OBSERVATION ON THE FEEDING OF NUDIBRANCH
Phyllidia varicosa LAMARCK, 1801 ON THE SPONGE Axinyssa cf. aculeata
WILSON, 1925 IN CORAL REEFS OF PRAMUKA ISLAND, THOUSAND ISLANDS NATIONAL PARK, INDONESIA

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Abstract
During three months period observations (November 16 to January 13, 2001) in coral reefs of Pramuka Island Indonesia, the nudibranch Phyllidia varicosa was observed while feeding on the sponge Axinyssa cf. aculeata. During feeding, P. varicosa extended externally the pharyngeal bulb onto the sponge causing visible somatic damage. The behaviors of contracting the body and retracting the rhinopores inside the rhinotube were also observed while feeding. Observations on the feeding of Phyllidia varicosa on the sponge Axinyssa cf. aculeata and the presence of a visible somatic damage on the prey due to predation are reported for the first time.

Keywords: Phyllidia varicosa, Axinyssa cf. Aculeata, feeding observation

1. Introduction
Phyllidiids are sponge-specialist predator [1-3]. They possess a small mouth without radula or other buccal hard part (e.g. jaws), oral glands, and pharyngeal retractor muscular. They can extend externally the pharyngeal bulb onto the sponge [2]. They are also known as a suctorial sponge feeder. Nevertheless, publications on the feeding observation of phyllidiids are a bit poor [2].

One of the most frequently encountered phyllidiids in tropical Indo-Pacific waters is Phyllidia varicosa [2,4]. This species is also quite abundant in Thousand Islands area, Indonesia [5]. In the field, P. varicosa can easily be recognised by the colouration and patterning of the live animal. The body size of P. varicosa is relatively big (57 mm) and of an elongate-ovate shape. The notum of the species has many conical or angular, sometimes compound tubercles which form three to six longitudinal ridges. The ridges may be broken or continuous. A central ridge is always present. The ridges are blue grey in color as well as the bases of the tubercles, but are capped in yellow. Toward the margins of the notum there are usually numerous, short, transverse ridges interspersed by black rays. The foot and gills are grey in colour. Phyllidia varicosa possesses yellow rhinophores and a black longitudinal foot stripe at the foot sole [2,4,6-8]. According to Brunckhorst [2], the external morphology of P. varicosa looks quite similar to the species of P. tula and P. coelestis but still it is easy to distinguish them in the field. Even though P. tula also has a longitudinal black stripe on its foot sole, but the foot is very dark grey and the notum possesses single rounded tubercles and is without ridges. Phyllidia coelestis is a smaller species which has neither a foot stripe nor a median ridge. The body shape is also more oval than P. varicosa and P. tula.

Prior to the present research, Shintosari [8] who performed her research in Thousand Islands Indonesia reported that P. varicosa was found on sponge of the genera Axinyssa and Lissodendoryx. Even though she did not give any comments whether they were considered as a prey or not, but the sponge may be assumed as a potential prey for the species as stated by Swennen [9] and Bloom [10]. Some years before, Burreson et al. [11] while diving off Pupukea on the north shore of Oahu Hawaii observed that P. varicosa was found feeding on the sponge Ciocalypta sp. (as Hymeniacidon sp.; see Karuso [12], Kassühlike [13], Cimino & Ghiselin [3]). Unfortunately, they also did not detail their observation. The most detailed feeding observation of Phyllidia varicosa was given by Brunckhorst [3] who observed P. varicosa feed on the sponge Halichondria sp. during his long field study in Australia, Thailand, and Papua New Guinea. He
reported that *P. varicosa* evert the pharyngeal bulb over the top of the sponge rather than into it. Nevertheless, visible somatic damage of the sponge was not reported. Allmon & Sebens [14] highlighted that only organisms with a visible somatic damage to the area directly beneath the nudibranch’s mouth are considered as a prey.

In the present paper, I report my observation on the feeding of *Phyllidia varicosa* on the sponge *Axinyssa cf. aculeata* Wilson, 1925 in coral reefs of Pramuka Island, Thousand Islands National Park, Indonesia. Some comments on the sponge prey from the former researchs are also reported. Record on the visible somatic damages of the prey due to predation of *P. varicosa* is also reported for the first time.

The aims of the present study are to recognise the substrate where *Phyllidia varicosa* feeding on, to know whether it has specificity in preying, and to know the behaviour of *Phyllidia varicosa* while feeding on the prey.

2. Materials and Methods

The study was performed in coral reefs of Pramuka Island, Thousand Islands National Park, Indonesia (Figur 1).

