Bulletin of Insectology ***77*** *(1): 124-136, 2024*
ISSN 1721-8861 eISSN 2283-0332

Morphology of the genitalia and internal reproduction organ of the adult black soldier fly *Hermetia illucens*

Wenzheng Tang[1], Jianing Zhang[1], Zhihui Shi[1], Xiaomin Chen[2], Fen Zhu[1]
[1]*Hubei International Scientific and Technological Cooperation Base of Waste Conversion by Insects, Huazhong Agricultural University, Wuhan, China*
[2]*Wuhan Center for Disease Control and Prevention, Wuhan, China*

Abstract

Larvae of the black soldier fly (BSF) *Hermetia illucens* L. (Diptera Stratiomyidae) are widely applied as a decomposition agent for organic waste, including manure, food waste and agricultural by-products. High-effective conversion of waste and industrialized BSF utilization need accumulation of basic data for BSF mass rearing. This research focused on the morphology of the adult reproductive system based on anatomy, which was limited previously. We provide the morphological identification of BSF reproductive system, especially their completely disassembled genitalia. Development grade of the female internal reproductive system was established according to the changes of the ovarian morphology. These results provided some basic data for the reproduction and development of the BSF.

Key words: copulatory organ, ovipositor, reproductive tracts, copulatory organ, spermathecae, ovarian development.

Introduction

The black soldier fly (BSF) *Hermetia illucens* L. (Diptera Stratiomyidae), is a saprophagous insect species widely distributed in tropical and warm temperate regions (Marshall *et al*., 2015; Zhang *et al*., 2019). This species got the attention due to its ability of waste bioconversion and the potential to be a new sustainable source of protein (Sheppard *et al*., 1994; Ji *et al*., 2017; Gasco *et al*., 2020). However, the available literature is mainly about the morphology and biological applications of the larvae and limited to mating and oviposition behaviours in their adults, though the adult stage shouldn't be ignored (Sheppard *et al*., 2002; Kortsmit *et al*., 2023; Lemke *et al*., 2023). Recent findings found the importance of adult feeding which enhances the fly performances and overall reproduction, as it provides additional energy aside from the accumulated nutrients during the larval stage (Bruno *et al*., 2019; Tettamanti *et al*., 2022). The necessary sunlight with specific wavelengths was also regarded as crucial factor of mating success, which could promote greater fertility and larval emergence (Tomberlin *et al*., 2002; Nakamura *et al*., 2016; Chen *et al*., 2023). These findings proved more attention on adults should be attached, including the basal systematic information, which may settle the concern about the low mating rate during controlled reproduction.

Males intercept females in a lekking arena where they gather. Upon the proper alignment of their genitalia, the newlyweds would fly to a convenient location to continue mating (Tomberlin and Sheppard, 2001). The males of this species exhibit polygynous behaviour, mating with multiple females (Samayoa *et al*., 2016). The morphologic differences in genitalia of both sexes have been preliminarily described in the research on mating and reproductive behaviours (Julita *et al*., 2020). Females are characterized by a sharp and flexible pseudovipositor consisting of 3 segments, terminating with two short strands. The genitalia of BSF males are more introverted and dilated in the basal portion, with the aedeagus positioned centrally and surrounded by a pair of hook-like parameres (Oliveira *et al*., 2016; Julita *et al*., 2020; Cai *et al*., 2022). However, these concerted descriptions mainly focused on shaping but lacked detailed entomic depictions based on anatomy. Additionally, the morphology of genitalia also served as clues in species taxonomy, particularly valuable within related species (Eberhard, 1990; Mews *et al*., 2010; Puniamoorthy *et al*., 2010; Hu *et al*., 2019). Considering the vast diversity of the genus *Hermetia* (comprising 75 species), it would be more feasible initially to identify individual species, although few anatomical structures of genitalia even in Stratiomyidae have been thoroughly documented previously (Rozkosny and Kozanek, 2006; Pujol-Luz *et al*., 2016).

In dipterans, the reproduction organ is conserved from the generalized structures in insects (Winterton *et al*., 1999; Klowden, 2013). However, specialized structures associated with common oviducts could still vary in shape, amount, and function, such as the spermathecae and the fertilization chamber for temporal sperm storage (Twig and Yuval, 2005). Distinctions in males also exist at the evolutionary level of the seminal vesicle (Wolfner, 1997; Name *et al*., 2012). As for BSF adults, the ovaries were initially documented by Tomberlin *et al*. (2002), and Malawey *et al*. (2019) further revealed some more characteristics of their reproductive systems. Munsch-Masset *et al*. (2023) continued to describe all the reproductive system from spermatogenesis and regarded them as well-differentiated for sexual selection. Nevertheless, notwithstanding diligent investigation, the unequivocal exhibition of their rudimentary morphology remains somewhat elusive, posing challenges to fully discerning the BSF tracts and providing a systematic delineation of their reproductive systems.

According to the recent studies of BSF as recycling resources and the barriers to BSF industrial rearing technology, more studies need to conduct on the adult productive systems (Meneguz *et al.*, 2023). First, we studied the morphology of bisexual genitalia based on anatomy, which would help to characterize this species on taxonomic basis. Moreover, we dissected the reproductive system of BSF adults to better describe their primitive morphology. Finally, we described the developmental morphology of the ovaries and eggs, and established a general criterion to grade sexual maturity.

Materials and methods

Black soldier fly colony

Experimental populations of *H. illucens* were reared in a laboratory of the Hubei International Scientific and Technological Cooperation Base of Waste Conversion by Insects. The colony was reared at a temperature of 27 ± 2 °C, with a relative humidity of 60 ± 5%. Adult flies were provided with powdered milk and water as food source with a photoperiod of 12L: 12D, while larvae were bred with moistened wheat bran (70%). Fifteen individuals of 3 days old were selected for dissection from each sex while characterizing the abdomen, genitalia, and reproductive system. Another group of BSF female adults was collected on each day from 1 to 6 days and dissected for their ovaries. They were separated into different groups (at least 10 individuals in each group) mainly based on the morphology of their ovaries and oocytes, which processed to be our developmental grading criteria.

External reproductive system

A group of 3 days old adults of BSF was selected for the morphological experiment. Initially, the legs and wings were carefully removed from each specimen. Intact abdomens were subsequently incised from the mesothorax region by precision forceps. These samples were applied to characterize the entire abdomen.

Another batch of 3 days old adult BSFs was collected for their abdomens in order to dissect and characterize the genitalia. Samples were softened in a boiling 10% KOH solution for 3-5 minutes, removed unwanted tissues, then washed 2-3 times with distilled water and dried on absorbent paper. Next, the prepared samples were mounted on glass slides and analysed under a stereomicroscope. Forceps were advised to exert controlled pressure on the anterior part of the abdomen as whole segments of genitalia gradually extruded from the terminal abdomen.

Photographs of each sample were taken with a Hirox RX-100 3D Digital Microscope, which was supplied by Wuhan Center for Disease Control & Prevention.

Internal reproductive system

We collected a group of 3 days old adults of BSF, removing their hind legs and wings, then fastened them on a wax tray, piercing through the midsection of the thorax. Dissections were performed under a stereomicroscope. The abdomens of female adults were horizontally incised through the transparent window on the sternum, whereas males were incised posteriorly. Then the abdominal cavities were revealed along the separation of the flank junction between the tergum and sternum. To immerse the entire abdominal cavity after the exposure, 2-3 drops of PBS solution were applied. We carefully separated the internal reproductive system from other organs and removed the fat bodies. The reproduction organ was then placed on glass slides for photography and documentation.

Additionally, another group of adult females were selected from 3 situations: 1-6 days old ones after eclosion, 1-2 days old ones after mating, and post-oviposition ones. Each situation comprised at least 10 individuals. The ovaries should be carefully separated for ovarian development observation, particularly the morphological changes. Grading criteria of ovarian development was established according with previous published studies on other insects (Mahmood and Crans, 1998; Raghu *et al.*, 2003).

Photographs were taken with a Nikon Digital Camera D5100 fixed on a MZ101 stereomicroscope.

