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# Spatiotemporal distribution of the magnetotactic multicellular prokaryote *Candidatus* Magnetoglobus multicellularis in a Brazilian hypersaline lagoon and in microcosms

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**Summary.** *Candidatus* Magnetoglobus multicellularis is an unusual morphotype of magnetotactic prokaryotes. These microorganisms are composed of a spherical assemblage of gram-negative prokaryotic cells capable of swimming as a unit aligned along a magnetic field. While they occur in many aquatic habitats around the world, high numbers of *Ca.* M. multicellularis have been detected in Araruama Lagoon, a large hypersaline lagoon near the city of Rio de Janeiro, in Brazil. Here, we report on the spatiotemporal distribution of one such population in sediments of Araruama Lagoon, including its annual distribution and its abundance compared with the total bacterial community. In microcosm experiments, *Ca.* M. multicellularis was unable to survive for more than 45 days: the population density gradually decreased coinciding with a shift to the upper layers of the sediment. Nonetheless, *Ca.* M. multicellularis was detected throughout the year in all sites studied. Changes in the population density seemed to be related to the input of organic matter as well as to salinity. The population density of *Ca.* M. multicellularis did not correlate with the total bacterial counts; instead, changes in the microbial community structure altered their counts in the environment. [**Int Microbiol** 2012; 15(3):141-149]

**Keywords:** *Candidatus* Magnetoglobus multicellularis · magnetotactic prokaryotes · spatiotemporal bacterial distribution · Araruama Lagoon

## Introduction

Magnetotactic bacteria are a group of prokaryotes characterized by the presence of magnetosomes. These intracellular, nano-sized, membrane-bound, magnetic iron-rich organelles are composed of either the iron oxide magnetite ($Fe_3O_4$), or the iron sulfide greigite ($Fe_3S_4$) [5,10,24]. The main characteristic of this group is their passive orientation along the lines of a magnetic field and their active migration propelled by flagella, a behavior called magnetotaxis [5]. Magnetotactic bacteria are morphologically, physiologically and phylogenetically diverse but they share several features such as their gram-negative staining and the fact that they are motile, microaerophilic or anaerobic, and able to synthesize magnetosomes [3,4,27]. These bacteria inhabit freshwater and marine environments characterized by a chemically stratified vertical concentration gradient [3].

One of the most intriguing and unusual morphotypes of magnetotactic bacteria is the uncultivated multicellular mag-

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netotactic prokaryote (MMP) named *Candidatus* Magnetoglobus multicellularis. This highly organized aggregate of genetically identical gram-negative bacteria is arranged in a sphere [1,7,12,18,26] composed of 10–40 cells that contain several greigite ($Fe_3S_4$) magnetosomes, and are multiflagellated. Interesting features of *Ca.* M. multicellularis include: (i) multicellularity in all stages of its life cycle [13], (ii) its high level of structural organization [12,14], (iii) the interdependence of the cells [2], and (iv) its complex swimming movement [14,28], which together suggest that this microorganism is a highly organized, prokaryotic multicellular organism [12]. Similar microorganisms have been described in different locations but thus far they have been only partially characterized [6,9,26,31,34,35].

Magnetotactic multicellular prokaryotes occur in many aquatic habitats around the world [6,9,14,26,34,35]. High numbers of *Ca.* M. multicellularis have been found in Araruama Lagoon, a large hypersaline lagoon near the city of Rio de Janeiro, in Brazil [1]. Like other greigite-producing magnetotactic bacteria, *Ca.* M. multicellularis mostly occurs in stratified saline environments and is detected in high numbers in anoxic to sub-oxic zones of the gradient [4,31,32]. *Candidatus* M. multicellularis prefers sediment areas with high iron and sulfur concentrations [32]. In fact, the ratio of iron and bioavailable sulfur concentrations regulates its population density; however, when iron is available, nutrients (e.g., nitrogen) regulate population growth [32].

The morphology, ultrastructure and local spatial distribution of *Ca.* M. multicellularis have been well described [1,12,14,18,28,32]. Ecological studies of uncultured species of greigite-producing magnetotactic bacteria such as *Ca.* M. multicellularis may provide helpful information about their role in natural habitats, about which so far little is known. The amount of iron accumulated by these microorganisms in magnetosomes and the fact that magnetosomes can be dissolved within the acidic vacuoles of ciliates that prey on *Ca.* M. multicellularis [20] suggest that *Ca.* M. multicellularis participates in the biogeochemical cycles of iron and sulfur in Araruama Lagoon. A correct interpretation of the potential role of *Ca.* M. multicellularis in this lagoon requires an expansion of our understanding of the distribution of this microorganism from the local environment to the whole lagoon and of the association of this distribution with that of other microorganisms in the lagoon. Such data may provide insights into the physiology of *Ca.* M. multicellularis, and thus leas to the development of cultivation techniques. Here, we report on the spatiotemporal distribution and community structure of a *Ca.* M. multicellularis population in the sediments of Araruama Lagoon. The annual distribution and abundance of *Ca.* M. multicellularis were also studied and compared with the total bacteria community.

