Geographical Characteristics of *Chrysomya bezziana* Based on External Morphology Study

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ABSTRAK

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Identifikasi berdasarkan morfologi karakter eksternal *Chrysomya bezziana* merupakan tahap penting untuk mengevaluasi keberhasilan program pemberantasan penyakit myiasis dengan tehnik pemandulan lalat. Kendati demikian, variasi geografi lalat *C. bezziana* masih menjadi kontroversial. Tujuan penelitian ini adalah untuk mengetahui pengaruh teknik penyimpanan sampel terhadap visualisasi morfologi karakter eksternal dan menganalisis variasi geografi populasi lalat tersebut di sepanjang daerah penyebarannya. Sebanyak 88 lalat yang berasal dari 7 populasi Indonesia, 2 populasi Afrika dan masing-masing 1 populasi dari Oman, India, Malaysia dan Papua New Guenia (PNG) digunakan pada studi ini. Larva lalat dikoleksi dari kasus myiasis alami dan dipelihara di laboratorium hingga menjadi lalat dewasa. Sampel disimpan dalam etanol 80% (penyimpanan basah) dan pin (penyimpanan kering). Sepuluh karakter eksternal dari kepala dan tubuh lalat digunakan sebagai parameter. Data diuji menggunakan analisis prinsip komponen and hirarki kelompok dalam program UNISTAT®. Adapun jarak antar kelompok dianalisis menggunakan UPGMA. Hasil penelitian menunjukkan bahwa metode pin (penyimpanan kering) memberikan visualisasi yang lebih jelas daripada metode 80% etanol (penyimpanan basah). Berdasarkan karakter eksternal, populasi *C. bezziana* terbagi dalam dua ras, yaitu ras Afrika dan ras Asia. Tidak ada variasi geografi untuk populasi Indonesia, kecuali populasi PNG yang membentuk kelompok sendiri.

Kata Kunci: Myiasis, *Chrysomya bezziana*, Morfologi, Variasi Geografi

ABSTRACT

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Correct identification of *Chrysomya bezziana* is a fundamental step to evaluate the successfulness of the eradication program based on Sterile Insect Techinque (SIT). However, geographical variation of the fly is being controversial among scientists. The aim of the study was to investigate the effect of preservation method on visualisation of characters of external morphology and to analyse geographical variation of *C. bezziana* populations throughout their distribution regions. A total of 88 flies collected from 7 populations in Indonesia, 2 populations in Africa and each 1 population from Oman, India, Malaysia and Papua New Guinea (PNG) were tested in the study. All larvae were removed from natural myiasis cases. The larvae were reared at laboratory until they became adult flies. The samples were preserved into two methods, wet (80% ethanol) and dried (pin) methods. Ten external characters of head and body were observed. Data were subjected to principal components and hierarchical cluster analyses in UNISTAT® software. The Euclid distance measure was used for the cluster analysis, and the linking method used was the unweighted pair-group method using arithmetic average (UPGMA), to compute the distance between clusters. Results indicated that dried preservation (pinned samples) provided better external character than ethanol preservation. Based on external morphology of adult stages revealed that *C. bezziana* occurs as two geographical races, African and Asian races. There was no geographical variation of *C. bezziana* throughout Indonesian archipelago, except for the population from PNG forming its own cluster.

Key Words: Myiasis, *Chrysomya bezziana*, Morphology, Geographical Variaton

INTRODUCTION

The Dipteran larvae of the Old World screwworm fly (OWSF), *Chrysomya bezziana*, cause myiasis and remain a major problem over African and Asian regions, including the archipelago country of Indonesia. The major negative impact of OWSF is on the livestock industry sector, because infestations can lead to abortion, reduction of milk production and losses in terms of weight gain, fertility, low meat quality and even mortality leading to substansial economic losses including costs of veterinarian fees and treatments

(TRAVERSA and OTRANTO, 2006). Many efforts to deal with screwworm myiasis have been conducted, but they have produced various results. The Sterile Insect Technique (SIT) is a promising programme to eradicate pest insects. The program was successfully applied to eradicate a primary myiasis agent, *Cochliomya hominivorax*, from the North American continent and more recently from Libya and from most countries of Central America. Trials in Papua New Guinea (PNG) revealed that the SIT should also be effective against *C. bezziana* (SPRADBERY *et al*., 1989; SPRADBERY, 1990; MAHON, 2002).

Correct identification of the Old World screwworm fly is a fundamental step in implementation of control and eradication programmes against it, such as SIT (IAEA/FAO, 2000). Misidentification could cause expensive and time consuming consequences due to misinterpretation of the efficiency of the eradication programme or surveillance (KIM *et al.*, 2009). Hall (2008) stated that identification of traumatic myiasis agents based on the morphology of eggs and first instar larvae is difficult because there are few good characters visible by light microscopy and because these stages live for only a relatively short time in the wound and, therefore, are seldom found in myiasis cases.

Mostly, the diagnosis of traumatic myiasis is focused on identification of morphological characters of the second and third instar larvae in the wound. These larval stages are more often and more easily found in the wound and they are most apparent to livestock owners and veterinary personnel (HALL, 2008). SUKONTASON *et al*. (2006) provided a key to identify the third instar larvae of *C. bezziana* from other blow flies such as *C. rufifacies, L. cuprina, C nigripes* and *C. megacephala*. In addition, they also observed the ultra structure of puparium of *C. bezziana* using both light microscopy (LM) and scanning electron microscopy (SEM). The investigation provided fine details of the palmate anterior spiracles, which clearly contain perforated sieve plates within that are covered with antler-like projections.

