Tropical Biomedicine 39(2): 226-230 (2022)
https://doi.org/10.47665/tb.39.2.008

RESEARCH ARTICLE

Potential application of Gustatory Receptor 1 (CmegGr1) gene as a molecular marker for identification of *Chrysomya megacephala* (Diptera: Calliphoridae)

Ghazali, S.N.A.[1], Emelia, O.[1], Hidayatulfathi, O.[2], Syamsa, R.A.[1]*

[1]Department of Parasitology and Medical Entomology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia
[2]Biomedical Science Programme, Diagnostic and Applied Health Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia
*Corresponding author: syamsarizal.abdullah@ppukm.ukm.edu.my

ARTICLE HISTORY

Received: 31 January 2022
Revised: 10 April 2022
Accepted: 10 April 2022
Published: 30 June 2022

ABSTRACT

Chrysomya megacephala larvae can easily be identified using cheap traditional microscopy techniques. Nevertheless, identification using taxonomy keys may be hampered, if the morphological characteristics of the larvae are incomplete, or immature for microscopic identification. To overcome the difficulty of species determination, molecular identification has gained relevance and is applied in forensic investigations. This study aimed to identify a novel target gene, known as the gustatory receptor 1 gene (CmegGr1), which has never been used for identification. The third instar larvae of *Ch. megacephala* (n = 30) and eight other forensically important fly species were obtained from two sources; rabbit carcasses and the Forensic Entomology Unit collection. Their DNAs were extracted and the CmegGr1 gene was amplified using polymerase chain reaction (PCR). The resulting sequences were subjected to phylogenetic analysis. A 209 bp fragment of the CmegGr1 gene was successfully amplified in 80% (24/30) of *Ch. megacephala* samples, while all of the non-*Ch. megacephala* species were not amplified. The phylogenetic analysis revealed that the evolutionary tree of CmegGr1 shares many traits with the 21a gustatory receptors of *Calliphora stygia* and *Lucilia cuprina* (Gr21a), which are also classified as necrophagous fly species. The high specificity of species identification was demonstrated in the present study using DNA barcoding, which led to the conclusion that the CmegGr1 gene could serve as an alternative marker for identifying *Ch. megacephala*.

Keywords: Forensic larvae; gustatory receptor 1 gene; *Chrysomya megacephala*; DNA identification; phylogenetic study.

INTRODUCTION

Blowflies play an important role in estimating minimum postmortem interval (PMI), because several species are often found in human corpses. In Malaysia, the most prevalent calliphorid species discovered in corpses are *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Macquart, 1843) (Diptera: Calliphoridae) (Syamsa *et al*., 2017). The strong association between *Ch. megacephala* larvae and decaying matter has led to its usefulness in assisting entomologists determine the time of death (Sukontason *et al*., 2008; Thevan *et al*., 2010). Its reputation as an early and dominant coloniser of corpses makes it a particularly attractive species for assisting forensic investigation (Kavitha *et al*., 2012; Syamsa *et al*., 2017).

The traditional identification of blowfly species in the field is based on morphological techniques that compare the differences in the adults, or matured larvae of forensic fly species across a wide range of features. However, the process of identifying and detecting this species is cumbersome, as the morphologies of the immature larvae are similar to other species. This problem can be overcome by rearing immature larvae until they are fully grown. However, in some cases, the larvae may end up dying, thus, hindering the identification process.

A significant number of studies that apply molecular approaches in identifying these flies has been conducted mainly by comparing known fly DNA sequences (Stojak, 2014). Previous studies have reported the potential of using a "barcoding region" within the mitochondrial DNA, namely, the cytochrome oxidase I (COI) gene, as a universal marker for molecular identification of the forensically important Diptera (Boehme *et al*., 2012). The COI gene has been established as a useful molecular marker in identifying blowfly species in Africa, Australia, Belgium, England, Germany, Lebanon, and Malaysia (Harvey *et al*., 2003; Boehme *et al*., 2010; Tan *et al*., 2010; Boehme *et al*., 2012; Jordaens *et al*., 2013; Shayya *et al*., 2018). However, the COI region was found to be inefficient in identifying *Ch. megacephala*, with recent studies demonstrating a possible hybridisation and an incomplete lineage sorting between this species and *Chrysomya saffranea* (Bigot, 1877) (Badenhorst & Villet, 2018). Therefore, it is crucial to find a new marker gene for this genus to avoid misinterpretations during DNA identification.

