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A New Species of Predatory Nudibranch (Gastropoda: Trinchesiidae) of the Coral *Pavona decussata*

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Some nudibranchs are predators of scleractinian corals, but little is known about their diversity. Here we describe *Phestilla fuscostriata* sp. nov., the first species of nudibranch that preys on *Pavona decussata*, a structure-forming agariciid species in the South China Sea. This new species has a white body with brown pigmentation on the dorsum and cerata, and exhibits excellent mimicry by matching the colour of its coral host. The nudibranch lays crescent-shaped egg masses on the coral surface, where the embryos develop and hatch in 2–3 weeks. This new species possesses a large number of cerata that are arranged in widely-spaced rows, with each row having one dorsal ceras and zero to several ventral cerata, which distinguishes it from all other congeneric species. A comparison of the mitochondrial *COI* and 16S rRNA genes and the nuclear H3 gene between *P. fuscostriata* sp. nov. and other *Phestilla* spp. found that their interspecific distances are large enough to justify the recognition of the new species.

Key words: Coral health, Coral-eating, Corallivory, *Phestilla*, Molecular phylogeny.

BACKGROUND

The nudibranch superfamily Fionoidea Nordsieck, 1972 is a highly diverse group of marine nudibranchs, containing 20 families, 52 genera and 324 species (MolluscaBase 2020). Like with many other nudibranchs, however, there has been some controversy with the systematics and phylogeny of Fionoidea. Cella et al. (2016) conducted a molecular phylogenetic analysis of several families of nudibranchs including Calmidae, Eubranchidae, Fionidae and Tergipedidae, and proposed that Fionidae include all these previously recognized families of Fionoidea. They recognized 11 genera of Fionidae: *Abronica*, *Calma*, *Cuthona*, *Cuthonella*, *Eubranchus*, *Fiona*, *Murmania*, *Tenellia*, *Tergipes*, *Tergiposacca*, and *Rubramoena*. Moreover, they expanded *Tenellia* to also cover several previously recognized genera including *Phestilla*, *Catriona*, *Trinchesia*, and most species of *Cuthona*. Two recent phylogenetic studies (Ekimova et al. 2017; Fritts-Penniman et al. 2020) supported the radical changes to the phylogenetic framework of Fionoidea proposed by Cella et al. (2016). However, Korshunova et al. (2017) considered morphological and ontogenetic characters to be important in the taxonomy of Fionoidea, and argued that the taxonomic changes proposed by Cella et al. (2016) based solely on molecular phylogeny were inappropriate; Korshunova et al. (2017) therefore restored the families Calmidae, Eubranchidae, Fionidae, and Tergipedidae, and reinstated the families Cuthonidae, Cuthonellidae, and Trinchesiidae (including the genera *Trinchesia*, *Tenellia*, *Phestilla*, *Diaphoreolis*, and *Catriona*). Given the controversy in the taxonomic of Fionoidea, this report of a new species of coral-eating

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nudibranch follows the taxonomy currently adopted by WoRMS (MolluscaBase 2020).

Within the family Trinchesiidae, although the majority of species are free-living, some form an obligate association with the prey species on which they settle, feed and lay eggs (Harris 1975; Faucci et al. 2007; Su et al. 2009; Cella et al. 2016). Among the most well-known examples of such obligate relationship are those between the genus *Phestilla* Bergh, 1874 and certain acroporid, agariciid, poritid and dendrophylliid coral species: the nudibranchs live on these corals and feed on them, and their larvae show host specificity in settlement (Robertson 1970; Harris 1975; Hadfield 1977; Rudman 1979 1981 1982; Miller and Hadfield 1986; Ritson-Williams et al. 2003; Faucci et al. 2007; Fritts-Penniman et al. 2020; Mehrotra et al. 2020; Wang et al. 2020). Unlike other genera of Trinchesiidae, *Phestilla* does not possess a cnidosac at the tip of its cerata, but instead has a glandular region in this location (Rudman 1981; Korshunova et al. 2017).

The genus *Phestilla* currently has nine recognized species (MolluscaBase 2020). Among them, eight are obligate corallivores: *P. lugubris*, *P. minor*, *P. panamica*, *P. sibogae* and *P. poritophages* all feed on *Porties* spp. (Harris 1975). *Phestilla melanobrachia* feeds on *Tubastraea* spp. (Harris 1968; Salvini-Plawen 1972; Harris 1975). *Phestilla subodiosa* feeds on *Montipora* spp., which are common in the aquarium industry (Wang et al. 2020). *Phestilla viei* feeds on *Pavona explanulata* (Mehrotra et al. 2020). In addition to these described coral-eating nudibranchs, an undescribed species of *Phestilla* has been reported to feed on *Goniopora* (Faucci et al. 2007). These previous studies thus have found various species of *Phestilla* to be predators of *Porites*, *Tubastraea*, *Montipora*, *Goniopora* and *Pavona*. Field observation and laboratory studies have revealed the specificity between corals and their nudibranch predators, with a particular species of *Phestilla* feeding on only one species or genus of coral, which implies a host shift associated with speciation (Faucci et al. 2007). Another species, described originally as *Tenellia chaetopterana* and currently recognized by WoRMS as *Phestilla chaetopterana* (MolluscaBase 2020), however, lives inside the tube of the annelid *Chaetopterus* sp. and is not known to be associated with a coral host (Ekimova et al. 2017).

The aims of the present study are thus to describe the morphology of a new species of *Phestilla* that is associated with the coral *Pavona decussata*, determine its phylogenetic relationship with other congeneric species, and provide information on its egg mass deposition and embryonic development.

