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Using the modern morphometric approach to determine sexual dimorphism of three medically important flies (Order: Diptera) in Thailand

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Abstract. *Chaiphongpachara T, Laojun S. 2019. Using the modern morphometric approach to determine sexual dimorphism of three medically important flies (Order: Diptera) in Thailand. Biodiversitas 20: 1482-1486.* This study assessed landmark-based geometric morphometric)GM(approach to determine sexual dimorphism of three medically important flies in Thailand, *Chrysomya megacephala* (Fabricius), *Musca domestica* (Linnaeus) and *Boettcherisca nathani* (Lopes). In the wing size analysis, the centroid size (CS) was computed to estimate the wing size. During wing shape analysis, shape variables were analyzed from principal components of partial warp scores calculated after generalized procrustes analysis of coordinates. Non-parametric permutation-based tests (1000 cycles) were used (after Bonferroni correction) at $p < 0.05$ for statistical comparisons of sizes and shapes between males and females in each fly species. The results of this study, analysis of wing size for sexual dimorphism based on wing CS did not find statistical differences in flies of any type ($p > 0.05$). However, not the size, the shape of the wings is a common factor used in identification of sexual dimorphism. The wing shape in all species was different between male and female sexes. These results have shown that the GM approach was effective in identifying the sexual dimorphism of *C. megacephala, M. domestica* and *B. nathani,* which is one way to help with sex differentiation in cases of incomplete specimens that cannot be classified by morphological methods.

Keywords: Geometric morphometric approach, *Chrysomya megacephala, Lucilia cuprina, Musca domestica, Boettcherisca nathani*

INTRODUCTION

Flies belonging to the order Diptera are insects of medical and veterinary importance and are a potential mechanical vector of serious and life-threatening diseases in humans worldwide, especially in developing countries (Service 2008; Khamesipour et al. 2018). Typical medically important flies include blow flies, house flies and flesh flies (Service 2008). These flies are important vectors of many human pathogens, such as bacteria (Sukontason et al. 2000; Nazni et al. 2005; Chaiwong et al. 2012), viruses (Tan et al. 1997), fungi and parasites (protozoans and helminth eggs((Graczyk et al. 2005; Maipanich et al. 2010). Mechanical transmission of pathogens by flies occurs when pathogens that stick to body surfaces or legs of flies are transferred to another organism without amplification or development of the pathogen (Service 2008; Khamesipour et al. 2018). Usually, medically important flies feed and reproduce in animal manure, human feces and other decaying organic substances that contain human pathogens (Chaiwong et al. 2012). Presently, control of flies is one way to reduce the number of diarrhea cases (Emerson et al. 1999; Chavasse et al. 1999). The most effective control of flies requires that biological information, such as habitat and behavior, be collected using entomological techniques.

Thailand is a developing tropical country in Southeast Asia, has a tropical monsoon climate and a variety of flies. Previous research has explored a checklist of medically important flies in the central region of Thailand and found that they have dense populations, especially *Chrysomya megacephala* (Fabricius) and *Musca domestica* (Linnaeus) (Chaiphongpachara et al. 2018). Correct species identification of flies is a crucial step for understanding their biology, which relates to formulating successful control vectors (Sontigun et al. 2017). Species identification of flies is quite challenging because many species have similar morphologies that easily cause identification errors. In addition, errors with identifying sexual dimorphism often occur because of incomplete specimen sampling from field collection. For example, organs or other body parts necessary for identifying a species or sex are damaged. Recently, the modern technique has been used to successfully identify 12 species of forensically important blowflies (Sontigun et al. 2017) and 12 species of flesh flies in Thailand (Sontigun et al. 2019).

The modern technique, or geometric morphometric (GM) technique, is a new approach (Dujardin 2008) that is commonly used to identify species in organisms with similar morphologies and to study morphological variations in different environments, especially with insects (Dujardin 2017). Recently, the landmark-based GM approach was used to distinguish the sex of *Aedes aegypti, Ae. albopictus*, and *Ae. scutellaris* as mosquito vectors in Thailand and found the approach to be highly accurate (Chaiphongpachara and Laojun 2019). Therefore, this research has used the landmark-based GM approach to efficiently determine the sexual dimorphism of three medically important flies in Thailand: *Chrysomya megacephala* (Fabricius), *Musca domestica* (Linnaeus) and *Boettcherisca nathani* (Lopes).

