Morphometric Studies of the Sweet Potato Weevil, *Cylas* Species-Complex in Southern Ghana

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ABSTRACT

The Sweet potato weevil, *Cylas* species, is a key pest of sweet potato, and widely distributed on the African continent. The management of the pest is limited because its taxonomic status is not clear. Populations of the same species occupying distinct localities experience different ecological and climatic pressures that might result in differentiation in traits. This study sought to identify and compare body sizes of *Cylas* species from four regions in southern Ghana – Central, Eastern, Greater Accra and Volta. Of the 6,686 samples collected from the four regions, two species were identified: *Cylas brunneus* Fabricius, and *Cylas puncticollis* Boheman. Twelve morphometric characters were examined and measured, of which four traits - elytra and rostrum lengths, pronotum and head widths contributed most to the variations observed. In *C. puncticollis*, individuals with the longest body were recorded in Greater Accra Region (7.084 ± 0.089 mm), while those in the Central Region had the smallest body size (6.786 ± 0.086 mm). Our findings suggest that distinct localities may influence changes in body size.

Keywords: Sweet potato, *Cylas brunneus, C. puncticollis,* morphometric studies, southern Ghana

Introduction

Sweet potato is one of the world's most important food crops in terms of human consumption, particularly in Sub-Saharan Africa, parts of Asia, and the Pacific Islands. First domesticated more than 5,000 years ago in Latin America, it is grown in more developing countries than any other root crop (Smit, 1997). In Sub-Saharan Africa, sweet potato is predominantly cultivated on small plots characterized by low fertility and drought-prone soils, producing relatively good yields with low inputs and minimal labour costs (Ewell and Mutuura, 1994). Historically, the production of roots and tubers in Africa has been restricted to assuring food security. The production of roots and tubers in developing countries is projected to increase by 58% (232 million tonnes) to 635 million tonnes between 2003 and 2020 (Bill and Melinda Gates Foundation, 2011).

The crop has recently become the focus of targeted bio-fortification for enhanced vitamin A. The Orange-fleshed varieties have been bred with 50-fold more ß-carotene than standard varieties, and these newly released varieties rank first among roots and tubers in Sub-Saharan Africa for their nutritional quality (Low *et al*., 2007; Low, 2013). Given its adaptability, low-external input requirements, nutritional quality, and improvement potential, it is not surprising that sweet potato has become a priority in crop-based strategies for enhancing food security in the tropics (Pfeiffer & McClafferty, 2007; Bill and Melinda Gates Foundation, 2011; Bouis and Islam, 2012).

Notable among the production constraints of sweet potatoes is the issue of insects, with the Sweet potato weevil as the main pest. With the different species

involved and their distribution in different habitats, the true identity of the pests needs to be ascertained, as it is of crucial essence in the development of strategies for their management. The identification process is usually exacerbated by variations in the morphology of the pest.

Variation is a natural feature of any morphological character (Plavcan, 2012). Populations of the same species inhabiting distinct localities can experience different ecological and climatic conditions, giving rise to variation in one or more traits (Bulgarella *et al.*, 2015). Size variations provide significant clues about the taxonomy, morphology, behaviour, and life history of species, yet they are also a major source of difficulty when attempting to establish species limits within a genus.

Cylas weevils are key pests of sweet potato worldwide, especially in drier agro-ecological zones. Nine species within the genus *Cylas* have been identified as pests that attack sweet potato and are placed in three species groups: *Cylas brunneus* Fabricius,1797*, Cylas puncticollis* Boheman, 1833, and *Cylas formicarius* (Fabricius, 1798) (Wolfe, 1991). The distribution of *Cylas* species varies between regions -*C. formicarius* is the most widespread, while *C. puncticollis* and *C. brunneus* are confined to Africa (Wolfe, 1991). In southern Ghana, *C. puncticollis* and *C. brunneus* co-occur geographically in sympatry, and previous studies on the genus *Cylas* revealed multiple sympatric species feeding on a single variety of *Ipomoea* (Agbessenou *et al.*, 2016). The fact that distinct species do occur in sympatry in southern Ghana is a good pointer to the potential problem of species identification in the field.

Wolfe (1991) proposed a taxonomic key to separate these three species groups. However, the most difficult systematic problems in *Cylas* involve the identification of members of each group. *Cylas puncticollis* is the most problematic species within the *C. puncticollis* species group, and some populations currently assigned to the *C. puncticollis* group may represent undescribed morphologically-similar cryptic species (Wolfe, 1991). Generally, cryptic species interfere with the recognition of taxonomic diversity and obscure levels of intra-specific variation. In addition, much attention has been focused on inter-specific patterns of variation, while intra-specific variation in *Cylas* species is unexplored. Many techniques have been used to explore intra-specific variation, with morphometrics being one of them. In this study, a within-group comparative method was applied to the taxonomy of the different populations of the genus *Cylas* sampled from different habitats, with the aim of comparing body size of adult weevils.

Methods

Field sites, sampling procedure and collection of *Cylas* spp.

The survey was conducted during the 2015 cropping season from July to December in the four leading sweet potato production regions in southern Ghana (Bidzakin *et al.*, 2014) -the Central, Eastern, Greater Accra and Volta regions (Table 1).

In the regions, potato farms of at least 0.5 acres were selected from at least two districts for the study. Sampling was carried out when crops were about three months old and ready for harvesting. This was based on the methodology reported by McSorley and Jansson (1991), with some modifications - where each field was divided into four parts (4 quadrants) and a total of 10 infested plant stands or hills selected in each quadrant. Vines and roots with signs of external feeding, oviposition punctures and damage were collected and placed in medium-sized (12 x 15 cm) grocery bags or envelopes, and transported to the laboratory.

Table 1: List of localities (with GPS coordinates) in the 4 regions where samples were collected.

