Title

Disease-associated aggregation of *Dactylopleustes yoshimurai* on sea urchins: host-level and lesion-level processes

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Host- and lesion-level aggregation in *Dactylopleustes*

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Abstract

Amphipods of the genus *Dactylopleustes* are specialized symbionts of sea urchins, and in some species aggregations on host lesions have been reported; however, the behavioural mechanisms underlying such lesion-associated aggregation remain poorly understood. We investigated host-level and within-host processes underlying lesion aggregation in the symbiotic amphipod *Dactylopleustes yoshimurai* on the short-spined sea urchin *Strongylocentrotus intermedius*. In paired host-selection trials with freely moving urchins, amphipods accumulated more on diseased than on healthy hosts. When hosts were held apart in net cylinders, this bias toward diseased hosts disappeared, and individuals that had settled on a host rarely switched hosts, suggesting that host-to-host transfer occurs mainly when urchins approach one another. In a time-series aggregation experiment on diseased hosts, most amphipods attached soon after introduction and gradually concentrated on the lesion surface within approximately 6 h. Qualitative observations suggested that repeated contacts with host pedicellariae may promote stepwise movements across the test, whereas amphipods that reached pedicellariae-inaccessible microhabitats, including long-spine tips or the lesion surface, became largely stationary. Finally, on otherwise healthy hosts, both experimental pedicellariae removal and surface wounding induced amphipod accumulation, indicating that lesion-like microhabitats can form via reduced defence, disturbance cues, or both. Together, our results support a stepwise model in which *D. yoshimurai* first attaches with limited host-level discrimination, switches hosts primarily via short-range transfer, and then is retained in lesion microhabitats on the host surface. This two-scale framework links experimental behaviour to field patterns and highlights host contact rates and lesion availability as key determinants of symbiont distribution.

Keywords

symbiosis, host selection, *Dactylopleustes yoshimurai*, *Strongylocentrotus intermedius*

Highlights

- Host-level bias appears only when urchins contact, enabling host-to-host transfer.
- Amphipods attach rapidly and concentrate on lesions within ~6 h after settlement.
- Pedicellariae removal and surface wounding each trigger local amphipod accumulation.
- Lesions act as retention microhabitats rather than primary host-attraction targets

1. Introduction

Sea urchins host a remarkable diversity of epibiotic and endobiotic symbionts spanning many animal groups from at least ten animal phyla (Hayes *et al.*, 2016), and these associates can range from parasites that consume host tissues to commensals or mutualists that gain shelter or food while exerting little cost, or even benefits, to their hosts. For example, some eulimid gastropods and echinoid-associated crabs can damage hosts through tissue feeding or boring (Castro, 1971; Warén, 1983; González-Vallejo & de Leon-Gonzalez, 2018; Dgebuadze *et al.*, 2020), whereas other crustaceans may reduce parasite loads or otherwise function as cleaners under certain situations (Sonnenholzner *et al.*, 2011). Such functional diversity makes sea urchins a useful system for asking not only 'what' symbionts do, but 'how' they choose hosts and microhabitats on host bodies, and how these behaviours shape host–symbiont outcomes.

Members of the genus *Dactylopleustes* Karaman & Barnard, 1979 are among the most specialized echinoid associates within the order Amphipoda and are thought to have evolved adaptations for living on sea urchin surfaces (Bousfield & Hendrycks, 1995). However, despite their close host association, the ecology and life history of *Dactylopleustes* remain poorly documented (Vader, 1978), and the mechanisms that generate their distribution across host individuals and host body regions are still unclear.

One member of the genus, *Dactylopleustes yoshimurai* Tomikawa, Hendrycks & Mawatari, 2004, occurs on several echinoids including the short-spined urchin *Strongylocentrotus intermedius*, and has repeatedly been observed forming dense aggregations on disease lesions of host urchins (Kodama et al., 2020). Recent long-term monitoring in Otsuchi Bay indicates that lesion-associated aggregation is not occasional but is a core feature of this symbiosis (Kodama et al., 2026). Over multiple years, *D. yoshimurai* remained at low densities on healthy urchins, whereas diseased urchins supported dense aggregations with pronounced winter peaks; these peaks broadly overlapped with seasonal increases in the prevalence of lesions in host urchins. Assemblages on diseased hosts were strongly dominated by small individuals, with recurring juvenile pulses in winter to early spring, whereas healthy hosts supported low numbers but relatively higher proportions of adults. These results highlight lesions as seasonally dynamic habitat patches that are particularly important for early life stages and suggest that amphipod population dynamics are shaped by the interaction between host disease phenology and amphipod reproduction/recruitment (Kodama et al., 2026).

Despite this clear field pattern, the proximate mechanism producing lesion-associated aggregation remains unresolved. One simple hypothesis is that amphipods aggregate to feed on exposed host tissues at lesions. However, DNA metabarcoding of gut contents suggests that urchin-derived material is only a limited component of the diet even for individuals collected from lesions, with non-host resources such as sediments being prominent (Kodama et al., 2024). This evidence shifts attention toward alternative mechanisms in which lesions are important primarily as microhabitats rather than as feeding sites. Lesions may (i) reduce the costs of living on the host surface (e.g., weakened pedicellarial defence), thereby increasing residence time, and/or (ii) offer localized microbial and chemical environments generated by surface disturbance and tissue damage that act as cues attracting amphipods to the lesion.

Here we build on the population-dynamic framework from Otsuchi Bay by testing mechanistic predictions that separate (1) host-level processes (host choice and host switching among urchin individuals) from (2) within-host processes (microhabitat selection and retention on the host surface). First, we compared the preference of *D. yoshimurai* for diseased versus healthy hosts under two arena designs: one allowing hosts to move and contact one another, and another in which hosts were held apart while amphipods could move freely between them. This contrast tests whether diseased-host bias requires close-range host–host transfer opportunities. Second, we quantified the timing of amphipod settlement and within-host redistribution on diseased hosts to test whether lesion aggregations form through an active behavioural sequence. Finally, to evaluate whether aggregation is driven primarily by avoidance of host defensive structures versus attraction to surface disturbance/damage-associated cues, we created lesion-like microhabitats on otherwise healthy hosts by removing pedicellariae in a defined area and by applying an explicit wounding treatment as a control. By integrating these experiments with long-term field patterns, we aim to identify the behavioural steps and proximate cues that make lesions disproportionately important habitat patches for *D. yoshimurai*.

2. Materials and Methods

2.1. Amphipod and sea urchin collection.

Dactylopleustes yoshimurai and its host urchin *Strongylocentrotus intermedius* were used in this study. All individuals used in the experiments were collected from a rocky subtidal area on the north coast of Otsuchi Bay, Japan. Specimens used in the host selection experiments (Sections 2.2–2.3) and the aggregation experiment (Section 2.4) were collected on 6–7 March 2023, and these experiments were conducted on 8–14 March 2023. Specimens used in the pedicellariae-removal experiment (Section 2.5) and the wounding experiment (Section 2.6) were collected on 11 February 2024, and these experiments were conducted on 12–21 February 2024.

Diseased sea urchins (mean ± SD = 46.37 ± 6.47 mm in test diameter) and healthy sea urchins (mean ± SD = 47.73 ± 5.26 mm in test diameter) were collected using SCUBA. For diseased urchins, photographs of the diseased area were taken, and its projected area was measured using ImageJ (mean ± SD = 129 ± 84 mm^2). Amphipods (mean ± SD = 4.66 ± 1.13 mm in body length) were collected from host urchins at the same locality and maintained in seawater-running tanks until use. Immediately before each experiment, sea urchins were rinsed with seawater to remove other symbiotic organisms from their body surfaces.

2.2. Host selection experiment 1: with freely moving sea urchin hosts.

The selectivity of diseased and healthy sea urchins by *D. yoshimurai* was experimentally investigated (n = 24). Transparent plastic tanks (425 × 245 × 285 mm) filled with natural seawater to a depth of 15 cm were used in the experiment. All the tanks used in the experiment were set up inside larger tanks in the open air and were water-bathed in running seawater to keep the temperature the same as in the natural condition.

One diseased and one healthy sea urchin were placed in the tank. The urchins in the same tank were selected so that their difference in diameter was as small as possible; the largest difference was 6.2 mm. After the introduction of the urchins, ten individuals of *D. yoshimurai* were gently put in the central part of the tank and left for 24 hours, allowing *D. yoshimurai* to settle freely on the sea urchin hosts (Fig. 1A). In this experimental design, both the urchin hosts and *D. yoshimurai* are free to move in the tank.

