Transglobal spread of an ecologically relevant sea urchin parasite

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Abstract

Mass mortality of the dominant coral reef herbivore *Diadema antillarum* in the Caribbean in the early 1980s led to a persistent phase shift from coral- to algal-dominated reefs. In 2022, a scuticociliate most closely related to *Philaster apodigitiformis* caused further mass mortality of *D. antillarum* across the Caribbean, leading to >95% mortality at affected sites. Mortality was also reported in the related species *Diadema setosum* in the Mediterranean in 2022, where urchins experienced gross signs compatible with scuticociliatosis. However, the causative agent of the Mediterranean outbreak has not yet been determined. In April 2023, mass mortality of *D. setosum* occurred along the Sultanate of Oman's coastline. Urchins displayed signs compatible with scuticociliatosis including abnormal behavior, drooping and loss of spines, followed by tissue necrosis and death. Here we report the detection of an 18S rRNA gene sequence in abnormal urchins from Muscat, Oman that is identical to the *Philaster* strain responsible for *D. antillarum* mass mortality in the Caribbean. We also show that scuticociliatosis signs can be elicited in *D. setosum* by experimental challenge with the cultivated *Philaster* strain associated with Caribbean scuticociliatosis. These results demonstrate the *Philaster* sp. associated with *D. antillarum* mass

mortality has rapidly spread to geographically distant coral reefs, compelling global-scale awareness and monitoring for this devastating condition through field surveys, microscopy, and molecular microbiological approaches, and prompting investigation of long-range transmission mechanisms.

Body Text

The long-spined sea urchin genus *Diadema* is ubiquitous in tropical reef habitats across the globe, where it exerts critical control on algal growth (1), allowing sufficient light and space for new corals to settle and thrive (2,3). The loss of these important herbivores can result in phase shifts from coral- to algal-dominated communities, with widespread ecosystem effects (1,4).

A mass mortality event of unknown etiology decimated Caribbean *Diadema antillarum* populations in the early 1980s, with very limited recovery in subsequent years (4–7). Another *D. antillarum* mass mortality event was reported in February 2022 in the U.S. Virgin Islands and by May 2022 abnormal urchins were observed across the Caribbean (7,8). The 2022 mass mortality was caused by a scuticociliate most closely related to *Philaster apodigitiformis* (8). Signs of the condition, termed *Diadema antillarum* scuticociliatosis, include abnormal behavior, loss of tube foot control, stellate spine orientation, spine drooping and loss, and finally tissue necrosis and death (7,8). In both the 1980s and 2022 die-offs, no other echinoid species were reportedly affected (4,8).

Beginning in July 2022, mass mortality was observed in clade b *Diadema setosum* in its invasive range in the Mediterranean Sea (9). Signs resembled scuticociliatosis (8,9), but the etiological agent was not determined. In April 2023, we observed abnormal clade b *D. setosum* in the Sea of Oman (Figure 1). Abnormal individuals were collected and preserved in RNALater until DNA was extracted from urchin samples (~1 mm body wall fragments, 1-3 spines with bases, or 200 µl coelomic fluid) using the Zymo Quick-DNA Tissue/Insect Kit (Irving, CA, USA) following manufacturer's instructions with the exceptions of omitting β-mercaptoethanol from the lysis buffer, bead-beating for 2 min, and eluting into sterile water. Urchin species and clade were confirmed through CO2b/ATP6b gene amplification and sequencing (10) (Figure 2A). We amplified the *Philaster* clade associated with *Diadema* scuticociliatosis from six abnormal urchins collected from the Capital Area Yacht Club in Muscat, Oman using nested PCR (11,12). Sequencing confirmed that these samples contained 18S rRNA gene sequences identical to *P. apodigitiformis* FWC2, the agent responsible for scuticociliatosis in the Caribbean (8) (Figure 2B). FWC2 was cultured from the coelomic fluid of a *D. antillarum* specimen with scuticociliatosis in the Florida Keys in June 2022, and has been maintained in xenic culture.