Results

Abdominal features in the black soldier fly

The adult abdominal cavity comprised 5 visible wide tergites (figure 1). Dull black mainly covered the flat and relatively slender abdomen, despite 2 notable translucent white patches at the base. Dorsally, the nearly rectangular patches were located on tergite II, while ventrally, the blocked spots merge into a window seizing the posterior margin of tergite I. The patches were smaller in females as a result of sexual dimorphism, for the crossing patches ended at the anterior of sternite I, and basally contracted inward sternite II occasionally (figure 1). Dense long hairs adhered closed to halters, along both sides of the abdomen (figure 1A-1B, figure 1E-1F).

The segments VI to X of the adult abdomen contracted into one visible section. In females, the socket segments of the abdomen were actually elongated as a pseudovipositor, which bears a leaf-shaped and plate-like tip terminals (figure 1B and 1D). In males, the copulatory organs exhibited an elongated structure that was more complex and dilated on the basial portion (figure 1F and 1H). A pair of lateral appendages called cerci occurred near the end of the abdomen, which has one or two subsegments, depending on the sex (figure 1).

The copulatory organ of the male adult

The condense segments VI, VII, and VIII were present from the terminal abdomen, under gentle pressure using forceps. Tergite VI sclerotized widely along the anterior edge, bending downward as horseshoes with slight extension to the lateral sides. Relatively slender sclerotization on tergite VII and VIII was located similarly, whereas curve more smoothly, and extent more aggravated on both sides (figure 2A-2C). Sticky sclerotization (red boxed area) centrally stands across the anterior of sternite VI to VIII (more flattening on sternite VI, figure 2B). Setae were distributed over the sclerites and the central region of the lateral sides. The remaining parts of these segments were membranous to facilitate the condensing of the copulatory organ. Segment VIII terminal was attached to the genital segment (figure 1F).

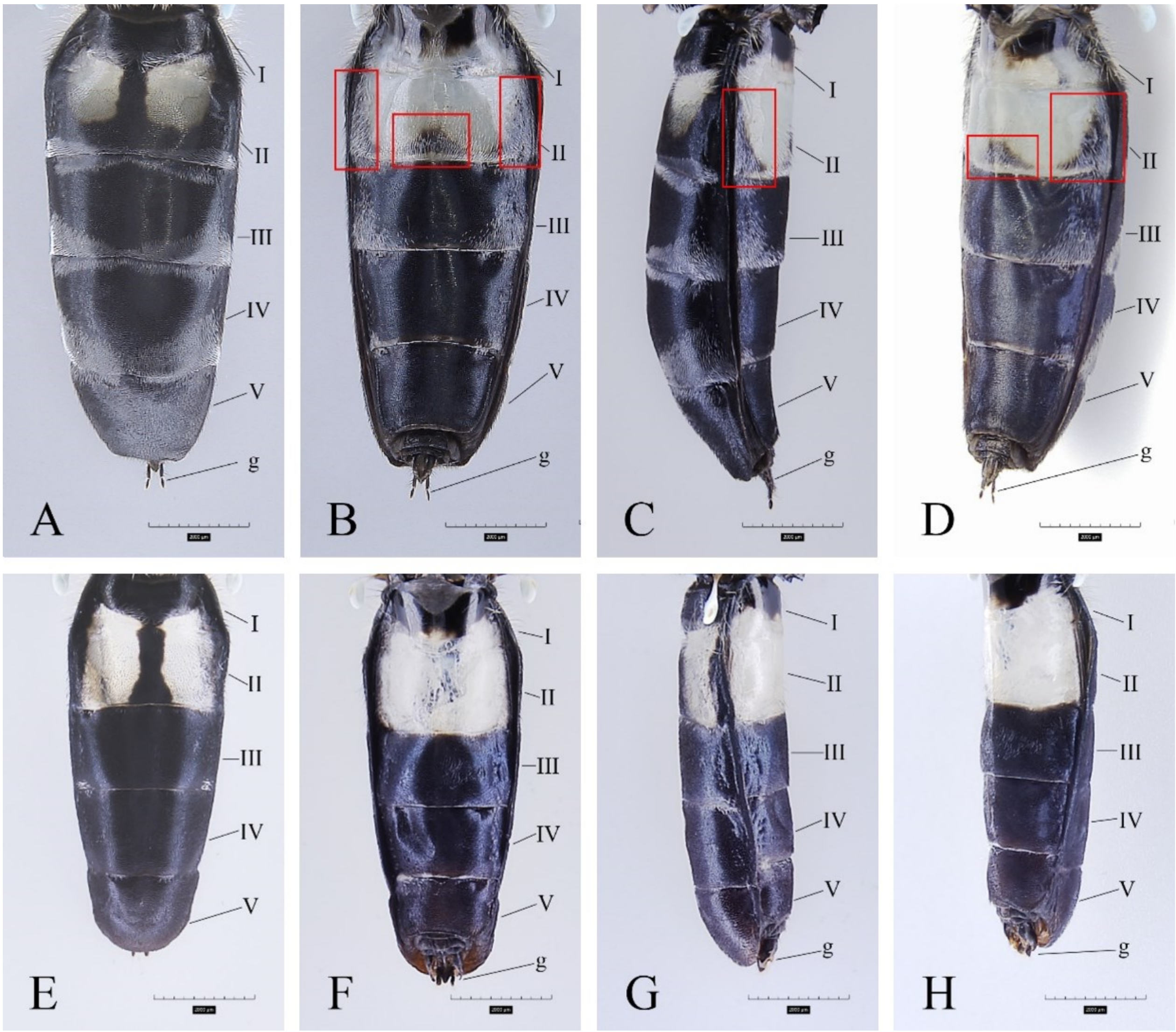

Figure 1. The abdomens of BSF. **A**: Dorsal view of the female, 2 translucent patches can be seen. **B**: Ventral view of the female. The red boxed area showed the sunken part of the patches. **C**-**D**: Lateral view of the female. The red boxed area showed the sunken part of the patches. **E**: Dorsal view of the male, 2 translucent patches can be seen. **F**: Ventral view of the male. **G**-**H**: Lateral view of the male. I-V are 5 visible abdominal segments, while g is the genitalia. Scale bars: A-H 2 mm.

The genitalia of the male adult were comprised of tergite IX, cerci, aedeagus, and genital appendages (figure 2D-2H). The basal corners of tergite IX extent almost cylindrical, combined with the flexible segments (VI to VIII), and terminally stretched out a triangular region, which left a slit anus (red boxed area) at the end (figure 2H-2J). Long hairs are present at the starting point of extension and the edged near the cerci on tergite IX. Segment X shared a similarly developed triangular terminal in tergite IX and was fitly adhibited on the end of tergite IX. On the lateral sides of segment X, a pair of cerci taper shape adhered to one side of the tip and ended with an obtuse telopodite. Hair sensillum densely distributed to the cerci (figure 2H-2J). The genital appendages originated from pronounced developed sternite IX and consisted of coxite and styli. A pair of furcal apophysis (red boxed area) was secondarily segmented in two, horizontally extending to segment VIII on the upper leading edge of sternite IX and ended sharply. The black styli enlarged basally and ventrally, showed a slight hook-like curvature distally, which directed inward (figure 2I-2J). It is notably that the paired verruca (red circled area) located inside the curvature on the dorsal side. Long hairs usually grew outside the styli as well as the verruca, especially the central region on both (figure 2F, 2J-2K). Coxite shared a thickness with styli more basally and structured a genital chamber with an acclivitous “M” on the trailing edge centre of coxite (figure 2I-2J). The genital chamber was closed by the inner side of tergite 9, and the phallus extended from the notch in the centre of “M”.

The phallus encompassed the aedeagus and phallic lateral lobes, which adopted slender and tubular configurations without apparent segmentation. The lobes were slightly longer than the aedeagus and forked outward based on the same horizontal plane (figure 2K-2L). The aedeagus and phallic lateral lobes unite at the base, which

Figure 2. Compositions of BSF male copulatory organs. **A**-**C**: Dorsal, ventral, and lateral views of segments VI, VII, and VIII, wide sclerotization can be seen in the anterior edge of each tergite (A, C), sticky sclerotization can be seen in the centre of each sternite (B). **D**-**F**: Dorsal, ventral, and lateral views of the male genitalia, aedeagus (adg), tergite IX (tIX), genital appendages (ga) and cerci (cer) can be observed. **G**-**H**: Dorsal and ventral views of tergite IX, tergite IX (tIX), segment X (X), and cerci (cer) can be observed. Segment X shared a similarly developed triangular terminal in tergite IX and was fitly adhibited on the end of tergite IX, a pair of cerci located on each side. Red boxed area shows the anus (H). **I**-**J**: The dorsal and ventral views of genital appendages, coxite (cxt), styli (stl) and aedeagus (adg) can be observed. The red boxed areas show a pair of furcal apophysis, and the red circled areas show a pair of verrucae. **K**-**M**: In the dorsal, ventral, and lateral views of the phallus, an aedeagus (adg), two phallic lateral lobes (pll), and a juxta (jxt) can be observed. Scale bars: A-C 1 mm; D-J 500 µm; K-M 200 µm.

combined a shortly columned juxta with an annular membranous band, gifted the aedeagus with stretch flexibility (figure 2K-2M).

