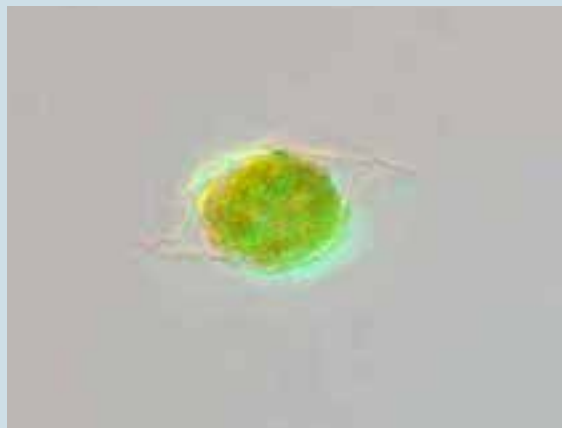


Epiphytic dinoflagellates on the seagrass *Thalassia testudinum*  
at Dzilam, southeastern Gulf of Mexico



# EPIPHYTIC DINOFLAGELLATES ON THE SEAGRASS *THALASSIA TESTUDINUM* AT DZILAM, SOUTHEASTERN GULF OF MEXICO

## DINOFLAGELADOS EPIFÍTICOS SOBRE EL PASTO MARINO *THALASSIA TESTUDINUM* EN DZILAM, SURESTE DEL GOLFO DE MÉXICO

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### ABSTRACT

To determine the epiphytic dinoflagellate taxocoenosis, to study the vertical distribution of dinoflagellates along the leaves of the seagrass *Thalassia testudinum* and to evaluate the potential risk of ciguatera, based on the year-round sampling at Dzilam, the northern Yucatan Peninsula, were the main objectives. Seagrass samples were taken monthly from 15 May 2012 to 20 May 2013, 100 m from the coastline. Water temperature varied between 22.3 °C (February) and 36.5 °C (April), and salinity varied between 29.6 (April) and 37.3 (May 2012). In total, 18 epiphytic dinoflagellate species were found. The genus *Prorocentrum* was predominant in terms of the number of species (7). *Prorocentrum lima* (up to 2228 cell/g of *Thalassia* wet weight, in April), *P. cf. sipadanensis* (up to 1111 cells/g, in August) and *Peridinium quinquecorne* (up to 987 cells/g, in January) largely determined the annual dynamics of the entire taxocoenosis. The dinoflagellate cell abundance usually increased towards the apices of the leaves throughout

the year, and the highest cell abundances were observed in the apical (the oldest) 12-20-cm part of the seagrass. The annual dynamics of the total dinoflagellate cell abundance was characterized by two peaks, in August 2012 and April 2013. The lowest cell abundance was observed in February and May 2013. Considering the data presently available on the toxicity and our results on the dominant species, *P. lima* may represent a major threat at Dzilam. Other potentially toxic species were: *Amphidinium carterae*, *Coolia* sp., *Prorocentrum concavum*, *P. foramosum*, *P. hoffmannianum* and *P. rathymum*.

**Keywords:** *dinoflagellates, epiphytes, Gulf of Mexico, microphytobenthos, Thalassia*

### RESUMEN

Los objetivos principales del presente estudio fueron determinar la taxocenosis de los dinoflagelados epifíticos, estudiar la distribución vertical de dinoflagelados de las hojas del pasto marino *Thalassia testudinum* así como evaluar el posible riesgo de la ciguatera con base en el muestreo anual en Dzilam,

en el norte de la Península de Yucatán. Muestras del pasto marino se tomaron mensualmente del 15 de mayo de 2012 al 20 de mayo de 2013, a la distancia de 100 m de la línea de la costa. La temperatura de agua varió entre 22.3 °C (febrero) y 36.5 °C (abril) y la salinidad entre 29.6 (abril) y 37.3 (mayo de 2012). En total, se registraron 18 especies de dinoflagelados epifíticos. El género *Prorocentrum* fue predominante en cuanto al número de especies (7). *Prorocentrum lima* (hasta 2228 cél./g del peso húmedo de *T. testudinum*, en abril), *P. cf. sipadanensis* (hasta 1111 cél./g, en agosto) y *Peridinium quinquecorne* (hasta 987 cél./g, en enero) determinaron en gran medida la dinámica anual de la taxocenosis entera. La abundancia celular de dinoflagelados usualmente se incrementó hacia los ápices de las hojas en el transcurso del año, y las abundancias celulares más altas se observaron en la parte apical (la más vieja) de 12-20 cm del pasto marino. La dinámica anual de la abundancia celular total de dinoflagelados se caracterizó por dos picos, en agosto de 2012 y abril de 2013. La abundancia celular más baja se observó en febrero y mayo de 2013. Considerando los datos disponibles sobre toxicidad hasta el presente y con nuestros resultados sobre las especies dominantes, *P. lima* representa la mayor amenaza en Dzilam. Otras especies potencialmente tóxicas fueron: *Amphidinium carterae*, *Coolia* sp., *Prorocentrum concavum*, *P. foraminosum*, *P. hoffmannianum* y *P. rathymum*.