The large dimension of Araruama Lagoon (about 220 $km^2$) contributes to the formation of different microenvironments where the behavior of the *Ca.* M. multicellularis population can be assessed. Accordingly, we chose three sampling sites at the lagoon that differ in their biogeochemical characteristics in order to observe the seasonal variation in population density and salinity changes and to study the microbial community structure. The latter was analyzed using quantitative (total cell counts) and qualitative (bacterial community fingerprints by PCR-DGGE) approaches during periods characterized by significant variations in *Ca.* M. multicellularis populations and salinity: (i) August 2005; when the salinity values differed between the three sites but the *Ca.* M. multicellularis population densities were similar; (ii) January 2006, when salinity values were similar but *Ca.* M. multicellularis counts reached the highest numbers at Baleia beach, a site used to evaluate the seasonal variation of *Ca.* M. multicellularis; (ii) February 2006, when, compared to the previous month, *Ca.* M. multicellularis numbers decreased drastically at Baleia beach. In addition, the population density and vertical distribution of *Ca.* M. multicellularis were studied in microcosms from Baleia beach. The microcosm experiments allowed us to reproduce the field conditions in the laboratory while avoiding the great variations that occur in the natural environment.

## Materials and methods

**Site description.** Araruama Lagoon is a large hypersaline coastal environment located near the city of Rio de Janeiro, Brazil (22°50′–22°57′ S, 42°00′–42°30′ W). This 220-$km^2$ lagoon has an average depth of 3 m and has been a stable and permanent saline environment for the last four or five centuries due to the unbalanced annual precipitation and evaporation [33]. Three sampling sites, with different salinity levels and anthropogenic influence, were chosen to evaluate the variation of *Ca.* M. multicellularis population during a year: (i) Baleia beach (at São Pedro d'Aldeia city; 22°52′ S, 42°07′ W), a highly urbanized area with high salinity (average of 5.0 g/l); (ii) Alkalis National Company (ANC) channel (next to Arraial do Cabo city; 22°55′ S, 2°05′ W), an environmentally protected area with a salinity similar to that of Baleia beach; and (iii) Rio das Moças estuary (next to Araruama city; 22°53′ S, 42°23′ W), an urbanized area with low salinity (average of 3.87 g/l).

**Sampling.** To determine the seasonal variations of *Ca.* M. multicellularis in Araruama Lagoon, samples were collected as previously described [21]. Briefly, the upper sediment layers and water were collected in tubular sediment cores. The top 5 cm of the sediment from the cores were removed and homogenized [1]. The sediment was immediately used for *Ca.* M. multicellularis and total bacterial cell counts. Monthly samples were collected the same day, in periods without precipitation to ensure that differences in salin-

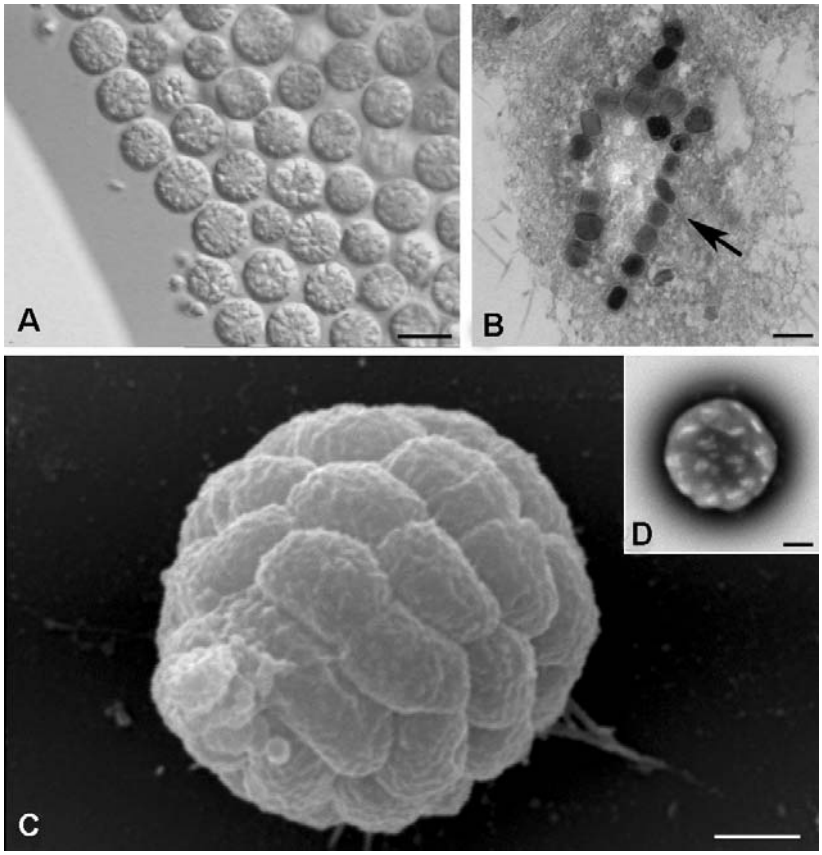


**Fig. 1.** (**A**) Differential interference contrast microscopy of magnetically concentrated *Ca.* M. multicellularis. (**B**) Transmission electron microscopy of *Ca.* M. multicellularis. Note the exclusive morphology and the magnetosome chains (arrow in B). (**C**) High-vacuum SEM and low-vacuum SEM (insert) of *Ca.* M. multicellularis. Scale bars: 10 µm in A, 200 nm in B, 2 µm in C and in the inset.

ity were not influenced by recent rainfall. Salinity was measured on pre-filtered water (through a 0.22-µm Millipore filter) from each sampling site with a refractometer. The sediment water content was measured as the difference between wet and dry weights (60 °C, 72 h).