Pseudovipositor of the female adult

The condensed part of the pseudovipositor consisted of 3 slender segments (VI, VII, and VIII+IX), which attenuated in turn (figure 3A-3B). Segments VI and VII bridged visceral segments with the genital segment (sternite VIII+IX), shared a membranous change on both ends and thickened from centre (figure 3A-3D). The tergites VIII and IX also integrated, followed by segment X and its appendage, cerci. Hairs were widely distributed outside the pseudovipositor, despite the membranous parts of segments VI and VII (figure 3).

The female external genitalia of BSFs was comprised of genital furca, tergite VIII+IX, sternite VIII+IX, segment X, and two cerci (figure 3E-3F). Folding structures were conspicuously recruited in the common area of both sternite and tergite VIII+IX (figure 3G-3H). Tergite VIII+IX assumed an almost square shape, ending with margins that were slightly concave (figure 3G-3H). Tergite

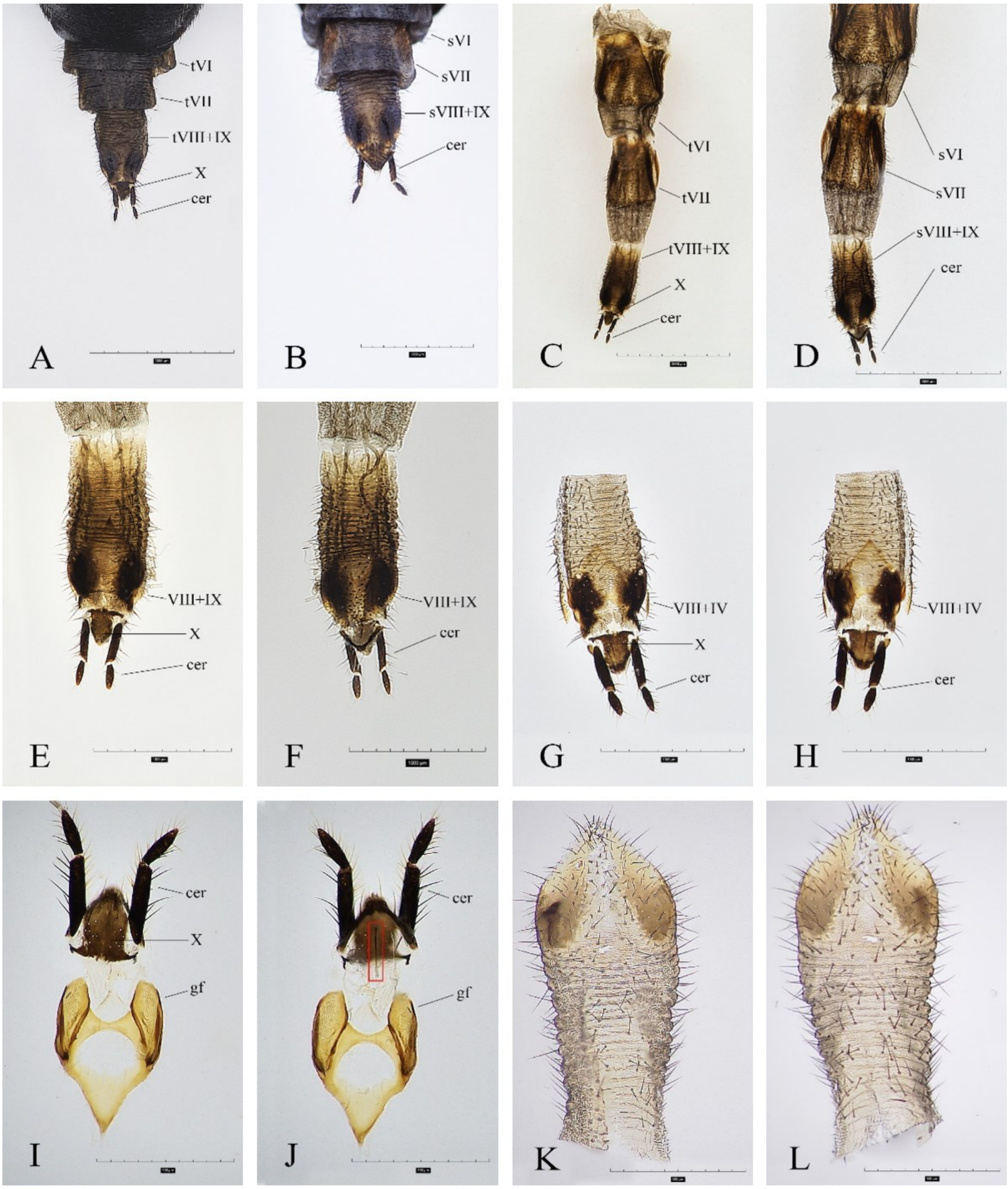

Figure 3. Compositions of BSF female pseudovipositor. **A**-**B**: Dorsal and ventral views of condensed part of pseudovipositor, tergite VI (tVI), VII (tVII), VIII+IX (tVIII+IX) and sternite VI (sVI), VII (sVII), VIII+IX (sVIII+IX), and cerci can be observed both dorsally and ventrally (A, B), while segment X only dorsally (B). **C**-**D**: Dorsal and ventral views of the stretched pseudovipositor. **E**-**F**: Dorsal and ventral views of genital segment and postgenital segment, tergite VIII+IX (tVIII+IX), sternite VIII+IX (sVIII+IX), segment X (X), and two cerci (cer) can be observed. **G**-**H**: Dorsal and ventral views of tergite VIII+IX, tergite VIII+IX (tVIII+IX) and cerci (cer) can be observed both dorsally and ventrally, and segment X (X) only dorsally. **I**-**J**: Dorsal and ventral views of the genital furca and segment X, the paired cerci (cer) located on the side of segment X. The red boxed area show the egg guide. **K**-**L**: Dorsal and ventral views of the sternite VIII+IX. Scale bars: A, C, D 2 mm; B, E-H 1 mm; I-L 500 µm.

VIII+IX terminally bore a leaf-shaped black spot on both sides, which were the attachment points of the lateral lobe of the genital furca (figure 3G-3H). There was an arrow-like needle at the middle inner end of tergite VIII+IX (figure 3G-3H). Post genital segment (segment X) is a black triangular area extending from the terminal part of tergite IX, dorsally fulfilled with villus. The white spots were the remaining bases of the hair roots on segment X. A pair of hairs were notably longer near the tip of segment X. The lateral sides of segment X centrally adhered a pair of cerci, which were segmented into 2 podites (figure 3I-3J). The coxopodite is almost a column, with a steep slant base and invagination end (figure 3E-3G). The telopodite embedded into the coxopodite with a shuttle shape. Hairy sensilla were densely distributed to cerci, with a notably elongated pair on the proximal side of both tip ends (figure 3I-3J). Apart from the attachment region of the cerci, segment X was a detachable sandwich plate resulting in an anus at the tip. The real genital segment (sternite VIII+IX) extended to form a diminutive and bluntly rounded central prominence (figure 3K-3L). The oblong region shared a folding structure with the tergite VIII+IX, while the prominence presented a heart-shape, featuring that the leap-shaped lateral black markings structured a V-like depression cross to the end. That was where the broadening genital furca abutted. The genital furca broadened on both lateral sides, while the sclerite on anterior lobe extent to a triangle closure towards the thorax, which was closed to the dorsal side. This specialized sclerotization formed a central hollow region as genital furca aperture (figure 3I-3J). This structure would be exposed with the terminal opening of segment VIII+IX, which was buried inside the genital chamber.