Published by Malaysian Society of Parasitology and Tropical Medicine.
All rights reserved.

The blowfly is naturally attracted to the odour of a decaying corpse and can reach one within minutes to lay eggs (Anderson, 2004; Wells & Stevens, 2008; Mahat & Jayaprakash, 2013; Mona *et al*., 2019). A gustatory gene in *Ch. megacephala*, which is classified under the chemoreceptor superfamily, is responsible for the sense of taste that corresponds to feeding behaviour, initiation of innate sexual responses, as well as reproductive responses (Sánchez-Gracia *et al*., 2009). According to Wang *et al*. (2013), the *Ch. megacephala* gustatory receptor 1 gene (CmegGr1) is highly expressed in the antennae and proboscises with maxillary palps in adult flies. As such, this is a promising target that could assist forensic scientists in identifying this species. The present study aimed to determine the potential of using the CmegGr1 gene as a suitable marker in the identification of *Ch. megacephala* larvae. This study has also compared the availability of this gene in a group of *Ch. megacephala* larvae and several forensically important larvae species.

MATERIALS AND METHODS

Sample collections

The third instar of *Ch. megacephala* larvae were collected from a simulation of rabbit carcasses at a forensic simulation site at Universiti Kebangsaan Malaysia (UKM), Selangor, Malaysia. The use of rabbit carcasses was approved by the UKM Ethics Committee (Approval No: PARAST/PP/2019/SYAMSA/30-JAN.2019-DEC.-2019-AR-CAT2.). The specimens were collected and directly preserved in 70% of ethanol. The collected samples were stored at 4°C in the Entomology Laboratory, Faculty of Medicine, UKM. All posterior and anterior spiracles were stored as vouchers.

DNA Extraction

The genomic DNA was extracted from 30 samples of *Ch. megacephala*, which consisted of 2–5 pooled larvae in each sample. Approximately 25 mg of body tissue samples were used for each extraction and the extracted DNAs were stored at -20°C. The genomic DNA was extracted using the QIAamp® DNA Mini Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions, with some modifications. After being incubated overnight in an ATL buffer, all samples were ground using sterile 1.5 mL tube plastic pestles. Next, the samples were incubated for 5 min at 56°C. The extracted DNA was then eluted with 200 µL of AE buffer, and eluted again after standing for 5 min in the AE buffer. The purity of the extracted DNA was quantified using a Nanodrop 2000c (Thermo Scientific®, USA) at an absorbance wavelength of A260/A280.

Polymerase Chain Reaction

The set of primers was designed using a Primer Explorer software (version 5) using the DNA template from *Ch. megacephala* gustatory receptor 1 (Gr1), with NCBI number of JQ365174.1. The purified DNA was subjected to PCR amplification of CmegGr1 gene following the protocol mentioned in Table 1. The target fragments were amplified with the primer pairs of CmegGr1-F/CmegGr1-B for CmegGr1 DNA fragment size of approximately 209 bp. The PCR mixtures contained ~150 ng DNA template, 1 unit of Taq DNA polymerase (Promega, USA), 1 × PCR reaction buffer (Promega, USA), 1.5 mM MgCl2 (Promega, USA), 200 µM of each dNTP (Promega, USA), 0.4 µM of each forward and reverse primer, and ddH2O to total up the mixture to 50 µL per reaction. The PCR was performed using an Eppendorf Mastercycler Pro Thermal Cycler. The temperature regime used was according to Tan *et al*. (2010) except for the annealing temperature which was optimised to be at 50°C for 1 min and 30 sec (Table 1). The amplified products were visualised by electrophoresis using 2% of agarose gel at 180 V for 30 min.

Sequence Analysis

Samples that showed positive results were sent for sequencing analysis using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) after undergoing purification using the Hiyield Plus Gel/PCR Mini Kit (Real Biotech Corp, Taiwan). Gene sequence analysis and database comparisons were performed using the BLAST programme (http://www.ncbi.nlm.nih.gov/blast/). Nucleic acid sequences of nine additional insect species have been retrieved from GenBank, as shown in Table 2. All sequences were aligned and trimmed using the BioEdit Sequence Alignment Editor, version 7.2.6 (Hall, 1999). Samples and database sequences were used to construct a phylogenetic tree to illustrate the relationships of each gene between species trees. The evolutionary tree was inferred using the Maximum-likelihood method with 1000 bootstrap replicates.

