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Establishing The Degree of Relationship in *Cryptolepis sanguinolenta* (Lindl.) Schter.: An Antimalaria Plant Using Cluster Analysis

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Cluster Analysis was used to determine the levels of similarities or the degree of resemblance in *Cryptolepis sanguinolenta* (Lindl.) Schter., an antimalarial plant. The results showed that within the populations, Pepease and Mamfe showed a closer association or resemblance than of Abonse in all the morphological characters. The degree of relationship or resemblance of the populations in terms of anatomical characters was much varied. With Pepease and Abonse populations closely related than Mamfe in terms of the root fibre length with reference to the root vessel element, Mamfe and Abonse populations were much related whiles Pepease was loosely related and finally, Mamfe and Pepease populations also closely related than Abonse in terms of the root prismatic crystal length.

Key Words: Cluster analysis, *Cryptolepis sanguinolenta*.

INTRODUCTION

Mist Nibima, prepared from the roots of *Cryptolepis sanguinolenta* is prescribed mainly for the management of malarial fever and urinary tract infections[1]. The plant, *C. sanguinolenta* is or will be a great potential in the quest by researchers, para-medicals and herbalists for an antimalarial drug.

In studying the plant species phenetically, all characters from any part of the plant body must be considered. This could be morphological, anatomical, cytological, physiological, biochemical, ecological, geographical, *etc*. and were employed throughout the plant species range of distribution as suggested by Davis and Heywood[2], Sneath[3], Sneath and Sokal[4,5], Jardine and Sibson[6] and Dunn and Everitt[7].

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Cluster analysis is an exploratory procedure, which is often helpful in understanding the complex nature of multivariate relationships. The objective of this technique is to group observations into clusters such that each cluster is a homogenous as possible with respect to the clustering variables. The identification of clusters enables one to know exactly which variables are close to each other. The technique does not make any distribution assumption. There are a number of computer programmes[8] (SPSS, SAS, Minitab, *etc*.) available for performing cluster analysis[9,10].

This study aims to establish a theoretical model for the categorization of the degree of relationship and the existence of variation in the plant species, *Cryptolepis sanguinolenta* using morphological and anatomical characters through the application of cluster analysis.

EXPERIMENTAL

C. sanguinolenta (Lindl.) Schtr. was collected from three sampling areas namely: Pepease, Mamfe and Abonse, and identified at the Centre for Scientific Research into Plant Medicine and the Environmental Science Department, University of Cape Coast. The voucher specimen has been deposited in the Department of Environmental Science, University of Cape Coast, Ghana.

Morphohistological studies: Morphological and histological studies were carried out in the plant species with the view to determine those characters (Figs. 1-3), which could be diagnostic for the delimitation of the plant species.

Morphological studies: Morphological characters were scored for the morphometric analysis. They were measured using a digimatic caliper and hand lens. Of these characters, radical leaf blade length (LfL) and width (LfW) and petiole length (LfPtL) (Fig. 1) were measured from fifty leaves were taken alongside the length and width of pod (PodL and PodW) were measured on ten randomly selected plant from each location (Fig. 2). The length and width of the seed (SeedL and SeedW) were measured on ten replicates alongside the length of the seed plus hair (SedhirL) as well as seed hair length (HairL) (Fig. 3) were measured on five mature specimen from each of the locations.

Histological studies: The anatomical studies of twenty plant species from each of the sample sites was carried out by macerating the root of the plant species.

Maceration of the root and stem: The root of the plant species were harvested from five different trees from each of the three sample locations (Pepease, Mamfe and Abonse). The root each of about 10 cm long were cut into small pieces to the thickness of match-sticks and put into beakers. A mixture of 2:1 (v/v) of absolute concentration each of hydrogen peroxide and acetic acid was added to the contents of the beakers and heated gently to boiling point on a water-bath in a fume chamber, until the tissues were softened and turned whitish. The beakers were sealed mesh of 212 μm and contents were then thoroughly washed with distilled water.