Internal reproduction organ of male adult

The internal reproductive system of male BSFs generally was comprised of a pair of testes, a pair of seminal ducts (vas deferens), a pair of accessory glands, and an ejaculatory duct (figure 4A).

A single testis was widely tubular and frequently snaked, ending flatly on one side and constricted to a translucent white seminal duct on the other side. The fibrous seminal ducts were little longer than the testes, and almost clustered primitively (figure 4A-4B). Each side of the seminal ducts were ended at the basal portion of a seminal gland, which expanded the width between testes and seminal ducts. The seminal glands were extremely prolonged, much longer than the other tubal structure in the internal reproductive system of male adults. The white glands, coloured most transparently, closed distally, and

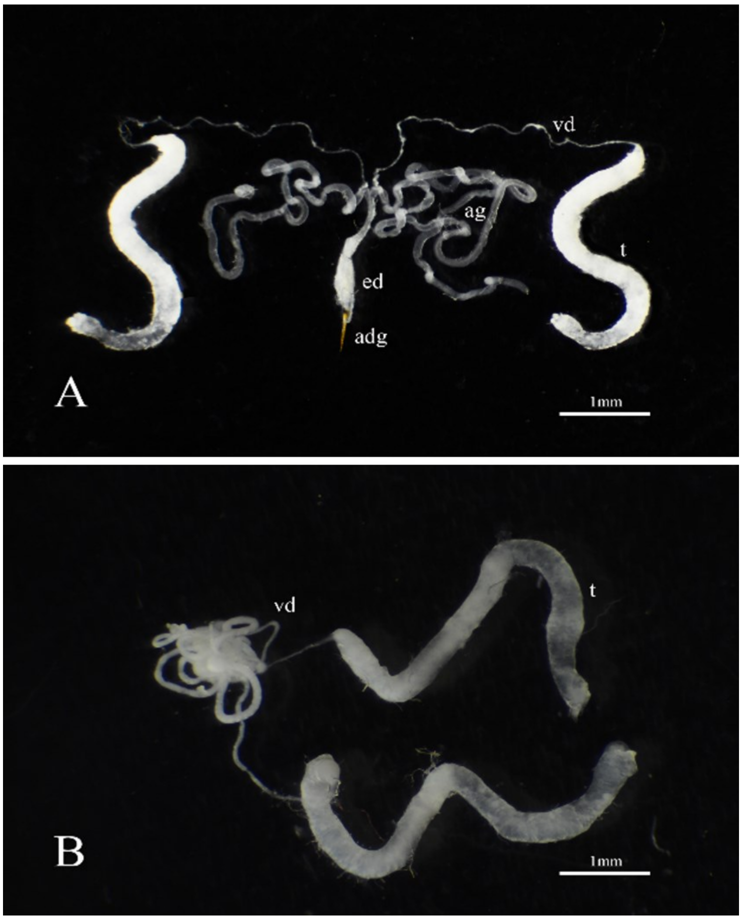

Figure 4. Compositions of BSF male internal reproduction organ. **A**: General composition of the male internal reproduction organ. A pair of testes (t), a pair of vas deferens (vd), a pair of accessory glands (ag), ejective duct (ed), and aedeagus (adg) can be observed. **B**: Paired testes, and vas deferens without separation. Vas deferens can be seen clustered with the attachment of lipid droplets. Scale bars: 1 mm.

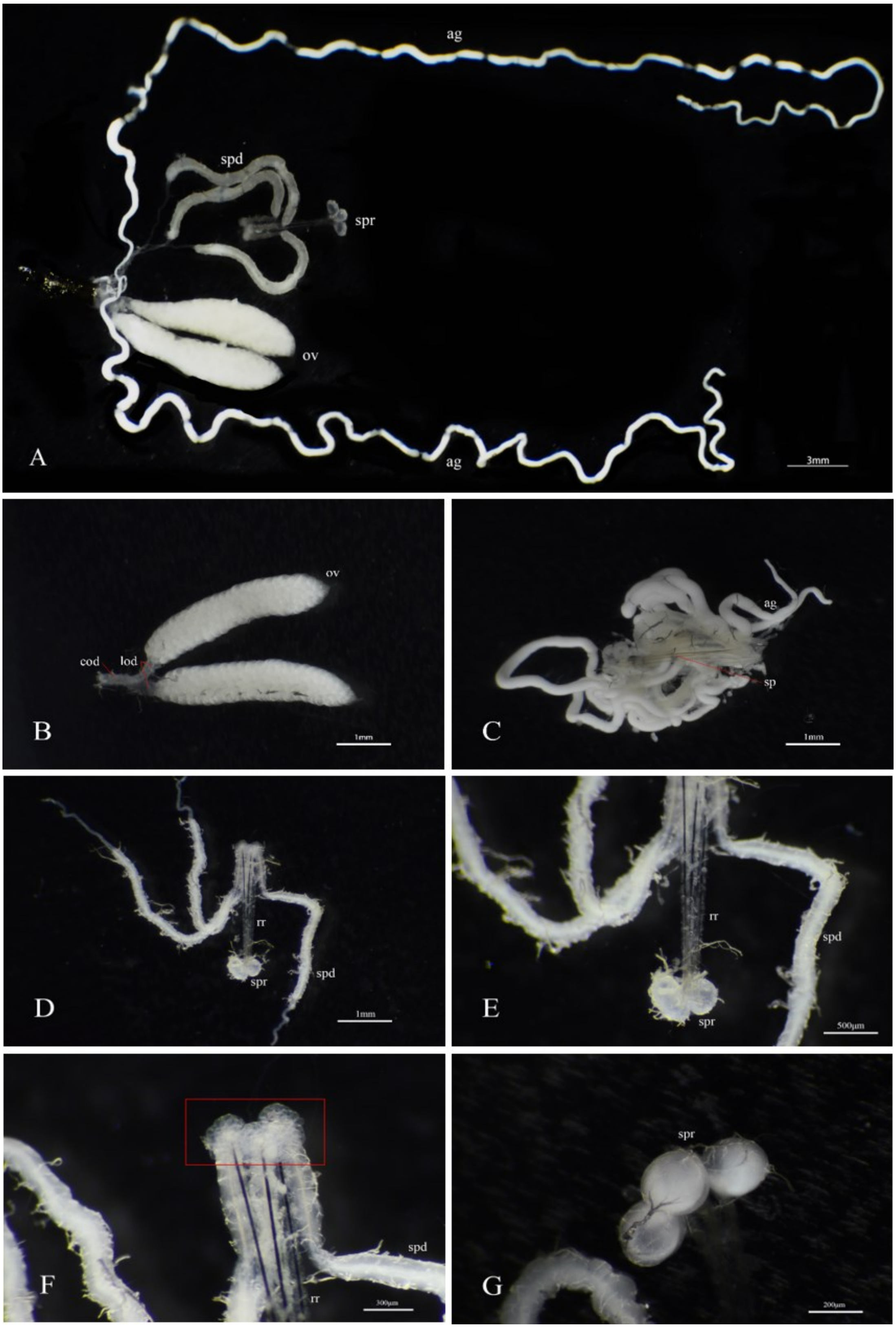

Figure 5. General composition of BSF female internal reproduction organ. **A**: Stretched view of female internal reproductive system, a pair of ovaries (ov), a pair of accessory glands (ag), and an evolved spermathecal complex (spr and spd) can be observed. **B**: Ovary along with lateral oviducts (lod) and common oviduct (cod). **C**: Accessory glands clustered with lipid droplets, and spermathecal complex. **D**: Full view of spermathecal complex, spermathecal reservoirs (spr), rigid rods (rr), spermathecal ducts (spd) can be seen. **E**: Rigid rods in details. **F**: Specialized spermathecal ducts in details. Red boxed area shows the turning sections surrounded with loads of little translucent vesicles. **G**: Spermathecal reservoirs after insemination, sperm can be observed inside. Scale bars: A 3 mm; B-D 1 mm; F 0.3 mm, G 0.2 mm.

were proximally converged into a single ejaculatory duct, whose width was similar to that of a single accessory gland. For a distance, the ejaculatory duct dilated as two continuous spindle structures near its connection to the copulatory organ with a whiter colour (figure 4A).

