Heliyon 10 (2024) e25207

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Digital microscope-assisted photography improves the accuracy of mosquito wing measurement

Zulhisham Zulzahrin [a], Meng Li Wong [a], Mohd Redzuan Ahmad Naziri [a], Yee-Ling Lau [a], Indra Vythilingam [a], Wenn-Chyau Lee [a,b,*]

[a] *Department of Parasitology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*

[b] *A*STAR Infectious Diseases Labs (A*STAR ID Labs), Agency for Science, Technology and Research (A*STAR), 8A Biomedical Grove, Immunos #05-13, Singapore 138648, Singapore*

ARTICLE INFO

Keywords:
External morphological characterization
Measurement
Callipers
Photography
ImageJ
Arthropods

ABSTRACT

Wing measurement is an important parameter in many entomological studies. However, the methods of measuring wings vary with studies, and a gold standard method was not available for this procedure. This in turn limits researchers from confidently comparing their research findings with published data collected by other means of measurement. This study investigated the interchangeability of three commonly available methods for wing measurement, namely the calliper method, stereomicroscope-assisted photography method, and digital microscope-assisted photography method, using the laboratory colony of *Aedes aegypti*. It was found that the calliper method and the photography-based methods yielded similar results, hence the good interchangeability of these methods. Nevertheless, the digital microscope-assisted photography method yielded more accurate measurements, due to the higher resolution of the captured photos, and minimal technical bias during the data collection, as compared to the calliper-based and stereomicroscope-assisted photography methods. This study served as a reference for researchers to select the most suitable measurement method in future studies.

1. Introduction

In entomological research, mosquito's physical attributes such as the body and wing measurements are often used as one of the physiologic indicators of mosquito fitness, which is associated with its vectorial capacity [1,2]. For instance, larger wing span has been shown to increase the survival of a female mosquito by expanding its flight range and allows better host-seeking capability [3,4]. Besides being associated with increased longevity and reproductivity, larger wings have been an indicator of increased gene and metabolite expression that are related to vitellogenesis [5,6]. Therefore, it is relevant that wing size of disease vectors like *Ae. aegypti* is measured when assessing the fitness of vectors following the implementation of novel vector control effort. Despite the importance of wing measurement in this field, there was no standardized method for wing measurement of mosquitoes and other insects. Several wing measurement protocols have been used by different studies [7–10]. In general, these techniques involve either the usage of calliper or the photography-based methods [11–13]. With its long history of application, calliper-based method has been considered as the reference technique to measure physical attributes of insects [14]. The usage of callipers allows quick measurement to be

* Corresponding author. Department of Parasitology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia

E-mail addresses: zzulhisham1999@um.edu.my (Z. Zulzahrin), mengli.wong0105@gmail.com (M.L. Wong), cerempet@um.edu.my (M.R.A. Naziri), lauyeeling@um.edu.my (Y.-L. Lau), indrav@um.edu.my (I. Vythilingam), wclee@um.edu.my (W.-C. Lee).

https://doi.org/10.1016/j.heliyon.2024.e25207
Received 16 August 2023; Received in revised form 7 December 2023; Accepted 23 January 2024
Available online 28 January 2024

performed at various working environment, including the field setting. Nevertheless, the accuracy of this method relies on the equipment, as well as the handler. Skilled, steady hands are required when measuring small and fragile objects with digital callipers, such as the wings of insects. Otherwise, the specimens may be damaged. On the other hand, photography-based methods were of shorter application history than the calliper method [9,11,12,15,16]. Most photography-based methods rely on an image processing software, such as ImageJ, for onscreen measurement of specimens. For wing measurement, the photography-based methods digitally preserve the wing structures through acquired images, allowing future investigation and referencing. However, photography-based methods may not provide on-the-spot measurements, and the accuracy of measurements relies heavily on the labelling and recording of photos, as well as the quality of the photos taken, which may only be revealed during the image processing stage. Importantly, there are different wing measuring protocols involving usage of various steps, tools, and software [11,12,16]. This may create confusion and challenges for cross reference, comparison and meta-analyses of data collected by different parties. Hence, a cross-examination of these wing measurement methods was in need to evaluate their interchangeability and more importantly, their performance and reliability. Here, three widely available wing measuring methods (calliper method, stereomicroscope-assisted photography method, and digital microscope-assisted photography method) were applied to measure the wings of *Aedes aegypti*, a highly domestic, medically important vector that is well-adapted to laboratory colonization. Two of the three methods here (stereomicroscope-assisted photography and digital microscope-assisted photography methods) were technically similar, as they were photography-based techniques. However, both methods used two different types of microscopes and photography approaches. Stereomicroscope and phone camera are more commonly available than the digital-microscope, despite the affordable cost of the latter. By comparing both photography-based methods as separate entities, we were able to evaluate the accuracy of the devices in measuring parts of small insect, despite the similar technical principles. The performance evaluation of these methods may serve as a reference for other researchers the future.

