occidentalis*		
114	*Wickerhamomyces anomalus*		
115	*Wickerhamomyces onychis*		
116	*Yamadazyma mexicana*		
117	*Yarrowia lipolytica*		
118	*Zygotorulaspora florentina*		

Table 4. Species occurrence in Mainland Ecuador and/or the Galápagos Islands

It is noticeable that in terms of biodiversity registered in Ecuador's mainland and the Galápagos Islands, the figures are very similar. Nevertheless, it should be understood that the total sample that is herein analyzed, represents only a fraction of the total yeasts species which may be found in this biodiverse region of the planet. At this point of the research the registers of yeast species in Ecuador are still in constant updating as well as the registers in the rest of the world. We believe that less than 5% of the world's yeast biodiversity has been described [21, 25]. A big effort and a long time of research are needed to try fulfilling the CLQCA database. Despite the data analyzed in this first overview of the biodiversity of yeasts in the four regions of Ecuador are still in process, we can certainly draw some features of the yeast biota of the Galápagos and its closest mainland territory.

6.3. A brief ecological approach to the most remarkable yeast species in Galápagos and Mainland

The yeast species that are part of the communities found both in the Galápagos Islands and the mainland are: *Aureobasidium pullulans, Barnettozyma californica, C. carvajalis, C. humilis, C. intermedia, C. oleophila, C. orthopsilosis, C. parapsilosis, C. pseudointermedia, C. saopaulensis, C. sinolaborantium, C. tropicalis, Debaryomyces nepalensis, Galactomyces geotrichum, Hanseniaspora* sp., *Pichia terricola, Rhodotorula glutinis*, and *Rh. mucilaginosa*.

Strains recovered of *B. californica* in other studies includes substrates such as soil, ox dung, insect frass, pond water, sewage water, river water, and stream water [25]. In the Ecuadorian collection this species was found in flowers, beetles captured in flowers of *Ipomoea alba*, giant turtle's dung, sugar cane bagasse, fungus, and rotten wood. Insects may have played a role in the dispersion of this species from mainland into the Galápagos.

Candida humilis in the Galápagos Islands has been found in rotten wood and flowers, but other registers of this species such as the strains CBS 6312 and CBS 6099 reported in The Yeasts a Taxonomic Studies [25] which were found in frass and gut of auger beetles. In this case we have maybe another evidence of insects as vectors of this yeast species and the occupation of the habitats in the Islands may not be so different than in mainland for this yeast species. In

Ecuadorian mainland *C. humilis* was isolated from mango fruit, chicha de jora (corn fermented beverage), spider network, rotten wood and some flowers from the Asteraceae family. As for *C. intermedia* and *C. parapsilosis* are considered widespread yeasts that can be found in clinical samples, caterpillar frass, beer contaminants and other substrates. In the case of Ecuadorian isolates of these both species, the origin is predominantly found in vegetal substrates and in archaeological fermentation pots from ancient cultures.

In the case of *C. saopaulonensis* we can find that in the mainland Ecuador this species was isolated from a Heliconiaceae as well as the type strain collected in Brazil [26]; in contrast, this yeast species was found in nine different flowers from Asteraceae family in Santa Cruz Island, which is an interesting case of adaptation to new ecological niches in oceanic islands [16].

C. sinolaborantium [27] is a yeast species that in the Ecuadorian mainland as well as in the Galápagos Islands was found chiefly in vegetal substrates except for one strain that was collected in *Drosophila* sp. Nevertheless the type strain was isolated from the gut of handsome fungus beetle in Panamá, and two other strains were isolated from a cerambycid larva. The original study was carried out in insect's guts. The results obtained by [27] suggest that this yeast species is part of the microbial community that can be found in the intestine of insects (beetles) whose excretions are dropped on flowers and other vegetal matter.

Another remarkable yeast species found in this survey both in mainland and the Galápagos Islands is *C. tropicalis.* This ubiquitous species is by far the most abundant in isolates number that has been collected since 2006 in our surveys in Ecuador; *C. tropicalis* is practically distributed in every kind of substrate, which is a sign of the high adaptability of this species. As part of a study on traditional fermented beverages we were able to find this yeast species even in unexpected substrates such as cassava fermented beverages which are produced in Yasuní National Park, located in the deep Ecuadorian Amazonia. This millenary beverage is still being produced in the same traditional way by autochthonous tribes of Waorani people [28]. In the literature *C. tropicalis* is reported to be collected from clinical samples. This species belongs to the *Lodderomyces* clade, where *C. albicans, C. theae* [29] and other potential pathogens have been accommodated. Nevertheless its presence by itself does not mean a hazard of infection in human, at least to those who possess a strong immune response due to permanent exposition to *Candida* sp. cell wall antigens [30].

A very few understood yeast species in terms of its ecology is *Debaryomyces nepalensis*, since it was isolated from a number of different substrates like soil, fermenting tobacco, spoiled sake and others [25]. The isolates from the Galápagos were found in rotten wood, but also in flowers, orchids, and leafs among others. This yeast species was also collected in a range of altitudes in Ecuadorian territory: from 150 to 1820 m.a.s.l.

Galactomyces geotrichum [31] is another yeast species which is widespread along the four natural regions of Ecuador. Its distribution has been registered from 75 to 3500 m.a.s.l. This fact demonstrates its high adaptability to a number of ecosystems and growth temperatures. It is a vigorous yeast species characterized by its fast growth in laboratory conditions. The strength of this yeast is a feature that has been useful in the prevention of microbial infections in greenhouse crops [25] because of its highly competitive way of occupying micro substrates and ecological niches. As for the substrates where this yeast species was found in this survey, these includes rotten wood, flowers, turtle's feces, insects, vegetal residues, fruits, etc. Remarkably, by 1970 just a few strains of this species were collected from soil samples in Puerto Rico [25].

The genus *Hanseniaspora* was also found in flower samples from the Galápagos and Mainland Ecuador. This genus is characterized by its bipolar budding, presenting apiculate and ovoid cells that can even be long ovoidal or elongate [32]. This genus is composed by a number of species: *H. clermontiae, H. guilliermondii, H. lachancei, H. meyeri, H. occidentalis, H. opuntiae, H osmophila, H. pseudoguilliermondii, H. uvarum, H. valbyensis, H. vinae, H. singularis and H. thailandica.* In Ecuador mainland it was found *H. meyeri*, *H. guilliermondii*, *H. valvyensis, H. opuntiae,* and *H. uvarum*. Nevertheless, we couldn't yet identify the Galápagos Islands isolates to the species level.

In terms of the substrates where the yeast species have been found both in mainland and the islands there is a wide range of sources: insects, flowers, leaves, feces, sugar cane mills, fungus, fruit, moss, and a number of samples taken from endemic plants in the Galápagos Islands, including *Miconia robinsoniana*, *Scalesia* sp., *Opuntia* sp. *Castela galapageia*, etc. This genus is widely represented in the CLQCA, where about 100 isolates were collected from all the regions in mainland and Galápagos. The isolates from Galápagos represent about 60% of the total isolates of this genus in the CLQCA.

