

NOTE

Blooms of a benthic ciliate, *Maristentor dinoferus* (Heterotrichida: Maristentoridae), on coral reefs of Guam, Mariana Islands

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Abstract—*Maristentor dinoferus* is visible in situ with the naked eye; it forms aggregations at several scales: individuals form dynamic clusters within colonies that are normally widely scattered, but in recurrent blooms during the dry seasons of 2005 through 2007, colonies often formed close together in patches (metapopulations). Patches were extremely abundant in Guam, both in Apra Harbor and on the outer coast. This is the first time that long-term changes in a benthic ciliate population have been documented in situ and that multi-scale patchiness has been reported for a benthic ciliate. Colonies were mapped and monitored; some patches over 1 m diameter were recorded. Since 2008, the distribution has again consisted of scattered colonies, as it did from 1999–2004.

Introduction

Patchiness of populations in space and time at multiple scales is well known and in the last 20 years has become important in the fields of spatial ecology (Kareiva 1994; Wu 1995) and metapopulation biology (Hanski & Gaggiotti 2004; Leibold et al. 2004; Holyoak et al. 2005). On coral reefs, patchiness is important in the study of factors that impact the health of reefs, such as outbreaks of crown-of-thorns (Birkeland 1982), macroalgae (Mumby et al. 2005; Ruiz & Ballentine 2009) and the colonial pelagophyte alga *Chrysocystis fragilis* (Schaffelke et al. 2004). It is important to reef management to distinguish natural population blooms from increases in an invasive species. In some of these reef population changes, and in phytoplankton patchiness (Turpin & Harrison 1979; Lehman & Scavia 1982), nutrient patchiness is a contributing factor, directly or indirectly. In the case of benthic ciliates, which are mostly heterotrophic, patchiness may likewise relate to nutrient (food) availability. However, the unicellular organisms on coral reefs are very poorly known, even at the biodiversity level, and few benthic ciliates have been studied in the field (Montagnes et al. 2002).

The heterotrich *Maristentor dinoferus* Lobban, Schefter, Simpson, Pochon, Pawlowski & Foissner, 2002, is an exceptional ciliate in being observable in situ. It is large (to 800 μm long), appears black, and its habit of clustering on the whitish brown seaweed *Padina* makes it easy to find and photograph even from a distance of a meter or more. The black appearance results from the presence of large amounts of maristentorin, a hypericin-like pigment that is probably toxic and used as a feeding deterrent (Mukherjee et al. 2006, Lobban et al. 2007). It is also unusual among marine ciliates in having symbiosis with *Symbiodinium* zooxanthellae, and presumably is largely autotrophic (Lobban et al. 2002). *M. dinoferus* exhibited spatial pattern formation at several scales, and for three years it underwent an apparently widespread bloom on Guam. The objective of this paper is to present our observations of *Maristentor* patches during that time. This is the first time that long-term changes in a benthic ciliate population have been documented in situ and that multi-scale patchiness has been reported for a benthic ciliate.

Materials and Methods

STUDY AREA

The main study site was at Outhouse Beach, on the north side of Apra Harbor, Guam, 13° 27' N, 144° 40' E. There is very little live coral. During the algal growth season in Guam, roughly January through May, the site has extensive *Padina* meadows but these tend to become loosely attached as the season progresses and be washed away by storms by July, leaving small remnant populations. *Maristentor* occurs from 2–5 m below low water on a variety of surfaces, but especially on *Padina*.

Additional populations were observed in 2006 at two deeper, outer coast sites: Cemetery Wall in War in the Pacific National Historical Park, Agat Bay (13° 23' N, 144° 39' E) at 12–15 m and at Double Reef in Haputo Ecological Reserve (13° 36' N, 144° 50' E) at 20 m. The Agat Bay site was in an area where the National Park Service was conducting a study of sedimentation and coral recruitment at the time and noticed the *Maristentor* bloom because it stained the divers' fingers with the pigment (A. Palmer, pers. comm.). Most southern and central reefs on Guam are heavily impacted by anthropogenic factors, but the northern reefs, including Double Reef, are healthier (Burdick et al. 2009).

