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Research article

Annual reproductive cycle of peanut worm, *Sipunculus nudus* from Thailand

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Abstract

Peanut worms have long been globally utilized as human food and fishing bait but their importance as a research animal to monitor marine environmental quality has only recently been exploited. There must be adequate knowledge of the breeding and culturing practices of a research species to provide reliable scientific data. At least 100 specimens of Sipunculus nudus Linnaeus, 1766 were collected from Mod Tanoy Beach, Kantang district, Trang province, Thailand each month from September 2012 to August 2013. The annual reproductive cycle was monitored of 60 specimens randomly selected and dissected for sex determination and inspection of male and female gamete stages. Data for 720 worms were collected and revealed a sex ratio of 1:1. Mature eggs and sperm plates were observed every month and the results indicated that spawning occurred throughout the year. S. nudus specimens from Mod Tanoy Beach, Thailand did not show a pattern of an annual reproductive cycle unlike S. nudus in China and other species of peanut worm which exhibited strong spawning patterns during the spring and summer months.

Introduction

Peanut worms are marine invertebrates with long cylindrical bodies without appendages or segmentation (Hsueh and Tan, 2016). They are found in all the oceans from intertidal zones to 900 m depth (Kawauchi and Giribet, 2013) with worldwide distribution in tropical, temperate and cold environments (Murina, 1975). Approximately 150 species are recognized globally (Cutler, 1994) with most research conducted on *Sipunculus nudus* Linnaeus, 1766 which is found in several countries around the world. In some areas of China and Vietnam, *S. nudus* is consumed as a high protein source and also used as fishing bait (Ha et al., 2007, Du et al., 2009)

Some peanut worms can reproduce asexually while most reproduce sexually; *S. robustus* produces new individuals by transverse fission and lateral budding (Rajulu and Krishnan 1969; Rajulu, 1975) while *Aspidosiphon brocki* reproduces by transverse fission into two unequal parts (Rice, 1970). Parthenogenesis by the development of unfertilized eggs into viable adults was reported in *Themiste lageniformis* (Pilger, 1987). With the exception of one species (*Nephasoma minutum*) which is a hermaphrodite (Paul, 1910; Akesson, 1958), the rest are dioecious and fertilization is external. Male and female gonads arise from coelomic epithelium cells as thin cords located along the bases of the ventral retractor muscles and maturation of gametes takes place in the coelomic fluid and they are then filtered through ciliated funnels into the excretory sacs of the nephridia and released into the environment through nephridiopores (Adrianov and Maiorova, 2010). Sexual identification was possible by observing gametocytes in the coelom (Ferrero-Vicente et al., 2014).

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Gametes were found all year round in some peanut worms while others produced sex cells for only a few months before spawning (Catalan and Yamamoto, 1994).

In Thailand, 19 species of peanut worm were reported by Hylleberg (1994, 2013). At Mod Tanoy Beach, personal observation found that peanut worms are collected by local fishermen and sold as sport-fishing bait in 10 g packets for USD 0.30. Peanut worms are also an ideal research animal for monitoring marine environment quality, particularly with regard to the bioaccumulation of heavy metals as concentrations of three heavy metals (Cd, Cr and Zn) in the gut juice extracted from wild-caught *S. nudus* were reported by Yan and Wang (2002) who identified the suitability of laboratory-produced peanut worms for use as research animals.

Basic information regarding the reproduction of peanut worms is essential to establish a breeding program and facilities. This requires preliminary data; therefore, the objective of this study was to determine their annual reproductive cycle.

Material and Methods

Description of area

The study area was selected as the intertidal zone of Mod Tanoy Beach, Kantang district, Trang province, Thailand, (7.307223º N, 99.416662º E). This area is located outside the mangrove belt with a submerged time ranging from 10–12 hr daily depending on tidal cycles (Fig. 1).

Fig. 1 Sampling site represented by the black star

Sampling method

At least 100 peanut worms were collected every month during low tide by a skilled local fisherman who located their burrow openings and used a shovel to dig them out (Fig2 .). Seventy peanut worms were randomly divided into two groups of 35 each. Each group was placed in a plastic bag (30 cm × 60 cm) and topped up with 1.5 L of 30 practical salinity units seawater. The bags were then filled with pure oxygen gas, tightly secured and placed in a styrofoam box which was air freighted to Don Mueang Airport, Bangkok, Thailand and transferred to the Aquatic Resources Research Institute, Chulalongkorn University, Bangkok, Thailand.