Contrary to the larvae, adult flies are rarely found around the wound or livestock housing. The females do come to livestock either to feed, when they require a protein source (e.g. blood or wound fluids), or to lay their eggs, usually on the edges of wounds. In contrast, males are usually found around vegetation (SPRADBERY, 1991; THOMAS, 1993). The identification of adults is required for flies captured in survey and monitoring traps. *Chrysomya bezziana* can be distinguished from other *Chrysomya* species by a combination of yellow face (rather than white), black-brown to dark anterior thoracic spiracles (rather than white) and waxy-white lower squamae rather than blackish-brown to dirty-grey (SPRADBERY, 1991).

A good knowledge of the morphology of adult screwworm flies not only can contribute to the effectiveness of a monitoring or surveillance program, but it also has potential to discriminate between geographical populations of the flies and, thereby, to assist in identifying the origin of flies in cases of introductions. Morphology studies of *C. bezziana* related to geographical variation of the species have been undertaken and produced various contrasting results. D. H. Colless described three geographical races of *C. bezziana* (Southeast Asia, Arabia and Africa races) according to external characters of the head, wings and body (SPRADBERY, 1991). By using diagnostic keys of the morphology of adult flies based on such races, a myiasis outbreak in Southwestern Iran was believed to have been introduced from Arabian countries of the Gulf region, such as Saudi Arabia, because morphological evidence demonstrated that *C. bezziana* populations causing myiasis in Iran were more similar to the putative Arabian race rather than to populations from southeast Asia and Africa (NAVIDPOUR *et al*., 2009). However, contrary to that result, HALL *et al*. (2001) found no evidence for such races, but some evidence that *C. bezziana* from PNG differed from flies from Southeast Asia, the Gulf region and South Africa, using the same characters as D.H. Colless and with 2 extra body characters.

The aim of the study is to determine the value of external morphological characters in defining geographical variation of *C. bezziana* populations in Indonesia, compared to the Gulf region, mainland Asia and African populations.

MATERIALS AND METHODS

Chrysomya bezziana specimens

Adults of Indonesian populations of *C. bezziana* used in this study were collected from West Sumatra (Agam), Southeast Sumatra (Lampung) South Sulawesi (Makassar), East Nusa Tenggara (East Sumba) and Banten (Lebak). In addition, adult flies were taken from the Indonesian Research Centre for Veterinary Science (IRCVS) laboratory colony, originally collected near Bogor, West Java. Other samples were obtained from the national collections of the Natural History Museum, London, collected from South Africa, Zimbabwe, Oman, Malaysia and PNG. The Indian adults were kindly provided by the Department of Primary Industry, Australia. In total, 88 adult flies were used in the morphology study, 36 females and 52 males (Table 1).

Medias for culturing of *C. bezziana* larvae

Adults of *C. bezziana* were obtained from larvae removed from natural wounds on livestock and reared in the laboratory using SUKARSIH *et al*. (2000) recipe, with a small modification made by replacing the dried blood with fresh beef blood collected from an abattoir, including adjusting the amount of each ingredient (Table 2). All ingredients were homogenized using a blender until they came to a gel-like consistency.

Larval and fly rearing procedure

Larvae removed from the natural wound were transferred into the artificial media. The larvae were reared at the temperature (30-32^{o}C) and relative humidity (75-80%) with low light intensity. After three to five days, the larvae became pupae and emerged as adult flies after being incubated for about seven days. The emerged adult fly room were transferred to another room and reared at ambient room temperature with the same relative humidity (75-80% RH). The room was darkened by placing UV car window film, about 40% light transmission, over the windows (SUKARSIH *et al.*, 2000).

There are two standard methods for preservation of adult flies, dried and wet preservation (Table 1). For wet preservation method, adult flies were starved for 24 hours to prevent contamination with digestion products. They were killed by immersing them alive into 80% ethanol (HALL *et al.*, 2001).

Table 1. Numbers of dry preservation (DP) and wet preservation (WP) specimens based on location sampled

Location	Number of specimens		Collector
	DP	WP	
South Africa	4	5	DP = Spradbery/NHM; WP = Hulley
Zimbabwe	-	3	WP =Malilangwe
Oman	4	-	DP = Spradbery/NHM
India	-	4	WP = Urech/DPI Australia
Malaysia	4	-	DP = Spradbery/NHM
Agam, West Sumatra	-	8	WP = Wardhana
Lampung, South Sumatra	1	-	WP = Wardhana
Banten, West Java	3	9	DP = Wardhana; WP = Wardhana
Bogor, West Java	-	9	WP = Wardhana
Gunung Kidul, Central Java	-	2	WP = Titik
Makassar, South Sulawesi	10	4	DP = Wardhana; WP = Wardhana
Matowai Maringu, East Sumba	2	8	DP = Wardhana; WP = Wardhana
PNG	8	-	DP = Spradbery/ NHM
Total	36	52	

Table 2. Ingredients of modified Larvae Rearing Media

Ingredients	Amount	Supplier
Fresh beef blood	450 g	Local
Low-fat milk powder	45 g	Local
Whole egg powder	45 g	Sunny Queen Products, Brisbane, Australia
Water lock® super-absorbent polymer gel	18 g	Grain Processing Corp. Muscaline, IA
Formalin 10 %	1 ml	AR Grade
Distilled water	645 ml	Local

Preservation of samples

The dried preservation specimens were pinned adult flies. After being starved for 24 hours, the flies were killed by placing in a freezer (-20^{o}C). When they were dead, they were pinned through the thorax, and then placed into a storage box until the morphological observation was conducted.

Character discrimination

The morphological characters examined in this study were adopted from HALL *et al.* (2001) with some additional scoring in body colour, lower squama and posterior setulae on subcostal sclerite (showed in highlight in Table 2; Figure 1).