In the case of *Pichia terricola* [33] has been scarcely represented in the mainland with only one isolate in the Andean province of Pichincha. The range of altitude in which this species is distributed in the Ecuadorian territory, according to the registers of the CLQCA, is from 100 to 1120 m.a.s.l., and it's found in a variety of habitats, from dry forests in the Galápagos Islands to cloudy forests in the mainland. The description of this species was based on soil samples from South Africa, but it was also found in cherry juice, from pressed grapes, spoiled figs, and wine [25]. This is an ethanol assimilating yeast, since it can use this compound as an alternative carbon source. This yeast can also use other carbon compounds such as glycerol and glucose, as well as succinate.

This yeast species was collected in flowers, rotten wood, fruit, insects, and exude of trees. In Galápagos the microhabitats for this species are quite different to those of the original description in 1957, where *P. terricola* was named based on the soil samples where it was found in South Africa by [33]. The appellative given to this species illustrates a frequent issue found in taxonomic accommodation; the species names derived from the substrates where the isolates were found, may not provide an accurate idea of its ecological niche. Frequently, yeasts isolates may represent allochthonous members of microbial communities [34].

Rhodotorula glutinis [35] and *Rhodotorula mucilaginosa* [36] are both basidiomycetous yeast species characterized by the production of carotenoid pigments which protect them from the UV irradiation that provokes damage in DNA [37-39]; especially in zones where the solar rays are particularly severe, like in the equatorial latitude, the need for a protective strategy can make the difference for yeast cells that are exposed to aggressive light stress [40].

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Both yeast species are ubiquitous being found in a wide range of latitudes, including in Antarctica glaciers (data not published). Some registers of *R. glutinis* are in atmosphere, trees, leaves, grapes, soil, spoiled leather, sea water, water supply of a brewery, sputum of pneumonia patient, exudates, limph nodes, feces, pasteurized beer and a number of other substrates [41]. These two yeast species are not fermentative, which is a trait of basidiomycetous yeast species.

From Ecuador we were able to collect 46 strains belonging to these two species. Astonishingly, 18 out of 46 were isolated from ancient-dormant yeast communities found in fermentation vessels and other utensils used by ancient cultures from the Andes used in their daily life. The studies of Microbial Archaeology [42] yielded other yeast species which will be taken later on in this chapter.

Other substrates where *R. glutinis* and *R. mucilaginosa* were isolated from are fermented beverages, sugar cane juice, insects (Orthoptera, Coleoptera, Hemiptera), as well as in flowers, and moss.

C. carvajalis [43] was the first yeast species described from Ecuador. This yeast was found in the course of a yeast biodiversity survey in the Amazonia. The substrate sampled was rotten wood and fallen leaf debris, collected around crude oil wells, close to Dayuma town. One Isolate of this species was collected in Santa Cruz Island from *Psidium guajava* mucilage. The closest relatives of *C. carvajalis* are *C. asparagi*, *C. fructus*, and *C. musae*. This group of yeasts belongs to the *Clavispora* clade. In Mainland Ecuador it has not collected any *C. asparagi* [44] isolate, but in the Galápagos Islands we have one register from Santa Cruz Island where this species was collected from a nitidulid beetle.

6.4. Ecology of the yeasts species in the Galápagos Islands and Mainland Ecuador

The yeast isolates that were identified, characterized and preserved in the CLQCA since 2006 represent an invaluable platform for studies in ecology of yeasts form Ecuador. Our data permit a better understanding of the situation of the yeast species and communities in natural environments of Ecuador, which is a contribution for the knowledge of the yeast ecology and biology. In this collaborative work other laboratories and centers have been involved, namely the Collection of Microorganisms and Cells at the Universidade Federal de Minas Gerais in Belo Horizonte, Brazil; the National Collection of Yeast Cultures in Norwich, England; the Department of Biology at the University of Western Ontario, In Ontario, Canada, among the more active collaborators in this survey.

In this part of the chapter we will analyze not only the yeast species occurrence in Ecuador, but also the relationships we have found respect to the substrates where the yeasts were collected; the ecological roles of the species as reported by other authors and contrasting information we got in our own work. Finally, we will present some ecological similitudes and differences in the roles and behavior of yeasts depending on their biogeographic zones where they occur.

6.5. Yeast species by substrate

We intended to compare the communities of yeasts that are present in the different substrates we have sampled in mainland and the islands. The most common substrates represented in this survey are: flowers, rotten wood, beetles, excrement, rotten vegetal matter, fruits, insects (*Drosophila* sp. and Nitidulid beetles), exudates, leaves, fungus, and human related substrates such as artisanal sugar cane mills. Some substrates like bodies of water, moss, wood, etc. are not analyzed due to the fact that in mainland we do not have the correspondent substrates to compare with.

The data were homogenized in order to get comparable ecological niches in Galápagos and Ecuador mainland. Consequently, a comparison of the yeast communities found in analogous substrates sampled both in the islands and the mainland is made in this section.

As a consequence of this grouping of substrates, the number of species having correspondence between mainland and the islands decreased from 38 to 22, since the variety of substrates in mainland is much larger than those sampled in the Galapagos, furthermore, it was not possible to match all the samples for a comparison.

Nevertheless, an independent analysis of the species found only in mainland and the Galápagos was also performed in order to get some biodiversity plots of the yeast communities. Based on that, we were able to analyze the situation of yeast biodiversity in Ecuador, taking into account the general characteristics of oceanic islands.

The substrates from which more samples were taken are flowers, insects and rotten wood. This fact responds to the specific objectives of the expeditionaries who centered their interest in the surveys of these substrates. Nonetheless, other substrates were also sampled with different intensity. The share of the samples taken in the expedition is shown in Figure 1.

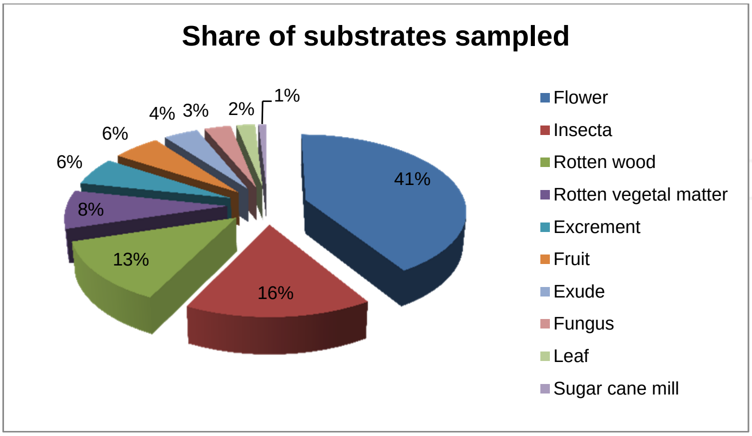

Figure 1. Substrates sampled in the basis of the number of samples taken in the Galápagos Islands.

Another issue is the number of isolates that were collected by substrate. Given the fact that the number of collections in the field made by each substrate varies substantially from one substrate to the other, a calculation was employed to obtain the average yeast isolates obtained by substrate. This calculation was done using the equation (1):

$$\overline{A} = \frac{N}{S} \tag{1}$$

Where $\overline{A}$ represents the average number of isolates per substrate, N represents the number of isolates with morphological differences gotten from each substrate; and, S corresponds to the number of samples taken from each substrate. In that way it was possible to determine the number of potentially different species based upon the macroscopic morphology. This approach provides an idea about the composition of the communities in terms of the complexity, based on the morphological differences from its members in a variety of substrates.