METHODS

Populations of *Maristentor* were studied in the field by scuba diving. We have observed the Apra Harbor populations periodically every year since 1999. Following a bloom in 2005, from which we were able to bulk harvest *Maristentor* for pigment extraction (Mukherjee et al. 2006), we made systematic observations at Outhouse Beach during the 2006 and 2007 growing seasons. Colonies of *Maristentor* were marked with numbered limestone ("marble") or ceramic tiles (see Fig. 3, below). Photographs of colonies were taken approximately weekly from mid March 2006 until most of the population (and *Padina* substratum) washed away in June to July 2006, periodically from August through October 2006, and again ca. weekly

from January through July 2007. Surveys were also conducted in February and April 2008. Marker tiles were still recognizable the second year, so that locations of populations could be compared. At another site, Cemetery Wall, colonies were tagged and observed on four dives from 15 May to 29 October 2006.

Underwater photographs were taken with a Sea&Sea 8000G (Ricoh) 7 MPx camera in a housing using natural light. The minimum distance from subject to the front of the housing was 1 cm, allowing individual cells to be distinguished; we also took overlapping frames from a distance of 1–2 m to show large patches. Photographs were processed and analyzed by hand in Adobe Photoshop®.

Terminology: The term *population* is used in different ways in different fields of biology and we wish to use it here without any implications of scale. We have defined three specific scales of patchiness in the results below — clusters, colonies, and patches — and we use population in a general sense.

Results

Maristentor occurred on a variety of substrata (Figs 1–6), including seaweeds of several different morphologies (large flat blades of *Padina*, small flat segments of *Halimeda*, narrow branched straps of *Dictyota*), sponges, rock, coralline algae, cyanobacterial mats, and sediment. It was most commonly observed on *Padina* (Figs 3, 8), but at the peak of the bloom was observed in abundance on sand (Fig. 1) and on cyanobacterial mats covering old *Padina* and other substrata (Figs 4–6).

Individuals typically formed **clusters** (Figs 2, 3, 5). Clusters most often had 25–100 cells but clusters with 150 or more individuals were common; several counts are indicated on Fig. 3 and the total number of cells in the photograph is estimated at 1,100. There was no upper size limit and the densest *Maristentor* formed continuous lawns on narrow surfaces, including the edges of *Padina* (Fig. 6). The lower end of the size range was a single standing individual. In the clusters, the bases of the cells were relatively close together, and the larger caps forced the cells to spread into a hemispherical cushion (Fig. 3, 5). Clusters were numerous on a *Padina* blade and a **colony** covered several to many blades (Figs 7–13). We define a colony as the individuals within a discrete area, surrounded by non-colonized substrata (see Hanski & Gaggiotti 2004), but we acknowledge that as colonies grew they sometimes became contiguous. Within a colony, the clusters sometimes occupied several substrata. Because of the topography of the *Padina* blades and the fact that on vertical blades *Maristentor* can colonize both sides, not all clusters were visible at once, so it was not possible to estimate the numbers of cells in a colony.

Apra Harbor

Spatial distribution of populations

Commonly during the bloom years, several colonies occurred nearby in **patches** at scales of both 1 m and 10 m (Fig. 14). For instance, the Coral Fingers main patch was about 0.9 m square at the end of April 2006 when the detail map was made, but there were several other colonies over a distance of 10 m E–W and