Specificity test

PCR was performed on the other species of larvae using a similar protocol used for CmegGr1 (Table 1). DNA isolated from *Ch. megacephala* was used as a positive control. The non-*Ch. megacephala* species were selected based on the availability of sources from the laboratory collections, namely, *Chrysomya rufifacies*; *Chrysomya nigripes* Aubertin, 1932; *Chrysomya villeneuvi* Patton, 1922; *Sarcophaga* spp., *Synthesiomyia nudiseta* Wulp, 1883; *Hypopygiopsis violacea* (Macquart, 1835); *Hemipyrellia ligurriens* (Wiedemann, 1830); and *Musca domestica* Linnaeus, 1758.

RESULTS

Detection of CmegGr1 from larvae

From the 30 samples of *Ch. megacephala* larvae, 80% (24 samples) were detected with the CmegGr1 gene (Figure 1). The DNA sequence analysis revealed that the extracted samples from the larvae contain 96% to 98% DNAs that are similar to the DNA of the adult CmegGr1 gene (see Figure 2A). Figure 2B shows that the phylogenetic tree of CmegGr1 has close characteristics as the 21a gustatory receptors (Gr21a) of *Calliphora stygia* and *Lucilia cuprina*.

Table 1. The sequenced primer used in this study and the temperature set for thermocycler

Primer	Sequences (5'-3')	Temperature
CmegGr1-F	CACCACTTAAGATACCTCCT	Pre-denaturation: 94°C [5 minutes] Denaturation: 94°C [1 minute] Annealing: 50°C [1 min 30 sec] Extension: 72°C [2 minutes] Final extension: 72°C [5 minutes] Hold: 4°C [∞]
CmegGr1-B	TACGAGCAAACTTTTGGTAG	

Table 2. GenBank ID used for sequence analysis

GenBank ID	Species	Country
MH750487.1	*Drosophila mojavensis*	United States of America
MH750458.1	*Drosophila arizonnae*	United States of America
MH750483.1	*Drosophila navojoa*	United States of America
AB042625.2	*Drosophila melanogaster*	Japan
KR674136.1	*Athetis dissimilis*	China
XM013241847.1	*Stomoxys calcitrans*	United States of America
JQ36177.1	*Musca domestica*	United States of America
XM005189934.2	*Musca domestica*	United States of America
KJ702098.1	*Calliphora stygia*	Australia
XM023443869	*Lucilia cuprina*	United States of America
JQ365174.1	*Chrysoma megacephala*	China

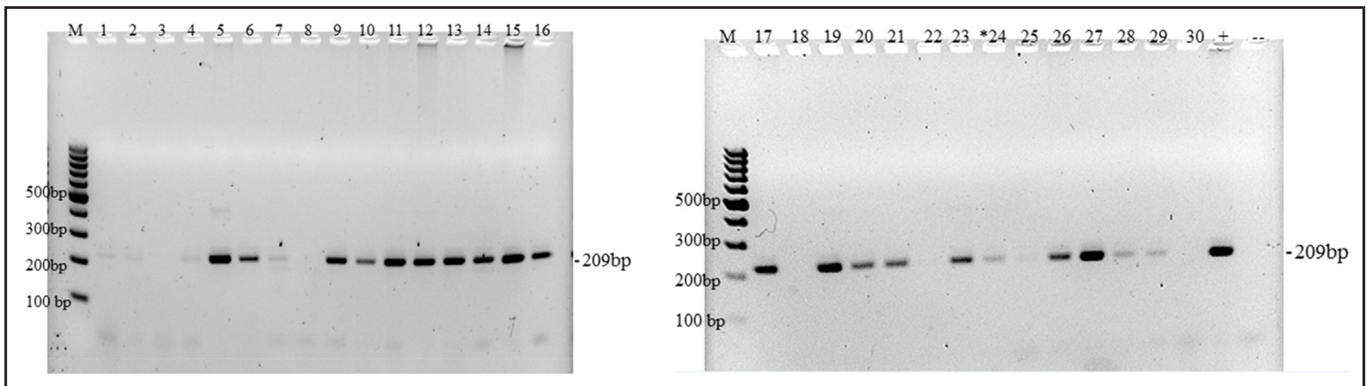

Figure 1. The electrophoresis gel analysis on 30 samples of *Ch. megacephala*'s larvae. The results showed that most of the larvae expressed CmegGr1 gene [100 bp M-DNA ladder; 1-30: *Ch. megacephala*'s larvae sample number; + : positive control (DNA of *Ch. megacephala*); - : negative control (ddH2O)].