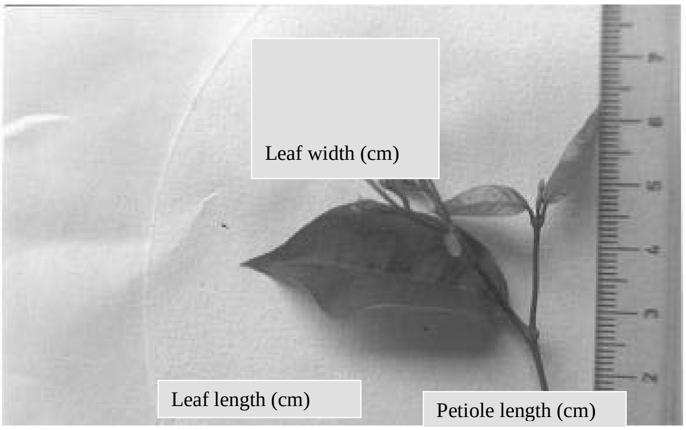

Fig. 1. The leaf characters of the plant species, *C. sanguinolenta* obtained from Pepease (X1)

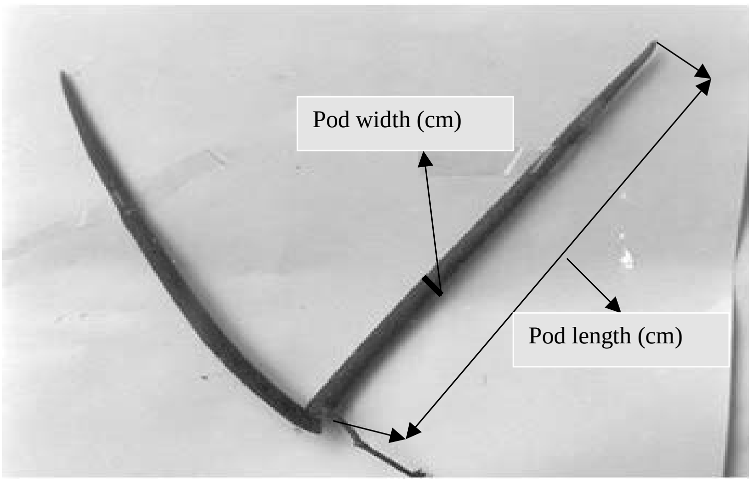

Fig. 2. The pod characters of the plant species, *C. sanguinolenta* obtained from Pepease (X1)

Drops of the macerated samples were then placed on glass slides and covered with cover slips and examined under high power magnification of a light microscope. The length and width of the following: fibres, vessel element and prismatic crystals (Figs. 4-6) were measured with the eyepiece graticule at a magnification of X40. Slides, which were found to be satisfactory, were photographed. In all 30 slides were prepared, 5 each for the root from each of the sample areas.

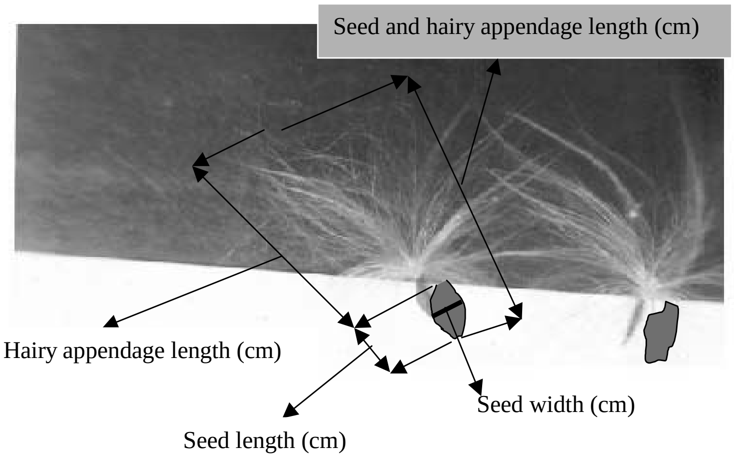

Fig. 3. The seed characters of the plant species, *C. sanguinolenta* obtained from Pepease (X1)

