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REVIEW ARTICLE

Black soldier fly (*Hermetia illucens*): Driving circular agriculture through organic waste recovery

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Abstract

The black soldier fly (BSF), *Hermetia illucens* has gained global attention for its diverse applications, particularly its efficiency in organic waste conversion, achieving 50-70 % waste reduction. The larvae produce high-value biomass containing 30-57 % protein and 21-42 % lipids, making them an excellent protein-rich feed ingredient. Additionally, BSF shows strong potential for biodiesel production, with conversion yields reaching up to 94 %. Key biological traits such as global adaptability, rapid development and efficient feed conversion make BSF suitable for scalable farming. Recent advances in genetic research, microbiome optimization and selective breeding are examined, highlighting their potential to enhance productivity and adaptability. The environmental and economic advantages of BSF-based bioconversion systems such as reduced greenhouse gas emissions, effective nutrient recycling and job creation; especially in developing regions are also discussed. Despite its promise, challenges persist, including the need for genetic diversity conservation, consistent regulatory policies and increased public acceptance. Future research priorities include optimizing organic waste substrates, developing targeted breeding strategies and exploring novel applications such as antimicrobial peptide production, chitin recovery and pigment extraction. In addition, progress in automation and bioprocessing technologies can significantly boost operational efficiency and scalability. By integrating innovation and encouraging cross-sector collaboration, BSF-based solutions can play a vital role in advancing global sustainability, food and feed security and environmental resilience.

Keywords: antimicrobial peptides; bioconversion; biodiesel; insect protein; microbiome optimization; waste management

Introduction

With the rising global population and increasing demand for animal protein, there is a pressing need for alternative, sustainable feed sources. Insects have emerged as a viable solution, offering high-quality protein, efficient biomass conversion and environmental resilience (1-3). Among these, Black Soldier Fly (BSF) have gained significant attention due to their short life, rapid growth and high adaptability to farming systems (4).

Hermetia illucens L., commonly known as BSF, belongs to the family Stratiomyidae, order Diptera (Fig. 1). BSF has a cosmopolitan distribution, thriving in tropical and warm temperate climates (5). However, due to its inability to tolerate cold temperatures, BSF cannot survive in regions like northwestern Europe and regions with temperatures below 5 °C (6). Genetic data suggests that captive populations from several continents trace back to a single North American origin, highlighting a common ancestry for many farmed strains (7).

BSF has emerged as a key candidate in sustainable agriculture and waste management. According to the European legislative framework (EG no. 1069/2009), BSF has been recognized as an insect for industrial rearing and granted the status of a farm animal (8). As the global population continues to grow, it becomes crucial to utilize available resources more efficiently. The European Union, Australia, Canada and the USA have established regulations allowing the trade and manufacture of Black soldier fly Larvae (BSFL) as animal feed under specific conditions. Ensuring legislative clarity and harmonization is essential for the successful industrial scaling of BSFL as a sustainable source of animal feed (9).

BSFL exhibit a high growth rate and efficiently convert low-grade organic waste into protein-rich biomass, outperforming broilers, pigs and fish in substrate protein conversion into body mass (4). Due to the high moisture content (up to 70 %) in BSFL substrates, the feed conversion ratio (FCR) is not suitable for comparing feed efficiency with other species. Instead, protein conversion ratio (PCR) and

Fig. 1. Black soldier fly.

gross energy conversion ratio (GECR) are used, demonstrating that BSFL efficiently utilize nutrients. BSF prepupae, rich in essential macro- and micronutrients for animal feed, also produce saturated fatty acids, creating opportunities for use in various industries, including biofuels and other non-food applications (10).

The year-round availability of BSF and its byproducts guarantees its reliability and sustainability as a resource (11). Using BSFL for waste composting is also an innovative, eco-friendly and cost-effective solution that promotes resource recovery and value-added products, gaining global attention for its self-sustaining nature. Current waste management technologies fail to fully utilize organic-rich waste, focusing mainly on disposal. Researchers are exploring sustainable methods to unlock its nutrient and economic value for more efficient waste management (5). BSFL significantly reduce nutrients and dry matter in organic material by 50 % or more and effectively decrease odorous volatile compounds by up to 100 % (12). This transformative potential has led to a shift in the perception of BSFL from being considered a nuisance to being recognized as a crucial component in promoting sustainability and waste management within the circular economy framework (13). Adult BSF typically rest on vegetation and avoid contact with humans or animals, with no evidence suggesting they act as vectors for pathogens. However, their larvae thrive in microbe-rich environments, including those containing pathogenic microorganisms (14).

Efficient insect production hinges on factors like fast growth, high survival rates, large body mass and robust reproductive capacity, all of which contribute to increased biomass production and the value of the produced biomass, particularly if the insects possess desired nutrients or valuable proteins. Managing healthy insect populations involves optimizing conditions across generations, minimizing inbreeding and genetic drift through well-designed breeding programs and ensuring environmental factors such as temperature and diet align with life stage requirements. This understanding of fundamental principles guides effective insect production practices (15). Despite the extensive utilization of BSF in the growing insect farming sector, there remains a significant gap in research regarding its genetics (13).

BSF exhibits high genetic diversity, a factor of considerable importance for both scientific exploration and commercial utilization. Understanding the genetic composition of fly cultures is essential for efficient management, enabling the utilization of diversity in breeding programs (16). Most commercial production currently relies on unimproved BSF populations without employing specific breeding strategies. Inbreeding is a concern in captive populations maintained for many generations under genetically isolated conditions (17). The availability of a comprehensive genetic inventory and microsatellite markers allows for exploring gene-environment interactions, studying phenotypic traits and developing effective breeding strategies. Next-generation sequencing and improved genomic resources have significantly advanced our understanding of BSF. Furthermore, synthetic biology holds promise in enhancing the capabilities of BSF by generating transgenic variants for improved feed, industrial biomolecule production and waste conversion (7).

This review aims to consolidate current knowledge on the biology, nutritional value, waste management potential, genetics and industrial applications of BSF, while highlighting knowledge gaps and opportunities for future research and innovation.

Life cycle and biology

Morphology and sexual dimorphism

The adult BSF is a large, slender insect with a segmented body (head, thorax and abdomen) and brownish wings. Males have a bronze-colored abdomen, while females exhibit a reddish-brown hue (14). Females (16.3 ± 0.91 mm) are larger than males (14.30 ± 0.19 mm) (18). The average male-to-female ratio is 0.98 (19).

Life cycle duration and environmental factors

The average life cycle duration of BSF was 45.08 ± 4.46 days for males and 46.15 ± 4.12 days for females under controlled conditions of 29.40 ± 1.77 °C, 68.25 ± 2.32 % relative humidity (RH) and a 14:10 (L:D) photoperiod, when reared on fruit and vegetable waste (18). However, the duration can extend up to 131 days, depending on the nutrient and energy content of the feeding substrates and the ambient temperature of the rearing conditions (20). BSF undergoes six larval instars from the first instar to the pupal stage. The mean durations of the developmental stages were 4.36 ± 0.24 days for egg hatching, 16.07 ± 2.59 days for the larval stage, 15.4 ± 2.50 days for the pupal stage, 9.95 ± 1.48 days for males and 10.33 ± 1.89 days for females, under the same rearing conditions (18) (Fig. 2).

Reproductive biology and mating dynamics

Males typically emerge two days before females, with mating occurring approximately two days after emergence. Being an eurygamous insect i.e., one that requires large open spaces for nuptial flight-the BSF engages in mating while flying (21). BSF adults are weak fliers and spend most of the day resting on plants (14). Females deposit their eggs in dry crevices located close to the larval substrate. The average weight of a single egg mass was 29.1 mg, containing approximately 998 eggs. Each egg had a weight of about 0.028 mg (22).

