INVITED REVIEW
doi: 10.2306/scienceasia1513-1874.2025.s010

ScienceAsia 51S (1): 2025: ID 2025s010: **1–12**

Pathogenesis and innate immune response to the microsporidian *Ecytonucleospora* (*Enterocytozoon*) *hepatopenaei* (EHP) infection in shrimp

Pattana Jaroenlak, Warumporn Yingsunthonwattana, Anchalee Tassanakajon[*,†]

Center of Excellence for Molecular Biology and Genomics of Shrimp, Department of Biochemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330 Thailand

[*]Corresponding author, e-mail: anchalee.k@chula.ac.th

Received 28 Jan 2025, Accepted 21 May 2025
Available online 28 May 2025

ABSTRACT: *Ecytonucleospora hepatopenaei* (EHP) is a microsporidian parasite that causes growth retardation and size variation, resulting in severe economic losses. In this review, we summarize the pathogenesis and host immune response to the EHP infection and highlight recent studies that are progressing our understanding on the EHP infection mechanism. EHP forms environmentally resistant spores as parts of its life cycle. The characteristics of the EHP spores vary between different shrimp hosts. EHP utilizes an invasion organelle called the polar tube to infect the host. The polar tube is used as a conduit to transfer EHP's nucleus and possibly other infectious cargos into host cytoplasm. EHP contains a compacted genome with ∼3.26 Mbp in size. Several metabolic genes are absent in EHP, making EHP rely solely on host for nutrients. EHP-infected shrimp are more susceptible for secondary infections. Transcriptomic and proteomic analyses of EHP-infected shrimp identified several immune genes and proteins that are differentially expressed after the parasite infection. Immune signaling pathways such as Toll, JAK/STAT pathways and the prophenoloxidase-activating cascade are mainly induced while apoptosis might be suppressed to facilitate the EHP invasion. Antimicrobial peptides such as a c-type lysozyme reduce EHP infection by inhibiting EHP spore germination while lectins promote spore aggregation. Oxidative stress contributes to the pathology of EHP and the antioxidant system balancing the excess reactive oxygen species to protect host cell damage. Further research on pathogenesis mechanisms and host-pathogen responses is required for developing and implementing strategies for prevention and control of EHP infection.

KEYWORDS: *Ecytonucleospora hepatopenaei*, EHP pathogenesis, hepatopancreatic microsporidiosis, polar tube firing, shrimp immunity

INTRODUCTION

Thailand is one of the world's largest shrimp producers which exports more than 603,995 tons in 2011. However, the shrimp production drastically dropped by ∼50% in 2020 [1]. The major cause of this reduction is due to infections from several pathogens, including viruses, bacteria, and parasites [2]. The infections of these pathogens lead to morbidity and mortality, resulting in severe economic losses [3]. Recently, a disease caused by a fungi-related parasite called hepatopancreatic microsporidiosis (HPM), has been found to be a serious threat to the shrimp industry worldwide [4]. The causative agent of HPM is a microsporidian parasite *Ecytonucleospora hepatopenaei* (EHP, formerly known as *Enterocytozoon hepatopenaei*) [5]. HPM is associated with serious size variation and growth retardation. Ponds infected with EHP showed reduction in feed intake, resulting in lower biomass production [6]. It has been estimated that EHP infection causes more than 76 million US dollars loss annually in Thailand, and over $571 million US dollars loss in India [3, 7]. Currently, EHP outbreaks have been reported in many major shrimp producing countries, for example Thailand, Indonesia, Vietnam, China, India, South Korea, and Venezuela [5, 8–11]. Typically, EHP and other microsporidian species develop environmentally resistant spores as a part of their life cycles [12]. These spores are equipped with a specialized invasion organelle called the polar tube [13]. The polar tube is a hundred-micron long tube which usually coils inside the spore like a spring [14]. When the spores are under suitable environments, the polar tube is rapidly fired out of the spores and later pierces the host cell membrane [14]. The polar tube serves as a conduit for the parasite to release its infectious materials into the host [13]. Once the parasite is in the host, it starts to undergo nuclear division and develop into new spores. Host cells fully loaded with newly synthesized spores bursts and these spores are released into external environments [15]. For EHP, the parasite uses a hepatopancreatic cell as a host. The infection causes sloughing of the hepatopancreatic tubules [5]. This results in malfunction of hepatopancreas – a major organ in digesting food and producing several enzymes [6]. As a result, EHP-infected shrimp experience growth retardation.

Although the EHP infection has led to huge economic losses to the shrimp aquaculture industry, the knowledge on shrimp's immune response to this fungal-related parasite has been poorly understood.

[†]2024 Outstanding Scientist of Thailand.

Lacking an adaptive immune system, shrimps mainly depend on innate immunity to fight against invading pathogens [16, 17]. The innate immune system serves as the first line of defense which is composed of cellular and humoral responses. The cellular immune responses are cell-mediated immunity such as phagocytosis, nodulation, encapsulation, and apoptosis [18] while the humoral immune responses include antimicrobial peptide (AMPs), melanization and blood clotting system [19]. Invading pathogens are recognized through the pattern recognition receptors (PRRs), which bind to pathogen-associated molecular patterns (PAMPs) and activate several immune signaling pathways to secrete immune effectors such as AMPs, to eliminate the pathogens. Integrated multi-omics analyses provide insights into molecular responses of shrimps to various stressors including pathogen infections [20–22]. Transcriptomic and proteomic analyses of EHP-infected shrimp reveal differentially expressed genes and proteins after the parasite infection, but the mechanisms involved are not well-documented [23–26]. Immune signaling pathways such as Toll, JAK/STAT pathways and the prophenoloxidase (proPO)-activating cascade are mainly induced while others are suppressed to facilitate EHP invasion.

In this review, we summarize recent studies on pathogenesis and the immune response to EHP infection which provide basic information essential for understanding and preventing the infection.

EHP PATHOGENESIS

Characteristics of the EHP spores

Like other microsporidian species, EHP exists outside the host in the form of spores which are environmentally resistant [27]. The shape of the EHP spore is oval, and composed of two spore wall layers, including a proteinaceous, electron-dense exospore and a chitin rich, electron-lucent endospore [5] (Fig. 1). EHP employs a harpoon-like invasion organelle called the polar tube in order to infect host cells. The polar tube of EHP has approximately 20–30 µm in length and is typically coiled inside the spores [5]. Ultrastructural studies of the EHP spores showed that the EHP spore characteristics vary depending on shrimp host species [5, 28]. Comparison between the EHP spores isolated from *P. monodon* and *L. vannamei* reveals variations in spore size, numbers of polar tube coils, and thickness of the spore wall layers [28]. EHP spores from *L. vannamei* (EHP_{Pv}) are slightly bigger than those from *P. monodon* (EHP_{Pm}). The average size of the spores is 1.65 ± 0.15 µm $\times$ 0.92 ± 0.05 µm in EHP_{Pv}, while it is 1.1 ± 0.2 µm $\times$ 0.6 ± 0.2 µm in EHP_{Pm} [5, 28]. In addition, the numbers of polar tube coils are different. It is reported that the polar tube of EHP_{Pm} is organized into 5–6 coils, while it is 4–5 coils in EHP_{Pv} [5, 28]. However, it is important to note that these numbers of the polar tube coils are visualized from 2D electron micrographs which greatly depend on how the sections were cut. The accurate polar tube coils should be quantified from 3D reconstruction of the spores [14]. Surprisingly, the thickness of the spore wall layers in EHP_{Pv} is ∼4 times thicker than those of EHP_{Pm}. The exospores layer is 10–15 nm thick for EHP_{Pv} and ∼2 nm thick for EHP_{Pm}. While the endospore thickness ranges between 40–60 nm in EHP_{Pv}, compared to ∼10 nm in EHP_{Pm} [5, 28]. Inside the mature EHP spores, they contain several organelles, and they seem to be similar between EHP_{Pv} and EHP_{Pm} [5, 28].

