

ORIGINAL ARTICLE

Adaptation of reef and mangrove sponges to stress: evidence for ecological speciation exemplified by *Chondrilla caribensis* new species (Demospongiae, Chondrosida)

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Caribbean Sea; *Chondrilla caribensis* new species; *Chondrilla nucula*; morphological and molecular characteristics; Porifera ecology; salinity; sedimentation; temperature.

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Abstract

Sponges (Porifera) in mangroves have adapted to a wide range of environmental parameters except for extended periods of exposure to freshwater or air. Many marine mangrove islands are located in the shallow backwaters of coral reefs in Belize and elsewhere in the Caribbean and have a mean tidal range of only 15 cm. They are densely populated by sponges, mostly attached to subtidal red-mangrove stilt roots and peat banks lining tidal channels. Some species are endemic to mangroves, others are immigrants from nearby reefs. Mangrove endemics endure environmental hardships, such as occasional exposure to air during spring tides, temperature and salinity extremes, fine sediments, even burial in detritus. Reef immigrants into mangroves enjoy protection from spongivores that do not stray into the swamp but they eventually succumb to environmental stress. There is evidence exemplified by the common demosponge *Chondrilla* aff. *nucula*, that sponges flourishing in both mangrove and reef habitats may develop separate ecologically specialized and reproductively isolated populations. Such processes can lead to genetic modifications and thus serve as mechanisms for ecological speciation. Because *Chondrilla nucula* Schmidt was first described from the Mediterranean Sea, it was long suspected that the western Atlantic population may be a separate species. New morphological and molecular evidence prompt us to describe it under a new name, *Chondrilla caribensis*, with two ecological forms, forma *caribensis* from mangroves and lagoons, and forma *hermatypica* from open reefs.

Problem

Sponges (Porifera) are a diverse phylum of an estimated 15,000 species, most of which live in our oceans, between the intertidal and hadal zones. They have unusual morphological, anatomical, and ontogenetic plasticity and are therefore difficult to classify with conventional taxonomic methods using color, shape, consistency, skeleton structure, spicules, and histology. Sponges are potent suspension feeders, filtering water up to 160 times their body volume per hour and removing most organic particles

smaller than 5 μm . They harbor a wealth of symbionts and endobionts, from bacteria to fishes, some of which play an important role in the benthos nutrient cycle. In many ecosystems, such as coral reefs, sponges develop substantial biomass and therefore play an important role in the ecology of the communities (for general references see Sarà & Vacelet 1973; Bergquist 1978; Diaz & Rützler 2001; Hooper & van Soest 2002; Rützler 2004).

Basic environmental parameters such as substratum availability, light, temperature, water movement, salinity, sediments, and nutrients have been identified as

determinants of geographic and local distributions (Sarà & Vacelet 1973) but few reports deal with the short- and long-term stress extremes that are common occurrences in tropical shallow-water environments, particularly enclosed inshore waters or lagoons.

Twin Cays in southern Belize are a model marine mangrove ecosystem. This kind of island is common in the Belize barrier-reef lagoon, and throughout the tropical and subtropical western Atlantic. It is also ecologically distinctive from mangroves lining the mainland, which are impacted by freshwater land runoff more than by the sea. Twin Cays and other nearby mangrove islands have been under investigation by scientists associated with the Caribbean Coral Reef Ecosystems Program (CCRE), Smithsonian Institution (Washington, DC, USA) since the early 1980s (Rützler & Feller 1996; Macintyre *et al.* 2004). Several studies addressed specifically the mangrove sponge fauna, its systematics (de Weerd *et al.* 1991; Rützler & Smith 1993; Hajdu & Rützler 1998), distribution (Rützler *et al.* 2000, 2004), and ecology (Rützler 1988, 1995; Wulff 2000, 2004, 2005; Diaz *et al.* 2004), among others. Nearly 180 sponge species were recorded from lagoon environments near Carrie Bow Cay (location of CCRE's Carrie Bow Marine Field Station). Of these, 51% occur only in mangrove channels and ponds, 12% are exclusive to lagoon reefs (patch-reefs, back-reef zones), and 37% are found in both habitats.

The environment in mangrove channels and ponds is generally harsh. Substrata for sponge settlement are rare and exposed to fine sediments, competitive pressure among sessile organisms is high, water movement and

exchange rates are poor, and water quality is impacted by dissolved organics (tannins), suspended sediments, intense solar radiation, and precipitation. On the other hand, some conditions are favorable to sponge growth, such as food supply (bacterioplankton) and protection from storm surge and some predators.

One objective of this study was to identify sponge species that are particularly suited to cope with the mangrove environment and to find the limits of their ecological tolerance. The other was to determine the significance of morphological variation expressed by sponge species that have both mangrove and reef populations, particularly in view of their limited dispersal ability through larvae, which are short-lived and poor swimmers. For the latter aim, the common and ubiquitous demosponge *Chondrilla* aff. *nucula* was singled out for a detailed analysis.

Material and Methods

Study area

This work was conducted at the Smithsonian Carrie Bow Marine Field Station on the Mesoamerican barrier reef in southern Belize (16°48.1' N, 88°04.9' W) and included adjacent reef and lagoon areas and the nearby Twin Cays mangrove (16°49.8' N, 88°06.2' W) (Rützler & Macintyre 1982) (Fig. 1). Twin Cays is a mangrove swamp of nearly 90 ha area with a great variety of aquatic habitats, from deep lagoon and tidal channels to extended ponds, lakes, and intertidal mud flats (Rützler *et al.* 2004). The surrounding lagoon water has full oceanic salinity, like the

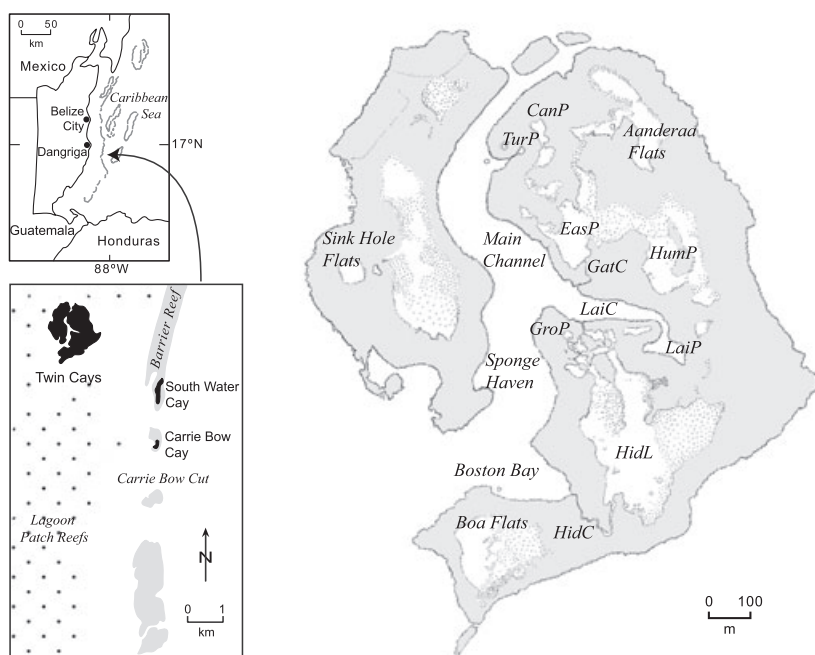


Fig. 1. Location of study sites on Twin Cays, Belize (Central America). For abbreviations see caption of Table 1.

open reef, a mean tide range of 15 cm, and a mean annual temperature range of 26–30 °C. Environmental conditions in the habitats inside the islands vary considerably and are strongly affected by geomorphology, precipitation, evaporation, solar radiation, air temperature, and high rate of sediment production and accumulation. Some observations and collections of sponges were made in the Pelican Cays, a mangrove archipelago about 16 km south of Carrie Bow Cay (16°39.8' N, 88°11.5' W), mainly Cat and Fisherman's cays (Rützler *et al.* 2000).

Sampling and experiments

Sponges described herein are deposited in the collection of the Department of Invertebrate Zoology, National Museum of Natural History (USNM). Observations of anatomy are based on microscope preparations of spicules separated by boiling in concentrated nitric acid and of tissue sectioned after embedding in epoxy resin (Rützler 1978). Scanning electron micrographs of cleaned spicules were taken on a Leica Stereoscan 440 W microscope at primary magnifications of up to 5000×. Terminology follows Boury-Esnault & Rützler (1997).

Habitats were surveyed by wading (at low tide) or snorkeling. Sponge species were recorded and collected noting their substrata and climatic habitat conditions, and position relative to mean low tide level. Tidal signatures were determined *in situ* with calibrated gauges or portable conventional recorders (float and strip chart) and related to data (times and heights) recorded by a pressure probe that is part of the meteorological and oceanographic monitoring system at the Carrie Bow Marine Field Station (Opishinski 2005). Salinity and temperature under extreme conditions (low tide combined with rain or full exposure to solar radiation) were spot-checked using digital thermometer and refractometer. Some locations were monitored at 10 min intervals during entire tidal cycles using a Knick (Berlin) Portamess 913 conductivity and temperature recorder.

