

## Research Article

## Abundance and diversity of ascidians in the southern Gulf of Chiriquí, Pacific Panama

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### Editor's note:

This paper is a contribution to the proceedings of the 3rd International Invasive Sea Squirt Conference held in Woods Hole, Massachusetts, USA, on 26–28 April 2010. The conference provided a venue for the exchange of information on the biogeography, ecology, genetics, impacts, risk assessment and management of invasive tunicates worldwide.

### Abstract

Little is known about the ascidian fauna of Pacific Panama. Ascidian surveys were conducted in the southern Gulf of Chiriquí on the Pacific coast of Panama in January 2008 and 2009. Surveys along linear transects at 2–3 m depth (snorkel, 2008) and 5 and 12 m depth (SCUBA, 2009) were conducted at multiple sites within a chain of islands extending out from the mainland. Twelve different ascidian taxa were observed with mean densities of up to ~17 ascidians·m<sup>-2</sup>. The most abundant species was *Rhopalaea birkelandi*. Two of the most abundant taxa (*Ascidia* sp., *Pyura* sp.) appear to represent previously undescribed species. Several species of didemnids were also abundant. Ascidians were most abundant near the coast of the mainland and were less abundant near the islands farthest offshore. These data on Panamanian ascidian communities provide a baseline of local biodiversity against which it will be possible to determine whether the communities change over time, if additional species become introduced to the region, or if native Panamanian species become invasive in other parts of the world.

**Key words:** diversity, Panama, reef, tunicates

### Introduction

Ascidians are a significant component of many benthic systems (Petersen and Riisgård 1992; Yakovis et al. 2004; Ribes et al. 2005), yet have historically have been less well studied than many other benthic invertebrates (e.g., corals, barnacles, etc.). Despite growing concerns about the spread and impact of invasive ascidians (Bullard and Carman 2009), many ascidian communities are poorly known. To address this lack of knowledge, researchers have begun to describe ascidian communities in widely separated geographical regions, including southern

Africa (Primo and Vázquez 2004), Australia (Kott 2005), Hawaii (Carlton and Eldredge 2009), and Venezuela (Rocha et al. 2010). Little is known about the ascidians of Central America, especially on the Pacific coast of Panama. In his seminal work on ascidian taxonomy, Van Name (1945) listed eight species as occurring in Pacific Panama, but to date there has not been a systematic effort to assess the abundance and diversity of ascidians in the region.

Some ascidians are highly invasive (Lambert 2002; Lambert and Lambert 2003) and recent outbreaks of ascidian species have caused significant ecological and economic damage

around the world (reviewed in Bullard and Carman 2009). Valuable time can be lost in controlling invasive ascidians as researchers attempt to identify pest species and determine their place of origin (Lambert 2009; Stefaniak et al. 2009). More knowledge is therefore needed about ascidian taxonomy and community structure in regions where information is lacking. The availability of such data will aid researchers in tracking the origin of new outbreaks and in identifying which environmental or ecological factors control invasive ascidians in their native habitats.

