



Morphometric characterization and comparison of the African rhinoceros beetle (AfRB) *Oryctes monoceros* Olivier (Coleoptera: Dynastidae) populations from different agro-ecological zones in Ghana

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Abstract

The African rhinoceros beetle (AfRB), *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae), is a very destructive coconut pest in Africa and Asia. Proper identification of a target pest is essential for developing integrated pest management strategies. Therefore, we morphologically characterized and compared AfRB populations from Coastal savannah, Transitional, Evergreen, and Deciduous forest agro-ecological zones in Ghana. Linear measurements of 24 morphological traits were selected, measured, and analyzed using t-test, ANOVA, MANOVA, PCA and the UPGMA method. The results showed variations in AfRB males and females, with males consistently larger than females. The AfRBs differed across the agro-ecological zones, with those from the Coastal savannah being the smallest. Beetles from the Transitional zone appeared larger, whereas those from the Deciduous forest were intermediate. Principal component analyses of the males and females from the agro-ecological zones showed overlaps of AfRB populations, with no clear separation. However, dendrogram formed by the UPGMA method using squared Euclidean distance of female AfRB populations showed that the Deciduous and Evergreen samples were similar but distinct from Coastal savannah and Transitional zones. Here we report that three subgroups can be morphologically separated within female AfRB populations in this study: (1) Deciduous and Evergreen, (2) Coastal savannah and (3) those from the Transitional zone. Our findings show that the morphometric variations of AfRB are influenced by the agro-ecological zones, which may effect the fitness parameters and dispersal potential of the pest.

Keywords AfRB · African rhinoceros beetle · Coconut · Morphometry · *Oryctes monoceros*

Introduction

Coconut (*Cocos nucifera* L.) belongs to the palm family Arecaceae (Palmaceae), and the only existing species classified into the genus *Cocos*. It is found in tropical and subtropical regions of the world, with over 62.45 million tonnes cultivated in 93 countries, including Central and South America, East and West Africa, Southeast Asia and the Pacific Islands, with a total cultivated area of more than 12 million hectares (Oyoo et al. 2015; Gurr et al. 2016; FAOSTAT 2020). Coconut and coconut-related activities generate income for millions of farmers and play a crucial role in wealth generation and improving the quality of life, especially among rural farmers (Gurr et al. 2016). The health benefits of coconut include the provision of vitamins, minerals, fatty acids, and dietary fibre (Enig 2010; Gurr et al. 2016).

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Despite the economic importance of coconut, arthropod pests, diseases, trunk cutting, settlement expansion, coastal erosion, lack of technical knowledge, inadequate certified planting materials, lack of funds, poor agronomic practices, low-quality planting materials, narrow genetic base, and other as biotic and abiotic factors continue to affect the profitability and sustainability of the industry (Cambell 2006; Pole et al. 2014). However, among the challenges associated with coconut, arthropod pests are a major constraint to the sustainable production of the crop, of which the African rhinoceros beetle (AfRB), *Oryctes monoceros* (Olivier) (Coleoptera: Scarabaeidae: Dynastinae) is the most economically important one (Allou et al. 2006; Tettey et al. 2022; Aidoo et al. 2022a, b).

Globally, AfRB is distributed in Africa and Asia. In Africa, West African countries with AfRB include Mali, Ghana, Côte d'Ivoire, Benin, Guinea, Burkina Faso, Nigeria, Niger, Senegal, Sierra Leone, Togo and Liberia (Philippe and Dery 2004; Allou et al. 2006; EPPO 2020). In East Africa, AfRB has been reported from Kenya, Somalia, Uganda, Malawi, Zanzibar Island and Seychelles (EPPO 2020). In Central Africa, countries with AfRB include Cameroon, Angola, and Congo. It has also been reported in Sudan in North Africa (EPPO 2020). The pest is also present in Mozambique and South Africa (EPPO 2022; Aidoo et al. 2022b). AfRB has been reported in Asia from Saudi Arabia and Yemen (EPPO 2020). In Ghana, AfRB was discovered in 1924, and has established in all coconut-growing regions, posing a serious threat to the coconut industry (Philippe and Dery 2004).

The African rhinoceros beetle is a major pest in Africa's coconut and oil palm, *Elaeis guineensis* Jacq. (Arecaceae) plantations (Bedford 2014), and attacks coconut and oil palm of all ages. However, young palms are the most severely affected (Young and Longworth 1981). The adults bore into the tops of the growing point of the palm through the basal parts of the leaves and enter the heart of the unfolded leaves, or center spike, working downwards, causing damage to the growing points (Philippe and Dery 2004). The youngest expanded leaves then die off, unfold in the central bud, showing triangular cuts at each side of the central rib. The damage to the growing point can serve as an infection court for bacteria and fungi, leading to the rotting of the growing spear (Bedford 2014). The damage also serves as an entry point for the African palm weevil, *Rhynchophorus phoenicis* Fabricius (Coleoptera: Curculionidae). In some cases, the inflorescence becomes badly damaged when the attack occurs on the unopened spathe. Under heavy infestation, damage to the whole shoot may lead to the death of the affected palm. About 40% of AfRB damage to coconut has been reported in tropical Africa (Allou et al. 2006).

Morphometric approaches have been useful in differentiating closely related species, populations, and haplotypes

among insects (Billah et al. 2008; Lashkari et al. 2015; Paris et al. 2016; Aidoo et al. 2019). For example, populations of Asiatic rhinoceros beetle (AsRB), *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae), a close relative of AfRB, have been studied using morphometric traits like the body length, Elytra length, Pronotal length, Pronotal width, and Cephalic horn length (Manjeri et al. 2013), and observed that body length, Pronotal width, and Pronotal length were the most important traits that contributed to the separation of the populations. In a similar study, seven morphological characters, including Elytron length, Body width, Body length, Wing length, Tibia length and Cephalic horn length were used to separate species of AsRB in India (Ramesh et al. 2020). Additionally, Elytra and Pronotum were used by Zhang et al. (2019) to provide insight into the ecology and evolution of stag beetles.

There is a close relationship between Rhinoceros beetles and tracheophyte angiosperms. In the case of AfRB, the species has evolved with coconut and oil palm but feeds on other plants, probably changing its physiognomy, physiology, and phenology, in response to host and environmental changes (Mariau 1967; Lomer 1985; Hallett 1996). AfRB has limited host plants including Angiosperms; Sisal hemp, *Agave sisalana* Perrine (Asparagales: Asparagaceae), Areca palm, *Areca catechu* L. (Arecaceae), *Cocos nucifera*, *Dracaena* Vand. ex L. (Asparagales: Asparagaceae), *El. guineensis*, Date-palm, *Phoenix dactylifera* (Arecaceae), *Raphia* palms, *Raphia hookeri* (CABI 2020). It also feeds on Banana, *Musa sapientum* L. (Zingiberales: Musaceae) and sugarcane, *Saccharum officinarum* L. (Poales: Poaceae) (CABI 2020).