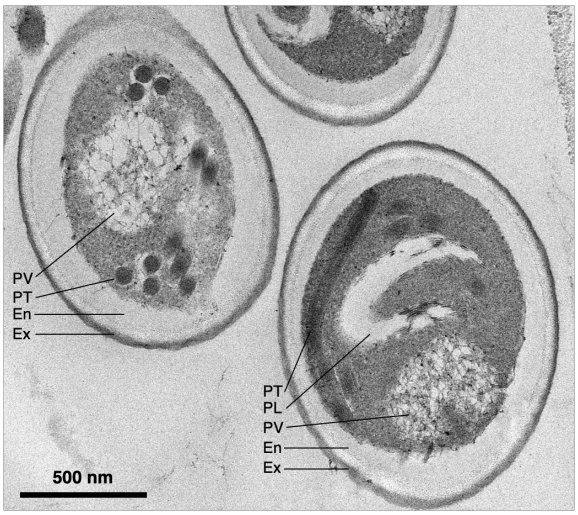

Fig. 1 The ultrastructures of the EHP mature spores. Mature EHP spores were isolated from hepatopancreas of EHP-infected *L. vannamei*. Ex = exospore layer, En = endospore layer, PT = polar tube, PV = posterior vacuole, and PL = polaroplasts.

The polar tube is connected to the anchoring disk which is located at the anterior end of the spore [28]. The thickness of the spore wall at this anterior end is the thinnest compared to other regions. It is suggested that the thinnest part of the spore wall determines where the polar tube is protruded out of the spore [14]. Similar to other species, EHP polar tube can be divided into two parts, including a straight segment which is connected to the anchoring disk, and a polar tube coil located to the posterior part of the spore [28]. The straight part of the EHP polar tube is surrounded by lamella polaroplasts and posterior polaroplasts [28]. It is hypothesized that the polaroplast might provide new membranes during infectious material transport [29]. The EHP spores contain a single nucleus, situated at the lower right of the spore center [28]. Polyribosomes are also clearly observed in the mature spores. They are tightly associated with polaroplasts and the polar tube coils [28]. The function of these polyribosomes is unclear. It is plausible that the polyribosomes are important for parasite protein translation once it is in-

side the host cell. In addition, posterior vacuole can be observed at the posterior end of the EHP spores [28]. In other microsporidian species, the posterior vacuole is expanded during the spore germination. However, the function of the posterior vacuole in EHP remains unclear.

EHP infection biology and its life cycle

In order to start the infection process, microsporidian spores need to fire their polar tubes, followed by the translocation of infectious materials into host cells (Fig. 2A). The primary infection site of EHP in shrimp is found in cytoplasm of the hepatopancreatic cells, including B-cells, E-cells, F-cells, and R-cells [4, 5, 28]. Two possible mechanisms have been proposed on how the microsporidian spores gain an entry into the host cells. First, the spore germination is triggered by suitable stimuli inside the host. Due to high forces generated during the polar tube firing and its rapid time scale, it is hypothesized that the polar tube may pierce the host cell membrane [14, 30]. Later, the infectious materials or sporoplasms travel through the polar tube into the host cell cytoplasm where it is deposited. The second mechanism suggests that the spores possibly enter the cells by phagocytosis [27]. The study was done in human-infecting microsporidian *Encephalitozoon cuniculi*. The phagocytosed spores are encapsulated in the phagosome compartment, which later be fused with lysosomes. Lower pH in the phagolysosome potentially triggers the polar tube germination. The polar tube then transfers the infectious materials into the neighboring cell's cytoplasm [12, 27]. It is still largely unclear what mechanism EHP takes to gain an entry into shrimp hepatopancreatic cells.

The polar tube firing or spore germination in microsporidia is a one of the fascinating, ultrafast processes in biology [30]. The spores typically germinate when they encounter host cells or under suitable environments (Fig. 2A). It remains unknown what physiological conditions within the host can induce the germination process. Many studies have been focused on the *in vitro* germination conditions [31–34]. Typically, conditions to trigger spore germination vary greatly among microsporidian species, for example *Encephalitozoon* spp. require hydrogen peroxide and high salt concentrations [35]. While dehydration and pH shift are common triggers for *Nosema* spp. [35]. In EHP, a red, water-soluble dye, phloxine B can be used to initiate polar tube firing [36]. The polar tube firing happens on an extremely fast timescale (less than 2 s) [14]. Due to its fast nature, it is challenging to study the germination process. Recent works have utilized high-speed live cell imaging to better understand the kinetics of polar tube firing in three microsporidian species that infects mosquitoes and humans, including *Anncaliia algerae*, *Encephalitozoon hellem*, and *Encephalitozoon intestinalis* [14]. The results showed that the polar tube firing can be divided into 3 phases, including (1) polar tube elongation, (2) polar tube static phase, and (3) emergence of the infectious material or sporoplasm [14]. In the first phase, the polar tube elongates to its maximum length, which takes ∼200 ms for *Encephalitozoon* spp. and 500 ms for *A. algerae* [14]. Velocity of the polar tube elongation is extremely high, reaching 235 µm/s in *A. algerae* and 336 µm/s in *E. hellem* [14]. At this velocity, the polar tube possibly generates enough forces to penetrate the host cell membrane. After the polar tube reaches the maximum length, the polar tube enters the second phase where the length of the polar tube is stable. It is possible that the infectious material is transported through the polar tube at this time [14]. In phase 3, the infectious cargo emerges at the distal end of the polar tube [14]. The sporoplasm appears as a membrane bound structure [37]. Composition of the sporoplasm is not known. However, it has been shown that nucleus and ribosomes are found in this compartment [37] (Fig. 2B). From this kinetics study, variations between species are clearly observed, even between closely related-species such as *E. hellem* and *E. intestinalis* [14]. For EHP as well as other microsporidian species that infects aquatic animals, the kinetics of the polar tube firing remain largely unexplored.

Once the parasite is inside the host, it starts to divide its nucleus by binary fission. This stage of EHP is called a plasmodial stage. Branched plasmodia typically grow around the host nucleus [28, 38]. Since EHP and other microsporidian species are well known to have a compact genome, many of the genes involved in metabolic pathways have been lost [39]. These gene losses are compensated with the expansion of transporter proteins, possibly facilitating the parasite to uptake energy and nutrients from the host [40]. It has been shown that EHP contains at least four nucleotide transporters and two of them highly expressed in the plasmodial stage [40]. Moreover, the growing plasmodia have been found to tightly associate with host mitochondria [28], possibly facilitating energy exploitation from the host. In the late plasmodial stage, the parasite starts to form new organelles, such as polar tube, anchoring disks, and endoplasmic reticulum [5]. Later, evagination of the parasite's plasma membrane occurs to form individual spores. This stage is called sporogony. Finally, chitin – a major component of the parasite spore wall begins to pack outside the plasma membrane. When the spore wall layer is built to ∼10 nm thick for EHP_{Pm} or ∼50 nm thick for EHP_{Pv}, the parasite becomes mature [5, 28]. In the next step, newly developed spores need to be released from the host cell. It is still unclear what might be the exit mechanism of EHP mature spores. One possibility is that the hepatopancreatic cells fully loaded with spores burst, releasing newly developed spores into