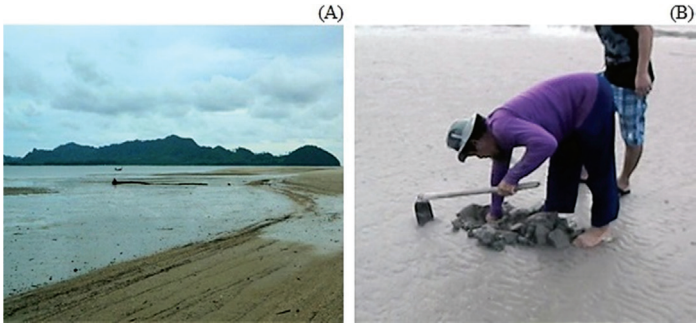

Fig. 2 Sampling site and collection method: (A) Mod Tanoy Beach, Libong subdistrict, Kantang district, Trang province, Thailand; (B) Digging up peanut worms

Species identification

All specimens were identified following the taxonomic keys of Cutler (1994). Morphological study was conducted based on dorsal dissection and observation under an compound microscope (CX23; Olympus Corp.; Tokyo, Japan) and a scanning electron microscope (JSM 5410LV; JEOL Ltd; Tokyo, Japan).

Determination of sex and stage of male and female gametes

Sixty peanut worms were randomly selected and dissected lengthwise from the posterior part of the body up to the head side. Gametes were collected from the coelom using a needle and pipette. Female gamete sizes (egg diameter) were measured using the phase contrast microscope with an ocular micrometer eyepiece (40×) attached to a digital camera. Sperm plates were photographed using the scanning electron microscope. Male and female gametes were observed and classified as follows.

Spermatogenesis in the coelom was classified into three stages based on the size and shape of the sperm plates and their component cells (Catalan and Yamamoto, 1994).

Stage I Spermatocytes break off from the testis into the coelom to increase cell numbers and develop into spermatocyte clusters (sperm plate) 25–30 µm in diameter.

Stage II The spherical cells within the sperm plate increase in number and develop into spermatocyte clusters 70–80 µm in diameter.

Stage III The spermatocytes are transformed into spermatids by meiosis and develop tails that extend outward in every direction from the sperm plate. Spermatozoa are fully grown when the head is about 1–1.5 µm in diameter and the tail is about 80–100 µm in length.

Five stages of oogenesis were described following Lan and Yan (2002) and Wang et al. (2005).

Stage I Cytoplasm growth (25–30 µm)
Stage II Follicle coat formation (60–70 µm)
Stage III High growth (120–150 µm)
Stage IV Jelly coat (150–160 µm)
Stage V Maturation (170–180 µm)

Length of study

The study lasted 12 mth from September 2012 to August 2013.

Results

Habitat

Mod Tanoy Beach is a sandy expanse of fine sand. Surface sediments are mainly composed of 95–97% sand with a sediment grain size of about 0.2 mm. Salinity is 30–35 practical salinity units and the average organic matter content is 0.53%. Mod Tanoy Beach is bordered by mangrove swamps and seagrass beds (Fig. 3).

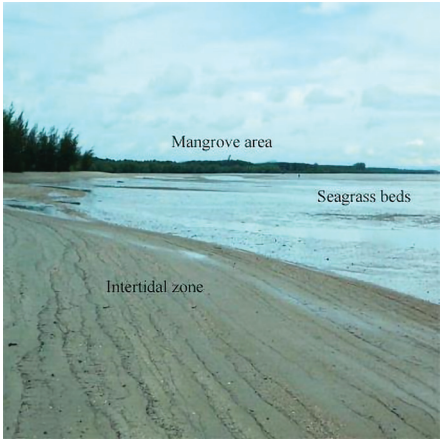

Fig. 3 Ecological habitat of peanut worms at Mod Tanoy Beach

Species identification

The external and internal morphology of a peanut worm from Mod Tanoy Beach is shown in Fig. 4A and 4B. Morphological examination of peanut worms collected from Mod Tanoy Beach, Trang, Thailand revealed close similarity with *S. nudus*, particularly the nephridia that was partially attached to the body wall. The brain was bi-lobed and *brain processes were dorsal and digitate or short* with *sponge-like mass* (Cutler, 1994). The digestive system was a recurved gut twisted into a *double helix*. The number of longitudinal muscle bands confirmed species identity as *Sipunculus nudus*.