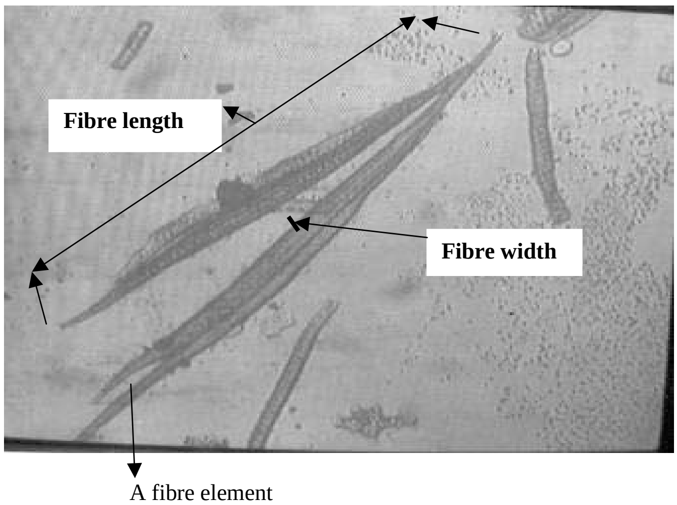

Fig. 4. Nature of fibres isolated from the root of *C. sanguinolenta* obtained from Pepease (X100)

Statistical analysis

A series of linear models (SPSS 10.0 and Minitab 13.32 for WINDOWS, Ver. 6.1) were fitted to the dataset to determine whether there is variation in the plant, *Cryptolepis sanguinolenta* collected from the populations (Systat. 2000). The variables used in this analysis were leaf morphological characters, fruit pod characteristics, fruit seed characteristics and anatomical characters. A cluster analysis using average linkage (Ward's method) was performed on the modified data to evaluate the degree of similarity, association, closeness or resemblance among the variables within the populations.

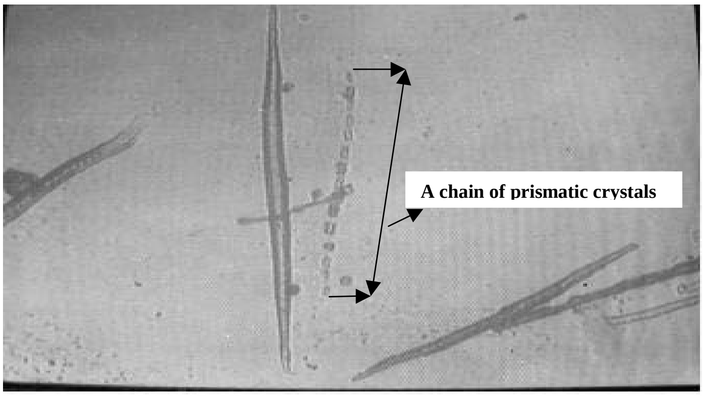

Fig. 5. A fibre and a chain of prismatic crystals isolated from the root of *C. sanguinolenta* obtained from Pepease (X100)

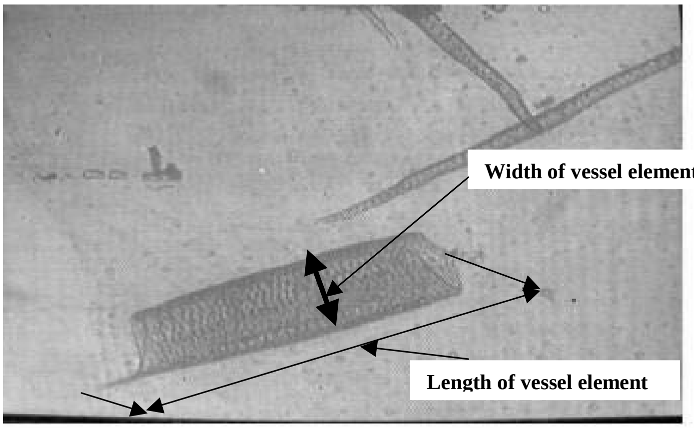

Fig. 6. Nature of vessel element showing bordered pits isolated from the root of *C. sanguinolenta* obtained from Pepease (X100)

RESULTS AND DISCUSSION

The results presented show the phentic relationships of the locations using morphological characters such as, leaf characters (Fig. 1), fruit pod characters (Fig. 2), fruit seed characters (Fig. 3) and anatomical characters; fibre (Fig. 4), vessel element (Fig. 5) and prismatic crystal (Fig. 6) of the plant species, *C. sanguinolenta* as OTUs.