Region	District	Agro-eco zone	Community	Lat	Long	Alt (m)	Weevils
Volta	Ohawu	Coastal Savannah	Bedjame	05° 39′ 17 N	00° 11′ 04 W	113	340
		Coastal Savannah	Kpotavi	06° 11′ 19 N	00° 50′ 58 E	63	1338
	Akatsi South	Coastal Savannah	Tadzevu	06° 07′ 35 N	00° 48′ 13 E	55	134
		Coastal Savannah	Awalavi	06° 07′ 35 N	00° 48′ 13 E	55	640
		Coastal Savannah	Ative	06° 07′ 35 N	00° 48′ 13 E	55	665
Central	Komenda Edna Eguafo Abirem	Coastal Savannah	Komenda	05° 04′ 14 N	00° 14′ 31 W	16	880
	Cape Coast	Coastal Savannah	Mpaesem	05° 07′ 17 N	01° 16′ 06 W	18	567
Eastern	Ayensuano	Semi-Deciduous Forest	Marfo	05° 51′ 91 N	00° 26′ 78 W	155	651
	Begoro	Semi-Deciduous Forest	Ehiamakyene	06° 23′ 29 N	00° 22′ 46 W	470	76
	Upper Manya	Semi-Deciduous Forest	Poponyafantem	06° 06′ 06 N	00° 00′ 57 W	98	310
	Kwahu East	Semi-Deciduous Forest	Akwasiho	06° 32′ 18 N	00° 45′ 19 W	260	105
Greater-Accra	Accra Municipality	Coastal Savannah	University Farm	05° 10′ 21 N	01° 17′ 49 W	76	655
	Ga-West	Coastal Savannah	Manchie	05° 45′ 57 N	00° 00′ 57 W	67	325

Sweet potato root incubation and adult collection

Field samples were incubated in rearing boxes (17 x 17 x 9.5 cm) and held till pupae and/or adult emergence in the laboratories of the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana (Plate 1). Set ups were monitored for development of pupae and/or adults. Pupae were collected from rearing boxes and kept in small boxes until adults emerged. After emergence, weevils were allowed to feed for 5 days on freshly-introduced sweet potato roots for full adult development and to attain full body colouration. At maturity, weevils were killed by freezing, and samples preserved in vials containing 70% alcohol.

A)  B) 

Plate 1: Incubation set up for collecting sweet potato weevils, A = incubation boxes with samples from the field, and B = split sweet potato roots in an incubation box

Insect identification

Identification was made with a Leica EZ4 D stereo microscope based on the morphological characteristics of the collected specimens. Voucher specimens were deposited at ARPPIS-UG and the Royal Belgian Institute of Natural Sciences (RBINS) in Brussels, Belgium.

Morphometric measurements

The procedure followed the general processes of slide preparation of Billah *et al.* (2005; 2008). Field collected samples were dissected using a Leica EZ4D Stereomicroscope. Imaging of mounted specimens was done using video microscopy – the Leica EZ4D Stereomicroscope fitted with an inbuilt camera and connected to a laptop. Body parts were measured using the Leica Application Software (Version 2.1). They included body length (*bl*); elytra length (*el*); elytra width (*ew*); elytra width at base (*ewb*); elytra width at apex (*ewa*); head length (*hl*); head width (*hw*); rostrum length (*rl*); pronotum length (*pl*); pronotum width (*pw*); pronotum width at apex (*pwa*); and pronotum width at base (*pwb*) (Figure 1).

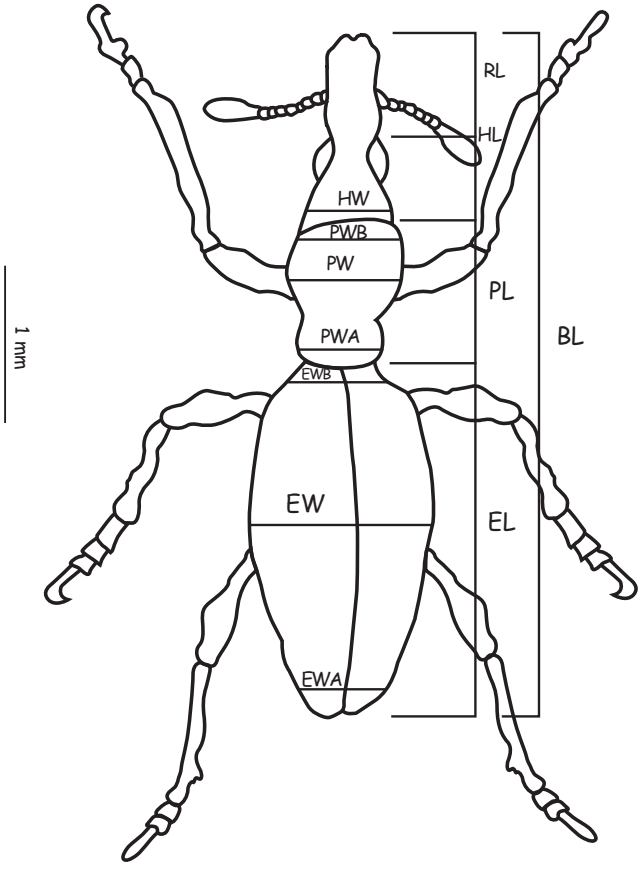


Fig. 1: Drawing of adult sweet potato weevil showing linear measurements of the 12 variables taken for morphometric analyses - body length (*bl*), elytra length (*el*), elytra width (*ew*), elytra width at base (*ewb*), elytra width at apex (*ewa*), head length (*hl*), head width (*hw*), rostrum length (*rl*), pronotum length (*pl*), pronotum width (*pw*), pronotum width at apex (*pwa*), and pronotum width at base (*pwb*)

Data analyses

All morphometric analyses were performed using Statistical Analysis System software version 8.2 (SAS Institute Inc., 2003). When ANOVAs were significant (P = 0.05), means were separated using the Student-Newman-Keuls (SNK) test. Multivariate statistical analyses, i.e. analysis of variance, principal components analysis and canonical analysis, were used to detect any possible variations (Kimani-Njoku *et al.*, 2001; Billah *et al.*, 2005; 2008). Principal Component Analysis (PCA) and Canonical Variate Analysis (CVA) were performed in the variance-covariance matrix of the 12 variables measured on the weevil populations. Mahalanobis squared distances (D^2) between the various populations were obtained as a measure of the relatedness between the populations based on means, variances and covariances.

Results

Two species were identified from the field materials – *C. puncticollis* and *C. brunneus* (Plate 2). Preliminary analyses of the data showed differences in the body sizes of male and female individuals of the same species (results not shown here), with the females being slightly bigger than the males. Based on this observation, all further analyses were conducted on same-sex basis.

Males of C. puncticollis

Table 2 shows mean linear measurements of males of *C. puncticollis* populations from the four regions. Among the measurements, elytra width at the base (*ewb*) and pronotum width at the apex (*pwa*) were two variables which showed significant differences big enough (P = 0.0002 and P < 0.0001, respectively) to separate the populations into three clusters. With *ewb*, individuals from the Central Region were shown to have the largest values, followed by those from the Eastern and Volta (which did not differ from each other), and Greater Accra had the smallest base elytra width. With pronotum width at the apex, the largest values were from the Eastern, followed by Central and Volta regions (which did not also differ from each other); the least were from Greater Accra. Five other variables, *bl, ewa, pl, pw* and *pwb,* could only separate the populations into two clusters, while the last five variables showed no significant differences in variation.