Internal reproduction organ of female

The internal reproductive system of female BSFs was comprised of a pair of ovaries, a pair of lateral oviducts, a common oviduct, a pair of accessory glands, and an evolved spermathecal complex (figure 5A-5C). The accessory glands and the spermathecal complex were clustered together with lipid droplets in a primitive status (figure 5C). The internal reproductive system of the female entered the genital chamber with a few separate tubes, as the median oviduct, accessory glands, and spermathecal ducts (figure 5A).

The mature ovaries are resembling with a banana shape, and shared a transparent wall with the shortly stalked lateral duct (figure 5B). The mature eggs were symbolized with their granulated shape and opaline colour (figure 6). The ovaries closed distally, while the other end connected to the short lateral oviducts. Paired lateral oviducts were converted into a single common oviduct, whose width was a little wider than the initially constrictive part of the lateral duct (figure 5B). Accessory glands were extremely prolonged and slowly constricted to the genital chamber, apart from the oviducts. The tube wall of accessory glands was translucent in males, whereas the cement inside giving a milky white appearance.

The evolved spermathecal complex was hardly tightened in the glands cluster (figure 5C). It can be initially described as 3 prolonged tubes and mainly slender transparent slivers with three reservoirs (figure 5A). Three adjacent spermathecal reservoirs formed a vesicle-shape and carried highly evolved spermathecal ducts (figure 5D). After fertilization, sperm were observed as milky-white fluid in the spermathecal vesicle, which was thickly crusted by membranes (figure 5G). The reservoirs with lacked of sperm were coloured more translucent. Three black spermathecal ducts stretched up from the storage and were arrayed as rigid rodes (figure 5D). The basial part of the solidifying tubes, combined with spermathecae, formed a transitional phase enlarged and coloured translucent yellow (figure 5E). The median part seemed to be protected vaguely by a translucent membrane and completely blackened. The terminal part was sharply bended as the gothic roof and tubes continued to extend more supply in a yellow colour. The turning sections (boxed area) were surrounded by loads of little translucent vesicles (figure 5F). Subsequently, the spermathecal ducts were surrounded with enlarged membranes for a long distance, as the main part of the canal. Near the base of spermathecae, this elastic part of canals was converted to a more flexible and translucent tube without dilation and finally connected to the fertilization chamber without integration (figure 5D).

The reproductive development of BSF adults

We used the development of the ovaries and oocytes to evaluate the reproductive development of the BSF adults. The development of the ovaries and eggs in BSFs was influenced by the stage of development, fertilization, and oviposition. Upon eclosion, the ovaries appeared as transparent and slender rods, while the internal oocytes were immature, observed as small and semi-transparent with an irregular shape (figure 6A, 6F). Small funnel-shaped openings were found on tips of each oocyte, which would atrophy during the development. During the transitive process, these oocytes carried the opening larger as a gap, and shaped more plumped than grade I. These oocytes in transitive stages could partially remain translucent on one side and fill another region with milky white (figure 6B, 6G). The ovaries also gradually enlarged, and the whole oocytes transformed to oval shapes with milky-white colour until ovaries were filled with plump oocytes, which brought a bumpier outline to the ovary (figure 6C, 6H).

Ovarian development was further improved with fertilization as a prerequisite. Finally, ovaries were fully expanded as bananas, with the transition of fertilized eggs into rice-like shapes (figure 6D, 6I). It is notable that eggs were spill into lateral oviducts, which also occasionally occurred in unfertilized females. In late oviposition, the ovaries shaped as deflated, slender rods (figure 6E). Few mature eggs were found in oviducts and the distal region of the ovaries, while oocytes were seen in ovaries, shaped like stage I, but more oval. According to present studies on morphological changes and ovary development stages established by Mahmood and Crans (1998) and Raghu *et al*. (2003), we characterized the ovarian development of BSF into 5 distinct stages (figure 6 and table 1).

Table 1. Grading of ovarian development of *H. illucens*.

Grade	Developmental period	Shape of single ovary	Ovarian colour	Egg shape	Egg colour
I	Previtellogenic development	Short stick	Translucent	Small and irregular	Translucent
II	Vitellogenin depositing	Stick	Translucent to opaline	Irregular or ellipsoid	Almost translucent partially with opaline
III	Expectant of egg mature	Embryo banana	Opaline	Ellipsoid	Opaline
IV	Peak of oviposition	Banana	Opaline	Granulated	Opaline
V	After oviposition	Atrophic stick	Translucent	Irregular or granulated	Transparent

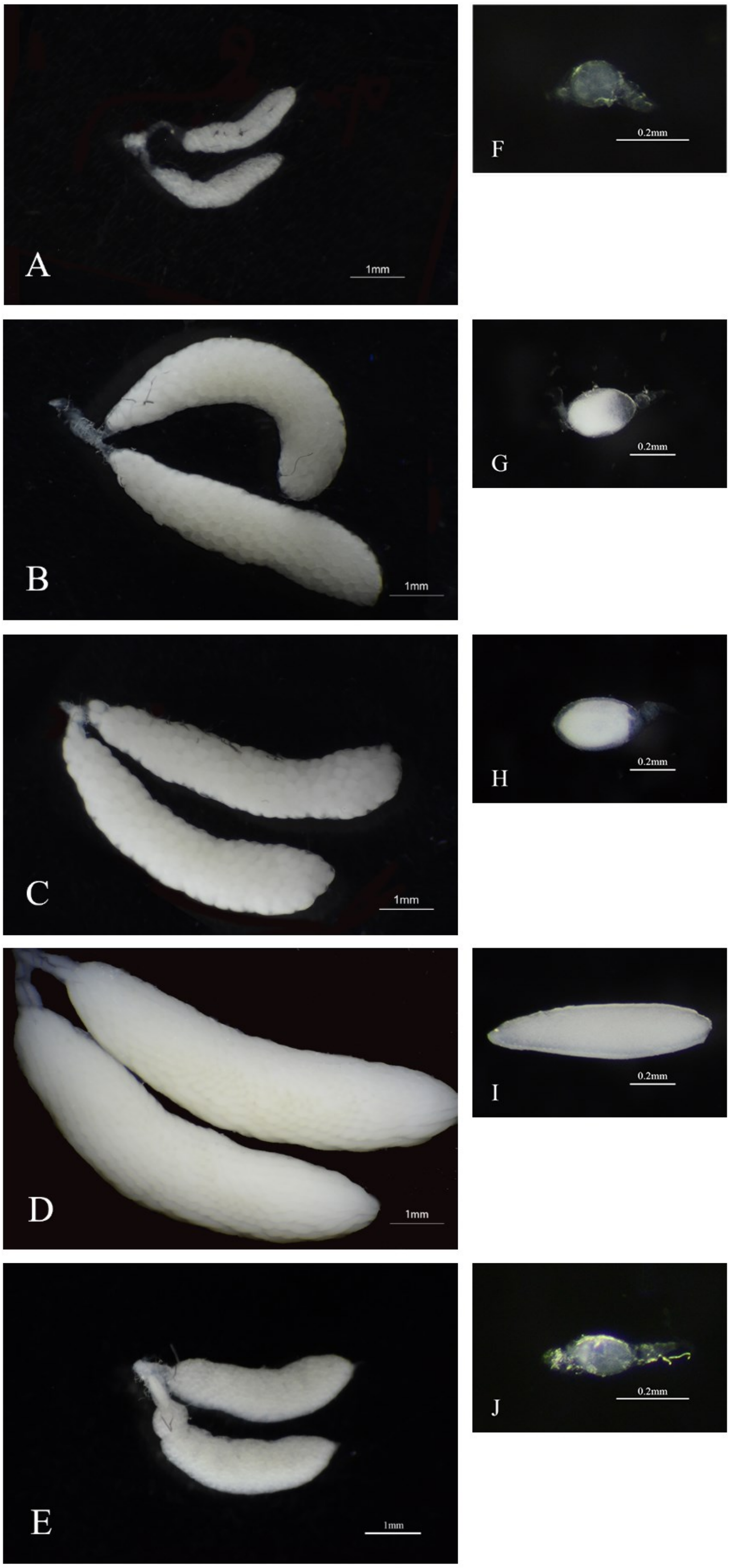

Figure 6. Five ovary development grades of BSF. (**A**) Previtellogenic development; (**B**) Vitellogenin depositing; (**C**) Expectant for egg mature; (**D**) Peak of oviposition; (**E**) Late oviposition. Aside from oocytes, few remained eggs at different grade, (**F**) Previtellogenic development; (**G**) Vitellogenin depositing; (**H**) Expectant for egg mature; (**I**) Peak of oviposition; (**J**) After oviposition. Scale bars: A-E 1 mm; F-J 0.2 mm.

