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Morphology, Feeding Rate and Larval Settlement Preference of the Corallivorous Nudibranch *Phestilla subodiosa* (Nudibranchia: Trinchesiidae) from Hong Kong

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We studied the morphology, host specificity, feeding rate and larval settlement preference of the corallivorous nudibranch *Phestilla subodiosa* collected from the field. Morphologically, these specimens collected from the scleractinian coral *Monipora peltiformis* in Hong Kong waters are different from the holotype and paratypes collected from an aquarium culture of *Montipora* spp. in having a diamond-shaped swollen bulbs, and brown spots on cerata, bulbs and the body immediately posterior to cerata. In experiments where *P. subodiosa* individuals were placed on the surface of several species of common scleractinan corals collected from Hong Kong waters, the nudibranchs were found to feed on *M. peltformis* at a rate of 0.05 cm^2 $individual^{-1}$ d^{-1}; however, they were killed and eaten by other tested coral species (*Pavnoa decussata*, *Porites lutea* and *Duncanopsammia peltata*). When cultured in seawater conditioned with *M. peltiformis,* the veliger larvae required six days to become competent for settlement, and at day 9 could reach a maximum metamorphic rate of 31.1%. At competence, the veliger larvae could be induced to settle, indicating the presence of a larval settlement cue released by the host coral. Other coral species or their conditioned seawater did not induce settlement of the *P. subodiosa* larvae. Overall, our study expands the distribution record of *P. subodiosa,* adds this species to the list of corallivorous nudibranchs in Hong Kong waters, provides morphological features that were not included in the original description of this species, reveals the host specificity, and provides the feeding rate of this species. These results contribute to a better understanding of the diversity and potential impact of corallivorous nudibranchs in coral ecosystems.

Key words: Coral health, Corallivory, *Montipora*, *Phestilla*, Predation, Subtropical Reef.

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BACKGROUND

Phestilla Bergh, 1874 is a small group of nudibranchs that feed on scleractinian corals (Mehrota et al. 2020) except *P. chaetopterana*, which lives inside the tube of an annelid (Ekimova et al. 2017). Absence of a cnidosac at the cerata tip is the key character that distinguishes *Phestilla* from other genera of Trinchesiidae (Rudman 1981). Currently there are 11 valid species of *Phestilla* (Mollucabase 2022) including *P. chaetopterana* (Ekimova et al., 2017), *P. fuscostriata* Hu et al., 2020, *P. goniophaga* Hu et al., 2020, *P. lugubris* (Bergh, 1870), *P. melanobrachia* Bergh, 1874, *P. minor* Rudman, 1981, *P. panamica* Rudman, 1982, *P. poritophages* (Rudman, 1979), *P. sibogae* Bergh, 1905, *P. subodiosa* Wang et al., 2020, and *P. viei*, Mehrota et al., 2020. Member of *Phestilla* have been widely found in the tropical and subtropical waters of the Indo-Pacific region (Faucci et al. 2007; Goodheart et al. 2017; Fritts-Penniman et al. 2020). Previous studies have reported the corallivory of *Phestilla* on seven genera of scleractinian corals: *Dendrophyllia*, *Gardineroseris*, *Goniopora*, *Montipora*, *Pavona*, *Porites* and *Tubastraea* (Gosliner et al. 2018). Four *Phestilla* spp. have been recorded in Hong Kong, with *P. lugubris* feeding on *Porites* spp., *P. melanobrachia* feeding on members of Dendrophylliidae*, P. fuscostriata* feeding on *Pavona deccussata,* and *P. goniophaga* feeding on *Goniopora* spp. (Morton and Morton 1983; Hu et al. 2020a b; Yiu et al. 2021). While *P. melanobrachia* and *P. lugubris* have been widely reported from the tropical Pacific (Harris 1968; Faucci et al. 2007), the other two species have been reported from only few localities: *P. goniophaga* from Guam as *Phestilla* sp. 2 (Ritson-Williams et al. 2007 2009), and *P. fuscostriata* from Singapore (Chew 2021) and Indonesia (Ritson-Williams, personal communication).

Previous studies on host specificity (Ritson-Williams et al. 2003 2007 2009) revealed that several *Phestilla* species including *P. melanobrachia*, *P. minor, P. sibogae*, *P. goniophaga* and one undescribed *Porites* eating species (*Phestilla* sp. 1 in Ritson-Williams et al. 2003) favoured a particular coral species or genus. Veliger larvae of these nudibranchs would settle or terminate their swimming phase of the life cycle in response to chemical signals of the particular coral host (Ritson-Williams et al. 2003 2007 2009). After settlement, some larvae also require chemical cues to induce metamorphosis – the physiological and morphological transformation from a larva to a juvenile (Ritson-Williams et al. 2007 2009). Very little is known about the larval development and ecology of *Phestilla*, except for *P. sibogae* (Hadfield 1977; Hadfield and Paul 2001), whose settlement is initiated in response to a water-soluble cue released from the host coral *Porites*

compressa (Hadfield and Scheuer 1985; Hadfield and Pennington 1990); it can detect the inductive cue in the water column and stop swimming and settle on the coral surface (Hadfield and Koehl 2004).

Given the lack of data on corallivorous nudibranchs in Hong Kong's fringing coral communities (Yeung et al. 2021), we proposed a project to study their diversity, host specificity and potential impact to scleractian corals (Hu et al. 2020a b; Yiu et al. 2021). While implementing this project in January 2021, we found a tiny nudibranch species feeding on *Montipora peltiformis* colonies in Bluff Island, Hong Kong. This was the first record of a corallivorus nudibranch associated with *Montipora* in the field. Our specimens look somewhat different in the gross shape and coloration pattern from the original description of *P. subodiosa* – the only currently known nudibranch that feeds on *Montipora* spp. (Wang et al. 2020). *Phestila subodiosa* was described based on the holotype and ten paratypes were collected from *Montipora* fragments brought from an aquarium shop, whereas a paratype was collected from Koh Tao, Thailand. The aims of the present study are therefore to determine the species identity of the *Phestilla* specimens collected from *Montipora* in Hong Kong waters by morphological and molecular analyses, and to provide information on the biology and ecology of this nudibranch, especially on the substrate preference of the adults and their feeding rate on the coral hosts, the larval development pattern, and their selectivity for settlement.

MATERIALS AND METHODS

Sample collection

Samples of *Phestilla* were collected from *Montiopora peltiformis* fragments at Bluff Island (22°19'30.0"N 114°21'14.8"E) at 5 m depth by SCUBA diving in January 2021. The nudibranch samples were preserved in 95% ethanol for molecular and morphological analyses. All specimens examined in this study are deposited in the Marine Biodiversity Collections of the South China Sea (SCSMBC), Chinese Academy of Sciences, Guangzhou.