Effects of geographical area on insect's body size have been studied (Chown and Gaston 2010; Heino 2015; Bai et al. 2016; Scriven et al. 2016). However, little is known about the morphometry of AfRB. Therefore, this study aimed to morphometrically characterize and compare populations of AfRB from different agro-ecological zones in Ghana, as well as to determine important morphometric traits that contribute to variation in the populations.

Materials and methods

Data collection and sample preparation

There are six agro-ecological zones in Ghana (Antwi-Agyei et al. 2012; Kemausuor et al. 2013; Rhebergen et al. 2016) Fig. 1.

However, coconut is cultivated in the Transitional zone, Deciduous forest, Evergreen/Rainforest, and Coastal Savannah zones. Sixty-eight AfRB adult females from Transitional zone (11), Deciduous forest (25), Evergreen (11), and Coastal savannah (21), and 37 adult males from Coastal

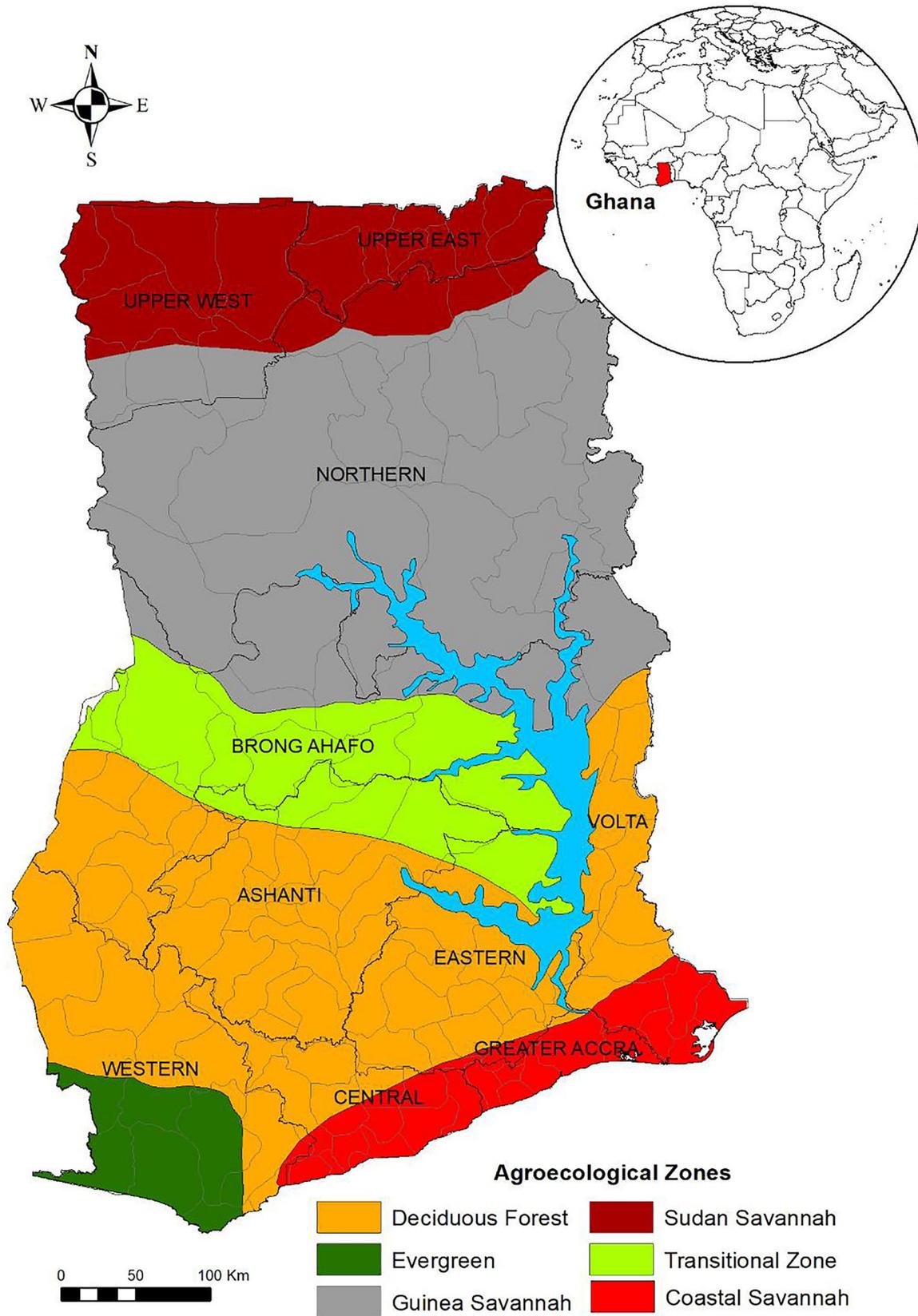


Fig. 1 Map showing the six agro-ecological zones in Ghana

savannah (13), Deciduous forest (10), and Transitional zone (14), collected from coconut plantations, were used for morphological analysis (Table S1). The number of samples collected depended on the availability of AfRB in each agro-ecological zone. Adult beetles were preserved in 75% ethanol, and sent to University of Ghana's African Region Postgraduate Programme in Insect Science (ARPPIS), West Africa Sub-Regional Centre laboratory in Accra, Ghana for further analysis. The description of body parts used for the morphometric analysis is shown in Fig. 2 (1–23). In the present study, we measured the Body length, Pronotal width, Pronotal length, Body width, Wing length, and Wing width. The other body parts measured were Body height, Hind tibia + Tarsals length, and Cephalic horn length. Total Body length was measured as the longest length of the body, and the longest length and width of the elytron were considered Elytral length and width, respectively. The Pronotal length was measured as the longest part of the Pronotum, and the widest part of the Pronotum was considered the Pronotal width. Cephalic horn length was measured from the base to the apex of the horn. The body parts were selected based on taxonomically-stable features of historical use (Billah et al. 2008, 2017; Manjeri et al. 2013; Zhang et al. 2019; Ramesh et al. 2020). All other measurements are illustrated in Fig. 2.

Morphometric traits of each hind wing of AfRB were measured for characterization and comparison. The wings were removed using a pair of forceps from the point of attachment to the body, ensuring they were whole and unbroken or torn. Afterwards, each part was placed in glycerin using a camel hair brush to soften and slide-mounted using Euparal as the mounting medium. Images were captured and digitized using a Leica EZ4 HD Stereomicroscope with an inbuilt digital camera connected to a computer. Only wings and legs from the left side of each insect were

