Journal of Mosquito Research
2014, Vol.4, No.14, 1-11 http://jmr.biopublisher.ca

Research Article Open Access

Morphometric Differentiation of Sympatric Populations of *Anopheles arabiensis* Patton and *Anopheles gambiae* Giles from Republic of Southern Sudan

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Journal of Mosquito Research, 2014, Vol.4, No.14 doi: 10.5376/jmr.2014.04.0014
Received: 08 Jun., 2014
Accepted: 13 Jul., 2014
Published: 23 Aug., 2014

Preferred citation for this article:
Hamza et al., 2014, Morphometric Differentiation of Sympatric Populations of *Anopheles arabiensis* Patton and *Anopheles gambiae* Giles from Republic of Southern Sudan, Journal of Mosquito Research, Vol.4, No.14 1−11 (doi: 10.5376/jmr.2014.04.0014)

Abstract Mosquitoes of the *Anopheles gambiae* complex, namely *Anopheles arabiensis* (Patton, 1905) and *Anopheles gambiae* (Giles, 1902) are the major vectors of human malaria in the African continent. The study is mainly conducted to investigate the morphometrics of members of *An. gambiae* complex in Republic of Southern Sudan. A morphometric multivariate analysis was carried out to investigate the morphological variations among sympatric populations of *An. arabiensis* and *An. gambiae* and to assess the degree of discrimination of the two fresh water species. Members of the *An. gambiae* complex were collected by hand capture from Wau town, Republic of Southern Sudan during the rainy season of 2010. Sixty nine morphometric characters were examined. A discriminant function analysis correctly identified *An. arabiensis* and *An. gambiae* to a confidence level reaching 85.2%. The best discriminating characters (in a decreasing order) selected by the analysis were: sector pale spot, tarsomere 4 of the fore leg, pre-sector pale spot, tarsomere 5 of the hind leg and tarsus of the fore leg. This morphometric method showed that females *An. arabiensis* and *An. gambiae* were not significantly different in the body size measurements. The morphometric analysis revealed the existence of a considerable degree of differentiation for some metric morphological characters among the females *An. arabiensis* and *An. gambiae*.
Keywords *Anopheles arabiensis*; *Anopheles gambiae*; Morphometric; Principal component analysis; Discriminant function analysis; Republic of Southern Sudan

Background

Members of the *Anopheles gambiae* complex are the most important malaria vectors in the African continent. It is a group of seven morphologically undistinguishable species of tropical mosquitoes (Service, 1985; Hunt et al., 1998). Within this species complex the most important malaria vectors are: *Anopheles gambiae* (Giles, 1902) and *An. arabiensis* (Patton, 1905) which are distributed over 70% of the Sub-Saharan Africa with *An. gambiae* being restricted to the more humid and forested localities and *An. arabiensis* being distributed over the dry savanna and semi- arid parts of the African continent (Service, 1980; Bryan, 1983; Lindsay et al., 1998). The two species are more adapted to the human environment; they are sympatric and synchronic over most of their distribution range (Petrarca et al., 1998). Female *An. gambiae* complex mosquitoes have slender body, (5mm) in size with three sections: head, thorax and abdomen. The morphological description of the complex had been given by Evans (1938), cited in Gillies and De-Meillon (1968).

Due to the observed differences in vectorial capacities and behavioural habits of the siblings in species complexes, efforts have been geared towards identification of the siblings of mosquito species. One of these efforts is the use of multivariate methods such as morphometrics (Coluzzi, 1964; White, 1977; Lambert and Coetzee, 1982; Service, 1988; Schmidt et al., 2003). Morphometrics is the field concerned with variation and changes in the body form or size of an organism. It transforms the complex body forms into quantitative series of numbers that can be analyzed and used for comparisons between the different forms (Daly, 1985).

Since the discovery that *An. gambiae* is not a single species but a complex of sibling species, many researchers attempted to find morphological variations between the siblings of the complex. These attempts