DNA extraction, sequencing and analysis

Genomic DNA was extracted from two specimens (SCSMBC030983-84) using the CTAB method (Stewart and Via 1993). DNA quantity was measured, and purity determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, US). DNA quality was checked by 1% agarose gel electrophoresis. The products were processed and submitted to Novogene

(Beijing, China) for commercial sequencing on an Illumina Novaseq 6000 platform to produce 10 GB paired-end sequencing data with an average read length of 150 bp.

The sequences were assembled *de novo* using CLC v7. BLAST+ v2.2.26 was used to extract the scaffolds that matched the mitochondrial cytochrome *c* oxidase subunit I (*COI*), mitochondrial 16S rRNA subunit (*16S rRNA*) and nuclear Histone H3 (*H3*) genes from a query file containing the corresponding gene sequences of *P. melanobrachia* downloaded from NCBI's GenBank. The sequences of these three genes in 35 nudibranch species belonging to 12 genera of Trinchesiidae were downloaded from GenBank (Table S4) for determination of the phylogenetic position of the collected specimens. Alignments of the three genes were conducted separately and trimmed manually to 548 bp for *COI*, 435 bp for *16S rRNA* and 327 bp for *H3* using MEGA 7. Sequences were concatenated using SequenceMatrix v.1.7.8 (Vaidya et al. 2011) and then imported to the website version of IQ-Tree (http://iqtree.cibiv.univie.ac.at/; Nguyen et al. 2015) for Maximum Likelihood tree reconstruction with 1,500 ultrafast bootstrap pseudoreplicates (Hoang et al. 2017). ModelTest (Kalyaanamoorthy et al. 2017) incorporated in IQ-Tree was applied for each partition of the concatenated sequences, which detected TVM+F+I+G4 as the best model for *COI* and *16S rRNA* and TIM2e+G4 for *H3* based on Bayesian Information Criterion. MrBayes v.3.2.7a (Ronquist and Huelsenbeck 2003) was used to perform the Bayesian Inference analysis with four Metropolis-coupled Markov Chain Monte Carlo applied to 10 million generations, sampled at every 1,000 generations with a 25% burn-in. Since the model detected for the concatenated dataset by ModelTest was not available in MrBayes, it was substituted by GTR+I+G –the closest over-parameterized model (Huelsenbeck and Rannala 2004). The phylogenetic trees were visualized and edited using FigTree v1.4.4. Pairwise p-distances for the respective *COI*, *16S rRNA* and *H3* genes were estimated using MEGA 7 separately using the bootstrap method with 10,000 pseudoreplicates for variance estimation. Rates among sites were gamma distributed with invariant sites (G+I) and the gamma parameter was set to four.

Morphological analysis

Specimens and their egg masses were photographed using an Olympus OM-D EM1markII with a M. Zuiko Digital ED 60mm f2.8 Macro lens. Morphological characteristics were examined under a Motic SMZ-171 stereomicroscope (Motic, China). Four specimens were dissected to extract the buccal mass and reproductive system. Cerata were also collected from the specimens and the inner contents were examined. The buccal masses were dissolved in 20% diluted bleach for 30 minutes at room temperature to remove connective tissues and muscles. The jaws and the radula were

examined and photographed under a Motic BA210 compound microscope. The reproductive system was observed under the stereomicroscope and line-drawing was made to show the key structures.

Natural history

The egg masses laid on the underside fragments of *M. peltiformis* that were kept in a laboratory at Hong Kong Baptist University were taken and kept in artificial seawater at 30 psu to observe the early development. When the embryos developed into swimming veliger larvae, the transparent membrane enclosing the egg masses were gently broken using forceps to allow the larvae to escape. The larvae, collected using pipettes, were maintained in a 1-L plastic container with ~ 25% of the wall replaced with 50 μm mesh placed in a 10-L glass tank with filtered seawater and aeration. A 12.5 cm^2 fragment of *M. peltiformis* was put in that beaker to encourage larval settlement. The water temperature was kept at 24 ± 0.5°C. The status of the larvae was monitored daily.

Rate of nudibranch consumption of host coral

A colony of *M. peltiformis* without *P. subodiosa* was cut into fragments of 5 cm × 2.5 cm. The coral fragments were kept in aquaria until the wounds healed. Four recovered coral fragments were used to determine the feeding rate of the nudibranch in separate 10 L aerated tanks. Twenty nudibranchs from infested *M. peltiformis* colonies were transferred to the surface of each of the test fragments. Photographs were taken from both sides of the fragment daily for four days. At the end of the experiment, the total consumed area of the fragment was measured using CPCe 4.0, and the feeding rate (*i.e.*, area consumed per day) were calculated.

Tests using non-host corals

Three species of common corals in Hong Kong (*Duncanopsammia peltata, Pavona decussata and Porites lutea*) were used. For each species, a colony was cut into several fragments for standard size (5 cm x 5cm). One nudibranch was placed on the surface of one fragment of each of three coral species in separate 10 L aerated tanks. In each species, there were four replicates.

Observations on larval development and testing of larval settlement preference

Membranes of newly laid egg masses were broken with needles, and the eggs were transferred into a small plastic tank (250 ml) with the wall replaced with 50 μm mesh. The beaker was placed

in a bigger glass tank (10 L) filled with 33 psu artificial seawater and fitted with a recirculated filtration system. The development of the eggs was observed daily until they reached the veliger stage and hatched.

Coral conditioned seawater was used to test for the presence of chemical cues that are attractive for nudibranch larval metamorphosis. The host coral conditioned seawater was prepared by placing a 5 cm^2 fragment of *M. peltiformis* into an aerated beaker containing 1 L artificial water with a salinity of 33 psu and kept at 24°C. After 72 h, the water was filtered through a 0.22 μm membrane and stored in a freezer at -20°C until use. Coral conditioned seawater prepared with a 5 cm^2 fragment of *Porites lutea* the same way as for *M. peltiformis* was used as non-host control. In addition, filtered artificial seawater without prior contact with coral was used as seawater control. The coral conditioned water and control seawater were spiked with antibiotics (90 μg ml^{-1} penicillin G and 75 μg ml^{-1} streptomycin sulfate) to suppress the bacterial growth (Hadfield and Scheuer 1985; Hadfield and Pennington 1990).

Six-well plates (Thermo Scientific™ 145380, USA) were used to test for the presence of coral released cues. Six ml of host or non-host coral conditioned seawater, or control seawater was added into each well. 10 to 15 veliger larvae were then transferred into each well. Each of the three treatments contained three replicate wells. The well plates were incubated at 26°C, and the number of settled individuals was determined by counting the empty shells daily. A previous study indicated that the shell would detach after a *Phestila* veliger larva metamorphose into a juvenile (Hadfield 1977). The metamorphic rate was expressed in percentage. The differences in metamorphic rate among the three treatments at different time point were compared using one-way analysis of variance (ANOVA) followed by the *post hoc* SNK tests.