Sediment covering live sponges was noted and samples were collected for analysis. Most of the sediment (mud and detritus) was wet-sieved through a series of four sieves with 63 µm to 4 mm mesh diameter; the fraction smaller than 63 µm was concentrated by settling in a measuring cylinder. After rinsing in distilled water, the fractions were dried (on pre-weighed Whatman no. 2 filters) to constant weight at 60 °C. Representative subsamples were fixed in 10% formalin-seawater for microscope examination of the organic and mineral components.

Field and laboratory experiments were carried out to test differences in stress resistance of the sponge *Chondrilla* aff. *nucula* Schmidt (Chondrillidae), a species that has morphologically distinctive populations in mangroves

(and seagrass meadows) of the lagoon and on reefs, <4 km apart. Twenty specimens were collected from each locality, cut to pieces of about the same size (*c.* 1.5 cm³ for the encrusting, 3.5 cm³ for the lobate form) and tied to microscope slides with monofilament fishing line (following the method of Rützler 1995). The slides with sponges were attached to 60 cm long pieces of PVC plumbing pipe and returned to their original habitat for 3 days to heal. After this period, 10 cuttings from the reef were transplanted to the mangrove and 10 from the mangrove to the reef; they were checked daily for a 10-day period. The other set was tested for resistance to sediment burial by placing it into a shallow tray in the laboratory's running seawater system and covering the sponges with a 1-cm layer of mangrove detritus (floc, collected at Grouper Gardens; Fig. 1). Cuttings of other locally abundant sponge species (three each, 3.5 cm³ volume; identically treated and covered by detritus) served as control: *Lissodendoryx* aff. *isodictyalis* (Carter) (Coelosphaeridae), *Biemna caribea* Pulitzer-Finali (Desmacellidae), and *Spongia tubulifera* Lamarck (Spongiidae), which are mangrove species known to us as highly resistant to sedimentation; *Cliona caribbaea* Carter (Clionidae) and *Ircinia felix* (Duchassaing and Michelotti) (Irciniidae), which are reef species (*I. felix*, like *Chondrilla*, has a counterpart in mangroves but the specimens used were collected on the reef, next to one of the experimental *Chondrilla*). Water flow to the experimental tray was just a trickle from an acrylic pipe punctured by small holes not to disturb the detritus cover and to simulate the condition in mangrove ponds. The sponge specimens were examined every morning for signs of decay by briefly lifting them out of the sediment. Water flow from the oscula, a demonstration of pumping activity, was confirmed by placing (by pipette) a drop of fluorescein dye dissolved in seawater over the incumbent pores and observing its expulsion through the nearby oscula.

Results

Field observations

Surveys of 10 principal enclosed swamp habitats, excluding the major channels that are flushed by lagoon water, resulted in the collection and observation of 25 species of common and large-growing sponges (Table 1). These were mainly attached to red-mangrove stilt-root or peat-bank substrata; only a few small crusts were found on calcified seaweed (*e.g.*, *Halimeda* spp.) or covering seagrass blades. Habitats are shallow (10–50 cm; exceptionally to 2 m) enclosed ponds and lakes, *c.* 150 m² to 1.75 ha in area, and narrow (1–2 m) channels connecting them with each other and with the open lagoon. Some channels

Table 1. Common mangrove sponges at Twin Cays (locations see Fig. 1) from habitats with stressful conditions, such as extremes in temperature, salinity, and sediment/detritus exposure (++ buried in flock, + silt-covered, ± partially covered/close proximity).

species	Twin Cays locations	temperature [°C]	salinity [ppt]	sediment/detritus
<i>Cinachyrella apion</i> (Uliczka)	GatC, GroP, HidC	19–30, 20–38, 39	20–38	++
<i>Geodia papyracea</i> Hechtel	GroP, HidC	19–30, 20–38, 39	20–38	±
* <i>Chondrilla</i> aff. <i>nucula</i> ^a Schmidt	LaiP, HidC	19–30, 20–38	20–38	++
<i>Suberites aurantiaca</i> (Duch. & Mich.)	HumP	18, 34, 39	41	++
* <i>Tethya actinia</i> de Laub.	GatC, HidC	19–30, 20–38, 37	20–38	+
<i>Lissodendoryx</i> aff. <i>isodictyalis</i> ^b (Carter)	GatC, GroP, LaiC, LaiP, HidC	19–30, 20–38, 39	20–38	++
<i>Biemna caribea</i> Pul.-Fin.	TurP, EasP, GatC, LaiC, HidC	18–30, 20–38	20–38	++
* <i>Clathria venosa</i> (Alcolado)	HidC	19–30, 20–38,	20–38	±
<i>Mycale microsigmatosa</i> Arndt	GroP, LaiP	31–33, 39	36–37, 38	±
<i>Tedania ignis</i> (Duch. & Mich.)	GatC, LaiC, LaiP, HidC	27–36, 30.5–33, 37	30–34, 36–37	±
<i>Scopalina ruetzleri</i> (Wiedenmayer)	GroP, HidC	19–30, 20–38, 39	20–38, 38	±
<i>Halichondria magniconulosa</i> Hechtel	EasP, HidL, HidC	18–30, 20–38	20–38	+
<i>Chalinula molitba</i> (de Laubenfels)	GatC, HidC	37	20–38	±
<i>Haliclona curacaoensis</i> (van Soest)	TurP, GatC, LaiC, HidC	19–30, 20–38	20–38	+
<i>Haliclona implexiformis</i> (Hechtel)	CanP, GatC, GroP, LaiC, HidC	19–30, 20–38, 39	20–38	++
<i>Haliclona magnifica</i> de Weerd et al.	GatC, HidL, HidC	18–30, 20–38	20–38	++
<i>Haliclona manglaris</i> Alcolado	LaiC, HidC	19–30, 20–38	20–38	+
<i>Haliclona ruetzleri</i> de Weerd	EasP, GatC	18, 37, 38	20–38	+
<i>Haliclona tubifera</i> (George & Wilson)	GatC, GroP, LaiC, LaiP, HidC	19–30, 20–38, 39	30–34, 20–38	++
* <i>Hyrtios proteus</i> Duch. & Mich.	GatC, HidC	19–30, 20–38	20–38	+
* <i>Ircinia felix</i> Duch. & Mich.	TurP, LaiP	30.5–33, 36	36–37	±
<i>Spongia tubulifera</i> Lamarck	HidC	20–38	20–38	+
<i>Dysidea etheria</i> de Laubenfels	GatC, HidC	19–30, 20–38	20–38	+
<i>Dysidea</i> ^b sp.	GatC, HidC	19–30, 20–38	20–38	+
<i>Halisarca</i> ^b sp.	HumP, GroP, HidC	18–30, 20–38, 39, 41	20–41	++

^aNew species, described herein.

^bNew species, to be described in the future.

Average year-around conditions on nearby reefs are taken as reference standard (26–30 °C temperature range, 35–36 ppt salinity range, low rate of fine-carbonates sedimentation, clear oceanic water). Measurements and ranges listed are from spot-checks and short-term recordings (rounded to the nearest °C temperature and unit salinity) made over the past 20 years during or shortly after low tide when water in shallow (<0.5 m) ponds and lakes became exposed to evaporation, freshwater (from rain), or high/low air temperatures and passed through tidal channels.

Species preceded by an *asterisk have populations (or close relatives) on reefs.

Habitat abbreviations are: CanP = Candy's Pond, EasP = East Pond, GatC = Gator Creek, GroP = Grouper Gardens Ponds, HidC = Hidden Creek, HidL = Hidden Lake, HumP = Hummingbird Pond, LaiC = Lair Channel, LaiP = Lair Pond, TurP = Turtle Pond (see Fig. 1).

(Gator and Hidden creeks) are subjected to rapidly moving tidal currents, with water temperature and salinity equal to that of the large ponds (usually quite extreme) when the tide flows out.

Temperatures measured in these extreme sponge habitats range from 19 to 41 °C, salinity from 20 to 38 ppt. Continuous recordings in tidal channels show temperature and salinity increases or drops of 0.5–5° and 1–5 parts per thousand within 5 min when the water flows back from a pond that was subjected to full sun or cold air, evaporation or precipitation during high tide. Normalization can take up to 2 h, after which time the new tidal cycle starts.

Sediment exposure is generally very high, with 1–5 mm thick silt coatings over part of or the entire specimens. Specimens of 10 of the investigated species (marked by ++ in Table 1) were regularly found dislodged from their substrata, fully or partially buried in detritus (floc) but

healthy. Comparison of mangrove bottom types show that sediments of inshore habitats are rich in decomposing plant matter, with small amounts of limestone and inorganic fine silt particles. Lagoon and open channel habitats are more exposed to clay-like mud (see below; Table 2). Although dislodged sponges were regularly found live embedded in floc, they do not survive buried in fine mud.

