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Morphomeric Variability among Samples of *Callosobruchus Subinnotatus* (Pic) (Coleoptera:Chrysomelidae) in Northwestern Nigeria

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Keywords: callsobruchus subinnotatus, morphoclusters, races, centroids, diagnostic features.

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MORPHOMERICVARIABILITYAMONGSAMPLESOFCALLOSOBRUCHUSSUBINNOTATUSPICCOLEOPTERACHRYSOMELIDAEINNORTHWESTERNNIGERIA

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Morphomeric Variability among Samples of *Callosobruchus Subinnotatus* (Pic) (Coleoptera:Chrysomelidae) in Northwestern Nigeria

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I. Introduction

Many members of the subfamily Bruchinae are closely related morphologically but may have unique geographical distributions, life histories and ecological relationships. Sometimes, a population will physically alter over time to suit the needs of its environment and thus, can make members of the same species look different (Anon., 2011). Polymorphism is common among Bruchinae populations as previously described for C. *maculatus* (Fabricius) and C. *chinensis* Linnaeus in which adults were separated into 'normal' and 'active' morphs. *Callosobruchus subinnotatus* (Pic) is a primary field-to-store pest of bambara groundnuts only in West Africa, although its host *Vigna subterranea* (L.) Verdcourt is grown throughout the arid zones of Africa and parts of Asia but little is currently known about its biology, morphs or how it may be controlled (Appleb and Credland, 2001). The morphological significance of some major diagnostic characteristics of C. *subinnotatus* is being investigated to see the variability that exists among samples collected in different ecological zones of Northwestern Nigeria.

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II. Materials and Methods

a) Sampling Sites and Culturing of C.subinnotatus

Samples were collected from three locations each of Kebbi (Birnin Kebbi, Kangiwa and Zuru), Kaduna (Kagarko, Kaduna South and Giwa), Kano (Danbata, Gaya and Kano), Katsina (Bakori, Charanchi and Daura) and Zamfara (Gusau, Kaura Namoda and Talata Mafara) States of Northwestern Nigeria. Cultures of adult bruchids were raised in 1- litre capacity, clear plastic containers (9 cm diameter, 16 cm high) with 8 cm diameter screw- type lids. Each container contained about 200 g seeds of a susceptible bruchid host (cowpea (*Vigna unguiculata* (Walp)), bambara groundnut (*Vigna subterranea* (Bandare and Saxena, 1995). The lid of each container had a central circular perforation (3 cm diameter) covered with fine muslin cloth for aeration. These were kept under laboratory conditions and observed daily until adult emergence. The lid and side walls of the container with active species were tapped repeatedly so that the adults gathering around the lid or the side walls dropped back unto the seeds and chilled by refrigeration.

The newly -emerged bruchinae were sieved and transferred into vials (7.5 cm high and 2.5 cm diameter) containing 70% ethanol until use. *C. subinnotatus* was identified by the use of keys on adult morphological characters (Southgate, 1958; Haines, 1991) and comparison with preserved specimens in the reference collections of the Insect Museum of Crop Protection Department, Ahmadu Bello University Zaria where the research was conducted.

b) Morphometrics

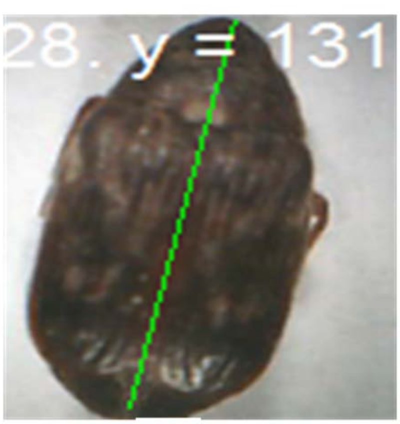
Five pairs of C. species each per location were randomly subinnotatus selected and subjected to morphometric studies. Diagnostic features used by Southgate et al., (1957) and Kingsolver (2004) which are now consistently used in bruchids classification and comparison were adopted. A total of (13)diagnostic characters were used in which measurements were carried out on ten and three ratios were used as explanatory variables.

c) *Measurements of Diagnostic features and their codes*

i. Body length (bl) was measured from the anterior margin of pronotum to apex of abdomen (Plate a).
ii. Body width (bw) was measured across greatest width of elytra (Plate b).
iii. Eye width (ew) was measured across greatest width of eye (Plate c).
iv. Distance between eyes (dbe) was measured across the narrowest distance between eyes (Plate d).
v. Antennal length (al) was measured when antenna was fully extended with head in hypognathous position and covered the distance from the socket at base of antennal scape to apex of last abdominal segment (Plate e).
vi. Hind femoral width (hfw) was measured across greatest width of hind femur (Plate f).
vii. Width of coxa (wc) was measured across greatest width of coxa (Plate g).
viii. Length of pronotum (lp) was measured centrally from the anterior margin to the base margin of pronotum (Plate h).
ix. Width of pronotum anterior (wpa) was measured across the narrowest width of pronotum anterior (Plate i).
x. width of pronotum base (wpb) was measured across the greatest width of pronotum base (Platej).

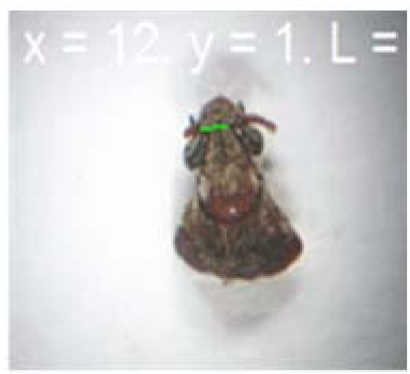
All measurements were made in pixels using a calibrated handheld digitalized MiScope microscope. Values obtained were converted to millimeter using a factor 2.54/DPI (Dots per inch). For instance, if the measured pixel value is 45, then the millimeter equivalent will be 45x 2.54/300 =0.39 mm (DPI for computer screen is 300) (Dallin, 2008).

Plate's a-j: Measurements of diagnostic features


a


b


c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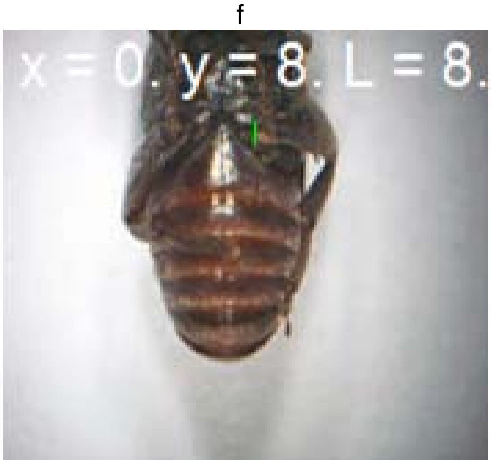
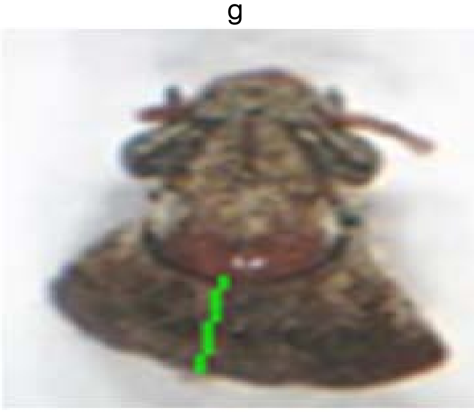


g



h

i

j

III. Results

Cluster analysis on morphor data obtained from *C. subinnotatus* in Northwestern Nigeria gave two morphoclusters with morphocluster 2 having the highest percentage distribution across the States (53.3%) than morphocluster 1 (46.7%) as shown in table 1 below.

Table 1 : Morphocluster Distribution of *C. subinnotatus* in Northwestern States of Nigeria.

	Clusters	N	% of Combined	% of Total
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
	[illegible]	[illegible]	[illegible]	[illegible]
Total		[illegible]		[illegible]

Distribution of the species among states studied showed that morphocluster 1 was recorded in all locations of Kebbi and Kaduna States as well as Charanchi (CRC) in Katsina State (100%) in the Northern Guinea savannah whereas morphocluster 2 were recorded in all locations of Zamfara and Kano States as well as Bakori and Daura locations of Katsina State in the Sahel zone as illustrated in Table 2.

Table 2 : Morphocluster Percentage of States of C. subinnotatus in Northwestern Nigeria.