2. Materials and methods

Ethical approval

Materials and equipment used, along with their sources, were provided separately (Supplementary Table 1). Experiments of this study were conducted with protocols approved by the Medical Research Ethics Committee of University of Malaya Medical Centre (MRECID # 2022822-11491) and Institutional Biosafety and Biosecurity Committee, Universiti Malaya (UMIBBC/PA/R/FOM/PARA-023/2022).

2.1. Rearing of laboratory-adapted Aedes aegypti

Mosquitoes were reared in the insectary at 25 °C and relative humidity of 75 %, with 12 h light/12 h dark photoperiod. The adults were maintained with a diet of 10 % sucrose solution fortified with Vitamin B complex. The immature stages were reared in a plastic container filled with dechlorinated water and fed with a blend of dried beef liver powder, baking yeast and mice pellet (1:1:2 wt ratio).

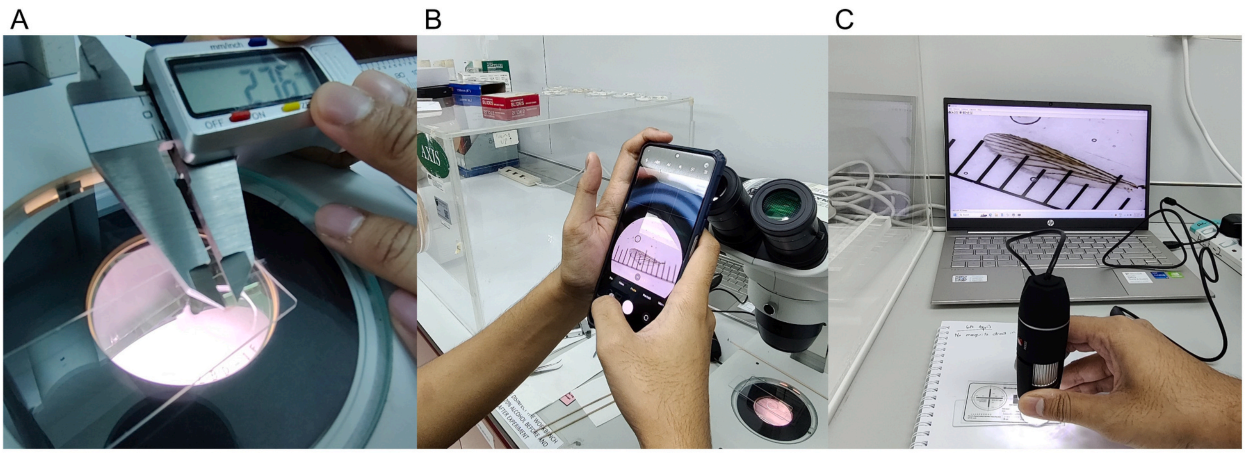


Fig. 1. Set up for wing measurement by three different methods. **(A)** The dissected wing is placed on a glass slide and covered with immersion oil and cover slip before being measured with a digital calliper. This was done under the stereomicroscope so length of apical notch and auxiliary margin can be measured as accurately as possible. **(B)** A phone camera is used to capture the image of the prepared wing slide through the stereomicroscope eyepiece. **(C)** A digital microscope connected to a laptop is used to capture image of the dissected wing against a micrometre slide on a white paper.

2.2. Wing measurement

Each wing was dissected off the mosquito and placed on a glass slide, covered with immersion oil and a glass cover slip. They were measured from the apical notch to the axillary margin [1], using the methods under evaluation, as elaborated in the subsequent segments. For each wing, eight technical replicates (reads) were made to obtain an average value for the recording.

2.2.1. Calliper method

Digital callipers (Precision Measuring, product code #9070554-001001, China) were used to measure *Ae. aegypti* wing (Fig. 1A The callipers were closed and zeroed before the measurement was taken under stereomicroscope. The calliper must be handled gently to avoid any damage to the specimen. The measurement values of the calliper method were used as the reference method values for subsequent analyses.

2.2.2. Stereomicroscope-assisted photography method

Glass slide with a dissected mosquito wing was placed on top of a plastic micrometre slide, and examined under the stereomicroscope (Olympus, SZ51, Japan), with viewing field adjusted to confer the highest possible resolution for both the wing outline and the micrometre scales. Subsequently, a digital image was taken using a phone camera through the stereomicroscope's eyepiece (Fig. 1B). The image was transferred to a computer for further analyses using ImageJ. For the technical replicates of measurement, eight images were taken for each wing at different orientations relative to the micrometre slide, to minimize bias arising from any image distortion.

2.2.3. Digital microscope-assisted photography method

Micrometre slide was placed between the glass slide with the mosquito wing and a white paper. The digital microscope (Gloptics, 1600×, China) was connected to a computer to which the images were captured (Fig. 1C). Eight images were taken for each wing at different orientations relative to the micrometre slide. Subsequently, the images were analysed with ImageJ.