had been done to some extents by Ribbauds (1944), Muirhead-Thomoson (1951) and Coluzzi (1964). Subsequently, many researchers have attempted to find morphological differences between the three fresh water species: *An. gambiae*, *An. arabiensis* and *An. quadriannulatus* (Ismail and Hammoud, 1968; Zahar et al., 1970; White and Muniss 1972; Reid, 1975a) without success. A subspecies *An. quadriannulatus davidsoni*, from Cape Verde Island was described based on morphological criteria only by Ribeiro et al. (1979). Ribeiro (1980) proposed a phylogeny for the complex, *An. gambiae* using the morphological data from *An. quadriannulatus davidsoni* and published data for other members of the complex. Coetzee (1989) carried out a morphometric analysis on all life stages of *An. gambiae*, *An. arabiensis*, *An. quadriannulatus* and *An. merus* occurring in southern Africa. He revealed that the length of the hind leg pale band at the junction of tarsomeres 3 and 4 is a good character for grouping *An. gambiae*/ *An. arabiensis* and *An. quadriannulatus*/ *An. merus* and he was able to separate *An. quadriannulatus*/ *An. merus* in the base of palpal index. He found that separation of individuals *An. gambiae* from *An. arabiensis* was not reliable. Petrarca et al. (1998) carried out a morphometric multivariate analysis on field and laboratory specimens of *An. gambiae* and *An. arabiensis* from different areas of Sub-Saharan Africa and he found that all the measures were significantly larger for *An. arabiensis*.

An. gambiae complex showed variation in the female palpal banding, *An. gambiae* Giles has 3-banded palps (Gillies and De-Meillon, 1968; Coetzee, 1989). Other members of the group have greater tendency to 4-banded palps in the adult female (Muirhead-Thomoson, 1951; Holstein, 1952; Paterson, 1964b; Coetzee, 2000).

Separation between the species of *An. gambiae* complex is much less reliable owing to the existence of a considerable overlap between the different character distributions (Ribeiro et al., 1979; Bryan 1980; Bushrod, 1981).

In the present study we carried out a morphometric analysis on field specimens of *An. arabiensis* and *An. gambiae* (previously identified by molecular polymerase chain reaction (PCR) techniques) in an attempt to find reliable morphological features for distinguishing between the two species.

1 Materials and methods

1.1 Collection site

Adult female *Anopheles* mosquitoes were collected from Wau town, Republic of Southern Sudan which is situated in the eastern part of the African continent. Wau is located in mid western part of Bahr El Ghazal region between 7°: 42″ N and 28°: 283″ E. The region is an area of natural swamps and ironstone plateaus characterized by equatorial (humid-tropical) climate. The climate in this region is marked by high temperature, high rainfall and very high humidity.

1.2 Mosquito collection and preservation

In door resting wild adult *Anopheles* mosquitoes were collected by hand capture using sucking tube (aspirator) during the rainy season 2010. *Anopheles* mosquitoes collected were preserved individually in 70% ethanol and kept at −20 ℃ for subsequent processing in the laboratory. Samples were transported to the laboratory well protected to minimize any damage. The processing of the materials for this study was carried out at the Department of Zoology, Faculty of Science, University of Khartoum, Sudan.

1.3 Identification and mounting of females *An. gambiae* complex

An. gambiae species complex were identified to the complex level using morphological identification keys described by Gillies and De-Mellion (1968) and Gillies and Coetzee (1987). Subsequently, females *An. gambiae* complex were dissected for morphological and molecular identification. For morphological analysis, permanent slides were made for detailed examination of external body structures of females *An. arabiensis and An. gambiae*. The head, wings and legs were carefully separated from the mosquito body and mounted in Puri's mounting medium on glass microscopic slides as described by WHO (1975) with minor modifications. The mosquitoes were mounted directly into Puri's mounting medium without prior clearing because gum-chloral mountant continues to clear specimens after mounting. The corresponding carcasses were preserved in 70% alcohol, kept at −20 ℃ and used for molecular identification.

1.4 Selection of morphological characters for morphometrics analysis

For terminology and morphological characters and abbreviations of palps, antennae, legs and wings, the nomenclature of Evans (1938) and Gillies and De-Mellion (1968) were adopted, except for wing spots, the nomenclature adopted by Gillies and Coetzee (1987) was followed.

A preliminary list was prepared from all morphological characters of the adult female *An. gambiae* complex include characters which were used by the previous workers in an attempt to discriminate between the members of the complex. Out of this list, all characters of external body parts that fixation and transport could not have changed were selected. Sixty nine characters (2 non metric and 67 metric) were finally retained and were measured on 53 females *An. arabiensis* and 35 females *An. gambiae*. The measured characters include 2 characters associated with antennae, 11 characters with palps (Table 1), 14 characters with wings (Table 2) and 14 characters of each fore, mid and hind legs (Table 3).

1.5 Morphological characters measurements

Measurements were done by the projection method (Zahar et al., 1970). Selected morphological characters of females *An. arabiensis* and *An. gambiae* were measured using a Wild MII binocular calibrated compound microscope fitted with a 1.25× phototube. Characters were measured using 3.5× and 40× objective lens depending on the size of the measured characters. The slide mounted specimens were projected with a phototube on a microscopic field using 8× eye piece. After an excellent view of the projected image of the measured character was obtained, the projected image was drawn and subsequently measured to the nearest half millimeter. Then the lengths were calibrated to the real lengths using a micrometer stage (1mm-Erma, Tokxc).