MATERIALS AND METHODS

Sample collection

Colonies of the scleractinian coral *Pavona decussata* were collected from Sharp Island, Hong Kong (22°21'32.9"N, 114°17'47.8"E, water depth ~2 m) in August 2018 by SCUBA diving and then cultured in the laboratory in an aquarium system. The nudibranchs and their egg masses, found on the surface of the *P. decussata* in October 2018, were collected from the coral surface (Fig. 1). The specimens were preserved in 95% ethanol for molecular study or 4% formaldehyde in seawater for morphological analysis. All specimens examined in this study are deposited in The Swire Institute of Marine Science, The University of Hong Kong (catalogue numbers SWIMS-Mol-19-001 to SWIMS-Mol-19-009).

Morphological analysis

The morphological characteristics of *P. fuscostriata* sp. nov., including adults, juveniles, egg masses and veliger larvae, were studied under a Motic SMZ-171 stereomicroscope. Photographs of these different life stages were taken using a Canon 77D digital camera attached to the stereomicroscope through a phototube. The cerata and digestive gland were examined under a Motic BA210 compound microscope. The buccal masses of selected specimens were extracted and soaked in 25% bleach solution for 20 min at room temperature (~ 24°C) to dissolve the connective and muscle tissues; they were then rinsed in deionized water three times. The radula and jaw were then dried, gold-plated and mounted on a stub for examination under a LEO 1530 FESEM scanning electron microscope (SEM). Selected specimens were also dissected, and the morphology of the reproductive system was drawn under the stereomicroscope.

Molecular analysis

Molecular analysis was conducted according to Wang et al. (2018 2019). Two specimens (SWIMS-Mol-19-008, SWIMS-Mol-19-009) were used for molecular analyses and dissection. For both specimens, genomic DNA was extracted from a piece of the foot tissue using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0. The concentration and purity of the extracted DNA were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and the DNA integrity was checked by electrophoresis using 1.0% agarose gel. Extracted DNA was used as a template to amplify

of the *COI*, 16S rRNA and H3 genes. The following primers were used in the PCR reactions: HCO2198 and LCO1498 (Folmer et al. 1994), 16SarL (Palumbi et al. 1991), 16SR (Puslednik and Serb 2008), H3AF and H3AR (Colgan et al. 1998). The following polymerase chain reaction (PCR) program was used for the *COI* gene: 1 min at 95°C (initial denaturation); 35 cycles of 30 s at 95°C (denaturation), 30 s at 52°C (annealing), and 45 s at 72°C (elongation); and 7 min at 72°C (final extension). The PCR reactions for the 16S and H3 genes were conducted using a similar programme, except that the annealing temperature was 50°C. The PCR products were sent to BGI Hong Kong for sequencing on an ABI 310 Genetic Analyzer. All new sequences were deposited into GenBank (Table S1).

Fragments of *COI*, 16S rRNA and H3 genes from 92 nudibranch species belonging to 31 genera (MolluscaBase 2020) were downloaded from GenBank (Table S1) for analyses to reveal the phylogenetic position of the new species. The species names used in this paper conformed to those adopted in WoRMS (MolluscaBase 2020). *COI*, 16S rRNA and H3 gene alignments were carried out separately using MUSCLE v.3.8.31 (Edgar 2004) under default settings. The aligned sequences in each dataset were then manually trimmed to remove gaps. For each individual, the sequences of *COI*, 16S and H3 were concatenated using SequenceMatrix v.1.7.8 (Vaidya et al. 2011). Phylogenetic analysis was performed using the Maximum Likelihood (ML) method for the concatenated dataset. The ML analysis was conducted via raxmlGUI v.1.3.1 (Silvestro and Michalak 2012) with bootstrap supports estimated for analyses of 1000 pseudoreplicates. The GTR+I+G nucleotide-substitution model was selected using jModelTest v. 2.1.1 (Darriba et al. 2012) as the best model for the phylogenetic analysis, based on Akaike information criteria (AIC). The phylogenetic tree was rendered using FigTree 1.4.0.

RESULTS

Class GASTRAPODA
Order NUDIBRANCHIA
Family Trinchesiidae F. Nordsieck, 1972

Phestilla fuscostriata sp. nov.

(Figs. 1–5)

urn:lsid: zoobank.org:act:3C3AE4E9-414F-4D05-A674-E4580B7F1ED2

Type material: Holotype SWIMS-Mol-19-001, living specimen 8 mm in length; Paratypes SWIMS-Mol-19-002 to SWIMS-Mol-19-007, living specimens

