Porpostoma guamensis n. sp., a Philasterine Scuticociliate Associated With Brown-Band Disease of Corals

CHRISTOPHER S. LOBBAN,* LAURIE M. RAYMUNDO§ and DAVID J. S. MONTAGNES†

Division of Natural Sciences, College of Natural & Applied Sciences, University of Guam, Mangilao, GU 96923, USA, and
Marine Laboratory, University of Guam, Mangilao, GU 96923, USA, and Institute of Integrative Biology, University of Liverpool, Crown Street, Liverpool L69 7ZB, United Kingdom

ABSTRACT. Brown band disease of coral is caused by a ciliate that consumes the tissue of the corals in the genus Acropora. We describe the ciliate associated with this disease on Guam, based on general morphology, division stages, and ciliature observed on live and protargol-stained specimens; modification of the oral structures between divisional stages, observed on protargol-stained specimens; and some aspects of behavior in field and laboratory studies. Porpostoma guamensis n. sp. is elongate and has ciliature typical for the genus; live cells are 70–500 × 20–75 μm; the macronucleus is sausage-like, elongate but often bent, positioned centrally along the main cell axis; the oral ciliature follows a basic pattern, being composed of three adoral polynucleid regions, as described for other species in the genus, although there is variability in the organization, especially in large cells where the three regions are not easily distinguished. Ciliates fed on coral with their oral region adjacent to the tissue, which they engulfed, leaving the coral a bare skeleton. Both zooxanthellae and nematocysts from coral occurred in the ciliates. Zooxanthellae appeared to be ingested alive but deteriorated within 2–3 days. Ciliates formed thin-walled division cysts on the coral and divided up to 3 times. Cysts formed around daughter cells within cysts. We provide some observations on the complex division pattern of the ciliate (i.e. tomont–trophont–cyst) and propose a possible complete pattern that requires further validation.

Key Words. Acropora, ciliate, coral reefs, division pattern, zooxanthellae.

DISEASE is a major driver of coral reef community structure, at times causing significant mortality (Aronson and Precht 2001; Harvell et al. 1999). Ciliates are not usually implicated as causative organisms in coral diseases, although the folliculinid heterotrich, Halofolliculina corallasia Antonius & Lipscomb, 2001 has been linked to “skeletal eroding band” in the Red Sea and Indo-Pacific (Antonius and Lipscomb 2001; Winkler, Antonius, and Renegar 2004), and another folliculinid has been associated with coral disease in the Caribbean (Croquer et al. 2006; Croquer, Bastidas, and Lipscomb 2006). Brown-band disease of corals, caused by a ciliate, was first noted to affect the coral genus Acropora on the Great Barrier Reef (Bourne et al. 2008; Dinsdale 1994). Since then, similar diseases by mobile ciliates causing brown bands around Acropora corals have occurred on reefs in the Philippines (Raymundo et al. 2009), Guam (Myers and Raymundo 2009), Palau (Page et al. 2009), Zanzibar (Tanzania), East Africa (Wel and Jordán-Dahlgren 2005), and Australia (Bourne et al. 2008).

Brown-band ciliates feed on coral tissue and can result in rapid tissue loss, potentially causing mortality of newly settled coral recruits (Cooper et al. 2007) and corals that have been depredated by Crown-of-thorns starfish (Nugues and Bak 2009). Although this disease is well documented, there has been a lack of rigorous taxonomic analysis of the taxa involved. Here, based on morphology, division stages and pattern modification of the oral structures between divisional stages, and some aspects of behavior, we provide a description of a new species associated with brown-band disease. We also provide observations of its aetiology and distribution in the waters around Guam, where it can infest Acropora, one of the dominant reef-building genera.

MATERIALS AND METHODS

Field observations. To estimate the extent of the brown-band ciliates on Guam reefs, surveys were conducted on 13 near-shore reefs (Fig. 1). For each reef, three 20 × 2 m belt transects were examined along the reef flat or shallow slope, between 1 and 9 m depth. Tumon Bay is subjected to natural nutrient enrichment from groundwater seepage and anthropogenic enrichment from coastal development (Denton et al. 2005). The nutrient status of LUMINAO is under investigation, but the abundance of cyanobacteria (Raymundo, pers. observ.) suggests that this is also a high-nutrient site. All other sites were considered relatively low nutrient. Coral colonies were counted, identified to genus, and examined for presence of the brown-band ciliate. Disease prevalence for each coral genus was determined as: number of colonies with brown band/total number of colonies × 100; data are presented as percentage ± one standard deviation.