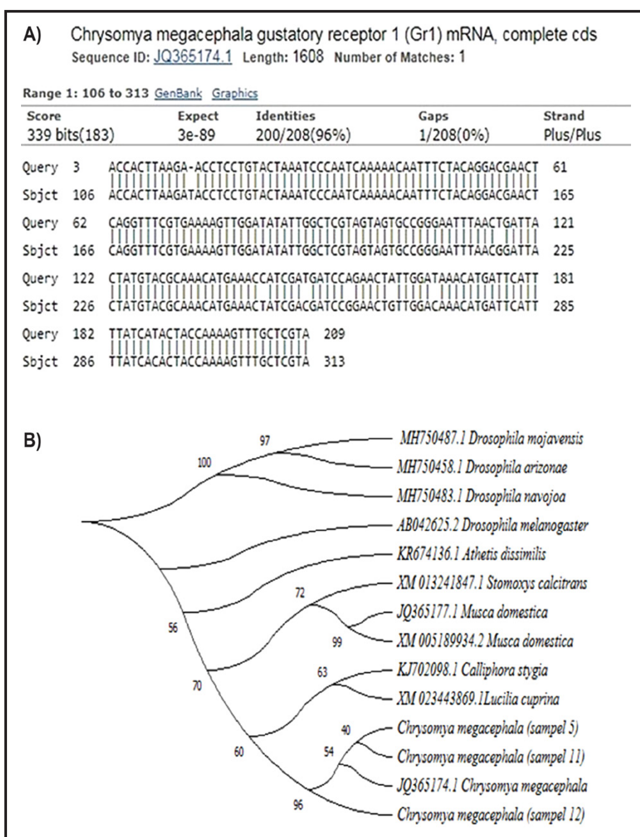

Figure 2. The sequence analysis results: A) from BLAST; and B) from the MEGA software. The extracted DNA showed 96% similar identities with *Ch. megacephala* gustatory receptor 1 (Gr1), with NCBI number: JQ365174.1 only, because there were no other hit sequences other than this. The maximum parsimony tree on the right side of the figure shows that CmegGr1 has a close relationship with two forensic flies, which are *Calliphora stygia* and *Lucilia cuprina.* The bootstrap values were located at each node.

Detection of CmegGr1 in different species of larvae

Figure 3 illustrates the specificity results of CmegGr1 towards *Ch. megacephala* species only. CmegGr1 was exclusively present in *Ch. megacephala* samples, but not in the other necrophagous forensic larvae. All samples from *Ch. rufifacies*, *Ch. nigripes*, *Ch. villeneuvi*, *Sarcophaga* sp., *Synthesiomyia nudiseta*, *Hypopygiopsis violacea*, *Hemipyrellia ligurriens*, and *Musca domestica* were found to be absent of CmegGr1.

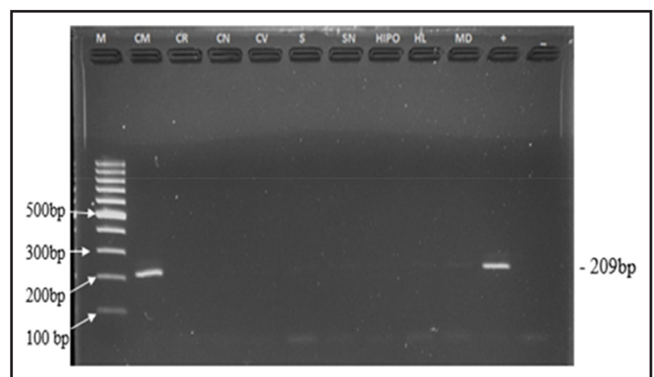

Figure 3. The electrophoresis gel analysis of CmegGr1. Specificity test using different species of forensic larvae. [100 bp M- DNA ladder; CM: *Ch. megacephala*; CR: *Ch. rufifacies*; CN: *Ch. nigripes*; CV: *Ch. villeneuvi*; S: *Sarcophaga* sp.; SN: *Synthesiomyia nudiseta*; HIPO : *Hypopygiopsis violacea*; HL: *Hemipyrellia ligurriens*; MD: *Musca domestica*; + : positive control (DNA of *Ch. megacephala*); - : negative control (ddH2O)].