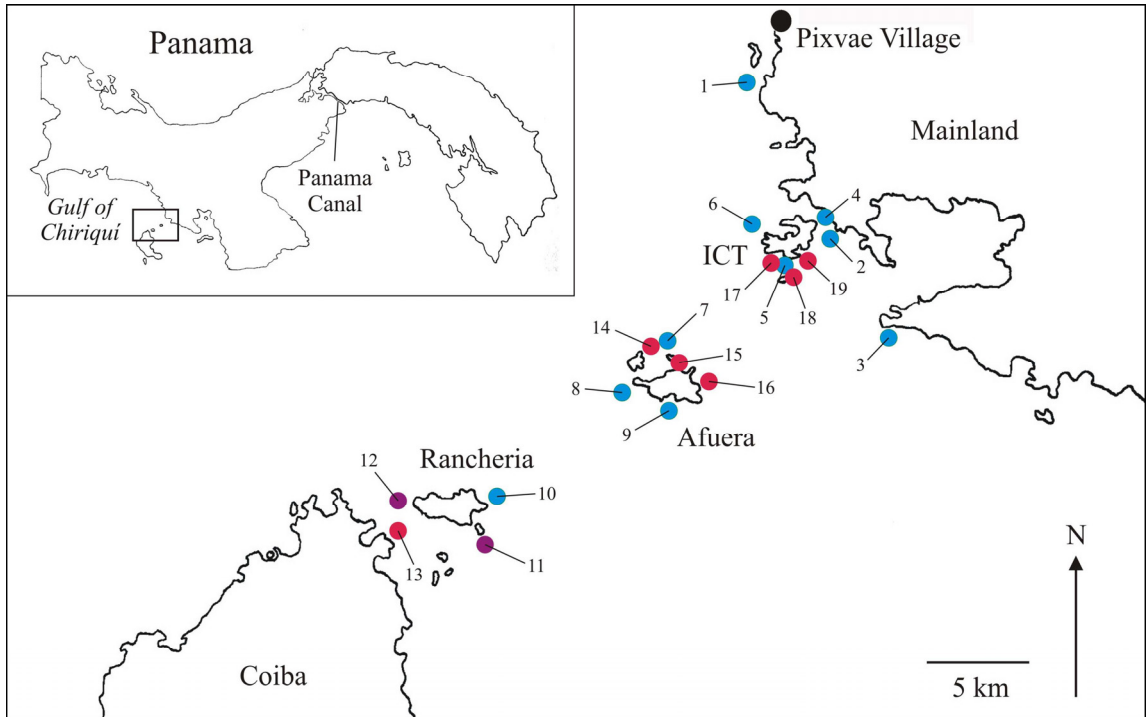
Understanding the ascidian communities of Panama is particularly important because the Panama Canal is a critical link to global trade and ships travelling through it may act as transport vectors (e.g., Fofonoff et al. 2003; Cohen 2006; Minchin et al. 2006). Comprehensive data about Panamanian ascidian communities are necessary to determine whether new species become introduced to the region or if Panamanian ascidians become invasive in other parts of the world.

Baseline data on Panamanian ascidian communities will also make it possible to monitor local changes over time, especially as coastal areas are developed. Currently, the human population of Panama is concentrated in several major cities, with much of the rest of the country remaining undeveloped. The province of Veraguas, on the Pacific coast of Panama (the location of the present study), currently has a population of ~3,000, mostly concentrated in the coastal village of Pixvae (Ibáñez 2005). Other populated sites in the region include a small tourist resort on Isla de Coiba and the Liquid Jungle Lab (LJL) research station on Isla Canales de Tierra (ICT). All other islands are uninhabited. Additional development may occur in this region in the near future. As a result, new ascidian species could be brought to the region through anthropogenic transport vectors (Minchin et al. 2006; Darbyson et al. 2009) and ascidian populations could flourish due to increased pollution and runoff associated with coastal development (Naranjo et al. 1996; Lambert and Lambert 2003; Carman et al. 2007). It is therefore important to assess ascidian communities (as well as other marine communities) in the region while it is still undeveloped. Here, we report on the diversity and abundance of shallow water ascidians found in the southern Gulf of Chiriquí, Panama, in 2008 and 2009.

## Methods

Ascidian surveys were conducted in the southern Gulf of Chiriquí on the Pacific coast of Panama, ~300 km southwest of the Panama Canal (Figure 1; Table 1). In this region, several small islands form a chain extending westward from the mainland to the island of Coiba. The islands are mostly bordered by rocky submarine slopes and are separated from each other by deepwater sand plains (~50 m). Shallow water benthic communities near the islands are dominated by coral, with coral abundance ranging from patchy distribution to well developed reefs (Glynn et al. 1972). The Gulf of Chiriquí is a non-upwelling region with relatively stable water temperatures of 27°C to 29°C (D'Croz and Maté 2004) and a tidal range of 3 to 4 m. The climate is humid-tropical with marked seasonality, including a dry season from mid-December to mid-April and a rainy season from mid-April to mid-December (Guzman et al. 2004).