Laboratory observations. The initial observations on ciliate behavior were made opportunistically, in the course of an unrelated laboratory experiment. On August 10, 2005, an experiment was set up in laboratory aquaria involving pair-wise allorecognition interactions between Acropora surculosa fragments (N = 30 fragments from four source colonies). All fragments were clinically healthy with intact tissue at the set up of this experiment. However, on August 13, patches of tissue loss were noticed at the base of several fragments. Hourly observations showed that tissue loss was progressing rapidly and examination under a dissecting microscope revealed high densities of swarming ciliates, which appeared to be consuming coral tissue. Fragments were observed periodically for rates of tissue loss over a 2-day period, after which the majority of the fragments were completely devoid of tissue. This study constituted the first recorded observation of brown-band disease impacting Guam reef corals. To study ciliate feeding behavior under more controlled conditions, samples of diseased corals (Fig. 2, 3), identified using a magnifying lens, were collected from LUMINAO Reef and TUMON BAY, in October 2005, December 2006, December 2007, and April 2008. Diseased coral branches were transported in fresh seawater and then maintained in an aerated open-system aquarium at 28 °C. These samples were monitored for progress of the infestation.

Ciliates could not be removed from coral branches; they were apparently stuck in thin-walled cysts (see “Description of Porpostoma guamensis n. sp.”) and wedged into the coral skeleton. Therefore, coral fragments were placed in beakers of seawater, and after ~ 2h, ciliates left the cysts and moved to the bottom or the surface of the beaker. Photomicroscopy of live ciliates was done with Olympus CZ51 trinocular dissection microscope (Olympus America Inc.) and a Nikon E600 DIC compound
microscope (Nikon Instruments Inc.); digital still and video images were recorded with Olympus 7070 consumer cameras fitted to the trinocular heads. Scales were applied using photographs of a stage micrometer taken at the same resolution, and all measurements are from photographs. Samples were fixed in Bouin’s solution (Foissner 1991) and protargol stained by the QPS method (Montagnes and Lynn 1987). Observations of stained specimens were made using a Zeiss Axiovert microscope (100X oil immersion and 40X objectives, Carl Zeiss MicroImaging, UK) equipped with a video camera (model KY-F55B, 3-CCD, 750 horizontal/C24 480 vertical lines) interfaced with an image analysis-image capturing program (Scion Image for Windows, Scion Corp., Frederick, MD) and a high-resolution frame grabber (CG-7, Scion Corp.). Illustrations were made using a Zeiss standard microscope, equipped with a drawing tube (100X oil immersion and 40X objectives). Illustrations are based on representative individuals chosen from observations of >100 cells (20–50 cells for each cell type).

To determine size classes and then to assess the division pattern, length and width data were collected and plotted. For this analysis data were obtained from a range of observations after cells had been placed in culture vessels at 28 °C. Length and width were determined from photographs of live cells that were either swimming or in cysts.

RESULTS

Description of Porpostoma guamensis n. sp. (Fig. 4–39, 41–47). Cells were free-swimming or active in thin-walled cysts (i.e. thin membranous enclosures of unknown composition) attached to substrate; cysts may form adherent masses of cells. Cell size was variable, in vivo 70–500 × 20–75 μm, depending on division stage (Fig. 4–12, 40). Protargol-stained cell size ranged widely, 75–350 × 25–75 μm (Fig. 16–20, 22–31). Cells of all sizes occurred in and out of cysts. Cysts of smaller cells sometimes occurred within cysts of larger cells. Except for the largest cells, cells within and out of cysts exhibited similar oral structure. Cell shape was also variable, cylinder-like, with rounded or tapering anterior, smaller specimens often having narrowed anterior. The oral depression was conspicuous and deeply invaginated, with right-posterior in-pocketing supported by fibers; the buccal field was ~30–40% of cell length. In all but some of the largest cells, the cytostome was clearly delineated by fibers, leading to a cytopyge, extending ~30% of the cell length (Fig. 21). The largest cells (~200–500μm) in cysts appeared to lack or have a reduced cytostomal region; this was not so for free-swimming large cells. One contractile vacuole was present terminally, but was not always observed in stained and live specimens and was never observed to contract in live specimens. The macronucleus was sausage-like, elongate but often bent, positioned centrally along the main cell axis (Fig. 22–31), and often enclosed by a distended membrane in protargol stains. Micronuclei were not observed, as prey nuclei obfuscated identification. Somatic cilia were ~5 μm long; oral cilia ~5–10 μm long, forming conspicuous polykinetids. Somatic kinety number was ~50–110, varying with cell size; free-swimming cells had ~50–60 kineties, encysted cells ~80–110 kineties. Somatic kineties were composed of densely arranged dikinetids, although largest cells in cysts had some monokinetids. All kineties terminated at both cell poles. There was clear displacement of kineties around the cytopyge, where a contractile vacuole pore may be located.

