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The cnidome and ultrastructural morphology of late planulae in *Lophelia pertusa* (Linnaeus, 1758)—With implications for settling competency

Susanna M. Strömberg[1] | **Carina Östman**[2] | **Ann I. Larsson**[1]

[1]Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, Strömstad, Sweden

[2]Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden

Correspondence
Susanna M. Strömberg, Department of Marine Sciences, Tjärnö Marine Laboratory, University of Gothenburg, Strömstad, Sweden.
Email: susanna.stromberg@marine.gu.se

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Abstract

The larval pre-competency period and competency window are important in delimiting the potential dispersal distance for pelagic larvae of sessile marine fauna. Here, we provide evidence for morphological changes in the late planulae of *Lophelia pertusa* that have implications for their dispersal potential. Three weeks after spawning, the planulae gain functional cnidocysts, indicating that they are competent to settle at this time. Cnidae have been shown to be used for primary anchoring during settling, and before this time point, the larvae most probably do not have the ability to attach to a substrate in high flow conditions. The appearance of functional cnidae coincides with larvae gaining a flexible mouth that can be opened to the full width of the larva. The larval isorhizas differ the most from the adult polyps isorhizas, while the p- and b-mastigophores bear more resemblance to the adult homologues of similar size. The external and internal morphology of late planulae is further described with demonstration of long apical cilia and its effect on swimming agility, morphological changes of the ciliated cells in the larval mouth region and an internal nerve plexus. This study also indicates that *L. pertusa* planulae seek out cryptic spaces for settling.

KEYWORDS

cnidocyst, cold-water coral larvae, competency window, scanning electron microscopy, settling

1 | INTRODUCTION

The onset of settling and metamorphic competency is the beginning of the end of the pelagic phase for the dispersing larvae of sessile marine organisms. The length of the pre-competency period that precedes this has been shown to significantly affect dispersal distance (Treml, Ford, Black, & Swearer, 2015). The pre-competency period for larvae of tropical corals is usually very short, with larvae often ready to settle within 2.5–5 days after spawning (Chui et al., 2014; Hayashibara, Ohike, & Kakinuma, 1997; Krupp, 1983; Miller & Mundy, 2003; Schwarz, Krupp, & Weis, 1999). The "competency window"—during which the larvae are able to make the transfer from larva to juvenile—of a number of shallow water corals has been established experimentally and can extend from a couple of days to over 100 days (Graham, Baird, & Connolly, 2008; Nozawa & Harrison, 2002; Richmond, 1987), giving larvae ample time to find a suitable substrate. In contrast, knowledge on embryo and larval development rates and the timing for settling competency in deep-sea species is almost non-existing. The only true deep-sea coral larvae that have been observed from release to settling are larvae of brooding soft corals (Octocorallia), releasing fully developed demersal planulae. These larvae were either

crawling over the substrate or swimming just above the seafloor and were observed to settle between 1 and 30 days, or 3 and 70 days post-release (Sun, Hamel, Edinger, & Mercier, 2010; Sun, Hamel, & Mercier, 2010, 2011).

Two species of semi-deep broadcast spawning corals have been observed all the way from spawning until settling: *Caryophyllia smithii*, a temperate solitary cup coral with a depth distribution of 40–400 m (Hoeksema, 2015; Tranter, Nicholson, & Kinchington, 1982); and *Oculina varicosa*, a facultative zooxanthellate framework forming coral (Brooke & Young, 2003,2005). The latter has a split depth range: the shallow water zooxanthellate form is found at 3 to 30 m depth, while the deep azooxanthellate form is found at 70–100 m depth. Settling in larvae of shallow *O. varicosa* was observed by days 21–27 in a rearing temperature of 25°C (Brooke & Young, 2005). Larvae of the deep *O. varicosa*, reared at 20°C, were never observed to settle during the 42 days the experiment lasted. The rearing temperature for embryo and larval cultures of *C. smithii* was 15°C, with settling occurring after 8–10 weeks (Tranter et al., 1982). Neither of the studied species′ larval development reflects true deep-sea (or cold-water) corals thriving between 4 and 12°C, although *C. smithii* is close.

Lophelia pertusa is one of six species of framework forming, deep-sea scleractinian corals, building extensive reefs on topographic highs on continental slopes and seamounts (Freiwald, Fosså, Grehan, Koslow, & Roberts, 2004; Roberts, Wheeler, Freiwald, & Cairns, 2009). The current status of the knowledge of the early life history of *L. pertusa* is that individual colonies within reefs have separate sexes (gonochoric), and fecundity is estimated to 3,300 oocytes per cm^2 (Waller & Tyler, 2005). Oogenesis takes a full year or more, while spermatogenesis takes less than a year, ending with a seasonal spawning of which the timing differs depending on geographic location (Brooke & Järnegren, 2012; Larsson et al., 2014; Pires, Silva, & Bastos, 2014; Waller & Tyler, 2005). According to laboratory observations, spawning is extended over a period of 2 months (Larsson et al., 2014), which has yet to be validated in situ. Fertilization is external (broadcast spawning), and the resulting larva is a uniformly ciliated, round to bullet-shaped planula (Larsson et al., 2014) typical of anthozoans (Martin & Koss, 2002). No settling has yet been observed in this species, but laboratory reared larvae have a longevity of up to a year in 7–8°C (Strömberg & Larsson, 2017).

Anthozoan planulae are more histologically differentiated than larvae of the other cnidarian classes (Martin & Koss, 2002). They consist of an outer ectoderm and inner endoderm, separated by a thin layer of mesoglea, and a neural plexus between ectoderm and mesoglea. They usually have a well-developed mouth and pharynx, and gastrovascular cavity. Planktotrophy (a feeding larval stage) is common, but lecithotrophic (non-feeding) planulae also occur. They have a distinct polarity of oral and aboral end, swimming with the aboral end first, spinning around the oral-aboral axis. Some species have larvae with an apical tuft of clustered cilia at the aboral pole. The apical tuft is swept or propelled in front of the planula while swimming (Martin & Koss, 2002).

The apical tuft is part of the sensory structures of the apical organ, which is a highly conserved trait in marine larvae of cnidarians as well as bilaterians (Marlow et al., 2014). A tuft of long cilia is a common feature, and the apical tuft has been hypothesized to have a function in settling. In the cup-coral *C. smithii*, the tuft is however lost 2–3 weeks prior to settling (Tranter et al., 1982), and thus, a function in food detection or detection of other environmental cues during the pelagic phase is more probable. In other marine larvae (e.g., gastropod veligers), there is nevertheless clear evidence of a presence of receptors for chemical settling cues in the apical sensory organ (Hadfield, Meleshkevitch, & Boudko, 2000). In the case of the veligers, the cue for settling is in fact the metabolites of their prey: the adult nudibranch feed on certain coral species, so feeding and substrate are tightly coupled. Settling cues in coral planulae is not related to feeding, but to suitable substrates and habitats (Tebben et al., 2011). Tranter et al. (1982) suggested that the tuft might enhance swimming agility, with rapid changes of direction as one effect. After losing the tuft, *C. smithii* larvae develop "a blister-like swelling" at the aboral end, undergoing morphological changes to prepare for settling, which is done by the aboral end. Some anthozoan planulae metamorphose after settling, but some start metamorphosis prior to settling with tentacle buds or small tentacles already present when settling takes place (Martin & Koss, 2002).

Grasso et al. (2011) have mapped the transition from searching to settling and metamorphosis at a molecular level and described the changes occurring in planulae of the tropical coral *Acropora millepora*. The sensory organ at the aboral end disappears and calicoblastic cells start to develop; thus, the apical tuft has to be gone for the larva to be able to settle. There is an upregulation of genes involved in apoptosis, and the cells specialized for the pelagic phase die off and are replaced. Metamorphosis in marine organisms is extremely swift compared to their terrestrial counterparts, and separate chemical cues have been identified for metamorphosis and settling, respectively, in tropical corals (Grasso et al., 2011; Hadfield, Carpizo-Ituarte, Carmen, & Nedved, 2001; Hadfield & Paul, 2001; Tebben et al., 2011).

Larsson et al. (2014) established that the time period for embryo development in *L. pertusa*, in which the embryos are primarily transported as passive particles, is 9 days at a rearing temperature of 7–8°C. This is the normal in situ temperature at the local reefs in the Skagerrak during spawning season. Embryogenesis is thus extended over several days, rather than hours as for tropical corals. The embryos are fully ciliated swimming blastulae at day 5,

followed by another passive period during gastrulation that occurs asynchronous during days 6–9 (Larsson et al., 2014). Consequently, the planulae are not fully developed and competent swimmers until day 10. This can be compared to the larvae of *C. smithii*, which were observed to become fully developed and feeding planulae after 48 hr at a rearing temperature of 15°C (Tranter et al., 1982). When the rearing temperature was increased to 11–12°C for *L. pertusa* embryos, development rate doubled, thus shortening the time for embryogenesis approximately by half (Strömberg & Larsson, 2017). Onset of bottom-probing behaviour in *L. pertusa* was observed three weeks after spawning, indicating readiness for settling, and cnidocyst[1] discharge then started at day 30 (Larsson et al., 2014).

The discharged cnidocysts observed included atrichous isorhizas, a cnida type not present in adult polyps (Strömberg & Östman, 2016), indicating a unique function for this cnida type in the planulae. The actinula larva of the hydrozoan *Tubularia mesembryanthemum* (now accepted as *Ectopleura crocea*) has been shown to use atrichous isorhizas for primary anchoring during settling (Yamashita, Kawaii, Nakai, & Fusetani, 2003). Hayashibara, Kimura, and Hatta (2000) found that the maximum abundance of cnidocysts coincided with a peak of settling in larvae of the tropical coral *Acropora nasuta* and suggested cnidae to be a good marker for larval maturity. Other studies have also shown a unique cnida complement in cnidarian planulae, in comparison to adults (Holst, Sötje, Tiemann, & Jarms, 2007; Paruntu, Hidaka, & Hidaka, 2000; Zenkert, Takahashi, Diesner, & Özbek, 2011). In addition, Abelson, Weihs, and Loya (1994) showed that planulae of coral species that colonize protruding bodies are using mucus strings (or some kind of adhesive filament) to be able to settle in this preferred habitat. Mucus strings have been coupled to feeding (Schwarz et al., 1999; Schwarz, Weis, & Potts, 2002; Tranter et al., 1982), but could well have dual function. Harii and Kayanne (2002) showed that coral planulae that attached by means of cnidocysts (*Pocillopora damicornis*) managed to stay attached at higher flow speed than planulae attaching by means of mucus (*Heliopora coerulaea*), indicating that attaching by cnidae is a possible adaptation for planulae that prefer habitats with higher flow speed.

