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REPORT

Pathology of lesions in corals from the US Virgin Islands after emergence of stony coral tissue loss disease

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Abstract Stony coral tissue loss disease (SCTLD) was first documented in Florida in 2014 and has since spread through the Caribbean causing unprecedented mortality in more than 20 species of corals. The cause of SCTLD is unknown, but bacteria are suspected based on regression of gross lesions in some corals treated with antibiotics. Limited pathology studies on SCTLD exist, but it is likely that 'SCTLD' is a general term encompassing tissue loss disease of unexplained origin. Here, we examined pathology of lesions in corals from the US Virgin Islands where SCTLD has recently emerged. The typical histologic lesion of SCTLD in Florida corals was lytic necrosis comprising vacuolation and necrosis of mucus cells with erosion of mesoglea and misshapen endosymbionts with variably sized intracytoplasmic granules and common occurrence of filamentous viral-like particles in endosymbionts visible on electron microscopy (EM). In contrast, USVI corals had mainly lytic mucus cell hypertrophy and necrosis with no involvement of mesoglea, endosymbiont pathology at the light microscopy level was less evident, and VLP were rarely seen on EM. We suspect SCTLD is likely more complex with multiple presentations and potential etiologies depending on geographic region. Further pathological studies from other regions might help refine the case definition of SCTLD.

Keywords Acute and subacute tissue loss · Scleractinian corals · Histology · Electron microscopy

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Introduction

Coral reef ecosystems in the Caribbean have been under anthropogenic pressure for generations mainly due to eutrophication and overexploitation (Jackson 1997). Disease in the Caribbean became prominent when an unexplained tissue-loss disease (white-band disease) of the reef-building coral, *Acropora palmata* emerged in the early 1980s (Gladfelter 1982). Subsequent studies confirmed this, and at least in Florida, core samples reveal that coral assemblage has shifted from formerly massive or highly branching *Acropora* spp. and *Orbicella* spp. to smaller species such as *Porites astreoides* and *Siderastrea siderea* (Aronson and Precht 2001; Toth et al. 2019). Since the early descriptions of tissue loss by Gladfelter (1982), the Caribbean has seen various diseases in stony corals (Weil 2004). Presently, the Caribbean is experiencing a new wave of mortality caused by an unexplained tissue loss disease termed stony coral tissue loss disease (SCTLD) that is affecting > 20 species of corals across a broad area (Papke et al. 2024). Stony coral tissue loss disease is considered infectious because it appears to be transmissible between diseased and healthy fragments in experimental aquarium settings (Aeby et al. 2019). The disease has also been transmitted experimentally using ballast water (Studivan et al. 2022b) and sediments (Studivan et al. 2022a). Lesions attributed to SCTLD often stop progressing when corals are treated with antibiotics, although new lesions often appear (Neely et al. 2020). This, along

with differences in bacterial flora between healthy and diseased corals (Rosales et al. 2023) has led some to assume that SCTLD may have a bacterial etiology, but no causative pathogen has been identified. Moreover, it is unclear whether lesion regression in corals treated with antibiotics is due to antibacterial effects of antibiotics or other factors. Some antibiotics have properties above and beyond killing bacteria that could be causing regression of lesions in corals. For instance, the antibiotic doxycycline used to treat skin lesions in humans has anti-inflammatory properties that could aid resolution of lesions (Henehan et al. 2017).

Gross and microscopic pathology are fundamental to understanding pathogenesis and sometimes causes of diseases in animals (Cheville 1988). Pathology might shed light on SCTLD; however, whilst microscopic pathology is commonly used to understand disease in vertebrates (Cheville 1988) and even shellfish (Carella et al. 2015), its use in coral disease investigations continues to be uncommon (Work and Meteyer 2014). In a recent review of papers on SCTLD (Papke et al. 2024), only three presented information on microscopic pathology. One study from Florida suggested SCTLD was the result of a dysfunction between host cells and zooxanthellae in deeper tissues leading to cell death and gross lesions of SCTLD (Landsberg et al. 2020); no evidence of bacteria or other causative microorganisms were visible on light microscopy. Subsequent studies using electron microscopy of corals from Florida showed damage to endosymbionts associated with filamentous viral-like particles (VLP) (Work et al. 2021). Molecular evidence of filamentous viruses was confirmed from corals in the US Virgin Islands (USVI) (Veglia et al. 2022). However, since that study, VLP similar to those seen in endosymbionts of Florida corals were seen in apparently healthy (disease-free) corals in the Caribbean and Pacific (Howe-Kerr et al. 2023), so a viral etiology for SCTLD remains to be confirmed.

The lack of clarity on exactly what constitutes SCTLD persists. For instance, in the original description, the disease was defined by corals manifesting tissue loss (Precht et al. 2016), but more recent studies now define SCTLD to include tissue loss with bleaching response (Aeby et al. 2021), or even discoloration (Lucas et al. 2024). From a pathology perspective, the current case definition of SCTLD stipulates acute to subacute tissue loss with microscopic evidence of lytic necrosis of gastrodermis of basal body wall, and endosymbiont degeneration and necrosis (Landsberg et al. 2020) Given the opacity of how SCTLD is defined in the western Atlantic Ocean, additional studies on pathology of corals in regions outside of Florida might help refine the case definition.

The USVI are an important area for coral reef ecosystems in the Caribbean, because they harbor several important marine protected areas and other locations of particular concern dedicated to protecting coral reefs (Pittman et al. 2014). Diseases in corals have been seen in the USVI since at least the 1980s when white band disease was first noticed by Gladfelter (1982), with subsequent observations of the coral disease white plague by Miller et al. (2003). In 2005, the USVI and other areas in the eastern Caribbean underwent a major bleaching event concomitant with disease that accelerated coral reef declines (Miller et al. 2009; Rogers et al. 2009). SCTLD was first noticed in the USVI at southwest St. Thomas in early 2019 and spread east to St. John by 2020 affecting mainly *Colpophyllia natans, Eusmilia fastigiata, Montastraea cavernosa, Orbicella* spp., and *Pseudodiploria strigosa* (Brandt et al. 2021). Experimental trials to transmit SCTLD showed that *Orbicella annularis, Colpophyllia natans,* and *Siderastrea siderea* were most susceptible, and histology of experimentally induced lesions had vague resemblance to those of wild collected Florida corals (Meiling et al. 2021). To gain a better understanding of the case definition of SCTLD and how it might apply to corals in the USVI, we described the gross and microscopic pathology of corals from that region.