DISCUSSION

In the context of using molecular techniques for identifying necrophagous larvae, it is crucial to choose an appropriate DNA target to facilitate and complement the morphology-based species identification. In general, the COI gene, which consists of ~1588 bp nucleotides, is the most common target amplicon used for molecular identification of the *Ch. megacephala* (Nelson *et al*., 2012; Sharma & Singh, 2015). However, previous studies found that the COI gene was not specific for the detection of *Ch. megacephala* (Kavitha *et al*., 2012; Badenhorst & Villet, 2018). Although this region is used extensively, the lack of amplification specificity against other carrion-breeding blowflies, such as *Chrysomya pinguis* (Walker, 1858), *Chrysomya bezziana* Villeneuve, 1914, *Chrysomya pacifica* Kurahashi, 1991, and *Chrysomya chani* Kurahashi, 1979 highlights the shortcoming of this sequence for exclusively identifying *Ch. megacephala* (Kavitha *et al*., 2012; Bharti & Singh, 2017; Pedales & Fontanilla, 2018).

Thus, the current study has focused on identifying a potential gene marker, as an alternative for the current use of barcoding markers. A new set of primers targeting the specific amplification of CmegGr1 gene was designed. The PCR results in this study showed a high specificity (100%) of the gene in distinguishing *Ch. megacephala* apart from other fly species. Hence, the application of CmegGr1

as a specific genetic marker could be used to specifically identify this fly species from a sample containing a large species of forensic flies. Nevertheless, the detection of this gene was noticeably less sensitive (80%) when the conventional PCR method was used, as it originates from nuclear markers, which only have a few copies within the blowfly's genome (Mona *et al*., 2019). Fortunately, this shortcoming can be circumvented by utilising other methods, such as real-time PCR or microarrays, which are known to possess higher sensitivity.

The phylogenetic analyses revealed that the 209 bp of CmegGr1 gene could differentiate *Ch. megacephala* from other species, as the sequence did not hybridise with the gustatory receptors of other insects. This study also found that the closest molecular characteristics of CmegGr1 were connected to the 21a gustatory receptors of *Calliphora stygia* and *Lucilia cuprina*, namely, CstyGr21a and LcupGr21a. The molecular analysis conducted by Leitch *et al*. (2015) also showed that CmegGr1 has molecular characteristical connections with CstyGr21a and the gustatory gene from *Drosophila melanogaster* Meigen, 1830, which is known as DmelGr21a. These receptors were found to be important carbon dioxide receptors, which might function as one of the important receptors for detecting volatile organic compounds for *Ch. megacephala.* Wang *et al*. (2013) have also determined the function of CmegGr1 gene extracted from adult *Ch. megacephala* as being one of the carbon dioxide receptors. Carbon dioxide is a type of odorant, cuticular hydrocarbon gas, which is highly volatile and important as a long-distance cue for carrion detection (Yang & Shiao, 2012; Dong *et al*., 2016). Interestingly, carbon dioxide is the main gas produced by a carcass at 80%, followed by hydrogen at 10% at a constant temperature of 25°C (Sakata *et al*., 1980).

The species-specific characteristics of carbon dioxide receptors in other fly species and the selection of partial CmegGr1 gene as an amplification target in this study have contributed to the high specificity in molecular identification of *Ch. megacephala*. The location of the amplicon was at the N-terminal of the protein and at intracellular (Wang *et al*., 2013). As the location of the sequences was not at transmembrane, the amino acids can easily adapt to the selective preference of the species. According to Robertson and Kent (2009), some insects were born without carbon dioxide receptors (e.g., honeybee, parasitoid wasp, human louse, pea aphid, water flea, and blacklegged tick), while others have carbon dioxide receptors (e.g., *Anopheles gambiae, Aedes aegypti*, and *Culex pipiens*). The behaviour of these insects that are attracted to either humans or corpses was paralleled to the presence of carbon dioxide receptors.

In addition, a study by Sánchez-Gracia *et al*. (2009) supported that most gustatory receptor families are unique and species-specific based on the evolutionary genom of chemosensory gene families using data from fully sequenced insect genomes from the 12 newly available Drosophila genomes. The sequence alignment of the gustatory receptors of *Ch. megacephala* has shown that the CmegGr1 and CmegGr2 amino acid sequences had different lengths in their N- and C-terminal regions compared to other insect species, thus, providing distinguishing points for *Ch. megacephala* against other insects (Wang *et al*., 2013). This observation supports the results obtained in this study. Overall, the gustatory gene has shown high specificity for insect identification through molecular detection methods and has the potential as a reliable marker for genetic analysers in the fieldwork.