![Figure 1. Map of Thousand Islands National Park (inside dashed lines: boxes inside the National Park indicates the core zone). The arrow number 4 shows the location of Pramuka Island. Inset: Map of South East Asia, a: location of Thousand Islands National Park.](image)

While SCUBA diving, nine individuals of *Phyllidia varicosa* were collected along with the sponges they were upon and then kept in aquarium (60 x 35 x 40 cm).

The aquarium was connected with a plastic tube (inside $\theta$ is 5 mm) to 50 L plastic tank, which was continuously refilled with fresh sea water. In order to get a good running sea water into the aquarium, the plastic tank was placed in a position of higher than the aquarium. A plastic tube with the same diameter as the inlet was also fixed as an outlet of the sea water from the aquarium. In addition, a commercial aquarium bubble air was also fixed to aerate the sea water in the aquarium. On November 16, 2000 the feeding behaviour was observed to see if *P. varicosa* selects a particular species of sponge out of three sponge species of each one specimen (recognised only from the morphology and colour) offered in the aquarium. The observations were done at 10 am till 2 pm and were then continued at 8 till 10 pm.
Further in situ observations were done during two SCUBA dives on November 18-19, 2000; December 12, 2000; and January 13, 2001. The behaviour of *Phyllidia varicosa* which was found crawling or resting on sponge was observed carefully. Behaviour observation were concentrated on the rhinophores and the mantle. The specimens were abruptly taken away from the sponge to see if any signs of mouth part as reported by Brunckhorst [2] were visible. Off several specimens, only *P. varicosa* which extended externally its pharyngeal bulb was recognised feeding on the sponge. Additional observations on the sponge were done to record any visible somatic damages due to predation by *P. varicosa*. General morphology including the colour of the sponge was recorded and recognised. Both the nudibranch *P. varicosa* and the sponge were taken for identification.

The sponge samples were preserved with alcohol 70%. Identification of the sponge was done by Dr. Rob van Soest, Department of Coelenterates and Porifera, Institute for Biodiversity and Ecosystem Dynamics (Zoologisch Museum), University of Amsterdam. The voucher of sponge specimen was deposited in Zoology Museum, Amsterdam. The code is ZMA POR. 16587.

*Phyllidia varicosa* was dissected immediately after collection to recognise its internal anatomy. The results were then compared to the description of Brunckhorst [2]. The description of both the external morphology and internal anatomy of *P. varicosa* was also discussed with Dr. Brunckhorst to ensure that the description was correct for the species of *P. varicosa* (pers. communication).

3. Results and Discussion

The feeding observations in aquarium did not give any suspecting results. All individuals just resting or crawling either on aquarium glass-wall or on the sponges. A pair of them showed copulatory activity, which lasted for about one hour. Some of them also lifted their mantle edge so that the gill leaflets which are laid between foot and mantle disc (ventrolateral) were clearly seen. Interestingly, some of them also show unusual activity: they crawled to the water-air interface, clung upside-down to the water and did not react, as if they were dead. The foot, which is laid pararel to the sea water surface secreted mucous along the foot sole. They did not show any feeding activity. One juvenile of *P. varicosa* (2 mm length) was observed crawling and resting on one of sponge species in aquarium that was later identified as Axinyssa cf. aculeata and constitutes the only sponge species *P. varicosa* feeding upon.

During 3 months in situ feeding observations, as much as 35 individuals of *P. varicosa* are observed. Eleven of them were observed to extend externally the pharyngeal bulb and recognised feeding on the sponge. All individuals contracted their body (mantle mass) and retracted their rhinophores inside rhinotubes while feeding. Inversely, they did contract their body but still extended the rhinophores outside the rhinotubes while resting (observation on 7 individuals).

The remaining individuals were observed crawling either on rubble of dead corals or muddy sand sea floor. Their bodies were relaxed and seemed to be flexible above the substrates. The rhinophores actively searched around. While collecting the sponge *Axinyssa* cf. aculeata and *P. varicosa* for chemical investigation purposes, as much as 15 individuals of *P. varicosa* (from the total 45 individuals) were also observed to extend externally the pharyngeal bulb feeding on the sponge.

