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Boettcherisca peregrina (Diptera: Sarcophagidae): A flesh fly species of medical and forensic importance

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Abstract. *Boettcherisca peregrine*, as a fly with the necrophagous habits found on human corpses and a vector of disease or parasitic, myiasis-producing agent, is a significant flesh fly species in forensic entomology and medical context. This study reviewed the various aspects of this fly species, including morphology, bionomics, molecular analysis, medical and forensic entomology involvement, such as morphological characteristics of larva, puparia and adult, developmental rate of larvae, the effects of heavy metal (such as Cd and Cu) on the growth and developmentin of larvae, and the impact of some specific stimulis on the labellar chemosensory hair of *B. peregrina*. Species identification, gene and functions, myiasis and forensic case of this species were also outlined. Therefore, the paper has an important implication for improving the role of *B. peregrina* in medicine and forensic science.

INTRODUCTION

Boettcherisca peregrina (Robineau-Desvoidy, 1830) (Diptera: Sarcophagidae) is a significant flesh fly species for medical and veterinary management, due to its ability of causing myiasis in human and other mammals as an ectoparasite, and potential used to estimate the minimum postmortem interval (PMImin) as colonizer in the early stage of corpse decomposition in forensic investigations (Byrd & Castner, 2009; Greenberg, 1971; Wells *et al*., 2001). Additionally, the species is beneficial to the biosphere and many ecosystems as carrion decomposers, due to their important role in the food chain (Ferrar, 1987). The adult of *B. peregrina* are robust and large-sized flies, with a red-tipped grid pattern on abdomen, gray and black of the background. *B. peregrina* has a pair of red eyes, and the reproductive cycle is of larviparous (Majumder *et al*., 2012). Geographically, this fly has been found in many parts of the world including Oriental, Palaearctic, and Australasian regions (Wang *et al*., 2017). *Boettcherisca.peregrina* has been recorded in many countries of the Oriental region, such as Thailand (Samerjai *et al*., 2014), India (Sharma *et al*., 2015) and Malaysia (Tan *et al*., 2010). Previous investigations on *B. peregrina* have focused on molecular analysis (Guo *et al*., 2010a), larval morphology (Sukontason *et al*., 2010), using pteridine fluorescence to deduce the age of adults (Zhu *et al*., 2013), The influence of drugs on flesh flies larval development (Goff, 1991), the growth and development rate of *B. peregrina* at various temperatures in different parts of the world (Wang *et al*., 2017), and cuticular hydrocarbon composition of pupal exuviae for species taxonomy (Ye *et al*., 2007). Therefore, we review its morphology, bionomics, molecular analysis,

medical and forensic entomology involvement to increase worldwide attention of this sarcophagid species.

Morphology

One of the important tasks in forensic entomology, medical, and veterinary entomology is to identify the species involved, thus gathering the morphology information of all stages in *B. peregrina*'s life cycle is the core for species identification (Sukontason *et al.*, 2014).

The life cycle of *B. peregrina* is comprises of larva, pupa and adult in accordance that the reproductive cycle of this sarcophagids species is ovoviviparous (Szpila *et al.*, 2015) (Fig. 1). The larva of *B. peregrina* has three instars, each instar shed off its exuvium to transform into the next instar (Majumder *et al.*, 2012). Sukontason *et al.* (2010), Chen (2013) has provided some features for species identification of *B. peregrina* larval. Additionally, Singh *et al.* (2012) summarised the characteristics that can be used for the larval identification of common Sarcophagidae. Erzinçlioğlu (2007), Szpila (2010) has published several significant papers on larval characters of Calliphoridae for species identification, however, the characters used for distinguishing the larvae of sarcophagids have not been critically revised.

Puparia are common remnants of carrion-breeding flesh flies which present on the decomposed cadavers involving forensic investigations (Mazzanti *et al.*, 2010). Samerjai *et al.* (2014) developed the key most commonly used to identify puparia of some flesh fly species, including *B. peregrina*, *B. nathani*, *L. pattoni*, *L.*

