

Article

Being Safe, but Not Too Safe: A Nudibranch Feeding on a Bryozoan-Associated Hydrozoan

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Abstract: Nudibranchs have a mostly carnivorous diet, and they prey on a wide variety of other animal taxa. Many species, mainly belonging to the Cladobranchia suborder, feed on cnidarians, including member of the class Hydrozoa. Several hydrozoan species display a symbiotic lifestyle, being associated with other benthic invertebrates, including for instance bryozoans, corals, octocorals, and sponges. In our knowledge, no record of nudibranch predation on symbiotic hydrozoans has been reported so far, possibly thanks to the protective action by the host towards its symbiotic hydrozoan. Here, we show the unexpected case of a nudibranch belonging to the recently described species *Sakuraeolis marhe* (Fernández-Simón and Moles, 2023) feeding on *Zanclaea* sp. 2, a hydrozoan associated with the cheilostome bryozoan *Celleporaria* sp. This trophic association is confirmed by the presence and storage of the nematocysts into the nudibranch cnidosacs. Moreover, the nudibranch appears to selectively store mostly a single type of nematocyst, that is large size stenotele. The observation here reported represents the first well-documented record of a nudibranch feeding on a symbiotic hydrozoan and the first confirmed case of predation on *Zanclaea* polyps. Moreover, we provide additional genetic information and the first description of the internal anatomy of *S. marhe*.

Keywords: *Sakuraeolis*; *Zanclaea*; *Celleporaria*; kleptocnidae; nematocysts; predation; symbiosis



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1. Introduction

Nudibranchs have a highly diversified, mostly carnivorous diet, preying on a wide range of animal taxa, including ascidians, bryozoans, cnidarians, crustaceans, echinoderms, entoprocts, fishes, other mollusks, polychaetes, and sponges [1,2]. However, most of the species are stenophagous and show a highly specialized diet, being able to feed exclusively on a single or a reduced number of benthic species [3–6]. Recently, a strong phylogenetic correlation with prey type has been demonstrated for one of the two nudibranch suborders, namely the Cladobranchia, revealing that most of the larger clades in the group feed on specific organisms [7]. In the Cladobranchia suborder, cnidarians are the most common prey items, especially those belonging to the classes Hydrozoa and Anthozoa [8], and the most recent common ancestor of the group has also been hypothesized to prey on them (and specifically on hydrozoans), with only a few subsequent dietary shifts [7]. Among cnidarian-eating cladobranchs, some taxa show a peculiar strategy, that is the sequestration of unfired nematocysts from their prey, made possible by multiple adaptations including behavior, cuticular epithelia, intracellular cuticular structures, and mucus secretions. Sequestered nematocysts, called kleptocnidae, are stored in specific structures in the upper portion of the cerata, the cnidosacs, where they remain functional [9]. There, they may play different

roles, including attacking prey items during feeding activities, or they may be stored to prevent damage to the nudibranch itself, even though different studies have shown that the kleptocnidae seem to be most effective for defensive purposes [9]. Some species also appear to selectively store specific types of nematocysts among those available from the prey [10], possibly those that are most effective against predation [11].

Hydrozoan-feeding nudibranchs prey on a variety of species, mostly with a large or erect colony, belonging to the Leptothecata and Anthoathecata orders, and they may be both generalist and specialist in their preferred preys [8,12,13]. Nudibranchs generally feed on the benthic colonial polyp stage, even though some species are known to feed on planktonic polyp colonies belonging to the family Porpitidae Goldfuss, 1818 and the order Siphonophorae [13,14], or even on the medusa stage, such as the curious case of *Phylliroe bucephala* Lamarck, 1816 parasitizing and preying the medusa of *Zanclaea costata* Gegenbaur, 1857 [15]. Recently, a peculiar trophic interaction between a nudibranch and a hydroid, termed kleptopredation, has also been characterized, where *Cratena peregrina* (Gmelin, 1791) selectively feeds on polyps of *Eudendrium racemosum* (Cavolini, 1785) that have recently captured planktonic preys, thus increasing the energy content of its meal [16].

Hydrozoan polyps are generally considered as substrate generalists, but several cases of symbioses with other benthic organisms are known [17], especially in the anthoathecates, with the hydrozoan colonies using the host as a substrate. An example is represented by the capitate family Zancleidae Russel, 1953, whose members associate with multiple organisms, including algae, bivalves, bryozoans, and corals [18–20]. Some of these associations have been characterized as mutualistic, with the hydrozoan polyps enhancing the competitive abilities of the host [21,22] and providing protection against predators, including platyhelminthes, nudibranchs, and other gastropods [21,23]. Interestingly, no report of nudibranch predation on these symbiotic hydrozoans has been reported so far. The only record of a nudibranch feeding on zancleid polyps involves the sea slug *Pteraeolidia ianthina* (Angas, 1864) and an undetermined *Zanclaea* species [8], but, except the names of the two organisms involved, no additional information was reported by the authors, including for instance whether the *Zanclaea* polyps were associated to other organisms or not. Rao and Alagarswami [24] also hypothesized that the nudibranch *Anteaeolidiella orientalis* (Bergh, 1888) may feed on *Zanclaea* polyps, but they did not observe the interaction and based their assumption solely on the co-occurrence of the two organisms in the same aquarium, leaving this trophic interaction doubtful.