2.2.4. Post-photography analyses with ImageJ

ImageJ [17] was used to measure the photographed wings from the two photography-based methods. For each image, a scale was created by measuring the pixels between two points on the micrometre slide with known and fixed distance. The scale (X pixels/Y mm) was set. After that, the distance between the apical notch and the axillary margin of a wing was measured.

2.3. Data analyses

Data were analysed using Graphpad Prism 9. By setting the calliper method as the reference method, Bland-Altman analysis was used to compare the two photograph-based methods with the calliper method. For correlation analyses, Spearmen's correlation test was performed. Kruskal-Wallis test with Dunn's post-test was used to compare the measurements done with these methods.

3. Results

In total, 70 *Ae. aegypti* wings were measured with the three methods. Overall, the wings of *Ae. aegypti* were found to be 2.8131 ± 0.3656 mm by the calliper method, 2.8405 ± 0.3474 mm by the stereomicroscope-assisted photography method, and 2.8962 ± 0.3582 mm by the digital microscope-assisted photography method.

Both the stereomicroscope- and digital microscope-assisted methods provided images of adequate quality (Fig. 2A and **B**, respectively) for measurement analyses, albeit of different resolution. The calliper method and the stereomicroscope-assisted

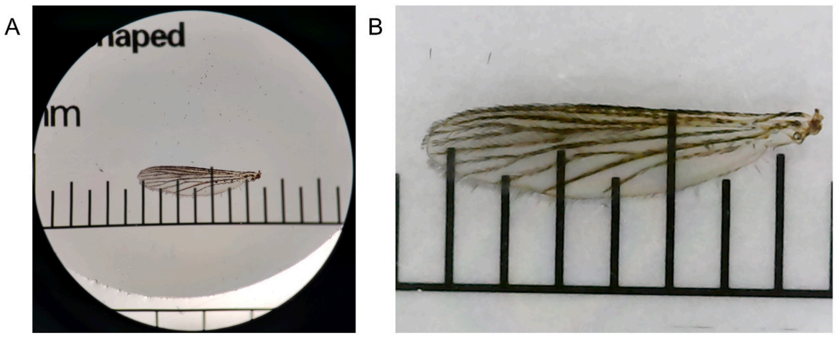
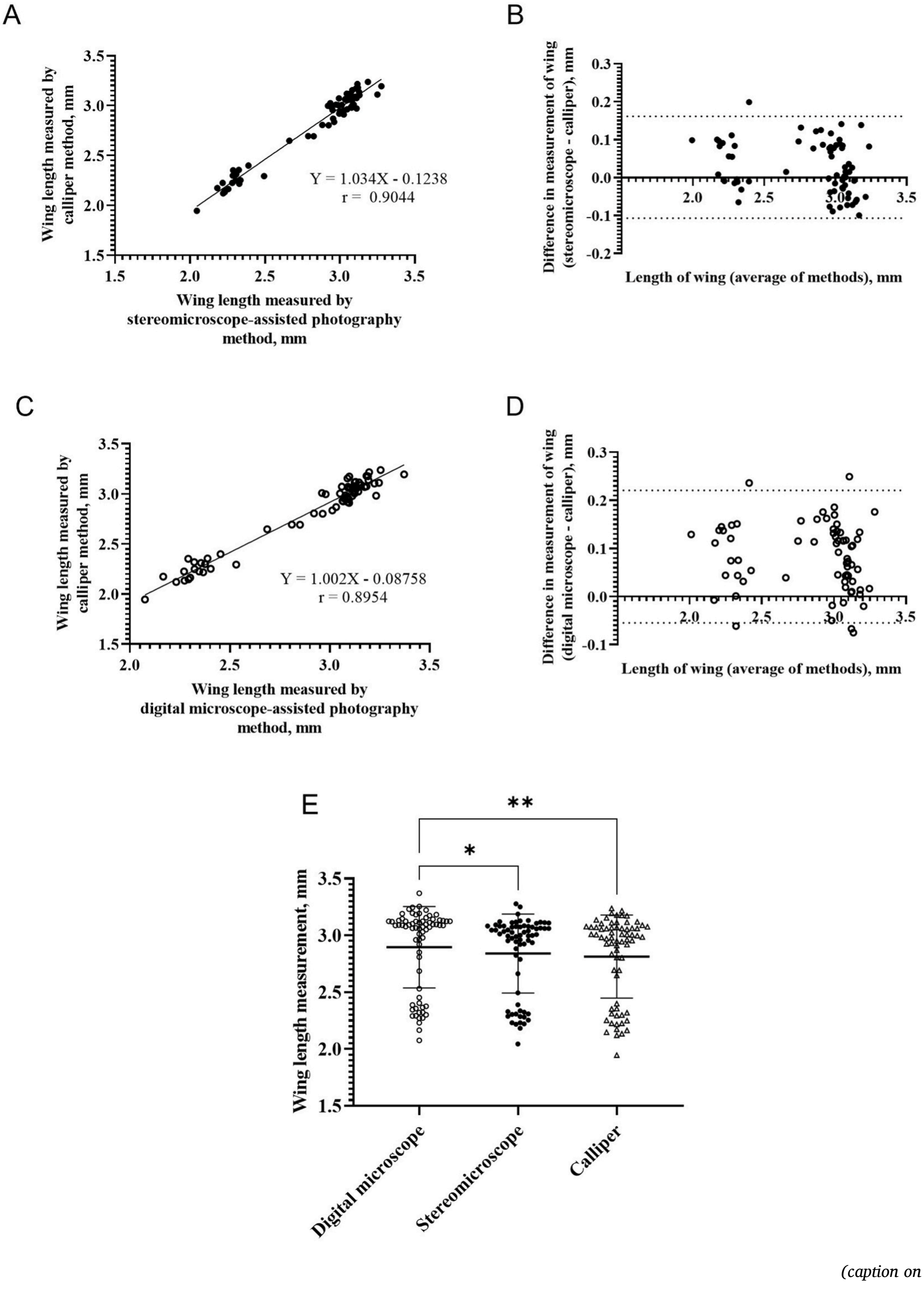