MATERIALS AND METHODS

Fly collection and identification

Male and female adults of *C. megacephala, M. domestica*, and *B. nathani* were captured at three sites (the geo-referenced locations of study sites: Site 1; 13°23'49.1°N 100°02'22.0°E, Site 2; 13°21'43.1°N 100°01'22.5°E, and Site 3; 13°22'54.9°N 99°58'49.1°E) in Samut Songkhram Province, Thailand in January 2019. Specimens were collected once a week between 06:00 and 18:00 h. In this study, fly traps were designed based on previous research (Chaiphongpachara et al. 2018) and were 30 × 30 × 50 cm. Two fly traps per site with pork liver as bait were used. They were placed near garbage dumps. Every evening at 18:00 h, flies were removed from the traps and were killed with ethyl acetate. Afterward, specimens were sent to the laboratory at the College of Allied Health Sciences, Suan Sunandha Rajabhat University, Samut Songkhram Provincial Education Center, Thailand for species and sex identification based on morphology using taxonomic keys (Tumrasvin and Shinonaga 1978; Carvalho and Mello-Patiu 2008; Kurahashi and Chaiwong 2013). All specimens were preserved at -20°C until further analysis.

Specimen preparation and landmark collection

The number of specimens per each species and each sex of flies used in this study were 35 individuals, which is more than twice the number of landmarks. Only the left wings of male and female *C. megacephala, M. domestica* and *B. nathani* were used for landmark-based GM analysis. The left wings were detached from each specimen with forceps and were mounted on labeled slides and coverslips by Hoyer's mounting medium. Then, wing slides were photographed using a Nikon DS-Ri1 SIGHT digital camera connected to a Nikon Eclipse E600 microscope (Nikon Corp., Tokyo, Japan) at 40x magnification.

For all wing photographs, the coordinates of 17 landmarks on the intersections of wing veins were digitized (Figure 1) to evaluate the sizes and shapes of males and females in each species of *C. megacephala, M. domestica* and *B. nathani.* Damaged wings were discarded to prevent errors in landmark positions during GM analysis.

After that, repeatability test was analyzed for evaluation of the accuracy of LMs repeatedly marked (Lorenz et al., 2017). Forty samples per species (20 male samples and 20 female samples) were randomly selected and landmark-digitized twice. In repeated measures, comparison of digitized samples between the first and the second time was used using the repeatability index, which is a Model II one-way ANOVA (Arnqvist and Märtensson).

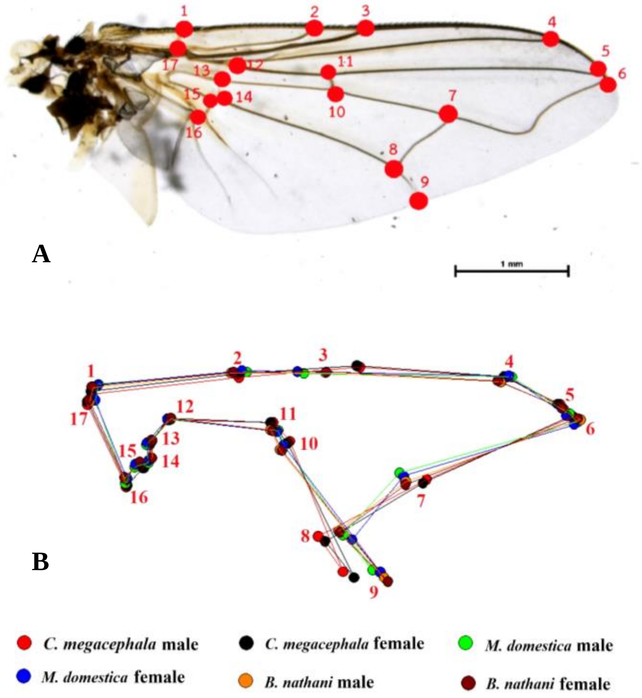

Figure 1. Positions of 17 landmarks digitized on the left wing)A(and superimposition of the mean landmark configuration in each species and sex of the flies)B(

Geometric morphometric analysis

In the wing size analysis, the centroid size (CS) was computed to estimate the wing size, which is calculated from the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein 1991). Then, non-parametric permutation-based tests (1000 cycles) were used (after Bonferroni correction) at $p < 0.05$ for statistical comparisons of sizes between males and females in each fly species.