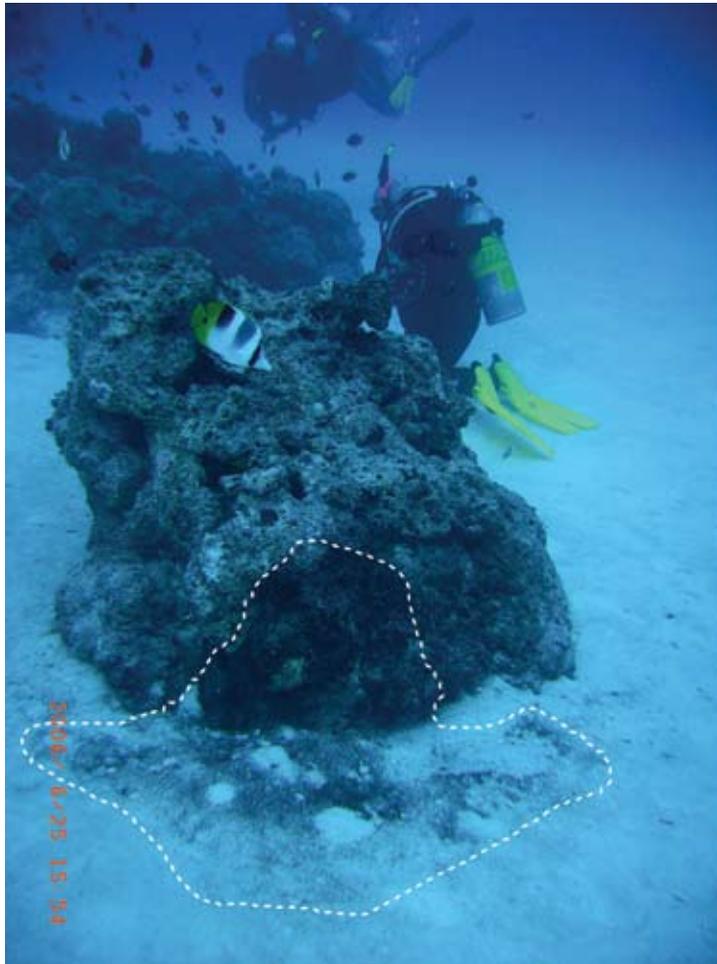


Fig. 1. Large, deep (20 m) colony on reef and sand at Double Reef during the 2006 bloom (no *Padina* present)—all the blackening within the dashed line is *Maristentor*.

9 m N–S. Along the 100 m length of Outhouse Beach we mapped over 40 patches in May 2006 (Fig. 14 shows only the western half since few of those patches were in the eastern half). Patches did not occur at the same places in 2007 or 2008.

Temporal changes in patches

Individuals and clusters are not static but migrate onto new substrate (Figs 7–10) by gliding (observed) and presumably also by swimming. We observed the following overall changes in the population and conditions at the Apra Harbor study site in 2006 and 2007. During the first several months of the year, when the *Padina* population was in good condition and growing, *Maristentor* was most