**Microcosm setup.** For microcosm experiments, samples of water and sediment were collected from Baleia beach in Araruama Lagoon. Sediments from the first 5-cm depth were homogenized and were placed in five 10-liter containers. The sediments were overlaid with *in situ* water (water-sediment ratio 1:2) in the laboratory and the microcosms were loosely covered and stored for several weeks at room temperature (28 °C) and under low-light conditions. To analyze the vertical distribution of *Ca.* M. multicellularis in microcosms, sub-samples of sediment were retrieved with 10-ml syringes, with tips removed (manually cut with a razor blade).

**Magnetic concentration and counting of *Ca.* M. multicellularis**. To analyze the vertical distribution of *Ca.* M. multicellularis in microcosms the top (1 cm) layer of the sediment was repeatedly removed (up to 5 cm) using a syringe, immediately diluted with sterile lagoon water, and placed onto a microscope slide. The slide was exposed to the magnetic field of a rare earth (Nd-B-Fe) magnet for 5 min, at which point *Ca.* M. multicellularis reached the edge of the drop and were directly counted with a Zeiss Axiostar Plus phase contrast microscope. For all other experiments, the concentration of magnetic bacteria was determined as described in [19]. The counts were done in a Neubauer chamber as described in [21]. The detection and counting of *Ca.* M. multicellularis by light microscopy were greatly simplified by the distinctive morphology of the microorganisms (Fig. 1). *Ca.* M. multicellularis counts were expressed as individuals (the whole microorganism) per gram of dry weight of sediment or per cm (or $cm^3$) of the sediment layer.

**Microscopy and fluorescent in situ hybridization (FISH).** Magnetically concentrated samples were imaged with light microscopy as well as transmission and scanning electron microscopy. For light microscopy, the samples were observed by differential interferential contrast under the influence of the magnetic field from a Nd-B-Fe magnet with an Axioplan 2 microscope (Carl Zeiss, Germany) attached to a digital camera. Transmission electron microscopy examination was done as described in [21]. Samples of *Ca.* M. multicellularis were imaged either in high-vacuum or low-vacuum modes using a FEI QUANTA 250 variable-pressure scanning electron microscope. For high-vacuum SEM, magnetically concentrated *Ca.* M. multicellularis were placed on glass coverslips and fixed in 2.5 % glutaraldehyde and 0.075 % ruthenium red in 0.1 M sodium cacodylate buffer, post-fixed in 1 % osmium tetroxide in 0.1 M sodium cacodylate buffer, dehydrated in ethanol series, dried by critical point method and sputtered with gold. The images were acquired using a standard Everhart-Thornley secondary electron detector. For the low-vacuum imaging, samples were prepared in the same way as the high-vacuum samples until the post-fixation step. The images were acquired under low-vacuum specimen chamber gas pressure of 80 Pa using a backscattered electron detector.

For fluorescent in situ hybridization (FISH) analysis, magnetically concentrated *Ca.* M. multicellularis were fixed in 4 % paraformaldehyde, washed in PBS and stored in a 1:1 mixture of PBS/ethanol at –20 °C. One microliter of the cell suspension was spotted onto gelatin-coated multi-well-slides [0.1 % gelatin, 0.01 % $KCr(SO_4)_2$], dried at 45 °C for 20 min, and finally dehydrated sequentially in 50, 70, and 100 % ethanol for 3 min (each). Hybridization was performed with a fluorescein-labeled probe designed for the 16S rRNA genes of similar organisms found in New England [6] and a rhodamine-labeled probe designed for the 16S rRNA genes of *Ca.* M. multicellularis collected in Baleia beach [1]. To each well,