Table 1 Mean, standard error and sample size of 2 antenna and 11 palp morphometric characters of females *An. arabiensis* and *An. gambiae*

Mosquito body part	Character	*An. arabiensis*	*An. gambiae*	P-value
Antenna	Flagellum	1.35±0.011 (37)	1.34±0.010 (21)	P>0.05
	No. co	26±0.000 (21)	26±0.000 (19)	P>0.05
Palp	Segment I	0.11±.006 (17)	0.10±0.005 (12)	P>0.05
	Segment II	0.70±0.010 (19)	0.69±0.009 (13)	P>0.05
	Segment III	0.73±0.007* (51)	0.71±0.006 (31)	P<0.05
	Segment IV	0.34±0.004* (52)	0.32±0.004 (31)	P<0.05
	Segment V	0.21±.003 (51)	0.20±.003 (31)	P>0.05
	Palp length	2.06±0.029 (17)	2.02±0.028 (12)	P>0.05
	Palpal index	0.75±0.004 (50)	0.74±0.007 (31)	P>0.05
	Apical pale spot	0.25±0.004 (50)	0.26±0.003 (30)	P>0.05
	Median pale spot	0.07±0.002 (47)	0.06±0.002 (29)	P>0.05
	Basal pale spot	0.07±0.002* (48)	0.06±0.003 (31)	P<0.05
	No. of pale spots	3±0.000 (47)	3±0.000 (29)	P>0.05

Note: Measurements are in (mm) except for number of coeloconic sensillae on antennal segment (No. co), palpal index and number of pale spots on palpus. Numbers between parentheses are sample sizes. P-values are based on t-test. *Significantly larger character

Table 2 Mean, standard error and sample size of 14 morphometric wing characters of females *An. arabiensis* and *An. gambiae*

Character	*An. arabiensis*	*An. gambiae*	P-value
Humeral pale spot	0.14±0.004	0.13±0.004	P>0.05
	(34)	(28)	
Pre-sector pale spot	0.10±0.005	0.12±0.005*	P<0.05
	(39)	(29)	
Pre-sector dark spot	0.40±0.008	0.39±0.008	P>0.05
	(43)	(30)	
Sector pale spot	0.15±0.006*	0.12±0.004	P<0.05
	(50)	(35)	
Median dark spot	0.69±0.010	0.70±0.009	P>0.05
	(50)	(35)	
Accessory sector pale spot	0.13±0.003	0.14±0.003	P>0.05
	(39)	(31)	
Sub-costal pale spot	0.27±0.009*	0.30±0.008	P<0.05
	(50)	(35)	
Pre-apical dark spot	0.49±0.010	0.50±0.013	P>0.05
	(50)	(35)	
P.I in 3rd D.A	0.09±0.003	0.10±0.004	P>0.05
	(37)	(27)	
Sub-apical pale spot	0.32±0.007	0.31±0.008	P>0.05
	(50)	(35)	
Apical dark spot	0.19±0.005	0.18±0.005	P>0.05
	(50)	(35)	
Apical pale spot	0.23±0.004*	0.21±0.006	P<0.05
	(48)	(33)	
Wing length including the fringe	3.59±0.032	3.55±0.026	P>0.05
	(49)	(35)	
Wing width including the fringe	0.99±0.009	0.99±0.010	P>0.05
	(47)	(30)	

Note: Measurements are in (mm). Numbers between parentheses are sample sizes. P-values are based on t-test. P.I in 3rd D.A = Pale interruption of third main dark area at first vein. *Significantly larger character

1.6 Statistical analyses

The soft-ware computer program package SPSS® version 16.0 for Windows was used for statistical aspects of the morphometric analysis. The measurements were not transformed to ratios so as to preserve the possible influence of differences in the body sizes of the two species. Box plots were used to check for the presence of outliers (Garson, 2012). Then the measured data were subjected to univariate and multivariate statistical analyses.

Univariate statistics like calculation of descriptives, Students t test (t-test) and Box and whisker plots were used. Descriptives analysis involved calculation of mean size measurements plus or minus their standard error and sample sizes. A t-test was carried out to test the significant differences of measured morphological characters among *An. arabiensis* and *An. gambiae* populations (Park, 2009). Box and whisker plot were used to summarize the univariate data.