Chondrilla aff. *nucula*, to be described below as a new taxon, is one of the five common mangrove species from extreme habitats that also occur on the nearby barrier reef and in many reef and lagoon locations throughout the tropical western Atlantic. The others are *Tethya actinia*, *Clathria venosa*, *Hyrtios proteus*, and *Ircinia felix*, which, unlike *Chondrilla*, display only slightly modified phenotypes for the different habitats. *Chondrilla* specimens encountered on the open reef are always thinly

Table 2. Percentage distribution of five size classes of two characteristic types of sediment making up the Twin Cays mangrove bottom.

type, location	weight [%] per fraction				
	>4 mm	>2 mm	>250 μm	>63 μm	<63 μm
floc, Grouper Gardens	18.1	14.1	38.3	27.9	3.6
mud, Sponge Haven	13.1	0.9	26.6	39.9	19.5

encrusting, yellowish green and in fully light- and current-exposed habitats, whereas those in mangrove and other lagoon locations are thickly lobate, cream to purplish- or chestnut-brown, and found on semi-obscure or dark substrata with moderate or nearly absent water-flow.

Experiments and microscopy

Chondrilla specimens from the reef were transplanted to a mangrove channel, adjacent to a peat bank and hanging red-mangrove stilt roots. They remained unchanged for the entire observation period except for one fragment that was lost when it presumably slipped out from under its tie. The PVC support rod kept the fragments away from the sediment bottom, as mangrove stilt roots typically do. Water flow and light levels (under the mangrove canopy) were low. In contrast, mangrove specimens placed on the reef (next to a big boulder of *Diploria* coral, encrusted at the base with a resident *Chondrilla*) were reduced to three over night, and entirely lost on day 4, ostensibly to fish feeding (which was not directly observed during the time it took to install the transplants).

Sponges buried under detritus in trays with slow-running seawater showed unambiguous responses. All three *Ircinia* (reef) fragments had stopped pumping after 24 h and showed signs of decay, putrid smell, and a coating of *Beggiatoa* bacterial filaments; they were discarded before

fouling the water for the other sponges. The remaining specimens of reef *Chondrilla* and *Cliona*, mangrove *Chondrilla*, *Lissodendoryx*, and *Biemna* were puffed up, smelled fresh, and were clearly pumping water, as was confirmed by exposure to fluorescein dye dissolved in seawater. On day 2, two of the *Cliona* (67%) and four of the reef *Chondrilla* (40%) developed the same condition as *Ircinia* and were removed. Day 3 did not reveal changes. On day 4, all remaining specimens of *Cliona* and reef *Chondrilla* were dead and discarded. Mangrove *Chondrilla* fragments seemed perfectly healthy but some of their pore fields were no longer active (no dye intake) and several oscula were contracted in favor of a few that were enlarged and clearly showed dye-marked water flow. The control fragments of *Lissodendoryx*, *Biemna*, and *Spongia* too were healthy and active: all specimens of the former two species had attached to or incorporated leaf fragments from the detritus cover. This condition remained unchanged until the last check on day 10.

Microscope observations revealed closure or inactivation of pore fields and oscula compromised by fine sediments. In buried *Lissodendoryx*, *Biemna*, and *Chondrilla*, new oscula were formed in areas of presumably better microclimatic conditions than those of the old, inactivated ones. *Biemna*, in places, developed short stolons with distal oscula to improve circulation. *Chondrilla*, both from the mangrove and from the reef, became blotchy in color from local loss of symbiotic cyanobacteria.

Microscope examination of sediment samples (Fig. 2; Table 2) revealed that floc from inshore ponds (Grouper Gardens; Fig. 3) is composed of large organic flakes (mangrove leaf and root fragments) and calcareous chips derived from *Halimeda* algae, with a medium and small fraction of detritus, with a few calcareous particles. The silt- and clay-sized fractions (<63 and <4 μm) contain detritus and calcareous debris. Sediment from the open

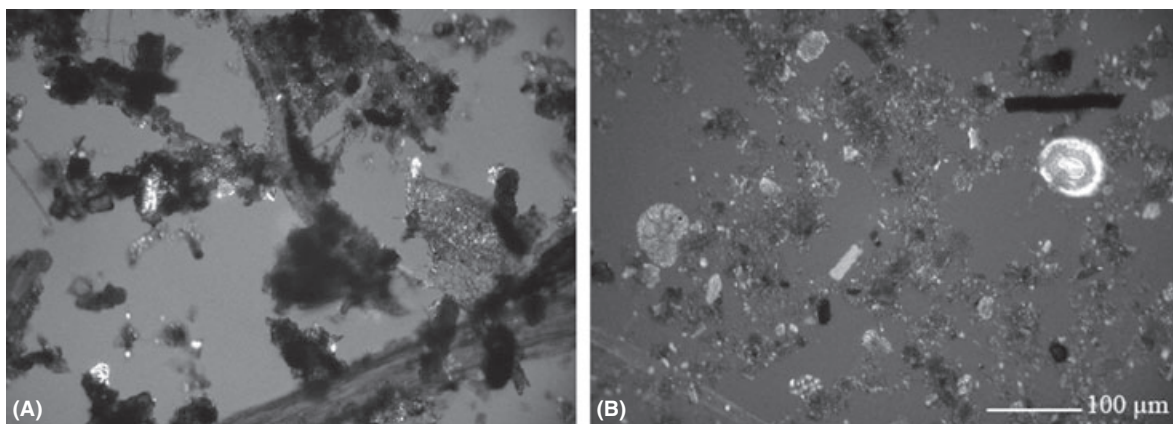


Fig. 2. Light micrographs (polarized) of typical mangrove-bottom sediments. (A): Detritus (floc) from Grouper Gardens, and (B): mud from the Main Channel (Sponge Haven).

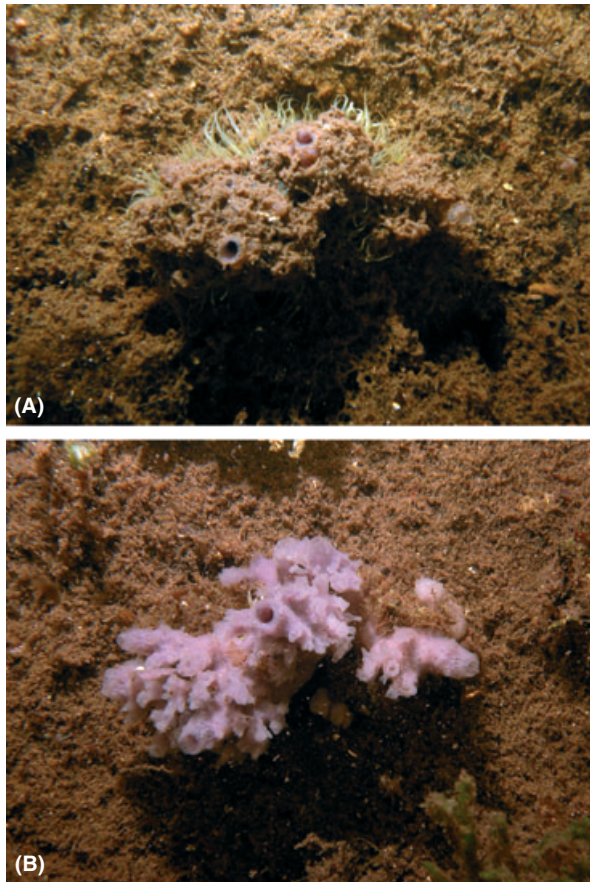


Fig. 3. Underwater photograph of *Haliclona* aff. *tubifera* at Grouper Gardens in 0.3 m; the skeleton of the sponge agrees with typical members of the species, the unusual lateral extensions may be a reaction to the buried state, to increase incurrent surface area. (A): Undisturbed specimen (tentacles of sea anemone, *Aiptasia tagetes*, are protruding in the rear), (B): the same sponge after clearing off sediment. (0.7 \times .)

channel and lagoon (Sponge Haven) consists of *Halimeda* chips and a large percentage of calcareous sand and silt, and calcareous and non-calcareous, possibly volcanic clay. Both sediment types are permeated by cyanobacterial and bacterial filaments, particularly in the surface layer where bacterial mats are formed; the channel mud in particular is rich in mucous substance.

Description of *Chondrilla caribensis* new species

Research by previous authors (Klautau *et al.* 1999; Usher *et al.* 2004) and our field and laboratory observations, including DNA analysis of reef and mangrove populations (Duran & Rützler 2006), convinced us that the common tropical western Atlantic *Chondrilla* species known as *Chondrilla nucula* Schmidt, a name first applied to a similar-looking sponge from the Adriatic Sea, is a separate taxon and deserves a new name.