Digramatically, Figs. 7b and 7c present a strong magnitude of association, closeness or resemblance between the leaf length of the plant obtained from Pepease (P-LfL) and that obtained from Mamfe (M-LfL), which falls within 80 - 89 % similarity level. The leaf length of the plant obtained from Abonse (A-LfL), which falls around 0 to 39 %, depicts a loose resemblance or weak association with the leaf lengths of Pepease and Mamfe, as reflected by the depth of shading in Fig. 7c.

Dendrogram using Ward Method

Rescaled Distance Cluster Combine

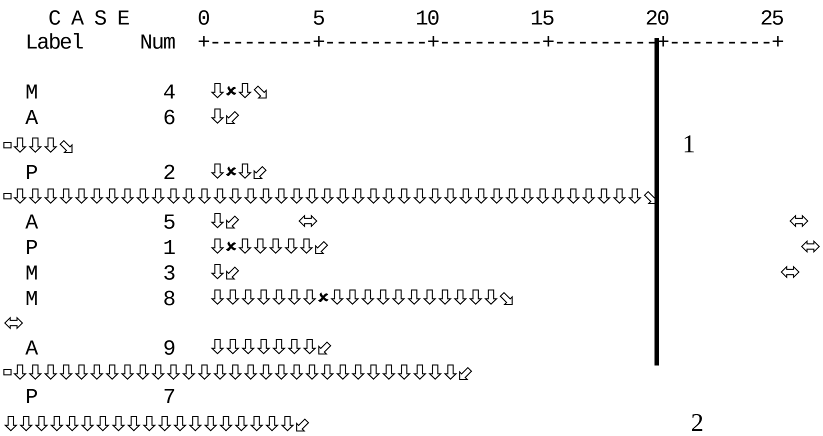

Fig. 7a. Dendrogram using Ward's method to show the degree of association within leaf morphological characters in the populations of individual score of *C. sanguinolenta*

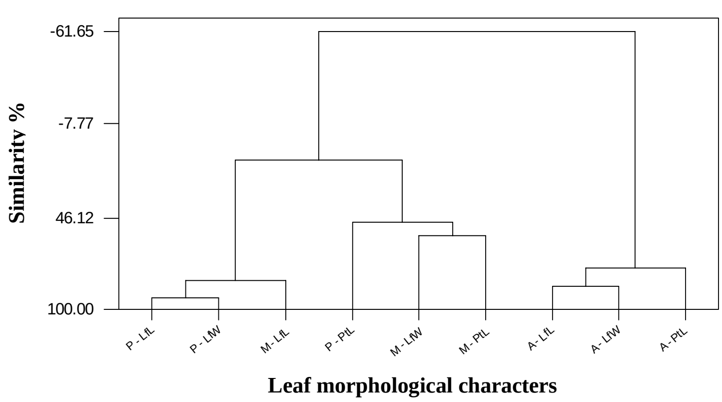

Fig. 7b. Dendrogram representing the degree of similarity obtained from some leaf morphological characters of the plant

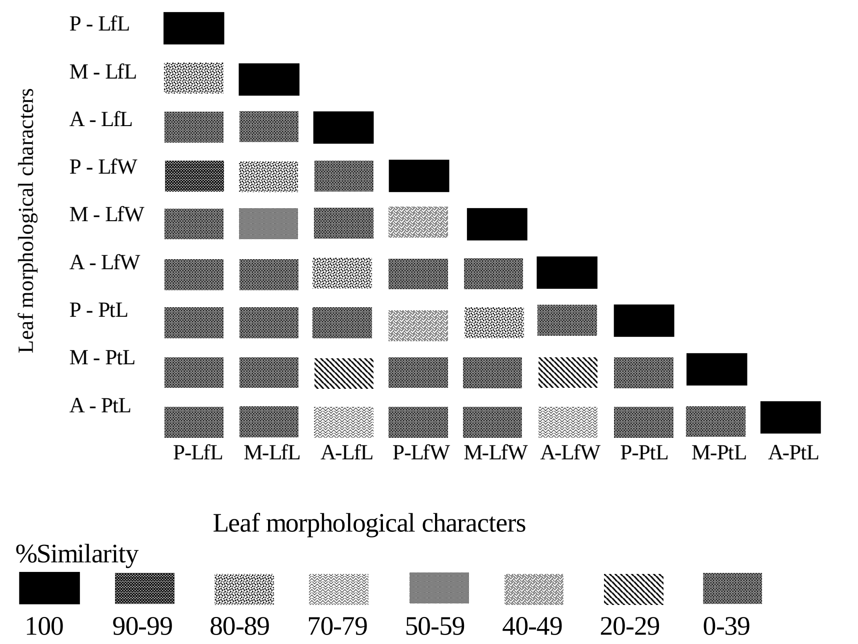

Fig. 7c. A schematic diagram showing a matrix of similarity coefficients between pairs of variables/leaf morphological characters of the plant in the three populations (Taxa -Operational Taxonomic Units). The magnitude of the coefficients is shown by the depth of shading