After 24 hours, each sea urchin was gently put into a plastic net (0.5 mm in mesh opening) along with the *D. yoshimurai* on their surface. The sea urchins were washed with seawater to detach *D. yoshimurai*, and the number of *D. yoshimurai* on each sea urchin was counted. For each trial, we recorded the numbers of amphipods associated with diseased urchin, healthy urchin, those remaining in the tank, dead individuals, and unrecovered individuals. The paired Wilcoxon signed-rank test was used to compare the number of *D. yoshimurai* between those on diseased urchin and on healthy urchin.

2.3. Host selection experiment 2: with sea urchin hosts prevented from moving.

The selectivity between diseased and healthy sea urchins by *D. yoshimurai* was experimentally investigated again, but under a condition in which host urchins are separated from each other (n = 20). The tanks were prepared in the same condition as experiment 1 except that the sea urchins are placed in cylinders made of plastic nets (3.0 × 3.0 mm in mesh opening; 90 mm in diameter; height extending above the water surface) to prevent the urchins from moving out of the cylinder. The two cylinders with healthy and diseased urchins were set 31.5 cm apart from each other in the tank. Twenty individuals of *D. yoshimurai* were gently put in the central part of the tank and left for 24 hours, allowing the amphipods to freely settle on the sea urchin hosts (Fig. 1B). In this experimental design, urchin hosts cannot approach each other, whereas *D. yoshimurai* can pass through the mesh of the plastic nets and thus can freely select the sea urchin hosts.

After 24 hours, the cylinders containing urchin are gently put into a plastic net separately (0.5 mm in mesh net) with the *D. yoshimurai* on their surface. The cylinders and sea urchins were washed with seawater to detach *D. yoshimurai*, and the number of *D. yoshimurai* was counted. For each trial, we recorded the numbers of amphipods associated with diseased urchin, healthy urchin, those remaining in the tank, dead individuals, and unrecovered individuals. The paired Wilcoxon signed-rank test was used to compare the number of *D. yoshimurai* between those on diseased and healthy urchins.

2.4. Aggregation experiment

Active aggregation of *D. yoshimurai* onto the diseased area of their host urchins was experimentally investigated in a light- and temperature-controlled laboratory. Cylindrical glass tanks (φ = 128 mm, seawater depth = 50 mm, n = 10) filled with natural seawater were placed on a stand for easy observation, as the diseased areas are located on the oral side and need to be observed from the bottom. A diseased sea urchin was placed in the central part of the tank, and ten individuals of *D. yoshimurai* were evenly placed around the marginal area of the tank (Fig. 2). Both the urchin and the *D. yoshimurai* are free to move in the tank.

During a 24-hour period after setting up, the urchin and the *D. yoshimurai* were observed every hour. At each observation, the number of *D. yoshimurai* settled on the urchins and that settled on the diseased area were counted separately. During the 24-hour period, the light was kept on, and the water temperature was kept at 7°C.

For visualization of temporal patterns, we added a smoothing curve using a generalized additive model (GAM) to the observed proportion of amphipods on the diseased area over time. The effect of the duration (hours) was assessed by a GAM using the ‘mgcv’ package in R version 4.2.0. In the GAM, binomial distributions with logit link functions were used (response: number of amphipods on the urchin or on the diseased area out of 10 individuals introduced).

2.5. Pedicellariae-removal experiment

The absence of pedicellariae (defensive organs of sea urchins) on diseased areas may be responsible for triggering amphipod aggregation. To examine this hypothesis, active aggregation of amphipods into an area without pedicellariae was experimentally examined by removing pedicellariae from a portion of the sea urchin body surface.

An experimental area was defined on healthy urchins as one ambulacral column and the two adjacent interambulacral columns surrounding it, spanning from the anal (aboral) side to 90° laterally. A control area of the same size (one ambulacral column and the two adjacent interambulacral columns, from the anal side to 90° laterally) was established on the opposite side. Within the experimental area, pedicellariae were removed as completely as possible using forceps.

Each sea urchin with pedicellariae removed was placed in a cylindrical glass container filled with natural seawater (φ = 128 mm, seawater depth = 50 mm, n = 20), and 10 individuals of *D. yoshimurai* were evenly placed around the marginal area of the tank (conditions identical to the aggregation experiment; Fig. 2). After setup, the containers were observed 12 h later, and the numbers of *D. yoshimurai* attached within the experimental and control areas were counted. The paired Wilcoxon signed-rank test was used to compare the number of *D. yoshimurai* between the experimental and control areas. Throughout the experiment, the lights were kept on continuously and room temperature was maintained at 7°C.

2.6. Wounding experiment

In the pedicellariae-removal experiment, the effect of pedicellariae removal cannot be separated from the effect of simply injuring a specific area of the sea urchin body surface. Therefore, we examined whether amphipod aggregation could be induced by wounding alone, without removing pedicellariae.

Experimental and control areas were established on the sea urchin body surface as in the pedicellariae-removal experiment. In the wounding experiment, the body surface within the experimental area was pricked repeatedly with a fine insect pin to create multiple small wounds, such that the density of punctures was approximately comparable to the density of pedicellariae present in the experimental area. All other experimental conditions were identical to those in the pedicellariae-removal experiment (Fig. 2; n = 20), and the numbers of *D. yoshimurai* attached within the wounded area and the control area were compared.

3. Results

3.1. Host selection experiment 1

In the host selection experiment with freely moving sea urchins, *D. yoshimurai* was found more frequently on diseased hosts than on healthy hosts (Fig. 3). The number of *D. yoshimurai* was significantly higher on diseased sea urchins (6.3 ± 2.6 individuals, mean ± SD) than on healthy sea urchins (1.2 ± 1.5 individuals) (paired Wilcoxon signed-rank test, $p < 0.001$).

Across trials, most individuals were recovered from either the diseased or healthy sea urchin (7.5 ± 2.0 individuals), whereas fewer remained in the tank (2.4 ± 1.9 individuals). Dead and unrecovered individuals were usually absent and occurred only occasionally (Dead: 0.08 ± 0.28; Lost: 0.21 ± 0.41 individuals), each recorded in 2/24 and 5/24 trials, respectively, and never exceeding two individuals per trial. No mortality was observed in any urchin during the experiment.

3.2. Host selection experiment 2

In the host selection experiment 2 using sea urchin hosts prevented from moving (and thus could not contact each other), *D. yoshimurai* showed no clear bias toward the diseased side (8.9 ± 3.4 individuals, mean ± SD) compared with the healthy side (7.3 ± 2.6 individuals). There was no significant difference in the numbers of *D. yoshimurai* on diseased and healthy urchins and/or their cylinders (Fig. 4; paired Wilcoxon signed-rank test, $p = 0.165$).

Across trials, most individuals were recovered from either urchin and/or cylinder (16.2 ± 3.1 individuals), whereas fewer remained in the tank (3.1 ± 2.9 individuals). Dead and unrecovered individuals were usually absent and occurred only occasionally (Dead: 0.4 ± 0.7; Lost: 0.45 ± 0.76), each recorded in 6/20 trials and never exceeding two individuals per trial. No mortality was observed in any urchin during the experiment.

3.3. Aggregation experiment

In the aggregation experiment, most amphipods attached to urchins soon after the start of the experiment, and within several hours many of the attached individuals became concentrated on the diseased area. Consistent with these observations, the GAM indicated that the number of *D. yoshimurai* on the host increased rapidly and reached a plateau at approximately 9 individuals within ~2 h (Fig. 5A). The number of amphipods on the diseased area also increased over time, reaching a plateau at approximately 7 individuals by ~6 h (Fig. 5B). No mortality was observed in any urchin during the experiment.

During these observations, host pedicellariae appeared to influence fine-scale movements of amphipods on the urchin surface. Pedicellariae surrounding settled amphipods were frequently oriented toward them (Fig. 6), and amphipods were repeatedly contacted by pedicellariae. Such contacts were often followed by small displacements, resulting in gradual, stepwise movements across the body surface. In contrast, individuals that reached microhabitats that seemed less accessible to pedicellariae (such as the tips of long spines or the lesion surface) generally showed little subsequent movement.

3.4. Pedicellariae-removal experiment

In the pedicellariae-removal experiment, *D. yoshimurai* was consistently more abundant in the experimental area (pedicellariae-removal area) than in the control area (Figs 7A, 8). The number of amphipods in the experimental area was 4.0 ± 3.2 individuals (mean ± SD), whereas that in the control area was 0.30 ± 0.57 individuals. Overall, the difference between the experimental and control areas was significant (paired Wilcoxon signed-rank test, $p < 0.001$). No mortality was observed in any urchin during the pedicellariae-removal experiment, indicating that the treatments did not cause lethal effects.