Recently, Cunha et al. [25] performed an extensive photographic and DNA barcoding survey of gastropods in the Central Maldives, and described the species *Sakuraeolis marhe* Fernández-Simón and Moles, 2023, a species currently known only from the Maldives and the Philippines, reporting that it was observed living and feeding on top of encrusting bryozoans associated with hydrozoans. This species belongs to the polyphyletic family Facelinidae Bergh, 1889, and to the genus *Sakuraeolis* Baba, 1965, which currently includes nine species, all of them distributed along the Indian and the west Pacific Ocean. *Sakuraeolis* species are known to feed on a variety of hydrozoan taxa, including the genera *Ectopleura* Agassiz, 1862, *Eudendrium* Ehrenberg, 1834, *Pennaria* Goldfuss, 1820, *Rhizophysa* Péron and Lesueur, 1807, and *Solanderia* Duchassaing and Michelin, 1846 [26–29], and on scyphozoan polyps of the genus *Aurelia* Lamarck, 1816 [30]. *Sakuraeolis marhe* could therefore represent the first clear case of nudibranch predation on symbiotic hydrozoans or bryozoans.

With this work, we provide new insights in both the recently described species *S. marhe* and its trophic interaction with the bryozoan and hydrozoan. Specifically, we provide a detailed description of the association between *S. marhe* and the bryozoan–hydrozoan symbiotic system, and we contribute additional genetic data of the nudibranch, including 16S rRNA and H3 sequences, assessing its phylogenetic position, we analyze its internal anatomy (buccal apparatus and reproductive system), complementing the original description of the species, and we show its ability to selectively store specific nematocyst types as kleptocnidae in the cnidosacs.

2. Materials and Methods

During surveys of the shallow reefs around Faafu Magoodhoo Island, Republic of the Maldives (3.087 N, 72.956 E), performed via SCUBA diving in March 2018, two nudibranchs were observed on a hydrozoan-associated bryozoan colony. A fragment of the bryozoan colony, together with associated hydrozoans and preying nudibranchs, was collected and brought to the facilities of the Marine Research and High Education (MaRHE) Center in Faafu Magoodhoo for further analyses. The interaction among the three groups of living organisms was observed under a Leica EZ4 D stereo microscope (Leica, Wetzlar, Germany) equipped with a Canon PowerShot G7 X Mark II camera (Canon, Tokyo, Japan). Subsequently, the organisms were anesthetized with menthol crystals and fixed in 10% formalin and 99% ethanol for morphological and molecular analyses, respectively. Fixed organisms were identified morphologically (all organisms) and genetically (hydrozoans and nudibranchs) by investigating the anatomical diagnostic characteristics, and by sequencing portions of informative DNA regions, namely the 16S rRNA and the H3 genes. An anatomical dissection of the nudibranch was carried out under a Nikon SMZ800N stereo microscope (Nikon, Tokyo, Japan) equipped with a Nikon Digital Sight 1000 camera (Nikon). The buccal apparatus was extracted using proteinase K solution (20 mg/mL) [31] for the digestion of the soft tissues and the observation of chitinous structures important for species identification, such as the radula and jaws. The reproductive system was also investigated, and a final schematic and descriptive drawing was reported. The bryozoan and associated hydrozoan species were studied under a Leica EZ4 D stereo microscope to identify them to the lowest level possible. Finally, hydrozoans and nudibranch cerata were analyzed under a Zeiss Axioskop 40 transmitted light compound microscope (Zeiss, Oberkochen, Germany) equipped with a Canon PowerShot G7 X Mark II camera (Canon) at a magnification of 40 \times and 100 \times , to identify and measure the nematocyst types. Nematocysts were measured using ImageJ 1.52p software [32], and were identified according to Östman [33].

DNA was extracted from one nudibranch individual using the QIAGEN DNeasy Blood & Tissue Kit, following the manufacturer's protocols, and from one hydrozoan polyp following the protocol described in Maggioni et al. [20], that is digesting the specimen in proteinase K and MilliQ water for 30 min at 50 °C, and for 10 min at 90 °C. A portion of the 16S rRNA was amplified for the nudibranch and hydrozoan, using the primer pair 16AR and 16BR described in Palumbi et al. [34], and the primer pair SHB and SHA described in Cunningham and Buss [35], respectively. Additionally, a portion of the H3 gene was also amplified for the nudibranch using the primer pair H3F and H3R, described in Colgan et al. [36]. All PCRs were performed following the protocols described in Maggioni et al. [37]: 5 min for the initial denaturation at 94 °C, 30 cycles of 30 s at 94 °C, 1 min at 50 °C (SHB–SHA), 53 °C (16AR–16BR), or 52 °C (H3F–H3R), 1.5 min at 72 °C, and a final elongation of 5 min at 72 °C. All PCR products were checked through electrophoretic runs in 1.5% agarose gels, purified with Illustra ExoStar (GE Healthcare, Chicago, IL, USA), and finally sequenced in both directions with an ABI 3730xl DNA Analyzer (Applied Biosystems, Waltham, MA, USA) at the MacroGen Europe Milan Genome Center (Milan, Italy). The obtained chromatograms were checked and corrected with Geneious 6.1.6, and the protein-coding gene H3 was translated to check for the presence of open reading frames. The sequences obtained in this study were deposited with the relative metadata in GenBank, with the accession numbers OQ110615, OQ145162, and OQ145163. Sequences were checked to avoid possible contamination using the Basic Local Alignment Search Tool (BLAST), and they were compared to those already present in GenBank. Moreover, for the nudibranch, related 16S sequences belonging to the family Facelinidae and the outgroup *Duvaucelia odhneri* J. Tardy, 1963 (GenBank accession number: OQ145163) were downloaded from GenBank and aligned to our sequence to assess its phylogenetic position. For comparison, a H3 dataset was also assembled and analyzed in the same way, even though this DNA region is known to be weakly informative at the species and genus level [38–40]. Sequences were aligned with MAFFT 7.110 [41] with the E-INS-i option; the substitution models