Methods

Three locations in St. John were selected for sampling in July 2021 (Fig. 1). SCTLD first appeared in USVI on the island of St. Thomas in January 2019 and had reached St. John and other islands in the Caribbean by January 2020 (Brandt et al. 2021), so our sampling effort was over a year after initial incursion of the disease on that island. Tektite and Yawzi reefs are adjacent to a relatively undeveloped watershed, and have a multi-decade history of coral monitoring with reefs dominated by *Montastraea cavernosa*, *Orbicella* spp., *Porites* spp., *Colpophylia* spp., and other coral species with known susceptibility to SCTLD that have undergone bleaching events in the past (Whelan et al. 2007; Miller et al. 2009) where sea surface temperatures at the time were 0.7–1.2C warmer than historical averages reaching to 30.2C. Hawksnest Bay reef has large well-developed patch reefs geographically distant (north vs. south side [Tektite Bay and Yawzi Point reefs]) from the other sites but adjacent to a more developed watershed. Samples from 79 coral colonies were haphazardly collected between 7–14 July 2021 from four sites (Table 1; Fig. 1) (Work 2013). Briefly, paired apparently normal and lesion samples from colonies manifesting gross lesions, and fragments from some corals not manifesting lesions, were processed for histopathology including routine paraffin embedding and staining with hematoxylin and eosin (Landsberg et al. 2020). Prior to collection, colonies were photographed and classified into six gross lesion types) (Work and Aeby 2006) including acute tissue loss identified by newly exposed skeleton (Fig. 2A), subacute tissue loss identified by a band of newly

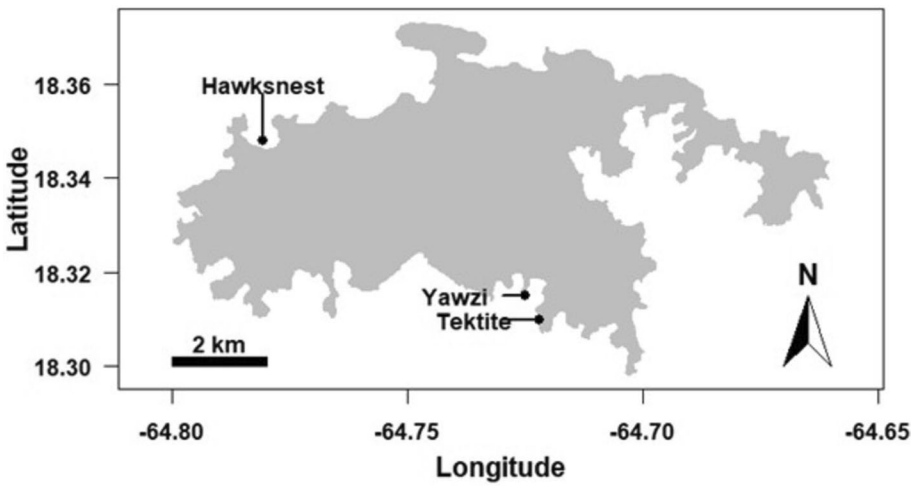

Fig. 1 Locations of corals collected on July 2021 on St. John, US Virgin Islands

Table 1 Location and number of colonies sampled for histopathology partitioned by location and species. Depths form deep and shallow for Tektite Bay ranged from 15–18 m and 8–12 m, respectively. Depths for Yawzi Point were about 6 m and for Hawksnest Bay ranged from 3–13 m

Species	Hawksnest Bay	Tektite Bay deep	Tektite Bay shallow	Yawzi Point	Total
Orbicella faveolata	12	8	1		21
Siderastrea siderea	5		7	3	15
Pseudodiploria strigosa	1	2		6	9
Montastraea cavernosa	1	2	3	1	7
Colpophyllia natans	1	3			4
Porites astreoides	1	3			4
Orbicella annularis	1	1	2		4
Agaricia agaricites	1	3			4
Stephanocoenia intersepta			1	2	3
Dendrogyra cylindrus		1		2	3
Acropora palmata	1			2	3
Diploria labyrinthiformis	1				1
Agaricia humilis			1		1
Total	25	23	15	16	79

exposed white skeleton near remaining tissue and discoloration from algal growth farther from the remaining tissue (Fig. 2B), chronic tissue loss identified by multifocal deposition of amorphous material on tissues sometimes bordered by bleaching (Fig. 2C), bleaching (Fig. 2D), bleaching with tissue loss (Fig. 2C, E), and discoloration (Fig. 2F). Lesions for sampling were chosen haphazardly to broadly reflect what was encountered on the reef at the time of the investigation (Table 2). A subset of tissues selected to represent the variety of light microscopic lesions encountered was also examined by transmission electron microscopy (TEM) as described (Work et al. 2021) (Table 3).

Changes at the cellular level were described separately for the coral host and endosymbionts. For host cells, changes included lytic mucus cell hypertrophy exemplified by enlarged mucus cells distorting tissue architecture (Fig. 3A) sometimes accompanied by necrosis characterized by clumping of cells with hypereosinophilic cytoplasm and pyknotic or karyorrhectic nuclei (Fig. 3B-D). Periodic acid Schiff-alcian-blue pH 2.5 (PAS-AB) stain was used to distinguish acidic and neutral mucin (Yamabayashi 1987) (Fig. 3E, F). Other host cell changes noted included atrophy characterized by decreased cell size (Fig. 4B; compare with Fig. 4A), and hyperplasia characterized by increased cell numbers (Fig. 4C). Changes in endosymbionts were noted as intracytoplasmic vacuolation (Fig. 4D). We also noted presence of non-host structures such as sponges, ciliates, cell-associated bacterial aggregates, coccidia (Fig. 4E) and fungi or algae (Fig. 4F) (Hawthorn et al. 2023). Host cell changes and non-host organisms were further partitioned as to their location in tissue compartments including epidermis/gastrodermis of surface or basal body wall, mesenterial filaments, calicodermis, or skeleton. For each coral genus manifesting a particular gross lesion type, we calculated the percent of each microscopic lesion type seen in apparently