Multivariate statistics such as principal component analysis and discriminant function analysis were used. Principal component analysis was conducted to simplify subsequent analysis of the data. The test is essentially a method of data reduction that aims to produce a small number of derived variables that explain most of the variance and can be used in place of the larger number of original variables (Pimentel, 1992). The analysis generates a set of principal components by weighting all the available variables. The first component explains the most variation; the second explains the next most variation, and so on. The amount of variance captures by one component represents by Eigen value. Investigation of the first few components will show which variables contribute

most to the variations between individuals (Dythan, 2003).

Discriminant function analysis concerns with classification and aims to obtain a small number of useful discriminating variables (Pimentel, 1992). Discriminant function analysis was carried out based on the results of the principal component analysis and t-test to assess the degree of discrimination among adult female *An. arabiensis* and *An. gambiae*.

2 Results

2.1 Morphometrics investigations

Tables 1, 2, 3 summarize the mean, standard error and sample size of the measured antenna, palp, wing and legs characters on females *An. arabiensis* and *An. gambiae*.

2.2 Student t-test

The t-test showed significant variations (P<0.05) between the sympatric populations of *An. arabiensis* and *An. gambiae* in 17% (13/69) of all the measured morphological characters (Table 1, 2, 3). Most of the significantly different characters (9/13) were larger in *An. arabiensis* individuals. Variations in the measured characters show promise for a discriminant function analysis in an attempt to distinguish between the two fresh water species.

2.3 Principal component analysis

Principal component analysis was carried out to simplify the analysis of Tables (1, 2, 3).The results of this analysis as presented in (Table 4) showed that three components with Eigen values more than 1 explained the data. Component one contributed the highest Eigen value of 0.52 with a percentage variance of 83.2%. It was largely influenced by the measurements of the mosquito body size. Component 2 and 3 showed Eigen values and percentages variance of 0.04, 6.3% and 0.02, 3.2%, respectively. They were mostly influenced by the variables measures of the body shape. These variables were less important due to the lower percentages of variance contributed by their components. Together, component 1, 2 and 3 account for 92.7% of the total variance. All the variables positively load on the first component except the: palpal index, humeral pale spot, pale band at the joint of tarsomeres 1and 2 of the hind leg and pale band at the joint of tarsomeres 3 and 4 of the hind leg.

The inspection of principal component 1 showed that individuals of *An. arabiensis* and *An. gambiae* were not significantly different (P>0.05). Box and whisker plot (Figure 1) summarize the scores of component 1.

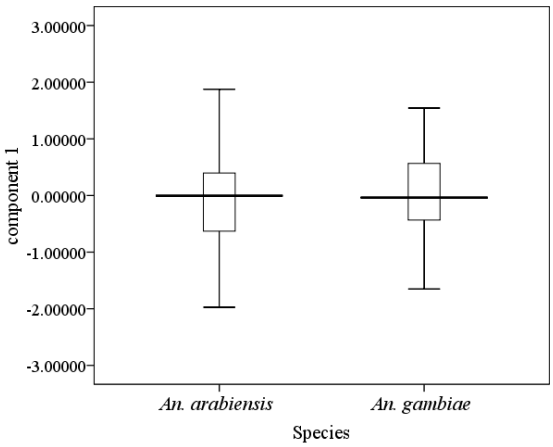

Figure 1 Box and Whisker plots of the scores of principal component 1 (83.2% explained variance) of females *An. arabiensis* and *An. gambiae* populations. Value zero on the Y-axis is the grand centroid (overall mean of the components scores) (P>0.05)

2.4 Discriminant function analysis

Discriminant function analysis was carried out using two different sets of variables. First, the analysis was applied on the characters linked with the mosquito body size. These characters comprised the five most influential characters for component 1, derived by the principal component analysis (Table 4). The characters used were: hind leg length, mid leg length, fore leg length, wing length including the fringe and wing width including the fringe. When these characters were subjected to discriminant function analysis, one significant function was derived. A P-value (Wilks Lambda test) bigger than 0.0001, indicates that the group centroids of the 2 species were not significantly different. The canonical correlation coefficient of the function was 0.401 and the related Chi-square ($\chi 2$) =6.766, this indicates a low correlation between the discriminant function and the original variables. The Chi-square was not significant (P>0.0001, df=5), indicating populations with no definite differences between the two species. Table 5 shows the standardized, unstandardized and loading coefficients for the discriminant function. The variables that mostly influenced the separation of the two species

Table 4 Results of principal component analysis: contribution of 37 variables to the first three principal components calculated from 53 females *An. arabiensis* and 35 females *An. gambiae*