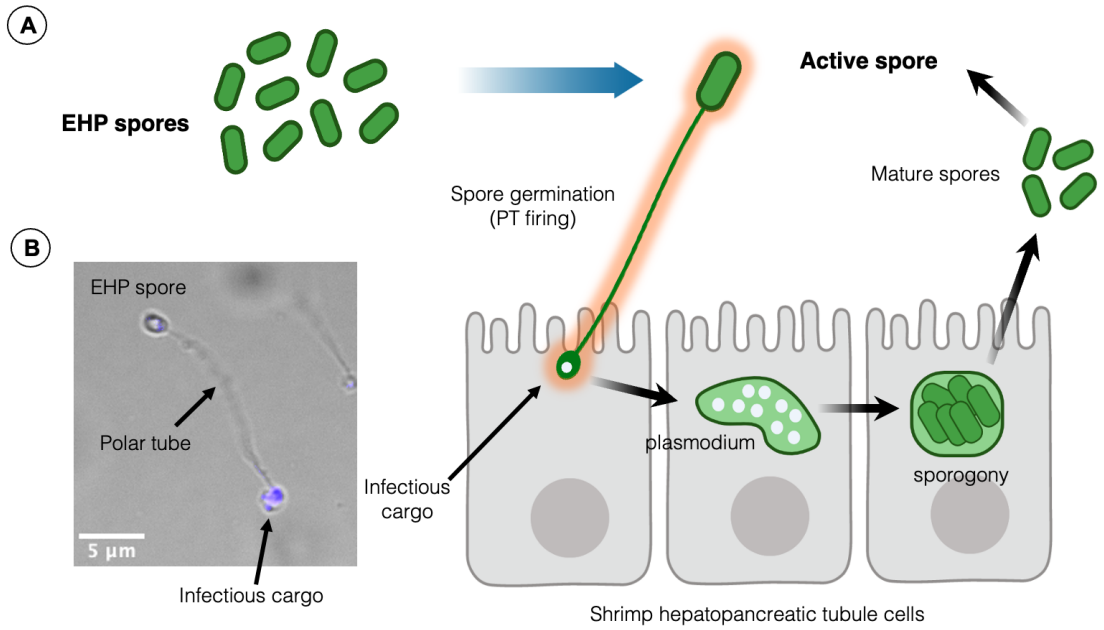

Fig. 2 The life cycle of EHP. (A) Mature EHP spores begin their infections by protruding the polar tube which is believed to penetrate the host cell membrane. The infectious cargo travels through the polar tube and is deposited into host cytoplasm. Later, the parasites start to divide by binary fission and develop into plasmodial and sporogony stages, respectively. Finally, the mature spores exit from the host cells into the external environment. (B) EHP polar tube firing process observed under a light microscope. Blue represents the nuclear content of EHP, which was stained with NucBlue dye. Note that (A) was illustrated using BioRender.

the environment. This hypothesis is supported by the fact that heavily infected hepatopancreas show signs of damaged hepatopancreatic tubules [38]. Alternatively, EHP spores may utilize the exocytosis process involving Rab protein families, similar to a *Caenorhabditis elegans*-infecting microsporidian *Nematocida parisii* [41]. The evidence supporting this idea is that some infected hepatopancreas show intact tubules with the parasites shaded into the hepatopancreatic lumen [28]. The released EHP spores can be transmitted into new, naive shrimp through an oral-fecal transmission [42]. So far, there is no report of a vertical transmission of EHP.

EHP genome and its reduced metabolic genes

EHP and other microsporidia have been recognized as minimalist organisms due to their compact genomes. Microsporidia have been widely used as a model to study genome reduction to the lowest limit of eukaryotic organisms [43]. The genomes of human-infecting microsporidian species in the Genus *Encephalitozoon* are among the smallest eukaryotic genomes known to date. Their genome sizes range between 2.3 to 2.9 Mbps. The 2.3 Mbp genome of *E. intestinalis* encodes only 1,833 genes, suggesting a massive gene loss [44]. To put this into perspective, *E. intestinalis* genome is ∼5 times smaller than the genome of a baking yeast *Saccharomyces cerevisiae* [45], and ∼10 times smaller when compared to another unicellular parasite *Plasmodium falciparum* [46]. For EHP, a whole genome sequencing study reveals 3.26 Mbp in size, which encodes ∼2,540 genes [39]. Typically, an organism with a large genome needs to pay the price for genome replication, in the form of time, nutrients, and space. All three costs increase with the genome size [43]. Hence, EHP and other microsporidia try to reduce these costs by reducing their genomes to rapidly proliferate and efficiently spend limited energy. Genomic studies of several microsporidian genomes reveal strategies that microsporidia use to reduce their genomes. These include loss of introns, shortening of intergenic spaces, gene minimization, gene deletion, and decreasing redundancy genes [43]. Even though genes involved in essential processes such as DNA replication, DNA repair, and protein synthesis are evolutionally maintained in microsporidia, several genes in metabolic and regulatory pathways are partially deleted [47]. Most of the genes involved in energy production (e.g. glycolysis, pentose phosphate pathway, fatty acid metabolism, amino acid and nucleotide biosyntheses and oxidative phosphorylation) are mainly lost. Almost all of the glycolytic enzymes in EHP are lost, except hexokinase and glyceraldehyde 3-phosphate dehydrogenase [39]. This suggests that EHP fully relies on hosts for energy.

To compensate for the loss of several genes in metabolic pathways, microsporidia expand families of

transporter proteins to import what is needed for their replications. For example, EHP and other microsporidian species contain at least 3 isoforms of nucleotide transports or ATP/ADP translocases [39, 40]. Duplication of the nucleotide transporter genes diversifies the functions of these transporters to not only import ATP, but also other substrates such as GTP and NAD^+ [48]. Localization studies of the nucleotide transporters from human-infecting microsporidian species *Trachipleistophora hominis* showed that they are expressed on the surface of the parasites, suggesting their functions in stealing host ATP and other purine nucleotides to support parasite growth [49]. For EHP, nucleotide transporter 1 and 2 (EhNTT1 and EhNTT2) were found to localize on both the parasite surface inside host cells and on the spore wall [40]. Knocking down of the EhNTT2 by RNA interference revealed that EHP copy numbers were significantly reduced, suggesting that EhNTT2 is important for EHP proliferation in shrimp [40]. In addition to nucleotide transporters, microsporidia also expand genes encoded for other transporters, including ATP-binding cassette (ABC) transporters, V/F type ATPases, UDP-N-acetylglucosamine transporters, and mechanosensitive ion channels. Interestingly, there are over 120 uncharacterized transporters found in EHP [39]. This highlighted the crucial roles of transporters to compensate for metabolic gene losses.

Another strategy that microsporidia use to recompense for the gene loss is manipulation of host metabolism by secreting proteins into host cytoplasm. Using a proximity labeling method to identify proteins from microsporidian *N. parisii* that come into contact with the *C. elagans* host, 72 proteins were identified [50]. Most of them contain signal peptides or transmembrane domains. Recent work on the EHP genome showed that there are 184 proteins of EHP with predicted signal peptide sequences [51]. However, the majority of these proteins have unknown functions. Microsporidia can also secrete metabolic enzymes into host cells. One of them is hexokinase [52]. It is proposed that microsporidia hexokinase could perform a classical function to phosphorylate glucose of the hosts, which is later taken up and metabolized by the parasites [53]. However, it is important to note that some microsporidian species, such as EHP, lack the complete set of glycolytic enzymes. Only hexokinase and glyceraldehyde 3-phosphate dehydrogenase remain, suggesting that these enzymes may contain alternate, unknown functions.

HPM and association of EHP with white feces syndrome

EHP is a causative agent of HPM. HPM is associated with growth retardation and severe size variation [4]. The primary infection site of EHP is shrimp hepatopancreas. This organ is a major organ for synthesizing digestive enzymes and plays a role in nutrient absorption [54]. Compared to healthy shrimp, EHP-infected shrimp show enlarged hepatopancreatic tubules and loosen muscle fibril connections, suggesting that the hepatopancreas of these infected shrimp might function abnormally [55]. In addition, EHP-infected shrimp show reductions in feed intake, average daily growth (ADG), and average body weight (ABW) [6]. These reductions impair shrimp growth, resulting in growth retardation. Transcriptomic analysis of hepatopancreas obtained from EHP-infected shrimp showed that genes involved in carbohydrate degradation and absorption (e.g. amylase and galactosidase) are down-regulated, as well as genes encoded for ABC transporters [55]. These transporters are important for cholesterol and lipid transport into the cells [56]. Hence, EHP-infected shrimp may impair in nutrient uptake and possibly result in growth retardation [55]. Moreover, it has been reported that the production of ecdysteroid-regulated protein (ERP) is higher in EHP-infected shrimp [23]. High level of ERP results in low production of ecdysteroid hormone, which is essential for the molting process [57]. Hence, EHP-infected shrimp would experience abnormal molting and result in stunting growth. Recently, a metabolomic study revealed that lipid peroxidation products in EHP-infected shrimp were significantly higher compared to healthy shrimp, indicating that EHP-infected shrimp are under oxidative stress [58]. In addition, immune-related genes are also down-regulated during EHP infection, including proPO 2 – a component of the proPO activating system, alkaline phosphatase (ALP), and pattern-recognition protein (PRP) genes, e.g. lectin A (LecA) and lectin D (LecD). This suggests that EHP infection reduces shrimp antimicrobial activity, and it may influence the ability of shrimp to recognize pathogens [55]. These reductions in immune-related genes lead to susceptibility of EHP-infected shrimp to other secondary infections such as viruses and bacteria [59, 60].