were determined using jModelTest 2 [42] under the Akaike Information Criterion, and maximum likelihood phylogenetic analyses were performed with RAxML 8.2.12 [43] with 1000 non-parametric bootstrap replicates.

3. Results

3.1. Morphological Characterization of the Organisms

The bryozoan and associated hydrozoan species were identified as *Celleporaria* sp. and *Zanclaea* sp. 2 (*sensu* Maggioni et al. [44]), respectively, following Maggioni et al. [19,44]. Specifically, the hydrozoan colony was characterized by a naked hydrorhiza partially embedded into the bryozoan skeleton, and by monomorphic, cylindrical, and transparent gastrozooids, with short oral and aboral tentacles with inclusions in the capitations. The cnidome was composed of stenoteles of three size classes (small size: $\sim 7 \times 5 \mu\text{m}$, medium size: $\sim 15 \times 12 \mu\text{m}$, and large size: $\sim 20 \times 16 \mu\text{m}$) abundant in the tentacle capitations, and also present in lower quantity in the hydrorhiza, and of macrobasic holotrichous euryteles ($\sim 20 \times 12 \mu\text{m}$) that were very abundant in the hydrorhiza. The encrusting bryozoan colony displayed a typical *Celleporaria* morphology, also showing a relatively large size, conspicuous pigmentation, and secondary calcification, but it was not possible to identify the specimen at the species level.

The morphological analysis of the nudibranch revealed that the external morphology and its internal anatomy perfectly matched with those reported for the genus *Sakuraeolis* (Figures 1 and 2), with the general shape of the radula and jaws resembling that described for *S. enosimensis* (Baba, 1930), the type species of the genus. In particular, it shares a muscular, tongue-shaped penis without an apical hook, the majority of the liver branches in the form of horseshoes, the anus cleioproctic, the rhinophores smooth, the foot-corners tentaculiform, the jaw-plates indented dorsally, and a central radula tooth broadly horseshoe-shaped with several rather strong lateral denticles (Figure 2). Moreover, the external morphology and coloration of our specimens also matched with that of *S. marhe*, as reported in Cunha et al. [25], whose internal anatomy has not been described yet and is provided here for the first time:

Facelinidae Bergh, 1889,

Sakuraeolis Baba, 1965,

Sakuraeolis marhe Fernández-Simón and Moles, 2023.

Buccal apparatus: The jaw-plates are horny yellow and show a dorsal indentation (Figure 2c,d). The jaw-edge bears a series of 35–40 well-defined and rounded regular denticles (Figure 2d). The radula (Figure 2e–i) has the formula $21 \times 0.1.0$. The teeth are broadly horseshoe shaped, bearing 7–9 rather strong denticles on either side of a produced median cusp. The teeth show a degree of intraindividual variability; in fact, the number of lateral denticles of the rachidian teeth may vary between 6 and 10, according to the position of the teeth throughout the radula (new or old teeth), and the median cusp can be differently pronounced according to this latter factor (Figure 2g–i).

Reproductive system: the penis is muscular, tongue-shaped, and unarmed. It protrudes from a large sheath visible in fresh and conserved specimens (Figure 2a,b). The prostatic portion is globose and glandular, and is connected to the vas deferens that is folded and that terminates in the ampulla. This is rounded and connected with a sac-shaped bursa copulatrix, and with a small tubular vagina (Figure 2b). A voluminous folded female gland mass is located ventrally between the female part of the reproductive system, and the ampulla and the ovotestis (Figure 2b).