Character	Component		
	1	2	3
Hind leg length	0.962	−0.268	0.010
Mid leg length	0.946	0.206	−0.245
Fore leg length	0.912	0.320	0.246
Wing length including the fringe	0.872	−0.098	0.229
Wing width including the fringe	0.797	−0.095	0.025
Length of palpal segment III	0.749	0.136	−0.055
Median dark spot	0.655	0.120	−0.081
Length of palpal segment IV	0.607	0.024	−0.070
Pre-apical dark spot	0.418	−0.031	−0.128
Palpal length	0.414	0.312	−0.150
Length of apical pale band on palpus	0.398	0.141	0.260
Length of palpal segment V	0.392	0.172	0.022
Antenna flagellum length	0.373	−0.074	0.033
Length of palpal segment II	0.350	0.159	−0.133
Apical dark spot	0.347	0.040	−0.140
Pre-sector dark spot	0.344	0.026	−0.005
Apical pale spot	0.234	−0.029	0.114
Accessory sector pale spot	0.212	0.052	0.181
Sub-apical pale spot	0.202	−0.129	0.188
Sub-costal pale spot	0.187	0.186	0.108
Pale band at the joint of tarsomeres 3&4 of the mid leg	0.186	0.028	0.134
Pale band at the joint of tarsomeres 2&3 of the hind leg	0.184	−0.029	0.083
Pale interruption of third main dark area of 1st vein	0.148	0.070	−0.006
Length of basal pale band on palpus	0.134	−0.113	−0.129
Length of median pale band on palpus	0.126	−0.117	0.078
Palpal index	−0.093	−0.057	−0.009
Pale band at the joint of tarsomeres 1&2 of the fore leg	0.054	0.017	0.032
Pale band at the joint of tarsomeres1&2 of the mid leg	0.039	−0.131	0.065
Pale band at the joint of tarsomeres 3&4 of the fore leg	0.072	−0.126	0.125
Pale band at the joint of tarsomeres 2&3 of the mid leg	0.060	−0.123	0.092
Pre-sector pale spot	0.043	0.050	−0.010
Humeral pale spot	−0.106	−0.074	−0.182
Sector pale spot	0.058	0.040	0.150
Pale band at the joint of tarsomeres 2&3 of the fore leg	0.110	−0.025	0.141
Length of palpal segment I	0.081	0.127	−0.129
Pale band at the joint of tarsomeres 1&2 of the hind leg	−0.061	−0.055	0.101
Pale band at the joint of tarsomeres 3&4 of the hind leg	−0.016	−0.017	0.071

Note: Relative percentages of explained variance for component 1, 2 and 3 are 83.2%, 6.3% and 3.2%, respectively. The coefficients are sorted by decreasing magnitude of component 1.

were (with a decreasing order of magnitude): wing length including the fringe, fore leg length and mid leg length. However, this discrimination was not a complete one, since only 67% of the original grouped specimens and 58% of the cross validated ones were correctly classified (Table 6).

Table 5 Canonical discriminant coefficients and loadings for the discriminant function. The analysis applied on the characters linked with mosquito body size to discriminate between females *An. arabiensis* and *An. gambiae*

Character	Unstandardized coefficient	Standardized coefficient	Loadings
Wing length including the fringe	13.995	3.140	0.267
Mid leg length	−5.876	−2.663	−0.073
Fore leg length	2.097	1.007	−0.028

Note: Standardized coefficient: represents the relative contribution of each variable to the discriminant function derived by the analysis. Loading: represents correlation between an independent variable and discriminant function derived by the analysis

Table 6 Leave-one-out cross validation for all specimens used in the discriminant analysis of morphometric measurements (characters linked with mosquito body size) of females *An. arabiensis* and *An. gambiae*

	Species	Predicted group membership		Total
		An. arabiensis	*An. gambiae*	
Original	*An. arabiensis*	45 (84.9%)	8 (15.1%)	53
	An. gambiae	21 (74.8%)	14 (25.2%)	35
Cross-validated	*An. arabiensis*	37 (69.8%)	16 (30.2%)	53
	An. gambiae	21 (60%)	14 (40%)	35

Note: In cross validation, each specimen is classified by the functions derived from all specimens other than that being classified. 67% of original grouped cases correctly classified. 58% of cross-validated grouped cases correctly classified

Second, the 13 characters that showed significant differences (using t-test) between the two populations of females *An. arabiensis* and *An. gambiae* (Tables 1, 2, 3) were subjected to the discriminant function analysis. One significant function was derived. A P-value (Wilks Lambda test) smaller than 0.0001, indicates that the group centroids of the 2 species were significantly different (P<0.0001). The canonical correlation coefficient of the function was 0.869 and the related Chi-square ($\chi 2$) =48.603, df =13, P =0.0000. The function explained the total variance among the two species, with (with a decreasing order of magnitude) sector pale spot, tarsomere 4 of the fore leg, pre- sector pale spot, tarsomere 5 of the hind leg and the tarsus of the fore leg having high discriminant loadings (Table 7).