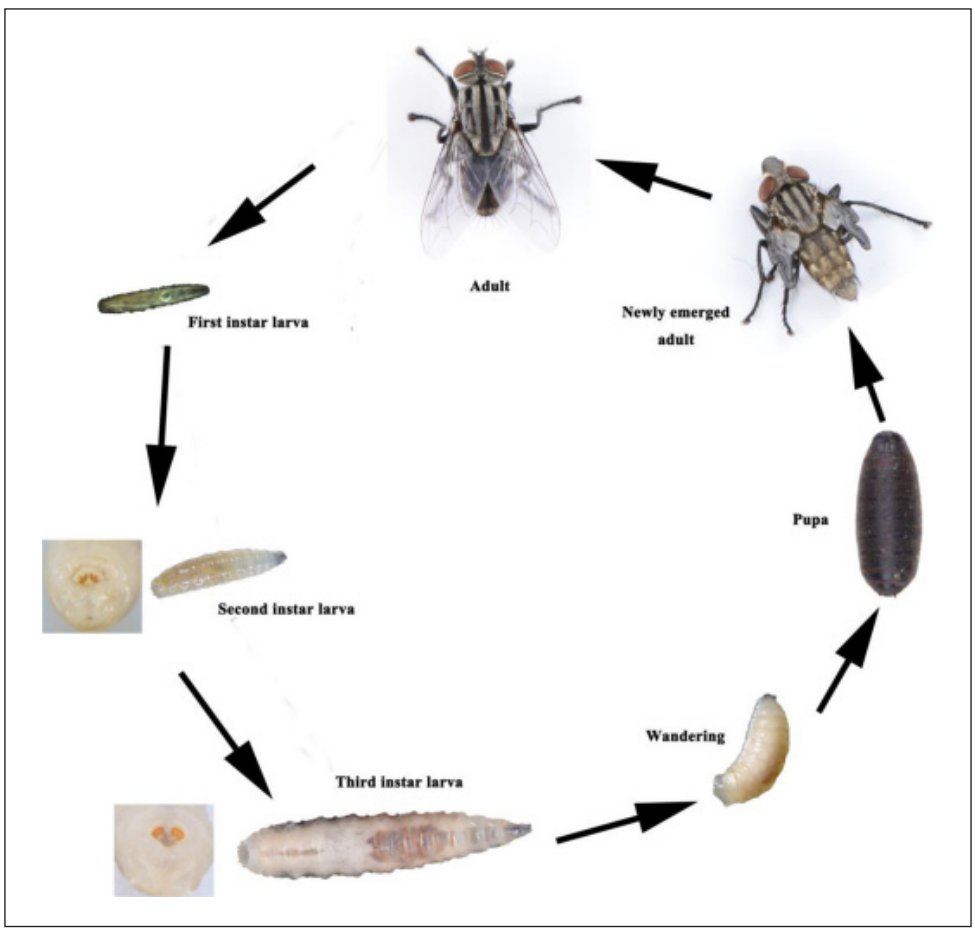

Figure 1. The life cycle of *B. peregrina*.

ruficornis and *S. dux*. Meanwhile, Samerjai *et al.* (2014) invented a cleaning apparatus for removing external sundries of the puparial with common laboratory appliance. It has been summarised by some papers that puparial features can be used for species identification (Sukontason *et al.*, 2006).

For the adult of *B. peregrina*, it is only the adult males that can be identified with certainty, with few suitable keys for distinguishing the adult female of this species (Smith, 1986; Wells *et al.*, 2001). The morphology key used to differentiate *B. peregrina* adult males from other forensically important flesh flies has been published (Chen, 2013; Lu, 2003). As for other sarcophagid of medical importance, the characteristic to identify adult males of *P. ruficornis* has been published in Thailand (Chaiwong *et al.*, 2009), while that for identifying the South American genera was updated (de Carvalho & de Mello-Patiu, 2008). Additionally, the review paper of the *S. dux* and *P. ruficornis* also has been reported (Suwannayod *et al.*, 2013).

Bionomics

The developmental rate of carrion-breeding flesh fly is crucial to estimate the PMI in forensic entomology (Wang *et al.*, 2017). In recent years, it has been emphasized to gather precise development data in specific regions to improve the accuracy of PMImin (Amendt *et al.*, 2011). The developmental durations of *B. peregrina* was recorded in Suzhou (Wang *et al.*, 2017), Beijing, Hangzhou, Guizhou (Chen, 2013) and Bangladesh (Majumder *et al.*, 2012) (Table 1). In addition, Goff (1993) found that the various drugs have an effect on the development of immature necrophagous insects. As early as 1989 and 1991, Goff reported that the residues and metabolites of cocaine and heroin can accelerate the development of the larvae of *B. peregrina* (Goff, 1989; Goff, 1991). Simultaneously, Goff also emphasized the significance of further analyses involving different classes of drugs, concentrations, and necrophagous fly species (Goff, 1993). Thus, there is a clear need for studying the impacts of different drugs on the developmental durations of different carrion-breeding flies to establish a systematic database for supporting criminal investigations.