Discussion

The abdominal sexual dimorph of BSF adults

Some morphological differences between both male and female adults of BSF have been observed based on the abdominal studies. It has been notices that, the dorsal side of cercus is discernible in female adults, while the cerci of males were failed to distinguish (figure 1A, 1E). The more visible cerci of the female adults actually consist of an extra subsegment compared to the males, which are privileged to explore a suitable oviposition gap more exteriorly. The distinctions between their abdominal spots were also clear. From a dorsal view, the paired spots were much smaller in females, while the spots in males significantly stretched across the intersegmental membrane between tergite I and II (Rozkosný, 1982). The ventral distinctions of the spots can only distinct partial females from others, due to the occasional absence of the window curvatures (red box area in figure 1B-1D). This absence hasn't been explained clearly, rendering the classification unreliable.

Other distinctions on the genital segment can be also attached from the process of our dissection. However, the condensed segments (VI-VIII) are commonly present in both sexes. In males, these segments are more significantly sclerotized, maintaining a similar shape in each segment, while the membranous sections remain shorter (figure 2A-2C, 3C-3D). The furcal apophysis (rex boxed area in figure 2B) creates some additional space for the central sclerotization, which may facilitate muscle attachment and strengthen the connection between genitalia and the condense segments. In addition to more membraned sections of condense segments, females even have more folded structures, which may commonly ensure a more flexible movement to genital segment (figure 3G-3H). Moreover, the terminal opening of the digestive system is located at segment X in female adults, while in male adults it has been observed at tergite VI. Hence, we regarded it as a retreat for copulation and oviposition.

Distinctive features of copulatory organs and pseudovipositors

Dissection of the copulatory organ could provide essential clues to identify species based on morphological taxonomy (Brammer and von Dohlen, 2010). In the Stratiomyidae family, paired sharp, spine-like processes on the ventral side of the parameral sheath were concurrent structures in copulatory organs (Rozkosný, 1982), which was described as styli and cerci (together with segment X) on the genital appendage in this article. The male BSF adults shared a similar shape of ventral genital appendage and cerci with *Sargus viridis* Say. However, the verruca inward was instead replaced by a more basally extensive furcula in *S. viridis*, and *H. illucens* monopolized a 'M' shape curvature as its phallothecal opening. The phallus could highly evolve in Stratiomyidae, displayed by the centrifugal extent and uneven height between the phallic lateral lobes and aedeagi (Sorenson and Fluke, 1953; Woodley, 2001; Brammer and von Dohlen, 2010). However, consistent feature was observed in BSF: three slender rods kept equal heights with slight branching end. In addition, the rods all compact together with a basal fusion, more so than Stratiomyidae's usual combinations of phallic lateral lobes.

Regarding the pseudovipositors of the female adults, we were unable to distinguish the characteristics of the BSF pseudovipositor from other species in Stratiomyidae, which has been not published on the characteristics of female terminalia in this family. The pseudovipositors of related species require more dissections, as the evolution of female genitalia was mainly selected by their own choice and occurred on genital furcae.

Sensilla are widely distributed on or in the genitalia of both sexes, as previous report (Li *et al*., 2018). They also characterized the regulatory function according to their findings. The sensilla on copulatory organs may function to sense the stimulation, both pressure and pheromones, suitable for their mating. However, the pseudovipositors of females were functioned to sense pressure, temperature, or humidity. This sensory capability may serve to confirm successful mating and help to locate a suitable site for oviposition.

The black arrow inside the genital segment of the BSF female adults has not been reported even in an ovipositor. It actually tightened to sternite VIII+IX (the hollow in figure 3K-3L) alone and the longer length and more swelling tip make it incompatible with a sensilla. Due to its unique location, we suspected it was an egg guide, but further verifications are required.

Distinctive features of internal reproductive systems

The whole internal reproductive system of male BSF adults dissected by us was aligned with the previous descriptions (Malawey *et al*., 2019; Munsch-Masset *et al*., 2023). The components were consistent with most brachyceran males, indicating a conservative nature. The tube-like and long paired accessory glands of BSF has been showed in figure 4A. The male accessory glands typically secret the protein and nutrition into the sperm flow, serving as a trigger of post-mated changes in females (Gillott, 2003). In the Mediterranean fruit fly, these glands can shift the female attraction from fruit odour to the volatile male pheromone (Jang, 2002). The elongated accessory glands in BSF male may also secret the similar substances to trigger post-mated behaviours.

The composition of the female internal reproduction system of BSF is conserved with the generalized female reproductive system (Snodgrass, 2018). The mature ovary is banana-shaped and filled with granulated eggs, which are similar with the previous findings (Tomberlin *et al*., 2002; Chen *et al*., 2023). Reports have suggested that the morphology of the ovaries was mainly conserved at a family level, with similarities observed across various families of dipterans, for the ovarian taxonomic similarity was not only found in Tephritidae and Phoridae (Sivinski *et al*., 2001; Kendra *et al*., 2006; Farder-Gomes *et al*., 2019), but also discovered in BSF and Asilidae (Owsley, 1946). Further verification is needed to determine if other species within Stratiomyidae family, exhibit similar ovarian characteristics, which could provide additional evidences to support the ovarian similarities among closely related species.

The accessory glands of BSF female were extremely prolonged and shared a tubular shape with Asilidae females (Owsley, 1946). In insects, the main function of female accessory glands was considered as secretion, gluing eggs together, providing food for larvae (after hatching), and helping form ootheca (Klowden, 2013; Kozeretska *et al*., 2022). The very prolonged accessory glands of BSF may help for gluing the massive number of eggs to a cluster.

The spermathecae has been described and functionally predicted from the vision of spermatozoa (Malawey *et al*., 2019; Munsch-Masset *et al*., 2023), and our results further unveiled their primitive role of the spermathecae. The morphologic features of the spermathecae ducts were also similarly described in several species of Hermetiinae (Malawey *et al*., 2019). In the turning section of the spermathecal duct in figure 5F, a triangle-like valve (supplemental material figure S1) was notably, clustered with translucent vesicles. The observation was consistent with previous study Munsch-Masset *et al*. (2023). They found that the vesicles as small glandular units, substitute partial function of the spermathecal glands, which was absent in BSF. The membranous structures attached on the rigid rods may act the similar function, as they also found holes on the rigid rods. The dilated membranes surrounding the spermathecal canals has been confirmed to be sperm pumps, which help the sperm transfer in the female reproductive tracts (Munsch-Masset *et al*., 2023).

The numbers of spermathecae vary ranging from one to four in females, despite the fact that a few species store sperm instead with analogue organs such as pseudospermathecae (Pascini and Martins, 2017). The morphological features of the spermathecal complex, especially on spermathecae ducts, can exhibit a significant diversity among species. The presence of spermathecae and its specialized structures can help in better understanding the evolution of sperm collection strategies, which partially favours the high fertility of hexapods.

The central spermathecal reservoir of the three was primarily full with sperm in figure 5G. This distribution has also been reported in other dipterans, like Culicidae (Barreto *et al*., 2008). However, we were unable to find the size differences among the three sperm storage organs under a stereomicroscope, and they may collectively store sperm without strict castes.

The morphologic decorations of the spermathecae ducts were also similarly described in several species of Hermetiinae (Malawey *et al*., 2019). In the turning section of the spermathecal duct in figure 5F, a triangle-like valve (supplemental material figure S1) was notably, clustered with translucent vesicles.

The reproductive development of the BSF adults

However, recent reports on the morphological variations in the BSF ovaries before and after oviposition (Munsch-Masset *et al*., 2023), but these reports were still insufficient for controlled reproduction. We further illustrated the sexual maturation process in adult BSF individuals based on the development of both ovaries and oocytes, and categorized the reproductive development of BSF into 5 distinct stages.