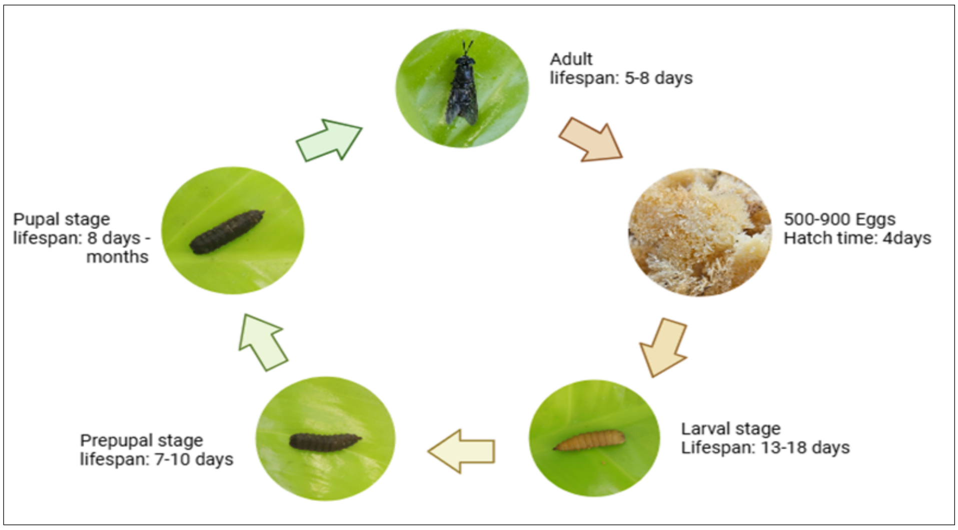

Fig. 2. The lifecycle of black soldier fly.

Male black soldier flies congregate at lekking sites, where they encounter and engage with flying females. BSF males rely on sunlight to detect females entering their mating sites, with most mating pairs occurring in direct sunlight (23). BSF adults were found to mate an average of three times, occasionally with different or previous partners and in some instances; they mated up to nine times (24). Sunlight is essential for mating, with 85 % of mating events occurring at an intensity of 110 µmol m^{2} s^{-1} (5).

Feeding behaviour and development

The antennae of BSFL are composed of two segments and are relatively short in the older instars, while proportionally longer in the younger larvae. When the head extends into the feeding substrate, the antennae are shielded from damage by a large, sturdy antennal pad (25).

The aggregation behaviour of BSFL, known as the larval mass effect, is typical of necrophagous Diptera and helps increase body temperature, thereby reducing developmental time. This behaviour has practical implications in accelerating bioconversion rates during industrial waste processing, making BSF a highly effective species for organic waste valorization (26).

BSFL feed in 5 min bursts, spending 44 % of their time near food, which causes congestion and slows consumption. This behaviour results in a 'fountain' effect, where larvae feed and are then pushed upward and out of the feeding mass, ensuring equitable access to food. This self-propagating flow increases the overall consumption rate; ensuring food is shared with those not actively feeding (27).

In the final larval stage, BSF larvae can reach up to 27 mm in length and 6 mm in width. They can weigh up to 220 mg, the highest among all developmental stages (28). At the end of the larval period, the prepupae migrate to a dry, suitable site for pupation and transform into pupae. Prepupae require a minimum of 10 days to pupate in a dry environment (29).

Digestion and microbial interaction

Digestive anatomy and enzyme activity

Findings revealed that the BSF larval head resembles that of campodeiform insect larvae, larvae with an elongated, flattened body adapted for active movement, typical of scavenger species. The highly developed mandibular-maxillary complex, characteristic of Stratiomyidae and like other scavenger larvae, enhances the intake of semiliquid food through a sweeping apparatus. The mouthparts function like a "tunnel boring machine," in which the hypopharynx helps sort finer organic particles from coarser inorganic ones, while the maxillary rasp and lacinial teeth, specialized structures in the maxilla used for scraping and tearing food, aid in breaking down material from semiliquid substrates for efficient digestion (25).

The alimentary canal of BSFL is composed of a short foregut, an elongated midgut divided into anterior (AMG), middle (MMG) and posterior (PMG) regions and a hindgut featuring the insertion of Malpighian tubules. Each midgut region exhibits distinct chemical and functional properties (Fig. 3). The anterior midgut (pH ~ 6) contains columnar cells with secretory activity and high amylase and lipase activity. The middle midgut (pH ~ 2) is characterized by copper cells, contributing to pathogen elimination and microbiota regulation through strong acidity and high lysozyme activity. The posterior midgut (pH ~ 8) facilitates protein digestion through serine proteases and exopeptidases, while also contributing to lipid and polysaccharide breakdown. The presence of elongated microvilli enhances nutrient absorption (30).

Gut extracts from BSFL show high amylase, lipase and protease activities, including strong trypsin-like protease activity, indicative of their polyphagous nature. These enzymatic activities, such as leucine arylamidase, α-galactosidase, β-galactosidase, α-mannosidase and α-fucosidase, suggest that BSFL can digest a wide range of food waste and organic materials more efficiently than other fly species, such as house flies (31).

Microbiome composition and functions

The gut microbiome of BSFL plays a crucial role in feed biodegradation and its bacterial composition is distinct from that of other insect species. The microbiota is primarily dominated by the phyla *Firmicutes* and *Proteobacteria*, with diet being a significant influence on its composition. Both host genetics and diet shape the microbial diversity, which, in turn, determines the metabolic potential of the larvae (32).

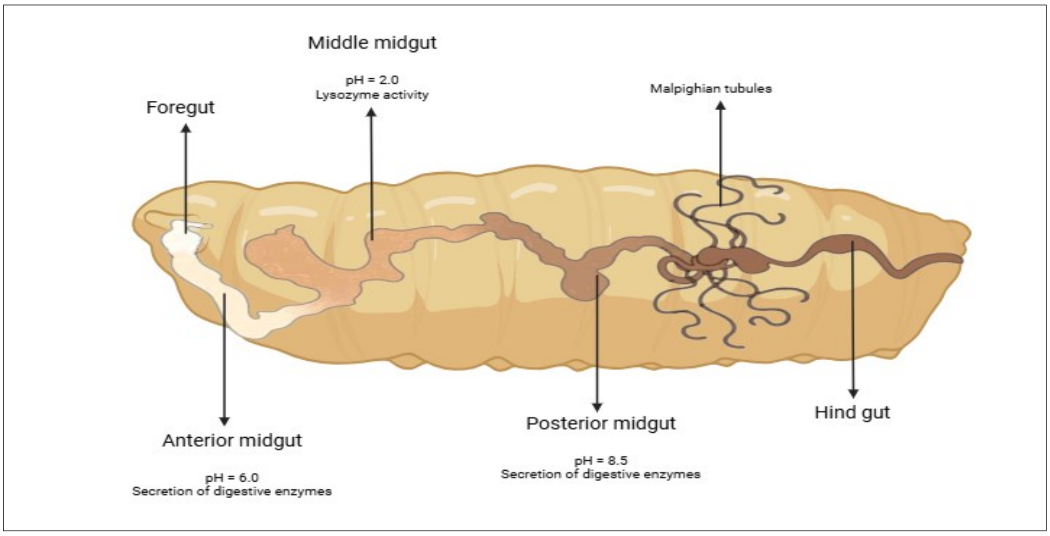

Fig. 3. Digestive system of black soldier fly.

The microbiota also varies across different regions of the gut, with distinct microbial communities in the anterior, middle and posterior parts due to the differences in luminal pH. This diverse microbial community is essential for insect health, as it affects the balance between advantageous and harmful bacterial populations. Core microbial species, including *Enterococcus*, *Klebsiella*, *Morganella*, *Providencia* and *Scrofimicrobium*, have been identified in the BSFL gut microbiome. Sequencing the BSF larval gut microbiome can help identify genes encoding enzymes capable of degrading complex waste. Additionally, microbial consortia with complementary metabolic functions can be identified and utilized for efficient waste degradation. DNA-based amplicon sequencing is used to identify bacterial and fungal taxa while also determining their relative abundance and community diversity. In contrast, RNA-based sequencing (transcriptomics), along with proteomics and phenotype microarrays, provides insights into the functional roles of the microbiome (33).