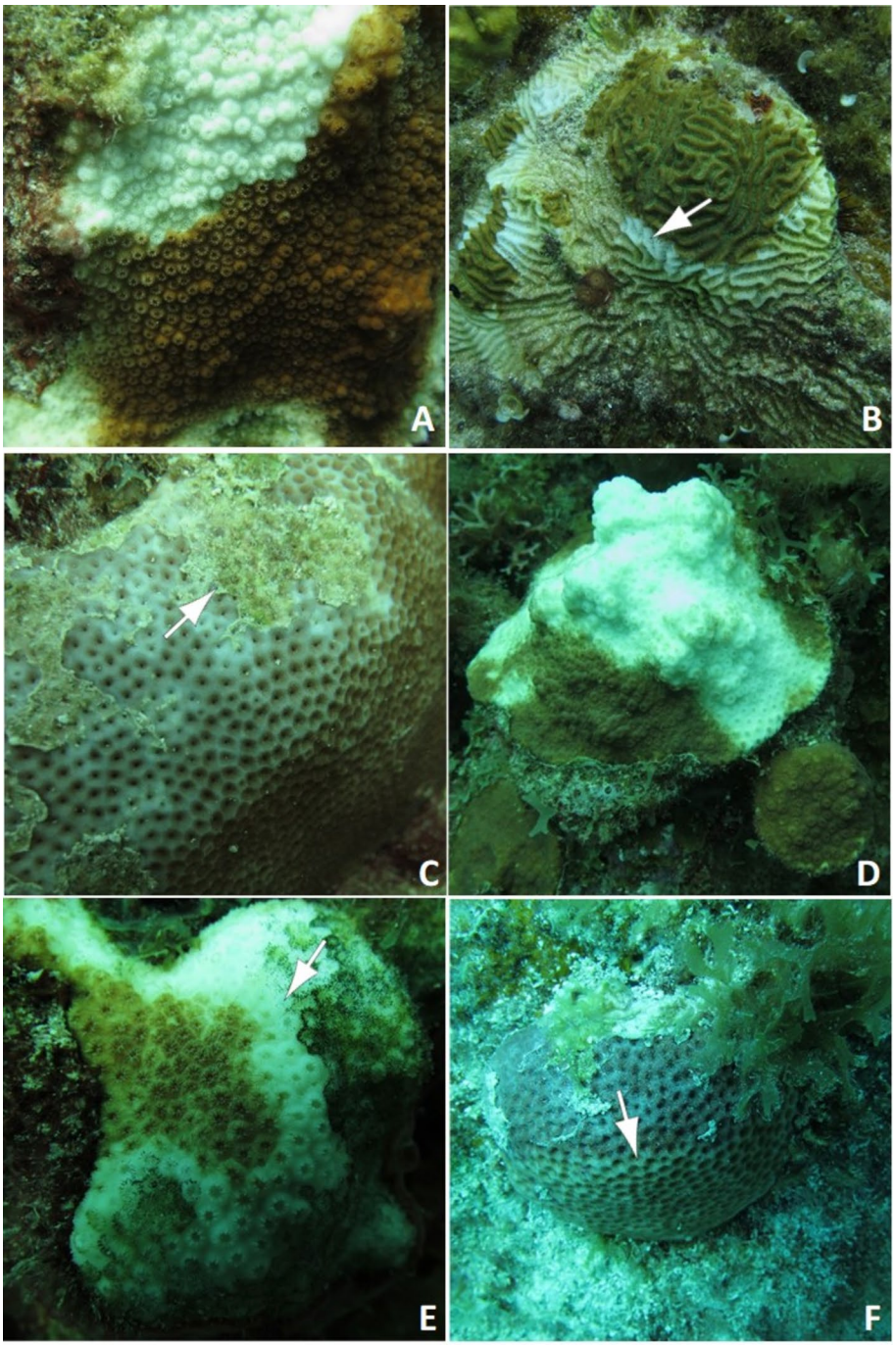

Fig. 2 Representative gross lesions seen in corals from St. John, US Virgin Islands (USVI). **A** Acute tissue loss in *O. faveolata*; note distinct area of tissue loss revealing intact bare white skeleton over. ca. 40% of colony. **B** Subacute tissue loss in *Pseudodiploria strigosa*; note island of intact tissue partially surrounded by intact bare skeleton (arrow) progressively covered by green patina (algal overgrowth). **C** Chronic tissue loss in *S. siderea*; note amorphous clumps of green material effacing skeleton (arrow) with bleaching of surrounding tissues. **D** Bleaching in *Orbicella faveolata*; note diffuse distinct area of white discoloration occupying > 50% of the colony. **E** Acute to subacute tissue loss with bleaching in *O. annularis*; arrow points to area of tissue loss with overgrowth of bare skeleton with speckled dark material to the right and bleached tissue separating brown coral tissue to the left and below. **F** Discoloration in *Siderastrea siderea*; note demarcation between two different colors (arrow)

normal vs lesion fragments and visualized with the R package pheatmap (R Core Team 2021). Colonies were graded as males, females, or both based on presence of oocytes, spermaries, or both gonads, respectively.

Results

Of lesions sampled, subacute tissue loss was the most common followed by bleaching, acute tissue loss, chronic tissue loss, bleaching with tissue loss, and discoloration (Table 2). Of the six gross lesion types sampled, five were sampled for *Siderastrea*, four for *Orbicella*, three for *Agaricia*, two each for *Pseudodiploria* and *Stephanocoenia*, and one each for *Colpophyllia*, *Dendrogyra*, and *Montastrea* (Table 2). Of colonies that could be sexed as male (M), female (F), or both (B) numbers of each sex and sample size (M:F:B; N) were 0:6:0; N = 25 for *Orbicella*, 2:1:0; N = 15 for *Siderastrea*, 0:1:0; N = 9 for *Pseudodiploria*, 1:1:0; N = 7 for *Montastraea*, 0:1:1; N = 5 for *Agaricia*, 1:2:0; N = 4 for

Table 2 Number of sampled colonies that were apparently normal or manifesting gross lesions from St. John USVI partitioned by genus

Genus	Normal	Tissue Loss-Acute	Tissue Loss-Subacute	Tissue Loss-Chronic	Bleaching	Bleaching-Tissue loss	Discoloration	Total
Acropora	3							3
Agaricia	1	1		1	2			5
Colpophyllia	1		3					4
Dendrogyra	1		2					3
Diploria	1							1
Montastraea	3		4					7
Orbicella	3	5	2		12	3		25
Porites	4							4
Pseudodiploria	2	1	6					9
Siderastrea	2	2	3	4		1	3	15
Stephanocoenia	1			1	1			3
Total	22	9	20	6	15	4	3	79

Table 3 Presence (x) of lesions on electron microscopy partitioned by species of coral, gross lesion, microscopic lesion, nd changes in host or endosymbiont cells

Species	Gross	Histology Summary	Mucus	Cavity	Spacing	Fusion
Orbicella faveolata	Bleaching	Lytic mucus cell hypertrophy, necrosis epidermis, mesenterial filaments and surface body wall,vacuolated endosymbionts	x	x	x	
Colpophyllia natans	Tissue loss-subacute	Lytic mucus cell hypertrophy, necrosis of gastrodermis of surface body wall, vacuolated endosymbionts	x		x	x
Montastraea cavernosa	Tissue loss-subacute	Lytic mucus cell hypertrophy, necrosis of gastrodermis of surface and basal body wall, vacuolated endosymbionts		x		
Porites astreoides	Normal	Vacuolation of endosymbionts				
Orbicella faveolata	Bleaching	No lesions		x		
Siderastrea siderea	Tissue loss-chronic	Lytic mucus cell hypertrophy, necrosis of surface and basal body wall, vacuolation of endosymbionts	x	x		x
Stephanocoenia intersepta	Normal	Necrosis of basal body wall, vacuolated endosymbionts		x		
Agaricia humilis	Tissue loss-acute	Lytic mucus cell hypertrophy, necrosis of basal body wall, vacuolation of endosymbionts		x		
Pseudodiploria strigosa	Tissue loss-subacute	No lesions		x		
Acropora palmata	Normal	No lesions				
Orbicella faveolata	Bleaching	Necrosis of basal body wall gastrodermis				
Diploria labyrinthiformis	Normal	Necrosis of basal body wall gastrodermis	x	x		

* Mucus refers to mucus cell hypertrophy. Cavity revers to intracytoplasmic cavity with debris, gigantism was seen only in *C. natans*, spacing refers to increased spacing between thylakoid membranes, Fusion refers to fusion of thylakoid membranes

Porites, 0:1:0; N = 3 for *Dendrogyra*, and 0:1:0; N = 3 for *Stephanocoenia*.