The aim of this study was to investigate the morphological changes of late planulae of *L. pertusa*, with special focus on the cnidae and their role in settling. The larval cnidome is described and compared to the cnidome of the adult polyps. We also describe the late larvae's external and internal morphology, with notes on the function of the apical cilia. The timing of the appearance of functional cnidae was of special interest since it indicates the onset of settling competency and thereby determines the minimum period for larval dispersal—the beginning of the end of the pelagic phase.

2 | MATERIAL AND METHODS

2.1 | Collections and maintenance

Five samples of *L. pertusa* for larval breeding were collected by means of a remotely operated vehicle (ROV, Ocean Modules V8 Sii) from the Tisler reef in Norway on 20 November 2014 (Table 1). The ROV was equipped with a landing net to get large fragments with minimal damage to gravid polyps. The samples were transported in containers with deep-water onboard the R/V Lophelia to the research station of University of Gothenburg at Tjärnö, (58°52′33.92″N, 11°8′46.60″E), *c*. 10 nmi south of the collection site.

All necessary permits were in place: permit to conduct research in Norwegian waters and for coral collections (2010/107-432.3; 2013-46) through the Ytre Hvaler National Park Board, the County Governor and the Norwegian Directorate of Fisheries; CITES export permit (EX-23-2014) through the Norwegian Environment Agency; and CITES import permit (Dnr: 4.10.18-10009/14 Nr: 51200-14) through the Swedish Board of Agriculture.

The corals were mounted on small concrete bases, attached with Aqua Medic Reef Construct epoxy putty, for an upright position. Dissections of a subsample of polyps from each of the larger samples for sex determination were done on November 24. The collected material consisted of two female and three male samples. The coral fragments were divided (mixing males and females) over four 18-L tanks supplied with flow-through of filtered deep-water, kept close to the seasonal in situ temperature of 7–8°C, and a salinity of 34–35 psu. A filter system consisting of one 50 µm and one 5 µm Ametek polypropylene cartridge mounted in sequence reduced particle inflow. Corals were fed twice a week with homogenized copepods (*Calanus* sp. from CALANUS AS, Norway), and by the end of December, closer monitoring to check for signs of spawning commenced.

The first spawning in 2015 took place on January 22, but only sperm were then released. On February 2, the simultaneous release of eggs and sperm gave the first batch of fertilized eggs. Several spawning events followed during February with the most fruitful occurring the 23rd, resulting in a plentitude of larvae. Gametes were collected in 2.5-L glass bowls, occasionally gently swirled with a spatula to increase fertilization

TABLE 1 Gender, positions and depths for *Lophelia pertusa* coral samples collected at the Tisler Reef in 20 November 2014

Gender	Positions		Depth (m)
Female	58°59.697′N	10°58.095′E	108
Male	58°59.714′N	10°58.041′E	114
Male	58°59.711′N	10°58.037′E	113
Female	58°59.694′N	10°58.080′E	109
Male	58°59.707′N	10°58.073′E	117

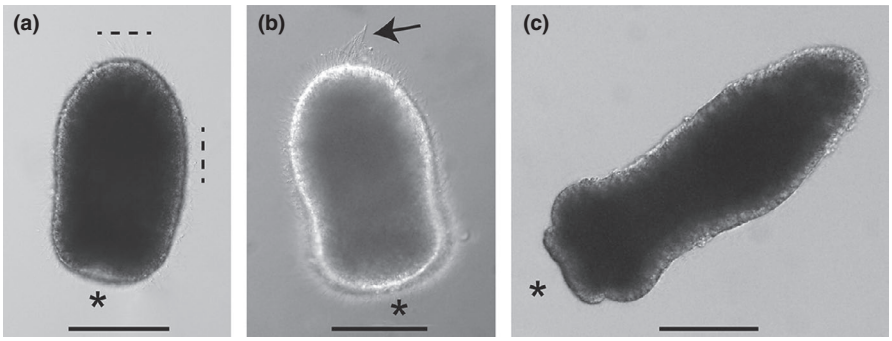

FIGURE 1 (a) Twenty-days-old *Lophelia pertusa* planula. Dashed lines indicate the length of the cilia: note that the apical cilia are almost twice as long as the cilia on the rest of the body. (b) Forty-three-days-old planula with tuft-like apical cilia. (c) Forty-four-days-old planula showing signs of metamorphosis: the bulging edge adjacent to the mouth is probably the beginning of tentacle buds. Asterisks indicate the oral ends. Scale bars 100 μm

rates. When embryogenesis was completed and the propagules turned into swimming blastulae (day 5), they were transferred to culture flasks, completely filled with water to avoid blastulae or planulae getting caught in the surface tension. Different types of flasks were used: 1- to 3-L glass or polycarbonate E-flasks, with or without ventilated caps. E-flasks were either laid down or put upside down, with the side or the wide bottom of the flasks facing upwards, since embryos and early larvae usually gather in the upper portion of the water volume. A fraction of the water was changed once or twice per week in smaller containers, less often in larger, and embryos and larvae were kept at low densities. Starting at 3 weeks of age, some of the larvae were fed; predominantly with the fine fraction of homogenized and centrifuged *Calanus* copepods, but some were fed with microalgae and other potentially suitable types of food such as microalgae and picoplankton.

2.2 | Larval behaviour and settling trials

The experimental set-up originally included a full settling experiment with 18 rotating 1-L beakers with several optional substrates available and six different treatments (including controls). Infestation by parasites added a random factor that made analysis futile, and no settling could be detected on the substrates at the final check. Instead, attachment trials were done under dissecting microscope in glass embryo bowls or standard Petri dishes, with water turbulence produced by squirting water with a pipette. Small pieces of coral skeleton were introduced as substrates. Observations of larval behaviours in contact with substrates in the attachment trials started when larvae were 43 days old and until 90 days.

2.3 | Documentation of larvae and cnidae under light microscopy

Samples of larvae were continuously taken for examination and documentation under light microscopy (LM), using an Olympus BX51 equipped with an Olympus DP70 camera. Some larvae were fixed with a drop of 4% zinc formaldehyde added to the seawater on the glass slides to get detailed images of larval morphology. When larvae had been observed to fire cnidocysts, squash preparations were done of live planulae to document cnidae. Some preparations were stained with nigrosin and eosin to enhance details. Cnidae were documented around live larvae from day 20, and in squashed larvae at days 30, 43 and 112. Squash preparations were done with 3–4 planulae on each slide, and 2–3 slides prepared per session, thus, 18–36 planulae in total. Not all prepared planulae contained cnidae suitable for documentation, and the exact number of planulae from which cnidae were documented was not noted. Measurements of capsule length and width, as well as length and width of everted shafts and tubules, were done with the image analysis software Image J (version 1.45s, Abràmoff, Magalhães, & Ram, 2004). Some results from the spawning season in 2013 are included in the present study, with collections and rearing described in Larsson et al. (2014).

2.4 | Preparation of embryos and larvae for scanning electron microscopy

Embryos and larvae were continuously sampled and fixed for scanning electron microscopy (SEM) using 1% Osmium Tetroxide. Samples were cleaned in filtered seawater and left in Osmium Tetroxide for 1 hr, rinsed in several baths of distilled H_2O and finally stored in 70% ethanol in 4°C until further processing. Before SEM, samples were dehydrated through a series of increasing concentrations of ethanol, and critical point dried in a Polaron CPD7501. After sputter coating with gold and titanium, the specimens were viewed under SEM (Zeiss, Supra 35VP, Gemini Tardis).

2.5 | Nomenclature

Cnidae were identified using the classification system and nomenclature established by Weill (1934) with modifications

made by Carlgren (1940), Cutress (1955), Mariscal (1974), Watson and Wood (1988), Östman (2000) and Östman, Kultima, and Roat (2010). Terminology on mucocytes was adopted from Goldberg (2002).

3 | RESULTS

3.1 | Observations on larval behaviour and general morphology

Studied under stereo magnifier with a lateral light source, the planulae had a translucent ectoderm, fully ciliated, with an opaque whitish endoderm inside. No permanently clustered apical tuft at the aboral pole was present at any age of planulae as described for other anthozoan planulae. The apical cilia were however almost twice as long as the cilia of the remaining body (Figure 1a,b). These apical cilia sometimes seemed bunched up and tuft-like during swimming, sweeping the water in front of the planula. Viewed under microscope, it was observed that when planulae shifted from standing on the spot to swimming, the apical cilia were crossed above the aboral pole: that is, when larvae were still, cilia were radiating from the aboral pole, and just prior to swimming away, the cilia on the right side of the aboral pole crossed over to the left and vice versa. The apical cilia were thus functioning like anaircraft propeller, used to propel the planula forward.

The oral pore opened after two weeks, consistent with previous observations, and in the mature planulae (≥20 days), the oral opening had a protruding peristome (rim) and was protractible and very flexible (Figure 1c). The mouth could open to the full width of the planula. Although no planulae metamorphosed into juvenile polyps, some of the planulae fed with homogenized copepods appeared to have started metamorphosis. The body shape was getting curved, with bulging edges adjacent to the mouth, indicating onset of tentacle bud formation (Figure 1c).

The body shape shifted somewhat with activity. A short bullet or completely spherical shape was usually associated with inactive larvae, while swimming usually was combined with a more prolonged bullet shape, but these shapes were not kept consistently. Starting at 20 days, but more frequent around 30 days, some planulae were almost vermiform. Especially at one occasion when a large number (*c*. 50) of 35-days-old larvae were collected in an embryo bowl, they were crawling along the bottom surface in an undulating