Table 7 Canonical discriminant coefficients and loadings for the discriminant function. The analysis applied on the characters selected by t-test to discriminate females *An. arabiensis* and *An. gambiae*

Character	Unstandardized coefficient	Standardized coefficient	Loading
Sector pale spot	−18.535	−0.551	−0.309
Tarsomere 4 of the fore leg	79.881	1.264	0.307
Pre-sector pale spot	8.555	0.217	0.288
Tarsomere 5 of the hind leg	21.247	0.318	0.262
Tarsus of the fore leg	−9.710	−1.531	0.258
Tarsomere 1 of the fore leg	17.195	0.318	0.250

The means of the discriminant scores (group centroids) of *An. arabiensis* and *An. gambiae* were 2.123 and −1.388, respectively. The difference between the centroids of the two species was highly significant (P<0.0001). However, this discrimination was a complete one, since only 85.2% of the original grouped specimens and 72.7% of the cross validated ones were correctly classified (Table 8), as indicated in Figures 2.

Table 8 Leave-one-out cross validation for all specimens used in the discriminant function analysis of morphometric measurements on females *An. arabiensis* and *An. gambiae*

	Species	Predicted Group Membership		Total
		An. arabiensis	*An. gambiae*	
Original	*An. arabiensis*	42 (79.2 %%)	11 (20.8%)	53
	An. gambiae	2 (5.7%)	33 (94.3 %)	35
Cross-validated	*An. arabiensis*	37 (69.8%)	16 (30.2%)	53
	An. gambiae	8 (22.9%)	27 (77.1%)	35

Note: 85.2% of original grouped cases correctly classified. 72.7% of cross-validated grouped cases correctly classified

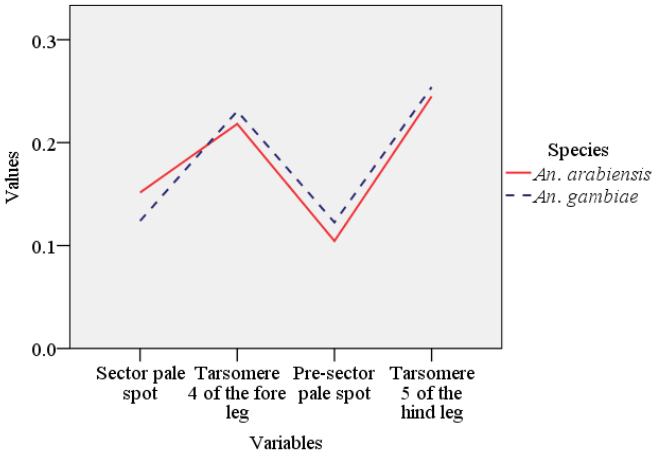

Figure 2 Characters showed significant differences between females *An. arabiensis* and *An. gambiae* using t-test (P<0.05). These characters were obtained 85.2% discrimination when applied to discriminant function analysis

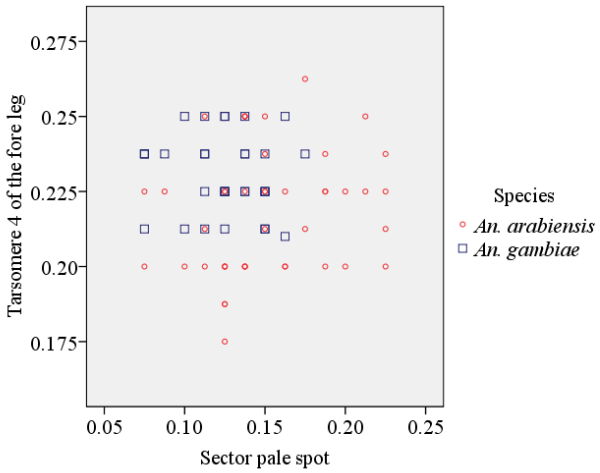

Figure 3 Scatter plot of the length of tarsomere 4 of the fore leg against the length of sector pale spot of females *An. arabiensis* and *An. gambiae*. The two characters mostly influenced the separation of the 2 species as revealed by 85.2% discrimination

The two species were previously identified by the molecular polymerase chain reaction (PCR) techniques. Using this morphometric method, 11 (20.8%) of the 53 *An. arabiensis* were misclassified as *An. gambiae*. Of the 35 *An. gambiae* 2 (5.7%) were misclassified as *An. arabiensis*. The mean error rate was 13.3%.

Figure 3 shows the scatter plot of tarsomere 4 of the fore leg against the sector pale spot of the wing, the two variables that mostly influenced the separation of the two species. The scatter plot showed separation between most individuals of the two species.

