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Infection by *Amoebophrya* spp. parasitoids of dinoflagellates in a tropical marine coastal area

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ABSTRACT: Infection of marine dinoflagellates by the parasitic dinoflagellate *Amoebophrya* spp. plays an important role in population dynamics and carbon flow in marine food webs. It has been extensively reported that *Amoebophrya* parasitoids occur in temperate coastal areas of the northern hemisphere; however, little is known about their distribution and importance in tropical areas and southern oceans. We used an rRNA-based, fluorescent *in situ* hybridization assay to detect *Amoebophrya* spp. infections during the decline of a late-summer dinoflagellate population dominated by *Ceratium falcatiforme* in a tropical coastal area of the southern Atlantic Ocean subjected to recurrent upwelling–downwelling cycles. Conditions during our survey were typical of downwelling when oligotrophic waters dominate the area. *C. falcatiforme* was the most infected host, with a prevalence averaging 2% over the study area at the beginning of sampling. At a fixed sampling station monitored over 4 wk, *Amoebophrya* prevalence escalated from 1 to 7% over a 6 d period, concomitant to a 94% decrease in host cell numbers. Infection by *Amoebophrya* was estimated to have killed ca. 11% of the host cell population within this period; thus, parasitism was not the main factor behind the *C. falcatiforme* population decline. Estimates based on biovolume calculations indicate that ca. 6.5% of the carbon in the decaying *C. falcatiforme* population was transformed into parasitoid dinospores, which became available to tintinnid ciliates that were abundant during our survey. Such a trophic link might be relevant in tropical oligotrophic waters when the system is characterized by a microbial food web structure.

KEY WORDS: *Amoebophrya* spp. · *Ceratium falcatiforme* · Parasitism · Dinoflagellates · FISH probes · South Brazil Bight

INTRODUCTION

Parasitism is a widespread inter-specific interaction in both terrestrial and aquatic habitats (Combes 2001). Heterotrophic dinoflagellates belonging to the genus *Amoebophrya* are remarkable, as they are able to infect not only several metazoans but also protists (Cachon & Cachon 1987). One such parasite, originally described as a single species, *Amoebophrya ceratii* Koepen, but currently recognized as a species complex (Coats et al. 1996, Coats & Park 2002, Gunderson et al. 2002, Salomon et al. 2003a, Kim et al. 2004, 2008), infects marine dinoflagellates. More than 40 marine dinoflagellate species from ca. 20 different genera are susceptible to *Amoebophrya* spp. (Park et al. 2004). The life cycle of *Amoebophrya* spp. displays 2 distinct phases: a free-swimming phase represented by small, flagellated forms called dinospores, and a parasitic, trophic phase whereby dinospores develop inside the host cells from which they derive their food (Cachon 1964, Cachon & Cachon 1987). Although aspects of the biology of free-living dinospores are known from cultured dinoflagellate–*Amoebophrya* systems (Coats & Park 2002), abundance and diversity of these free-living stages in natural marine waters have only recently been reported on with the help of molecular techniques (Chambouvet et al. 2008).

Infections of dinoflagellates by *Amoebophrya* spp. are lethal (Cachon 1964, Coats & Bockstahler 1994), rendering *Amoebophrya* spp. a typical parasitoid fea-

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ture. Therefore, for host-population dynamics, parasitism by *Amoebophrya* spp. is similar to predation by grazers, in that part of the host population will be killed.

Studies on cultured host–parasitoid systems have revealed important features of *Amoebophrya* spp. infection dynamics and interaction with their hosts at the cellular level (Coats & Bockstahler 1994, Park et al. 2002). For *Amoebophrya*–host systems that are not established in cultures, the biology of the interaction is best studied by direct observation of natural host populations. Field studies offer an opportunity to assess the host range and prevalence (i.e. the percentage of infected cells in the host population) of *Amoebophrya* spp. in the presence of several potential host species and other components of the microbial community that can interfere with the process. In this respect, molecular probes targeting ribosomal RNA (rRNA) have been successfully used to detect infection by morphologically similar but genetically distinct *Amoebophrya* spp. on natural dinoflagellate populations, helping to clarify the parasitoid's diversity and several host-specificity issues (Salomon et al. 2003a, Chambouvet et al. 2008).

The frequency of high *Amoebophrya* spp. prevalence events in nature seems to be different among different *Amoebophrya*–dinoflagellate systems. For example, infection of the dinoflagellate *Dinophysis norvegica* in the Baltic Sea is seldom above 10% (Gisselson et al. 2002, Salomon et al. 2003b, 2006a), whereas *Gyrodinium uncatenum* and *Scrippsiella trochoidea* populations in Chesapeake Bay frequently show localized, high-prevalence levels up to 80% (Coats et al. 1996). High virulence and the narrow host ranges of several *Amoebophrya* spp. observed both in laboratory and field studies support the idea that these parasitoids can contribute to the decline of a host population, making them potential candidates as mitigating agents against harmful dinoflagellate blooms (Coats & Park 2002, Chambouvet et al. 2008). Moreover, highly host-specific parasites influence phytoplankton community structure and species succession (Ibelings et al. 2004).

Infection of dinoflagellates by *Amoebophrya* can also alter the carbon flow within marine food webs. Dinospores released from dinoflagellate hosts at the end of the trophic phase can be ingested by ciliates (Maranda 2001, Johansson & Coats 2002) creating new, temporary trophic links within the microbial food web.

Amoebophrya spp. occur in marine coastal areas worldwide, although most records come from temperate regions in the northern hemisphere (see the review by Park et al. 2004). Coastal tropical waters, on the other hand, have received little attention (Salomon et al. 2006b). In the present study, we used an rRNA-based probe assay to investigate dinoflagellate infection by *Amoebophrya* spp. during the decline of a late-summer dinoflagellate assemblage in the coastal waters off Cabo Frio, 150 km north of Rio de Janeiro, in the South Brazil Bight. This area is of particular interest due to recurrent upwelling events that influence marine food webs in this region (Valentin 1984).