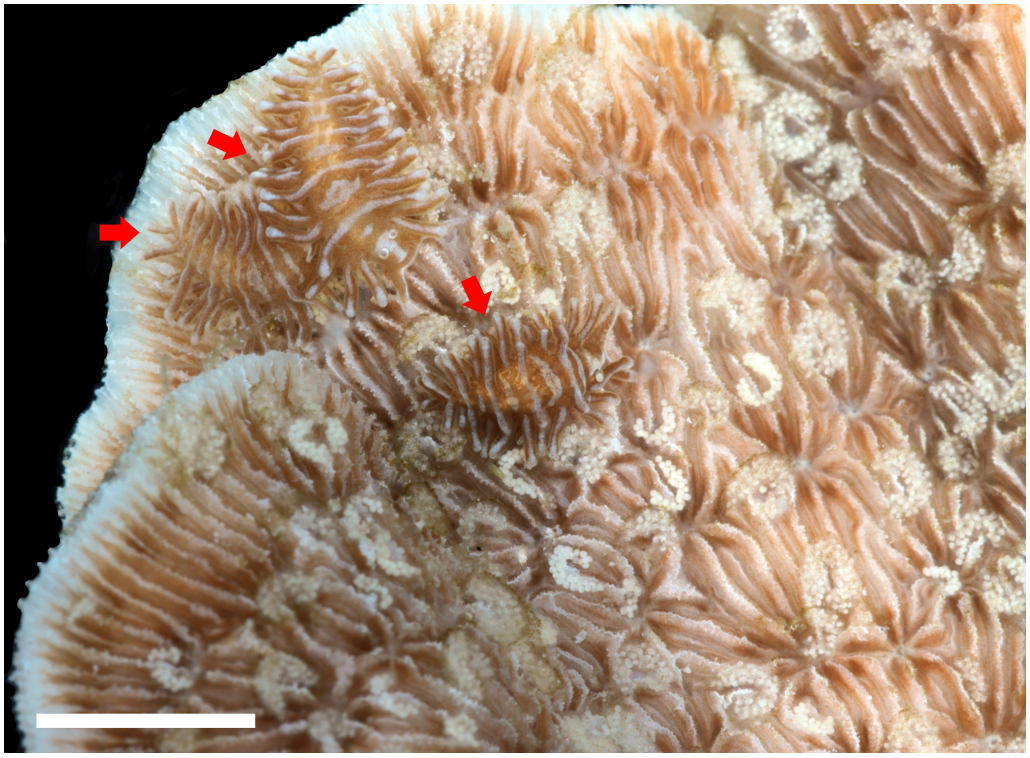

Fig. 1. A colony of the coral *Pavona decussata* showing three adults of the nudibranch *Phestilla fuscostriata* sp. nov. (indicated by red arrows) and many crescent-shaped egg masses of the nudibranch on the coral surface. Scale bar = 1.0 cm.

2–8 mm in length. All type specimens were collected on 26 October 2018 from the surface of *Pavona decussata* colonies cultured in an aquarium at Hong Kong Baptist University. The coral colonies were originally brought back from Sharp Island, Hong Kong on 21 August 2018 by James Xie.

Type locality: Sharp Island, Hong Kong.

Etymology: The species epithet *fuscostriata*, from the Latin fuscus (brown) and striatus (streaky), refers to the brown stripes on the body, which is a morphological character of the new species.

Geographical distribution: This species is currently known from eastern Hong Kong waters including the type locality Sharp Island (this study) and Chek Chau (Wong et al. 2017) in Hong Kong. Given the wide distribution of its host coral *P. decussata* in the Indo-Pacific region (Veron 2000) including Hong Kong (Xie et al. 2017 2020), we expect this species to have a wider distribution, especially along the northern coasts of the South China Sea.

Habitat: Shallow water, 1–4 m water depth, on the surface of *P. decussata* colonies (Fig. 1).

External morphology: Living specimens 2 mm to 8 mm in length (Fig. 2A–F). Body excluding cerata elongate, dorsal-ventrally flattened. General body colour white with dense brown pigmentation on dorsal side of head, tentacles, body and cerata. Ethanol preserved specimens white due to loss of brown pigmentation.

Oral tentacles and rhinophores digitiform; in adults the former approximately twice as the length and twice the diameter of the latter (Fig. 2A–D). A very small eye present behind rhinophore (Fig. 2F). Cerata digitiform, swollen distally, and arranged in seven transverse rows in holotype, each row consisting of 1 to 6 cerata attached laterally on a distinctly raised ridge on each side of the body, with number of cerata decreasing from anterior to posterior. Less rows of cerata and fewer number of cerata per row present in juveniles (Figs. 2E–F, 4F). Within a row, one single pair of dorsal cerata and zero to several pairs of ventral cerata present. In holotype, dorsal cerata seven pairs, ventral cerata six rows with 5, 5, 4, 3, 2 and 1 pair from first row to the sixth row, respectively. Longest cerata on the second row, approximately 1.5 times as long as body width. A translucent glandular region present at the tip of each ceras (Fig. 2A–F). Comparing adult and juvenile specimens (Figs. 2A–F, 4F) indicates that dorsal cerata develop earlier than ventral cerata.

Anus acleioproctic, located dorsally on right side of body between third and fourth rows of cerata. Reproductive opening located anterior to first row of cerata, on right side of body.

Internal morphology: Some internal structures such as white-coloured buccal mass, digestive gland, and oocytes clearly visible through translucent body wall (Figs. 2B, 3A–C). Digestive gland with branches extending to each oral tentacle and ceras throughout body (Figs. 2A, 3A–B). Digestive gland within cerata brown throughout the length, with a translucent outer body wall and apex (Fig. 3B). Colour of digestive gland and cerata reflects that of cells of the dinoflagellate *Cladocpium* sp. (LaJeunesse et al. 2018), presumably ingested from the coral host; many such dinoflagellate cells remain intact and clustered (Fig. 3C). Glandular region contains large glandular cells but lacks nematocysts (Fig. 3B).

A pair of jaws present in buccal mass, with a radula inside. Jaws thin and triangular, with no distinct serrations present along masticatory border (Fig. 3D). Radula formula 18 × 0.1.0. In each row tooth sizes different, with a medium-sized central cusp, three to four smaller secondary denticles on each side of central cusp, and seven to eight large primary denticles with one to two secondary denticles between them (Fig. 3E).

