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# Abundance and diversity of microfungi in three coastal beaches of Mexico

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**The abundance and diversity of species of microfungi was investigated on the beaches of Delfines, Km 24 Veracruz-Alvarado Highway, and El Coco, located on the coasts of the Caribbean Sea, Gulf of Mexico, and the Pacific Ocean, respectively. On each beach a sample composed of sand, subtidal wood or washed-up detritus with moist sand was collected. The samples were analyzed by three different methods, resulting in a total of 1,160 occurrences that fluctuated between 340 and 441 occurrences/sample. The number of species/sample fluctuated between 20 and 32. A total of 52 species was found, of which 12 were marine, and 40 nonmarine, of terrestrial origin, and of these 15 were ascomycetes, 34 were hyphomycetes, 2 were blastomycetes and one was a coelomycete. The abundance distribution showed few species with high or low values, with the greatest proportion having intermediate values. In order to compare species diversity among the samples frequency curves were utilized, based on the number of species expected from samples taken at random; the results showed that the beach at El Coco was richest in species.**

Key Words——arenicolous mycobiota; marine fungi; species richness.

The arenicolous micromycetes live between the grains of sand on marine beaches, an environment named the endopsammon (Lewis, 1977), where there coexist three ecological types of micromycetes: 1) marine, which grow and sporulate exclusively in a marine or estuarine habitat; 2) facultative marine, of terrestrial or fresh water origin, which grow and sporulate in marine as well as a terrestrial habitat; and 3) nonmarine, of terrestrial or fresh water origin, which neither grow nor sporulate in a marine environment (Kohlmeyer and Kohlmeyer, 1979). The majority of the studies on endopsammophilous micromycetes are on systematic aspects (Sundari et al., 1996; Kohlmeyer and Volkmann-Kohlmeyer, 1997) and to a lesser degree physiology (Grant et al., 1996), morphology (Jones, 1995) and ecology (Kohlmeyer, 1966). In Mexico little is known of the structure of the communities of microfungi that inhabit the beaches of the nearly 11,000 km of Mexican coasts on the Pacific Ocean, Gulf of Mexico and the Caribbean Sea (Kohlmeyer, 1968, 1980, 1984; Kohlmeyer and Kohlmeyer, 1971; Hyde, 1992; González and Herrera, 1993). This study is a preliminary contribution in which the endopsammophilous mycobiota of three beaches of Mexico is characterized.

## Materials and Methods

**Areas of study** Three beaches on the open sea were studied: Delfines Beach, Cancun, State of Quintana Roo, situated on the coast of the Caribbean Sea, (21°02′57″ N, 86°46′05″ W); the beach at Km 24 Veracruz-Alvarado Highway, State of Veracruz, located on the coast of the Gulf of Mexico (18°52′30″ N, 95°55′ W), and El Coco Beach, State of Colima, which is located on the coast of the Pacific Ocean (19°10′12″ N, 104°39′17″ W) (Fig. 1). These beaches were sampled from April 4–10, 1995, during low tide. In order to characterize the environment the zonification of the beach profile proposed by Carranza-Edwards and Caso-Chávez (1994) was followed, which recognizes four zones: 1) infrabeach, a zone always covered by water under normal conditions, 2) mesobeach, a zone continually covered by water and exposed to the air in a rythmic and alternate manner, 3) suprabeach, a zone that is dry under normal conditions and only becomes wet occasionally due to marine storms or extremely high waves, and 4) the terrestrial domain, a limited zone from the beach to the land, which can be composed of dunes stabilized and colonized by plants. In this study only the mesobeach was studied because it is the area where the major quantity of organic remains are accummulated.

**Collection of materials and processing of the samples** The samples were analyzed by three different methods, the first for marine micromycetes, the second to study the marine and nonmarine micromycetes, and the third for the nonmarine micromycetes. In the first method, incubation of plant remains in a damp chamber (Kohlmeyer and Kohlmeyer, 1979), in the mesobeach of each of the beaches, 50 samples of subtidal wood or washed-up detritus (wood pieces, algae and other debris) were col-


Fig. 1. Location of the three studied Mexican coastal beaches.