Tabel 2. Diagnostic characters of *C. bezziana* and their scoring in the morphology study based on HALL *et al.*, 2001. Character states highlighted were added in this study

Character	Character score						
	0	1	2	3	4	5	6
BC = Body Colour	-	Blue	Blue/green	Green	Blue/black	Green/black	Blue/purple
AB = Terminal bands on tergite	Absent	Obscure	Apparent	Distinct	-	-	-
WB = Wing base & cell R	-	Completely clear	Base & cell R slightly darkened	Base & cell R strongly darkened	-	-	-
TP = Soft hairs of thoracic pleura	-	All plae	Mixed pale & dark	All dark	-	-	-
LS = Lower squama	-	Pale + white pigmented + long white hairs	Clear white + 'variable' hairs*	Darker waxy white, pigmented along outer edge + black hairs	Darker waxy white + no pigmented edge + mix white & black hairs	-	-
FB = Frontal setular bristles	-	Pale except for dorsal patch	Lower 2/3 mixed pale & dark	All dark	-	-	-
VS = Sentulae around the vibrissa	-	All pale	A few dark immediately around the virbrissa	Dark extending around the vibrissa	-	-	-
GG = Genal groove	-	Only slightly intended	Normal/average indentation	Heavily identended	-	-	-
SC = Anterior setulae on subcostal sclerite	-	Patch of 'ginger' setulae	Mixed dark & 'ginger' setulae	Patch of dark setulae	-	-	-
PC = Posterior setulae on subcostal sclerite	Ginger'	One or two dark setulae	One/two/three ginger setulae	-	-	-	-

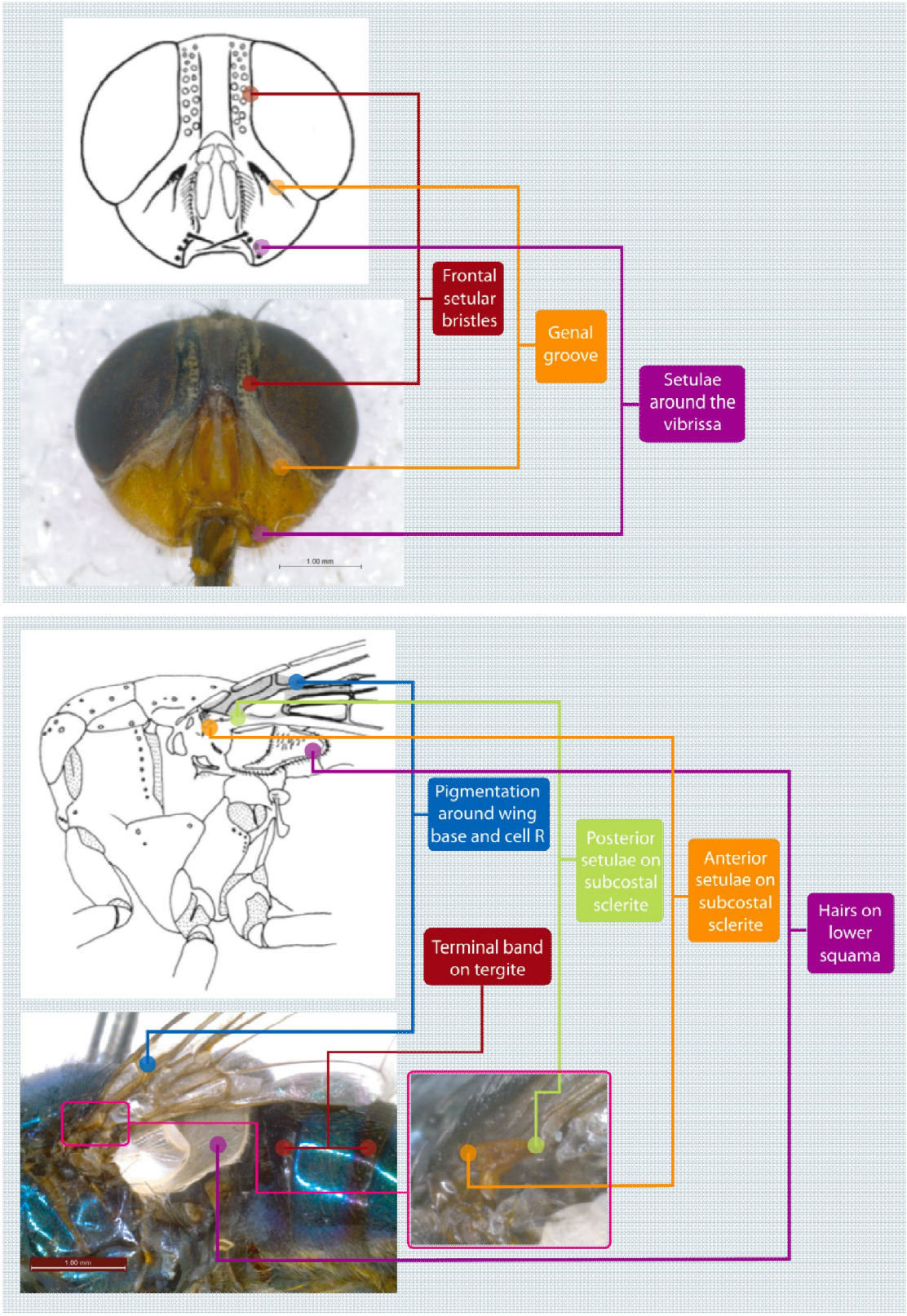


Figure 1. Characters used in external morphology analysis: head, thorax and wing characters

For wet specimen, the flies investigated were individually put into a cavity block containing 80% ethanol and their characters were observed under a microscope (x50 magnification). After that, the individual fly was placed into an Eppendorf (1.5 ml) tube labelled with the number of the specimen, its location, sex and collector.