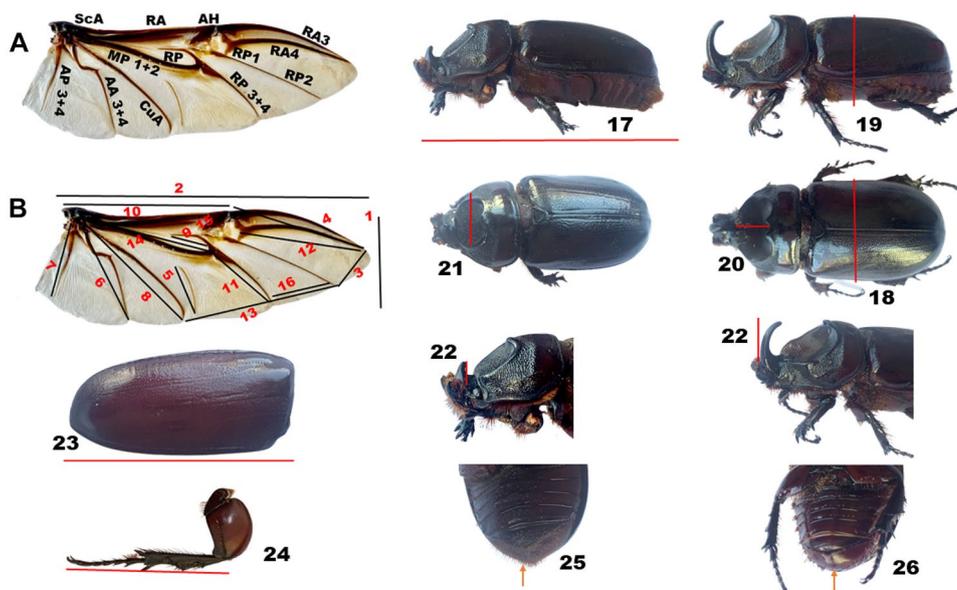
used for measurements to avoid possible negative allometric effects (Rohlf and Slice 1990; Woolley et al. 1994; Billah et al. 2008, 2017). Each specimen was measured three times and averaged for further analysis. All measurements were performed with a digital Mitutoyo calliper at 0.01 mm precision (Manjeri et al. 2013). The separation of males and females was based on abdominal tip hairs as shown in Fig. 2 (25–26). Males have a smooth abdominal tip, whereas females have a profuse growth of reddish-brown hairs on the abdominal tip. Measurements of morphometric traits consisted of 16 hind wing veins, 8 body parts, and 1 ratio value.

Statistical methods

Prior to analysis, the normality of data was tested using the Shapiro–Wilk test. This was followed by $\log_{10}(x + 1)$ transformation to ensure that the data were normally distributed while untransformed linear measurements are presented in the tables. The linear measurements were subjected to two-way multivariate analysis of variance (MANOVA) to determine whether there was variation between morphological characters, sexes due to sexual dimorphism, and agro-ecological zones, and as well as to determine if there is an interaction between agro-ecological zones and sexes due sexual dimorphism of AfRB. Wing and hind leg measurements were analyzed separately to allow covariance between distances, sexes, and species to interact more meaningfully (Woolley et al. 1994; Billah 2004; Billah et al. 2008, 2017).

Afterwards, morphological traits from different agro-ecological zones were compared using analysis of variance (ANOVA). In case a significant difference was detected, means were separated using Tukey's honestly significant difference (HSD) test. To determine any sex-related variations

Fig. 2 *Oryctes monoceros* traits measured for morphometric analysis (1–24). Veins of the hind wings measured (1–16), (17) body length, (18) body width, (19) body height, (20) pronotal length, (21) pronotal width, (22) cephalic horn length male and females, (23) elytron length, (24) hind leg + tarsals, and abdominal tip of male and female (25–26)



in the linear measurements, we used either Welch's t-test or the Student t-test, depending on whether the variance was equal or not. Student t-test was used when the variance was equal, and in case of inequality of variance, the comparison was made using Welch's t-test. The variance of the data was determined using Fligner-Killeen test. All analyses were conducted in R statistical software (version 4.0.3) (R Core Team 2020) at 95% confidence level.

To illustrate the morphological variation of males and females of *O. monoceros* across the different agro-ecological zones, we first submitted the 24 morphological traits recorded to the z-score standardization, then using the Paleontological Statistics (PAST; Version 3.09) software, we computed the Principal Component Analysis (PCA).

Results

The two-way MANOVA showed significant variation between morphological parameters and sexes due sexual dimorphism ($F=22.221$, $df=1(75, 24)$, $p<0.0001$), and agro-ecological zones ($F=2.801$, $df=3(231, 72)$, $p<0.0001$), but there was no interaction between agro-ecological zone and sexes due sexual dimorphism of AfRB

($F=0.960$, $df=2(152, 48)$, $p=0.551$). AfRB adult males were larger than females in most of the morphometric traits considered in the present study (Table 1). Of the morphological traits, 12 characters were significantly different ($p<0.05$). These characters were Cephalic horn length, Elytral length, Pronotal width, Pronotal length, Body height, Body length, MP 1 + 2 vein length of hind wing, RA4 vein length of hind wing, ScA + RA + AH vein length of hind wing length, RA3 vein length of hind wing and wing length of hind wing. However, we did not find a significant difference between male and female AfRB based on the following morphometric traits: Wing width, RP2 – RA3 vein length of hind wing, III-IV vein length of hind wing, AA 3 + 4 vein length of hind wing, AP 3 + 4 vein length of hind wing, CuA vein length of hind wing, RP vein length of hind wing, RP2 vein length of hind wing, CuA—RP2 vein length of hind wing, I-II vein length of forewing, RP3 + 4—RP2 vein length of hind wing and Hind tibia + tarsals (Table 1).

There was no significant difference between the wing aspect ratio (i.e., the ratio between wing length and wing width) (Table 1).

Subsequent comparisons of populations from the different agro-ecological zones showed that agro-ecological zone had effect on the morphology of the AfRB measurement

Table 1 Size of morphological traits (mean \pm standard error) (cm) from female and male *Oryctes monoceros* from different agro-ecological zones

