Rhizocephalans and how they turn crabs into zombies: the case of sacculinid *Sacculina beauforti* on orange mud crab *Scylla olivacea*

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Keywords: Rhizocephalans infection*, Sacculina beauforti, Scylla olivacea,* aquaculture, Infection diagnosis

Rhizocephalans are a group of parasitic barnacles that infect crustacean hosts, significantly altering their morphology, physiology, and behavior. Due to the lack of relevant knowledge, infected crabs can be inadvertently introduced into crustacean aquaculture systems, potentially causing irreversible damage. Therefore, the prevention, treatment, and dissemination of knowledge related to rhizocephalans are crucial. This study summarizes the distribution of common rhizocephalans and their hosts in Southeast Asia. One representative rhizocephalan parasite, *Sacculina beauforti*, has detrimental effects on the economically important orange mud crab *Scylla olivacea*, exemplifying how these parasites can turn crabs into "zombies." The study details the journey of a tiny barnacle, from the initial infection of the host to preparing the 'incubator,' and discusses the potential impacts of rhizocephalan infection on aquaculture and fisheries, including broodstock selection, crab culture, soft-shell crab production, and breeding. Additionally, the study reviews existing methods for diagnosing infected crabs, such as morphological observations, established discriminant function equations, and molecular screening. Rhizocephalan infections pose significant risks to the aquaculture of non-fully cultured crustacean species. Once detected, infected crabs should be immediately culled and removed to prevent further spread. In conclusion, this understanding has enabled us to develop a series of recommendations focusing on future research, management, and aquaculture development efforts.

The journey of a tiny parasitic barnacle

Crustaceans serve as a crucial food resource, offering high-quality protein and vital economic support to coastal fisheries and aquaculture communities globally (Waiho *et al*., 2021). However, the intensification of aquaculture has escalated the risks of stress and disease outbreaks, with parasites being a common cause of these diseases (Waiho *et al*., 2022). Among these parasites, Rhizocephalans (Crustacea, Cirripedia), or parasitic barnacles, lack most typical crustacean characteristics, such as segmentation and appendages, and specifically target other crustacean hosts, inducing significant morphological, physiological, and potentially behavioral alterations (Høeg, 1995). Due to the lack of specialized knowledge and improper handling, these parasites could be accidentally introduced into mud crab culture systems. There is still no known treatment for rhizocephalan infections, thus, culling the whole cultured population is the only option, leading to substantial losses (Waiho *et al*., 2021).

As one of the most diverse and advanced parasitic infraclasses in the Metazoa, Rhizocephalans have a unique way of infecting hosts. Traditionally, they were broadly classified into Akentrogonida and Kentrogonida based on different life history strategies, depending on the presence or absence of a kentrogon larva during the cypris metamorphosis stage (Glenner & Hebsgaard, 2006; Hiller *et al*., 2015). However, recent research using 18S gene sequences of rhizocephalans has shown that the previously established taxonomic classification of these two orders is obsolete, as both are polyphyletic (Høeg *et al*., 2020). In this review, we maintained the use of Akentrogonida and Kentrogonida for a more precise and informative depiction of the invasion process.

Throughout the whole life cycle of rhizocephalans, each female infects only a single host once (Glenner & Hebsgaard, 2006; Hiller *et al*., 2015). In general, the life cycle of Kentrogonid rhizocephalans (**Figure 1**) begins with the mature externa releasing free-swimming and non-feeding male and female nauplii, with males being significantly larger than females (Høeg & Rybakov, 1992; Høeg *et al*., 2020). Subsequently, the female nauplius molts into a cyprid and then into a kentrogon stage (Waiho *et al*., 2021). The free-swimming female cyprid then attaches to the newly molted, unhardened exoskeleton, or the exposed soft tissues between the joints of a crustacean host. At this stage, it transforms into a kentrogon equipped with a hollow, cuticle-reinforced probe that pierces the host's exoskeleton, directly transferring parasitic material into the host's hemolymph via the female cyprid's antennae (termed as vermigon; Høeg, 1985; Glenner, 2001). Upon successful infection, rhizocephalans develop a root system (interna) within the host's body, coiling around most of the host's internal organs to absorb nutrients and exert neuroendocrine regulation (Høeg, 1995; Waiho *et al*., 2021). The externa typically emerges within the brood chamber or within the interior part of the abdomen. At this stage, the virgin externa necessitates the entry of a male cyprid to ensure its continued growth, mature sexually, and reproduce (Høeg, 1985). When oocytes mature in the externa, they are fertilized by the sperm produced by the male within the sac. New sexually dimorphic nauplii will be released from the externa after they mature.