Reproductive system diaulic with both female and male ducts (Fig. 3F). Ovotestis large, consisting of a number of lobules located posterior in coelom (Fig. 2B). Hermaphroditic duct connects to ovotestis on one end and swollen ampulla on the other end. Prostate swollen and elongate. Vas deference narrow and winding. Penis simple, with a small stylet inside penial sac. Bursa copulatrix spherical.

Eggs, egg masses and early juveniles: Eggs white, 0.2 mm in diameter, clearly observable through translucent body wall on ventral side (Fig. 2B, 2D). Eggs masses crescent-shaped, ~0.25 cm in diameter (Fig. 1), with a translucent membrane enclosing around 20–50 eggs (Fig. 4A). At ~ 24°C, eggs would develop into veligers and break through membrane in 2–3 weeks (Fig. 4B and C). Veliger with a pair of black eyes and a well-developed swimming velum (Fig. 4D). Newly settled juveniles more elongate, velum lost, but oral tentacles or cerata not yet developed (Fig. 4E). After roughly one week, juveniles resemble adults, with black eyes, but with tentacle and cerata, although at this stage cerata few and small (Fig. 4F).

Ecology: *Phestilla fuscostriata* sp. nov. resembles its host coral *P. decussata* in the coloration pattern, therefore exhibiting excellent camouflage. We were unaware of its presence in our aquarium system until this nudibranch built up a dense population on *P. decussata*, which eventually killed some of the colonies. The only known food source for the new species is *P. decussata*. When other species of scleractinian corals such as *Platygyra carnosa* and *Acropora digitifera* were also present in the same aquarium, the nudibranch was found only on *P. decussata*, which indicates its host specificity. During reproduction, this nudibranch

deposits egg masses and glues them tightly on the surface of the coral colonies (Fig. 1).

Molecular Analysis

Six new gene sequences were obtained for three genes based on DNA extracted from two *P. fuscostriata* sp. nov. specimens. Removing the low-quality sites at the two ends of the raw sequences resulted in a 671-bp *COI* fragment, a 448-bp 16S rRNA fragment and a 349-bp H3 fragment. The concatenated DNA sequences of the three genes were used for phylogenetic analysis based on the Maximum Likelihood (ML) method (Fig. 5). Exploratory analyses were also conducted for each gene. The topology of the *COI* tree is similar to that of figure 5, although the former has lower bootstrap support values at several nodes (Fig. S1). The 16S rRNA and H3 trees also support the conclusion that *P. fuscostriata* sp. nov. is a distinct species, but compared with the *COI* tree their topology is more dissimilar with that of figure 5.