During wing shape analysis, shape variables were analyzed from principal components of partial warp scores (also called relative warps) calculated after generalized procrustes analysis of coordinates (Rohlf and Slice 1990). Mahalanobis distances were calculated from discriminant analysis to evaluate the difference in wing shape between sexes by non-parametric permutation-based tests (1000 cycles), after Bonferroni correction, at $p < 0.05$. A cross-validation test was used to assess the accuracy of classification between sexes in each species based on Mahalanobis distances. In addition, a neighbor-joining tree was constructed to examine the similarities among male and female *C. megacephala, M. domestica* and *B. nathani* based on the Procrustes distances. In this study, the allometric effect of wing size on wing shape was investigated, which evaluated by the linear correlation coefficient between size and the first discriminant factor (Dujardin 2008).

Geometric morphometric analysis software

Digitization of landmarks and GM analysis were performed using the CLIC (Collection of Landmarks for Identification and Characterization) package version 97, which is freely available at https://xyom.io. The neighbor-joining tree was constructed using R software, which is freely available at https://cran.r-project.org.

RESULTS AND DISCUSSION

In this study, the landmark-based GM approach was used to analyze the morphological differences between male and female *C. megacephala*, *M. domestica* and *B. nathani*, which were divided into size and shape analyses. The repeatability of the size (CS) and shape (relative warps) was as high values as 0.98 and 0.91, respectively.

The size comparison of flies based on CS between male and female sexes found that the male of *B. nathani*)mean: 8 .4 6 mm, min-max: 5.67-13.33 mm(and female of *C. megacephala* (mean: 8.01 mm, min-max: 6.69-8.81 mm) were the largest)Table 1). Comparisons of variation of wing CS in each species and sex of the flies is shown in Figure 2. A size difference between male and female sexes of *C. megacephala*)p = 0.35(, *M. domestica*)p = 0.87(and *B. nathani*) p = 0.13(was not found in this analysis after applying the Bonferroni test)Table 1).

After generalized procrustes analysis of coordinates, shape variables were calculated. The principal components of the partial warp were computed to illustrate the morphospace and found complete separation between sexes *in C. megacephala* but no good separation in *M. domestica* and *B. nathani* (Figure 3).

The wing shape in all species was different between male and female sexes based on Mahalanobis distances after applying the Bonferroni test (p < 0.01) (Table 2). Cross-validated reclassification scores were 71% to 94%, which were found to be excellent in males (94%) and females (91%) of *C. megacephala* (Table 3). A neighbor-joining tree based on the Procrustes distances in Figure 4 show pattern of wing shape similarity among male and female flies, which consolidates males and females in all species into the same cluster. In addition, the morphological tree pointed out that *C. megacephala* has fewer similarities than *M. domestica* and *B. nathani*. Allometry of all samples in this study accounted for 32% (p < 0.001) of wing size influencing variations in wing shape (Figure 5).

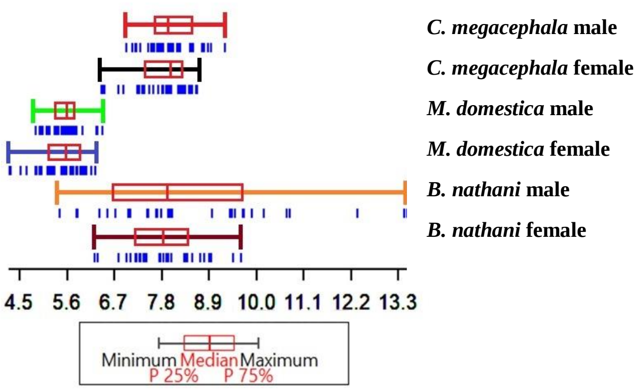

Figure 2. Comparisons of variation of wing CS in each species and sex of flies. The data in this study are converted from pixels to mm. Each box shows the group median separating the 25th and 75th quartiles. The vertical bars under the six boxes represent the wing sizes of individual specimens

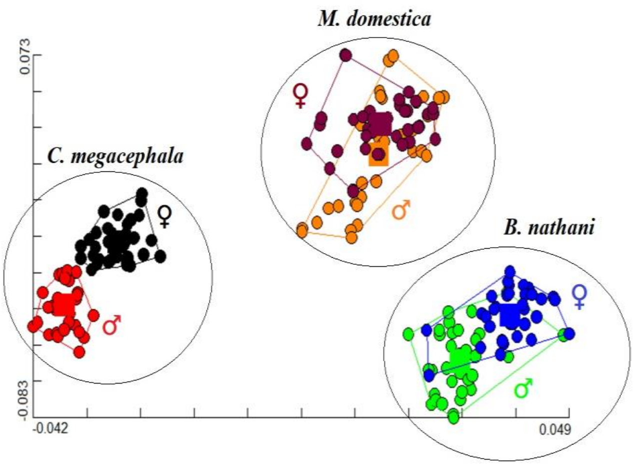

Figure 3. Morphospace based on shape variables between sexes of *C. megacephala*, *M. domestica* and *B. nathani*