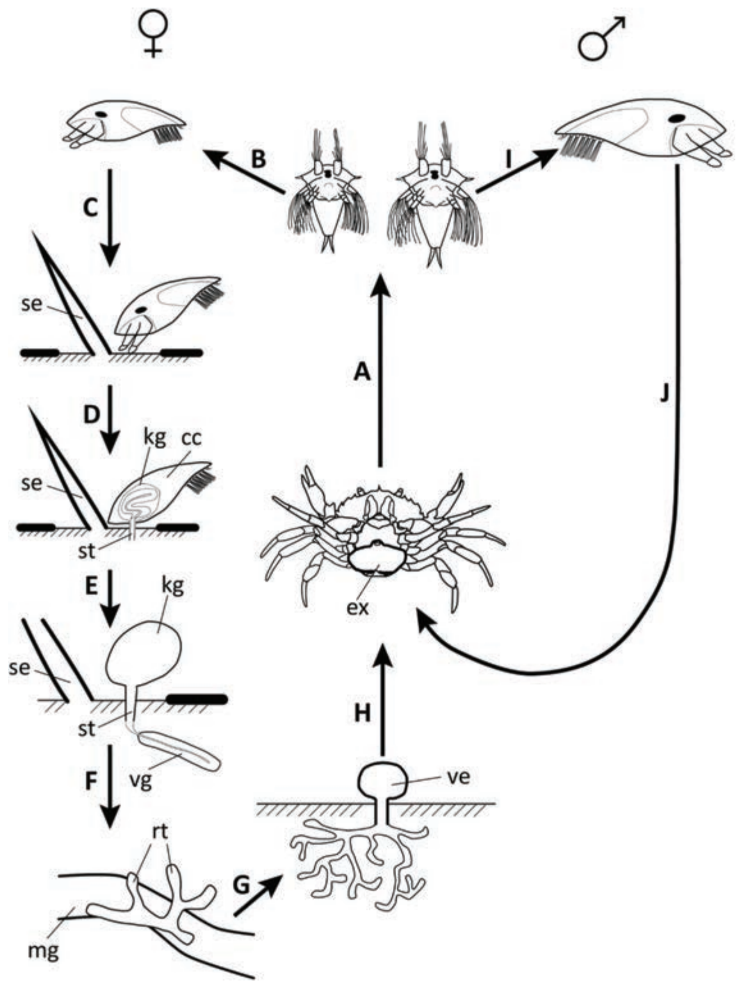

Figure 1. General life cycle of a kentrogonid rhizocephalan (ex: externa; se: setae; kg: kentrogon; cc: cyprid; st: stylet; vg: vermigon; mg: host's midgut; rt, rootlet; ve: virgin externa) Reprinted with permission from Waiho *et al*. (2021)

The prevalence of rhizocephalans in Southeast Asia

Rhizocephalans show a high degree of host specificity and typically parasitize one or only a few host species. However, the changes they brought about significantly impact aquaculture, particularly for potential commercially valuable species, in coastal countries such as those in Southeast Asia (Waiho *et al*., 2021) (**Table 1**). The prevalence of rhizocephalan parasites has been reported in recent years, including China (Yoshida *et al*., 2014; Yang *et al*., 2018), Indonesia (Bhagawati *et al*., 2021), India, Malaysia (Waiho *et al*., 2017; Waiho *et al*., 2020; Fazhan *et al*., 2020), Philippines (Jung *et al*., 2021), Singapore (Jung *et al*., 2021), Thailand (Moser *et al*., 2005), and Viet Nam (Glenner & Hebsgaard, 2006), (**Table 1**).

The documented rhizocephalan parasites in Southeast Asia can inform fishers in specific geographical regions about the potential occurrence of which rhizocephalans on which crustacean host species. This information can help bridge the knowledge gap for fishers and fish farmers in particular area (**Table 1**). For example, *S. olivacea*, a key species among *Scylla* species, plays a crucial role in supporting coastal livelihoods and contributing to the economic growth of Southeast Asian countries as well as Australia, Japan, and Pakistan (Keenan *et al*., 1998; Albert-Hubatsch *et al*., 2016). Parasitic sacculinid *S. beauforti* infections are prevalent in *S. olivacea* populations in East Malaysia and Thailand (Moser *et al*., 2005; Waiho *et al*., 2017). In this review, we use *S. beauforti* and its host *S. olivacea* as an example to elucidate the significant impacts that rhizocephalan parasites have on their host (**Figure 2**).