MATERIALS AND METHODS

Sampling. Sampling was performed onboard the RV 'Diadorim' (IEAPM-Brazilian Navy), off the Rio de Janeiro State coast (23° 00′ S, 42° 00′ W), in the Cabo Frio area, South Brazil Bight (Fig. 1). The first sample used to quantify parasitic infection of dinoflagellates was taken during routine monitoring in the area (Stn E; Fig. 1) on March 23, 2004, when several cells of *Ceratium falcatiforme* E. G. Jørgensen were observed to harbor *Amoebophrya* spp.-like trophonts. Afterwards, the plankton communities were collected on March 24 at 5 stations (Stns A to E; Fig. 1). Stns A, B, and C, depths between 26 and 57 m, were located on an upwelling area. Stns D and E, 10 and 5 m deep, respectively, were located in a protected area inside Anjos Bay. In order to follow the development of the planktonic communities and infection by *Amoebophrya* spp., sampling continued for several weeks at Stn E (Fig. 1). Water temperature, salinity, and chlorophyll fluorescence depth profiles were measured using a conductivity, temperature, and depth profiler. Water was collected with a 2 l Niskin bottle from the surface and from 1 intermediate depth (15 m at Stns A, B, and C; 5 m at

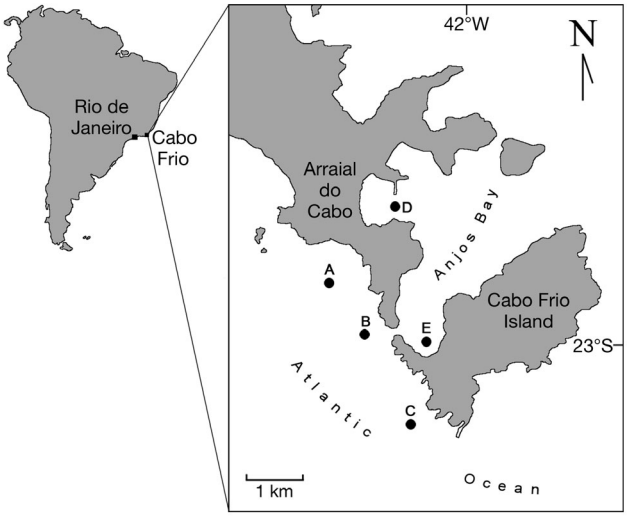

Fig. 1. Location of the 5 stations (A to E) sampled in the present study

Stn D and 3 m at Stn E). Vertical net hauls (mesh size: 20 µm) were also taken on every occasion from near-bottom to surface (from 30 m to surface at Stn C).

Sample preservation. Aliquots of 0.5 l of seawater were fixed with formalin (2% final concentration) for identification and quantification of planktonic cells. Net tow material was concentrated down to 7 ml in 2 size fractions, 20 to 70 µm and 70 to 300 µm, using nylon nets mounted on Plexiglas tubes. After fractionation, cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 8.0) for 1 h, rinsed once with PBS, twice with 70% (v/v) ethanol, transferred to 70% ethanol and stored at –20°C (Salomon et al. 2003a).

Cell counts. Identification and quantification of auto- and heterotrophic dinoflagellates, other phytoplankton species, and tintinnid ciliates were carried out using the formalin-fixed samples. Cells were analyzed using an inverted microscope at 400× magnification following Utermöhl's (1958) sedimentation technique. Only the fraction >20 µm was analyzed.

Probe hybridization. Detection of *Amoebophrya* spp. inside host cells was done by fluorescent (whole cell) *in situ* hybridization (FISH) with a fluorescein-tagged, rRNA-based oligonucleotide probe (5′-TTA TTA TGA (AG)TC ATC CAA AA-3′). The probe was designed based on 13 small subunit rDNA sequences of *Amoebophrya* spp. available in the GenBank database (www.ncbi.nlm.nih.gov; Accession Numbers: AF069516, AF239260, AF472553, AF472554, AF472555, AY208892, AY208893, AY208894, AY260467, AY260468, AY260469, AY775284, AY775285). These sequences are from *Amoebophrya* spp. that have been observed infecting the following dinoflagellates: *Gymnodinium sanguineum* (=*Akashiwo sanguinea*), *Dinophysis norvegica*, *Karlodinium micrum*, *Gymnodinium instriatum*, *Scrippsiella* sp., *Ceratium tripos*, *Prorocentrum micans*, *Prorocentrum minimum*, *Ceratium lineatum*, *Alexandrium affine*, and *Gonyaulax polygramma.* Hybridization of samples from various sea areas showed that the probe detects *Amoebophrya* in several different dinoflagellates species (P. S. Salomon et al. unpubl. data). Cells from the vertical net tows stored in 70% ethanol were used in the assays following Salomon et al. (2003a), except that hybridization was done overnight at 50°C instead for 5 h at 45°C. After hybridization, cells were counter-stained for ca. 15 min with 0.5 to 1 µg ml^{-1} DAPI, resuspended in an antifade solution (SlowFade Antifade, Molecular Probes), and mounted on microscope slides with cover slips sealed with nail polisher. Detection was carried out with an Olympus BX40 epifluorescence microscope, with filter sets suitable for fluorescein (λ_{ex} 470 to 490 nm, λ_{em} > 550 nm) and DAPI (λ_{ex} 360 to 370 nm, λ_{em} > 420 nm) absorption/emission spectra.

***Amoebophrya* infections.** At least 2000 cells of *Ceratium falcatiforme* and 1000 cells of both *Dinophysis acuminata* and *Prorocentrum minimum* were analyzed and scored as infected or non-infected. *Amoebophrya* spp. prevalence was expressed as a percentage of infected cells. Infections were further discriminated according to parasitoid developmental stage as 'early' (prior to circular configuration of parasitoid nuclei) or 'late' stage (all other stages). Parasitoid prevalence in dinoflagellate species other than the ones mentioned above could not be precisely estimated due to low cell densities. However, in order to verify whether these or any other cells in the samples were also infected, 3 to 5 slides $sample^{-1}$ were scanned for a probe signal without scoring cell numbers. Whenever a positive probe signal was detected, the host was identified and the parasitoid infection stage and location inside the host were determined.

To check the efficiency of the probe in detecting the *Amoebophrya*-infected dinoflagellates present in the samples, 1 slide from each sample was scanned for infection based on DAPI, which allows identification of middle to late infection stages based on hollow spaces in the host nucleus or the circular or multinucleated pattern of the parasitoids trophont nuclei, respectively. Cells found infected based on the DAPI signal were checked for a probe signal.