Heavy metal pollution has severely threatened people's health and the biological diversity and has become a global environmental problem (Sun *et al.*, 2007). In the aspect of environmental risk assessment, the flesh fly of *B. peregrina* is considered as a model organism to determine its response to heavy metal exposure, since they are commonly distributed in urban habitats and predominantly confronted with heavy metals in polluted sites (Wu *et al.*, 2013). The metabolism and distribution of cadmium (Cd) in immature of *B. peregrina* have been

Table 1. Developmental times of life stages of *B. peregrina* at different constant temperatures

No.	Temp, °C	Developmental duration, h						References
		First-instar	Second-instar	Third-instar	Wandering	Pupal stage	Total duration	
1	16	56.0±2.8	53.6±2.2	170.0±4.4	74.2±2.3	713.3±30.0	1064.7±34.8	Wang *et al.*, 2017
	19	40.5±5.3	43.0±2.0	121.3±4.7	61.3±7.2	490.0±16.2	756.0±19.0	
	22	29.0±1.0	28.6±3.0	95.2±1.8	40.0±2.8	366.8±2.7	559.6±5.5	
	25	20.3±0.5	19.5±1.0	70.0±1.6	34.5±1.9	270.0±5.2	414.3±3.9	
	28	16.8±1.8	15.6±0.9	59.6±2.2	22.4±2.2	200.6±0.9	315.0±2.0	
	31	14.5±1.7	13.6±2.2	53.5±2.3	19.4±1.9	177.0±1.7	278.0±4.0	
	34	12.4±0.9	12.2±0.4	48.4±3.0	16.0±2.8	170.0±3.8	258.0±3.5	
2	16	41	53	218	Unstated	648	960±40	Chen, 2013
	20	19	40	133	Unstated	408	600±25	
	24	14	31	75	Unstated	258	378±15.75	
	28	13.5	25	57.5	Unstated	192	288±12	
	32	6.3	8	65.9	Unstated	172	252.2±10.5	

reported (Aoki Y 1984). In addition, Wu *et al.* (2013, 2014) has reported the effects of heavy metal for the metabolism, growth and development, and the reproduction of *B. peregrina*.

The chemosensory hairs of the *B. peregrina* is considered as a model system in the aspect of studying invertebrate taste reception, due to its simpler structure and unique response to specific stimuli (Amakawa, 1990). Previous studies have reported the effect of some specific stimulis on the labellar taste receptor cells of *B. peregrina*, such as amiloride (Sadakata *et al.*, 2002), multiple receptor sites (Furuyama *et al.*, 1999), alkali metal ions (Kijima, 1997), pH and several sugars (glucose, mannose, sucrose, fructose, and maltose) in mixtures and single solutions (Amakawa, 1990), G Protein Modulators (GTPyS and GDP~S) and inositol 1,4,5-trisphosphate (IP3)-mediated transduction cascade (Koganezawa & Shimada, 2002a; Shimada, 1997).

This fly species lives closely related to the human environment. *B. peregrina* was collected on rabbit carcasses in Malaysia (Silahuddin *et al.*, 2015), in Brazil (de Souza & Von Zuben, 2016), in Australia (Farrell *et al.*, 2015). In addition, Moribayashi, Ohtaki and Kurahashi had done various researches for the factors affecting development and pupal diapause of *B. peregrina* (Moribayashi, 2002), including the effects of photoperiodic and arachidonic acid content on development rates of the larvae of *B. peregrina* (Moribayashi, 2016; Kurahashi & Ohtaki, 1979), the effects of geographic variation (Moribayashi, 2001), different profiles of ecdysone secretion (Moribayashi, 1988), the ring glands physiological differentiation of mature larvae (Moribayashi, 1992), chilling period (Moribayashi, 1999), the change of ecdysone titer (Ohtaki, 1972), photoperiod (Atsuko *et al.*, 2008), pupal diapause and nondiapause of *B. peregrina*.