Table 1. The geographic distribution and host species of general rhizocephalan species in Southeast Asia

Rhizocephalan species	Host species	Geographic distribution	Collection site	References
Sacculina beauforti	Orange mud crab, *Scylla olivacea*	Malaysia	Marud Bay-Sabah	Waiho *et al.*, 2017; Waiho *et al.*, 2020; Fazhan *et al.*, 2020
S. beauforti	Orange mud crab, *S. serrata*	Malaysia		Boschma, 1949
Loxolhyiacils ihlei	Orange mud crab, *S. serrata*	Malaysia; northern Australia		Boschma, 1949; Knuckey *et al.*, 1995
S. serenei	Crucifix crab, *Charybdis feriata*	Malaysia	Kedah	Unpublished
Diplothylacus sinensis	Blue swimming crab, *Portunus pelagicus*	Malaysia	Johor	Unpublished
S. beauforti	*S. olivacea*	Thailand		Moser *et al.*, 2005
Sacculina sp.	Mole crab, *Albunea symmysta*	Indonesia	Parangkusumo Beach, Yogyakarta	Bhagawati *et al.*, 2021
Thompsonia japonica	*Lophozozymus dodone*	Indonesia	Sanur, Bali	Glenner *et al.*, 2003
S. leptodiae Guérin-Ganivet	Xanthid crab, *Leptodius exaratus*	Singapore	Labrador Beach	Glenner *et al.*, 2003
Thompsonia littoralis	Xanthid crab, *L. exaratus*	Singapore	Labrador Park Beach	Glenner *et al.*, 2003
S. leptodiae	Xanthid crab, *L. affinis*	Singapore	Labrador	Jung *et al.*, 2021
Peltogaster plana	White-lined rock crab, *Grapsus albolineatus* (Lamarck)	Taiwan, China	Kenting National Park	Yoshida *et al.*, 2014
P. postica	Narrow-clawed hermit crab, *Pagurus angustus*	Taiwan, China	Chisi, Penghu, Shetoshan, Penghu	Yoshida *et al.*, 2014
Septosaccus cf. snelliusi	Hermit crab, *Diogenes tumidus*	Taiwan, China	Shetoshan, Penghu	Yoshida *et al.*, 2014
Dipterosaccus indicus	Morgan's hermit crab, *Calcinus morgani*	Taiwan, China	Haikou, Renting, Shizuwan, Kaohsiung, and Houwan	Yoshida *et al.*, 2014
Polyascus planus	Thukuhar shore-crab, *Metopograpsus thukuhar*	Taiwan, China		Liu & Lützen, 2000
S. sinensis	*Leptodius exaratus*	Hong Kong, China		Glenner & Hebsgaard, 2006
S. sinensis	Smooth coral crab, *Leptodius affinis*	Hong Kong, China		Jung *et al.*, 2021
Polyascus gregaria	Chinese mitten crab, *Eriocheir sinensis*	China	Yangtze Estuary, Shanghai	Yang *et al.*, 2021
S. lata	Swimming crab, *Charybdis miles*	China	Beibu Gulf, South China Sea	Yang *et al.*, 2018
Diplothylacus sinensis	Three-spotted crab, *Portunus sanguinolentus*	China	Honghai Bay, South China Sea	Yang *et al.*, 2014
D. sinensis	Three-spotted crab, *P. sanguinolentus*	Viet Nam	South of Nha Trang	Lützen & ThiDu, 1999; Yang *et al.*, 2014
Thylacoplethus squillae	Three-spotted crab, *P. s sanguinolentus*	Viet Nam	South of Nha Trang	Lützen & ThiDu, 1999
Pottsia serenei	Three-spotted crab, *P. s sanguinolentus*	Viet Nam	South of Nha Trang	Lützen & ThiDu, 1999
P. serenei	Mantis shrimp, *Squilla* sp. *(Squillidae)*	Viet Nam		Glenner & Hebsgaard, 2006
Octolasmis angulata	Swimming crab, *P. pelagicus*	Viet Nam	North, central, and south of Viet Nam	Dang *et al.*, 2021