Based on Song’s (2000) observations of Porpostoma notatum Möbius, 1888 and his designation of its oral structures, we have concluded that the oral ciliature followed two patterns. In free-swimming and most encysted cells the following pattern existed (Fig. 16–21, 32–35). Adoral polykinetid 1 extended almost to the anterior apex; it was composed of 15–35 irregularly shaped parts that gradually widened posteriorly and became composed of an increasing number of ciliary rows, each part containing 1 to ~10 longitudinal rows of kineties. Polykinetid 2 had ~20–25 tightly packed and highly organized longitudinal rows and was not distinctly separated from polykinetid 3; polykinetid 3 had, relative to polykinetid 2, a chaotic structure, sometimes forming distinct lateral-oriented kineties but often fragmented, consisting of ~15–20 kineties. The paroral kinety curved on the right under-side (or lateral wall) of the oral cavity, oblique to main body axis. Scutica, not always observed, had as few as three but in

Fig. 1. Map of Guam showing 13 sites surveyed for disease to establish baseline prevalence of diseases affecting Guam corals and the locations on Luminao Reef and Tumon Bay where brown-band diseased samples were collected. Inset globe: location of Guam in Western Pacific Ocean.

---

2 J. EUKARYOT. MICROBIOL., NO. II. III

Fig. 2–15. Porpostoma guamensis n. sp., live material. 2. Band of active cells feeding on Acropora, healthy coral tissue on the left. 3. Gorged, encysted cells on coral skeleton; cells appear as brown flecks on the white coral. 4–12. An indication of the size range of cells, with all images oriented with the anterior end to the left. 4. Gorged trophonts on coral fragment. 5. Moderately large cell without zooxanthellae. 6. Moderately large cell with zooxanthellae; 7. Smaller, free-swimming cell with few zooxanthellae. 8, 9. Smaller, swimming cell from dividing population and one daughter cell. 10–12. Very small cells without zooxanthellae. 13. Encysted cells with apparently healthy zooxanthellae (arrows), and ejected fecal material apparently with zooxanthellae remains (arrowhead). 14, 15. Very small cell at two orientations showing oral area (arrows) and residual color from consumed zooxanthellae. Scale bars: Fig. 2 = ca. 5 mm; Fig. 3 = 1 mm; Fig. 4–12 = 100 μm; Fig. 13 = 50 μm; Fig. 14, 15 = 20 μm.
Fig. 16–20. Line drawings of protargol-stained cells of Porpostoma guamensis n. sp., indicating variation in size and morphology. Note that the basic structure of the oral polykinetids is similar regardless of cell size. 16, 17. Details of oral region of P. guamensis n. sp.; 18–20. Whole cells; 16, 17, 20. Ventral views; 18, 19. Left-ventral views. cp, cytoproct; n, macronucleus; nm, macronuclear membrane; p, paroral kinety; s, scutica; v, contractile vacuole.
well-presented specimens up to 40 kinetosomes, arranged in a kinety, extending posterior to oral depression.

The oral structure differed from that described above in the largest cells found in cysts (Fig. 38, 39). It is unclear precisely how the oral structures of larger cells map onto the pattern described above, but we recognize three distinct regions and tentatively designate these: adoral polykinetid 1, extending almost to the anterior apex, was composed of 100–150 laterally oriented kineties that gradually widened posteriorly from 1 to 15 kinetosomes; polykinetid 2 was more tightly organized than polykinetid 1 and continuous with it, extending deep into the oral cavity; polykinetid 3 appeared as a less organized, less tightly packed extension of polykinetid 1, covering the entire oral cavity. The paroral kinety curved on the right under-side (or lateral wall) of oral cavity, oblique to main body axis. Scutica were not observed.