Of 248 microscopic lesions seen, mucus cell lytic hypertrophy was most common (73 or 29%) affecting gastrodermis of basal body wall (22 or 9%), gastrodermis of surface body wall (19 or 8%), epidermis (16 or 6%), or mesenterial filaments (16 or 6%). Necrosis was the second most common microscopic lesions (58 or 23%) affecting gastrodermis of basal body wall (22 or 9%), gastrodermis of surface body wall (13 or 5%), epidermis (11 or 4%), calicodermis (7 or 3%), or mesenterial filaments (5 or 2%). Lesions in endosymbionts were limited to depletion (35 or 9%) or vacuolation (8 or 5%) (Table 4). Overgrowth of skeleton by algae, fungi, or sponges was seen in 52 or 21%. We saw one instance each of coccidia (Keeling et al. 2021), bacterial aggregates, or ciliates none of which were associated with tissue invasion or cell pathology. Heatmaps revealed *Orbicella* with bleaching to have the highest number and variety of histologic lesions with atrophy of gastrodermis and epidermis particularly prominent, calicodermal necrosis was common in discolored *Siderastrea*, lytic mucus cell hyperplasia was common in *Orbicella* with subacute

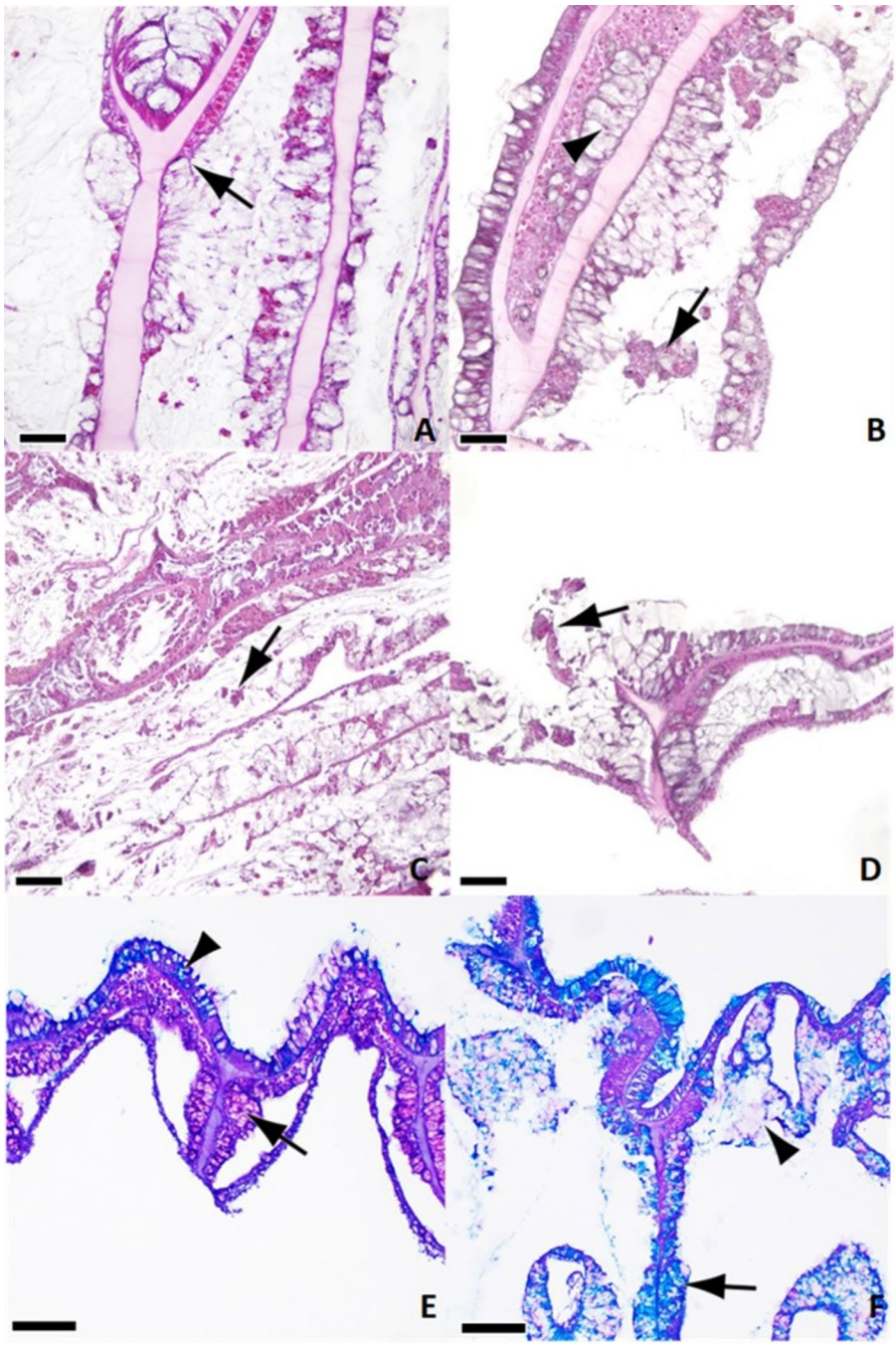

Fig. 3 Representative microscopic lesions in corals from the US Virgin Islands; Hematoxylin and eosin (A-D) and Periodic acid-Schiff's reagent and alcian-blue (E–F). **A** *C. natans* with subacute tissue loss; contrast junction (arrow) of gastrodermis of surface body wall with lytic mucus cell hypertrophy (below arrow) vs intact gastrodermis (above arrow); bar = 50 µm. **B** *P. strigosa* with subacute tissue loss; note mild lesion with lytic mucus cell hypertrophy in gastrodermis of surface body wall not extending into mesoglea (arrowhead) with clumps of necrotic gastrodermis sloughing into gastrovascular canals (arrow); bar = 50 µm. **C** *O. annularis* with diffuse acute tissue loss; severe lesion with marked lytic mucus cell hypertrophy and necrosis of gastrodermis of basal body wall with sheets of mucus and necrotic debris (arrow); bar = 50 µm. **D** *O. faveolata* with diffuse bleaching; necrosis of surface body wall with clumps of necrotic debris (arrow) contrasted with more intact surface body wall to right with epidermis, mesoglea, and gastrodermis; bar = 50 µm. **E** Apparently normal *O. faveolata*; note mucocytes of gastrodermis (arrow) that do not disrupt architecture and are dominated by magenta PAS-positive neutral mucins whereas mucocytes of epidermis are dominated by alcian-blue staining acidic mucins (arrowhead); bar = 100 µm. **F** *O. faveolata* with diffuse bleaching; note intact gastrodermal cells have more alcian-blue staining neutral mucins whereas lytic hypertrophic areas have magenta PAS-positive acid mucins with a paler hue than seen in intact gastrodermis (arrowhead); bar = 100 µm