Sex determination

Sixty peanut worms were placed under the light microscope for sex determination by observing the coelomic fluid through the semi-transparent body wall and then dissected for confirmation. Female peanut worms were easily identified by the presence of opaque white, free floating eggs. Male peanut worms were difficult to identify unless they were dissected (Fig. 5A–5D).

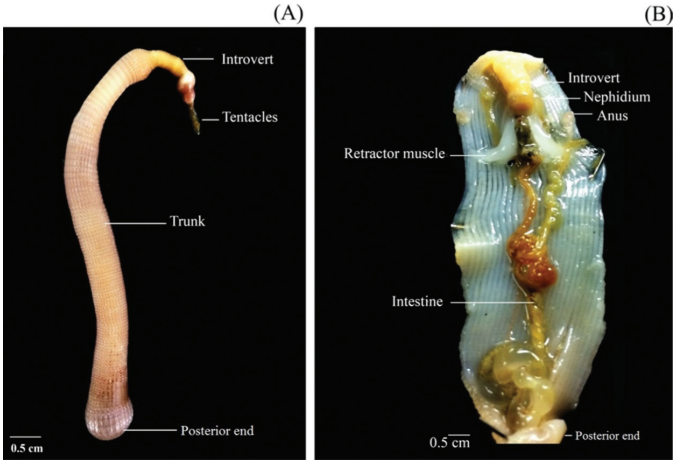

Fig. 4 Morphology of peanut worm Sipunculus nudus: (A) External characteristics; (B) Internal features (scale bar = 0.5 cm)

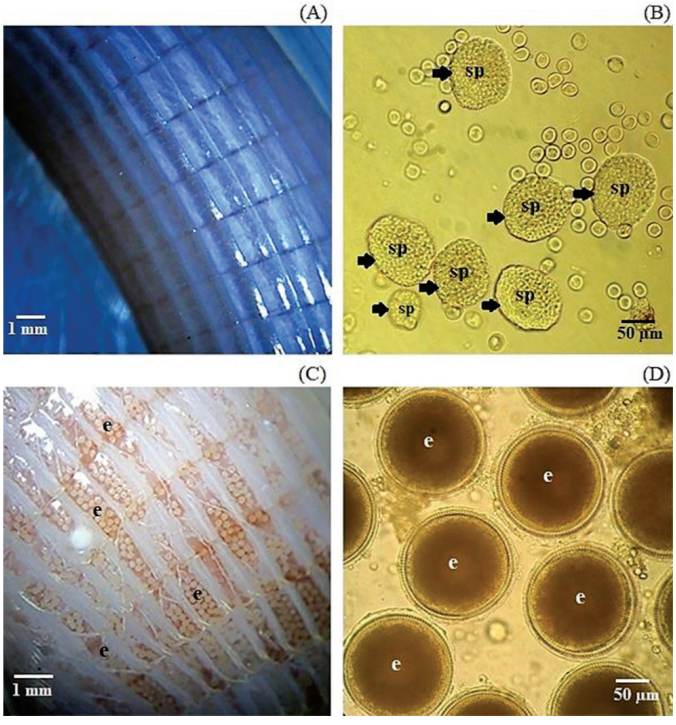

Fig. 5 Sex identification from external view and internal confirmation: (A) External body of male Sipunculus nudus, scale bar 1 mm; (B) Internal body, sperm plates freely floating in coelomic fluid (black arrows), scale bar 50 µm; (C) External body of female S.nudus, scale bar 1 mm; (D) Internal body, free floating eggs (e = egg; sp = sperm plate)

Sex ratio

The mean body lengths of male and female worms were 16.3 ± 2.2 cm and 16.8 ± 3.2 cm while mean body weights were 9.0 ± 1.7 g and 11.0 ± 2.1 g, respectively. From the total of 720 peanut worms examined, 49.16% were male and 50.84% were female (Fig. 6) with a sex ratio close to 1:1.

Stages of spermatogenesis

All three stages of spermatogenesis were found in each male *S. nudus* examined. Spermatocyte clusters (sperm plates) of various sizes were observed under the light microscope in male worms each month during the whole study period (Fig. 7A and 7B). Enlargements of spermatozoa *via* electron microscopy are shown as Fig. 7C and 7D.