Cell measurements, biovolume, and carbon content estimates. Material from the net tow collected at Stn E on March 24 was hybridized with the probe as described above. To avoid possible distortion effects from the cover slip, cells were mounted in a 100 µm deep chamber (Palmer Maloney) and analyzed at 200× magnification. Linear measurements of girdle diameter, total, hypothecal, and epithecal length were conducted in 20 uninfected *Ceratium falcatiforme* cells and in 20 cells infected with a mature trophont. Host biovolume was calculated using a double-cone geometrical approximation, whereas trophont biovolume was calculated as a prolate spheroid based on linear measurements of diameter and height (Hillebrand et al. 1999). Carbon contents were then estimated based on biovolume according to Menden-Deuer & Lessard (2000).

RESULTS

Oceanographic conditions

Surface water temperature at all 5 sampling stations on March 24 was ca. 24°C. Vertical temperature profiles indicated a homogeneous upper layer, with little temperature variation throughout the water column above 35 m depth. A well-defined thermocline was

observed at Stn C, with a sharp gradient from 24 to 18.5°C between 35 and 45 m depth (Fig. 2). At Stn E, water temperature did not vary much over time, decreasing by only 1°C (from 24 to 23°C) by the end of the 4 wk sampling period. Salinity at the surface was ca. 35.1 at Stns A to C, and slightly higher (ca. 35.2) at Stns D and E (Fig. 2). Depth profiles showed little variation in salinity, except at Stn C, where a layer of lower salinity water (ca. 31.5) was present at ca. 40 m depth. Salinity at Stn E remained at around 31.2 during the whole sampling period. Relative chlorophyll fluorescence was similar at the surface across the 5 sampling stations. At the 3 deepest sampling stations (Stns A, B, and C; see Fig. 1), chlorophyll fluorescence maxima were observed at ca. 15 m depth (Fig. 2).

Composition of the plankton community

Dinoflagellates dominated the plankton communities at all 5 locations on March 24, with cell densities reaching up to 7×10^3 l^{-1} at Stn B. On average, dinoflagellate cells accounted for 88% of the planktonic organisms larger than 20 µm (including auto- and heterotrophic dinoflagellates, other phytoplankton, and tintinnid ciliates). The most abundant species was *Ceratium falcatiforme*, with cell densities ranging from 1.3×10^3 cells l^{-1} at Stn C to 6.5×10^3 cells l^{-1} at Stn B (Fig. 3a). Overall, *C. falcatiforme* cells represented 61 to 92% of the dinoflagellates and 49 to 88% of the plankton community's (>20 µm) total cell numbers. Two other dinoflagellate species, *Dinophysis acuminata* and *Prorocentrum minimum*, were present in all samples at densities of up to 0.3×10^3 and 0.7×10^3 cells l^{-1}, respectively. Other dinoflagellates present included *C. furca*, *C. macroceros*, *C. tripos*, *Protoperidinium* spp. and several unidentified peridinoid forms, but in very low densities. The diatom communities, represented mainly by the genera *Chaetoceros*, *Coscinodiscus*, *Ditylum*, *Navicula*, and *Nitszchia*, were present at maximum cell densities of ca. 0.5×10^3 cells l^{-1} (Fig. 3c). Tintinnid ciliates were observed in all samples at densities between 0.08 and 0.56×10^3 cells l^{-1} (Fig. 3d). In general, cell densities of *C. falcatiforme* and other groups were similar between surface and deep samples, typically with less than a 2-fold difference between them (Fig. 3). Exceptions were the dinoflagellate communities — not including *C. falcatiforme* — and tintinnids at Stn E, where cell numbers were, respectively, 3.4 and 4.7 times higher at the surface than at 15 m depth (Fig. 3b,d).

A shift in dominance from dinoflagellates to diatoms occurred in the planktonic communities at Stn E during the 4 wk sampling (Fig. 4). *Ceratium falcatiforme* cell densities decreased by 94% — from 3.55×10^3 to 0.22×10^3 cells l^{-1} — between March 24 and March 30. By April 20, *C. falcatiforme* densities had decreased to 0.06×10^3 cells l^{-1} at 3 m depth and were below detection limit at the surface. Cell numbers of dinoflagellate species other than *C. falcatiforme* showed a similar trend, though with a less pronounced decrease (Fig. 4c). As a result, *C. falcatiforme* became less repre-

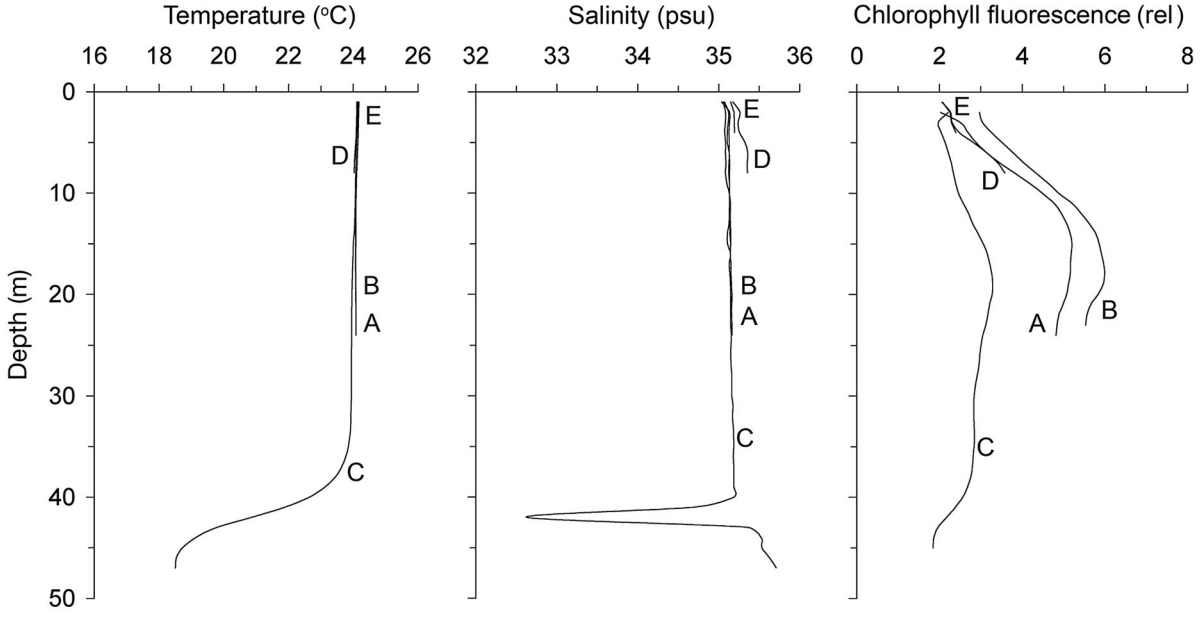

Fig. 2. Depth profiles of temperature, salinity and chlorophyll fluorescence (rel: relative units) at the 5 stations (A to E) in the Cabo Frio area shown in Fig. 1