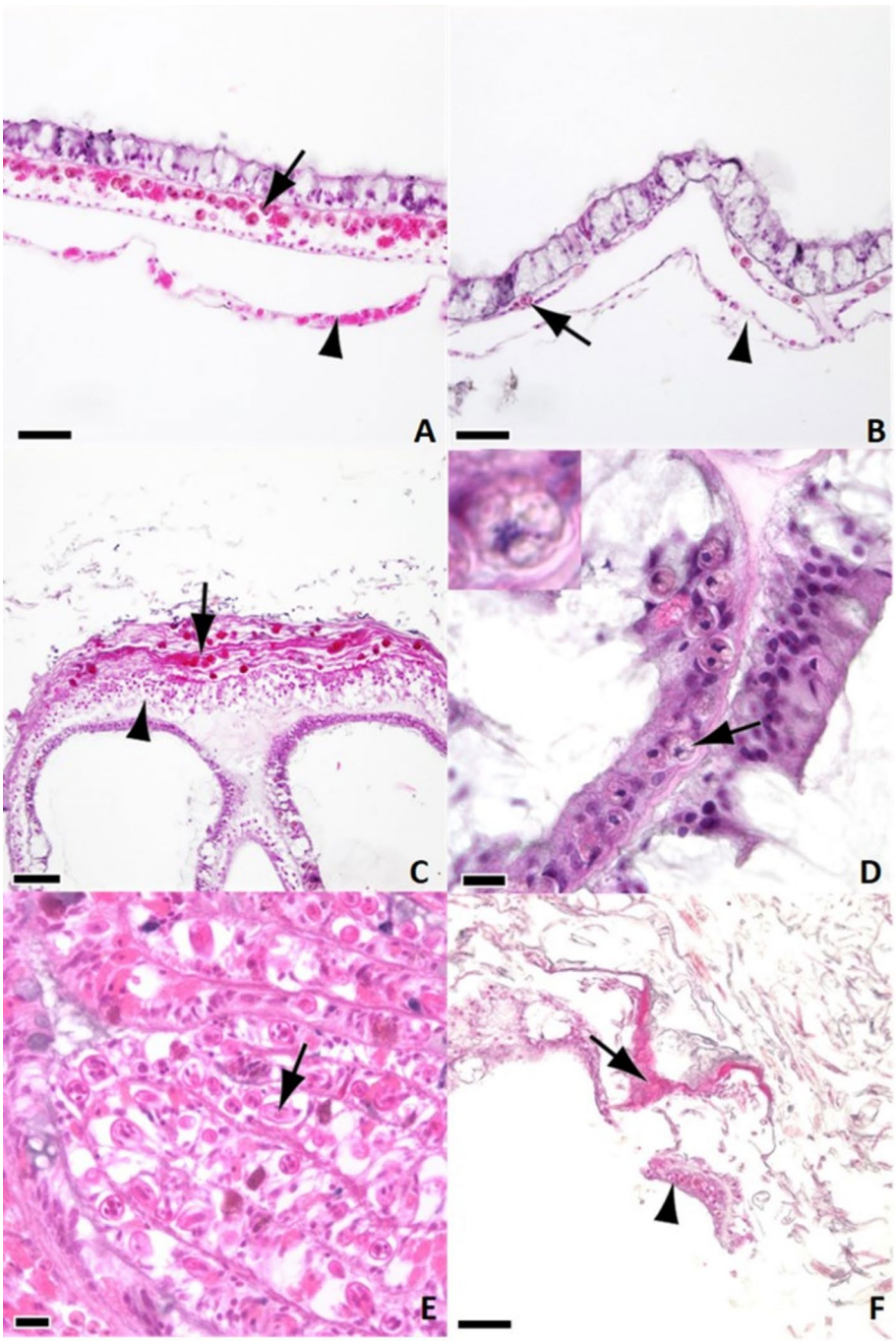

Fig. 4 Representative microscopic lesions in corals from the US Virgin Islands (continued); hematoxylin and eosin. **A** *A. agaricites* normal tissue; note gastrodermis replete with endosymbionts (arrow) and gastrodermis of basal body wall with granular cells (arrowhead); bar = 20 µm. **B** *A. agaricites* with diffuse bleaching; note atrophied gastrodermis with lack of endosymbionts in gastrodermis underlying epidermis (arrow points to singleton) and diffuse atrophy of gastrodermis of basal body wall with lack of granular cells (arrowhead); contrast with A; bar = 20 µm. **C** *C. natans* with subacute tissue loss; note endolithic organisms (top of photos) adjacent to hyperplastic calicodemis (arrowhead) with deposition of hyaline membranes and granules (arrow); bar = 50 µm. **D** *O. annularis* with diffuse bleaching; note endosymbionts with intracytoplasmic vacuole (arrow) with detail inset; bar = 10 µm. **E** *D. cylindrus* with diffuse subacute tissue loss; note coccidia (arrow) in gastrodermis of mesenteries with no host response; bar = 10 um. **F** *S. siderea* with diffuse subacute tissue loss; note mixed filamentous non-staining structures (fungi/filamentous algae) adjacent to necrotic basal body wall with hyaline membrane deposition (arrow) and remnant intact basal body wall (arrowhead); bar = 20 µm

tissue loss, and lesions were generally more severe for tissue loss versus bleaching (Fig. 5). When stained with PAS-alcian-blue, gastrodermis in diseased colonies shifted from magenta PAS positive (Fig. 3E) to alcian-blue positive acidic mucins (Fig. 3F), and cells with mucus cell lytic hypertrophy stained pale magenta PAS positive indicative of neutral mucins (Fig. 3F).

On electron microscopy, necrosis was exemplified by cellular disorganization, cytoplasmic blebbing and karyorrhexis (Fig. 6B compare with Fig. 6A). Mucus cell lytic hypertrophy was exemplified by variably sized accumulations of mucus (Fig. 6C) associated with cell membrane rupture (Fig. 6D). Endosymbiont pathology included variably sized intracellular cavities containing electron dense debris (Fig. 7A, B), gigantism of chloroplast, increased spacing between thylakoid membranes (Fig. 7B), dissociation of thylakoid membranes (Fig. 7C), or fusing of thylakoid membranes (Figs. 7D, E). Filamentous viral-like particles (Fig. 7F) were seen in only 1 of 12 cases examined by TEM and in that sample, only one endosymbiont. Of 12 samples

Table 4 Number (percent) of microscopic lesions number seen by genus for corals collected from US Virgin Islands. SBW, BBW, and LHM refer to surface body wall, basal body wall, and lytic mucus cell hypertrophy, respectively

	Orbicella	*Colpophyllia*	*Montastraea*	*Agaricia*	*Siderastrea*	*Stephanocoenia*	*Pseudodiploria*	*Dendrogyra*	*Diploria*
Atrophy									
Epidermis	3 (3%)	0	0	1 (6%)	1 (2%)	0	0	0	0
Gastrodermis SBW	6 (6%)	0	0	1 (6%)	1 (2%)	2 (40%)	0	0	0
Necrosis									
Epidermis	5 (5%)	0	0	0	5 (10%)	0	1 (4%)	0	0
Gastrodermis SBW	5 (5%)	2 (9%)	0	0	5 (10%)	0	1 (4%)	0	0
Gastrodermis BBW	0	3 (13%)	5 (24%)	2 (12%)	6 (12%)	0	3 (12%)	2 (29%)	1 (50%)
Mesenteries	3 (3%)	0	1 (5%)	0	1 (2%)	0	0	0	0
Calicodermis	0	1 (4%)	0	0	6 (12%)	0	0	0	0
LHM									
epidermis	9 (9%)	3 (13%)	0	0	2 (4%)	0	2 (8%)	0	0
Gastrodermis SBW	9 (9%)	3 (13%)	1 (5%)	0	1 (2%)	0	5 (20%)	0	0
Gastrodermis BBW	9 (9%)	5 (22%)	2 (10%)	0	0	0	6 (24%)	0	0
Mesenteries	9 (9%)	4 (17%)	0	1 (6%)	0	0	2 (8%)	0	0
Calicodermal hyperplasia	2 (2%)	0	0	1 (6%)	4 (8%)	0	0	0	0
Depletion endosymbiont	17 (17%)	0	2 (10%)	7 (41%)	3 (6%)	2 (40%)	0	4 (57%)	0
Vacuolation endosymbiont	5 (5%)	0	3 (14%)	0	0	0	0	0	0
Skeletal overgrowth	17 (17%)	2 (9%)	7 (33%)	4 (24%)	14 (29%)	1 (20%)	5 (20%)	1 (14%)	1 (50%)

examined by TEM, debris-filled cavities in endosymbionts were the most commonly seen lesion followed by lytic mucus cell hypertrophy in host cells (Table 3).