RESULTS

Molecular Analysis

Three gene sequences were obtained from each of the two specimens. Alignment and concatenation resulted in a dataset of 1419 bp (658 bp for *COI*, 433 bp for *16S rRNA* and 328 bp for *H3*). Our phylogenetic analyses showed that the two specimens we collected from the field are *P. subodiosa* (Fig. 1). The *p* distance among two specimens were identical, and exhibited very small genetic distances with other specimens of *P. subodiosa,* including two collected from *Montipora* spp. in an aquarium (0.4% for *COI*, 0% for *16S* and 0% for *H3*) (Wang et al. 2020), and one from an unknown habitat in Jeju Island, South Korea (1.1% for *COI*, 0.3 % for *16S*) (Cho et al. 2018). The clade that is most closely related to *P. subodiosa* consists of two species – *P. viei and P.*

fuscostriata. The *p* distance between *P. subodiosa* and these two species are much larger: 13.2–17.4% for *COI*, 12.0–12.8% for *16S* and 4.1–4.5% for *H3* (Table S1–S4).

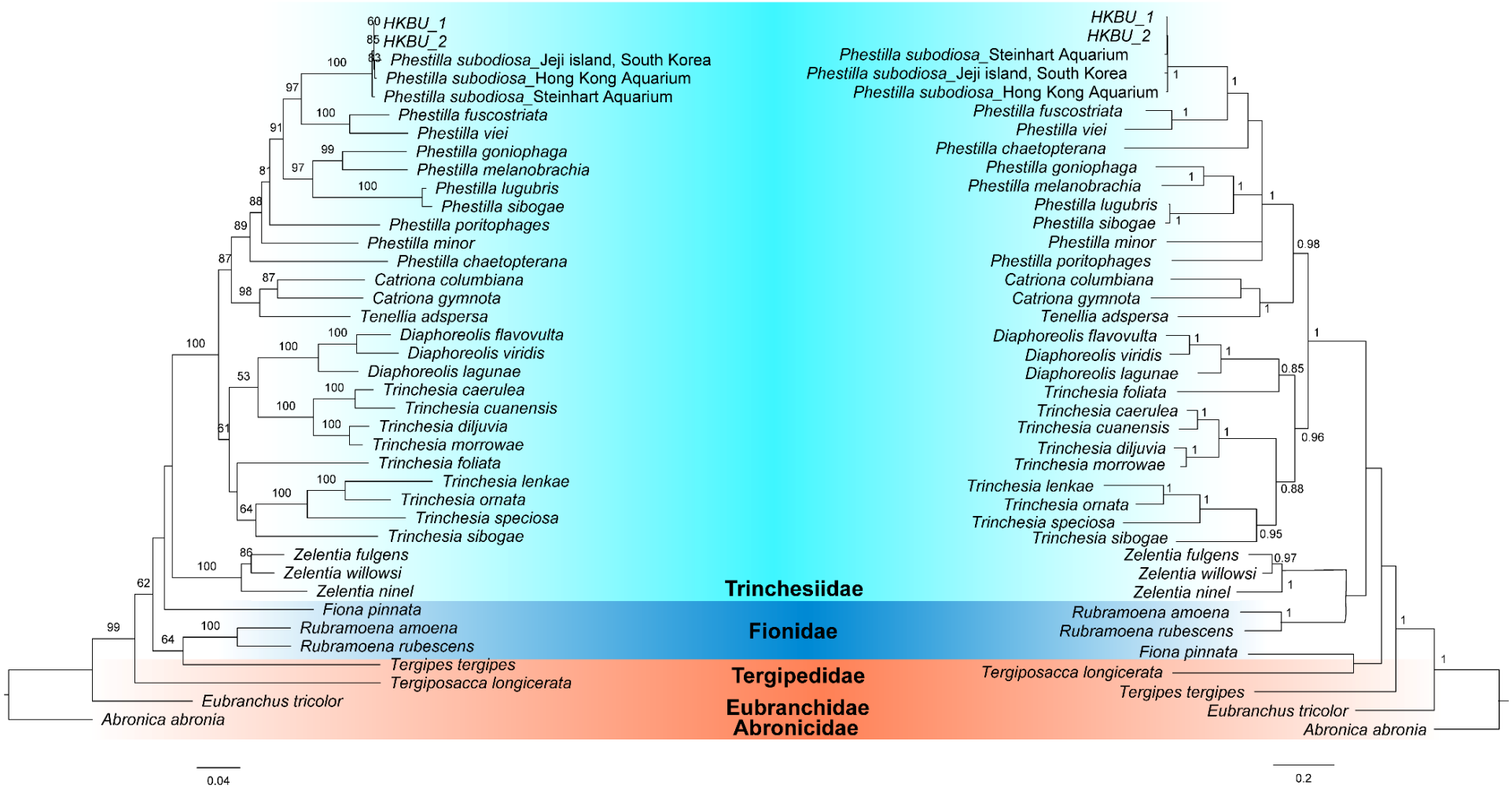

Fig. 1. Phylogenetic trees constructed using concatenated *COI/16S rRNA/H3* sequence. Left: Maximum Likelihood tree with bootstrap values > 50 shown in the node. Right: Bayesian Inference tree with posterior probability values > 0.7 shown in the node.

TANONOMIX ACCOUNT

Class Gastropoda Cuvier, 1795

Order Nudibranchia Cuvier, 1817

Family Trinchesiidae Nordsieck, 1972

***Phestilla subodiosa* Wang, Conti-Jerpe, Richards & Baker, 2020**

(Figs. 2–4)

Materials examined: SCSMBC030983-86, collected from *M. peltiformis* colonies at Bluff Island (22°19'30.0"N, 114°21'14.8"E), Eastern Hong Kong waters, at 2–6 m water depth.

External morphology (Fig. 3): Mature live specimens are elongated, measuring up to 5 mm in length and 0.5 mm in width. Body is white, with patches of rusty pigments inside cerata and the tissue next to cerata. Both rhinophores and oral tentacles are short digitiform. One pair of light black eyes are located slightly posterior to the base of rhinophore. There are 5 to 6 rows of cerata, and each row comprise one to three pairs of cerata, with larger individuals having more pairs. Cerata of bigger individuals have swollen bulbs that are spherical. Cerata tip lacks a cnidosac.

Internal morphology (Fig. 4): Inside the cerata and the body next to the cerata, there are many symbiotic dinoflagellate cells which give the rusty coloration of the nudibranch. Jaws are translucent and thin, around 0.3 mm in width in a 3 mm long individual. Radula are located inside the jaw, with a formula of 8 × 0.1.0. There are three to four primary denticles on each side of radula, and the denticles are of similar lengths. Reproductive system consists of a large female gland mass, a small penial gland, a vas deferens bridging penile gland and female gland, and a tubular ampulla on the opposite side of the penial gland.