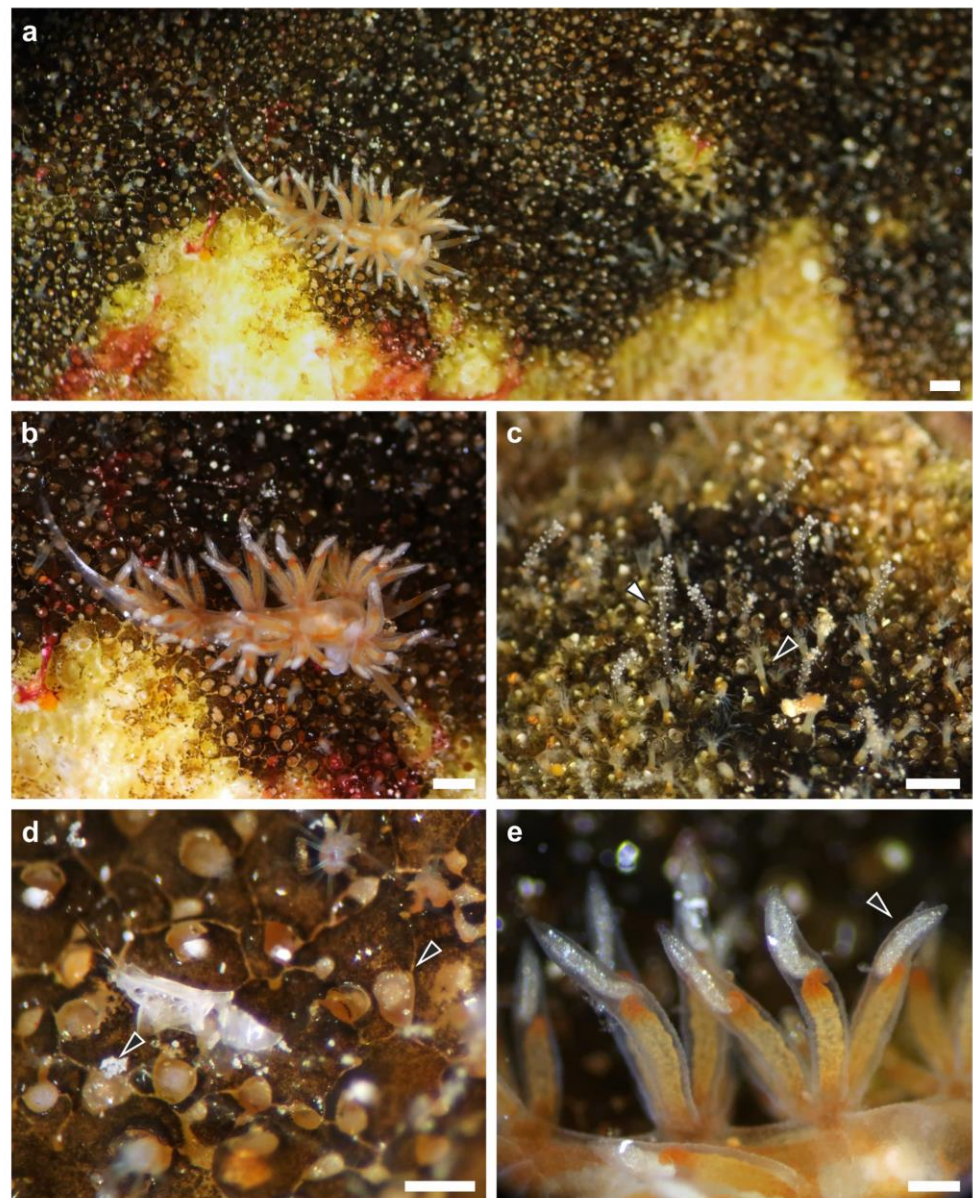


Figure 1. *Sakuraeolis marhe* feeding on *Zanclea* sp. 2 associated with *Celleporaria* sp. (a,b) An individual of *S. marhe* crawling on *Celleporaria* sp. while feeding on *Zanclea* sp. 2. (c) A detail of the association between *Celleporaria* sp. and *Zanclea* sp. 2, showing extended bryozoan zooids (black arrowhead) and hydrozoan polyps (white arrowhead). (d) Signs of predation of *S. marhe* on *Zanclea* sp. 2 polyps (two examples of consumed polyps are indicated by black arrowheads). (e) Detail of the cerata with terminal cnidosacs (arrowhead) containing kleptocnidae. Scale bars: (a–c) 0.5 mm, (d,e) 0.2 mm.