Many but not all cells contained prey (evidenced by zooxanthellae and coral nematocysts); larger cells contained more prey. No compound crystalline organelle "eyespot" was observed (Fig. 38, 39). Live, free-swimming cells were colorless to brownish yellow often with numerous food vacuoles or zooxanthellae (Fig. 4–15). Division was rarely noted in preserved specimens but commonly seen in both swimming and encysted live cells (see "Division").

**Field observations.** Brown-band disease on Guam reefs was observed only affecting corals of the genus *Acropora*, particularly staghorn growth forms, and appeared as an advancing brown band with a jelly-like consistency adjacent to clinically healthy tissue (Fig. 2), leaving bare skeleton behind it. In some cases, there was a thin band of white, exposed skeleton immediately preceding the ciliate band, separating it from healthy tissue. The brown band appeared to be composed of gorged (i.e. virtually full of zooxanthellae) cells that had settled in cysts (Fig. 3). The disease was observed on thicket-forming staghorn corals, *Acropora acuminata* (Verrill, 1864), *Acropora muricata* (Linnaeus, 1758), and *Acropora aspera* (Dana, 1846) in two out of 13 sites surveyed. Prevalence within the genus at these sites was: Luminao, 3.8 ± 3.8% (*Acropora* abundance: 13% of live hard coral population); Tumon: 4.7 ± 2.3% (*Acropora* abundance: 60%).

**Feeding behavior.** The opportunistic study of brown-band disease progression in *A. surculosa* (Dana, 1846) fragments in the laboratory indicated that ciliates at a density of ~120 cells/mm² in a band of 2–3 mm actively fed along exposed tissue margins from the fragment base upward, consuming tissue at an average rate of 1.85 mm/h. Within 2 days, 88% of the fragments, which averaged 7 cm in length, were devoid of tissue, and no ciliates were observed on the exposed skeleton. Although no field observations have been made in Guam of the ciliates affecting non-staghorn morphologies, these laboratory observations clearly indicate that the ciliates can affect other species, though data suggest it is rare.

On fragments collected from Luminao and Tumon Bay, cells feeding on coral were observed to form aggregates, with their oral region adjacent to the coral, which they engulfed, leaving bare coral skeletons. Both zooxanthellae and nematocysts occurred in the ciliates. Zooxanthellae appeared to be ingested alive but deteriorated or were digested within 2–3 days, as the ciliates divided and became smaller. Shriveled or fragmented zooxanthellae, presumably in food vacuoles, occurred inside the ciliates and extracellularly within cysts, possibly as egested material (Fig. 13, 47).

**Swimming behavior and movement in cysts.** Ciliates generally rotated around their long axis while swimming and within cysts, but not while feeding. Within cysts cells of all sizes exhibited ciliary beating, rotation, and forward-backward movement, resulting in cysts that were somewhat longer than the cells (Fig. 13, 43).

**Division (Fig. 40, 44–47).** Size categorization of dividing and/or recently divided cells suggested four size-classes of cells with
Illustrations of protargol-stained *Porpostoma guamensis* n. sp., indicating variation in size, morphology, cyst shape, and presence of zooxanthellae within cells. Note the specimen in Fig. 25 is the same cell used to illustrate the oral structure of some large cocooned cells (Fig. 37, 38). mn, macronucleus; s, surface of cyst; z, zooxanthellae.
three divisions between the largest and smallest cells (Fig. 47); this was determined from observation of live and preserved cells (e.g. Fig. 2–20, 22–31) and evaluation of size categories of live cells (Fig. 40). Division can occur within cysts, and these cells may form cysts within larger cysts. Alternatively, free-swimming cells can divide. Cyst formation was not observed.

**DISCUSSION**

**Comparison with similar species.** The species under investigation most closely resembles members of the genus *Porpostoma*, recently evaluated in detail by Song (2000) and Song and Wilbert (2000). Note, however, that based solely on earlier literature, Lynn (2008) considered the genus *Porpostoma* to be a subjective synonym of *Helicostoma*. Here, we have followed the direction of Song (2000) and Song and Wilbert (2000) but recognize that once a rigorous evaluation of the Philasterida is conducted, this designation may require revision.

