Zoological Studies **61:** 7 (2022)
doi:10.6620/ZS.2022.61-07

Zoological Studies

Open Access

Predation of Oysters Using an Autonomic Pharynx in the Oyster Leech *Cryptostylochus* sp. (Polycladida: Stylochidae)

Ching-Jung Teng[1], Ying-Jung Su[1], Chih-Yu Yeh[1], and Wei-Ban Jie[1,*]

[1]*National Experimental High School at Hsinchu Science Park, No.300, Jieshou Rd, East Dist., Hsinchu City 300, Taiwan.*
**Correspondence: E-mail: wbjie@nehs.hc.edu.tw (Jie).*
E-mail: ally901119@gmail.com (Teng); h611119@nehs.hc.edu.tw (Su); rubyyehmail@gmail.com (Yeh)

Received 27 May 2021 / Accepted 28 January 2022 / Published 5 May 2022
Communicated by Benny K.K. Chan

Oyster farming has been threatened by marine flatworms for over 40 years, but few studies have been done to address this issue. Here, we examine the predatory behavior of the polyclad flatworm *Cryptostylochus* sp. from Penghu, Taiwan. Its predation process consists of three parts: the attack, invasion, and ingestion period. During the attack period, which begins 4 or more hours before the invasion, protruding pharynges form fragments that we call "autonomic pharynges". The autonomic pharynx is translucent white, membrane-like, and variable in length. Using time-lapse photography, we show that the pharynx moves around slowly and independently for roughly 10 hours before losing mobility. The autonomic pharynx moved toward the edge of the oyster shell and increases the frequency at which the oyster or clam opens and closes compared to the control group. The passageway demonstrates negative phototactic behavior in petri dishes. Linear regression showed a positive correlation between the number of autonomic pharynges and the clam mortality rate. Histological dissections showed that clam mantle tissue is externally digested by the autonomic pharynx. The fragments of multi-branched ruffled pharynx from polyclad flatworms slowly detach and play a critical role during the period of the attack on bivalves.

Key words: Stylochid flatworms, Polyclad, Predatory behavior, External digestion, Oyster farming.

BACKGROUND

Most polyclad flatworms are benthic carnivores (Newman and Cannon 2003), some of which feed on bivalves (oysters and mussels) and cause severe long-term damage to oyster farming (Pearse and Wharton 1938; Shu and Lin 1980; Ventilla 1984; Newman et al. 1993). Stylochid flatworms—acotylean polyclads in the family Stylochidae that are also known as oyster leeches—are most commonly associated with bivalves. Field investigations in Taiwan show that the mortality rate for oysters is correlated with the number of stylochid flatworms (Shu and Lin 1980). Every year, the density of stylochid flatworms begins increasing in May and peaks in August. Shu and Lin (1980) noted that, in 1979, the mortality rate for oysters peaked in August at 53%. In the wild, some oyster leeches can be found on the surface of oyster shells and others spawn on the inner layer of open shells (Shu and Lin 1980; Newman et al. 1993; Gammoudi et al. 2009; personal observation).

Oyster leeches have been harming oyster farming in many countries for years. For example, *Stylochus ijimai* Yeri and Kaburaki, 1918 attacks cultivated oysters and inflicts heavy damage in several parts of Japan (Kato 1944). In Northern Tunisia, the stylochid flatworm *Imogine mediterranea* feeds on commercial mussels and poses a significant threat to mussel farming under natural conditions (Gammoudi et al. 2017). Stylochid flatworms represent a hazard to the farming industry.

Citation: Teng CJ, Su YJ, Yeh CY, Jie WB. 2022. Predation of oysters using an autonomic pharynx in the oyster leech *Cryptostylochus* sp. (Polycladida: Stylochidae). Zool Stud **61:**7. doi:10.6620/ZS.2022.61-07.

Studies mainly focus on the anatomy, reproductive and embryonic development of acotylean flatworms, but they rarely investigate their predatory behavior (Gammoudi et al. 2012). In 1944, Kato noted that one stylochid flatworm, *Stylochus orientalis* Bock, 1913, was found at 75 m deep in the Taiwan Strait. Since then, there have been no taxonomic classifications of oyster leeches that have addressed their impact on the oyster industry in Taiwan.

Newman and Cannon (2003) found that, when the oyster leech moves inside the oyster, it wraps its body around the mollusk's flesh, thrusts out its pharynx and consumes the tissue externally. Field experiments by Gammoudi et al. (2017) showed that it takes 4.5 days to kill half of a bivalve population, and this period decreases as the size of the polyclad flatworm population increases. The flatworm extends its pharynx and disables the mollusk's adductor muscle, which irreversibly changes the mollusk and eventually kills it (Gammoudi et al. 2017). Lee et al. (2006) found that when *Imogine lateotentare* inserts its pharynx into the barnacle *Amphibalanus variegatus*, the barnacle cannot close its opercular valves properly, so the authors suggested that these stylochids may paralyze their barnacle prey with toxins. Jie et al. (2013) reported that the acotylean flatworms *Ilyella gigas* preys on intertidal crabs and alpheid shrimps (*Alpheus strenuus*) in tidal pools using the membranous and highly ruffled pharynx to digest the crustacean externally. However, the method by which the actively moving crustaceans are captured by relatively slow and soft marine flatworms is unknown.