Clusters	KBBK		KBKGW		KBZR		KDGW		KDKGK		KDKS		KNDBT		KNGY		KNKN	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
1	10	100	10	100	10	100	10	100	10	100	10	100	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	10	100	10	100	10	100
Combined	10	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100	10	100

Clusters	KTBKR		KTCRC		KTDR		ZMGS		ZMKND		ZMTMF	
	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%	Freq.	%
1	0	0	10	100	0	0	0	0	0	0	0	0
2	10	100	0	0	10	100	10	100	10	100	10	100
Combined	10	100	10	100	10	100	10	100	10	100	10	100

Key

KBBK -Kebbi State – Birnin Kebbi
KBKGW - Kebbi State – Kangiwa
KBZR - Kebbi State – Zuru
KDGW - Kaduna State – Giwa
KDKGK – Kaduna State – Kagarko
KDKS - Kaduna State – Kaduna South
KNDBT – Kano State – Danbata
KNGY – Kano State – Gaya
KNKN - Kano State – Kano
KTBKR – Katsina State – Bakori
KTCRC – Katsina State - Charanchi
KTDR - Katsina State - Daura
ZMGS - Zamfara State - Gusau
ZMKND – Zamfara State – Kaura Namoda
ZMTMF – Zamfara State – Talata Mafara

Centroids of C. *subinnotatus* morphoclusters based on morphometric studies are shown in Figure 1. The result revealed that morphocuster 2 had higher centroid mean values at bl (4.74 mm), bw (2.40), hfw (0.73), lp (1.14 mm), wpa (0.75 mm) and wpb (1.60, mm) measurements as against 4.71 mm, 2.27 mm, 0.50 mm, 1.02 mm, 0.65 and mm, 1.35 mm on the same parameters of morphocluster 1 respectively. Conversely, morphocluster 1 had higher mean values at ew (0.51 mm), dbe (0.25 mm) and al (2.25, 2.05 mm) measurements than morphocluster 2.

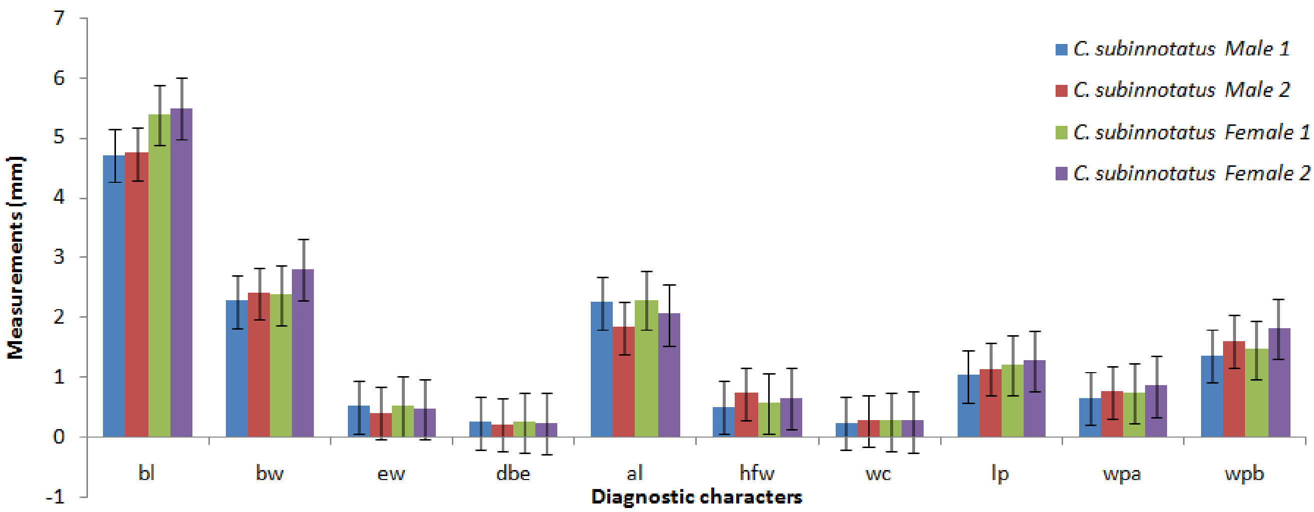


Figure 1 : Centroids derived from Cluster analysis of different variables of male and female Callosobruchus subinnotatus specimens collected from different locations in Northwestern Nigeria.

Key

bl – Body length
bw – Body width
ew – Eye width
dbe – Distance between eyes
al – Antennal length
hfw – Hind femoral width
wc – Width of coxa
lp - Length of pronotum
wpa – Width of pronotum anterior
wpb – Width of pronotum base

The within cluster variation simultaneous 95% confidence intervals of morphometric features showed that the means and mean ranges of bl, bw, hfw, lp, wpa and wpb in morphocluster 2 were significantly (P<0.05) higher than the same parameters in morphocluster 1 and the overall means. Conversely, morphocluster 1 were significantly (P<0.05) higher than the overall means and morphocluster 2 mean value at, ew, ew: dbe, as shown in Figures 2a-m below. For instance, bl of morphocluster 2 with mean value 4.74 mm ranging between 4.56-4.90 mm was significantly (P<0.05) higher than the mean of morphocluster 1 (4.71 mm, ranged 4.57-4.87 mm) and above the overall mean (4.72 mm). Similarly, bw of morphocluster 2 (2.40 mm, ranged between 2.33-2.45 mm) was significantly (P<0.05) higher than the mean value of morphocluster 1 (2.27 mm, ranged 2.22-2.35 mm) and the overall mean (2.32 mm). hfw mean and range values (0.70mm, o.5-1.0 mm of morphocluster 2 was significantly (P<0.05) higher than the mean and ranges of morphocluster 1 (0.50 mm, ranged 0.48- 0.55 mm) and the overall means (0.58 mm). Conversely, morphocluster 1 mean and range values at ew (0.51 mm, ranging 0.49-0.52 mm) was significantly (P<0.05) higher than the overall means (0.23 mm) and morphocluster 2 means and range values (0.40 mm, 0.30-0.42 mm). As in ew, the mean value and ranges of dbe and al were also significantly (P<0.05) higher than the same parameters in morphocluster 2 and the overall means.

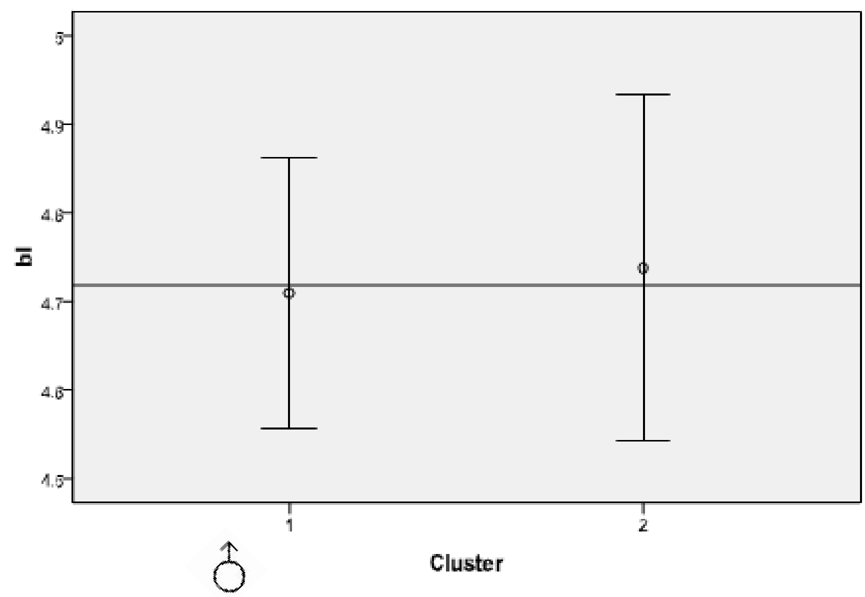
IV. Discussion

In common with several other successful insect pests, C. subinnotatus as a species shows great intraspecific variation in among large morphological traits often accompanied by a tremendous ability to adapt to localize environmental conditions (Ndong et al., 2012).