It is important to note that stage III represents the same vitellogenesis stage as stage II in BSF, but some oocytes begin developing into eggs at the end of stage III, which is also characteristic of stage IV. That was the main reason we separate this process into separate stages. However, the grading numbers of ovarian development stages could vary among insects. Barbosa *et al*. (2023) graded the ovary development of Calliphoridae and Sarcophagidae briefly into 3 stages: initial, premature, and mature or pregnant. The ovarian development was graded to 6 stages in *Anastrepha suspensa* (Loew) (Kendra *et al*., 2006) and 5 stages in *Culiseta melanura* (Coquillet) (Mahmood and Crans, 1998). Although the stage grades were slightly different, but they were primarily determined by the direct observation of oocyte and ovarian morphology. It has been noted that the copulation in BSF occurred even at stages I to II as early mating, result in sperm being stored in spermathecae until ovary development. This character enables them to mate before reaching ovarian maturity, having advantage the adult females of BSF to complete reproduction in a shorter time after eclosion.

We also observed that eggs of BSF remain opal while developing in the ovaries and continue to exhibit opal in primiparity eggs. However, these opal coloured eggs gradually turned into yellow and darken after 4-6 hours. From our perspective, the maintenance of opal should originate from the food-derived pigments in the vitellus, while the subsequent colour change could be influenced by the developing effect of protective function of egg sheath (Osanai-Futahashi *et al*., 2016). Therefore, we suggest that future research focus on the composition of the egg sheath in BSF, which could provide more information on their incubation process.

Conclusions

In summary, our study identified the sexual distinctions in abdominal morphology and systematically described the detailed structures of genitalia of both sexes. We also dissected the internal productive systems of BSFs with separated structures including a highly evolved spermatheca complex. A grading criterion for ovarian development in BSF was established to evaluate sexual maturity. Further studies should concentrate on the coevolution of genitalia and reproductive system, and the research related to reproductive development in BSF adults may be assisted by our results.

Acknowledgements

We would like to thank Wuhan Center for Disease Control and Prevention for providing access to Hirox 3D Digital Microscope and Jing Liu for technical support in photography capturing; Abdul Hafez, Jie Zhang, Yitin Wen, and Hui Liu for assistance in helping to revise this manuscript.

This research was partly supported by the Fundamental Research Funds for the Central Universities (No. 302/510323003).

Wenzheng Tang and Jianing Zhang contributed equally as co-first authors to this work. All authors declare that there are no conflicts of interest.

References

Barbosa T. M., Jales J. T., Medeiros J. R., Gama R. A., 2023.- Sarcosaprophagous dipterans associated with differentially-decomposed substrates in Atlantic Forest environments.- *Acta Brasiliensis*, 7 (1): 14-21.

Barreto M., Burbano M. E., Barreto P., 2008.- Gynandromorphs in mosquitoes (Diptera: Culicidae) from Valle del Cauca, Colombia.- *Neotropical Entomology*, 37 (3): 296-300.

Borkent A., Borkent C. J., Sinclair B. J., 2008.- The male genital tract of Chaoboridae (Diptera: Culicomorpha).- *Canadian Entomologist*, 140 (6): 621-629.

Brammer C. A., Von Dohlen C. D., 2010.- Morphological phylogeny of the variable fly family Stratiomyidae (Insecta, Diptera).- *Zoologica Scripta*, 39 (4): 363-377.

Bruno D., Bonelli M., Cadamuro A. G., Reguzzoni M., Grimaldi A., Casartelli M., Tettamanti G., 2019.- The digestive system of the adult *Hermetia illucens* (Diptera: Stratiomyidae): morphological features and functional properties.- *Cell and Tissue Research*, 378 (2): 221-238.

Cai M. M., Li L., Zhao Z. Z., Zhang K., Li F., Yu C., Yuan R. F., Zhou B. H., Ren Z. Q., Yu Z. N., Zhang J. B., 2022.- Morphometric characteristic of black soldier fly (*Hermetia illucens*) Wuhan strain and its egg production improved by selectively inbreeding.- *Life*, 12 (6): 873.

Chen B. H., Kou Z. Q., Jiang Y. G., Luo X. Y., Li P. L., Sun K. J., Wang W. W., Huang Y. P., Wang Y. H., 2023.- Intersex is required for female sexual development in *Hermetia illucens*.- *Insect Science*, 30 (4): 901-911.

Córdova-García G., Esquivel C. J., Pérez-Staples D., Ruiz-May E., Herrera-Cruz M., Reyes-Hernández M., Abraham S., Aluja M., Sirot L., 2022.- Characterization of reproductive proteins in the Mexican fruit fly points towards the evolution of novel functions.- *Proceedings of the Royal Society B: Biological Sciences*, 289 (1977): 20212806.

Eberhard W. G., 1990.- Animal genitalia and female choice.- *American Scientist*, 78 (2): 134-141.

Farder-Gomes C. F., Pinto Santos H. C., Oliveira M. A., Zanuncio J. C., Serrao J. E., 2019.- Morphology of ovary and spermathecae of the parasitoid *Eibesfeldtphora tonhascai* Brown (Diptera: Phoridae).- *Protoplasma*, 256 (1): 3-11.

Gasco L., Biancarosa I., Liland N. S., 2020.- From waste to feed: a review of recent knowledge on insects as producers of protein and fat for animal feeds.- *Current Opinion in Green and Sustainable Chemistry*, 23: 67-79.

Gillott C., 2003.- Male accessory gland secretions: modulators of female reproductive physiology and behavior.- *Annual Review of Entomology*, 48 (1): 163-184.

Hu G. L., Gao K., Wang J. S., Hebert P. D. N., Hua B. Z., 2019.- Molecular phylogeny and species delimitation of the genus *Dicerapanorpa* (Mecoptera: Panorpidae).- *Zoological Journal of the Linnean Society*, 187 (4): 1173-1195.

Jang E. B., 2002.- Physiology of mating behavior in Mediterranean fruit fly (Diptera: Tephritidae): chemoreception and male accessory gland fluids in female post-mating behavior.- *Florida Entomologist*, 85 (1): 89-93.

Ji Y., Ren D. Z., Ye M. Q., Luo C., An X. C., 2017.- Studies on optimum temperature for rearing black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidea).- *Journal of Environmental Entomology*, 39 (2): 390-395.

Julita U., Fitri L. L., Putra R. E., Permana A. D., 2020.- Mating success and reproductive behavior of black soldier fly *Hermetia illucens* L. (Diptera, Stratiomyidae) in tropics.- *Journal of Entomology*, 17 (3): 117-127.

Kendra P. E., Montgomery W. S., Epsky N. D., Heath R. R., 2006.- Assessment of female reproductive status in *Anastrepha suspensa* (Diptera: Tephritidae).- *Florida Entomologist*, 89 (2): 144-151.

Klowden M. J., 2013.- *Physiological systems in insects*, 3rd Edition.- Academic press, New York, USA.

Kortsmit Y., van der Bruggen M., Wertheim B., Dicke M., Beukeboom L. W., van Loon J. J. A., 2023.- Behaviour of two fly species reared for livestock feed: optimising production and insect welfare.- *Journal of Insects as Food and Feed*, 9 (2): 149-169.

Kozeretska I., Serga S., Kovalenko P., Gorobchyshyn V., Convey P., 2022.- *Belgica antarctica* (Diptera: Chironomidae): a natural model organism for extreme environments.- *Insect Science*, 29 (1): 2-20.

Lemke N. B., Dickerson A. J., Tomberlin J. K., 2023.- No neonates without adults: a review of adult black soldier fly biology, *Hermetia illucens* (Diptera: Stratiomyidae).- *BioEssays*, 45 (1): 2200162.

Li Z. Y., Zhou Q., Zhou K. L., Zhou J. H., Li Q., 2018.- Sensilla of adult *Hermetia illucens* observed with scanning electron microscope (II): labellum, haltere and externalia.- *Journal of Chinese Electronic Microscopy Society*, 37 (1): 183-189.

Mahmood F., Crans W. J., 1998.- Ovarian development and parity determination in *Culiseta melanura* (Diptera: Culicidae).- *Journal of Medical Entomology*, 35 (6): 980-988.

Malawey A. S., Mercati D., Love C. C., Tomberlin J. K., 2019.- Adult reproductive tract morphology and spermatogenesis in the black soldier fly (Diptera: Stratiomyidae).- *Annals of the Entomological Society of America*, 112 (6): 576-586.

Marchini D., Del Bene G., Cappelli L., Dallai R., 2003.- Ultrastructure of the male reproductive accessory glands in the medfly *Ceratitis capitata* (Diptera: Tephritidae) and preliminary characterization of their secretions.- *Arthropod Structure & Development*, 31 (4): 313-327.