Two clustering groups showing patterns of variations of individuals at a similarity or rescaled distance of 5 or 20 is indicated in Fig. 8a below:

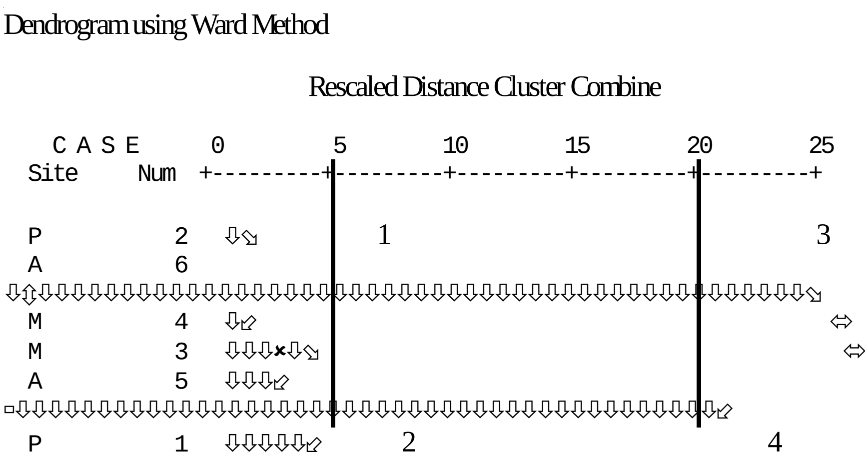

Fig. 8a. Dendrogram using Ward's method using fruit pod characteristics of *C. sanguinolenta* obtained from the locations

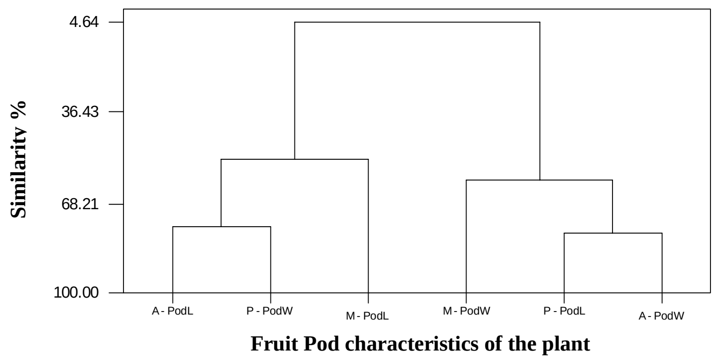

Fig. 8b. Dendrogram representing the degree of similarity of the fruit pod characteristics of the plant drawn in the manner

Figs. 8b and 8c present a stronger magnitude of association, closeness or resemblance between the fruit pod length of the plant obtained from Abonse (A-PodL) and that obtained from Mamfe (M-PodL), which falls within 50 - 59 % similarity level. The fruit pod length of the plant obtained from Pepease (P-PodL), which falls within 0 to 9 %, depicts a loose resemblance or weak association with the fruit pod lengths of Abonse and Mamfe, as reflected by the depth of shading in Fig. 8c.