To determine if clade a *D. setosum*, native to the Indo-Pacific, is also susceptible to scuticociliatosis, we ordered specimens from commercial aquarium suppliers for use in challenge experiments. One urchin (SL9) presented scuticociliatosis signs upon arrival and we observed ciliates resembling *P. apodigitiformis* swarming in dropped spines under microscopy. Following the protocols previously applied to culture FWC2 (8), we established the SL9 culture from the coelomic fluid of this urchin. Within 48 h of incubation at room temperature, the SL9 culture was densely populated by ciliates morphologically similar to FWC2 and dilution-to-extinction was performed. PCR and sequencing confirmed 100% identity between the 18S rRNA gene sequences of the FWC2 and SL9 cultures (Figure 2B).

Detecting this scuticociliate in an urchin obtained through the aquarium trade provided initial evidence for the ability of *P. apodigitiformis* to infect clade a *D. setosum*, leading us to conduct a controlled five-day experimental challenge. Eighteen urchins acquired through aquarium suppliers (Figure 2A) were placed into individual tanks filled with ~7 l of 5 µm-filtered offshore Florida

Keys seawater and an airstone bubbler. Twelve urchins were inoculated with ~250 ciliates each by addition to the water directly above the urchin (n=6 FWC2, n=6 SL9), and the remaining six urchins were treated with the same volume of 5 µm-filtered culture (n=3 FWC2, n=3 SL9) to control for bacteria within the media. Urchins were monitored for signs of infection and collected when disease was apparent or at experiment termination after five days; water used in challenge experiments was bleached for 24 h to kill any ciliates before disposal.

Upon collection, urchins were dissected to obtain coelomic fluid, spine/spine base, and body wall samples, which were frozen at -80^{o}C until DNA extraction and quantitative PCR for *P. apodigitiformis* following previously published protocols (8). Five of the six urchins treated with each ciliate culture lost spines and died, whereas only two of the six controls (one each of FWC2 and SL9 filtrate) exhibited signs of infection (Figure 2C), likely resulting from ciliate exposure prior to arrival at our facility. Grossly normal urchins had lower levels of *P. apodigitiformis* in body wall, spine, and coelomic fluid samples than abnormal urchins by quantitative PCR (qPCR) for the 28S rRNA gene, regardless of treatment (Figure 2D). Although the negative impacts of scuticociliatosis on *Diadema* spp. are clear, many questions remain about the factors that affect *P. apodigitiformis* growth and pathogenicity and *Diadema* immune responses and mechanisms for disease resistance. Although *D. antillarum* and *D. setosum* exhibit similar signs of scuticociliatosis, individuals of both species display variability in their responses to infection, including some experimentally infected specimens of each species that remained grossly normal.

Our experimental challenge results, combined with the detection of *P. apodigitiformis* in field samples from Oman, demonstrate that this parasite can cause scuticociliatosis in both clades of *D. setosum*, representing a significant threat to these important herbivores. Despite *D. setosum* being an invasive species in the Mediterranean Sea, the detection of scuticociliatosis in the Sea of Oman, part of its native Red Sea habitat, could have disastrous ecological effects on coral reef communities reminiscent of those seen in the Caribbean following the 1980s die-off (1,4,6). These results highlight the importance of monitoring urchin densities and ecosystem-level effects resulting from loss of these keystone herbivores in affected regions. Additionally, our experimental infection of clade a *D. setosum* indicates the vulnerability of this population to scuticociliatosis should the ciliate reach its native range, emphasizing the need for baseline benthic surveys. We also observed mortality of *Echinothrix* sp. in Fujairah and Al Fahal Island, suggesting that other species in the

Diadematidae family may be susceptible to scuticociliatosis. Similar to the ciliate *Philaster lucinda*, which is associated with many coral diseases (13), *P. apodigitiformis* has now been detected in geographically disparate locations. Therefore, it is critical to assess long-range transmission routes of this ciliate, including natural (e.g., currents, seabirds) and anthropogenic (e.g., shipping vessels, recreational diving, aquarium trade) pathways.