Figure Code	Trait	Male	Female	t-value	df	p-value
1Fig. 1	Wing width	1.55 \pm 0.01	1.52 \pm 0.01	-1.043	120.00	0.299
1Fig. 2	Wing length	4.47 \pm 0.03	4.34 \pm 0.04	-2.488	120.00	0.014
1Fig. 3	RP2 – RA3	0.63 \pm 0.01	0.63 \pm 0.01	0.030	120.00	0.975
1Fig. 4	RA3	1.98 \pm 0.02	1.91 \pm 0.02	-2.291	120.00	0.023
1Fig. 5	I – II	0.76 \pm 0.01	0.74 \pm 0.01	-1.055	120.00	0.293
1Fig. 6	AA 3 + 4	1.52 \pm 0.02	1.52 \pm 0.02	-0.113	120.00	0.909
1Fig. 7	AP 3 + 4	1.04 \pm 0.02	1.01 \pm 0.01	-1.354	120.00	0.178
1Fig. 8	CuA	2.14 \pm 0.02	2.09 \pm 0.02	-1.657	120.00	0.099
1Fig. 9	RP	0.60 \pm 0.01	0.59 \pm 0.01	-0.643	120.00	0.521
1Fig. 10	(ScA + RA + AH)	2.47 \pm 0.01	2.40 \pm 0.02	-2.268	120.00	0.025
1Fig. 11	RP2	1.11 \pm 0.01	1.10 \pm 0.01	-0.662	120.00	0.509
1Fig. 12	RA4	1.79 \pm 0.02	1.74 \pm 0.02	-2.163	120.00	0.032
1Fig. 13	CuA – RP2	2.01 \pm 0.02	1.98 \pm 0.02	-0.919	120.00	0.359
1Fig. 14	MP 1 + 2	1.93 \pm 0.02	1.86 \pm 0.02	-2.514	120.00	0.013
1Fig. 15	III-IV	0.49 \pm 0.01	0.48 \pm 0.01	-1.310	120.00	0.192
1Fig. 16	RP3 + 4 – RP2	0.79 \pm 0.01	0.77 \pm 0.01	-1.317	120.00	0.190
1Fig. 17	Body length	4.45 \pm 0.03	4.32 \pm 0.04	-3.00	118.88	0.003
1Fig. 18	Body width	1.92 \pm 0.01	1.85 \pm 0.02	-3.18	117.33	0.001
1Fig. 19	Body height	1.32 \pm 0.01	1.24 \pm 0.01	-3.65	120.00	<0.001
1Fig. 20	Pronotal length	0.90 \pm 0.02	0.65 \pm 0.01	-9.86	120.00	<0.001
1Fig. 21	Pronotal width	1.15 \pm 0.02	0.90 \pm 0.02	-10.08	120.00	<0.001
1Fig. 22	Cephalic horn length	0.78 \pm 0.02	0.33 \pm 0.01	-25.48	120.00	<0.001
1Fig. 23	Elytra length	2.63 \pm 0.02	2.51 \pm 0.02	-4.35	119.95	<0.001
1Fig. 24	Hind tibia + tarsals	1.69 \pm 0.02	1.67 \pm 0.01	-0.89	120.00	0.372
	Wing aspect ratio (L/W)	2.90 \pm 0.02	2.85 \pm 0.02	-0.685	120.00	0.094

as illustrated by the significant differences between agro-ecological zones and morphological traits. Female AfRB from the Transitional zone were larger than those from the other agro-ecological zones. Beetles collected from Coastal savannah were consistently the smallest, whereas those from the Deciduous and Evergreen were intermediary in size (Table 2). The morphological characters that significantly varied across the agro-ecological zones included Elytra length ($F = 10.9$, $df = 64(3)$, $p < 0.0001$), Pronotal width ($F = 3.21$, $df = 64(3)$, $p = 0.029$), Pronotal Length ($F = 4.99$, $df = 64(3)$, $p = 0.004$), Body height ($F = 6.15$, $df = 64(3)$, $p = 0.001$), Body length ($F = 6.15$, $df = 64(3)$, $p = 0.001$), I-II vein length of hind wing ($F = 4.04$, $df = 64(3)$, $p = 0.010$), MP1 + 2 vein length of hind wing ($F = -2.16$, $df = 64(3)$, $p = 0.032$), ScA + RA + AH vein length of hind wing length ($F = 3.21$, $df = 64(3)$, $p = 0.008$), CuA – RP2 vein length of hind wing ($F = 4.67$, $df = 64(3)$, $p = 0.005$), RA4 vein length of hind wing ($F = 4.33$, $df = 64(3)$, $p = 0.007$), RP3 + 4 vein length of hind wing ($F = 5.66$, $df = 64(3)$, $p = 0.002$), CuA vein length of hind wing ($F = 10.74$, $df = 64(3)$, $p < 0.0001$), AP3 + 4 vein length of hind wing ($F = 14.73$, $df = 64(3)$, $p < 0.0001$), III-IV vein length of hind wing ($F = 6.94$, $df = 64(3)$, $p = 0.004$), RA3 vein length of hind wing

($F = 4.81$, $df = 64(3)$, $p = 0.0004$), Wing length of hind wing ($F = 4.58$, $df = 64(3)$, $p = 0.005$), and Wing width of hind wing ($F = 8.81$, $df = 64(3)$, $p < 0.0001$).

Similarly, males AfRB differed across the different agro-ecological zones, with beetles from the coastal savannah being consistently smallest when compared to those from deciduous and transition zones (Table 3). Adults from transitional zone appeared largest, whereas those from deciduous forest zone were intermediate (Table 3). The morphometric characters that significantly differed across the agro-ecological zones for males were Elytra length ($F = 5.307$, $df = 34(2)$, $p = 0.009$), Hand tibia + Tarsals ($F = 18.01$, $df = 34(2)$, $p = 0.036$), Pronotal width ($F = 3.49$, $df = 34(2)$, $p = 0.041$), Body length ($F = 11.01$, $df = 34(2)$, $p < 0.001$), RA4 vein length of hind wing ($F = 6.98$, $df = 34(2)$, $p = 0.002$), RP vein length of hind wing ($F = 6.73$, $df = 34(2)$, $p = 0.049$), AP3 + 4 vein length of hind wing ($F = 5.91$, $df = 34(2)$, $p = 0.006$), Wing length ($F = 3.71$, $df = 34(2)$, $p = 0.034$) and Wing width ($F = 4.52$, $df = 34(2)$, $p = 0.018$). However, those that did not show a significant difference across the different agroecological zones were RP2 – RA3 vein length of hind wing ($F = 2.18$, $df = 34(2)$, $p = 0.129$), I-II vein length of hind wing ($F = 2.20$, $df = 34(2)$, $p = 0.126$), AA 3 + 4 vein length of hind wing

Table 2 Size (mean \pm standard error) (cm) of the 25 morphological traits in the female populations of *Oryctes monoceros* from different Agro-ecological zones in Ghana