For dried specimens, the individual fly was directly observed on the pin by adjusting its orientation under a microscope (x 50 magnification) to see the characters from the most suitable angle. A card label with the number of the specimen, its location, sex, and collector was pinned with the specimen together with any previous labels.

Statistical analysis

All scorings of morphological data were entered into 2008 spreadsheet. Principal components analysis and hierarchical cluster analysis were performed using UNISTAT® software. The Euclid distance measure was used for the cluster analysis, and the linking method used was the unweighted pair-group method using arithmetic average (UPGMA), to compute the distance between clusters. This clustering grouped taxa according to overall similarity and the results were presented in a dendrogram. This analysis is able to determine whether samples of specimens comprise one or more distinct entities (operational taxonomic units, OTUs) (QUICKE, 1993), which was the overall objective of the study.

RESULTS AND DISCUSSION

When a principal components analysis was undertaken to compare the effects of the two different preservation methods on scoring of the characters of adult males of *C. bezziana*, it was clear that there was indeed a subtle impact of preservation (Figure 2). Based on the first two components, approximately half of the specimens fell into a zone of overlap between the two populations (22/45 = 48.9%). However, the other half of the specimens (23/45 = 51.1%) was in two zones of clear separation, containing specimens that were either only pinned (5/17 = 29.4% of pinned specimens) or only ethanol preserved (18/28 = 64.3% of ethanol preserved specimens). Therefore, to avoid any confounding effects due to preservation, all further analyses of treated pinned and ethanol preserved specimens was done separately, in addition to the separation of males and females that followed the methodology of HALL *et al.* (2001).

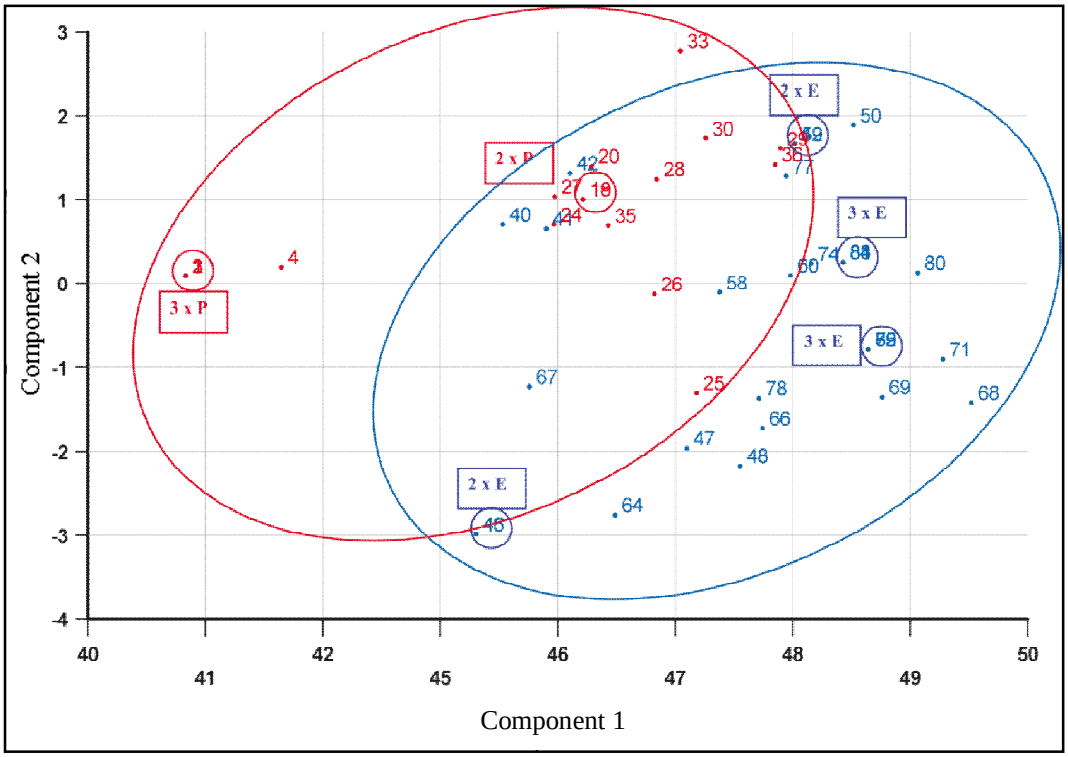
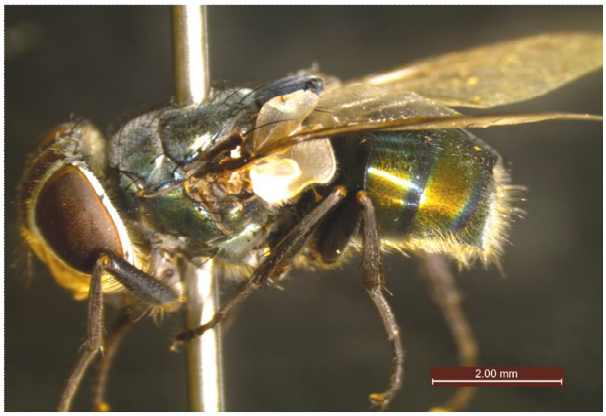


Figure 2. Plot of first two principal components for adult males either preserved in ethanol (E = blues) or pinned (P = red). The blue oval shape includes all ethanol preserved specimens and the red oval shape includes all pinned specimens. Note the area of overlap but also the areas of clear separation of the two populations. Due to identical character scores, six of the points included multiple (2 or 3) specimens