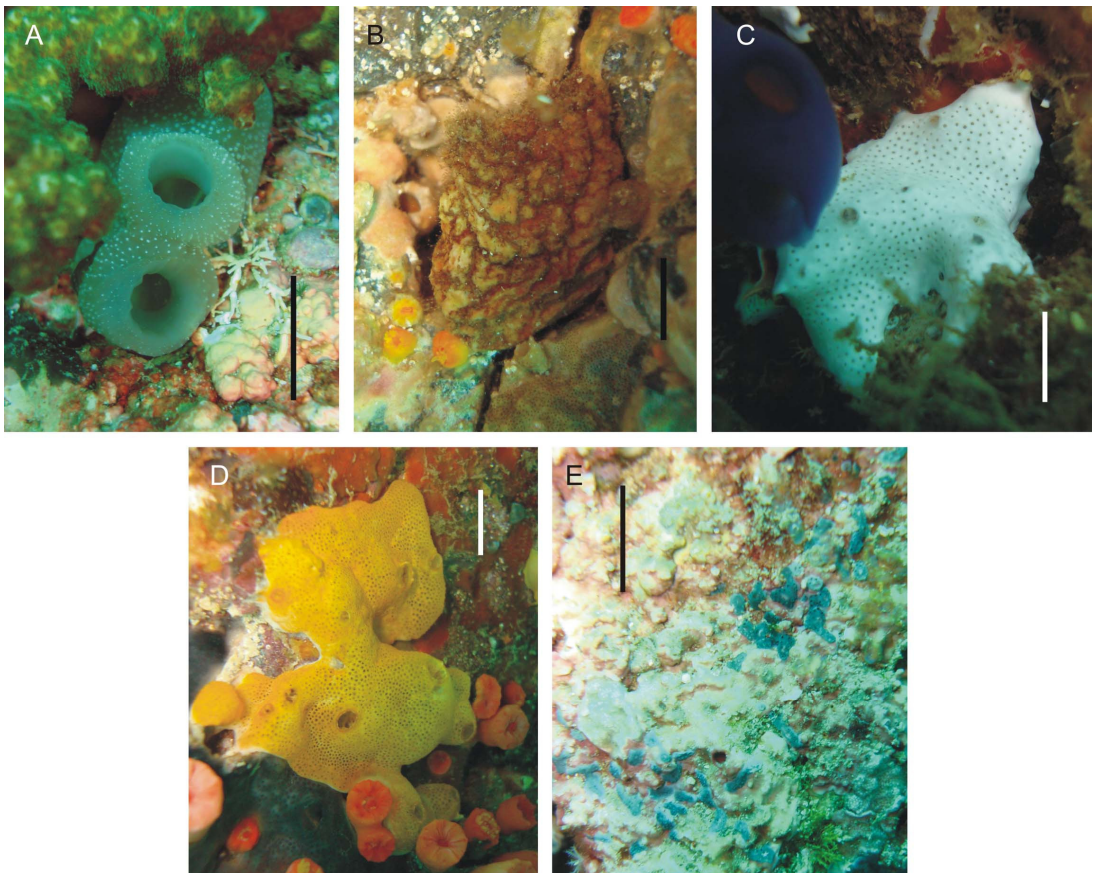
We conducted snorkel and SCUBA surveys in the dry season to assess ascidian communities in the region. In January 2008, we conducted snorkel surveys at nine separate sites at a depth of 2 to 3 m, with three sites each on the islands of ICT, Afuera and Rancheria/Coiba (Figure 1; Table 1). In January 2009, we conducted SCUBA surveys at twelve separate sites at depths of 5 and 12 m, with three sites on the mainland coast and three sites each on ICT, Afuera, and Rancheria (Figure 1; Table 1). Because the three principal islands are roughly the same size and equidistant from each other, our surveys represent a natural experimental transect extending from the mainland to the large offshore island of Coiba. Rancheria/Coiba was treated as a single location during snorkel surveys because the channel between these two islands is relatively shallow and the islands appear to have more subtidal connectivity than other islands in the region; to better match the sites surveyed on the other islands and to avoid potential biogeographical influences of the large island of Coiba, only sites on Rancheria were surveyed during 2009 SCUBA surveys. The Mainland and ICT were treated as distinct sites because even though they are physically close to each other (~0.5 km), they are separated by a deep channel with strong currents and because they may be ecologically distinct due to biogeographical processes. Latitude and longitude were recorded for each site with a handheld GPS unit.