Gammoudi et al. (2017) and Jie et al. (2013) proposed that marine flatworms feed through vitro predation using their highly ruffled pharynx. The feeding behavior of marine flatworms has been observed in the wild, but these observations still do not explain how the highly ruffled pharynx penetrates bivalves without the prey noticing. There are no long-term recordings of this behavior in the wild, probably because the flatworms live in areas with constant tidal changes and insufficient light. In this study, we create long-term recordings to closely examine the process of polyclad flatworm predation behavior in tanks. As oysters' shells are irregular in shape and covered with biofouling, it can be very difficult to observe the flatworm's transparent pharynx. Hence, this study also experimented with using clams as a substitute for oysters. Laboratory recordings allowed us to continuously observe the specimens at multiple angles and with a steady intensity of light to determine the role of the flatworm's ruffled pharynx.

MATERIALS AND METHODS

Sampling and identification

This study used 80 oyster leech samples that were similar in size collected from oyster farms in Baisha, Penghu (23.63°N 119.59°E), Taiwan, from April to September 2018–2020. These are the main months when oyster leeches prey (Shu and Lin 1980). Collection was suspended during both periods of October to April due to weather conditions. All flatworms were well-fed and mature enough for reproduction. The collectors noted that the flatworms were found perching on both the inner and outer surfaces of the oyster shells.

Specimens were collected, stored in a plastic bottle filled with seawater and transported to the laboratory. They were placed in a glass tank (45 × 25 × 32 cm) with a circulating filtration system with water at 28°C and fed oysters and clams every two weeks. Portuguese oysters, *Crassostrea angulata* now considered as *Magallana angulata* (Salvi and Mariottini 2021), and Asian hard clams (*Meretrix lusoria*) of similar sizes were purchased from Nanliao, Hsinchu and cultivated in glass tanks filled with seawater to allow us to observe them in captivity.

Seven individuals were set aside for identification using histology (Fig. 1). These specimens were fixed in 10% frozen formalin (diluted with seawater) for at least two days and then transferred into 70% ethanol prior to histological examination. All of the specimens from Penghu and their location data were deposited into the National Museum of Natural Science and the Chinese Culture University, Taiwan.

Predation study

The flatworms were starved for three days before the trials. In each set, 3–5 flatworms were placed into a transparent glass container (26 × 16 × 24 cm) with 2–3 live oysters or clams and videotaped. The entire predation process, which lasted for 12 hours, was recorded using time-lapse photography (GZ-RX500, JVC) from four different angles. The attack behavior of the flatworms and the reactions from the bivalves were analyzed using the video recording. These tests were replicated five times. Red light was used when light was insufficient during nighttime. We observed that the oyster shells vibrated, which is defined as closing, opening, then closing of the shell. The number of shell movements each hour within four hours before death was counted. The shell vibration frequency during the predation by flatworms calculated based on the cumulative shell valve openings and closing.

Collecting autonomic pharynges

The flatworm everted its pharynx when it was pressed onto the ventral surface with a paintbrush (Fig. 2). Multiple autonomic pharynges of similar sizes were collected from different flatworms in the following tests. The pharynges from different flatworms were then used in the preference test, histology study, and attack behavior test.

Preference test for autonomic pharynges

We conducted olfactory and dark preference tests to determine how autonomic pharynges oriented and moved. For the olfactory preference test, five pieces of autonomic pharynges were collected from different flatworms and placed in the middle of a test tube (148 mm long and 15 mm in diameter). A photometer (HR350, HiPoint) was used to ensure that both sides received an equal amount of light (5.2 lux). The clam meat was then placed at the closed end of the test tube. A video recorder was used to record the number of autonomic pharynges on both sides of the middle line. The experiment was replicated 15 times for a chi-square test. For each group, autonomic pharynges were defined as demonstrating an olfactory preference if more than two pieces of autonomic pharynges moved near the clam meat.

A dark preference experiment was also conducted to determine whether light intensity affects the autonomic pharynges' behavior. In the dark preference test, a petri dish (86 mm in diameter) was covered with a black bag and divided into two equal parts using a black plastic board. One side was illuminated by a white lamp and the other side remained in the dark (light side: 28.73 lux, dark side: 1.53 lux). The experiment was conducted in a dark room. For each experimental set, five pieces of autonomic pharynges were randomly collected from different flatworms and initially placed into the middle of the petri dish. The experiment was video recorded and the number of autonomic pharynges on each side was counted after one hour. For each group, autonomic pharynges were defined as showing a preference for darkness if more than two moved toward the dark side of the petri dish. The test was replicated 15 times for a chi-square test.