*Palabras clave:* dinoflagelados, epifitos, Golfo de México, microfitorobentos, *Thalassia*

## INTRODUCTION

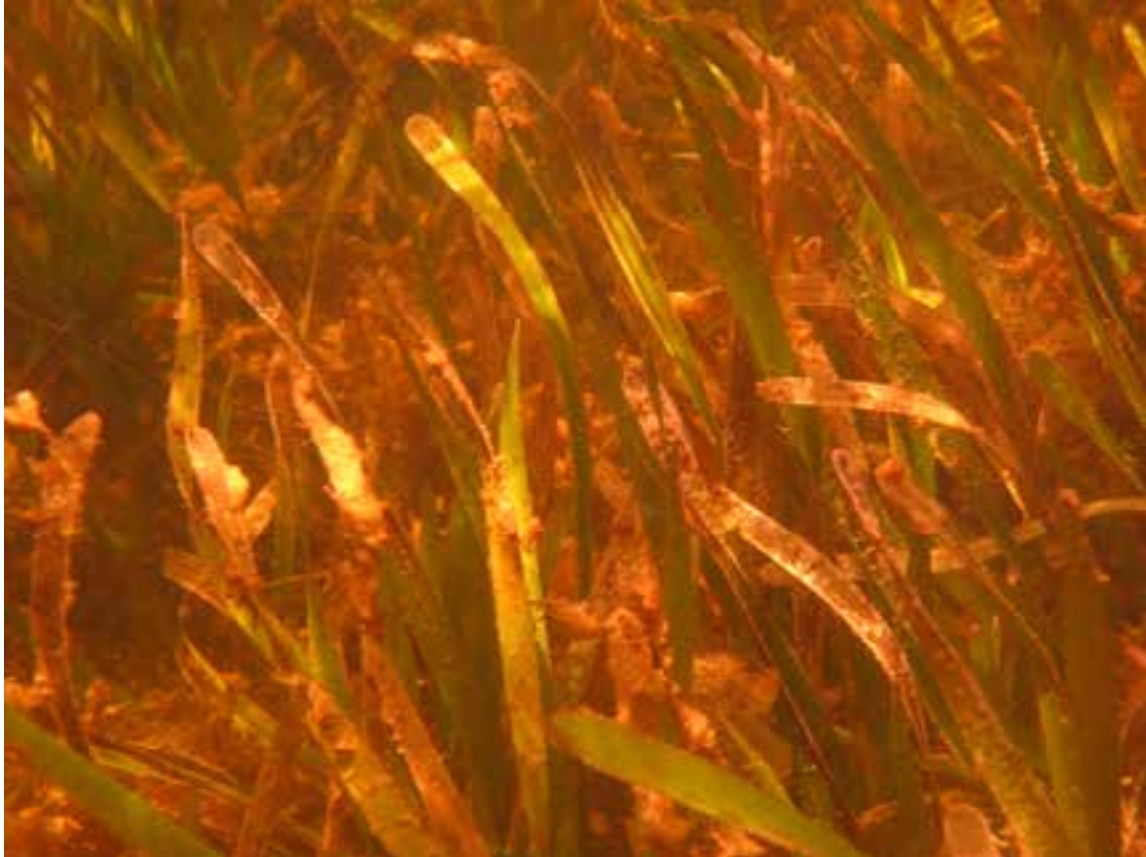
Seagrass beds are common in a shallow zone along the northern Yucatan Peninsula, and *Thalassia testudinum* Banks et Solander ex König (Angiospermatophyta: Hydrocharitaceae) is the most common species (Okolodkov, pers. obs.). As a habitat and a refuge for hundreds of species from microalgae to sea turtles and manatees, it also represents a risk for human health due to some toxic epiphytic dinoflagellates, including the species that cause ciguatera. Recently, there was a report on ciguatera-related biotoxins from the northern Yucatan coast found in humans poisoned by eating the great barracuda *Sphyraena barracuda* (Sphyraenidae) (Okolodkov *et al.* 2014). Earlier, in the period of 1984-2004, in the Yucatan Peninsula, 23 cases of ciguatera were reported; the carnivorous fishes (*Lutjanus* spp., *Epinephelus* spp, *Mycteroperca* spp. and the great barracuda) were