A)

B)

Plate 2: General habitus of adult sweet potato weevils. A) *Cylas puncticollis* (female) and B) *Cylas brunneus* (female). (Photos: Camille Locatelli, RBINS, Belgium)

Projection of the dataset of body measurements on the first two principal axes showed incomplete separation of the four populations, with the first two components accounting for 59.4% (PC1 = 36.8% + PC2 = 22.6%) of the total size variation (Fig. 2A). However, projection of the dataset on the first two canonical axes showed a much better separation trend between weevil populations, accounting for 90.2% (CV 1= 55.9% + CV 2 = 34.3%) of the variation (Fig. 2B). Clusters representing the population from the Greater-Accra, Central and Eastern regions were clearly separated, while the population from the Volta Region was only separated from that of the Central Region, but was found lying between the Greater Accra and Eastern Region populations. This separation pattern is in line with the outcome of the Mahalanobis squared distances (Table 6), where the largest separation value (D^2 = 7.28) was between populations of the Greater Accra and the Central regions, with those of the Eastern and Volta regions lying between the two. The smallest distance of D^2 = 3.00 was between populations of the Greater Accra and Volta regions (indicating the level of relatedness of the two populations), and thus corroborating the clustering seen in Fig 2B (Table 6A).

Table 2: Linear measurement (± SE) comparison of male populations of *C. puncticollis* from four regions (Central, Eastern, Greater Accra, and Volta)

Region	Mean linear measurements (mm) (± SE)											
	*bl	el	ew	ewb	ewa	hl	hw	Rl	pl	pw	pwa	pwb
Central	6.690 ± 0.082 b (10)	3.452 ± 0.036 a (10)	1.592 ± 0.019 a (10)	0.656 ± 0.016 a (10)	0.468 ± 0.019 a (10)	0.894 ± 0.017 a (10)	0.778 ± 0.008 a (10)	1.064 ± 0.023 a (10)	1.154 ± 0.015 b (10)	0.922 ± 0.012 b (10)	0.638 ± 0.014 b (10)	0.826 ± 0.009 b (10)
Eastern	6.900 ± 0.072 a (14)	3.497 ± 0.052 a (14)	1.586 ± 0.021 a (14)	0.617 ± 0.012 b (14)	0.420 ± 0.011 b (14)	0.887 ± 0.015 a (14)	0.777 ± 0.011 a (14)	1.071 ± 0.019 a (14)	1.196 ± 0.011 b (14)	0.950 ± 0.010 ab (14)	0.714 ± 0.010 a (14)	0.854 ± 0.009 a (14)
Greater Accra	6.929 ± 0.089 a (7)	3.571 ± 0.063 a (7)	1.583 ± 0.029 a (7)	0.554 ± 0.016 c (7)	0.400 ± 0.000 b (7)	0.886 ± 0.010 a (7)	0.783 ± 0.013 a (7)	1.079 ± 0.023 a (7)	1.263 ± 0.018 a (7)	0.966 ± 0.011 a (7)	0.589 ± 0.011 c (7)	0.857 ± 0.013 a (7)
Volta	6.664 ± 0.056 b (14)	3.411 ± 0.034 a (14)	1.537 ± 0.012 a (14)	0.600 ± 0.007 b (14)	0.397 ± 0.003 b (14)	0.897 ± 0.011 a (14)	0.763 ± 0.009 a (14)	1.054 ± 0.014 a (14)	1.187 ± 0.016 b (14)	0.919 ± 0.070 b (14)	0.630 ± 0.011 b (14)	0.825 ± 0.008 b (14)
F	3.47	1.85	1.87	8.60	7.75	0.14	0.72	0.30	6.59	3.84	19.60	3.17
Df	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41	3, 41
P	0.0245	0.1531	0.1504	0.0002	0.0003	0.9334	0.5478	0.8269	0.0010	0.0165	< 0.0001	0.0341

Means in the same column followed by different letters are significantly different (P = 0.05), using Student-Newman-Keuls (SNK) test, while those in same column with different letters together are not significantly different from each other. Figures in parentheses are numbers of replicates.

*bl-body length; el-elytra length; ew-elytra width; ewb-elytra width at base; ewa-elytra width at apex; hl-head length; hw-head width; rl-rostrum length; pl-pronotum length; pw-pronotum width; pwa-pronotum width at apex; pwb-pronotum width at base