lected, covered with moist sand, and incubated in sterile hermetic polyethylene bags under atmospheric conditions for 4–12 mo, at the end of which they were examined microscopically to locate fungal structures. When necessary, some samples were moistened with sterile artificial seawater to avoid dehydration. For the second method, direct inoculation of agar plates with sandy soil, the method of Barron (1971) was followed. In the mesobeach of each of the three beaches three sites were selected in a random manner and in each one two samples of sand were taken. In the laboratory sand was taken from each sample with a transfer needle and sprinkled on the surface of a culture medium. For isolation of marine micromycetes two media were used: cornmeal-seawater agar (CMA/SW): Difco corn meal agar 17 g, Instant Ocean artificial seawater 1 L (Hyde et al., 1987), and soluble starch-sea water agar (SSA/SW): soluble starch 10 g, yeast extract 1 g, agar 18 g, Instant Ocean artificial seawater 1 L (Nakagiri and Tubaki, 1982); for nonmarine micromycetes a medium for the primary isolation of fungi from soil was used (PIFS): dextrose 5 g, yeast extract 2 g, $NaNO_3$ 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $KH_2PO_4$ 1 g, bacto-oxgall 1 g, sodium propionate 1 g, agar 20 g, distilled water 1 L (Booth, 1971), and potato-dextrose agar (PDA): potatoes 200 g, dextrose 15 g, agar 20 g, distilled water 1 L (Booth, 1971). To all of the culture media was added, after sterilization, 50 $\mu$g/ml of thiabendazol to restrict the development of rapidly growing fungi, and 500 $\mu$g/ml of penicillin, 300 $\mu$g/ml of streptomycin, and 1 mg/ml of chloramphenicol to inhibit the growth of bacteria and yeasts. Plates of each medium were made in quadruplicate and incubated for 5 wk at 25°C at 60% relative humidity with a 12 h photoperiod under cool white lights. The plates were examined daily and each colony that developed was transferred to a tube with SSA/SW or PDA medium in order to isolate it. Once the colonies grew in the tubes, they were separated into morphologically different groups and transferred to 60 mm Petri dishes of specific agar media for identification and quantification. For the third method, agar plates with dilutions of sandy soil (Barron, 1971) was used. In the mesobeach of each of the beaches three sites were selected in a random manner and from each one two samples of sand were taken. In the laboratory 25 g dry-weight equivalent of sand from each sample was added to a sterile graduate cylinder and the volume made up to 250 ml with sterile 0.15% water agar; the soil-agar mixture was blended for 60 s; 5 ml of the supernatant was immediately transferred to 45 ml of the dilutant successively to obtain dilutions of $10^{-1}$ to $10^{-5}$. Afterwards, 1 ml of each dilution was pipetted on to the surface of the medium and distributed uniformly with a glass rod. Four plates of each dilution were prepared with the same PIFS and PDA media and antibiotics employed in the previous method and incubated under similar conditions. Subsequent procedures were the same as that mentioned for the third method. Cultures and slides of the species are deposited in the collection of fungi of the Instituto de Biología, Universidad Nacional Autónoma de México (MEXU).

**Analysis of the data** In order to estimate the abundance and diversity of the mycobiota obtained by the above three methods, the data were analyzed according to the statistical procedures utilized by Bills and Polishoot (1994) and the calculations were made utilizing the software program of Ludwig and Reynolds (1988). The abundance of species is expressed as the number of individual occurrences of a species. The percentage abundance is the number of occurrences of a species divided by the total number of occurrences recovered from the sample. In order to compare the relative abundance of the species and the abundance of the principal species that occurred in most of a sample, the species were arranged in descending order according to their abundance. To distinguish the dominant fungi, the total abundance of each species is presented in descending order. The number of species expected in a random sample of (n) individuals (E(Sn)), taken from a population of N total individuals distributed among S species, was calculated with the formula of Hurulbert (1971):

$$E(Sn)=\sum_{i=1}^{s}\left\{1-\left[\left(\frac{N-n_i}{n}\right)\Big/\left(\frac{N}{n}\right)\right]\right\}$$

where n, is the number of individuals of the species i. In addition, rarefaction curves were constructed in order to compare the richness of species among the samples of the three beaches. The similarity with reference to the composition of species among the samples was obtained by applying the community coefficient of Sørensen (1948):

$$CC=\frac{2a}{b+c}$$

where a is the number of common species in the two samples compared, b is the number of single species in the first sample and c is the number of single species in the second sample.