White Faces Syndrome (WFS) is characterized by a pathology that leads to shedded fecal strings floating on the surface of shrimp ponds [61]. Two types of WFS have been identified, including ATM-WFS and EHP-WFS [61]. ATM-WFS involves sloughing of tubules epithelial cells of hepatopancreas with aggregated microvilli and no bacterial infection [61]. The second type of WFS has been found to be associated with EHP infections in grow-out farms (EHP-WFS) [62]. High amounts of EHP spores, bacterial cells, and sloughed hepatopancreatic cells are detected in the white feces strings. Recent study showed that bacteria in the genus *Vibrio* and *Propionigenium* are abundant in shrimp with WFS [60]. Co-infection of EHP and specific strains of *Vibrio parahaemolyticus* results in WFS in laboratory and farm settings [63]. Through their combined actions, co-infection of *V. parahaemolyticus* and EHP

dramatically increases damages to the hepatopancreas, to the point of causing total tissue failure and morbidity. Apart from bacteria, co-infection with viruses has been reported [59, 64]. In India, there was a report of co-infection between EHP and infectious myonecrosis virus (IMNV) [59]. The co-infected shrimp exhibited clinical lesions of both IMNV and EHP which are white in the abdominal segment and slow growth [59]. In addition, screening of shrimp samples collected from grow-out ponds in India showed that 4 samples were tested positive with both EHP and white spot syndrome virus (WSSV) [64]. It is possible that EHP infection would lead to susceptibility to the WSSV infection. However, laboratory infection challenges need to be tested to reproduce this co-infection.

INNATE IMMUNE RESPONSE TO THE EHP INFECTION

Transcriptional and proteomic responses to the EHP infection

The host immune responses to the EHP infection have been primarily reported by transcriptomic and proteomic analyses. The differential expressed proteins (DEPs) and metabolites that were altered in the hepatopancreas of *L. vannamei* after EHP infection have been reported [23]. The results found that the innate immune system of *L. vannamei* was activated while the hormone regulation and energy metabolism pathway was downregulated. The DEPs involved in immune response include peritrophin-44-like protein, alpha2 macroglobulin, proPO-activating enzymes, ferritin, Rab11A, and cathepsin C. Furthermore, significant changes of apoptosis-related proteins were also found among differentially expressed proteins between EHP-infected and uninfected shrimp *L. vannamei* which indicated that the apoptosis pathway was activated upon the EHP infection [25]. Transcriptomic data of intestine and hepatopancreas of EHP-infected *L. vannamei* showed many differentially expressed genes (DEGs) [24]. Among the DEGs, several immune genes were significantly changed compared with the control of uninfected shrimp suggesting that the shrimp immune defense system was activated or suppressed after the EHP infection. In addition, the differential expression analysis showed that shrimp's intestines had more downregulated genes compared with the upregulated ones while the opposite was observed in the hepatopancreas of the EHP-infected shrimp. Many down regulated genes are related to digestion and absorption such as *PvTrypsin*, and genes in the pathways of vitamin digestion and absorption and ABC transporter, suggesting that EHP infection reduces the nutritional supply of shrimp. While many up-regulated genes were significantly enriched in O-glycan biosynthesis and mannose type O-glycan biosynthesis pathways, which reveals the alteration in the energy metabolism pathways [24]. This might indicate the reduction of the intestinal absorption capacity but the induction of the hepatopancreas metabolic energy consumption, resulting in growth retardation in the infected shrimp. *Minichromosome maintenance proteins* (*MCMs*) and *Interleukin-1 Receptor Associated Kinase1* (*IRAK1*) genes involved in phagocytosis and Toll signaling pathways, respectively, were among the upregulated immune-related genes suggesting that both cellular and humoral immune responses were activated upon EHP infection. A comparative transcriptomic analysis reveals that EHP infection considerably influences host gene expression including those involved in detoxification, apoptosis, and lipid metabolism, and unveils the dynamic molecular interactions between EHP and the white shrimp *L. vannamei*, during the early and late stages of infection [26]. These omics analyses reveal the overall changes of genes and proteins in the EHP-infected shrimp, but the mechanisms involved in host response and the interplay of host-pathogen interactions to regulate host immunity require further investigation.

Toll, JAK/STAT signaling pathways and antimicrobial peptides

Toll, IMD, and JAK/STAT pathways are the major immune pathways regulating the immune response of invertebrates including shrimps [65]. These signaling pathways act synergistically to activate the production of AMPs and other immune effector proteins leading to a broad-spectrum host response. Genes in the Toll and JAK/STAT pathways, were found to be upregulated upon the EHP infection in *L. vannamei* [66]. Meanwhile, gene silencing of *LvDOME* and *LvTLR2* resulted in increased EHP copy number, suggesting that both JAK/STAT and Toll pathways are important immune signaling pathways involved in EHP infection. The results were corresponded to those previously reports in *N. bombycis*-infected silkworms that the activation of the Toll and JAK/STAT pathways were found upon the microsporidia infection [67].

AMPs are the key components of the innate immune system in shrimps [68]. AMPs are mainly produced in shrimp hemocytes and rapidly released into the hemolymph to eliminate invading pathogens. In response to the EHP infection, it was found that the expression of *L. vannamei* AMPs including *anti-lipopolysaccharide1* (*LvALF1*), *penaeidin* 3 (*LvPEN3*) and *c-type lysozyme* (*LvLyz-c*), was significantly increased, indicating that these AMPs might play a key role in combating the EHP infection [66]. The function of a c-type lysozyme, *LvLyz-c*, in reducing the EHP infection was further elucidated and the results found that *LvLyzc*, regulating under the JAK/STAT pathway, can significantly inhibit the EHP spore germination possibly by its ability to digest a chitinous endospore layer [66]. It has been reported that AMPs can inhibit

spore germination and reduce enterocytes infection in *E. intestinalis* and *E. hellem*, and in the insect parasite *Nosema algerae* [69]. While many AMPs can directly kill microorganisms, some AMPs protect hosts against microsporidia infection by inhibiting spore germination [66, 69]. However, this inhibitory activity of AMPs is not applicable to all microsporidian species and is likely due to different germination mechanisms.

The crustin genes were also found to be induced after the EHP infection, in *L. vannamei* [70] but their antimicrobial actions against EHP have not been explored. Many AMP genes (e.g. *gloverins*, *lebocins*, *cecropin* and *moricins*) were also shown to defend the silkworm against *N. bombycis* [67]. In honeybees, the expression of some AMPs (abaecin, denfesin and hymenoptaecin) was increased after the *N. apis* infection. In contrast the transcript levels of these AMPs were significantly suppressed in *N. ceranae* infection [71]. This seems to correlate with a more prevalent and virulent of *N. ceranae* than *N. apis*. Transcriptional responses in shrimp *L. vannamei* showed downregulation of small open reading frame-encoded peptides (SEPs)-related genes during the early stages of EHP infection [26]. SEPs are involved in various biological processes and might be involved in activation of the NF-κB pathway and regulation of the expression of AMPs in shrimp. Therefore, down-regulation of SEPs might lead to a decrease in AMP levels to promote EHP invasion. However, the mechanism of AMP suppression by EHP remains unclear and requires further investigation.

Melanization response to the EHP infection

Melanization mediated by a proPO-activating cascade is an important innate immune response in invertebrates that produces melanin and toxic reactive intermediates against invading pathogens [72]. It has been reported that the proPO cascade plays a crucial role in defense against microsporidia infections in both vertebrates and invertebrates [73]. In shrimp *L. vannamei*, the proPO activating enzyme (PPAE), the key serine protease in the proPO activation cascade, was found among the upregulated immune protein in hepatopancreas of the EHP-infected shrimp [23]. Recently, it has been reported that the proPO-activating system-related genes were highly responded to the EHP infection, and the hemolymph PO activity was increased after the parasite infection [74]. Furthermore, the melanization products can inhibit the EHP spore germination. Suppression of the proPO-activating system resulted in increased EHP copy number, reduced expression of several genes in the JAK/STAT and Toll signaling pathways, as well as antimicrobial peptides and reduced hemocyte adhesion and encapsulation of the EHP spores. These results suggest that the proPO cascade plays a vital role in reducing EHP infectivity and cross-talks with other humoral and cellular responses to coordinately defend the EHP infection [74]. In silkworm, the key genes involved in melanization were also found to be upregulated among the differentially expressed genes. However, it has been reported that the hemolymph melanization is partially suppressed by a serpin, NbSPN6, secreted by microsporidian *N. bombycis* to reduce the activity of serine proteases, and inhibit host melanization [75]. In shrimps, there has been no evidence yet of a parasite serpin that could inhibit the proPO cascade.