Enzyme discovery and industrial applications of gut enzymes

BSFL gut hosts a diverse microbial community crucial for digestion and nutrient acquisition. One such enzyme CS10, a cellulase isolated from the BSF gut microflora, exhibits strong industrial potential. It shows optimal activity at 50 °C and pH 7.0 and functions as an endo-β-1,4-glucanase, efficiently degrading cellulose. CS10 remains stable between 20-50 °C and within a pH range of 4.0-10.0, making it highly suitable for industrial applications. Additionally, it is resistant to chemical inhibitors, further supporting its potential for use in biomass conversion, biofuel production and waste management (34). PulSS4 (Pullulanase Secretory System 4) is a novel pullulanase enzyme identified from the gut bacteria of BSF that facilitates the breakdown of complex polysaccharides, such as pullulan, contributing to carbohydrate digestion. It exhibits activity in a temperature range of 10-50 °C, with optimum activity at 40 °C and is active in a pH range of 6.5-10.5, with an optimum pH of 9.0. The enzyme retains more than 80 % of its original activity across a broad pH range of 5-11 when incubated for 24 hr at 30 °C. Additionally, PulSS4 demonstrates high chemical stability, remaining active in the presence of various chemical reagents, including 10 % polar organic solvents and 1 % non-ionic detergents (35). Additionally, *Hermetia illucens* Serine Protease 1 (Hi-SP1) functions as a chymotrypsin-like protease that is active specifically during the larval stage, while *Hermetia illucens* Serine Protease 2 (Hi-SP2) is a trypsin-like protease with broader expression throughout larval development. Both enzymes contribute significantly to protein digestion. The optimum temperature for the activity of Hi-SP1 and Hi-SP2 is approximately 37 °C, with an optimal pH of around 7.2 (36). Manipulating the gut microbiota and associated digestive enzymes is considered a promising strategy to improve productivity and sustainability in industrial BSF rearing systems (2).

Rearing and farming

Rearing system components

The BSFL system consists of the Larvero, where the larvae feed and develop and the fly house, where the adult flies live and breed (Fig. 4). A properly managed rearing facility is vital for maintaining a steady supply of healthy BSF larvae and adults for waste processing. Substrates and early instar larvae (4-6 days old) are placed in the Larvero of the BSFL treatment unit to initiate the composting process. The number of BSFL introduced is determined by the amount of waste relative to the available volume and surface area (37). Specialized harvesting equipment is often unnecessary, as BSFL prepupae exhibit a self-harvesting behaviour, migrating away from the feeding substrate in search of pupation sites. At this stage, prepupae have emptied their digestive tracts, no longer feed and use their mouthparts to move in search of a safe location (29).

Environmental and operational parameters

Optimal rearing temperatures (27-30 °C) enhance survival, development and longevity in BSF, while extreme heat (36 °C) severely impairs growth and survival, highlighting critical trade-offs in life history traits. These flies are native to (sub)-tropical and warm-temperate climates, with their mating behaviour being influenced by the availability of space and sunlight. However, small-scale indoor rearing has posed challenges due to their sensitivity to artificial light sources and cage dimensions. Larval competition strongly influences key life-history traits, such as development time, body weight and survivorship. Lower larval densities typically result in larger individuals and faster development. BSF growth is

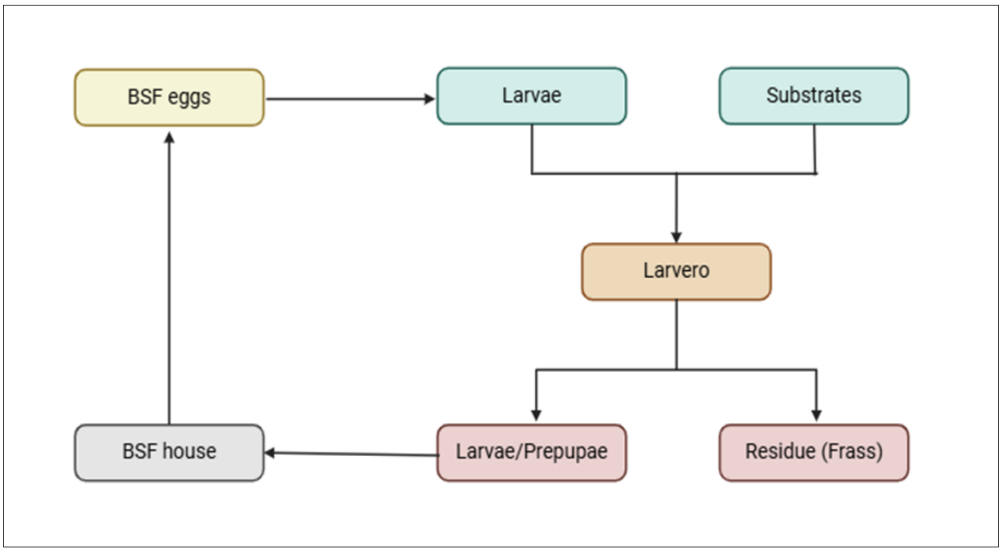

Fig. 4. Flowchart of a basic BSF treatment process.

shaped by various biotic and abiotic factors. These include the feeding substrate's type, depth and aeration, alongside temperature, moisture, pH, feeding rate and larval density. For adult BSF, factors like cage size, fly density, light exposure and ambient temperature and humidity are also crucial (38).

Adult nutrition

The adult BSF also has a functional digestive system, enabling it to ingest and digest food. During metamorphosis, the larval midgut is shed and replaced with a new epithelium, which forms through the proliferation and differentiation of midgut stem cells. These finding contrasts with earlier assumptions that adult BSF do not feed, highlighting a shift in our understanding of their biology and the potential to optimize reproduction via dietary manipulation. Furthermore, the type of food provided influences the fly's lifespan. These results not only confirm that the adult BSF can feed but also suggest that optimizing the fly's diet could enhance its performance in mass rearing systems (30). When sugar and water were provided to adults, it increased longevity for both sexes. A protein-rich diet maximizes egg production, extends the oviposition period and improves female longevity for industrial and research purposes, producing three times more eggs compared to protein-free diets (39).

Substrate quality

Substrate diversity and utilization

Despite constituting over half of total global waste, organic waste remains a largely untapped resource. BSF has emerged as a prominent species for addressing global agricultural and food waste issues, serving as a source of novel biomolecules with diverse applications. Still widespread adoption of BSF remains limited due to unclear regulations, low public awareness and negative perceptions of insect-based solutions. Technical challenges such as controlled rearing needs, inconsistent waste streams, lack of infrastructure, high startup costs and competition from traditional methods like composting also hinder progress. BSFL demonstrate significant potential for biotransformation, with the composition of the feeding substrate significantly influencing their growth. They efficiently convert organic waste into valuable byproducts, making them a sustainable waste management option. BSFL can consume various substrates, including formulated artificial diets and organic waste materials such as crop residue, dairy manure, poultry manure, human faeces, abattoir waste and sludges (19, 40, 41).