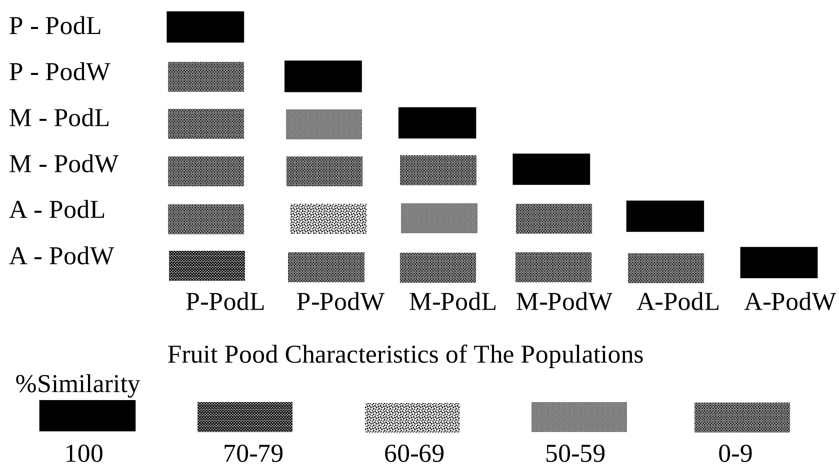

Fig. 8c. A schematic diagram showing a matrix of similarity coefficients between pairs of variables/fruit pod characteristics in the various plant organs (Taxa - Operational Taxonomic Units). The magnitude of the coefficients is shown by the depth of shading.

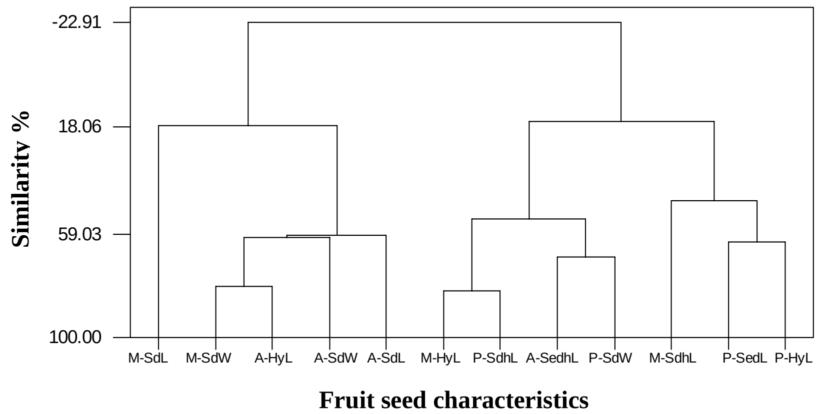

Fig. 9a. Dendrogram representing the degree of similarity of the fruit seed characteristics of the *C. sanguinolenta*

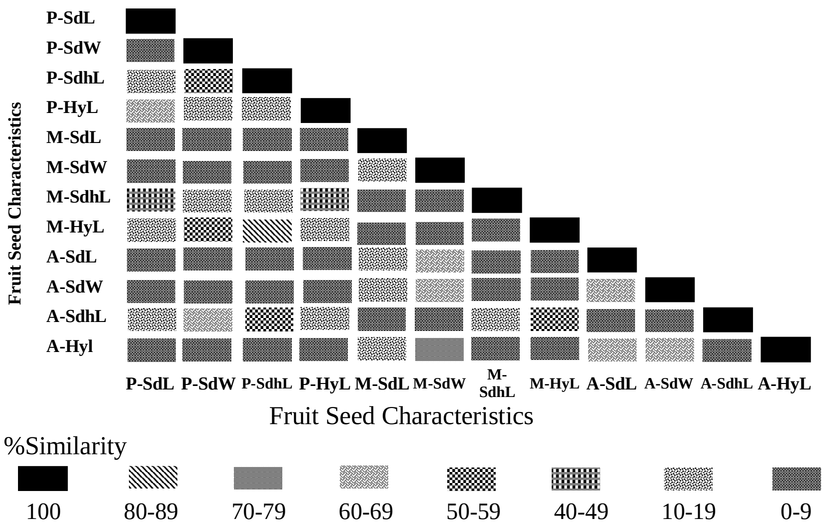

Fig. 9b. A schematic diagram showing a matrix of similarity coefficients between pairs of variables/fruit seed characteristics of *C. sangunolenta* obtained from the populations (Taxa Operational Taxonomic Units). The magnitude of the coefficients is shown by the depth

For example, in Figs. 9a and 9b above, there is a strong magnitude of association, closeness or resemblance between the fruit seed length of the plant, *C. sangunolenta* obtained from Mamfe (M-SdL) and that from Abonse (A-SdL), which falls within 10 - 19 % similarity level. The fruit seed length of the plant obtained from Pepease (P-SdL), which falls within 0 to 9 %, depicts a loose resemblance or weak association with the fruit seeds from Abonse and Mamfe, as reflected by the depth of shading in Fig. 9b.