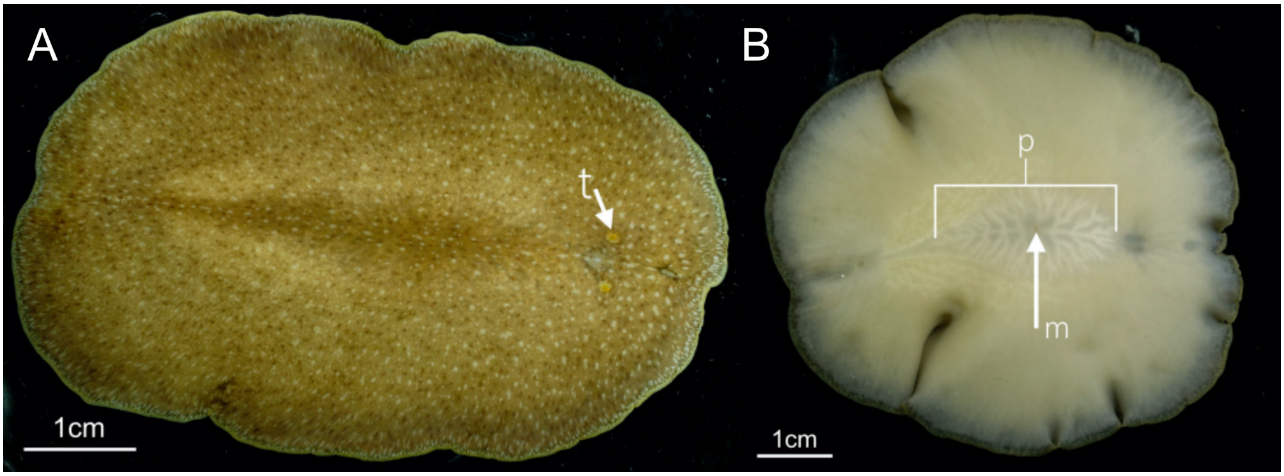

Fig. 1. *Cryptostylochus* sp. A, Dorsal view and B, ventral view. The pharynx is large and highly ruffled. The mouth is in the center of the pharynx ventrally. The head is on the right side. t, tentacles; p, pharynx; m, mouth.

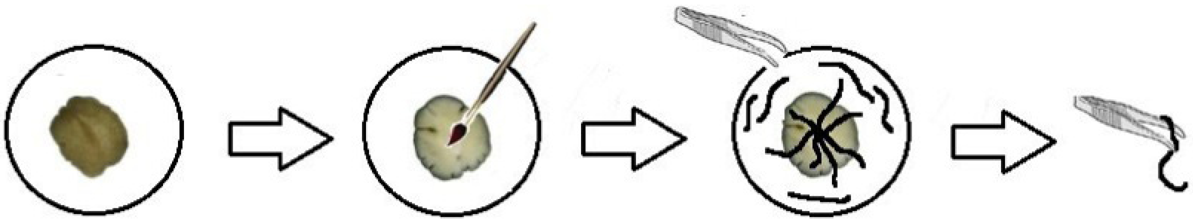

Fig. 2. Illustration of the collection of autonomic pharynges from *Cryptostylochus* sp. The flatworm is flipped over with its ventral surface facing upward. The highly ruffled pharynx is everted under stimulation by pressing the ventral surface with a paintbrush. The pharynx extends and spontaneously breaks into pieces of autonomic pharynges, which are collected by tweezers.

Attack behavior of autonomic pharynges

Attack behavior tests used clams but not oysters. Although oysters are the natural prey of the oyster leeches in oyster farms, the smooth shell surface of the clams allowed us to more clearly observe the autonomic pharynges, and thus it was easier to use clams as our model.

Five, 10, 15, 20, 25, and 30 pieces of autonomic pharynges were collected. Autonomic pharynges were placed on the surface of live clams in the experimental group, whereas in the control group nothing was placed on the clams. The clams were placed in glass containers and time-lapse photography was used to record for 12 consecutive hours. Clams were identified as alive or dead by evaluating whether the shell closed in response to external stimulus. The percent mortality was calculated once we determined how each individual reacted to the attack. The experiment was replicated five times. A linear regression model was created using Microsoft Excel 2019 23 VL 16.37 to determine the relationship between the presence of autonomic pharynges and clam mortality. Clams were determined to be alive or dead after 12 hours and the results were used for the chi-squared test of independence.

Histological study

Asian hard clam (*Meretrix lusoria*) mantles were used as a substitute for oyster tissue. To determine whether autonomic pharynges directly damaged the clams, histological sections were conducted to observe the microscopic anatomy of the damaged tissue of clam mantles. In the histological study, 15 clams were dissected into two halves. The clam mantles were kept and other viscera were removed. In the experiment group, 30 pieces of autonomic pharynges were placed on one of the halves, whereas no pieces were put on the other half (the control group). Both halves were then put in 35‰ seawater for observation. After 12 hours, the two halves were fixed and dehydrated by serial passage using 30%, 50%, 70%, 95% and 99.5% alcohol for two to four hours in total, then embedded in paraffin. These histological procedures were modified from the standard protocols of Jie et al. (2013).

RESULTS

Species identification

The specimens are oval-shaped, with a maximum length of 65 mm long and a maximum width of 42 mm. The Flatworms have brown dorsal backs scattered with dark and light brown dots. Specimens are fleshy with few marginal folds. Their cone-shaped head tentacles have light brown tips and eyespots at each base. The lateral sides are folded, with a translucent light brown rim. Their ventral sides are unmarked and the pharynx is large and ruffled and around one third the length of the body. The mouth is positioned in the center of the pharynx (Fig. 1).

Male and female gonopores are separated, drawing closer to the posterior end. Male copulation apparatus comprises seminal vesicles, a free prostatic vesicle and an unarmed penis papilla (Fig. 3A). The prostatic duct joins the ejaculatory duct at the base of the unarmed penis papilla. The elongated muscular prostatic vesicle is mono-glandular (Fig. 3B). The interior lining consists of numerous short tubes that are radially arranged close to each other along the inner border of the muscle wall. The seminal vesicles are anchor-shaped (Fig. 3C). Lang's vesicle is absent. Based on the morphology of paired tentacles, position of ruffled pharynx, arrangement of sexual apparatus and extravesicular prostatic glands, the Penghu oyster leech is a member of the family Stylochidae Stimpson, 1857 (Faubel 1983; Bulnes et al. 2005; Gammoudi et al. 2009). Bulnes et al. (2005) proposed that the genus *Cryptostylochus* be diagnosed by the presence of mono-glandular prostatic