Table 1. The geographic distribution and host species of general rhizocephalan species in Southeast Asia (*Cont'd*)

Rhizocephalan species	Host species	Geographic distribution	Collection site	References
O. angulata *O. alata* *O. warwicki* *Dianajonesia tridens*	Swimming crab, *Charybdis truncata*	Viet Nam	Nha Trang Bay	Ha *et al.*, 2024
S. beauforti	Box crab, *Calappa lophos*	India	Southeast Coast	Dayalane *et al.*, 2023
Sacculina sp.	Three-spotted crab, *P. sanguinolentus*	India	Parangipettai coastal waters, southeast coast	Raffi *et al.*, 2012
S. compressa	Tubercles crab, *Ozius tuberculosus*	Philippines	Panglao	Jung *et al.*, 2021
S. insueta	Riedel's fiddler crab, *Ptychognathus riedelii*	Philippines	Kawasan	Jung *et al.*, 2021
Sesarmaxenos gedehensis	Mangrove crab, *Sesarmops* sp.	Philippines	Kawasan	Jung *et al.*, 2021

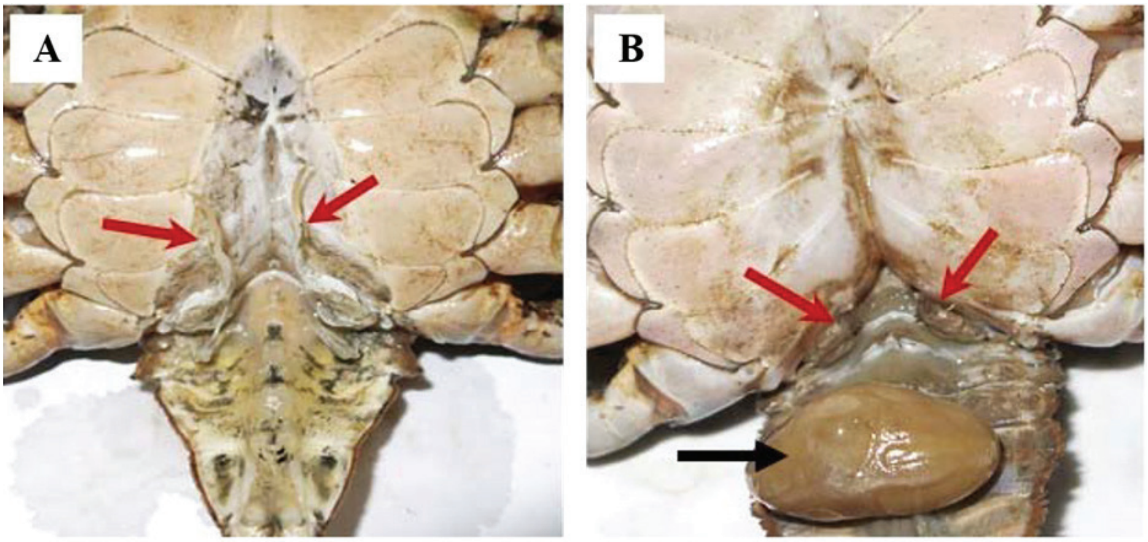

Figure 2. Differences in the abdomen and gonopod length of normal and infected crab, *S. olivacea* (A) normal male crab, (B) infected male crab with the presence of externa (black arrow) and the reduced gonopods (red arrows) with permission from Fazhan *et al.*, 2018

Getting the 'incubator' ready

Rhizocephalan barnacles, especially sacculinids, are known for their host-castrating effect after infection (Hartnoll, 1962). During the emergence of externa, they are capable of inducing morphological, physiological, and potentially behavioral changes in their hosts (Hartnoll 1967; Fazhan *et al.*, 2018; Fazhan *et al.*, 2020; Waiho *et al.*, 2020). One of the most notable transformations caused by *S. beauforti* in *S. olivacea* is the alteration of the male's narrow abdomen into a female-like broadened abdomen (Waiho *et al.*, 2017). Consequently, primary sexual characteristics such as the male's gonopods (copulatory appendages) and the female's gonopores/spermathecae (spermatophore storage) are significantly reduced, as they are less relevant to the reproduction of rhizocephalans (Waiho *et al.*, 2017). These changes ultimately lead to infertility in the infected hosts. In addition, compared to normal individuals, infected crabs show higher body weight and smaller body size (Waiho *et al.*, 2017; Fazhan *et al.*, 2018).