3.5. Wounding experiment

In the wounding experiment, *D. yoshimurai* was consistently more abundant in the experimental area (wounded area) than in the control area (Figs 7B). The number of amphipods in the experimental area was 4.1 ± 3.2 individuals (mean ± SD), whereas no amphipods were observed in the control area. Overall, the difference between the experimental and control areas was significant (paired Wilcoxon signed-rank test, $p < 0.001$). No mortality was observed in any urchin during the wounding experiment, indicating that the treatments did not cause lethal effects.

4. Discussion

This study provides experimental evidence that the sea urchin symbiont *Dactylopleustes yoshimurai* actively accumulates on diseased areas of its host, *Strongylocentrotus intermedius*, and clarifies the behavioural steps that can generate this pattern. Across experiments, the results consistently point to a stepwise process operating at two spatial scales: (1) host-level redistribution among urchin individuals and (2) lesion-level retention on the host body surface. The host selection experiments show that diseased hosts were more frequently used than healthy hosts only when sea urchins could freely move within the arena. When host urchins were spatially separated, amphipods did not preferentially occupy diseased hosts, and individuals that settled on a host tended to remain there. In parallel, the aggregation experiment demonstrated rapid within-host redistribution, with most amphipods attached to the host within a few hours and then concentrating on disease lesion within approximately six hours. Finally, pedicellariae removal and wounding both induced local accumulation on otherwise healthy hosts, indicating that amphipod aggregation can be triggered even in the absence of naturally developed lesions. Together, these results support a stepwise mechanism of host use in which amphipods first attach to a host with little discrimination, then move between hosts mainly when hosts approach each other, and finally concentrate on particular host microhabitats that provide attractive cues or reduced costs. By explicitly separating host-level redistribution from lesion-level retention, this framework provides a simple behavioural explanation for why diseased urchins can accumulate high local densities even if initial host encounter is only weakly discriminatory.

4.1. Host-level selection is mediated by host movement and host proximity

A key result is that amphipods showed a preference for diseased hosts only when hosts were allowed to move freely. This implies that host-level selection in *D. yoshimurai* depends strongly on opportunities for host-to-host transfer created by host movement and proximity. In experiment 1, diseased and healthy urchins could approach or contact each other, creating frequent opportunities for amphipods to transfer. In experiment 2, hosts were held apart by net cylinders, preventing close approaches, and amphipods showed no diseased host bias. This pattern is consistent with the broader principle that many symbionts require short-distance transfer routes and often depend on direct host contact or very close proximity for host switching (e.g., Proctor, 2003; Presley, 2011). Our results also suggest that once *D. yoshimurai* reaches a host, it generally remains on that host unless another host becomes available at very short range. Under natural conditions, therefore, host density and the frequency of inter-host contacts are likely to be important determinants of amphipod redistribution across individuals.

A key implication of the contrast between our experimental setups is that the realized rate of between-host movement should depend on how often conspecific urchins approach or contact one another. Although we did not quantify inter-individual spacing of S. intermedius in Otsuchi Bay, we frequently observed small clusters of conspecifics occupying the same crevices and feeding in close proximity on macroalgal patches (Fig. 9). Such clustering is consistent with time-lapse observations indicating that *S. intermedius* tends to group near food substrates under calm conditions when palatable kelp is available (Zhadan & Vaschenko, 2019). More broadly, dense aggregations of sea urchins including cohesive grazing/feeding fronts along macrophyte-bed boundaries are widely reported and are not unusual in echinoids (e.g., Lauzon-Guay & Scheibling, 2007; Abraham, 2007). In these contexts, host–host proximity and tactile encounters can be frequent, providing repeated opportunities for short-range symbiont transfer. We therefore suggest that even if initial host-level discrimination by *D. yoshimurai* is weak at first encounter, redistribution within a clustered host patch could increase the probability of reaching a diseased host, after which lesion-level retention would rapidly amplify host-level differences.

Alternative explanations for the absence of preference in experiment 2 should also be considered. The net cylinders may have reduced visual or tactile cues from the host, or altered local flow and the distribution of chemical cues. Many symbionts rely primarily on chemical information to detect and recognize hosts (e.g., Derby & Atema, 1980; Stevens, 1990; Ambrosio & Brooks, 2011; Williamson et al., 2012), but additional sensory modes, including vision, can become important at close range in some taxa (Mikheev et al., 2004; Ambrosio & Brooks, 2011; Williamson et al., 2012). Amphipods are often considered to have limited vision (Bellan-Santini, 2015), yet visual limitation cannot be excluded, particularly because the cylinders were black and may have partially obscured host outlines. At the same time, if chemical cues from both urchins rapidly permeated the still water of the tank, then a strong chemical gradient between hosts may not have formed, potentially weakening any chemical-based discrimination. Regardless of the specific sensory mechanism, the experimental contrast indicates that host proximity is a necessary condition for a host-level diseased–healthy bias to emerge at the scale of whole hosts.

More generally, our results fit a two-scale framework that is broadly applicable to directly transmitted ectosymbionts and ectoparasites: between-host redistribution is often constrained by opportunities for close-range transfer, whereas within-host distributions are shaped by microhabitat-level retention (White et al., 2017). In such systems, host-to-host transfer frequently depends on direct host contact or very short-range proximity, so the realized dispersal rate can scale with host density and contact structure. Once on a host, many ectosymbionts exhibit microhabitat specialization on the host body and concentrate in regions that reduce exposure to host defenses or disturbance, thereby increasing residence time and local density (Johnson et al., 2012; Pilosof et al., 2012). Within this framework, the strong association of *D. yoshimurai* with lesions can be interpreted primarily as a retention process: lesions (and experimentally disturbed areas) function as "retention microhabitats" that retain individuals after initial attachment, amplifying host-level differences even when initial host encounter is only weakly discriminatory.

4.2. Rapid within-host redistribution supports lesion-level retention

Consistent with this interpretation, the aggregation experiment directly demonstrated that amphipods do not merely remain where they first land on the host. Instead, they attached quickly and then redistributed across the host surface, concentrating on lesions within hours. The rapid timing is informative because it constrains possible mechanisms. A response occurring within several hours is consistent with immediate sensory cues on the host surface, such as cues associated with damaged tissues, microbial films, or host secretions, and is less consistent with explanations requiring long-term host deterioration or prolonged feeding on tissue.

The within-host process revealed here provides a mechanistic bridge to population-level patterns reported previously. Long-term field surveys in Otsuchi Bay showed that diseased urchins support much higher densities of *D. yoshimurai* and that assemblages on diseased hosts are strongly dominated by juveniles, particularly in winter (Kodama et al., 2026). Our experiments demonstrate how strong microhabitat concentration can arise rapidly after settlement and how aggregation can occur without requiring strong initial preference at the moment of host encounter. Under this framework, diseased hosts can accumulate high densities primarily because they offer microhabitats that retain amphipods once individuals have arrived.

4.3. What cue drives aggregation on lesions

A central question is why *D. yoshimurai* aggregates on diseased areas. One hypothesis is direct feeding on exposed host tissues at lesions (Kodama et al., 2020). However, DNA metabarcoding indicates that urchin-derived material is a minor dietary component even for individuals collected from lesions, whereas non-host resources such as sediments are prominent (Kodama et al., 2024). This makes it unlikely that consumption of host tissue alone explains lesion-associated aggregation, although opportunistic feeding on host material may still occur.

A second hypothesis is defence-related habitat selection. Sea urchins use pedicellariae as defensive organs, and pedicellariae can deter or remove small epibionts and can deliver bioactive compounds (Campbell & Rainbow, 1977; Mebs, 1984; Nakagawa et al., 2003; but see also Guenther et al., 2007). Diseased areas often exhibit reduced defensive function, including loss or reduced activity of pedicellariae. Under this hypothesis, amphipods would be expected to avoid intact, highly defended surfaces and accumulate in microhabitats where defensive pressure is reduced. Our manipulative experiments partly support the idea that defence- and damage-related cues are important. Amphipods attached in higher numbers to pedicellariae removal areas than to control areas on the same host. However, amphipods also accumulated on experimentally wounded areas where pedicellariae were not deliberately removed. This result indicates that pedicellariae absence alone is not required to elicit aggregation. Instead, wounding or disturbance to the host surface can itself generate attractive conditions.