**Figure 1.** Sites surveyed for ascidian diversity and abundance in the southern Gulf of Chiriquí, on the Pacific coast of Panama. The study area is indicated by the box on the inset map of the country of Panama. Within the Gulf of Chiriquí, red circles indicate sites surveyed by snorkel (2 to 3 m depth, 2008), blue circles indicate sites surveyed by SCUBA (5 m and 12 m depths, 2009), purple circles indicate sites surveyed both by snorkel in 2008 and SCUBA in 2009. ICT = Isla Canales de Tierra.

**Table 1.** Locations of tunicate surveys conducted in the southern Gulf of Chiriquí, Pacific Panama (all records are from the present study).

Location	Location in Figure 1	Coordinates		Type of Sampling
Mainland 1	1	7°48'54.0" N	81°35'20.4" W	SCUBA
Mainland 1	2	7°45'06.9" N	81°33'46.1" W	SCUBA
Mainland 1	3	7°43'05.3" N	81°32'13.9" W	SCUBA
ICT 1	4	7°45'12.5" N	81°34'06.8" W	SCUBA
ICT 2	5	7°44'18.0" N	81°34'46.8" W	SCUBA
ICT 3	6	7°45'02.6" N	81°35'25.4" W	SCUBA
Afuera 1	7	7°42'14.0" N	81°37'30.0" W	SCUBA
Afuera 2	8	7°41'20.4" N	81°38'13.2" W	SCUBA
Afuera 3	9	7°41'15.1" N	81°37'23.9" W	SCUBA
Rancheria 1	10	7°38'44.0" N	81°41'22.9" W	SCUBA
Rancheria 2	11	7°37'56.5" N	81°41'43.9" W	both
Rancheria 3	12	7°38'36.2" N	81°43'19.5" W	both
Rancheria/Coiba	13	7°37'50.0" N	81°43'44.1" W	snorkel
Afuera 1 – Snorkel	14	7°42'19.9" N	81°37'59.3" W	snorkel
Afuera 2 – Snorkel	15	7°41'54.3" N	81°37'26.5" W	snorkel
Afuera 3 – Snorkel	16	7°41'36.6" N	81°36'50.3" W	snorkel
ICT 1 – Snorkel	17	7°44'37.6" N	81°34'59.0" W	snorkel
ICT 2 – Snorkel	18	7°44'03.9" N	81°34'58.9" W	snorkel
ICT 3 – Snorkel	19	7°44'39.7" N	81°34'45.2" W	snorkel



**Figure 2.** Ascidians from the southern Gulf of Chiriqui, Panama. Photographs of previously undescribed species and didemnid color morphs. Scale bars indicate 1 cm. **A.** *Ascidia* sp., probably an undescribed species. **B.** *Pyura* sp., probably an undescribed species. **C.** White didemnid. **D.** Orange didemnid. **E.** Grey didemnid. High quality photos were not available for the black colonial (Table 2) or the yellow didemnid morph. The yellow didemnididae looked very much like the white didemnid with bright yellow highlights on portions of its tunic.

At each study site, the ascidian community was assessed by counting the types and numbers of ascidians present along benthic transects. All transects were linear and composed of ten 1-m<sup>2</sup> quadrats. Starting points for transects were determined arbitrarily by a submerged researcher who selected a topographical feature as the starting point. Each transect was completed by sequentially laying ten quadrats (side-to-side) from the starting point along a line of constant depth parallel to the shoreline. A single transect surveyed an area 10 m<sup>2</sup> in size. To survey quadrats, one person held the quadrat in place while a second person recorded data. Quadrat inspection was visual; organisms (e.g., coral) in the quadrats were not disturbed and rocks were not overturned during surveys. Thus, transect data represent a surface layer estimate of

ascidian abundance and diversity. To ensure consistency of depth relative to tidal state, we sampled for three to four hours per day with low tide occurring during the middle of the sampling period. For identification purposes, representative ascidian specimens were photographed *in situ* and voucher specimens collected when possible.