## Results

From the samples of sandy soil from the beaches of Delfines, Km 24 Veracruz-Alvarado Highway and El Coco, a total of 1,160 occurrences was obtained that fluctuated between 340 and 441 occurrences /sample (average=387) (Tables 1, 2). The number of species/sample fluctuated between 20 and 32. A total of 52 fungi was found, of which 12 were marine and 40 nonmarine of terrestrial origin; 15 belonged to the ascomycetes, 34 to the hyphomycetes, two to the blastomycetes and one to the coelomycetes. On comparing the mycobiota of the samples between pairs of beaches, the most similar were the beaches of Delfines and El Coco, and the least similar were the beaches at Km 24 Veracruz-Alvarado Highway and El Coco (Table 3). The distributions of the abundances in the three samples indicated the presence of few abundant species (11.6%–7% of the total occurrences) and a high proportion of species with intermediate abundance (5%–0.5%) as well as species of low abundance (0.4%–0.1%) (Table 2). The dominant species that were found in one or more of the samples, with an abundance above 5% of total occurrences, were *Cladosporium cladosporioides*, *Corollospora maritima*, *Lindra thalassiae*, *Emericella nidulans* and *Aspergillus niger*. The nonmarine species *Cladosporium cladosporioides* and the marine *Corollospora maritima* were the most abundant of the three samples with an abundance percentage of 11.6% and 11.3% of the total of occurrences, respectively. The species that were isolated only once (0.1% of the total occurrences) were *Alternaria longipes*, *Gilmaniella humicola*, *Graphium penicillioides*, *Lindra marinera*, *Cirrenalia tropicalis*, *Emericella violacea*, *Chaetomium globosum* and *Lasiodiplodia theobromae*. The results of the rarefaction indices based on the number of species expected from a subsample of standard size taken at random from 200 occurrences of each sample indicated that the El Coco beach was richest

Table 1. Number of occurrences and of species from three samples of sandy soil from the beaches of Delfines, Cancun, State of Quintana Roo (DCQ), Km 24 Carretera Veracruz-Alvarado, State of Veracruz (VAV), and El Coco, State of Colima (ECC).

| Sample | Total occurrences | Total species | E(s200)[a] | A[b] | B[c] | H[d] | C[e] | U[f] |
|---|---|---|---|---|---|---|---|---|
| DCQ | 340 | 20 | 19 | 6 | 2 | 12 | 0 | 0 |
| VAV | 441 | 28 | 26 | 7 | 0 | 19 | 0 | 2 |
| ECC | 379 | 32 | 28 | 8 | 0 | 23 | 1 | 1 |
| Mean | 387 | | | | | | | |
| Total | 1160 | | | | | | | |

Total number of different species from the three samples: 52

a) E(s200) is the expected number of species in a random sample of 200 occurrences taken from the total population of occurrences from each sample.
b) Ascomycetes.
c) Blastomycetes.
d) Hyphomycetes.
e) Coelomycetes.
f) Sterile mycelium.

Table 2. Abundance of the micromycetes from samples of sandy soil from three beaches in Mexico on the coasts of the Caribbean Sea (Delfines Beach, Cancun, Quintana Roo) (DCQ), Gulf of Mexico (beach at Km 24 Veracruz-Alvarado Highway, Veracruz) (VAV), and the Pacific Ocean (El Coco Beach, State of Colima) (ECC), respectively.