The combined outcome of these two experiments narrows the likely mechanisms to at least two plausible scenarios. The first is that amphipods respond directly to cues associated with damage, such as host body fluids, damaged epidermis, or rapidly developing microbial films on injured surfaces, and therefore accumulate wherever such cues are present regardless of whether a natural lesion exists. This explanation is consistent with the observation that both pedicellariae removal and pin pricking promoted aggregation, because both treatments likely caused some degree of surface damage and exposure of host-derived substances. The second scenario is that amphipods do avoid pedicellariae and that the wounding treatment indirectly reduced defensive pressure by impairing pedicellariae function in the treated area. In other words, the wounding manipulation may have unintentionally weakened pedicellariae activity enough that amphipods no longer avoided the area, leading to an apparent preference. Distinguishing these alternatives will require experiments that separately manipulate pedicellariae activity and tissue damage, for example by removing pedicellariae with minimal tissue disruption, or by producing chemical cues from damage without changing physical defence structures. Direct observations of pedicellariae attacks and amphipod responses would also strengthen inference.

Importantly, under field conditions it is difficult to envisage situations in which pedicellariae alone are removed while the surrounding body surface remains entirely undamaged. In most naturally occurring lesions or injuries, surface disturbance and a reduction in effective pedicellarial defence are likely to co-occur. This point matters because it implies that moving toward "damage-associated" signals and avoiding pedicellariae attacks are not mutually exclusive explanations, but can operate simultaneously on the urchin body surface. On an intact surface, repeated pedicellariae contacts may impose a behavioural cost (e.g., displacement or interruption), whereas damaged patches may simultaneously provide disturbance-associated cues and represent areas where pedicellariae defence is weakened. Therefore, orientation toward damage-associated signals could be adaptive not only because such cues are attractive per se, but also because it brings amphipods into zones of reduced defensive interference. Even if the primary driver were avoidance of pedicellariae attacks, amphipods could still be expected to move in the direction of damage-associated cues insofar as those cues reliably indicate safer microhabitats. In this sense, attraction toward damage-associated signals and defence-related habitat selection are not contradictory but can reinforce each other on the sea urchin body surface.

At present, therefore, our data show that *D. yoshimurai* is not strictly attracted to naturally formed lesions as a unique habitat. Rather, amphipods can be induced to aggregate on experimentally created microhabitats associated with surface disturbance. This interpretation aligns with the dietary evidence (Kodama et al., 2024) because it implies that aggregation is more likely driven by microhabitat safety or by locally available non-host resources that accumulate in disturbed areas, rather than by feeding on host tissues alone.

4.4. Individuals that did not settle on hosts

Across host selection experiments 1 and 2, a fraction of amphipods did not settle on either host and remained in the tank. Two explanations are plausible. Individuals may have been excluded by the host, potentially via pedicellariae attacks, resulting in failure to settle. A small number of individuals also died during the trials, although the cause is unknown. Alternatively, some amphipods may have failed to recognize or orient to the host and adopted immobility as a risk reduction strategy. A similar pattern has been reported for the symbiotic shrimp *Gnathophylloides mineri*, which moves toward hosts when it recognizes them but becomes stationary when host cues are absent or inadequate, potentially reducing predation risk (Williamson et al., 2012). Both mechanisms may operate simultaneously in our system, and future work that records the fate of individuals after contact with host surfaces, including direct observation of pedicellariae interactions, would help resolve whether non-settlers are primarily repelled, killed, or behaviourally inactive.

4.5. A behavioural model for lesion-associated aggregation

Synthesizing the results, we propose a three-step behavioural model to explain lesion-associated aggregation in *D. yoshimurai*. The process can terminate at any point once individuals reach lesion-like retention microhabitats. First, amphipods recognize and attach to sea urchin hosts with limited discrimination between diseased and healthy individuals. Second, after settlement they move across the host surface, likely in part due to repeated interference from host pedicellariae, until they encounter microhabitats with reduced pedicellariae access (including lesions). Upon reaching such microhabitats, movement decreases and residence time increases, resulting in rapid local accumulation. Third, if retention microhabitats are not reached, host-to-host transfer can occur mainly when urchins approach or contact one another. After switching hosts, individuals repeat the within-host search and retention process described above. This iterative framework provides a mechanistic explanation for the strong field association between amphipods and diseased hosts and for the rapid emergence of lesion-level aggregations.

4.6. Implications and future directions

The dependence of host switching on host proximity suggests that host density and host contact rates may influence the spatial distribution and local population dynamics of *D. yoshimurai*. This perspective is consistent with field observations showing that diseased hosts act as key habitat patches for juveniles and that amphipod occurrence and density peak in winter when disease prevalence is high (Kodama et al., 2026). By enabling rapid concentration of juveniles onto favourable microhabitats, lesion and damage associated cues may be particularly important during recruitment periods.

At the same time, the proximate cue driving aggregation remains unresolved. Our experiments demonstrate active microhabitat concentration and show that both pedicellariae removal and wounding can induce aggregation, but they do not yet separate the roles of reduced defence, tissue damage cues, and other correlated factors. Future experiments that independently manipulate defensive structures and tissue damage, combined with measurements of chemical cues and direct observation of pedicellariae interactions, will be critical to identify the primary drivers. Clarifying these mechanisms will also help evaluate the potential reciprocal effect of amphipods on host condition, including whether amphipods are neutral, beneficial, or detrimental to lesion development and healing.

In conclusion, this study experimentally confirms that *D. yoshimurai* actively concentrates on diseased areas of *S. intermedius* and reveals that lesion-associated aggregation can emerge through a combination of limited host-level discrimination, proximity-dependent host switching, and rapid within-host microhabitat selection. These findings provide a behavioural foundation for understanding the strong association between this amphipod and host disease observed in the field and identify key mechanisms that should be targeted in future work to resolve why aggregations form and what ecological consequences they have for both symbiont and host.

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Statements and Declarations

Author Contributions statement

Masafumi Kodama: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. Ryoga Yamazaki: Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. Ko Tomikawa: Investigation, Validation, Writing – review & editing. Gen Kume: Resources, Supervision, Writing – review & editing. Toru Kobari: Resources, Supervision, Writing – review & editing. Jun Hayakawa: Conceptualization, Methodology, Investigation, Resources, Supervision, Writing – review & editing.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Ethical approval

All applicable international, national and/or institutional guidelines for sampling, care and experimental use of organisms for the study have been followed and all necessary approvals have been obtained. The study did not involve human participants. No animal welfare approval was required as we were working with common invertebrates.

Conflict of interest

The authors declare that they have no competing interests.

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Figure legends

Fig. 1. Schematic illustrations (upper) and photographs (lower) of the host selection experiments 1 and 2. (A) Host selection experiment 1: urchins and *Dactylopleustes yoshimurai* are free to move in the tank; urchins often contact each other. (B) Host selection experiment 2: urchins are placed in cylinders made of plastic nets to prevent them from moving out of the cylinders, and thus urchins are always separated; *D. yoshimurai* are free to move in the tank and can pass through the mesh of the plastic nets.

Fig. 2. Schematic illustrations (A) and photographs (B) of the aggregation experiment. One individual of host urchin *S. intermedius* and ten individuals of *D. yoshimurai* are free to move in the tank.

Fig. 3. Result of the host selection experiment 1 using freely moving urchin hosts. Boxplots with overlaid jittered points showing the number of *Dactylopleustes yoshimurai* attached to the diseased and healthy urchins. Each box indicates the upper and lower quartiles. Black bold line in each box indicates the median. Whiskers indicate minimum and maximum values excluding outliers. Grey dots represent all the raw data. Values outside 1.5 times the interquartile range above the upper quartile and below the lower quartile are treated as outliers. The p value was calculated by the paired Wilcoxon signed-rank test.

Fig. 4. Result of the host selection experiment 2 using sea urchin hosts prevented from moving. Boxplots with overlaid jittered points showing the number of *Dactylopleustes yoshimurai* attached to the diseased and healthy urchins. Each box indicates the upper and lower quartiles. Black bold line in each box indicates the median. Whiskers indicate minimum and maximum values excluding outliers. Grey dots represent all the raw data, with jittering to better visualize overlapping data. The p value was calculated by the paired Wilcoxon signed-rank test.