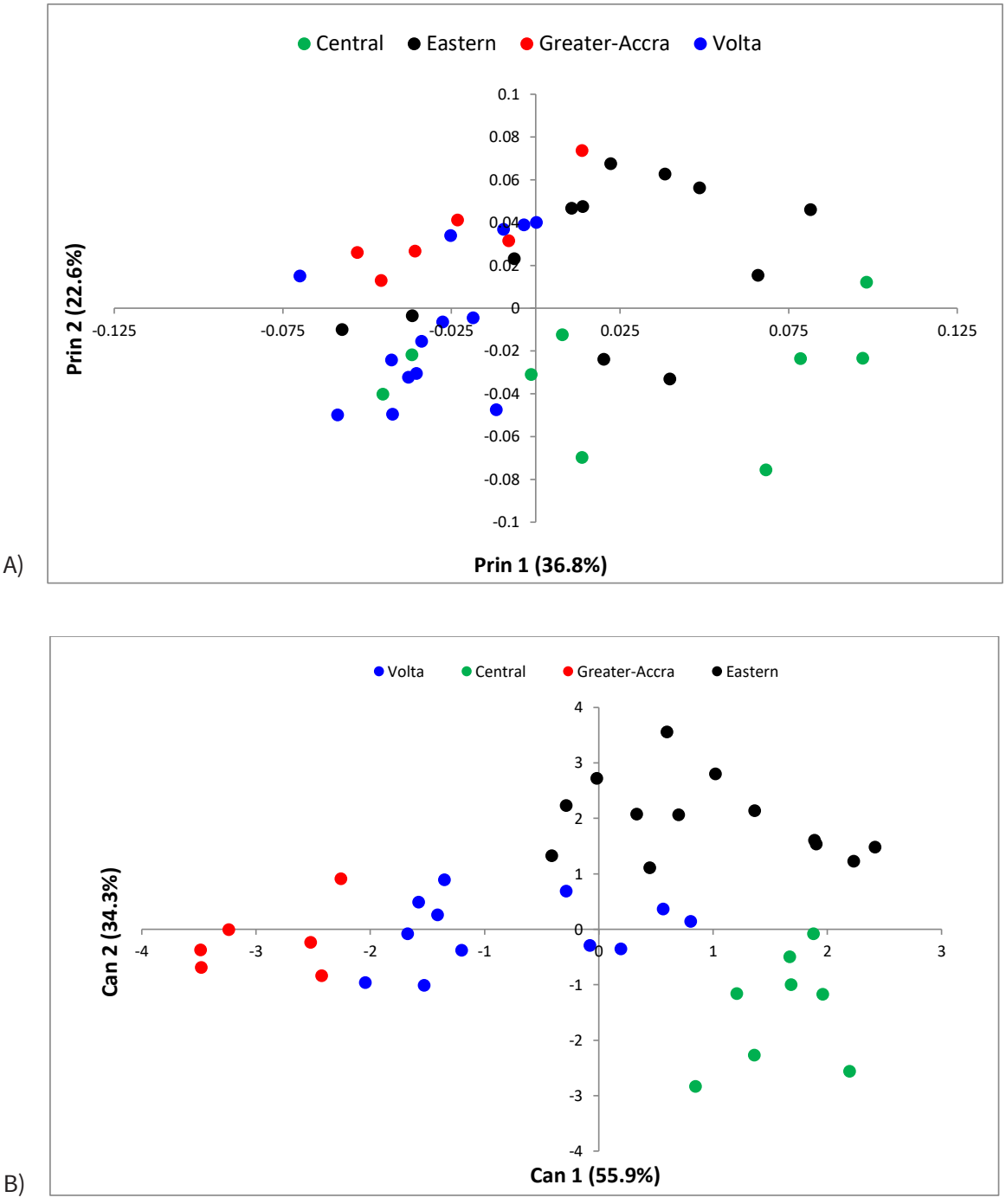


Fig. 2: Projection of linear body measurement dataset of male individuals of *Cylas puncticollis.* A) First two principal components, B) First two canonical variates.

Females of C. puncticollis

The only variable that could tell differences in the population was *pwa*, which put them in three clusters, while four other variables (*el, hw, rl* and *pwb*) could separate them into two clusters (Table 3). Projection of the dataset on the first two principal component axes produced a fuzzy separation of the populations, accounting for 64.8% (PC 1 = 44.0% + PC 2 = 20.8%) of the variation (Fig 3A). However, when the dataset was projected on the first two canonical axes, variation as high as 90.6% (CV 1= 74.3% + CV 2 = 16.3%) was accounted for, thus separating the populations into three clusters – Eastern, Greater Accra and Volta, with individuals from the Central Region lying between, and not clearly separated from the others (Fig 3B). Values in Table 6A show the most distant groups as the Greater Accra and Eastern populations (D^2 = 6.14), followed by the Volta and Eastern (D^2 = 3.36). The smallest value (and the most closely related) was between populations from the Volta and Central regions (D^2 = 1.43; Table 6A).

Table 3. Linear measurement (± SE) comparison of female populations of *C. puncticollis* from four regions (Central, Eastern, Greater Accra, and Volta).

Region	Mean linear measurements (mm) (± SE)											
	*bl	El	Ew	ewb	ewa	hl	hw	rl	pl	pw	pwa	pwb
Central	6.810 ± 0.156 b (10)	3.472 ± 0.063 b (10)	1.584 ± 0.027 a (10)	0.624 ± 0.026 a (10)	0.408 ± 0.014 a (10)	0.690 ± 0.016 a (10)	0.804 ± 0.014 b (10)	1.211 ± 0.039 b (10)	1.290 ± 0.032 b (10)	1.028 ± 0.025 a (10)	0.666 ± 0.013 b (10)	0.864 ± 0.013 b (10)
Eastern	7.092 ± 0.092 ab (13)	3.594 ± 0.046 ab (13)	1.585 ± 0.033 a (13)	0.615 ± 0.010 a (13)	0.415 ± 0.010 a (13)	0.720 ± 0.011 a (13)	0.826 ± 0.010 b (13)	1.302 ± 0.020 a (13)	1.318 ± 0.025 ab (13)	1.020 ± 0.017 a (13)	0.712 ± 0.015 a (13)	0.903 ± 0.010 ab (13)
Greater Accra	7.267 ± 0.133 a (6)	3.740 ± 0.063 a (6)	1.667 ± 0.027 a (6)	0.600 ± 0.000 a (6)	0.393 ± 0.007 a (6)	0.710 ± 0.013 a (6)	0.870 ± 0.015 a (6)	1.295 ± 0.019 a (6)	1.403 ± 0.034 a (6)	1.073 ± 0.028 a (6)	0.603 ± 0.013 c (6)	0.923 ± 0.025 a (6)
Volta	6.960 ± 0.072 ab (10)	3.520 ± 0.040 b (10)	1.576 ± 0.019 a (10)	0.584 ± 0.015 a (10)	0.400 ± 0.000 a (10)	0.708 ± 0.015 a (10)	0.816 ± 0.008 b (10)	1.230 ± 0.012 b (10)	1.342 ± 0.018 ab (10)	1.012 ± 0.012 a (10)	0.638 ± 0.010 bc (10)	0.878 ± 0.015 ab (10)
F	2.346	3.718	1.458	1.143	0.850	0.911	4.316	3.057	2.362	1.310	10.600	2.784
Df	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35	3, 35
P	0.090	0.020	0.243	0.345	0.476	0.446	0.011	0.041	0.088	0.287	< 0.0001	0.055

Means in the same column followed by different letters are significantly different (P = 0.05), using Student-Newman-Keuls (SNK) test, while those in same column with different letters together are not significantly different from each other. Figures in parentheses are numbers of replicates.