Body colour - *C. bezziana* from Papua New Guinea - Lab. strain (score 6)

Body colour - *C. bezziana* from Malaysia (score 2)

Body colour - *C. bezziana* from Oman (score 3)



Body colour - *C. bezziana* from South Africa (score 3)

Body colour - *C. bezziana* from East Sumba Indonesia (ethanol preservation; score 2)

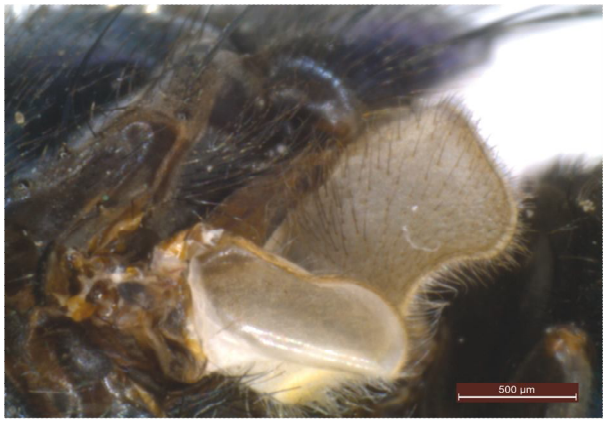
Comments:
Score 0 : -
Score 1 : Blue
Score 2 : Blue/green
Score 3 : Green
Score 4 : Blue/black
Score 5 : Green/black
Score 6 : Blue/purple

Figure 3. Examples of *C. bezziana* from various geographical regions to illustrate the range of body colour recorded

Lower Squama - *C. bezziana* from Oman (Score 1)

Lower Squama - *C. bezziana* from South Africa (Score 1)

Lower Squama - *C. bezziana* from Banten - Indonesia (Score 4)



Lower Squama - *C. bezziana* from Malaysia (Score 3)

Lower Squama - *C. bezziana* from Southeast Sumatra (Score 3)

Comments:
Score 0 : -
Score 1 : Brilliant white + long white hairs
Score 2 : white + variable hairs
Score 3 : Darker waxy white, pigmented along outer edge + black hairs
Score 4 : Darker waxy white, no pigmented along outer edge + mix black and white hairs

Figure 4. Examples of lower squama of *C. bezziana* from various geographical regions to illustrate the variation observed in this character

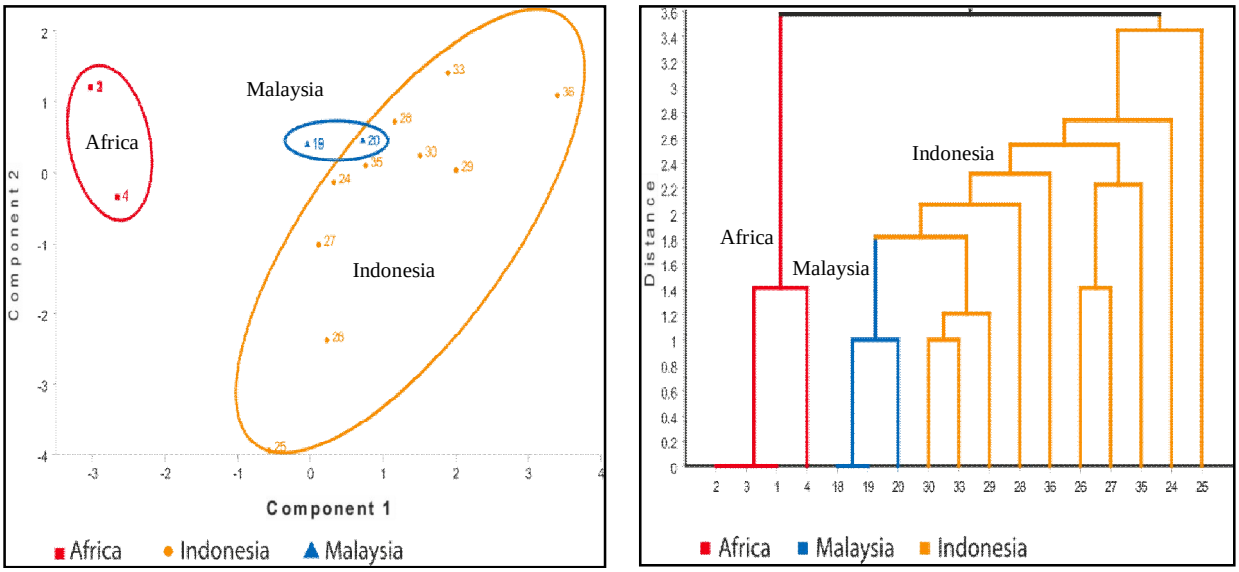
Pinned adults

A total of 18 pinned males and 18 pinned females from different geographical regions were analysed. Morphological analyses were performed separately between males and females (HALL *et al*., 2001). For pinned males, based on principal components analysis, the characters that contributed most to the first component were VibSet (setulae around the vibrissa) and LwSqm (lower squama), while those that contributed most to the second component were AbBand (terminal bands on tergite) and FrStBr (Frontal setular bristles).

For pinned females, the characters that contributed most to the first component were LwSqm (lower squama) and BodCol (Body colour), while those that contributed most to the second component were AbBand (Terminal bands on tergite) and WingB (wing base and cell R). The first two components accounted for 61.99% of the variance in males and 79.38% in females. Differences observed in the character states of body colour and lower squama of flies collected from different regions are shown in Figures 3 and 4, respectively.