This study has highlighted the exclusivity of CmegGr1 gene as a new identification marker for *Ch. megacephala,* which can be beneficial in forensic investigations. The uniqueness, high specificity, and expression of the gene at the immature stages of the fly enables it to be further developed as a diagnostic marker to ease the identification of *Ch. megacephala* at forensic sites. This study can provide validation for future studies to reinvestigate similar genes in other species.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Faculty of Medicine, Universiti Kebangsaan Malaysia for allowing the use of their research facilities and for funding this project under grant number GGPM-2017-047. In addition, special thanks to Dr. Wong Kon Ken from the Department of Medical Microbiology and Immunology, Faculty of Medicine, UKM for assisting in perming the phylogenetic analysis, and for guiding the authors in interpreting the results.

Conflict of Interests

The authors declare that they have no conflict of interests. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

REFERENCES

Anderson, G.S. (2004). Determining time of death using blow fly eggs in the early postmortem interval. *International Journal of Legal Medicine* **118**: 240-241. https://doi.org/10.1007/s00414-004-0443-6

Badenhorst, R. & Villet, M.H. (2018). The uses of *Chrysomya megacephala* (Fabricius, 1794)(Diptera: Calliphoridae) in forensic entomology. *Forensic Sciences Research* **3**: 2-15. https://doi.org/10.1080/20961790.2018.1426136

Bharti, M. & Singh, B. (2017). DNA-based identification of forensically important blow flies (Diptera: Calliphoridae) from India. *Journal of Medical Entomology* **54**: 1151-1156. https://doi.org/10.1093/jme/tjx084

Boehme, P., Amendt, J., Disney, R.H.L. & Zehner, R. (2010). Molecular identification of carrion-breeding scuttle flies (Diptera: Phoridae) using COI barcodes. *International Journal of Legal Medicine* **124**: 577-581. https://doi.org/10.1007/s00414-010-0429-5

Boehme, P., Amendt, J. & Zehner, R. (2012). The use of COI barcodes for molecular identification of forensically important fly species in Germany. *Parasitology Research* **110**: 2325-2332. https://doi.org/10.1007/s00436-011-2767-8

Dong, J., Song, Y., Li, W., Shi, J. & Wang, Z. (2016). Identification of putative chemosensory receptor genes from the *Athetis dissimilis* antennal transcriptome. *PLoS ONE* **11**: e0147768. https://doi.org/10.1371/journal.pone.0147768

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

Harvey, M.L., Mansell, M.W., Villet, M.H. & Dadour, I.R. (2003). Molecular identification of some forensically important blowflies of southern Africa and Australia. *Medical and Veterinary Entomology* **17**: 363-369. https://doi.org/10.1111/j.1365-2915.2003.00452.x

Jordaens, K., Sonet, G., Richet, R., Dupont, E., Braet, Y. & Desmyter, S. (2013). Identification of forensically important Sarcophaga species (Diptera: Sarcophagidae) using the mitochondrial COI gene. *International Journal of Legal Medicine* **127**: 491-504. https://doi.org/10.1007/s00414-012-0767-6

Kavitha, R., Nazni, W.A., Tan, T.C., Lee, H.L., Isa, M.N. & Sofian-Azirun, M. (2012). Molecular identification of blow flies recovered from human cadavers during crime scene investigations in Malaysia. *Malaysian Journal of Pathology* **34**: 127-132.

Leitch, O., Papanicolaou, A., Lennard, C., Kirkbride, K.P. & Anderson, A. (2015). Chemosensory genes identified in the antennal transcriptome of the blowfly *Calliphora stygia*. *BMC Genomics* **16**: 255. https://doi.org/10.1186/s12864-015-1466-8

Mahat, N.A. & Jayaprakash, P.T. (2013). Forensic Entomology in Malaysia: A Review. *Malaysian Journal of Forensic Science* **4**: 1-6.

Mona, S., Khalid, M., Jawad, M., Noreen, S. & Rakha, A. (2019). Forensic entomology: A comprehensive review. *Advancements in Life Sciences* **6**: 48-59.