Molecular analysis

Species identification

Rapid and accurate species identification is significant in biological sciences and legal medicine (Cai, 2010). Traditional morphological ways have encountered many challenges for identifying many females and immature stages of sarcosaprophagous species (Smith, 1986; Wells *et al.*, 2001). DNA-based method can supplement morphological identification by distinguishing species credibly and rapidly with low requirement for sample preservation (Guo *et al.*, 2012a). Animal mitogenome has been widely used for the research of species identification (Harrison, 1989). The complete mitochondrial genome of *B. peregrina* (GenBank accession number: KF921296) has been sequenced by Zhong *et al.* (2016). Previous several studies had reported the species identification of the *B. peregrina* with other flesh flies. Such as the application 465 bp fragments of mitochondrial cytochrome c oxidase subunit I (COI) gene in India (Sharma *et al.*, 2015), the application 637 bp cytochrome coxidase subunit II (COII) and 555 bp 16S rRNA fragments (Guo *et al.*, 2012b), the 189 bp fragments of COII gene (Guo *et al.*, 2010a), the rDNA internal transcribed spacer 2 (ITS2) (Song *et al.*, 2008), the single nucleotide polymorphisms (SNPs) of the COII sequences (Zhang *et al.*, 2015a), the complete COI and COII gene (Zhang *et al.*, 2015b) in China. The species of *B. peregrina* and *Sarcophag similis* forms two single clade respectively with high support value of 72% in the NJ tree based on a 272 base pair region of COI indicated the similarity of these two species in China (Guo *et al.*, 2010b). In addition, the identification power of the COI gene was evaluated using a minibarcode region of 127 bp, standard barcode region of 658 bp and the entire COI region 1,535 bp on 99 *Sarcophaga* species including *B. peregrina* (Jordaens *et al.*, 2013). The differences within *B. peregrina* species were revealed based on a 278-bp segment of the COI gene and a 289-bp segment of the 16 ribosomal RNA (16S rDNA) gene (Guo *et al.*, 2011).

The dangers of relying on a single gene for species identification have been illustrated by recent researchs, while combing use of multiple genes is more valuable for evolutionary analysis and species identification (Guo *et al.*, 2014). To raise the identification efficiency of certain

genes, the molecular markers are required to be further screened and optimized. Meanwhile, it is necessary to explore accurate, rapid and reliable species identification methods that have relatively low requirement to the samples preservation so as to improve the application of flesh flies in forensic investigation.

Gene and functions

The gene of insect provides rich information for studying the mechanisms of anti-microbial peptides, sex-determination, chemoreceptors, and insecticide resistance (Adams *et al.*, 2000).

Many chemoreceptors and associated proteins played a very important role in the gustatory and olfactory abilities of insects, these proteins were encoded by at least four major gene families (Touhara & Vosshall, 2009). Koganezawa and Shimada (2002b) reported a cDNA library of taste tissue of the *B. peregrine*. They found seven OBP genes named gustatory PBP-related proteins (GPBPRPs) 1-7, the GPBPRP6 and 7 genes were expressed mainly in labellum, and GPBPRP2, 3 and 5 genes were expressed in tarsus, which reflecting different functions in different taste tissues.

In general, insects have a diverse innate immunity pathway to inhibit the survival of various microbes (Stokes *et al.*, 2015), and to resist bacterial and fungal infections, which can activate an antimicrobial defense system by expressing the related genes (Ferrandon *et al.*, 2007). Ando (1987) purified three antibacterial proteins from the hemolymph of *B. peregrina* third instar larvae named sarcotoxin IIA, IIB, and IIC. Natori (2010) discussed and summarized the functions of the immune molecules in the hemolymph of *B. peregrina* larvae, and especially emphasised the dual roles of some immune molecules in development and defense, including antibacterial proteins, humoral lectin, antifungal protein (AFP) and small antibacterial compound (5-S-GAD).

Typically, the heteromorphic XX/XY system determined the sex of insects, and Y-linked is male determining genes (Andere *et al.*, 2016). This phenomenon has been confirmed in the olive fruit fly (*Bactrocera oleae*), the common house fly (*M. domestica*) and the Mediterranean fruit fly (*Ceratitis capitata*) (Dubendorfer *et al.*, 2002). Agrawal *et al.* (2010) reported that *B. peregrina* have 12 chromosomes, including 5 pairs of meta/submetacentric autosomes and a pair of small dot-like sex chromosomes-XY in the males and XX in the females. Some genes are involved in the sex determination pathway in dipteran insects, including the *daughterless* (*da*), *doublesex* (*dsx*), *sex lethal* (*sxl*), *transformer* (*tra*), *transformer 2* (*tra2*), *maleless* (*mle*), and *fruitless* (*fru*) (Andere *et al.*, 2016).

The metabolism and xenobiotic resistance, detoxification genes have been reported in *P. regina* (Andere *et al.*, 2016), in *M. domestica* (Scott *et al.*, 2014), in *D. melanogaster* (Adams *et al.*, 2000). For *B. peregrina*, these aspects remain undefined and needs to be studied in the future. The genome sequence of *B. peregrina* was expected to provide more information about this species in the relationship between gene and proteins functions.