Figure Code	Trait	CoastalSavannah	DeciduousForest	Evergreen Forest	Transition zone
1Fig. 1	Wing width	1.43 \pm 0.02b	1.53 \pm 0.01a	1.60 \pm 0.05a	1.59 \pm 0.03a
1Fig. 2	Wing length	4.18 \pm 0.06b	4.34 \pm 0.05ab	4.27 \pm 0.07ab	4.53 \pm 0.08a
1Fig. 3	RP2 – RA3	0.59 \pm 0.02b	0.64 \pm 0.01a	0.66 \pm 0.02a	0.66 \pm 0.02a
1Fig. 4	RA3	1.89 \pm 0.04	1.90 \pm 0.04	1.89 \pm 0.04	2.00 \pm 0.04
1Fig. 5	I – II	0.69 \pm 0.02b	0.76 \pm 0.01a	0.75 \pm 0.04ab	0.80 \pm 0.02a
1Fig. 6	AA 3 + 4	1.47 \pm 0.04	1.53 \pm 0.03	1.55 \pm 0.04	1.57 \pm 0.02
1Fig. 7	AP 3 + 4	0.91 \pm 0.02c	1.04 \pm 0.01ab	0.98 \pm 0.08bc	1.13 \pm 0.03a
1Fig. 8	CuA	1.96 \pm 0.03b	2.11 \pm 0.03a	2.10 \pm 0.05a	2.24 \pm 0.04a
1Fig. 9	RP	0.58 \pm 0.01	0.59 \pm 0.01	0.60 \pm 0.02	0.63 \pm 0.01
1Fig. 10	ScA + RA + AH	2.32 \pm 0.03b	2.42 \pm 0.02ab	2.35 \pm 0.07ab	2.52 \pm 0.04a
1Fig. 11	RP2	1.04 \pm 0.02b	1.11 \pm 0.02a	1.11 \pm 0.03ab	1.16 \pm 0.02a
1Fig. 12	RA4	1.68 \pm 0.03b	1.73 \pm 0.02ab	1.75 \pm 0.05ab	1.84 \pm 0.02a
1Fig. 13	CuA – RP2	1.89 \pm 0.03b	1.96 \pm 0.03ab	1.97 \pm 0.04ab	2.09 \pm 0.03a
1Fig. 14	MP 1 + 2	1.74 \pm 0.04b	1.88 \pm 0.03ab	1.88 \pm 0.06ab	1.99 \pm 0.05a
1Fig. 15	III-IV	0.45 \pm 0.01b	0.48 \pm 0.01ab	0.50 \pm 0.02a	0.50 \pm 0.00a
1Fig. 16	RP3 + 4 – RP2	0.74 \pm 0.02	0.76 \pm 0.01	0.78 \pm 0.03	0.78 \pm 0.02
1Fig. 17	Body length	4.17 \pm 0.06b	4.37 \pm 0.06a	4.28 \pm 0.07ab	4.55 \pm 0.06a
1Fig. 18	Body width	1.85 \pm 0.03	1.85 \pm 0.02	1.81 \pm 0.03	1.90 \pm 0.05
1Fig. 19	Body height	1.23 \pm 0.02b	1.26 \pm 0.03a	1.17 \pm 0.01ab	1.32 \pm 0.04a
1Fig. 20	Pronotal length	0.60 \pm 0.03b	0.66 \pm 0.02ab	0.62 \pm 0.02b	0.76 \pm 0.04a
1Fig. 21	Pronotal width	0.85 \pm 0.04b	0.91 \pm 0.03ab	0.85 \pm 0.03ab	0.99 \pm 0.03a
1Fig. 22	Cephalic horn length	0.35 \pm 0.02	0.33 \pm 0.01	0.30 \pm 0.02	0.34 \pm 0.02
1Fig. 23	Elytra length	2.41 \pm 0.04c	2.57 \pm 0.03ab	2.43 \pm 0.04bc	2.68 \pm 0.04a
1Fig. 24	Hind tibia + tarsals	1.64 \pm 0.02b	1.67 \pm 0.03ab	1.73 \pm 0.02ab	1.67 \pm 0.01a

Means with different letters within the same row are statistically different (Tukey's HSD test, $P < 0.05$)

Table 3 Size (mean \pm SE) of the 25 morphological traits in male populations of *Oryctes monoceros* from different Agro-ecological zones in Ghana

Figure Code	Trait	Coastal Savannah (cm)	Deciduous Forest (cm)	Transition Zone (cm)
1Fig. 1	Wing width	1.49 \pm 0.02b	1.57 \pm 0.02ab	1.56 \pm 0.02a
1Fig. 2	Wing length	4.35 \pm 0.04b	4.48 \pm 0.06ab	4.56 \pm 0.06a
1Fig. 3	RP2 – RA3	0.6 \pm 0.02	0.65 \pm 0.02	0.66 \pm 0.02
1Fig. 4	RA3	1.92 \pm 0.02b	2.00 \pm 0.03ab	2.03 \pm 0.03a
1Fig. 5	I – II	0.73 \pm 0.01	0.78 \pm 0.03	0.77 \pm 0.01
1Fig. 6	AA 3 + 4	1.48 \pm 0.04	1.58 \pm 0.04	1.53 \pm 0.02
1Fig. 7	AP 3 + 4	0.95 \pm 0.03 b	1.04 \pm 0.04ab	1.09 \pm 0.03a
1Fig. 8	CuA	2.07 \pm 0.02	2.13 \pm 0.04	2.19 \pm 0.05
1Fig. 9	RP	0.57 \pm 0.02b	0.61 \pm 0.01ab	0.62 \pm 0.01a
1Fig. 10	ScA + RA + AH	2.42 \pm 0.02	2.49 \pm 0.03	2.49 \pm 0.03
1Fig. 11	RP2	1.07 \pm 0.02	1.12 \pm 0.02	1.14 \pm 0.02
1Fig. 12	RA4	1.72 \pm 0.02b	1.78 \pm 0.03ab	1.85 \pm 0.03a
1Fig. 13	CuA – RP2	1.96 \pm 0.02	2.00 \pm 0.04	2.05 \pm 0.04
1Fig. 14	MP 1 + 2	1.92 \pm 0.01	1.93 \pm 0.05	1.93 \pm 0.03
1Fig. 15	III-IV	0.47 \pm 0.01b	0.50 \pm 0.01ab	0.50 \pm 0.00a
1Fig. 16	RP3 + 4 – RP2	0.77 \pm 0.02	0.77 \pm 0.03	0.81 \pm 0.01
1Fig. 17	Body length	4.33 \pm 0.04b	4.41 \pm 0.04a	4.58 \pm 0.04a
1Fig. 18	Body width	1.91 \pm 0.04	1.91 \pm 0.03	1.94 \pm 0.02
1Fig. 19	Body height	1.28 \pm 0.02	1.34 \pm 0.03	1.33 \pm 0.02
1Fig. 20	Pronotal length	0.84 \pm 0.04	0.91 \pm 0.03	0.93 \pm 0.02
1Fig. 21	Pronotal width	1.09 \pm 0.03b	1.16 \pm 0.03ab	1.19 \pm 0.02a
1Fig. 22	Cephalic horn length	0.72 \pm 0.03	0.78 \pm 0.04	0.81 \pm 0.03
1Fig. 23	Elytra length	2.56 \pm 0.03 b	2.64 \pm 0.04ab	2.69 \pm 0.03a
1Fig. 24	Hind tibia + tarsals	1.62 \pm 0.05b	1.73 \pm 0.04ab	1.74 \pm 0.02a

Means with different letters within the same row are statistically different (Tukey's HSD test, $P < 0.05$)

($F = 1.98$, $df = 34(2)$, $p = 0.154$), CuA vein length of hind wing ($F = 3.07$, $df = 34(2)$, $p = 0.059$), ScA + RA + AH vein length of hind wing ($F = 2.18$, $df = 34(2)$, $p = 0.129$), RP2 vein length of hind wing ($F = 3.08$, $df = 34(2)$, $p = 0.058$), CuA – RP2 vein length of hind wing ($F = 0.252$, $df = 34(2)$, $p = 0.252$), MP 1 + 2 vein length of hind wing ($F = 0.01$, $df = 34(2)$, $p = 0.995$), III-IV vein length of hind wing ($F = 3.03$, $df = 34(2)$, $p = 0.061$), RP3 + 4 – RP2 vein length of hind wing ($F = 1.82$, $df = 34(2)$, $p = 0.177$), Body length ($F = 0.265$, $df = 34(2)$, $p = 0.769$), body height ($F = 1.318$, $df = 34(2)$, $p = 0.281$), Pronotal length ($F = 2.743$, $df = 34(2)$, $p = 0.078$) and Pronotal width ($F = 2.972$, $df = 34(2)$, $p = 0.064$).