The two concatenated sequences of the new

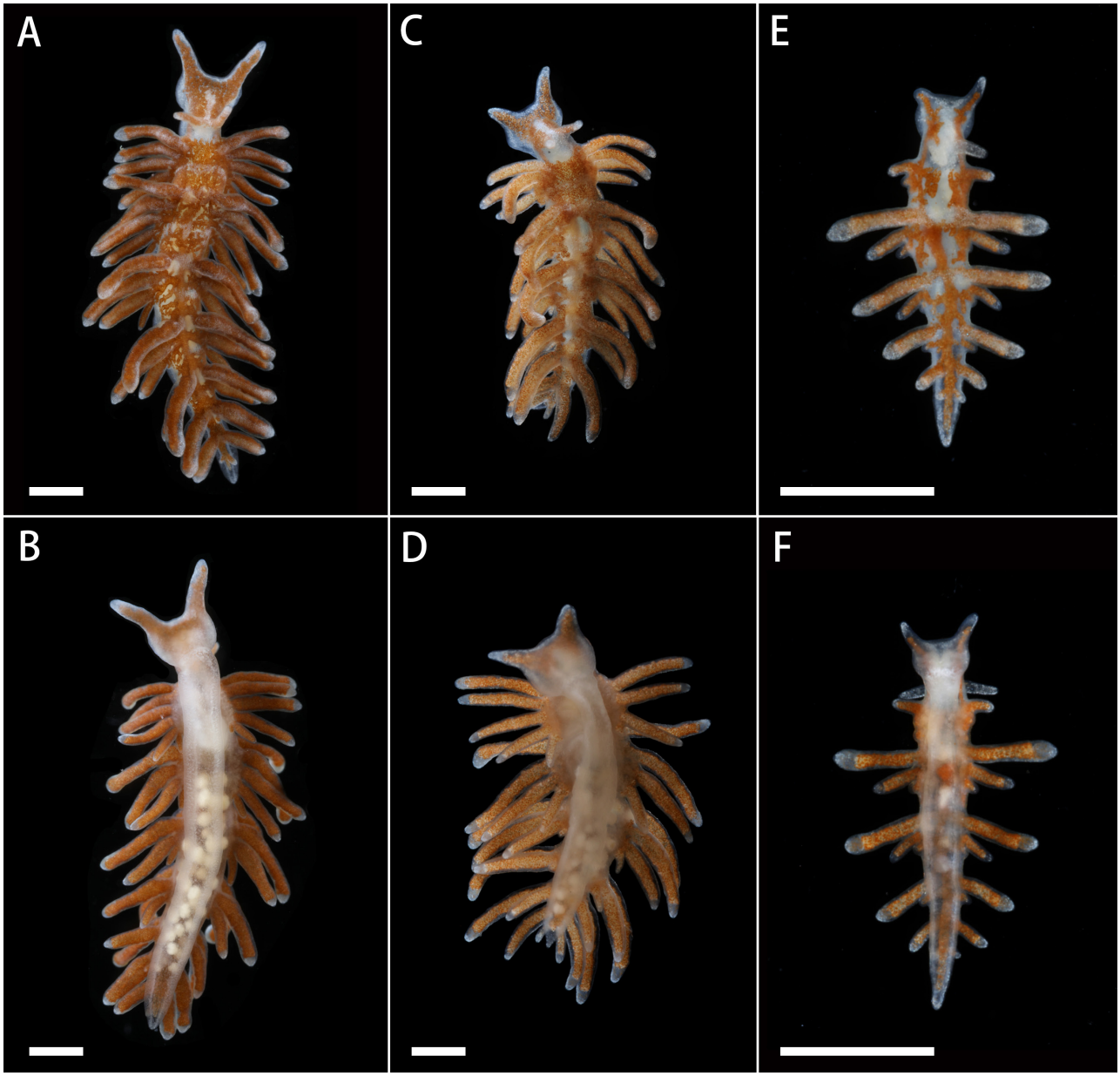

Fig. 2. Living specimens of *Phestilla fuscostriata* sp. nov. A, Holotype, SWIMS-Mol-19-001, dorsal view. B, Holotype, SWIMS-Mol-19-001, ventral view; C, Paratype, SWIMS-Mol-19-002, dorsal view. D, Paratype SWIMS-Mol-19-002, ventral view. E, Paratype, SWIMS-Mol-19-005, dorsal view. F, Paratype, SWIMS-Mol-19-005, ventral view. Scale bars = 1.0 mm

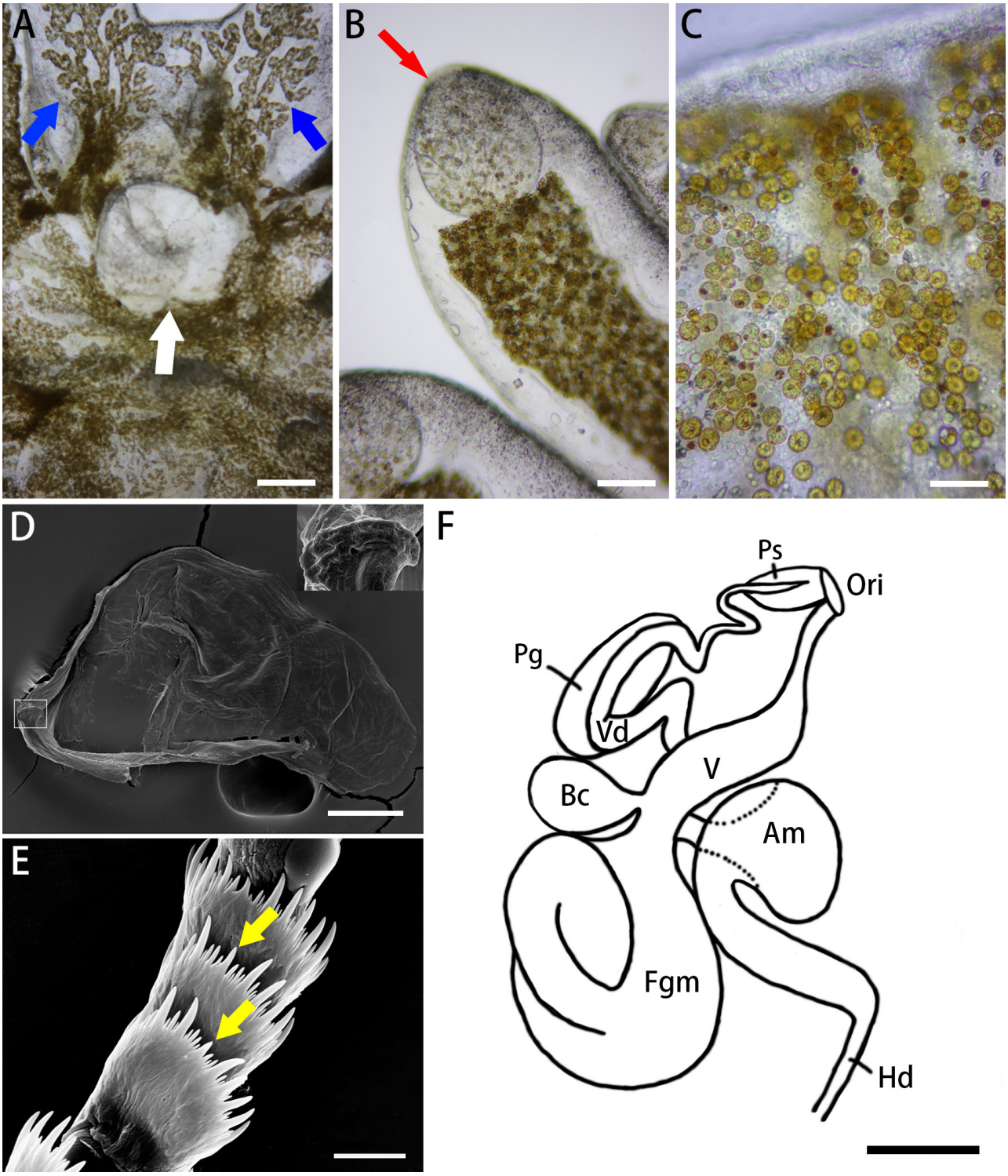

Fig. 3. Internal structures of *Phestilla fuscostriata* sp. nov. A, Ventral view of the head showing the buccal mass (white arrow) and the highly branched digestive gland (blue arrows) extending to the oral tentacles. B, Glandular region in the tip of a ceras (red arrow). C, An enlarged part of ceras showing some intact *Cladocpium* sp. cells. D, A SEM micrograph of the jaw (lateral view) with a section of the masticatory border enlarged in the inset. E, Radula with the central cusps indicated by yellow arrows. F, Reproductive system. Am, ampulla; Bc, bursa copulatrix; Fgm, female gland mass; Hd, hermaphroditic duct; Pg, penial gland; Ori, orifice; Ps, penial sac; Vd, vas deferens. Scale bars: A, C, E = 10 μm; B, D = 100 μm; F = 1.0 mm.