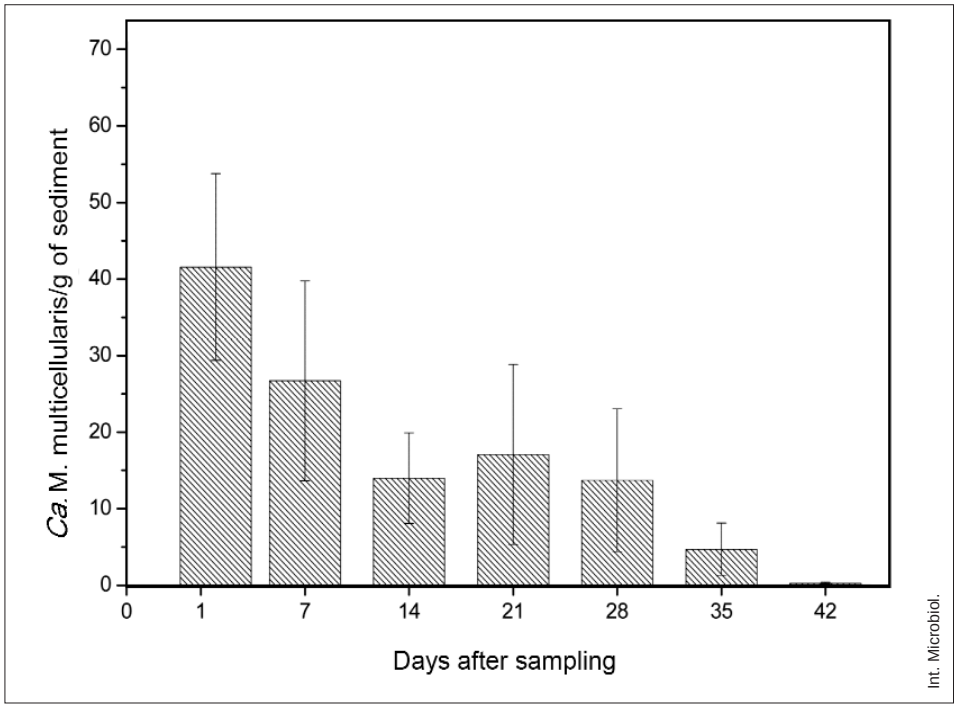


**Fig. 2.** Population densities of *Candidatus* M. multicellularis in a microcosm over a period of 42 days after sampling. The bars are averages from five replicas and the standard deviation. The microorganisms tended to decrease in number until finally none were detected.

8 µl of hybridization buffer (0.9 M NaCl, 20 mM Tris-HCl, 5 mM $Na_2EDTA$, 0.01 % SDS, pH 7.2), 1 µl of probe (25 ng/ml) and 1 µl of 4′,6-diamino-2-phenylindole (DAPI) (100 ng/ml) were added. Hybridization was carried out at 45 °C for 1h in a humidified chamber. The slides were washed in buffer containing 112 mM NaCl, 20 mM Tris-HCl, 5 mM $Na_2EDTA$, 0.01 % SDS, pH 7.2, at 48 °C for 20 min, rinsed in distilled water, and air dried. Slides were mounted with *n*-propyl gallate (0.2 M in glycerol:PBS, 9:1), and the preparations were examined with a Zeiss Axioplan 2 fluorescence microscope equipped with filter sets 02, 09 and 15.

**Total bacterial cell counts.** The total bacterial cell counts were done as previously described [11,21]. The samples were examined with a Zeiss Axioplan 2 fluorescence microscope. Total bacterial numbers were expressed per gram (dry weight) of sediment.

**DNA extraction and PCR amplification.** The sediment samples were kept frozen at –20 °C until DNA extraction using the Bio101 FAST DNA Spin Kit for soil (QBioGene). The DNA was analyzed by electrophoresis in 0.8 % (w/v) agarose gels in Tris–borate–EDTA buffer to check its purity and molecular size.

DNA was amplified by PCR with 16S rDNA gene-based universal primers (968 F and 1401 R) [23]. A GC clamp [22] was added to the forward primers (F). The reaction mixture (50 µl) contained 1 µl of template DNA (sediment total DNA), 1× PCR buffer (20 mM Tris-HCl, pH 8.4, and 50 mM KCl), 3.75 mM $MgCl_2$, 2.5 mM of each dNTP, 0.5 µM each primer, 0.45 µg/µl of bovine serum albumin (BSA), 1 % (w/v) formamide, 0.5 U/µl Taq polymerase and sterile MilliQ water.

All PCR amplifications were done with a thermal cycler (Mastercycler Personal, Eppendorff, Hamburg, Germany). The thermal cycle involved an initial denaturing step of 94 °C for 3 min, followed by 35 cycles of 1 min at 94 °C, annealing for 1 min at 55 °C and extension for 1 min at 72 °C, followed by a final extension at 72 °C for 10 min. Five µl aliquots of the PCR products were analyzed by electrophoresis on 1.5 % agarose gels to check the sizes and amounts of amplicons.

**Denaturing gel gradient electrophoresis (DGGE) analysis.** For denaturing gel gradient electrophoresis (DGGE), 15 µl of PCR products were loaded onto 6 % (w/v) polyacrylamide gels (40 % acrylamide stock solution, 2 % bis solution 37.5:1) containing a 45-70 % gradient of denaturants [100 % denaturants consisting of 40 % (v/v) formamide and 7 M urea]. DGGE was done with the Dcode universal mutation detection system (Bio-Rad Dcode, Richmond, VA, USA) at 100 V and 60 °C for 15 h, in TAE buffer (20 mM Tris, 10 mM acetate, 1 mM EDTA pH 7.4).

Gels were stained with SYBR green nucleic acid gel stain (1:10000 dilution; Molecular Probes, Carlsbad, CA, USA), and photographed under UV light with a Gel Doc 2000 system (Bio-Rad Laboratories, Carlsbad, CA, USA). The DGGE gel images were used to construct dendrograms, with the DGGE banding patterns converted into a binary matrix using presence–absence data. Cluster analysis, represented as similarity dendrograms, was done with Statistica 6.0 software (Stat Soft Co). The dendrogram was constructed with unweighted pair-group method with arithmetic averages (UPGMA). The similarities calculation was based on the Pearson coefficient.