Fig. 2. Wing measurements with different methods. Scale bars represent 0.5 mm. Dissected wings laid flat on a glass slide with immersion oil and cover slip on top against a micrometre slide. **(A)** Picture captured by digital microscope showing a zoomed-in wing compared to **(B)** picture captured by phone camera through the stereomicroscope showing a more zoomed-out picture of the wing in order to get a sharp detail of the wing and the micrometre slide hash marks.



(caption on next page)

Fig. 3. Graphs of statistical analyses performed. **(A)** Spearmen's Correlation and Linear Regression plot of wings measured via the stereomicroscope-assisted photography method against calliper method ($r = 0.9044$; 95 % CI = 0.8481–0.9405; $p < 0.0001$; df = 68). **(B)** Bland-Altman plot of average vs difference between wings measured stereomicroscope-assisted photography method and calliper method (95 % limit of agreement between −0.1070 and 0.1617; mean bias = 0.02733). **(C)** Spearmen's Correlation and Linear Regression plot of wings measured via the digital microscope-assisted photography method against calliper method ($r = 0.8954$; 95 % CI = 0.8343–0.9348; $p < 0.0001$; df = 68). **(D)** Bland-Altman plot of average vs difference between wings measure by digital microscope-assisted photography method and calliper method (95 % limit of agreement between −0.05472 and 0.2209; mean bias = 0.08310). **(E)** Kruskal-Wallis with Dunn's multiple comparison test was conducted to evaluate the difference between values obtained through three method tested. Significant differences were found between values obtained by digital microscope-assisted method with stereomicroscope-assisted and calliper method (adjusted $p = 0.0094$ and 0.0385 respectively). Clustering of data are due to size sexual dimorphism as female mosquitoes are bigger than their male counterparts.

photography method were significantly correlated ($r = 0.9044$; 95 % CI = 0.8481–0.9405; $p < 0.0001$; df = 68) (Fig. 3A). Bland-Altmann analysis revealed good agreement between the two methods (95 % limit of agreement between −0.1070 and 0.1617), with insignificant bias (mean bias = 0.02733) (Fig. 3B). Likewise, the digital microscopy-assisted photography method demonstrated significant correlation with the calliper method ($r = 0.8954$; 95 % CI = 0.8343–0.9348; $p < 0.0001$; df = 68) (Fig. 3C). Via the Bland-Altmann analysis, good agreement was found between both methods (95 % limit of agreement between −0.05472 and 0.2209) with insignificant bias (mean bias = 0.08310) (Fig. 3D). Nevertheless, comparison of the measurements on the same wing structures by these methods revealed a different picture. The measurements recorded by the calliper method were of insignificant difference with the values obtained from the stereomicroscope-assisted photography method. The measurements collected with the digital microscope-assisted photography method were significantly different from data collected with the stereomicroscope-assisted photography method (adjusted $p = 0.0385$) and calliper method (adjusted $p = 0.0094$) (Fig. 3E).

4. Discussion

Of the various protocols available to measure wings, three methods were recruited for this study due to the cost, practicality, and convenience of these methods, which are suitable for both the laboratory and field settings. Despite the need for a camera and computer, the photography-based methods recruited in this work can be applied in field settings, as they do not require large-sized equipment or computer with large capacity to operate.

Technically, the calliper and photography-based methods employed the same technique of positioning and securing the wing prior to data acquisition, where the wing was mounted to a glass slide and covered with a glass coverslip to secure the position of the wing and prevent the loss of the wing specimens by wind. The usage of immersion oil allowed flattening of the wing structure between the glass slide and coverslip, without the need to apply excessive force, which may damage the structure and induce artefact to the measurement.