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Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Data Availability Statement

All ciliate sequences generated in this study are available in GenBank (Accession Numbers: OR730962-OR730978).

References

1. Carpenter RC. Mass mortality of *Diadema antillarum*: I. Long-term effects on sea urchin population-dynamics and coral reef algal communities. Mar Biol. 1990 Feb;104(1):67–77.

2. Williams SM. The reduction of harmful algae on Caribbean coral reefs through the reintroduction of a keystone herbivore, the long-spined sea urchin *Diadema antillarum*. Restor Ecol. 2022 Jan;30(1):e13475.

3. Idjadi J, Haring R, Precht W. Recovery of the sea urchin *Diadema antillarum* promotes scleractinian coral growth and survivorship on shallow Jamaican reefs. Mar Ecol Prog Ser. 2010 Mar 22;403:91–100.

4. Lessios HA. The great *Diadema antillarum* die-off: 30 years later. Annu Rev Mar Sci. 2016 Jan 3;8(1):267–83.

5. Lessios HA, Robertson DR, Cubit JD. Spread of *Diadema* mass mortality through the Caribbean. Science. 1984 Oct 19;226(4672):335–7.

6. Lessios HA. Mass mortality of *Diadema antillarum* in the Caribbean: What have we learned? Annu Rev Ecol Syst. 1988 Nov;19(1):371–93.

7. Hylkema A, Kitson-Walters K, Kramer PR, Patterson JT, Roth L, Sevier MLB, et al. The 2022 *Diadema antillarum* die-off event: Comparisons with the 1983-1984 mass mortality. Front Mar Sci. 2023 Jan 5;9:1067449.

8. Hewson I, Ritchie IT, Evans JS, Altera A, Behringer D, Bowman E, et al. A scuticociliate causes mass mortality of *Diadema antillarum* in the Caribbean Sea. Sci Adv. 2023 Apr 21;9(16):eadg3200.

9. Zirler R, Schmidt LM, Roth L, Corsini-Foka M, Kalaentzis K, Kondylatos G, et al. Mass mortality of the invasive alien echinoid *Diadema setosum* (Echinoidea: Diadematidae) in the Mediterranean Sea. R Soc Open Sci. 2023 May;10(5):230251.

10. Lessios HA, Kessing BD, Pearse JS. Population structure and speciation in tropical seas: Global phylogeny of the sea urchin *Diadema*. Evolution. 2001 May 9;55(5):955–75.

11. Vilanova-Cuevas BY, Reyes-Chavez B, Breitbart M, Hewson I. Design and validation of a PCR protocol to specifically detect the clade of *Philaster* sp. associated with *Diadema antillarum* scuticociliatosis. BioRxiv [Internet]. 2023 Sep 11; Available from: http://biorxiv.org/lookup/doi/10.1101/2023.09.11.557215

12. Dopheide A, Lear G, Stott R, Lewis G. Molecular characterization of ciliate diversity in stream biofilms. Appl Environ Microbiol. 2008 Mar 15;74(6):1740–7.