The first two loadings of the principal components (PC) of female AfRB contributed to 59.87% of the variation (PC1 = 51.82%, PC2 = 8.05%) (Table 4). In the first principal component, the morphometric traits that contributed most to the variation of the data were the Wing length (0.25), body length (0.24), ScA + RA + AH of the hind wing (0.24), and CuA of the hind wing (0.25). In the second principal component loadings, III-IV vein of the hind wing (-0.33), width of hind wing (-0.37), I-II vein of the hind wing (-0.36), and Cephalic horn length (0.36) contributed most the observed variations. The negative PC loadings for III-IV vein of the

hind wing, width of hind wing, and I-II vein of the hind wing showed negative correlations between the morphometric traits and the second component.

The first two coefficients of the principal components (PC) of males contributed to 52.11% of the variation (PC1 = 43.42%, PC2 = 8.69%) (Table 5). In the first principal component, the morphometric traits that contributed most to the variation of the male data were the wing length (0.28), CuA of the hind wing (0.25), ScA + RA + AH of the hind wing (0.26), and RP2 vein of the hind wing (0.25), RA4 vein of the hind wing (0.27), Elytra length (0.28). In the second principal component, RP2-RA3 vein of the hind wing (-0.31), III-IV vein of the hind wing (0.37), RP3 + 4 – RP2 vein of the hind wing (-0.28), and Body height (0.27) and Pronotal width (0.28) contributed most to the variation.

The cluster analysis for female AfRB using Euclidean distance similarity formed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) showed that samples from Deciduous and Evergreen forest are closely related, and are different from those collected in the Coastal savannah and Transitional zones (Fig. 3A). In the present study, females of AfRB can be morphologically separated into three sub-groups, (i) Deciduous and Evergreen, (2) Coastal, and (3)

Table 4 Standardized loadings on the first eight (8) principal component variables for females

Figure code	Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
1Fig. 1	Wing width	0.20	-0.37	-0.07	-0.01	0.11	0.23	-0.00	-0.08
1Fig. 2	wing length	0.25	0.12	-0.07	-0.08	-0.08	0.05	0.05	0.08
1Fig. 3	RP2 – RA3	0.17	-0.20	-0.37	0.12	-0.04	0.32	-0.06	-0.17
1Fig. 4	RA3	0.21	0.09	-0.27	-0.04	-0.13	-0.06	-0.01	-0.22
1Fig. 5	I – II	0.17	-0.33	0.22	-0.03	0.16	-0.02	0.34	-0.19
1Fig. 6	AA 3 + 4	0.18	0.01	-0.14	0.39	0.15	-0.13	-0.49	0.30
1Fig. 7	AP 3 + 4	0.21	-0.07	-0.07	-0.08	0.14	0.25	-0.36	-0.05
1Fig. 8	CuA	0.25	-0.09	0.13	-0.04	0.11	-0.12	-0.08	0.07
1Fig. 9	RP	0.19	-0.05	0.13	0.38	0.02	0.17	0.14	0.51
1Fig. 10	ScA + RA + AH	0.24	0.09	-0.00	-0.04	0.00	-0.00	0.01	0.11
1Fig. 11	RP2	0.21	-0.20	0.31	0.00	0.04	-0.16	-0.11	-0.15
1Fig. 12	RA4	0.22	-0.17	-0.07	0.01	0.09	-0.11	0.09	0.03
1Fig. 13	CuA – RP2	0.21	0.09	-0.05	-0.16	-0.34	0.29	0.01	-0.19
1Fig. 14	MP 1 + 2	0.23	-0.07	0.18	-0.00	0.01	0.14	0.12	-0.00
1Fig. 15	III-IV	0.18	-0.36	0.10	-0.05	-0.13	-0.31	0.12	0.08
1Fig. 16	RP3 + 4 – RP2	0.17	-0.06	-0.28	0.12	-0.55	0.03	0.18	0.23
1Fig. 17	Body length	0.24	0.08	-0.01	-0.06	0.02	-0.21	0.04	-0.07
1Fig. 18	Body width	0.17	0.27	-0.09	0.10	-0.19	-0.46	0.20	-0.04
1Fig. 19	Body height	0.10	0.17	-0.34	-0.43	0.47	0.08	0.36	0.34
1Fig. 20	Pronotal length	0.23	0.23	0.03	-0.02	0.03	0.06	-0.10	-0.25
1Fig. 21	Pronotal width	0.22	0.25	0.11	0.03	0.08	-0.07	-0.06	-0.20
1Fig. 22	Cephalic horn length	0.09	0.36	0.28	0.45	0.12	0.33	0.32	-0.12
1Fig. 23	Elytra length	0.22	0.20	-0.03	-0.09	0.19	-0.15	-0.18	0.01
1Fig. 24	Hind tibia + tarsals	0.11	0.14	0.45	-0.44	-0.30	0.19	-0.20	0.34
Eigen values		12.43	1.93	1.27	1.08	0.95	0.81	0.69	0.67
% variance		51.82	8.05	5.30	4.51	3.96	3.41	2.88	2.79

Transitional zone. The cluster analysis based on the Euclidean distance similarity of male AfRB showed that populations from Deciduous and Transitional were more similar than those from the Coastal savannah agro-ecological zones (Fig. 3B). The results showed that populations from Transition and Deciduous were similar but different from those collected from Savannah agro-ecological zones.

Discussion

Development of ecologically sound management strategies requires accurate identification of the targeted pests (Augustinos et al. 2018; Konje et al. 2019). Hence, we used a morphometric approach based on linear measurements of body parts and wing veins to morphologically characterize, compare and provide insight into AfRB populations from different agro-ecological zones in Ghana. Morphometric approach is essential for assessing phenotypic variation caused by a variety of biotic (e.g., predation and competition) and abiotic (e.g., temperature, rainfall and humidity) influences (Daly 1985; Nakazawa et al. 2013; Paris et al. 2017; Beukeboom 2018). The method has been used to

classify adults and immature stages of insects, as well as compare genetic, environmental, and phenotypic variations (Daly 1985; García-Pérez et al. 2013; Paris et al. 2016, 2017; Billah et al. 2017; Aidoo et al. 2019). It has been used to study the morphology of the Asian rhinoceros beetle, a close relative of the African rhinoceros beetle from Oil palm plantations (Manjeri et al. 2013).

Our findings showed that on average, males were larger than females in most of the measured morphometric traits. For example, the Cephalic horn length in males (0.78 ± 0.02) was larger than that of females (0.33 ± 0.01). This is consistent with that of Doane (1913), which showed that on average, AsRB males had a longer Cephalic horn than females. The observed phenotypic variations in male and female body sizes of Rhinoceros beetles could be associated with sexual and natural selection (i.e., might have evolved to be different in sizes in order to exploit resources such as food) (Cassini 2020). This variation in body size may come from phenotypic plasticity, but it can also be due to variation across populations, as observed along latitudinal gradients (Horne et al. 2019). The morphometric traits considered in our study included Wing veins, Body length and width, Elytral length, Hind tibia + Tarsal length and Pronotal length and width.