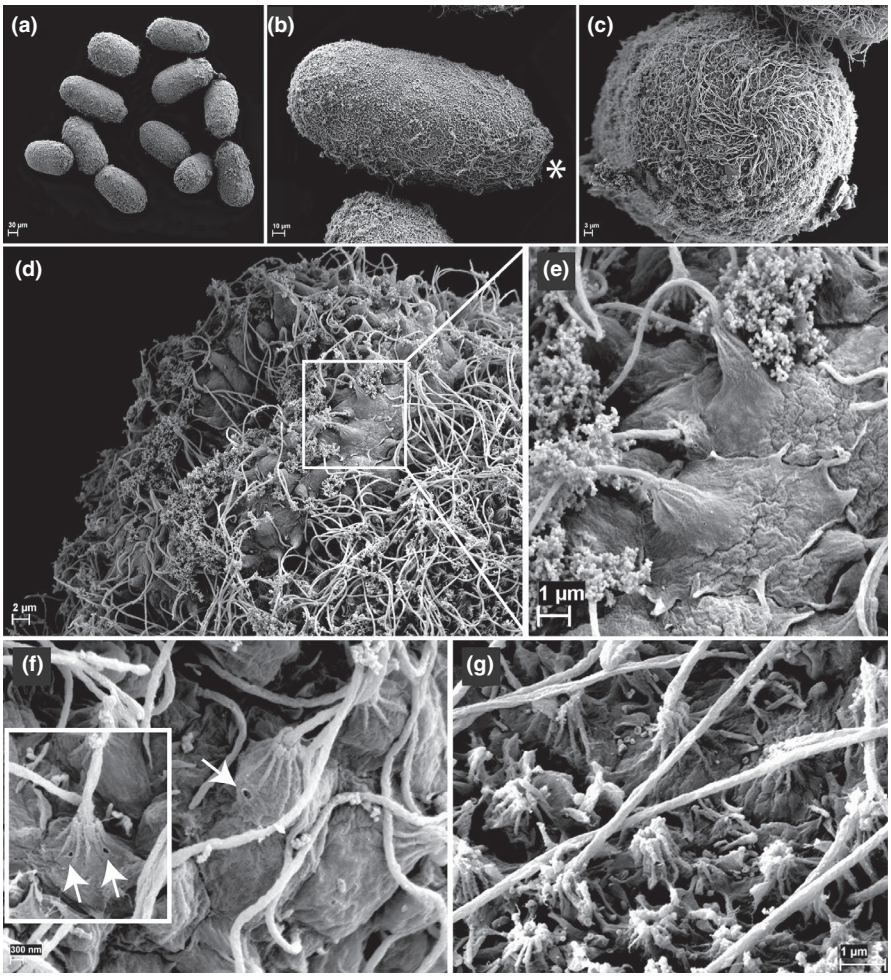

FIGURE 2 Scanning electron micrographs (SEM) of 90-days-old *Lophelia pertusa* planulae. (a) A collection of bullet-shaped planulae. (b) A planula with a protruding rim around the mouth (asterisk indicates the oral end). (c) The aboral end: no apical tuft with permanently clustered cilia. (d) The mouth area with a protruding rim. (e) Close-up of the mouth area as outlined with a square in (d): the collars of microvilli encircling the base of the motile cilia are embedded, contrary to the cells of the general body surface. Only their upper parts are visible. (f) One or two pores (white arrows) were observed at the bases of the embedded microvilli on some of the cells. (g) Ciliated cells of the general body surface: note that the collars of microvilli are superficially rooted on the cell surface and not embedded as in (d–f)

snake-like manner, more than three times as long as wide. The vermiform shape and undulating movement were connected solely to tactile substrate inspections. Leaving the substrate to resume swimming, the larvae became bullet-shaped again. During fixations, they would contract and lose the vermiform shape, and the longest of the fixed 90-days-old planulae documented under SEM were merely twice as long as wide (Figure 2a,b).

3.2 | External ultrastructure of late planulae

The 90-days-old planulae studied under SEM were mostly bullet shaped with a more or less prominent peristome (rim around the mouth, Figure 2a,b). The aboral pole showed no signs of bearing an apical tuft of permanently clustered cilia (Figure 2c). The ultrastructure of the planula body surface was mostly consistent with other studies, although the ciliated

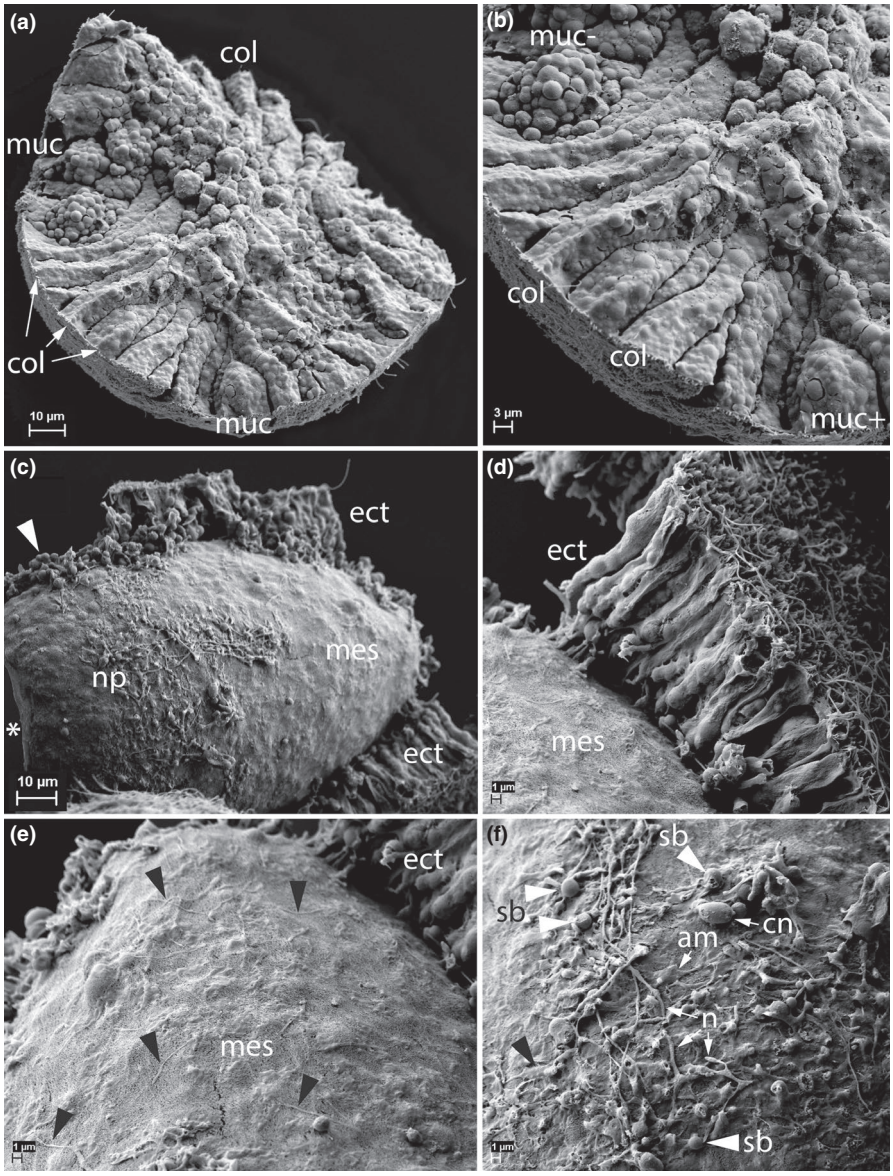

FIGURE 3 (a) The internal morphology of an early blastula (3–4 days old) with slender columnar cells (col) and mucocytes (muc, large clusters of spherical bodies = mucus filled loculae). (b) Closer view of the columnar cells (col) and mucocytes: mucocyte without cell membrane (muc−); mucocyte with an intact cell membrane (muc+). (c) 26-days-old planula with the ectoderm (ect) partially removed, and the mesoglea (mes) exposed. The mouth is marked out with an asterisk (*). Part of the nerve plexus (np) is visible on the surface of the mesoglea on the oral half of the planula. Arrowhead points out broken part of ectoderm with scattered spherical bodies. (d) Close-up of a part of the ectoderm on the aboral end with tall, narrow columnar cells rising over the mesoglea—the same cells that are visible in the lower right corner in Figure 4c (note the rotated orientation). (e) Close-up of the aboral end of the planula with exposed mesoglea (mes) and neurons (arrowheads) crisscrossing over the surface. (f) Close-up of the oral half of the planula with the nerve plexus with neurons (n), spherical bodies (sb) and a possible cnidocyst (cn). The spherical bodies could be cell content from broken ectodermal cells (see arrowhead in [c]). Possible amoebocytes (am) attach to the mesoglea. The black arrowhead (lower left) points out what could be a part of a neuron connecting the ectoderm with the mesoglea

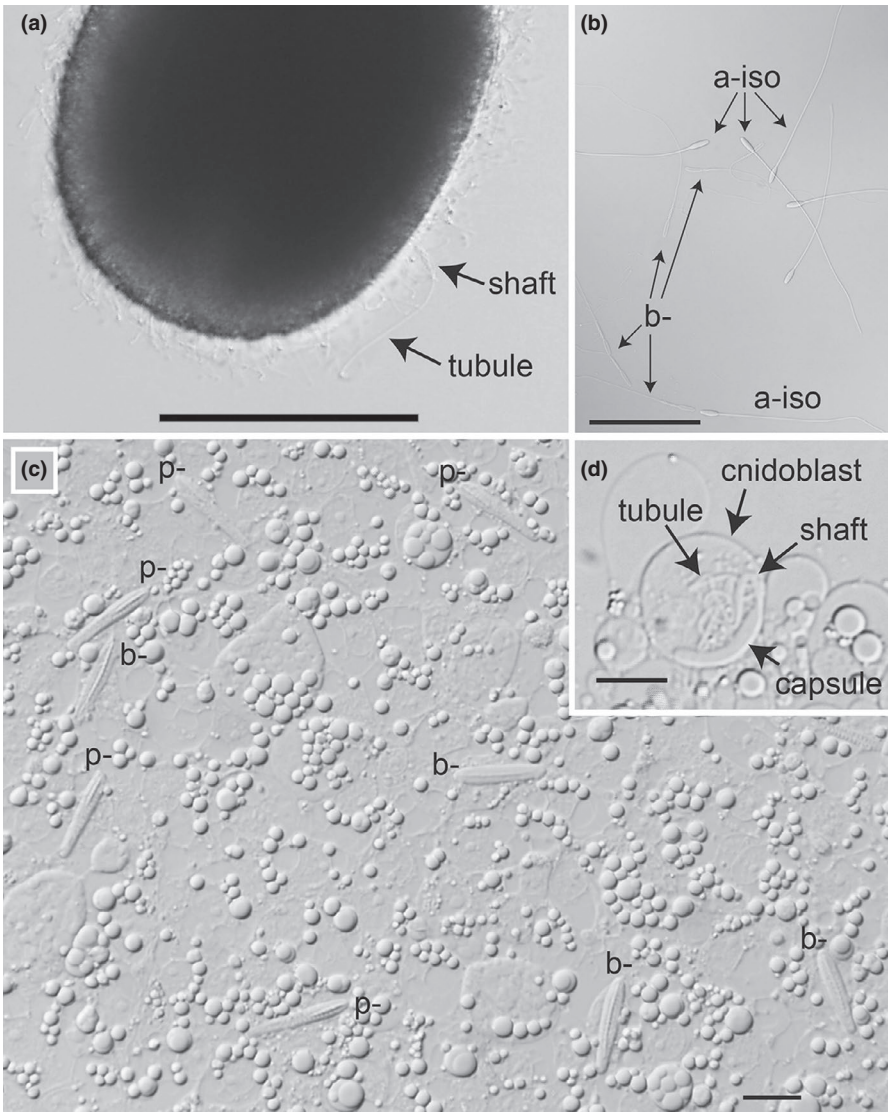

FIGURE 4 (a) Planula with a discharged nematocyst (p- or b-mastigophore) with visible shaft and tubule extending from the ectoderm. (b) Atrichous isorhizas (a-iso) with straight and stiff tubules, and b-mastigophores (b-) discharged by a planula under the coverslip. (c) Squash preparation of a planula with p- and b-mastigophores sparsely distributed in the tissue. The letters marking the cnidae types are all placed at the apical end. (d) A spherical cnidoblast with developing b- or p-mastigophore: note that the external shaft (not yet invaginated into the capsule) is of wider diameter than the tubule. The capsule is bent at this stage. Scale bars in (a) and (b) 100 μm, and 10 μm in (c–d)

cells around the mouth differed from the cells of the general body surface. In the mouth area, the basal parts of the collars of microvilli encircling the motile cilia were embedded in the here swollen cell surface, with only the upper parts visible (Figure 2d,f). This characteristic was not observed around the oral pore of younger planulae and is to our knowledge not described previously in the literature. On some of the cells, one or two pores could be observed between the embedded bases of the microvilli (Figure 2f). On the general body surface, the collars surrounding the motile cilia were usually exposed, fully visible all the way down to the cell surface where they were superficially rooted (Figure 2g).