Stages of oogenesis

Stage IV and V oocytes were most frequently observed in each female *S. nudus* examined throughout the study period. Stage I and II oocytes were also observed along with higher stages of development (Fig. 8A–8E). Combinations of Stages IV and V accounted for more than 80% of total oocytes observed each month. The size of Stage V coelomic oocytes was 180 µm and in only one case were mature oocytes found inside the nephridium (Fig. 9A).

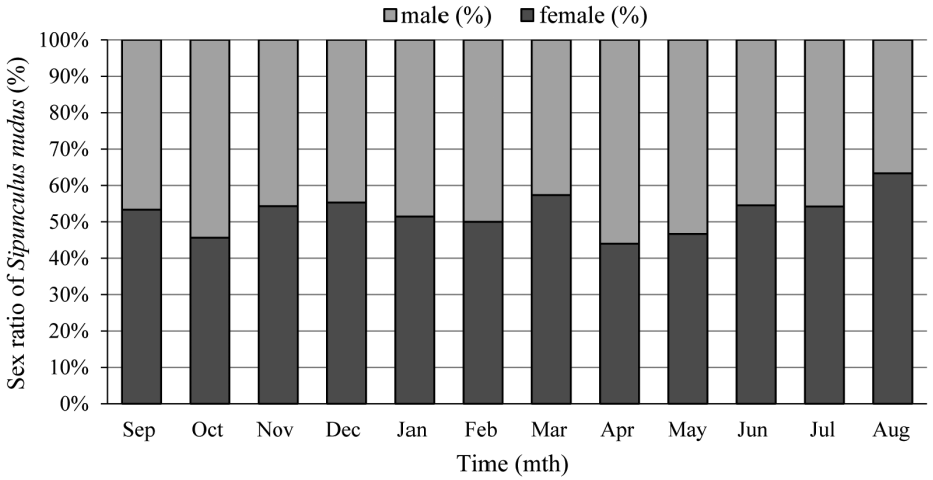

Fig. 6 Percentage of male and female S. nudus specimens collected monthly from September 2012 to August 2013

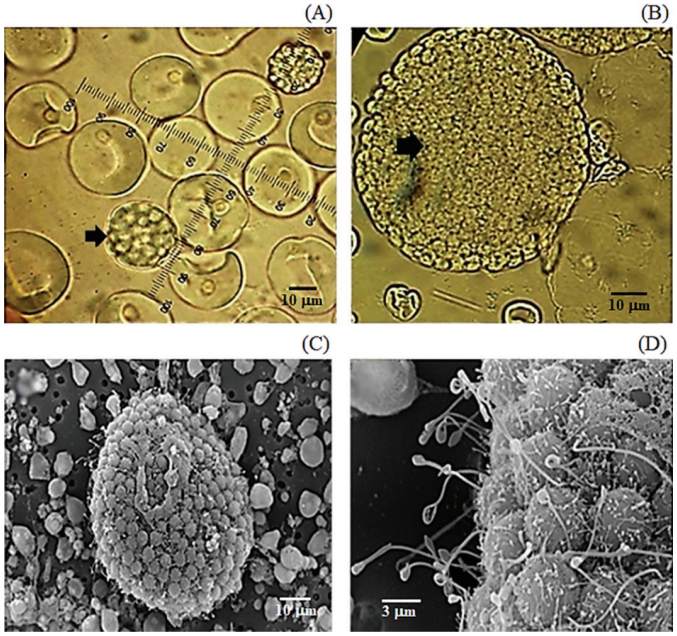

Fig. 7 Stages of spermatogenesis in Sipunculus nudus (black arrows) in coelom: (A) Stage I; (B) Stage II, light microscopy; (C–D) stage III, scanning electron microscopy