Influence of substrate composition on larval traits

BSFL growth, pupal size, sex ratio, reproductive success and fatty acid profiles are all influenced by the type and composition of the substrate-particularly its sugar and protein content. Factors such as food quality, quantity, temperature, pH, moisture content and larval density further impact BSFL performance (42). The optimal initial pH for BSFL production ranges from 6.0 to 8.0, promoting larval biomass and favourable life-history traits, whereas extremely acidic conditions (pH 2.0 and 4.0) negatively affect growth. Higher substrate pH increases larval output and reduces greenhouse gas emissions, optimizing BSFL growth and minimizing environmental impact (43). Moisture content is another critical factor influencing BSFL growth and survival. Stagnant liquid in the substrate can limit larval food access, affecting waste reduction rates (19). Larvae favouring substrates with up to 70 % moisture content (4).

Pre-treatment and fermentation technologies

Effective pre-processing of substrates, such as municipal solid waste (MSW), through the segregation of biodegradables, inerts and metals is crucial for composting technologies (14). The high moisture content in MSW and the elevated lignocellulosic composition of agricultural waste can hinder composting efficiency. However, pre-treatment methods such as hydrothermal treatment, ionization, pulsed electric field discharge and microbial treatment can enhance biodegradability and digestibility, thereby facilitating BSFL consumption and growth by improving waste recovery efficiency and increasing biomass surface area (14, 44). Certain organic wastes, such as sewage sludge and lignocellulosic materials, often lack essential nutrients required for optimal BSFL development. To support larval growth, these substrates can be blended with nutrient-rich, low-cost feedstocks such as palm kernel expeller or soybean curd residue. Moreover, microbial fermentation such as that using *Aspergillus oryzae* can break down complex lignocellulosic structures, improving nutrient accessibility and overall bioconversion efficiency. For instance, fermenting maize straw with *A. oryzae* was shown to enhance protein content and fatty acid composition in BSFL biomass, although it also prolonged larval development and reduced adult lifespan and fecundity (40). The presence of heavy metals in the feed material can adversely impact BSFL life-history traits, leading to the accumulation of toxic elements in the prepupa. High zinc levels cause larval mortality and infertility in eggs, significantly reducing production efficiency (45).

Diverse applications of BSF (Fig. 5)

Chitin

BSF is a rich source of chitin, a multifunctional biopolymer with significant biological properties and diverse applications in biomedicine, antimicrobial agents and cosmetics (11). Byproducts of BSF farming, such as cocoons and sheddings, are abundant sources of chitin (11) and even dead flies can be utilized for extracting chitin as a primary product (46). The chitin content in BSF ranges from 8 % to 24 % depending on developmental stage, processing method and whether larvae, pupae, or sheddings are used (47). Additionally, chitin extracted from BSFL improves in quality and yield as the insect progresses through its life stages with increased crystallinity, enhanced thermal stability and a higher acetylation level of up to 94 %. This is primarily due to increased sclerotization and structural development of the exoskeleton. As larvae mature, they deposit more chitin and associated proteins (48). Among the various life stages of BSF, the pupal stage accumulates the highest amount of chitin. The high fat content in BSF makes fat separation an essential step before chitin extraction (11). However, the extraction and purification of chitin from BSF remain challenging due to the strong binding between chitin and proteins, requiring efficient processing techniques (49). Co-fermentation has emerged as a cost-effective and eco-friendly technique that enhances chitin yield. Optimizing extraction processes is crucial for producing high-quality chitin and chitosan products (11).

Manure

Ineffective recycling of organic waste leads to environmental pollution and the loss of potential fertilizers (50). BSF composting is a sustainable and cost-effective solution, requiring minimal technical skills, low land use and offering a reduced ecological footprint (5). This process efficiently recycles nutrients, increasing nitrogen and phosphorus levels in treated residues while significantly reducing zoonotic bacteria such as *Salmonella* spp., to undetectable levels in harvested prepupae (50).

BSF frass, the residual by-product from larval digestion is a nutrient-rich organic fertilizer characterized by a balanced N-P-K ratio of 1:0.9:1.1 and a high dry matter content of around 69.62 %, making it suitable for agricultural use (Table 1). Its slow-release properties, indicated by a low ammonium nitrogen content (15.78 % of total nitrogen) and a moderate carbon-to-nitrogen (C:N) ratio of 14.71, support long-term soil fertility. However, the frass shows high variability in micronutrient composition-particularly in iron (Fe), copper (Cu) and zinc (Zn) highlighting the need for feed-specific analysis for precision agriculture applications. With a slightly alkaline pH of 7.46, BSF frass can benefit acidic soils, although pH levels should be monitored to avoid imbalances. However, these values influenced by the larval feed substrate Additionally, post-processing methods such as composting may be required to stabilize the material and reduce potential phytotoxicity risks before field application (51). The high nitrogen fertilizer equivalence (NFE) of BSF manure was demonstrated its strong potential to enhance crop production sustainably in previous experiments (52). This study also compared the performance of BSF manure and a commercial organic fertilizer (SAFI) on maize (H513) under varying application rates. The application of BSF manure resulted in taller maize plants and higher chlorophyll concentrations than other treatments. Notably, grain yield increased by 14 % when 7.5 t ha^{-1} of BSFFF was applied, compared to the same rate of SAFI (52). However, frass is primarily phosphorus-dominated and may not provide a well-balanced nutrient composition for all crops. Incorporating nitrogen-rich inputs could improve its nutritional profile, making frass-based fertilizers more effective (53) (Table 2).

Antimicrobial peptides

The BSF has one of the largest antimicrobial peptides (AMP)

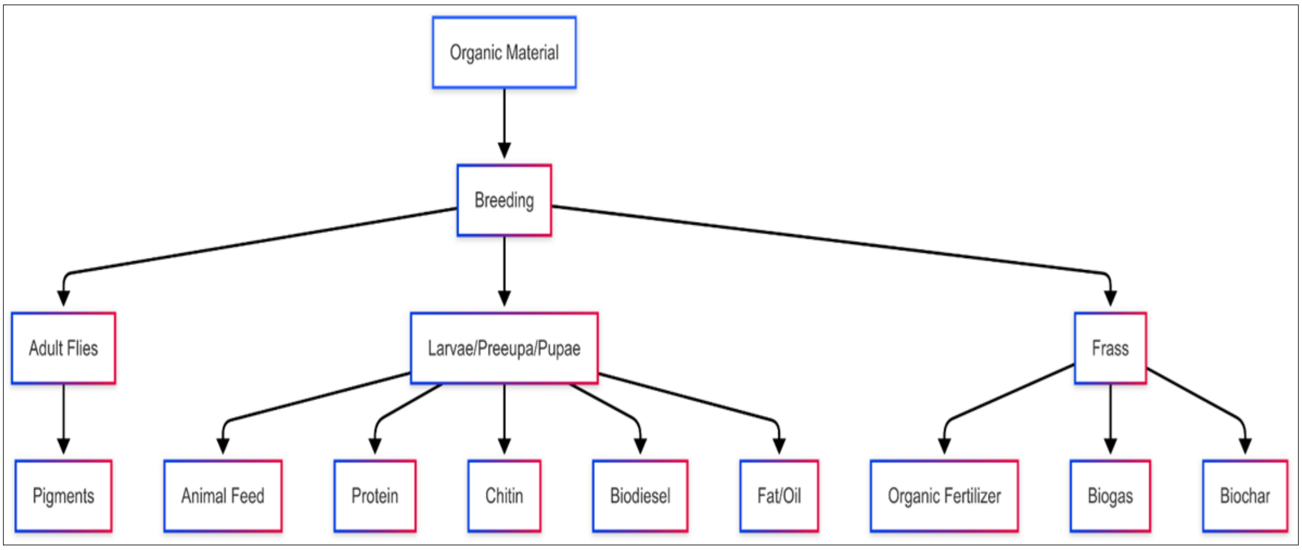

Fig. 5. A flowchart of diverse applications of black soldier fly.