Table 1. Statistical analyses of mean wing CS differences between sexes

Species and sex	Mean±S.D. (mm)	Min-max (mm)	p-value
C. megacephala male	8.13±0.28	7.14-9.32	0.35
C. megacephala female	8.01±0.34	6.69-8.81	
M. domestica male	5.66±0.12	5.02-6.49	0.87
M. domestica female	5.64±0.24	4.54-6.42	
B. nathani male	8.46±3.92	5.67-13.33	0.13
B. nathani female	7.90±0.65	6.44-9.67	

Note: All pairwise CS differences were not statistically significant)p > 0.05(. Min, minimum; Max, maximum; mm, millimeters; Mean, average CS; S.D., standard deviation

Table 2. Statistical analyses of Mahalanobis distance scores between sexes

Species	Mahalanobis distance scores	*p*-value
C. megacephala male	6.33	< 0.01
C. megacephala female		
M. domestica male	3.56	< 0.01
M. domestica female		
B. nathani male	2.85	< 0.01
B. nathani female		

Note: All pairwise Mahalanobis distances were statistically significant (p < 0.05)

Table 3. Cross-validated reclassification scores based on wing shape

Species and sex	Percent of correctly assigned individuals	Assigned/ observed
C. megacephala male	94	33/35
C. megacephala female	91	32/35
M. domestica male	82	29/35
M. domestica female	77	27/35
B. nathani male	77	27/35
B. nathani female	71	25/35

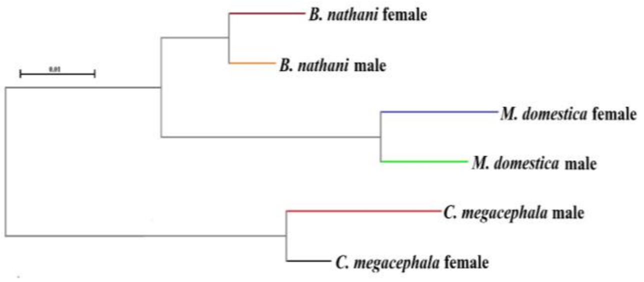

Figure 4. Neighbor-joining tree based on the Procrustes distances

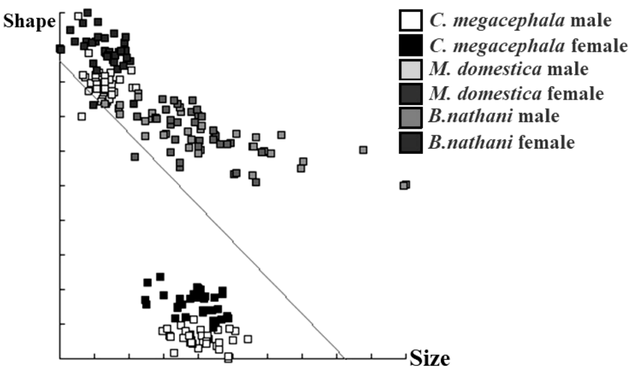

Figure 5. An allometric plot which a point represents an individual sample

Discussion

Increasing the knowledge of vector insects is important for understanding vectors, which will lead to effective vector control. Accurate sex identification of flies is an important first step in their study. Although sex identification of adult flies by the morphological method is relatively easy, sometimes the specimens obtained from the field are incomplete, resulting in false identification. In this study, the landmark-based GM approach was used to analyze the sexual dimorphism of three types of flies that are common in Thailand: *C. megacephala*, *M. domestica* and *B. nathani*. The estimation of sexual dimorphism of the flies was divided into two analyses, size and shape, both of which were found to be inconsistent.

The analysis of wing size for sexual dimorphism based on wing CS did not find statistical differences in flies of any type. The results of this study were consistent with previous research that used the landmark-based GM approach to identify 12 species of blowflies in Thailand: *Chrysomya megacephala, C. chani, C. pinguis, C. rufifacies, C. villeneuvi, C. nigripes, Lucilia cuprina, L. papuensis, L. porphyrina, L. sinensis, Hemipyrellia ligurriens* and *H. pulchra*. That study found that size could not be used for species identification (Sontigun et al. 2017). The size of the wing is a factor that has been recognized as inappropriate for species identification and sexual dimorphism because it is easily variable from environmental conditions, especially in breeding sites (Aytekin et al. 2009; Virginio et al. 2015; Lorenz et al. 2017; Chaiphongpachara et al. 2018; Chaiphongpachara et al. 2019).