Egg mass and veliger larvae: Egg masses are oval shaped, roughly 1 mm × 0.5 mm in size, with a translucent membrane enclosing around 20 eggs. Each egg is light yellow in colour, roughly 0.2 mm in diameter (Fig. 5A). At 24°C, eggs developed into embryos (Fig. 5B) and then veliger larvae (Fig. 5C) in 2 days without feeding. Veliger larvae have a pair of black eyes and a well-developed swimming velum. After 8–10 days, the veliger larvae lost the velum and shell, and metamorphosed into more elongated juveniles that do not yet have tentacle or cerata (Fig. 5D).

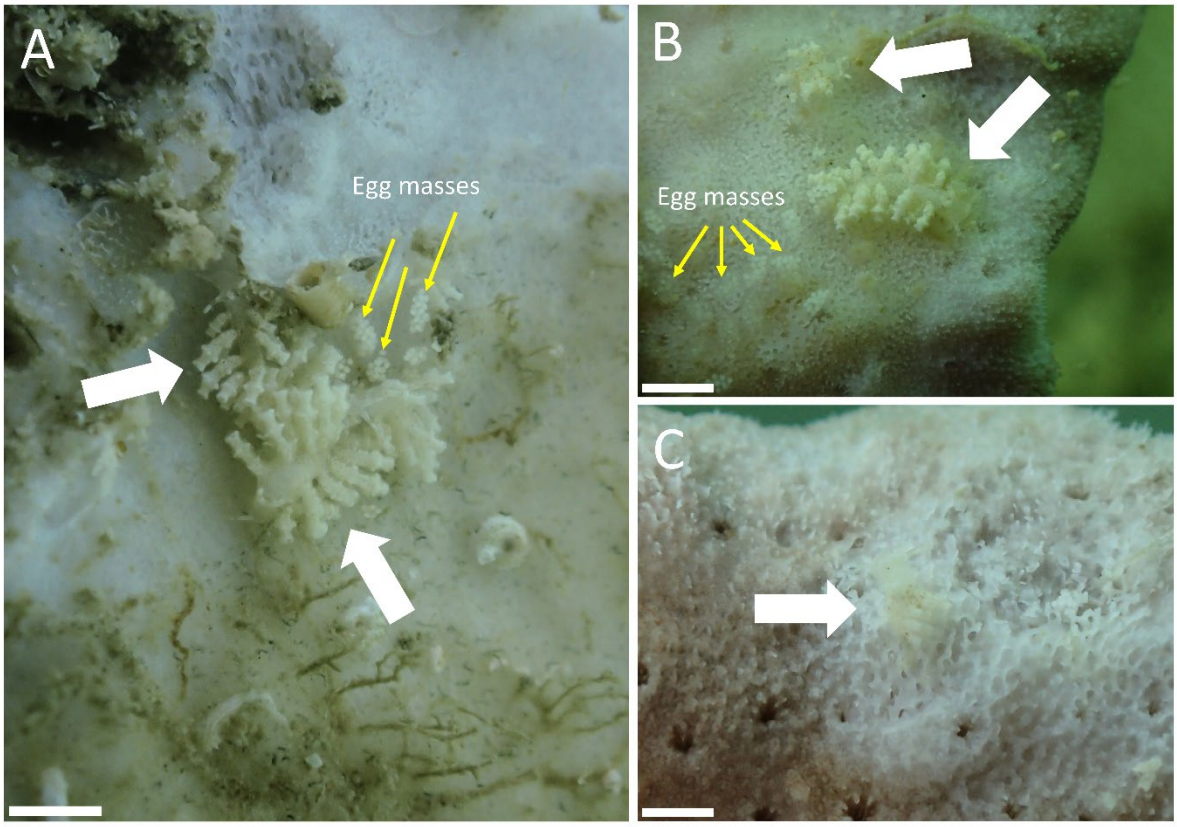

Fig. 2. Selected photographs showing *Phestilla subodiosa* in the field. A, several adult individuals of *Phestilla subodiosa* feeding on a fragment of *Montipora peltiformis*. White arrows indicate the nudibranchs, yellow arrows indicate their egg masses. B, One juvenile and one adult individuals of *Phestilla subodiosa* feeding on a fragment of *Montipora peltiformis*. White arrows indicate the nudibranchs, yellow arrows indicate their egg masses. C, A juvenile individuals of *Phestilla subodiosa* feeding on a fragment of *Montipora peltiformis*. White arrows indicate the nudibranchs. Scale bars: A–C = 1 mm.