The two recent assessments of species of *Porpostoma* provide a rigorous review for comparison: *P. notatum* by Song (2000) and *Porpostoma grassei* (Corliss and Snyder, 1986) by Song and Wilbert (2000). Respective differences of *P. guamensis* from *P. notatum* are: the general shape and structure of the oral polykinetids (OP), with OP 2 and 3 being larger in *P. guamensis*; maximum cell size (500 vs. 180 \(\mu\)m); pigmentation (clear-brown vs. dark grey-black); macronuclear shape (sausage-like vs. elongate and highly twisted); and habitat (ectoparasite on coral vs. free-living/histophage). *Porpostoma guamensis* also lacks the

---

**Fig. 32–37.** Oral structure of *Porpostoma guamensis* n. sp. 32–35. Protargol-stained specimen, indicating oral ciliature. 32–34. View at three focal planes. 35. Same focal plane as 32, frame shifted posteriorly. 36, 37. Photomicrographs of a live cell in ventral view (36) and right three-quarter view, showing the absence of a crystalline eyespot. The oral region extending upward from the arrow in each photo. Differential interference contrast. Scale bars: Fig. 32–35 = 10 \(\mu\)m; Fig. 36, 37 = 20 \(\mu\)m.
“eyespot” described for *P. notatum* (Kuhlmann, Brücker, and Schepers 1997).

*Porpostoma guamensis* appears nothing like *P. grassei*: maximum cell size (500 vs. ~185 μm long); larger OP 2 and 3; macronuclear shape (sausage-like vs. irregularly shaped or globular macronucleus that is sometimes fragmented); and habitat (ectoparasite on coral vs. exclusively collected from Antarctic waters where hard-corals are absent). Because these are the only two other species assigned to the genus *Porpostoma*, we, therefore, conclude that *P. guamensis* represents a new species.

Besides the features above, there are other behavioral factors that support our designation of a new species. The division pattern of *P. guamensis* n. sp. differs from that of *P. notatum*, as described by Kuhlmann et al. (1997), Naeem and Fenchel (1994), and Mugard (1949), who indicated that tomites of *P. notatum* divide to form four tomites; they also suggest that in cysts there was only one or two divisions, while we observed up to three divisions for *P. guamensis* within and likely outside of thin-walled cysts. These researchers also observed no ciliary movement in the encysted tomites of *P. notatum*, while *P. guamensis* exhibited ciliary beating, rotation, and forward–backward movement within cysts. Such behavior suggests that these might not be resting cysts but...
a protective mechanism, possibly to allow the ciliates to remain in the light on corals while actively using the zooxanthellae for photosynthesis (i.e. mixotrophy), if the zooxanthellae remain active as observed for an undescribed brown-band ciliate in Australia (Ulstrup, Kühl, and Bourne 2007). Such mixotrophic behavior has not been observed in other species of Porpostoma. The feeding behavior of P. guamensis also differed from that of P. notatum. As we indicated, P. guamensis is an ectoparasite of coral polyps, consuming living tissue and zooxanthellae. In contrast, P. notatum is histophagic, feeding on damaged tissue of invertebrates found in marine intertidal regions (e.g. Kuhlmann et al. 1997; Naeem and Fenchel 1994).

TAXONOMIC SUMMARY
Class: Oligohymenophorea de Puytorac et al., 1974
Subclass: Scuticociliatia Small, 1967
Order: Philasterida Small, 1967
Porpostoma guamensis n. sp.

Diagnosis. Ciliate shape and ciliature typical for the genus. Live cells 70–500 × 20–75 μm. Ciliates form thin-walled cysts in which the cells may be active; cells divide up to 3 times in cysts and form cysts within cysts. Macronucleus sausage-like, elongate, but often bent, positioned centrally along main cell axis. Ciliates contain zooxanthellae prey that they obtain from consuming coral polyps. Association with the host coral is possibly restricted to the genus Acropora. Masses of ciliates form brown bands several millimeters wide on coral branches.

Type locality. Luminao Reef, Guam, Mariana Islands, 13°27′53.8″N, 144°38′50.58″E.

Type material. Holotype is a slide of protargol-stained cells deposited in the Natural History Museum, London, UK, accession numbers 2010:11:5:1 (holotype) and 2010:11:5:2 (paratype).

Etymology. The specific epithet refers to the island of Guam, where the species was first noted.