3.3 | Internal ultrastructure of blastula and planula

A few of the embryos and larvae studied under SEM were partially broken, exposing the internal morphology (Figure 3). A blastula was found broken in half, showing slender columnar cells and large clusters of spherical bodies, the latter being mucocytes with their mucus-filled loculae (Figure 3a-b). Some of the mucocytes still had an intact cell membrane, while some had lost the membrane and the loculae (vesicle-like spumous chambers) were exposed and more conspicuous (compare mucocytes in the upper left and lower right corner of Figure 3b). The columnar cells contain spherical bodies as well, but probably with other contents than mucus. The polarity of the blastula cannot be determined, but the columnar cells radiate out in two directions, facing forward and backward in the image, while mucocytes are mainly seen laterally, radiating left–right. The blastula still had very few cilia, so it is an early blastula, only 3–4 days old. Day 5 blastulae are usually fully ciliated (each cell ciliated) and capable of swimming.

Figure 3c shows a 26-days-old planula with the ectoderm removed from the entire side facing forward. The oral-aboral axis runs from the lower left to the mid-right with the rim of the mouth visible at the left corner. The remaining

ectoderm is seen lining the planula. In a girdle at the oral half of the planula, a nerve plexus is visible on the otherwise smooth surface of the mesoglea. A part of the ectoderm is broken, leaving scattered spherical bodies on the mesoglea (arrowhead, Figure 3c). Figure 3d,f is close-ups of parts of that same planula. The ectoderm is *c*. 20 μm thick and has tall and narrow cells (Figure 3d). On the mesoglea (exposed on the aboral half of the planula), long fine neurons are crisscrossing the smooth net-like surface (Figure 3e). In the area of the nerve plexus, it is difficult to identify the different components (Figure 3f). Neurons are visible (n), and small spherical bodies (sb) that could be cell contents of some kind from broken ectodermal cells (see arrowhead in Figure 3a), for example loculae from mucocytes. The larger oval object is, although small (*c*. 3.5 μm), possibly a mature cnidocyst (cn) capsule with a small protruding tip, or the base of an everted tubule broken off. Amoeboid cells can be seen smeared onto the mesoglea (am, Figure 3f). Another cell type is seen in the lower left corner (black arrowhead, Figure 3f). It is elongate, with extensions that attach it to the mesoglea or connect it with other cells, and a broken upward facing connection. This could be a nerve connection between ectoderm and mesoglea, or another type of cell anchored to the mesoglea.

3.4 | Observations of cnida discharge

Live planulae observed under LM were vigorous swimmers, darting back and forth under the coverslip. In contact with the edges of the coverslip, and only here, planulae were sometimes observed to fire cnidae, starting when larvae were 20 days old. Figure 4a shows a planula that has fired a nematocyst (b- or p-mastigophore). Usually, they would linger at the edge while firing, and the discharged cnidae were dominated by atrichous isorhizas with straight and stiff tubules, however, a few b-mastigophores were also observed (Figure 4b). In contrast, when larvae were stained with nigrosin and eosin (including ethanol) before squash preparations, the discharged cnidae comprised all types, and mucus was also released.

When small pieces of coral skeleton were introduced to planulae (44 and 69 days old), they temporarily inspected the surfaces, especially interested in any crevices present. When a pipette was used to create water movement in the dish, the water turbulence induced cnida discharge, resulting in that planulae close to the substrate attached. Sometimes, larvae also attached to each other during turbulence. Spontaneous attachment to the substrate by means of cnidae was also observed. While some planulae did attach, they were never seen to make direct physical contact with the surface of smooth substrates. The planulae were in those cases observed hovering above the substrate, with only a cnida tubule holding it in place. The fast discharge and attachment by cnidae is clearly distinguishable from the slow production of a mucus string, so it was certain that the attachment was by cnidae and not mucus strings.

When planulae were 90 days old, a piece of coral skeleton with the septa (skeletal lamellae) facing the bottom of the dish was introduced. One larva was observed to swim in under the substrate, not emerging again. When the piece of skeleton was turned over a while later, the larva had wedged itself in between the lamellae, making full physical contact with the substrate. The substrate and planula were immediately fixed for SEM. The planula was lost in the process, but the cnidocyst tubules by which it had attached were still there and were documented under SEM.

3.5 | Larval cnidome under light microscopy

The larval cnidome consisted of two or three types of isorhizas, microbasic b-mastigophores and microbasic p-mastigophores, all sparingly distributed over the planula ectoderm (Figure 4b–c). Developing cnidoblasts were also observed (Figure 4d). Figure 5 shows a complete collection of the cnida complement of the planulae, both intact capsules and discharged capsules with shafts and tubules evaginated. In Table 2, all the capsule metrics are compiled, and Figure 6 shows histograms with the frequency distributions of capsule lengths in three of the cnida types. Only measurements of intact capsules were included.

3.5.1 | Atrichous isorhizas with broad, smooth tubules

The most common isorhiza was atrichous, with an inverted tubule of wide diameter. The intact capsules were 18–26 μm, broad oval, with *c*. 10 coils of tubule that entirely filled up the capsule (Figures 5a,b and 6a).

Everted atrichous isorhizas of this type viewed under LM had broad, smooth tubules, 102–335 μm long and 1.3–3.0 μm wide (Figure 5i). No spines, helical pattern or other texture was visible. In fresh preparations, the tubules, when everted, were straight and stiff (Figure 4b), while in dried preparations, they became flattened and less stiff, undulating over the slides (Supporting Information Figure S1). The tubule diameter tapered slightly from proximal to distal end, from 3 to 2 μm in anisorhiza with a 29.5-μm-long capsule. The transition between capsule and tubule was inconspicuous. In some of the everted capsules, there was a "hook" (Supporting Information Figure A,B). These isorhizas will hereafter be referred to as "smooth atrichous isorhizas," with smooth referring to the everted tubule.

3.5.2 | Atrichous isorhizas with pleated tubules

This type of atrichous isorhiza was less common than the smooth type (Figure 5c–e). Intact capsules were elongate