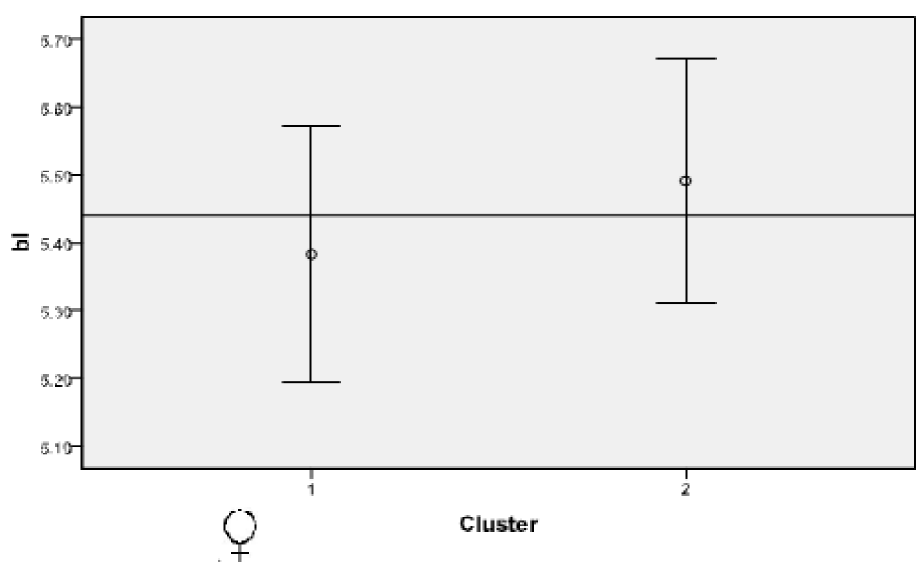
The occurrence of intraspecific variation will have a significant impact on the development and implementation of effective, long term and sustainable control method against C. subinnotatus and, potentially, other bruchids species.

Morphometric studies on the species gave two distinct morphoclusters (1 and 2) and possibly suggesting the existence of two morphs or races of C. subinnotatus in Northwestern Nigeria. Centroids of morphocluster 2 are significantly (P<0.05) higher than morphocluster 1 at body length; body width; length of pronotum; width of pronotum anterior and with of pronotum base while at eye width; distance between eyes and antennal length Morphocluster 1 had higher values. These findings have shown that morphocluster 2 are relatively larger in size than morphocluster 1 and both forms studied were uniformly dark brown to black with elytra relatively longer than wide.. This corroborates the work of Credland and Appleby (2001) which reported the existence of two adult morphs of C. *subinnotatus* termed 'active' and 'normal' forms. They differed in morphology, physiology and behavior and that variation in their characteristics suggests their adaptation to different environments of field and seed stores.

The hind femoral width, eye width and width of pronotum base were both twice the width of coxa, distance between eyes and width pronotum anterior respectively and thus may serve as additional taxonomic tool for identifying C. *subinnotatus*. The explanatory variables revealed hind femur to be bicarinate with inner tooth acutely triangular and slightly longer than the outer tooth concurring with the report of Southgate, (1958).

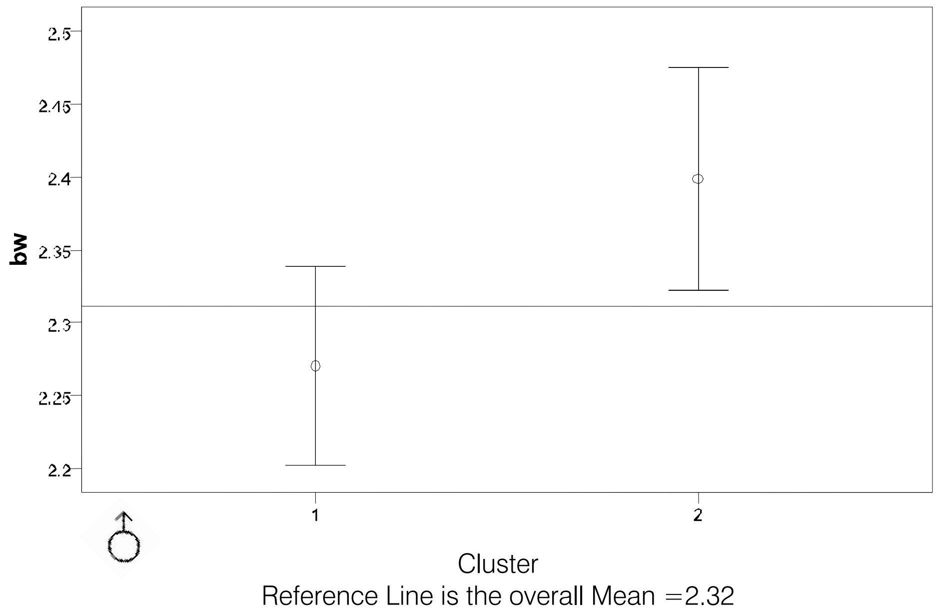


Cluster
Reference Line is the overall Mean =4.72



Cluster
Reference Line is the overall Mean =5.44

Figure 2a : Means and Simultaneous 95% Confidence Intervals of body length (bl) in *Callosobruchus subinnotatus* male and female respectively.



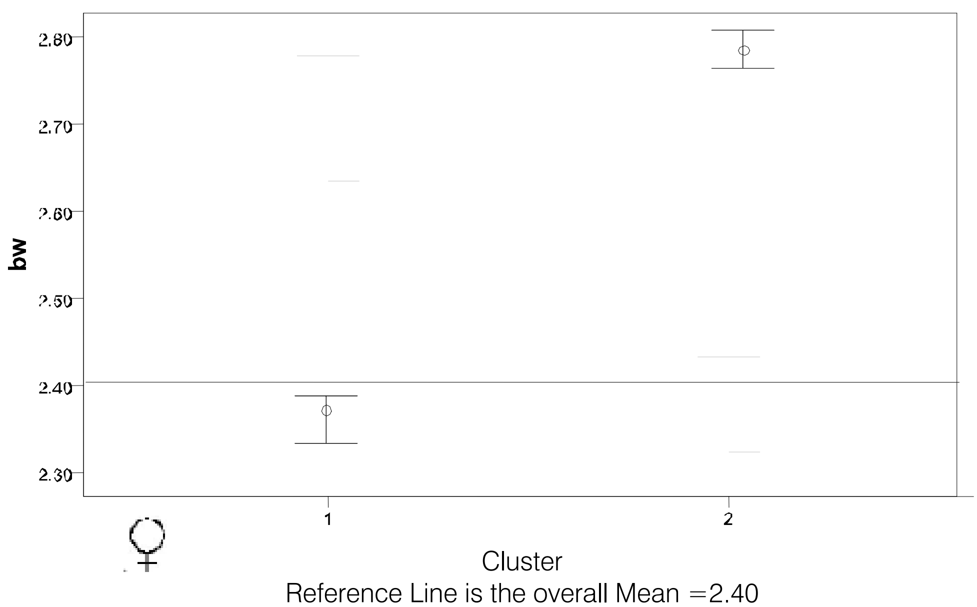


Figure 2b : Means and Simultaneous 95% Confidence Intervals of body width (bw) in *Callosobruchus subinnotatus* male and female respectively.