Lectins

Lectins are proteins that recognize carbohydrates on the surface of pathogenic microorganisms and mediate immune responses through several mechanisms, such as neutralization, agglutination and opsonization [76]. C-type lectins (CTLs) recognize and bind microbial carbohydrates through a carbohydrate recognition domain (CRD) – and play important roles in invertebrate immunity [77]. Previous studies in invertebrates showed that microsporidian infections induce the expression of various immune proteins including C-type lectins [53]. In contrast, some C-type lectins in shrimp have been found to be downregulated after the EHP infection and are possibly involved in controlling homeostasis of gut microbiome during EHP infection [78]. Likewise, *Aedes aegypti* C-type lectins, mosGCTLs, have been shown to facilitate bacterial colonization by coating the bacterial surface and counteract AMP-mediated elimination to enabling eco-adaption of the gut microbiome in mosquitoes [79].

Hemocytin is a lectin with a unique structure homolog to hemolectin in *Drosophila melanogaster* and a von Willebrand factor (vWF), the hemostasis-related protein in human blood [80]. Hemocytin plays a vital role in insect immunity by mediating the agglutination of hemocytes, pathogens, and various immune factors, which promote nodulation or encapsulation around pathogens and also participate in wound healing [81]. In shrimp, *L. vannamei* hemocytin (LvHCT), plays a vital role in shrimp innate immunity against the EHP infection by promoting EHP spore aggregation and possibly activating the proPO-activating cascade [82]. Similarly, silkworm hemocytin combats *N. bombycis* by binding onto the surface of the pathogen and facilitating pathogen agglutination, together with hemocyte aggregation and melanization [81].

Oxidative stress

Oxidative stress, particularly reactive oxygen species (ROS), are a vital part of the innate immune system's defense against pathogens. ROS can directly attack pathogens, activate inflammation, and regulate immune signals [83]. However, an excess of ROS can damage cellular components including lipids, proteins, and DNA, alter immune functions, inflammatory responses and induce organ and tissue dysfunction. The activities of many antioxidant enzymes (e.g.,

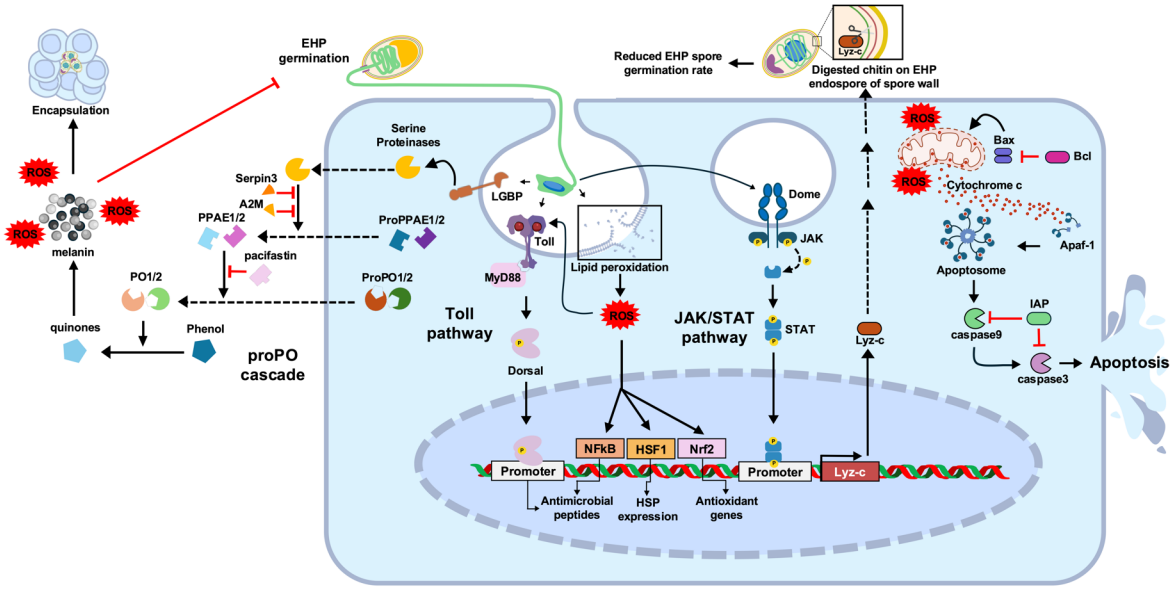

Fig. 3 Schematic overview of the innate immune response to the invasion of *Ecytonucleospora hepatopenaei* in shrimp. Following the EHP infection, shrimps activate Toll and JAK/STAT signaling pathways to secrete many antimicrobial peptides and immune effector proteins [19, 66]. The proPO-activating cascade is activated leading to synthesis of melanin and cytotoxic intermediates to encapsulate the parasite and inhibit spore germination [72, 74]. Oxidative stress [58] and apoptosis [25, 86] are also influenced by the parasite infection.

manganese superoxide dismutase (MnSOD), catalase (CAT), glutathione peroxidase (GPX), glutathione-S-transferases (GST) can neutralize free radicals to avoid oxidative stress. During EHP infection, the antioxidant genes, *MnSOD*, *CAT*, *GPX*, *GST*, and a nuclear factor erythroid 2p45-related factor2 (Nrf2) were upregulated in the EHP challenged shrimp. In addition, the EHP infection also induces accumulation of the lipid peroxidation products, lipid peroxide (LPO) and malondialdehyde (MDA) that causes oxidative damage to the cell membrane of epithelial cells of the hepatopancreas corresponding to the histopathological features that the hepatopancreas was significantly damaged, with vacuoles and membrane damage between the hepatic tubules [38] and may lead to the growth retardation of *L. vannamei* [58]. Transcriptomic analysis of EHP infected *L. vannamei* also found that the glutathione antioxidant system is suppressed in the early phase of the infection but becomes activated in the later stages that could facilitate the early invasion of EHP while assisting the host in mitigating oxidative damage caused by the late-stage infection [26]. Previous studies reported that microsporidian infections can upregulate proteins involved in oxidative stress and energy metabolism in the host. In silkworms, *N. bombycis* infection induces changes in oxidative stress-related proteins in the fat body [84]. In addition, GST and thiol peroxiredoxin were significantly upregulated after *N. bombycis*-infected *Bm*N cells [85].

Apoptosis

Apoptosis is the process of programmed cell death that is also one of the host responses to pathogen infection. It is recognized as an important defense mechanism against viral, bacterial and parasitic pathogens during innate and adaptive immunity. Interestingly, pathogens including some microsporidia can modulate apoptosis by either inducing or inhibiting apoptosis allowing successful pathogen proliferation [86]. Likewise, shrimp pathogens such as white spot syndrome virus (WSSV) and yellowhead virus (YHV), can influence the host apoptosis system and the balance between the pro- and anti-apoptosis activation processes is crucial to viral pathogenesis [87, 88]. Proteomic analysis showed that apoptosis-related proteins were significantly upregulated after the EHP infection, which indicated that the apoptosis pathway was activated in *L. vannamei* hepatopancreas. In addition, the expression level of *caspase3* gene was upregulated in EHP-infected shrimp [25]. In contrast, analysis of cell apoptosis during the first and second weeks of EHP infection showed no significant difference in apoptosis of hepatopancreas cells [89]. The honeybees' microsporidia *N. apis* and *N. ceranae* infections induce regulation of genes involved in apoptosis and the cell cycle. Interestingly, *N. bombycis* can inhibit host apoptosis by downregulating the expression of apoptotic protease activating factor-1 (*apaf1*) and cytochrome c (*cyt-c*) and upregulating the expression of *buffy*,

confirming that the inhibition of apoptosis is a common response to benefit the parasite infection [90]. Besides, *Encephalitozoon* infection can suppress apoptosis of Vero cells by inhibiting the cleavage of caspase-3, phosphorylation and translocation of p53 [91]. Nonetheless, the regulation of apoptosis by EHP remains to be further elucidated.