All the sponge *P. varicosa* were feeding upon had a similar colouration and outer surface morphology. Most sponges are criptic and encrusting among dead coral rubble. They were always found covered by sediment, which made them difficult to recognise unless there were preyed upon by *P. varicosa*. The sponge species was Identified as *Axinyssa* cf. aculeata. The sponge has a laterally flattened club-shaped growth form but is usually found in irregular shape and an irregular shaggy-conulose surface, with interconnected grooves an gullies. No other *Axinyssa* species have been described with such a characteristic shaggy surface. Most other species being smooth or microconulose. A shallow depression is present on the upperside of the sponge. No apparent oscules are visible. The colour of live animal is purple to whitish purple but is pale yellow after being preserved in alcohol. The skeleton consists of largely confusedly arranged spicules, with vague
Figure 2. Similarity pattern between pharyngeal bulb and the visible damage on sponge due to predation of *Phyllidia varicosa* (see the arrows).

Figure 3. Anatomy of Digestive and reproductive organ of *Phyllidia varicosa*: a. visceral sheet; b. anus; c. digestive track (ventral view); d. nidamental gland mass; e. Bursa copulatrix and ampulla; f. digestive gland; g. ovotestis; h. pharyngeal bulb.

Parallel tracts running at right angles to the surface. The ectosomal skeleton is a continuation of the choanosomal skeleton, no special tangential spicules are recognized. Spongin is not visible, but in between the scattered spicules a collagenous groundsubstance is apparent.
Spicules oxeads in a large size range, 570-1080x5-30µm, usually sharply pointed, straight, but more often curved, occasionally flexuous. All of these characteristics reveal that the sponge specimen refers to the species of *Axinyssa aculeata* Wilson, 1925 [15].

A visible somatic damage of the sponge due to predation of *P. varicosa*, was recorded. Instead of colour-changed of the sponge (from purple or whitish purple to green in colour), it was also recorded that the visible damages on sponge due to predation looked similar to the internal pharyngeal bulb which is folded glandular disc in shape (Figure 2).

The internal anatomy of the species is similar to the description given by Brunckhorst [2]. The visceral envelope is a dark grey-black sheet. Inside the pharyngeal bulb there is a thick, folded, glandular epithillium forming an orange disc-like which often protude posteroventrally. The digestive organ is laid dorsally. The dominant mass part is digestive gland which is brown in color. It occupied two thirds of the body cavity. In general, the reproductive system is cream to yellowish in colour. The bursa copulatrix and ampulla are tan-brown. The nidamental gland mass is very large and spherical. The ovotestis overlies the anterior portion of the digestive glands and is yellow in colour (Figure 3).

Such unusual behaviour of *P. varicosa* as crawled to the water-air interface; clung upside-down to the water and did not react anyway as if they were die; lifted the mantle edge, were never found in the field. These phenomenon, however, were also observed by Cronin et al. [16] which worked with the species *Tritonia hammerorum* (*tritoniid nudibranch*). They suspected that it may be due to the stress of stagnant water conditions in aquaria. Agreeing with their opinion, I consider the stress of stagnant water conditions leading to anoxic condition is the main reason why they did such unusual behaviour. Even though I kept the species in a running sea water aquarium, but in fact the flow rate was very low. In the experiment, the reaction to anoxic condition was shown by lifting their mantle edge followed by crawled to the water-air interface, clung upside-down to the water and secreted mucous along the foot sole. By lifting their mantle edge, the gill leaflets which is laid between foot and mantle disc (ventrolateral) were exposed directly to circumstance sea water so that the chance to get soluble oxygen will increase. When the anoxic condition continued, the present of mucous along the foot sole while clung upside-down to the water make possibly for them to absorb oxygen directly from the air. This kind of reaction is also shown by other kind gastropods e.g. terrestrial and intertidal gastropods (personal observation). Unconvenient situation was also proven as they did copulatory process just a few hour after they kept into the aquarium. Rudman [17] reported that such reproduction process as copulation and egg-laying in aquarium are not a sign of well-being, in fact it is often a sign of stress.