involved in all cases reported for Mexico, on the whole (Nuñez-Vásquez *et al.* 2008).

In the southern Gulf of Mexico, the epiphytic dinoflagellates have been the object of the taxonomic and ecological studies performed in Veracruz and Yucatan (Okolodkov *et al.* 2007, 2014, Aguilar-Trujillo *et al.* 2014), and both seagrasses and seaweeds were sampled. However, more detailed studies on selected seagrass species are still lacking, and the vertical distribution of the cell abundance and the species are unknown. To determine the epiphytic dinoflagellate taxocenosis, to study the vertical distribution of dinoflagellates along the *T. testudinum* leaves and to evaluate the risk of ciguatera based on year-round sampling were the main objectives of this study.

## MATERIAL AND METHODS

Samples of *T. testudinum* (Fig. 1) were taken monthly, at 10:30-12:30 a.m., from 15 May 2012 to 20 May 2013, 100 m from the coastline, at Dzilam de Bravo (Dzilam, for short), the northern Yucatan Peninsula in the southeastern Gulf of Mexico (21°23'36.86"N, 88°53'46.98"W). At a site with a depth of 1.0-1.3(1.7) m, 10 to 15 leaves were separated with a knife and placed into a plastic 500-ml bottle. On shore, leaves of 35-60 cm length were immediately cut into three equal (basal without rhizome, medium and apical) parts and placed in three bottles with seawater taken from the sampling site and fixed with 37% formalin to a final concentration of 4%. Water temperature and salinity were measured with a YSI-Professional Plus (Yellow Springs, Ohio, USA). In the laboratory, the epiphytes were separated by vigorous agitation for one minute, stained with a 0.2% Trypan Blue water solution and counted in a Sedgwick-Rafter chamber under an inverted Olympus CKX-41 microscope in a bright field following the procedure described earlier (Okolodkov *et al.* 2007). The cell counts were recalculated so that the results could be presented as cells per gram of *T. testudinum* wet weight (cells/g TWW). Dinoflagellate species were identified under an inverted microscope, considering our previous, more thorough morphological observations on cells obtained from the same study area (Okolodkov *et al.* 2009, 2014, Aguilar-Trujillo *et al.* 2014) using a compound Olympus BX51 microscope and a JEOL JSM5310LV scanning electron microscope. Okolodkov *et al.* (2009) erroneously reported *Gambierdiscus caribaeus* as *G. toxicus*. Some species were identified tentatively because data were lacking for some diagnostic features such as the periflagellar area in *Prorocentrum* species.