Myiasis

Myiasis is caused by the larvae of necrophagous flies invaded the organs and tissues of warm-blooded vertebrate animals and humans (Ren *et al.*, 2018). Those parasites larvae mainly stayed in the mouth, eyes, nose, subcutaneous tissues, skin, stomach, intestines, urinogenital system, ears, and other soft tissues of the host (Hall *et al.*, 2016). The human myiasis caused by *B. peregrina* was very rare and, only several myiasis cases available, which occurred in different sites, such as nasal (Kamimura, 1986), oral (Matsuzaki, 1987) and the eyes (Miura *et al.*, 2005). Such cases suggest that proper control measures for flies involved in myiasis in humans are necessary. In addition to the medical importance of flesh flies as mechanical vectors of parasitic disease agents, they can cause myiasis in the hospital environment which are also called nosocomial myiasis, and can be considered as an indicator of wound care neglect, either by the nurses or by oneself (Nazni, 2011). Miura *et al.* (2005) reported that the larvae of *B. peregrina* were found at the left conjunctival

sac of a patient in a Japan hospital, which further confirmed the potential of this species in nosocomial invasion.

Forensic entomology

The role of forensic entomology in forensic investigation has become increasingly important. The larvae and adults of necrophagous insects found on decomposed corpses can provide significant information for PMI estimations, especially for the corpses with intervals more than 72h (Byrd & Castner, 2009; Cai, 2010). Case studies have been described in research articles (Arnaldos *et al.*, 2005), books (Cai, 2010), which demonstrated the ability of forensic entomology to accurately estimate the PMI. *Boettcherisca peregrina* is one of the dominant species in some case studies and the most widespread sarcophagid species found on human corpses in China (Chen, 2013; Wang *et al.*, 2017). The potentiality of *B. peregrina* for estimating the PMI in forensic entomology has been well demonstrated by many studies, such as in Switzerland (Cherix *et al.*, 2012), in Thailand (Sukontason *et al.*, 2007), in Hawaiian Islands (Goff, 1987). In China, Ying *et al.* (2013) reported that a decomposing female corpse in Xiang River of Hunan province was found, and the main larvae found on the corpse were identified as *B. peregrina* by both morphologic observation and mitochondrial DNA sequence. PMI was estimated between 90 and 120 hours based on the entomological evidence of experimentally obtained. However, according to the forensic investigation, the interval was up to 128 hours. Although the knowledge of local fauna, climatic and micro-environment had been considered in this case, the PMI was inaccurately estimated. This case indicated that ecological, evolutionary, and genetic mechanisms of carrion decomposition require further investigation (Amendt *et al.*, 2011; Smith, 1986).

Compared with blowflies, flesh flies are rarely applied in forensic investigations to estimate the PMI, despite they have obvious advantages for decomposed corpses (Cherix *et al.*, 2012), which is mainly due to the difficulties in identifying the larvae or adults of sarcophagid species associated with human remains (Sukontason *et al.*, 2014; Suwannayod *et al.*, 2013). In addition, the practical application of necrophagous insects in forensic investigations indicated that the developmental duration and local succession data is necessary, as the more similar between the experiment and the real case in climatic and micro-environmental conditions, the more accurate the PMI can be obtained (Wang *et al.*, 2017). On the other hand, great efforts are needed by the forensic scholars to introduce standards protocols when handling cases in order to determine the time of death accurately (Ying *et al.*, 2013).

CONCLUSIONS

Although the information of *B. peregrina* is relatively limited, the importance of this sarcophagid species is improving, particularly in the area of forensic entomology. In addition to the PMI estimation, further exploring of necrophagous habits of *B. peregrina* in view of the role in carrion decomposition such as the potential mechanisms driving the colonization patterns is also important. This study reviewed various aspects of this fly species, including morphology, bionomics, molecular analysis, medical and forensic entomology involvement. However, further study on various bionomic of this species is necessary, such as developmental rates in fluctuating temperature conditions, thermal parameters, lower developmental threshold, the relevant factors effecting development durations, flight activity, seasonal prevalence and all aspects involved in the application in medical and forensic entomology. The molecular markers for species identification are still required to be further screened and optimized. Gene and functions of *B. peregrina* need to be studied further in the future to understand the mechanisms of the evolution, population structure, behavior, and physiology of this species. Although such studies are time-consuming, efforts including

resources and expertise should be either maintained or initiated since the significant meaning of this species to humans.

Competing interests
The authors have declared no competing interests exist.

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