species formed a monophyletic clade and were deeply nested within the clade containing most of the *Phestilla* sequences (Fig. 5), which is in accordance with the results of previous studies (Cella et al. 2016; Ekimova et al. 2017; Mehrotra et al. 2020; Wang et al. 2020). *Phestilla fuscostriata* sp. nov. is sister to *P. viei*, which is associated with another species of *Pavona* (*i.e.*, *P. explanulata*) (Mehrotra et al. 2020). Except for *P. sibogae*, all recognized *Phestilla* species are included in a clade, but the maximum likelihood bootstrap value (62) was not very high. *Phestilla sibogae*, however, is different from all other *Phestilla* spp. in that it is nested in a clade with some *Trinchesia* species, as well as several undescribed species assigned to *Tenellia* by Cella et al. (2016); this topology is consistent with the results of previous studies (Cella et al. 2016; Ekimova et al. 2017; Mehrotra et al. 2020; Wang et al. 2020).

The results of the uncorrected pairwise distances for the three genes showed that the minimum interspecific distances of *COI*, 16S rRNA and H3 were 12.5%, 4.8% and 3.2%, respectively, which are substantially larger than the intraspecific differences between the two sequenced specimens of *P. fuscostriata* sp. nov. (0.5% for *COI*, 1.5% for 16S rRNA and 0.6% for H3 (Table S2). Among these intraspecific distances, the smallest values for the three genes came from *P. viei*, which is consistent with figure 5 in identifying *P. fuscostriata* sp. nov. and *P. viei* as sister species.

DISSCUSION

In this paper, we described the morphology of *Phestilla fuscostriata* sp. nov., provided information on its life history and determined its phylogenetic position within the superfamily Fionoidea based on the sequences of three genes. This species is unique in that it lives on the surface of, feeds on the tissue of and lays eggs on the agariciid coral *Pavona decussata*. It has a white body with a brown pigmentation pattern that exhibits excellent mimicry against the background color of its host. The brown pigmentation comes from the