Order Chondrosida Boury-Esnault & Lopès

Family Chondrillidae Gray

Demospongiae with cortex of fibrillar collagen and pori arranged in sieves. All genera rich in collagen, with or without skeleton; when present, skeleton made up by siliceous astrose microscleres or nodular spongin fibers (see also Boury-Esnault 2002).

Genus *Chondrilla* Schmidt

Chondrillidae with a siliceous skeleton of astrose spicules located in the cortex and lining the aquiferous canals. Type species: *Chondrilla nucula* Schmidt 1862, Gulf of Kvarner (Quarnero), Adriatic Sea.

Chondrilla caribensis new species

(Table 3, Figs 4–7)

Diagnosis: *Chondrilla* of drab to dark brown color and globular to cushion-like, lobate growth (lagoon and mangrove population), or yellow-green, thinly encrusting (reef form). With spherasters and spheroxyasters as spicules, ornated by secondary spination (microspination) on the tips of the primary spines. Spicule total diameter (means for the entire population) is 28.5 μm , with spherasters and oxyspherasters (combined) having a mean center diameter of 65.5%, and a mean number of 25 spines. Spherasters concentrated in the cortex and along choanosomal canals, dispersed in the choanosome. Spheroxyasters, if present, dispersed in the choanosome.

Distribution: Tropical and subtropical western Atlantic, from Bermuda to Brazil.

Etymology: Named after the Caribbean Sea (*sensu lato*) where most observations on the species were made.

Comments: The closest relative is *Chondrilla nucula* Schmidt, described from many locations in the Mediterranean Sea. This species looks very similar to the calm-water form of *C. caribensis*. Molecular evidence (see Discussion), subtle morphological differences, and geographic separation suggest that the Caribbean population belongs to a different species. Specimens of *C. nucula* do not express a very thin encrusting form, have only spherasters, no spheroxyasters for spicules (some spherasters may be reduced to lumpy spheres), a smaller average spicule size (*c.* 24 μm , compared with 29 μm in *C. caribensis*), and fewer spines (average 20, *versus* 25); spicules of *C. nucula* lack secondary microspination.

We distinguish two infraspecific forms based on subtle morphological differences specified below: forma *caribensis*, the typical expression in the mangroves, and forma *hermatypica*, the crustose type found on the reefs. Molecular genetic characterization of these taxa is presented elsewhere (Duran & Rützler 2006).

Table 3. Comparison of taxonomic characters in different populations of *Chondrilla*. Range of spicule dimensions was measured from light-microscope slides or SEM images (indicated); mean values and SD are calculated where possible. The ratio of core to total diameter is given, the number of spines estimated from counts on SEM images (including estimated number of hidden spines from the lower surface of each spicule). Microspination consists of minute secondary spines that often can only be resolved by SEM; any uncertainties are indicated by a question mark (?). Spheroxyasters (with a centrum of <50% of total diameter) are present in some specimens. (+ present; – absent; n.a. = not applicable.)

species/form	USNM, field no., locality	spherasters			oxy-spherasters	
		diameter [μm] range (mean \pm SD)	ratio [%] range (mean)	spines [n] range (mean)	micro- spination	
Mediterranean Sea						
<i>Chondrilla nucula</i> Schmidt	Gulf of Kvarner (Schmidt 1862)	20.3–27.0	n.a.	n.a.	–	–
	Lesina (= Hvar, Croatia) (von Lendenfeld 1897)	23–28 (25)	57–64 (n.a.)	~24 (n.a.)	–	–
	Lesina (= Hvar, Croatia) (slides von Lendenfeld collection)	17.5–27.5 (24.1 \pm 0.6)	56–70 (62)	n.a.	–	–
	Gulf of Naples (Vosmaer 1933)	2–34 (25)	n.a.	n.a.	–	–
	USNM 23955 ^a Rovinj, Croatia	13.0–35.0 (27.3 \pm 1.2)	55–77 (65)	17–28 (22)	–	–
	USNM 23895 Rovinj, Croatia	12.5–30.0 (23.7 \pm 0.6)	54–90 (61)	n.a.	–	–
	Portofino, Italy (Bavastrello <i>et al.</i> 1993)	20–28 (25)	50	n.a.	–	–
	Portofino, Italy (Bavastrello <i>et al.</i> 1993, Fig. 3) ^a	10.5 ^b –25.0 (14.8 \pm 2.0)	100–98 (67)	0–23 (18)	–	–
	mean of means	23.5 \pm 1.5	64	20	–	–
Caribbean Reef						
<i>Chondrilla aff. nucula</i> (= <i>Chondrilla caribensis</i> n. sp. forma <i>hermatypica</i>)	USNM 1092268 – Florida Keys, Coral Gardens	22.5–28.75 (24.4 \pm 0.4)	55–84 (73)	n.a.	+	–
	USNM 1092269 – Florida Keys, Coral Gardens	15.0–37.5 (29.5 \pm 1.6)	59–80 (69)	n.a.	+	–
	USNM 1092270 – Bimini, Turtle Rocks	17.5–32.5 (26.2 \pm 0.9)	38–80 (66)	n.a.	+	+
	USNM 1092271 ^a – Harrington Sd., Bermuda	19.4–30.0 (25.1 \pm 0.8)	53–90 (62)	15–26 (19)	+	–
	USNM 1092272 ^a – Virgin Islands, St John	30.0–32.9 (30.3 \pm 1.3)	66–77 (70)	22–31 (28)	+	–
	USNM 1083003 – Belize, Carrie Bow	23.7–36.25 (32.8 \pm 0.7)	61–75 (68)	n.a.	+	–
	USNM 1083002 ^a – Belize, Carrie Bow	12.8–36.1 (29.3 \pm 1.6)	64–82 (76)	21–31 (27)	+	–
	USNM 1083001 – Belize, Carrie Bow	22.5–35.0 (29.2 \pm 0.8)	62–80 (67)	n.a.	+	–
	USNM 1083000 ^a – Belize, Carrie Bow	12.0–31.0 (26.9 \pm 0.9)	35–87 (65)	12–30 (25)	+	+
mean of means	28.2 \pm 0.9	68	25	9+/0–	2+/7–	
Caribbean Mangrove						
<i>Chondrilla aff. nucula</i> (= <i>C. caribensis</i> n. sp. forma <i>caribensis</i>)	USNM 1092273 – Florida Keys, Mangroves KML	22.5–27.5 (24.9 \pm 0.3)	45–67 (58)	n.a.	+	+
	USNM 1092274 – Florida Keys, Mangroves KML	22.5–27.5 (25.3 \pm 0.4)	50–78 (56)	n.a.	+	+
	USNM 1092276 – Panama, Bocas del Toro	15.0–28.8 (25.1 \pm 0.8)	41–78 (66)	n.a.	+	+
	USNM 1092277 – Panama, Bocas del Toro	22.5–30.0 (27.1 \pm 0.5)	47–70 (61)	n.a.	+	+
	USNM 1092278 – Panama, Bocas del Toro	30.0–40.0 (34.7 \pm 0.5)	60–77 (67)	n.a.	+	–
	USNM 1092275 – Belize, Manatee Lagoon	25–37.5 (32.6 \pm 0.7)	58–77 (66)	n.a.	+	–
	USNM 1082998 ^a – Belize, Twin Cays	21.5–38.0 (31 \pm 1.3)	61–78 (69)	22–30 (25)	+	–

Table 3. (Continued).

species/form	USNM, field no., locality	spherasters			oxyspherasters	
		diameter [μm] range (mean \pm SD)	ratio [%] range (mean)	spines [n] range (mean)	micro- spination	
	USNM 1082999 – Belize, Boston Bay	17.5–30.0 (24.7 \pm 0.7)	41–71 (57)	n.a.	?	+
	USNM 1083004 – Belize, Manatee Lagoon	25–37.5 (31.9 \pm 0.7)	50–82 (64)	n.a.	+	+
	USNM 1083005 – Belize, Cat Cay	22.5–40 (34.0 \pm 1.2)	60–89 (73)	n.a.	+	–
	USNM 1083006 – Belize, Cat Cay	17.5–32.5 (26.0 \pm 0.7)	43–78 (52)	n.a.	+	+
	USNM 1092279 – Belize, Cat Cay	17.5–36.3 (28.8 \pm 1.1)	64–81 (70)	n.a.	+	–
mean of means		28.8 \pm 1.1	63	25	11+/0–	7+/5–

^aFrom SEM images.^bMany are mere spheres with bumps.