13. Sweet MJ, Séré MG. Ciliate communities consistently associated with coral diseases. J Sea Res. 2016 Jul;113:119–31.

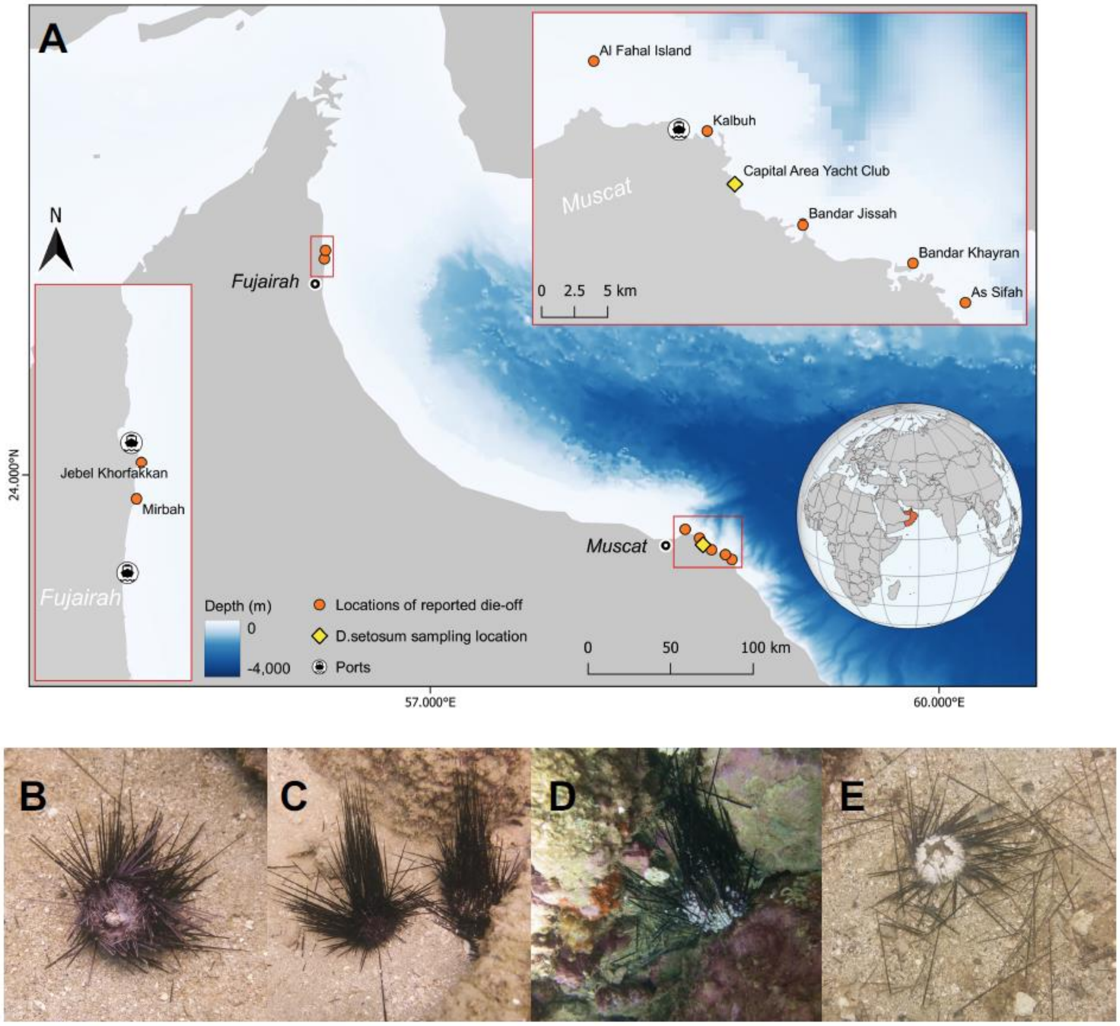

Figure 1. Abnormal *Diadema setosum* reported from the Sea of Oman.

(A) Graphical depiction of locations in the Sea of Oman where *D. setosum* die-offs were reported (orange points) and confirmed in collected individuals via PCR (yellow diamond). Inlaid images show more detailed maps of sites in Fujairah (left) and Muscat (top). Basemap created in QGIS using data freely available from General Bathymetric Chart of the Ocean (www.gebco.net) and

EOX (maps.eox.at). (B-E) Images depicting signs seen in urchins considered “grossly abnormal”, including unusual behavior (B), stellate spine arrangement (C), spine loss and tissue necrosis (D), and eventual death (E). Photo credit: Kaileigh Cornfield.