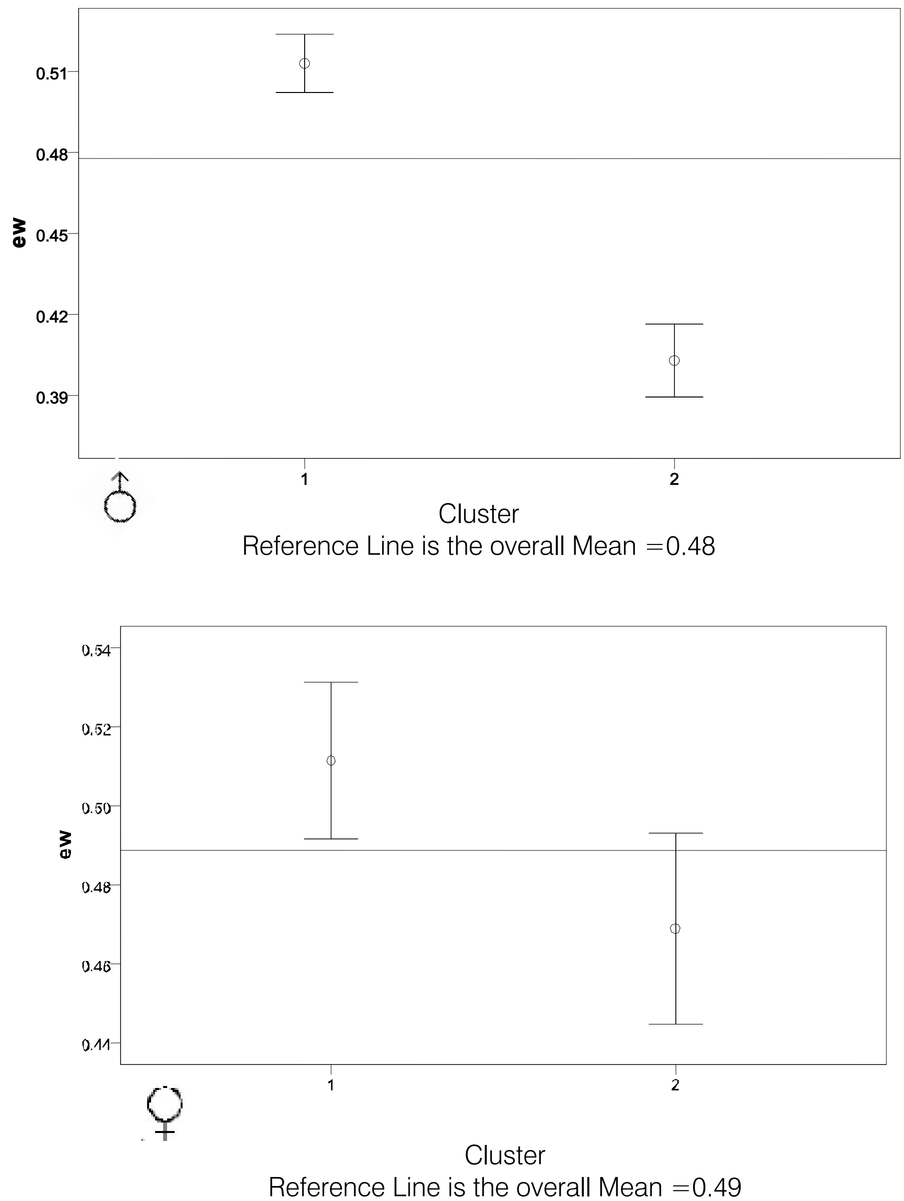
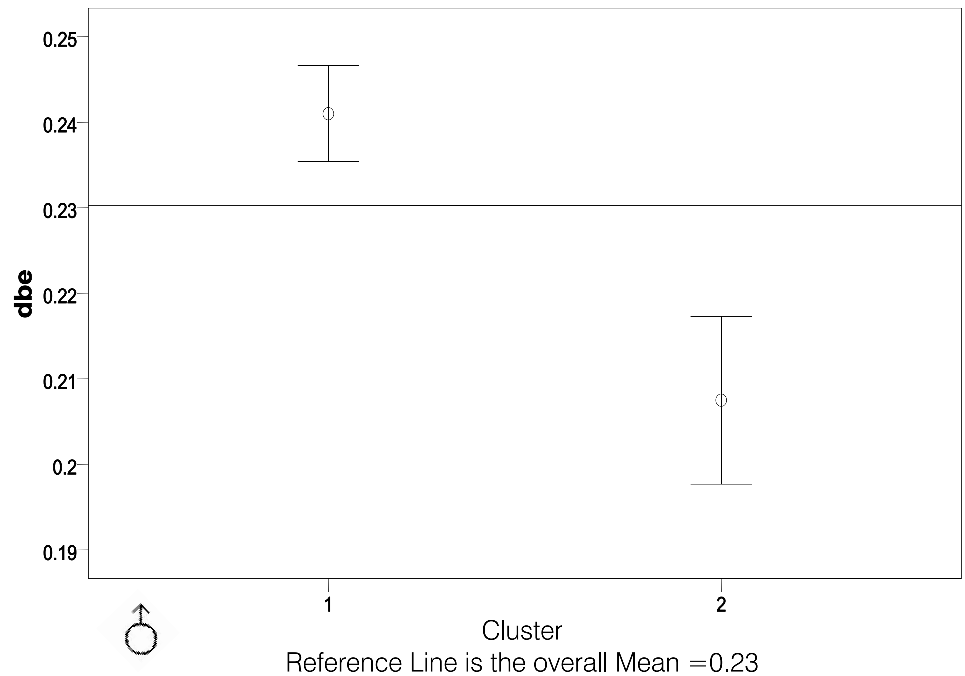


Figure 2c : Means and Simultaneous 95% Confidence Intervals of eye width (ew) in *Callosobruchus subinnotatus* male and female respectively.



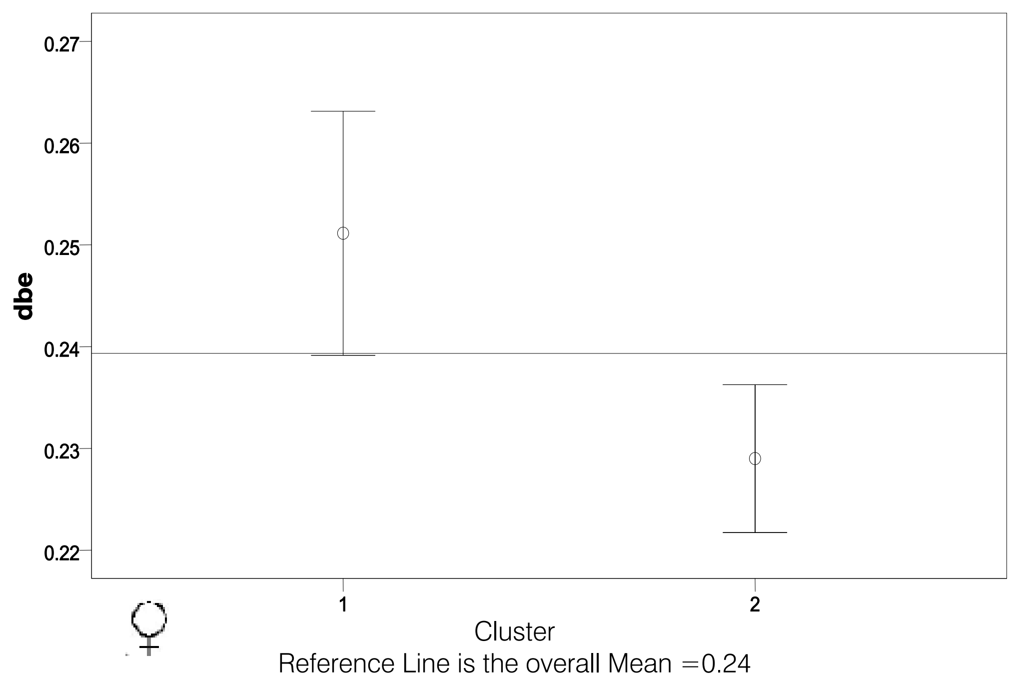
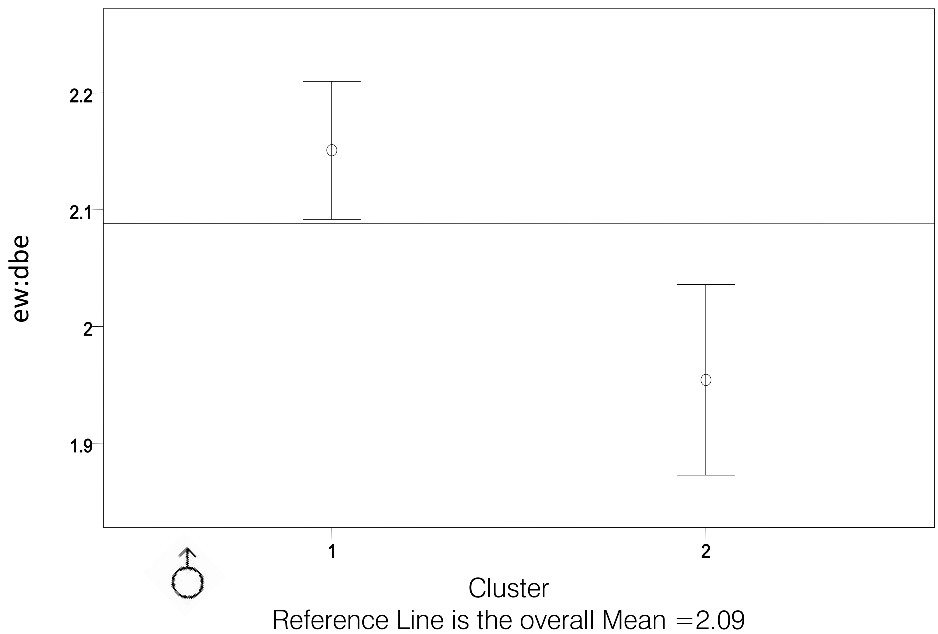


Figure 2d : Means and Simultaneous 95% Confidence Intervals of distance between eyes (dbe) in *Callosobruchus subinnotatus* male and female respectively.