Holotype: USNM 1082998 (forma *caribensis*); Twin Cays, Belize (16°49.8' N, 88°06.2' W); coll. K. Ruetzler, 17 Apr 2005; color: dark brown; habitat: Sponge Haven cove, on red-mangrove stilt root, 0.5 m. *Paratypes*: USNM 1082999 (forma *caribensis*); Twin Cays, Belize (16°49.8' N, 88°06.2' W); coll. K. Ruetzler, 27 July 2003; color: dark brown to drab; habitat: Boston Bay, on red-mangrove stilt root, 0.5 m. USNM 1083000 (forma *hermatypica*); Carrie Bow Cay, Belize (16°48.1' N, 88°04.9' W); coll. K. Ruetzler, 16 Apr 2005; color: yellowish green; habitat: patch reef off southern point of Carrie Bow Cay, on the base of coral boulder, 1.0 m. USNM 1083001 (forma *hermaty-*

pica); Carrie Bow Cay, Belize (16°48.1' N, 88°04.9' W); coll. K. Ruetzler, 25 July 2003; color: yellowish green; habitat: patch reef off southern point of Carrie Bow Cay, on the base of coral boulder, 3.0 m.

Other material: USNM 1092271; Hall Island, Harrington Sd., Bermuda (32°21.9' N, 64°40.7' W); coll. K. Ruetzler, 17 Aug 1973; color: chocolate brown with purple fringe, whitish along sides, whitish oscula; habitat: cave, west of island, 1 m. USNM 1092270; Turtle Rock, Bimini, Bahamas (25°67' N, 79°15' W); coll. M. Becerro & S. Duran, 22 Jun 2004; color: green; habitat: coral rock, 5 m. USNM 1092268; Coral Gardens, Florida Keys (24°50.229' N,

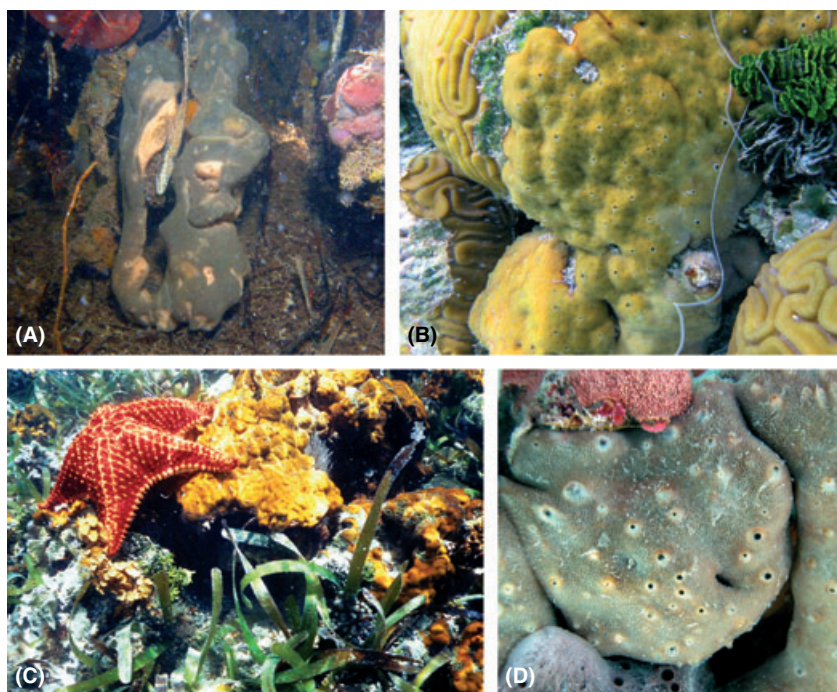


Fig. 4. Underwater photographs of *Chondrilla caribensis* new species. (A): Forma *caribensis* attached to red-mangrove stilt root (0.5 \times). (B): Forma *hermatypica* overgrowing *Diploria* coral head (0.5 \times). (C): Forma *hermatypica* cementing recently dead *Agaricia* coral rubble in the Pelican Cays, southern Belize; with *Oreaster reticulatus*, a predator starfish (0.4 \times). (D): Forma *hermatypica*, close-up of oscula and pore fields (1.0 \times).

Fig. 5. Light micrographs of *Chondrilla caribensis* new species. (A): Osculum surrounded by incurrent pore field. (B): Cross-section (sponge surface to the left). Ca: aquiferous canal, Ch: choanocyte chambers, Co: cortex with spherasters, Cy: cyanobacteriocytes.

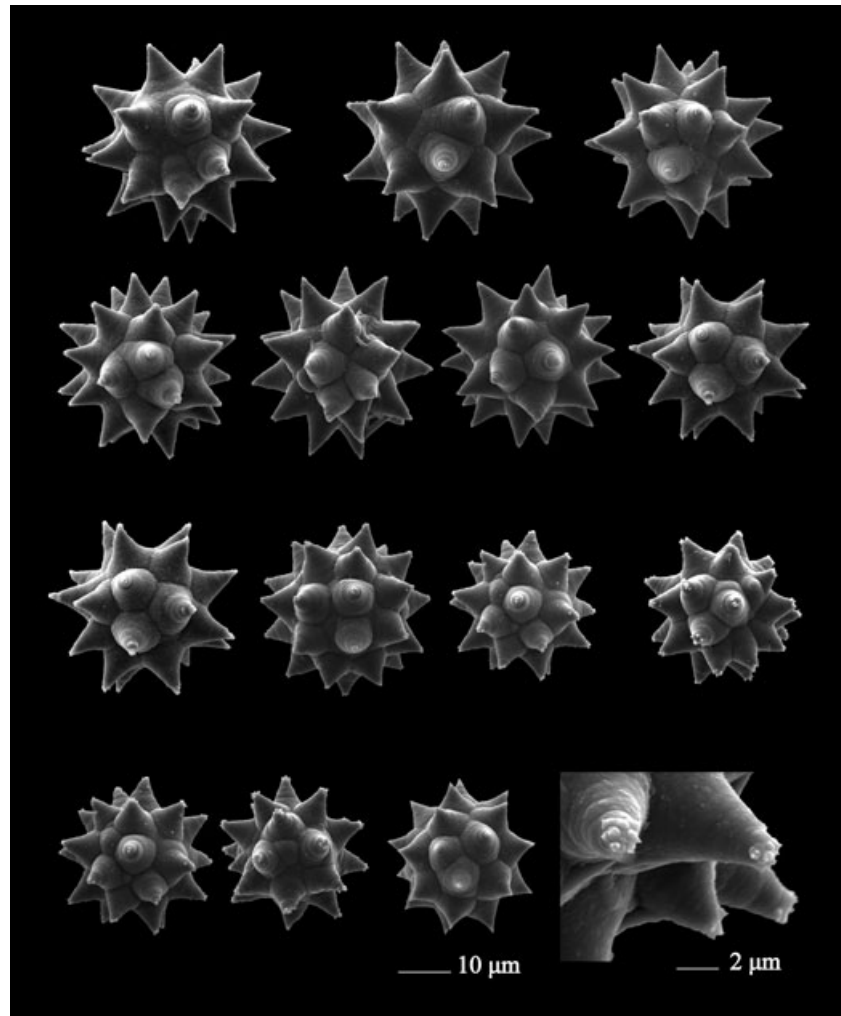
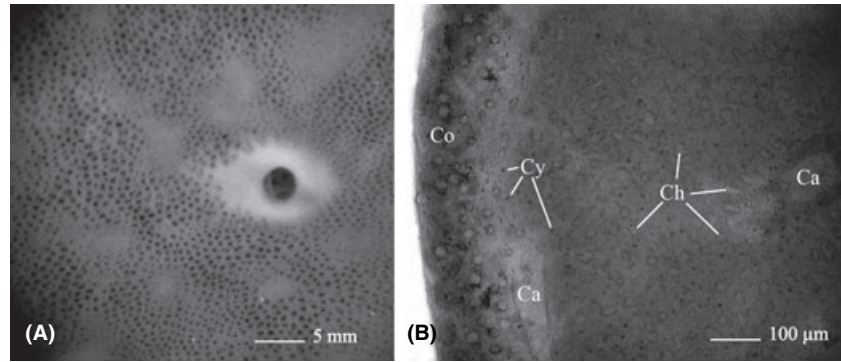


Fig. 6. SEM micrographs of spicules, *Chondrilla caribensis* new species, forma *caribensis* (holotype, USNM 1082998); inset: magnified tips of microspined rays.