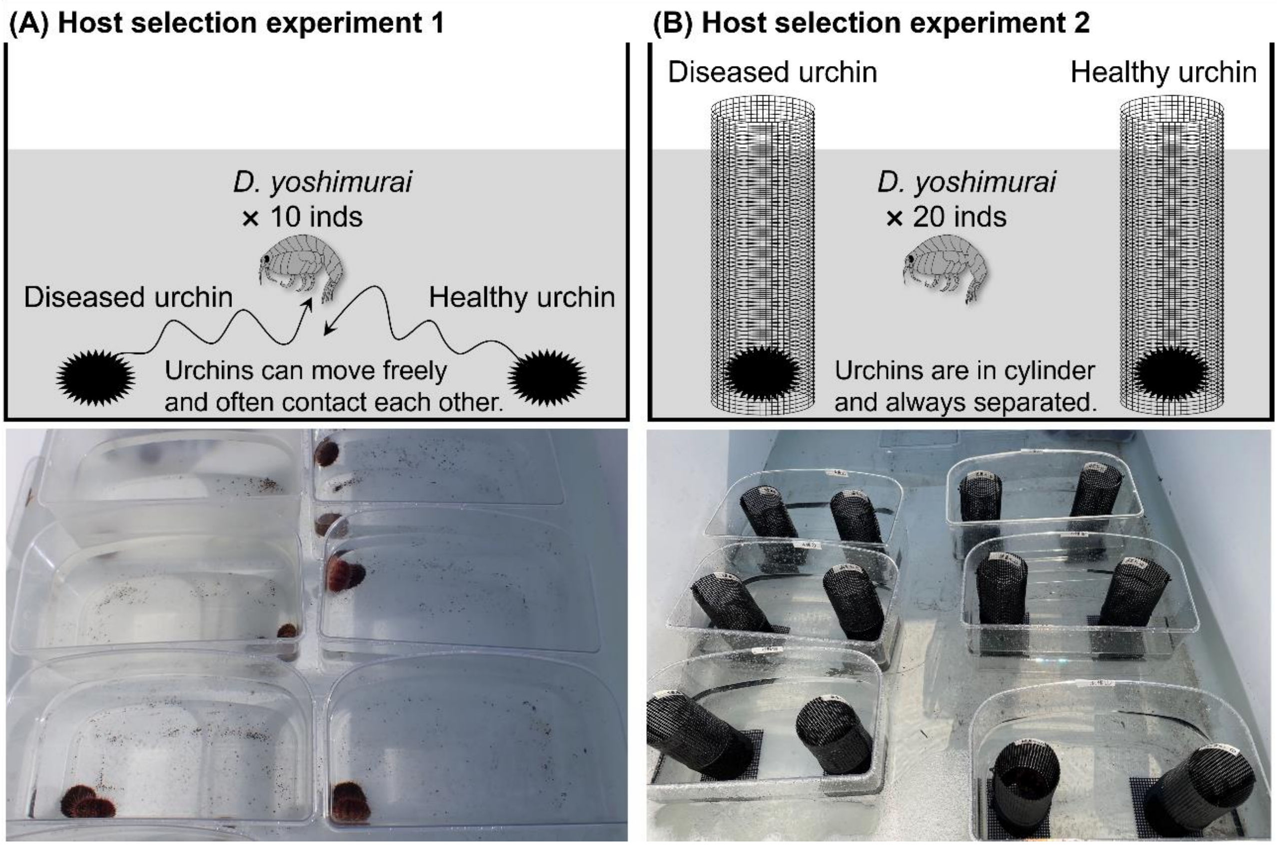
Fig. 5. Result of the aggregation experiment. Changes in the number of *Dactylopleustes yoshimurai* over time: (A) *Dactylopleustes yoshimurai* on the host urchin, (B) *Dactylopleustes yoshimurai* on the diseased area of the host urchin. Grey dots represent all the raw data, with jittering to better visualize overlapping data. Red solid lines and shaded areas represent the generalized additive model (GAM) fitted line and 95% confidence intervals, respectively.

Fig. 6. Photograph from the aggregation experiment: *Dactylopleustes yoshimurai* that actively aggregated on the lesion of a sea urchin during the experiment. Pedicellariae are abundant on the urchin surface except on the lesion, and pedicellariae adjacent to the aggregated amphipods appear to be oriented toward the amphipods.

Fig. 7. Result of the (A) pedicellariae-removal experiment and (B) Wounding experiment. Boxplots with overlaid jittered points showing the number of *Dactylopleustes yoshimurai* attached to the experimental and control areas. Each box indicates the upper and lower quartiles. Black bold line in each box indicates the median. Whiskers indicate minimum and maximum values excluding outliers. Black dots represent all the raw data, with jittering to better visualize overlapping data. The p value was calculated by the paired Wilcoxon signed-rank test.

Fig. 8. Photograph from the pedicellariae-removal experiment: *Dactylopleustes yoshimurai* that actively aggregated on the experimental area (Pedicellariae-removal area). Yellow arrows indicate individuals of *D. yoshimurai*.

Fig. 9. Examples of conspecific aggregation of the sea urchin *Strongylocentrotus intermedius* in Otsuchi Bay. (A) High-density aggregation on a macroalgal patch (feeding-associated aggregation). (B) Several individuals sheltering in a sand patch between boulders and carrying pieces of seaweed and/or shell fragments on the body surface, apparently as camouflage.



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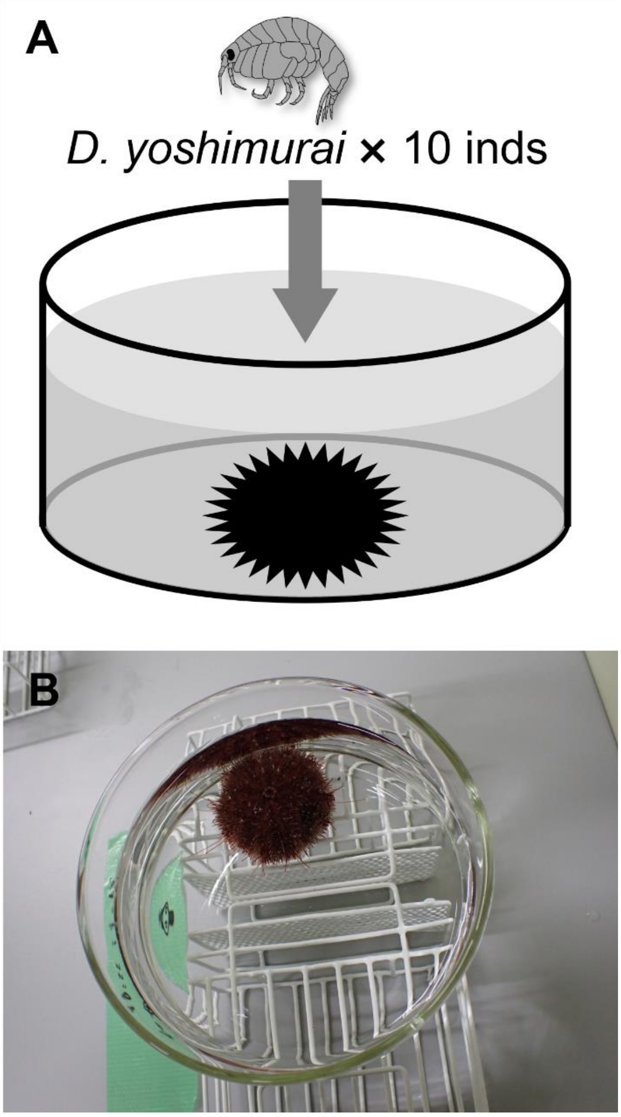