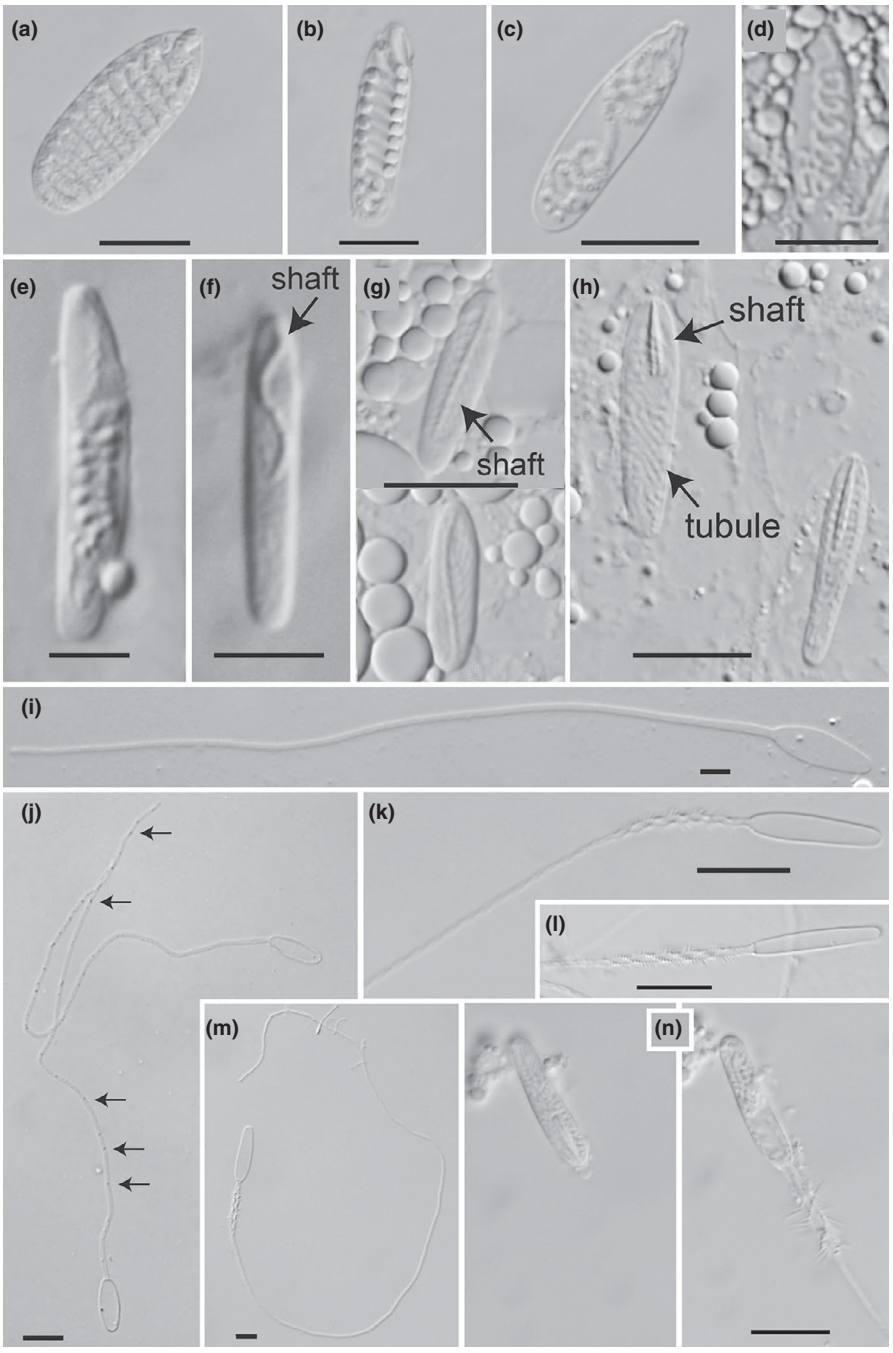

FIGURE 5 Cnidae complement of *Lophelia pertusa* planulae. (a, b) Atrichous isorhizas with broad tubules. In (b), the tubule is seen in cross-section showing the tubule diameter. Note how the tubule fills up the entire capsule (c–e). Atrichous isorhizas with thinner, pleated, tubules: note that the tubules do not fill the capsules. E is probably the same type as (c) but immature, while (d) shows a possible third type of isorhiza. (f) Mature b-mastigophore with a thread-like undulating shaft. (g) Two immature b-mastigophores, in the lower the immature shaft reaches all the way to the basal end of capsule. (h) Two p-mastigophores: the left with the deep v-notch (bifurcation) visible on the simple shaft, the right viewed from another angle. (i) An everted atrichous isorhiza in full length showing the broad, smooth tubule (correlating with the type in [a,b]). (j) Two everted atrichous isorhizas with the tubules seen in full length. The tubule wall has a vague pleated texture without helical pattern (correlating with the type in [c, e]), and dark dots (arrows). (k) An everted b-mastigophore: the shaft has short spines in five helical rows and is just slightly broader than the tubule. The everted tubule has a visible helical pattern, but no visible spines. (l) A b-mastigophore with a narrower capsule and microbasic shaft with six spine rows. (m) An everted b-mastigophore showing the tubule in full length. (n) A p-mastigophore before and after discharge. The shaft, equipped with long spines in three rows, is markedly wider than the tubule. The shaft doubles its length from inverted to everted stage. Note the difference in magnification between images. All scale bars 10 μm

Cnida type	mean ± *SD*	range	l/w ratio
Atrichous isorhizas with smooth tubules ($n = 32$) inv. tubule 1.1–2.7µm wide in 10 coils, ev. tubule 1.3–3.0 µm wide, up to 335 µm long	22.5 ± 1.9	17.6–26.1	2.6–3.9
Atrichous isorhizas with pleated tubules ($n = 8$) inv. tubule 1.0–1.2 µm wide ev. tubule. 1.0–1.5 µm wide, up to 200 µm long	24.1 ± 8.1	18.7–43.5	2.7–5.0
b-mastigophores ($n = 31$) inv. shaft 0.4–0.9 of capsule length ev. shaft slightly longer than capsule, tubule *c*. 0.8 µm wide, up to 340 µm long	16.1 ± 4.0	12.4–28.6	2.9–5.7
p-mastigophores ($n = 48$) inv. shaft 0.3–0.5 of capsule length ev. shaft shorter than capsule, tubule *c*. 0.9 µm wide, full length of tubule unknown	18.0 ± 2.4	14.4–28.9	3.2–7.3

Note. ev.: everted; inv.: inverted.

TABLE 2 Capsule metrics for cnidae in *Lophelia pertusa* larvae: number of measured capsules (n), mean length (µm), standard deviation (±*SD*), range and length/width (l/w) ratios

oval, mostly about 20 µm long, but two extremes at 30 and 44 µm were measured in 43-days-old planulae. Since only eight intact capsules were measured, no histogram with frequency distributions of capsule lengths was produced. The capsules sometimes had a protruding apical tip (Figure 5c). The inverted tubule had a texture that made it look like it had spines and was first mistaken for a holotrichous isorhiza. The tubule did not fill up the capsule, making irregular coils (Figure 5c). Capsules in Figure 5c,e are likely the same type despite the difference in distribution of the tubules.

Everted atrichous isorhizas of this second type had thinner (1.0–1.5 µm wide) and less stiff tubules than the smooth atrichous isorhizas: that is, the tubules were undulating freely over the slides. The transition between capsule and tubule was more marked than in the smooth type. The emptied capsules never had the internal hook seen in smooth atrichous isorhizas (Supporting Information Figure A,B). The tubule wall had a vague pleated texture with no helical pattern. Only at the base of the tubule, close to the capsule, there was sometimes a faint helical pattern (Supporting Information Figure B). There were small dark dots sparingly distributed along the tubule and no visible spine armature (Figure 5j). The tubule never flattened against the slide in dry preparations as the tubules of the smooth atrichous isorhizas. The full length was 117–202 µm; nota bene, this was measured from tubules discharged from capsule sizes up to only 28 µm and could thus potentially be longer in specimens with larger capsules. This type of isorhiza will hereafter be referred to as "pleated atrichous isorhizas," with pleated referring to the texture of the everted tubule.

Some capsules, probably belonging to a third type of isorhiza, had a short pointed oval shape. The capsule in Figure 5d was 19.5 µm long and 6.3 µm wide, with an inverted tubule of 0.7 µm width in six regular coils. They had a broad mid-part, pointed at both ends, and the coiled tubule was seemingly stretched between the apical and basal end of the capsule (Figure 5d). This type of isorhiza was very rare and only seen within tissues, never isolated or everted, so a more detailed description warrants further studies.

3.5.3 | Microbasic b-mastigophores

The intact capsules were 12–29 µm long, narrow, with parallel sides (Table 2; Figures 5f,g and 6a).The inverted shaft was thread-like, sometimes undulating, and reaching 0.4–0.9 of the capsule length. In immature b-mastigophores, before the tubule and shaft were fully developed and properly packed, the capsules were broader and the shafts longer than in mature capsules. The shafts were then reaching the basal end of the capsule (Figure 5g).

Everted b-mastigophore shafts were microbasic, approximately as long as the capsules or slightly longer, with 5–6 spine rows (Figure 5k,l).The diameter of the shaft was not markedly wider than that of the tubule. The everted tubules had a texture revealing helical pleats, but no visible spines (Figure 5k). Some tubules had dark dots, similar to those of the pleated atrichous isorhiza tubules. In the distal end of the tubule, there were larger bits of substance visible inside the tubule (Supporting Information Figure B). The full length of everted tubules was up to *c*. 340 µm (Figure 5m), and the diameter was *c*. 0.8 µm.

3.5.4 | Microbasic p-mastigophores

Intact capsules were narrow to broad elongate, broadest along the shaft, and 14–29 µm long (Table 2; Figures 5h and 6c). Shafts were short and simple, reaching 0.3–0.5 of the

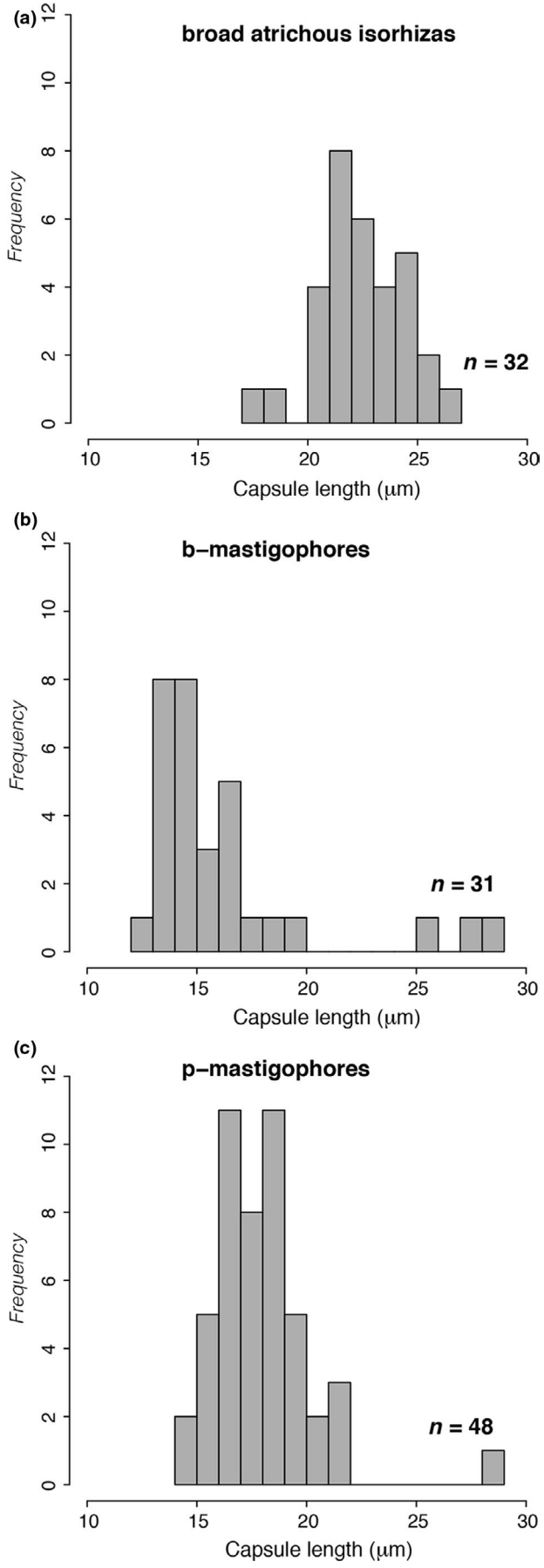

FIGURE 6 Frequency distributions of capsule lengths (μm) of three of the larval nematocyst types: (a) smooth atrichous isorhizas (i.e. smooth everted tubules); (b) b-mastigophores; (c) p-mastigophores. Only intact capsules were included. The frequency distribution of the atrichous isorhizas with pleated tubules is not shown since only eight intact capsules were measured. Note that the ranges of the *y*- and *x*-axes are the same on all three for easy comparison. (*n* = number of measured capsules)

capsule length, and with the v-notch (bifurcation) usually extending half the shaft length. Seen from the side, the shaft was pointed at both ends and the v-notch not visible. The inverted tubule was almost filling the entire capsule, except for the area around the proximal shaft (at the apical end of the capsule).

Everted p-mastigophore shafts were microbasic, shorter than the capsule, with three spine rows, and the shaft doubled its length from inverted to everted stage (Figure 5n).The shaft was twice as wide as the tubule, with a marked transition between the two. Although more intact capsules of p-mastigophores than b-mastigophores were found, and measured, everted p-mastigophores were almost non-existing in the studied material. Only one certain everted p-mastigophore was found. The full length of the p-mastigophore tubule is thus unknown.