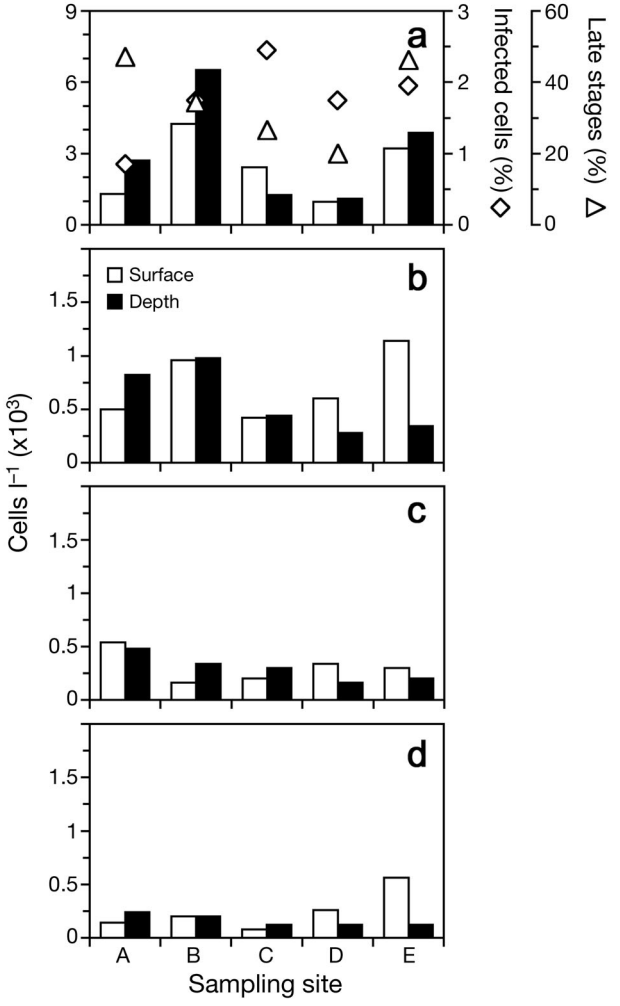

Fig. 3. Abundance of components of the plankton community and *Amoebophrya* spp. prevalence on *Ceratium falcatiforme* at the 5 stations (A to E). Cell densities are shown for surface and intermediate depth samples (see 'Materials and methods' for details). (a) Left *y*-axis: *C. falcatiforme* cell densities; right *y*-axis: parasite prevalence (percentage of infected cells) and percentage of late infection stages of the parasite relative to the total infected host cells. (b) Dinoflagellates other than *C. falcatiforme*, (c) diatoms and (d) tintinnid ciliates

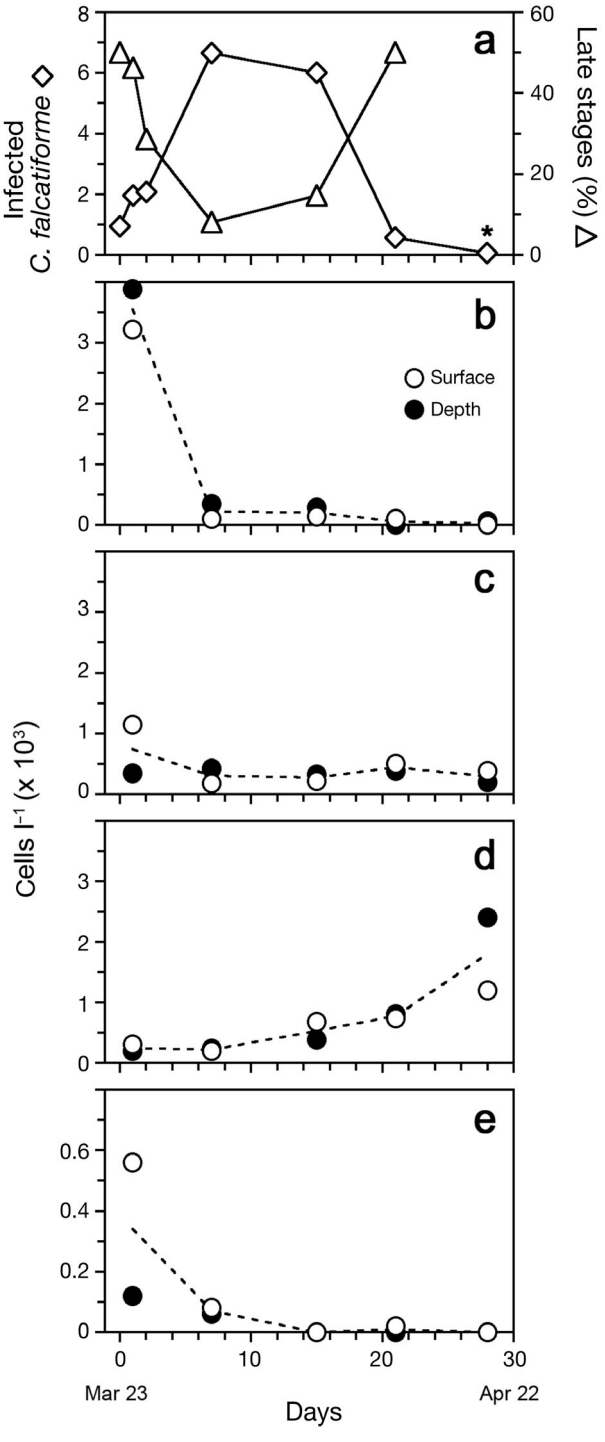

Fig. 4. Development of the plankton community and infection by *Amoebophrya* spp. of *Ceratium falcatiforme* at sampling Stn E over a 4 wk period. (a) Parasitoid prevalence and percentage of late infection stages relative to the total infected host cells (*: estimate of percentage of late stages was not possible because prevalence was below detection limit, i.e. no infected cells out of 2000 cells analysed). (b) *C. falcatiforme* cell density, (c) other dinoflagellates, (d) diatoms and (e) tintinnid ciliates. Dashed line: average of surface and intermediate depth sample values