| Species[a)] | Sample | | | Abundance | Percentage of abundance |
|---|---|---|---|---|---|
| | DCQ | VAV | ECC | | |
| *Cladosporium cladosporioides* | 34 | 21 | 80 | 135 | 11.6 |
| **Corollospora maritima* | 21 | 52 | 58 | 131 | 11.3 |
| **Lindra thalassiae* | 51 | 44 | 0 | 95 | 8.2 |
| *Emericella nidulans* | 10 | 45 | 30 | 85 | 7.3 |
| *Aspergillus niger* | 55 | 10 | 16 | 81 | 7.0 |
| *Aspergillus flavus* | 14 | 41 | 2 | 57 | 4.9 |
| *Fusarium solani* | 2 | 5 | 40 | 47 | 4.1 |
| *Stachybotrys chartarum* | 0 | 44 | 0 | 44 | 3.8 |
| **Corollospora pulchella* | 30 | 0 | 14 | 44 | 3.8 |
| *Drechslera biseptata* | 28 | 0 | 0 | 28 | 2.4 |
| *Aspergillus terreus* | 7 | 10 | 10 | 27 | 2.3 |
| *Trichoderma viride* | 7 | 2 | 18 | 27 | 2.3 |
| *Curvularia pallescens* | 0 | 25 | 0 | 25 | 2.2 |
| *Scopulariopsis brevicaulis* | 0 | 0 | 23 | 23 | 2.0 |
| *Fusarium semitectum* | 20 | 0 | 2 | 22 | 1.9 |
| *Nigrospora sphaerica* | 12 | 4 | 6 | 22 | 1.9 |
| *Cladosporium sphaerospermum* | 15 | 4 | 1 | 20 | 1.7 |
| *Curvularia tuberculata* | 0 | 20 | 0 | 20 | 1.7 |
| **Halosphaeria salina* | 20 | 0 | 0 | 20 | 1.7 |
| **Varicosporina ramulosa* | 0 | 18 | 0 | 18 | 1.6 |
| **Corollospora angusta* | 0 | 15 | 0 | 15 | 1.3 |
| *Curvularia intermedia* | 0 | 15 | 0 | 15 | 1.3 |
| *Myrothecium roridum* | 0 | 0 | 15 | 15 | 1.3 |
| *Aureobasidium pullulans* | 5 | 0 | 8 | 13 | 1.1 |
| **Corollospora gracilis* | 0 | 12 | 0 | 12 | 1.0 |
| *Curvularia senegalensis* | 0 | 0 | 12 | 12 | 1.0 |
| *Acremonium rutilum* | 0 | 11 | 0 | 11 | 0.9 |
| *Curvularia lunata* | 0 | 0 | 10 | 10 | 0.9 |
| *Exserohilum rostratum* | 0 | 10 | 0 | 10 | 0.9 |
| Sterile mycelium A | 0 | 10 | 0 | 10 | 0.9 |
| *Myrothecium verrucaria* | 0 | 8 | 0 | 8 | 0.7 |
| *Chrysonilia sitophila* | 0 | 0 | 6 | 6 | 0.5 |
| *Aspergillus pulverulentus* | 0 | 5 | 0 | 5 | 0.4 |
| *Microascus trigonosporus* | 0 | 5 | 0 | 5 | 0.4 |
| *Alternaria alternata* | 0 | 0 | 4 | 4 | 0.3 |
| *Cladosporium herbarum* | 2 | 0 | 2 | 4 | 0.3 |
| **Torpedospora radiata* | 0 | 0 | 4 | 4 | 0.3 |
| *Neocosmospora vasinfecta* var. *africana* | 0 | 0 | 4 | 4 | 0.3 |
| **Arenariomyces parvulus* | 3 | 0 | 0 | 3 | 0.3 |
| *Zygosporium masonii* | 0 | 0 | 3 | 3 | 0.3 |
| **Dendryphiella arenaria* | 0 | 0 | 3 | 3 | 0.3 |
| *Alternaria citri* | 0 | 2 | 0 | 2 | 0.2 |
| *Rhodothorula rubra* | 2 | 0 | 0 | 2 | 0.2 |
| *Candida lusitania* | 2 | 0 | 0 | 2 | 0.2 |
| *Alternaria longipes* | 0 | 0 | 1 | 1 | 0.1 |
| *Gilmaniella humicola* | 0 | 1 | 0 | 1 | 0.1 |
| *Graphium penicillioides* | 0 | 0 | 1 | 1 | 0.1 |
| **Lindra marinera* | 0 | 1 | 0 | 1 | 0.1 |
| **Cirrenalia tropicalis* | 0 | 0 | 1 | 1 | 0.1 |
| *Emericella violacea* | 0 | 0 | 1 | 1 | 0.1 |
| *Chaetomium globosum* | 0 | 0 | 1 | 1 | 0.1 |
| *Lasiodiplodia theobromae* | 0 | 0 | 1 | 1 | 0.1 |
| Sterile mycelium B | 0 | 1 | 1 | 1 | 0.1 |
| Sterile mycelium C | 0 | 1 | 0 | 1 | 0.1 |
| *Thielavia terricola* | 0 | 0 | 1 | 1 | 0.1 |
| Totals | 340 | 441 | 379 | 1160 | 100 |

a) The species are arranged in decreasing order of the total abundance obtained; *Marine species.

in species (Fig. 2) (Table 1).