Discussion

The main host response in corals from the USVI was multifocal to locally extensive lytic hypertrophy of mucus cells sometimes associated with necrosis of gastrodermis of the surface and basal body wall. Endosymbionts also manifested pathology in the form of variably sized cavities filled with unidentified debris. Notably pathology of corals from the USVI differed from those in Florida in terms of histology and electron microscopy (Table 5). On histology, the main lesion in Florida corals was multifocal lytic necrosis of gastrodermis of surface and basal body wall (Landsberg et al. 2020), but this lesion often extended into the mesoglea, something which was not evident in corals from the USVI. In this context, the lesions we observed were more in alignment with those seen on histology during experimental transmission trials of SCTLD in corals from the USVI (Meiling et al. 2021). Pathology of endosymbionts also differed from that seen in corals from Florida in that we did not see intracytoplasmic refractile vacuoles (Landsberg et al. 2020). Rather, endosymbiont pathology seen in USVI corals manifested as varying degrees of intracytoplasmic vacuolation. On electron microscopy, corals from the USVI, like those of Florida, showed host cell response of excessive mucus production, gigantism of chloroplasts with thylakoid membrane abnormalities, and presence of intracytoplasmic cavities containing varying amounts of electron dense material. However, in contrast to Florida corals, there was a notable absence of asymmetric viral-like particles (Work et al. 2021). This brings into doubt just what role viruses play in causing SCTLD, at least outside of Florida. As in Florida, corals from USVI had microscopic lesions in apparently normal fragments from lesioned colonies, and there appeared to be little relationship between gross and microscopic lesions, a phenomenon also noted in Florida corals with SCTLD (Landsberg et al. 2020). Many corals also had overgrowth of skeleton by a mix of filamentous algae and fungi to which coral hosts responded by calicodermal hypertrophy or necrosis (Fig. 4F). The presence of a host response to those endolithic organisms suggests they were having some adverse effect on coral health, a phenomenon that has been documented elsewhere. For instance, dark spots in *Siderastrea* in the Caribbean are associated with overgrowth of fungi (Work and Weil 2015). Rich et al. (2021) saw endolithic organisms with similar associated basal body wall changes in *Pseudodiploria* (and other species) with and without growth anomalies in St Kitts suggesting that endolithic organisms may not be an essential

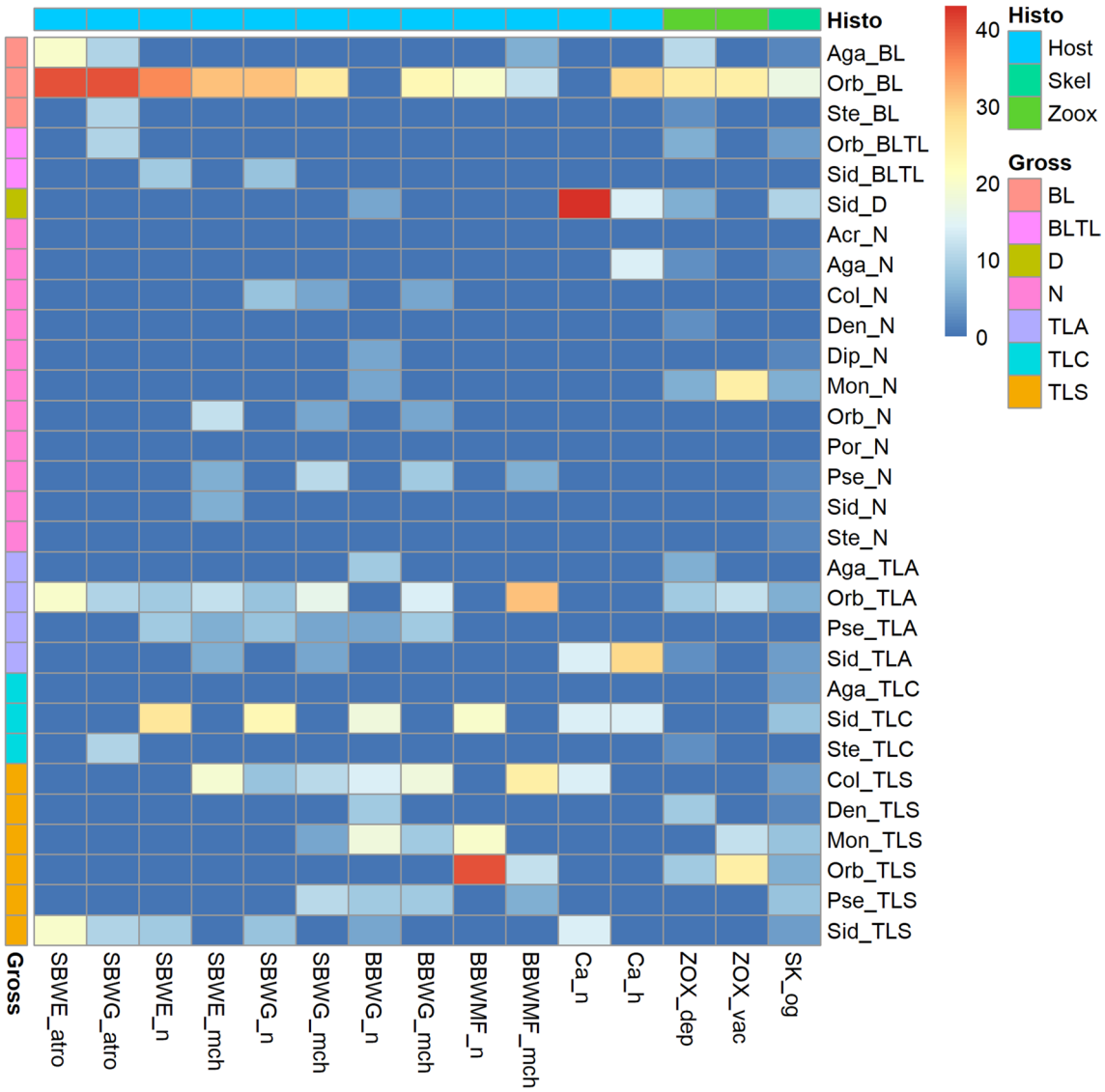

Fig. 5 Heatmap of gross vs histologic lesions in samples partitioned by genus. Y-axis labels are Genus-GrossLesion-Status with the following genera (Acr-*Acropora*, Aga-*Agaricia*, Col-*Colpophyllia*, Den-*Dendrogyra*, Dip-*Diploria*, Mon-*Montastrea*, Orb-*Orbicella*, Sid-*Siderastrea*, Ste-*Stephanocoenia*) partitioned by color-coded (left) gross lesion (BL-Bleaching, BLTL-Bleaching with tissue loss, D-Discoloration, N-Apparently normal, TLA-Acute tissue loss, TLC-chronic tissue loss, TLS-subacute tissue loss). X labels are histologic lesions (BBWG/E-basal body wall gastrodermis/epidermis, SBWG/E-surface body wall gastrodermis/epidermis, ZOX-Zooxanthellae, SK-Skeleton, Ca-Calicodermis followed by letter after underscore: _atro-atrophy _dep-depletion, _h-hypertrophy, _mch- lytic mucus cell hypertrophy, _n-necrosis, _og overgrowth of skeleton, _vac vacuolation) color coded (top) by host, skeleton, or zooxanthellae. Colors of individual cells are percentages of a given histology lesion type ranging from low (blue) to high (red)

component of the case definition of SCTLD. Evaluating corals elsewhere in the Caribbean might shed additional light on the role of endolithic organisms in coral health in the region. The absence of TEM lesions in apparently normal corals (Table 3) suggests the changes seen here both at the light- and electron-microscope level are true pathology and not mere sampling artifact as implied elsewhere (Papke et al. 2024). Moreover, if all of what we are seeing is collection artifact, it should affect tissues uniformly and we would not have segmental distribution of lesions as seen here. As an example, fish gills are prone to collection artifacts, but those generally manifest as a uniform change (Wolf et al. 2015).