Sampling protocols were slightly different for snorkel and SCUBA surveys. In 2008, snorkel transects were conducted at depths of 2 to 3 m on three islands, with three sites per island and three transects per site, giving a total of 27 transects. For analysis, the mean number of ascidians·m<sup>-2</sup> was determined for each site and an ANOVA was used to assess differences in abundance between islands. In 2009, SCUBA transects were conducted at two depths (5 m and 12 m) on three

**Table 2.** Occurrence of ascidians in the southern Gulf of Chiriquí, Pacific Panama. Ranch = Rancheria and Rancheria/Coiba. A.a. - *Ascidia archaia*, A.sp. - *Ascidia* sp., D.sp1 - *Didemnum* spp. (white), D.sp2- *Didemnum* sp. (orange), D.sp3- *Didemnum* spp. (grey), D4 - Didemnidae (yellow), P.l. - *Pyura lignosa*, P.sp. - *Pyura* sp., R.b. - *Rhopalaea birkelandi*, S.r. - *Symplegma rubra*, E.m. -*Eusynstyela misakiensis*, U.b.c. - unidentified black colonial.

Site	A.a.	A.sp.	D.sp1	D.sp2	D.sp3	D4	P.l.	P.sp.	R.b.	S.r.	E.m.	U.b.c.
<b>SCUBA</b>												
Mainland												
1		•	•	•	•	•		•	•		•	
2		•	•	•	•			•	•		•	
3		•	•	•		•		•	•		•	
ICT												
4							•		•		•	
5		•	•	•	•			•	•			•
6		•	•	•			•	•	•	•	•	•
Afuera												
7	•		•	•				•		•	•	
8			•	•	•							
9			•		•			•				
Rancheria												
10			•	•								
11			•	•	•			•				
12			•	•	•			•	•			
<b>Snorkel</b>												
ICT												
17		•						•	•			
18		•							•			
19		•						•	•			
Afuera												
14												
15												
16												
Rancheria												
11												
12		•						•	•			
13		•							•			

islands and on the mainland coast (four locations in all), with three sites per location and one transect at each depth per site, giving a total of 24 transects. Two-factor ANOVAs were used to examine differences in total ascidian abundance and abundances of individual ascidian species among islands and depths.

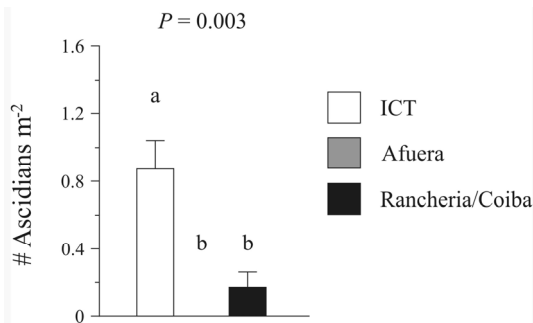
**Results**

Ascidian diversity and abundance varied among sites (Tables 2, 3 and 4). Twelve ascidian taxa were found. Some of these species were previously undescribed (Figure 2). Colonial ascidians in the family Didemnidae were common and were found in several distinct color morphs. Due to the difficulty of identifying didemnids to the species level, it is unclear

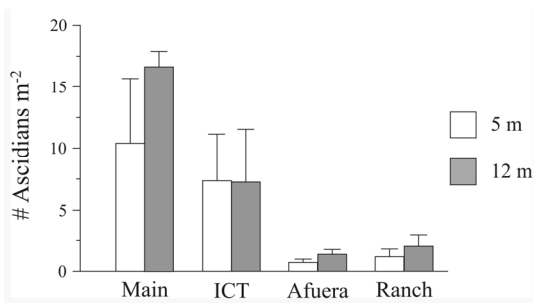
whether these are separate species or different varieties of the same species.