sentative among the dinoflagellates over the 4 wk sampling period. On the 2 last sampling occasions, *C. falcatiforme* represented <20% of the dinoflagellate cells in the samples. Moreover, increasing amounts of *C. falcatiforme* cells with low pigmentation, empty theca, as well as fragments of *C. falcatiforme* theca were found in the samples towards the end of the sampling period. Diatoms, on the other hand, were found at their highest numbers at the end of the sampling period (2.4×10^3 cells l^{-1}) at the surface, when they represented 90% of the plankton community (Fig. 4d). Tintinnids decreased in numbers to almost undetectable levels from April 4 and onward (Fig. 4e).

Amoebophrya infections

Probe hybridization unveiled at least 9 dinoflagellate species infected by *Amoebophrya* spp. (Table 1). A positive probe signal was seen almost exclusively inside dinoflagellates, including the genera *Ceratium*, *Prorocentrum*, *Protoperidinium*, *Dinophysis*, and several non-identified peridinoid forms. To an overwhelming degree infections were found in the dominant dinoflagellate species, *C. falcatiforme* (Fig. 5). The only non-dinoflagellate cells with positive probe signals were from a tintinnid ciliate (Table 1). In some cases, the round bodies unveiled by the probe signal inside tintinnid cells were larger than the smallest forms found in dinoflagellate hosts (Fig. 6). Fully developed trophonts were never observed inside tintinnids. In dinoflagellates, probe signal showed the presence of a wide range of *Amoebophrya* development stages inside the host cells. The late developmental stage represented by mature trophonts appeared as a large, green mass occupying almost the whole host intracellular space (Fig. 5a–c). At this infection stage, host chloroplasts and the nucleus were no longer present. Instead, the multinucleated, pattern of the parasitoid syncytium was evident by the DAPI signal (Fig. 5c). The earliest infection stages were detected as small (ca. 5 µm) round bodies, both in the cytoplasm and in the nucleus of the host (Fig. 5d). In many cases, the exact infection in the host nucleus could be seen in the DAPI signal as an empty space matching in size and location the round bodies unveiled by the probe signal (Fig. 5f). All infected cells detected based on the DAPI signal had positive probe signals.

Prevalence of *Amoebophrya* spp. on *Ceratium falcatiforme* on March 23 averaged ca. 2%. Maximum estimated prevalence was 2.5% at Stn C. Despite differences in prevalence levels among sites, no correlation was found between prevalence and host densities. Percentage of late infection stages ranged from 20% at sampling Stn D up to 50% at Stn A (Fig. 3a). The time course of infection measured at Stn E showed a 7-fold, nearly linear increase in parasitoid prevalence from 1 to 7% in 1 wk (Fig. 4a). This increase in prevalence occurred simultaneously with the decline in the *C. falcatiforme* population. Parasitoid prevalence remained at ca. 6% for another week, but thereafter decreased to <1%, and then fell under the detection limit. The percentage of late infection stages showed an opposite trend compared to prevalence (Fig. 4a). Percentage of late stages decreased from 50 to <10%, while prevalence increased during the first week of sampling, and increased again to initial levels as prevalence decreased towards the end of the sampling period.

Biovolume and carbon content

Ceratium falcatiforme cells infected with mature *Amoebophrya* trophonts were on average 25% wider (mean girdle diameter = 25.4 µm, CV = 8.1%) than non-infected cells (mean girdle diameter = 20.3 µm, CV = 7.8%). Cell length was not significantly different between the 2 groups (*t*-test: p = 0.8472). Average cell volume for uninfected *C. falcatiforme* was 22 926 µm^3 (CV = 17%), whereas cells infected with mature *Amoebophrya* spp. trophonts had an average biovolume of 36 616 µm^3 (CV = 21%). Carbon content based

Table 1. Infection of *Amoebophrya* spp. on dinoflagellates as detected by the FISH probe signal. Presence of infection (x) is shown for various host species at different locations (see Fig. 1) and on different dates. Observations of nuclear infections (o) are also given. Observations of the probe signal inside tintinnid ciliates are indicated in the last line

Host	Sampling location and date									
	A	B	C	D	E					
	Mar 24	Mar 24	Mar 24	Mar 24	Mar 24	Mar 25	Mar 30	Apr 7	Apr 13	Apr 20
Ceratium falcatiforme	xo	xo	xo	xo	xo	xo	xo	xo	xo	
Ceratium furca	x	x	x	x	x	x	xo	xo		
Ceratium tripos				x	x		x	x		
Ceratium macroceros			x	xo	xo	x	x			
Prorocentrum minimum	x	x	x		x		x	x	x	
Prorocentrum sp.	x	x	x		x		x			
Protoperidinium steinii[a]	x	x	x	x	xo					
Protoperidinium sp.[a]	x	x		x	x		x			x
Dinophysis acuminata										x
Unidentified dinoflagellates[b]	x	x			x			x	x	x
Tintinnid cilliates	x				x		x			