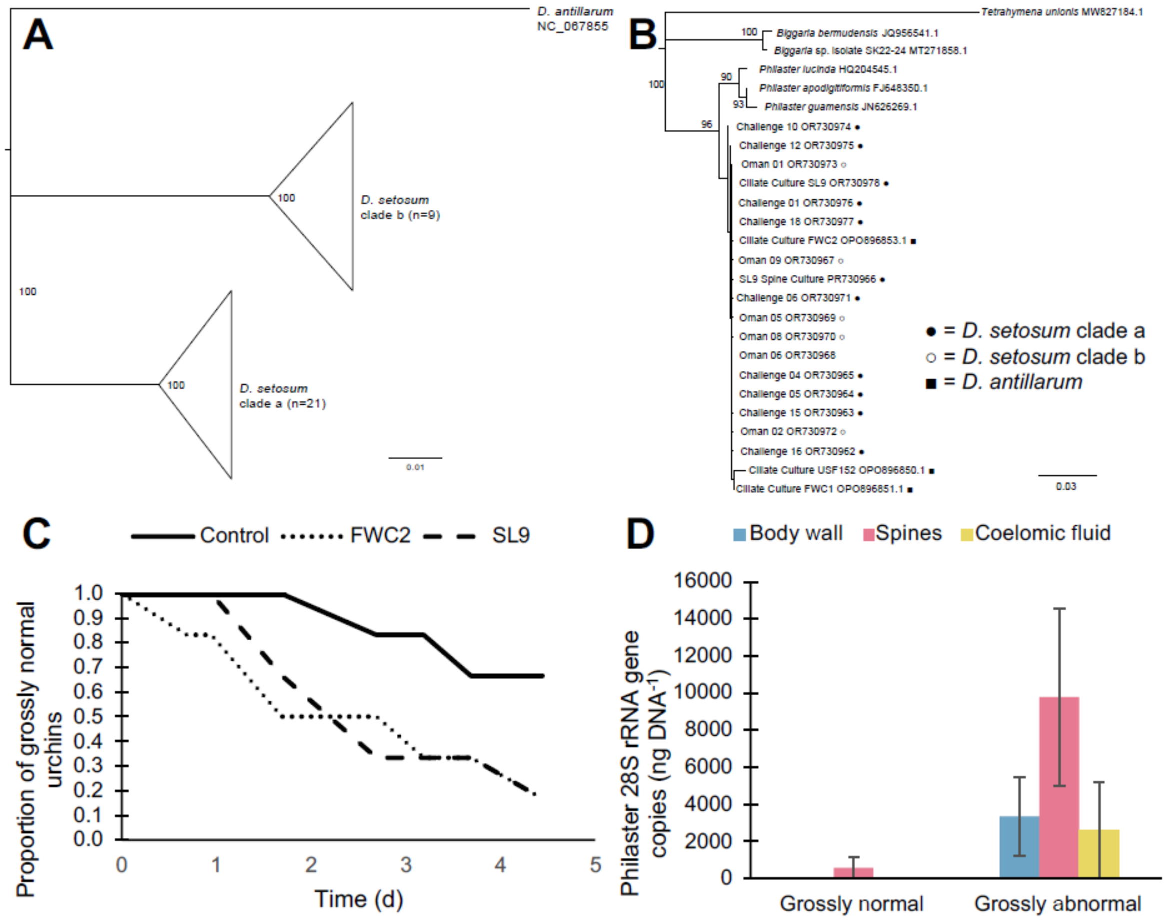

Figure 2. *Diadema* and scuticociliate phylogenies and results of challenge experiments.

(A) Phylogenetic representation of *D. setosum* clades a and b sampled during this experiment, as well as *D. antillarum*. (B) Phylogenetic representation of scuticociliate sequences from *D. setosum* (clade a = filled circle, clade b = empty circle), *D. antillarum* (filled square), and close relatives identified by BLASTn searches. All phylogenetic analyses were performed in Geneious Prime using the Geneious Tree Builder and Tamura-Nei genetic distance model with the neighbor-joining. (C) Survivorship curve showing the decrease in grossly normal individuals over time for urchins treated with two *Philaster* cultures (FWC2 and SL9) and the controls. (D) Quantitative PCR results for body wall, spines, and coelomic fluid samples from the 18 experimental challenge urchins, classified by animal state at the end of the experiment.