From a general point of view, we can say that the potential number of different species isolated is predominant in those substrates where the conditions favor the yeast reproduction and activity. Thus, in the sugar cane mill we could recover an average of 5 yeast isolates per sampling (regardless its biodiversity), while in flowers we could recover an average of 1.9 yeast isolates per sample. The abundance of yeasts also seems to be higher in insect's guts as well as in rotten wood: from the guts of beetles staying within *Datura* sp. and *Ipomoea* sp. flowers we could recover 3.1 and 4 isolates in average, which is slightly lower than the number of isolates from the sugar cane mill. In rotten wood the number of isolates was relatively high since we could recover an average of 3.5 yeast colonies. Table 5 resumes the average of isolates recovered by substrate in the Galápagos Islands. Nevertheless, these data does not provide enough information about the biodiversity of yeasts living in the substrates.

A further analysis of these figures reveals that the occurrence of yeast isolates in the samples has not a direct relationship with the biodiversity that can be found in each substrate, considering that macroscopic differences of yeast colonies does not necessarily represent different yeast species, but strains of a same species. A clear example of that is the biodiversity of yeasts found in the sugar cane mill (5 possible different species) which reports the highest average of isolates per sample (5 isolates), compared to flowers which present a rather low average of isolates per sample (1.9 isolates) but the highest biodiversity (14.2 species per sample). In Figure 2 the correlation between the number of samples, the number of isolates per sample, and the number of species by sample is drawn.

The insects were the substrates that yielded the highest biodiversity of yeasts in the Galápagos Islands (38 species), followed by rotten wood (35 species) samples and flowers (28 species). The substrates like trees exudates, rotten plant matter (leaves and fruits), fruits, fungus, leaves, and excrement, yielded from 7 to 11 different yeast species, while the sugar mill and fermented sugar cane juice sampled presented 5 isolates. Nonetheless, the highest average number of species per sample was registered in flowers, followed by insects and rotten wood.

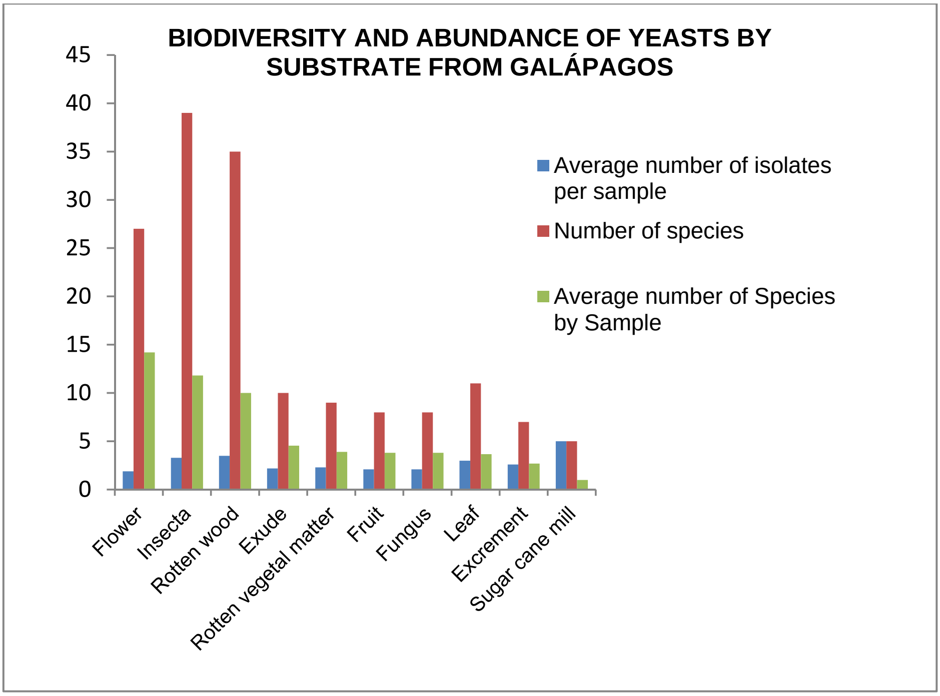

Figure 2. Correlation between number of samples from substrates, number of isolates per sample, and biodiversity of yeasts in substrates

On the other hand, the fermentation vessels and utensils where sugar cane juice is extracted and then transformed into fermented liquor containing high ethanol degree, constitutes a restrictive environment for yeast, where only a few species such as *Saccharomyces cerevisiae* can be found, owing to its high tolerance to ethanol provided by the fatty acids contained in the cells [45]. The rather poor biodiversity of this kind of yeast community is inversely proportional with the population abundance of the community. This inversely proportional relationship confirms that the highest biodiversity of yeasts can be found in those substrates where yeast communities play a fundamental ecological role. Most yeast species isolated from flowers are supposedly nectar-inhabiting yeasts. Dense yeast communities often occur in the floral nectar of animal-pollinated plants, where they can behave as parasites of plant-pollinator mutualisms [46-50].

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SUBSTRATE	AVERAGE OF ISOLATES BY NUMBER OF SUBSTRATE SAMPLED
Flower	1.9
Rotten wood	3.5
Beetle in *Datura* sp./*Ipomoea* sp.	3.1
Turtle´s excrement	2.6
Rotten leaf/fruit	2.3
Fruit	2.1
Drosophila sp. in *Datura* sp./*Ipomoea* sp./*Psidium guajava*	4
Exude	2.2
Leaf	3
Fungus	2.1
Sugar cane mill	5

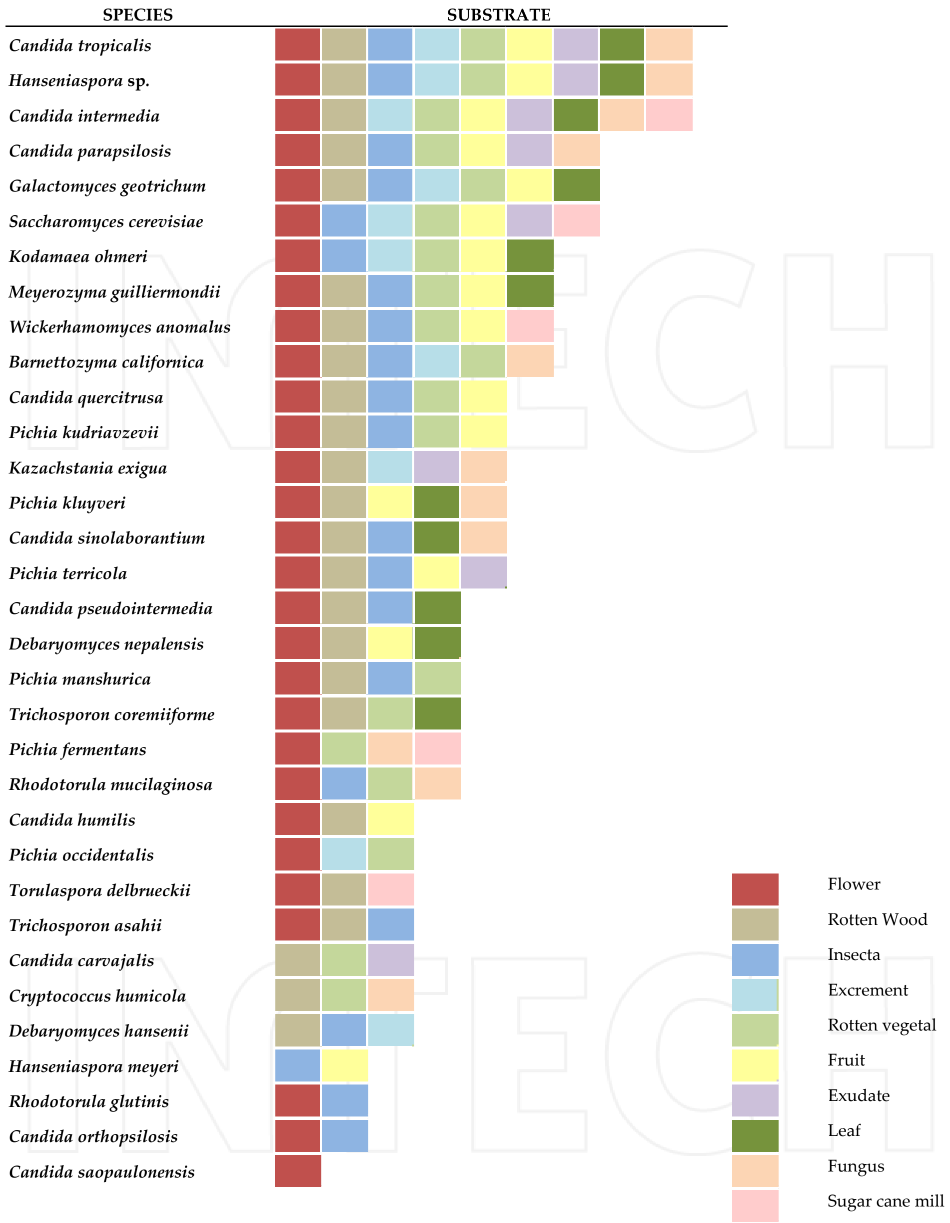
Table 5. Abundance of yeast isolates recovered from different substrates in the Galápagos Islands