The results presented in Figs. 9a and 9b above, indicate a strong magnitude of association, closeness or resemblance between the fruit seed plus hairy appendage length of the plant, *C. sangunolenta* obtained from Mamfe (M-SdhL) and that from Abonse (A-SdhL), which falls within 10-19 % similarity level. The fruit seed length of the plant obtained from Pepease (P-SdL), which falls within 0 to 9 %, depicts a loose resemblance or weak association with the fruit seeds from Abonse and Mamfe.

Again, in Figs. 9a and 9b above, there is a strong magnitude of association, closeness or resemblance between the fruit seed hairy appendage only length of the plant, *C. sangunolenta* obtained from Mamfe (M-SdhL) and that from Abonse (A-SdhL), which falls within 10-19 % similarity level. The fruit seed length of the plant obtained from Pepease (P-SdL), which falls within 0 to 9 %, depicts a loose resemblance or weak association with the fruit seeds from Abonse and Mamfe, as reflected by the depth of shading in Fig. 9b.

The level of relationship of anatomical characters within the locations. Anatomical characters within the locations were utilized to determine the patterns of relationship at the intraspecific relationship within the locations by applying the Clarke and Warwick method. At a rescaled distance of 20, two clusters were identified at 1 and 2 (Fig. 10a) below:

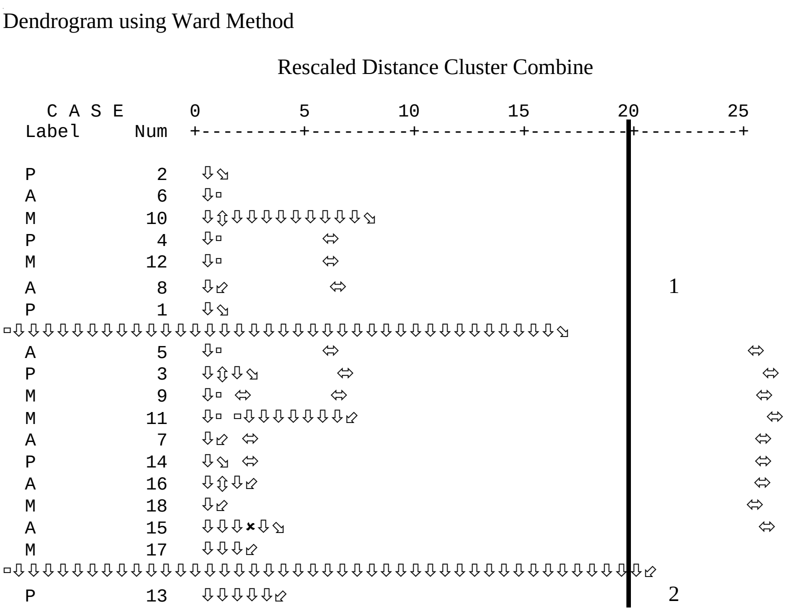

Fig. 10a. Dendrogram showing Ward's method using anatomical characters of *C. sanguinolenta* obtained from the populations

Figs. 10b and 10c presented below show low or weak magnitude of association, closeness or resemblance between the root fibre length of the plant obtained from Pepease (P-FiRL) and that obtained from Abonse (A-FiRL) which falls within 10-19 % similarity level. The root fibre length of the plant obtained from Mamfe (M-FiRL), which falls around 0 to 9 %, reflects a much looser or weaker resemblance with those of Pepease and Abonse, as reflected by the depth of shading in Fig. 10c.