3 Discussion

Measurements of various morphological characters of females *An. arabiensis* and *An. gambiae* from Republic of Southern Sudan were in accordance with the published data of the same species from other sites along the distribution range of the two species (Coluzzi, 1964; Petrarca et al., 1998; Adeleke et al., 2008).

The results of the principal component analysis confirmed the report of (Dythan, 2003) that the individuals of females *An. arabiensis* and *An. gambiae* differed mainly in the mosquito body size measurements. The morphometric analysis (discriminant function analysis and t-test) showed that females *An. arabiensis* and *An. gambiae* were not significantly different in the body size measurements. In contrast Petrarca et al. (1998) found that *An. arabiensis* had mean body size greater than *An. gambiae*.

Using the significantly different characters between the two species, as revealed by t-test- the discriminant function analysis revealed differentiation between the two species to a confidence level approaching 85.2%. Characters that mostly influenced differentiation were: sector pale spot, tarsomere 4 of the fore leg, pre-sector pale spot, tarsomere 5 of the hind leg and tarsus of the fore leg. In other groups of insects a morphological discriminability bigger than 75-80% is sufficient to

propose the separation to species of populations of differentiated morphs in dragon flies (Carrison, 1992).

The best morphological character selected by the morphometric analysis for discrimination of adult females *An. arabiensis* and *An. gambiae* was the sector pale spot of the wing. The spot vary significantly between the two species. Petrarca et al. (1998) stated that this spot is important for discriminating the laboratory strains of *An. arabiensis* and *An. gambiae* as observed by Coronel (1962) but at the same time the character failed to discriminate the field strains of the two species.

In this morphological investigation, all the individuals of *An. gambiae* and *An. arabiensis* examined for the number of pale bands on the palpus were 3-banded. The same finding has been reported by Gillies and De-Mellion (1968) and Bryan (1980). The palpal index was used by Coluzzi (1964) and Bryan (1980) in separating the salt-water species from fresh-water species of the *An. gambiae* complex. The results of the morphometrics analysis indicated that this character did not discriminate the two species, as observed by Petrarca et al. (1998).

Coetzee (1986, 1989) examined the use of hind leg banding patterns for identifying members of the *An. gambiae* group of mosquitoes. They showed that the pale band at the apex of hind tarsus 3 and the base of hind tarsus 4 was separated *An. gambiae* and *An. arabiensis* from *An. merus* and *An. quadriannulatus*. In our study, the character failed to distinguish the sympatric populations of *An. gambiae* and *An. arabiensis*.

Coluzzi (1964) stated that the number of coeloconic sensillae of the antenna might prove useful for the differentiation *An. gambiae* and *An. arabiensis* in East Africa. Also the partially discrimination value of the number of coeloconic sensillae is confirmed by the observations of other authors (Ismail and Hammoud, 1968; Petrarca et al., 1984; Coetzee, 1989). In our study, the mean number of antennal coeloconic sensillae did not vary significantly between the two species as pointed out by Petrarca et al. (1998).

Adeleke et al. (2008) carried out a morphometric analysis of *An. gambiae* complex in Abeokuta, Metropolis. The results suggest that the antennal length and wing length may be of significant value in separating *An. gambiae* and *An. arabiensis*. This results contrast sharply with the finding of our study that the two characters did not vary significantly between the two species.

The morphometric analysis conducted in this study confirmed the existence of variability within several characters among the populations of *An. arabiensis* and *An. gambiae*. This study suggests that a considerable degree of differentiation for some metric morphological characters exists among the females of the two species. More studies are needed on morphometric studies on *An. gambiae* complex so as to document as many discriminating characters as possible. Further studies are still recommended by comparing the measured morphological characters with genetic composition in order to give valuable clues for identifying the sibling species.

Authors' contributions

Hamza A.M. participated in field sampling, performed the technical work, analyzed the results and drafted the manuscript. Abukashawa S.M. contributed to the reading and revising the draft manuscript. El Rayah El A. supervised the research group and participated in revising the draft manuscript. All authors read and approved the final manuscript

Acknowledgements

The authors thank Dr. Noteila Mustafa Khalid, Department of Zoology, Faculty of Science, Khartoum College of Medical Sciences for her collaboration and assistance in the statistical analysis of the results. We thank Mr Abd El Gader Morwak, Ministry of Health, Western Bahr El Ghazal State, Republic of Southern Sudan for his assistance in the collection of mosquito samples from Republic of Southern Sudan. We thank Department of Zoology, Faculty of Science, University of Khartoum for the institutional support and use of the facilities of the Zoology Laboratory. This study was supported by a grant from Kassala University.