Table 5 Standardized loadings on the first eight (8) principal component values for males

Figure code	Trait	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
1Fig. 1	Wing width	0.22	0.01	0.27	-0.06	0.16	0.10	0.32	0.05
1Fig. 2	Wing length	0.28	-0.07	-0.01	-0.18	0.01	0.00	0.04	0.05
1Fig. 3	RP2 – RA3	0.16	-0.31	-0.22	-0.24	0.21	0.04	-0.08	-0.27
1Fig. 4	RA3	0.22	-0.18	0.02	0.18	-0.35	0.25	0.01	0.12
1Fig. 5	I – II	0.18	-0.21	0.27	0.31	0.04	-0.19	-0.01	-0.20
1Fig. 6	AA 3 + 4	0.13	0.25	0.32	-0.11	-0.08	0.43	-0.41	0.26
1Fig. 7	AP 3 + 4	0.18	-0.09	0.29	0.18	0.31	0.00	-0.25	0.16
1Fig. 8	CuA	0.25	0.01	0.16	-0.10	-0.13	0.11	-0.09	-0.12
1Fig. 9	RP	0.14	-0.02	0.01	-0.38	0.49	-0.31	0.06	0.38
1Fig. 10	ScA + RA + AH	0.26	0.07	-0.02	-0.10	-0.06	0.19	-0.02	-0.32
1Fig. 11	RP2	0.25	-0.17	0.03	-0.16	0.01	-0.17	-0.23	-0.05
1Fig. 12	RA4	0.27	-0.20	0.08	-0.05	-0.06	-0.06	-0.07	0.04
1Fig. 13	CuA – RP2	0.19	-0.24	0.20	0.07	-0.05	0.01	0.54	0.09
1Fig. 14	MP 1 + 2	0.14	0.20	0.23	-0.35	-0.37	-0.15	0.16	-0.22
1Fig. 15	III-IV	0.08	0.37	0.25	0.40	0.02	-0.28	0.06	0.10
1Fig. 16	RP3 + 4 – RP2	0.12	-0.28	-0.31	0.30	-0.05	0.24	0.20	0.30
1Fig. 17	Body length	0.23	0.11	-0.14	0.10	0.13	0.00	0.19	-0.27
1Fig. 18	Body width	0.16	0.29	-0.21	0.04	0.41	0.48	0.09	-0.17
1Fig. 19	Body height	0.13	0.27	-0.36	-0.01	-0.21	-0.27	0.05	0.06
1Fig. 20	Pronotal length	0.22	0.24	-0.04	0.31	0.18	-0.09	-0.04	-0.11
1Fig. 21	Pronotal width	0.23	0.28	-0.22	0.01	-0.04	-0.12	-0.02	0.15
1Fig. 22	Cephalic horn length	0.23	0.08	-0.19	-0.13	-0.18	0.06	0.03	0.45
1Fig. 23	Elytra length	0.27	0.09	-0.12	0.04	-0.04	-0.07	-0.06	-0.14
1Fig. 24	Hind tibia + tarsals	0.18	-0.22	-0.22	0.21	-0.05	-0.19	-0.42	-0.03
Eigen values		10.43	2.08	1.86	1.55	1.22	0.97	0.93	0.77
% variance		43.42	8.69	7.77	6.49	5.10	4.04	3.86	3.22

These traits are reflective body shapes that are associated with habitat, locomotion, burrowing, and flying (Talarico et al. 2011).

Body size of AfRB varied across the different agro-ecological zones, with larger beetles sampled from the Transitional zone. The variation in morphometric traits could be associated with environmental conditions. However, effects of environmental conditions on morphological traits of insects, especially beetles are poorly documented. Notwithstanding, in the Ground Beetle *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae), Sukhodolskaya and Savelev (2014), indicated that latitude, anthropogenic disturbance, climate, and landscape features affect its morphometric variation. Body size can affect life history traits including reproduction rate, dispersal ability, ontogeny length, competition, and size of offspring, which is useful for understanding the interactions between an organism and its surroundings (Stearns 1992). Knapp and Uhnava (2014) studied the effects of body size and nutrition intake on fecundity and overwintering success in *Anchomenus dorsalis* (Coleoptera: Carabidae), and showed that structural body size affects its egg production. The size of an insect may also affect its dispersal powers, fitness, and survival in general. For example, in a flight mill study to test the effects of beetle size, sex, and age

on flight capacity of the Mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), the findings showed that beetle pre-flight weight affected flight; initiation, distance, and duration, with larger-bodied Mountain pine beetles likely to fly longer distances and stay longer in flight than their smaller-bodied counterparts (Evenden et al. 2014). Also, Shegelski et al. (2019), studied morphological variation associated with dispersal capacity in *Dendroctonus ponderosae* and found that beetles that flew longer distances (> 11 km) had a greater body weight and larger wings than those that flew shorter distances (< 1 km).

The first two principal components analysis showed overlaps of AfRB populations for both males and females. However, morphometric traits that contributed most to PCAs varied by sexes. Wing length, Body length, ScA + RA + AH of the forewing, and CuA of the forewing, III-IV vein of the hind wing, wing width of the hind wing, I-II vein of the hind wing, and Cephalic horn length contributed most to variations in female populations based on the first and second components. For the males, wing length, CuA of the hind wing, ScA + RA + AH of the hind wing, and RP2 vein of the hind wing, RA4 vein of the hind wing, Elytra length, RP2 – RA3 vein of the hind wing, III-IV vein of the hind wing (0.37), RP3 + 4 – RP2 vein of the hind wing, Body height,