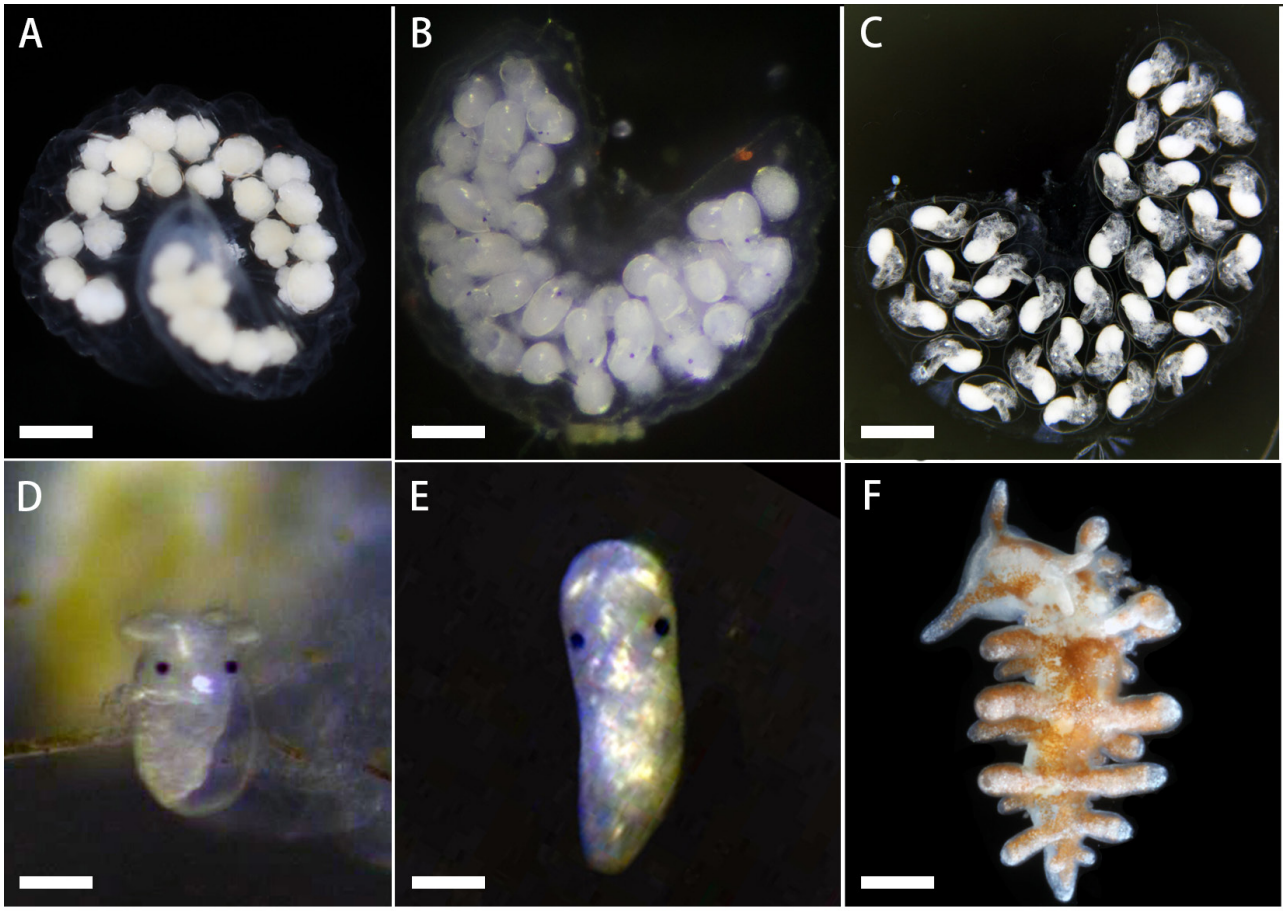

Fig. 4. Early developmental stages of *Phestilla fuscostriata* sp. nov. A, Embryos inside egg membrane. B, Rudimentary veligers without well-formed shells inside egg membrane. C, Veligers with well-formed shells. D, Hatched veliger with well-developed velum for swimming. E, Postlarva, dorsal view. F, Early juvenile, with the head turned to the left when the photograph was taken. Scale bars: A–C, F = 500 µm; D–E = 40 µm.

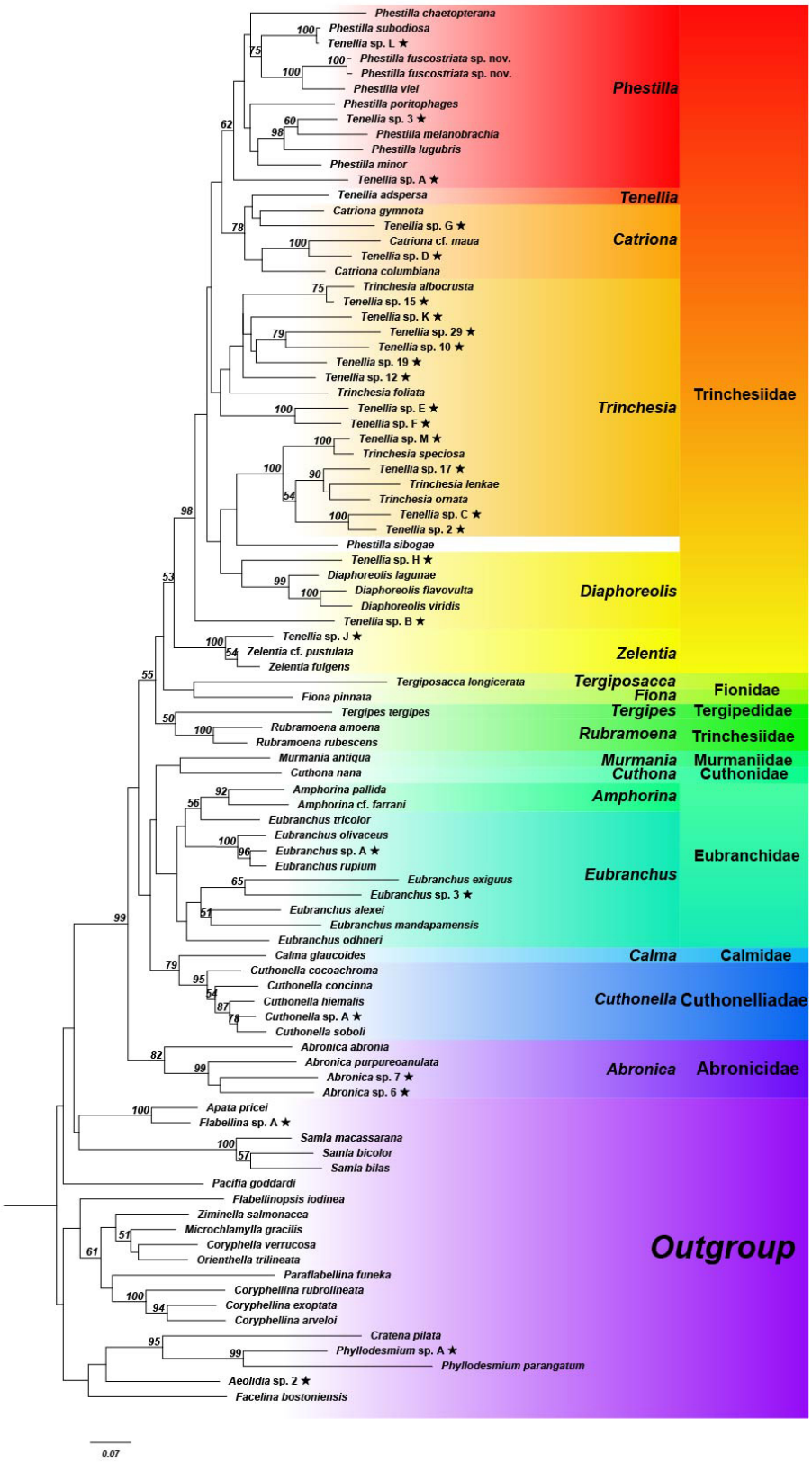

Fig. 5. Maximum likelihood (ML) tree constructed based on the concatenated sequences of *COI*, 16S rRNA and H3 genes. Numbers on the branches represent ML bootstrap values (maximum 100) based on 1000 replicates. Only ML bootstrap values > 50 are shown. All the *Tenellia* spp. with black asterisks are those considered to belong to *Tenellia*, but was not formally described by Cella et al. (2016).

color of the *Cladocpium* sp. cells they ingested, which belong to clade C1, based on our analysis of the ITS2 gene of the symbiotic algae associated with *P. decussata* collected from the same location (Zhang et al. 2019).