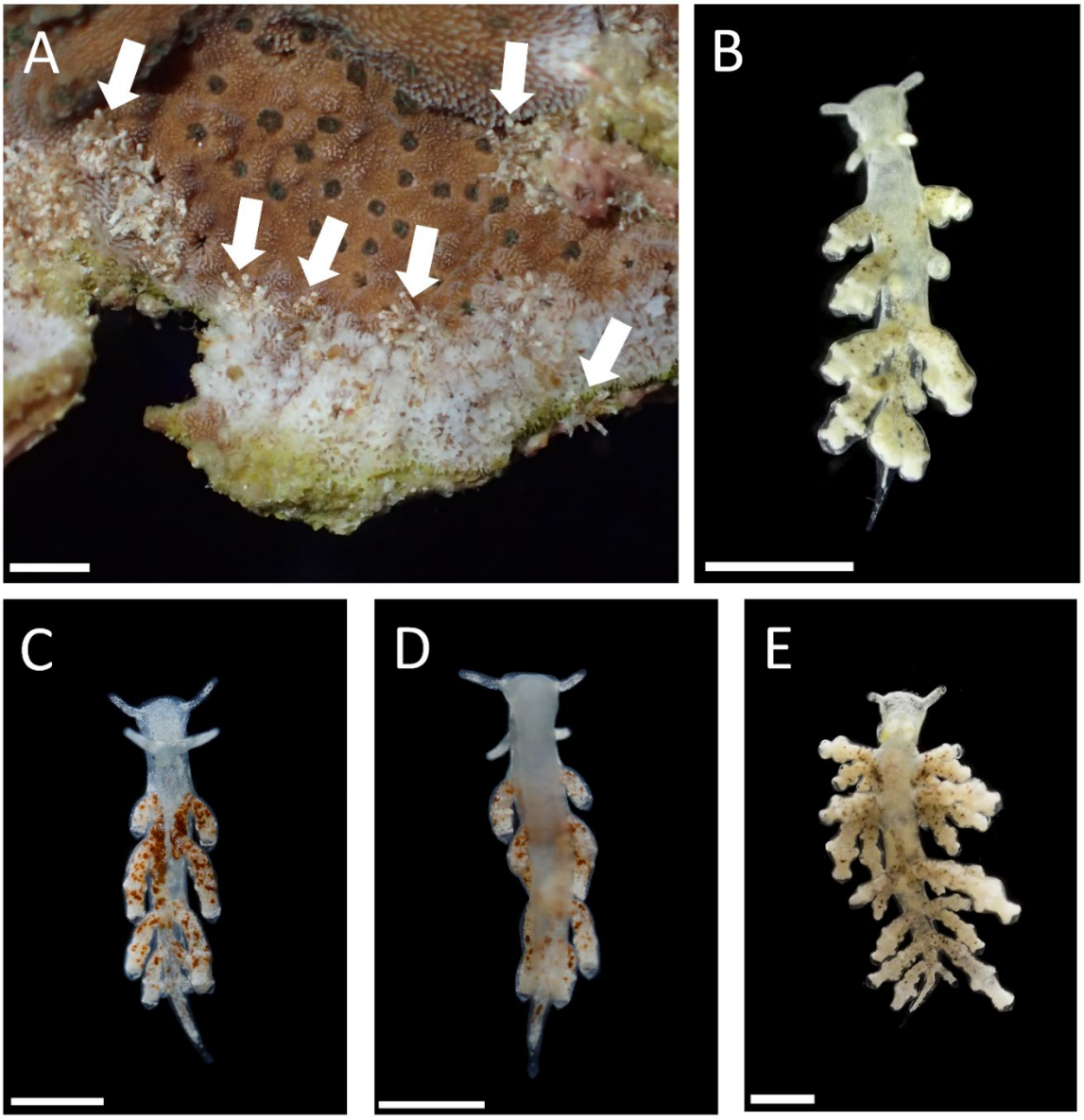

Fig. 3. Selected photographs showing *Phestilla subodiosa*. and its coral host. A, several individuals of *Phestilla subodiosa*. feeding on a fragment of *Montipora peltiformis*, with a clear feeding scar along the lower edge. B, Dorsal view of SCSMBC030984. C–D, Dorsal view and ventral view of SCSMBC030985, respectively; E: Dorsal view of SCSMBC030986. Scale bar: A = 5 mm; B–E = 1 mm.

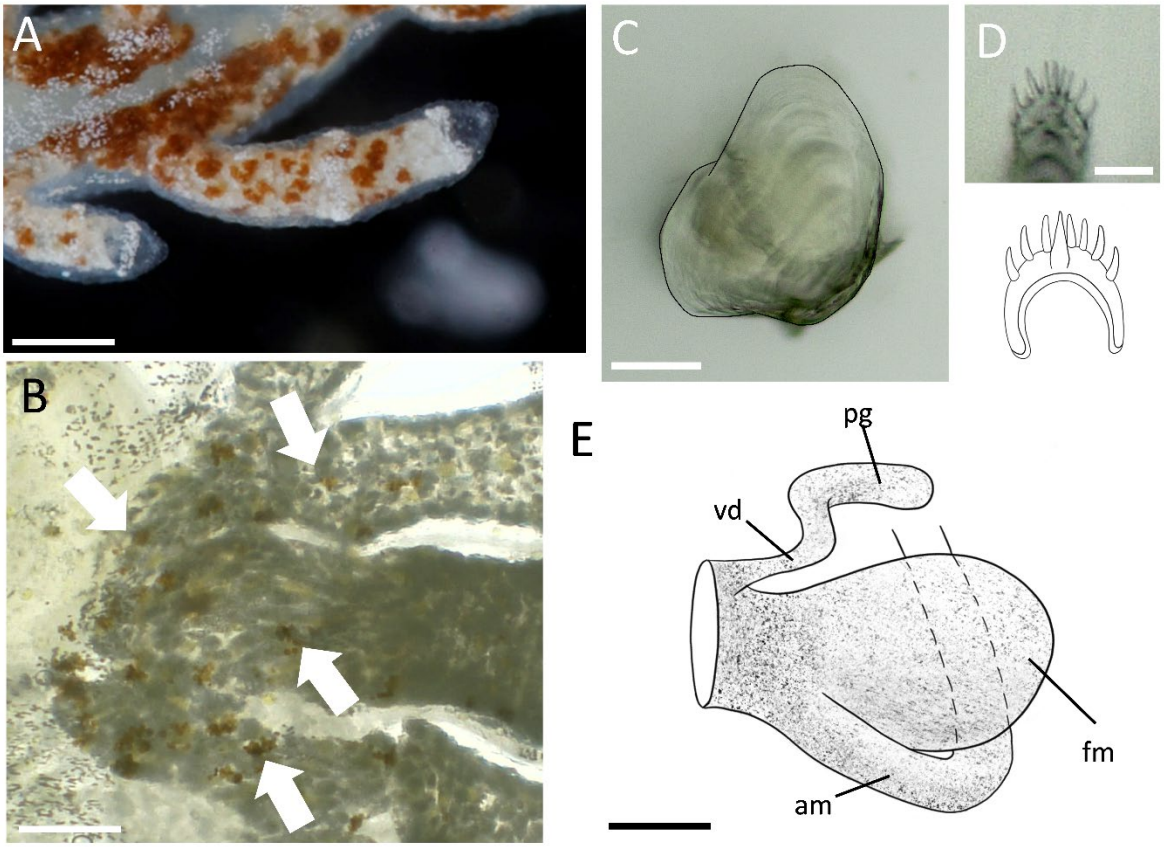

Fig. 4. Internal morphology of *Phestilla subodiosa*. A, Close-up of a section of the dorsum and two cerata; note that there is no cnidosac at the tip. B, dinoflagellates inside the cerata and the body (indicated by white arrows indicated). C, Jaw. D, Radula. E, Drawing of reproductive system; fm: Female gland; am: ampulla; pg: penile gland; vd: vas deferens. Scale bars: A = 500 µm; B = 250 µm; C = 150 µm; D = 25 µm; E = 120 µm.