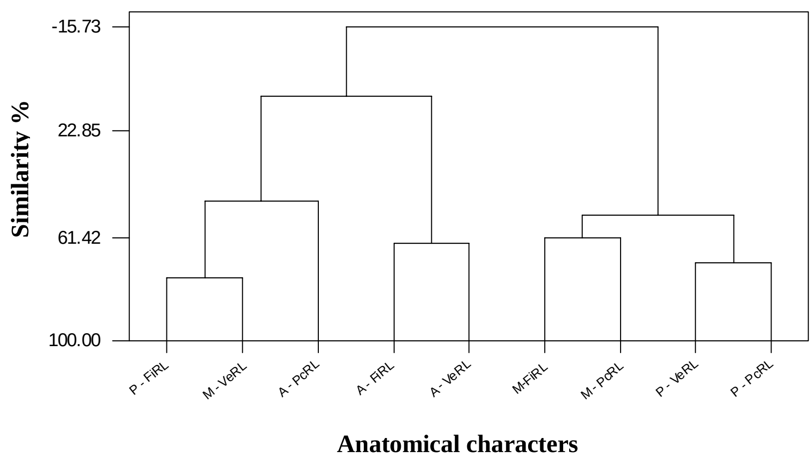

Fig. 10b. Dendrogram representing the hypothetical hierarchy obtained from root an anatomical characters

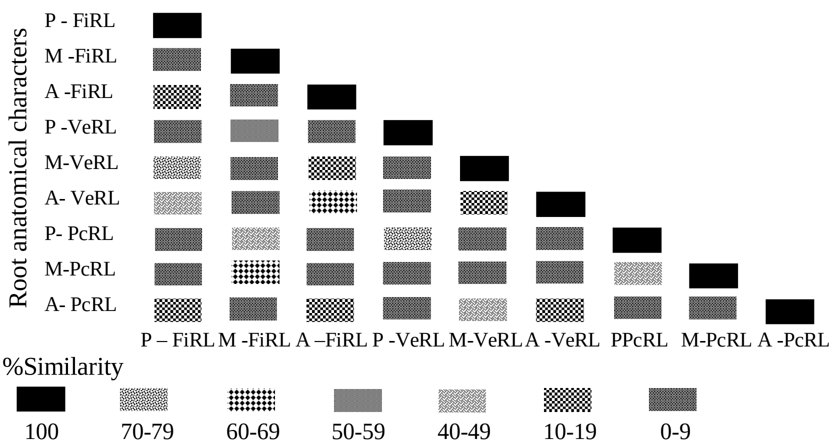

Fig. 10c. A schematic diagram showing a matrix of hypothetical similarity coefficients between pairs of variables/root anatomical characters of the plant in the three populations (Taxa-Operational Taxonomic Units). The magnitude of the coefficients is shown by the depth of shading

There was turn of events when the root vessel elements were considered; Mamfe and Abonse indicated a loose or weak resemblance within 10-19 % similarity level, whiles Pepease showed even a looser or weaker resemblance with the above pair (Figs. 10b and 10c).

Referring to the root prismatic length of the plant of the populations, the magnitude of shading proves the association, closeness or resemblance between Mamfe (M-PcRL) and that of Pepease (P-PcRL) at a similarity level of 40 to 49 % to be better than what was obtained from Abonse (A-PcRL) to be a weak or loose resemblance at 0 to 9, as shown in Figs. 10b and 10c above.

Addae-Mensah *et al.*[1], Sneath and Sokal[4,5], Sibson and Jardine[6], Sneath[3,5], Davis and Heywood[2] and Hatheway[11] have all reported on the use of a wide-range of characters, such as, morphology, anatomy, phytochemical constitutes, *etc.* in establishing the degree of association, resemblance or closeness (phenetic relationship) in order to create taxonomic groupings within populations. Similarly, the results presented in Figs. 7a-c, 8a-c, 9a-c (morphological characters) and 10a-c (anatomical characters) based on the dendrograms and the schematic presentations all tend to delimit the plant species, *C. sanguinolenta* into two distinct taxonomic groupings.

Conclusion

The application of morphological and anatomical characters also led to the establishment of two phenetic patterns. Generally, there was much phenetic relationship between Mamfe and Abonse populations than with Pepease in terms of the following characteristics; fruit pod, seed and hairy appendage and hairy appendage. An exceptional situation was with the leaf characteristics where Pepease and Mamfe populations are phenetically related with Abonse being loosely associated.

Two phenetic groupings were observed with reference to the total alkaloid content of the plant organs of the plant species, with Pepease and Abonse establishing a closer relationship in terms of the total alkaloid content in the root and stem with Mamfe being loosely associated. A different relationship was encountered with the leaf total alkaloid where the degree of relationship between Pepease and Mamfe was very strong, with Abonse showing a loose relationship.

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