Morphologically, *P. fuscostriata* sp. nov. can be distinguished from other congeneric species by several unique features. First, its cerata are arranged in well separated rows along the body, unlike most species of *Phestilla* whose rows of cerata are close together. Several species of *Tenellia*, including *Tenellia adspersa* (Thompson and Brown 1984; Evertsen et al. 2004), *Tenellia fuscata* (Harris et al. 1980) and *Tenellia ivetteae* (Gosliner and Bertsch 2017), also have well separated rows of cerata. However, this is considered a paedomorphic feature retained in the adults of different clades of nudibranchs (Korshunova et al. 2017). Second, the radula of *P. fuscostriata* sp. nov. is also unique among the reported *Phestilla* spp. in that it has three to four secondary denticles on the left and right side of the central cusp that is smaller than the primary denticles. In a few other species of *Phestilla*, such as *P. lugubris*, *P. poritophages*, *P. subodiosa* and *P. melanobrachia*, the central cusp is larger than other denticles (Rudmen 1979 1981; Cella et al. 2016). Although in *P. chaetopterana* the central cusp is smaller than the primary denticles, there are no secondary denticles between them. In *P. viei*, there is no central cusp in the radula (Mehrotra et al. 2020). Third, the egg masses of *P. fuscostriata* sp. nov. are special in that they are crescent-shaped, each with fewer eggs (20–50) inside. In a few other species of *Phestilla*, such as *P. chaetopterana*, *P. lugubris* and *P. poritophages*, the egg masses are long sausage-like, often forming spiral coils with large numbers of eggs inside (Rudmen 1979 1981; Ekimova et al. 2017). However, in *P. viei* the egg masses are also crescent-shaped and appear longer (Fig. 1 in Mehrotra et al. 2020), and each egg mass contains more eggs (80–120) (Mehrotra et al. 2020).

Our molecular analysis indicates that *P. fuscostriata* sp. nov. is deeply nested within a clade consisting of all valid *Phestilla* species with the relevant DNA sequences, except *P. sibogae*. This result, together with a key morphological similarity to the species of *Phestilla* (*i.e.*, replacement of cnidosac with a glandular region), supports the placement of *P. fuscostriata* sp. nov. in this genus. The *COI* sequence divergence between *P. fuscostriata* sp. nov. and its most closely related species, *P. viei* (*i.e.*, 12.5%), are larger than the intraspecific sequence differences in most molluscan species (*i.e.*, < 2%, Layton et al. 2014), which justifies the recognition of the new species. *Phestilla sibogae*, however, is nested within a clade with some species of *Trinchesia*, which agrees with Cella et al. (2016) and Ekimova et al. (2017), and indicates the need to transfer this species to *Trinchesia* to make the genus monophyletic.

Our phylogenetic analysis (Fig. 5) shows that the coral-eating species form two clades: one includes only one species (*P. sibogae*) and another includes all other species (*P. subodiosa*, *P. fuscostriata* sp. nov., *P. viei*, *P. poritophages*, *P. melanobrachia*, *P. lugubris* and *P. minor*, together with *Tenellia* sp. 3 and *Tenellia* sp. A, which have not been formally described). This result indicates that corallivory in fionid nudibranchs has evolved at least twice, but on one occasion there was substantial speciation and host shift, which is consistent with the results of a previous study (Faucci et al. 2007). However, despite the potential benefit of its obligate nutritional association with corals, it is puzzling that, within this second clade, *P. chaetopterana* appears to have lost this association, and now lives inside the tube of the annelid *Chaetopterus* sp. (Ekimova et al. 2017).

CONCLUSIONS

We report the nudibranch *Phestilla fuscostriata* sp. nov.—a predator of the coral *Pavona decussata*. Morphologically, this new species has a white body with brown pigmentation on the dorsum and cerata, which exhibits excellent mimicry to the color of its coral host. This new species possesses a large number of cerata that are arranged in widely-spaced rows with each row having one dorsal ceras and zero to several ventral cerata, which distinguishes it from all other congeneric species. The adults lay crescent-shaped egg masses on the coral surface. The embryos develop and hatch in 2–3 weeks. Analyses of the mitochondrial *COI* and 16S rRNA genes and the nuclear H3 gene sequences showed that *P. fuscostriata* sp. nov. is distinct from all other species of *Phestilla*. Its most closely related species is *P. viei*—a predator of the coral *Pavona explanulata* from the Gulf of Thailand.

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Authors' contributions: JWQ initiated the study. JH conducted the morphological analysis and drafted

the manuscript, JH and YZ conducted the molecular analysis, YJX discovered the nudibranchs. All authors revised the manuscript.

Competing interests: JH, YZ, YJX and JWQ declare that they have no conflict of interests.

Availability of data and materials: Six DNA sequences of *Phestilla fuscostriata* sp. nov.—two from the *COI* gene, two from the 16S rRNA gene and two from the H3 gene—are deposited in GenBank (accession numbers in Supplementary Material Table S1). The type specimens are deposited in Swire Institute of Marine Science, The University of Hong Kong (catalog numbers SWIMS-Mol-19-001 to SWIMS-Mol-19-009).

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Supplementary materials

Fig S1. Individual genes trees showing the phylogenetic relationships in Fionidae: *COI* (A), 16S rRNA (B), 3H (C). (download)

Table S1. GenBank accession numbers of the sequences used to reconstruct the phylogeny of *Phestilla* spp. and their related genera/species. (download)

Table S2. Uncorrected *COI*/16S rRNA/H3 *p*-distances (%) among all species of described *Phestilla* with available sequences. (download)