The differences in pathology of SCTLD in Florida vs USVI, summarized in Table 5, could be explained by temporal or geographic variation. Florida corals were sampled between 2016 and 2018 whereas USVI corals were sampled in 2021. Possibly, manifestation of disease has changed over this time interval. Changes in manifestation of disease over time is not unusual. For instance, highly pathogenic avian

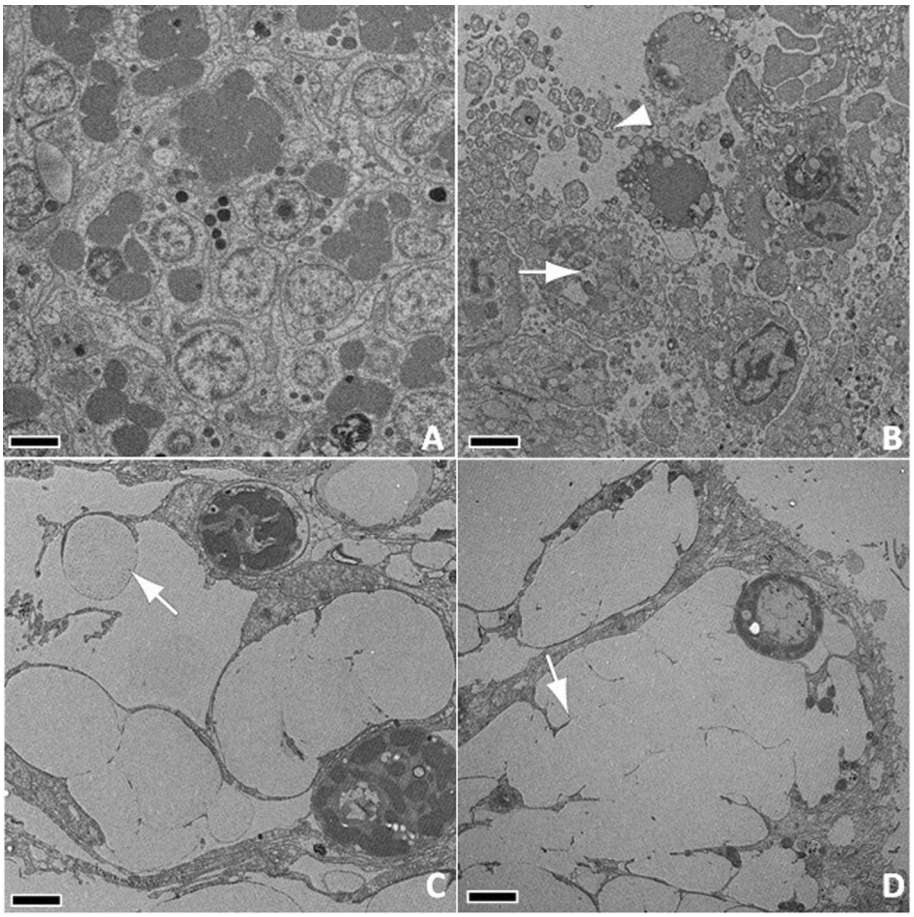

Fig. 6 Electron micrographs of host cell pathology in corals collected from the US Virgin Islands stained with osmium. **A** Apparently normal *S. interscepta*; note uniform cells; bar = 2 µm. **B** *O. faveolata* with diffuse bleaching; note necrotic cells with blebbed cytoplasm (arrowhead) and karyorrhexis (arrow); bar = 2 µm. **C** *O. faveolata* with diffuse bleaching; note mucus vacuoles coalescing and generation of smaller vacuoles within larger ones (arrow) and two relatively normal endosymbionts nearby; bar = 4 µm. **D** Same as C; terminal phase of mucus production with massive disruption of cell architecture with remnant cell membranes (arrow). Note endosymbiont with large intracytoplasmic cavity; bar = 8 µm

influenza H5N1 is known to circulate in wild waterfowl and rarely causes disease in those birds. In the early 2000s, H5N1 caused limited mortality in bar-headed geese when it emerged in China in the early 2000s (Chen et al. 2006) however, in the early 2020s, it has caused unprecedented and significant mortality in marine mammals and seabirds in South America (Leguia et al. 2023). Geographic variation could also explain the differences between USVI and Florida. Even though both regions contain similar assemblages of coral species, these animals may manifest disease in different ways. An example is fibropapillomatosis, a disease associated with Chelonid herpesvirus 5 that causes tumors in green turtles (*Chelonia mydas*) in Florida and Hawaii. In Hawaii, the disease has been declining (Chaloupka et al. 2009), and turtles there commonly manifest oral tumors (Aguirre et al. 2002) whereas in Florida, the disease continues to persist at high prevalence and oral tumors are rare (Foley et al. 2005). Turtles in Hawaii and Florida also have a different immunological response to the virus (Work et al. 2020).

Both above examples referring to temporal or spatial variation in disease manifestation apply to gross presentation of diseases with known etiologies (avian influenza associated with orthomyxovirus and fibropapillomatosis associated with herpesvirus). However, at the cellular level, the tissue pathology for these diseases is invariant. For example, histology of fibropapillomatosis in Florida green turtles (Herbst et al. 1999) is similar to that of Hawaiian green turtles (Aguirre et al. 1998). Likewise, histology of avian influenza in poultry from South America is similar to that seen in similar birds from North America (Jones and Swayne 2004; Zhou et al. 2006; Neufeld et al. 2009). Given this, the difference in microscopic pathology seen in corals from Florida vs USVI prompts the question of whether SCTLD in both regions might have different etiologies.