## Results

**Microcosm experiments.** Variations over 42 days in the *Ca.* M. multicellularis population densities in microcosms containing water and sediment from the Baleia beach (São

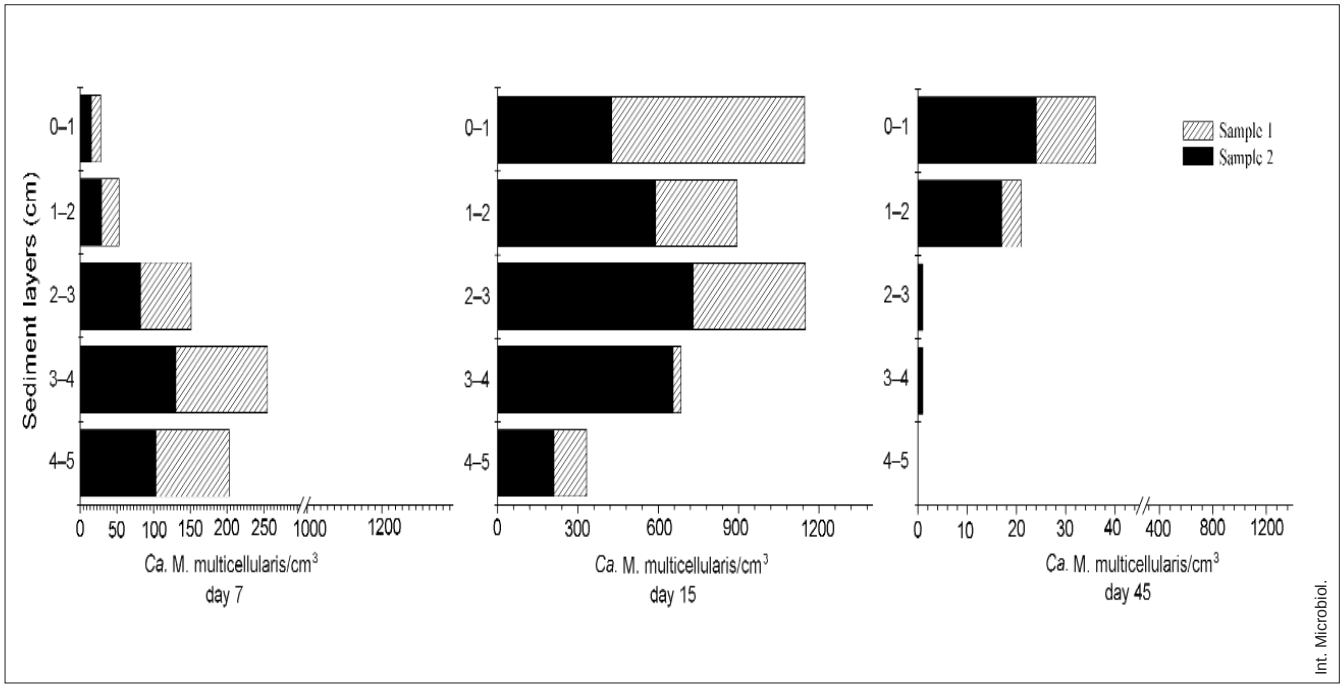


**Fig. 3.** Vertical distribution of *Ca.* M. multicellularis within the sediment of a microcosm. The bars show the behavior of the populations obtained from two core samples from the same microcosm at 7, 15 and 45 days after sampling. The microorganisms tend to move up to the water–sediment interface over time.

Pedro d'Aldeia) are shown in Fig. 2. The highest concentrations were observed the first day after sampling (40 individuals/g of dry weight of sediment). Thereafter, the population density gradually decreased until day 42 after sampling, when it was almost zero. The vertical distribution of *Ca.* M. multicellularis within the sediment in the microcosms of Baleia beach over time is shown in Fig. 3. Initially, the microorganisms were distributed all along the upper 5 cm of the microcosms sediment, but their preferred zone seemed to be within the 3.0–5.0 cm layer, where more than $2 \times 10^2$ *Ca.* M. multicellularis/cm$^3$ were observed. Gradually, the population of *Ca.* M. multicellularis moved to the upper layers of the microcosms sediment; 45 days after sampling it reached the sediment–water interface but its density had decreased almost ten-fold.

A preliminary subset of the data obtained from Baleia beach has already been published to address the salinity dependence of *Ca.* M. multicellularis [21]. They are presented herein for the sake of comparison with the data obtained from two other different microenvironments.

**Direct sampling in the lagoon.** FISH analysis revealed that all *Ca.* M. multicellularis (and also all cells from *Ca.* M. multicellularis) collected from the three sites (Baleia beach, ANC channel, and Rio das Moças estuary) hybridized with the two probes described in Materials and methods (Fig. 4). These results indicate that the populations from those sites are identical and also that the cells that make up the multicellular microorganisms belong to the same phylotype.

Both the salinity and the numbers of *Ca.* M. multicellularis were measured in core samples collected directly at the three sites throughout a year. The lowest salinity occurred in Rio das Moças estuary (average 4.4 g/l; max. 5.0 g/l and min. 3.9 g/l). In the ANC channel and at Baleia beach the salinity values were very similar and almost identical (averages 5.3 g/l and 5.5 g/l, respectively).