The use of callipers is convenient as it provides on-the-spot reading. Nevertheless, this method may yield higher inter-personal variation. Furthermore, the specimens may be damaged due to the mishandling of callipers by inexperienced handlers, resulting in wastage of research resources. On the other hand, the photography-based methods minimize the risk of damaging specimens. The images of the wings are captured and catalogued, allowing objective reassessment of the measurements by different individuals, even after the experiments, provided that the labelling and recording of photos are conducted systematically, and the images taken are of good quality. Importantly, the archived digital images can be used for future analyses such as characterization of the wing outline via a geometric morphometric study [18]. Succinctly, the classic calliper approach allows instantaneous data acquisition, whereas the photography-based methods allow digital preservation of data. The advantages and disadvantages of each method are summarised in Table 1.

Despite the high similarity of the measurements obtained, hence the interchangeability between the calliper method and both photography-based methods, significant difference in readings was noted between the digital microscope-assisted photography method and the other two methods (Fig. 4A–H). In fact, the digital microscope-assisted photography method was the better, more objective approach among the three methods tested. When measurements were made with the calliper, the calliper jaws were adjusted and placed above the specimen that was covered by the coverslip. In other words, the calliper jaws and the specimens to be measured were not of the same depth of view. Besides, the calliper jaws were directed at the specimen in an angular manner, instead of superimposing over the specimen to be measured. This would give rise to parallax error, compromising the accuracy of measurement

Table 1
Comparison of the three evaluated methods.

Method	Digital calliper	Stereomicroscope-assisted	Digital microscope-assisted
Data preservation	No	Yes, sample is preserved as digital images	Yes, sample is preserved as digital images
Specimen handling	Higher risk of damaging specimens	Low risk of damaging specimens	Low risk of damaging specimens
Speed of data acquisition	on-the-spot reading	Post-photography processing via software is required	Post-photography processing via software is required
Likelihood of bias due to technical errors	High, due to interpersonal variation	High, due to parallax error	Less likely
Price	RM40-RM50 (~9–10 USD)	RM7,000-RM9,000 (~1500–2000 USD)	RM40-RM80 (~9–17 USD)