What is causing lytic mucus cell hypertrophy in corals from the USVI is unclear. Cnidaria often produce excessive mucus as a host response to chemical insults such as exposure to drilling muds, crude oil, dredge spoils, or heavy metal, a topic reviewed in Brown and Bythell (2005). However, we have no evidence that corals sampled here were exposed to such compounds. Mucus in corals is secreted in a condensed form that subsequently swells upon hydration (Brown and Bythell 2005). Perhaps increase in cell or vesicle membrane permeability is allowing excessive influx of water and hydration of mucus, leading to cell lysis and necrosis of mucus cells. Gastrodermis in diseased colonies stain PAS positive compared to apparently normal tissues suggesting a metabolic shift

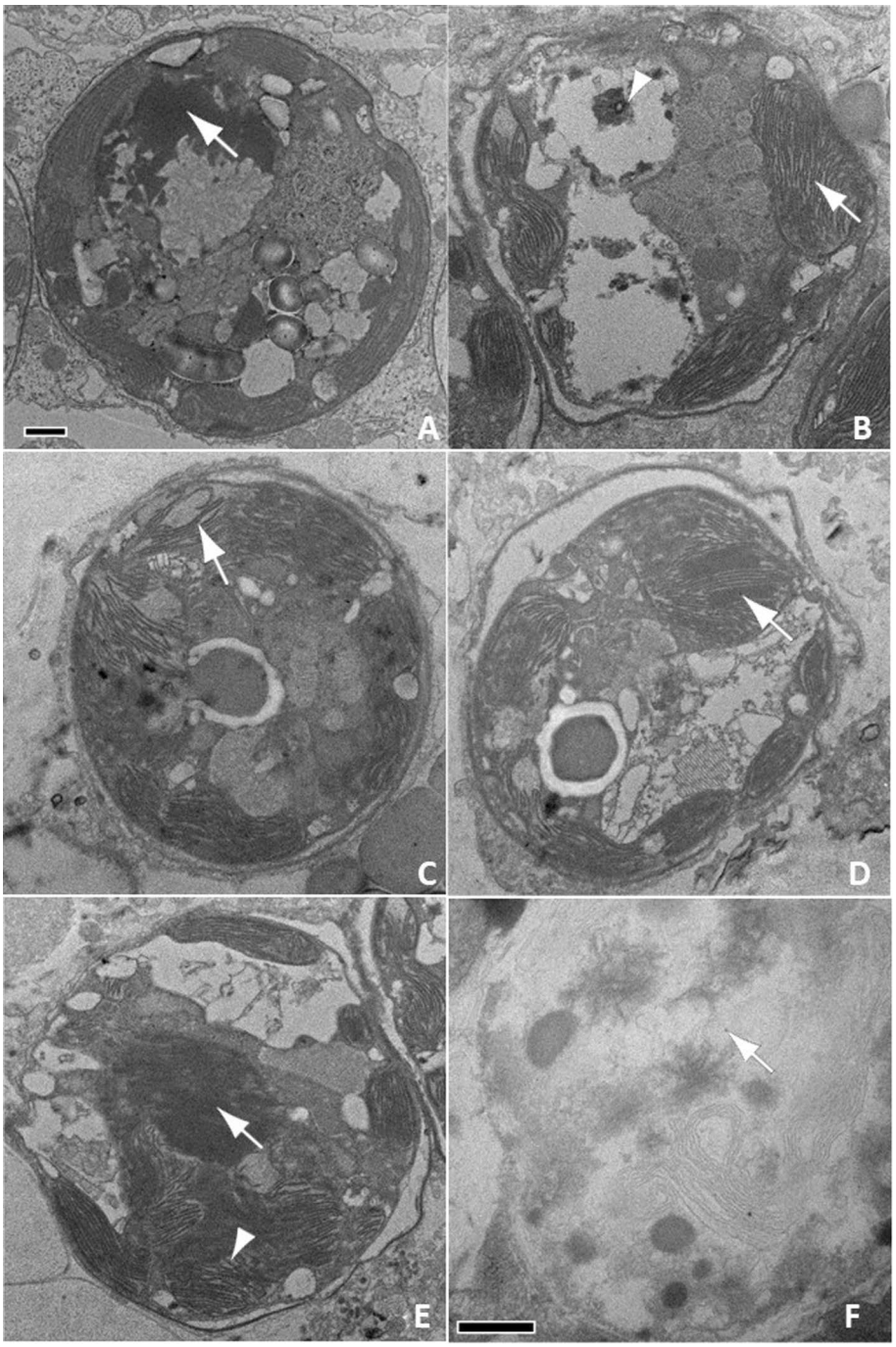

Fig. 7 Electron micrographs of endosymbiont pathology in corals collected from the US Virgin Islands stained with osmium. Bar in A is one µm and applies for all but F (400 nm). **A** Bleaching in *A. humilis*. Note cavity partially filled with electron-dense material (arrow). B–E) *C. natans* with subacute tissue loss. **B** Note variably sized cavities with electron dense debris (arrowhead) and gigantism of chloroplasts with increased spacing between thylakoid membranes (arrow). **C**) Chloroplasts with dissociation of thylakoids (arrow). **D**) Deformed endosymbiont with chloroplast gigantism with segmental fusion of thylakoids (arrow). **E** Pleomorphic cavities with debris and enlarged chloroplasts with fused thylakoids (arrow) or increased spaces between thylakoid membranes (arrowhead). **F** *P. strigosa* with subacute tissue loss; note stellate viral-like particles (arrow) in cavity of endosymbiont

Table 5 Summary of regional differences in manifestation of stony coral tissue loss disease (SCTLD) pathology between Florida and US Virgin Islands

Microscopic lesion	Florida	USVI
Lytic mucus cell hypertrophy	Rare	Common
Lytic necrosis with incursion into mesoglea	Common	Not seen
Endolithic overgrowth with calicodermal hypertrophy	Common	Rare
Deformed endosymbionts with intracytoplasmic refractile vacuoles	Common	Rare
Electron microscopy lesion		
Gigantism of chloroplasts in endosymbionts	Present	Present
Cavities with debris in endosymbionts	Present	Present
Asymmetric viral like particles	Common	Rare

from acidic alcian-blue positive mucins in normal tissues (Fig. 3E) to more neutral PAS-positive mucins (Fig. 3F) (Yamabayashi 1987). The paler staining in areas of mucus cell lytic hypertrophy (Fig. 3F) would suggest dilution of those mucins with water which would fit altered membrane permeability and influx of water into the cells with swelling and rupture of membranes (Fig. 6D). Confirming such a mechanism would require more systematic investigations of cell membrane integrity, something beyond the purview of microscopy. Finally, it is possible that given its widespread occurrence, lytic mucus cell hypertrophy could be a non-specific host response incidental to tissue loss. Resolving this could be done by sampling corals before and after incursion of SCTLD at a cite to see if this lesion is present at both time points.

Given the lack of infectious agents evident on light and electron microscopy, infectious agents do not appear responsible for the host response seen here and would contrast with the accepted belief that bacteria are causing SCTLD (Papke et al. 2024). As in Florida, corals had notable pathology in endosymbionts that differed only in the absence of viral-like particles. Based on TEM findings in Florida, Work et al. (2021) surmised that endosymbiont pathology in Florida corals could be caused by either viruses or toxicosis. In addition to viruses, Work et al. (2021) hypothesized that corals afflicted with SCTLD may be responding to toxins produced by endosymbiotic dinoflagellates. Toxins from coral endosymbionts (Nakamura et al. 1993) have been implicated in causation of coral bleaching (McConnaughey 2012). It is well known that many algal toxins interfere with membrane ion channels and cell membrane permeability (Wang 2008). Interestingly, cell swelling is a common denominator of cellular damage in cases of marine toxin poisoning. For instance, in mammals, domoic acid causes vacuolation of nerve cells (Pulido 2008) whereas yessotoxin causes fluid accumulation and swelling of heart cells (Terao et al. 1990). Perhaps compromised endosymbionts generate toxins that lead to inability of the coral host to maintain cell membrane homeostasis leading to lytic mucus cell hypertrophy. The diverse pathologies seen herein suggest that SCTLD is more complex than a "single etiology", and evaluating the pathology of the condition elsewhere might help refine our case definition.

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Declarations

Conflict of interests None to declare.

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