*bl-body length; el-elytra length; ew-elytra width; ewb-elytra width at base; ewa-elytra width at apex; hl-head length; hw-head width; rl-rostrum length; pl-pronotum length; pw-pronotum width; pwa-pronotum width at apex; pwb-pronotum width at base

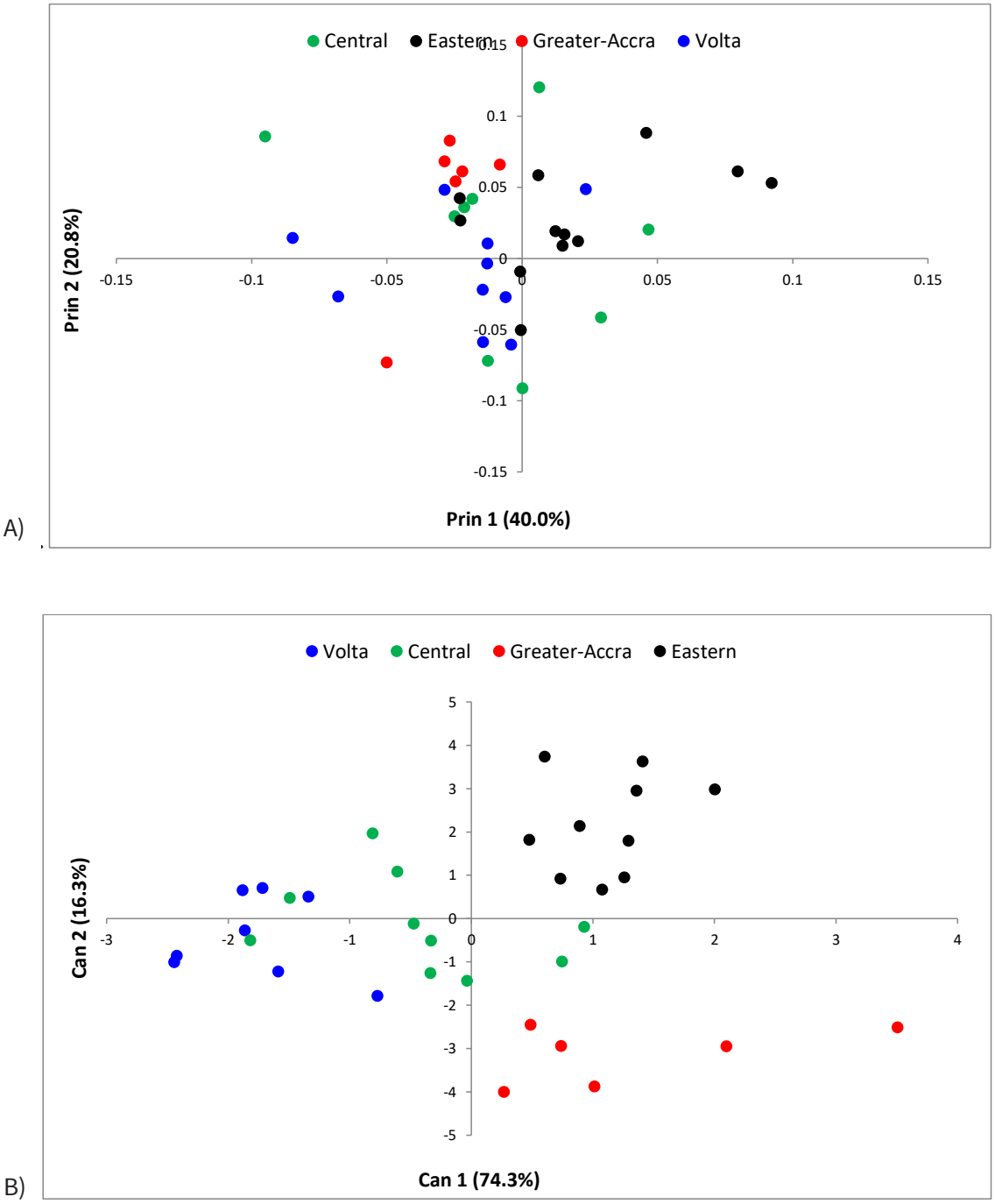


Fig. 3: Projection of linear body measurement dataset of female individuals of *Cylas puncticollis.* A) First two principal components, B) First two canonical variates

Males of C. brunneus

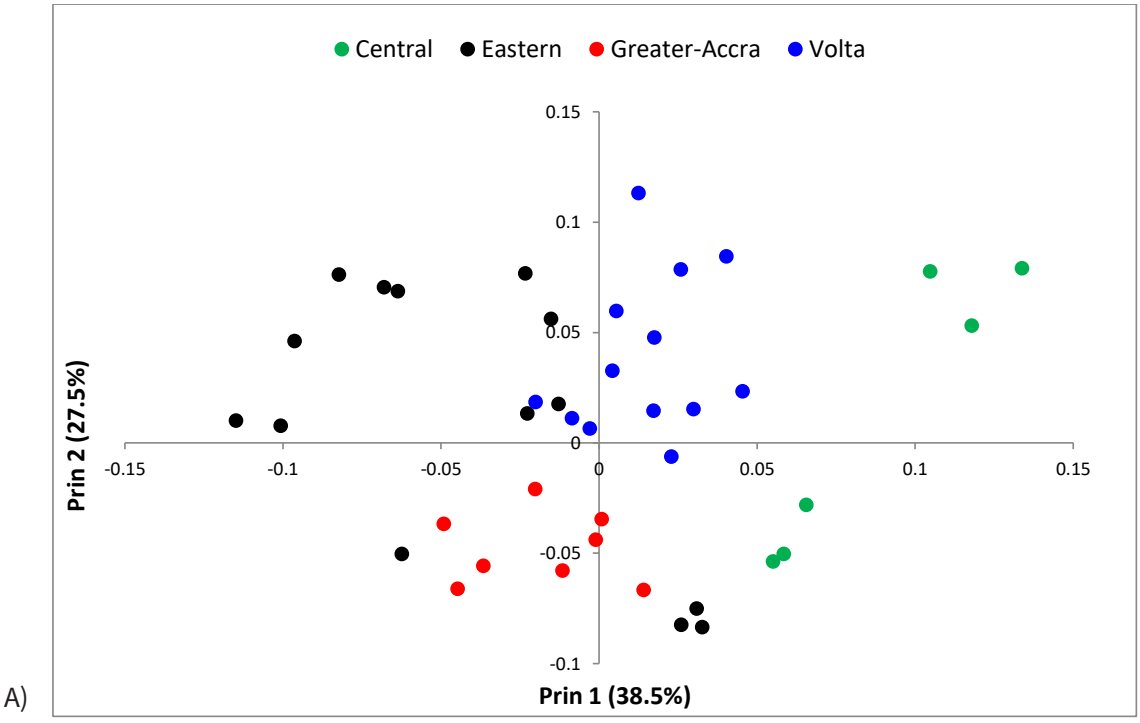
Table 4 shows that only variables *ewa* and *hl* had significant differences that could separate the populations into three groups, while *ewb, rl, pwa* and *pwb* could only identify them as two groups. Projection of the data on the first two principal component and canonical variate axes shows 66.0% (PC 1 = 38.5% + PC 2 = 27.5%) and 91.1% (CV 1 = 70.1% + 21.0%) of variations being accounted for in Figs. 4A and 4B, respectively. The canonical plot (Fig. 4B) shows populations from the Eastern, Greater Accra and Central regions almost in different quadrants, with those of the Volta Region lying in the middle. However, the largest distance between the centroids of the clusters was seen in populations of the Eastern and Central regions ($D^2 = 4.97$), followed by $D^2 = 4.44$ between the Greater Accra and Central regions. The closest was between the Greater Accra and Eastern regions ($D^2 = 1.60$), followed by $D^2 = 1.62$ between the Greater Accra and Volta regions (Table 6B).