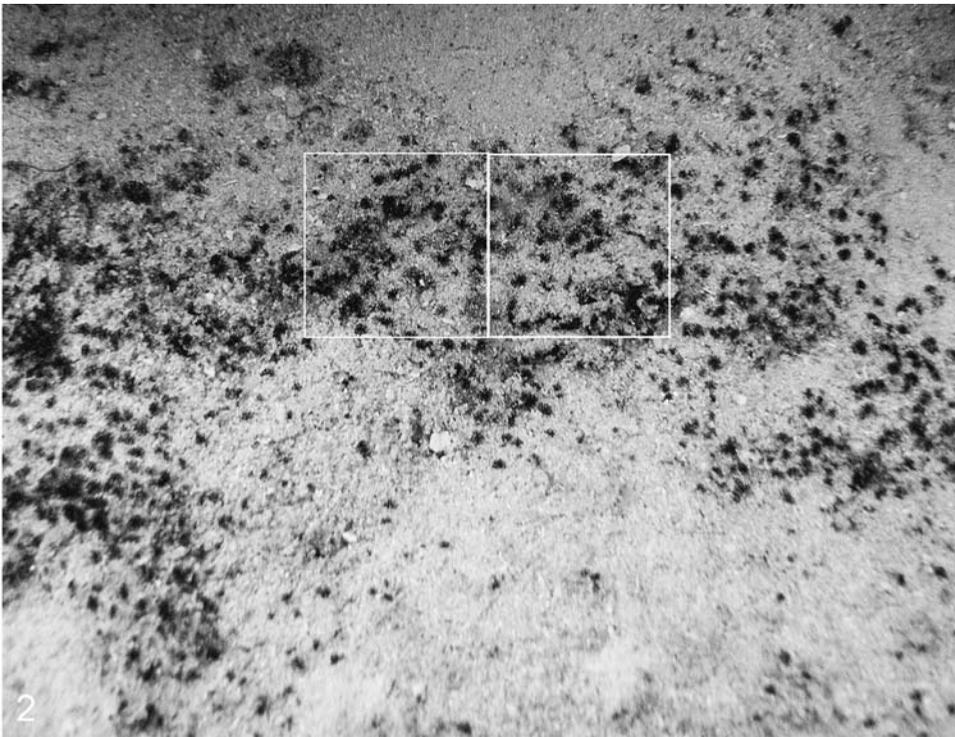
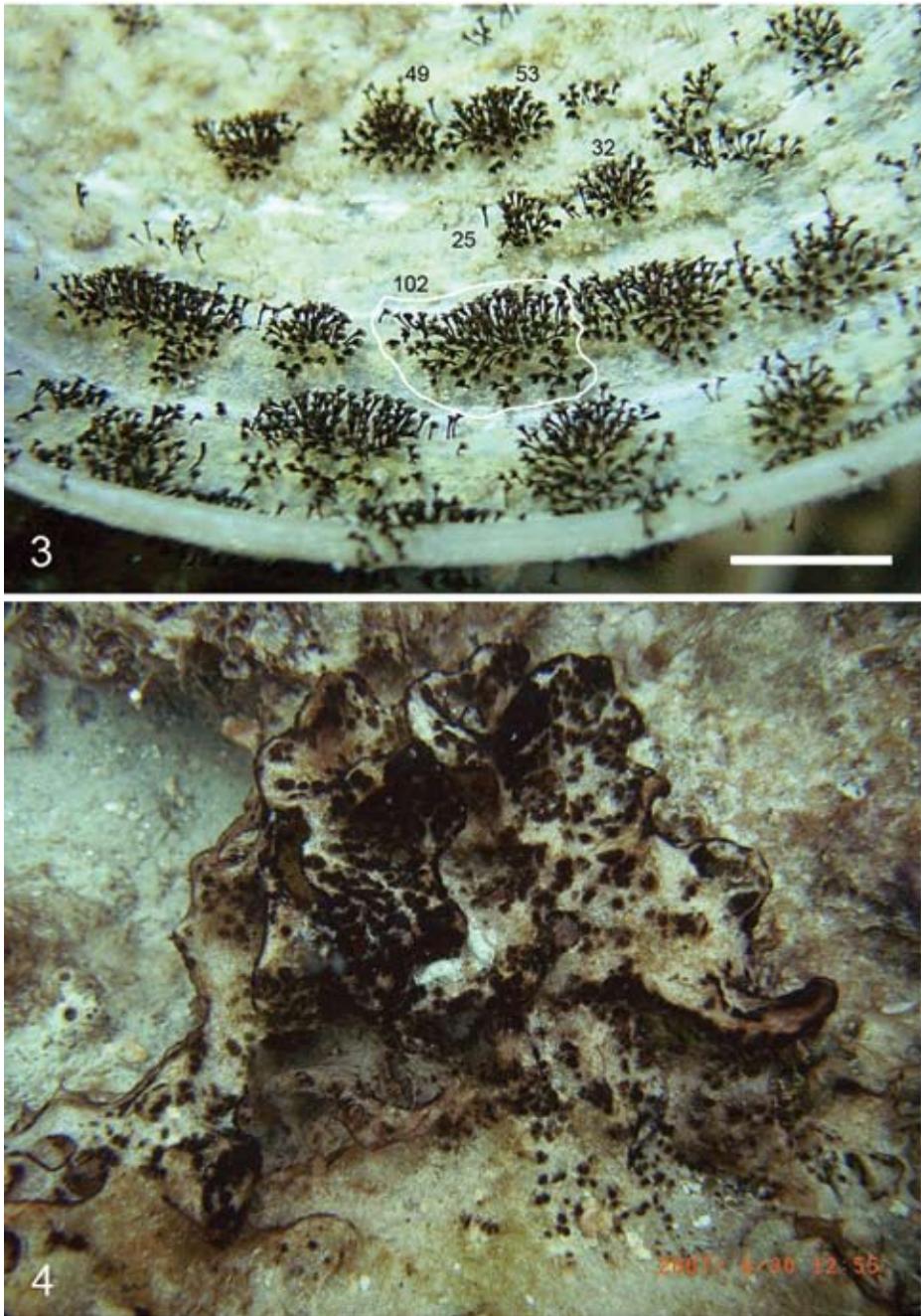