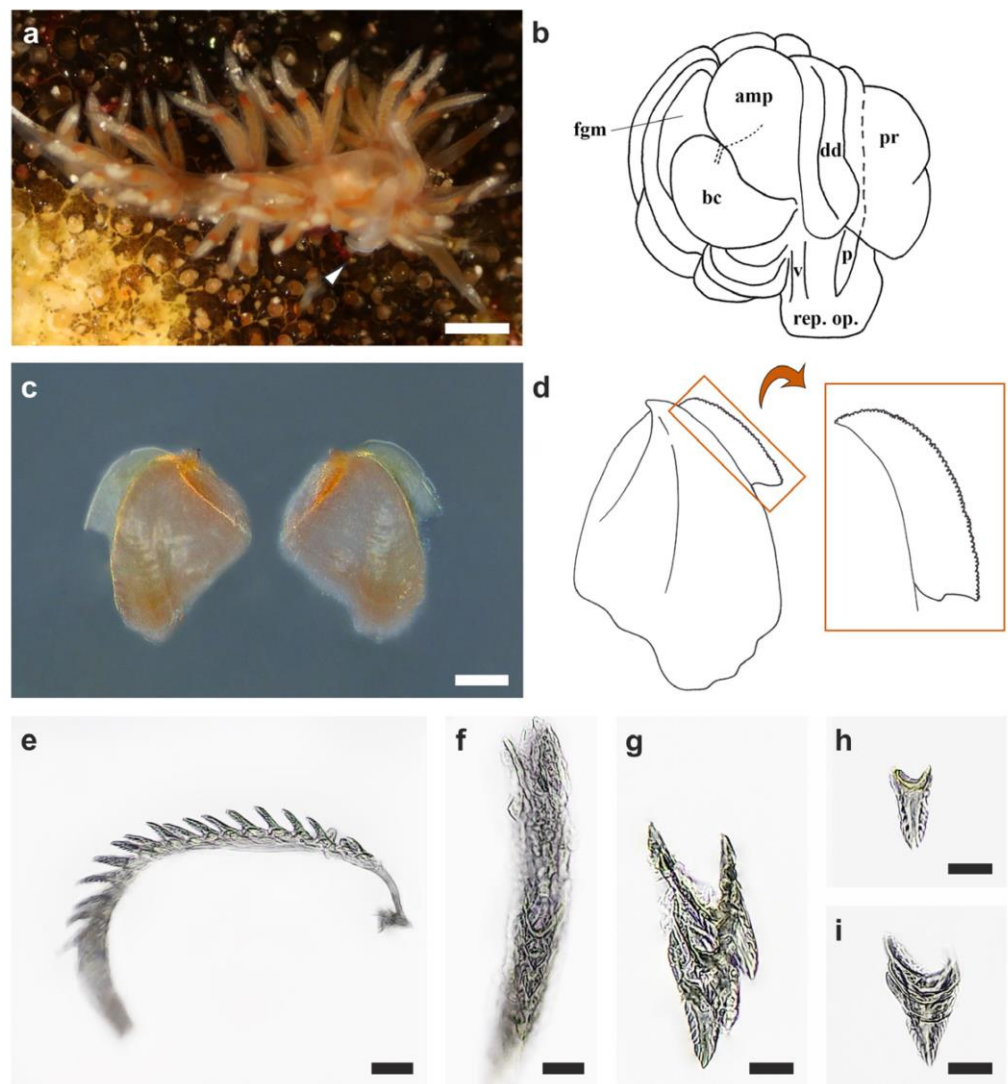


Figure 2. Anatomical diagnostic characteristics of *Sakuraeolis marhe*. (a) In vivo picture of an *S. marhe* individual with everted male reproductive structures (arrowhead). (b) Schematic drawing of the reproductive system. (c) Picture of the two jaws of the buccal apparatus, and (d) schematic drawing of the jaws and the jaw-edge, drawn at a different magnification scale (orange rectangle). (e,f) Lateral and frontal view of the radula, respectively, and (g–i) intra-individual variability among the teeth. amp: ampulla, bc: bursa copulatrix, dd: vas deferens, fgm: female gland mass, p: penis, pr: prostate, rep. op.: reproductive opening, v: vagina. Scale bar: (a) 0.5 mm, (c) 0.75 mm, (e,f) 25 μ m, (g–i) 10 μ m.

3.2. Molecular Phylogenetics

The obtained sequences showed no contamination and further confirmed the morphological identifications. The hydrozoan 16S sequence (GenBank accession number: OQ145162) perfectly matched the deposited sequences of *Zanclaea* sp. 2 from the Maldives (percentage identity of up to 100%). The nudibranch 16S sequence (GenBank accession number: OQ145163) showed a percentage identity of 95.65% with a sequence ascribed to a *Facelina* sp. (GenBank accession number: MK478697) collected in Indonesia [45], and it resulted 93.44% identical with one *S. enosimensis* sequence (GenBank accession number: HM162682) from California. Similarly, the latter specimen (GenBank accession number: HQ010472) showed a percentage identity of 95.27% with the obtained nudibranch H3 sequence (GenBank accession number: OQ110615), whereas the highest match was with *Caloria* sp. 4 (96.96%; GenBank accession number: JQ996965) from the Philippines. The 16S phylogenetic hypothesis revealed a topology that was broadly concordant with the

COX1 tree produced by Cunha et al. [25] (Figure 3). The *Sakuraeolis marhe* sequence was closely related to the *Facelina* sp. sequence from Indonesia, followed by two Favorinidae sp. sequences from the Philippines. This group was then closely related to *Sakuraeolis arcana* Ellis-Diamond, Picton, Tibiriçá & Sigwart, 2021 from Mozambique, *S. enosimensis* from California, *S. japonica* (Baba, 1937) from Korea, and *Caloria* sp. 4 from the Philippines. Finally, this clade was sister to a clade composed of the *Cratena* Bergh, 1864 and *Myja* Bergh, 1896 sequences, together with two Facelinidae sp. 2 sequences, again similarly to the data from Cunha et al. [25]. Overall, the nodes were moderately to well-supported, especially regarding the relationships of *S. marhe* with *Facelina* sp. and Favorinidae sp., similarly to what was shown in Cunha et al. [25]. The H3 phylogenetic tree (Figure S1) was characterized by a lower resolution, similar to what was shown in previous studies [38–40], and the relationships between the *S. marhe* sequence and other facelinid sequences remained unclear, according to this DNA region. Overall, the results obtained from both morphological and molecular analyses confirmed that the nudibranch belongs to the species *S. marhe*, the bryozoan to *Celleporaria* sp., and the hydrozoan to *Zanckea* sp. 2.