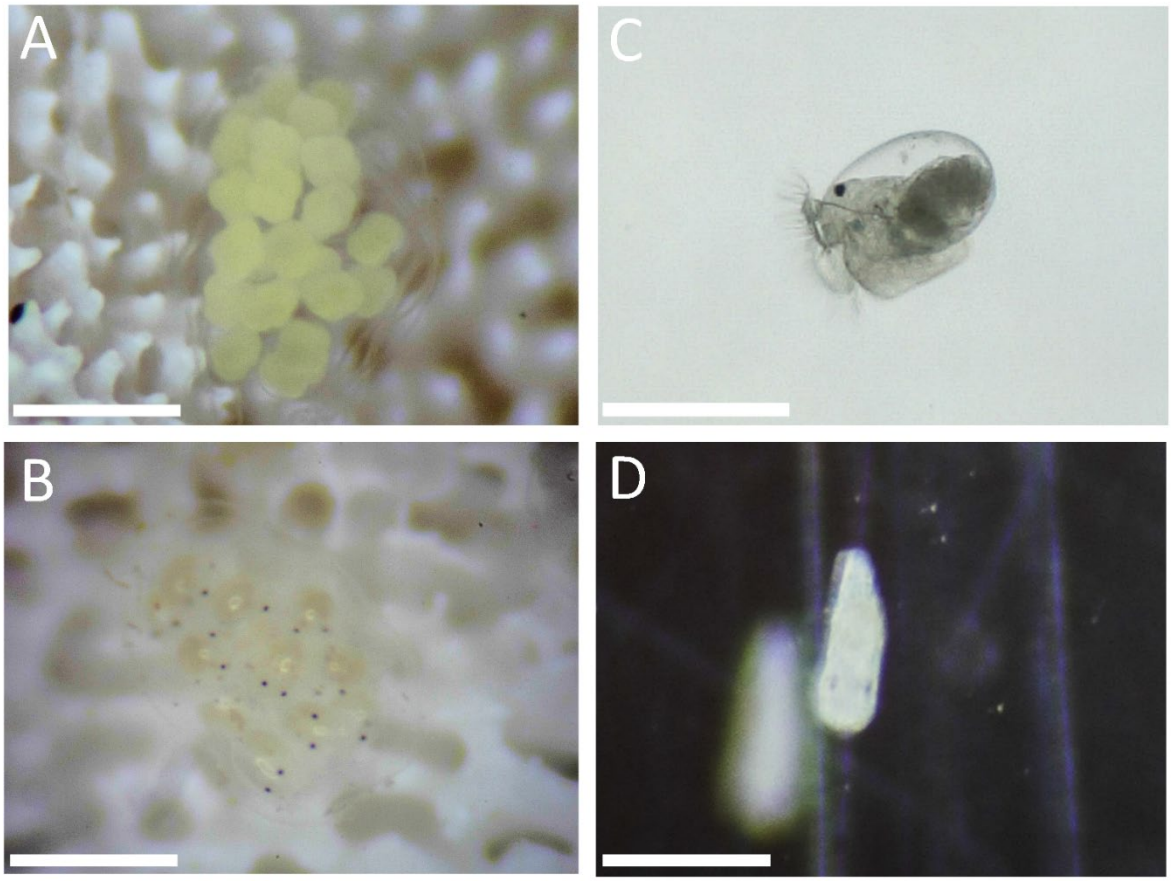

Fig. 5. Early development in *Phestilla subodiosa*. A, Newly laid egg mass attached to denuded coral skeleton. B, Developing embryos in the egg mass. C, A veliger. D, a post-metamorphosis juvenile. Scale bars: A–B = 500 μm; C = 200 μm; D = 300 μm.

Feeding rate on host coral: Twenty individuals of *P. subodiosa* consumed 0.57 ± 0.24 cm^2, 1.48 ± 0.95 cm^2, 2.70 ± 1.48 cm^2 and 3.80 ± 2.03 cm^2 of *M. peltiformis* fragment when measured after 24 h, 48 h, 72 h, and 96 h of exposure, respectively (Fig. 6). The mean feeding rate was 0.95 ± 0.51 cm^2 per day.

Non-host coral test: All nudibranchs transferred to the surfaces of the other three coral species were found dead within one hour, but the method of nudibranch killing appeared to differ. *Pavona deccussta* killed the nudibranch by extruding the mesenterial filaments entangling the prey (Fig. 7A). *Porites lutea* killed the prey using the tentacles outside the body, then slowing digested the prey after it became a slurry (Fig. 7B). *Duncanopsammia peltata* killed the prey after secreting mucus to trap the nudibranch (Fig. 7C, D).

Larval metamorphosis: There were no or very few metamorphosed larvae in the first five days, and the metamorphic rates were not significantly different among the three treatments (Fig. 8, Table S5–6). Starting from day 6, there were significant differences among the three treatments, with the metamorphic rate in the *M. peltiformis* conditioned seawater being

significantly higher than in the other two treatments. By the end of the experiment at day 10, the metamorphic rate in the *M. peltiformis* conditioned seawater reached 31.11%, compared to only 11.11% in the *P. lutea* conditioned seawater, and 6.06% in the control seawater.

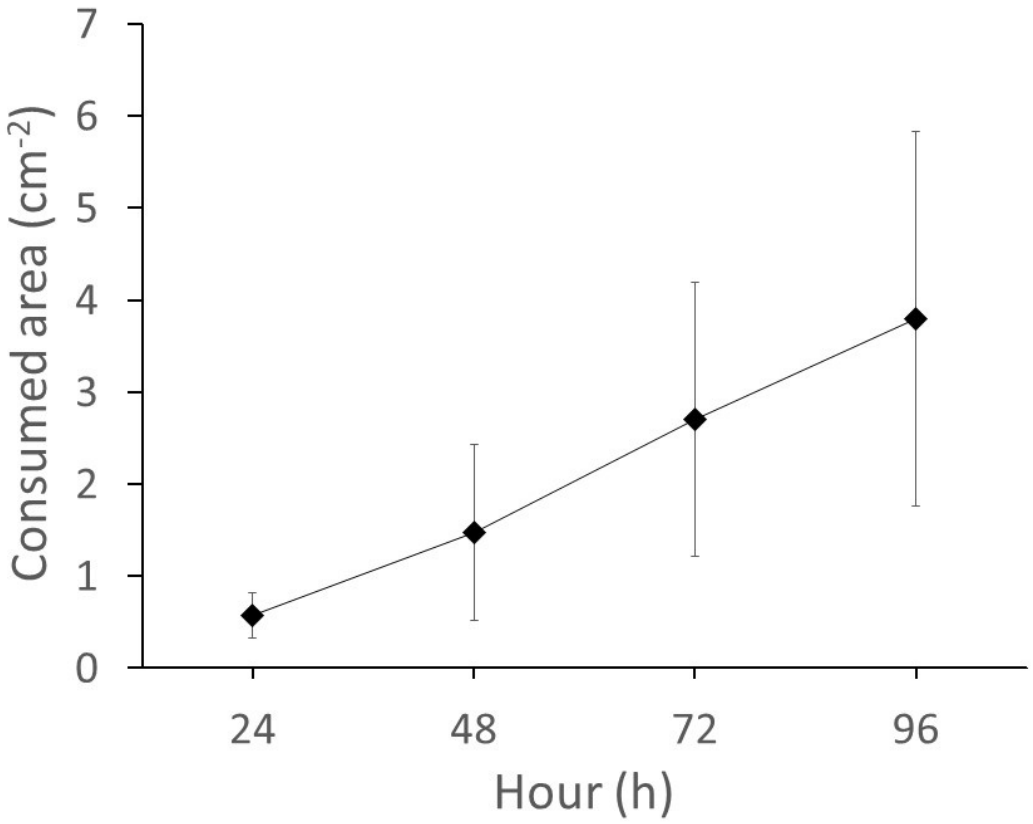

Fig. 6. Feeding rate of *Phestilla subodiosa* on *Montipora peltiformis*. Each datum represents mean ± SD of four replicates.