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Table 3 Mean, standard error and sample size of 14 morphometric fore leg, mid leg and hind leg characters of females *An. arabiensis* and *An. gambiae*

Character	Fore leg			Mid Leg			Hind Leg		
	An. arabiensis	*An. gambiae*	P-value	*An. arabiensis*	*An. gambiae*	P-value	*An. arabiensis*	*An. gambiae*	P-value
Coxa	0.29 ±0.004	0.30 ±0.003	P>0.05	0.18 ±0.003	0.18 ±0.003	P>0.05	0.28 ±0.003	0.27 ±0.005	P>0.05
	(42)	(31)		(42)	(35)		(43)	(31)	
Trochanter	0.18 ±0.03	0.17 ±0.004	P>0.05	0.12 ±0.002	0.11 ±0.002	P>0.05	0.12 ±0.002	0.11 ±0.002	P>0.05
	(43)	(32)		(43)	(35)		(43)	(31)	
Femur	1.60 ±0.016	1.59 ±0.012	P>0.05	1.93 ±0.020	1.94 ±0.019	P>0.05	2.00 ±0.020	2.01 ±0.019	P>0.05
	(44)	(35)		(45)	(32)		(44)	(33)	
Tibia	1.930.019	1.94 ±0.020	P>0.05	2.12 ±0.022	2.14 ±0.021	P>0.05	2.20 ±0.023	2.20 ±0.019	P>0.05
	(44)	(35)		(45)	(32)		(43)	(33)	
Tarsus	2.72 ±0.029	2.80.022*	P<0.05	3.25 ±0.035	3.30 ±0.027	P>0.05	5.12 ±0.059	5.15 ±0.054	P>0.05
	(44)	(33)		(42)	(31)		(40)	(28)	
Leg Length	6.73 ±0.068	6.77 ±0.062	P>0.05	7.61 ±0.083	7.65 ±0.062	P>0.05	9.73 ±0.108	9.75 ±0.099	P>0.05
	(41)	(29)		(39)	(31)		(37)	(26)	
t1	1.42 ±0.015	1.46 ±0.014*	P<0.05	1.55 ±0.018	1.58 ±0.012	P>0.05	2.51 ±0.033	2.53 ±0.025	P>0.05
	(44)	(34)		(42)	(32)		(42)	(32)	
t2	0.54 ±0.007	0.55 ±0.005	P>0.05	0.69 ±0.009	0.71 ±0.008	P>0.05	1.03 ±0.012	1.05 ±0.012	P>0.05
	(44)	(34)		(45)	(32)		(43)	(31)	
t3	0.37 ±0.005	0.38 ±0.004	P>0.05	0.51 ±0.006	0.51 ±0.006	P>.05	0.80 ±0.010	0.80 ±0.010	P>0.05
	(44)	(34)		(45)	(32)		(43)	(29)	
t4	0.22 ±0.003	0.23 ±0.002*	P<0.05	0.31 ±0.004	0.32 ±0.003	P>0.05	0.52 ±0.007	0.53 ±0.007	P>0.05
	(44)	(33)		(45)	(32)		(41)	(28)	
t5	0.17 ±0.002	0.18 ±0.002	P>0.05	0.18 ±0.002	0.18 ±0.003	P>0.05	0.24 ±0.003*	0.25 ±0.002	P<0.05
	(44)	(33)		(45)	(31)		(41)	(28)	
t1-t2	0.19 ±0.003	0.18 ±0.005	P>0.05	0.09 ±0.003	0.09 ±0.004	P>0.05	0.09 ±0.003	0.08 ±0.004	P>0.05
	(44)	(33)		(42)	(26)		(42)	(29)	
t2-t3	0.17 ±0.002	0.17 ±0.005	P>0.05	0.09 ±0.002*	0.07 ±0.004	P<0.05	0.08 ±0.002*	0.07 ±0.003	P<0.05
	(43)	(33)		(41)	(26)		(41)	(28)	
t3-t4	0.04 ±0.001	0.04 ±0.001	P>0.05	0.09 ±0.022	0.06 ±0.003	P>0.05	0.07 ±0.002	0.07 ±0.003	P>0.05
	(42)	(31)		(38)	(24)		(42)	(28)	

Note: Measurements are in (mm). Numbers between parentheses are sample sizes. P-values are based on t-test. t1-t5= Tarsomere 1-5. t1-t2= Pale band at the joint of tarsomeres 1 and 2. t2-t3= Pale band at the joint of tarsomeres 2 and 3. t3-t4= Pale band at the joint of tarsomeres 3 and 4. *Significantly larger character