In summary, the host responses to the EHP infection have recently been studied but are far from fully elucidated. To combat the EHP infection, shrimps induce the innate immune signaling pathways such as Toll and JAK/STAT signaling to produce lysozyme and antimicrobial peptides and the proPO-activating cascade is also induced to activate melanization and encapsulation to reduce the parasite infectivity. Meanwhile, oxidative stress and antioxidant enzymes as well as pro- and anti-apoptosis processes are strictly regulated to maintain host cell homeostasis (Fig. 3).

CONCLUSION AND PERSPECTIVES

EHP has emerged as a serious threat to the shrimp aquaculture causing growth retardation and potential susceptibility to other diseases resulting in severe economic losses. EHP infects the hepatopancreas of shrimp through a polar tube firing mechanism, allowing the sporoplasm to enter into host cells where they infect, replicate and eventually leading to hepatopancreatic cell damages and malfunction. The EHP infection disrupts shrimp's metabolism and suppresses the immune system, making shrimp more susceptible to other infections. Understanding of EHP pathogenesis and shrimp's innate immunity are crucial fundamental knowledge that will contribute to a proper and effective management to prevent and control the disease such as development of therapeutic substances that inhibit the EHP life cycle or modulate shrimp's immune responses against the pathogen.

Acknowledgements: This Research is funded by Thailand Science Research and Innovation Fund Chulalongkorn University (FOOD_FF_68_016_2300_004). The authors also thank the support from Chulalongkorn to the Center of Excellence for Molecular Biology and Genomics of Shrimp University under the Ratchadaphisek Somphot Endowment Fund.

REFERENCES

1. Jatuporn C (2022) Impact assessment of the COVID-19 pandemic on shrimp exports in Thailand: an empirical study using time series analysis. *Sustain Sci Pract Policy* **14**, 16518.
2. Flegel TW (2006) Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. *Aquaculture* **258**, 1–33.
3. Shinn AP, Pratoomyot J, Griffiths D, Trong TQ, Vu NT, Jiravanichpaisal P, Briggs M (2018) Asian shrimp production and the economic costs of disease. *Asian Fish Sci* **31**, 29–58.
4. Thitamadee S, Prachumwat A, Srisala J, Jaroenlak P, Salachan PV, Sritunyalucksana K, Flegel TW, Itsathitphaisarn O (2016) Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture* **452**, 69–87.
5. Tourtip S, Wongtripop S, Stentiford GD, Bateman KS, Sriurairatana S, Chavadej J, Sritunyalucksana K, Withyachumnarnkul B (2009) *Enterocytozoon hepatopenaei* sp. nov. (Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): fine structure and phylogenetic relationships. *J Invertebr Pathol* **102**, 21–29.
6. Kongplong S, Kanjanasopa D, Pongtippatee P, Vanichviriyakit R, Withyachumnarnkul B (2023) 5-Aminolaevulinic acid reduced the mortality of the Pacific white shrimp *Litopenaeus vannamei* infected with *Enterocytozoon hepatopenaei*. *Aquaculture* **568**, 739322.
7. Patil PK, Geetha R, Ravisankar T, Avunje S, Solanki HG, Abraham TJ, Vinoth SP, Jithendran KP, et al (2021) Economic loss due to diseases in Indian shrimp farming with special reference to *Enterocytozoon hepatopenaei* (EHP) and white spot syndrome virus (WSSV). *Aquaculture* **533**, 736231.
8. Ha NT, Ha DT, Thuy NT, Lien VTK (2010) Occurrence of microsporidia *Enterocytozoon hepatopenaei* in white feces disease of cultured black tiger shrimp (*Penaeus monodon*) in Vietnam has been detected. *Agric Rural Dev Sci Technol* **12**, 45–50. [In Vietnamese with English abstract]
9. Tang KFJ, Pantoja CR, Redman RM, Han JE, Tran LH, Lightner DV (2015) Development of *in situ* hybridization and PCR assays for the detection of *Enterocytozoon hepatopenaei* (EHP), a microsporidian parasite infecting penaeid shrimp. *J Invertebr Pathol* **130**, 37–41.
10. Liu YM, Qiu L, Sheng AZ, Wan XY, Cheng DY, Huang J (2018) Quantitative detection method of *Enterocytozoon hepatopenaei* using TaqMan probe real-time PCR. *J Invertebr Pathol* **151**, 191–196.
11. Kim BS, Jang GI, Kim SM, Kim YS, Jeon YG, Oh YK, Hwang JY, Kwon MG (2021) First report of *Enterocytozoon hepatopenaei* infection in Pacific whiteleg shrimp (*Litopenaeus vannamei*) cultured in Korea. *Animals* **11**, 3150.
12. Franzen C, Müller A, Hartmann P, Salzberger B (2005) Cell invasion and intracellular fate of *Encephalitozoon cuniculi* (Microsporidia). *Parasitology* **130**, 285–292.
13. Weidner E (1976) The microsporidian spore invasion tube. The ultrastructure, isolation, and characterization of the protein comprising the tube. *J Cell Biol* **71**, 23–34.
14. Jaroenlak P, Cammer M, Davydov A, Sall J, Usmani M, Liang FX, Ekiert DC, Bhabha G (2020) 3-Dimensional organization and dynamics of the microsporidian polar tube invasion machinery. *PLoS Pathog* **16**, e1008738.
15. Balla KM, Luallen RJ, Bakowski MA, Troemel ER (2016) Cell-to-cell spread of microsporidia causes *Caenorhabditis elegans* organs to form syncytia. *Nat Microbiol* **1**, 16144.
16. Tassanakajon A, Somboonwiwat K, Supungul P, Tang S (2013) Discovery of immune molecules and their crucial functions in shrimp immunity. *Fish Shellfish Immunol* **34**, 954–967.
17. Bachere E (2000) Shrimp immunity and disease control. *Aquaculture* **191**, 3–11.