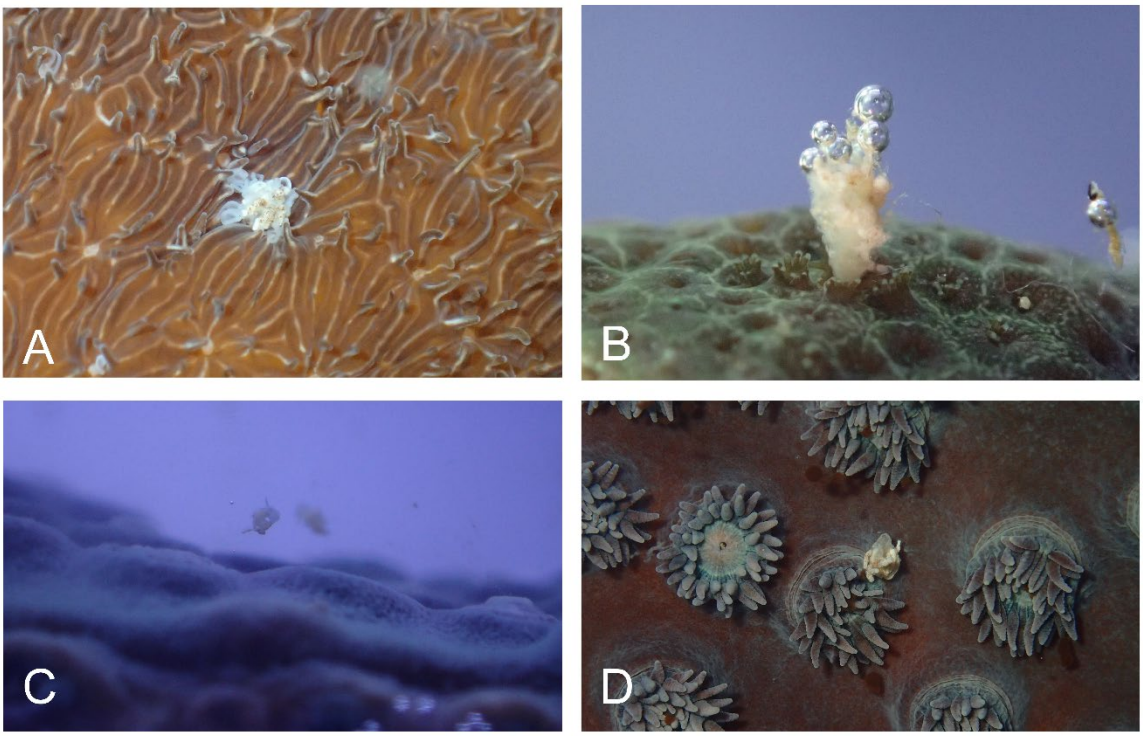

Fig. 7. Photographs showing the individuals of *Phestilla subodiosa* were killed by non-host coral species. A, *Pavona deccussata*. B, *Porites lutea*. C-D: *Duncanopsammia peltata*.

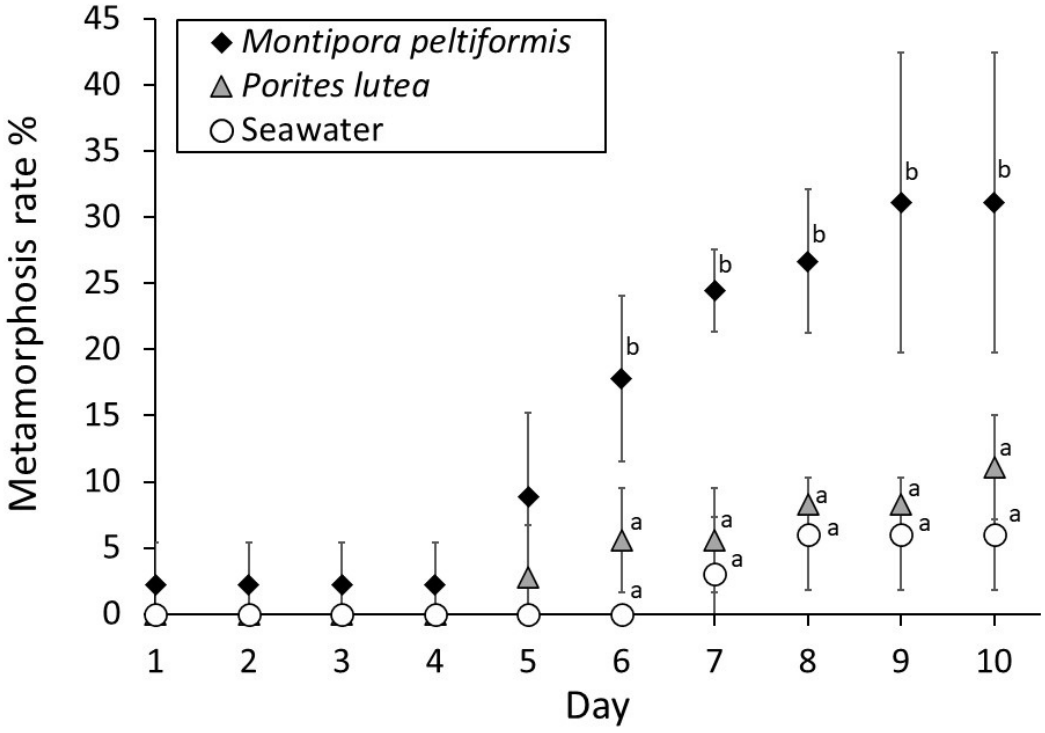

Fig. 8. The metamorphic rate of larvae response to chemical cue released by host and non-host coral species. Three replicates of each treatment were scored for cumulative metamorphosis. Error bars represent ± 1 SD.

DISCUSSION

Based on phylogenetic and morphological analyses, we provide evidence that the *Phestilla* samples we collected from *M. peltiformis* colonies from the field in Hong Kong belong to *P. subiodiosa.* The DNA sequences of our specimens are very closely related to the sequences from the type specimens of *P. subiodiosa,* and such differences should be considered as intraspecific. Another nudibranch collected from Jeju Island, South Korea (Cho et al. 2018) also has very small genetic distances with the types and our samples, which also indicates that they are conspecific. Although *Montipora millepora* has been reported from Jeju (Sugihara et al. 2014), the coral host of this nudibranch remains unknown as this was not reported (Cho et al. 2018).