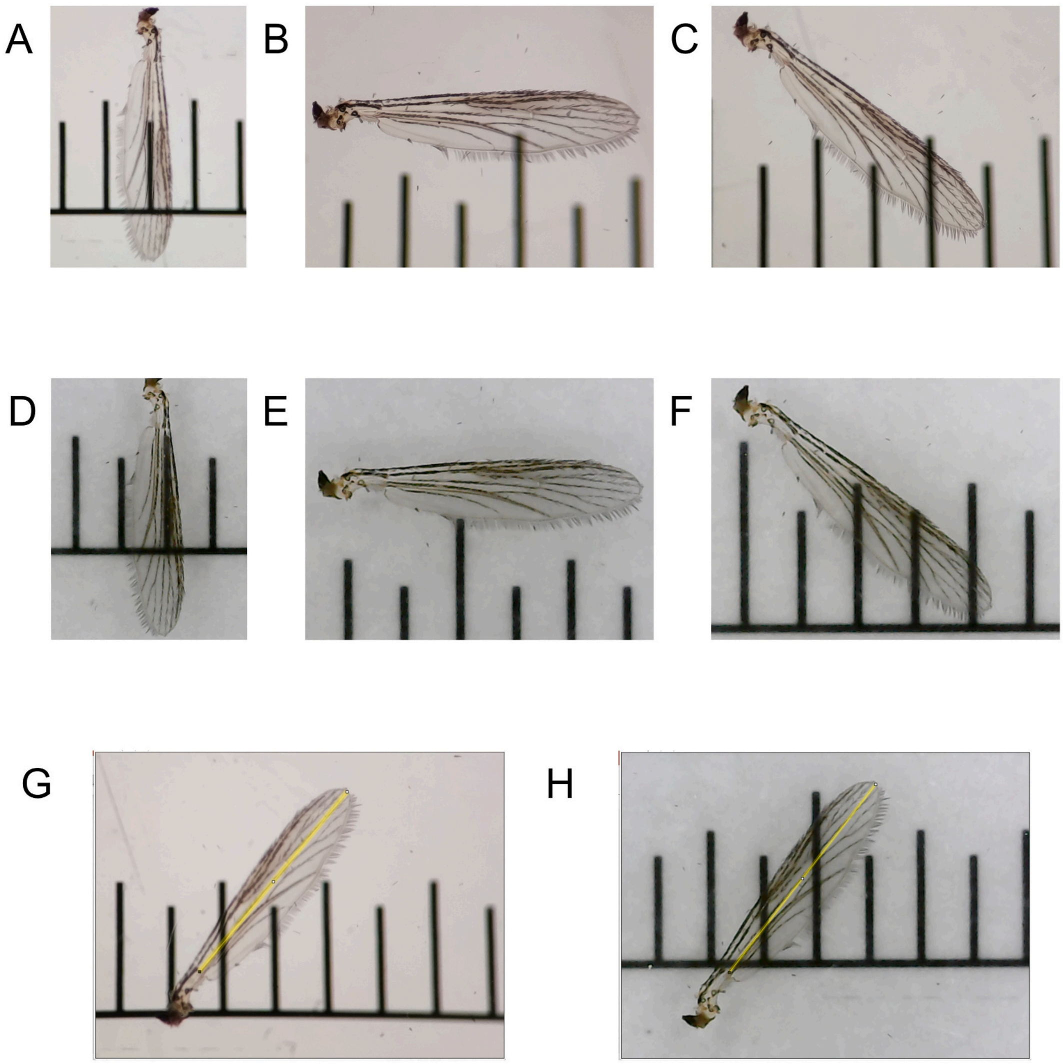


Fig. 4. Comparison of images captured by stereomicroscope-assisted photography method and digital microscope-assisted photography method. **(A, B and C)** Images obtained by stereomicroscope-assisted photography method have blurry hash marks compared to **digital microscope-assisted photography (D, E and F)** that has clear and distinctive hash marks. Distance (in pixels) between two hash marks are measured and converted to mm. **(G and H)** Screen capture of ImageJ image processing of stereomicroscope-assisted and digital microscope-assisted photography methods respectively.

made. Likewise, parallax error happened to the stereomicroscope-assisted photography method, as the lens of the phone camera might not aligned correctly with the eyepiece of the stereomicroscope (Fig. 4A–C). In addition, the resolution of the photos captured with phone camera was compromised by the stability of the photographer. Consequently, the distance calculated by the image processor was not as accurate (Fig. 4G). Nevertheless, this limitation can be resolved with a built-in camera system for the stereomicroscope, although such set up may not be convenient for field setting. Alternatively, a phone stand secured properly to the microscope may improve the situation. In contrast, limitation associated with parallax error can be overcome by the digital microscope-assisted photography method (Fig. 4D–F). The microscope is secured upright and perpendicular to the specimen, instead of being held and manoeuvred by hands. The photos captured were of consistently high resolution, allowing precise distance measuring by the image processor (Fig. 4H). Despite the significant difference in the collected readings with those collected with the calliper method that has long application history, the newer digital microscope-assisted photography method demonstrated superior performance at wing measurement. Notably, this measurement method should be applicable to the measurement of other body parts of insects.

The performance of photography-based methods relies on the availability of image processing software. In fact, image processor-assisted data acquisition is the trend in research involving morphology characterization. To date, image processing software has been used across different biological fields such as dentistry, radiology and agriculture [19–23]. Due to its open-sourced nature, the application continues to expand via compatible plugins developed by its user-based community. The availability of these digital materials allows the establishment of artificial intelligence-based platforms that can perform automated identification of specimens

[24]. Hence, the photography-based measurement methods allow scientists to explore and discover more with the specimens studied. Importantly, user-friendly devices with reliable built-in photography feature potentiate the usefulness of such platform.

With a reliable measurement method for physical attributes of insects, the fitness of an insect population can be evaluated more reliably. For instance, when a new vector control strategy is implemented, the fitness of the targeted vector can be evaluated via measurement performed on key body parts such as the wings [11]. Besides, the efficacy of population displacement-based vector control strategies (such as the release of genetically or biologically modified mosquitoes) can be evaluated with a reliable wing measurement methods [2]. Despite being a simple technique, crucial information on the vector's fitness can be collected rapidly, allowing a reliable and quick glance into the degree of adaptation and dispersal by the newly released insects. As mentioned earlier, a larger wing size is correlated to a wider dispersal of vector [3,4]. Wing size comparison between the wild type vectors and the released biologically modified vectors will provide critical information to predict and monitor the success of such strategies. As wing size is associated with the flight distance of malaria vectors [25], a reliable wing measurement method may assist researchers to decipher the transmission picture of emerging zoonotic malaria, which is transmitted by simio-anthropophilic *Anopheles* mosquitoes in forested areas [26,27]. In addition, the availability of a reliable measurement method may facilitate the investigations of divergence evolution or development of geographic races in insect population [28], as well as morphometric-based classification of insects [29].

In conclusion, photography assisted by digital microscope delivered good performance at measuring the external morphological features of small organisms like insects. Besides, the good portability, user-friendly nature, and low operating cost of digital microscope allows this method to be applied in the field setting as well. Our study demonstrated improved accuracy of research enabled by the improved imaging technique and image processing technology. The findings here may inspire researchers to invest in additional equipment that is affordable and relatively accessible to collect important data. Such reliable and affordable research method will enable seamless data comparison among different research groups.