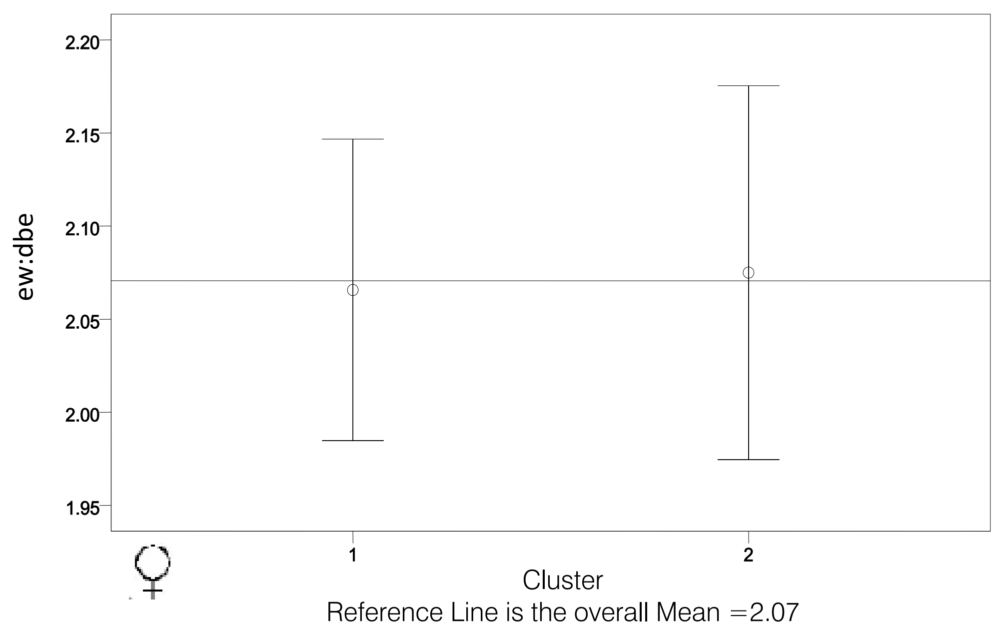


Figure 2e : Means and Simultaneous 95% Confidence Intervals of eye width ratio distance between ey (ew:dbe) *Callosobruchus subinnotatus* male and female respectively.

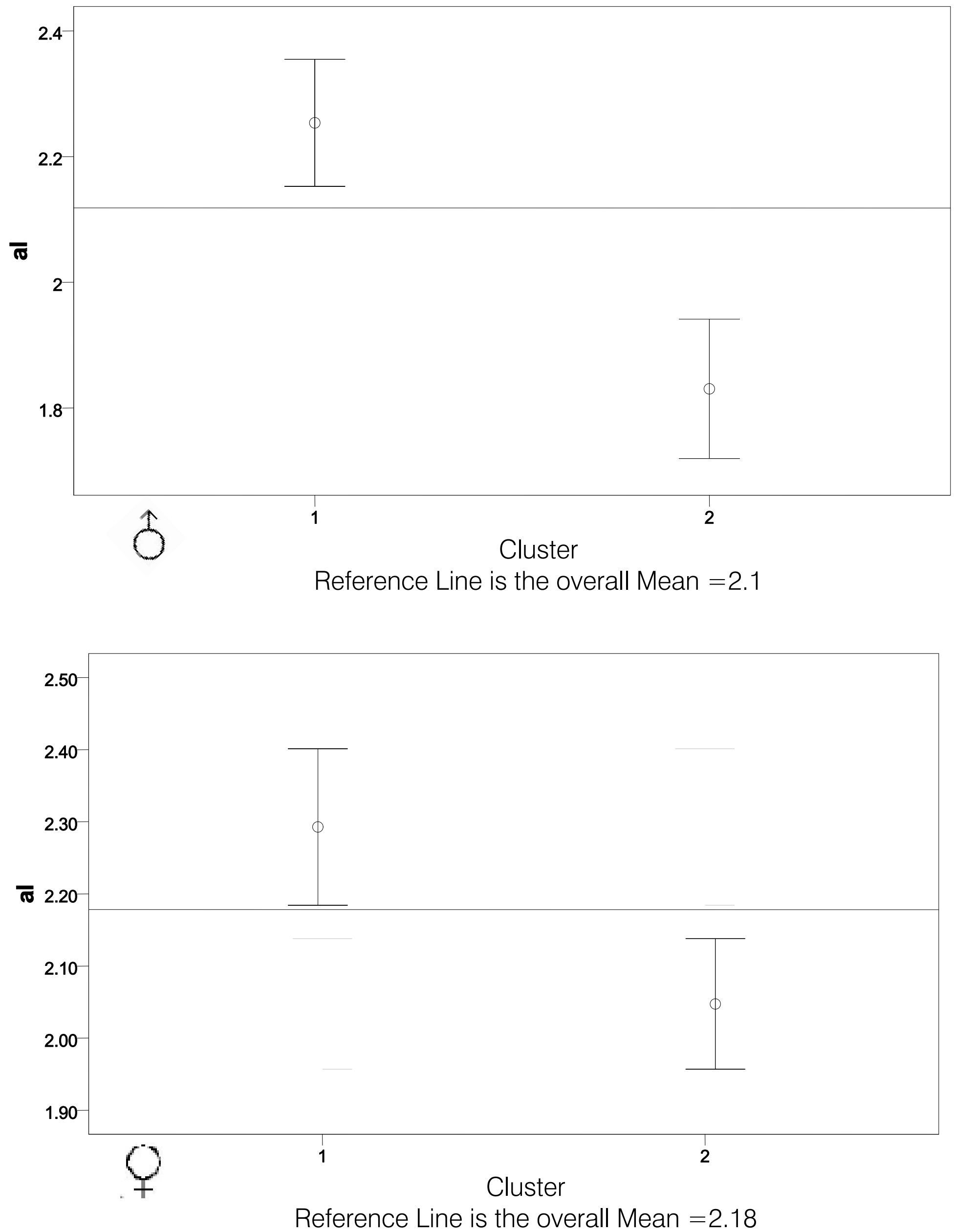
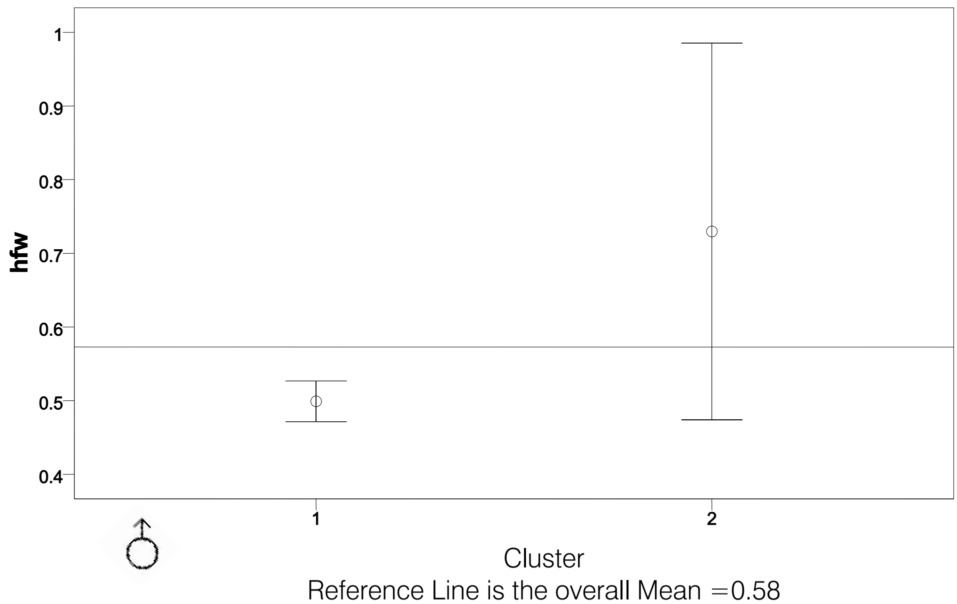


Figure 2f : Means and Simultaneous 95% Confidence Intervals of antennal length (al) in *Callosobruchus subinnotatus* male and female respectively.