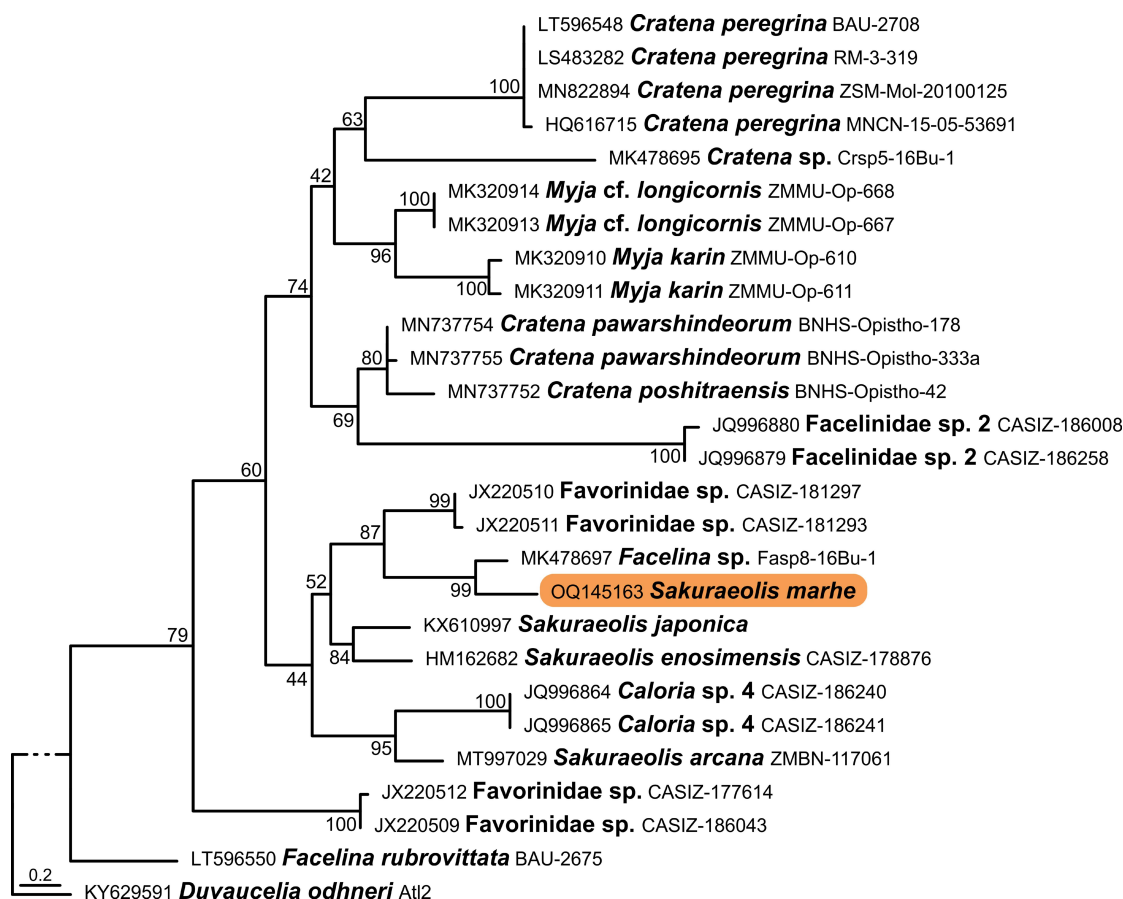


Figure 3. Maximum likelihood 16S phylogenetic reconstruction of *Sakuraeolis marhe*, highlighted in orange, and closely related species. Codes before the species name are the GenBank accession numbers, whereas codes after the species names refer to the voucher numbers or sample IDs. Numbers at nodes are bootstrap values.

3.3. Behavioral Observations and Kleptocnidae Assessment

The two *S. marhe* individuals were observed moving on top of the bryozoan colony and feeding specifically on *Zanckea* polyps (Figure 1a–c), but not on *Celleporaria* zooids, which were observed to be intact after the passage of the nudibranchs. Specifically, the nudibranchs were observed crawling on the *Celleporaria* surface, leaving behind consumed

or partially consumed hydrozoan polyps (Figure 1d). The cerata were filled with kleptocnidae (Figures 1e and 4a,b) that were identified as large size stenoteles (Figure 4c) and macrobasic holotrichous euryteles (Figure 4d), the latter being very rare. The analysis of the hydrozoan cnidome revealed the presence of stenoteles (Figure 4e) and macrobasic holotrichous euryteles (Figure 4f), in agreement with Maggioni et al. [19,44]. Only large size stenoteles ($\sim 20 \times 16 \mu\text{m}$) were observed in the cnidosacs, whereas *Zanclaea* sp. 2 polyps also possessed small and medium size stenoteles, and euryteles were rare, despite being common in the *Zanclaea* hydrorhiza, as shown in this work and in Maggioni et al. [44].