CRediT authorship contribution statement

Wenn-Chyau Lee: Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. Indra Vythilingam: Writing – review & editing, Resources, Funding acquisition. Yee-Ling Lau: Writing – review & editing, Supervision, Resources, Funding acquisition. Mohd Redzuan Ahmad Naziri: Writing – review & editing, Resources, Project administration. Meng Li Wong: Methodology, Investigation. Zulhisham Zulzahrin: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation

Funding

We would like to thank the Ministry of Higher Education, Malaysia, for the Fundamental Research Grant Scheme (FRGS/1/2022/SKK12/UM/02/9) awarded to WL. ZZ was also supported by FRGS/1/2022/SKK12/UM/02/9. MLW was supported by the Long-Term Research Grant Scheme (LRGS) also by the Ministry of Higher Education, Malaysia (LRGS/1/2018/UM/01/1/1). YL was supported by LRGS of the Ministry of Higher Education, Malaysia (LRGS/1/2018/UM/1/1/4). IV was supported by LRGS of the Ministry of Higher Education, Malaysia (LRGS/1/2018/UM/01/1/3). The funders had no role in the study design, data collection, analysis, decision to publish or preparation of the manuscript.

Data availability

The data associated with this study were not deposited into a publicly available repository. All data have been included in the text and supplementary files of this article. Raw data will be made available upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

We would like to thank Prof. Dr. Fong Mun Yik for the support and assistance provided during the execution of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e25207.