Table 1. Physico-chemical composition and nutrient profile of BSF manure (51)

Category	Parameter	Unit	Value
Physical	Dry Matter (DM)	%	69.62 ± 18.80
	Organic Matter (OM)	% DM	86.22 ± 5.17
	pH	-	7.46 ± 1.12
	Electrical conductivity	mS cm^{-1}	4.03 ± 1.69
Macronutrients	Total Nitrogen (N)	g kg^{-1} DM	32.20 ± 8.37
	Ammonium-N (NH_4+-N)	g kg^{-1} DM	5.60 ± 3.08
	C:N Ratio	-	14.71 ± 4.56
	Phosphorus (P)	g kg^{-1} DM	12.40 ± 4.34
	Potassium (K)	g kg^{-1} DM	29.30 ± 10.26
	Magnesium (Mg)	g kg^{-1} DM	4.70 ± 2.12
	Calcium (Ca)	g kg^{-1} DM	8.80 ± 8.27
	Sulfur (S)	g kg^{-1} DM	6.30 ± 3.02
Micronutrients	Copper (Cu)	mg kg^{-1} DM	43.76 ± 64.33
	Boron (B)	mg kg^{-1} DM	34.54 ± 26.94
	Zinc (Zn)	mg kg^{-1} DM	136.32 ± 98.15
	Manganese (Mn)	mg kg^{-1} DM	79.54 ± 35.00
	Iron (Fe)	mg kg^{-1} DM	1808.40 ± 1736.06

repertoires ever recorded in insects (55). BSF larvae thrive in microbe-rich environments and serve as a potential source of AMPs, with their antibacterial activity increasing as the larvae age. AMPs play a crucial role in the innate immune defense against pathogens, offering an alternative to antibiotics due to the limited ability of microorganisms to develop resistance, making them promising candidates for next-generation antimicrobials. Peptides derived from BSF larvae have demonstrated strong antibacterial properties, positioning them as a novel source for antibacterial drug development (56). The Duox-TLR3 gene in BSF larvae exhibits antimicrobial activity by suppressing pathobionts through immune responses involving free radicals and AMPs. Duox and TLR3 regulate AMP production in BSF larvae by mediating reactive oxygen species (ROS) generation and activating immune responses against pathogens, with silencing these genes leading to decreased AMP levels and dysbiosis (57). Additionally, diet influences AMP expression in BSFL, shaping their antibacterial activity against various bacteria. Moreover, BSF larvae secrete bactericidal compounds that inhibit house fly (*Musca domestica*) egg-laying and reduce foodborne pathogens such as *Escherichia coli* and *Salmonella enterica*. As a result, large-scale BSF farming does not pose a significant risk of disease transmission (37).

Feed

Food futurists anticipate that sustainability-focused societies will progressively embrace insects as alternative protein sources (14). By 2050, the consumption of animal products is projected to increase by 60-70 %, resulting in a higher demand for feed resources. However, traditional feed ingredients like soymeal and fishmeal are both expensive and may become scarce in the future. In this context, insect farming emerges as a sustainable alternative, offering a viable solution to meet the growing need for animal feed while reducing reliance on conventional resources (28) (Table 3).

The proteins found in BSFL are easily digestible, with protein digestibility ranging from 72.78 % to 78.67 % and are rich in essential amino acids, which are vital for the growth and development of ruminants (58). BSF larvae also have a high apparent metabolizable energy (AME) value (59). The prepupal biomass is rich in essential amino acids, with lysine, valine and arginine being the most abundant. Despite variations in the substrate, amino acid levels remained stable. Threonine, isoleucine, methionine and tryptophan were also present in consistent amounts, highlighting the nutritional reliability of BSF prepupae (6), though it is deficient in methionine, cysteine and tryptophan (59). Proteins can be extracted from BSF larvae using conventional methods such as aqua-based, salt, detergent and alkali solvent extraction, as well as non-conventional techniques like microwave, ultrasound, enzyme and pulsed electric field-assisted extraction (60).

Table 3. Comparison of nutritional composition of BSF larvae, fishmeal and soymeal (28)

Nutrient	BSF Larvae	Fishmeal	Soymeal
Chemical composition (% in dry matter)			
Crude Protein	42.1	70.6	51.8
Lipid	26.0	9.9	2.0
Calcium	7.56	4.34	0.39
Phosphorus	0.90	2.79	0.69
Ca:P Ratio	8.4	1.56	0.57
Essential amino acids (g/16g Nitrogen)			
Methionine	2.1	2.7	1.32
Cystine	0.1	1.2	0.80
Valine	8.2	4.9	4.50
Isoleucine	5.1	4.2	4.16
Leucine	7.9	7.2	7.58
Phenylalanine	5.2	3.9	5.16
Tyrosine	3.6	3.1	3.50
Histidine	3.0	2.4	3.05
Lysine	6.6	7.5	6.18
Threonine	3.7	4.1	3.78
Tryptophan	0.5	1.0	1.36
Non-essential amino acids (g/16g Nitrogen)			
Serine	3.1	3.9	5.18
Arginine	5.6	6.2	7.64
Glutamic Acid	13.2	12.6	19.99
Aspartic Acid	11.0	9.1	14.14
Proline	6.6	5.6	5.40
Glycine	5.7	6.4	4.52
Alanine	7.7	6.3	4.54

Table 2. Comparative nutrient composition of different manures (51, 54)

Manure	Organic C (%)	N (%)	C:N	P (%)	K (%)	Ca (%)	Mg (%)
BSF manure	50.0	3.22	14.71	1.24	2.93	0.88	0.47
Rabbit manure	30.1	1.01	29.8	0.54	1.95	1.15	0.40
Cow dung	26.5	1.86	14.24	0.82	2.11	1.01	0.51
Poultry manure	17.8	2.91	6.12	0.84	3.79	3.34	0.64
Green manure	23.6	2.51	9.40	0.52	3.04	3.01	0.10
Pig manure	20.1	2.16	9.77	0.80	2.16	1.45	0.54

BSF can partially synthesize fatty acids through *de novo* biosynthesis, utilizing carbohydrates as a primary source of acetyl-CoA. However, it cannot produce polyunsaturated fatty acids; instead, it accumulates them from the diet and metabolizes them into saturated forms (61). Saturated fatty acids (SFA) constitute up to 76 % of the total fatty acids in larvae, followed by monounsaturated fatty acids (MUFA) at up to 32 and polyunsaturated fatty acids (PUFA) at up to 23 %. Regardless of dietary composition, lauric acid (C12:0) is the most abundant SFA, making up as much as 52 % of the total fatty acid content, while palmitic acid (C16:0) and oleic acid (C18:1 n-9) contribute 12-22 % and 10-25 %, respectively (10) (Table 4).

BSF biomass is nutritionally rich in protein, fat and minerals, while having relatively low fiber content. Its crude protein content ranges from 30 % to 57 %, while fat content varies between 21 % and 42 % of dry matter based on the substrate (Table 4). A study on metabolic changes in the nutritional composition of BSF from egg to adult revealed significant shifts in both crude fat and protein content. During larval development, a rapid increase in crude fat was observed from 4 to 14 days, reaching a maximum of 28.4 % in dry mass. At the same time, crude protein content continuously decreased, hitting a minimum of 38 % at the 12-day larval stage before peaking at 46.2 % in the early pupa stage. A sharp decrease in crude fat occurred from early prepupae (24.2 %) to late pupae (8.2 %). Crude protein, however, reached its highest value of 57.6 % at the postmortem adult stage, with a fat content of 21.6 % (6).

Studies indicate that BSF larvae can be a partial substitute for traditional animal feed in poultry, pig and fish diets. However, replacing feed entirely or excessively may lead to reduced performance due to the larvae's high fat content, ash content and the effects of processing (42). BSF larvae have the potential to be processed into a textured protein with a unique flavor for human consumption. However, their acceptance is limited by social stigmas, legal restrictions on waste-fed organisms and existing taboos surrounding insect consumption (14). Large-scale biorefineries could significantly enhance the sustainability of BSFL for human consumption in the future (3).