The shape of the wings is a common factor used in identification of species and sexual dimorphism because it is influenced directly by genetics and has less environmental variation than size factors (Virginio et al. 2015; Cavaignac et al. 2016; Dujardin 2017). In this study, shape analysis based on Mahalanobis distances revealed differences between male and female sexes in all types of flies. The results of cross-validated reclassification used to verify the accuracy of the individual in classifications ranged from 71% to 94%. In addition, a neighbor-joining tree based on the Procrustes distances showed similarities between sexes, which supports the potential of the GM approach for identifying sexual dimorphism in flies. Recently, the GM approach has been used to separate genera and species, and distinguish between the sexes of 12 species of flesh flies in Thailand: *Boettcherisca nathani, B. peregrine, Lioproctia pattoni, L. ruficornis, L. saprianovae, Parasarcophaga brevicornis, P. dux, P. scopariiformis, Sarcorohdendorfia antilope, S. multivillosa, S. seniorwhitei* and *Seniorwhitea princeps* (Sontigun et al. 2019). In this study, the relationship between wing size and wing shape (allometry) was found. Usually, allometry is found in insects for morphological effects of sexual dimorphism (Lorenz et al., 2017). However, the allometric effect was not removed from this study because the effect of size and shape can be an important species-specific characteristic (Dujardin JP. 2008).

The GM approach is effective at separating male and female sexes of files, especially in *C. megacephala*, which can be separated by more than 89%. This was consistent with previous studies that found that the *C. megacephala* wing can easily separate males and females (87.5% of males and 100% of females were correctly classified between sexes) (Sontigun et al. 2017). In addition, this approach has succeeded in separating sexes of mosquitoes belonging to order Diptera with the same level of accuracy as for flies.

This study has shown that the GM approach was effective in identifying the sexual dimorphism of *C. megacephala*, *M. domestica* and *B. nathani*, which is one way to help with sex differentiation in cases of incomplete specimens that cannot be classified by morphological methods.

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REFERENCES

Arnqvist G, Märtensson T. 1998. Measurement error in geometric morphometrics: empirical strategies to assess and reduce its impact on measure of shape. Acta Zool Acad Sci Hung 44: 73-96.

Aytekin S, Aytekin AM, Alten B. 2009. Effect of different larval rearing temperatures on the productivity (Ro) and morphology of the malaria vector *Anopheles superpictus* Grassi (Diptera: Culicidae) using geometric morphometrics. J Vector Ecol 34 (1): 32-42.

Bookstein FL. 1991. Morphometric tools for landmark data: geometry and biology. Cambridge University Press, United Kingdom.

Cavaignac E, Savall F, Faruch M, Reina N, Chiron P, Telmon N. 2016. Geometric morphometric analysis reveals sexual dimorphism in the distal femur. Forensic Sci Intl 259: 246.e1-5.

Carvalho CJB, Mello-Patiu CA. 2008. Key to the adults of the most common forensic species of Diptera in South America. Rev Bras Entomol. DOI: 10.1590/S0085-56262008000300012.

Chaiphongpachara T, Laojun S. 2019. Landmark-based geometric morphometric analysis of wings to distinguish the sex of *Aedes* mosquito vectors in Thailand 20)2(: 419-424.

Chaiphongpachara T, Laojun S, Jongvisuttisan N, Tubsamut P, Dasom A. 2018. A checklist of medically important flies (Order: Diptera) in the central region of Thailand. Biodiversitas 19 (6): 2134-2139.

Chaiphongpachara T, Sriwichai P, Samung Y, Ruangsittichai, Ruangsittichai J, Morales Vargas RE, Cui L, Sattabongkot J, Dujardin JP, Sumruayphol S. 2019. Geometric morphometrics approach towards discrimination of three member species of Maculatus group in Thailand. Acta Trop 192: 66-74.

Chaiphongpachara T, Juijayen N, Chansukh KK. 2018. Wing geometry analysis of *Aedes aegypti* (Diptera, Culicidae), a dengue virus vector, from multiple geographical locations of Samut Songkhram, Thailand. Iran J Arthropod Borne Dis 12 (4): 351-360.