6.6. Analyzing the yeast communities in Ecuador Mainland and The Archipelago of Galápagos

Disharmony and relictualism are both characteristics of oceanic islands [16]. The yeast diversity in the Galápagos Islands seems to be highly influenced by Ecuador mainland: the yeast species that are represented in the Galápagos and mainland accounts for 31% of the total species represented in mainland. Coincidently, this is the figure that corresponds to the iterative plant species in the mainland and the islands [18]. Another feature is the competence for ecological niches in the Galápagos which is not as severe as in mainland, where the biodiversity is much higher and the communities are richer.

With the data we got, it is not possible to analyze relictualism in the islands, since there is not any fossil register of ancient yeasts in the mainland which now occurs in the islands. Nevertheless, we do have one example of what we could consider a relictual species that has collected in Galápagos from the surface of a leaf. The same yeast species was resuscitated from ancient chicha (corn beer) fermentation vessels during a Microbial Archaeology survey in Quito in 2008 [21, 42]. The substrate is catalogued as an archaeological piece from 680 a.D. and the yeast species is *Candida theae* [29]. After intensive sampling in Ecuador mainland, we did not found any other isolate of this yeast species.

To analyze the state of the yeast communities in the islands and in mainland, we performed a series of comparisons in order to find the composition of the communities in the correspondent substrates in mainland, islands and a combination of both. The results are shown in Figures 3a, 3b, and 3c.


SPECIES
SUBSTRATE
Candida tropicalis
Hanseniaspora sp.
Candida intermedia
Candida parapsilosis
Galactomyces geotrichum
Saccharomyces cerevisiae
Kodamaea ohmeri
Meyerozyma guilliermondii
Wickerhamomyces anomalus
Barnettozyma californica
Candida quercitrusa
Pichia kudriavzevii
Kazachstania exigua
Pichia kluyveri
Candida sinolaborantium
Pichia terricola
Candida pseudointermedia
Debaryomyces nepalensis
Pichia manshurica
Trichosporon coremiiforme
Pichia fermentans
Rhodotorula mucilaginosa
Candida humilis
Pichia occidentalis
Torulaspora delbrueckii
Trichosporon asahii
Candida carvajalis
Cryptococcus humicola
Debaryomyces hansenii
Hanseniaspora meyeri
Rhodotorula glutinis
Candida orthopsilosis
Candida saopaulonensis
Flower
Rotten Wood
Insecta
Excrement
Rotten vegetal
Fruit
Exudate
Leaf
Fungus
Sugar cane mill

(a)