References

[1] A. Mohammed, D.D. Chadee, Effects of different temperature regimens on the development of *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes, Acta Trop. 119 (1) (2011 Jul) 38–43.
[2] H.L. Yeap, N.M. Endersby, P.H. Johnson, S.A. Ritchie, A.A. Hoffmann, Body size and wing shape measurements as quality indicators of *Aedes aegypti* mosquitoes destined for field release, Am Soc Trop Med Hyg [Internet] 89 (1) (2013) 78–92. Available from: https://www.ajtmh.org/view/journals/tpmd/89/1/article-p78.xml.
[3] R.S. Nasci, The size of emerging and host-seeking *Aedes aegypti* and the relation of size to blood-feeding success in the field, J. Am. Mosq. Control Assoc. 2 (1) (1986 Mar) 61–62.
[4] H. Briegel, Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*, J Insect Physiol [Internet] 36 (3) (1990) 165–172. Available from: https://www.sciencedirect.com/science/article/pii/002219109090118Y.
[5] D.P. Price, F.D. Schilkey, A. Ulanov, I.A. Hansen, Small mosquitoes, large implications: crowding and starvation affects gene expression and nutrient accumulation in *Aedes aegypti*, Parasit Vectors [Internet] 8 (1) (2015) 252, https://doi.org/10.1186/s13071-015-0863-9. Available from:.
[6] E.H. Jeffrey Gutiérrez, K.R. Walker, K.C. Ernst, M.A. Riehle, G. Davidowitz, Size as a proxy for survival in *Aedes aegypti* (Diptera: Culicidae) mosquitoes, J. Med. Entomol. 57 (4) (2020 Jul) 1228–1238.
[7] R.J. Wootton, K.E. Evans, R. Herbert, C.W. Smith, The hind wing of the desert locust (*Schistocerca Gregaria* Forskål): I. Functional morphology and mode of operation, J Exp Biol [Internet 203 (19) (2000 Oct 1) 2921–2931, https://doi.org/10.1242/jeb.203.19.2921. Available from:.
[8] S. Wecker, T. Hörnschemeyer, M. Hoehn, Investigation of insect morphology by MRI: assessment of spatial and temporal resolution, Magn. Reson. Imaging 20 (1) (2002 Jan) 105–111.
[9] P.J. DeVries, C.M. Penz, R.I. Hill, Vertical distribution, flight behaviour and evolution of wing morphology in Morpho butterflies, J Anim Ecol [Internet 79 (5) (2010 Sep 1) 1077–1085, https://doi.org/10.1111/j.1365-2656.2010.01710.x. Available from:.
[10] G.D. Powney, S.J. Brooks, L.J. Barwell, P. Bowles, R.N.L. Fitt, A. Pavitt, et al., Morphological and geographical traits of the british odonata, Biodivers. Data J. 2 (2014) e1041.
[11] M. Boumaza, B. Merabti, Y. Adjami, M.L. Ouakid, T.M. Carvajal, Geometric morphometric wing analysis of avian malaria vector, *Culiseta longiareolata*, from two locations in Algeria, Insects 13 (11) (2022 Nov).
[12] S.O. Weiland, M. Detcharoen, B.C. Schlick-Steiner, F.M. Steiner, Analyses of locomotion, wing morphology, and microbiome in *Drosophila nigrosparsa* after recovery from antibiotics, Microbiologyopen 11 (3) (2022 Jun) e1291.
[13] T. Ivorra, S.M. Khorri, R. Rahimi, C.C. Hoe, New developmental data of *Chrysomya megacephala* (Diptera: Calliphoridae) in tropical temperatures and its implications in forensic entomology, Trop. Biomed. 40 (1) (2023 Mar) 1–6.
[14] J.R. De La Torre Bueno, Practical and popular entomology.—No. 8.: a method of measuring insects, 2012/05/31, Can Entomol [Internet] 37 (8) (1905) 269. Available from: https://www.cambridge.org/core/article/practical-and-popular-entomologyno-8-a-method-of-measuring-insects/834FD04FFB10D442FC6C2A6C37048134.
[15] I.M. Soto, V.P. Carreira, E.M. Soto, E. Hasson, Wing morphology and fluctuating asymmetry depend on the host plant in cactophilic Drosophila, J Evol Biol [Internet] 21 (2) (2008 Mar 1) 598–609, https://doi.org/10.1111/j.1420-9101.2007.01474.x. Available from:.
[16] T. Chaiphongpachara, S. Laojun, Annual variability of wing morphology in *Culex sitiens* Wiedemann (Diptera, Culicidae) mosquito vectors from the coastal area of Samut Songkhram Province, Thailand, J Parasitol Res 2019 (2019) 3978965.
[17] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, Nat Methods [Internet] 9 (7) (2012) 671–675, https://doi.org/10.1038/nmeth.2089. Available from:.
[18] C. Lorenz, F. Almeida, F. Almeida-Lopes, C. Louise, S.N. Pereira, V. Petersen, et al., Geometric morphometrics in mosquitoes: what has been measured? Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis 54 (2017 Oct) 205–215.
[19] G. Polder, G. Blokker, G. van der Heijden, An ImageJ Plugin for Plant Variety Testing, 2012 Jan 1.
[20] S.J. Brookes, in: P. Papagerakis (Ed.), Using ImageJ (Fiji) to Analyze and Present X-Ray CT Images of Enamel BT - Odontogenesis: Methods and Protocols, Springer New York, New York, NY, 2019, pp. 267–291, https://doi.org/10.1007/978-1-4939-9012-2_26. Available from:.
[21] S. Hisham, N. Abdullah, M.H. Mohamad Noor, D. Franklin, Quantification of secondary dentin formation using dental orthopantomographs in a contemporary Malaysian population, Aust J Forensic Sci [Internet 51 (sup1) (2019 Jul 29) S180–S183, https://doi.org/10.1080/00450618.2019.1569130. Available from:.
[22] N. Andrialovanirina, D. Ponton, F. Behivoke, J. Mahafina, M. Léopold, A powerful method for measuring fish size of small-scale fishery catches using ImageJ, Fish Res [Internet] 223 (2020) 105425. Available from: https://www.sciencedirect.com/science/article/pii/S0165783619302802.
[23] S. Satir, M.H. Buyukcavus, K. Orhan, A novel approach to radiographic detection of bucco-palatal/lingual dilacerations: a preliminary study with ImageJ, Proc. Inst. Mech. Eng. Part H J Eng Med [Internet 235 (11) (2021 Jul 6) 1310–1314, https://doi.org/10.1177/09544119211030717. Available from:.
[24] M. Manderfield, Seek, Picture Insect, Google Lens: an Analysis of Popular Insect Identification Apps Using Photos of Realistic Quality, 2022.
[25] R. Faiman, A.S. Yaro, M. Diallo, A. Dao, S. Djibril, Z.L. Sanogo, et al., Quantifying flight aptitude variation in wild *Anopheles gambiae* in order to identify long-distance migrants, Malar J [Internet] 19 (1) (2020) 263, https://doi.org/10.1186/s12936-020-03333-2. Available from:.
[26] Y.L. Lau, W.C. Lee, J. Chen, Z. Zhong, J. Jian, A. Amir, et al., Draft genomes of *Anopheles cracens* and *Anopheles maculatus*: comparison of simian malaria and human malaria vectors in Peninsular Malaysia, PLoS One [Internet 11 (6) (2016 Jun 27) e0157893, https://doi.org/10.1371/journal.pone.0157893. Available from:.
[27] W.C. Lee, F.W. Cheong, A. Amir, M.Y. Lai, J.H. Tan, W.K. Phang, et al., *Plasmodium knowlesi*: the game changer for malaria eradication, Malar J [Internet] 21 (1) (2022) 140, https://doi.org/10.1186/s12936-022-04131-8. Available from:.
[28] A. Gafur, A. Ajizah, The use of female wing measurements for discrimination of *Aedes aegypti* (L.) (Diptera: Culicidae) populations from South Kalimantan, HAYATI J Biosci [Internet] 15 (1) (2008) 8–12. Available from: https://www.sciencedirect.com/science/article/pii/S1978301916302601.
[29] J.P. Martinet, H. Ferté, P. Sientzoff, E. Krupa, B. Mathieu, J. Depaquit, Wing Morphometrics of Aedes Mosquitoes from North-Eastern France, vol. 12, Insects, 2021.