## Discussion

The results of these studies suggest that the endopsammophilous mycobiota was composed of nonmarine and to a lesser extent marine species, which agrees with the results of other studies on communities of the same type of environment (Koehn, 1979; Rees and Jones, 1985). The distributions of the abundances of the species showed few species with high or low abundance and many species with intermediate abundance. The nonmarine species *Cladosporium cladosporioides* and the marine species *Corollospora maritima* were the most abundant in the three beaches, with an abundance of 11.6% and 11.3%, respectively. In general, the abundance and composition of the marine and nonmarine species was similar to that recorded in a temperate beach in Gronhoj, Denmark by Rees and Jones (1985), who also found *Cladosporium cladosporioides* (10.9%) and *Corollospora maritima* (10.5%) as the most abundant species. The nonmarine mycobiota of terrestrial origin that was recorded in this work was similar in its composition to that of the endopsammon of Enewetak Atoll, Marshall Islands (Dunn and Baker, 1983), as well as that reported in Mexico (González and Herrera, 1993); with respect to the marine micromycetes, some species found in this study were also registered from beaches in Japan (Tokura, 1982, 1984), the United States (Wagner-Merner, 1972), Canada (Booth, 1981), Denmark (Koch, 1974; Farrant et al., 1985) and Spain (Genilloud et al., 1994).

A comparison of the mycobiota of the samples among the pairs of beaches showed moderate similarity in their species composition and, although the samples had species in common, the distinctive composition and the dissimilarity in the relative abundance among the species suggest a variation among the communities that possibly is a reflection of the different abiotic and biotic characteristics of these beaches. The sand samples of the beaches in Delfines and El Coco were the most similar

Table 3. Similarity among the mycobiota of the beaches at Delfines, Cancun, State of Quintana Roo (DCQ), Km 24 Veracruz-Alvarado Highway, State of Veracruz (VAV), and El Coco, State of Colima (ECC).

| Pairs of beaches compared | Number of species from the beaches compared | Common species | Community coefficient of Sørensen |
|---|---|---|---|
| DCQ-VAV | 20–28 | 11 | 0.46 |
| VAV-ECC | 28–32 | 10 | 0.33 |
| ECC-DCQ | 20–32 | 14 | 0.54 |