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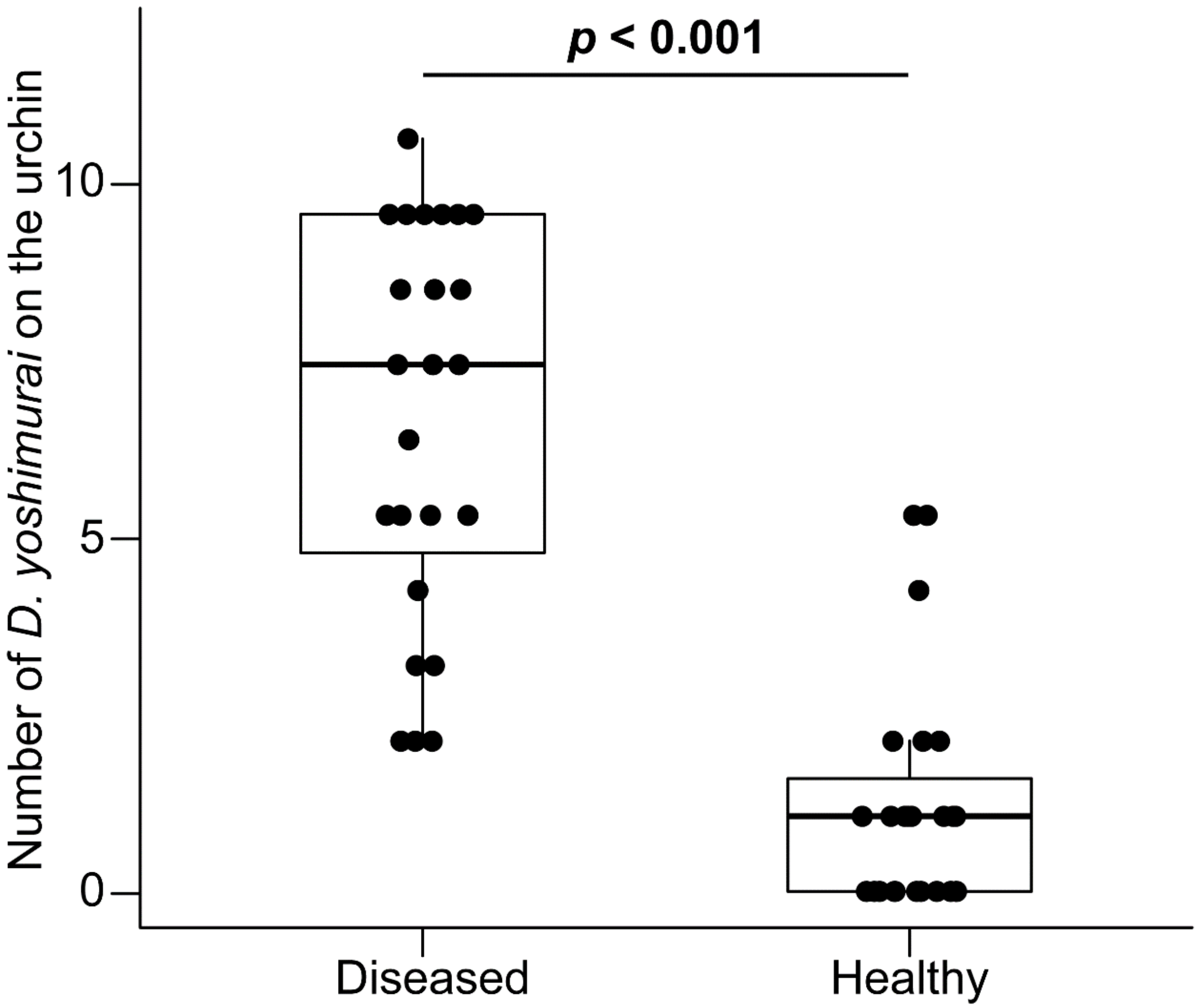

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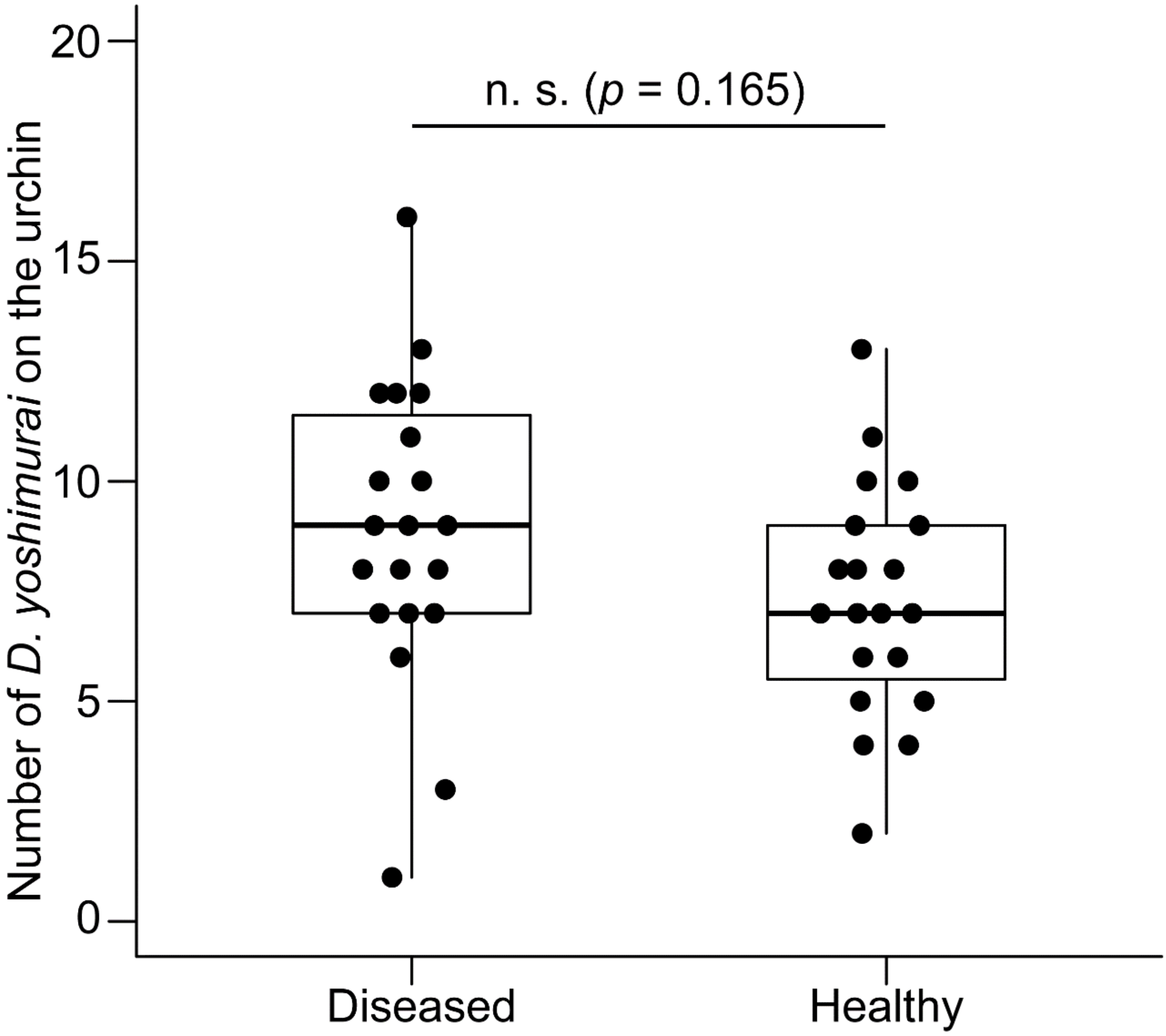

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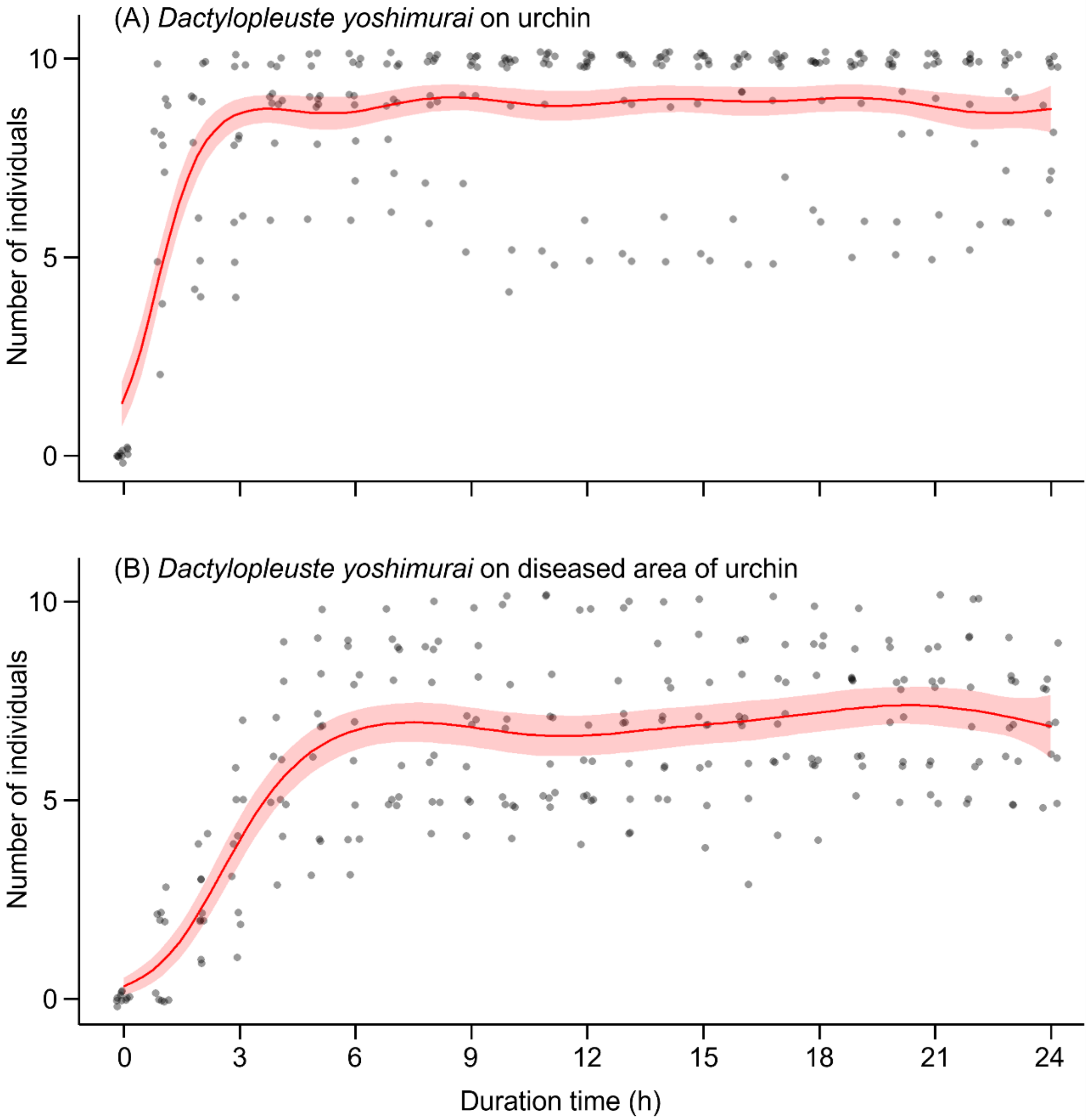