Table 4. Crude Protein (CP) and Crude Fat (CF) composition of black soldier fly larvae and prepupae reared on different substrates

Substrate	Growth stage	% CP	% CF	References
Abattoir waste	larvae	56.3	NA	(62)
Human feces	larvae	35.5	NA	(62)
Primary sludge	larvae	16.9	NA	(62)
Digested sludge	larvae	14.7	NA	(62)
Cow manure	Prepupae	41.2 ± 2.1	35.7 ± 2.9	(41)
Poultry manure	Prepupae	41.7 ± 4.0	36.2 ± 3.5	(41)
Pig manure	Prepupae	42.8 ± 4.49	36.5 ± 3.9	(41)
Restaurant waste	Prepupae	43.1	38.6	(6)
Biogas digestate	Prepupae	42.2	21.8	(6)
Chicken feed	Prepupae	41.2	33.6	(6)
Vegetable waste	Prepupae	39.9	37.1	(6)
Fruits	Prepupae	37.8	41.7	(63)
Horse manure	Prepupae	40.9	12.9	(63)

Table 5. Biodiesel production from black soldier fly larvae reared on different organic waste sources

Organic waste	Biodiesel produced (g/1000 BSFL)	Reference
Cattle manure	35.5 g	(67)
Pig manure	57.8 g	(67)
Chicken manure	91.4 g	(67)
Rice straw (30 %) + Restaurant solid waste (70 %)	21.9 g	(68)
Food waste	94.0 g	(69)

Biodiesel

The bioconversion of waste by BSF not only supports effective waste management but also serves as a sustainable source of biodiesel, positioning it as a valuable alternative feedstock for renewable energy (64). Biodiesel production from BSFL demonstrates significant potential across different scales. A small-scale urban biofuel plant can generate approximately 122.73 L/day of biodiesel from 1 ton of organic waste, while medium-scale rural production increases to 1678.85 L/day. Large-scale industrial plants can achieve an impressive output of 22272.73 L/day, underscoring the scalability and economic feasibility of BSFL as a biofuel source (65).

Lignocellulosic biomass, derived from agricultural waste, is another promising resource for biofuel production. This approach offers a sustainable alternative to fossil fuels, addressing global energy shortages while mitigating climate change impacts (47). Additionally, BSFL biodiesel production produces fewer carbon emissions than other biodiesel feedstocks, such as microalgae, used cooking oil and non-edible oils, making it an environmentally friendly alternative. The diet of BSFL plays a crucial role in biodiesel yield, as it directly influences fat accumulation (Table 5). BSFL contains substantial amounts of fatty acids, contributing up to 70 % extractable oil, which can be efficiently converted into biodiesel (66).

The extracted lipids undergo transesterification using sulfuric acid as a catalyst in methanol, facilitating the conversion into biodiesel (64). Despite its promise, research on biodiesel production from invertebrates, particularly insect larvae, remains scarce. Compared to traditional catalytic methods, BSFL biodiesel production is highly efficient, achieving up to 94 % yield through non-catalytic transesterification. This process eliminates the need for costly catalysts and simplifies downstream purification, significantly reducing both production costs and energy input (66).

Harvesting fifth instar BSFL is more beneficial for biodiesel production due to its higher lipid content, greater FAME yield and lower chitin content, which reduces processing challenges (70) (Table 6).

Table 6. Comparison of biochemical composition and biodiesel yield between fifth and sixth instar black soldier fly larvae (70)

Parameter	Fifth instar BSFL	Sixth instar BSFL
Lipid content (%)	34.23 ± 0.65	25.88 ± 0.36
Chitin content (%)	7.61 ± 0.93	18.62 ± 1.25
Protein content (%)	34.66 ± 0.31	37.70 ± 0.14
Nitrogen content (%)	6.07 ± 0.01	7.32 ± 0.06
FAME yield (%)	~33	~25
Biodiesel composition (FAME %)	C12:0 (~60 %), C14:0 (~15 %), C16:0 & C18:1 (~10 %)	Similar

FAME: Fatty Acid Methyl Ester.

Pigments

BSF adult flies are a potential source of natural pigments, including melanin and ommochromes (71). Eumelanin-type pigments are synthesized throughout all life stages of BSF, including larvae, pre-pupae, pupae and adult flies (dead flies), with the highest melanin content found in the cuticles, where it remains even after adult emergence. Melanin is also present in the insect body in a complex with lipids. It can be extracted in two forms: as a melanin-chitin complex and as water-soluble melanin, while ommochromes are specifically extracted from BSF eyes (71, 72). These pigments exhibit strong antioxidant and antiradical properties. It can also be used as natural antioxidants and as food colorants. These pigments hold significant industrial and biotechnological potential in biomedical applications as natural antioxidants, in environmental remediation for contaminant sorption and in biodegradable pigment production (71). Melanin is also known for its distinct physical and chemical properties. These attributes make it a promising alternative biopolymer with potential applications in environmental sustainability (73).

Economic and environmental potential of BSF farming

BSFL offer environmental sustainability, economic efficiency and industrial applications, creating new economic opportunities for industries and entrepreneurs, especially in developing countries (5). BSFL efficiently utilize organic waste, often at little or no cost, enabling effective waste-to-feed conversion (11). Low-cost insect-based feed production can generate employment and improve livelihoods for both farmers and urban entrepreneurs. Inclusive business models can integrate smallholder farmers into BSFL-based agribusiness, enhancing income opportunities and food system resilience (1). The use of BSF-derived animal feed can help offset waste collection costs, fostering small-scale entrepreneurial ventures in organic waste management. With a well-established market, BSFL production could further enhance profitability for small-scale entrepreneurs (19). Inclusive business models that incorporate insects in animal feed can help address socio-economic and environmental challenges in developing countries. This approach aligns with the United Nations' Sustainable Development Goals by promoting sustainability and economic resilience. Additionally, organizing farmers into cooperatives can enhance supply consistency and strengthen their position within the value chain (44). Centralized BSFL plants yield the highest financial returns, while decentralized approaches offer benefits such as job creation, reduced transport costs and smaller footprints. A hybrid model, with decentralized waste treatment and centralized larvae processing, can optimize both financial viability and community benefits. Local government support is a key to strengthening decentralized networks. Key improvements needed for the expansion of BSF farming include enhanced government support, cost-effective investments, lower operational expenses, improved coordination among regulatory bodies and increased consumer acceptance (74). A lack of harmonized regulations across countries remains a major bottleneck for international trade and scalability of BSF-derived products. Developing globally accepted safety standards and feed regulations is critical (9).

BSFL significantly mitigate environmental pollution by reducing volatile emissions from decomposing animal waste, a major contributor to environmental degradation. BSFL systems also demonstrate high water use efficiency and require significantly less land compared to conventional livestock systems, making them ideal for sustainable protein production in resource-limited settings (12). The life cycle assessment (LCA) of BSFL-based food waste conversion indicates a low global warming potential (GWP) of 17.36 kg CO_2 per ton of processed waste (44). Minimal waste retention time is crucial for effective pathogen reduction, with antibacterial effects potentially stemming from interactions between larval secretions and bacterial structures (50).