A predicted division pattern (Fig. 48). A close relative to P. guamensis n. sp., the histophagic ciliate P. notatum exhibits a variable division pattern (Naeem and Fenchel 1994): when provided substantial food, ciliates increase in size to form large trophonts or feeding cells; they then encyst when food becomes scarce, and later they excyst and undergo two divisions to produce four tomites as a dispersal stage. When less food is available, only one division occurs after excystment, producing two tomites. Two divisions of P. notatum may occur when cells are encysted (Mugard 1949).

We have not been able to assess the entire division pattern of P. guamensis, but from some direct observations and circumstantial evidence, we can speculate on the complete pattern. For instance, we are confident that large well-fed cells form cysts, within which up to three divisions can occur (Fig. 48). We are also confident that free-swimming cells will divide. However, of particular note, we did not determine which factors stimulate cells to

Fig. 44–47. Dividing Porpostoma guamensis n. sp. 44. First division in cyst showing plane of cell division perpendicular to cell axis. 45. Swimming cell in division. 46. Daughter cells in a cyst changing shape. 47. Cysts containing cells that have undergone multiple divisions, with individual sheaths around each cell. Scale bars = 100 μm.
A note on the variable oral morphology with division stage. There was a reduction in the oral region only in large, gorged cells within cysts; the oral area appeared to be fully developed in swimming cells of all sizes and dividing cells in cysts. Kuhlmann et al. (1997) describe a similar reduction in the oral cavity and cytostome in the protomont stage through the tomites of *P. notatum*, with redevelopment when theronts were released. While we could not see the oral area adequately in the smallest tomites, the oral area appears to be well developed in the first-division cells in the cysts. Furthermore, the oral area was fully developed in even the smallest swimming cells, which would presumably be interpreted as theronts, no matter what their size. Possibly the reduction of the oral region is linked to an autotrophic phase of mixotrophy within the cysts, but this too requires further study, possibly using techniques outlined by Ulstrup et al. (2007).

Molecular identification. Brown-band disease, caused by a ciliate that appears morphologically similar to the one we describe here, has been characterized by an 18S rRNA gene sequence (Bourne et al. 2008); their work placed the brown-band ciliate within the scuticociliates. Our attempts to obtain a gene sequence failed, so we cannot provide a comparison. There is clearly scope to continue this work, possibly examining several markers and many isolates from distinct locations, to assess the phylogenetic position of this ciliate, beyond our morphology-based assessment.

Occurrence and prevalence. Brown-band disease has been noted on *Acropora* corals across the southern Pacific and Indian Oceans (see Introduction). Although the rarity of the disease among the sites surveyed made it difficult to draw conclusions regarding links with water quality or other environmental drivers, our observations and those of others (Nugues and Bak 2009; Willis, Page, and Dinsdale 2004) suggest that the disease targets corals in the genus *Acropora*.

Given the ability of *P. guamensis* n. sp. to denude coral branches rapidly and its low but apparent persistent abundance in both pristine and nutrient-enriched environments, we suggest that, like many diseases, under appropriate conditions it may have a significant impact on the ecosystem. Furthermore, the genus *Acropora*, an important and dominant reef component, is under threat from other infectious diseases (Myers and Raymundo 2009; Willis et al. 2004), is highly susceptible to bleaching (Loya et al. 2001; van Woesik, Irikawa, and Loya et al. 2004), and is a favored food of the highly destructive sea star *Acanthaster planci* (Burdick et al. 2010). An additional cause of rapid mortality targeting this genus is, therefore, a cause for concern. Thus, we recommend that studies continue on the autecology and identification of *P. guamensis*, using field and laboratory experimental approaches and morphological and molecular methods of identification.

ACKNOWLEDGMENTS

We thank Wayne Coats and Denis Lynn for advice on the identity and phylogenetic position of the ciliate. We also thank Gaytha Langlois for dialogue on the Australian material. We acknowledge support from the Global Environment Facility/World Bank Coral Reef Targeted Research Program for L. Raymundo’s work. Microscopic observations of live cells were made on microscopes funded by grants to C. Lobban from the National Institute of General Medical Science RISE grant R25 GM063682 and Department of Education Title III MSEIP grant P120A040092. Work conducted by D. Montagnes was unfunded.

LITERATURE CITED


Received: 09/11/10, 11/01/10; accepted: 11/04/10