Table 4: Linear measurement (± SE) comparison of male populations of *C. brunneus* from four regions (Central, Eastern, Greater Accra, and Volta).

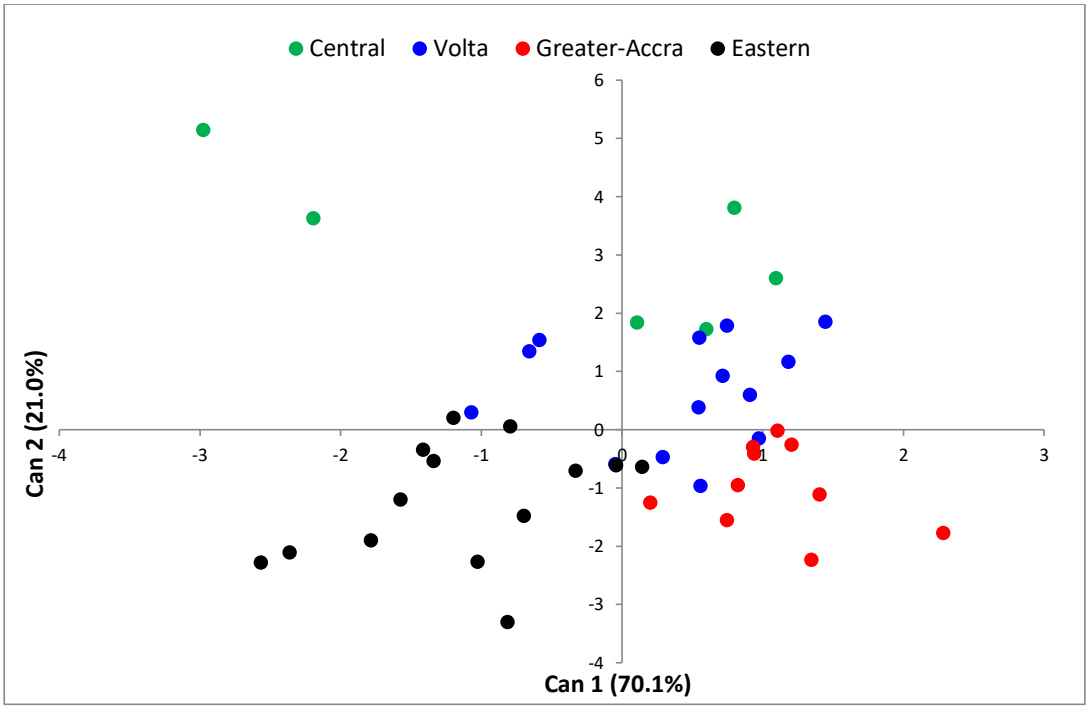
Region	Mean linear measurements (mm) (± SE)											
	*bl	el	Ew	ewb	ewa	hl	hw	rl	pl	pw	pwa	pwb
Central	5.583 ± 0.065 a (6)	2.893 ± 0.041 a (6)	1.273 ± 0.022 a (6)	0.527 ± 0.028 a (6)	0.427 ± 0.013 a (6)	0.840 ± 0.023 a (6)	0.613 ± 0.012 a (6)	0.738 ± 0.013 b (6)	1.017 ± 0.012 b (6)	0.777 ± 0.012 a (6)	0.453 ± 0.012 b (6)	0.650 ± 0.015 ab (6)
Eastern	5.653 ± 0.054 a (15)	2.888 ± 0.023 a (15)	1.245 ± 0.016 a (15)	0.483 ± 0.011 ab (15)	0.355 ± 0.008 c (15)	0.728 ± 0.014 c (15)	0.617 ± 0.006 a (15)	0.803 ± 0.013 a (15)	1.091 ± 0.016 a (15)	0.789 ± 0.009 a (15)	0.507 ± 0.018 a (15)	0.667 ± 0.006 a (15)
Greater Accra	5.500 ± 0.082 a (10)	2.796 ± 0.050 a (10)	1.212 ± 0.023 a (10)	0.448 ± 0.009 b (10)	0.352 ± 0.005 c (10)	0.750 ± 0.017 bc (10)	0.598 ± 0.010 a (10)	0.781 ± 0.014 a (10)	1.066 ± 0.020 ab (10)	0.770 ± 0.017 a (10)	0.466 ± 0.009 b (10)	0.634 ± 0.010 b (10)
Volta	5.700 ± 0.049 a (15)	2.915 ± 0.029 a (15)	1.261 ± 0.010 a (15)	0.469 ± 0.009 a (15)	0.389 ± 0.005 b (15)	0.793 ± 0.012 ab (15)	0.615 ± 0.005 a (15)	0.798 ± 0.012 a (15)	1.075 ± 0.012 ab (15)	0.793 ± 0.008 a (15)	0.507 ± 0.006 a (15)	0.656 ± 0.005 ab (15)
F	1.974	2.242	1.978	5.060	17.157	8.450	1.392	3.098	2.607	0.904	3.617	3.049
Df	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42	3, 42
P	0.133	0.097	0.132	0.004	< 0.0001	< 0.0001	0.259	0.037	0.064	0.447	0.021	0.039

Means in the same column followed by different letters are significantly different (P = 0.05), using Student-Newman-Keuls (SNK) test, while those in same column with different letters together are not significantly different from each other. Figures in parentheses are numbers of replicates.