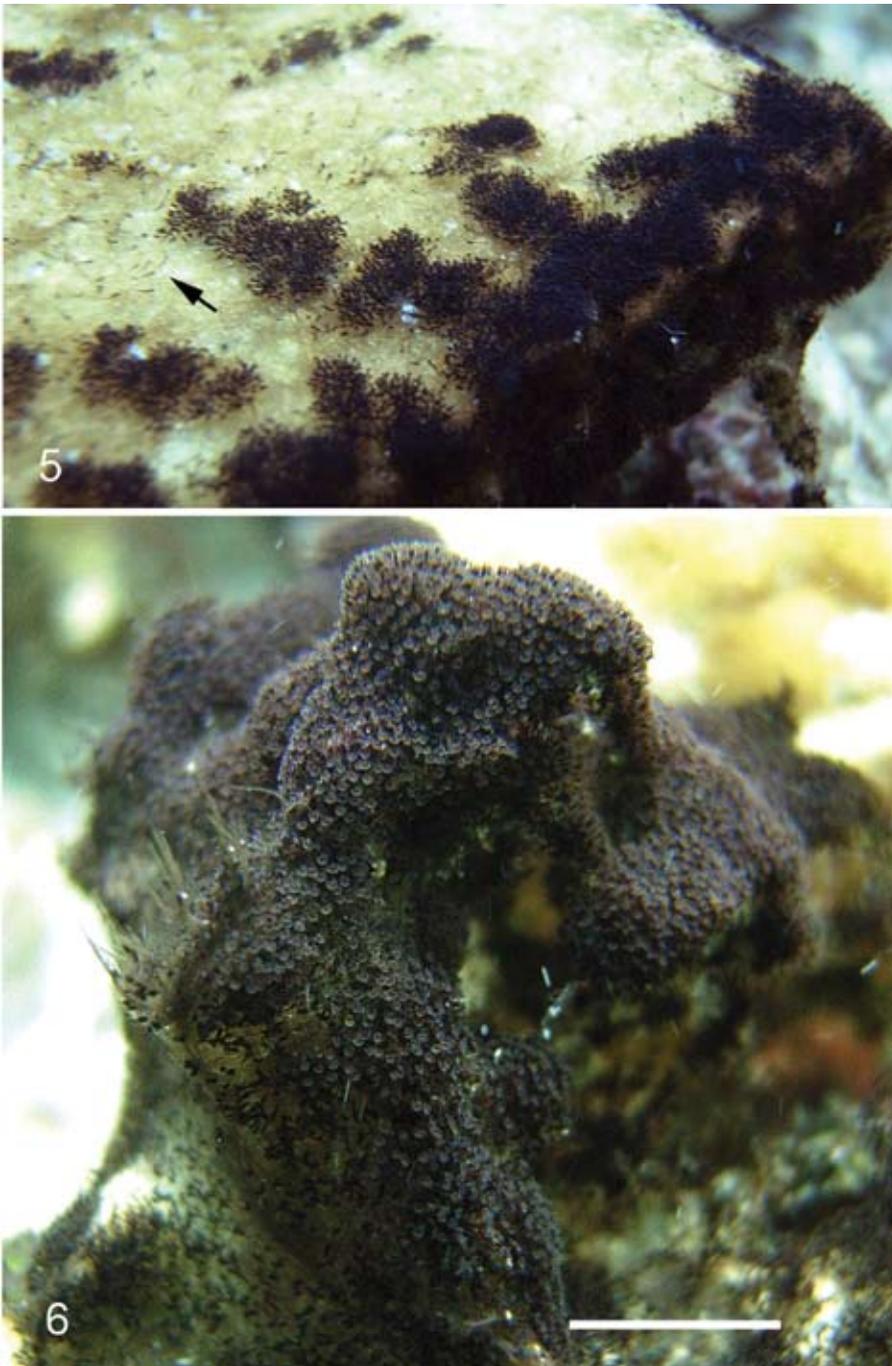
Fig. 2. Closer photo of part of the Double Reef colony on sand, the two squares indicating the ca. 50 x 50 mm regions in which clusters were counted.