80°43.769' W); coll. M. Becerro & S. Duran, 29 May 2004; color: dark brown; habitat: coral rock, 3 m. USNM 1092269; Coral Gardens, Florida Keys (24° 50.229' N, 80°43.769' W); coll. M. Becerro & S. Duran, 29 May 2004; color: dark brown; habitat: coral rock, 3 m. USNM 1092274; Florida Keys; coll. M. Becerro & S. Duran, 30

May 2004; color: green; habitat: mangrove, near Florida Keys Marine Lab, 0.5 m. USNM 1092272; Current Hole, St. John, Virgin Islands (18°20' N, 64°44' W); coll. K. Ruetzler, 4 Apr 1967; color: grayish brown; habitat: coral rock, 15–20 m. USNM 1083002; Carrie Bow Cay, Belize (16°48.1' N, 88°04.9' W); coll. K. Ruetzler, 23 Apr 2005; color: blackish

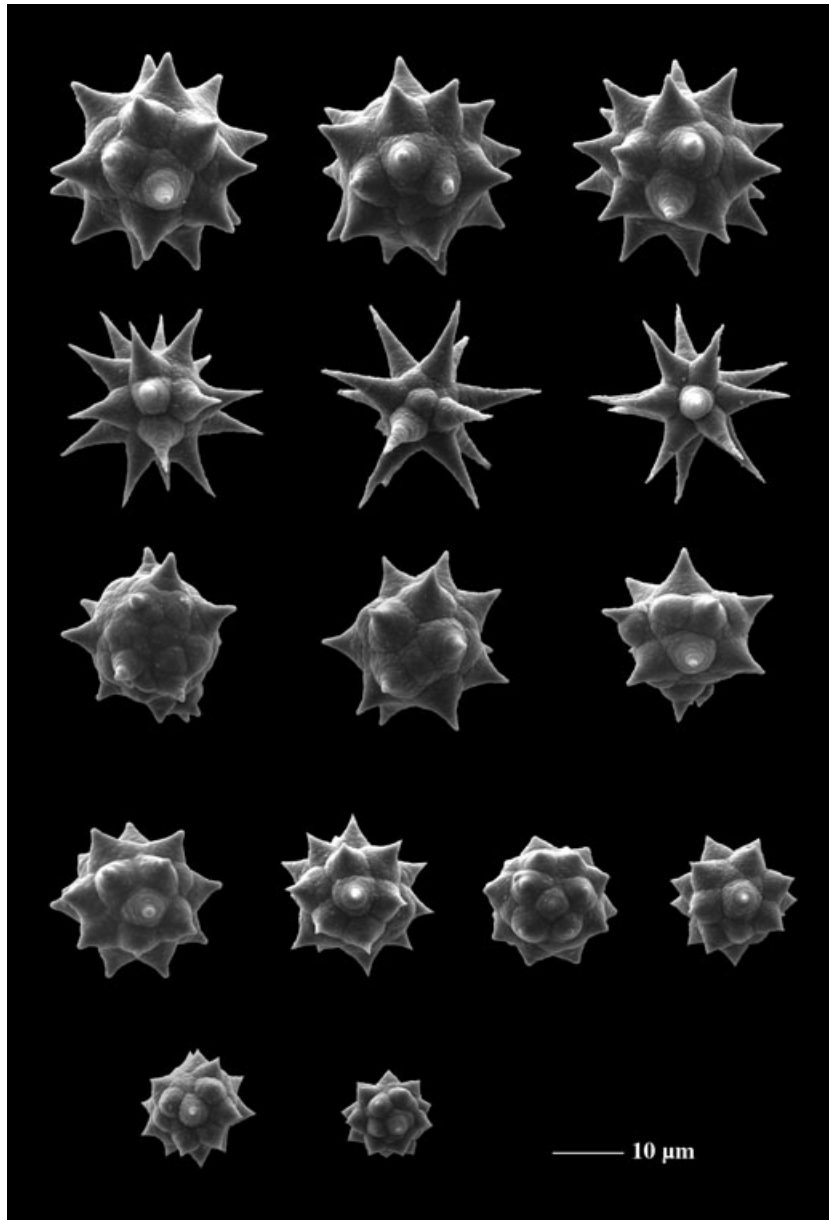


Fig. 7. SEM micrographs of spicules, *Chondrilla caribensis* new species, forma *hermatypica* (paratype, USNM 1083000); inset: magnified tips of microspined rays.

brown; habitat: dock, west Carrie Bow Cay, on concrete pillar, 0.8 m. USNM 1083003; Carrie Bow Cay, Belize (16°48.1' N, 88°04.9' W); coll. K. Ruetzler, 24 July 2003; color: blackish brown; habitat: dock, west Carrie Bow Cay, on concrete pillar, 0.8 m. USNM 1092275; Manatee Cay, Pelican Cays, Belize (16°40.047' N, 88°11.530' W); coll. M. Becerro & S. Duran, 3 Aug 2004; color: gray; habitat: mangrove pond, 0.5 m. USNM 1083004; Manatee Cay, Pelican Cays, Belize (16°39.8' N, 88°11.5' W); coll. K. Ruetzler, 28 July 2003; color: dark brown to drab; habitat: mangrove pond, on red-mangrove stilt root, 0.7 m. USNM 1092279; Cat Cay, Pelican Cays, Belize (16°39.8' N, 88°11.5' W); coll. K. Ruetzler, 4 Aug 1997; color: reddish brown; habitat:

coral rock, <1 m. USNM 1083005; Cat Cay, Pelican Cays, Belize (16°39.8' N, 88°11.5' W); coll. K. Ruetzler, 28 July 2003; color: light greenish brown; habitat: coral ridge, on coral rock and rubble, 1.0 m. USNM 1083006; Cat Cay, Pelican Cays, Belize (16°39.8' N, 88°11.5' W); coll. K. Ruetzler, 28 July 2003; color: blackish (mottled) to greenish brown; habitat: coral ridge, on coral rock and rubble, 1.0 m. USNM 1092276; Bocas del Toro, Panama (8°56.6' N, 82°01' W); coll. S. Duran, 8 February 2005; color: gray; habitat: mangrove, 0.5 m. USNM 1092277; Bocas del Toro, Panama (08°56.6' N, 82°01' W); coll. S. Duran, 8 February 2005; color: gray; habitat: mangrove, 0.5 m. USNM 1092278; Bocas del Toro, Panama (08°56.6' N,

82°01' W); coll. S. Duran, 8 February 2005; color: gray; habitat: mangrove, 0.5 m.

Chondrilla caribensis forma *caribensis*

(Table 3, Figs 4A and 6)

Color and morphology: Generally grayish to chestnut and purplish brown, some specimens are cream to greenish gray; darkest where illumination is strongest and symbiotic cyanobacteria numerous and dense (cream-colored specimens occur in dark caves). Chlorophyll and phycobilin concentrations in these symbionts determine the color variations. Thickly encrusting (to 1.6 cm) and lobate, commonly extending over an area of 1–200 cm² (a few as much as 900 cm²) and covering mangrove roots, peat banks, or lagoonal rock, or bases of seaweed such as *Halimeda* spp. Surface smooth and shiny; extended pore-sieve areas; sporadic oscular openings (fewer than 1 per 3 cm² surface area) on highest surface elevations (lobes), often distinctive because of paler areas around them. Undisturbed oscula measure 0.5–4 mm in diameter and may show a delicate membranous collar; many are closed during periods of high sediment exposure. Individual pori are 100–500 µm wide when observed alive; they are confined to pore sieves where they are 60–1500 µm apart.

Consistency: Slippery to the touch; firm but elastic, like cartilage, tough to tear; similar to firm liver (hence the vernacular name, chicken liver sponge).

Anatomy: A 5-µm cuticle covers the ectosome which is 200–350 µm thick. The outer 50–80 µm ectosomal layer is a cortex reinforced by a concentration of spicules (100–340/mm² cross section), spherulous cells, and symbiotic cyanobacteria. The inner layer has fewer spicules and scattered spherulous cells and cyanobacteria. Pore canals (fixed material) measure 20–25 µm in diameter. Choanocyte chambers (in fixed 1 µm sections) have a range of 33 × 25–50 × 30 µm; they show 17–25 cells per section.

Spicules: Spherasters (centrum >50% of total diameter) average 29 µm in overall diameter. Their spines (15–31, average 25) may be rough or serrated, or beset by secondary microspines. Spheroxyasters (centrum <50%) are present in 22% of specimens examined; they too are microspined. Spherasters are condensed in the cortex and along the linings of the aquiferous canals. Spheroxyasters occur in the choanosome, by themselves or mixed-in with spherasters.

Habitat: Solid substrata such as rock, conch shells, red-mangrove stilt roots, peat banks, and seaweed in the shallow barrier-reef lagoon (0.2–4 m). Specimens can survive for months after becoming dislodged from their support and lying on the mangrove bottom covered by detritus. Specimens are found in locations with great sediments exposure and substantial salinity and temperature fluctuations.

Comments: Wiedenmayer (1977) called this form the amorphous habit *a* of the Western Bahamas population of '*C. nucula*'.

Chondrilla caribensis forma *hermatypica* new form

(Table 3, Figs 4B–D and 7)

Color and morphology: Yellowish brown to olive, darker brown along the growth edge, lighter areolas surrounding the oscula. Very thinly (0.8–3 mm) encrusting, continuously and uniformly covering coral-rock areas of 0.1–0.5 m² and more. Shiny slick surface interrupted by fairly regularly spaced oscula, *c.* 1 per cm² and all open (0.5–2.5 mm diameter) and apparently continuously active. Pori (100–300 µm diameter each) occur in extended sieve patches between the oscular mounds.