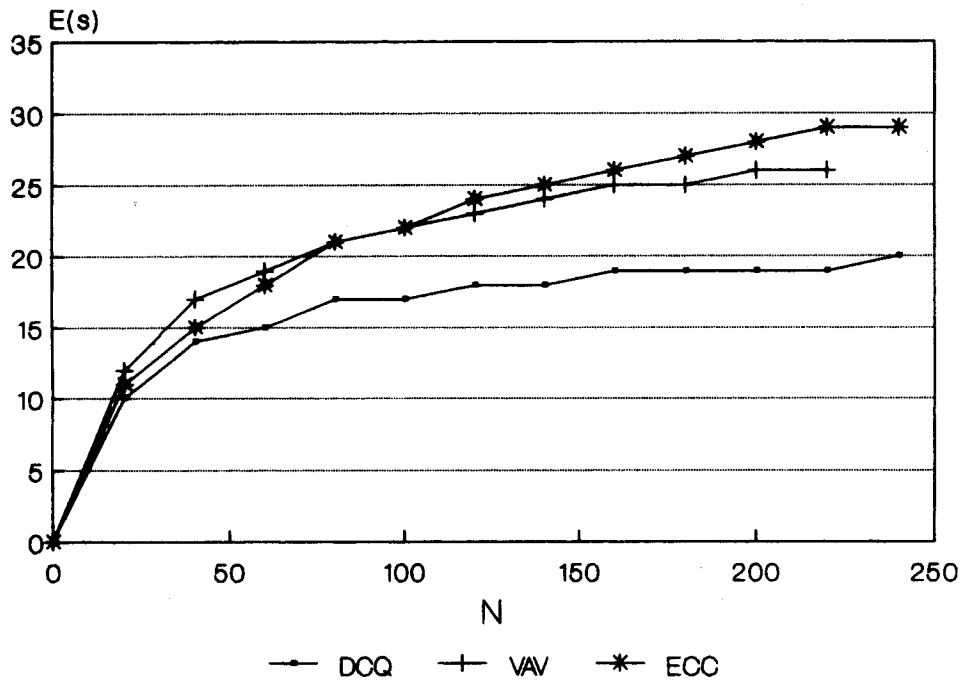


Fig. 2. Rarefaction curves for microfungi of the beaches at Delfines, Cancun, State of Quintana Roo (DCQ), Km 24 Veracruz-Alvarado Highway, State of Veracruz (VAV), and El Coco, State of Colima (ECC).
E(s) is the expected number of species in a random sample of n individuals taken from the total population of isolates from each sample.

despite their being geographically most distant.

The well-being of the endopsammon depends on the stable structure of the communities of organisms that inhabit this environment (Dighton, 1995). The stable biological communities adapted to their medium have a given level of species diversity, and communities that have too many or too few species, or that have a disproportionate number of certain species, indicates that the community is in crisis (Ludwig and Reynolds, 1988). The alpha-diversity values that were obtained in the samples of the three beaches studied were high and characteristic of this type of environment and indicate that probably the communities of micromycetes are stable. In order to determine the appropriate sample size one can enumerate the species as a function of the number of occurrences and with these data trace a curve of species-occurences. Frequently, an asymptote of the species-occurences is considered indicative of a sufficient sample size (Heck et al., 1975). The asymptote species-occurence curves of the samples of sand from the three beaches rose to just slightly below the maximum number of occurrences, which indicates that the size of the samples was adequate to characterize the endopsammon. It must be noted that the results of this study on the abundance and diversity of the endopsammophilous mycobiota are imprecise because at present the application and reliability of the methods to study and evaluate fungal diversity have serious limitations due to the difficulty of defining and delimiting a fungus in nature (Miller, 1995). Few investigators have developed specific techniques for studying the micromycetes of the endopsammon (Kohlmeyer and Kohlmeyer, 1979; Kirk, 1983; Rees and Jones, 1985). The use of different methodologies for the extraction of species from an ecosystem gives different values of fungal diversity (Miller, 1995). However, in this work, three different methods for studying fungal communities in the endopsammon were followed with the object of extracting the largest number possible of fungi, since at present, there is no one specific method for obtaining marine and nonmarine micromycetes. Likewise, it is appropriate to collect a wide variety of natural subtrates as this has the advantage of yielding the greatest number of species (Kohlmeyer and Kohlmeyer, 1979). In nature it is difficult to differentiate which fungi are living and which are dead and, of the living, to know which are active and which inactive in this environment (Miller, 1995). With the methods employed in this study it was not possible to estimate all of the fungi that live and are active in the endopsammon, as only those in a state of sporulation were recovered. Many fungi cannot be counted or identified because they do not develop in vitro and if they grow but do not sporulate they cannot be identified with conventional keys (Bills and Polishoot, 1994). In this work three fungi were not identified because they did not sporulate in vitro. In humid environments with high temperatures the aspergilli predominate over the penicilli (Pirozynski, 1968). Dunn and Baker (1983) found few penicilli with respect to the aspergilli in the endopsammon of Enewetak Atoll, Marshall Islands. In the three beaches studied in this work no penicilli were found, but various species of aspergilli were observed and some developed their ascigerous state, such as *Emericella nidulans*, which was very abundant (7.3%); on the other hand, the cultures of *Aspergillus flavus* formed numerous sclerotia. The nonmarine fungi, under the influence of certain ecological factors, can be partially active in the endopsammon and sometimes can become permanently active, and when this occurs, these fungi become facultatively marine species. A study on the descomposition of *Thalassia testudinum* Konig showed that the nonmarine micromycetes are active in the endopsammon (Newell and Fell, 1980). The high number of nonmarine fungi found in this work perhaps indicates that in this environment they are more active than has been previously thought in the process of remineralization and nutrient recycling and that probably many are facultatively marine species, although this status has not been demostrated.

In subsequent investigations, in order to characterize more precisely the mycobiota of the endopsammon, it will be necessary to develop methodology that will permit the extraction of a greater number of species from this environment.

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