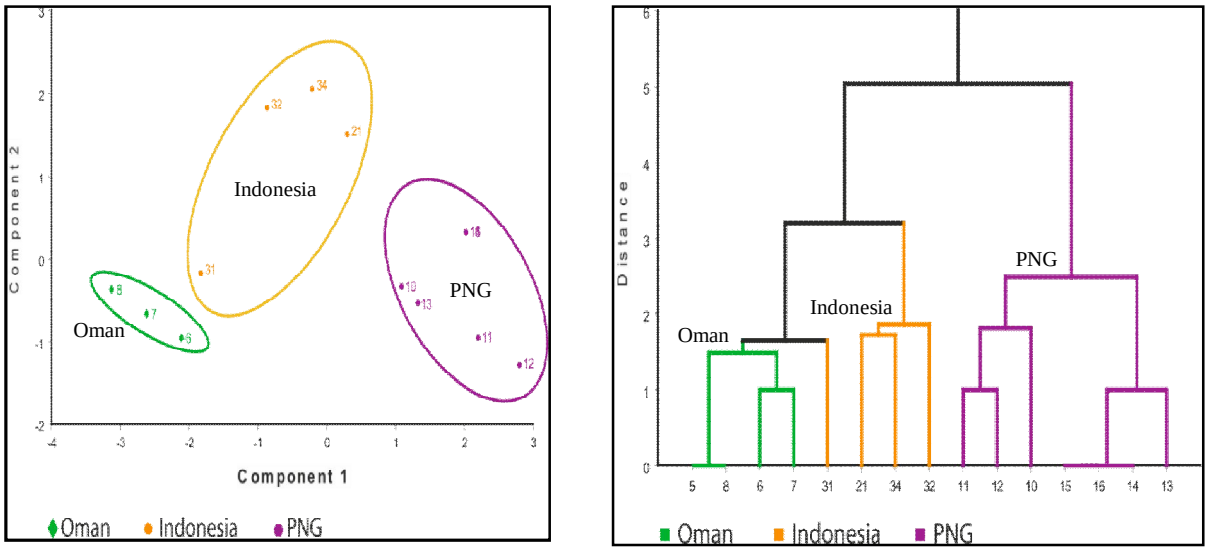
Principal component analysis of character states of pinned males demonstrated that flies from Africa were different from other populations (Figure 5A), however, the others could not be divided into geographical groups. The dendrogram constructed by UPGMA cluster showed a concordant result, with the African specimens placed in a single branch, separate to the Asian specimens (Malaysia and Indonesia) (Figure 5B). Similarity of *C. bezziana* population from Malaysia and Indonesia could be understood because they are geographically close cluster. The result was concordant with HALL *et al*. (2001) demonstrating that *C. bezziana* population from Gulf and Indian flies tended to cluster together based on the same characters in this study.

As no female African specimens were available for study, the results of analysis of the female specimens could not be directly compared with those of the males. However, principal component analysis of the character states of pinned females indicated that flies from PNG (both wild type and laboratory colony) were different from Oman and other Indonesian groups (Figure 6A). This result was reflected in the dendrogram constructed by UPGMA, which clustered flies from PNG (both wild type and laboratory colony) on a different branch to those from elsewhere in Asia and the Gulf region (Figure 6B).



(A) **(B)**

Figure 5. Plot of first two principal components for pinned males specimens of *C. bezziana*. Red oval shapes encloses all African specimens, blue oval from Malaysia and the orange those from Indonesia (A). Dendrogram from cluster analysis (UPGMA; Euclid measure; average between groups method) of morphological data of pinned males of *C. bezziana*. The number on the horizontal axis is the individual code of each fly specimen (B).



(A) **(B)**

Figure 6 . Plot of first two principal components for pinned female specimens of *C. bezziana*. The purple oval encloses flies from PNG, the orange encloses those from Indonesia (Asian, except PNG) and the green those from Oman (Gulf) (A). Dendrogram from cluster analysis (UPGMA; Euclid measure; average between groups method) of morphological data of pinned females of *C. bezziana*. The number on the horizontal line is the individual code of each fly specimen (B).

Ethanol specimens

Using the same statistical procedures as for the pinned specimens, the character states of 32 males and 20 females from different countries preserved in 80% ethanol were analysed. Based on principal components analysis, the characters that contributed most to the first component for male specimens were PoSubCost (posterior setulae on subcostal selerite) and LwSqm (lower squama) while those that contributed most to the second component were WingB (wing base and cell R) and AbBand (terminal bands on tergite). For females, the characters that contributed most to the first component were VibSet (Setulae around the vibrissae) and LwSqm (lower squama), while those that contributed most to the second component were BodCol (Body colour) and SubCost (Anterior setulae on subcostal sclerite). The first two components accounted for 56.46% of the variance in males and 67.52% in females.

Principal component analysis of character states of ethanol preserved males also showed, as for pinned specimens, that flies from Africa were different from other populations. Other specimens could not be defined into geographical groups. The dendrogram constructed by UPGMA cluster analysis provided the same result, with the African specimens clusterd within a single branch, however, the African branch was contained within other branches rather than being a completely separate branch as observed in the pinned males.

For ethanol preserved females, the result of principal component analysis agreed with those for the pinned and ethanol preserved males. Thus the African specimens were split away from the Asian specimens (Figure 7A). This result was shown also in the dendrogram constructed by UPGMA cluster analysis, where the African specimens were clustered within a completely separate branch to the other specimens (Figure 7B).

Morphological characters of *C. bezzziana*

The suite of external morphological characters used in this study were adopted from HALL *et al*. (2001), which gave some indication of value in showing geographical variation of *C. bezziana*. Basically, HALL *et al*. (2001) used external characters of adult flies that had been identified previously (SPRADBERY, 1991) and they added two further characters located on the thorax of adult flies: anterior setulae on subcostal sclerite (SC) and posterior setulae on subcostal sclerite (PC). The result revealed that the first two components accounted for 56% of the variance in females and 52% of the variance in male. This observation was lower compared to the current study.