Consistency: Slippery and tough cartilaginous; tougher than forma *caribensis*.

Anatomy: The anatomical structure is very similar to that described above but the cortex is only 15–20 µm thick, with 35–50 spicules mm⁻² cross section, and at least some specimens lack spicules in the choanosome. Chamber size and number of choanocytes per cross section are within the ranges of the above.

Spicules: Spherasters have a mean diameter of 28 µm, very similar to the mangrove form. They too have secondary microspination. Spheroxyasters, also microspined, occur in the choanosome of 58% of specimens. Spine number (from one specimen with SEM images) ranges from 15 to 30, mean 25.

Habitat: Coral rock, 0.5–6 m depth, in shallow, exposed reef areas.

Comments: Wiedenmayer (1977) called this the thinly encrusting habit *b* of '*C. nucula*' in the Western Bahamas. Many coral heads, for instance species of *Diploria* and *Montastraea*, are partly covered by this form and progress in overgrowth was noted during periods of unusual water warming which apparently stressed the scleractinians but not the sponge. Following a water-warming and coral bleaching event in the Pelican Cays in 1998, encrusting *Chondrilla caribensis* overgrew large stands of recently dead corals (species of *Agaricia*, *Porites*, *Acropora* and *Millepora*; Macintyre, by Macintyre *et al.* 2000). One noticeable benefit was that the sponge solidified the substrata and facilitated recolonization by coral recruits (authors' unpublished observations).

Discussion

Ecological observations on mangrove sponges

Firm substrata suitable for sponge colonization and relatively clear of sediments are rare in the detritus-based mangrove swamp ecosystem. They are primarily provided

by red-mangrove stilt roots and peat banks lining tidal channels and inshore ponds and lakes; only one species, the r-strategist *Mycale microsigmatosa*, was occasionally found encrusting *Thalassia* seagrass blades. Usable surface area is limited by the muddy channel bottom below and the low-tide level above, because most of these sessile filter feeders seem unable to protect themselves from clogging sediments and from desiccation at low tide. Closer study of resistance to temporary exposure to air, however, revealed unexpected morphological and physiological adaptations (Rützler 1995). And now we find that certain sponges that settled on plant matter or had fallen off rotted roots and became buried in mud developed mechanisms that allow their survival despite submergence in sediment.

Sponges in calm-water environments are particularly sensitive to fine sediments because they inevitably clog inhalant pores, and even exhalant oscules; hydraulic pressure generated by the choanocytes is generally not strong enough to clear particles covering the openings or lodged in the vestibules. The detritus (floc) material characteristic of many muddy bottoms in inshore mangrove channels and ponds has much lower specific gravity than the carbonate silt or clay particles that abound in the main channel and the surrounding lagoon. Although mineral fine sediments occur in small amounts mixed in with floc, the detritus particles tend to cake much less and allow water to flow among them. Furthermore, some indigenous mangrove sponges seem to be able to develop, when buried, complex surface protuberances which increase the inhalant surface area (e.g., *Haliclona* aff. *tubifera*; Fig. 3). Others, such as *Chondrilla* change their anatomy by closing compromised pores and oscula in favor of new functional canals and openings in other parts of the body. This organizational plasticity is due to the capacity for cell transformation and mobility and to the absence of permanent histological systems in sponges (Bond 1992; Gaino *et al.* 1995). More specialized morphological and physiological adaptations to prevent clogging, such as branched or cylindrical body plan, special localization of pores and oscula, and strong water-pumping pressure enhanced by cyclic on-off flow (Reiswig 1971; Rützler 1997; Bell 2004), were not present in the species we studied. One possible escape mechanism from a threatening condition, in *Chondrilla* at least, is fragmentation and reattachment elsewhere, and some active although restricted locomotion (Bond & Harris 1988; Pronzato 2004; Usher *et al.* 2004).

Temperature and salinity are important factors determining geographic distribution of sponges and can be limiting at any given location. A few cold-water haplosclerid species form asexual reproductive bodies to overcome periods of adverse condition, as freshwater sponges

do, but little reliable information is available about the function of 'gemmules' in the few tropical marine species from which they were reported; some are probably a misinterpretation of large, incubated larvae (e.g., in *Ulosa ruetzleri*; Wiedenmayer 1977). An exception is the occurrence of 'resting bodies' in certain hadromerids (Clionidae, Suberitidae), encapsulated clusters of archaeocytes (Simpson 1984). From a few older studies we know that some tropical sponges can tolerate a temperature range of 18.6–35 °C, for instance, the reef sponge *Iotrochota birotulata* in the Dry Tortugas (Florida) (de Laubenfels 1932), and salinity extremes with a long-term low of 20 ppt (some species of Clionidae and Suberitidae) and a high of 46 ppt (the commercial sponge *Hippospongia lachne*, Spongiidae) (Hartman 1958; Storr 1976). Interestingly, the highest tolerance for low salinity occurs among the Hadromerida (Clionidae, Suberitidae), whereas the pure freshwater sponges (Spongillida) belong to the order Haplosclerida. Suberitids (with resting bodies) are also known to occur under extreme hydrochemical conditions in a tropical marine volcano lake (Satonda, Indonesia), an enclosed water body with double normal alkalinity (compared with Standard seawater), and low salinity (31 ppt) and high temperature (31 °C) in the top 5 m zone where sponges occur (Arp *et al.* 1996).

The temperature extremes registered by us for mangrove habitats (18–41 °C) are records of highs and lows for sponges but are only of brief duration, several hours at a time. The same is true for salinity (20–41 ppt), only exceeded (at the low end) by near-freshwater conditions in Chilka Lake (Bay of Bengal, India) where clionids and suberitids co-exist with spongillids (Annandale 1915), and (at the high end) by a value of 46 ppt at commercial sponge grounds in the Bahamas (Storr 1976); the latter may have triggered a commercial-sponge epidemic.

Comparing our 25 species with those from other locations with extreme environmental regime (low salinity, primarily), there is little overlap. Of our 25 taxa, none have close relatives in other stressful habitats; only at the genus level, members of *Suberites*, *Halichondria*, *Haliclona* and *Halicarca* were encountered elsewhere flourishing under unfavorable conditions (Sarà & Vacelet 1973; Rützler, unpublished observations). Species of *Cliona*, common on all reefs and in many temperate and boreal brackish-water lagoons have not been encountered in the Twin Cays mangrove. Only four of the typical Twin Cays mangrove sponges have conspecific populations on the reef.

Morphology and systematics of *Chondrilla*

The great similarity of *Chondrilla* specimens collected at both sides of the Atlantic over nearly 150 years clearly explains why the name of the Mediterranean *Chondrilla*

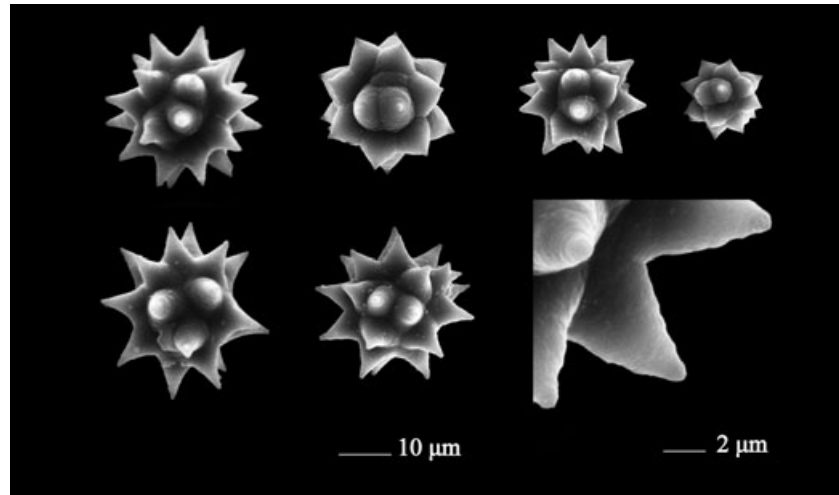


Fig. 8. SEM micrographs of spicules, *Chondrilla nucula* from near the type locality, off Rovinj (Istria), Croatia (USNM 2395); inset: magnified tips of rays.

nucula was applied to both the populations. Until SCUBA diving started to be widely used, it was rare to find the more distinctive, thinly encrusting reef form that is unique to the western Atlantic.

Chondrilla nucula was first described from the Gulf of Quarnero (now Kvarner, Croatia) in the Adriatic Sea (Schmidt 1862: 39, Pl. 3: 22, 22a) (Fig. 8). The description was very brief but a few years later, Schmidt (1870) reported a sponge from the Antilles which he included with *C. nucula* although he acknowledged its predominately flat, crustose shape and a deviating morphology of part of the spicules, with more elongate spines than the spherasters.