3.6 | SEM preparations of cnidae

On the surface of a planula in the SEM preparations, an isorhiza tubule was observed stretching over almost the full length of the planula (Figure 7a). Stretched out, it would probably be longer than the planula. Small knobs were visible along the tubule, between fine helical furrows (Figure 7b-d). The tubule was *c*. 1 μm wide in its flattened condition, thinner than that of the broad, smooth type and congruent with the tubule of the atrichous isorhiza with pleated tubule (Figure 5j).We suggest the knobs to be agglutinant substance. The presence of helical furrows also speaks in favour of the type that had a vague pleated texture on its everted tubule. If this pairing of tubules is correct, the lack of regular helical appearance under LM could be due to the small knobs that confound the fine helical furrows seen under SEM. What would speak against this pairing is that the tubule in Figure 7a–d is flattened, while the tubules of the pleated atrichous isorhizas never seemed to be flattened on the slides in dry preparations viewed under LM.

Also found on the surface of the planulae were two everted p-mastigophores, with the capsules still rooted in the ectoderm (Figure 7e,f).There are slight differences between the two shafts: the spines on the shaft in Figure 7e are 1.7–1.9 μm, and in Figure 7f 2.3–2.6 μm. The spines of the shaft of b-mastigophores measured from LM images (Figure

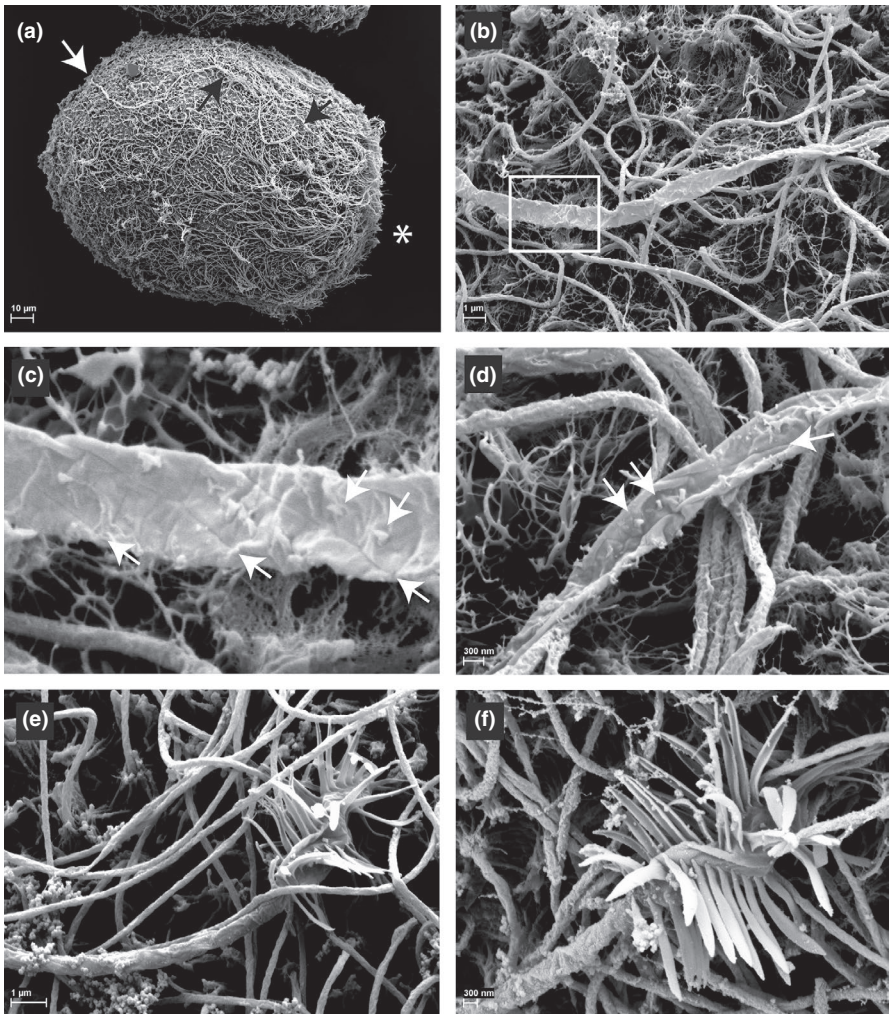

FIGURE 7 (a) Discharged atrichous isorhiza with its tubule almost as long as the planula. Arrows mark the tubule, with the right arrow pointing to the place where the capsule is embedded in the ectoderm. The asterisk (*) marks the oral end. (b) Closer view of the isorhiza tubule in (a), the white rectangle marks the zoomed in area in (c). (c) A part of the isorhiza tubule showing helical furrows (three lower arrows), and the small knobs (possibly agglutinant substance) centrally placed between furrows (upper right arrows). The tubule is *c*. 1 µm broad in its flattened condition (see scale bar in [b]) and congruent with the isorhizas in Figure 5c,j. (d) Another part of the same tubule as in (a–c) with visible small knobs (central arrows) and a furrow (upper right arrow). (e,f) The shafts and tubules of two discharged p-mastigophores, with the capsules still embedded in the ectoderm. The shafts bear prominent spine armature, but no spines are visible along the tubule. Spines are inserted in helical rows around the central part of the shaft

5k,l) were 0.9–1.4 µm, while the spines on the shaft of the p-mastigophore in Figure 5n were 2.3–3.0 µm. Potentially, the mastigophore in Figure 7e could thus be a b-mastigophore rather than a p-mastigophore, but more likely is that both are p-mastigophores. Measuring small details in LM is precarious due to refraction, and the uncertainty of the measurements in the LM images is large. The spines in Figure 5k are long enough to belong to a p-mastigophore. The long spines of the shafts were pointed linear in both mastigophores, inserted into the shaft in their full width at the base, while the margins of the spines are rolled in (Figure 7f), giving them a slender canoe-shape. Both tubules had a pleated surface and no visible spines.

On the substrate where a planula had wedged itself in between the skeletal lamellae of the piece of coral skeleton introduced to the planulae (Figure 8a), two different types of possible cnidatubules were found. Another planula had wedged itself in under the free edge of a sheet of glue (used to attach the substrate to a coverslip) in a similar way as the larva between the lamellae (inset in Figure 8a), indicating that *L. pertusa* planulae seek out cryptic spaces for settling.

The first type of tubule is thin, between 0.5 and 1 µm (Figure 8b–d). There were spines present, although not congruent with spines previously observed from cnida tubules. Thin adhesive strands could be seen in close proximity to the spines at approximately equal distances; therefore, we suggest that the "spines" are undischarged adhesive strands, and not spines per se. As seen in Figure 8b, two "spines" follow on the two discharged adhesive strands. The thin adhesive strands project in both directions, perpendicular from the tubule, attaching it very closely to the substrate (Figure 8c). Comparing the tubule in the area with the discharged adhesive strands and the area with undischarged strands ("spines"), the discharge has changed the morphology of the tubule. The tubule looks flattened where the spines are intact, and more round—and thereby less wide from this angle—where they are discharged. This type of tubule is not congruent with the tubules documented on the larvae, and difficult to associate with any of the tubules documented under LM.

The second type of tubule found on the substrate was completely smooth with no visible knobs, spines or furrows. The entire tubule is attached flat against the substrate, with

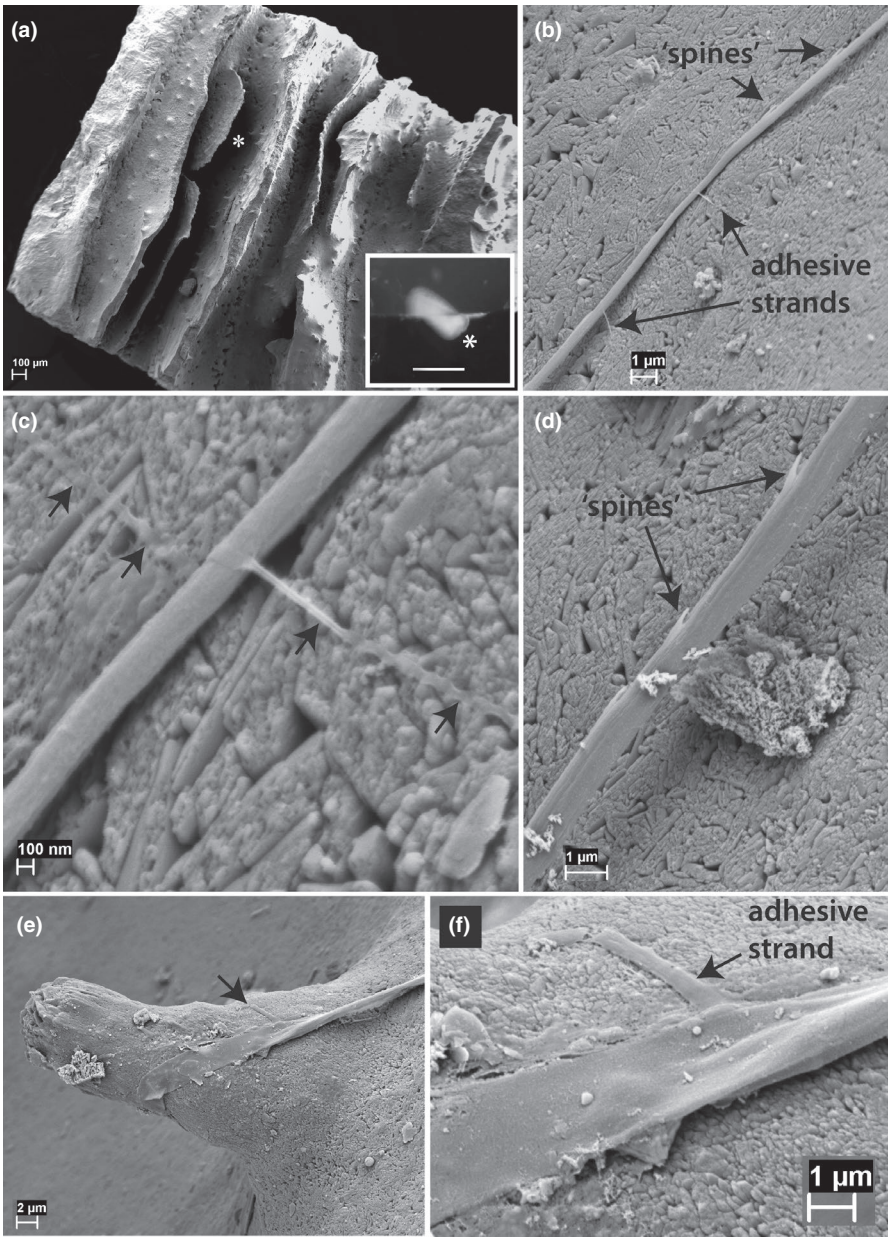

FIGURE 8 (a) A piece of coral skeleton with the interior septa (skeletal lamellae) facing forward. An asterisk indicates the site where the planula had wedged itself in between the lamellae. The inset shows a planula that had wedged itself in under a sheet of glue that attached a piece of substrate to the coverslip, in a similar way as the planula found between the lamellae (scale bar in inset 100 µm). (b) Part of a tubule with thin adhesive strands and what looks like spines but could be undischarged adhesive strands. (c) A close-up of the part of the tubule in (b) with thin adhesive strands attached to the substrate. Note that the strand runs in both directions from the tubule (arrows). (d) Distal part of the same tubule as in (b and c) with a close-up of the "spines." (e, f) Possibly, the tubule of an atrichous isorhiza with broad, smooth tubule attached to a skeletal projection on the substrate. An adhesive strand runs from the tubule. No helical furrows are visible, congruent with the light microscopy images of the smooth atrichous isorhiza in Figure 5i

an additional perpendicular adhesive strand (Figure 8e-f). It correlates with the everted tubule of the smooth atrichous isorhiza seen in the LM preparations (Figure 5i) and is of similar width (*c*. 2 µm).