18. Xu D, Liu W, Alvarez A, Huang T (2014) Cellular immune responses against viral pathogens in shrimp. *Dev Comp Immunol* **47**, 287–297.
19. Tassanakajon A, Rimphanitchayakit V, Visetnan S, Amparyup P, Somboonwiwat K, Charoensapsri W, Tang S (2018) Shrimp humoral responses against pathogens: antimicrobial peptides and melanization. *Dev Comp Immunol* **80**, 81–93.
20. Duan Y, Xing Y, Zhu X, Li H, Wang Y, Nan Y (2023) Integration of transcriptomic and metabolomic reveals carbonate alkalinity stress responses in the hepatopancreas of *Litopenaeus vannamei*. *Aquat Toxicol* **260**, 106569.
21. Li W, Tang X, Xing J, Sheng X, Zhan W (2014) Proteomic analysis of differentially expressed proteins in *Fenneropenaeus chinensis* hemocytes upon white spot syndrome virus infection. *PLoS One* **9**, e89962.
22. Zhang H, Yu C, Li Y, Liu Q (2024) Transcriptome profiles of the hepatopancreas of the Chinese swamp shrimp (*Neocaridina denticulata*) under different diurnal rhythms. *ScienceAsia* **50**, ID 2024043.
23. Ning M, Wei P, Shen H, Wan X, Jin M, Li X, Shi H, Qiao Y, et al (2019) Proteomic and metabolomic responses in hepatopancreas of whiteleg shrimp *Litopenaeus vannamei* infected by microsporidian *Enterocytozoon hepatopenaei*. *Fish Shellfish Immunol* **87**, 534–545.
24. Yang LG, Wang Y, Wang Y, Fang WH, Feng GP, Ying N, Zhou JY, Li XC (2021) Transcriptome analysis of pacific white shrimp (*Penaeus vannamei*) intestines and hepatopancreas in response to *Enterocytozoon hepatopenaei* (EHP) infection. *J Invertebr Pathol* **186**, 107665.
25. Liu H, Shen M, He Y, Li B, Pu L, Xia G, Yang M, Wang G (2023) Analysis of differentially expressed proteins after EHP-infection and characterization of caspase 3 protein in the whiteleg shrimp (*Litopenaeus vannamei*). *Fish Shellfish Immunol* **135**, 108698.
26. Zhang L, Zhang S, Qiao Y, Cao X, Cheng J, Meng Q, Shen H (2023) Dynamic interplay of metabolic and transcriptional responses in shrimp during early and late infection stages of *Enterocytozoon hepatopenaei* (EHP). *Int J Mol Sci* **24**, 16738.
27. Franzen C (2004) Microsporidia: how can they invade other cells? *Trends Parasitol* **20**, 275–279.
28. Wang Y, Chen J, Na Y, Li XC, Zhou JF, Fang WH, Tan HX (2023) *Ecytonucleospora hepatopenaei* n. gen. et comb. (Microsporidia: Enterocytozoonidae): a redescription of the *Enterocytozoon hepatopenaei* (Tourtip et al, 2009), a microsporidian infecting the widely cultivated shrimp *Penaeus vannamei*. *J Invertebr Pathol* **201**, 107988.
29. Weidner E, Byrd W, Scarborough A, Pleshinger J, Sibley D (1984) Microsporidian spore discharge and the transfer of polaroplast organelle membrane into plasma membrane. *J Protozool* **31**, 195–198.
30. Chang R, Davydov A, Jaroenlak P, Budaitis B, Ekiert DC, Bhabha G, Prakash M (2023) Energetics of the microsporidian polar tube invasion machinery. *eLife* **12**, RP86638.
31. Frixione E, Ruiz L, Undeen AH (1994) Monovalent cations induce microsporidian spore germination *in vitro*. *J Eukaryot Microbiol* **41**, 464–468.
32. Hashimoto K, Sasaki Y, Takinami K (1976) Conditions for extrusion of the polar filament of the spore of *Plistophora anguillarum*, a microsporidian parasite in *Anguilla japonica*. *Bull Jpn Soc Sci Fish*, 837–845.
33. Ishihara R (1967) Stimuli causing extrusion of polar filaments of *Glugea fumiferanae* spores. *Can J Microbiol* **13**, 1321–1332.
34. Jaroenlak P, Usmani M, Ekiert DC, Bhabha G (2022) Mechanics of microsporidian polar tube firing. *Exp Suppl* **114**, 215–245.
35. Weiss LM, Delbac F, Hayman JR, Pan G, Dang X, Zhou Z (2014) The microsporidian polar tube and spore wall. In: Weiss LM, Becnel JJ (eds) *Microsporidia: Pathogens of Opportunity*, 1st edn, Wiley, Hoboken, NJ, pp 261–306.
36. Aldama-Cano DJ, Sanguanrut P, Munkongwongsiri N, Ibarra-Gámez JC, Itsathitphaisarn O, Vanichviriyakit R, Flegel TW, Sritunyalucksana K, et al (2018) Bioassay for spore polar tube extrusion of shrimp *Enterocytozoon hepatopenaei* (EHP). *Aquaculture* **490**, 156–161.
37. Cali A, Weiss LM, Takvorian PM (2002) *Brachiola algerae* spore membrane systems, their activity during extrusion, and a new structural entity, the multilayered interlaced network, associated with the polar tube and the sporoplasm. *J Eukaryot Microbiol* **49**, 164–174.
38. Chaijarasphong T, Munkongwongsiri N, Stentiford GD, Aldama-Cano DJ, Thansa K, Flegel TW, Sritunyalucksana K, Itsathitphaisarn O (2021) The shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP): biology, pathology, diagnostics and control. *J Invertebr Pathol* **186**, 107458.
39. Wiredu Boakye D, Jaroenlak P, Prachumwat A, Williams TA, Bateman KS, Itsathitphaisarn O, Sritunyalucksana K, Paszkiewicz KH, et al (2017) Decay of the glycolytic pathway and adaptation to intranuclear parasitism within Enterocytozoonidae microsporidia. *Environ Microbiol* **19**, 2077–2089.
40. Thepmanee O, Munkongwongsiri N, Prachumwat A, Saksmerprome V, Jitrakorn S, Sritunyalucksana K, Vanichviriyakit R, Chanarat S, et al (2023) Molecular and cellular characterization of four putative nucleotide transporters from the shrimp microsporidian *Enterocytozoon hepatopenaei* (EHP). *Sci Rep* **13**, 20008.
41. Szumowski SC, Botts MR, Popovich JJ, Smelkinson MG, Troemel ER (2014) The small GTPase RAB-11 directs polarized exocytosis of the intracellular pathogen *N. parisii* for fecal-oral transmission from *C. elegans*. *Proc Natl Acad Sci USA* **111**, 8215–8220.
42. Tangprasittipap A, Srisala J, Chouwdee S, Somboon M, Chuchird N, Limsuwan C, Srisuvan T, Flegel TW, et al (2013) The microsporidian *Enterocytozoon hepatopenaei* is not the cause of white feces syndrome in whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei*. *BMC Vet Res* **9**, 139.
43. Jespersen N, Monrroy L, Barandun J (2022) Impact of genome reduction in microsporidia. *Experientia Suppl* **114**, 1–42.
44. Corradi N, Pombert J-F, Farinelli L, Didier ES, Keeling PJ (2010) The complete sequence of the smallest known nuclear genome from the microsporidian *Encephalitozoon intestinalis*. *Nat Commun* **1**, 77.
45. Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, et al (1996) Life with 6000 genes. *Science* **274**, 546–567.
46. Gardner MJ, Hall N, Fung E, White O, Berriman M, Hyman RW, Carlton JM, Pain A, et al (2002) Genome sequence of the human malaria parasite *Plasmodium falciparum*. *Nature* **419**, 498–511.
47. Nakjang S, Williams TA, Heinz E, Watson AK, Foster PG,