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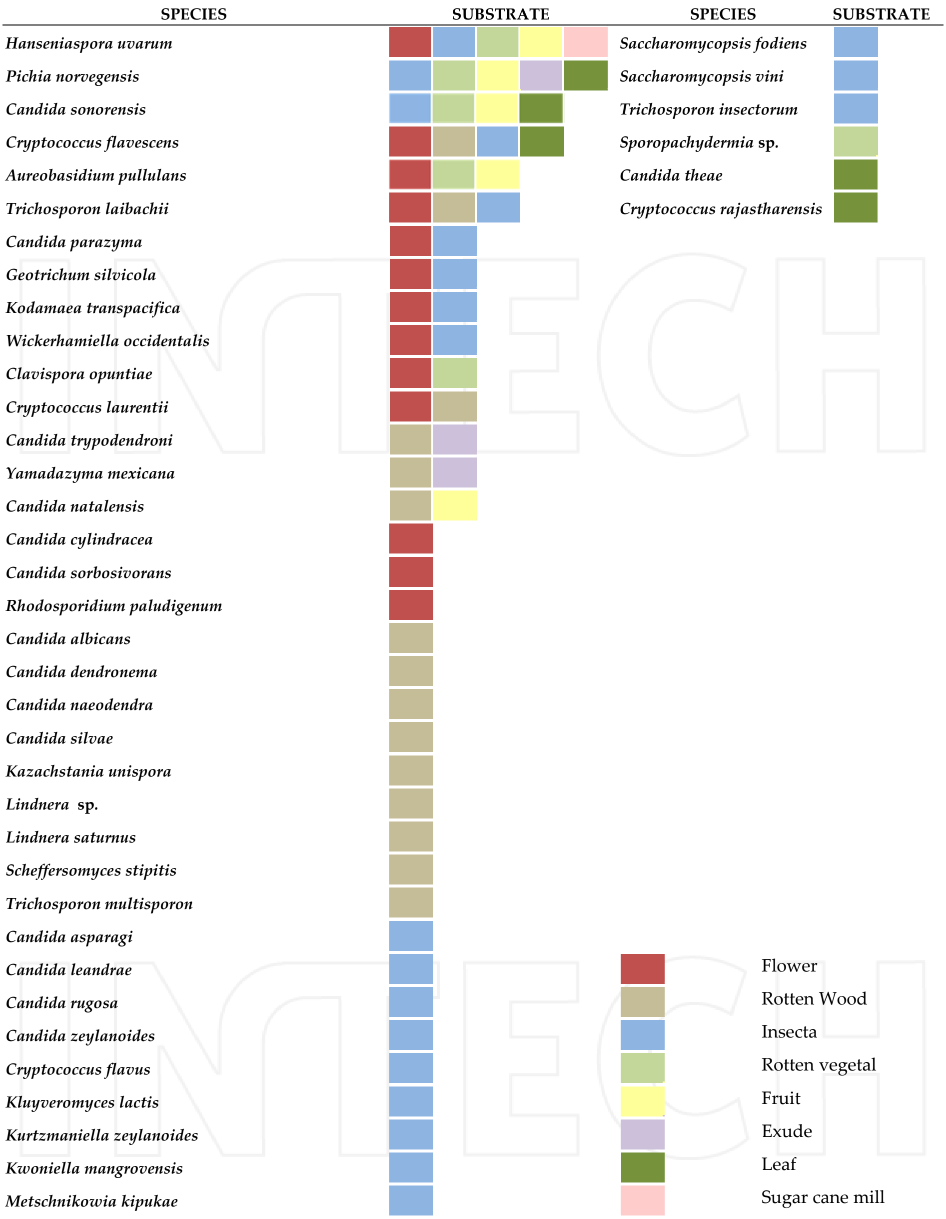
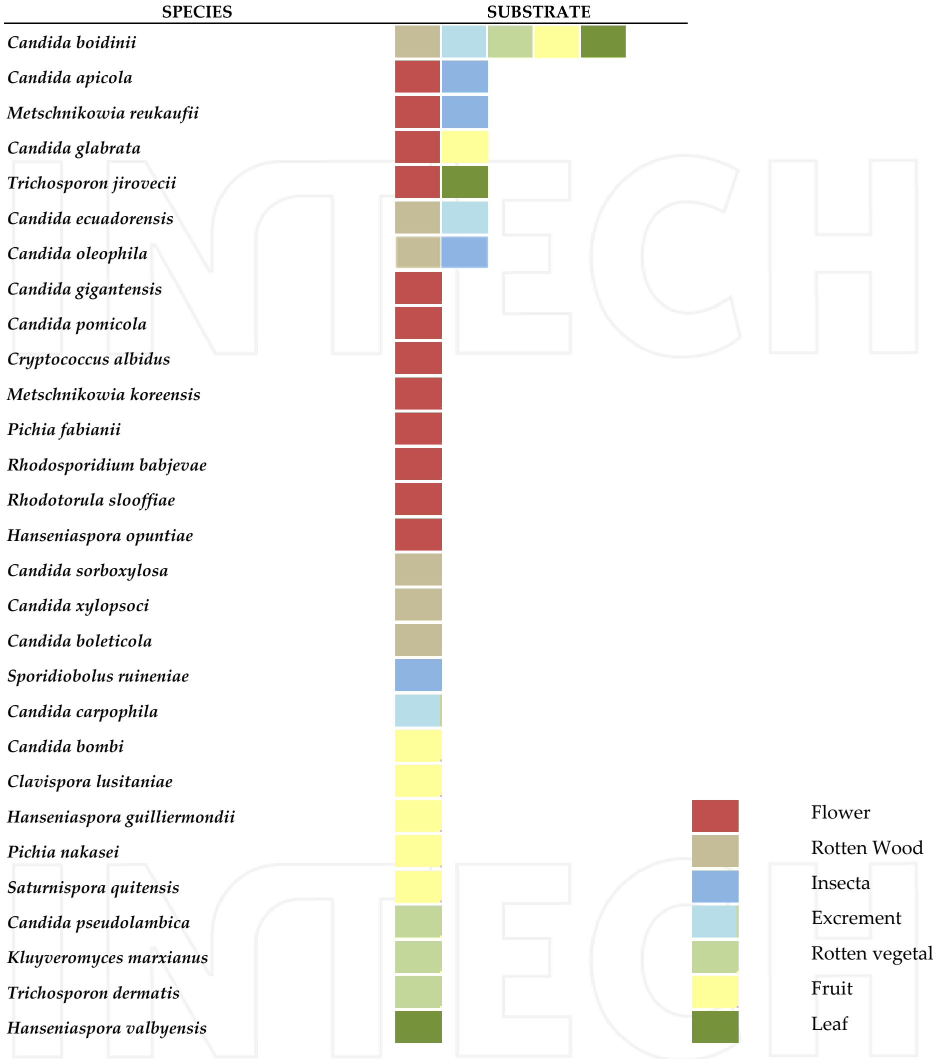


(b)



(c)

Figure 3. (a) Shared species between Galápagos Islands and Mainland Ecuador and its occurrence in different substrates; (b) Species found in Galápagos and the substrates these yeast colonize; (c) Species found in Ecuador Mainland and the substrates these yeasts colonize

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7. Ecological parameters and models developed

The degree of specialization of a yeast species is given by its metabolic abilities and tolerance to environmental factors such as UV radiation [40], inhibition substances and even microbial predators [34], etc. Leaves are exposed to fluctuations of temperature and relative humidity values, which may have an impact on the yeast communities. Large fluxes of UV radiation are also one of the most prominent features of the leaves, fruits and other substrates in the environment to which microorganisms have presumably had to adapt [21, 51]. Many plants contain a number of compounds whose adaptive significance may be a defense against invertebrates and microorganisms; for instance, all parts of *Datura* sp. contain toxic belladonna alkaloids, the concentration of which is highest in the petioles of the flowers [52]. These compounds also act, in some cases, as selective agents which shape the yeast community composition [53].

The yeasts which are highly adaptable to different ecological niches—like *Candida tropicalis*—are the ones that display a wide range of responses and developed defense strategies [45]. This particularly abundant and cosmopolitan yeast species is the most frequently isolated in mainland and in the islands according to CLQCA database. This yeast species has been found in flowers, rotten wood, insects, excrement, rotten vegetal matter, and fungus (Figure 3a). *Candida tropicalis* has been reported to grow even at pH so high as 10 [41], which is a remarkable feature in terms of tolerance facing unusually hard environmental conditions.

On the other hand there are yeasts that show a narrower repertoire of metabolism and even metabolic deficiencies such as *Saccharomycopsis fodiens*, a predacious yeast species that is deficient in sulfate uptake and require supplementation of organic sulfur sources [54]. This yeast species is highly specialized in predation of other yeasts and fungi. Nevertheless it appears to be quite rare in the environment and very few is known about its natural history and ecology. Only three isolates from Costa Rica, Australia and the Galápagos islands were found so far. A further expedition in Taiwan registered other isolates of this species associated to *Drosophila* flies [34].

7.1. How the ecological niches are occupied by yeasts in mainland and the islands?

It is certainly ventured to establish in an accurate way the ecological relationships of yeasts in the islands and the mainland with general data and relatively few samples. For this report we have addressed this issue by means of a global comparison of yeast species number that has been found in different substrates both in mainland and the islands. Despite the kind of substrate, we marked the repetitions of registers in substrates from each one of the species. This analysis gave us a general scheme of the degree of adaptation that is currently facing the yeasts.

In Figure 4 it is seen the general state of the yeasts based on the number of substrates they occur. This analysis was performed on those species found in mainland and the islands and those which are represented in both ecosystems.