**Figure 1.** A fragment of *Thalassia testudinum* bed at Dzilam.

## RESULTS

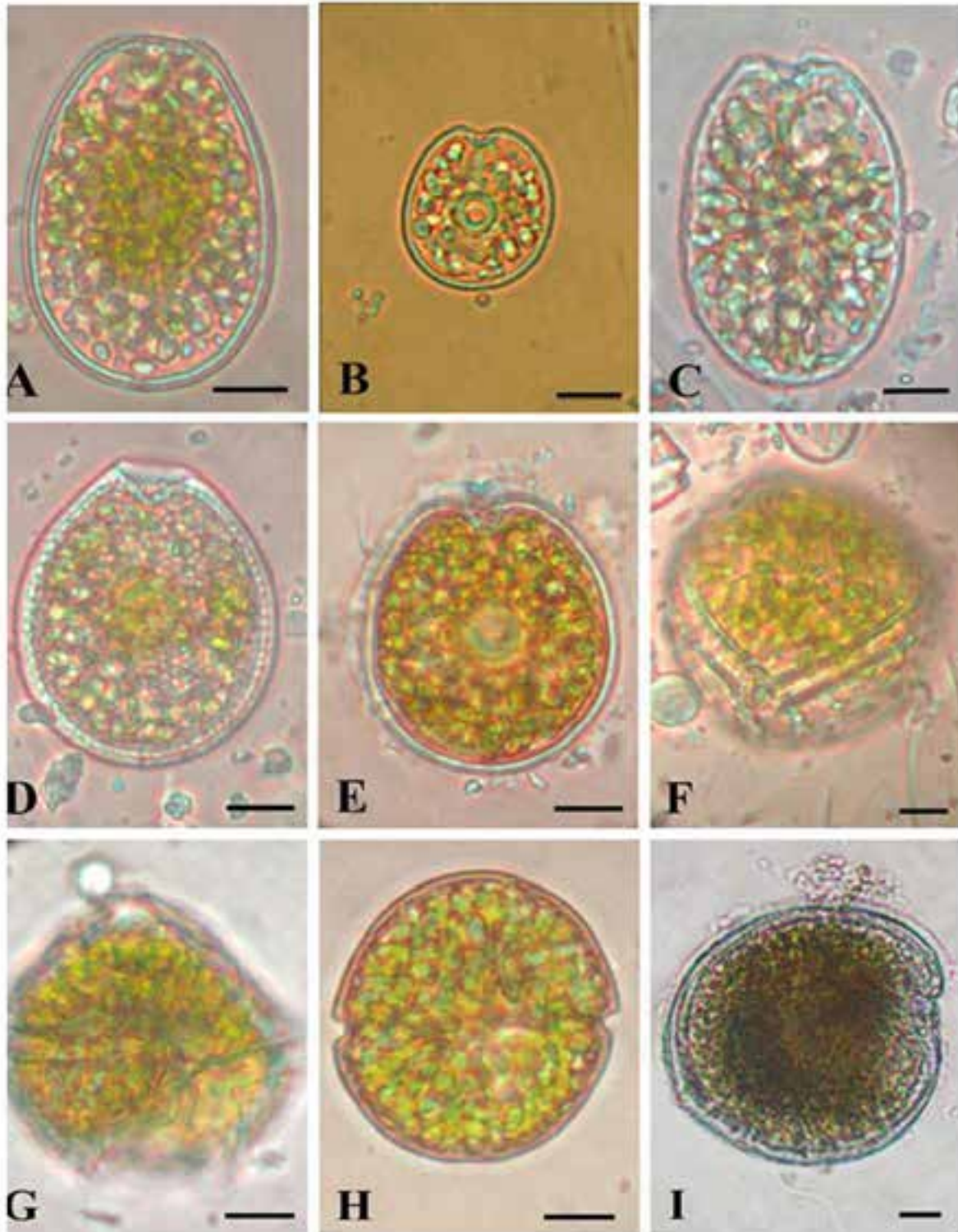
During the study period, water temperature varied between 22.3 °C (February) and 36.5 °C (April) and salinity between 29.6 (April) and 37.3 (May 2012). In total, 18 dinoflagellate species were found: *Amphidinium carterae* Hulburt, *Bysmatrum caponii* (Horiguchi et Pienaar) Faust et Steidinger, *Cabra* cf. *aremonica* Chomérat, Couté et Nézan, *Coolia* sp., *Durinskia* cf. *capensis* Pienaar, Sakai et Horiguchi, *Peridinium quinquecorne* T.H. Abé, *Prorocentrum concavum* Fukuyo, *P. foraminosum* Faust, *P. hoffmannianum* Faust, *P. lima* (Ehrenberg) Dodge, *P. rathymum* Loeblich III, Sherley et Schmidt, *P. sculptile* Faust, *P. cf. sipadanensis* Mohammad-Noor, Daugbjerg et Moestrup, *Prorocentrum* sp., *Sinophysis ebriola* (Herdman) Balech, *S. microcephala* Nie et Wang, *S. stenosoma* Hoppenrath and (?) *Togula* sp. The genus *Prorocentrum* was predominant in terms of the number of species (7).