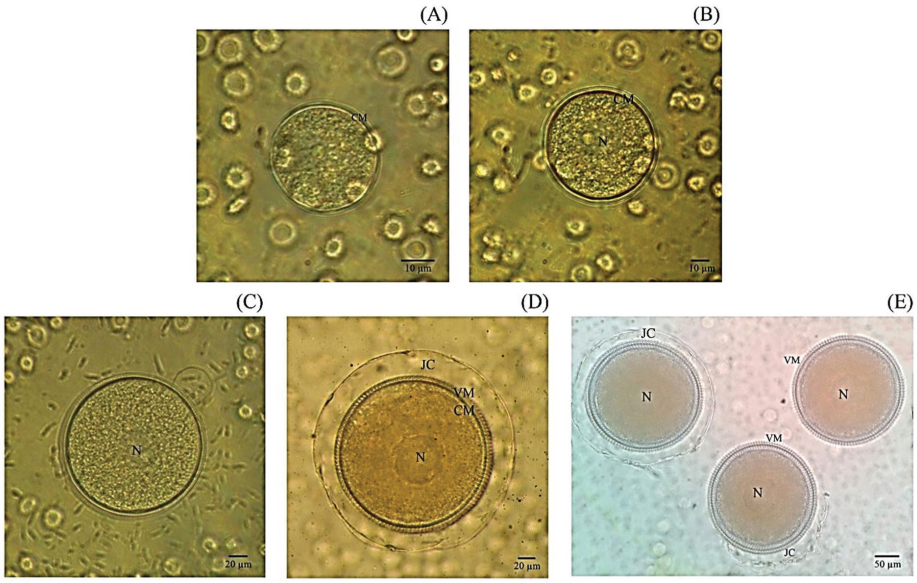

Fig. 8 Representative stages of coelomic oocytes of S. nudus under light microscopy: (A) Stage I (25–30 µm); (B) Stage II (60–70 µm); (C) Stage III (120–150 µm); (D) Stage IV (150–160 µm); (E) Mixed stage of jelly coat and maturation (jelly coat break down) (170–180 µm). Scale = 10 µm (A, B); 20 µm (C, D); 50µm (E). CM = Cell membrane; JC = Jelly coat; N = Nucleus; VM = Vitelline membrane

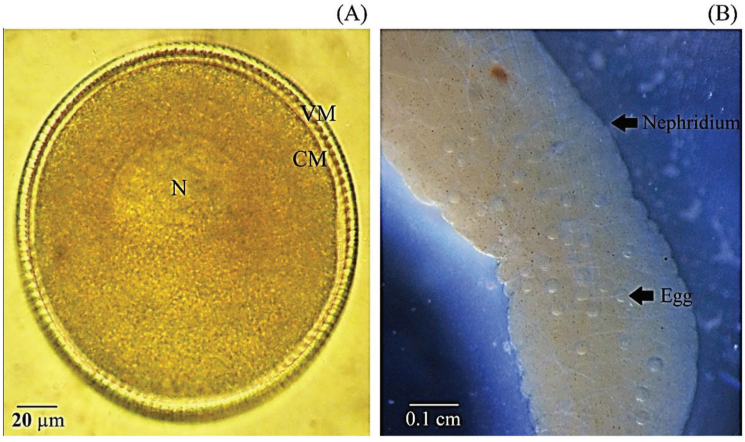

Fig. 9 Images showing: (A) Jelly coat of egg broken down (stage of maturation) 170–180 µm, electron micrograph scale bar 20 µm. (B) Eggs in nephridium, stereo micrograph scale bar 0.1 cm. CM = Cell membrane; N = Nucleus; VM = Vitelline membrane

Discussion

Mod Tanoy Beach is an ideal habitat for peanut worms due to the surrounding environment with mangroves onshore and sea-grass beds offshore. The mangroves continuously produce organic nutrients (Tantanasiriwong and Bhatia, 1976) and the seagrass beds provide a complex habitat for peanut worm larvae (Erftemeijer et al. 1993).

S. nudus has several genetically different populations and is found on all continents (Kawauchi and Giribet, 2013). Nineteen species of peanut worm were reported in Thailand by Hylleberg (1994, 2013) but *S. nudus* was not included. Therefore, this study represents the first record of *Sipunculus nudus* in Thailand.

S. nudus individuals were collected using a unique method because the conventional benthos method cannot be used to remove peanut worms from their burrows. This constraint yielded the result that only broodstock of the peanut worm were caught. Female worms were similar in size to male worms lengthwise but were heavier. Female worms were easy to identify since their coelomic oocytes could be seen from outside without visual aids. Identification of males required dissection to verify sex gametes under the microscope. The results of the current study confirmed a sex ratio of 1:1 in *S. nudus* from Thailand, similar to findings reported by Rice (1989). A sex ratio of 1:1 is advantageous for breeding programs especially if artificial fertilization is employed.

A comparison of mature peanut worm egg sizes is summarized in Table 1. The mature oocyte diameter was similar among and within species.