abundant on these surfaces but also occurred on other substrata nearby. By late May–June a thick cyanobacterial mat had overgrown much of the *Padina* and other surfaces (that also seems to have been a bloom event) and *Maristentor* populations quickly became very abundant on these mats particularly at the east end of Outhouse Beach (Figs 4–6). In July 2006 and 2007 SE winds destroyed most of the *Padina* and the cyanobacterial mat and coated much of the substratum with sediment for 1–2 weeks. However, loss of *Maristentor* was only partly related to substrate removal, since we observed some examples of loss and/or dispersal of *Maristentor* without loss of *Padina* in 2006 (see below) and noticed in 2007 that much *Maristentor* persisted in well-anchored *Dictyota* and *Halimeda* clumps. Because of the structure and darker color of the latter two seaweeds, small numbers of *Maristentor* would be almost impossible to find, and we suspect that substantial scattered remnants were present between the three blooms.

Growth and decline of populations can be indicated by the following example (Figs 7–13). Patch CF-M (see Fig. 14) was observed for 16 weeks and photodocumented for the last 9 weeks in May–June. Growth of population CF-M1 is indicated in Figs 7–10 as the clusters spread and perhaps dispersed; the *Padina* was also growing and a marker tile also proved to be an acceptable new substrate. In early June 2006 we noted many additional “satellite” populations springing up



Figs 3–4. *Maristentor* clusters. Fig. 3. Close-up of typical clusters and standing individuals on *Padina*, with counts of individuals for several clusters. Fig. 4. Late-season scene at Outhouse Beach, 30 June 2007, showing thick cyanobacterial mat over old *Padina* with dense and abundant *Maristentor* clusters. Scale bar on Fig. 3 = ca. 5 mm.



Figs 5–6. *Maristentor* clusters. Fig. 5. Large and merging clusters plus some gliding individuals (arrow) on cyanobacterial mat. Fig. 6. Dense lawn of *Maristentor* on cyanobacteria-covered *Padina*, Outhouse Beach 19 June 2007. Scale bar on Fig. 6 = ca. 5 mm.

near this patch and Patch 2. Initially we tagged and mapped these but the number of them along the study site became overwhelming. Simultaneously, population CF-M3A declined over a period of two weeks, so that by mid-June the remaining *Padina* was bare (Figs 11–13). Populations elsewhere in the patch remained a few weeks longer but all were gone by mid-July.

Cemetery Wall

The reef slope here had extensive *Padina* and the pattern of *Maristentor* patches seemed to be similar to that observed at Outhouse Beach. However, we were only able to observe here on three occasions in May–June 2006, near the peak of the bloom. On 28 October 2006 we observed no *Maristentor*.

Double Reef

The substrate was largely *Halimeda*, rock, and sand, and the presence of a population of *Maristentor* extending over all these (Figs 1, 2), observed on 25 June 2006, was surprising. In this case it is not clear whether the population fits within the definition of a colony or a patch. Evidently at this depth and time there was little disturbance of the sand except by burrowing animals. From close photos we counted the number of clusters in the colonized areas (squares on Fig. 2) and estimated the population density at 1×10^6 cells m^{-2} . On a second dive 9 September 2006 no *Maristentor* was found.