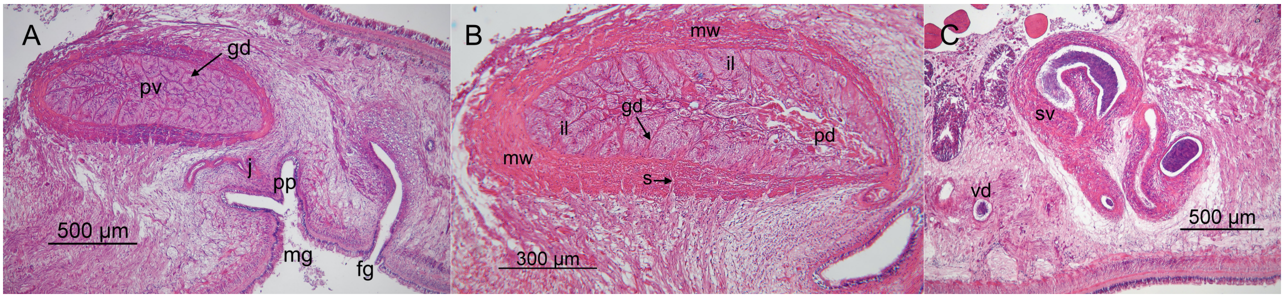

Fig. 3. Sagittal section of *Cryptostylochus* sp. A, Genital apparatus. B, Monoglandular prostate gland. C, Anchor-shaped seminal vesicle. fg, female gonopore; gd, glandular duct; il, interior lining; j, joint of prostatic duct and ejaculatory duct; mg, male gonopore; mw, muscular wall; pd, prostatic duct; pp, penis papilla; pv, prostatic vesicle; s, secretion; sv, seminal vesicle; vd, vas deferens.

glands. The Penghu oyster leech specimens have monoglandular prostatic vesicles so it was identified as a species of the genus *Cryptostylochus* Faubel, 1983.

Attack period before invasion

The entire predation process of *Cryptostylochus* sp. in Portuguese oysters (*Magallana angulata*) was observed in five replicate tanks over 12 hours. Each tank followed the same process: attack period, invasion, and ingestion period (Fig. 4). In the beginning, the Penghu oyster leech extended its white translucent pharynx towards the ventral edge of the oyster shell. The attack lasted for 4 to 8 hours. The attack from the flatworms using protruding pharynges did increase the oysters' shell movement until 4 hours before they died (Fig. 5). One to two hours before the oyster died, its shell vibrated to the maximum extent and then gradually failed to close (Fig. 4A). Although the flatworm moved near the oyster while extending its pharynx towards the shell edge, its range of motion was not restricted throughout the attack period in the tank. Polyclad flatworms may also extend their pharynx some distance away from the oyster. The stylochid flatworms invaded their prey after the oysters gradually lost the ability to close the shell. The polyclad flatworms crawled into the shell during this invasion period (Fig. 4B). Finally, the polyclad flatworms wrapped around the entire prey, not leaving any flesh in the shells. They stayed in the empty shells during the ingestion period until the digestion process was complete (Fig. 4C).

The attack period before the invasion was a long, slow process. It was very difficult to detect the attack, and observers have rarely been seen it in the wild. The

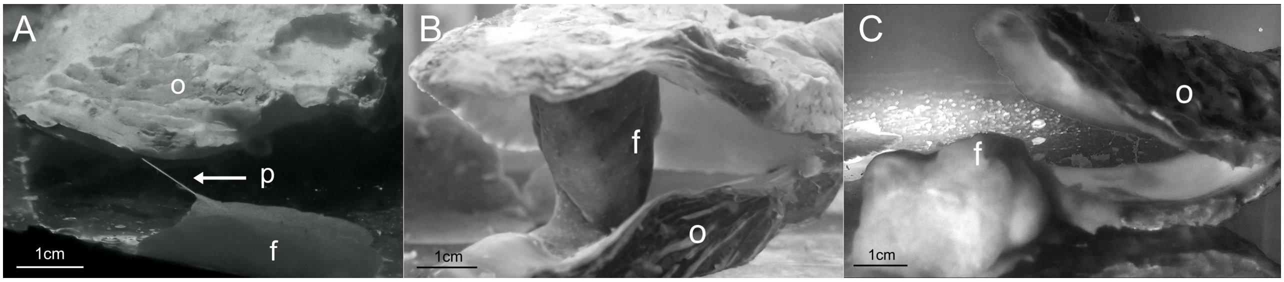

Fig. 4. The three predatory processes for *Cryptostylochus* sp. on Portuguese oysters (*Magallana angulata*). A, Attack period: flatworms extend their pharynx into the oyster from the shell opening for 4 to 8 hours and some autonomic pharynges are formed. B, Invasion period: the stylochid flatworms crawl into the shell after the oyster cannot resist and the oyster gradually loses its ability to close its shell. C, Ingestion period: the flatworm wraps the entire oyster flesh and separates it from the shell. f, flatworm; o, oyster; p, protruding pharynx.