Genetic diversity and population structure

The BSF is a useful model for studying genetic differentiation, dispersal patterns and geographic lineages (7). Recent phylogenetic analyses have revealed a close relationship among certain haplotypic populations of BSFL across different biogeographic regions, suggesting a cosmopolitan distribution potentially influenced by anthropogenic factors (75). The assembly of the BSF genome was achieved using advanced sequencing technologies such as Pacific Bioscience, 10X Genomics linked read and high-throughput chromosome conformation capture sequencing, resulting in a highly contiguous genome spanning 1.01 gigabases (Gb) with 99.75 % of scaffolds successfully assembled into pseudochromosomes representing the organism's seven chromosomes. Notably, chromosomes one to six in BSF resemble autosomes, while chromosome seven exhibits' characteristics typical of an X chromosome in males, highlighting the unique sex determination system of BSF (76). The '677 CO1 sequences' refer to genetic data obtained from analysing the cytochrome oxidase subunit 1 (CO1) gene in BSF. The CO1 gene is commonly used as a DNA barcode marker due to its high variability among species, universal primer design, established reference in molecular taxonomy, ease of sequencing, ability to provide phylogenetic insights and its application in biodiversity studies. This gene is commonly utilized in molecular studies for genetic diversity analysis and species identification due to its relatively conserved nature and informative data about evolutionary relationships. Analysis of 677 CO1 sequences globally identified 52 haplotypes, including ten major ones, showcasing significant genetic diversity. Phylogenetic analyses of 60 complete mitochondrial genomes revealed evolutionary relationships among major haplotypes, estimating separation events to over 2 million years and suggesting complex migration patterns (77) (Fig. 6).

Research investigating genetic and phenotypic changes during the early domestication of BSF revealed rapid differentiation and eventual collapse due to inbreeding depression, emphasizing the importance of maintaining genetic diversity and phenotypic variation for long-term population viability and productivity. Effective genetic management, including the maintenance of genetic diversity through broad founder populations and the introduction of wild flies, is crucial for sustainable and profitable entomofarming, particularly with species like the BSF that face challenges such as founder effects and loss of genetic

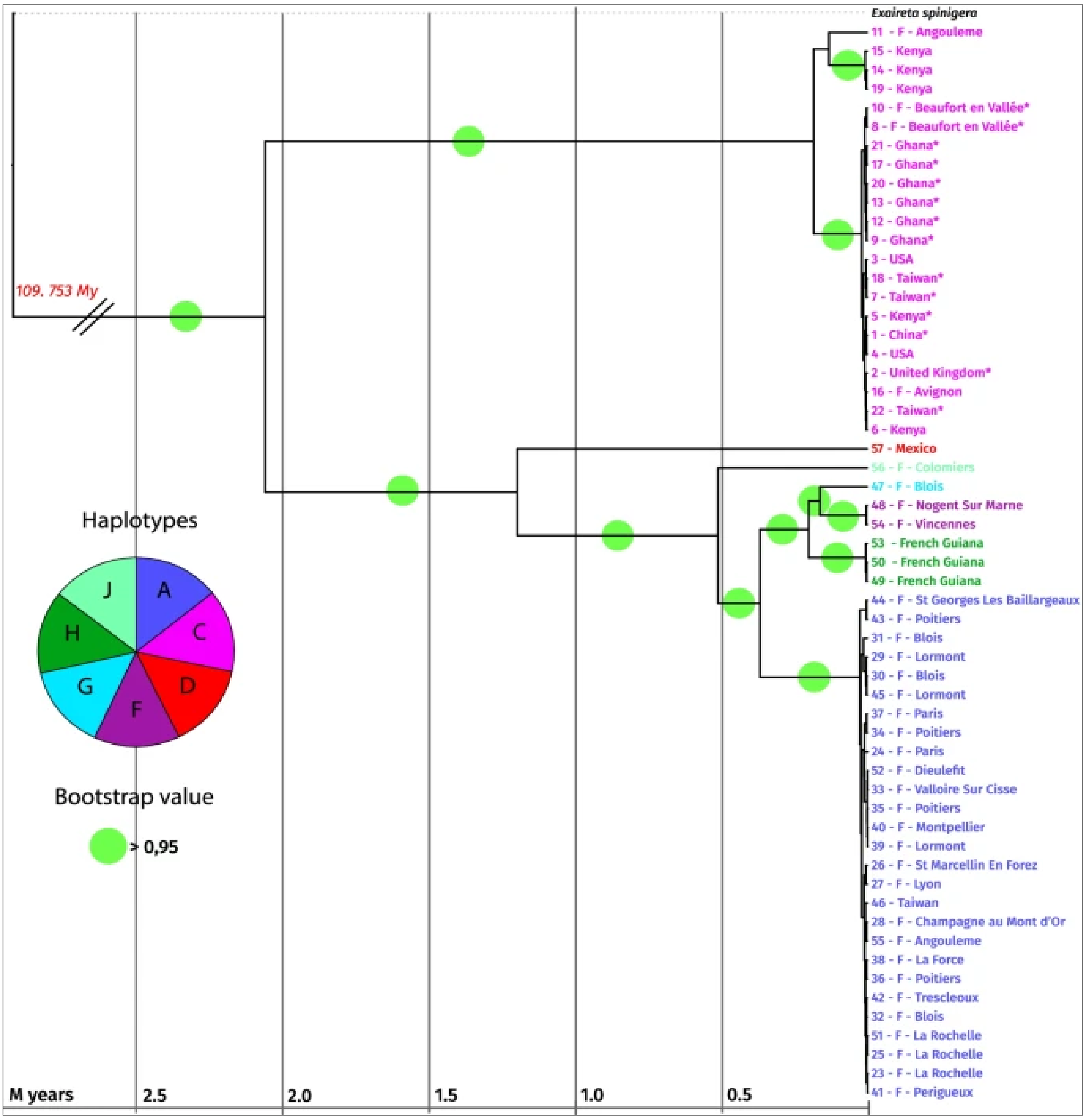

Fig. 6. Bayesian time tree of 57 mitochondrial genomes of *Hermetia illucens*, aligned using MAFFT and analyzed using BEAST. The D-loop regions were excluded and the tree was rooted using *Exaireta spinigera* (Stratiomyidae). Haplotypes based on CO1 sequences are color-coded; commercial individuals are marked with asterisks. Green circles indicate bootstrap support values >0.95 from maximum likelihood analysis (500 replicates) (77).

diversity over generations in captivity (17). The laboratory colony exhibits a severe lack of genetic diversity across generations when compared to natural populations, which is associated with phenotypic divergence, potentially influenced by a founder effect, genetic processes and inbreeding within the captive population. Notable declines in genetic diversity and rapid divergence of captive BSF populations from their wild counterparts have been observed, emphasizing the necessity for effective genetic management strategies in BSF farming. The wild BSF population has shown high genetic diversity, characterized by low relatedness and high heterozygosity, suggesting a recently admixed population. In contrast, the mass-reared colony exhibited a substantial decline in genetic diversity across generations, showing signs of inbreeding and relatedness coefficients comparable to full siblings (78). Non-metric dimensional scaling analysis revealed phenotypic and genetic variations across generations, highlighting the impact of domestication on traits such as clutch size, pupae weight and exclusion rates (17).

The variability in conversion efficiency of the same feed or feeds with similar nutrient composition, observed in different laboratories, can be attributed to genetic differences among colonies, like differences between genetic lines in livestock species like chickens and pigs (79). Genetic drift, mutations, or selection occurring after the initial colony establishment are factors affecting genetic diversity and performance in captive insect populations. Monitoring inbreeding, genetic drift and introducing genetic diversity through outbreeding are crucial practices for maintaining captive insect populations' performance. Molecular genetic markers like microsatellites are valuable tools for evaluating genetic diversity and homozygosity in BSF colonies. Understanding details such as the number of generations since colony establishment, founder population size and any genetic bottlenecks is crucial for comprehending genetic variability within BSF populations (80). Successive generations of BSF colonies show increasing population differentiation and declining genetic diversity, driven by both natural and human-mediated selection. Creating barcodes for cultures is suggested as an affordable method for genetic characterization and preserving DNA and specimens for later studies is recommended to bolster genetic research (16).