Discussion

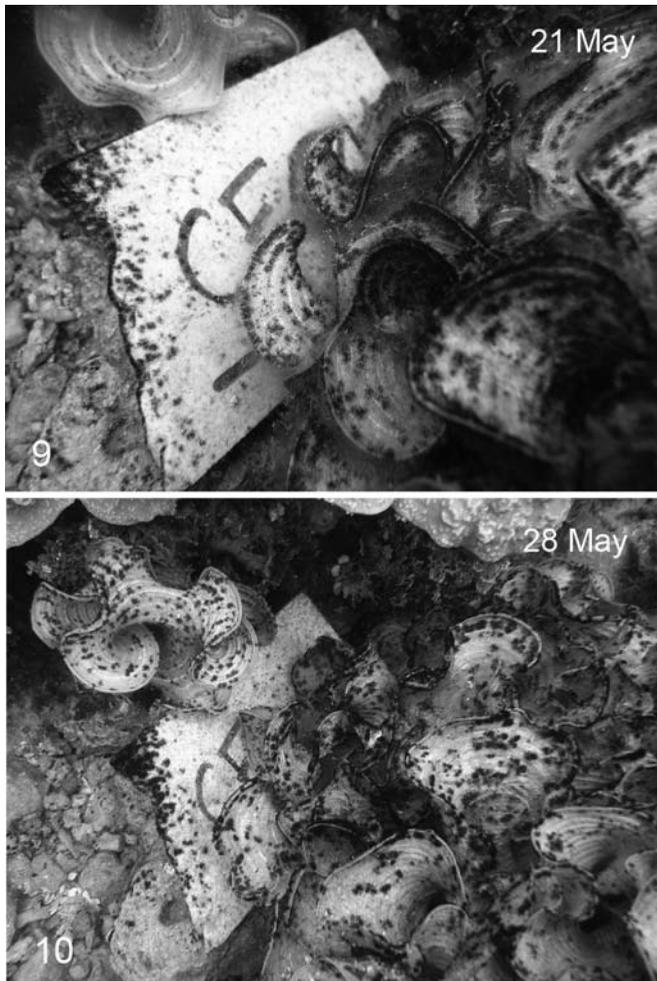
Maristentor dinoferus was known from the original description (Lobban et al. 2002) to form populations that were patchily distributed on the reef. From subsequent observations we have a better sense of the existence of populations that persist over their growing season, and the impression that during the bloom years colonies multiply to form patches that may be metapopulations. Metapopulations were defined by Hanski & Gaggiotti (2004) as assemblages of discrete local populations (in our case, colonies) with migration and gene flow among them. However, the distance between colonies was very variable and we have no evidence to show that migration does occur between them. Migration would be possible because *Maristentor* cells regularly move by gliding in and out of clusters (observed in situ) and are capable swimmers (observed in vitro). Colonies are potentially clonal, and even an entire patch could be a single clone; more likely there are many genetic individuals at all scales.

While we have observed population growth on a gross scale, we have not yet been able to measure growth rate. The three-dimensional topography of the *Padina* blades precludes quantitative photography except at the scale of clusters. Moreover, it is clear that we are seeing only a portion the total distribution of *Maristentor* at the site, since we can rarely see colonies on dark backgrounds, while individual cells and clusters anywhere would go unnoticed. Nevertheless, we had a rare glimpse of extensive blooms that recurred over three years before returning to the normal pattern of isolated colonies.



Figs 7–10, above and facing page. *Maristentor* colony growth over weekly intervals near the peak of the 2006 bloom. Colonies were part of CF-M = “Coral fingers main” patch (see Fig. 14). Figs 7–10. Growth of colony CF-M1 showing migration of *Maristentor* onto and across the marker tile May 14 and 21, and onto the newly growing *Padina* on May 21 and 28. Notice growth of *Padina* both from upper left and lower right over the marker. Scale bar on tile (almost entirely visible in a) = 50 mm.

It is clear that the series of blooms of *Maristentor* was a temporary event and there is no reason to suppose that they were due to local anthropogenic effects, nor that *Maristentor* is invasive. However, the reasons for the bloom can barely be guessed and the return of these blooms cannot yet be predicted. There was a three-year (2006–2008) stand of high seawater in the region as a result of El Niño conditions (Chowdhury et al. 2010). Burdick et al. (2008) reported coral bleaching events in Guam in 2006 and 2007 after a decade without them, but Paulay &



Benayahu (1999) had found no correlation between prior coral bleaching events and water temperature, so that it is not clear how environmental conditions may have been different during the bloom years. *Maristentor* has the potential to be a model organism for metacommunity studies but knowledge of when to expect these blooms is prerequisite.