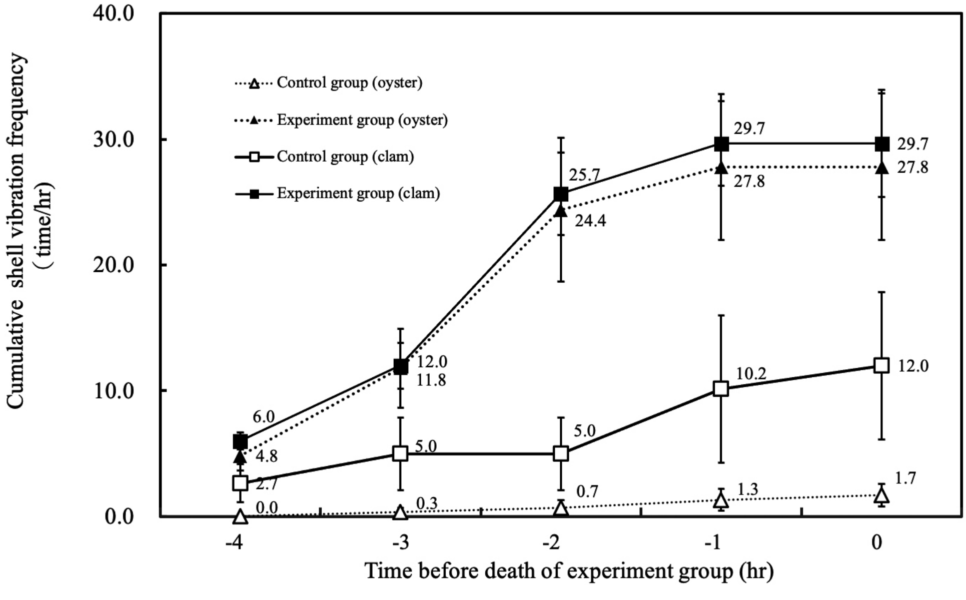

Fig. 5. Cumulative shell valve openings and closures frequency for Portuguese oysters (*Magallana angulata*) and Asian hard clams (*Meretrix lusoria*) during the attack period by *Cryptostylochus* sp. (each test used five each of oysters and clams).

autonomic pharynx was first found using time-lapse video recording. *Cryptostylochus* sp. has an extremely large and muscled ruffled pharynx in the center of its ventral surface (Fig. 1B). Time-lapse photography showed that the ruffled pharynx radiated white translucent branches outward and the polyclad flatworm controlled the protrusion and contraction of its pharynx. A protruding pharynx gradually formed fragments (Fig. 6). The autonomic pharynges were translucent white, membrane-like, and variable in length. It slowly moved around for roughly 10 hours before losing mobility. When the pharynx detached from the body, the movements and trajectories can be detected by time-lapse recording (Video S1). The formation of autonomic pharynges occurred at least 4 to 8 hours before the polyclad crawled into the shell of the prey.

Orientation preference of autonomic pharynges

Pharynx fragments, herein also called autonomic pharynges, can also be collected by physical stimulation in a petri dish. Although they have the ability to move independently in all directions, autonomic pharynges displayed no significant preference ($\mathcal{X}^2 = 2.25, P > 0.05$) for clam meat in the olfactory preference test. However, the autonomic pharynges demonstrated significant negative phototaxis ($\mathcal{X}^2 = 22.41, P < 0.01^{**}$)—when isolated pharynges move away from light and toward darker places (Table 1). This result showed that autonomic pharynges can automatically move toward and enter the shell of prey through the shell opening.

Behavior of the autonomic pharynx during the attack period

Various numbers of autonomic pharynges were placed on the surface of live clams and time-lapse photographed for 12 hours. The autonomic pharynges were observed to scatter randomly, with some moving under the shell or to the bottom of the container.

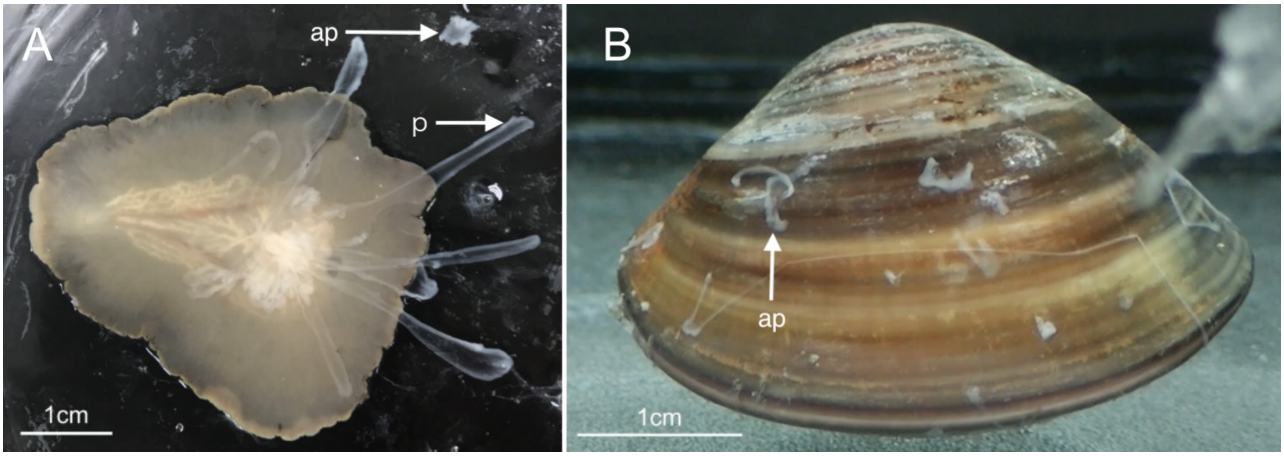

Fig. 6. A, Protruding pharynx of *Cryptostylochus* sp. and the autonomic pharynx shown by arrows. B, Collected autonomic pharynx placed on the surface of a clam shell to easily record and analyze the video results. Using the time-lapse video, we found that some autonomic pharynges slowly crawled around for roughly 10 hours before losing mobility. ap, autonomic pharynx; p, protruding pharynx.