*Candidatus* M. multicellularis was detected during the whole year in core samples collected at the three sites for the seasonal variation studies, with the lowest counts usually found in Rio das Moças estuary. At Baleia beach, the *Ca.* M. multicellularis population increased from 10 to $10^2$ individuals/g of dry sediment in periods when the salinity ranged from 4.0 g/l to around 6.0 g/l (between October 2005 and January 2006; when salinities ranged from 5.0 to 5.7 g/l) and decreased by the same proportion when the salinity was greater than 5.9 g/l (July 2005 to September 2005 and February 2006). Throughout the study period, *Ca.* M. multicellularis counts were usually lower in ANC channel (below $10^2$ individuals/g of dry sediment) than in Baleia beach, with

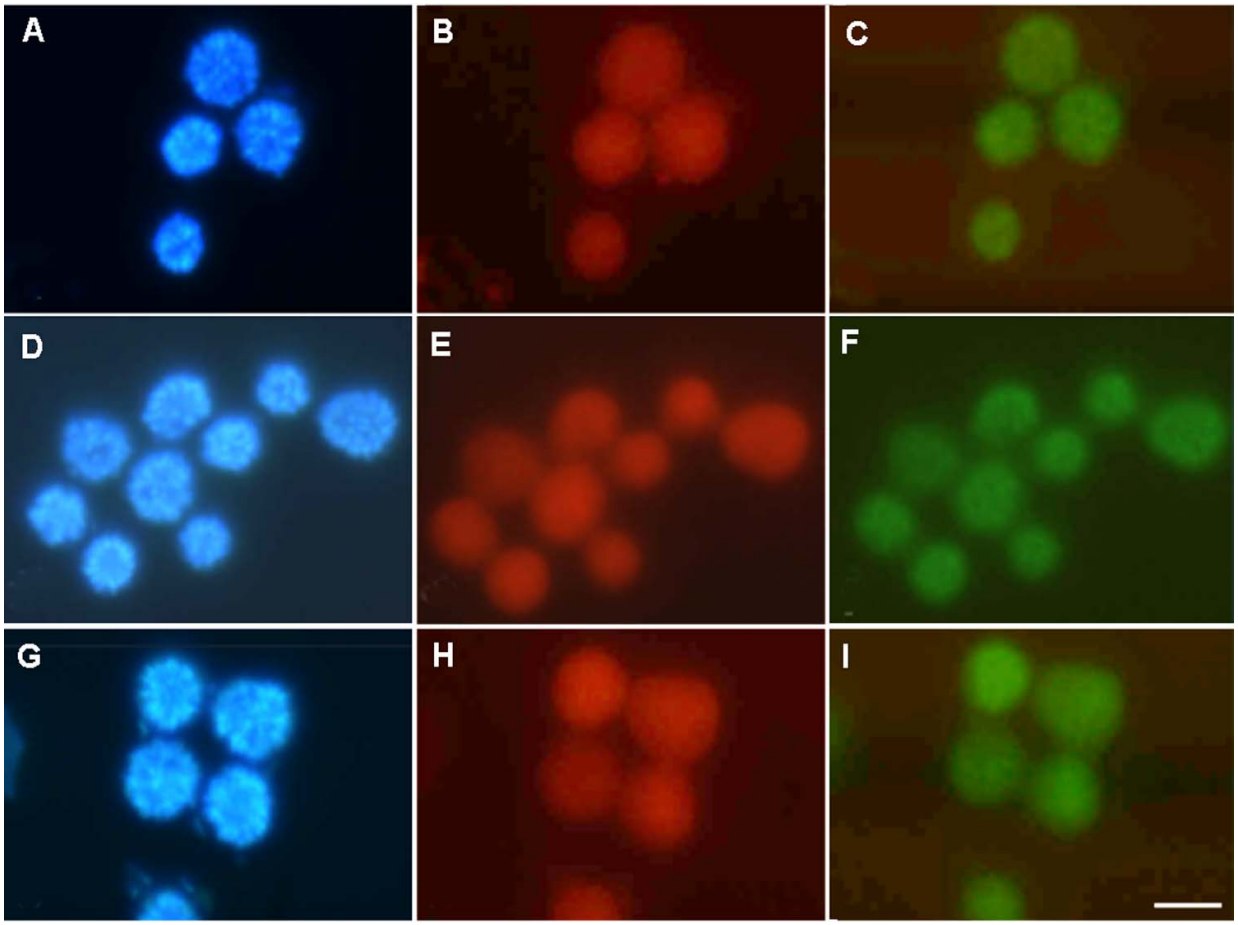


**Fig. 4.** Fluorescent in situ hybridization (FISH) of *Ca.* Magnetoglobus multicellularis collected from: (**A–C)** Rio das Moças estuary; (**D–F)** ANC channel; and (**G–I**) Baleia beach sites at Araruama Lagoon. Fluorescence microscopy images of the same microorganisms stained with 4′,6-diamidino-2-phenylindole (DAPI; A, D, G); hybridized with a probe specific for *Ca.* M. multicellularis (rhodamine; B, E, H), and with a probe used for MMPs described in New England (fluorescein; C, F, I). Bars: 5 μm.

the greatest difference occurring between October and March (ten times more individuals/g dry weight of sediment at Baleia beach). *Candidatus* M. multicellularis population reached its maximum abundance ($7.7 \times 10^2$ individuals/g dry weight of sediment) at Baleia beach in January 2006. In February, at the same site, the numbers of *Ca.* M. multicellularis decreased abruptly (to $2.5 \times 10$ individuals/g dry weight of sediment).