Apart from extreme morphological alterations, the internal physiological changes induced by rhizocephalans are also noteworthy. The gonads (testes and ovaries) are primary targets of infection. In infected testes, apart from remnants of spermatozoa, no viable germ cells are found, indicating arrested spermatogenesis. Rootlets were also observed in the testes of infected individuals. The infected ovaries are similarly damaged, showing no discernible cellular structure (**Figure 3**) (Fazhan *et al.*, 2020). As the primary tissue responsible for nutrient absorption, storage, vitellogenesis during growth, and ovarian development, the hepatopancreas suffers severe damage from the parasite (Fazhan *et al.*, 2020).

Although the diameter of the hepatopancreatic tubules is unaffected, there is a significant reduction in their number in infected individuals. The hepatopancreatic tissue of infected crabs appears loosely packed, with rootlets observed in the intertubular spaces.

Besides morphological and physiological alterations, rhizocephalan infection also induces distinctive behavioral changes in hosts (Waiho *et al*., 2021). Especially in males, they exhibit spawning behaviors typically associated with females following parasite infection (Rasmussen, 1959). Rhizocephalans exert control over their hosts, causing them to accept the invading parasite's externa as a part of themselves, exhibiting maternal-like care behaviors such as regular abdominal ventilating and grooming until parasite hatching occurs (Takahashi *et al*., 1997). Upon maturation, nauplii are expelled through the aperture of externa, while the host engages in spawning behaviors like tiptoeing and abdominal ventilation to help disperse the larvae (Yamaguchi *et al*., 1999). Due to the inability to molt after the emergence of the externa, infected hosts are more susceptible to epizoic infestation, leading to increased grooming behavior (Bishop & Cannon, 1979; Mouritsen & Jensen, 2006) and reduced engagement in burying or hiding, which has been shown to significantly decrease epibiont colonization (Becker & Wahl, 1996; Innocenti *et al*., 1998; Innocenti *et al*., 2003).

Additionally, the protective and territorial behavior typically exhibited by males, such as aggression, is significantly reduced in rhizocephalan-infected males (Wardle & Tirpak, 1991; Innocenti *et al*., 2003; Vázquez-López *et al*., 2020). This reduction in aggression is believed to increase the lifespan of both the host and the parasite by minimizing the risk of host mortality due to aggression-related injuries. In contrast,