Table 1. Preference of the autonomic pharynx of *Cryptostylochus* sp. n: number of groups (five pieces per group), total pieces, total number of autonomic pharynges

A, Olfactory preference test for 15 groups of autonomic pharynges		
	n (total pieces)	Expected value
Close to the clam meat	10 (44)	7.5 (37.5)
Away from the clam meat	5 (31)	7.5 (37.5)
B, Dark preference test for 15 groups of autonomic pharynges		
	n (total pieces)	Expected value
Dark side	14 (58)	7.5 (37.5)
Light side	1 (17)	7.5 (37.5)

These wandering pharynges did not attack, but some pharynges entered the clam through the shell opening, gradually increasing the openings and closures of the clams' valves. When there were more than 15 autonomic pharynges placed on the surface of the clam shell, there was a substantial increase in clam mortality (Fig. 7). The shells of attacked clams were gradually opened, clam mantles were atrophied, and viscera was separated from the shell. The clam eventually died (Video S2).

The highest mortality percentage was achieved when 30 pieces of autonomic pharynges were used. The number of autonomic pharynges was linearly correlated with clam mortality ($R^2 = 0.82$) (Fig. 7). The chi-squared test of independence showed that autonomic pharynges significantly affected clam mortality ($\mathcal{X}^2 = 17.36$, $P < 0.01^{**}$), but the control group showed no effects (Table 2). Autonomic pharynges independently opened the shells of clams, shrank mantles, and separated viscera, similarly to intact stylochid flatworms in clams or oysters in tanks.

For the histological observations, 30 autonomic pharynges were collected and placed on the surface of the dissected clam mantles for 12 hours in seawater at room temperature. The tissue of the mantle muscle displayed a large rupture, with pores unevenly distributed and muscle fibers loosened and torn apart, whereas the control samples had neatly arranged muscle fibers (Fig. 8). Based on the differences in muscle fiber arrangement, the histological sections clearly showed that the autonomic pharynges damaged the muscle tissue of the clam mantle.

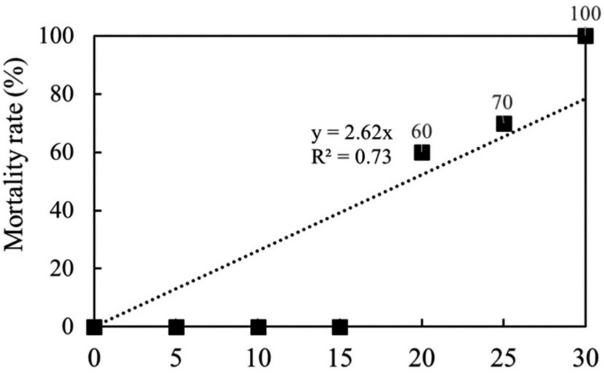

Fig. 7. Linear regression for the clam (*Meretrix lusoria*) mortality percentage on the number of autonomic pharynges of *Cryptostylochus* sp.

DISCUSSION

Oyster leeches like those in the family Stylochidae have a ruffled pharynx. During predation, the polyclad extends its pharynx and detaches the adductor muscle of bivalves (Gammoudi et al. 2017). However, the low mobility and translucent appearance of the autonomic pharynges makes observing the entire predation process almost impossible in the wild. In this study, the entire

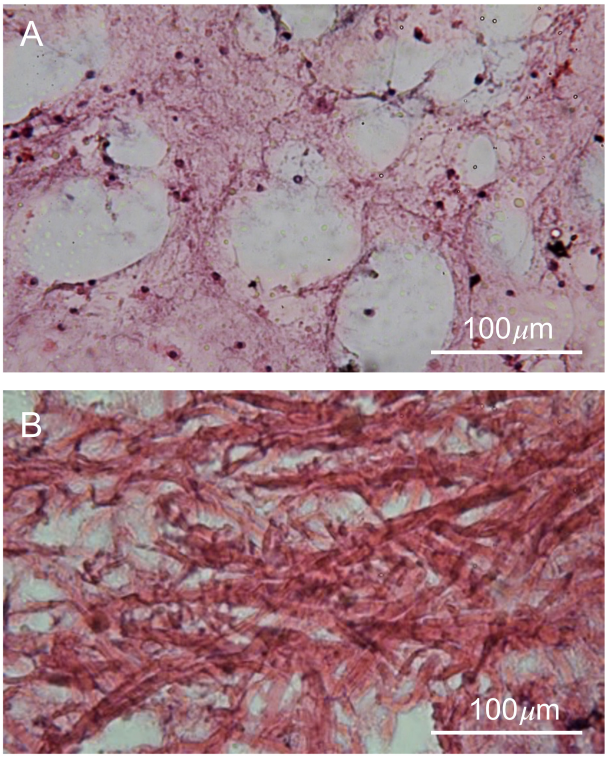

Fig. 8. Histological micrograph of Asian hard clam (*Meretrix lusoria*) mantle tissue. A, *Cryptostylochus* sp. after 12 hours of decomposition by 30 autonomic pharynges. B, Control group without autonomic pharynges.

Table 2. Relationship between the presence of autonomic pharynx of *Cryptostylochus* sp. and clam (*Meretrix lusoria*) mortality

	Dead	Alive
Control Group	0 (0%)	15 (100%)
Experimental Group	11 (73.3%)	4 (26.67%)

predation process for flatworms was recorded using time-lapse photography in the laboratory. A clearer observation of the pharynx was achieved by recording for a longer time and from multiple angles. The attack period is a long, slow process that is very difficult to detect, but our study tracks it for the first time.