In general, keeping nudibranch alive -in particular *P. varicosa* together with sponge or other organisms is extremely difficult (Johannes [18], Burreson et al. [11], Brunckhorst [2]). Instead of the stress condition, the failure of keeping nudibranch alive for feeding observation in aquarium is due to the lack of knowledge of the species’s prey. Offering all kind of sponge near or where the species crawling on is just a trial experiment. Rudman [17] said that the major problem is that we do not know which prey (usually sponge) they feed on. Even if we know which sponges to feed them, keeping sponges alive in aquarium is difficult. In situ observation on the extending externally of the pharyngeal bulb of *Phyllidia* spp. was firstly report by Brunckhorst [2]. While Brunckhorst [2] reported that he could observe directly the extending of the pharyngeal bulb of *P. varicosa* while feeding on the sponge, in the present study I have to take out suddenly *P. varicosa* from the sponge to see the extending pharyngeal bulb. While feeding, *P. varicosa* contracted their body (mantle mass) and retracted their rhinophores inside rhinotubes. The contraction of the body may be related to feeding *modus operandi* of phyllidiids proposed by Brunckhorst [2]. The arrangements of the phyllidiids's foregut operates as a sieve-plate or coarse filter. The glandular secreations being expelled through the mouth and the muscular pharynx which would then relax producing a vacuum to take up the food materials from the sponge. It seems that *P. varicosa* did predigestion of the sponge and just took up the products of predigestion. Brunckhorst [2] who observed the feeding of *Phyllidia* spp. did not find calcareous spicules in the faeces. In addition, I observed that the faeces of *P. varicosa* is not a solid-compact material. They are liquid, dirty gray in colour and very soluble in the sea water.

Even though Brunckhorst [2] has extensively observed the feeding of phyllidiids species, but he did not report any visible somatic damages on the sponge due to the predation. According to Allmon & Sebens [14] the present of a visible somatic damage to the area directly beneath the nudibranchs’s mouth are strongly help to considere any organisms as a prey. In the present study, the present of a visible somatic damage on the sponge due to the predation of *P. varicosa* is reported for the first time. It is no surprise if the scars have similar pattern to the inner side of the
pharyngeal bulb of *P. varicosa* (Figure 2). It is assumed that *P. varicosa* do predigestion of the sponge and just take up the products of predigestion. The present of both strong acid and weak acid in pharyngeal bulb make this predigestion process possibly [2]. The interesting one is the present of green colour at and around the scars on the sponge due to predation. This green colour was never found at the area which not attacked by *P. varicosa* nor inside the sponge mass. It is considered that it should be the colour of the sponge symbiotic microalgae. The extracts of the sponge which contain a large amount of chlorophyle (red in colour under the UV 366 nm) support this assumption.

The sponge *Axinyssa* cf. *aculeata* constitutes the only sponge that was eaten by *P. varicosa* during three months observation. Identification of the specimen reveal that all characteristics of the species close to *Axinyssa aculeata* Wilson, 1925, but the growth form. The growth form of typical specimens of *Axinyssa aculeata* is a thick-walled irregular cup of 12-15cm diameter, and its spicules may be up to 1500x40µm in size. These discrepancies with the present specimen prevent a definitive identification, but it is quite conceivable it is a small not fully grown individual of *A. Aculeata* [15].

The species of *A. aculeata* belongs to the order Halichondrida where the species of *Ciocalypta* sp. and *Halicondria* sp. are included in. These two sponges are known to be the prey of *P. varicosa* from the former research (Burreson [11], Brunckhorst [2]). According to Bloom [10] the order of Halichondrida is the non-reticulated sponges which has less difficulty of fragmentation than reticulated one. It is therefore no surprise if *A. aculeata, Ciocalypta* sp., and *Halicondria* sp. appear as a suitable foot for *P. varicosa* which as all other phyllidiids species has no radula. It is considered that the reason why the sponge prey of *P. varicosa* geographically varies is due to the limited geographic distribution of the sponge species [19]. The sponge *A. aculeata* originally described from the Philippines, but it is also reported commonly from Indonesian waters. By making a generalization, *Ciocalypta* sp. and *Halichodria* sp. should be also common in Hawaiian water and Australian water. In addition to the degree of fragmentation difficulty of the sponge, it is considered that the sesquiterpene isocyanides which always present in the order of Halichodrida attract *P. varicosa* to feed on. The sesquiterpene isocyanides has been known as an effective chemical defense for phyllidiids species, which sequestered from the prey (Cimino & Ghiselin [3]). Nevertheless, the fact that *P. varicosa* is a specialist predator for the sponge *A. cf. aculeata* in Thousand Islands area is quite amazing. According to Van Soest [15] *Axinyssa* has the spicules sticking out a bit, whereas *Ciocalypta* and *Halichondria* have them pararel.

Even *A. cf. aculeata* has been described with such a characteristic shaggy surface, whereas most others being smooth or microconulose. Perhaps the sponge *A. cf. aculeata* has a more active secondary metabolites which can be sequestered by *P. varicosa* for defense purposes. Research on the investigation of secondary metabolites both from the sponge *A. cf. aculeata* and the nudibranch *P. varicosa* followed by fish feeding experiments will help to support this assumption.

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**References**