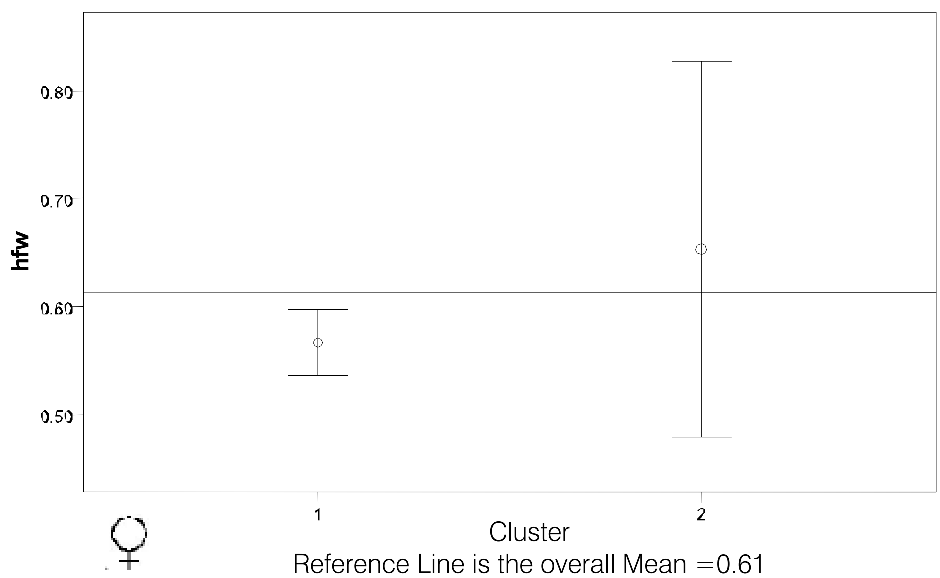
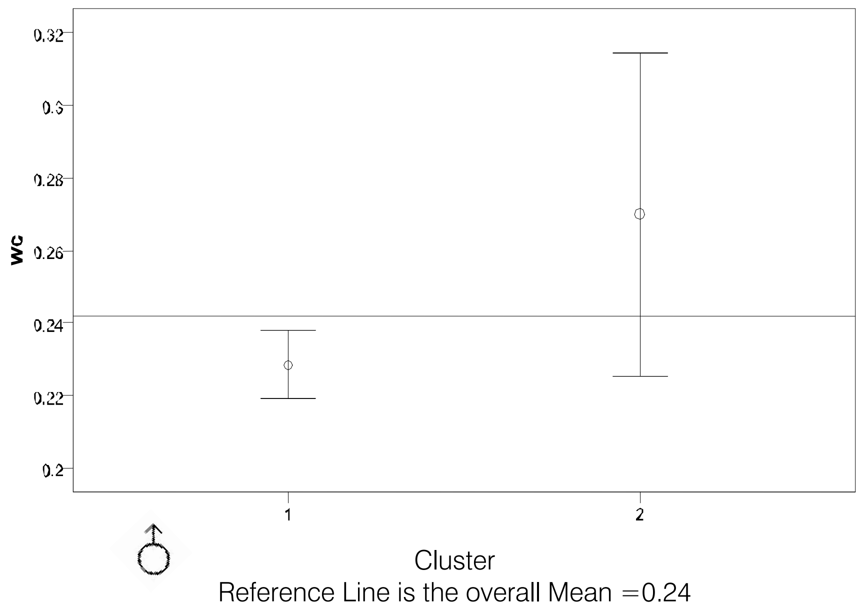


Figure 2g : Means and Simultaneous 95% Confidence Intervals of hind femoral width (hfw) in *Callosobruchus subinnotatus* male and female respectively.



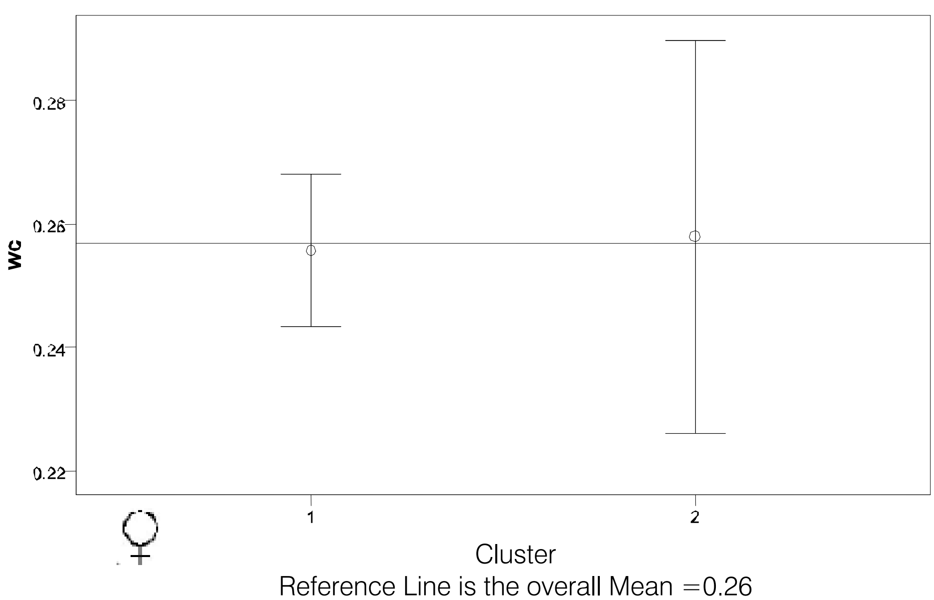
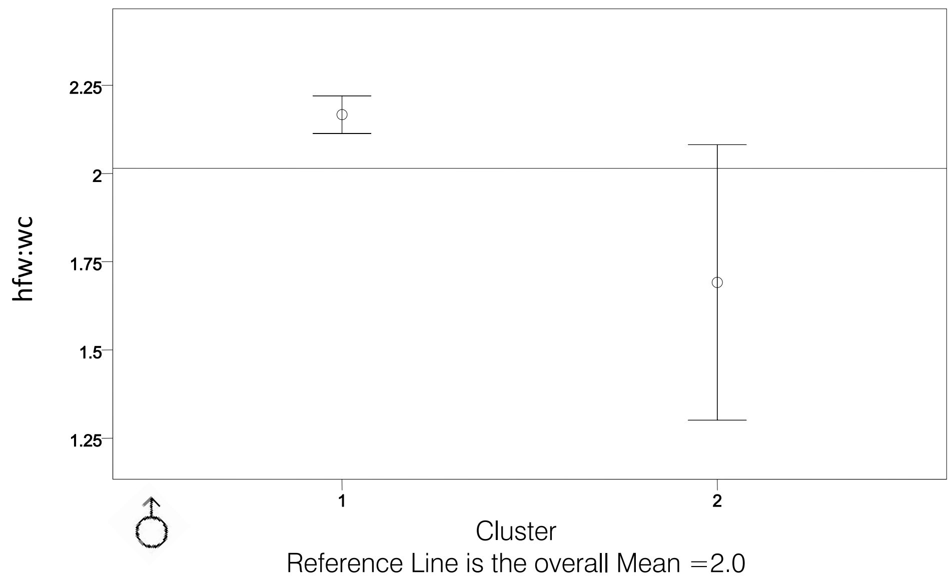


Figure 2h : Means and Simultaneous 95% Confidence Intervals of width of coxa (wc) in *Callosobruchus subinnotatus* male and female respectively.