*bl-body length; el-elytra length; ew-elytra width; ewb-elytra width at base; ewa-elytra width at apex; hl-head length; hw-head width; rl-rostrum length; pl-pronotum length; pw-pronotum width; pwa-pronotum width at apex; pwb-pronotum width at base



A)



B)

Fig. 4: Projection of linear body measurement dataset of male individuals of *Cylas brunneus.* A) First two principal components, B) First two canonical variates

4.4. Females of C. brunneus

In this group, only two of the 12 variables made a significant difference - elytra width at apex (*ewa*) and pronotum width at apex (*pwa*) (Table 5). Elytra width at apex showed populations from Central and Volta being significantly different from those from the Eastern and Greater Accra regions. Based on *pwa*, populations from the Eastern and Central regions are significantly different from each other, but not any different from those of the Greater Accra and Volta regions (Table 5). With the close nature of the group, projection of the dataset on the first two principal component axes did not show much separation, accounting for only 60.5% (PC 1 = 36.4% + PC 2 = 24.1%) of the variation (Fig 5A). Projection on the first two canonical axes accounted for 90.6% (CV 1 = 74.3% + 16.3%) of the variation observed in the two variables – *ewa* and *pwa* (Fig 5B).Mahalanobis squared distance (D^2 = 4.23) was largest between Eastern and Volta, followed by that between Eastern and Central (D^2 = 4.11). The most closely related populations were Volta and Central (D^2 =1.35), followed by Volta and Greater Accra (D^2 =1.47) (Table 6B).

Table 5: Linear measurement (± SE) comparison of female populations of *C. brunneus* from four regions (Central, Eastern, Greater Accra and Volta)

Region	Mean linear measurements (mm) (± SE)											
	*bl	El	ew	ewb	ewa	hl	hw	rl	pl	pw	pwa	pwb
Central	5.520 ± 0.107 a (5)	2.896 ± 0.041 a (5)	1.280 ± 0.013 a (5)	0.512 ± 0.015 a (5)	0.400 ± 0.000 a (5)	0.680 ± 0.011 a (5)	0.636 ± 0.009 a (5)	0.817 ± 0.019 a (5)	1.148 ± 0.027 a (5)	0.844 ± 0.009 a (5)	0.444 ± 0.018 b (5)	0.692 ± 0.008 a (5)
Eastern	5.664 ± 0.072 a (11)	2.902 ± 0.035 a (11)	1.269 ± 0.014 a (11)	0.476 ± 0.014 a (11)	0.349 ± 0.008 b (11)	0.655 ± 0.012 a (11)	0.636 ± 0.008 a (11)	0.856 ± 0.008 a (11)	1.189 ± 0.012 a (11)	0.840 ± 0.010 a (11)	0.529 ± 0.021 a (11)	0.691 ± 0.009 a (11)
Greater Accra	5.511 ± 0.096 a (9)	2.809 ± 0.058 a (9)	1.249 ± 0.033 a (9)	0.467 ± 0.009 a (9)	0.360 ± 0.009 b (9)	0.667 ± 0.011 a (9)	0.629 ± 0.008 a (9)	0.841 ± 0.015 a (9)	1.153 ± 0.021 a (9)	0.822 ± 0.017 a (9)	0.482 ± 0.017 ab (9)	0.676 ± 0.008 a (9)
Volta	5.593 ± 0.07 a (15)	2.883 ± 0.028 a (15)	1.251 ± 0.012 a (15)	0.501 ± 0.009 a (15)	0.395 ± 0.007 a (15)	0.677 ± 0.007 a (15)	0.624 ± 0.005 a (15)	0.834 ± 0.009 a (15)	1.163 ± 0.014 a (15)	0820 ± 0.007 a (15)	0.497 ± 0.008 ab (15)	0.669 ± 0.007 a (15)
F	0.671	1.038	0.480	2.655	9.883	1.224	0.820	1.369	1.035	1.138	3.720	1.978
Df	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36	3, 36
P	0.575	0.387	0.698	0.063	< 0.0001	0.315	0.492	0.268	0.389	0.347	0.020	0.135

Means in the same column followed by different letters are significantly different (P = 0.05), using Student-Newman-Keuls (SNK) test, while those in same column with different letters together are not significantly different from each other. Figures in parentheses are numbers of replicates

*bl-body length; el-elytra length; ew-elytra width; ewb-elytra width at base; ewa-elytra width at apex; hl-head length; hw-head width; rl-rostrum length; pl-pronotum length; pw-pronotum width; pwa-pronotum width at apex; pwb-pronotum width at base