Genotype-by-diet interactions in BSF necessitate tailored breeding strategies, as the genetic background of BSF and environment-mediated interactions significantly impact larval traits, challenging the notion of broad conspecific plasticity. Tailored BSF breeding strategies are essential for efficient agricultural support, enabling precision breeding and feeding schemes that optimize production outcomes. Diversified selective breeding strategies can create resilient, multipurpose BSF breeds capable of consistent performance across various waste streams, thereby balancing profitability and sustainability. The influence of genotype on larval production further highlights the importance of selecting appropriate genetic backgrounds for optimal production (81). Additionally, maintaining genetic soundness and optimizing breeding conditions are crucial for sustaining healthy breeding stocks and enhancing the efficiency and quality of insect production. High-quality insect populations depend on both genetic health maintenance and optimized rearing environments, emphasizing the interconnectedness of genetic and environmental factors in successful BSF production (15).

Genomic resources and tools

Next-generation genome-wide DNA sequencing has also proven beneficial for insect farming, allowing for the selection of desirable traits and enhancing efficiency and productivity. Notably, advancements in genome editing techniques such as CRISPR/Cas9 have revolutionized genetic manipulation in non-model organisms like the BSF. Utilizing tools like CRISPR/Cas9, researchers have successfully modified the genetic makeup of BSF, creating flightless and enhanced feeding capacity phenotypes. Furthermore, CRISPR/Cas9 has become instrumental in precise genome engineering across diverse organisms, offering versatility and precision in gene editing (82, 83). Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) also play vital roles in precise genome engineering (84). Additionally, DNA pool sequencing has been employed to identify single nucleotide polymorphisms (SNPs) in BSF populations, providing valuable insights for developing genotyping chips and supporting advanced genomic selection and breeding programs (85). Transcriptomics and metagenomics are essential fields in understanding gene expression patterns, regulatory mechanisms and the genetic content of entire organism communities, particularly in entomology studies (86, 87). Technologies like sequencing, CRISPR/Cas9 genome editing and DNA pool sequencing, have revolutionized research in entomology, particularly concerning the BSF and insect farming. These technologies enable precise selection of desirable traits, identification of genetic variations and development of genotyping chips for advanced breeding programs. Transcriptomics and metagenomics studies further contribute to understanding gene expression patterns and the genetic content of insect communities (88). A transgenic system established for BSF using specific promoters and transposases has shown enhanced transformation rates, providing essential tools for genetic manipulation and basic research in BSF (89).

Genetic improvements for optimizing traits

Assessing genotype-by-diet interactions in the BSF emphasizes the need for tailored breeding strategies. The significant impacts of BSF genetic background and environment-mediated interactions on larval traits challenge the notion of broad conspecific plasticity in BSF. Establishing tailored BSF breeding is crucial for efficiently supporting the agricultural sector and indicates opportunities for precision breeding and feeding schemes. Diversified selective breeding strategies show potential for creating resilient, multipurpose BSF breeds that perform consistently across various waste streams, aiming to balance profitability and sustainability in the sector. The influence of genotype on larval production in BSF stresses the critical importance of selecting appropriate BSF genetic backgrounds for optimal production outcomes (81). BSF larvae have the ability to accumulate heavy metals like cadmium, lead, mercury and arsenic (45). Three metallothionein genes (BSFMT1, BSFMT2A and BSFMT2B) were identified in BSF, suggesting their involvement in cadmium (Cd) tolerance. This research may indirectly contribute to utilizing BSF for organic waste conversion, highlighting the practical applications of understanding Cd detoxification mechanisms in BSF (90).

Genetic modifications, such as the use of CRISPR/Cas9 to disrupt the PTTH gene, have been employed to extend the larval stage of BSF, resulting in delayed pupation and increased larval size and weight. This genetic manipulation improves waste consumption efficiency (82). Artificial selection led to substantial improvements in larval body weight after 10, 13 and 16 generations of selection (91). Genetic soundness and optimization are crucial for maintaining healthy breeding stocks and optimizing insect production. Utilizing both genetic and environmental factors is essential for enhancing production efficiency and quality by understanding the genetic basis of desired traits and their constraints. Breeding and maintaining high-quality insect populations, including maintaining genetically healthy populations and optimizing rearing environments, are crucial for successful insect production (15). Manipulating the fatty acid content in the substrate of BSF larvae allows control over the resulting fatty acid profile of BSF oil (92). The main drawback to BSF as a food source is their deficiency in fat-soluble vitamins like Vitamins A, D and E. An alternative is to genetically engineer BSF that can synthesize these essential vitamins. A BSF line has been engineered with the two main carotenoid biosynthetic genes, CarRA and CarB, for the production of provitamin carotenoids within the Vitamin A family. This manipulation involves inserting transgenes into the BSF genome for the expression of functional protein products (93).

Temperature plays a crucial role in the development of BSF, influencing traits like adult longevity, weight and larval development time, which are essential for fitness and reproductive success. BSF showed tolerance to potential overwintering, with prepupae and pupae exhibiting the highest cold tolerance (6). The Bioforte colony underwent selective breeding at 12 °C and 16 °C to develop cold-tolerant BSF with improved production performance. After nine generations, there were significant improvements in larvae weight, survival rate and dry matter conversion rate (94). Genetic diversity and gut microbiome of BSF from various global locations are explored. Using genetic analysis and metagenomics, distinct genetic branches and predominant bacterial families in BSF samples are identified. Haplotype mapping reveals shared genetic connections among samples from different countries, providing key insights into BSF's global genetics and gut microbiome (95).

The microbiota plays a pivotal role in BSF larvae growth, affecting organic substrate utilization and conversion into protein, fat and chitin (2). Specific bacteria like *Enterococcus*, *Klebsiella*, *Morganella*, *Providencia* and *Scrofimicrobium* are crucial in substrate breakdown and influence growth patterns, longevity and reproduction (44). The gut biome of BSF larvae is genetically adapted for decomposing organic matter, leading to higher levels of antimicrobial peptides (AMPs) and improved survival rates when consuming organic matter (96).

Identification of genes related to immune responses and chemoreception systems indicates adaptations to pathogen-rich environments and host specialization in BSF (82). The genes responsible for encoding AMPs derived from BSF were cloned in other organisms like silkworms, imparting disease resistance (97). A total of 180 antibiotic resistance genes (ARGs) and 10 mobile genetic elements (MGEs) were identified in the larval gut, with tetracycline resistance genes being predominant (98). Lastly, lignin content has been found to negatively impact larval growth, suggesting the need for lignin-degrading microorganisms in substrates rich in lignin (99). The continued optimization of the microbiome is expected to play a significant role in improving BSF traits and expanding its potential for industrial-scale rearing and biotechnological applications (2).

Conclusion

BSF (*Hermetia illucens*) farming presents a promising solution for sustainable waste management, alternative protein production and renewable energy generation. Advances in genetic research, microbiome optimization and selective breeding can further enhance its productivity and adaptability, unlocking significant environmental and economic benefits. However, to maximize its potential, challenges such as genetic diversity conservation, regulatory harmonization and public acceptance must be addressed. Future research should focus on waste substrate optimization, tailored breeding programs and novel applications, including antimicrobial peptides, chitin extraction and pigment production. Additionally, bioprocessing advancements and automation can improve efficiency and scalability, making BSF farming a viable solution for global sustainability, food security and environmental resilience. Integrating innovative technologies and fostering collaborations will allow BSF to play a critical role in closing nutrient loops, reducing waste and supporting circular bioeconomy initiatives. As research progresses and these technologies are scaled, BSF has the potential to become a key player in transforming waste management systems, contributing to sustainable food production and enhancing environmental sustainability on a global scale.

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Authors' contributions

AES conducted the literature review and drafted the manuscript. GS conceptualized the study, supervised the writing process and contributed to manuscript revisions. MS, SS and MLM provided critical insights and guidance. All authors reviewed and approved the final manuscript.

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Declaration of generative AI and AI-assisted technologies in the writing process

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