[a]Heterotrophic
[b]Mostly peridinoid forms

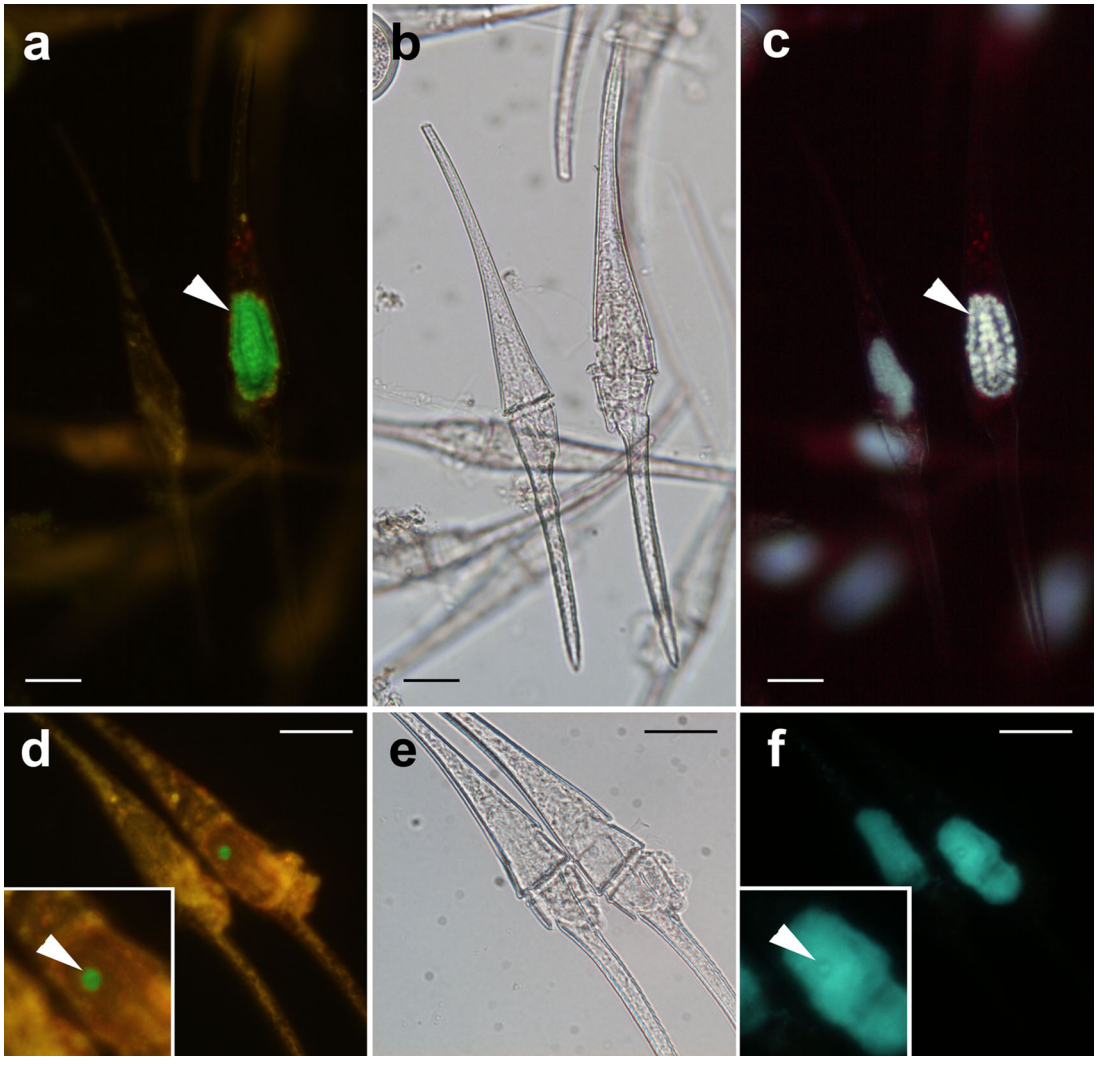

Fig. 5. *Ceratium falcatiforme* infected by *Amoebophrya* spp. (a–c) Late infection stage showing a mature parasitic trophont (arrowheads) occupying a large portion of the host's intracellular space; several non-infected cells are also seen (compare panels a and b). The multinucleated pattern characteristic of *Amoebophrya* spp. is evident in panel c (arrowhead). (d–f) Early infection stage showing a ca. 5 µm parasitoid (arrowheads) attached to the host's nucleus; a non-infected cell is also shown. The parasitoid location appears as a hollow space in the host's nucleus (f, arrowhead). Micrographs in each row represent the same specimens observed with different microscopic techniques. Left panels: epifluorescence microscopy unveiling the probe's signal (fluorescein dye); center panels: bright field microscopy; right panels: epifluorescence microscopy unveiling the DAPI signal. Scale bar = 20 µm

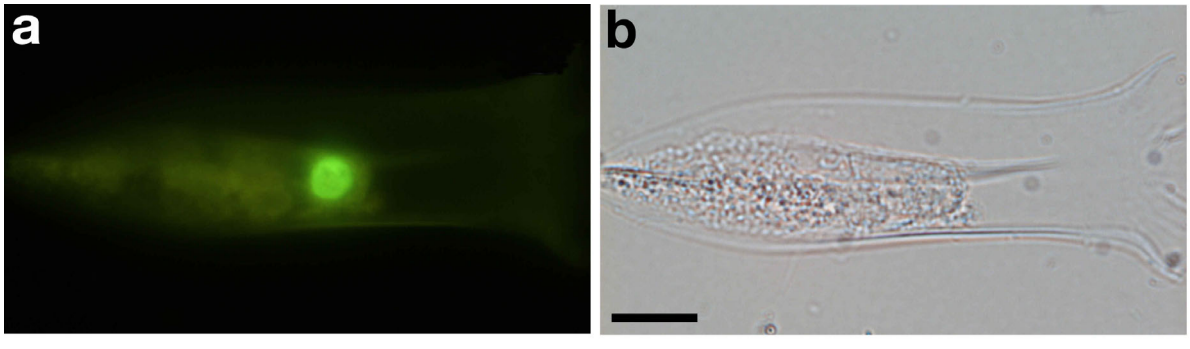

Fig. 6. A positive probe signal in a tintinnid ciliate. (a) Round body unveiled under epifluorescence microcopy with a filter set for fluorescein; (b) the same specimen as seen under bright-field microscopy. Scale bar = 20 µm

on cell biovolume was 46% higher in infected hosts (average 4144 pg C $cell^{-1}$, CV = 17%) compared to uninfected host cells (average 2827 pg C $cell^{-1}$, CV = 14%). Mature trophont biovolume averaged 12 062 μm^3 (CV = 19%). The estimated carbon content was 1670 pg C $trophont^{-1}$, which corresponds on average to 41% of the carbon in an infected cell.