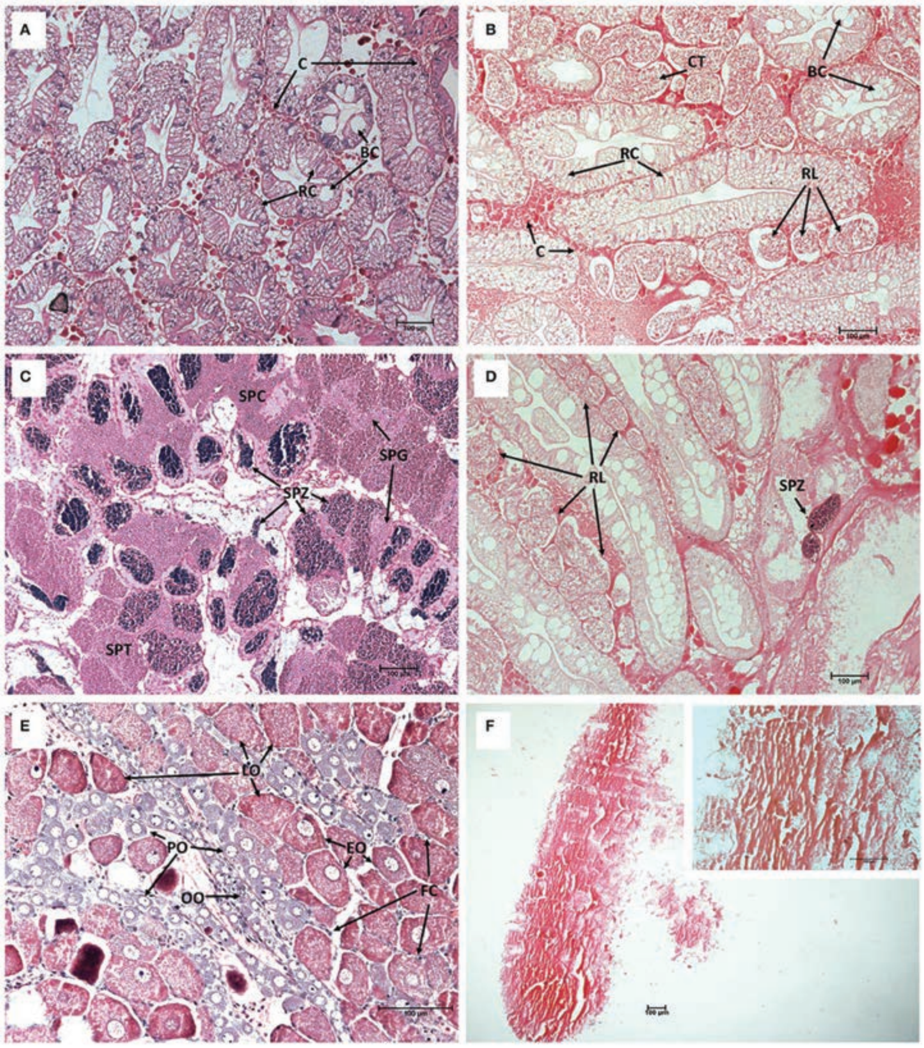

Figure 3. Histological cross-sections (Mayer's Hematoxylin-Eosin staining) of hepatopancreas (A), testis (C), and ovary (E) of healthy crab *S. olivacea* versus hepatopancreas (B), testis (D), and ovary (F) of infected *S. olivacea*. A: Healthy hepatopancreas with normal tubule shape and arrangement, magnification 10 ×; B: Infected hepatopancreas with the presence of rhizocephalan rootlets (RL) and collapsed tubules (CT), magnification 10 ×; C: Healthy testis with the presence of spermatogonia (SPG), spermatocytes (SPC), spermatids (SPT) and spermatozoa (SPZ), magnification 10 ×; D: Infected testis with the presence of RL, almost no spermatogenesis activity, cell lysis and only remnants of SPZ could be found, magnification 10 ×; E: Healthy ovary with the presence of oogonia (OO), primary oocytes (PO), early-maturing oocytes (EO), and late-maturing oocytes (LO), with follicle cells (FC) surrounding oocytes, magnification 20 ×; F: Infected ovary appeared degenerated and no rigid cell structures were observed, magnification 4 × (large panel) and 20 × (upper right panel). Connective tissues (C), R-cells (RC), B-cells (BC). Reprinted with permission from Fazhan *et al*., (2020).

aggression in females often increases following infection (Innocenti *et al*., 2003). Besides aggression, rhizocephalan infection also impacts the host's respiratory and feeding behaviors (Waiho *et al*., 2021). Ultimately, all these changes induced by the rhizocephalan *S. beauforti* aim to transform the host (*S. olivacea*) into an incubator for the development of the externa.

Impact on biodiversity, fishery, and aquaculture

Rhizocephalan infections can severely impact the health of mud crab populations, as mud crab aquaculture still heavily relies on wild populations for broodstock. The most significant impact of rhizocephalan infections on crustacean aquaculture is the reduction in the reproduction and growth of crustaceans. Notably, there is a prolonged latency period during which rhizocephalans remain inside the host before any visible external morphological changes and/or the emergence of the externa. Crustacean hosts in the early stages of infection, where the externa has just appeared, or infected females with minimal externa, often go unnoticed and are easily mistaken as healthy individuals (Waiho *et al*., 2021). Additionally, infected male individuals may be mistaken for berried females by fishers due to severe feminization characteristics (Li *et al*., 2011; Waiho *et al*., 2017). These misidentifications can have serious implications for broodstock selection in aquaculture. In most crabs, individuals infected by rhizocephalans exhibit smaller body size and weight, as well as reduced feeding behavior, making fattening efforts difficult to succeed (Belgrad & Griffen, 2015; Waiho *et al*., 2017).

Another profitable aspect of crab aquaculture is the cultivation of soft-shell crabs. This practice capitalizes on the growth of crustaceans during molting, where within a brief period of hours after shedding their old exoskeleton, the new exoskeleton remains soft and expands by absorbing water (Shafer *et al*., 1995; Waiho *et al*., 2015). Several species such as *S. olivacea* (Eubion *et al*., 2020), *Portunus pelagicus* (Azra & Ikhwanuddin, 2015), and *Callinectes sapidus* (Oesterling 1988) are commonly used in soft-shell crab production. These crabs are all documented to be susceptible to rhizocephalan infections (Alvarez *et al*., 1999; Yang *et al*., 2014; Waiho *et al*., 2017). Although the molting process may still occur, reduced feeding activity, pronounced feminization characteristics post-molt, and the potential for rhizocephalan larvae to spread through the aquatic system if not promptly detected can lead to the production of smaller, lower-quality soft-shell crabs with longer production cycles, ultimately reduces profitability for crab farmers.