Other dominant species (those contributing more than 10% of the total dinoflagellate cell abundance in a sample) were as follows: *Prorocentrum lima* (up to 2228 cell/g TWW, in April), *P. cf. sipadanensis* (up

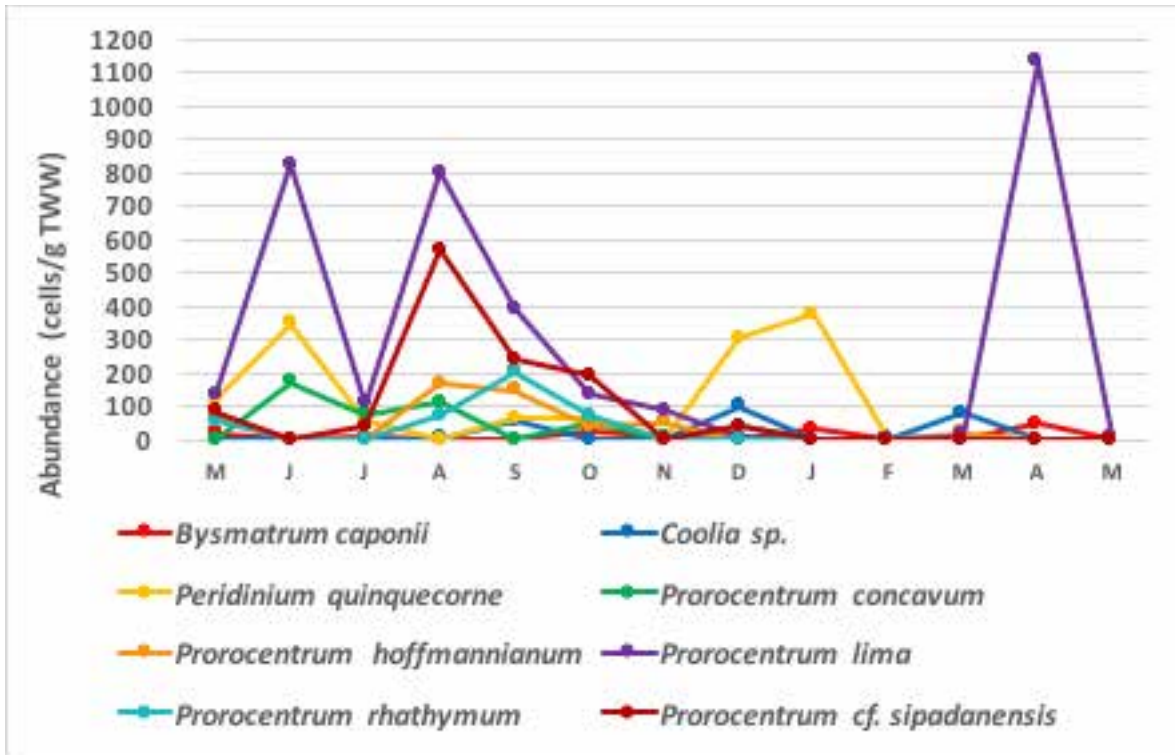
to 1111 cells/g, in August), *Peridinium quinquecorne* (up to 987 cells/g, in January), *Prorocentrum rathymum*, *P. hoffmannianum*, *P. concavum*, *Coolia* sp. and *Bysmatrum caponii* were the most abundant species (Fig. 2 and 3). The former three largely determined the annual dynamics of the entire taxocoenosis, and the highest cell abundances per sample were observed in the apical 12-20 cm part of the seagrass. The annual dynamics of the total dinoflagellate cell abundance was characterized by two peaks, in August and April (Fig. 4). The lowest cell abundances were observed in February and May 2013. Comparing the cell abundances found in May 2012 and May 2013, the difference is significant; thus interannual changes in physical-chemical conditions must be involved.

The dinoflagellate cell abundance usually increased towards the apices of the leaves throughout the year, with two pronounced peaks of 3841 and 2584 cells/g TWW, in August and April, respectively (Fig. 5). Only in July was it significantly higher (2229 cells/g TWW) in the medium part and in September in the basal part.