In future studies of *Maristentor* colonies, a measure of the increase in numbers of individuals might be obtained by combining non-destructive photographic transects with sampling for pigment spectrometry, since one can produce a standard curve for maristentorin versus cell numbers. One might also search for cryptic clusters this way. Our time spent closely observing *Maristentor* in situ during the bloom years led to some interesting results on cell behavior at a small scale that will be described elsewhere.



Figs 11–13. Decline of colony CFM-3A (part of CF-M patch, as Figs. 7–10) in June from the peak at May 28. *Maristentor* disappeared but there was still *Padina* present on June 16. (Marker tile was replaced June 2.) Scale bar on tile = 50 mm.

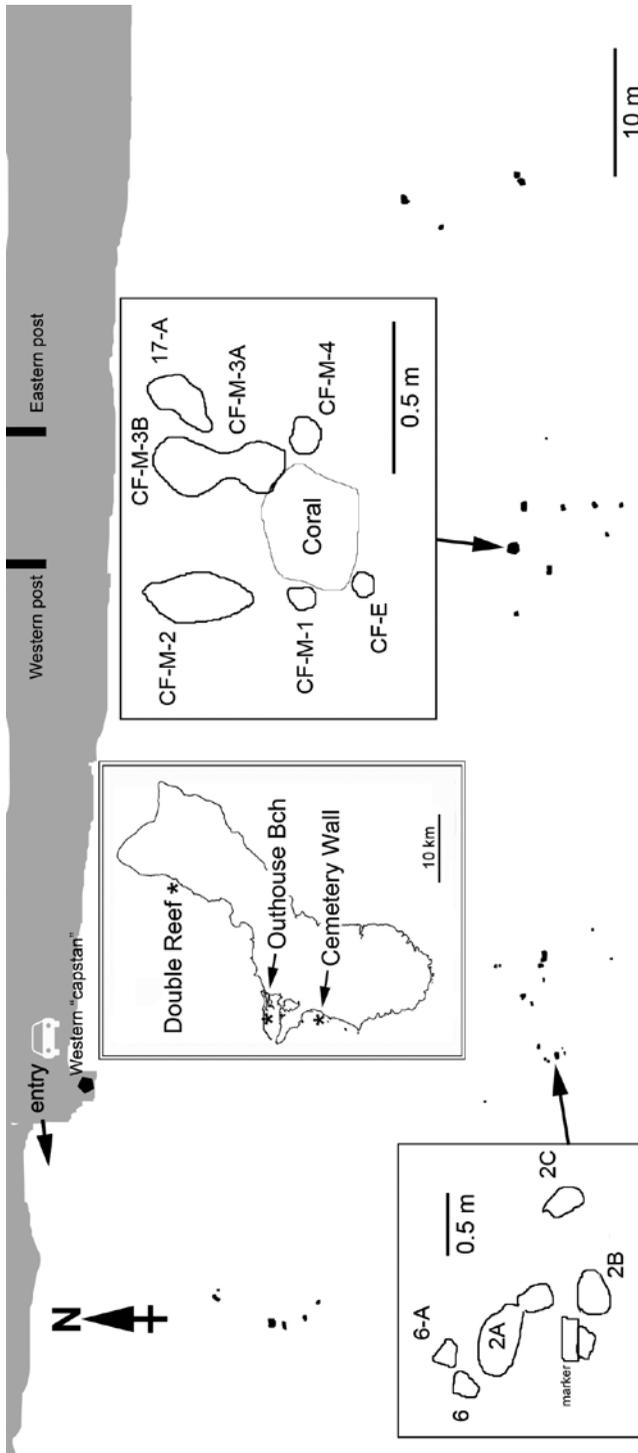


Fig. 14. Maps of colonies and patches along western half of Outhouse Beach. Large map (scale bar = 10 m) simplified from a scale map that included the underwater landmarks and designations of all colonies. Shore (gray) and several terrestrial landmarks indicated. Colonies indicated by black shapes. Insets with single borders are schematic diagrams, approximately to the scales indicated, showing the colonies within Patch 2 and “Coral Fingers” Main Patch. Inset with double border is a map of Guam indicating the locations of the three study sites.

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