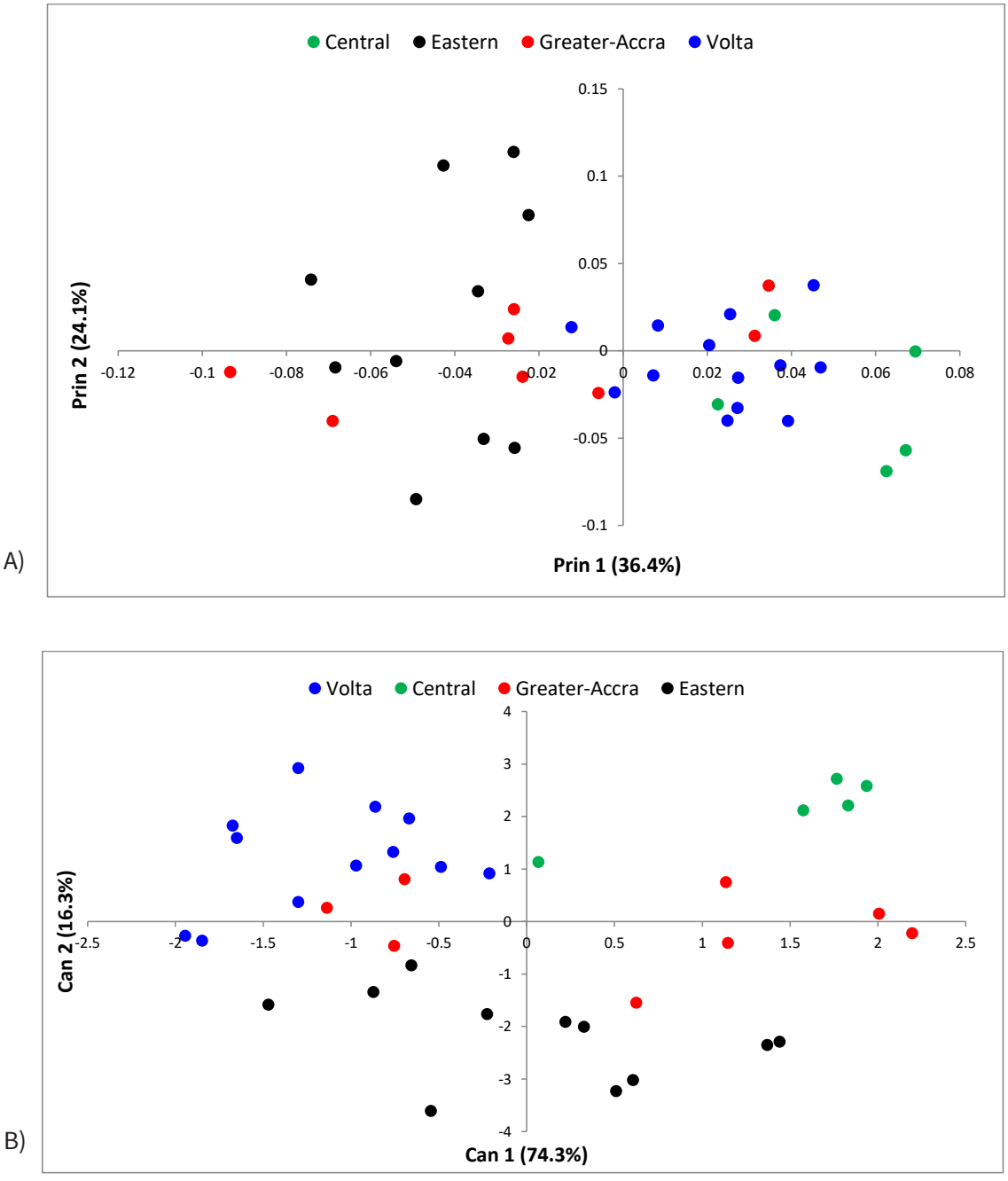


Fig. 5: Projection of linear body measurement dataset of female individuals of *Cylas brunneus.* A) First two principal components, B) First two canonical variates

Table 6. Mahalanobis squared distances (D^2) between sweet potato weevil populations from the four regions (Central, Eastern, Greater Accra and Volta). A = *Cylas puncticollis* **(male and female), and B =** *Cylas brunneus* **(male and female).**

A)

Region	Central	Eastern	Greater Accra	Volta
		Males		
Central	0			
Eastern	4.92	0		
Greater Accra	7.28	6.35	0	
Volta	3.84	3.50	3.00	0
		Females		
Central	0			
Eastern	2.60	0		
Greater Accra	3.06	6.14	0	
Volta	1.43	3.36	3.02	0

B)

Region	Central	Eastern	Greater Accra	Volta
		Males		
Central	0			
Eastern	4.97	0		
Greater Accra	4.44	1.60	0	
Volta	2.33	2.34	1.62	0
		Females		
Central	0			
Eastern	4.11	0		
Greater Accra	1.78	1.55	0	
Volta	1.35	4.23	1.47	0

Discussion

This study has expanded our appreciation of size variation in both *C. puncticollis* and *C. brunneus* and provided us solid information about geographical size variation in both species. Traditional morphometrics represents one of the most commonly used ways of investigating intra- and inter- specific body size variation among organisms within a given population (Rohlf & Bookstein, 1990; Umphrey, 1996; Billah, 2004). Morphometric studies revealed differences in traits in the population of the two weevil species across regions. In the males of *C. puncticollis,* the two variables that put the population into three clusters were *ewb* (elytra width at base) and *pwa* (pronotum width at apex). The population from the Central Region had the largest trait, followed by those from Eastern and Volta (which did not differ significantly); Greater Accra had the smallest size. In terms of *pwa,* Greater Accra still had the smallest pronotum width at the apex, and Eastern the highest value, while Central and Volta were found between the extremes (and did not differ from each other). In the females, however, only one variable (*pwa*) was able to put the populations into three clusters, and the separation was as observed in the males – Eastern, Central (together with Volta) in the middle, and Greater Accra with the smallest size.

In *C. brunneus,* two variables (*ewa* and *hl*) were responsible for putting the males into three clusters. In terms of elytra width at apex, Central had the biggest size, followed by Volta, and then Eastern and Greater Accra (as one cluster). In the head length too, Central had the biggest value and Eastern the smallest value, with Volta and Greater Accra falling between the two values. Interestingly, Volta did not differ significantly from the high Central values, but neither did Greater Accra differ significantly from the low Eastern values. In the females, however, no variable could separate the populations into more than two clusters, and only two variables (*ewa* and *pwa*) had significant differences. While *pwa* was common in both the male and female populations of *C. puncticollis, ewa* was common in both the male and female populations of *C. brunneus.*

Some of these characteristics have been reported in previous studies to be the source of variation and were used to describe individuals. Although the presence of cryptic species cannot be ruled out, the variation in traits could be the result of the polytypic status of some of the species such as the *C. puncticollis* group, to which species have been assigned to the group (Wolfe, 1991), and also due to the effect of the environment/habitat (Chown & Gaston, 2010). Traditional morphometrics represents one of the most commonly used ways of investigating intra- and inter- specific body size variation among organisms within a given population. This study has revealed differences in traits in the population of the two weevil species across regions.

For the 12 variables used in this study, the multivariate analyses were able to rearrange and rank those that played principal roles in the separation of the individuals into the population identities assigned them. There were 2-7 variables that were used in the four *Cylas* populations (*C. puncticollis* males + females and *C. brunneus* males + females). In the *C. puncticollis* males, two of the variables (*ewb* and *pwa*) could be used to separate populations from the four regions into three clusters, while five other variables (*bl, ewa, pl, pw* and *pwb*) separated the populations into two clusters. In the females of the same species, one variable (*pwa*) separated the populations into three clusters, while four others (*el, hw, rl* and *pwb*) could only separate them into two clusters. In the *C. brunneus* males, two variables (*ewa* and *hl*) separated the populations into three clusters, while four others (*ewb, rl, pwa,* and *pwb*) could only separate them into two clusters. In the females, only two variables (*ewa* and *pwa*) were of significant differences to separate them into two clusters. In all cases, the variables which contributed most in grouping of the populations were *pwa, pwb, pl, pw, ewa, ewb,*and occasionally, *bl, hl, hw, rl,* and *el*, representing measurements of different parts of the pronotum, elytra, head, rostrum, and body length. These have thus become the principal variables that contribute most to the variations observed in the populations.

Conclusion

Identified as a powerful tool in species delimitation, the morphometric study has provided an in-depth knowledge of the status of the sweet potato weevil. It has been demonstrated in this study that variations in body size exist among individuals within each species across and within the four regions. It is therefore suggested that more studies be conducted on the two common species, *C. puncticollis* and *C. brunneus*, to ascertain their true taxonomic status, which has important practical implications for the effective development and use of management strategies against such pest complexes, and which can also result in the incorrect establishment of trade barriers for agricultural commodities that serve as hosts of the sweet potato pests.

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