Chaiwong T, Srivoramas T, Sukontason K, Sanford MR, Moophayak K, Sukontason KL. 2012. Survey of the synanthropic flies associated with human habitations in Ubon Ratchathani province of northeast Thailand. J Parasitol Res 2012: 613132. DOI: 10.1155/2012/613132

Chaiwong T, Srivoramas T, Sukontason KOM, Sanford MR, Sukontason KL. 2012. Bacterial fauna associated with the blowfly, *Chrysomya megacephala* (F.) in Ubon Ratchathani province of NorthEast Thailand. Intl J Parasitol Res 4)1(: 71-74.

Chavasse DC, Shier RP, Murphy OA, Huttly SRA, Cousens SN, Akhtar T. 1999. Impact of fly control on childhood diarrhoea in Pakistan: Community-randomised trial. Lancet 353 (9146): 22-25.

Dujardin JP. 2017. Modern Morphometrics of Medically Important Arthropods, Genetics and Evolution of Infectious Diseases. 2nd ed. Elsevier, Nederlands.

Dujardin JP. 2008. Morphometrics applied to medical entomology. Infect Genet Evol 8: 875-890.

Emerson PM, Lindsay SW, Walraven GEL, Faal H, Bøgh C, Lowe K, Bailey RL. 1999. Effect of fly control on trachoma and diarrhoea. Lancet 353 (9162): 1401-1403.

Graczyk TK, Knight R, Tamang L. 2005. Mechanical transmission of human protozoan parasites by insects. Clin Microbiol Rev 18 (1): 128-132.

Khamesipour F, Lankarani KB, Honarvar B, Kwenti TE. 2018. A systematic review of human pathogens carried by the housefly (*Musca domestica* L.). BMC Public Health 18 (1): 1049. DOI: 10.1186/s12889-018-5934-3.

Kurahashi H, Chaiwong T. 2013. Keys to the flesh flies of Thailand, with description of a new species of *Robineauella enderlein* (Diptera: Sarcophagidae). J Entomol Zool Stud 64: 83-101

Lorenz C, Almeida F, Almeida-Lopes F, Louise C, Pereira SN, Petersen V, Vidal PO, Virginio F, Suesdek L. 2017. Geometric morphometrics in mosquitoes: What has been measured. Infect Genet Evol 54: 205-215.

Maipanich W, Sa-nguankiate S, Pubampen S, Kusolsuk T. 2010. Intestinal parasites isolated from house-flies in the tourist attraction areas in Thailand. J Trop Med Parasitol 33)3(: 532-546.

Nazni WA, Seleena B, Lee HL, Jeffery JT, Rogayah TA, Sofian MA. 2005. Bacteria fauna from the house fly, *Musca domestica* (L.). Trop Biomed 22 (2): 225-231

Rohlf FJ, Slice D. 1990. Extensions of the procrustes method for the optimal superimposition of landmarks. Syst Zool 39: 40-59.

Service M. 2008. Medical Entomology for Students. 4th ed. Medical Entomology for Students, Fourth Edition. Cambridge University Press 20 (8): 1428.

Sontigun N, Samerjai C, Sukontason K, Wannasan A, Amendt J, Tomberlin JK, Sukontason KL. 2019. Wing morphometric analysis of forensically important flesh flies (Diptera: Sarcophagidae) in Thailand. Acta Trop. 190: 312-319.

Sontigun N, Sukontason KL, Zajac BK, Zehner R, Sukontason K, Wannasan A, Amendt J. 2017. Wing morphometrics as a tool in species identification of forensically important blowflies of Thailand. Parasit Vectors 10: 229.

Sukontason K, Bunchoo M, Khantawa B, Piangjai S, Sukontason K, Methanitikorn R, Rongsriyam Y. 2000. Mechanical carrier of bacterial enteric pathogens by *Chrysomya megacephala* (Diptera: Calliphoridae) in Chiang Mai, Thailand. Southeast Asian J Trop Med Public Health 31)1(: 157-161

Tan SW, Yap KL, Lee HL. 1997. Mechanical transport of rotavirus by the legs and wings of *Musca domestica* (Diptera: Muscidae). J Med Entomol 34 (5): 527-31.

Tumrasvin W, Shinonaga S. 1978. Studies on medically important flies in Thailand. V. On 32 to the subfamilies Muscinae and Stomoxyinae including the taxonomic keys (Diptera: Muscidae). Bull Tokyo Med Dent Univ 2: 77-81.

Virginio F, Oliveira Vidal P, Suesdek L. 2015. Wing sexual dimorphism of pathogen-vector culicids. Parasit Vectors 14)8(: 159.