Nelson, L.A., Lambkin, C.L., Batterham, P., Wallman, J.F., Dowton, M., Whiting, M.F., Yeates, D.K. & Cameron, S.L. (2012). Beyond barcoding: A mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). *Gene* **511**: 131-142. https://doi.org/10.1016/j.gene.2012.09.103

Pedales, R.D.C. & Fontanilla, I.K.C. (2018). Forensic entomology in the Philippines: establishing baseline data on the forensically important blow fly species *Chrysomya megacephala* (Fabricius, 1794). *Philippine Journal of Science* **147**: 17-25.

Robertson, H.M. & Kent, L.B. (2009). Evolution of the gene lineage encoding the carbon dioxide receptor in insects. *Journal of Insect Science* **9**: 9-19. https://doi.org/10.1673/031.009.1901

Sakata, M., Miki, A., Kazama, H., Morita, M. & Yasoshima, S. (1980). Studies on the composition of gases in the post-mortem body: animal experiments and two autopsy cases. *Forensic Science International* **15**: 19-29. https://doi.org/10.1016/0379-0738(80)90191-7

Sánchez-Gracia, A., Vieira, F.G. & Rozas, J. (2009). Molecular evolution of the major chemosensory gene families in insects. *Heredity* **103**: 208-216. https://doi.org/10.1038/hdy.2009.55

Sharma, S. & Singh, D. (2015). Mitochondrial DNA of two forensically important species of Chrysomya (Diptera: Calliphoridae) from India. *Oriental Insects* **48**: 316–321. https://doi.org/10.1080/00305316.2015.1013181

Shayya, S., Debruyne, R., Nel, A. & Azar, D. (2018). Forensically relevant blow flies in Lebanon survey and identification using molecular markers (Diptera: Calliphoridae). *Journal of Medical Entomology* **55**: 1113-1123. https://doi.org/10.1093/jme/tjy068

Stojak, J. (2014). Use of molecular methods in the identification of the species, age and sex of insects useful in forensic entomology. *Problemy Kryminalistyki* **286**: 66-69. http://doi.org/10.34836/pk.2014.286.4

Sukontason, K., Piangjai, S., Siriwattanarungsee, S. & Sukontason, K.L. (2008). Morphology and developmental rate of blowflies *Chrysomya megacephala* and *Chrysomya rufifacies* in Thailand: application in forensic entomology. *Parasitology Research* **102**: 1207-1216. https://doi.org/10.1007/s00436-008-0895-6

Syamsa, R.A., Omar, B., Ahmad, F.M.S., Hidayatulfathi, O. & Shahrom, A.W. (2017). Comparative fly species composition on indoor and outdoor forensic cases in Malaysia. *Journal of Forensic and Legal Medicine* **45**: 41-46. https://doi.org/10.1016/j.jflm.2016.12.002

Tan, S.H., Rizman-Idid, M., Mohd-Aris, E., Kurahashi, H. & Mohamed, Z. (2010). DNA-based characterisation and classification of forensically important flesh flies (Diptera: Sarcophagidae) in Malaysia. *Forensic Science International* **199**: 43-49. https://doi.org/10.1016/j.forsciint.2010.02.034

Thevan, K., Ahmad, A.H., Md. Rawi, C.S. & Singh, B. (2010). Growth of *Chrysomya megacephala* (Fabricius) maggots in a morgue cooler. *Journal of Forensic Sciences* **55**: 1656-1658. https://doi.org/10.1111/j.1556-4029.2010.01485.x

Wang, X., Zhong, M., Liu, Q., Aly, S.M., Wu, C. & Wen, J. (2013). Molecular characterization of the carbon dioxide receptor in the oriental latrine fly, *Chrysomya megacephala* (Diptera: Calliphoridae). *Parasitology Research* **112**: 2763-2771. https://doi.org/10.1007/s00436-013-3410-7

Wells, J.D. & Stevens, J.R. (2008). Application of DNA-based methods in forensic entomology. *Annual Review of Entomology* **53**: 103-120. https://doi.org/10.1146/annurev.ento.52.110405.091423

Yang, S.T. & Shiao, S.F. (2012). Oviposition preferences of two forensically important blow fly species, *Chrysomya megacephala* and *C. rufifacies* (Diptera: Calliphoridae), and implications for postmortem interval estimation. *Journal of Medical Entomology* **49**: 424-435. https://doi.org/10.1603/ME11133