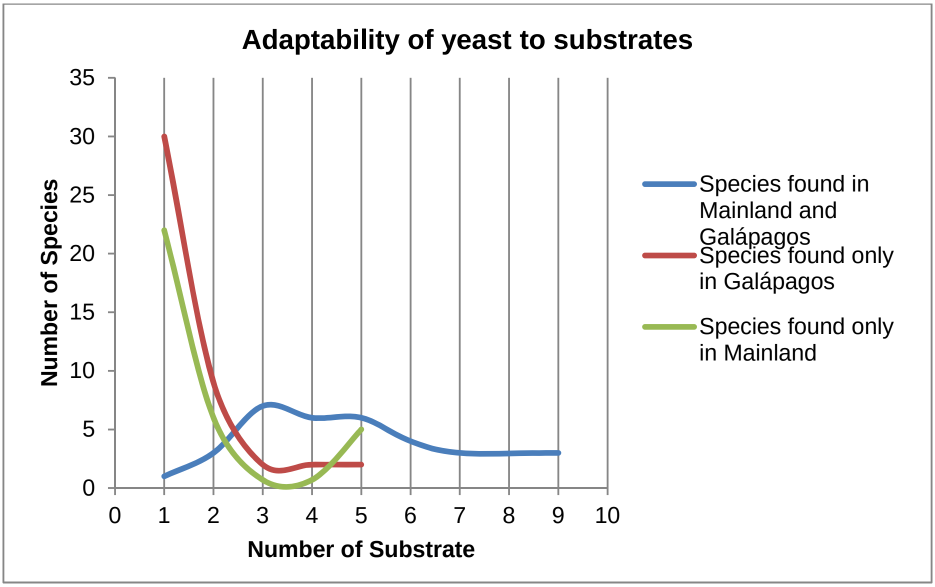

Figure 4. Global comparison in terms of adaptability to different substrates of yeast species.

Noteworthy, those yeast species which are common to Galápagos and mainland are represented by various yeasts that are able to colonize up to 9 different substrates. Only 1 yeast species looks confined to one kind of substrate. In the case of the species found in mainland exclusively, the figures are different, since the adaptability to various substrates looks lower, while the colonization of a single substrate is higher than the previous case, namely 22 yeasts occupy only one kind of substrate. As for the Galápagos yeasts the degree of adaptability is seen in more species but the specialization is much higher than in the before mentioned cases, this is 30 yeast species are confined to one specific substrate.

The yeast species shared between Galápagos and the mainland are mostly generalistic and show a wide range of substrates as ecological niches occupied. In contrast, the majority of the yeast species which have been found exclusively, both in the islands and the mainland, is less generalistic and occupy a narrow range of substrates. In other words, we can see that those yeast species which are more adapted to the mainland and the island ecosystems will probably find some ecological barriers that may impede the colonization of those ecosystems which they do not belong to. This can be regarded as a disharmony example.

No endemism has been studied or detected in this survey. There is only one yeast isolate representing a hitherto non described species, this is *Lindnera* sp. that was collected from *Scalesia* sp. rotten wood. This genus of tree is endemic to the Galápagos Islands.

The fact that in mainland the competition for substrates may be much more intensive than in the islands [16] could lead to shape more generalist yeast communities, while in the islands the yeasts species show a different trend that may be explained by a lower competence level and lower diversity in terms of ecological niches as compared to mainland.

7.2. Yeast specialization in Ecuadorian environments

From the data obtained in this work, it has been developed a simple model of calculation which allows us to establish the Index of Abundance (I_a) which is understood as the product of the total ecosystems where the species has been registered and the total different substrates that the species is able to colonize. The index maximum value is 1 and minimum is 0. The equation (2) to calculate such index is as follows:

$$I_a = \frac{(S_c * O)}{(S_m * O_m)} \tag{2}$$

Where I_a is the Index of Abundance; S_c is defined as the substrates colonized by the species; O is the occurrence in the different ecosystems that are being analyzed; S_m is the maximum number of substrates analyzed in the survey; and, O_m represents the maximum occurrence and is equal to the total number of ecosystems analyzed (seven ecosystems in the case of this study).

The I_a provides figures to compare the relative abundance of each yeast species in the ecosystems. Moreover it gives an idea about the generalist species, where the higher the I_a, the lower the specialization (more generalist species).

On the other hand, it is also necessary to define the extent of specialization of each yeast species. The Index of Specialization (S_i) is calculated from the following equation (3):

$$S_i = \frac{-\log_{10} I_a}{2} \tag{3}$$

Where S_i is defined as the Index of Specialization; and I_a is the Index of Abundance.

The values of S_i are inversely proportional to those of I_a given that the higher the abundance, the lower the specialization.

In Table 6 the I_a and S_i calculations can be seen based on the data from CLQCA and the analysis of seven ecosystems and 10 substrates (no yeast have been collected in all the substrates). It can be seen that *C. tropicalis* has been found in nine of 10 substrates compared in this survey, being the species with the higher I_a and lower S_i. The number 1 represents the positive occurrence and 0 means the lack of register of the species in the particular substrate. The number of substrates colonized by the species was taken from Figure 3a, 3b, and 3c.

SPECIES	AMAZON REGION	COASTAL REGION	HIGHLAND REGION	FLOREANA ISLAND	ISABELA ISLAND	SAN CRISTOBAL ISLAND	SANTA CRUZ ISLAND	OCCURRENCE IN ECOSYSTEMS	SUBSTRATES COLONIZED	INDEX OF ABUNDANCE (I_a)	SPECIALIZATION INDEX (S_i)
Candida tropicalis	1	1	1	1	1	1	1	7	9	0.90	0.02
Candida intermedia	1	1	1	1	1	0	1	6	9	0.77	0.06
Hanseniaspora sp.	1	1	1	0	1	1	1	6	9	0.77	0.06
Galactomyces geotrichum	1	1	1	1	1	0	1	6	7	0.60	0.11
Barnettozyma californica	0	0	1	1	1	1	1	5	6	0.43	0.18
Candida quercitrusa	0	1	1	1	1	1	1	6	5	0.43	0.18
Kodamaea ohmeri	1	1	1	0	1	1	0	5	6	0.43	0.18
Meyerozyma guilliermondii	0	1	1	0	1	1	1	5	6	0.43	0.18
Candida parapsilosis	0	1	1	0	1	0	1	4	7	0.40	0.20
Saccharomyces cerevisiae	1	1	1	0	0	0	1	4	7	0.40	0.20
Pichia kluyveri	1	1	1	1	0	0	1	5	5	0.36	0.22
Pichia kudriavzevii	1	1	1	0	1	1	0	5	5	0.36	0.22
Wickerhamomyces anomalus	0	1	1	1	0	0	1	4	6	0.34	0.23
Debaryomyces nepalensis	1	0	1	1	0	1	1	5	4	0.29	0.27
Hanseniaspora uvarum	0	0	0	1	1	1	1	4	5	0.29	0.27
Pichia terricola	0	0	1	1	1	0	1	4	5	0.29	0.27
Rhodotorula mucilaginosa	0	1	1	1	1	0	1	5	4	0.29	0.27
Candida pseudointermedia	0	0	1	1	1	0	1	4	4	0.23	0.32
Pichia manshurica	1	1	1	1	0	0	0	4	4	0.23	0.32
Candida sinolaborantium	0	0	1	1	0	0	1	3	5	0.21	0.33
Kazachstania exigua	0	0	1	0	1	0	1	3	5	0.21	0.33
Pichia norvegensis	0	0	0	1	0	1	1	3	5	0.21	0.33
Torulaspora delbrueckii	1	1	1	1	0	0	1	5	3	0.21	0.33
Candida sonorensis	0	0	0	1	0	1	1	3	4	0.17	0.38
Pichia fermentans	0	1	1	0	0	0	1	3	4	0.17	0.38
Trichosporon coremiiforme	0	0	1	1	0	0	1	3	4	0.17	0.38
Candida boidinii	0	1	1	0	0	0	0	2	5	0.14	0.42