The pharynx of the stylochid flatworms spontaneously breaks off and forms autonomic pharynges. Gammoudi et al. (2017) noted that stylochid flatworms move their protruding pharynges along the edges of the mussel's valves while attacking. This study shows that stylochid flatworms attack oysters and clams from a much farther distance. Our video recording shows that flatworms did not remain near the edge of the valves of the oysters and clams. Long before the polyclad started feeding, the polyclad flatworm might have sent autonomic pharynges piece by piece into the shell of the prey, causing the oyster shell vibration to gradually increase.

Oysters regularly open their shells for respiration, feeding and excretion (Bayne 2017), so small, slow and imperceptible autonomic pharynges can invade oysters and clams without triggering immediate noticeable changes in the prey's behavior. Our predation study shows that the frequency of oyster shell valve openings and closings do not increase until four hours before the oyster and clam dies, which implies that the autonomic pharynges can autonomically attack their prey long before the flatworms invade (Fig. 5). The presence of autonomic pharynges is critical for determining whether flatworm predation succeeds (Fig. 7).

Shell movements are proposed to be a means to assess how mussels respond a) to their environment and b) to predation (Robson et al. 2007; Robson et al. 2010). During the attack period, the autonomic pharynges crawled into the shell and the frequency of the clam shell valve movement increased. Our study shows that autonomic pharynges can elicit anti-predation behavior in clams and separate clam meat without the presence of intact flatworms (Fig. 7; Table 2).

The autonomic pharynges independently undergo negative phototaxis and crawl into the interior of the shell. Our results imply that negative phototactic behavior in autonomic pharynges may explain how the pharynges enter the shell interior instead of moving into the surrounding environment. Further experiments are required to confirm the phototactic behavior of autonomic pharynges in real situations. This behavior might also be a reason why oyster leeches are mostly found on the outer surface of oyster shells in oyster farms (collectors narrated).

The pharynx of polyclad flatworms incapacitates mussels by opening their valves and detaching their adductor muscle, which results in mussel mortality (Gammoudi et al. 2017). This study also showed that the collected autonomic pharynges can damage other tissue than just the adductor muscle. The histological damages caused by autonomic pharynges might damage and eventually kill the clams. This external digestive function of autonomic pharynges might be a new pattern of predation behavior in polyclad flatworms.

Ritson-Williams et al. (2006) noted that some flatworms use deadly neurotoxins to capture mobile prey, chiefly mollusks. We do not, however, know if *Cryptostylochus* species bear neurotoxins (personal preliminary test). Histological sections of the mantle also atrophied and muscle tissues ruptured, so autonomic pharynges may contain protease enzymes that a) decompose muscles and b) have external digestion functions. The clams' mortality rate is positively correlated with the number of autonomic pharynges on the clams' surfaces, so the more autonomic pharynges generated during the attacking period, the higher the chance of successful predation. This report determines the role of autonomic pharynges and shows the importance of autonomic pharynges during predation by stylochid flatworms.

In tanks, flatworms can prey on both oysters and clams through the exact same attack, invasion and ingestion periods, even though oysters and clams are different in many ways; this does not, however, suggest that stylochid flatworms are a natural predator of clams in the wild. Many polyclads feed on small gastropods, sessile ascidians, bivalves and barnacles (Galleni et al. 1980; Littlewood and Marsbe 1990; Murina et al. 1995; Newman and Cannon 2003; Ritson-Williams et al. 2006; Gammoudi et al. 2017). No cases have been reported of flatworms preying on Asian hard clams (*Meretrix lusoria*) in the wild. However, our study used clams as a new model animal and substitute for oysters to more clearly observe the predation behavior of flatworms. Although clams and oysters live in different habitats and differ in ingestion behavior, laboratory observations showed that the oyster leech also preyed on clams. Clams have anterior and posterior adductor muscles, while oysters only have anterior adductor muscles (Bayne 2017). Despite the many differences between oysters and clams, both increase shell movement and produce white debris when attacked. Clams are more common than oysters in local markets. Clam shells are much smoother than oysters' and it is easier to obtain a similar shell size than other bivalves, making clams suitable for statistics. Using clams as a substitute allows us to create a preliminary model for flatworm behavior studies. Replacing oysters with clams allowed us to clearly observe the entire flatworm attack process. Our use of clams as a substitute for oysters is also a novel discovery in this study.

CONCLUSIONS

In conclusion, this study agrees with Gammoudi et al.'s (2017) description of the flatworm attack period. We confirm that autonomic pharynges break off from the flatworms and crawl into the clam host's shell. Our results show that autonomic pharynges can decompose the clam mantle and further prevent the clams from closing their shells, eventually causing the clam to die when enough autonomic pharynges are present. The present work indicates that autonomic pharynges play a critical role during the predation process of the stylochid flatworm *Cryptostylochus* sp.

Acknowledgments: The authors express their sincere appreciation to Guo's Blessed Oyster in Penghu for their generosity collecting and transporting flatworms. The authors are also deeply grateful to Shih-Chieh, Kuo at the Chinese Culture University and Kwen-Shen, Lee at the National Museum of Natural Science for specimen storage. The authors are also grateful to Ping-Chiang, Lyu and I-Chen, Hu of the National Tsing Hua University for their advice on chemical analysis.

Authors' contributions: CJD conducted statistical analysis of data and analyzed videotape recordings. YJS was responsible for ensuring a stable source of flatworms and oysters. CYY contributed to setting up the environment for time-lapse photography. WBJ provided support in the identification of flatworm species. All authors cooperated on the experimental design and worked equally on the manuscript.