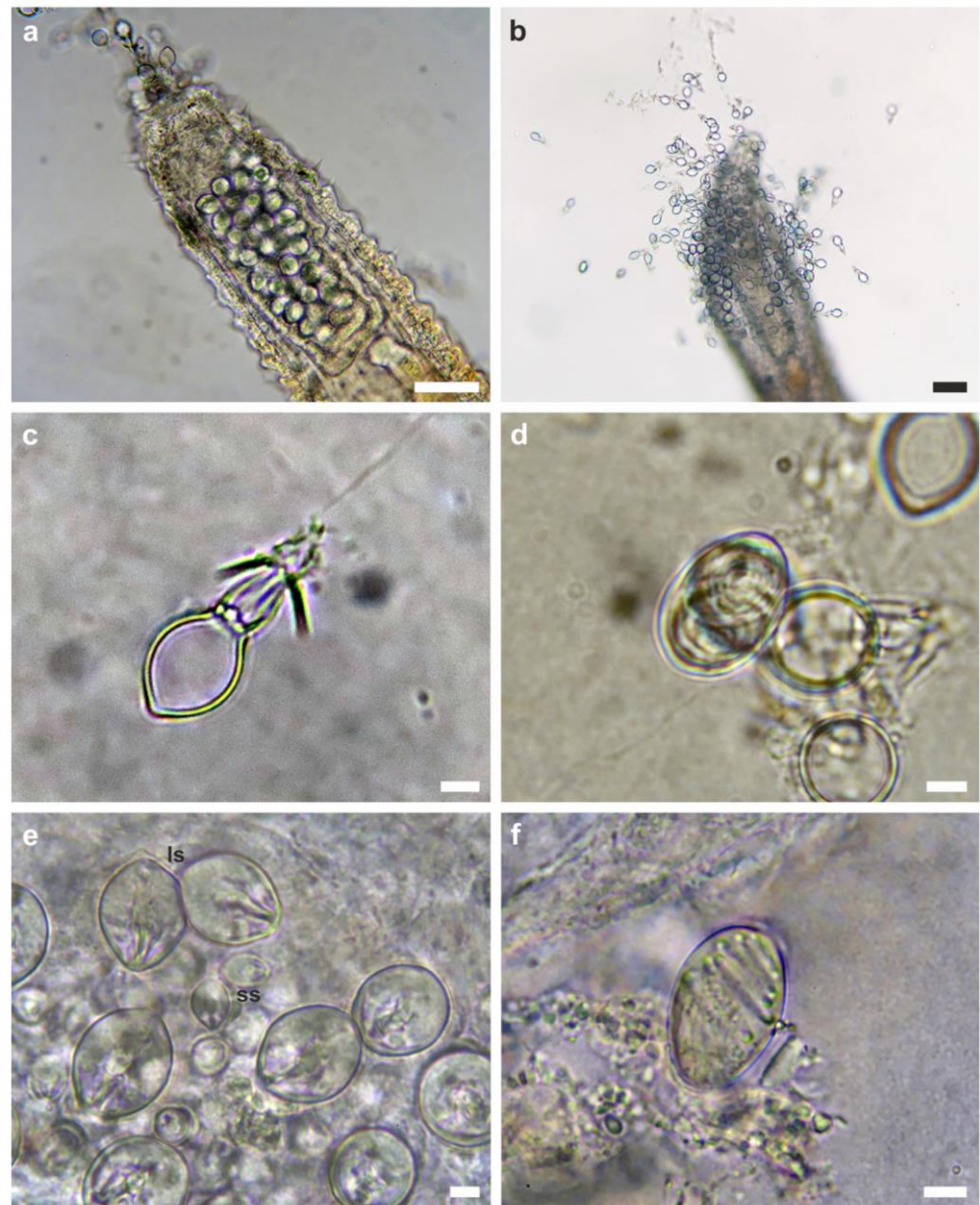


Figure 4. Kleptocnidae and nematocysts of *Sakuraeolis marhe* and *Zanclaea* sp. 2. Detail of a cnidosac with (a) undischarged and (b) discharged kleptocnidae. (c) Discharged stenotele and (d) undischarged eurytele from the cnidosac. (e) Small size stenoteles (ss) and large size stenoteles (ls), and (f) eurytele isolated from *Zanclaea* sp. 2 polyps. Scale bars: (a,b) 50 μm ; (c–f) 5 μm .

4. Discussion

Maldivian coral reefs are home to an extraordinary diversity of organisms, with new species being frequently described from different areas of the archipelago [46–49]. In addition, previously unknown or understudied inter-specific associations and interactions have been recently described [50–52], such as the case of an amphipod associated with the gastropod *Coriocella hybiae* Wellens, 1991 [53], revealing that the diversity and ecology of these ecosystems strongly need further research, especially given the current coral reef crisis [54].