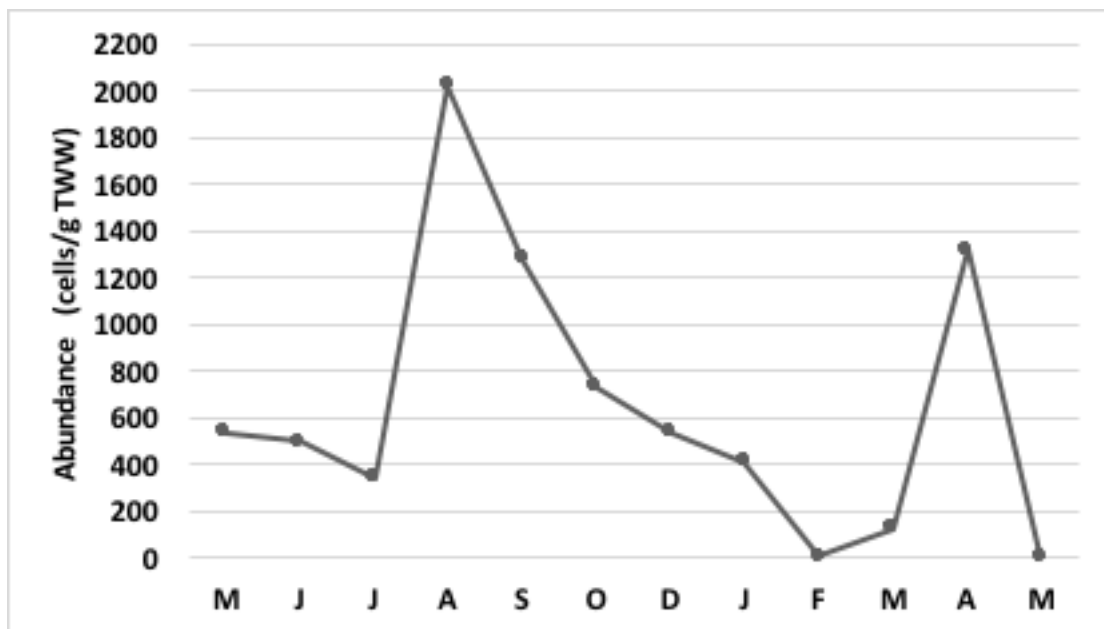




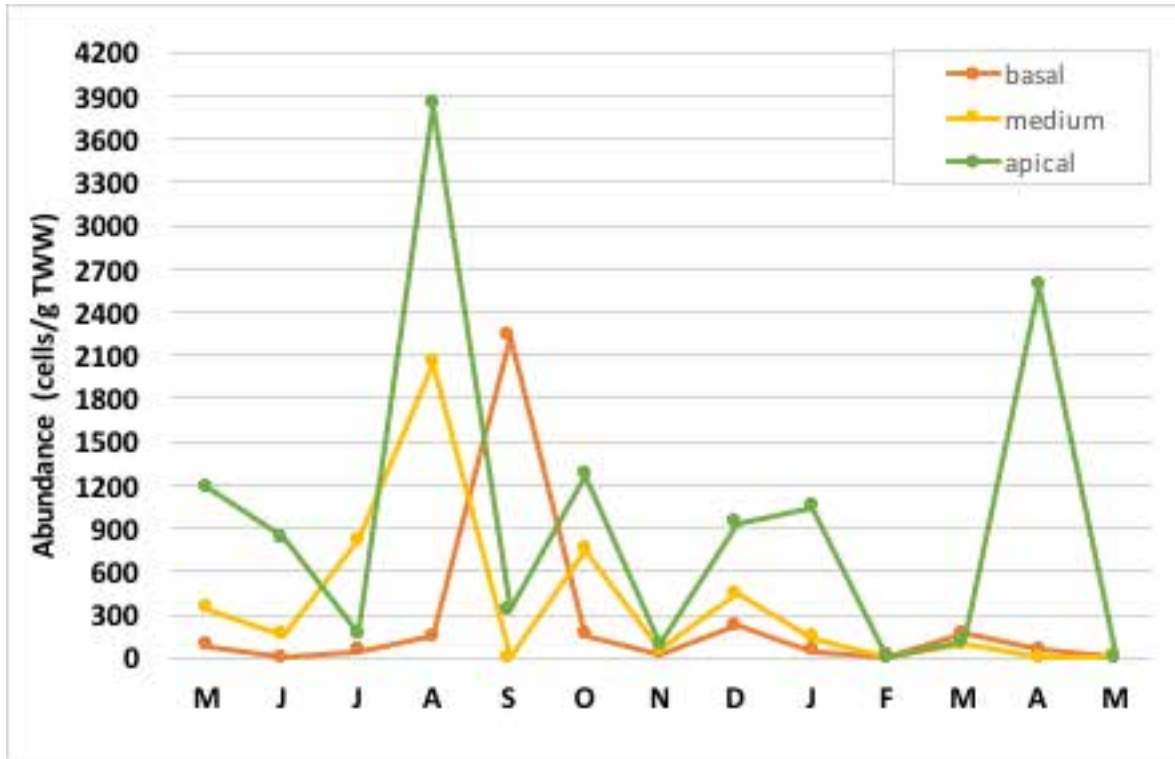
**Figure 2.** Some epiphytic dinoflagellates inhabiting *Thalassia testudinum* leaves at Dzilam: **A** – *Prorocentrum lima*; **B** – *Prorocentrum* cf. *sipadanensis*; **C** – *Prorocentrum rathymum*; **D** – *Prorocentrum hoffmannianum*; **E** – *Prorocentrum concavum*; **F** – *Coolia* sp.; **G** – *Bysmatrum caponii*; **H** – *Durinskia* cf. *capensis*; **I** – *Gambierdiscus caribaeus*. Scale bar: 10  $\mu$ m.