More detailed observations on specimens dredged near the type locality in the northern Adriatic were added by Schulze (1877) and von Lendenfeld (1897). The color was described as chestnut to purplish gray, the shape as nearly spherical when young, later spreading into lobate cushions of 1 cm height and 3–5 cm diameter. The inconspicuous oscula are slightly elevated on top of papillae. The ectosome is about 0.3 mm thick, the choanocyte chambers oval, $25\text{--}33 \times 15\text{--}20 \mu\text{m}$ in diameter. The skeleton is composed of spherasters, $23\text{--}28 \mu\text{m}$ in diameter with a 13 to 18- μm center, concentrated in the ectosome and less numerous in the choanosome where they are located along the walls of the aquiferous canals.

The above description reflects also the morphology of the western Atlantic *Chondrilla* that is commonly encountered in seagrass meadows, mangroves, and other lagoon habitats. Despite the surprising observation of the encrusting habit made by the original author of *C. nucula* in the Antilles (Schmidt 1870), it was not until a century later that observant reef divers elaborated on the differences. Two habits of *C. nucula* were described from shallow habitats in the Bahamas (Wiedenmayer 1977): (i) amorphous, lobate, dark brown or beige, attached to

lagoon vegetation, other sponges, and rock; and (ii) thinly encrusting, yellowish brown or fulvous, covering large areas of rock substrata (on reefs). Distinguishing features concerned the cortex, which was measured as 0.5–1 mm thick in (i), only 150–200 μm in (ii), but not the spicules, spherasters, 10–40 μm in diameter (including 2.5–10 μm long spines). Aggressive overgrowth potential of the encrusting habit (ii) was discovered and studied on several Caribbean reefs where it was the dominant aggressor among encounters with corals (Glynn 1973; Vicente 1990).

Comparing a number of taxonomic features among specimens of *Chondrilla* from the same and different localities in the western Atlantic and in the Mediterranean, we found that neither color, surface structure, consistency, distribution of oscula and pori, nor spicule density, abundance of spherulous cells, and thickness of the cortex offered consistent differences usable for characterizing a new taxon. Microbial symbionts too were compared and appear to be identical (Gaino *et al.* 1976; Rützler 1990; Maldonado *et al.* 2005). Statistical assessment of spheraster size yielded better results: the spicules of Mediterranean sponges were about 18% smaller on average. Note, however, that year-around sampling among a population of Mediterranean *C. nucula* demonstrated that spicule size, shape, and density are not stable taxonomic characters but depend on environmental parameters, such as seasonal fluctuations in dissolved silica content and temperature of the sea (Bavestrello *et al.* 1993).

The latest authoritative definition of the genus *Chondrilla* is found in Boury-Esnault (2002). The paper describes and illustrates the type species, *C. nucula*, based on type material from the Adriatic and specimens from other Mediterranean locations. The author notes that spheraster morphology and size are poor characters for discriminating between species, and that only

Mediterranean and adjacent Atlantic populations (Spain, Portugal, Morocco) should be assigned to this species.

Careful examination of light and SEM micrographs convinced us not only of a spheraster size difference between Mediterranean *C. nucula* and our *C. caribensis*, but also of a difference in microanatomical details. Spicules of forma *caribensis* are similar to those of *C. nucula* but differ in size (larger) and microspination (present). Forma *hermatypica*, although closest to forma *caribensis* in spicule size, lacks microspination; it can be distinguished from both by the addition of oxyspherasters.

Molecular, chemical, and ecological separation of *Chondrilla* species

Applying allozyme analysis to *Chondrilla nucula* specimens collected from a number of localities ranging from the Mediterranean to Caribbean Panama and the southwestern Atlantic (Brazil), Klautau *et al.* (1999) determined an unexpectedly high level of genetic differentiation. *C. nucula* is not a cosmopolitan species rather there may be as many as five cryptic species in the western Atlantic, at least one of them in the Caribbean (Klautau *et al.* 1999; Wörheide *et al.* 2005). The paucity of phenotypic characters in *Chondrilla* was also considered in a study of Australian species involving DNA sequence analyses (Usher *et al.* 2004). For comparison, these authors sequenced DNA from a Bermuda specimen (preserved since 1948 at The Natural History Museum, London), and from Adriatic and other Mediterranean *C. nucula*. This molecular work revealed three *Chondrilla* species in Australian waters, distinct from the Adriatic *C. nucula* as well as from the Bermuda sponge which clustered differently from all others. The Australian species could also be distinguished by mean size and presence or absence of two size classes of spherasters, as well as by presence or absence of oxyasters. The authors comment on a further complication, that asexual reproduction (through fragmentation) and rafting could account for wide dispersal of a species in some cases.

Our own genetic assessment of *Chondrilla* using mitochondrial DNA was restricted to the Caribbean region but showed that geographical distance of hundreds of kilometers between populations (such as between Florida Keys, Belize, and Atlantic Panama) is less of a genetic barrier than the habitat diversity separating mangrove and reef sponges that are only a few kilometers apart (Duran & Rützler 2006). These data too support our finding that Mediterranean and western Atlantic *Chondrilla* belong to different species.

Similar ecological observations were made on *Cliona varians* (as *Anthosigmella*), another common sponge in Caribbean calm-water (lagoon) and exposed reef envi-

ronments (Hill & Hill 2002). The lagoon specimens from turtle-grass habitats are branching (forma *variens*) and do not survive when transplanted to the open reef where they are consumed by angelfish. The reef specimens are thinly encrusting (and excavating), resist grazing, and survive transplantation to low-wave-energy environments, although they do not develop branches there (nor do they on the reef when caged). The authors deduce that *Cliona incrustans* is a distinct species. Contrary to our observations on *Chondrilla*, *C. incrustans* develops a thicker cortex with higher spicule concentration in response to fish predation to protect the choanosome because chemical defense may not be a sufficient deterrent. Strong wave action may also induce stronger, skeleton-reinforced tissue, as was shown for intertidal *Haliclona* and shallow *Cinachyrella* species (Palumbi 1984; McDonald *et al.* 2002). However, this trend is not reflected by the *Chondrilla* example, which always has a distinctive cortex of fibrillar collagen whose enrichment with varying amounts of spicules is not correlated with water movement.

Numerous studies on predation and chemical defense (Pawlik 1998; Wulff 2006) demonstrate that certain sponge predators are quite reliable in separating very similar species. In investigating feeding deterrence properties of *Chondrilla* 'nucula' from Florida and the Bahamas, Swearingen & Pawlik (1998) found no differences within each locality (reef or mangrove), but mangrove specimens transplanted to reefs were quickly consumed by fishes, as also observed by us. When caged on the reef, the massive, lobate mangrove sponges became thinner, whereas encrusting reef specimens did not gain thickness when transplanted to the mangrove. The authors interpreted this to mean that encrusting growth on the reef reflected hydrodynamics, not grazing. Nevertheless, we now have evidence that mangrove and reef forms are genetically different (Duran & Rützler 2006).

Ecological separation of closely related species with different morphologies is not without precedent. For instance, in the Mediterranean, calcareous sponges show great intra-specific variability, even in restricted areas, with distinct subspecific entities localized in different ecological niches (Sarà 1961). In another example, the encrusting *Spongia virgultosa* occurs in shallow habitats with strong water currents and wave action, whereas closely related flabellate or cup-shaped *S. agaricina* is found only in low-energy environments, where it withstands high levels of fine-particle sedimentation (Pronzato *et al.* 1998). Short life span and limited swimming capability of larvae can easily separate and genetically isolate populations that are only short distances apart. Such microgeographic isolations may lead to fractionation into

subspecific entities (demes, races, forms), with subsequent formation of species (Sarà 1961).

Conclusion

Snorkeling or diving on a Caribbean off-shore reef and in nearby mangrove tidal channels immediately reveals the extremely different biotic and abiotic factors that characterize these two environments. Sponge specialists can tell that the faunas are also different and that species, as defined by traditional means, have different morphological qualities in both the ecosystems. The key question is whether environmental influences are strong enough to not only influence distributional patterns but also to cause genetic change. Moreover, can the lack of larval mobility and other means of dispersal, and of population mixing, lead to ecological speciation?

Sponges in Caribbean island mangroves are diverse and quantitatively important despite adverse conditions, such as lack of solid substrata, heavy sedimentation, and temperature-salinity extremes. The most remarkable tolerance is survival by some species after being buried in sediment. Here, morphological plasticity helps overcome the clogging of pores by fine particles. A few mangrove residents have sibling taxa on nearby coral reefs that differ somewhat in morphology. One example is a species known as *Chondrilla* 'nucula'. Recent molecular work shows that the Caribbean sponge differs from the Mediterranean *C. nucula*; hence, we describe it as *C. caribensis*. Further DNA sequencing and skeleton study by SEM indicated that the mangrove and reef specimens differ not only in shape and color but also genetically and in details of spiculation. They are therefore defined as separate subspecific taxa (forma *manglaris*, forma *hermatypica*) that emerged in ecological separation.

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