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SPECIES	AMAZON REGION	COASTAL REGION	HIGHLAND REGION	FLOREANA ISLAND	ISABELA ISLAND	SAN CRISTOBAL ISLAND	SANTA CRUZ ISLAND	OCCURRENCE IN ECOSYSTEMS	SUBSTRATES COLONIZED	INDEX OF ABUNDANCE (I_a)	SPECIALIZATION INDEX (S_i)
Candida humilis	0	1	1	0	0	0	1	3	3	0.13	0.45
Cryptococcus humicola	1	0	1	0	0	0	1	3	3	0.13	0.45
Debaryomyces hansenii	0	0	1	0	1	0	1	3	3	0.13	0.45
Pichia occidentalis	0	1	1	0	1	0	0	3	3	0.13	0.45
Trichosporon asahii	0	1	1	0	0	0	1	3	3	0.13	0.45
Cryptococcus flavescens	0	0	0	1	1	0	0	2	4	0.11	0.47
Yamadazyma mexicana	0	1	0	1	1	0	1	4	2	0.11	0.47
Aureobasidium pullulans	0	0	0	1	0	0	1	2	3	0.09	0.53
Candida carvajalis	1	0	0	0	0	0	1	2	3	0.09	0.53
Candida orthopsilosis	0	1	1	0	1	0	0	3	2	0.09	0.53
Candida parazyma	0	0	0	1	1	1	0	3	2	0.09	0.53
Cryptococcus laurentii	0	0	1	0	1	0	1	3	2	0.09	0.53
Kodamaea transpacifica	0	0	0	1	1	1	0	3	2	0.09	0.53
Trichosporon laibachii	0	0	0	1	1	0	0	2	3	0.09	0.53
Candida ecuadorensis	1	0	1	0	0	0	0	2	2	0.06	0.62
Candida natalensis	0	0	0	1	0	0	1	2	2	0.06	0.62
Candida oleophila	0	0	1	0	0	0	1	2	2	0.06	0.62
Geotrichum silvicola	0	0	0	0	1	1	0	2	2	0.06	0.62
Hanseniaspora meyeri	0	0	1	1	0	0	0	2	2	0.06	0.62
Rhodotorula glutinis	0	0	1	0	1	0	0	2	2	0.06	0.62
Wickerhamiella occidentalis	0	0	0	0	1	1	0	2	2	0.06	0.62
Candida apicola	0	0	1	0	0	0	0	1	2	0.03	0.77
Candida glabrata	0	0	1	0	0	0	0	1	2	0.03	0.77
Candida saopaulonensis	0	0	1	0	0	0	1	2	1	0.03	0.77
Candida theae	0	0	1	0	0	1	0	2	1	0.03	0.77
Candida trypodendroni	0	0	0	0	0	0	1	1	2	0.03	0.77
Clavispora opuntiae	0	0	0	1	0	0	1	2	1	0.03	0.77

SPECIES	AMAZON REGION	COASTAL REGION	HIGHLAND REGION	FLOREANA ISLAND	ISABELA ISLAND	SAN CRISTOBAL ISLAND	SANTA CRUZ ISLAND	OCCURRENCE IN ECOSYSTEMS	SUBSTRATES COLONIZED	INDEX OF ABUNDANCE (I_a)	SPECIALIZATION INDEX (S_i)
Kazachstania unispora	0	0	0	1	0	0	1	2	1	0.03	0.77
Kluyveromyces lactis	0	0	0	0	1	1	0	2	1	0.03	0.77
Metschnikowia reukaufii	0	0	1	0	0	0	0	1	2	0.03	0.77
Rhodosporidium paludigenum	0	0	1	0	0	0	1	2	1	0.03	0.77
Trichosporon dermatis	1	0	1	0	0	0	0	2	1	0.03	0.77
Trichosporon jirovecii	0	0	1	0	0	0	0	1	2	0.03	0.77
Candida albicans	0	0	0	1	0	0	0	1	1	0.01	0.92
Candida asparagi	0	0	0	0	0	1	0	1	1	0.01	0.92
Candida boleticola	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida bombi	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida carpophila	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida cylindracea	0	0	0	0	0	0	1	1	1	0.01	0.92
Candida dendronema	0	0	0	1	0	0	0	1	1	0.01	0.92
Candida gigantensis	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida leandrae	0	0	0	0	0	1	0	1	1	0.01	0.92
Candida naeodendra	0	0	0	1	0	0	0	1	1	0.01	0.92
Candida pomicola	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida pseudolambica	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida rugosa	0	0	0	0	1	0	0	1	1	0.01	0.92
Candida silvae	0	0	0	1	0	0	0	1	1	0.01	0.92
Candida sorbosivorans	0	0	0	0	0	0	1	1	1	0.01	0.92
Candida sorboxylosa	1	0	0	0	0	0	0	1	1	0.01	0.92
Candida xylopsoci	0	0	1	0	0	0	0	1	1	0.01	0.92
Candida zeylanoides	0	0	0	0	1	0	0	1	1	0.01	0.92
Clavispora lusitaniae	0	0	1	0	0	0	0	1	1	0.01	0.92
Cryptococcus albidus	0	0	1	0	0	0	0	1	1	0.01	0.92
Cryptococcus flavus	0	0	0	0	1	0	0	1	1	0.01	0.92

SPECIES	AMAZON REGION	COASTAL REGION	HIGHLAND REGION	FLOREANA ISLAND	ISABELA ISLAND	SAN CRISTOBAL ISLAND	SANTA CRUZ ISLAND	OCCURRENCE IN ECOSYSTEMS	SUBSTRATES COLONIZED	INDEX OF ABUNDANCE (I_a)	SPECIALIZATION INDEX (S_i)
Cryptococcus rajastharensis	0	0	0	1	0	0	0	1	1	0.01	0.92
Hanseniaspora guilliermondii	0	0	1	0	0	0	0	1	1	0.01	0.92
Hanseniaspora opuntiae	0	0	1	0	0	0	0	1	1	0.01	0.92
Hanseniaspora valbyensis	0	0	1	0	0	0	0	1	1	0.01	0.92
Kluyveromyces marxianus	0	0	1	0	0	0	0	1	1	0.01	0.92
Kurtzmaniella zeylanoides	0	0	0	0	1	0	0	1	1	0.01	0.92
Kwoniella mangrovensis	0	0	0	0	1	0	0	1	1	0.01	0.92
Lindnera sp.	0	0	0	0	0	0	1	1	1	0.01	0.92
Lindnera saturnus	0	0	0	0	0	0	1	1	1	0.01	0.92
Metschnikowia kipukae	0	0	0	0	1	0	0	1	1	0.01	0.92
Metschnikowia koreensis	0	0	1	0	0	0	0	1	1	0.01	0.92
Pichia fabianii	0	0	1	0	0	0	0	1	1	0.01	0.92
Pichia nakasei	0	0	1	0	0	0	0	1	1	0.01	0.92
Rhodosporidium babjevae	0	0	1	0	0	0	0	1	1	0.01	0.92
Rhodotorula slooffiae	0	0	1	0	0	0	0	1	1	0.01	0.92
Saccharomycopsis fodiens	0	0	0	0	0	1	0	1	1	0.01	0.92
Saccharomycopsis vini	0	0	0	0	0	0	1	1	1	0.01	0.92
Saturnispora quitensis	0	0	1	0	0	0	0	1	1	0.01	0.92
Scheffersomyces stipitis	0	0	0	0	0	0	1	1	1	0.01	0.92
Sporidiobolus ruineniae	0	0	1	0	0	0	0	1	1	0.01	0.92
Sporopachydermia sp.	0	0	0	1	0	0	0	1	1	0.01	0.92
Trichosporon insectorum	0	0	0	0	1	0	0	1	1	0.01	0.92
Trichosporon multisporon	0	0	0	1	0	0	0	1	1	0.01	0.92

Table 6. Occurrence in different ecosystems and different substrates of yeast species in Galápagos and Mainland and calculation of I_a and S_i.