A comparison of mature coelomic eggs found during the year among *S. nudus* and other species of peanut worm is presented in Table 2. The breeding period of *S. nudus* in China was from April to September while the other four species (*Phascolosoma scolops, P. arcuatum*, *Siphonosoma cumanense* and *Aspidosiphon muelleri* Diesing, 1851) spawned during the summer (July–August). *Golfingia margaritacea* from Ushuaia, Argentina spawned from the end of February until April when the annual temperature was highest (Amor, 1993). Lan and Yan (2002) and Wang et al. (2005) reported the spawning season of *S. nudus* in the Gulf of Tonkin as April to September when water temperature is between 27 and 34°C. These phenomena agreed with conclusions by Cutler (1994) and Ying et al. (2009) that in temperate waters, marine invertebrates usually exhibit two-to-three-month peaks in reproductive activity during the summer depending on latitude.

Unexpectedly, mature oocytes of *S. nudus* from Mod Tanoy Beach were detected every month during the current study, indicating that spawning occurred throughout the year. This might have been due to the latitude of the study site in the tropical waters of the Andaman Sea. Moreover, natural food and nutrients for peanut worms were abundant since Mod Tanoy Beach is close to both mangroves and seagrass beds.

Unfortunately, mature oocytes were detected in only one nephridia. Rice (1983) suggesting that prior to spawning, fully grown oocytes were accumulated in the nephridia and stored for a short time before expulsion into the surrounding seawater to be fertilized. The lack of detection in the current study might have been due to oocytes being expelled during transportation from Trang to Bangkok or perhaps mature oocytes are not drawn into the nephridia until spawning time which might occur according to lunar phases.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 1 Summary of adult length and mature egg diameter in peanut worms

Family	Species	Size of adult (length mm)	Size of mature egg (μm)	Locality	Authors
Golfinglidae	*Golfingia vulgaris*	155	177	Roscoff, France	Gerould (1906)
Themistidae	*Themiste pyroides*	180	190	Vancouver Island, Canada	Rice (1967)
	Themiste lageniformis	26	145	Florida, USA; Hawaii, USA	Williams (1972)
Sipunculidae	*Phascolopsis gouldii*	165	180	Newport, RI, USA	Gerould (1906)
	Siphonosoma cumanense	200	122	Puerto Rico	Rice (1988)
	Sipunculus nudus	200	178	Puerto Rico; Florida, USA	Rice (1988)
	Sipunculus nudus	-	170	Xiamen, China	Quo and Li (1993)
	Sipunculus nudus	-	175	Beihai, China	Wu (1999)
	Sipunculus nudus	-	180	Gulf of Tonkin, China	Lan and Yan (2002)
	Sipunculus nudus	-	190	Zhanjiang, China	Wang et al. (2005)
	*Sipunculus nudus**	150	180	Trang, Thailand	this study

* Peanut worms in the current study

Table 2 Spawning months of peanut worms

Species	Month												Locality	Author
	J	F	M	A	M	J	J	A	S	O	N	D		
Golfingia margaritacea	-	+	+	+	-	-	-	-	-	-	-	-	Ushuaia, Argentina	Amor (1993)
Phascolosoma scolops	-	-	-	-	-	-	+	-	-	-	-	-	Kuroshima, Japan	Catalan and Yamamoto (1994)
Siphonosoma cumanense	-	-	-	-	-	-	+	+	-	-	-	-	Nishiwaki Beach, Japan	Catalan and Yamamoto (1994)
Phascolosoma arcuatum	-	-	-	-	-	-	+	+	-	-	-	-	Zhejiang, China	Ying et al. (2009)
Aspidosiphon muelleri	-	-	-	-	-	-	-	+	+	-	-	-	Southeastern Spain	Ferrero–Vicente et al. (2014)
Sipunculus nudus	-	-	-	-	+	+	+	+	+	-	-	-	Xiamen, China	Quo and Li (1993)
Sipunculus nudus	-	-	-	-	+	+	+	+	+	-	-	-	Beihai, China	Wu (1999)
Sipunculus nudus	-	-	-	+	+	+	+	+	+	-	-	-	Southern Beihai, china	Lan and Yan (2002)
Sipunculus nudus	-	-	-	-	+	+	+	+	+	-	-	-	Zhanjiang, China	Wang et al. (2005)
*Sipunculus nudus**	+	+	+	+	+	+	+	+	+	+	+	+	Trang, Thailand	This study

(+) = Mature eggs detected (spawning period); (-) = Mature eggs not found

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