Results of snorkel surveys in 2008 indicated there were differences in ascidian abundance among islands (Figure 3). Ascidians were most abundant on ICT, the island nearest to the mainland, and significantly less abundant on Rancheria/Coiba, the area farthest offshore. No ascidians were found at sites on Afuera. A total of 93 individual ascidians representing three different taxa were observed in snorkel surveys, including *Ascidia* sp. (55% of observed individuals), *Rhopalaea birkelandi* Tokioka, 1971 (40%) and *Pyura* sp. (5%).

SCUBA surveys in 2009 also revealed significant differences in ascidian abundance among locations (Table 5; Figure 4). Ascidians were most abundant on the mainland coast, less



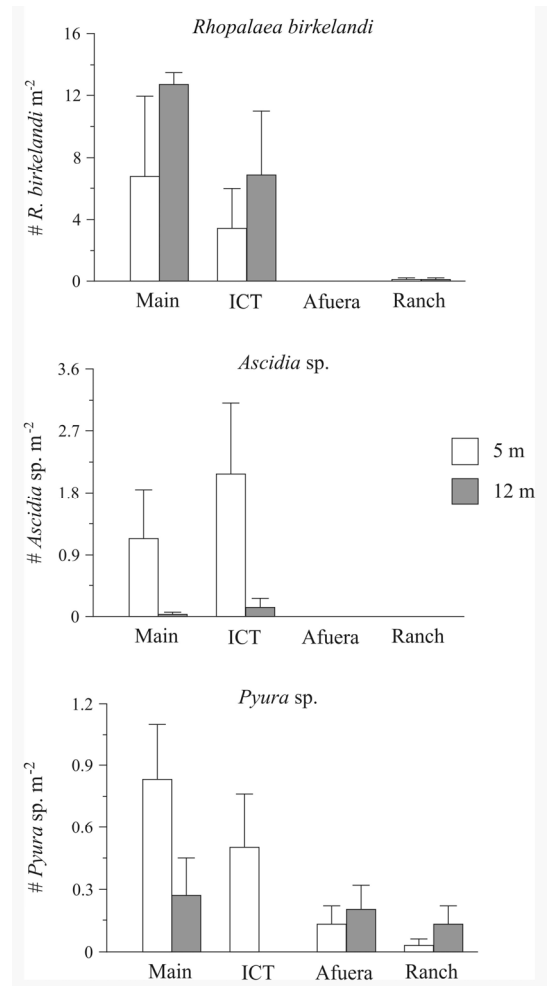
**Figure 3.** Mean abundance of ascidians in year 2008 snorkel surveys (2 to 3 m depth) in the southern Gulf of Chiriquí, Panama. Three sites were surveyed on each island and three 10 m<sup>2</sup> transects were surveyed per site. Statistical analysis = ANOVA.



**Figure 4.** Mean abundance of ascidians in year 2009 SCUBA surveys (5 m and 12 m depths) in the southern Gulf of Chiriquí, Panama (n = 3 sites location<sup>-1</sup>). Three sites were surveyed at each location and one 10 m<sup>2</sup> transect was surveyed at each depth at each site. Ranch = Rancheria. Statistical analysis = two-factor ANOVA.

**Table 3.** Ascidian diversity and abundance observed in snorkel transects in the southern Gulf of Chiriquí, Pacific Panama in year 2008 (9 sites, three 10 m<sup>2</sup> transects surveyed per site).

Species	# of sites observed	# of individuals found
<i>Ascidia</i> sp.	5	51
<i>Pyura</i> sp.	3	5
<i>Rhopalaea birkelandi</i> Tokioka, 1971	5	37



**Figure 5.** Mean abundance of individual species of ascidians in year 2009 SCUBA surveys (5 m and 12 m depths) in the southern Gulf of Chiriquí, Panama. Three sites were surveyed at each location and one 10 m<sup>2</sup> transect was surveyed at each depth at each site. Ranch = Rancheria. Statistical analysis = two-factor ANOVA.