Morphologically, our specimens are similar with most of the type specimens in being associated with *Montipora* and being small in body size. Nevertheless, the type specimens used from morphological description were collected from *Montipora* spp. from an aquarium whose origin could not be determined (Wang et al. 2020), while our specimens were collected from *M. peltiformis* were collected from the field in Hong Kong. One of the paratypes was collected from Koh Tao, Thailand, but there is no specific morphological description of this specimen in Wang et al. (2020). Our fully grown specimens differed substantially from the described types in that the ceratal bulbs are diamond-shaped and there are brown spots on cerata, ceratal bulbs and the body immediately posterior to cerata. In contrast, the type specimens have spherical swollen ceratal bulbs, and light brownish speckles on cerata only (Wang et al. 2020). Moreover, the radula formula and number of denticles on each side of a tooth row are slightly different between our specimens and the types.

Our feeding experiments provide the first consumption rate of *P. subodiosa* (~ 0.05 cm^2 $individual^{-1}$ day^{-1} = 3.2 polyps $individual^{-1}$ day^{-1}) on *M. peltiformis*. *P. sibogae* which can reach a maximum size of more than 30 mm has been reported to consume up to 6.4 cm^2 *Porites* tissues per day (Haramaty 1991). *Phestilla goniophaga* (former name: *P*. sp. 2), which is similar in size with *P. sibogae*, also has a high consumption rate of up to 30 polyps per day when feeding on *Porites* (Ritson-Williams et al., 2003). *Phestilla* sp. 1, whose maximum length is 5 mm, has a low consumption rate of < 3 Porites polyps $individual^{-1}$ day^{-1} (Ritson-Williams et al. 2003). Therefore, due to its small body size, *P. subodiosa* has a low consumption rate. This results indicates that *P. subodiosa* should not be able cause substantial tissue loss in its host in the field. In fact, our field observation of *M. peltiformis* colonies did

not find obvious wounds caused by nudibranchs, although we found occasional tissue damage of this coral by the corallivorous snails *Drupella* spp. Nevertheless, under laboratory conditions, *P. subodiosa* outbreaks have been observed, causing whole colony damage to *M. peltiformis*, which supports the hypothesis that the populations of such nudibranchs may be controlled by predators in the field (Gochfeld and Aeby 1997). Nevertheless, *P. melanobrachia* and *P. goniophaga* are much bigger than *P. subodiosa* and their feeding scars on their respective coral hosts are often easy to see in Hong Kong waters (Yiu et al. 2021; Hu et al. 2020b), therefore the potential of a corallivorous nudibranch to cause substantial tissue damage to its coral hosts depend largely on its body size. Furthermore, our non-host coral tests indicated that *P. subodiosa* has a high host specificity, and will not be able to survive on non-*Monipora* corals. This result is consistent with that of other studies showing high host specificity of *Phestilla* species (Ritson-Williams et al. 2003; Faucci et al. 2007).

Our larval development experiments indicate that a chemical cue released from host coral *M. peltiformis* is an inducer to the larval metamorphosis of *P. subodiosa.* The larvae take 6 days to reach metamorphic competence. In other species of *Phestilla*, both shorter (3 days in *P. minor* 5 days in *P. sibogae* and *P. goniophaga*) and longer (> 12 days in *P. melanobrachia*) periods to reach the metamorphic competence have been reported (Ritson-Williams et al. 2003). Since the veliger larvae of *Phestilla* develop in the water column, the ability to remain competent to undergo metamorphosis after six days indicates that the *P. subodiosa* can travel with current to places tens of kilometres from its natal locality. Nevertheless, field populations of *P. subodiosa* have only been reported from Thailand (Wang et al. 2020), South Korea (Cho et al. 2018) and Hong Kong, and it is not sure how populations of this species are connected by ocean currents. Based on *COI* sequences showing substantial (*i.e.*, 1.1%) divergence between the Hong Kong and Jeju specimens, we can conclude the there is no contemporary gene flow between these two populations.

CONCLUSIONS

The *Phestilla* of specimens collected from Bluff Island was found to be *P. subodiosa* based on phylogenetic and *p*-distance analyses. It is the second report of this species in the field. Our specimens are similar with the type specimens in size but differ slightly from them in the shape of the cerata and pigmentation pattern. Our laboratory experiment shows that *P. subodiosa* has a high host specificity, which feeds on *M. peltiformis*, and was killed and eaten

by other genera of corals tested. Our larval settlement experiment indicates that a chemical cue released from *M. peltiformis* can trigger the settlement and the metamorphosis of the veliger larvae of the nudibranch. Although feeding by *P. subodiosa* will not likely cause substantial tissue loss in the host under normal field conditions when the nudibranch density is likely controlled by predatory fish, in aquarium without natural predators, its outbreak can cause whole coral colony death. Studies are needed to better understand the distribution of the nudibranch, the genetic connectivity among its populations, and whether the small wounds caused by its feeding will affect coral health by inviting pathogen attack. Furthermore, since larger corallivorous nudibranchs (*i.e.*, *P. melanobrachia* and *P. goniophaga*) are common among Hong Kong's coral communities (Hu et al. 2020b; Yiu et al. 2021), their populations should be monitored, along with other coralliviores such as *Diadema setosum* and *Drupella rugosa* that have been reported to cause colony- to community-level coral destruction (Morton et al. 2002; Lam et al. 2007; Dument et al. 2013; Qiu et al. 2014).

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Authors' contributions: JWQ initiated the study. SKFY conducted sampling, molecular and morphological analysis as well as drafted the manuscript.

Competing interests: SKFY and JWQ declares they have no conflict of interest.

Availability of data and materials: Voucher specimens are deposited in the in the Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, and gene sequences used for phylogenetic analyses are deposited on the GenBank.

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Supplementary Materials

Table S1. Uncorrected *COI p*-distances calculated based on individual gene of 10 described *Phestilla* species. (download)

Table S2. Uncorrected *16S p*-distances calculated based on individual gene of 10 described *Phestilla* species. (download)

Table S3. Uncorrected *H3 p*-distances calculated based on individual gene of 8 described *Phestilla* species. (download)

Table S4. GenBank accession numbers of the sequences used in phylogeny reconstruction and species delimitation retrieved from NCBI. (download)

Table S5. ANOVA results showing the difference of the means between the three treatments each day for the larval settlement experiment. (download)

Table S6. Post hoc Student-Newman-Keuls test results showing the difference of the means between the three treatments each day for the larval settlement experiment. (download)