Samples obtained directly from cores collected in Rio das Moças estuary contained the highest numbers of total bacteria in all months studied, with a slight decrease in February 2006 (Table 1). At Baleia beach and ANC channel the bacteria counts were very similar in the months analyzed. Significant variation among these sites could not be detected nor was there any apparent correlation between total bacterial counts and *Ca.* M. multicellularis numbers.

**Table 1.** Total bacterial cells counts from the first 5 cm of sediment layers (expressed as numbers of bacteria/g dry weight of the sediment)

| Location | August 2005 | January 2006 | February 2006 |
|---|---|---|---|
| Rio das Moças estuary | $1.2 \times 10^9 \pm 0.1 \times 10^9$ | $1.5 \times 10^9 \pm 0.5 \times 10^9$ | $6.4 \times 10^8 \pm 0.3 \times 10^8$ |
| Baleia beach | $8.4 \times 10^8 \pm 3.5 \times 10^8$ | $6.9 \times 10^8 \pm 1.6 \times 10^8$ | $4.0 \times 10^8 \pm 0.5 \times 10^8$ |
| ANC channel | $6.4 \times 10^8 \pm 1.6 \times 10^8$ | $5.4 \times 10^8 \pm 1 \times 10^8$ | $4.9 \times 10^8 \pm 0.5 \times 10^8$ |

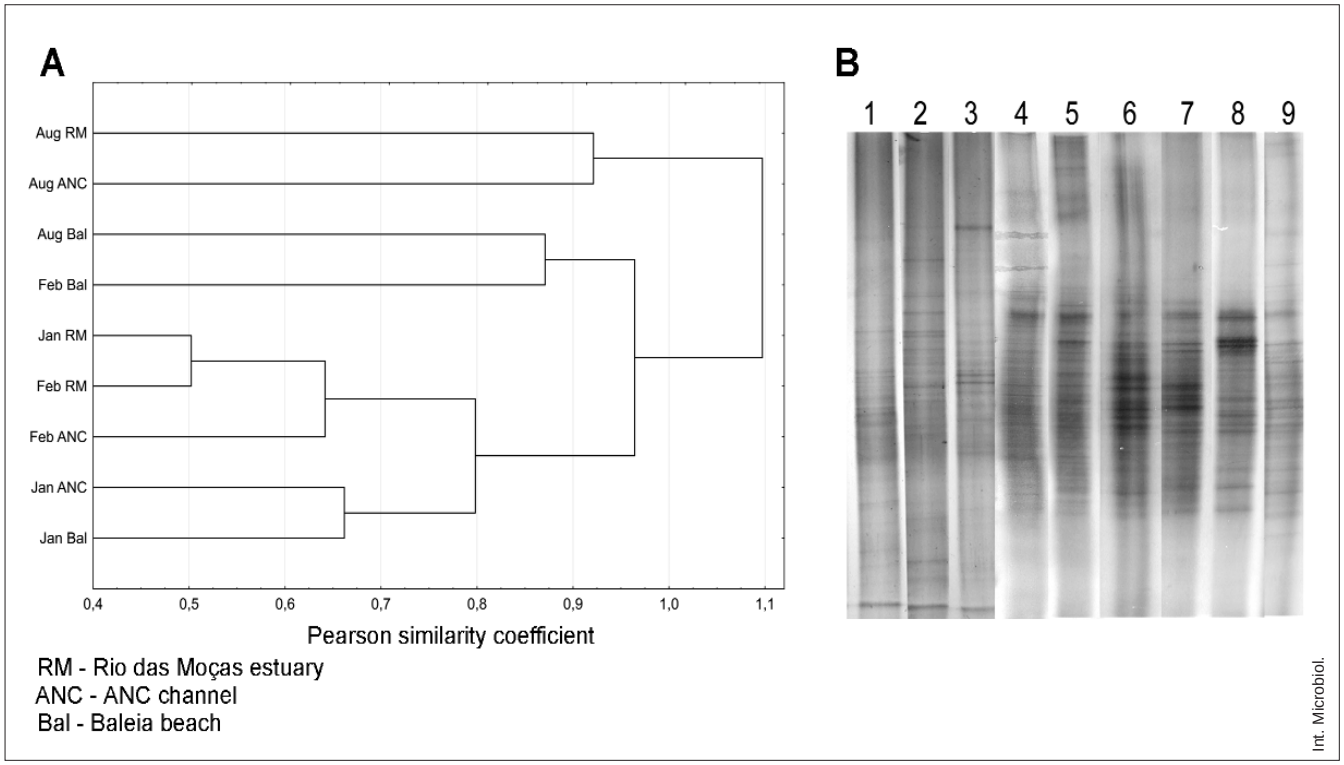


**Fig. 5.** Dendogram (**A**) and DGGE gel (**B**). The DGGE profile was used to construct the dendogram using the Pearson similarity coefficient and employing an unweighted pair group method with arithmetic averages (UPGMA). The DGGE gel lanes show the following: 1 - Jan RM; 2 - Jan ANC; 3 - Jan Bal; 4 - Feb RM; 5 - Feb ANC; 6 - Feb Bal; 7 - Aug RM; 8 - Aug ANC; 9 - Aug Bal.