Sendra KM, Heaps SE, Hirt RP, et al (2013) Reduction and expansion in microsporidian genome evolution: new insights from comparative genomics. *Genome Biol Evol* **5**, 2285–2303.
48. Dean P, Sendra KM, Williams TA, Watson AK, Major P, Nakjang S, Kozhevnikova E, Goldberg AV, et al (2018) Transporter gene acquisition and innovation in the evolution of microsporidia intracellular parasites. *Nat Commun* **9**, 1709.
49. Dean P, Hirt RP, Embley TM (2016) Microsporidia: why make nucleotides if you can steal them? *PLoS Pathog* **12**, e1005870.
50. Reinke AW, Balla KM, Bennett EJ, Troemel ER (2017) Identification of microsporidia host-exposed proteins reveals a repertoire of rapidly evolving proteins. *Nat Commun* **8**, 14023.
51. Wang L, Li H, Shi W, Qiao Y, Wang P, Yu Z, Zhao R, Hu R, et al (2022) Whole-genome sequencing and comparative genomic analysis of a pathogenic *Enterocytozoon hepatopenaei* strain isolated from *Litopenaeus vannamei*. *Aquac Int* **31**, 523–546.
52. Cuomo CA, Desjardins CA, Bakowski MA, Goldberg J, Ma AT, Becnel JJ, Didier ES, Fan L, et al (2012) Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res* **22**, 2478–2488.
53. Tamim El Jarkass H, Reinke AW (2020) The ins and outs of host-microsporidia interactions during invasion, proliferation and exit. *Cell Microbiol* **22**, e13247.
54. Wang W, Wu X, Liu Z, Zheng H, Cheng Y (2014) Insights into hepatopancreatic functions for nutrition metabolism and ovarian development in the crab *Portunus trituberculatus*: gene discovery in the comparative transcriptome of different hepatopancreas stages. *PLoS One* **9**, e84921.
55. Duan Y, Chen H, Wang J, Zeng S, Wang Y, Mo Z, Dan X, Li Y (2021) Response signatures of *Litopenaeus vannamei* to natural *Enterocytozoon hepatopenaei* infection revealed by the integration of the microbiome and transcriptome. *Aquaculture* **542**, 736885.
56. Young J, Holland IB (1999) ABC transporters: bacterial exporters—revisited five years on. *Biochim Biophys Acta* **1461**, 177–200.
57. Keller R (1992) Crustacean neuropeptides: structures, functions and comparative aspects. *Experientia* **48**, 439–448.
58. Cao Z, Chen C, Wang C, Li T, Chang L, Si L, Yan D (2023) *Enterocytozoon hepatopenaei* (EHP) infection alters the metabolic processes and induces oxidative stress in *Penaeus vannamei*. *Animals* **13**, 3661.
59. Jithendran KP, Navaneeth Krishnan A, Jagadeesan V, Anandaraja R, Ezhil Praveena P, Anushya S, Bala Amarnath C, Bhuvaneswari T (2021) Co-infection of infectious myonecrosis virus and *Enterocytozoon hepatopenaei* in *Penaeus vannamei* farms in the east coast of India. *Aquac Res* **52**, 4701–4710.
60. Munkongwongsiri N, Prachumwat A, Eamsaard W, Lertsiri K, Flegel TW, Stentiford GD, Sritunyalucksana K (2022) *Propionigenium* and *Vibrio* species identified as possible component causes of shrimp white feces syndrome (WFS) associated with the microsporidian *Enterocytozoon hepatopenaei*. *J Invertebr Pathol* **192**, 107784.
61. Sriurairatana S, Boonyawiwat V, Gangnonngiw W, Laosutthipong C, Hiranchan J, Flegel TW (2014) White feces syndrome of shrimp arises from transformation, sloughing and aggregation of hepatopancreatic microvilli into vermiform bodies superficially resembling gregarines. *PLoS One* **9**, e99170.
62. Sathish Kumar T, Makesh M, Alavandi SV, Vijayan KK (2022) Clinical manifestations of White feces syndrome (WFS), and its association with *Enterocytozoon hepatopenaei* in *Penaeus vannamei* grow-out farms: a pathobiological investigation. *Aquaculture* **547**, 737463.
63. Aranguren Caro LF, Mai HN, Cruz-Florez R, Marcos FLA, Alenton RRR, Dhar AK (2021) Experimental reproduction of White Feces Syndrome in whiteleg shrimp, *Penaeus vannamei*. *PLoS One* **16**, e0261289.
64. Thamizhvanan S, Sivakumar S, Santhosh Kumar S, Vinoth Kumar D, Suryakodi S, Balaji K, Rajkumar T, Vimal S, et al (2019) Multiple infections caused by white spot syndrome virus and *Enterocytozoon hepatopenaei* in pond-reared *Penaeus vannamei* in India and multiplex PCR for their simultaneous detection. *J Fish Dis* **42**, 447–454.
65. Li FH, Xiang JH (2013) Signaling pathways regulating innate immune responses in shrimp. *Fish Shellfish Immunol* **34**, 973–980.
66. Sangklai N, Supungul P, Jaroenlak P, Tassanakajon A (2024) Immune signaling of *Litopenaeus vannamei* c-type lysozyme and its role during microsporidian *Enterocytozoon hepatopenaei* (EHP) infection. *PLoS Pathog* **20**, e1012199.
67. Ma Z, Li C, Pan G, Li Z, Han B, Xu J, Lan X, Chen J, et al (2013) Genome-wide transcriptional response of silkworm (*Bombyx mori*) to infection by the microsporidian *Nosema bombycis*. *PLoS One* **8**, e84137.
68. Tassanakajon A, Amparyup P, Somboonwiwat K, Supungul P (2010) Cationic antimicrobial peptides in penaeid shrimp. *Mar Biotechnol (NY)* **12**, 487–505.
69. Leitch GJ, Ceballos C (2009) A role for antimicrobial peptides in intestinal microsporidiosis. *Parasitology* **136**, 175–181.
70. Karthikeyan K, Sudhakaran R (2019) Experimental horizontal transmission of *Enterocytozoon hepatopenaei* in post-larvae of whiteleg shrimp, *Litopenaeus vannamei*. *J Fish Dis* **42**, 397–404.
71. Antúnez K, Martín-Hernández R, Prieto L, Meana A, Zunino P, Higes M (2009) Immune suppression in the honey bee (*Apis mellifera*) following infection by *Nosema ceranae* (Microsporidia). *Environ Microbiol* **11**, 2284–2290.
72. Amparyup P, Charoensapsri W, Tassanakajon A (2013) Prophenoloxidase system and its role in shrimp immune responses against major pathogens. *Fish Shellfish Immunol* **34**, 990–1001.
73. Pan G, Bao J, Ma Z, Song Y, Han B, Ran M, Li C, Zhou Z (2018) Invertebrate host responses to microsporidia infections. *Dev Comp Immunol* **83**, 104–113.
74. Sukonthamarn P, Wongvises P, Sangklai N, Jaroenlak P, Tassanakajon A (2024) Prophenoloxidase-activating system plays a crucial role in innate immune responses to *Enterocytozoon hepatopenaei* infection in shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol* **154**, 109925.
75. Bao J, Liu L, An Y, Ran M, Ni W, Chen J, Wei J, Li T, et al (2019) *Nosema bombycis* suppresses host hemolymph melanization through secreted serpin 6 inhibiting the

prophenoloxidase activation cascade. *J Invertebr Pathol* **168**, 107260.
76. Kennedy J (1995) Lectins, versatile proteins of recognition: a review. *Carbohydr Polym* **26**, 219–230.
77. Janeway CA, Medzhitov R (2002) Innate immune recognition. *Annu Rev Immunol* **20**, 197–216.
78. Ning M, Huang Y, Cao X, Shen H, Gu W, Ren X, Meng Q (2024) The shrimp C-type lectins modulate intestinal microbiota homeostasis in microsporidia infection. *Aquaculture* **581**, 740435.
79. Pang X, Xiao X, Liu Y, Zhang R, Liu J, Liu Q, Wang P, Cheng G (2016) Mosquito C-type lectins maintain gut microbiome homeostasis. *Nat Microbiol* **1**, 16023.
80. Lenting PJ, Christophe OD, Denis CV (2015) von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. *Blood* **125**, 2019–2028.
81. Ni W, Bao J, Mo B, Liu L, Li T, Pan G, Chen J, Zhou Z (2020) Hemocytin facilitates host immune responses against *Nosema bombycis*. *Dev Comp Immunol* **103**, 103495.
82. Sukonthamarn P, Nanakorn Z, Junprung W, Supungul P, Tassanakajon A (2023) Role of hemocytin from *Litopenaeus vannamei* in immune response against microsporidian *Enterocytozoon hepatopenaei*. *Fish Shellfish Immunol* **136**, 108710.
83. Yang Y, Bazhin AV, Werner J, Karakhanova S (2013) Reactive oxygen species in the immune system. *Int Rev Immunol* **32**, 249–270.
84. Li Z, Pan G, Ma Z, Han B, Sun B, Ni Q, Chen J, Li T, et al (2017) Comparative proteomic analysis of differentially expressed proteins in the *Bombyx mori* fat body during the microsporidia *Nosema bombycis* infection. *J Invertebr Pathol* **149**, 36–43.
85. He X, He X, Liu H, Li M, Cai S, Fu Z, Lu X (2014) Proteomic analysis of BmN cells (*Bombyx mori*) in response to infection with *Nosema bombycis*. *Acta Biochim Biophys Sin* **46**, 982–990.
86. Martín-Hernández R, Higes M, Sagastume S, Juarranz Á, Dias-Almeida J, Budge GE, Meana A, Boonham N (2017) Microsporidia infection impacts the host cell's cycle and reduces host cell apoptosis. *PLoS One* **12**, e0170183.
87. Leu JH, Lin SJ, Huang JY, Chen TC, Lo CF (2013) A model for apoptotic interaction between white spot syndrome virus and shrimp. *Fish Shellfish Immunol* **34**, 1011–1017.
88. Chintapitaksakul L, Udomkit A, Smith DR, Panyim S, Sonthayanon B (2008) Expression analysis of selected haemocyte transcripts from black tiger shrimp infected with yellow head virus. *ScienceAsia* **34**, 327–333.
89. Ma R, Zhu B, Xiong J, Chen J (2024) The pathogenic mechanism of *Enterocytozoon hepatopenaei* in *Litopenaeus vannamei*. *Microorganisms* **12**, 1208.
90. He X, Fu Z, Li M, Liu H, Cai S, Man N, Lu X (2015) *Nosema bombycis* (Microsporidia) suppresses apoptosis in BmN cells (*Bombyx mori*). *Acta Biochim Biophys Sin* **47**, 696–702.
91. del Aguila C, Izquierdo F, Granja AG, Hurtado C, Fenoy S, Fresno M, Revilla Y (2006) *Encephalitozoon* microsporidia modulates p53-mediated apoptosis in infected cells. *Int J Parasitol* **36**, 869–876.