Furthermore, in recent years, intra- and inter-species breeding of crabs, especially mud crabs, has received significant attention and has become a possibility (Fazhan *et al*., 2017). However, rhizocephalan infections significantly hinder these efforts by inducing profound changes in hosts, thereby impeding attempts to enhance desirable traits in crabs (Waiho *et al*., 2020; Miroliubov *et al*., 2023). Because infected individuals become sterile or experience significantly reduced reproductive performance, even in primary reproductive organs (*i.e*. males' gonopods and females' pleopods), this greatly hinders normal mating behaviors and successful reproduction (Waiho *et al*., 2017).

Methods of identifying an infected crab

Up to now, there are no known treatments for rhizocephalan infections. However, proper management and prevention strategies can be employed to mitigate the impact of these parasites (Waiho *et al*., 2021). To prevent the inadvertent introduction of rhizocephalans into aquaculture facilities, the first step is the accurate identification of infected individuals to avoid selecting them. The most evident characteristic of rhizocephalans infestation is the presence of an externa on the abdomen (Sherman *et al*., 2008). Additionally, the size and length of secondary sexual organs, such as gonopods and pleopods, below the abdomen may be reduced. While this method is simple and quick, it requires species-specific expertise to accurately identify the rhizocephalan parasite.

Based on the visible morphological alterations from rhizocephalans, Fazhan *et al*. (2018) developed two functions to predict the infection status of *S. beauforti* in *S. olivacea*, achieving success rates of over 93 % in males and 100 % in females. The functions utilized abdomen width (AW), carapace width (CW), gonopod length (GL), and pleopod length (PL) as morphological data references and serve as a simple, quick, and non-invasive method for detecting *S. beauforti* infections in *S. olivacea*. Specifically, two functions, *i.e*. function 1 = $(94.027 \times AW / CW) - (115.906 \times GL / CW) + (33.561 \times GL / AW) + (1.117 \times CW / GL) - (3.812 \times AW / GL) - 29.932$ for males and function 2 = $(31.8 \times PL / CW) - (0.164 \times CW / PL) - 5.793$ for females.

Another method involves molecular screening through polymerase chain reaction (PCR) for detecting infections, as demonstrated in identifying *Loxothylacus texanus* in *Callinectes sapidus* and *Sacculina carcini* (Sherman *et al*., 2008; Mouritsen *et al*., 2018). This method allows for the detection of rhizocephalans infection in the early stages before externa formation. However, it involves sample disinfection, followed by extraction of hepatopancreas or digestive gland tissues, DNA extraction, PCR detection, and sequencing (Mouritsen *et al*., 2018). These steps are relatively cumbersome and impractical for large-scale screening in aquaculture systems.

For the sustainable development of fisheries and aquaculture industries, any crustaceans found infected with rhizocephalans in the wild or aquaculture systems should be promptly euthanized rather than discarded into the environment or left unattended (Basson, 1994). This is because infected crabs are sterile and malnourished, serving as living incubators for parasites. They are unable to contribute to spawning and, if the parasites' larvae hatch into the aquaculture system, the consequences can be severe. These larvae may parasitize any potential host available, ultimately causing irreversible economic losses to fish farmers. Additionally, in daily aquaculture operations, strict water quality control and regular sanitation measures are essential (Prema *et al*., 2020). Maintaining optimal water quality helps reduce stress on marine organisms, potentially lowering their susceptibility to infections (Prema *et al*., 2020). Regular cleaning and disinfection of aquaculture equipment and facilities help prevent the spread of parasites (Prema *et al*., 2020).

Conclusion

The interaction and relationship between rhizocephalans and their crustacean hosts is intriguing. These parasites regulate not only the morphology and physiology of their hosts but also induce behavioral changes, offering novel insights and potential solutions for challenges in crustacean aquaculture. Current methods to detect parasite infections include morphological observations, established discriminant function equations, and molecular screening. However, developing solutions that target host-parasite interactions—such as immune response, host finding, genetics, and pharmacological aspects—could potentially offer a long-term solution.

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