With these data it is possible to obtain a plot S_i versus I_a which provides the curve of Adaptability of yeast species in the ecosystems herein analyzed. Figure 5 shows the curve:

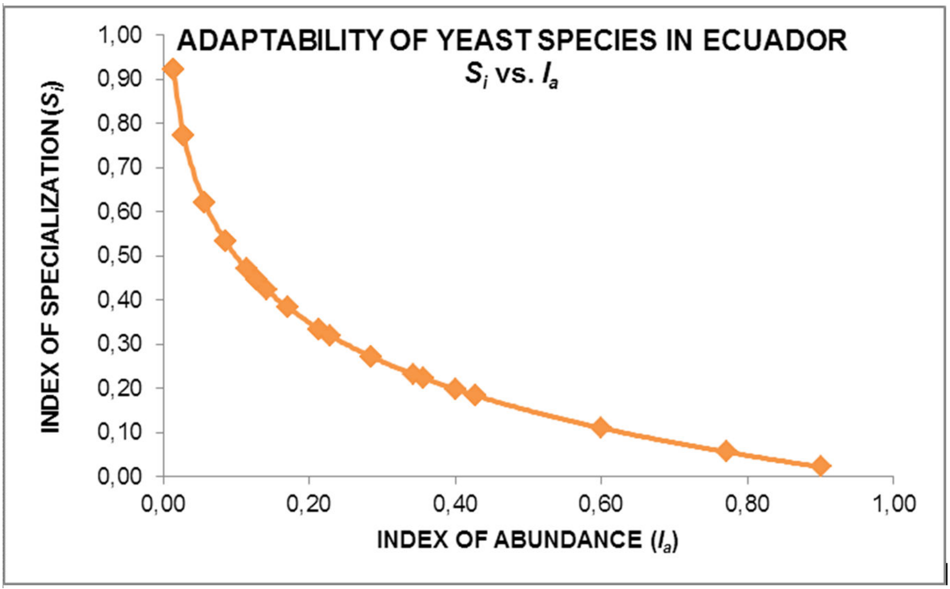

Figure 5. Curve of adaptability of the yeasts species studied in Mainland Ecuador and four Islands in the Galápagos Archipelago.

Analyzing this curve it is possible to infer that the higher the slope, the more specialized is the yeast species and vice versa. This kind of modeling is useful to have a general understanding of the behavior of yeast species in the ecosystems. As the information of new collects and characterization of yeasts is updated in the data base of the CLQCA this model will become more robust and accurate.

8. Concluding remarks

Despite the current appearance of human-disturbing habitats, especially in those islands that are inhabited, where this survey took place, the Galápagos Archipelago are the world's most pristine, best preserved and protected, tropical oceanic island ecosystem where more than the 95% of the land area in the islands is part of the Galápagos National Park [55].

The present work is a first approach to the study of the yeasts biodiversity and occurrence in the Galápagos Islands and Mainland Ecuador. The data herein presented show, nevertheless, some interesting relationships in both environments. Adaptation to the new conditions as well as the disharmony in term of biodiversity between both ecosystems appears to be aligned to the situation of some macro organisms such as plants. We have found a coincidence of 31% of the yeast species between the explored islands and the mainland; remarkably, 30% of plant species represented in the Galápagos Islands has also been registered in mainland. We could not proof endemism in the present work.

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Endangered plant species may also put in danger some insects associated to those endemic plants, since the insect-plant associations generates different degrees of dependence. Studies carried out between 2001 and 2002 showed that 19 endangered plant species exhibit a range of interactions (with different degrees) with 108 different insect species that use the plants as refugee or food. The study carried out by [56] shows that 77% of the insects are endemic. From our studies, insects have shown to be the second source of yeasts biodiversity after flowers. Moreover, studies focused on insect-yeast interactions, have shown a remarkable role of insects acting as "wet nurses" for yeasts during certain periods during the year [57]. Consequently, some yeast species could be endangered along with their hosts who have a tight mutualism relationship with the endangered plant species.

To date, two novel yeast species collected in this survey were already described (i.e. *Saccharomycopsis fodiens* and *Kodamaea transpacifica*). Both yeasts species were isolated from nitidulid beetles in ephemeral flowers of *Ipomoea* sp. and *Datura* sp. These species are regarded as biomarkers for ancient migrations of Polinesyan sailors, who took the sweet potato plants (*Ipomoea* sp.) from the Andes (currently Ecuador and Peru) and introduced them into Polynesia and beyond [58].

In this chapter we have developed a new ecological approach by means of a mathematical model which is useful for a better understanding of the adaptability of yeasts as well as the specialization degree of these microorganisms in Ecuadorian ecosystems. The data herein processed will be completed in future expeditions, but constitutes a base for the upcoming ecological studies of the yeasts in the Galápagos Islands and Ecuadorian Mainland. The mathematical model shows an inverse correlation between the "Index of Specialization" (S_i) and the "Index of Abundance" (I_a). Moreover, it can be seen that the trend is towards the specialization since 70 out of 104 yeasts species analyzed (c.a. 67%) showed a S_i between 0.92 to 0.53, which means that they were isolated from a maximum of three out of seven ecosystems and a maximum of three out of nine substrates; 30 yeast species (c.a. 29%) showed an intermediate S_i, between 0.18 and 0.47, meaning that these yeasts species were found in a maximum of six different ecosystems and six different substrates; finally, only four yeast species (c.a. 4%) showed a very low S_i, between 0.02 and 0.11, which means that these species were found in up to seven ecosystems and nine substrates analyzed. These four yeast species are considered the more generalist and exhibit the highest adaptability, but represents a minority in the complete pool of yeast species studied. The total number of ecosystems analyzed was seven and the total number of substrates studied were 10. No yeast species were found in all the 10 substrates.

Moreover, we have found that in the Galápagos Islands the percentage of yeast species that are colonizing a single substrate is about a 30% higher than the correspondent figure in mainland (Figure 4). We can hypothesize that in mainland the ecosystems have had much more exchanges along the natural history, furthermore, the yeasts species were able to adapt to a wider range of substrates. In the Galápagos archipelago, a rather young group of volcanic islands, the exchange as well as the opportunity to colonize new substrates and—in evolutionary terms—to adapt to new micro environments, has been certainly lower than in the interconnected mainland ecosystems. In other words, we can see that adaptability of yeasts in mainland and the archipelago is clearly different.

Undoubtedly there is much work to do in order to attain a better understanding on ecology of yeasts in the archipelago, since the sampling was done in 4 of the 21 islands. Future expeditions may focus on substrates such as flowers, insects and rotten vegetal matter, in order to fill the shortfalls from the previous collections, such as more novel yeast species as well as endemic species from the Galápagos Islands.

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