**Table 4.** Ascidian diversity and abundance observed in SCUBA transects in the southern Gulf of Chiriquí, Pacific Panama in year 2009 (12 sites, one 10 m<sup>2</sup> transect surveyed at 5 m and one at 12 m at each site).

	# of sites observed	# of individuals or colonies found
<i>Ascidia archaia</i> Sluiter, 1890	1	3
<i>Ascidia</i> sp.	5	101
<i>Didemnum</i> spp. (white)	11	210
<i>Didemnum</i> sp. (orange)	10	40
<i>Didemnum</i> spp. (grey)	7	48
Didemnidae (yellow)	2	8
<i>Pyura lignosa</i> Michaelsen, 1908	2	2
<i>Pyura</i> sp.	9	63
<i>Rhopalaea birkelandi</i> Tokioka, 1971	7	897
<i>Symplegma rubra</i> Monniot, 1972	2	2
<i>Eusynstyela misakiensis</i> (Watanabe and Tokioka, 1972)	6	28
Unidentified black colonial	2	2

**Table 5.** P-values for two-factor ANOVAs of ascidian abundance in 2009 SCUBA surveys of Pacific Panama (data presented in Figures 4 and 5).

	Location	Depth	Location*Depth
Total Ascidiens	0.001	0.350	0.666
<i>Rhopalaea birkelandi</i>	0.004	0.207	0.587
<i>Ascidia</i> sp.	0.073	0.029	0.126
<i>Pyura</i> sp.	0.049	0.063	0.094

abundant on nearby ICT, and significantly less abundant on Afuera and Rancheria. There was no significant difference in total ascidian abundance between depths (5 m and 12 m) and no significant depth by location interaction. In total, 1404 individual ascidiens were observed in SCUBA surveys. The most abundant species was *R. birkelandi*, which accounted for 63.9% of all ascidiens found and exhibited a significant difference in abundance among locations (Table 5; Figure 5). Other abundant taxa were *Ascidia* sp. and *Pyura* sp. (Figures 2 and 5); *Ascidia* sp. was significantly more abundant at a depth of 5 m than at 12 m and had a near-significant trend ( $P = 0.073$ ) in abundance among locations (Table 5). There was also a near-significant trend ( $P = 0.063$ ) for *Pyura* sp. to be more abundant at 5 m than at 12 m (Table 5). Not all taxa could be identified to the species level, so direct comparisons of species richness among sites was not conducted. In all, eight taxa were found on the mainland, ten on ICT, seven at Afuera and five on Rancheria.

## Discussion

Twelve ascidian taxa were found at island and mainland sites in the southern Gulf of Chiriquí, Panama, with mean densities of up to ~17 ascidiens·m<sup>-2</sup>. Most taxa were found in SCUBA surveys, with only three being found in snorkel surveys, likely because of the shallower depths sampled in snorkel surveys (2 to 3 m) compared to SCUBA surveys (5 m and 12 m) (Menge et al. 1983). The overall diversity found in the present study is similar to that found by earlier studies conducted in Central America. Van Name (1945) documented eight species in Pacific Panamanian waters, mainly on islands in the area of Panama Bay. Tokioka (1972) documented thirteen species in neighboring Pacific Costa Rica, from one location in northern Costa Rica at Playas del Coco. The current work represents the first systematic assessment of ascidian abundance and diversity in Pacific Panama.

The most abundant ascidian in the region was *Rhopalaea birkelandi*, a large (several cm tall),

bright blue solitary ascidian in the family Diazonidae. The formal description of the species is based on specimens from Costa Rica (Tokioaka 1971), suggesting that *R. birkelandi* may be native to the Pacific side of Central America. In the southern Gulf of Chiriquí, the species was very abundant on the mainland coast and on the nearshore island of ICT, but was absent farther offshore on Afuera and present in very small abundances at the farthest offshore sites on Rancheria. There was a tendency for *R. birkelandi* to be more abundant at 12 m depth than 5 m ( $P = 0.207$ ) and SCUBA divers frequently saw larger numbers of *R. birkelandi* at deeper depths than at shallow depths. However, due to the small sample size these observations were not statistically significant.