Bacterial DGGE fingerprinting obtained from the top 5 cm of sediment from cores collected at the three sites showed a high number of bands well spread throughout the gel. DGGE patterns of PCR products rerun or re-amplified showed reproducible fingerprints (data not shown). The dendrogram (Fig. 5) revealed three clusters, one of which corresponded to all the samples from January and February 2006, with the exception of the sample from Baleia beach in February 2006. This sample grouped within the August 2005 cluster and was very distant from the sample from Baleia beach in January 2006, indicating that a significant change in community structure had occurred at this location between these two periods.

## Discussion

There are few reports on the temporal variation of magnetotactic bacteria in sediment microcosms [8,9,15,25]. Here we determined the spatiotemporal distribution of *Ca.* M. multicellularis in microcosms rather than, as in most studies, removing these microorganisms from their environment. An understanding of the factors that regulate the *Ca.* M. multicellularis population density may provide the necessary information to understand their physiology and the importance of this organism in the microbiota of Araruama Lagoon. Although high numbers of magnetotactic bacteria were detected in microcosms sediments several months after sampling, the community composition undergoes drastic succession upon prolonged incubation in microcosms [8,9,25]. Aged microcosms are dominated by magnetotactic coccoid bacteria [8,9,15,25]; MMPs have occasionally been reported, but only in fresh samples [8,25]. The populations of *Ca.* M. multicellularis studied in the present work could not survive for many weeks in the microcosms of Baleia beach. We have observed that these microorganisms are frequently detected in freshly collected sediments from the same environment and then tend to disappear [21, and this work]. Changes over time in physicochemical factors, such as redox potential, sulfide, sulfate and iron concentrations, in the microcosms [25] are probably the main causes for this disappearance.

Magnetotactic bacteria are adapted to particular niches in vertical geochemical gradients and thus are very sensitive to changes in the microenvironment [8,15]. In the microcosms,

the decline in *Ca.* M. multicellularis populations over time was associated with changes in their vertical distribution—most likely, movement to the upper layers of the sediments in microcosms in search of a new micro-niche. This behavior indicates that the gradients in microcosms are not stable and gradually become hostile environments for *Ca.* M. multicellularis. Flies et al. [8] also suggested that the difficulty in developing stable magnetotactic bacteria populations in microcosms is due to the lack of stability in the gradients of these systems. We observed a change in the appearance of the microcosms sediments few weeks after the sampling incubation, perhaps reflecting changes in stratification. The migration of *Ca.* M. multicellularis populations also could be related to the availability of organic matter, as in the upper layers of the sediments nutrients are more abundant [32].

Due to its large size, the Araruama Lagoon accommodates different geological formations and receives different anthropogenic influences. Souza et al. [33] grouped 14 different sites in Araruama Lagoon into five clusters with different biogeochemical characteristics. This biogeochemical diversity makes the Araruama Lagoon an excellent environment to study the spatiotemporal variation of *Ca.* M. multicellularis. Our work involved locations with significant differences in biogeochemical characteristics, salinity, and urbanization levels as well as in *Ca.* M. multicellularis abundances (according to [21]). An influence of salinity on *Ca.* M. multicellularis population densities was observed during the months and at the sites studied, corroborating data previously reported [21]. Yet, salinity differences alone cannot fully explain the behavior of *Ca.* M. multicellularis; rather, recent works have shown that the composition of magnetotactic bacteria communities is influenced by salinity [16] and temperature [17]. However, similar shifts in the cores collected at the sites were not observed, since Araruama Lagoon is a tropical to semi-arid environment where the temperature accordingly remains relatively constant and *Ca.* M. multicellularis is the only magnetotactic microorganism found therein.

Sobrinho et al. [32] suggested that iron and nutrient availability regulates the growth of *Ca.* M. multicellularis at Araruama Lagoon. At the ANC channel and Rio das Moças estuary, the availability of organic matter is high [33]. Rio das Moças estuary is located in a region with the lowest salinity, such that salinity could be the main factor controlling *Ca.* M. multicellularis populations. At Baleia beach, iron is not a limiting factor in the regulation of population density [32].

Salinity values at ANC channel and Baleia beach were very similar; however, the *Ca.* M. multicellularis counts were always very different. The main difference observed between these two sites is the urbanization level. Baleia beach is situated in a highly urbanized area, while the ANC channel is located in an environmentally preserved area. The highest abundances of *Ca.* M. multicellularis were determined in cores collected at the sites at the beginning of the Brazilian summer (January 2006). In summer, the human population increases drastically due to tourism in the region, increasing the disposal of sewage and consequently adding organic matter into the lagoon. Souza et al [33] reported that the highest values for chlorophyll-*a* occur in January. Our data suggest that the input of organic matter is related to the increase in the *Ca.* M. multicellularis population at Araruama Lagoon. Simmons and Edwards [30] likewise observed a relationship between organic matter and the presence of MMPs. The highest values determined in our study were similar to the maximum abundances found in the same season in the sediment of Salt Pond (MA, EUA) [29].

We also analyzed the total bacterial communities from the three sites to determine whether the changes observed in the *Ca.* M. multicellularis population correlated with those in other bacterial populations in the same environment. However, the factors that influenced the *Ca.* M. multicellularis did not have any apparent effect on the number of total bacteria, although they might have led to changes in the bacterial community structure. Comparison of DGGE patterns suggested a major change in bacterial community structure between January and February 2006 at Baleia beach, which in turn might have caused the abrupt reduction in the population of *Ca.* M. multicellularis.

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**Competing interests.** None declared.

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