DISCUSSION

In the present study, we successfully used an rRNA-based FISH assay to detect and quantify infection by the parasitoid dinoflagellate *Amoebophrya* spp. on photosynthetic and heterotrophic dinoflagellates in a tropical coastal area of the southern Atlantic Ocean. Our results, together with those of a previous study (Salomon et al. 2006b), indicate that infection of dinoflagellate populations by *Amoebophrya* might be a common phenomenon in this area.

Records on the infection of free-living dinoflagellate species by the parasitoid *Amoebophrya* spp. have accumulated since early reports at the end of the 19th century (see review by Park et al. 2004). The first and hitherto only report on the occurrence of *Amoebophrya* spp. in southern Atlantic coastal waters comes from the same area as the present study where cells of *Protoperidinium* spp. were found infected (Salomon et al. 2006b). The present study, besides expanding the knowledge on the distribution and importance of *Amoebophrya* spp. in tropical waters, constitutes the first account of infection of *Ceratium falcatiforme* by such parasitoids. Several other *Ceratium* species, including *C. fusus*, which is morphologically similar to *C. falcatiforme*, are among the dinoflagellate species reported to be infected by these parasitoids (Elbrächter 1973, Nishitani et al. 1985, Park et al. 2004 and references therein).

DNA sequencing of marine microbial communities from both northern and southern hemispheres, including coastal and oceanic areas, systematically retrieves eukaryote rDNA gene sequences similar to *Amoebophrya* spp. (López-García et al. 2001, Romari & Vaulot 2004). Although *Amoeborhya* has not been reported to infect dinoflagellates in freshwater, similar rDNA sequences were recently found in a lake (Lepere et al. 2008). These findings have led to speculation on whether the actual distribution and abundance of *Amoebophrya* spp. parasitoids could be much larger than what is known based on observations of infected hosts. Nonetheless, while DNA sequencing of whole microbial communities is enlightening, in that it helps to unveil the diversity of marine eukaryotes, the alleged parasitic nature of some of these small eukaryotes from which the DNA sequences originate would be confirmed if they were actually found infecting potential hosts in nature. In this sense, FISH probes applied to whole-cell assays, like the one used in the present work, are one of the most suitable tools (Salomon et al. 2003a, Chambouvet et al. 2008).

Because the probe used in the present study was not designed to be specific for 1 single *Amoebophrya* sp., we cannot yet conclude whether there was only 1 *Amoebophrya* parasitoid with a broad host range or several different parasitoids with narrow host ranges (or a combination of both) infecting the various dinoflagellate species we observed. However, given the genetic diversity recently reported in *Amoebophrya* spp. originating from different dinoflagellate hosts (Gunderson et al. 2002, Salomon et al. 2003a, Chambouvet et al. 2008, Kim et al. 2008), the presence of multiple species/strains is a likely hypothesis. Confirmation on the genetic diversity of *Amoebophrya* spp. infecting different dinoflagellates in the Cabo Frio area must await DNA sequencing or the use of FISH probes designed towards genetically distinct parasitoids. In the present study, the probe's wide target range (within *Amoebophrya* spp.) assured that the maximum number of infected host species were recorded, giving a more complete picture of *Amoebophrya* spp. infection within the dinoflagellate populations present during our sampling. The probe specificity/range was tested in our study as we hybridized it against the natural communities containing a plethora of microorganisms, including several dinoflagellates. The effectiveness of the probe was confirmed by the fact the all parasitoid stages unmistakably identified by the DAPI signal, i.e. medium to mature multinucleated trophonts, as well as other features like hollow spaces in the host nucleus (see Fig. 5) or small round bodies in the host cytoplasm that are indicative of *Amoebophrya* infections (Salomon et al. 2003b, 2006a), were positively stained by the probe. Moreover, the fact that the probe did not stain any other cells in the samples reflects its degree of specificity towards *Amoebophrya* spp.

Amoebophrya spp. infecting primarily athecate dinoflagellate hosts have been suggested to have a narrower host range compared to those that use thecate dinoflagellates as primary hosts (Coats & Park 2002, Kim 2006). However, high specificity by *Amoebophrya* spp. infecting thecate hosts has been observed in the field (Chambouvet et al. 2008). In the present study, thecate forms dominated the dinoflagellate communities. Although up to 9 different thecate dinoflagellate species were found infected in a single sample, parasitoid prevalence indicates a strong preference towards the genus *Ceratium*. *Dinophysis acuminata* was detected during the entire sampling period, in some cases at cell densities similar to those of *C. falcatiforme*, but infection was always below the

detection limit. On the other hand, cells of 3 *Ceratium* species (*C. tripos*, *C. furca*, and *C. macroceros*), which were present in very low numbers, were found infected at all stations sampled on March 24, as well as throughout the first 2 wk of the studies at Stn E (see Table 1). This pattern is in agreement with previous observations by, e.g., Nishitani et al. (1985), who found up to 8 dinoflagellate species infected by *Amoebophrya* spp. in Quartermaster Harbour, Washington, USA, with parasitoid prevalence differing among different host species.

Although *Ceratium falcatiforme* dominated the plankton community at the beginning of the sampling period, maximum cell densities at Stn E were not extremely high (maximum ca. 3.5×10^3 cells l^{-1}). For infection to occur, contact between free-living parasitoid stages (dinospores) and host cells is necessary, and the encounter rate is dependent on both host and free-living parasitoid cell densities. High *Amoebophrya* spp. prevalence in natural dinoflagellate populations is usually observed when host cell densities are on the order of 10^6 l^{-1} (e.g. Nishitani et al. 1985, Coats et al. 1996). It is possible that the *C. falcatiforme* cell densities during our sampling were not high enough to allow prevalence to escalate above the observed 7% of *Amoebophrya* spp. Reproductive output (i.e. number of dinospores produced per infected host cell) and dinospore success in establishing new infections are negatively affected when the hosts are grown in nutrient-limiting conditions, retarding parasitoid epidemics throughout the population (Yih & Coats 2000). Cells in the decaying *C. falcatiforme* population were probably nutrient limited, which might have reduced parasitoid reproductive output. Moreover, grazing of *Amoebophrya* spp. free-living dinospores by ciliates takes place in nature and has been shown to strongly suppress infection (Maranda 2001, Johansson & Coats 2002). The presence of tintinnids with a positive probe signal during our sampling strongly suggests such a trophic link, i.e. the ingestion of dinospores. The fact that a probe signal was only sporadically found in tintinnids is probably due to fast digestion of dinospores after ingestion.