Marshall S. A., Woodley N. E., Hauser M., 2015.- The historical spread of the black soldier fly, *Hermetia illucens* (L.) (Diptera, Stratiomyidae, Hermetiinae), and its establishment in Canada.- *Journal of the Entomological Society of Ontario*, 146: 51-54.

Men Q. L., Sun M. W., Starkevich P., 2018.- Comparative morphological study of reproductive system between Tipulidae and Limoniidae (Diptera: Tipuloidea).- *Zoological Systematics*, 43 (1): 89-102.

Meneguz M., Miranda C. D., Cammack J. A., Tomberlin J. K., 2023.- Adult behaviour as the next frontier for optimising industrial production of the black soldier fly *Hermetia illucens* (L.) (Diptera: Stratiomyidae).- *Journal of Insects as Food and Feed*, 9 (4): 399-414.

Mews C. M., Szinwelski N., Sperber C. F., 2010.- A new genus and new species of Brazilian Luzarinae crickets (Grylloidea: Phalangopsidae).- *Studies on Neotropical Fauna and Environment*, 45 (3): 159-174.

Munsch-Masset P., Labrousse C., Beaugeard L., Bressac C., 2023.- The reproductive tract of the black soldier fly (*Hermetia illucens*) is highly differentiated and suggests adaptations to sexual selection.- *Entomologia Experimentalis et Applicata*, 171 (11): 857-866.

Nakamura S., Ichiki R. T., Shimoda M., Morioka S., 2016.- Small-scale rearing of the black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in the laboratory: low-cost and year-round rearing.- *Applied Entomology and Zoology*, 51 (1): 161-166.

Name K. P. O., Barros-Cordeiro K. B., Filho J. B. G., Wolff M., Pujol-Luz J. R., Báo S. N., 2012.- Structure and ultrastructure of spermatozoa and spermiogenesis in three species of *Lucilia* Robineau-desvoidy, 1830 (Diptera: Calliphoridae).- *Journal of Morphology*, 273 (2): 160-172.

Oliveira F. R., Doelle K., Smith R. P., 2016.- External morphology of *Hermetia illucens* Stratiomyidae: Diptera (L. 1758) based on electron microscopy.- *Annual Research & Review in Biology*, 9 (5): 1-10.

Osanai-Futahashi M., Tatematsu K. I, Futahashi R., Narukawa J., Takasu Y., Kayukawa T., Shinoda T., Ishige T., Yajima S., Tamura T., Yamamoto K., Sezutsu H., 2016.- Positional cloning of a *Bombyx* pink-eyed white egg locus reveals the major role of cardinal in ommochrome synthesis.- *Heredity*, 116 (2): 135-145.

Owsley W. B., 1946.- The comparative morphology of internal structures of the Asilidae (Diptera).- *Annals of the Entomological Society of America*, 39 (1): 33-68.

Pascini T. V., Martins G. F., 2017.- The insect spermatheca: an overview.- *Zoology*, 121: 56-71.

Pujol-Luz J. R., De Godoi F. S. P., Barros-Cordeiro K. B., 2016.- Description of the puparium of *Hermetia pulchra* (Diptera: Stratiomyidae) from Brazil.- *Zootaxa*, 4205 (4): 357-364.

Puniamoorthy N., Kotrba M., Meier R., 2010.- Unlocking the "Black box": internal female genitalia in Sepsidae (Diptera) evolve fast and are species-specific.- *BMC Evolutionary Biology*, 10: 275.

Raghu S., Halcoop P., Drew R. A. I., 2003.- Apodeme and ovarian development as predictors of physiological status in *Bactrocera cacuminata* (Hering) (Diptera: Tephritidae).- *Australian Journal of Entomology*, 42: 281-286.

Rozkošný R., 1982.- *A biosystematic study of the European Stratiomyidae (Diptera): Volume 1 - introduction, Beridinae, Sarginae and Stratiomyinae*.- Springer, The Hague, The Netherlands.

Rozkosny R., Kozanek M., 2006.- A review of the Oriental *Hermetia bicolor* group with descriptions of two new species (Diptera: Stratiomyidae).- *Insect Systematics & Evolution*, 37 (1): 81-90.

Samayoa A. C., Chen W. T., Hwang S. Y., 2016.- Survival and development of *Hermetia illucens* (Diptera: Stratiomyidae): a biodegradation agent of organic waste.- *Journal of Economic Entomology*, 109 (6): 2580-2585.

Sheppard D. C., Newton G. L., Thompson S. A., Savage S., 1994.- A value added manure management system using the black soldier fly.- *Bioresource Technology*, 50 (3): 275-279.

Sheppard D. C., Tomberlin J. K., Joyce J. A., Kiser B. C., Sumner S. M., 2002.- Rearing methods for the black soldier fly (Diptera: Stratiomyidae).- *Journal of Medical Entomology*, 39 (4): 695-698.

Sinclair B. J., Borkent A., Wood D. M., 2007.- The male genital tract and aedeagal components of the Diptera with a discussion of their phylogenetic significance.- *Zoological Journal of the Linnean Society*, 150 (4): 711-742.

Sivinski J., Vulinec K., Aluja M., 2001.- Ovipositor length in a guild of parasitoids (Hymenoptera: Braconidae) attacking *Anastrepha* spp. fruit flies (Diptera: Tephritidae) in southern Mexico.- *Annals of the Entomological Society of America*, 94 (6): 886-895.

Snodgrass R. E., 2018.- *Principles of insect morphology*.- Cornell University Press, New York, USA.

Sorenson J. S., Fluke C. L., 1953.- The male genitalia of the Stratiomyidae with special reference to Wisconsin species.- *Annals of the Entomological Society of America*, 46 (3): 337-342.

Tettamanti G., Van Campenhout L., Casartelli M., 2022.- A hungry need for knowledge on the black soldier fly digestive system.- *Journal of Insects as Food and Feed*, 8 (3): 217-222.

Tomberlin J. K., Sheppard D. C., 2001.- Lekking behavior of the black soldier fly (Diptera: Stratiomyidae).- *Florida Entomologist*, 84 (4): 729-730.

Tomberlin J. K., Sheppard D. C., Joyce J. A., 2002.- Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets.- *Annals of the Entomological Society of America*, 95 (3): 379-386.

Twig E., Yuval B., 2005.- Function of multiple sperm storage organs in female Mediterranean fruit flies (*Ceratitis capitata*, Diptera: Tephritidae).- *Journal of Insect Physiology*, 51 (1): 67-74.

Valdez J. M., 2001.- Ultrastructure of the testis of the Mexican fruit fly (Diptera: Tephritidae).- *Annals of the Entomological Society of America*, 94 (2): 251-256.

Winterton S. L., Merritt D. J., O'Toole A., Yeates D. K., Irwin M. E., 1999.- Morphology and histology of the spermathecal sac, a novel structure in the female reproductive system of Therevidae (Diptera: Asiloidea).- *International Journal of Insect Morphology & Embryology*, 28 (4): 273-279.

Wolfner M. F., 1997.- Tokens of love: functions and regulation of *Drosophila* male accessory gland products.- *Insect Biochemistry and Molecular Biology*, 27 (3): 179-192.

Woodley N. E., 2001.- *A world catalog of the Stratiomyidae (Insecta: Diptera)*.- Bachuys, Leiden, The Netherland.

Zhang J., Wen Y. T., Gao Z. H., Lei C. L., Zhu F., 2019.- Progress in research on, and the utilization of, *Hermetia illucens*.- *Chinese Journal of Applied Entomology*, 56 (5): 997-1006.

Authors' addresses: Fen Zhu (corresponding author: zhufen@mail.hzau.edu.cn), Wenzheng Tang (wenzheng.t@webmail.hzau.edu.cn), Jianing Zhang (jhonnyzhang@webmail.hzau.edu.cn), Zhihui Shi (zhihuishi@webmail.hzau.edu.cn), Hubei International Scientific and Technological Cooperation Base of Waste Conversion by Insects, Huazhong Agricultural University, Wuhan, 430070, China; Xiaomin Chen (174818235@qq.com), Wuhan Center for Disease Control and Prevention, Wuhan, 430024, China.

Received October 3, 2023. Accepted April 4, 2024.

(Supplemental material available at http://www.bulletinofinsectology.org/Suppl/vol77-2024-124-136tang-suppl.pdf)