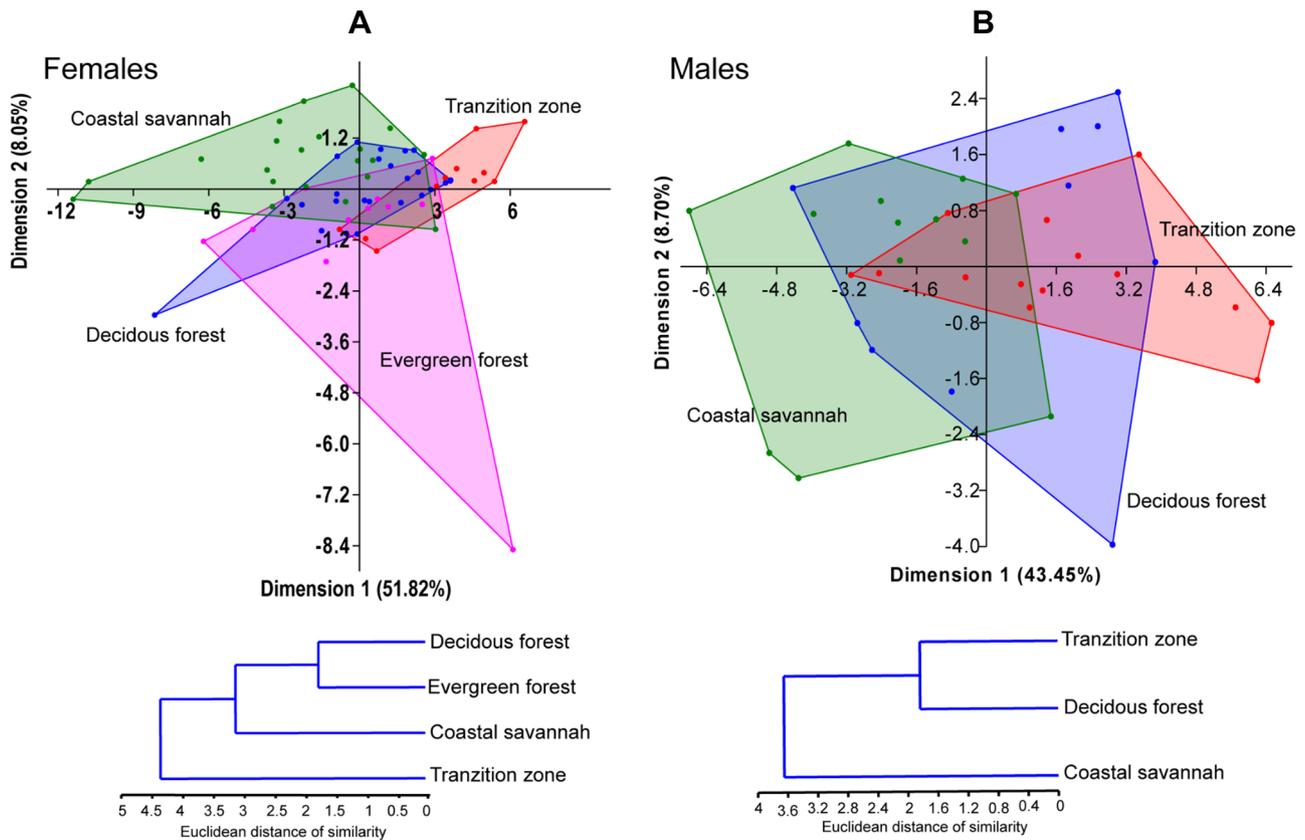


Fig. 3 Scatterplot showing the first two principal components of distance variables, and dendrogram formed by UPGMA method using squared Euclidean distance of female **A** and male **B** *Oryctes monoc-*

eros populations from Deciduous forest, Evergreen forest, Coastal savannah and Transitional zones

and Pronotal width contributed most to the first and second components. Body length, Pronotal width, Pronotal length, length of the Cephalic horn, and Elytral length contributed most to the first two principal components in the AsRB populations from Oil palm plantations (Manjeri et al. 2013).

However, the dendrograms plotted by UPGMA method based on squared Euclidean distance of AfRB females from the different agro-ecological zones showed existence of two main groups, which can further be separated into three subgroups within the AfRB populations. The first group included samples from the Deciduous, Evergreen, and Coastal savannah zones, whereas the second group came from the Transitional zone. The three subgroups consisted of Deciduous and Evergreen forests, which were distinct from the Coastal savannah zone, and separate from the Transitional zone. The observed phenotypic variation in the present study may be due to variations in habitat, as well as physiological and behavioural characteristics (Den Boer 1986; Manjeri et al. 2013). The observed differences in the populations can also be attributed to the following occurrences; geographical barriers including mountain ranges or rivers, and any region that excludes vital resources, such as

a region devoid of water, a burned area devoid of food, or migration, a new host or environment (or both), landscape changes (bottleneck effect), and genetic changes caused by stochastic events like gene transfer, genetic drift, mutation, or natural selection (Berlocher and Feder 2002; Lashkari et al. 2015). However, future AfRB molecular studies are required to establish the connection between morphological features and phenotypic plasticity for its management.

A positive correlation exists between plasticity in structural body size (e.g., leg length, elytron length, head width, or wingspan) and environmental conditions experienced during development, with no change in structural body size over the adult lifetime (Irwin and Lee 2000). In contrast, body mass appears to be highly variable throughout an adult lifetime depending on the environmental conditions experienced during the adult stage (Chaabane et al. 1997; Bommarco 1998; Knapp et al. 2013).

The adult beetles found in the Coastal savannah were consistently the smallest, whereas those from the Transitional zone were the largest. This could be due to food availability, geographic range size, and energy consumption (Brown et al. 2004; Chown and Gaston 2010; White et al. 2007). Several

authors have linked resource availability and predation with insect body size (Scriber and Lederhouse 1992; Scriber 2002; Berner et al. 2005; Billah et al. 2005; Stoks et al. 2006; Chown and Gaston 2010). In part, the observed phenotypic variation may be associated with variations in rainfall and temperature conditions in the different agro-ecological zones as described by Asare-Nuamah and Botchway (2019). A study on beetle body size linked to climate change and warming temperatures showed that increased rearing temperature reduced the size of 95% of laboratory-reared beetles, with larger-bodied species shrinking disproportionately more than smaller-bodied species (Tseng et al. 2018). Similarly, high temperatures affect body size and weight of *Ophraella communa* LeSage (Coleoptera: Chrysomelidae). A study on effects of temperature, photoperiod, and rainfall on morphometric variation of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) found a strong relationship between temperature and photoperiod, and changes in wing shape under laboratory conditions (Paris et al. 2017).

Several studies have shown that proper identification of a target species is critical for developing appropriate management strategies, while incorrect identification of a species could result in wrong management tactics, thereby leading to financial losses and time wasting (Moritz et al. 2000; Bonants et al. 2013; Tyagi et al. 2017; Tahir et al. 2018; McCarty and Elliott 2020). According to Begum et al. (2017), proper identification of an agricultural pest is required to select appropriate pesticides for the target pest. Moreover, detection of crop pests using artificial intelligence is vital for developing integrated pest management strategies. However, this requires accurate identification of the target species (Demirel and Kumral 2021). The characterization of the field populations of AfRB will serve as a guide to enable biologists, ecologists and pest managers to direct biological control programmes (Arora and Dhawan 2017). Furthermore, identification and implementation of correct resistance management measures demands a clear understanding of the genetic and morphometric variations in natural populations of a pest (Dogac et al. 2015). Here we show a detailed morphometric analysis of AfRB populations collected from different agro-ecological zones and provide a theoretical basis for developing integrated pest management strategies against AfRB.

Conclusion

In the present study, we report that AfRB males are larger than females. The principal component analysis showed overlaps of AfRB populations based on agro-ecological zones. However, dendrogram formed by UPGMA method using squared Euclidean distance of AfRB female

populations showed that the Deciduous and Evergreen forests and Coastal savannah zones were distinct from each other but separate from the Transitional zone populations. Here we report that three subgroups can be morphologically separated within AfRB species in this study, (i) the Deciduous and Evergreen, (2) the Coastal, and (3) the Transitional zone populations. Further molecular studies are required to better understand the impact of agro-ecological zones on AfRB populations. Also, there is a need for further studies to understand if there is a relationship between the observed phenotypic expressions and phenotypic plasticity within AfRB populations.

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Declarations

Ethical standard Not applicable.

Conflict of interest The authors declare that they have no conflicts of interest.

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