4 | DISCUSSION

4.1 | The role of cnidae and substrate microtopography in larval settling

The results of the present study establish that the onset of settling competency in *L. pertusa* most likely starts three weeks (20 days) after spawning, as planulae at this time have developed functional cnidae. This sets the limit for the pre-competency period, that is, the period during which planulae disperse with no means of settling. The appearance of functional cnidae coincides with the onset of bottom-probing behaviour and development of a flexible mouth (Larsson et al., 2014). Since *L. pertusa* larvae are planktotrophic (Strömberg & Larsson, 2017), it is likely that they need to feed for a while before achieving metamorphic competence, and thus, the competency period could be delayed until they have gained the necessary nutrients. The bottom-probing behaviour did not comprise all larvae even after five weeks (Larsson et al., 2014; Strömberg & Larsson, 2017), also speaking for a period of maturation and feeding before complete readiness for settling. A minimum dispersal period of three to five weeks secures along dispersal distance and lessens the chance for retention in the natal reef, and is an important trait to add to dispersal models.

The first observation of cnida discharge in this study was earlier than the previously observed occurrence at 30 days (Larsson et al., 2014). Planulae were now 20 days old when they first were observed to fire cnidae. In 2013, documentation under LM was carried out on day 20, 21, 26, 28, and 30, with cnida discharge not observed until day 30. In 2015, we started to look more closely for cnidae presence from day 20 to get a more precise timing for cnida discharge capacity, only to find it had already started. In 15-days-old planulae, however, no cnida discharge was observed. Larval development rate is affected by temperature, with higher temperature in general causing the development rate to speed up (O'Connor et al., 2007; Strathmann, 1987), also documented in *L. pertusa* (Strömberg & Larsson, 2017). The larval cultures were reared at the same temperature in both 2013 and 2015 (7–8°C), ruling out this effect to cause the difference in timing of first observed cnida discharge in this particular case. The batch of embryos reared in 11–12°C, as described in Strömberg and Larsson (2017), was not used for cnidae studies in the present study since the cultures crashed before the embryos had developed into mature planulae.

Hayashibara et al. (2000) found a coinciding maximum abundance of cnidae and peak in settling at 8 days post-fertilization in the tropical coral *A. nasuta*. Cnidae first appeared after 3–4 days, simultaneous with first observed settlement, but with a later peak for both cnidae abundance and settling. Especially, spirocysts decreased following settling, indicating that *A. nasuta* use spirocysts for settling. The authors suggested that presence of cnidae is a sign that the larvae are mature and ready to settle, which seem to be the case also for *L. pertusa* planulae in this study. Other *Acropora* species has been observed to have abundant spirocysts at the aboral pole, with increased abundance of spirocysts coinciding with the peak in settling (Okubo & Motokawa, 2007).

For a more certain onset of cnidae development, it is necessary to use molecular tools that can indicate when cnidoblasts start to differentiate and produce the minicollagens and proteins associated with cnidae. This has been done for the starlet anemone *Nematostella vectensis* (Wolenski, Layden, Martindale, Gilmore, & Finnerty, 2013), and several studies have been done on *Hydra* (Adamczyk et al., 2008; Beckmann & Özbek, 2012; David et al., 2008). Wolenski et al. (2013) traced gene expression of the Nv-NF-κB gene associated with cnidae and found expression to start at 30 hr post-fertilization, while mature cnidae began appearing after 2–3 days and were more abundant after one week. At day 3, the animals had developed into juvenile polyps.

Our observations on cnidae function in settling, as a means of temporary attachment by adhesive cnida tubules, verify the importance of cnidae presence for functional readiness to settle (competency).The live observations under stereo magnifier clearly showed that larvae attached by means of cnidae tubules, both spontaneously and induced by turbulence. The documented material was however scarce, and all types of cnidae were not observed under SEM. We were thus not able to completely corroborate the different cnida types with both LM and SEM images, or those found on planulae with those found on the substrate. This is a weakness of the present study, especially considering the cnidae tubules observed on the substrate. These are different from the tubules observed on the planulae, and therefore, we cannot claim for certain that these truly are cnidae tubules from the planula. Nevertheless, the tubules were found on the exact spot where the planula had been observed to wedge in, and no tubules of these kinds were found anywhere else on the piece of coral skeleton examined. The broader type of tubule (Figure 8f) correlated well with the broad, smooth atrichous isorhiza tubule documented under LM (Figure 5i), while the thin tubule on the substrate is not possible to assign to any of the other documented cnidae. There were other structures present on the substrate, although clearly recognized as emanating either from hydrozoans or diatoms.

The differences between the tubules found on the planulae and on the substrate could in part be due to that the tubules on the substrate were discharged in seawater from a live planula in response to a proper stimuli, while discharge of the tubules on the planulae was due to the chemicals used in the SEM fixation process and thus possibly incomplete. Chemical and morphological changes of the tubule occur during discharge, with rapid swelling of capsules and tubules after stimuli. Berking and Herrmann (2006) suggested that a lowered pH in the mature and ready-to-discharge capsules produces strong hydrogen bonds between components of the capsule contents, thereby causing a tight packaging. During cnidogenesis, and during discharge, the pH is higher, resulting in a release of hydrogen bonds and the volume of the capsule contents therefore increase. At discharge, this swelling together with an increased osmotic pressure is suggested to cause the burst of the capsule operculum and provide the driving force for the shaft and tubule evagination. The cnidae found on planulae seemed to be a bit more dried up than those on the substrate. On the substrate, tubules could have reacted to the substrate and due to this be more fully discharged and stretched out over the surfaces.

The agglutinant fibrillae of spirocysts have been found to discharge only in contact with a substrate and not just because the main tubule is discharged (Mariscal, McLean, & Hand, 1977). This seems to be the case also for the adhesive strands on the thin tubule found on the substrate, with both undischarged ("spines") and discharged strands along the tubule as seen in Figure 8b-d.

The here studied larvae had a preference for cryptic spaces, although the full settling trial failed and no larval settling could be detected on the substrates in the rotating beakers. A preference for crevices in dimensions closely matching larval widths was found for two tropical corals, *A. millepora* and *Ctenactis crassa*, in a study by Whalan, Abdul Wahab,

Sprungala, Poole, and Nys (2015), with successful settling and metamorphosis in crevices without any chemical cues present. Other marine larvae have also been found to prefer to settle in crevices with higher survival for some of the species as a result (Walters & Wethey, 1996).

Although we did not get SEM images of settled planulae with tubules still attached, to see what types of cnidae were used for primary anchoring as we aimed, we still provide a conceptual illustration of the hypothesized attachment mode of a larva. Figure 9 shows a planula within a crevice, with the aboral pole inserted into the crevice, and the oral pole facing the opening. Cnidae tubules are anchoring the larva to the walls of the pit. Both the tendency of larvae to fire cnidae under the coverslips—only at the very edge of the slip with "open water" to one side—and the observations of larvae squeezing in between lamellae of coral skeleton, or under the sheet of glue that held a piece of substrate in place, indicate that the larvae seek out cryptic spaces for settling. The larvae's preference for cryptic spaces is important to consider when constructing artificial reefs and settling substrates for cold-water coral restoration efforts.

4.2 | Comparison of larval and adult cnidomes and their functions

The cnida composition of *L. pertusa* larvae is different from the cnidome of the adult polyps. Neither of the two (or three) types of isorhizas present in planulae are present in adult polyps (Strömberg & Östman, 2016). The adult polyp has one type of holotrichous isorhiza in two size classes, a broad, oval in the tentacles, and a longer in the acontia. Despite the difference in sizes, the morphological characters are otherwise identical. The adult's isorhizas have three very regular helices of small but conspicuous spines along the entire tubule. Their positions in the tentacles and acontia suggest a role in prey capture and defence. Their function in adult polyps should be entangling, with the small spines acting as barbs, or possibly penetrating.

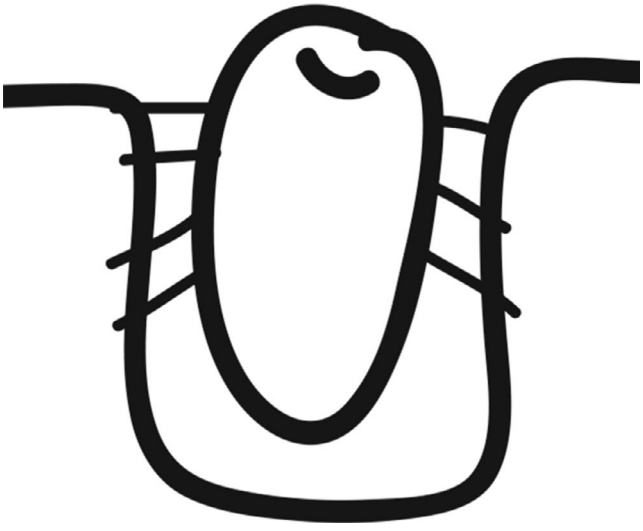

FIGURE 9 A conceptual illustration of a settled planula in a crevice, with cnida tubules attaching it to the walls of the crevice. The oral pole is facing the opening, and the aboral pole is facing the bottom

The atrichous isorhiza with pleated tubules has possible agglutinant substances present, visible as dark dots along the tubule in Figure 5j. The corresponding tubule from the SEM preparations had small knobs of what could be droplets of agglutinant substance, similar to those of spirocysts (Figure 7c-d), although this needs to be verified with further studies. We have no documented evidence of these tubules attaching by means of agglutinant fibrillae. Both the knobs (visible in SEM) and the dark dots (visible in LM) were irregularly spaced along the tubules, but not correlating completely as the dark dots were more sparsely distributed than the knobs. That atrichous isorhizas have agglutinant substances is, however, shown in other studies. Amano, Koizumi, and Kobayakawa (1997) found that a monoclonal antibody (AE03) for mucus granules in the basal disc of *Hydra* also recognized atrichous isorhizas, leading them to suggest that the atrichous isorhizas contain the same type of adhesive substance as the sticky mucus of the pedal disc with which the animals attach to a substrate. *Hydra* uses the adhesive atrichous isorhizas for temporary attachment when they move over a substrate using the tentacle tips.