The relatively large trophonts sporadically observed inside tintinnids might represent successful initial infection by *Amoebophrya*, either by the same strain/species infecting dinoflagellates or by another one more specific to tintinnids. Cachon (1964) described one *Amoebophrya* species, *Amoebophrya tintinni*, infecting the tintinnid *Xystonella lohmanni*. We cannot rule out the possibility that the FISH probe used in the present study hybridizes to other parasitoids closely related to the *Amoebophrya* spp. infecting dinoflagellates. Gene sequences from such parasitoids are needed to clarify their identity.

Similar to our observations of *Ceratium falcatiforme*, enlargement of dinoflagellate host cells upon infection by *Amoebophrya* spp. has also previously been reported (Taylor 1968, Kim et al. 2004, Chambouvet et al. 2008). This has been interpreted to reflect the maintenance of photosynthetic activity by the host during *Amoebophrya* spp. maturation and the simultaneous parasitoid assimilation of host photosynthetic products for growth (Park et al. 2004).

An important aspect of the infection of natural dinoflagellates by *Amoebophrya* spp. is the extent to which parasitism contributes as a loss factor for host populations. Significant *Amoebophrya*-induced mortality has been reported in, e.g., *Gyrodinium uncatenum* and *Gonyaulax catenella* populations when prevalence levels were as high as 81 and 30%, respectively (Nishitani et al. 1985, Coats et al. 1996). Our data showing the concurrent decline in *Ceratium falcatiforme* cell numbers and increase in parasitoid prevalence suggest some influence of parasitism. Moreover, the temporal pattern in the percentage of late infection stages indicates a more-or-less synchronized wave of infection throughout the host population. If the generation time of the parasitoid inside the host is known, the fraction of cells in a given host population killed per day can be estimated by dividing prevalence by the generation time of the parasitoids (Coats & Bockstahler 1994). The generation time for the *Amoebophrya–C. falcariforme* system has not been estimated, but existing data from other cultured *Amoebophrya*–dinoflagellate systems show development times ranging from 1.4 to 3 d at 20°C (Coats & Park 2002). High temperature, as observed during our sampling occasions, is expected to speed up parasitoid growth, thus shortening development time. Assuming that there was a linear increase in parasitoid prevalence from 0.9 to 6.7% during the first week of our sampling period and an intracellular generation time of 2 d for the parasitoid (which is reasonable considering the *in situ* water temperature), *Amoebophrya* would have killed ca. 11% of the host cell population within this 6 d period. The actual decrease in *C. falcatiforme* cell densities for that period was ca. 94% (from 3550 to 220 cells l^{-1}). Thus, parasitism was not the main factor behind the *C. falcatiforme* population decline during our survey, though it likely accelerated the process. From the above estimates, it becomes evident that loss factors other than parasitism by *Amoebophrya* spp. contributed to the decline in *C. falcatiforme* between March 24 and March 30. Sampling Stn E is situated in a protected area inside Anjos Bay. Furthermore, water temperature suggests we were sampling in the same water mass over the entire period. Therefore, losses due to advection were probably not very high. Copepods were in very low abundance in the net

tows from the location where the dinoflagellates were collected, indicating that grazing was not a relevant loss factor either, even if the copepods in the area would have been able to graze on such large *C. falcatiforme* cells. The increase in numbers of low-pigmented cells and intact, empty *C. falcatiforme* theca during the sampling period suggests that the cells were metabolically damaged and were dying. Exhaustion of nutrients, which are usually scarce in the area during downwelling conditions (Valentin 1984), was probably the main cause for the *C. falcatiforme* population decline.

The 94% decrease in *Ceratium falcatiforme* cell numbers between March 24 and March 31, including the 11% killed by *Amoebophrya* spp., corresponds to ca. 9.91 µg C l^{-1}. According to our estimates, a mature trophont, which differentiates into dinospores shortly after emergence from its host, accounts for 41% of the carbon in an infected *C. falcatiforme* cell. Such a value is in line with results from Yih & Coats (2000), who estimated, based on number and size of dinospores produced per host, that *Amoebophrya* sp. infecting *Gymnodinium sanguineum* (=*Akashiwo sanguinea*) converts between 31 and 48% of its host's biomass into parasitoid biomass, depending on the host's nutritional status. Assuming all infections observed in the *C. falcatiforme* population during our survey reached maturation and produced dinospores, host biomass transformed into *Amoebophrya* dinospore biomass during the above-mentioned decline amounts to ca. 0.64 µg C l^{-1}, or 6.5% of the total value. In other words, 6.5% of the carbon previously in the form of *C. falcatiforme* cells, which would otherwise be lost as dissolved organic material or cell debris upon cell death, was instead transformed into parasitoid dinospores. These dinospores were then available to microzooplankton, potentially creating a new trophic link. The magnitude of such a link would depend on the microzooplankton grazing potential, and its duration is probably short, since *Amoebophrya* spp. dinospores only survive for hours to days when hosts are absent (Coats & Park 2002).

Oceanographic conditions during our survey are typical of a downwelling state. During such periods, oligotrophic waters dominate in the Cabo Frio area and the plankton community experiences extensive nutrient limitation (Valentin 1984). The system is then characterized by a microbial food web structure, whereby carbon produced by bacteria and small phytoplankton cells is channeled via flagellates and ciliates up to the mesozooplankton and higher trophic levels (Guenther et al. 2008). Occasional development of large dinoflagellate cells — like *Ceratium falcatiforme* — during these periods might restrict carbon flow up the food web. The temporary trophic link created by the presence of *Amoebophrya* spp. dinospores would then be important in such cases, since it retains part of the carbon that would otherwise be lost to deep layers upon host population decline. Infection of large host cells is expected to make such a process relevant even at relatively low parasitoid prevalence levels, because parasitoid biomass produced per host is higher relative to the biomass of small hosts. The importance of parasitism on dinoflagellate populations in oligotrophic coastal areas might generally be underestimated.

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