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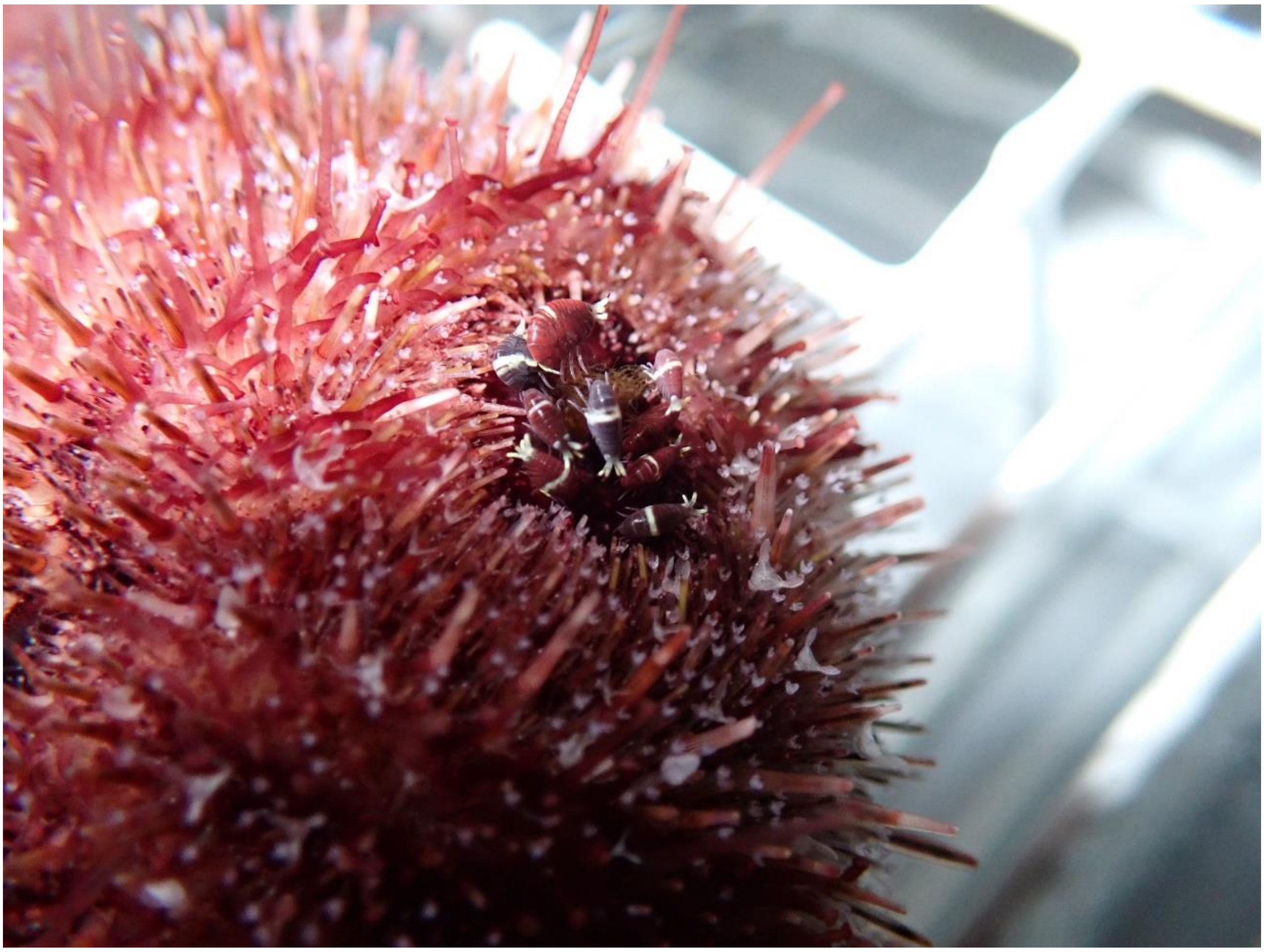

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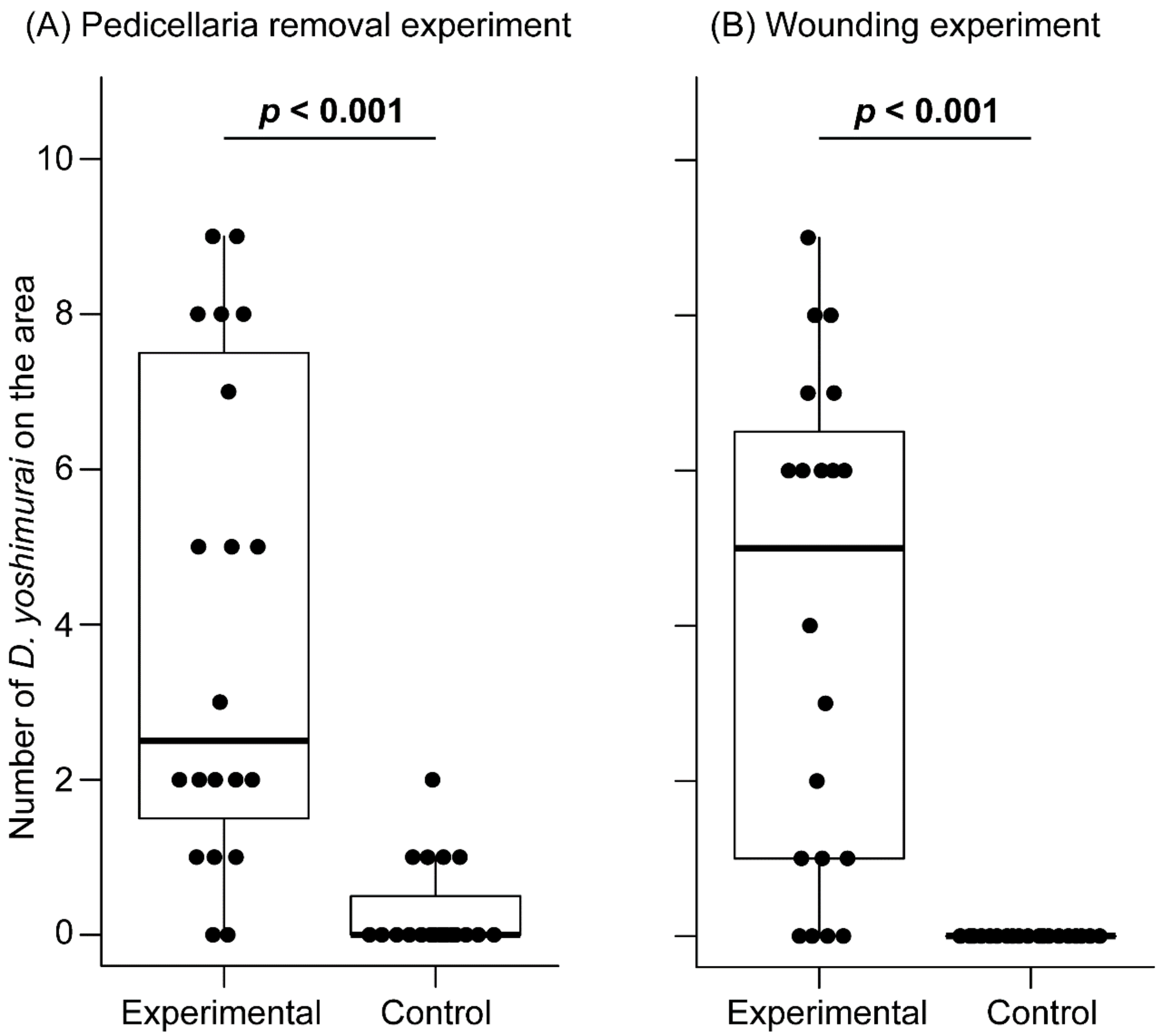