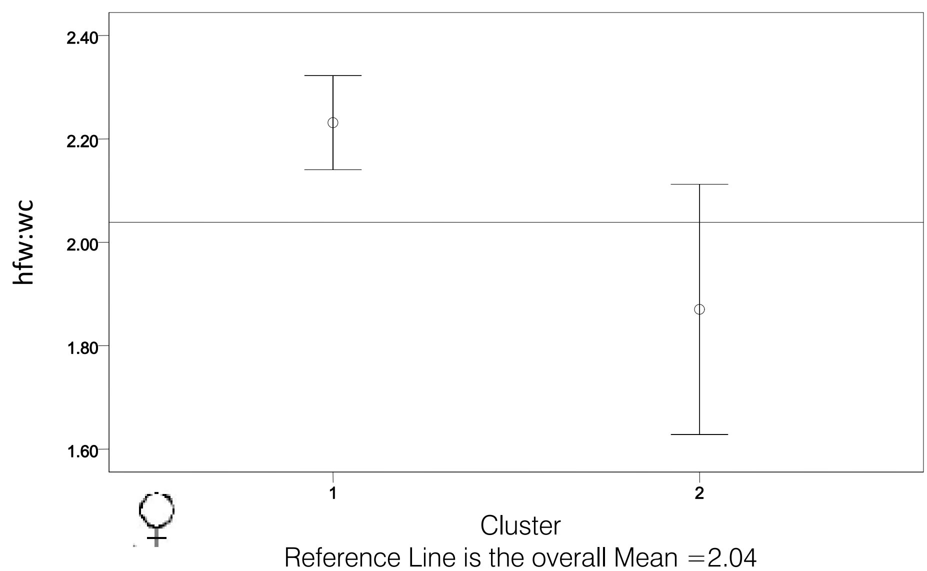
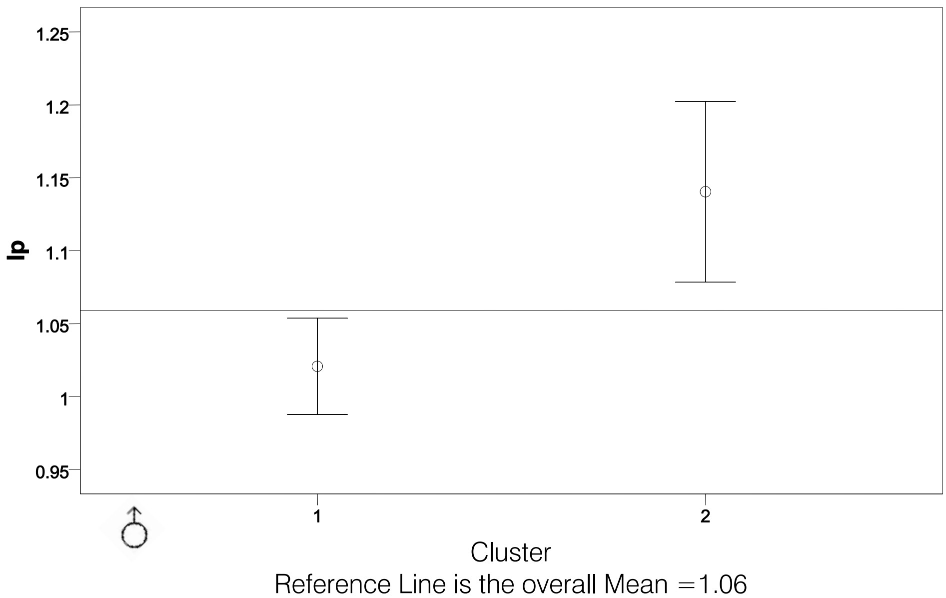


Figure 2i : Means and Simultaneous 95% Confidence Intervals of hind femoral width ratio width of coxa (wc) in *Callosobruchus subinnotatus* male and female respectively.



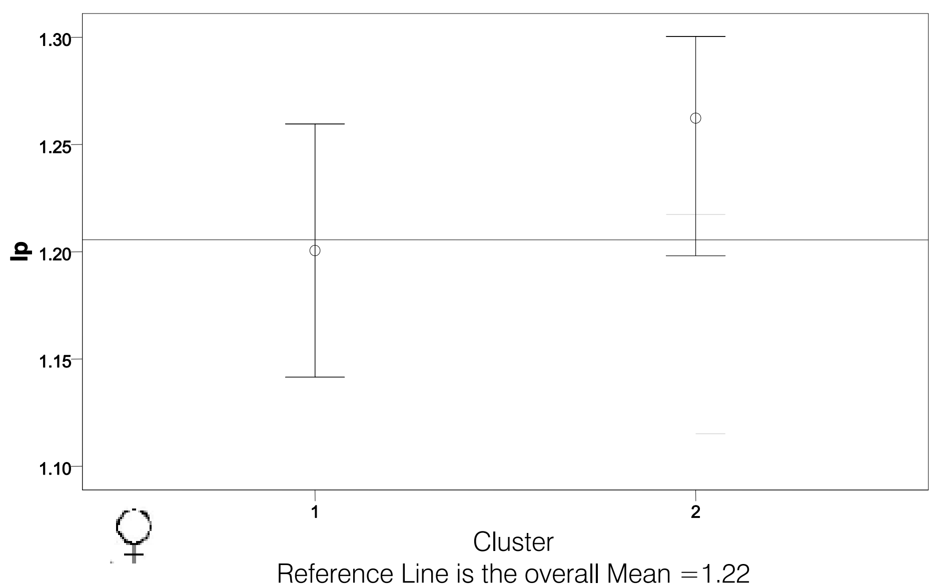
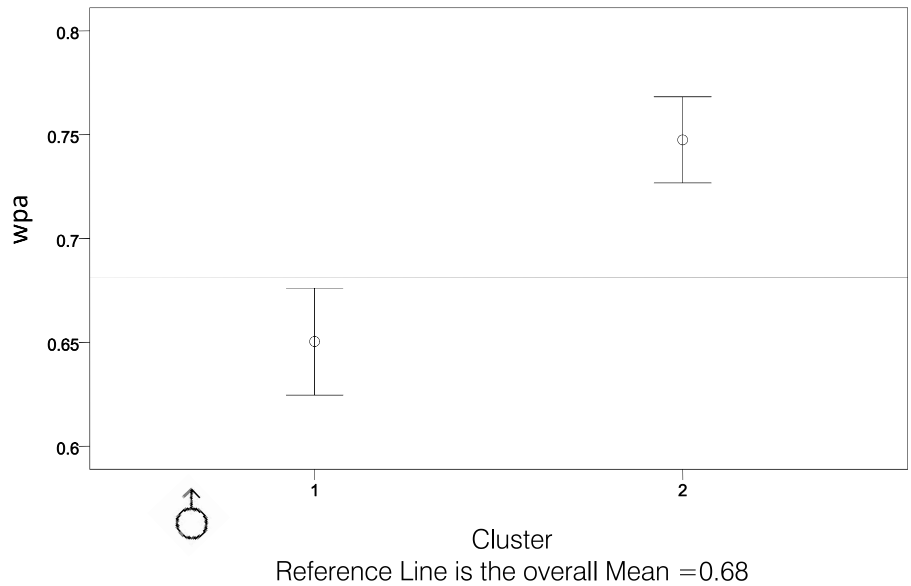


Figure 2j : Means and Simultaneous 95% Confidence Intervals of length of pronotum (lp) in *Callosobruchus subinnotatus* male and female respectively.



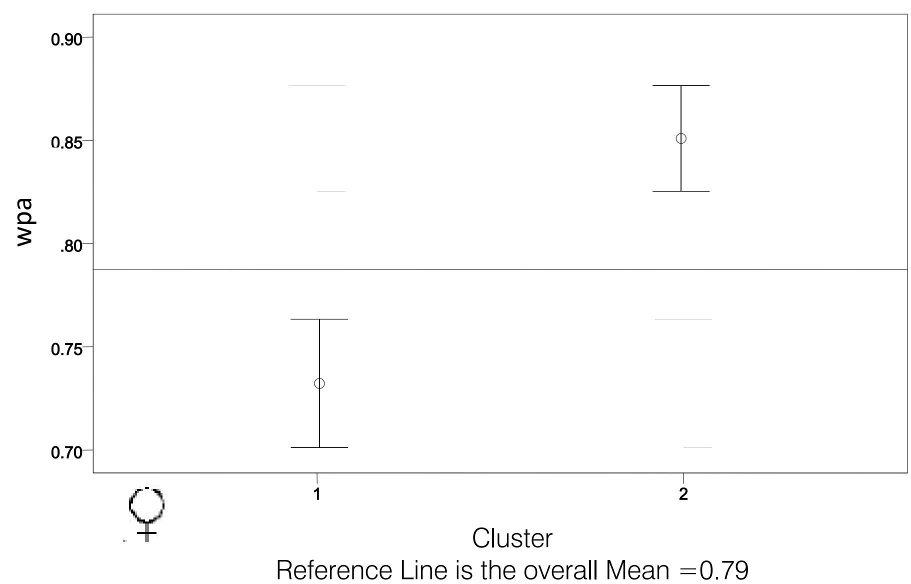


Figure 2k : Means and Simultaneous 95% Confidence Intervals of width of pronotum anterior (wpa) in *Callosobruchus subinntatus* male and female respectively.