Two previously undescribed species, *Ascidia* sp. and *Pyura* sp. (Figure 2), were very common (present at 75% and 42% of sites respectively in 2009 surveys) and abundant ( $> 2 \text{ m}^{-2}$  at some sites) in the region. *Ascidia* sp. was absent from one of the three island sites (Afuera), and was significantly more abundant at shallow depths (2 m to 5 m) than at deeper depths (12 m). It is striking that these two large (several cm tall) and abundant species have remained undescribed. This suggests that much more taxonomic work is needed on Panamanian ascidians.

Didemnids were common in the region and were represented by several color morphs (Table 2; Figure 2). Some of these morphs are likely different species, but others (such as the white and yellow didemnids) may be variations of the same species. More work is needed on this complex group.

Ascidian abundances ranged from 0.0 to 1.3 ascidians  $\text{m}^{-2}$  at a depth of 2 to 3 m in snorkel surveys (2008), to 0.4 to 20.9 ascidians  $\text{m}^{-2}$  at 5 and 12 m depths in SCUBA surveys (2009). The sites surveyed were consistently rocky with coral cover and seemed structurally similar, so these differences are most likely due to depth rather than to habitat type (Menge et al. 1983). However, as snorkel and SCUBA surveys are logistically very different from each other, direct comparisons of snorkel versus SCUBA data may be of limited value. The percent cover of ascidians was not specifically determined, but it appeared very low. Most ascidians observed were either isolated solitary individuals or very small colonies. This is not surprising given that ascidian abundance in the tropics is often low. For example, Bailey-Brock (1989) found that ascidians were a minor component of fouling

communities on artificial reefs in Hawaii, and Bak et al. (1996) found only  $\sim 2.5$  colonies  $\text{m}^{-2}$  of *Trididemnum solidum* during an outbreak on reefs in Curaçao. Tropical ascidian abundances can be higher, especially when invasive species become established, as happened in American Samoa (Vargas-Ángel et al. 2008) and in the Netherland Antilles (Sommer et al. 2009).

In both snorkel and SCUBA surveys, ascidians were most abundant on the mainland coast and less abundant on the distant offshore islands of Afuera and Rancheria. This distribution pattern was also true for *R. birkelandi* and *Pyura* sp. These results suggest that island biogeographic processes may be at work in the region with mainland populations serving as the source for island species. This conclusion is further supported by the fact that only six of the twelve taxa observed were present at the farthest offshore sites. Ascidians produce short-lived larvae that do not readily disperse long distances (Svane and Young 1989; Petersen and Svane 1995). Thus, it may be relatively easy for larvae from the mainland to reach ICT, which lies  $\sim 0.5$  km offshore, but difficult for them to reach the more distant islands. Human-mediated transport associated with boat traffic to the tourist resort on Coiba may have helped establish some ascidians at Rancheria. This could explain why some species (e.g., *R. birkelandi*) were present in low abundances on Rancheria, but were absent from Afuera, which is uninhabited and rarely visited.

Increased anthropogenic development along the Pacific coast of Panama may lead to changes in the ascidian communities of the region (Wonham and Carlton 2005). Additional shipping could lead to the introduction of nonnative ascidians (Carlton and Geller 1993; Minchin et al. 2006; Locke et al. 2007), while increased maritime construction (e.g., docks and pilings) could facilitate their establishment (Glasby et al. 2007). The southern Gulf of Chiriquí could be especially prone to invasion because species-poor systems (e.g., islands) are often more susceptible to invasions than species-rich systems (Stachowicz et al. 2002; Stachowicz and Tilman 2005). It is therefore important to monitor the region's habitats and transport vectors so that control efforts can be undertaken if the community structure begins to change or if new species are introduced.

The present study represents a first step in documenting the ascidian communities in Pacific Panama. Similar studies, as well as detailed

taxonomic investigations, should be undertaken in other parts of Central America. Additionally, a monitoring program should be developed to periodically reassess ascidian communities in the region. Future work could also focus on manipulative ecological experiments to determine what underlying mechanisms drive the ascidian distribution patterns found in this study.

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