In this context, *Sakuraeolis marhe* was recently described from Central Maldives by Cunha et al. [25], based on the external morphology and genetic data. The specimens herein analyzed matched the morphological description of this species, and their identification was further supported by the newly produced genetic data, the overlapping distribution, and the substrate on which they were found. Additionally, the characterization of the internal anatomical diagnostic features (i.e., the buccal apparatus and the reproductive system) complemented the original description of the species. The anatomical description here provided is congruent with the characters considered diagnostic for the genus *Sakuraeolis*, except for the features of the penial sheath that, however, are also lacking in the newly described congeneric *S. arcana* [29], suggesting that this structure may not be diagnostic at the genus level. This morphological observation sheds some lights on the gap of knowledge still existing on the synapomorphies characterizing this apparently non-monophyletic genus, for which an integrative systematic assessment is indeed desirable. With this work, we also provided ecological information on the species, regarding the substrate and the feeding preferences. Indeed, *S. marhe* was observed living on the bryozoan *Celleporaria* sp. and selectively preying on its associated symbiotic hydrozoan *Zanclaea* sp. 2.

Celleporaria species are known to host different hydrozoan species in the Indo-Pacific, including *Zanclaea divergens* Boero, Bouillon & Gravili, 2000, *Zanclaea* sp. 1, and *Zanclaea* sp. 2 [20,37,55,56], and these associations are supposed to be intimate, with the hydrozoans having lost the perisarc covering the hydrorhiza, and being protected by the bryozoan skeleton through secondary calcification. Advantages for bryozoan-associated hydrozoan colonies may be increased food availability, thanks to water currents produced by the host, and protection from predators, thanks to the bryozoan skeleton [17]. On the other hand, *Celleporaria* zooids were observed stimulating the release of mucus particles from hydrozoan polyps, which were then ingested [56]. Osman and Haugsness [21] reported on a *Celleporaria*-associated *Zanclaea* species that enhanced the competitive abilities of the host in the Eastern Pacific, by protecting it from competitors and predators thanks to nematocysts. Even though the latter *Zanclaea* species remains undetermined, it looks like *Celleporaria* and all associated hydroids live in intimate and possibly mutualistic associations with positive trophic and defensive outcomes for both symbiotic partners. A similar situation was also hypothesized for coral-associated *Zanclaea* species [23], where hydroids might repel predators, including corallivore gastropods and pathogens, thanks to their nematocysts.

The predatory behavior of *S. marhe* here reported was therefore unexpected, revealing that the beneficial roles of symbiotic hydrozoans seem not to apply when the predators are specialized hydrozoan eaters. Even if the nudibranch does not directly feed on the bryozoan, it may affect the structural integrity of zooids by crawling on the surface, and may reduce its competitive ability by removing the associated hydrozoans. This questions the defensive function of symbiotic hydrozoans, at least in the *Zanclaea*–*Celleporaria* association here examined. At the same time, the possible protective function of the bryozoan skeleton towards associated hydrozoans is also challenged by the observations here reported, at least for what concerns *S. marhe* predatory activities.

The analysis of the cnidosacs content confirmed the presence of kleptocnidae previously belonging to *Zanclaea* sp. 2 polyps, namely, large size stenoteles, and very rarely, euryteles, both with sizes concordant with those of *Zanclaea* sp. 2 nematocysts. The modulation of kleptocnidae storage has been demonstrated in a few cases, being, for instance, governed by both the diet and the presence of predators in *Flabellina verrucosa* (M. Sars,

1829) [57]. The uptake of specific types of nematocysts among those available from the prey has also been shown in other nudibranch species such as *Cratena pilata* (Gould, 1870), storing only mastigophores from the prey *Pennaria disticha* Goldfuss, 1820 [58]. The occurrence of kleptocnidae mainly of the stenotele type in *S. marhe* may suggest that this species is selective in the types of nematocysts that it stores in the cnidosacs or in the hydrozoan structures that it feeds on. Euryteles are concentrated in the hydrorhiza of *Zanclaea* sp. 2 and are not found in polyps, suggesting that *S. marhe* may selectively feed on the polyps, possibly avoiding hydrorhizal structures. On the other hand, small and medium size stenoteles were not observed in the cnidosacs, and these nematocysts are very common in polyps, indicating that *S. marhe* selectively stores large size stenoteles in its cnidosacs and leaving open the hypothesis that the nudibranch feeds on the hydrorhiza as well, but does not store euryteles.

In conclusion, the morphological and anatomical descriptions of *S. marhe* were provided here for the first time, together with useful insights on the diagnostic power of the reproductive structures that are nowadays considered useful to delimit nudibranchs at the genus taxonomic level. Finally, the interaction here described represents the first well-documented record of a nudibranch feeding on a symbiotic hydrozoan and storing the nematocysts in its cnidosacs. This also represents the first record of nudibranch predation on *Zanclaea* polyps, highlighting the need for further research to better characterize and comprehend the roles of these hydrozoans in the many symbiotic systems in which they are involved.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15040484/s1>, Figure S1: Maximum likelihood H3 phylogenetic reconstruction of *Sakuraeolis marhe*.

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Conflicts of Interest: The authors declare no conflict of interest.

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