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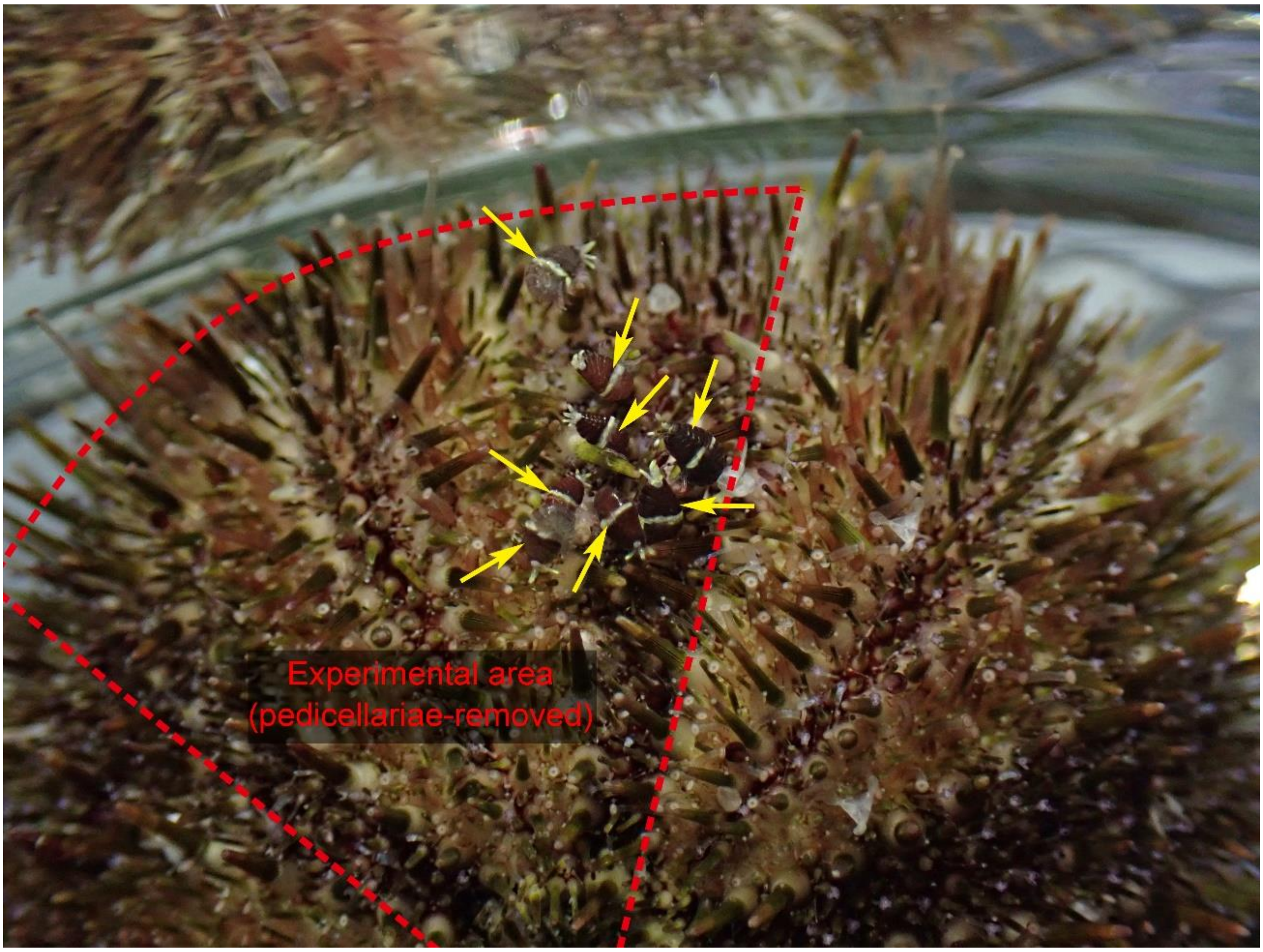

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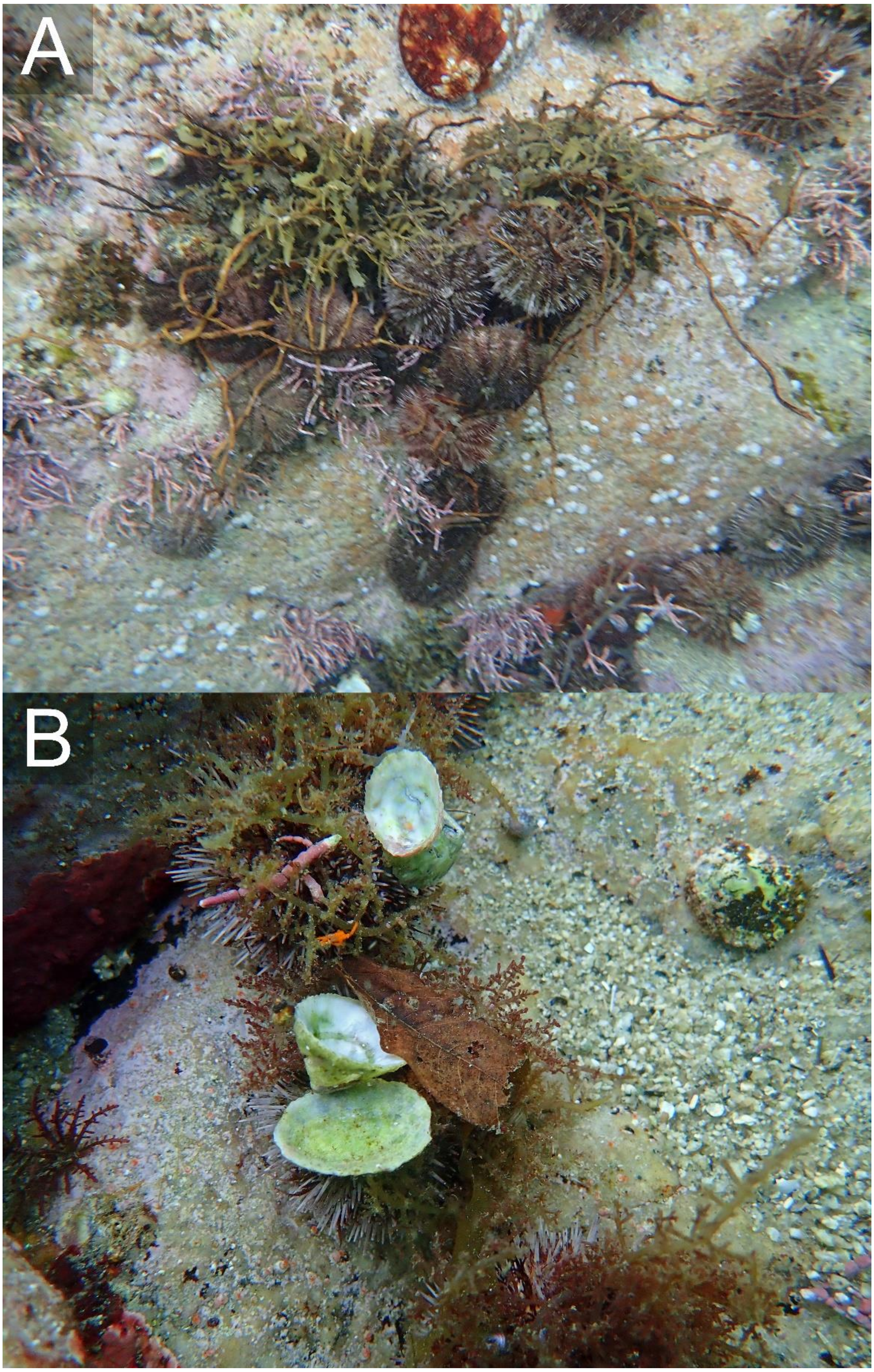

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