**Figure 3.** Monthly changes in the abundance of the dominant species of the epiphytic dinoflagellates of *Thalassia testudinum* at Dzilam from May 2012 to May 2013 (TWW: *Thalassia* wet weight). *Prorocentrum lima*, *Prorocentrum* cf. *sipadanensis* and *Peridinium quinquecorne* largely determined seasonal changes of the whole epiphytic dinoflagellate taxocoenosis.



**Figure 4.** Monthly changes in the total abundance of the dominant species of the epiphytic dinoflagellates of *Thalassia testudinum* at Dzilam from May 2012 to May 2013 (TWW: *Thalassia* wet weight).



**Figure 5.** Monthly changes in the abundance of the epiphytic dinoflagellates on three parts of *Thalassia testudinum* leaves at Dzilam from May 2012 to May 2013 (TWW: *Thalassia* wet weight). Almost always the apical (older) part harbors more dinoflagellate cells.

Although *Gambierdiscus caribaeus* Vandersea, Litaker, Faust, Kibler, Holland et Tester (Fig. 2I) was not observed on *T. testudinum* leaves, rare cells of this species were found in seaweed samples taken from July through December at sampling sites located 75, 100 and 200 m from the coastline (this study).

## DISCUSSION

Unlike in earlier studies on the epiphytic dinoflagellates performed along the northern coast of the Yucatan Peninsula, the benthic-planktonic *Peridinium quinquecorne* was observed for the first time among the dominant species in the taxocoenosis of epiphytic dinoflagellates. This species has been known to be a blooming species in the state of Veracruz, southwestern Gulf of Mexico (Aké-Castillo *et al.* 2014, Pérez-Morales *et al.* 2015, Rodríguez-Gómez *et al.* 2015). It is curious that *P. quinquecorne* was not observed as a part of the epiphytic dinoflagellate taxocoenosis in Veracruz whereas in the northern Yucatan it was. Similar to Veracruz, *Prorocentrum lima* was the

principal dominant species in our study. Unlike our study, in previous studies in the northern Yucatan waters, *Prorocentrum rathymum* was frequently the predominant species responsible for the annual dynamics of the entire taxocoenosis of epiphytic dinoflagellates (Aguilar-Trujillo *et al.* 2014, Okolodkov *et al.* 2014). Earlier, it was shown that *P. rathymum* prefers low nutrient concentrations and that *P. lima* is correlated with high ammonium concentrations (Okolodkov *et al.* 2014), so the shift from *P. rathymum* to *P. lima* as the dominant species can be explained by the eutrophication of the coastal waters at Dzilam after a long-term red tide that occurred in the study area in 2011 and maintained itself for several months (Merino-Virgilio *et al.* 2014).