Competing interests: The authors have no competing interests.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent for publication: Not applicable.

Ethics approval consent to participate: Ethics approval does not apply.

REFERENCES

Bayne B. 2017. Biology of oyster. Academic Press, London, UK.

Bock S. 1913. Studien über Polycladen. Zool Bidr Uppsala **2:**31–344.

Bulnes VN, Faubel A, Park JK. 2005. Two new marine species from South Korea with remarks on the family Stylochidae (Acotylea, Polycladida, Platyhelminthes). J Nat Hist **39(23):**2089–2107. doi:10.1080/00222930500081997.

Faubel A. 1983. The Polycladida, Turbellaria. Proposal and establishment of a new system. Part I. The Acotylea. Mitt hamb zool Mus Inst **81:**189–259.

Galleni L, Tongiorgi P, Ferrero E, Salghetti U. 1980. *Stylochus mediterraneus* (Turbellaria: Polycladida), predator on the mussel *Mytilus galloprovincialis.* Mar Biol **55:**317–326. doi:10.1007/BF00393784.

Gammoudi M, Ben Ahmed R, Bouriga N, Ben-Attia M, Harrath AH. 2017. Predation by the polyclad flatworm *Imogine mediterranea* on the cultivated mussel *Mytilus galloprovincialis* in Bizerta Lagoon (northern Tunisia). Aquac Res **48:**1608–1617. doi:10.1111/are.12995.

Gammoudi M, Noreña C, Tekaya S, Prantl V, Egger B. 2012. Insemination and embryonic development of some Mediterranean polyclad flatworms. Invertebr Reprod Dev **56:**272–286. doi:10.1080/07924259.2011.611825.

Gammoudi M, Tekaya S, Noreña C. 2009. Contribution to the knowledge of acotylean polyclads (Platyhelminthes, Polycladida) from Tunisian coasts. Zootaxa **2195:**43–60. doi:10.11646/zootaxa.2195.1.3.

Jie WB, Kuo SC, Wu SC, Lee KS. 2013. Unreported predatory behavior on crustaceans by *Ilyella gigas* (Schmarda, 1859) (Polycladida: Ilyplanidae), a newly-recorded flatworm from Taiwan. Platax **10:**57–71.

Kato K. 1944. Polyclads of Japan. J Sigenkagaku Kenkyusyo **1:**257–318.

Lee KM, Beal MA, Johnston EL. 2006. A new predatory flatworm (Platyhelminthes, Polycladida) from Botany Bay, New South Wales, Australia. J Nat Hist **39:**3987–3995. doi:10.1080/00222930500485263.

Littlewood DTJ, Marsbe LA. 1990. Predation on cultivated oysters, *Crassostrea rhizophorae* (Guilding), by the polyclad turbellarian flatworm, *Stylochus* (*Stylochus*) *frontalis* Verrill. Aquaculture **88:**145–150. doi:10.1016/0044-8486(90)90289-Y.

Murina GV, Grintsov V, Solonchenko A. 1995. *Stylochus tauricus*, a predator of the barnacle *Balanus improvisus* in the Black Sea. Hydrobiologia **305:**101–104. doi:10.1007/BF00036369.

Newman LJ, Cannon LRG, Govan H. 1993. *Stylochus* (*Imogene*) *matatasi* n. sp. (Platyhelminthes, Polycladida): pest of cultured giant clams and pearl oysters from Solomon Islands. Hydrobiologia **257:**185–189. doi:10.1007/BF00765011.

Newman LJ, Cannon LRG. 2003. Marine Flatworms. The World of Polyclads. CSIRO Publishing, Australia.

Pearse AS, Wharton GW. 1938. The oyster "leech" *Stylochus inimicus* Palombi, associated with oysters on the coast of Florida. Ecol Monogr **8:**605–655. doi:10.2307/1943085.

Robson AA, Garcia De Leaniz C, Wilson RP, Halsey LG. 2010. Behavioural adaptations of mussels to varying levels of food availability and predation risk. J Mollus Stud **76:**348–353. doi:10.1093/mollus/eyq025.

Robson A, Wilson R, de Leaniz CG. 2007. Mussels flexing their muscles: a new method for quantifying bivalve behaviour. Mar Biol **151:**1195–1204. doi:10.1007/s00227-006-0566-z.

Ritson-Williams R, Yotsu-Yamashita M, Paul VJ. 2006. Ecological functions of tetrodotoxin in a deadly polyclad flatworm. Proc Natl Acad Sci **103:**3176–3179. doi:10.1073/pnas.0506093103.

Salvi D, Mariottini P. 2021. Revision shock in Pacific oysters taxonomy: the genus *Magallana* (formerly *Crassostrea* in part) is well-founded and necessary. Zool J Linnean Soc **192:**43–58. doi:10.1093/zoolinnean/zlaa112.

Shu JU, Lin YS. 1980. Biological studies on the oyster predator, *Stylochus inimicus*. CAPD Fisheries Series **3:**39–51.

Ventilla RF. 1984. Recently development in the Japanese oyster culture industry. Adv Mar Biol **21:**1–57. doi:10.1016/S0065-2881(08)60098-X.

Supplementary Materials

Video S1. Time-lapse video converted from 5 minutes of continuous taping of the collected flatworm autonomic pharynxes crawling on the surface of a clam shell. (download)

Video S2. Time-lapse video converted from 27 minutes of continuous taping of the collected flatworm autonomic pharynxes causing the clam's shell valve to open, close, and eventually die. (download)