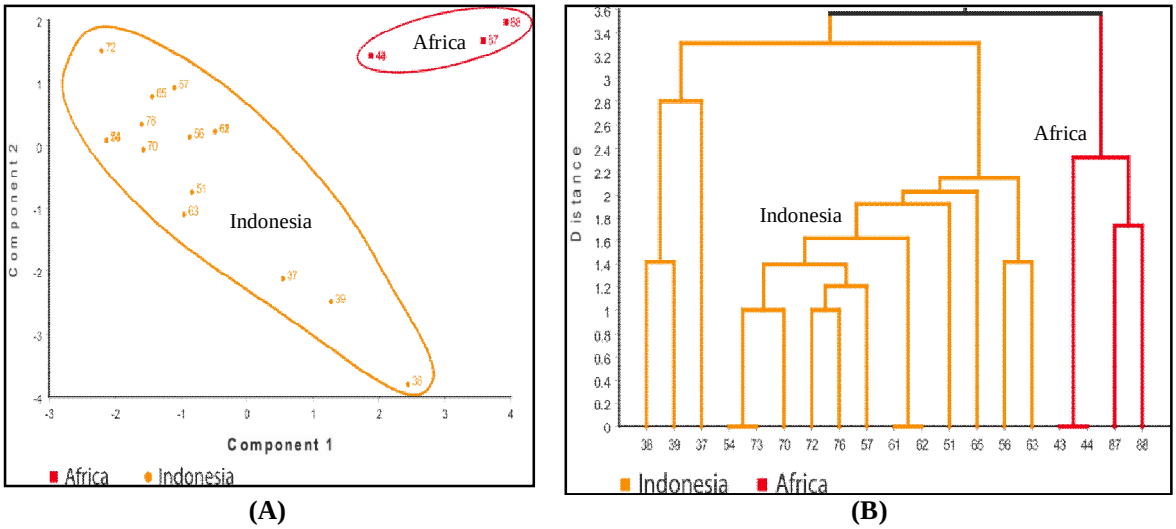


Figure 7 . Plot of first two principal components for ethanol preserved female specimens of *C. bezziana*. The red oval encloses all African specimens and the orange oval is from Indonesia (A). Dendrogram from cluster analysis (UPGMA; Euclid measure; average between groups method) of morphological data of ethanol preserved females of *C. bezziana.* The number on the horizontal axis is the individual code of each fly specimen (B).

In this study, some additional character states were added to the characters of body colour (BC), lower squama (LS) and posterior setulae on subcostal sclerite (PC), because some of the characters observed on the specimens examined did not match the states described in HALL *et al's* character matrix (Table 2). For BC, two extra character states were added: green/black and green/purple scoring 5 and 6, respectively. To LS a single extra character state was added, scoring 4, i.e., darker waxy white with black hair and no pigmented edge. One extra character state was also added to PC, i.e., one/two/three ginger setulae.

Geographical variation of *C. bezziana* according to morphology study

Among characteristics investigated, genal grove (GG) had a score of 2 for all specimens and therefore had no value in separating specimens and was not used in the statistical analyses. So these results are based on 9 characters. The results demonstrated that, based on 9 morphological characters, *C. bezziana* collected from different regions showed clear geographical variation. For pinned males and ethanol preserved males and females, all African specimens were clustered into a generally distinct group apart from all other populations (PNG, Southeast Asia and Gulf). The other clear cluster was of PNG specimens among pinned females. The discovering was concordant with molecular studies using both mitochondrial and nuclear DNA (HALL *et al*., 2001; HALL *et al*., 2009; READY *et al*., 2009).

These finding have some similarity but also some contradiction to those of HALL *et al.* (2001). There was evidence that flies from PNG populations were somewhat distinctive from other populations in HALL *et al*. (2001) and in the present study. However, HALL *et al*. (2001) found that African and most Asian populations were similar to each other whereas the result from this study showed African flies to be distinct due to two additional characters. SPRADBERY (1988 unpublished) stated that according to size, colour and some important taxonomic characters, South African and Gulf *C. bezziana* differed from South Asian and PNG populations.

The present result is a significant encouragement to further studies on the morphological distinction between African and Asian lineages, to look for further key characters that will enable their unambiguous separation, for example, (1) characters on the genitalia of adults, especially males, are presently unexplored but could provide a wealth of characters for identification, because the genitalia morphology is crucial to mating success (HALL *et al*., 2009) and, (2) wing morphometry. LYRA *et al*. (2009) revealed significant differences in wing size and shape among *C. hominivorax* and *C. macellaria* population in Brazil and in Urugay. In addition, a set of chemical characters that merit study are cuticular hydrocarbons, compounds that make up the contact sex pheromones of *C. bezziana* that are key to mate identification and, like the genitalia, are vital to mating success (BROWN, *et al*., 1998; CARLSON *et al*., 2007).

The different finding between HALL *et al*. (2001) and the present study could be explained at least in part by the additional characters assessed. According to principal components analysis, taking into account males and females of both pinned and ethanol preserved specimens, three of the four characters that contributed more frequently than any others to the first component as either the first or second most important character (i.e. LS x 4, BC x 1 and PC x 1) were those to which new character states were added in the present study. This is good evidence that the additional character states could provide better resolution in separating geographical populations of *C. bezziana*.