As for the vertical distribution of the dinoflagellate cell abundance along the seagrass leaves, it is obvious that fish species that prefer feeding on the apices (Lobel & Ogden 1981), as does the permanent dweller of the seagrass beds in the eastern Caribbean, the herbivorous bucktooth parrotfish *Sparisoma radians* (Scaridae), have a greater



potential for accumulating dinoflagellate toxins through the food web. In the islands of the West Indies, among marine spermatophytes, only the seagrasses *Thalassia testudinum* and *Syringodium filiforme* Kütz. (syn.: *Cymodocea manatorum* Asch.) are eaten by reef fishes in significant quantities (Rundall 1967). Some fishes from the families Kyphosidae, Scaridae and Acanthuridae had plant material constituting more than 50% by volume of the stomach contents; in addition, some omnivorous fishes fed heavily on marine plants. Four seagrass species, including *T. testudinum*, were found in the stomachs of more than 30 species of mainly coral reef-dwelling fishes primarily from the families Scaridae and Acanthuridae, but also from the Sparidae, Monacanthidae, Tetrodontidae and Ostraciontidae (Rundall 1967). In addition, the families Syngnathidae, Gobiidae, Labridae, Gerreidae, Scorpaenidae, Sciaenidae and Blennidae are also among typical seaweed grazers in many geographical areas around the world (Pollard 1984). In the Lobos Reef, in the northern part of the state of Veracruz, the most abundant coral reef fishes associated with *T. testudinum* are *Scarus iseri* and *Sparisoma radians* (Scaridae), *Halichoeres bivittatus* (Labridae), *Stegastes adustus* and *Stegastes leucostictus* (Pomacentridae) (González-Gándara & Trinidad-Martínez 2006). In south Florida and the Caribbean Sea, apart from fishes, sea urchins and green turtles constitute the major groups that graze seagrass meadows (Ogden *et al.* 1973, Ziemann *et al.* 1984). Additionally, gastropods, crustaceans and sea mammals should be added to this list (Valentine & Heck 1999). Seagrasses were shown to be a significant food source for juvenile fish in and offshore from south Texas lagoons (Fry & Parker 1979). In general, seagrass meadows provide shelter from predators and an abundant food source (“board and lodging”), functioning as a nursery ground (Rundall 1967, Pollard 1984). Valentine & Heck (1999) estimated that about 3 to 100% of seagrass net primary production enters food webs via the grazing pathway. These results are crucial to the understanding of the scale of the ciguatera transfer through food webs.

Also, it is clear that the annual dynamics of the epiphytic dinoflagellate taxocoenosis, at least in terms of the maximal cell abundances, depend primarily on the growth of the dominant species, *Prorocentrum lima*, *P. cf. sipadanensis* and *Peridinium quinquecorne* on the apical (the oldest) part of the seagrass leaves.

*Gambierdiscus caribaeus* was recently shown to be a ciguateric species (Holland *et al.* 2013). Pre-

viously, it was found in May and November 2008 in abundance in shallow waters at two localities near Dzilam (Okolodkov *et al.* 2014). Therefore, the study area can be considered potentially ciguateric. The following dinoflagellate species found in the study area on *T. testudinum* may represent a threat to human health: *Amphidinium carterae* (produces haemolytic substances, ichthyotoxins, and it was also implicated as a causative agent of ciguatera; Baig *et al.* 2006), *Coolia* sp. (in tropical waters, at least two species, *C. tropicalis* Faust and *C. malayensis* Leaw, Lim *et al.* 2013), *Prorocentrum concavum* (okadaic acid and ichthyotoxins), *P. foraminosum* (dinophysistoxin-1), *P. hoffmannianum* (okadaic acid and fast-acting toxins), *P. lima* (okadaic acid, dinophysistoxin-1, dinophysistoxin-2, prorocentrolides and fast-acting toxins) and *P. rathymum* (okadaic acid). Some dinoflagellates that had been considered non-toxic have been shown to produce toxins, specifically *P. foraminosum* (dinophysistoxin-1; Kameneva *et al.* 2015). In addition, there are some species whose toxicity is yet unknown (e.g., *P. sipadanensis*). Considering the data presently available on the toxicity and our results on the dominant species, *P. lima* and *P. rathymum* may represent a major threat at Dzilam. Application of both inverted light and scanning electron microscopy allows the abundance estimation and identification to species or generic level. However, this does not guarantee an absolute resolution of the quantification vs. identification problem, due to the impossibility of applying both types of microscopy to the same cells, a common problem remaining to be solved in ecological studies.

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### **CRÉDITOS A LAS FOTOS DE LA PORTADA**

*Pteromonas aculeata* Lemmermann. Cantera Oriente,  
Reserva Ecológica del Pedregal de San Ángel, CU, UNAM, Ciudad de México.

Fotos de E. Novelo. Vistas frontal, apical y lateral respectivamente de un ejemplar vivo.

### **CINTILLO LEGAL**

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