The smooth type of larval atrichous isorhiza had broad and stiff tubules, with no visible surface structures such as helical furrows, spines or knobs. If the tubule on the substrate in Figure 8e-f belongs to this type, it also has adhesive properties on the tubule wall itself and attaches to a substrate.

The thin tubule in Figure 8b-d cannot be assigned to any specific type of the documented cnidae, but its thin adhesive strands are similar to the agglutinant fibrillae of spirocysts, although sparsely distributed along the tubule. The sparse distribution of adhesive strands along the tubules is in contrast with the dense fibrillae of spirocysts found in the tentacles of adult polyps. In polyps, the spirocysts function in food capture, aggression and substrate attachment, the latter function in mobile sea anemones (Mariscal et al., 1977; Thorington & Hessinger, 1990). A larger attachment area is beneficial when handling large prey, but for primary attachment of a planula, a smaller attachment surface area (fewer fibrillae/adhesive strands) is probably sufficient and more economic to produce, and also easier to detach from.

Paruntu et al. (2000) investigated the difference in cnida complement between larvae and polyps in the tropical coral *P. damicornis*, and found that planulae had abundant spirocysts that had decreased significantly during the process of settling, and the authors suggested these to be involved in substrate attachment. Hayashibara et al. (2000) found spirocysts to be used in settling also in the coral *A. nasuta*, while the actinula larva of the hydrozoan *T. mesembryanthemum* (syn.

E. crocea) used atrichous isorhizas (Yamashita et al., 2003). No spirocysts were found in the *L. pertusa* planulae. Only one possible spirocyst was observed, although not included in this documentation, as it was not certain that it was a spirocyst since surrounding tissue material obscured it. Spirocysts are found in the tentacles of adult polyps, and possibly, they do not form in *L. pertusa* planulae until the tentacles are forming. It is clear though, that different species of corals and hydrozoans use their cnidae differently during the larval stage and have a unique cnida complement in comparison with the adult polyp. Interesting to note is that spirocysts are the main cnida type used for coral planulae attachment, while hydrozoan larvae and polyps use atrichous isorhizas, but that *L. pertusa* planulae have atrichous isorhizas and no spirocysts.

The microbasic b- and p-mastigophores present in planulae are similar to the adult forms, with slight differences in the relation between shaft and capsule lengths. The b-mastigophores of the planula has a relatively long shaft with five spine rows compared to shafts of b-mastigophores in adult polyps of similar capsule size that normally have three spine rows. In contrast, the p-mastigophores have shorter shafts with three spine rows in the planulae compared to corresponding p-mastigophores in adult polyps, with 7–9 spine rows. The most significant difference lies in the lack of spine armature on the tubules of the larval types, while the tubules of the adults' p- and b-mastigophores are spined, at least the larger ones. The presence of spines on the tubules on small size p- and b-mastigophores in the adults is not certain. The largest b- and p-mastigophores were found in 43-day-old planulae (the "outliers" in Figure 6b,c). Most of the measured capsules were from 30- and 43-day-old, and a few from 112-day-old planulae. There was, however, neither enough material nor enough subsampling done at critical points in time, to make any conclusions about cnida sizes at different ages.

The lack of everted p-mastigophores in the material despite that the intact capsules of p-mastigophores outnumbered the b-mastigophores could be because many of the everted cnidae were measured on images of dried preparations, where the spines on the shaft sometimes had fallen off and thus some everted p-mastigophores might have been taken for b-mastigophores. The spines on p-mastigophore shafts are, however, much longer and more conspicuous than the spines on b-mastigophore shafts, so a mistake of that kind should not have been made. We have no good explanation for this observation.

Everted b-mastigophores and p-mastigophores are usually distinguished from each other by the shorter spines on the everted shafts, and a shaft diameter barely wider than the tubule in the b-mastigophores. This is in contrast to the prominent spines of the p-mastigophore shaft that also have a more conspicuous change of diameter between shaft and tubule (Strömberg & Östman, 2016). In adult polyps, these nematocysts have a role in prey capture, toxin delivery and defence, while the function in planulae needs further investigation. Slattery, Hines, Starmer, and Paul (1999) identified two defensive metabolites from larvae of the soft coral *Sinularia polydactyla* that was found to deter pufferfish from eating the larvae. The fish quickly spat out both larvae and pellets spiked with the two compounds (pukalide and 11β-acetoxypukalide), but not pellets without it. The authors did not identify the source of the compounds—if connected to cnidae or not—but since nematocysts do deliver toxins and probably cause general nuisance the effect could well be caused by cnida discharge. The b- and p-mastigophores found in the larvae could thus be for defence. If larvae are capable of using them for prey, capture is still unknown.

4.3 | External morphological larval features and their function

The observations of the apical cilia of *L. pertusa* planulae are congruent with observations of the apical tuft of other anthozoan planulae, although they were not permanently clustered as seen in some other species (Martin & Koss, 2002). Chia and Koss (1979) also reported apical cilia that were not fixed together but still moved as a unit in the sea anemone *Anthopleura elegantissima*. It has been suggested that the tuft is involved in substrate choice since sea anemone larvae have been observed sweeping the tuft along the bottom surface of a culture dish (Chia & Koss, 1979). In *C. smithii*, however, the tuft is lost 2–3 weeks prior to settling (Tranter et al., 1982). Since the apical organ and tuft are lost and exchanged for calicoblastic cells (Grasso et al., 2011) prior to settling, a function in substrate choice seems unlikely. More plausible is that the planulae sweep the water in front of them searching for food patches or to detect other environmental cues. Tranter et al. (1982) suggested the apical tuft to enhance swimming agility, enabling swift changes of swimming direction. Here, we also observed that they have a clear function in shifting from motionless to swimming by crossing over of the apical cilia.

The difference in morphology of the collars of microvilli at the base of the motile cilia in the mouth area as seen in our Figure 2d–f, with embedded microvilli, compared to those of the general body surface of late planulae could have two functions. Firstly, a function of flexibility—mouth movement likely requires that cells need to be able to contract and relax. Secondly, the motile cilia around the mouth might need a higher degree of control than cilia on the remaining body. If cilia are involved in food particle recognition, and the planulae are able to reject incoming particles not recognized as food before ingested, the cilia should have a quick response time. The cilia must rapidly be able to change beat direction, with greater innervation and muscle control to accommodate this. It is difficult to find reference material in the necessary resolution to make a comparison. Figure 3 in Chia and Koss (1979) shows collars of microvilli of the general ectodermal cells of planulae of the sea anemone *A. elegantissima* that is congruent with the

corresponding general body cells in this study. The microvilli are visible in their full length and superficially rooted on the cell surface. To our knowledge, there are no previous studies showing embedded collars of microvilli.

The pores found on some of the embedded microvilli could potentially be part of the chemosensory apparatus that aid in food versus non-food recognition. This needs to be verified through a more detailed examination by transmission electron microscopy thin sections that can reveal the composition and ultrastructure of the tissue within the embedded microvilli. While different prey-specific substances have been identified to bind to chemoreceptors on supporting cells adjacent to cnidocysts in sea anemones (Thorington & Hessinger, 1988), there has been no previous record of specific morphological features associated to the chemoreceptors. Pores could also be an artefact produced in the drying process during the preparations for SEM, as water vapour leaves the tissues. The consistent position of the pores at the cell surface of the embedded collars of microvilli, and no other similar pores observed elsewhere, nevertheless suggests that they are not mere artefacts.

4.4 | On the ultrastructure of the internal morphology

The internal morphology of the 26-days-old planula exposed a nerve plexus. The definition of nerve plexus is "a branching network of intersecting nerves," and in van Marle (1977), it is stated that nerve connections with cnidocytes and gland cells only occur within a plexus. With this in mind, we interpret the network of nerve fibres and cellular bodies on the surface of the mesoglea as a nerve plexus, where cnidocytes and neurons are interconnected. This nerve plexus is restricted to the oral half of the planula, and on the apical half, there is only scattered neurons crisscrossing the smooth surface of the mesoglea. The small oval object in Figure 3f could be a cnida capsule, despite the small size (3.5 µm). Adult polyps have tiny cnidae as small as 6 µm, measured in LM images (Strömberg & Östman, 2016).Cnidae as small as this could easily be overlooked in fresh squash preparations.

Visible in Figure 3f is also what looks like amoebocytes, smeared onto the mesoglea. Amoebocytes, sometimes with filipodial extensions, have been identified in anthozoans and might have a function in for example regeneration, wound healing and secreting connective mesogleal fibres (Gold & Jacobs, 2013; Meszaros & Bigger, 1999; Tucker, Shibata, & Blankenship, 2011). The latter function correlates well with its position on the mesoglea in our study.

The elongate cell type in Figure 3f (arrowhead) could be part of a nerve or other cell type connecting the ectoderm and mesoglea. Chia and Koss (1979) described the nerve plexus in the sea anemone *A. elegantissima* and defined two types of sensory cells with extensions attaching to the mesoglea. The elongate cell has a broken process in one end, pointing up from the surface, suggesting an extension of the cell into the ectoderm.

5 | CONCLUSIONS

The present study has established the minimum dispersal period of *L. pertusa* planulae to three weeks (reared at 7–8°C). Before this period, planulae have no means of settling since primary anchoring is achieved by means of cnida tubules, and functional cnidae are present from a larval age of 20 days. From this age on, an increasing fraction of the larval cohort will start to search for a substrate to settle on. The limits for the pre-competency period are valuable information to include in biophysical dispersal models since it has significant influence on dispersal distance (Treml et al., 2015) and is equally important as the competency window that follows for the dispersal dynamics of marine larvae.

The cnida composition of *L. pertusa* larvae is different from the cnidome of the adult polyps with at least two types of isorhizas present in planulae that are not found in adult polyps. This study suggests that these cnidae are involved in the preliminary anchoring to the substrate preceding permanent attachment and metamorphosis in this coral, although more studies on larval cnida function need to be carried out to fully resolve the specific functions of the different cnidae. It is interesting that *L. pertusa* planulae lack the cnida-type spirocysts, used for primary attachment by other coral planulae, while possessing atrichous isorhizas as used by hydrozoan larvae and polyps.

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ENDNOTE

[1]Nomenclature: the cnido*cyst* is the stinging organelle produced by the cnido*cyte,* the specialized cell containing the cnidocyst. Cnidae is a general term for cnidocysts, and the cnidome is the complete set of cnidae types present within an organism. Anthozoan cnidarians have three types of cnidocysts: nematocysts, spirocysts and ptychocysts.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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