CONCLUSION

Dried preservation (pinned samples) provided reliable external character than ethanol preservation. Based on external morphology of adult stages revealed that there was no geographical variation of *C. bezziana* throughout Indonesian archipelago, except for the population from PNG forming its own cluster. Additional characters in this study supported the differentiation of African populations of *C. bezziana* from Asian. In addition, the current investigation was the first study demonstrated that African populations are morphologically distinct from Asian population. Clearly, these are areas where a great deal more research is needed, specifically to identify new characters for population discrimination (e.g. genital morphology, chemical taxonomy).

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REFERENCES

BROWN, M.V., R. MORTON, M.J. LACEY, J.P. SPRADBERY and R.J. MAHON. 1998. Identification of the geographical source of adults of the Old World screwworm fly, *Chrysomya bezziana* Villeneuve (Diptera, Calliphoridae), by multivariate analysis of cuticular hydrocarbons – a tale of two species. Comparative Biochemistry and Physiology part B, Biochemist. *Mol. Biol*. 119: 391-399.

CARLSON, D.A., D.R. BERKEBILE, S.R. SKODA, K. MORI and S. MIHOK. 2007. Candidate sex pheromones of the New World screwworm *Cochliomyia hominivorax*. *Med. Vet. Entomol.* 2: 93-96.

HALL, M.J.R., W. EDGE, J.M. TESTA, Z.J.O. ADAMS and P.D. READY. 2001. Old World screwworm fly, *Chrysomya bezziana*, occurs as two geographical races. *Med. Vet. Entomol.* 15: 393-402.

HALL, M.J.R. 2008. New world screwworm (*Cochliomyia hominivorax*) and Old world screwworm (*Chrysomya bezziana*). Chapter 2.1.10. OIE Terrestrial Manual 2008. pp. 265-275.

HALL, M.J.R., A.H. WARDHANA, G. SHAHHOSSEINI, Z.J.O. ADAMS and P.D. READY. 2009. Genetic diversity of populations of Old World screwworm fly, *Chrysomya bezziana*, causing traumatic myiasis of livestock in the Gulf region and implications for control by sterile insect technique. *Med. Vet. Entomol.* 23 (Spl.1): 51-58.

IAEA/FAO, International Atomic Energy Agency/Food and Agriculture Organization. 2000. Genetic sexing and population genetic of screwworms. International Atomic Energy Agency, 7-11 August 2000, Vienna, Austria. 31 p.

KIM, K.S., Z. SZENDREI, C. RODRIGUEZ-SONA, P.G. JR. MULDER and T.W. SAPPINGTON. 2009. Molecular diagnostic for Boll weevil (Coleoptera, Curculionidae) based on amplification of three species-specific microsatellites. *J. Econom. Entomol*. 102: 759-766.

LYRA, M.L., L.M. HATADANI, A.M. DE AZEREDO-ESPIN and L.B. KLACZKO.Wing morphometry as a tool for correct identification of primary and secondary New World screwworm fly. *Bull. Entomol. Res.* 100: 19-26.

MAHON, R.J. 2002. A trial of the sterile insect release method against the Old World Screwworm fly in Malaysia. *In*: Pro. of the International Conference on Control of Old World Screwworm Fly in some Countries of the Middle East. Bahrain, April 4, 2002. AOAD, Bahrain.

NAVIDPOUR, S., N. HOGHOOGHI-ROAD, H. GOODARZI and A.R. POOLADGAR. 2009. Outbreak of *Chrysomya bezziana* in Khoozestan Province, Iran. *Vet. Rec*. 139: 217-222.

QUICKE, D.L.J. 1993. Principles and Techniques of Contemporary Taxonomy. Black Academic and Professional, Glasgow.

READY, P.D., J.M. TESTA, A.H. WARDHANA, M. AL-IZZI, M. KHALAJ and M.J.R. HALL. 2009. Phylogeography and recent emergence of the Old World Screwworm Fly, Chrysomya bezziana, based on mitochondrial and nuclear gene sequences. *Med. Vet. Entomol.* 23 (Spl.1): 43-50.

SPRADBERY, J.P. 1988. Screw-worm fly (*Chrysomya bezziana*) hybridisation studies in the United Kingdom. Personal report.

SPRADBERY, J.P., R.S. TOZER, J.R. ROBB and P. CASSELLS. 1989. The screw-worm fly *Chrysomya bezziana* Villeneuve (Diptera, Calliphoridae) in a sterile insect release trial in Papua New Guinea. *Res. Pop. Eco.* 31: 353-342.

SPRADBERY, J.P. 1990. Australian Screwworm Fly Unit. Manual of Operations. Canberra, CSIRO Division of Entomology Technical Report. No. 49.

SPRADBERY, J.P. 1991. A Manual for the Diagnosis of Screwworm Fly. CSIRO Division of Entomology. Canberra. Australia.

SUKARSIH., S. PARTOUTOMO, R. TOZER, E. SATRIA, G. WIJFFELS and G. RIDDING. 2000. Establishment and maintenance of a colony of the old world screwworm fly *Chrysomya bezziana* at BALITVET in Bogor, West Java, Indonesia. *JITV Special Edition,* 5: 144-149.

SUKONTASON, K.L., S. PIANGJAI, W. BOONSRIWONG, N. BUNCHU, R. NGERN-KLUN, R.C. VOGTSBERGER and K. SUKONTASON. 2006. Observations of the third instar larva and puparium of *Chrysomya bezziana* (Diptera, Calliphoridae). *Parasitol. Res.* 99: 669-674.

THOMAS, D.B. 1993. Behavioral aspects of screwworm ecology. *J. Kansas Entomol. Soc.* 66: 13-30.

TRAVERSA, D. and D. OTRANTO. 2006. A new approach for the diagnosis of myiasis of animals: The example of horse nasal myiasis. *Vet. Parasitol.* 14: 186-190.