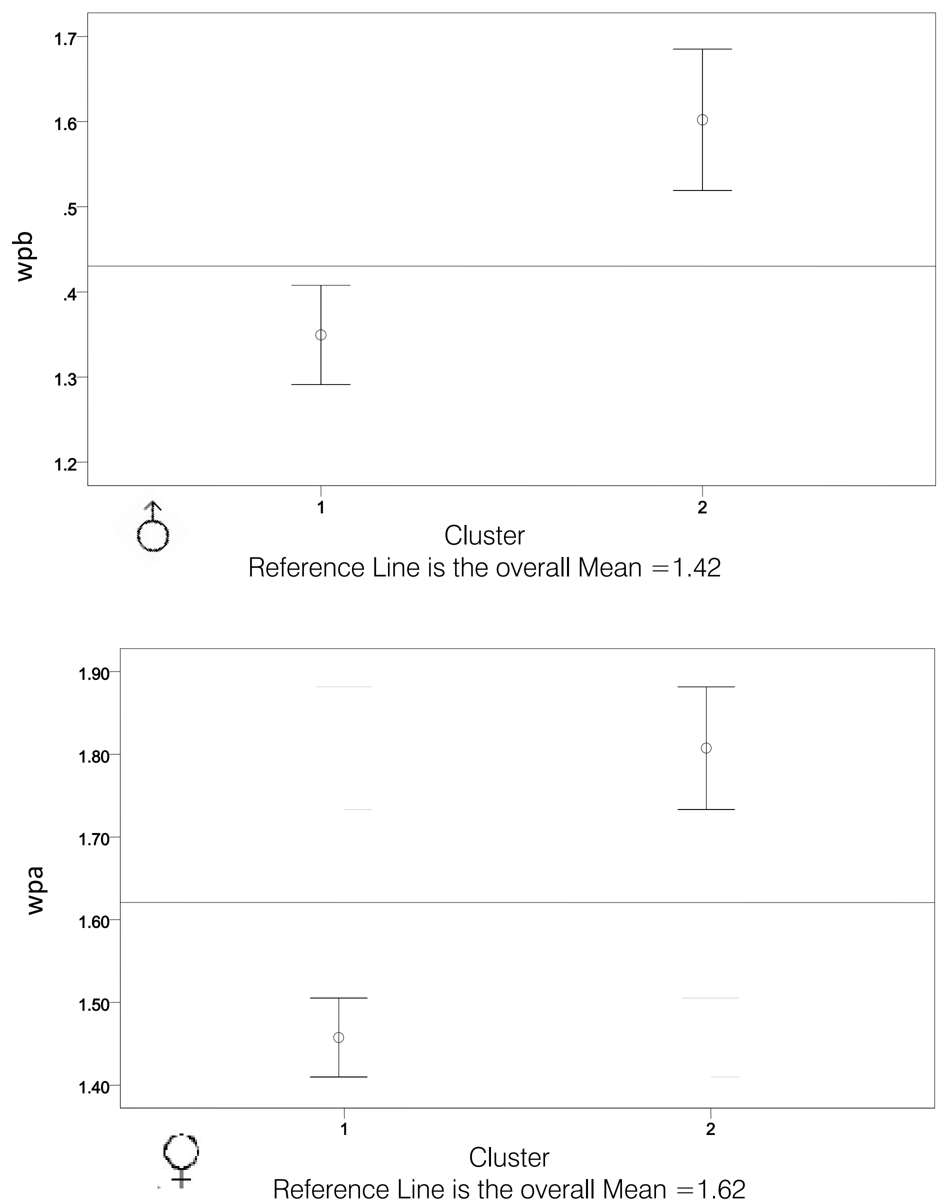
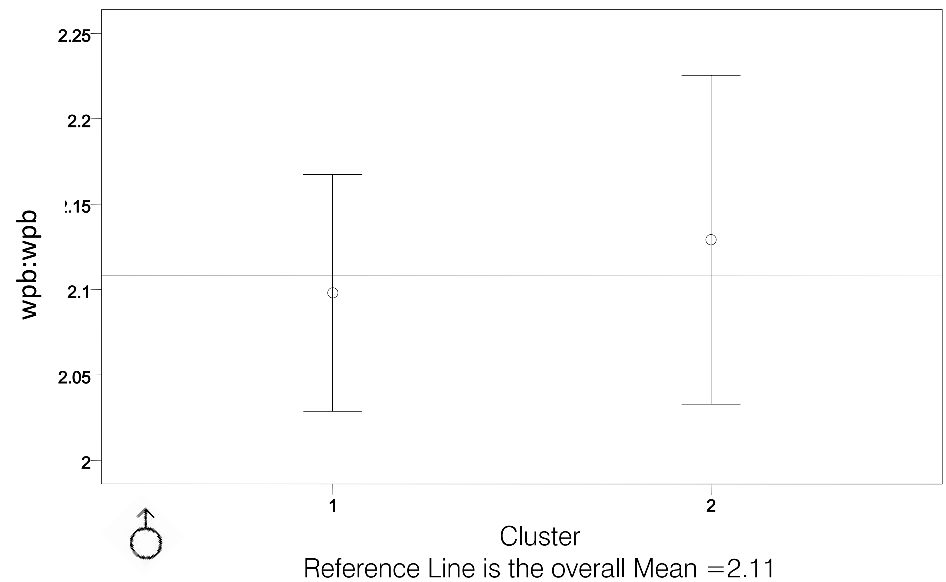


Figure 2l : Means and Simultaneous 95% Confidence Intervals of width of pronotum base (wpb) in *Callosobruchus subinnotatus* male and female respectively.



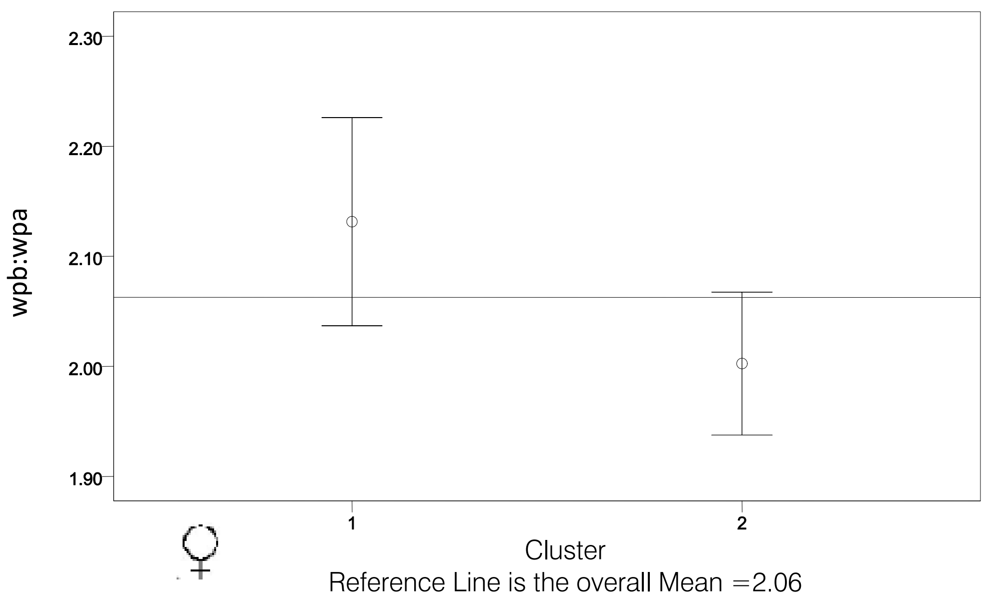


Figure 2m : Means and Simultaneous 95% Confidence Intervals of width of pronotum base ratio width of pronotum anterior (wpb:wpa) in Callosobruchus subinnotatus male and female respectively.

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